Cambridge Monographs on Cancer Research

Cyclopenta[a]phenanthrenes
Cambridge Monographs on Cancer Research

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Books in this Series
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Cyclopenta[a]phenanthrenes

Polycyclic aromatic compounds structurally related to steroids

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Cyclopenta(a)phenanthrenes: polycyclic aromatic compounds structurally related to steroids.

1. Cyclopentaphenanthrenes
I. Title II. Bhatt, Tarlochan S.
547'.5 QD395

Library of Congress cataloguing in publication data
Coombs, Maurice M.
Cyclopenta(a)phenanthrenes: polycyclic aromatic compounds structurally related to steroids.
(Cambridge monographs on cancer research)
Includes bibliographies and index.
2. Carcinogens. I. Bhatt, Tarlochan S. II. Title.
III. Series. [DNLM: 1. Carcinogens. 2. DNA metabolism. 3. Gonanes. QU 85 C775c]
RC268.7.C95C66 1987 616.9'4071 86-34339

ISBN 0 521 30123 8
This book is dedicated to the memory of our colleague and friend Dr David Campbell Livingston, 1936–1983, late of the Chemistry Laboratory, Imperial Cancer Research Fund, London.
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Abbreviations employed in this book

Journal titles

*Acta Crystallogr.*  Acta Crystallographica
*Acta Unio Int. Cancrum*  Acta Unio Internationalis contra Cancrum
*Adv. Lipid Res.*  Advances in Lipid Research
*Am. J. Cancer*  American Journal of Cancer
*Am. J. Physiol.*  American Journal of Physiology
*Annalen*  Justus Liebigs Annalen der Chemie
*Arch. Biochem. Biophys.*  Archives of Biochemistry and Biophysics
*Arch. Geselwulstforsch.*  Archiv für Geselwulstforschung
*Ber.*  Berichte der Deutschen Chemischen Gesellschaft
*Biochem. J.*  Biochemical Journal
*Biochem. Biophys. Acta*  Biochimica et Biophysica Acta
*Br. J. Cancer*  British Journal of Cancer
*Cancer Biochem. Biophys.*  Cancer Biochemistry and Biophysics
*Cancer Lett.*  Cancer Letters
*Cancer Res.*  Cancer Research
*Chem. Abst.*  Chemical Abstracts
*Chem. Ber.*  Chemische Berichte
*Chem. Ind. (London)*  Chemistry and Industry (London)
*Chem. Geol.*  Chemical Geology
Abbreviations

Edinburgh Med. J.  Edinburgh Medical Journal
FEBS Lett.  Federation of European Biochemical Societies Letters
Indian J. Phys.  Indian Journal of Physics
Int. J. Cancer  International Journal of Cancer
J. Am. Chem. Soc.  Journal of the American Chemical Society
J. Biol. Chem.  Journal of Biological Chemistry
J. Heterocyclic Chem.  Journal of Heterocyclic Chemistry
J. N. C. I.  Journal of the National Cancer Institute
Mutation Res.  Mutation Research
Nucleic Acids Res.  Nucleic Acids Research
Prog. Exp. Tumor Res.  Progress in Experimental Tumor Research
Science  Science (Washington, D.C.)
Z. Ernahrungswiss.  Zeitschrift für Ernährungswissenschaft
Abbreviations

Z. Krebsforsch. Zeitschrift für Krebsforschung
Z. Kristallogr. Zeitschrift für Kristallographie
Z. Naturforsch. Zeitschrift für Naturforschung
Z. Physiol. Chem. Zeitschrift für Physiologische Chemie

Some other common abbreviations

Å Ångstrom unit (10⁻¹⁰ metre)
bp boiling point
Ci Curie (3.7 x 10¹⁰ nuclear transformations/second)
Et ethyl (C₂H₅)
h hour
hplc high-pressure liquid chromatography
Hz Hertz (frequency unit, 1 cm⁻¹ ≈ 3 x 10¹⁰ Hz)
J coupling constant (in Hz)
L litre
Me methyl
min minute
mL millilitre (10⁻³ L)
mp melting point
m/z ratio of mass to charge
n normal
NADPH nicotinamide-adenine dinucleotide phosphate, reduced
n₀ refractive index (at sodium D-line)
nm nanometre (10⁻⁹ metre)
nmr nuclear magnetic resonance
ppm parts per million
s second, singlet
s.d. standard deviation
sec. secondary
tert. tertiary
tlc thin-layer chromatography
Tris tris(hydroxymethyl)aminomethane hydrochloride (buffer)
σₒ optical rotation (at sodium D-line)
δ chemical shift (in ppm from tetramethylsilane)
λₒ ultraviolet maxima (in nanometres)
νₒ infrared maxima (in microns, µm)
µL microlitre (10⁻⁶ L)
µm micron (micrometre, 10⁻⁶ metre)
Acknowledgements

There has been an interest in cyclopenta[a]phenanthrenes in the laboratories of the Imperial Cancer Research Fund in London for 20 years, beginning with G. F. Marrian's suggestion of a possible metabolic route leading to the complete aromatization of steroids. We wish to acknowledge the continued generous financial support afforded by this organization throughout this period.

We are also indebted to all those listed below whom we have been fortunate to have as collaborators on this project, and who have contributed so many ideas and years of hard work.

*Imperial Cancer Research Fund*

Peter Abbott
Jeffrey Allen
Francis Crawley
Frances Crew
Charles Croft
Cynthia Dixon
Stanley Fisher
Sheila Furn
John Gilbert
Stephen Hadfield
Maureen Hall
Andrea Harper
S. Bala Jaitly
Joseph Jircny
Michael Jones
Anna-Maija Kissenerghis
Campbell Livingston
Andrew McEwen
Odarty Ribeiro
James Richards

Veronica Siddle
Clive Smith
Murugesu Thanikasalam
David Thomas
Colin Vose
Jill Welsh
Stretton Young

*Northern Polytechnic, London*

Alexandra Clayton
Kim Hendrick
Mary McPartlin
Jill Trotter

*Fox Chase Center, Philadelphia*

Jenny Glusker
Setsuo Kashino
Richard Peck
David Zacharias
Acknowledgements

Wistar Institute, Philadelphia
Leila Diamond
John DiGiovanni
Francis Kruszewski

University of Surrey
John Elvidge
John Jones
Jeremy Russell
Alan Wiseman

University College, Swansea
Dianne Kelly
James Parry
J. L. Wiebers

King's College, London
Alex Drake

University of Uppsala, Sweden
Kirsten Lindahl-Kiessling
Inga Karlberg

We are also greatly indebted to Audrey Becket who typed the manuscript of this book and helped us in many ways with its preparation.
Extensive world-wide studies in cancer epidemiology throughout most of this century have led to a generally accepted view that as much as 80–90% of all human cancer has its origins in environmental factors in our diet and lifestyle. Although in most cases several factors are likely to combine to produce a particular disease, the real significance of the above conclusion is its corollary, that identification and elimination of these environmental factors should reduce the incidence of the disease. During this time period the incidence of most forms of cancer has remained essentially constant with one notable exception, lung cancer. Mortality from this disease has risen inexorably to its present epidemic proportions during the last seventy years and now accounts for the death of some 26,000 men and 15,000 women each year in England and Wales alone. There is now no doubt that this is a direct result of cigarette smoking, and it is reliably predicted that abandonment of the habit would reduce this death rate ten-fold. The causes of other major forms of cancer, such as breast cancer in women and colon cancer in both sexes, have proved to be much more elusive. Their origins are probably complex and they are more difficult to study because there are no natural ‘controls’ as there are with lung cancer, where direct comparisons may be made between smokers and non-smokers. All possibilities for their causation are now under active consideration, and among them the influence of endogenous carcinogens generated by aberrant metabolism within the host is unknown, but cannot be discounted. It is now established that carcinogens of varied types are elaborated by both plants and microorganisms, and there seems to be no a priori reason why animals should not be similarly afflicted. Since most cancers are primarily diseases of old age it would seem unlikely that there would be strong evolutionary pressure against this happening. The idea that carcinogens, arising from steroids by incorrect metabolism, might be
important in the induction of human cancer was a view commonly held in
the past, but which has been neglected in recent times.

Over fifty years ago, at the very outset of research into chemical
carcinogenesis, the potent polycyclic aromatic hydrocarbon carcinogen
3-methylcholanthrene was prepared by simple chemical transformations
from a bile acid derivative. The correct structures for the bile acids and
sterols were themselves established at about the same time, and the
synthesis of this carcinogen took on an exaggerated importance in the
minds of scientists. The enduring idea that methylcholanthrene formed
by incorrect bile acid or sterol metabolism in the body might prove to be a
cause of human cancer led to numerous attempts to identify this
carcinogen in tumours and other tissues, but without success. The reason
for this failure now seems obvious because experience has shown that
cyclization of the sterol side-chain does not occur to provide a fifth fused
ring. Instead this side-chain tends to be lost with migration of the angular
methyl groups, leading to compounds of the simpler four-ring cyclo-
penta[a]phenanthrene series. These compounds thus possess the same
basic carbon ring system as the steroids, but lack the angular methyl
groups which characterize the latter. They are therefore capable of
complete dehydrogenation to fully aromatic phenanthrene derivatives,
and compounds of this type do in fact occur widely in petroleum and in
oil-bearing shales as well as in river and lake sediments; they are also
found in cooking oils that have been overheated. Advances in our
understanding of oestrogen biosynthesis and metabolism have moreover
suggested a plausible route by which cyclopenta[a]phenanthrenes might
be formed in the body, but so far no attempts have been made to test this
possibility. The need for further investigations in this area using modern
techniques is now apparent, because numerous cyclopenta[a]phenan-
threnes have since been synthesized and some have been shown to be
strong carcinogens, similar in potency to the classical polycyclic
hydrocarbon carcinogen benzo[a]pyrene.

This book is concerned with the occurrence, chemical synthesis,
physical and chemical properties, and biological attributes of cyclo-
penta[a]phenanthrenes. These are discussed in detail, as is their history
which is intimately connected with the establishment of the correct
structure of steroids. Interesting structure/carcinogenicity relationships,
similar to those found among related polycyclic aromatic systems such as
the chrysenes and benzo[a]anthracenes, are examined in relation to the
metabolism of these compounds and the interactions of their metabolites
with biological macromolecules. The book also lists physical data for
nearly 350 cyclopenta[a]phenanthrene derivatives and contains exhaus-
Preface

tive references to the original literature. It is hoped that it will provide a useful point of departure for those concerned with the possibility of the involvement of cyclopenta[a]phenanthrenes in the aetiology of human disease.

M.M.C.
T.S.B.
Six possible isomers can be formed by fusion of a cyclopentane ring with the angular tricyclic aromatic hydrocarbon phenanthrene, as shown in Fig. 1. Of these the [a] isomer occupies a special position because it possesses the same carbon ring system as members of the large and important group of natural products, the steroids. Indeed, cyclopen-ta[a]phenanthrenes became of importance during the elucidation of the structures of these natural products over 50 years ago. At about the same time it first became evident that carcinogenic properties were associated
Introduction

with many polycyclic aromatic hydrocarbons which result from incomplete combustion of most carbonaceous materials, and that the great majority of these could also be considered as derived from phenanthrene. The coincidence in time between the fruition of these two quite independent lines of research led many to feel that there might be a connection between them, and for a time there was considerable interest in the possible relationship between these two classes of compounds. Although most of the carcinogenic polycyclic aromatic hydrocarbons discovered at that time contained four or more fused benzene rings, the simpler cyclopenta[a]phenanthrenes with the same carbon skeleton as the C18 and C19 steroids (with the exception of the angular methyl groups in these steroids) were also examined, and these studies have continued until the present day.

In writing this monograph the purpose is to review the subject fully including both the earlier work as well as the more recent developments. A careful search has been made in Chemical Abstracts back to 1907, but a problem has been encountered in that the term ‘cyclopenta[a]phenanthrene’ includes numerous partially hydrogenated derivatives which are better considered as steroids. In general we have neglected these if they include in their structure angular methyl groups, unless they have some direct relevance to the phenanthrene-derived compounds which are the main topic here. This work now spans more than half a century and in reviewing it almost inevitably some omissions will have occurred; the authors wish to apologize for these in advance. In the text individual compounds are assigned arabic numbers (in heavy type) sequentially so that they can be referred to without ambiguity, and the structures of most are shown in the figures. The latter and also the tables are numbered sequentially throughout the monograph, but literature references are collected at the end of each chapter in alphabetical and chronological order.

1.1 Nomenclature of cyclopenta[a]phenanthrenes

In common with many other polycyclic aromatic ring systems, the nomenclature of cyclopenta[a]phenanthrenes has undergone changes since the early work, and this will lead to confusion unless it is dealt with here. Originally compound (1) in Fig. 2 was named 1,2-cyclopentenophenanthrene and was numbered as shown 1(A). With the advent of the more facile method of designating the edge of each ring by a letter, as shown for phenanthrene in Fig. 1, the prefix denoting the fused five-membered ring becomes cyclopenta[a]; however, cyclopenta[a]phenanthrene itself now refers to the fully unsaturated hydrocarbon
of which (2) is the $17^H$-isomer. $15^H$-cyclopenta[a]phenanthrene is there­fore compound (3), and the compound (1) containing only one double bond in the five-membered ring is correctly named 16,17-dihydro-$15^H$-cyclopenta[a]phenanthrene. In the older literature these two unsaturated hydrocarbons, (2) and (3), were named $\Delta^{1(2)}$ and $\Delta^{2(3)}$-1,2-cyclopent-dienophenanthrene, respectively. When the position of the ‘extra’ hydrogen atom (on the carbon not doubly bonded) is fixed by a substituent, for example by the ketone oxygen atom in the ketone (4), its position need not be designated; e.g., the correct name for this ketone is 15,16-dihydrcyclopenta[a]phenanthren-17-one or 15,16-dihydro-17-oxocyclopenta[a]phenanthrene. Numbering of the ring system follows the steroid convention as indicated on formula 1(B) in Fig. 2. The term cyclopenta[a]phenanthrene is therefore properly reserved for the fully unsaturated compounds containing a double bond in the five-membered ring. However, in this monograph it will be also used more loosely as a general term for compounds such as (1)-(4) of both types, except of course, in formal chemical names.

1.2 Cyclopenta[a]phenanthrenes, steroids, and carcinogenic polycyclic hydrocarbons

Sterols isolated from plant and animal tissues and the bile acids from animal bile had been studied for over a century when in 1928 Windaus and Wieland received the Nobel Prize for their outstanding work on these natural products. Extensive degradative experiments utilizing the classical methods of organic chemistry, purification by
crystallization to a constant melting point or constant optical rotation and identification by elemental analysis, were unassisted by the chromatographic and spectroscopic techniques so widely used today. By 1928 these experiments had culminated in the structures shown in Fig. 3 for the bile acid deoxycholic acid and the typical sterol cholesterol. However, in 1932 these formulae were criticized on what at that time were quite esoteric grounds. In an X-ray crystallographic investigation of the vitamin D precursor ergosterol Bernal (1932b) determined its molecular dimensions to be $7.2 \times 5 \times 17-20\, \text{Å}$, and pointed out that these figures did not fit well with those ($8.5 \times 7 \times 18\, \text{Å}$) calculated for this sterol from the Wieland–Windaus structure. This supported a previous observation based on the results of experiments with surface films of sterols by Adam and Rosenheim (1929). Several years previously Diels et al. (1927) had obtained chrysene (5) and two other unidentified hydrocarbons by dehydrogenation of sterols by prolonged heating at 360°C with selenium, but it was at that time generally considered that these drastic conditions might well lead to deep-seated rearrangements within the molecule, so that the products would be of little use as indicators of structure. Discarding this view, Rosenheim and King (1932) proposed the novel structure (6a) for deoxycholic acid based on perhydrochrysene, and Bernal (1932a) calculated that an analogous structure for ergosterol would have the dimensions ($7.5 \times 4.5 \times 20\, \text{Å}$), in good agreement with those found. Within a few months chemical considerations led to the revised structure (6b) for deoxycholic acid based on perhydrocyclopenta[a]phenanthrene; this formula has stood the test of time and is accepted

Fig. 3

Deoxycholic acid (1928) Cholesterol (1928) Chrysene

Deoxycholic acid (May, 1932) Chrysene (September, 1932)
Carcinogenic compounds

today. In order to confirm this new structure for the bile acids and sterols the other hydrocarbons obtained by Diels, particularly the one melting at 124–125°C, were reinvestigated. This hydrocarbon, which has since become known as Diels' hydrocarbon, was originally obtained, together with chrysene and another hydrocarbon C_{25}H_{24}, by selenium dehydrogenation of cholesterol and cholesteryl chloride; it was also isolated from the products of similar dehydrogenation of ergosterol and cholic acid. The following year Rosenheim and King (1933) proposed that Diels' hydrocarbon was 16,17-dihydro-17-methyl-15H-cyclopenta[a]phenanthrene (7) on the basis of its ultraviolet spectrum, another use of a novel technique in those early days. The problem was taken up by several groups and all three (15-, 16-, and 17-methyl) hydrocarbons as well as the parent hydrocarbon 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) were synthesized. It was not, however, until 1935 that Diels' hydrocarbon was finally and unambiguously assigned structure (7) by careful comparison with the synthetic specimen (Hillemann, 1935), thus finally confirming the steroid ring structure previously arrived at on quite different grounds.

At the same time momentous discoveries were being made in the field of carcinogenesis. Over 100 years ago occupational skin cancer was reported among oil and tar workers both in Germany (von Volkmann, 1875) and in Scotland (Bell, 1876). Attempts to induce skin tumours in animals by topical application of these materials failed at first, but success was finally achieved by two Japanese workers, Yamagiwa and Ichikawa (1915). They succeeded where others had failed by persisting in their treatment, thus simulating human exposure; they found that tumours could be induced on the ears of rabbits by repeated, frequent applications of coal tar over a protracted period. It was then discovered that the dorsal skin of mice offered a more convenient test system, and using this technique a systematic search for the active carcinogenic agent in coal tar was initiated under the direction of Sir Ernest Kennaway at the Royal Cancer Hospital, London (for a short history of this work see Kennaway, 1955). It was soon established that carcinogenic tars resulted from the pyrolysis of many carbon-containing materials as widely different as cholesterol, yeast, human hair and tissue, and even acetylene. It was also noted (Hieger, 1930) that all tars contained characteristic lines in their fluorescence spectra similar to those displayed by the polycyclic aromatic hydrocarbon benz[a]anthracene (Fig. 4), which is itself essentially non-carcinogenic. These lines, however, were of shorter wavelength in benz[a]anthracene, suggesting that the carcinogenic compound giving the characteristic lines was of similar type, but of higher molecular
weight. The homologue dibenz[a,h]anthracene first prepared by Clar (1929) now holds the distinction of being the first pure chemical compound to be shown to be carcinogenic, as demonstrated by Kennaway and Heiger (1930). The Royal Cancer Hospital group then undertook the fractionation of two tons of gas-works pitch and, using the fluorescence spectrum as a guide, eventually (Cook et al., 1933) obtained seven grams of yellow crystals consisting mainly of benzo[a]pyrene. This polycyclic aromatic hydrocarbon was found to be a potent carcinogen in the mouse skin-painting test, more active than dibenz[a,h]anthracene and having a fluorescence spectrum closely similar to that previously observed to be associated with carcinogenicity in the crude tars. Later work established that most polycyclic aromatic carcinogens were derived from the angular system of phenanthrene, whereas its linear isomer anthracene did not generally give rise to biologically active derivatives.

In the same year another carcinogenic polycyclic aromatic hydrocarbon was isolated, not from tar but from the products of pyrolysis of a steroid. During the elucidation of the structure of the cholic acids, the degradation product 12-ketocholanic acid (8) was found to undergo cyclization and decarboxylation at 330°C to yield dehydrocholocholine (9). Dehydrogenation of this hydrocarbon with selenium led to loss of the two angular methyl groups and aromatization of the four six-membered rings to give 3-methylcholanthrene (Wieland and Dane, 1933) which was subsequently shown to be an even more potent carcinogen than benzo[a]pyrene (Cook and Hazelwood, 1933, 1934). This simple,
Carcinogenic compounds

straightforward four-step transformation of a natural steroid into a potent carcinogen was important on several grounds. Firstly, selenium dehydrogenation was accompanied by the loss of the angular methyl groups, but the ring system remained intact. Thus it became more probable that the cyclopenta[a]phenanthrene skeleton of Diels' hydrocarbon correctly represented the steroid ring system. Secondly, the position of the carbon side chain was established at C-17 in cholic acids and thus through chemical correlations in all the sterols, as well as in other physiologically important steroids such as the progestogens and corticoids to be discovered later. Finally, this remarkable preparation of a carcinogen from a natural steroid for a time led to the feeling that an important and exciting breakthrough in our understanding of the origin of human cancer was at hand. For example, Fieser (1936) in his book *Chemistry of Natural Products Related to Phenanthrene* wrote,

‘While proof is entirely lacking, it appears possible that many forms of cancer may originate in the metabolic production of methylcholanthrene or related substances from the bile acids, or perhaps from the sterols or sex hormones, of the body'.

Fifty years later we have to say that this possibility has not been realized, although it has not been disproved. There have been a number of new twists to the story among which the cyclopenta[a]phenanthrenes find an important place. The chemistry and biological attributes of these compounds have been studied extensively partly because of their close relationship with the steroids, but also because of the attraction of this possibility of the endogenous formation of carcinogens from normal steroid hormones.

Early work on the synthesis of cyclopenta[a]phenanthrenes was largely aimed at proving, through the provision of pure synthetic specimens of known structure, the nature of the steroid dehydrogenation products. It was soon established that Diels’ hydrocarbon, its 15- and 16-methyl isomers, and the parent hydrocarbon (1) (Fig. 2) were not carcinogenic (Hartnell, 1951). Interest then centred on the possibility of the formation of cholanthenes from steroids under conditions approximating to physiological (Inhoffen, 1953). The discovery of the C₁₈ and C₁₉ steroid hormones lacking a side chain at C-1₇, required to provide the extra carbon atoms for construction of the fifth ring, seemed to rule these out as precursors, although Fieser (1941) suggested that 17-ketones might condense with biological ketoacids to provide the necessary atoms. Parallel with this, work on the synthesis and testing of numerous polycyclic aromatic hydrocarbons went on apace, and it rapidly became clear
that nearly all these were based upon a phenanthrene ring system. Moreover, methyl substituents often had a dramatic effect in increasing the activity of weakly carcinogenic hydrocarbons. Thus the feeble carcinogenicity of chrysene (Fig. 3) is considerably enhanced by methyl substitution at C-5, whereas substitution at other ring positions has little effect (Dunlap and Warren, 1943). In the benz[a]anthracene (Fig. 4) series the hydrocarbon itself is essentially inactive, whilst carcinogenic activity of the monomethylbenz[a]anthracenes decreases in the order 7-Me > 12-Me > 9-Me (Cook and Kennaway, 1938); 7,12-dimethylbenz[a]anthracene (DMBA) is one of the most potent carcinogens known (Bachmann et al., 1938). It therefore became of importance to study the eight possible derivatives of 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) bearing methyl groups on the aromatic rings. Butenandt (1942) began by synthesizing the 6-methyl derivative from the androgen dehydroandrosterone (3-hydroxyandrost-5-en-17-one); during the following decade he and his group obtained the rest of the isomers by total synthesis and tested them for carcinogenicity in the mouse skin-painting test (Butenandt and Dannenberg, 1953). All were inactive with the exception of the 7- and 11-methyl hydrocarbons which were extremely weak carcinogens, thus strengthening the opinion held at that time that four aromatic rings were essential for high potency. In later work Dannenberg showed that introduction of a double bond into the five-membered D-ring also led to weak activity in the absence of methyl substitution. He found (Dannenberg, 1970) that dehydrogenation of sterols with high-potential quinones led to compounds of this class.

1.3 Oestrogens and cyclopenta[a]phenanthrenes

Continuing this short account of those exciting and eventful early years we turn to the female sex hormones, the oestrogens. A convenient and reliable assay for oestrogenic activity was devised by Allen and Doisy (1923) based on the ability of the test compound to induce oestrus in castrated female mice, as indicated by characteristic morphological changes in the cells lining the vagina. Using this test it was discovered that human pregnancy urine was a good source of oestrogenic activity, and six years later two groups led by Doisy (1929) in America and Butenandt (1929) in Germany isolated the main oestrogenic hormone, oestrone (Fig. 5). This hormone differs from most other steroids in possessing a phenolic A-ring and in lacking the angular methyl group at C-10. Soon afterwards a second, less active oestrogen, oestriol, was obtained from the same source by Marrian (1930) in England, while in France, Girard et al. (1932) using the water-solubilizing ketone reagent (trimethylamino-
acetohydrazine hydrochloride) which now bears his name fractionated over 50,000 L of pregnant mares' urine to isolate the naphtholic oestrogen equilenin.

During work aimed at establishing the structure of oestrone, the methyl ether of this hormone was submitted to Wolff–Kishner reduction to yield the 17-deoxy derivative (10) (Fig. 5) which on selenium dehydrogenation yielded 16,17-dihydro-3-methoxy-15H-cyclopenta[a]phenanthrene (11) identical with a synthetic specimen (Cook and Girard, 1934). Again selenium dehydrogenation led to elimination of the angular methyl group, but retention of the ring system. On the other hand, when oestrone methyl ether was treated with the methyl Grignard reagent and the resulting tertiary alcohol (12) was dehydrogenated the product was the 17,17-dimethyl derivative (14), not as expected the 17-monomethyl compound (17) or either of its 15- and 16-methyl isomers, all of which were synthesized for comparison (Cohen et al., 1935). Thus migration of the angular methyl group occurs during dehydrogenation and dehydration of this carbinol (12). The reason for this would appear to be the
Introduction

presence of the hydroxyl group vicinal to the quaternary carbon at C-13, because migration also occurs when oestradiol methyl ether (15) is similarly dehydrogenated to yield 16,17-dihydro-3-methoxy-17-methyl-15H-cyclopenta[a]phenanthrene (17). Migration also occurs when these alcohols are dehydrated under less drastic conditions to give the 17,17-dimethyl- and 17-methyl-18-nor-steroids (13) and (16), respectively. The structure of equilenin was also confirmed by selenium dehydrogenation of the tertiary alcohol derived from the methyl ether (analogous to 12) to furnish 15,16-dihydro-17,17-dimethyl-3-methoxycyclopenta[a]phenanthrene (14).

In the body oestrogens are biosynthesized from male sex hormones, the androgens. This was conclusively proved when it was shown that $^{14}$C-labelled testosterone was converted into $[^{14}\text{C}]$ oestradiol (Heard et al., 1955; Baggett et al., 1955). Aromatization of the A-ring in the androgen androst-4-ene-3,17-dione (18) (Fig. 6) requires the loss of the C-19 methyl group together with two of the hydrogen atoms at C-1 and C-2. Meyer (1955a) isolated the 19-hydroxy derivative (19) as a metabolite of this androgen, and showed that on incubation with enzyme preparations from the adrenal gland, the ovary, and the placenta it gave rise to oestrogens more readily than the original hormone (Meyer, 1955b). It is now established that further oxidation at this carbon gives the 19-aldehyde (20) from which one hydrogen atom at C-2 is lost by enolization and the other at C-1 by concerted oxidative elimination of the aldehyde group as formic acid to give the phenol oestrone as shown in Fig. 6 (Stevenson et al., 1985). Working on human urinary oestrogen metabolites at Edinburgh, Marrian and his group isolated a new and unusual oestrone metabolite from pregnancy urine by partition chromatography on Celite. This compound was characterized (Loke et al., 1959) as 18-hydroxyoestrone (21) by its ready loss of formaldehyde when treated with dilute alkali, and by the formation of 18-noroestrone characterized as its methyl ether (22) identical with a synthetic sample (Loke et al., 1958) (Fig. 7). At that time compound (21) was unique, being the first instance of a steroid hydroxylated at C-18. Later, of course,
it was found that similar 18-hydroxylation occurs during the biosynthesis of the adrenocortical hormone aldosterone when the substrate is probably corticosterone (Eisenstein, 1967). Thus 18-hydroxylation is established as a normal adrenal function, at least for C_{21} steroids. After his move to London at about this time to take up the position of Director of Research at the Imperial Cancer Research Fund, Marrian pointed out to one of the present authors (M.C.) that should elimination of the 18-methyl group from oestrone occur in vivo, there would arise the possibility of complete aromatization of the steroid to 15,16-dihydro-3-hydroxycyclopenta[a]phenanthren-17-one (23). An oestrogen, equilenin, was already known with both A- and B-rings aromatic, but aromatization of ring-C in this compound is normally blocked by the angular 18-methyl group. Furthermore, 3-deoxyequilenin (25) had been isolated from mares' pregnancy urine by two groups (Marker and Rohrmann, 1939; Prelog and Fuhrer, 1945) under conditions which apparently precluded its formation as an artefact. The structure of this compound was later confirmed by its synthesis from equilenin (Bachmann and Dreiding, 1950); they submitted the steroid to the Bücherer reaction to obtain its 3-amino analogue, the diazonium salt of which was reduced with hypophosphorous acid to give the 3-deoxy compound with properties identical with those of 3-deoxyequilenin isolated from natural sources. The biosynthesis of this compound is unknown, but if it is formed like normal 3-oxygenated steroids via lanosterol, presumably elimination of this oxygen must occur before the final aromatization step takes place. In yeast squalene cyclization first gives enzyme-bound lanosta-8,24-diene which is subsequently 3-hydroxylated (Barton and Moss, 1966). If the enzyme-bound, fully cyclized hydrocarbon became free before the final step it could conceivably give rise to 3-deoxy steroids. It is interesting that
equilenin itself is apparently not derived from oestrone by further biological dehydrogenation (Gallagher et al., 1958). Non-oxidative cyclization of squalene to several triterpenes which lack oxygen at C-3 is well known, but has been little investigated (Manitto, 1981). Elimination of the 18-methyl group and C-ring aromatization in the 3-deoxy compound (25) would furnish the parent ketone 15,16-dihydrocyclopenta[a]phenanthren-17-one (4).

It was therefore decided to synthesize both these cyclopenta[a]phenanthren-17-ones (4) and (23) in order to examine them for both oestrogenic and carcinogenic activity. In addition, preparation of the series of 17-ketones corresponding to Butenandt's isomeric methyl hydrocarbons was also undertaken. When it was discovered that the 11-methyl-17-ketone (26) (15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one) was a strong carcinogen (Coombs and Croft, 1966), much more potent than the corresponding hydrocarbon tested by Butenandt, interest in cyclopenta[a]phenanthrenes was renewed; work in this area has continued until the present day, and numerous related 17-oxocyclopenta[a]phenanthrenes have been synthesized and tested for mutagenic and carcinogenic activity. Recent work has been aimed at understanding the observed structure/activity relationships in terms of the metabolism of these compounds and their ability to modify biological macromolecules. In quite another sphere a number of cyclopenta[a]phenanthrene derivatives have very recently been shown to occur naturally in petroleum and other mineral oils, as well as in river and lake sediments, thus again giving rise to renewed interest in this series of compounds.

1.4 References

References


Introduction


Chemical synthesis of cyclopenta[a]phenanthrenes: hydrocarbons

2.1 Early synthesis of hydrocarbons in connection with steroids

As has already been outlined, syntheses of cyclopenta[a]phenanthrenes lacking oxygen substitution in ring-D were originally undertaken 50 years ago to provide rigid structural proof for steroid dehydrogenation products. These syntheses were greatly assisted by previous investigations on the preparation of phenanthrene derivatives by Haworth and his school in connection with elucidation of the structures of the resin acids (Fieser, 1936). The parent hydrocarbon 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) was obtained by both Kon (1933) and by Ruzicka et al. (1933) by essentially the same route, outlined in Fig. 8. Condensation of 2-(1-naphthyl)ethyl bromide with ethyl cyclopentanone-2-carboxylate gave the β-keto-ester (40) which was decarboxylated, and the
resulting ketone was reduced to the secondary alcohol (41). This was cyclized to the tetracyclic compound (44), without isolation of the intermediate cyclopentene (42), by heating it with phosphorus pentoxide, and the synthesis was completed by dehydrogenation with selenium. Ruzicka discovered that the final hydrocarbon (1) could be obtained directly by heating the keto-ester (40) with sulphuric acid. A somewhat different route was followed by Cook and Hewett (1933) who were interested in this compound in connection with their work on polycyclic aromatic hydrocarbon carcinogens. These workers first obtained the tertiary alcohol (43) by treating cyclopentanone with the Grignard reagent from 2-(1-naphthyl)-ethyl chloride, and this was cyclized directly to the final cyclopenta[a]phenantrrene with hot sulphuric and acetic acids. In both cases the unsaturated hydrocarbon (42) was probably an intermediate in these cyclizations because it could be formed from either of the alcohols (41) or (43) by dehydration under mild conditions. Presumably the sulphuric acid acts as an oxidizing agent in these reactions to remove the extra hydrogen atoms after dehydrating the alcohol and forming the carbonium ion. By employing the two isomeric 4-methyl and 5-methyl homologues of ethyl cyclopentanone-2-carboxylate Ruzicka also synthesized the 15- and 16-methyl derivatives (45 and 46, respectively) of the parent hydrocarbon; both proved to be distinctly different from Diels' hydrocarbon (7) obtained from sterol dehydrogenations. In later work Cook and Hewett (1934) investigated the cyclization of the naphthylethylcyclopentene (42) with aluminium chloride and stannic chloride, isolating two spirans in addition to the expected product. The spiran rings in these two hydrocarbons were unstable to the high-temperature conditions during selenium dehydrogenation which aromatized them to chrysofluorene and 2-methylpyrene as shown in Fig. 9. Spiran formation was suppressed by placing a methyl group at the other.

Fig. 9
end of the double bond in (42). Thus in a synthesis of Diels’ hydrocarbon (7) (Harper et al., 1934) shown in Fig. 10, 2,5-dimethylcyclopentanone was treated with the Grignard reagent from 2-(1-naphthyl)ethyl bromide to give the tertiary carbinol (47), which on being heated with phosphorus pentoxide led to a good yield of the cyclized product (48). The angular methyl group was eliminated, but the 17-methyl group was retained on selenium dehydrogenation to yield Diels’ hydrocarbon (7). A year earlier Bergmann and Hillemann (1933) had obtained the same compound for the first time, from 2-acetylnaphthalene by way of a Reformatsky reaction with methyl bromoacetate, followed by reduction of the double bond, hydrolysis, ring closure, and Clemmensen reduction of the 15-ketone (49) (Fig. 10). Both samples melted at 125°C, like Diels’ hydrocarbon itself, but for a time there was some confusion between this compound and the parent hydrocarbon (1), although the latter melted some 10 degrees higher. The two compounds cannot be distinguished readily from their elementary compositions:

Calculated for C_{18}H_{16}: C, 93.1; H, 6.9%
Calculated for C_{17}H_{14}: C, 93.5; H, 6.5%

owing to the limits of accuracy of combustion analysis (Ruzicka and Thomann, 1933); also mixed melting points failed to be helpful in this instance. In order to resolve this question, Hillemann (1935) prepared another sample of the hydrocarbon, this time by the method of Harper, and carefully compared the purified material with Diels’ hydrocarbon with which it proved to be identical. In particular the synthetic sample gave a characteristic nitroso compound, mp 238–239°C, identical with that given by Diels’ hydrocarbon, whereas the hydrocarbon (1) did not, as had been noted by Diels previously (Diels and Clar, 1934). Thus the structure of Diels’ hydrocarbon was settled at last.

Another synthesis of Diels’ hydrocarbon, together with its 17-ethyl and 17-isopropyl derivatives, was described by Riegel et al. (1943) (Fig. 11).
The 3-acylphenanthrenes were converted in three steps into the propionic acids which were cyclized to yield the 15-ketones (49 and 50). The 17-alkyl-16,17-dihydro-15H-cyclopenta[a]phenanthrenes (7, 52, and 53) were then obtained by reduction. The 17-ethyl hydrocarbon (52) was also isolated in 40% yield from selenium dehydrogenation of pregnanediol at 300–350°C (Schöntube and Janak, 1968). A similar synthesis of Diels' hydrocarbon via its 15-ketone was reported by Tatta and Bardhan (1968).

Condensation of diethyl 2-(1-naphthyl)-ethylmalonate with ethyl crotonate in the presence of base, followed by hydrolysis and decarboxylation gave the di-acid (54). Ring closure was effected with 85% sulphuric acid, and the resulting ketone was reduced and aromatized by being heated with sulphur; the overall yield was over 70%. Cyclization of the acid chloride to give the 17-methyl-15-ketone (49) was accomplished with aluminium chloride in nitrobenzene; reduction of this ketone gave Diels' hydrocarbon (7). The authors pointed out three advantages of this synthetic route: (i) the easy availability of starting materials; (ii) good yields throughout; and (iii) the avoidance of dehydrogenation in the final stage of the synthesis.

This first phase of cyclopenta[a]phenanthrene synthesis concluded with the synthesis of several methoxycyclopenta[a]phenanthrenes in connection with structural studies on the oestrogens, as discussed in Chapter 1. For the synthesis of 16,17-dihydro-3-methoxy-15H-cyclopenta[a]phenanthrene (11) (Fig. 12) the Grignard reagent, prepared from the chloride (55), itself secured in six steps from 2-naphthylamine, was added to 2-methylcyclopentanone; dehydration of the resulting alcohol gave the cyclopentene which was cyclized with aluminium chloride to the angular methyl compound (56). The angular methyl group was elimin-
ated as usual on selenium dehydrogenation to furnish the desired 3-methoxy hydrocarbon (11) (Cohen et al., 1935). The overall yield from 2-aminonaphthalene was only 0.02%, but the pure sample was identical with the sample prepared by selenium dehydrogenation of 17-deoxyoestrone methyl ether. The 17,17-dimethyl derivative (14) was obtained in an analogous manner from 2,5,5-trimethylcyclopentanone, as was the 17-methyl compound (17) from 2,5-dimethylpentanone. For the preparation of the 16- and 15- isomers (57 and 58) the bromide corresponding to (55) was condensed with ethyl 4-methyl- and 5-methylcyclopentanone-2-carboxylate, respectively, following the original synthesis shown in Fig. 10. The 4- and 6-methoxy isomers (59), (61), and (62) were prepared by Kon and Ruzicka (1935) in an analogous manner from the corresponding methoxy-1-naphthylethyl bromides. The 3-methoxy hydrocarbon (11) was obtained in better yield by a different route (Chuang et al., 1939). Condensation of the acid chloride of the methoxynaphthylbutyric acid with the sodium salt of 2-acetyl-diethyl-succinate gave the diketo-diester (64) which on ring closure with sodium ethoxide and decarboxylation

![Fig. 12](image-url)
yielded the 1,3-cyclopentanedione (65). Cyclization of the latter with phosphorus pentoxide then gave 3-methoxy-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (66), converted by Clemmensen reduction and selenium dehydrogenation to the desired 3-methoxy hydrocarbon (11). The 3-methoxy-17-methyl compound (17) was later obtained from the corresponding 17-ketone via a Grignard reaction (Coombs, 1966), and by dehydrogenation of a byproduct (63) isolated from the demethylation of oestrone methyl ether by fusion with pyridinium hydrochloride at 200°C (Hoffsommer et al., 1966). The way in which these methoxycyclopenta[a]phenanthrenes were used to establish the structures of oestrone and equilenin have been discussed in Chapter 1 (see Fig. 5).

2.2 Syntheses of the isomeric aryl methyl hydrocarbons

The next synthetic phase, in which all the possible monomethyl and several dimethyl derivatives of 16,17-dihydro-15H-cyclopenta[a]phenanthrene were prepared, led to the first detection of carcinogenicity among compounds of this class. However, the first step in this direction was taken in connection with the structure of the cardiac
aglycone strophanthidin. Like the sterols, this gave Diels' hydrocarbon on selenium dehydrogenation, although a suggested formula for the aglycone should have led to its 6-methyl homologue (68) (Fig. 13). Both this cyclopenta[a]phenanthrene and the 6-monomethyl derivative (67) were synthesized by Gamble and Kon (1935) from 1-(4-methyl-1-naphthyl)-ethyl bromide and the requisite cyclopentanone following the general method of Harper (see Fig. 10). Neither compound was identical with Diels' hydrocarbon and the suggested formula for strophanthidin had to be abandoned. The same two compounds together with the 6,17,17-trimethyl analogue (69) were later obtained by Butenandt and Suranyi (1942) from the androgen dehydroepiandrosterone (DHA, 3-hydroxy-androst-5-en-17-one). The 6-methyl group was introduced into the steroid by opening the 5,6-epoxide, prepared by oxidizing the 5(6)-double bond with perbenzoic acid, by means of methyl magnesium iodide. Introduction of the 17-methyl group was achieved by using the known propensity of the steroid 18-methyl group vicinal to a hydroxyl group to migrate to C-17 during selenium dehydrogenation. Diels' hydrocarbon (7) was also synthesized by dehydrogenation of 3,17-dihydroxyandrost-5-ene, omitting the epoxidation step. Yet two more cyclopenta[a]phenanthrenes were synthesized while establishing the structure of the plant sapogenin, sarsasapogenin (Kon and Woolman, 1939) (Fig. 14). To locate the hydroxyl group in this molecule the alcohol was oxidized to the ketone and the position of the oxygen was marked by converting it to the tertiary carbinol, methylsarsasapogenin, by treatment

Fig. 14

3-methylcholesterol

methy1sarsasapogenin

3-methylcholesterol
with methyl magnesium iodide. Dehydrogenation gave a hydrocarbon thought to be 16,17-dihydro-3-methyl-15H-cyclopenta[a]phenanthrene (70), rather than the initially expected 3,17-dimethyl analogue (71). In order to confirm this, both these hydrocarbons were prepared as outlined in Fig. 14. 2-Acetyl-6-methylnaphthalene was transformed into 11-acetoxy-15,16-dihydro-3-methylcyclopenta[a]phenanthren-17-one (72) by Robinson's method (discussed later in this chapter), and the derived methyl ether was treated with the methyl Grignard reagent to give 3,17-dimethyl-11-methoxy-15H-cyclopenta[a]phenanthrene (73), dehydration of the intermediate tertiary carbinol having occurred readily during the reaction. Mild hydrogenation of this compound saturated the 16(17)-double bond yielding the dihydro derivative (74), while prolonged, vigorous catalytic reduction (Adams catalyst in acetic acid at 80–85°C for some days) of both the 11-acetoxy-17-ketone (72) and the 11-methoxy-16(17)-ene (73) removed the oxygen functions and partially reduced the aromatic rings. The crude reduction products were heated at 320°C with equal weights of 10% palladized charcoal, and the hydrocarbon fractions were isolated by percolation of petroleum extracts through columns of activated alumina. The 3-methyl hydrocarbon (70), which was obtained in about 20% yield, was found to be identical with the dehydrogenation product of methylsarsasapogenin, showing that migration of the 18-methyl group to C-17 does not always occur during dehydrogenation of steroids. The 3,17-dimethyl hydrocarbon (71) was obtained in a similar way from (73) by vigorous reduction followed by dehydrogenation, and was shown to be identical with a major product from dehydrogenation of 3-methylcholestanol; a minor product appeared to be the 3-methyl hydrocarbon (70). During elucidation of the structure of the alkaloid cyclobuxine C_{25}H_{42}ON_{2}, thought to possess the formula (75) shown in Fig. 14, selenium dehydrogenation yielded 15,16-dihydro-4,17-dimethyl-17-ethyl cyclopenta[a]phenanthrene (76) (Brown and Kupchan, 1962) thus confirming the nature of the carbon skeleton.

The rest of the methyl isomers and a number of dimethyl homologues were prepared by Butenandt and his co-workers by total synthesis over the next 10 years. For the synthesis of the 11-methyl and 11,12-dimethyl derivatives (83 and 87, respectively) use was made of a method for cyclopenta[a]phenanthrene synthesis elaborated by Robinson in his extensive work directed at the synthesis of steroids and which resulted in an early total synthesis of an equilenin stereoisomer. In number XXI of a remarkable series of papers from Oxford entitled ‘Experiments on the synthesis of substances related to the sterols’ published in the Journal of the Chemical Society, Robinson (1938) adapted a little-known reaction
Isomeric arylmethyl hydrocarbons

discovered by Kehrer and Igler (1899) to the naphthalene series (Fig. 15). The furfurylidene derivative of 1-acetyl-naphthalene (77), obtained in high yield by base-catalysed condensation of 1-acetyl-naphthalene with furfuraldehyde, on being boiled with ethanolic hydrochloric acid yielded the diketooctanoic acid (78). The mechanism of this intriguing reaction, in which the furan ring is opened by the formal addition of two molecules of water, is obscure; however, the acid is obtained reproducibly in about 50% yield, the other product being a black insoluble tar. Intramolecular ring closure of the side chain brought about by hot 2% potassium hydroxide gave the naphthylcyclopentenone acetic acid (79) which was itself cycled to yield 1-acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (80) by boiling it with acetic anhydride. Both of these cyclizations occur essentially quantitatively and the whole sequence provides an elegant entry into the cyclopenta[a]phenanthrene series.

Butenandt et al. (1946a) modified this route by first converting the cyclopentenone (79) into the cyclopentane (81) by mild hydrogenation of the tetrasubstituted double bond (Koebner and Robinson, 1938), followed by heating the derived semicarbazone with sodium ethoxide at 180°C (Fig. 15). Cyclization in this acid is less facile because it is not favoured by the driving force provided by the creation of a new aromatic ring; it was achieved by treating the acid chloride of (81) with aluminium chloride to give the ketone (82) in about 50% yield. The 11-methyl group was introduced by a Grignard reaction with methyl magnesium iodide; dehydration and dehydrogenation with platinized charcoal at 300-310°C.

Fig. 15
then led to 16,17-dihydro-11-methyl-15H-cyclopenta[a]phenanthrene (83). Methylation at C-12 in the 11-ketone (82) was achieved in two ways; direct methylation with methyl iodide and potassium tert.-butoxide yielded a monomethyl derivative (85) of mp 85–86°C. Alternatively, condensation of the ketone (82) with dimethylmalonate in the presence of sodium methoxide, followed by pyrolysis of the glyoxalate (84) on glass powder at 180°C gave the 12-carboxylic ester (85); base-catalysed methylation of the latter with methyl iodide and decarboxylation of the intermediate led to a stereo-isomeric 12-methyl-ketone, mp 117–118°C, which could be converted into the lower-melting isomer with aqueous methanolic potassium hydroxide. Treatment of (86) with methyl lithium and dehydration–dehydrogenation of the intermediate tertiary carbinol finally yielded 16,17-dihydro-11,12-dimethyl-15H-cyclopenta[a]phenanthrene (87). The 12-methyl hydrocarbon was also prepared in low yield from the 12-methyl-11-ketone (86) by way of Clemmensen reduction, and proved to be identical with a specimen prepared by another route (see below).

This general synthetic route to cyclopenta[a]phenanthrenes was also employed for the synthesis of the 7-methyl isomer (Butenandt et al., 1949a), starting from 2-acetyl-3-methyl-5,6,7,8-tetrahydronaphthalene (88) (Fig. 16). This was converted into the furfurylidene derivative (89) which underwent acid-catalysed furan ring opening to the heptanoic acid (90). However, on ring closure with dilute potassium hydroxide Butenandt reported that the cyclopentenone acetic acid (91) was accompanied by the furan propionic acid (95) (although in the experimental part of their paper, under the description of the acid (90) they state, 'In manchen Ansätzen wurde ein Gemisch der Dioxoheptansäure und der nachstehend beschriebene Furan-propionsäure erhalten'). In connection with the synthesis of the 17-ketone corresponding to (94) Coombs and Jaitly (1971) repeated the acid treatment of the fur-
Isomeric arylmethyl hydrocarbons

furylidene compound (89), obtaining similar amounts of the furan acid (95) and the required heptanoic acid (90); however, cyclization of the latter with dilute alkali led exclusively to (91). The reason for this apparent discrepancy is not known. The German preparation continued by vigorous catalytic reduction of (91), saturating the double bond and removing the ketone oxygen to yield the acid (92). Cyclization of the corresponding acid chloride with stannic chloride to the ketone (93) (70.5%), Clemmensen reduction of the latter, and dehydrogenation gave 16,17-dihydro-7-methyl-15H-cyclopenta[a]phenanthrene (94).

Another intermediate (96) discovered by Robinson during the early approaches to steroid synthesis was adopted by Butenandt for the preparation of several other cyclopenta[a]phenanthrenes (Fig. 17). This ketone was obtained by Hawthorne and Robinson (1936) by condensation of tetralone with acetylcyclopent-l-ene in the presence of sodium amide. Elimination of the ketone oxygen by heating the semicarbazone with sodium ethoxide and a trace of hydrazine hydrate, followed by dehydrogenation provided a new synthesis of 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) (Butenandt et al., 1946c). Introduction of a methyl group at C-12 by treatment of the ketone (96) with methyl magnesium iodide, followed by dehydration and dehydrogenation gave the 12-methyl hydrocarbon (97). In a similar manner 7-methyltetralone

Fig. 17
gave rise to the 2-methyl and 2,12-dimethyl compounds (98 and 99, respectively), while the 4-methyl (100) and 4,12-dimethyl (101) isomers were prepared from 5-methyltetralone (Butenandt et al., 1949b).

Oxidation of the parent hydrocarbon (1) with chromic acid did not lead, as expected for a phenanthrene derivative, to the 6,7-quinone (Butenandt et al., 1946b). Instead oxidation occurred at the five-membered ring to afford the 15-ketone (102) (Fig. 18) previously synthesized by Bachmann (1935). On the other hand, osmium tetroxide added across the 6,7-double bond in the hydrocarbon (1) to yield the 6,7-cis-dihydrodiol (103), mild oxidation of which with chromic acid in acetic acid solution gave the desired quinone (104). Reaction of this quinone with an excess of methyl magnesium iodide followed by heating the product with a mixture of zinc chloride and zinc dust then furnished 16,17-dihydro-6,7-dimethyl-15H-cyclopenta[a]phenanthrene (105) in 12.5% yield based on the quinone.

For the synthesis of the 1-methyl hydrocarbon (107) (Butenandt et al., 1950) 1-bromo-8-methylnaphthalene, obtained in several steps from 1,8-diaminonaphthalene (Fieser and Seligman, 1939), was treated with
phenyl lithium at $-10^\circ C$ and ethylene oxide was added to yield the naphthylethyl alcohol (106). The scheme then followed the original method of Cook and Hewett (1933) shown in Fig. 8. In the final cyclization, addition of some acetic anhydride together with acetic and sulphuric acids was found markedly to improve the yield of 16,17-dihydro-1-methyl-15$H$-cyclopenta[a]phenanthrene (107). In its absence a second hydrocarbon, $C_{18}H_{20}$ having a naphthalene chromophore, was also isolated. 1-Methyl compounds are also obtainable from steroids by migration of the 19-methyl group to C-1 by the dienonephenol rearrangement (Kirk and Hartshorn, 1969). Thus 1-methyl-19-norcholasta-1,3,5(10),6-tetraen-3-ol (108) is readily available from cholesta-1,4,6-trien-3-one by treatment with hot acid. Dehydrogenation of this compound with selenium was accompanied, however, by loss of the 1-methyl group with formation of Diels’ hydrocarbon (7) (Butenandt et al., 1954). A dimethyl hydrocarbon thought to be 16,17-dihydro-1,17-dimethyl-15$H$-cyclopenta[a]phenanthrene was obtained earlier (Inhoffen et al., 1949) by similar dehydrogenation of cholesta-1,4-dien-3-one or its rearrangement product, but was later shown to be the 4,17-isomer (110).

Butenandt’s synthesis of the 1-methyl hydrocarbon concluded the acquisition of the 11 possible monomethyl hydrocarbons derived from 16,17-dihydro-15$H$-cyclopenta[a]phenanthrene. A number of 17-methyl hydrocarbons carrying in addition aryl methyl groups were later obtained from 17-ketones via the Grignard reaction; these are discussed in the next section.

Three syntheses of 16,17-dihydro-15$H$-cyclopenta[a]phenanthrenes have been described employing the Diels–Alder reaction to construct this ring system (Fig. 19). Sen Gupta and Bhattacharyya (1954) condensed 1-vinylnaphthalene with cyclopentane-1,2-dicarboxylic acid anhydride in boiling xylene to obtain a crystalline adduct thought to be (111). Treatment with alkali or acid converted it to the naphthyl isomer (112) which resisted dehydrogenation with selenium. Conversion of this to its calcium salt followed by heating the latter with lime caused decarboxylation, and the crude product could then be dehydrogenated to the parent hydrocarbon (1). In the second approach Tamayo and Martin (1952) formed ring-B by condensation of 4-vinylindan (113) with $p$-benzoquinone. The starting material was obtained from 4-chloroindan via the lithium derivative which reacted with ethylene oxide to yield 4-(2-hydroxyethyl)indan, readily dehydrated to (113). Condensation of this with $p$-benzoquinone at 100$^\circ C$ for 20 h gave the adduct (114) which was converted into the dihydroxy-tetrahydro compound (115) and thence into 16,17-dihydro-1,4-dihydroxy-15$H$-cyclopenta[a]phenanthrene (116). In the third
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method (Butz et al., 1940) the acetylene (117) was heated together with maleic anhydride at 130°C to yield the crystalline adduct (118), mp 249–251°C, which could be converted into the parent hydrocarbon (1) in low yield by dehydrogenation over palladium on charcoal at an elevated temperature.

Yet another way of constructing the cyclopenta[a]phenanthrene system by finally closing ring-C was devised by Birch and Robinson (1944) (Fig. 20). Condensation of the 1-acetyl-3,4-dihydronaphthalenes (119 and 120) with lithium 2-isopropylcyclopentenone gave the diketones (121 and 122) which could be cyclized by sodium ethoxide to the 11-keto-12(13)-enes (123 and 124). Attempted methylation of (123) at the angular position (C-13) by addition of methyl magnesium iodide in the presence
of cuprous bromide failed, giving instead the product of addition to the carbonyl group and dehydration (6,7,8,14,16,17-hexahydro-17-isopropyl-11-methyl-15H-cyclopenta[a]phenanthrene, 125). Direct dehydrogenation of (123) with palladium on charcoal gave the 11-phenol (126) whilst reduction of both (123) and (124) with sodium and ethanol followed by selenium dehydrogenation led to 16,17-dihydro-17-isopropyl-15H-cyclopenta[a]phenanthrene (53) and its 3-methoxy derivative (127).

2.3 Hydrocarbons containing an extra double bond in ring-D (15H- and 17H-cyclopenta[a]phenanthrenes)

Although heating with either selenium or noble metal catalysts causes efficient dehydrogenation of the six-membered rings, a double bond is not introduced into the five-membered ring of the steroid or dihydrocyclopenta[a]phenanthrene systems by these reagents. This can, however, be readily accomplished starting from the ring-D ketones, the syntheses of which are described in Chapter 3. Thus treatment of 15,16-dihydrocyclopenta[a]phenanthren-15-one (102) and -17-one (4) with methyl magnesium iodide in boiling benzene, followed by chromatography of the products on active alumina yielded 15-methyl-17/Z-cyclopenta[a]phenanthrene (128) and its 17-methyl-15H isomer (133), respectively (Dannenberg et al., 1960) (Fig. 21). These compounds were found to be carcinogenic in the mouse skin-painting test, rather more active than the very weakly carcinogenic 7- and 11-methyl-16,17-dihydro-15H-derivatives previously tested, and the question arose as to whether carcinogenicity would be higher in molecules containing both these structural features (Dannenberg, 1960). This was later found to be the case by Coombs and Croft (1969) who investigated the series of six 17-methyl-16(17)-enes (133-138) prepared from the corresponding 17-ketones by the same method (Coombs, 1966). Dannenberg noted that the formation of (133) was accompanied by the appearance of a dimer C_{36}H_{28}, and suggested the structure (129) for this substance. This was confirmed by Coombs by a nuclear magnetic resonance study; he also reported that the yield of the monomer varied appreciably with the structure of the ketone used as substrate, as shown in Table 1. Dimerization apparently occurred during chromatography although neutral alumina was employed, for the yield of the 11-methoxy-17-methyl-16(17)-ene (138) was only 18% when it was obtained in this way, whereas it was 85% when it was isolated directly by crystallization before chromatography. The yield was also low for the 12-methyl (136) and 11,12-dimethyl (137) compounds prepared by the general procedure.
Hydrogenation of the D-ring double bond occurred readily to give the corresponding dihydro derivatives (7, 17, 139–142), but the corresponding double bond in the dimer (129) was inert, as anticipated from its sterically hindered nature. Dannenberg also obtained the 17-methyl-16(17)-ene (133) when 15,16-dihydrocyclopenta[a]phenanthrene-17-one

![Chemical structures](image)

**Table 1. Yields of 17-methyl-15H-cyclopenta[a]phenanthrenes isolated after chromatography on alumina**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted (133)</td>
<td>60</td>
</tr>
<tr>
<td>3-methoxy (134)</td>
<td>55</td>
</tr>
<tr>
<td>12-methyl (136)</td>
<td>28</td>
</tr>
<tr>
<td>11-methyl (135)</td>
<td>74</td>
</tr>
<tr>
<td>11,12-dimethyl (137)</td>
<td>32</td>
</tr>
<tr>
<td>11-methoxy (138)</td>
<td>18</td>
</tr>
</tbody>
</table>
was treated with the methyl Wittig reagent. Coombs, however, isolated the expected 17-methylene derivative (143) without difficulty, but showed that it isomerized very readily to (133) in the presence of traces of acid.

Two isomers of the parent hydrocarbon cyclopenta[a]phenanthrene can exist (Fig. 22), depending upon whether the ring-D double bond is at 16,17- (i.e., 15H) (3) or at 15,16- (i.e., 17H) (2). The theoretically possible third 16H-isomer (144) is unknown, although intermediates with this quinoid bond structure seem to be involved in certain reactions at ring-D (Chapter 3). The 17H-isomer, mp 164–165°C, was prepared by Badger et al. (1952) by elimination from the 15-benzoate (146) in boiling dimethylaniline and by Süß (1953) by decarboxylation of 17H-cyclopenta[a]phenanthrene-17-carboxylic acid (148). In an attempt to prepare the then-unknown 15H-isomer (3) Coombs (1966) reduced the 17-ketone (4) with sodium borohydride and converted the 17-alcohol into its p-toluene sulphonate (145). However, base-catalysed elimination from this ester in boiling collidine gave the 17H-isomer (2) of mp 164–165°C; the structure of this compound was confirmed by preparing it in the same manner from the 15-tosylate (147). Since the 16(17)-ene (3) had presumably been formed initially from the 17-tosylate (145) and had subsequently undergone isomerization at 170°C in the reaction mixture, use was made of dimethylsulphoxide as solvent for this elimination (Nace, 1959). After 30 min at 100°C in this solvent the hydrocarbon isolated in low yield by rapid chromatography on silica gel was different from (2); it was charac-

![Fig. 22](image-url)
Chemical synthesis: hydrocarbons

terized as the 16(17)-ene (3) by the similarity of its ultraviolet spectrum with that of 17-methyl-15\(H\)-cyclopenta[a]phenanthrene (133). In particular it lacked the small maximum at 239 nm characteristic of the 15(16)-ene chromophore. It was rapidly isomerized at 180°C, and even at 100°C when the elimination in dimethyl sulphoxide was prolonged; it melted at the same temperature as its 17\(H\)-isomer, no doubt due to the fact that it isomerized during heating up to 165°C. Kon (1933) had obtained an isomeric hydrocarbon, mp 182–183°C, by selenium dehydrogenation of the phosphoric acid cyclization product of the hydrocarbon (42) (Fig. 9) and thought it to be either 15\(H\)- or 17\(H\)-cyclopenta[a]phenanthrene, but this hydrocarbon was shown later to be chrysofluorene (Cook and Hewett, 1934). More recently Kotlyarevskii and Zanina (1961) isolated a compound of the same melting point and empirical formula from the products of pyrolysis of 1-(1,2,3,4-tetrahydro-1-hydroxy-1-naphthyl)-2(1-hydroxycyclopentyl)-acetylene over a magnesia–chromia–alumina catalyst at 400–500°C. They claimed it to be a 1,2-cyclopen-tadienophenanthrene by reference to Kon’s paper, and by the fact that it consumed two atoms of hydrogen to yield 16,17-dihydro-15\(H\)-cyclopenta[a]phenanthrene. The structure of this compound is obscure, but it does not appear to be either 15\(H\)- or 17\(H\)-cyclopenta[a]phenanthrene. In a later study, Coombs and Hall (1973) treated 15\(H\)-cyclopenta[a]phenanthrene (3) with osmium tetroxide to obtain the cis-16,17-diol. Similar treatment of the 17\(H\)-isomer (2), prepared by elimination from the 17-tosylate by boiling with collidine as described above, unexpectedly gave two diols, the anticipated cis-15,16-diol and in addition the cis-16,17-diol identical with that obtained from the 15\(H\)-hydrocarbon. When 17\(H\)-cyclopenta[a]phenanthrene, prepared from the 15-tosylate by elimination by the more gentle method (in dimethyl sulphoxide at 100°C for 1 h), was similarly oxidized only the cis-15,16-diol was formed. It was therefore evident that the sample of the hydrocarbon previously thought to be the 17\(H\)-isomer was in fact a mixture of the 15\(H\)- and 17\(H\)-hydrocarbons; the pure hydrocarbon differed from this mixture only in that the 239-nm band in its ultraviolet spectrum was somewhat more intense, and that it gave rise to only one diol. Dehydrogenation of 16, 17-dihydro-15\(H\)-cyclopenta[a]phenanthrene with 2,3-dichloro-5,6-dicyanobenzoquinone in boiling benzene furnished a good yield of the mixture of 15\(H\)- and 17\(H\)-hydrocarbons, as disclosed by the osmium tetroxide test. By contrast the tosylate derived from 16,17-dihydro-11-methyl-15\(H\)-cyclopenta[a]phenanthren-17-ol (149) on being boiled with collidine, gave only the 15\(H\)-hydrocarbon (150), 11-methyl-15\(H\)-cyclopenta[a]phenanthrene; it showed no ultraviolet absorption at
239 nm and gave only one diol with osmium tetroxide. It therefore seems that an 11-methyl group stabilizes the 16(17)-double bond in the five-membered ring in this compound.

Introduction of a double bond into the five-membered D-ring of 15,16-dihydrocyclopenta[a]phenanthrenes cannot be accomplished directly by dehydrogenation by selenium or noble metal catalysts, but dehydrogenation with high-potential quinones is successful in this respect. Dannenberg et al. (1956) found that the ultraviolet spectra of a mixture of cyclopenta[a]phenanthrenes isolated from the products of dehydrogenation of cholesterol with chloranil (tetrachloro-p-benzoquinone) in boiling xylene indicated the presence of compounds having an extra double bond in ring-D (Fig. 23). Under these conditions three new cyclopenta[a]phenanthrenes (151)–(153) were isolated in 32% yield. In all these the cholesterol side chain was retained, but the 18-methyl group had migrated to C-17 giving optically active products. In addition the 19-methyl group had been eliminated in the case of (151), but had migrated...
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to C-1 in (152) and C-4 in (153). The structures of these hydrocarbons were confirmed by their preparation by similar dehydrogenation of 19-norcholesterol (154), 1-methyl-19-norcholesta-1,3,5(10),6-tetraene (155) (Dannenberg et al., 1964), and 4-methyl-19-norcholesta-1,3,5-triene (156) (Dannenberg and Neumann, 1961) in yields ranging between 30 and 45%. The best yields and shortest reaction times were obtained with DDQ (2,3-dichloro-5,6-dicyanobenzoquinone) in boiling anisole. Quinone dehydrogenation reactions are known to involve a two-step ionic mechanism (Jackman, 1960). Abstraction of a hydride ion (H⁻) from cholesta-3,5-diene (157) by the quinone is followed by loss of a proton (H⁺) or carbonium ion (CH⁺) with the formation of the hydroquinone and a double bond in the substrate (Fig. 23). Dannenberg (1970) proposed that this diene is first dehydrogenated to the 1,3,5-triene (158); addition of a proton then induces a diene-phenol type of rearrangement (symbolized by $\text{\textbullet\textbullet}$) leading eventually to the 4-methylcyclopenta[a]phenanthrene (153). Hydride abstraction from C-7 in this triene gives the carbonium ion (159) which undergoes another diene-phenol rearrangement to yield finally the 1-methyl hydrocarbon (152). Loss of a proton from (159) followed by hydride ion abstraction from C-9 in the 1,3,5,7-tetra-ene results in loss of the methyl group as a carbonium ion and formation of a naphthalene, finally converted to (151) by further dehydrogenations.

Dehydrogenation of 17β-methyl-Δ⁹(13)-testosterone (160) (Fig. 24) with DDQ in boiling dioxan containing p-toluene sulphonlic acid led to the

![Fig. 24](image_url)
1,17,17-trimethyl-3-phenol (162) by way of the 1,4,6,9(11),16(17)-penta-ene (161) (Brown and Turner, 1971). Isolation of a 16(17)-ene from a 17-methyl-17-ol is unusual because dehydration is normally accompanied by Wagner–Meewein rearrangement of the 18-methyl group to C-17. In this case it was considered that relief of the usual 11β-H, 18-methyl diaxial interaction of about 0.8 kcal/mol by the presence of the 9,11-double bond accounts for this. The final step, involving a diene-phenol rearrangement of the C-19 methyl group to C-1 as well as rearrangement of the C-18 methyl group to C-17, occurred simply by boiling the penta-ene (161) with acetic anhydride and p-toluene sulphonic acid, possibly by the mechanism shown, the last double bond being provided by aerial oxidation. By contrast the 1,4,6-trien-3-one (163) lacking the 9(11)-double bond underwent the usual diene-phenol rearrangement to give the naphthol (164), dehydrogenation of which with DDQ yielded a mixture of (162) and the 15(16)-ene, 3-hydroxy-1,17,17-trimethylcyclopenta[a]phenanthrene (165), probably via the sequence shown. This compound was not obtained from its 15,16-dihydro derivative (162) by treatment with DDQ.

Ring-D cleavage accompanied dehydrogenation of oestrone with chloranil in a mixture of boiling dioxan and tert.-butanol (Cross et al., 1963) (Fig. 25). Dehydrogenation proceeded via the 9(11)ene (166) to yield the dihydrophenanthrene acid ester (167); this was not formed in the absence of tert.-butanol. The 17,17-dicycloethylene ketal of oestrone
methyl ether gave a similar product in 77% yield under very mild conditions with an excess of DDQ in benzene at room temperature for 5 min (Boots and Johnson, 1966). In the case of the 17β-ol (168) cleavage occurred with the formation of the corresponding aldehyde (169) (Dannenberg, 1970). Quinone dehydrogenation is often accompanied by Diels–Alder addition to yield quinones, and sometimes by chlorination. Thus chloranil dehydrogenation of ergosterol gave, besides the expected product 17-methyl-17-(1,4,5-trimethyl-2-hexenyl)-cyclopenta[a]phenanthrene (170), also the product (171) of 7,15- addition of the quinone. Similar dehydrogenation of 3β,17β-dihydroxy-17α-methylandrostan-5-ene gave 3-chloro-15,16-dihydro-17,17-dimethylcyclopenta[a]phenanthrene (172) (Dannenberg and Hebenbrock, 1966).

A novel route to cyclopenta[a]phenanthrenes has recently been described by Lee-Ruff and co-workers based on the ready acid-catalysed ring cleavage of cyclobutanones and cyclobutanol. Thus condensation of cyclopentadiene with 1-naphthyl ketene, prepared in situ from 1-naphthylacetyl chloride and triethylamine in boiling benzene, gave the cyclobutanone (173) (Fig. 26). Treatment of the latter with trifluoroacetic acid then led to 11,12,13,14-tetrahydro-17H-cyclopenta[a]phenanthren-12-one (174) by way of the enol cation or cyclopropyl cation in about 75% yield (Lee-Ruff et al., 1981). Reduction of the cyclobutanone to the cyclobutanol (175) and acid treatment gave phenyl-1-naphthylmethane (60%), 13,14-dihydro-17H-cyclopenta[a]phenanthrene (176) (25%), and 17H-cyclopenta[a]phenanthrene (2) (5%) (Lee-Ruff et al., 1982). Both the tetrahydroketone (174) and the dihydrohydrocarbon (176) were converted into (2) by conventional means.

Fig. 26
References

2.4 References


Cohen, A., Cook, J. W. & Hewett, C. L. (1935). The synthesis of
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Chemical synthesis of cyclopenta[a]-phenanthrenes: ring-D ketones

Early cyclopenta[a]phenanthrene syntheses were directed at the preparation of hydrocarbons and were not in the main readily adaptable to the synthesis of the corresponding ring-D ketones. However, parallel synthetic endeavour at that time was also aimed at the synthesis of 17-ketosteroids such as equilenin. Thus Bardhan (1936) condensed 2-(1-naphthyl)ethyl bromide with dimethyl 2-ketoadipate to give the product (177) which was cyclized with cold sulphuric acid to the dihydrophenanthrene (178) (Fig. 27). Formation of the five-membered ring by heating with acetic anhydride followed by decarboxylation at 210°C gave 11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (179). The last step of dehydrogenation to give the corresponding phenanthrene was not undertaken, but the structure of (179) was established by converting it by way of Clemmensen reduction and selenium dehydrogenation into the known hydrocarbon (1). A similar synthesis was reported by Bachmann et al. (1943); in this the final cyclization of the di-acid (178) was brought about under Dieckmann conditions with sodium methoxide in dry benzene. Bachmann and Kloetzel (1937) obtained the parent 17-ketone (4), 15,16-dihydrocyclopenta[a]phenanthren-17-one by cyclization of the acid chloride of 3-(1-phenanthyl)propionic acid (180) with aluminium chloride in nitrobenzene at 0°C, several other cyclization methods having failed. This ketone, mp 195–196°C, was isolated in very low yield, the major product being its isomer (181) in which cyclization had occurred in the opposite direction. The isomeric 15-ketone (102) (15,16-dihydrocyclopenta[a]phenanthren-15-one), mp 183–184°C, was the only product when the chloride of 3-(2-phenanthyl)propionic acid (182) was cyclized in nitrobenzene solution with stannic chloride at 80°C (Fig. 27). The structures of both these ketones were confirmed by reduction to the
known hydrocarbon (1). Finally, the synthesis of the 15,17-diketone (185) was described by Fieser et al. (1936) who simply condensed dimethyl phenanthrene-1,2-dicarboxylate (183) with ethyl acetate in the presence of sodium, and hydrolysed and decarboxylated the product to give the pure diketone in 74% yield. 15,16-Dihydrocyclopenta[a]phenanthren-15,17-dione (185) melted at 240.5–241.5°C, and behaved as a typical diketone, dissolving in dilute alkali to give a red solution. It was recovered unchanged when the solution was immediately acidified, but on standing hydrolysis to a mixture of isomeric acetyl phenanthroic acids took place. 15,16-Dihydro-1,6-dimethoxy-cyclopenta[a]phenanthren-17-one (186) was also prepared in a similar manner (Fieser and Hershberg, 1936).

Two other syntheses of the 11,12-dihydro-17-ketone (179) have been described. Johnson and Petersen (1945) employed the Stöbbe reaction to

![Chemical synthesis: ring-D ketones](image-url)
add ring-D to 1,2,3,4-tetrahydrophenanthren-1-one (187) as shown in Fig. 28; the final ring closure was effected with anhydrous zinc chloride in acetic acid. This method was later used extensively by Coombs and his co-workers for the preparation of methyl and other homologues. Bachmann and Holman (1951) used the same tetrahydrophenanthrene ketone (187) and its 3-methoxy derivative (188), but built up the three-carbon chain by a Reformatsky reaction with bromoacetic ester followed by Arndt–Eistert chain extension, and ring closure as before.

Robinson (1938) in searching for synthetic routes to 17-keto steroids devised the remarkable cyclopenta[a]phenanthrene synthesis already discussed in the previous chapter (see Fig. 29). In this reaction scheme closure of the six-membered ring-C in the precursors (79) and (189) (Robinson and Rydon, 1939) was brought about almost quantitatively by boiling them with acetic anhydride. The products (80) and (190),
however, contained a phenolic acetate group at C-11, and methods for removing this without concomitant loss of the 17-oxygen function were unknown at that time. This problem was circumvented (Koebner and Robinson, 1938) by first reducing the 13(14)-double bond, then cyclizing the cyclopentanone acid (191) to give the 11,17-diketone (193). The two ketone groups in this compound differ markedly in reactivity, the 11-ketone being relatively inert owing to steric hindrance, and use was made of this property to eliminate the unwanted oxygen at C-11. Unfortunately, neither the reduction nor the cyclization proceed smoothly in this case; the catalytic reduction of the tetra-substituted double bond over palladium on charcoal is sluggish and over-reduction with loss of the 17-oxygen is difficult to minimize. Ring closure proceeds with difficulty; most conventional methods fail, but yields of about 40% can be obtained under strictly controlled conditions (exposure to polyphosphoric acid at 125°C for 3 min) (Birch et al., 1945). The present authors experienced these difficulties when employing this route for the preparation of 17-ketones of the cyclopenta[a]phenanthrene series, but found that the diketone (193) was most valuable starting material for the synthesis of a number of compounds. Two recent developments have made this route potentially more attractive (Fig. 30). Cyclization of a closely related compound (195) to the diketone (196) was achieved in 65% yield (75% based on the recovered starting material) by the use of
hydrogen fluoride in dry tetrahydrofuran (Posner et al., 1979). Also the diketone (193) has been obtained (Jung and Hudspeth, 1978) by a new method. The bicyclic enone (197), readily obtainable in 67% yield in several steps from vinyl acetate and dimethoxytetra-chlorocyclopentadiene, reacted with 1-naphthyl magnesium bromide to give a high yield of the exo-alcohol (198). Treatment of this in tetrahydrofuran with sodium amide caused a base-catalysed Cope rearrangement to the hexahydrocyclopenta[a]phenanthrene (199) in 75% yield. This compound was readily converted by partial hydrogenation, diketalization, and dehydrogenation into the diketone (193) via the decahydro compound (200). The C/D ring junction in this important diketone (193) is trans (Chinn et al., 1962), and it has been resolved into its optically active (+) and (−) forms making use of stereospecific reduction with Rhodotorula mucilaginosa (Siewinski et al., 1969). The diketone was first reduced to the diol with lithium aluminium hydride (Mejer and Kalinowska, 1969), then selectively oxidized back to the 17α- and 17β-hydroxy-11-ketones with chromium trioxide in pyridine. On fermentation of the 17α-hydroxy-ketone with this organism only half was reduced stereospecifically to the 17α, 11β-diol, which on re-oxidation yielded (+) (193); re-oxidation of the residual ketoalcohol led to (−) (193) of opposite and equal specific rotation. The melting point of both these optical isomers was 138°C, 20 degrees higher than that of the racemic diketone ordinarily encountered.

Following Marrian’s suggestion regarding the possibility of aromatization of oestrone to its phenanthrene analogue already described in Chapter 1, a reappraisal of methods for the synthesis of 17-ketocyclopenta[a]phenanthrenes was undertaken. Robinson’s 11,17-diketone (193) was initially taken as a point of departure (Fig. 31) (Coombs, 1965), and it was found that by heating it with 2-methyl-2-ethyl-1,3-dioxolan in the presence of a catalytic quantity of p-toluene sulphonic acid this diketone gave the oxo-ketal (201) in good yield. Prolonged heating led to the 11,17-diketal (203), but it was found that the benzylic ketal group in this compound was very sensitive to acid so that it could be readily converted back into the monoketal with a trace of hydrogen chloride in damp chloroform (Coombs, 1966). When this oxo-ketal (201) was reduced with sodium borohydride to the 11-ol and the latter was boiled with acetic acid containing hydrochloric acid in the presence of an excess of nitrobenzene 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) was obtained directly in 86% yield melting at 197°C; after recrystallization it formed very pale yellow needles, melting at 203–204°C. In a similar way the 3-methoxy-oxo-ketal (202) furnished the methyl ether of the phenanthrene
analogue of oestrone, 15,16-dihydro-3-methoxycyclopenta[a]phenanthren-17-one (24), in the same yield. In the absence of nitrobenzene the yield of (4) was halved and the tetrahydro derivative (205) was also formed. A detailed examination of the reaction led to the conclusion that it proceeds via the enol by abstraction of hydrogen from C-14 as shown in Fig. 31. Nitrobenzene had been employed previously by Koebner and Robinson (1941) to induce aromatization of ring-C. Thus when the 11,17-diketone (193) was treated with sodium hydroxide in the presence of nitrobenzene the phenol 15,16-dihydro-11-hydroxycyclopenta[a]phenanthren-17-one (206) was formed directly, presumably via the enols by a similar mechanism. The tendency for dehydrogenation of ring-C to occur in suitable compounds was illustrated by these authors who found that the 16-benzylidene derivative of x-norequilenin methyl ether (207), dissolved in methanolic sodium hydroxide, on exposure to air for two days gave a bright yellow precipitate of 16-benzylidene-15,16-dihydro-3-methoxycyclopenta[a]phenanthren-17-one (208) by the formal loss of four hydrogen atoms from this ring.

In a similar manner (Fig. 32) treatment of the oxo-ketal (201) with methyl or ethyl magnesium iodide, or with n-butyl lithium (Coombs et al., 1973), and submission of the products to acid in the presence of nitrobenzene led to good yields of the 11-methyl-, 11-ethyl-, and 11-n-butyl-derivatives (26, 211, and 212). Methylation of the oxo-ketal (201)
with methyl iodide in the presence of potassium tert.-butoxide gave a mixture of mono- and dimethyl derivatives (209) and (210), separated by column chromatography. Reduction of the 12-methyl-oxo-ketal (209) or treatment of it with methyl lithium followed by acid and nitrobenzene led to the 12-methyl- and 11,12-dimethyl-17-ketones (130 and 131), again in good yield. Treatment of the oxo-ketal (201) with the ylide from trimethylsulphonium iodide (Coombs et al., 1975) and acidification of the intermediate epoxide to pH3 afforded the diol (213) which was acetylated and dehydrated with phosphoryl chloride in pyridine. Quinone dehydrogenation of the product (214) and removal of the protecting groups finally yielded 15,16-dihydro-11-hydroxymethyl-cyclopenta[a]phenanthren-17-one (215). Condensation of the oxo-ketal (201) with ethyl formate in the presence of sodium ethoxide gave the bright yellow formyl-ketone (216) which was reduced to the diol with lithium aluminium hydride. When this diol was boiled with acetic acid containing hydrochloric acid and nitrobenzene, the 12-methyl-17-ketone (130) was formed together with the dimer (217).

Whilst this route was most successful for the preparations of 11- and 12-substituted-17-ketones, several difficulties beset its application to the
synthesis of $15,16$-dihydro-7-methylcyclopenta[a]phenanthren-17-one (230) (Fig. 33). The furyrylidene derivative of 2-acetyl-3-methylnaphthalene (218) gave not only the expected dioxoheptanoic acid (220) on acid hydrolysis, but the naphthylfuran propionic acid (223) was also formed in similar yield (Coombs and Jaitly, 1971). The analogous methoxy compound (219) obtained from 2-acetyl-3-methoxynaphthalene gave mostly the furan acid (224) with only a small quantity of the dioxo acid (221), whereas the unsubstituted compound (77) gave the dioxo acid (78) almost exclusively (Coombs and Vose, 1975). An nmr study confirmed the previous suggestions (Butenandt et al., 1949; Short and Rockwood, 1969) that a substituent at C-3 in the naphthalene ring decreases conjugation between this ring system and the adjacent side chain by preventing co-planarity of these two systems. This in turn diminishes charge delocalization in the protonated intermediate (225) thereby promoting cyclization to the furan. The dioxo acid (220) underwent smooth ring-closure in dilute alkali to the cyclopentenone (226) in which again the methyl group prevents co-planarity of the naphthalene and cyclopentone rings. Hydrogenation of this compound gave a mixture of at least six products, and was not pursued further. Cyclization of (226) with boiling acetic anhydride occurred normally to give $11$-acetoxy-$15,16$-dihydro-7-methylcyclopenta[a]phenanthren-17-one (227) in good yield. After several failures it was discovered that reduction of the corresponding diethylphosphate (229) with sodium in a mixture of tetrahydrofuran and liquid ammonia (Kenner and Williams, 1955) led to the desired 7-methyl-17-ketone (230), surprisingly in 47% yield. Presumably the
ketone is protected as the enol during this reduction. When this scheme was applied to the synthesis of the unsubstituted ketone (4), the yield in the reduction of the phosphate (228) was 27%; overall the yield from the cyclopentenone acid (79) was 18%, similar to that obtained in the longer synthesis from this intermediate previously described (see Figs. 29 and 31). Desulphurization of the tosylate of 15,16-dihydro-11-hydroxy-cyclopenta[a]phenanthren-17-one with Raney nickel to the corresponding hydrocarbon was later reported (Kawarura et al., 1974), but this procedure, of course, also removes the 17-carboxyl group.

Another kind of difficulty arose when syntheses of 1,2,3,4,15,16-hexhydrocyclopenta[a]phenanthren-17-one (237) and its 11-methyl homologue (238) were attempted using this general approach (Coombs and Bhatt, 1973) (Fig. 34). 6-Acetyl-1,2,3,4-tetrahydronaphthalene was converted into its furfurylidene derivative which with hot acid yielded the corresponding dioxoheptanoic acid without difficulty, and ring closure of this to give the cyclopentenone acid (231) occurred smoothly under alkaline conditions. Over-reduction was difficult to avoid during the hydrogenation of the double bond in this compound, and a better method of obtaining the desired cyclopentanone acid (232) was by reduction with lithium in liquid ammonia. The difficulty arose in the next cyclization step because, lacking the strong directing influence of a naphthalene ring system, ring closure occurred in both directions leading to the angular (233) and linear (234) diketones in the ratio 1:4. Cyclization of the cyclopentenone acid (231) with boiling acetic anhydride gave a similar mixture of angular (235) and linear (236) acetoxy ketones. The angular diketone (233) was converted into its 17-ketal which on being either reduced or treated with the methyl Grignard reagent, followed by
aromatization with nitrobenzene and acid, yielded the tetrahydro-17-ketones (237) and (238).

The above-described synthetic scheme is not convenient for the preparation of certain other ring-substituted 17-oxocyclopenta[a]phenanthrenes owing to the difficulty of obtaining suitably substituted 2-acetyl naphthalenes. Attention was therefore directed towards the method of Johnson and Petersen (1945) utilizing the Stobbe reaction to build up the five-membered ring-D from suitable 1,2,3,4-tetrahydrophenanthren-1-ones. This method was also used by Riegel et al. (1948) for the synthesis of 12-substituted derivatives (Fig. 35). These authors condensed 1-naphthyl magnesium chloride with ethylidene, propylidene, and isobutylidene malonic diester and hydrolysed and decarboxylated the products to furnish the 3-alkyl-4-(1-naphthyl)butyric acids (239-241). Cyclization of the latter (phosphorus pentachloride and stannic chloride) gave the required three-ring ketones (242-245) which were converted into the half-esters (246-248) by means of the Stobbe reaction with diethyl succinate in the presence of potassium tert.-butoxide. Closure of the five-membered ring (zinc chloride in acetic acid) followed by hydrolysis and decarboxylation gave the tetrahydro ketones (249-251), from which the 12-alkyl-15,16-dihydrocyclopenta[a]-phenanthren-17-ones (130, 253, and 254) were obtained on dehydrogenation by heating with palladium on charcoal. Clemmensen reduction of these ketones gave the 12-alkyl hydrocarbons (97, 255, and 256) in which they were interested. This sequence was also employed by Woodward et

Fig. 35

\[
\begin{align*}
R & = CH_3 \quad 239 \quad 242 \quad 246 \\
R & = CH_2 CH_3 \quad 240 \quad 243 \quad 247 \\
R & = CH(CH_3)_2 \quad 241 \quad 244 \quad 248 \\
R & = CH(CH_3) \quad 249 \quad 250 \quad 251 \\
& 253 \quad 254 \quad 97 \\
& 255 \quad 256 \quad 110
\end{align*}
\]
al. (1953) to construct the 4,17-dimethyl hydrocarbon (110) from 5-methyl tetralone via 4-methyl-11,12,15,16-tetrahydro-cyclopenta[a]phenanthren-17-one (257).

For the synthesis of the 2-, 3-, 4-, and 6-methyl- and 6-methoxy-17-ketones (Coombs et al., 1970), the 2-methoxy-17-ketone (Coombs et al., 1975), the 1-methyl, 7,11-dimethyl-, and 1,11-methano-17-ketones (Ribeiro et al., 1983), and 1-methyl-4-hydroxy-17-ketone (Coombs et al., 1985) the required 1,2,3,4-tetrahydrophenanthren-1-ones were prepared by several routes (Fig. 36). Thus reaction of Grignard reagents prepared...
Chemical synthesis: ring-D ketones

from the 1-bromonaphthalenes (258-260) with ethylene oxide gave the alcohols (261-263, X = OH), converted to the bromides (261-263, X = Br) with phosphorus tribromide and thence to the naphthyl butyric acids (264-266) by means of the malonic acid synthesis. Ring closure then gave the required tricyclic ketones (187, 267, and 268). In a second method the readily available 1-tetralones (269-273) reacted with methyl 3-bromo-crotonate under Reformatsky conditions to form the 2,4-dienoic acids (274-278); isomerization to the naphthyl acids (279-283) was achieved with palladium black at 280-300°C, followed by saponification. Cyclization as before gave the ketones (187 and 284-287). 1,2,3,4-Tetrahydro-4-methylphenanthren-1-one (290) was obtained either from 2-(1-naphthyl)-propionaldehyde (288) by condensation with malonic acid followed by decarboxylation and catalytic hydrogenation of the double bond and cyclization, or from methyl 2-(1-naphthyl)-propionate (289) by reduction to the alcohol, conversion of this into the bromide, and chain extension by the malonic acid route to yield the same naphthyl valeric acid. For the synthesis of 15,16-dihydro-1-methylcyclopenta[a]phenanthren-17-one (Ribeiro et al., 1983) the keto-ester (291) was converted by a Reformatsky reaction with methyl bromoacetate into the lactone (292), which underwent acid-catalysed hydrogenolysis to the acid ester (293). This was cyclized and the ketone was transformed in several steps into the naphthyl acetic ester (294), which was chain-extended and cyclized as before to yield the tricyclic ketone. In a similar manner Reformatsky reaction with 3-methyltetralone (296) and ethyl 2-bromopropionate led to the ester (297) from which 1,2,3,4-tetrahydro-4,10-dimethylphenanthren-1-one (298) was obtained as before. The tetracyclic ketone (300) was synthesized from 3-acenaphthyl acetic acid (299) which was reduced to the alcohol, converted into the bromide with triphenylphosphine dibromide, and thence into the nitrile with potassium cyanide. Hydrolysis to the acid and cyclization gave the desired ketone (300). 8-Methoxy-5-methyl-1,2,3,4-tetrahydrophenanthren-1-one (301) was prepared from the corresponding tetralone by Reformatsky reaction with ethyl bromoacetate, followed by dehydration and aromatization to the naphthalene. The side chain was extended and cyclized as before. These ketones were converted into 15,16-dihydrocyclopenta[a]phenanthren-17-ones by the sequence illustrated in Fig. 35. The Stöbbe reaction proceeded in fair to good yield, except in the case of the dimethyl ketone (298) where the yield of half-ester was poor; presumably steric hindrance by the peri-7-methyl group accounts for this. Closure of the five-membered ring was satisfactory provided strictly anhydrous conditions were maintained by the use of freshly fused zinc chloride in a
Ring-D ketones

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mixture of acetic acid and acetic anhydride. Dehydrogenation, the final step in this synthesis, was best achieved with DDQ in boiling benzene or (probably better) by palladium in boiling p-cymene. The former method fails with the 11-methyl compounds, probably because the 11-methyl group is axial in the 11,12-dihydro-derivative and therefore does not possess a pair of trans-diaxial protons required for quinone dehydrogenation. Fifteen 17-ketones prepared in this way from 1,2,3,4-tetrahydrophenanthren-1-ones are summarized in Table 2.

The 1-methyl-17-ketone (302) was also obtained in very low yield from testosterone as shown in Fig. 37. Initially A-ring aromatization of a suitable steroid via the dienone-phenol rearrangement appeared to

Table 2. 15,16-Dihydrocyclopenta[a]phenanthren-17-ones prepared by the Stobbe reaction

<table>
<thead>
<tr>
<th></th>
<th>1,2,3,4-tetrahydrophenanthrene</th>
<th>15,16-dihydrocyclopenta[a]-phenanthren-17-one</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted</td>
<td>(187)</td>
<td>(4)</td>
</tr>
<tr>
<td>1-CH₃</td>
<td>(295)</td>
<td>(302)</td>
</tr>
<tr>
<td>2-CH₃</td>
<td>(284)</td>
<td>(303)</td>
</tr>
<tr>
<td>3-CH₃</td>
<td>(285)</td>
<td>(304)</td>
</tr>
<tr>
<td>4-CH₃</td>
<td>(286)</td>
<td>(305)</td>
</tr>
<tr>
<td>6-CH₃</td>
<td>(267)</td>
<td>(306)</td>
</tr>
<tr>
<td>11-CH₃</td>
<td>(290)</td>
<td>(26)</td>
</tr>
<tr>
<td>2-OCH₃</td>
<td>(287)</td>
<td>(307)</td>
</tr>
<tr>
<td>6-OCH₃</td>
<td>(268)</td>
<td>(308)</td>
</tr>
<tr>
<td>7,11-(CH₃)₂</td>
<td>(298)</td>
<td>(309)</td>
</tr>
<tr>
<td>1,11-methano</td>
<td>(300)</td>
<td>(310)</td>
</tr>
<tr>
<td>1-CH₃,4-OCH₃</td>
<td>(301)</td>
<td>(311)</td>
</tr>
<tr>
<td>12-CH₃</td>
<td>(242)</td>
<td>(130)</td>
</tr>
<tr>
<td>12-CH₂CH₃</td>
<td>(243)</td>
<td>(253)</td>
</tr>
<tr>
<td>12-CH₂(CH₃)₂</td>
<td>(245)</td>
<td>(254)</td>
</tr>
</tbody>
</table>

Fig. 37
Chemical synthesis: ring-D ketones

promise a convenient way of placing a methyl group at C-1 in a cyclopenta[a]phenanthrene. Testosterone acetate was transformed in four steps by known methods (Burgess et al., 1962) into 1-methyloestra-1,3,5(10)-trien-17-one (312). After having rejected several possible methods for the elimination of the 18-methyl group, the method devised by Cohen et al. (1971) for the removal of a 4-methyl group from a tetracyclic triterpene was selected, and investigated first with the readily available oestrone methyl ether. Abnormal Beckmann rearrangement of the oxime (314) in dimethylsulphoxide with dicyclohexylcarbodiimide and trifluoroacetic acid gave the cyano-ene (315) which was epoxidized with m-chloroperbenzoic acid. Cyclization of the epoxide with boron trifluoride in boiling toluene then afforded 18-noroestrone methyl ether (22) in 12.5% yield, from which both 13α and 13β epimers were isolated in the pure state (Coombs and Vose, 1974). When this reaction sequence was applied to the oxime of the steroid (312) its 18-nor derivative (313) was isolated in 14% yield, apparently as the single 13β epimer. Unfortunately, complete dehydrogenation of this compound with DDQ in boiling dioxan proceeded with difficulty, but a small amount of 15,16-dihydro-1-methylcyclopenta[a]phenanthren-17-one (302) identical with that prepared by total synthesis was isolated (Ribeiro et al., 1983). Of no use practically, this transformation again stresses the close relationship between steroids and cyclopenta[a]phenanthrenes.

Isotopically labelled 15,16-dihydrocyclopenta[a]phenanthren-17-ones required for biochemical and biological studies were prepared (Fig. 38) by adapting the various methods already described. A methyl group labelled with either carbon-14 or tritium was readily introduced at C-11 by means of a Grignard reaction between the oxo-ketal (201) and the appropriately labelled methyl magnesium iodide followed by the usual aromatization procedure. The five-membered ring was also labelled at C-15 and C-16 with carbon-14 by the use of diethyl [2,3-14C]succinate in the Stöbбе reaction (Coombs et al., 1970). Reduction of the oxo-ketal (201) with tritiated sodium borohydride and removal of the easily exchangeable tritium gave the [11-3H]alcohol (317) which yielded [11-3H]-15,16-dihydrocyclopenta[a]phenanthren-17-one without further loss of label, showing that as expected the 11-hydroxyl group is axial in this alcohol. Comparatively low to moderate specific activities were obtainable by these methods. For higher specific activities (in excess of 1 Curie per millimole) catalytic debromination of readily available 15-bromo-17-ketones such as (318) over palladium on calcium carbonate in tritium gas readily gave 15-[3H]-17-ketones at this activity. Even higher specific activities (5–30 Ci/mmol) were obtained by generally labelling with
tritium by acid-catalysed exchange with \[^3\text{H}\]acetic acid at 160°C over Adam's catalyst (Coombs, 1979). All positions were similarly labelled by this method with the exception of the sterically hindered bay region (C-1 and C-11), as disclosed by tritium nuclear magnetic spectroscopy (see Table 3). Tritium is readily lost from C-16 in these compounds by acid-catalysed exchange via the enol, but is stable at other positions under normal conditions (Russell et al., 1985).

Badger et al. (1952) proposed an interesting route to 17-ketocyclopenta[a]phenanthrenes via ring-enlargement of the fluorenone (319) shown in Fig. 39. Treatment of this ketone with diazomethane in ether at room temperature, followed by addition of methanol and a trace of sodium carbonate gave the methoxyphenanthryl propionic ester in about 35% yield. Cyclization of the corresponding acid (320) with anhydrous hydrogen fluoride occurred, however, in the wrong direction to give the methoxy-ketone (321). In order to block this cyclization, the methoxyphenanthrene (320) was chlorinated with phosphorus pentachloride to the o-chloro-methoxy acid which on ring closure with phosphorus pentachloride and stannic chloride yielded 7-chloro-15,16-dihydro-6-methoxycyclopenta[a]phenanthren-17-one (322).

Fig. 38
Another general method for the synthesis of cyclopenta[a]-phenanthrenes based on double acylation of naphthalenes has been described by Rahman and co-workers in several publications (Rahman and Perl, 1968; Rahman and Rodriguez, 1969, 1971; Rahman and Vuano, 1971). For example, ethyl 3-(2-naphthyl)propionate was acylated with succinic anhydride and aluminium chloride at C-6 to give the ketodiester (323) and was converted into the cyclopenta[a]-phenanthrenes (324), (325), (326), and (1) by standard methods. An attractive feature of this route is the good yield at each step, as shown in Fig. 39; many adaptations of this method can be visualized. A straightforward preparation of 15,16-dihydro-15-phenyl-cyclopenta[a]phenanthren-17-one (328) from 2-acetyl-naphthalene has also been reported (Shotter et al., 1973). Conversion of the latter into the styryl ketone (327) in 91% yield was followed by cyclization with polyphosphoric acid at 130°C (47% yield). Treatment of the phenanthylhydroxypropionic acid (329) with 97% sulphuric acid gave 17-methyl-16-phenylcyclopenta[a]phenanthren-15-one (330) (Mladenova-Orlinova et al., 1970).

A recent cyclopenta[a]phenanthren-17-one synthesis employs the Diels–Alder reaction to construct the C-ring (Corey and Estreicher, 1981). Reaction of 3-nitro-2-cyclopentenone with the dimethoxy-diene (331) in boiling toluene gave the adduct which, without isolation, was treated with 1,5-diazabicyclo-[4.3.0]non-5-ene forming 3,11-dimethoxy-

Table 3. Distribution of tritium in 15,16-dihydrocyclopenta[a]-phenanthren-17-ones generally labelled by catalytic exchange with \([^3]H\)acetic acid as disclosed by tritium nmr spectroscopy

<table>
<thead>
<tr>
<th>Relative ([^3]H) incorporation at:</th>
<th>Compound</th>
<th>unsubstituted</th>
<th>11-methyl</th>
<th>12-methyl</th>
<th>11,12-dimethyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-15</td>
<td>14.2</td>
<td>15.3</td>
<td>8.4</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>C-16</td>
<td>12.3</td>
<td>16.6</td>
<td>47.0</td>
<td>17.9</td>
<td></td>
</tr>
<tr>
<td>C-2</td>
<td>26.9</td>
<td>7.3</td>
<td>7.8</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>7.3</td>
<td>7.3</td>
<td>7.9</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>9.7</td>
<td>5.7</td>
<td>8.4</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>C-6</td>
<td>13.1</td>
<td>8.3</td>
<td>7.4</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>C-7</td>
<td>10.4</td>
<td>6.4</td>
<td>1.9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>C-12</td>
<td>4.1</td>
<td>4.5</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>4.1</td>
<td>1.2</td>
<td>1.9</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>C-11</td>
<td>5.2</td>
<td>—</td>
<td>0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-CH(_3)</td>
<td>—</td>
<td>27.4</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>12-CH(_3)</td>
<td>—</td>
<td>9.3</td>
<td>—</td>
<td>31.9</td>
<td></td>
</tr>
</tbody>
</table>
6,7,15,16-tetrahydrocyclopent[a]phenanthren-17-one (332) in 51% yield. This approach clearly merits further attention.

3.1 References
Chemical synthesis: ring-D ketones


References


Chemical synthesis: ring-D ketones


All cyclopenta[a]phenanthrenes contain an aromatic phenanthrene ring system fused to a five-membered ring, and not unexpectedly chemical reactions due to both these structural moieties are observed. In the D-ring ketones the carbon-oxygen dipole induces electron withdrawal from the whole system, and often reactions at the five-membered ring tend to dominate the chemistry of these compounds.

Oxidation of the parent hydrocarbon (1) with chromic acid (Fig. 40) was found to give mainly the 15-ketone (102) (Hoch, 1938; Butenandt et al., 1946; Badger et al., 1952) together with a trace of the expected 6,7-quinone (104) (Badger et al., 1952). The 11-methyl hydrocarbon (83), conveniently prepared by hydrogenolysis of the 11-methyl-17-ketone (26) with Adam's catalyst and palladium on charcoal in acetic acid containing hydrochloric acid, also gave the 11-methyl-15-ketone (334) in 37% yield (Coombs, 1969). By contrast, the five-membered ring in the 17-ketone, 15,16-dihydrocyclopenta[a]phenanthren-17-one (4), resisted further attack; oxidation with chromic acid in acetic acid led to a nearly quantitative yield of the bright yellow 6,7-quinone (335) (15,16-dihydrocyclopenta[a]phenanthrene-6,7,17-trione). Similar oxidation of the 11-methyl-17-ketone (26) gave the corresponding 11-methyl-6,7,17-trione (336) in 51% yield, together with unchanged starting material and small amounts of three other oxidation products. Butenandt et al. (1946) obtained the 6,7-quinone (104) from the parent hydrocarbon (1) indirectly by first treating it with osmium tetroxide to give the cis-6,7-diol (103) in good yield; this was then further oxidized to the quinone with chromic acid. Coombs found that the 17-ketones (4) and (26) also reacted smoothly with osmium tetroxide in pyridine to furnish the keto-diols (337) and (338) which could be further oxidized with chromic acid to the quinones in high yield. The keto-diols were dehydrated when they were
heated with 2.5-m sulphuric acid; the unsubstituted keto-diol (337) gave mainly the phenol 15,16-dihydro-6-hydroxycyclopenta[a]phenanthren-17-one (339) with a small amount of a second phenol, probably the 7-hydroxy isomer; this 6-phenol (339) was identical with the sample obtained from total synthesis (see Fig. 36). The 11-methyl keto-diol (338) was less readily dehydrated, and gave only the 6-phenol (340). Oxidation of the two 17-ketones with cerium (IV) ammonium nitrate in aqueous acetic acid at ambient temperature, conditions reported to oxidize the methyl group in 1-methyloestrone methyl ether to a formyl group in 90% yield (Laing and Sykes, 1968), led to mixtures containing the 6,7,17-triones and several other unidentified oxidation products in low to moderate yield.

Oxidation of the parent hydrocarbon (1) with lead tetraacetate gave the 15-acetoxy compound (333) (Badger et al., 1952). Similar oxidation of the two ketones (4) and (26) took a different course, leading to the 16-
acetoxy derivatives, but better yields of the latter were obtained by similar oxidation of the enol acetates (341) and (342). These were readily prepared in high yield from the ketones with isopropenyl acetate, catalysed with p-toluene sulphonic acid. Oxidation with lead tetraacetate in a mixture and acetic acid and acetic anhydride with irradiation with visible light from a tungsten filament lamp then afforded the 17-keto-16-acetates (343) and (344) in excellent yield. Based on infrared evidence the initial oxidation products appeared to be the 16,17,17-triacetates, but these gave the 17-keto-16-acetates on recrystallization of the crude reaction products. The 17-ketone-16-ols (345) and (346) were readily obtained by acid hydrolysis; alkaline caused decomposition.

Friedel-Crafts acylation of 16,17-dihydro-15H-cyclopenta[a]-phenanthrene (1) (Dannenberg et al., 1965) in nitrobenzene with acetyl chloride in the presence of aluminium chloride led to substitution at C-12 (Fig. 41). The 12-acetyl compound (347), obtained in 38% yield, was converted via Beckmann rearrangement of its oxime into the 12-amide (348) and thence into 12-amin-16,17-dihydro-15H-cyclopenta[a]-phenanthrene (349). This amide (348) was synthesized independently from Robinson's 12-ketone (96); the oxime (350) of this ketone was acetylated, then heated with acetic anhydride and acetyl chloride (Semmler-Wolff aromatization) to give the 6,7-dihydro-12-diacetylamino compound (351) in 60% yield. Treatment with potassium hydroxide followed by dehydrogenation with platinum on charcoal at 300°C furnished the same amide. Friedel-Crafts acylation of the naphthyl
compound (44) (11,12,13,14,16,17-hexahydro-15H-cyclopenta[a]-phenanthrene), on the other hand, occurred at C-6 to give the 6-acetyl derivative (353), which was dehydrogenated and converted into the 6-amide (354) as before. Electrophilic bromination of the 17-ketones (4) and (26) with bromine in chloroform or acetic acid, even in the absence of light or in the presence of silver nitrate and acid (Derbyshire and Waters, 1950), conditions known to favour aryl bromination, did not lead to aryl substitution, but gave instead the 15-bromo (318) and (355) and 15,15-dibromo derivatives (356) and (357) (Fig. 42) by attack at the benzylic position in the five-membered ring (Coombs et al., 1973). With a large excess of bromine addition occurred at the 6,7-position of (4) to form the 6,7-dihydro-6,7,15,15-tetram bromide (360), which on being heated lost hydrogen bromide to yield 15,16-dihydro-6(or 7),15,15-tribromocyclopenta[a]phenanthren-17-one (361) (Coombs, unpublished work). At no point did electrophilic bromination of the aromatic phenanthrene ring system occur, emphasizing the overriding effect of the carbonyl group at C-17 on the reactivity of the aromatic ring system. Free-radical
bromination of the ketones (4) and (26) with N-bromosuccinimide in carbon tetrachloride during irradiation with visible light furnished the 16-bromo-17-ketones (358) and (359) in high yield. Similar bromination of the 15-bromo-17-ketone led to 15,16-dibromo-15,16-dihydrocyclopenta[a]phenanthren-17-one (362), different from the dibromide obtained by direct bromination.

Dehydrobromination of 16-bromo-15,16-dihydrocyclopenta[a]phenanthren-17-one (358) with triethylamine in tetrahydrofuran gave bright orange crystals of the fully unsaturated ketone, cyclopenta[a]phenanthren-17-one (363). Debromination occurred readily, being complete in a few minutes at room temperature, and could be followed conveniently by ultraviolet spectroscopy. The purification of this compound was complicated by the ease with which it gave rise to insoluble material on attempted recrystallization. In this respect it resembled the analogous compound indenone which is known to undergo spontaneous polymerization (Marvel and Hinmann, 1954). 11-Methylocyclopenta[a]phenanthren-17-one (364), obtained from 16-bromo-15,16-dihydro-11-methylocyclopenta[a]phenanthren-17-one (359) by the same method (Coombs et al., 1975), appeared to be somewhat more stable. Again by analogy with indenone (Lacey and Smith, 1971) acid-catalysed hydration of the 15(16)-double bond in the enone (363) using aqueous sulphuric acid in tetrahydrofuran, followed by acetylation, yielded the 15-acetoxy-17-ketone (365) from which the secondary alcohol (369) was obtained by acid hydrolysis. Hydration of the corresponding double bond in the 11-methyl homologue (364) proceeded more slowly, and recourse was made to sulphuric acid-catalysed addition of acetic acid which occurred much faster; the 11-methyl-15-acetoxy-17-ketone (366) was isolated directly by crystallization in 40% yield. As before the alcohol (370) was prepared by acid hydrolysis, alkali being detrimental. The 15-methoxy-17-ketones (367) and (368) were similarly made by acid-catalysed addition of methanol to the double bond (Bhatt et al., 1982).

In an effort to direct electrophilic bromination of these ketones to the aromatic phenanthrene rings, bromination in the presence of thallium triacetate (McKillop et al., 1972) was investigated (Coombs et al., 1973). Under these conditions (Fig. 43) with the parent, unsubstituted ketone 15,16-dihydrocyclopenta[a]phenanthren-17-one (4), the main product was a compound C_{19}H_{14}O_3 devoid of bromine; this was identified as the 15-acetoxy-17-ketone (365) already prepared as described above. It was not formed when either the 17-ketone (4) or its 15-bromo derivative (318) was treated with thallium triacetate in the absence of bromine, the best yield of the 15-acetate being secured when one equivalent of bromine was
added to the original ketone and thallium triacetate in carbon tetrachloride at ambient temperature. A bromo derivative of thallium therefore appears to be involved, and it was proposed that esterification by it of the 17-enol to form the thallic ester (371) was followed by attack at C-15 by acetate, giving (318) as shown. This mechanism is similar to that proposed previously for the formation of 10β-trifluoroacetoxy-19-norandrosta-1,4-diene-3,17-dione (373) by the action of thallium trifluoroacetate on oestrone via the phenolic thallic ester (372) (Coombs and Jones, 1972). The proposed intermediate (371) involves bond rearrangement in the five-membered ring to a quinoid structure; this has been observed in other reactions involving substitution in ring-D. Thus attempted nucleophilic replacement of bromine in the 15-bromo-17-ketone (318) by acetate led smoothly not to the expected 15-acetate, but to the 16-acetoxy-17-ketone (343) identical with the specimen prepared by lead tetraacetate oxidation (Coombs et al., 1973). Also attempted demethylation of the 15-methoxy-17-ketone (367) with boron tribromide gave instead the 16-bromo-17-ketone (Coombs, unpublished work). In both these reactions quinoid intermediates (374) and (375), arising from nucleophilic attack at C-16 in the enol accompanied by bond migration, account reasonably for these unexpected results.

Reduction of the 16-hydroxy-17-ketones (345) and (346) with sodium borohydride (Fig. 44) readily furnished the 16,17-trans-diols (376) and
(377), whilst addition of osmium tetroxide to the D-ring double bond in 15\textit{H}-cyclopenta[\textit{a}]phenanthrene (3) and its 11-methyl homologue (150) gave the 16,17-cis-diols (378) and (379). In a similar way oxidation of 17\textit{H}-cyclopenta[\textit{a}]phenanthrene (2) with osmium tetroxide led to the 15,16-cis-diol (382) (Coombs and Hall, 1973). On acid-catalysed dehydration all these diols gave rise to the previously unknown 16-ketones (380) and (381), analogous to the unconjugated ketone similarly formed on dehydration of indan-1,2-diol (Brooks and Young, 1956). These ketones are easily distinguished from their 15- and 17-isomers by their ultraviolet absorption spectra which are similar to those of the hydrocarbons, and by their infrared carbonyl stretching frequencies at 1750 cm\textsuperscript{-1} as opposed to those of the conjugated ketones at 1690 cm\textsuperscript{-1}. Presumably formation of 16-ketones occurs with gain in energy due to relief of strain in ring-D outweighing that which would have been gained by conjugation of the carbonyl group at either C-15 or C-17. Synthesis of partially hydrogenated 16-ketocyclopenta[\textit{a}]phenanthrenes have been reported in two papers summarized in Fig. 44. Wilds (1942) brominated

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig44}
\caption{Fig. 44}
\end{figure}
1,2,3,4-tetrahydrophenanthren-1-one and condensed the 2-bromo-1-ketone with sodioacetoacetic ester to form the diketone (383). Treatment of the latter with dilute potassium hydroxide led to cyclization with the formation of 11,12,13,17-tetrahydro-16H-cyclopenta[a]phenanthren-16-one (384) in high yield. On Clemmensen reduction followed by dehydrogenation with palladium on charcoal it was converted into the known hydrocarbon (1). In the second report (Turner, 1949) the same tricyclic ketone was transformed almost quantitatively into its furfurylidene derivative (385), which with hot acid yielded the diketo acid (386) by a reaction analogous to that reinvestigated by Robinson and described previously. Treatment of this diketone with dilute alkali caused ring closure to the 16-ketone (387) which had ultraviolet absorption very similar to that described by Wilds (1942) for (384).

Grignard reactions with the 17-ketone (4) (Fig. 45) yielded 17-methyl- and 17-isopropyl-15H-cyclopenta[a]phenanthrenes (133) and (388) which on treatment with osmium tetroxide yielded the corresponding 16,17-cis-diols (389) and (400) (Dannenberg et al., 1960). Oxidation of the methyl-diol (389) with periodate cleaved ring-D to form the phenanthrene ketoaldehyde, whereas oxidation of this diol or of the precursor hydrocarbon (133) with chromic acid led to the corresponding ketoacid; 15-methyl-17H-cyclopenta[a]phenanthrene (128) likewise gave the isomeric ketoacid on similar oxidation. The diol (401) resulted from ozonolysis of the 17-methyl-15H-hydrocarbon. Dehydration of the 17-methyl-16,17-cis-diol (389) afforded the 17-methyl-16-ketone (402)
closely similar in properties to the parent 16-ketone (380). The 17-ketones also react normally in the Reformatsky reaction as first shown by Robinson and Slater (1941) who condensed the readily available 11-methoxy- and 3,11-dimethoxy-17-ketones with ethyl bromoacetate in the presence of zinc to give the unsaturated esters (403) and (404) or their Δ11(17) isomers (Fig. 46). In the course of the synthesis of cholanthenes Dannenberg (1950) prepared the unsubstituted ester (405) and, by employing ethyl 2-bromopropionate, also the 20-methyl homologue (406) (Dannenberg and Dannenberg-von Dresler, 1964). The double bonds in these two esters were reduced by catalytic hydrogenation and the side chains in the products (407) and (408) were extended by means of the Arndt–Eistert reaction to (409) and (410) before cyclization at C-12 and after further transformations to furnish the cholanthenes (411) and (412). For the synthesis of the isomeric ring system 16,17-benzo-15H-cyclopenta[a]phenanthrene [inden(2',3':1,2)phenanthrene] (416), derivatives of which have been isolated as minor products during
General chemistry

dehydrogenation of various sterols (Ruzicka and Goldberg, 1937), Nasipuri and Roy (1961) prepared the sodium salt of methyl 15,16-dihydro-17-oxo-cyclopenta[a]phenanthrene-16-carboxylate by Dieckmann cyclization of the phenanthrene-1,2-diester (413). Acid hydrolysis of the 16-carboxylate led to 15,16-dihydrocyclopenta[a]phenanthren-17-one (4), mp 200–201°C, whilst condensation with the Mannich base 4-piperidinobutan-2-one methiodide or with 1-chloropentan-3-one gave the diketo-esters (414) and (415), converted in several steps to the desired pentacyclic hydrocarbons (416) and (417). This ring system was also obtained (Buchta and Kraetzer, 1966) via Michael addition of ethyl vinyl ketone to the 16-hydroxymethylene-17-ketone (418) to form 15,16-dihydro-16-(3-oxopentyl)cyclopenta[a]phenanthren-17-one (419) which was cyclized and aromatized as before.

The 17-methylene group in cyclopenta[a]phenanthrenes, situated between two double bonds, is acidic. Thus the parent 17H-hydrocarbon (2) (Fig. 47) condensed readily with benzaldehyde and with p-dimethylaminobenzaldehyde in the presence of sodium hydroxide to yield the yellow 17-benzylidine compounds (420) and (421); hydrogenation of (420) gave the 17-benzyl derivatives (423) (Coombs, 1966). The yellow isopropylidene derivative (422) was similarly obtained by condensation with acetone in the presence of piperidine; in the presence of sodium hydroxide there was isolated a colourless isomer (424) in which the terminal double bond had rotated out of conjugation with the aromatic ring system, thereby presumably relieving the steric interaction between the side chain and hydrogen at C-12. Complete hydrogenation of this
diene readily gave 15,16-dihydro-17-isopropyl-17H-cyclopenta[a]-phenanthrene (53), but when hydrogenation was interrupted after two atom-equivalents had been absorbed, the starting material was recovered along with the fully reduced hydrocarbon (53) and both the 17-isopropyl-16(17)-ene (388) and its 15(16)-isomer (425), demonstrating that isomerization of the double bond in the five-membered ring from C-16(17) to C-15(16) had occurred in the course of the reduction. Partial reduction of the aromatic system in 17-ketocyclopenta[a]phenanthrenes cannot be conveniently accomplished by direct catalytic hydrogenation because the benzylic carbonyl group is eliminated comparatively readily (Robinson and Rydon, 1939). For example, reduction of the easily accessible 3,11-dimethoxy-17-ketone (426) in acetic acid over Adam's catalyst at 70°C gave 11,12,13,14,16,17-hexahydro-15H-11-hydroxy-3-methoxy-cyclopenta[a]phenanthrene (427) among other products. In order to preserve the 17-carbonyl function, present in oestrogens and many C_19 steroids, Robinson first opened the five-membered D-ring prior to hydrogenation as depicted in Fig. 48. The 16-hydroxymethylene derivative (429) was obtained by condensation of the dimethoxy-17-ketone with ethyl formate in pyridine, catalysed by sodium ethoxide; other methods gave more or less of the dimer (428). Conversion to the nitrile (430) was brought about with hydroxylamine in acetic acid, and without purification it was hydrolysed to the di-acid (431) with hot aqueous alcoholic potassium hydroxide. This di-acid was esterified and reduced catalytically as before; three partially hydrogenated products (432-434) were isolated from the reduction-product mixture. Re-formation of the D-ring was achieved by heating the lead salts of these di-acids in vacuo. In this way di-acid (432) gave 3,11-dimethoxy-6,7,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (332) whilst 18-noroestrone methyl ether, 6,7,8,9,11,12,13,14,15,16-decahydro-3-methoxy-cyclopenta[a]phenanthren-17-one (22) of unknown stereochemistry was obtained from the di-acid (434); the isomeric di-acid (433) resisted cyclization by this method. Two years later Robinson and Slater (1941) approached this problem from a different angle. Starting with the readily available 12-ketones (96) and (437), reduction by the Pondorff procedure gave the corresponding alcohols (438) and (439), dehydration of which with potassium hydrogen sulphate yielded the required 9(11),12(13)-dienes (440) and (441) together with the products of their further dehydrogenation, the 6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrenes (442) and (443). The latter were the only products obtained when oxidation of the dienes to the corresponding 17-ketones with selenium dioxide was attempted. More recently Jacob et al.
General chemistry

(1971) found that reduction of the 12-ketone (96) with lithium aluminium hydride gave, in addition to the diene (440) and triene (442) reported by Robinson, also the isomeric 11,12,13,14,16,17-hexahydro-15\(H\)-hydrocarbon (44) as well as the alcohol containing only one aromatic A-ring (444). Robinson and Slater also found that the Reformatsky product (405) already described, on vigorous reduction (with Raney nickel at 200–220\(^\circ\)C under 65 atm. pressure) was hydrogenated to the 18-nor-oestrogen derivative (445) with elimination of the 11-methoxy group.

In a recent study (Elvidge et al., 1985) the relative rates of proton exchange at C-16 in a series of 17-ketocyclopenta[a]phenanthrenes were measured. These compounds were labelled at C-16 specifically by acid-
catalysed or hydroxide-catalysed exchange with tritiated water, and the position of the label in the product was checked by tritium nmr ($\delta 2.70-2.86$ in deuterated chloroform). Hydroxide-catalysed detritiation in 9:1 (v/v) water–dioxane was followed by measuring the radioactivity remaining in aliquots of the substrate at fixed time intervals; the results are shown in Table 4. The rate constants for the unsubstituted 17-ketone (4) and its 1-methyl homologue (302) were essentially the same, but as expected methyl groups elsewhere in the molecule decreased this rate, the effect being greater the nearer the methyl group was to C-16. The rate for the 12-methyl isomer (130) was the lowest for the monomethyl compounds and was decreased further by introduction of a second methyl group at C-11 (131). The 7-methyl-17-ketone (230) was, however, anomalous because the rate was markedly increased, and this was true also of the other two 7-methyl derivatives (309) and (447). The 7-methyl group also had an effect on H-15 because in its nmr spectrum these protons were deshielded $0.42-0.46$ ppm compared with the other isomers (see Table 4). These effects of 7-methyl substitution can be attributed to the strain it introduces into these molecules. This was indicated by the unexpected difficulties encountered in the synthesis of both the 7-methyl- and 7,11-dimethyl-17-ketones, and is fully confirmed by the results of X-ray crystallography discussed in Chapter 8.

Table 4. Hydroxide-catalysed second order rate constants ($k_{OH}^2$) at 298.2°K for detritiation of various [16-$^3$H]-15,16-dihydrocyclopenta[a]-phenanthren-17-ones, and their nmr H-15 resonance signals ($\delta$) measured in ppm from tetramethylsilane

<table>
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<tr>
<th>Compound</th>
<th>$10^3k_{OH}^2$ mol$^{-1}$ s$^{-1}$</th>
<th>H-15 ($\delta$)</th>
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<tr>
<td>unsubstituted-17-ketone (4)</td>
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<td>1-methyl-17-ketone (302)</td>
<td>1.85±0.05</td>
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<td>3-methyl-17-ketone (304)</td>
<td>1.42±0.02</td>
<td>3.25</td>
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<td>4-methyl-17-ketone (305)</td>
<td>1.47±0.19</td>
<td>3.26</td>
</tr>
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<td>6-methyl-17-ketone (306)</td>
<td>1.15±0.14</td>
<td>3.27</td>
</tr>
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<td>7-methyl-17-ketone (230)</td>
<td>3.17±0.08</td>
<td>3.70</td>
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<tr>
<td>11-methyl-17-ketone (26)</td>
<td>1.47±0.06</td>
<td>3.24</td>
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<tr>
<td>12-methyl-17-ketone (130)</td>
<td>0.67±0.07</td>
<td>3.28</td>
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<tr>
<td>11,12-dimethyl-17-ketone (131)</td>
<td>0.30±0.05</td>
<td>3.22</td>
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<tr>
<td>7,11-dimethyl-17-ketone (309)</td>
<td>2.45±0.19</td>
<td>3.63</td>
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<tr>
<td>11-hydroxy-17-ketone (206)</td>
<td>0.23±0.02</td>
<td>—</td>
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<tr>
<td>11-methoxy-17-ketone (132)</td>
<td>2.06±0.06</td>
<td>3.22</td>
</tr>
<tr>
<td>7-methyl-11-methoxy-17-ketone (447)</td>
<td>2.50±0.17</td>
<td>3.58</td>
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<tr>
<td>11-ethyl-17-ketone (211)</td>
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<tr>
<td>1,11-methano-17-ketone (310)</td>
<td>0.87±0.07</td>
<td>3.25</td>
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</table>
4.1 References


Jacob, G., Cagniant, D. & Cagniant, P. (1971). Recherches dans le domaine
Some 350 individual cyclopenta[a]phenanthrenes have so far been reported in the chemical literature, and at least some physical characteristics of the majority of these have been described. In the earlier papers often only the melting point of the compound and possibly those of certain readily prepared derivatives were given. In somewhat later reports it is usual also to find ultraviolet absorption data, while in papers published during the last 25 years infrared, nuclear magnetic resonance, and mass spectra are frequently encountered. In this chapter melting points and spectroscopic data are summarized for the great majority of these cyclopenta[a]phenanthrenes, and this is followed by a separate section on the mass spectrometry of a few of these compounds. Consideration of structural data derived from X-ray crystallographic studies appears in a separate chapter.

5.1 Melting points and spectral data

In the compilation which follows cyclopenta[a]phenanthrenes have been listed by molecular formula according to the convention adopted by Chemical Abstracts. The molecular formula is followed by the serial (arabic) number, when one has been assigned to it in the text, tables and figures, and by the complete chemical name. The Chemical Abstracts Registry Number [enclosed in square brackets] is also quoted when this is available. The compilation ends with a reverse index, relating serial number to molecular formula to assist location of individual compounds referred to elsewhere in the book. Melting points are quoted in degrees Centigrade, and ultraviolet spectra as $\lambda_{\text{max}} (\log_{10} \varepsilon)$ where $\varepsilon$ is the molecular absorption coefficient, usually measured in ethanolic solution. Infrared absorption wavelengths ($\mu$m), especially of the strong aromatic bands in the 11–14$\mu$m region, are tabulated to provide an additional
Melting point and spectral data

means of identification; measurements are for Nujol mulls unless otherwise specified. Nuclear magnetic (proton) resonance chemical shifts (δ) measured in parts per million (ppm) relative to tetramethylsilane in deuterated chloroform are also quoted where available.

The ultraviolet absorption spectra of 16,17-dihydro-15H-cyclopenta[a]phenanthrene hydrocarbons were discussed by Dannenberg and Steidle (1954) who pointed out that these compounds, like simple phenanthrenes, possess four groups of absorption bands, shown in Fig. 49 for Diels' hydrocarbon. The most intensive band with a maximum around 260 nm (log10 e 4.7–4.8) was designated the β-group, that with the shortest wavelength around 220 nm (log10 e 4.5) the β'-group, the three maxima between 280 and 310 nm (log10 e 4.0–4.2) the p-group, and the three weak maxima between 320 and 360 nm (log10 e 2.5–3.2) the α-group in accordance with the notation originated by Clar (1952). As expected addition of one or even two methyl groups to the five-membered ring has little effect on the chromophore, whereas substitution of one methyl group in the phenanthrene ring system causes a bathochromic shift of the β-band of up to 7 nm, depending upon the position of substitution. Substitution of a second methyl group causes a further shift in the same direction of 4–11 nm, from the parent hydrocarbon. By contrast the p- and α-band groups do not undergo this red shift to the same extent.

Fig. 49. Ultraviolet spectra recorded in ethanol of Diels' hydrocarbon (7) showing the β', β, p and α bands, and of 15,16-dihydronaphthalen-17-one (4) and its borohydride reduction product, the 17-ol.
Conjugation of a double bond at C-15 or C-17 in the five-membered ring also causes a bathochromic shift of the \( \beta \)-band (10.0–14.5 nm); the 17H-hydrocarbons are readily distinguished by the presence of an additional absorption band at 239 nm absent in their 15H-isomers (Coombs, 1966b). Conjugation of an exocyclic carbon–carbon double bond at C-17 results in a similar shift of the \( \beta \)-band, but conjugation of a carbonyl oxygen at this position has less effect. Thus 15,16-dihydrocyclopenta[a]-phenanthren-17-one (4) absorbs at 265 nm; however, the \( \alpha \)-bands are shifted 10–15 nm in the same direction (compared with the 16,17-dihydro hydrocarbon) whereas the \( \beta \)-bands are not and one of these \( (\lambda_{\text{max}} \sim 280 \text{ nm}) \) is lost (see Fig. 49). Aryl methyl substitution is accompanied by the usual red shift in these 17-ketones. Surprisingly, conjugation of a carbonyl group at C-15 to give the isomeric ketone (102) causes a blue shift of the \( \beta \)-band to 251.5 nm, and introduction of a C-15(16) double bond into the 17-ketone to yield cyclopenta[a]phenanthren-17-one (363) is also accompanied by a similar blue shift to 253 nm. In this compound the three maxima constituting the \( \beta \)-group are not greatly displaced, but the three weak \( \alpha \) maxima are replaced by one broad maximum at 380–390 nm which imparts a yellow colour to this ketone and its 11-methyl homologue (364). The ultraviolet spectra of all these ketones become very similar to those of the corresponding hydrocarbons after mild reduction in situ with sodium borohydride to the secondary alcohol as shown in Fig. 49. The spectra of the various tetrahydro, hexahydro, etc., hydrocarbons are generally similar to those of the analogous phenanthrene derivatives.

Little needs to be said about the infrared and nmr spectra of these compounds. In the former the conjugated carbonyl group in the 15- and 17-ketones absorbs at about 5.92 \( \mu \text{m} \) as expected and slightly lower in the

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<td>1,2,3,4,5</td>
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Mass spectrometry

D-ring bromo ketones, but at 5.71 μm in the unconjugated 16-ketone (380). The strong out-of-plane deformation bands in the 11.0–14.0 μm region are characteristic of each structure and give information regarding the substitution patterns. Dannenberg et al. (1953) discussed these absorption bands in the spectra of 16 variously substituted mono- and dimethyl derivatives of 16,17-dihydro-15H-cyclopenta[a]phenanthrene measured in both the solid state (potassium bromide discs) and in solution in carbon disulphide. With few exceptions bands occurred within the wavelength ranges shown in Table 5 for the various ring-substitution patterns for spectra obtained in solution.

In the nmr spectra of the 15,16-dihydro-17-ketones the aromatic protons resonate between δ 7.3 and 7.8, but the bay-region protons at C-1 and C-11 are deshielded by about 1 ppm. The C-15 methylene group resonates as a multiplet at δ 3.24–3.28 for the unsubstituted 17-ketone (4) and all its isomeric arylmethyl derivatives with the exception of the 7-methyl compound (δ 3.70), disclosing steric compression in this region of the molecule confirmed by X-ray crystallographic studies (Chapter 8).

5.2 Mass spectrometry of cyclopenta[a]phenanthrenes

The use of mass spectrometry for the determination of molecular weights of organic compounds including cyclopenta[a]phenanthrenes is commonplace, and it has recently led to the identification of several hydrocarbons of this series from a number of natural sources. Generally no difficulty is experienced with relatively simple aromatic compounds because often the molecular ion is also the most abundant ion in the spectrum. The technique is particularly valuable for the study of metabolites, usually obtainable only in very limited quantities. However, trans-1,2-dihydrodiols which commonly occur as metabolites derived from aromatic compounds often give problems because they undergo facile dehydration, so that the M⁺-18 ion is abundant and the molecular ion is weak or non-existent.

As a preliminary to employing mass spectrometric fragmentation patterns to assist in the elucidation of the structures of unknown metabolites of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) and related compounds, the mass spectrometry of several assorted synthetic compounds shown in Fig. 50 was studied in detail (Vose and Coombs, 1977). Table 6 shows the ions of abundance greater than 2% of the base peak produced from these compounds under comparable conditions (AEI MS-902 mass spectrometer with an ionizing potential of 70 eV; samples inserted directly into the source). The first four compounds, the parent 17-ketone (4), its
Table 6. Compound (see Fig. 50) and ionic abundance

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Physical and spectral properties

11-methyl (26) and 11-hydroxymethyl (215) derivatives, and the 6-phenol (339) have the molecular ion as the base peak, behaviour characteristic of aromatic compounds. All show $M^+\cdot28$ ions arising from loss of the carbonyl group, and other ions which follow from the general degradative pathways proposed in Fig. 51. This is similar to the scheme reported by

Fig. 50. Synthetic cyclopenta[a]phenanthrenes studied by mass spectrometry (Vose and Coombs, 1977).

Fig. 51. Proposed origin of ions formed by fragmentation of ring-D in 15,16-dihydrocyclopenta[a]phenanthren-17-ones, and the loss of ketene involving a concerted six-membered hydrogen shift.
Bowie (1966) for the related ketone indan-1-one. The 11-methyl and 11-hydroxymethyl ketones yield in addition weak ions at M⁺-15 and M⁺-31, respectively, indicating the loss of these substituents from the molecular ion. By contrast the molecular ion is of low abundance with the other four cyclopenta[a]phenanthrenes. The M⁺-60 ion arising from the loss of acetic acid from the molecular ion is the base peak in the spectrum of the 16-acetoxy-17-ketone (343), with a less-abundant ion at M⁺-88 due to further loss of CO; the reverse is true for the isomeric 15-acetoxy-17-ketone (365). The ion at M⁺-42 in the spectrum of the latter is absent in that of its 16-isomer and a metastable ion (m* at 212.2) confirms that this ion arises by one-step loss of ketene from the molecular ion, probably by loss of the 17-carbonyl and 16-methylene groups. The ion at M⁺-42 constitutes the base peak in the spectrum of the enol acetate (341); again it is shown to arise by one-step loss of ketene from the molecular ion by the presence of a metastable ion m* 196.5. This may occur via a concerted six-membered shift as shown in Fig. 51, leading to the molecular ion of the 17-ketone which then undergoes further fragmentation as already described. The enol acetate (341) is homologous with the M⁺-60 ion in the spectrum of the diacetate (446), and in this compound the base peak arises by loss of 42 mass units from the molecular ion, so that this rearrangement is probably general for compounds and ions of this series possessing or capable of forming an enol acetate structure.

The use of mass spectrometry in the identification of various metabolites is described in the chapter on the metabolism of cyclopenta[a]phenanthrenes. However, a rather unusual use of this technique is described here because it illustrates the power of this method to help solve otherwise intractable problems. After metabolic activation the carcinogen (26) binds covalently to DNA if it is added to the incubation mixture. As will be described later, reaction occurs mainly (~80%) with the guanine moieties to the extent of about one base in 10⁴ bases in the DNA. From separate considerations it was thought that this reaction occurred between the exocyclic amino group in the deoxyguanine bases and a 3,4-dihydrodiol,hydroxy-1,2-epoxide derived from the carcinogen to yield an adduct of the type shown as the ion of m/z 447 in Fig. 52. When DNA treated in this way was submitted to pyrolysis electron-impact mass spectrometry without further derivatization (Wiebers et al., 1981), in addition to the usual ion products derived from the four common DNA component bases (adenosine, guanine, cytosine, and thymine) other peaks of low abundance (about one-hundredth of the peak heights of the normal components) were seen. It was readily possible, in view of the previous studies, to fit these into the postulated fragmentation scheme.
shown in which the numbers represent the masses of the ions observed. The structures proposed for the molecular ion and two fragment ions are also displayed in this figure. The most abundant ion $m/z$ 368 was selected for further scrutiny by mass-analysed ion kinetic-energy spectrometry (MIKES), a technique which allows the further fragmentation of a single ion to be followed in detail. Ideally the molecular ion would be selected for this purpose, but in this case it was of extremely low abundance and it was felt that fragmentations leading to the relatively stable $m/z$ 368 ion were reasonably well understood. Major daughter ions in the MIKE spectrum of this ion are shown in Table 7 and fully substantiate the structures proposed. In particular the ion at $m/z$ 218 demonstrates loss of guanine (150 mass units), whilst the ion at $m/z$ 233 (loss of 135 mass units) indicates that the entity of mass 218 derived from the carcinogen is attached to the NH of the exocyclic $N^2$-amino group of this purine. This confirmation of the supposed structure of the major DNA adduct is

Fig. 52. Proposed molecular ion ($m/z$ 447) formed from the DNA adduct, and fragmentation pathways leading to the ions $m/z$ 368 and 365.
important because, owing to the extremely minute amount available, it is
doubtful whether the information could have been obtained in any other
way.

It has been known for a long time that during selenium dehydrogena-
tion of steroids at elevated temperatures the long sterol side chain at C-17
is lost, although it is retained on quinone dehydrogenation at substan-
tially lower temperatures. It is therefore interesting to observe that in the
mass spectra of cyclopenta[a]phenanthrenes bearing a sterol side chain
the molecular ion is of very low abundance; the base peak is the ion
formed by loss of this side chain (Ludwig et al., 1981).

5.3 Compilation of physical and spectroscopic data for most of the
cyclopenta[a]phenanthrenes reported in the chemical literature
up to 1985

**C_{17}H_{10}Br_{2}O**

(356) 15,15-Dibromo-15,16-dihydrocyclopenta[a]phenanthren-17-one
[50905-49-2] [74495-83-3]
MW 390; mp 213–213.5°C
\( \lambda_{\text{max}} \) 279 (4.70), 298 (4.24), 314.5 (4.18), 360 (3.30), 377 (3.27) nm
\( \nu_{\text{max}} \) 5.85, 12.45, 14.0 μm
δ 4.69 s (H-16) (Coombs et al., 1973a)

**C_{17}H_{10}Br_{2}O**

(362) 15,16-Dibromo-15,16-dihydrocyclopenta[a]phenanthren-17-one
[50905-50-5]
86  Physical and spectral properties

MW 390;  mp 179–180°C
\( \lambda_{\text{max}} \) 283 (4.60), 311.5 (4.33), 361 (3.19), 380 (3.18) nm
\( \nu_{\text{max}} \) 5.85, 12.04, 13.10\( \mu \text{m} \)
\( \delta \) 5.05 (H-15), 6.10 (H-16)  (Coombs et al., 1973a)

\( \text{C}_{17}\text{H}_{10}\text{O} \) (363) Cyclopenta[a]phenanthren-17-one [50905-54-9]
MW 230;  Orange prisms, mp 202–208°C
\( \lambda_{\text{max}} \) 253, 286, 298, 310, 382 nm
\( \nu_{\text{max}} \) 5.85, 12.25, 12.80, 13.26\( \mu \text{m} \)  (Coombs and Hall, 1973)

\( \text{C}_{17}\text{H}_{10}\text{O} \) (185) Cyclopenta[a]phenanthren-15,17-dione
MW 246;  mp 240.5–241.5°C  (Fieser et al., 1936)

\( \text{C}_{17}\text{H}_{10}\text{O} \) (335) 6,7,15,16-Tetrahydrocyclopenta[a]phenanthrene-6,7,17-trione
MW 262;  mp 245–247°C
\( \lambda_{\text{max}} \) 271–279 (4.03), 331 (3.31), 406 (3.21) nm
\( \nu_{\text{max}} \) 5.84, 5.97, 6.30, 10.45, 11.63, 11.90, 12.90\( \mu \text{m} \)  (Coombs, 1969)

\( \text{C}_{17}\text{H}_{11}\text{BrO} \) (318) 15-Bromo-15,16-dihydrocyclopenta[a]phenanthren-17-one
MW 311;  mp 196–197°C
\( \lambda_{\text{max}} \) 267 (4.78), 286.5 (4.41), 298.5 (4.36), 310 (4.13), 354 (3.32),
372 (3.31) nm
\( \nu_{\text{max}} \) 5.85, 12.26, 13.25\( \mu \text{m} \)
\( \delta \) 4.83 (J 3,7) (15-H), 4.19 (J 18,7) and 3.68 (J 18,3) (H-16)  (Coombs et al., 1973a)

\( \text{C}_{17}\text{H}_{11}\text{BrO} \) (358) 16-Bromo-15,16-dihydrocyclopenta[a]phenanthren-17-one
MW 311;  Golden laths, mp 175°C (decomp.)
\( \lambda_{\text{max}} \) 230.5 (4.28), 273 (4.73), 300.5 (4.27), 335 (3.00), 354 (3.13),
371 (3.13) nm
\( \nu_{\text{max}} \) 5.88, 12.26, 13.25\( \mu \text{m} \)
\( \delta \) 3.20 (J 18,2) and 3.48 (J 18,6) (H-15), 5.96 (J 6,2) (H-16)
(Coombs et al., 1973a)

\( \text{C}_{17}\text{H}_{12} \) (3) 15H-Cyclopenta[a]phenanthrene [219-07-8]
MW 216;  mp 165–167°C
\( \lambda_{\text{max}} \) 220 (4.45), 269 (4.79), 273 (4.81), 292 (4.23), 302 (4.17),
314 (3.96) nm
\( \nu_{\text{max}} \) (CS\textsubscript{2}) 12.48, 13.40, 14.50\( \mu \text{m} \)  (Coombs, 1966b)

\( \text{C}_{17}\text{H}_{12} \) (2) 17H-Cyclopenta[a]phenanthrene [219-08-9]
MW 216;  mp 164–165°C (Coombs, 1996b); 163–164°C (Badger et al., 1952)
\( \lambda_{\text{max}} \) 224 (4.41), 239 (4.24), 269 (4.72), 273.5 (4.70), 292 (4.17),
302.5 (4.16), 314.5 (4.04) nm
\( \nu_{\text{max}} \) (CS\textsubscript{2}) 12.49, 13.25, 13.30, 14.48\( \mu \text{m} \)  (Coombs, 1966b)

\( \text{C}_{17}\text{H}_{12}\text{O} \) (102) 16,17-Dihydrocyclopenta[a]phenanthren-15-one [32425-83-5]
MW 232;  mp 184–186°C (Coombs, 1966b); 183–184°C (Bachmann
Compilation of data from the literature

and Kloetzel, 1937); pale yellow needles, mp 183–184°C (Badger et al., 1952)

\[ \lambda_{max} 216 (4.41), 251.5 (4.58), 288 (4.06), 319 (4.08), 344 (3.65), 362 (3.49) \text{ nm} \]

\[ \nu_{max} 5.9, 10.32, 10.4, 11.4, 11.76, 11.9, 12.26, 12.92, 13.2, 13.94 \mu \text{m} \]

(Coombs, 1966b)

\( \text{C}_{17}\text{H}_{12}\text{O} \) (380) 15,17-Dihydrocyclopenta[a]phenanthren-16-one
MW 232; mp 172–174°C [42123-03-5]

\[ \lambda_{max} 258 (4.77), 269 (4.21), 278 (4.11), 300 (4.21), 319 (3.14), 335 (3.10), 350 (3.03) \text{ nm} \]

\[ \nu_{max} 5.88, 11.92, 12.49, 13.10, 13.30 \mu \text{m} \]

(Coombs and Hall, 1973)

\( \text{C}_{17}\text{H}_{12}\text{O} \) (4) 15,16-Dihydrocyclopenta[a]phenanthren-17-one [786-66-3]
[74495-81-1] [74495-82-2]
MW 232; mp 203–204°C (Coombs et al., 1970); 200–201°C (Nasipuri and Roy, 1961)

\[ \lambda_{max} 265 (4.89), 284 (4.52), 296 (4.38), 334 (3.24), 350 (3.40), 367 (3.44) \text{ nm} \]

\[ \nu_{max} 5.92, 11.48, 11.82, 12.40, 12.86, 13.22, 14.18 \mu \text{m} \]

\[ \delta 3.28 (H-15), 2.70 (H-16) \]

(Coombs, 1966a)

\( \text{C}_{17}\text{H}_{12}\text{O}_{2} \) (396) 15,16-Dihydro-2-hydroxycyclopenta[a]phenanthren-17-one
[51651-45-1]
MW 248; mp 340°C (decomp.)

\[ \lambda_{max} 274.5 (4.74), 366 (3.40), 385 (3.45) \text{ nm} \]

\[ \nu_{max} 4.59, 296 (4.62) \]

(Coombs et al., 1975)

\( \text{C}_{17}\text{H}_{12}\text{O}_{2} \) (23) 15,16-Dihydro-3-hydroxycyclopenta[a]phenanthren-17-one
MW 248

\[ \lambda_{max} 278, 291, 324, 367 \text{ nm} \]

\[ \nu_{max} 234, 277, 288, 371 \text{ nm} \]

(Coombs, unpublished)

\( \text{C}_{17}\text{H}_{12}\text{O}_{2} \) (339) 15,16-Dihydro-6-hydroxycyclopenta[a]phenanthren-17-one
[24684-45-5]
MW 248; mp 280°C

\[ \lambda_{max} 270 (4.81), 287 (4.48), 302 (4.28), 361 (3.37), 378 (3.42) \text{ nm} \]

\[ \nu_{max} 3.20, 5.96, 11.54, 11.80, 12.18, 12.26, 13.08 \mu \text{m} \]

(Coombs, 1969)

\( \text{C}_{17}\text{H}_{12}\text{O}_{2} \) (206) 15,16-Dihydro-11-hydroxycyclopenta[a]phenanthren-17-one
[83053-63-8]
MW 248; mp 310–315°C (Robinson, 1938); 306–310°C (Bhatt et al., 1982)

\[ \lambda_{max} 261 (4.98), 293 (4.72), 368 (4.05), 387 (4.16) \text{ nm} \]

(Bhatt et al., 1982)

\( \text{C}_{17}\text{H}_{12}\text{O}_{2} \) (369) 15,16-Dihydro-15-hydroxycyclopenta[a]phenanthren-17-one
[55081-28-2]
Physical and spectral properties

MW 248; Isolated as its acetate (365), mp 197-198°C (see $\text{C}_{17}\text{H}_{14}\text{O}_3$) (Coombs et al., 1973a)

$\text{C}_{17}\text{H}_{12}\text{O}_2$ (345) 15,16-Dihydro-16-hydroxycyclopenta[a]phenanthren-17-one [24684-54-6]
MW 248; mp 186-187°C, 197°C (decomp.)
$\lambda_{\text{max}}$ 265 (4.95), 284 (4.49), 296 (4.39), 335 (3.15), 351 (3.32), 369 (3.33) nm
$\nu_{\text{max}}$ 2.96, 5.92, 12.16, 12.44, 13.30 μm (Coombs, 1969)

$\text{C}_{17}\text{H}_{13}\text{BrO}$
MW 313
$\lambda_{\text{max}}$ 256.5, 278, 299 nm
$\nu_{\text{max}}$ 3.01 μm (Coombs et al., 1973a)

$\text{C}_{17}\text{H}_{14}$ (104) 6,7,16,17-Tetrahydro-15H-cyclopenta[a]phenanthrene-6,7-dione
MW 248; Bright red needles, mp 209-211°C (Badger et al., 1952); 213°C (Butenandt et al., 1946b)

$\text{C}_{17}\text{H}_{14}$BrO
16,17-Dihydro-16-hydroxy-16,17-dihydro-15H-cyclopenta[a]phenanthrene
MW 218
$\nu_{\text{max}}$ 3.22, 12.80, 13.25 μm
δ 2.1-5.4 (m, 3H), 4.0 (m, 1H), 5.7 (m, 3H), 7.0-8.0 (m, 7H)
(Coombs et al., 1973a)

$\text{C}_{17}\text{H}_{14}$ (1) 16,17-Dihydro-15H-cyclopenta[a]phenanthrene [482-66-6]
MW 218; mp 133-134°C (Kon, 1933); 134-135°C (Cook and Hewett, 1933); 132-133°C (Butz et al., 1940); 134-135°C (Coombs, 1966b)
$\lambda_{\text{max}}$ 216 (4.48), 259 (4.77), 280 (4.16), 288 (4.07), 300 (4.71) nm
$\nu_{\text{max}}$ (CS$_2$) 12.32, 13.27 μm (Dannenberg et al., 1953)
$\nu_{\text{max}}$ 10.62, 11.56, 12.3, 12.95, 13.4, 14.08 μm (Coombs, 1966b)
$m/z$ 218 (100%), 219 (19%), 189 (28%), 188 (7%), 163 (6%)
(Chaffee and Jones, 1983)

$\text{C}_{17}\text{H}_{14}$O (38) 16,17-Dihydro-3-hydroxy-15H-cyclopenta[a]phenanthrene
MW 234; mp 184-188.5°C (Hoffelmer et al., 1964)

MW 234; mp 166-167°C acetate, mp 127-128°C
(Badger et al., 1952)

$\text{C}_{17}\text{H}_{14}$O 16,17-Dihydro-15H-cyclopenta[a]phenanthren-11-ol
MW 234; mp 174-175°C
$\lambda_{\text{max}}$ 228, 246, 279, 303, 349, 366 nm (Bhatt, unpublished data)

$\text{C}_{17}\text{H}_{14}$O 16,17-Dihydro-15H-cyclopenta[a]phenanthren-17-ol
MW 234; mp 183-184°C
$\lambda_{\text{max}}$ 259 (4.77), 280 (4.09), 288 (3.98), 300 (4.12) nm
$\nu_{\text{max}}$ 3.15, 9.55, 11.50, 12.28, 12.92, 13.40, 13.85 μm
(Coombs, 1966b)

$\text{C}_{17}\text{H}_{14}$O (384) 11,12,13,17-Tetrahydro-16H-cyclopenta[a]phenanthren-16-one
Compilation of data from the literature

MW 234; mp 185–185.5°C
oxime, mp 247–250°C (Wilds, 1942)

C_{17}H_{14}O (179) 11,12,15,16-Tetrahydro-cyclopenta[a]phenanthren-17-one
MW 234; Pale yellow scales, mp 210°C (Bardhan, 1936); colourless rhombohedral prisms, mp 214–216°C (Bachmann et al., 1943); pale yellow crystals, mp 219–221°C (Johnson and Peterson, 1945); 218–220°C (Coombs et al., 1970)
λ_{max} 272 (4.61), 282 (4.70), 325 (3.18), 337 (3.21), 367 (2.97) nm
ν_{max} 11.50, 11.94, 12.14, 13.24, 13.85 μm (Coombs et al., 1970)

C_{17}H_{14}O (174) 11,12,13,14-Tetrahydro-17H-cyclopenta[a]phenanthrene-12-one
[67279-07-6] [80299-44-1]
MW 234; mp 84–85°C (Lee-Ruff et al., 1981)

C_{17}H_{14}O 13,14,15,16-Tetrahydrocyclopenta[a]phenanthrene-17-one
[7421-33-2] [5836-87-3]
MW 234; mp 95–96°C
λ_{max} 239 (4.73), 303 (3.88), 316 (4.01), 330 (3.88), 336 (3.86) nm
ν_{max} 5.75 μm (Coombs, 1966a)

C_{17}H_{14}O_2 (116) 16,17-Dihydro-1,4-dihydroxy-15H-cyclopenta[a]phenanthrene
MW 252; mp 210°C (Tamayo and Martin, 1952)

C_{17}H_{14}O_2 (382) 16,17-Dihydro-cis-15,16-dihydroxy-15H-cyclopenta[a]phenanthrene
MW 250 (characterized as its diacetate (109) (see C_{17}H_{14}O_2)
(Coombs and Hall, 1973)

C_{17}H_{14}O_2 (378) 16,17-Dihydro-cis-16,17-dihydroxy-15H-cyclopenta[a]-phenanthrene [42122-94-1]
MW 250; mp 226–228°C
λ_{max} 250.5 (4.71), 257 (4.83), 279 (4.16), 287 (4.06), 299 (4.16), 320 (2.71), 327.5 (2.56), 334.5 (2.87), 343 (2.49), 350 (2.86) nm
ν_{max} 3.05, 12.15, 12.24, 13.33, 13.48 μm (Coombs and Hall, 1973)

C_{17}H_{14}O_2 (376) 16,17-Dihydro-trans-16,17-dihydroxy-15H-cyclopenta[a]-phenanthrene [42122-95-2]
MW 250; mp 227–278°C
λ_{max} 250.5 (4.71), 257 (4.82), 279 (4.16), 287 (4.05), 299 (4.17), 320 (2.65), 327.5 (2.49), 335 (2.84), 343 (2.44), 350 (2.84) nm
ν_{max} 3.08, 12.10, 13.44 μm (Coombs and Hall, 1973)

C_{17}H_{14}O_2 (324) 1,2,3,4,16,17-Hexahydrocyclopenta[a]phenanthrene-1,15-dione
MW 250; mp 173–175°C (Rahman and Rodriguez, 1969)

C_{17}H_{14}O_2 (±) (193) 11,12,13,14,15,16-Hexahydrocyclopenta[a]phenanthrene-11,17-dione [23462-83-1] [24808-98-8] [67530-16-9]
MW 250; Colourless needles, mp 115°C (Koebner and Robinson, 1938); 120°C (Coombs, 1965)
λ_{max} 216 (4.61), 248 (4.33), 315 (3.82)
ν_{max} 5.78, 5.98 μm (Coombs, 1965)
Physical and spectral properties

C_{17}H_{14}O_2
(+) (193) (+)-11,12,13,14,15,16-Hexahydrocyclopenta[a]-
phenanthrene-11,17-dione
MW 250; mp 138°C, [α]_D° +266° (CHCl₃)
λ\text{max} 248 (4.32), 314 (3.80) nm
ν\text{max} 5.75, 5.95 μm
(Cagara and Siewinski, 1975)

C_{17}H_{14}O_2
(−) (193) (−)-11,12,13,14,15,16-Hexahydrocyclopenta[a]-
phenanthrene-11,17-dione
MW 250; mp 138°C, [α]_D° −258° (CHCl₃)
λ\text{max} 248 (4.33), 314 (3.86) nm
ν\text{max} 5.75, 5.95 μm
(Cagara and Siewinski, 1975)

C_{17}H_{15}O_3
(337) 17-Oxo-6,7,15,16-tetrahydrocyclopenta[a]-phenanthrene-cis-6,7-diol
MW 266; mp 232–234°C
λ\text{max} 245 (4.02), 309–317 (4.43) nm
ν\text{max} 2.86, 2.96, 5.78, 10.31, 11.91, 12.90, 13.51 μm
(Coombs, 1969)

C_{17}H_{15}N (349) 12-Amino-16,17-dihydro-15H-cyclopenta[a]-phenanthrene
[3036-49-5]
MW 233; mp 250°C
λ\text{max} 255.5 (4.64), 314 (3.98), 360 (3.11)
ν\text{max} (KBr) 3.03, 3.47–4.41, 10.87, 12.20, 13.25 μm
C_{17}H_{15}N.HCl [2960-80-7] (Dannenberg et al., 1965)

C_{17}H_{16} (442) 6,7,16,17-Tetrahydro-15H-cyclopenta[a]-phenanthrene
MW 220; mp 60°C (Jacob et al., 1971); 61–62°C (Robinson and Slater, 1941)
λ\text{max} 272 (4.30) nm
ν\text{max} 12.20, 13.07 μm
δ 7 (m), 7.5 (m), 2.1 (m), 2.8 (m)
(Jacob et al., 1971)

C_{17}H_{16} (7421-32-1) 13,14,16,17-Tetrahydro-15H-cyclopenta[a]-phenanthrene
MW 220; mp 79–80°C
λ\text{max} 239 (4.76), 303 (3.84), 315 (3.91), 337 (3.63) nm
ν\text{max} 10.55, 11.54, 12.15, 12.42, 13.25, 13.50, 13.80 μm
(Coombs, 1966a)

C_{17}H_{16}O (325) 1,2,3,4,16,17-Hexahydrocyclopenta[a]phenanthren-15-one
MW 236 (Rahman and Rodriguez, 1969)

C_{17}H_{16}O (237) 1,2,3,4,15,16-Hexahydrocyclopenta[a]phenanthren-17-one
MW 236; mp 145–146°C
λ\text{max} 256.5 (3.98), 282 (3.25), 292.5 (3.27), 333.5 (2.86), 348 (2.91) nm
ν\text{max} 5.89, 9.55, 11.8, 12.1, 12.5 μm
(Coombs and Bhatt, 1973)

C_{17}H_{16}O (82) 11,12,13,14,16,17-Hexahydro-15H-cyclopenta[a]phenanthren-11-one
MW 236; mp 119–120°C (Butenandt et al., 1946a)

C_{17}H_{16}O (205) 11,12,13,14,15,16-Hexahydrocyclopenta[a]phenanthren-17-one
[786-64-1]
MW 236; mp 114–114.5°C (Coombs, 1966a)
Compilation of data from the literature

C_{17}H_{16}O 12-Hydroxy-11,12,13,14-tetrahydro-17H-cyclopenta[a]phenanthrene
[67279-08-7] [80299-45-2]
MW 236 (Dao et al., 1978)

C_{17}H_{16}O 13,14,16,17-Tetrahydro-15H-cyclopenta[a]phenanthren-17-ol
[788-65-2] [7421-34-3]
MW 236; mp 117–118°C
λ_{max} 239 (4.75), 301.5 (3.85), 315 (3.95), 330 (3.79), 336 (3.69) nm
ν_{max} 3.00, 9.35 μm (Coombs, 1966a)

C_{17}H_{16}O
(103) 6,7-cis-Dihydroxy-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrene
MW 252; mp 193°C (Butenandt et al., 1946b)

C_{17}H_{16}O
(115) 1,4-Dihydroxy-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrene
MW 252 (Tamayo and Martin, 1952)

C_{17}H_{16}O 11,12,13,14,15,16-Hexahydro-11-hydroxycyclopenta[a]phenanthren-17-one [23462-85-3] [23462-84-2]
(Mejer and Kalinowska, 1969)

C_{17}H_{16}O
11,12,13,14,15,16-Hexahydro-11α-hydroxycyclopenta[a]phenanthren-17-one [23462-84-2]
MW 252; mp 115°C
λ_{max} 229 (4.91), 278 (3.78) nm
ν_{max} (Nujol) 2.93, 5.75 μm
ν_{max} (CCl₄) 2.77 μm (Mejer and Kalinowska, 1969)

C_{17}H_{16}O
11,12,13,14,15,16-Hexahydro-11β-hydroxycyclopenta[a]phenanthren-17-one [23462-85-3]
MW 252; mp 201°C
λ_{max} 230 (4.96), 278 (3.84) nm
ν_{max} (Nujol) 2.88, 5.78 μm
ν_{max} (CCl₄) 2.77 μm (Mejer and Kalinowska, 1969)

C_{17}H_{16}O
11,12,13,14,16,17-Hexahydro-17α-hydroxy-15H-cyclopenta[a]-phenanthren-11-one [23462-86-4]
MW 252; mp 119°C
λ_{max} 243 (4.35), 310 (3.88)
ν_{max} (Nujol) 2.93, 6.02 μm
ν_{max} (CCl₄) 2.76 μm (Mejer and Kalinowska, 1969)

C_{17}H_{16}O
11,12,13,14,16,17-Hexahydro-17β-hydroxy-15H-cyclopenta[a]-phenanthren-11-one [23462-87-5]
MW 252; mp 132°C
λ_{max} 243 (4.35), 310 (3.93) nm
ν_{max} (Nujol) 2.93, 6.02 μm
ν_{max} (CCl₄) 2.76 μm (Mejer and Kalinowska, 1969)
Physical and spectral properties

\( \text{C}_{11}\text{H}_{16}\text{O}_2 \)

(+)11,12,13,14,16,17-Hexahydro-17\( \beta \)-hydroxy-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthren-11-one [23462-88-6]

MW 252; mp 111-112°C, \([\alpha]\)\( ^{20} \) –13° (CHCl₃)

Spectra as for (±) compound (Mejer and Kalinowska, 1969)

\( \text{C}_{11}\text{H}_{16}\text{O}_2 \)

(114) 1,4,5,6,9,10,16,17-Octahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene-1,4-dione

MW 252 (Tamayo and Martin, 1952)

\( \text{C}_{11}\text{H}_{18} \)

(326) 1,2,3,4,16,17-Hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene

MW 222; mp 94-96°C (Rahman and Rodriguez, 1969)

\( \text{C}_{11}\text{H}_{18} \)

(440) 6,7,8,14,16,17-Hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene-12-one

MW 222; mp 79°C (Robinson & Slater, 1941); 78°C (Jacob et al., 1971)

\( \lambda_{\text{max}} \) 230 (3.78), 242 (3.75), 250 (3.66), 321 (4.09), 335 (4.15), 352 (3.97) nm

\( \nu_{\text{max}} \) 13.25 μm

\( \delta \) 8(m), 7.4(m), 6.76(d), 6.15(d), 3.2(m), 2.2(m) (Jacob et al., 1971)

\( \text{C}_{11}\text{H}_{18} \)

(44) 11,12,13,14,16,17-Hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene

[31301-56-1]

MW 222; mp 91-92°C (Coombs, 1966a); 85-87°C (Buchta and Ziemer, 1956)

\( \lambda_{\text{max}} \) 230 (4.28), 275 (3.63), 285 (3.76), 292 (3.54) nm (Coombs, 1966a)

\( \text{C}_{11}\text{H}_{18}\text{O} \)

(96) 6,7,8,12,13,14,16,17-Octahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthren-12-one

MW 238; mp 171°C (Robinson and Slater, 1941); 173°C (Jacob et al., 1971); 169-170°C (Hawthorne and Robinson, 1936)

\( \lambda_{\text{max}} \) 295 (4.20) nm

\( \nu_{\text{max}} \) 5.99 μm

\( \delta \) 7.7(m), 7.2(s), 6.5(s), 1.8(m) (Jacob et al., 1971)

\( \text{C}_{11}\text{H}_{18}\text{O} \)

11\( \alpha \),17\( \alpha \)-Dihydroxy-11,12,13,14,16,17-hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene [23462-89-7]

MW 254; mp 164°C

\( \lambda_{\text{max}} \) 229 (5.12), 279 (3.86) nm

\( \nu_{\text{max}} \) (CCl₃) 2.77 and 290μm (Mejer and Kalinowska, 1969)

\( \text{C}_{11}\text{H}_{18}\text{O} \)

11\( \beta \),17\( \alpha \)-Dihydroxy-11,12,13,14,16,17-hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene [23462-92-2]

MW 254; mp 192°C

\( \lambda_{\text{max}} \) 229 (5.11), 279 (3.81) nm

\( \nu_{\text{max}} \) (CCl₃) 2.77μm (Mejer and Kalinowska, 1969)

\( \text{C}_{11}\text{H}_{18}\text{O} \)

(±)11,17\( \beta \)-Dihydroxy-11,12,13,14,16,17-hexahydro-15\( \beta \)-cyclopenta[\( \alpha \)]phenanthrene

MW 254; mp 115°C [23462-90-0]
Compilation of data from the literature

\[ \lambda_{\text{max}} 229 (5.03), 278 (3.79) \text{ nm} \]
\[ \nu_{\text{max}} (\text{CCl}_4) 2.77 \mu\text{m} \]

(Meje and Kalinowska, 1969)

\[ \text{C}_{17}\text{H}_{20}\text{O}_2 \]
\[ (+) 11,17\beta\text{-Dihydroxy-11,12,13,14,16,17-hexahydro-15H-cyclopenta[a]phenanthrene [23462-91-1]} \]
MW 254; mp 170–175°C, \([\alpha]_D^0 +63^\circ \text{ (EtOH)}\)
\[ \lambda_{\text{max}} 299 (5.08), 279 (3.87) \text{ nm} \]
\[ \nu_{\text{max}} (\text{CHCl}_3) 2.77 \mu\text{m} \]

(Meje and Kalinowska, 1969)

\[ \text{C}_{17}\text{H}_{20}\text{O}_2 \]
\[ (233) 1,2,3,4,11,12,13,14,15,16-Decahydrocyclopenta[a]-phenanthrene-11,17-dione \]
MW 254; mp 111–113°C
\[ \lambda_{\text{max}} 200 (4.25), 257 (3.91), 308 (3.33) \text{ nm} \]
\[ \nu_{\text{max}} 5.75, 5.95, 10.1, 10.7, 11.9 \mu\text{m} \]

(Coombs and Bhatt, 1973)

\[ \text{C}_{17}\text{H}_{18}\text{O} \]
\[ 12\text{-Hydroxy-6,7,8,12,13,14,16,17-octahydro-15H-cyclopenta[a]-phenanthrene} \]
MW 240; mp 131–132°C

(Robinson and Slater, 1941)

\[ \text{C}_{18}\text{H}_{12}\text{Br}_2\text{O} \]
\[ 15,15\text{-Dibromo-15,16-dihydro-11-methyl-cyclopenta[a]-phenanthren-17-one [50905-51-6] [74495-85-5]} \]
MW 234; Orange crystals, mp 206–207°C
\[ \lambda_{\text{max}} 267.5 (4.68), 302.5 (4.32), 315 (4.12), 369 (3.29), 384 (3.31) \text{ nm} \]
\[ \delta 4.69 \text{ (H-16)} \]

(Coombs et al., 1973a)

\[ \text{C}_{18}\text{H}_{12}\text{O} \]
\[ 11\text{-Methylcyclopenta[a]phenanthren-17-one} \]
MW 244 [55651-29-1]
\[ \lambda_{\text{max}} 256, 288, 298, 312, 389 \text{ nm} \]

(Coombs et al., 1975)

\[ \text{C}_{18}\text{H}_{12}\text{O}_2 \]
\[ 17H-Cyclopenta[a]phenanthrene-17-carboxylic acid \]
MW 260; mp 250–270°C

(Süss, 1953)

\[ \text{C}_{18}\text{H}_{12}\text{O}_2 \]
\[ 15,16\text{-Dihydro-16-formylcyclopenta[a]phenanthren-17-one} \]
MW 260; mp 197–198°C

(Buchta and Kraetzer, 1962)

\[ \text{C}_{18}\text{H}_{12}\text{O}_3 \]
\[ 11\text{-Methyl-6,7,15,16-tetrahydrocyclopenta[a]phenanthrene-6,7,17-trione} \]
### Physical and spectral properties

MW 276; mp 230°C
\[ \lambda_{\text{max}} \] 263 (4.15), 345 (3.44), 410 (2.94) nm
\[ \nu_{\text{max}} \] 5.80, 6.00, 6.24, 10.30, 11.91, 12.08, 13.70, 13.86 \( \mu \)m

(Coombs, 1969)

### C_{18}H_{13}BrO

(355) 15-Bromo-15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one [50905-52-7]
MW 325; mp 211.5–212°C

\[ \lambda_{\text{max}} \] 267.5 (4.68), 290 (4.37), 303 (4.29), 363 (3.31), 381 (3.35) nm
\[ \nu_{\text{max}} \] 5.88, 12.20, 13.18, 13.85, 14.29 \( \mu \)m

\[ \delta \] 4.85 (J 7.3) (H-15), 3.72 (J 18.3) and 4.23 (J 18.7) (H-16)

(Coombs et al., 1973a)

### C_{18}H_{13}BrO

(359) 16-Bromo-15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one [55651-28-0]
\[ \lambda_{\text{max}} \] 275.4, 359, 377 nm
\[ \nu_{\text{max}} \] 5.81, 11.49, 12.09, 12.50, 12.99, 13.19, 14.81 \( \mu \)m

\[ \delta \] 5.94 (J 2.6) (H-16), 3.49 (J 6.2) and 3.30 (J 2.2) (H-15)

(Coombs et al., 1975)

### C_{18}H_{13}ClO

(322) 7-Chloro-15,16-dihydro-6-methoxycyclopenta[a]phenanthren-17-one
MW 296.5; mp 220°C

(Coombes et al., 1952)

### C_{18}H_{14}

(143) 15,16-Dihydro-17-methylenecyclopenta[a]phenanthrene
MW 230; mp 217–218°C

\[ \lambda_{\text{max}} \] 215 (4.22), 273.5 (4.69), 287 (4.44), 299 (4.34), 307 (4.09), 333 (3.08), 349 (3.28), 367 (3.34) nm
\[ \nu_{\text{max}} \] 3.25, 6.18, 10.62, 11.6, 12.04, 12.42, 13.42, 13.9 \( \mu \)m

\[ \delta \] 5.56 and 5.13 (olefinic protons), 3.25 and 3.05 (methylene protons)

(Coombs et al., 1976)

### C_{18}H_{14}

MW 230; mp 87–89°C

\[ \lambda_{\text{max}} \] 221.5 (4.47), 267 (4.65), 274 (4.65), 294 (4.08), 306 (4.08), 317 (2.83), 348 (2.85), 364 (2.74) nm
\[ \nu_{\text{max}} \] 12.27, 13.32, 14.27 \( \mu \)m

(Coombs and Hall, 1973)

### C_{18}H_{14}

(128) 15-Methyl-17H-cyclopenta[a]phenanthrene
MW 230; mp 135°C

\[ \lambda_{\text{max}} \] 221 (4.68), 241 (4.45), 267.5 (4.60), 293 (4.02), 305 (4.17), 317.5 (4.15), 344 (3.08), 361 (2.99) nm
\[ \nu_{\text{max}} \] (KBr) 12.29, 12.40, 13.24, 13.41 \( \mu \)m

(Dannenberg et al., 1960)

### C_{18}H_{14}

(133) 17-Methyl-15H-cyclopenta[a]phenanthrene [3353-08-0]
MW 230; Cream needles, mp 209–212°C

\[ \lambda_{\text{max}} \] 223 (4.30), 270 (4.70), 276 (4.73), 293 (4.14), 316 (3.74), 331 (2.71), 348 (2.69), 366 (2.46) nm
\[ \nu_{\text{max}} \] 11.0, 11.55, 12.0, 12.3, 12.8, 13.4, 13.82 \( \mu \)m

(Coombs, 1966b)

### C_{18}H_{14}O

(302) 15,16-Dihydro-1-methylcyclopenta[a]phenanthren-17-one
MW 230; mp 230°C

\[ \lambda_{\text{max}} \] 263 (4.15), 345 (3.44), 410 (2.94) nm
\[ \nu_{\text{max}} \] 5.80, 6.00, 6.24, 10.30, 11.91, 12.08, 13.70, 13.86 \( \mu \)m

(Coombs, 1966b)
Compilation of data from the literature

MW 246; mp 189–190°C
λ_max 266 (4.84), 288 (4.53), 303 (4.30), 359 (3.77), 375 (3.77) nm
ν_max 5.92, 12.09, 12.99 µm
δ 2.73 (H-16), 3.08 (methyl), 3.38 (H-15) (Ribeiro et al., 1983)

C_{18}H_{14}O (303) 15,16-Dihydro-2-methylcyclopenta[a]phenanthren-17-one
[27343-46-0]
MW 246; mp 221–222°C
λ_max 267 (5.05), 284 (4.22), 298 (4.12), 336 (3.11), 352 (3.34), 370 (3.27) nm
ν_max 5.85, 11.72, 11.90, 12.10, 12.50, 12.95, 14.08 µm (Coombs et al., 1970)

C_{18}H_{14}O (304) 15,16-Dihydro-3-methylcyclopenta[a]phenanthren-17-one
[27363-65-1]
MW 246; mp 203–204°C
λ_max 267 (5.02), 285 (4.67), 298 (4.41), 344 (3.28), 366 (3.25) nm
ν_max 5.88, 11.10, 11.60, 11.90, 12.36, 13.05, 13.90, 14.10 µm (Coombs et al., 1970)

C_{18}H_{14}O (305) 15,16-Dihydro-4-methylcyclopenta[a]phenanthren-17-one
[27343-45-9]
MW 246; mp 265–266°C
λ_max 266 (4.98), 284 (4.67), 300 (4.36), 336 (3.06), 352 (3.22), 370 (3.23) nm
ν_max 5.92, 11.10, 11.88, 12.05, 12.34, 12.60, 13.85, 14.15 µm (Coombs et al., 1970)

C_{18}H_{14}O (306) 15,16-Dihydro-6-methylcyclopenta[a]phenanthren-17-one
[27343-44-8]
MW 246; mp 210.5–212°C
λ_max 266 (5.06), 284 (4.69), 298 (4.45), 336 (3.10), 352 (3.32), 370 (3.33) nm
ν_max 5.86, 11.44, 12.12, 13.00, 14.25 µm (Coombs et al., 1970)

C_{18}H_{14}O (230) 15,16-Dihydro-7-methylcyclopenta[a]phenanthren-17-one
[30835-65-5]
MW 246; mp 198–199°C
λ_max 268 (4.79), 286 (4.42), 301 (4.28), 343 (3.23), 360 (3.41), 378 (3.42) nm
ν_max 5.91, 11.3, 11.98, 12.92, 13.34 µm (Coombs and Jaitly, 1971)

C_{18}H_{14}O (381) 15,17-Dihydro-11-methylcyclopenta[a]phenanthren-16-one
[42123-04-6]
MW 246; mp 152.5–153°C
λ_max 256.5 (4.77), 282 (4.06), 295 (4.08), 307 (4.16), 324 (2.99), 339 (306), 355 (3.04) nm
ν_max 5.75, 12.08, 12.58, 13.46 µm (Coombs and Hall, 1973)

C_{18}H_{14}O (26) 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one
[892-17-1] [74495-84-4] [74524-21-3]
MW 246; mp 171–172°C
λ_max 222 (4.11), 264 (4.83), 288 (4.49), 301 (4.32), 342 (3.11), 358 (3.38), 376 (3.43) nm
Physical and spectral properties

\[ \nu_{\max} \ 5.92, 11.40, 12.28, 13.34, 14.00, 14.52 \mu m \]
\[ \delta \ 2.68 (H-16), 3.00 (methyl), 3.23 (H-15) \quad (Coombs, 1966b) \]

**C\textsubscript{18}H\textsubscript{16}O (130)** 15,16-Dihydro-12-methylcyclopenta[a]phenanthren-17-one

\[ [789-46-8] \]
MW 245; mp 233°C (Coombs, 1966b; Riegel et al., 1948)
\[ \lambda_{\max} \ 219 (4.18), 268 (4.85), 286 (4.42), 299 (4.36), 340 (2.96), 355 (3.22), 375 (3.28) \text{ nm} \]
\[ \nu_{\max} \ 5.92, 11.36, 12.32, 12.70, 13.20 \mu m \]
\[ \delta \ 2.70 (H-16), 2.82 (methyl), 3.28 (H-15) \quad (Coombs, 1966b) \]

**C\textsubscript{18}H\textsubscript{16}O (49)** 16,17-Dihydro-17-methylcyclopenta[a]phenanthren-15-one

MW 246; mp 138–139°C (Tatta and Bardhan, 1968)

**C\textsubscript{18}H\textsubscript{16}O (402)** 15,17-Dihydro-17-methylcyclopenta[a]phenanthren-16-one

\[ [42123-02-4] \]
MW 246; mp 231–233°C (Dannenberg et al., 1960)

**C\textsubscript{18}H\textsubscript{16}O (334)** 16,17-Dihydro-11-methylcyclopenta[a]phenanthren-15-one

\[ [24684-42-2] \]
MW 246; mp 182–183°C
\[ \lambda_{\max} \ 217 (4.57), 253 (4.63), 284 (4.15), 323 (4.14), 363 (3.41) \text{ nm} \]
\[ \nu_{\max} \ 5.94, 11.60, 12.22, 13.28, 13.72, 14.02 \mu m \quad (Coombs, 1969) \]

**C\textsubscript{18}H\textsubscript{16}O\textsubscript{2} (458)** 15,16-Dihydro-4-hydroxy-1-methylcyclopenta[a]phenanthren-17-one

MW 262.0994; found, 262.0992
\[ \lambda_{\max} \ 270, 305 \text{ nm} \]
\[ \lambda_{\max} \ \text{(anion)} \ 260, \text{ sh} 284, 325 \text{ nm} \quad (Coombs et al., 1985) \]

**C\textsubscript{18}H\textsubscript{16}O\textsubscript{2} (340)** 15,16-Dihydro-6-hydroxy-11-methylcyclopenta[a]phenanthren-17-one

\[ [24684-47-7] \]
MW 262; Golden yellow needles, mp 335°C
\[ \lambda_{\max} \ 265 (4.76), 289 (4.51), 306 (4.27), 368 (3.37), 384 (3.41) \text{ nm} \]
\[ \nu_{\max} \ 3.08, 5.95, 11.46, 12.05, 12.86, 13.04, 13.74, 14.50 \mu m \quad (Coombs, 1969) \]

**C\textsubscript{18}H\textsubscript{16}O\textsubscript{2} (215)** 15,16-Dihydro-11-hydroxymethylcyclopenta[a]phenanthren-17-one

\[ [55651-36-0] \]
MW 262; mp 190–192°C
\[ \lambda_{\max} \ 264.5 (4.85), 285 (4.51), 300 (4.32), 356 (3.29), 372.5 (3.41) \text{ nm} \]
\[ m/z \ 262; (M+, 100%), 234 (M+ - CO, 20%), 235 (M+ - CHO, 45%), 233 (M+ - CH\textsubscript{2}OH, 18%) \quad (Coombs et al., 1975) \]

**C\textsubscript{18}H\textsubscript{16}O\textsubscript{2} (370)** 15,16-Dihydro-15-hydroxy-11-methylcyclopenta[a]phenanthren-17-one

\[ [55651-31-5] \]
MW 262; mp 182–183°C
Compilation of data from the literature

MW 262
$\lambda_{\text{max}}$ 265 (4.84), 300 (4.53), 356 (3.29), 374 (3.21) nm
(Coombs et al., 1975)

C$_{18}$H$_{14}$O$_2$

(346) 15,16-Dihydro-16-hydroxy-11-methylcyclopenta[a]phenanthren-17-one [24684-56-8]
MW 262; mp 205–207°C
$\lambda_{\text{max}}$ 264 (4.88), 288 (4.52), 301 (4.35), 345 (3.14), 361 (3.36), 379 (3.39) nm
$\nu_{\text{max}}$ 3.00, 5.92, 11.44, 11.62, 12.06, 12.28, 12.55, 12.80, 13.56, 14.10 μm
(Coombs et al., 1975)

C$_{18}$H$_{14}$O$_2$

(307) 15,16-Dihydro-2-methoxycyclopenta[a]phenanthren-17-one [55651-43-9]
MW 262; mp 180–180.5°C
$\lambda_{\text{max}}$ 271 (4.81), 360 (3.45), 377 (3.51) nm
$\nu_{\text{max}}$ 5.87–5.95, 9.59, 10.47, 12.14, 12.66, 13.16, 13.89 μm
(Coombs et al., 1975)

C$_{18}$H$_{14}$O$_2$

(24) 15,16-Dihydro-3-methoxycyclopenta[a]phenanthren-17-one [24684-46-6]
MW 262; mp 209 and 230°C
$\lambda_{\text{max}}$ 218–220 (4.21), 269 (4.78), 277 (4.83), 319 (4.22), 347 (3.62), 366 (3.28) nm
$\nu_{\text{max}}$ 5.92, 11.42, 12.38, 13.04 μm
(Coombs, 1966b)

C$_{18}$H$_{14}$O$_2$

(308) 15,16-Dihydro-6-methoxycyclopenta[a]phenanthren-17-one
MW 262; mp 196–197°C
$\lambda_{\text{max}}$ 270 (4.98), 287 (4.69), 302 (4.37), 361 (3.36), 378 (3.33) nm
$\nu_{\text{max}}$ 5.90, 11.86, 12.24, 12.76, 13.04, 13.78, 14.12 μm
(Coombs et al., 1970)

C$_{18}$H$_{14}$O$_2$

(132) 15,16-Dihydro-11-methoxycyclopenta[a]phenanthrene-17-one [5836-85-1]
MW 262; mp 179°C (Robinson, 1938); 184°C (Coombs, 1966b)
$\lambda_{\text{max}}$ 214 (4.21), 261 (4.86), 292 (4.42), 303 (4.29), 346 (3.42), 363 (3.72), 381 (3.82) nm
$\nu_{\text{max}}$ 5.88, 11.30, 12.30, 13.32 μm
(Coombs, 1966b)

C$_{18}$H$_{14}$O$_2$

(367) 15,16-Dihydro-15-methoxycyclopenta[a]phenanthren-17-one [83053-56-9]
MW 262; mp 155°C
$\lambda_{\text{max}}$ 268, 297, 352, 369 nm
(Bhatt et al., 1982)

C$_{18}$H$_{14}$O$_3$

(465) 15,16-Dihydro-2,15-dihydroxycyclopenta[a]phenanthren-17-one [55081-26-0]
MW 278; Yellow solid, mp 278°C
$\lambda_{\text{max}}$ 276; anion 250, 300 nm
(Coombs and Crawley, 1974)
Physical and spectral properties

\( \text{C}_{18}\text{H}_{14}\text{O}_3 \)
15,16-Dihydro-11-hydroxy-3-methoxycyclopenta[a]phenanthren-17-one
MW 278; mp 293-299°C (Robinson, 1938)

\( \text{C}_{18}\text{H}_{14}\text{O}_4 \)
(463) 8,9-Epoxy-2,15-dihydroxy-11-methyl-8,9-seco[1,3,5,7,9,11,13-heptaen-17-one
MW 294; mp 280-282°C
\( \lambda_{\text{max}} \) 278 (4.55); anion 255 (4.50), 299 (4.50)nm
(Coombs and Crawley, 1974)

\( \text{C}_{18}\text{H}_{15}\text{NO} \)
16,17-Dihydro-17-methyl-15\( H \)-cyclopenta[a]phenanthren-15-one, oxime [17981-86-1]
MW 261; mp 169-171°C (Tatta and Bardhan, 1968)

\( \text{C}_{18}\text{H}_{16} \)
(107) 16,17-Dihydro-1-methyl-15\( H \)-cyclopenta[a]phenanthrene [63020-74-6]
MW 232; mp 75-76°C (Butenandt et al., 1950)
\( \lambda_{\text{max}} \) 256 (4.80), 285 (4.05), 297 (4.03), 306 (4.11), 323 (2.68), 339 (2.69), 355 (2.54)nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS2) 12.22, 12.77, 13.28\( \mu \)m (Dannenberg et al., 1953)

\( \text{C}_{18}\text{H}_{16} \)
(98) 16,17-Dihydro-2-methyl-15\( H \)-cyclopenta[a]phenanthrene [3988-20-3]
MW 232; mp 106-107°C (Butenandt et al., 1946c)
\( \lambda_{\text{max}} \) 261 (4.81), 282 (4.16), 291 (4.07), 303 (4.16), 323 (2.85), 338 (3.08), 354 (3.10)nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS2) 12.03, 12.26, 13.21\( \mu \)m (Dannenberg et al., 1953)

\( \text{C}_{18}\text{H}_{16} \)
(70) 16,17-Dihydro-3-methyl-15\( H \)-cyclopenta[a]phenanthrene
MW 232; mp 132°C (Kon and Woolman, 1939)

\( \text{C}_{18}\text{H}_{16} \)
(100) 16,17-Dihydro-4-methyl-15\( H \)-cyclopenta[a]phenanthrene [63020-75-7]
MW 232; mp 151-152°C (Butenandt et al., 1949a)
\( \lambda_{\text{max}} \) 255 (4.69), 263 (4.78), 283.5 (4.14), 294.5 (4.12), 306.5 (4.22), 324 (2.80), 339 (2.85), 355 (2.73)nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS2) 12.33, 12.72, 13.29\( \mu \)m (Dannenberg et al., 1953)

\( \text{C}_{18}\text{H}_{16} \)
(67) 16,17-Dihydro-6-methyl-15\( H \)-cyclopenta[a]phenanthrene [63020-26-8]
MW 232; mp 109-110°C (Gamble and Kon, 1935); 98-101°C (Butenandt and Suranyi, 1942)
\( \lambda_{\text{max}} \) 262 (4.80), 282 (4.16), 290 (4.06), 303.5 (4.09), 322 (2.83), 337 (2.92), 354 (2.87)nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS2) 11.52, 12.26, 13.27\( \mu \)m (Dannenberg et al., 1953)

\( \text{C}_{18}\text{H}_{16} \)
(94) 16,17-Dihydro-7-methyl-15\( H \)-cyclopenta[a]phenanthrene [63020-76-8]
MW 232; mp 106-108°C (Butenandt et al., 1949a)
\( \lambda_{\text{max}} \) 261 (4.77), 281 (4.24), 291 (4.06), 304 (4.08), 323.5 (2.91), 339 (3.15), 355 (3.19)nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) 11.42, 12.22, 13.44\( \mu \)m (Dannenberg et al., 1953)
Compilation of data from the literature

C_{18}H_{16} (83) 16,17-Dihydro-11-methyl-15H-cyclopenta[a]phenanthrene
[24684-41-1]
MW 232; mp 80–81°C (Butenandt et al., 1946a); 81–82°C (Coombs, 1969)
\( \lambda_{\text{max}} \) 256 (4.81), 285 (4.05), 297 (4.03), 306 (4.10), 323.5 (2.91), 340 (3.05), 356 (3.07) nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS) 11.58, 12.28, 13.43 μm (Dannenberg et al., 1953)

C_{18}H_{16} (97) 16,17-Dihydro-12-methyl-15H-cyclopenta[a]phenanthrene
[63020-73-5]
MW 232; mp 86–87°C (Butenandt et al., 1946a); 85–86°C (Riegel et al., 1948)
\( \lambda_{\text{max}} \) 261 (4.79), 282 (4.16), 291 (4.05), 303 (4.15), 321 (2.70), 337 (2.70), 353 (2.56) nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) (CS) 11.53, 12.27, 13.39 μm (Dannenberg et al., 1953)

C_{18}H_{16} (45) 16,17-Dihydro-15-methyl-15H-cyclopenta[a]phenanthrene
MW 232; mp 76–77°C (Ruzicka et al., 1933)
Trinitrobenzene deriv., mp 143–144°C

C_{18}H_{16} (46) 16,17-Dihydro-16-methyl-15H-cyclopenta[a]phenanthrene
MW 232; mp 106–107°C; bp 160–170°C/0.3 mm (Ruzicka et al., 1933)
Trinitrobenzene deriv., mp 140–141°C

C_{18}H_{16} (7) Diels' hydrocarbon [549-38-2]
16,17-Dihydro-17-methyl-15H-cyclopenta[a]phenanthrene
MW 232; mp 125–126°C (Diels and Gadke, 1927; Diels and Rickert, 1935; Bergmann and Hilleman, 1933); 126–127°C (Riegel et al., 1942); 125.5–126°C (Tatta and Bardhan, 1968); 126.5–127.5°C (Coombs, 1966b)
\( \lambda_{\text{max}} \) 259 (4.80), 280 (4.14), 288.5 (4.04), 300.5 (4.11), 321 (2.74), 336 (2.94), 351.5 (2.33) nm (Dannenberg and Steidle, 1954)
\( \nu_{\text{max}} \) 11.6, 12.06, 12.36, 13.06, 13.4, 13.9 μm (Coombs, 1966b)

C_{18}H_{16}O (11) 16,17-Dihydro-3-methoxy-15H-cyclopenta[a]phenanthrene
MW 248; mp 76–77°C (Cook and Girard, 1934)
Orange picrate, mp 135–136.5°C
Trinitrobenzene deriv., mp 160–161°C

C_{18}H_{16}O (59) 16,17-Dihydro-4-methoxy-15H-cyclopenta[a]phenanthrene
MW 248; mp 153°C
Trinitrobenzene deriv., mp 160–161°C

C_{18}H_{16}O (61) 16,17-Dihydro-6-methoxy-15H-cyclopenta[a]phenanthrene
MW 248; mp 129°C
(Kon and Ruzicka, 1935)

C_{18}H_{16}O (448) 16,17-Dihydro-11-methyl-15H-cyclopenta[a]phenanthren-17-ol
[40951-13-1]
MW 248; mp 140–141°C
Physical and spectral properties

\[ \lambda_{\text{max}} 227 (4.22), 255 (4.78), 281 (4.02), 293 (4.02), 304.5 (4.10), 323 (2.79), 338.5 (2.96), 354 (2.98) \text{ nm} \]

COOBS and Hall, 1973

\[ \nu_{\text{max}} 3.08, 12.12, 12.24, 13.33 \mu \text{m} \]

C_{16}H_{10}O 1-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[85616-49-5]

MW 248; mp 138–140°C

\[ \lambda_{\text{max}} 276 (4.55), 286 (4.62), 330 (4.09), 343 (4.11), 376 (3.87) \text{ nm} \]

Ribeiro et al., 1983

C_{16}H_{10}O 2-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[27343-51-7]

MW 248; mp 139–140°C

\[ \lambda_{\text{max}} 272.5 (4.56), 283 (4.68), 327 (3.29), 338 (3.31), 370 (2.94) \text{ nm} \]

Coombs et al., 1970

C_{16}H_{10}O 3-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[257] MW 248; mp 180.5–181.5°C

\[ \lambda_{\text{max}} 276 (4.50), 287 (4.65), 327 (3.06), 342 (3.12), 375 (2.99) \text{ nm} \]

Coombs et al., 1970

C_{16}H_{10}O 4-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[27343-48-2]

MW 248; mp 177–178°C

\[ \lambda_{\text{max}} 271 (4.58), 282 (4.68), 327 (3.14), 341 (3.19), 368 (3.00) \text{ nm} \]

Coombs et al., 1970

C_{16}H_{10}O 5-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[27343-47-1]

MW 248; mp 156.5–157°C

Riegel et al., 1948

C_{16}H_{10}O 6-Methoxy-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[55561-42-8]

MW 264; mp 154–154.5°C

\[ \lambda_{\text{max}} 273.5 (4.39), 284 (4.44), 328 (4.30) \text{ nm} \]

Coombs et al., 1975

C_{16}H_{10}O 2-Methoxy-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[27343-47-1]

MW 264; mp 210–211°C (Chuang et al., 1939)

Colourless plates, mp 211–212°C (Bachmann and Holman, 1951)

C_{16}H_{10}O 6-Methoxy-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

[27343-47-1]
Compilation of data from the literature 101

MW 264; mp 175–176°C
$\lambda_{max}$ 280 (4.53), 290 (4.64), 327 (2.92), 342 (2.94), 375 (2.99) nm
$\nu_{max}$ 11.70, 12.20, 13.15$\mu$m

(Coombs et al., 1970)

$\text{C}_{16}\text{H}_{10}\text{O}_{2}(379)$ 16,17-Dihydro-cis-16,17-dihydroxy-11-methyl-15$H$-cyclopenta[a]phenanthrene [42123-00-2]
MW 264; mp 181–182°C
$\lambda_{max}$ 227 (4.19), 255 (4.83), 281 (4.07), 292.5 (4.03), 305 (4.11), 323.5 (2.80), 338.5 (2.96), 354 (2.94) nm
$\nu_{max}$ 3.02, 9.11, 12.20, 13.46$\mu$m (Coombs and Hall, 1973)

$\text{C}_{16}\text{H}_{10}\text{O}_{2}(377)$ 16,17-Dihydro-trans-16,17-dihydroxy-11-methyl-15$H$-cyclopenta[a]phenanthrene
MW 264; mp 224–226°C [42123-09-1]
$\lambda_{max}$ 227 (4.22), 256 (4.85), 281 (4.07), 292.5 (4.04), 305 (4.13), 323 (2.82), 338.5 (2.97), 354 (2.98) nm
$\nu_{max}$ 3.05, 9.36, 12.12, 13.42$\mu$m (Coombs and Hall, 1973)

$\text{C}_{16}\text{H}_{10}\text{O}_{2}(389)$ 16,17-Dihydro-cis-16,17-dihydroxy-17-methyl-15$H$-cyclopenta[a]phenanthrene [42202-82-4]
MW 264; mp 171–172°C
$\lambda_{max}$ 215 (4.56), 251 (4.73), 257.5 (4.84), 279 (4.18), 287.5 (4.08), 299 (4.19), 320 (2.65), 334 (2.84), 351 (2.82) nm
$\nu_{max}$ (KBr) 2.94, 8.56, 8.63, 8.95, 9.30, 12.15, 13.30$\mu$m

(Dannenberg et al., 1960)

$\text{C}_{16}\text{H}_{10}\text{O}_{3}(194)$ 11,12,13,14,15,16-Hexahydro-3-methoxycyclopenta[a]phenanthrene-11,17-dione
MW 250; mp 126–127°C (Koebner and Robinson, 1938)

$\text{C}_{16}\text{H}_{10}\text{O}_{3}(338)$ 11-Methyl-17-oxo-6,7,15,16-tetrahydrocyclopenta[a]phenanthrene-cis-6,7-diol
MW 280; mp 244–245°C
$\lambda_{max}$ 232 (4.13), 308–314 (4.22) nm
$\nu_{max}$ 2.90, 3.05, 5.96, 10.20, 10.38, 11.14, 12.04, 12.78, 13.10, 13.40, 13.80, 14.46$\mu$m

(Coombs, 1969)

$\text{C}_{16}\text{H}_{10}\text{O}_{3}(457)$ 3a,4$\beta$-Dihydroxy-11-methyl-3,4,15,16-tetrahydrocyclopenta[a]phenanthren-17-one
MW 280 (see Chap. 7 for spectral data) (Coombs et al., 1979; 1980)

$\text{C}_{16}\text{H}_{10}\text{O}_{3}(459)$ 1a,2$\beta$-Dihydroxy-11-methyl-1,2,15,16-tetrahydrocyclopenta[a]phenanthren-17-one
MW 280 (see Chap. 7 for spectral data) (Coombs et al., 1979; 1980)

$\text{C}_{16}\text{H}_{10}\text{O}_{4}(454)$ 11-Methyl-1,2,15,16-tetrahydro-1a,2$\beta$,15-trihydroxy-cyclopenta[a]phenanthren-17-one
MW 296 (see Chap. 7 for spectral data)

(Coombs and Crawley, 1974; Coombs et al., 1980)
Physical and spectral properties

\( \text{C}_{18} \text{H}_{16} \text{O}_4 \) (456) 11-Methyl-3,4,15,16-tetrahydro-3\( \alpha \),4\( \beta \),16-trihydroxy-cyclopenta[a]phenanthren-17-one
MW 296 (see Chap. 7 for spectral data) (Coombs et al., 1980)

\( \text{C}_{18} \text{H}_{18} \text{O}_4 \) (455) 11-Methyl-3,4,15,16-tetrahydro-3\( \alpha \),4\( \beta \),15-trihydroxy-cyclopenta[a]phenanthren-17-one
MW 296 (see Chap. 7 for spectral data) (Coombs et al., 1980)

\( \text{C}_{18} \text{H}_{16} \text{O} \) (460) 8,9-Epoxy-1\( \alpha \),2\( \beta \),15-trihydroxy-11-methyl-8,9-secogon-3,5,7,9,11,13-hexaen-17-one
MW 312; mp 120°C (decomp.), [\( \alpha \)]\( \text{D} \) -214° (c. 0.134, EtOH)
\( \lambda_{\text{max}} \) 267 (4.57), 320 (4.03), 332 (4.04), 352 (3.67), 370 (3.61) nm
\( \nu_{\text{max}} \) 2.94-3.16, 5.91, 6.23, 9.62, 9.95 \( \mu \)m
\( \delta \) H-1, 5.85 (J\( \text{J}_{1,2} \); H-2, 4.36 (J\( \text{J}_{1,2} \), J\( \text{J}_{1,6} \), J\( \text{J}_{2,6} \)); H-3, 6.30 (J\( \text{J}_{4,9} \), J\( \text{J}_{6,7} \)); H-4, 6.85 (J\( \text{J}_{9,4} \), J\( \text{J}_{6,7} \)); H-5, 7.52 (J\( \text{J}_{3,4} \), J\( \text{J}_{7,8} \)); H-7, 8.48 (J\( \text{J}_{5,6} \); H-12, 7.52;
H-15, 5.76 (J(\( \text{J}_{13,15} \), J(\( \text{J}_{15,16} \)); H-16, 5.19 (J(\( \text{J}_{17,18} \), J(\( \text{J}_{16,17} \)); H-16,
2.60 (J(\( \text{J}_{17,18} \), J(\( \text{J}_{16,17} \)); 11-CH\( \text{CH} \), 3.22 (Coombs and Crawley, 1974)

\( \text{C}_{18} \text{H}_{18} \text{O} \) (443) 3-Methoxy-6,7,16,17-tetrahydro-15H-cyclopenta[a]phenanthrene
MW 250; mp 101-102°C (Robinson and Slater, 1941)

\( \text{C}_{18} \text{H}_{18} \text{O} \) (238) 1,2,3,4,15,16-Hexahydro-11-methylcyclopenta[a]phenanthren-17-one
MW 250; mp 150-152°C
\( \lambda_{\text{max}} \) 223 (4.23), 261 (4.77), 287 (3.97), 297.5 (4.02), 308 (3.87),
344 (3.69), 354 (3.74) nm
\( \nu_{\text{max}} \) 5.95, 11.5, 12.45 \( \mu \)m
m/z 250.13574 (M\( ^+ \)), 235, 222, 207, 194, 179
(Coombs and Bhatt, 1973)

\( \text{C}_{18} \text{H}_{18} \text{O} \) (86) 11,12,13,14,16,17-Hexahydro-12-methyl-15H-cyclopenta[a]-phenanthren-11-one
MW 250
12\( \alpha \)-methyl, mp 85-86°C
12\( \alpha \)-methyl, mp 117-118°C (Butenandt et al., 1946a)

\( \text{C}_{18} \text{H}_{16} \text{O}_4 \) (462) 1,2,3,4,15,16-Hexahydro-1\( \alpha \),2\( \beta \),15-trihydroxy-11-methylcyclopenta[a]phenanthren-17-one
MW 298; mp 225-227°C
\( \lambda_{\text{max}} \) 260 (4.70), 285 (3.89), 295 (3.94), 342 (3.46), 354 (3.49) nm
(Coombs and Crawley, 1974)

\( \text{C}_{18} \text{H}_{18} \text{O}_4 \) (441) 6,7,8,14,16,17-Hexahydro-3-methoxy-15H-cyclopenta[a]-phenanthrene
MW 252; mp 82-85°C (Robinson and Slater, 1941)

\( \text{C}_{18} \text{H}_{18} \text{O}_4 \) (427) 11,12,13,14,16,17-Hexahydro-11-hydroxy-3-methoxy-15H-cyclopenta[a]phenanthrene
MW 268; mp 141-142°C (Robinson and Rydon, 1939)
Compilation of data from the literature

C_{18}H_{20}O_{2} (437) 3-Methoxy-6,7,8,12,13,14,16,17-octahydro-15H-cyclopenta[a]phenanthren-12-one
MW 268; mp 192°C (Robinson and Slater, 1941)

C_{18}H_{20}O_{2}
3-Methoxy-6,7,8,12,13,14,15,16-octahydrocyclopenta[a]phenanthren-15-one
MW 268; mp 142°C (Nazarov et al., 1953)

C_{18}H_{20}O_{2}
3-Methoxy-6,7,8,12,13,14,15,16-octahydrocyclopenta[a]phenanthren-17-one
MW 268; bp 210–215°C/0.5 mm (Nazarov et al., 1953)

C_{18}H_{20}O (313) 6,7,8,9,11,12,13,14,15,16-Decahydro-1-methylcyclopenta[a]phenanthren-17-one
MW 254; mp 85–88°C (Coombs and Vose, 1974)

C_{18}H_{20}O (93) 1,2,3,4,11,12,13,14,15,16-Decahydro-7-methyl-15H-cyclopenta[a]phenanthren-11-one
MW 254; mp 102–103°C (Butenandt et al., 1949a)

C_{18}H_{20}O (22) 6,7,8,9,11,12,13,14,15,16-Decahydro-3-methoxycyclopenta[a]phenanthren-17-one
MW 270
mp 13β, 149–150°C; 13α, 120–121°C (Loke et al., 1958)
mp 13β, 161–163°C; 13α, 121–122°C (Johns, 1958)
mp 13β, 163–164°C; 13α, 120–122°C (Coombs and Vose, 1974)

C_{18}H_{22}O (439) 12-Hydroxy-3-methoxy-6,7,8,12,13,14,16,17-octahydro-15H-cyclopenta[a]phenanthrene
MW 270; mp 157–161°C (Robinson and Slater, 1941)

C_{18}H_{22}O
3-Methoxy-6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[a]phenanthren-15-one
MW 270; two isomers, mp 160–160.3°C and 116–117°C (Nazarov et al., 1953)

C_{19}H_{18}O_{2} (341) 17-Acetoxy-15H-cyclopenta[a]phenanthrene [24684-52-4]
MW 274; mp 210–211°C
λ_{max} 222 (4.88), 269 (4.91), 273 (4.92), 291 (4.28), 312 (3.89), 329 (2.91), 345 (2.83), 363 (2.66) nm
ν_{max} 5.72, 8.24, 11.00, 12.02, 12.28, 12.85, 13.34, 13.88 μm (Coombs, 1969)

C_{19}H_{14}O_{2} (397) 2-Acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one
[55651-44-0]
MW 290; mp 248–249°C
λ_{max} 265.5 (5.84), 282.5 (4.46), 296 (4.36), 352 (3.37), 369 (3.39) nm
Physical and spectral properties

\[^{\text{v}_{\text{max}} \text{ 5.70 (acetate), 5.90 (aryl CO), 9.85, 10.75\text{\mu m}}\]
\[^{\text{C}_{19}H_{16}O_3}\]
\[^{(80) \text{11-Acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one \[24684-58-0\]}}\]
\[^{\text{MW 290; mp 207\text{°C} \text{ (Robinson, 1938)}}\]

\[^{\text{C}_{19}H_{16}O_3}\]
\[^{(343) \text{16-Acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one \[24684-53-5\]}}\]
\[^{\text{MW 290; mp 178\text{°C} \text{ (Coombs et al., 1973a)}}\]

\[^{\text{C}_{19}H_{16}O_3}\]
\[^{(365) \text{15-Acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one \[50905-55-0\]}}\]
\[^{\text{MW 290; mp 197–198\text{°C} \text{ (Coombs et al., 1973a)}}\]

\[^{\text{C}_{19}H_{16}O_3}\]
\[^{(186) \text{1,6-Dimethoxy-cyclopenta[a]phenanthrene-15,17-dione \[Fieser and Hershberg, 1936\]}}\]
\[^{\text{MW 306; Deep yellow needles, mp 281–283\text{°C} \text{ (Fieser and Hershberg, 1936)}}\]

\[^{\text{C}_{19}H_{16}\text{O}\_3}\]
\[^{(135) \text{11,17-Dimethyl-15H-cyclopenta[a]phenanthrene \[5831-10-7\]}}\]
\[^{\text{MW 244; mp 149–150\text{°C} \text{ (Coombs et al., 1966b)}}\]

\[^{\text{C}_{19}H_{16}\text{O}\_3}\]
\[^{(136) \text{12,17-Dimethyl-15H-cyclopenta[a]phenanthrene \[5831-09-4\]}}\]
\[^{\text{MW 244; mp 161–162\text{°C} \text{ (Coombs et al., 1966b)}}\]

\[^{\text{C}_{19}H_{16}\text{O}\_3}\]
\[^{(347) \text{12-Acetyl-16,17-dihydro-15H-cyclopenta[a]phenanthrene \[2960-75-0\]}}\]
\[^{\text{MW 260; mp 128\text{°C} \text{ (Coombs et al., 1975)}}\]

\[^{\text{C}_{19}H_{16}\text{O}\_3}\]
\[^{(309) \text{15,16-Dihydro-7,11-dimethylcyclopenta[a]phenanthren-17-one \[85616-56-4\]}}\]
\[^{\text{MW 260; mp 208–210\text{°C} \text{ (Dannenberg et al., 1965)}}\]
Compilation of data from the literature

δ 2.65 (H-16), 2.82 and 2.98 (methyls), 3.63 (H-15)
(Ribeiro et al., 1983)

C₁₉H₁₆O (131) 15,16-Dihydro-11,12-dimethylcyclopenta[a]phenanthren-17-one
[894-52-0]
MW 260; mp 149–150°C
λ max 218 (4.09), 264 (4.88), 292 (4.45), 304 (4.32), 349 (3.05), 365 (3.34), 383 (3.40) nm
ν max 5.94, 11.40, 12.38, 13.26, 14.00 μm
δ 2.70 (H-16), 2.75 (methyls), 3.17 (H-15) (Coombs, 1966a)

C₁₉H₁₆O (211) 15,16-Dihydro-11-ethylcyclopenta[a]phenanthren-17-one
[42028-27-3]
MW 260; mp 129–130°C
λ max 264.5 (4.91), 289 (4.47), 302 (4.33), 360 (3.51), 378 (3.54) nm
ν max 5.88, 12.38, 13.36, 13.90, 14.54 μm (Coombs et al., 1973b)

C₁₉H₁₆O (253) 15,16-Dihydro-12-ethylcyclopenta[a]phenanthren-17-one
MW 260; mp 206.5–207°C (Riegel et al., 1948)

C₁₉H₁₆O (50) 16,17-Dihydro-17-ethylcyclopenta[a]phenanthren-15-one
MW 260; mp 110–111.2°C (Riegel et al., 1943)
Oxime, mp 169–170.8°C

C₁₉H₁₆O (134) 3-Methoxy-17-methyl-15//-cyclopenta[a]phenanthrene
MW 260; mp 204–205°C
λ max 223 (4.28), 273 (4.84), 294 (4.22), 304 (4.16), 318 (4.10), 338 (2.92), 355 (3.04), 374 (3.03) nm
ν max 10.94, 11.64, 12.22, 13.10, 13.82 μm (Coombs, 1966b)

C₁₉H₁₆O (138) 11-Methoxy-17-methyl-15//-cyclopenta[a]phenanthrene
[5831-12-9]
MW 260; mp 159–160°C
λ max 221 (4.37), 272 (4.70), 280 (4.74), 287 (4.63), 324 (3.69), 339 (3.29), 356 (3.49), 374 (3.57) nm
ν max 10.92, 11.95, 12.10, 12.20, 12.55, 13.10, 13.35, 13.90 μm
(Coombs, 1966b)

C₁₉H₁₆O₂ (333) 15-Acetoxy-16,17-dihydro-15//-cyclopenta[a]phenanthrene
MW 276; mp 127–128°C (Badger et al., 1952)

C₁₉H₁₆O₂ (390) 15,16-Dihydro-11-ethoxycyclopenta[a]phenanthren-17-one
[83053-57-0]
MW 276; mp 191–193°C
λ max 261 (4.91), 291 (4.54), 362 (3.98), 381 (4.00) nm (Bhatt et al., 1982)

C₁₉H₁₆O₂ (311) 15,16-Dihydro-4-methoxy-1-methylcyclopenta[a]phenanthren-17-one
MW 276; mp 199–200°C
λ max 262, 302, 366, 384 nm
δ 2.80 (H-16), 3.07 (methyl), 3.40 (H-15), 4.03 (O-methyl)
(Coombs et al., 1985)

C₁₉H₁₆O₂ (468) 15,16-Dihydro-6-methoxy-11-methylcyclopenta[a]phenanthren-17-one [24684-49-9]
Physical and spectral properties

MW 276; mp 203-205°C
\( \nu_{\text{max}} \) 5.86, 12.26, 13.18, 13.78 \( \mu \)m (Coombs, 1969)

C\(_{19}\)H\(_{16}\)O\(_2\)

(447) 15,16-Dihydro-11-methoxy-7-methylcyclopenta[a]phenanthren-17-one [30835-61-1]
MW 276; mp 215-216°C
\( \lambda_{\text{max}} \) 267 (4.81), 293.5 (4.37), 321 (3.92), 355 (3.44), 373 (3.74), 393 (3.81) nm (Coombs and Jaitly, 1971)

C\(_{19}\)H\(_{16}\)O\(_2\)

(368) 15,16-Dihydro-15-methoxy-11-methylcyclopenta[a]-phenanthren-17-one [83053-62-7]
MW 276; mp 158°C
\( \lambda_{\text{max}} \) 265, 299.5, 355, 373 nm (Bhatt et al., 1982)

C\(_{19}\)H\(_{16}\)O\(_2\)

(407) 17-(16,17-Dihydro-15\( H \)-cyclopenta[a]phenanthryl)-acetic acid
MW 276; mp 164-166°C
\( \lambda_{\text{max}} \) 215 (4.56), 259 (4.74), 280 (4.19), 288 (4.10), 300 (4.10), 320 (2.65), 327.5 (2.47), 333 (2.83), 342.5 (2.41), 351 (2.83) nm
\( \nu_{\text{max}} \) 3.32, 3.75, 3.85, 5.87, 12.35, 13.37 \( \mu \)m

(Dannenberg and Dannenberg-von Dresler, 1964)

C\(_{19}\)H\(_{16}\)O\(_3\)

(111) 8,12,13,14,16,17-Hexahydro-15\( H \)-cyclopenta[a]phenanthrene-13,14-dicarboxylic acid anhydride
MW 292; mp 172-173°C (Sen Gupta and Bhattacharyya, 1954)

C\(_{19}\)H\(_{16}\)O\(_3\)

(112) 11,12,13,14,16,17-Hexahydro-15\( H \)-cyclopenta[a]phenanthrene-13,14-dicarboxylic acid anhydride
MW 292; Flakes, mp 183°C (Sen Gupta and Bhattacharyya, 1954)

C\(_{19}\)H\(_{16}\)O\(_3\)

(426) 15,16-Dihydro-3,11-dimethoxycyclopenta[a]phenanthren-17-one
MW 292; mp 200-201°C (Robinson, 1938)

C\(_{19}\)H\(_{16}\)O\(_3\)

(387) 15-(16-Oxo-11,12,13,17-tetrahydro-16\( H \)-cyclopenta[a]-phenanthryl)-acetic acid
MW 292; mp 221-223°C (Turner, 1949)

C\(_{15}\)H\(_{17}\)Cl

(172) 3-Chloro-15,17-dihydro-17,17-dimethylcyclopenta[a]-phenanthrene [13914-44-8]
MW 280.5; mp 134°C
\( \lambda_{\text{max}} \) 220 (4.66), 224 (4.67), 255 (4.78), 261 (4.84), 283 (4.24), 292 (4.18), 304 (4.26), 323 (2.99), 331 (2.74), 338 (3.24), 347 (2.75), 355 (3.31) nm
\( \nu_{\text{max}} \) (KBr) 11.10, 11.62, 11.94, 12.09 \( \mu \)m

(Dannenberg and Hebenbrock, 1966)

C\(_{19}\)H\(_{17}\)NO

(354) 6-Acetamido-15,17-dihydro-15\( H \)-cyclopenta[a]phenanthrene [2960-73-8]
Compilation of data from the literature

MW 275; mp 273°C
$\lambda_{\text{max}}$ 262 (4.68), 291 (3.97), 304 (4.02), 338 (2.96), 350 (2.81) nm
$\nu_{\text{max}}$ 3.09, 6.01, 6.46, 11.52, 12.26, 13.45 μm

(C Dannenberg et al., 1965)

$\text{C}_{19}\text{H}_{17}\text{NO}$

(348) 12-Aacetamido-16,17-dihydro-15H-cyclopenta[a]phenanthrene
[2960-79-4]
MW 275; mp 248°C
$\lambda_{\text{max}}$ 222.5 (4.48), 242.5 (4.49), 262.5 (4.81), 303 (4.17), 322 (2.75),
339 (2.87), 355 (2.84) nm
$\nu_{\text{max}}$ (CS$_2$) 11.60, 12.05 μm

(Dannenberg et al., 1953)

$\text{C}_{19}\text{H}_{18}$

(99) 16,17-Dihydro-2,12-dimethyl-15H-cyclopenta[a]phenanthrene
[3974-81-0]
MW 246; mp 136-137°C (Butenandt et al., 1946c)
$\lambda_{\text{max}}$ 264 (4.71), 284 (4.10), 292 (3.98), 305 (4.08), 322 (2.75),
339 (2.87), 355 (2.24) nm (Dannenberg and Steidle, 1954)
$\nu_{\text{max}}$ (CS$_2$) 11.62, 12.34 μm

(Dannenberg et al., 1965)

$\text{C}_{19}\text{H}_{18}$

(101) 16,17-Dihydro-4,12-dimethyl-15H-cyclopenta[a]phenanthrene
[63020-70-2]
MW 246; mp 130-131°C (Inhoffen et al., 1949); 134.5-136°C
(Butenandt et al., 1949b)
$\lambda_{\text{max}}$ 250 (4.59), 264 (4.70), 286 (4.03), 296.5 (4.02), 308.5 (4.13),
339 (2.63), 356 (2.24) nm (Dannenberg and Steidle, 1954)
$\nu_{\text{max}}$ (CS$_2$) 11.62, 12.35, 13.27 μm

(Dannenberg et al., 1953)

$\text{C}_{19}\text{H}_{18}$

(68) 16,17-Dihydro-6,17-dimethyl-15H-cyclopenta[a]phenanthrene
MW 246; mp 80°C (Gamble and Kon, 1935); 78-78.5°C
(Butenandt and Suranyi, 1942)
$\lambda_{\text{max}}$ 271 (4.82), 282 (4.18), 290 (4.06), 303.5 (4.10), 323 (2.75),
337 (2.88), 354 (2.84) nm (Dannenberg and Steidle, 1954)
$\nu_{\text{max}}$ (CS$_2$) 11.47, 12.22, 13.27 μm

(Dannenberg et al., 1953)

$\text{C}_{19}\text{H}_{18}$

(105) 16,17-Dihydro-6,7-dimethyl-15H-cyclopenta[a]phenanthrene
[63020-72-4]
MW 246; mp 98-99°C (Butenandt et al., 1946a)
$\lambda_{\text{max}}$ 263.5 (4.78), 286 (4.15), 296.5 (4.04), 307 (3.95), 327 (2.86),
342 (3.02), 358 (3.12) nm (Dannenberg and Steidle, 1954)
$\nu_{\text{max}}$ (CS$_2$) 12.25, 13.00, 13.31 μm

(Dannenberg et al., 1953)

$\text{C}_{19}\text{H}_{18}$

(87) 16,17-Dihydro-11,12-dimethyl-15H-cyclopenta[a]phenanthrene
[63020-69-9] MW 246; mp 105-106°C (Butenandt et al., 1946a)
Physical and spectral properties

$\lambda_{\text{max}}$ 261 (4.73), 288 (3.96), 300 (3.98), 311 (4.01), 343 (2.83), 360 (2.72) nm  (Dannenberg and Steidle, 1954)

$\nu_{\text{max}}$ 12.32, 13.37 $\mu$m  (Dannenberg et al., 1953)

C$_{19}$H$_{18}$ (139) 16,17-Dihydro-11,17-dimethyl-15H-cyclopenta[a]phenanthrene [5831-16-3]
MW 246;  mp 65°C
$\lambda_{\text{max}}$ 219 (4.45), 227 (4.19), 257 (4.80), 294 (4.02), 306 (4.07), 324 (2.85), 339 (3.00), 355 (3.03) nm

$\nu_{\text{max}}$ 11.40, 12.02, 12.24, 13.28, 13.40, 13.94, 14.60 $\mu$m (Coombs, 1966b)

C$_{19}$H$_{18}$ (140) 16,17-Dihydro-12,17-dimethyl-15H-cyclopenta[a]phenanthrene [5831-15-2]
MW 246;  mp 56-57°C
$\lambda_{\text{max}}$ 217 (4.63), 261 (4.83), 290 (4.06), 303 (4.13), 321 (2.57), 337 (2.64), 353 (2.41) nm

$\nu_{\text{max}}$ 11.50, 12.22, 13.42 $\mu$m (Coombs, 1966b)

C$_{19}$H$_{18}$ (255) 16,17-Dihydro-12-ethyl-15H-cyclopenta[a]phenanthrene [7478-25-3]
MW 246;  mp 64.5-65°C (Riegel et al., 1948)

C$_{19}$H$_{18}$ (52) 16,17-Dihydro-17-ethyl-15H-cyclopenta[a]phenanthrene [17290-34-5]
MW 246;  Irregular platelets, mp 85-86°C (Riegel et al., 1943); 78-79°C (Schontube and Janak, 1968)
Picrate, mp 94.8-96.4°C (Coombs, 1966b)

C$_{19}$H$_{20}$O (58) 16,17-Dihydro-3-methoxy-15-methyl-15H-cyclopenta[a]phenanthrene
MW 262;  mp 97.5-98°C (Cohen et al., 1935)

C$_{19}$H$_{18}$O 12,12-Dimethyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one [5837-18-3]
MW 262;  mp 186-187°C
$\lambda_{\text{max}}$ 219 (4.87), 241 (4.11), 251 (4.16), 262 (4.57), 270 (4.89), 281 (5.00), 324 (4.48), 356 (4.51), 362 (4.25) nm

$\nu_{\text{max}}$ 5.90, 10.10, 12.30, 12.78, 13.22 $\mu$m
6 1.32 (6H,s,two geminal methyls), 314 (2H,s,C-11 methylene), 2.90 (2H,m,C-15 methylene), 2.7 (2H,m,C-16 methylene) (Coombs, 1966a)

C$_{19}$H$_{20}$O (57) 16,17-Dihydro-3-methoxy-16-methyl-15H-cyclopenta[a]phenanthrene
MW 262;  mp 136.5-137.5°C (Cohen et al., 1935)

C$_{19}$H$_{18}$O (17) 16-Dihydro-3-methoxy-17-methyl-15H-cyclopenta[a]phenanthrene
MW 262;  mp 147.5-148.5°C (Cohen et al., 1935); 149-150°C (Coombs, 1966b)
$\lambda_{\text{max}}$ 212 (4.42), 225 (4.30), 236 (4.36), 261 (4.82), 282 (4.20), 291 (4.12), 302 (3.95), 326 (2.83), 341 (3.16), 358 (3.28) nm

$\nu_{\text{max}}$ 11.64, 12.28, 12.45, 13.80 $\mu$m (Coombs, 1966b)

C$_{29}$H$_{20}$O (62) 16-Dihydro-6-methoxy-17-methyl-15H-cyclopenta[a]phenanthrene
Compilation of data from the literature

MW 262; mp 111°C (Kon and Ruzicka, 1935)

\( \text{C}_{19}\text{H}_{18}\text{O} (142) \) 16,17-Dihydro-11-methoxy-17-methyl-15H-cyclopenta[a]-phenanthrene

MW 262; Viscous syrup, bp 80°C/10⁻³ mm

\( \lambda_{\text{max}} \) 218-220 (4.42), 228 (4.45), 246.5 (4.60), 252.5 (4.59), 276 (4.44), 298 (3.95), 330 (3.25), 345 (3.55), 362 (3.65) nm

\( \nu_{\text{max}} \) 11.92, 12.26, 12.56, 13.30, 13.96 μm (Coombs, 1966b)

\( \text{C}_{19}\text{H}_{18}\text{O} (711) \) 7,11-Dimethyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one [85616-55-3]

MW 262; mp 145-146°C

\( \lambda_{\text{max}} \) 265 (4.47), 274 (4.75), 285 (4.84), 335 (4.39) nm

\( \nu_{\text{max}} \) 5.94, 6.13, 6.20 μm

δ 1.06 (3H,d,J 7 Hz, CHMe), 2.68 (3H,s, aryl methyl) (Ribeiro et al., 1983)

\( \text{C}_{19}\text{H}_{18}\text{O} (250) \) 12-Ethyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

MW 262; mp 102-103°C (Riegel et al., 1948)

\( \text{C}_{19}\text{H}_{18}\text{O} (332) \) 3,11-Dimethoxy-6,7,15,16-tetrahydrocyclopenta[a]phenanthren-17-one

MW 294; mp 143°C (Robinson and Rydon, 1939); 142-143°C (Corey and Estreicher, 1981)
dinitrophenylhydrazone, mp 242-243°C (Robinson and Rydon, 1939)

\( \text{C}_{19}\text{H}_{18}\text{O} (235) \) 11-Acetoxy-1,2,3,4,15,16-hexahydrocyclopenta[a]phenanthren-17-one

MW 294; mp 207-208°C

\( \lambda_{\text{max}} \) 222 (4.27), 257.5 (4.84), 286 (4.00), 296 (4.04), 307 (3.91), 340 (3.72), 352 (3.81) nm

\( \nu_{\text{max}} \) 5.69, 5.89, 8.30, 9.60, 10.50, 12.40 μm (Coombs and Bhatt, 1973)

\( \text{C}_{19}\text{H}_{18}\text{O} (201) \) 17,17-Ethylenedioxy-11,12,13,14,15,16-hexahydrocyclopenta[a]phenanthren-11-one

MW 294; mp 98°C

\( \lambda_{\text{max}} \) 216.5, 244, 310 nm

\( \nu_{\text{max}} \) 6.05 μm (Coombs, 1965)

\( \text{C}_{19}\text{H}_{18}\text{O} (85) \) 12-Carbomethoxy-11,12,13,14,16,17-hexahydro-15H-cyclopenta[a]phenanthren-11-one

MW 294; mp 149-150°C (Butenandt et al., 1946a)

\( \text{C}_{19}\text{H}_{18}\text{O} (335) \) 6-Acetyl-11,12,13,14,16,17-hexahydro-15H-cyclopenta[a]-phenanthrene [2960-76-1]

MW 264; mp 73°C

\( \lambda_{\text{max}} \) 228 (4.64), 312 (3.84), 333 (3.78) nm

\( \nu_{\text{max}} \) 5.96, 11.30, 13.08 μm

δ 2.73, 3.24, 7.1-7.9, 8.65 (Dannenberg et al., 1965)
Physical and spectral properties

**C_{19}H_{20}O_{3}**

(199) 17,17-Dimethoxy-8,9,11,12,13,14-hexahydrocyclopenta[a]-phenanthren-11-one
MW 296
\[ v_{\text{max}} 5.85 \mu m \]
\[ \delta 6.9-7.3 (4H, m), 5.8-6.3 (4H, m), 3.17 (3H, s), 3.09 (3H, s), 2.3-3.5 (6H, m) \]
\[ m/z 296 (M^+), 264 (M^+ -MeOH), 262 (M^+ -MeOH-H_2) \]
(Jung and Hudspeth, 1978)

**C_{19}H_{22}**

(48) 11,12,13,14,16,17-Hexahydro-14,17-dimethyl-15H-cyclopenta[a]-phenanthrene
MW 250; bp 160°C/0.4 mm, nD \[ 1.60681 \] (Harper et al., 1934)

**C_{19}H_{22}O**

(56) 11,12,13,14,16,17-Hexahydro-3-methoxy-14-methyl-15H-cyclopenta[a]phenanthrene
MW 266; Thick yellowish gum which refused to crystallize
Nitrobenzene derivative scarlet needles, mp 110-110.5°C
(Cohen et al., 1935)

**C_{19}H_{24}O**

(63) 11,12,13,14,16,17-Hexahydro-3-methoxy-17-methyl-15H-cyclopenta[a]phenanthrene
MW 268; mp \[ 73-77°C \] (Hoffsommer et al., 1966)

**C_{20}H_{25}O_{2}**

(200) 17,17-Dimethoxy-6,7,8,9,11,12,13,14,16,17-decahydro-15H-cyclopenta[a]phenanthren-11-one
MW 300
\[ \delta 3.95 (2H, d, J_{\alpha,\beta} 10 Hz) \]
(Jung and Hudspeth, 1978)

**C_{20}H_{16}NO_{2}**

(430) 15,16-Dihydro-3,11-dimethoxy-16-cyanocyclopenta[a]-phenanthren-17-one
MW 317
(Robinson and Rydon, 1939)

**C_{20}H_{16}**

(422) 17-Isopropylidendecyclopenta[a]phenanthrene [5830-65-9]
MW 256: Bright yellow leaflets, mp 188-189°C
\[ \lambda_{\text{max}} 214 (4.44), 228 (4.50), 270 (4.63), 302 (4.67), 310 (4.77), 347 (3.61), 366 (3.50) nm \]
\[ \nu_{\text{max}} 6.10, 11.66, 12.00, 12.14, 12.50, 13.20 \mu m \] (Coombs, 1966b)

**C_{20}H_{16}O_{2}**

(424) 17-(2-Propenyl)-15H-cyclopenta[a]phenanthrene [5837-12-7]
MW 256: Colourless needles, mp 255°C
\[ \lambda_{\text{max}} 223 (4.40), 271 (4.74), 276 (4.75), 295 (4.30), 3.16 (3.40) nm \]
\[ \nu_{\text{max}} 10.20, 10.90, 12.08, 12.38, 12.86, 13.48, 14.60 \mu m \] (Coombs, 1966b)

**C_{20}H_{16}O_{2}**

(342) 17-Acetoxy-11-methyl-15H-cyclopenta[a]phenanthrene
MW 288
(Coombs, 1969)

**C_{20}H_{16}O_{3}**

(469) 6-Acetoxy-15,16-dihydro-11-methycyclopenta[a]phenanthren-17-one [24684-48-8]
Compilation of data from the literature

MW 304; mp 225-227°C (Coombs, 1969)

C_{20}H_{16}O_{3}

(227) 11-Acetoxy-15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-one
MW 304; mp 243-244°C
δ 2.92 (3H, s, aryl methyl), 2.49 (3H, s, acetate), 2.80 (2H, m, C-16 methylene), 3.76 (2H, m, C-15 methylene)
(Coombs and Jaitly, 1971)

C_{20}H_{16}O_{3}

(72) 11-Acetoxy-15,16-dihydro-3-methylcyclopenta[a]phenanthren-17-one
MW 304; mp 224°C (Kon and Woolman, 1939)

C_{20}H_{16}O_{3}

MW 304; mp 209-210°C
λ_{max} 266 (4.81), 301 (4.30), 356 (3.11), 374 (3.19) nm
(Coombs et al., 1975)

C_{20}H_{16}O_{3}

(344) 16-Acetoxy-15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one [24684-55-7]
MW 304; mp 156-157°C
λ_{max} 263 (4.87), 288 (4.52), 301 (4.35), 342 (3.12), 357 (3.37), 375 (3.42) nm
ν_{max} 5.72, 5.81, 8.10, 12.02, 12.14, 12.90, 13.18, 13.30, 13.96 μm
(Coombs et al., 1975)

C_{20}H_{16}O_{3}

MW 304; mp 175-176°C
λ_{max} 265 (4.88), 285 (4.50), 300.5 (4.36), 356 (3.32), 373 (3.44) nm
δ (CD_{3}SOCD_{3}), 2.08 (s, acetate), 2.82 (m, 16-H_{2}), 3.12 (m, 15-H_{2}), 5.32 (s, 11-CH_{2})
m/z 304 (M^{+}, 100%), 245 (M^{+}-CH_{3}CO_{2}, 20%), 231 (M^{+}-CH_{3}COCH_{3}, 30%)
(Coombs et al., 1975)

C_{20}H_{16}O_{4}

(190) 11-Acetoxy-15,16-dihydro-3-methoxycyclopenta[a]phenanthren-17-one
MW 320; mp 241-242°C (Robinson and Rydon, 1939)

C_{20}H_{16}O_{4}

(429) 15,16-Dihydro-3,11-dimethoxy-16-formylcyclopenta[a]-phenanthren-17-one
MW 320; mp 195°C (decomp.) (Robinson and Rydon, 1939)

C_{17}H_{18}

17-Ethyl-17-methylcyclopenta[a]phenanthrene
MW 258; mp 117°C, [α]D^20 = -63.8° (ethanol)
λ_{max} 221 (4.78), 242 (4.51), 265.5 (4.63), 295 (4.01), 307 (4.16), 319 (4.15), 345 (3.03), 361 (2.62) nm
(Dannenberg and Neumann, 1961b)
Physical and spectral properties

$\text{C}_{20}\text{H}_{18}$ (388) 17-Isopropyl-15$H$-cyclopenta[a]phenanthrene
MW 258; mp 153°C (Coombs, 1966b); 154–156°C (Dannenberg et al., 1960)
\[\lambda_{\text{max}} 224 (4.31), 271 (4.70), 275 (4.71), 316 (4.02) \text{ nm}\]
\[\nu_{\text{max}} 10.26, 10.90, 11.50, 12.22, 12.90, 13.34, 13.66 \mu\text{m}\]
(Coombs, 1966b)

$\text{C}_{20}\text{H}_{18}$ (425) 17-Isopropyl-17$H$-cyclopenta[a]phenanthrene [5830-63-7]
MW 258; mp 106–107°C
\[\lambda_{\text{max}} 221 (4.66), 240 (4.33), 269 (4.65), 274 (4.60), 292.5 (4.07), 303 (4.08), 317 (3.95) \text{ nm}\]
\[\nu_{\text{max}} 10.30, 10.52, 10.76, 10.90, 11.50, 12.30, 13.00, 13.26, 13.65, 14.00 \mu\text{m}\]
(Coombs, 1966b)

$\text{C}_{20}\text{H}_{18}$ (137) 11,12,17-Trimethyl-15$H$-cyclopenta[a]phenanthrene [5831-11-8]
MW 258; mp 126–126.5°C
\[\lambda_{\text{max}} 219 (4.35), 280 (4.93), 311 (4.14), 355 (2.78) \text{ nm}\]
\[\nu_{\text{max}} 10.90, 12.30, 13.18, 13.34, 13.64, 14.90 \mu\text{m}\]
(Coombs, 1966b)

$\text{C}_{20}\text{H}_{18}\text{O}$ (254) 15,16-Dihydro-12-isopropylcyclopenta[a]phenanthren-17-one
MW 274; mp 183.5–184°C  
(Riegel et al., 1948)

$\text{C}_{20}\text{H}_{18}\text{O}$ (73) 3,17-Dimethyl-11-methoxy-15$H$-cyclopenta[a]phenanthrene
MW 274; mp 130–131°C  
(Kon and Woolman, 1939)

$\text{C}_{20}\text{H}_{18}\text{O}$ (51) 16,17-Dihydro-17-isopropoxycyclopenta[a]phenanthren-15-one
MW 274; mp 143.6–144.4°C  
(Riegel et al., 1943)

Cyclic oxime, mp 205–211°C

$\text{C}_{20}\text{H}_{18}\text{O}$ (165) 3-Hydroxy-1,17,17-trimethylcyclopenta[a]phenanthrene
[isolated as its acetate $\text{C}_{22}\text{H}_{20}\text{O}_{2}$]
MW 316; mp 121–122°C
\[\lambda_{\text{max}} 263, 297, 310, 322\]
\[\nu_{\text{max}} 5.65, 8.13 \mu\text{m}\]
\[\delta 1.42 (s, 17-gem-diMe), 2.38 (s, 3-OAc), 3.15 (s, 1-Me), 6.75 (d, J 6, 16-H), 7.2–8.8 (m, olefinic and Ar H)\]
\[m/z 316 (44\%, M^+), 275 (23\%), 274 (100\%, M^+\text{-CH}_2\text{CO}), 259 (29\%), 244 (15\%), 215 (15\%)\]
(Brown and Turner, 1971)

$\text{C}_{20}\text{H}_{18}\text{O}_2$ (391) 15,16-Dihydro-11-n-propoxycyclopenta[a]phenanthren-17-one
[83053-58-1]
MW 290; mp 181–182°C
\[\lambda_{\text{max}} 262.5 (4.92), 292 (4.66), 363 (4.05), 382 (4.12) \text{ nm}\]
(Bhatt et al., 1982)

$\text{C}_{20}\text{H}_{18}\text{O}_2$ (392) 15,16-Dihydro-11-isopropoxycyclopenta[a]phenanthren-17-one
[83053-59-2]
MW 290; mp 172–173°C
\[\lambda_{\text{max}} 261 (4.78), 292 (4.41), 364 (4.01), 384 (4.05) \text{ nm}\]
(Bhatt et al., 1982)

$\text{C}_{20}\text{H}_{18}\text{O}_2$ (408) 17-(16,17-Dihydro-15$H$-cyclopenta[a]phenanthyl)-2-propionic acid
Compilation of data from the literature

MW 290; mp 235-240°C
$\lambda_{\text{max}}$ 256 nm

(C Dannenberg, 1950)

C$_{20}$H$_{18}$O$_2$

(409) 17-(16,17-Dihydro-15H-cyclopenta[a]phenanthyl)-3-propionic acid
MW 290; mp 187-190°C
$\lambda_{\text{max}}$ 215 (4.54), 259 (4.80), 280 (4.18), 288 (4.08), 300 (4.06), 320 (278), 327.5 (2.58), 335 (2.94), 342.5 (2.50), 350 (2.88) nm
$\nu_{\text{max}}$ 3.00, 3.80-3.85, 5.78, 13.20, 13.24 $\mu$m

(Dannenberg and Dannenberg-von Dressler, 1964)

C$_{20}$H$_{18}$O$_4$

(84) 11,12,13,14,16,17-Hexahydro-12-methoxyoxalyl-15H-cyclopenta[a]phenanthren-11-one
MW 322; mp 157-158°C

(Butenandt et al., 1946a)

C$_{20}$H$_{20}$

(36) 15,16-Dihydro-17-ethyl-17-methylcyclopenta[a]phenanthrene
MW 260; mp 88-89°C
$\nu_{\text{max}}$ (CS$_2$) 12.33, 13.39 $\mu$m

(Dannenberg et al., 1953)

C$_{20}$H$_{20}$

(467) 16,17-Dihydro-17-n-propyl-15H-cyclopenta[a]phenanthrene
MW 260; mp 88-89°C
$\nu_{\text{max}}$ (CS$_2$) 12.33, 13.39 $\mu$m

(C Dannenberg et al., 1953)

C$_{20}$H$_{20}$

(256) 16,17-Dihydro-12-isopropyl-15H-cyclopenta[a]phenanthrene
MW 260; mp 88-89°C

(Riegel et al., 1948)

C$_{20}$H$_{20}$

(53) 16,17-Dihydro-17-isopropyl-15H-cyclopenta[a]phenanthrene
MW 260; Thin plates, mp 97.6-98.4°C (Riegel et al., 1943); 85°, 93°C (Coombs, 1966b)
Picrate, mp 108-113°C

(Riegel et al., 1943)

C$_{20}$H$_{20}$

(69) 16,17-Dihydro-6,17,17-trimethyl-15H-cyclopenta[a]phenanthrene
MW 260; mp 96-97°C (Butenandt and Suranyi, 1942)
$\lambda_{\text{max}}$ 262 (4.82), 282 (4.18), 290 (4.06), 303.5 (4.10), 323 (2.76), 337 (2.87), 354 (2.80) nm (Dannenberg and Steidle, 1954)
$\nu_{\text{max}}$ (CS$_2$) 11.47, 12.22, 13.26 $\mu$m

(Dannenberg et al., 1953)

C$_{20}$H$_{20}$

(141) 16,17-Dihydro-11,12,17-trimethyl-15H-cyclopenta[a]phenanthrene
MW 260; mp 75-77°C
Physical and spectral properties

λ\textsubscript{max} 218 (4.33), 224.9 (4.26), 261 (4.71), 287 (3.98), 301 (3.97), 312 (4.01), 343 (3.17), 360 (3.07) nm
ν\textsubscript{max} 11.50, 12.12, 12.56, 13.30, 13.86\,\mu m  \quad (Coombs, 1966b)

C\textsubscript{20}H\textsubscript{20}O (74) 16,17-Dihydro-3,17-dimethyl-11-methoxy-15H-cyclopenta[a]-phenanthrene
MW 276; mp 83–84°C  \quad (Kon and Woolman, 1939)

C\textsubscript{20}H\textsubscript{20}O (162) 15,16-Dihydro-3-hydroxy-1,17,17-trimethylcyclopenta[a]-phenanthrene
MW 276; mp 142–144°C  \quad (Brown and Turner, 1971)

C\textsubscript{20}H\textsubscript{20}O (14) 15,16-Dihydro-17,17-dimethyl-3-methoxycyclopenta[a]phenanthrene
MW 276; mp 106–107°C  \quad (Cohen \textit{et al.}, 1935)

C\textsubscript{20}H\textsubscript{20}O (126) 16,17-Dihydro-11-hydroxy-17-isopropyl-15H-cyclopenta[a]-phenanthrene
MW 276; mp 162°C  \quad (Birch and Robinson, 1944)

C\textsubscript{20}H\textsubscript{20}O (251) 12-Isopropyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthrene-17-one
MW 276; mp 115–116°C  \quad (Riegel \textit{et al.}, 1948)

C\textsubscript{20}H\textsubscript{20}O (400) 16,17-Dihydro-cis-16,17-dihydroxy-17-isopropyl-15H-cyclopenta[a]phenanthrene
MW 292; mp 183°C

C\textsubscript{20}H\textsubscript{20}O (209) 17,17-Ethlenedioxy-11,12,13,14,15,16-hexahydro-12-methylcyclopenta[a]phenanthren-11-one
MW 308; mp 123–124°C

C\textsubscript{20}H\textsubscript{20}O (202) 17,17-Ethlenedioxy-11,12,13,14,15,16-hexahydro-3-methoxy-cyclopenta[a]phenanthren-11-one
MW 324; mp 114°C

C\textsubscript{20}H\textsubscript{20}O (213) 17,17-Ethlenedioxy-11,12,13,14,15,16-hexahydro-11-hydroxy-11-hydroxymethylcyclopenta[a]phenanthrene
MW 326; needles, mp 168–170.5°C

δ 3.85 (1H, d, J 11.5, H\textsubscript{A}CH\textsubscript{A}OH), 4.33 (1H, d, J 11.5, H\textsubscript{B}CH\textsubscript{B}OH), 3.96 (4H, s, OCH\textsubscript{2}CH\textsubscript{2}O)
Compilation of data from the literature

m/z 326.1511 (M⁺, 35%), 380 (M⁺-H₂O, 20%), 295 (M⁺-CH₃OH, 100%), 99 (acetal ion, 89%)  (Coombs et al., 1975)

C₂₀H₂₄O (164) 3-Hydroxy-11,12,13,14,15,16-hexahydro-1,17,17-trimethylocyclopenta[a]phenanthrene
MW 280; mp 158-160°C
λ_max 263, 283, 294, 307 nm
ν_max 3.13 μm
δ 0.90 (s, methyl), 1.10 (s, methyl), 2.88 (s, 1-methyl), 6.80-7.75 (4H, aromatic)  (Brown and Turner, 1971)

C₂₀H₂₄O (123) 17-Isopropyl-6,7,8,9,11,14,16,17-octahydro-15H-cyclopenta[a]phenanthren-11-one
MW 280; bp 193-195°C/0.08 mm  (Birch and Robinson, 1944)

C₂₀H₂₆O (13) 17,17-Dimethyl-3-methoxy-6,7,8,9,11,12,15,16-octahydrocyclopenta[a]phenanthrene
MW 282; mp 58-60°C  (Cohen et al., 1935)

C₂₀H₂₈O₆ (416) 16,17-Benzooctahydrocyclopenta[a]phenanthrene
MW 266; mp 331-332°C  (Nasipuri and Roy, 1961; Ruzicka and Goldberg, 1937)

C₂₁H₁₈O₂
(405) Ethyl 17-(cyclopenta[a]phenanthrylidene)-acetate
MW 302; mp 183-184°C
λ_max 215 (4.34), 274 (4.68), 285 (4.63), 299 (4.67), 314-324 (4.53), 355 (3.85), 373 (3.80) nm
ν_max (KBr) 5.88, 6.13, 8.5-8.7, 9.63, 9.74, 11.65, 12.12, 12.89, 13.28 μm  (Dannenberg et al., 1964)

MW 334; mp 189-191°C
λ_max 223 (4.29), 255 (4.76), 279 (4.13), 287.5 (4.06), 299.5 (4.11), 319 (2.58), 325.5 (2.49), 334 (2.59), 340 (2.35), 350 (2.23) nm
ν_max 5.90, 12.26, 13.25 μm  (Coombs and Hall, 1973)

C₂₁H₂₀O₄ cis-16,17-Diacetoxy-16,17-dihydro-15H-cyclopenta[a]phenanthrene [42122-93-0]
MW 344; mp 173-175°C
λ_max 249.5 (4.74), 256.5 (4.87), 278.5 (4.20), 286 (4.09), 298 (4.18), 319.5 (2.63), 327.5 (2.48), 333.5 (2.82), 343 (2.43), 350 (2.82) nm
ν_max 5.75, 12.24, 13.26 μm
δ H₂-15, 3.42 (J 16.5); H₆-15, 3.70 (J 16.7); H-16, 4.28 m; H-17, 6.42 d (J 6)  (Coombs and Hall, 1973)

C₂₁H₂₀ 1,17-Dimethyl-17-ethylocyclopenta[a]phenanthrene
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MW 272; mp 123–124°C, [α]"²⁺ −56.2 (alcohol)
λ_max 224 (4.70), 244 (4.40), 266 (4.64), 297.5 (4.01), 309 (4.32),
322 (4.23), 347 (2.91), 364 (2.63) nm
(Dannenberg and Neumann, 1961b)

C21H20O (212) 11-n-Butyl-15,16-dihydrocyclopenta[a]phenanthren-17-one
[63642-51-3]
MW 288; mp 113–114°C
λ_max 264.5 (4.90), 289.5 (4.45), 302 (4.32), 360 (3.41), 377 (3.46) nm
ν_max 5.90, 11.44, 12.72, 13.40, 14.08 μm
(Coombs et al., 1973b)

C21H20O2 (393) 11-n-Butoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one
[83053-60-5]
MW 304; mp 156–157°C
λ_max 261.5 (4.87), 292 (4.44), 363.5 (3.76), 382 (3.82) nm
(Bhatt et al., 1982)

C21H22O2 (410) 17-(16,17-Dihydro-15H-cyclopenta[a]phenanthryl)-3-butyric
acid
MW 304; mp 157–159°C
λ_max 257 (4.79) nm
(Dannenberg, 1950)

C21H22O4 (118) 1,2,3,4,5,6,7,11,12,13,16,17-Dodecahydro-15H-cyclopen-
ta[a]phenanthrene-6,7,11,12-tetracarboxylic-acid-6,7,11,12-dian-
ydride
MW 368; mp 249–251°C
λ_max 255.5 (4.28) nm
(Butz et al., 1940)

C21H22NO3 (351) 12-Diacetylamino-6,7,16,17-tetrahydro-15H-cyclopenta[a]-
phenanthrene
MW 319; mp 151°C
λ_max 211 (4.77), 273 (4.31), 284 (4.18), 301 (3.55) nm
ν_max 5.85, 13.03 μm
δ 2.3 (10 aliphatic), 7.15–7.35 (5 aromatic)
(Dannenberg et al., 1965)

C21H22O3P (228) 15,16-Dihydro-17-oxocyclopenta[a]phenanthren-11-ol diethyl-
phosphate [50835-59-7]
λ_max 262 (4.83), 288.5 (4.48), 302 (4.32), 340 (3.27), 357 (3.52),
375 (3.59) nm
ν_max 5.90, 7.9, 9.8 μm
(Coombs and Jaitly, 1971)

C21H22 (76) 15,16-Dihydro-4,17-dimethyl-17-ethylocyclopenta[a]phenanthrene
MW 274; mp 145–147°C
(Brown and Kupchan, 1962)

C21H22 (37) 16,17-Dihydro-15-isopropyl-4-methyl-15H-cyclopenta[a]-
phenanthrene [72814-88-1] [77327-08-3]
MW 272
(Laflamme and Hites, 1979)

C21H22O (127) 16,17-Dihydro-17-isopropyl-3-methoxy-15H-cyclopenta[a]-
phenanthrene
MW 290; mp 129°C
(Birch and Robinson, 1944)
Compilation of data from the literature

\( \text{C}_{21}\text{H}_{22}\text{O}_3 \)

(210) 17,17-Ethylenedioxy-12,12-dimethyl-11,12,13,14,15,16-hexahydrocyclopenta[a]phenanthren-11-one
MW 322; mp 167°C
\( \lambda_{\text{max}} \) 217 (4.35), 244 (4.14), 306 (3.59) nm
\( \nu_{\text{max}} \) 5.95 μm
(Coombs, 1966a)

\( \text{C}_{21}\text{H}_{22}\text{O}_4 \)

(203) 11,11:17,17-Bis(ethylenedioxy)-11,12,13,14,15,16-hexahydrocyclopenta[a]phenanthrene
MW 338; mp 170°C
(Coombs, 1966a)

\( \text{C}_{21}\text{H}_{26} \)

(125) 6,7,8,14,16,17-Hexahydro-17-isopropyl-11-methyl-15H-cyclopenta[a]phenanthrene
MW 278; Colourless oil, bp 190°C/0.1 mm
(Birch and Robinson, 1944)

\( \text{C}_{22}\text{H}_{18}\text{O}_6 \)

(466) 2,15-Diacetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one
MW 362
\( \lambda_{\text{max}} \) 267 nm
\( \nu_{\text{max}} \) 5.76, 5.81, 5.88 μm
(Coombs and Crawley, 1974)

\( \text{C}_{22}\text{H}_{20}\text{O}_3 \)

(464) 2,15-Diacetoxy-8,9-epoxy-11-methyl-8,9-secogona-1,3,5,7,9,11,13-heptaen-17-one
MW 378; mp 208–210°C
\( \lambda_{\text{max}} \) 267, 298sh, 356, 374 nm
\( \nu_{\text{max}} \) 5.68, 5.76, 5.83 μm
(Coombs and Crawley, 1974)

\( \text{C}_{22}\text{H}_{20}\text{O}_3 \)

(406) Ethyl 17-(cyclopenta[a]phenanthrylidene)-2-propionate
MW 316; mp 92–93°C
(Dannenberg, 1950)

\( \text{C}_{22}\text{H}_{20}\text{O}_3 \)

(419) 15,16-Dihydro-16-(3-oxopentyl)cyclopenta[a]phenanthren-17-one
MW 316; mp 165–166°C
(Buchta and Kraetzer, 1962)

\( \text{C}_{22}\text{H}_{20}\text{O}_3 \)

(403) Ethyl 17-(11-methoxycyclopenta[a]phenanthrylidene)-acetate
MW 332; mp 144°C
(Robinson and Slater, 1941)

\( \text{C}_{22}\text{H}_{20}\text{O}_4 \)

(446) 16,17-Dihydro-11-methyl-15\( H \)-cyclopenta[a]phenanthrene-cis-16,17-diacetate [42123-07-9]
MW 348; mp 192.5–193°C
\( \lambda_{\text{max}} \) 226 (4.15), 254.5 (4.88), 281 (4.11), 292 (4.06), 303.5 (4.14), 323.5 (2.81), 338.5 (2.97), 354 (2.98) nm
\( \nu_{\text{max}} \) 5.75, 7.97, 8.08, 12.23 μm
Physical and spectral properties

\[ C_{22}H_{22}O_4 \]
\[ \delta \text{ H-15, 3.35 (J 16.4); H-15, 3.64 (J 16.6); H-16, 5.69;} \]  
\[ \text{H-17, d 6.39 (J 6)} \]  
\[ (\text{Coombs and Hall, 1973}) \]

\[ 16,17-\text{Dihydro-11-methyl-15H-cyclopenta[a]phenanthrene-trans-16,17-diaceate [42123-08-0] [63780-58-5]} \]
\[ \text{MW 348; mp 156–157°C} \]
\[ \lambda_{\text{nm}} 224 (4.14), 254 (4.88), 280.5 (4.09), 292 (4.05), 304 (4.15), 323 (2.80), 338 (2.97), 354 (2.97) \text{ nm} \]
\[ \nu_{\text{nm}} 5.79, 7.95, 8.07 \mu m \]
\[ \delta \text{ H-15, 3.16 (J 17.4); HB-15, 3.90 (J 17.8); H-16, 5.58;} \]  
\[ \text{H-17, d 6.42 (J 4)} \]  
\[ (\text{Coombs and Hall, 1973}) \]

\[ (394) 15,16-\text{Dihydro-11-n-pentoxy-cyclopenta[a]phenanthrene-17-one [83053-61-6]} \]
\[ \text{MW 318; mp 138–138.5°C} \]
\[ \lambda_{\text{nm}} 262 (4.88), 293 (4.52), 363 (4.09), 382 (4.13) \text{ nm} \]
\[ (\text{Bhatt et al., 1982}) \]

\[ (214) 11-\text{Acetoxy-methyl-17,17-ethylenedioxy-13,14,15,16-tetrahydrocyclopenta[a]phenanthrene} \]
\[ \text{MW 350} \]
\[ \lambda_{\text{nm}} 237.5 (4.81), 303 (3.88), 315 (3.87), 336 (3.59) \text{ nm} \]
\[ (\text{Coombs et al., 1975}) \]

\[ (229) 15,16-\text{Dihydro-7-methyl-17-oxocyclopenta[a]phenanthren-11-ol diethylphosphate [30835-64-4]} \]
\[ \text{MW 397; Brown crystals, mp 140–142°C} \]
\[ \lambda_{\text{nm}} 266, 291, 305, 350, 368, 396 \text{ nm} \]
\[ \nu_{\text{nm}} 5.88, 7.82, 9.70 \mu m \]
\[ (\text{Coombs and Jaitly, 1971}) \]

\[ (39) 16,17-\text{Dihydro-17-isopentyl-15H-cyclopenta[a]phenanthrene [21549-34-8]} \]
\[ \text{MW 288; mp 115°C} \]
\[ (\text{Wilk and Taupp, 1969}) \]

\[ (204) 11,11:17,17-Bis(ethylenedioxy)-11,12,13,14,15,16-hexahydro-3-methoxy-cyclopenta[a]phenanthrene \]
\[ \text{MW 368; mp 174°C} \]
\[ (\text{Coombs, 1966a}) \]

\[ (445) 17-(6,7,8,9,11,12,13,14,16,17-decahydro-3-methoxy-15H-cyclopenta[a]phenanthryl)acetate \]
\[ \text{MW 342; bp 208–215°C/0.4 mm} \]
\[ (\text{Robinson and Slater, 1941}) \]

\[ (328) 15,16-\text{Dihydro-15-phenylcyclopenta[a]phenanthrene-17-one [50558-60-6]} \]
\[ \text{MW 308; White needles, mp 222°C} \]
\[ \nu_{\text{nm}} 5.87, 12.05 \mu m \]
\[ \delta 1.25–3.10 (13H, aromatic), 5.05 (q, CH), 6.46–7.57 \text{ [H-15 and H-16, 2q, J}_{15,16} \text{ (trans) 7.7, J}_{15,16} \text{ (cis) 2.6, J}_{16,16} 19.2}] \]
\[ (\text{Shotter et al., 1973}) \]
Compilation of data from the literature

\( \text{C}_{23}\text{H}_{20}\text{O}_4 \) (414) Methyl 15,16-dihydro-17-oxo-16-(3-oxobutyl)cyclopenta\[a\]-phenanthrene-16-carboxylate
MW 360; mp 170°C (Nasipuri and Roy, 1961)

\( \text{C}_{23}\text{H}_{20}\text{O}_4 \) (404) Ethyl 17-(3,11-dimethoxycyclopenta[a]phenanthrylidene)-acetate
MW 362; mp 192°C (Robinson and Slater, 1941)

\( \text{C}_{23}\text{H}_{20}\text{O}_3 \) (395) 15,16-Dihydro-11-n-hexoxycyclopenta[a]phenanthren-17-one
MW 332; mp 136-137°C
\( \lambda_{\text{max}} \) 246 (4.96), 279 (4.78), 303 (4.30), 349 (4.07) (Bhatt, unpublished data)

\( \text{C}_{24}\text{H}_{16}\text{NO}_3 \)
MW 365; mp 237-238°C
\( \lambda_{\text{max}} \) 229, 263, 285, 300, 338, 355, 373 nm (Coombs and Jaitly, 1971)

\( \text{C}_{23}\text{H}_{16} \) (420) 17-Benzylidendecyclopenta[a]phenanthrene [5830-62-6]
MW 292; Orange needles, mp 262-263°C
\( \lambda_{\text{max}} \) 249 (4.52), 328 (4.65), 384 (4.07) nm
\( \nu_{\text{max}} \) 12.05, 12.47, 13.12, 13.38, 14.44 nm (Coombs, 1966b)

\( \text{C}_{23}\text{H}_{16}\text{O}_3 \)
MW 320 (Mladenova-Olinova et al., 1970)

\( \text{C}_{23}\text{H}_{16} \)
MW 338; mp 176-177°C (Badger et al., 1952)

\( \text{C}_{23}\text{H}_{16}\text{O}_3 \)S
11-Hydroxy-17-oxo-cyclopenta[a]phenanthrene tosylate [54206-57-4] (Kawarura et al., 1974)

\( \text{C}_{23}\text{H}_{20} \) (423) 17-Benzyl-16,17-dihydro-15H-cyclopenta[a]phenanthrene [5830-61-5]
MW 260; mp 156-157°C
\( \lambda_{\text{max}} \) 216 (4.46), 260 (4.81), 281 (4.20), 289 (4.08), 301 (4.14), 320 (2.77), 336 (2.98), 352 (3.00) nm
\( \nu_{\text{max}} \) 12.10, 12.36, 12.96, 13.30, 13.48, 14.28 μm (Coombs, 1966b)

\( \text{C}_{23}\text{H}_{20}\text{O}_3 \)S
MW 388 (Coombs and Hall, 1973)

\( \text{C}_{24}\text{H}_{20}\text{O}_3 \)S
MW 388; Leaflets, mp 178-179°C
\( \lambda_{\text{max}} \) 220 (4.48), 259 (4.83), 279 (4.23), 288 (4.09), 300 (4.15) nm
Physical and spectral properties

\( \nu_{\text{max}} \) 11.40, 12.24, 12.80, 12.95, 14.00, 14.30, 14.68 \mu m  

(Coombs, 1966b)

C_29H_22O_4

(415) Methyl 15,16-dihydro-17-oxo-16-(3-oxopentyl)cyclopenta[a]phenanthrene-16-carboxylic acid
MW 374; mp 157°C  

(Coombs, 1966b)

C_27H_22O

(461) 1α,2β,15-Triacetoxy-1,2,15,16-tetrahydro-11-methylcyclopenta[a]phenanthren-17-one
MW 422; mp 170-171°C
\( \lambda_{\text{max}} \) 268 (4.64), 322 (4.05), 333 (4.07), 370 (3.50) nm
\( \nu_{\text{max}} \) 5.75, 5.85, 9.80 \mu m
H-1, 7.11 (J 1 2 2); H-2, 5.47 (J 1 2 2, J 2 3 6); H-3, 6.28 (J 2 3 5, J 3 4 9); H-4, 6.95 (J 3 4 9); H-6, 7.55 (J 6 7 9); H-7, 8.11 (J 6 7 9); H-12, 7.56; H-15, 6.70 (J 15,16 1.5, J 15,16 6); H-16, 3.28 (J 15,16 1.5, J 16,16 19); H-16, 2.62 (J 15,16 1.5, J 16,16 19); 11-CH_3, 2.93
(Coombs and Crawley, 1974)

C_25H_30

(208) 16-Benzylidene-15,16-dihydro-3-methoxycyclopenta[a]-phenanthren-17-one
MW 350; Yellow needles, mp 223-224°C
(Koebner and Robinson, 1941)

C_25H_22O_3

(207) 16-Benzylidene-11,12,13,14,15,16-hexahydro-3-methoxy-cyclopenta[a]phenanthren-17-one
MW 354
(Koebner and Robinson, 1941)

C_25H_22O_3S

(149) 16,17-Dihydro-17-hydroxy-11-methyl-15H-cyclopenta[a]-phenanthrene tosylate
MW 402
\( \nu_{\text{max}} \) 9.71, 9.90 \mu m
(Coombs and Hall, 1973)

C_26H_21N

(421) 17-p-Dimethylaminobenzylidene cyclopenta[a]phenanthrene
MW 347; Yellow crystals, mp 248-250°C
\( \lambda_{\text{max}} \) 371 (4.18), 434 (4.58) nm
\( \nu_{\text{max}} \) 12.15, 12.32, 12.58, 13.24, 13.50 \mu m
(Coombs, 1966b)

C_26H_30

(151) 17-Methyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene
MW 342; mp 138-139°C, [α]_D^20 = -75° (CHCl_3)
\( \lambda_{\text{max}} \) 221 (4.83), 241.5 (4.56), 265 (4.66), 294.5 (4.05), 306.5 (4.18), 319.5 (4.18), 345 (3.07), 362 (2.98) nm
\( \nu_{\text{max}} \) (KBr) 12.18, 12.63, 13.24 \mu m
δ 0.59 (d, J 6.5), 0.88 (d, J 6.0), 1.38 (s), 6.4-8.8 (m)
(Dannenberg et al., 1964)

C_26H_32

(27) 15,16-Dihydro-15-methyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]-phenanthrene [80382-29-2]
MW 344
δ 0.74 (3H,d, J 6.5), 0.88 (6H,d, J 6.5), 1.33 (3H,s), 2.29 (2H,m), 3.23 (2H,m), 7.43-8.58 (8H,m)
Compilation of data from the literature

m/z 344 (M⁺, 4%), 231 (M⁺-C₇H₈, 100%), 216 (M⁺-C₇H₉-CH₃, 8%) (Ludwig et al., 1981)

C₂₇H₃₉ (170) 17-Methyl-17-[2(5,6-dimethylhepta-3-enyl]-cyclopenta[a]phenanthrene [13914-42-6]
MW 354; mp 131°C
λmax 222 (4.84), 242.5 (4.58), 267.5 (4.67), 295 (4.06), 307 (4.19), 320 (4.18), 346 (3.08) nm
νmax (KBr) 12.17, 12.61, 13.21 μm

C₂₇H₃₉ (171) 17-Methyl-17-[2(5,6-dimethylhepta-3-enyl]-cyclopenta[a]phenanthrene
MW 354; mp 131°C
λmax 222 (4.84), 242.5 (4.58), 267.5 (4.67), 295 (4.06), 307 (4.19), 320 (4.18), 346 (3.08) nm
νmax (KBr) 12.17, 12.61, 13.21 μm

C₂₇H₃₂ (152) 1,17-Dimethyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene
MW 356; mp 152°C, [a]F -57.8° (CHC{l}Cl)
λmax 224 (4.75), 237 (4.43), 244 (4.56), 272.5 (4.59), 280 (4.46), 298 (4.06), 310 (4.24), 323 (4.23), 347 (3.01), 364 (2.79) nm
νmax 12.14, 13.27 μm
(6.4-8.8 (aromatic and olefinic protons), 3.12 (1-methyl), 1.38 (17-methyl) (Dannenberg et al., 1964)

C₂₇H₃₄ (153) 15,16-Dihydro-1,17-dimethyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene [26231-18-5]
MW 358; mp 140°C, [a]F -22.4° (CHC{l}Cl)
λmax 213 (4.45), 226.5 (4.25), 257 (4.89), 282 (4.13), 293 (4.08), 305.5 (4.18), 337 (2.70), 353 (2.60), 364 (2.64) nm
νmax (CS₂) 12.19, 13.23, 13.96 μm (Dannenberg et al., 1964)
δ 0.73 (3H,d,J 6.5), 0.87 (6H,d,J 6.5), 1.34 (3H,s), 2.34 (2H,m), 3.17 (3H,s), 392. (2H,m), 7.50-8.72 (7H,m)
m/z 358 (M⁺, 4%), 245 (M⁺-C₇H₈, 100%), 230 (M⁺-C₇H₉-CH₃, 10%), 215 (M⁺-C₇H₈-2CH₃, 10%) (Ludwig et al., 1981)

C₂₇H₃₄O₂ (30) 15,16-Dihydro-4,17-dimethyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene [80382-27-0]
MW 358; mp 140°C, [a]F -11.8° (CHC{l}Cl)
λmax 216 (4.58), 255 (4.71), 263 (4.79), 283.5 (4.13), 294 (4.10), 306 (4.22), 322 (2.76), 337.5 (2.81), 354 (2.69) nm (Dannenberg and Neumann, 1961b)
δ 0.73 (3H,d,J 6.8), 0.87 (6H,d,J 6.5), 1.34 (3H,s), 2.31 (2H,m), 2.75 (3H,s), 3.29 (2H,m), 7.50-8.57 (7H,m)
m/z 358 (M⁺, 4%), 245 (M⁺-C₇H₈, 100%), 230 (M⁺-C₇H₉-CH₃, 10%), 215 (M⁺-C₇H₈-2CH₃, 8%) (Ludwig et al., 1981)

C₂₇H₃₄O₂ (33) 15,16-Dihydro-4,17-dimethyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene [80382-25-8]
MW 358; mp 140°C, [a]F -11.8° (CHC{l}Cl)
λmax 216 (4.58), 255 (4.71), 263 (4.79), 283.5 (4.13), 294 (4.10), 306 (4.22), 322 (2.76), 337.5 (2.81), 354 (2.69) nm (Dannenberg and Neumann, 1961b)
δ 0.73 (3H,d,J 6.8), 0.87 (6H,d,J 6.5), 1.34 (3H,s), 2.31 (2H,m), 2.75 (3H,s), 3.29 (2H,m), 7.50-8.57 (7H,m)
m/z 358 (M⁺, 4%), 245 (M⁺-C₇H₈, 100%), 230 (M⁺-C₇H₉-CH₃, 10%), 215 (M⁺-C₇H₈-2CH₃, 8%) (Ludwig et al., 1981)

C₂₇H₃₄O₂ (15,16-Dihydro-15,16-dihydoxy-4,17-dimethyl-17-[2(6-methyl)-heptyl]-cyclopenta[a]phenanthrene
MW 390; mp 87°C
λmax 216 (4.53), 229 (4.23), 253 (4.72), 261 (4.80), 282 (4.16),
Physical and spectral properties

292.5 (4.13), 305 (4.23), 320 (2.76), 336 (2.74), 352 (2.42) nm

\( \text{v}_{\text{max}} \) (KBr) 2.96, 9.40 μm (Dannenberg and Neumann, 1961a)

C<sub>30</sub>H<sub>39</sub> (32) 15,16-Dihydro-1,17-dimethyl-17-[2-(5-ethyl-6-methyl)-heptyl]cyclopenta[a]phenanthrene [80382-28-1]

MW 386

\[ \delta 0.75 (\text{3H}, \text{d}, J 6.8), 0.84 (\text{6H}, \text{d}, J 6.8), 0.86 (\text{3H}, \text{t}, J 7.6), \]

1.35 (3H,s), 2.31 (2H,m), 3.13 (3H,s), 3.28 (2H,m), 7.44–8.78 (7H,m)

\( m/z \) 386 (M+, 3%), 245 (M+-C<sub>10</sub>H<sub>21</sub>, 100%), 230 (M+-C<sub>10</sub>H<sub>21</sub>-CH<sub>3</sub>, 7%), 215 (M+-C<sub>10</sub>H<sub>21</sub>-2CH<sub>3</sub>, 7%) (Ludwig et al., 1981)

(35) 15,16-Dihydro-4,17-dimethyl-17-[2-(5-ethyl-6-methyl)heptyl]cyclopenta[a]phenanthrene

MW 386

\[ \delta 0.75 (\text{3H}, \text{d}, J 6.5), 0.86 (\text{3H}, \text{t}, J 6.5), 0.87 (\text{6H}, \text{d}, J 6.5), \]

1.33 (3H,s), 2.72 (3H,s), 3.30 (2H,m), 7.30–8.25 (7H,m)

\( m/z \) 386 (M+, 4%), 245 (M+-C<sub>10</sub>H<sub>21</sub>, 100%), 230 (M+-C<sub>10</sub>H<sub>21</sub>-CH<sub>3</sub>, 7%), 215 (M+-C<sub>10</sub>H<sub>21</sub>-2CH<sub>3</sub>, 7%) (Ludwig et al., 1981)

C<sub>15</sub>H<sub>30</sub>O<sub>1</sub>

15,16-Diacetoxy-15,16-dihydro-4,17-dimethyl-17-[2-(6-methyl)-heptyl]cyclopenta[a]phenanthrene

MW 474; \([\alpha]^{D}+29^o \) (ethanol)

\( \lambda_{\text{max}} \) 216 (4.58), 221 (4.40), 253 (4.73), 261 (4.80), 282.5 (4.20), 293 (4.17), 305.5 (4.25), 320 (2.94), 336 (2.93), 352 (2.83) nm

\( v_{\text{max}} \) 5.76, 8.10, 9.5, 12.17, 12.55, 13.10 μm

(Dannenberg and Neumann, 1961a)

C<sub>36</sub>H<sub>32</sub>O<sub>2</sub>

(217) Bis(15,16-dihydro-17-oxocyclopenta[a]phenanther-12-ylmethyl) ether [5837-22-0]

MW 506; mp 229–233°C

\( \lambda_{\text{max}} \) 219, 267.5, 286, 298, 338, 353, 371 nm

\( v_{\text{max}} \) 5.92, 12.32, 13.36, 13.68, 14.50 μm

(Coombs, 1966a)

C<sub>30</sub>H<sub>28</sub> (129) 16-[(16,17-Dihydro-17-methyl-15H-cyclopenta[a]-phenanthryl)]-17-methyl-15H-cyclopenta[a]phenanthrene

\( \lambda_{\text{max}} \) 261.5 (4.76), 273 (4.80), 283.5 (4.92), 300.5 (4.44), 324 (4.13) nm

\( v_{\text{max}} \) 11.60, 12.32, 13.40, 13.88 μm

(Coombs, 1966b)

C<sub>39</sub>H<sub>38</sub>O<sub>2</sub>

(428) 16,16'-Di(17-oxo-3,11-dimethoxycyclopenta[a]phenanthryl)-methine

MW 594; mp 301–302°C (Robinson and Rydon, 1939)

5.4 Index relating serial numbers assigned to cyclopenta[a]-phenanthenes appearing in the text, tables, and figures to their molecular formulae

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5.5 References


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References

Physical and spectral properties


References


Carcinogenicity and mutagenicity of cyclopenta[a]phenanthrenes

6.1 Structure/carcinogenic activity relationships

As has been discussed in Chapter 1, simple cyclopenta[a]-phenanthrene hydrocarbons became of interest during the establishment of the correct structure of the sterols in the 1930s. This coincided with the isolation of benzo[a]pyrene from coal tar in 1933, and the intense interest in carcinogenic polycyclic hydrocarbons which followed soon showed that their structures were based mainly upon the phenanthrene ring system extended with one or more fused aromatic ring(s). Diels' hydrocarbon (16,17-dihydro-17-methyl-15H-cyclopenta[a]phenanthrene,7) was shown to be inactive as a carcinogen (Craciun et al., 1939; Butenandt and Suranyi, 1942), and this reinforced the idea that additional fused benzo rings, as for example in the benz[a]anthracenes, were essential for carcinogenic activity. However, it also became apparent that correct methyl substitution can have dramatic effects on activity; thus whilst benz[a]anthracene is essentially inactive, its 7,12-dimethyl derivative is one of the most potent carcinogens known. This prompted Butenandt to synthesize the aryl methyl isomers of Diels' hydrocarbon and to test them, together with six dimethyl and trimethyl homologues, for carcinogenicity in the mouse (Butenandt and Dannenberg, 1953) both by skin painting and injection experiments. For the former, mice of the B1.H1. strain of known cancer incidence (Kaufmann et al., 1942) as well as mice of mixed strain from a commercial source were employed. The animals received two drops of a 0.4% solution of the test compound in benzene twice weekly on their dorsal skin, and the experiments lasted from 275 to 714 days with the results shown in Table 8. Only compounds bearing methyl groups at C-7 or C-11 were carcinogens; the 7-methyl (94) and 11-methyl (83) isomers were of similar activity with tumour incidences of 14.3 and 15.4%, respectively, whereas the 11,12-dimethyl hydrocarbon
Table 8. Skin tumour induction in mice with methyl homologues of 16,17-dihydro-15H-cyclopenta[a]phenanthrene

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. and (strain of mice used)</th>
<th>Tumours at site of application</th>
<th>No. of mice with tumour/Total no. of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted (1)</td>
<td>22 (Bl.HI.)</td>
<td>0 papillomas 0 carcinomas</td>
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</tr>
<tr>
<td>1-methyl (107)</td>
<td>21 (mixed)</td>
<td>0</td>
<td>—</td>
</tr>
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<td>2-methyl (98)</td>
<td>18 (Bl.HI.)</td>
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</tr>
<tr>
<td>2-methyl (98)</td>
<td>10 (mixed)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>4-methyl (100)</td>
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</tr>
<tr>
<td>6-methyl (67)</td>
<td>10 (Bl.HI.)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>7-methyl (94)</td>
<td>35 (mixed)</td>
<td>1 papillomas 4 carcinomas</td>
<td>5/35</td>
</tr>
<tr>
<td>11-methyl (83)</td>
<td>20 (Bl.HI.)</td>
<td>1 papillomas 1 carcinomas</td>
<td>2/13</td>
</tr>
<tr>
<td>12-methyl (97)</td>
<td>13 (Bl.HI.)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>17-methyl (7)</td>
<td>25 (Bl.HI.)</td>
<td>0</td>
<td>—</td>
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<tr>
<td>(Diels' hydrocarbon)</td>
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</tr>
<tr>
<td>4,12-dimethyl (101)</td>
<td>10 (mixed)</td>
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<td>—</td>
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<td>—</td>
</tr>
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<td>2,12-dimethyl (99)</td>
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<td>—</td>
</tr>
<tr>
<td>6,7-dimethyl (105)</td>
<td>14 (mixed)</td>
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<td>—</td>
</tr>
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<td>6,7-dimethyl (105)</td>
<td>10 (mixed)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>11,12-dimethyl (87)</td>
<td>10 (Bl.HI.)</td>
<td>0 papillomas 1 carcinomas</td>
<td>1/10</td>
</tr>
<tr>
<td>6,17,17-trimethyl (69)</td>
<td>11 (Bl.HI.)</td>
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Table 9. Skin-painting and injection experiments with unsaturated D-ring cyclopenta[a]phenanthrenes

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. and (strain of mice used)</th>
<th>Route of application</th>
<th>Tumours at the site of application</th>
<th>No. of mice with tumour</th>
</tr>
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<tr>
<td></td>
<td></td>
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<td>papillomas</td>
<td>carcinomas</td>
</tr>
<tr>
<td>17-methyl-15H- (133)</td>
<td>15 (Swiss)</td>
<td>topical</td>
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<td>1</td>
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<td></td>
<td>10 (mixed)</td>
<td>topical</td>
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<td>6</td>
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<tr>
<td>15-methyl-17H- (128)</td>
<td>15 (Swiss)</td>
<td>topical</td>
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<td>8</td>
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<tr>
<td>17-isopropyl-15H- (388)</td>
<td>15 (Swiss)</td>
<td>topical</td>
<td>0</td>
<td>3</td>
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<tr>
<td>17-methyl-15H- (133)</td>
<td>10 (Swiss)</td>
<td>injection</td>
<td>3</td>
<td>spindle cell sarcomas</td>
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<tr>
<td></td>
<td>10 (mixed)</td>
<td>injection</td>
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<td></td>
</tr>
<tr>
<td>15-methyl-17H- (128)</td>
<td>10 (Swiss)</td>
<td>injection</td>
<td>1</td>
<td>carcinoma</td>
</tr>
<tr>
<td>17-isopropyl-15H- (388)</td>
<td>10 (Swiss)</td>
<td>injection</td>
<td>0</td>
<td></td>
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</table>

a, mean latent period, 73 weeks; b, mean latent period, 83 weeks.
(87) was less active (tumour incidence 10%). No latent periods were
given and the use of two different strains of mice complicates interpreta-
tion, but it is evident that these hydrocarbons are only weakly carcino-
genic. This was supported by the observation that no local tumours were
induced by injection of these compounds into Bl.Hl. mice (5 mg in oil).

Three cyclopenta[a]phenanthrenes containing a conjugated double
bond in ring-D, namely 17-methyl-15H- (133), 17-isopropyl-15H- (388),
and 15-methyl-17H-cyclopenta[a]phenanthrene (128) (Table 9), were
also tested for carcinogenicity in mice of mixed strain and Swiss mice with
low spontaneous tumour incidence (Dannenberg, 1960) by the methods
already described. Introduction of a conjugated D-ring double bond was
accompanied by the appearance of weak carcinogenicity; all three com-
pounds were active by topical application, although their activity was low
as judged by their long mean latent periods. The least active was the
isopropyl hydrocarbon, and this failed to induce tumours on injection.

Quinone dehydrogenation of sterols (Dannenberg et al., 1956) leads to
this type of hydrocarbon, and Dannenberg felt that their carcinogenicity
might be connected with the possibility of the reactive D-ring double
bond behaving as a 'K-region'. Thus two independent structural factors
give rise to carcinogenicity in the cyclopenta[a]phenanthrene series,
namely methyl substitution at C-7 or C-11 and extension of the conjuga-
tion of the phenanthrene ring system into the D-ring. However, it was not
established whether these two influences were additive.

A chance came to test this using a series of 17-ketones which were
originally synthesized for another purpose (see below). As shown in Fig.
21 these were converted by means of the Grignard reaction into the
corresponding 17-methyl-15H-cyclopenta[a]phenanthrenes which were
further reduced catalytically to their 16,17-dihydro derivatives. The 12
compounds thus obtained, together with three related hydrocarbons
(Fig. 53), were tested for carcinogenicity (Coombs and Croft, 1966,
1969). A standardized method was set up employing Swiss mice of the
T.O. (Theiler's Original) strain outbred within a closed colony in the
laboratories of the Imperial Cancer Research Fund for many years. They
were housed 10 to a cage on sawdust bedding and had free access to a
standard solid laboratory diet and water at all times. At the beginning of
the experiment they were three months old, and they were used in
formally randomized groups of 20 (10 male and 10 female) for each
compound. One drop of a toluene solution (0.5% w/v) of the compound
was applied to the shaved dorsal skin (1 drop = 6 μL = 30 μg = 120 nmol)
twice weekly for one year. The mice were observed for a second year and
experiments were terminated at two years (~730 days). Survival was
Carcinogenicity and mutagenicity

excellent; more than 40% of the animals were still alive at this time in the untreated and solvent control groups, and 90% survived to 18 months. No skin tumours were seen on any mice painted with toluene alone and their survival was the same as those of the untreated group. The date of appearance, position, and size of all skin tumours were recorded both when the animals were painted during the first year, and once a week during the second year. The times of appearance of the first skin tumours were used to calculate mean latent periods; at this time the tumour was less than 1 mm in diameter, but it was scored only if it persisted and grew in size. When the tumour exceeded 1 cm in diameter the animal was killed and autopsied, as were all the mice remaining at the end of the experiment. Occasionally seriously sick animals were killed and autopsied to avoid loss of the tumour material. All skin tumours and surrounding tissue were examined by routine histology and classified as papillomas unless there was evidence of penetration of the panniculus carnosus muscle, when they were classed as carcinomas.

Table 10 summarizes the results of this experiment. The last column lists $I_{ball}$ indices calculated for these compounds from the observed mean latent periods and tumour incidences as follows:

$$I_{ball} = \frac{\text{percentage of animals with tumour}^*}{\text{mean latent period in days}} \times 100$$

* based on the number of animals alive at the time of the appearance of the first skin tumour in that group

Fig. 53. Cyclopenta[a]phenanthrenes tested for carcinogenicity by skin painting (Coombs and Croft, 1969).
Table 10. Skin tumour induction with some cyclopenta[a]phenanthrene hydrocarbons and methoxy analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with skin tumours at site of application</th>
<th>Percentage tumour incidence</th>
<th>Ibail index</th>
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<tr>
<td></td>
<td>papillomas</td>
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<td></td>
</tr>
<tr>
<td>16,17-dihydro-15H-cyclopenta[a]phenanthrenes</td>
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<td></td>
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<tr>
<td>17-methyl (Diels' hydrocarbon 7)</td>
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<td>11,12,17-trimethyl (141)</td>
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<td>30</td>
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<td>4</td>
<td>25</td>
</tr>
<tr>
<td>17H-cyclopenta[a]phenanthrene (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17-methylene-15,16-dihydro (143)</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>17-isopropylidenecyclopenta[a]phenanthrene (422)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
For this index 'tumours' include both papillomas and carcinomas at the site of application, and the latent period is the time elapsed between the beginning of the treatment and the first appearance of the first skin tumour on each mouse. The mean latent period is the average latent period for all the animals in that group. This index is a better measure of carcinogenicity than tumour incidence alone because it also takes latent periods into account, although, of course, it does not include other important parameters such as the total number of tumours per animal, the carcinoma/papilloma ratio, or the rate of tumour growth. However, it has the merit of allowing comparisons to be made readily, and is probably a valid procedure for a series of closely related compounds such as the one at present under discussion because these compounds all have very similar physical properties, especially solubility.

Substitution of a methyl group at C-17 in 16,17-dihydro-15H-cyclopenta[a]phenanthrene does not lead to activity because Diels' hydrocarbon (7) fails to induce skin tumours, as others have found. In agreement with Butenandt and Dannenberg, introduction of a methyl group at C-11 into Diels' hydrocarbon leads to weak carcinogenicity (compounds 139 and 141) whereas introduction of this group at C-12 does not (compound 140). A methoxy group at the biologically important C-3 position is ineffective, but again it is weakly activating at C-11. Also in agreement with the latter author (Dannenberg, 1960), introduction of a 16(17)-double bond into Diels' hydrocarbon to yield 17-methyl-15H-cyclopenta[a]phenanthrene (133) is accompanied by the appearance of weak activity; the tumour incidence of this compound reached 30%, but its mean latent period was very long resulting in a low Iball index. The isomeric 17-methylene hydrocarbon (143) was of similar activity, but as it is known to isomerize readily to the 16(17)-isomer (133) the meaning of this is unclear. 17H-Cyclopenta[a]phenanthrene (2) lacking a D-ring methyl group was inactive as was the isopropylidene hydrocarbon (422). However, introduction of a methyl group at C-11 in these unsaturated ring-D compounds dramatically enhanced activity; thus 11,17-dimethyl-15H-cyclopenta[a]phenanthrene (135) and its 11,12,17-trimethyl homologue (137) were moderately strong carcinogens. It is interesting that in this series a methoxy group at C-11 has only a marginally activating effect, while at C-3 it appears to cause deactivation. Thus it is clear that ring-D unsaturation and 11-methyl group substitution are additive vis-à-vis induction of carcinogenicity in this series.

The 17-ketones corresponding to these hydrocarbons were also tested in this experiment to determine whether conjugation by a carbonyl group at C-17 would have an enhancing effect similar to that shown by a 16(17)-
Structure/activity relationships

double bond. A carbonyl group at this position is, of course, a feature of many naturally occurring steroids. The results of this test are shown in Table 11. It is immediately obvious that conjugation of a carbonyl group has a considerable enhancing effect on carcinogenicity. In fact 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) was the most potent carcinogen to be tested, and was more active than the analogous ring-D unsaturated hydrocarbon (135). The 11,12-dimethyl and 11-methoxy ketone (131 and 132) were also strong carcinogens whilst the unsubstituted ketone (4) and its 12-methyl (130) and 3-methoxy (24) derivatives were devoid of activity. The 11-acetoxy-17-ketone (80) appeared to be marginally active, but the 11-phenol (206) was too insoluble to be tested in this way. The high activity of the 11-methyl-17-ketones was unexpected because introduction of oxygen substituents into polycyclic aromatic hydrocarbons often has the reverse effect. This series also differs from the 16(17)-ene series in that the parent 17-ketone (4) is inactive, as are its 12-methyl and 3-methoxy derivatives, whilst the 11-methoxy derivative is considerably more carcinogenic than the corresponding hydrocarbon derivative (138).

The high activity of the 11-methyl-17-ketone (26) prompted a much more thorough study of the structure/carcinogenicity relationships in this series of 17-ketones. All the aryl methyl isomers were synthesized and tested, as were a range of related compounds listed in Table 12 which shows the results of testing these compounds by the standard method (Coombs et al., 1973, 1979; Hadfield et al., 1984; Kashino et al., 1986). The 11-methyl-17-ketone (26), used as a positive control, was again shown to be a potent carcinogen, whereas the 2-, 3-, 4-, and 6-methyl

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with skin tumours at site of application</th>
<th>Percentage tumour incidence</th>
<th>Ibull index</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted</td>
<td>0 0 0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>11-methyl</td>
<td>0 0 0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>12-methyl</td>
<td>0 0 0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>11,12-dimethyl</td>
<td>2 11 65</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3-methoxy</td>
<td>0 0 0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>11-methoxy</td>
<td>2 9 55</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>11-acetoxy</td>
<td>1 1 10</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Table 11. Skin tumour induction with some 15,16-dihydrocyclopenta[a]phenanthren-17-ones
Table 12. Skin tumour induction with some further derivatives and analogues of 15,16-dihydrocyclopenta[a]phenanthren-17-one by repeated application

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with skin tumour(s) at the site of application</th>
<th>Percentage tumour incidence</th>
<th>In all index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>papillomas</td>
<td>carcinomas</td>
<td></td>
</tr>
<tr>
<td>1-methyl-17-ketone (302)</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>2-methyl-17-ketone (303)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3-methyl-17-ketone (304)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4-methyl-17-ketone (305)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6-methyl-17-ketone (306)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7-methyl-17-ketone (230)</td>
<td>0</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>0</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>11-ethyl-17-ketone (211)</td>
<td>3</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>11-n-butyl-17-ketone (212)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,11-methano-17-ketone (310)</td>
<td>6</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>7,11-dimethyl-17-ketone (309)</td>
<td>2</td>
<td>11</td>
<td>65</td>
</tr>
<tr>
<td>6-methoxy-11-methyl-17-ketone (468)</td>
<td>2</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>11-methoxy-7-methyl-17-ketone (447)</td>
<td>0</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>11,12-dihydro-11-methyl-17-ketone (252)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydro-11-methyl-17-ketone (238)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>unsubstituted-15-ketone (102)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-methyl-15-ketone (334)</td>
<td>3</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydrochrysene-1-one (449)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydro-11-methylchrysene-1-one (450)</td>
<td>1</td>
<td>16</td>
<td>85</td>
</tr>
<tr>
<td>11-methyl-17-ol (448)</td>
<td>2</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

All compounds were applied as before at a dose of 120 nmol twice weekly for one year with observation for a second year except: a, the dose was 200 nmol twice weekly for one year; and b, the dose was 200 nmol twice weekly for 10 weeks only. Observation was continued for up to two years in both cases as usual.
isomers were inactive. The 1-methyl-17-ketone (302) appeared to possess marginal carcinogenic activity because one mouse developed a papilloma at the site of application; this has never been observed with mice in control groups treated repeatedly with the vehicle (toluene) alone. The 7-methyl-17-ketone (230) was moderately active, so that these structure/activity relationships resemble those found by Butenandt and Dannenberg for the corresponding hydrocarbons, with the exception that in the ketone series the 11-methyl compound is much more active than its 7-methyl isomer. The 11-ethyl-17-ketone (211) was much less carcinogenic than its methyl homologue, and the 11-n-butyl-17-ketone (212) was inactive. This order of decreasing carcinogenicity with increasing carbon chain length in a bay-region substituent has been observed in several other series of polycyclic aromatic hydrocarbons. As anticipated, the 7,11-dimethyl-17-ketone (309) proved to be a powerful carcinogen, probably more active than its 11-methyl homologue. Unfortunately, owing to shortage of material repeated applications of this dimethyl ketone were continued for only 10 weeks instead of the 50 weeks in the standard regime, but this, nevertheless, resulted in 30 skin tumours, a third of which were malignant, in 13/20 mice with a short mean latent period of 19 weeks. There is little doubt that both the number of tumours and the tumour incidence would have been higher if topical applications had been continued as usual for one year. Introduction of a 6-methoxy group into the 11-methyl ketone to give compound (468) considerably diminished its activity, whereas introduction of an 11-methoxy group into the 7-methyl-17-ketone to give compound (447) led to an increase in activity, although not to the extent that might have been predicted. Interruption of conjugation in the phenanthrene ring system abolished carcinogenicity; thus neither the 11,12-dihydro-11-methyl-17-ketone (252) nor the 1,2,3,4-tetrahydro derivative (238) were active. It is, therefore, probable that an intact phenanthrene system is the smallest polycyclic aromatic ring system that can give rise to carcinogenicity upon correct substitution. Transposition of the 17-carbonyl group to C-15 (compound 334) markedly reduced activity; in fact this ketone was a weak carcinogen, like the corresponding saturated D-ring hydrocarbon (139). This is surprising because Dannenberg (1960) demonstrated that in the unsaturated D-ring hydrocarbon series both 15-methyl-17H- and 17-methyl-15H-cyclopenta[a]phenanthen-17-one (310) is moderately carcinogenic, although it...
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does not possess a bay-region. Expansion of the five-membered ring by one carbon atom to yield 11-methyl-1,2,3,4-tetrahydrochrysen-1-one (450) retained activity unchanged; thus the Iball indices of this ketone and of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) were essentially the same. The unsubstituted 1,2,3,4-tetrahydrochrysen-1-one (449) was inactive.

The unexpectedly high activity of this chrysenone (450) was further investigated by converting it into a series of hydrocarbons including 1,11-dimethylchrysen (453) by means of the Grignard reaction followed by dehydration, dehydrogenation, or reduction as outlined in Fig. 54. These compounds were then tested for carcinogenicity by the standard procedure (Coombs et al., 1974) with the results shown in Table 13. The ketone (450) was by far the most active, and the least active was the tetrahydrohydrocarbon (452); the Iball indices of both were similar to those of the analogous cyclopenta[a]phenanthrenes. However, the 3,4-dihydro hydrocarbon (451) was distinctly less carcinogenic than the corresponding 11,17-dimethyl-15//-cyclopenta[a]phenanthrene (135), and in fact it was the fully aromatic 1,11-dimethylchrysen (453) that possessed activity similar to that of (135) (see Table 10). This tetrahydro ketone (450) is therefore markedly more active than the corresponding aromatic hydrocarbon containing four condensed benzene rings.

The tests so far described all involved repeated application of the test compound and therefore each test with 20 mice required about 100 mg of sample. While this was usually readily possible with synthetic compounds, testing of metabolites, obtained by in vitro metabolism and separation by high-pressure liquid chromatography, posed a problem in this respect. Tests by repeated application measure 'complete carcinogenicity', i.e., the sum total of the initiation and promotion caused by the test compound, but it is possible to test for initiation alone using a two-stage initiation–promotion regime. With an initiating dose of 1.6μmol
Table 13. *Skin tumours induced in mice by repeated application of some 11-methylchrysene derivatives*

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with skin tumour(s) at site of application</th>
<th>Iball index</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-methyl-1,2,3,4-tetrahydrochrysen-1-one (450)</td>
<td>17/20</td>
<td>45</td>
</tr>
<tr>
<td>1,11-dimethylchrysene (453)</td>
<td>15/19</td>
<td>22</td>
</tr>
<tr>
<td>1,11-dimethyl-3,4-dihydrochrysene (451)</td>
<td>8/20</td>
<td>10</td>
</tr>
<tr>
<td>1,11-dimethyl-1,2,3,4-tetrahydrochrysene (452)</td>
<td>3/18</td>
<td>4.5</td>
</tr>
</tbody>
</table>
Carcinogenicity and mutagenicity

(400 μg) applied once, followed by twice-weekly promotion of the treated area of skin by the application of 10 μL of a 1% v/v solution of croton oil in toluene, it was established that the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) produced a tumour incidence and mean latent period similar to those produced by the standard repeated-application method. However, the two-stage method used less than one-tenth of the amount of sample required by the latter, and proved to be useful for studies with metabolites. It was also employed to determine the initiating activity of a range of synthetic compounds (Coombs and Bhatt, 1978; Coombs et al., 1979; Bhatt et al., 1982; Hadfield et al., 1984; Kashino et al., 1986) shown in Table 14.

As was to be expected, the 11-methyl-17-ketone (26) and its 7,11-dimethyl homologue (309) proved to be potent tumour initiators whilst the parent unsubstituted ketone (4) and its 3-methyl derivative (304) were inactive. In line with the results from repeated application of these compounds, the dimethyl compound appeared to be the more active as shown by its shorter mean latent period, and consequently its higher Iball index. Of course, these indices cannot be compared directly with those derived from repeated-application experiments, but they serve the same purpose of providing a convenient guide to the relative initiating potential of a series of closely related compounds. Predictably the 11-methylchrysenone (450) was also a very active tumour initiator whereas the unsubstituted chrysenone (449) was inactive. The 1,11-methano-17-ketone (310) was a moderately active initiator, and again the 1-methyl-17-ketone (302) appeared to be marginally active. However, occasionally a papilloma is induced in uninitiated control mice painted with croton oil, so that the meaning of a low Iball index in this system is questionable. Among the 11-alkoxy-17-ketones the methoxy compound (132) was the most active, followed by the 11-ethoxy-17-ketone (390) which was somewhat less so, whilst the n-pentoxy, and n-hexoxy derivatives were not tumour initiators. Unexpectedly, both the 11-isopropoxy and 11-n-butoxy-17-ketones appeared to be very weak tumour initiators, but again these low indices are questionable. The 15-methoxy-17-ketone (367) was not an initiator, but its 11-methyl derivative (368) was active. In this Table the three hydroxy derivatives and the parent 11-methyl-17-ketone used as a positive control are placed together separately. The reason for this is that they were tested together in a separate experiment (Coombs and Bhatt, 1982) in which for solubility reasons acetone–toluene (1:1 v/v) was employed in place of toluene to administer the initiating dose of these compounds. This led to the unexpected result of effectively increasing the initiating activity of this carcinogen, as can be seen from the increased
Table 14. Initiating activity of some 15,16-dihydrocyclopenta[a]phenanthren-17-ones in the two-stage regime using croton oil as a tumour promoter

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with skin tumours at site of application</th>
<th>Percentage tumour incidence</th>
<th>Ibail index&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>papillomas</td>
<td>carcinomas</td>
<td></td>
</tr>
<tr>
<td>unsubstituted-17-ketone (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-methyl-17-ketone (302)</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>3-methyl-17-ketone (304)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>14</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>1,11-methano-17-ketone (310)</td>
<td>7</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>7,11-dimethyl-17-ketone (309)</td>
<td>10</td>
<td>6</td>
<td>80</td>
</tr>
<tr>
<td>11-methoxy-17-ketone (132)</td>
<td>9</td>
<td>2</td>
<td>55</td>
</tr>
<tr>
<td>11-ethoxy-17-ketone (390)</td>
<td>6</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>11-n-propoxy-17-ketone (391)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-isopropoxy-17-ketone (392)</td>
<td>2</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>11-n-butoxy-17-ketone (393)</td>
<td>4</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>11-n-pentoxy-17-ketone (394)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-n-hexoxy-17-ketone (395)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-methoxy-17-ketone (367)</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>15-methoxy-11-methyl-17-ketone (368)</td>
<td>3</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydrochrysene-1-one (449)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydro-11-methylchrysene-1-one (450)</td>
<td>11</td>
<td>7</td>
<td>90</td>
</tr>
<tr>
<td>16-hydroxy-11-methyl-17-ketone (346)</td>
<td>14</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>11-hydroxymethyl-17-ketone (215)</td>
<td>7</td>
<td>2</td>
<td>45</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26) (positive control)</td>
<td>13</td>
<td>6</td>
<td>95</td>
</tr>
</tbody>
</table>

<sup>a</sup> These figures cannot, of course, be compared directly with Ibail indices derived from repeated-application experiments.

<sup>b</sup> Acetone-toluene (1:1 v/v) used to deliver the initiating dose.
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Iball index. However, within this small group it is clear that the 16-hydroxy derivative is almost as active as the original carcinogen whereas the 15-hydroxy and 11-hydroxymethyl derivatives are much less active. These hydroxy derivatives are of interest because they also occur as metabolites of this 11-methyl-17-ketone.

In summary it is clear that in the 17-ketone series carcinogenicity is favoured by small electron-releasing groups at C-11 and to a lesser degree at C-7, but not elsewhere in the molecule. On these grounds the 11-pheno(15,16-dihydro-11-hydroxycyclopenta[a]phenanthren-17-one, 206) was expected to be carcinogenic, but it was not possible to test it by topical application as usual because it was very sparingly soluble in most organic solvents. It was therefore finely ground and suspended in olive oil for subcutaneous injection into T.O. mice at 10 mg/animal. Subsequent promotion of the dorsal skin remote from the site of injection (shoulder) with 1% v/v croton oil in toluene then led, in two separate but identical experiments, to tumour incidences of 60 and 65% with mean latent periods of 25 and 30 weeks, respectively. Under these conditions of systemic initiation the 11-methyl-17-ketone used as a positive control was very active, giving a 95% dorsal skin tumour incidence with a short mean latent period of 21 weeks.

6.2 The carcinogenicity of 15,16-dihydro-11-methylcyclopenta[a]-phenanthren-17-one

From the foregoing it is apparent that this 11-methyl-17-ketone (26) is a potent carcinogen in T.O. mice. This was further studied in two dose/response studies and direct comparisons with the classical polycyclic aromatic hydrocarbon carcinogen benzo[a]pyrene. In the first (Coombs et al., 1979) this ketone was painted on to the dorsal skin twice weekly, using groups of 20 T.O. mice as usual, at doses of 50, 25, 10, and 5 μg per mouse. A further group was treated in the same way with benzo[a]pyrene (5 μg/mouse), and the last group received the 11-methyl-17-ketone at this dosage together with 15 μg of the aryl hydrocarbon hydroxylase inhibitor 7,8-benzo[flavone (Kinoshita and Gelboin, 1972). The results of this comparison are displayed in Table 15 and Fig. 55. Comparison of the 11-methyl-17-ketone and benzo[a]pyrene at the same twice-weekly dose (5 μg = 20 nmol) showed that these two compounds are of comparable potency as complete carcinogens on T.O. mouse skin. The figure also shows the curves formed by plotting the time of first appearance of the tumours, and the best line for points given by plotting mean latent periods (Lp) against the logarithms of the doses (d) using the well-known relationship
Table 15. Dose/response study with the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one by repeated application, and the effect of co-administration of the aryl hydrocarbon hydrolysis inhibitor 7,8-benzoflavone

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg twice weekly)</th>
<th>Percentage tumour incidence</th>
<th>Mean latent period ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-methyl-17-ketone</td>
<td>50</td>
<td>85</td>
<td>23.3 ± 8.1</td>
</tr>
<tr>
<td>11-methyl-17-ketone</td>
<td>25</td>
<td>90</td>
<td>28.4 ± 7.7</td>
</tr>
<tr>
<td>11-methyl-17-ketone</td>
<td>10</td>
<td>80</td>
<td>31.2 ± 6.8</td>
</tr>
<tr>
<td>11-methyl-17-ketone</td>
<td>5</td>
<td>45</td>
<td>34 ± 8.2</td>
</tr>
<tr>
<td>11-methyl-17-ketone plus 7,8-benzoflavone (15µg)</td>
<td>5</td>
<td>15</td>
<td>36.2 ± 8.6</td>
</tr>
<tr>
<td>benzo[a]pyrene</td>
<td>5</td>
<td>50</td>
<td>37.5 ± 11.2</td>
</tr>
</tbody>
</table>
Carcinogenicity and mutagenicity

\[ L_p = a - b (\log_{10} d + c) \]

(Bryan and Shimkin, 1941) where \(a\), \(b\), and \(c\) are constants. In this case the data fit the equation

\[ L_p = 29.40 - 10.803 (\log_{10} d - 1.199) \]

where \(L_p\) is in weeks and \(d\) in \(\mu\)g; the correlation coefficient is 0.96. Also shown are the tumours induced by this carcinogen administered together with 7,8-benzoflavone. As found previously (Coombs et al., 1975) this flavone markedly decreased the tumour incidence and increased the mean latent period. When the latter (36.1 weeks) was fitted to this line, it was found that this inhibitor effectively reduced the dose to about half of that administered.

In the second dose/response experiments (Coombs and Bhatt, 1982) single applications of the carcinogen (26) were made at doses of 1600, 400, and 200 nmol/mouse to groups of 20 animals which were then promoted with croton oil–toluene twice weekly as usual, with the results shown in Fig. 56. Even at the lowest dose (200 nmol = 50 \(\mu\)g) tumour incidence was 50%; at the highest dose (1600 nmol = 400 \(\mu\)g) both the tumour incidence and mean latent period were similar to those observed on repeated application of 100–200 nmol of this compound twice weekly.

Fig. 55. Induction of skin tumours in T.O. mice by twice-weekly application of benzo[a]pyrene (BaP) (+ + +), the 11-methyl-17-ketone (26) (o — o), and the relationship between mean latent period and dose for the latter. The solid circles (● — ●) represent tumours produced by this carcinogen when administered together with the aryl hydrocarbon hydroxylase inhibitor 7,8-benzoflavone (BF).
The 11-methyl-17-ketone

A very similar result was obtained in a previous experiment (Coombs et al., 1979) in which the 11-methyl-17-ketone (26) was again compared with benzo[a]pyrene in the two-stage system, with both compounds at a dose of 1600 nmol and subsequent twice-weekly promotion. The effects of the aryl hydrocarbon hydroxylase inhibitor 7,8-benzoflavone and the epoxide hydratase inhibitor 1,1,1-trichloropropene oxide (TCPO) on tumour induction by (26) under these conditions were also studied (Table 16). It is apparent that benzo[a]pyrene is somewhat less active as a tumour initiator than the cyclopenta[a]phenanthrene (26) in this system. Unexpectedly, co-administration of 7,8-benzoflavone together with the initiating dose of (26) had little effect on either tumour incidence or latent period, in marked contrast to its effect upon repeated twice-weekly applications already discussed. It seems probable that the reason for this may reside in the comparatively high initiating dose (1600 nmol) used, in comparison with the twice-weekly dose (20 nmol) employed before (see Fig. 55). In a preliminary experiment (Coombs et al., 1975) it was found that this inhibitor had less effect at a higher dose of (26) even though the same ratio of carcinogen:inhibitor was maintained. Appreciable shortening of the mean latent period was obtained when the epoxide hydratase inhibitor TCPO was given with the initiating dose of the

Fig. 56. Appearance of first skin tumours in T.O. mice (groups of 20) treated with a single dose of the carcinogen (as shown) followed by promotion with croton oil applied twice weekly.
Table 16. Comparison of tumour induction by 15,16-dihydro-11-methylcyclopenta[a]phenanthrene and benzo[a]pyrene in the two-stage system, and the effects of 7,8-benzo[flavone (BF) and 1,1,1-trichloropropene oxide (TCPO) on the former

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice with tumours at site of application</th>
<th>Percentage tumour incidence</th>
<th>Mean latent period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>papillomas</td>
<td>carcinomas</td>
<td></td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>14</td>
<td>4</td>
<td>90</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26) plus BF</td>
<td>12</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26) plus TCPO</td>
<td>17</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>benzo[a]pyrene</td>
<td>10</td>
<td>3</td>
<td>65</td>
</tr>
</tbody>
</table>
The 11-methyl-17-ketone

151
carcinogen (26). Similar effects have been observed with 3-methylcholanthrene (Berry et al., 1977; Burki et al., 1974) and benzo[a]pyrene (Berry et al., 1977). With the latter the inhibitor prevents hydration of the initial 7,8-oxide to the 7,8-diol (Selkirk et al., 1974), but the ultimate carcinogen, the 7,8-dihydroxy-9,10-epoxide is not a substrate for this enzyme (Wood et al., 1976). This suggests that the 11-methyl-17-ketone (26) may be activated in a similar manner, via its 3,4-hydroxy-1,2-epoxide.

This ketone (26) also behaved like benzo[a]pyrene when croton oil was used as a co-carcinogen. The latter is defined as 'all forms of augmentation of tumour induction, usually brought about by concurrent administrations of the carcinogen and the added factor' (Berenblum, 1969). As shown in Table 17, T.O. mice in the usual groups of 20 were treated topically twice weekly with 20 or 200 nmol of the 11-methyl-17-ketone (26) or 20 nmol of benzo[a]pyrene dissolved in either toluene or toluene-croton oil (99:1 v/v). At both dose levels of (26) 1% croton oil in the solvent markedly reduced the mean latent periods, the difference again being the larger with the lower dose of the carcinogen (26). Benzo[a]pyrene behaved in a similar manner, but again appeared to be rather less active than the cyclopenta[a]phenanthrene (26) on the basis of tumour incidence, although not on mean latent period (Vose et al., 1981).

In the work described so far skin tumours induced with the 11-methyl-17-ketone (26) at the site of application were studied after topical application to the dorsal skin. The first experiment (Table 11) was supported by a small injection experiment shown in Table 18, employing T.O. mice (Coombs and Croft, 1969). Of the three compounds tested, the unsubstituted 17-ketone (4) gave no tumours in line with its lack of activity after topical application. The 11-methyl-17-ketone (26) was the only compound to yield local sarcomas at the site of injection, although the dose required was high. Both this compound and its 11,12-dimethyl homologue (131) gave rise to both papillomas and carcinomas on the skin remote from the site of injection, mainly on the ventral surfaces, without apparent promotion. Ventral skin is thicker and contains considerably more fat than dorsal skin, and it seems probable that this may retard the loss of this lipophilic ketone, thereby allowing it to act over a prolonged period at this site. It is interesting that the more lipophilic benzo[a]pyrene [which dissolves in hexane – the cyclopenta[a]phenanthrenone (26) does not], like other polycyclic aromatic hydrocarbons, readily gives rise to local sarcomas after subcutaneous injection. It has already been mentioned, in connection with testing the 11-phenol (206), that the 11-methyl-17-ketone acts as a potent systemic initiator after subcutaneous
Table 17. Co-carcinogenicity experiments with benzo[a]pyrene or the 11-methyl-17-ketone and croton oil

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (nmol)</th>
<th>Solvent</th>
<th>Percentage of mice with skin tumours at the site of application</th>
<th>Mean latent period (weeks)</th>
<th>Difference in mean latent period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>20</td>
<td>toluene</td>
<td>70</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>20</td>
<td>toluene/croton oil</td>
<td>95</td>
<td>31.4</td>
<td>11.3</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>200</td>
<td>toluene</td>
<td>90</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>200</td>
<td>toluene/croton oil</td>
<td>100</td>
<td>21.4</td>
<td>4.9</td>
</tr>
<tr>
<td>benzo[a]pyrene</td>
<td>20</td>
<td>toluene</td>
<td>30</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>benzo[a]pyrene</td>
<td>20</td>
<td>toluene/croton oil</td>
<td>70</td>
<td>26.0</td>
<td>8.8</td>
</tr>
</tbody>
</table>
Table 18. *Subcutaneous injection experiment with 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) and its 11-methyl (26) and 11,12-dimethyl (131) derivatives*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose injected (mg)</th>
<th>No. of mice injected</th>
<th>No. of mice with local sarcomas</th>
<th>No. of mice with remote skin tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted -17-ketone (4)</td>
<td>50</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>50</td>
<td>18</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>8</td>
<td>21</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11,12-dimethyl-17-ketone (131)</td>
<td>8</td>
<td>21</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>


Carcinogenicity and mutagenicity

injection of 10 mg of this compound in olive oil, giving a high yield of dorsal skin tumours after promotion of this site remote from the site of injection with croton oil. In order to carry out the long-term experiment to be described (Coombs et al., 1979) it was necessary to avoid the induction of local sarcomas. This was easily achieved by reducing the injected dose to 3 mg; subsequent twice-weekly dorsal promotion with croton oil then led to dorsal skin tumours at the site of promotion in 65% of the animals (see Table 19), but no local sarcomas. The mean latent period for these skin tumours was 33 weeks; no dorsal skin tumours were observed in the absence of croton oil promotion of this area. When mice were injected but otherwise left untreated for six months before the usual promotion was begun, a tumour incidence of 45% was still obtained with the shorter mean latent period of 24 weeks. Ibalt indices calculated from these tumour incidences and mean latent periods (from the start of promotion) were almost identical (28 for immediate promotion and 27 for delayed promotion), indicating that initiation with this carcinogen is a permanent and irreversible state. These injected animals also developed a number of other tumours, whether they were promoted or not. The eyelids were particularly affected, and head and ventral surface tumours were also noted. This useful property of the carcinogen (26) of initiating skin effectively after subcutaneous injection without the induction of local sarcomas has been put to use in two other experiments. In the first (Bhatt et al., 1984), concerned with demonstrating that silica fibre isolated from the seeds of the grass Phalaris canariensis can act as a tumour promoter in T.O. mice, among a total of 70 mice injected there were 10 with eyelid and 22 with ventral trunk tumours. It has also made possible skin-transplantation experiments in Balb C mice, which are highly susceptible to the carcinogenicity of this ketone (J. Cox, private communication). Also Abbott (1983) demonstrated that this compound induced skin tumours in C57Bl mice, but not DBA/2 mice, in both the repeated-application (200 nmol twice weekly) and two-stage (1600 nmol once, followed by twice-weekly promotion) regimes. The resistance of DBA/2 mice is difficult to understand because for all three strains (T.O., C57Bl, and DBA/2) total DNA adducts in the treated skin, as well as the pattern of adducts as disclosed by high-pressure liquid chromatography of the derived nucleosides, were very similar. Moreover, the persistences of these DNA adducts in vivo were also similar for all three strains. It would be interesting to investigate whether DBA/2 mice lack an oncogene which is possessed in common by T.O., C57Bl, and Balb C mice, making them susceptible to cancer.

Little work has been carried out with cyclopenta[a]phenanthrenes in
<table>
<thead>
<tr>
<th>Group(a)</th>
<th>Promotion</th>
<th>No. of mice with dorsal skin tumours</th>
<th>Mean latent period ± s.d. (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total</td>
<td>papillomas</td>
</tr>
<tr>
<td>1</td>
<td>none</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>begun 8 days after injection</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>begun 6 months after injection</td>
<td>9</td>
<td>8</td>
</tr>
</tbody>
</table>

Tumours other than those appearing on promoted skin

<table>
<thead>
<tr>
<th>Group</th>
<th>Eyelid</th>
<th>Ear</th>
<th>Head</th>
<th>Ventral surface</th>
<th>Mice with lung adenomas(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 (2 pap.)</td>
<td>—</td>
<td>2 pap.</td>
<td>2 (1 mammary carc.)</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4 pap.</td>
<td>1 carc.</td>
<td>—</td>
<td>2 pap.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2 anaplastic carc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 sebaceous adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1 carc.</td>
<td>1 carc.</td>
<td>—</td>
<td>1 pap.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1 pap.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) 20 mice per group.

(b) i.e., 24 weeks from beginning of promotion.

c No lung adenomas among control mice injected with olive oil alone.
pap. = papilloma; carc. = carcinoma.
Table 20. Mammary tumours induced in rats after intragastric instillation of the 11-methyl-17-ketone (26)

<table>
<thead>
<tr>
<th></th>
<th>No. of rats</th>
<th>at 20 weeks</th>
<th>at 30 weeks</th>
<th>at 50 weeks</th>
<th>at 75 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at time of treatment</td>
<td>with tumour</td>
<td>alive</td>
<td>with tumour</td>
<td>alive</td>
</tr>
<tr>
<td><strong>Adenocarcinomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in rats after (26)</td>
<td>27</td>
<td>0</td>
<td>94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in control rats</td>
<td>96</td>
<td>(P = 0.455)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fibroadenomas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in rats after (26)</td>
<td>27</td>
<td>0</td>
<td>26</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>in control rats</td>
<td>96</td>
<td>(P = 0.5270)</td>
<td>94</td>
<td>92</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
animals other than mice. However, intragastric instillation of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) into 27 virgin female Sprague-Dawley rats (30 mg/rat) induced mammary adenocarcinomas in six of them, as shown in Table 20 (Coombs et al., 1979). Mammary tumours were detected during the 18th week after treatment, but a single spontaneous adenocarcinoma did not appear among control rats until the 42nd week. Differences in tumour incidence between the two groups were significant at 30 weeks and highly significant at 50 weeks as indicated by the P values calculated by the exact method of Yates (Fisher, 1954). By contrast, benign fibroadenomas occurred later and to a similar extent in both the carcinogen-treated and the untreated control groups. In its ability to induce mammary carcinomas after a single intragastric instillation this compound resembles other potent known carcinogens such as 3-methylcholanthrene (Shay et al., 1949) and 7,12-dimethylbenz[a]anthracene (Huggins, 1961). However, it is less potent than either of the latter in this regard and also in its ability to induce skin tumours in mice after topical application (Coombs and Croft, 1969). The suggestion that the carcinogenicity of aromatic hydrocarbons increases as their structure approaches that of steroids (Yang et al., 1961) is therefore not substantiated. Interperitoneal injection of the cyclopenta[a]-phenanthrenone into 57 male Sprague-Dawley rats (10 mg/rat, in oil) did not induce local sarcomas in any of these animals. In this behaviour it differs markedly from polycyclic aromatic hydrocarbons such as benzo[a]pyrene (Flesher, 1976). However, a third of these animals later developed leukaemia; this disease is caused in rats by hydrocarbons such as 7,12-dimethylbenz[a]anthracene only after injection directly into the bloodstream via the caudal vein (Huggins et al., 1978). In addition 25% also suffered liver degeneration, while 50% developed cirrhosis of the kidney including one animal with a reticulosarcoma of this organ (Bhatt, 1986).

6.3 The mutagenicity of cyclopenta[a]phenanthrenes

Over the last decade there has been a great deal of interest shown in the relationship between carcinogenicity and mutagenicity, chiefly in search of short-term tests for carcinogenicity that could supplant the tedious and expensive animal experiments of the type already described. During the intervening period it has become apparent that there is in fact a good correspondence between these two types of biological activity (Ames et al., 1973; Purchase et al., 1976). However, previously this was not obvious, and it then seemed of interest to re-test the 36 cyclopenta[a]phenanthrenes and two chrysenes listed in Table 21 for mutagenicity
Table 21. Mutagenicity and carcinogenicity of some cyclopenta[a]phenanthrenes and related chrysenes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutagenicity (no. of revertant TA100 colonies/nmol)</th>
<th>Carcinogenicity (Iball index)</th>
<th>Repeated application</th>
<th>Initiation-promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopenta[a]phenanthrenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-methyl-16,17-dihydro-15H-(Diels' hydrocarbon, 7)</td>
<td>0.7*</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,17-dimethyl-16,17-dihydro-15H- (139)</td>
<td>1.5</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-methoxy-17-methyl-16,17-dihydro-15H- (17)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17H- (2)</td>
<td>4.0*</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-methyl-15H- (133)</td>
<td>1.6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,17-dimethyl-15H- (135)</td>
<td>1.0</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12,17-dimethyl-15H- (136)</td>
<td>1.0</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,12,17-trimethyl-15H- (137)</td>
<td>1.2</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-methoxy-17-methyl-15H- (134)</td>
<td>0.7</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-methoxy-17-methyl-15H- (138)</td>
<td>0.9</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15,16-dihydro-17-one (4)</td>
<td>9.5*</td>
<td>&lt;1</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>1-methyl-15,16-dihydro-17-one (302)</td>
<td>&lt;0.2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2-methyl-15,16-dihydro-17-one (303)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-methyl-15,16-dihydro-17-one (304)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-methyl-15,16-dihydro-17-one (305)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>6-methyl-15,16-dihydro-17-one (306)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-methyl-15,16-dihydro-17-one (230)</td>
<td>12.3</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Oral LD₅₀ (mg/kg)</td>
<td>Oral LD₅₀ (mg/kg)</td>
<td>Oral LD₅₀ (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>11-methyl-15,16-dihydro-17-one (26)</td>
<td>21.7</td>
<td>46</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>12-methyl-15,16-dihydro-17-one (130)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11,12-dimethyl-15,16-dihydro-17-one (131)</td>
<td>1.1</td>
<td>30</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>1,11-methano-15,16-dihydro-17-one (310)</td>
<td>1.9</td>
<td>(16)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>11-ethyl-15,16-dihydro-17-one (211)</td>
<td>1.8</td>
<td>8</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-n-butyl-15,16-dihydro-17-one (212)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>3-methoxy-15,16-dihydro-17-one (24)</td>
<td>0.2</td>
<td>&lt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-methoxy-15,16-dihydro-17-one (132)</td>
<td>3.1</td>
<td>25</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>11-ethoxy-15,16-dihydro-17-one (390)</td>
<td>1.8</td>
<td>—</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>11-n-propoxy-15,16-dihydro-17-one (391)</td>
<td>0.3*</td>
<td>—</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>11-isopropoxy-15,16-dihydro-17-one (392)</td>
<td>1.1</td>
<td>—</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11-n-butoxy-15,16-dihydro-17-one (393)</td>
<td>&lt;0.2</td>
<td>—</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>11-n-pentoxy-15,16-dihydro-17-one (394)</td>
<td>&lt;0.2</td>
<td>—</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>11-methoxy-7-methyl-15,16-dihydro-17-one (447)</td>
<td>34.5</td>
<td>17</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>6-methoxy-11-methyl-15,16-dihydro-17-one (468)</td>
<td>1.7</td>
<td>14</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>16-hydroxy-11-methyl-15,16-dihydro-17-one (346)</td>
<td>18.3</td>
<td>11</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-methyl-11,12,15,16-tetrahydro-17-one (252)</td>
<td>&lt;0.2</td>
<td>&lt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>16,17-dihydro-15-one (102)</td>
<td>6.7*</td>
<td>&lt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-methyl-16,17-dihydro-15-one (334)</td>
<td>3.7</td>
<td>5</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

**Chrysenes**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oral LD₅₀ (mg/kg)</th>
<th>Oral LD₅₀ (mg/kg)</th>
<th>Oral LD₅₀ (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3,4-tetrahydro-1-one (449)</td>
<td>7.2*</td>
<td>&lt;1</td>
<td>—</td>
</tr>
<tr>
<td>11-methyl-1,2,3,4-tetrahydro-1-one (450)</td>
<td>7.1</td>
<td>45</td>
<td>—</td>
</tr>
</tbody>
</table>

* indicates mutagenic, but not carcinogenic.
since there existed approximately quantitative data on the relative potency of these compounds as carcinogens in mice of a single strain. Initially, it was found that the compounds were not mutagenic in Ames' original *Salmonella typhimurium* strains TA1535 and TA1538, but when these bacteria became available carrying the R-factor plasmid (pKM101) as TA98 and TA100 (McCann *et al*., 1975), making the bacteria more sensitive to polycyclic aromatic hydrocarbons, experiments became possible with cyclopenta[a]phenanthrenes. It was soon found that the carcinogenic 11-methyl-17-ketone (26) was readily detected using TA100 and a standard homogenate obtained from the livers of rats induced with the chlorinated biphenyl mixture Aroclor 1254, but it was less readily detected with TA98. Thereafter, *Salmonella typhimurium* TA100 was used exclusively (Coombs *et al*., 1976; Bhatt *et al*., 1982; Hadfield *et al*., 1984) in a standardized protocol. Each compound was first tested with three concentrations of the enzyme preparation (homogenate) to ascertain the concentration needed to give the highest mutation rate. As can be seen for the 7-methyl-17-ketone (230) in Fig. 57, 20μL or 50μL of homogenate give the same initial slope of the dose/response curve, but this slope is less steep with 100μL, although the maximum number of revertant colonies is larger at a higher dose. The compound was then re-tested, at least in triplicate, using the optimum enzyme concentration and at 1, 5, 10, 50, and 100μg/plate; the mutagenicity was expressed as the

![Fig. 57. Induction of his+ mutations in *Salmonella typhimurium* TA100 with 15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-one (230) at increasing doses and at three levels of enzyme (μL of homogenate).](image-url)
number of revertant colonies/nmol calculated from the initially straight part of the dose/response curve where cytotoxicity is minimal. Compounds exhibiting less than 0.2 colony/nmol were considered non-mutagenic; this number is approximately equivalent to the spontaneous mutation rate of these bacteria under these conditions. Also collected in this Table are Ibail indices of carcinogenicity for skin tumours induced in T.O. mice under the standard repeated-application (120 nmol twice weekly) and two-stage (1600 nmol once, twice-weekly promotion with croton oil) procedures, as already outlined. Compounds for which this index is <1 were not carcinogenic in these tests, i.e., the tumour incidence was less than 5% (<1 in 20 animals) in 700 days.

In this study all the carcinogens were mutagenic with the possible exception of the 1-methyl-17-ketone (302) and the 11-n-butoxy-17-ketone (393); the former may possess marginal carcinogenicity whilst the latter appears to be a very weak carcinogen. However, the reverse was not true, for out of the 15 non-carcinogens six (marked with asterisks) were mutagenic. Also there was no consistent relationship between carcinogenic and mutagenic potency, although several strong carcinogens were also strong mutagens, giving dose/response curves similar to that shown in Fig. 58 for the 11-methyl-17-ketone (26). The dramatic

Fig. 58. Dose/response showing the effect of the increasing toxicity of the carcinogenic 11-methyl-17-ketone (26) above 10 μg/plate, and the inhibitory effect of a constant amount (30 μg) of 7,8-benzoflavone (BF) on mutation induction.
Table 22. Mutagenicity of 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) and four related ketones in V79 cells mediated by HepG2 cells in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg/mL)</th>
<th>Treatment time (h)</th>
<th>Percentage cloning efficiency&lt;sup&gt;(a)&lt;/sup&gt;</th>
<th>Mutation frequency&lt;sup&gt;(b)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,16-dihydrocyclopenta[a]phenanthren-17-one (4)</td>
<td>1.0</td>
<td>48</td>
<td>4.5</td>
<td>7.8</td>
</tr>
<tr>
<td>2-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (303)</td>
<td>1.0</td>
<td>48</td>
<td>10.7</td>
<td>1.2</td>
</tr>
<tr>
<td>3-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (304)</td>
<td>1.0</td>
<td>48</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>11-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (26)</td>
<td>0.5</td>
<td>24</td>
<td>26.2</td>
<td>34.4</td>
</tr>
<tr>
<td>11-methyl-1,2,3,4-tetrahydrochrysene-1-one (450)</td>
<td>0.5</td>
<td>24</td>
<td>20.31</td>
<td>28.2</td>
</tr>
</tbody>
</table>

<sup>(a)</sup> Percentage of reduction in cloning efficiency compared with untreated control cells.
<sup>(b)</sup> Number of 6-TG-resistant mutants/10⁸ viable V79 cells at the end of a four-day expression period; the average mutation frequency in control cells was 0.37.
Mutagenicity of cyclopenta[a]phenanthrenes 163
decrease in the number of mutant colonies observed with doses above 20μg/plate probably reflects the considerable toxicity of this compound. This figure also demonstrates the effect of adding 7,8-benzoflavone (300μg) to the plate; in agreement with its inhibitory effect on tumour induction, this flavone also considerably reduced the apparent mutagenicity of this compound, presumably by blocking metabolism. It is noticeable that of the six mutagenic non-carcinogens, five are parent unsubstituted compounds which become carcinogens upon 11-methyl substitution. Of these 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) is of particular interest for it has been tested side-by-side with its 11-methyl homologue (26) on many occasions, but has never been found to give tumours.

In order to carry this investigation a little further, these two compounds were also tested for mutagenicity in a mammalian cell culture system (Bhatt et al., 1983), using V79 Chinese hamster cells and scoring for mutations conferring 6-thioguanine resistance (Huberman and Sachs, 1974). It was unexpectedly discovered that hamster embryo cells, used by these authors metabolically to activate polycyclic aromatic hydrocarbon carcinogens, did not appear to activate the cyclopenta[a]phenanthrene (26). The method of Diamond et al. (1980) was therefore employed, making use of human hepatoma cells (Hep G2) to cause activation. Under these conditions the 11-methyl-17-ketone (26) and its strongly carcinogenic chrysenone analogue (450) were both potent mutagens whilst the 2- and 3-methyl-17-ketones (303 and 304, respectively, both non-carcinogens) were inert. However, the non-carcinogenic parent ketone (4) was still moderately mutagenic in this system (Table 22). Again substantial toxicity was encountered with the carcinogen (26) in these cells, the cloning efficiency being reduced 50% at a dose of 1μg/mL, at which the highest mutation frequency was observed (Fig. 59). These two cyclopenta[a]phenanthrenes (4) and (26) were also compared for their ability to increase the frequency of sister chromatid exchanges in human lymphocytes, again using Hep G2 cells to cause biological activation (Lindahl-Kiessling et al., 1984). In this case it was found that the carcinogen (26) was able consistently to increase this frequency whereas the non-carcinogen (4) was not.

Several of these compounds have also been tested in yeast (Kelly, 1983; Kelly and Parry, 1983). Gene conversion at the trp5 locus was measured in Saccharomyces cerevisiae strain JD1, whilst strain D6 was used to detect mitotic aneuploidy. The experiments were carried out in ignorance of the identities of the compounds until completion of the series. Results are summarized in Table 23 in which a positive response (+) denotes at
least a two-fold increase over control levels in white, cycloheximide resistant, monosomic colonies in strain D6 or trp+ prototrophs in strain JD1. For reference carcinogenicity is displayed alongside, again either positive or negative, without any indication of potency. There is a remarkably good correlation between genetic activity and carcinogenicity in both strains of this yeast. Of the carcinogens only the 11-phenol (206) was not detected; this compound is almost insoluble in water and most organic solvents, so that a sufficient concentration was probably not achieved in the incubation medium. The 11-isopropanyl-17-ketone (392) was active in D6, but not in JD1 thus confirming its apparent very weak carcinogenicity, whereas the 11-n-butoxy-17-ketone, also a very weak carcinogen, was active in both, although it was not detected in the Ames’ test. All the non-carcinogens were inactive in both D6 and JD1, including the unsubstituted parent ketone (4) which was mutagenic in both the Ames’ test and in V79 cells in culture. The 1-methyl-17-ketone (302) was not mutagenic in any of these tests, thus throwing further doubt on its questionable carcinogenicity. Sensitivity for these compounds was poor.

Fig. 59. Toxicity (reduction in plating efficiency) and mutagenicity (induction of 6-thioguanine-resistant colonies) with the carcinogenic 11-methyl-17-ketone (26) in V79 cells mediated by human HepG2 liver cells.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Strain D6 (mitotic aneuploidy)</th>
<th>Strain JD1 (gene conversion)</th>
<th>Carcinogenicity (see Table 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,16-dihydrocyclopenta[a]phenanthren-17-one (4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (302)</td>
<td>-</td>
<td>-</td>
<td>- (?</td>
</tr>
<tr>
<td>2-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (303)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (304)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (230)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (26)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11,12-dimethyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (131)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11,11-methano,15,16-dihydrocyclopenta[a]phenanthren-17-one (310)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-methoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (132)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-ethoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (390)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-n-propoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (391)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11-isoproxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (392)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-n-butoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (393)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11-n-pentoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (394)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11-hydroxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (206)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydro-11-methylchrysen-1-one (450)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Carcinogenicity and mutagenicity

the maximum increase in genetic activity over background never reaching more than three-fold, and there was little indication of potency. Addition of rat liver homogenate to the plates generally increased the observed frequency of mitogenic conversion and aneuploidy events, but the yeasts were to some extent capable of activating these compounds themselves, especially when they had been grown on low glucose (0.5%) to the exponential phase and then transferred to high glucose (20%) to suppress mitochondrial function. Under these conditions a marked increase in the P450/448 content of the yeast could be demonstrated.

Of these methods for measuring genetic toxicity the Ames’ test is to be preferred on grounds of operational simplicity as well as sensitivity. In this cyclopenta[a]phenanthrene series the test is capable of detecting virtually all the carcinogens, but suffers chiefly from being too sensitive in that it also detects some non-carcinogens as mutagens. This is of little consequence when the test is used to distinguish the biologically active metabolites of these compounds among the many separable by hplc, as is described in Chapter 7.

6.4 References


References


Carcinogenicity and mutagenicity


Wood, A. W., Wislocki, P. G., Chang, R. L., Yagi, H., Hernandez, O.,
References


Metabolic activation of cyclopenta[a]-phenanthrenes and their interaction with DNA

7.1 Activation of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one

In the various mutation tests described in the last chapter, without exception none of the cyclopenta[a]phenanthrenes was active in the absence of an added source of metabolism. Since there is a good parallelism between mutagenicity and carcinogenicity in this series, it seemed reasonable to suppose that metabolism was required before these compounds could act to cause tumours in animals. Dannenberg (1960) found that introduction of a double bond between C-16 and C-17 in Diels' hydrocarbon (7) to give 17-methyl-15H-cyclopenta[a]phenanthrene (133) led to weak carcinogenicity (see Table 9). He noted that this double bond was the most chemically reactive in the molecule, adding, for example, to osmium tetroxide to give the 16,17-cis-diol in preference to the K-region 6,7-double bond, and he suggested that the carcinogenicity of this hydrocarbon and its 15-methyl-17H-isomer (128) might be due to the reactivity of these double bonds in the five-membered ring-D. It has already been shown that some of the 17-ketones of this series, lacking a carbon–carbon double bond in the ring-D, are more active than the corresponding unsaturated ring-D hydrocarbons (see Tables 10 and 11), and in these ketones the most chemically reactive double bond is between C-6 and C-7 (i.e., in the K-region). Metabolism of cyclopenta[a]-phenanthrene hydrocarbons such as (128) and (133) does not appear to have been studied yet, although structure/activity relationships established since Dannenberg's suggestion do indicate interesting differences between the two series (see Table 24). Thus comparing the Iball indices obtained under the same standard conditions for several identically substituted pairs of 17-methyl-16,17-enes and 15,16-dihydro-17-ketones it is clear that carcinogenicity among the latter is confined to 11-
substituted derivatives, whereas all the hydrocarbons appear to be active to some extent. Analogously substituted 17-ketones are more active than the corresponding hydrocarbons, particularly in the case of the 11-methoxy derivatives.

At the outset the 11-methyl-17-ketone (26) was chosen for metabolic study because it was at that time the most carcinogenic cyclopenta[a]phenanthrene known; in many experiments the inactive unsubstituted parent 17-ketone (4) was examined alongside. An indication that simple metabolic experiments employing the microsomal fraction from rat liver in incubations in phosphate buffer containing an excess of NADPH in air would yield meaningful results came from an early experiment (Coombs et al., 1975). In this it was established that the aryl hydrocarbon hydroxylase inhibitor 7,8-benzoflavone not only inhibited tumour production with this 11-methyl ketone (26), but also considerably diminished the binding of this 3H-labelled carcinogen to calf thymus DNA under these in vitro metabolic conditions (Table 25). The carcinogen administered twice weekly at a total dose of 60μg/week gave the expected skin tumour incidence (85%) and mean latent period (29 weeks). At one-tenth of this weekly dose, in two identical experiments the tumour incidence was approximately halved, and the mean latent period increased about 50%; these results illustrate the good reproducibility achievable in this type of tumour-induction experiment. No skin tumours were produced at one-hundredth of the original weekly dose. When a mixture of the carcinogen at the highest weekly dose was painted together with 7,8-benzoflavone at three times this dose, tumour incidence was reduced to 70% and the mean latent period was prolonged by almost 30%, but when both were repeatedly administered together at one-tenth

Table 24. Comparison of Iball indices (T.O. mice, repeated application) of 17-methyl-15H-cyclopenta[a]phenanthrene hydrocarbons with the corresponding 15,16-dihydrocyclopenta[a]phenanthren-17-ones

<table>
<thead>
<tr>
<th>Substitution</th>
<th>Iball index (see Table 21)</th>
<th>17-methyl-16,17-ene</th>
<th>17-ketone</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted</td>
<td>(133) 6 (4) &lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-methyl</td>
<td>(135) 27</td>
<td>(26) 46</td>
<td></td>
</tr>
<tr>
<td>12-methyl</td>
<td>(136) 7 (130) &lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,12-dimethyl</td>
<td>(137) 23</td>
<td>(131) 30</td>
<td></td>
</tr>
<tr>
<td>11-methoxy</td>
<td>(138) 11</td>
<td>(132) 25</td>
<td></td>
</tr>
<tr>
<td>3-methoxy</td>
<td>(134) 2 (24) &lt;1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 25. Effect of the aryl hydrocarbon hydroxylase inhibitor 7,8-benzoflavone on tumour induction (A), and on in vitro binding (B) of the carcinogen 15,16-dihydro-11-methyl-cyclopenta[a]phenanthren-17-one (26) to DNA

(A) Tumour induction (T.O. mice in groups of 20, painted twice weekly)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage skin tumour incidence</th>
<th>Mean latent period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(26) 60μg/week</td>
<td>85</td>
<td>29</td>
</tr>
<tr>
<td>(26) 6μg/week</td>
<td>45</td>
<td>43</td>
</tr>
<tr>
<td>(26) 6μg/week</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>(26) 0.6μg/week</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>(26) 60μg/week plus 7,8/benzoflavone (180μg/week)</td>
<td>70</td>
<td>37</td>
</tr>
<tr>
<td>(26) 6μg/week plus 7,8-benzoflavone (18μg/week)</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>(26) 6μg/week plus 15,16-dihydrocyclopenta[a]phenanthren-17-one (54μg/week)</td>
<td>50</td>
<td>45</td>
</tr>
</tbody>
</table>

(B) DNA binding (covalent binding to calf thymus DNA added to the in vitro incubation)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Microsomal preparation</th>
<th>DNA binding index (μmol ketone/mol DNA phosphorus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(26)</td>
<td>active</td>
<td>46.5 ± 2.5</td>
</tr>
<tr>
<td>(26) + 3 molar equiv. of 7,8-benzoflavone</td>
<td>heat-inactivated</td>
<td>0</td>
</tr>
<tr>
<td>(26) + 3 molar equiv. of 7,8-benzoflavone</td>
<td>active</td>
<td>0</td>
</tr>
<tr>
<td>Preparation 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(26)</td>
<td>active</td>
<td>18.1 ± 2.0</td>
</tr>
<tr>
<td>(26) + 3 molar equiv. of 7,8-benzoflavone</td>
<td>absent</td>
<td>0</td>
</tr>
<tr>
<td>(26) + 3 molar equiv. of 7,8-benzoflavone</td>
<td>active</td>
<td>0.87</td>
</tr>
</tbody>
</table>

a, average of 5 replicate determinations ± s.d.
of these amounts no skin tumours were observed. Thus this inhibitor is relatively more active at lower doses of the carcinogen as has been noted already. Administration of a nine-fold quantity of the non-carcinogenic parent ketone (4) together with the carcinogen (26) had no effect on the tumour induction by the latter. This carcinogen (26) bound covalently to calf thymus DNA added to the in vitro metabolic incubation, but this was largely prevented by inclusion of the inhibitor in the incubation mixture. No DNA binding occurred in the absence of the microsomal preparation, or if the enzyme was deactivated by heat treatment (2 min at 100°C) prior to incubation with the substrate and DNA. This whole experiment clearly supports the view that metabolism is involved in tumour induction with this carcinogen, and that this simple metabolic system is a valid model for its study.

Under similar in vitro metabolic conditions in the absence of added DNA and using microsomes isolated from the livers of uninduced Sprague-Dawley rats (Coombs et al., 1976), the main metabolites extracted from the incubation mixture with ethyl acetate appeared to be mono-ols on examination by thin-layer chromatography (tlc). These were readily identified as the 15-ols (369 and 370) and 16-ols (345 and 346), from the unsubstituted -17-ketone (4) and its carcinogenic 11-methyl homologue (26), respectively, by direct comparison of \( R_f \) values and ultraviolet spectra of material recovered from the plates with those of the synthetic compounds. When \(^3\)H-labelled compounds were used these identities were confirmed by dilution analysis of the derived acetates. The carcinogen also gave a small amount of its 11-hydroxy-methyl derivative (215) identified in the same way, and by the fact that as expected it retained only about 65% of the radioactivity of the original compound when the latter was labelled with tritium in the 11-methyl group. Other more polar metabolites were also detected by tlc, and these were increased relative to the mono-ols when the rats were induced by injection with 3-methylcholanthrene or phenobarbitone before sacrifice. While tlc gave a good separation of these metabolites and the overall structures of some could be tentatively deduced from their relative \( R_f \) values and their ultraviolet spectra, particularly after in situ reduction of the carbonyl group with sodium borohydride, not enough could be obtained in this way for further examination. Moreover, it was difficult to avoid aerial oxidation using this technique.

Rapid progress was made when high-pressure liquid chromatography (hplc) was introduced because it overcame both of these difficulties. Also developments in high-field Fourier transform nuclear magnetic resonance (nmr) spectroscopy at this time greatly increased the sen-
sitivity of this analytical technique so that it became applicable to the amounts of metabolites obtainable by hplc. Finally, the renewed interest in the metabolism of polycyclic aromatic hydrocarbons had led to the identification of a bay-region diol-epoxide as the active form of benzo[a]pyrene (Sims et al., 1974), and it was subsequently demonstrated that a number of other phenanthrene-derived hydrocarbons followed a similar activation route (Jerina et al., 1978). It seemed probable that the carcinogenic 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) might undergo analogous metabolism, and this was reinvestigated using these new techniques with this in mind.

Figure 60 shows a typical hplc separation of in vitro metabolites of this compound (26) extracted with ethyl acetate after incubation with NADPH and a microsomal preparation obtained from the livers of male Sprague-Dawley rats previously injected with 3-methylcholanthrene (Coombs et al., 1980). Under these conditions most of the conjugating enzymes normally present in the supernatant are absent, thus largely preserving the products of primary metabolism. After being shaken in the presence of atmospheric oxygen for 30 min at 37°C, extraction with ethyl acetate routinely led to recovery of about 80% of the material incubated, as determined by recovered radioactivity when a labelled substrate was metabolized. A reverse-phase column was employed to separate the complex mixture formed using a gradient of aqueous methanol increasing in methanol concentration, so that the more polar metabolites eluted first and last peak to emerge was the unreacted substrate (26). The profile shown in the figure was monitored continuously at 254 nm, but the profile of eluted radioactivity was very similar. The properties and the structures

Fig. 60. Profile of in vitro metabolites of the carcinogen (26) separated by reverse-phase hplc and monitored by their ultraviolet absorption at 254 nm; the first peak m is of microsomal origin, and the last is the unchanged substrate (26).
Activation of 11-methyl-17-ketone

Deduced for the main metabolites (a–g) are listed in Table 26 (Coombs et al., 1980). The peak denoted M, of microsomal origin, was always present and acted as a useful chromatographic marker. Molecular formulae were based on molecular weights obtained from mass spectrometry; the patterns of fragment ions were in all cases those expected from previous work with synthetic compounds (see Chapter 5). With the

### Table 26. Structures and properties of the main metabolites a–g (Fig. 60) of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 11-methyl-1,2,15,16-tetrahydro-1,2,15-trihydroxycyclopenta[a]phenanthren-17-one (454) C_{18}H_{16}O_4</td>
<td><strong>Found:</strong> M⁺ 296.1043; <strong>Calculated</strong> 296.1049</td>
</tr>
<tr>
<td></td>
<td>λ_{max} 265 (4.60), 322 (4.10), 334 (4.14), 354 (3.71), 373 (3.68) nm</td>
</tr>
<tr>
<td></td>
<td>λ_{max} (reduced) 259 (4.65), 267 (4.69), 320 (3.80) nm</td>
</tr>
<tr>
<td>(b) 11-methyl-3,4,15,16-tetrahydro-3,4,15-trihydroxycyclopenta[a]phenanthren-17-one (455) C_{18}H_{16}O_4</td>
<td><strong>Found:</strong> M⁺ 296; <strong>Calculated</strong> 296</td>
</tr>
<tr>
<td></td>
<td>λ_{max} 273 (4.70), 327sh (3.83), 335 (3.84), 370sh (3.54) nm</td>
</tr>
<tr>
<td></td>
<td>λ_{max} (reduced) 248 (4.74), 327 (3.97), 347 (3.85) nm</td>
</tr>
<tr>
<td>(c) 11-methyl-3,4,15,16-tetrahydro-3,4,16-trihydroxycyclopenta[a]phenanthren-17-one (456) C_{18}H_{16}O_4</td>
<td><strong>Found:</strong> M⁺ 296; <strong>Calculated</strong> 296</td>
</tr>
<tr>
<td></td>
<td>λ_{max} 272 (4.70), 327sh (3.89), 333 (3.90), 370sh (3.61) nm</td>
</tr>
<tr>
<td></td>
<td>λ_{max} (reduced) 248 (4.76), 326 (4.03), 347 (3.90) nm</td>
</tr>
<tr>
<td>(d) 15,16-dihydro-15-hydroxy-11-hydroxymethylcyclopenta[a]phenanthren-17-one C_{18}H_{16}O_3</td>
<td><strong>Found:</strong> M⁺ 278.0948; <strong>Calculated</strong> 278.0943</td>
</tr>
<tr>
<td></td>
<td>λ_{max} 269 (4.78), 300 (4.27), 358 (3.26), 376 (3.24) nm</td>
</tr>
<tr>
<td></td>
<td>λ_{max} (reduced) 255 (4.82), 279 (4.09), 290 (4.05), 301 (4.09), 334 (4.06), 350 (2.93) nm</td>
</tr>
<tr>
<td>(e) 3,4-dihydroxy-11-methyl-3,4,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (457) C_{18}H_{16}O_4</td>
<td><strong>Found:</strong> M⁺ 280.1109; <strong>Calculated</strong> 280.1100</td>
</tr>
<tr>
<td></td>
<td>λ_{max} 273 (4.71), 332sh (3.84), 357 (3.57), 371 (3.55) nm</td>
</tr>
<tr>
<td></td>
<td>λ_{max} (reduced) 250 (4.70), 306 (3.58), 330 (3.63), 349 (3.59) nm</td>
</tr>
<tr>
<td>(f) 15,16-dihydro-15-hydroxy-11-methylcyclopenta[a]phenanthren-17-one (370)</td>
<td>Identified by reference to the synthetic compound (see Chapter 5 for physical data)</td>
</tr>
<tr>
<td>(g) 15,16-dihydro-16-hydroxy-11-methylcyclopenta[a]phenanthren-17-one (346)</td>
<td>Identified by reference to the synthetic compound (see Chapter 5 for physical data)</td>
</tr>
</tbody>
</table>
vicinal trans-dihydro diols the molecular ions were weak, but the M⁺-18 ions (loss of water) were prominent, and ions due to the ready loss of carbon monoxide (28 mass units) from ring-D confirmed the retention of this double-bonded oxygen atom in these metabolites. The ultraviolet spectra of these cyclopenta[a]phenanthrene derivatives after mild reduction of this conjugated double bond with sodium borohydride resembled those of the corresponding known phenanthrene derivatives, and allowed assignment of the points of saturation and hence the position of the trans-dihydridiol systems. This was further checked by dehydration of these diols by heating them with 50% sulphuric acid for 30 min, and examining the resulting phenols (Table 27). Metabolites (b), (c), and (e) gave 4-phenols with almost identical ultraviolet spectra, similar to those of synthetic 15,16-dihydro-4-hydroxy-1-methylcyclopenta[a]phenanthren-17-one (458), whereas the 2-phenol from metabolite a resembled synthetic 15,16-dihydro-2-hydroxycyclopenta[a]phenanthren-17-one (396). The inter-relationships between these various metabolites are shown in Fig. 61. The mono-ols (215), (346, g), (370, f), and the A-ring diols (459) and (457, e) suffer further conversion into the triols (454, a), (455, b) (456, c), and the diol (d). This was confirmed by similar metabolism of the synthetic mono-ols which gave rise to these further-hydroxylated derivatives as expected. The minor

<table>
<thead>
<tr>
<th>Phenol</th>
<th>Neutral</th>
<th>Alkaline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derived from (a)</td>
<td>278 (4.71)</td>
<td>250 (4.53), 301 (4.55)</td>
</tr>
<tr>
<td>15,16-dihydro-2-hydroxycyclopenta[a]phenanthren-17-one (396)</td>
<td>274.5 (4.74)</td>
<td>245 (4.59), 296 (4.62)</td>
</tr>
<tr>
<td>Derived from (b)</td>
<td>269 (4.61), 274 (4.62), 301.5 (4.48)</td>
<td>261 (4.70), 327 (4.37)</td>
</tr>
<tr>
<td>Derived from (c)</td>
<td>269 (4.68), 274 (4.67), 301 (4.57)</td>
<td>262 (4.76), 328 (4.46)</td>
</tr>
<tr>
<td>Derived from (e)</td>
<td>271 (4.66), 301 (4.45), 270, 305</td>
<td>261 (4.64), 325 (4.32)</td>
</tr>
</tbody>
</table>

Table 27. Ultraviolet spectra of phenols obtained by acid-catalysed dehydration of the metabolites (a), (b), (c), and (e) in comparison with those of two synthetic phenols
metabolites (215) and (459) were not resolved with a 10-\textmu m reverse-phase column, but using a similar 5-\textmu m column the 11-hydroxymethyl-17-ketone (215) ran as a small peak just ahead of the 15-ol (370), whilst the 1,2-diol (459) appeared just before the 3,4,15-triol (455) and possessed ultraviolet characteristics almost identical with those of the 1,2,15-triol (454). Thus the 1,2-diols are noticeably more polar than the corresponding 3,4-diols; it later appeared that this was due to the fact that the 1,2-diols were diaxial, whereas the 3,4-diols were diequatorial. It therefore seems that this ketone (26) suffered oxidative metabolic attack at rings -A and -D and at the methyl group, but not elsewhere in the molecule. In particular the 6,7-double bond, the most reactive chemically, was unaffected.

The kinetics of the formation of certain of these various metabolites were investigated, chiefly in order to optimize the amount of the 3,4-dihydropseudo since this was required for further study. Aliquots were withdrawn from an in vitro incubation of the ^{14}C-labelled carcinogen at intervals, immediately cooled in ice and extracted with ethyl acetate. Separation of the metabolites by hplc and quantitation were carried out

\begin{itemize}
  \item \textbf{Fig. 61. Structures proposed for the main metabolites formed in vitro from the carcinogen (26).}
\end{itemize}
as usual to generate the curves shown in Fig. 62. About 75% of the substrate was consumed, but at a decreasing rate during 45 min, by shaking in air at 37°C. The 3,4-dihydrodiol increased in amount up to about 15 min and then remained fairly constant, decreasing somewhat only at times after 30 min. Presumably this plateau resulted from the rate of its formation just matching its loss by further metabolism until the amount of substrate was diminished substantially. The 15-ol, on the other hand, increased rapidly in amount at first, reaching a maximum accounting for about 25% of the substrate at 10 min, but thereafter decreased sharply. Conversely, the 1,2,15-triol after a sluggish start increased in amount roughly linearly up to about 30 min, confirming that it was formed largely from the 15-ol by further metabolic attack in ring-A. Subsequently, incubations were generally maintained for 30 min to allow consumption of about 70% of the substrate and to maximize the quantity of the 3,4-dihydrodiol under these conditions of substrate and enzyme concentration.

Identification of the biologically active metabolite(s) of this carcinogen (26) was tackled in two ways – by measuring the ability of metabolite fractions (a–g) to bind to calf thymus DNA in vitro after further metabolism, and by investigating their mutagenicity in the Ames' test. It has already been mentioned (Table 25) that after metabolism this compound binds covalently to DNA added to the incubation mixture.

Fig. 62. Kinetics of the formation of certain metabolites of the carcinogen (26) on in vitro incubation.
When DNA exposed in this way to the $^{14}$C-labelled 11-methyl-17-ketone (26) was isolated, purified, hydrolysed enzymatically to its constituent nucleosides, and the latter were separated by chromatography on a column of Sephadex LH20 (hydroxypropyl cellulose) using a water-methanol gradient, two radioactive peaks A and B eluted following the natural nucleosides (detected by their ultraviolet absorption) as shown in Fig. 63. This carcinogen, generally labelled with tritium at very high specific activity (13.9 Ci/mmol), was also applied to mouse skin in vivo as in a tumour-initiation experiment; 24 h later the mice were killed and the treated skin was removed. Isolation of the DNA was achieved by initial digestion of the skin with proteinase K according to the method of Blin and Stafford (1976). After purification and enzymatic hydrolysis, the constituent nucleosides were separated as before by LH20 chromatography to give radioactive peaks A and B with the same retention times as those obtained from calf thymus DNA in vitro. In this experiment the amount of peak A relative to peak B was less, and owing to the lability of the tritium due to metabolism, the normal nucleosides also became

Fig. 63. Sephadex LH20 profiles of DNA hydrolysates: (a) profile from calf thymus DNA treated in vitro with the $^{14}$C-labelled carcinogen (26) (the dotted line represents ultraviolet absorption of the eluate continuously monitored at 254 nm and the solid line radioactivity); (b) similar profile from DNA isolated from the skin of mice treated topically in vivo with the $^{3}$H-labelled carcinogen (26); (c) co-chromatography of the in vitro $^{14}$C-adduct B and in vivo $^{3}$H-adduct B, establishing their identity.
labelled to some extent. When the main adduct peak B, labelled with $^{14}$C from the first in vitro experiment, was co-chromatographed on LH20 with the $^3$H-labelled adduct peak B from the in vivo experiment they co-eluted together precisely. Thus it seemed that the simple in vitro model adequately represented the in vivo situation in mouse skin. The binding of the $^{14}$C-labelled metabolic fractions (a–f) to DNA was next studied in vitro using hepatic microsomes and calf thymus DNA as before. In each case the recovered DNA was degraded to its constituent nucleosides which were separated by LH20 chromatography, and the radioactivity in peaks A and B from each was measured. The results obtained from this experiment are displayed in Table 28 which also lists the mutagenicity of these fractions a–f in the Ames' test. In both tests the metabolite (e) (3,4-dihydrodiol) was by far the most active, being two and a half times more mutagenic and binding to DNA nearly four times as much as the original carcinogen (26). It was also the only metabolite to yield adduct B; the fact that it also gave some adduct A proved that it underwent further hydroxylation to metabolites (b) or (c). Adduct A is formed from the 3,4,15- and 3,4,16-triols (b and c); the mono-ols (g) and (f) also gave this adduct, no doubt after their conversion into (b) and (c). The 1,2,15-triol (a) and the 11-hydroxymethyl-15-ol (d) were inactive in both tests. Thus the primary metabolite responsible for the biological activity of the carcinogen (26) is its 3,4-dihydrodiol (457, e). That this was also mainly responsible for its carcinogenicity was investigated in a direct, straightforward manner (Coombs and Bhatt, 1982). Repeated large-scale (10$\mu$mol) in vitro incubation of this carcinogen and separation of the

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mutagenicity (revertants/ nmol)</th>
<th>DNA-binding index ($\mu$mol of metabolite/mol of DNA phosphorus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adduct A</td>
</tr>
<tr>
<td>(a)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(b)</td>
<td>8.9</td>
<td>0</td>
</tr>
<tr>
<td>(c)</td>
<td>14.8</td>
<td>74</td>
</tr>
<tr>
<td>(d)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(e)</td>
<td>45.6</td>
<td>0</td>
</tr>
<tr>
<td>(f)</td>
<td>6.6</td>
<td>58</td>
</tr>
<tr>
<td>(g)</td>
<td>13.6</td>
<td>31</td>
</tr>
<tr>
<td>Carcinogen (26)</td>
<td>17.5</td>
<td>30</td>
</tr>
</tbody>
</table>
metabolites produced by hplc gave enough of these main metabolites (a-g) for them to be tested for carcinogenicity in the standard two-stage initiation/promotion experiment with T.O. mice. Groups of 20 mice were employed as usual, and each received a topical dose of 100 nmol [in toluene–acetone (1:1 v/v) because this solvent was known to enhance the carcinogenicity of (26)]; twice-weekly promotion with 1% croton oil in toluene then led to the result illustrated in Fig. 64. Again the 3,4-diol (457) was more active than the original carcinogen both in respect to higher skin tumour incidence and to a shorter mean latent period. The 3,4,15- and 3,4,16-triols (455 and 456) were much less active as tumour initiators, and the 1,2,15-triol (454) and 11-hydroxymethyl-15-ol were inactive. This firmly established that 3,4-dihydroxy-11-methyl-3,4,15,16-tetrahydrocyclopenta[a]phenanthren-17-one is the main proximate form of the carcinogen (26).

The nature of the adduct peaks A and B was further investigated by hplc (Abbott and Coombs, 1981). When the total radioactive DNA adducts (peaks A and B) derived from mouse skin treated in vivo with the carcinogen (26) as already described were chromatographed on a 10-μm reverse-phase column in water–methanol (9:1 v/v) six discrete peaks were seen (Fig. 65). By chromatographing peaks A and B separately it
was found that A gave rise to I, II, and III, while peak B separated into IV, V, and VI. The DNA recovered from an \textit{in vitro} binding experiment gave a similar result, and the origin of the individual peaks was determined by incubating each of the main metabolites \((b, c, e, g)\) and \((f)\) with DNA in the presence of rat liver microsomes \textit{in vitro}. In this way it became clear that the proximate carcinogen (metabolite e) gave rise to the main adduct V accounting for over 80\% of the total adducts, as well as the minor adduct IV, while the other peaks arose from the triols \((b)\) and \((c)\). By incubating DNA labelled with tritiated deoxyguanosine with the \(^{14}\text{C}\)-labelled carcinogen \((26)\) it was readily established that the major adduct V as well as II (probably the 16-hydroxy derivative of V) both involved binding to this base. By using poly(deoxyadenosine-thymidine) labelled with tritiated deoxyadenosine it was likewise shown that adduct VI (and possibly I) involved binding to adenosine. Thus it appears that

Fig. 65. hplc Profile of DNA adducts derived from the skin of mice treated topically with the \(^{3}\text{H}\)-labelled carcinogen.
over 85% of the DNA binding observed with this carcinogen is to deoxyguanosine. Thus in this respect, as well as its activation via the 3,4-dihydrodiol, this 11-methyl-17-ketone closely resembled benzo[a]pyrene.

Since further metabolism was required before the 3,4-dihydrodiol (457, e) bound to DNA in vitro or caused mutations in bacteria it was tempting to assume that this, like benzo[a]pyrene, was mediated via a bay-region diol-epoxide. However, this was difficult to prove in the absence of the synthetic diol epoxide, although two separate pieces of evidence pointed in this direction. As has already been discussed in Chapter 5, a detailed and extensive mass spectral study of DNA treated in vitro with the carcinogen (26) established that a 2,3,4-trihydroxy derivative was bound to deoxyguanosine via its exocyclic N\textsuperscript{2}-amino group (Wiebers et al., 1981). The other evidence comes from LH20 elution volume data with the nucleoside peak B, which as we have seen, consists mainly (>80%) of this deoxyguanosine adduct. In Tris buffer at pH 8.7 this adduct has an elution volume of about 250 mL, whereas in borate buffer of the same pH and ionic strength it elutes earlier, with an elution volume of about 200 mL (Coombs et al., 1979). This is consistent only with an intermediate anti-1,2-epoxy-trans-3,4-dihydrodiol, nucleophilic opening of which would yield a 2,3,4-trihydroxy derivative in which the vicinal C-2 and C-3 hydroxy groups are cis with respect to one another, and therefore are able to complex with the borate. A syn-1,2-epoxy-trans-3,4-dihydrodiol would give rise to an all-trans arrangement unable to form a complex, and thus elute earlier (Fig. 66). The absolute stereochemistry was based on an nmr and circular dichroism study of the 3,4-diol (457, e) (Hadfield et al., 1984b). Chemical shifts (δ values) and coupling constants for the ring-A protons in this 11-methyl-3,4-dihydroxy-3,4-dihydro-17-ketone and its unsubstituted homologue, both obtained from metabolism of (26) and (4) in vitro, together with the borohydride reduction product of the latter diol are shown in Table 29. From the large coupling constants between H-3 and H-4 in these co-

![Fig. 66. Nucleophilic opening of 3,4-dihydro-3,4-dihydroxy-anti- and syn-1,2-oxides at C-1; only the anti-oxide can give a vicinal cis-diol which can complex with borate.](image-url)
Table 29. Chemical shifts (ppm) and coupling constants (Hz) of the A-ring protons in the metabolically derived 3,4-diol (457), its unsubstituted homologue and the borohydride reduction product of the latter

<table>
<thead>
<tr>
<th>Compound</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-methyl-3,4-dihydroxy-17-ketone (457)</td>
<td>7.58 double doublet</td>
<td>6.32 double doublet</td>
<td>4.6 multiplet</td>
<td>4.9 doublet</td>
</tr>
<tr>
<td></td>
<td>$J_{1,2} = 10.5$</td>
<td>$J_{2,3} = 2$</td>
<td>$J_{3,4} = 12$</td>
<td></td>
</tr>
<tr>
<td>unsubstituted-3,4-dihydroxy-17-ketone</td>
<td>7.3 doublet (broad)</td>
<td>6.19 double doublet</td>
<td>4.35 multiplet</td>
<td>4.75 doublet</td>
</tr>
<tr>
<td></td>
<td>$J_{1,2} = 10$</td>
<td>$J_{2,3} = 2$</td>
<td>$J_{3,4} = 10$</td>
<td></td>
</tr>
<tr>
<td>unsubstituted-3,4,17-triol</td>
<td>7.24 double doublet</td>
<td>6.12 double doublet</td>
<td>4.30 multiplet</td>
<td>4.69 doublet</td>
</tr>
<tr>
<td></td>
<td>$J_{1,2} = 10$</td>
<td>$J_{2,3} = 2$</td>
<td>$J_{3,4} = 11$</td>
<td></td>
</tr>
</tbody>
</table>
Activation of 11-methyl-17-ketone

pounds it is clear that the 3,4-diol system is trans and diequatorial. Both 3,4-dihydroxy-17-ketones had similar circular dichroism curves of the same sign; the unsubstituted 3,4,17-triol had a circular dichroism curve similar to and of the same sign as that of trans-1R,2R-dihydroxy-1,2-dihydrophenanthrene. Taken together these findings point to [3R,4R]-dihydroxy-3,4,15,16-tetrahydrocyclopenta[a]phenanthren-17-one as the structure of the proximate carcinogen (457) which is further oxidized at C-1, C-2 and then interacts with DNA mainly as illustrated in Fig. 67.

The in vitro metabolism of this carcinogen (26) has also been studied with the human hepatoma cell line HepG2 (Bhatt, 1986) in connection with the ability of these cells to activate this compound to a mutagen in the V79 Chinese hamster cell system as described in Chapter 6. These cells are highly induceable (Dawson et al., 1985) and after pretreatment with benzanthracene or Arochlor microsomes prepared from them gave the range of metabolites seen with rat liver microsomes, including a large amount of the proximate carcinogen (457). However, in addition products from enzymatic reduction of the 17-ketone function were also observed. Thus with uninduced microsomes the major metabolite was the 17-alcohol (16,17-dihydro-11-methyl-15H-cyclopenta[a]phenanthren-17-ol, 448), while with induced microsomes the 16,17-diol and a phenol, probably the 4,15,17-triol, were also identified. The reductase responsible for 17-ketone reduction appeared to be largely present in the supernatant for when the microsomes were carefully washed these products were minimized. After exposure to these cells the carcinogen (26) bound to added DNA to give the adduct peaks A and B on LH20 chromatography, apparently identical with those found after activation with rat liver preparations. Little metabolism was seen when hamster embryo cells were used, whether they were induced or not, and this

Fig. 67. Proposed metabolic route leading to the ultimate carcinogen and its main deoxyguanosine adduct.
explains why these cells fail to activate this carcinogen to a mutagen in V79 cells. In this respect this carcinogenic cyclopenta[a]phenanthrenone appears to differ from hydrocarbons such as benzo[a]pyrene, but the reason for this is not clear at present.

Mouse embryo cells derived from T.O. mice also activate this carcinogen (26). In a study of the binding of a number of polycyclic hydrocarbon carcinogens to nuclear and mitochondrial DNA (Allen, 1979; Allen and Coombs, 1980) in these cells, the familiar patterns of adduct peaks were again observed. However, the mitochondrial DNA binding index (\(\mu\)mol of compound bound per mol of DNA phosphorus) was over 500 times higher than was this index for the nuclear DNA. This was found to be common also for the other carcinogens, where the ratio ranged between 50-fold and 250-fold.

7.2 In vivo metabolism of 15,16-dihydro-11-methylcyclopenta[a]-phenanthren-17-one in the rat

Prior to the work on in vitro metabolism just described urinary metabolites of this carcinogen (26) and its inactive parent ketone (15,16-dihydrocyclopenta[a]phenanthren-17-one (4) were investigated in the rat. Adult Sprague-Dawley males were injected (100 mg/animal) intraperitoneally with these compounds, labelled with \(^{14}\)C or tritium, and urine was collected for three days when excretion of radioactivity was largely completed. It was found advantageous to extract the metabolites from the crude urine by first absorbing them on to neutral charcoal, from which they could be recovered by elution with methanol containing ammonia. The extract was then evaporated to a syrup which was mixed with water and re-extracted with ethyl acetate. This work was, of course, carried out before the advent of hplc, and the metabolites obtained in this way were compared by careful semiquantitative tlc on silica gel-coated glass plates. Each compound gave over a dozen metabolites which formed discrete bands on the plate, and could be located by their radioactivity (after autoradiography on X-ray film), their fluorescence, and by various colour reactions. By removal of the material comprising these bands from the plate, and eluting it with ethanol ultraviolet spectra could be obtained. The result of this survey is shown in Table 30. The most noticeable feature is the remarkable similarity between the patterns of urinary metabolites of these two ketones. In most cases this similarity extended to a number of colour reactions (not shown) carried out by spraying the plates. The amounts in the bands, as judged by their radioactivity, were also similar for each pair with the exceptions shown in the second column. With the two pairs of phenols (bands 4 and 5) in each
Table 30. Properties of urinary metabolites of ketones (4) and (26) separated by tlc

<table>
<thead>
<tr>
<th>Radioactive band derived from ketone (4) or (26)</th>
<th>Percentage of total radioactivity</th>
<th>Rp</th>
<th>Fluorescence</th>
<th>Ultraviolet spectra</th>
<th>Anion</th>
<th>After reduction (neutral)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neutral [λmax (nm)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (4)</td>
<td>—</td>
<td>0.80</td>
<td>purple</td>
<td>265, 286, 297</td>
<td>unchanged</td>
<td>255, 284, 296</td>
</tr>
<tr>
<td>2 (4)</td>
<td>—</td>
<td>0.79</td>
<td>blue-purple</td>
<td>264, 286, 297</td>
<td>unchanged</td>
<td>256, 283, 294</td>
</tr>
<tr>
<td>3 (26)</td>
<td>—</td>
<td>0.64</td>
<td>yellow</td>
<td>262</td>
<td>unchanged</td>
<td>256</td>
</tr>
<tr>
<td>4 (26)</td>
<td>15-20</td>
<td>0.64</td>
<td>yellow</td>
<td>267, 288, 299</td>
<td>unchanged</td>
<td>255</td>
</tr>
<tr>
<td>5 (4)</td>
<td>15-20</td>
<td>0.61</td>
<td>purple</td>
<td>266, 296</td>
<td>280</td>
<td>260</td>
</tr>
<tr>
<td>6 (26)</td>
<td>15-20</td>
<td>0.53</td>
<td>yellow</td>
<td>267, 300</td>
<td>281</td>
<td>260</td>
</tr>
<tr>
<td>7 (26)</td>
<td>5</td>
<td>0.52</td>
<td>yellow</td>
<td>263</td>
<td>276</td>
<td>259</td>
</tr>
<tr>
<td>8 (4)</td>
<td>5</td>
<td>0.50</td>
<td>green</td>
<td>264</td>
<td>278</td>
<td>259</td>
</tr>
<tr>
<td>9 (26)</td>
<td>5</td>
<td>0.50</td>
<td>green</td>
<td>262</td>
<td>unchanged</td>
<td>—</td>
</tr>
<tr>
<td>10 (4)</td>
<td>50</td>
<td>0.45</td>
<td>yellow</td>
<td>268, 288, 301</td>
<td>unchanged</td>
<td>255, 296, 259</td>
</tr>
<tr>
<td>11 (4)</td>
<td>—</td>
<td>0.40</td>
<td>purple</td>
<td>261, 269</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12 (4)</td>
<td>—</td>
<td>0.32</td>
<td>yellow</td>
<td>261, 269</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13 (26)</td>
<td>—</td>
<td>0.20</td>
<td>purple</td>
<td>254, 260</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
case 3–4 times as much was obtained from the parent ketone (4) as from
its 11-methyl homologue (26), accounting for 30–40% of the total
radioactivity for the former. By contrast the amounts of the similar
metabolites in band 10 differed greatly; only a trace was obtained from
(4), whereas it was a major metabolite from (26), accounting for about
50% of the total. The structure of this metabolite was therefore
determined since this difference between the carcinogen and its inactive
parent seemed possibly of significance.

To this end a total of 20 g of the 11-methyl-17-ketone (26) labelled with
tracer amounts of tritium were injected into rats, and metabolites were
isolated from their urine as already described. The mixture was then
chromatographed on silica gel columns, finally to give the band-10
metabolite, after further purification by recrystallization, as 120 mg of
pale fawn needles, mp 120°C decomp. It was optically active, \([\alpha]_D^{25°} -214°
(c. 0.134, ethanol), and its ultraviolet characteristics, \(\lambda_{\text{max}}\) 267 (4.57),
320 (4.03), 332 (4.04), 352 (3.67), 370 (3.61) nm, were very similar
to those of 11-methyl-1,2,15,16-tetrahydro-1,2,15-trihydroxy-cyclo-
penta[a]phenanthren-17-one (454, metabolite a, Table 26) later
isolated from in vitro metabolism of (26). Combustion analysis estab-
lished the formula \(C_{18}H_{16}O_4\) (see Fig. 68); the mass spectrum was
informative, although the molecular ion was absent:

<table>
<thead>
<tr>
<th>(m/z)</th>
<th>% abundance</th>
<th>Derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>294.0892</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Found</td>
<td>294.0893</td>
<td>M* - H_2O</td>
</tr>
<tr>
<td></td>
<td>278</td>
<td>M* - H_2O-O</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>M* - 2H_2O-O</td>
</tr>
<tr>
<td></td>
<td>232</td>
<td>M* - 2H_2O-O-CO</td>
</tr>
</tbody>
</table>

Fig. 68. The structure (460) of the main urinary metabolite of the carcinogen
(26) in the rat, and some of its chemically derived products.
The fifth 'non-functional' oxygen atom was therefore thermally labile; it was also eliminated on acetylation with acetic anhydride in pyridine at room temperature, giving the triacetate C_{24}H_{22}O_{7} (461) and on mild hydrogenation yielding the dihydro-deoxy derivative C_{19}H_{19}O_{4} (462). Surprisingly, it was, however, retained when the metabolite was submitted to acid-catalysed dehydration (2.5-M sulphuric acid at 100°C for 1 h) to give the phenol C_{18}H_{14}O_{4} (463). A full nmr study finally established the structures shown in Fig. 68 for these compounds (Coombs and Crawley, 1974, 1975) (see Chapter 5 under the appropriate molecular formula for details). Precedents exist in the chemical literature both for loss of the bridge oxygen in oxepins of this type (460) both thermally and on hydrogenation as well as its retention in strongly acidic media, but not for its loss on mild acetylation; the mechanism by which this occurs remains a mystery. The occurrence of a structure such as (460) as a metabolite of a polycyclic aromatic compound is, to the best of our knowledge, unique. Its structure is closely related to that of the major metabolite (454) encountered during in vitro metabolism with hepatic preparations, and it is tempting to postulate that it is formed from this metabolite by further microbiological oxidation in the gut. It has not been tested for carcinogenicity, but in view of its close relationship to (454) which is itself inactive, and the fact that the epoxide-oxepin structure appears to be stable enough to withstand the long and complicated extraction and purification procedures used in its isolation, it seems unlikely that it would prove to be active.

7.3 Biological activation of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one

It has been known for 40 years that nearly all carcinogenic polycyclic aromatic hydrocarbons are related to the angular hydrocarbon phenanthrene rather than the linear hydrocarbon anthracene, but the reason for this has become apparent only recently. It now seems that for activity the hydrocarbon needs a 'bay region' (the extramolecular area bounded by C-1, C-10, C-9, and C-11 in a cyclopenta[a]phenanthrene) in order to allow metabolic formation of a bay-region diol-epoxide of the type already discussed. This is the ultimate carcinogenic species and it is perhaps hardly surprising that the 1-, 2-, 3-, and 4-methyl derivatives are inactive, because these A-ring substituents would be expected to interfere with the formation of the 3,4-diol-1,2-oxide. However, on these grounds it is less clear why the unsubstituted structure is inactive and becomes carcinogenic only on substitution at C-7 or C-11; an analogous situation also exists in the related chrysene and benz[a]anthracene series.
It was reasoned that the 1,11-methano compound (310), in which C-1 is joined covalently to C-11 by a methylene group, should not be carcinogenic for although it has a small substituent at C-11 favouring activity, the substituent at C-1 was expected to obstruct the formation of a bay-region diol-epoxide. The first indication that this reasoning was not sound came when it was discovered that this compound was weakly mutagenic towards *Salmonella typhimurium* TA100. It was therefore tested more thoroughly than usual for carcinogenicity, and in two identical initiation/promotion experiments appeared to possess initiating activity approaching that of its 11-methyl-17-ketone analogue (26), although it proved to be only moderately active as a complete carcinogen in two repeated-application experiments. An investigation into the metabolism of this compound (310) was therefore undertaken in an attempt to account for this unexpected result (Coombs *et al.*, 1982; Hadfield *et al.*, 1984a). The same strategy and tactics were employed as those that successfully unravelled the activation of the 11-methyl-17-ketone (26). After *in vitro* metabolism of the generally labelled [3H]-1,11-methano-17-ketone (310) metabolites were separated by hplc (Fig. 69) and the fractions (i)–(x) were assayed for mutagenicity in the Ames' test. This ketone is only weakly active in this test with TA100, giving under optimum conditions from two to three times the background number of revertants; nevertheless, the results of this comparison were clear (Table 31). Only fraction (iv) elicited a higher reversion rate than the starting material which was

Fig. 69. hplc Separation of *in vitro* metabolites of 15,16-dihydro-1,11-methanocyclopenta[al]phenanthren-17-one (310); equimolar amounts of fractions (i)–(x) were tested for mutagenicity (see Table 31).
Activation of 1, 11-methano-17-ketone

eluted in fraction (x). The structure of metabolite (b) contained in this fraction and purified to homogeneity by hplc was therefore studied, as were those of the other metabolites (a–g) obtained pure in sufficient quantity. Molecular formulae based on their mass spectra are shown in Table 32 together with selected ions of diagnostic value. All metabolites gave molecular ions except metabolite (b) which was, however, known to be a vicinal diol from other evidence (see below), and gave an abundant [M+H2O] ion; the analogous ion was in fact the base peak in the spectrum of the diol (a). Both metabolites also gave abundant ions due to

Table 31. Relative mutagenic response from equimolar amounts of metabolites contained in fractions (i)–(x) (Fig. 69)

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Relative mutagenicity (as percentage of fraction x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i)</td>
<td>18</td>
</tr>
<tr>
<td>(ii)</td>
<td>24</td>
</tr>
<tr>
<td>(iii)</td>
<td>23</td>
</tr>
<tr>
<td>(iv)</td>
<td>160</td>
</tr>
<tr>
<td>(v)</td>
<td>44</td>
</tr>
<tr>
<td>(vi)</td>
<td>28</td>
</tr>
<tr>
<td>(vii)</td>
<td>80</td>
</tr>
<tr>
<td>(viii)</td>
<td>59</td>
</tr>
<tr>
<td>(ix)</td>
<td>43</td>
</tr>
<tr>
<td>(x) (310)</td>
<td>100</td>
</tr>
</tbody>
</table>

a, based on radioactivity.

Table 32. Molecular formulae of metabolites (a–g) and selected mass spectral ions of diagnostic value

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Selected diagnostic ions [observed (calculated) (relative abundance, %)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) C_{18}H_{14}O_{3}</td>
<td>M⁺ 278.0954 (278.0943) (69.2%); M⁻H₂O (100%); M⁺-H₂O-C₇H₆O (80.7%)</td>
</tr>
<tr>
<td>(b) C_{18}H_{14}O_{3}</td>
<td>M⁺-H₂O 260.0711 (260.0837) (58.6%); M⁺-H₂O-C₇H₆O (58.6%)</td>
</tr>
<tr>
<td>(c) C_{18}H_{14}O_{3}</td>
<td>M⁺ 276.0794 (276.07864) (62%); M⁺-H₂O (100%)</td>
</tr>
<tr>
<td>(d) C_{18}H_{14}O_{3}</td>
<td>M⁺ 258 (33%); M⁺-CHO (17%)</td>
</tr>
<tr>
<td>(e) C_{18}H_{14}O_{3}</td>
<td>M⁺ 260 (100%); M⁺-C₇H₆O (70%)</td>
</tr>
<tr>
<td>(f) C_{18}H_{14}O_{3}</td>
<td>M⁺ 260 (100%)</td>
</tr>
<tr>
<td>(g) C_{18}H_{14}O_{3}</td>
<td>M⁺ 258.06808 (258.06770) (100%); M⁺-CO (28%); M⁺-2(CO) (91%)</td>
</tr>
<tr>
<td>(310) C_{18}H_{14}O_{3}</td>
<td>M⁺ 244 (57.3%); M⁺-C₇H₆O (100%)</td>
</tr>
</tbody>
</table>
Table 33. nmr Spectra of ring-A protons of metabolites (a) and (b) of the 1,11-methano-17-ketone (310)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>4.48 doublet ( (J_{3,4} = 5.5) )</th>
<th>6.46 double double ( (J_{3,4} = 9.5; J_{3,3} = 5.5) )</th>
<th>7.00 doublet ( (J_{3,4} = 9.5) )</th>
<th>4.18 broad singlet</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>6.14 multiplet ( (J_{2,3} = 3.5; J_{2,18} = 2.0) )</td>
<td>4.78 multiplet ( (J_{3,4} = 5.5; J_{3,3} = 3.5) )</td>
<td>6.02 doublet ( (J_{3,4} = 5.5) )</td>
<td>4.15 unresolved doublet</td>
</tr>
</tbody>
</table>
Activation of 1, 11-methano-17-ketone

further loss of 42 mass units, as did the original 1,11-methano-17-ketone (310). This is not a common degradative pathway in 17-ketocyclopenta[α]phenanthrenes, nor was it seen with 4,5-methylene-phenanthrene itself. The loss seems to be due to the formal elimination of ketene from the five-membered ring, and appears to be characteristic of this ring lacking further substitution in these 1,11-methano compounds. Although the ultraviolet spectrum of the 1,11-methano-17-ketone (310) [$\lambda_{max}$ 266.5 (4.77), 277 (4.76) nm] differs appreciably from that of (say) the 11-methyl-17-ketone (26) [$\lambda_{max}$ 264 (4.83) nm], the spectra of metabolites (a) and (b) strongly resembled those of, respectively, the 1,2- and 3,4-dihydrodiols of the latter, and this was fully confirmed by their nmr spectra listed in Table 33. The structures of the other metabolites were deduced from similar combinations of mass and ultraviolet spectral information. For example, the mono-ol (e) had a strong M$^+$-42 ion (unsubstituted 17-keto D-ring) and ultraviolet spectrum similar to that of the original ketone (310); after borohydride reduction the spectra of (e) and the diketone (g) were identical. These are therefore both 18-substituted-17-ketones. The ultraviolet spectrum of (d) differed from all the others in that it was altered on addition of alkali (enolizable β-diketone). The two hydroxyl groups in the diol (e) (ultraviolet spectrum similar to the original ketone) were at C-15 and C-18 because this diol had an intense M$^+$-H$_2$O ion, a characteristic of the 15-hydroxy-, but not 16-hydroxy-11-methyl-17-ketone. The proposed structures of all these metabolites are illustrated in Fig. 70. Thus again with this compound (310) the most mutagenic metabolite was a trans-3,4-dihydrodiol, but here the coupling constant, 5.5 Hz, was smaller than that associated with trans-3,4-dihydrodiols from other cyclopenta[α]phenanthren-17-ones (10–12 Hz) indicating that it possessed a conformation mid-way between diaxial and diequatorial. After metabolic activation in vitro (rat liver microsomes) the 1,11-methano-17-ketone (310) bound to added DNA which, after isolation and enzymatic hydrolysis, gave essentially a single nucleoside adduct. An identical result was obtained with DNA isolated from the skin of mice treated topically with this compound in vivo. As with the main adduct from the 11-methyl-17-ketone (26), this eluted from an LH20 column after the common nucleosides, and appeared significantly earlier in the presence of borate. This strongly suggests that the 3,4-dihydrodiol (metabolite b) is the proximate carcinogen, and that it is further converted by metabolism into an anti-3,4-dihydroxy-1,2-oxide. That the 1,2-double bond in (310) is capable of metabolism via a 1,2-oxide is proved by the isolation of the 1,2-dihydrodiol (metabolite a). The bay region in the 1,11-methano-17-ketone (310) is obstructed
mainly within the plane of the molecule. The bridge methylene protons are magnetically equivalent because they resonate as a singlet, and this part of the molecule is therefore essentially flat (as will be described later, this was also proved by X-ray crystallography). It therefore seems that the active oxygen species is inserted into the 1,2-double bond of this molecule from either above or below this plane in the active site of the oxidase. This helps to account for the rapid fall-off in carcinogenic activity with chain length at C-11 observed in both the alkyl and alkoxy series because these substituents would not be confined to this plane. The carcinogenicity of (310) further emphasizes the role of small substituents at C-11 in endowing cyclopenta[a]phenanthrenes with biological activity. In retrospect it is not entirely surprising that this compound is a carcinogen because as long ago as 1946 it was shown that the corresponding bridged analogue of 7,12-dimethylbenz[a]anthracene, namely 1,12-methano-7-methylbenz[a]anthracene, yielded tumours in mice after injection (Dunlap and Warren, 1946). Much more recently metabolic oxidation at methyl-substituted double bonds in both 7-methylbenzo[a]pyrene (Kinoshita et al., 1982) and 8-methylbenz[a]anthracene (Yang et al., 1981) has been reported.

Fig. 70. Structures proposed for the in vitro metabolites of the 1,11-methano-17-ketone (310).
Comparative in vitro metabolism

7.4 Comparative in vitro metabolism of 15,16-dihydrocyclopenta[a]phenanthren-17-one and some of its methyl homologues

The marked structure/biological activity relationships among cyclopenta[a]phenanthrenes are, of course, mirrored in other polycyclic aromatic systems. For example, in both the chrysene and benz[a]anthracene series methyl substitution at the bay region in the position other than on the terminal benzo ring leads to a considerable enhancement in carcinogenicity, but there does not yet seem to be any general agreement on the reason for this. In the series of 17-keto-cyclopenta[a]phenanthrenes the isomers are very similar to one another in most of their physical properties, including solubility which is likely to be of importance in their pharmacodynamics. Unlike polycyclic aromatic hydrocarbons these polycyclic ketones are not appreciably soluble in hexane; they are, however, reasonably soluble in aromatic hydrocarbons and in alcohols, and are readily soluble in dipolar solvents such as chloroform and dimethylsulphoxide. Their solubility in water is less than 0.5 μg/mL, but is increased somewhat by the presence of protein as, for example, in tissue culture media. No quantitative comparison of their solubility has been made, but the virtual identity of their chromatographic properties in a number of solvent systems suggests that this is closely similar for the isomeric members of this series, and probably their tissue distribution in animals will not therefore be dissimilar. In their chemistry, too, the inactive 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) and its carcinogenic 11-methyl homologue show close similarity (see Chapter 4). However, an apparent major difference in the urinary metabolites of these two ketones has already been noted, and made a comparative study of the in vitro metabolism of compounds of this series imperative since it was evident that metabolism was intimately connected with the expression of their biological effects. In contemplating this it seemed initially that the 1-, 2-, 3-, and 4-methyl derivatives could be excluded, for it appeared likely that these ring-A substituents would block diol-epoxide formation. However, when it was discovered that the C-1 methylene group in 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one (310) was unable to do this it was decided to include in the study the 1-methyl-17-ketone (302), especially as the cancer tests with this compound were not convincingly negative. The unsubstituted parent ketone (4) and its 6- and 12-methyl derivatives (306 and 130), all non-carcinogens, and the carcinogenic 7- and 11-methyl-17-ketones (230 and 26) and the 11,12-dimethyl-17-ketone (131) have also been investigated using the in vitro method with hepatic microsomes from 3-methylcholanthrene-induced rats as already described.
Table 34. Ultraviolet maxima of the main in vitro metabolites of the unsubstituted 17-ketone (4), and its 11-methyl (26), 12-methyl (130), and 11,12-dimethyl (131) derivatives separated by hplc as shown in Fig. 71

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>unsubstituted 17-ketone (4)</th>
<th>11-methyl-17-ketone (26)</th>
<th>12-methyl-17-ketone (130)</th>
<th>11,12-dimethyl-17-ketone (131)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>266, 320, 332, 336 (255, 264, 310, 347)</td>
<td>265, 322, 334, 373 (259, 267, 305)</td>
<td>268, 321, 333, 375 (257, 266, 310)</td>
<td>273, 323, 336, 366, 386 (261, 270, 328)</td>
</tr>
<tr>
<td>(b)</td>
<td>266, 316, 328, 365 (257, 265)</td>
<td>262, 306, 319, 332 (259, 267, 305)</td>
<td>261, 303, 315, 327 (257, 266)</td>
<td>—</td>
</tr>
<tr>
<td>(d)</td>
<td>278, 330, 360, 376 (253, 312, 328)</td>
<td>272, 327, 333, 370 (250, 326, 347)</td>
<td>268, 362, 374 (246, 322)</td>
<td>275, 320, 338, 372 (254, 320, 334, 350)</td>
</tr>
</tbody>
</table>

$\lambda_{\text{max}}$ (nm); peaks of highest intensity are underlined and spectra in parentheses were obtained following in situ reduction of the carbonyl group to the secondary alcohol.
As was anticipated from earlier tlc work it was found (Hadfield et al., 1984a) that the main metabolites of the unsubstituted ketone (4) were analogous to those of its 11-methyl derivative already described. Lacking the methyl group they were somewhat more polar, eluting about five minutes earlier from the reverse-phase hplc column than their 11-methyl counterparts. They were readily identified by their ultraviolet spectra (Table 34) which were very similar to those of the metabolites derived from the carcinogen (26). The most polar (Fig. 71) was again the 1,2,15-trihydroxy-17-ketone (a) followed by the 1,2-diol (b); the latter was formed in larger amount from this compound (4) than from its 11-methyl derivative (26), possibly because the methyl group in the latter partially hinders approach of the active site in the enzyme to the 1,2-double bond. After removal of the extra conjugation by reduction of the ketone to the secondary alcohol, the circular dichroism spectrum of the product had the same sign as phenanthrene-[1R,2R]-diol (Fig. 72); the absolute stereochemistry of this metabolite is therefore also [1R,2R] or 1α,2β as

Fig. 71. hplc Profiles of the in vitro metabolites formed from the unsubstituted 17-ketone (4) and its 11-methyl (26) and 12-methyl (130) derivatives.
shown. Next in order of decreasing polarity were the two 3,4,15- and 3,4,16-triols (c and d), followed by the 3,4-dihydrodiol (e) shown by its nmr spectrum (Table 29) to be diequatorial and by its circular dichroism spectrum (after borohydride reduction) to be \([3R,4R]\) or \(3α,4β\). It was further characterized by its mass spectrum which showed a molecular ion (\(M^+\) found 266.09379; \(C_{17}H_{14}O_3\) requires 266.09429) (81.4%), and \textit{inter alia} ions at 248 (\(M^+\)-H$_2$O, 100%), 200 (\(M^+\)-H$_2$O-CO, 72.8%). Finally a large amount of the 15-ol (f, 369) and a smaller quantity of the 16-ol (g, 345) were eluted, and characterized by comparison with the synthetic materials.

The 12-methyl-17-ketone (130) yielded a similar range of \textit{in vitro} metabolites (Fig. 71), and the 3,4-dihydrodiol (e) was again conspicuous. It was characterized by its ultraviolet spectrum and elution time (both virtually identical to those of its 11-methyl isomer) and its mass spectrum (\(M^+\) found 280.10949; \(C_{18}H_{16}O_3\) requires 280.10994, 8.6%; \(M^+\)-H$_2$O, 100%; \(M^+\)-H$_2$O-CO, 16.5%). Its nmr spectrum shown in Table 35 demonstrated that this diol was \textit{trans}-diequatorial, and again the circular dichroism spectrum showed that it was \([3R,4R]\) or \(3α,4β\). The additional methyl group in the 11,12-dimethyl-17-ketone (131) led to further retention on the reverse-phase column so that this ketone and its metabolites were eluted about five minutes later than the 11- or 12-methyl-17-ketone and their analogous metabolites. The range of major metabolites formed was the same as those (a–f) obtained from the other compounds, but the

Fig. 72. Circular dichroism spectra of phenanthrene-[1R,2R]-dihydrodiol and of the borohydride reduction product of the 1,2-dihydro-1,2-dihydroxy metabolite of (4).
Table 35. Chemical shifts (ppm) and coupling constants (Hz) of A-ring protons in trans-3,4-dihydrodiols derived from the 6-methyl- and 12-methyl-17-ketones by metabolism

<table>
<thead>
<tr>
<th>3,4-diol from:</th>
<th>H-1</th>
<th>H-2</th>
<th>H-3</th>
<th>H-4</th>
</tr>
</thead>
</table>
| 6-methyl-17-ketone (306) | 7.68 double doublet  
\(J_{1,2} = 9; J_{1,3} = 0.5\) | 6.52 double doublet  
\(J_{1,2} = 9; J_{1,3} = 4\) | 4.46 double doublet  
\(J_{2,3} = 4; J_{1,3} = 0.5\) | 5.06 singlet |
| 12-methyl-17-ketone (130) | 7.29 double doublet  
\(J_{1,2} = 10; J_{1,3} = 2\) | 6.21 double doublet  
\(J_{1,2} = 10; J_{2,3} = 2.5\) | 4.46 multiplet  
\(J_{3,4} = 11; J_{3,3} = 2.5; J_{1,2} = 2\) | (obscured by methyl signal) |
extra methyl group also caused the expected bathochromic shift in the ultraviolet maxima observed (see Table 34). From its elution time the 3,4-dihydriodiol formed from the 11,12-dimethyl compound (i.e., metabolite e) was trans-diequatorial, and its absolute stereochemistry was in all probability [3R,4R] like all the others. Under identical conditions of incubation, extraction, and chromatographic separation, the four 17-ketones gave these metabolites in the relative amounts shown in Table 36. Thus at very similar total percentage conversion, all four compounds (two carcinogens, and two non-carcinogens) yielded commensurate amounts of 3,4-dihydriodiol, which appeared to be trans and of the same conformation and absolute stereochemistry. Thus metabolism cannot account for the lack of carcinogenicity of the ketones (4) and (130).

The 6- and 7-methyl-17-ketones (306 and 230, respectively) have not been studied in such detail (Coombs et al., 1981), but examination of the hplc profiles of their in vitro metabolites showed a marked difference. Whereas the 6-methyl compound gave just three main metabolites, the 7-methyl ketone yielded many metabolites, all in small amounts. Amongst these was a trans-3,4-dihydriodiol with retention time and ultraviolet characteristics similar to those of the analogous 11-methyl-17-ketone-3,4-diol. It was further characterized by its mass spectrum: $M^+$, 280 (31.5%); $M^+\cdot H_2O$, 262 (100%); $M^+\cdot H_2O\cdot CO$, 234 (14%). After metabolic activation in vitro the 7-methyl-17-ketone (230) bound to calf thymus DNA added to the incubation to about half the extent of the 11-methyl isomer; this seems to account satisfactorily for its weaker carcinogenicity. The 6-methyl-17-ketone (306), on the other hand, failed to exhibit appreciable binding to DNA in vitro or in vivo (mouse skin) in line with its failure to

Table 36. Relative amounts of major metabolites formed from the unsubstituted -17-ketone (4), its 11-methyl (26), 12-methyl (130), and 11,12-dimethyl (131) derivatives under identical incubation conditions in vitro

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>(Compound) amount of metabolite as percentage of the total ethyl acetate extractable radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a)</td>
<td>(4) 11.2 (26) 8.0 (130) 5.2 (131) 0.9</td>
</tr>
<tr>
<td>(c)</td>
<td>(4) 9.2 (26) 5.7 (130) 12.1 (131) 6.3</td>
</tr>
<tr>
<td>(d)</td>
<td>(4) 4.2 (26) 4.0 (130) 6.6 (131) 5.3</td>
</tr>
<tr>
<td>(e)</td>
<td>(4) 11.6 (26) 9.2 (130) 7.7 (131) 11.1</td>
</tr>
<tr>
<td>(f)</td>
<td>(4) 4.3 (26) 10.8 (130) 1.7 (131) 9.2</td>
</tr>
<tr>
<td>Other metabolites</td>
<td>(4) 35.7 (26) 38.1 (130) 39.1 (131) 42.1</td>
</tr>
<tr>
<td>Unchanged compound</td>
<td>(4) 23.8 (26) 24.2 (130) 27.6 (131) 25.1</td>
</tr>
</tbody>
</table>
Comparative in vitro metabolism

Compounds were tested for their ability to evoke tumours in mice. Nevertheless, the major metabolite of this ketone, accounting for about 30% of the material in the extract, was a trans-3,4-dihydrodiol, $\lambda_{\text{max}}$ 271.5, 309, 322.5, 358, 373 nm. However, the total lack of magnetic coupling between H-3 and H-4 in its nmr spectrum (Table 35; see also Fig. 73) demonstrated that the bonds connecting these protons to C-3 and C-4 in this metabolite were at right angles to one another, and consequently the diol hydroxyl groups were diaxial. This was also confirmed by the polarity of this diol which eluted about 10 minutes earlier than the diequatorial 3,4-dihydrodiols formed by the other compounds. Further microsomal metabolism of this diol led to several more polar metabolites with ultraviolet spectra characteristic of 1,2,3,4-tetrahydro derivatives. However, if a 3,4-diol-1,2-epoxide was involved its half-life must have been exceedingly short because the 6-methyl-17-ketone did not bind significantly to DNA added to the incubation medium. Introduction of a methoxy group at C-6 in the 11-methyl-17-ketone (26) to yield (468) diminished its carcinogenicity drastically (Iball indices 46 and 14, respectively – see Table 12), again demonstrating the inhibitory effect of a peri substituent. In the hydrocarbon series 16,17-dihydro-7-methylcyclopenta[a]phenanthrene (94) is a weak carcinogen, but its 6,7-dimethyl analogue (105) is inactive (see Table 7). Also 5,12-dimethylchrysene (which has methyl groups in the bay region and the peri

Fig. 73. nmr Signals due to H-3 and H-4 of the metabolically derived 3,4-dihydroadiol of 15,16-dihydro-6-methycyclopenta[a]phenanthren-17-one (306).
positions equivalent to C-11 and C-6 in a cyclopenta[a]phenanthrene) is much less carcinogenic than 5-methylchrysene itself (Hecht et al., 1979). Probably any bulky group at the peri position (C-6 in cyclopenta[a]phenanthrenes) would force an adjacent 3,4-diol into the diaxial conformation, and this appears to have a deleterious effect on carcinogenicity.

The in vitro metabolism of the 1-methyl-17-ketone (302) has also been examined recently (Coombs et al., 1985) for the reasons already given. The profiles of metabolites (Fig. 74) obtained in the usual way, although not separated in quite the same way as shown in Fig. 71, did not bear much resemblance to those of the other isomers. In particular no less than six metabolites (hatched in the figure) were phenolic, whereas only traces of phenols were found before. Phenolic metabolites, even if their structure is unknown, are easy to identify because addition of a little alkali to the methanolic solution in the cuvette generates the anion with its own characteristic ultraviolet absorption spectrum. As can be seen from Table 37 three of these phenols (q, r, and x) were extremely similar, and the latter (x) was positively identified as 15,16-dihydro-4-hydroxy-1-methylcyclopenta[a]phenanthren-17-one (458) by comparison of its elution time and ultraviolet spectra (neutral, anion, and reduced) with those of the synthetic compound which had been completely characterized. The spectra of the other phenolic metabolites (n, s, and t) also shared some similarities with this compound. It therefore appears that a major pathway in the metabolism of the 1-methyl-17-ketone (302) involves epoxidation of the 3,4-double bond followed by rapid isomerization of 3,4-epoxide to the 4-phenol (metabolite x), rather than enzymatic opening to give a 3,4-dihydrodiol as observed with all the other compounds.

Fig. 74. hplc Profile of the in vitro metabolites of 15,16-dihydro-1-methylcyclopenta[a]phenanthren-17-one (302); the hatched peaks are phenolic.
Table 37. Ultraviolet maxima of metabolites (k–x) formed from the 1-methyl-17-ketone (302) (Fig. 74) ($\lambda_{\text{max}}, \text{nm}$; peaks of highest intensity underlined)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Neutral</th>
<th>Anion</th>
<th>Reduced</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k)</td>
<td>259, 277, 290, 301</td>
<td>—</td>
<td>236, 259</td>
<td>1,2,3,4-tetraol</td>
</tr>
<tr>
<td>(l)</td>
<td>269, 331, 337, 368</td>
<td>—</td>
<td>258, 267, 305</td>
<td>1,2,15(or 16)tritol</td>
</tr>
<tr>
<td>(m)</td>
<td>269, 279, 298</td>
<td>—</td>
<td>257, 267, 294, 303</td>
<td>CPP-triol&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(n)</td>
<td>264, 297</td>
<td>258, 293</td>
<td>248, 267, 273, 284, 293</td>
<td>Phenol</td>
</tr>
<tr>
<td>(o)</td>
<td>270, 302, 359, 367</td>
<td>—</td>
<td>259, 282, 292, 304</td>
<td>CPP-diol&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(p)</td>
<td>268, 302, 369</td>
<td>—</td>
<td>259, 303, 363</td>
<td>CPP-diol&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(q)</td>
<td>254, 278, 303</td>
<td>261, 290, 324</td>
<td>249, 282, 296, 318, 346, 365</td>
<td>Phenol</td>
</tr>
<tr>
<td>(r)</td>
<td>253, 277, 301</td>
<td>262, 291, 325</td>
<td>249, 255, 281, 344, 361</td>
<td>Phenol</td>
</tr>
<tr>
<td>(s)</td>
<td>243, 267, 293</td>
<td>262, 291</td>
<td>248, 258, 281, 291</td>
<td>Phenol</td>
</tr>
<tr>
<td>(t)</td>
<td>252, 276, 299</td>
<td>268, 290, 301</td>
<td>247, 258, 266, 292</td>
<td>Phenol</td>
</tr>
<tr>
<td>(u)</td>
<td>266, 284, 302, 357, 375</td>
<td>—</td>
<td>258, 281, 292, 304</td>
<td>CPP-o&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(v)</td>
<td>270, 285, 303, 358, 375</td>
<td>—</td>
<td>256, 281, 292, 303</td>
<td>CPP-o&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(w)</td>
<td>268, 290, 303</td>
<td>—</td>
<td>256, 282, 292, 305</td>
<td>CPP-o&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(x)</td>
<td>270, 305</td>
<td>260, 284, 324</td>
<td>259, 285, 300, 320</td>
<td>4-phenol</td>
</tr>
<tr>
<td>(302)</td>
<td>266, 288, 303, 359, 375</td>
<td>—</td>
<td>256, 282, 293, 305, 338, 353</td>
<td>1-methyl-17-ketone</td>
</tr>
</tbody>
</table>

<sup>a</sup> CPP indicates that the metabolite has a typical 15,16-dihydrocyclopenta[a]phenanthrene chromophore.
Further hydroxylation of this metabolite then leads to the other, more polar, phenols. Presumably isomerization is promoted by electron release by the p-methyl group for the reverse, stabilization of epoxides towards isomerization, is known to be favoured by electron withdrawal (Chiasson and Berchtold, 1977). Another six metabolites (designated ccp-diol, etc. in the Table) appeared to be hydroxylated derivatives retaining the intact phenanthrene chromophore; their extents of hydroxylation can be guessed from their relative elution times, but an extensive nmr and mass spectral investigation would be needed to establish their exact structures. Only two metabolites, (k) and (l), had spectra suggesting partial unsaturation. Metabolite (l) was obviously a 1,2-dihydrodiol from the characteristic splitting of the ultraviolet maximum into two peaks of similar intensity (λ\text{max} 258, 267 nm) on borohydride reduction; from its polarity it was probably further hydroxylated at C-15, C-16, or at the methyl group. Thus again there is evidence for enzymatic attack at the carbon-substituted double bond at C-1,2. The spectra of metabolite (k) were those typical of a 1,2,3,4-tetrahydro-17-ketone; this compound may therefore be a 1,2,3,4-tetrahydro-tetraol formed either by further hydroxylation of 1,2-diol or of a 3,4-diol. The former seems the more likely because there is no evidence of a 3,4-dihydro-diol, and this seems to account adequately for the 1-methyl-17-ketone (302) being essentially inactive as a carcinogen and mutagen.

Thus to summarize, metabolic studies have shown that the carcinogenic 11-methyl-, 7-methyl-, and 11,12-dimethyl-17-ketones all yield trans-diequatorial 3,4-dihydrodiols, presumably capable of further transformation to diol-epoxides since they bind to DNA after metabolism. The 1-methyl and 6-methyl-17-ketones are non-carcinogens because, for different reasons, they do not form diol-epoxides of this sort. This leaves the unsubstituted and 12-methyl-17-ketones which are anomalous because they form trans-[3R,4R]diequatorial diols and yet are not carcinogens.

7.5 Formation of DNA adducts, and their persistence

Further progress in understanding these structure/activity relationships came from examining the binding of some of these compounds to DNA. The unsubstituted-17-ketone (4) is a bacterial mutagen although it is not carcinogenic. After \textit{in vitro} metabolic activation it bound to added DNA, albeit to a lesser extent than the 11-methyl ketone. The pattern of nucleoside adducts separated by Sephadex chromatography was similar to those given by the latter (see Fig. 63). Originally (Table 21) the 12-methyl-17-ketone was classed as a non-mutagen; however, a
more recent study (Hadfield, 1983) disclosed weak activity with *Salmonella typhimurium* TA100. After metabolic activation *in vitro* this compound also bound to added DNA, but to only about 15% of the extent of the 11-methyl isomer (Russell *et al.*, 1985). It yielded the familiar pattern of nucleosides except that peak A was almost the same size as peak B. When the binding of these compounds to mouse skin DNA was measured *in vivo* as before the following results were obtained, where DNA binding is quoted as nmol of compound/mol of DNA phosphorus in peaks A and B:

<table>
<thead>
<tr>
<th>Compound</th>
<th>DNA binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted-17-ketone (1)</td>
<td>19</td>
</tr>
<tr>
<td>11-methyl-17-ketone (26)</td>
<td>458</td>
</tr>
<tr>
<td>12-methyl-17-ketone (130)</td>
<td>155</td>
</tr>
<tr>
<td>11,12-dimethyl-17-ketone (131)</td>
<td>974</td>
</tr>
</tbody>
</table>

Thus the unsubstituted-17-ketone essentially failed to bind covalently to DNA *in vivo* in the target tissue, and this seems to explain its failure to act as a carcinogen. The 12-methyl-17-ketone, however, bound to DNA *in vivo* to about one-third of the extent of the 11-methyl isomer, i.e., to a greater extent than it did *in vitro*. When in each case the major adduct B was chromatographed on Sephadex LH20 using borate buffer (Hadfield *et al.*, 1984a), the 12-methyl adduct showed the usual early elution expected if it were derived from an anti-diol-epoxide. By contrast the adduct from the unsubstituted ketone showed no such effect (Fig. 75). This experiment was repeated several times, always with the same result, and on the face of it seems to indicate that this adduct is derived from a syn-diol-epoxide as outlined in Fig. 66. However, further experiments are needed to confirm this surprising result. By analogy with benzo[a]pyrene only the anti-diol-epoxides might be expected to exhibit carcinogenicity (Levin *et al.*, 1977; Buening *et al.*, 1978) although syn-diol-epoxides are mutagenic to bacteria (Wood *et al.*, 1977).

This still left the lack of carcinogenicity of the 12-methyl-17-ketone unexplained for at the topical dose of 1000 nmol on mouse skin this compound gave a DNA binding ratio equivalent to that given by about 340 nmol of its 11-methyl isomer, and at this dose on mouse skin the latter is strongly carcinogenic (Russell *et al.*, 1985). However, an interesting result was obtained when the persistence of these DNA adducts was investigated.

The rate of formation and persistence of DNA adducts of the carcinogenic 11-methyl-17-ketone was studied by Abbott and Crew (1981). They found that after topical application (400 µg per mouse, to the dorsal skin)
or intramuscular injection (3 μg per mouse, into the shoulder) binding to the DNA of skin, lung, and liver reached a maximum at 1–2 days, and then declined. The half-life in skin and lung, targets for this carcinogen, was about 7 days, whereas it was only 2.5 days for liver which is not a target (Fig. 76). By labelling the skin with tritiated thymidine it was shown that labelled DNA was lost from this tissue with a half-life of about 6 days. Hence there is little evidence for active repair of the 11-methyl lesions in skin whereas they are rapidly eliminated from liver. When this type of experiment was extended to the 12-methyl- (Russell et al., 1985) and 11,12-dimethyl-17-ketones (Furn, personal communication, 1985) (1000 nmol/mouse, topical), maximum binding was again found at about 2 days, but loss of adducts from the skin occurred with a half-life of 3.5

Fig. 75. Elution of the main DNA adducts formed by the unsubstituted (4) and 12-methyl-17-ketones (130) from Sephadex LH20 columns with Tris and borate buffers of the same pH and molarity.
days for the former while it was about 7 days for the latter, like the result for the 11-methyl-17-ketone. Thus it appears the 12-methyl-17-ketone is not carcinogenic because the DNA damage it causes in skin is largely repaired before cell division occurs. This is another unexpected result that needs confirmation.

7.6 Summary

Carcinogens of the 17-oxocyclopenta[a]phenanthrene series are not mutagenic in the absence of prior metabolism. This suggests that the observed carcinogenicity/chemical structure relationships might, at least in part, be related to the correct metabolic activation of these molecules and, as has been outlined above, this indeed proves to be the case. These compounds are biologically activated like polycyclic aromatic hydrocarbons, through benzo-ring oxidation. Specifically, epoxidation of the 3,4-double bond followed by enzyme-induced hydrolytic ring opening to give a diequatorial \textit{trans}-3α,4β-dihydrodiol is required. This metabolite must then be further oxidized at C-1,2 to an \textit{anti}-3α,4β-diol-1α,2α-epoxide, which must be stable enough to avoid reaction with the nucleophiles such as water present in the environment in which it is

Fig. 76. Loss of \textsuperscript{3}H-labelled DNA adducts of the carcinogen (26) from mouse skin, lung, and liver; total amounts of the adducts were estimated from the adduct peaks A and B eluting from Sephadex columns (see Fig. 63). The half lives (\(t_1\)) were not adjusted for the rate of DNA turnover in these tissues, about six days for skin and lung, and much longer for liver (Abbott and Crew, 1981).

![Graph showing the binding ratio in liver and lung vs. days after treatment]
produced. It must also survive transport through the nuclear membrane and into the cell’s genetic material in the nucleus. There it must be capable of reacting covalently with specific nucleoside bases to give adducts that resist removal by the efficient DNA repair enzymes, and be able to cause mutations that ultimately lead to abnormal and uncontrolled cell division, possibly by activation of a cellular oncogene. All these requirements must be met in turn, and it is consequently clear why most of the methyl isomers (for instance) are inactive. On the other hand, this information does not help us to understand why 7- and 11-methyl substitution are uniquely able to lead to diol-epoxides which fulfil all these requirements. This problem, which applies also to other polycyclic aromatic systems and has recently been discussed (DiGiovanni et al., 1983), will be mentioned again in Chapter 8.

7.7 References
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formed from the metabolically activated carcinogen 15,16-dihydro-11-

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X-ray crystallography of some cyclopenta[a]-phenanthrenes: an apparent correlation between molecular strain and carcinogenicity

8.1 Early work

We usually think of X-ray crystallography as being a technique which has been developed to its present high state of sophistication over the last 25 years. However, its origins go back much further, and in fact one of its earliest uses was to help establish the structure of sterols. In 1932 Bernal studied the X-ray diffraction patterns of crystals of several sterols, and from their experimentally established cell dimensions deduced that these molecules were 17–20 Å long, 7–8 Å wide, and 5 Å thick. The original Windaus formula (Chapter 1, Fig. 3) would have required these dimensions to be approximately 18.0 × 7.0 × 8.5 Å, making the molecules too thick to fit into the unit cell derived from the X-ray diffraction data. The same conclusion was also reached from a study of the molecular cross-section sizes derived from the surface area of monolayer films by Adam (1930). Diels' hydrocarbon had, of course, previously been isolated from the products of selenium dehydrogenation of cholesterol and other sterols and its identification as a cyclopenta[a]phenanthrene had strengthened this evidence for a revised steroid structure. However, at that time uncertainty still existed as to whether this hydrocarbon was 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) or its 17-methyl homologue (7) (Fig. 77), and Bernal set out to examine
this question by X-ray crystallography (Bernal and Crowfoot, 1935). For comparison the synthetic parent hydrocarbon (1) and Diels' hydrocarbon prepared by dehydrogenation of cholesterol (Diels et al., 1927), as well as the two specimens of the 17-methyl hydrocarbon synthesized by different methods by Bergmann and Hillemann (1933) and by Harper et al. (1934) were available. Suitable crystals were obtained by crystallization from the melt by the hot-wire method (Bernal and Crowfoot, 1933) because in many cases the crystals which separated from solution were too small and imperfect for X-ray examination.

All four crystal samples were found to consist of 'lath-shaped molecules of width approximately 6 Å and thickness 4 Å packed together in parallel bundles'. Two characteristic crystalline forms were observed: monoclinic in which the planes of the rings were inclined to the basal plane, and orthorhombic with them approximately parallel to it. The parent hydrocarbon (1) crystallized in both forms and both were examined, while all three samples of the 17-methyl homologue formed only monoclinic crystals and appeared to be crystallographically identical with one another within the limits of the experimental method (Table 38). It was therefore concluded that Diels' hydrocarbon was definitely not the parent compound (1), and it was observed (correctly) that the small differences in melting points and crystal habit found between the three samples of the 17-methyl derivative were probably due 'to the presence of impurities, possibly in very small quantities'. For this compound the unit cell had a density = 1.185 ± 0.005 g/cm³ indicating that it contained four molecules of molecular weight 232 ± 3 (C_{18}H_{16} requires 232); the molecules were arranged in the crystal as shown in Fig. 78.

The parent hydrocarbon (1) was also studied by Iball (1935) who pointed out that the X-ray intensities found were remarkably similar to those observed for crystals of chrysene, and proposed the structure of the

<table>
<thead>
<tr>
<th>Hydrocarbon</th>
<th>Space group</th>
<th>Cell dimensions (Å)</th>
<th>Probable molecular length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) C_{17}H_{14}</td>
<td>B2/a</td>
<td>18.2 6.05 21.2</td>
<td>11.6</td>
</tr>
<tr>
<td>(1) C_{17}H_{14}</td>
<td>Aba</td>
<td>8.10 6.4 22.8</td>
<td>11.4</td>
</tr>
<tr>
<td>(7) C_{18}H_{16}</td>
<td>Aba</td>
<td>8.50 6.25 24.3</td>
<td>12.1</td>
</tr>
</tbody>
</table>

Table 38. Crystal data for 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) and Diels' hydrocarbon (7)
former on this basis. However, it was not until 20 years later that the positions of the carbon atoms could be located in this structure using the Fourier transformation (Entwhistle et al., 1954) which, because of the extensive computation required, became practicable only after the introduction of electronic digital computers. The definitive paper did not appear for another seven years (Entwhistle and Iball, 1961) and in the meantime another X-ray structure for this compound had been published (Basak and Basak, 1959). Iball's structure was based on Fourier synthesis with 50 terms and subsequent refinement of the atomic co-ordinates by four cycles of least-squares calculations, reducing the reliability factor finally to $R = 0.108$. The reliability factor (discrepancy index) is an estimate of the agreement between the measured diffraction pattern intensities and those calculated for a particular model of arrangement of atoms in the repeat unit; values of 0.06–0.02 are considered good for present-day structural determinations (Glusker and Trueblood, 1985).

Basaks' results were obtained from a study restricted to Fourier synthesis

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig78.png}
\caption{Arrangement of the molecules of 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) in the orthorhombic and monoclinic crystals (Bernal and Crowfoot, 1955).}
\end{figure}
in two zones with no refinement of the co-ordinates \((R = 0.28)\) and in many instances the bond lengths differed appreciably from those of Iball, although the overall structure was the same (see Table 39). The K-region C–C bond C(6)–C(7) was short, as found for other aromatic compounds containing a phenanthrene nucleus. The bonds joining the five-membered ring to the aromatic system C(14)–C(15) and C(17)–C(13) were intermediate in length between a single aliphatic single bond and an aromatic bond, whereas the other cyclopentene bonds C(15)–C(16) and C(16)–C(17) were rather longer than a normal aliphatic single bond. The aromatic rings in this molecule were essentially flat, but carbon atoms C(15) and C(17) bonded to them were 0.1 Å below the plane while C(16) was 0.18 Å above. Bond angles are shown in Table 40. The exocyclic angle at C(9) is unusually large, no doubt relieving the interaction between the protons H(1) and H(11) in the bay region. No other X-ray crystallographic studies of cyclopenta[a]phenanthrenes seem to have been published since 1961 until the recent work on the 17-ketones described below.

Table 39. Carbon–carbon bond lengths for 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) obtained from X-ray crystallography measurements

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond length (Å)</th>
<th>Iball</th>
<th>Basak</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(1)–C(2)</td>
<td>1.39</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>C(2)–C(3)</td>
<td>1.34</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>C(3)–C(4)</td>
<td>1.40</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>C(4)–C(5)</td>
<td>1.40</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>C(5)–C(6)</td>
<td>1.45</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>C(6)–C(7)</td>
<td>1.36</td>
<td>1.39</td>
<td></td>
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<tr>
<td>C(7)–C(8)</td>
<td>1.44</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>C(8)–C(9)</td>
<td>1.45</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C(9)–C(10)</td>
<td>1.43</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>C(10)–C(5)</td>
<td>1.45</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C(9)–C(11)</td>
<td>1.41</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>C(11)–C(12)</td>
<td>1.41</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>C(12)–C(13)</td>
<td>1.44</td>
<td>1.42</td>
<td></td>
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<tr>
<td>C(13)–C(14)</td>
<td>1.36</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>C(14)–C(15)</td>
<td>1.52</td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>C(15)–C(16)</td>
<td>1.55</td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td>C(16)–C(17)</td>
<td>1.57</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>C(17)–C(13)</td>
<td>1.51</td>
<td>1.46</td>
<td></td>
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</table>
Table 40. Carbon–carbon bond angles (°) in 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1)

<table>
<thead>
<tr>
<th>Endocyclic angles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C(10)–C(1)–C(2)</td>
<td>120</td>
</tr>
<tr>
<td>C(1)–C(2)–C(3)</td>
<td>121</td>
</tr>
<tr>
<td>C(2)–C(3)–C(4)</td>
<td>122</td>
</tr>
<tr>
<td>C(3)–C(4)–C(5)</td>
<td>120</td>
</tr>
<tr>
<td>C(4)–C(5)–C(10)</td>
<td>118</td>
</tr>
<tr>
<td>C(5)–C(6)–C(7)</td>
<td>118</td>
</tr>
<tr>
<td>C(6)–C(5)–C(10)</td>
<td>121</td>
</tr>
<tr>
<td>C(6)–C(7)–C(8)</td>
<td>124</td>
</tr>
<tr>
<td>C(7)–C(8)–C(9)</td>
<td>118</td>
</tr>
<tr>
<td>C(8)–C(9)–C(10)</td>
<td>118</td>
</tr>
<tr>
<td>C(8)–C(9)–C(11)</td>
<td>119</td>
</tr>
<tr>
<td>C(9)–C(11)–C(12)</td>
<td>124</td>
</tr>
<tr>
<td>C(11)–C(12)–C(13)</td>
<td>116</td>
</tr>
<tr>
<td>C(12)–C(13)–C(14)</td>
<td>123</td>
</tr>
<tr>
<td>C(13)–C(14)–C(8)</td>
<td>121</td>
</tr>
<tr>
<td>C(13)–C(14)–C(15)</td>
<td>112</td>
</tr>
<tr>
<td>C(14)–C(15)–C(16)</td>
<td>102</td>
</tr>
<tr>
<td>C(15)–C(16)–C(17)</td>
<td>101</td>
</tr>
<tr>
<td>C(16)–C(17)–C(13)</td>
<td>101</td>
</tr>
<tr>
<td>C(17)–C(13)–C(14)</td>
<td>113</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exocyclic angles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C(4)–C(5)–C(6)</td>
<td>121</td>
</tr>
<tr>
<td>C(7)–C(8)–C(14)</td>
<td>123</td>
</tr>
<tr>
<td>C(8)–C(14)–C(15)</td>
<td>127</td>
</tr>
<tr>
<td>C(1)–C(10)–C(9)</td>
<td>121</td>
</tr>
<tr>
<td>C(10)–C(9)–C(11)</td>
<td>124</td>
</tr>
<tr>
<td>C(12)–C(13)–C(17)</td>
<td>124</td>
</tr>
</tbody>
</table>

8.2 The molecular structures of thirteen 15,16-dihydro-17-ketones derived from X-ray crystallography: the shapes of the molecules

As part of an in-depth study of 15,16-dihydrocyclopenta[a]phenanthren-17-one and its isomeric methyl homologues aimed at improving understanding of the structure/carcinogenicity relationships found among them, thirteen 17-ketones have been studied by X-ray crystallography. The compounds, chosen to cover the widest possible range of biological activities from inactive compounds to the strongest carcinogen, are shown in Fig. 79. Two quite separate investigations have been made; in the first (Clayton et al., 1983) the 11-methyl-17-ketone (26) and the six ketones shown in the top half of the figure were studied at the Northern Polytechnic, London, under the supervision of Dr Mary McPartlin using Cu-Kα radiation for (4) and Mo-Kα radiation for the rest. The six compounds shown in the bottom half of this figure, together with
ketone (26), were later investigated in the laboratory of Dr Jenny Glusker at the Institute for Cancer Research, Fox Chase, Philadelphia, using Mo-Kα radiation for the 11,12-dihydro compound (252) and Cu-Kα radiation for all the rest (Kashino et al., 1986). The carcinogen (26) (15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one) was therefore common to both groups and was studied using both types of radiation. The sample used at Fox Chase was synthesized there by a variation of the published method, and the structure of the compound was solved independently in the two laboratories without prior knowledge of one another’s results. It is satisfying to observe close agreement between the two sets of data; for example, the average differences between C–C distances and angles were within ±0.002 Å and ±0.11°, less than the quoted experimental errors. For this reason the two sets of

Fig. 79. The 13 cyclopenta[a]phenanthrenes studied by X-ray crystallography (Clayton et al., 1983; Kashino et al., 1986).
Table 41. Crystal data for the thirteen cyclopenta[a]phenanthrenes shown in Fig. 79

<table>
<thead>
<tr>
<th>Compound</th>
<th>Unsubstituted</th>
<th>1-Me</th>
<th>2-Me</th>
<th>6-Me</th>
<th>7-Me</th>
<th>11-Me</th>
<th>12-Me</th>
<th>7,11-diMe</th>
<th>11,12-diMe</th>
<th>11-Et</th>
<th>11-OMe</th>
<th>1,11-CH$_2$</th>
<th>11-Me-11,12-H$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
<td>C$<em>{22}$H$</em>{22}$O2</td>
<td>C$<em>{22}$H$</em>{22}$O</td>
</tr>
<tr>
<td>Mol. wt</td>
<td>232.35</td>
<td>246.31</td>
<td>246.31</td>
<td>246.31</td>
<td>246.31</td>
<td>246.31</td>
<td>246.31</td>
<td>260.33</td>
<td>260.33</td>
<td>260.33</td>
<td>260.33</td>
<td>260.33</td>
<td>262.31</td>
</tr>
<tr>
<td>mp (°C)</td>
<td>205-204</td>
<td>189-190</td>
<td>221-222</td>
<td>210.5-212</td>
<td>198-199</td>
<td>170-172</td>
<td>223</td>
<td>209-210</td>
<td>149-150</td>
<td>129-130</td>
<td>179</td>
<td>195</td>
<td>177-178</td>
</tr>
<tr>
<td>Space group</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>Pbca</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>P2$_1$/c</td>
<td>Pm</td>
<td>Fdd</td>
</tr>
<tr>
<td>β (°)</td>
<td>109.20</td>
<td>93.71</td>
<td>103.98</td>
<td>92.97</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>90.68</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>95.92</td>
<td></td>
</tr>
<tr>
<td>Calc. density (g cm$^{-3}$)</td>
<td>1.146</td>
<td>1.303</td>
<td>1.294</td>
<td>1.337</td>
<td>1.282</td>
<td>1.308</td>
<td>1.314</td>
<td>1.304</td>
<td>1.284</td>
<td>1.257</td>
<td>1.329</td>
<td>1.316</td>
<td>1.264</td>
</tr>
<tr>
<td>Volume (Å$^3$)</td>
<td>1089.2</td>
<td>2510.9</td>
<td>1263.6</td>
<td>1223.4</td>
<td>1275.8</td>
<td>2488.5</td>
<td>2489.2</td>
<td>1326.3</td>
<td>1346.5</td>
<td>2752.3</td>
<td>10.491</td>
<td>1304.5</td>
<td>2464.3</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Final R (%)</td>
<td>8.3</td>
<td>5.2</td>
<td>8.4</td>
<td>5.8</td>
<td>8.1</td>
<td>5.1</td>
<td>7.1</td>
<td>5.6</td>
<td>5.9</td>
<td>7.0</td>
<td>6.6</td>
<td>6.3</td>
<td>8.1</td>
</tr>
</tbody>
</table>

The angle β (°) is the angle between the a and c crystal axes; where it is not quoted it is 90.0° and the crystals are orthorhombic.
results are considered together for the purpose of the discussion which follows. Some fundamental crystal data for the thirteen 17-ketones shown in Fig. 79 are presented in Table 41. The structures were refined by full matrix least squares, and in all cases the final reliability factors were between 0.051 and 0.084. These, and the complete agreement between the two structures independently derived for the 11-methyl-17-ketone (26), give us reason to believe that the differences in bond lengths and angles calculated from the X-ray data are meaningful. They lead to an interesting correlation.

Carbon–carbon and carbon–oxygen bond lengths for the 13 compounds are listed in Table 42 together with estimated standard deviations in parentheses; Table 43 similarly displays the bond angles. It is noticeable that equivalent C–C bonds in these molecules differ from the average values by not more than 0.03 Å. This applies equally to the 1,11-methano compound (310) although it is obviously strained owing to the five-membered ring fused in the bay region. All have long (1.418–1.470 Å) C(9)–C(10) bay-region bonds, and short (1.320–1.371 Å) C(6)–C(7) K-region bonds, very similar to those observed in the hydrocarbon (1), 1.433 and 1.355 Å (Entwhistle and Iball, 1961) and in phenanthrene itself, 1.460 and 1.352 Å, respectively (Kay et al., 1971). In the 11,12-dihydro derivative (252) C(11)–C(12) is a single bond (1.538 Å) while C(9)–C(11) and C(13)–C(14) fall within the range of the aryl–methyl bond lengths (1.503–1.531 Å). The aryl–methylene C(1)–C(18) and C(11)–C(18) bond lengths in the 1,11-methylene compound (310) are similar. In contrast to the bond lengths, the bond angles show more variability among these 17-oxocyclopenta[a]phenanthrenes. For example, the endocyclic angles in six-membered rings (120.0° for a regular hexagon) vary between 115.8° and 128.1°. With two exceptions (the 7-methyl and 1,11-methano compounds, 230 and 310) the exocyclic bay-region angles C(1)–C(10)–C(9) and C(10)–C(9)–C(11) are greater than 120° (range 120.5–126.0°); this is particularly marked in compounds with substituents at C-1 or C-11 in the bay region. The 1,11-bridged compound (310) is quite anomalous in that these two angles are only 110.7° and 109.9°, resulting in considerable distortion from the normal pattern. This can readily be seen by superimposing it upon the parent molecule 15,16-dihydrocyclopenta[a]phenanthren-17-one (4) as shown in Fig. 80. To compensate for these small bay-region angles, the angles at the other side of the molecule, C(4)–C(5)–C(6) and C(7)–C(8)–C(14), are exceptionally large, 129.5° and 131.2°. The perturbing effect this distortion has on the electronic distribution in this molecule is demonstrated by comparing their ultraviolet absorption spectra. That of the unsubstituted compound
Table 42. Bond lengths (Å) with estimated standard deviations (in parentheses) for the thirteen cyclopenta[a]phenanthrenes shown in Fig. 79

<table>
<thead>
<tr>
<th>Bond</th>
<th>Unsubt.</th>
<th>1-Me</th>
<th>2-Me</th>
<th>6-Me</th>
<th>7-Me</th>
<th>11-Me</th>
<th>12-Me</th>
<th>7,11-Me1</th>
<th>11,12-Me2</th>
<th>11-Et</th>
<th>11-OMe</th>
<th>1,11-CH₂</th>
<th>11-Me-H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(11)-C(12)</td>
<td>1.36(8)</td>
<td>1.39(1)</td>
<td>1.40(1)</td>
<td>1.36(1)</td>
<td>1.37(1)</td>
<td>1.37(1)</td>
<td>1.37(1)</td>
<td>1.38(1)</td>
<td>1.35(1)</td>
<td>1.39(1)</td>
<td>1.40(1)</td>
<td>1.37(1)</td>
<td>1.36(1)</td>
</tr>
<tr>
<td>C(12)-C(13)</td>
<td>1.36(1)</td>
<td>1.39(1)</td>
<td>1.39(1)</td>
<td>1.37(1)</td>
<td>1.36(1)</td>
<td>1.40(1)</td>
<td>1.38(1)</td>
<td>1.38(1)</td>
<td>1.40(1)</td>
<td>1.40(1)</td>
<td>1.39(1)</td>
<td>1.40(1)</td>
<td>1.40(1)</td>
</tr>
<tr>
<td>C(13)-C(14)</td>
<td>1.32(3)</td>
<td>1.32(3)</td>
<td>1.34(3)</td>
<td>1.37(1)</td>
<td>1.35(1)</td>
<td>1.35(1)</td>
<td>1.37(1)</td>
<td>1.37(1)</td>
<td>1.35(1)</td>
<td>1.35(1)</td>
<td>1.35(1)</td>
<td>1.35(1)</td>
<td>1.37(1)</td>
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<tr>
<td>C(14)-C(15)</td>
<td>1.39(1)</td>
<td>1.41(1)</td>
<td>1.42(1)</td>
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<td>1.42(1)</td>
<td>1.41(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
</tr>
<tr>
<td>C(15)-C(16)</td>
<td>1.41(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.42(1)</td>
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<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
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<tr>
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<td>1.33(1)</td>
<td>1.33(1)</td>
<td>1.33(1)</td>
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<tr>
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<td>1.42(1)</td>
<td>1.42(1)</td>
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<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
<td>1.42(1)</td>
</tr>
<tr>
<td>C(18)-C(19)</td>
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<td>1.42(1)</td>
<td>1.41(1)</td>
<td>1.41(1)</td>
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<td>1.41(1)</td>
<td>1.41(1)</td>
<td>1.41(1)</td>
<td>1.41(1)</td>
</tr>
<tr>
<td>C(19)-C(20)</td>
<td>1.39(1)</td>
<td>1.42(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
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<td>1.43(1)</td>
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<td>1.43(1)</td>
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<td>1.43(1)</td>
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<tr>
<td>C(20)-C(21)</td>
<td>1.39(1)</td>
<td>1.42(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
<td>1.43(1)</td>
</tr>
</tbody>
</table>
Table 43. Bond angles (°) with estimated
phenanthrenes shown in Fig. 79
U

IS

II
121.6(8)
123.7(8)

S S S S S S S S S S S S S S

dsSsSsssSsBS”^
22SSSS

3§SSS§SSSSSS§§S5s22S

S§§SSSS§S§SSS2dd§SS2ll§

2gg22ggg2222^2:gj?jS2Sfe§§

II

S 'C o

ss§§dssdsdss^sas2§§§s
118.5(2)
117.2(2)

SaSSS§SS§BSS§SSSSas2ss
120.4(2)
120.1(1)

i
§=
122.0(4)
123.4(4)

asassaaaasassaaaag^s

120.1(9)
122.3(9)

ii

119.9(2)
119.2(1)

sgsssssassssas^assl^s

122.0(6)
121.6(6)

li

120.3(6)
122.4(10)

(in parentheses) for the non-hydrogen atoms in the thirteen cyclopenta[a]-

1 st

117.8(2)
1 20 . 5 ( 1 )

.

1 2 0 .1 (2 )
1 1 8 .3 (2 )

S.

.7(6)
.7(6)

s .d

III

5s

§s


(4) (dotted line) is characteristic of all its methyl homologues, and
different from that of the 1,11-methano-17-ketone (310) (solid line). In
the compounds lacking substituents in the bay region the long C(9)–C(11)
bond and large bay-region bond angles help to minimize the repulsive
interaction between H(1) and H(11). However, this is still marked as can
be seen from their proton magnetic resonance spectra in which these
hydrogens are deshielded by about 1 ppm compared with the other
aromatic protons in these molecules. As was previously found by Ent-

Fig. 80. Superimposed plan views of 15,16-dihydrocyclopenta[a]phenanthren-
17-one (4) (dotted lines) and its 1,11-methano derivative (310) (solid lines)
and their ultraviolet absorption spectra.

Table 44. Dihedral angles between planes of rings

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dihedral angle (°) between rings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/B</td>
</tr>
<tr>
<td>unsubstituted (4)</td>
<td>1.5</td>
</tr>
<tr>
<td>1-methyl (302)</td>
<td>2.4</td>
</tr>
<tr>
<td>2-methyl (303)</td>
<td>2.2</td>
</tr>
<tr>
<td>6-methyl (306)</td>
<td>0.9</td>
</tr>
<tr>
<td>7-methyl (230)</td>
<td>2.5</td>
</tr>
<tr>
<td>11-methyl (26)</td>
<td>7.0</td>
</tr>
<tr>
<td>12-methyl (130)</td>
<td>1.0</td>
</tr>
<tr>
<td>7,11-dimethyl (309)</td>
<td>9.7</td>
</tr>
<tr>
<td>11,12-dimethyl (131)</td>
<td>7.9</td>
</tr>
<tr>
<td>11-ethyl (211)</td>
<td>4.0</td>
</tr>
<tr>
<td>11-methoxy (132)</td>
<td>1.3</td>
</tr>
<tr>
<td>1,11-methano (310)</td>
<td>1.3</td>
</tr>
</tbody>
</table>
whistle and Iball (1961) for the parent hydrocarbon 16,17-dihydro-15H-cyclopenta[α]phenanthrene (1), these 17-ketones are essentially planar. The exceptions are the compounds with methyl or ethyl groups in the bay-region, particularly at C-11. This is best demonstrated by consideration of the dihedral angles observed between the planes of the aryl rings listed in Table 44 [the dihydro derivative (252) is obviously not comparable and is omitted from this Table]. The angle between rings -A and -C is roughly the sum of those between rings -A and -B and rings -B and -C. Thus severe steric interactions in the bay region in those compounds bearing a methyl or ethyl group at C(11) are avoided by considerable out-of-plane deformation, as illustrated by side views of these molecules (Fig. 81). In the 11-methyl-11,12-dihydro-17-ketone (252) the methyl group is axial, with the bond joining it to C(11) at right angles to the plane of the rings (Fig. 82). This compound is not dehydrogenated by quinones which require the substrate to possess a trans-diaxial arrangement of the protons to be abstracted, and this axial methyl conformation had previously been correctly assigned to it (Coombs et al., 1970) on these grounds. The approximate planarity of the 1,11-methano-17-ketone (310) was suggested by its nmr spectrum in which the bridge methylene protons are equivalent, resonating as a singlet (Ribeiro et al., 1983).

The effects these factors have on the geometry of the bay region are summarized in Table 45, in which the distances are in Å and the interbond and torsion angles are given in degrees. The torsion angle C(1)–C(10)–C(9)–C(11) is defined as the angle between the bonds C(1)–C(10) and C(9)–C(11), looking along the central bond C(10)–C(9). A carbon substituent at C(11) causes the molecule to twist about the central ring so that this substituent and the proton at C(1) are not in the same plane, thus minimizing their interaction. When the substituent is a methyl group this leads to a bay-region torsion angle of 13.5–20.5° and all these 17-ketones are strong carcinogens. In the benz[a]anthracene and chrysene series 7,12-dimethylbenz[a]anthracene and 5,6-dimethylchrysene, both potent carcinogens, have torsion angles of this order. The weakly carcinogenic 11-ethyl-17-ketone (211) has a smaller torsion angle (8.9°) whilst the inactive compounds range between 6.8° (2-methyl) to 0.1° (12-methyl). Interestingly, a methyl group at the other bay-region position, at C(1), does not have this effect; the torsion angle is only 5.2°. However, the C(10)–C(1)–C(2) angle in this compound is narrowed by comparison with that in the other compounds, and the associated bonds C(1)–C(2) and C(1)–C(10) are longer than average. In plan view these molecules closely resemble steroids; for example, as is shown in Fig. 83 in which the 11-methyl-17-ketone (26) is superimposed on oestrone. However, this
Fig. 81 (i) and (ii). Views of structures of twelve 15,16-dihydrocyclopenta[a]phenanthren-17-ones calculated from X-ray crystallography data; all are shown as plan and side views, and tilted at 30°.

Unsub-\textsuperscript{7}-ketone (4)

1-Methyl-17-ketone (302)

2-Methyl-17-ketone (303)

6-Methyl-17-ketone (306)

7-Methyl-17-ketone (230)

11-Methyl-17-ketone (26)
Molecular structure of 15,16-dihydro-17-ketones
Table 45. Bay-region geometry

<table>
<thead>
<tr>
<th>Bond length (Å) C(1)–C(10)</th>
<th>Bond length (Å) C(9)–C(10)</th>
<th>Bond length (Å) C(9)–C(11)</th>
<th>Angle (°) C(1)–C(10)–C(9)</th>
<th>Angle (°) C(10)–C(9)–C(11)</th>
<th>Torsion angle (°) C(1)–C(10)–C(9)–C(11)</th>
<th>Non-bonded distances (Å) C(1)–C(11)</th>
<th>C(1)–C(18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H at C(1) and C(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unsubstituted (4)</td>
<td>1.392</td>
<td>1.445</td>
<td>1.395</td>
<td>123.4</td>
<td>123.8</td>
<td>2.6</td>
<td>2.988</td>
</tr>
<tr>
<td>2-Methyl (383)</td>
<td>1.395</td>
<td>1.454</td>
<td>1.434</td>
<td>124.2</td>
<td>122.5</td>
<td>6.8</td>
<td>3.012</td>
</tr>
<tr>
<td>6-Methyl (396)</td>
<td>1.407</td>
<td>1.459</td>
<td>1.414</td>
<td>121.6</td>
<td>122.3</td>
<td>2.7</td>
<td>2.953</td>
</tr>
<tr>
<td>7-Methyl (320)</td>
<td>1.419</td>
<td>1.455</td>
<td>1.434</td>
<td>123.5</td>
<td>119.0</td>
<td>2.0</td>
<td>2.935</td>
</tr>
<tr>
<td>12-Methyl (130)</td>
<td>1.409</td>
<td>1.455</td>
<td>1.417</td>
<td>122.9</td>
<td>122.4</td>
<td>0.1</td>
<td>2.970</td>
</tr>
<tr>
<td>(average)</td>
<td>(1.404)</td>
<td>(1.453)</td>
<td>(1.419)</td>
<td>(123.1)</td>
<td>(122.0)</td>
<td>(2.8)</td>
<td>(2.971)</td>
</tr>
<tr>
<td>CH₃ at C(1), H at C(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Methyl (392)</td>
<td>1.428</td>
<td>1.469</td>
<td>1.423</td>
<td>126.0</td>
<td>123.5</td>
<td>5.2</td>
<td>3.095</td>
</tr>
<tr>
<td>H at C(1), CH₂ at C(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Methyl (26)</td>
<td>1.409</td>
<td>1.462</td>
<td>1.442</td>
<td>124.6</td>
<td>125.0</td>
<td>13.5</td>
<td>3.102</td>
</tr>
<tr>
<td>7,11-Dimethyl (309)</td>
<td>1.410</td>
<td>1.449</td>
<td>1.446</td>
<td>124.6</td>
<td>121.8</td>
<td>20.5</td>
<td>3.042</td>
</tr>
<tr>
<td>11,12-Dimethyl (131)</td>
<td>1.411</td>
<td>1.470</td>
<td>1.437</td>
<td>124.7</td>
<td>124.3</td>
<td>13.8</td>
<td>3.096</td>
</tr>
<tr>
<td>(average)</td>
<td>(1.410)</td>
<td>(1.457)</td>
<td>(1.441)</td>
<td>(124.6)</td>
<td>(123.7)</td>
<td>(15.6)</td>
<td>(3.08)</td>
</tr>
<tr>
<td>H at C(1), CH₂CH₃ at C(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Ethyl (211)</td>
<td>1.423</td>
<td>1.475</td>
<td>1.441</td>
<td>124.9</td>
<td>124.8</td>
<td>8.9</td>
<td>3.118</td>
</tr>
<tr>
<td>H at C(1), OCH₃ at C(11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-Methoxy (132)</td>
<td>1.407</td>
<td>1.470</td>
<td>1.435</td>
<td>124.4</td>
<td>124.8</td>
<td>0.7</td>
<td>3.084</td>
</tr>
<tr>
<td>Carcinogenic hydrocarbons (using numbering analogous to that used above)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMBA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.410</td>
<td>1.470</td>
<td>1.402</td>
<td>123.0</td>
<td>123.5</td>
<td>22.1</td>
<td>3.044</td>
</tr>
<tr>
<td>5,6-DMC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.403</td>
<td>1.483</td>
<td>1.408</td>
<td>123.5</td>
<td>121.8</td>
<td>21.2</td>
<td>3.047</td>
</tr>
<tr>
<td>(average)</td>
<td>(1.408)</td>
<td>(1.477)</td>
<td>(1.405)</td>
<td>(123.3)</td>
<td>(122.7)</td>
<td>(21.7)</td>
<td>(3.045)</td>
</tr>
</tbody>
</table>

<sup>a</sup> DMBA, 7,12-Dimethylbenzo[a]anthracene (Glusker, 1981).

<sup>b</sup> 5,6-DMC, 5,6-Dimethylchrysene (Zacharias <i>et al.</i>, 1984).
Carcinogenicity and molecular strain

Fig. 82. Three views (as in Fig. 81) of 11-methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (252).

Fig. 83. Plan and side views of oestrone (dotted lines) superimposed on 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26) (solid lines).

similarity in two dimensions is lost in the third dimension because rings -C and -D in the steroid are not quasi planar.

8.3 An apparent correlation between carcinogenicity and molecular strain

Thus there seems to be a rough correlation between out-of-plane deformation and carcinogenicity, but in this respect the 7-methyl-17-ketone (230) is anomalous since it is essentially flat. Molecular strain is, however, introduced also into this structure by the 7-methyl group which is locked in the plane of the ring system. Non-bonding distances between the two C(15) hydrogen atoms and one of the 7-methyl hydrogens are exceptionally short (1.82 and 1.84 Å), even shorter than those found for
the two hydrogen atoms in the bay region (1.94 Å). Moreover, movement of the methyl group out of the plane of the rings would further decrease one of these. This leads to several consequences; for example, the bay-region angle C(10)–C(9)–C(11) is 119.0°, 3° less than the average for the compounds lacking a bay-region substituent, and results in the H(1) to H(11) non-bonding distance being the shortest observed (Table 46). In the five-membered ring the protons at C(15) are deshielded by 0.42–0.46 ppm, and the rate of proton exchange at C(16) is approximately

Table 46. Non-bonding H(1) . . . H(11) distances, rate constants for detritiation at C(16), and nmr chemical shifts at C(15) for six 17-ketones

<table>
<thead>
<tr>
<th>Compound</th>
<th>H(1) . . . H(11) distance (Å)</th>
<th>Rate constant (×10^5 K^T^OH– nmol^-1 s^-1)</th>
<th>Chemical shift (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted (4)</td>
<td>1.999</td>
<td>1.83 ± 0.14</td>
<td>3.28</td>
</tr>
<tr>
<td>2-methyl (303)</td>
<td>2.019</td>
<td>(not measured)</td>
<td>3.25</td>
</tr>
<tr>
<td>6-methyl (306)</td>
<td>1.973</td>
<td>1.15 ± 0.14</td>
<td>3.27</td>
</tr>
<tr>
<td>7-methyl (230)</td>
<td>1.942</td>
<td>3.17 ± 0.08</td>
<td>3.70</td>
</tr>
<tr>
<td>11-methyl (26)</td>
<td>—</td>
<td>1.47 ± 0.06</td>
<td>3.24</td>
</tr>
<tr>
<td>12-methyl (130)</td>
<td>2.004</td>
<td>0.67 ± 0.07</td>
<td>3.28</td>
</tr>
</tbody>
</table>

Table 47. Molecular strain and carcinogenicity

<table>
<thead>
<tr>
<th>Compound</th>
<th>General state of molecule</th>
<th>Iball index(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted (4)</td>
<td>planar, unstrained</td>
<td>&lt;1</td>
</tr>
<tr>
<td>1-methyl (302)</td>
<td>essentially planar, some bond and angle distortion in ring-A</td>
<td>1</td>
</tr>
<tr>
<td>2-methyl (303)</td>
<td>essentially planar, unstrained</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6-methyl (306)</td>
<td>planar, unstrained</td>
<td>&lt;1</td>
</tr>
<tr>
<td>7-methyl (230)</td>
<td>essentially planar, evidence of strain in bay region and ring-D</td>
<td>10</td>
</tr>
<tr>
<td>11-methyl (26)</td>
<td>out-of-plane deformation, bay-region torsion angle 13.5°</td>
<td>46</td>
</tr>
<tr>
<td>12-methyl (130)</td>
<td>planar, unstrained</td>
<td>&lt;1</td>
</tr>
<tr>
<td>7,11-dimethyl (309)</td>
<td>out-of-plane deformation, bay-region torsion angle 20.5°</td>
<td>&gt;49(b)</td>
</tr>
<tr>
<td>11,12-dimethyl (131)</td>
<td>out-of-plane deformation, bay-region torsion angle 13.8°</td>
<td>30</td>
</tr>
<tr>
<td>11-ethyl (211)</td>
<td>some out-of-plane deformation, bay-region torsion angle 8.9°</td>
<td>8</td>
</tr>
<tr>
<td>11-methoxy (132)</td>
<td>planar, unstrained</td>
<td>25</td>
</tr>
<tr>
<td>1,11-methano (310)</td>
<td>planar, but severe bond angle distortions</td>
<td>16</td>
</tr>
</tbody>
</table>

(a) Figure for repeated application of the compound twice weekly for one year.
(b) Twice-weekly applications for 10 weeks only.
doubled compared with the other five compounds. This strain built into the 7-methyl-17-ketone (230) was reflected originally in the difficulty experienced in the synthesis of this structure.

The general condition of 12 compounds examined by X-ray crystallography and shown in Fig. 79 (neglecting the 11,12-dihydro compound (252) which is not comparable) are summarized in Table 47 alongside their potencies as skin carcinogens in T.O. mice, as indicated by their Iball indices taken from Tables 11 and 12. It is evident that there is (with one exception) a direct correlation between molecular strain and carcinogenicity among these 17-oxocyclopenta[a]phenanthrenes. Newman (1983), of course, has also noted a similar relationship in the benz[a]anthracene series, but the way in which these two properties are connected is quite obscure at the present time. There is one exception to this general rule among the compounds studied by X-ray crystallography; the 11-methoxy-17-ketone (132) is a fairly strong carcinogen although it is planar and its bond angles are quite normal. Unlike the carbon substituents at C(11), the ether oxygen at C(11) is in the molecular plane and only 1.98 Å from the hydrogen at C(1), suggesting that this contact is attractive. This is reflected in its nmr spectrum in which H(1) is further deshielded, resonating at δ 9.8 compared with δ 8.8 for the 11-methyl-17-ketone (26) (Abraham and Loftus, 1978). There is a marked difference between the 11-alkoxy and 11-alkyl series in regard to their ability to induce skin tumours, summarized in Table 48. This comparison is

<table>
<thead>
<tr>
<th>Compound</th>
<th>Iball index</th>
<th>Repeated application</th>
<th>Initiation/promotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-Alkyl series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-methyl (26)</td>
<td>46</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>11-ethyl (211)</td>
<td>8</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11-n-butyl (212)</td>
<td>&gt;1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>7,11-dimethyl (309)</td>
<td>49(a)</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>11-Alkoxy series</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-methoxy (132)</td>
<td>25</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>11-ethoxy (390)</td>
<td>—</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>11-n-butoxy (393)</td>
<td>—</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7-methyl-11-methoxy (447)</td>
<td>17</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

(a, Incomplete treatment, see Table 47.)
somewhat confused because two methods of skin painting have been employed, giving rise to two series of Iball indices (see Chapter 6 for a discussion of this), and not all the compounds have been tested by both methods. However, it is obvious that in the alkyl series carcinogenic activity falls off rapidly with increase in alkyl chain length so that the ethyl derivative is only weakly active and the butyl homologue is inactive. In the 11-alkoxy series, on the other hand, the ethoxy compound is not much less active than its methoxy homologue, and even the n-butoxy compound is a weak carcinogen. Moreover, whereas introduction of a 7-methyl group into the 11-methyl compound to give the 7,11-dimethyl-17-ketone (309) increases its potency so that this ketone is the most active so far encountered in this series, the 7-methyl-11-methoxy-17-ketone (447) is appreciably less carcinogenic than the 11-methoxy analogue (132). It therefore seems probable that the way in which 11-alkyl and 11-alkoxy groups endow the parent molecule with carcinogenic potential may be fundamentally different.

8.4 Investigation of the diol-epoxides of 15,16-dihydrocyclopenta[a]-phenanthren-17-ones by computer modelling

The enhancing effect of methyl substitution at the non-benzo bay-region position (the ‘bay-region methyl effect’) is, of course, well known among several polycyclic aromatic hydrocarbon systems, and has been noted by many authors (DiGiovanni et al., 1983; Hecht et al., 1979; Iyer et al., 1980). However, no really satisfactory explanation for this effect has been advanced. With this large amount of exact X-ray data to hand it seemed worth while investigating the effects of the methyl group on the structures of the diol-epoxides of the strongly carcinogenic 11-methyl-17-ketone (26) by computer construction (Kashino et al., 1986). These metabolites have not been synthesized, but they are known to be concerned in the expression of biological activity; it is probable that they are very reactive and this would further complicate their study by X-ray crystallography, although the diol-epoxides of benzo[a]pyrene have been studied in this way (Neidle et al., 1980; Neidle and Cutbush, 1983).

An interesting result which follows from the dihedral angle of 12.5° between rings -A and -C in the 11-methyl-17-ketone (26) is that this molecule can exist in right- and left-handed mirror-image forms, and as normally prepared consists of the racemate. This can be seen in the crystal structure disclosed by X-ray analysis and is illustrated in Fig. 84, in which both forms are clearly visible. The energy required to flip one form to the other is not known and cannot be reliably calculated, so that it is unclear whether the two forms exist as stable conformers in solution. A
study of the temperature factors from the X-ray structure (Table 49) indicates that the atoms having the lowest temperature factors, and hence least motion in the crystalline state, are those in the bay region. That is, there is no disorder of the 11-methyl group in the crystal, and no evidence for interconversion of the two forms in the crystalline state.

In constructing the diol-epoxides this has therefore been borne in mind, and the four structures, two syn and two anti, shown in Fig. 85 have been considered although as described in Chapter 7 the biologically derived diol-epoxide appears to be exclusively anti, with the diol system $[3R,4R]$ (i.e., $3\alpha, 4\beta$). These four structures were obtained by superimposing the X-ray co-ordinates of the syn- and anti-diol-epoxides of benzo[a]pyrene on those of the two conformers of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26). Four further $[3S,4S]$ optical isomers are possible, and are precise mirror images of those shown. The diol-epoxides of 15,16-dihydro-11-methoxycyclopenta[a]-phenanthren-17-one (132) were similarly constructed although here, of

Fig. 84. Mirror-image forms of the 11-methyl-17-ketone (26) disclosed by X-ray crystallography; the two forms have been superimposed with the bond joining C(13) to C(14) in common.
course, only one conformer exists because the oxygen at C(11) is in the plane of the flat aromatic rings. Selected interatomic distances measured from these computer-simulated diol-epoxides are shown in Table 50. Some of these distances are unacceptably short, as indicated, and this leads to the conclusion that the most likely structure for the diol-epoxide of the 11-methyl-17-ketone (26) is an anti structure with the epoxide oxygen and 11-methyl group on the same side of the molecular plane [(a) in Fig. 85]. Neither syn conformers would appear to be likely. This therefore confirms the same conclusion previously drawn from entirely different reasoning. In the absence of the 11-methyl group either diol-epoxide may be formed without generating unacceptably short non-bonding distances; in practice only the syn-diol-epoxide is observed for

Table 49. Temperature factors (B) for the oxygen and carbon atoms in 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one.

<table>
<thead>
<tr>
<th>Atom</th>
<th>B</th>
<th>Atom</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.3</td>
<td>C(9)</td>
<td>4.0</td>
</tr>
<tr>
<td>C(1)</td>
<td>6.3</td>
<td>C(10)</td>
<td>4.6</td>
</tr>
<tr>
<td>C(2)</td>
<td>7.7</td>
<td>C(11)</td>
<td>4.1</td>
</tr>
<tr>
<td>C(3)</td>
<td>7.7</td>
<td>C(12)</td>
<td>4.4</td>
</tr>
<tr>
<td>C(4)</td>
<td>6.7</td>
<td>C(13)</td>
<td>4.3</td>
</tr>
<tr>
<td>C(5)</td>
<td>5.2</td>
<td>C(14)</td>
<td>4.2</td>
</tr>
<tr>
<td>C(6)</td>
<td>5.8</td>
<td>C(15)</td>
<td>5.4</td>
</tr>
<tr>
<td>C(7)</td>
<td>5.0</td>
<td>C(16)</td>
<td>6.3</td>
</tr>
<tr>
<td>C(8)</td>
<td>4.1</td>
<td>C(17)</td>
<td>5.3</td>
</tr>
</tbody>
</table>

Fig. 85. The two anti- and two syn-diol epoxides of the 11-methyl-17-ketone (26) studied by computer construction.
Table 50. Interatomic distances (Å) in models of diol-epoxides

<table>
<thead>
<tr>
<th>Diol-epoxides of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (26)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O(epoxide)---H(11-methyl)</td>
<td>anti</td>
<td>(a)</td>
<td>3.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b)</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>syn</td>
<td>(c)</td>
<td>2.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d)</td>
<td>2.38 (rather short)</td>
<td></td>
</tr>
<tr>
<td>H(1)(epoxide)---H(11-methyl)</td>
<td>anti</td>
<td>(a)</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b)</td>
<td>1.56 (too short)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>syn</td>
<td>(c)</td>
<td>1.31 (too short)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d)</td>
<td>1.83</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diol-epoxides of 15,16-dihydro-11-methoxycyclopenta[a]phenanthren-17-one (132)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O(epoxide)---O(methyl)</td>
<td>anti</td>
<td>3.81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>syn</td>
<td>3.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H(1)(epoxide)---O(methyl)</td>
<td>anti</td>
<td>1.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>syn</td>
<td>1.77</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 50. Interatomic distances (Å) in models of diol-epoxides

the parent unsubstituted 17-ketone (4) (Hadfield et al., 1984). In the case of the 11-methoxy-17-ketone (132) the computer model indicates that there is little differentiation between syn- and anti-diol-epoxides except for a rather short O---O distance in the syn-isomer.

8.5 Molecular orbital calculations

Ground-state molecular orbital wave functions have been calculated by the CNDO/2 all valence approximation (Pople et al., 1965; Pople and Segal, 1966) for the seven structures examined in London (Clayton, 1982). Bond indices, defined as the number of electrons statistically localized between a pair of atomic centres (Wiberg, 1968), are shown in Table 51. The values for aromatic C-C bonds vary considerably from that for benzene, 1.425e (Ibata et al., 1975), from the highest values (1.660—1.720e) for C(6)-C(7) to the lowest (1.14—1.20e) for the adjacent C(5)-C(6) bond. The sum of the bond indices for each aromatic ring (Table 52) is less than that for benzene owing to migration of electronic charge to the carbon-oxygen dipole at C(17). As a consequence of this aromatic bromination, for example, occurs at C(15) and not at aromatic carbon in these ketones (see Chapter 4). There are three other notable features apparent in this Table. Firstly, the sum of bond indices for rings -A and -C are in all cases greater than for ring-B. Thus this ring acts as an electron donor towards the two outer aromatic rings; a similar pattern is also observed in phenanthrene (Dixon et al., 1978). Secondly, the sum of the bond indices for ring-A is always greater than that for ring-C, emphasizing the electron-acceptor characteristics of the
<table>
<thead>
<tr>
<th>Bond</th>
<th>Unsubstit. (4)</th>
<th>2-Methyl (303)</th>
<th>6-Methyl (306)</th>
<th>7-Methyl (230)</th>
<th>11-Methyl (26)</th>
<th>12-Methyl (130)</th>
<th>1,11-Methano (310)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(10)–C(1)</td>
<td>1.315</td>
<td>1.315</td>
<td>1.320</td>
<td>1.250</td>
<td>1.295</td>
<td>1.325</td>
<td>1.275</td>
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<tr>
<td>C(1)–C(2)</td>
<td>1.540</td>
<td>1.505</td>
<td>1.555</td>
<td>1.550</td>
<td>1.565</td>
<td>1.535</td>
<td>1.485</td>
</tr>
<tr>
<td>C(2)–C(3)</td>
<td>1.350</td>
<td>1.305</td>
<td>1.355</td>
<td>1.340</td>
<td>1.310</td>
<td>1.350</td>
<td>1.345</td>
</tr>
<tr>
<td>C(3)–C(4)</td>
<td>1.555</td>
<td>1.580</td>
<td>1.535</td>
<td>1.555</td>
<td>1.590</td>
<td>1.535</td>
<td>1.550</td>
</tr>
<tr>
<td>C(4)–C(5)</td>
<td>1.290</td>
<td>1.280</td>
<td>1.315</td>
<td>1.295</td>
<td>1.280</td>
<td>1.320</td>
<td>1.270</td>
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<tr>
<td>C(5)–C(6)</td>
<td>1.165</td>
<td>1.165</td>
<td>1.140</td>
<td>1.200</td>
<td>1.200</td>
<td>1.175</td>
<td>1.190</td>
</tr>
<tr>
<td>C(6)–C(7)</td>
<td>1.720</td>
<td>1.725</td>
<td>1.680</td>
<td>1.660</td>
<td>1.700</td>
<td>1.695</td>
<td>1.700</td>
</tr>
<tr>
<td>C(7)–C(8)</td>
<td>1.175</td>
<td>1.165</td>
<td>1.175</td>
<td>1.140</td>
<td>1.165</td>
<td>1.145</td>
<td>1.175</td>
</tr>
<tr>
<td>C(8)–C(9)</td>
<td>1.340</td>
<td>1.345</td>
<td>1.345</td>
<td>1.345</td>
<td>1.330</td>
<td>1.350</td>
<td>1.350</td>
</tr>
<tr>
<td>C(9)–C(10)</td>
<td>1.125</td>
<td>1.135</td>
<td>1.125</td>
<td>1.145</td>
<td>1.165</td>
<td>1.140</td>
<td>1.150</td>
</tr>
<tr>
<td>C(10)–C(11)</td>
<td>1.340</td>
<td>1.330</td>
<td>1.335</td>
<td>1.315</td>
<td>1.320</td>
<td>1.310</td>
<td>1.335</td>
</tr>
<tr>
<td>C(11)–C(12)</td>
<td>1.310</td>
<td>1.305</td>
<td>1.305</td>
<td>1.265</td>
<td>1.255</td>
<td>1.320</td>
<td>1.250</td>
</tr>
<tr>
<td>C(12)–C(13)</td>
<td>1.555</td>
<td>1.560</td>
<td>1.560</td>
<td>1.560</td>
<td>1.530</td>
<td>1.520</td>
<td>1.560</td>
</tr>
<tr>
<td>C(13)–C(14)</td>
<td>1.305</td>
<td>1.310</td>
<td>1.305</td>
<td>1.290</td>
<td>1.320</td>
<td>1.280</td>
<td>1.280</td>
</tr>
<tr>
<td>C(14)–C(15)</td>
<td>1.460</td>
<td>1.460</td>
<td>1.455</td>
<td>1.465</td>
<td>1.450</td>
<td>1.480</td>
<td>1.480</td>
</tr>
<tr>
<td>C(15)–C(16)</td>
<td>1.280</td>
<td>1.300</td>
<td>1.280</td>
<td>1.280</td>
<td>1.300</td>
<td>1.320</td>
<td>1.270</td>
</tr>
<tr>
<td>C(16)–C(17)</td>
<td>1.015</td>
<td>1.005</td>
<td>1.020</td>
<td>1.010</td>
<td>1.005</td>
<td>1.010</td>
<td>1.005</td>
</tr>
<tr>
<td>C(17)–C(18)</td>
<td>1.015</td>
<td>1.010</td>
<td>1.000</td>
<td>1.015</td>
<td>1.025</td>
<td>1.020</td>
<td>1.020</td>
</tr>
<tr>
<td>C(aryl)–C(18)</td>
<td>1.015</td>
<td>1.005</td>
<td>1.020</td>
<td>1.010</td>
<td>0.995</td>
<td>1.005</td>
<td>1.010</td>
</tr>
<tr>
<td>C(1)–C(18)</td>
<td>—</td>
<td>1.035</td>
<td>1.030</td>
<td>1.030</td>
<td>1.025</td>
<td>1.045</td>
<td>—</td>
</tr>
<tr>
<td>C(11)–C(18)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.040</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 51. Bond indices for seven 17-oxocyclopenta[a]phenanthrenes
adjacent five-membered ring. Finally, the sum of the bond indices for any ring with a methyl or methano substituent is less than that for the corresponding unsubstituted ring. Thus the methyl groups show electron-acceptor character, and this is reflected in the aryl C–CH₃ bond indices which average 1.033 for these ketones. This somewhat surprising feature has been observed previously for benzene–toluene by Libit and Hoffmann (1974) who concluded that the ‘classic’ electron-donor character of the methyl group is realized through a polarization mechanism resulting in a decrease and increase in electron density at the ipso and ortho carbon atoms, respectively. The expected changes in charge distribution are evident in the molecular fragments surrounding the methyl group in these substituted ketones. For example, the bond indices for C(1)–C(2) and C(2)–C(3) in the 2-methyl-17-ketone (303) are less than those in the unsubstituted compound (4), whilst the reverse is true for C(3)–C(4).

Orbital energies of the three highest occupied molecular orbitals (HOMO) and three lowest unoccupied molecular orbitals (LUMO) of these seven ketones are listed in Table 53. Two important features are apparent. Firstly, LUMO and LUMO + 1 orbitals are energetically similar and well separated from the remaining unoccupied orbitals, with energy differences between LUMO + 1 and LUMO + 2 of 67.0–80.0 au × 10⁻³. Energy differences between the highest occupied orbitals are smaller, the differences between HOMO and HOMO-1 varying between 22.5 and 31.0 au × 10⁻³. Secondly, the introduction of a methyl or methano group slightly increases the energy of HOMO and decreases that of LUMO. This is consistent with the dual character of the methyl group, as an electron acceptor and as electron donor via an hyperconjugative mechanism. Pure electron donors increase the energy of both HOMO and LUMO, whereas pure conjugative groups increase the

### Table 52. Sum of bond indices for each aromatic ring in seven 17-oxocyclopenta[a]phenanthrenes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ring-A</th>
<th>Ring-B</th>
<th>Ring-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>unsubstituted (4)</td>
<td>8.390</td>
<td>7.865</td>
<td>8.250</td>
</tr>
<tr>
<td>2-methyl (303)</td>
<td>8.315</td>
<td>7.865</td>
<td>8.275</td>
</tr>
<tr>
<td>6-methyl (306)</td>
<td>8.395</td>
<td>7.805</td>
<td>8.255</td>
</tr>
<tr>
<td>7-methyl (230)</td>
<td>8.365</td>
<td>7.825</td>
<td>8.225</td>
</tr>
<tr>
<td>11-methyl (26)</td>
<td>8.365</td>
<td>7.880</td>
<td>8.185</td>
</tr>
<tr>
<td>12-methyl (130)</td>
<td>8.380</td>
<td>7.815</td>
<td>8.190</td>
</tr>
<tr>
<td>11,12-methano (31)</td>
<td>8.260</td>
<td>7.900</td>
<td>8.175</td>
</tr>
</tbody>
</table>

[benzene, 8.550e (Ibata et al., 1975)].
Table 53. *Orbital energies (au × 10⁻³) of the three highest occupied (HOMO) and three lowest unoccupied (LUMO) orbitals for seven 17-oxo-cyclopenta[a]phenanthrenes*

<table>
<thead>
<tr>
<th>Orbital</th>
<th>Unsubst. (4)</th>
<th>2-Methyl (303)</th>
<th>6-Methyl (306)</th>
<th>7-Methyl (230)</th>
<th>11-Methyl (26)</th>
<th>12-Methyl (130)</th>
<th>1,11-Methano (310)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMO + 2</td>
<td>161.5</td>
<td>150.5</td>
<td>154.5</td>
<td>154.5</td>
<td>159.0</td>
<td>153.0</td>
<td>142.5</td>
</tr>
<tr>
<td>LUMO + 1</td>
<td>81.5</td>
<td>78.0</td>
<td>74.5</td>
<td>76.0</td>
<td>79.0</td>
<td>73.0</td>
<td>75.5</td>
</tr>
<tr>
<td>LUMO</td>
<td>80.5</td>
<td>70.5</td>
<td>71.0</td>
<td>64.5</td>
<td>65.5</td>
<td>55.5</td>
<td>69.5</td>
</tr>
<tr>
<td>(LUMO–HOMO</td>
<td>489.5</td>
<td>472.0</td>
<td>474.0</td>
<td>460.5</td>
<td>462.0</td>
<td>451.0</td>
<td>468.5</td>
</tr>
<tr>
<td>HOMO</td>
<td>-409.5</td>
<td>-401.5</td>
<td>-403.0</td>
<td>-395.0</td>
<td>-396.5</td>
<td>-395.5</td>
<td>-399.0</td>
</tr>
<tr>
<td>HOMO-1</td>
<td>-432.0</td>
<td>-426.5</td>
<td>-426.5</td>
<td>-426.0</td>
<td>-425.5</td>
<td>-422.5</td>
<td>-417.0</td>
</tr>
<tr>
<td>HOMO-2</td>
<td>-439.5</td>
<td>-440.5</td>
<td>-439.5</td>
<td>-438.5</td>
<td>-436.0</td>
<td>-439.0</td>
<td>-442.5</td>
</tr>
</tbody>
</table>

au = atomic units.
energy of HOMO while they decrease the energy of LUMO (Fleming, 1976).

8.6 References

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9

Conclusion

9.1 The occurrence of cyclopenta[a]phenanthrenes

From the beginning cyclopenta[a]phenanthrenes have been associated on the one hand with sterols and steroids, and on the other with polycyclic aromatic hydrocarbons. Related to this is the possibility of their occurrence in nature, particularly in animal tissues as a result of aberrant steroid metabolism. This idea was raised at an early stage (Cook, 1933) and has reappeared periodically on a number of occasions since (Steiner, 1943; Haddow, 1958; Dannenberg, 1960; Bischoff, 1969; Coombs and Croft, 1969; Wilk and Taupp, 1969; Coombs et al., 1973). However, to the best of the present authors' knowledge no definitive report of a cyclopenta[a]phenanthrene (i.e., a steroid derivative lacking both angular methyl groups) arising from such a source has yet appeared.

In contrast to this, recent studies indicate that cyclopenta[a]-phenanthrene hydrocarbons are widely distributed in several natural environments where they have probably arisen from sterols by microbiological dehydrogenation. This unexpected discovery has come to light as a result of the use of modern sophisticated analytical techniques such as gas chromatography–mass spectrometry with computer-assisted peak sorting. Nevertheless, Diels' hydrocarbon (7) was first identified in petroleum over 20 years ago (Mair and Martinez-Pico, 1962). In recent work (Ludwig et al., 1981) aromatic components of shale oil were separated into classes containing 1,2,3... aromatic rings, and these fractions were further separated by hplc. The nine cyclopenta[a]phenanthrenes shown in Fig. 86 were identified on the basis of their mass spectra, and the identity of five of them (27, 30, 32, 33, 35) was confirmed by comparison with authentic samples prepared from sterols by the method of Dannenberg, namely chloranil dehydrogenation to the
Conclusion

17\(H\)-cyclopenta[a]phenanthrene followed by catalytic hydrogenation to its 15,16-dihydro derivative. These hydrocarbons appear to be widespread constituents of petroleum-bearing sediments where they are formed by long-term degradation of sterols in the subsurface; they do not appear to occur in recent sediments. In an investigation of the hydrocarbons present in samples of Toarcian shales at different sites in the Paris basin, Mackenzie et al. (1981) identified the cyclopenta[a]phenanthrene (33) and also 15,16-dihydro-17-ethyl-17-methylcyclopenta[a]phenanthrene (36), again by comparison with authentic specimens. The methyl ethyl hydrocarbon (36) has also been reported to occur in Chinese shales (Shi et al., 1982). Mackenzie et al. found that the extent of steroid aromatization increased with burial depth, and therefore with maturity of the sample. The extent of carbon–carbon bond cracking in the side chain was found to be small in the shallow samples, and became significant only in the deepest specimens. It was suggested that these two reactions – aromatization and side-chain cracking – probably occur during petroleum formation and that steroid hydrocarbon distribution might serve as a useful indication of maturity in exploration studies. The parent hydrocarbon 16,17-dihydro-15\(H\)-cyclopenta[a]phenanthrene (1) has recently been identified in Australian brown coal where it was obtained as a minor component of the triaromatic hydrocarbon fraction separated by hplc. Its occurrence together with numerous other tetra- and pentacyclic hydrocarbons derived from phenanthrene establishes its probable origin from triterpene precursors (Chaffee and Johns, 1983).

A different cyclopenta[a]phenanthrene hydrocarbon, namely 16,17-dihydro-15-isopropyl-4-methyl-15\(H\)-cyclopenta[a]phenanthrene (37), has been identified by several groups in river and lake sediments. Wakeham et al. (1980) examined cores of recent sediment from Lake Lucerne, Lake Zurich and Griefensee (Switzerland), and Lake Washington (north-west U.S.A.). Aromatic components were separated by hplc into groups according to the number of aromatic rings and these were subsequently analysed by gas chromatography–mass spectrometry; the cyclopenta[a]phenanthrene (37) was identified in the phenanthrene fraction. These authors considered that the variety of phenanthrenes and

Fig. 86. Cyclopenta[a]phenanthrenes which occur naturally in mineral oils.
Occurrence of cyclopenta[a]phenanthrenes

chrysenes found pointed to short-term alteration of biogenic precursors and could in no way be linked to anthropogenic combustion or pyrolysis processes. It was proposed that the hydrocarbon probably arose as a result of dehydrogenation of sterols or triterpenes mediated by microorganisms. The same cyclopenta[a]phenanthrene was also identified in sediment cores collected from two Adirondack lakes in New York state, remote from industrial activities (Tan and Heit, 1981), and from the Amazon river and Cariaco Trench (Laflamme and Hites, 1979). These authors suggested that it could be formed from a pentacyclic triterpene such as lupeol via cleavage of the oxygenated A-ring, followed by aromatization of the remaining six-membered rings, together with migration and loss of the angular methyl groups as shown in Fig. 87.

Whereas these cyclopenta[a]phenanthrenes can be considered as naturally occurring, others occur in edible oils which have been overheated. Heating above 200°C causes the formation of a small amount of fluorescent material, arising from aromatization of sterols which are always present naturally in both animal and vegetable fats. Schmid (1962) and Schmid and Waitz (1963) heated the potential sterol precursors, cholesterol, phytosterol, and ergosterol, at 400°C in a slow stream of nitrogen, and isolated from the products 16,17-dihydro-15H-cyclopenta[a]phenanthrene (1) and Diels' hydrocarbon (7) among other phenanthrene and chrysene derivatives. Pyrolysis of ergosterol (Hof-"einer, Lisbet and Schmid, 1964) gave in addition 16,17-dihydro-3-hydroxy-15H-cyclopenta[a]phenanthrene (38). All these cyclopenta[a]phenanthrenes were obtained in the crystalline state and fully

Fig. 87. Suggested derivation of the cyclopenta[a]phenanthrene (37) found naturally in river and lake sediments from the triterpenoid lupeol. The phenol (38) was isolated from the products formed by heating sterols at 400°C in nitrogen, while the hydrocarbon (39) was obtained by exposing cholesterol adsorbed on silica gel to iodine vapour at ambient temperature.
characterized. Earlier Falk et al. (1949) had studied the products obtained from heating cholesterol in air at 360°C, in connection with the report of Hieger (1947) that commercially obtained samples of this sterol were weakly carcinogenic. The products were separated chemically into acidic, basic, ketonic neutral, and non-ketonic neutral fractions, the last two fractions accounting for 23% and 75% of the whole, respectively. The non-ketonic neutral fraction was further separated by column chromatography, and the individual substances were identified by their ultraviolet absorption spectra. The phenanthrene derivative of cholesterol (33) and Diels' hydrocarbon (7) occurred in the fluorescent fractions from the column, but no trace of 3-methylcholanthrene was observed. The latter was carefully sought because theoretically it could have arisen through cyclization of the cholesteryl side chain; instead this side chain was apparently lost to yield Diels' hydrocarbon (7). Compounds (7) and (33) are not carcinogens, and Fieser considered that cholesterol itself, so widely distributed in nature, was most unlikely to be active. He therefore sought carcinogenic activity among the many known oxidation products of this sterol, eventually discovering definite activity in the 6β-hydroperoxy derivative of cholest-4-en-3-one (Fieser et al., 1955).

The methods for producing cyclopenta[a]phenanthrenes from sterols all involve pyrolysis or quinone oxidation at elevated temperatures, conditions far removed from those encountered in living systems. It is therefore of considerable interest and possible significance that Wilk and Taupp (1969) found that 16,17-dihydro-17-isopentyl-15H-cyclopenta[a]phenanthrene (39), obtainable from cholesterol by selenium dehydrogenation at 350°C, was also formed when this sterol was adsorbed on silica gel and exposed to iodine vapour at room temperature for 20 h. The authors considered that the oxidation potential required in the adsorbed state was substantially lower than that normally required for dehydrogenation, and that this might be a model for biological dehydrogenation of steroids by naturally occurring quinones found in the cell.

A considerable amount of data has now been accumulated on the relationship between chemical structure and carcinogenicity among cyclopenta[a]phenanthrenes. On this basis it seems unlikely that any of the hydrocarbons so far identified from natural sources or as pyrolysis products would be active. However, the recently discovered widespread occurrence of cyclopenta[a]phenanthrenes indicates that this class of compound is rather prone to arise from sterols and steroids under a variety of conditions, and this again raises the unresolved question of
whether they occur in animal tissues as a result of incorrect steroid biosynthesis or metabolism.

9.2 Do cyclopenta[a]phenanthrenes occur as a result of aberrant steroid metabolism in animals?

It is now well established that carcinogens are produced by a variety of microorganisms and plants, and there seems no a priori reason why animals should not also possess similar capabilities. The incidences of most common forms of cancer rise steeply with age, and in Great Britain about one in five people eventually die from one of the many forms of this disease. We now have extensive knowledge of the long latent periods encountered in animal experiments with weak carcinogens, or with very low doses of more potent carcinogens, and it seems entirely possible that at least some of this human disease might result from the prolonged action of minute amounts of an endogenous carcinogen formed in the body by aberrant steroid metabolism. The amount needed might only be small, and the potency not particularly high to produce this high cancer incidence observed in elderly people. This view, fashionable 40 years ago, led to experiments that failed to detect such a carcinogen, but in retrospect two reasons can now be advanced to account for this failure. The first has to do with the experimental methods available then. Quite astonishing advances have been made in analytical procedures in the intervening years, and as we have seen these are now beginning to disclose a whole range of unexpected cyclopenta[a]phenanthrenes in natural sources. The application of these new sensitive methods to animal tissues and extracts has not yet been undertaken. The second reason is undoubtedly connected with the history of this subject. As has already been pointed out, the straightforward synthesis in 1933 of the extremely potent carcinogen 3-methylcholanthrene from a simple derivative of cholic acid, coupled with the observations that Diels’ hydrocarbon and other simple cyclopenta[a]phenanthrenes were inactive, for two decades strongly directed interest away from cyclopenta[a]phenanthrenes and towards the cholanthrenes. However, it now seems to be firmly established that sterol dehydrogenation does not lead to compounds of the latter class, but instead side-chain cleavage occurs to generate a variety of cyclopenta[a]phenanthrenes. The discovery of potent carcinogens among this class of sterol degradation products inevitably redirects attention to the possibility of the endogenous formation of carcinogenic cyclopenta[a]phenanthrenes by incorrect steroid metabolism in mammals.
In the older literature, reviewed by Steiner in 1943, there are a number of reports on the production of tumours in animals with various crude tissue extracts. Activity was generally found in association with lipids, and in most instances with the unsaponifiable fraction of these lipids. Crude extracts reported to be active were prepared from human cancer tissues, bile, urine, lung, and liver, but it was the latter source that was studied in greatest detail. Later, in an extensive comparison of extracts of human liver and other human and animal organs (Steiner et al., 1947), considerable carcinogenic potential was detected with extracts of human liver from both normal and cancer patients. Extracts of the minced, frozen tissue were made by alkaline hydrolysis followed by extraction with ethylene dichloride; after removal of the solvent the unsaponifiable lipid was dissolved in warm tricaprylin and injected into C57Bl mice in groups of about 50. Sarcomas were induced at the site of injection in up to half the animals treated with some, but not all, of these liver extracts from both normal and cancer patients. Extracts prepared in the same way from human spleen, and even from livers of stillborn infants also gave some sarcomas, although fewer in number. It was felt these two sources reduced the possibility that all the carcinogenic activity was due to extrinsic, dietary carcinogens. Hieger (1946) also successfully induced sarcomas in mice with unsaponifiable lipid material from human liver, and from pooled lung, kidney, and muscle. He fractionated this lipid and determined that the most active fraction was the crystalline material obtained which consisted largely (85%) of cholesterol. The latent periods for these tumours were long (14–24 months) and he suggested that the carcinogen was probably of low potency. Bischoff (1963), in a thorough review of sterol carcinogenesis, pointed out that the cholesterol oxidation products 5α,6α-epoxy-3β-cholesterol and 6β-hydroperoxycholest-4-en-3-one were known to produce local sarcomas on injection into mice. He felt that this should be taken into account when considering Hieger’s results. With this in mind Higginson et al. (1964) attempted to reproduce the experiments of Des Ligneris (1940) who had found that non-saponifiable extracts obtained from the livers of Bantu subjects, both with and without primary liver cancer, were more carcinogenic in mice than similar extracts from white subjects. The extracts were prepared by the former authors by saponification of the livers in alcohol with potassium hydroxide, followed by extraction of the non-saponifiable lipid with peroxide-free ether, and care was taken to avoid aerial oxidation by carrying out the whole procedure under nitrogen. An extract was prepared from pooled mouse liver in the same way. None of these extracts yielded tumours when mice were repeatedly painted with them, but after injec-
A result of aberrant steroid metabolism?

...tion all gave a low incidence of fibrosarcomas at the injection site, and this was not seen with the solvent (sesame oil or tricaprylin) alone. The authors concluded that while this experiment confirmed the existence of low-grade carcinogens in non-saponifiable extracts from human and mouse liver, it did not support the racial difference claimed by Des Ligneris. Deliberate exposure of these extracts to air and light for prolonged periods to produce peroxides, the presence of which was confirmed by analysis, did not increase this low sarcoma incidence, thus casting doubt on Bischoff's suggestion. It remains difficult to understand the reason for these results, but they cannot be ignored because they are the outcome of quite extensive experiments conducted by serious, capable workers. A whole battery of powerful methods is now available that could be employed to determine whether these crude extracts contain true endogenous carcinogens, or whether the activity is a result of artefacts, either exogenous carcinogens or carcinogens produced under the drastic conditions used in the extraction processes. For example, bacterial mutation assays could be used rapidly to pinpoint active fractions which could then be investigated by gas chromatography–mass spectrometry, a technique which has recently had spectacular success in identifying small molecules, including cyclopenta[a]phenanthrenes, in a variety of natural sources.

The net result of the various structure/carcinogenicity studies among cyclopenta[a]phenanthrenes outlined in this book can be readily summarized as follows:

(i) Carcinogenicity is conferred on the parent hydrocarbon 16,17-dihydro-15\(H\)-cyclopenta[a]phenanthrene (1) by methyl substitution at C-7, and especially at C-11;
(ii) Potency is increased by extending conjugation of the phenanthrene nucleus into the five-membered D-ring, either as an endocyclic carbon–carbon double bond or as an exocyclic carbon–oxygen double bond at C-17;
(iii) The 11-methyl group can be replaced by a methoxy group without marked loss of carcinogenicity, but longer side chains at this position reduce or abolish activity.

In contemplating possible structures for a hypothetical carcinogenic cyclopenta[a]phenanthrene which might be derived from a steroid in vivo, a difficulty is apparent. Whilst a 17-carbonyl group is a common feature of both androgens and oestrogens, rearrangements leading to an 11-methyl or 11-methoxy structure are without precedent; in all known examples rearrangement of the C-19 methyl group in steroids leads to 1-
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or 4-methyl derivatives. It is therefore of considerable interest that introduction of an 11-hydroxy group was also found to lead to carcinogenicity in a cyclopenta[a]phenanthrene. Injection of the sparingly soluble phenol 15,16-dihydro-11-hydroxycyclopenta[a]phenanthren-17-one (206) suspended in olive oil into mice followed by repeated application of croton oil to their dorsal skin caused the appearance of skin tumours, both carcinomas and papillomas, at the site of promotion (Bhatt et al., 1982), thus proving that this phenol is capable of systemic initiation. This compound was less potent than its 11-methyl analogue, but essentially the same result was obtained in two identical experiments separated in time by about a year. 11-Hydroxy steroids are, of course, well known and arise mainly if not entirely from the adrenal cortex. As we have already seen the 3-deoxy steroid androsta-3,5-dien-17-one has been isolated from the urine of both male and female patients with adrenal tumours (Burrows et al., 1937; Wolfe et al., 1941). This is important because the 3-hydroxy group present in the vast majority of steroids would probably inhibit activity in a cyclopenta[a]phenanthrene since the analogous synthetic phenol derived from 3-methylcholanthrene is not a carcinogen (Shear and Leiter, 1941). In this connection Marker and Rohrmann (1939) in their study which disclosed the presence of 3-deoxy equilenin in mares' pregnancy urine, also described a diketone $C_{18}H_{16}O_2$, mp 214°C. This compound was isolated from the non-crystalline carbinol fraction after mild oxidation with chromic acid and formed only a monosemicarbazone, from which the 11,17-diketone structure (see Fig. 88) was proposed for it. The close relationship between this 3-deoxy steroid and the carcinogenic cyclopenta[a]phenanthrene phenol (206) is apparent especially when one considers that the adrenal cortex is not only the centre of steroid 11-hydroxylation, but also of oxygenation at C-18, as in aldosterone biosynthesis. C-18 hydroxylation of this 11,17-diketone could lead to the loss of the angular methyl group and conversion to the 11-phenol (206). Whether this or any other cyclopenta[a]phenanthrene occurs in adrenal tissue or in tumours of this organ is, of course, at present unknown. Adrenal extracts were studied intensively during the isolation

![Fig. 88](image)
of the cortical hormones 40 years ago, but in almost all of this work only the more polar fractions were examined (Fieser and Fieser, 1959). In this work finely chopped frozen tissue was extracted with acetone, the latter was removed by evaporation, and the aqueous residue was extracted with petroleum ether to leave the adrenocortical hormone in the aqueous phase (Cartland and Kuizenga, 1936). Hieger (1946) tested adrenal extracts (prepared by the saponification and solvent extraction method used for liver) for carcinogenicity, but failed to induce sarcomas. It seems unlikely that either of these extraction methods would have isolated the phenol (206), although the petroleum extract might have contained cyclopenta[al]phenanthrene hydrocarbons, if they were present.

Modern cancer research is beginning to uncover the cause of some human tumours. By far the most important discovery is that cigarette smoking is causally related to the tragic increase in lung cancer observed during this century, a disease that now claims some 30,000 men each year in this country alone. Several other minor forms of cancer have been traced to industrial exposure to carcinogens. For example, bladder cancer is associated with exposure to aromatic amines previously used as dyestuff intermediates and as antioxidants in the manufacture of rubber. However, it still remains true that the cause of the majority of human tumours remains completely unknown. The case for the initiation of at least some of these by endogenous carcinogens formed in the body from steroids now seems as strong as it did 50 years ago. It is clear that a fresh approach to this whole question now needs to be made, fully utilizing both the splendid new analytical techniques and our deeper understanding of steroid biochemistry.

9.3 References


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Throughout this Index the word 'cyclopenta[a]phenanthren(e)' is abbreviated to 'cpp', but is indexed as the full word. The prefixes cis, trans, syn, anti, and \( n \) (= normal) are ignored for the purpose of indexing. Besides more general entries, the index also contains the chemical names of approximately 350 cpps, of which over 280 bear the serial numbers (in bold type) assigned to them in the text, tables, and figures. There is a brief guide to cpp nomenclature on pages 2-3 of this book, but in case of difficulty look up the serial number in the serial number-molecular formula index (pp. 122-5). Then using the molecular formula find the compound in the physical and spectral data compilation (pp. 85-122) which is arranged according to the Chemical Abstracts system and also contains the full chemical name used here. Author indexes are to be found at the end of each chapter.
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D.Sc. Presentation

I wish to base my presentation for the degree of Doctor of Science on my work in the general area of chemical carcinogenesis over the last 25 years (please see 1959-1987 section (viii) in my Curriculum Vitae). This research is largely summarised in a recent book entitled "Cyclopenta[a]phenanthrenes" published last year by Cambridge University Press, and which I now include as the main part of my submission. It is supported by 24 scientific papers and reviews, of which I am author or co-author, which seem to me to provide a fair overview of my interests and endeavours in this field. These together represent about a quarter of my publications which are listed in full in my c.v. Those now submitted consist of:-


(ii) a review on chemical carcinogenesis commissioned by the Journal of Pathology in 1980, and a chapter written by invitation for the book "Target Organ Toxicity" published in 1987;

(iii) 11 assorted papers on carcinogen metabolism, mutagenicity and carcinogenicity testing, and chemical structure/carcinogenicity relationships, published mainly in cancer research journals.

I am the sole author of the first few of these papers, but later, as head of the Chemistry Laboratory in the Imperial Cancer Research Fund, I had the benefit of collaboration with a series of excellent postdoctoral research fellows, postgraduate Ph.D. students, and senior technical staff whose names appear in the various publications. I directed all the research described in these papers and had a substantial personal involvement at the practical level in all of it. Although most of the animal work was carried out by my colleague, Dr. T.S. Bhatt, I possessed a Home Office animal licence until I moved to Surrey a year ago.

M. M. Coombs

M.M. Coombs
September, 1988
Announcing a new series

CAMBRIDGE MONOGRAPHS ON CANCER RESEARCH

Chemical Carcinogens

Scientific Editors: Dr. Maurice M. Coombs, Imperial Cancer Research Fund Laboratories, London; Dr. John Ashby, Imperial Chemical Industries Plc, Cheshire; Professor R. Marian Hicks, United Biscuits (UK) Ltd, R. & D. Centre, High Wycombe, formerly at the Middlesex Hospital Medical School, London.

Executive Editor: Herbert Baxter, formerly at the Laboratory of the Government Chemist, London.

This major series will examine in detail the mechanisms of carcinogenesis. Initially the series has concentrated on chemical (environmental) carcinogenesis, where there is a great quantity of data which is in need of presentation in a coherent and systematic format. The series is now broadening to include other aspects of carcinogenesis, such as cell and molecular biology.

An understanding of the chemistry and metabolism of chemical carcinogens will help to provide valuable insight into this area, as well as pointing the way for more effective safety measures. The series has therefore been initiated with a number of monographs on the chemistry and biological effects of chemical carcinogens. The monographs will be in-depth studies that bring together and evaluate the published data, and present a coherent, integrated account of the cited literature. Volumes of approximately 200-300 pages are envisaged, each involving the following subject areas:

Nomenclature and structure
Occurrence; Synthesis
Industrial preparation and use (for compounds used commercially)
Physical and spectral properties (including X-ray studies)
Analysis
General chemistry (and synthesis if not included above)
Metabolism, including identification of metabolites
Interaction of the compounds, and their metabolites, with biological macromolecules
Toxic, mutagenic and carcinogenic properties.

In a series of this nature it is not easy to define areas precisely, and it is recognized that authors will require a certain amount of latitude in their approach to both the style and content of these volumes. Since the subject matter of the books covers a range of scientific disciplines, some of the books may require more than one author.

We think that the series will be popular as it will provide comprehensive and integrated reviews of the chosen topics to both chemists and biologists. The appearance of the series is timely because of the high current interest in the role of environmental factors, including chemical pollution, in the aetiology of human cancer.
Published titles:

M. Osborne & N. Crosby: *Benzopyrenes*

M.M. Coombs & T.S. Bhatt: *Cyclopenta(a)phenanthrenes*

M. Newman, S. Veeraraghavan & B. Tierney: *Chemistry and biology of benz(a)anthracenes*

The following titles are now in preparation for the series:

J. Jacob: *Sulphur analogues of polycyclic aromatic hydrocarbons*

M.J.E. Hewlins & J.C. Phillips: *Nitrogen analogues of polycyclic aromatic hydrocarbons*

W.F. Karcher: *Dibenzanthracenes*

J. Jacob & W.F. Karcher: *Benzofluoranthenes and fluoranthene*

C.W. Vose and S.T. Hadfield: *Cholanthrenes*

H. Rosenkranz & P. Howard: *Nitrated polycyclic aromatic hydrocarbons*

S.K. Yang: *Chrysenes*

W. Lijinsky: *Chemistry and biology of N-nitroso compounds*

R.G. Harvey: *Polycyclic aromatic hydrocarbons: chemistry and carcinogenesis*

T. Sugimura & S. Takayama: *Heterocyclic amines: mutagens and carcinogens in cooked and pyrolysed foods*

J.P. Glusker: *Structural studies in cancer research*

J. Higginson et al.: *The epidemiology and causes of human cancer*

B.M. Wagner & R.M. Leader: *Animal models of carcinogenesis*

R.W. Tennant: *Biological aspects of identifying chemical carcinogens*

G.B. Leslie, C. Joseph & F.J.C. Roe: *Hormones, diet and cancer*

G. H. Pigott: *Solid state carcinogenesis*

A.R. Kinsella: *Colorectal carcinoma: a scientific perspective*
More than twenty books in this series are in preparation and the first three are now published. The series originated with books on chemical carcinogenesis, and from the beginning authors have been encouraged to write in depth about fields in which they have been personally involved and have a special interest. About ten volumes covering various classes of polycyclic aromatic hydrocarbons and their heterocyclic analogues, N-nitroso compounds, and aromatic nitro compounds, are being written. More recently the series has widened in scope and more general monographs, for example on animal models in carcinogen testing, biological aspects of short-term tests, the effects of diet and hormones on human cancer incidence, and modern perspectives in cancer aetiology, are now under contract.

Organ-specific carcinogenesis

We wish now to propose further monographs on the separate diseases which are loosely classed together as cancer, seen primarily from the scientific viewpoint. For example, bladder cancer, leukaemia, and cancer of the colon, breast and lung are distinct, common human diseases which immediately spring to mind. There is clearly scope for a number of useful volumes of this type, and with these we suggest that the following points, among others, should be considered:

Occurrence of the particular human disease and what is known of its causes through epidemiology, etc.

A short review of its salient pathology, and a brief indication of methods used in its treatment including a realistic view of what is achievable by these means. This will include inter alia a concise discussion of the anatomy and physiology of the organ in question for the benefit of non-medical readers.

Animal models for the disease, induction of the disease with particular carcinogens, possibly with a résumé of what is known about the mechanism of action of the carcinogen at the molecular level; effects seen experimentally in the use of promoters and tumour inhibitors in these situations, with particular respect to possible human exposure to these and similar agents.

Like the earlier volumes of this series, these new monographs of about 300 pages will be aimed largely at graduates who need to obtain an authoritative, up-to-date review of the present situation in these areas.

Cambridge University Press is taking much care in presenting these books attractively in hard-back edition at an affordable price and we hope that the series will become established world-wide in many medical and scientific libraries. Of course we realise that individual authors will want to approach their subject in their own way and may want to involve colleagues in view of the wide range of subject matter to be covered. However we hope that each volume will be a well-integrated, in-depth study of the disease in question with adequate references to the original literature for those who want to delve further. The editors would welcome suggestions for subject areas, with or without synopses, and any other comments on this proposed new venture.

John Ashby Scientific
Maurice Coombs Editors
Marian Hicks
Herbert Baxter Executive Editor
Publications


42.* Potentially carcinogenic cyclopenta[a]phenanthrenes. Part X. Oxygenated derivatives of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one of metabolic interest. M.M.


CHEMICAL CARCINOGENESIS: A VIEW AT THE END OF THE FIRST HALF-CENTURY*

M. M. COOMBS
Chemistry Laboratory, Imperial Cancer Research Fund, London

Reviews of chemical carcinogenesis commonly begin with the London surgeon Sir Percival Pott, who in 1775 concluded that the high incidence of epithelioma of the scrotum among chimney sweeps was due to the soot to which they were daily exposed from an early age. In his "Chirurgical Observations" he wrote "It is a disease which always makes its first attack on, and its first appearance in the inferior part of the scrotum; where it produces a superficial, painful, ragged, ill-looking sore with hard and rising edges. The trade call it soot-wart. . . . The disease, in these people, seems to derive its origin from a lodgement of soot in the rugae of the scrotum." Probably the first preventive measure against chemically induced cancer was taken in 1778 when the Danish chimney-sweeps' guild urged its members to bathe daily (Clemmesen, 1951). A hundred years later the same disease occurred among workers in the Lancashire cotton spinning industry where it became known as "mule spinner's cancer". About this time occupational skin cancer among oil and tar workers was reported by von Volkmann (1875) in Germany and by Bell (1876) in Scotland, and it was suspected that prolonged contact of the spinners with clothes impregnated with mineral oil used to lubricate their machines was responsible for the disease. Towards the end of the century attempts were made to produce skin cancer experimentally in animals by topical application of these oils and tars. Success was finally achieved in 1915 by two Japanese workers, Yamagiwa and Ichikawa, who induced tumours on the ears of rabbits by repeated application of coal tar; they succeeded where others had failed by persisting with their treatment, thus imitating the human exposure. Using the dorsal skin of mice as a more convenient test system, Sir Ernest Kennaway and his associates at the Royal Cancer Hospital in London soon showed that strongly carcinogenic tars resulted from pyrolysis of many carbonaceous materials such as cholesterol, yeast, and human hair, skin, and muscle, as well as non-carcinogenic oils and even acetylene (HC = CH) (Kennaway, 1955). Examination of these tars revealed that they all possess characteristic lines in their fluorescence spectra, similar to those displayed by the polycyclic aromatic hydrocarbon benz[a]anthracene (1:2-benzanthracene, 1, fig. 1), itself essentially non-carcinogenic. In 1929 Clar synthesised a derivative, dibenz[a,h]anthracene (1:2:5:6-dibenzanthracene, 2), which has the distinction of being the first pure chemical compound to be shown to be carcinogenic (Kennaway and Hieger, 1930).
The study of chemical carcinogenesis can therefore be considered to begin with these momentous discoveries. It is the purpose of this review to outline briefly the progress which has been made in our understanding of this important subject during the intervening half-century. The viewpoint is of necessity that of an organic chemist whose interest is focused on this area.

Types of chemical compounds having carcinogenic properties

Over 3000 pure chemical compounds are now known to be carcinogenic, in experimental animals and it seems certain that this number will increase since at present about 200,000 new organic compounds are reported yearly (Arcos, 1978). Chemical carcinogens are now known to belong to widely diverse structural types and include a number of natural products, thus dispelling the idea current some years ago that carcinogenic activity would be found only among "artificial" compounds for which detoxifying enzymes had not been developed during the course of evolution. In this section the various types of chemical carcinogens will be discussed and the bearing they have on human disease will be noted.

Polycyclic hydrocarbons

Using the characteristic fluorescence spectrum as a guide, Kennaway and his associates fractionated 2 tons of gas-works pitch, eventually to obtain 7 grams of yellow crystals consisting mainly of benzo[a]pyrene (BaP, 3 : 4-benzpyrene, 3, fig. 1). This compound is strongly carcinogenic and it is now known to be very widely distributed because it is formed whenever organic matter undergoes incomplete combustion. It is present in urban air in measurable quantities; in fact it is estimated that in America no less than 1300 tons of benzo[a]pyrene is emitted into the atmosphere each year. Even so, Shubik (1972) has suggested that the lower incidence of lung cancer in the USA compared with this country, when allowance is made for the different smoking habits, may be due to the 12,000-fold higher content of benzo[a]pyrene in the coal tar used in road-making here in Britain, compared with the petroleum asphalts used for this purpose in the USA. Benzo[a]pyrene is also detectable in everyday materials such as charcoal-broiled steak, whisky, and tobacco smoke (Nagao and Sugimura, 1978). Compounds 1–3 belong to a group of...
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polycyclic compounds, structurally related to phenanthrene (4), many of which are carcinogenic (fig. 2).

Phenanthrene itself is inactive, but a relative, 7,12-dimethylbenz[a]anthracene (DMBA, 9:10-dimethyl-1:2-benzoanthracene, 5), is one of the most potent carcinogens known. Topical application of micrograms of this compound is sufficient to induce skin tumours in the mouse. Simpler compounds of the cyclopenta[a]phenanthrene series structurally related to steroids are active; for example the ketone (6) studied by ourselves is similar in activity to benzo[a]pyrene as a carcinogen (Coombs, Bhatt and Croft, 1973). 3-Methylcholantherene (7), another "classical" carcinogen, was first produced by pyrolysis of a bile acid derivative (Cook and Haslewood, 1933; Wieland and Dane, 1933). At one time there was a suspicion that such compounds might arise in the body by aberrant steroid metabolism (Inhoffen, 1953); however, no evidence has come to light to support this idea. Many synthetic heterocyclic analogues of these polycyclic aromatic hydrocarbons made by Buu-Hoi and his associates (Lacassagne, Buu-Hoi, Dandel and Zajdela, 1956; Buu-Hoi, 1964) are also strong carcinogens.

Aromatic amines and azo compounds (fig. 3)

Another large group of related compounds comes under this heading. The greatly elevated incidence of bladder cancer formerly found among workers in dyestuff factories (Wignall, 1929) was traced to their daily contact with aromatic amines such as benzidine (8) and 2-naphthylamine (β-naphthylamine, 9), produced as intermediates in dye-stuff manufacture (Case, Hosker, McDonald and Pearson, 1954). Benzidine, of course, was commonly employed in medical laboratories as a test for occult blood. Bladder cancer was also a health problem in sections of the rubber industry where aromatic amines were used as anti-oxidants (Case and Hosker, 1954). The manufacture and use of these materials is now prohibited in this country under the Carcinogenic Substances Regulations, 1967, No. 879. Another aromatic amine which has achieved considerable importance in experimental cancer research is 2-acetamidofluorene (AAF, 10). This compound was proposed as an insecticide during the 1939–45
war, but luckily it was tested (Wilson, De Eds and Cox, 1941) before it was employed on a large scale. It is a powerful carcinogen, producing a variety of tumours in rodents, including those of the liver, external acoustic meatus, and mammary glands. Very recently Sugimura and co-workers (1977) in Japan, investigating the mutagenicity of partly charred fish and meat, made the important discovery that pyrolysates of the amino-acid tryptophan led to compounds of the type (11), superficially related to AAF. Compound 11, 3-amino-1-methyl-5H-pyrido[4,3-b]indole, was intensely mutagenic for bacteria and transformed cells in culture; it would therefore be expected to be carcinogenic in animals when proper tests are completed.

Closely related to the aromatic amines are the aromatic azo compounds which are important as dyes. Some, such as o-aminazotoluene (12), give liver tumours on prolonged feeding to rats (Yoshida, 1934a). Of this class the most notorious is butter yellow (13), (Yoshida, 1934b; Kinoshita, 1936) so called because up to about 1935 it was used to colour margarine yellow, like butter. Many food colouring agents are azo compounds, but today they are submitted to extensive animal tests before they are certified safe for use in food.

Fig. 3.—Carcinogenic aromatic amines and azo compounds; (11) is the recently discovered pyrolysis product of the amino-acid tryptophan.

Nitrosamines and nitrosamides, long familiar to organic chemists as synthetic intermediates, form another large and more recently discovered group of potent carcinogens (Magee and Swann, 1969). Formulae of typical nitrosamines are shown in the figure; the common structural feature associated with carcinogenicity seems to be the possession of the moiety CH-N(NO)R (Lijinsky, 1977).

Dimethyl nitrosamine, (CH₃)₂N-NO, was formerly used as an industrial solvent, and it was the observation of liver cirrhosis in exposed workers that led Magee and Barnes (1954) to investigate it in animals. It proved to be highly toxic, and in chronic experiments in rats it gave 100 per cent incidence of liver
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Cancer. Of over 100 nitrosamines tested, 90 per cent. are carcinogens, and many display remarkable organotropic properties. Thus while the liver is the main target, followed by lung and kidney with the majority of the compounds, certain derivatives reproducibly give rise to cancer of the oesophagus, forestomach, and nasal cavity (Druckrey, 1975). Another notable feature of nitrosamine carcinogenesis is that it has been shown to occur in each of the 16 species in which tests have been made—in fish, birds, and mammals, including guinea-pigs which are resistant to most chemical carcinogens, and in monkeys. It can hardly be doubted that man is also susceptible.

In contrast to the nitrosamines and the other types of carcinogens so far discussed, nitrosamides such as N-methyl-N-nitrosourea (MNU 14) and the related N-methyl-N-nitrosourethane (MNUT, 15) and N-methyl-N°-nitro-N-nitrosoguanidine (MNNG, 16) are direct acting carcinogens, i.e., they do not require biological activation before they can exert their biological potential, although tissue thiols markedly enhance the rate of methylation of DNA by MNNG (Lawley and Thatcher, 1970). Like the nitrosamines, they elicit tumours at several sites (Druckrey, 1975). Oral administration of MNNG to guinea-pigs produced squamous-cell carcinomas of the forestomach while MNUT gave adenocarcinomas of the pancreas and gastric carcinomas resembling human stomach cancer. Intravenous injection of MNU into rats, dogs, and rabbits yielded neural tumours of the brain and spinal cord. The latter were induced in young rats after treating their mothers with ethylnitrosourea at the 15th day of gestation; transplacental carcinogenesis was also observed with other nitroso compounds.

Nitrosamines are readily formed by interaction of amines with nitrous acid ($HNO_2$) at acid $pH$ (Walters, 1977). Suitable amines, of course, occur widely in foodstuffs, particularly meat and fish. Apart from atmospheric nitrogen, the most widespread form of this element is nitrate, but many bacteria and some fungi and plants are capable of reducing this to nitrite; a nitrate reducing enzyme is also present in human saliva (Tannenbaum et al., 1977). Sodium nitrate is widely used as a preservative in cured meat and fish, bacon, cheese, etc.
It is therefore not surprising that trace amounts of nitrosamines can be detected in samples of these foods; levels of 100 \( \mu \text{g/kg} \) of dimethylnitrosamine have been observed consistently in fried bacon (Scanlan, 1975). No human disease is yet definitely associated with nitrosamines, but the presence of nitrosamines, including a new compound \( N\text{-I-methylacetonyl-N-3-methylbutyl nitrosamine} \), in corn bread inoculated with common fungi of the \( \text{Fusarium}, \text{Geotrichan} \), and \( \text{Aspergillus} \) species and encountered as food in the Henan province of China, has recently been discussed with reference to the high incidence of oesophageal cancer in this area (Shixin, Mingxin, Chuan, Mingyao, Yinglin, and Liang, 1979). At present it is impossible to judge the effects of exposure of human beings to trace levels of carcinogens, for it is not certain whether we may extrapolate from animal experiments or whether there is a low threshold dose below which no effect is found (Cornfield, 1977). However, it has been pointed out (Walters, 1977) that the steady decline in gastric cancer in the western world may be due to the use of refrigeration to store nitrate-containing vegetables, thus inhibiting its enzymatic conversion to nitrite. It is clearly essential to aim at minimisation of the amounts of these versatile carcinogenic nitroso compounds in the environment.

**Biological alkylating agents (fig. 5)**

Nitrosamines and nitrosamides are alkylating agents, but here we consider other types which like the latter (but not the former) are direct acting carcinogens. \( 2,2'\text{'-Dichlorodiethyl sulphide} \) (17), used as the vesicant mustard gas in the 1914–18 war, causes cancer of the bronchus, larynx and nasal sinuses (Doll, 1977a). Replacement of the sulphur atom by nitrogen gives a nitrogen mustard...
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(18), the progenitor of hundreds of anti-cancer drugs such as cyclophosphamide (19), chlorornaphazin (20), and the more distantly related myleran (21).

Many of these drugs have been shown to be weak to moderately strong carcinogens in animal tests, and there is evidence for the involvement of some in the induction of human tumours. Chlorornaphazin, for example, caused bladder cancer in patients with other forms of cancer treated with it and its use has been discontinued (Thiede, Chieritz and Christenson, 1964); treatment with cyclophosphamide is associated with the induction of leukaemia. Medically unimportant monofunctional alkylating agents, such as methyl methanesulphonate (19), β-propiolactone (20) used in fumigation, and vinyl chloride (21) are also carcinogens. The last, produced industrially on a vast scale in the manufacture of polyvinylchloride (PVC), produces a rare type of liver tumour (angiosarcoma) in workers exposed to it (Waxweiler et al., 1976).

![Chemical structures]

Fig. 6.—Some naturally occurring carcinogens.

Fortunately PVC itself appears to be safe, but there has been considerable concern about the possible release of occluded monomer from this plastic which is used in food packaging.

Carcinogenic natural products (fig. 6)

One of the newer aspects of chemical carcinogenesis is the lengthening list of naturally occurring compounds which have been shown to be active. These include the weak liver carcinogens safole (25), present in oil of sassafras, pyrrolizidine alkaloids (26) which occur in senecio leaves used in Africa to prepare a medicinal tea, and capsaicin (27), a constituent of chili peppers.

The aflatoxins such as aflatoxin B1 (28) are a family of complex lactones produced by the common yellow mould Aspergillus flavus which contaminates peanuts and certain grains. This compound (28) is perhaps the most potent carcinogen known, producing liver tumours in rats fed with 1 μg per day, and local sarcomas in mice after subcutaneous injection of 10 μg (Butler, Greenblat and Lijinsky, 1969). It is also extremely toxic and was, in fact, discovered as a
result of the investigation in 1960 of the death of a large number of young turkeys fed on contaminated peanut meal (Lancaster et al., 1961). The aflatoxins are widely distributed especially in hot, humid parts of the world such as central Africa and South East Asia where it is thought that they may be implicated in the abnormally high incidence of primary liver cancer found in these areas (Linsell and Peers, 1977). Another carcinogenic natural product is the glycoside cycasin (29a) present in cycad nuts, used as a foodstuff in Guam. It is hydrolysed by the intestinal flora to the aglycone methylazoxymethanol (29b) which behaves as a methylating agent like the methylnitrosamines, causing liver and kidney tumours in experimental animals (Lacqueur, 1964). Bracken ferns, common in Great Britain, contain an unidentified carcinogenic principle (Evans and Mason, 1965) which may account for the frequent observation of stomach tumours in cattle in certain parts of Scotland. Young bracken fronds are eaten as a vegetable in Japan.

\[
\begin{align*}
\text{HO} & \quad \text{HO} \\
\text{30} & \quad \text{31}
\end{align*}
\]

**Fig. 7.**—Oestrogens: oestrone (30) and stilboestrol (31).

### Steroids (fig. 7)

In 1932 Lacassagne induced mammary cancer by injection of oestrone (30) into male mice of a strain in which only the females normally develop this tumour. This natural sex hormone, which is essential for the full development of all female animals, was therefore the first naturally occurring compound whose administration appeared to cause cancer.

At about this time the newly discovered carcinogenic hydrocarbon benzo[a]pyrene was found to possess weak oestrogenic activity. Dodds (1947), in extensive work aimed at investigating the connection between these two biological activities, synthesised the compound stilboestrol (31) which has exceptional oestrogenic potency, surpassing even that of the natural hormone. This compound has since gained importance as a drug, and commercially as an aid in fattening of cattle for slaughter. Used therapeutically to control prostatic cancer, it not unexpectedly causes breast development with a risk of breast cancer in the men treated. It is now evident that the use of large doses of stilboestrol to avert threatened abortion has resulted in vaginal cancer in some of the daughters of the woman treated 15–20 years previously (Bibbo, Gill and Azizi, 1977). The more recent treatment of menopausal symptoms by hormone replacement therapy using oestrogens has led to the development of endometrial cancer (Smith, 1975).
The bile acids, too, have been under suspicion as carcinogens ever since the early pyrolytic conversion into 3-methylcholanthrene. More recently Hill (1975) has proposed that they may be involved through anaerobic metabolism by intestinal flora, in the causation of colon cancer.

Miscellaneous organic compounds (fig. 8)

The preceding sections mentioned the main classes of chemical carcinogens without, of course, being exhaustive. There are many other compounds which do not fall into these categories; a few of these of particular interest are mentioned here.

Colon cancer has until recently been difficult to study experimentally due to the lack of an animal model for the disease, but now colon tumours are readily obtainable in rodents by gastric instillation of 1,2-dimethylhydrazine (33) (Newberne and Rogers, 1973). Another powerful, direct-acting carcinogen which has found much use in research is 4-nitroquinoline-N-oxide (32) (Nakahara, Fukuoka and Sugimura, 1957). The drugs isoniazid (34), important in the treatment of tuberculosis (Toth and Shubik, 1966), and the useful trichomonacide metronidazole (35) (Rustia and Shubik, 1972), increase the incidence of lung adenomas in mice, although there is no evidence that they are active in man (Goldman, Ingelfiner and Friedman, 1977). Urethane (C₂H₅O·CONH₂) previously employed as a hypnotic, is more active, giving occasional liver tumours as well as lung adenomas (Tannenbaum and Silverstone, 1958). Hycanthone (36), widely used as a schistosomicide, is a weak liver carcinogen in mice, and is interesting because it probably represents the first instance where a bacterial mutagen was subsequently found to be carcinogenic (Hartman and
Ethionine (37), a synthetic homologue of the natural amino-acid methionine, is a hepatocarcinogen in the rat. Several common solvents are active on chronic exposure. Carbon tetrachloride previously used in dry-cleaning is a weak liver carcinogen, as is chloroform employed at one time as an anaesthetic and commonly as an ingredient in cough syrups. Dioxan, a widely used solvent, induces tumours in the liver in rodents at relatively high doses (Argus, Arcos and Hoch-Ligeti, 1965).

Inorganic carcinogens

Chemical carcinogens are not confined to organic compounds. A number of metallic elements are known to be active in producing various types of tumours in man and in animals, either as the pure metal or in the form of its salts or oxide (Hemberg, 1977). Thus it has been known for a long time that workers in the chromate and nickel industry suffer from a high incidence of lung cancer, while beryllium salts cause this disease in animals of several species, including primates. Injections of cadmium or its salts, or cobalt powder, induce sarcomas at the site of injection, and kidney tumours follow renal degeneration caused by lead poisoning in experimental animals. Arsenic is interesting because whereas lung and skin cancer has resulted from its therapeutic use in humans, its carcinogenicity has not been confirmed in animal studies (Ott, Holder and Gordon, 1974).

By far the most important inorganic carcinogen is asbestos; of the various forms of this mineral crocidolite (blue asbestos) is apparently the most active (Doll, 1955). Asbestos has been used widely in construction, insulation, etc., and an important use is as a component in brake linings. Blue asbestos is no longer imported, but it is still encountered during the demolition of older buildings. Workers in those industries where exposure to asbestos dust occurs (Selikoff, 1977) suffer from a rare tumour, mesothelioma, which appears to be caused by the ability of the extremely thin mineral fibres to penetrate into the pleura. Mesothelioma can be induced in rats by implantation of asbestos or even glass fibres of comparable dimensions in the pleura (Stanton and Wrench, 1972). An elevated incidence of bronchial carcinoma also occurs among these asbestos workers. In addition there appears to be a synergistic relationship between asbestos inhalation and cigarette smoking, because the incidence of lung cancer among asbestos workers who smoke is higher than that expected if the two exposures were purely additive (Saracci, 1977). The carcinogenicity of asbestos seems to be related to its physical shape and size, rather than to its physiochemical properties and therefore comes into the province of physical carcinogenesis, rather than chemical carcinogenesis.

Metabolic activation and the somatic mutation theory of cancer

The bewildering variety of chemical compounds outlined above leads us to ask what the underlying reason is for their special ability to cause cancer. Subtle factors are involved because frequently delicate structure/activity relationships are observed. To quote one example, with one exception the
positional methyl isomers of the strongly carcinogenic ketone (6) are all inactive (Coombs, Bhatt, and Croft, 1973). Although it is true that for many compounds we are still unable to answer the above question in detail, important conceptual and practical advances have been made in this area during the last two decades. In order to understand these it is first necessary to discuss the somatic mutation theory of cancer.

Sixty-five years ago Boveri (1914) suggested that cancer might arise as a result of mutations in somatic cells. This theory accounts for the most characteristic feature of cancer, namely that tumour cells give rise to daughter tumour cells with identical properties, distinct from those of the normal tissue cells from which the tumour has developed. Altered genetic information is inherited, so that tumours seldom regress. The theory also readily explains the wide variety of tumours found in animals and man, and that most tumours appear to be clonal in origin (Fialkow, 1976). More recently it has been supported by investigations of the hereditary disease xeroderma pigmentosum which occurs about once in 250,000 births (Arlett and Lehmann, 1978). The skin of sufferers with this affliction is susceptible to damage by sunlight and artificial sources of ultraviolet light; irregular pigmented lesions appear early, followed by moles and later by malignant epithelial skin tumours. Those affected seldom survive long into adulthood. The disease is caused by a genetically controlled lack of the enzymes which normally repair DNA damage caused by ultraviolet light, thus demonstrating a clear relationship between induced DNA damage and cancer. Analysis of human cancer incidences, which mostly increase steeply with age, suggests that mutations in several (four to five) genes are necessary to transform a normal into a tumour cell (Cairns, 1975). Spontaneous mutations occur at random throughout life, thus providing a reason for the long latent period often seen between the application of a carcinogen and the appearance of a tumour.

If cancer is caused in general by somatic mutation, it follows that all chemical carcinogens should also be mutagens. It was the apparent failure of the potent classical carcinogens to act as mutagens in the standard bacterial test systems that previously discredited the somatic mutation theory. The reason for this failure is easy to see in retrospect; no allowance was made for the crucial role played by mammalian metabolism in mutagenesis and carcinogenesis. Traditionally, metabolism of compounds foreign to the body was looked upon purely as the method by which the animal disposed of this material by rendering it water soluble prior to excretion. The notion that this process might involve reactive intermediates which can react covalently with DNA and thereby cause mutations did not take root easily. Nevertheless, the essential correctness of this idea is readily demonstrated experimentally by adding a source of oxidative enzymes, such as that contained in freshly prepared rat liver homogenate containing the correct co-factors, to the bacterial test system. Under these conditions the classical carcinogens are strong mutagens. The key to modern work on mutagenesis and carcinogenesis is therefore biological activation.

It is now difficult to see why it took so long for this concept to gain general acceptance. Already in 1947 Boyland stressed the importance of metabolism
in carcinogenesis with polycyclic hydrocarbons, and the covalent interaction of carcinogens with proteins of target tissues had been noted (Miller and Miller, 1952). Demonstration of the binding of the nitrogen mustard (18, fig. 5) to nucleic acids (Wheeler and Skipper, 1957) was followed by Brookes and Lawley (1964), who in a classical paper showed that the carcinogenic potency of five polycyclic hydrocarbons correlated with their extents of binding to the DNA, but not to the RNA or protein of mouse skin in vivo. A clear demonstration of the part played by metabolism in the activation of the carcinogenic aromatic amine, 2-acetamido fluorene (AAF, 10), was provided by the pioneering work of J. and E. Miller (Miller, 1970). They found (fig. 9) that AAF, which after

![Diagram of AAF activation](image)

Fig. 9.—Biological activation of the carcinogen 2-acetaminofluorene (AAF). Enzymatic hydroxylolation at nitrogen is followed by conversion to the sulphate ester (40) which is the proximate carcinogen. The latter decomposes to yield the electrophilic ultimate form (41) which reacts, for example, with deoxyguanosine (dG), to form the major adduct (42). Compound (39) is a synthetic ester which is also a proximate carcinogen.

injection gives rise to distant liver tumours in most species except the guinea-pig, undergoes biological N-hydroxylation to N-hydroxy-AAF in the liver (38). This metabolite which is more carcinogenic than AAF particularly at the site of injection, and is also active in guinea-pigs, is termed the "proximate carcinogen." It is not itself capable of reacting spontaneously with biological macromolecules (proteins and nucleic acids), whereas simple synthetic esters such as the acetate (39) have this property. In the intact animal the ester probably involved is the sulphate (40) formed through esterification of (38) by the enzyme PAPS (3'-phosphoadenine-5'-phosphosulphate). Breakdown of this sulphate ester by cleavage of the N-O bond (with acquisition of the two electrons it contains by the oxygen atom, as symbolised by the curved arrow) generates the electrophilic species (41). The latter, which is electron deficient, readily attacks electron-rich (nucleophilic) centres in molecules forming, for example with deoxyguanosine,
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the covalent adduct (42) in neutral, aqueous solution. The net result is that the carcinogen AAF becomes chemically bound to the purine base at C-8 through its nitrogen atom, providing a model for the way in which AAF reacts with DNA.

The concept of the reactive form ("ultimate carcinogen") as an electrophilic reagent has proved to be fruitful (Miller and Miller, 1971). It is applicable to direct acting carcinogens, such as the nitrogen mustard (15) (fig. 10) which does not require metabolic activation, and by cleavage of a C-Cl bond gives the electrophilic species (43). Dimethylnitrosamine (44) undergoes biological C-hydroxylation with subsequent loss of this carbon atom to give the azo compound (45) which acts as a source of the electrophilic methylating group [CH$_3^+$. The second part of this pathway is shared by MNU which does not need intermediate metabolic activation (Druckrey, 1975).

The first chemical carcinogens to be discovered, the polycyclic aromatic hydrocarbons, are currently the last in which the pathway of biological activation has been delineated (fig. 11). Following the original suggestion by Boyland (1950) that epoxides might be important in the metabolism of aromatic compounds, Sims (1974) at the Chester Beatty Research Institute in London showed that the proximate form of benzo[a]pyrene (3) is its 7,8-trans-diol (46), and that this is further metabolised to the ultimate carcinogen, the bay-region diol-epoxide (47). The strained epoxide ring in this metabolite acts as an electrophilic reagent. It combines for example with the electron-rich exocyclic amino group of deoxyguanosine to give the adduct (48) in which a trihydroxy derivative of the hydrocarbon is covalently linked to deoxyguanosine through

![Figure 10](image_url)

**Fig. 10.**—Activation of a nitrogen mustard (spontaneous), a nitrosamine (44, through biological hydroxylation to a proximate carcinogen), and a nitrosamide (14, spontaneous) to ultimate electrophilic carcinogens.
C-10 of the benzo ring (Weinstein, 1976). Since 1974 there has been intense interest in this field, particularly in the USA, and no less than eight phenanthrene-related polycyclic carcinogens including the ketone (6) (Coombs, Kissongerhis, Allen, and Vose, 1979) are now known to be activated in an analogous manner.

The extremely potent carcinogen aflatoxin B1 (28) is also activated through an epoxide formed at the double bond in the terminal furan ring, with subsequent opening to give an electrophilic form that substitutes N-7 in deoxyguanosine (Swenson et al., 1977). It seems very probable that when the metabolism of other types of carcinogens is studied it will be found that these, too, give rise to reactive forms that are electrophilic reagents.

The enzymes that carry out these oxidations occur in the microsomal fraction of tissues as members of the "mixed function oxidase" system. This is a complex of numerous mono-oxygenases which require NADPH and molecular oxygen, and which carry out aromatic ring oxidation, aliphatic hydroxylation, oxidative demethylation, N-hydroxylation, etc., on a great variety of organic compounds (Gillette, 1969). Relatively high levels of these enzymes are present in liver, and they are detectable in most other tissues. Enzyme levels are capable of being raised by prior administration of many types of compound, including polycyclic hydrocarbons and drugs such as phenobarbital, by a process known as enzyme induction. Responsiveness to the induction of the enzymes which oxidise aromatic double bonds (aryl hydrocarbon hydroxylase, AHH) is under strict genetic control in mice (Nebert, Robinson, Niwa, Kumaki, and Poland, 1975), and mice of inducible strains are
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more susceptible to carcinogenesis by polycyclic hydrocarbons than mice of non-inducible strains (Nebert and Felton, 1975). In humans it seems probable that the variation in the outcome of exposure to chemical carcinogens, for example among smokers exposed to roughly the same carcinogenic dose, may be due to this phenomenon. Thus it has been shown that the induced levels of AHH in the placenta of women who smoke varies considerably between individuals (Nebert, Winkler, and Gelboin, 1969). Moreover, there is now strong evidence that there is a greater proportion of high inducers among patients with squamous-cell lung cancer than among matched patients with other forms of cancer, or among healthy controls (Emery, Danford, Arrand, Duncan, and Paton, 1978). The biological role of the mixed function oxidase system is to provide detoxification of compounds foreign to the animal; hydroxylation increases the polarity of these lipid soluble materials, thus facilitating their excretion. All aromatic compounds appear to be metabolised by the same process involving these reactive electrophilic intermediates, yet only a few are carcinogens. Evidently the latter must generate intermediates with some special property, the nature of which is not clear at present. This may be no more than the correct degree of stability to allow the intermediate to survive the aqueous medium within the cell for long enough for it to migrate from its site of formation in the cytoplasm into the nucleus, and there react with the DNA.

Animal tests

Animal tests for carcinogens are still of great importance. They do not differ fundamentally from the methods originally used; the need for persistence in treatment and patience in observation are still paramount. Tumours at most sites have been induced in animals of many species, using practically every route of administration (Argus, Arcos, and Wolf, 1968).

Skin “painting” using mice is still a method of choice for locally-acting carcinogens such as polycyclic hydrocarbons. Skin tumours (papillomas) can readily be detected at an early stage so that this method naturally lends itself to the study of dose-response relationships. The latent period (i.e., the time which elapses between the first dose of the carcinogen and the appearance of the tumour) is as definite a parameter of carcinogenicity as tumour incidence, and a useful index of activity includes both factors:

\[ \text{Iball index} = \frac{\text{percentage tumour incidence}}{\text{latent period in days}}. \]

The mean latent period (L) of a particular carcinogen is related to the dose (d) by an equation of the type: \( L = a - b \log_{10} d + c \), where a, b, and c are constants characteristic of that carcinogen (Bryan and Shimkin, 1941). Relatively simple theoretical mathematical models have been proposed which account for this relationship (Brodsky, 1979). Quantitative experiments are obviously difficult when internal tumours are used as the end point, but similar data can be obtained; for example Druckrey (1967) obtained good quantitative...
data for the induction of malignant tumours in rats given different doses of diethylnitrosamine in their drinking water.

Skin painting tests usually involve repeated, twice or thrice weekly, applications of the carcinogen at a fairly low dose. Berenblum (1941) made a discovery of fundamental importance when he found that skin tumours could be induced equally well with one topical dose of the carcinogen, itself ineffective, when this was followed by repeated treatments with a “promoting agent”. The most important promoter is croton oil, obtained from the seeds of the tropical plant *Euphorbiaea Croton tiglium*. However, phenols especially anthranil (1,8,9-trihydroxyanthracene) (Bock and Burns, 1963), long chain hydrocarbons such as *n*-dodecane (Shubik *et al.*, 1956), and certain other compounds are also active. Structure/promoter activity relationships nevertheless are precise. Thus Hecker (1971) isolated numerous derivatives of the diterpene phorbol (49a) (fig. 12) from croton oil and has synthesised others. Promoting activity

\[ \text{49a, } R^1 = R^2 = H \]
\[ \text{b, } R^1 = R^2 = \text{CO(CH}_2\text{)}_3 \text{CH}_3 \]
\[ \text{c, } R^1 = \text{CO(CH}_2\text{)}_{12} \text{CH}_3, R^2 = \text{COCH}_3 \]

Fig. 12.—The naturally occurring phorbol (49a) isolated from croton oil, and synthetic (49b) and natural (49c) diesters which are promoting agents.

ranges from the inert parent, phorbol, through the weakly active 12,13-dibutyrate (49b), to the 12-myristate, 13-acetate (49c) which is the most potent promoter known.

The importance of Berenblum’s discovery is that it establishes that tumour induction is a two- or multi-stage process, consisting of “initiation” and “promotion” (Berenblum, 1975). It has been shown by appropriate experiments that initiation is a rapid and irreversible process, being completed within a few hours of the administration of the single dose of carcinogen. The modern view is that this time is an indication of the time taken for the carcinogen to reach its target, undergo metabolism to its ultimate form, and cause specific DNA damage essential for the eventual appearance of the tumour. Promotion, on the contrary, is a prolonged and less understood concept. On the practical level, the two-stage test has advantages: it uses much less test compound, an important consideration when valuable natural products and metabolites are to be evaluated, and experiments are less hazardous because they avoid the repeated handling of the carcinogen.
Subcutaneous injection of locally-acting carcinogens yields sarcomas at the site of injection, often together with other tumours at distant sites, such as the ear ducts where these lipophilic compounds can accumulate and undergo metabolism over a prolonged period. In this connection Huggins (1961) made the observation that mammary cancer can be induced regularly and quickly by gastric instillation of strong carcinogens into virgin female rats 50 days of age; presumably a similar mechanism operates through accumulation in the mammary fat pads, although here the hormonal status of the animals also is of importance. Chronic feeding experiments are necessary to induce liver tumours, for example, with aromatic amines (Yoshida, 1934).

Whatever the experiment, work with animals suffers from a number of inherent drawbacks. The long latent period for tumour induction means that the animals may have to be kept for most or the whole of their lifespan, ranging from 2 years for mice, 3–4 years for rats, to about 10–15 years for dogs. To make matters worse, even if rather large groups of animals are employed the test will still be comparatively insensitive. A chemical would be generally agreed to be non-carcinogenic if it failed to increase the low spontaneous tumour incidence in several hundred test animals of two or more species over their entire lifespan. However, if this chemical is subsequently used, as a food additive for example, millions of human beings would be exposed to it for prolonged periods. The sheer difference in the size of the groups at risk makes adequate testing virtually impossible and certainly extremely expensive.

It is therefore understandable that over the last few years great efforts have been made to devise rapid, simple, and economic bioassays to supplement animal tests. This work has led to important practical advances, and at the same time has greatly strengthened the somatic mutation hypothesis.

Carcinogenicity and mutagenicity

The realisation that metabolism is essential for the activation of the majority of chemical carcinogens, together with the increasingly likely hypothesis that DNA damage is involved in tumour initiation, has led many people to re-examine mutagenesis by carcinogens in microbiological and cell culture systems. As a result of this work, so-called short-term tests for carcinogenicity (Bridges, 1976) have been proposed which employ a wide range of organisms, from bacteriophage and bacteria, through yeasts and insects, to mammalian cells in culture. The great attraction of these methods is that they circumvent the two main drawbacks to animal tests; the long latent period is avoided, and the statistics are readily improved because it is easy to grow millions of individual bacteria or cells on a single petri dish. Bioassays have been devised (Anderson, 1978), that involve not only mutagenesis (Ames et al., 1973), but also look at the damaging effects of carcinogens on DNA by other means, such as chromosome aberrations including sister chromatid exchange (Perry and Evans, 1975), DNA fragmentation (Heddle, 1973), and DNA repair (Sand and Stich, 1975). The main objection to all these tests is that they are even further removed from the human situation than the animal tests they are designed to supplement.
Probably the most important results have come from the study of bacterial mutation brought about by chemical carcinogens. In these tests the rationale is invariably the same: the organism is exposed to the test compound, if necessary together with an appropriate system for biological activation, and the progeny is subsequently examined for some inherited alteration. Biological activation is provided \textit{in vitro} by the inclusion of mammalian (usually rat liver) microsomes, which are a good source of oxidative enzymes, plus NADPH. Originally "host mediated" assays were devised in which the micro-organisms were incubated within the animal treated with the test compound; metabolism was provided by the tissues of the host, and later the organisms were recovered, plated out, and examined for mutants (Garridge and Legator, 1969). Mutagenesis is also investigated in mammalian cells (Arlett, 1977) in culture; here the cells themselves may be capable of the necessary metabolism for biological activation, or this may be provided by a "feeder-layer" of irradiated, but metabolically competent rat cells. Mutation is usually detected as a change in growth requirements; e.g., Chinese hamster cells are screened for the ability of test compounds to produce resistance to the lethal effect of azaguanine by a forward mutation (Chu and Malling, 1968). Observed mutations are of two main types, frameshift and point mutations (base-pair substitution); while both occur through alterations in the normal nucleotide base sequence in the DNA, different mechanisms are involved (Streisinger \textit{et al.}, 1966). Both usually lead to loss of a protein or impairment of its function. Mutants which have lost an essential function can also be used to look for back mutations to sensitivity of the wild type. Here the mutation causes restoration of a functional protein, although only rarely will this be chemically identical with the protein having the same function in the wild type.

The principle of back mutation has been employed extensively by Ames (McCann and Ames, 1977) in California, whose influence in this area has been considerable. Using a strain of \textit{Salmonella typhimurium} unable to synthesise the amino acid histidine, compounds are tested for their capacity to cause reversion to histidine non-requirement by a back mutation. Sensitivity is achieved by selecting his- bacteria which further are unable to repair DNA damage (uvr\(^{-}\)), and which lack the usual lipopolysaccharide coat thus improving their permeability to the test chemical (Ames, Lee and Durston, 1973). Introduction of a bacterial plasmid (R-factor) carrying an ampicillin resistance marker enhances the sensitivity of some strains to reversion by particular classes of mutagens (McCann, Springarn, Kobori and Ames, 1975). The most important tester strains are:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type of mutation causing reversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA 1535</td>
<td>base-pair substitution</td>
</tr>
<tr>
<td>TA 1537</td>
<td>frameshift (-C-C-C-C-)</td>
</tr>
<tr>
<td>TA 1538</td>
<td>frameshift (-C-G-C-G-C-G-)</td>
</tr>
<tr>
<td>TA 98</td>
<td>+R factor, as for 1538</td>
</tr>
<tr>
<td>TA 100</td>
<td>+R factor, as for 1538</td>
</tr>
</tbody>
</table>
Thus TA 1535 and TA 100 detect reversions caused by base-pair substitutions while the rest are reverted by frameshift mutations specific for tracts in the DNA composed of repeated deoxycytosine (C) or deoxycytosine-deoxyguanosine (C-G) units. Standard bacteriological techniques are employed and a test is complete within 3 days.

Examination of almost 300 compounds, about half of which were known carcinogens, by these methods showed that 90 per cent. of the carcinogens were mutagenic while 87 per cent. of the non-carcinogens were inactive (McCann, Choi, Yamasaki and Ames, 1975; McCann and Ames, 1976). Essentially the same result was obtained with a different 120 chemicals at the I.C.I. Central Toxicology Laboratory during extensive work carried out there to find reliable short-term assays (Purchase et al., 1976). The conclusion drawn from this work was that the Ames' test, together with a cell transformation test, are able to detect a high percentage of a wide range of carcinogens, while generating a low level of false positives. This cell transformation test (Styles, 1977) involves exposure of BHK21/C13 hamster cells to the test compound in tissue culture medium, followed by selection of the transformed cells by their ability to form colonies when grown in semi-solid agar under strictly specified conditions. No claim is made that the transformed colonies are composed of tumour cells; however it is claimed that transformation correlates about as well as mutagenesis does in the Ames' system with carcinogenicity. These tests are now in everyday use for screening new chemicals. Among 54 closely related polycyclic compounds tested against TA 100, we found that without exception all 38 carcinogens were mutagenic, but that in addition there were seven mutagens that seem not to be carcinogenic (Coombs, Dixon and Kissonerghis, 1976).

It is clear from these and many similar results that the various tests using Salmonella typhimurium are invaluable as primary screens when looking for carcinogens among compounds of unknown biological propensity (fig. 13). Ames et al., (1975) tested 169 permanent hair dyes commercially available in the USA, and found that 89 per cent. of them were mutagenic. 2,4-Diaminotoluene (50) is mutagenic; formerly used as a hair dye, it was banned on account of its carcinogenicity in rats, but it is still used in the synthesis of hair dyes. The flame retardant tris(2,3-dibromopropyl) phosphate (51), widely used as a textile additive, particularly in children's pyjamas, is mutagenic (Blum and Ames, 1977) and has recently been shown to be carcinogenic (Gold, Blum and...
The food additive AF-2 (furylfuramide, 52) was used in Japan from 1965 as a food preservative until it was found to be mutagenic in bacterial tests including TA 98 and TA 100 (Yahagi et al., 1967). It had previously failed to produce tumours, but on re-testing it was shown to be carcinogenic in mice, rats, and hamsters (Nagao, Sugimura and Matsushima, 1978). Many cancer chemotherapeutic drugs are mutagenic in this test system (Benedict et al., 1977).

These short-term tests are rapid, relatively cheap, and easy to carry out. They will be used increasingly, but of course they cannot displace the animal tests. On the theoretical side this recent correlation of mutagenicity and carcinogenicity seems to put the somatic mutation theory into an unassailable position.

**DNA repair and carcinogenesis**

From the foregoing it is clear that there is abundant proof that carcinogens react chemically with DNA. If animals are treated with a chemical carcinogen which is labelled with a radioactive isotope so that its fate can be followed, it is usually found that binding of the carcinogen to DNA in the target cells (e.g., liver cells for a nitrosamine or aromatic amine, skin cells for a polycyclic compound) reaches a maximum within the first 24 hr. The amount bound per unit of DNA then decreases, rapidly at first then more slowly, and often binding can be demonstrated even after several weeks have elapsed. It is thought that this rapid decrease is due to excision repair brought about by repair enzymes essentially similar to those that remove thymidine dimers from u.v.-irradiated DNA. This process is best understood in bacteria, such as *Escherichia coli* (Lehmann and Bridges, 1977). An exonuclease first cuts the DNA at a point adjacent to the bound molecule, and an endonuclease then removes the altered base together with a tract of several adjacent bases. This tract is then re-synthesised by a DNA polymerase, and the completed chain is finally joined by a ligase. Repair by this multi-enzyme system is usually error-free. A second type of repair, often termed post-replication repair, operates when the excision enzymes are unable to remove the blocking adduct, and probably also when the excision repair system is overwhelmed. In this type of repair replication of the damaged DNA occurs, but gaps are left corresponding to the altered bases. These gaps are later filled, but this process is prone to error leading to mispairing of bases, and to mutations. It seems to be the lack of one or more enzymes of the excision repair complex by xeroderma pigmentosum patients and the operation of the second type of repair that leads to their u.v. light-induced skin lesions.

Most of the work in animals has so far been carried out with nitrosamines because they alkylate DNA to a greater extent than these other types of carcinogens. Dimethylnitrosamine gives rise mainly to N-7-methyldeoxyguanosine (53) with smaller amounts of 0-6-methyldeoxyguanosine (54) and N-3-methyldeoxyguanosine (55) (fig. 14). Of these the 0-6-methyl nucleoside (54) miscodes (Lawley and Martin, 1975) and is thought to be responsible for the carcinogenicity of dimethylnitrosamine (Loveless, 1969). A recent and unexpected
development has been the discovery of yet a third type of DNA repair process which is relatively error-free and is inducible by low doses of methylating agents. First found in bacteria (Samson and Cairns, 1977), this inducible repair system appears to be active also in the liver of rats treated with dimethylnitrosamine (Montesano, Bresil, and Margison, 1979). The precise mechanism of this type of repair is not yet clear; it appears to differ from the previous two types not only in that it is inducible, but also in that it involves *in-situ* alteration of the 6^-methylguanine residues (Karran, Lindahl, and Griffin, 1979; Robins and Cairns, 1979).

As we have already seen, AAF attaches itself mainly to the C-8 position of deoxyguanosine (adduct 42, fig. 9), but this adduct is lost rapidly by repair. It

![53](image)

![54](image)

![55](image)

R = 1-deoxyribose

![56](image)

**Fig. 14.**—The three methylation products (53–55) of deoxyguanosine, and the minor adduct it forms with AAF (56).

is the minor adduct (56, fig. 14), in which deoxyguanosine is linked through its exocyclic nitrogen to C-3 of AAF that persists and may be involved in carcinogenesis (Westra, Kriek, and Hittenhausen, 1976). Benzo[a]pyrene also attaches to the exocyclic amino group of deoxyguanosine (adduct 48, fig. 11), but it is not known at present whether it is this or one of the minor adducts (Jeanette *et al.*, 1977) which persists. It is well established on the basis of the total dose required to produce tumours that polycyclic hydrocarbons like benzo[a]pyrene are in general more active than aromatic amines such as AAF. Perhaps it is of significance that binding through the exocyclic amino group of deoxyguanosine appears to give rise to the major product formed from benzo[a]pyrene, but the minor adduct from AAF (56). It has been shown by Yamasaki *et al.* (1977) that the latter does not cause local denaturation, in contrast to the major adduct (42) which does; it is thought that it is the recognition of denaturation by the endonuclease that initiates excision repair.
It is evident that there is a great deal to be discovered concerning the precise types of lesions in the DNA that are important, and research in this area is in progress in many laboratories. The much more important problem of which mutations are related to carcinogenesis has yet to be approached in depth. From the practical point of view of cancer prevention this research, essential for our understanding of the process of tumour initiation, may be irrelevant because we now have convenient short-term tests which for the first time make screening the environment for potential carcinogens feasible. Coupled with detailed statistical knowledge of cancer incidences throughout the world, this promises to allow us to identify the cause of several types of cancer, and we hope, to eradicate them by prevention. Prevention is always better than cure, and this is never more true than for the diseases known as cancer.

Cancer as an environmental and social disease

A most important development in cancer research has been the gradual realisation as a result of epidemiology that much cancer appears to be of environmental origin, and therefore in theory largely preventable. The first director of research of the Imperial Cancer Research Fund, E. F. Bashford, in a “Draft of Scheme for Enquiring into the Nature, Cause, Prevention, and Treatment of Cancer” written in 1902 and published in 1908 proposed that statistics of incidence should be collected both for general causes and for special groups. These proposals have been realised to an extent that Bashford could not have envisaged. We have already seen that cancers among numerous occupational and “special groups” have been investigated, causes identified, and prevention instituted. However, these constitute only a small proportion of all cancer cases, and it is true that with the exception of lung cancer, the causes of the other major cancers remain unknown. However, the first steps towards their discovery have been taken, so that today we have a great wealth of detailed information on the incidence of various forms of cancer for most of the countries throughout the world (Doll, 1977b).

In this country cancer accounts for one death in five, and of these deaths about 50 per cent. occur as a result of one of the three major diseases, cancer of the lung, colon, or breast. When appropriate adjustments are made for the changing age structure of the population, it is found that mortality from most forms of cancer has remained fairly constant during this century, with two exceptions. As already mentioned cancer of the stomach has shown a slow, but steady decline, whereas lung cancer has increased enormously, almost 100-fold in men since 1910. The latter is now the most common cancer in man and it is becoming increasingly common in women among whom smoking became socially acceptable only in the 1930’s. The evidence that this disease is associated with smoking tobacco, especially cigarettes, is now overwhelming and so well known (Doll, 1977b) that it need not be reiterated here. However, it must be mentioned that although this evidence has been common knowledge for almost 20 years, negligible impact has been made on prevention by effectively discouraging the habit.
CHEMICAL CARCINOGENESIS

It is when we look at world-wide cancer incidences that marked differences become discernible. For example the incidence of lung cancer among American men is about half that found among British men, despite similar smoking habits. Breast cancer is much less common among Japanese women than among British or American women. In northern Iran cancer of the oesophagus is very common; about 20 per cent. of people over the age of 50 die from this disease. The incidence is also abnormally high in certain areas of France where it is associated with the consumption of cider-based liqueurs (Doll, 1977b). Primary liver tumours are much more frequent in underdeveloped parts of the world than in Western Europe and the USA, whereas the reverse is true of cancer of the colon. Worldwide incidence of the latter is closely correlated with meat consumption (Wynder and Reddy, 1975), although since this in turn is a good indicator of relative affluence, it is not at all certain what factor is involved here. In Japan meat consumption is low and so is colon cancer, but cancer of the stomach is very high although the incidence of this disease is declining there also. The incidence of both colon and stomach cancer among Japanese who have emigrated to California tends towards that of the white population, particularly in the second and third generations. This indicates that whilst these diseases probably have environmental causes, these are connected with cultural differences rather than general factors to which the whole population is exposed.

Consideration of epidemiological data of this nature led Sir Richard Doll (1977b) to conclude that most cancers have environmental causes, and if these were known and were prevented incidence rates should drop by 80–90 per cent. For example, in 1976 there were over 33,000 deaths from lung cancer in England and Wales (Mortality Statistics, 1976); of these about 30,000 would have been avoided if smoking had not become a widespread habit. The economic consequences of stopping smoking have been estimated by the Department of Health and Social Security (1972). At first the increase in the population, coupled with the decrease in absence from work and the amount of health care required, would increase the gross national product. However, within two decades this would be cancelled by the large number of old people who would require support, but who would not contribute to the production of national wealth. It seems inevitable that considerations such as these must influence government's attitude to cigarette sales. Diet is implicated in large bowel cancer, and in particular the consumption of animal fat is under suspicion (Hill, 1977). The presence of a mutagen in the faeces of individuals on a full Western diet is now established (Bruce et al., 1977), and would seem to give hope that the cause of this disease, which killed nearly 11,000 people in England and Wales in 1976, will be found. Dietary fat is also correlated with breast cancer in women (Armstrong and Doll, 1975), but here other factors are also important. About half the 12,000 deaths (in England and Wales in 1976) would have been avoided if these women had had their first pregnancy soon after puberty (Stoll, 1976). The reason for this is not understood at present, but it suggests lines of research which may ultimately lead to preventative measures.

Although great advances have been made in the treatment of clinical cancer,
further improvements in therapy are becoming more and more difficult to achieve. In the field of chemotherapy the drugs employed, with a few exceptions, are designed to kill dividing cells by causing massive DNA damage. Not unexpectedly many of these agents can be shown to be carcinogenic in animals, and in fact Haddow (1945) postulated that substances which inhibit tumour growth may be expected to be also carcinogenic, and vice versa. Soon after our discovery in 1966 of the strong carcinogenicity of the cyclopenta[a]phenanthrene (6) it was, at Professor Haddow's suggestion, tested at the Chester Beatty Research Institute for its capacity to inhibit the growth of the Walker 256 tumour in rats. In agreement with his postulate it was found that 6 was active in this respect whereas the homologue of 6 lacking the methyl group, which is not a carcinogen in mice and rats (Coombs and Croft, 1969), was without tumour-inhibiting activity (personal communication). Despite this well-known relationship a number of carcinogenic anti-cancer agents are currently in use not only in the alleviation of terminal disease, but also in treatments designed to effect long-term “cures”. Thus a growing number of apparent cures of childhood leukaemia have been claimed for various combinations of intensive chemotherapy using them over the last 10 years. Undoubtedly it is right to use even known carcinogens to save life, but there must be misgivings about the ultimate outcome of this treatment. All our experience with animals tells us that carcinogens always have a latent period which is frequently a significant proportion of the animal's lifespan; only time will disclose whether the treatment received by these patients will give rise to other cancers. An entirely new approach to cancer chemotherapy is clearly needed. Agents must be discovered which can control the growth and invasive properties of tumours without damaging cells. This, after all, is a natural process. After fertilisation, the zygote produces trophoblastic cells which temporarily resemble malignant tumour cells (Manes, 1974); they multiply vigorously and invade the uterine wall, implanting the ovum. Having completed this task, these cells lose their invasive quality and become part of the fetus. Almost nothing is at present known of the factors controlling this process, but it is encouraging that an active interest is now being shown in chemical agents which can trigger off differentiation (Marks et al., 1978). However, no useful drugs have so far been described which utilise this idea.

In contrast to the severe problems facing improvements in the chemotherapy of cancer, the chances of dramatically improving cancer prevention have never seemed brighter. It seems entirely possible, given the investment of adequate funds and facilities throughout the world, that the cause of several of the major forms of cancer may now be established within the foreseeable future. The real problems of prevention may then be tackled. These will undoubtedly be formidable, for judging from the difficulty experienced in trying to change smoking habits it will be extremely difficult to change dietary or other cultural factors, if these are finally implicated. Already lung cancer must be considered as a major social disease of this century and every effort must be sustained to reduce its incidence.
CHEMICAL CARCINOGENESIS

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Target Organ Toxicity

Volume II

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Chapter 10

ORGAN SPECIFICITY IN TUMOR INITIATION AND COMPLETE CARCINOGENESIS (INCLUDING SPECIES VARIATION)

Maurice M. Coombs

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1. INTRODUCTION

The origin of the study of cancer induction by chemicals is often ascribed to the London surgeon Sir Percival Pott, who over 200 years ago postulated that the high incidence of epithelioma of the scrotum among chimney sweeps was due to their excessive exposure to soot. The same disease made its appearance again in England 100 years later, among the Lancashire cotton spinners, who called it "mule spinner’s cancer." At about this time other forms of skin cancer were making their appearance following industrialization as it became established in northern Europe. Thus, occupational skin cancer among oil and tar workers was commented upon by Volkmann in Germany as early as 1875, and it became apparent that exposure to mineral oil was the cause of both their and the mule spinners' disease. The latter used oil liberally to lubricate their machines, so much so that their clothes became impregnated with it. However, many attempts to produce skin tumors in animals by exposing them to tar and oils did not meet with success until 1915, when two Japanese workers, Yamagiwa and Ichikawa, simulated human exposure by persisting in their treatment. They found that skin tumors could be produced on the ears of rabbits by frequently repeating applications of coal tar over several months. It was soon discovered that the dorsal skin of mice was also sensitive to tumor induction by similar treatment with coal tar, and offered a more convenient test system. Using this test, Kennaway and his associates in London identified aromatic materials as the carcinogetic component of these tars, culminating in 1930 in the demonstration of the carcinogeticity of dibenz[a,h]anthracene, the first pure, synthetic organic compound shown to be active. This was soon followed by the isolation of a few grams of benzo[a]pyrene by fractionation of two tons of gas-works pitch. The study of chemical carcinogenesis was then well under way, and it continues at an ever-increasing spate today.

However, already a new form of occupational cancer was appearing, namely bladder cancer among workers in dyestuff factories. It later transpired that certain dyestuff intermediates, particularly aromatic amines such as 2-naphthylamine and benzidine, were to blame for this disease. Some workers were heavily exposed; bladder cancer incidences approaching 100% appeared among men who had previously been engaged in the large-scale purification of these compounds by distillation, and an elevated incidence was found even among office staff employed in these factories. This problem also later turned up in the rubber industry where these aromatic amines were used as anti-oxidants.

Thus, at the very outset it was obvious, unfortunately as a result of human exposure, that chemical carcinogetins are markedly organ specific. The great mass of animal data subsequently generated during the 50 years following these early observations has shown this to be a general rule among nearly all classes of chemical carcinogens. This work has moreover demonstrated that many carcinogetins are, in addition, species specific, and that in some cases the type of cancer induced is dependent upon the route of administration. These complications, together with the fact that cancer induction is usually a protracted process often occupying a substantial proportion of the animal’s normal life span, make the testing of new compounds for carcinogeticity difficult. A recent review of comparisons of carcinogeticity of 250 chemicals in two species, in most cases rats and mice, revealed that 38% were noncarcinogenic, and 44% were carcinogetic, in both species; 8% were active in mice only and 7% in rats only. Moreover, only 64% of the carcinogetins produced cancer at the same site in the two species. Animals of more than one species must therefore be employed, in sufficient numbers to give the tests statistical significance, and treatment must be continued indefinitely. Mice live about 2 years, rats 3 to 4, and other test animals even longer, so that thorough testing of a new chemical for its capacity to produce tumors is a very expensive operation, frequently by far the most expensive part of the complete toxicological examination. Furthermore, the routes of administration will probably have been chosen with regard
to previous experience with other compounds of the type under study, and this can itself lead to errors of omission.

For all these reasons knowledge of organ and species specificity in carcinogenesis is for the most part fragmentary and unsatisfactory for many carcinogens. Humans are for obvious reasons the least studied, virtually all the information coming from retrospective epidemiology, and of course this makes risk assessment for a new chemical particularly difficult to evaluate. Nevertheless, the broad outlines of the subject are now clear and in this chapter we will explore these phenomena in a general way, always bearing in mind the limitations already mentioned. The main types of chemical compounds which tend to give rise to carcinogens (aromatic hydrocarbons, arylamines, nitroso compounds, alkylating agents) will be discussed separately, and these will be followed by some miscellaneous and inorganic carcinogens, and carcinogenic natural products. Suggested reasons for specificity, usually depending upon differential tissue distribution and metabolism, will be discussed where appropriate. Two recent publications review this subject in detail and are recommended for further reading; the first is a report of a symposium on organ and species specificity in carcinogenesis held at Raleigh, N.C. in 1981, and the second is a computer-generated compilation of the target organ specificity of 811 selected chemical compounds. These include many chemicals of industrial and medicinal importance, and no less than 97 organ sites are considered; much recent mutagenic and other genotoxic data is also tabulated. This publication is particularly useful because it contains an enormous amount of data in an easily accessible form together with over 750 references to the original literature. Other extensive sources of data are the International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risk of chemicals to man and the National Cancer Institute’s Survey of Compounds Which Have Been Tested for Carcinogenic Activity.

II. POLYCYCLIC HYDROCARBONS

Following from the early work on the production of cancer by oils and tars, the chemistry and biology of polycyclic aromatic hydrocarbons has become a major part of the study of chemical carcinogenesis. Of some 350 polycyclic hydrocarbons and simple heterocyclic analogues tested just over 50% show evidence of carcinogenicity. Most, but not all, of the active compounds contain four or more fused aromatic rings, three of which are fused in an angular configuration as in phenanthrene (Figure 1 (1)). This hydrocarbon is itself inactive although its 1,2,4-trimethyl derivative is a weak carcinogen; by contrast the cyclopenta[a]phenanthrene ketone (Figure 1 (2)), which is a 1,2,4-trisubstituted phenanthrene derivative formally related in structure to the steroids, is a potent carcinogen with an activity similar to that of benzo[a]pyrene (Figure 1 (3)) on mouse skin. Benz[a]anthracene with four fused rings gives rise to many strong carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA, Figure 1 (4)) and 3-methylcholanthrene, both of which have been studied extensively. Benzo[a]pyrene, of course, is a widespread pollutant, occurring whenever organic materials undergo incomplete combustion and for this reason it has been intensively studied over the last decade.

Carcinogens of this class are effective locally, and have often been tested by the classical method of skin painting using mice. They are activated by metabolism to bay-region diol epoxides, as shown here for benzo[a]pyrene. The 7,8-double bond in this hydrocarbon is enzymatically oxidized to the epoxide which is rapidly and stereospecifically opened by another enzyme, epoxide hydrolase, to yield the [7R,8R]-trans-diol (Figure 1 (6)). The olefinic 9,10-double bond in the latter is further oxidized, mainly to the anti-diol epoxide (Figure 1 (7)), which acts as an electrophilic reagent, reacting inter alia with nucleophilic sites in DNA such as the exocyclic amino group in deoxyguanosine to yield the adduct (Figure 1 (8)). These oxidations are brought about by microsomal monooxygenases (aryl hydrocarbon
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FIGURE I. Polycyclic aromatic carcinogens derived from phenanthrene (1): 15,16-dihydro-11-methoxycyclopenta[a]phenanthrene-17-one (2), benzo[a]pyrene (3), 7,12-dimethylbenz[a]anthracene (4), and 3-methylcholanthrene (5). These compounds are biologically activated by oxidation of their terminal benzo-ring, as shown for benzo[a]pyrene (3), first to the non-bay region diol (6), followed by formation of the diol-epoxide (7) which then attacks the exocyclic amino group in deoxyguanosine in DNA to form trihydroxy adducts of type (8).

hydroxylases) and it is not clear whether different oxidative enzymes are active in epoxidizing these two different double bonds. Thus, at least two enzymes (epoxide hydrase and a monoxygenase) are involved in the activation of this hydrocarbon; in addition other enzymes such as UDP-glucuronosyl transferases, glutathione-S-epoxide transferases and sulfotransferases are involved in benzo[a]pyrene metabolism. Aryl hydrocarbon hydroxylase is present in most tissues including skin, although its level is highest in liver, and it is inducible by a variety of compounds including polycyclic aromatic hydrocarbons, flavones, and polychlorinated biphenyls. Both its constitutive and induced levels are genetically regulated, and differ between animals of different species, between tissues of the same species, and even between individuals of the same species. The latter is particularly noticeable for humans. Consequently, observed organ and species differences in chemical carcinogenesis with polycyclic hydrocarbons are at least partly dependent upon these factors.

Polycyclic aromatic hydrocarbons as a class act locally, i.e., tumors arise at the site of application. Mouse skin is particularly sensitive to these agents, papillomas, and later carcinomas developing on the treated areas after topical application. Initially this gave rise to a sensitive test for carcinogens of this class, and the reproducibility of the test made possible the establishment of the two-stage nature of cancer induction. To illustrate, we will take examples from our own work with the cyclopenta[a]phenanthrene ketone (Figure 1 (2)). Although this compound is not a simple hydrocarbon, it is metabolized to a trans[3R,4R]-diol-anti-1,2-epoxide which reacts predominantly with deoxyguanosine in DNA in precisely the same manner as benzo[a]pyrene, a typical polycyclic aromatic hydrocarbon. This ketone efficiently induces skin tumors in mice; at a topical dose of 50 µg given twice weekly to T.O. (Theiler's Original, derived from Swiss) mice 90% of the animals develop skin tumors with a short mean latent period of about 25 weeks. Essentially the same result is obtained if a single initial topical dose of 400 µg is followed twice weekly by topical doses of the promoter croton oil. Note that this dose is only 16% of the cumulative dose administered.
over 25 weeks in the first experiment; if it is not followed by repeated treatments with the
promoter it is ineffective, producing skin tumors in only 15% of the mice with a long mean
latent period of 65 weeks.\textsuperscript{4} No tumors are found on repeated topical treatment of the mice
with the promoter in the absence of the initiating dose of carcinogen. The two-stage ex­
periment can also be carried out by giving the initiating dose by injection; a larger dose (10
mg) is administered in oil by subcutaneous injection and this is followed twice weekly by
the promoter applied topically to the dorsal skin, remote from the injection site. Tumors
appear on the treated skin in 95% of the mice with a mean latent period of 21 weeks,
whereas no dorsal skin tumors occur in animals injected with the carcinogen, but not
promoted.\textsuperscript{4} Thus, a single dose of this carcinogen applied topically or systemically initiates
the skin; moreover, this initiation persists, for even if promotion with croton oil is delayed
for 6 months similar tumor incidences and latent periods are observed when promotion is
eventually begun.\textsuperscript{17} Histologically the skin tumors are diagnosed as a mixture of papillomas
and carcinomas, the proportion of the latter usually being higher when repeated applications
of the carcinogen are used. In all these respects this cyclopenta[\textit{a}]phenanthrene (Figure 1
(2)) behaves like a classical polycyclic aromatic hydrocarbon, but it is less potent than either
DMBA (Figure 1 (4)) or 3-methylcholanthrene (Figure 1 (5)). Like them\textsuperscript{26-27} it induces
mammary adenocarcinomas in virgin Sprague-Dawley rats after a single intragastric
instillation.\textsuperscript{17}

In general, rat skin is not susceptible to carcinoma induction with polycyclic aromatic
hydrocarbons; however, injection of either rats or mice with compounds of this class usually
gives rise to sarcomas at the injection site. In the latter respect, the carcinogenic polycyclic
ketone (Figure 1 (2)) differs because local sarcomas are not frequently observed in mice\textsuperscript{28}
or rats. In an ongoing experiment similarly injected Sprague-Dawley rats have given no
local sarcomas, but a number have developed leukemia and skin tumors on the face and
trunk.\textsuperscript{16} These experiments perhaps provide a clue as to the importance of tissue distribution
in polycyclic hydrocarbon carcinogenesis. This ketone is less lipophilic than hydrocarbons
such as (3) to (5) of Figure 1; for example, it is almost insoluble in aliphatic hydrocarbon
solvents such as hexane that readily dissolve the latter. It is therefore probably more rapidly
cleared from the injection site than these hydrocarbons, especially when oil is used as the
vehicle. On the other hand, it is moderately lipophilic, as shown by the fact that after
intraperitoneal injection into Balb/C mice traces of the unchanged compound can still be
detected 1 month later in dorsal skin, especially in the fatty dermal layer. Mice so initiated
are highly susceptible to promotion with phorbol esters.\textsuperscript{197}

Benzo[\textit{a}]pyrene (Figure 1 (3)) can be considered as a typical polycyclic hydrocarbon
carcinogen; it has been studied extensively\textsuperscript{29} and sensitive methods for its detection and
analysis\textsuperscript{30} in the environment have been developed. It is always found when other polycyclic
aromatic hydrocarbons are present, and has often been used as an indicator of their presence.
It has been tested in nine species (mouse, rat, hamster, rabbit, guinea pig, duck, newt, dog,
and monkey) and found to be carcinogenic in each.\textsuperscript{29} Besides skin tumors already discussed,
it induces malignant tumors of the forestomach in mice,\textsuperscript{31} rats,\textsuperscript{32} and hamsters\textsuperscript{33} after oral
administration. After subcutaneous or intramuscular injection local sarcomas have been
observed in mice,\textsuperscript{34} rats,\textsuperscript{35} hamsters,\textsuperscript{36} guinea pigs,\textsuperscript{37} and in the primates, tree shrews,\textsuperscript{38}
and cottontail marmosets.\textsuperscript{39} There are very little data concerning the effects of benzo[\textit{a}]pyrene
itself in man; in two reports\textsuperscript{40,41} topical exposure caused warts which later regressed, and
in another a squamous epithelioma developed in a man while carrying out an animal ex­
periment with this carcinogen.\textsuperscript{42}

Polycyclic aromatic hydrocarbons are suspected of being involved in one major human
disease, namely lung cancer. Large amounts of these compounds find their way into the
atmosphere as a result of industrialization, especially power generation utilizing the combu­
stion of fossil fuels. This may account for the higher incidence of this disease generally
observed for urban compared with rural areas. Much more striking is the very high incidence of lung cancer among heavy cigarette smokers. Cigarette consumption and lung cancer mortality have increased steadily year by year since the early part of this century, and only recently has this increase been checked. It is thought that this is due to the considerable reduction of tar yield achieved with many brands of cigarettes within the last few years. Cigarette tar contains many polycyclic aromatic compounds, including benzo[a]pyrene in amount (1.6 μg/100 cigarettes) insufficient by itself to account for the disease. It is thought that other active compounds in the tar, carcinogens, promoters, and co-carcinogens, may account for this discrepancy. In this connection it is known that heavy smokers employed in the asbestos industry are at particularly high risk, and that alcohol consumption increases the risk of cancer of the mouth and esophagus in this group. Attempts to induce lung cancer in animals by intratracheal administration of benzo[a]pyrene were unsuccessful until the carcinogen was given together with a dust such as hematite that could fix it in the lung, or by using the carcinogen in a particulate form. Lung tumors in dogs, similar to human lung tumors, have been induced by fixation of the carcinogen in the bronchial wall.

In summary, polycyclic aromatic hydrocarbons appear to be carcinogenic to most species and most tissues where they can accumulate and act over a prolonged period. The one notable exception is liver; there are almost no reports of liver tumors induced with these agents. Possibly the high level of many enzymes, including the conjugating enzymes, in this tissue ensures that carcinogens are rapidly metabolized to harmless excretion products. Moreover, in a comparison of the binding of the carcinogenic cyclopenta[c]phenanthrene (Figure 1 (2)) to the DNA of mouse skin, lung, and liver, loss of adducts from the former two could not be measured above the normal rate of DNA turnover, whereas adducts were removed rapidly from liver, with a half-life of 2.5 days. This suggests that liver may possess a specially active DNA repair system to help protect it from damage by the multitude of foreign chemicals it has to metabolize.

That differential tissue distribution and metabolism, or even DNA repair are not always the determining factors in carcinogenesis by polycyclic aromatic compounds is shown by the following recent experiment. The carcinogen (Figure 1 (2)) was applied topically to mice of the T.O., C57BL, and DBA/2 strains in an identical manner. In both repeated application and initiation-promotion experiments, a high incidence of skin tumors was induced in T.O. and C57BL mice, although the mean latent period for the latter was longer in each case. By contrast, in both types of experiment, the DBA/2 mice were completely resistant to tumor induction, although metabolism of the carcinogen was similar in terms of the total DNA adducts isolated from the treated skin of mice of all three strains, and in the pattern of individual adducts. In addition, the rate of removal of these adducts from the skin DNA was not different for the three strains. It therefore appears that in this case, strain specificity is related to a step(s) in the promotional stage common to both the carcinogen and to the phorbol ester used in the repeated application and initiation-promotion experiments, respectively.

III. ARYLAMINES

The history of synthetic dyestuffs goes back to the beginnings of organic chemistry as we know it today. Perkin's early synthesis of the violet dye mauveine by the oxidation of impure aniline was soon followed by the discovery of diazonium coupling of aromatic amines, and the chemistry of aromatic systems was rationalized by Kekulé by his proposal for the ring structure of benzene in 1863. Within a few years commercial manufacture of synthetic dyestuffs began in Germany, and 25 years later, Rehn first noted three cases of bladder cancer among chemists in a dyestuff works. Reporting a further 23 cases a few years later, he suggested that . . . aniline, its derivatives, and related compounds such as naphth
FIGURE 2. Human bladder carcinogens 2-naphthylamine (9), benzidine (10), and 4-aminobiphenyl (11), and the strong carcinogen 2-acetamidofluorene (AAF, 12). Aromatic amines are enzymatically activated as shown here for AAF; N-hydroxylation to (13) followed by esterification gives the active ester (O-acetate 14, or O-sulfate 15), which undergoes fission to the resonance-stabilized electrophile (16). Reaction of this through nitrogen with C-8 of deoxyguanosine, or through carbon with the exocyclic amino group leads to the DNA adducts (17) and (18), respectively.

lamine..." might have been responsible for this disease. However, it was not until 1938 that bladder cancer was produced in an animal model, the dog. Human bladder cancer is a comparatively rare disease so that cases caused by occupational exposure are more readily identified, and many case reports appeared during the first half of this century. In 1947, a thorough epidemiological survey of the chemical industry in the U.K. was initiated; it established that 2-naphthylamine (Figure 2 (9)) and benzidine (Figure 2 (10)) were associated with the disease, whereas aniline and 1-naphthylamine were not. During the course of this investigation, a new source of bladder cancer was discovered in the rubber industry, where a complex of the two naphthylamines with acetaldehyde was used as a compounding ingredient. Subsequently 4-aminobiphenyl (Figure 2 (11)) was also identified as a potent human bladder carcinogen.

Extensive animal studies with aromatic amines have since been carried out and many new structures and analogues have been tested. Among these, one of the most studied is 2-acetamidofluorene (Figure 2 (12)). This compound, originally proposed as an insecticide, was found to produce several types of tumors in rats, and has subsequently achieved a prominent place in cancer research, although, unlike amines (9) to (11) of Figure 2, it has never become an important intermediate manufactured on a large scale. In general, polynuclear aromatic amines are more active than single-ring arylamines, and ortho-methyl or -methoxy substituents often increase carcinogenicity of the parent amine, usually also diversifying its organ selectivity. N-Acetyl derivatives are equally or sometimes more active than the parent amines. Numerous metabolic studies with various carcinogens of this type point to N-hydroxylation as being implicated in their activation. This was first established for 2-acetamidofluorene (Figure 2 (11)); like most aromatic compounds this amide is extensively ring-hydroxylated, and the resulting phenols are excreted largely as their sulfate and glucuronide conjugates. In addition, most species excrete N-hydroxy-AAF (Figure 2 (13)), except the guinea pig which is also resistant to the induction of distant liver tumors.
by administration of this carcinogen. N-Hydroxy-AAF is also carcinogenic, but gives tumors at the site of injection and is active in the guinea pig. This metabolite was therefore termed the "proximate carcinogen" by the Millers. However, it does not itself react spontaneously with biological macromolecules such as proteins and nucleic acids, whereas simple esters such as the O-acetate (Figure 2 (14)) possess this capability. In the intact animal, probably the O-sulfate (Figure 2 (15)) is the active species although N,O-acyltransferases present in many tissues catalyze the transfer of the N-acetyl group to form the O-acetate which may also be involved in binding to DNA. Heterolytic fission of the N-O bond in (Figure 2 (15)) yields the resonance-stabilized electrophile (Figure 2 (16)) which reacts with nucleophilic centers, including both C-8 and the exocyclic amino group of deoxyguanosine in DNA, to yield the adducts (17) and (18), both found in Figure 2.

In man, exposure to the aromatic amines (9) to (11) of Figure 2 during manufacture (among others) was probably mostly via skin absorption and ingestion, and led to bladder cancer. Apparently monkeys are also susceptible to induction of bladder tumors with 2-naphthylamine, but not with 2-acetamidofluorene, the activity of which in man is unknown. This disease may be induced in dogs, and more conveniently in hamsters; certain strains of rats and mice are also susceptible. Most animal tests have been carried out by feeding these arylamines over prolonged periods, and tumors of the liver and ear duct are commonly observed in addition to those of the bladder. Mammary tumors occur in females of certain hamster strains. Skin tumors are not usually observed, although repeated treatment of the skin of mice with croton oil after feeding the animals with AAF (Figure 2(12)) was successful in this respect. Prior feeding with N-hydroxy-AAF (Figure 2 (13)) produced fewer skin tumors, suggesting that a different form of activation might be involved in skin.

While arylamines have proved to be carcinogenic in all species in which they have been tested, with the exception of the guinea pig and steplemming, marked organ specificity exists among closely related members of this class. Thus, 2-naphthylamine induces mainly liver and bladder tumors, whereas 3-methyl-2-naphthylamine yields tumors of the large intestine in certain rat strains, and in hamsters suffering from intestinal enteritis. The latter is interesting as a possible model for the human disease colon cancer, for which ulcerative colitis is a predisposing condition. 3-Nitro-2-naphthylamine gives rise to mammary and forestomach tumors. Again, 2-acetamidofluorene induces mainly liver cancer in rats, while 2,7-diacetamidofluorene reliably induces carcinomas of the glandular stomach. Higher homologues, such as 2-aminoanthracene and 4-amino- and 4-dimethylaminostilbene give mainly ear duct tumors and mammary tumors in young adult female rats. Similar results were obtained with 3-acetamidophenanthrene, but the 2-acetamido isomer in addition induced leukemia.

Closely related to these polycyclic amines are several heterocyclic amines (Figure 3 (19 to 21)). These occur in charred parts of cooked fish and meat, and were first detected by their high mutagenic activity in bacterial tests. Trp-P-1 (Figure 3 (19)) and Trp-P-2 (Figure 3 (20)) were isolated from pyrolysates of DL-tryptophan, as was Glu-P-1 (Figure 3 (21)) from glutamic acid. Fibrosarcomas were obtained in rats and hamsters after injection of Trp-P-1, and feeding both Trp-P-1 and Trp-P-2 induced hepatomas in mice. These results are of importance because these amino acids occur commonly in cooked food.

As already mentioned aniline was exonerated from blame for occupational bladder cancer and proved inactive in animal tests. Recent interest in single-ring arylamines has arisen because Ames et al. discovered that many hair dyes are mutagenic in tests with Salmonella typhimurium. Apparently some 40% of adult women in the U.S. regularly use hair dyes, and concern was felt because aromatic dianimes are commonly used in these preparations together with an oxidizing agent such as hydrogen peroxide. Long-term feeding experiments in both rats and mice established that 2,4-diaminotoluene, 2,4-diaminoanisole, and o-phenylenediamine were carcinogenic in both species, inducing mostly bladder and liver cancer;
FIGURE 3. Carcinogenic heterocyclic amines (19—21) formed by the pyrolysis of amino acids in charred food, and two azo-dye carcinogens, butter yellow (22) and aminoazotoluene (23).

the anisole also gave a high incidence of thyroid tumors.\(^7\) Recently, chronically high-level intakes of aniline itself have given rise to hemangiosarcomas in the spleens of male rats; it has been suggested\(^6\) that prolonged stress by aniline on the hematopoietic system may have indirectly caused these tumors.

On the whole there is still little understanding of the reasons for the observed organ and species selectivity in carcinogenesis by aromatic amines.\(^6\) Undoubtedly, tissue distribution must play a role, but this seems to have been little studied. The \(N\)-hydroxylation mechanism of activation already discussed accounts satisfactorily for a number of observations; thus, animals in which \(N\)-hydroxylation is low, such as the guinea pig, are generally resistant to arylamine carcinogenesis. Also, compounds in which the amino group is ortho to a second aromatic ring, such as 1-naphthylamine and 1-AAF, do not undergo \(N\)-hydroxylation and are not carcinogens.

Related to the aromatic amines are the azo dyes which originated the interest in this area of chemical carcinogenesis. These are large in number, and have been widely studied in the past.\(^6\) Feeding dyes such as butter yellow (Figure 3 (22)),\(^1\) so called because it was formerly added to margarine to color it yellow like butter, and \(o\)-aminoazotoluene (Figure 3 (23)) to rats induced hepatomas in good yield with a relatively short latent period.\(^2\) Related compounds cause a variety of tumors (bladder and liver in the dog,\(^3\) ear duct, intestine, and skin in the rat\(^4\)) similar to those seen with amines. Azo dyes appear to be activated by \(N\)-hydroxylation, after metabolic loss of an \(N\)-methyl substituent if necessary, followed by formation of the sulfate ester.\(^5\) Enzymatic cleavage of azo bonds may in some cases lead to detoxification by giving single ring fragments, but in others it may lead to activation.\(^6\)

IV. NITROSAMINES AND NITROSAMIDES

Nitrosamines (\(R_N-N=O\)) have long been familiar to organic chemists as useful and versatile intermediates, but it was not until 1956 that their carcinogenicity was first demonstrated. Following up studies on the toxic effects of dimethylnitrosamine among workers exposed to it during its manufacture as a solvent, Magee and Barnes\(^7\) showed 19/20 rats fed on a diet containing 50 ppm of this nitrosamine developed liver tumors within 26 to 40 weeks. At higher doses severe liver damage was observed. Since then interest in this class of carcinogens has increased year by year, and the last 5 years have witnessed a veritable explosion in research and publications on this subject. Some 1400 papers now appear annually on all aspects of nitrosamines and the related nitrosamides (\(R-CO.NH.N=O\)), and no fewer
than 8 monographs on this subject have been published by the International Agency for Research on Cancer since 1972. Several recent reviews also summarize this field from both the biological and chemical angles. There is a number of reasons for this intense interest; many nitrosamines and nitrosamides are potent carcinogens with remarkably organotropic properties; certain of them occur naturally in the environment, and the possibility of their in vivo formation from dietary amines is all too apparent.

At the present time about 300 nitroso compounds have been tested for carcinogenicity, and about 90% have proved to be active. These compounds include symmetrical, unsymmetrical, and cyclic nitrosamines, nitrosoureas, nitrosourea derivatives, and nitrosoureas. They are carcinogenic in 39 species belonging to 36 genera, 25 families, 17 orders, and 5 classes of animals ranging from fish to primates. Sites at which tumors appear include brain and nervous system, oral cavity, esophagus, stomach, gut, liver, kidney, urinary bladder, pancreas, hematopoietic system, heart, and skin. There is still no unequivocal evidence for their carcinogenicity in man, although there can be little doubt that he, too, is susceptible. Two cases of acute nonlymphocytic leukemia in brain cancer patients treated with the cytostatic N-nitrosourea derivatives, N,N'-bis-2-chloroethyl)-N-nitrosourea and N-cyclohexyl-N-2-chloroethyl-N-nitrosourea have been reported, and both these drugs were subsequently shown to be carcinogenic in the rat. Severe liver damage and cirrhosis leading to death occurred recently in a victim of criminal poisoning with dimethylnitrosamine, and almost identical toxic symptoms were previously seen in laboratory rodents after high doses of nitrosamines. Also, similar DNA methylation patterns occur in human liver after dimethylnitrosamine poisoning as occur in rat liver after exposure to this carcinogen.

Nitrosamines (unlike nitrosamides) are not direct-acting carcinogens, and must be metabolically activated. It now appears that this is achieved by α-hydroxylation as shown in Figure 4 for the two resonance extremes (24) and (25) of dimethylnitrosamine. Both lead, via α-hydroxylation followed by fragmentation of a six-membered, partially hydrogen-bonded transition state, ultimately to methyl diazonium hydroxide (Figure 4 (26)), which is a powerful electrophile, readily methylating nucleophilic sites in DNA and other biological macromolecules. On the other hand, the nitrosamide, N-nitroso-N-methylurea (Figure 4 (27)), is already hydrogen-bonded for spontaneous fragmentation to yield the diazo hydroxide hydroxide; it does not therefore need biological activation and consequently behaves as a direct-acting carcinogen. Breakdown of the corresponding nitrosourethane (Figure 4 (28)), in which hydrogen bonding is absent, is considerably enhanced by thiol compounds by formation of the thio-half-acetal (Figure 4 (29)) as an intermediate, although the nitrosourethane is usually considered as a direct-acting carcinogen.

Probably no group of carcinogens has been more widely tested than these nitroso compounds. Diethylnitrosamine has received the most attention, having proved to be active in all 25 species in which it has been tested. Dimethylnitrosamine and N-nitroso-N-methylurea are likewise carcinogenic in each of the 16 species tested. Organ specificity in nitrosamine and nitrosamide carcinogenesis depends mainly on the chemical structure of the carcinogen, although the animal species, route of application, and duration of treatment often play a part.

As Magee and Barnes were the first to show, dimethylnitrosamine induces primary liver tumors in rats after chronic feeding for some months. However, the same authors found that after a single high dose of this carcinogen malignant kidney tumors were observed exclusively. Inhalation (100 ppm in air for 30 min twice weekly) induced tumors of the nasal cavity in rats, with no liver cancer, indicating that the enzyme system responsible for α-hydroxylation is present also in this tissue. In feeding experiments this carcinogen induced liver cancer in Syrian hamsters, but in mice it gave rise mainly to lung cancer, presumably due to its high volatility.

Diethylnitrosamine was found to be a potent liver carcinogen in the rat, but after low
FIGURE 4. Biological activation of dimethylnitrosamine (resonance extremes 24 and 25) occurs by α-hydroxylation; the 6-membered hydrogen-bonded intermediates fragment to yield methylazidonium hydroxide (26), which is a powerful electrophile. The same product is formed by spontaneous fragmentation of methyl nitrosourea (27), while thiols enhance fragmentation of methyl nitrosoureas (28) via formation of the intermediate thio hemiacetal (29).

In a recent comparison of over 65 different nitroso compounds in BD rats, Druckrey et al.\textsuperscript{108} reached the following conclusions regarding the relationship between chemical structure and organ specificity of nitrosamines. Symmetrically substituted dialkyl nitrosamines produced liver cancer, the exceptions being di-n-butyl nitrosamine which caused bladder tumors and diamyl nitrosamine which selectively induced lung cancer. Unsymmetrically dialkyl nitrosamines, especially methyl amyl-, cyclohexyl-, phenyl-, benzyl-, or phenylethyl-nitrosamines selectively produced carcinomas of the esophagus. However, methylallyl nitrosamine gave malignant tumors of the kidney. Nitrosamines in which α-hydroxylation is impaired (ethyl tert-butyl-, dibenzyl-, dicyclohexyl-) or is impossible (diphenyl, diallyl-) are not carcinogenic.

In a recent review, Lijinsky\textsuperscript{91} has given species and target organ data for 2 homologous series of nitrosamines as shown in Tables 1 and 2. The interesting differences between straight chain nitrosamines with odd and even numbers of carbon atoms may be due to omega oxidation followed by successive beta oxidations, leading to different final products.
Among the cyclic nitrosamines differences in target organ are accompanied by large differences in potency, e.g., the compound in which \( n = 4 \) is a much weaker carcinogen than the one in which \( n = 6 \). They are also unusual in that they bind to DNA in vivo much less than their aliphatic counterparts.

In another publication\(^{17}\) Lijinsky compared the carcinogenicity of three cyclic nitrosamines in four species, again with marked organotropic results (Table 3). The cumulative oral doses administered over 6 months or more needed to induce these tumors in 50% or more of the animals ranged between 0.5 and 8 g/kg. Lijinsky suggested that these profound differences between species might be explained “by large differences in metabolism or by kinetic differences in the balance of various pathways of metabolism”.

In contrast to the dependence upon metabolism displayed by nitrosamines, nitrosamides (Figure 5) decompose spontaneously to give alkylating intermediates and are therefore direct-acting carcinogens. In line with this idea, subcutaneous injections of nitrosamides into rats
Table 3
CARCINOGENICITY OF THREE CYCLIC NITROSAMINES IN ANIMALS OF FOUR SPECIES

<table>
<thead>
<tr>
<th>Species, and organs bearing tumors</th>
<th>Rat</th>
<th>Syrian hamster</th>
<th>European hamster</th>
<th>Guinea pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>Esophagus</td>
<td>Pancreas</td>
<td>Pancreas</td>
<td>Liver</td>
</tr>
<tr>
<td>2.6-Dimethylnitrosomorpholine</td>
<td>Esophagus</td>
<td>Lung</td>
<td>Lung</td>
<td>Liver</td>
</tr>
<tr>
<td>2.6-Dimethyldinitrosopiperazine</td>
<td>Lung</td>
<td>Forrestomach</td>
<td>Lung</td>
<td>No tumors</td>
</tr>
<tr>
<td>Nitrosoheptamethyleneimine</td>
<td>Esophagus</td>
<td>Esophagus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Chemical structures](image)

FIGURE 5. Direct-acting carcinogenic nitroso compounds: nitrosamides (30), nitrosourethanes (31), nitrosoureas (32), nitrosobiurets (33), and nitrosonitroguanidines (34).

produced local sarcomas, while topical application of N-nitroso-N-methyleurea to mice led to skin tumors. As is to be expected, the potency of these carcinogens roughly parallels their chemical reactivity (Figure 5); most active are alkynitrosocarboxamides (30), followed in order by urethanes (31), ureas (32), biurets (33), and nitroguanidines (34). Mainly tumors of the forestomach were obtained after oral administration of these nitrosamides to rats and to hamsters, but gastric carcinomas occurred in the guinea pig. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) (34) was found, in rats and dogs, to be particularly suitable for the induction of gastric tumors which resemble human stomach tumors, and now provide an important model for this disease. On intravenous injection into rats methylnitroso urethane (MNUT) (Figure 5 (31)) produced lung carcinomas exclusively, whereas ethynitrosourethane gave carcinomas of the forestomach. Unexpectedly N-methyl-N-nitrosoureia (MNU) (Figure 5 (32)) gave neither, but led to a high yield of neurogenic tumors of the brain and spinal cord; similar results were obtained in rabbits and in dogs. Higher alkyl homologues of MNU tended to give fewer neurogenic tumors, but induced other types of cancer, particularly of the digestive tract and leukemia. In the hamster n-propyl-nitrosoureia gave tumors of the spleen, forestomach, duodenum, and vagina.
N-Nitroso compounds have also been shown to cause cancer transplacentally; dimethyl- and diethylnitrosamines given to pregnant rats led to the appearance of a variety of tumors in their offspring. However, ethyl-nitrosourea, a carcinogen that exerts its effects independently of metabolism, afforded the most interesting results; administration of a single dose on the 15th day of gestation produced malignant and mostly multiple tumors of the brain, spinal cord, trigeminal nerves, and peripheral nervous system in all the offspring. No tumors were observed when the carcinogen was administered before the 12th day of gestation, indicating that a certain degree of differentiation is required before tumor induction can become effective. Administration to the progeny 30 days after birth led to fewer neurogenic and an increasing proportion of kidney and female genital tumors.

Reasons for the remarkable organotrophy shown by nitroso compounds are for the most part obscure, but are usually attributed to organ-specific metabolic effects as far as the nitrosamines are concerned. With the direct-acting nitrosamides, the route of application is more important, and organotrophy depends in a more understandable way on the relative stability of the carcinogen. Thus, by the oral route the reactive N-nitrosomethylurethane gives tumors of the forestomach, while the more stable MNNG (Figure 5) induces gastric cancer. Also, the very reactive acetoxymethyl-methylnitrosamine induces local tumors after oral, subcutaneous, intravenous, and intrarectal application to rats; systemic administration also led to tumors of the heart, a most unusual site. The great variety of animal tumors now available by the use of specific nitroso compounds has opened up new areas of cancer research, and ultimately may help to improve treatment for the analogous human diseases.

On the other side of the coin, however, the ready formation of nitrosamines by the simple reaction of amines with nitrous acid means that these compounds are ubiquitous in our environment. The development over the last 15 years of extremely sensitive methods for the detection of nitrosamines in household and environmental materials has brought this problem into focus. Nitrosamines are present in trace amounts in many foodstuffs, being particularly high in preserved meat and fish where no doubt they form by reaction of the constituent secondary and tertiary amines with sodium nitrite used as the preservative. Probably more important is the formation of nitrosamines in vivo, particularly in the stomach where the acid conditions are ideal for their rapid production. Many animal experiments have been carried out in which several types of tumor have been induced by deliberately feeding various amines and sodium nitrite. It is very difficult to gauge the extent of human exposure, although the recent method of measuring the amount of the noncarcinogenic N-nitrosoproline formed from orally administered proline should help in the future. Unfortunately, there is also a lack of animal data on the effects of chronic feeding with very low doses of nitrosamines, as might be expected to occur in the human situation, because in the past most experiments have been designed to produce tumors in a relatively short time. For the rat the minimum dose for tumor induction seems to be about 1 ppm in the diet. There has recently been much interest in nitrosamine levels in various parts of the world, especially those such as northern Iran and areas in China where there is known to be an abnormally high incidence of esophageal cancer. However, so far it has not been proved possible to implicate nitrosamines in human disease with certainty, although clearly it is prudent to ensure that human exposure to these agents is always as low as is practicable.

V. ALKYLATING AGENTS

Although nitrosamines and nitrosamides are alkylating agents of a particular type, some halogenated hydrocarbon derivatives and similar compounds that readily undergo nucleophilic displacement can also alkylate biological macromolecules, and many are carcinogenic. These compounds are, of course, direct acting carcinogens, tending to give local tumors at
FIGURE 6. Carcinogenic antitumor drugs: cyclophosphamide (35), chlomaphazin (36), and chlorambucil (37), and the fumigant \( \beta \)-propiolactone (38).

the site of application. The group is large, including a number of industrial intermediates and the medically important class of anticancer agents known as the nitrogen mustards.

Soldiers exposed to the vesicant mustard gas \((2,2'-\text{dichloroethyl sulfide, S(CH}_2\text{CH}_2\text{Cl})_2\)) in World War I later suffered from cancer of the bronchus, larynx, and nasal sinuses. Replacement of the sulfur atom in this compound by nitrogen gives the nitrogen mustard \(\text{NH(CH}_2\text{CH}_2\text{Cl)}_2\), the simplest member of a series of hundreds of anticancer drugs such as cyclophosphamide (35), chlomaphazin (36), and chlorambucil (37) (Figure 6). These agents have in common the ability to kill dividing cells by incapacitating their DNA; from the present vantage point of understanding the general correlation between DNA damage and carcinogenesis it is not surprising that many have been shown to be weak to moderately strong carcinogens in animal tests. In man cyclophosphamide has apparently induced a whole range of cancers (reticulum cell sarcoma, chronic lymphocytic leukemia, Hodgkin's disease, cervical cancer, malignant melanoma, astrocytoma, glioblastoma, and bladder cancer.) Bladder cancer was also noted in patients with other forms of cancer treated with chlomaphazin. Chlorambucil produces lymphomas in rats, and lymphosarcomas and ovarian and lung cancer in mice after intraperitoneal injection. Several case reports have implicated this drug in the induction of leukemia and other cancers in man.

The reactive compound \(\text{H}_2\text{O.C.-(CH}_2\text{)}_3\text{NCH}_2\text{CH}_2\text{Cl}\) is a powerful carcinogen, producing skin tumors in mice, after skin painting and lung and nasal tumors in rats after inhalation. \(\beta\)-Propiolactone (Figure 6 (38)), previously used as a medical sterilant, is similarly active in mice, rats, hamsters, and guinea pigs. Vinyl chloride (CH\(_2\)=CHCl), produced on a huge scale in the manufacture of polyvinylchloride (PVC), is toxic to animals and man. In mice, rats, hamsters, and rabbits exposure to this compound in air leads to angiosarcomas (malignant tumors affecting blood vessel walls) of the liver and other organs together with tumors of the lung, intestines, kidney, skin, mammary gland, and forestomach. Liver angiosarcomas have also been diagnosed in a number of PVC workers exposed on an average of 20 years previously to vinyl chloride at high concentrations in air.

VI. SOME NATURAL PRODUCTS

At one time it was felt that naturally occurring compounds would not be carcinogenic because animals would have evolved efficient means to deal with these threats to their existence. We now know that is not true, and in fact certain enzyme systems designed for this purpose can act to the detriment of the animal, causing biological activation. The lengthening list of carcinogenic natural products includes a few of the more important compounds discussed below (Figure 7).
Aflatoxin B₁ (Figure 7 (39)) is one of a number of complex lactones formed by the yellow mold Aspergillus flavus which widely contaminates foodstuffs in warm, humid parts of the world. It was, in fact, discovered in 1960 as a result of an investigation into the death of a number of turkeys fed contaminated peanut meal. Fed to rats at 1 μg/kg in the diet, liver cell carcinomas occurred in 10% of the animals, while all the animals succumbed to this tumor at 100 times this dose. Aflatoxin B₁ is therefore one of the most potent liver carcinogens known. Low dietary doses induced liver cancer in trout, salmon, guppies, and ducks, and in rhesus monkeys, marmosets, and tree shrews. Administered to rats in dimethylsulfoxide solution either by stomach tube or by intraperitoneal injection, it induced hepatocellular carcinomas; similar results were also obtained with rhesus monkeys. There is convincing circumstantial evidence that primary liver cancer in man may be caused by this agent. Aflatoxins are activated by epoxidation of the terminal furanoid double bond, and it is interesting that like the hydrocarbons which are also activated in a similar manner local sarcomas result after injection of aflatoxin B₁ (Figure 7 (39)) in oil into both mice and rats. However, the similarity stops here, because aflatoxins are powerful liver carcinogens, whereas hydrocarbons almost never cause tumors at this site.

Aflatoxins are members of a group of mycotoxins, many of which are mutagenic and teratogenic. A related structure, sterigmatocystin (Figure 7 (40)), also produces liver tumors in rats and lung tumors in mice after oral administration. Patulin (Figure 7 (41)), another cyclic lactone antibiotic, induces sarcomas at the site of injection in rats. Chloramphenicol (Figure 7 (42)), an antibiotic metabolite of Streptomyces venezuelae used clinically for various infectious diseases, has caused severe bone marrow depression in patients followed by leukemia.

Liver and other tumors have been induced in rodents also with several alkaloids of the pyrrolizidine group, of general structure (Figure 7 (43)). These undergo enzymic dehydrogenation in the liver to yield highly reactive alkylating agents (Figure 7 (44)) which are probably the proximate carcinogens. These alkaloids are found in senecio leaves used in
FIGURE 8. 4-Nitroquinoline-N-oxide (48), a powerful carcinogen, is activated by reduction to the oxime (49); acylation of the latter gives a reactive ester, fission of which leads to an electrophile. Thiouracil (51), an antithyroid substance, is carcinogenic, as are the antibacterial nitrofurans (52 and 53).

various parts of Africa to prepare medicinal tea. The glycoside cycasin (Figure 7 (45)) occurs as a constituent of cycad nuts used as a foodstuff in Guam. Hydrolysis by intestinal flora yields the aglycone methylazomethanol (Figure 7 (45b)), which behaves as a methylating agent like the methylnitrosamines. Similarly, cycasin produces a variety of malignant tumors mainly of the liver, kidney, and intestine in rats, hamsters, guinea pigs, rabbits, and fish.

Carcinogenicity associated with the natural and synthetic estrogens, estrone (Figure 7 (46)) and diethylstilbestrol (Figure 7 (47)), is probably not due to their conversion to electrophilic agents as seems to be the case for most carcinogens. Lacasagne was the first to use estrone to induce breast cancer in male mice of a strain in which only the females normally developed this tumor; this sex hormone was therefore probably the first natural product shown to be carcinogenic. Stilbestrol, synthesized by Dodds in 1947, has exceptional estrogenic potency surpassing that of the natural hormone, and has found importance both as a drug and commercially as an aid to fattening cattle for slaughter. Large doses used formerly to prevent threatened abortions unfortunately led to vaginal tumors in some of the daughters of women treated 15 to 20 years previously. More recently, treatment of older women for the alleviation of menopausal symptoms by estrogen replacement therapy has given rise to endometrial cancer. Today there is concern lest the prolonged use of contraceptives containing estrogens should raise the incidence of breast cancer among the women taking them. The estrogens are extremely powerful hormones and their use in unnatural situations presumably upsets the delicate balance controlling tissue growth and replacement. However, the detailed mechanism by which this leads to cancer is still obscure.

VII. MISCELLANEOUS ORGANIC COMPOUNDS

The preceding sections have catalogued the main types of chemical carcinogens without, of course, being in any way exhaustive. There are still many other miscellaneous organic compounds which are carcinogenic, and here we shall mention just a few of these which are interesting for various particular reasons (Figure 8).

Colon cancer has until recently been difficult to study experimentally due to the lack of an adequate animal model. Now 1,2-dimethylhydrazine (CH₃NH₂NHCH₃) is found readily to induce adenocarcinomas of the colon and rectum when fed to rats; at low doses liver
tumors are observed. Oral administration to mice and hamsters gives high incidences of angiosarcomas mainly localized in muscle, liver, lung, heart, and pancreas. However, after subcutaneous injection colon carcinomas were found in mice as well as rats, but in hamsters hepatocellular carcinomas were seen in addition. Dimethylhydrazine probably acts as a methylating agent since azoxymethane, a possible metabolite, also gave a high incidence of colon and rectal carcinomas in rats and mice.

4-Nitroquinoline-N-oxide (Figure 8 (48)) is a powerful synthetic carcinogen which has found much use in cancer research. Enzyme systems necessary for its activation occur in most prokaryotic and eukaryotic cells and for this reason it tends to act locally. Thus, unlike most carcinogens it frequently induces tumors of the mouth, tongue, and salivary glands in mice, rats, and hamsters when given orally; however, tumors of the esophagus, forestomach, stomach, and lung have also been observed in these animals and in rabbits. In skin painting experiments it has been shown to induce skin tumors in mice, hamsters, and guinea pigs. Biological activation involves reduction of the nitro group to yield the oxime (Figure 8 (49)), followed by acylation of the latter forming the ester (Figure 8 (50)) which acts as a potent electrophile.

It has been known for a long time that thyroid tumors can be induced by low iodine diets. Unavailability of inorganic iodide suppresses the synthesis of thyroxine resulting in a hormonal imbalance of the hypothalamo-pituitary-thyroid system, and leads to thyroid cancer by an indirect mechanism, as is probably also the case of cancer caused by the female sex hormones. Antithyroid substances such as thiourea (S=C(NH₂)₂) and thiouracil (Figure 8 (51)) not unexpectedly are carcinogenic in this situation. Thus, thiourea produced liver, thyroid, and Zymbal gland tumors in rats both by oral administration and by intraperitoneal injection; mice, however, were resistant. Similarly, oral thiouracil also produced thyroid tumors in several strains of rats.

The 5-nitrofurans have been widely used as food preservatives and clinically as antibacterial agents since 5-nitro-2-furaldehyde semicarbazone (Figure 8 (52)) was shown to be active in 1944. A large number of related compounds have been synthesized and many show antibacterial activity; some also appear to be carcinogenic. Thus, (Figure 8 (52)) produced benign mammary tumors in a high proportion of female rats after feeding it in their diet. A similar compound AF-2, furylfuramide (Figure 8 (53)), was used in Japan as a food additive until it was found to be strongly mutagenic in bacterial tests. It had previously failed to produce tumors in an animal test, but on subsequent retesting was shown to be carcinogenic in mice, rats, and hamsters.

Finally, a number of chlorinated hydrocarbons of great technical and industrial importance appear to be weakly carcinogenic in animal tests. Both chloroform (CHCl₃) and carbon tetrachloride (CCl₄) give rise to liver tumors in rodents; chloroform, once a constituent of cough syrups, also induced malignant kidney tumors in male rats and thyroid tumors in females. Tetrachloroethylene (Cl₂C·CCl₂), used mainly in dry cleaning, also produced hepatocellular carcinomas in mice.

VIII. INORGANIC CARCINOGENS

Carcinogens are by no means confined to organic compounds; carcinogenicity is associated with the elements arsenic, beryllium, cadmium, chromium, nickel, and lead in the form of the pure metals or more usually their salts or oxides. Several forms of the mineral asbestos (complex fibrous magnesium and iron silicates) are also known to be carcinogenic in man and in animals. Much of the information regarding the carcinogenicity of these materials comes from human epidemiology because they are all of considerable industrial importance, and exposure to them has occurred during their mining, refinement, and use. Arsenic is of particular interest because in the past numerous attempts to demonstrate its carcinogenicity in a number of animal species (mice, rats, rabbits, and dogs) have met with
failure, although more recently intratracheal instillation of a pesticide containing calcium arsenate has been shown to induce lung tumors in rats. Human exposure has occurred through the medicinal use of Fowler’s solution (potassium arsenite) for a variety of ailments including skin diseases such as psoriasis, leading to chronic arsenicism (peripheral neuropathy) and skin cancer. The tumors often appear at unusual sites such as the palms and soles, different from the skin tumors induced by other known carcinogens; ingested arsenic is known to concentrate in the skin and hair. Epidemiology shows the occurrence of an excess of respiratory cancer among workers engaged in the manufacture of arsenical pesticides and those engaged in smelting copper, lead, and zinc, metals which in the crude state contain arsenic.

Beryllium and its compounds find technical application mostly in electrical equipment, and in the nuclear industry; the use of beryllium in fluorescent tubes has been discontinued. Beryllium compounds are carcinogenic in rats, rabbits, and monkeys; lung tumors result from inhalation or intratracheal instillation, and osteocarcinomas from intravenous or intramuscular administration (rabbits). There is limited epidemiological evidence that exposure to beryllium and its compounds may lead to an increased lung cancer risk in man. Cadmium and a few of its salts are used mainly in electroplating, although they are important in a number of other technical applications. In the rat they give rise to sarcomas at the injection site, and testicular tumors of interstitial-cell origin follow testicular atrophy in both the rat and mouse. There is a suggestion that heavy industrial exposure to cadmium oxide may cause prostate cancer in man.

Chromium is used widely in the metallurgical industry, particularly in the production of ferrochromium alloys and stainless steel, and in electroplating. It is also employed in the manufacture of refractory materials, and many highly colored chromates are used as pigments. A number of Cr(VI) compounds (chromates, chromium trioxide) produce sarcomas at their sites of subcutaneous, intramuscular, and intrapleural administration in the rat. Lead chromate, the main component of the pigment chrome yellow used widely in paints and printing inks, also induced renal carcinomas after intramuscular injection in rats. Numerous worldwide epidemiological studies have firmly implicated Cr(VI) compounds in the causation of occupational lung cancer among chromate production workers; increased risk of other forms of cancer (esophagus, prostate, maxillary sinus) has also been reported.

The transition metal, nickel, also finds many uses in the modern industrial world. In the past, human exposure occurred mainly in nickel refineries where cancer of the lung and nasal cavities was found to be, respectively, 5 to 10 times and 100 to 900 times higher than that expected from the average national rates. Nickel carbonyl, involved in one refining process, may not have been to blame because similarly increased risks were associated with refineries using the electrolytic process. Suspicion falls upon nickel oxide and subsulfide present in the crude nickel ore, the dust of which leads to heavy exposure to these compounds during the early parts of the refining process. Nickel subsulfide and oxide produced local and rhabdomyosarcomas after intramuscular injection into rats and mice; other nickel compounds were also active by this route. Inhalation of nickel carbonyl produced the occasional lung tumor in rats, while after intravenous injection it gave rise to a range of malignant tumors (liver, kidney, breast, lymphomas, and leukemia). Extensive experiments on the carcinogenicity of nickel subsulfide in mice, rats, rabbits, and hamsters of several strains has recently been reviewed.

Lead is one of the oldest metals known and has many uses: in electrical storage batteries, solders, alloys (brass and bronze), as tetraethyl lead as an anti-knock additive for motor fuels, as the oxides in paint, etc. Lead acetate and phosphate produced kidney tumors in rats and mice after oral or parenteral administration. Subcutaneous administration of tetraethyl lead in tricaprylin to neonatal mice induced lymphomas in 5/41 females, but not in the males. Of three epidemiological studies of workers who were exposed to lead and
inorganic lead compounds and to tetraethyl lead, two indicated slight, but not significant increases in deaths from cancer of the digestive and respiratory systems, and of the skin (tetraethyl lead workers). 185

Suspicion of an association between the disease asbestosis and lung cancer among industrial asbestos workers was voiced in 1935. 187 First reports of pleural and peritoneal tumors appeared 10 years later, but the occurrence of mesotheliomas in these workers was not recognized until 1963. 188 Mesothelioma is a rare tumor in the general population, and this has aided epidemiological surveys of asbestos-linked cancer. Worldwide surveys have confirmed the original findings, and all industrially important forms of asbestos have been shown to induce mesotheliomas in rats following intrapleural administration. 189 Mesotheliomas have also been induced in hamsters 190 and rabbits. 191 The long latent periods observed in these experiments are in agreement with the average of 40 years estimated for the production of these tumors in humans. Risk of carcinoma of the lung in asbestos workers is greatly increased in those who smoke cigarettes, 43 whereas risk of mesothelioma is not. 192 Fiber dimensions appear to be important with regard to the latter disease, and in fact glass fibers of the same optimum dimensions also induce mesotheliomas. 193 This suggests that the aerodynamic properties of these fibers are critical in ensuring that they reach their target, but the mechanism by which they cause cancer is still unknown. Asbestos is, of course, an important commercial material, used mainly in the building industry for insulation; it is also an essential constituent of brake linings and clutch plates. It is, therefore, widely distributed and the cause of concern today. 194

IX. CONCLUSION

Perhaps the most striking feature disclosed by a review of this sort is the remarkable organ specificity shown by many carcinogens. Thus, in general polycyclic hydrocarbons tend to produce skin cancer, whereas many aromatic amines induce bladder tumors; in humans asbestos causes mesotheliomas while vinyl chloride exposure is associated with angiosarcomas. Frequently, organ specificity differs between animals of different species; for example 2-aminonaphthalene gives liver tumors in mice, but is a bladder carcinogen in man.

Where they have been examined sufficiently it is often clear that several factors combined to give rise to the observed species or organ specificity. Tissue distribution is obviously important, and is sufficient to explain the effects of very reactive direct-acting carcinogens. The alkylating agent bis(chloromethyl)ether induces skin tumors when applied topically, but tumors of the lung and nasal cavities when inhaled. Nitrosomethylurea, a chemically more stable direct-acting carcinogen capable of being distributed intact throughout the body, gives a variety of tumors (kidney, intestine, stomach, lung, brain) after intravenous injection, as well as local tumors of the blood vessels; skin tumors again result from topical application of this compound. The clearest correlation between DNA damage induced in specific tissues and subsequent tumor formation, comes, however, from the study of radiation carcinogenesis. Both UV light and ionizing radiation possess wavelengths associated with sufficient energy to break most chemical bonds, and not unexpectedly are known to cause macroscopic chromosome damage and to be mutagenic. UV radiation is the less energetic and is incapable of penetrating far into tissue; in agreement with this it is found to give rise to skin cancer. X- and γ-rays are much more energetic; they can penetrate tissue and deliver their energy internally. Leukemia developed in certain of those (atomic bomb survivors, patients irradiated for diseases such as ankylosing spondylitis) who suffered general irradiation. Radioactive metals such as strontium-90 and plutonium, on the other hand, become incorporated into bone where their intense local irradiation gives rise to osteosarcomas.

The majority of chemical carcinogens require metabolic activation before they can act,
and this leads to a second important factor; not only has the carcinogen to reach the target organ, but there it must undergo the correct metabolic transformation. Activation of several major classes of carcinogens, such as polycyclic hydrocarbons and aromatic amines, requires more than one metabolic step; moreover, a number of detoxifying enzymes compete with this process. A further complication is that at least some of the enzymes involved are capable of being induced by a variety of substances. Since relatively little is known concerning tissue distribution and inducibility of these enzymes it is usually not possible to predict the target organ(s) for these carcinogens, except by analogy. However, there does appear to be a positive correlation between high aryl hydrocarbon hydroxylase activity in primary lung cancer patients compared with patients with other pulmonary disease, as would be expected if polycyclic aromatic hydrocarbons were the cause of their cancer.195

Other important considerations also apply to the determination of species and organ specificity in chemical carcinogenesis. Different tumors proliferate at different rates; a particularly rapidly growing tumor may therefore kill the animal before other tumors become visible. This can sometimes be disclosed by varying the dose; thus, chronic feeding of rats with dimethylnitrosamine leads to liver cancer, whereas a single, high dose gives rise to kidney tumors. Closely related to this is simply the adequacy of the experimental observations; it is easy to overlook or neglect small tumors when the postmortem examination is focused on large tumors at an expected site. Also related is the question of dormant cells. A great mass of animal data firmly establishes that tumor induction occurs in two or more distinct steps; at subthreshold doses animals treated with a variety of carcinogens show no signs of cancer until subsequently treated with a promoting agent. Tumor promoters are themselves organ specific so this must add to organ specificity in carcinogenesis. Finally, DNA repair is clearly of importance and this, too, is reviewed in Chapter 9, this volume.

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A comparative examination of the in vitro metabolism of five cyclopenta[a]phenanthrenes of varying carcinogenic potential

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Metabolites of 15,16-dihydrocyclopenta[a]phenanthrene-17-one and its 1- and 12-methyl homologues (all non-carcinogens) along with those from the 11-methyl and 11,12-dimethyl-17-ketones (carcinogens), produced in vitro by hepatic microsomes from methylcholanthrene induced rats, were separated by reverse phase h.p.l.c. Identifications of individual metabolites were based upon elution times, u.v. spectra, and in some cases by mass spectrometry, circular dichroism, and identity with synthetic derivatives. All five compounds were biologically oxidised at their terminal A and D rings to yield 1,2-dihydrodiols and 15- and 16-ols; with the exception of the 1-methyl compound, all gave similar amounts of 3, 4-dihydrodiols. The 1-methyl compound by contrast failed to produce this metabolite, furnishing instead the 4-phenol and five other, probably related phenolic derivatives. Previous work has established that for the 11-methyl-17-ketone, the 3,4-dihydrodiol is the proximate carcinogen. Thus, whereas lack of biological activity with the 1-methyl compound can be ascribed to its failure to produce a 3,4-dihydrodiol, in the case of the unsubstituted parent ketone and its 12-methyl derivative other determining factors must come into play.

Introduction

Derivatives of 15,16-dihydrocyclopenta[a]phenanthrene (I Figure 1) which are related structurally to both steroids and polycyclic hydrocarbons provide an interesting series for the study of the correlation between carcinogenicity and chemical structure (1). Thus, whilst the 11-methyl derivative (II) is a potent carcinogen, similar in its behaviour to benzo[a]pyrene on mouse skin (2), its 7-methyl isomer is less active and the 1-, 2-, 3-, 4-, 6- and 12-methyl isomers are not carcinogenic (3,4). The 11,12-dimethyl compound (V) is also strongly active and the 7,11-dimethyl analogue (15,16-dihydro-7,11-dimethylcyclopenta[a]phenanthrene-17-one) is the most carcinogenic member of this series so far tested. The Iball indices for these compounds, tested by repeated application to the dorsal skin of mice, are listed in Table I. The activating effect of a bay region methyl group [at C(11) in the cyclopenta[a]phenanthrene series] is well known in similar polycyclic hydrocarbons, but the reason for this is still not clear.

It is now firmly established that metabolism is involved in the activation of the 11-methyl ketone (III). Thus, this compound is not mutagenic towards Salmonella typhimurium TA 100 in the Ames test, and fails to bind covalently to DNA in vitro unless it is first exposed to biological oxidation. Under these conditions the major nucleoside adduct formed with DNA added to the incubation is identical to that isolated from the DNA of mouse skin after topical application of (III), as in a tumour induction experiment (5). Detailed examination of the metabolites of (III), isolated by ethyl acetate extraction after incubation in vitro with rat liver microsomes and NADPH, demonstrates that this molecule is metabolically oxidised in ring A to form both 1,2- and 3,4-dihydrodiols; oxidation occurs in addition at the methyl group and at both methylene groups at C(15) and C(16) to give mono-ols. Metabolites derived from oxidation in both ring A and ring D are also observed, but enzymic attack does not apparently occur at C(6), C(7) or C(12) in this molecule (6). Of the in vitro metabolites the 3,4-dihydrodiol is the most carcinogenic, being about 10 times more active than the original compound (III) (7). Moreover, after further metabolism in vitro this metabolite binds to exogenous DNA to form the same adduct, via further oxidation to the [R,R]trans-3,4-dihydro-3,4-dihydroxy-anti-1,2-epoxide, analogous to the most potent diol epoxide stereoisomer of benzo[a]pyrene (8).

More recently examination of in vitro metabolism has been extended to the unsubstituted parent ketone (I) and its 12-methyl derivative (IV), both non-carcinogens (8). It was found that both compounds give rise to [R,R]trans-3,4-dihydropyrenes analogous to the proximate carcinogen from (III). The present report extends this work in that it attempts to identify other metabolites of these two non-carcinogens. In addition the 1-methyl-(II) and 11,12-dimethyl-17-ketone (V) are examined in a similar manner for the first time.

Materials and methods

Chemistry

The compounds (I) – (V) were prepared previously in this laboratory (9,10) and their purity was established by standard methods. For the quantitative comparison they were generally labelled with tritium by Pt-catalysed exchange with tritiated water, and repurified by column chromatography (11).

Fig. 1. Cyclopenta[a]phenanthrenes referred to in the text.
For the synthesis of 15,16-dihydro-4-hydroxy-1-methylcyclopenta[a]phenan-then-17-one (VI, Figure 1) the phenolic function was protected throughout as its methyl ether, and the multi-step route followed that used previously (2) into 15,16-dihydro-4-methoxy-1-methylcyclopenta[a]phenanthren-17-one, m.p. 276.1148. C19H19O3 requires M+ 262.0994. Other intermediates in this synthesis also had spectral characteristics consistent with their expected structures.

Metabolism

Incubation were carried out using microsomes derived from the livers of male Sprague Dawley rats induced by l.p. injection of methylcholanthrene 24 h before sacrifice, essentially as previously described (6). Briefly, the compound (1 μmol) dissolved in dimethyl sulphoxide (50 μl) was added to 0.1 M Tris buffer (3 ml, pH 7.2) containing NADPH (20 mg), followed by microsomes equivalent to 5 mg of protein suspended in Tris buffer (1 ml). The mixture was shaken gently in air at 37°C for 30 min, after which it was cooled in ice and extracted six times with an equal volume of ice-cold ethyl acetate. The extracts were dried over anhydrous sodium sulphate, evaporated to dryness in an atmosphere of nitrogen, and finally redissolved in methanol (100 μl). Separation was achieved by h.p.l.c. using a Whatman Partisil 10/30 reverse phase ODS column (Whatman, Maidstone, Kent) and a Waters h.p.l.c. system (Waters Associates, MA, USA). A solvent gradient, 15% v/v methanol/water/pure methanol over 80 min at a flow rate of 4 ml/min, was usually employed; other conditions are as described in the text. Metabolites were detected by continuous u.v. monitoring at 254 nm, collected as appropriate in clean glass vials, and stored at −20°C until utilised, usually within a few hours.

Examination by u.v. spectroscopy

The u.v. spectra of peaks isolated by h.p.l.c. were examined using a Perkin-Elmer Coleman 572 recording spectrophotometer over the wavelength range 200—400 nm, adjusting the water/methanol ratio in the reference cell to match approximately that of the sample. Reduction of the ketone to the corresponding alcohol was achieved by adding a few mg of sodium borohydride in zine and recording the reduced spectrum after 15—20 min. When phenols were suspected, the u.v. spectrum of the anion was produced by adding one drop of N sodium hydroxide to the original solution in the cell.

Results and discussion

Figure 3 illustrates the h.p.l.c. profiles of the ethyl acetate soluble metabolites obtained from in vitro incubations of the unsubstituted (I), 12-methyl (IV) and 11,12-dimethyl (V) compounds. On this reverse phase column the most polar metabolites elute first, and the last major peak to appear is the unchanged starting material. Allowing for the increased lipophilicity following the introduction of one or two methyl groups into the molecule, indicated by the increasing elution times for the unchanged ketones, the metabolic profiles for these three compounds show general similarity. On the other hand the profile for the 1-methyl-17-ketone (II) shows many differences (Figure 5, where the h.p.l.c. conditions were 30—100% gradient).

Table II lists the u.v. spectra of the metabolites derived from compounds (I), (IV) and (V) together with data previously obtained for metabolites of the 11-methyl-17-ketone (III), the structures of which have been confirmed by mass spectrometry (5,6).

Elution times of these were very similar to those shown here for the 12-methyl compound. The simple u.v. maxima shown in Figure 5, where the h.p.l.c. conditions were 30—100% MeOH/water over 3 h at 4 ml/min, although this was also a linear gradient).

Examination of metabolite Ib by circular dichroism

A solution of metabolite (Ib) (Figure 3) in methanol was reduced by the addition of sodium borohydride. The solvent was removed in vacuo, and the residue was extracted with ethyl acetate which was washed with water, dried over anhydrous sodium sulphate, and evaporated to dryness under nitrogen. H.p.l.c. of the residue, redissolved in methanol (100 μl), disclosed equal amounts of two reduction products with identical u.v. spectra (λmax 257,265 nm), and absence of (Ib). The two fractions were combined for the circular dichroism measurement. Phenanthrene.[3R,4R]-dihydriodiol was obtained by metabolism of phenanthrene using the method described above, followed by h.p.l.c. separation. C.d. spectra were recorded in methanol with 1 cm path length cells using a JASCO 340 Cs spectrometer at King's College, University of London.
Metabolism of five cyclopenta[a]phenanthrenes

Retainion time (min)

Fig. 3. H.p.l.c. profiles of the products of \textit{in vitro} metabolism of compounds (I), (IV) and (V); u.v. trace monitored at 254 nm. For u.v. maxima of peaks see Table II. Linear gradient, 15—100% methanol in water, over 80 min at 4 ml/min. M represents a peak of microsomal origin.

Independent spectrum for each compound by \textit{in situ} reduction of the ketone group also greatly facilitates identification. The structure of the 3,4-diols (Ie) and (IVe) have in addition been established already by n.m.r. and mass spectrometry (8), while the structures of (Ih) and (Ig) follow from their identity with synthetic samples of the 15-ol and 16-ol of (I) (13). Mass spectra of selected metabolites of compounds (I) and (IV) (Table III) confirm these assignments.

In each case the first major peak (M) to emerge from the column is of microsomal origin since it contains no radioactivity when tritiated compounds are employed. The next peak (a) consists of \textit{trans}-1,2-dihydrodiols further hydroxylated in ring D, probably largely at C(15). As with bay-region vicinal diols of polycyclic hydrocarbons, the 1,2-diol system in these metabolites is diaxial, thus accounting for their polarity. Other minor metabolites eluting before (b) have 1,2,3,4-tetrahydro chromophores (\(\lambda_{\text{max}} 260\), and 240 nm after reduction to the 17-alcohol) and are therefore tetra-ols and penta-ols derived from metabolic saturation of ring A. Peaks (b) represent the \textit{trans}-1,2-dihydrodiols, again diaxial and much more polar than the isomeric \textit{quasi}-diequational \textit{trans}-3,4-dihydrodiols (e). Circular dichroism was used to establish the absolute stereochemistry of the unsubstituted 1,2-diol (Ib). Reduction of this metabolite with sodium borohydride in methanol gave an equimolar mixture of epimeric 17\(\alpha\)- and 17\(\beta\) alcohols, separable by h.p.l.c. It has recently been shown for the corresponding 3,4-dihydro-3,4-dihydroxy-17-one that the epimeric 17-ols have similar c.d. spectra of the same sign, demonstrating that, as expected, the 17-ols has little effect on the chiral dihydrophenanthrene chromophore (8). Figure 4 shows that the mixture of epimeric 1,2,17\(\alpha\) and 1,2,17\(\beta\)-trios derived from (I) has a c.d. spectrum similar to, and of the same sign as that of phenanthrene-[3R,4R]-dihydridiol isolated as a metabolite of phenanthrene using hepatic microsomes from methylcholanthrene-induced rats (14). The 1,2-diol system in metabolite (Ib) is therefore also [1R,2R], (i.e., 1a,2\(\alpha\)) as shown.

The u.v. spectra of the 3,4-dihydrodiols (e) are very similar to those of metabolites (c) and (d) which are 3,4-diols further hydroxylated at ring D, and possibly also at the methyl groups for (IV) and (V). In the mass spectrometer the 3,4-dihydrodiols undergo facile dehydration and molecular ions are not always

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\lambda_{\text{max}}) (nm) of metabolites a—f (see Figure 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>373</td>
</tr>
<tr>
<td>Unsubstituted (I)</td>
<td>266,320,332, 266,277,316,328</td>
</tr>
<tr>
<td></td>
<td>366</td>
</tr>
<tr>
<td></td>
<td>355sh,375</td>
</tr>
<tr>
<td></td>
<td>(257,266,310)</td>
</tr>
<tr>
<td>11,12-dimethyl (V)</td>
<td>273,323,336, 267,320sh,338sh</td>
</tr>
</tbody>
</table>

\(a\)Refs. 6 and 7.

\(b\)The figures in brackets represent the u.v. maxima after reduction of the 17-carboxyl group with sodium borohydride \textit{in situ}. The peaks of highest intensity are underlined; sh indicates a well-defined shoulder.
observed (6), the ion of highest mass often being the [M-H₂O]⁺ ion. Ions due to the loss of CO, CO + H₂O, and 2H₂O (from 15-hydroxy-A-ring diols) are also commonly observed (15). Metabolite (VIc) decomposed further, but the ions at m/z 170, 155 and 127 in its mass spectrum occur also in the more normal spectrum of the related 3,4-diol (VId). The mass spectrum of metabolite (IVf), which retains the phenanthrene chromophore, is notable in having the molecular ion as the base peak, as has been observed previously for other D-ring mono-ols of this series (1). The metabolites (IVf) and (Vf), and (IVg) and (Vg) having u.v. spectra similar to (If) and (Ig), respectively, are assigned 15-ol and 16-ol structures by analogy. Peaks labelled (h), (i), and (j) in the figure all have u.v. spectra which closely resemble those of metabolites (f) and (g); from their elution times these appear to be 15,16-diols and/or hydroxymethyl-ring D monools.

Table III. Mass spectra of selected metabolites of compounds (I) and (IV) shown in Figure 3

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Molecular formula</th>
<th>m/z (relative abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Ia) C₁₇H₁₄O₄</td>
<td>Found M⁺, 282.0894; calc. 282.0892, 282 (32, M⁺), 264 (53, M-H₂O), 254 (20, M-CO), 246 (12, M-2H₂O), 236 (41, M-CO-H₂O)</td>
<td></td>
</tr>
<tr>
<td>(Ib) C₁₇H₁₄O₃</td>
<td>Found M⁺, 266.0943, calc. 266.0943, 266 (13, M⁺), 248 (60, M-H₂O), 220 (25, M-CO-H₂O)</td>
<td></td>
</tr>
<tr>
<td>(Ic) C₁₇H₁₄O₄</td>
<td>Found M⁺, 282.0891; calc. 282.0892, 282 (65, M⁺), 264 (57, M-H₂O), 246 (35, M-CO), 236 (21, M-CO-H₂O)</td>
<td></td>
</tr>
<tr>
<td>(IVa) C₁₈H₁₆O₄</td>
<td>296 (6, M⁺), 278 (14, M-H₂O), 262 (11), 260 (9, M-2H₂O), 170 (50)</td>
<td></td>
</tr>
<tr>
<td>(IVe) C₁₈H₁₄O₄</td>
<td>223 (8), 170 (67), 155 (100), 127 (79)</td>
<td></td>
</tr>
<tr>
<td>(IVd) C₁₈H₁₄O₄</td>
<td>278 (13, M-H₂O), 263 (10), 262 (18), 260 (20, M-2H₂O), 232 (M-2H₂O-CO), 170 (65), 155 (100), 127 (58)</td>
<td></td>
</tr>
<tr>
<td>(IVf) C₁₈H₁₄O₂</td>
<td>263 (23), 262 (100, M⁺), 245 (11, M-OM), 244 (13, M-H₂O), 233 (14), 216 (23, M-H₂O-CO), 215 (28), 203 (23), 202 (35)</td>
<td></td>
</tr>
</tbody>
</table>

Of the six possibilities for the dimethyl compound (V) at least three (Vh, i, j) appear to occur.

An attempt was made to compare the amounts of the main metabolites (a) – (f) quantitatively using compounds (I) and (III) – (V) labelled with tritium. The compounds were incubated under identical conditions using microsomes from the same preparation, and the amounts of the various metabolites were estimated from the radioactivity found in the appropriate h.p.l.c. peaks. Table IV summarises the average of two experiments. Recovery of tritium in the ethyl acetate extracts was in all cases better than 80%, the remainder presumably representing water-soluble metabolites and tritiated water formed by exchange. All four compounds were metabolised to similar extents and, more significantly, they all gave 3,4-dihydrodiols (e) in not dissimilar amounts. Since (IIIe) is the proximate form of the carcinogen (III) (5), lack of this metabolic step is not the reason for the non-carcinogenicity of compounds (I) and (IV).

It is evident from Figure 5 that the profile of metabolites from the 1-methyl-17-ketone (II) bears little resemblance to those from compounds (I) and (III) – (V), although the in vitro metabolism of all these compounds was carried out under similar conditions. No fewer than six of the peaks (n, q – t, x) proved to be phenolic, whereas phenols are not found as metabolites of the other compounds. Table V lists the u.v. maxima of peaks (k) – (x) together with tentative structural assignments. Of the non-phenolic peaks
Retention time (min)

Fig. 5. H.p.l.c. profile of in vitro metabolism of compound (II); u.v. trace monitored at 254 nm. The hatched peaks represent phenolic metabolites; for u.v. maxima of peaks k—x see Table V. Linear gradient, 30—100% methanol in water, over 3 h at 4 ml/min. M represents a peak of microsomal origin.

Table V. U.v. spectra of metabolites (k) — (x) derived from the 1-methyl-17-ketone (II). \( \lambda_{\text{max}}, \text{nm} \); peaks of highest intensity underlined

<table>
<thead>
<tr>
<th>Peak</th>
<th>Neutral</th>
<th>NaBH(_4)</th>
<th>Alkaline</th>
<th>Tentative assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>259,277,290,301</td>
<td>236,259</td>
<td>—</td>
<td>1,2,3,4-tetraol</td>
</tr>
<tr>
<td>l</td>
<td>269,304sh,333,337,368</td>
<td>258,267,305</td>
<td>—</td>
<td>1,2,15(or 16) triol</td>
</tr>
<tr>
<td>m</td>
<td>268,279sh,298</td>
<td>257,266,282sh,294,303</td>
<td>258,293</td>
<td>phenan-triol(^a)</td>
</tr>
<tr>
<td>n</td>
<td>264,297</td>
<td>248,267,273,284,293</td>
<td>258,293</td>
<td>phenol</td>
</tr>
<tr>
<td>o</td>
<td>270,302,359,376</td>
<td>259,282,292,304</td>
<td>—</td>
<td>phenan-diol</td>
</tr>
<tr>
<td>p</td>
<td>268,302,369</td>
<td>259,303,363</td>
<td>—</td>
<td>phenan-diol</td>
</tr>
<tr>
<td>q</td>
<td>254,278,303</td>
<td>249,282,296sh,318sh,346,365</td>
<td>261,290,324</td>
<td>phenol</td>
</tr>
<tr>
<td>r</td>
<td>253,277,301</td>
<td>249,255sh,281,344,361</td>
<td>262,291,325</td>
<td>phenol</td>
</tr>
<tr>
<td>s</td>
<td>243,267,293</td>
<td>248,258sh,281,291</td>
<td>262,291</td>
<td>phenol</td>
</tr>
<tr>
<td>t</td>
<td>252,276,299</td>
<td>247,258,265sh,292sh</td>
<td>268,290sh,301sh</td>
<td>phenol</td>
</tr>
<tr>
<td>u</td>
<td>266,284sh,302,357,375</td>
<td>258,281sh,292sh,304</td>
<td>—</td>
<td>phenan-ol</td>
</tr>
<tr>
<td>v</td>
<td>270,285sh,303sh,358,375</td>
<td>256,281,292,303</td>
<td>—</td>
<td>phenan-ol</td>
</tr>
<tr>
<td>w</td>
<td>268,290sh,303sh</td>
<td>256,282,292,305</td>
<td>—</td>
<td>phenan-ol</td>
</tr>
<tr>
<td>x</td>
<td>270,305</td>
<td>250,285,300sh,320,349,365</td>
<td>260,284sh,324</td>
<td>4-phenol (\text{VI})</td>
</tr>
</tbody>
</table>

\(^a\)Phenan- denotes an intact phenathrene chromophore.

\(^b\)sh denotes a shoulder.

Another six (m, o, p, u — w) retain chromophores indicating an intact phenanthrene conjugated to a 17-carbonyl group. The three peaks (u — w) have retention times expected for mono-ols; these therefore probably represent the 15- and 16-hydroxy and the 1-hydroxymethyl derivatives of (II), although it is not possible to specify which is which. The two very similar peaks (o) and (p) appear to be diols from their polarity, and there are at least three other minor metabolites which may be other isomers in this region. Peak (m) has the mobility of a triol, and may consequently be the 15,16-dihydroxy-1-hydroxymethyl derivative of (II). Peak (I) is also a triol, but from its u.v. spectrum is quite clearly a 1,2-dihydro diol further hydroxylated in ring D; splitting of the main peak at 269 nm into two peaks of similar intensity at 258 and 267 nm on reduction of the carbonyl group is most characteristic of the 1,2-diol system (see Table II). This metabolite (I) thus corresponds to metabolite (a) from compounds (I) and (III) — (V). However it is less polar than the latter because the diol system is quasi-diequatorial, the 1-methyl group taking up a quasi-axial conformation. A precedent exists (16) for oxidation at C(1) already carrying a carbon substituent in these compounds; 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one is also metabolised to form a 1,2-diol, but here the diol is quasi-diaxial due to constraint by the 1,11-methylene bridge in the plane of the aromatic rings.

No evidence of a prominent 3,4-dihydrodiol or further hydroxylated derivative, characteristic of compounds (I) and (III) — (V), was observed among the metabolites (k — x) of the 1-methyl-17-ketone (II). However peak (k) exhibits chromophores which strongly suggest a 1,2,3,4-tetrahydro-tetraol structure; this could of course have arisen by further metabolism of either a (diequatorial) 1,2-dihydrodiol or a transient 3,4-dihydrodiol. A clue to the apparent absence of 3,4-diols comes from identifica-
tion of the least polar phenol (x) as the 4-phenol (15,16-dihydro-4-hydroxy-1-methylcyclopenta[a]phenanthren-17-one, VI). Both the elution time and the u.v. spectra (neutral, alkaline, and reduced) of this metabolite were indistinguishable from those of the synthetic 4-phenol. Presumably (x) arises from facile isomerisation of the initially formed 3,4-epoxide of (II) before it can undergo enzymic hydration. Isomerisation is probably promoted by electron release by the \(\beta\)-methyl group because the reverse, stabilisation of epoxides towards isomerisation, known to be favoured by electron withdrawal (17). Of the other phenols, (q) and (r) have u.v. spectra similar to that of the 4-phenol (x), but have the polarity of diols and are therefore probably the 15- and 16-hydroxy derivatives of (VI). Peaks (n, probably a triol) and (s, probably a diol) are similar, but somewhat different from (q) and (r) in that their anions lack the strong absorption at \(\sim 325\) nm. They could possibly be 4-phenols additionally hydroxylated at the methyl group (r), and also in the D ring (n). Finally the phenol (l) shows general similarity to both sets of phenols.

In summary, the 1-methyl-17-ketone (II) appears to be metabolised at the A and D rings, like the other four compounds, but differs from them in that the 3,4-epoxide rapidly isomerises to the 4-phenol (VI) which itself gives rise to a range of hydroxy phenols. The C(1)-methyl group is also extensively hydroxylated, and despite its presence the C(1), C(2) double bond is attacked to yield a 1,2-dihydrodiol. The lack of a 3,4-dihydrodiol for conversion to a bay-region diol epoxide appears to offer a satisfactory reason for the non-carcinogenicity of 15,16-dihydro-1-methylcyclopenta[a]phenanthren-17-one (II).

By contrast, the non-carcinogens (I) and (IV) form trans-3,4-dihydrodiols in amounts commensurate with those given by the two carcinogens (III) and (V). Thus the general conclusion to be drawn from this study is that although correct initial metabolism is obviously necessary, other factors, among which are the necessity for further metabolism to a diol epoxide of the right stereochemistry and stability, are also essential in causing these compounds to be carcinogenic.

Acknowledgement

We are indebted to Dr. A. F. Drake (King's College) for the c.d. measurements.

References


Received on 22 March 1985; accepted on 31 May 1985
The biological activity and activation of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one, a carcinogen with an obstructed bay region

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The proposal that an unobstructed bay region is a prerequisite for tumorigenic activity in cyclopenta[a]phenanthrene-17-ones is not supported by the observation of the tumorigenicity of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthrene-17-one towards the skin of T.O. mice. The title compound is oxidised in vitro by a mixed function oxidase to produce, inter alia, a trans-3,4-dihydrodiol, postulated as the proximate tumorigen. Unequivocal identification of a second metabolite as a trans-1,2-dihydrodiol derivative demonstrates the potential for enzymatic oxidation within the obstructed bay region and supports the proposal that the ultimate tumorigen is a trans-3,4-dihydrodiol-anti-1,2-oxide. This is further substantiated by the chromatographic behaviour of the major hydrocarbon-nucleoside adduct derived from mouse skin treated with the parent compound in vivo. The structures of certain others of the metabolites produced in vitro are also considered.

Introduction

Extensive studies on members of the cyclopenta[a]phenanthrene-17-one series (Figure 1, I) have shown that tumorigenic activity is critically dependent upon both the position and nature of substituents within the aromatic nucleus (I). Table I illustrates that small electron-releasing groups at C-7 or C-11 potentiate activity in the otherwise inactive series, while terminal ring substituents (C-1 - C-4) fail to do so. Furthermore the tumorigenic activity of C-11 substituted analogues diminishes rapidly as a function of increasing chain length, as demonstrated by both the 11-alkyl and 11-alkoxy series (1,2). The most active cyclopenta[a]phenanthrene-17-one (II) is metabolised to a trans-[3R],[4R]-dihydrodiol-anti-1,2-oxide (II) (3,4), and the binding to DNA of this species is believed to be responsible for the observed biological activity (5).

Recognition that the terminal benzo ring is the principal site of activation within this series as it is with many other polycyclic compounds (6), taken in conjunction with the observed structure-activity relationships, prompts this study of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthrene-17-one (III). This compound retains an essential electron-releasing substituent at C-11 but also has a rigidly obstructed bay region and a terminal ring substituent, both latter features postulated as being deleterious to biological activity. The aim of this study is to evaluate the relative importance and possible roles of such structural modifications in determining activity.

Materials and methods

Chemistry

The title compound (III), the synthesis of which has been reported (7), was labelled with tritium by catalytic exchange at the Radiochemical Centre, Amersham, UK, and purified here (8) to a final specific activity of 1.935 Ci/mmol.

Skin tumour induction

The title compound (III) was tested twice in both the initiation promotion and repeated application assays, using formally randomised T.O. mice in groups of 20 (10 male and 10 female). Conditions were generally as described previously (9). Mice in group 1 received twice-weekly applications to their dorsal skin of a toluene solution of the compound (III) (50 µg in 10 μl) for 50 weeks; group 2 received similar treatment for 39 weeks. Mice in groups 3 and 4 were treated topically with a single dorsal dose of the compound (400 µg in 40 μl toluene). After one week, twice-weekly applications of croton oil in toluene (1:99 v/v; 10 μl) were undertaken and continued for the two year duration of the experiment. Groups 5—7 were controls: 5, toluene twice weekly; 6, croton oil twice weekly; 7, clipped and otherwise untreated. Animals in all groups were observed for two years.

Table I. Carcinogenicity of isomeric methyl cyclopenta[a]phenanthren-17-ones (see Figure 1)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Carcinogenicity (^a,b,c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IB</td>
<td>46</td>
</tr>
<tr>
<td>IC</td>
<td>&lt;1</td>
</tr>
<tr>
<td>ID</td>
<td>10</td>
</tr>
<tr>
<td>IE (all isomers)</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

\(^a\)Expressed as Iball index.
\(^b\)Tested as complete carcinogens by skin-painting.
\(^c\)See reference (1).
Preparation of metabolites and their reaction with DNA in vitro

The title compound (III), labelled with tritium at 0.74 mCi/mmol, was metabolised using hepatic microsomes prepared as described previously (5) from male Sprague-Dawley rats (± 200 g) induced with a solution of Aroclor 1254 in corn oil (120 mg in 3 ml; i.p., 4 days before sacrifice). Details of incubation procedures, isolation of metabolites by h.p.l.c., and methods used to study binding to exogenous DNA in vitro have been described (5).

Binding to DNA in vivo

Male T.O. mice, 7–9 weeks old, were used in groups of 20. All protocols involved closely followed those previously described (5).

Mutagenicity assays

Metabolites were tested for mutagenicity towards Salmonella typhimurium TA 100 using a standard plate incorporation assay (10) with a rat liver homogenate activating system. Adenosine-5'-triphosphate (ATP, 20 mmol/ml; Sigma Chemical Co., London, UK) was added to the supplement on occasions as specified in the text.

Analysis of metabolites

Isolated metabolites were purified to homogeneity by further reverse-phase h.p.l.c. prior to analysis. Proton magnetic resonance (p.m.r.) spectra were recorded on a Bruker WH 400 spectrometer (Queen Mary College, London, UK) in CD3 OD using tetramethylsilane as the internal standard. U.v. spectra were recorded on a Bruker WH 400 spectrometer (Queen Mary College, London, UK) in CD3 OD using tetramethylsilane as the internal standard. U.v. spectra were recorded on a Perkin-Elmer Coleman 572 spectrophotometer, using 1 cm path length cells. Electron-impact mass spectra were measured at 70 eV by direct insertion into an AEI MS 902 mass spectrometer coupled to a MSS data acquisition unit (Queen Mary College).

Results

Skin tumour induction by compound III

The results of the duplicate experiments (Table II) indicate that the title compound (III) is substantially active in both the initiation-promotion and repeated application assay, approaching the activity of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (IB) as a tumour initiator but being considerably less active as a complete tumorigen.

Bacterial mutagenicity of compound III

In contrast to its behaviour as a relatively strong tumorigen, the title compound (III) elicits only a very low mutation frequency in S. typhimurium TA 100 (Table III). Although showing dose-response behaviour the mutation frequency is only approximately twice background at its optimum. This contrasts strongly to compound IB which produces over 25 times the background number of revertant colonies at an optimum dose of 10 μg/plate when employed as a positive control. Addition of ATP to the activating system, stimulating sulphotransferase activity and facilitating conjugation of putative hydroxyl functions (11), fails to enhance the mutation frequency. This result offers no support to the postulate that the activity of the title compound (III) in vivo relies upon an activation pathway normally redundant in vitro leading to, for example, a reactive benzyl carbamion ion intermediate (12).

The profile of the ethyl acetate-extractable metabolites formed by incubation of compound (III) with rat liver microsomes in vitro, and separated by reverse phase h.p.l.c. is shown in Figure 2. When equivalent amounts of fractions (i) – (x), based on radioactivity, are examined for their mutagenicity, only fraction (iv) elicits a higher reversion rate than the parent compound (III, fraction x). Relative average reversion rates are: (i) 18, (ii) 24, (iii) 23, (iv) 160, (v) 44, (vi) 28, (vii) 80, (viii) 59, (ix) 43 and (x) 100%. Fraction (iv) apparently consists of a single metabolite (b) with a retention time expected for a diol derived from (III).

DNA binding in vivo and in vitro

Treatment of mouse skin with high specific activity [H]compound (III) and subsequent isolation and hydrolysis of DNA gives a peak of radioactivity eluting from Sephadex LH 20 later than the least mobile unmodified nucleoside, adenosine. Further examination of this fraction by h.p.l.c. discloses a pattern of adducts as shown in Figure 3. In keeping with 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (IB) a single adduct accounts for over 80% of the radioactivity bound (5). The pattern of adducts obtained from in vitro microsomal oxidation of compound (III) in the presence of exogenous DNA proves essentially identical: this is verified.

Table II. Skin tumour incidences of formally randomised TO mice treated with 15,16-dihydro-11-methanocyclopenta[a]phenanthren-17-one

<table>
<thead>
<tr>
<th>Group (10♂ + 10♀ per group)</th>
<th>Treatment</th>
<th>No. of tumourless survivors at (months)</th>
<th>No of mice with squamous papillomas</th>
<th>Carcinomas</th>
<th>Total no. of mice with tumours</th>
<th>Mean latent period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>18</td>
<td>11</td>
<td>8</td>
<td>2</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>19</td>
<td>14</td>
<td>7</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>14</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>29</td>
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<tr>
<td>4</td>
<td>c</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>d</td>
<td>19</td>
<td>17</td>
<td>15</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>6</td>
<td>e</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>f</td>
<td>20</td>
<td>19</td>
<td>16</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

aMice received 50 μg of (III) in 10 μl toluene twice weekly for 50 weeks.

bMice received 50 μg of (III) in 10 μl toluene twice weekly for 39 weeks.

Mice received 400 μg of (III) in 40 μl toluene once, then 10 μl of a 1% v/v solution of croton oil in toluene twice weekly for 50 weeks.

Mice received 10 μl of toluene twice weekly for 50 weeks.

Mice were clipped but otherwise untreated.

Histology unavailable for two mice.

S. T. Hadfield, T. S. Bhattacharyya, and M. M. Coombs

Table III. Mutagenicity of (III) towards S. typhimurium TA 100 using a rat liver homogenate activating system additionally fortified with ATP

<table>
<thead>
<tr>
<th>Number of revertant colonies/platea,b</th>
<th>− ATP</th>
<th>+ ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test 1 (20 μg) 205 ± 22</td>
<td>256 ± 13.3 p &gt;0.9 (t-test)</td>
<td></td>
</tr>
<tr>
<td>Test 2 (20 μg) 273 ± 18.2</td>
<td>297 ± 25 p &gt;0.9 (t-test)</td>
<td></td>
</tr>
</tbody>
</table>

aBackground, not subtracted, was unaffected by ATP: 116 ± 2.5, test 1; 114 ± 20.2, test 2.

bMean of 5 plates ± 1 SD.
Biological activity of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one

Fig. 2. Separation of metabolites of the 1,11-methano-17-ketone (III) by reverse-phase h.p.l.c. Conditions used: Whatman Partisil M9 10/50 ODS column eluted with a linear gradient of methanol in water commencing at 15% and increasing at a rate of 1%/min.

Fig. 3. Separation by h.p.l.c. of deoxynucleoside adducts of compound (III) derived in vivo. Conditions used: Whatman Partisil 10/25 ODS column equilibrated in 10% methanol in water and eluted with an increasing methanol gradient of 0.2%/min.

by the observation that equal amounts of the major in vivo and in vitro adducts co-elute, giving rise to a single sharp peak. Incubation of metabolite b (Figure 2) with microsomes in the presence of DNA generates a single adduct chromatographically indistinguishable from the major adduct derived from the parent compound (III) and eluting slightly ahead of the major adduct derived from compound IB. On the basis of the high level and identity of binding to DNA both in vivo and in vitro, metabolite b is proposed as the proximate tumorigen/mutagen.

Analysis of metabolites
Metabolite b. The u.v. spectra of this metabolite specifically resemble the corresponding spectra of the trans-3,4-dihydrodiol derivative of the 15,16-dihydro-11-methanocyclopenta[a]-phenanthren-17-one (IB) (Figure 4). The hypsochromic shift upon in situ reduction with sodium borohydride indicates an intact, conjugated carbonyl function.

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The mass spectrum (Table V) has an apparent molecular ion at m/z 260.0711 (C_{18}H_{12}O_{2} requires m/z 260.0837). The trans-3,4-dihydrodiol function is clearly identified by other means (vide infra) therefore this ion is believed to arise through facile loss of the elements of water from the true molecular ion, as can be seen to occur with analogous trans-3,4-dihydrodiols leading in each case to the base peak (3,4).
irradiation of this signal causes the signal at constant between the carbinol protons, selective proton spin-decoupling experiments. The coupling metabolite b (Table V) is consistent with the putative incorporation of strain into the molecule.

3,4-dihydrodiol structure, assignments being substantiated by compound the molecular ion or base peak appears exclusive to the title compound (218). Predominant loss of 42 atomic mass units from either significantly smaller than analogous coupling constants presence of the methylene bridge and concomitant introduction of strain into the molecule. Therefore this peak may represent loss of a ketene moiety within the probe of the spectrometer. Subsequent fragmentation of the molecular ion and thermal dehydration expected from breakdown of the unmodified five-membered ring (13). In addition, a major fragment ion occurs at m/z 1488 (204 9.3) 244 17.3 188 17.8 1488

Available evidence does not distinguish between an authentic fragmentation of the molecular ion and thermal dehydration within the probe of the spectrometer. Subsequent fragmentation: m/z 258, [M-H₂O-H₂]⁺; 232 [M-H₂O-CO]⁺ or [M-H₂O-C₅H₄]⁺, and 204, [M-H₂O-C₅H₄-CO]⁺ are those expected from breakdown of the unmodified five-membered ring (13). In addition, a major fragment ion occurs at m/z 218. Predominant loss of 42 atomic mass units from either the molecular ion or base peak appears exclusive to the title compound (III) and certain of its metabolites and is not commonly observed among other cyclopenta[α]phenanthren-17-ones. These lie typically in the range of 10—12 Hz (1A—C) (4) indicating that in each case the hydroxyl functions are in a predominantly quasi-diequatorial conformation. In this instance, however, the average dihedral angle between the hydroxyl groups is larger leading to a loss of hydrogen bonding and a consequent increase in polarity. This is reflected in the smaller elution volumes (reverse-phase h.p.l.c.) of both the metabolite itself and the derived nucleoside adduct as compared to the respective species generated from 15,16-dihydro-11-methycyclopenta[α]phenanthren-17-one (II). It is interesting to speculate that the weak mutagenicity of the title compound (III), mediated via a further derivative of metabolite b, may also reflect the increase in polarity, since the test relies upon the ability of metabolites generated extracellularly to permeate the lipophilic bacterial cell wall prior to expression of mutagenicity.

Metabolite a. Evidence for metabolic epoxidation of the C-1,2 double bond in the title compound (III) follows from identification of metabolite a as a trans-1,2-dihydriodiol derivative. The ultraviolet spectra resemble those reported for a 1,2-dihydriodiol derivative of compound IB (Figure 4). The mass spectrum has a molecular ion at m/z 278.0954 (C₁₅H₁₄O₃ requires 278.0943) and fragment ions consistent found for trans-3,4-dihydrodiols derived from other cyclopenta[α]phenanthren-17-ones.
Table VI. Comparison of the predominant fragmentations observed in the electron impact mass spectra of metabolites c—g and the synthetic compounds, 15-hydroxy and 16-hydroxy-15,16-dihydro-l,l-methanocyclopenta[a]phenanthrene-17-one

<table>
<thead>
<tr>
<th>% Intensity</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>15-OH-(IB)</th>
<th>16-OH-(IB)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M + 1</strong></td>
<td>13</td>
<td>-</td>
<td>21</td>
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<td>19</td>
</tr>
<tr>
<td>M</td>
<td>62</td>
<td>33</td>
<td>100</td>
<td>100</td>
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<td>11</td>
<td>-</td>
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</tr>
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<tr>
<td>M - 16</td>
<td>40</td>
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<td>7</td>
<td>6</td>
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</tr>
<tr>
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<td>7</td>
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<td>14</td>
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</tr>
<tr>
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<td>12</td>
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<td>M - 45</td>
<td>22</td>
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<td>30</td>
<td>-</td>
<td>38</td>
<td>28</td>
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<tr>
<td>M - 47</td>
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<td>21</td>
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<td>3</td>
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<td>M - 56</td>
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<td>6</td>
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<td>26</td>
<td>32</td>
<td>14</td>
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<td>14</td>
</tr>
</tbody>
</table>

Fig. 6. U.v. spectra of metabolites c—g and the parent compound (III). Broken lines refer to spectra of metabolites after in situ reduction with sodium borohydride. The spectrum of metabolite d after treatment with base is also shown (λ_max 262, 310 nm).

with the proposed trans-1,2-dihydrodiol structure (Table IV). The p.m.r. spectrum (Table V) confirms this assignment. In the absence of a benzylic carbinol proton, the conformation of the dihydrodiol system is inferred from a comparison of the other available coupling constants with those reported for trans-3,4-dihydroxy-3,4-dihydrophenanthrene in which the bay region hydroxyl groups adopt a quasi-diaxial conformation (14). The close agreement of the two sets of coupling constants indicates that the hydroxyl groups of metabolite a have the expected quasi-diaxial conformation.

Further metabolism of metabolite b

The implication of a trans-3,4-dihydrodiol as the promutagenic/protumorigenic form of the title compound (III) and identification of a trans-1,2-dihydrodiol derivative suggest that the ultimate form of compound (III) is probably a bay region diol-epoxide by comparison with the established route of activation of the carcinogen IB. This idea is supported by the chromatographic behaviour of the major nucleoside adduct. The elution volume of this species from Sephadex LH20 is reduced specifically by addition of a borate buffer to the eluent (Figure 5). This is consistent with formation of a cyclic borate complex involving a vicinal cis-diol system as would be produced at C-2/C-3 by nucleophilic attack at C-1 of an anti-diol-epoxide (15). Since there is insufficient material to allow direct use of the appropriate spectrometric methods, confirmation of this finding awaits synthesis of the pertinent intermediates.

Other metabolites of 15,16-dihydro-1,11-methanocyclopenta[a]phenanthrene-17-one

U.v. and mass spectral data for metabolites c-g are presented in Figure 6 and Table VI, respectively. The u.v. spectra of metabolites c, e, and f (Figure 6) are closely similar and resemble that of the parent compound (III); these metabolites therefore appear to retain the intact methanophenanthrene system conjugated to a C-17 carbonyl group. After in situ treatment with sodium borohydride the ultraviolet spectra of metabolites e and g become identical and very closely resemble that of similarly reduced metabolite c. Reduction of metabolite f leads to a spectrum resembling that of the reduced parent, i.e., 15,16-dihydro-1,11-methanocyclopenta[a]phenanthrene-17-ol. The observation of bathochromic shift
in the ultraviolet spectrum of metabolite d upon treatment with base suggests the presence of a potentially acidic function.

The mass spectra (Table VI) suggest that metabolites d and g are diketone derivatives of the title compound (III), while e and f are monohydroxylated derivates and c a dihydroxylated derivative. The uncharacteristically long wavelength absorbance and red shift (u.v.) and relatively short retention time (b.p.l.c.) for an alleged diketone suggest that the second carbonyl function of metabolite d may be situated at C-15. These observations may then be rationalised in terms of a preferred enol tautomer. Metabolite g, also a diketone, shows none of the anomalies associated with d. Therefore, the second carbonyl function is assigned to the C-18 position.

On the basis of these deductions, the hydroxyl function in e is assigned to the C-18 position (congruence of reduced u.v. spectra of e and g). The similarity of the reduced spectrum of metabolite c with that of e and g suggests that one hydroxyl group in c is at C-18; the second must therefore be at C-15 or C-16. Further information comes from a comparison of the fragmentation patterns of metabolites c and f with those of synthetic 15- and 16-hydroxy derivatives of the 11-methyl-17-ketone (IB). Loss of the elements of water from the former is a major fragmentation pathway, but this is not so for the latter. By analogy therefore, metabolite c is the 15,18-diol whereas metabolite f is the 16-ol. The same conclusion is reached concerning the latter by consideration of the non-identity of the reduced spectra of metabolites d and f. The proposed structures are summarised in Figure 7.

Discussion

The tumorigenicity of the title compound (III) is not entirely without precedent. Dunlap and Warren (16) found that the corresponding methano-analogue of dimethylbenz[a]anthracene, 1,12-methano-7-methylbenz[a]anthracene, elicited tumours in mice after a single subcutaneous injection in tricaprylin. Furthermore, recent reports have also described metabolic oxidation at substituted aromatic double bonds in both 7-methylbenzo[a]pyrene (17) and 8-methylbenzo[a]-anthracene (18).

The evidence presented strongly implicates a trans-3,4-dihydrodrol-anti-1,2-oxide analogous to structure (II) as the active form of 15,16-dihydro-11,11-methanocyclopenta[a]phenanthren-17-one (III). This result emphasises the role of C-11 substituents in endowing cyclopenta[a]phenanthren-17-

ones with carcinogenic activity and further demonstrates that substitution at an aromatic double bond is not sufficient to prevent metabolic oxidation.

The obstruction to the bay-region provided by the methylene bridge is mainly within the plane of the molecule. It is probable that the active oxygen species is inserted from either above or below this plane in the active site of the enzyme; this would help to explain the inhibitory effect of increasing size and chain length of C-11 substituents which are not confined to this plane. Finally, bay-region dihydrodiol-epoxides of terminal ring methyl-substituted (inactive) cyclopenta[a]phenanthren-17-ones would experience different conformational constraints to those of the title compound (III). Work in progress is exploring the role of such conformational differences in determining carcinogenic potential.

Acknowledgement

This work was presented in part at the 7th International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, 1982.

References

15. King, H.W.S., Osborne, M.R., Beland, F.A., Harvey, R.G. and Brookes, P. (1976), (+)-α,β-Dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene is an intermediate in the metabolism and binding to


(Received on 23 May 1984; accepted on 3 September 1984)
High skin tumour initiating activity of the metabolically derived trans-3,4-dihydro-3,4-diol of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthen-17-one

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(Received on 21 December 1981; accepted on 5 February 1982)

Abstract
Eight main metabolites of the parent carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthe-17-one (I) were assayed for their ability to initiate skin tumours in T.O. mice after topical application in two-stage experiments with croton oil used as the promoter. All were less active than I with the exception of the trans-3,4-dihydro-3,4-diol which was more than ten times as active. This diol is therefore confirmed as the proximate carcinogen, a conclusion reached previously on different evidence.

Introduction
We have previously (1) identified a trans-3,4-dihydro-3,4-diol as the probable proximate form of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanther-17-one (I) (Figure 1), which is itself similar in potency to benzo[a]pyrene in inducing skin tumours in mice (2). Of the eight main metabolites of I isolated after in vitro metabolism with rat liver microsomal preparations (1,3,4) this 3,4-diol reacted the most extensively with DNA; moreover it was the only metabolite to yield the main nucleotide adduct isolated from the DNA of the skin of mice following topical application of I in vivo (1,5). It was also more mutagenic towards Salmonella typhimurium TA100 than the carcinogen itself or any of the other metabolites (1). It was therefore of interest to investigate the skin tumour initiating potential of this compound in comparison with that of the carcinogen I and its other metabolites, employing T.O. mice known to be susceptible to I.

Materials and Methods

Chemicals
Compounds I–IV (Figure 2) were prepared here as previously described (6), and their purity was established by standard methods. 14C-methyl-labelled carcinogen I (7) with a specific activity of 9.34 mCi/mmol was used in the metabolic experiments to facilitate quantitation of the metabolites. Acetone and toluene were Analar grade from Fisons Ltd., Loughborough, and croton oil (95:5 v/v) (400 nmol) and fat were removed from this solution by one extraction with an equal volume of hexane. The clear yellow methanol solution was injected on to a Whatman Partisil M9 10/50 ODS column (Whatman Ltd., Maidstone) fitted to standard Waters Associates h.p.l.c. equipment, and eluted with methanol/water (15:85 v/v) changing to methanol alone in a linear gradient at 1%/min at a flow rate of 120 ml/h. The major metabolites and unchanged ketone (I) were collected separately; they amounted to 73% of the radioactivity used. From a total of 85 μmol of I processed in this manner were obtained the 1,2,15-triol (9.06 μmol, 10.7% yield), 3,4,16-triol (5.2, 6.1%), 3,4,15-triol (2.99, 3.5%), 11-hydroxymethyl-15-ol (3.27, 3.8%), 3,4-diol (2.53, 3.0%), monohydroxy compounds (6.78, 9%), and unchanged I (4.22, 5.0%) after purification by further h.p.l.c. The five polyhydroxy metabolites and recovered carcinogen (I) were dissolved in toluene/acetonitrile (1:1 v/v) at 5 μmol/ml for skin painting.

Skin painting experiments
Randomly bred Theiler's Original (T.O.) Swiss mice from a closed colony maintained in these laboratories were divided by a formal randomisation procedure into groups of 10 mice of either sex for each experiment shown in Tables I–III. Animals were maintained and treated essentially as previously described (8), and as indicated in these tables. Mice were killed when their largest skin tumour reached 1 cm in diameter, or when they died naturally; a few sick animals were killed earlier in order to avoid loss of material. Tumours were examined histologically, and those that involved the panniculus carnosus muscle were classified as carcinomas whilst those which did not were classified as papillomas.

Results and Discussion
As a preliminary to this comparative experiment a dose response study with the carcinogen (I) was undertaken in order to judge the minimum dose of the metabolically derived compounds that would be needed to give a meaningful result.

Fig. 1. The initiating dose of the carcinogen (I) shown was administered in 40 μl of toluene to groups of 20 mice, and this was followed by twice-weekly promotion with toluene/croton oil (99:1 v/v, 10 μl). The curves represent the first appearance of the first skin tumour/mouse in Figures 1–3.
Figure 1 and Table I show the time of first appearance of skin tumours in groups of 20 mice initiated with either 1600, 400, or 200 nmol of I dissolved in toluene, followed by twice-weekly promotion with croton oil. Even at the lowest dose the tumour incidence reached 50% with a mean latent period of 31.4 weeks. It was therefore considered that a dose of about this value would be suitable for the proposed comparison of metabolites.

All three mono-hydroxy derivatives of I [i.e., the 15-ol (II), 16-ol (III), and 11-hydroxymethyl compound (IV)] occur as microsomal metabolites in the approximate ratio 12:1:3 (4). However, these are difficult to separate by h.p.l.c., especially on a large scale as required here. It was therefore decided to test the three synthetic mono-ols (II, III and IV), although it was appreciated that the two former were racemic whereas the corresponding metabolites themselves were probably optically active. Compounds II—IV, together with the parent carcinogen (I), were tested in groups of 20 mice with an initiating dose of 1600 nmol/mouse (Figure 2 and Table II). For solubility reasons the solvent employed was acetone/toluene (1:1 v/v), and as before promotion was carried out twice weekly with croton oil. The initiating potential of the 16-ol (III) (100% tumour incidence, mean latent period 26.6 weeks) was found to be not far short of that of the carcinogen (I) (95%, 21.4 weeks). The two other mono-ols II and IV were less active as demonstrated both by their tumour incidences (40% and 50%) and their longer mean latent periods (45 and 41 weeks, respectively). The use of acetone/toluene (1:1 v/v) in place of toluene alone had the unexpected effect of shortening the mean latent period: 21.4 weeks for 1600 nmol of I in acetone/toluene (Figure 2) compared with 30.3 weeks for the same dose in toluene (Figure 1).

For comparison of the polyhydroxy metabolites it was finally decided to use a dose of 100 nmol/mouse because this required <1 mg of each metabolite, an amount conveniently obtainable from several in vitro metabolic preparations and h.p.l.c. separations. The carcinogen (I) recovered from these preparations was used as a standard, and the solvent was again acetone/toluene followed by twice-weekly promotion as before. The results of this comparison (Figure 3 and Table III) are readily appreciated; the 3,4-diol is highly carcinogenic. Both on the basis of tumour incidence (90%) and mean latent period (24.0 weeks) it is clearly much more active than the parent compound (I) (60%, 33.8 weeks); in fact its activity approaches that of I at 16 times the dose (Figure 2, 95%, 21.4 weeks). By contrast none of the other polyhydroxy metabolites is as active as I. The 3,4,16-triol produced five skin tumours (one regressed) with a mean latent period of 53.6 weeks, while the 3,4,15-triol gave two papillomas (66 weeks) and the 11,15-diol only one (38 weeks).

It is therefore clear that all the metabolites of the carcino-

![Figure 2. First appearance of skin tumours after an initiating dose of 1600 nmol of compounds I—III in 40 µl toluene/aceton (1:1 v/v) followed by twice-weekly promotion with toluene/croton oil (99:1 v/v, 10 µl).](image)

### Table I. Dose response in the two-stage system with the carcinogen (I).

<table>
<thead>
<tr>
<th>Initiating dose (nmol)</th>
<th>No. of tumourless survivors at (months)</th>
<th>No. of squamous papillomas</th>
<th>Total no. of mice with dorsal tumours</th>
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</thead>
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<tr>
<td></td>
<td>6 12 18 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>12 5 1 0</td>
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<td>3</td>
</tr>
<tr>
<td>400</td>
<td>14 8 3 1</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>200</td>
<td>19 12 5 2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Croton oil control</td>
<td>19 18 15 5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Toluene control</td>
<td>20 18 16 7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The initiating dose in each case was administered to the shaved dorsal skin in 40 μl of toluene. Twice-weekly promotion was begun one week later using 10 µl of toluene/croton oil (99:1 v/v)/mouse. The initiating dose of carcinogen was omitted with the croton oil control mice, and both carcinogen and promoter were omitted with the toluene control animals.

### Table II. Skin tumour initiation with the ketone (I) and three synthetic monohydroxy derivatives (II—IV).

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of tumourless survivors at (months)</th>
<th>No. of mice with squamous carcinomas</th>
<th>Total no. of mice with dorsal tumours</th>
<th>Mean latent period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 12 18 24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketone (I)</td>
<td>5 0 0 0</td>
<td>13</td>
<td>19</td>
<td>21.4</td>
</tr>
<tr>
<td>15-ol (II)</td>
<td>20 17 10 3</td>
<td>6</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>16-ol (III)</td>
<td>9 0 0 0</td>
<td>11</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>11-CH₃OH (IV)</td>
<td>17 13 5 2</td>
<td>6</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>Toluene/aceton control</td>
<td>19 19 11 4</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

aThe initiating dose in each case was 1600 nmol administered once in 40 μl of toluene/aceton (1:1 v/v). Twice-weekly promotion was begun one week later using 10 µl of toluene/croton oil (99:1 v/v) for each mouse. Control mice received 40 μl of toluene/aceton initially. b Three tumours were unavailable for histology. c One tumour was unavailable for histology, and one tumour (2 mm) regressed.
gen (I) are less active than the parent compound with the exception of the \textit{trans}-3,4-dihydro-3,4-diol, which at a conservative estimate is >10 times as active. Thus, this diol is confirmed as the proximate carcinogen, a conclusion reached earlier on different grounds (1). The ultimate carcinogen, the \textit{anti}-3,4-dihydro-3,4-trans-dihydroxy-1,2-dihydro-1,2-ep oxide has not been isolated, but its structure has also been previously suggested (1), and recently confirmed by a mass spectral examination of the adduct it forms with DNA (9). The carcinogen 15,16-dihydro-11-methylcyclopenta[a]-phenanthrene-17-one (I) is therefore metabolically activated via a bay-region diol epoxide, like a number of related hydrocarbons (10) including those with a methyl group at an analogous position in the bay (11–13).

Table III. Skin tumour initiation with the ketone (I) and its main microsomal metabolites.

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of tumourless survivors at (months)</th>
<th>No. of mice with squamous papillomas</th>
<th>No. of mice with carcinomas</th>
<th>Total no. of mice with dorsal tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,15-triol</td>
<td>6</td>
<td>12</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>3,4,16-triol</td>
<td>20</td>
<td>19</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>3,4,15-triol</td>
<td>20</td>
<td>18</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>11-hydroxy-15-methyl-15-diol</td>
<td>20</td>
<td>19</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>3,4-diol</td>
<td>6</td>
<td>1</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>recovered</td>
<td>17</td>
<td>8</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>6</td>
<td>12b</td>
</tr>
</tbody>
</table>

The initiating dose in each case was 100 nmol/mouse administered once in 20 \(\mu l\) of toluene/acetone (1:1 v/v). Twice weekly promotion was begun one week later using 10 \(\mu l\) of toluene/acetone oil (99:1 v/v) for each mouse.

Tumours were unavailable for histology (because death occurred and autolysis was too far advanced) as follows: 3,4,16-triol; 1; 11-hydroxy-15-methyl-15-diol; 1; 3,4-diol; 3; 1; 6; *One tumour regressed after it had reached 3 mm diam.

References

DNA adducts of the carcinogen, 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one, in vivo and in vitro: high pressure liquid chromatographic separation and partial characterization

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(Received on 24 March 1981; accepted on 15 April 1981)

Abstract
The 11-methyl derivative (11-methyl ketone) is the most carcinogenic of the series of methylated derivatives based on 15,16-dihydro-cyclopenta[a]phenanthren-17-one. The nucleoside adducts derived from [3H]-11-methyl ketone-modified mouse skin DNA have been separated by both Sephadex LH-20 chromatography and reverse-phase h.p.l.c. and compared to those derived from DNA modified in vitro with the [14C]-11-methyl ketone using rat liver microsomes. The in vivo modified DNA separated to give 6 adducts (designated I–VI) on h.p.l.c. The major in vivo adduct (80% total adducts) co-chromatographed with the major in vitro adduct. The metabolites of the 11-methyl ketone (designated a–g) have been separated by h.p.l.c., and the adducts derived from each of these individual metabolites determined by further metabolism in the presence of DNA. H.p.l.c. separation of these adducts has allowed characterization of the in vivo adducts. The major adduct (V) and possibly one of the minor adducts (IV) were derived from the 3,4-dihydro-3,4-diol of the 11-methyl ketone (metabolite c). Adducts II and III were derived from the 16- and 15-monohydroxylated derivatives of the 11-methyl ketone and also from their corresponding 3,4-diols and therefore are likely to be the 16- and 15-hydroxy derivatives, respectively, of adduct V. Adduct VI, however, although derived from the 15-hydroxy-3,4-diol had a late retention time on h.p.l.c., suggesting either a non-diol-epoxide adduct or a deoxyadenosine adduct. The use of [3H-G]-DNA has established that the major adduct (V) and the 16-hydroxy-derived adduct (II) contain deoxyguanosine. Reaction of the carcinogen with [3H-G]-DNA showed that the major adduct peak and DNA, therefore, is important for a fuller understanding of their mode of action. The compound, 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (11-methyl ketone*, Figure 1, structure II) is the most carcinogenic member of the series of mono-methylated derivatives based on the compound, 15,16-dihydro-cyclopenta[a]phenanthren-17-one (Figure 1, structure I). The series contains compounds with a gradation of carcinogenic effectiveness resulting only from altering the position of the methyl group (1). The 11-methyl ketone is carcinogenic on mouse skin after topical application of a low dose or after i.m. injection of a higher dose (2). It is of equivalent carcinogenicity to benzo[a]pyrene on topical application to mouse skin (2) and is mutagenic to Salmonella typhimurium TA 100 (3). It is activated via a vicinal diol-epoxide in the bay-region (4) although hydroxylation can also occur in the 11-, 15- and 16-positions of the molecule (5). A covalent bond with DNA is formed both in vitro after metabolic activation (6) and in vivo on mouse skin (4). Partial separation of the DNA adducts has been achieved on Sephadex LH-20 allowing quantitation of the extent of total carcinogen binding (4, 6).

In this paper, we present the separation by h.p.l.c. of the carcinogen—DNA adducts formed both in vivo and in vitro. The pattern of adducts formed in vitro, using rat liver microsomes, resembles that found in vivo from mouse skin. By analysis of the DNA adducts formed from individual metabolites of the 11-methyl ketone the origin of specific adducts has been elucidated and a better understanding of the metabolism of the carcinogen has been achieved. The major adducts formed in vivo involve covalent attachment of the carcinogen to either deoxyguanosine or deoxyadenosine.

*Abbreviations: 11-methyl ketone, 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one; poly (dA-dT), alternating co-polymer of deoxyadenylc and deoxythymidylc acids.
Materials and Methods

Radiochemicals and enzymes

The 11-methyl-14C-labelled carcinogen (Figure 1; 9.3 mCi/mmol) was prepared in these laboratories (7). General tritium labelling of the carcinogen (4.63 Ci/mmol) was performed by the Radiochemical Centre, Amersham, UK, followed by purification in these laboratories (8). Tritium-labelled deoxynucleoside triphosphates were obtained from the Radiochemical Centre. Specific base-labelled DNA was prepared according to Kelly et al. (9), using the appropriate tritium-labelled triphosphates and E. coli DNA polymerase I.

The [3H-A]-poly(dA-dT) was prepared according to Schachman et al. (10), using [3H]dATP, dTTP and E. coli DNA polymerase I.

Pancreatic DNase I (type I, E.C.3.1.45), snake venom phosphodiesterase (type II, E.C.3.1.4.1), E. coli alkaline phosphatase (type II, E.C.3.1.3.1), and pancreatic RNase (type III-A, E.C.3.1.3.22) were obtained from Sigma, London, UK. E. coli DNA polymerase I (E.C.2.7.7.7) was obtained from Boehringer, Lewes, UK.

In vitro metabolism and separation of metabolites

The general procedure for the isolation of metabolites has been described previously (4, 5). For the large scale preparation of metabolites required in these experiments, the following incubation mixture was used: 10 μmol [14C]-11-methyl ketone, 125 mg NADPH, microsomes from 40 g rat liver in 0.1 M Tris-HCl, pH 7.2, in a total volume of 25 ml. The mixture was incubated at 37°C for 30 min before ethyl acetate extraction and separation of the metabolites on a Whatman Partisil M9 10/50 ODS column (Whatman Ltd., Maidstone, UK) using a Waters h.p.l.c. system (Waters Associates, MA, USA).

In vitro reaction of 11-methyl ketone and its metabolites with DNA

The preparation of microsomes and their use in in vitro studies on the binding of the 11-methyl ketone to DNA have been previously described (6). A normal reaction mixture contained the following: 2 mg labelled or unlabelled DNA, 0.4 μmol [14C]-11-methyl ketone, or one of its metabolites in DMSO, microsomes from 2 g rat liver, 6 mg NADPH, in total volume of 2 ml of 0.1 M Tris-HCl, pH 7.2, 0.04% agar. Incubation was carried out at 37°C for 1–2 h before extraction with chloroform-saturated 0.1 M Tris-phenol, pH 7.2. The sample was dialysed extensively before enzymatic hydrolysis (Method 1).

In the analysis of DNA bases associated with the adducts, the labelled DNA was contained in a small dialysis sac which was placed in the flask containing the microsomal enzymes and the carcinogen. Following incubation, the dialysis sac was transferred to a large flask of distilled water for several hours before enzymatic hydrolysis (Method 2).

[3H-A]-Poly(dA-dT), after modification with the 11-methyl ketone by Method 1 was separated from any endogenous DNA associated with the microsomes by CsCl density gradient centrifugation. Solutions of CsCl containing modified [3H-A]poly(dA-dT) were prepared in 0.01 M potassium phosphate buffer, pH 7.4, to a density of 1.600 g/cm³. Centrifugation was carried out at 45,000 r.p.m. in a MSE Superspeed 65 centrifuge for 40 h at 25°C and the position of the poly(dA-dT) was monitored by both acid-insoluble radioactivity and absorbance at 254 nm. Poly(dA-dT)-containing fractions were collected, dialysed and enzymatically hydrolysed.

Carcinogen treatment of mouse skin and isolation of DNA

Male T.O. Mice (Theiler’s Original, derived from Swiss), 7 – 9 weeks old were used in groups of 10. For the analysis of carcinogen – DNA adducts in mouse skin, the dorsal skin of the mice was shaved with electric clippers (approx. area, 2.5 cm x 2.5 cm) and [G-3H]-11-methyl ketone (4.63 Ci/mmol, 400 μg in 40 μl toluene) applied to the shaved area of each mouse (2, 4). Three days after treatment, the shaved section of dorsal skin was removed and frozen in liquid nitrogen.

Frozen tissue was homogenized in cold 5% p-aminosalicylic acid, 1% NaCl using a Polytron P-20 (Northern Media Supplies, N. Humberside, UK), and DNA isolated by repeated extraction with phenol mixture (500 g phenol, 70 g m-cresol, 0.5 g 8 hydroxyquinoline, 50 ml water) followed by exhaustive dialysis. The dialysate was reduced to 5 ml, made 0.4% in sodium acetate, 1 mg heat-treated RNase added, and incubated at 37°C to remove contaminating RNA. After 2 h, the mixture was again extracted with phenol mixture, followed by dialysis before enzymatic hydrolysis.

Enzymatic hydrolysis and Sephadex LH-20 chromatography

Modified DNA was hydrolysed in 0.01 M Tris-HCl buffer, pH 7.2, 0.01 M MgCl₂, with 1000 units of pancreatic DNase I for 2 h at 37°C, followed by hydrolysis in 0.1 M Tris HCl, pH 9.0, with 0.05 units of snake venom phosphodiesterase and 10 units of alkaline phosphatase for 16 h. Carcinogen – DNA adduct separation was essentially according to the method of Baird and Brookes (11). The hydrolysed DNA solution was made 20% in methanol and applied to a Sephadex LH-20 column (90 x 1.5 cm) (Pharmacia, Sweden). The column was eluted with a gradient of 20% to 100% methanol and 5 ml fractions collected. The positions of the four deoxynucleosides, which elute early, were monitored at 254 nm while the carcinogen adducts, eluting after approximately 40 fractions, were monitored by sampling the fractions for radioactivity. The amount of DNA hydrolysed was determined from the absorbance of the deoxyadenosine peak which is the last deoxynucleoside to elute. Fractions corresponding to carcinogen – DNA adducts were pooled and reduced in volume before further analysis by h.p.l.c.

H.p.l.c. separation of carcinogen – DNA adducts

Analysis of the carcinogen – DNA adducts by analytical h.p.l.c. was performed on Waters Associates
DNA adducts of 15,16-dihydro-11-methylcyclo-penta[a]phenanthren-17-one

(MA, USA) equipment fitted with a Partisil 10/25 ODS column (25 cm x 4.6 mm) from Whatman Ltd., (Maidstone, UK). The column was equilibrated in 10% methanol–water and elution of adducts at a constant flow rate of 1 ml/min was achieved with an increasing methanol gradient (0.2%/min). One ml fractions were collected directly into scintillation vials, 5 ml of Aquasol scintillation fluid (New England Nuclear, MA, USA) added and the radioactivity determined in a LKB 1215 Rackbeta liquid scintillation counter.

Results and Discussion

The carcinogenicity of 11-methyl ketone on mouse skin has been well established. It is effective by single dose followed by croton oil promotion as well as by repeated administration and appears to have equivalent potency to the ubiquitous carcinogen, benzo[a]pyrene (2). Although binding of this carcinogen to DNA has been reported previously, a more detailed analysis of individual adducts separated by both Sephadex LH-20 and h.p.l.c. has now been achieved.

Sephadex LH-20 separation and analysis of radioactive peaks

The separation of the DNA adducts of the carcinogen, 11-methyl ketone, on Sephadex LH-20 has been described previously (4, 6). In vitro, two peaks of radioactivity corresponding to carcinogen–nucleotide adducts were consistently eluted following the four normal deoxynucleosides (peaks A and B, Figure 2). In some cases, a third radioactive peak (peak C, Figure 2) was found when the DNA was directly in contact with the microsome mixture (Method I). When the DNA was separated by a dialysis sac (Method 2), this peak was never present. In vivo, peak C has never been found and peak A was greatly reduced (4). These peaks have been collected and further analysed by h.p.l.c. (see later).

Three other peaks of radioactivity which eluted before the normal deoxynucleosides have been observed after reaction of the carcinogen with DNA both in vivo and in vitro. Early eluting peaks have been reported in the analysis of several hydrocarbons (11, 12, 13, 14, 15). The first peak, eluting after approximately 45 ml, was observed only in the case of incomplete enzymatic hydrolysis and represents modified polymer or nucleoside monophosphates. The second, eluting after approximately 70 ml, contained only a very small amount of u.v. absorbing material and co-eluted with small oligonucleotide markers of 2-4 nucleotides which lack a terminal phosphate. The radioactive counts may be derived from intercalated carcinogen or from carcinogen-bound protein associated with these oligomers. The third peak of radioactivity, the X peak (Figure 2) eluting after approximately 90 ml, contained a sharp peak of u.v. absorbing material and a variable but high proportion of radioactive counts. This material was resistant to further hydrolysis by either phosphodiesterase or proteinase K and was completely dialysable. The u.v. spectra suggested that it contained largely thymidine which was confirmed by the use of 3H label.

Fig. 2. Sephadex LH-20 profile of in vitro modified calf thymus DNA treated with [14C]-11-methyl ketone __________, radioactivity in each of the fractions; ............., u.v. absorption by continuous monitoring. The positions of the X peak and the four deoxynucleosides as well as the adduct peaks A, B and C are shown.

Fig. 3. H.p.l.c. profile of X peak of mouse skin DNA. Separation was on a reverse-phase Partisil 10/25 ODS column, eluted with 10% methanol–water. The position of the normal deoxynucleosides is shown.

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specific base-labelled DNA. The peak was further analysed on reverse-phase h.p.l.c. using a Partisil 10/25 ODS (25 cm x 4.6 mm) column which was eluted with 10% methanol–water. The X peak derived from carcinogen-modified mouse skin DNA separated into three components as shown in Figure 3. The major peak (retention time 7.4 min) co-eluted with thymidine, while the second largest (retention time 5.4 min) co-eluted with deoxycytidine. The radioactivity eluted as a diffuse band not associated with any of the u.v. peaks (Figure 3). This is consistent with the fact that the X peak can be observed after hydrolysis of normal DNA which has not been modified with carcinogen. The early elution of the thymidine and deoxycytidine appears to be associated with a specific interaction with the Tris buffer used in the hydrolysis of the DNA. Separate chromatographic analysis of thymidine in 0.1 M Tris buffer, pH 9, led to the appearance of an X peak. Similar chromatographic analysis in the absence of Tris buffer or in the presence of MgCl₂ did not produce an X peak. The radioactivity associated with this peak may be non-covalently bound carcinogen co-eluting at the same position. The possibility of non-covalent interaction of the 11-methyl

Fig. 4. H.p.l.c. profile of radioactive 11-methyl ketone–DNA adducts derived from mouse skin. Separation was on a reverse-phase Partisil 10/25 ODS column eluted with an aqueous methanol gradient. The relative percentage of each of the peaks is shown.

Fig. 5. H.p.l.c. profile of radioactive 11-methyl ketone–DNA adducts derived in vitro using rat liver microsomes. Separation was on a reverse-phase Partisil 10/25 ODS column eluted with an aqueous methanol gradient. A. DNA free in reaction mixture; B. DNA enclosed in a dialysis sac. Co-chromatography with in vivo-derived adducts has allowed the assignment of the peak numbers shown.

Fig. 6. H.p.l.c. profile of 11-methyl ketone metabolites derived in vitro using rat liver microsomes. u.v. absorption at 254 nm. Separation was on a reverse-phase Partisil M9 10/50 ODS column eluted with an aqueous methanol gradient.
DNA adducts of 15,16-dihydro-11-methylcyclo-penta[a]phenanthren-17-one

ketone with DNA was tested by the incubation of [14C]-11-methyl ketone in the absence of microsomes. After phenol extraction of the DNA, enzymatic hydrolysis and Sephadex LH-20 chromatography, as described in Methods, radioactive counts were found under the X peak only. Although the radioactive counts in peak X were not as high as would be found after metabolic activation, non-covalent interaction with the DNA does take place. These observations suggest that the early eluting peaks contain only a very minor amount, if any, of covalently bound carcinogen. Our major effort, therefore, has concentrated on the separation and identification of the adduct peaks eluting after the normal deoxynucleosides on Sephadex LH-20.

In vivo, a small amount of metabolic incorporation of tritium was found in the normal deoxynucleosides, especially deoxyadenosine and deoxyguanosine. No such incorporation was found using 14C-labelled carcinogen.

H.p.l.c. analysis of adducts

Figure 4 shows the h.p.l.c. pattern of adducts derived from the DNA of carcinogen-treated mouse skin. The two peaks derived from Sephadex LH-20
Fig. 8. H.p.l.c. profiles of [14C]-11-methyl ketone-DNA adducts derived in vitro from [3H-G]DNA using Method 2 (see Methods) and of [14C]-11-methyl ketone-DNA adducts derived in vitro from [3H-A]poly(dA-dT) using Method 1 (see Methods). •, 14C d.p.m.; ○, 3H d.p.m.

chromatography each separated into several adducts. Peak A gave rise to peaks I, II and III (retention times 54, 68 and 75 min) on h.p.l.c., while peak B separated to peaks, IV, V and VI (92, 105 and 115 min). The h.p.l.c. pattern of adducts derived from DNA treated in vitro with carcinogen in the presence of rat liver microsomes is shown in Figure 5A. Figure 5B shows the pattern of adducts derived from DNA which had been separated from the microsomes by a dialysis sac (Method 2, see Methods); both peaks IV and VI have been greatly reduced or eliminated. Generally the pattern of adducts for in vitro modified DNA is essentially the same as for in vivo modified DNA, with the addition of some very minor adduct peaks. The major adducts from both in vivo and in vitro modified DNA have been shown to co‐chromatograph.

Adducts derived from carcinogen metabolites

The origin of the individual adduct peaks was determined by incubating each of the metabolites derived from the 11-methyl ketone (Figure 6) with DNA in the presence of rat liver microsomes. Metabolite peak g has been previously shown to co‐chromatograph with an authentic marker compound, the 16-hydroxy-11-methyl ketone (5), while metabolite peak f co‐chromatographs with both marker compounds, the 15-hydroxy-11-methyl and the 11-hydroxymethyl ketones (5). Metabolite e has been shown by u.v. and mass spectrometry to be the 3,4-diol of the 11-methyl ketone (4). As previously observed only metabolites b, c, e, f and g covalently bind to DNA upon re-incubation in the presence of microsomes (4). H.p.l.c. of the DNA adducts derived separately from each of these metabolites is shown in Figure 7. In each case, the major adducts co-chromatograph with adducts derived from in vivo carcinogen-modified DNA.

Metabolite e, the 3,4-dihydroxy-11-methyl ketone, gave rise to an adduct with a retention time of 105 min which corresponded to the major DNA adduct derived from the 11-methyl ketone (4) both in vivo (Figure 4) and in vitro (Figure 5). A small adduct peak with a retention time of 92 min corresponded to peak IV found in vivo (Figure 4) and appears to be derived only from this 3,4 diol (e). Metabolite g, the 16-hydroxy-11-methyl ketone, and metabolite b both gave rise to an adduct with retention time 68 min, corresponding to peak II in vivo (Figure 4). Metabolite b is known to be a 3,4,16-triol (5) and therefore is most likely to be the 3,4,16-trihydroxy-11-methyl ketone. Similarly, metabolites f and c both gave rise to peaks with retention times of approximately 30 min, 75 min and 115 min (Figure 7). Metabolite f is known to consist of the 15-hydroxy-11-methyl ketone mixed with a small proportion of its 11-hydroxymethyl isomer (5). Metabolite c is a 3,4,15-triol (5) and, therefore, likely to be the 3,4,15-trihydroxy-11-methyl ketone. The structure of the adduct in the first peak (retention time, 30 min; Figure 7) is unknown but chromatographic behaviour suggests a higher degree of hydroxylation; it is not found in vivo.
DNA adducts of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one

Fig. 9. Proposed metabolic pathways of the carcinogen, 11-methyl ketone, leading to the metabolites shown. Upon further metabolism in the presence of DNA, the individual metabolites gave rise to adducts which correspond to the in vivo-derived adducts shown.

The second peak (retention time, 75 min; Figure 7) is probably derived from the 3,4,15-hydroxy-1,2-epoxide and corresponds to peak III after in vivo reaction of the 11-methyl ketone (Figure 4). The third peak (retention time, 115 min; Figure 7) is unexpected since it cannot be a deoxyguanosine adduct derived from the 15-hydroxy-diol-epoxide due to its late retention time. It is retained longer than the adduct derived from the metabolite e (3,4-diol). Two explanations are possible: firstly, it could be derived from a non-diol-epoxide leading to an adduct containing 3 or less hydroxyl groups in its polycyclic moiety; or secondly, it could represent a deoxyadenosine adduct, which are known to elute more slowly from reverse-phase h.p.l.c. columns (16). Base analysis of the adducts supports the latter explanation (see later). Figure 9 shows the metabolic pathways which lead to the formation of the adducts described.

Base analysis of adducts

The analysis of the bases associated with the major adducts was attempted by employing specific base labelled DNA or polynucleotides. The use of labelled DNA in direct contact with the microsomal mixture made interpretation of the data difficult, but by placing the labelled DNA within a dialysis sac floating in the microsomal mixture (see Methods) identification of the guanine adducts could be achieved (Figure 8). The major adduct (peak V) and the 16-hydroxylated adduct (peak II) were both found to contain a guanine base (Figure 8). This procedure, however, as noted above, leads to the loss of peaks IV and VI and identification of the bases associated with these peaks could not be achieved in this way. No meaningful information could be obtained using [H-adenine]DNA or [H-cytosine]DNA owing to the presence of endogenous DNA within the microsomal preparation leading to contamination. The lower buoyant density of [H-A]poly(dA-dT), however, allowed separation of this polymer from contaminating DNA by CsCl density gradient centrifugation. Subsequent hydrolysis and separation of adducts by h.p.l.c. led to the identification of a deoxyadenosine-containing adduct peak (Figure 8) corresponding to peak VI from in vivo modified DNA (Figure 4). Minor contamination of the polymer by DNA was seen by a minor peak at retention time 105 min, the position of the major guanine adduct. The bases associated with the mouse skin DNA adduct peaks I, III and IV remain unknown.

Conclusion

The carcinogen, 11-methyl ketone, reacts with mouse skin DNA in vivo to produce six distinct adduct peaks which can be separated by h.p.l.c. Figure 9 shows the probable metabolic pathways which lead to the formation of these adducts. The major pathway is via a 3,4-diol which leads to both the major in vivo and in vitro adducts, through the formation of a 3,4-dihydroxy-1,2-epoxide (4). Metabolic activation through a vicinal diol-epoxide appears to be a common pathway for a number of carcinogenic polycyclic compounds (15, 17, 18, 19, 20, 21). Hydroxylation of the 11-methyl ketone at either the 11-, 15- or 16-positions in addition to the formation of the 3,4-diol leads to the formation of the minor adducts, peaks II and III, which represent approximately 4 and 1%, respectively, of the total in vivo adducts. At least 2 of the adducts, the major adduct (peak V) and the 16-hydroxy adduct (peak II) have been shown to contain deoxyguanosine, while the last adduct to elute (peak VI) contains deoxyadenosine. This adduct, which represents approximately 9% of the total in vivo adducts, is derived from the 15-hydroxy-3,4-diol. No corresponding deoxyadenosine adduct derived from the 3,4-diol has been found. The impor-
tance of these adducts in carcinogenic transformation has yet to be resolved.

Acknowledgement
The authors wish to thank Miss Frances Crew for excellent technical assistance.

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Covalent binding of polycyclic aromatic compounds to mitochondrial and nuclear DNA

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Since the pioneering work of the Millers1 it has become clear that most chemical carcinogens require metabolism to reactive electrophiles and then exhibit their carcinogenic potential by reacting chemically with, and modifying, cellular macromolecules. At first modification of proteins was considered most likely to be of importance in carcinogenesis. Later, Brookes and Lawley2 demonstrated that the extent of binding of several polycyclic hydrocarbons to DNA, but not to RNA or protein isolated from the skin of mice treated topically with these compounds, correlated with their known carcinogenic potency to this tissue. Mammalian cells, particularly mouse embryo cells, treated with chemical carcinogens have often been used, and DNA has been involved almost exclusively from whole cells. However, mitochondria possess unique DNA which accounts for 0.1-1% of the total DNA present in mammalian cells3, and three studies have shown that carcinogenic alkylating agents modify the mitochondrial DNA by a factor about five times greater than the nuclear DNA from the same cells4-6. We demonstrate here that with six polycyclic aromatic compounds, all of which require metabolic activation and bind to DNA to a much smaller extent than direct-acting alkylating agents, the binding to mitochondrial relative to DNA is dramatically increased by a factor of nearly 50 to over 500.

Whole mouse embryo cells in their third passage from specific pathogen-free Tyler's Original (TO) mice were labelled in culture with [2-14C]thymidine (60 mCi mmol-1) and grown to confluency. Tritium-labelled polycyclic compounds (0.16-1.25 μg ml-1, depending on toxicity) were added as shown in Table 1 and 24-48 h later the cells were collected, washed, and gently disrupted mechanically by hand using a Potter-Elvehjem homogenizer. Nuclei and mitochondria were isolated by the methods of Prescott et al.7 and Miyaki et al.8, respectively, and their purity was checked by light and electron microscopy. Nuclear DNA was extracted using Diamond's modification of the Kirby method9 which involves isolation of the DNA from SDS lysates by phenol extraction, subsequent treatment with RNase, and a second isolation by phenol extraction. Mitochondrial DNA was obtained basically by the method of Hill10,11 because mitochondrial DNA was lost during phenol extraction, the latter step and RNase treatment were omitted. The resultant solution of mitochondrial DNA therefore still contained mitochondrial RNA and residual protein which were also labelled with tritium through binding to the hydrocarbons to these macromolecules.

The amount of nuclear DNA (usually 1-2 mg) was determined by UV spectrophotometry, having calibrated this by phosphoros analysis using the colorimetric method of King and Wotton14. Mitochondrial DNA was estimated, following enzymatic hydrolysis and separation of the deoxynucleosides, by relating the 14C d.p.m. in the thymidine peak to the specific 14C activity of the nuclear DNA, and was found to fall into the expected range (about 2 μg). On one occasion, however, a contamination by direct estimation of the mitochondrial DNA by the fluorimetric method of Hill and Whatley13 using mithramycin, thus establishing that the 14C specific activities of the nuclear and mitochondrial DNA were similar. After dilution with carrier calf thymus DNA, both samples were hydrolysed enzymatically with DNase which had been carefully checked for absence of RNase activity, and the liberated deoxyribonucleosides were separated by Sephadex LH20 chromatography (Fig. 1). The binding index (μmol of compound bound per μmol of DNA phosphorous) was calculated from the total amount of tritium in the modified deoxyribonucleoside peaks which elute after the natural deoxyribonucleosides, assuming the specific activity of the bound compound was unaltered. As Phillips et al.15 have shown, residual protein and RNA do not interfere with this separation because they are eluted in the first 100 ml, immediately before the natural nucleosides. Six polycyclic aromatic compounds were examined in this way, the results of which are summarized in Table 1.

All six compounds modified both nuclear and mitochondrial DNA, and in each case the binding index for the latter was considerably higher than for the former. Although experimental difficulties made the precision of the values rather uncertain, the large differences observed cannot be explained by the small deviations in purine and pyrimidine base ratios known to exist between mammalian nuclear and mitochondrial DNA. These differences in binding index may occur because both the mitochondrial inner16 and outer17 membranes contain the enzymes required to metabolize polycyclic aromatic compounds which probably tend to accumulate in these lipophilic membranes18. Furthermore, mitochondrial DNA is 'naked', being devoid of the histone and non-histone proteins that are intimately associated with DNA in the nucleus. The highest DNA-binding indices, both nuclear and mitochondrial, are shown by the two most potent carcinogens: 7,12-dimethylbenz[a]anthracene (DMBA) and 3-methylcholanthrene (MCA). Benzo[a]pyrene and 1,5,6-dihydro-11-methylcyclopenta[a]phenanthren-17-one (11-MeCPP) are similar to each other in potencies19, but are less active as carcinogens than DMBA and MCA; benz[a]anthracene is an extremely weak carcinogen whereas 12-MeCPP is inactive18. The nuclear DNA-binding indices of these last four compounds do not reflect where they are bound, but there is good correspondence with the mitochondrial binding indices. In all cases, the nucleoside adducts seen in the nuclear DNA hydrolysates are also present in the hydrolysates of the mito-

Fig. 1 LH20 chromatography of hydrolysates of nuclear (a) and mitochondrial (b) DNA from mouse cells treated in culture with benzo[a]pyrene. Hydrolysates were run on Sephadex LH20 columns of 90 x 1.5 cm with a linear gradient of 30% methanol in water increasing to 100% methanol. Fractions (5 ml) were collected and aliquots (1.5 ml) were counted for 14C and 3H activities in a LKB Wallac 1215 Rackbeta liquid scintillation counter. The amounts of the nucleosides were corrected for the efficiency of the hydrolysis, calculated from the total 14C in the DNA hydrolysed and the 14C in the thymidine peak. The large amount of tritium eluted in the first 100 ml of the mitochondrial DNA hydrolysate arises from the tritiated hydrocarbon bound to the RNA and residual protein. ———, UV absorption at 254 nm; ⋅ ⋅ ⋅ ⋅ ⋅ ⋅ ⋅, 3H d.p.m.; — — — — —, 14C d.p.m.
Table 1 Binding of polycyclic aromatic compounds to nuclear and mitochondrial DNA

<table>
<thead>
<tr>
<th>Compound</th>
<th>Specific activity (Ci mmol⁻¹)</th>
<th>Dose (µg ml⁻¹)</th>
<th>Incubation time (h)</th>
<th>Binding index (µmol of compound bound per mol DNA phosphorous)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear DNA (nuc)</td>
</tr>
<tr>
<td>DMBA</td>
<td>19.6</td>
<td>0.212</td>
<td>24</td>
<td>12.1±0.2</td>
</tr>
<tr>
<td>MCA</td>
<td>9.0</td>
<td>0.496</td>
<td>24</td>
<td>21.9±0.3</td>
</tr>
<tr>
<td>B[α]P</td>
<td>18.0, 27.0</td>
<td>0.235</td>
<td>24</td>
<td>7.5±1.9</td>
</tr>
<tr>
<td>11-MeCPP</td>
<td>13.9</td>
<td>1.250</td>
<td>48</td>
<td>0.53</td>
</tr>
<tr>
<td>B[α]A</td>
<td>5.9</td>
<td>0.644</td>
<td>24</td>
<td>0.44±0.11</td>
</tr>
<tr>
<td>12-MeCPP</td>
<td>27.5</td>
<td>1.250</td>
<td>48</td>
<td>0.28±0.03</td>
</tr>
</tbody>
</table>

All compounds were generally labelled with tritium. DMBA, 7,12-dimethylbenz[a]anthracene; MCA, 3-methylcholanthrene; B[α]P, benzo[α]pyrene; 11-MeCPP, 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one; B[α]A, benz[a]anthracene; 12-MeCPP, 15,16-dihydro-12-methylcyclopenta[a]phenanthren-17-one.

* Duplicate experiments ±s.d.
† Single experiment.

amounts of slower-running adducts. In the case of mitochondrial DNA, but in addition the latter also exhibit substantial damage caused by this modification will also be in this ratio, because the efficiency of DNA repair may be different at these two sites. However, mitochondrial DNA codes for several essential subunits of ATPase, cytochrome bc, and cytochrome c oxidase, and it is therefore interesting that derangements of energy metabolism often characterize tumours (see ref. 22). Recently Wilkie et al. found that most carcinogens they tested caused the cytoplasmic 'petite' mutation in the yeast Saccharomyces cerevisiae. Moreover, effects on the cell surface were observed in non-fermentable conditions where mitochondrial function is required for growth. These authors suggest a possible role for mitochondrial mutations in cancer through a heritable disturbance either directly or by modulation of a nuclear gene.
Mutagenic and Carcinogenic Metabolites of the Carcinogen 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one

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ABSTRACT

Microsomal metabolites of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (Structure I) were separated by high-pressure liquid chromatography, and their structures were established on the basis of their ultraviolet and mass spectra, together with considerations of their general chemical properties. This was assisted by comparisons with metabolites formed in the same way from the synthetic 15-hydroxy (Structure II), 16-hydroxy (Structure II), and 11-hydroxymethyl (Structure IV) derivatives, which themselves occur as metabolites of Structure I. Products derived from attack at the two benzo-ring double bonds occurred, but no K-region products were found. Only metabolites having a non-bay region 3,4-dihydriodiol system were mutagenic and bound to DNA after in vitro microsomal activation, and it was concluded that the 3,4-dihydriodiol system was most mutagenic and bound to DNA after in vitro microsomal activation, and it was concluded that the 3,4-dihydro-3,4-diol (Metabolite e) was the main form and that the 3,4,5-diols of the monools (Structures II to IV) were minor proximate forms of this carcinogen. In a two-stage experiment, the synthetic 16-ol (Structure II) was shown to be almost as carcinogenic as was Structure I itself in mice; the 15-ol (Structure III) and 11-hydroxymethyl derivative (Structure IV) were much less active. The same order was also observed in the mutagenicity of these compounds in the Ames test.

INTRODUCTION

Recently, we (9) described the separation of microsomal metabolites of the strong carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (Structure I) by HPLC (Chart 1); the 7 main metabolites (a to g) were examined for mutagenicity in the Ames test and for their ability to bind to DNA on reincubation with rat liver microsomes and NADPH. Of the 7, Metabolite e was the most mutagenic, being 2 to 3 times more active than the carcinogen itself. Moreover, it was the only metabolite to give rise in vitro to the major DNA Adduct B obtained from the skin of mice treated in vivo with the carcinogen. Metabolite e was identified on the basis of its mass and UV spectra, together with its general chemical properties, as the trans-3,4-dihydro-3,4-dihydroxy derivative of Structure I and was proposed as the proximate carcinogen. Of the other metabolites, Metabolites a and d were biologically inert, but Metabolites b, c, f, and g were all mutagenic, and all gave rise to the more polar, minor DNA Adduct A, but not to Adduct B. This paper is concerned with the nature of these metabolites and consequently supplements our original communication.

MATERIALS AND METHODS

These were generally as described recently (9); the 11-methyl-14C-labeled carcinogen (Structure I) was used to allow quantitation. The unlabeled synthetic monools, Structure II (2), Structure III, and Structure IV (7), were metabolized, and the metabolites were separated by HPLC essentially as already described for Structure I (9).

The compound (500 µg) was incubated for 30 min at 37° under gentle shaking in air with 0.1 ml Tris buffer (10 ml), pH 7.2, containing NADPH (37.5 mg) and microsomes equivalent to 10 g of liver obtained from rats previously induced by i.p. injection of 20-methylcholanthrene (15). The solution was extracted 5 times with ethyl acetate (10 ml each); the latter was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure. The residue was redissolved in 250 µl of methanol:water (95:5, v/v), and fat was removed by 2 extractions with hexane (250 µl each); negligible radioactivity was lost from the aqueous methanolic phase which now formed a clear pale yellow liquid. This solution (100 µl) was injected to a Whatman Partisil M9 10/50 ODS column (Whatman Ltd., Maidstone, England) fitted to a Varian 4200 liquid chromatography system and was eluted with 15% methanol in water changing to methanol alone in a linear gradient at 1% min at a flow rate of 120 ml/hr (Chart 3).

Owing to slight variations in retention times in individual HPLC runs, the identity of certain metabolites of these monools with metabolites of the 14C-labeled carcinogen of similar retention time was checked by cochromatography of the corresponding unlabeled and radioactive fractions. In practice, it was not difficult to assign peaks as shown in Chart 3 with certainty. The UV spectra of certain metabolites were found to alter on storage, the extent of the change being dependent on both storage time and conditions. The UV data in Table 2 were obtained within 2 to 3 hr of the metabolites having been extracted from the incubation and separated by HPLC. Reduction with sodium borohydride and dehydration with hot 5 N sulfuric acid was carried out as described (9).

Animal Experiment. One hundred formally randomized TO mice were divided into 5 groups each consisting of 10 males and 10 females, 3 months old. One week after their backs were shaved with electric clippers, each of the mice in Groups 1 to 4 was treated with 400 µg of the test compound dissolved in 80 µl of toluene:acetone (1:1, v/v). The compounds used were: Group 1, Structure I; Group 2, Structure II; Group 3, Structure III; and Group 4, Structure IV. Twice-weekly promotion with 10 µl of croton oil:toluene (1:99 v/v) was begun 8 days after initiation. Mice in Group 5 were treated with toluene:acetone alone; no tumors were produced by this treatment; neither were tumors induced previously when control mice were treated as above with croton oil:toluene. General conditions were as described previously (3).

1 This work was supported by the Imperial Cancer Research Fund.
2 To whom requests for reprints should be addressed.
3 The abbreviation used is: HPLC, high-pressure liquid chromatography.
Received June 28, 1979; accepted November 20, 1979.
Mutagenic and Carcinogenic Metabolites

Table 1
Mass spectra of Metabolites a to f

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Mass spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>C_{15}H_{10}O_2, M+ 298 (21), 297 (28), 296 (100), M&quot;, 280 (16), 279 (16), 278 (61), M—H_2O, 262 (13), 254 (24), 253</td>
</tr>
<tr>
<td>Requires: 296.1043</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>C_{15}H_{10}O_2, M+ 296 (13), 252 (69), 251 (11), 250 (16), M—CO—H_2O, 248 (11), 220 (11), 219 (23), 203 (11), 202 (22)</td>
</tr>
<tr>
<td>Requires: 296.1049</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>C_{15}H_{10}O_2, M+ 296 (25), 280 (21), 279 (21), 278 (100), M—H_2O, 263 (11), 262 (33), 261 (11), 260 (44), M—2H_2O, 250 (11), M—CO—H_2O, 248 (11), 220 (11), 219 (23), 203 (11), 202 (22)</td>
</tr>
<tr>
<td>Requires: 280.1043</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>C_{15}H_{10}O_2, M+ 280 (18), 279 (19), 278 (100), M&quot;, 260 (15), M—H_2O, 231 (12), M—H_2O—CHO, 203 (16), 202 (11)</td>
</tr>
<tr>
<td>Requires: 278.0948</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>C_{15}H_{10}O_2, M+ 280 (28, M&quot;), 279 (12), 264 (19), 263 (22), 262 (100), M—H_2O, 234 (25), M—CO—H_2O, 219 (16), M—H_2O—CHO, 202 (10)</td>
</tr>
<tr>
<td>Requires: 280.1100</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>C_{15}H_{10}O_2, M+ 264 (19), 263 (20), 262 (100), M&quot; , 245 (17), 244 (31), M—H_2O, 233 (11), 231 (11), 223 (17), 219</td>
</tr>
<tr>
<td>Requires: 262.0995</td>
<td></td>
</tr>
<tr>
<td>Requires: 262.0994</td>
<td></td>
</tr>
</tbody>
</table>

Only ions with m/e above 200 of 10% or more relative abundance compared with the base peak are listed.

RESULTS

A typical HPLC separation of metabolites produced by incubation of Structure I with a microsomal system is shown in Chart 1 as a UV trace (254 nm); the 14C trace was almost identical except that the peak marked m was absent. This is therefore due to material of microsomal origin. Under these conditions, about 75% of the radioactivity was extracted from the incubation mixture with ethyl acetate, and Metabolites a to g plus unchanged Structure I accounted for about 85% of this. The least polar material was readily shown to be unchanged (Structure I) by its elution volume and UV spectrum. In a similar manner, the minor Metabolite c was identified as the 16-hydroxy derivative of Structure I by comparison with the synthetic ketol (Structure II) (2). The major Peak f had the same elution volume as do both the synthetic 15-hydroxy and 16-hydroxy-methyl derivatives (7) of Structure I, which are not resolved on this column. The molecular ion (m/e 262) was the base peak in the mass spectrum of Metabolite f, and an accurate mass determination gave its formula as C_{15}H_{10}O_2, a monool (Table 1). The UV data for Metabolite f (Table 2) were almost identical with those of the synthetic 15-ol (Structure III), in line with the expectation that this would be the major monol formed (8).

Metabolites a to e were separately purified by further HPLC, and their mass spectra established that Metabolites a, b, and c were triols (C_{15}H_{10}O_3) and that Metabolites d and e were diols (C_{15}H_{10}O_2). All with the exception of Metabolite d exhibited prominent M—H_2O ions, suggesting that they contained vicinal diol systems capable of being readily dehydrated. In contrast, the molecular ion was the only major ion in the mass spectrum of Metabolite d, behavior characteristic of cyclopenta[a]phenanthrenes retaining an intact phenanthrene ring system (17). This conclusion was supported by its UV spectral data (Chart 2; Table 2) which showed a conjugated phenanthrene chromophore similar to that of the 15-ol (Structure III). Metabolite a had a UV spectrum almost identical with that of the major urinary metabolite (Structure V) of the carcinogen and gave the known 15-hydroxy-2-phenol on acid dehydration (4). This Metabolite a was therefore the 1,2-dihydro-1,2,15-triol (Chart 5).

The UV spectra of Metabolites b and c were almost identical and were similar to that of Metabolite e; these metabolites were therefore 3,4,x-triols, where x denotes attachment of the third hydroxyl group at C-15, at C-16, or to the 11-methyl group. Information on the position of the third hydroxyl group in Metabolites b and c was provided by a study of the metabolites formed by microsomal incubation of the 3 synthetic monools, Structures II to IV, under identical conditions (Chart 3). Since the synthetic 15- and 16-ols are racemic mixtures whereas the corresponding biologically derived monools are probably optically active, introduction of a second chiral center in the racemic monools, such as an optically active trans-3,4-diol system, will then result in 2 chromatographically distinct enantiomers. It should also be borne in mind that previously it was found that microsomal metabolism of the carcinogen (Structure I) gave rise to the 16-ol (Structure II), 15-ol (Structure III), and 11-hydroxymethylketone (Structure IV) in the approximate ratio 1:2:3 (8).

Referring to Chart 3, i, it is seen that metabolism of the 16-ol produces equal amounts of 2 metabolites, the retention time of the more polar being identical with that of Metabolite c. The UV spectra of these 2 metabolites were identical with that of Metabolite c and similar to that of the 3,4-diol (Metabolite e); they are therefore probably 3,4,16-triols, enantiomeric at C-16. The 15-ol (Chart 3, i) yielded unequal amounts of 2 metabolites chromatographically and spectrally indistinguishable from Metabolites b and c. These are therefore probably the 2 enantiomeric 3,4,15-triols, the reason for their inequality...
being that one of the optically active 15-ols is a better substrate for the enzyme(s) leading to 3,4-diol formation. The major metabolite of the 11-hydroxymethylketone (Chart 3, iii) was also chromatographically and spectrally identical with metabolite b; as expected, this optically inactive substrate gave a single peak which is most probably the 11-hydroxymethyl-3,4-diol. To summarize, Metabolite b (Chart 1) may be composed of the 3,4,15-triol and 11-hydroxy-3,4-diol, while Metabolite c may be a mixture of 3,4,15- and 3,4,16-triols.

Of the monools, the 15-ol (Structure III) is the best substrate, and its major metabolite is the 1,2,15-triol (Metabolite a) which occurs as a single peak, again probably due to optical selectivity. In agreement with this assignment of Metabolites a, neither the 16-ol (Structure II) nor 11-hydroxymethyl compound (Structure IV) gives a metabolite with the retention time of Metabolite a. The 16-ol (Structure II) gives a minor metabolite with a slightly longer retention time, which probably accounts for the shoulder on the trailing edge of Metabolite a (Chart 1). Metabolite d is formed from both the 15-ol and 11-hydroxymethyl ketone but not from the 16-ol. This diol has a UV spectrum similar to that of the 15-ol and is therefore the 11-hydroxymethyl-15-ol. As expected, none of the 3 monools gave rise to Metabolite e. The main in vitro metabolic pathways for the carcinogen (Structure I) and the structures of the metabolites are summarized in Chart 5.

The results of testing the synthetic monools for carcinogenic activity in a 2-stage experiment is shown in Chart 4, which indicates the time of first appearance of the first skin tumor on each animal in Groups 1 to 4 up to 1 year; this experiment is still in progress, and full details will be published later. The 16-ol (Structure II) proves to be almost as carcinogenic as is Structure I; at 50 weeks, tumor incidence for Structure I is 95%, and for Structure II it is 100%; mean latent periods are 21.2 and 26.8 weeks, respectively. By contrast, the 15-ol (Structure III) (24%, 35.8 weeks) and 11-hydroxymethylketone (Structure IV) (35%, 34.5 weeks) are much less active. The mutagenic activity of these compounds towards S. typhimurium TA100 with metabolic activation is shown in Table 3. The 16-ol is two-thirds as active as is Structure I, while the 15-ol and 11-hydroxymethylketone are only 15 and 7% as active, respectively.

**DISCUSSION**

The carcinogen (Structure I) is metabolized by 2 main routes as shown in Chart 5. Aliphatic hydroxylation at the 15- and 16-methylene groups and at the 11-methyl group gives the monools II to IV (Metabolites f and g) and the diol d. Epoxidation of the non-bay-region double bond in the benzo ring of Structure I, followed by enzymatic opening, leads to the 3,4-diol (Metabolite e) which is the proximate carcinogen (9). Secondary metabolism of the monools yields the 1,2,15-triol (Metabolite a) and the 3,4,16-triols (Metabolites b and c). The metabolism of Structure I therefore resembles that of other purely polycyclic hydrocarbons in that both the benzo-ring double bonds are attacked, while K-region (C-6,7) products are not found. As with other phenanthrene-based carcinogens,1, 10, 11, 13-16, 19), the 3,4-diol system gives rise to mutagenic and carcinogenic activity. Presumably, the 3,4,15-triols, like the 3,4-diol (Metabolite e) (9), are further oxidized at the 1,2-double bond to epoxides which are therefore the ultimate forms of the carcinogenic monools (Structures II to IV).

The much higher carcinogenic and mutagenic activity of Structure II compared with Structure III appears to be caused by the 16-hydroxy group in the former compound directing aryl oxidation to the non-bay-region double bond. The 15-ol (Structure III), on the other hand, is largely converted into the 1,2,15-triol (Metabolite a). Like the bay-region diol of benz[a]pyrene (18), Metabolite a is biologically inert and its 3,4-double bond is not apparently oxidized further in vitro. However, in the intact rat it seems likely that Metabolite a is converted into the oxepin (Structure V) because this is a major metabolite in the urine of the animals given injections of the carcinogen (Structure I) (5). The comparatively low biological
activity of the 11-hydroxymethylketone (Structure IV) is anomalous because the main microsomal metabolite of this compound is the 3,4-dihydroxy-11-hydroxymethyl ketone (Metabolite b). After further metabolic activation, this metabolite binds very strongly to DNA in vitro (9), suggesting that epoxidation of its bay-region double bond occurs normally. The reason for its low mutagenicity and carcinogenicity may be that the adjacent hydroxymethyl group is strategically placed to open the 1,2-epoxide by rear attack at C-1, thereby reducing the half-life of this ultimate carcinogen.

In addition to these main metabolic transformations, it is clear from the number of minor metabolites that several other pathways are also operative. Thus, the fact that Metabolite e, on further metabolism in the presence of DNA, gives a small amount of the faster-running DNA Adduct A in addition to the main Adduct B (9), shows that Metabolite e is hydroxylated to one or more of the triols b and c which are known to give rise solely to Adduct A (9). Examination of the minor metabolites more polar than Metabolite a (Charts 1 and 3) reveals that they all possess UV spectra characteristic of the 1,2,3,4-tetrahydro
The theory of biological activation of polycyclic aromatics (epoxides). This work therefore fully supports the bay region theory of biological activation of polycyclic aromatic carcinogens (12).

REFERENCES

Identification of the Proximate and Ultimate Forms of the Carcinogen 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one

Maurice M. Coombs, Anna-Maija Kissongerhis, Jeffrey A. Allen, and Colin W. Vose

ABSTRACT

Enzymatic hydrolysis of calf thymus DNA treated in vitro with the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one and a microsomal enzyme system followed by column chromatography disclosed two adduct fractions, A and B, eluting after the natural nucleosides. Isolation and hydrolysis of the DNA from the skin of mice treated topically with 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one or from mouse embryo cells exposed to 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one in culture gave mainly Adduct B identical with that obtained in vitro. The seven main metabolites formed from 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one with the microsomal system were isolated by high-pressure liquid chromatography and were individually incubated with DNA and the activating system; the DNA was subsequently recovered and analyzed. Adduct B arose from a single metabolite identified on the basis of its ultraviolet and mass spectra together with its general chemical behavior as a 3,4-dihydro-trans,3,4-dihydroxy derivative of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one. In addition, this metabolite was the most mutagenic in the Ames test, being more active than the carcinogen itself. This metabolite is therefore the proximate form of the carcinogen.

The ultraviolet spectrum of Adduct B resembled that of a 1,2,3,4-tetrahydro derivative of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one, while the chromatographic mobility of the adduct on Sephadex LH-20 was markedly increased by inclusion of borate in the eluant, indicating a cisdial diol structure. Since the 3,4-diol is trans, this suggests further metabolism of the benzo ring. It is therefore proposed that the ultimate carcinogen is a 1,2-dihydro-1,2-epoxy-3,4-dihydro-3,4-diol of 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one.

INTRODUCTION

After metabolic activation, the strong carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (Structure I) (9) (Chart 1) binds to DNA in vitro (16) and is mutagenic to Salmonella typhimurium TA100 (13). Both these activities are suppressed by the aryl hydrocarbon hydroxylase inhibitor 7,8-benzo[flavone, which strongly inhibits formation of metabolites derived from enzymatic attack on the aromatic rings, but not those produced by hydroxylation at saturated carbon atoms (C-15 and C-16 methylene groups and 11-methyl group) (16). Skin tumor production by topical application of this carcinogen (Structure I) is also reduced by a coadministration of 7,8-benzo[flavone (11). Taken together with the observed correlation between mutagenicity and carcinogenicity in the cyclopenta[a]phenanthrene series (13), this suggests that the metabolite responsible for the carcinogenic activity of Structure I is derived from the aromatic part of this molecule. The ability of Structure I to bind to DNA in vitro after metabolic activation decreases rapidly under ambient conditions and is lost immediately on acidification, suggesting that the active metabolite is probably an aryl oxide (16). This paper describes experiments which have led to its identification as a 3,4-dihydroxy-1,2-epoxide of Structure I. A preliminary report has appeared (10).

MATERIALS AND METHODS

Labeled Compounds. 11-methyl-14C-labeled carcinogen (Structure I) (9.3 mCi/mmol) was synthesized by the method described previously (15). Generally tritiated Structure I (13.9 Ci/mmol) was prepared by platinum metal-catalyzed exchange in 70% [3H]acetic acid (6). Tritium nuclear magnetic resonance revealed that, with the exception of C-1 and C-16, all positions were labeled as follows: C-2 and C-3, together, 23.6; C-4, 18.2; C-6, 8.2; C-7, 4.5; C-12, 3.1; C-15, 16.4; 11-methyl, 25.5%. The structures of these compounds are shown in Chart 1.

Enzymes. DNase I (DN-CL), snake venom phosphodiesterase type II (P-6877), alkaline phosphatase (Escherichia coli) type III (P-4252), and RNase A (R5125) were obtained from Sigma, London, England. Proteinase K (fungal) and NADPH were from British Drug Houses, Poole, Dorset, England.

In Vitro Experiments with DNA. Binding of the carcinogen (Structure I) and its isolated metabolites to DNA in vitro and enzymatic hydrolysis of the DNA followed by separation of its constituents by chromatography on Sephadex LH-20 was carried out as described previously (16).

Isolation of DNA from Mouse Skin. Male TO (Tyler’s Original) mice, 7 to 9 weeks old, were used in groups of 15. Their backs were shaved with electric clippers, and 24 hr later G-3H-labeled Structure I (400 μg in 40 μl toluene) was applied to each mouse; the area covered was approximately 2.5 x 2.5 cm. Groups of mice were killed by cervical dislocation at selected times after treatment (Chart 3); the treated area of skin was removed and frozen in liquid nitrogen in an earthenware mortar. The frozen skin fragments were ground to a powder by hand, using a pestle, and added in small portions to a lysing solution of proteinase K at 50° according to the general method of Blin and Stafford (2) for isolation of DNA. This consists of solubilization of the tissue, isolation of the nucleic acids by phenol extractions, reincubation with RNase, and reisolation of the DNA by further phenol extractions. The DNA solution thus obtained was exhaustively dialyzed against tap water and finally against distilled water before concentration for enzymatic hydrolysis.

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Received March 12, 1979; accepted June 11, 1979.
Isolation of DNA from Mouse Embryo Cells. Secondary whole-mouse-embryo cells were seeded at $5 \times 10^6$ cells/150-mm tissue culture dish (Falcon Plastics, Oxnard, Calif.) in Dulbecco's modification of Eagle's medium containing 10% fetal calf serum (Gibco Bio-cult, Paisley, Scotland) and grown to confluency over 3 days. Each of 15 dishes (total $4 \times 10^6$ cells) was treated with 50 $\mu$g of the G-3H-labeled carcinogen (Structure I) in 50 $\mu$l of dimethyl sulfoxide to give a final concentration of 1.25 $\mu$g/ml, and incubation was continued for a further 48 hr. The medium was removed, the dishes were washed twice with phosphate-buffered saline containing NaCl (10 g), KCl (0.25 g), Na$_2$HPO$_4$ (1.44 g), KH$_2$PO$_4$ (0.25 g), CaCl$_2$, 2H$_2$O (0.17 g), and MgCl$_2$, 6H$_2$O (0.13 g) per liter of sterile distilled water, pH 7.2, and the cells were scraped off, suspended in phosphate-buffered saline, and centrifuged at 900 $\times$ g for 5 min. The cell pellet was washed twice with phosphate-buffered saline by resuspension and centrifugation; it was finally added to the proteinase K lysing solution, and the DNA was isolated as already described.

In Vitro Metabolism and Separation of Metabolites. 11-methyl-14C-labeled Structure I (500 $\mu$g), microsomes from 10 g of rat liver, and NADPH (37.5 mg) in 0.1 M Tris buffer (10 ml), pH 7.2, were shaken gently in air at 37°C for 30 min, and the metabolites formed were extracted with ethyl acetate essentially as already described (16).

Metabolites were separated by HPLC as described previously (16) except that a Whatman Partisil M9 10/50 ODS column (Whatman Ltd., Maidstone, England) was used with a gradient initially of 15% methanol in water changing to methanol alone at 1%/min and a flow rate of 120 ml/hr (Chart 4).

Identification of Metabolite e. HPLC fractions containing Metabolite e were pooled and rerun, collecting only the central part of the peak. The aqueous methanolic solution thus obtained was evaporated to dryness in a vacuum at 35°C, and the residue was dissolved in a small volume of methanol. An aliquot containing about 50 $\mu$g (based on its radioactivity) was evaporated in a small capillary tube for direct insertion into the inlet of a A.E.I. MS 902 instrument, and the mass spectrum was recorded at 70 eV and 1500 (10% valley) resolving power (Table 2).

An aliquot was diluted with methanol for UV spectroscopy (Table 2; Chart 5), sodium borohydride (10 mg) was added, and the spectrum was rerecorded after 30 min. For dehydrogenation, another aliquot (1 ml, 10 absorbance units) was heated in a boiling water bath for 30 min with 5 $n$ sulfuric acid (4 ml). The cooled solution was extracted 3 times with ethyl acetate, and the latter was washed with saturated sodium hydrogen carbonate solution and with water and dried over anhydrous sodium sulfate. After evaporation to dryness in a vacuum, the residue was redissolved in neutral methanol, and its UV spectrum was recorded before and after addition of sodium hydroxide (0.1 ml). A further aliquot (2 ml, 10 absorbance units) was stirred with 5% palladium-on-charcoal catalyst (1 mg) in an atmosphere of hydrogen for 1 hr, and the UV spectrum was recorded after removal of the catalyst by centrifugation. This solution was further reduced with sodium borohydride as already described, and its spectrum was recorded.

The elution volume of Metabolite e (428 ml) run on a Sephadex LH-20 column with Tris buffer (pH 8.7) was unchanged when the eluting solvent was replaced by sodium borate buffer (pH 8.7).

Determination of Mutagenicity of Metabolites a to g. Metabolites a to g (Chart 4) were isolated by HPLC, and their ability to cause reversion to prototrophy in S. typhimurium TA100 grown on a histidine-deficient medium was examined essentially as described previously (13).

Characterization of Adduct B. LH-20 fractions containing this adduct (Chart 2) were pooled and concentrated in a vacuum to 2 ml. The aqueous solution, $\lambda_{\text{max}}$ 260, 281 nm, was reduced with sodium borohydride, $\lambda_{\text{max}}$ 240, 285 nm; intensity at 260 nm was much decreased (for UV spectra of 1,2,3,4-tetrahydro derivatives of Structure I, see Table 2 and Chart 5). The elution volume of Adduct B on a column of Sephadex LH-20 equilibrated with Tris buffer, pH 8.7, was 256 ml. When the buffer was replaced by sodium borate buffer, pH 8.7, this volume was reduced to 205 ml (Chart 6).

RESULTS

Binding of Structure I to DNA in Vitro and in Vivo. As shown previously (16), the 14C-labeled carcinogen (Structure I) binds to DNA when the latter is present during the incubation with rat liver microsomes and NADPH. Enzymatic hydrolysis of this DNA followed by chromatography of the resulting nucleosides on Sephadex LH-20 columns gives 2 radioactive peaks, A and B, eluting after the natural nucleosides (UV trace) as shown in Chart 2a. The same pattern was observed with calf thymus, salmon sperm, and E. coli DNA and with DNA prepared from mouse skin, provided in vitro incubations were used. In addition to Adducts A and B, a sharp coincident peak (Peak X) of radioactivity and UV absorption always appeared at about 85 ml, the approximate elution volume of polydeoxyribonucleotides. The size of this peak was not altered by further incubation with the various enzymes used in the isolation and hydrolysis of the DNA.

In order to investigate the pattern of DNA binding in vivo, mice were treated, each on its dorsal region, with 400 $\mu$g of the high-specific-activity 3H-labeled carcinogen. This is known to be a powerful initiating dose which, when followed by twice-weekly promotion with croton oil, gave rise to tumors in 90% of the animals with a mean latent period of 35 weeks (8). After 24 hr and at subsequent times up to 36 days mice were killed, DNA was isolated from the treated skin and was hydrolyzed and chromatographed as before. In each case, a pattern similar to that shown in Chart 2b was found, consisting of Adduct B and a trace of Adduct A. Identity of this in vivo 3H-labeled Adduct B with the 14C-labeled Adduct B formed in vitro was established by cochromatography, when both isotopes eluted....
incubation with rat liver microsomes and NADPH were separated by HPLC and examined for biological activity by means of the Ames test. Previously, we have demonstrated (13) that there is a good correlation between mutagenicity, as measured by the reversion to prototrophy in S. typhimurium TA100, and carcinogenicity among 29 cyclopenta[α]phenanthrenes which had also been tested for their ability to elicit skin tumors in mice. Like the carcinogen itself, none of the metabolites was active in the absence of further metabolism; mutagenicity in its presence is summarized in Table 1. Mutabolite e was the most active, being more than twice as active as the carcinogen (Structure I). Metabolites b, c, f, and g were also mutagenic, but less so than Structure I, while Metabolites a and d were inert.

Binding of these 14C-labeled metabolites to DNA in vitro by the method already described is also shown in Table 1. Metabolite e was unique in that it was the only metabolite to give rise to the nucleoside Adduct B, the main adduct produced by the carcinogen in vivo. Its extent of binding was also several times together (Chart 2c). In addition to these adducts, Chart 2b also shows the presence of the early Peak X and an even faster-running peak. The latter, but not Peak X, was decreased when the DNA was isolated by precipitation before hydrolysis; however, this step was usually omitted to ensure that no loss of DNA occurred. The faster-running peak may therefore consist of nondialyzable, carcinogen-modified peptides not amenable to complete digestion with the protease used. A small amount of labeling of the natural nucleosides is also evident in Chart 2b. This arises by metabolic incorporation of tritium displaced from the 3H-labeled carcinogen during oxidative metabolism. This in vivo pattern of radioactivity bound to DNA is similar to those reported for other carcinogens (28).

Chart 3 shows the time course of binding of the 3H-labeled carcinogen (Structure I) to mouse skin DNA in vivo. Maximum radioactivity in Peaks A, B, and X occurred at 48 hr. Peak X declined rapidly during the subsequent week, then more slowly and was barely present at 36 days. By contrast, the amount of Adduct B remained constant until the fifth day and then decreased, rapidly at first, to about 10% of its original value at 36 days.

Binding of the 3H-labeled carcinogen to the DNA of mouse embryo cells in culture was also studied. Again, maximum binding was found at 48 hr; the pattern of radioactivity in the hydrolysis products was the same as that for mouse skin shown in Chart 2b.

**Mutagenicity and Binding to DNA of Metabolites.** Metabolites a to g (Chart 4) formed from 14C-labeled Structure I by
greater than that of Structure I. Of the other metabolites, Metabolites b, c, f, and g all gave only Adduct A, while Metabolites a and d did not bind to DNA. The parallelism between mutagenicity and total amount of the metabolite bound to DNA is noticeable. The nature of these other metabolites is under investigation and will form the subject of a separate communication.

The Structure of Metabolite e (Table 2; Chart 5). The retention volume of metabolite e suggested a diol, and this was confirmed by an accurate mass determination which showed its composition to be C_{18}H_{16}O_{3}. The third oxygen atom is present as the original ketone function because mild reduction caused a shift in the UV maximum from 273 to 250 nm. These spectra are unlike the spectra of Structure I and its reduction product (\lambda_{\text{max}} 264 and 255 nm, respectively), indicating that the diol oxygens are situated in a conjugated ring. The intense ion in the mass spectrum of metabolite e at m/e 262, corresponding to a loss of water from the molecular ion, confirms the presence of a readily dehydrated vicinal diol system. The UV spectrum of Metabolite e was different from those of the known 1,2- (12), 6,7- (5), 11,12-, or 13,14-dihydro (4) derivatives of 15,16-dihydrocyclopenta[a]phenanthrene-17-one; it therefore probably represents the unknown 3,4-dihydro chromophore. Acid-catalyzed dehydration of Metabolite e gave a phenol, the UV spectrum of which was not the same as those of the known 2- (14), 3-, 6-, and 7-phenols (5) of this series; the 4-phenol is the expected product from dehydration of the 3,4-dihydro-3,4-diol, by comparison with the product (1-naphthol) of dehydration of 1,2-dihydro-1,2-dihydroxynaphthalene (17, 19). The presence of the 3,4-dihydro system in Metabolite e was confirmed by catalytic hydrogenation, the product of which had the known chromophore of a 1,2,3,4-tetrahydro compound (7). The trans stereochemistry expected of a diol formed metabolically with mammalian enzymes was also suggested by the failure to decrease the elution volume of this metabolite by addition of sodium borate to the eluting buffer. Metabolite e is therefore the 3,4-dihydro-3,4-trans-dihydroxy derivative and is the proximate carcinogen (Chart 7); the absolute stereochemistry, or indeed whether it is a single enantiomorph, is not known at present.

Identification of the Ultimate Carcinogen. The nature of the reactive metabolite formed from the 3,4-dihydro-3,4-diol (Metabolite e) is suggested by consideration of the nucleoside Adduct B which arises from it. The UV spectrum of this material is that expected of an adduct consisting of a 1,2,3,4-tetrahydro derivative of Structure I attached to a nucleoside base without conjugation of either chromophore. Adduct B eluted from a Sephadex LH-20 column earlier and as a single peak when borate buffer (23) was used in place of Tris buffer (Chart 6). This is indicative of a cis-diol system, and because the 3,4-diol is trans the implication is that the adduct contains a third hydroxyl group at C-2, cis with respect to that at C-3. This in
threne, namely, benz(a)pyrene (26, 29, 37), benzo(a)pyrene (23, 37), both syn- and anti-diol epoxides are found; only the anti-diol-epoxide (Structure III) is formed from the carcinogen (Structure I). 7,12-Dimethylbenz[a] anthracene, with an analogous methyl group in the bay region, seems also to give mainly the anti-isomer (18). While Structure I and the corresponding 11-methoxy-17-ketone are strong carcinogens, the 11-ethyl-17-ketone is much less active, and the 11-butyl-17-ketone is inactive as a carcinogen (9) and as a mutagen (16). Possibly, these larger groups hinder approach of the oxidase to the 1,2-double bond and hence inhibit epoxidation.

In the present experiments, binding to DNA has been estimated from the sum of the radioactivity in Adducts A and B. However, there is evidence that the early Peak X also consists at least partly of carcinogen-modified nucleotides (18, 28). Although this peak is small in vitro (Chart 2a), in vivo it is appreciable, especially at early times (Chart 3). Peak X from the DNA of the skin of treated mice disappears more quickly and completely than does Adduct B which is still easily demonstrable even after 36 days. In studying the binding of tritiated 7,12-dimethylbenz[a]anthracene to the DNA of mouse embryo cells in culture, Dipple and Nebzydoski (18) also observed that the peak eluting at 85 ml and the later adduct peaks were similar in amount at 24 hr but were at a ratio of 1:10 at 48 hr.

Nearly all potent polycyclic carcinogens possess a phenanthrene ring system together with additional, fused aromatic ring(s). 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one, with potency similar to that of benzo(a)pyrene, is therefore one of the simplest molecules capable of being activated through a bay-region diol-epoxide to display high carcinogenic activity. The importance of the carbonyl group at C-17 lies in its conjugation with the phenanthrene system, for it may be replaced by a carbon-carbon double bond at C-16(17) with retention of strong activity, while the hydrocarbon with a saturated 5-membered ring is only a weak carcinogen (9). The 11-methyl ketone (Structure I) is the most active of its positional methyl isomers; the 7-methyl ketone is less active, and the 2-, 3-, 4-, 6-, and 12-methyl ketones, like the parent ketone 15,16-dihydrocyclopenta[a]phenanthren-17-one, are not carcinogens. Since all these compounds have similar bay regions, this of itself is not sufficient to ensure carcinogenicity; correct positional substitution by a small electron-releasing group is also critical. The same pattern of activation by methyl substitution is also seen among the methylbenz[a]anthracenes and methylchrysesenes, which may be viewed as analogs of Structure I in which conjugation by the 17-carbonyl group is replaced by the conjugation of a further, fused benzene ring. Comparison of the metabolic products formed from Structure I with those from its inactive isomers may lead to a better understanding of the role of the 11-methyl group in inducing carcinogenic properties in these compounds.

REFERENCES
Proximate-Ultimate Forms of a Cyclopenta[α]phenanthrene


THE CARCINOGENICITY OF 15,16-DIHYDRO-11-METHYL-CYCLOPENTA[A]PHENANTHREN-17-ONE

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Received 11 June 1979  Accepted 14 August 1979

Summary.—Direct comparison of skin-tumour induction by 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (I) and by benzo[a]pyrene on mouse skin, both by repeated application or by initiation with a single dose followed by promotion with croton oil, demonstrated that these two carcinogens have similar potency. After repeated application of (I) the mean latent period for skin-tumour induction was linearly related to the logarithm of the dose over a 10-fold dose range. Under these conditions, application of the aryl-hydrocarbon-hydroxylase inhibitor 7,8-benzo-flavone together with (I) inhibited tumour induction by about 40%. By contrast, in the 2-stage experiment, little effect on tumour incidence or latent period was observed when this inhibitor was applied with the single initiating dose of (I). Co-administration of the epoxide-hydratase inhibitor 1,1,1-trichloropropene oxide caused enhancement by shortening the latent period.

After s.c. injection of (I) into mice, a similar number of tumours was induced on skin remote from the site of injection by promotion with croton oil begun either one week or 6 months after initiation. Gastric instillation of (I) into female rats induced mammary adenocarcinomas.

We have previously outlined our interest in compounds of the cycloptenta-[a]phenanthrene series as aromatic analogues of steroids (Coombs & Croft, 1969). Comparisons of the carcinogenicity of some 40 closely related members of this series have revealed that the title compound (I) (see Fig. 1) is the most active, and that its activity depends upon 2 structural features: the presence of a small electron-releasing group at C-11, and further conjugation of the phenanthrene ring system at C-17. Of the monomethyl isomers, only the 11-methyl-17-ketone (I) is strongly carcinogenic; the 7-methyl-17-ketone is a weak carcinogen whilst the 2-, 3-, 4-, 6-, and 12-methyl isomers are inactive, as is the unsubstituted parent ketone (V) (Coombs et al., 1973). The 11-methoxy-17-ketone (II) is almost as active as (I), but other methoxy isomers lack activity. Strong carcinogenicity is also associated with the 11,17-dimethyl-16(17)-ene (III), in which conjugation by the 17-carbonyl group is replaced by conjugation by the 16(17)-double bond; the corresponding hydrocarbon with a saturated 5-membered ring is much less active, as are ring-methyl isomers of (III) (Coombs & Croft, 1969).

These comparisons were all made by the same method: groups of 20 mice were treated topically with 50 µg of the compound twice weekly for one year, and observed for a second year. First appearance of skin tumours at the site of application (dorsal region) was scored, and tumours were subsequently classified histologically. In this paper, the carcinogenicity of (I) is compared with that of the classical carcinogen benzo[a]pyrene, both by this method and by the 2-stage system.

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The original method is also used to test the 1,2,3,4-tetrahydro derivative (IV) for carcinogenicity, and to investigate the effect of the dose of (I) on latent period. The effects of the enzyme modifiers 7,8-benzoflavone (BF) and 1,1,1-trichloropropene oxide (TCPO) on its carcinogenicity are presented, as is the production of mammary tumours in rat-feeding experiments with (I).

MATERIALS AND METHODS

Chemicals.—The cyclopenta[a]phenanthrenes (I), (IV) and (V) were synthesized here, as already described (Coombs, 1966; Coombs & Bhatt, 1973); their structures are shown in Fig. 1. Benzo[a]pyrene, 7,8-benzoflavone and 1,1,1-trichloropropene oxide were obtained from the Aldrich Chemical Co., Milwaukee, and croton oil from the Sigma Chemical Co., St Louis. Toluene was Analar grade from Fisons Ltd., Loughborough, and was used throughout as the vehicle.

Mouse experiments.—Formally randomized TO (Tyler's Original) albino mice (10 males and 10 females) were used for each group, 1–3 and 6–26; Groups 4 and 5 each consisted of 20 males and 20 females. Conditions were generally as previously described (Coombs & Croft, 1969).

In Groups 1–10 (Table I) compounds or mixtures were applied to the shaved dorsal skin twice weekly as toluene solutions (10 μl at each administration) for 50 weeks, and the mice were observed for up to 100 weeks. Mice in Groups 11–16 (Table II) each received 400 μg of carcinogen in 80 μl of toluene; in addition, mice in Group 13 each received BF (1,200 μg) dissolved in the carcinogen solution. Twice-weekly promotion with croton oil (10 μl of a 1% v/v solution in toluene) as indicated in the Table was begun 8 days later. Animals in Group 14 were treated topically with TCPO (10 μl of a 10% v/v solution in toluene) at 30 min before and again immediately before the initiating dose of carcinogen. Negative control Groups 17 and 18 were each treated initially with toluene (800 μl); mice in Group 17 were subsequently treated twice weekly with this solvent (10 μl), whereas those in Group 18 were “promoted” with croton oil in the usual way.

Mice in Groups 19–23 (Table III) were injected, each in the right shoulder with the carcinogen (I) in olive oil (0.2 ml); Groups 24 and 25 were similarly injected with the ketone (V) (3 mg), while Group 26 was injected with olive oil alone. Promotion of the shaved dorsal region as already described was started 8 days later (Groups 20, 22, and 25) and 6 months later (Groups 21 and 23). In all cases promotion was continued for the whole experiment, up to 2 years.

Animals were killed when their skin tumour reached 1 cm in diameter; a few sick mice were killed earlier to avoid loss of material. All animals were opened and examined macroscopically for abnormal tissues; tumours other than those appearing on promoted skin for mice injected with (I) (Groups 19–21) are shown in Table IV. All tumours were examined histologically except in a few cases, as noted in the Tables, when they were unavailable because the animal had died, and autolysis was too far advanced, and also when more than one skin tumour per animal was obtained. In the latter case only the first-appearing tumour, which was usually also the first to reach 1 cm in diameter, was examined histologically. Classification of skin tumours as papillomas or carcinomas was carried out as previously described (Coombs et al., 1973). Usually 1 cm tumours were carcinomas when induced by repeated application of the carcinogen (Table I), but papillomas were more common when produced by initiation and promotion (Tables II and III). A complete list of experimental and control groups appears in Tables I–III, which also show the rate of tumourless survival.

Rat experiment.—Virgin Sprague-Dawley
rats, random-bred in this Institute, were divided into 2 groups by a formal randomization procedure. They were housed 3-4 to a cage, fed on pelleted GR 3 diet (Dixon and Son, Ware, Herts) and allowed free access to water. At 50 days of age, 30 mg of carcinogen (I), suspended and partly dissolved in corn oil (2 ml), was given intragastrically to each of 27 rats; a further 96 rats were left untreated. From the fourth week of treatment all rats were examined weekly to detect developing tumours. These were removed surgically when they had grown to about 1 cm in diameter, and were classified histologically (Table V) as malignant adenocarcinomas or benign fibroadenomas (Young & Hallowes, 1973).

Statistical methods.—Latent periods shown in Figs. 2-4 refer to the first appearance of the first skin tumour on each animal. Mean latent periods are listed in Tables I-III together with their standard deviations. Estimation of the probability of the curves of latent period for Groups 4 and 5, 12 and 13, and 12 and 14 (Figs. 2 and 3) being different is made by the summary chi-square procedure advocated by Mantel (1966) for comparing 2 sets of life tables in their entirety. This statistical method takes both differences in latent period and tumour incidence into consideration over the whole experimental period, rather than at any single time. The test tends to be conservative, in that the latent periods are considered only as the order in which the first tumour on each animal appears in time, and not as actual time values. The probabilities of the number of rat mammary tumours being significant (Table V) are calculated by the exact method of Yates (Fisher, 1954).

RESULTS

Direct comparison of the tumour incidence and mean latent period of skin tumours produced by the carcinogen (I) at its lowest dose (Group 4, 34-7 weeks, 45%) with benzo[a]pyrene at the same dose (Group 8, 37-5 weeks, 50%) (Fig. 2) demonstrates that they are similar as complete carcinogens on mouse skin. Also shown in Fig. 2 are curves formed by plotting the time of first appearance of skin tumours with (I) at 50, 25, 10, and 5 µg twice weekly. Using the well known empirical relationship between mean latent period (L, in weeks) and dose (d, in µg),

\[ L = a - b \left( \log_{10} d + c \right) \]

where \( a, b, \) and \( c \) are constants (Bryan & Shimkin, 1941), the plot of \( L \) against \( -\log_{10}d \) approximates to the straight line, and the mean latent period = 29-40 - 10-803(log10d - 1-199) with a correlation coefficient, \( r = 0-96 \). Also shown are the 6 tumours induced by twice-weekly applications of (I) (5 µg) plus 7,8-benzoflavone (15 µg), with a mean latent period of 36-2 weeks. When this latent period is fitted on an extrapolation of the dose-response curve, it appears that under these conditions the inhibitor effectively reduces the administered dose to about 3 µg. Finally, Table I shows no evidence for carcinogenicity for the 1,2,3,4-tetrahydro derivative (IV) in this test involving repeated applications.

Fig. 3 and Table II illustrate the results of the initiation-promotion experiments. A single application of 400 µg of (I) per mouse followed by twice-weekly promotion with croton oil (Group 12, 30-0 weeks,
CARCINOGENESIS BY A CYCLOPENTA[a]PHENANTHRENE 917

Table I.—Skin-tumour production in mice by repeated topical application

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound(s)</th>
<th>Dose (µg, 2x/week)</th>
<th>No. of tumour-free survivors at (months)</th>
<th>No. of mice with tumours</th>
<th>No. of squamous papillomas</th>
<th>No. of squamous carcinomas</th>
<th>Mean latent period ± s.d.</th>
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<tr>
<td>1</td>
<td>I</td>
<td>50</td>
<td>6 12 18 20</td>
<td>17</td>
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<tr>
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<td>I</td>
<td>25</td>
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<td>18</td>
<td>2</td>
<td>16</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>10</td>
<td>15 2 1</td>
<td>16</td>
<td>1</td>
<td>15</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>5</td>
<td>5 18 12 6</td>
<td>18*</td>
<td>3</td>
<td>13</td>
<td>34 ± 8</td>
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<td>5</td>
<td>I+BF</td>
<td>5+15</td>
<td>38 31 20 8</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>36 ± 6</td>
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<tr>
<td>6</td>
<td>BF</td>
<td>15</td>
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<td>0</td>
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<td>16 9 5 0</td>
<td>10*</td>
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</tr>
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<td>10</td>
<td>—</td>
<td>—</td>
<td>19 18 10 4</td>
<td>0</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

* Two tumours were unavailable for histology in each of Groups 4, and 8.

P 4 vs 5 < 0.01, estimated by the method of Mantel (1966)—see Materials and Methods.

90%) gave a result similar to that observed recently (Coombs & Bhatt, 1978) when the initiating dose was subdivided and given on 4 subsequent days (35 weeks, 90%). Comparison of Groups 12 and 16 reveals that (I) is somewhat more active than benzo[a]pyrene as an initiator (Group 16, 33 weeks, 65%). Both carcinogens at this dose gave some skin tumours without promotion (Groups 11 and 15) after comparatively long latent periods.

Unexpectedly, topical application of 7,8-benzoflavone (1,200 µg/mouse) together with the initiating dose (400 µg) of (I) (Group 13) appears to have no effect on latent period or tumour incidence. This is in marked contrast to the result with repeated twice-weekly administration, already discussed. Application of the epoxide-hydratase inhibitor TCPO before the initiating dose of (I) (Group 14) caused enhancement, evident as shortening of the latent period.

After i.m. injection of (I) (3 mg/mouse), this carcinogen initiated skin tumours remote from the site of injection. As shown in Fig. 4 and Table III, promotion by croton oil started 8 days after initiation led to skin tumours in 65% of the mice, with a mean latent period of 33 weeks (Group 20). When promotion was delayed for 6 months, 50% of the mice developed skin tumours, with a mean latent period of 24 weeks from the start of croton-oil treatment (Group 21). No tumours appeared on the dorsal skin without promotion (Group 19); however, other tumours were found in animals of all 3 groups (Table IV). Injection of 300 µg of (I) (Groups 22 and 23) was ineffective, as was injection of 3 mg of the unsubstituted ketone (V) (Groups 24 and 25, Table III).

Mammary adenocarcinomas were de-
Table II.—Skin-tumour production in mice in the initiation-promotion experiments

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound(s)†‡</th>
<th>Promotion (twice weekly croton oil)</th>
<th>No. of tumour-free survivors at (months)</th>
<th>No. of mice with squamous papillomas</th>
<th>Mean latent period ± s.d. (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>I</td>
<td>—</td>
<td>6</td>
<td>18 17 10 6 5</td>
<td>48-0 ± 10-7</td>
</tr>
<tr>
<td>12</td>
<td>I</td>
<td>+</td>
<td>12</td>
<td>5 1 0 1 16*</td>
<td>29-9 ± 13-9§</td>
</tr>
<tr>
<td>13</td>
<td>I + BF</td>
<td>+</td>
<td>15</td>
<td>1 1 0 16</td>
<td>12 4 30-6 ± 9-5§</td>
</tr>
<tr>
<td>14</td>
<td>I + TCPO</td>
<td>+</td>
<td>9</td>
<td>2 1 0 16*</td>
<td>23-6 ± 9-7§</td>
</tr>
<tr>
<td>15</td>
<td>B(a)P</td>
<td>—</td>
<td>19</td>
<td>15 12 10 7</td>
<td>42-9 ± 17-7</td>
</tr>
<tr>
<td>16</td>
<td>B(a)P</td>
<td>+</td>
<td>14</td>
<td>13 4 4 13*</td>
<td>3 3 33-5 ± 17-3</td>
</tr>
<tr>
<td>17</td>
<td>toluene</td>
<td>—</td>
<td>20</td>
<td>18 16 7 0</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>toluene</td>
<td>+</td>
<td>19</td>
<td>18 15 5 0</td>
<td>—</td>
</tr>
</tbody>
</table>

* Histology unavailable for one animal.
† One tumour was a spindle-cell sarcoma.
‡ The initiating dose of (I) and B(a)P was 400 µg/mouse; in Group 13, 7,8-benzoflavone (BF) (1,200 µg/mouse) was applied simultaneously; mice in Group 14 received 1,1,1-trichloropropene oxide (TCPO) (10 µl of a 10% v/v solution) at 30 min before and again immediately before the initiating dose of (I).
§ P12 vs 13 = 0-62; P12 vs 14 = 0-17, estimated by the method of Mantel (1966)—see Materials and Methods.

![Fig. 4.—Induction of dorsal skin tumours after injection of the ketone (I) (3 mg/mouse) followed by promotion with croton oil 8 days (O—O), or 6 months (●—●) later.](image)

In both the repeated-application and initiation-promotion experiments 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (I) is comparable with benzo[a]pyrene as a carcinogen, despite the former having only 3 fused aromatic rings. Practically all known carcinogenic polycyclic hydrocarbons have 4 or more fused aromatic rings, 3 of which form a phenanthrene ring system. The ketone (I), in common with a number of other phenanthrene-derived carcinogens (Jerina et al., 1978) is metabolically activated by conversion to its bay-region, 3,4-dihydro-3,4-dihydroxy-1,2-dihydro-1,2-epoxide (Coombs et al., 1979). It therefore seems probable that (I) represents the smallest conjugated system that can be activated by this mechanism to display strong carcinogenic activity. No evidence for carcinogenicity was found for its 1,2,3,4-tetrahydro derivative (IV), in keeping with the proposed activation mechanism, for this compound lacks the necessary double bonds for metabolic formation of a bay-region diol-epoxide.

At the lowest dose (5 µg twice weekly) co-administration of the aryl-hydrocarbon-
CARCINOGENESIS BY A CYCLOPENTA[A]PHENANTHRENE

Table III.—Skin tumours appearing on promoted skin following injection of (I) into mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Promotion (twice weekly)</th>
<th>No. of tumour-free survivors at (months)</th>
<th>No. of mice with dorsal tumours</th>
<th>No. of squamous papillomas</th>
<th>No. of squamous carcinomas</th>
<th>Mean latent period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound dose (mg)</td>
<td>croton oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>I 3 none</td>
<td>20 16 5 0 0 0 9 4</td>
<td>33 ± 11.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>I 3 6 months later</td>
<td>11 7 2 9 8 1</td>
<td>40 ± 7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>I 0.3 8 days later</td>
<td>19 16 10 5 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>I 0.3 6 months later</td>
<td>19 14 11 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>V 3 none</td>
<td>19 19 14 8 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>V 3 8 days later</td>
<td>19 16 12 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>olive oil none</td>
<td>20 19 18 14 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>olive oil none</td>
<td>20 19 18 14 0</td>
<td>— — — —</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 24 weeks from beginning of promotion.

Table IV.—Tumours other than those appearing on promoted skin, after injection of (I) into mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Eyelid</th>
<th>Ear</th>
<th>Head</th>
<th>Ventral surface</th>
<th>Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>4 (2 pa.)*</td>
<td>—</td>
<td>2 sq. pa.</td>
<td>2† (1 mammary ca.)</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>4 sq. pa.</td>
<td>1 sq. ca.</td>
<td>—</td>
<td>1 sq. pa.</td>
<td>7</td>
</tr>
<tr>
<td>21</td>
<td>1 sq. pa.</td>
<td>1 sq. ca.</td>
<td>—</td>
<td>1 sq. pa.</td>
<td>8</td>
</tr>
</tbody>
</table>

* Histology not available for 2 tumours.
† Histology not available for 1 tumour.
sq. pa.=squamous papilloma.
sq. ca.=squamous carcinoma.
‡ No lung adenomas among animals in the olive-oil control (Group 26).

Table V.—Mammary tumours induced in rats after intragastric instillation of (I) (30 mg/rat)

<table>
<thead>
<tr>
<th></th>
<th>No. of rats at time of treatment</th>
<th>No. of rats with tumour alive at 20 weeks</th>
<th>No. of rats with tumour alive at 30 weeks</th>
<th>No. of rats with tumour alive at 50 weeks</th>
<th>No. of rats with tumour alive at 75 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinomas</td>
<td>27</td>
<td>2 26</td>
<td>4 26</td>
<td>6 20</td>
<td>—</td>
</tr>
<tr>
<td>In rats after I</td>
<td>In untreated rats</td>
<td>96</td>
<td>0 104</td>
<td>0 94</td>
<td>1 4</td>
</tr>
<tr>
<td>P*</td>
<td>0-0055</td>
<td>0-0019</td>
<td>0-0001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibroadenomas</td>
<td>In rats after I</td>
<td>27</td>
<td>0 26</td>
<td>0 26</td>
<td>0 20</td>
</tr>
<tr>
<td>In untreated rats</td>
<td>96</td>
<td>0 94</td>
<td>0 92</td>
<td>3 85</td>
<td>7 70</td>
</tr>
<tr>
<td>P**</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0-0270</td>
<td>0-2845</td>
</tr>
</tbody>
</table>


The hydroxylase inhibitor, 7,8-benzoflavone (15 µg) reduced the carcinogenicity of (I), in agreement with previous observations (Coombs et al., 1975). Using the dose-response curve shown in Fig. 2, BF apparently causes about 40% inhibition of tumour production under these conditions. It was therefore surprising that inhibition was lacking when BF (1,200 µg) was given together with the initiating dose of I (400 µg) in the two-stage experiment. The reason for this difference is not clear. Several workers have shown that BF inhibits hydrocarbon-induced aryl
hydrocarbon hydroxylase more than the constitutive enzyme (Grundin et al., 1973; Hill & Shih, 1975; Wiebel et al., 1971). It seems possible that promotion experiments using a relatively large single dose, as described here, might differ from experiments in which a small dose is administered repeatedly, in that the majority of the dose would be activated by the constitutive enzyme in the first situation, but not in the second.

Shortening of the mean latent period was obtained when the epoxide-hydratase inhibitor TCPO was given topically together with the initiation dose of (I). Enhancement of tumour production with TCPO has also been reported for 3-methylcholanthrene (Berry et al., 1977; Burki et al., 1974) and benzo[a]pyrene (Berry et al., 1977). With the latter, the inhibitor in vitro prevents hydration of the initially formed 7,8-oxide to the 7,8-diol (Selkirk et al., 1974) but the ultimate carcinogen, the 7,8-dihydroxy-9,10-epoxide, is apparently not a substrate for this enzyme (Wood et al., 1976). The carcinogen (I) is activated in a manner analogous to that of benzo[a]pyrene, so it seems probable that the mechanism by which TCPO causes enhancement is the same in both cases. Possibly, by inhibiting cytoplasmic epoxide hydratase, TCPO allows the initially-formed non-bay region oxide to escape to a site where it can be more advantageously further converted into the ultimate carcinogen.

In the experiments so far described involving topical application of (I), tumour formation is confined to the treated skin. The injection (mouse) and feeding (rat) experiments demonstrate that this carcinogen is also active systemically, and in more than one animal species. Previously, injection of (I) (8 and 50 mg) into mice led to ventral skin tumours as well as sarcomas at the site of injection (Coombs & Croft, 1969). Tumours at several sites, including the ventral surfaces, were seen in the present experiment (Table IV). Injection of 3 mg per mouse, but not one tenth of this dose, was sufficient to initiate the dorsal skin (Fig. 4 and Table III) so that subsequent promotion yielded a skin-tumour incidence of 65% and a mean latent period of 33 weeks. After injection initiation persisted, for when promotion was started 6 months later skin tumours occurred in 50% of the mice, but with a shorter mean latent period of 24 weeks (calculated from the beginning of promotion). No tumours were induced with the parent unsubstituted ketone (V) when injection of this compound (3 mg) was followed by topical treatment with croton oil. This agrees with the failure to induce tumours in mice by injection (50 mg) or by skin painting (50 μg twice weekly) (Coombs & Croft, 1969) or in the two-stage system with an "initiating" dose of 400 μg (Coombs & Bhatt, 1975).

In its ability to induce mammary carcinomas after a single intragastric instillation of 30 mg, compound (I) resembles other known potent carcinogens such as 3-methylcholanthrene (Shay et al., 1949) and 7,12-dimethylbenz[a]anthracene (Huggins, 1961). However, it is less potent than either of these, both in this regard and also as judged by their relative mean latent periods for induction of skin tumours in mice (Coombs & Croft, 1969). The suggestion that the carcinogenicity of aromatic hydrocarbons increases as their structure approaches that of the steroids (Yang et al., 1961) is therefore not substantiated. The ketone (I) not only possesses the same carbon-ring system as the steroids, but also bears an oxygen atom at C-17, a position which is oxygenated in virtually all natural C18 and C19 steroids. This carcinogen, with potency similar to that of benzo[a]pyrene, is best considered as a simple member of the large group of polycyclic hydrocarbon carcinogens whose structures are based on phenanthrene.

We thank Dr L. Pang for confirming the pathology of the mouse tumours, Miss J. Macdonald for valuable assistance, and Mr G. D. Everitt for providing computer facilities.

REFERENCES

Berry, D. L., Slaga, T. J., Viaje, A. & 4 others (1977) Effect of trichloropropene oxide on the


Evaluation of the Mutagenicity of Compounds of Known Carcinogenicity, Belonging to the Benz[a]anthracene, Chrysene, and Cyclopenta[a]phenanthrene Series, Using Ames’s Test

Maurice M. Coombs, Cynthia Dixon, and Anna-Maija Kissonergehis

Summary

Fifty-four polycyclic compounds, 29 of the cyclopenta[a]phenanthrene series, 11 chrysenes, and 14 benz[a]anthracenes, have been tested for mutagenicity by Ames’s method, using Salmonella typhimurium TA100. Without exception all 37 carcinogens and a known initiator were mutagens. Of the 16 noncarcinogens 7 were mutagenic, but none of these has yet been tested for initiating, as opposed to carcinogenic, activity. There appeared to be little quantitative correspondence between carcinogenic and mutagenic potency, however, and possible reasons for this are discussed. The aryl hydrocarbon hydroxylation inhibitor, 7,8-benzoflavone strongly inhibited the mutagenicity of certain compounds when it was added to the incubations.

Introduction

The somatic mutation theory of cancer requires carcinogens to be mutagens. Although previously most polycyclic hydrocarbon carcinogens appeared to be inactive when tested in standard mutagenesis assays, it is now established that the biological activity of these molecules is mediated by oxidative metabolism, a fact that had not been taken into consideration hitherto. Using histidine-deficient mutants of Salmonella typhimurium in a back-mutation system with mammalian liver preparations to provide metabolic activation, Ames (12) has recently claimed a 90% correspondence between carcinogenicity and mutagenicity in over 300 compounds of widely diverse chemical types. These included a series of 38 polycyclic aromatic compounds which were mostly tested against a new strain, S. typhimurium TA100. The latter was derived from an earlier strain (TA1535, his-, excision repair deficient, deep rough) by inclusion of an R-factor plasmid (pKM101, carrying ampicillin resistance) which makes the bacteria more sensitive to mutagenesis by polycyclic compounds (13). Among this series of 38 compounds, the correlation between carcinogenicity and mutagenicity was better than 90%.

Our interest in this area arose from the need to distin-

"Numbers in italics refer to compounds listed in Table 1."
following this published procedure closely. Liver “S-9” was prepared from the livers of 150-g male Sprague-Dawley rats, random bred in the I.C.R.F. closed colony and maintained on Diet GR3EK (E. Dixon and Sons, Ware, Hertfordshire, England) and water ad libitum. The rats were induced with Aroclor 1254 as described (2).

Compounds were added in 100 μl of dimethyl sulfoxide. They were first screened at 2 or 3 dose levels with 20, 50, or 100 μl of S-9 per plate. When mutagenic activity was observed, a dose-response curve was obtained, using the optimum amount of liver preparation and the compounds at doses up to 100 μg/plate. All compounds were also tested in the absence of liver enzymes. Controls included dimethyl sulfoxide alone (100 μl) to provide the spontaneous background mutation rate and 3 positive controls, benz(a)pyrene (5 μg), acetamidofluorene (10 μg), and the ketone (7) (10 μg/plate). The bacteria were routinely checked for ampicillin resistance, and the sterility of the S-9 was also tested. All experiments were carried out in duplicate, and mutagenesis was measured as the average number of revertant TA100 colonies per plate less the average spontaneous mutation rate (usually about 70/plate). In the inhibition experiments (Chart 3), 7,8-benzoflavone (300 μg) in dimethyl sulfoxide (50 μl) was added to each plate together with the carcinogen, also in dimethyl sulfoxide (50 μl).

RESULTS

The carcinogenic and mutagenic activities of the 54 compounds tested are presented in Table 1. For Compounds 1 to 34, for which comparable mouse data obtained here by skin painting are available, carcinogenicity is expressed as a single figure (Iball index) embracing both tumor incidence, including papillomas and carcinomas, and latent period (Table 1). Compounds marked <1 were noncarcinogens, i.e., the tumor incidence was less than 5% (1 in 20 animals) in 700 days (3, 4, 7). Spontaneous skin tumors have never been observed, even among control animals painted with toluene. The carcinogenicity data for the methylichrysenes (35 to 40) represent the results of a recent study of both the carcinogenic and initiating activities of these hydrocarbons to mouse skin (11). The data for the benz(a)anthracenes (41 to 54) are taken from summaries (10) of both skin painting (mouse) and injection (rat) experiments. These benz(a)anthracenes have not been tested systematically for initiating activity nor have Compounds 1 to 34.

Mutagenicity is expressed both as the number of revertant TA100 colonies per μg of compound incorporated in the plate and as the number of revertant colonies per nmole (12). Compounds exhibiting less than 0.2 colony/nmole were classed as nonmutagens; this number is approximately equivalent to the spontaneous mutation rate. Chart 2 shows typical dose-response curves for the ketone (6) obtained with various amounts of liver enzymes (S-9) in the same experiment. Mutagenicity figures in Table 1 are calculated from the initially straight parts of the dose-response curves, using the optimum amount of S-9; i.e., for Compound 6 mutagenicity is given as the number of revertant colonies with a dose of 10 μg/plate, using 50 μl of S-9. At these low doses of test compounds, cytotoxicity is negligible as evidenced by the appearance of a normal background “lawn” of unreverted bacteria. This grows by utilizing the trace of histidine added to the plate to allow the several rounds of replication necessary to “fix” mutations induced by the compounds. Mutagenicity is therefore given by the slope of the initially straight part of the dose-response curve and, as can be seen from Chart 2, this slope becomes less (i.e., apparent mutagenicity is diminished) when too much enzyme is used. Although we have attempted to use the optimum amounts of S-9 for each compound, it is obvious that the precision of these data is open to some question. Also the number of revertant colonies in the positive controls varied somewhat (± 10 to 20%) from day to day, even when the same bacterial suspension was used. We have tested a number of compounds that appear in Ames’s table, namely the positive controls benz(a)pyrene and acetamidofluorene and Compounds 3, 7, 40, and 54. In all cases our results are qualitatively similar to those of Ames, but we regularly observe less than one-half the number of colonies reported by him. We attribute this to a difference in the strain of rats used or to the effect of the different diet. These problems do not, however, invalidate the conclusions discussed below. Several representative cyclopenta[al]phenanthrenes were also tested with other strains of S. typhimurium developed by Ames. The mutagenicity of these carcinogens was detected with much reduced sensitivity with TA98, and not at all with strains TA1555 to 1558 which lacked the R factor.

Chart 3 illustrates the effect of the aryl hydrocarbon hydroxylase inhibitor, 7,8-benzoflavone, on the mutagenicity of the carcinogen (7); at a dose of 10 μg/plate about 95% inhibition is observed.

DISCUSSION

Examination of Table 1 discloses that without exception all 37 carcinogens and the known initiator (35) are mutagens. The 7 noncarcinogens marked with asterisks are also mutagenic, whereas the remaining 9 noncarcinogens are inactive. There are no carcinogens that are not mutagens; even the very weak carcinogens, characterized by low tumor incidence and long latent period (e.g., hydrocarbons 28 and 34), are readily detected as mutagens.

On the other hand it is disappointing that there is little evidence of correlation between carcinogenic and muta-
Carcinogenicity and Mutagenicity

generic potency, except perhaps in the 2 isomeric series (2 to 8 and 35 to 40). Correlation was no better than when papillomas and carcinomas were considered separately for Compounds 1 to 34. A decrease in the number of revertant colonies is commonly seen with increase in dose (Charts 2 and 3), and this is ascribed to toxicity (1). Both in Ames’s table (12) and in this work, it is noticeable that strong carcinogens often appear to be less mutagenic than are weaker carcinogens of the same structural type, e.g., compare 7,12-dimethylbenz[a]anthracene (54) with the less active 7-methyl compound (46) or the 2 ketones (7 and 16). Species and organ variability may contribute to the lack of quantitative correspondence between carcinogenicity and mutagenicity, for whereas rat liver was used as the source of microsomes for the bacterial mutagenicity tests carcinogenic activity was measured by tumor induction on mouse

### Table 1

<table>
<thead>
<tr>
<th>Compound Description</th>
<th>C*</th>
<th>A</th>
<th>B</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopent[a]phenanthrenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1) 15,16-Dihydro-17-one</td>
<td>&lt;1</td>
<td>820/20</td>
<td>9.5*</td>
<td>7</td>
</tr>
<tr>
<td>(2) 2-Methyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>23/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(3) 3-Methyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>69/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(4) 4-Methyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>82/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(5) 6-Methyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>44/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(6) 7-Methyl-15,16-dihydro-17-one</td>
<td>10</td>
<td>1000/20</td>
<td>12.3</td>
<td>3</td>
</tr>
<tr>
<td>(7) 11-Methyl-15,16-dihydro-17-one</td>
<td>46</td>
<td>1760/20</td>
<td>21.7</td>
<td>3, 7</td>
</tr>
<tr>
<td>(8) 12-Methyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>72/100</td>
<td>&lt;0.2</td>
<td>7</td>
</tr>
<tr>
<td>(9) 3-Methoxy-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>71/100</td>
<td>0.2</td>
<td>7</td>
</tr>
<tr>
<td>(10) 11-Methyl-15,16-dihydro-17-one</td>
<td>25</td>
<td>240/20</td>
<td>3.1</td>
<td>7</td>
</tr>
<tr>
<td>(11) 11-Ethyl-15,16-dihydro-17-one</td>
<td>8</td>
<td>350/50</td>
<td>1.8</td>
<td>3</td>
</tr>
<tr>
<td>(12) 11-n-Butyl-15,16-dihydro-17-one</td>
<td>&lt;1</td>
<td>4/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(13) 11,12-Dimethyl-15,16-dihydro-17-one</td>
<td>30</td>
<td>220/50</td>
<td>1.1</td>
<td>7</td>
</tr>
<tr>
<td>(14) 11-Methyl-6-hydroxy-15,16-dihydro-17-one</td>
<td>11</td>
<td>700/10</td>
<td>18.3</td>
<td>3</td>
</tr>
<tr>
<td>(15) 11-Methyl-6-methoxy-15,16-dihydro-17-one</td>
<td>14</td>
<td>310/50</td>
<td>1.7</td>
<td>3</td>
</tr>
<tr>
<td>(16) 7-Methyl-11-methoxy-15,16-dihydro-17-one</td>
<td>17</td>
<td>2500/20</td>
<td>34.5</td>
<td>3</td>
</tr>
<tr>
<td>(17) 11-Methyl-11,12,15,16-tetrahydro-17-one</td>
<td>&lt;1</td>
<td>80/100</td>
<td>&lt;0.2</td>
<td>3</td>
</tr>
<tr>
<td>(18) 16,17-Dihydro-15-one</td>
<td>&lt;1</td>
<td>290/10</td>
<td>6.7*</td>
<td>7</td>
</tr>
<tr>
<td>(19) 11-Methyl-16,17-dihydro-15-one</td>
<td>5</td>
<td>750/50</td>
<td>3.7</td>
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<tr>
<td>(20) 17-Methyl-15H</td>
<td>6</td>
<td>350/50</td>
<td>1.6</td>
<td>7</td>
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<tr>
<td>(21) 11,17-Dimethyl-15H</td>
<td>27</td>
<td>200/50</td>
<td>1.0</td>
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<tr>
<td>(22) 11,17-Dimethyl-15H</td>
<td>7</td>
<td>250/50</td>
<td>1.0</td>
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<tr>
<td>(23) 11,12,17-Trimethyl-15H</td>
<td>23</td>
<td>90/20</td>
<td>1.2</td>
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<tr>
<td>(24) 3-Methoxy-17-methyl-15H</td>
<td>&lt;1</td>
<td>140/50</td>
<td>0.7*</td>
<td>7</td>
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<tr>
<td>(25) 11-Methoxy-17-methyl-15H</td>
<td>12</td>
<td>170/50</td>
<td>0.9</td>
<td>7</td>
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<tr>
<td>(26) 17H</td>
<td>&lt;1</td>
<td>930/50</td>
<td>4.0*</td>
<td>7</td>
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<tr>
<td>(27) 17-Methyl-15,16-dihydro- (Diels’ hydrocarbon)</td>
<td>&lt;1</td>
<td>150/50</td>
<td>0.7*</td>
<td>7</td>
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<tr>
<td>(28) 11,17-Dimethyl-15,16-dihydro</td>
<td>3</td>
<td>125/20</td>
<td>1.5</td>
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<tr>
<td>(29) 3-Methoxy-17-methyl-15,16-dihydro</td>
<td>&lt;1</td>
<td>34/100</td>
<td>&lt;0.2</td>
<td>7</td>
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Chrysenes

<table>
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<tr>
<th>Compound Description</th>
<th>C*</th>
<th>A</th>
<th>B</th>
<th>Ref.</th>
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<tr>
<td>(30) 1,2,3,4-Tetrahydro-1-one</td>
<td>&lt;1</td>
<td>1460/50</td>
<td>7.2*</td>
<td>4</td>
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<td>(31) 11-Methyl-1,2,3,4-tetrahydro-1-one</td>
<td>45</td>
<td>1370/50</td>
<td>7.1</td>
<td>4</td>
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<tr>
<td>(32) 1,11-Dimethyl</td>
<td>23</td>
<td>160/20</td>
<td>2.1</td>
<td>4</td>
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<td>(33) 3,4-Dihydro-1,11-dimethyl</td>
<td>10</td>
<td>210/20</td>
<td>2.7</td>
<td>4</td>
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<tr>
<td>(34) 1,2,3,4-Tetrahydro-1,11-Dimethyl</td>
<td>4</td>
<td>55/20</td>
<td>0.7</td>
<td>4</td>
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<tr>
<td>(35) 1-Methyl</td>
<td>Initiator</td>
<td>285/10</td>
<td>6.9</td>
<td>11</td>
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<tr>
<td>(36) 2-Methyl</td>
<td>Weak</td>
<td>155/10</td>
<td>3.7</td>
<td>11</td>
</tr>
<tr>
<td>(37) 3-Methyl</td>
<td>Weak</td>
<td>340/20</td>
<td>4.1</td>
<td>11</td>
</tr>
<tr>
<td>(38) 4-Methyl</td>
<td>Weak</td>
<td>445/10</td>
<td>10.8</td>
<td>11</td>
</tr>
<tr>
<td>(39) 5-Methyl</td>
<td>Moderate</td>
<td>1245/10</td>
<td>30.1</td>
<td>11</td>
</tr>
<tr>
<td>(40) 6-Methyl</td>
<td>Weak</td>
<td>92/10</td>
<td>2.2</td>
<td>11</td>
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</table>
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Table 1—Continued

<table>
<thead>
<tr>
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<th>A</th>
<th>B</th>
<th>Ref.</th>
</tr>
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<tbody>
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<td>Benz[a]anthracenes</td>
<td></td>
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<td></td>
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<tr>
<td>(41) Benz[a]anthracene</td>
<td>Weak</td>
<td>994/20</td>
<td>11.3</td>
<td>10</td>
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<tr>
<td>(42) 1-Methylbenz[a]anthracene</td>
<td>±</td>
<td>500/20</td>
<td>6.0</td>
<td>10</td>
</tr>
<tr>
<td>(43) 2-Methylbenz[a]anthracene</td>
<td>±</td>
<td>313/10</td>
<td>7.6</td>
<td>10</td>
</tr>
<tr>
<td>(44) 3-Methylbenz[a]anthracene</td>
<td>Inactive</td>
<td>194/5</td>
<td>9.4</td>
<td>10</td>
</tr>
<tr>
<td>(45) 4-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>2462/20</td>
<td>30.2</td>
<td>10</td>
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<tr>
<td>(46) 5-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>659/10</td>
<td>16.9</td>
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</tr>
<tr>
<td>(47) 6-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>318/10</td>
<td>7.7</td>
<td>10</td>
</tr>
<tr>
<td>(48) 7-Methylbenz[a]anthracene</td>
<td>Strong</td>
<td>1275/20</td>
<td>15.4</td>
<td>10</td>
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<tr>
<td>(49) 8-Methylbenz[a]anthracene</td>
<td>Moderate</td>
<td>330/20</td>
<td>4.0</td>
<td>10</td>
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<tr>
<td>(50) 9-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>348/20</td>
<td>4.4</td>
<td>10</td>
</tr>
<tr>
<td>(51) 10-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>122/10</td>
<td>3.0</td>
<td>10</td>
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<tr>
<td>(52) 11-Methylbenz[a]anthracene</td>
<td>Weak</td>
<td>464/50</td>
<td>2.3</td>
<td>10</td>
</tr>
<tr>
<td>(53) 12-Methylbenz[a]anthracene</td>
<td>Moderate</td>
<td>460/20</td>
<td>5.7</td>
<td>10</td>
</tr>
<tr>
<td>(54) 7,12-Dimethylbenz[a]anthracene</td>
<td>Very strong</td>
<td>437/20</td>
<td>5.6</td>
<td>10</td>
</tr>
</tbody>
</table>

* C, carcinogenicity; M, mutagenicity; *, compounds that are mutagens, but not known carcinogens; ±, compounds for which evidence of carcinogenicity rests on the observation of single papillomas.

Chart 2. Dose-response curves for the carcinogen (5) at 3 liver enzyme levels (volumes of S-9). For details see text.

Chart 3. Inhibition of the mutagenicity of the ketone (7) with the aryl hydrocarbon hydroxylase inhibitor 7,8-benzoflavone. Each plate contained the inhibitor (300 µg) and S-9 (100 µg). Similar results were also obtained with the chrysene (41), benz[a]pyrene, and dibenz[a,h]anthracene.

skin. Experiments are in progress to examine this possibility. However, a more fundamental reason for these discrepancies may be connected with the specificity shown by carcinogens for the target sequence in the bacterial DNA, a mutation that causes reversion to prototrophy. Ames (13) has shown that certain classes of chemical carcinogens are especially effective in causing reversions in particular strains of *S. typhimurium*, whereas they are much less active in other strains. In this work it was found that acetamidofluorene was very efficient in causing frame-shift mutations in both TA98 and TA1538 but was markedly less able to revert TA100 by base-pair substitution. The opposite behavior was noted for a number of cyclopenta[a]phenanthrenes, whereas benzo(a)pyrene showed less specificity. It seems that each carcinogen has its own inherent specificity for the different base sequences involved in these mutations, and hence it is unlikely that a quantitative relationship between mutagenicity and carcinogenicity would hold among a series of different compounds tested with a particular bacterial strain.

Among the 6 monomethyl chrysenes, all of which have been carefully tested both as initiators and as complete carcinogens (11), the 1-, 2-, 3-, 4-, and 6-isomers (35 to 38, and 40) are comparable mutagens, all possess similar initiating activity, and all are complete carcinogens except 1-methylchrysen (35). On this basis one might expect 1-, 2-, and 3-methylbenz[a]anthracenes (42 to 44) to be initiators; evidence for the carcinogenicity of Compounds 42 and 43 is meager and it is negative for that of 44, but all the other isomers appear to be carcinogenic to varying extents (10). Of the other 6 mutagens that are not carcinogens, 4 (Compounds 1, 18, 27, and 30) are parent molecules, which are unsubstituted in the phenanthrene ring system but which become carcinogenic when substituted with a methyl group at C-11. In Series 1 to 8 it appears that a methyl group can either enhance or diminish mutagenicity, depending upon the position of its substitution. It is not known whether the 7 noncarcinogenic mutagens are initiators. An experiment is in hand to test certain of them by the classical skin painting method, using croton oil as the promoting agent.
The marked inhibition of the mutagenicity of the carci-
ogen (7) in the presence of 7,8-benzoflavone (Chart 3) is in
line with the strong inhibition also observed in the covalent
binding of this ketone to DNA \textit{in vitro}, after metabolism in
the presence of the flavone (5). This inhibitor also sup-
pressed tumor formation by Compound 7 when both were
painted together on to the backs of mice (5). We have also
found marked inhibition of mutagenicity with the chrysen-
one (31), benzo[a]pyrene, and dibenz[a,h]anthracene. Skin
tumor production is suppressed to varying extents when
this inhibitor is repeatedly painted together with these car-
cinogens (unpublished observations).

In summary, Ames’s test, using \textit{S. typhimurium} TA100, is
found to provide a sensitive and convenient qualitative
method for the detection of carcinogens among polycyclic
compounds by virtue of their mutagenic activity.

ACKNOWLEDGMENTS

We wish to thank Dr. B. N. Ames for the samples of bacteria, Dr. S. S.
Hecht and Dr. R. F. Newbold for gifts of the isomeric methylchrysenes (35 to
40) and benzanthracenes (42 to 53), respectively, and Dr. Stonard for a
sample of Aroclor 1254. Dr. R. Dubeczko suggested the use of Ames’s test,
and it is a pleasure to acknowledge his stimulating help and encouragement.

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The Relative Carcinogenic Activities of a Series of 5-Methylchrysene Derivatives

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Chemistry Department, Imperial Cancer Research Fund, Lincoln’s Inn Fields, London WC2A 3PX [M. M. C., T. S. B., M. H., and the National Institute for Medical Research, Mill Hill, London, NW7 1AA [C. J. C.], England

SUMMARY

The carcinogenicity of 1,11-dimethylchrysene (\(\equiv 5,7\)-dimethylchrysene) and its 1,2,3,4-tetrahydro-, 3,4-dihydro-, and 1,2,3,4-tetrahydro-1-oxo derivatives was tested by mouse skin painting. Each mouse received 30 \(\mu\)g of the chrysene on the dorsal skin twice weekly for one year, and the animals were observed for a second year.

The 1-ketone was much more active than any of the hydrocarbons. Of the latter, the fully aromatic 1,11-dimethylchrysene was fairly active, the 3,4-dihydro derivative was less so, while the 1,2,3,4-tetrahydro compound was a weak carcinogen.

These results are discussed in connection with the carcinogenicity of the corresponding cyclopenta[a]phenanthrenes, which exhibit a similar structure-activity relationship.

INTRODUCTION

The polycyclic hydrocarbon chrysene (I), like its isomer benz[a]anthracene (II), has generally been found to lack or to possess at most marginal carcinogenic activity (14a, 16). However, methyl substitution in the latter at certain, specific ring positions, particularly C-7 (14b) and C12 (14c), promotes activity so that 7,12-dimethylbenz[a]anthracene is one of the most potent carcinogens known (Chart 1).

The methyl derivatives of chrysene have been less extensively studied. Like the parent hydrocarbon the 1-methyl (13), the 4- and 6-methyl (12), the 2,3-dimethyl (15), and the 4,5-dimethyl derivatives (12) either lack or possess very weak carcinogenic activity. By contrast, the 5-methyl (12) and 5,6-dimethyl derivatives (1, 12) of chrysene are moderately strong carcinogens. C-5 (\(\equiv C-11\)) in chrysene is in the same position relative to the 3 rings comprising the phenanthrene system as is C-12 in benz[a]anthracenes.

A similar situation exists with regard to the associated structure 15,16-dihydrocyclopenta[a]phenanthrene (IIIa), which is of interest because it possesses the same ring system as do the natural steroids. Both the parent hydrocarbon (IIIa) and its 17-methyl derivative (Diel’s hydrocarbon, IIIb) are without activity, as are the majority of the isomeric methyl and dimethyl derivatives. Weak carcinogenicity is conferred by methyl substitution at C-11 (5, 7) to give IIIc and \(X\) in which the aryl methyl groups are positionally equivalent to C-5 in chrysenes and C-12 in benz[a]anthracenes. It seems probable that the correlation between carcinogenicity and methyl substitution at these equivalent ring positions is not fortuitous and that these methyl groups therefore exert some common effect that endows these hydrocarbons with activity.

Introduction of a conjugated \(\Delta^{14}\)-double bond into Diel’s hydrocarbon also confers weak activity (7, 11), and it reinforces the effect of 11-methyl substitution so that \(IX\) is a much stronger carcinogen than either IIIC or \(X\). Thus, with the use of the same strain of mice and the same skin painting technique used in the experiment described in this paper, \(IX\) induced skin tumors at the site of application in 17 of 18 animals (based on the number of animals alive at the appearance of the 1st tumor), with a mean latent period of 48 weeks (7). More interesting was the discovery that introduction of a carbonyl group at C-17 also greatly augments the activity conferred by 11-methyl substitution, so that 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (\(VIIb\)) (100% tumor incidence, 31 weeks, compared with 21 weeks for 20-methyl-cholanthrene used as a positive control in the same experiment) (6) is more active than the most active hydrocarbon (\(IX\)). The parent ketone (\(VIIa\)) was without activity.

On exploration of the consequences of structural alterations of \(VIIb\) on its carcinogenicity, it was found that the homolog with 1 extra methylene group in the nonaromatic ring, namely 11-methyl-1,2,3,4-tetrahydrochrysen-1-one (\(IVb\)), retained the high potency of \(VIIb\). This methylchrysenone induced skin tumors in 17 of 18 mice with a mean latent period of 30 weeks. As in the cyclopenta[a]phenanthrene series, the parent, unsubstituted ketone (\(Ib\)) was devoid of activity.

With this methylchrysenone (\(Ib\)) to hand, 1,11-dimethylchrysenone (\(VII\)) and the 3,4-dihydro (\(V\)), and 1,2,3,4-tetrahydro (\(V\)) derivatives were readily available (Chart 2). It was of interest to determine whether the carcinogenic potency of these compounds would fall into the order \(IPb > V > VII\), i.e., the same order as their cyclopenta[a]phenanthrene counterparts \(VIIb\), \(IX\), and \(X\). The relative activity of the fully aromatic analog (\(VII\)) was also of interest for, of course, this can have no counterpart in the series bearing a 5-membered ring. This paper describes the preparation and testing of these compounds by our standard skin painting technique.
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Chart 1. Chemical structures mentioned in the text.

Chart 2. Chrysenes tested by skin painting (IVb to VII), and their cyclopenta[a]phenanthrene analogs (VIIb to X).

MATERIALS AND METHODS

Chemistry

The preparation of the methylchrysenone (IVb) has been reported previously (10). Equipment and general methods used are as recently described (6). Wåselm Grade I alumina (Koch-Light Laboratories Ltd., Colnbrook, SL3 OBZ, Buckinghamshire, England) was used for the purification of hydrocarbons.

Preparation of 3,4-Dihydro-1,11-dimethylchrysene (V). The ketone (IVb) (2.60 g) in benzene (26 ml) was added gradually to a solution of magnesium iodide [from magnesium turnings (2.0 g), methyl iodide (3.0 ml), and dry ether (50 ml)], and the mixture was heated under reflux for 1 hr. The ether was removed by distillation while more benzene (50 ml) was added, and heating was continued for a further 2 hr. The cooled reaction mixture was poured into aqueous ammonium chloride (10%, w/v) (125 ml) containing 5 N HCl (2.5 ml), more benzene was added, and the whole was shaken vigorously. The organic layer was separated without delay, washed with aqueous NaHCO₃, and dried over anhydrous Na₂SO₄. The residue left on removal of the solvent was purified by chromatography on a column of alumina (100 g) using hexane as the eluant. Fractions homogeneous by thin-layer chromatography were combined to yield a cream-colored solid (1.46 g). Recrystallization from methanol gave 3,4-dihydro-1,11-dimethylchrysene (V), m.p. 104–104.5°.

\[
\text{C}_{20}\text{H}_{18} \\
\text{Required: C 93.0; H 7.0} \\
\text{Found: C 92.8; H 6.85}
\]

\[
\lambda_{\text{max}} \text{H}_{\text{max}} 223.5 (\log \epsilon 4.33), 272 (4.70), 284.5 (4.70), 298 (4.28), 308 sh (4.13), 321 (3.72), 355 (2.89), and 372 nm (2.87); \nu_{\text{max}} 874, 822, 749 \text{ cm}^{-1}
\]

Preparation of 1,11-Dimethylchrysene (VII). The above dihydro compound (345 mg) in dry benzene (135 ml) was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (335 mg) at room temperature for 24 hr. Precipitated hydroquinone was collected, and the filtrate was evaporated to leave a brown solid (431 mg). Chromatography on a column of alumina (100 g) with hexane gave a white solid (270 mg) which on recrystallization from methanol yielded 1,11-dimethylchrysene (VII) (150 mg), m.p. 168.5–169°.

\[
\text{C}_{20}\text{H}_{16} \\
\text{Required: C 93.7; H 6.3} \\
\text{Found: C 93.6; H 6.4}
\]

\[
\lambda_{\text{max}} 225 (4.43), 265 sh (4.84), 272 (4.96), 304 (3.97), 317 (4.10), 330.5 (4.10), 350 (3.99), and 369 nm (3.79); \nu_{\text{max}} 864, 798, and 778 \text{ cm}^{-1}
\]

Preparation of 1,11-Dimethyl-1,2,3,4-tetrahydrochrysene (VI). A solution of the dihydro compound (V) (305 mg) in ethyl alcohol (150 ml) was stirred with 5% palladium-on-charcoal catalyst (150 mg) in an atmosphere of hydrogen for 90 min. After removal of the catalyst by filtration, the solution was evaporated to leave a white solid. Recrystallization from methanol (50 ml) gave 1,11-dimethyl-1,2,3,4-tetrahydrochrysene (VI) (170 mg), m.p. 69–69.5°.

\[
\text{C}_{20}\text{H}_{20} \\
\text{Required: C 92.3; H 7.7} \\
\text{Found: C 92.5; H 7.5}
\]

\[
\lambda_{\text{max}} 484 (4.30), 256.5 (4.80), 282 (4.00), 294.5 (4.02), 306 (4.09), 338.5 (2.76), and 354 nm (2.69); \nu_{\text{max}} 813 and 748 \text{ cm}^{-1}
\]

Skin Painting Experiment

This was carried out as previously described (6). Groups of 10 male and 10 female mice were used for each compound, 30 µg of which were applied as a 0.5% w/v solution in toluene twice weekly for 50 weeks. The animals
Carcinogenicity of 5-Methylchrysenes

were observed for a 2nd year or killed when their induced tumor had reached 8 to 10 mm in diameter. Tumors were examined histologically except in 8 cases in which tissue was unavailable because death had occurred and autolysis was too far advanced. The results of this experiment are summarized in Table 1 and in Chart 3.

RESULTS

Of the 4 chrysenes tested, the 1-ketone (IVb) was by far the most active, producing tumors in 17 of the 20 mice alive at the appearance of the 1st tumor. The mean latent period, 27 weeks, was similar to that (31 weeks) previously reported (6) (the mean latent for the control, 20-methylcholanthrene, was 22 weeks compared with 21 weeks found previously). The least active chrysene was the tetrahydro hydrocarbon (VII) (3 of 18 mice, 55 weeks). In both respects, these chrysenes are similar to the corresponding cyclopenta[a]phenanthrenes. However, the dihydrochrysene (V) was distinctly less active (8 of 20, 57 weeks) than the hydrocarbon (IX) (7) (17 of 18, 48 weeks), with regard to both the tumor incidence and latent period. Indeed, it was the fully aromatic chrysene (VII) (15 of 19, 50 weeks) that had activity similar to that of IX.

DISCUSSION

These results indicate that the size of the 4th, partially saturated ring has little influence on the relative carcinogenicity of the ketones VIIb and IVb, and of the tetrahydro hydrocarbons X and VII. This is not the case with the dihydrohydrocarbons IX and V. Here the sterically strained, 5-membered cyclopentadiene ring in IX induces carcinogenic activity to an extent similar to that induced by the fully aromatic, 6-membered ring in the chrysene (VII), whereas the corresponding dihydrochrysene (V) resembles the less active tetrahydrochrysene (VII) in latent period, although its tumor incidence was higher. The electrons associated with the partially unsaturated ring in IX are less localized than those in V. In certain chemical reactions (8, 9), cyclopenta[a]phenanthrenes react with bond migration as shown in Chart 4A, whereas this does not occur with 3,4-dihydrochrysenes (for example, V). The fused cyclopentadiene ring therefore resembles, in some respects, an aromatic ring, and this seems to be reflected in the similar carcinogenic activity possessed by IX and VII.

Chart 3. Skin tumor production by chrysenes (IVb to VII) and methylcholanthrene (MC). Experimental details are given in "Materials and Methods." The unsubstituted chrysenone VIIa was inactive in this system (6). All visible skin tumors grew progressively.

Table 1

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>No. of tumorless survivors at</th>
<th>No. of mice with Squamous papilloma</th>
<th>Squamous carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 mo.</td>
<td>12 mo.</td>
<td>18 mo.</td>
</tr>
<tr>
<td>11-Methyl-1-oxo-1, 2, 3, 4-tetrahydro-(IVb)</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,11-Dimethyl-1, 2, 3, 4-tetrahydro-(V)</td>
<td>20</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>3,4-Dihydro-1, 11-dimethyl-(V)</td>
<td>20</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>1, 11-Dimethylchrysene-(VII)</td>
<td>19</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Methylcholanthrene</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Toluene control</td>
<td>19</td>
<td>18</td>
<td>14</td>
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<tr>
<td>Clipping control</td>
<td>20</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

* Tumors were unavailable for histology for these compounds as follows: VII, 3; V, 2; VII, 3.

* An additional animal had a tumor (spindle-cell sarcoma) at the site of application.

Chart 4. A, bond migration in cyclopenta[a]phenanthrenes; B, possible incorporation of the angular 18-methyl group into the D-ring in plant steroids from N. physaloides.
In both the cyclopenta[a]phenanthrene and chrysene series, introduction of a doubly bonded ketone oxygen atom at the benzylic position equivalent to C-17 in a steroid augments the activity of the hydrocarbon. Again, the effect is similar to the effect of fusion of an additional aromatic ring, now at C-12 and C-17. In the case of cyclopenta[a]phenanthrene, this would lead to cholangrene which is a strong carcinogen, somewhat less active than its well-known 20-methyl homolog (14d). Since both the ketone and fused aromatic ring can be regarded as electron-withdrawing substituents, it seems probable that both function by altering the electron distribution in the phenanthrene system.

Formally, the chrysene ring system can be derived from a C18 steroid by incorporation of the carbon of the 18-methyl group into the D-ring, as indicated in Chart 4B, XI → XIII. A particularly clear indication that this may occur naturally is afforded by recent investigations (2–4) on the family of insect-repellent plant steroids isolated from Nicandra physaloides. Certain of these are normal steroids bearing a 18-methyl group and having a side chain at C-17. Others lack the 18-methyl group and possess an aromatic 6-membered D-ring bearing the same side chain, but now situated at C-2 in this ring, C-1 having presumably been supplied by incorporation of the methyl group.

The isolation of naturally occurring derivatives of chrysene adds interest and importance to the examination of the relationship between structure and carcinogenic activity in the chrysene series.

ACKNOWLEDGMENTS

The authors wish to thank W. A. Dick and F. P. Wharton for technical assistance.

REFERENCES

Carcinogenic cycloPenta[a]phenanthrenes

M. M. Coombs and C. J. Croft

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I. Introduction

Interest in a possible relationship between carcinogenic polycyclic hydrocarbons and the natural steroids originated from the early work on chemical carcinogenesis [Cook, 1933]. Following the dramatic chemical transformation of the bile acid derivative, deoxycholic acid, into the potent carcinogen 20-methylcholanthrene (20-MC) independently by Wieland and Dane [1933] and Cook and Haslewood [1933], speculation in this field centered upon the possibility of in vivo production of cholanthenes and this was extensively reviewed by Inhoffen [1953] and by Haddow [1958]. The early concepts were extended by Fieser [1941] who suggested that if endogenous carcinogens do arise as a result of abnormal steroid metabolism, the process might be closely related to the biosynthesis of the partially aromatic steroids, the oestrogens. In order

to provide a fifth fused ring, at that time-considered essential for carcinogenic activity, he suggested that such steroids might condense with pyruvic acid and subsequently undergo cyclization to compounds of the cholanthrene series.

Subsequent work revealed that carcinogenic properties are associated with a great number of polycyclic hydrocarbons, and these results tended to overshadow the earlier interest in cholanthrenes. It was noted that the majority of these hydrocarbons were derivatives of phenanthrene having additional, fused aromatic rings and much effort was expended in attempts to correlate chemical structure and reactivity with the carcinogenic activity shown by these compounds. In particular, attempts to use quantum mechanical indices of electron density of the so-called K-regions as a measure of carcinogenic potency met with success for certain limited series of closely related hydrocarbons [DAUDEL and DAUDEL, 1966], but failed to account for the effects of alkyl substitution. Thus, in the benzanthracene series, methyl substitution at C-1 to C-4 has little effect on the marginal activity of the parent hydrocarbon, whereas methyl substitution at C-7 and C-12 produces extremely active carcinogens. In the cholanthrene series, lengthening or branching the hydrocarbon substituent at C-20 drastically lowers activity. Neither of these effects can be accounted for on purely electron density grounds, and the unavoidable conclusion is that while high electron density in the K-region is of undoubted importance in conferring carcinogenic properties, its action may be cancelled by steric effects of the ring substituents. This has been emphasized by YANG, CASTRO, LEWIS and WONG [1961], who pointed out the close spatial relationship between several classes of steroid hormones and some of the strongest polycyclic hydrocarbon carcinogens. They suggested that the latter might act by occupying the same receptor sites as these hormones.

While considerable knowledge of the carcinogenicity of polycyclic hydrocarbons containing four or more condensed aromatic
rings has been gained, less attention has been paid to members of the cyclopenta[a]phenanthrene series having the same ring system as the steroids, with only three fused aromatic rings. The lack of activity of the parent hydrocarbon (I) and its 17-methyl homologue (Diels' hydrocarbon), both of which were isolated from the products of dehydrogenation of a number of steroids at an early stage [Fieser and Fieser, 1959], is probably a factor which diverted interest from the cyclopenta[a]phenanthrenes as potential endogenous carcinogens. Also, the large number of different chemical types now known to possess carcinogenic properties has tended to diminish interest in polycyclic hydrocarbon carcinogenesis. However, in a systematic study of the methyl homologues of (I), Butenandt and Dannenberg [1953] established that while the 1-, 2-, 4- and 6-monomethyl, the 4,17-, 6,17-, 2,12-, 4,12- and 6,7-dimethyl, and 6,17, 17-trimethyl derivatives of (I) were inactive, weak carcinogenic activity was conferred by methyl substitution at C-7 and C-11. In later work on the nature of the carcinogenic constituent of the hydrocarbon mixture obtained by chloranil dehydrogenation of cholesterol, Dannenberg [1960] showed that the 17-methyl derivative of the A14-hydrocarbon (II) and its 15-methyl- A15 isomer also possessed weak carcinogenic activity. In view of the results of his previous work mentioned above, he suggested that 11-methyl substitution might increase the activity of these compounds.

II. Biosynthetic Considerations

The earlier search for endogenous carcinogens related to the steroids was carried out at a time when little was known about the biosynthesis of the latter. In particular, a difficulty encountered in

\[ \text{Note on chemical nomenclature. The term cyclopenta[a]phenanthrene is properly reserved for the fully unsaturated hydrocarbon of which (II) is the 15-H-isomer; (I) is therefore its 16,17-dihydro derivative. The term will be employed loosely in this paper to imply both types of structure. These compounds are numbered according to the steroid convention, as shown.} \]
effecting the *in vivo* transformation of a C_{18} or C_{19} steroid into a cyclopenta[10]phenanthrene was the presence of the angular 18- and 19-methyl groups, which block aromatization. Recent work on the metabolism and biosynthesis of steroids has largely removed these difficulties, so that *in vivo* formation of cyclopenta [a] phenanthrene derivatives now appears distinctly feasible.

Complete aromatization of a typical C_{19} steroid such as androstosterone requires removal of the two angular methyl groups and would lead to the phenolic ketone (III); simultaneous elimination of the 3-oxygen function would give the corresponding 3-deoxyketone (IV).

![Chemical structures](image)

Precedents for loss of the 19-methyl group and aromatization of rings A and B are provided by the well-known, partially aromatic steroids such as oestrone and equilenin, while a precedent for the additional loss of the 3-oxygen function is afforded by 3-deoxyequilenin. This compound was isolated by two groups of workers, Marker and Rohrmann [1939] and Prelog and Führer [1945], from mare's pregnancy urine under conditions which preclude its formation as an artefact. The biosynthetic derivation of this compound is not known. If it is formed in common with the 3-oxygenated steroids by way of lanosterol, elimination of the 3-oxygen atom must occur before the final aromatization step. However, another possibility exists, because Barton and Moss [1966] have recently indicated that in yeast, squalene cyclization follows the route: squalene $\rightarrow$ lanosta-8,24-diene (enzyme bound) $\rightarrow$ lanosterol. If the enzyme-bound hydrocarbon became free, presumably it could lead to a series of 3-deoxy steroids. In this connection it is interesting that many triterpenoids lack oxygen at C-3, although they are also derived from squalene.
All C\textsubscript{18} oestrogens lack the 19-methyl group and it is now firmly established that they are biosynthesized from C\textsubscript{19} androgens by oxidative elimination of this methyl group followed by aromatization of ring-A [Talalay, 1965]. Recent work suggests that the 18-methyl group may also be eliminated by a similar mechanism. Thus, the biosynthesis of aldosterone is now known to involve hydroxylation of the 18-methyl group, probably of corticosterone [Eisenstein, 1967], so that 18-hydroxylation is established as a normal adrenal function, at least for C\textsubscript{21} steroids. In 1958, Marnian isolated a new oestrogen metabolite, 18-hydroxyoestrone, from human pregnancy urine and showed that under mild conditions \textit{in vitro} it readily underwent \(\beta\)-elimination of formaldehyde to yield 18-nor-oestrone. The way for complete aromatization to the \textit{cyclopenta}[\alpha]\textit{phenanthrene} analogue (III) by straightforward biological dehydrogenation is thus theoretically open. In order to establish whether compounds of this type are carcinogenic, and if so, what the structural requirements for such action are, the \textit{cyclopenta}[\alpha]\textit{phenanthrenes} shown in figure 1 were synthesized and tested for carcinogenicity in mice.

\[ \text{III. Methods} \]

\textbf{A. Chemistry}

The \textit{cyclopenta}[\alpha]\textit{phenanthrenes} used in this study are shown in figure 1. They include compounds bearing oxygen substituents at the biologically interesting 3-, 11- and 17-positions, as well as methyl at C-11 which, on the basis of Butenandt and Dannenberg’s results [1953], was expected to confer carcinogenicity in certain derivatives.

The ketones 2\textsubscript{a}–f were prepared by variations on the general route devised by Robinson [1938] for the construction of the steroid skeleton. The ketones were converted by means of the
methyl Grignard reagent to the 17-methyl-Δ7 hydrocarbons 1a–f which were hydrogenated to the 17-methyl saturated D-ring derivatives 3a–f. Details of the synthesis of these compounds and of compounds 4–6 have been published [Coombs, 1966].

![Chemical structures](image)

Fig. 1. Compounds tested by skin painting.

### B. Animal experiments

Two skin-painting experiments were performed. The methods and preliminary results of the first experiment have been reported previously [Coombs and Croft, 1966]. The final results of this experiment are included in table I, where they are indicated by an asterisk*. The second experiment was performed in an identical manner to the first, except that toluene was used as the solvent instead of benzene. As in the first experiment, one drop (6 μl) of a 0.5% (w/v) solution of each chemical was applied twice a week for 52 weeks to the dorsal skin of 20 T.O. random-bred mice, and they were observed for a second year. Two negative control groups consisted of animals painted with the solvent (toluene) only, and animals which were clipped but otherwise untreated. In addition, two positive control groups consisting of animals painted with
Table 1. Number of mice surviving without tumors and histology of induced tumors at site of application in the skin painting experiments

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Number of tumorless survivors at:</th>
<th>Number of mice with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 months 12 months 18 months 24 months Squamous papilloma Squamous carcinoma Spinal-cell carcinoma</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>17 10 5 0 0 1 14 0</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>18 9 0 0 0 1 16 0</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td>18 15 10 2 1 4 0 0</td>
<td></td>
</tr>
<tr>
<td>1d*</td>
<td>19 13 10 0 3 3 0 0</td>
<td></td>
</tr>
<tr>
<td>1e</td>
<td>19 16 12 0 0 1 2 0</td>
<td></td>
</tr>
<tr>
<td>1f</td>
<td>19 19 18 7 0 0 1 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18 16 13 4 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>17 11 4 0 1 4 0 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16 16 14 5 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14 6 0 0 2 1 11 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17 2 0 0 1 1 13 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18 0 0 8 5 2 0 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>17 8 3 0 2 9 0 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>18 9 6 4 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18 7 6 3 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>15 14 13 3 0 0 1 0</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>20 17 15 6 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2b*</td>
<td>18 0 0 8 5 2 0 0</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>17 8 3 0 2 9 0 0</td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>18 9 6 4 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2d*</td>
<td>20 15 4 1 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>2f</td>
<td>15 14 13 3 0 0 1 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20 17 15 6 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18 13 9 2 0 6 0 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15 13 8 2 0 6 0 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>14 13 9 3 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17 17 12 5 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>20 13 7 3 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>16 15 13 7 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>19 19 17 7 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>DMBA</td>
<td>17 13 8 3 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>9 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Solvent (toluene)</td>
<td>20 20 18 8 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Clipping</td>
<td>20 20 18 8 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Solvent* (benzene)</td>
<td>17 13 8 3 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Clipping*</td>
<td>20 18 16 13 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>

* Results of first experiment (see page 74).

7,12-dimethylbenz[a]anthracene (DMBA) and 20-methylcholanthrene (MC) were included.

Compounds 2a, 2b and 2d were also tested by subcutaneous injection into formally randomized groups of T.O. mice from the
### Table II. Tumours following subcutaneous injection of ketones 2a, 2b and 2d

<table>
<thead>
<tr>
<th>Compound injected</th>
<th>Dosage</th>
<th>Number of mice injected</th>
<th>Number of tumourless survivors at end of experiment</th>
<th>Number of mice with local spindle cell sarcoma</th>
<th>Number of mice with remote skin tumours</th>
<th>Histology of largest skin tumour in each animal: Number of mice with:</th>
<th>Squamous papilloma</th>
<th>Squamous carcinoma</th>
<th>Sebaceous adenoma</th>
<th>Mixed sebaceous adenoma and squamous papilloma</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>8 mg in 0.2 ml olive oil</td>
<td>21</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>2b</td>
<td>8 mg in 0.2 ml olive oil</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>0.2 ml olive oil</td>
<td>21</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>50 mg in 0.25 ml olive oil</td>
<td>18</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2d</td>
<td>50 mg in 0.25 ml olive oil</td>
<td>18</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.25 ml olive oil</td>
<td>18</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
same source as those used in the skin-painting experiments. The compounds were suspended in olive oil and injected in the region of the right scapula. Control groups were injected with the olive oil only. The dosages and number of animals injected are indicated in table II.

The size and position of all skin tumours were recorded when the animals were painted (i.e., twice a week) during the first year of both skin-painting experiments, and fortnightly during the second year. The animals which had been injected subcutaneously were observed fortnightly.

In the first skin-painting experiment tumours were biopsied for histology, but this procedure was not followed in the second experiment in which the tumours were allowed to grow until they were about 1 cm in diameter, when the animals were killed and autopsies performed.

Whenever possible, autopsies were performed on dead animals, and sick animals were killed and autopsied. In the skin-painting experiments, at the end of two years, surviving animals were killed and autopsied. Because of the high mortality of the subcutaneously injected animals, all survivors were killed after one year in the case of the 8 mg dosage, and after 9 months in the case of the 50 mg dosage.

Tumours arising at the application site in the skin-painting experiments were examined histologically, and those which involved the panniculus carnosus muscle were classified as carcinomas. If the latter muscle was intact they were classified as papillomas. In 25 cases no skin tumours were available for histology (in 10 of these cases, because the tumours had regressed; in 15, because the animals had died and autolysis was too far advanced). Regressions occurred only with tumours less than 4 mm in diameter.

In the subcutaneous experiments the largest skin tumour and the subcutaneous tumours at the site of injection were examined histologically.

IV. Results

A. Hydrocarbons

In the series of hydrocarbons possessing a 17-methyl-saturated D-ring (compounds 3a-f), Diels' hydrocarbon (3d) was inactive, in
agreement with several previous groups of workers [HARTWELL, 1951], as were the 3-methoxy, 11-methoxy and 12-methyl derivatives. The 11-methyl derivative gave 2 tumours, thus confirming BUTTNER and DANNENBERG's result [1953] that 11-methyl substitution gave rise to very weak activity in the parent hydrocarbon. The 11,12-dimethyl homologue was rather more active, producing four tumours during the period of painting and three more subsequently. It is interesting to note that BADES [1942] demonstrated weak carcinogenicity with the related tricyclic hydrocarbon, 1,2,3, 4-tetramethylphenanthrene.

The results of this test are presented diagrammatically in figure 2, where the number of animals with tumours are plotted against time in weeks; Isbell's [1939] indices, calculated from these results, are shown in parentheses. Histology of induced tumours and the survival of tumour-free mice are shown in table I.

The 17-methyl-A\textsuperscript{4} hydrocarbons (compounds 1a-f) displayed higher carcinogenic activity, as demonstrated in figure 3. Again in agreement with DANNENBERG [1960], the unsubstituted compound produced several tumours after a long latent period, as did its 17-methylene isomer (7). The 11-methoxy and 12-methyl derivatives had similar activity, but tumours tended to appear earlier. Distinctly higher activity was observed with the 11-methyl and 11,12-dimethyl derivatives (1a and 1b), which produced tumours

![Diagram of tumour production](image-url)
Carcinogenic cyclopenta[a]phenanthrenes

Fig. 3. Tumour production by the 17-methyl-\( \Delta^1 \) compounds. 3-OMe (If) produced one tumour at 99 weeks. The 17-methylene isomer (7) produced 6 tumours (63, 67, 83, 102, 102 and 102 weeks).

- methylcholanthrene (MG)
- 11-methyl (Id)
- 11,12-dimethyl (Id)
- 11-methoxy (Ie)
- 12-methyl (Ie)
- unsubstituted (Id)

earlier and in a larger proportion of the animals. Thus, the effects of 11-methyl substitution and a double bond in the 5-membered D-ring are additive, and give rise to hydrocarbons with about half the activity of 20-methylcholanthrene, on the basis of the Iball’s indices. All the compounds in this series showed activity, for even the 3-methoxy compound (If) produced one tumour towards the end of the experiment.

The lack of activity in 17H-cyclopenta[a]phenanthrene itself (\( \delta \)) is surprising because Dannenberg [1960] found activity in the 15-methyl derivative of this hydrocarbon (as well as in its \( \Delta^{14} \)-17-methyl isomer \( \theta \)). A possible reason may be that the presence of a methyl group adjacent to the double bond prevents partial delocalization of the double bond over the 5-membered ring [Coombs, 1966]. This would result in maintenance of the high electron density at the double bond, presumably of importance if, as Dannenberg [1960] suggested, this is the K-region in these molecules. Alternatively, a methyl group attached to the unsaturated D-ring may confer activity by some other mechanism. Similar considerations may explain the inactivity of the \( \Delta^{10} \)-17-isopropylidene compound \( \delta \).
B. Ketones

Since the introduction of oxygen substituents into carcinogenic hydrocarbons usually diminishes their activity, the high level of carcinogenicity exhibited by both the 11-methyl and 11-methoxy substituted 17-ketones (2a, b and c), summarized in figure 4, was unexpected. The 11-methyl ketone (2b) appeared to be the most active compound tested in the present work. In two independent experiments all the survivors at one year had tumours, giving survival indices of 31 and 36 and a mean latent period of about 35 weeks, compared with an index of 48 and a mean latent period of about 25 weeks for 20-methylcholanthrene, tested at the same time. The 11,12-dimethyl ketone displayed similar carcinogenicity, while the 11-methoxy ketone had a somewhat longer mean latent period, but still produced tumours in the majority of the animals and was as active as the most active A17-hydrocarbon. In this series carcinogenicity appeared to be confined to these 11-substituted derivatives. Even the 11-acetoxy-17-ketone (f) produced tumours in two animals, whereas the unsubstituted ketone and its 15-isomer (5) were all inactive. The 17-ketones. The unsubstituted (2d), 12-Me (2e) and 3-Ome (2f) -17-ketones and the 15-ketone (5) were all inactive. The 11-acetoxy-17-ketone (f) produced 2 tumours (42 and 63 weeks).

Fig. 4. Tumour production by the 17-ketones. The unsubstituted (2d), 12-Me (2e) and 3-Ome (2f) -17-ketones and the 15-ketone (5) were all inactive. The 11-acetoxy-17-ketone (f) produced 2 tumours (42 and 63 weeks).
Carcinogenic cycloPenta[a]phenanthrenes

Table III. Iball's indices

<table>
<thead>
<tr>
<th>Compound</th>
<th>11-Me</th>
<th>11,12-diMe</th>
<th>11-OMe</th>
<th>Unsubst.</th>
<th>12-Me</th>
<th>3-OMe</th>
<th>11-OAc</th>
<th>20-Me</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>27</td>
<td>31,36</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>23</td>
<td>30</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>11</td>
<td>25</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

devoid of activity, as were the 12-methyl- and the 3-methoxy-17-ketones.

These skin painting results were confirmed by subcutaneous injection of three of the ketones into mice. In the two experiments shown in table II, the 11-methyl ketone yielded local sarcomas and multiple skin tumours remote from the injection site, and the latter were also given by the 11,12-dimethyl ketone. No skin tumours were produced by the unsubstituted ketone, which was also inactive by skin painting. Histologically, some of these remote skin tumours were mixtures of squamous papillomas and sebaceous adenomas which appear to be similar to those observed by KLENNENBERG, NEUFACH and SHABAD [1940] following subcutaneous injection of human liver extracts into mice, and by ROE, ROWSON and SALAMAN [1961] after injection of newborn mice with dimethylbenzanthracene.

Discussion

In table III Iball's indices, derived from the skin-painting experiments, clearly demonstrate that 11-methyl substitution promotes carcinogenicity in all three series of cyclopenta[a]phenanthrenes. In the ketone series only 11-substituted compounds are active, whereas all the 17-methyl-16 hydrocarbons possess carcinogenic properties to some extent. It is tempting to suggest that possibly these two series of compounds exert their biological action in different ways. DANNENBERG [1960] has proposed that in these hydrocarbons the double bond in the five-membered ring should
be designated the K-region, since it is chemically the most reactive
double bond, undergoing preferential reaction with oxidizing agents
such as osmium tetroxide and chromic acid. The 17-ketones, on the
other hand, interact with these reagents mainly at the Δ5 (7)
'phenanthrene' double bond, which might therefore be considered
as the K-region in 17-oxo-15,16-dihydro[α]phenanthrenes. It is hoped that metabolic studies now in progress will help
to illuminate these problems, since Boyland [1964] has shown that,
contrary to previously held opinion, polycyclic hydrocarbons
appear to suffer preferential biological oxidation at their K-regions.

Recently, Sims [1967] reported that the 15- and 16-ketones
derived from 20-methylcholanthrene were strong carcinogens,
although not as potent as the hydrocarbon itself. He suggested that
these ketones might well be carcinogens in their own right, with
their activity unrelated to that of the parent hydrocarbon. In the
present work the 11-substituted-17-ketones in the structurally
related [α]phenanthrenes series are much more active
than the hydrocarbons from which they are derived.

Although carcinogenic activity has been demonstrated in certain
methoxy derivatives of several polycyclic hydrocarbon carcinogens
(e.g., 12-methoxydibenz[a,h]anthracene [Barry, Cook, Hasle-
wood, Hieger, Hewitt and Kennaway, 1935]; 8-methoxybenzo-
[a]pyrene [Cook and Schoental, 1952]), in the present case,
11-methoxy substitution induces carcinogenicity in an otherwise
inactive ketone; moreover, the 11-acetoxy ketone is also weakly
active. Steroid-like compounds bearing an additional methyl group
at C-11 are unknown as natural products and biosynthetic pathways
leading to such compounds, for instance by abnormal cyclization
of squalene or by one-carbon methylation of a preformed tetracyclic
structure, are without precedent. On the other hand, 11-oxygenated
steroids are of widespread occurrence, and our attention is focussed
upon the 11-methoxy- and 11-acetoxy-17-ketones which not only
possess the steroid ring system, but also bear oxygen atoms at two
of the positions commonly oxygenated in several classes of steroid
hormones. It would seem quite possible, on the basis of the bio-
synthetic scheme outlined above, that such 11-oxygenated steroids
might give rise to 11-oxygenated [α]phenanthrenes in vivo.

\[\text{Coombs: Unpublished observations.}\]
Carcinogenic cyclopenta[a]phenanthrenes

Conclusions

It is now well established that chemical carcinogens are produced by a variety of plants and microorganisms [Roe and Lancaster, 1964], and it seems possible that a similar state of affairs may also exist in animals. This could explain the carcinogenicity shown by extracts of various animal and human tissues [see, for example, Higginson, Dunn and Sutton, 1964]. The hypothesis advanced by Cook [1933], that endogenous carcinogens produced by abnormal steroid metabolism could explain the occurrence of spontaneous animal neoplasms, is still valid. If such an abnormality were under genetic control like other inborn errors of metabolism, it could account for the strong genetic influence found in inbred strains of mice on spontaneous tumour production, a fact which cannot be entirely accounted for on the basis of known environmental, hormonal or viral factors. The present work clearly shows that strong carcinogenic properties are associated with relatively simple aromatic compounds which are closely related in chemical structure to the natural steroids; moreover, modern steroid biochemistry indicates possible pathways by which such compounds could arise in vivo. While cyclopenta[a]phenanthrenes from natural sources have so far not been identified, it seems important to enlarge our knowledge of the structural limits associated with carcinogenicity in this field as a first step towards ascertaining whether compounds of this type have significance in spontaneous malignant disease.

Acknowledgements

The authors are greatly indebted to the Director of Research of the Imperial Cancer Research Fund, Dr. G. F. Marrian, F.R.S., for the suggested metabolic pathway which initiated this work. They also wish to thank him and other members of this Institute for subsequent interest and encouragement; in particular, they thank T. S. Bhatt and J. A. Smith for valuable technical assistance.

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Carcinogenic cyclePenta[a]phenanthrones


Received November 1967

By M. M. Coombs

Reprinted from

JOURNAL
OF
THE CHEMICAL SOCIETY

SECTION C
Organic Chemistry

1955

By M. M. Coombs

15,16-Dihydro-17-oxocyclopenta[a]phenanthrene and its 3-methoxy, 11- and 12-methyl, and 11,12-dimethyl derivatives have been synthesised by aromatisation of the 3-unsubstituted and 3-methoxy-11,17-diketones in which rings C and D are both saturated: the mechanism of the aromatisation is discussed.

RENEWED interest in 1,2-cyclopentenophenanthrene (16,17-dihydro-15H-cyclopenta[a]phenanthrene) (IX) has arisen in connection with the carcinogenic properties of certain members of this series structurally related to the natural steroids,1,2 and it became of interest to devise syntheses of derivatives oxygenated at positions 3 and 17 (steroid numbering). Although several syntheses of the 17-oxo-derivative (VIA) have been described, they are not readily adapted to the preparation of nuclear-substituted derivatives. It has now been found that the diketones (Ia) and (Ib),* obtained by

Selective ketalisation of the 17-carbonyl group in the diketone (Ia) was achieved by acid-catalysed exchange with ethylmethylidioxolan to give the oxo-ketal (IIa) in good yield. Under comparable conditions the 3-methoxy-diketone (Ib) was converted into a mixture of the 17-monoketal (IIb) and the 11,17-diketal (IVb); the corresponding diketal (IVA) was also obtained from (Ia) by prolonged exchange. These diketals were readily partially hydrolysed to the 17-monoketals with chloroform containing a trace of hydrochloric acid. The infrared absorption at 620 µ and have ultraviolet spectra closely similar to those of the parent diketones. The monoketals (IIa) and (IIb) exhibit typical aryl ketone infrared absorption at 600 µ and have ultraviolet spectra closely similar to those of the parent diketones. The diketals (IVA) and (IVb), on the other hand, lack infrared carbonyl absorption, while (IVA) has λmax 229 and 280 µ, characteristic of the unconjugated naphthalene chromophore in cyclopent[a]phenanthrenes having rings c and d saturated. Reduction of the oxo-ketals (IVA) and (IVb) with sodium borohydride furnished the corresponding 11-hydroxy-17-ketals (IIIA) and (IIIB) as epimeric mixtures which were not further separated.

When (IIIA) was boiled with acetic acid containing hydrochloric acid, instead of the expected product of dehydrogenation and deketalisation, namely (Va), a mixture of (VIa) and its tetrahydro-derivative (VII) was obtained. Since this result pointed to the mutual oxidation-reduction of a common intermediate, the dehydration was repeated in the presence of a large excess of nitrobenzene; as expected, the fully oxidised product (VIa) was now obtained as the sole isolated product, in 86% yield. Under these conditions, with nitrobenzene and acid, the 11-hydroxy-3-methoxy-compound (IIIb) gave the ketone (Vib), the phenanthrene analogue of 18-nor-oestrone methyl ether, in 84% yield.

The diketone (Ia) was reduced with borohydride to the corresponding diol which was then heated with nitrobenzene and acid, as above. The product, however, was a complex mixture, chromatography of which yielded the unsaturated alcohol (Vb) as the main component (approximately 30% yield). A small amount of (IX) contaminated with the corresponding 16,16-dehydro-compound was also isolated, but only a trace of the expected 15,16-dihydro-17-hydroxycyclopenta[a]phenanthrene was detected. Compound (Vb) had λmax 239 and 315 µ, characteristic of structure (IX) having additional hydrogen atoms at the c/d ring fusion positions (13,14); on catalytic reduction, followed by reoxidation of the saturated alcohol with the Jones reagent, the ketone (VII) was isolated, identical with that obtained above. Oxidation of the alcohol (Vb) with the Jones reagent gave the ketone (Va) in low yield, while oxidation with an excess of chromium trioxide in pyridine did not afford (Va), but (VIa) was readily isolated (68%). The unsaturated ketone (Va) was better prepared from the hydroxy-ketal (IIla) through its tosylate which, on elimination, gave the ketal (Vc). Mild acid hydrolysis of the latter then led to (Va). This compound was very sensitive to acids and disproportionated completely to (VIa) and other products when the mild acid hydrolysis was prolonged. When the unsaturated ketal (Vc) was heated with nitrobenzene and acid, as described above, the sole product was (VIa) (91% yield); in the absence of nitrobenzene the yield was 43%. The compound (VII) was isolated (36%), together with its 13,14-dehydro-derivative (22%), presumably formed by acid-catalysed isomerisation of the 13,14-dihydro-17-ketone to the fully conjugated 11,12-dihydro structure.

It therefore appears that a 17-carbonyl group is necessary for the easy dehydrogenation of the 13,14-dihydro-compounds. In order to investigate this further, compound (VIIIa) was prepared and reduced to the

\[ \text{Va} : X = \text{O} \]
\[ \text{Vb} : X = \text{CH}_2\text{OH} \]
\[ \text{Vc} : X = \text{CHO} \]
\[ \text{Vd} : X = \text{H}_2 \]

corresponding alcohol. The latter was then heated with acid, both with and without added nitrobenzene. The products from both reactions were similar complex mixtures consisting of about ten separate compounds as judged by thin-layer chromatography. The three least polar compounds were isolated and identified; from both

![Diagram](VII)

![Diagram](VIII-a)

![Diagram](VIII-b)

![Diagram](VIII-c)

experiments these were (VIIIc), (Vd), and (IX), in yields of 14, 3, and 20% (from reaction with PhNO₂), and 14, 10, and 20% (without PhNO₂). Thus, although limited aromatisation occurs, the extent to which it does so is not influenced by the addition of nitrobenzene, as is the case with the ketone (Va). With this compound it is suggested that dehydrogenation is initiated by abstraction of a hydride ion from C-14 in the enol (XIII), followed by bond rearrangement and the eventual loss of the second hydrogen atom as a proton. Further evidence for this mechanism is presented below.

![Diagram](XIII)

Methylation of the oxo-ketal (Ila) with methyl iodide and potassium t-butoxide gave mixtures of the 12-methyl (Iic) and 12,12-dimethyl (Xa) derivative depending on the ratios of reagents employed; methylation with 3 equivalents was most satisfactory, producing (Iic) in 62% yield. The ultraviolet spectra of (Iic) and (Xa) closely resembled that of the unsubstituted compound (Iia). The structure of (Xa) was further substantiated by the presence of its nuclear magnetic resonance (n.m.r.) spectrum of two unsplit methyl signals at δ 8.64 and 8.82. Both 12-methyl derivatives exhibited a marked tendency, probably associated with their cage-like structures, to occlude solvents, and reproducible analytical figures were obtained only after sublimation. These ketones also resisted reduction by borohydride, but were smoothly reduced to the corresponding alcohols (IIIc) and (Xb) with lithium aluminium hydride. In line with previous results, when (IIIc) was heated with nitrobenzene and a mixture of acetic and hydrochloric acids, the ketone (VIc) was produced in 75% yield.

Condensation of the oxo-ketal (Ila) with ethyl formate in the presence of sodium ethoxide afforded the formyl compound (IId) in high yield. This bright yellow substance behaved as a typical β-dicarbonyl compound, dissolving readily in aqueous sodium hydroxide and exhibiting infrared carbonyl absorption at 6.24 and 6.3 μ. Attempted 12-methylation of its potassium salt with methyl iodide was unsuccessful, giving only the retro-aldol product (Ila). Reduction with lithium aluminium hydride gave the diol (IId), while catalytic hydrogenation stopped after the uptake of 1 mol. of hydrogen, with the formation of the 12-hydroxymethyl-11-ketone (Ile). The reaction of the diol (IIId) with acids was studied in the expectation that double dehydrogenation would occur to provide an alternative route to (VIc). When the diol was boiled with acetic and hydrochloric acids this compound was indeed formed as the main product (45%). However, when the reaction was conducted in the presence of an excess of nitrobenzene, a second major product, C₂₀H₂₂O₂, was isolated in addition to (VIc). This showed a single aryl ketone infrared band, and its ultraviolet spectrum was almost identical with that of (VIc). It is therefore assigned the dimeric ether structure (XVI), and its formation is considered to involve the carbonium ion (XV). In the absence of an oxidising agent, a prototropic shift from C-14 in the initially formed ion (XIV), followed by bond rearrangement and the expulsion of a proton, leads to (VIc). In the presence of nitrobenzene, rapid abstraction of a hydride ion from C-14 in (XIV) leads, by the previously discussed mechanism, to the relatively stable ion (XV) which then attacks a second molecule of hydroxymethyl compound to give (XVI).

Treatment of the oxo-ketal (Ila) with an excess of methylmagnesium iodide, followed by chromatography of the product on alumina, gave two substances, identified by spectroscopy as the 11-methyl-11-en-17-one and its ethylenedioxy-derivative. By heating these with nitrobenzene and acid as usual, the ketone (VId) was formed in 69% yield.

For the preparation of the dimethyl compound (Vie), the methyl-oxo-ketal (Iic) was treated with methyl-lithium, and the crude product heated with nitrobenzene and acid. Chromatography of the reaction product then furnished the ketone (Vie) in 72% yield, together with a small amount of a compound C₁₉H₂₀O. The structure (XI) is assigned to the latter on the following grounds.
The ultraviolet spectrum showed, in addition to maxima at 271 and 281 μm characteristic of the unconjugated naphthalene chromophore, strong absorption at 255 μm. The latter was attributed to the â-unsaturated ketone which was also indicated by strong infrared bands at 5.88 (conjugated C=O) and 6.1 μ (conjugated double bond, intensity enhanced by cis configuration). The n.m.r. spectrum confirmed this structure, with two 3-proton doublets at δ 8.63 (J = 8 c./sec.; secondary 11-methyl group) and at 7.55 (J = 3 c./sec.; 12-methyl group, adjacent to double bond). As expected, (XI) resisted hydrogenation over palladised charcoal; with Adams catalyst, rapid reduction of the ketone was accompanied by disappearance of the 255 μm absorption band and followed by very slow saturation of the tetrasubstituted double bond without further change in the ultraviolet spectrum.

The ketal (Xb) was also treated with nitrobenzene and acetic-hydrochloric acid in the expectation that (Vie) would be formed through dehydration accompanied by a 1,2-shift of a methyl group to C-11. Compound (Vie) was, in fact, obtained in low yield on prolonged heating, while the C-16 protons absorb at δ 7.75. The ultraviolet spectrum of this compound was closely similar to that of 11,12-dihydro-(IX). In addition, the non-aromatic region of the n.m.r. spectrum consisted of a 6-proton singlet at δ 6.88 (two geminal methyl groups) and a 2-proton singlet at δ 6.92 (benzyllic methylene group adjacent to a quaternary carbon atom), together with asymmetrical multiplets at approximately 7.1 and 7.3 arising from the C-15 and C-16 methylene groups, respectively. The equivalence of the two methyl groups indicates that rapid inversion occurs at C-11 and at C-12. The fact that the dihydro-ketones (XI) and (XII), in which enolisation cannot occur towards the C-13 bridgehead, are not further dehydrogenated by nitrobenzene in acetic-hydrochloric acid provides further support for the mechanism proposed above for this aromatization. The formation of these compounds as by-products in reactions leading to (Vie) probably reflects the strain introduced by steric compression of the methyl groups in the intermediate 11,12-dimethyl-11-ene. That this strain persists in (Vie) is demonstrated by the relative shielding of the methyl resonance in this compound with that in both the 11- and 12-methyl compounds. Cross and Durham recently reported that in 15,16-dihydro-3-hydroxy-1,17,17-trimethylcyclopenta[a]phenanthrene absorption of the protons of the 11-methyl group, which is sterically equivalent to the 11-methyl group in (VId), also occurs at δ 7.00, and they attribute this low-field absorption to strong deshielding by all three proximate aromatic rings. The methylene

### Table 1

| N.m.r. spectra (δ-values) of 17-oxocyclopenta[a]phenanthrenes (VI) |
|--------------------------|------------------|------------------|
| CH₃ protons               | C-16             | C-16             |
| (Vla)                    | 6.72t            | 7.3m             |
| (Vlb)                    | 6.72t            | 7.3m             |
| (Vlc)                    | 6.72t            | 7.3m             |
| (Vld)                    | 6.72t            | 7.3m             |

s = singlet, t = triplet, m = multiplet.
*Partly obscured by methyl singlet.

### Table 2

| Infrared and ultraviolet absorption maxima of 17-oxocyclopenta[a]phenanthrenes (VI) |
|---------------------------------|------------------|------------------|
| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

λmax. (μ) log e

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

| (Vla)                           | 218(4.18)        | 265(4.69)        |
| (Vlb)                           | 218(4.18)        | 265(4.69)        |
| (Vlc)                           | 218(4.18)        | 265(4.69)        |
| (Vld)                           | 218(4.18)        | 265(4.69)        |

Protons are assigned to the 15- and 16-positions as shown by analogy with those in (IX). This compound exhibits two multiplets in the methylene region, at δ 6.85 and 7.75, the areas of which are in the ratio 2 : 1. It therefore follows that the lower figure is associated with the magnetically near-equivalent C-15 and C-17 protons, while the C-16 protons absorb at δ 7.75. The ultraviolet and infrared absorption maxima of the ketones (Vla—e) and the 11-methoxy-derivative (VII) are in Table 2. Introduction of a 12-methyl group produces the expected small bathochromic shift of the maximum, but the 11-methyl and 11-methoxy-ketones appear to be anomalous in this respect.

**Experimental**

Thin-layer chromatography (t.l.c.) was carried out using plates coated with silica gel (Merck, Kieselgel G) and dried by A. D. Cross and L. J. Durham, *J. Org. Chem.*, 1965, 30, 3190.
overnight in air. Melting points were determined mostly on a hot-stage apparatus. Solutions were dried over anhydrous sodium sulphate. Ether refers to AnalaR diethyl ether, and silica gel was the MFC grade supplied by Hopkin and Williams. Ultraviolet spectra (log ε in parentheses) were recorded for ethanol solutions using a Perkin-Elmer 137UV instrument. Infrared spectra were recorded on a Perkin-Elmer 137 Infracord, and refer to Nujol mulls unless otherwise stated. N.m.r. spectra were recorded on a Varian A60 spectrometer for deuteriochloroform solutions with tetramethylsilane as internal standard.

17,17-Ethyleneoxy-11,12,13,14,15,16-hexahydro-11-oxo-cyclopenta[a]phenanthrene (IIa).—The diketone (Ia) (46 g.), m. p. 120° (lit.1 116°), λ_max 216 (4-61), 245 (4-33), 315 (3-82) μν, ν_max 6-78, 6-88 μ, was heated under reflux with ethyl-methyldioxolan (220 ml.; b. p. 117—118°) and toluene-β-sulphonic acid (100 mg.) using a short fractionating column equipped with a take-off head. The volatile products were very slowly distilled off through the side-arm, and small amounts of fresh catalyst were added from time to time. After about 20 hr. the temperature of the vapour was constant at 117°. The reaction mixture was cooled, extracted with sodium hydroxide carbonate solution, washed with water, and dried. After removal of the excess of the dioxolan in vacuo, the brown gum was crystallised from benzene (60 ml.) and hexane (60 ml.), giving a first crop of crystals (31-9 g.), m. p. 93—95°, and a second crop (11-3 g.), m. p. 84—90°, of less pure material. The compound (Ia) crystallised from ethanol in prisms, m. p. 98° (Found: C, 77-85; H, 6-25. C_{19}H_{16}O requires C, 77-5; H, 6-15%). λ_max 216 (4-58), 245 (4-31), 313 (3-91) μν, ν_max 6-05 μ. The bisethyleneoxy-compound (Iva) was also isolated by fractional crystallisation from the products of a reaction in which the heating had been prolonged. This compound, m. p. 170° (from ethanol) (Found: C, 74-5; H, 6-8. C_{19}H_{16}O requires C, 74-35; H, 6-55%). λ_max 229 (4-92), 280 (3-75) μν, showed no carbonyl absorption in the infrared. When (Iva) (3-5 g.) was shaken for 3 min. with chloroform (50 ml.) containing 5 drops of conc. hydrochloric acid and worked up as before, (Iia) (1-95 g.), m. p. 95°, was recovered.

17,17-Ethyleneoxy-11,12,13,14,15,16-hexahydro-11-oxo-cyclopenta[a]phenanthrene (Ib).—The diketone (Ib) (10-8 g.), m. p. 133° (lit.1 126—127°), λ_max 221 (4-60), 249 (4-32), 315 (3-72) μν, ν_max 5-8, 6-0 μ, was treated with ethylmethyldioxolan as described above. Fractional crystallisation of the product from ethanol—ether afforded the derivative (Iib) (4-0 g.), m. p. 114° (from ethanol) (Found: C, 74-3; H, 6-1. C_{19}H_{16}O requires C, 74-0; H, 6-29%). λ_max 221 (4-65), 246 (4-48), 310 (3-76) μν, ν_max 6-0 μ, together with the bisethyleneoxy-compound (Ivb) (3-8 g.), m. p. 174° (Found: C, 71-55; H, 6-7. C_{19}H_{16}O requires C, 71-7; H, 6-55%). When (Ivb) (2-68 g.) was shaken with chloroform containing hydrochloric acid as before, crystallisation of the crude product from ethanol furnished (Iib) (1-96 g.), m. p. 108°.

**Methylation of the Oxo-ketal (Iia).**—The oxo-ketal (Iia) (20—25 g.) was boiled under reflux for 15 min. with t-butyl alcohol (640 ml.) containing potassium (8-06 g., 3 equiv.) under an atmosphere of pure, dry nitrogen. After being cooled to 25°, methyl iodide (13 ml., 3 mol.) was added in one lot with vigorous stirring, and cooling was maintained so that the temperature of the mixture did not rise above 27°. After being kept at room temp. for 5 hr., the mixture was poured into water (1-5 l.) and extracted with ether (total, 1 l.). The extracts were washed with water, dried, and evaporated, to give a brown syrup (22 g.) which was chromatographed on a column of alumina (1 kg.); 100-ml. fractions were collected. Fractions 17—41 (ether) were combined (14-56 g.); crystallisation from a mixture of benzene (20 ml.) and hexane (30 ml.) yielded the methyl-oxo-ketal (Iic) (11 g.), m. p. 120—121°. A specimen sublimed at 120°/10° mm. had m. p. 123—124° (Found: C, 77-6; H, 6-6. C_{22}H_{26}O requires C, 77-9; H, 6-55%). λ_max 217 (4-61), 240sh (4-32), 310 (3-81) μν, ν_max 6-0 μ. Fractions 46—78 (ether) (2-3 g.), on recrystallisation from hexane, gave the starting material (Iia) (2 g.), m. p. 93—94°. Fractions 11—15 (1:1 ether—hexane) crystallised from hexane to yield the dimethyl-oxo-ketal (Xa) (1-45 g.), m. p. 162—164°. After two recrystallisations this substance had m. p. 167°, λ_max 217 (4-35), 244 (4-14), 306 (3-59) μν, ν_max 5-94 μ; a correct analysis was obtained only after sublimation at 150°/10° mm. although this did not alter this m. p. (Found: C, 78-2; H, 6-85. C_{22}H_{26}O requires C, 78-2; H, 6-9%). Recrystallisation of fraction 16 (0-93 g.) and various mother-liquid residues (3-58 g.) afforded additional (Iic) (2-1 g.) and (Xa) (0-24 g.). The yield of (Iic) (61-7%) was lower when less than 3 mol. of potassium butoxide and methyl iodide were employed; correspondingly more starting material was recovered. The amount of (Xa) increased at the expense of (Iic) with molar ratios greater than 3.

17,17-Ethyleneoxy-12-formyl-11,12,13,14,15,16-hexahydro-11-oxo-cyclopenta[a]phenanthrene (IId) —To methanol-free sodium methoxide (from 0-5 g. of sodium) was added the oxo-ketal (Iia) (2-04 g.), ethyl formate (1-61 ml.), and dry benzene (15 ml.). The flask was filled with dry nitrogen, and the mixture was boiled gently for 22 hr. The almost colourless solution containing pale fawn solid was poured into water and extracted with ether. Evaporation of the dried extract left a brown syrup (1 g.) which on crystallisation from hot n-butanol. The formyl derivative (IId) was obtained as large yellow prisms (2-74 g.), m. p. 144—145° (Found: C, 74-45; H, 5-75. C_{22}H_{26}O requires C, 74-5; H, 5-65%). λ_max 218 (4-54), 254s (4-13), 362 (3-87) μν, ν_max 6-24, 6-5 μ. For attempted methylation, (IId) (1-07 g., 3 mmoles) was heated with t-butyl alcohol (30 ml.) containing potassium (0-375 g.). To the bright yellow suspension of the potassium salt was added methyl iodide (1-42 g., 10 mmoles), and the mixture was boiled gently for 22 hr. The almost colourless solution containing pale fawn solid was poured into water and extracted with ether. Evaporation of the dried extract left a brown syrup (1 g.) which on crystallisation from hexane and ethanol gave the unmethylated oxo-ketal (Iia) (0-68 g., m. p. and mixed m. p. 96—98°. 15,16-Dihydro-17-oxocyclopenta[a]phenanthrene (Vla). —By the general procedure. The oxo-ketal (Iia) (6-2 g.), tetrahydrofuran (40 ml.), water (10 ml.), and sodium borohydride (2 g.) were heated under reflux with stirring for 5 hr., then left at room temp. for 20 hr. The excess of reducing agent was destroyed by the careful addition of dilute acetic acid to bring the pH to 6-5, and, after the addition of more water, the mixture was extracted with ether, the extract was washed with sodium hydrogen carbonate solution and water, and dried. Removal of the solvent left a semi-crystalline mass (6-15 g.) which showed no infrared carbonyl absorption.
This material was dissolved in a mixture of glacial acetic acid (120 ml.) and nitrobenzene (30 ml.); addition of conc. hydrochloric acid (30 ml.) produced a green coloration which became yellow-brown after being heated for a few minutes. Heating under reflux continued for 30 min., the mixture was diluted with water, and the nitrobenzene was removed by distillation in steam. When cold, the brown solid was dried (4.5 g., 85-88%), m. p. 197°C. Recrystallisation from benzene (150 ml.; charcoal) yielded the ketone (VIa) as very pale yellow needles (3-6 g.), m. p. 203-204°C (lit. 200-201°C) (Found: C, 87-8; H, 5-2%).

**By the general procedure, but with omission of nitrobenzene.**

The oxo-ketal (IIa) (1-0 g.) was reduced with sodium borohydride as described above. The product was dissolved in acetic acid (80 ml.) and boiled for 1 hr. with conc. hydrochloric acid (20 ml.), to give a yellow solution. After removal of the solvent in vacuo the residue was dissolved in benzene (20 ml.) and chromatographed on alumina (90 g.). The initial fractions, eluted with 0-1% methanol in benzene and together weighing 146 mg., were recrystallised from light petroleum (b. 60-80°C) (20 ml.), to give the ketone (VII) (88 mg.), m. p. 115°C (lit. 111-112°C) (Found: C, 66-65; H, 6-65%). Calc. for C₂₁H₂₂O₂: C, 82.5; H, 5-4%

The crude product (5-0 g.) and 96% sulphuric acid (3-76 ml.) was heated for 15 min. at 40°C. After dilution with water, the solution was neutralised with dilute sodium carbonate, and the brown solid was filtered free from catalyst and used as such. The solution was evaporated to give a crystalline mass, X, ν₃ 280 μ. The crystals were dissolved in acetone (10 ml.), and an aqueous solution (0.5 ml.) of chromic acid (from potassium dichromate (5-0 g.) and 96% sulphuric acid (3-75 ml.) made up to 25 ml. with water) was added dropwise. After 15 min. at room temperature, the mixture was diluted with water and extracted with ether, and the solution was washed, dried, and evaporated, to leave a crystalline residue (220 mg.). Recrystallisation from hexane (40 ml.) afforded the ketone (VII) as rosettes of needles, m. p. 114-114.5°C alone and 114°C-115°C when mixed with the analytical specimen.

The unsaturated alcohol (Vb) (229 mg.) was hydrogenated in ethyl acetate with Adams catalyst (10 mg.). When the calculated volume of hydrogen had been consumed (15 min.), the solution was filtered free from catalyst and evaporated to give a crystalline mass, ρ₀ 280 μ. The crystals were dissolved in acetone (10 ml.), and an aqueous solution (0.5 ml.) of chromic acid (from potassium dichromate (5-0 g.) and 96% sulphuric acid (3-75 ml.) made up to 25 ml. with water) was added dropwise. After 15 min. at room temperature, the mixture was diluted with water and extracted with ether, and the solution was washed, dried, and evaporated, to leave a crystalline residue (220 mg.). Recrystallisation from hexane (40 ml.) afforded the ketone (VII) as rosettes of needles, m. p. 114-114.5°C alone and 114°C-115°C when mixed with the analytical specimen.

Oxidation of (Vb) (240 mg.) with chromic acid in aceton, essentially as described above for the saturated alcohol, gave a product which was shown to consist of at least eight substances by t.l.c. Careful column chromatography on silica gel with hexane-dichloromethane (2:1) gave a chromatographically homogeneous fraction (42 mg.), ρ₀ 316 μ, which, on crystallisation from hexane, yielded (Va) (10 mg.), m. p. 96-98°C, the infrared and ultraviolet spectra of which were identical with those of the analytical sample described below.

13,14,15,16-Tetrahydro-17-oxocyclopenta[a]phenanthrene (Va).—The hydroxy-ketal (IIa) (2-5 g.) in dry pyridine (6 ml.) was allowed to stand at room temp. for 72 hr. with toluene-p-sulphonyl chloride (1-8 g.) and 0.6% of toluene. After dilution with saturated sodium chloride solution (200 ml.) and extraction with dichloromethane, the crude tosylate was obtained as a brown gum (3-7 g.) by evaporation of the solvents. This material was boiled for 1 hr. with redistilled collidine

* See Part II (following Paper).

The cooled solution was diluted with ether (200 ml.) and quickly extracted with N-hydrochloric acid (200 ml.) and with sodium hydrogen carbonate solution, and dried. Removal of the solvent under reduced pressure left a crystalline solid (2 g.) which on crystallisation from ethanol furnished cream prisms (1-12 g.), m. p. 92—95°. The ethylenedioxy-compound (Va) formed leaflets (from ethanol), m. p. 96° (Found: C, 82-0; H, 6-65. C10H14O requires C, 82-0; H, 6-65%). 239 (4-81), 302 (3-89), 314 (4-00), 319 (3-84), and 336 (3-72) μm.

This ketal (250 mg.) was dissolved in tetrahydrofuran (25 ml.) containing 5N-hydrochloric acid (0-8 ml.). After 8 hr. at room temp. the mixture was diluted with water (100 ml.), extracted with ether, and the extract was washed with aqueous sodium hydrogen carbonate. Chromatography of the semi-solid residue (obtained by concentration of the dried extract) on silica gel with dichloromethane. The first fractions afforded pale yellow leaflets (50 mg.) of the 11,12-dihydro-

ketone (Va) (48 mg.), m. p. 95—96° (Found: C, 87-5; H, 6-0%). 239 (4-73), 303 (3-88), 316 (4-01), 330 (3-86), and 336 (3-86) μm. 11*55, 12*15, 12*42, 13*25, and 13*5 p.

Recrystallisation from (Va) (200 ml.) and with sodium hydrogen carbonate solution, and dried. Removal of the solvent under reduced pressure left a yellow gum (236 mg.) which was chromatographed on silica gel in dichloromethane. The first fractions yielded (VII) (85 mg.), m. p. 103—108° (109—112° when mixed with an authentic specimen of m. p. 114—115°). Recrystallisation of (VII) (from both experiments, total was 141 mg.) from ethanol gave needles (84 mg.), m. p. 91—92°, not raised by further recrystallisation.

The infrared and ultraviolet spectra of (IX) from both experiments were identical with those of a pure sample. 16,16-Dihydro-12-methyl-17-oxycyclopenta[a]phenanthrene (VIC).—By the general procedure. The methyl-oxo-ketal (Iic) (3-0 g.) in tetrahydrofuran (90 ml.) was reduced by the addition of lithium aluminium hydride (3-0 g.) in small portions with stirring during 3 hr. at room temperature, and the mixture was left overnight. Excess of hydride was destroyed by the cautious addition of ethyl acetate, and the mixture was diluted with water and brought to pH 7 with 2N-acetic acid. The reduction product was extracted with ether and the extract was washed and dried. Evaporation of the solvent left a gum (2-37 g.) devoid of infrared carbonyl absorption. Treatment of this with nitromethane and acetic acid by the general procedure gave the methyl-ketone (VIC) (1-80 g.), m. p. 233° (from benzene) (Found: C, 88-1; H, 5-65. C10H14O requires C, 87-5; H, 6-75%).

From the 12-formyl compound (VId). (a) The formyl compound (906 mg.) was suspended in ethanol (40 ml.) and shaken in hydrogen with Adams catalyst (100 mg.). Uptake of hydrogen was rapid at first, but became very slow. After filtration from the catalyst, the solution was evaporated, leaving a pale yellow gum, which was reduced with lithium aluminium hydride (500 mg.) in tetrahydrofuran (10 ml.) as previously described. Infrared carbonyl absorption was virtually absent from the spectrum of the product (842 mg.). Treatment of the latter with nitromethane and acetic acid by the general procedure gave a dark brown solid (656 mg.), t.l.c. (1:1 dichloromethane—benzene) of which revealed (VIC) (RP 0-44) and a second major product (RP 0-50), together with several less polar spots. Fractional crystallisation from benzene afforded (VIC) (51 mg.), m. p. 208—213° (infrared spectrum identical with that of the authentic material) and the RP 0-5 material, δ 15-16-dihydro-17-oxycyclopenta[a]phenanthrol-12-methyl ether (XVI) (70 mg.), m. p. 229—233° (Found: C, 85-55; H, 5-15%)

The formyl compound (800 mg.) was reduced with lithium aluminium hydride (500 mg.) in tetrahydrofuran (10 ml.) under gentle reflux for 8 hr., and worked up as before to give an amber gum (377 mg.), the infrared spectrum of which was identical with that of the 842 mg. (above). This gum was dissolved in glacial acetic acid (10 ml.) and boiled with conc. hydrochloric acid (2-5 ml.) for 30 min. After dilution with water, the mixture was extracted with dichloromethane, and the extract was washed with 5\% sodium hydroxide solution, dried, and evaporated, to yield a brown gum which exhibited weak 11-ketone absorption at 5-9 p. This was heated with stirring under nitrogen. When the addition was complete, the mixture was boiled under reflux for 4 hr., then set aside at room temperature overnight. The reaction mixture was shaken with ice-cold ammonium chloride solution, and the organic layer separated, washed with water, and dried. The residue (61 g.), obtained on evaporation of the solvents and still containing some unchanged 11-ketone (weak v_max 6-0 p), was chromatographed on alumina. Two main fractions, together weighing 3-54 g., were not further investigated. On evaporation, fractions 96–127 gave brown crystals (180 mg.) which were not reduced when shaken with 5\% palladium-charcoal in an atmosphere of hydrogen.

From the dimethyl-oxo-hetal (Vla).—To a solution of dimethylmagnesium iodide [from lithium chips (3-5 g.), methyl iodide (20 ml.), and ether (600 ml.)] freshly prepared solution of methyl-lithium [from lithium (4-25 g.) (4-57), 270 (4-89), 281 (5-00), 324 (4-48), 336 (4-51), 362sh (4-25) μ, v_max 5-90 (C=O), 10-1, 12-3, 12-78, 13-22 μ. This alcohol was dissolved in a mixture of acetic acid (40 ml.) and nitrobenzene (10 ml.). Addition of conc. hydrochloric acid (10 ml.) produced a brilliant emerald green solution, the appearance of which was unchanged after being boiled for 0-5 hr. On further heating, the colour gradually became brown, and after 22 hr. a portion showed strong infrared absorption at 5-9 μ (conjugated C=O). The solution was diluted with water, and the brown oil which remained after removal of the nitrobenzene in steam was chromatographed on a column of silica gel (200 g.) with hexane containing increasing concentrations of dichloromethane; 50-ml fractions were collected. T.l.c. revealed that fractions 75–85 contained almost pure dimethyl-ketone (Vie); evaporation yielded fawn needles (101 mg.) which, after one recrystallisation from ethanol, had m. p. 148–149°. Fractions 86–95 were mixtures and were not further investigated. On evaporation, fractions 96–127 gave brown crystals (180 mg.) which were recrystallised from ethanol to yield 11,12,13,14-tetrahydro-12,17-dimethyl-17-oxocyclonapta[a]phenanthrene (XII) as prisms (112 mg.), m. p. 186–187° (Found: C, 86-9; H, 6-8\%); M, 252. C_{19}H_{16}O requires C, 87-0; H, 6-9\%; M, 262-3, v_max 219 (4-87), 241 (4-11), 251sh (4-16), 262sh (4-57), 270 (4-89), 281 (5-00), 324 (4-48), 336 (4-51), 362sh (4-25) μ, v_max 5-90 (C=O), 10-1, 12-3, 12-78, 13-22 μ.

The author thanks Dr. G. F. Marrian, F.R.S., for his interest, and T. S. Bhatt and D. M. Carr for skilful technical assistance.

Division of Chemistry and Biochemistry, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2.

[6/1233 Received, November 18th, 1965]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes (1,2-Cyclopentenophenanthrenes). Part II.\textsuperscript{1} Derivatives Containing Further Unsaturation in Ring D

By M. M. Coombs

Reprinted from
JOURNAL
OF
THE CHEMICAL SOCIETY

SECTION C
Organic Chemistry

1966
Potentially Carcinogenic Cyclopenta[a]phenanthrenes (1,2-Cyclopentenophenanthrenes). Part II. Derivatives Containing Further Unsaturation in Ring d

By M. M. Coombs

Substituted 17-methyl-15H-cyclopenta[a]phenanthrenes have been prepared from the corresponding 15,16-dihydro-17-ketones by the Grignard reaction, and reduced to the analogues of the Diels hydrocarbon. The 15,16-dihydro-17-methylene-derivative (unsubstituted) was obtained by the Wittig reaction. Elimination from the 17-toluene-p-sulphonyloxy-compound at 170° gave the \( \Delta^4 \) - 17H-compound (A), but at 100° the \( \Delta^6 \) - 15H-isomer was formed. Base-catalysed condensation of (A) with acetone and aryl aldehydes yielded 17-substituted-methylene derivatives. The chemical shifts of protons attached to ring D in these compounds are listed.

Although 1,2-cyclopentenophenanthrene (16,17-dihydro-15H-cyclopenta[a]phenanthrene) (I) is not itself carcinogenic, weak activity has been reported for the 11-methyl and 11,12-dimethyl compounds\(^1\) and for derivatives of (I) containing further unsaturation in the D-ring.\(^2\) It was therefore of interest to investigate whether carcinogenic potency would be enhanced by the presence of both of these structural features in the same molecule. The recent demonstration\(^3\) of the presence of 16,17-dihydro-3-hydroxy-15H-cyclopenta[a]phenanthrene in the pyrolysis products of natural fats makes the study of compounds oxygenated at positions 17, 11, and 3 of particular relevance. The availability of a number of the methyl and methoxy-17-ketones by a new route\(^4\) prompted the present investigation.

The yields of the latter varied considerably (see Table 3); since no attempt was made to isolate the dimers [except from the reactions with (IIa) and (IIIc)], these yields probably provide a rough indication of the amount of dimer formed. In the case of (IIIc), crystallisation of the crude Grignard reaction product from boiling alcohol gave the 11-methoxy-monomer (IIIc) in 85% yield, whereas the yield was only 18% when the crude product was chromatographed in the usual way, and much 11-methoxy-dimer was also isolated. It therefore appears that dimerisation accompanied dehydration on the chromatographic column, although neutral alumina was employed. The structure (V) suggested by Dannenberg for the unsubstituted dimer was confirmed by its nuclear magnetic resonance (n.m.r.) spectrum, which exhibited methyl absorption (total of 6 protons) at \( \delta = 8.23 \) (singlet) and \( \delta = 8.12 \) (closely spaced doublet, \( J = 2 \) c./sec.) together with a well-resolved 2-proton multiplet at \( \delta = 2.4 \) assigned to the C-15 methylene protons adjacent to the 16-double bond. In addition, absence of absorption in the olefinic proton region and absorption in the aromatic proton region closely similar to that of the monomer (IIa) strengthened this evidence. hydrogenation of the monomers (IIib—f) furnished the 17-methyl compounds (IVb—f), derivatives of the Diels hydrocarbon (IVa); the dimer (V) resisted hydrogenation, as expected from the sterically hindered nature of its 16-double bond.

The ultraviolet absorption maxima of these compounds are summarised in Table 1. Conjugation of the 16-double bond causes a bathochromic shift of about 15 m\( \mu \) in the main peak, and further small shifts are caused by the introduction of methyl substituents into the phenanthrene nucleus. Methyl and methoxy substitution at C-11 is anomalous as noted\(^4\) for the ketones (IIId) and (IIIf). The moderate-to-weak absorption bands in the 280—380 m\( \mu \) region are particularly useful for the identification of the compounds in these various series. The strong infrared bands in the 10—15 m\( \mu \) region are also listed in Table 1.

Dannenberg et al.\(^5\) obtained the 17-methyl compound (IIIa) from the 17-ketone (IIa) by a Grignard reaction followed by chromatography of the crude tertiary alcohol on alumina, and reported the concomitant formation of a dimer, \( \text{C}_{18}\text{H}_{20}\). This has now been confirmed, and the method applied to the ketones (IIib—f) to prepare the corresponding 17-methyl-16-enes (IIib—f).

However, in our hands the 17-methylene compound (VI) was isolated without difficulty, but it isomerised to (IIIA) in the presence of traces of acid. The ultraviolet absorption maximum of (VI) (273-5 μ) was near that of (IIIA) (276 μ), but in addition (VI) also exhibited a peak at 315, and a well-resolved 2-proton triplet at 34-35 while the C-15 and C-16 methylene protons were represented by multiplets at 34-36, derived from the C-15 methylene group.

In order to prepare the unknown 15H-cyclopenta[a]phenanthrene, compound (IIA) was reduced with sodium borohydride to the 17-ol (VIIA) and converted into the tosylate (VIIb). Base-catalysed elimination with boiling collidine then afforded a compound, m. p. 164—165°, in high yield, having the same ultraviolet absorption characteristics as 17H-cyclopenta[a]phenanthrene reported for a sample of the same m. p. prepared 


Table 1
Ultraviolet and infrared absorption maxima of cyclopenta[a]phenanthrenes (III) and (IV)

<table>
<thead>
<tr>
<th>λmax (μ)</th>
<th>λmax (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(IIIIA)</td>
<td>...234(4-14), 316(3-74), 331(2-71), 349(2-69), 366(2-46)</td>
</tr>
<tr>
<td>(IIIB)</td>
<td>...234(4-28), 273(4-84), 294(4-22), 304sh(4-16), 318(4-10), 338(2-92), 355(3-04), 374(3-03)</td>
</tr>
<tr>
<td>(IIIC)</td>
<td>...222(4-38), 274(4-90), 278(4-80), 290(4-27), 318(2-88), 349(2-71), 367(4-49)</td>
</tr>
<tr>
<td>(IIID)</td>
<td>...222(4-32), 270(4-70), 276(5-81), 295sh(4-13), 306h(4-13), 317(3-69), 350(2-93), 365(2-83)</td>
</tr>
<tr>
<td>(IIIE)</td>
<td>...219(4-55), 250(4-93), 311(4-14), 355sh(2-78)</td>
</tr>
<tr>
<td>(IIIF)</td>
<td>...221(4-37), 272(4-70), 280(4-74), 287(4-03), 324(3-69), 339(3-29), 356(3-49), 374(3-67)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>λmax (μ)</th>
<th>λmax (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(IV)</td>
<td>...218(4-39), 259(4-69), 280(4-07), 285(3-99), 300(4-05), 320(2-76), 336(2-66), 352(2-87)</td>
</tr>
<tr>
<td>(IVb)</td>
<td>...212(4-42), 225(4-30), 236(4-36), 241(4-82), 234(4-20), 231(2-95), 240(2-83), 241(3-16), 318(3-28)</td>
</tr>
<tr>
<td>(IVc)</td>
<td>...217(4-03), 261(4-83), 290(4-06), 304(3-15), 312(2-57), 337(2-64), 353(2-41)</td>
</tr>
<tr>
<td>(IVd)</td>
<td>...219(4-45), 227(4-19), 257(4-60), 294(4-02), 306(4-07), 324(2-88), 339(3-00), 356(3-03)</td>
</tr>
<tr>
<td>(IVe)</td>
<td>...218(4-33), 224(9-26), 241(7-71), 297(3-98), 301(3-07), 312(4-01), 343(3-17), 356(3-07)</td>
</tr>
<tr>
<td>(IVf)</td>
<td>...218—220(4-42), 239(4-45), 249(5-40), 252(5-60), 276(4-44), 289(3-95), 350(5-25), 345(3-05), 362(3-65)</td>
</tr>
</tbody>
</table>

* Liquid film

through decarboxylation of the 17-carboxylic acid. In particular, it showed the small peak at 240 μ which was claimed to be diagnostic of the Δ15 system. In order to confirm the identity of our compound, 16,17-dihydro-15-oxocyclopenta[a]phenanthrene was prepared and reduced with borohydride. Conversion of the 15-ol into its tosylate, and elimination from the latter as before, then furnished the Δ15-compound (VIIIa) identical with this compound derived from the 17-ketone.

An isomeric compound, m. p. 182—183°, obtained by Kon by selenium dehydrogenation of the phosphoric acid cyclisation product of 2-(2'-naphthylethyl)cyclopentanol and claimed to be the 15H- or 17H-compound was shown by Cook and Hewett to be chrysfluorene. Recently Kotlyarevskii and Zanina isolated a compound, m. p. 182—182.5°, from the products of

O. Söss, Annalen, 1953, 579, 133.
Condensation of (VIIIa) with benzaldehyde in ethanol containing potassium hydroxide gave the 17-benzylidene-15-ene (Xa). On hydrogenation, this compound, which showed strong light absorption at 269 and 273 μm, absorbed 2 mol. of hydrogen, to yield (IXa), which showed strong light absorption at 240 and 383 μm, absorbed 2 mol. of hydrogen, to yield (I). However, these authors make no reference to the later work of Cook and Hewett. The 15-ene (VIIIa), m. p. 164—165°, obtained in the present work also absorbed 2 atoms of hydrogen to yield (I), but its ultraviolet and infrared spectra differed from those of the Russian compound. It appeared unlikely that the latter was the unknown Δ16-isomer because this must have been formed initially during the elimination of the 17-tosylate, but apparently rearranged even at 170° to the 15-ene. In order to effect this elimination under milder conditions, use was made of dimethyl sulphoxide. The hydrocarbon, C₉H₁₆, isolated in very low yield after the 17-tosylate had been heated at 100° in this solvent for a short time, exhibited an ultraviolet spectrum (see Figure) very similar to that of the 17-methyl-16-ene affording the p-dimethylaminobenzaldehyde compound (Xc), of interest because the corresponding 1-p-dimethylaminobenzaldehyde is claimed to exhibit marked anti-tumour activity. Condensation of (VIIa) with acetone in the presence of potassium hydroxide did not lead to the isopropylidene derivative (Xb), but this substance was readily secured as a yellow compound (λₘₐₓ. 310 μm) when the condensation was catalysed by piperidine. A colourless isomer isolated from the potassium hydroxide catalysed reaction had ultraviolet absorption very similar to that of the 17-methyl-16-ene (IIIa) and is consequently assigned the structure (XII). Presumably rotation of the isopropenyl group about the 17-single bond relieves steric compression with the C-12 hydrogen atom and deconjugates the terminal double bond from the ring-system chromophore. The structures proposed for (Xb) and (XII) were confirmed by their n.m.r. spectra; in the methyl and methylene regions the former consisted of two proton singlets at 7-52 and 7-69 arising from the two non-equivalent methyl groups, while the latter revealed the presence of one methyl group adjacent to a double bond (3-proton singlet at 8-1) and the C-15 methylene group (2-proton multiplet at 6-18). Hydrogenation of (Xb) readily gave (Xib), but when the reduction was interrupted after the uptake of 2 atoms of hydrogen the expected 17-isopropylidene analogue of (VI) was not obtained. The product instead consisted of a mixture of four compounds, present in comparable amounts. After chromatographic separation these proved to be starting material (Xb), the tetrahydro-derivative (Xib), and two dihydro-compounds. One of the latter was (IXb), from its ultraviolet absorption characteristics and melting point. The ultraviolet spectrum of the other was similar to that of (VIIa), with λₘₐₓ 269 and a small maximum at 240 μm, and it must therefore have the Δ15-17-isopropyl structure (VIIib). The ease of reduction of the tetrasubstituted 16-double bond suggests that this also is a result of the relief of steric strain.

Chemical shifts of the p-ring protons in representatives of the various classes of compound encountered in the present work are summarised in Table 2.

![Diagram](image)

**Table 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-17</th>
<th>C-16</th>
<th>C-15</th>
<th>Other protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>6-85m(2H)</td>
<td>7-75m(2H)</td>
<td>6-85m(2H)</td>
<td></td>
</tr>
<tr>
<td>(VIIa)</td>
<td>6-85m(2H)</td>
<td>(3-3 *)</td>
<td>6-75m(2H)</td>
<td></td>
</tr>
<tr>
<td>(IXa)</td>
<td>[7-0—8-5(3H) ]*</td>
<td>6-75m(2H)</td>
<td>Me, 8-63d(3H)</td>
<td></td>
</tr>
<tr>
<td>(IIia)</td>
<td>3-55s</td>
<td>6-33t(2H)</td>
<td>Me, 7-75d(3H)</td>
<td></td>
</tr>
<tr>
<td>(IVa)</td>
<td>6-95m(2H)</td>
<td>6-75m(2H)</td>
<td>CH₃ protons</td>
<td>4-44s, 4-57s</td>
</tr>
<tr>
<td>(Xb)</td>
<td>7-3m(2H)</td>
<td>7-6m(2H)</td>
<td>Me groups</td>
<td>7-52s(3H), 7-69s(3H)</td>
</tr>
<tr>
<td>(XII)</td>
<td>3-25s</td>
<td>6-18d(2H)</td>
<td>Me, 8-3s(3H)</td>
<td></td>
</tr>
</tbody>
</table>

* Unresolved; s = singlet, d = doublet, m = multiplet.
EXPERIMENTAL

Experimental conditions are generally as described in Part I (preceding Paper).

Grignard Reactions with 17-Ketones.—To a solution of methylmagnesium iodide [from magnesium turnings (0-8 g.), methyl iodide (1-2 ml.), and dry ether (20 ml.)] was added the ketone (1-0 g.) in dry benzene, and the mixture was heated under reflux for 1 hr. The ether was then removed by distillation, more benzene (20 ml.) was added, and heating was continued for 1 hr. further. The cooled reaction mixture was poured into water (1 l.), and the cream precipitate was dried at room temperature over phosphorus pentoxide for 30 min., and the mixture was boiled under nitrogen for 30 min., to give a clear orange solution. After addition of the ketone (IIa) (4-04 g.), the mixture was boiled under reflux in nitrogen for 4 hr. and left overnight at room temperature. Most of the solvent (150 ml.) was removed in vacuo, the residue was treated with water (700 ml.), the dark oil was extracted with chloroform, and the solution was dried and evaporated. The residual gum was rapidly chromatographed on alumina (200 g.) with benzene (1 l.); on evaporation, the eluate furnished the 17-methylene compound (VI) (2-05 g.) as a cream solid which crystallised from ethanol (350 ml.) as large pale amber leaflets (1-7 g.) (Found: C, 93-65; H, 6-9%; M, 240). Calc. for C_{18}H_{22}O requires C, 93-9; H, 6-1%; M, 240. The formula C_{18}H_{22}O requires C, 93-9; H, 6-1%; M, 240. The formula C_{18}H_{22}O requires C, 93-9; H, 6-1%; M, 240. The formula C_{18}H_{22}O requires C, 93-9; H, 6-1%; M, 240.

Hydrogenation of Δ^{14}-Compounds.— The unsaturated compound (500 mg.) in ethanol (100 ml.) was shaken with Adams catalyst (50 mg.) in an atmosphere of hydrogen until the calculated volume of hydrogen had been absorbed. The catalyst was removed by filtration and washed with ethanol, the solution was evaporated to dryness and the residue was recrystallised from ethanol or otherwise as specified in Table 4.

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The above alcohol (1 g.), toluene-p-sulphonyl chloride (1·7 g.), and dry pyridine (25 ml.) were kept at room temperature for 24 hr. The mixture was diluted with water (250 ml.), and the pale brown precipitate was washed with water and dried (200 g.), m. p. 167—185°. The tosyl derivative (VIIb) formed leaflets from (ethanol), m. p. 178—179° (Found: C, 74·4; H, 5·3%). $C_{17}H_{14}O_2S$ requires C, 74·2; H, 5·2%. $\lambda_{\text{max}}$ 224 (4·41), 271 (4·70), 276 (4·71), 316 (4·02) mp, $\alpha_{D}$ 290° (4·07), 295 (4·07), 300 (4·17), 308 (4·17) mp, 10·63, 11·57, 12·3, 12·96, 13·14, 13·76 μ. $\lambda_{\text{max}}$ (CS) 695, 746, 752, 810 cm.$^{-1}$ (measured as a 2% solution on a Perkin-Elmer 237 grating spectrometer). When the reaction was allowed to proceed at 100° for 6 hr., the material recovered exhibited $\lambda_{\text{max}}$ 239 and 269 mp. The same result was observed when a small sample of (IXa) was heated at 180° in a sealed, evacuated glass tube for 30 min.

17-Isopropenyl-15H-cyclopenta[a]phenanthrene (XII).—The compound (VIIa) (100 mg.) was dissolved in acetone (20 ml.) and treated with a solution (0·001 ml.) containing potassium hydroxide (250 mg.) in methanol (10 ml.). An orange colour rapidly developed, and within 2 hr. crystals filled the liquid. The crystals (40 mg.), m. p. 250—255°, were recrystallised from benzene (2 ml.), to furnish the isopropenyl compound (XII) as needles, m. p. 255° (Found: C, 93·95; H, 6·35. $C_{17}H_{14}O_2S$ requires C, 93·7; H, 6·3%). $\lambda_{\text{max}}$ 223 (4·40), 271(4·74), 276(4·75), 295(4·30), 316(3·40) mp, $\alpha_{D}$ 10·2, 10·9, 12·8, 12·8, 12·8, 14·6 μ.

17-Isopropylidenecyclopenta[a]phenanthrene (Xb).—The 15-olefin (VIIa) (250 mg.) was dissolved in acetone (6·5 ml.) containing piperidine (100 mg.) and left at room temperature in a sealed flask for 2 days. The solution became yellow and yellow crystals separated. The solvent was evaporated in vacuo and the residue was recrystallised from n-butanol. The isopropylidene compound (Xb), formed bright yellow leaflets (251 mg.), m. p. 188—189° (Found: C, 93·6; H, 6·4%). $\lambda_{\text{max}}$ 214(4·44), 228(4·50), 270(4·63), 302(4·67), 310(4·77), 345—349μ (3·01), 364—369μ (3·50) mp, $\alpha_{D}$ 6·1 (conjugated C=C) 11·66, 12·0, 12·4, 15·0, 13·2 μ. The isopropylidenecompound (23·8 g.) in ethyl acetate (5 ml.) was hydrogenated using 5% palladium-charcoal (21 mg.). Uptake of 2 mol. of hydrogen was complete within 15 min. Evaporation of the colourless, filtered solution, and recrystallisation of the residue from methanol (1 ml.), gave needles (15 mg.), m. p. 97—98° (lit. 97—98° of (Xb)) (Found: C, 92·6; H, 6·0). Calc. for $C_{17}H_{14}O_2S$: C, 92·25; H, 7·75%. $\lambda_{\text{max}}$ 214(4·61), 250(4·78), 281(4·17), 283(4·86), 300(4·14) mp, $\alpha_{D}$ 11·54, 12·38, 13·0, 13·4 μ. The isopropylidene compound (25·1 g.) was hydrogenated as above with 2 mg. of catalyst, and shaking was interrupted when 1 mol. of hydrogen had been absorbed (17 min.). The filtered solution was evaporated, and the pale yellow residue was chromatographed on a column of silica gel (20 × 1 cm.; 10 g.) with hexane, collecting 10-ml. fractions. Fractions 10—16 gave crystals (4·2 mg.), m. p. 85—93°, of the saturated compound (Xb). Fractions 17—23 were combined, and yielded crystals (7·1 mg.), m. p. 105—107°, raised to 106—107° after one crystallisation from methanol, of the isopropyl compound (VIIb) (Found: C, 93·2; H, 6·9. $C_{17}H_{14}O_2S$ requires C, 93·0; H, 6·9%). $\lambda_{\text{max}}$ 221(4·66), 240(4·33), 269(4·65), 274(4·60), 292(4·07), 305(4·07), 317(3·95) mp, $\alpha_{D}$ 10·30, 10·52, 10·76, 10·8, 11·5, 12·3, 13·0, 13·26, 13·65, 14·0 μ. Further elution with hexane (fractions for 40—60) gave crystals (6·0 mg.), m. p. 135—142°, raised to 160—165° (lit. 144—156° by recrystallisation from methanol of (Xb)), $\lambda_{\text{max}}$ 224(4·31), 271(4·70), 276(4·71), 316(4·02) mp, $\alpha_{D}$ 10·26, 10·9, 11·5, 12·22, 12·9, 13·34, 13·66 μ. Finally, elution of the yellow band with benzene-hexane (1:1) gave yellow crystals (4·0 mg.), m. p. 172—176°, of the starting material (Xb).

17-Benzylidenecyclopenta[a]phenanthrene (Xb).—To a solution of the 15-olefin (VIIa) (210 mg.) in ethanol (15 ml.) containing benzaldehyde (120 mg.) was added a saturated
solution (0.5 ml.) of potassium hydroxide in ethanol. An orange crystalline solid rapidly separated; after 30 min. on a steam-bath, the solution was cooled and the solid was dried (231 mg.), m. p. 257—259°. The benzylidene compound (Xa) formed orange, feathery needles (from toluene,) m. p. 262—263° (Found: C, 94.0; H, 5.55. C21H14 requires C, 94.7; H, 5.3%). \( \lambda_{\text{max}} \) 249(4.52), 328(4.63), and 384(4.07) \( \mu \mu \), \( \nu_{\text{max}} \) 12.05, 12.47, 13.12, 13.38, and 14.44 \( \mu \mu \).

Hydrogenation of this compound (100 mg.) in ethyl acetate (20 ml.) over 5% palladium-charcoal (100 mg.) stopped with the uptake of 2 mol. of hydrogen. After filtration, the colourless solution was evaporated, and the residue was recrystallised from ethanol, to furnish the benzyl compound (XIa) (73 mg.), m. p. 156—157° (Found: C, 93.4; H, 6.7. C24H20 requires C, 93.45; H, 6.55%). \( \lambda_{\text{max}} \) 216(4.40), 260(4.81), 281(4.20), 289(4.08), 301(4.14), 320(2.77), 330(2.98), 352(3.00) \( \mu \mu \), \( \nu_{\text{max}} \) 12.1, 12.36, 12.96, 13.3, 13.48, 14.28 \( \mu \mu \).

17-p-Dimethylaminobenzylidene cyclopenta[a]phenanthrene (Xc).—Condensation of (VIIa) (170 mg.) with p-dimethylaminobenzaldehyde (117 mg.) in the presence of potassium hydroxide as described above gave the compound (Xc) (165 mg.) as a bright yellow microcrystalline solid, m. p. 248—250° (Found: C, 90.05; H, 6.25. C26H21N requires C, 89.9; H, 6.1%). \( \lambda_{\text{max}} \) 317(4.18), 434(4.58) \( \mu \mu \), \( \nu_{\text{max}} \) 12.15, 12.32, 12.58, 13.24, 13.5 \( \mu \mu \).

The author thanks Dr. G. F. Marrian, F.R.S., for his interest, and Dr. C. J. Croft of the Pathology Unit for testing many of the compounds described in Parts I and II for carcinogenic activity, the results of which will be published elsewhere. It is also a pleasure to acknowledge the valuable technical assistance of T. S. Bhatt and D. M. Carr.

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[5/1234 Received, November 18th, 1963]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part III.¹ Oxidation Studies

By M. M. Coombs, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part III.\textsuperscript{1} Oxidation Studies

By M. M. Coombs, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2

Oxidation of 15,16-dihydrocyclopenta[a]phenanthren-17-one and of its strongly carcinogenic 11-methyl homologue with chromic acid, osmium tetroxide, lead tetra-acetate, and ammonium cerium(IV) nitrate was studied. The position of attack, either on the phenanthrene nucleus or on the five-membered ring \textit{D}, depended mainly upon the oxidising agent and only to a minor extent upon the substrate. Both the biologically inactive hydrocarbon, 16,17-dihydro-15\texttext{-}cyclopenta[a]phenanthrene, and its weakly carcinogenic 11-methyl homologue gave the corresponding 15-ketones as the main products on chromic acid oxidation.

Skin-painting experiments have revealed the surprising fact that 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (\textit{lb}) possesses strong carcinogenic properties whereas as expected the unsubstituted ketone (\textit{la}) is devoid of such activity.\textsuperscript{2} The chemical reactivities of these two compounds have therefore been compared, and various oxygenated derivatives which might be expected to be encountered in metabolic work now in progress have been synthesised.

Oxidation of the parent hydrocarbon 16,17-dihydro-15\texttext{-}cyclopenta[a]phenanthrene (\textit{IIa}) with chromic acid\textsuperscript{3,4} was found to give mainly the 15-ketone (\textit{Xa}), together with a trace of the 6,7-quinone.\textsuperscript{4} The weakly carcinogenic 11-methyl hydrocarbon (\textit{IIb}),\textsuperscript{5} conveniently prepared by hydrogenolysis of the 11-methyl ketone (\textit{Ib}), on similar oxidation gave the 11-methyl-15-ketone (\textit{IIIb}) (37\%) with recovery of starting material (\textit{IIb}) (12\%). In contrast to these results, similar oxidation of the 17-ketone (\textit{la}) resulted in an almost quantitative yield of the bright yellow 17-oxo-6,7-quinone (\textit{IVa}), while oxidation of the 11-methyl-17-ketone (\textit{Ib}) under these conditions gave several products. The major compound (51\%) was the 11-methy1-17-oxo-6,7-quinone (\textit{IVd}), but starting material (16\%) was also recovered and t.l.c. disclosed three other minor oxidation products. Addition of osmium tetroxide across the 6,7-double bond in the hydrocarbon (\textit{IIa}) was investigated by Dannenberg and his co-workers\textsuperscript{6} who isolated the 6,7-diol (\textit{XIa}) after treatment in benzene solution containing pyridine. The ketones (\textit{Ia}) and (\textit{Ib}) under these conditions, or better in pyridine solution without added benzene, gave the corresponding keto-diols (\textit{Va}) and (\textit{Vb}) in good yield. These compounds exhibited strong, broad u.v. absorption bands around 310 mp which shifted on borohydride reduction to maxima around 275 mp, similar to that of the diol (\textit{Xla}). A notable feature in the i.r. spectra of the keto-diols (\textit{Va} and \textit{b}) was enhanced absorption at 1600 cm.\textsuperscript{-1}; this was not observed with the diol (\textit{Xla}). Oxidation of the keto-diols with chromic acid afforded high yields of the keto-quinones (\textit{IVa} and \textit{b}), thus confirming the structure of the latter. The keto-diol (\textit{Va}) was dehydrated smoothly when heated with sulphuric acid, but the 11-methyl keto-diol (\textit{Vb}) was dehydrated less readily. The main product from (\textit{Va}) was the 6-ol (\textit{VI}; \textit{R} \textsuperscript{1} = \textit{H}); the derived methyl ether (\textit{Vla}; \textit{R} \textsuperscript{1} = \textit{Me}) was identical with a specimen synthesised from 1-methoxynaphthalene.\textsuperscript{6} A minor phenolic

\textsuperscript{3} A. Butenandt, H. Dannenberg, and D. von Dresler, Z. Naturforsch., 1946, 1, 222.
\textsuperscript{5} A. Butenandt and H. Dannenberg, Arch. Geschwulstforsch., 1953, 6, 1.
\textsuperscript{6} M. M. Coombs and S. B. Jaitly, unpublished work.
product was probably the isomeric 7-hydroxy-17-ketone (VII); it possessed an increased \( R_p \) value, as did also the 11-hydroxy-17-ketone in which the hydroxy-group is sterically hindered (see Experimental section). By analogy, the dehydration product from (Vb) was assumed to be the 6-ol (VIIb; \( R' = H \)), and this was supported by its \( R_p \) value. Moreover, in the n.m.r. spectrum of the derived acetate (VIIb; \( R' = Ac \)) the chemical shift of the multiplet ascribed to the C-15 methylene protons was the same as that of the C-15 methylene protons in the unsubstituted ketone (Ia), whereas distinct alterations in the position of this signal are noted with 7-substituted derivatives.\(^7\)

Oxidation of the ketones (Ia and b) with lead tetra-acetate was also studied, because the potent carcinogen 9,10-dimethylbenzanthracene is oxidised to the 9,10-bis-acetoxyethyl derivative by this reagent,\(^8\) and it was hoped that the 11-acetoxyethyl-17-ketone could be obtained in this way. Little reaction was observed when the unsubstituted ketone (Ia) was stirred with the reagent in glacial acetic acid at room temperature, but irradiation of a boiling mixture gave the 16-acetoxy-ketone (VIIa; \( R' = Ac \)) in moderate yield. This acetoxy-ketone was better prepared from the enol acetate (IXa), itself obtained in high yield from (Ia) with isopropenyl acetate and toluene-\( p \)-sulphonic acid, by oxidation with lead tetra-acetate at room temperature. The 17-oxo-group in (VIIa; \( R' = Ac \)) absorbs at 1720 cm\(^{-1}\), 30 cm\(^{-1}\) to higher frequency than in the parent ketone, probably as a result of interaction with the acetate carbonyl group. Many instances of elevation of normal carbonyl frequencies in 21-acetoxy-20-keto- and 12-acetoxy-11-keto-steroids are recorded.\(^9\) Acid hydrolysis of (VIIa; \( R' = Ac \)) furnished the 16-hydroxy-17-ketone (VIIa; \( R' = H \)), which had 'normal' carbonyl absorption at 1690 cm\(^{-1}\). The u.v. spectra of (VIIa; \( R' = H \) or Ac) were identical and closely resembled that of the ketone (Ia), with the difference that all the maxima were shifted 2—4 m\( \mu \) to longer wavelengths.

The 11-methyl-16-hydroxy-17-ketone (VIIIb; \( R' = H \)) was also readily prepared by oxidation of the enol acetate (IXb) with lead tetra-acetate as already described, and had properties similar to (VIIa). Treatment of the 11-methyl ketone (Ib) with this reagent at room temperature caused little reaction, but in boiling, irradiated acetic acid it gave as main product the 11-methyl-16-acetoxyketone (VIIIb; \( R' = Ac \)); no evidence for appreciable oxidation of the 11-methyl group was found.

Conversion of the aromatic methyl group in 1-methyl-oestrone methyl ether into a formyl group, by oxidation with cerium(iv) ammonium nitrate in 90% acetic acid at room temperature, has recently been reported\(^{10}\) to occur smoothly without concomitant attack at the aliphatic (C-18) or O-methyl groups, or at the activated methylene groups at C-6 and C-16. However, under these conditions the unsubstituted ketone (Ia) was rapidly oxidised, and the keto-quinone (IVA) was isolated in 28% yield; unchanged (Ia) was also recovered (23%). A third substance (ca. 15%) was not obtained pure, but possessed spectral properties suggesting the enolic \( \beta \)-diketone structure, cyclopenta[a]phenanthrene-15(16H),17-dione. The several minor oxidation products included an acidic fraction (10%) which appeared to consist of a mixture of phenanthrene-carboxylic acids produced by cleavage of the 5-membered ring d. Oxidation of the 11-methyl ketone (Ib) with cerium(iv) ammonium nitrate followed by t.l.c. of the reaction mixture disclosed a similar variety of products, although the amount of quinone was considerably less, as also noted in the chromic acid oxidation of this ketone. The

\(^7\) M. M. Coombs, unpublished work.
cyclopenta[a]phenanthrene ring system is thus markedly more susceptible to oxidation by cerium(iv) ions than is the oestrane system, and these experiments were discontinued.

**EXPERIMENTAL**

Reagents and apparatus were generally as described in Part I. The medium-to-strong i.r. bands in the 10—15 μ region are quoted in addition to other salient bands of diagnostic relevance.

15,16-Dihydro-11-methylcyclopenta[a]phenanthrene (IIb).

—The 11-methyl ketone (Ib),11 glacial acetic acid (100 ml) containing conc. hydrochloric acid (4 ml), and a mixture of Adams catalyst (50 mg) and 5% palladium-charcoal (100 mg) were shaken in hydrogen. Absorption of gas ceased when the calculated volume had been consumed (60 hr.). The catalyst was filtered off and the solution furnished an oil which was chromatographed on alumina in benzene-hexane (1:1). The hydrocarbons (IIb) was obtained as a colourless oil (1.13 g) which crystallised and gave needles (510 mg), m.p. 81—82° from ethanol (lit.,13 80.5°—91.5°).

16,17-Dihydro-11-methylcyclopenta[a]phenanthrene-15-one (IIIB).—The hydrocarbon (IIb) (1.26 g) was stirred with glacial acetic acid (14 ml) while a solution of chromium trioxide (0.882 g) in 80% acetic acid (2.2 ml) was added during 5 min. Stirring was continued at room temperature for 40 hr., at the end of which the brown suspension was poured into water and extracted with chloroform. The extract was washed with sodium hydrogen carbonate solution and water, and dried. Evaporation left a yellow solid which was redissolved in benzene and chromatographed on a column of alumina. Elution with benzene-cyclohexane (1:1) gave the starting material (IIb) (150 mg).

Further elution with benzene yielded the ketone (IIIB) (485 mg, 37%), which gave pale yellow needles, m.p. 182—183° (from hot ethanol) (Found: C, 88.6; H, 5.7. C17H1404 requires C, 87.8; H, 5.75%). λmax 217 (log ε 4.57), 235 (4.63), 284 (4.15), 323 (4.14), and 363 (3.41) μ, υmax 5.94 (15-CO), 11.6-9, 11.9, 12.23, 13.28, 13.72, and 14.02 μ.

15,16-Dihydrocyclopenta[a]phenanthrene-6,7,17-trione (IVA).—The unsubstituted ketone (Ia) (92 mg) in glacial acetic acid (10 ml) was stirred with chromium trioxide (150 mg) at room temperature for 24 hr.; t.l.c. then showed the absence of starting material. The yellow solid which had separated was collected, washed with a little acetic acid, then with water, and dried, giving the ketoquinone (IVA) (67 mg), m.p. 245—246° (decomp.) [m.p. 250° (decomp.) from toluene] (Found: C, 77.7; H, 4.2. C14H12O4 requires C, 77.6; H, 3.96%). υmax 5.84 (17-CO), 6.97 and 6.30 (quinone), 10.45, 11.63, 11.90, and 12.90 μ.

The acetic acid mother liquors were diluted with water and the green solution was extracted with chloroform to yield yellow crystals (20 mg) shown by t.l.c. to be (IVA) contaminated with traces of four other substances.

This keto-quinone was also obtained by oxidation of the keto-diol (VA) (20 mg) with chromium trioxide (10 mg) in acetic acid (4 ml) for 2 hr. at room temperature. The mixture was treated as before to give yellow crystals (16 mg), m.p. 246—249°, i.r. spectrum identical with that of the analytical specimen of (IVA).
Methylation of the phenol (VIIa; \( R' = H \)) (150 mg.) under nitrogen with 10% aqueous sodium hydroxide (50 ml.) and dimethyl sulphate (10 ml.) gave a solid (125 mg.) which gave pale yellow needles of the methyl ether (VIIa; \( R' = Me \)) (55 mg.), m.p. 199—205° (from ethanol) (Found: C, 82-7; H, 5-5. \( C_{19}H_{14}O_2 \) requires C, 82-4; H, 5-4%). \( \nu_{max} \) 5-90 (17-CO), 10-22, 12-74, 13-02, and 13-76 \( \mu \). Mixed m.p. with a sample (m.p. 196—197°C) synthesised from 1-methoxynaphthalene showed no depression.

6-7,15,16-Tetrahydro-6,7-dihydroxy-11-methylecyclopenta[a]phenanthren-17-one (Vb).—The 11-methyl ketone (Ib) (2-0 g.) was oxidised with osmium tetroxide (2-05 g.) in pyridine (30 ml.) as already described. The crude grey solid was boiled with ethanol (100 ml.), filtered while hot, and concentrated to yield discoured crystals (1-37 g.); these gave the 11-methyl keto-diol (Vb) as prisms, m.p. 245—246° (from ethanol) (Found: C, 76-9; H, 5-7. \( C_{19}H_{14}O_2 \) requires C, 78-6; H, 4-85%).

The yellow rosettes of needles (Found: C, 81-85; H, 5-1. \( C_{20}H_{16}O_3 \) requires C, 81-85; H, 5-3%). C, 78-8; H, 4-65. \( C_{19}H_{12}O_2 \) requires C, 78-6; H, 4-85%). 14-46 μ.

15-16-Dihydro-6-hydroxy-11-methylecyclopenta[a]phenanthren-17-one (Vb; \( R' = H \)).—The diol (Vb) (1-16 g.) was heated in a sealed tube with 85% sulphuric acid (60 ml.) for 2 hr. The yellow solid was collected and washed with aqueous \( N \)-sodium hydroxide, leaving a grey solid (0-73 g.) which proved to be starting material, m.p. 245—247°. The yellow solid was collected and washed with 3-05 (OH), 5-90 (17-CO), 10-20, 10-38, 11-12, 12-04, 12-78, 13-10, 13-40, 13-80, and 14-46 μ.

When the time of heating was extended to 17 hr., the diol (Vb) (0-04 g.) gave the phenol (VIIb) (0-76 g.) to give a solution which was cooled to yield pale yellow needles, m.p. 205—207° (decomp.) (Found: C, 78-8; H, 4-65. \( C_{17}H_{12}O_2 \) requires C, 78-6; H, 4-85%). \( \nu_{max} \) 5-70 and 8-16 (acetate), 5-81 (17-CO), 12-14, 12-44, 13-10, 13-40, 13-80, and 14-46 μ.

Hydrolysis of this acetoxy-ketone (600 mg.) in methanol (25 ml.) with 5N-sulphuric acid (10 ml.) under reflux for 2 hr. gave a solution which was cooled to yield pale yellow prisms of the hydroxy-ketone (VIIIa; \( R' = H \)) (342 mg.) m.p. 186—187°. Recrystallisation from ethanol-chloroform (2:1; 30 ml.) gave a sample, m.p. 195° (decomp.) (Found: C, 82-1; H, 4-65. \( C_{19}H_{12}O_2 \) requires C, 82-4; H, 4-85%). \( \nu_{max} \) 2-96 (16-OH), 5-92 (17-CO), 12-16, 12-44, and 13-30 μ. Attempted alkaline hydrolysis led to a mixture of products.

Acetylation of the pure phenol (200 mg.) with acetic anhydride-pyridine gave the acetate (VIIb; \( R' = Ac \)) (158 mg.), m.p. 225—227° (from n-butanol (20 ml.) (Found: C, 78-85; H, 5-0. \( C_{20}H_{16}O_3 \) requires C, 78-95; H, 5-3%).

Methylation of the phenol (560 mg.) as before gave the methyl ether (VIIb; \( R' = Me \)) (564 mg.) which separated from ethanol as prisms, m.p. 209—213° (Found: C, 78-8; H, 5-2. \( C_{19}H_{14}O_2 \) requires C, 78-6; H, 4-85%). 14-10 μ.

15-16-Dihydro-3-hydroxy-3-methoxycyclopenta[a]phenanthren-17-one (VIIIb; \( R' = Ac \)).—The 11-methyl ketone (Ib) (6-65 g.) was treated with isopropenyl acetyl and toluene- \( \beta \)-sulphonic acid (20 mg.) during 6 hr. while the solvent (200 ml.) was slowly distilled off. Dichloromethane (100 ml.) was added and the solution was washed with aqueous sodium hydrogen carbonate and water, and dried. The pale fawn crystals obtained on removal of the solvent yielded the enol acetate (IXa) (3-23 g.), m.p. 210—211° (Found: C, 78-3; H, 5-0. \( C_{19}H_{14}O_2 \) requires C, 78-2; H, 5-3%). C, 78-8; H, 4-65. \( C_{18}H_{14}O_2 \) requires C, 78-6; H, 4-85%). 5-90 (17-CO), 12-14, 12-44, 13-10, 13-40, 13-80, and 14-10 μ.

15-16-Dihydro-4-chloro-11-methylecyclopenta[a]phenanthren-17-one (VIIIb; \( R' = H \)).—The unsubstituted ketone (Ia) (3-30 g.) was heated under reflux with isopropenyl acetate (250 ml.) and toluene- \( \beta \)-sulphonic acid (20 mg.) during 6 hr. while the solvent (200 ml.) was slowly distilled off. Dichloromethane (100 ml.) was added and the solution was washed with aqueous sodium hydrogen carbonate and water, and dried. The pale fawn crystals obtained on removal of the solvent yielded the enol acetate (IXa) (3-23 g.), m.p. 210—211° (Found: C, 78-3; H, 5-0. \( C_{19}H_{14}O_2 \) requires C, 78-2; H, 5-3%). C, 78-8; H, 4-65. \( C_{18}H_{14}O_2 \) requires C, 78-6; H, 4-85%). 5-90 (17-CO), 12-14, 12-44, 13-10, 13-40, and 13-88 μ.

Acetylation as before gave 3-acetoxy-15,16-dihydroxy-cyclopenta[a]phenanthren-17-one, pale yellow needles from n-butanol, m.p. 209—213° (Found: C, 78-8; H, 5-2. \( C_{19}H_{14}O_2 \) requires C, 78-6; H, 4-85%).
Oxidation of the 17-Ketones with Lead Tetra-acetate.—Unsubstituted ketone (Ia). This ketone (230 mg.) was boiled under reflux with acetic acid (10 ml.) and lead tetra-acetate (500 mg.) with irradiation for 16 hr. The brown solution was diluted with chloroform and treated as in the similar oxidation of the enol acetate to afford a yellow gum, t.l.c. (dichloromethane) of which disclosed the presence of starting material (Ia), the 16-acetoxy-ketone (VIIIa; R' = Ac), and small amounts of several other products more polar than the latter. This gum was absorbed on a small column of silica gel. Elution with hexane-dichloromethane (4:1) gave a brown material (93 mg.) which yielded (Ia), m.p. 199–201° (30 mg.) from benzene. Later fractions contained the acetoxy-ketone, m.p. 178–180° (55 mg.). The i.r. and u.v. spectra of this material were identical with those of the crude lead gel. Elution with hexane-dichloromethane (4:1) gave the latter. This gum was absorbed on a small column of silica gel. Evaporation left a yellow gum (475 mg.) which was rechromatographed to yield an amorphous solid (235 mg.), not obtained crystalline although it appeared to consist of a single substance (t.l.c.). This solid had i.r. bands at 6-10, 5-83, and 5-75 μ (CO), suggesting a partially enolised β-diketone system, and this was confirmed by the production of a green colour when a dilute ethanolic solution of the material was mixed with ethanolic copper(II) acetate. The u.v. and visible absorption maxima of 15,16-dihydrocyclopenta[a]phenanthren-17-one derivatives

![Absorption Maxima Table]

I thank T. S. Bhatt and Miss J. Y. Comben for technical assistance, and J. F. Richards and D. W. Thomas for the microanalyses.

[9/855 Received, May 21st, 1969]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part IV. Synthesis of 17-Ketones by the Stobbe Condensation

By M. M. Coombs,* (Mrs.) S. B. Jaitly, and F. E. H. Crawley, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2
Potentially Carcinogenic Cyclopenta[α]phenanthrenes. Part IV.\textsuperscript{1} Synthesis of 17-Ketones by the Stobbe Condensation

By M. M. Coombs,\textsuperscript{*} (Mrs.) S. B. Jaitly, and F. E. H. Crawley, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2

2-, 3-, 4-, and 6-Methyl- and 6-methoxycyclopenta[α]phenanthren-17-ones were prepared from appropriately substituted naphthalenes or tetralones, through Stobbe condensation of the corresponding 1,2,3,4-tetrahydrophenanthren-1-ones. The synthesis of ketones labelled with \textsuperscript{14}C in ring D and in the 11-methyl group is also described.

In Part I\textsuperscript{2} we described the use of the intermediate 11,12,13,14,15,16-hexahydrocyclopenta[α]phenanthrene-11,17-dione\textsuperscript{3} in a simple synthesis of 15,16-dihydrocyclopenta[α]phenanthren-17-ones. However, this scheme was not suitable for providing \textsuperscript{14}C-labelled ketones for biochemical studies, nor did it offer a convenient route to several methyl homologues required to extend our skin-painting experiments.\textsuperscript{4} We have therefore used the scheme described by Johnson and Peterson\textsuperscript{5} in which an appropriate 1,2,3,4-tetrahydrophenanthren-1-one is condensed by the Stobbe reaction with diethyl sodiosuccinate to provide the extra carbon atoms with which to construct the five-membered ring D. We now describe the synthesis of a number of cyclopenta[α]phenanthren-17-ones by this method.

1,2,3,4-Tetrahydrophenanthren-1-ones were prepared by two general routes. In the first, the 1-bromo-naphthalenes (Ia—c) were converted into the 1-(2-hydroxyethyl) derivatives (IIa—c; X = OH) by condensation of the derived Grignard reagents with ethylene bromide to yield the bromides (IIa—c; X = Br) from which the diesters [IIa—c; X = CH(CO_2Et)] were obtained by condensation with sodiomalonic ester.

\[
\text{(II)} \quad \text{(II)} \quad \text{(III)} \quad \text{(IV)}
\]

Saponification followed by decarboxylation gave the acids (IIIa—c), which were cyclised with tin(iv) chloride\textsuperscript{7} to give the tricyclic ketones (IVa—c).

In the second method the readily available 1-tetralones (Va—d) reacted with methyl γ-bromocrotonate under

\textsuperscript{6} W. E. Bachmann and W. L. Wilds, \textit{J. Amer. Chem. Soc.}, 1942, 64, 1424.
\textsuperscript{7} W. E. Bachmann and W. L. Wilds, \textit{J. Amer. Chem. Soc.}, 1940, 62, 2084.
Reformatsky conditions \(^8\) to yield the 2,4-dienoic esters (VIIa–d). Isomerisation of the latter by heating with palladium black followed by saponification gave the acids (VIIa–d), which were cyclised as before to give the ketones (VIIia–d). The m.p.s of 1,2,3,4-tetrahydro-7-methylphenanthren-1-one (VIIic) (70.5–71.5°) and its precursor, the acid (VIIc) (134–135°), differed appreciably from those reported by Orcutt and Bogert \(^9\) (92–94 and 116–118°, respectively), who obtained these compounds from a Friedel-Crafts succinoylation of 2-methyl-naphthalene. However, the m.p. of (VIIc) agreed with that quoted by Nasipuri and Roy, \(^10\) who made this compound by the route we used. Since the starting material for this synthesis, 3,4-dihydro-6-methylnaphthalen-1(2H)-one (Vc), was prepared by cyclisation of 4-(m-tolyl)butyric acid, evidence was sought to demonstrate that cyclisation had not led to the isomeric 8-methyltetralone. The n.m.r. spectra of each of the methyltetralones (Vb–d) in the aromatic proton region exhibited a one-proton signal at \(\tau 2.0–2.2\), ascribed to the C-8 proton, downfield from those of the other two aromatic protons (2.8–3.0). Moreover, similar deshielding of the protons of an 8-methyl group is to be expected in view of the magnetic anisotropy induced by the carbonyl group, \(^11\) but the methyl signals of (Vb–d) all had similar \(\tau\) values (7.66, 7.65, and 7.75, respectively). The structure of (Vc) and thus that of (VIIc), is therefore assured. Since Friedel-Crafts acetylation of 2-methylnaphthalene gives mixtures of the 6- and 8-acetyl derivatives \(^12\) it seems possible that the minor isomer used by Orcutt and Bogert was 2-methyl-8-succinoylnaphthalene, not the 5-succinoyl compound as claimed. This would have led \(\#\) to the naphthylbutyric acid (VIIb) and tetrahydrophenanthren-1-one (VIIib), both of which have been synthesised during the present work and which have m.p.s 125–126 and 89–90°, respectively, similar to those reported by the American workers.

\(^*\) We thank a Referee for this suggestion.


catalytic dehydrogenation under controlled conditions. Hydrogenolysis to the hydrocarbon, 15,16-dihydro-17H-cyclopenta[a]phenanthrene, was encountered as a side-reaction. Despite considerable experimentation we have been unable to secure satisfactory results with either 20% platinum on charcoal, as used by this author, or with a commercially available 10% sample. Although conditions were varied widely, the reaction at best led to (XIVa) in about 25% yield with considerable recovery of starting material. The 11-methyl homologue (XIVf) was similarly prepared (see Experimental section). Better yields were obtained by dehydrogenation of the tetrahydro-compounds with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), and this method was adopted for the majority of the reactions. The

11-methyl compound (XIVf) was not obtained from (XIIIif) and DDQ. Presumably steric factors ensure that (XIIIIf) exists largely as the isomer in which the 11-methyl group is axial; consequently it does not possess a pair of trans-diaxial protons, a stereochemical requirement for quinone dehydrogenation. [15,16-14C2] labelled ketones (XIVa and f) were also obtained by slight modifications of the method described, and the 11-[methyl-14C] ketone was prepared from the keto-acetal (XV) with [14C]methylmagnesium iodide.

Spectral data for the 15,16-dihydrocyclopenta[a]phenanthren-17-ones (XIVa—g) are collected in Table 4. Introduction of a methyl substituent at position 2, 3, 4, or 6 in the parent ketone (XIVa) caused a bathochromic shift of the main u.v. absorption band of 1—2 nm., but the 11-methyl ketone (XIVf) was anomalous in this respect, as previously observed. The 6-methoxy-ketone (XIVg) was identical with that derived from the acid-catalysed dehydrogenation of 6,7,15,16-tetrahydro-6,7-dihydrocyclopenta[a]phenanthren-17-one.1

The six-membered ring homologue of the carcinogen (XIVf), namely 1,2,3,4-tetrahydro-11-methylchrysene-1-one (XVIII), was readily prepared from the ketone (XI) by application of the Reformatsky reaction with methyl y-bromocrotonate, following the method of Cook and Schoental8 for the unsubstituted ketone. The resulting ester (XVI) was isomerised and saponified to yield the methylphenanthrylbutyric acid (XVII), which on cyclisation gave (XVIII), identical with this ketone previously prepared by a less convenient route.10

EXPERIMENTAL

Reagents and apparatus were generally as described in previous Parts of this series. Alumina used for column chromatography was Woelm, grade I, unless otherwise specified. Radioactivity was determined with a Nuclear Chicago Liquid Scintillation System, Mark I, or by counting thin films with a Nuclear Chicago C116 gas-flow counter.

1-(2-Hydroxyethyl)naphthalenes (Ila—c; X = OH).—These alcohols were prepared by the general method of Bachmann and Wilds: 1-(2-hydroxyethyl)naphthalene (Ila) (85%), m.p. 62°, b.p. 140—141°/2 mm. (lit., b.p. 160°/0-2 mm.); 1-(2-hydroxyethyl)-4-methylnaphthalene (Iib) (80%), b.p. 152—155°/1-6 mm. (lit., b.p. 155°/0-5 mm.); 1-(2-hydroxyethyl)-4-methoxynaphthalene (Iic) (90%), m.p. 84—86°, b.p. 190°/2 mm. (lit., m.p. 87°).

1-(2-Bromoethyl)naphthalenes (Ila—c; X = Br).—The alcohol (25 g.) and redistilled phosphorus tribromide (10 ml.) were heated together on a steam-bath for 2 hr. After dilution with benzene the solutions were washed with 2N-sodium carbonate and with water. Evaporation of the dried solutions gave oils which were distilled under reduced pressure: 1-(2-bromoethyl)naphthalene (Ila) (77%), m.p. 109—110° (lit., m.p. 109—110°); 4-(4-methyl-1H-pyridinyl)cyclopentene (Ilb) (63%), m.p. 148—149°.

The bromides (Ila—c) were condensed with sodiomalic ester by the procedure of Bachmann and Wilds7 to yield the acids (IIia—c) as follows: 4-(1-naphthyl)butyric acid (IIia) (77%), m.p. 110—112° (lit., m.p. 109—110°); 4-(4-methyl-1H-pyridinyl)butyric acid (IIib) (63%), m.p. 148—149°.


(Found: C, 78-7; H, 6-9. C_{15}H_{14}O_4 requires C, 78-9; H, 7-0%). 4-(4-methoxy-1-naphthyl)butyric acid (IIIc) (70%), m.p. 125—130° (lit. 11 m.p. 131°).

4-(1-Naphthyl)butyric acids (VIIa—d) from Tetralones (V).

These compounds were all prepared as described here for (VIIa).

A mixture of 1-tetralone (9-4 g.), zinc wool (3-6 g.), methyl γ-bromocrotonate (10 g.), absolute ether (40 ml.), and dry benzene (40 ml.), containing a small crystal of iodine, was heated under reflux. Fresh zinc wool (0-5 g.) was added four times during 2 hr.; more methyl γ-bromocrotonate (2 ml.) was then added and heating was continued for 4 hr. The cooled mixture containing much yellow solid was poured into 2N-hydrochloric acid and was shaken with more benzene. The organic layer was evaporated and the residue was hydrolysed by boiling with methanol (60 ml.), potassium hydroxide (6 g.), and water (10 ml.) for 2 hr. After dilution with water the solution was acidified and extracted with benzene. The extract was shaken with saturated sodium hydrogen carbonate solution and the aqueous layer was acidified to give the crude dienoic acid as a gum. This was re-esterified by heating for 1 hr. with methanol (80 ml.) to which acetyl chloride (10 ml.) had been cautiously added. After removal of the solvents under reduced pressure the methyl 2,4-dienoate (V) was obtained as a brown syrup (9-68 g., ν_{max} 5-855 (conj. C=O) and 6-2s (conj. diene), μ (strong, broad) 330 nm.

This ester was isomerised with palladium black (0-5 g.) at 280—300° for 1 hr. The product was distilled in vacuum, filtered from catalyst, and saponified as before. 4-(1-Naphthyl)butyric acid (VIIa), m.p. 109—110° (47-5% overall yield from 1-tetralone), was identical with the sample prepared by the first route (i.e., (IIIa)).

In a similar way 7-methyl-1-tetralone 21 gave 4-(7-methyl-1-naphthyl)butyric acid (VIIIb) (70%), m.p. 125—126° (Found: C, 78-4; H, 6-85. C_{16}H_{14}O_2 requires C, 78-9; 7-06%). 6-methyl-1-tetralone 20 gave 4-(6-methyl-1-naphthyl)butyric acid (VIIc) (66%), m.p. 134—135° (lit. 13 136°). 6-methyl-1-tetralone 20 led to 4-(5-methyl-1-naphthyl)butyric acid (VIId) (60%), m.p. 128—129° (lit. 24 m.p. 127—129°).

4-(1-Naphthyl)pent-2-enoic Acid (IXa).—Malonic acid from Tetralones (VIIa—c), (VIIb—d), and (X).—These ketones were obtained from the corresponding naphthylbutyric acids (IIIa—c), (VIIb—d), and (X) by cyclisation with thionyl chloride and tin(IV) chloride; 4 redistillation of the latter immediately before use improved yields. When necessary the crude product was chromatographed over alumina with toluene—dichloromethane to purify it before recrystallisation from methanol.

Yields, m.p.s, and spectroscopic data are collected in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>1,2,3,4-Tetrahydrophenanthren-1-ones</td>
</tr>
<tr>
<td>Yield (%)</td>
</tr>
<tr>
<td>(IVA)</td>
</tr>
<tr>
<td>(IVb)</td>
</tr>
<tr>
<td>(VIIb)</td>
</tr>
<tr>
<td>(VIIc)</td>
</tr>
<tr>
<td>(VIIId)</td>
</tr>
<tr>
<td>(XI)</td>
</tr>
<tr>
<td>* C_{15}H_{14}O_2 requires C, 85-7; H, 6-6%</td>
</tr>
<tr>
<td>λ_{max} (nm.) (log ε)</td>
</tr>
<tr>
<td>(IVA)</td>
</tr>
<tr>
<td>(IVb)</td>
</tr>
<tr>
<td>(IVc)</td>
</tr>
<tr>
<td>(VIIb)</td>
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<tr>
<td>(VIIc)</td>
</tr>
<tr>
<td>(VIIId)</td>
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<tr>
<td>(XI)</td>
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<tr>
<td>υ_{max} (μ)</td>
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<tr>
<td>(IVA)</td>
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<tr>
<td>(IVb)</td>
</tr>
<tr>
<td>(IVc)</td>
</tr>
<tr>
<td>(VIIb)</td>
</tr>
<tr>
<td>(VIIc)</td>
</tr>
<tr>
<td>(VIIId)</td>
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<tr>
<td>(XI)</td>
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</table>

(bromide (IXg) (70%), b.p. 160—165°/10 mm. (Found: Br, 32-2. C_{15}H_{14}Br requires Br, 32-1%). This bromide (47-5 g.) was condensed with diethyl sodiomalonate and the product was decarboxylated as described before to afford (X) (19-9 g.), m.p. 76°. A Retorovsky reaction between 1-tetralone and methyl 4-bromopent-2-enoate 22 followed by heating with palladium black failed to yield the acid (X). 1,2,3,4-Tetrahydrophenanthren-1-ones (IVa—c), (VIIb—d), and (XI).—These ketones were obtained from the corresponding naphthylbutyric acids (IIIa—c), (VIIb—d), and (X) by cyclisation with thionyl chloride and tin(IV) chloride; redistillation of the latter immediately before use improved yields. When necessary the crude product was chromatographed over alumina with toluene—dichloromethane to purify it before recrystallisation from methanol.

Yields, m.ps, and spectroscopic data are collected in Table 1.

3-(3,4-Dihydro-1-phenanthryl)-3-ethoxycarbonylpicrylic Acids (XIa—g) prepared by the Stobbe Reaction.—General Procedure. The ketone (IVA) (17-9 g.) and diethyl sodiomalonate (30 g., 1-5 mol.) were heated under reflux for 1 hr. with a solution of potassium (5 g.) in dry t-butanol (80 ml.) under dry nitrogen. The cooled solution was acidified with 2N-hydrochloric acid and extracted with ether, which was then re-extracted with N-ammonium hydroxide. The half-

ester which precipitated on acidification of the alkaline extract was collected, washed with water, and dried to give a brown solid (22-7 g., 77% based on the ketone). This yield was raised to 97% by twice recycling the ketone recovered from the original ether extracts. When the
ketone–ester ratio was varied the following yields were obtained:

<table>
<thead>
<tr>
<th>Molar ratio</th>
<th>% Product based on ketone ester ketone ester</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1:1</td>
<td>2</td>
</tr>
<tr>
<td>1:9</td>
<td>7</td>
</tr>
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Half-esters prepared as described here are shown in Table 2; analytical samples were purified by recrystallisation from methanol.

Table 2

<table>
<thead>
<tr>
<th>Half-esters (XIIa—g) prepared by the Stobbe reaction</th>
<th>Found (%)</th>
<th>Req'd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketone ester M.p. C H C H</td>
<td>(IVa) (XIIa)</td>
<td>148—149 74-2 6-35 C_{18}H_{14}O_{4} 74-05 6-2</td>
</tr>
<tr>
<td></td>
<td>(VIIib) (XIIb)</td>
<td>135—136 74-3 6-5</td>
</tr>
<tr>
<td></td>
<td>(VIIIic) (XIIbc)</td>
<td>120—122 74-35 6-4</td>
</tr>
<tr>
<td></td>
<td>(VIIIId) (XII)</td>
<td>164—165 74-4 6-5</td>
</tr>
<tr>
<td></td>
<td>(IVb) (XIIe)</td>
<td>Not obtained crystalline</td>
</tr>
<tr>
<td></td>
<td>(XI) (XIIe)</td>
<td>47—50</td>
</tr>
<tr>
<td></td>
<td>(IVc) (XIIg)</td>
<td>Not obtained crystalline</td>
</tr>
</tbody>
</table>

11,12,15,16-Tetrahydrocyclopenta[a]phenanthren-17-ones (XIIIa—g).

The half-ester (XIIa) (6-3 g.) and catalyst (20% Pt~C 17 or 10% Pt~C; Koch–Light) were heated together in a small hard glass test tube in a metal bath. The cooled mass was dissolved in toluene–dichloromethane, filtered from catalyst, and chromatographed on alumina.

By dehydrogenation with platinum-charcoal. The ketone (XIIIa) (50 mg.) and catalyst (20% Pt–C 17 or 10% Pt–C; Koch–Light) were heated together in a small hard glass test tube in a metal bath. The cooled mass was dissolved in toluene–dichloromethane, filtered from catalyst, and chromatographed on alumina.

The experiments listed in Table 5 are representative of a number performed.

Table 3

<table>
<thead>
<tr>
<th>Yield Found (%)</th>
<th>Yield Req'd (%)</th>
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<tbody>
<tr>
<td>(XIIIa)</td>
<td>C H C H</td>
</tr>
<tr>
<td>69</td>
<td>130—140 87-5 6-45</td>
</tr>
<tr>
<td>61</td>
<td>222—223 86-8 6-4</td>
</tr>
<tr>
<td>55</td>
<td>180—181 87-1 6-35</td>
</tr>
<tr>
<td>87</td>
<td>177—178 87-2 6-3</td>
</tr>
<tr>
<td>30</td>
<td>175—176 81-45 6-05 C_{18}H_{14}O_{4} 81-8 6-1</td>
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</table>

Table 4

<table>
<thead>
<tr>
<th>Yield Found (%)</th>
<th>Yield Req'd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(XIVa)</td>
<td>C H C H</td>
</tr>
<tr>
<td>34</td>
<td>203—204 87-8 5-2</td>
</tr>
<tr>
<td>32</td>
<td>221—222 87-7 5-8</td>
</tr>
<tr>
<td>30</td>
<td>203—204 87-45 5-65</td>
</tr>
<tr>
<td>265—266 87-55 5-75</td>
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<tr>
<td>29</td>
<td>210—212 87-7 5-7</td>
</tr>
<tr>
<td>10</td>
<td>196—197 82-65 5-75 C_{18}H_{14}O_{4} 82-6 5-85</td>
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Table 5

<table>
<thead>
<tr>
<th>Yield Found (%)</th>
<th>Yield Req'd (%)</th>
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<tbody>
<tr>
<td>(XV)</td>
<td>C H C H</td>
</tr>
<tr>
<td>265 (4-89)</td>
<td>334 (3-24)</td>
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<tr>
<td>296 (4-58)</td>
<td>330 (3-40)</td>
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<td>334 (3-34)</td>
<td>370 (3-37)</td>
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<tr>
<td>265 (4-98)</td>
<td>330 (3-36)</td>
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<tr>
<td>266 (4-06)</td>
<td>332 (3-32)</td>
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<tr>
<td>264 (4-83)</td>
<td>335 (3-38)</td>
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<tr>
<td>270 (4-98)</td>
<td>370 (3-43)</td>
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λ max (nm.) (log e)

<table>
<thead>
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<th>Lambda max (nm.)</th>
<th>(log e)</th>
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</thead>
<tbody>
<tr>
<td>(XIVa)</td>
<td>203—204</td>
</tr>
<tr>
<td>(XIVb)</td>
<td>221—222</td>
</tr>
<tr>
<td>(XIVc)</td>
<td>203—204</td>
</tr>
<tr>
<td>(XIVd)</td>
<td>265—266</td>
</tr>
<tr>
<td>(XIVe)</td>
<td>210—212</td>
</tr>
<tr>
<td>(XIVf)</td>
<td>196—197</td>
</tr>
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λ max (nm.) (log e)

<table>
<thead>
<tr>
<th>Lambda max (nm.)</th>
<th>(log e)</th>
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<tbody>
<tr>
<td>(XV)</td>
<td>4-89</td>
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<tr>
<td>(XVI)</td>
<td>4-52</td>
</tr>
<tr>
<td>(XVII)</td>
<td>5-05</td>
</tr>
<tr>
<td>(XVIII)</td>
<td>5-06</td>
</tr>
<tr>
<td>(XIX)</td>
<td>4-69</td>
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<tr>
<td>(XX)</td>
<td>4-49</td>
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<tr>
<td>(XXI)</td>
<td>4-96</td>
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<tr>
<td>(XXII)</td>
<td>4-37</td>
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λ max (μ)

<table>
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<th>Lambda max (μ)</th>
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</thead>
<tbody>
<tr>
<td>(XV)</td>
</tr>
<tr>
<td>(XVI)</td>
</tr>
<tr>
<td>(XVII)</td>
</tr>
<tr>
<td>(XVIII)</td>
</tr>
<tr>
<td>(XIX)</td>
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</tbody>
</table>

* Prepared by catalytic dehydrogenation; m.p. 171—172° (lit.2 171—172°).
Table 5

<table>
<thead>
<tr>
<th>Temp. (min.)</th>
<th>Catalyst (mg.)</th>
<th>Products (%)</th>
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</thead>
<tbody>
<tr>
<td>220—225°</td>
<td>3</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>250—255</td>
<td>15</td>
<td>10 (10%)</td>
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<tr>
<td>250—255</td>
<td>60</td>
<td>10 (10%)</td>
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<tr>
<td>220—225</td>
<td>3</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>220—225</td>
<td>3</td>
<td>5 (20%)</td>
</tr>
<tr>
<td>250—255</td>
<td>15</td>
<td>10 (10%)</td>
</tr>
</tbody>
</table>

* Heated under dry nitrogen. † Heated under carbon dioxide.

Similar results were obtained with the 11-methyl tetrahydro-ketone (XIII).

15,16-Dihydro[15,16-14C]cyclopenta[a]phenanthren-17-one. Diethyl [2,3-14C]succinate (5-72 g., 1 mCi) was condensed with (VIIa) (3-92 g.) and the product was cyclised and dehydrogenated with DDQ as already described. The ketone, [15,16-14C]-[XIVA] (1-54 g., 24 μ Ci/mmole, 33% overall chemical yield), m.p. 205—206°, ran as a single spot on t.l.c. and had u.v. and i.r. spectra identical with those of an authentic specimen. Radiochemical purity determined by dilution analysis was 98.6%.

15,16-Dihydro-11-[14C]methylcyclopenta[a]phenanthren-17-one. With use of a vacuum-line technique to exclude atmospheric moisture and oxygen, the Grignard reagent prepared from [14C]methyl iodide (0-71 g., 1 mCi) was treated with the ketoacetat1 (XV) (1-47 g.), and the product was dehydrated and dehydrogenated by boiling with nitrobenzene and acetic and hydrochloric acids as previously described.2 After chromatography of the crude product [methyl-14C]-[XIVf] (0-66 g., 208 μ Ci/mmole, 55% overall chemical yield) was obtained as pale yellow needles, m.p. 175—177°, the i.r. and u.v. spectra of which were identical with those of an analytical sample. The radiochemical purity was determined by dilution analysis to be 98%.

4-[(4-Methyl-1-phenanthryl)butyric Acid (XVII).—1,2,3,4-Tetrahydro-4-methylphenanthren-1-one (XI) (1-72 g.) was treated with zinc wool and methyl γ-bromocrotonate as described for the tetralones. The crude ester (XVI) was isomerised with palladium black (200 mg.) at 240—260° for 2 hr. under dry nitrogen. After separation from the catalyst the saturated ester was saponified to yield 4-(4-methyl-1-phenanthryl)butyric acid (XVII) (800 mg.), m.p. 100—102° (from benzene) (lit.13 102—102.5°).

1,2,3,4-Tetrahydro-11-methylchrysen-1-one (XVIII).—A mixture of the acid (XVII) (500 mg.) and thionyl chloride (0-3 ml.) in anhydrous ether (5 ml.) containing 2 drops of pyridine was left for 1 hr., then evaporated under reduced pressure. The residue was dissolved in dry benzene (10 ml.) and treated with cooling in an ice-bath with redistilled tin(iv) chloride (0-5 ml.). After 20 min. at room temperature the mixture was hydrolysed with ice-cold hydrochloric acid and the benzene layer was washed with aqueous sodium carbonate and water, dried, and evaporated. The residue gave 1,2,3,4-tetrahydro-11-methylchrysen-1-one (XVIII) (195 mg.), m.p. 139.5—140° (from ethanol-acetone) (lit.,19 138—140°), λmax 287 (log ε 5-32), 298 (4-84), 302 (4-64), 344 (2-73), 363 (3-06), and 380 (3-10) nm.

We thank the Imperial Cancer Research Fund for a Fellowship (to S. B. J.) and a Bursary (to F. E. H. C.). We also thank T. S. Bhatt and Miss J. Y. Comben for technical assistance, and J. F. Richards and D. W. Thomas for the microanalyses.

[9/2135 Received, December 15th, 1969]
Potentially Carcinogenic Cyclopenta[α]phenanthrenes. Part V.¹ Synthesis of 15,16-Dihydro-7-methylcyclopenta[α]phenanthren-17-one

By M. M. Coombs and (Mrs.) S. B. Jaitly, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

Reprinted from

JOURNAL
OF
THE CHEMICAL SOCIETY

SECTION C
Organic Chemistry

1971
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part V. Synthesis of 15,16-Dihydro-7-methylcyclopenta[a]phenanthren-17-one

By M. M. Coombs and (Mrs.) S. B. Jaitly, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

15,16-Dihydro-7-methylcyclopenta[a]phenanthren-17-one has been prepared by sodium and liquid ammonia reduction of the diethyl phosphate of its readily available 11-hydroxy-derivative. Among the monomethyl derivatives of the hydrocarbon 15,16-dihydrocyclopenta[a]phenanthrene, of interest on account of its structural resemblance to the natural steroidal sex hormones, the 7- and 11-methyl derivatives were found by Butenandt and Dannenberg to display weak carcinogenic activity. In more recent work in the 17-ketone series we have shown that 11-methylcyclopenta[a]phenanthren-17-one is a potent carcinogen, while the unsubstituted ketone and its 12-methyl and 6-methyl derivatives are without activity. It was, therefore, of interest to synthesise and test the 7-methyl-17-ketone, but a number of unexpected difficulties were encountered when this was attempted, apparently resulting from the strategic position occupied by the methyl group in relation to the five-membered ring in these compounds.

Employing the scheme described in Part I, 2-acetyl-3-methylnaphthalene was converted into the furfurilidene derivative (I; R = Me). Treatment of the latter with acid gave the expected dioxoheptanoic acid (III; R = Me) together with considerable amounts of a non-ketonic acid, C_{18}H_{16}O_{3}, to which the naphthylfuran structure (II) was assigned. The n.m.r. spectrum of (II) consisted of six aromatic protons (7.1-9.28), four methylene protons (multiplet centred at 7.05), an aryl methyl singlet (7.38), and a pair of one-proton doublets (3.47 and 3.82, each with J 7 Hz), assigned to the two furan hydrogen atoms. Although this compound was stable to hot hydrochloric acid, treatment with nitric acid in cold acetic acid cleaved the furan ring to give the dioxoheptanoic acid (III; R = Me), together with a second acid with similar chromatographic properties. Since these conditions are those under which 2,5-diaryl-1,2,3,4-tetrahydro-7-methylnaphthalene was formed, we were unable to account for this discrepancy.

In the unsubstituted series treatment of the furfurilidene compound (I; R = H) with acid gives the dioxo-acid (III; R = H) as the only isolated product. In the present case conjugation of the carbonyl group with the aryl rings is sterically inhibited by the methyl group, and, as Butenandt et al. have demonstrated for the tetrahydro-analogue, this favours cyclisation involving this carbonyl group leading to the furan-acid.

Treatment of the dioxoheptanoic acid (III; R = Me) with base afforded the cyclopentenone (IV; R = Me) in high yield.

The German authors carried out a series of reactions similar to those described above, starting with 6-acetyl-1,2,3,4-tetrahydro-7-methylnaphthalene. They reported that acid treatment of the furfurilidene derivative (V) led to one product, the tetrahydroheptanoic acid (VI), while on cyclisation of the latter with base two compounds, the cyclopentenone (VII) and the furan (VIII), were isolated. Since this seemed improbable on the basis of our results with the naphthyl analogues, this work was repeated. In our hands acid treatment of (V) gave both (VI) and (VII) as expected, while with base the dioxo-acid (VI) gave only the cyclopentenone (VII). We are unable to account for this discrepancy.

The cyclopentenone acid (IV; R = Me) absorbed at 259 nm. compared with 268 and 306 nm. for the unsubstituted acid (IV; R = H) showing that, as in Butenandt's compound (VII), resonance of the enone moiety with the naphthalene rings is inhibited. Hydrogenation of the double bond in (IV; R = Me) did not occur smoothly, as it did with the unsubstituted homologue (IV; R = H). More than the calculated volume of hydrogen was absorbed and the product consisted of six compounds, as judged by t.l.c. The compound...
present in the largest amount proved to be the desired cyclopentanone acid (IX) required for cyclisation to the 11,17-diketone in order to complete the synthesis by the previously described method. Unfortunately, separation of (IX) from the hydrogenation mixture proved impracticable except on a small scale by t.l.c.

As in the unsubstituted series, the acid (IV; R = Me) readily underwent cyclisation when boiled with acetic anhydride to give a high yield of 11-acetoxy-15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-one (X; R = Ac), from which the parent phenol (X; R = H) and methyl ether (X; R = Me) were prepared. The availability of this phenol encouraged attempts to remove the 11-oxygen function from it in order to arrive at the title compound.

addition, a benzylic ketone function. Difficulty was experienced in preparing the phenyltetrazoyl ether, probably as a result of the steric overcrowding, but the benzoazolyl ether (XII) was obtained under rather vigorous conditions. Hydrogenation of this ether occurred sluggishly at a pressure of 6 atmospheres over 5% palladium–charcoal to give only a trace of the required ketone (XI; R = H). The major product was chromatographically and spectroscopically distinct from the latter and was not the urethane (XI; R = PhNH–COO) which would have arisen through cleavage of the benzoxazoyl aryl–oxygen bond. The failure of this reaction can probably also be attributed to steric factors.

Attention was therefore directed towards reducing agents with low steric requirements. Reduction with sodium in liquid ammonia has this characteristic and was employed to reduce aryl diethyl phosphates to hydrocarbons by Kenner and Williams. These authors reported that methyl benzoate could be obtained in 20% yield from the diethyl phosphate of methyl salicylate by this method, and this encouraged us to attempt to reduce [XI; R = PO(OEt)_2] without prior protection of the ketone group. This phosphate was secured in good yield by prolonged treatment of a suspension of the phenol (XI; R = OH) in carbon tetrachloride with diethyl phosphite and triethylamine. Reduction with two atoms of sodium per molecule of phosphate in a mixture of liquid ammonia and tetrahydrofuran led to a mixture of products from which the required ketone (XI; R = H) was readily isolated by column chromatography in 27% yield. Although this yield was low, the overall yield from the acid (IV; R = H) was 18%, similar to the overall yield obtained in the seven-stage synthesis partially described in Part I.

This scheme was applied to the 7-methyl-11-phenol (X; R = H). Reduction of the derived diethyl phosphate [X; R = PO(OEt)_2] with sodium in liquid ammonia–tetrahydrofuran occurred smoothly to furnish the required 15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-one (XIII) in 43% yield. The u.v. absorption spectrum of this ketone closely resembled those of

N.m.r. spectra (r-values) of 15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-ones

<table>
<thead>
<tr>
<th>CH₄ protons</th>
<th>C-15</th>
<th>C-16</th>
<th>Me protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X; R = Ac)</td>
<td>6-24t</td>
<td>7-2m*</td>
<td>7-08s, 7-51s (Ac)</td>
</tr>
<tr>
<td>(X; R = Me)</td>
<td>6-5t</td>
<td>7-35m</td>
<td>7-18s, 5-9s (OMe)</td>
</tr>
<tr>
<td>(XIII)</td>
<td>6-5t</td>
<td>7-28m</td>
<td>7-08s</td>
</tr>
<tr>
<td>[Unsubstituted]</td>
<td>6-5t</td>
<td>7-08s</td>
<td></td>
</tr>
<tr>
<td>(XI; R = H)</td>
<td>6-72t</td>
<td>7-3m</td>
<td>* Partly obscured by methyl singlet.</td>
</tr>
</tbody>
</table>

s = Singlet, t = triplet, m = multiplet.

its positional isomers, particularly that of the strongly carcinogenic 11-methyl-17-ketone, with the exception

that the main band absorbed at a slightly higher wavelength. The n.m.r. spectra of the 7-methyl-17-ketones (XII) and (X; R = Ac, Me) differed from those of other cyclopenta[a]phenanthren-17-ones \(^5\) not bearing a 7-methyl group, in demonstrating that the 15-methylene protons were deshielded.

**Experimental**

Reagents and apparatus were generally as described in previous Parts of this series except that most of the u.v. spectra were recorded for ethanol solutions on a Perkin-Elmer Model 402 spectrophotometer.

**Acid Treatment of 7-Furfurylideneacetyl-1,2,3,4-tetrahydro-6-methylnapthalene (V).** This compound (8 g), prepared according to Butenandt,\(^7\) b.p. 180°/1-5 mm., m.p. 75°–77° (lit., 69°–72°), was boiled with ethanol (33 ml) and conc. hydrochloric acid (8 ml) for 9 hr., when the alcohol was removed under reduced pressure. The dark gum was again boiled for 2 hr. with a mixture of water (47 ml), glacial acetic acid (47 ml), and conc. hydrochloric acid (19 ml), then decanted while hot from the insoluble tar through a pad of glass wool. The crystals which separated when the filtrate was cooled were collected, the mother liquor was returned to the flask, again boiled for 2 hr., and the whole procedure was repeated nine times until only a trace of further crystals was obtained from the cooled extract. Each of the 10 crops (total, 443 g) had m.p. 110°–115° and was shown by t.l.c. (toluene–ethanol, 5:1) to consist of two compounds, present in similar amounts. Recrystallisation of a portion (0-5 g) from 80° aqueous acetic acid gave the furfurylphthyl acid (VIII; 0-1 g), m.p. 144°–

145° (lit., 143°). After dilution of the mother liquor with an equal volume of water there crystallised the dioxo-acid (VI) (0-2 g), m.p. 115°–116°, which after two recrystallisations from ethanol had m.p. 123°–124° (lit., 120°–122°) (Found: C, 71-6; H, 7-45. Calc. for C\(\text{H}_8\)O\(\text{O}_4\): C, 71-8; H, 7-36). This acid (0-19 g) was heated under reflux with 1-9° aqueous potassium hydroxide (19°5 ml) for 3 hr. The cooled solution was acidified, extracted with ether, and the ether was washed with water and dried. On evaporation it yielded crystals (0-19 g), t.l.c. of which showed the presence of some starting material (V), \(R_p 0-50\), and a new acid (VII), \(R_p 0-58\), but no trace of the furyl-phthyl-acid (VIII), \(R_p 0-66\). Recrystallisation from acetone-light petroleum afforded (VII) (40 mg), m.p. 128°–130° (lit., 130°–132°).

**3-Furfurylideneacetyl-2-methylnapthalene (I; R = Me).**—Contrary to a published report,\(^\text{13}\) hydrogenation of 2-methylnapthalene over W2 Raney nickel at 130 atm./130° gave a mixture of 1,2,3,4-tetrahydro-6-methyl- and 1,2,3,4-tetrahydro-2-methyl-napthalenes [n.m.r. Me, \(\tau 7-79\) s and 8-90 d (\(/ 5\) Hz), respectively] which was completely separable only by g.l.c. A pure sample of this former was therefore prepared by Clemmensen reduction \(^\text{14}\) of 1,2,3,4-tetrahydro-7-methylnapthalen-1-one and submitted to a Friedel–Crafts reaction \(^\text{16}\) with acetyl chloride to yield 6-acetyl-1,2,3,4-tetrahydro-7-methylnapthalene, b.p. 142°–144°/4 mm., m.p. 31°–32° (lit.,\(^\text{15}\) oil b.p. 156°–157°/11 mm.). Dehydrogenation with sulphur \(^\text{19}\) afforded 2-acetyl-3-methylnapthalene, b.p. 184°–188°/23 mm., m.p. 82°–83° (lit.,\(^\text{15}\) oil b.p. 164°/11 mm.) (from light petroleum, then ethanol) (Found: C, 85-0; H, 6-7. Calc. for C\(\text{H}_8\)O\(\text{O}_4\): C, 84-75; H, 6-6%). methyl singlets \(\tau 7-30\) and 7-53.


This ketone (46-1 g) was dissolved in a solution containing furfuraldehyde (17-1 ml), ethanol (125 ml), and 2N-sodium hydroxide solution (5 ml) and left at room temp. for 18 hr. The yellow crystals were collected and washed with cold ethanol. The furfurylphthyl compound (I; R = Me) (41-1 g) had m.p. 103°–105°, raised to 109°–110° by four recrystallisations from methanol (Found: C, 82-7; H, 5-75. C\(\text{H}_8\)O\(\text{O}_4\) requires C, 82-4; H, 5-45%). \(\lambda_{\text{max}}\) (log e) 223-5 (4-64), 255 (4-88) nm. (4-13), 333.

**Acid Treatment of 3-Furfurylideneacetyl-2-methylnapthalene (I; R = Me).**—The furfurylphthyl compound (I; R = Me) (47 g) was heated under reflux for 9 hr. with ethanol (175 ml) containing concentrated hydrochloric acid (45 ml). The ethanol was removed under reduced pressure and the resulting black gum was extracted repeatedly by boiling with a mixture of glacial acetic acid (250 ml), water (250 ml) and concentrated hydrochloric acid (100 ml), as described for the tetrahydro-analogue. The product, obtained in 10 crops (total, 22-7 g) all of which had m.p. 105°–130° and consisted of two compounds as judged by t.l.c., was dissolved in hot 80° acetic acid (180 ml). Brown crystals (9-57 g), m.p. 145°–153°, separated which after further recrystallisation from ethyl acetate and then n-butanol gave 5-2-[2-(3-methylphthyl)]-furan-2-propionic acid (II), m.p. 157°–158° (Found: C, 76-95; H, 5-95. C\(\text{H}_8\)O\(\text{O}_4\) requires C, 77-1; H, 5-75%). \(\lambda_{\text{max}}\) 255 (4-49), 274 (4-36), 310 (4-22), 338 nm. (3-28). The mother liquor was heated and diluted with hot water (100 ml); on cooling 7-2-[3-methylphthyl]-4,7-dioxohexoic acid (III; R = Me) (10-1 g), m.p. 116°–120°, separated as fawn needles. Recrystallisation from aqueous methanol gave a sample m.p. 123°–124° (Found: C, 72-65; H, 6-0. C\(\text{H}_8\)O\(\text{O}_4\) requires C, 72-45; H, 6-1%). \(\lambda_{\text{max}}\) 241 (4-63), 248 (4-62), 283 (3-88), 332 nm. (3-13); \(\nu_{\text{max}}\) 5-88, 5-90, 5-97 μ; \(\tau 7-42\) (5, Me).

The furan (II) (12 g) was suspended in acetic acid (100 ml) while concentrated nitric acid (5 ml) was added dropwise with cooling so that the temp. of the mixture did not exceed 20°. After 5 min. the clear, pale orange solution was poured into water (500 ml), and the precipitate was crystallised from ethanol (50 ml). The first crop (5-72 g) was shown by t.l.c. (toluene–ethanol, 1:5) to consist of the dioxoheptanoic acid (III; R = Me) together with a second, slightly more-polar compound; the second crop (0-40 g) was almost pure (III; R = Me). Recrystallisation of a specimen from ethanol gave needles, m.p. 125°–126°, not depressed on admixture with pure (III; R = Me), m.p. 123°–124°, described above. A portion (0-5 g) of the first crop was dissolved in ethyl acetate (20 ml) and shaken with 5% palladium–charcoal in an atmosphere of hydrogen. No uptake of gas was observed and t.l.c. demonstrated that the mixture was unchanged.

[2-(3-Methylphthyl)]-2-oxo-5-cyclopent-1(5)-eneacetic acid (IV; R = Me).—The dioxo-acid (III; R = Me) (5-0 g) dissolved in 2% aqueous sodium hydroxide solution (500 ml) was kept at 95° for 80 min. The gum which separated when the cooled solution was acidified was extracted with dichloromethane and the extract was washed with water, and dried. Evaporation yielded a gum (4-0 g) which was crystallised from n-butanol (8 ml) to give the acid (IV; R = Me) (2-4 g), m.p. 182°–184° raised to 185°–186° by three recrystallisations from ethanol (Found: C, 76-9; 76-8) 232.


H, 5-65. C₁₈H₁₈O₃ requires C, 77-1; H, 5-75%. λₘₐₓ 222 (4-78), 259 (4-21), 321 nm. (3-00), νₘₐₓ 5-85 (carboxyl and ketone C=O) and 6-04 μ (C=C). The methyl ester, prepared from the acid with methanol and dry hydrogen chloride, formed an oil νₘₐₓ 5-77 (ester C=O), 5-88 (ketone C=O), and 6-10 μ (C=C).

[2-3-Methylnaphthyl]-2-oxo-5-cyclopentaene-acetic Acid (IX).—The above ester (3-76 g.) in methanol (20 ml.) was shaken with 5% palladium-charcoal (1 g.) in hydrogen. After 165 hr. the calculated volume of hydrogen had been taken up, but gas was still being absorbed. T.l.c. (dichloromethane) showed the presence of starting material and one major and 5 minor products. Attempts to separate this mixture by chromatography on silica-gel columns with several solvent systems failed, and a portion was finally resolved by preparative t.l.c. The zone bearing the major product was eluted to give an oil, λₘₐₓ 273 nm., which was saponified with 10% aqueous sodium hydroxide containing some ethanol at the boil for 30 min. Acidification gave a solid which was crystallised from ethanol to form prisms of C₁₈H₁₈O₃ requires C, 76-55; H, 6-45%, X mₐₓ 229 (4-96), 243 (4-24), 257 (4-39), 300, 305, 338, 355 nm. (3-79, 3-86). The methyl ether (X; R = Me), prepared by methylation of the phenol with dimethyl sulphate and sodium hydroxide in ethanol, formed needles (from ethanol), m.p. 335—340° (decomp.) (Found: C, 82-9; H, 5-4%, 267 (4-81), 293-5 (4-37), 321 (3-92), 355 (3-44), 373 (3-74), 393 nm. (3-81). 11-Benzoxazol-2-yl-15,16-dihydropentacyclo[a]phenanthren-17-one (X; R = PhNH*CO*O) formed yellow needles of the sodium salt from the phenol (XII).—To the sodium salt from the phenol (X; R = H) was added isocyanatobenzene (1 ml.) and dry pyridine (5 ml.) in a sealed tube at 100° for 18 hr. The crystals which filled the flask fitted with a solid carbon-dioxide cold-finger condenser were filtered off and recrystallised from n-butanol. The urethane (XI; R = PhNH-CO-O) formed

This acetate (6-14 g.) was hydrolysed by heating it under reflux with ethanol (240 ml.), water (5 ml.), and sodium hydroxide (2-5 g.) for 1-6 hr. Acidification with 2n-hydrochloric acid gave a yellow precipitate which was collected, washed with water, and dried in vacuo (4-62 g.). An analytical sample of the phenol (X; R = H) was prepared, by crystallisation from n-butanol, as pale yellow prisms, m.p. 335—340° (decomp.) (Found: C, 82-9; H, 5-4%). The sodium salt (3-52 g.) of this phenol was methylated with diethyl phosphate (2-58 ml.) while triethylamine (2-8 ml.) was added drop wise. The mixture became warm. After being allowed to stand at room temp. for 6 days protected from moisture, the paste was dissolved by the addition of dichloromethane and extracted with 0-1% sodium hydroxide. The organic layer was washed with water, dried, and evaporated to give a brown solid which was crystallised from ethanol (50 ml.). There separated fawn needles (5-97 g.) of 

This phosphate (5-97 g.) was suspended in liquid ammonia (300 ml.) and sodium-dried tetrahydrofuran (100 ml.) in a flask fitted with a solid carbon-dioxide cold-finger condenser and sodamide drying tube. Sodium (0-69 g., 2 atom per molecule of phosphate) cut into ca. 60 pieces, was added slowly with magnetic stirring during 1 hr. so that the blue colour from each fresh addition was discharged before the next addition was made. A clear, dark brown solution was formed when ca. 20% of the sodium had been added. When addition was complete the solution was stirred for a further 15 min. After which the ammonia was evaporated in a stream of nitrogen using a warm water-bath. Water was added and the mixture was extracted with dichloromethane. The organic solution was washed with water until the washings were neutral and evaporated to give a brown gum shown by t.l.c. to contain the required product (XI; R = H), a small quantity of the starting material, and several other compounds more polar than the former. This gum, dissolved in toluene-ethyl acetate was added to a column of alumina (Woelm Grade II, 250 g.). Elution with toluene containing 5% ethyl acetate gave fractions which on evaporation furnished 15,16-dihydropentacyclo[a]phenanthren-17-one (XI; R = H) as pale yellow needles (0-945 g.), m.p. 202—204°, with i.r. and u.v. spectra identical with those of an authentic specimen. The overall yield from the unsaturated acid (IV; R = H) was 18%. 15,16-Dihydro-11-hydroxy-7-methylcyclopenta[a]phenanthren-17-one (X; R = H).—The unsaturated acid (IV; R = Me) (5-95 g.) was heated under reflux with acetic anhydride (45 ml.) for 3-5 hr. The solution was cooled and crystals separated and were collected and dried (5-57 g.), m.p. 238—240°; a further quantity (0-77 g.) was obtained by hydrolysis of the mother liquor. 11-Acetyl-15,16-dihydro-11-hydroxy-7-methylcyclopenta[a]phenanthren-17-one (X; R = Ac) formed straw-coloured laths (from n-butanol), m.p. 243—244° (Found: C, 78-8; H, 5-4. C₂₀H₁₆O₃ requires C, 78-95; H, 5-3%).

This acetate (6-14 g.) was hydrolysed by heating it under reflux with ethanol (240 ml.), water (5 ml.), and sodium hydroxide (2-5 g.) for 1-6 hr. Acidification with 2n-hydrochloric acid gave a yellow precipitate which was collected, washed with water, and dried in vacuo (4-62 g.). An analytical sample of the phenol (X; R = H) was prepared, by crystallisation from n-butanol, as pale yellow prisms, m.p. 335—340° (decomp.) (Found: C, 82-9; H, 5-4%). The sodium salt (3-52 g.) of this phenol was methylated with diethyl phosphate (2-58 ml.) while triethylamine (2-8 ml.) was added drop wise. The mixture became warm. After being allowed to stand at room temp. for 6 days protected from moisture, the paste was dissolved by the addition of dichloromethane and extracted with 0-1% sodium hydroxide. The organic layer was washed with water, dried, and evaporated to give a brown solid which was crystallised from ethanol (50 ml.). There separated fawn needles (5-97 g.) of diethyl 11-(15,16-dihydro-17-oxocyclopenta[a]phenanthryl) phosphate [XI; R = OPO(OEt)₂], m.p. 144—145° (Found: C, 65-65; H, 5-4; P, 7-8 C₁₈H₁₈O₅P requires C, 65-65; H, 5-4; P, 7-8%). 2 n - 263 (4-83), 388-5 (4-48), 390 (4-32), 340 (3-27), 357 (3-53), 375 nm. (3-59), νₘₐₓ 5-90 (aryl ketone), 7-9 (P=O-aryl), 9-8 μ (P=O-alkyl). This phosphate (5-97 g.) was suspended in liquid ammonia (300 ml.) and sodium-dried tetrahydrofuran (100 ml.) in a flask fitted with a solid carbon-dioxide cold-finger condenser and sodamide drying tube. Sodium (0-69 g., 2 atom per molecule of phosphate) cut into ca. 60 pieces, was added slowly with magnetic stirring during 1 hr. so that the blue colour from each fresh addition was discharged before the next addition was made. A clear, dark brown solution was formed when ca. 20% of the sodium had been added. When addition was complete the solution was stirred for a further 15 min. After which the ammonia was evaporated in a stream of nitrogen using a warm water-bath. Water was added and the mixture was extracted with dichloromethane. The organic solution was washed with water until the washings were neutral and evaporated to give a brown gum shown by t.l.c. to contain the required product (XI; R = H), a small quantity of the starting material, and several other compounds more polar than the former. This gum, dissolved in toluene-ethyl acetate was added to a column of alumina (Woelm Grade II, 250 g.). Elution with toluene containing 5% ethyl acetate gave fractions which on evaporation furnished 15,16-dihydropentacyclo[a]phenanthren-17-one (XI; R = H) as pale yellow needles (0-945 g.), m.p. 202—204°, with i.r. and u.v. spectra identical with those of an authentic specimen. The overall yield from the unsaturated acid (IV; R = H) was 18%.

The above ether (100 mg.) and 5% palladium-charcoal (50 mg.) in ethyl acetate (25 ml.) was shaken in hydrogen at 6 atm. and 30—35° for 24 hr. (the starting material was largely recovered after less drastic treatment). T.l.c. (dichloromethane) revealed some starting material (Rₚ 0-22) and a trace of the expected ketone (Rₚ 0-65) together with a slightly less-polar material (Rₚ 0-30). After elution with ethanol this material had λₘₐₓ 261 nm. and no sharp peaks in the 300—400 nm. region characteristic of a cyclopenta[a]phenanthrene.

The phenol (XI; R = OH) (1-0 g.) was heated with isocyanatobenzene (1 ml.) and dry pyridine (5 ml.) in a sealed tube at 105° for 18 hr. The crystals which filled the dark liquid were filtered off and recrystallised from n-butanol. The urethane (XI; R = PhNH-CO-O) formed

18 Neth. Pat. Appl. 6,505,511 (Chem. Abs., 1966, 64, 12,679h).
cream needles, $\lambda_{\text{max}}$ 263, 288, 301-5, 339, 353, 375 nm.; $\nu_{\text{max}}$ 3-00 (NH), 5-86 (amide I), 5-90 (aryl ketone), 6-51 $\mu$ (amide II); $R_f$ 0-14 (dichloromethane).

15,16-Dihydro-7-methylcyclopenta[c]phenanthren-17-one (XII).—The phenol (X; $R = \text{OH}$) (4-52 g.) was suspended in carbon tetrachloride (20 ml.) and treated with diethyl phosphate (3-25 ml.) and triethylamine (3-55 ml.) at room temperature for 13 days. Work-up as before yielded brown crystals of the diethyl phosphate [X; $R = \text{PO(OEt)}_2$] (3-02 g.), m.p. 140—142°, 266, 291, 305, 350, 368, and 396 nm.; $\nu_{\text{max}}$ 5-88 (aryl ketone), 7-82 (P=O-aryl), and 9-70 $\mu$ (P=O-alkyl).

This phosphate (3-00 g.) in liquid ammonia (150 ml.) and tetrahydrofuran (50 ml.) was reduced with sodium (0-36 g.) as described above. Chromatography of the crude product on alumina gave fractions, homogeneous by t.l.c., which were combined and evaporated to yield pale yellow crystals (0-822 g.). Recrystallisation from ethanol gave 15,16-dihydro-7-methylcyclopenta[a]phenanthren-17-one (XII) (0-665 g.), m.p. 198—199° (Found: C, 88-05; H, 5-85. C$_{18}$H$_{14}$O requires C, 87-75; H, 5-75%). $\lambda_{\text{max}}$ 268 (4-79), 286 (4-42), 301 (4-28), 343 (3-23), 360 (3-41), 378 nm. (3-42); $\nu_{\text{max}}$ 5-91 (aryl ketone), 11-3, 11-9,12-9, 13-34 $\mu$.

We thank the Imperial Cancer Research Fund for a Fellowship (to S. B. J.); we are indebted to Dr. M. Wilkinson for valuable discussions. We also thank T. S. Bhatt and Mrs. M. C. Hall for technical assistance, and D. W. Thomas for the microanalyses.

[0/1076 Received, June 24th, 1970]
Potentially Carcinogenic Cyclopenta[α]phenanthrenes. Part VI.1
1,2,3,4-Tetrahydro-17-ketones

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Reprinted from
JOURNAL
OF
THE CHEMICAL SOCIETY
PERKIN TRANSACTIONS I
1973
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part VI.1
1,2,3,4-Tetrahydro-17-ketones

By Maurice M. Coombs * and Tarochan S. Bhatt, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

Cyclisation of 2-oxo-5-(6,7,8-tetrahydro-2-naphthyl)cyclopentaneacetic acid led to both angular and linear ring-fused products from which were derived 1,2,3,4,15,16-hexahydrocyclopenta[a]phenanthren-17-ones and 1,2,7,8,9,10-hexahydrocyclopenta[a]anthracen-3-ones.

Essential structural features associated with a high degree of carcinogenicity in compounds of the cyclopenta[a]phenanthrene series appear to be the presence of a methyl substituent at C-11 and a C=C or C=O double bond at C-17.2 Reduction of the 11,12-double bond in the potent carcinogen 11-methyl-15,16-dihydrocyclopenta[a]phenanthren-17-one (I) abolishes activity, but the effect of reduction elsewhere in this molecule has not been studied. 1,2,3,4-Tetrahydrodibenz[a,A]anthracene retains carcinogenic activity comparable with that of the fully aromatic hydrocarbon,3 and it was therefore of interest to prepare the 1,2,3,4-tetrahydro-derivative of compound (I), namely the ketone (IIb). Additionally, knowledge of the u.v. absorption of this ketone was of importance in connection with the structural elucidation of a major metabolite of the carcinogen (I) in the rat.4

The syntheses of the ketones (IIa and b) followed the general scheme employed in Part I.5 6-Acetyl-1,2,3,4-tetrahydro-1-naphthalene6 was converted into its furfurylidene derivative (III), which with hot acid gave the dioxoheptanoic acid (IV). Cyclisation of the latter under alkaline conditions led to the cyclopentenic acid (Va), which was reduced with lithium in liquid ammonia to the cyclopentane acid (VIa). Over-reduction was difficult to avoid when catalytic hydrogenation over palladium was employed to reduce this tetrastubstituted double bond, and the cyclopentane acid (VIb) was isolated as a by-product.

In the naphthalene series, phosphoric acid-catalysed cyclisation of the analogue of (VIa) occurs exclusively in the angular sense to yield a phenanthrene derivative. In the present case lack of the strongly directing, fused aromatic ring led to a 1:4 mixture of the angular

4 F. E. H. Crawley, unpublished work.
(VIIa) and linear (VIIIa) diketones. Each selectively gave a monoacetal [(VIIb) and (VIIIb)] on acid-catalysed exchange with 2-ethyl-2-methyl-1,3-dioxolan.

The structures of these compounds followed from their i.r. spectra, which in both cases indicated a conjugated carbonyl group, and from the characteristic n.m.r. signals of their aromatic protons. Those of (VIIb) consisted of an AB quartet centred at $\tau = 2.88$, whereas those of (VIIIb) formed two rather broad 'singlets' at $\tau = 2.22$ and 2.98. Ring closure of the cyclopentane acid (VIIb) with phosphoric acid gave mainly the linear monoketone (VIIIc), with evidence of a second ketone, probably the angular isomer (VIIc).

Cyclisation of (Va) by heating with acetic anhydride led to a mixture of two isomeric acetoxy-ketones, (IX) and (Xa), readily distinguished by their n.m.r. spectra, the aromatic region of the latter consisting of three well resolved one proton singlets. In confirmation, dehydrogenation of (IX) with 2,3-dichloro-5,6-dicyanobenzo-1,4-quinone (DDQ) yielded the known 11-acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one, and similar dehydrogenation of the tetrahydrothracen-3-one (Xa) gave 5-acetoxy-1,2-dihydrocyclopenta[a]anthracen-3-one (XIa). The u.v. absorption of this compound was similar to that of its 5-deacetyl derivative (XIIb) described later.

The acetal (VIIb) was converted into the ketone (IIb) by reaction with methylmagnesium iodide followed by treatment with acid and nitrobenzene. The unsubstituted tetrahydro-ketone (IIa) was prepared from (VIIb) by reduction with sodium borohydride and subsequent treatment with acid and nitrobenzene. In contrast to the high yields of phenanthrenes previously obtained with this dehydration-dehydrogenation reaction, the yields of naphthalenes obtained in the present work were poor. Since it was essential to conserve for biological evaluation the small quantity of (IIb) obtained, this ketone was characterised by mass spectrometry. The base peak was the molecular ion, $m/e$ 250. The next most abundant ion was at $m/e$ 222 (19-5%), corresponding to $M - CO$, and for which a metastable peak at $m/e$ 197.3 was observed.

In a similar manner the linear acetal (VIIb) furnished 1,2,7,8,9,10-hexahydrocyclopent[a]anthracen-3-one (Xb). Dehydrogenation with DDQ gave the corresponding 1,2-dihydro-compound (XIIb), with u.v. absorption characteristics almost identical with those of 2-acetylanthracene.

EXPERIMENTAL

Reagents and apparatus were generally as described in previous Parts of this series.

6-Furfurylideneacetyl-1,2,3,4-tetrahydronaphthalene (III).

To a solution of 6-acetyl-1,2,3,4-tetrahydronaphthalene 5 (202 g; b.p. 146° at 10 mmHg) in 2-furaldehyde (99 g) and ethanol (720 ml) was added aqueous sodium hydroxide (8% w/v; 2 ml) dropwise during 20 min. After 18 h at room temperature the pale yellow crystals were collected and recrystallised from ethanol to yield the furfurylidene compound (III), m.p. 68—69° (Found: C, 80.8; H, 6.45. C$_{17}$H$_{14}$O$_3$ requires C, 80.9; H, 6.4%). $\lambda_{max}$ (EtOH) 308-5 nm.


(log ε 3-60) and 342 nm (4-23), ν_{\text{max}} (Nujol) 1670 (CO), 1015, 980, 868, 820, 808, 730, and 700 cm⁻¹.

4,7-Dioxo-7-(5,6,7,8-tetrahydro-2-naphthyl)heptanoic Acid (IV).—The furfurylidenone compound (III) (210 g) was heated under reflux with conc. hydrochloric acid (195 ml) and ethanol (750 ml) for 9 h. The ethanol was removed under diminished pressure, and the dark gum which remained was boiled with a mixture of conc. hydrochloric acid (432 ml), glacial acetic acid (1073 ml) and water (1073 ml) for 2 h. The hot solution was decanted from the tar through a pad of glass wool and the crystals which separated on cooling were collected. The mother liquor was returned to the flask and boiled with the tar for 2 h. After seven repetitions of this cycle the crystalline material amounted to 165 g; recrystallisation from ethyl acetate gave the diketo-acid (IV) as needles, m.p. 115—116° (Found: C, 70-75; H, 6-8. C_{10}H_{14}O_{3} requires C, 70-8; H, 7-0%). ν_{\text{max}} (EtOH) 215-5 (log ε 4-14) and 258 nm (4-06), ν_{\text{max}} (Nujol) 1680—1740 (CO), 918, and 802 cm⁻¹.

5-Oxo-2-(5,6,7,8-tetrahydro-2-naphthyl)cyclopent-1-enecarboxylic Acid (Va).—A solution of the diketo-acid (IV) (10 g) in water (960 ml) containing potassium hydroxide (20 g) was maintained at 95° for 1 h. Charcoal was added and the hot solution was filtered, cooled, and acidified with conc. hydrochloric acid (128 ml). The precipitated acid (V) was collected, washed with water, and dried (9-7 g); it was sufficiently pure for the next stage. A sample crystallised from n-butanol had m.p. 132—134° (Found: C, 76-05; H, 7-1%). ν_{\text{max}} (EtOH) 225-5 (log ε 3-39) and 284 nm (3-60).

Reduction of the Unsaturated Acid (Va).—(a) With lithium in liquid ammonia. The acid (Va) (1-0 g) in dry tetrahydrofuran (10 ml) was added to a solution of lithium (400 mg) in liquid ammonia (375 ml) during 5 min, with cooling in acetone—solid carbon dioxide. Ammonium chloride (40 g) was then added during 20 min, followed by tetrahydrofuran (50 ml), and the mixture was stirred at room temperature for 14 h. Water was added and the aqueous layer was acidified and extracted with ether. Removal of the solvent left 2-oxo-5-(5,6,7,8-tetrahydro-2-naphthyl)cyclopent-1-ene-1-carboxylic acid (Vb) (8-5 g), m.p. 91-5—92-5 (Found: C, 76-3; H, 6-75. C_{17}H_{20}O_{3} requires C, 76-8; H, 7-0%). ν_{\text{max}} (Nujol) 1765 and 1190 (acetate), 1695 (conj. CO), 1050, and 908 cm⁻¹, ν = 3-21, 2-40, and 2-62 (each 1H, s, aromatic).

(c) Hydrogenation of the methyl ester (Vb). This ester (29-4 g) was reduced over palladium—charcoal as in (b) until t.l.c. showed absence of the starting material. The product was saponified to yield a brown gum (27-5 g), t.l.c. (dichloromethane) of which showed the presence of the cyclopentanone acid (Vla), R_{f} 0-65, accompanied by the cyclopentane acid (Vlb), R_{f} 0-85.

11-Acetoxy-1,2,3,4,15,16-hexahydrocyclopenta[a]phenanthren-17-one (IX) and 5-acetoxy-1,7,8,9,10-hexahydrocyclopenta[a]anthracene-3-one (Xa).—The cyclopentanone acid (Va) (5 g) was heated under reflux with acetic anhydride (40 ml) for 1 h. The needles (1-5 g) which separated on cooling were collected and crystallised repeatedly from benzene and from n-butanol to give the acetoxyanthracenone (Xa), m.p. 228—229° (Found: C, 77-45; H, 6-0. C_{18}H_{14}O_{3} requires C, 77-55; H, 6-15%). ν_{\text{max}} (EtOH) 263 (log ε 4-57), 287 (3-91), 297-5 (3-96), 308 (3-86), 338 (3-67), and 352 nm (3-69). ν_{\text{max}} (Nujol) 1765 and 1190 (acetate), 1695 (conj. CO), 1050, and 908 cm⁻¹, ν = 2-31, 2-40, and 2-62 (each 1H, s).

The acetic anhydride mother liquor was poured into water (500 ml) and extracted with dichloromethane; the extract was washed with water, dried, and evaporated. The crystalline residue (3-4 g) was recrystallised from benzene and sublimed to yield the acetoxyphenanthrenone (IX), m.p. 207—208° (Found: C, 77-1; H, 5-6. C_{18}H_{14}O_{3} requires C, 77-55; H, 6-15%). ν_{\text{max}} (EtOH) 222 (log ε 4-57), 287-5 (4-84), 288 (4-00), 296 (4-04), 307 (3-91), 340 (3-72), and 352 nm (3-81). ν_{\text{max}} (Nujol) 1270, 1206 (acetate), 1700 (conj. CO), 1040, 907, and 810 cm⁻¹.

The acetoxy-ketone (IX) (75 mg), DDQ (141 mg), and dry benzene (4 ml) were boiled together for 21 h. The solution, containing precipitated hydroquinone, was diluted with ether (10 ml) and filtered. Removal of solvent from the washed filtrate and sublimation of the residue gave 11-acetoxy-16-dihydrocyclopenta[a]phenanthren-17-one, m.p. and mixed m.p. 215—216°.

The acetoxy-ketone (Xa) (290 mg) and DDQ (567 mg) were boiled together in dry benzene (8 ml) for 24 h. The product, isolated as already described, was chromatographed on silica gel, with ethyl acetate—dichloromethane (1: 20) as eluant. Fractograms were homogeneous by t.l.c. were pooled and submitted to yield 5-acetoxy-1,2-dihydrocyclopenta[a]anthracene-9-one (Xia), m.p. 291—293°, ν_{\text{max}} (EtOH) 264-5 (log ε 4-48), 277 (4-45), 299 (3-53), 347 (3-58), 364 (3-61), 378 (3-55), and 398 nm (3-47).

1,2,3,4,13,14,15,16-Octahydrocyclopenta[a]phenanthrene-11(12H),17-dione (VIIa) and 1,3a,4,7,8,9,10,11b-Octahydrocyclopenta[janthracene-3(2H),6-dione (VIIia).—Phosphoric acid (20 g) and phosphorus pentoxide (20 g) were heated together at 120° for 2-5 h to give a clear solution. The keto-acid (Vla) (5-4 g) was reduced over palladium—charcoal as in (b) until t.l.c. showed absence of the starting material. The product was saponified to yield 11-acetoxy-16-dihydrocyclopenta[a]phenanthren-17-one, m.p. and mixed m.p. 215—216°.
165 were combined and the product (6-4 g) was recrystallised twice from ethanol to yield the anthracenedione (VIIIA), m.p. 102—103° (Found: C, 80-45; H, 6-95%). \( \lambda_{\text{max}}(\text{EtOH}) \) 221 (log e 4-36), 264 (4-12), and 302 nm (3-35); \( \lambda_{\text{max}}(\text{Nujol}) \) 1733 (five-membered ring ketone). 1675 (conj. ketone). 1605 (strong, aromatic C=C), 935, and 841 cm\(^{-1}\), \( \delta \ 2-30 \) and 2-89 (aromatic singlets).

17,17-Ethylidene-1,2,3,4,12,13,14,15,16,17-decachlorocyclopenda[a]phenanthren-1-one (VIIIB) and 3,3-Ethylenedi-1,2,3,3a,4,7,8,9,10,11-b-decachlorocyclopenda[a]anthracen-5-one (VIIIBa).—A crude mixture of the diketones (VIIA) and (VIIIA) (7-0 g) was heated with 2-ethyl-2-methyl-1,3-dioxol-an (4-0 ml) and toluene-p-sulphonic acid (20 mg) while the more volatile products (10 ml) were slowly removed by distillation. After 5 h the solution was diluted with ether, washed with sodium hydrogen carbonate solution and with water, and dried. The gum (7-0 g) left on evaporation was chromatographed on a column of alumina (Woelm, grade II) with hexane—ether (1:1) and the more volatile products were removed. Evaporation gave a brown crystalline solid which showed no i.r. carbonyl absorption. This material was treated with acid in the presence of nitrobenzene and acid as already described above to give a gum (300 mg).

1,2,3,4,15,16-Hexachlorocyclopen-ta[a]phenanthren-1-one (VIIIB).—The o xo-acetal (VIIIB) (300 mg), dissolved in dry benzene (5 ml), was added to a solution of methylmagnesium iodide (from magnesium turnings 120 mg), methyl iodide (0-30 ml), and dry ether (5 ml) under dry nitrogen. The mixture was boiled under reflux for 6 h, cooled, poured into a saturated solution of ammonium chloride containing a few drops of conc. hydrochloric acid, and extracted with benzene. The organic layer was washed with sodium hydrogen carbonate solution and dried. Evaporation left a gum (320 mg) which showed only weak i.r. carbonyl absorption.

This material was treated with acid in the presence of nitrobenzene as described above to give a gum (300 mg). The products from two reactions were combined (620 mg) and chromatographed twice on columns of silica gel containing 1% v/v ethyl acetate. Fractions containing the desired 11-methylphenanthren-1-one (IIb), readily detected by its strong purple fluorescence in u.v. light, were homogeneous by t.l.c., but still deeply coloured. Evaporation gave a brown crystalline solid (110 mg) which on sublimation gave colourless material, m.p. 150—152°, \( \lambda_{\text{max}}(\text{EtOH}) \) 223 (log e 4-23), 287 (3-97), 297-5 (4-02), 308 (3-87), 344 (3-69), and 354 nm (3-74), \( \lambda_{\text{max}}(\text{Nujol}) \) 1690 (conj. CO), 807, and 808 cm\(^{-1}\), m/e 260-13754 (M+) (C\(_{18}\)H\(_{13}\)O requires 250-13576), 235, 222, 207, 194, and 179.

Cyclisation of the Cyclopentane Acid (VIIIB).—This acid (900 mg) was added to a solution of phosphorus pentoxide (3 g) in phosphoric acid (3 ml) which had been equilibrated at 120° for 2-5 h. After 6 min the solution was poured into ice and the green gum was extracted with ethyl acetate. The extract was washed with sodium hydrogen carbonate solution and dried; removal of the solvent left a gum which crystallised. Repeated recrystallisation from ethanol yielded 1,2,3,4,7,8,9,10,11-b-decachlorocyclopenta[a]anthracen-5-one (VIIIC), m.p. 70—77° (Found: C, 85-2; H, 8-85. \( C_{18}H_{13}O \) requires C, 84-85; H, 8-4%). \( \lambda_{\text{max}}(\text{EtOH}) \) 219 (log e 4-24), 262 (3-99), and 306 nm (3-72), \( \lambda_{\text{max}}(\text{Nujol}) \) 1670 (conj. CO), 1605s (aromatic C=C), 912, 872, 862, and 818 cm\(^{-1}\), \( \tau \ 2-32 \) and 3-02 (one-proton singlets). T.l.c. (dichloromethane) of the mother liquors revealed a second spot just ahead of (VIIIC). Elution with ethanol gave a solution with \( \lambda_{\text{max}} \) 258 and 300 nm, showing that this was probably the angular isomer (VIIIC).

We thank D. W. Thomas for the microanalyses and C. W. Vose for the mass spectrum of (IIb).

[2]2618 Received, 23rd November, 1972]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part VII.¹ Ring-D Diols and Related Compounds

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Reprinted from JOURNAL OF THE CHEMICAL SOCIETY
PERKIN TRANSACTIONS I
1973
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By Maurice M. Coombs * and (Mrs.) Maureen Hall, Chemistry Department, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

Elimination from 16,17-dihydro-17-tosyloxycyclopenta[a]phenanthrene and from its 15-tosyloxyl isomer in dimethyl sulfoxide at 100° gave, respectively, 15//- and 17//-cyclopenta[a]phenanthrenes whereas, contrary to a previous report, elimination from the former in boiling collidine led to a 1:1 mixture of the two olefins. Dehydrogenation of 16,17-dihydro-15//-cyclopenta[a]phenanthrene with DDQ also led to a mixture of the 15//- and 17//-olefins. Elimination from 16,17-dihydro-11-methyl-17-tosyloxy-15//-cyclopenta[a]phenanthrene in boiling collidine yielded only the 11-methyl-15//-olefin. Oxidation of these olefins with osmium tetroxide gave cis-diols, whereas trans-diols were obtained by reduction of the corresponding 18-hydroxy-17-ketones with sodium borohydride. Unconjugated 16-ketones resulted from acid-catalysed dehydration of these diols.

Metabolic studies on the potent, carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one 2 (Ib) have implicated attack at the five-membered ring and prompted the work on synthetic ring-D oxygenated compounds described here.

In Part II 3 we claimed the synthesis of 15//-cyclopenta[a]phenanthrene (IIa) and the 17//-isomer (III), the former from the 17-tosylate in dimethyl sulfoxide at 100°, and the latter by elimination under more vigorous conditions in boiling collidine. The latter olefin was also prepared 3 by elimination from the 15-tosylate in boiling collidine. Both samples of olefins had the same m.p. and u.v. absorption characteristics as 17//-cyclopenta[a]phenanthrene reported by Sis 4 from the decarboxylation of the 17-carboxylic acid. The former olefin (IIa) had u.v. absorption similar to that of the related 17-methyl-16(17)-ene. 3 In order to prepare the 16,17- and 15,16-diols these olefins were treated with osmium tetroxide. As expected, (IIa) gave the cis-16,17-diol (IVa) as sole product, but the supposed 17//-isomer yielded roughly equal amounts of two diols, one of which was identical with (IVa). The second compound was formulated as the cis-15,16-diol (Va), since its u.v. spectrum bore a strong resemblance to that of 15-hydroxy-16,17-dihydro-15//-cyclopenta[a]phenanthrene. 1 The identity of (Va) was confirmed by the preparation of a specimen of 17//-cyclopenta[a]phenanthrene by elimination from the tosylate in dimethyl sulfoxide at 100°. Addition of osmium tetroxide to this olefin gave a diol characterised as (Va) on the basis of mixed m.p. determinations of the derived diacetates. The u.v. spectra of the 16//- and 17//-isomers of cyclopenta[a]phenanthrene differ as previously described, 2 but the purer sample of the latter displays more intense absorption at 239 and 269 nm than reported. 3 It is therefore apparent that boiling either 15//- or 17//-cyclopenta[a]phenanthrene in collidine (b.p. 175°) leads to an equilibrium mixture containing roughly equal proportions of the two isomers and that the compound described previously as (III) was in reality a mixture of the two olefins. Dehydrogenation of 16,17-dihydro-15//-cyclopenta[a]phenanthrene with 2,3-dichloro-5,6-dicyanobenzo-1,4-quinone (DDQ) in boiling benzene also gave a mixture of the two olefins in good yield, but in this case the 15//-isomer predominated.

Reduction of 16-hydroxy-15,16-dihydrocyclopenta[a]phenanthren-17-one 5 with sodium borohydride in methanol led to a high yield of a third diole, more polar and less soluble than (IVa). This was therefore assigned the trans-16,17-diol structure (VIa). A small quantity of the cis-diol (IVa) was identified chromatographically in the crude reduction products. Presumably coordination of the 16-hydroxy-group in the a-ketol with the borohydride directs attack by the latter on the carbonyl group from the side of the ring bearing the 16-oxygen atom, thus leading to a trans product.

16,17-Dihydro-11-methyl-15//-cyclopenta[a]phenanthren-17-ol (VIIa) was prepared by borohydride reduction of the ketone (Ib). Elimination from the

† Unpublished work.

1 Part VI, M. M. Coombs and T. S. Bhatt, preceding paper.
4 O. Sis, Annalen, 1953, 579, 133.
derived 17-tosylate \( \text{VIIb} \) in boiling collidine gave an olefin which displayed no u.v. absorption at 238 nm; the spectrum was similar to that of the known 11,17-dimethyl-16(17)-ene. That this olefin was 11-methyl-

\[
\begin{align*}
\text{Me} & \quad \text{(VII)} \\
\text{Me} & \quad \text{(VIII)} \\
\text{Me} & \quad \text{(IX)} \\
\text{Me} & \quad \text{(XI)}
\end{align*}
\]

15H-cyclopenta[a]phenanthrene \( \text{IIb} \), unaccompanied by its 17H-isomer, was established by oxidation with osmium tetroxide to yield a single cis-diol \( \text{VIIIa} \). is substantially smaller for the cis-diacetates than for the trans-isomers.

Acid catalysed dehydration of the mixture of 15,16- and 16,17-cis-diols \( \text{IVa} \) and \( \text{Va} \) furnished a single product, the 16-ketone \( \text{Xa} \), analogous to the unconjugated ketone formed on similar dehydration of indane-1,2-diol. The structure of \( \text{Xa} \) follows from its u.v. absorption characteristics, which are similar to those of 16,17-dihydro-15H-cyclopenta[a]phenanthrene. In addition, the i.r. stretching frequency of the 16-carbonyl double bond occurs at 1750 cm\(^{-1} \), similar to that of the 16-carbonyl group in the 17-methyl-16-ketone \( \text{XI} \) prepared by dehydration of the 17-methyl-16,17-diol. Dehydration of the 11-methyl-16,17-diol \( \text{IXa} \) gave the 11-methyl-16-ketone \( \text{Xb} \), a positional isomer of the potent carcinogen \( \text{lb} \).

**EXPERIMENTAL**

Reagents and apparatus were generally as described in previous Parts of this series. T.l.c. was performed on plates coated with Kieselgel G (Merck) and dried overnight in air. Solutions in organic solvents were dried over anhydrous sodium sulphate.

Reactions of 15H- and 17H-Cyclopenta[a]phenanthrenes with Osmium Tetroxide.—Comparative experiment. Samples \( \text{mg} \) of 15H-cyclopenta[a]phenanthrene and the olefin designated as the 17H-isomer in Part II were separately dissolved in portions \( \text{ml} \) of a solution in benzene of OsO\(_4\),2py \( \text{containing osmium tetroxide (254 mg, mmol) and pyridine (160 mg, mmol in 100 ml). After 20 h}

Thus the presence of the 11-methyl group appears to fix the position of the ring-D double bond in this olefin \( \text{IIb} \). The more polar and higher melting 11-methyl-trans-16,17-diol \( \text{IXa} \) was obtained as before by borohydride reduction of 15,16-dihydro-16-hydroxy-11-methylcyclopenta[a]phenanthrene-17-one. In this case also the cis-diol \( \text{VIIIa} \) was a minor reduction product.

The two pairs of cis- and trans-16,17-diols are readily distinguished by comparison of the n.m.r. spectra of their diacetates, as shown in the Table. In particular, separation of the chemical shifts between the two quartets due to the two non-equivalent C-15 protons the supernatant liquids were pipetted off and the brown precipitates were heated for 1.5 h on a steam-bath, each with pyridine \( \text{ml} \), water \( \text{ml} \), and sodium pyrosulphite \( \text{g} \). Addition of more water \( \text{ml} \) gave grey suspensions which were extracted with ether, yielding, in both cases, white solids. That from the 15H-olefin gave, on t.l.c. (ethanol–toluene, 1:5), one spot, \( \text{R} \) 0-43; the solid from the 17H-olefin exhibited two spots, \( \text{R} \) 0-40 and 0-46, of similar intensity. These solids were separately acetylated with acetic anhydride \( \text{ml} \) in pyridine

from n-butanol gave the trans-16,17-diol (Via), m.p. 165°, contaminated with a small quantity of the cis-16,17-diol, m.p. 168—171°, with the same Rₚ. The isolated diol (8 mg) gave a further quantity of (III) (5 mg). Crystallisation from dichloromethane-hexane (1 : 10) on three 20 × 20 cm plates furnished 17H-cyclopenta[a]phenanthrene (33 mg), X max (EtOH) 227 (log e 4-74), 256 (4-86), 269 (4-98), 281 (4-81), 302 (4-91), 313-5 (4-82), 334-5 (2-80), 355 nm (2-80), v max (Nujol) 3285 (OH), 825, 817, and 757 cm⁻¹.

17H-Cyclopenta[a]phenanthrene (III).—16,17-Dihydro-15H-cyclopenta[a]phenanthrene-15-ol (175 mg), dissolved in dry pyridine (5 ml), was kept with toluene-p-sulphonyl chloride (142 mg) for 3 h. After dilution with water (50 ml), the mixture was extracted with dichloromethane and the extract was washed successively with 2% sulphuric acid, aqueous sodium hydrogen carbonate, and water, and dried. Removal of the solvent gave the crude tosylate as a gum (137 mg) which was dissolved in dimethyl sulphoxide (0-2 ml) and heated on a steam-bath for 1 h. The solvent was removed in vacuo, and the residual gum was triturated with hexane-dichloromethane (10 : 1). The extract (40 mg) was purified by preparative t.l.c. (hexane—benzene, 4 : 1) on three 20 × 20 cm plates to furnish 17H-cyclopenta[a]phenanthrene (III) (12 mg) as a white solid, Rₚ 0-44, together with a small amount (cf. the cis-16,17-diol, Rₚ 0-50) of the trans-16,17-diol (Vib), prepared by treatment with acetic anhydride—pyridine at room temperature for 20 h, crystallised from ethanol in prisms, m.p. 159—160° (Found: C, 75-3; H, 5-1: C₂₁H₁₈O₄ requires C, 75-45; H, 5-43%).

Dehydrogenation of 16,17-Dihydro-15H-cyclopenta[a]-phenanthrene with 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone. —The dihydro-compound (80 mg) was heated under reflux with a solution of DDQ (100 mg) in dry benzene (10 ml) for 20 h. The precipitate was filtered off and the filtrate was evaporated to dryness. The resulting solid was chromatographed on a column of alumina (Woelm, grade IV; 5 g) with hexane—benzene (4 : 1) to yield 15H- and 17H-cyclopenta[a]phenanthrene (53-3 mg), Rₚ 0-44 (EtOH) 239 (log e 4-026), 269 (4-798), and 273-5 nm (4-803). A sample (10 mg) of this olefin was treated with osmium tetroxide as described in the comparative experiment. The isolated diol (8 mg) gave a major spot, Rₚ 0-43, and a minor spot at Rₚ 0-46, on t.l.c. as already described. After acetylation the product (6 mg) melted at 165—166° (cf. with mixed m.p.s in the previous section).

16,17-Dihydro-11-methyl-15H-cyclopenta[a]phenanthren-17-ol (VIIa).—15,16-Dihydro-11-methoxycyclopenta[a]phenanthren-17-one (1-20 g) was stirred in methanol (40 ml) during the addition of sodium borohydride (1 g). After 1 h, more sodium borohydride (1 g) was added and stirring was continued for a further 2 h. Dilution with water (60 ml) gave a precipitate which was collected, washed with water, and dried. The cream solid (0-951 g) was shown by t.l.c. (ethanol—toluene, 1 : 4) to consist mainly of a substance with Rₚ 0-44, together with a small amount of the cis-16,17-diol, Rₚ 0-50. Crystallisation of this solid from n-butanol gave the trans-16,17-diol (Vib), prepared by treatment with acetic anhydride—pyridine at room temperature for 20 h, crystallised from ethanol in prisms, m.p. 159—160° (Found: C, 75-3; H, 5-1: C₂₁H₁₈O₄ requires C, 75-45; H, 5-43%).
11-Methyl-15H-cyclopenta[a]phenanthrene (IIb).—The 17-ol (VIIa) (424 mg), toluene-p-sulphonyl chloride (330 mg), and dry pyridine were kept together at room temperature for 2 h. After dilution with chloroform (30 ml) the solution was washed as already described to yield the tosylate (VIIb) as a resinous brown solid (355 mg), \( \nu_{\text{max}} \) (Nujol) 1030 and 1010 cm\(^{-1}\), characteristic of tosylates. This derivative, dissolved in collidine (5.0 ml), was boiled under reflux for 30 min, and worked up as already described to give an oil (360 mg) showing no u.v. absorption at 238 nm. After filtration through a short column of alumina (Woelm, grade II) in benzene–hexane (1:1) the product was extracted with chloroform and the solid left on removal of the solvent was recrystallised twice from benzene with hexane to furnish the 17-dihydro-15H-olefin (Hb), m.p. 87—89° (Found: C, 93-6; H, 6.3. \( \text{C}_{18} \text{H}_{14} \text{O} \) requires C, 93-8; H, 6.1%). \( \nu_{\text{max}} \) (EtOH) 221-5 (log e 4-47), 267 (4-65), 274 (4-65), 294 (4-08), 306 (4-06), 317 (2-83), 348 (2-85), and 364 nm (2-74), \( \nu_{\text{max}} \) (Nujol) 815, 751, and 701 cm\(^{-1}\).

16,17-Dihydro-11-methyl-15H-cyclopenta[a]phenanthrene-16,17-diol (VIIa).—A solution of the 15H-olefin (IIb) (115 mg, 0-5 mmol) in benzene (11-5 ml) was treated with sodium pyrosulphite as already described to remove the 16,17-diol (VIIa), m.p. 184—185° (Found: C, 87-6; H, 6.1. \( \text{C}_{18} \text{H}_{14} \text{O} \) requires C, 87-7; H, 5-7%). \( \lambda_{\text{max}} \) (EtOH) 255 (log e 4-80), 278 (4-23), 284 (4-10), 319 (3-00), 334 (3-03), and 350 nm (2-06), \( \lambda_{\text{max}} \) (Nujol) 1740 (CO), 818, and 748 cm\(^{-1}\).

16,17-Dihydro-11-methyl-15H-cyclopenta[a]phenanthrene-15,16-diacetoxy-trans-16,17-diol (IXa).—A mixture (107 mg) of the 15,16-diacetoxy- and 16,17-diacetoxyl-compounds from the osmium tetroxide oxidation was dissolved in methanol (10 ml) and water (2 ml) and heated on a steam-bath with potassium hydroxide (200 mg). After 75 min, 8-n-sulphuric acid (50 ml) was added and heating was continued for 6 h. The cooled mixture was extracted with chloroform and the solid left on removal of the solvent was recrystallised twice from benzene with the addition of hexane to yield the ketone (Xa) as pale yellow rosettes of needles (10 mg), m.p. 172—174° (Found: C, 87-65; H, 5.1. \( \text{C}_{17} \text{H}_{10} \text{O} \) requires C, 87-9; H, 5.2%). \( \lambda_{\text{max}} \) (EtOH) 259 (log e 4-76), 279 (4-21), 283 (4-11), 300 (4-21), 319 (3-14), 335 (3-10), and 350 nm (3-08), \( \lambda_{\text{max}} \) (Nujol) 1750 (CO), 833, 802, 768, and 748 cm\(^{-1}\).

We thank C. W. Vose for discussions, and D. W. Thomas for the microanalyses.
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part VIII.¹
Bromination of 17-Ketones

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Reprinted from
JOURNAL
OF
THE CHEMICAL SOCIETY
PERKIN TRANSACTIONS I
1973
Bromination of phenanthrene under a variety of conditions leads to substitution at the 9- and 10-positions.\(^2\) We considered that, by analogy, bromination of 15,16-dihydrocyclopenta[\(a\)]phenanthren-17-one (Ia) would yield 6- or 7-bromo derivatives, especially since these positions (as in phenanthrene) are attacked by reagents such as osmium tetroxide and chromic acid.\(^3\) It was hoped that the expected 6- and 7-bromo derivatives, by reductive debromination in tritium gas, would furnish these ketones labelled specifically with tritium at very high specific activity,\(^4\) making them available for biological experiments in connection with the high carcinogenic activity of the 11-methyl-17-ketone (Ib).\(^5\) In the event this aim was not achieved, and the results described in this paper emphasize the substantial reactivity of the five-membered ring in compounds of this series.

Treatment of the unsubstituted ketone (Ia) with 1 equiv. of bromine in chloroform or acetic acid gave a mono- and a dibromo-derivative. The former proved to be the 15-bromo-17-ketone (IIa) since its n.m.r. spectrum exhibited signals of an ABX system, corresponding to two non-equivalent methylene protons coupled to a strongly deshielded methine proton (see Table). Moreover, reductive debromination in a mixture of tritium and hydrogen gas gave the [\(15^{3}H\)]=[\(17\) ketone (Vc) which retained its label both on treatment with alkali and on oxidation to the 6,7-quinone,\(^9\) proving that the tritium was not at C-6, -7, or -16.

Bromination of the ketone (Ia) with N-bromosuccinimide gave a different monobromo-derivative in high yield. This bromo-ketone also exhibited an ABX system in its n.m.r. spectrum and is thus the 16-bromo-17-ketone (III). Reduction of this compound with sodium borohydride gave the cis-bromohydrin, since the product was stable to alkali. Dehydrobromination of (III) occurred readily, being essentially complete within 10 min at room temperature on treatment with triethylamine in tetrahydrofuran, or with 2 equiv. of aqueous sodium hydroxide in dioxan. Cyclopenta[\(a\)]phenanthren-17-one (IV) formed bright orange crystals with characteristic u.v. absorption, but purification was complicated by the ease with which it gave rise to insoluble material on storage or attempted recrystallisation. In this respect it resembles the analogous system indenone.\(^6\) Treatment of the 16-bromide (III) with sodium acetate in acetic acid also gave insoluble, intract-
able material, presumably through prior elimination to the enone (IV).

Similar attempted displacement of bromine from the 15-bromo-17-ketone (IIa) led smoothly, not to the expected 15-acetoxy-compound, but to the 16-acetoxy-17-ketone (VIIa) identical with the compound previously obtained by oxidation of the parent ketone (Ia) with lead tetra-acetate.\(^3\) Formation of this compound probably occurs by nucleophilic attack by an acetate anion at C-16 in the enol with concomitant expulsion of bromide from C-15 (Scheme 1), since the 15-bromide (IIa) showed no evidence of rearrangement to the isomeric 16-bromide (III) under the reaction conditions employed, but without potassium acetate. The 15-acetoxy-17-ketone (Vb) was prepared, again by analogy with indenone,\(^7\) by acid-catalysed hydration of the 15(16)-double bond in the enone (IV) using aqueous sulphuric acid in tetrahydrofuran at room temperature, followed by acetylation. The structure (Vb), rather than the isomeric 17-acetoxy-15-ketone structure, was assigned to this compound on the basis of its u.v. absorption characteristics which are very similar to those of the 17-ketones (Ia and b) and quite different from those of the 15-ketone of this series.\(^3\) A substantial quantity of insoluble pink solid precipitated out during this reaction, and a minor reaction product was the ketone (la). This compound was also formed when the elimination of the 16-bromide with triethylamine was carried out in boiling benzene. It would therefore appear that (la) is produced from the enone (IV) by abstraction of hydrogen, presumably from the solvent or a second molecule of the phenanthrene.

Bromination of the 15-bromo-17-ketone (IIa) with \(N\)-bromosuccinimide led to a dibromo-compound which was different from that obtained by bromination of (Ia) with molecular bromine. The n.m.r. spectrum of the former showed two one-proton singlets arising from two chemically and magnetically different protons, and was therefore the 15,16-dibromide (VI). The product from the reaction with bromine was assigned as the 15,15-dibromide (VIIa) because it had a two-proton singlet at \(\tau 5.31\) in its n.m.r. spectrum.

Bromination of the carcinogenic ketone (Ib) with \(N\)-bromosuccinimide led to a dibromo-compound which was different from that obtained by bromination of (Ia) with molecular bromine. The n.m.r. spectrum of the former showed two one-proton singlets arising from two chemically and magnetically different protons, and was therefore the 15,16-dibromide (VI). The product from

bromine in chloroform also gave mono- and di-bromo-
derivatives, the structures of which were readily estab-
lished as the 15-bromo- and 15,16-dibromo-17-ketones, (IIb) and (VIIb), by n.m.r. spectroscopy.

In an attempt to direct bromination of (Ia) to the
aromatic rings, the reaction with bromine in acetic acid
was carried out with complete exclusion of light, but
the same mixture of 15-bromo- and 15,16-dibromo-derivatives
was isolated. Bromination with bromine in the
presence of silver nitrate and acid, a method reported to
favour electrophilic bromination owing to the liberation
of Br⁺ cations, also gave the same mixture of bromoketones
together with a variety of other products which
was not investigated.

Recently thallium triacetate has been recommended 9
as a good catalyst for aromatic bromination. Treatment
of the ketone (Ia) with one equivalent of bromine in
carbon tetrachloride in the presence of thallium triacetate
gave small amounts of the usual bromoketones, together
with a compound C₁₉H₁₄O₃ as major product. The
latter was characterised as the 15-acetoxy-17-ketone
(Vb), identical with the product of acetylation of the
15-alcohol, described above. It was not formed when
either the ketone (Ia) or the 15-bromoketone (IIa)
was treated with thallium triacetate in carbon tetrachloride,
and the best yield of (Vb) was secured when the bromine
was added in one lot to the mixture of (Ia) and thallium
triacetate. It therefore seems probable that a bromo-
derivative of TH₃ is involved, possibly allowing
nucleophilic attack of acetate at C-15 by a mechanism
involving an enolic thallic ester (Scheme 2), similar to
that recently proposed to account for the formation of
10β-trifluoroaceoxy-19-norandrosta-1,4-diene-3,17-dione
by the action of thallium tris(trifluoroacetate) on
oestrone. 1₀

EXPERIMENTAL

Materials and methods were generally as described in
previous parts of this series.

15-Bromo- and 15,16-Dibromo-15,16-dihydrocyclopenta[a]phenanthren-17-one, (IIa) and (VIIa).—A solution of bromine
(2-09 g) in dry chloroform (50 ml) was added drop-
wise with stirring during 2 h to a solution of 15,16-dihydro-
cyclopenta[a]phenanthren-17-one (3-00 g) in chloroform
(200 ml), and after stirring at room temp. for 20 h, the
solvent was removed under reduced pressure to leave a
brown solid (3-58 g). Chromatography on Wöelm Grade II
alumina using toluene gave two substances, from first
fractions an orange solid (0-597 g) and from later fractions
a cream solid (1-603 g). Crystallisation of the latter from
n-butanol gave 15-bromo-15,16-dihydrocyclopenta[a]phenanthren-17-one (IIa) (1-455 g, m.p. 196—197° (Found:
3-35; Br, 25-7%). λₑₒₘ (EtOH) 267-5 (log e 4-78), 298-5 (4-36),
310 (4-13), 354 (3-32), and 372 nm (3-31), λₑₒₘ (Nujol) 1711 (C=O), 820, and 757 cm⁻¹. Several
crystallisations of the orange solid from n-butanol gave
15,16-dibromo-15,16-dihydrocyclopenta[a]phenanthren-17-one
(VIIa) (0-428 g, m.p. 213—213-5° (Found: C, 52-25; H,
2-25; Br, 41-2. C₁₉H₁₀Br₂O requires C, 52-35; H, 2-23; Br,
41-0%). λₑₒₘ (EtOH) 279-5 (log e 4-70), 298 (4-24), 314-5
(4-18), 366 (3-30), and 377 nm (3-27), λₑₒₘ (Nujol) 1710
(C=O), 816, and 716 cm⁻¹. Use of glacial acetic acid in place of chloroform in this
bromination gave the same two products in a similar ratio,
even when light was carefully excluded.

Bromination in the presence of silver ions was carried out
as follows. To a stirred solution of the ketone (Ia) (116 mg)
in a mixture of glacial acetic acid (30 ml) and water (10 ml)
containing silver nitrate (85 mg) was added a solution of
bromine (80 mg) in glacial acetic acid (0-57 ml) dropwise
with exclusion of light. After 5 h water was added and the
mixture was extracted with dichloromethane. T.l.c. disclosed
at least five products, prominent among which was the
15-bromoketone (IIa).

Bromination of the 15-Bromoketone (Ia) with N-Bromo-
succinimidine.—The 15-bromoketone (IIa) (200 mg) in
carbon tetrachloride (10 ml) was boiled with N-bromosuccinimide
(114 mg) for 1 h adjacent to a 150 W lamp. After dilution
with more carbon tetrachloride, the cooled solution was
washed with water, dried, and evaporated to yield an
orange solid (235 mg). Recrystallisation from carbon
tetrachloride gave 15,16-dibromo-15,16-dihydrocyclopenta-
a[phenanthren-17-one (VI), m.p. 179—180°; the mixed
m.p. with the 15,15-dibromoketone (m.p. 213—213-5° was
165—175° (′Found: C, 52-65; H, 2-3; Br, 41-35%). λₑₒₘ
(EtOH) 283 (log e 4-60), 311-5 (4-33), 361 (3-19), and
380 nm, (3-18), λₑₒₘ (Nujol) 1717 (C=O), 831, and 760 cm⁻¹.

Bromination of 15,16-Dihydro-11-methylcyclopenta[a]
phenanthren-17-one.—This ketone (Ib) (436 mg) in chloroform
(15 ml) was treated with a solution of bromine (279 mg) in
chloroform (20 ml) as already described. After 20 h the solvent
was removed to leave a brown solid (605 mg), chromatography of which on silica gel (100 g) with toluene-
dichloromethane (3:1) gave two substances. From the
first fractions was obtained an orange solid (134 mg) and
from later fractions a yellow solid (395 mg). Several
recrystallisations of the former from n-butanol gave orange
crystals (38-5 mg) of 15,16-dibromo-15,16-dihydro-11-methyl-
cyclopenta[a]phenanthren-17-one (VIIb), m.p. 206—207°
(Found: C, 53-6; H, 2-8; Br, 39-1. C₁₉H₁₂BrO requires C,
53-5; H, 3-0; Br, 39-55%). λₑₒₘ (EtOH) 267-5 (log e
4-68), 302 (4-32), 315sh (4-12), 369 (3-39), and 384 nm
(3-31), λₑₒₘ (Nujol) 1712 (C=O), 825, 744, 718, and
708 cm⁻¹. Recrystallisation of the yellow solid from the same solvent
furnished yellow needles (247 mg) of 15-bromo-15,16-dihydro-
11-methylcyclopenta[a]phenanthren-17-one (IIb), m.p.
211—212° (Found: C, 66-8; H, 4-0; Br, 24-55%). C₁₉H₁₂-
BrO requires C, 66-5; H, 4-05; Br, 24-55%). λₑₒₘ (EtOH)
267-5 (log e 4-68), 290 (3-37), 303 (3-29), 363 (3-31), and

1972, 37, 88.

The bromoketone (III) (100 mg) was treated with sodium borohydride (15 mg) in a mixture of dioxan (2.0 ml) and ethanol (1.0 ml) for 40 h at room temp. Addition of water precipitated an orange solid which was collected, washed with water, and dried. Evaporation left a buff solid (183 mg), recrystallised from benzene solution to give a sample of (Vc) with a specific activity of 72.3 pCi mmol⁻¹.

Radioinactive ketone (Ia) was added to a portion of the benzene solution to give a sample of (Vc) with a specific activity of 78.6 µCi mmol⁻¹. This ketone (92 mg) was stirred with a solution of chromium trioxide (150 mg) in glacial acetic acid (10 ml) until the reaction mixture became completely yellow, specific activity 72.3 pCi mmol⁻¹. Radioinactive ketone (Ia) was added to a portion of the benzene solution to give a sample of (Vc) with a specific activity of 72.3 pCi mmol⁻¹. This ketone (92 mg) was stirred with a solution of chromium trioxide (150 mg) in glacial acetic acid (10 ml). After 24 h the yellow precipitate of the 6,7-quinone was collected, washed with water, and dried. Recrystallisation from boiling toluene gave golden yellow leaflets, specific activity 72.3 µCi mmol⁻¹.

The u.v. spectrum of the recovered material was identical with that of (Ia).

With triethylamine in boiling benzene. The bromoketone (III) (675 mg) was heated under reflux with a mixture of triethylamine (10 ml) and dry benzene (10 ml) for 30 min, giving a deep red solution and a brick-red solid. After addition of further benzene the solid was filtered off, and the solution was washed with water and dried. Removal of the solvent left a deep orange syrup (206 mg) which crystallised, m.p. 175—190°, λ max (EtOH) 290nm, 265, 284, 297, and 310 nm. Attempts to purify this material by recrystallisation or chromatography led to further quantities of insoluble red solid, and the only pure compound isolated was a small amount of the ketone (Ia), m.p. 198—199°, u.v. spectrum identical with that of authentic (Ia).

With potassium acetate in acetic acid. The bromoketone (III) (1.0 g) in acetic acid (20 ml) was heated on a steam-bath with potassium acetate (2.0 g) for 6 min to give a deep red solution filled with flocculent solid. The mixture was diluted with ethyl acetate, insoluble material (283 mg) was collected, and the solution was washed with water, and dried. The orange oil obtained on evaporation no longer dissolved completely in ethyl acetate. Filtration of the solution and re-evaporation left an orange gum, λ max (EtOH) 209, 297, and 310 nm, which on storage in the refrigerator became partly insoluble in this solvent.

Reaction of the 15-Bromo-17-ketone (Iia) with Potassium Acetate in Acetic Acid.—The 15-bromoketone (Iia) (290 mg), acetic acid (20 ml), and potassium acetate (1.0 g) were heated under reflux for 20 h. The acetic acid was removed under reduced pressure, the residue was shaken with saturated sodium chloride solution and ethyl acetate, and the latter was washed with aqueous sodium hydrogen carbonate, and dried. Evaporation left a buff solid (183 mg), recrystallisation of which from benzene gave 16-acetoxy-15,16-dihydrocyclopenta[a]phenanthren-1-one (VII), m.p. 175—178°, mixed m.p. 178—179° with an authentic sample. The i.r. and u.v. spectra of this material were also identical with those of the authentic material. The 15-bromoketone (Iia), m.p. and mixed m.p. 192—193°, unchanged when a sample (20 mg) was boiled under reflux with glacial acetic acid (20 ml) for 2 h and worked up as described above. The u.v. spectrum of the recovered material was identical with that of (Iia).

[15-3H]-15,16-Dihydrocyclopenta[a]phenanthren-1-one (Vc).—The 15-bromo-17-ketone (Iia) (3.1 mg) and 5% Pd—CaCO₃ (3.5 mg) in ethanol (1.5 ml) were stirred together in an atmosphere of hydrogen containing ca. 0.5% v/v of tritium gas. After 30 min, when absorption of gas had ceased, the mixture was added to water (10 ml) and extracted with benzene (2 x 5 ml). The benzene extract was washed with 5% (w/v) aqueous sodium hydroxide (5 x 5 ml), then twice with water, and finally diluted to 100.0 ml with benzene. This solution contained 840 µCi of tritium and on t.i.c. ran as a single spot with Rf values, u.v. absorption, and fluorescence under u.v. light indistinguishable from those of authentic (Ia). Further extraction of the benzene solution with aqueous alkali removed no radioactivity.

Radioactive ketone (Ia) was added to a portion of the benzene solution to give a sample of (Vc) with a specific activity of 78.6 µCi mmol⁻¹. This ketone (92 mg) was stirred with a solution of chromium trioxide (150 mg) in glacial acetic acid (10 ml). After 24 h the yellow precipitate of the 6,7-quinone was collected, washed with water, and dried. Recrystallisation from boiling toluene gave golden yellow leaflets, specific activity 72.3 µCi mmol⁻¹.
washed with water, dried, and evaporated to yield a partly solid orange gum (187 mg). This material was acetylated with acetic anhydride (2 ml) in dry pyridine (2 ml) over night. After addition of water, the mixture was extracted with dichloromethane and the extract was washed with 2n-H₂SO₄, then with water until neutral, and dried. Evaporation gave a dark brown gum (188 mg) which was chromatographed on silica gel (60 g). Fractions eluted with dichloromethane containing 2% v/v ethyl acetate and shown by t.l.c. to contain the 15-acetate were combined (55 mg). Two recrystallisations of this solid from hot n-butanol gave the 15-acetoxy-17-ketone (Vb) as cream needles, m.p. 197—198°, and 197—199° when mixed with a sample of this compound obtained by treatment of (Ia) with thallium triacetate and bromine (see below).

Treatment of the Ketone (Ia) with Thallium Triacetate and Bromine.—The ketone (Ia) (464 mg, 2 mmol) and thallium triacetate (763 mg, 2 mmol) were stirred together in carbon tetrachloride (100 ml), a solution of bromine (320 mg, 2 mmol) in carbon tetrachloride was added, and the mixture was stirred at room temp. for 22 h. The solid was collected and washed with the same solvent. The solution and washings were shaken with aqueous sodium hydrogen carbonate solution and with water, and dried; evaporation of the solvent left a yellow gum (297 mg).

This gum and material from a similar experiment (total, 615 mg) were chromatographed on a column of silica gel (60 g), eluting with dichloromethane. The first fractions contained a mixture (62 mg) of the bromoketones (IIa) and (VIIa), identified by their Rp values and u.v. light absorption characteristics. Subsequent fractions consisted of the starting material (Ia) and the product (Vb) (together, 236 mg) in the approximate ratio 1:2. Rechromatography of this material gave the 15-acetoxy-17-ketone (Vb) which after repeated recrystallisation from n-butanol, then ethanol, had m.p. 198.5—199° (Found: C, 78.9; H, 4.6; M⁺, 290.0949. C₁₉H₁₄O₃ requires C, 78.6; H, 4.85%; M 290.0943), νₚₓₓ (Nujol) 1740 (acetate), 1710 (ketone), 1022, 855, 820, and 757 cm⁻¹, λₚₓₓ (EtOH) 269 (log ε 4.76), 297 (4.26), 330sh (3.10), 349 (3.09), 366 (3.07) nm.

When this procedure was repeated, but with omission of bromine, or when the 15-bromoketone (IIa) (10 mg) and thallium triacetate (12.5 mg) were stirred together in carbon tetrachloride (2 ml) for 22 h, the starting materials appeared unchanged and no new compounds had appeared, as judged by t.l.c.

We thank the Imperial Cancer Research Fund for a Bursary (to C. W. V.). We are indebted to Dr. R. B. Boar for helpful criticism of the manuscript, and to D. W. Thomas for the microanalyses. 

[3/994 Received, 16th May, 1973]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part IX.¹
Characterisation of a 5,10-Epoxybenzocyclodecene as a Major Urinary Metabolite of the Carcinogen 15,16-Dihydro-11-methylcyclopenta[a]-phenanthren-17-one

By Maurice M. Coombs * and Francis E. H. Crawley, Department of Chemistry, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX

Reprinted from
JOURNAL
OF
THE CHEMICAL SOCIETY
PERKIN TRANSACTIONS I
1974
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part IX.¹
Characterisation of a 5,10-Epoxybenzocyclodecene as a Major Urinary
Metabolite of the Carcinogen 15,16-Dihydro-11-methylcyclopenta[a]-
phenanthren-17-one

By Maurice M. Coombs * and Francis E. H. Crawley, Department of Chemistry, Imperial Cancer Research
Fund Laboratories, Lincoln’s Inn Fields, London WC2A 3PX

The major urinary metabolite of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]-phenanthren-17-one (Ib)
produced by the rat is shown by chemical and spectral examination to possess the novel structure 8,9-epoxy-
1α,2α,2β,15β,15γ,tri hydroxy-11-methyl-8,9-secogona-3,5,7,9,11,13-hexaen-17-one (II).

There is a marked difference² in the urinary metabolism of 15,16-dihydrocyclopenta[a]phenanthren-17-one (Ia)
and its strongly carcinogenic² 11-methyl homologue (Ib) in the rat. In both cases, after injection of the [14C]-
labelled ketones, 40—50% of the radioactivity present in the urine was extractable by ethyl acetate, and t.l.c.
disclosed the presence of ca. 12 metabolites. However, the extract from the urine of rats which had been
administered the carcinogen (Ib) contained a metabolite which accounted for ca. 50% of the radioactivity in this
extract, whereas only a trace of material with a similar Rf value, u.v. absorption, and fluorescence was present
in the extract from rats which had received the non-

² M. M. Coombs and F. E. Crawley, in preparation.
cancerous parent ketone (Ia). This paper describes the chemical identification of this major metabolite.

On acetylation this metabolite (C_{18}H_{16}O_6) yielded a triacetate (III), C_{24}H_{22}O_7, indicating that acetylation was accompanied by the loss of an oxygen atom. Hydrogenation of the metabolite occurred with the uptake of 2 moles of hydrogen to give the dihydrotriol (IVA), C_{18}H_{18}O_4, again with loss of an oxygen atom. Acetylation of (IVA) gave the dihydrotriacetate (IVb) (C_{24}H_{24}O_9) identical with the product of hydrogenation of the triacetate (III). Treatment of the metabolite with hot mineral acid did not result in loss of oxygen, but gave each of these ketones the carbonyl group in conjugated, absorbing at 1690 cm^{-1} in the hydroxy-ketones (II) and (Va) and at 1720 cm^{-1} in the acetates (III) and (Vb). A similar difference in the carbonyl frequency has been observed between the 15-hydroxy-ketone (VIIa) and its acetate (VIIb). That one hydroxy-group in these compounds is at C-15, rather than at C-16, is demonstrated by the very similar chemical shifts and coupling constants of the 15- and 16-protons in the n.m.r. spectra of the acetates (III) and (Vb) when compared with those of the synthetic 15-acetoxy-17-ketone (VIIb), in contrast to those of the 16-acetoxy-17-ketone (VIIIb) (Table 1).

The u.v. spectra of (II) and (III) are very similar, despite the loss of the non-functional oxygen atom in the latter, but they differ from those of the other chromophores so far encountered with these metabolites. The u.v. spectra of the compounds formed on reduction with borohydride resemble that of 3,4-dihydro- (but not 1,2-dihydro-)phenanthrene. Thus ring B must also be aromatic and the conjugated ethylenic double bond must be between C-3 and C-4. The second and third OH groups are, therefore, attached at C-1 and C-2. Selective hydrogenation of the 3,4-double bond in (III) and in (II) (accompanied in this case by loss of the extra oxygen atom), gave products with u.v. spectra very similar to that of the synthetic ketone (IX), thus confirming that ring A is partially unsaturated.

The position and conformation of the secondary hydroxy-groups at C-1 and C-2 are further established by consideration of the CHO H n.m.r. signals for C-1 and C-2 (Table 2). H-2 in metabolite (II) resonates at δ 6.4 and

\[ R = H \quad b; R = Me \]

\[ R = Ac \quad b; R = Ac \]

\[ R = Me, OAc \quad Me, OAc \]

\[ R = Ac \quad b; R = Ac \]

\[ R = H \quad b; R = Ac \]

\[ R = Ac \quad b; R = Ac \]

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\[ R = Ac \quad b; R = Ac \]
is deshielded by 1.1 p.p.m. in the triacetate (III); it thus behaves as a typical secondary alcohol. H-1 resonates 1.5 p.p.m. lower in (II), at γ 4.15, and is deshielded by 1.26 p.p.m. on passing to (III). The environment of H-1 is similar to that experienced by H-1 in the cyclopenta[a]phenanthrene (Ib), in which H-1 resonates at 1—1.5 p.p.m. to lower field than the rest of the aromatic protons.

p.p.m. to lower field than the rest of the aromatic protons in this molecule. H-1 in (II) and (III) is therefore equatorial, in almost the same plane as the aromatic rings, and within the deshielding zone of the aromatic

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<td>N.m.r. data (τ values, J in Hz) for compounds (VIIa), (VIII), (III), and (Vb) in CDCl₃</td>
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<td>N.m.r. data * (τ values, J in Hz) for compounds (II) and (III) in CD₂OD</td>
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* Some assignments were checked by spin-spin decoupling experiments.

The similarity of the u.v. spectra of (II) and (III), and of their borohydride reduced derivatives indicates that (II) and (III) have the same overall structure. The 'extra' oxygen atom in (II) can then be accommodated only as an internal epoxy-bridge, since treatment with acid does not open the ring to give a diol, as would be expected if a 1,2-epoxide were present. Several pieces of evidence suggest that (II) contains an internal epoxy-bridge. The presence of ions showing the loss of an oxygen atom in the mass spectrum of (II) is the most significant. The valence tautomer (X) of pyrene 15,16-epoxide gave the expected M⁺ ion at m/e 218 and an even more intense signal at m/e 202 (M — O). In strong acid this molecule is protonated at oxygen with formation of the stable 3-membered ring. Treatment of 1,6-epoxy-[10]annulene (XI) with acid gave 1-benzoxepin (XII), while mild hydrogenation led to naphthalene by elimination of the epoxy-bridge. In a similar way treatment of (II) with acid yields (Va) with retention of the 'extra' oxygen atom, while hydrogenation causes its elimination. An n.m.r. study of sym-1,6-epoxy-8,13-methano[14]-annulene (XIII) led to the conclusion that this molecule tolerates compression of the bridges rather than bending.

of the ring. The close similarity of the u.v. spectra of (II) and (III) indicates that here, also, the molecule of (II) is not substantially bent by the oxygen bridge.

Consideration of the n.m.r. spectra of (II) and (III) obtained under identical conditions (Table 2) shows that H-1, -2, and -16 in (III) are deshielded, as expected, by ca. 1 p.p.m. The chemical shifts of H-3, -4, -6, -12, and -15 are similar in (II) and (III), indicating that these protons occupy very similar conformations in the two molecules. The slight deshielding of H-4, -6, and -12 probably results from the greater coplanarity, and hence greater ring current, associated with the aromatic rings a and c in (III). By contrast, H-7 and the protons of the 11-methyl group are substantially shielded in (III). This suggests loss of an electronegative substituent from the adjacent 8- and 9-positions on passing from (II) to (III), and therefore that the epoxy-bridge is $8 \rightarrow 9$. The structure of the metabolite is therefore as shown in (II).

Since the ready loss of the epoxy-bridge on acetylation under mild conditions was unexpected, the acetylation conditions were investigated. Omission of the acid wash of the ether extract or examination of the reaction mixture before addition of water gave products indistinguishable by t.l.c. from (III); also the metabolite (II) was recovered unchanged after dissolution in dry pyridine. It was therefore concluded that elimination of the oxygen occurred during the acetylation reaction itself. This may involve rear-side attack by the carbonyl oxygen of the C-1 acetyl group at C-9, since a model demonstrates that these two atoms can approach one another closely. Also the $8 \rightarrow 9$ epoxy-bridge in the dehydraidation product (Va) is stable to acetylation, and the product does not possess a 1α-acetoxy-group. The n.m.r. chemical shifts and coupling constants (Table 1) of H-15 and -16 of this acetate (Vb) are very similar to those of (III). The low field region of this spectrum includes a one-proton doublet at $\tau 1-33 (J 0 \text{ Hz})$ while the rest of the aromatic protons resonate in the range $\tau 2-0-2-85$. This proton is therefore probably H-1, and in agreement with this it is not ortho-coupled since the phenolic OH group must then be at C-2. Two pairs of one-proton quartets at $\tau 2-58 (J_8 8, J_m 2 \text{ Hz})$ and $2-04 (J_8 8 \text{ Hz})$ can then be assigned to H-3 and -4, respectively. Signals due to the three protons in rings b and c occur between $\tau 2-0$ and 2-4 as generally observed in cyclopenta[a]phenanthrenes. There is little evidence to suggest the position of the epoxy-bridge (Vb). However, the protons of the 11-methyl group in this compound resonate at a lower field than those in 11-methylcyclopenta[a]phenanthrenes ($\tau 7-0$), so that the bridge is probably $8 \rightarrow 9$. If it were $8 \rightarrow 10$, H-1 would not be deshielded to the extent observed. The dehydration product therefore has structure (Va). The product of dehydration and deacetylation of (III) with acid is similar to (Va) but contains one less oxygen atom, and is therefore probably the corresponding cyclopenta[a]phenanthrene (Vla).

It seems improbable that metabolism of the ketone (Ib) could lead directly to insertion of the 8,9-epoxybridge in the metabolite (II). This compound is more likely to have arisen by rearrangement of a reactive intermediate. Chemical oxidation of (Ia) and (Ib) occurs at the K-region, osmium tetraoxide yielding the 6,7-dihydro-6,7-diols and chromic acid giving the 6,7-quinones. It thus seems likely that this region will also be the site of major metabolic attack leading to 6,7-dihydro-6,7-epoxides. Rearrangement of the latter with migration of the oxygen atom at a position bridging C-8 and C-9 seems possible. Recently a rearrangement of this type has been proposed by Bruce to account for the formation of indan-4-ol (XV) and indan-5-ol (XVI) from 3α,7α-epoxy-3α,7α-dihydroidan (XIV). In this rearrangement the arene oxide opens to give a zwitterion which collapses to an isomeric oxide, resulting in movement of the oxygen atom round the ring, finally to yield the two phenols. Possibly the marked difference in urinary metabolism between the carcinogenic 11-methylketone (Ib) which gives (II), and the inactive unsubstituted ketone (Ia) which gives only a trace of the unsubstituted homologue, is that the electron-releasing 11-methyl group favours this type of rearrangement, or that oxepin formation allows release of the 1-H-11-Me interaction.

**EXPERIMENTAL**

Procedures are generally as described in the preceding parts of this series. M.p.s were obtained using a Kofler hot stage. 8 E. Vogel, U. Haberland, and J. Ick, Angew. Chem. Internat. Edn., 1970, 9, 517.
stage microscope (RCH, Reichert, Austria), or glass capillary tubes and an Electrothermal electrically heated block. I.r. spectra were recorded as mulls in Nujol on a Perkin-Elmer model 257 spectrometer. N.m.r. spectra were measured on a Varian A60 (60 MHz) or a Perkin-Elmer R32 (90 MHz, Me6Si lock) spectrometer. Mass spectra were obtained using an A.E.I. MS902 instrument.

**Preparation of 15,16-Dihydro-11(3H)methylcyclopenta[a]phenanthren-17-one.**—This compound was prepared from 17,17-ethylenedioxy-12,13,14,16,17-hexahydrocyclopenta[a]phenanthren-11-one (588 mg, 2 mmol) and [3H]-methyl iodide (282 mg, 2 mmol, 200 mCi) by a Grignard reaction as previously described for the [4H]compound. The product (248 mg, 26-5 mCi) obtained after chromatography as fawn needles, m.p. 202°, ran as a single spot on t.l.c. (dichloromethane) and had u.v. and i.r. spectra identical with those of the authentic, unlabelled ketone.

**Characterisation of the Metabolite** 8,9-Epoxy-1a,2,15,16-trihydroxy-11-methyl-8,9-secogona-3,5,7,9,11,13-hexaen-17-one (II).—This material was soluble in ethanol and moderately soluble in water, and was best purified by recrystallisation from ethyl acetate with addition of a little charcoal. After repeated recrystallisation from this solvent the metabolite (II) was obtained as pale fawn needles which decomposed without melting at 120° (in vacuo). [α]D26 214° (c 0-134, EtOH). For analysis, samples were dried at 40° in vacuo after recrystallisation from ethanol (Found: C, 69-3; H, 5-7%), and from ethyl acetate (Found: C, 70-3; H, 5-2%; m/e 294-0893. C16H12O4 requires C, 69-2; H, 5-16%). M+ — H2O requires m/e 294 (6%), 278 (M — H2O — O, 34), 277 (100), 261 (30), 260 (21), 259 (50), 248 (20), 234 (36), and 232 (50), λmax (EtOH) 264 (log ε 4-57), 320 (4-03), 332 (4-04), 352 (3-87), and 370 nm (3-61), unchanged by the addition of aqueous NaOH; treatment of the ethanolic solution with NaBH4 for 30 min gave λmax (EtOH) 268 (log ε 4-60), 207 (4-67), 3-18 (3-78) nm; vmax 3400—3160 (O—H str., probably involving intermolecular hydrogen bonding), 1690 (ArCO), 1670 (acetate CO) and 1710 (ketone CO), and 1020 cm-1 (possibly C—O—C), r (CD3 OD) in Table 2, r (CDCl3) in Table 1, m/e 422 (M+), no M — O peak.

1,2,3,4,15,16-Hexahydro-1a,2,15,16-trihydroxy-11-methylcyclopenta[a]phenanthren-17-one (IVA).—The metabolite (II) (15 mg), dissolved in ethanol, was stirred in an atmosphere of hydrogen with 10% palladium-on-charcoal (Johnson–Matthey). Uptake of gas ceased after 80 min when 1-95 mol. equiv. of hydrogen had been consumed. The catalyst was removed by filtration and the product was crystallised from ethanol to yield cream needles of the hydrogenated derivative (IVA) (Found: M+, 298-1199. C16H12O4 requires M+, 298-1208), m.p. 225—227° (from ethanol), λmax (EtOH) 280 (log ε 4-70), 255 (3-80), 295 (3-94), 342 (3-40), and 354 nm (4-40). [α]D26 (c 4-77), 287 (3-97), 279-5 (4-02), 344 (3-69), and 354 nm (3-74). Acetylation of (IVA) as previously described gave a triacetate, m.p. 123—124°, identical with the triacetate obtained by hydrogenation of (III) (see below).

**Hydrogenation of the Triacetate** (III).—Hydrogenation of (III) (5 mg) as already described yielded crystalline 1a,2,15,16-triaceetoxo-1,2,3,4,15,16-hexahydro-11-methylcyclopenta[a]phenanthren-17-one (IVb) (Found: M+, 424-1523. C24H20O4 requires M+, 424-1522), m.p. and mixed m.p. with the foregoing sample 123—125°, λmax as for (IVA), vmax (CHCl3) as for (IVA), with increased intensity at 2925 and 2850 cm-1 (aliphatic C—H).

8,9-Epoxy-2,15-di-hydroxy-11-methyl-8,9-secogona-1,3,5,7,9,11,13-heptaen-17-one (Va).—The metabolite (II) (20 mg) was heated on a steam-bath with 5m-H2SO4 (10 ml); after 1 h the yellow solid was collected, washed with water, and dried to give the phenol (Va), homogeneous by t.l.c. [tulene—ethyl acetate—methanol (2:1:1 and 15:5:1, v/v), yellow fluorescence in u.v. light], m.p. 280—282° (Found: C, 73-25; H, 4-4%; m/e, 278-0935. C16H12O4 requires C, 73-45; H, 4-8%). M+ — O requires m/e, 278-0943, m/e 294 (M+ 7%), 278 (M — O, 22), 277 (M — OH, 8), 275 (8), 261 (100), 260 (10), 259 (29), 247 (10), 244 (10), and 232 (14), vmax (Nujol) 1680 (conjugated C=O), 3180—3265 (OH), and 1005 cm-1, λmax (EtOH) 278 nm (log ε 4-55), λmax (EtOH soln. (2-5 ml) + 5m-NaOH (0-1 ml)) 255 (4-50) and 299 nm (4-50); after treatment with NaBH4, λmax (EtOH) 258, 310, 347, and 366 nm, λmax (EtOH + NaOH) 251 and 344 nm. The phenolic nature of (Va) was confirmed by the production of an immediate deep blue colour with Gibb’s reagent. Compound (Va) could not be hydrogenated under the conditions described for (II) and (III).

Acetylation of (Va) by the method described previously gave cream crystals of the diacetate (Vb), m.p. 208—210° (from ethanol) (Found: C, 70-0; H, 4-6%; M+, 378-1008. C18H16O6 requires C, 69-65; H, 4-8%; M, 378-1103, λmax (EtOH) 287, 298bs, 356, and 374 nm, vmax (Nujol) 1734, 1734, and 1715 cm-1 (C=O str.), no OH absorption, τ (CDCl3) in Table 1, m/e [cf. (Va)] 378 (M+, 1%) and 382 (M — O, 48).

**Treatment of the Triacetate** (III) with Acid. Compound (III) (5 mg) was heated at 100° with 5m-H2SO4 for 2 h, cooled, and extracted with ethyl acetate. Evaporation of the washed and dried extract gave 15,16-dihydro-2,15,16-dihydroxy-11-methylcyclopenta[a]phenanthren-17-one


(VIa) as a yellow solid, \( \lambda_{\text{max}} \) (EtOH) 276 nm, \( \lambda_{\text{max}} \) (EtOH + NaOH) 250 and 300 nm, \( \lambda_{\text{max}} \) (EtOH + NaBH\(_4\)) 260 and 315 nm. This substance further resembled (Va) in its yellow fluorescence in u.v. light, blue colour with Gibb’s reagent, and similar \( R_f \) value; m/e 278 \((M^+)\), 277, and 276.

Acetylation of this material in the usual way yielded the diacetate (VIb) (Found: \( M^+ \), 362-1139. \( C_{43}H_{44}O_2 \) requires \( M \), 362-1154). \( \lambda_{\text{max}} \) (EtOH) 267 nm, \( \nu_{\text{max}} \) (Nujol) 1735, 1720, and 1700 cm\(^{-1}\), no OH absorption.

We thank Dr. C. W. Vose for his interest and help, especially in the interpretation of the mass spectra, Mr. T. S. Bhatt for assistance with the animal work, Mr. G. Combarides for n.m.r. spectra, and Mr. P. Cook for mass spectral measurements. We are indebted to Mrs. R. E. M. Jones and Dr. R. Spragg (Perkin-Elmer Ltd.) who recorded the 90 MHz spectra. F. E. H. C. thanks the Imperial Cancer Research Fund for a research bursary.

[4/993 Received, 10th May, 1974]
Potentially Carcinogenic Cyclopenta[a]phenanthrenes. Part X.¹ Oxygenated Derivatives of the Carcinogen 15,16-Dihydro-11-methylcyclopenta[a]phenanthren-17-one of Metabolic Interest

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Reprinted from

JOURNAL
OF
THE CHEMICAL SOCIETY

PERKIN TRANSACTIONS I

1975
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penta[a]phenanthren-17-one of Metabolic Interest

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Two metabolites of the title compound, namely the 15-hydroxy- and 11-hydroxymethyl derivatives, and also 15,16-dihydro-2-hydroxycyclopenta[a]phenanthren-17-one have been synthesised by adaptations of routes previously described for compounds of this series.

After injection of the carcinogen 15,16-dihydro-11-
methylcyclopenta[a]phenanthren-17-one (Ib) into rats, the major metabolite isolated from the urine was the epoxytrihydroxybenzocyclodecene (II), whereas the non-carcinogenic ketone (Ia) was not metabolised to a similar structure in significant amounts. The epoxy-compound (II) was not found among the in vitro metab-
olites when the carcinogen was incubated with various rat liver preparations in the presence of NADPH and oxygen, but here the major metabolite was the 15-
hydroxy-derivative (IIa), together with smaller amounts of the 16-hydroxy- (IVa) and 11-hydroxymethyl- (Va) ketones. This paper describes the chemical synthesis of (IIa) and (Va), and of 15,16-dihydro-2-hydroxycyclo-
penta[a]phenanthren-17-one (VIIa) which was required as a model during the elucidation of the structure of the phenolic epoxybenzocyclodecene (VI) formed on treat-
ment of the urinary metabolite with acid.

The 15-acetoxy-ketone (IIIb) was readily obtained by either of the methods described for the corresponding derivative of the parent ketone. Treatment of (Ib) with bromine and thallium(III) acetate led directly to the 15-acetate (IIIb), but isolation from the reaction mixture by chromatography was not readily accomplished. Attention was therefore directed towards the second method, acid-catalysed addition to the enone (VIIIb). The carcinogen was readily brominated in good yield with N-bromosuccinimide to give the 16-bromo-ketone (IX), the ring D protons of which possessed n.m.r. chemical shifts and coupling constants almost identical with those of 16-bromo-15,16-dihydrocyclopenta[a]-phenanthren-17-one, and different from those of the


known 15-bromo-derivative of (Ib).\(^*\) Dehydrobromination with triethylamine readily generated the 16-en-17-
\[
\begin{align*}
&\text{(I)} \quad R = H \\
&\text{(II)} \quad R = Me \\
&\text{(III)} \quad R = H \\
&\text{(IV)} \quad R = Ac \\
&\text{(V)} \quad R = H \\
&\text{(VI)} \quad R = Ac \\
&\text{(VII)} \quad R = H \\
&\text{(VIII)} \quad R = Ac \\
&\text{(IX)} \quad R = Br
\end{align*}
\]
temperature. Recourse was therefore made to acid-catalysed addition of acetic acid to this double bond, for it is known that this occurs some 700 times faster than acid-catalysed hydration of the double bond in the analogous system indene.\(^6\) By using acetic acid containing 5% of sulphuric acid, addition was essentially complete within 1 h and the 15-acetoxy-17-ketone (IIIb) was obtained directly by crystallisation in about 40% yield. It was essential to remove the triethylamine hydrobromide before the addition of the acid; failure resulted in regeneration of the 16-bromoketone (IX). The reason for this surprising result is not clear, for acid attacks as expected at C-16. There was evidence for a small proportion of the 15-bromide in the material recovered. Attempted alkaline hydrolysis of the acetate (IIIb) led to degradation, but hydrolysis with sulphuric acid in tetrahydrofuran gave the 15-hydroxy-compound (IIIa) in moderate yield.

Hydroxymethyl derivatives of methyl substituted polycyclic aromatic hydrocarbons have been prepared directly by oxidation with lead tetra-acetate,\(^6\) but under these conditions the carcinogen (Ib) is converted largely into the 16-acetate (IVb).\(^7\) For synthesis of the 11-hydroxymethyl derivative (Va) we therefore used the convenient intermediate (X).\(^8\) Grignard reagents prepared from benzyl chloromethyl ether and chloromethyl methyl ether failed to react with this ketone, presumably owing to steric hindrance, but addition to the carbonyl group occurred in reasonable yield with the ylide from trimethylsulphonium iodide.\(^9\) The expected epoxide was not isolated, for when the reaction was worked up by acidification to pH 2–3 with dilute acetic acid the product obtained was the 11-hydroxy-11-hydroxymethyl acetal (XIa). Absence of i.r. carbonyl absorption and of a methyl singlet in its n.m.r. spectrum indicated this structure. In addition, the methylene protons of the hydroxymethyl group appeared as an AB quartet with geminal coupling of 11-5 Hz. The

base peak in the mass spectrum, m/e 295, corresponds to the loss -CH₂OH from the molecular ion.

Protection of the primary hydroxy-group by mild acetylation, followed by dehydration with phosphoryl chloride and pyridine gave the Δ¹-acetel (XII) in which the methylene protons of the acetoxymethyl group were equivalent, appearing as a singlet. Aromatisation of ring C was completed by dehydrogenation with dichlorodicyanobenzoquinone (DDQ); removal of the acetel function with acid led to the fully aromatic acetoxymethyl ketone (Vb) with u.v. absorption very similar to that of the ketone (Ib). Mild alkaline hydrolysis finally yielded the required 11-hydroxymethyl ketone (Va).

For the synthesis of the \(2\)-phenolic-17-ketone (Vila) the route previously employed was followed, starting from the tricyclic ketone (XVI). The latter was prepared from anisole by a six-step sequence, after abandoning a potentially shorter route by way of Friedel–Crafts succinylation of \(2\)-methoxynaphthalene. Although this reaction was stated to yield solely 3-(2-methoxy-1-naphthoyl)propionic acid (XIIIa) in unstated yield when carbon disulphide was used, employing these conditions Bachmann and Horton isolated the required 7-methoxy-1-naphthoyl isomer as the ethyl ester (XV) in ca. 10% yield, but obtained none of the 2-methoxy-isomer (XIIIa). In our hands, repetition of the succinylation according to these authors led to a small amount of (XV) together with a much larger quantity of the phenolic ester (XIVb). The latter was readily methylated with diazomethane to the 2-methoxy-1-naphthoyl ester (XVII), but attempted methylation with dimethyl sulphate and alkali failed, giving only the phenolic acid (XIVa). This partly explains the above discrepancy, for the former authors stated merely that the reaction was 'worked up in the usual way,' whereas the latter specify treatment of the crude Friedel–Crafts product with dimethyl sulphate and alkali before esterification and distillation. Nevertheless, it is not clear why the original authors did not also observe extensive demethylation with aluminium chloride in boiling carbon disulphide.

Stobbe condensation of the tricyclic ketone (XVI) with diethyl succinate yielded the half-ester (XVII), which was cyclised with acetic anhydride and anhydrous zinc chloride. The product (XVIII), readily identified by its u.v. spectrum characteristic of 11,12,16,17-tetrahydro-17-ketones of this series, was dehydrogenated by prolonged heating with chloranil in toluene. The yield of the cyclopenta[\(a\)]phenanthrene (XIX) was ca. 60%: reaction with DDQ occurred more quickly, but the yield was less. Demethylation with boron tribromide below 0° yielded the 2-hydroxy-17-ketone (VIIa), isolated as its more soluble acetate (VIIb). The free 2-phenol resembled in most respects the known 3-, 6-, and 7-phenols, except that with alkali the u.v. maximum at 274.5 nm gave place to two maxima of similar intensity at 245 and 296 nm, instead of the simple bathochromic shift shown by the maxima of these other phenols. This behaviour was also exhibited by the metabolite derivative (VIIa), with a transition from 278 nm (neutral) to maxima of equal intensity at 255 and 299 nm in alkaline solution. The n.m.r. signals due to the aromatic protons in these two compounds are compared in the Table. Very similar chemical shifts are observed for H-3, -4, and -12, while the presence of the 11-methyl group in (VIIb) causes H-1 to resonate at ca. 0.3 p.p.m. to lower field than that in (VIIb), as has been previously observed with similar compounds. However, H-6 and -7 in (VIIb) are accidentally equivalent, appearing as a strong singlet at 2.17 while in (VIIb) they form an AB quartet. One proton, probably observed for H-3, -4, and -12, while the presence of the 11-methyl group in (VIIb) causes H-1 to resonate at ca. 0.3 p.p.m. to lower field than that in (VIIb), as has been previously observed with similar compounds. However, H-6 and -7 in (VIIb) are accidentally equivalent, appearing as a strong singlet at 2.17 while in (VIIb) they form an AB quartet. One proton, probably
H-6, resonates at $\tau$ ca. 2.20 whereas the other (H-7) appears at ca. 2.02 indicating attachment of the electron-negative ring oxygen at the adjacent carbon atom (C-8). A similar deshielding of H-7 in the metabolite (II) was observed when compared with its triacetate lacking this ring oxygen atom.

**EXPERIMENTAL**

Materials and methods were generally as described in previous parts of this series. I.r. spectral data for compounds marked with an asterisk are listed in Supplementary Publication No. SUP 21216 (2 pp.).

16-Bromo-15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (IX).—The carcinogen (Ib) (2.46 g, 10 mmol) in carbon tetrachloride (100 ml) was boiled under reflux with N-bromosuccinimide (2.14 g) for 3 h adjacent to a 150 W lamp. After cooling, the precipitated succinimide was filtered off, and the orange solution was washed with water, dried, and evaporated to give a solid (2.21 g). Recrystallisation from benzene yielded the 16-bromo-11-methylketone (IX) (1.55 g), m.p. 168° (unchanged by further recrystallisation) (Found: $M^+$, 324.0141. $C_{18}H_{22}BrO$ requires $M^+$, 324.0149). $\lambda_{\text{max}}$(Nujol) 1720 (C-O), 820, 892, 800, 770, 768, and 675 cm$^{-1}$; $\lambda_{\text{max}}$(EtOH) 274, 5, 359, and 377 nm, $\tau$ (CDCl$_3$) 4-06 (q. $J$ 2 and 6 Hz, H-16), 6-7 (q, $J$ 2 and 20 Hz, H-15), and 6-51 (q, $J$ 6 and 20 Hz, H-15).

16-Acetoxy-15,16-dihydro-11-methylcyclopenta[a]phenanthren-17-one (IIIb).—From the enone (VIIIb). The bromoketone (IX) (1.06 g) was dissolved in pure tetrahydrofuran (THF) (100 ml) and treated with triethylamine (2.0 ml). Aliquot portions were removed at intervals and diluted with ethanol for u.v. spectrophotometry; after ca. 30 min, conversion into the enone (VIIIb), $\lambda_{\text{max}}$ 266, 288, 298, and 312 nm, appeared to be complete. Dichloromethane (100 ml) was added and the solution was washed with water to remove triethylamine hydrobromide. After addition of a mixture of glacial acetic acid (180 ml) and conc. sulphuric acid (20 ml) aliquot portions were removed, diluted with acetic acid, and their u.v. spectra observed. After 1 h, when conversion into the product, $\lambda_{\text{max}}$ 266 nm, was complete, more dichloromethane was added and the reaction mixture was washed several times with water, with sodium hydrogen carbonate solution, again with water, and dried. Evaporation and crystallisation of the residue from ethanol gave red needles (0.4 g), m.p. 196—204°. Several recrystallisations from this solvent with the addition of charcoal yielded the 16-acetoxy-11-methyl-17-ketone (IIIb)* as pale pink needles, m.p. 209—210° (Found: C, 78.86; H, 5.15. $C_{18}H_{22}O_2$ requires C, 78.95; H, 5.3%). $\lambda_{\text{max}}$(EtOH) 266 (log $\varepsilon$ 4.81), 301 (4.30), 356 (3.11), and 374 nm (3.19).

In a preliminary experiment the bromo-ketone (IX) (50 mg) in THF (1.0 ml) was treated with triethylamine (0.1 ml) for 30 min, when the above spectral change had occurred. Addition of 5% sulphuric acid in acetic acid caused an almost immediate change to $\lambda_{\text{max}}$(HOAc) 277 nm. After 30 min water was added and the product was extracted with dichloromethane as before to yield a pale brown crystalline solid (30 mg), m.p. 146—155°, $\lambda_{\text{max}}$(Nujol) as for (IX), with weak bands at 820, 760, 722, and 700 cm$^{-1}$ possibly caused by the presence of a small quantity of the 15-bromo-17-ketone.4

This acetate (IIIb) (130 mg) was boiled for 20 h with 2$n$-H$_2$SO$_4$ (7.5 ml) in THF (15 ml). The dark brown solution was diluted with water and extracted with ethyl acetate. The crude product, which showed several spots on t.l.c., was purified by this method, recovering the material of $R_F$ 0.60 by elution of the silica gel with ethanol. Evaporation of the solvent under reduced pressure gave the 15-hydroxy-11-methyl-17-ketone (IIIA) as a pale greenish solid (20 mg), $\lambda_{\text{max}}$(EtOH) 265 (log $\varepsilon$ 4.84), 300 (4.63), 356 (3.29), and 374 nm (2.21). This compound appeared to be rather unstable; rechromatography after keeping it at ambient temperature again showed several spots, and for this reason no attempt was made at further purification.

From (Ib) with thallium(III) acetate and bromine. The carcinogen (Ib) (150 mg) and thallium(III) acetate (234 mg) were stirred together in carbon tetrachloride (35 ml). Bromine (100 mg) in this solvent (2 ml) was added in one lot, and the mixture was stirred at room temperature for 23 h, when t.l.c. showed that most of the ketone (Ib) had been consumed with the formation of a more polar material identified as the 15-acetate (IIIb) by its $R_F$ value and the greenish blue colour developed at 10° after spraying with ethanolic H$_2$SO$_4$. After filtration from the dark precipitate, removal of the solvent gave a bright yellow gum (194 mg) which was chromatographed on a column of silica gel (Hopkins and Williams M.F.C.) (20 g), eluting with dichloromethane containing increasing proportions of ethyl acetate. Fractions containing the acetate (IIIb), but still contaminated with starting material (Ib) (60 mg) were purified further by preparative t.l.c. (silica gel, 1 mm layers; CH$_2$Cl$_2$ giving finally an orange solid (11 mg) with i.r. and u.v. spectra identical with those of the pure 15-acetate (IIIb) described above. The chromatographic column remained strongly fluorescent when no more material could be eluted.

11,17-Ethylendioxy-11-hydroxy-11-hydroxymethyl-15,12,13,14,15,16-hexahydrocyclopenta[a]phenanthrene (XIa).—Sodium hydride (60% dispersion in oil; 0.35 g) was washed by decantation with light petroleum (b.p. 40—60°). Dry dimethyl sulphoxide (30 ml) was added under nitrogen and the mixture was stirred at 70° until hydrogen evolution ceased. After cooling, dry tetrahydrofuran (30 ml) was added and the mixture was further cooled to $-5^\circ$ before trimethylsulphonium iodide (3.69 g) dissolved in dimethyl sulphoxide was added slowly with stirring, keeping the temperature below 0°. When the addition was complete the oxo-acetol (X) (3.5 g) in a mixture of dimethyl sulphoxide (16 ml) and THF (15 ml) was run in slowly below 0°, and the solution was stirred at this temperature for 2 h. When the reaction mixture had attained room temperature it was poured into water (260 ml) and acidified to pH 2—3 with glacial acetic acid to facilitate extraction with dichloromethane. The extract was washed with water, saturated sodium hydrogen carbonate solution, water, and dried. The gum (3.4 g) left on removal of the solvent under reduced pressure crystallised from benzene—n-hexane as fawn needles (1.3 g); the mother liquor contained mostly starting material. Recrystallisation gave the 11-hydroxy-11-hydroxymethyl acetal (XIa)* as needles (1.12 g), m.p. 168—170° (Found: C, 73.73; H, 6.8%; $M^+$, 326.1511. $C_{18}H_{20}O_2$ requires C, 73.66; H, 6.9%). $M^+$ (EtOH) 230, 5 (log $\varepsilon$ 4.92), 272, 8 (3.74), 282, 7 (3.76), and 291 nm (3.62); $\lambda_{\text{max}}$(CDC$_3$) 6-13 (d, $J$ 11.5 Hz, H$_{\text{aCHbOH}}$), 5-67 (d, $J$ 11.5 Hz, H$_{\text{aCHbOH}}$), and 6-04 (s, OCH$_2$CH$_2$O).

* For details of Supplementary Publications, see Notice to Authors No. 7 in J.C.S. Perkin I, 1974, Index issue.
This diol (1:12 g) was acetylated with acetic anhydride and pyridine overnight at room temperature. Chromatography of the product (1:1 g) on alumina (Merck grade II–III) with toluene-ethanol (200:1 v/v) gave the 11-acetoxyethyl-11-hydroxy acetel (XIIb) as a pale yellow glass (0.78 g) (Found: C, 71-7; H, 6-95%; m/z 305 (M+ - CH2OAc, 15), and 99 (acetol ion, 100); \( \lambda_{max} \) (log \( e \)) 273.4 (7-47), 282 (7-36), and 291 nm (3-39); \( \tau \) 7-95 (s, OAc), 6-05 (s, OCH2CH2O), 5-98 (d, \( J = 11-5 \) Hz, H2A3OAc), 4-92 (d, \( J = 11-5 \) Hz, H2A3OAc).

16,16-Dihydro-11-hydroxymethylcopenta[a]phenanthren-17-one (Va). — Redistilled phosphoryl chloride (394 mg, 0.24 ml) was added to a vigorously stirred solution of the acetoxyethyl acetel (XIIb) in pyridine (15 ml), cooled in ice. The solution was stirred at 0° for 30 min and then at 78° for 6 h in the absence of moisture. After addition of ice and extraction with ether, a pale orange-yellow gum (211 mg) was obtained which ran as one spot on t.c., \( R_f \) 0-73 (ethyl acetate–dichloromethane, 1:9 v/v) and appeared to be the required 11-acetoxyethyl-11-acetal (XII), \( \lambda_{max} \) (EtOH) 237.5 (log \( e \)) 4-81, 303 (3-88), 315 (3-87), and 328 nm (3-80), similar to the spectrum of the corresponding 11-methyl-11-acetal; \( \tau \) 7-90 (s, OAc), 7-32 (m, H-13 and -14), 6-00 (s, OCH2CH2O), and 4-75 (s, CH3OAc). Chromatography of a sample of this XII-acetal on alumina (Grade III) resulted in decomposition to a number of products, and the acetel was therefore used without further purification.

This material (60 mg), dissolved in benzene (10 ml), was treated with dichlorodicanoquinone (DDQ) (40 mg) at room temperature for 48 h. The precipitated hydroquinone was collected and the filtrate was washed with 2N-sodium hydroxide to yield an orange glass (49 mg) (Found: C, 70-6; H, 6-05; \( M^+ \), 368-1623), \( \lambda_{max} \) (Nujol) 3295 (OH), 1729 (ester 0=0), and 1670 cm-1 (C=O). Methylation of (XIIb) (1-0 g) with an excess of diazomethane in ether gave an oil which rapidly crystallised to the methoxy-ester (XIIIb) (0-95 g), m.p. 72-74-6° (lit. 12 79-5—81°). Evaporation of the methanol and crystallisation of the residue from benzene yielded bright yellow prisms (20-5 g) of ethyl 3-(2-hydroxy-1-naphthyl)propionate (XIVb), m.p. 95-96° (Found: C, 70-6; H, 6-05; \( C_{13}H_{16}O_4 \) requires C, 70-5%; H, 5-9%).

Saponification of (XIVb) (0-5 g) with warm 2N-NaOH gave the acid (XIVA) (0-34 g), m.p. 105-106° (Found: C, 68-65; H, 5-16. \( C_{13}H_{16}O_4 \) requires C, 68-85; H, 5-9%).

This diol (10-0 g) with hexane-dichloromethane (1:1 v/v) gave an oil which rapidly crystallised to the methoxy-ester (XIIIb) (0-95 g), m.p. 72-74-6° (lit. 12 79-5—81°). Evaporation of the methanol and crystallisation of the residue from benzene yielded bright yellow prisms (20-5 g) of ethyl 3-(2-hydroxy-1-naphthyl)propionate (XIVb), m.p. 95-96° (Found: C, 70-6; H, 6-05; \( C_{13}H_{16}O_4 \) requires C, 70-5%; H, 5-9%).

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(XIX).—The tetrahydro-ketone (XVIII) (0.88 g) and chloranil (1.20 g, 1.6 mol) were boiled together in dry toluene (100 ml) for 1 week when t.l.c. showed that little of the starting material remained. The dark crystalline precipitate of the hydroquinone was collected, washed with toluene, and the combined toluene solutions were evaporated to a small volume, and placed on a column of silica gel (Hopkins and Williams, MFC). Elution with toluene containing increasing proportions of dichloromethane gave fractions (total 0.63 g) homogeneous by t.l.c. Crystallisation from ethanol yielded the 2-methoxy-ketone (XIX) (0.40 g), m.p. 164—165°, raised to 180—180.5° by two more recrystallisations from this solvent (Found: C, 82.6; H, 5.5. C18H14O2 requires C, 82.4; H, 5.4%). vmax (EtOH) 271 (log e 4.81), 360 (3.45), and 377 nm (3.51); νmax (Nujol) 1705—1680 (aryl C=O), 1043, 955, 825, 790, 760, and 720 cm⁻¹.

15,16-Dihydro-2-hydroxycyclopenta[a]phenanthren-17-one (VIIa).—The methoxy-ketone (XIX) (0.54 g) in dichloromethane (50 ml) was cooled to −80° and boron tribromide (5 ml) was added. After 1 h the solution was gradually warmed to 0° during 15 min, then poured into ice. The cream coloured precipitate was collected, washed with water, dried, and stirred with dichloromethane. After filtration, acetylation of the solid (0.38 g) gave a product which was crystallised from benzene to yield pale fawn needles of 2-acetoxy-15,16-dihydrocyclopenta[a]phenanthren-17-one (VIIb), m.p. 248—249° (Found: C, 78.65; H, 4.85. C14H10O4 requires C, 78.6; H, 4.85%), λmax (EtOH) 265-5 (log e 4.85), 282-5 (4.66), 296 (4.36), 352 (3.37), and 369 nm (3.36); νmax (Nujol) 1675 (acetate C=O), 1095 (aryl C=O), 1015, and 930 cm⁻¹; τ (CDCl3) 6.66 (t, 16-H2), 7.17 (t, 16-H2), and 7.61 (CH3COs).

This acetate (100 mg) was hydrolysed by boiling with ethanol (20 ml) containing aqueous 5% NaOH (2 ml) for 1 h. Dilution with n-HCl (40 ml) gave a cream coloured precipitate which was washed with dichloromethane and recrystallised from butan-1-ol. The 2-hydroxy-17-ketone (VIIa) formed pale yellow needles (29 mg), m.p. 340° (decomp.; sealed, evacuated capillary), λmax (EtOH) 274-5 (log e 4.74), 366 (3.40), and 383 (3.45) (neutral); 245 (4.59) and 296 (4.62) (alkaline); 257 (4.69) and 310 (3.95) (neutral, after reduction with NaBH4); and 249 (4.73) and 334 nm (4.18) (alkaline, after reduction with NaBH4).

We thank Mr. D. Thomas for the microanalyses and Mr. P. Cook for the mass spectral measurements. C. W. V. thanks the Imperial Cancer Research Fund for a bursary.

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15,16-Dihydro-1-methylcyclopenta[a]phenanthren-17-one has been prepared starting from testosterone, and in addition by a multi-stage synthesis from a correctly substituted naphthalene. The latter route has also been employed for the synthesis of the 7,11-dimethyl-17-ketone, and the 1,11-methano-17-ketone containing a bridged bay region.

In previous parts of this series we have described the synthesis of the 2-, 3-, 4-, 6-, 11-, and 12-methyl derivatives of 15,16-dihydrocyclopenta[a]phenanthren-17-one (1a), as well as numerous other compounds related to this parent polycyclic ketone. Of these monomethyl derivatives the 11-methyl-17-ketone (1b) is a strong carcinogen, similar in potency to benzo[a]pyrene. The 7-methyl isomer is less active and all the other isomers are inactive, as is the parent ketone (1a). The carcinogen (1b) is biologically activated to its anti-1,2,3,4-tetrahydro-trans-3,4-dihydroxy-1,2-epoxide, and it was therefore anticipated that like the 2-, 3-, and 4-methyl isomers, the unknown 1-methyl-17-ketone would lack carcinogenic activity. The 1,11-methano-derivative (2) was also expected to be inactive since, although it still retains a small electron-releasing substituent at C-11, the bay region is apparently blocked by the 1,11-methylene group. This paper describes the syntheses of these two compounds, the former by two independent routes; a preliminary communication has appeared in connection with the latter (2). Also reported is the synthesis of the 7,11-dimethyl-17-ketone (1d); the potency of this compound was of interest because it bears methyl groups at both positions known to confer carcinogenic activity in this series.

Initially A-ring aromatisation of a suitable steroid via the dienone-phenol rearrangement seemed to offer a convenient means of placing the methyl group at C-1 in a cyclopenta[a]-phenanthrene. Dannenberg’s method was selected, since ring-A aromatisation and removal of the 3-oxygen function are effected simultaneously. Thus testosterone acetate was converted by known methods in four steps into 1-methyl-17-ketone (1a);11 several methods were considered for removal of the blocking 18-methyl group, but finally an adaption of the method devised by Pinhey and his co-workers13 for the removal of a 4-methyl group from a tetracyclic triterpenoid was chosen. An abnormal Beckmann rearrangement of the derived oxime (2b) yielded the methylene nitrile (4). Epoxidation with m-chloroperbenzoic acid led to the epimeric epoxynitriles (5) which, without separation, were cyclised with boron trifluoride to give the 18-norketone (6). The yield in the cyclisation step (14%) was much lower than that obtained by Pinhey and his co-workers for cyclisation of the six-membered ring A, and the overall yield of (6) from (2a) was only 8%. Unfortunately, complete dehydrogenation of the 18-norketone (6) to the desired cyclopenta[a]phenanthrenone also proved difficult, but as finally achieved, albeit in poor yield, by prolonged reaction with dichlorodiacyanobenzquinone (DDQ) in boiling dioxan to furnish a small sample of 15,16-dihydro-1-methylcyclopenta[a]phenanthren-17-one (1c) identical with the material from the second synthesis (see below). Although of no use practically, this route is nevertheless of interest as a new way of converting a natural steroid into a member of the cyclopenta[a]phenanthrene series.

A more practical synthesis of this compound was accomplished by adding rings C and D sequentially to a suitable substituted naphthalene, a strategy previously adopted by us for the synthesis of cyclopenta[a]phenanthrenes. Treatment of the keto-ester (7) with methyl bromoacetate in the presence of zinc gave the lactone (8), readily reduced by catalytic hydrogenolysis to the acid-ester (9). Cyclisation of the latter with stannic chloride was followed by borohydride reduction of the resulting tetralone (10); subsequent dehydration, and dehydrogenation of the product by heating with sulphur, yielded 8-methyl-1-naphthylacetic acid (11a). Chain extension was achieved by reduction to the corresponding naphthylacetaldehyde, bromination, and application of the malonic acid synthesis. Cyclisation of the resulting naphthylbutyric acid (12a) gave the tricyclic ketone (13a) which was converted, via the Stobbe half-ester (14a) and further cyclisation with zinc chloride, into the 11,12-dihydro-compound (15a) by the previously described methods. Catalytic dehydrogenation
with palladium in boiling p-cymene then led smoothly to the 1-methyl-17-ketone (1c).

A similar sequence of reactions was employed in the synthesis of the 7,11-dimethyl-17-ketone (1d). 2-(3-Methyl-1-naphthyl)propionic acid (11b) (obtained via Reformatsky reaction between 3-methyltetralone and ethyl 2-bromo-2-propionate) was chain-extended and cyclised as before to yield 4,10-dimethyl-1,2,3,4-tetrahydropyranthren-1-one (13b) in good yield. Unfortunately the Stobbe reaction with this ketone and the subsequent cyclisation step occurred with difficulty, presumably as a result of steric hindrance by the adjacent methyl group. Similar problems were encountered in the synthesis of 15,16-dihydro-7-methylocyclophanthrene-17-one (1e).\(^1\) The final catalytic dehydrogenation, however, occurred in 85% yield despite the presence of the methyl group at C-11. This dehydrogenation method with palladium in boiling p-cymene appears to be superior to the methods previously used (DDQ in boiling benzene, or fusion with palladium at 220 °C).\(^1\)

Originally \(^4\) in the synthesis of the 1,11-methano-compound (2), acenaphthen-1-ylacetic acid (16) was converted into the corresponding propionic acid (17) by the Arndt-Eistert reaction.\(^5\) On a larger scale it was however found preferable to carry out this transformation in several stages, by reduction of the acid (17) and bromination of the resulting alcohol, conversion into the nitrile and hydrolysis to (17). Cyclisation to the tetracyclic ketone (18) followed by elaboration of the five-membered ring via the Stobbe half-ester (19) was carried out as before.

The cyclophanthreneones (1c) and (1d) possessed spectral and other properties similar to those of the other positional isomers of this series. Noticeable differences in the u.v. spectrum of the methano-compound (2) probably reflect the considerable distortions in the phenanthrene system induced by bridging the bay region, as disclosed by the previous X-ray study.\(^8\) As expected, the 7,11-dimethyl-17-ketone is found to be a potent bacterial mutagen, whilst the 1-methyl-17-ketone is essentially inactive; experiments to evaluate their carcinogenicity are in progress. The methano-ketone (2) proves to be both mutagenic to Salmonella typhimurium TA 100 (after biological activation) and a moderately strong skin tumour inducer in mice. An investigation of its metabolism is in hand to attempt to account for this.

**Experimental**

Unless otherwise stated, m.p.s were determined for samples in open capillary tubes and are uncorrected, as are the b.p.s. I.r. spectra were recorded using either Nujol mulls or liquid films. N.m.r. data are for deuteriochloroform solutions with tetramethylsilane as internal reference and were recorded at 60 MHz. U.v. spectra were recorded for solutions in 98% ethanol. ‘Drying’ refers to the use of magnesium sulphate; ‘evaporation’ implies the use of reduced pressure.

1-Methyl-17-hydroxyiminooestra-1,3,5(10)-triene (3b).—1-Methyloestra-1,3,5(10)-triene-17-one \(^1\) (2 g), hydroxylamine hydrochloride (1.04 g), sodium hydrogen carbonate (1.25 g), water (2.5 ml), and methanol (45 ml) were heated together under reflux for 1.5 h, and the mixture then cooled and poured into water (100 ml). The precipitate was collected by filtration, washed with water, and crystallised from ethanol to yield the oxime (3b) as needles (2.1 g), m.p. 96-98 °C, \[\text{[a]}_D \] +178°; \(\lambda_{\text{max}}\) 265 nm; \(\nu_{\text{max}}\) 3 260, 945, 930, 767, and 740 cm\(^{-1}\); \(\delta_{\text{H}}\) 1.02 (3 H, s, 18-Me), 2.36 (3 H, s, ArMe), 2.80 (2 H, m, ArCH\(_2\)), 7.00 (3 H, s, ArH), and 8.60 (1 H, broad s, NOH) (Found: C, 80.0; H, 8.7; N, 4.8%; M, 283.1936. C\(_{20}\)H\(_{25}\)NO requires C, 80.5; H, 8.9; N, 4.9%; M, 283.1938).
dicyclohexylcasbo-di-imide (3.92 g) and trifluoroacetic acid (0.32 ml). After 3 h at room temperature the reaction mixture was added to water, the benzene layer was separated, and the aqueous layer was extracted with more benzene. The combined organic fractions were washed with saturated brine, dried, and evaporated to yield a honey-coloured oil (5 g). Chromatography on silica with benzene elution gave the seco-nitrile (4) as an oil (1.2 g) which did not crystallise, λ<sub>max</sub> 265 nm; ν<sub>max</sub> 2 <sup>2</sup> 46 (C=N), 1 580, 895, 772, and 745 cm<sup>-1</sup>; δ 2.37 (3 H, s, ArMe), 4.72 (1 H, s, C=CH<sub>2</sub>), 4.96 (1 H, s, C=CH<sub>2</sub>) and 7.00 (3 H, s, ArH) (Found: N, 5.3%; M<sup>+</sup> 265.1830).

13,18-L-Epoxy-1-methyl-1,3,17-seco-oestr-1,3,5(10)-trien-17-carbonitrile (5).—An ice-cold solution of m-chloroperbenzoic acid (1.45 mmol) in chloroform (3 ml) was added to a vigorously stirred solution of the nitrile (4) (1.32 mmol) in chloroform (9 ml) at 0 °C. The mixture was stirred at this temperature for 15 h, after which it was added to ice-cold water. The chloroform layer was washed with cold aqueous 2M-sodium hydroxide and with water, dried, and evaporated to give a gum which was chromatographed on silica gel. Elution with toluene yielded the 1-methyl-17-ketone (6) (95 mg) which failed to crystallise, λ<sub>max</sub> 3400 v,br and 1735 cm<sup>-1</sup>; ν<sub>max</sub> 2246 (C=N), 1 645, 1 220, 840, 772, and 745 cm<sup>-1</sup>; δ 2.37 (3 H, s, ArMe), 4.72 (1 H, s, C=CH<sub>2</sub>), 4.96 (1 H, s, C=CH<sub>2</sub>) and 7.00 (3 H, s, ArH) (Found: C, 80.6; H, 8.5; N, 4.65%; M<sup>+</sup> 281.1774. C<sub>19</sub>H<sub>23</sub>N requires C, 80.6; H, 8.5; N, 4.65%; M<sup>+</sup> 281.1780).

1-Methyl-18-nor-oestr-1,3,5(10)-trien-17-one (6).—The epoxy-nitrile (5) (300 mg) and boron trifluoride-ether (0.75 ml) were heated under reflux in toluene (145 ml) for 54 h. The chloroform to give the seco-nitrile (4) (15 g) in dichloromethane (50 ml) was added dropwise. After being stirred at room temperature for 2 h the reaction mixture was poured onto an excess of ice and hydrochloric acid. Separation of the organic layer was followed by extraction of the aqueous layer with dichloromethane, and the combined extracts were washed with water, dried, evaporated, and distilled to yield methyl 8-methyl-4-oxo-1,2,3,4-tetrahydro-1-naphthylacetate (10) (15 g) as a mobile oil, b.p. 175 °C/1 mmHg, ν<sub>max</sub> 1 730 (ester) and 1 685 cm<sup>-1</sup> (unsaturated ketone); δ 2.32 (3 H, s, ArMe), 1.8—2.8 (7 H, m, aliphatic H), 3.68 (3 H, s, CO<sub>2</sub>Me), 6.75—7.30 (2 H, m, 6-H and 7-H), and 7.75 (1 H, dd, J<sub>meta</sub> 7 and J<sub>meta</sub> 2 Hz, 5-H) (Found: C, 72.2; H, 7.2. C<sub>14</sub>H<sub>18</sub>O<sub>4</sub> requires C, 72.4; H, 6.9%).

8-Methyl-1-naphthylacetic Acid (11a).—The tetralone (10) (20 g) was stirred with sodium borohydride (5 g) in ethanol (100 ml) overnight at room temperature. After dilution with water the organic material was extracted with ethyl acetate and the extract was washed with water, dried, and evaporated to yield the hydroxyster (20 g), ν<sub>max</sub> 3400 v,br, and 1 735 cm<sup>-1</sup>. A solution of this hydroxy-ester in benzene (50 ml) containing a small quantity of tolulene-p-sulphonic acid was heated under reflux for 0.5 h; the cooled solution was then washed with water, dried, and evaporated to leave an oil. Sulphur (3.0 g) was added and the resultant mixture was heated at 230—235 °C for 0.5 h. When cold, the reaction mixture was dissolved in ethanol (20 ml) and heated under reflux for 1 h with sodium hydroxide (10 g) and water (30 ml). After dilution with more water (200 ml), the solution was extracted once with ether and the clear aqueous layer acidified with concentrated hydrochloric acid and extracted several times with ethyl acetate. The combined extracts were dried and evaporated to give the naphthylacetic acid (11a) as a slightly greenish solid (12.1 g), m.p. 173—174 °C (ligroin), ν<sub>max</sub> 1 710 cm<sup>-1</sup>; δ 2.95 (3 H, s, ArMe), 4.38 (2 H, s, CH<sub>2</sub>), and 7.35—8.20 (6 H, m, ArH) (Found: C, 78.5; H, 6.1. C<sub>14</sub>H<sub>16</sub>O<sub>2</sub> requires C, 78.0; H, 6.0%).

8-Methyl-1-naphthylbutyric Acid (12a).—A solution of the acid (11a) (15 g) in dichloromethane (50 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (2 g) in ether (200 ml); when the exothermic addition was complete...
plete the reaction mixture was heated under reflux for 1 h and then cooled in ice. Water was added, initially dropwise, followed by concentrated hydrochloric acid; the organic fraction was then separated and the aqueous layer extracted with ether. The combined organic solutions were washed with water, dried, evaporated, and the residue distilled at 130—132 °C/0.9 mmHg to yield 1-(8-methylphenyl)ethanol (12 g) as an oil which slowly crystallised, m.p. 57—58 °C; \( \nu_{\text{max}} \) 3, 200 br cm\(^{-1} \); \( \delta \) 2.83 (3 H, s, Me), 3.2—3.9 (4 H, m, (CH\(_2\))\(_2\)), and 6.9—7.8 (6 H, m, ArH) (Found: C, 83.3; H, 7.8. C\(_{16}\)H\(_{18}\)O requires C, 83.8; H, 7.6%).

The naphthylethanol (10 g) in acetonitrile (50 ml) was added to a suspension of triphenylphosphine dibromide (17 ml) in water, dried, evaporated, and the residue distilled at 130—132 °C/0.9 mmHg to yield 1-(8-methyl)naphthyl)ethanol (12 g) in the same solvent (100 ml), and the mixture was maintained at 60 °C for 2 h. After evaporation of the solvent, the solid residue was triturated with benzene and the solution filtered through a short column of silica. Removal of the benzene left the bromide as a mobile oil which, without further purification, was treated with diethyl sodiomalonate [from diethyl malonate (17 ml) and sodium hydride (50%) (3.8 g) in dry dimethylformamide (100 ml)]; the reaction mixture was stirred at 60 °C for 5 h. After evaporation, the residue was dissolved in ethanol (50 ml) and heated under reflux with sodium hydroxide (100 ml) for 5 h. After evaporation, the residue was dissolved in the same solvent (100 ml), and the mixture was maintained at 60 °C for 2 h. After evaporation of the solvent, the solid residue was triturated with benzene and the solution filtered through a short column of silica. The pyridine was removed by evaporation to leave an oil, \( \nu_{\text{max}} \) 1, 675 cm\(^{-1} \), \( \delta \) 2.50 (2 H, m, CH\(_2\) Ar), 7.48—7.95 (4 H, m, ArH), and 8.37 (1 H, d, \( J \) 6 Hz, 15-CH\(_2\)) (Found: C, 79.2; H, 7.8. C\(_{16}\)H\(_{18}\)O requires C, 79.7; H, 7.6%).

5-Methyl-1,2,3,4-tetrahydrophenanthren-1-one (13a).—Cyclisation of the acid (12a) (7 g) was effected by the method used to prepare the tetralone (10), but employing phosphorus-chloride (9.5 g) and tin(iv) chloride (7.0 ml). The mixture was then heated under reflux for 6 h. After dilution of the cooled reaction mixture with water (200 ml) the products were extracted with ether, and the extracts were dried and evaporated to leave an oil which was chromatographed on a column of silica gel, with chloroform-light petroleum as eluant. 1-Methyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (15a) was isolated as a buff solid (0.8 g, m.p. 138—140 °C (ethanol), \( \lambda_{\text{max}} \) 276 (4.55), 286 (4.62), 330 (4.09), 343 (4.11), and 376 (3.87) nm; \( \nu_{\text{max}} \) 1, 685 (17-\( CO \)) and 1, 650 cm\(^{-1} \) [13(14) double bond] (Found: C, 87.6; H, 6.3. C\(_{16}\)H\(_{16}\)O requires C, 87.1; H, 6.5%).

4-(3-Methylphenyl)pentanoic Acid (12b).—This acid was prepared by methods similar to those used to convert (11a) into (12a). The intermediate 2-(3-methyl-1-naphthyl)propionic acid (11b) \(^{14} \) was reduced with lithium aluminium hydride to 4-(3-methylphenyl)propionaldehyde in 85% yield, b.p. 155—157 °C/0.9 mmHg, \( \nu_{\text{max}} \) 3, 560, 3, 480, and 1, 608 cm\(^{-1} \); \( \delta \) 1.34 (3 H, d, CHMe), 2.44 (ArMe), 3.33—3.92 (3 H, m, CH-), and 7.05—8.27 (6 H, m, ArH) (Found: C, 83.5; H, 8.2. C\(_{16}\)H\(_{18}\)O requires C, 84.0; H, 8.1%). This alcohol was readily converted into 4-(3-methylphenyl)pentyl bromide (73%), b.p. 166 °C/2.0 mmHg, \( \nu_{\text{max}} \) 1, 680 and 1, 608 cm\(^{-1} \); \( \delta \) 1.55 (2 H, d, J 6 Hz, CHMe), 2.46 (3 H, s, ArMe), 3.2—4.25 (3 H, m, CHClH), and 7.00—8.23 (6 H, m, ArH) (Found: C, 63.9; H, 6.0. C\(_{16}\)H\(_{18}\)Br requires C, 63.9; H, 5.8%). Conversion of the latter into the pentanoic acid (12b) (70%) was best conducted in absolute ethanol; the product was not obtained crystalline; \( \nu_{\text{max}} \) 1, 705, 1, 660, and 1, 608 cm\(^{-1} \); \( \delta \) 1.40 (3 H, d, J 6 Hz, CHMe), 1.90—2.33 [4 H, m, (CH\(_2\))] 2.98 (3 H, s, ArMe), 3.60 (1 H, m, CH), and 7.10—8.20 (6 H, m, ArH) (Found: C, 79.2; H, 7.8. C\(_{16}\)H\(_{16}\)O requires C, 79.3; H, 7.5%).

4,10-Dimethyl-1,2,3,4-tetrahydrophenanthren-1-one (13b).—Cyclisation with tin(v) chloride as for (13a) gave the phenanthrene (13b) (65%), m.p. 65 °C (light petroleum), \( \nu_{\text{max}} \) 1, 670 cm\(^{-1} \); \( \delta \) 1.45 (3 H, d, J 7.5 Hz, CHMe), 1.95—3.00 [4 H, m, (CH\(_2\))] 3.92 (1 H, m, CH), and 7.23—8.17 (5 H, m, ArH) (Found: C, 85.3; H, 7.2. C\(_{16}\)H\(_{18}\)O requires C, 85.7; H, 7.2%).

7,11-Dimethyl-11,12,15,16-tetrahydrocyclopenta[a]phenanthren-17-one (15a).—The Stobbe reaction with the ketone (13b), carried out as for (13a), gave the half-ester (14b) (20%), as an oil, \( \nu_{\text{max}} \) 1, 735 and 1, 710 cm\(^{-1} \). Without further purification (1.5 g) was cyclised with zinc chloride as described for (15a) to yield the ketone (15b) (15%), m.p. 145—146 °C (ethanol), \( \lambda_{\text{max}} \) 265sh (4.47), 274 (4.75), 285 (4.84), 335br (4.39) nm; \( \nu_{\text{max}} \) 1, 685, 1, 632, and 1, 614 cm\(^{-1} \); \( \delta \) 1.06 (3 H, d, J 7 Hz, CHMe), 2.68 (3 H, s, ArMe), and 7.15—8.17 (5 H, m, ArH) (Found: C, 87.2; H, 7.2. C\(_{16}\)H\(_{18}\)O requires C, 87.0; H, 6.9%).
15,16-Dihydro-7,11-dimethylcyclopenta[a]phenanthren-17-one (1d).—Dehydrogenation of (1b) was performed as in the preparation of the 1-methyl compound to yield 15,16-dihydro-7,11-dimethylcyclopenta[a]phenanthren-17-one (1d) (85%), m.p. 208—210 °C (ethanol); \( \lambda_{max} \), 275 (4.76), 293 (4.48), 307 (4.29), 368 (3.46), and 389 nm (3.51); \( \nu_{max} \), 1 678 cm\(^{-1}\); \( \delta \), 2.65 (2 H, t, J 5 Hz, 15-CH\(_2\)), 2.82 (3 H, s, ArMe), 2.98 (3 H, s, ArMe), 3.63 (2 H, t, J 5 Hz, 15-CH\(_2\)), and 7.30—8.85 (6 H, m, ArH) (Found: C, 88.7; H, 5.1. \( \text{C}_{18}\text{H}_{12}\text{O} \) requires C, 88.5; H, 5.7%).

Dehydrogenation of this tetrahydro-compound (0.76 g) with Pd/c in p-cymene under reflux for 0.5 h, and recrystallisation of the product from methanol led to 15,16-dihydro-1,11-methanocyclopenta[a]phenanthren-17-one (2) as a pale yellow solid (0.64 g), m.p. 195 °C; \( \lambda_{max} \), 266.5 (4.77), 277 (4.76), 303 (3.34), 348 (3.03), and 365.5 nm (2.86); \( \nu_{max} \), 1 685 (CO), 1 634, 1 400, 1 292, 820, and 805 cm\(^{-1}\); \( \delta \), 2.8 (2 H, m, CH\(_2\)C=O), 3.25 (2 H, t, CH\(_2\)), 3.63 (2 H, t, CH\(_2\)), and 7.35 (6 H, m, ArH) (Found: C, 88.7; H, 5.1. \( \text{C}_{18}\text{H}_{14}\text{O} \) requires C, 88.5; H, 4.95%).

References