DEVELOPMENT OF REGIOSPECIFIC AROMATIC IODINATION METHODOLOGIES

A Thesis

presented to the University of Surrey
for the Degree of Doctor of Philosophy
in the Faculty of Science

by

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ABSTRACT

Various aromatic iodination methodologies have been examined, with regard to the incorporation of radioisotopes of iodine into pharmaceutical molecules.

The thesis is divided into six chapters:

Chapter One presents a literature survey of applications of iodine isotopes in nuclear medicine, and an overview of the methodologies which have been used to incorporate iodine isotopes into aromatic systems.

Subsequent chapters describe work carried out in specific areas. Iodination via thalliation and mercuriation is dealt with in Chapter Two, while Chapter Three is concerned with some oxidative iodination reactions, principally those due to Radner and Sugiyama. The oxidation of iodine by electrochemical means is the subject of Chapter Four, which describes experiments conducted with the reactive positive iodine species so produced. This work is extended in Chapter Five by using electrochemically generated positive iodine in the demetallation of silylated arenes.

In the final chapter, Chapter Six, the utility of the electrochemical method is further examined by applying it to some substrates of biological significance. General conclusions are then made, considering all the various iodination techniques.
ACKNOWLEDGEMENTS

Firstly, I would like to thank my three supervisors at the University of Surrey; Professor J.R. Jones for initiating and coordinating the project, and for his constant support; Dr. R. Bolton, for his encouragement and enthusiasm, and for his thorough proof-reading, and Dr. I.D. Cunningham, for co-supervising the work described in Chapters Two and Three.

Thanks must also go to SERC and Fisons Pharmaceutical Division for financial support. Dr. W.J.S. Lockley of Fisons acted as a co-supervisor and directed the work described in Chapter Six.

It is with great pleasure that I acknowledge the help of Professor J.H.P. Utley of Queen Mary and Westfield College, University of London. I am indebted to him for advice on electrochemical matters and for the loan of an electrochemical cell.

Assistance with practical matters has been supplied by a number of individuals. In particular, I would like to thank Mr. W. Brench and colleagues at the university glassblowing unit for the construction of platinum electrodes, Mr. J.P. Bloxsidge for assistance with NMR kinetic experiments, Dr. A.S. Culf for helping with the one gas-line tritiation performed, and Ms. J.M. Paul for advice and the loan of equipment for organolithium work.

Numerous others have played diverse roles in making the last three years more enjoyable. Special thanks go to Mr. S.G. Watts and Mrs. L. Matley for their hospitality during my time at Fisons, Adrian Culf and Terry Smith for pints and philosophy, and the co-residents of 34, Lynwood, Guildford during 1991-93.
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To my mother, and to the memory of my father.
Abbreviations used in this thesis

Chemicals:

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<th>Name</th>
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<tbody>
<tr>
<td>CAN</td>
<td>Cerium (IV) Ammonium Nitrate</td>
</tr>
<tr>
<td>CAT</td>
<td>Chloramine-T</td>
</tr>
<tr>
<td>DCT</td>
<td>Dichloramine-T</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
</tr>
<tr>
<td>IMP</td>
<td>N-Isopropyl-2-(4-iodophenyl)isopropylamine</td>
</tr>
<tr>
<td>mUBG</td>
<td>meta-Iodobenzylguanidine</td>
</tr>
<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>NCS</td>
<td>N-Chlorosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-Iodosuccinimide</td>
</tr>
<tr>
<td>TBHC</td>
<td>tertiarry-Butyl Hypochlorite</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>TFAA</td>
<td>Trifluoroacetic anhydride</td>
</tr>
<tr>
<td>TMF</td>
<td>Trimethyl Orthoformate</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethysilane</td>
</tr>
<tr>
<td>TTFA</td>
<td>Thallium (III) trifluoroacetate</td>
</tr>
</tbody>
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Miscellaneous

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>EII</td>
<td>Electron Ionization</td>
</tr>
<tr>
<td>ESR</td>
<td>Electron Spin Resonance</td>
</tr>
<tr>
<td>FAB</td>
<td>Fast Atom Bombardment</td>
</tr>
<tr>
<td>GC</td>
<td>Gas Chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IRMA</td>
<td>Immunoradiometric assay</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>n.c.a.</td>
<td>No-carrier-added</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>RIA</td>
<td>Radiimmunoassay</td>
</tr>
<tr>
<td>SCE</td>
<td>Saturated Calomel Electrode</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single-Photon-Emission Computed Tomography</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-Layer Chromatography</td>
</tr>
</tbody>
</table>

NMR spectra are summarised using standard notation as follows:

s - singlet  
d - doublet  
t - triplet  
m - multiplet  
dd - double doublet  
b - broad
Structures of Commonly Used *In Situ* Oxidants

(i) Chloramine-T (CAT)

(ii) Iodogen

(iii) Iodobeads

(iv) N-Halosuccinimides
CHAPTER ONE

A Survey Of Aromatic Iodination And Radioiodination
The initial purpose of this work was to develop methods for the regiospecific incorporation of halogen isotopes into pharmaceutical molecules. At an early stage it was decided to concentrate on iodine, more specifically, on aromatic iodination. A survey of recent literature suggested that the isotopes of iodine were amongst the most widely used in nuclear medicine. The lack of efficient and regiospecific methods for the preparation of iodoaromatic compounds, together with the synthetic utility of non-radioactive iodo compounds, were further reasons for focusing the study on iodination. The iodination of aromatic compounds was studied in preference to that of aliphatic compounds, as the aryl-iodine bond is generally less susceptible to cleavage in vivo.

Many isotopes of the halogens find application in nuclear medicine. Fluorine-18 is used extensively in the imaging technique known as Positron Emission Tomography (PET). This involves the use of F-labelled agents which bind at specific sites in the body. The radiation emitted by F is sufficiently energetic to be detected externally, and, by using a suitable array of cameras, images can be built up from the localisation of the isotope. Fluorine-18 has a relatively short half-life of 109.8 mins., thus the labelling of imaging agents presents a considerable synthetic challenge. Improved methodologies are constantly being sought as the isotope becomes increasingly popular. Fluorine-18 is a popular label by virtue of its small size (almost isosteric with hydrogen) and because of the strength and *in vivo* stability of the carbon-fluorine bond.
Potential uses of chlorine isotopes in nuclear medicine remain largely unexplored. Several isotopes have sufficiently long half-lives to be used as tracers, e.g. $^{36}\text{Cl} - 37.2\text{ mins.}$, $^{37}\text{Cl} - 57\text{ mins.}$ It is however metastable chlorine-34 which is perhaps the most interesting. It is a positron emitter with a half-life of 32 mins. which could possibly find application in PET studies. The principal reason for the paucity of data on $^{34m}\text{Cl}$ is that, until recently, no method for its production afforded suitable quantities for use in research or clinical evaluation.

The isotopes of bromine cover a wider range of nuclear properties than those of chlorine and have been used much more extensively. Bromine-75 is a positron emitter with a half-life of 1.6 hours which has been used as a tracer. Bromine-77 is a $\gamma$-emitter (239, 521 keV) with a half-life of 56 hours which has been employed for some in vivo applications. Nuclear reactions leading to $^{77}\text{Br}$, and the preparation and applications of $^{77}\text{Br}$-labelled radiopharmaceuticals have been reviewed by Stocklin.

It is however the isotopes of iodine which have found the most widespread use in the field of nuclear medicine. Their applications are discussed in detail in Section 1.2.

For radiolabelling purposes, the isotopes of iodine have both advantages and disadvantages over carbon-14 or tritium. The relatively short half-lives allow the possibility of attaining much higher specific activities (Table 1.1). A result of this is that very small amounts of radiiodinated materials give very high count rates and can be used in very sensitive assays.
Table 1.1: Properties of Commonly Used Radioisotopes (from ref. 5)

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H</td>
<td>12.2 y</td>
<td>29 Ci mmol$^{-1}$</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>5600 y</td>
<td>62 mCi mmol$^{-1}$</td>
</tr>
<tr>
<td>$^{125}$I</td>
<td>60 d</td>
<td>2000 Ci mmol$^{-1}$</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>8 d</td>
<td>2600 Ci mmol$^{-1}$</td>
</tr>
</tbody>
</table>

Another advantage over carbon-14 and tritium is ease of detection. Iodine isotopes are $\gamma$-emitters and thus can be counted directly; this avoids the need for preparing scintillant solutions as is the practice with $\beta$-emitters.

The principal disadvantage of labelling with iodine is that it is usually a foreign label. Replacement of a proton by a bulky iodine atom may have a significant effect on the properties of the substrate. Reaction rates may be affected, or, in the case of peptides, a conformational change may result.

Another disadvantage is a consequence of the shorter half-lives. Radiation decomposition occurs more rapidly, iodine is lost and purity of radiolabelled substrates cannot be guaranteed.

The fifth halogen, astatine, is produced artificially in cyclotron reactions. The longest lived isotope, $^{210}$At, has a half life of just 8.3 hours. It is however $^{211}$At which has received the most attention. Numerous papers have been published exploring the potential use of this isotope in cancer therapy.$^{6-10}$ It is an $\alpha$-emitting isotope with a half-life of 7.21 hours. The decay occurs via two pathways; by direct $\alpha$-particle emission to give $^{207}$Bi (42%), and by electron capture to form the very short-lived $^{211}$Po, which decays by $\alpha$-particle emission to $^{207}$Pb (58%).
energies of these two α-emissions are 5.87 MeV and 7.45 MeV respectively, corresponding to ranges of 55μm and 80μm in unit density tissue. This is ideal for destroying cells, thus 211At-labelled agents with a high affinity for tumours are potentially very useful for radiotherapeutic purposes.

To some extent, the chemical properties of astatine and its compounds resemble those of iodine. Thus, astatides are readily oxidised to molecular astatine, and oxidation of the molecule to a positive species proceeds more readily than is the case with iodine. In this respect, it is reasonable to assume that the oxidative iodination methodologies described in this thesis would be broadly applicable to astatination. In fact, there have been reports of aromatic astatination by direct electrophilic attack, via diazonium salt intermediates and via demetallation reactions of stannylated, mercuriated and thalliated derivatives.

A further reason for focusing this work on iodination is that there is considerable interest in the preparation of simple iodoaromatic compounds. Quite apart from the interest in radiolabelled compounds, the development of effective methods for iodinating arenes is particularly worthwhile owing to their great versatility as synthetic intermediates. Iodoaromatic compounds can be used as precursors to a wide range of functionalised derivatives, as detailed in the reviews of Merkushev. The most important reactions are perhaps those due to Ullmann and Heck.

The Ullmann reaction involves copper promoted coupling of aryl halides (equation 1.1). It is particularly effective when
iodoarenes are used owing to the good leaving group properties of iodide. The mechanism is thought to involve an arylcopper intermediate.

\[ \text{Cu} \quad \text{2 ArI} \xrightarrow{\Delta} \text{Ar-Ar} \quad 1.1 \]

Fanta has written several reviews on the reaction.\(^{21,22}\) It has been used to prepare many biaryls, both symmetrical and unsymmetrical. The preparation of an unsymmetrical biaryl requires the use of two different aryl iodides, and hence there exists the possibility of forming three different products. However, in such cases it is often found that only one product is formed.

Biaryl coupling is not only brought about by copper. Certain nickel complexes have been shown to be effective, as have activated nickel, zinc-nickel complexes, sodium formate / Pd-C, and several other reagents.\(^{24}\) Most of these reactions appear to be unique to aryl iodides. Arylation reactions may also be brought about by photochemical means since the aryl-iodine bond is readily cleaved in a homolytic fashion by U.V. light.

The Heck reaction\(^{25}\) involves the arylation of alkenes by treatment with an arylpalladium reagent (equation 1.2).

\[ \text{R}_2\text{C}≡\text{CH}_2 + \text{ArPdX} \rightarrow \text{R}_2\text{C}≡\text{CH-Ar} \quad 1.2 \]

The arylpalladium intermediate can be prepared in several ways, one of which is the treatment of the corresponding aryl iodide with Pd(OAc)\(_2\) and base.
Other uses of aromatic iodo compounds include their conversion to alkylated aromatics via coupling with lithium dialkyl-copper reagents (alkyldehalogenation). \(^{26}\)

\[
\text{Arl} + R_2\text{CuLi} \rightarrow \text{ArR} \quad \text{(1.3)}
\]

Similarly, alkyne-substituted arenes may be formed from the reaction of aryl iodides with alkynyl copper derivatives. \(^{27}\)

\[
\text{Arl} + \text{RC}≡\text{CCu} \rightarrow \text{ArC}≡\text{CR} \quad \text{(1.4)}
\]

Recently, Hiura and coworkers\(^{29}\) have described the coupling of aryl iodides with terminal alkynes, using a copper (I) iodide-triphenylphosphine catalyst in the presence of potassium carbonate.

Further details on these and many other reactions of iodoarenes can be found in Merkushhev's review\(^{19}\) and March's textbook. \(^{24}\)

An additional use of regiospecifically iodinated derivatives, and other halogenated compounds, is as precursors for site-specific deuteration or tritiation. Dehalogenation is readily brought about by deuterium or tritium gas, in the presence of a base and a suitable catalyst. Much of the work described in Chapter Six was carried out with this goal in mind.
1.2 Isotopes of Iodine

Naturally occurring iodine is composed exclusively of the isotope iodine-127. However, a series of radionuclides has been prepared ranging from mass 117 to mass 139. Three of these, namely iodine-123, iodine-125 and iodine-131 have found extensive use in nuclear medicine. More recently, a fourth isotope, iodine-122, has found application in PET/SPECT work. Other isotopes have been used, albeit much less widely. These include those with mass 121, 124, 128, 129 and 132. The properties of the important isotopes are listed in Table 1.2.

(*- - SPECT = Single Photon Emission Computed Tomography)

Table 1.2: Properties of Important Iodine Isotopes

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Half-life</th>
<th>Decay Mode</th>
<th>Principal $\gamma$-emissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{122}$I</td>
<td>3.63 min</td>
<td>$\beta^+$, EC</td>
<td>511 KeV</td>
</tr>
<tr>
<td>$^{123}$I</td>
<td>13.3 h</td>
<td>EC</td>
<td>159 KeV (86%)</td>
</tr>
<tr>
<td>$^{128}$I</td>
<td>60.1 d</td>
<td>EC</td>
<td>35.5 KeV (100%)</td>
</tr>
<tr>
<td>$^{131}$I</td>
<td>8.07 d</td>
<td>$\beta^-$</td>
<td>364.5 KeV (79%)</td>
</tr>
</tbody>
</table>

1.2.1 Iodine-125

Iodine-125 was one of the earliest used isotopes in the life sciences. It is a $\gamma$-emitter with a relatively long half-life of 60.1 days. Its $\gamma$-emissions are described as "soft" and are relatively easy to protect personnel from, while the long half-life is convenient for the storage and transportation of labelled preparations.
The major use of iodine-125 lies in the field of radio-immunoassay (RIA). This technique was developed over 30 years ago by Yalow and Berson for the accurate estimation of specific substances (antigens) at picomolar \((10^{-12} \text{M})\) or even femtomolar \((10^{-15} \text{M})\) concentrations in biological fluids. The requirements for RIA are an antiserum (antibodies raised against the antigen by injecting animals), and labelled and unlabelled forms of the antigen. The technique depends on competition between the labelled antigen and the unlabelled antigen for a fixed, limited amount of antibody. Thus, antiserum of known dilution is incubated with a known excess of labelled antigen so that approximately half of the antigen is bound. A known amount of unlabelled antigen is then added, which, by competing for the limited number of binding sites, displaces a certain amount of the labelled antigen. The antibody-bound and free antigens are then separated and either one, or both, of the fractions are counted (i.e. assayed for radioactivity). This procedure is repeated at several concentrations of unlabelled antigen, and a calibration curve is set up. By reference to this curve, the concentration of antigen in unknown samples can be estimated. The labelled and unlabelled antigens do not need to be identical; the requirement is that they are similarly recognised by the antibody binding site. Because of the great sensitivity required, labelled antigens need to be prepared at very high specific activities. Iodine-125 has been the isotope of choice for this purpose as it is available at specific activities as high as 2000 Ci/mmol.
RIA is not restricted to immunologically active proteins. Antibodies can be raised against smaller molecules such as steroids, by coupling these to immunologically active proteins. To do this, bridging units are required. The practicalities and pitfalls of such procedures are described in the review articles by Seevers and Counsell\(^\text{1}\) and by Bolton\(^\text{3}\). Complications can arise due to recognition of the bridging units, etc.

Iodine-125 is also employed in the related, but distinct technique of immunoradiometric assay (IRMA). In this case, it is the antibody, not the antigen which is radiolabelled. Concentrations of antigens are estimated by incubating the biological fluid under investigation with an excess of antibody, then separating antigen-bound from unreacted antibody, and counting. In the preparation of labelled antibodies, radiiodination is carried out in the presence of the antigen in order to protect the active site. An early example of IRMA is the work of Miles and Hales\(^\text{32}\) with insulin.

The \(\gamma\)-emissions of iodine-125 are too weak to be detected outside the body. Thus, iodine-125 does not have any applications in imaging. However, it is often used in preliminary studies with imaging agents as a tracer to monitor tissue distribution. The longer half-life is advantageous for such applications. Such studies are by no means restricted to imaging agents; the isotope is used on a broad basis in autoradiography and in \textit{in vivo} tissue disposition experiments.
1.2.2 Iodine-131

Iodine-131 is a β-emitter with a half-life of 8.1 days. It has found application in radiotherapy where the β-radiation is used to destroy malignant cells. The earliest work in this area made use of the affinity of the thyroid gland for iodide ions. Thus, $^{131}$I-iodide ions were administered for the treatment of thyroid cancer.\(^3\)

As has already been mentioned in the context of therapy with $^{211}$At (p.4), research in this field is aimed at producing compounds which can deliver the radioisotope to malignant tumours, with maximum tumour to normal tissue concentration ratios. Other considerations are low toxicity and minimal in vivo cleavage of iodine, to avoid unwanted thyroid accumulation.

A representative substrate is tamoxifen (5), a non-steroidal antiestrogen employed in the treatment of breast tumours.\(^1\)\(^2\)\(^3\)\(^4\) Its affinity for estrogen receptors also makes the radioiodinated material a potential imaging agent for the detection of tumours.

\[\text{NMe}_2\]

(5)
Tamoxifen has been labelled at various aromatic positions; destannylation procedures (see Chapter Five) generally result in labelling ortho to the aminoethoxy substituent, while Hunter and Strickland\textsuperscript{34} inserted \textsuperscript{131}I at one of the other phenyl rings via a diazonium salt intermediate.

Perhaps the most widely studied of all \textsuperscript{131}-labelled radio-pharmaceuticals is \textit{meta}-iodobenzylguanidine (\textit{mIBG}) (6). This compound, an adrenergic neuron-blocking agent, was introduced by Wieland et al.\textsuperscript{35} in 1980. It has since been shown to localise in many types of malignant tumours.\textsuperscript{36,37} The location of tumours is also commonly performed using \textsuperscript{131}I-labelled monoclonal antibodies.

1.2.3 Iodine-123

The short half-life and 159 KeV gamma emissions of iodine-123 make it the isotope of choice for many in vivo applications. The intensity of its emissions, and the absence of any $\beta$-particle emissions, are ideally suited for diagnostic $\gamma$-imaging. A short half-life reduces the radiation exposure to patients treated with a radionuclide.

The advantages of iodine-123 were pointed out as early as 1962, by Myers and Anger.\textsuperscript{38} In the development of nuclear medicine, iodine-123 has often been described as expensive and not widely available.\textsuperscript{39,40} However, it has increasingly replaced iodine-131, which has more hazardous, stronger radiation.
Some of the earliest uses of $^{123}$I-labelled compounds were the evaluation of kidney function (renography) with $^{123}$I-hippuric acid$^{41}$, liver function tests with iodinated Rose Bengal dye$^{42}$ and myocardial imaging with long-chain fatty acids containing $^{123}$I-labelled aromatic moieties.$^{43-47}$ Work carried out up to 1976 has been reviewed by Stöcklin.$^2$

One of the major uses of $^{123}$I-labelled compounds during the 1980's has been in brain imaging.$^{48}$ Perhaps the most widely used of all $^{123}$I-radiopharmaceuticals are amphetamine analogues, principally N-isopropyl-2-(4-iodophenyl)isopropylamine (IMP) (7). This compound, developed by Winchell and coworkers$^{49,50}$ is now used routinely in monitoring brain blood flow by SPECT.

![Chemical structures](image)

Amphetamine derivatives of this kind are highly lipophilic and rapidly cross the blood-brain barrier. Once inside the brain, they are bound at specific sites and are retained long enough for SPECT image acquisition.$^{31}$
Clinical studies of regional cerebral perfusion in patients suffering from stroke, epilepsy and dementia have been reported using $^{123}$I-IMP. A recent publication has described significant differences in the cerebral perfusion of $^{123}$I-IMP in the brains of crack abusers.

Another amine which is widely used in brain imaging is $N,N',N'$-trimethyl-$N$-[2-hydroxy-3-methyl-5-$^{123}$I-iodobenzyl]-1,3-propane diamine (HIPDM) (8), first described by Kung et al. in 1983.

Iodine-123 must be of high isotopic purity for imaging applications. Several of the most straightforward nuclear reactions leading to iodine-123 also produce intolerable amounts of iodine-124. This has high-energy emissions (603 KeV) and a half-life of 4.3 days. Contamination of iodine-123 with more than $\leq 1\%$ of iodine-124 is unacceptable for SPECT studies since the high radiation background degrades the image. Its presence also results in an undesirable additional radiation dose for the patient.
1.2.4 Iodine-122

The positron emitting isotope, iodine-122, has in recent years found application in PET/SPECT work. The development of a generator system for $^{122}$I from $^{122}$Xe was described in the mid-1980's by Mathis and coworkers.\textsuperscript{53,59}

The most significant feature of the isotope is its very short half-life (3.5 min). This makes its incorporation into suitable molecules extremely challenging. The labelling chemistry, plus any subsequent purification step that may be necessary, cannot require more than a few minutes. The rapid decay also puts constraints on the imaging procedures. Koerlein et al.\textsuperscript{60} have reported on a study of $^{122}$I-labelling of various simple aromatic substrates using demetallation techniques. Some success was achieved by using tin and mercury compounds as precursors.

There are of course advantages of a short half-life. Patients receive a low absorbed radiation dose and PET procedures can be repeated in the same individual within short intervals.\textsuperscript{59}
1.3 Aromatic Iodination Methodologies

Iodine differs from the lighter halogens in that the diatomic molecule is generally unreactive towards aromatic systems as an electrophile. Only the most electron-rich arenes such as phenols and anilines may be attacked by I₂, and, even with these substrates, a base such as KHCO₃ is required in order to drive the reaction by the removal of HI. Less electron-rich substrates are completely inert towards I₂. As a result, a wide variety of reagents and experimental conditions have been developed in order to facilitate iodination reactions. The strategies can be divided into two broad categories, namely direct and indirect methods.

1.3.1 Direct Iodination

In order to achieve direct aromatic iodination, a source of positive, electrophilic iodine is required. Positive iodine can be obtained by simply oxidising iodine or iodide, and a variety of reagents capable of doing this have been described. These are summarised below in part (a). Other reagents, such as the silver (I) ion, are believed to promote iodination by polarising the diatomic iodine molecule. These can also be regarded as oxidants, and are thus also included in part (a). In many cases, reagents are likely to serve a dual purpose and are thus difficult to categorise.

Iodine is also rendered electropositive when it is bound to a more electronegative element, most commonly chlorine (as in iodine monochloride), or nitrogen (as in N-iodoamides). Compounds in this category are described in part (b).
(a) Oxidising Agents

(i) Nitric Acid / Sulphuric Acid Mixtures

The use of nitric acid as an iodine oxidant was studied extensively by Datta and Chatterjee. They described the iodination of benzene, toluene, xylene, mesitylene, thiophene and naphthalene. Reactions were carried out by heating the substrates with iodine and adding concentrated nitric acid in small portions. Yields were generally in the range of 50 – 80%. The nitric acid method was subsequently used by Dains and Brewster for the large scale preparation of iodosobenzene.

Addition of a mixture of nitric and sulphuric acids to iodine and an aromatic substrate in acetic acid is often referred to as the Tronov-Novikov method. It has been shown to be effective for the iodination of benzene, alkylbenzenes, halobenzenes and several other compounds. Deactivated compounds such as benzoic acid and nitrobenzene were meta-iodinated at elevated temperatures. The best results were obtained when a mixture of nitric acid of density 1.4 and sulphuric acid of density 1.84 was used. Competing nitration was only observed at elevated temperatures.

Oleum, or fuming sulphuric acid, has been found to be a useful solvent for the iodination of compounds deactivated towards electrophilic attack. Allen et al. used such a medium for the tetraiodination of phthalic anhydride. Much kinetic work in the medium has been conducted by Arotsky and coworkers. In 1970, they reported on a study of the iodination of aromatic nitro-compounds in 20% oleum. Experiments were carried out using 0.4 M
solutions of iodine in commercial oleum, at room temperature and at elevated temperatures. Nitrobenzene, the nitrotoluenes, the dinitrobenzenes and two dinitrotoluenes were used as substrates. All were iodinated in moderate yield except 1,4-dinitrobenzene. Throughout the work, no iodination occurred at positions ortho- to a nitro group. This effect could not satisfactorily be accounted for. The active iodinating species was thought to be $I_3^+$ at room temperature and $I_2^+$ at high temperature.

(ii) Iodic Acid ($HIO_3$) / Periodic Acid ($HIO_4$)

The use of iodic acid as an oxidant in iodination was mentioned as early as 1866 by Kekulé. However, it was not until the 1960's that its usefulness was studied in detail.

In 1966, Suzuki et al. compared a variety of oxidants for the iodination of some polyalkylbenzenes bearing bulky groups, for example, isomeric $t$-butyl-dimethylbenzenes. The highest yields, and the cleanest products, were obtained using periodic acid as the oxidant, in 17:3 (v/v) acetic acid - water.

By dissolving a 3:1 mixture of iodine and periodic acid in concentrated sulphuric acid, Mattern generated a powerful reagent capable of polyiodinating deactivated substrates. Benzene was readily tetraiodinated, and under more forcing conditions (a 2-fold excess of $I^+$; 100°C) was converted to hexaiodobenzene. Levitt and Iglesias had apparently carried out the same reaction somewhat earlier. In 1991, Mattern and Chen reported on an extensive study of the polyiodination of benzenesulfonic acid. It was suggested that sulfonic acid intermediates may have played a role in the
polyiodination of benzene in sulphuric acid, but the results suggested otherwise. Nevertheless, tetra- and pentaiodobenzenes were found amongst the products after prolonged exposure of benzene-sulfonic acid to the reagent. It was postulated that three iodine atoms could be directly substituted into the ring positions of the substrate, but that a fourth substitution was not possible on steric grounds. Instead, a fourth equivalent of the iodine electrophile brought about iododesulfonation, yielding tetraiodobenzene.

(iii) Peracetic Acid \((\text{CH}_3\text{CO}_3\text{H})\)

The iodination of simple aromatic substrates using iodine and peracetic acid has been studied in considerable detail by Ogata et al. Reactions were carried out by adding solutions of peracetic acid in acetic acid to mixtures of iodine and the aromatic substrate. Benzene and toluene were iodinated in high yield, but nitrobenzene and benzoic acid failed to react. Kinetic studies led the authors to propose a mechanism involving rate-determining formation of hypoiiodous acid, HOI:

\[
\text{CH}_3\text{CO}_3\text{H} + \text{I}_2 + \text{H}_2\text{O} \rightleftharpoons \text{CH}_3\text{CO}_2\text{H} + 2\text{HOI} \quad 1.5
\]

At this time, the actual iodinating species was thought to be acetyl hypoiodite, present because of the equilibrium:

\[
\text{CH}_3\text{CO}_2\text{H} + \text{HOI} \rightleftharpoons \text{CH}_3\text{CO}_2\text{I} + \text{H}_2\text{O} \quad 1.6
\]

Subsequent investigations showed that reaction yields for the iodination of benzene and less electron-rich substrates were increased by the addition of sulphuric acid. From this and other
observations the authors proposed protonated acetyl hypoiodite as the reactive species:

$$\text{CH}_3\text{CO}_2\text{H} + \text{HOI} + \text{H}^+ \rightleftharpoons (\text{CH}_3\text{CO}_2\text{H})^+ + \text{H}_2\text{O}$$  \hspace{1cm} 1.7

Iodic acid, HIO₃, was invariably found as a by-product, suggesting that the desired iodination reaction occurred in competition with further oxidation of hypoiodous acid:

$$\text{HOI} + \text{CH}_3\text{CO}_3\text{H} \rightleftharpoons \text{HIO}_3 + 2\text{CH}_3\text{CO}_2\text{H}$$  \hspace{1cm} 1.8

In subsequent kinetic studies with more reactive substrates an autocatalytic effect was observed. As a possible explanation, Ogata and Aoki postulated an additional reaction pathway involving aryl iodine diacetate intermediates:

$$\text{ArI} + \text{CH}_3\text{CO}_3\text{H} \longrightarrow \text{ArIO} + \text{CH}_3\text{CO}_2\text{H}$$  \hspace{1cm} 1.9

$$\text{ArIO} + 2\text{CH}_3\text{CO}_2\text{H} \rightleftharpoons \text{ArI(O}_2\text{CCH}_3)_2 + \text{H}_2\text{O}$$  \hspace{1cm} 1.10

$$\text{I}_2 + \text{ArI(O}_2\text{CCH}_3)_2 \longrightarrow 2\text{CH}_3\text{CO}_2\text{I} + \text{ArI}$$  \hspace{1cm} 1.11

In 1970, Ogata and Urasaki reported on the use of peracetic acid in the iodination of acenaphthene and fluorene. These electron-rich hydrocarbons are readily oxidised by peracetic acid, hence concurrent oxidation was expected when iodination was attempted in this way. This was found to be the case, but by adding the peracetic acid solution dropwise to a solution of the hydrocarbon and excess iodine, oxidation was minimised and iodinated products were isolated in good yields.
Further mechanistic investigations were carried out using biphenyl, diphenylmethane and bibenzyl. Steric constraints resulted in exclusive para-iodination in the case of biphenyl, but small amounts of ortho-isomers were seen with diphenylmethane and bibenzyl. Also formed with diphenylmethane and bibenzyl were α-hydroxy and α-acetoxy derivatives, indicative of competing radical pathways. Iodination was favoured when sulphuric acid was added, while the addition of water increased the amounts of α-substituted by-products. This was seen as further evidence for protonated acetyl hypojodite being the effective iodinating agent.

Moerlein and coworkers have reported on the usefulness of peracetic acid in no-carrier-added (n.c.a.) radioiodination and radiobromination. The acid, generated in situ from acetic acid and hydrogen peroxide, was examined as an oxidant for radioactive ions in both direct halodeprotonation and in halodemetalation reactions. The direct halogenation reactions were found to be slow unless aromatic substrates were activated by electron-releasing groups. The demetalation studies are described in Section 5.1 (see p. 240).

(iv) Bis(acetoxy)iodobenzene (Diacetoxyphenyliodine) (PhI(OAc)₂) / Bis(trifluoroacetoxy)iodobenzene (PhI(O₃CCF₃)₂)

As mentioned in the preceding subsection, Ogata and Aoki proposed that aryl iodine diacetates could react with iodine to generate acetyl hypojodite (eq. 1.11). Subsequently, direct iodination using such organic compounds of polyvalent iodine was studied by Merkushev and coworkers. These reagents were found to be very convenient and effective, particularly the trifluoroacetyl
analogue, PhI(O₂CCF₃)₂. Typically, the aromatic substrate was stirred
with a stoichiometric amount of iodine and a slight excess of
PhI(O₂CCF₃)₂ in chloroform or tetrachloromethane at room temperature
for 15 - 120 minutes. A wide range of substrates were iodinated in
good to excellent yields. Iodobenzene is produced as a by-product,
hence the method is restricted to substrates more activated
towards electrophilic attack than iodobenzene.

(v) Transition Metal Compounds - Cobalt (III), Manganese (III), etc.

There have been numerous reports of aromatic iodination
using transition metal compounds as oxidants. Generally speaking,
such reactions have been carried out under quite harsh conditions
and are not appropriate for radiochemical work. A summary of work
involving transition metals is provided in Section 3.5.

(vi) Electrochemical Oxidation

Several workers have described the production of positive
iodine species by electrochemical means. Anodic oxidation of iodine,
particularly in acetonitrile, has been shown to generate an
iodine (I) reagent which rapidly and efficiently iodinates a wide
variety of aromatic substrates. Work of this nature forms the
basis of Chapter Four.

In addition, electrolysis has also been used to oxidise
iodide ions in aqueous solution for the iodination of proteins.
This is described in Section 1.4.1 (vii), p.48.
Efficient aromatic iodination using catalytic amounts of nitrosonium tetrafluoroborate was reported in 1988 by Radner. This interesting reaction was selected for further study and a detailed account of Radner's work is given in Section 3.1.

(viii) Fluorine gas (F₂ + I₂ → IF)

The use of fluorine gas in direct aromatic iodination was first reported in 1988 by Rozhen et al. A full account was published in 1990.

The method involves the in situ formation of IF from its elements. This was generally carried out at -78°C, by passing nitrogen-diluted F₂ through a suspension of iodine in CFCl₃. Iodinations with the IF so produced were carried out by adding precooled solutions of the aromatic substrates in CHCl₃. Reactions were slow at -78°C; only relatively electron-rich compounds such as toluene were iodinated within a few hours. At room temperature, deactivated compounds such as benzonitrile and ethyl benzoate were iodinated at their meta-positions, while polyiodination occurred with more reactive substrates. For many compounds, the optimum temperature for reaction was found to be around -20°C. For example, acetanilide reacted only very slowly at -78°C, while tars were produced when the temperature exceeded 0°C. At -20°C, a reasonable yield (55%) of p-iodoacetanilide was obtained.

The limitations to the method are similar to those of other similar techniques. Nitrobenzene failed to react, even after 24 hours at room temperature. At the other end of the reactivity...
scale, the reagent caused the complete destruction of substrates such as phenol or anisole. A notable advantage is that sensitive functionalities such as aromatic aldehydes were not affected by the reagent. Benzaldehyde was converted to 3-iodobenzaldehyde in 85% yield, after 3 hours at 25°C.

(ix) Koser's Reagent

Recently, McNeilis and coworkers have reported on the use of Koser's reagent, \( \text{[hydroxy(tosyloxy)iodobenzene]} \), in the iodination of polyalkylbenzenes. The reagent was used in catalytic quantities with N-iodosuccinimide (NIS), or in stoichiometric quantities with iodine to iodinate \( p \)-xylene, mesitylene, 1,3,5-triethylbenzene and durene. Reactions were conducted in methanol at room temperature. Reaction of equimolar quantities of mesitylene, iodine and Koser's reagent produced 2,4-di-iodomesitylene as the principal product, indicating that both atoms of the iodine molecule were rendered electrophilic by the reagent.

Lewis Acids

A number of reagents have been described which promote iodination by polarising the iodine molecule (Fig. 1.1).

\[
\text{I}^\delta^+ \stackrel{-\delta^0}{\text{I}} \underline{\text{Ag}}^\oplus
\]

Fig. 1.1: Polarisation of Molecular Iodine by the Silver (I) Cation
The promotion of iodination by silver (I) ions has been known, and used successfully in preparative work, for a great many years. Generally speaking, the method is restricted to moderately electron-rich compounds in the absence of mineral acids, but less activated substrates can be iodinated by systems such as I$_2$/AgSO$_4$/H$_2$SO$_4$. The most commonly used silver salt in early work was the perchlorate (Birkenbach-Goubeau method$^{33}$), but its use has diminished probably for reasons of safety.

A number of studies of iodination with silver compounds were published in the early 1950's. Derbyshire and Waters$^{34}$ showed that benzoic acid was iodinated at the meta-position by treatment with I$_2$/AgSO$_4$/H$_2$SO$_4$. Further substrates were treated similarly by Barker and Waters$^{33}$; conc. H$_2$SO$_4$ was required for the iodination of deactivated substrates such as nitrobenzene, while a lower acid concentration was optimal for naphthalene, to avoid concurrent sulphonation and oxidative dimerisation.

Silver trifluoroacetate has proved to be a very effective reagent in aromatic iodination. Hasszeldine and Sharpe$^{36}$ published a comprehensive study of its use in 1952. When the salt is added to iodine in a suitable solvent (nitrobenzene was used), silver iodide is precipitated, leaving CF$_3$CO$_2$-I$^+$ in solution. The method is thus related to the work of Ogata$^{30-34}$ and Merkushev$^{35-37}$, described in parts (iii) and (iv). Bergmann and Shahak$^{37}$ also studied the trifluoroacetate, as well as reactions with silver fluoride. They reported low to moderate yields from simple mono-substituted
aromatics, while experiments with various naphthols and methoxy-naphthalenes produced iodinated derivatives and products from oxidative coupling in various proportions.

In 1977, Kobayashi and coworkers reported on the greater reactivity of silver trifluoromethanesulphonate. This was used in chloroform solution, presumably generating $\text{CF}_3\text{SO}_3^-\cdot\text{I}^-$ in situ.

More recently, Sy and Lodge showed that $\text{I}_2/\text{AgNO}_3$ was an effective system for the iodination of alkylbenzenes. When this method was applied to the iodination of aromatic amines, nitrated by-products were found. Sy later reported that $\text{Ag}_2\text{SO}_4$ suspended in ethanol was more appropriate for this purpose. In a subsequent publication, Sy reported yields of 77 - 88% when the $\text{I}_2/\text{Ag}_2\text{SO}_4$ system was applied to the iodination of methoxy- and dimethoxy-amphetamines. Al-Loherdan has reported on related work in which silver sulphate, nitrate and carbonate were compared for the iodination of simple substrates. All three behaved similarly.

(xi) Copper salts

Copper salts have found application in aromatic iodination under a variety of experimental conditions. Baird and Surridge reported on the general applicability of copper (II) chloride, which, in conjunction with iodine, or iodides of iron, aluminium and other metals, proved to be a very effective catalyst. A good variety of substrates were iodinated using this approach. The role of Cu(II) was shown to be twofold; activation of iodine via a Lewis acid interaction, and recycling of the hydrogen iodide produced via redox changes. Further details are given in Section 3.5, p.165.
The system iodine / copper (II) acetate / acetic acid has been studied by Horiuchi and coworkers\textsuperscript{104,105} for the iodination of electron-rich substrates. The method allowed the regiospecific preparation of 2-iodoestradiol-17β-acetate.

Copper ions also find application in halogen-for-halogen exchange reactions. These are summarised in the context of radio-iodination on p.57.

(xii) Alumina

In 1986, Kabalka and coworkers\textsuperscript{106} showed that the dehydrated surface of γ-alumina (chromatography alumina) was effective in the promotion of aromatic iodination. Its activity was accounted for in terms of polarisation of I\textsubscript{2} by partially exposed Al\textsuperscript{3+} ions, and removal of the product HI by reaction with O\textsuperscript{2-} ions. Furthermore, it was suggested that the electrophilic iodine species, OI\textsuperscript{-} and HOI may have been formed by reaction of I\textsubscript{2} with O\textsuperscript{2-} ions and surface hydroxyl groups respectively.

Two experimental procedures were used - a solution method in which iodine and alumina were slurried in an excess of the aromatic substrate, and a dry method in which iodine and the substrate were separately absorbed onto alumina and these solids were intimately mixed. Both were carried out at room temperature for 24 hours. Such procedures resulted in low to moderate yields of iodo products when applied to simple aromatic substrates (benzene, toluene, naphthalene, etc). Anisole was also iodinated, but underwent competing demethylation. The reaction failed with aniline due to complexation, and with acetanilide which was preferentially
cleaved to give aniline. In contrast, N,N-dimethylaniline reacted smoothly, giving the p-iodo isomer. The highest yields were from azulene; using excess \( I_2 \) at room temperature a 100% yield of 1,3-diiodoazulene was obtained, while at 100°C, with excess azulene, 1-iodoazulene was obtained in 73% yield.

The reaction is restricted to moderately electron-rich substrates and is clearly of no application in radiiodination. However, the use of a solid-state reactant is attractive for obvious reasons.

Recent work by Kodomari and coworkers\(^{107} \) has combined the alumina and copper (II) ion methodologies. At 80°C, in carbon tetrachloride solution, copper (II) chloride supported on alumina was shown to be very effective for the iodination of alkylbenzenes and aromatic ethers. Polycyclic hydrocarbons, such as anthracene, were chlorinated rather than iodinated under such conditions, but this was readily overcome by using copper (II) sulphate in place of copper (II) chloride.

(xii) Mercury (II) Oxide / Tetrafluoroboric Acid on Silica

In 1984, Barleunaga et al.\(^{108} \) reported that the title system was effective in promoting aromatic iodination. The exact mechanism involved is open to some debate, although the authors favoured \textit{in situ} aromatic mercuriation, effectively by \( \text{Hg(BF}_4\text{)}_2 \), followed by iodo-demercuration. Considering these steps as separate events would classify the reaction as indirect (Section 1.3.2), but the "one-pot" nature of the process, and the apparent promotion by silica, would seem to link it with the procedures outlined in part (xii) above.
Kajigaeshi and coworkers have found that the quaternary ammonium salt PhCH$_2$(CH$_3$)$_3$N$^+$.ICl$_2^-$, in conjunction with zinc chloride, is a very effective iodinating agent for phenols$^{109}$, anilines$^{110}$ and aromatic ethers$^{111}$. For example, by treating various aromatic ethers with one or two equivalents of this reagent in acetic acid, mono- or di-iodinated products were obtained in yields of 87 - 98%.$^{111}$ Reaction via "I⁻" was proposed, according to the equations:

\[
\text{PhCH}_2(\text{CH}_3)_3\text{N}^+\text{ICl}^- + \text{ZnCl}_2 \rightarrow \text{PhCH}_2(\text{CH}_3)_3\text{N}^+\text{Cl}^- + \text{I}^+ + \text{ZnCl}_3
\] 1.12

\[
\text{ArH} + \text{I}^+ + \text{ZnCl}_3^- \rightarrow \text{ArI} + \text{ZnCl}_2 + \text{HCl}
\] 1.13

The authors emphasised the simplicity, mild conditions and high yields of the process, although its effectiveness was only demonstrated for electron-rich substrates.
(b) Compounds Containing Iodine Bound to Electronegative Elements

(i) Iodine monochloride, ICl

The interhalogen compound, iodine monochloride, is perhaps the most convenient source of electrophilic iodine. The molecule is polarised such that the iodine atom bears a partial positive charge. It has been used very widely in preparative work. Instances of its use in non-radioactive work are too numerous to mention; its use in radioiodination is described in Section 1.4 (ii), p.42.

(ii) N-Iodoamides (R-C-(O)-NHI)

N-Iodoamides, particularly in acidic environments, have the ability to iodinate aromatic compounds. Work in this area was mainly carried out by Goosen and coworkers, who studied the reactions of N-iodoacetamide, N-iodo-4-nitrobenzamide and N-iodo-2,4-dinitrobenzamide. Initial experiments were conducted in the absence of acid catalysts, using the aromatic substrates as solvents. This approach afforded only low to moderate yields of the iodoaromatic products. Subsequent studies, using sulphuric acid as a catalyst resulted in better results, and optimal conditions of 2 hours at 50°C, in 5M acid were established. However, the method remained restricted to substrates activated towards electrophilic attack. It was catalysis by TFA which resulted in vastly improved yields and even halobenzenes were readily iodinated. This was attributed to the formation of trifluoroacetyl hypoiodite, as in eq. 1.14:

\[ R-C-NHI + CF_3COOH \rightleftharpoons R-C-NH_2 + CF_3COOI \]
This, of course, is the same intermediate as Haszeldine and Sharpe\textsuperscript{96} postulated to be formed from silver trifluoroacetate and iodine.

Analogous work using \(\text{N-iodosuccinimide (NIS)}\) and trifluoro-methanesulfonic acid has recently been reported by Olah et al.\textsuperscript{11\text{\textemdash}E}\ NIS itself has not found widespread use in aromatic iodination, although it has been used in the iodination of carbonyl compounds and alkenes. Olah and coworkers\textsuperscript{11\text{\textemdash}E} have found that, in the presence of 2\,-\,5 molar equivalents of triflic acid, NIS produces a reagent capable of iodinating even the most deactivated substrates.

(iii) Miscellaneous N-Iodo Compounds

Dissolution of iodine in morpholine proceeds with loss of the iodine colouration to produce some sort of complex, possibly of type (9). This reagent has been shown to be effective in the iodination of electron-rich substrates, particularly phenols.\textsuperscript{11\text{\textemdash}E}

\[
\begin{align*}
\text{(9)} & \quad \text{(10)} \\
\text{O} & \quad \text{Me} \\
\text{NH} & \quad \text{Me} \\
\text{I} & \quad \text{r} \\
\end{align*}
\]

The \(\text{N-iodo derivative, 1,3-diiodo-5,5-dimethylhydantoin(10), has been shown to be a very convenient carrier of positive iodine and used in the iodination of various anilines, aminonaphthalenes and electron-rich heterocycles.}\textsuperscript{11\text{\textemdash}7}
1.3.2 Indirect Iodination

The principal drawback of direct iodination is a lack of regiospecificity. The preparation of a single regioisomer generally requires an indirect approach. These fall into two categories; those involving diazonium salts or their equivalents, and those involving metallated aromatics.

(a) Sandmeyer Type Reactions - Decomposition of Triazenes

Early work in this area involved the diazotization of aromatic amines followed by decomposition of the diazonium salt with iodide or an iodine radical source. The actual reacting species has been shown to be I$_3^-$; iodide is oxidised to iodine by the diazonium salt or nitrous acid.

\[ \text{R-NH}_2 + \text{HNO}_2 \rightarrow \text{R-N} = \text{N} \rightarrow \text{R-I} \]

More useful are pyrrolidine-triazenes (11) or 1-aryl-3,3-di-alkyltriazenes (12), formed by trapping diazotised amines with an appropriate secondary amine. These are stable to air and light and can be stored for long periods.

\[ \text{R-N} = \text{N} \rightarrow \text{R} \]

(11)  

\[ \text{R-N} = \text{N-} \rightarrow \text{R} \]

(12)
Almost a century after Wallach's initial report\textsuperscript{124}, Heindal et al.\textsuperscript{120} reported that the decomposition of 1-ary lazopyrrolidines by potassium iodide in hydrochloric acid or TFA gave good yields of aryl iodides. The reaction was studied using a series of five different aniline derivatives as precursors. These were converted to the corresponding iodo compounds in yields of 19-66%. The triazene intermediate derived from \( p \)-nitroaniline was not decomposed by \( KI \) in \( HCl \); TFA was found to be necessary for its conversion to \( p \)-iodonitrobenzene.

Heindal and coworkers\textsuperscript{125} later extended this work to radioiodination. Their study involved three simple aniline derivatives and two more complex pharmaceutical molecules. Iodinations were carried out under various experimental conditions, first with \( ^{127}I \) and then with n.c.a. \( ^{123}I \). It was concluded that optimum conditions were dependent on the substrate and no generalisations could be made. N.c.a. radiiodinations were of limited success, resulting in numerous by-products and only moderate radiochemical yields. However, the method allowed the preparation of two \( ^{123}I \)-labelled drug molecules which were not accessible via the classical Sandmeyer route. Imaging agents analogous to IMP (p.12) have been labelled via the triazene method. Elmaleh et al.\textsuperscript{126} labelled phentermine (13) with iodine-123 by decomposition of a piperidinotriazene derivative:

\[
\begin{array}{c}
\text{N} \quad \text{N} \quad \text{N} \\
\text{NH}_2 \\
\rightarrow \\
\text{H}^+ \\
\text{123I}^- \\
\end{array}
\]

(13)
The use of a triazene derivative in the $^{125}$I-labelling of p-iodophenyltriphenylphosphonium nitrate has recently been described by Byon et al.\textsuperscript{127}

Several related methods are worthy of note. Aryldiazonium tetrafluoroborates are stable and can be converted to aryl iodides by treatment with iodine or iodomethane in chloroform, in the presence of catalytic amounts of crown-ethers.\textsuperscript{113,120}

Another method involves the conversion of aryldiazonium tetrafluoroborates to aryl arylazo sulfones using sodium p-toluenesulfinate in dichloromethane. These can then be converted to aryl iodides by treating with potassium iodide and 18-crown-6 as catalyst in acetonitrile at room temperature.\textsuperscript{129}

The most obvious limitation to this kind of approach is that the appropriate aniline precursor must be available, or at least, readily accessible. Another consideration which needs to be taken into account is that functional groups on the substrate need to be stable to, or protected from, the reagents used in the diazotization.
Iodine can also be introduced into aromatic compounds by the displacement of various metals. The use of organometallic intermediates in radiolabelling has been reviewed by Kabalka and Varma. Boron, silicon, germanium, tin, mercury and thallium have all been used in this context. The reactions involving each of these metals are summarised below. More detailed accounts of the use of thallium and mercury and the Group IV metals are given in Chapters Two and Five respectively.

(i) Boranes

The reaction of organoboranes with molecular iodine in the presence of base was developed by Brown and coworkers. 

\[
R_3B + 3I_2 \xrightarrow{\text{NaOMe}} 3RI + 3NaI + B(OMe)_3
\]

In its original form, as in equation 1.16, the reaction is inappropriate for radiiodination. Molecular iodine is required, whereas radioisotopes are generally supplied as sodium iodide. Furthermore, the use of a strong base such as sodium methoxide puts severe limitations on the functional groups which can be present.

Alternative iododeboronation reactions, employing electrophilic iodine species, were subsequently developed by Kabalka and coworkers. Much of the early work was concerned with the preparation of alkyl and vinyl iodides from alkenes and alkynes respectively, but the formation of iodobenzene from triphenylborane was also
described.\textsuperscript{122, 125} This was achieved via the \textit{in situ} oxidation of iodide by chloramine-T (CAT):

\[
\text{Ph}_3\text{B} + I^- \xrightarrow{\text{CAT}} \text{PhI}
\]

A further development was the use of arylboronic acids.\textsuperscript{127} These were produced from bromo derivatives by a transmetallation process, involving Grignard intermediates (equation 1.18):

\[
\text{ArBr} \xrightarrow{1. \text{Mg, BH}_3} \text{ArB(OH)}_2 \xrightarrow{2. \text{H}_3\text{O}^+}
\]

This type of strategy has been used for $^{123}\text{I}$-labelling of the imaging agent, N-isopropyl iodoamphetamine. The required precursor, N-isopropyl p-bromoamphetamine, was obtained from p-bromoanaldehyde via the scheme set out below\textsuperscript{137}:

\begin{align*}
\text{CHO} & \xrightarrow{\text{a. EtNO}_2, \text{NH}_4\text{OAc}} \text{NO}_2 & \text{a. EtNO}_2, \text{NH}_4\text{OAc} \\
\text{Br} & \xrightarrow{\text{b. BH}_3, \text{NaBH}_4} \text{NH}_2 & \text{b. BH}_3, \text{NaBH}_4 \\
\text{Br} & \xrightarrow{\text{c. NaBH}_3\text{CN, CH}_3\text{COCH}_3} & \text{c. NaBH}_3\text{CN, CH}_3\text{COCH}_3
\end{align*}
(ii) Silanes

Organosilanes have been used quite extensively in region-specific halogenation and radiohalogenation. Electrophilic iodine can be used to displace trialkylsilyl groups in a manner analogous to the deboronation reaction described in the preceding subsection:

\[
\begin{align*}
\text{SiMe}_3 & \quad \text{CAT} \\
\longrightarrow \\
\text{X} & \quad \text{I}^-
\end{align*}
\]

This type of reaction was initially studied by Eaborn\(^{139}\) and developed for radiiodination by Wilbur et al.\(^{140}\) and by Coenen and Moerlein.\(^{141}\) Radiiodide is oxidised \textit{in situ}, by agents such as \(N\)-chlorosuccinimide (NCS - see page x). The reaction proceeds most rapidly in protic solvents such as acetic acid. Electrophilic iododesilylation reactions of this type are the subject of Chapter Five.

Another method involving silicon is the cleavage of arylpentfluorosilicates by halogens. In 1983, Wilbur and Svitra\(^{142}\) reported on a study of the usefulness of these compounds in n.c.a. radiobromination and radiiodination. A series of alkyl- and arylpentfluorosilicates was synthesized and reacted with electrophilic halogen species produced by \textit{in situ} oxidation of \(^{77}\text{Br}\) bromide and \(^{129}\text{I}\) iodide:

\[
\text{R-SiF}_5\text{K}_2 \quad \text{I}^- \quad \text{R-I} \quad \text{oxid.}
\]

1.19
The method proved to be very effective and radiochemical yields in the range 74 - 96% were obtained. In contrast to the reactions involving aryltrimethylsilanes, this process was unaffected by a change of solvent (AcOH to MeOH) and, significantly, could be carried out using commercial radicidalide solutions containing NaOH. Arylpentafluorosilicates are prepared by the action of potassium fluoride on aryltrichlorosilanes. These in turn are obtained from the reaction of tetrachlorosilane with the appropriate Grignard reagents. The arylpentafluorosilicates are produced as heavy white precipitates which are insoluble in most solvents. This makes them easy to purify, but is also their principal disadvantage. Whereas -SiMe₃ groups can be carried through a synthetic sequence, the insolubility of arylpentafluorosilicates prevents their introduction until the final step. Their precursors, aryltrichlorosilanes, are too reactive to be carried through multi-step syntheses.

(iii) Germanes

 Aryltrialkylgermanes can be cleaved by halogens in the same way as their silicon counterparts:

\[ \begin{array}{c}
\text{GeMe}_3 \\
\text{CAT} \\
\text{I} \\
\end{array} \rightarrow
\begin{array}{c}
\text{X} \\
\text{X} \\
\end{array} \]
The carbon-germanium bond is somewhat weaker than the carbon-silicon bond, thus the reaction proceeds more readily.\textsuperscript{141} The use of iododegermylation in the radiiodination of simple model compounds has been extensively studied by Hoerlein.\textsuperscript{143} Despite the promising results, germylated derivatives do not appear to have been used in the preparation of radiopharmaceuticals. One factor may be the high cost of germanium compounds, compared to their silicon and tin analogues, although in radiolabelling the cost of the isotope would outweigh the cost of any chemical reagents.

(iv) Stanmanes

Stannylated precursors have been used very extensively in radiiodination.\textsuperscript{144} Tributyltin derivatives are preferred to trimethyltin derivatives as they are less toxic. Aryl-tin bonds are readily cleaved by halogens, even when the aromatic ring is deactivated to electrophilic attack:

\[
\begin{align*}
\text{X} \quad \text{SnBu}_3 & \quad \text{I}^- \quad \text{CAT} \quad \text{X} \quad \text{I} \\
\end{align*}
\]

In radiolabelling, the iodine electrophile is most commonly generated \textit{in situ} from sodium iodide and chloramine-T (\textit{vide infra}). An extensive review of applications of such methodology is given in Section 5.1.
(v) Thallates

Aromatic compounds can be thalliated using thallium (III) trifluoroacetate in TFA. The resulting arylthallium bis(trifluoroacetates) yield aromatic iodides upon treatment with inorganic iodide.\textsuperscript{145} Such methodology is dealt with in considerable detail in Section 2.1.

\[
\begin{array}{c}
\text{X} \quad \text{TTFA} \quad \text{TFA} \\
\downarrow \\
\text{Tl(OCOCF}_3\text{)}_2 \\
\downarrow \\
\text{I} \\
\end{array}
\]

Gilliland \textit{et al.}\textsuperscript{145} have explored the utility of the above reaction in the production of radiopharmaceuticals, achieving radiochemical yields as high as 81\%. (For further details, see Section 2.1.2, p.76).

(vi) Mercurials

A similar transformation to that outlined in part (v) above can be carried out via arylmercury intermediates. This reaction is discussed in Section 2.2. Mercuriated derivatives have been little used in the radiiodination of aromatic compounds, but a great number of radiohalogenations have been performed via aliphatic and vinylic mercurials.\textsuperscript{130}
(vii) Plumbanes

Organolead compounds, analogous to the silicon, germanium and tin compounds described in parts (ii) - (iv), are known, although these are very reactive and not of any utility.

In addition, there have been reports of electrophilic plumbylation reactions analogous to those described for thallium and mercury in parts (v) and (vi). For example, aromatic ethers react with lead tetraacetate in benzene or acetic acid at elevated temperatures to give the para-aryllead triacetate\(^{147}\):

\[
ArH + Pb(OAc)_4 \rightleftharpoons ArPb(OAc)_3 + HOAc
\]

Sergachev et al.\(^{148}\) have described the iodination of a series of simple arenes using Pb(OAc)_4 and I_2 in TFA solution.
1.4 Radioiodination Techniques

Much of the work on developing radioiodination procedures has been concerned with the labelling of peptides and proteins. As a consequence, this section is largely a summary of the techniques used to radioiodinate such large biomolecules. However, notes on the radioiodination of smaller aromatic molecules are included where appropriate.

In the context of protein labelling, the terms "direct" and "indirect" have different meanings than those in the case of simple aromatic iodination. "Direct" iodination of a protein refers to the electrophilic attack of iodine on tyrosine or histidine residues; "indirect" refers to the attachment of a radiolabelled moiety to the protein, usually via a free -NH₂ group.

1.4.1 Direct Radioiodination of Biomolecules

(i) Molecular Iodine

The tyrosine residues in proteins are relatively reactive and may, under suitable conditions, be attacked by molecular iodine. However, radioiodine is almost invariably supplied as sodium iodide, so an oxidation step is required. Numerous oxidising agents have been used to bring about this conversion, for example, iron (III) sulphate⁴⁹, nitrous acid⁵⁰, potassium iodate⁵¹, hydrogen peroxide⁵² and ammonium persulphate.⁵³ In theory, all the oxidants described in Section 1.3.1 could be used in radiochemical work, but there are two important reasons why oxidation to molecular iodine is not favoured.
The first disadvantage is that only 50% of the isotope can be incorporated into the substrate; heterolytic cleavage of the I-I bond means that the other 50% ends up as radioiodide. A further disadvantage is that the volatility of molecular iodine makes it much more hazardous to handle than iodide. The oxidation step also takes time, thus lengthening the radiation exposure time of workers and resulting in some loss of radioactivity.

(ii) Iodine Monochloride

As mentioned in Section 1.3.1, iodine monochloride has been used as a source of positive iodine for a great many years. For radiolabelling purposes, the technique was introduced in 1958 by McFarlane. The molecule is polarised so that the iodine bears a partial positive charge; this circumvents the problem of isotope loss mentioned in the preceding section since all the iodine is able to act as an electrophile. However, in its original form, the reaction was only useful for trace labelling since non-radioactive ICl was used to generate the labelled reagent via exchange with radioiodide.

A somewhat improved technique was reported by Helmkamp et al. in 1960. They found that hydrogen peroxide in commercial Na\(^{31}\)I interfered with the labelling reaction, and so reduced it by the addition of sulphite ions. The method allowed \(\gamma\)-globulin to be labelled to a level of 10mCi / mg of protein. In 1961, Samols and Williams reported improved results for the trace-labelling of insulin by the iodine monochloride method.
In 1967, Helmkamp et al.\textsuperscript{157,158} reported on a modification of the technique known as the oxidative ICl method. This involved the formation of the ICl\textsubscript{2}\textsuperscript{-} anion by oxidation of iodide ions with periodate in hydrochloric acid:

\begin{equation}
2\text{I}^- + \text{IO}_3^- + 6 \text{HCl} \rightleftharpoons 3\text{ICl}_2^- + 3\text{H}_2\text{O} \tag{1.21}
\end{equation}

From the stoichiometry of equation 1.21, it is apparent that two-thirds of the positive iodine is derived from the iodide while the remaining third comes from the periodate. Thus, using n.c.a. radioiodide, up to 66\% of the iodine in the product may be radioactive, and, in theory, all of the isotope is available for reaction.

This technique was investigated further by Doran and Spar who used it for the iodination of various proteins for RIA and the localisation of tissue antigens.\textsuperscript{159} The labelled proteins were of a high quality, and showed improved retention of biological activity compared to proteins labelled by oxidative iodination with chloramine-T (\textit{vide infra}).

The production and use of carrier-free \textsuperscript{123}I-iodine monochloride was first described in 1972 by Lambrecht et al.\textsuperscript{57} Carrier-free \textsuperscript{123}I was produced by the decay of \textsuperscript{123}Xe, which in turn was produced by the \textsuperscript{4}He bombardment of \textsuperscript{122}Te. By condensing \textsuperscript{123}Xe in ampoules containing excess Cl\textsubscript{2}, \textsuperscript{123}I-ICl was produced in yields of around 90\%, after several hours incubation at ambient temperature. Excess Cl\textsubscript{2} was removed \textit{in vacuo} to leave carrier-free \textsuperscript{123}I-ICl. The authors demonstrated that the ICl prepared in this way could
be used to iodinate salicylic acid, human serum albumin and other substrates at very high specific activities.

(iii) Chloramine-T

Chloramine-T is the N-chloro- derivative of the sodium salt of the amide of p-toluenesulfonic acid. Its use in radioiodination was introduced by Hunter and Greenwood in 1962.\textsuperscript{161} The reagent has since become the most widely used oxidant in radioiodination. Chloramine-T breaks down slowly in aqueous solution, releasing HOCl, which is believed to be the actual oxidising species. This reacts with iodide to form some sort of electrophilic iodine species, possibly $\text{HOI}^\cdot$. All of the available radioiodide can be converted into such an electrophilic form, thus high specific activities are accessible.

Protein labelling with chloramine-T is carried out in buffered aqueous media. The optimum pH for tyrosine labelling is around 7.2, while histidine requires a pH of 8.1.\textsuperscript{5} Reactions are terminated, typically after a few minutes, by the addition of a reducing agent. This reduces not only chloramine-T, but unreacted iodine as well. Carrier KI and a further protective protein are then added. The KI facilitates removal of unreacted radioiodide by gel filtration, while the second protein acts as a carrier and serves to minimise losses of the labelled material, for example, by adhesion to glass surfaces. Because of the tendency of proteins to adhere to glass, polystyrene reaction vessels are generally used for work of this nature.
The principal problem associated with chloramine-T labelling of proteins is that oxidation damage may occur. Methionine residues are especially susceptible, being easily converted to sulphotides. In addition, tryptophanyl peptide bonds may be cleaved. A further undesirable side reaction is chlorination. As chloramine-T is used in excess with respect to iodide ions, significant substitution by electrophilic chlorine species may occur.

Competing side reactions are minimised by keeping reaction times as short as possible and by using the minimum amount of chloramine-T. The conditions are nevertheless quite harsh for proteins and numerous reports have described loss of structural integrity and decreased biological activity following exposure to chloramine-T.

Several modifications of the chloramine-T method have been published. Aqueous chlorine and sodium hypochlorite have been used to oxidise radiiodide via essentially the same chemistry. Proteins labelled in this way have shown enhanced retention of biological and immunological activity compared to those labelled by the chloramine-T method.

(iv) Iodobeads

Since the early 1980's, the Pierce Chemical Company have marketed "Iodobeads". These are non-porous polystyrene beads, 2.8mm in diameter, derivatised with N-chlorobenzenesulphonamide. Chemically, they are equivalent to immobilised chloramine-T. The usefulness of Iodobeads in radiiodination was first reported by Markwell in 1982, who used them in the ¹²⁵I-labelling of antiporcine insulin
antiserum. Notable results were an almost quantitative incorporation of radiiodide into the protein (99%) and an excellent recovery of the protein (95%). Reactions were terminated by simply removing the reaction solution from the Iodobeads with a Pasteur pipette, so a reducing agent was not required.

Iodobeads have since found favour with many workers in the radiolabelling field. For example, Tsomides and Risen\textsuperscript{165} used them in the stoichiometric labelling of peptides, while Culbert and Hunter\textsuperscript{166} used them in the iododestannylation of an IMP precursor bound to an organotin polymer (see p.249).

(v) Iodogen

Iodogen is the commercial name of 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril, a reagent introduced by Fraker and Speck\textsuperscript{167} in 1978 (structure on p.\textit{x}). It is an oxidising agent structurally similar to chloramine-T. It is however essentially insoluble in water and is used in the form of a coating on the inside of radiiodination vessels. A solution of the compound in dichloromethane is transferred to the reaction vessel and the solvent is allowed to evaporate slowly so as to produce a coating. Radiolabelling is then carried out by adding buffered peptide or protein solution and radioactive sodium iodide to the treated vessel. As the reaction is a heterogeneous process, longer reaction times are required. This may cause problems with more sensitive proteins.
The principal advantage of iodogen is obvious: being a solid phase reactant it is rapidly separated from the protein solution. Furthermore, the heterogeneous nature of the reaction significantly reduces the oxidation problems associated with chloramine-T. A recent publication claims optimisation of the method.

(vi) Enzymatic Oxidation

Peroxidase enzymes, in the presence of nanomolar quantities of hydrogen peroxide and iodide ions, have the ability to iodinate tyrosine, and to a lesser extent, histidine residues. Most effective is lactoperoxidase which has been used quite extensively in the labelling of proteins. Early work with lactoperoxidase was aimed at the trace-labelling of proteins, for example immunoglobulins. Higher specific activities were reported in 1971 by Thorell and Johansson, for the $^{125}$I-labelling of various polypeptide hormones.

Enzymatic oxidation is perhaps the mildest of all the techniques used in radiiodination. This is particularly true when the hydrogen peroxide required is generated in situ by another enzyme, i.e. glucose oxidase. Because of the mild conditions, damage to proteins is minimal. Migachi and Chambrauch iodinated various hormones (gonadotropins) by both enzymatic means and chloramine-T oxidation, then examined the products by gel electrophoresis. Much less damage occurred when the enzyme was used, and, in contrast to some reactions involving chloramine-T, full biological activity was
preserved. A second example is the n.c.a. labelling of fibrinogen, a compound which is very sensitive to chemical effects that can destroy its biological activity.\textsuperscript{174}

A further development in this field is the use of solid-phase lactoperoxidase.\textsuperscript{175} The enzyme has been immobilised on various supports such as sepharose\textsuperscript{176} and phenoxyacetylcellulose (Enzorb A).\textsuperscript{177} Reactions involving such systems are carried out using suspensions of the solid reagents in buffered media, and terminated simply by diluting or centrifuging the reaction mixture. Iodinated products can then be rapidly separated from the immobilised enzyme by gel filtration.\textsuperscript{178}

In summary, enzymatic oxidation provides an extremely mild alternative to chemical oxidation and is thus particularly suited to the iodination of sensitive substrates. However, full use of the isotope is never realised since the desired reaction is always accompanied by some self-iodination of the enzyme.

(vii) Electrolytic Oxidation

There have been numerous reports on the radiiodination of peptides by electrolytic oxidation of iodide ions. Such processes have invariably been carried out in aqueous media, and thus differ considerably from the work on positive iodine species described in Chapter Four.
Among the earliest reports of electrolytic iodination were those of Rosa et al.179-181. Fibrinogen179, human albumin180 and insulin181 were labelled with either 125I or 127I by electrolysis of potassium radioiodide solutions, in a medium of 0.9% aqueous sodium chloride. Oxidation damage to the proteins was minimal, in contrast to reactions involving potassium iodate or chloramine-T as oxidants. Another advantage was that the rate of iodination could be controlled by varying the current. The relative amounts of mono and di-iodinated tyrosine were estimated in the case of human albumin180 following enzymatic hydrolysis. When low currents were used a more uniform distribution of the iodine throughout the tyrosine residues was obtained. Iodinations were carried out at pH 7, on a very small scale, and theoretical yields were achieved.

Welch et al.182 also used enzymatic hydrolysis to analyse iodine distribution in labelled peptides. They compared electrolytic, enzymatic, iodine monochloride and chloramine-T methods for labelling fibrinogen and found a variation in the labelling pattern between the methods. The electrolytic technique was perhaps the cleanest.

One of the drawbacks of the early work was that the procedures required relatively large amounts of protein, i.e. 100μg quantities. However, both Donabedian et al.183 and Sammon et al.184 have developed micro-electrolytic procedures for the labelling of smaller amounts (1 - 5μg). Donabedian et al.183 used a Teflon micro-cell in the high specific activity labelling of polypeptide hormones for RIA, while Sammon et al.184 carried out very similar work using a small platinum crucible as the anode and reaction
vessel. In both cases good retention of immunological properties was reported.

The principal disadvantage associated with the electrolytic technique is that the protein is exposed to the potentially damaging radiiodide solution for a considerable length of time. In the aforementioned work on human albumin\textsuperscript{130}, electrolysis was carried out over a period of 12 hours, although times of 30 - 40 minutes seem more typical.\textsuperscript{179, 181, 183} Electrolysis is performed in a dilute aqueous solution, usually at room temperature, and such conditions are conducive to the denaturation of sensitive proteins.

Use of electrolysis has not been restricted to protein labelling. The technique has also been used for the \textsuperscript{125}I-labelling of estradiol.\textsuperscript{125} Electrolysis was performed in aqueous dioxan containing the substrate, Na\textsuperscript{125}I and phosphate buffer (pH 7.4). Maximum iodination yields of 55 - 60\% were attained after 25 - 30 minutes and preparative TLC permitted the separation of 2-iodo- and 4-iodoestradiols. These were formed in roughly equal amounts.

In a further example, Stanko and Slavin\textsuperscript{136} used electrolysis to incorporate \textsuperscript{125}I and \textsuperscript{131}I into some polyiodophenols, such as Rose Bengal dye, via an isotopic exchange process. Electrolysis of aqueous sodium iodide, in the presence of radiiodide and the substrate, led to high incorporations of radiiodine. Radiochemical yields exceeding 90\% were quoted.
1.4.2 Indirect Radiolabelling - Prosthetic Groups

(1) Bolton and Hunter Reagent

The most widely used indirect method of protein labelling is that introduced by Bolton and Hunter in 1973. The technique involves the conjugation of the active ester, N-succinimidyl-3-(4-hydroxy-5-[\(^{125}\)I]iodophenyl)propionate, with the protein, usually via the free amino group of a lysine residue. The reagent itself is labelled prior to conjugation using the chloramine-T method. The overall process is illustrated in Fig. 1.2:

![Diagram of Bolton and Hunter Reagent]

Fig. 1.2: Use of Bolton and Hunter Reagent
This type of indirect approach is used when the protein itself is not stable under the conditions of oxidative iodination, or when unlabelled tyrosine residues are essential for the protein's biological activity. It is also used for the labelling of smaller molecules which do not contain suitably reactive moieties for direct iodination.

Conjugation of the Bolton and Hunter reagent is generally performed in buffered solution at around pH 8.5. The temperature is kept low to reduce the rate of hydrolysis of the reagent to the corresponding propionic acid derivative. Concurrent hydrolysis of the reagent limits the efficiency of the conjugation reaction, but this can be offset by using an excess. For higher specific activities, the 3,5-di-iodo derivative can be used.

(ii) Other Prosthetic Groups

Numerous other prosthetic groups have been described in the literature. The reagent, methyl 4-hydroxybenzimidate hydrochloride (14), developed by Wood et al., has also been used for labelling proteins. It is used in the same manner as the Bolton and Hunter reagent, but differs slightly in that it preserves a positive charge near the point of conjugation.

![Chemical structures](14) (15)
Prosthetic groups have also been used in the labelling of many smaller molecules, primarily for the production of $^{125}$I-labelled antigens for RIA. The common features are an activated aromatic moiety which can be readily iodinated, and some sort of bridging unit for attachment to the substrate. The groups most commonly used are histamine (15), tyramine (16) and tyrosine methyl ester (17), which are generally attached via (carboxymethyl)oxime or hemisuccinate bridges. Substrates are first derivatised with (carboxymethyl)hydroxylamine or succinic anhydride so that free carboxyl groups are available. These are then used to couple the activated aromatic units via amide linkages. In work with smaller molecules, radiiodination is often carried out after the conjugation step.

The iodination of such prosthetic groups is not restricted to the chloramine-T method. For example, Massaglia et al. employed electrolytic oxidation for the $^{125}$I-labelling of tyrosine moieties. Tyrosine methyl ester was iodinated in yields of 60–80% and then coupled to various steroid derivatives for use in RIA. Heindal and Van Dort used a Sandmeyer/Wallach approach (see p.31) to prepare $[^{125}$I]-3-iodobenzoyl hydrazide. This was then directly coupled to the antitumour antibiotic, doxorubicin.
Recent publications on indirect radiolabelling have shown a trend towards developing more stable prosthetic groups for in vivo applications. Ortho-iodinated tyrosine moieties are quite susceptible to in vivo deiodination, leading to decreased localisation of the isotope and enhanced thyroid uptake. This is especially undesirable in cancer therapy with $^{131}$I-labelled monoclonal antibodies (MAbs), where the aim is to concentrate the isotope in tumours. To this end, various researchers have developed conjugating agents in which radiiodine is bound to non-activated aromatic rings. Wilbur et al. $^{191}$ used $\text{H-succinimidyld-4-iodobenzoate}$, while Zalutsky et al. $^{192-194}$ studied the meta-iodinated analogue. More recently, Garg et al. $^{195}$ have reported on the use of the analogous compound containing a pyridine ring, $\text{H-succinimidyld-5-iodo-3-pyridinecarboxylate}(18)$. The radioiodination of such deactivated prosthetic groups has been carried out via iododestannylation of tributyltin derivatised precursors, while conjugation is carried out via amino groups as in Fig. 1.2.

Other approaches have been used. For example, Srivastava et al. $^{196}$ developed $\text{N-(p-iodophenyl)maleimide}$, which was coupled to MAbs via free sulphydryl groups. In this case, radiiodine was introduced via a mercuriated intermediate. Recently, Kurth et al. $^{197}$ have reported the preparation and use of $[^{125}\text{I}]-\text{N-(4-iodophenylethyl)-(aminooxy)acetamide}(19)$. This was coupled to a MAb via an aldehyde function produced by periodate oxidation of the antibody's sugar moiety. The resulting labelled MAb showed significantly higher tumour concentration than the chloramine-T iodinated MAb, while thyroid uptake of released iodine was up to 25 times lower.
1.4.3 Exchange Labelling

A commonly used method for the incorporation of isotopes in general is by exchange. Molecules have been labelled with iodine isotopes by both iodine for iodine exchange, and by iodine for bromine exchange. Such reactions have been performed under a wide variety of chemical and physical conditions, some of which are outlined below. (N.B. - *I is used to denote radioiodine)

(i) Exchange In Solution

The simplest method of trace-labelling many small organic molecules is to iodinate them with stable $^{127}$I, and then to carry out exchange with radiiodide by refluxing in a suitable solvent. Obviously, only very low specific activities can be obtained in this way, and the rate of exchange is very much dependent on the nature of the substrate. Nevertheless, several radiopharmaceuticals have been prepared by this method, as indicated by Seevors and Counsell in their review of radioiodination.
(ii) Exchange In Melt

Numerous exchange reactions have been performed in molten media. Severs and Counsell divided these into three categories, namely those conducted in a melt of the non-radioactive substrate, exchange in molten acetamide, and exchange in a blend of substrate and ammonium sulphate. The first of these is the most straightforward; labelled sodium iodide is simply added to the iodoaromatic above its melting point. The method is restricted to compounds of high dielectric constant which can dissolve sodium iodide. The compound also needs to be stable at elevated temperature.1

The acetamide melt technique was introduced in 1976 by Elias and Lotterhos.133 Acetamide melts at 82°C and provides a convenient medium for the exchange labelling of compounds of low dielectric constant. Elias and Lotterhos133 showed that all three isomeric iodo benzoic acids readily underwent exchange with Na131I at 140 - 180°C.

The ammonium sulphate method, as described by Mangner et al.133 involves the heating of the substrate with ammonium sulphate and radiiodide at 120 - 160°C. This was shown to be effective for the labelling of many aromatic compounds, including the iodo benzoic acids and several iodo benzyl guanidines. The success of the method is believed to be related to the acidity of the medium, which increases at high temperature as ammonia is driven off. Recently, El-Shaboury and Farah200 described the efficient radiiodination of 4-iodoantipyrine in melts containing ammonium hydrogen orthophosphate.
Other media for exchange in melt have been described. Counsell and coworkers\textsuperscript{201} used pivalic acid (trimethylacetic acid) for the \(^{125}\text{I}\)-labelling of a wide range of radiopharmaceuticals. Reactions were carried out at 155°C, typically for 1 - 2 hours. Substrate decomposition was negligible and radiochemical yields of 55 - 99% were obtained.

(iii) Exchange For Bromine

There have been several reports of radioiodination by substitution of bromine.\textsuperscript{7} The majority of these seem to involve aliphatic compounds, but some aromatic compounds have been labelled in this way. In their publication on exchange in molten acetamide, Elias and Lutterhos\textsuperscript{133} reported a 92% yield for the \(^{131}\text{I}\) for Br exchange of 3-bromobenzoic acid. An advantage of exchange for Br over \(^{81}\text{I}\) for \(^{127}\text{I}\) replacement is that chromatographic separation of high specific activity iodinated product from brominated precursor may be possible.

(iv) Copper Ion Promoted Exchange

It has long been known that copper (I) ions can assist nucleophilic and/or homolytic substitution processes involving the halogens. Stanko et al.\textsuperscript{202, 203} have shown that copper ions greatly accelerate iodine exchange in compounds such as \(\sigma\)-iodobenzoic acid and \(\sigma\)-iodohippuric acid, both in DMSO and in aqueous solutions. The addition of Cu(I)Cl to aqueous solutions of substrate and Na\(^{127}\text{I}\) resulted in a reduction of reaction times from 2 - 3 hours to 5 - 10 minutes for 80 - 90% isotopic exchange.
In 1990, Noerlein reported on a detailed study of copper ion assisted iododebromination. Experiments were carried out using n.c.a. Na$^{123}$I and a series of 11 simple para-substituted bromoaromatics. Iodine for bromine exchange was performed in DMSO at 140°C, in the presence of copper (I) chloride. Each reaction was allowed to proceed for a fixed period of 30 minutes, before the mixture was analysed to assess the extent of iodination. High radiochemical yields were obtained for all the substrates, irrespective of the degree of electronic activation, and the process was completely regiospecific in all cases. The highest yields were obtained from substrates bearing strongly electron-donating or strongly electron-withdrawing substituents, suggesting the process was a homolytic rather than a nucleophilic one. A significant observation, with regard to rapid n.c.a. radiiodination was that radiochemical yields were unaffected by up to 25% water in the reaction mixtures.

In recent years there have been numerous reports on the use of copper(I) assisted iodine exchange or iododebromination in the labelling of radiopharmaceuticals. Cu(I) is generally produced in situ from Cu(II) and a reducing agent. Some preparations have been formulated in kit form, an example being a kit for the generation of $^{123}$I-meta-iodobenzylguanidine ($\text{mIBG}$).
1.4.4 Decay Induced Labelling ("Recoil" or "Excitation" Labelling)

There have been several reports of successful radiiodine labelling using "recoil" or "hot-atom" techniques. This involves the production of highly positive iodine species (recoil atoms) from the electron capture decay (and ensuing Auger electron loss) of xenon isotopes. Both iodine-123 and iodine-125 can be obtained from the corresponding isotopes of xenon.

Early work with $^{123}$Xe has been reviewed by Stöcklin. Details were given of several instances where biomolecules were labelled with $^{125}$I, simply by exposure to $^{123}$Xe. Such direct decay-induced labelling is inherently non-regiospecific and the decay-induced formation of iodinating reagents is perhaps more useful. One example of this has already been described, namely the in situ generation of $^{123}$I-ICl by Lambrecht et al. (see p.43).

El-Garhy and Stöcklin described an alternative method in which KIO$_3$ was exposed to $^{123}$Xe, then used to iodinate aromatic systems such as tyrosine residues in acidic solution. Labelling patterns were consistent with wholly electrophilic substitution, in contrast to the more general labelling obtained by exposing aromatics to $^{123}$Xe.
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CHAPTER TWO

Some Studies of Metallation-Iododemetallation Reactions Involving Thallium and Mercury
2.1 Thalliation - Iododethalliation

The metal thallium has the somewhat unusual property of readily forming bonds to carbon, without a need for the stringent conditions normally associated with organometallic chemistry. Under favourable conditions, direct thalliation of aromatic compounds can be brought about resulting in stable organothallium derivatives. Exposure of such compounds to iodine or iodide ion results in rapid cleavage of the carbon-metal bond, thus providing a route to the corresponding iodoarenes.

2.1.1 Aromatic Thalliation

The direct replacement of hydrogen in aromatic compounds by thallium (III) was first reported in 1943 by Gilman and Abbott. They obtained a low yield of di(4-dibenzo furyl)thallium chloride from the reaction of dibenzofuran with thallium (III) chloride at elevated temperature. Somewhat later, Glushkova and Kocheshkov reported the thalliation of benzene and electron-rich arenes using thallium (III) triisobutyrate. From the effects of aromatic structure on rate and product distribution, the reaction was found to be electrophilic in nature.

By far the most comprehensive study of the thalliation of aromatic compounds has been that of Taylor, McKillop and co-workers. They made the crucial breakthrough of finding a powerful yet relatively stable thalliating reagent, namely thallium (III) trifluoroacetate (TTFA). This could be prepared and used in situ by heating a suspension of thallium (III) oxide in trifluoroacetic acid (TFA). Using this reagent, rapid and convenient thalliation of
a large number of aromatics was achieved, yielding arylthallium (III) bis(trifluoroacetates).\(^6\) E.g., with toluene:

\[
\text{Me} \quad + \quad \text{Me} \quad + \quad \text{O} \quad + \quad \text{CF}_3\text{COOH} \quad 2.1
\]

Kinetic and mechanistic studies (see Section 2.3) have indicated the active electrophile to be \(\text{Tl}(\text{OCOCF}_3)^2+\), formed by the loss of one trifluoroacetate ligand from TTFA.

The reaction with substrates such as toluene was found to be very straightforward. Thalliation occurred almost exclusively at the \(\text{para}\)-position resulting in \(p\)-tolythallium bis(trifluoroacetate). Similarly, the thalliation of other \(\text{mono}\)-substituted arenes (anisole, halobenzenes, etc.) was found to proceed with high \(\text{para}\)-selectivity.

For compounds bearing substituents such as \(-\text{COOH}, -\text{COOR}, -\text{CH}_2\text{OH}, \text{etc.}\), \text{ortho}\-thalliation was observed. For example, the reaction of methyl benzoate with TTFA in TFA gave 95\% of the \text{ortho}\-thalliated product.\(^6\) This was explained in terms of an intermediate substrate-electrophile complex, resulting in chelate controlled \text{ortho}\-delivery of the thallium.
Fig. 2.1: Chelate controlled ortho-thalliation of methyl benzoate

Ortho-thalliation of the kind described above occurs with high specificity only when the substituents are directly bound to the aromatic ring, or are connected via a single methylene unit. This illustrates a requirement for 5- or 6-membered chelate rings in the substrate-electrophile complex. When the chelating group is separated from the aromatic ring by two methylene units, as in 3-phenylpropanoic acid, only limited chelation can occur and a mixture of isomers is produced. A further increase in chain length eliminates ortho-thalliation via chelation, so that the product isomer distribution from reaction with 5-phenylpentanoic acid is similar to that with alkylbenzenes.  

The regiospecificity of thalliation can also be affected by temperature. Alkylbenzenes which are thalliated exclusively at the para-position at room temperature may be thalliated at the meta-position by performing the reaction at 73°C, the temperature of refluxing TFA. The thermodynamic product accumulates at the expense of the kinetic product since thalliation is reversible. Taylor et al. obtained a 17-fold increase in the amount of m-iodocumene formed at 73°C compared to that formed at 25°C.
Another aspect of arylthallium compounds is their tendency to undergo disproportionation to produce diarylthallium (III) species:

\[ 2 \text{ArTlX}_2 \rightleftharpoons \text{Ar}_2\text{TlX} + \text{TlX}_3 \]

This behaviour was discussed by Henry, and by McKillop et al. A related property is the ability of thallium substituents to migrate. Such transthalliation processes (2.3, 2.4) have been studied in detail by Kooyman et al.

\[
\begin{align*}
\text{ArTlX}_2 + \text{Ar'X} & \rightarrow \text{Ar'}\text{TlX}_2 + \text{ArH} \\
\text{ArTlX}_2 + \text{Ar'H} & \rightarrow \text{ArAr'TlX} + \text{HX}
\end{align*}
\]

Since these reactions are generally quite slow, they are unlikely to be of great significance in simple preparative work and are thus deemed to be beyond the scope of this review.

As stated previously, a wide range of aromatics can be thalliated using TTFA in TFA. There are however limitations, most significantly with more reactive compounds. The reagent has powerful oxidising capacity and with electron-rich arenes, this leads to side reactions through the generation of radical cations.

In the case of aromatic amines, phenols, methoxy compounds, naphthalenes, etc, treatment with TTFA in TFA results in oxidative coupling to form symmetrical biaryls. However, Taylor et al. have demonstrated that competing radical processes of this kind may be suppressed by carrying out the thalliations in TFA diluted with diethyl ether. Using 50 : 50 TFA - diethyl ether as the solvent,
the clean thalliation of a series of anisoles, dimethoxybenzenes and naphthalenes was achieved. Aniline is readily oxidised to form diazobenzene under the conditions of thalliation. Nevertheless, Braun et al.11 were able to obtain p-iodoaniline via thalliation in 92-94% yield. Oxidation products were avoided by restricting reaction times to 2-10 minutes. An atmosphere of dry argon was used for this work.

At the other end of the reactivity scale, Deacon and Tunaley12 have shown thallium (III) trifluoromethanesulfonate to be a more powerful thalliating agent. This was prepared in situ by adding trifluoromethanesulfonic acid to a solution of TTFA in TFA. Using this reagent, thalliation of some polyfluoroarenes (2,3,5,6-tetrafluoranisole, 2,3,5,6-tetrafluorotoluene, 1,2,3,5-tetrafluorobenzene and pentafluorobenzene) was achieved.

2.1.2 Iododethalliation and Applications

The treatment of arylthallium bis(trifluoroacetates) with aqueous potassium iodide was shown to be a very facile synthetic method for the production of iodoarenes, the iododethalliation reaction being effectively instantaneous.5-6

Despite the apparent simplicity of the process, it is not well defined in terms of mechanism. Dethalliation of arylthallium compounds would appear to be a unique property of iodide ions. When an arylthallium bis(trifluoroacetate) is treated with aqueous chloride ions, dethalliation does not occur. Instead, trifluoroacetate is merely displaced by chloride to yield the corresponding arylthallium dichloride. The situation with bromide is less clear-cut.
Treatment of an arylthallium bis(trifluoroacetate) with aqueous KBr at 0 - 5°C results in ligand exchange to form the arylthallium dibromide, but these are very unstable and decompose to give aryl bromides on storage or with heating. By analogy, it was proposed that iododethalliation might proceed via arylthallium di-iodides (equation 2.5) although no such intermediates could be isolated or intercepted.

\[
\text{ArTl(OOCF}_3\text{)}_2 + 2\text{KI} \rightarrow [\text{ArTlI}_2] \rightarrow \text{ArI} + \text{TlI} + 2\text{CF}_3\text{COOK} \tag{2.5}
\]

An alternative mechanism was proposed by Ishikawa and Sekiya to account for their observations of direct iodination of benzene using I\(_2\)/TTFA. A quantitative yield was obtained using only 0.5 equivalents, suggesting oxidation of HI by Tl(III) (equations 2.6 - 2.8) :

\[
\text{ArTl(OOCF}_3\text{)}_2 + \text{I}_2 \rightarrow \text{ArI} + \text{Tl(OOCF}_3\text{)}_2 \tag{2.6}
\]

\[
\text{Tl(OOCF}_3\text{)}_2 + \text{CF}_3\text{COOH} \rightarrow \text{Tl(OOCF}_3\text{)}_3 + \text{HI} \tag{2.7}
\]

\[
2\text{HI} + \text{Tl(OOCF}_3\text{)}_3 \rightarrow \text{I}_2 + \text{TlOCOCF}_3 + 2\text{CF}_3\text{COOH} \tag{2.8}
\]

Application of iododethalliation to the production of iodine labelled radiopharmaceuticals has been described by Gilliland et al. Thalliation of 3,4-bis(4-methoxyphenyl)hexane and subsequent deethalliation using Na\(^{131}\)I and then non-radioactive KI resulted in \(^{131}\)I-3,4-bis(3-iodo-4-methoxyphenyl)hexane. The product needed only minimal purification and was obtained in 81% radiochemical yield. Radiiodination of benzoic acid was carried out via isolation of
the ortho-thalliated derivative. A portion of this, suspended in water was treated with Na$^{131}$I, again followed by non-radioactive KI to complete the reaction. The $^{131}$I-o-iodobenzoic acid so produced was of high radiochemical purity.$^{14}$

Experiments performed on a dopamine derivative were largely unsuccessful. The electron-rich system could not be thalliated owing to oxidative coupling. Its nitrogen and catecholic -OH groups were protected as trifluoroacetyl derivatives and thalliation of the tris(trifluoroacetyl) dopamine was attempted. This proved to be very slow and many impurities were produced.$^{14}$

Long-chain fatty acids bearing terminal phenyl groups have been widely studied as potential myocardial imaging agents (Section 1.2.3, p.12). Such compounds are well suited to the conditions used in thalliation and the high para-selectivity offered by the reaction makes it an attractive method for their radiiodination. Goodman et al.$^{12}$ used the technique to label some methyl-branched fatty acids with $^{125}$I for tissue distribution studies. Kulkarni and Parkey$^{16,17}$ prepared $^{123}$I- and $^{128}$I-p-iodophenylpentadecanoic acid in the same manner.

In a subsequent report, Kulkarni and Parkey$^{16}$ described a more detailed study of the thalliation of phenylpentadecanoic acid. The time required for the reaction to reach equilibrium was determined at various temperatures. Figures of 3 hours at room temperature, 20 - 25 minutes at 50°C and 5 minutes at 100°C were quoted, although no experimental details were given and it is not clear how TFA was maintained at 100°C.
A further example of the use of thallium in radiiodine labelling is provided by a recent paper by Abbas et al. They used iododethalliation to prepare $^{[123I]}$- and $^{[131I]}$-2-(5-(4-iodo-phenyl)pentyl)oxirane-2-carboxylic acid ethyl ester (20), another fatty acid derivative used in the assessment of myocardial metabolism.

![Chemical structure of compound 20](image)

The use of organothallium intermediates in radiolabelling has not been restricted to iodine isotopes. For example, Visser and Diemer have described the preparation of o-astatobenzoic acid and p-astat analogs by incubating the appropriate thalliated precursors with $^{211}$At$^-$. 
2.2 Mercuriation - Iododemercuration

2.2.1 Aromatic Mercuriation

Aromatic compounds can undergo direct mercuriation in a manner analogous to the thalliation process described in Section 2.1. Thus, the addition of mercury (II) trifluoroacetate solution to a solution of an aromatic compound in TFA results in the rapid formation of the mercuriated arene, ArHgOCOCF₃.

Arylmercury derivatives were known as early as the 19th century. For example, Dimroth described the mercuriation of phenol and thiophene in the late 1890's. These reactions were conducted using mercury (II) chloride; it was only after the kinetic studies of the 1950's and 1960's that the greatly superior reactivity of the trifluoroacetate was established (vide infra). Historically, most mercuriation reactions have been performed using the chloride, or the acetate, formed in situ by dissolving mercury (II) oxide in acetic acid.

There are a number of significant differences between the mercuriation and thalliation reactions. One of the most important is the nature of the active electrophile. As mentioned previously, a trifluoroacetate anion needs to be lost from TFA in order to produce [Tl(OCOCF₃)₂]⁻, the reacting species. This is not the case with mercury (II) trifluoroacetate which reacts as the neutral compound. A consequence of this is that mercuriation proceeds in non-polar solvents, dichloromethane being convenient. The fact that mercury compounds react in an undissociated form also explains why mercury (II) acetate and chloride are effective mercuriating agents.
whereas the corresponding thallium (III) compounds are not effective thalliating agents.

A further important difference between mercuriation and thalliation is that polymercuration occurs quite readily. Whereas an aromatic ring can only be mono-thalliated, all aromatic protons can be substituted by mercury. The reason for this contrasting behaviour is not absolutely clear. Steric arguments have often been invoked, but such a distinction between mercury and thallium seems unlikely. An electrostatic explanation would seem more plausible.

Mercury (II) trifluoroacetate is not a powerful oxidising agent as TTFA is. Consequently, the difficulties encountered in the thalliation of electron-rich compounds are not encountered in mercuriation. The early examples of the mercuriation of phenol and thiophene have already been mentioned; such compounds are difficult to thalliate without concurrent oxidation.
2.2.2 Iododemercuration and Applications

One of the earliest reports of iododemercuration would appear to be contained within a further paper by Dimroth. Salicylic acid was mercuriated with mercury (II) acetate in AcOH and subsequent reaction of the product with potassium iodide was reported to yield the potassium salt of o-iodosalicylic acid.

Generally speaking, the displacement of mercury by iodine proceeds much less readily than the displacement of thallium. In an extreme case, Deacon and Farquaharson quoted time periods of up to 14 days to ensure the complete iododemercuration of some polymermercuriated arenes. The reaction was brought about by stirring with excess iodine and sodium iodide in DMF.

It is perhaps because of the slow demercuration reaction that literature examples of radiohalogenation via mercuriated intermediates are not as numerous as might be expected. Also, the fact that mercuriation is less regiospecific, and is complicated by multiple substitution, makes it less appealing than thalliation for many applications. The majority of literature iododemercuriations seem to involve electron-rich, often phenolic-type compounds such as estradiol. Non-radioactive work with estradiol has been reported by Santaniello et al. and by Tsukamoto and Yada. Santaniello's study involved mercuriation with mercury (II) acetate, conversion to the chloromercury derivative with chloride ions, and subsequent demercuration with iodine or bromine in chloroform. This procedure afforded 2-substituted estradiols regiospecifically. Tsukamoto and Yada treated estradiol directly with iodine and mercury (II) acetate.
in acetic acid, and obtained a mixture of the 2- and 4-iodinated products.\textsuperscript{27}

Estradiol was also amongst the compounds studied by Visser and coworkers.\textsuperscript{20,29} They described the preparation of aromatic astatine compounds via organomercury intermediates. Initially, only the astatination of simple model compounds was described\textsuperscript{20}, but in a follow-up publication the method was applied to various steroids and pyrimidines.\textsuperscript{29} Substrates were mercuriated using either HgSO\textsubscript{4} in 0.4 M H\textsubscript{2}SO\textsubscript{4} or Hg(OAc)\textsubscript{2} in NaOAc buffer. The initial products were then converted to chloromercury derivatives by exposure to excess NaCl. Astatination was then performed by adding \textsuperscript{211}At\textsuperscript{-} in buffered media to the chloromercury compounds in chloroform. Reaction times were typically of the order of an hour and radiochemical yields in the range 70 - 95\% were obtained.

An example which highlights the relevance of the kinetic and mechanistic studies described in Section 2.3, is the work of Dougan et al.\textsuperscript{30,31} They used mercuriation - ioddodemercuriation in the preparation of \textsuperscript{123}I-(15-p-iodophenyl)pentadecanoic acid\textsuperscript{30}, but were concerned about the possibility of interference from other isomers in studies of heart disease. In a subsequent investigation, phenylpentadecanoic acid ethyl ester was mercuriated with mercury (II) trifluoroacetate in TFA, with reaction times of 3.5 minutes and 3.5 days.\textsuperscript{31} After the short reaction time, an isomer distribution of 15.2\% o, 8.7\% m and 76.1\% p was obtained, while after 3.5 days, this had become 32.5\% o, 43.3\% m and 24.2\% p.
2.3 Kinetic and Mechanistic Studies of Thalliation and Mercuriation

This section details some of the kinetic studies that have been conducted on the thalliation and mercuriation reactions. It is largely chronological, hence mercuriation is discussed first.

Much of the early kinetic work on aromatic mercuriation was performed by Westheimer et al.\textsuperscript{32-34} and by Brown et al.\textsuperscript{35-42}. This work has been well-reviewed by Kitching\textsuperscript{43} and by Taylor.\textsuperscript{44,45}

Surveying these early reports reveals how the complexities of the subject have only gradually been clarified. The earliest studies on the mercuriation of benzene\textsuperscript{32,33,35,36}, with $\text{Hg(OAc)}_2$ in aqueous acidic media, established fundamental facts such as the acceleration of the reaction by perchlorate ions and the retardation by water. It was only after a decade of continued research that all the observations were satisfactorily explained.\textsuperscript{34,35,40}.

Perchloric acid showed a strong catalytic effect on mercuriation with $\text{Hg(OAc)}_2$, owing to the formation of the much more electrophilic acetoxymercury (II) perchlorate (equation 2.9):

$$\text{Hg(OAc)}_2 + \text{HClO}_4 \rightleftharpoons \text{Hg(OAc)}^+\text{ClO}_4^- + \text{AcOH} \quad \text{2.9}$$

The addition of sodium perchlorate also accelerated the reaction, since this generated perchloric acid in situ (equation 2.10):

$$\text{NaClO}_4 + \text{AcOH} \rightleftharpoons \text{NaOAc} + \text{HClO}_4 \quad \text{2.10}$$

Water was found to retard the reaction, owing to its protonation by perchloric acid (equation 2.11). The acid was thus unavailable for catalysis.
Further details on these points are given in the reviews by Taylor.\textsuperscript{44,48}

Brown's first studies on the \textit{Hg(OAc)}\textsubscript{2} / AcOH / HClO\textsubscript{4} system, were performed on benzene and toluene.\textsuperscript{35,36} Reactions were followed by removing aliquots, evaporating the unreacted arene and converting the mercuriated derivatives to the corresponding bromo- compounds using bromine in carbon disulphide. These were then quantified by means of infrared spectroscopy. At 25°C, the toluene / benzene rate ratio was found to be around 8, and the isomer distribution \((o : m : p)\) in the reaction with toluene was estimated as 21 : 9.5 : 69.5. However, this was not constant with time; the amounts of the \(o\)- and \(m\)-isomers increased over a period of days as though the system was tending towards a statistical 40 : 40 : 20 distribution (36 : 31 : 33 after 28 days\textsuperscript{36}). The reaction was less selective at higher temperatures, both in terms of the toluene / benzene rate ratio and the toluene isomer distribution.

Subsequent studies were carried out using alkylbenzenes\textsuperscript{36} and the halobenzenes.\textsuperscript{39} In the case of alkylbenzenes, observations were readily explained on steric grounds. For example, no ortho-mercuriation was seen with \(t\)-butylbenzene, as would be expected in a reaction involving a bulky electrophile. Halobenzenes reacted more slowly, the relative rates (PhH = 1) being 0.702 (PhF), 0.100 (PhCl) and 0.090 (PhBr). Reaction rates were measured by following the loss of Hg\textsuperscript{2+} by titration with standard thiocyanate solution.
Results had to be corrected for the reaction of mercury (II) acetate with AcOH. In order to determine isomer distributions, mercuriated derivatives were converted to bromo-derivatives and the resulting dihalobenzenes were analysed by g.c.

Rate data for all substrates studied was collated and a Hammett plot was constructed. Results showed a good correlation with $\sigma^+$ values and a $\rho$ value of -4.00 was determined.

In 1966, Brown and Wirkkala reported on mercuriation by mercury (II) trifluoroacetate in TFA. Reactions were very much faster in this medium and the kinetics were not complicated by reactions with the solvent. The mercuriation of benzene at 25°C was found to proceed $6.9 \times 10^2$ times faster in TFA than in acetic acid. As in the earlier work with the acetate, rate data were collected for alkyl- and halobenzenes. Despite the greatly enhanced reactivity of the trifluoroacetate reagent, reactions were found to be more selective. For example, mercuriation of toluene at 25°C resulted in an $o:m:p$ ratio of 12.2:8.6:79.2. The reaction in TFA was also found to be more sensitive to substituents. The toluene/benzene rate ratio was found to be 9.89 (cf. 8 in AcOH). The logarithms of the partial rate factors correlated with $\sigma^+$ values giving a $\rho$ value of -5.68.

These results appear to contradict the general reactivity-selectivity principle but are explained by the second step of the substitution process being rate-limiting, i.e. $k_1 > k_2$. This arises because of the relatively low strength of the C-Hg bond.
Another consequence of $k_1$ being greater than $k_2$ is that considerable kinetic isotope effects are observed; $k_{i+}/k_{i-}$ values in the range 4.7 - 6.8 have been reported.\textsuperscript{34,41}

Most of the more recent kinetic studies of mercuriation have been accompanied by a similar study of thalliation. Thus, it is difficult to divide this review into separate sections for the two mettallation processes. Instead, the work of the various research groups is summarised in approximately chronological order.

Since about 1970, numerous attempts have been made to study the kinetics of aromatic thalliation. Amongst the earliest work was that of Henry.\textsuperscript{7} He examined the thalliation of simple aromatic compounds in various acidic media, using polarographic analysis to monitor the composition of the mixtures. Experiments carried out in aqueous perchloric acid indicated that thalliation rates increased with increasing acid concentration and with temperature. Relative rates were consistent with an electrophilic aromatic substitution, toluene reacting faster than benzene, and benzoic acid reacting only slowly at elevated temperature. The use of alternative solvents led to the conclusion that TFA was by far the best solvent for thalliation.
Kinetic experiments using benzene and toluene were also carried out by Briody and Moore. They found that thalliation with TTFA in TFA was too fast to study by conventional kinetic procedures. Dilution of the TFA with acetic acid caused a marked decrease in the reaction rates and allowed measurements to be made. Toluene was found to react around seven times as fast as benzene. Primary isotope effects of 3.6 and 3.7 were recorded for the two substrates.

Thalliation by thallium (III) acetate in 4:1 acetic acid-water was also studied by Briody and Moore, using perchloric and sulphuric acids as catalysts. The reactions were followed by monitoring the loss of thallium (III). This was done by quenching aliquots on ice, adding bromate reagent and estimating the bromine liberated with KI and thiosulphate titration. The kinetic data did not conform to a simple rate law presumably due to the numerous thallium (III) species existing in the mixtures, \( \text{Tl(OAc)}_{3-n} \), where \( X = \text{ClO}_4 \) or \( \text{HSO}_4 \). Toluene to benzene rate ratios varied from 1.9 to 15.5.

In 1977, Olah and coworkers reported on kinetic studies of both the thalliation and mercuriation of aromatic compounds, using the metal trifluoroacetates in TFA. They pointed out that the relatively high percentage of the meta-isomer obtained in the mercuriation of toluene by Brown and Nelson was probably due to the longer reaction time, compared to that used by Klapproth and Westheimer. It was confirmed that isomer distributions in mercuriations were not constant, but that the percentages of the
ortho- and meta-isomers increased with the time of reaction. The toluene / benzene rate ratio decreased from 15 after the shortest reaction times to 8 after 160 minutes.

In view of these findings, Olah et al. carried out competitive mercuriation experiments with other substrates at 0°C to minimize the isomerisation process. For fast reactions, a flow-quenching apparatus was employed to allow short reaction times (of the order of 1 sec.). Precooled solutions of the aromatic substrate and mercury (II) trifluoroacetate were mixed via two syringes. After specific time intervals, reaction mixtures were quenched with sodium bromide solution. The precipitated arylmercury (II) bromides were then dried and converted to bromoarenes using bromine in chloroform. Bromoarenes were subsequently analysed by gas/liquid chromatography. The relative rates measured for nine different mono-substituted aromatics correlated reasonably well with Hammett-Brown $\sigma^+$ constants giving a $\rho$ value of -6.31 (correlation coefficient 0.95). (Improved correlation (0.98) was found by using the Yukawa-Tsuno treatment, $\rho = -5.82$.) The results were indicative of the $\sigma^*$-complex nature of the transition state for mercuriation. This was also the implication of some additional experiments with polymethylbenzenes. The relative mercuriation rates of these were also determined by competitive methods, and the results compared with the relative stabilities of $\sigma^*$- and $\pi$-complexes of the substrates. A better correlation was found with $\sigma^*$-complex stabilities.

Olah et al. also studied the thalliation process in a similar manner. A toluene / benzene rate ratio of 35 was measured.
at 15°C, while the toluene isomer distribution was found to be 9% ortho, 5% meta and 86% para. These results remained approximately constant with reaction time at temperatures of 15°C and below. However, at higher temperatures (50°C, 73°C), the amounts of ortho- and meta-isomers increased with reaction time, at the expense of the kinetically favoured para-isomer, and the toluene/benzene rate ratio decreased dramatically. As with mercuriation, the relative reactivities of a series of mono-substituted aromatics were determined. Correlation of these with Hammett-Brown $\sigma^+$ constants resulted in a $\rho$ value of -6.92 (correlation coefficient 0.96). Interestingly, in the alkylbenzene series, the order of reactivity for thalliation ($t$-Bu > $i$-Pr > Et > Me) was found to be the reverse of that seen for mercuriation (Me > Et > $i$-Pr > $t$-Bu). Thalliation also proceeded with a greater para-selectivity, indicative of a greater steric requirement. The relative thalliation rates of poly-methylbenzenes were also measured. Comparison of these with $\sigma$- and $\pi$-basicities of the substrates resulted in similar correlations, so that this criterion was not conclusive in determining the nature of the transition state. However, the authors favoured a $\sigma$-complex intermediate, citing as evidence the aforementioned isotope effect data of Briody and Moore.46

The widely varying toluene/benzene rate ratios reported by Briody and Moore46 and by Olah et al.49 prompted Stock and coworkers49 to carry out some similar measurements. They studied competitive thalliation of benzene and toluene under the standard preparative conditions, i.e. reaction with TTFA in TFA at 25°C.
A series of experiments was conducted in which the toluene / benzene concentration ratio was varied from 0.05 to 0.2. The TTFA concentration was also varied by a factor of two. The reactions were allowed to proceed for 7 mins., then quenched with excess potassium iodide. The resulting iodoarenes were analysed by g.c., while toluene isomer distributions were determined by NMR. The toluene / benzene rate ratio was found to be reasonably constant at $43.5 \pm 5$ and the toluene isomer distribution was found to be $9.7\%$ ortho, $3.5\%$ meta and $86.8\%$ para. Partial rate factors were evaluated and the authors concluded that the results were wholly consistent with a conventional electrophilic substitution process via a Wheland intermediate.

In 1980, Roberts published the findings of an NMR study of aromatic thalliation and mercuriation. The large Tl-H couplings (see p.97) made it relatively straightforward to study thalliation by this method. Rate constants were evaluated by monitoring the appearance or disappearance of well-resolved signals in the $^1H$ spectrum, when arenes and TTFA were mixed in d-TFA. Experiments were conducted with benzene, toluene and t-butylbenzene and the reactivity order, $t$-Bu $>$ Me $>$ H was found, in accordance with Olah's results. The measured toluene / benzene rate ratio, 24, was however somewhat lower than in the earlier studies. Experiments with $d_5$-toluene, $C_6D_5CH_3$, resulted in a primary isotope effect of 5.0, indicating rate-determining proton transfer.

The parallel NMR study on mercuriation did not generate rate constants, but served to confirm Olah's observations of
isomerisation. The mercuriation of toluene, by mercury (II) trifluoroacetate in TFA, was conducted at 18°C. Initially, the para-isomer predominated, but after 26 hours, ortho-, meta- and para-isomers were present in comparable amounts. When the reaction was allowed to proceed for a longer time, the meta-isomer became predominant.

In further studies, Roberts and coworkers studied aromatic thalliation and mercuriation by spectrophotometric and titrimetric methods, as well as by NMR.51-62 On mixing aromatic substrates and the metallating agents in TFA, fleeting yellow colours were seen due to intermediate π-complexes. It was found possible to study the fate of these by monitoring the U.V./vis. spectrum in the range 300 - 350nm. Evidence pointed to a mechanism of the type described by equation 2.13:

\[
\text{ArH} + \text{Hg(OOCOCF}_3\text{)}_2 \xrightleftharpoons[k_1]{k_2} \text{[π-complex]} \rightarrow \text{products} \quad 2.13
\]

Using conditions where [ArH] >> [Hg(OOCOCF}_3\text{)}_2], equation 2.14 holds, where \( k_{obs} \) is the observed pseudo-first-order rate constant:

\[
\frac{1}{k_{obs}} = \frac{1}{k_1 k_2} \cdot \frac{1}{[\text{ArH}]} + \frac{1}{k_2} \quad 2.14
\]

The components of \( k_{obs} \) were thus separable. The results obtained from the U.V. and titrimetric studies were in excellent agreement. Values of \( k_1 \) and \( k_2 \) for mercuriation were determined for a series of simple aromatic substrates. Experiments with benzene and \( d_6 \)-benzene allowed isotope effects to be evaluated.
Ki values varied as expected for the formation of a π-complex, while the effect of substituent on $k_2$ was more marked. Taken in combination with Brown's partial rate factors, the results were used to construct a Hammett plot, correlating well with $\sigma^+$ values ($\rho = -5.8$). However, Roberts' results were in closer agreement with those of Olah, and using Olah's isomer ratios to calculate partial rate factors, a $\rho$ value of -6.4 was obtained from $\sigma^+$ correlations.

Isotope effects ($k_R/k_D$) were found to be 6.4 and 5.6 from the titrimetric and spectrophotometric methods respectively. Dissection of the isotope effect into those on $K_i$ and $k_2$ revealed that most of the effect was apparent on the $k_2$ values.

Analogous experiments carried out with TTFA showed the reactions to be very much faster than the earlier NMR study had indicated. A great anomaly was apparent, which Roberts attributed to impurities in commercial TTFA. In 1982, he effectively dismissed the 1980 NMR study, as the TTFA used was found to contain large amounts of thallium (I). Results were only consistent within a particular batch; variations in thallium (III) content were found between batches. Recrystallisation from TFA did not remove the impurities.

As a result, Roberts carried out subsequent work using thallium (III) acetate, which could be obtained in a high state of purity. It was acknowledged that the reactive species in TFA solution was likely to be $\text{Tl}((\text{Ac})_n(\text{OCOCF}_3)_{3-n})$, but this system allowed the collection of reproducible data. Rates of thalliation
were measured for a series of alkyl- and halobenzenes. These correlated well with $\epsilon^+$ values, yielding a $\rho$ value of -8.3, indicative of greater charge development during the reaction than in the case of mercuriation. However, the stability of TTFA-arene $\pi$-complexes showed a greater sensitivity towards substituent effects than the corresponding mercury complexes.\textsuperscript{52}

In 1984, Lau and Kochi\textsuperscript{53} reported on an extensive kinetic and mechanistic study of thalliation, using a series of polymethylbenzenes. Preparative work with these substrates was indicative of three major reaction types, namely nuclear substitution (A), biaryl coupling (B) and side-chain substitution (C):

\[
\text{ArH} + \text{TI(OCOCF}_3\text{)}_3 \rightarrow \text{ArTi(OCOCF}_3\text{)}_2 \quad \text{(A)}
\]

\[
\text{ArH} + \text{TI(OCOCF}_3\text{)}_3 \rightarrow \frac{1}{2}\text{Ar-Ar} \quad \text{(B)}
\]

\[
\text{ArH} + \text{TI(OCOCF}_3\text{)}_3 \rightarrow \text{ArCH}_2-\text{OCOCF}_3 \quad \text{(C)}
\]

Fig. 2.2: Reactions of Polymethylbenzenes with TTFA (from Ref.53).

In the case of mesitylene (1,3,5-trimethylbenzene), pathway (A), leading to mesitylthallium bis(trifluoroacetate), was the only significant reaction. Likewise, only one product was formed from the reaction of hexamethylbenzene, derived from exclusive side-chain substitution (C). The intermediate substrates, durene (1,2,4,5-tetramethylbenzene) and pentamethylbenzene, gave rise to a mixture of products, derived from all three reaction types, (A), (B) and (C).
Despite these apparently disparate pathways, each reaction proceeded via a π-complex intermediate, as indicated by a transient yellow colour. Kinetic studies were based upon spectrophotometric measurements of the decay of the associated charge-transfer bands, as the π-complexes collapsed to products. Quantitative measurements indicated that $\text{Tl(OCOCF}_3\text{)}^2$ was the sole species involved in π-complex formation, derived from dissociation of TTFA.

$$\text{Tl(OCOCF}_3\text{)}_3 \xrightarrow{K_1} \text{Tl(OCOCF}_3\text{)}^2 + \text{CF}_3\text{CO}_2^-$$

2.15

It was established that $K_1$, the dissociation constant for equation 2.15, was around $10^{-3}$M, and that the reactions of TTFA could be regulated by the addition of lithium trifluoroacetate. Lau and Kochi questioned Roberts' dismissal of his NMR study, suggesting that the discrepancy between the rates measured by NMR and those measured spectrophotometrically was due to differential dissociation at the concentrations involved, rather than impurities in the TTFA.

ESR studies showed that the processes leading to biaryls (B) and side-chain substituted products (C) involved radical cation intermediates, whereas no radical intermediates were detected in the reaction with mesitylene (A). The authors made the point that the thalliation of substrates such as durene provided a somewhat unusual example of circumstances in which competing electrophilic (2 electron) and electron-transfer (1 electron) processes occurred under the same experimental conditions. It was also noted that
the seemingly very different processes (A) and (C) proceeded at very similar rates.

In a subsequent publication, Lau and Kochi compared their results on thalliation with those from a parallel study of mercuriation, using the same series of substrates. Transient yellow colours were again seen, although the colour was permanent in the case of hexamethylbenzene. The products obtained were quite different in that only pathway (A) was in operation. In other words, the sole products were nuclear mercuriated derivatives and no products derived from radical cation intermediates could be detected. Since nuclear mercuriation is not possible in the case of hexamethylbenzene, the reaction apparently stopped at the π-complex stage.

Spectrophotometric studies confirmed that dissociation of mercury (II) trifluoroacetate, in an analogous way to equation 2.15, was not involved. The addition of lithium trifluoroacetate had no effect on the reaction rates. Thus the electrophiles in thalliation and mercuriation were shown to be Tl(OCOCF₃)₂⁺ and Hg(OCOCF₃)₂ respectively, isostructural species differing only in charge. Both metallations were shown to occur via π-complexes, but only in the case of thalliation were radical cations shown to be involved. Mercuriation appears to be a straightforward electrophilic reaction but thalliation of electron-rich substrates is complicated by competing electron-transfer processes (equation 2.16).
\[ \text{ArH} + \text{Ti(OCOCF}_3\text{)}_2^+ \rightleftharpoons [\text{Ti(OCOCF}_3\text{)}_2, \text{ArH}^+] \]

The ion-pair, \([\text{Ti(OCOCF}_3\text{)}_2, \text{ArH}^+]\), can then collapse to a \(\sigma\)-complex, leading to electrophilic substitution, or can undergo diffusive separation, allowing further reactions of the radical cation. This model has been used to account for the observation that the dilution of TFA with diethyl ether suppresses radical reactions.\(^{10}\) Solvation by TFA is believed to facilitate the separation of the ion-pair.
2.4 Experimental

2.4.1 Thalliation and Mercuriation - Simple Preparative Work

(a) Thalliation of Simple Arenes

Thallium (III) trifluoroacetate (TTFA) was obtained from Aldrich. The compound was found to be very sensitive to air and moisture. A portion of the off-white powder turned brown within seconds on exposure to air. In view of this, all manipulations involving the compound were carried out in a glove bag (Aldrich AtmosBag) under an atmosphere of dry nitrogen.

(i) Toluene

Mixing equimolar (0.1 M) solutions of toluene and TTFA in TFA resulted in the immediate formation of a white precipitate, p-tolylthallium bis(trifluoroacetate). This was filtered off, washed with a little dichloromethane and then dried in vacuo.

The 'H NMR spectrum was recorded in de-DMSO solution and matched published examples. Coupling constants (J_{Th-H}) were found to be 1014 Hz for the ortho protons and 368 Hz for the meta protons. McKillop et al. obtained values of 1025 and 376 Hz for the same system. They also found the para-methyl group signal to be split by 66 Hz. Such a measurement was not made from the spectrum shown in Fig. 2.3, since this part of the spectrum would appear to be complicated by additional signals. Nevertheless, the difference in shift between the two peaks does seem consistent with a splitting of the order of 60 Hz.
(ii) Other Methylbenzenes

White precipitates were also produced when TTFA / TFA was mixed with TFA solutions of m-xylene, p-xylene and mesitylene. The $^1$H NMR spectra of these products were essentially as reported by McKillop et al., although considerable amounts of impurities were evident.
(b) Mercuriation of Simple Arenes

Mercury (II) trifluoroacetate was obtained from Aldrich as a white powder. It was found to be stable in air and the conditions used to handle TTFA were not found to be necessary.

(i) Toluene

The mercuriation of toluene was carried out by adding a small amount of toluene (0.1g) to ca. 5mLs of a 0.5M solution of mercury (II) trifluoroacetate in TFA. The mixture was stirred for ca. 16 hours. The TFA was then cautiously removed by a slow stream of nitrogen gas. Fig. 2.4 shows the \(^1\)H NMR spectrum of the crude product in CDCl\(_3\) solution:

![NMR Spectrum of Mercuriated Toluene in CDCl\(_3\)](image)

Fig. 2.4: \(^1\)H NMR Spectrum of Mercuriated Toluene in CDCl\(_3\)

The presence of three discrete methyl signals at around 2.2 ppm is clearly an indication of three different products being present. When the methyl signals were given an arbitrary integral of 3, the integral of the aromatic region was found to be 3.8, indicating at least one mercuriation per toluene molecule.
Iododemercuration was performed using a method similar to that employed by Deacon and Farquaharson. The mercuriated toluene was stirred in DMF for several days with excess potassium iodide and iodine. The products were ultimately obtained by pouring the reaction mixture into a stirred solution of sodium metabisulphite containing some chloroform. The layers were separated and mercuric ions were precipitated from the aqueous portion by treatment with sodium sulphide. The aqueous solution was extracted with further portions of chloroform and combined chloroform extracts were washed of DMF using large amounts of water. Evaporation of the dried extracts afforded a mixture of iodotoluenes, the NMR spectrum of which is shown in Fig. 2.5:

![Fig. 2.5: $^1$H NMR Spectrum (Aromatic Region) of Iodotoluenes Obtained from Demercuration of Mercuriated Toluene](image)

Integration of the spectrum reproduced in Fig. 2.5 resulted in an estimated isomer distribution of 35% ortho, 15% meta and 50% para-iodotoluene.
A portion of anisole (0.1g) was added to a solution of mercury (II) trifluoroacetate in TFA (5mls, ca. 0.5M), and the mixture was left overnight. The volatiles were then removed by a slow stream of nitrogen gas, to leave a solid with a slight purple colouration. A portion of the crude residue was taken up in CDCl₃ and the 'H NMR spectrum was recorded (Fig.2.6):

Fig. 2.6 : 'H NMR Spectrum of Mercuriated Anisoles in CDCl₃

The mercuriated anisole was first washed of excess mercury (II) trifluoroacetate. It was then treated with excess iodine and potassium iodide in DMF, in a similar manner to the mercuriated toluene described above. After ca. 3 days, chloroform extraction was carried out as before. Evaporation of the dried extracts afforded a mixture of iodoanisoles, as is evident from the spectrum shown in Fig. 2.7. Integration of the appropriate peaks resulted in an estimated isomer distribution of 75-80% α-iodoanisole and 20-25% p-iodoanisole.
Fig. 2.7: $^1$H NMR Spectrum (Aromatic Region) of Iodoanisoles Obtained from Demercuriation of Mercuriated Anisoles

(iii) Chlorobenzene

A portion of chlorobenzene (0.15g) was added to a 0.26 M solution of mercury (II) trifluoroacetate in TFA (ca. 10 mls). The mixture was allowed to stand for 72 hours at room temperature. Excess potassium iodide was then added to precipitate chlorophenyl-mercury (II) iodides. The white solid was filtered off and washed with dichloromethane. After drying, it was treated with a solution of iodine in chloroform. After six hours of stirring, excess iodine was removed by treatment with aqueous sodium metabisulphite. The chloroform solution was washed with water and then dried over anhydrous sodium sulphate. Evaporation of the solvent afforded a yellow paste. This was taken up in CDCl$_3$ and the $^1$H NMR spectrum was recorded. The only significant product was $p$-chloroiodobenzene; only a trace of the $o$-isomer was detected.
2.4.2 NMR Studies of Aromatic Thalliation and Mercuration

(a) $^1$H NMR

Initial kinetic experiments were attempted using $^1$H NMR spectroscopy to monitor reactions. Several attempts were made to repeat Roberts' experiment of following the thalliation of toluene by NMR. For this purpose an NMR job file was set up to record a series of $^1$H spectra in $d$-TFA solution at specified intervals. Solutions of toluene and TTFA were prepared in $d$-TFA, and then equimolar quantities were mixed in NMR tubes. Spectra were recorded as soon as possible after mixing. At concentrations in excess of 0.1M, precipitation of the product tolylthallium bis(trifluoroacetate) occurred. At lower concentrations, the characteristic pattern of meta and ortho $^{203,205}$Tl-H coupling developed, as depicted in Fig 2.3.

Roberts monitored the reaction with toluene over a period of 40 minutes. In an attempt to repeat this experiment, ten spectra were recorded at intervals of 5 minutes. On examining the results it was found that only the first two spectra were in any way different. Equilibrium had apparently been reached within 10 minutes so that spectra numbers 3-10 were essentially identical.

The thalliation of anisole in $d$-TFA was also studied by $^1$H NMR, albeit only qualitatively. On mixing the reagents a set of four signals at $\delta = 9.0, 7.6, 6.5$ and 5.7 ppm appeared, similar to those seen with toluene. However, over a period of 48 hours, the pattern became much more complex, consistent with the observation that ortho-thalliated anisole accumulates over long periods.56
Initial experiments in this area were carried out using a sample of low activity $^3$H-toluene which had been tritiated in all ring positions. A $^3$H NMR spectrum in $d$-TFA solution showed three well-resolved signals from the three ring positions. When mercury (II) or thallium (III) trifluoroacetate was added, a sharp decrease in the signal-to-noise ratio was observed, consistent with rapid metallo-detrinitiation. The relative integrals of the three signals remained approximately constant, suggesting detrinitiation at all ring positions at similar rates. No new $^3$H signals appeared in the spectra, even when the spectral window was increased to cover shifts of 15 ppm. A broad $^3$H-TFA signal had been expected.

Fig. 2.8: Examples of $^3$H NMR Spectra of Low-Activity Toluene; (a) Before, and (b) After the Addition of Hg(OOCF$_3$)$_2$
Little useful information could be gleaned from the above experiments with low-activity [3H]-toluene. The next approach was to employ specifically-labelled toluene of high specific activity, so that 3H NMR signals could be obtained within a matter of minutes.

Catalytic Dehalogenation of 4-Bromotoluene:

High specific activity (4-3H)-toluene was prepared by the catalytic tritiodehalogenation of 4-bromotoluene using tritium gas. A mixture of 4-bromotoluene (45mg), 5% palladium on charcoal (50mg) and triethylamine (0.3mls) was stirred under tritium gas (2Ci, 0.8cm³) for 3½ hours. The mixture was then left overnight in the presence of excess hydrogen to ensure complete dehalogenation. The reaction mixture was then taken up in diethyl ether (5mls) and the catalyst was removed by filtration. The ether solution was then washed with 2N hydrochloric acid (2 x 2mls) and water (2 x 2mls), then dried over anhydrous magnesium sulphate.

The ether solution was found to contain around 700µCi. Since the maximum amount of toluene recoverable was 22mg, the minimum specific activity was 2.8 Ci/mmol.

NMR Experiments:

A 0.5ml portion of the aforementioned ether solution was evaporated and the residue was taken up in 200µl of d-TFA. A 3H NMR signal with adequate signal-to-noise could be obtained within 2 - 3 minutes (Fig. 2.9 (a)). Addition of mercury (II) trifluoroacetate to this solution caused a marked decrease in signal-to-noise, but even with 70µCi present in an NMR tube, no signal from [3H]-TFA
appeared. Clearly, a standard was required to enable the rate of
loss of tritium from the toluene to be measured. In order to
preserve the simplicity of the system, an external standard was
sought rather than an internal one.

A sealed capillary was prepared containing 20mCi of 5Ci/ml
tritiated water. A tritiated aprotic solvent (e.g. DMSO) would have
been preferable as a standard, but the specific activity required
made this impractical. The water-containing capillary was inserted
into a Teflon NMR tube and held centrally by two Teflon rings.
The tube was then filled with a suitable amount of [4-3H3]-toluene
in d-TFA and spectra were accumulated. Unfortunately, it was found
that a sharp 3H signal could not be obtained from both components
simultaneously. The inclusion of the capillary caused the single
sharp line from the toluene to become a broad, indefinite feature.
When the spectrometer was tuned to give a reasonably good signal
from the toluene, the water signal became broad (Fig. 2.9 (b)).

From these findings, it was concluded that an internal
standard was necessary. Tritiated dichloromethane would have been
desirable, but labelling of this solvent is by no means trivial.
Instead, chloroform was chosen because of the ease with which it
can be tritiated via base-catalysed exchange. This was carried out
by stirring d-chloroform (1ml), dioxan (0.5ml) and tritiated water
(5µl,20Ci/ml) for 28 hours in the presence of a KOH pellet. In
this manner, 13mCi was incorporated into the chloroform. This was
mixed with a solution of [4-3H]-toluene in d-TFA and the 3H
spectrum was recorded. Only one signal was observed, suggesting that
the resonances from the labelled chloroform and the 4-position of the toluene were non-resolvable (Fig. 2.9 (c)).

Despite this setback, several experiments were carried out in which mercury (II) trifluoroacetate was added to such mixtures. Fig. 2.9 (d) shows an example in which a minor $^3$H signal appeared at $\delta = 6.88$ ppm. However, the spectrum did not develop further, and so the ultimate aim of the work, to monitor the progress of the reaction over 10 or more spectra, was not achieved.

Further work in this area was not permitted because of problems encountered in the disposal of waste containing both tritium and thallium.
Fig. 2.9: Examples of $^3$H Spectra Obtained with High Specific Activity [4-3H]-Toluene:

(a) $^3$H NMR Spectrum of [4-3H]-Toluene in d-TFA (less than 3 minutes accumulation)

(b) As Above, with Capillary of HTU

(c) $^3$H NMR Spectrum of [4-3H]-Toluene/[3H]-Chloroform in CDCl$_3$

(d) $^3$H NMR Spectrum of [4-3H]-Toluene/[3H]-Chloroform in CDCl$_3$, After the Addition of Mercury (II) Trifluoroacetate
On mixing TFA solutions of arenes and thallium (III) or mercury (II) trifluoroacetates, a yellow colour appears which then fades over a period of minutes. This is due to the formation of an intermediate π-complex. Roberts et al. and Lau and Kochi have carried out quantitative spectroscopic studies of the decay of such transient species, which are described in some detail in Section 2.3. A feature of Lau and Kochi's work is the degree of sophistication of their apparatus and the stringent precautions they took to protect reactions from air and moisture. It was not practicable to impose such rigorous precautions during the course of this study, but it was nevertheless decided to carry out a limited investigation with more modest apparatus.

TFA was first purified by distillation from phosphorus (V) oxide. A middle cut, b.pt. 73°C, was collected. Stock solutions of thallium (III) and mercury (II) trifluoroacetates in TFA were then prepared by dispensing the compounds into volumetric flasks under nitrogen in a glove bag. Stock solutions of aromatics were also prepared in TFA, generally at ten times the concentration of the metallating agent. This allowed a simple experimental procedure to be adopted in which 0.1 ml of arene solution was added to 1 ml of thallium (III) or mercury (II) solution in a 5mm U.V. cell, to give an equimolar mixture of the two reactants. The mixture was rapidly shaken and then the automated collection of absorbances at a fixed wavelength at timed intervals was initiated.
Reactions were generally complete within two minutes and intervals of 2 - 5 seconds were used to obtain 16 measurements. Mixtures were then left for 30 - 60 minutes in order to obtain an "infinity" absorbance reading. This infinity value, $A_\infty$, was then subtracted from the measured absorbances, $A$, and $\ln(A - A_\infty)$ was plotted against time. Linear first order plots were obtained in most cases.

(a) Thalliation

Experiments of this nature were first carried out using $m$- and $p$-xylenes. The first step was to locate the position of the absorbance maxima of the $\pi$-complexes with TTFA. These were found at 326nm and 311nm for $m$- and $p$-xylene respectively, in agreement with the results of Lau and Kochi.$^{54}$

Exploratory experiments were conducted with $m$-xylene. Mixing equimolar amounts of this substrate and TTFA resulted in a series of absorbances, yielding a very good linear plot of $\ln(A - A_\infty)$ vs. time. However, reproducibility between experiments was very poor, and it was soon realised that TTFA stock solutions had a very short shelf-life. In fact, such solutions, even if tightly stoppered, became completely ineffective when stored overnight.

In further experiments, it was found that the use of excess TTFA resulted in curved plots. When reactions were attempted in 1 : 1 TFA - AcOH, no $\pi$-complex absorption could be detected.
Table 2.1 shows a sample of results from initial studies on the reaction of TTFA with \( p \) and \( m \)-xylenes. The \( m \)-xylene - TTFA \( \pi \)-complex is seen to decay ca. 3 times faster than the \( p \)-xylene-TTFA complex.

Table 2.1: Rates of Xylene - TTFA \( \pi \)-Complex Decay, 25°C

(i) \( p \)-Xylene - \( \lambda = 311 \)nm

<table>
<thead>
<tr>
<th>( [p-Xylene]/M )</th>
<th>( [TTFA]/M )</th>
<th>( k/s^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 9.0 \times 10^{-3} )</td>
<td>( 9.0 \times 10^{-3} )</td>
<td>0.034</td>
</tr>
<tr>
<td>( 9.0 \times 10^{-3} )</td>
<td>( 9.0 \times 10^{-3} )</td>
<td>0.033</td>
</tr>
</tbody>
</table>

(ii) \( m \)-Xylene - \( \lambda = 326 \)nm

<table>
<thead>
<tr>
<th>( [m-Xylene]/M )</th>
<th>( [TTFA]/M )</th>
<th>( k/s^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 9.0 \times 10^{-3} )</td>
<td>( 9.0 \times 10^{-3} )</td>
<td>0.087</td>
</tr>
<tr>
<td>( 9.0 \times 10^{-3} )</td>
<td>( 9.0 \times 10^{-3} )</td>
<td>0.107</td>
</tr>
</tbody>
</table>

(b) Mercuriation

Experiments analogous to those described in part (a) above were performed using mercury (II) trifluoroacetate. In exploratory experiments, it was found that steady "infinity" absorbances were not obtained if excess mercury salt was used. This effect seems most likely to be attributable to polymermercuriation.

The results with \( p \)-xylene (Table 2.2 (i)) seem to indicate that the rate of \( \pi \)-complex decay is independent of the reactant concentrations. This is consistent with the rapid formation of an intermediate complex which then decays more slowly.
Table 2.2: Rates of Xylene - Mercury (II) Trifluoroacetate $\pi$-Complex Decay, 25°C

(i) $p$-Xylene - $\lambda$ = 310nm

<table>
<thead>
<tr>
<th>[p-Xylene]/M</th>
<th>[Hg(OCOCF$\text{$_3$}$)$_2$]/M</th>
<th>k/s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.027</td>
<td>0.027</td>
<td>0.019</td>
</tr>
<tr>
<td>0.05</td>
<td>0.01</td>
<td>0.023</td>
</tr>
<tr>
<td>0.05</td>
<td>0.005</td>
<td>0.022</td>
</tr>
</tbody>
</table>

(ii) $m$-Xylene - $\lambda$ = 320nm

<table>
<thead>
<tr>
<th>[m-Xylene]/M</th>
<th>[Hg(OCOCF$\text{$_3$}$)$_2$]/M</th>
<th>k/s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>0.006</td>
<td>0.195</td>
</tr>
<tr>
<td>0.06</td>
<td>0.006</td>
<td>0.196</td>
</tr>
<tr>
<td>0.06</td>
<td>0.012</td>
<td>0.233</td>
</tr>
<tr>
<td>0.06</td>
<td>0.012</td>
<td>0.215</td>
</tr>
</tbody>
</table>

Experiments with $m$-xylene (Table 2.2 (ii)) generated rate constants an order of magnitude greater than those obtained with $p$-xylene. These relative rates compare favourably with the relative reactivities estimated by Lau and Kochi$^{54}$, which were 360 and 41 for the two compounds, compared to 1 for benzene.
Chlorobenzene

On mixing TFA solutions of mercury (II) trifluoroacetate and chlorobenzene a small but measurable absorbance appeared at around 320nm in the UV spectrum, seemingly representing a low, equilibrium concentration of a π-complex. The absorbance at this wavelength was measured at intervals over a period of 30 minutes and a linear plot of \( \ln(A - A_\infty) \) against \( t \) was obtained.

Table 2.3: Rate of Decay of Absorbance at 320nm from Chlorobenzene – Mercury (II) Trifluoroacetate Mixture; 25°C.

<table>
<thead>
<tr>
<th>[chlorobenzene]/M</th>
<th>[Hg(COCF₃)₂]/M</th>
<th>k/s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.011</td>
<td>0.011</td>
<td>1.11 \times 10⁻³</td>
</tr>
</tbody>
</table>
2.4.4 Detritiation Experiments

The replacement of an aromatic proton by thallium or mercury in the metallation process presents the possibility of measuring rates of reaction by a detritiation method.

\[
\begin{align*}
\text{Hg}(\text{OCOF}_3)_2 & \quad \rightarrow \quad \text{CF}_3\text{COOT} \\
\text{HgOCOF}_3
\end{align*}
\]

The UV experiments described in Section 2.4.3 indicated that the metallation of compounds such as toluene and xylene is fast and therefore not amenable to the detritiation technique. In view of this, initial experiments in this area were carried out using chlorobenzene, on the assumption that this would react much more slowly. The measurement of the decay of its \( \pi \)-complex with mercury (II) trifluoroacetate was consistent with this point of view. Chlorobenzene was also chosen because samples of specifically labelled \( [2-^3\text{H}]^-, [3-^3\text{H}]^- \) and \( [4-^3\text{H}]^- \)-chlorobenzene were available in the radiochemistry laboratory.*

The procedure adopted was as follows. Mercury (II) trifluoroacetate was weighed into a volumetric flask and then made up with TFA to give a solution of 0.1 - 0.2 M. This solution was then thermostatted at 25°C. A droplet of tritiated substrate was added, small enough to give pseudo first order conditions. Aliquots

* - These were prepared by Dr. J.R. Brewer
were removed at timed intervals and pipetted into test tubes containing 10 ml of aqueous sodium hydroxide solution and 10 ml of toluene based scintillator (3.4g dm⁻³ PPO). After vigorous shaking the layers were allowed to separate. Most of the toluene layer was then pipetted off and dried over anhydrous sodium sulphate. The extract was then assayed for tritium by means of a Beckmann LS-100 liquid scintillation counter.

Counts were seen to decrease with each successive extract, although the results were far from perfect. Anomalous counts were quite common, and large "infinity" counts had to be subtracted from the measured counts in order to obtain a linear ln (DPM) vs. time plot.

Despite these potential sources of error, a reasonably high percentage of experiments led to acceptable linear plots, and the results derived from these are listed in Tables 2.4 - 2.11. All experiments were conducted at 25 ± 0.1°C.

Table 2.4 : Results of Detritiations of [4⁻³H]-Chlorobenzene in TFA

<table>
<thead>
<tr>
<th>[Hg(OCOCF₃)₂]/M</th>
<th>k/s⁻¹</th>
<th>k₂/M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.109</td>
<td>3.47 x 10⁻⁵</td>
<td>3.18 x 10⁻⁴</td>
</tr>
<tr>
<td>0.145</td>
<td>5.05 x 10⁻⁵</td>
<td>3.49 x 10⁻⁴ t</td>
</tr>
<tr>
<td>0.145</td>
<td>5.39 x 10⁻⁵</td>
<td>3.72 x 10⁻⁴ t t</td>
</tr>
<tr>
<td>0.145</td>
<td>4.59 x 10⁻⁵</td>
<td>3.17 x 10⁻⁴</td>
</tr>
<tr>
<td>0.152</td>
<td>5.55 x 10⁻⁵</td>
<td>3.65 x 10⁻⁴</td>
</tr>
</tbody>
</table>

 t - with [2⁻³H]-chlorobenzene; t t - with [3⁻³H]-chlorobenzene.
Table 2.5: Results of Detritiation of [4-3H]-Anisole in TFA

<table>
<thead>
<tr>
<th>[Hg(OCOCF₃)₂]/M</th>
<th>k/s⁻¹</th>
<th>k₂/M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.122</td>
<td>4.27 x 10⁻³</td>
<td>0.035</td>
</tr>
<tr>
<td>0.179</td>
<td>5.76 x 10⁻³</td>
<td>0.032</td>
</tr>
<tr>
<td>0.220</td>
<td>7.45 x 10⁻³</td>
<td>0.034</td>
</tr>
<tr>
<td>0.231</td>
<td>7.66 x 10⁻³</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Table 2.6: Results of Detritiation of [2-3H]-Anisole in TFA

<table>
<thead>
<tr>
<th>[Hg(OCOCF₃)₂]/M</th>
<th>k/s⁻¹</th>
<th>k₂/M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.081</td>
<td>3.29 x 10⁻⁴</td>
<td>4.06 x 10⁻³</td>
</tr>
<tr>
<td>0.097</td>
<td>3.95 x 10⁻⁴</td>
<td>4.07 x 10⁻³</td>
</tr>
<tr>
<td>0.116</td>
<td>4.42 x 10⁻⁴</td>
<td>3.81 x 10⁻³</td>
</tr>
<tr>
<td>0.118</td>
<td>4.29 x 10⁻⁴</td>
<td>3.64 x 10⁻³</td>
</tr>
<tr>
<td>0.256</td>
<td>8.37 x 10⁻⁴</td>
<td>3.27 x 10⁻³</td>
</tr>
<tr>
<td>0.786</td>
<td>8.38 x 10⁻⁴</td>
<td>(1.07 x 10⁻³)</td>
</tr>
</tbody>
</table>

Table 2.7: Results of Detritiation of [4-3H]-Toluene in TFA

<table>
<thead>
<tr>
<th>[Hg(OCOCF₃)₂]/M</th>
<th>k/s⁻¹</th>
<th>k₂/M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.114</td>
<td>1.82 x 10⁻³</td>
<td>0.016</td>
</tr>
<tr>
<td>0.114</td>
<td>2.31 x 10⁻³</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Table 2.8: Results of Detritiation of [2-3H]-Bromobenzene in TFA

<table>
<thead>
<tr>
<th>[Hg(OCOCF₃)₂]/M</th>
<th>k/s⁻¹</th>
<th>k₂/M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.120</td>
<td>1.21 x 10⁻⁴</td>
<td>1.0 x 10⁻³</td>
</tr>
<tr>
<td>0.151</td>
<td>1.52 x 10⁻⁴</td>
<td>1.0 x 10⁻³</td>
</tr>
</tbody>
</table>
Table 2.9: Results of Detritiation of [4-{\textsuperscript{3}H}]-Anisole in Dichloromethane

<table>
<thead>
<tr>
<th>[Hg(OCOCF\textsubscript{3})\textsubscript{2}]/M/M</th>
<th>k/s\textsuperscript{-1}</th>
<th>k\textsubscript{2}/M\textsuperscript{-1}s\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.038</td>
<td>1.33 \times 10^{-4}</td>
<td>3.55 \times 10^{-2}</td>
</tr>
<tr>
<td>0.060</td>
<td>2.17 \times 10^{-4}</td>
<td>3.61 \times 10^{-2}</td>
</tr>
<tr>
<td>0.075</td>
<td>2.58 \times 10^{-4}</td>
<td>3.44 \times 10^{-2}</td>
</tr>
</tbody>
</table>

Table 2.10: Result of Detritiation of [4-{\textsuperscript{3}H}]-Toluene in Dichloromethane

<table>
<thead>
<tr>
<th>[Hg(OCOCF\textsubscript{3})\textsubscript{2}]/M/M</th>
<th>k/s\textsuperscript{-1}</th>
<th>k\textsubscript{2}/M\textsuperscript{-1}s\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.029</td>
<td>4.56 \times 10^{-5}</td>
<td>1.6 \times 10^{-3}</td>
</tr>
</tbody>
</table>

Table 2.11: Detritiation of [4-{\textsuperscript{3}H}]-Chlorobenzene in Dichloromethane

No detritiation observed over a period of ≈100 hours.

Table 2.12: Comparison of Detritiation Results in TFA and Dichloromethane (k\textsubscript{2} values, M\textsuperscript{-1}s\textsuperscript{-1})

<table>
<thead>
<tr>
<th></th>
<th>TFA</th>
<th>Dichloromethane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisole</td>
<td>0.033</td>
<td>3.5 \times 10^{-2}</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.02</td>
<td>1.6 \times 10^{-3}</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>3.4 \times 10^{-4}</td>
<td>No Reaction</td>
</tr>
</tbody>
</table>
2.5 Discussion

The thalliation and mercuriation of aromatic compounds has been extensively studied, resulting in a vast body of literature. As noted in the introductory sections, the important differences between the two metallation processes are that polymermercuriation can occur whereas only mono-thalliation can be achieved, and that mercuriation is more versatile in terms of the solvents which can be used.

Since the metallation processes, and the subsequent iodode-metallation processes have been well-studied, the simple preparative experiments described in Section 2.4.1 could do no more than confirm previous observations. Thus, when the thalliation of toluene was carried out using suitably high concentrations of reactants, only the para-substituted product, p-tolythallium bis(trifluoroacetate), was produced. This high regiospecificity arises because of the insolubility of the product, not because the Tl(OCOCF₃)₂⁺ electrophile possesses extreme selectivity. In dilute TFA solution at 25°C, Stock et al.⁴⁷ obtained an o : m : p ratio of 9.7 : 3.5 : 86.8 for the thalliation of toluene and similar percentages were reported by Olah et al.⁴⁸ Nevertheless, the reaction is one of the most para-selective of all electrophilic aromatic substitutions on toluene.

Preparative-scale thalliation was not performed on anisole, but qualitative NMR experiments (p.103) were also consistent with literature reports. The initial spectrum showed four broad, well-separated peaks, diagnostic of ortho and meta Tl-H coupling, and
thus consistent with \textit{para}-thalliation. However, spectra recorded at
intervals over a 48 hour period became progressively more complex,
as isomerisation to the thermodynamically more stable \textit{ortho}-isomer
occurred.\textsuperscript{46} It is also likely that the spectra became more complex
due to oxidation of the anisole, and its consequent polymerisation.
As noted earlier, the system TTFA / TFA possesses considerable
oxidising power ($\text{TI}^{3+} + 2e^- \rightarrow \text{TI}^+; E^0 = -1.25\text{v}^{\circ}$). This is another
factor which must be borne in mind when considering possible
radiopharmaceutical applications.

Preparative mercuriations did not produce any unexpected
results. After a reaction time of 16 hours, mercuriated toluenes
were converted to the corresponding iodo- derivatives, revealing
an isomer distribution, \(o : m : p\), of 35 : 15 : 50. This is wholly
consistent with the results of Olah \textit{et al.}\textsuperscript{46}, who obtained an
\(o : m : p\) ratio of 23.3 : 7.9 : 68.8 after 1 minute, 32.5 : 10.1 :
57.4 after 15 minutes and 37.4 : 12.6 : 50 after 160 minutes (22°C).

Anisole gave a preponderance of the \textit{ortho}-mercuriated
isomer, when the reaction was allowed to proceed overnight. As in
the case of thalliation, this can be accounted for in terms of
accumulation of the thermodynamically favoured product, formed with
the assistance of an intramolecular interaction between the metal
and the oxygen of the methoxy group (cf. Fig 2.1, p.73).

Chlorobenzene was mercuriated, and hence iodinated, almost
exclusively at the \textit{para}-position. Again, this is in good agreement
with the findings of Olah \textit{et al.}\textsuperscript{46}, whose \(p\)-chloroiodobenzene was
contaminated by no more than 1-2\% of the \textit{ortho} and \textit{meta}-isomers.
During the course of the aforementioned work, the iodode-
mercuriation process appeared to be less problematic than some
literature reports, e.g. Ref. 24, had suggested. In Section 2.4.1 (b),
it was reported that the demercuriation reactions with mercuriated
toluene and anisole were allowed to proceed for up to 3 days.
However, subsequent experiments suggested that this was unnecessary.
Complete demercuriation was seemingly achieved within a few hours,
using excess iodine and potassium iodide in DMF. Of course, such a
procedure could not be used in high-activity radiodiodination, and,
in this respect, the rapid iododethalliation reaction with iodide
ions would seem particularly advantageous.

Section 2.4.2 describes some experiments aimed at monitoring
the progress of thalliation and mercuriation by NMR spectroscopy.
The 'H NMR experiment with toluene (p. 103) supported Roberts' 1982
statement52 regarding his 1980 NMR publication.50 Thus, the rate of
thalliation was found to be much faster than the NMR study had
indicated. Attempts were then made to study the thalliation of
toluene using tritium-labelled material. However, as described in
Section 2.4.2 (b), these experiments were largely unsuccessful. Work
in this area had to be terminated because waste containing both
tritium and thallium or mercury was deemed to be unacceptable.

Sections 2.4.3 and 2.4.4 describe further attempted kinetic
studies of the metallations. Yellow-coloured π-complexes were seen
to accompany the reactions of m- and p-xylenes, and the maxima of
these corresponded to the wavelengths reported by Lau and Kochi.54
First-order rate constants were measured, and the results are given
in Tables 2.1 and 2.2. In view of the high sensitivity of TTFA solutions towards air and moisture, the accuracy of the results in Table 2.1 is questionable. In contrast, mercuriation did not seem to be adversely affected by air and moisture, hence the results in Table 2.2 are thought to be somewhat more reliable. As noted earlier, \( m \)-xylene reacted about an order of magnitude faster than \( p \)-xylene, consistent with the data of Lau and Kochi.\(^{54}\)

Section 2.4.4 describes some detritiation experiments which were carried out in a further attempt to get some appraisal of metallation rates. These were conducted exclusively with mercury (II) trifluoroacetate, in view of the instability of TTFA.

Considering first the results in Table 2.4, it can be seen that \([2-{^2}\text{H}]-, [3-{^3}\text{H}]-\) and \([4-{^3}\text{H}]-\) chlorobenzene were all detritiated at the same rate. This result was somewhat unexpected, in view of the high para-selectivity seen in the preparative-scale experiments described earlier. A possible explanation is isomerisation of the Wheland intermediate, as described by Olah and coworkers.\(^{58}\) Studies of such arenemercurinium ions by NMR suggested that they were in rapid exchange via \( \pi \)-complex intermediates, as shown in Fig 2.10:

\[
\text{Fig. 2.10: Isomerisation of Arenemercurinium Ions (Ref. 58)}
\]
Alternatively, the lack of distinction between the three regioisomers may have been due to the conditions employed. Under pseudo first-order conditions, with only a trace of substrate, poly- or even permurcuriation may have occurred. Another factor, which was not properly considered at the time of the experiments, was the kinetic isotope effect that was almost certainly involved. As noted in Section 2.3, deuterium isotope effects in the range 4.7 - 6.8 have been reported. Thus, a tritium isotope effect as high as 18 is not inconceivable.

In contrast to chlorobenzene, [2-3H]- and [4-3H]-anisoles were not detritiated at similar rates. By comparing the results in Tables 2.5 and 2.6, it can be seen that [4-3H]-anisole was detritiated an order of magnitude faster than its [2-3H]-counterpart. The results in Table 2.6 also show that the rate constants, k, did not continue to increase when the Hg({OCOCF}_3)_2 concentration was increased beyond about 0.2 M; the values of k obtained with [Hg({OCOCF}_3)_2] = 0.256 M and 0.786 M were virtually identical.

Table 2.12 compares the averaged k_2 values for detritiation in TFA and dichloromethane. For anisole and toluene, the reactions are seen to be an order of magnitude faster in TFA, but for chlorobenzene, the reaction appeared not to proceed at all in the less polar solvent.

In retrospect, it is fair to say that the kinetic work described in Sections 2.4.2 - 2.4.4 was carried out without a full appreciation of the degree of complexity of the kinetics. Articles such as the overview of mercuriation by Taylor as illustrate how
the various equilibria involved can be modified by a host of factors. Although the reaction in TFA is less complex than in aqueous acetic acid, it is still subject to factors such as the water content of solutions, and to considerable isotope effects. Kinetic work on thalliation is additionally complicated by the instability of the reagent. As Roberts found, TFA is by no means a suitable reagent for quantitative investigations. It is sold as a technical grade reagent and its purification by recrystallisation is said to be ineffective.

As a result of the practical difficulties outlined above, the study provided little in the way of additional mechanistic information. With regard to radioiodination, the suitability or otherwise of procedures involving metallated intermediates is also well-documented. As mentioned earlier, thalliation possesses the considerable advantages of high regiospecificity and rapid iodode-thalliation with iodide ions. The principal disadvantage is the high oxidising capacity of Tl(III) reagents. Mercuration has the advantage that the reagents are not strong oxidising agents and that reactions do not require TFA as a solvent. However, it has the disadvantages of lower selectivity and that demercuration occurs less readily than dethalliation. Finally, no consideration of using organomercury or organothallium compounds would be complete without due regard to their toxicity. Clearly, any preparation of a radiopharmaceutical for *in vivo* use, via a thalliated or mercuriated precursor would need to involve rigorous purification steps to ensure that all traces of the metals were removed.
2.6 References


CHAPTER THREE

The Radner Process and Other Oxidative Iodination Reactions.
3.1 The Radner Method

In 1988, Radner published a paper describing the use of nitrosonium tetrafluoroborate (NO\textsuperscript{+}BF\textsubscript{4}\textsuperscript{-}) as a catalyst in direct iodination reactions. Arenes covering a wide range of reactivities were iodinated in high yields using ammonium iodide as the iodine source in conjunction with NOBF\textsubscript{4} and oxygen. The most striking aspect of the work was that only catalytic quantities of the reagent were required. For example, 0.6 mmol of NOBF\textsubscript{4} was sufficient for the production of 45 mmol of 2-iodo-p-xylene, corresponding to an effective yield of 7500% based on NO\textsuperscript{+}. In this respect the Radner process differs from other iodination techniques, which tend to require stoichiometric quantities of reagents.

The reactions were carried out at 25°C in TFA/acetic acid or TFA/dichloromethane mixtures. Radner was able to tailor the solvent composition to suit the reactivities of substrates. For the less reactive arenes, such as halobenzenes, TFA containing 3 - 6% trifluoroacetic anhydride (TFAA) was used. For more reactive aromatics such as 1-methoxynaphthalene, TFA-rich solvent mixtures were found to be unsuitable. Under such conditions substrate oxidation occurred in preference to iodine oxidation, resulting in biaryl coupling. This was eliminated by using acetic acid as a diluent. In 1:10 TFA: AcOH, 1-methoxynaphthalene was mono-iodinated in 83% yield. Similarly, when iodination of thiophene was attempted in TFA, many by-products were produced. A clean reaction, giving a 91% yield of 2-iodothiophene, was obtained by diluting the TFA with dichloromethane.
The reaction was rationalised in terms of the generation of "I++" from I₂ and NO⁺:

\[ I^- + NO^+ \rightarrow 0.5I_2 + NO \]  \hspace{1cm} 3.1

\[ 0.5I_2 + NO^+ \rightarrow I^+ + NO \]  \hspace{1cm} 3.2

Radner demonstrated the requirement for oxygen by carrying out a series of iodinations of p-xylene under various conditions. In TFA containing a small amount of TFAA, 100% conversion was achieved within 2 hours under an atmosphere of O₂. In the same time period, only 11% conversion was achieved in air and just 0.1% under argon. The role of oxygen would seem to lie in the conversion of some NO to NO₂. This is thought to combine with further NO to give N₂O₃. In acid solution this is converted to NO⁺ and water, thus regenerating the catalyst:

\[ NO + 0.5O_2 \rightarrow NO_2 \]  \hspace{1cm} 3.3

\[ NO + NO_2 \rightarrow N_2O_3 \]  \hspace{1cm} 3.4

\[ N_2O_3 + 2H^+ \rightarrow 2NO^+ + H_2O \]  \hspace{1cm} 3.5

Equations 3.3 - 3.5 can be combined as equation 3.6, to describe the overall process whereby the NO produced by reactions 3.1 and 3.2 is re-oxidised to NO⁺:

\[ 2NO + 0.5O_2 + 2H^+ \rightarrow 2NO^+ + H_2O \]  \hspace{1cm} 3.6

A role for TFAA would thus seem to be the removal of the water formed by the forward reaction in equation 3.6.
Radner also found that other nitrogen oxide species showed some catalytic activity; NO$_2$/N$_2$O$_4$, NaNO$_2$, NaNO$_3$ and HNO$_3$ were all shown to be effective catalysts in the iodination of mesitylene. Reactions were carried out in various solvent mixtures of TFA, TFAA, AcOH and CH$_2$Cl$_2$, with NO$_x$: NH$_4$I molar ratios in the range 0.01 - 0.1. Iodomesitylene was produced in yields of 92 - 98%, with only small amounts (2 - 3%) of nitromesitylene as a by-product. Toluene was also iodinated using NaNO$_3$ as the catalyst, but only when concentrated sulphuric acid was present. The latter reaction would seem to be related to the Tronov-Novikov$^2$ reaction outlined in Chapter One (p.16).

It is uncertain whether the catalytic activity of NaNO$_3$ and HNO$_3$ is due to NO$_2^+$ acting in the same manner as NO$_2^-$, or due to lower NO$_x$ species derived from reduction of N(V). Radner$^1$ found that the addition of sodium azide, a powerful nitrous acid scavenger, effectively inhibited the iodination of mesitylene, which could be evidence for the latter possibility. However, azide ions would also be expected to react with NO$_2^+$ or I$^+$, thereby rendering the evidence inconclusive.

Radner$^1$ acknowledged that there were several mechanistic uncertainties regarding his reaction, yet offered a number of suggestions to account for his observations. The favoured scheme is encompassed by equations 3.7 - 3.10, where the transformation designated as 3.10 seems most likely to proceed as indicated by equation 3.6.
Mechanistic studies of the Radner reaction have also been carried out by Galli. He investigated the iodination of durene and mesitylene, with a view to differentiating between classical electrophilic substitution, A (3.11), and a pathway involving radical-ion-pairs, B (3.12), as championed by Kochi (vide infra):

A. \[ \text{ArH} + \text{E}^+ \longrightarrow \left[ \text{Ar}_{\text{H}}_{\text{E}} \right]^+ \longrightarrow \text{ArE} + \text{H}^+ \] 3.11

B. \[ \text{ArH} + \text{E}^+ \longrightarrow \left[ \text{ArH}_{\text{E}}_{\text{E}} \right] \longrightarrow \left[ \text{ArH}^+_{\text{E}}, \text{E}^+ \right] \longrightarrow \left[ \text{Ar}_{\text{H}}_{\text{E}} \right]^+ \] 3.12

Mesitylene (1,3,5-trimethylbenzene) and durene (1,2,4,5-tetramethylbenzene) were chosen as a mechanistic probe because of their contrasting reactivities. The \( \alpha/\beta \) directing effect of the methyl groups makes mesitylene very reactive towards electrophiles, thereby favouring pathway A. Durene is more readily oxidised to the radical cation, \( \text{ArH}^{**} \), thus a pathway of type B could be expected to be more prevalent.
Galli obtained a mesitylene / durene reactivity ratio of 50 when the substrates were iodinated by the Radner method. From this it was concluded that reaction was occurring via the classical electrophilic substitution pathway. Identical mesitylene / durene reactivity ratios were obtained when various iodination techniques were compared (the Ag+, S2O52- and Ce(IV) methods). This was taken as evidence for a common reactive intermediate, namely the I⁺ cation.

Galli also offered an explanation for the catalytic activity of NaNO₃ and other NOₓ species reported by Radner. He pointed to trifluoroacetic anhydride (TFAA) which Radner included in his solvent mixtures. Nitrate anions are known to react with TFAA to generate the mixed anhydride, O₂NO₃(O)CF₃:

\[
\text{NO}_3^- + (\text{CF}_3\text{CO})_2\text{O} \rightleftharpoons \text{O}_2\text{NO}(\text{O})\text{CF}_3 + \text{CF}_3\text{CO}_2^- \quad 3.13
\]

In polar media this can dissociate to release NO₂⁺:

\[
\text{O}_2\text{NO}(\text{O})\text{CF}_3 \rightleftharpoons \text{NO}_2^+ + \text{CF}_3\text{CO}_2^- \quad 3.14
\]

Galli proposed that I⁺ can be generated from NO₂⁺ via a reaction analogous to that represented by equation 3.7 (3.15):

\[
\text{I}_2 + \text{NO}_2^+ \rightarrow [\text{I}-\text{I}/\text{NO}_2]^+ \rightarrow \text{I}^+ + \text{I}^+/	ext{NO}_2 \quad 3.15
\]

Again, this would seem to be of relevance to the Tronov-Novikov method and related iodination methods employing nitric acid. In fact, Radner himself commented that the success of the Tronov-Novikov method may be due to reactions between NO₂⁺ and I₂, analogous to those proposed between NO⁺ and I₂, as outlined...
above. He also made the suggestion that the levels of nitrated by-products produced in such reactions were low because NO$_2^+$ was rendered less electrophilic by complexation with I$_2$.

The complete absence of nitrated by-products in Radner's work is at first sight somewhat surprising, particularly in the light of some reports by Kochi et al.$^4$-$^6$. During the course of the past decade, Kochi and coworkers have published a great number of papers, a common theme of which is Kochi's belief that charge-transfer complexes, as in equation 3.12, are ubiquitous intermediates in electrophilic aromatic substitutions. A considerable portion of this work has been concerned with complexes of arenes, ArH, with the nitrosoonium cation, NO$^+$. On addition of arenes to solutions of NOBF$_4$ in solvents such as acetonitrile, bright colours were produced owing to the formation of 1:1 π-complexes, [ArH, NO$^+$]. In 1989, Kim and Kochi$^4$ reported that the exposure of such complexes to oxygen provided an efficient route to the corresponding nitroarenes, a process which they termed oxidative aromatic nitration. Since oxygen is deliberately admitted in Radner's process, small amounts of nitrated by-products may be anticipated if the steps leading to oxidative aromatic nitration are feasible in the presence of iodine.

In assigning a mechanism to the process, Kim and Kochi$^4$ first pointed out that, under the conditions used, NO$^+$ was inert towards oxygen and that nitrosoarenes could not be converted directly to nitroarenes. Thus, it was concluded that the formal transformation of N(III) reagent to N(V) product must occur via a
reactive intermediate, namely the 1:1 charge-transfer complex:

\[
\text{ArH} + \text{NO}^+ \iff [\text{ArH}, \text{NO}^+] \quad 3.16
\]

\[
[\text{ArH}, \text{NO}^+] \iff [\text{ArH}^{++}, \text{NO}] \quad 3.17
\]

\[
[\text{ArH}^{++}, \text{NO}] \xrightarrow{O_2} [\text{ArH}^{++}, \text{NO}_2] \xrightarrow{\text{ArH}} [\text{ArNO}_2] \quad 3.18
\]

The process was studied under a variety of experimental conditions. In brief, oxidative aromatic nitration proceeded most cleanly in polar aprotic solvents (MeCN, MeNO₂). In less polar media (CH₂Cl₂), or in the presence of added base (2,6-di-t-butyl-4-methylpyridine), side-chain substitution became significant as a competing reaction.

As delineated by equations 3.16 – 3.18, it may be thought that oxidative aromatic nitration is restricted to electron-rich substrates. However, Kim and Kochi found that toluene and p-xylene were readily nitrated by exposure to NOBF₄ and oxygen. This was explained in terms of the oxidation of the complexed NO being very much faster than the reverse reaction of equation 3.17. In other words, although there is only a very small degree of charge-transfer in complexes of toluene with NO⁺, this may be sufficient for reaction to proceed, if the follow-up reaction is very rapid.

In a subsequent publication, Kochi and coworkers reported on a study of the redox equilibria of NO⁺ and its non-bonded complexes by cyclic voltammetry. The reversible redox potential, E°, for the NO⁺/NO couple was found to be highly solvent dependent, as befits a small, coordinatively unsaturated cation. Values of E°
vs. SCE were measured as 1.28V, 1.33V and 1.48V in acetonitrile, nitromethane and dichloromethane respectively. This work was extended by performing cyclic voltammetry on the charge-transfer complexes of NO⁺ with various polymethylbenzenes. Complexes of durene, pentamethylbenzene and hexamethylbenzene were studied in acetonitrile solution, generating $E^\circ$ values of 1.13V, 1.10V and 1.02V vs. SCE respectively. The large shift of redox potential is illustrative of the powerful stabilizing effect that arene complexation has on the NO⁺ cation.

In 1991, Kim and Kochi⁶ reported further on the nature of the [ArH,NO+] complexes. In particular, the complexes with polymethylbenzenes were found to be readily crystallised and were thus isolated and subjected to detailed analysis. U.V./vis., infra-red and NMR spectroscopic studies were conducted, in addition to the collection of X-ray crystallographic data. The picture that emerged was one of increasing electron-transfer within the complexes with decreasing ionization potential of the arene. Thus, on going from benzene to hexamethylbenzene, the increase in the number of methyl groups was accompanied by extension of the N-O bond length and a concomitant decrease in the stretching frequency of the bond. In the case of hexamethylbenzene, the N-O bond length approximated to that in nitric oxide while the ¹H NMR spectrum was that of the radical cation, C₆Mes⁺⁺.

Several points arise from the aforementioned work which may be of relevance to Radner's reaction. Firstly, the fact that the nitrososionium cation is capable of generating radical cations
in media such as acetonitrile raises the question as to whether a reaction pathway involving radical intermediates could contribute to the iodination process. Galli's results would seem to suggest otherwise, but the possibility cannot be ruled out. Secondly, Kochi's measurement of redox potentials for NO$^+$ in various environments appear to be quite pertinent to Radner's experience with different solvents. The example of 1-methoxynaphthalene has already been mentioned; Radner found that this compound was almost completely oxidised by NOBF$_4$ / NH$_4$I / O$_2$ in 3 : 1 TFA - AcOH, but was iodinated in high yield in 1 : 10 TFA - AcOH. The contrasting behaviour may be attributed to the variation of oxidising power of NO$^+$ in relation to its solvation.

Charge-transfer complexes of the type [ArH,NO$^+$], as studied by Kochi et al., may also be invoked in attempting to account for some additional observations by Radner. For example, Radner reported that his method was largely unsuccessful when applied to polycyclic aromatic hydrocarbons such as pyrene. These difficulties were accounted for in terms of reduction of the arene reactivity due to complexation with iodine. However, Radner later reported that the formation of 1-iodopyrene was accompanied by some 1-nitropyrene and sometimes 1-acetylpyrene. The generation of such by-products may be an indication of the involvement of radical intermediates, as in the oxidative aromatic nitration process proposed by Kim and Kochi. In order to achieve the successful iodination of substrates such as pyrene, Radner developed an alternative method involving iodine cyanide and aluminium trichloride.
Nitrosonium tetrafluoroborate was obtained from Aldrich as a white solid. The compound is known to be very hygroscopic and when exposed to air, was found to emit pungent fumes, presumably of oxides of nitrogen. As a result, the reagent was invariably dispensed into pre-weighed vessels, under an atmosphere of dry nitrogen in a glove bag. When not in use, the compound was kept refrigerated in a desiccator.

3.2.1 Preparative Scale Iodinations

Initial experiments on the NOBF$_4$ catalysed iodination of simple aromatic substrates were conducted in a solvent system of 1:1 TFA - dichloromethane. Subsequently, similar experiments were performed in 9:1 acetic acid - acetic anhydride, the medium chosen for detritiation experiments (see Section 3.2.2).

(i) Iodination of Toluene

(a) In 1:1 TFA - Dichloromethane

Toluene (0.5g) was placed in a small round-bottomed flask equipped with a magnetic stirrer bar, together with iodine (ca. 3g, 2-3 equivs.). The mixture was taken up in the title solvent system (ca. 20mls) and when all the iodine had dissolved, NOBF$_4$ (5mg) was added. The flask was fitted with a CaCl$_2$-filled drying tube to allow exposure to air, and the mixture was stirred overnight at room temperature.
After ca. 20 hours, the solution was poured into ca. 200mls of water and solid sodium bicarbonate was added in small portions to neutralise the TFA present. Extraction was then carried out using dichloromethane (3 x 50mls). Excess iodine was removed from the combined extracts by washing with aqueous sodium metabisulphite solution (2 x 50mls). The dichloromethane solution was further washed with portions of sodium bicarbonate solution and water, and then dried over anhydrous magnesium sulphate. Evaporation of the solvent afforded an almost colourless oil which partially solidified on cooling. Analysis by 'H NMR showed the product to be a mixture of around 40% 2-iodotoluene and 60% 4-iodotoluene. This is in reasonable agreement with the result of Radner1, who obtained 37% and 63% respectively. Fig. 3.1 shows the aromatic region of the spectrum.

Fig. 3.1 : 'H NMR Spectrum (Aromatic Region) of Iodotoluenes Obtained from the Radner Reaction in 1 : 1 TFA - CH₂Cl₂
(b) In 9 : 1 Acetic Acid - Acetic Anhydride

The above procedure was repeated using ca. 25mls of 9 : 1 AcOH - Ac₂O as the solvent. Dichloromethane extraction afforded a mixture of iodotoluenes in essentially the same isomer distribution as seen in part (a), 40% ortho and 60% para. However, additional minor peaks are apparent in the aromatic region of the spectrum (Fig. 3.2), at ca. $\delta = 7.95$, 7.48 and 7.32 ppm.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{iodotoluenes_nmr_spectra.png}
\caption{\textsuperscript{1}H NMR Spectrum (Aromatic Region) of Iodotoluenes Obtained from the Radner Reaction in 9 : 1 AcOH - Ac₂O}
\end{figure}
(ii) Iodination of Anisole

(a) In 1 : 1 TFA - Dichloromethane

A portion of anisole (0.5g) was stirred at room temperature with iodine (2 - 3 equivalents) in the title solvent system. NOBF₄ (5mg) was added and the mixture was stirred for around 20 hours, exposed to air via a drying tube.

Extraction was carried out as described for toluene in the preceding section. Evaporation of the solvent from the dried, combined extracts afforded a black oily solid. Despite its appearance, the residue gave a very clean 'H NMR spectrum when dissolved in CDCl₃. Integrals were indicative of 9 - 10% 2-iodoanisole and 90 - 91% 4-iodoanisole (Fig. 3.3).

![Fig. 3.3: 'H NMR Spectrum (Aromatic Region) of Iodoanisoles Obtained from the Radner Reaction in 1 : 1 TFA - CH₂Cl₂](image-url)
(b) In 9:1 Acetic Acid - Acetic Anhydride

A similar experiment was conducted in the title solvent system. Subsequent extraction and NMR analysis showed the product in this case to be almost exclusively 4-iodoanisole. The amount of 2-iodoanisole was estimated to be no more than 2 - 3%. Fig. 3.4 shows the aromatic region of the spectrum.

Fig. 3.4: 'H NMR Spectrum (Aromatic Region) of Iodoanisoles Obtained from the Radner Reaction in 9:1 AcOH - Ac₂O

A blank experiment was also conducted in 9:1 acetic acid - acetic anhydride. Anisole (0.2g) was stirred with a large excess of iodine (6g) in the medium, but in the absence of NOBF₄. After four days, the mixture was extracted and the product was subjected to 'H NMR analysis. The resultant spectrum was that of pure anisole; no trace of p-iodoanisole had been produced.
A tube seems worthy of mention at this point. It was carried out as part of the collection of some general observations on the properties of the nitrosonium cation and its possible relevance was only realised in retrospect.

It had been noticed that intense blue-green colours were produced when anisole and NOBF₄ were mixed in various solvents. At first, it was thought that impurities and moisture in the anisole had contributed to the formation of the species responsible. Thus, anisole was thoroughly dried by refluxing over sodium metal and then carefully distilled. Even after such purification, an intense blue colouration still resulted when NOBF₄ was added to a dilute solution of anisole in chloroform. Subsequently, the reagents were mixed in CDCl₃ solution and ¹H NMR spectra of the blue solution were recorded. These featured only the peaks one would expect from a clean solution of anisole; ie. despite the colour, no additional or modified signals were apparent. An excess of iodine (unweighed) was then added and spectra were recorded at intervals. During the course of an hour, a pair of doublets at δ = 7.55 / 7.52 and δ = 6.67 / 6.64 ppm increased in intensity, attributable to p-iodoanisole. Thus, with an electron-rich substrate such as anisole, the Radner reaction appears to proceed, albeit slowly, in chloroform.

(c) In Chloroform

An exploratory experiment conducted in CDCl₃ in an NMR
The iodination of aromatic compounds is in simplest terms the replacement of hydrogen by iodine. A consequence of this is that iodination can be followed by a detritiation technique. Using specifically labelled substrates, attempts have been made to monitor the Radner reaction in this way.

A solvent system of 10% acetic anhydride in acetic acid was chosen for this work. The anhydride was included as a water regulator. In view of the high para-selectivity of its iodination in this solvent mixture, anisole was chosen as the tritiated substrate.

Preparation of $[4-^3H]$-Anisole (and $[4-^2H]$-Anisole)

Anisole was tritiated at the para-position via a Grignard reaction starting with 4-iodoanisole. An ethereal solution of 4-iodoanisole (9.33g, 40mmol) was added slowly to excess magnesium turnings (≈ 1.2g) with gentle heating. The Grignard solution so produced was divided so that deuterated, as well as tritiated materials, could be produced from the same experiment. Around 90% of the reagent was quenched with D$_2$O to provide $[4-^2H]$-anisole for use in subsequent experiments (see Section 3.2.3). To the other 10% was added tritiated water (5μl, 20Ci/ml), followed by a series of droplets of H$_2$O to quench the remainder. Excess water was then added and the product was extracted into portions of ether. The tritiated product, ca. 0.4g, was found to contain around 30mCi; this corresponds to a specific activity of ≈ 8mCi/mmol.
Detritiation - Experimental Procedure

A 0.0287M stock solution of iodine was prepared in 9 : 1 acetic acid - acetic anhydride (1.820g/250ml). In each kinetic run, a 10ml aliquot of this solution was used. It was first thermostatted at 25°C in an oil bath in order to reach thermal equilibrium. A trace amount of [4-3H]-anisole was then added. It was established that there was no loss of tritium over a 24 hour period in the absence of the catalyst. Kinetic runs were initiated by adding a small, pre-weighed quantity of NOBF₄ to the stirred solution. In a first series of experiments, brisk magnetic stirring was maintained throughout the reactions in view of the need for oxygen.

Aliquots (0.2 or 0.5ml) were removed at timed intervals and transferred to tubes containing 10mls of toluene-based scintillant and 10mls of 1M aqueous sodium hydroxide solution. The hydroxide carried out the dual function of neutralising the acetic acid and removing the iodine colour which would otherwise have affected the counting efficiency. Toluene extracts were removed and then dried over anhydrous sodium sulphate before counting.

The radioactivity in the extracts decreased regularly with time until a constant level of around 5% of the initial count was reached, possibly reflecting the small amount of 2-iodoanisole seen in the preparative work. This "infinity" value was subtracted from the measured counts and plots of ln (DPK - DPK₀) versus time were constructed. Some satisfactory linear plots were obtained, the gradients of which yielded the first order rate constants listed in part (i) of Table 3.1.
Table 3.1: RESULTS OF DETRITIATION OF [4-^3H]-ANISOLE BY IODINE/NOBF₄
IN ACETIC ACID SOLUTION CONTAINING 10% (v/v) ACETIC ANHYDRIDE

(i) 25°C, $[I₂] = 0.0287$ M, stirred and stoppered:

<table>
<thead>
<tr>
<th>[NOBF₄]/M</th>
<th>$k$/s$^{-1}$</th>
<th>$k_2$/M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0050</td>
<td>$1.5 \times 10^{-4}$</td>
<td>0.030</td>
</tr>
<tr>
<td>0.0082</td>
<td>$3.1 \times 10^{-4}$</td>
<td>0.038</td>
</tr>
<tr>
<td>0.0087</td>
<td>$2.7 \times 10^{-4}$</td>
<td>0.031</td>
</tr>
<tr>
<td>0.0109</td>
<td>$2.6 \times 10^{-4}$</td>
<td>0.024</td>
</tr>
<tr>
<td>0.0141</td>
<td>$3.9 \times 10^{-4}$</td>
<td>0.027</td>
</tr>
<tr>
<td>0.0413</td>
<td>$9.5 \times 10^{-4}$</td>
<td>0.023</td>
</tr>
</tbody>
</table>

(ii) 25°C, $[I₂] = 0.0143$ M, stirred and stoppered:

<table>
<thead>
<tr>
<th>[NOBF₄]/M</th>
<th>$k$/s$^{-1}$</th>
<th>$k_2$/M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0069</td>
<td>$1.8 \times 10^{-4}$</td>
<td>0.026</td>
</tr>
<tr>
<td>0.0145</td>
<td>$2.3 \times 10^{-4}$</td>
<td>0.019</td>
</tr>
</tbody>
</table>

(iii) 25°C, $[I₂] = 0.0143$ M, stirred rapidly, left unstoppered:

<table>
<thead>
<tr>
<th>[NOBF₄]/M</th>
<th>$k$/s$^{-1}$</th>
<th>$k_2$/M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0066</td>
<td>$2.6 \times 10^{-4}$</td>
<td>0.040</td>
</tr>
</tbody>
</table>

(iv) 25°C, $[I₂] = 0.0287$ M, no stirring, stoppered:

<table>
<thead>
<tr>
<th>[NOBF₄]/M</th>
<th>$k$/s$^{-1}$</th>
<th>$k_2$/M$^{-1}$s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0139</td>
<td>$1.7 \times 10^{-4}$</td>
<td>0.012</td>
</tr>
</tbody>
</table>
The observed first order rate constants, k/s⁻¹, were divided by the NOBF₄ concentrations to give, perhaps somewhat arbitrarily, second order values, k₂/N⁻¹s⁻¹, as listed in the right-hand column of Table 3.1. The values of k₂ listed in part (i) are seen to vary over a wide range, 0.023 - 0.038 M⁻¹s⁻¹, although the observed rates are clearly related to the NOBF₄ concentration. Halving the iodine concentration did not seem to affect k₂ significantly (Table 3.1, part (ii)), although comparing the fifth entry in (i) with the second entry in (ii) may give a different impression.

The degree of experimental error inherent from the scatter of points was estimated to be of the order of 10%. This alone was not thought to be large enough to account for the great variance in the rate constants, and it was suspected that variable oxygen content of the solutions may have been the cause. In order to investigate this, further runs were conducted under different physical conditions, leading to the results in parts (iii) and (iv) of Table 3.1. Part (iii) relates to an experiment in which the rate of magnetic stirring was maximised and the reaction vessel was left unstoppered between sampling, thereby increasing exposure to atmospheric oxygen. The k₂ value so obtained is seen to be larger than any of those listed in parts (i) or (ii). In part (iv), the stirrer was switched off and the flask was kept well stoppered between sampling. The derived k₂ value is considerably lower than those obtained for the stirred reaction mixtures.

These results were a clear indication that the degree of exposure to atmospheric oxygen had a significant effect on the
rate of detritiation of [4-$^3$H]-anisole. Consequently, efforts were made to carry out further experiments under identical conditions. For this purpose, a U-tube was used, with one arm connected to an oxygen gas cylinder, as depicted in Fig. 3.5.

The apparatus depicted in Fig. 3.5 was used in a second series of detritiation experiments, in which reactions were carried out under an overpressure of oxygen. As before, 9:1 acetic acid-acetic anhydride was used as the reaction solvent; stock solutions of iodine were prepared at 0.0152 M and 0.0248 M in this medium. Runs were conducted by placing a 10ml aliquot of such a solution in the U-tube, which was thermostatted in oil at 25°C. One arm of the tube was then stoppered and the solution was displaced with oxygen. The oxygen cylinder was regulated so that bubbles escaped to the open arm of the U-tube at a rate of approximately 1 per second. A trace of tritiated substrate, followed by a pre-weighed amount of NOBF$_4$ were added as in the previous experiments, and sampling was carried out as before.
Using these modified conditions, plots of ln DPH vs. time were found to be somewhat different from the straight-line plots which generated the results in Table 3.1. Instead, curved plots were obtained, of the general form depicted in Fig. 3.6.

Fig. 3.6: Plot of ln (DPH - DPM<sub>n</sub>) vs. time (mins); [I<sub>2</sub>] = 0.0248M, [N<sub>2</sub>B<sub>4</sub>Cl<sub>4</sub>] = 0.0112M; under O<sub>2</sub>; 25°C.

As is apparent from Fig. 3.6, the use of oxygen causes very rapid detritiation during the initial 15 - 20 minutes of the reaction. Thereafter, counts decrease more slowly until an infinity value, 5 - 10% of the initial value, is reached.

The data was deemed too unreliable for the evaluation of meaningful rate constants, although estimates of the fast initial rates were made on the basis of four or five points. Observed rate constants were found in the range $1.2 \times 10^{-3} - 3.3 \times 10^{-3} \text{ s}^{-1}$. 
Dividing these by the NOBF₄ concentration used in each particular run resulted in \( k_2 \) values in the range 0.23 - 0.39 M⁻¹s⁻¹, an order of magnitude greater than the values shown in Table 3.1.

Possible reasons for the curvature of plots, as in Fig. 3.6 were then considered. The fault did not lie with the [4-²H]-anisole, since a fresh sample gave consistent results. Other factors considered were an isotope effect giving rise to curvature, and competing nitrosation and/or nitration. Experiments aimed at testing these possibilities are described in Sections 3.2.3 and 3.2.4, respectively.

A possibly noteworthy observation was that the appearance of the plots was not affected by changing the order of addition of the reagents. Thus, when NOBF₄ was added 15 minutes before the tritiated substrate, the resultant detritiation plot was of the same general form as that depicted in Fig. 3.6. This rules out a mechanism in which a species, formed in a pre-equilibrium between NOBF₄ and iodine, slowly reacts with ArH, and is consistent with a process in which ArH forms an equilibrium with one of the inorganic reagents to form a complex which is slowly attacked by the other.
3.2.3 Examination of Deuterium Isotope Effects

In the context of the deuteriation work described in Section 3.2.2, it was suggested that a tritium isotope effect from [4-\textsuperscript{3}H]-anisole may have led to enhanced ortho-iodination and thus contributed to the curvature of the plots. In order to test this, samples of [4-\textsuperscript{2}H]-anisole and [4-\textsuperscript{2}H]-toluene were prepared and used in preparative scale Radner iodinations.

The preparation of [4-\textsuperscript{2}H]-anisole via a Grignard reagent has already been described (see p.142). In a similar manner, [4-\textsuperscript{2}H]-toluene was prepared from 4-bromotoluene. The crude product was purified by micro-distillation from a droplet of molten sodium.

Preparative scale Radner iodination was first performed on the deuterated anisole. Using the general procedure described in Section 3.2.1, a portion of [4-\textsuperscript{2}H]-anisole (0.1g) was stirred overnight with a slight excess of iodine and ca. 5mg of \textit{NOBF}_4 in 9:1 AcOH - Ac\textsubscript{2}O. Subsequently, the products were extracted and assayed by \textsuperscript{1}H NMR.

The resultant spectrum suggested that the deuteration of anisole at the para-position had little or no effect on the isomer distribution of its iodination products. As in Fig. 3.4, the only significant signals in the aromatic region were those due to \textit{p}-iodoanisole. The amount of 2-iodo-[4-\textsuperscript{2}H]-anisole was estimated to be no more than a few percent, although an accurate determination was not possible owing to the presence of many minor peaks in the spectrum.
On a separate occasion, the experiment was repeated using a different, perhaps purer, sample of [4-\textsuperscript{2}H\textsubscript{2}]-anisole. The integrals of the NMR signals from the two products led to an estimate of the proportion of 2-iodo-[4-\textsuperscript{2}H\textsubscript{2}]-anisole of 5.3%. Comparing this with the 2 - 3% quoted on p.140 for the iodination of non-deuterated anisole suggests that there may be a very small isotope effect, although the experimental error in measuring integrals of minor signals could accommodate such a variation.

Experiments with [4-\textsuperscript{2}H\textsubscript{3}]toluene produced contrasting results. Firstly, the iodination was performed in a 1 : 1 mixture of TFA and dichloromethane. NOBF\textsubscript{4} (15mg), [4-\textsuperscript{2}H\textsubscript{3}]toluene (0.15g) and iodine (1g) were stirred for 24 hours in this solvent mixture, exposed to air. The extracted products were analysed by \textsuperscript{1}H NMR, the results of which indicated that there was no deviation from the approximately 2 : 1 ratio of para to ortho-substituted products seen in other experiments with toluene.

However, a later experiment conducted in 9 : 1 AcOH - Ac\textsubscript{2}O generated a quite different result. On this occasion, the reaction was carried out by stirring [4-\textsuperscript{2}H\textsubscript{3}]toluene (0.25g), iodine (0.25g) and NOBF\textsubscript{4} (5mg) in 15mls of the solvent mixture. The products were extracted after 20 hours and from the integrals in the aromatic region of the \textsuperscript{1}H NMR spectrum, it was estimated that the product consisted of 2-iodo-[4-\textsuperscript{2}H\textsubscript{3}]toluene (58%) and 4-iodotoluene (42%).

Results in 9 : 1 AcOH - Ac\textsubscript{2}O :

<table>
<thead>
<tr>
<th></th>
<th>\textsuperscript{orth}</th>
<th>\textsuperscript{para}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluene</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>[4-\textsuperscript{2}H\textsubscript{3}]Toluene</td>
<td>58</td>
<td>42</td>
</tr>
</tbody>
</table>
3.2.4 Radio - TLC Study

In an attempt to identify the products from the Radner reaction on anisole, under the conditions used in the detritiation experiments, the process was monitored by radio - TLC. In view of the ortho / para specificity of the reaction, anisole was labelled at the meta-position so that all ortho / para substituted reaction products would remain tritium-labelled.

Anisole was specifically tritiated at the 3-position via the Grignard intermediate derived from 3-bromoanisole. To magnesium turnings (~ 0.4g) was added 3-bromoanisole (1.87g, 1mmol) in a few ml's of dry diethyl ether, and the mixture was refluxed for four hours. Tritiated water (5µl, 20Ci/ml⁻¹) was then added to the cooled solution, followed by a series of droplets of H₂O. The extracted product, ca. 1g, was found to contain around 30mCi. It was subjected to ¹H and ³H NMR analysis, the results of which were satisfactory.

The first experiment carried out with the [3-³H]-anisole was to add a small amount to a solution of I₂ / NOBF₄ in AcOH - Ac₂O, as in the kinetic studies described previously. As expected, no detritiation was observed. This result not only rules out the possibility of any rearrangements involving the meta-position, but also confirms the effectiveness of the extraction procedure.

Further experiments were then carried out under similar conditions, in a U-tube as described in Section 3.2.2. Instead of extracting aliquot taken from the solution, it was found that the process could be monitored by simply spotting small amounts of the reaction mixture onto a TLC plate at timed intervals.
Evaporation of the solvent halted the reaction, and development of the plate by a suitable solvent mixture separated the tritiated components. In a typical experiment, nine samples were taken and spotted at 2 cm intervals on a 20 cm x 20 cm silica TLC plate. The plate was then developed using 9:1 hexane-ethyl acetate as the eluent. After drying, the nine vertical tracks were assayed for tritium using a Berthold linear TLC analyser. The [3-\textsuperscript{3}H]-anisole prepared for this work was of sufficiently high activity such that the use of 1-2 \mu l per run provided enough radioactivity for the analysis of 10 \mu l samples of the 10 ml reaction solution.

Fig. 3.7 shows a series of results obtained at the times indicated from an experiment with \([\text{NORB}_4] = [I_2] = 0.0152 M\). This degree of signal-to-noise was obtained after 4 minutes of scanning of each vertical track. The results show the appearance of three tritiated products as the signal from the anisole diminishes. The print-outs shown in Fig. 3.7 represent the distribution of radioactivity over the whole 20 cm TLC plate. The reaction mixture was applied 1 cm from the lower edge of the plate and the solvent front was allowed to reach 1 cm from the top. Hence, the actual TLC experiment is represented by the central 90% of the x-axis.

The \(R_f\) value for the starting material, [3-\textsuperscript{3}H]-anisole was calculated to be around 0.70. The three tritiated products appear at \(R_f\)'s of 0.30, 0.45 and 0.61. Scan (c) in Fig. 3.7 shows that only a small amount of anisole is left unreacted after half an hour, while (d) indicates complete reaction within 45 minutes.
Numerous experiments were carried out aimed at assigning the peaks in Fig. 3.7, but with only limited success. Problems were encountered with reproducing the radio-TLC separations with non-radioactive anisole. The quantities of substrate involved in the tracer experiments are extremely small and attempts to separate larger amounts of reaction products did not result in efficient separation. Eventually, using a preparative silica TLC plate, and 9:1 hexane-ethyl acetate as eluent, a portion of a concentrated reaction mixture was chromatographed and 1cm wide bands of silica were scraped from the plate around the aforementioned Rf values.
Although the radio-TLC results, as depicted in Fig. 3.7, are suggestive of the formation of comparable amounts of the three products, most material on the preparative TLC plate was found at around \( R_r = 0.45 \). In fact, insufficient material was washed from the silica scraped from \( R_r = 0.30 \) for any meaningful NMR signals to be detected. The material at \( R_r = 0.45 \) was taken up in CDCl\(_3\) and the resultant NMR spectrum is shown in Fig. 3.8:

![NMR Spectrum](image)

**Fig. 3.8**: \(^1H\) NMR Spectrum (Aromatic Region) of Extract from TLC Plate \((R_r = 0.45)\).

Inspection of Fig. 3.8 indicates that the material found at \( R_r = 0.45 \) contains no \( p \)-idoanisole \((\delta = 7.58 / 7.55 \text{ and } 6.70 / 6.67 \text{ ppm})\). The pair of doublets at \( \delta = 8.23 / 8.20 \) and \( 6.99 / 6.96 \text{ ppm} \) would seem most likely to be attributable to \( p \)-nitroanisole. The other signals, at \( 8.12, 7.83 / 7.80 \) and \( 6.89 / 6.86 \text{ ppm} \), exhibit the characteristic pattern of a 1,2,4-tri-substituted benzene, with the downfield signals showing meta coupling. A likely contender for the compound responsible is 4-ido-2-nitroanisole.
Only a trace of material was washed from the silica at $R_f = 0.61$. The resulting dilute solution in CDCl$_3$ produced the poor quality NMR spectrum reproduced in Fig. 3.9.

Despite the low signal-to-noise, Fig. 3.9 clearly indicates that the major components of the material are $p$-nitroanisole and $p$-iodoanisole.

The radio-TLC results reproduced in Fig. 3.7 seemingly show a successful separation of three reaction products. However, the spectra shown in Fig. 3.8 and Fig. 3.9 clearly indicate that the conditions used failed to separate the components on a larger scale. Despite these difficulties, the main point is clear—under the conditions used in the detritiation experiments, iodination was not the principal reaction. The relative concentrations of the reactants were such that nitration and di-substitution were also significant reactions.
3.2.5 Replacement of Nitrosonium Tetrafluoroborate (NOBF₄) by Nitronium Tetrafluoroborate (NO₂BF₄).

As mentioned in Section 3.1, one of the ideas postulated by Radner was that nitration products were not seen in his iodination reactions employing HNO₃ or other N(V) species because NO₂⁺ was bound up in a complex with molecular iodine. In order to test this hypothesis, Radner-type reactions were carried out using NO₂BF₄ in place of NOBF₄.

A 0.5M solution of NO₂BF₄ in sulfolane was found to be available in the laboratory. This was used in preparative scale experiments with toluene and anisole, analogous to those described in Section 3.2.1. The reactions were conducted in 1 : 1 CH₂Cl₂ - TFA, using large excesses of iodine and 0.1ml portions of the aforementioned NO₂BF₄ solution.

Products were extracted after ca. 16 hours and analysed by 'H NMR as before. Fig. 3.10 shows the aromatic region of the spectrum of the products derived from toluene.

![Fig. 3.10: 'H NMR Spectrum (Aromatic Region) of Products Obtained from Reaction of Toluene with I₂ / NO₂BF₄.](image-url)
Fig. 3.10 shows that a multi-component mixture of products was produced, but that the mixture contained no trace of any iodonitrobenzenes. The major components appear to be two para-substituted compounds, on the basis of the pairs of doublets at $\delta = 7.99 / 7.96$ and $7.38 / 7.35$ ppm and at $\delta = 7.88 / 7.86$ and $7.28^* / 7.26$ ppm.

(* - Coincident with CHCl$_3$ signal).

The reaction with anisole generated fewer products. The aromatic region of the $^1$H NMR spectrum is shown in Fig. 3.11:

![Fig. 3.11: $^1$H NMR Spectrum (Aromatic Region) of Products Obtained from Reaction of Anisole with I$_2$ / NO$_2$BF$_4$.](image)

$p$-Iodonitroanisole is seen as a pair of doublets at $\delta = 7.56 / 7.53$ and $6.68 / 6.65$ ppm, but the major product is another para-substituted derivative with signals at $\delta = 7.96 / 7.93$ and $6.96 / 6.93$ ppm.
Another oxidant which has been used to promote iodination is ammonium cerium (IV) nitrate (CAN). This was described in 1981 by Sugiyama. Good yields of iodinated products were obtained with polymethylbenzenes. The general procedure is that the appropriate aromatic substrate is incubated with iodine (or an alkali metal iodide) and CAN in a solvent such as acetonitrile. Reaction times tend to be long; Sugiyama maintained his reaction mixtures at 60°C for 24 hours. In contrast to the Radner process, stoichiometric rather than catalytic amounts of the reagent are required.

The range of aromatic compounds that can be iodinated by the Sugiyama method is somewhat limited. Its usefulness has only been demonstrated for methylbenzenes and other activated compounds such as methoxybenzenes and naphthalenes. No reaction occurred with deactivated substrates such as nitro- or chlorobenzene. Electron-rich compounds such as phenols or anilines were completely consumed but not iodinated.

The iodination of methylbenzenes was shown to occur with ortho-para orientation. For example, the iodination of o-xylene gave para- and ortho- substituted products in the ratio 6:1. From this and other results, oxidation of iodine to produce an electrophilic, positive iodine species was postulated. Another piece of evidence came from the fact that no side-chain substituted products were obtained with polymethylbenzenes, ruling out the involvement of radical intermediates.
This view was supported by the work of Galli.\textsuperscript{3} As well as investigating the Radner reaction, Galli also studied Sugiyama's process, using his mesitylene / durene mechanistic probe. Mesitylene / durene reactivity ratios were consistently found to be around 50, as seen with other iodination techniques. Thus, as in the case of the Radner reaction (see p.131), a mechanism involving the \textit{I}^+ cation was proposed.

Galli's work\textsuperscript{3} also highlighted another important point. In a mixed solvent system of 60 : 8 : 8 : 24 - AcOH : TFA : TFAA : CH\textsubscript{3}CN, a higher than stoichiometric conversion to products was observed (210%), based on the amount of Ce(IV). This was attributed to the generation of NO\textsubscript{2}+ from TFAA via equations 3.14 and 3.15 (see p.131), with subsequent formation of \textit{I}^+ via equation 3.16 (p.133). Significant amounts of nitrated by-products were produced, as well as nuclear acetylated durene and side-chain substituted durene. This emphasises the important difference between the Radner and Sugiyama methods, that of stoichiometry. Electrophiles such as NO\textsubscript{2}+ are most probably present under the conditions of the Radner process, but only in catalytic quantities. Hence, no nitrated by-products are detected. In contrast, Sugiyama's use of a molar equivalent of CAN, with its six nitrate anions, makes competing nitration much more significant, particularly when TFAA is present. Galli\textsuperscript{3} found that the amounts of the by-products were significantly decreased when TFAA was omitted, but were not eliminated entirely.
In 1991, Muathen described a slight modification of the Sugiyama method. Instead of ammonium cerium (IV) nitrate, he used tetrabutylammonium cerium (IV) nitrate, which is more soluble in organic media. Aromatic iodinations were carried out by refluxing aromatic substrates, tetrabutylammonium iodide and the cerium (IV) reagent, in a molar ratio of 1:1:2, for 24 hours in dichloromethane. In this way, anisole, 1,2-dimethoxybenzene and some di- and trimethylbenzenes were mono-iodinated in isolated yields of 72-78%.

Literature accounts of iodinations employing CAN are not numerous, but some successful applications are worthy of note. Asakura and Robins used the reagent in the iodination of uracil nucleosides. Treatment of the protected uracil derivatives with iodine or metal iodides and CAN at 80°C in acetonitrile gave the corresponding protected 5-iodouracil nucleosides in very good yields. Reaction times were generally quite short, typically 1 hour. The direct iodination of some unprotected uracil derivatives was also achieved. In these reactions acetic acid was used as the solvent and reaction times of 30 minutes were typical. Lithium iodide was found to be very effective as the iodine source.

Catlin and Heldrich employed Sugiyama's reaction in the di-iodination of lipophilic α,ω-bis-para-anisylalkanes. In order to determine the optimum reaction conditions beforehand, an extensive GC study was carried out using anisole as a model substrate. Some important observations were made. The best results were obtained by adding equimolar excesses of both CAN and iodide (Bu₄N⁺ I⁻) to the arene in refluxing acetonitrile. The use of a further excess of
CAN was found to be detrimental, since this destroyed the product, 4-iodoanisole. An excess of iodide also had a negative effect; it effectively inhibited the reaction, presumably by preventing the formation of positive iodine species.

Iodination at the 3-positions of flavones, thioflavones and thiochromones using CAN has been reported by Zhang and Li.¹³
3.4 Experiments with Ammonium Cerium (IV) Nitrate

Ammonium cerium (IV) nitrate (CAN) was obtained from Aldrich as an orange crystalline solid. Portions were dried overnight at 100°C before use.

3.4.1 Preparative Experiments

Preparative iodinations using CAN were carried out on some simple aromatic substrates. The solvent system chosen was that used by Galli², 10 : 3 (v/v) glacial acetic acid - acetonitrile.

(i) Iodination of Toluene

A 0.09M solution of CAN was prepared in the aforementioned solvent system. To 25mls of this solution were added toluene (0.2g) and iodine (0.57g), to make the resultant mixture 0.09M in each component. After stirring overnight at room temperature, the mixture was poured into 200mls of water, and solid sodium bicarbonate was added in small portions to neutralise the AcOH. The solution was then extracted with chloroform (3 x 30mls). Excess iodine was removed from the combined extracts by treatment with aqueous sodium metabisulphite solution. The chloroform solution was subsequently washed with water, sodium bicarbonate solution and further water, before being dried over anhydrous magnesium sulphate. Filtration of the desiccant and evaporation of the solvent afforded a brown oily residue which partially solidified on cooling. The 'H NMR spectrum was recorded in CDCl₃ solution and was indicative of clean and complete conversion to iodotoluenes. Integrals corresponded to an isomer distribution of 35% ortho and 65% para-iodotoluene. The aromatic region of the spectrum is reproduced in Fig. 3.12.
(ii) Iodination of Anisole

As with toluene, a solution 0.09M in CAN, iodine and the aromatic substrate was stirred overnight at room temperature. The products were extracted in exactly the same way as in part (i). The \(^1\)H NMR spectrum was recorded in CDCl\(_3\) solution, the aromatic region of which is shown in Fig. 3.13:

![Fig. 3.12: \(^1\)H NMR Spectrum (Aromatic Region) of Iodotoluenes from the Sugiyama Reaction](image)

![Fig. 3.13: \(^1\)H NMR Spectrum (Aromatic Region) of Products from the Sugiyama Reaction on Anisole](image)
Inspection of the spectrum reproduced in Fig. 3.13 shows the principal product to be p-iodoanisole (doublets at $\delta = 7.55 / 7.52$ and $6.67 / 6.64$ ppm). However, a second set of doublets at $\delta = 8.17 / 8.14$ and $6.93 / 6.90$ ppm can be attributed to p-nitroanisole. Additional minor signals suggest there may be some di-substitution.

3.4.2 Deuterium Isotope Effect

As in the case of the Radner reaction (Section 3.2.3), the Sugiyama reaction was performed on a sample of $[4-^{2}H]$-toluene in order to assess the effect of para-deuteration on the product isomer distribution.

The reaction was carried out using CAN (1.1g, 2mmol), iodine (0.5g, 2mmol) and $[4-^{2}H]$-toluene (0.25g, 2.7mmol). These were mixed in 25mls of 10 : 3 acetic acid - acetonitrile and the solution was stirred for 20 hours. The mixture was then poured into water and extraction was performed as described in Section 3.4.1.(i). NMR analysis led to an estimated isomer distribution of 57% 2-iodo-$[4-^{2}H]$-toluene and 43% 4-iodotoluene.
3.5 Transition Metals in the Oxidation of Iodine

The Radner and Sugiyama methods described in the preceding sections rely on the oxidising ability of NO⁺ and Ce(IV) for the conversion of iodide to a positive species. Many transition metal compounds possess comparable or even greater oxidising capacity and their use in aromatic iodination has been investigated by numerous researchers. In most cases, the role of the metal ion seems to be more complex than simply that of an oxidant, but, for the sake of simplicity, such complications are largely ignored in this review. What follows is a chronological summary of aromatic iodination reactions involving various transition metal compounds. Silver salts are omitted here since they have already been discussed in some detail (Section 1.3.1, p.24).

In 1970, Baird and Surridge reported that copper (II) chloride, in conjunction with various iodine sources, was effective in the promotion of aromatic iodination. It was found that many aranes could be iodinated simply by refluxing with CuCl₂ and I₂. In this way, p-iodoacetanilide and p-iodoanisole were obtained in respective yields of 76% and 80%, although the reaction times were quite long (28 and 6 hrs., respectively). The role of copper ions was thought to be twofold; promoting the iodination reaction via Lewis acid catalysis, and recycling by-product hydrogen iodide to the reaction as iodine, via a redox reaction.

Less activated substrates failed to react when molecular iodine was used. However, iodination was achieved by using various metal iodides as the iodine source. The best results were obtained
using FeI₂.₄H₂O although the iodides of Co, Bi, Sn and Al were also effective. The precise role of the second metal was unclear, but the blend of metal halides clearly produced a more effective catalyst. A mixture of AlI₃ and CuCl₂ was sufficiently reactive to iodinate chlorobenzene. This mixture was in fact too acidic for most other substrates; t-butylbenzene underwent disproportionation to give benzene and di-t-butylbenzenes, while xylenes underwent many undesirable side reactions leading to arylmethanes, biaryls and polymeric products. Such side-reactions were not encountered with FeI₂.₄H₂O, hence this was the iodine source of choice.¹⁴

During the course of the reaction, the metal iodide is converted to the chloride, the overall stoichiometry being:

$$\text{M}^{n+} + 2n\text{CuCl}_2 + n\text{ArH} \rightarrow n\text{ArI} + n\text{HCl} + 2n\text{CuCl} + \text{MCl}_n$$

The fact that the iodides of Fe and Al are the most effective in this process is clearly an indication of the Lewis acid nature of the interaction. Closely related work using SbCl₅ was published in 1974 by Uemura et al.¹⁵ Although it is not a transition metal, Sb(V) can be reduced to Sb(III) with concurrent aromatic iodination:

$$2\text{ArH} + \text{SbCl}_5 + I_2 \rightarrow 2\text{ArI} + \text{SbCl}_3 + 2\text{HCl}$$

Reactions were carried out by refluxing I₂ and SbCl₅ with aromatic substrates for 30 minutes in carbon tetrachloride or 1,2-dichloroethane. Good to excellent yields of iodinated products were obtained, with, in most cases, very high para selectivity. The only
exceptions were toluene, which gave 58%  o- and 42% p-iodotoluene, and the deactivated substrate, ethyl benzoate, which gave a 48% yield of the meta-iodinated product after 5 hours. From the results of some competitive iodinations using benzene and toluene, Uemura et al.\textsuperscript{15} postulated that the reaction involves the in situ formation of ICl, the polarisation of which is enhanced through interaction with SbCl\textsubscript{5}.

Following the publication by Baird and Surridge\textsuperscript{14}, several other examples of the use of copper (II) compounds were reported. In 1973, Nefedov\textsuperscript{16} described the iodination of azulene using iodine and copper (II) acetate in DMF, suggesting that a single-electron transfer mechanism was in operation, rather than electrophilic attack. The use of three equivalents of iodine resulted in a 98% yield of 1,3-di-iodoazulene\textsuperscript{16}. Somewhat more recently, Horiuchi and Satoh\textsuperscript{17} reported on the iodination of various electron-rich substrates using copper (II) acetate and iodine. The reactants were refluxed for 6-9 hours in glacial acetic acid, resulting in high yields with polymethylbenzenes, aniline, phenetole and anisole. The method was subsequently applied to the iodination of estradiol-17\beta-acetate, affording the 2-iodo- derivative.\textsuperscript{18}

These reactions involving copper salts would seem to be more closely related to silver-ion promoted iodination than to the oxidative processes of Radner and Sugiyama. Consequently, they will not be mentioned further. More akin to the Sugiyama reaction are iodinations involving oxo anions containing metals in high oxidation states.
Potassium permanganate, in acidic media, has been shown to be effective in this context. Using iodine, KMnO₄, and H₂SO₄ in 80% acetic acid, a wide range of simple aromatic substrates were iodinated in yields of 49 - 98%. The method was subsequently applied to the iodination of a slightly more complex biphenyl-substituted diene.

In 1985, Shimizu et al. reported on the iodination of benzene using various metal salts in acidic solvents. It was found that compounds such as sodium vanadate(V) promoted iodination when heated with iodine and benzene at 100°C in trifluoromethanesulfonic acid under oxygen. In some respects, the reaction resembles Radner's process, although the conditions are clearly much more severe. The catalytic activities of a wide variety of other metal compounds were tested using methanesulfonic acid as the solvent. Compounds showing activity included CuCl₂, VCl₃, TiCl₄, and MnO₂.

Reactions proceeding under much milder conditions were described by Makhon'kov et al. in 1986. They reported on the iodination of various simple aromatics using cobalt (III) acetate, manganese (III) acetate and ammonium cerium(IV) sulphate in aqueous TFA. Reactions were carried out at room temperature, using iodides (NaI, KI, Bu₄NI) as the iodine source. Interesting observations were made, some of which may have a wider relevance to other parts of this thesis (e.g. Chapter Four).

It was shown that the metal salts in TFA were effective for the iodination of benzene and mildly deactivated derivatives such as halobenzenes. Stoichiometric quantities of reagents were
used, according to equation 3.21:

\[
\text{ArH} + \text{I}^- + 2\text{M}^{n+} \rightarrow \text{ArI} + \text{H}^+ + 2\text{M}^{(n-1)+}
\]

3.21

The iodination of chlorobenzene using cobalt (II) acetate and potassium iodide was then studied in detail, under various conditions. The reaction was found to be very slow in anhydrous TFA, producing only a 54% yield of chloroiodobenzenes after five days. However, in 9 : 1 TFA-water, a 92% yield was obtained after just 30 minutes. This solvent composition was found to be optimal since the addition of further water resulted in greatly reduced yields. The addition of water also reduced the specificity of the reaction; the substitution pattern was 93% para - 7% ortho in dry TFA, 85% para - 15% ortho in 9 : 1 TFA-water, and 82% para - 18% ortho in 4 : 1 TFA-water. When potassium iodide was replaced by molecular iodine, the reaction proceeded more slowly owing to the low solubility of the latter. This could be circumvented by using 10% dichloromethane in the reaction mixture (i.e. 8 : 1 : 1 TFA - H₂O - CH₂Cl₂). However, this approach was not favoured since it made recycling of the expensive TFA more difficult.

The other compounds, manganese (III) acetate and ammonium cerium(IV) sulphate, behaved similarly and thus 9 : 1 TFA-water was used in all subsequent work. The latter compound was chosen as an alternative source of cerium(IV), in order to avoid the possible complications which may arise from the six nitrate ligands of ammonium cerium(IV) nitrate (vide supra).
All three oxidants facilitated near quantitative iodinations of benzene and halobenzenes. Reaction times as short as 2 minutes were quoted for manganese (III) acetate. With toluene, complications arose due to competing radical reactions. Iodotoluenes were produced in the familiar 2 : 1 \textit{para-ortho} ratio, but were contaminated by varying amounts of benzyl trifluoroacetate, benzaldehyde and bitolyls. A further indication of the powerful oxidising capacity of the reaction solutions was that anisole was completely polymerised, and so its iodination could not be realised.

Despite the apparently high reactivity of the $\text{I}^+$ species involved, deactivated arenes (PhCF$_3$, PhNO$_2$, PhCO$_2$H) were inert under the reaction conditions. Trifluoroacetyl hypoiodite was proposed as the reactive species, formed according to equation 3.22:

\[
2\text{M}^{n+} + \text{I}^- + \text{CF}_3\text{CO}_2\text{H} \rightarrow 2\text{M}^{(n-1)+} + \text{CF}_3\text{CO}_2\text{I} + \text{H}^+ \quad 3.22
\]

In all of the above work, the role of the oxidant has been assumed to be the oxidation of $\text{I}^-$ to $\text{I}^+$. Another possibility is that it is the aromatic which is oxidised to generate the radical cation, which is then susceptible to nucleophilic attack. Reactions of this type were reported in 1977 by Kurz and Hage.\textsuperscript{25} They reacted various aromatics with cobalt (III) trifluoroacetate, in the presence of various nucleophiles. For example, benzene with cobalt (III) trifluoroacetate, in the presence of Cl\textsuperscript{-}, Br\textsuperscript{-} and I\textsuperscript{-} generated reasonable yields of the respective halobenzenes. Although nucleophilic substitution was almost certainly occurring with Cl\textsuperscript{-}, iodination was thought much more likely to occur via I\textsuperscript{-} oxidation.
3.6 Experiments with Transition Metal Compounds

As mentioned in the introduction, Section 3.5, Shimizu et al.\textsuperscript{22} have described the use of sodium metavanadate, and other transition metal compounds, in the iodination of benzene. Strongly acidic media were used and reactions were carried out under an atmosphere of oxygen. The use of oxygen is reminiscent of the Radner reaction, and by analogy with Radner's process it was felt that less severe conditions would be needed to iodinate more activated substrates. Exploratory experiments were therefore carried out using anisole.

3.6.1 Exploratory Experiments

An initial series of experiments was carried out using some readily available transition metal compounds. Ammonium metavanadate (NH\textsubscript{4}VO\textsubscript{3}), ammonium molybdate (NH\textsubscript{4})\textsubscript{2}MoO\textsubscript{4} and sodium tungstate (Na\textsubscript{2}WO\textsubscript{4}.2H\textsubscript{2}O) were selected for investigation. Unfortunately, these initial experiments were conducted in a solvent mixture containing TFA, acetic acid and acetic anhydride. All attempts to iodinate anisole in this medium resulted in para-acetylation rather than iodination. It is however interesting to note that this reaction only proceeded cleanly in the presence of iodine. Reaction of anisole, ammonium metavanadate and acetic anhydride in TFA, in the absence of iodine, produced a mixture of many by-products. No reaction occurred when anisole, iodine and ammonium metavanadate were stirred in TFA alone. These observations are summarised in equations 3.23 - 3.25:
Each experiment was performed in ca. 20mls of solvent at room temperature, using 5mmol of each component. The products were obtained by pouring the reaction mixture into water and extracting with chloroform. The other compounds studied, ammonium molybdate and sodium tungstate, produced essentially the same results.

A similar experiment with toluene was also performed in the mixed solvent system (containing acetic anhydride). The reaction was terminated after 60 hours and the extracted products were examined by 'H NMR. The principal products were 2- and 4-iodo-toluenes, in the ratio 37 : 63. Additional signals were also apparent in the spectrum, but the contaminants were not readily identified and not studied further.
Further experiments with NH₄VO₃ were performed in 9 : 1 TFA - water, the solvent mixture found to be optimal in the work of Makhon'kov et al.²⁴

(i) Anisole

Anisole (0.541g, 5mmol), NH₄VO₃ (0.585g, 5mmol) and I₂ (1.268g, 5mmol) were stirred at room temperature in ca. 25mls of 9 : 1 TFA - water. The experiment was terminated after 60 hours by pouring the mixture into water. Subsequent chloroform extraction and product analysis (by NMR) showed that the reaction was only 25% complete. The extract was found to contain ≈25% p-iodoanisole and ≈75% unreacted anisole. No other compounds were evident.

(ii) Toluene

The above procedure was repeated using toluene (0.461g, 5mmol). After 60 hrs reaction, the composition of the mixture was estimated, by 'H NMR, to be 41.5% p-iodotoluene, 25.2% o-iodotoluene and 33.3% unreacted toluene. Again, no by-products were detected. The o/p ratio (0.61) is similar to that seen in other experiments, e.g. 37 / 63 = 0.59.

(iii) Chlorobenzene

Repeating the above procedure using chlorobenzene (0.563g, 5mmol) produced a small amount (∼10%) of p-chloroiodobenzene after 60 hours. Most of the chlorobenzene was extracted unchanged.
3.7 Discussion

Of the direct iodination techniques summarised in Chapter One, the Radner method was selected for further study largely because it seemed relatively unexplored. Since Radner's initial report, the only mechanistic study of the reaction appears to be that of Galli.

Preparative-scale iodination experiments with toluene and anisole have confirmed the remarkable catalytic effect of the nitrosonium cation, as reported by Radner. A few mg of NOBF₄ has been shown to be sufficient for the complete iodination of 0.5g quantities of aromatic substrates. Product isomer distributions were in good agreement with those quoted by Radner and consistent with the attack of an electrophilic I⁺ species.

Following the success of the simple preparative experiments some detritiation experiments were carried out, the principal aims of which were to get some appreciation of the rate of reaction of various substrates, and to determine the optimum concentrations for rapid iodination. Viewed in retrospect, these experiments seem ill-conceived and inherently flawed, but nevertheless the results do highlight certain points. The main fault in the experimental procedure lies in the relative quantities of reagents used. In his large-scale preparative work, Radner used approximately equal quantities of arene and iodine source, and initiated the reaction with a catalytic amount of NOBF₄. However, in the detritiation experiments described in Section 3.2.2, only a trace of tritiated aromatic substrate was added to the reaction mixture. The arene
concentration was thus of the same order of magnitude as the NOBF$_4$ concentration, and in the presence of excess iodine, multiple iodination of anisole would clearly have occurred. Despite this, the rate of tritium loss from [4-²H]-anisole should be a good indication of the rate of the initial iodination, given the high para-specificity seen with anisole in preparative experiments.

The first set of experiments, which generated the results in Table 3.1 (p.144), gave an indication of the effect of oxygen on the reaction rate. As mentioned previously (p.145), measured rates were not reproducible and the variation in rate constants could not be accounted for in terms of experimental error alone. The discrepancies were attributed to variable oxygen concentration in the reaction solutions, and the results of experiments performed under various physical conditions (Table 3.1, parts (iii) and (iv)), strongly supported this hypothesis.

Consequently, further detritiation experiments were conducted under regulated conditions, using an overpressure of oxygen from a cylinder. Under such conditions, curved detritiation plots were produced (Fig. 3.6), the initial rates being an order of magnitude greater than in the absence of added oxygen. However, it is very doubtful that iodination was the actual reaction being monitored. With regard to the report by Kim and Kochi, the conditions and relative concentrations employed would appear to be very conducive to oxidative aromatic nitration. With NO$^+$ and anisole concentrations of the same magnitude, a significant concentration of the charge-transfer complex, [ArH, NO$^+$] = [ArH$^{**}$, NO], was likely to be present.
Since oxygen was deliberately admitted to the reaction mixtures, and was thus available in abundance, oxidative aromatic nitration, as described by equations 3.16 - 3.18 (p.133) was most likely to occur. The results of radio-TLC experiments, described in Section 3.2.4, supported this, indicating the formation of at least three products. NMR analysis of fractions removed from TLC plates showed that p-nitroanisole was in fact the major product.

Two reaction schemes can thus be envisaged to account for the aforementioned observations. Firstly, in the absence of added oxygen (i.e. reactions merely exposed to air), Radner iodination can be envisaged as proceeding via the following scheme:

(i) Radner Reaction

\[ 0.5I_2 + NO^+ \xrightarrow{O_2} I^+ + NO_2 \]  \hspace{1cm} (3.26)

Here, the generation of \( I^+ \) would be expected to be the rate-limiting step, and hence the reaction exhibits a marked dependence on the concentration of dissolved oxygen.
Under the conditions used in the detritiation experiments, a second scheme is likely to be operative, involving the radical cation of anisole:

(ii) Oxidative Aromatic Nitration

\[
\begin{align*}
\text{OMe} + \text{NO}^+ & \rightleftharpoons [\text{OMe}, \text{NO}^+] \rightleftharpoons [\text{OMe}, \text{NO}] \\
\text{diffusive separation, I}_2 & \quad \text{O}_2, \text{ collapse}
\end{align*}
\]

With an abundance of oxygen, oxidation of NO in the charge-transfer complex occurs, ultimately leading to p-nitroanisole. The charge-transfer complex can also undergo diffusive separation, thereby allowing the anisole radical cation to interact with iodine, resulting in some iodinated products.

In the context of possible radical pathways, the experiment involving anisole in chloroform solution (p.141) seems particularly interesting. Despite the fact that Radner used only acidic media for his iodinations, the NMR experiment conducted in CDCl₃ clearly
indicated that anisole could be iodinated in such a medium. One can envisage formation of the charge-transfer complex, as in the above scheme, but with a high concentration of iodine and a low concentration of oxygen (stoppered NMR tube; minimal surface area exposed to air), the diffusive separation pathway, leading to iodoanisole would be favoured.

Although the aforementioned detritiation experiments have failed to provide the desired results, the findings suggest that the Radner reaction is inappropriate for radiolabelling purposes. In n.c.a., or high specific activity radiodiiodination procedures, sodium radiodiiodide is generally employed at concentrations of $10^{-9}$ or $10^{-10}$ M. It seems inconceivable that Radner iodination could be brought about using an NOBF$_4$ concentration several orders of magnitude lower than this. On the other hand, if NOBF$_4$ was used at a concentration comparable to concentrations of substrate and iodide, competing nitration, as seen in the detritiation experiments would be expected. Thus it can be concluded that the utility of the Radner method lies solely in the large scale iodination of aromatic compounds. In the context of radiodiiodination of molecules of biological significance, the method would seem to be restricted to the preparation of large amounts of iodo precursors for use in halogen-for-halogen exchange reactions (Section 1.4.3) and in ipso metallation reactions (Chapter Five). It may also be useful in the preparation of precursors for tritium-labelling with T$_2$ gas. The use of NOBF$_4$ in conjunction with radiodiiodide solutions seems very unlikely, except perhaps for very low specific activity work.
The Sugiyama reaction, as described in Section 3.3, is also considered inappropriate for radiolabelling purposes. As noted previously, the reaction is restricted to moderately electron-rich substrates and is prone to nitrination occurring as a side-reaction. Reaction times are also quite long; too long to be of use in radioiodination with shorter-lived isotopes.

Section 3.4 describes some preparative experiments carried out with toluene and anisole. Toluene was cleanly and completely converted to iodotoluenes, which were produced in an approximately 2 : 1 para-ortho ratio. No nitrated by-products were detected from the reaction with toluene, but in the case of anisole, some p-nitroanisole was apparent. The principal product was nevertheless, p-idoanisole. Galli's conclusion that the Radner and Sugiyama methods involve a common reactive species was supported in this study in that the two methods produced iodinated products in the same isomer distributions. This is also true of the experiments with [4-³H]-toluene.

Finally, experiments with other compounds containing metals of variable valence have indicated that these may indeed be capable of generating electrophilic iodine. The compounds examined in this study, ammonium metavanadate, ammonium molybdate and sodium tungstate, all showed some promoting activity, but were much less effective than CAN. Clearly, these reactions are too slow to be of relevance to radioiodination. A multitude of metal salts are capable of oxidising iodine to I⁺, but these have no advantages over other oxidants.
3.8 References


CHAPTER FOUR

Iodination With Electrochemically Generated Positive Iodine
4.1 Electrochemical Oxidation Of Iodine

The Radner and Sugiyama methods described in Chapter Three involve the oxidation of iodide to produce electrophilic, positive iodine species. Both reactions utilise nitrogen-containing oxidants and there is a possibility of producing nitrated by-products. A cleaner, and in many respects, a more straightforward, method for iodine oxidation is by electrochemical means.

For many years, electrochemistry was largely overlooked by organic chemists. However, it has since become a very powerful and versatile synthetic tool. Recent reviews have highlighted the synthetic utility and environmental cleanliness of electrochemical techniques. It is very much a "clean technology" and will clearly find much wider application in the future.

Electrochemistry is based on oxidations and reductions and in view of the ease with which iodine can change its oxidation state, it is no surprise that its reactions have been studied electrochemically. As has already been described in Chapter One (Section 1.4.1 (vii)), peptides have been iodinated by electrolysis of aqueous iodide solutions. Such methodology involves the oxidation of iodide ions to produce an electrophilic species, possibly $\text{H}_2\text{IOI}^+$. The work to be described in this chapter however, is concerned with the electrochemical oxidation of iodide or iodine, in aprotic media to produce very reactive "$\text{I}^+$" species.

Work in this area was pioneered in the late 1960's by Miller and coworkers. They found that iodine could be oxidised at a platinum sheet anode in acetonitrile solution containing...
lithium perchlorate as the supporting electrolyte. In initial work⁶, mixtures of iodine and aromatic substrates were simply oxidised at an anode. Procedures of this type resulted in low yields of mono-iodinated products. Competing oxidation of the aromatic substrates occurred, resulting in tar-like polymerisation products from anisole and side-chain acetalisation in the case of p-xylene. These side-reactions were eliminated by oxidising the iodine independently and adding the aromatic substrate subsequently.

Oxidation of iodine in acetonitrile - lithium perchlorate resulted in a pale yellow solution of "I⁺". This was found to react cleanly with benzene and more reactive arenes to give mono-iodinated products in high yields (80 - 100%). Isomer distributions were 50:50 ortho:para for toluene and 30:70 for anisole.⁶ Using more than one equivalent of the iodinating agent, di-iodination was achieved. In the case of p-xylene, stepwise conversion to 2,3,5,6-tetraiodo-p-xylene was demonstrated.

The positive iodine species regenerated iodine instantly when iodide ion was added. This was used as a test to get some appraisal of the rate of reaction with arenes. When iodide was added immediately after the addition of p-xylene, only a trace of iodine was liberated, indicating that the iodination of the xylene had occurred very rapidly. Another indication of the reactivity of the species was that a higher percentage of polyiodinated product was formed when the xylene was added slowly (over a period of 30 seconds).
Miller et al. proposed that the iodinating species was CH₂C⁺=NI, the iodonium cation solvated by acetonitrile. In their studies involving lithium perchlorate, the coulometric n value for the iodine oxidation was found to be considerably greater than 2 F / mol I₂. In other words, the quantity of electricity passed corresponded to the removal of more than two electrons from each iodine molecule. This was attributed to residual water in the electrolyte. In all experiments using lithium perchlorate a white precipitate was produced, which was identified as N-iodoacetamide (equations 4.1, 4.1a).

\[
\begin{align*}
\text{CH}_3\text{C}=\text{NI} + \text{H}_2\text{O} \rightarrow & \quad \text{CH}_3\text{C}=\text{NI} + \text{H}^+ \quad \text{4.1} \\
& \quad \text{H} \\
\text{CH}_3\text{C}=\text{NHI} \quad \text{4.1a}
\end{align*}
\]

It was postulated that N-iodoacetamide could act as a second iodinating agent since the white precipitate disappeared on addition of aromatic substrates. This was confirmed by synthesizing the compound independently and testing its reactivity with anisole. It proved to be an effective iodinating agent, provided that a strong acid was present.

When tetraethylammonium perchlorate was used in place of the lithium salt, the oxidation was complete after the passage of 2.3 F / mol, and no precipitate was observed. This reflects the difficulty of obtaining lithium salts free from moisture.
In a subsequent publication, Miller and Watkins reported on a detailed study of the reactivity of the electrochemically generated iodine (I), aimed at elucidating the mechanism of aromatic iodination. Using a system of acetonitrile - tetraethylammonium perchlorate to generate the reactive species, competition experiments were conducted using pairs of aromatic substrates and relative rates were evaluated. These led to a linear Hammett plot with a $\rho^+$ value of -6.27. From this, it was concluded that the classical mechanism of electrophilic substitution was in operation, as set out in equation 4.2, where B is a base of unknown identity. High positional and substrate selectivity was indicative of rate-limiting formation ($k_1$) or destruction ($k_2$) of the $\sigma$-complex via transition states with considerable positive charge localised in the ring.

Experiments were also conducted with specifically deuterated substrates to gain information on kinetic isotope effects. The changes in isomer distribution between reactions involving anisole, toluene and chlorobenzene and their para-deuterated counterparts led to estimates of $k_1/k_2$ of 1.45, 1.54 and 4.46 respectively. Similar experiments with $d_6$-benzene and 3-$d$-ethyl benzoate provided values of 2.25 and 1.50. These results indicated that the C-H bond is broken in the rate-limiting step, hence it was concluded that $k_2$
was partially or totally rate-limiting in all cases. The non-linear variation of $k_n/k_o$ with $r^*$ was discussed in terms of variation of transition state structure as a function of substituent, i.e. in terms of the extent of proton transfer. The large value observed for chlorobenzene was associated with a high degree of symmetry in the transition state.

The other possible interpretation of the variable isotope effect, i.e. a change in the rate-limiting step between activated and deactivated substrates was effectively dismissed by Miller and Watkins. Assuming a steady-state concentration of the $r$-complex and no secondary isotope effect on $k_1$ or $k_-1$, they set up equations to express the dependence of $k_n/k_o$ on $k_2''/k_2^1$ and $k_2/k_-1$. Solving such equations with the measured $k_n/k_o$ values for chlorobenzene and toluene led to an unrealistically large $k_2''/k_2^1$ value (>12) in the case of toluene. Furthermore, a change in rate-limiting step would have been manifested as a non-linear Hammett plot, which was not observed.

Miller and Watkins also generated electropositive iodine in dichloromethane, using tetraethylammonium tetrafluoroborate as the supporting electrolyte. Kinetic experiments performed in this medium resulted in strikingly different relative rates, compared to those found in acetonitrile. The associated Hammett plot produced a $p^*$ value of -2.85 (cf. -6.27 in MeCN). This difference was attributed to a number of factors, e.g. differences in solvent basicity, the ability to complex $I^-$ and the nature of the base $B$ in equation 4.2. Although the reaction in dichloromethane was found to be less
selective between pairs of substrates, the regioselectivity of the substitution was somewhat higher. The halobenzenes were iodinated with significantly greater para-selectivity in dichloromethane than in acetonitrile.

The I⁺ species produced by Miller and Watkins was found to be most effective with moderately electron-rich compounds. Nevertheless, reasonable yields of meta-iodinated products were obtained when the reagent was added to benzoic acid or ethyl benzoate. Attempts to iodinate even more electron-deficient compounds failed. The problem of iodinating deactivated aromatic compounds was later addressed by Lines and Parker. They found that anodic oxidation of iodine in solvents containing trifluoroacetic acid produced a more reactive iodinating species. Using a mixture of 1 : 9 TFA - 1,2-dichloroethane, a reagent capable of iodinating even the most deactivated compounds, such as nitrobenzene and benzonitrile, was produced. Tetrabutylammonium tetrafluoroborate was used as the electrolyte in this work.

In contrast to the work of Miller et al., Lines and Parker found their reagent to be unstable and higher yields were obtained when the aromatic substrate was present during the electrolysis. The nature of the iodinating species is less clear than in the case of acetonitrile, but CF₃CO₂⁻ I⁺ is an obvious candidate. However, as Lines and Parker pointed out, this compound has been characterised and used as an iodinating agent. The reagent produced in 1,2-dichloroethane was apparently much more reactive.
Some interesting results were obtained by performing the electrolysis in different solvent mixtures. The iodination of nitrobenzene failed completely in 10% TFA in acetonitrile and in dichloromethane. When 10% TFA was added to dichloromethane, a 47% yield of 3-iodonitrobenzene was obtained. In 1,2-dichloroethane - 10% TFA, the yield increased to 78%.

Lines and Parker also carried out experiments to examine the effect of TFA content on the iodination of nitrobenzene in 1,2-dichloroethane. Their results are tabulated below.

<table>
<thead>
<tr>
<th>% TFA</th>
<th>Yield of 3-iodonitrobenzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
</tr>
<tr>
<td>10</td>
<td>78</td>
</tr>
<tr>
<td>20</td>
<td>65</td>
</tr>
</tbody>
</table>

A decrease in yield was seen when the TFA content was increased above 10%. This was attributed to a reduction of iodine solubility in the electrolyte.

Electrochemical studies of iodine oxidation have also been conducted by Kargin and coworkers. They oxidised iodine at a platinum anode in acetonitrile containing 0.1 M tetraethylammonium tetrafluoroborate as the electrolyte. The anolyte was then used in competitive iodinations of benzene and toluene. In dry acetonitrile, an iodotoluene / iodobenzene molar ratio of 170 was determined, with an iodotoluene isomer distribution (by g.c.) of 43% ortho and 57% para. Addition of 0.5 M water to the anolyte caused a marked drop in current efficiency (i.e. in reaction yields), but also made the
reaction much more selective. The iodotoluene / iodobenzene ratio was increased to 530. With reference to the work of Miller et al., this was attributed to N-iodoacetamide formation (equation 4.1a), the covalently bound I$^{2+}$ being less reactive and hence more selective.

In a separate study in which toluene and benzene were apparently present during the course of the electrolysis, the rate ratio was estimated as 315, much higher than that found by Miller and Watkins. The authors suggested that the radical cation, I$_2$$^2+$, might be involved in the reaction, thereby accounting for the greater selectivity on the grounds of lower electrophilicity.

In the same publications, the preparation of I$^+$ using sodium perchlorate in acetonitrile as electrolyte was described. Following electrolysis, evaporation of the solvent afforded a cream-coloured powder, which caused spontaneous ignition when added to benzene. Indobenzene was nevertheless detected in the products, as was acetonitrile. Analysis of the powder by i.r. spectroscopy showed the absence of a C≡N stretching band at ≈2100cm$^{-1}$, from which it was concluded that the structure proposed by Miller et al., CH$_3$C$^-$=NI, was correct.

This view was shared by Shono et al. who reported on the possibility of electricity storage using the iodine-nitrile system. Iodine was oxidised in various solvents to test them as donors for the formation of stable iodonium complexes. Electrolysis of the various systems was conducted in a two-compartment cell using lithium tetrafluoroborate as the supporting electrolyte. The passage of 0.2 F / mol I$_2$ caused the potential between anode and
-190-
cathode to increase from \(V_1\) to \(V_2\). The cell was then discharged from \(V_2\) to \(V_1\), using a load of 10\(\Omega\) to measure the efficiency of recovery of electricity. Recoveries in excess of 70\% were obtained from systems involving nitriles, and the authors proposed that such systems could possibly be used as storage batteries.

Returning to the subject of aromatic iodination, Shono and coworkers\(^{14}\) reported in 1989, that higher para-selectivity could be achieved by generating "I\(^+\)" in trimethyl orthoformate (TMOF) solution. Hydrated lithium perchlorate (LiClO\(_4\).3H\(_2\)O) was used as the supporting electrolyte. In the same manner as Miller et al.\(^6,7\) used their I\(^+\) - acetonitrile reagent, Shono et al.\(^{14}\) found it necessary to preform their I\(^+\) - TMDF reagent, and then to add it to solutions of the substrates to be iodinated. The reagent was found to be effective in the iodination of benzene, toluene, \(t\)-butylbenzene and anisole, but it failed to react with chlorobenzene. Ortho-para ratios were found to be 30 : 70 for toluene and 13 : 87 for anisole; while \(t\)-butylbenzene gave essentially only the para-substituted product.

The iodonium cation, solvated by acetonitrile, has also been produced by chemical means. Winfield et al.\(^{15,16}\) showed that, in the solvent, iodine was oxidised by the hexafluorides of molybdenum and uranium to yield "I\(^+\) MoF\(_6\)\(^-\)" and "I\(^+\) UF\(_6\)\(^-\)" respectively. The reactions were carried out by adding the metal hexafluorides to frozen solutions of iodine in acetonitrile, then allowing the mixtures to warm to room temperature. The brown solutions became pale yellow as the iodine was oxidised and solid products were obtained on removal of the volatiles. Elemental analysis showed the compounds
to contain two molecules of acetonitrile per iodanium cation, consistent with the formulae \([\text{I}(\text{NCMe})_2][\text{MoF}_6]\) and \([\text{I}(\text{NCMe})_2][\text{UF}_6]\). Supporting evidence for these structures was provided by low temperature Raman and i.r. spectroscopic data which implied the presence of a centrosymmetric \(\Pi-\Pi-\Pi\) unit.

In studying the chemistry of these compounds, Anderson and Winfield \(^{16}\) found that they readily iodinated aromatics such as benzene, toluene, anisole, aniline and salicylic acid when mixed in acetonitrile. A familiar ortho/para orientation of attack was found. It is therefore reasonable to assume that the reagent produced electrochemically by Miller and Watkins \(^{7}\) was in fact the same species, although cross-referencing between "organic" and "inorganic" publications seems negligible. Further studies on \([\text{I}(\text{NCMe})_2][\text{MoF}_6]\) showed that it oxidised \(\text{Cu}(I)\) to \(\text{Cu}(II)\), \(\text{NO}\) to \(\text{NO}^-\) and thallium metal to a mixture of \(\text{TI}(I)\) and \(\text{TI}(III)\). It was also shown that thallium (III) hexafluoromolybdate in acetonitrile solution oxidises iodide to iodine, and then to \(\text{I}^-\), thus generating \([\text{I}(\text{NCMe})_2][\text{MoF}_6]\) in situ. It was suggested that such a process could possibly be involved in iododethalliation (see Section 2.1.2, p.75).

Klapötke and coworkers \(^{17-18}\) have also prepared compounds in which the iodonium cation is solvated by acetonitrile, with hexafluoroarsenate(V) as the counterion. By mixing \(\text{I}_3\text{AsF}_6^-\) with one equivalent of acetonitrile in sulphur dioxide solution, the mono-solvated compound, \([\text{MeCNI}]^+\text{[AsF}_6^-\text{]}\), was produced:

\[
\text{MeCN} + \text{I}_3\text{AsF}_6^- \xrightarrow{\text{SO}_2} \left[\text{MeCNI}\right]^+\text{[AsF}_6^-\text{]} + \text{I}_2
\]

\(^{4.3}\)
The solvent, and by-product iodine, were removed in vacuo to give a 95% yield of the product. When a second equivalent of acetonitrile was added, \([(\text{MeCN})_2\text{I}]^+\text{[AsF_6]}^-\) was obtained.\(^{17}\)

Such reactions were found to be very solvent dependent. The driving force behind equation 4.3 seems to be the insolubility of iodine in SO\(_2\). The reaction did not occur in SO\(_2\)Cl\(_2\) or SO\(_2\)ClF in which iodine is soluble. When the product, \([(\text{MeCN})\text{I}]^+\text{[AsF_6]}^-\) (formed in SO\(_2\)) was added to iodine in SO\(_2\)Cl\(_2\), the reverse reaction took place, and pure I\(_3^+\text{AsF_6}^-\) was isolated.\(^{13}\)

Reactions between I\(_3^+\text{AsF_6}^-\) and other nitriles have also been examined.\(^{12}\) Whereas MeCN and ICN react with I\(_3^+\) to give \([(\text{MeCN})\text{I}]^+\) and \([(\text{ICN})\text{I}]^+\) respectively, CF\(_3\)CN and BrCN do not react. This gives an indication of the ligand basicity required for stabilisation of I\(^+\) and explains why acetonitrile is the solvent of choice for electrochemical work.

To conclude this section, it would seem appropriate to mention another positive iodine compound produced chemically rather than electrochemically, namely bis(pyridine)iodonium(I) tetrafluoroborate (IPyzBF\(_4\)). This reagent has been studied extensively by Barleunga et al., originally in the iodo-fluorination of alkenes, but more recently in aromatic iodination.\(^{20,21}\)

The compound was used in conjunction with acids (HBF\(_4\) or CF\(_3\)SO\(_2\)H) to iodinate a wide variety of simple arenes. Reactions were performed at room temperature in dichloromethane using arenne, IPyzBF\(_4\) and acid in a 1:1.1:2.2 stoichiometry. Iodination was accompanied by the precipitation of large amounts of pyridinium.
salt, i.e.:

\[
\text{IPy}_2\text{BF}_4 + 2\text{CF}_3\text{SO}_3\text{H} \rightarrow \text{I}^+\text{BF}_4^- + 2\text{CF}_3\text{SO}_3\text{[PyH]}^+ \quad \text{(4.4)}
\]

With regard to equation 4.4, the acidified reagent would seem to behave as a solution of I^+BF_4^- in dichloromethane and can thus be considered equivalent to the reagent produced electrochemically by Miller and Watkins\(^7\), using the CHCl_2 - Bu_4N^+ BF_4^- system (see p.186).

Barleunga et al.\(^{31}\) found that the HBF_4 - IPyzBF_4 reagent was quite adequate for the iodination of most aromatic substrates, giving yields of 90% or more within minutes with alkylbenzenes. However, yields were considerably lower with less electron-rich substrates and the reagent failed to react with methyl benzoate or nitrobenzene. For the iodination of deactivated compounds, the CF_3SO_3H - IPyzBF_4 reagent proved to be greatly superior; benzoic acid, methyl benzoate, benzaldehyde and nitrobenzene were all meta-iodinated with yields in excess of 80% after 10 - 14 hours.

The activation of electrophilic iodine by the addition of triflic acid has also been reported by Olah and coworkers.\(^{22}\) They found that a mixture of CF_3SO_3H and N-iodosuccinimide (NIS) produced an iodine electrophile capable of reacting with even the most deactivated of substrates. Direct comparison with Barleunga et al.'s IPyzBF_4 may however be somewhat inappropriate since NIS was used in neat CF_3SO_3H solution. The reactions did not proceed in aprotic solvents such as dichloromethane.
4.2 Experimental

4.2.1 Production of Iodine (I) Species

(a) Apparatus and Materials.

Experiments on the electrochemical oxidation of iodine were carried out using a two-compartment cell as depicted in Fig. 4.1. The anode chamber, with a capacity of around 40 mls, was separated from the smaller cathode chamber by a Grade 4 glass sinter disc with a diameter of 25mm. The anode chamber was furnished with a gas inlet tube, so that the anolyte could be maintained under an atmosphere of dry nitrogen. The passage of nitrogen gas in the vicinity of the electrode also helped to agitate the solution.

![Fig. 4.1 - Electrochemical Cell](image)

A platinum electrode was constructed by spot-welding a Pt wire to a piece of Pt foil (25mm x 30mm x 0.025mm). The Pt wire was in turn spot-welded to a Ni wire and then embedded in a 6mm o.d. glass tube. The electrode was held in position in the anode chamber using a standard Quickfit thermometer holder.
Power was supplied by means of standard laboratory D.C. supply, capable of delivering 30 V at up to 1 A. Electrolytes were purchased from Aldrich (Bu₄N BF₄, LiBF₄, CF₃SO₂Li) or Fluorochem (LiPF₆, LiSbF₆) and used without further purification.

(b) Exploratory Experiments with the Acetonitrile-Tetrabutylammonium Tetrafluoroborate System

Initial experiments were conducted using a 0.1 M solution of tetrabutylammonium tetrafluoroborate (Aldrich) in dry (distilled from CaH₂) acetonitrile. Perchlorates were avoided for reasons of safety. In a typical experiment, iodine (0.5 g) was placed in the anode chamber, together with a small magnetic stirrer bar. The cell was then filled with the electrolyte solution so that the anode chamber contained ca. 40 mls. A slow stream of dry nitrogen was introduced and the solution was stirred until all the iodine had dissolved. A potential difference of 30 volts was then applied between the platinum anode described above, and a graphite rod which served as the cathode. In the initial stage of such an experiment, a current of 0.2 - 0.3 A was typical. This decreased steadily as the iodine colour faded from brown to pale yellow during the course of the electrolysis.

It rapidly became apparent that several modifications had to be made to the experimental procedure. Firstly, it was found necessary to immerse the cell in an ice-bath while electrolysis was in progress. The passage of relatively high currents had a significant heating effect; the glass sinter disc separating anode and cathode chambers became quite hot and in some cases local boiling of the acetonitrile was observed.
It was also found necessary to use a nickel spatula as the cathode in place of a graphite rod. Discharge of the tetra-butylammonium cation at the graphite caused it to fracture and resulted in the rod being reduced to a powder within minutes. No such problems were encountered using a nickel cathode and pale yellow or colourless solutions of "I⁺" were prepared routinely.

Solutions of the "I⁺" reagent were found to be unstable at room temperature, rapidly turning brown as iodine was liberated. However, when protected from moisture and kept in a freezer, they could be stored for long periods without noticeable decomposition. In order to estimate the coulometric \( n \) value for the oxidation, 0.2536g (1 mmol) portions of iodine were completely oxidised and the number of coulombs required was evaluated. This was achieved by recording the current at timed intervals, plotting current against time and estimating the area under the curve. An example is given below:

<table>
<thead>
<tr>
<th>Time / mins.</th>
<th>I / A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.19</td>
</tr>
<tr>
<td>1</td>
<td>0.20</td>
</tr>
<tr>
<td>8</td>
<td>0.19</td>
</tr>
<tr>
<td>23</td>
<td>0.13</td>
</tr>
<tr>
<td>27</td>
<td>0.10</td>
</tr>
<tr>
<td>32</td>
<td>0.09</td>
</tr>
<tr>
<td>38</td>
<td>0.07</td>
</tr>
<tr>
<td>44</td>
<td>0.06</td>
</tr>
<tr>
<td>60</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*background 0.045*

**Fig. 4.2 : Plot of Current vs. Time for the Oxidation of I₂ (1mmol) in MeCN**
Amps x seconds = coulombs (C)

Area under curve ≈ 208.5 C

1 Faraday (F) = 96487 C

\[ 208.5 \text{ C} = 2.16 \times 10^{-3} \text{ F} \]

\[ \div 2.16 \text{ F/mole I}_2 \]

Further estimates of the number of coulombs passed gave consistent results in the range 2.0 - 2.2 F/mole I₂. Thus it is clear that iodine is completely oxidised to the +1 state in accordance with equation 4.5:

\[ \text{I}_2 \rightarrow 2\text{I}^+ + 2e^- \quad 4.5 \]

(c) Use of Iodide Ions as the Iodine Source

If these electrochemical methods are to be used in radio-labelling, it must also be possible to generate I⁺ by the anodic oxidation of iodide ions, since radioisotopes are usually supplied as NaI. To demonstrate this, an experiment was carried out using Bu₄N⁺ I⁻ (0.739g, 2 mmol) as the iodine source. As in part (b) above, 0.1 M Bu₄N⁺ BF₄⁻ in acetonitrile was used as the electrolyte. The application of 30 V resulted in the instantaneous formation of brown I₂ in the vicinity of the anode. On prolonged electrolysis, the brown solution gradually became pale orange as I⁺ was formed, but a white suspension, thought to be N-iodoacetamide, was also produced. Complete oxidation was not realised in this experiment; the current fell to zero while the anolyte was still orange and a flocculent precipitate was observed in both cell compartments.
Subsequently, complete oxidation of I\(^-\) to I\(^+\) was achieved using a slightly modified procedure. The electrolyte concentration was doubled to 0.2 M and 1ml of glacial acetic acid was added to the cathode compartment to facilitate hydrogen evolution as a cathode reaction. In this way, 2 mmol of I\(^-\) was fully oxidised in around 2 hours. This result would seem to indicate that starting from iodide ions presents no special problems.

(d) U.V. Spectrophotometric Monitoring of Iodine Oxidation

Anodic oxidation of iodine, under the conditions described in section (b), was followed by U.V. spectrophotometry. Aliquots of 0.1 ml were removed from the anode compartment during the course of the electrolysis and added to 2ml portions of dry acetonitrile in a U.V. cell. Before commencing electrolysis, a solution of iodine and tetrabutylammonium tetrafluoroborate in acetonitrile exhibited absorbances at 213, 288, 362 and 460 nm (Fig. 4.3). Within seconds of applying a voltage to the system, the absorbances at 288 nm and 362 nm were lost. This could possibly be explained in terms of some iodide in the initial solution, since the absorbance at 362nm could be attributed to the triiodide ion. During the subsequent oxidation of iodine, typically over 30 - 45 minutes, the absorbance at 213 nm intensified, the absorbance at 460 nm disappeared and a new, intense absorbance appeared at 260 nm. (Fig. 4.3.)
Fig. 4.3 - U.V. Spectra Recorded at Timed Intervals During Anodic Oxidation of Iodine

- a - prior to commencement of electrolysis.
- b - ca. 30 secs. after the application of 30 V.
- c - after 9 mins.
- d - after 15 mins.

Subsequently, the absorbances at 213 and 260nm continued to intensify, and were off-scale after 30 minutes.
4.2.2 Preparative Experiments

As described in the preceding section, the anodic oxidation of iodine in the acetonitrile-\text{Bu}_4\text{N}^+\text{BF}_4^- system yielded a pale yellow or colourless solution of I*. The concentration of I* was estimated by assuming complete oxidation of the iodine placed in the anode chamber, in accord with equation 4.5. Portions of such a solution were then reacted with approximately equimolar amounts of various arenes and the products were examined by $^1\text{H}$ NMR. The results of these experiments are summarised as follows:

(i) Toluene

Toluene (1 mmol) was stirred overnight with one equivalent of I* solution. The mixture was then poured into water and the organic products were extracted into chloroform. NMR analysis showed iodonitocenes to be the sole products, with an isomer distribution of 33\% ortho and 67\% para.

(ii) Anisole

Using the same procedure as outlined above, anisole also reacted cleanly, yielding only iodoanisoles. The para-isomer was the principal product; the amount of o-iodoanisole was estimated to be less than 5\%.

(iii) Chlorobenzene

Repeating the procedure with chlorobenzene also resulted in complete conversion to iodinated products. The isomer distribution was similar to that seen with anisole, i.e. 95\% p-chloroiodobenzene and 5\% o-chloroiodobenzene.
(iv) Benzoic Acid

The reaction of I⁺ with benzoic acid was attempted by stirring 1 mmol of the substrate with a twofold excess of the iodinating agent for 24 hours. The organic products were obtained by pouring the reaction mixture into 4M aqueous HCl and washing with ethyl acetate. The combined organic extracts were extracted with portions of 3M aqueous NaOH, which were subsequently pooled, acidified and re-extracted with an ethyl acetate–diethyl ether mixture. NMR analysis (d₆-DMSO solution) showed the benzoic acid to be extracted unchanged.

It proved difficult to remove all traces of the tetra-butylammonium cation from the products. All ¹H NMR spectra of organic products featured weak but persistent signals from Bu₄N⁺ protons. To avoid this, and to study the reactions of I⁺ directly by ¹H NMR and without isolation processes (vide infra), anodic oxidation of iodine was studied using an inorganic electrolyte, lithium tetrafluoroborate. The salt was obtained from Aldrich as an off-white powder, and was used at a concentration of 0.5 M in dry acetonitrile in exploratory experiments. Oxidation of iodine at the anode was accompanied by the deposition of a dark grey coating of lithium metal on the nickel cathode. In some experiments, particles of Li became suspended in the solution and rapidly reacted with atmospheric moisture and/or iodine. In order to avoid such complications, it was found convenient to remove the Li coating at intervals by immersion of the cathode in water.
The lithium tetrafluoroborate - acetonitrile system proved to be very convenient for the generation of I⁺ and was used in many subsequent experiments. The preparative experiments outlined above were repeated with I⁺ generated in the presence of LiBF₄, and, as expected, the results were unaffected by the change of cation.

4.2.3 Other Solvents

Anodic oxidation of iodine was attempted in a variety of alternative solvents, using in each case 0.2 M tetrabutylammonium tetrafluoroborate as the electrolyte. Iodine was apparently oxidised in DMF and DMSO, but the resulting pale yellow anolytes did not iodinate toluene. Very little current flowed when the electrolysis was attempted in propylene carbonate or in trimethyl orthoformate (TMOF), the solvent used by Shono et al. However, the addition of a small amount of water to the TMOF solution allowed a moderate current to flow.

(i) The 5% Water in TMOF - Lithium Tetrafluoroborate System

A solution of lithium tetrafluoroborate (2.5g) in 50 mls of TMOF was prepared. To this was added 2.5 mls of water. This medium was used for the oxidation of 0.5g of iodine.

Application of 30 V resulted in an initial current of 0.20 A. Hydrogen was of course evolved at the cathode. After ca. 90 minutes a pale yellow solution was obtained, which was used to test Shono's claim of higher para-selectivity in the iodination of
toluene. The analyte was added to 0.2 g of toluene in a few ml of THF and the mixture was stirred overnight.

Chloroform extraction, followed by analysis of the products by 'H NMR spectroscopy, revealed complete conversion to iodotoluenes. Integration led to an estimated isomer distribution of 70 - 71% para- and 29 - 30% ortho-iodotoluene, in agreement with the result of Shono¹⁴, but only marginally more para-selective than was found in acetonitrile (67% : 33%).

(ii) The Pivalonitrile - Tetrabutylammonium Tetrafluoroborate System

In view of literature accounts of positive iodine species and the findings of the aforementioned experiments using other solvents, it would seem that nitriles are the best solvents for the iodonium cation. Pivalonitrile (trimethylacetonitrile) was chosen as an alternative nitrile in order to ascertain whether the properties of "I⁺" could be modified by a change of solvent. It was thought that solvation of the iodonium ion by the bulkier nitrile may have led to higher para-selectivity in reactions such as that with toluene.

Iodine was oxidised in pivalonitrile using 0.1 M tetrabutylammonium tetrafluoroborate as the supporting electrolyte. The current on applying 30 V was low, only 0.03 A. Despite this, the iodine was completely oxidised when the electrolysis was allowed to proceed overnight. An almost colourless "I⁺" solution was obtained. This was split into two portions and used in reactions with anisole and toluene.
Iodinations were allowed to proceed overnight. Subsequent extraction and NMR analysis showed that both substrates had been cleanly iodinated. In the case of anisole, p-iodoanisole was the only significant product. A small amount of o-iodoanisole was detected, but this amounted to no more than 2 - 3%. Iodotoluenes were obtained in the familiar isomer distribution of 67% para and 33% ortho. The bulkier solvent was thus shown not to have any effect on the regioselectivity of the iodine electrophile.

4.2.4 Other Electrolytes - The Effect of the Counterion

Following the successful use of lithium tetrafluoroborate as a supporting electrolyte, several other lithium salts were examined to ascertain whether the properties of "I+" could be modified by a change of anion.

(i) Lithium trifluoromethanesulfonate

Following the observation of Barleunga et al.\textsuperscript{27} that the addition of trifluoromethanesulfonic acid to IPy\textsubscript{2} BF\textsubscript{4} produces a more powerful iodinating agent, experiments were carried out with lithium trifluoromethanesulfonate to find out whether the change of anion would have a similar effect on electrochemically produced positive iodine. The lithium salt was obtained from Aldrich as a white powder. A solution of \approx 0.25 M was prepared by dissolving 2.4 g of the compound in 60 ml of dry acetonitrile.

Electrolysis was carried out in the usual manner with 1 mmol of iodine in the anode chamber. A reasonable current was obtained, 0.11 A. The iodine colour faded slowly as lithium metal was deposited at the cathode. In the latter stages of the process
the lithium metal appeared to be attacked and a yellow suspension was produced. This would suggest some migration of iodine to the cathode compartment resulting in lithium iodide formation.

The positive iodine species obtained from this system is perhaps best described as CF₃SO⁻[MeCN-I⁺]. Its reactivity was tested in reactions with toluene and benzoic acid.

The reaction with toluene was carried out in the same manner as the experiments described in the preceding section. The subsequent extraction of the mixture afforded only iodotoluenes, but with a reduced para-selectivity. From NMR integration, the isomer distribution was estimated as 41% ortho and 59% para.

The reaction with benzoic acid was also performed as described previously (Section 4.2.2 - part (iv), p.201). Whereas the earlier experiment, using LiBF₄, had not shown any reactivity of I⁻ towards the deactivated substrate, the experiment using CF₃SO₃Li was indicative of some reaction. The main component of the reaction mixture was still unreacted benzoic acid, but additional signals in the NMR spectrum were suggestive of ca. 40% conversion to m-iodo-benzoic acid. The signals were however poorly resolved and the identity of the product was not confirmed by further experiments.

(ii) Lithium Hexafluorophosphate

Lithium hexafluorophosphate was obtained from Fluorochem as a white solid. Fumes were evolved when the compound was weighed in air and it was noticed that a solution in acetonitrile etched the inner surface of a Pyrex beaker. In this respect, the compound behaved quite differently to lithium tetrafluoroborate.
In an initial experiment, 2.3g of LiPF\textsubscript{6} was dissolved in 60 mls of dry acetonitrile to give a solution of ≈ 0.25 M. Oxidation of 1 mmol of iodine was attempted in this medium. When a potential difference of 30 V was applied, an initial current of 0.40 A was observed. This decreased swiftly to 0.30 A within five minutes. A brown suspension was produced in the cathode compartment while the contents of the anode compartment became black. In order to determine whether any iodine oxidation had taken place, the anolyte was stirred overnight with ≈ 1 mmol of toluene. The toluene was extracted unchanged.

Interaction of the PF\textsubscript{6} anion with the nickel cathode was a possible reason for this failure. When the nickel spatula was replaced by a second platinum foil electrode, some improvement was noticed; the brown colour of the iodine solution faded somewhat during the early stages of the electrolysis. However, a brown suspension/precipitate was again seen in the cathode chamber and eventually the anolyte turned black. These problems were finally overcome by adding 3-4 mls of glacial acetic acid to the cathode chamber to facilitate hydrogen evolution as the cathodic reaction. In this manner, a pale yellow solution of "I\textsuperscript{-}" was produced.

The reactivity of the reagent was tested by stirring half the contents of the anode chamber with toluene (92mg, 1 mmol) for ca. 14 hrs. Chloroform extraction was then performed in the usual manner, and subsequent NMR analysis led to an estimated product isomer distribution of 52.5% ortho and 47.5% para-iodotoluene.
(iii) Lithium Hexafluoroantimonate

Lithium hexafluoroantimonate was obtained from Fluorochem as a black solid. In an initial investigation, 3.75g of the solid was weighed into a beaker and 60mls of dry acetonitrile were added. This would have made a solution of $= 0.25 \text{ M}$, but the compound did not dissolve fully. The fine suspension of black particles was transferred to the electrochemical cell, which contained 1 mmol of iodine in the anode chamber. Application of 30 V resulted in a large current of 0.43 A. Lithium metal was deposited on the nickel cathode. A faint lilac colour developed in the catholyte at first, but subsequently the solution around the cathode turned yellow and ultimately, was rendered opaque by a brown suspension. As in part (ii) above, the anolyte from the failed experiment was stirred with some toluene to see if any $\text{I}^+$ had been generated, but as before, no iodotoluenes were detected.

As with lithium hexafluorophosphate, these problems were remedied by adding a few mls of glacial acetic acid to the cathode chamber. Using platinum foil for both electrodes, a solution of 3.5g $\text{LiSbF}_6$ in 60mls acetonitrile, and 5 mls of AcOH, a current of 0.28 A was observed. As hydrogen was evolved at the cathode, the iodine colour faded in the anode chamber leaving a dull orange solution of $\text{I}^+ \text{SbF}_6^-$. Once again, toluene was used as a substrate in examining the reactivity of the $\text{I}^+$ species. The experiment was conducted on the 1 mmol scale and terminated after ca. 14 hrs. Iodotoluenes were produced with an isomer distribution of 50.5% ortho : 49.5% para.
Following on from the Radner and Sugiyama reactions performed on para-deuterated substrates (Sections 3.2.3 and 3.4.2, respectively), some of the I⁺ solutions produced as described in the preceding sections were used analogously in reactions with [4-²H] toluene. The results of these experiments are summarised below:

(i) Acetonitrile - LiBF₄

As described in Section 4.2.2, iodine was fully oxidised in acetonitrile containing 0.5 M LiBF₄. To [4-²H] toluene (93mg, 1 mmol) was added ca. 1.1 equivalents of the I⁺ solution and the mixture was stirred for 2 hours. The organic products were then extracted in the usual manner. NMR analysis showed the only products to be iodo toluenes, but in a significantly altered isomer distribution of 79.5% ortho and 20.5% para. This marked deviation from the normal 33% ortho : 67% para was somewhat unexpected, although Miller and Watkins⁷ reported a similar result with [4-²H] toluene (77.2% ortho : 22.8% para). A correlation with Miller and Watkins' result for the deuterated substrate was however not anticipated, since they found a 50 : 50 mixture of isomers with normal toluene, not the 33 : 67 encountered throughout this work.

The iodination of [4-²H] toluene under the aforementioned conditions was carried out for a second time, but rather than terminating the reaction after 2 hours, the mixture was allowed to stir for 100 hours. Extraction afforded iodo toluenes in the ratio 81% ortho : 19% para.
(ii) Acetonitrile - LiPF₆

The $I^+$ solution produced using LiPF₆ as the electrolyte (Section 4.2.4 - (ii)) was also used in a reaction with 1 mmol of [4-$^3$H]-toluene. After ca. 16 hours the products extracted were 77% 2-iodo-[4-$^3$H]-toluene and 23% 4-iodotoluene.

(iii) Acetonitrile - LiSbF₆

The "I-SbFe-" generated as described in Section 4.2.4 - (iii) was also reacted with [4-$^3$H]-toluene, as in part (ii), above. The products were extracted in exactly the same isomer distribution as from the "I-PF₆-" experiment; 77% ortho- and 23% para-iodotoluene.
In order to follow the reactions of the positive iodine species by $^1$H NMR spectroscopy of the reaction mixture, some electrochemical oxidations were carried out in $d_5$-acetonitrile, CD$_3$CN. All experiments conducted in this solvent were performed under identical conditions. Lithium tetrafluoroborate was used as the supporting electrolyte.

In each experiment, 1 mmol of iodine (0.254 g) was placed in the anode compartment of the cell as described previously. A solution of lithium tetrafluoroborate (0.5 M) in CD$_3$CN was added so that the anode compartment contained 25 mls. Complete oxidation was assumed to give a solution of 0.08 M "I⁺".

(a) Equimolar Conditions

Solutions of various aromatic substrates were prepared in CD$_3$CN at 0.08 M, so that 0.5 ml portions contained $\approx 4 \times 10^{-5}$ moles. Such 0.5 ml portions were combined with 0.5 ml portions of the "I⁺" solution, to give reaction mixtures of 1 ml. After shaking, half of the mixture was pipetted into an NMR tube and spectra were accumulated at timed intervals. The results of these experiments are summarised below:

(i) Toluene

Iodination occurred before the sample could be transferred to the spectrometer. Iodotoluenes were produced with the familiar isomer distribution of 67% para and 33% ortho. With a deficiency of toluene, polyiodination occurred readily; the presence of 2,4-di-
iodotoluene was apparent from the spectrum. With a greater excess of iodinating agent, 2,4,6-triiodotoluene (δ = 8.28 ppm) was produced.

(ii) Anisole

As in the case of toluene, the reaction was complete before a spectrum could be recorded. The only significant product was p-iodoanisole; the amount of the o-isomer was minimal, the signals being barely detectable in the spectral noise. With an excess of I⁺, p-iodoanisole readily underwent further iodination to give 2,4-diiodoanisole.

(iii) 2-Phenylpropanoic Acid

Not unexpectedly, this compound showed reactivity similar to that of toluene, but with increased para-selectivity due to steric constraints. It was completely iodinated within minutes to give predominantly the para-substituted product. The amount of the o-isomer was estimated to be less than 10% (cf. toluene - 33%).

(iv) Fluorobenzene

The iodination of fluorobenzene was also found to be very rapid and was complete within a few minutes. The product was almost exclusively p-fluoriodobenzene, with only a trace of the o-isomer.
(v) Chlorobenzene

The iodination of chlorobenzene was found to be much more convenient to study on an NMR timescale, taking around an hour at the concentrations employed. Over such a time-period the conversion to chloroiodobenzenes could be readily followed, the products being formed with an isomer distribution of around 95% para and 5% ortho.

(vi) Iodobenzene

Iodobenzene was found to react at qualitatively the same rate as chlorobenzene. The principal product was p-diiodobenzene, resulting in a single peak at $\delta = 7.46$ ppm.

(vii) Benzoic Acid

No reaction was observed.

(viii) Triphenylgermane, Ph₃GeH

Three equivalents of the iodinating agent were added to a solution of triphenylgermane in $d_5$-acetonitrile. Rapid demetallation occurred resulting in the formation of iodobenzene. This reacted further to give some 1,4-diiodobenzene. This reaction is discussed in greater detail in Chapter Five (p. 258).
(b) Pseudo First-Order Conditions

Solutions of the iodinating species in $d_3$-acetonitrile were prepared using 0.25 M lithium tetrafluoroborate as described in section (a), and used in a series of kinetic experiments with simple aromatic compounds. Stock solutions of benzene, fluorobenzene, chlorobenzene, iodobenzene and toluene were prepared at 0.02 M in CD$_3$CN. Reaction mixtures were prepared by placing 0.25ml aliquots of these solutions in vials and making up to 1ml with various amounts of the "I*" solution and CD$_3$CN. For example, 0.25ml 0.02 M ArH + 0.5ml CD$_3$CN + 0.25ml 0.08 M "I*" gave a solution with [ArH] = 5 x 10$^{-3}$ M and ["I*"] = 0.02 M.

Reactions were then followed by $^1$H NMR using an automated kinetics routine. When the reaction time was conveniently long (ie. with chlorobenzene and iodobenzene) standard tuning procedures were used and 16-scan spectra were recorded at suitable intervals. This was not possible for the faster reactions. The problem was solved by tuning the spectrometer on an identical solution, replacing this in the magnet by the mixture under investigation and recording spectra unlocked. In this way, spectra could be obtained within a few minutes of mixing the reactants, at intervals of 1 minute (16 scans) or 30 seconds (8 scans).

The reaction with toluene was found to be too fast for study even with these modifications. Experiments were carried out using single 90° pulses so that spectra could be obtained every 2 seconds. In some cases the final few % of the reaction was seen in the initial spectra, but usually the reaction was complete.
before the sample could be transferred to the spectrometer. This was also the case when very low, equimolar concentrations of toluene and the iodinating species were used.

In the more convenient experiments, with the halobenzenes, 12 - 15 spectra were typically recorded. An integral file was set up on a representative spectrum and all the other spectra were integrated between the same limits. From the relative integrals, the amounts of starting material, ArH, and iodinated product, ArI, were calculated. The observed first-order rate constants, $k_{obs}$, were then evaluated from plots of $\ln [ArH]$ vs. time. These results are listed in the central column of Table 4.1. The values in the left-hand column refer to the maximum theoretical $I^+$ concentration, assuming complete oxidation of the available iodine to the $+1$ state. The actual values are likely to be somewhat lower, due to possible leakage of iodine to the cathode chamber during electrolysis and subsequent decomposition of the reagent on storage.

It must be emphasised that the aim of these experiments was to get some appraisal of the relative reactivities of various arenes towards the $I^+$ reagent, not to determine absolute rate constants. The errors associated with the experimental procedures are too large to make such measurements worthwhile. As well as the uncertainty regarding the $I^+$ concentration, errors also arose because of poor temperature control. Although the NMR probe was accurately maintained at 25°C, the reagent solution was not pre-equilibrated at this temperature owing to its inherent instability. Reaction mixtures were probably a few degrees cooler at first.
Table 4.1: Results of Kinetic Experiments Followed by $^1$H NMR.
$25^\circ C$; $[\text{ArH}] = 5 \times 10^{-3}$ M throughout.

(i) Chlorobenzene

<table>
<thead>
<tr>
<th>$[\text{I}^+]/$M</th>
<th>$k_{\text{obs}}/s^{-1}$</th>
<th>$k/M^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>$1.96 \times 10^{-4}$</td>
<td>0.0098</td>
</tr>
<tr>
<td>0.032</td>
<td>$4.63 \times 10^{-4}$</td>
<td>0.0145</td>
</tr>
<tr>
<td>0.040</td>
<td>$6.03 \times 10^{-4}$</td>
<td>0.0151</td>
</tr>
<tr>
<td>0.040</td>
<td>$6.33 \times 10^{-4}$</td>
<td>0.0158</td>
</tr>
<tr>
<td>0.052</td>
<td>$9.65 \times 10^{-4}$</td>
<td>0.0186</td>
</tr>
<tr>
<td>0.060</td>
<td>$1.05 \times 10^{-3}$</td>
<td>0.0175</td>
</tr>
</tbody>
</table>

(ii) Iodobenzene

<table>
<thead>
<tr>
<th>$[\text{I}^+]/$M</th>
<th>$k_{\text{obs}}/s^{-1}$</th>
<th>$k/M^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.040</td>
<td>$6.26 \times 10^{-4}$</td>
<td>0.0156</td>
</tr>
</tbody>
</table>

(Some additional iodobenzene runs gave consistently slow reactions. Cloudiness was observed and N-iodoacetamide formation is suspected)

<table>
<thead>
<tr>
<th></th>
<th>$k/H^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>$9.1 \times 10^{-5}$</td>
</tr>
<tr>
<td>0.020</td>
<td>$6.2 \times 10^{-5}$</td>
</tr>
<tr>
<td>0.060</td>
<td>$1.96 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

(iii) Fluorobenzene

<table>
<thead>
<tr>
<th>$[\text{I}^+]/$M</th>
<th>$k_{\text{obs}}/s^{-1}$</th>
<th>$k/M^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>$2.2 \times 10^{-3}$</td>
<td>0.11</td>
</tr>
<tr>
<td>0.030</td>
<td>$3.3 \times 10^{-3}$</td>
<td>0.12</td>
</tr>
</tbody>
</table>

(iv) Benzene

<table>
<thead>
<tr>
<th>$[\text{I}^+]/$M</th>
<th>$k_{\text{obs}}/s^{-1}$</th>
<th>$k/M^{-1}s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020</td>
<td>$3.3 \times 10^{-3}$</td>
<td>0.167</td>
</tr>
<tr>
<td>0.020</td>
<td>$7.5 \times 10^{-3}$</td>
<td>0.375</td>
</tr>
<tr>
<td>0.020</td>
<td>$4.3 \times 10^{-3}$</td>
<td>0.216</td>
</tr>
<tr>
<td>0.020</td>
<td>$4.5 \times 10^{-3}$</td>
<td>0.227</td>
</tr>
<tr>
<td>0.024</td>
<td>$5.1 \times 10^{-3}$</td>
<td>0.211</td>
</tr>
<tr>
<td>0.024</td>
<td>$6.6 \times 10^{-3}$</td>
<td>0.276</td>
</tr>
<tr>
<td>0.030</td>
<td>too fast for study</td>
<td></td>
</tr>
</tbody>
</table>
(c) Recovery of $d_5$-Acetonitrile

In view of the high cost of the $d_5$-acetonitrile used in this work, some recycling of the solvent was carried out. Residues from each experiment were pooled, and when a volume > 50mls had been collected, the solvent was cautiously distilled. The first distillate was pink due to iodine and NMR analysis indicated the presence of aromatic impurities. However, subsequent distillation from calcium hydride, through a 20cm Vigreux column, afforded dry and essentially pure CD$_3$CN. Benzene (b.pt. 80°C) could not be removed from CD$_3$CN (b.pt. 81°C) by distillation. This problem was overcome by adding fresh iodinating agent to the contaminated solvent to convert the benzene to much less volatile iodobenzene.

(d) Evidence for $^1$H - $^1$H Coupling?

An interesting observation was that all $^1$H NMR spectra of positive iodine species in CD$_3$CN featured three sharp lines at around 6ppm. These minor but persistent signals were separated by 53Hz (Fig. 4.4):

![Fig. 4.4: Expansion Of Some Minor Signals Seen In All $^1$H NMR Of Iodine (I) Species in CD$_3$CN](image-url)
4.2.7 Kinetic Experiments by Titrimetric Methods

To complement the kinetic studies by NMR described in section 4.2.6., and because of the high cost of $d_2$-acetonitrile, iodination by electrochemically produced $I^+$ was also followed using a simple titrimetric method.

In such experiments, a known quantity of $I^+$ was added to an excess of an aromatic substrate in acetonitrile solution, thermostatted at 25°C in a volumetric flask. Aliquots were then withdrawn at timed intervals and added to flasks containing an excess of acidified potassium iodide solution. Combination of unreacted $I^+$ with iodide ions thus liberated molecular iodine, which was estimated by thiosulphate titration.

From the results of the NMR studies, it was clear that only the relatively slow reactions, i.e. with the halobenzenes, could be monitored in this way. Thus, the iodination of bromobenzene was the first reaction to be studied.

Iodine was completely oxidised in acetonitrile solution, containing 0.2M LiBF$_4$ as the supporting electrolyte. The quantities used (0.2536g I$_2$ in 40mls MeCN) were such that complete oxidation would produce a solution 0.05M in $I^+$. A solution of bromobenzene in acetonitrile was prepared at the same concentration (0.7851g / 100ml ≈ 0.050M) in a volumetric flask. Kinetic experiments were then carried out by adding 10mls of the $I^+$ solution to 40mls of the bromobenzene solution, thermostatted at 25°C. Samples (5mls) were taken at intervals of 6 minutes and added to conical flasks containing 50mls of 1.5M aq. hydrochloric acid and ca. 2g of potassium iodide.
Several precautions were taken to minimise aerial oxidation of iodide ions. Freshly distilled, deionized water was used to make up the acid solution and solid potassium iodide was added shortly before the addition of the aliquot of reaction mixture. Furthermore a small amount of solid sodium bicarbonate was added to each flask before the iodine was titrated, in an attempt to create a blanket of carbon dioxide over the solution. The iodine was then estimated by titration with 0.005M sodium thiosulphate solution, using starch as indicator. A representative set of results is listed in Table 4.2:

Table 4.2: Iodination of Bromobenzene
(25°C, [PhBr] = 0.04M; [I⁻] = 0.01M)

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Titre (0.005M S₂O₃²⁻)</th>
<th>[I₂]/10⁻²M</th>
<th>ln[I₂]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30.2</td>
<td>1.510</td>
<td>-4.19</td>
</tr>
<tr>
<td>6</td>
<td>27.3</td>
<td>1.365</td>
<td>-4.29</td>
</tr>
<tr>
<td>12</td>
<td>25.7</td>
<td>1.285</td>
<td>-4.35</td>
</tr>
<tr>
<td>18</td>
<td>23.5</td>
<td>1.175</td>
<td>-4.44</td>
</tr>
<tr>
<td>24</td>
<td>22.5</td>
<td>1.125</td>
<td>-4.49</td>
</tr>
<tr>
<td>30</td>
<td>20.6</td>
<td>1.030</td>
<td>-4.57</td>
</tr>
<tr>
<td>36</td>
<td>19.7</td>
<td>0.985</td>
<td>-4.62</td>
</tr>
<tr>
<td>42</td>
<td>18.4</td>
<td>0.920</td>
<td>-4.69</td>
</tr>
<tr>
<td>48</td>
<td>17.5</td>
<td>0.875</td>
<td>-4.74</td>
</tr>
</tbody>
</table>

A plot of ln[I₂] vs. time resulted in a good straight line fit, with a measured gradient of 1.83 x 10⁻⁴s⁻¹. Dividing this by the bromobenzene concentration, 0.04 M, generated a second-order rate constant, k, of 4.58 x 10⁻⁵M⁻¹s⁻¹. A duplicate run led to a result of 4.81 x 10⁻⁵M⁻¹s⁻¹.
Further experiments of this kind were performed using fluorobenzene as the substrate. As expected, the reaction was found to be much faster and reaction mixtures had to be sampled at least every minute. The rapid decrease in thiosulphate titre was consistent with the reaction being around an order of magnitude faster than that with bromobenzene. However, the titres were too irregular for the meaningful evaluation of rate constants.

Because of the severely limited range of substrates that could be studied by the titrimetric method, the matter was not pursued further. The experiments did however raise some puzzling questions. On inspection of Table 4.2, it can be seen that titres fell from 30.2ml to 17.5ml during the course of the experiment, volumes which were much larger than had been anticipated. Since 1 mmol of iodine was anodically oxidised in 40mls of acetonitrile, the maximum I⁺ concentration was 0.05 M. Titration of 1ml of such a solution with 0.005 M thiosulphate solution (following its addition to excess iodide) would be expected to require a volume of 20ml, if equations 4.6 and 4.7 were the only reactions occurring.

$$\text{I}^+ + \text{I}^- \rightarrow \text{I}_2 \quad 4.6$$

$$\text{I}_2 + 2\text{S}_2\text{O}_3^{2-} \rightarrow 2\text{I}^- + \text{S}_4\text{O}_6^{2-} \quad 4.7$$

However, when such a titration was carried out, the volume of 0.005 M thiosulphate solution required was consistently recorded as 31.2ml. The implication of this is that the concentration of I⁺ was 0.078 M rather than 0.05 M, or that the solution contained other oxidants capable of oxidising iodide ions.
4.3 Discussion

Aromatic iodination with electrochemically generated positive iodine has not received the degree of attention that it possibly deserves. The mechanistic study of Hiller and Watkins⁷ remains to this day the most comprehensive investigation of the topic, after almost two decades. In review articles, or in general introductions to papers on iodination, the subject is often relegated to a few lines towards the end of a summary, or, more commonly is totally ignored. The related topic of electrolytic iodination of peptides and proteins in aqueous media (Section 1.4.1(vii)) is also dismissed by many authors. The principal reason offered for this apparent lack of interest in electrochemical techniques is that expensive, specialised equipment is required. Some authors have additionally expressed reservations about the timescale of anodic oxidation, particularly with regard to the half-lives of radioisotopes.

The work described in this chapter has shown that the electrochemical oxidation of iodine can be easily achieved, using relatively simple equipment. The two-compartment cell depicted in Fig. 4.1 (p. 194) proved to be quite adequate for the purpose, once initial difficulties such as the destruction of graphite cathodes had been overcome. Admittedly, slightly more sophisticated equipment would have been preferable, such as a constant current supply. However, a knowledge of the number of coulombs passed during the oxidation was not essential, since the reaction was self-indicating. Complete oxidation was signified by the loss of the brown colour of I₂ in the anode chamber.
This assumption was supported by estimating the number of coulombs passed over the time period required to fully decolourise the anolyte (Section 4.2.1 (b), p.196). Areas under plots of current against time invariably corresponded to the passage of 2.0 - 2.2 F / mole I2, thus there can be little doubt that iodine is fully oxidised to the +1 state as in equation 4.5 (p.197).

The colourless, or more usually, very pale yellow solutions of the iodine (I) reagent were found to be very unstable at room temperature. Iodine was liberated within minutes if a portion of the anolyte was left in an open beaker. In contrast, the reagent appeared to be quite stable when stored in a tightly-stoppered vessel at -20°C. The favoured mode of storage was however as a frozen solid in a dry-ice container.

The preparative experiments described in Section 4.2.2 led to results which were generally comparable to those of Miller and Watkins. However, a few discrepancies arose which will be addressed later. The range of compounds which could be iodinated using the I+ reagent was essentially as described by Miller and Watkins. A notable exception was benzoic acid; whereas Miller and Watkins obtained a reasonable yield of m-iodobenzoic acid (40%), the I+ species generated in this study did not react with the substrate. The only difference between the two systems is the counterion; ClO4- in Miller and Watkins' work and BF4- in this study. The greater reactivity of Miller and Watkins' "I+ ClO4-" is also manifested in a lower regioselectivity. Inspection of Table 4.3 shows that "I+ BF4-" was much more selective.
Table 4.3: Product Isomer Distributions

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Ref. 7</th>
<th>This work</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ortho</td>
<td>meta</td>
</tr>
<tr>
<td>PhOme</td>
<td>33</td>
<td>10</td>
</tr>
<tr>
<td>PhMe</td>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>PhF</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>PhCl</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>PhI</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>PhCOOH</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

In the context of equation 4.2 (p. 185), these differences can be attributed to the role of the counterion in removing the proton from the Wheland intermediate, and to the overall effect of the medium (i.e. its dielectric constant) on the relative magnitudes of the various rate constants involved.

Further work was then carried out using different solvents and electrolytes (Sections 4.2.3 and 4.2.4, respectively). Wet trimethyl orthoformate (TMOF) was used as a solvent to emulate the work of Shono et al.\textsuperscript{14}, and indeed, the same iodotoluene isomer distribution (30\% ortho, 70\% para) was obtained. The main reason for Shono's publication was to claim a high para-selectivity, but, as shown in Table 4.3, toluene was iodinated with almost the same degree of selectivity in this study, using I\textsuperscript+ BF\textsubscript{4}\textsuperscript{−} in acetonitrile.

Another solvent investigated was pivalonitrile. The logic behind this selection has already been outlined (p. 203); regarding the nitrile as a "vehicle" for the I\textsuperscript+ cation, it was thought that
the use of a bulkier nitrile may also have led to high para-selectivity for steric reasons. In the event, the change of solvent had absolutely no effect on the isomer distributions of products.

Section 4.2.4 describes some experiments in which iodine was oxidised in acetonitrile using some alternative electrolytes. A common theme of literature on iodination, and electrophilic aromatic substitutions in general, is that the reactivity of electrophiles is enhanced by the addition of TFA, and enhanced still further by the addition of triflic acid. With this in mind, lithium triflate was used as an electrolyte in order to generate \( \text{CF}_3\text{SO}_3^-\text{I}^- \). The greater reactivity expected of this species was borne out by experiment. Thus, toluene was iodinated with reduced para-selectivity (41% ortho, 59% para) and benzoic acid, inert towards \( \text{I}^+\text{BF}_4^- \), showed some degree of reaction with the reagent.

Two further lithium salts, the hexafluorophosphate (LiPF₆) and the hexafluoroantimonate (LiSbF₆), were also studied. As described in the experimental section, unforeseen complications arose with these salts. The problems were eventually solved by adding acetic acid to the cathode compartment of the cell during electrolysis, so as to facilitate hydrogen evolution as a cathode reaction. In this way, solutions of iodine(I) were generated which were then tested in reactions with toluene. Iodotoluenes were produced much less selectively, the o/p isomer distribution in both cases being around 50 : 50. These results must however be treated with some caution. In retrospect, it would seem that the lower selectivity was more likely to be attributable to acetic acid which had diffused into
Section 4.2.5 describes some experiments performed with [4-2H]-toluene so as to investigate isotope effects. This work was carried out with I+ generated in acetonitrile, using LiBF₄, LiPF₄ and LiSbF₆ as electrolytes. Although these systems gave rise to widely differing product isomer distributions with ordinary toluene, the results with [4-2H]-toluene were curiously similar. In each case, the amount of ortho-substituted product was in the range 77-81%, while the amount of 4-iodotoluene was only 19-23%. Miller and Watkins⁷ obtained the same result with their "I+ ClO₄-" system (77.2% ortho, 22.8% para), despite the 50:50 distribution they obtained with non-deuterated toluene.

It is not clear how Miller and Watkins determined their \( k_\text{d} / k_\text{o} \) values; different rules appear to have been applied to different substrates. In the case of toluene, the value of 1.54 seems to have been derived simply by comparing the percentage of ortho-substituted product from deuterated and non-deuterated material ie. 77/50 = 1.54. Applying this approach to the results of this study gives a value of 2.41 (79.5/33).

Section 4.2.6 describes some experiments utilising iodine (I) generated in \( d_5 \)-acetonitrile solution. These were carried out so that reactions could be monitored directly by \(^1\)H NMR, thereby avoiding the need to extract products and allowing assessment of reaction rates to be made. Exploratory experiments using equimolar quantities of reactants (4.2.6 (a)), served to confirm the results of the preparative experiments performed earlier.
Subsequent NMR experiments (4.2.6 (b)), conducted under pseudo-first-order conditions, were aimed at getting some appreciation of relative rates. For the reasons outlined on p. 214, it was not possible to obtain accurate rate constants. In addition to the factors already mentioned (uncertainty regarding the I\(^+\) concentration and a lack of temperature control), it was not possible to fully protect solutions from aerial moisture and some conversion to N-iodoacetamide was suspected.

The initial aim of these experiments was to construct a Hammett plot. However, it transpired that only the halobenzenes were iodinated at a rate convenient for NMR study. The reaction with benzene was too fast for study, while less reactive substrates (PhCOOH, PhCN) failed to react. Thus the range of compounds which could be studied was severely limited. Measured rate constants are given in Table 4.4, with an estimated value for toluene.

Table 4.4: Rates of Iodination by I\(^+\) in \(d_5\)-Acetonitrile, as Estimated From Sequential NMR Spectra (25°C)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(k/M^{-1}s^{-1})</th>
<th>(\log k)</th>
<th>Hammett (\sigma^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PhCl</td>
<td>0.0152</td>
<td>-1.82</td>
<td>+0.114</td>
</tr>
<tr>
<td>PhI</td>
<td>0.0156</td>
<td>-1.81</td>
<td>+0.135</td>
</tr>
<tr>
<td>PhF</td>
<td>0.12</td>
<td>-0.92</td>
<td>-0.073</td>
</tr>
<tr>
<td>PhH</td>
<td>0.245</td>
<td>-0.61</td>
<td>0.000</td>
</tr>
<tr>
<td>PhMe</td>
<td>ca. 200 ?</td>
<td>+2.3</td>
<td>-0.311</td>
</tr>
</tbody>
</table>

Clearly, these data are inadequate for the construction of a Hammett plot; all that can be concluded is that the \(\rho\) value is large and negative, most probably in the range -5 to -10.
Additional kinetic studies were attempted by a titrimetric method (Section 4.2.7), but again problems were encountered. Like thallium (III) trifluoroacetate described in Chapter Two, it was concluded that the inherent instability of the I+ reagent makes it unsuitable for kinetic work.

Considering the possible application of electrochemical oxidation to radiiodination, there would not appear to be any reasons why it should not be possible. The experiment with Bu₄NI (p.197) showed that it was possible to generate iodine (I) starting from iodide ions. The presence of water would not seem to be a problem; both Miller and Watkins⁷ and Shono et al.¹⁴ used wet solvents for optimal results. Perhaps the biggest problems in applying the method to n.c.a. radiiodination would be engineering ones in scaling down the apparatus for use with µl volumes of solutions. However, it is unlikely that such problems would be insurmountable.

In summary, anodic oxidation of iodide or iodine provides a method whereby complete conversion to electrophilic iodine (I) can be achieved. The reagent so produced, using the acetonitrile-lithium tetrafluoroborate system, is capable of the clean and efficient iodination of substrates as deactivated as halobenzenes. Less reactive arenes may be iodinated by using other counterions. As with all direct iodination methods, the technique does not offer regiospecificity. This potential drawback is addressed in the following chapter by combining the method with the use of organo-metallic precursors.
4.4 References


CHAPTER FIVE

Iodination Via Group IVb Organometallic Intermediates
The cleavage of aryl-SR\(^+\) bonds by electrophilic reagents, where M = Si, Ge or Sn, provides a method for the regiospecific incorporation of a wide variety of functionalities into aromatic rings. Demetallation reactions of this type have been extensively studied by Eaborn\(^1\), and the synthetic applications of electrophilic desilylation have been reviewed by Chan and Fleming.\(^2\)

The preparation of aryl halides from aryltrimethylsilanes was first described in 1948 by Pray et al.\(^3\) Phenyltrimethylsilane was cleaved by refluxing with molecular iodine for 12 hours, in the presence of aluminium, to give iodobenzene and iodotrimethylsilane.

The displacement of an -MR\(^+_3\) group by an electrophile always proceeds in an \textit{ipsa} fashion, thus the position of the metal substituent determines the position of the incoming group. In this way, only one isomer is produced. Eaborn\(^1\) listed many other advantages of demetallation over conventional aromatic substitutions. Isomers may be obtained which are formed only to a very small extent in direct electrophilic attack. Furthermore, the reactivity of the carbon-metal bond can often allow substitution to occur in systems too unreactive to react as ArH. For compounds which are not stable to conventional substitution conditions, the demetallation reaction allows much milder conditions to be used. For aromatic compounds with a side-chain susceptible to electrophilic attack, an -MR\(^+_3\) group may direct reaction to a ring position and away from the side-chain.
Compounds of the type ArMR₃ also have advantages over the corresponding ArMgX and ArLi compounds. They can be used to make derivatives with substituents incompatible with Mg and Li, such as -COR and -NO₂. Furthermore, lithiation to produce ArLi compounds generally results in a mixture of isomers. Conversion to ArMR₃ organometallics makes it possible to separate the isomers.

The mechanism of electrophilic demetallation is analogous to that of simple aromatic substitution, proceeding via a Wheland type σ-complex as shown in Fig. 5.1:

![Fig. 5.1: Mechanism of Electrophilic Desilylation](image)

In most reactions of this type, step 1 is found to be rate determining. This was demonstrated for cases where E⁺ = H⁺ (protodesilylation) by Baborn et al. Various compounds of the type ArMR₃ (M = Si, Ge, Sn, Pb) were cleaved by HCl in aqueous dioxane. In all cases, the reaction was slower in D₂O than in H₂O. A further study on the cleavage of X-C₆H₄SiMe₃ compounds by TFA and X-C₆H₄SnMe₃ compounds by AcOH showed ca. 6-fold decreases in rate when the corresponding deuterated acids were used."
The cleavage of $X-\text{C}_8\text{H}_6\text{SiMe}_3$ compounds by halogens has also been studied kinetically. The kinetics were also found to be consistent with rate-determining formation of a Wheland-type intermediate ($E = \text{Br}$ or $\text{Cl}$). However, the results of some studies have highlighted certain anomalies. For example, Stock and Spector studied the iododesilylation of aryltrimethylsilanes by iodine monochloride in acetic acid solution. Iodine monochloride was found to react around 8 times faster than chlorine, quite the reverse of the case with direct halogenation, where chlorine is by far the more reactive electrophile. The result could possibly be interpreted in terms of a four-centre transition state, of type (I), but other results do not favour this.

![Fig. 5.2: Postulated Transition States in Halodesilylations](image)

A mechanism involving a transition state of type (I) would proceed with retention of configuration at silicon, but as Baborn and Steward showed, using optically active silanes, bromodesilylation proceeds with inversion of configuration. A possible mechanism which would probably involve inversion at silicon is one which involves a six-centre intermediate of type (II).
Kinetic and mechanistic studies have also been carried out on iododesalnnylation. Eaborn et al. studied the reaction of iodine with ArSnMe derivatives in carbon tetrachloride, whereas Buchman et al. used methanol as the solvent. All results from such studies have been consistent with the aforementioned mechanism of electrophilic aromatic substitution, but again, certain anomalies have been highlighted. For example, Hasielski et al. observed that iododesalnnylation with iodine in methanol was faster than protodesalnnylation with perchloric acid, whereas the reverse was found to be true in the case of desilylation.

The synthetic utility of trimethylsilylated aromatics in regiospecific iodination was studied in the 1970's by Calas and coworkers. They found that compounds such as the three isomeric trimethylsilyltoluenes could be converted to the single iodinated products by refluxing with iodine in the presence of a catalytic amount of aluminium. In the same way, bis(trimethylsilyl)benzenes were converted to the corresponding di-iodobenzenes.

In order to achieve iododesilylation of less activated aryltrimethylsilylalanes, such as those bearing chlorine substituents, a source of positive iodine (ICl, IBr) was found to be necessary. In a subsequent paper, the use of this method to iodinate very deactivated compounds was described. Isomeric iodonitrobenzenes were produced from bis(trimethylsilyl)benzenes by first displacing one -SiMe group with NO^+, then using ICl to displace the other.
Early studies on the radio bromination and radioiodination of aromatics via aryltrimethylsilanes were carried out by Wilbur and coworkers. They made a systematic study of the reactions of ortho, meta and para-trimethylsilyltoluenes, first with stable bromine and iodine, and subsequently with $^{22}$Br and $^{131}$I. Reactions were conducted in a variety of solvents, using N-chlorosuccinimide (NCS) and t-butylhypochlorite (TBHC) as in situ oxidants, to oxidise bromide and iodide to electrophilic species.

The work showed that desilylation was an effective method for the preparation of specific halogenated products, although the reaction times were quite long for radioiodination. Iodinations were found to be very much slower than the corresponding brominations, and because of the very low iodide ion concentrations involved, no -carrier-added (n.c.a.) iodination with $^{131}$I failed to proceed under the conditions used with stable $^{127}$I. All reactions were found to be very much faster when glacial acetic acid was used as solvent compared to the rates in methanol. N.c.a. radioiodination was thus achieved by carrying out the desilylation in acetic acid at 60°C, using Na$^{131}$I and NCS. An acidic solvent also has the advantage of neutralising the base present in radioiodide preparations. In other solvents, p-toluenesulfonic acid was added for this purpose.

A subsequent study focused on the halogenation of phenols via silylated derivatives. The ortho / para directing property of electron donating groups such as -OH and -OMe is so strong that ipso substitution of m-trimethylsilyl derivatives does not readily occur. For example, Baborn and Webster reported that the bromination
of \( m \)-\((\text{trimethylsilyl})\)anisole resulted in \( 4\)-bromo-\( 3\)-\((\text{trimethylsilyl})\)anisole, and not \( m \)-bromoanisole. For radiopharmaceutical applications, meta-iodination of activated rings is desirable, since this position would be less susceptible to dehalogenation \textit{in vivo}.

Wilbur et al. studied the bromination and iodination of isomeric \((\text{trimethylsilyl})\)phenols, \((\text{trimethylsilyl})\)anisoles and \((\text{trimethylsilyl})\)phenol acetates. Reaction conditions were the same as those employed in the previous study, i.e. the reactions were carried out in acetic acid at 60°C, in the presence of NCS.

In the case of the \((\text{trimethylsilyl})\)phenols, all three regioisomers underwent some substitution by bromine without cleavage of the \(-\text{SiMe}_3\) moiety. This was minimal in the case of the para-isomer, the principal product being \( p \)-bromophenol. Reaction of the \( o \)-isomer resulted in an approximately 1:1 mixture of \( o \)-bromo-phenol and \( 4\)-bromo-\( 2\)-(trimethylsilyl)phenol. The \( m \)-isomer underwent bromination without any loss of the \(-\text{SiMe}_3\) moiety, to give one major product. Whether this was \( 4\)-bromo-\( 3\)-(trimethylsilyl)phenol or \( 2\)-bromo-\( 5\)-(trimethylsilyl)phenol was not determined.

In the case of the \((\text{trimethylsilyl})\)anisole derivatives, the \( o \) and \( p \) isomers underwent clean \textit{ipso} desilylation to yield the specific halogenated products. This was demonstrated for both \( ^{77}\text{Br} \) and \( ^{125}\text{I} \). In contrast, the \( m \)-isomer did not undergo \textit{ipso} desilylation, but gave one of the two possible \( \text{bromo(\text{trimethylsilyl})anisoles} \). Again, its identity was not determined.

Successful \textit{meta}-desilylation was achieved by converting the \((\text{trimethylsilyl})\)phenol to its acetate, using acetic anhydride and
pyridine. All three (trimethylsilyl)phenol acetates were efficiently converted to the specific bromo- and iodo- products by treatment with X⁻/NCS in acetic acid at 60°C.¹³

Various aspects of radiohalogenation using Group IVb organo-metallics have been addressed in a series of papers by Moerlein and coworkers.¹⁹⁻²⁵ In 1985, Moerlein and Coenen¹⁹,²¹ reported on an extensive and systematic study of radiohalogenation, using not only aryltrimethylsilanes, but their germanium and tin counterparts as well. For each metal, a series of para-substituted compounds, p-X-C₆H₄-MMe₃, was prepared with X = -CF₃, -Br, -F, -H, -CH₃ and -OCH₃. These substrates were treated at room temperature with solutions of n.c.a. Na¹²⁷Br and Na¹³¹I in several different solvents, using N,N-dichloramine-T (DCT) as the in situ oxidant.

All experiments were terminated after a fixed period of 30 minutes and radiochemical yields were compared. A comparison of the three metals showed radiochemical yields to increase in the order Si < Ge < Sn. This is what one would expect in view of the accepted reaction mechanism¹, the intermediate σ-complexes being increasingly stabilised by hyperconjugative (σ-π conjugative) electron release from the C-M bond on descending the group.

A comparison was also made between three solvent types, acidic (acetic acid), polar (methanol) and non-polar (carbon tetrachloride). Reactions were found to be most rapid in acetic acid. Two reasons were given for this. Firstly, a low pH is favourable for the production of positive halogen species and suppresses their reduction. Secondly, since the intermediate σ-complex is a
charge-separated structure, the energy of its transition state is decreased in more polar solvents.

The cleavage of the aryl-tin bond was found to be very rapid, irrespective of any substituents on the ring. Destannylation was also largely unaffected by changing the solvent. In the case of halogenodegermylation, such factors were more important. Radiocchemical yields (after 30 minutes) decreased with solvent polarity and were low for the deactivated substrate (X = -CF₃). This trend continued on moving to the series of aryltrimethylsilanes. Radiocchemical yields in excess of 10% were obtained only with the more activated substrates (X = -OMe, -Me, -F). In the non-polar solvent carbon tetrachloride, only the most activated substrate (X = -OMe) underwent significant demetallation.

Radiocchemical yields for iodination (¹²³I) were found to be higher than those for bromination (¹²⁷Br) throughout this study. This was explained in terms of the relative instability of BrCl. Once formed, the halogen electrophile, BrCl or ICl, can either react with the aromatic substrate or undergo reduction by reaction with water in the solvent. Positive bromine species are reduced more readily than iodine species, hence water oxidation is more important.

Also examined was the effect of added water. This is clearly of great relevance to radiohalogenation since isotopes are supplied as aqueous halide solutions. Although it is a simple matter to freeze-dry aqueous preparations, it does take time and in the case of short-lived isotopes, time is the dominant factor.
The yields from destannylations were unaffected by up to 50% water in the reaction mixtures. In contrast, the degemtylation and desilylation reactions were adversely affected by relatively small amounts of water, resulting in greatly reduced yields. These observations can also be attributed to redox reactions between water and the electrophilic halogen species. The rates of such competing pathways are comparable to rates of degemtylation or desilylation, but are insignificant relative to the rapid rate of destannylaton.21

Based on these factors alone, it might be thought that tin compounds are the most useful for the rapid incorporation of radiohalogens into aromatic systems. However, there are some other considerations which need to be taken into account. The ease with which the aryl-tin bond is cleaved results in competing chlorodemetalation when dichloramine-T is used as the in situ oxidant. Under n.c.a. radiolabelling conditions the oxidant is present in high concentrations relative to the radiohalide and chlorination becomes a very serious problem. It is especially undesirable for radiopharmaceutical applications since chlorinated contaminants are difficult to separate chromatographically from radiobromine or radiiodine labelled products.

Moerlein and Coenen21 showed that chlorodemetalation was minimal for germanium and silicon compounds. Germanium and silicon compounds also have greater general chemical stability than their tin counterparts, for example, towards acids and alkalis. A further advantage, certainly in the case of trimethylmetal derivatives, is
that the germanium and silicon compounds are much less toxic than the tin compounds.

The conclusion that is drawn from this piece of work is that the choice of metal depends on the degree of activation of the aromatic ring. Stannylated derivatives are clearly required for the halogenation of deactivated systems, but for more electron-rich systems, the more stable silylated derivative may be the substrate of choice.

In a subsequent paper, Moerlein\textsuperscript{22} presented a case for the general usefulness of germanium compounds in halogenodemetalation. This study was carried out using phenyltrimethylgermane, two para-substituted derivatives (p-fluoro-, p-hydroxy-) and two meta-substituted derivatives (m-chloro-, m-methoxy-). Preparative scale halogenations were carried out using chlorine, bromine, iodine and iodine monochloride. The substrates were chosen to highlight certain points. For example, as has already been noted, m-(trimethylsilyl)anisole does not undergo demetalation with positive halogen species, but reacts at the para-position.\textsuperscript{7} This is not the case with m-(trimethylgermyl)anisole. The more reactive aryl-germanium bond is cleaved more readily than the para-C-H bond, and clean ipso halogenodemetalation occurs. In fact, Moerlein\textsuperscript{22} observed only de-germylation for all the substrates studied; products from competing halogenodeprotonation were non-existent.

The reaction with chlorine was very rapid with all of the substrates in each of three solvents. This was also true for bromination, although longer reaction times were required for the
meta-substituted compounds, particularly in the non-polar solvent. Reactions were very much slower when iodine was used although high yields were obtained eventually. Rapid iododegermylation was achieved by using iodine monochloride as a source of positive iodine. The yields of iodinated products were 96-99% with this reagent after one minute in the polar solvents.

The applicability of demetallation reactions to labelling with the short-lived isotope, iodine-122 ($t_{1/2} = 3.6$ min.), was examined in a subsequent study. Organometallic derivatives containing boron, silicon, germanium, tin and mercury were compared in a series of experiments in which reaction times were limited to 30 seconds or 1 minute. Two approaches were used; in situ oxidation of $^{122}\text{I}^-$ with dichloramine-T and exchange labelling of iodine monochloride. The isotope was produced from $^{122}\text{Xe}$ (see Section 1.2.4) and rinsed from a steel generator loop with the reaction solvent.

Most experiments with $^{122}\text{I}$ were carried out in ethanol because of its physiological compatibility. In this medium, only the reactions involving tin and mercury gave acceptable yields of iodinated products within the time constraints set by the isotope. Additional experiments in acetic acid solution showed that high yields could also be obtained from the germylated derivative in this medium.

The problem of competing chlorodemetallation in reactions involving dichloramine-T was also encountered in this study. It was circumvented by using peracetic acid to oxidise iodide. This was generated in situ from hydrogen peroxide and glacial acetic acid.
In 1988, Moerlein et al.\textsuperscript{24,25} reported on a detailed study of \textit{in situ} oxidation using peracetic acid. The reagent was used in both halogenodeprotonation reactions with simple arenes and halogenodemetalation reactions with Group IVb organometallics. Initial experiments indicated that commercial preparations of peracetic acid were too strongly oxidising and so, as noted above, the reagent had to be prepared \textit{in situ}:

\[
\text{CH}_3\text{CO}_2\text{H} + \text{H}_2\text{O}_2 \xrightarrow{H^+} \text{CH}_3\text{CO}_3\text{H} + \text{H}_2\text{O} \quad 5.1
\]

Experiments were carried out to determine the optimal composition of the mixture for halogenation.\textsuperscript{25} This was found to be a 2:1 mixture of 11.4 M aqueous hydrogen peroxide and glacial acetic acid. For \textsuperscript{131}I labelling, additional sulphuric acid (0.2 M in the mixture) was found to be necessary, to neutralise the base present in Na\textsuperscript{131}I.

It was postulated that the halogenating agent was the hypohalous acid, HOX, formed by oxidation of halide ions by the peracid (eq. 5.2). However, the reaction is probably more complex (see p.18).

\[
\text{CH}_3\text{CO}_3\text{H} + X^- \xrightarrow{} \text{HOX} + \text{CH}_3\text{CO}_2^- \quad 5.2
\]

Although the use of peracetic acid prevents competing chlorodemetalation, the acidic environment does result in acid hydrolysis, most notably of the tin compounds, X-C\textsubscript{6}H\textsubscript{4}SnMes. This is not a great problem as the products are generally less difficult
to separate than those bearing other halogens. The highest yields of radiohalogenated products (after a fixed reaction time of 30 mins) were obtained from non-activated substrates (X = -H, -F). Electron-deficient arenes (X = -CF₃) reacted more slowly as seen in earlier work¹⁹-²³, but yields were also low from electron-rich substrates (X = -OMe), owing to the aforementioned protiolysis being faster than iododestannylation.

The studies of radioiodination via Group IVb organometallics summarised in the preceding pages have involved only simple model compounds. There have however been numerous reports on the use of such techniques in the radioiodination of compounds of biological importance.

Wilson and Jacob²⁶ showed that a number of more complex molecules could be regioselectively iodinated by using trimethylsilylated precursors. Silver salts were used to activate the iodine molecule; AgBF₄ was found to be the most effective. The work thus represents a combination of two iodination methodologies. The method was optimized using simple compounds such as phenyltrimethylsilane, 4-tolyltrimethylsilane and 4-(trimethylsilyl)diphenylmethane. Having established that the sole products were those of ipso iododesilylation, the authors used the method to iodinate some compounds of biological significance. An amino acid derivative, N-acetyl phenylalanine methyl ester (21), and a dipeptide, N-formylphenylalanylglycine ethyl ester (22), were successfully iodinated at the 4-position of their phenyl rings, starting from the corresponding 4-trimethylsilyl-derivatives. However, an attempt to iodinate a penicillin derivative
in a similar fashion failed, presumably due to attack on the sulphur or \(\beta\)-lactam ring.

\[
\begin{align*}
\text{(21)} & \quad \begin{array}{c}
\text{NHCOCH}_3 \\
\text{CH}_2\text{CHCO}_2\text{Me}
\end{array} \\
\text{(22)} & \quad \begin{array}{c}
\text{NHCHO} \\
\text{CH}_2\text{CHCONHCH}_2\text{CO}_2\text{Et}
\end{array}
\end{align*}
\]

In a subsequent paper, Jacob and coworkers\(^{27}\) described some similar reactions employing iodine monochloride in conjunction with silver salts to bring about iododesilylation. A carbobenzyloxy protected 4-trimethylsilylphenylalanine ester was iodinated in good yield. Other experiments were focused on 1,4-bis(trimethylsilyl)benzene; using one equivalent of silver tetrafluoroborate / iodine monochloride, it was shown that one trimethylsilyl group could be selectively cleaved, leading to a yield of \(> 99\%\) of p-iodophenyltrimethylsilane. It must be emphasised that this work was conducted using only stable iodine-127.

The radioiodination of some phenoxyacetic acid derivatives via trimethylsilylated intermediates was described by Ohmomo et al. in 1989.\(^{23}\) The interest in these compounds lies in their potential use as brain imaging agents. Using Na\(^{125}\)I and NCS in acetic acid at 65\(^\circ\)C, aryltrimethylsilyl derivatives of some dimethylaminooethyl phenoxyacetates, dimethylaminooethyl phenoxyacetamides, phenoxyethyl ethylenediamines and phenoxyethylpiperazine were efficiently converted to the corresponding \(^{125}\)I-labelled derivatives.
One further example of the use of trimethylsilylated derivatives is provided by the work of Pert and Ridley.\textsuperscript{29} They devised a strategy for the regiospecific preparation of 2-bromo- and 2-iodoestradiol from the parent compound. This was achieved by protecting the -OH groups as methoxymethyl- derivatives, lithiating the protected compound with sec-BuLi in THF, and quenching with chlorotrimethylsilane. Exposure of the silylated derivatives to the electrophilic halogen reagents, NBS and NCS/I\textsuperscript{-} afforded the 2-bromo- and 2-iodo- derivatives respectively.

Over the last 10 - 15 years, destannylation has become much more extensively used than desilylation for the incorporation of iodine isotopes. Work in this area was pioneered by Hanson, Seitz and coworkers\textsuperscript{30-40} and an excellent review was published by Hanson in 1988.\textsuperscript{41} Three main reasons were given for the preferred use of organotin intermediates.\textsuperscript{41} The first of these, which is also true for silicon compounds, is that methods for the synthesis of the organometallic derivatives are well documented in the literature. Secondly, as has already been discussed, carbon-tin bonds are more readily cleaved than carbon-silicon bonds. The third reason for favouring tin compounds was related to the ease of separation of the radiohalogenated product from the precursor. It was pointed out that the displacement of a Bu\textsubscript{3}Sn- group by iodine would result in a greater difference in lipophilicity than would desilylation, or ipso halogenation of diazonium salts, triazenes or boronic acids. Hence, a radiopharmaceutical produced by destannylation would be more easily separated from its precursor by reversed-phase HPLC.
One of the first compounds studied by Hanson's group was tamoxifen (23), the antiestrogen described on p.10.30-34. 

The dimethylaminoethoxy side chain of this compound made it ideally suited for ortho-directed lithiation, and subsequent transmetallation with n-tributyltin chloride afforded the stannylated intermediate (24) in 98% yield. This was readily converted to the iodinated product (25) on exposure to iodine in CCl₄, ¹²⁵I-iodine monochloride or Na¹²⁵I / chloramine-T. The highest yield of labelled product was however obtained using hydrogen peroxide / acetic acid as the in situ oxidant. This was achieved by incubating such an oxidising mixture with Na¹²⁵I and n-tributylstannyltamoxifen for 5 minutes at ambient temperature. The reaction was quenched by the addition of sodium metabisulphite, and the product was isolated by means of reversed-phase HPLC. The product was eluted well ahead of the unreacted stannylated precursor. This lithiation – stannylation strategy was later used by Bloomer et al. for the astatination of tamoxifen, using Na₂¹¹At.
Following their work with tamoxifen, Hanson and coworkers explored the possibilities of radiiodinating a variety of other biologically significant molecules by the attachment of stannylated thiophene moieties.36-40

Thiophene was first 2,5-di-lithiated and converted to 2,5-bis(trimethylstannyl)thiophene using trimethyltin chloride. In this instance, the trimethyltin derivative was used in preference to the tributyltin analogue because the substituted thiophene was a solid and could be recrystallised. The purified intermediate was then mono-lithiated to give the trimethylstannyliothienyllithium shown in the above scheme. This was reacted with cyclic ketones as shown, thereby attaching the reactive stannylated thiophene moiety as a prosthetic group, ultimately allowing very facile iodination.

The reaction was used to prepare analogues of haloperidol, a dopamine D-2 receptor with applications in neuropathology33, some cycloalkanols with potential as cerebral imaging agents and some 17-α-thienyl-estradiol derivatives, which were potential estrogen
receptor localising agents.\textsuperscript{35-40} Hanson's review\textsuperscript{41} also describes a
great deal of research concerned with the iodination of vinyl
substituted radiopharmaceuticals \textit{via} the tributyltin route.

In essence, Hanson's work employs phenyl, thieryl or vinyl
moieties as prosthetic groups for the attachment of radiiodine to
pharmaceuticals. This can be represented by the generalised scheme
below:

\[
R - X - \text{SnBu}_3
\]

During the past few years, iododestannylatian has seemingly
become the most popular technique for the radiiodination of small
aromatic molecules. A multitude of prosthetic groups for protein
labelling, SPECT imaging agents, receptor antagonists, etc, have been
labelled in this way, resulting in a vast body of literature. As
a consequence, this section is concluded with a small number of
selected examples rather than a comprehensive survey.

The preparation of simple \textit{p}-iodophenyl- prosthetic groups
for indirect protein labelling has already been mentioned (see
Section 1.4.2, p.52-54). Further to this, Wilbur et al.42-45 have published several papers on the stannylation and iodoxostannylation of various non-activated prosthetic groups, their aim being the production of labelled monoclonal antibodies (MAb's), resistant to in vivo deiodination. In a subsequent publication46, the use of this methodology in the 211At-labelling of MAb's was described. Wilbur and coworkers47 have also used the tributyltin route to prepare a radiolabelled phenylalanine derivative for use in peptide synthesis.

A great deal of recent literature involving destannylation has been concerned with the labelling of substrates for SPECT studies. Imaging agents such as IMP (see p.12) and antagonists used in receptor mapping studies have received the most attention. A study of 123I-labelling of IMP-type compounds has recently been reported by Dao-Boulanger and coworkers.48 They prepared tributyltin derivatives of IMP, N,N-dimethylphenetermine and N-(2-diethylaminoethyl)-benzamide and used these in n.c.a. radioiodination experiments. The displacement of bulky Bu3Sn- groups from these relatively small molecules caused such a change in lipophilicity that purification by HPLC was not found to be essential. The use of a Sep-Pak column allowed the isolation of the high-specific-activity products.

Other classes of compounds which are frequently labelled via the destannylation route are dopamine D-2 receptor antagonists such as epidepride (29) and related compounds49-51, and benzodiazepine derivatives such as (30), used for in vivo imaging and quantitation of receptor sites in the brain.52
In all of the aforementioned examples, very similar procedures have been adopted. In fact there would appear to be a well-established, tried and tested routine for labelling compounds in this way. The first step is the synthesis of the bromo-, or sometimes iodo-, derivative of the substrate, usually on a fairly large scale. This is then converted to the tributyltin intermediate by one of two procedures. For simple molecules, which are stable towards base, reaction with n-butyllithium followed by tributyltin chloride can be used. However, the more commonly used method is that developed by Azizian et al., in which the bromo precursor is stannylated using bis(tributyltin) (Bu₃Sn₂) in the presence of palladium acetate and a zerovalent palladium catalyst (tetrakis triphenylphosphine palladium (0); [Ph₃P]₄Pd).

The iododestannylation step is generally performed using a considerable excess of the tin derivative, which is ultimately removed by chromatography. The most commonly used in situ oxidant is chloramine-T, although many of the other oxidants described in
Section 1.4.1 have also been used. For example, in their work on benzodiazepines, McBride et al.\textsuperscript{52} used Iodogen to oxidise n.c.a. radiiodide. The final step, that of chromatographic purification, is generally performed by means of reversed-phase HPLC, although, as noted above, this may not be essential for some smaller molecules.

The established procedure outlined above has been applied to a wide range of radiopharmaceuticals. Further examples are a penicillin derivative\textsuperscript{54}, a prostaglandin receptor antagonist\textsuperscript{55}, a retinoid\textsuperscript{56} and clorgyline, an aromatic imaging agent containing an alkyne function.\textsuperscript{57} These examples illustrate how the facile cleavage of the carbon-tin bond allows the regiospecific iodination of complex molecules containing sensitive functionalities.

A notable departure from the routinely used procedures has recently been reported by Culbert and Hunter.\textsuperscript{55} They described an elegant piece of work using polymer-supported tin in the preparation of labelled IMP.

One of the major problems associated with radiolabelling \textit{via} demetallation at the n.c.a. level is the separation of nanomolar quantities of product from unreacted metallated precursor. In order to avert such difficulties, Culbert and Hunter\textsuperscript{55} prepared the organotin polymer (31) from divinylbenzene. This was coupled, as shown, with a lithiated \textit{N}-isopropyl-amphetamine derivative, formed by treating the \textit{p}-bromo- analogue with two equivalents of \textit{n}-BuLi. The aryl-tin bond was readily cleaved by iodine in chloroform, leaving IMP in solution. Radiiodination was then attempted using Na\textsuperscript{131}I and Iodobeads (see p.45) as the oxidant. The product, obtained in
44% radiochemical yield, was readily separated from the polymeric precursor and the Iodobeads.

Labelling strategies of this nature seem set to gain in popularity in future years. The preparation and uses of polymer supported tin reagents have recently been reviewed by Neumann.59
5.2 Experimental

The work of Moerlein et al.\textsuperscript{19-25} described in Section 5.1 involves the \textit{in situ} oxidation of iodine to an electrophilic species in order to bring about demetallation. In view of the findings in Chapter Four, that electrochemical oxidation of iodine produces a reactive electrophilic iodine species, it was felt worthwhile to examine the action of such a species on aromatic compounds bearing silyl substituents.

Trimethylsilyl derivatives of a number of simple aromatic substrates were prepared via Grignard reactions. The reaction of these with positive iodine species generated in $d_5$-acetonitrile was examined by $^1$H NMR spectroscopy. A number of reactions were also carried out on a preparative scale.

5.2.1 Preparation of Silylated Arenes

 Aryltrimehtylsilanines were prepared via Grignard reactions from the corresponding bromo- derivatives. The method used was similar to that described by Moerlein.\textsuperscript{60} The following procedure is typical:-
Preparation of m-Anisyltrimethylsilane

A crystal of iodine was sublimed through Mg turnings (2.6g, 0.11 mol) in a three-necked flask equipped with a magnetic stirrer. A solution of m-bromoanisole (18.7g, 0.10 mol) in 60 mls of dry THF was added slowly, with heating to maintain steady boiling. The mixture was maintained under nitrogen and refluxed for 4½ hours. It was then allowed to cool to ca. 30°C, and with rapid stirring, a solution of chlorotrimethylsilane (11.0g, 0.1 mol) in dry THF (50 mls) was added dropwise. Following this addition, the mixture was refluxed for a further 3½ hours, then allowed to cool. It was subsequently poured into ca.200 mls of 5% aqueous ammonium chloride solution and the product was extracted using dichloromethane (3 x 30mls). The combined extracts were washed with aqueous sodium bicarbonate solution and with water, then dried over anhydrous magnesium sulphate. The desiccant was filtered off after 24 hours. Rotary evaporation of the filtrate afforded the product, m-anisyltrimethylsilane as a colourless oil (14.6g, 81%), essentially pure on the basis of its ¹H NMR spectrum.

¹H NMR (CDCl₃) : δ = 7.47 - 7.42 (t, 1H), 7.26 - 7.20 (m, 2H), 7.06 - 7.02 (dd, 1H), 3.97 (s, 3H), 0.40 (s, 9H).
Cognate Preparations of Aryltrimethylsilanes:

(i) Phenyltrimethylsilane

The above procedure was carried out using bromobenzene, (15.7g, 0.10 mol). Extraction afforded phenyltrimethylsilane as a colourless oil. The product was purified by distillation, resulting in an isolated yield of 11.0g, 73%.

$^1$H NMR (CDCl$_3$) : $\delta = 7.72 - 7.68$ (m, 2H), 7.55 - 7.51 (m, 3H), 0.43 (s, 9H).

(ii) p-Chlorophenyltrimethylsilane

The procedure was repeated using 4-bromochlorobenzene (19.2g, 0.10 mol). The product was obtained as a pale yellow oil (15.9g, 86%). Its $^1$H NMR spectrum featured only the desired signals, and the compound was not purified further.

$^1$H NMR (CDCl$_3$) : $\delta = 7.33/7.30$ (d, 2H), 7.22/7.19 (d, 2H), 0.14 (s, 9H).

(iii) m-Chlorophenyltrimethylsilane

Reaction of 3-bromochlorobenzene (15.3g, 0.08 mol) with chlorotrimethylsilane (8.7g, 0.08 mol) resulted in a pale yellow oil, m-chlorophenyltrimethylsilane (8.7g, 0.08 mol).

$^1$H NMR (CDCl$_3$) : $\delta = 7.4 - 7.21$ (m, 4H), 0.17 (s, 9H).
(iv) p-Tolytrimethylsilane

Repeating the above procedure with 4-bromotoluene (17.3 g, 0.10 mol) afforded p-tolytrimethylsilane as a pale brown oil (13.9 g, 85%). It was noticed that the product darkened on storage.

$^1$H NMR (CDCl$_3$): $\delta = 7.55/7.52$ (d, 2H), 7.30/7.27 (d, 2H), 2.45 (s, 3H), 0.37 (s, 9H).

(v) m-Trimehylsilylbenzotrifluoride

Starting with 3-bromobenzotrifluoride (11.3 g, 0.05 mol), the procedure was repeated (using 5.5 g, 0.05 mol of Me$_3$SiCl) to yield m-trimehylsilylbenzotrifluoride as a colourless oil (10.3 g, 94%).

$^1$H NMR (CDCl$_3$): $\delta = 7.82 - 7.79$ (m, 2H), 7.67 - 7.64 (bd, 1H), 7.57 - 7.51 (m, 1H), 0.28, (s, 9H).
5.2.2 Reaction of Silylated Arenes with Electrochemically Generated Positive Iodine

Electrochemically generated iodine (I) was prepared in $d_5$-acetonitrile solution as described in Section 4.2.2. Thus, I$_2$ (0.254g, 1 mmol) was completely oxidised in 25mls of a 0.5M solution of lithium tetrafluoroborate in CD$_3$CN. Aliquots of this solution were added to solutions of aryltrimethylsilanes in the same solvent and the ensuing reactions were monitored by $^1$H NMR spectroscopy. All such experiments were conducted at 25°C.

Stock solutions of the aryltrimethylsilanes were prepared at 0.02 M by dissolving $1 \times 10^{-4}$ moles of each substrate in 5mls of CD$_3$CN. Typically, 0.5 ml portions of these solutions were placed in NMR tubes, and the iodinating agent and further CD$_3$CN were added in varying proportions to give, for example 1.0, 1.5 or 2.0 molar equivalents of I$^+$.  

(i) p-Tolyltrimethylsilane

Mixing molar equivalents of I$^+$ and p-tolyltrimethylsilane in CD$_3$CN resulted in the rapid formation of p-iodotoluene. This is illustrated in Fig. 5.3, which shows the aromatic regions of the spectra before and after mixing. The pair of doublets due to the starting material (at surprisingly different shifts from those in CDCl$_3$) were replaced by the characteristic doublets of p-iodotoluene at $\delta = 7.57/7.55$ and 6.94/6.91 ppm. No trace of the ortho-isomer was evident, which is invariably formed in direct electrophilic attack on toluene.
Fig. 5.3: Reaction of $I^+$ with $p$-Tolyltrimethylsilane: Aromatic Region of $^1H$ NMR Spectra Before and After Mixing.

The reaction was apparently instantaneous, even at greater dilution. Single scan spectra at 2 second intervals showed the transformation to be too fast for kinetic study by NMR.

By way of comparison, a qualitative experiment was carried out in which a considerable excess of molecular iodine was added to a portion of the $p$-tolyltrimethylsilane solution. Iododesilylation did occur, but to an extent of less than 10% over a period of 30 minutes.

(ii) Phenyltrimethylsilane

The addition of a molar equivalent of $I^+$ to phenyltrimethylsilane resulted in rapid iododesilylation yielding iodobenzene. This was clear from the appearance of NMR signals at $\delta = 7.71/7.69$, 7.35/7.33/7.30 and 7.13/7.10/7.08 ppm. No trace of the starting
material remained. When an excess of I⁺ was used, an additional peak appeared in the spectrum at δ = 7.46 ppm, attributable to 1,4-di-iodobenzene.

(iii) p-Chlorophenyltrimethylsilane

Exposure of the title compound to an equivalent of I⁺ in CD₃CN resulted in rapid conversion to p-iodochlorobenzene. In terms of NMR signals, this was apparent from the replacement of the doublets of the starting material (7.33/7.30 and 7.22/7.19 ppm) by new doublets at δ = 7.70/7.67 and 7.17/7.14 ppm.

(iv) m-Trimethyleisylbenzotrifluoride

Addition of a slight excess of I⁺ to m-trimethyleisyl-benzotrifluoride in CD₃CN yielded m-iodobenzotrifluoride. With such a deactivated substrate, it had been anticipated that the metallation may have been considerably slower. However, using standard ¹H NMR procedures, requiring ca. 6 minutes between sample preparation and spectrum acquisition, the reaction was seen to be complete within this time. The silylated derivative (NMR signals at 7.82/7.79, 7.68/7.65 and 7.57/7.55/7.52 ppm) was converted to m-iodobenzotrifluoride, identified by signals at δ = 8.05 – 7.97 (m, 2H), 7.71/7.68 (d, 1H) and 7.36/7.33/7.30 (t, 1H). Thus, the combination of electrochemical oxidation with regioselective silylation allows the preparation of a derivative which is not accessible by direct attack of I⁺ on benzotrifluoride.
of Wilbur et al., regarding ortho/para halogenodeprotonation of meta-silylated anisole and phenol derivatives (p. 233-4), complications were anticipated with this compound. Thus, para-iodination was expected rather than meta-iododesilylation. In the event, a further problem was encountered in that radical cation intermediates were apparent. When the iodinating agent was added to the substrate in CD$_3$CN, an intense, dark-green colour was produced and a multitude of signals were seen in the $^1$H NMR spectrum.

(vi) Triphenylgermane

A sample of triphenylgermane, Ph$_3$GeH (33), was found to be available in the laboratory, in the form of a white crystalline solid.
The compound was used in several NMR experiments using various quantities of the I⁺ reagent. The 'H NMR spectrum of the starting material in CD₃CN featured two groups of signals from the aromatic protons, centred at δ = 7.53 and 7.41 ppm. On mixing with ca. 3 equivalents of a CD₃CN solution of I⁺, these signals were rapidly replaced by signals attributable to iodobenzene (a doublet and two triplets, centred at δ = 7.71, 7.37 and 7.15 ppm, respectively). An additional peak appeared at δ = 7.46 ppm, due to some p-di-iodobenzene.

When the experiment was performed using a further excess of I⁺, the signal at δ = 7.46 ppm became the dominant peak in the spectrum. In addition, minor signals at δ = 7.92 and 7.10 ppm showed the presence of some o-diiodobenzene. In addition to these expected signals, two small triplets were seen at δ = 7.79 and 7.64 ppm, which were not identified.

In summary, all three phenyl substituents of (33) were readily cleaved from the metal centre by the action of I⁺, thus producing iodobenzene. In the presence of excess iodinating agent, this reacted further to give di-iodobenzenes.
5.2.3 Investigation of a Direct Silylation Method -
Trimethylsilylation via Ortho-Lithiation

A serious constraint on the use of silicon, germanium, tin
or boron in the radiiodination of pharmaceuticals is that the
metallated derivatives are generally prepared via Grignard reactions
which require halogenated precursors. The overall process is thus
halogen for halogen exchange. In view of this, a possible method
of producing metallated derivatives without a need for halogenated
starting materials has been examined.

In 1983, Krizan and Martin\textsuperscript{51} reported on the preparation
of silylated derivatives and arylboronic acids via the \textit{in situ}
trapping of ortho-lithiated aromatics. This was achieved using a
sterically encumbered base, lithium 2,2,6,6-tetramethylpiperidide, and
chlorotrimethylsilane (TMSCl) or trimethyl borate to trap the aryl-
lithium intermediates before they could undergo undesirable self-
condensation reactions. The success of the approach was dependent
on two criteria; it was necessary that the base deprotonated the
aromatic substrate more rapidly than it reacted with the trap, and
that the resulting aryllithium intermediate reacted more rapidly
with the trap than with the aromatic substrate.

This section describes some experiments in which such
an approach was used in an attempt to silylate, and ultimately
iodinate, some aromatic substrates deactivated towards electrophilic
attack. A $1:3:6$ substrate - base - TMSCl stoichiometry was used,
in accordance with the method of Krizan and Martin.\textsuperscript{51}
Preparation of Lithium 2,2,6,6-Tetramethylpiperide (LiTMP)

To 2,2,6,6-tetramethylpiperidine (0.828g, 6mmol) in ca. 5mls of THF was added n-BuLi (3.8mls, 1.6 M in hexane, 6mmol) at room temperature under nitrogen. The solution, referred to hereafter as LiTMP, was cooled to -78°C prior to use, by means of a dry ice - acetone bath.

Reactions with Selected Substrates

(1) 1,4-Dicyanobenzene

A solution of LiTMP (6mmol) was prepared as described above and cooled to -78°C. To this was added freshly distilled chlorotrimethylsilane (1.35g, 12mmol) in ca. 20mls of THF, followed by the substrate, 1,4-dicyanobenzene (0.256g, 2mmol), in a further 20mls of THF. A red colour developed as the substrate was added. The mixture was stirred at -78°C for 15 - 20 minutes, then allowed to warm slowly to room temperature. After ca. 1 hour, the apparatus was dismantled; the red solution instantly became pale yellow on exposure to moist air. It was then poured into a large volume (ca. 200mls) of water and the products were extracted with diethyl ether (3 x 50mls). It was found necessary to add sodium chloride to aid separation of the phases. Ether extracts were pooled, washed with 1.5M hydrochloric acid (2 x 50mls), and with water (2 x 50mls), then dried over anhydrous potassium carbonate. Evaporation of the solvent afforded a yellow solid. This was dissolved in boiling methanol and the solution was transferred to an evaporating basin. White crystals were produced which were filtered off by suction.
The 'H NMR spectrum was recorded in CD$_3$CN solution and featured two lines only; at $\delta = 7.95$ and $\delta = 0.41$. Integrals were found to be in the ratio 1 : 9. This is consistent with any one of the three possible isomeric disilylated derivatives, (34), (35) or (36):

![Chemical Structures](image)

On steric grounds, (34) seems to be most likely.

The melting point of the product was measured as 135-137°C.

Elemental analysis: Calcd. for C$_{14}$H$_{20}$N$_2$Si$_2$: C 61.7%, H 7.4%, N 10.3%, found C 60.9%, H 7.5%, N 10.1%.
(ii) 1,4-Bis(trifluoromethyl)benzene

The procedure described in part (i) was carried out using 1,4-bis(trifluoromethyl)benzene (0.428g, 2mmol). The solution remained colourless throughout the process. Extraction with diethyl ether, followed by evaporation, afforded a colourless oil from which crystals were deposited on cooling. Recrystallisation from methanol resulted in white crystals with melting point 44-46°C. As in (i), the $^1$H NMR spectrum (CD$_3$CN solution) featured just two lines, at $\delta = 8.03$ and $\delta = 0.34$ ppm. The product is thought most likely to be the 2,5-disilylated derivative, (37):

![Chemical Structure](image)

(37)

The yield, after two recrystallisations, was 0.322g (45%).

Elemental analysis: Calcd. for C$_{14}$H$_{20}$FeSi$_2$, C 46.9%, H 5.6%, found C 46.7%, H 5.7%.
Attempted Reaction of Silylated Derivatives with Iodine (I) Species

On the basis of elemental analyses and NMR spectral data, the products of the previous experiments are thought most likely to be the 2,5-disilylated derivatives of 1,4-dicyano- and 1,4-bis-(trifluoromethyl)benzene, (34) and (37). The next stage of the study was the attempted desilylation of these derivatives, using electrochemically generated iodine (I).

For this purpose, "I+ triflate" was produced in acetonitrile solution, as described in Section 4.2.4 (1), p.204. Oxidations were performed in 0.25 M lithium trifluoromethanesulfonate, using 0.254g (1 mmol) portions of iodine. Complete oxidation thus resulted in batches of I+ solution containing a maximum of 2 mmol. These were added to flasks containing 2,5-di(trimethylsilyl)-1,4-dicyanobenzene (0.136g, 0.5 mmol) and 2,5-di(trimethylsilyl)-1,4-bis(trifluoromethyl)-benzene (0.179g, 0.5 mmol), respectively. Each reaction mixture was allowed to stir overnight at room temperature, before pouring into water and extracting with diethyl ether. Subsequent NMR analysis revealed that the desired reactions had not occurred. The starting materials were extracted unchanged.

The conclusion that is drawn from this result is that the presence of two electron-withdrawing groups, such as -CF3 or -CN, renders the ring too electron-deficient for attack by I+. Thus the substituents which facilitate ortho-lithiation and subsequent silylation do not allow electrophilic attack to occur, despite the fact that m-trimethylsilylbenzotrifluoride rapidly underwent desilylation with the I+ reagent. (Section 5.2.2 (v)).
In a bid to increase the electrophilicity of the reagent, TFA was added to the reaction mixture. Once again, 1mmol of iodine was exhaustively oxidised in 0.25 M CF₃SO₃-Li⁺ in acetonitrile. The complete anolyte was then added to a further portion of 2,5-di-(trimethylsilyl)-1,4-dicyanobenzene (100mg), dissolved in 30mls of TFA. The reaction mixture was allowed to stir for 4 days, but as above no reaction took place.
5.3 Discussion

As in the case of methodologies involving thalliated and mercuriated derivatives (Chapter Two), iodination via Group IVb organometallic intermediates is a well-established technique. This is reflected in the lengthy review of the topic, Section 5.1.

The work described in this chapter was undertaken for three main reasons. Firstly, it seemed a logical extension of the work described in Chapter Four, to make the substitution process more regiospecific by the use of metallated precursors. The second reason was to address the problem of competing reactions in iodo-demetalation procedures. As noted in Section 5.1, the efficiencies of existing methodologies are often restricted by the presence of other electrophiles, leading to competing chlorodemetalation with NCS and related reagents, and competing protiodemetalation with acidic reagents. With control of the number of coulombs passed, anodic oxidation of iodine can be expected to generate a solution of I⁻ free from other electrophiles, which may therefore be advantageous in iodo-demetalation.

The third reason for performing the work described in Section 5.2 was to ascertain whether the use of more reactive iodine electrophile would extend the range of utility of silylated precursors towards more electron-deficient arenes. As described in the introduction (p.244 - 250), the vast majority of radiiodinations involving demetalizations are performed using the very reactive stannylated derivatives. Silylated derivatives are generally not considered useful for such applications owing to their relatively
slow reactions with \textit{in situ} generated iodine electrophiles. However, the use of a preformed I\(^+\) species, such as that produced by anodic oxidation, may make rapid iododesilylation a feasible option. This would be desirable for some applications since silylated derivatives possess the considerable advantage of general chemical stability. Whereas trialkyltin substituents generally have to be introduced immediately prior to the halodemetalation step, it may be possible to carry trimethylsilyl groups through synthetic procedures. The stability of silylated derivatives also allows them to be stored for long periods.

A series of simple trimethylsilylated aromatic substrates was prepared via Grignard reactions from the corresponding bromo-derivatives (Section 5.2.1). These reactions proceeded with generally good yields, the highest yields being obtained on days of low humidity. The reactions of these compounds with electrochemically generated I\(^+\) were studied directly by recording \(^1\)H NMR spectra of the reaction mixtures. As expected, \(p\)-tolyltrimethylsilane and \(p\)-chlorophenyltrimethylsilane underwent complete and regiospecific \textit{ipso} demetalation, yielding \(p\)-iodotoluene and \(p\)-chloriodobenzene respectively. In both cases, the \textit{ortho}-isomers, which are invariably formed by direct iodination of the parent compounds, were not detectable.

\(m\)-Trimethylsilylbenzotrifluoride provides an example in which metallation extends the range of compounds that can be iodinated by electrophilic attack. The parent compound, benzotrifluoride is inert towards anodically produced I\(^+\). However, as described in
Section 5.2.2 (iv), \textit{m}-trimethylsilylbenzotrifluoride undergoes rapid iododemetallation when exposed to the reagent, yielding the otherwise inaccessible \textit{m}-iodobenzotrifluoride.

At the other end of the reactivity scale, the use of I\textsuperscript+ was found to be incompatible with electron-rich substrates such as \textit{m}-anisyltrimethylsilane. As noted in Section 5.2.2 (v), the addition of I\textsuperscript+ to this compound in acetonitrile solution produced a dark green colouration, typical of reactions involving radical cations of anisole derivatives. Subsequent work-up of the reaction mixture yielded a complex mixture of products, and none of the desired \textit{m}-iodoanisole.

The results discussed above illustrate that, overall, the use of silylated precursors shifts the range of compounds that can be iodinated in the direction of less electron-rich arenes. In the broader context of radiiodination of substrates of biological interest, the method would seem to be useful over a reasonably broad spectrum of reactivities, ranging from moderately activated compounds (e.g. toluene) to quite deactivated compounds (e.g. benzotri fluoride).

Perhaps the biggest restriction on the use of Group IVb organometallic intermediates is that halogenated precursors are generally required to generate them. It was for this reason that the work described in Section 5.2.3 was undertaken. Direct trimethylsilylation of some deactivated substrates was attempted employing the method of Krizan and Martin. The technique involves ortholithiation of the arene as the first step, and is thus restricted
to substrates with acidic ortho-protons, i.e. those adjacent to substituents such as -CN and -COOR. In this study, 1,4-dicyano-
benzene and 1,4-bis(trifluoromethyl)benzene were used to examine the reaction. Early experiments, not reported in Section 5.2.3, were carried out using only a small excess of the base (LiTMP) and trap (TMSCl) over the amount of substrate. These led to complex mixtures of products which were not readily separated. However, using a 1:3:6 substrate-base-trap ratio, as used by Krizan and Martin\textsuperscript{61}, both substrates were cleanly disilylated. It seems likely that the considerable excess of reagents ensures complete disilylation and that a third substitution does not occur, possibly for steric reasons. The situation with 1,3-bis(trifluoromethyl)benzene was not so clear-cut; multi-component mixtures were produced and the matter was not pursued further.

As noted in Section 5.2.3, the favoured structures for the disilylated products are (34) and (37), respectively. These were then used in attempted iododesilylation reactions with electrochemically generated I\textsuperscript{+}. It was anticipated that the deactivated substrates may have been resistant to electrophilic attack, hence, the more reactive "I\textsuperscript{+} triflate" reagent (see Chapter Four) was generated by using CF\textsubscript{3}SO\textsubscript{3}Li as the electrolyte. In the event, even this reagent did not cleave the trimethylsilyl-groups and so the ultimate goal of the experiments was not achieved. One may speculate that the desired reactions would have been feasible with trimethylgermyl-substituents, or failing that, trimethylstanny-substituents, but time did not permit a confirmation of this to be made. Even if
such reactions were feasible, the utility of the method would be restricted by the nature of the silylation procedure, i.e. the functional groups present would have to be compatible with the use of an organolithium reagent.

A possible application of such methodology, assuming that germylated or stannylated derivatives can be cleaved by I⁺, may be in the area of deactivated prosthetic groups for protein labelling (see p.54). A benzonitrile derivative, bearing a suitably protected bridging unit, could perhaps be metallated ortho to -CN, and then radiiodinated to provide a prosthetic group highly resistant to \textit{in vivo} dehalogenation.

In summary, the use of silylated aromatic substrates introduces regiospecificity into the reactions of electrochemically generated I⁺. The familiar $2:1$ para-ortho isomer ratio found in direct iodinations of toluene can be transformed to 100% $p$-ido-toluene by the use of $p$-tolyltrimethylsilane as the starting material. Silylation also extends the range of compounds which can be iodinated electrophilically, $m$-trimethylsilylbenzotrifluoride providing an example of this.

Viewed from a different angle, anodic oxidation of iodine produces an I⁺ species capable of rapidly displacing trimethylsilyl groups from even moderately deactivated arenes. The unsuccessful experiments with disilylated 1,4-dicyanobenzene and 1,4-bis(trifluoromethyl)benzene have however indicated the limitations of this reaction. As mentioned earlier, the iodine electrophile produced by anodic oxidation can be expected to be free from other electro-
philes, unlike in existing methodologies. In the context of radio-
iodination, prior electrochemical oxidation of NaI, followed by rapid
reaction with a metallated substrate, may be preferable to a slow
reaction of NaI and substrate, in the presence of an \textit{in situ}
oxidant.
5.4 References


CHAPTER SIX

Application Of Methodologies To Selected Molecules of Biological Interest and General Conclusions
6.1 Introduction

The work described in Chapters Two to Five has been concerned with the iodination of simple model compounds. In order to pass judgement on the applicability of various methodologies to the labelling of biologically active compounds, it was deemed necessary to carry out some preparative experiments using somewhat more appropriate substrates.

In Section 2.5, it was concluded that metallation-iodode-metallation processes involving thallium and mercury are reasonably well understood. Both metals have been employed in regiospecific radiolabelling procedures and their limitations, e.g. the oxidation of electron-rich substrates by TTFA, is well documented. Likewise, the use of Group IV organometallic precursors, especially stannylated derivatives, is well established, as is evident from the review in Section 5.1. It was felt that there was little that could be added to existing knowledge on these procedures.

In contrast, the Radner process described in Chapter Three has not been used in the iodination of more complex molecules. However, as noted in Section 3.7, it would appear that the method is best suited to large-scale preparative work and its use in n.c.a. radiolabelling seems unlikely.

It was thus decided to focus on the synthetic utility of electrochemically generated positive iodine species. Although there have been several reports on the electrolytic radiiodination of peptides and proteins (see Section 1.4.1 (vii), p.48), it would appear that the type of work described in Chapter Four has not been
applied to radiohalogenation. As a result, the first part of this chapter is primarily concerned with the use of electrochemically generated positive iodine in the iodination of some molecules of interest to Fisons Pharmaceuticals Ltd.

6.2 Iodination of Aromatic Amino Acid Derivatives

6.2.1 Introduction

Methodologies for the iodination of peptides and proteins are well established (Section 1.4). However, all of the direct radioiodination methods (Section 1.4.1, parts (i)-(vii)) are restricted to the labelling of tyrosine, or sometimes histidine, residues. In some circumstances this may not be particularly desirable, for example, when tyrosine residues play a key role at the active site of the protein, or under conditions which are conducive to in vivo deiodination. Furthermore, there may be applications for which a labelled peptide containing no electron-rich tyrosine or histidine residues may be useful.

With these factors in mind, labelling with radiiodine at less reactive phenylalanine or tryptophan residues may sometimes be advantageous. The results of Chapter Four suggested that I\textsuperscript{-} generated in acetonitrile was sufficiently reactive to achieve this. The ethyl esters of N-acetyl-L-phenylalanine and N-acetyl-L-tryptophan were chosen as model substrates, the ultimate aim being the iodination of a hexapeptide of interest to Fisons, the precise structure of which is confidential. The iodinating agent used in this work was produced by anodic oxidation as described in Section 4.2.1, using a two-compartment cell as depicted in Fig. 4.1.
6.2.2 Iodination of N-Acetyl-L-Phenylalanine Ethyl Ester

\[
\text{CH}_2\text{CHCO}_2\text{Et} \quad \text{NHCOCH}_3
\]

(I)

Iodination of this compound was attempted using an excess of the iodinating agent, the aim being to tri-iodinate the phenyl ring. Iodine (0.51g, 2 mmol) was completely oxidised at a platinum anode using 0.2 M lithium tetrafluoroborate in acetonitrile as the supporting electrolyte. The pale yellow iodine (I) solution (40mls) was then added slowly to a solution of N-acetyl-L-phenylalanine ethyl ester (38) (0.15g, 0.64 mmol) in acetonitrile (10mls). The mixture was stirred overnight, then poured into water (70mls). Products were extracted into chloroform (4 x 25mls). Combined extracts were washed with aqueous sodium metabisulphite (2 x 30mls), then with brine, and finally with water. After drying over anhydrous magnesium sulphate, the solvent was removed to leave a yellow solid.

Mass spectral analysis (EI+) showed the molecular weight to be 613, consistent with tri-iodination. A large peak at \( m/z = 486 \) suggested that di-iodinated material was also present. This was supported by TLC investigations; a 3 : 2 mixture of toluene and diethyl ether separated the product into two components having \( R_f \) values of 0.30 and 0.26, compared to \( R_f = 0.24 \) for the starting material.
6.2.3 Iodination of N-Acetyl-L-Tryptophan Ethyl Ester

The iodination of N-acetyl-L-tryptophan ethyl ester (39) was attempted using a slight excess of the iodinating agent. The reagent was prepared at the same concentration as above, and 6mls of the solution (corresponding to 0.5 mmol I²) were added dropwise to 0.137g (0.5 mmol) of (39) in a few ml's of acetonitrile. When the addition was half complete, the solution became opaque, almost black in colour, but this colouration disappeared on addition of the complete 6ml aliquot. The mixture then took on the appearance of a dilute iodine solution, being dull orange in colour. It was stirred for 30 minutes, then poured into water (100mls). Extraction was performed with chloroform (4 x 20mls).

Not unexpectedly, in view of the visual evidence of radical cation intermediates, a complex mixture of products was obtained. TLC analysis of the crude product, using 2:2:1 toluene - ethyl acetate - n-butanol as the developing solvent, revealed the presence of at least five components (R₄'s : 0.95, 0.84, 0.76, 0.66 and 0.62). An experiment with the hexapeptide mentioned in the introduction was also unsuccessful, presumably because it contained a tryptophan residue. Consequently, work in this area was not pursued further.
6.3 Iodination of Sodium Cromoglycate Analogues

6.3.1 Introduction

Since the late 1960's, sodium cromoglycate (40) has been used very extensively in the prophylactic treatment of asthma. It was developed from a plant extract, khellin, which contains just one chromone unit, fused to a furan ring. Of the many analogues of khellin which were investigated, the bischromone (40) was found to have outstanding protective activity against asthma when inhaled before antigen challenge.\(^1\,2\) The development and evaluation of sodium cromoglycate was reviewed in considerable detail by Cox et al.\(^3\) in 1970.

Although developed principally for the treatment of asthma, sodium cromoglycate has also been found to be effective for the relief of various other allergic disorders. For example, administered as a nasal sufflation, it is used in the prophylactic treatment of allergic rhinitis, while solutions of the drug are available as eye-drops for the relief of allergic eye irritations. In addition, the compound has been administered orally for the treatment of ulcerative colitis, various food allergies, etc.
Despite its widespread use, the precise mode of action of sodium cromoglycate remains unclear and is the subject of much ongoing research. In the elucidation of drug metabolism, a great deal of information can often be obtained by using radiolabelled forms of the molecule. Labelling of a compound to high specific activity with tritium enables its fate in very complex biological systems to be monitored. This type of approach has been used with sodium cromoglycate, but its tritiation has not proved trivial. 

This section describes the attempted iodination of some chromones using electrochemically generated iodine (I). The aim of the work was to prepare polyiodinated derivatives which could possibly be used as precursors in catalytic tritiodeiodination procedures with tritium gas.

As in the work with the amino acid derivatives described above, I⁺ was generated in acetonitrile using the apparatus shown in Fig. 4.1. However, for the work with chromones, lithium triflate was used as the electrolyte instead of lithium trifluoroacetate, in view of the somewhat enhanced reactivity observed with this counterion.
6.3.2 Iodination of Various Chromones

(i) Attempted Iodination of FPL 60521 XX, FPL 60571 KP, FPL 61191 KP and FPL 51587.

Iodine (0.253g, 1 mmol) was placed in the anode compartment of an electrochemical cell and dissolved in ca. 40mls of a 0.2 M solution of lithium triflate in acetonitrile. An additional 10mls of electrolyte was sufficient to fill the cathode chamber. On application of 30 V, an initial current of 0.83A was observed. This
decreased steadily as the iodine was oxidised. Complete oxidation would have resulted in a 0.05 M solution of I⁺, but the solution remained pale orange, suggesting that this was not fully achieved. Nevertheless, the solution of the reagent was frozen in dry ice and aliquots were used subsequently in iodination experiments.

Portions of FPL 60521 XX, FPL 60571 KP, FPL 61191 KP and FPL 51587 (10 mg of each) were placed in small flasks, along with small magnetic stirrer bars. Aliquots of 5 ml of the aforementioned I⁺ solution were then added to each flask. Dicyclohexyl-18-crown-6 was added to FPL 60571 KP, FPL 61191 KP and FPL 51587 in an attempt to solubilise these sodium salts. This was successful for the former two, but failed in the case of FPL 51587.

The reactions were monitored by regular TLC analysis. Only in the case of FPL 60521 XX were any significant changes observed within a few hours. Applying a sample of the mixture to a TLC plate and eluting with 1:1 toluene-diethyl ether, revealed the presence of a new component having an Rₜ value of 0.68, compared to 0.63 for the starting material.

A second series of experiments was then carried out, again using 10 mg portions of the four substrates, but with a tenfold excess of I⁺ in each case. As before, FPL 60521 XX reacted quite rapidly, generating the aforementioned product with a somewhat larger Rₜ value than the starting substrate. Close inspection of the TLC plate showed the presence of additional components with even higher Rₜ values (vide infra).
The reactions of the sodium salts, FPL 60571 KP, FPL 61191 KP and FPL 51587, were followed by TLC using 10:7:6 ethanol - propan-2-ol - water as the developing solvent. In the case of FPL 60571 KP, new components having Rf values of 0.77 and 0.63 were evident, the starting material having a value of 0.71. Some degree of reaction was also apparent from the analysis of the FPL 61191 KP mixture, although the situation was much less clear-cut. Attempts to iodinate FPL 51587 were apparently futile, possibly because of its low reactivity towards electrophiles, but more probably because of its insolubility in acetonitrile. The addition of several equivalents of dicyclohexyl-18-crown-6 had no solubilising effect on the compound, nor did the addition of various amounts of DMF as a co-solvent.

The reaction with FPL 60521 XX was terminated after around 2½ hours by pouring the mixture into water (20mls). Extraction was carried out using chloroform (3 x 5mls) and the combined extracts were washed with aqueous sodium metabisulphite solution (2 x 10mls), then with water (2 x 10mls). The crude product, ca. 10mg, was then dissolved in a little dichloromethane and applied to two 20 x 20cm x 250μm analytical TLC plates. Elution was carried out using 3:2 toluene - diethyl ether, resulting in separation into two principal bands at Rf values of around 0.5 and 0.7. Silica was scraped from the plates in 1cm wide bands around these Rf's and the products were removed by repeated washing with ethanol - chloroform mixtures. (In an earlier experiment, methanol was used for this purpose, but it was found that trans-esterification, leading to the mixed methyl ethyl ester occurred readily).
The two isolated products were subjected to mass spectral analysis (EI*), which showed them to be mono- and di-iodinated derivatives of FPL 60521 XX respectively (m/z = 560 and 686). It was assumed that iodination had occurred exclusively on the phenyl substituent and that the two products corresponded to the para- and ortho/para-iodophenyl derivatives, (45) and (46). However, NMR results suggested otherwise:

Lower Band :

{\textsuperscript{1}H NMR (CDCl\textsubscript{3}) : } \delta = 9.10 (d, finely split, 1H), 7.90/7.88 (d, 2H), 7.37/7.35 (d, 2H), 7.15/7.13 (d, 2H), 4.45 - 4.37 (q, 4H), 1.37 - 1.34 (t, 6H). Additional minor signals were seen in the aromatic region (\(\delta = 8.3 - 7.3\)) and at \(\delta = 2.63\) and 2.18.

Upper Band :

Insufficient material was available for a quality \({\textsuperscript{1}H NMR}\) spectrum to be obtained. The recorded spectrum contained the same groups of signals as above, plus several other unidentified peaks.
The spectrum of the mono-iodinated product (lower band) featured a pair of doublets at \( \delta = 7.90 / 7.88 \) and \( 7.37 / 7.35 \) ppm, characteristic of a para-substituted phenyl substituent, and is thus consistent with structure (45). However, the spectrum of the di-iodinated material (m/z = 686) also contained these signals, suggesting that the second iodination took place on the chromone ring, rather than at the ortho- or meta- positions of the phenyl ring. Further evidence for this is given in part (ii) below, which describes the polyiodination of FPL 60521 XX.

The reactions with the sodium salts, FPL 60571 KP and FPL 61191 KP, were terminated after somewhat longer time periods, in excess of 24 hours. The reaction mixtures were poured into 50ml portions of 10\% (v/v) aqueous hydrochloric acid and products were extracted into 1 : 1 chloroform - propan-2-ol. The isolated materials were examined by TLC, using 12 : 3 : 5 n-butanol - acetic acid - water as the developing solvent. In both cases, some degree of reaction was evident, although resolution on the plates was not particularly good. Subsequently, preparative TLC was attempted using 20cm \( \times \) 20cm \( \times \) 200\( \mu \)m Whatman KC 18F reversed-phase plates. The developing solvent consisted of a 60 : 40 mixture of methanol and 0.1M aqueous ammonium acetate solution. This resolved the product from FPL 60571 KP into two bands having \( R_f \) values of 0.53 and 0.38, compared to > 0.9 for the starting material. It does not seem unreasonable to assign these to mono- and di-iodinated products, although their isolation was not achieved. When the bands were scraped from the plates and washed with methanol, a gum-like material was apparently
stripped from the silica from which the products could not be separated. Because of these difficulties it was decided to focus attention on the diethyl ester, FPL 60521 XX.

(ii) Iodination of FPL 60521 XX (large excess of I<sup>-</sup>)

In this experiment, a large excess of electrochemically produced iodine (I) was used, the aim being to tri-iodinate the substrate. Iodine (0.51g, 2mmol) was placed in the anode compartment of the cell, dissolved in ca. 40mLs of a 0.2M lithium triflate solution in acetonitrile. Application of 30v resulted in a current of around 0.8A. When the oxidation was complete, the anolyte was pipetted from the cell and added to a flask containing FPL 60521 XX (19mg, 0.044mM). Assuming essentially complete oxidation of the iodine, these quantities represent a molar excess of the iodinating agent of the order of 50. The mixture was stirred for 72 hours, then poured into water (50mLs). Extraction was carried out using chloroform (5 x 10mLs) and the extracts were combined. The organic phase was washed sequentially with water, sodium metabisulphite solution, sodium bicarbonate solution and again with water, before being left to dry over anhydrous magnesium sulphate.

Subsequent filtering of the dessicant and evaporation of the solvent afforded a yellow solid, which was subjected to TLC analysis using 3 : 2 toluene - diethyl ether as the solvent. The product was separated into three components having R<sub>v</sub> values of 0.51, 0.69 and 0.84, compared to 0.49 for the starting material. These were tentatively assigned as the mono-, di- and tri-iodinated
derivatives respectively. Preparative TLC was performed by applying
the redissolved product to two 20cm x 20cm x 250μm silica analytical
TLC plates and eluting with 3:2 toluene–diethyl ether. As in
part (i), bands of silica were scraped from the plates at the
appropriate R\textsubscript{r} values, and the products were obtained by washing
the silica with ethanol–chloroform mixtures. Since the aim of the
experiment was to tri-iodinate the substrate, only the uppermost
two bands were collected, i.e. those thought to correspond to di-
and tri-iodinated material. These assignments were supported by mass
spectral results which indicated the masses to be 686 and 812
respectively. The FAB spectrum of the upper band material featured
an additional peak at m/z = 939 (M + H\textsuperscript{+}). This mass of 938 clearly
indicates the presence of some tetra-iodinated material.

\textbf{NMR Data :–}

\textbf{Lower band :}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) : δ = 9.13 (s, 1H\textsuperscript{+}), 7.89/7.86 (d, 2H), 7.36/7.34
(d, 2H), 7.14 (s, 1H), 4.44 – 4.37 (q, poorly defined, 4H), 1.37 – 1.35
(t, poorly defined, 6H).

(* – includes additional small signals at δ = 9.16 and 9.14 ppm.)

\textbf{Upper band :}

\textsuperscript{1}H NMR (CDCl\textsubscript{3}) : δ = 9.17 (s, 1H), 7.90/7.88 (d, 2H), 7.32/7.30
(d, 2H), 4.44 – 4.38 (q, 4H), 1.37 – 1.33 (t, 6H).
Inspection of the above data indicates that a pair of doublets in the aromatic region is common to all the spectra. The implication is that only the para-position of the phenyl ring is iodinated; ortho-iodination is not observed, presumably because of the large steric requirement of the chromone moiety. Instead, the second and third iodine atoms appear to be incorporated at the chromone pyran rings. Evidence for this comes from the fact that the doublet at $\delta = 7.15 / 7.13$ ppm in the spectrum of the mono-iodinated product (see part (i)) is halved in intensity in the spectrum of the di-iodo-derivative, and is completely absent from that of the tri-iodo-derivative. Furthermore, the inherent asymmetry of the di-iodinated product accounts for the observed complication of the ethyl group signals, which do not appear as a clearly resolved quartet/triplet pattern. Symmetry is restored by tri-iodination, hence the environments of the two ethyl groups become identical once more and a simple pattern is observed. The proposed structures for the two products are thus (47) and (48), below:

![Chemical structures](image-url)
The indication of tetra-iodination provided by the mass spectral results suggests that under forcing conditions, a second iodine substitution can occur on the phenyl ring. Since ortho-iodination is apparently ruled out on steric grounds, a small amount of meta-substitution, leading to the 3,4-di-iodophenyl-derivative, seems the most likely explanation for the peak at m/z = 939.

6.3.3 Iodination of Cromoglycate Diethyl Ester

\[
\begin{align*}
\text{EtO}_2\text{C} & \quad \text{OH} \\
\text{CO}_2\text{Et} &
\end{align*}
\]

(49)

Following the work with the chromone derivatives described above, attention was turned to the iodination of cromoglycate itself. Experiments were carried out using the diethyl ester (49) rather than sodium cromoglycate (40), for reasons of solubility and ease of extraction. Iodine (0.51g, 2 mmol) was completely oxidised in the anode compartment of an electrochemical cell, containing 40mLs of a 0.2M solution of lithium triflate in dry acetonitrile. During
the course of the electrolysis, ca. 1 ml of glacial acetic acid
was added to the cathode chamber to facilitate hydrogen production
as a cathode reaction. When the anolyte was almost colourless, it
was removed and added to cromoglycate diethyl ester (49), (0.131g,
0.25 mmol). The resulting mixture was stirred overnight, then poured
into ca. 50mls of aqueous sodium bicarbonate solution. Extraction
was carried out using chloroform (3 x 25 mls). Combined extracts were
washed of iodine with sodium metabisulphite solution, then washed
with water. The yellow chloroform layer became cloudy and brine
was found to be necessary to achieve a good separation. After
washing with brine (2 x 30 mls), the chloroform solution was dried
over magnesium sulphate. The desiccant was subsequently filtered off
and the solvent was removed by rotary evaporation. TLC analysis on
silica, using 3 : 2 toluene - diethyl ether, was indicative of three
products, having Rf values 0.66, 0.52 and 0.35 (cf. 0.03 for the
starting material). The reaction products were then separated by
preparative TLC on two 20 x 20 cm x 2 mm silica PLC plates (Merck).
The two upper bands (Rf = 0.65 and Rf = 0.5) were collected. Products
were removed from the silica by repeated washes with 1:1 ethanol
- dichloromethane, with the assistance of sonication. Pooled extracts
were filtered and rotary evaporated to give yellow powders, 75 mg
from the upper band and 52mg from the central band.

The two products were subjected to mass spectral analysis
(FAB), which indicated their respective masses to be 1070 and 944.
These values are 42 mass units greater than would be expected
for tetra- and tri-iodo- cromoglycate diethyl ester. In the light
of the NMR results presented below, it is apparent that this extra mass arises from O-acetylation of the secondary alcohol function on the aliphatic bridging unit. \((CH_3CO = 43\) mass units).

**NMR results:**

**Upper band:**

\(^1H\) NMR (CDCl\(_3\)) : \(\delta = 8.54\) (s, 2H), 7.04 (s, 2H), 5.75 (m, 1H), 4.58 - 4.44 (m), including q at 4.47 (12H in total), 2.26 (s, 3H) and 1.44 (t, 6H).

\(^{13}C\) NMR shifts : \(\delta = 175.8, 171.0, 159.7, 157.7, 156.4, 151.8, 151.1, 119.7, 115.8, 90.0, 80.8, 74.0, 72.5, 63.1, 21.7\) and 14.0.

![Chemical Structure](image)

(50)

Evidence for the O-acetyl group comes from the \(^1H\) signal at 2.26 ppm and the fact that there are 16 discrete \(^{13}C\) signals rather than 14.

When the iodination of (49) was attempted without adding acetic acid to the cathode compartment, an intractable, unidentified product was obtained. It is possible that ring opening of the chromone occurs under basic conditions and that the presence of acetic acid facilitates the smooth iodination of the substrate.
6.4 Iodination of Desglycine and Remacemide

6.4.1 Introduction

Remacemide, or FPL 12924 (52), is a novel anticonvulsant, currently undergoing Phase II clinical trials. It was discovered during the screening of a large number of glycinate derivatives. The results of tests on mice were published in 1990.\(^6\) These indicated the compound to be a potent, safe, non-sedating, orally acting anticonvulsant, specific for the prevention of seizures elicited by electric-shock. The findings were predictive of clinical utility for patients with generalised tonic / clonic seizures.

This section describes some attempts of direct iodination of remacemide (52) and its analogue, desglycine (51), using anodically generated I\(^{-}\). The purpose of these experiments was to investigate the direct synthesis of tritiation and multi-tritiation precursors. Tritiated derivatives are desirable for microautoradiography, tissue localisation and distribution studies, and for receptor binding work. Furthermore, \(^{125}\)I-labelled compounds may also be required for specific radiolmmunoassay and receptor studies.
6.4.2 Experiments with Desglycine

Desglycine hydrochloride was first converted to the free base by dissolving a portion in aqueous sodium hydroxide solution and extracting with diethyl ether. Subsequent evaporation of the ether afforded desglycine as a colourless, viscous liquid.

(i) Using a Two-fold Excess of Iodine (I)

Iodine (0.50g, 1.97 mmol) was completely oxidised in an electrochemical cell, using 0.2 M lithium triflate in acetonitrile as the supporting electrolyte. The anolyte, containing a theoretical maximum of 3.94 mmol of positive iodine, was then added slowly to desglycine (51) (0.41g, 1.94 mmol) in a small flask.

After stirring overnight, the reaction mixture was diluted with water (ca. 1:1), and with continued stirring, solid sodium metabisulphite was added in small portions until the iodine colour was discharged. A few mls of aqueous sodium hydroxide solution was added to ensure basic conditions, and the mixture was extracted with chloroform (3 x 30mls). Combined extracts were washed once with water (20mls), then extracted with 15% (v/v) aqueous hydrochloric acid (3 x 30mls). Combined acid extracts were cooled in ice, made basic by the addition of sodium hydroxide solution, then re-extracted with chloroform (3 x 30 mls). The chloroform solution was washed with water and sodium bicarbonate solution, before being dried over magnesium sulphate. Filtering off the desiccant, followed by rotary evaporation of the solvent, afforded the product as a brown gum.
Mass spectral analysis (FAB) of the crude product showed peaks at m/z = 212 and 338, corresponding to the starting material and its mono-iodinated derivative respectively.

(ii) Using an Eight-fold Excess of Iodine (I)

The above procedure was repeated using the same quantity of iodine (I), but with less desglycine. A solution containing ca. 4 mmol of "I+" was added to desglycine (106 mg, 0.5 mmol) and the mixture was stirred overnight.

Extraction was carried out in essentially the same manner as described in part (i), although ethyl acetate was used in preference to chloroform as the organic phase. As previously, the product was obtained as a brown gum.

TLC analysis was carried out using a 4:1 mixture of a 5% solution of triethylamine in chloroform and ethyl acetate. The mixture was separated into two major components having Rs values of 0.98 and 0.63. Fainter spots were seen with values of around 0.95 and 0.66.

The mass spectrum (FAB) contained peaks at m/z = 212 and 338 as in part (i), plus further peaks at m/z = 464 and 590, corresponding to di- and tri-iodinated desglycine. Each peak was accompanied by a larger one, 17 mass units smaller, indicative of ammonia loss.
6.4.3 Experiments with Remacemide

As with desglycine, the hydrochloride was first converted to the free base. In the case of remacemide, the free base was obtained as a white solid.

(1) Using a Two-fold Excess of Iodine (I)

A further 0.5g of iodine was oxidised in a 0.2M solution of lithium triflate in acetonitrile, as described in the case of desglycine. The iodine (I) solution so produced (40mls, 3.9 mmol I⁺ max.) was then added to remacemide (52) (0.50g, 1.9 mmol), dissolved in 10mls of acetonitrile.

After ca. 2 hours, the reaction mixture was subjected to TLC analysis, using ethyl acetate as the developing solvent. Results suggested that the starting material (Rₛ = 0.04) had been completely converted to two products having Rₛ values of 0.52 and 0.93. These were isolated using the same technique as described in the case of desglycine, ie. chloroform extraction, followed by extraction into aqueous acid, neutralisation with base and then re-extraction into chloroform. Rotary evaporation afforded only a small amount of a colourless oil. In view of this, the initial chloroform extract was extracted further, using somewhat more concentrated hydrochloric acid and subsequent work-up afforded a further product in the form of a brown gum.

TLC analyses of the two products suggested that the first extract, the colourless oil, was largely composed of the starting material. Following ethyl acetate elution, the largest spot on the
TLC plate was seen at \( R_f = 0.08 \), corresponding to desglycine. Only traces of material were seen at higher \( R_f \)'s of 0.58 and 0.95. In contrast, the second extract gave large spots at these \( R_f \)'s and only a trace of material at the lower value.

The colourless oil obtained from the first extraction was subjected to mass spectral analysis. The FAB spectrum featured the expected peak at \( m/z = 395 \), due to the mono-iodinated product. In addition, minor peaks at \( m/z = 269 \) and 521 revealed the presence of both the starting substrate and some di-iodinated material.

(ii) Using an Eight-fold Excess of Iodine (I)

The experiment described in part (i) was repeated, but with less remacemide (134 mg, 0.5 mmol). The reaction mixture was allowed to stir overnight. Extraction was carried out as in part (a)-(ii), with ethyl acetate. The reaction products were obtained in the form of a light brown gum.

Using a solvent system of 4 : 1 5% Et\(_3\)N in chloroform-ethyl acetate, TLC examination of the product revealed it to be a multi-component mixture. Whereas the starting substrate had an \( R_f \) of 0.36 in the medium, the light-brown product was split into at least four components having \( R_f \)'s of 0.04, 0.14, 0.49 and 0.65. The material at \( R_f = 0.49 \) appeared to be the most abundant.

The FAB mass spectrum contained none of the desired peaks but instead indicated the presence of desglycine (\( m/z = 212 \)), formed by the loss of the aminoacetyl side-chain from remacemide.
In this concluding section, the work described in Sections 6.2 - 6.4 will be briefly discussed. Thereafter, the discussion will be widened by considering the applicability of various iodination methodologies to radiolabelling in general.

The first experiments to be considered are those performed on the amino acid derivatives, N-acetyl-L-phenylalanine ethyl ester (38) and N-acetyl-L-tryptophan ethyl ester (39). As mentioned in the introductory section (6.2.1), these experiments were carried out with a view to iodinating a small peptide and also to assess the utility of the I⁺ reagent in the iodination of peptides and proteins in general. From the outset, it was felt that the I⁺ reagent would be too reactive (i.e. too oxidising) for application in this area. This view was borne out by experiment; although the relatively robust phenylalanine derivative (38) was readily iodinated without any problems, the more sensitive tryptophan derivative (39) was decomposed by the reagent. The target peptide, which contained a tryptophan residue, was also unstable under the conditions used. It was therefore concluded that only those peptides devoid of any sensitive amino acids could be iodinated in this way.

The experiment with the phenylalanine derivative (38) showed that the phenyl ring could be di- or tri-iodinated with anodically generated I⁺, as was expected in the light of the findings of Chapter Four. Obviously, direct iodination of phenylalanine residues could not be achieved in the presence of more reactive aromatic units, e.g. tyrosines. An interesting experiment in this context...
might be to compare the reactivity of tyrosine with that of a germylated or stannylated phenylalanine unit, although the synthetic steps required to produce such derivatives may be too troublesome to make such an experiment worthwhile.

Generally speaking, iodine (I) produced by anodic oxidation in acetonitrile would seem to be too reactive for use in peptide and protein labelling. Instead, the method would seem to be more applicable to the iodination of smaller, less electron-rich, aromatic substrates. Chromones, as described in Section 6.3, fit this category and indeed, some of these were iodinated with a reasonable degree of success. Discussion of these experiments will be limited to the diethyl ester, FPL 60521 XI (41), in view of the problems encountered in isolating the products from the reactions of the three sodium salts (FPL 60571 KP, FPL 61191 KP and FPL 51587).

As described in Section 6.3.2, the phenyl-substituted chromone derivative (41) was readily mono- and di-iodinated, and with a large excess of I⁺, was tri- or even tetra-iodinated. The orientation of substitution was somewhat unexpected; NMR evidence indicated that the first iodination took place as expected at the para-position of the phenyl ring, but that the second and third iodine atoms were incorporated at free positions on the chromone pyran rings.

Following the successful iodination of (41), attention was turned to sodium cromoglycate itself, or rather its diethyl ester (49). Fortuitously, acetic acid was added to the electrochemical cell during production of I⁺ for these initial experiments. The reagent so produced was found to be capable of the tetra-
iodination of (49) with concurrent O-acetylation of the secondary alcohol function. When the acetic acid was omitted, the substrate was decomposed by the I⁺ reagent, resulting in a multi-component mixture of unidentified products. The reason for this is not clear. It was suggested that the acid may have inhibited base-catalysed opening of the pyran ring. An alternative explanation may simply be that O-acetylation prevents oxidation of the secondary alcohol by I⁺.

The final pair of substrates examined were desglycine (51) and remacemide (52). These compounds, bearing unsubstituted phenyl groups, were clearly substrates which would be iodinated by I⁺ in acetonitrile. In the first instance, both compounds were reacted with a small excess of I⁺ for a short time. Analysis of products by mass spectroscopy confirmed that the mono-iodinated derivatives were present. Since the principal aim of the work was to produce precursors for multiple tritiation, these studies were followed by further experiments in which the substrates were exposed to a greater excess of I⁺, for a longer time. Under these conditions, some decomposition of both substrates, but especially remacemide, was observed.

The reasons for selecting electrochemical oxidation for further study have already been outlined in the introduction to this chapter (p 276). In retrospect, it can additionally be claimed that the method was the most appropriate with regard to the aims of the experiments. With the exception of the work with amino acid derivatives (Section 6.2), the aim of all experiments described
in this chapter was to prepare polyiodinated derivatives which could be used as precursors for high specific activity tritium-labelled analogues. Clearly, ipso-demetallation procedures leading to mono-iodinated products would not have been suitable for this purpose. Mercuration may have resulted in multiple substitution under forcing conditions, but such reactions are not convenient, nor are the subsequent iododemetallation steps. Direct iodination methods such as Radner's or Sugiyama's may have generated the same I⁺ species as anodic oxidation, but much less rapidly, and it seems unlikely that the same degree of substitution (e.g. tetra-iodination of cromoglycate diethyl ester) would have been attainable.

To conclude a thesis, it would seem customary to propose avenues for further research. In this instance, one avenue of investigation is obvious, namely the application of electrochemical iodine oxidation to actual radiiodination. As mentioned above, the substrates used in Sections 6.3 and 6.4 have been studied with tritiation as the main goal. To some extent, the work described in this thesis has lacked direction because there have been no specific target molecules for radiiodination. Various methods have been examined with only simple model compounds and assessments of the utility of these have been made solely on the basis of large-scale work with non-radioactive iodine-127. As many literature reports suggest, work with non-radioactive iodine often bears little resemblance to work on the n.c.a. scale with radiiodine. The problems are not just a matter of scaling-down reactions, but are more commonly due to the various contaminants which are found in
radiiodide preparations. Numerous species may be found in radio-
iodide solutions, some of which are added deliberately (e.g. NaOH
to prevent oxidation to volatile I₂), while others arise from
impurities in the targets employed in the nuclear reactions used
to generate the radioisotopes. As Baldwin⁷ pointed out, these
contaminants vary considerably between the various iodine isotopes,
and also vary between batches of the same isotope, thereby making
n.c.a. labelling as much an art as a science. In order for a
complete comparison of iodination methodologies to be made, it
would seem of prime importance to compare their sensitivities to
commonly encountered contaminants. Clearly, this can only be done
by experimentation with actual radiiodide preparations and actual
target molecules.
6.6 References


