KINETIC AND MAGNETIC RESONANCE

STUDIES OF

TRITIATED HETEROCYCLIC

COMPOUNDS

A Thesis

submitted to the University of Surrey
for the degree of Doctor of Philosophy in the
Faculty of Biological and Chemical Sciences:

by

Conor O'Brien

The Cecil Davies Laboratory,
Department of Chemistry,
University of Surrey. September 1972
SUMMARY

The rates of detritiation of benzimidazole-2-T, 1-alkylbenzimidazole-2-T, 1,3-dimethylbenzimidazolium-2-T bromide, imidazo [4,5-b] pyridine-2-T, purine-8-T, 9-alkylpurine-8-T, adenine-8-T, adenosine-8-T, guanine-8-T, guanosine-8-T, hypoxanthine-8-T, inosine-8-T and 9-methyl-hypoxanthine-8-T have been measured as a function of pH at 85°. The pH-rate profiles obtained showed that the reactive species for exchange are the protonated and neutral forms of the substrate. The exchange was specifically catalysed by hydroxide ion. The mechanism proposed involves parallel rate determining deprotonation of the conjugate acid by hydroxide ion, giving a ylide intermediate, and deprotonation of the neutral compound producing a carbanion intermediate. The latter pathway was not observed for benzimidazoles, purine, adenine and imidazo [4,5-b] pyridine and reasons for this behaviour are given. Evidence in support of the proposed mechanism includes pH-rate profiles, primary kinetic isotope effects, substituent effects, exchange from model compounds and the realisation of similar base-catalysed hydrogen exchange mechanisms in other heterocyclic systems. The variation of rate with pH for guanosine is in close agreement with the very recent findings of Tomasz et al. [42].

Triton magnetic resonance spectroscopy is being developed as a non-destructive method for elucidating the position and exact distribution of the label in tritiated organic compounds. A series of tritiated heterocyclic
compounds were prepared by base catalysed exchange using tritiated water as the source of label, and their triton magnetic resonance spectra were obtained. A direct correlation between triton and proton chemical shifts was obtained thus enabling the prediction and assignment of triton magnetic resonance spectra by using the wealth of published proton chemical shift data.
ACKNOWLEDGEMENTS

This work was carried out under the supervision of Dr. J. R. Jones and Professor J. A. Elvidge, Chemistry Department, University of Surrey. I wish to express my deepest gratitude to Dr. Jones and Professor J. A. Elvidge for advice and guidance throughout the work.

I would like to thank the Radiochemical Centre, Amersham, for a grant and for providing some tritiated purines and tritiated water. Thanks are also due to Dr. J. R. Catch, Dr. E. A. Evans, Dr. H. C. Sheppard and Dr. J. C. Turner of the Radiochemical Centre for helpful comments.

I wish to thank Mr. D. W. Earls for many useful discussions during the course of the work.

I would also like to thank Professor G. K. Helmkamp for a gift of 9-i-propylpurine and 9-t-butylpurine; Professor G. T. Newbold of Fisons for samples 5, 6-dichloro-, 4,5,6-trichloro-, and 4, 5, 6, 7-tetrachlorobenzimidazole and Dr. Nitya Anand, Central Drug Research Institute, Lucknow, India for a sample of imidazo [4,5-b] pyridine. Thanks are also due to Professor F. Bergmann and Dr. D. Lichtenberg, Department of Pharmacology, Hebrew University, Jerusalem and Dr. R. T. Walker, Chemistry Department, University of Birmingham for a gift of purine derivatives and for pointing out key references relating to hydrogen exchange of purines.

Finally I would like to thank my wife Olive and daughter Hazel for support and encouragement during the course of this work.
TO OLIVE
Summary ..................................  2
Acknowledgements  ........................  4
General Introduction ........................  8
PART 1. Kinetics and Mechanism of the base catalysed detritiation from the 2-
position of benzimidazoles and the 8-
position of purines  ........................  12
1.1. Introduction
Review of base catalysed hydrogen exchange of
imidazoles and benzimidazoles ............... 13
Review of base catalysed hydrogen exchange of
purines ...................................... 17
1.2. Experimental
Materials .................................... 24
Preparation of tritiated compounds .......... 25
Preparation of reaction solutions .......... 27
Ionisation Constants ........................ 27
Measurement of the rate of detritiation
(A) Separation Technique .................. 29
(B) Liquid Scintillation Counting ........ 33
(C) Conventional Method .................. 34
(D) Initial Rate Method .................... 38
(E) Stability of Purines and Benzimidazoles
in acid and alkali ........................ 41
Measurement of the rate of hydrogen-deuterium
exchange of purine ....................... 43
(A) Measurement of the rate of deuteriation
of purine .................................. 43
(B) Measurement of the rate of deuteriation of purine-8-D.

1.3. Results

Benzimidazoles

Purine and 9-substituted purines

Imidazo [4,5-b] pyridine

Adenine and Adenosine

Guanine and Guanosine

Hypoxanthine, Inosine, and 9-methylhypoxanthine

Effect of Temperature on the rate of detritiation of Adenine-8-T and Guanine-8-T

1.4. Discussion

Exchange of the protonated substrate

Exchange of the neutral substrate

PART 2. Triton Magnetic Resonance spectra of some labelled heterocyclic compounds

2.1. Introduction

2.2. Experimental

Equipment

Materials

Synthesis of labelled compounds

2.3 Results

Referencing

Spectra

2.4 Discussion

PART 3. References
GENERAL INTRODUCTION
Heterocyclic compounds are characterised by structures in which one or more of the ring atoms are of elements other than carbon. Their importance is apparent from the number and variety that occur naturally – many fulfill important physiological functions in plants, animals and man, or are prepared by the drug and dye industry.

Many of these compounds can be labelled with the isotopes of hydrogen and subsequently employed to determine detailed biosynthetic and metabolic pathways. For such investigations the stability of the label must be known and it is in this context that studies of reaction mechanisms play an important part. The information can frequently be supplemented by studies of primary, secondary and solvent isotope effects.

Of the two isotopes of hydrogen, only tritium ($^3$H,T) is radioactive, being a beta-emitter (Emax = 18 Kev) with a half life of 12.26 years.[1]. The very weak beta-radiation minimises the need for shielding and the difficulty of detection has been overcome by the development of liquid scintillation counting [2]. Concentrations of tritium as low as $10^{-8} - 10^{-10}$ atom % can be readily detected and in this respect as well as others, the use of tritium is advocated. Thus for compounds of low solubility the nuclear magnetic resonance method of following hydrogen – deuterium exchange is not practical; in many cases the accuracy compares unfavourably with that obtained using tritium. The extreme sensitivity of the tritium tracer technique also makes possible, by means of an initial rate
method, the measurement of very slow rates of reaction. Finally the tritium labelled compound is only required at tracer concentrations whereas the corresponding deuterium labelled compound is usually prepared with high isotopic content.

The heterocyclic compounds studied in this work were mainly benzimidazoles (1) and purines (2). These compounds have the imidazole ring (3) in common and isotopic hydrogen exchange from the aromatic C - H bond between the imino and the tertiary nitrogen of the imidazole ring was studied.

![Chemical structures](image)

The benzimidazole ring system is found naturally in vitamin B<sub>12</sub> where it is complexed to the central cobalt atom and the imidazole ring itself is found in the amino acid histidine (a normal constituent of most proteins) and in the pilocarpine alkaloids.

The purine ring system has an even more important role in the vital processes of the body where adenine and guanine are constituents of nucleic acids. The nucleic acids are macromolecules present in all living cells and the purine bases provide the means of holding the two helical chains of the DNA and RNA systems together by specific hydrogen bonding between adenine and thymine (or uracil) and between guanine and cytosine. This specificity of hydrogen bonding allows the exact replication of the DNA or RNA molecule by unwinding of
the double strand into two single strands, so that each single strand acts as a template for the formation of a new and complimentary strand that binds to it by hydrogen bonding.

A number of alkaloids are purine derivatives, including caffeine and theobromine which occur in the tea plant and coffee bean and have a physiologically stimulating action in the body. Coenzyme A and nicotinamide adenine dinucleotide contain the purine derivative adenine and exhibit important enzymatic activity in the body.

The objects of the present work were threefold:

1. to devise satisfactory methods of labelling the compounds with tritium
2. to study the stability of the label under various conditions and hence formulate a mechanism that would account for the observed behaviour,
3. to propose conditions for storing the tritiated compounds.

Attention has already been drawn [3] to the fact that the instability of tritium atoms in labelled purines may lead to erroneous conclusions being drawn when such compounds are employed as tracers.

In the second part of the thesis many of the tritiated heterocyclic compounds studied in the first part are prepared at much higher tritium concentrations to study their triton magnetic resonance spectra. Tritium is an excellent isotope for nuclear magnetic resonance studies having a spin of $\frac{1}{2}$ and a sensitivity to detection higher than that of the proton. This work was undertaken with the object of providing accurate chemical shifts and coupling constants for comparison with the proton magnetic resonance data.
PART I

KINETICS AND MECHANISM OF BASE-CATALYSED HYDROGEN EXCHANGE FROM THE 2-POSITION OF BENZIMIDAZOLES AND THE 8-POSITION OF PURINES.
I.I. INTRODUCTION

Base-catalysed hydrogen exchange of Imidazoles and benzimidazoles.

The deuteriation of imidazole (3) was first studied by Gillespie et al.[4] who reported that exchange took place at the 4- and 5- position in neutral D₂O at 250°. Deuteriation of the 2- position was observed in NaOD at 150°, it was suggested that exchange in this case took place on the conjugate base of imidazole while exchange at the 4- and the 5- positions involved the neutral molecule. Mannschreck, Seitz and Staab [5] however found that the 2- H of imidazole disappears with an approximate half-life of 700 minutes in D₂O at 37°. Furthermore, 1-benzylbenzimidazole (4) exchanges its 2-H in CH₃OD at 60° with a half-life of 110 minutes [5]. As this cannot exist in the form of a conjugate base, the positional reactivity order found by Gillespie [4] must be in error and may be due to loss of deuterium from the 2- position during their isolation procedure.

Staab et al.[6] also found that 1,3-dibenzylimidazolium chloride (5) deuteriates very rapidly in CH₃OD at 37° (t₁/₂ < 3 mins.), exchange of the 2- H only was observed. Olofson et al.[7] found that 1,3- dimethylimidazolium ion (6) also exchanged at the 2 - position, the rate being first-order in deuterioxide ion concentration and apparently independent of both buffer concentration and buffer type.
\[ \text{Chemical structures}\]

1. \[ \text{Structure 1} \]
2. \[ \text{Structure 2} \]
3. \[ \text{Structure 3} \]
4. \[ \text{Structure 4} \]
5. \[ \text{Structure 5} \] \( R = \text{C}_6\text{H}_5\text{CH}_2 \)
6. \[ \text{Structure 6} \] \( R = \text{CH}_3 \)
7. \[ \text{Structure 7} \]
8. \[ \text{Structure 8} \]
9. \[ \text{Structure 9} \] \( R = \text{H} \)
10. \[ \text{Structure 10} \] \( R = \text{CH}_3 \)
11. \[ \text{Structure 11} \] \( R = \text{H}_2\text{N} \)
12. \[ \text{Structure 12} \] \( R = \text{H} \)
13. \[ \text{Structure 13} \]
14. \[ \text{Structure 14} \]
15. \[ \text{Structure 15} \]
16. \[ \text{Structure 16} \] \( R = \text{H} \)
17. \[ \text{Structure 17} \] \( R = \text{RIBOSE} \)
18. \[ \text{Structure 18} \]
In a preparation of imidazole-1-D by exchanging the N-H in D$_2$O, Joop and Zimmermann [8] noted exchange also at the 2- position. Bellocq et al. [9] repeated the earlier work of Gillespie [4] and found that heating imidazole (3) in D$_2$O at 250° gave imidazole-2,4,5-D$_3$. Imidazole was also deuteriated specifically in the 2- position in NaOD at 60°. Thus the 2-H of imidazoles is more reactive than the 4(5) - H in hydrogen-deuterium exchange in neutral or basic conditions.

Harris and Randall [10] studied the rate of deuterium exchange from the 2- position of 1-methylimidazole-2,4,5-D$_3$ (7) as a function of pH. The rate increases with increasing pH but in the alkaline region becomes independent of pH. Such pH dependence was consistent with the rate determining attack by hydroxide ion on the conjugate acid of 1-methylimidazole, to form a ylide intermediate (Scheme 1.1). A subsequent fast reaction of the carbanion with the solvent H$_2$O gave the exchanged product.

\[
\begin{align*}
\text{D} & \quad \text{N} & \quad \text{+ OH}^- & \quad \text{slow} & \quad \text{D} & \quad \text{N} & \quad \text{+ HDO} \\
\text{D} & \quad \text{D} & \quad \text{CH}_3 & \quad \text{N} & \quad \text{+ OH}^- & \quad \text{fast} & \quad \text{D} & \quad \text{H} & \quad \text{N} & \quad \text{CH}_3 & \quad \text{+ OH}^- \\
\text{D} & \quad \text{N} & \quad \text{+ H}_2\text{O} & \quad \text{fast} & \quad \text{D} & \quad \text{N} & \quad \text{+ OH}^- \\
\text{D} & \quad \text{D} & \quad \text{CH}_3 & \quad \text{N} & \quad \text{+ OH}^- & \quad \text{fast} & \quad \text{D} & \quad \text{H} & \quad \text{N} & \quad \text{CH}_3 & \quad \text{+ OH}^- \\
\end{align*}
\]

Scheme 1.1
Further evidence for this mechanism was provided by Haake, Bausher and Miller [11] who measured the rates of deuteriation of 1,3,4-trimethylimidazolium iodide (8). Exchange of the 2-H was specifically catalysed by deuteroxide ion, with a large second order rate constant \( (1.3 \times 10^2 \text{ M}^{-1}\text{sec}^{-1} \text{ at } 33^\circ) \). As this molecule can only exist in the cationic form and undergoes rapid base-catalysed exchange, the conjugate acid of the unsubstituted compound is the reactive species in hydrogen exchange reactions.

Vaughan, Mughrabi and Chung Wu [12] measured the rates of deuteriation of imidazole at various pD values at 65° and 70° for the 2-position, and at 180° and 190° for the 4(5)-position. From the variation of rate of exchange with pD, the suggested mechanism involved deprotonation of the 2-position of the conjugate acid by deuteroxide ion and by \( \text{D}_2\text{O} \), leading to a ylide intermediate. The variation of rate with pD for exchange of the 4(5)-position was consistent with these pathways, with an additional pathway at very high pD involving deprotonation of the neutral imidazole by deuteroxide ion.

Hydrogen exchange of benzimidazoles has been investigated to a smaller extent. Fritzsche [13] observed that benzimidazole (9) was deuteriated in the 2-position on heating at 300° in \( \text{D}_2\text{O} \) for 36 hours. Zatsepina, Kaminsky and Tupitsyn [14] measured the rates of exchange of the 2-position of benzimidazole (9) and its 1-methyl derivative (10) in \( \text{C}_2\text{H}_5\text{OD} \), the latter exchanging faster. The exchange of 1-methylbenzimidazole was faster in 0.6M \( \text{C}_2\text{H}_5\text{OK} - \text{C}_2\text{H}_5\text{OD} \) than in pure \( \text{C}_2\text{H}_5\text{OD} \), but the exact nature of the catalysis was not investigated.
Base-catalysed hydrogen exchange of purines

Eidinoff and Knoll [15] prepared tritiated adenine (11) by heating adenine and tritiated water in the presence of platinum catalyst at 100°C for 18 hours. The distribution of tritium in the molecule was not ascertained. The position of exchange became clear only when the proton magnetic resonance spectrum of purine (12) was assigned. Schweizer et al. [16] found that purine exchanged its 8-H on heating in D₂O at 105°C for 4 hours (t½ ~ 45 min.). The position of exchange was assigned because desulphurisation of 8-mercaptopurine (13) with deuteriated Raney nickel gave the same product as the exchange reaction, viz. purine-8-D.

Bullock and Jardetzky [17] confirmed these findings by unambiguously synthesising purine-8-D by ring closure of 4,5-diamino-pyrimidine (14) with D₄C₆D₂. They also found that adenine, adenosine (15), hypoxanthine (16), inosine (17) and 6-chloropurine (18) readily exchange the 8-proton for deuterium by heating in D₂O at 90-100°C for 10-20 mins.

Fox [18] also showed that the various purines exchanged at the 8-position on refluxing in D₂O or D₂O-dimethylformamide mixtures. Examples include 7-benzyl- and 9-benzyladenine, adenosine and 6-chloropurine. However when 3-benzyladenine (19) was heated in D₂O - DMF for 27 hours at 100°C, exchange of the 2-H as well as the 8-H was noted. Pugmire et al. [19] also confirmed that purine exchanges its 8-H on heating in D₂O at 100°C for 10 mins., partial exchange was also observed at the 6-position after 72 hrs. at 100°C.

Fritzsche [20] obtained infra-red spectral evidence for exchange at the 8-H of guanosine (20) and adenosine (15) by comparison of the C-H and C-D stretching frequencies with those of imidazole, benzimidazole, imidazole-2-D and benzimidazole-2-D.
\( \text{HN} \) \( \text{NH} \) \( \text{HOCH} \) \( \text{CH} \) \( \text{OH} \) (20) \( R = H \)

\( \text{HN} \) \( \text{NH} \) \( \text{OH} \) \( \text{COCH(NH}_2\text{)CH}_2\text{C}_6\text{H}_4\text{CH}_3 \) (22)

\( \text{HN} \) \( \text{OH} \) \( \text{H}_2\text{POOCH}_2\text{O} \) (23)

\( \text{O} \) \( \text{CH}_3 \) \( \text{H} \) (24)

\( \text{X} = O, R = \text{RIBOSE} \)

\( \text{X} = S, R = \text{RIBOSE} \)

\( \text{CH}_3 \) \( \text{RIBOSE} \) (27)

\( \text{H}_2\text{N} \) \( \text{RIBOSE} \) (28)
Shelton and Clark [21] reported incorporation of tritium into purines on heating at 100° for 5.5 hours in tritiated water. By analogy with earlier work on the deuteriation of purines, exchange at the 8-position was assumed. By this method labelled adenosine, guanosine, guanosine monophosphate (21), puromycin (22) and adenosine monophosphate (23) and a number of purine containing dinucleoside monophosphates of high specific activity were produced. The approximate rates of exchange of tritium from adenosine and guanosine were determined in H₂O at 30°. The rate of exchange of adenosine -8-T was approximately constant at pH 2, 7.5 and 11 whereas the rate of exchange of guanosine -8-T increased with increasing pH. At 100° in H₂O, both compounds exchanged with a t½ = 30 minutes. These results show that the platinum catalyst used by Eidinoff and Knoll [15] was not necessary to bring about exchange.

The lability of the 8-position of purine nucleosides under alkaline conditions was also found by Wilt [22] who reported exchange of guanosine monophosphate -8-T (21) in 0.3N KOH at 35° for 16 hours. Similarly, McDonald and Philips [23] found that adenosine monophosphate -8-T (23) exchanged in H₂O at 92° (t½ = 90 mins.). Also Ostermann et al. [24] noted that various nucleoside diphosphates could be tritiated by heating in THO at 100°. Maslova et al. [25] studied the rate of incorporation of tritium into the 8-position of adenosine monophosphate under various conditions and found that the rate of exchange at pH = 4 was the same as that at pH = 7. Manor et al. [26] also reported that tritium was lost from guanosine and guanosine monophosphate
labelled at the 8- position when kept at 37° in 0.4N KOH for 37 hours. Van Dyke et al. [27], however reported no detectable loss of tritium from adenosine-8-T in 1.0N KOH over a period of 20 hours at 37°.

An important application of the known lability of the 8-position of the purine system is the in vitro synthesis of tritiated DNA [24,28,29], RNA and polynucleotides [25,29,30] by heating the inactive material in THO for a short period. The use of tritiated nucleic acids which are labelled by this method, has also provided information about the stability of purines, labelled in the 8-position, under various conditions. For example, tritium labelled DNA was subjected to acidic hydrolysis and the purine bases were separated and found to be of high specific activity [32]. This suggests that the 8-position does not exchange appreciably in 70% hydrochloric acid at 100°, which was one step in the hydrolytic procedure. Similarly, during the alkaline hydrolysis of tritiated RNA (labelled by in vivo incorporation of guanosine-8-T) some of the tritium exchanged from the guanosine [22]. Hydrolysis in 1N HClO₄ at 25° for 20 hours did not exchange any tritium, indicating the stability of the label under acidic conditions [22].

Waterfield et al. [33] and Evans et al. [34] have found that adenine-8-T, adenosine-T(G) and guanosine-8-T lose a very large fraction of the tritium on heating under various conditions at 121°. The data for adenine [34] is shown in Table 1.1.
Table 1.1 Exchange of tritium from Adenine-8-T and Adenine-2-T

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Adenine-2-T</th>
<th>Adenine-8-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>121</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>121</td>
<td>7.4</td>
<td>20</td>
<td>97</td>
</tr>
<tr>
<td>0.3</td>
<td>121</td>
<td>9-10</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>24</td>
<td>37</td>
<td>5.1</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>24</td>
<td>37</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>7.4</td>
<td>6</td>
<td>48</td>
</tr>
</tbody>
</table>

Exchange at the 2-position was always slower than the 8-position and increases with increasing pH, whereas exchange from the 8-position is slow at acidic pH but does not increase at high pH.

Hydrogen-deuterium exchange in hypoxanthine (16) and substituted hypoxanthines was studied by Bergmann et al. [35,36]. Hypoxanthine exchanged at the 8-position but the rate at very high pH was much slower than at pH 10.5. Substituted hypoxanthines with an alkyl group at the 1- or the 3-position exchanged at the 2-position rather than the 8-position in 0.1N hydroxide at 70°C. Hypoxanthines with an alkyl group at the 7- or 9-position exchanged exclusively at the 8-position in the same medium. No explanation was advanced for the different positions of exchange. The pH-dependence of the rate of deuteriation of 3-methylhypoxanthine (24) showed that the rate of exchange was independent of pH in the acidic region studied (pH > 3) while in basic pH the rate increased markedly until a constant pH-independent value was reached at pH > 10. No attempt to
interpret the pH-rate dependence was made [35].

In an attempt to develop methods for preparing specifically labelled nucleosides and nucleotides, Wechter [37] reported that the rate of deuteriation of the 8-position of adenosine increased with pH at 95° until it becomes immeasurably fast in 0.1N NaOH.

Broom and Robins [38] found that the 8-proton of 7-methylguanosine (25) and 7-methyl-6-thioguanosine (26) does not show up when the NMR spectrum is run in D₂O as solvent. Tomasz [39] made a more detailed study of this very rapid exchange and found \( t_{1/2} \) of 5.5 min. at pH 4.1 at 28°. This was interpreted as rate determining attack of lyate anion leading to a ylide intermediate as shown in Scheme 1.2, by analogy

![Scheme 1.2](https://example.com/scheme12.png)

with thiazolium and imidazolium exchange. Powerful evidence for the existence of the ylide intermediate came from an observation that 7-methylguanosine catalysed the benzoin condensation [39]. A key step in this condensation involves
addition of a carbonion to a carbonyl group and hence provides proof of a carbonion intermediate.

The 8-H proton of 7-methylinosine (27) also exchanged instantaneously in D₂O and could not be detected by NMR even within a few minutes of dissolving the compound in D₂O [40,41].

The kinetics of exchange of tritium from the 8- position of guanosine-, 1-methylguanosine and adenosine have been determined at various pH values at 37°C [42]. The pH-dependence of the rate of exchange was consistent with hydroxide catalysed abstraction of the tritium from the 7-protonated form of the purines, producing a ylide intermediate in a slow step. The ylide is then reprotonated by the solvent H₂O in a fast step. For guanosine an additional exchange pathway involving hydroxide catalysed exchange of the guanosine zwitterion (28) was invoked to explain the increase in rate at basic pH [42].
1.2 EXPERIMENTAL

Materials

Adenine-8-T (500 mCi/mM, solid), adenosine-T(G) (843 mCi/mM aqueous solution), guanine-8-T sulphate (132 mCi/mM, solid), guanosine-8-T (500 mCi/mM, aqueous solution), hypoxanthine-T(G) (1000 mCi/mM, solid) and inosine-T(G) (500 mCi/mM, aqueous solution) were supplied by the Radiochemical Centre and were used without further purification.

Benzimidazole, purine and 9-methylhypoxanthine were commercially available. 1-Methyl-, 1-ethyl- and 1-isopropylbenzimidazole were prepared by alkylation of benzimidazole in an alcoholic KOH solution, by a known method [43].

1,3-Dimethylbenzimidazolium bromide was prepared by refluxing 1-methylbenzimidazole in xylene with an excess of bromomethane for 1 hr. The white crystals that were precipitated and isolated in 60% yield, were recrystallized three times from ethanol-ether mixtures: mp 245-6°C, λ max (pH7) 263 mµ (ε5,600). (Found: C, 44.1; H, 5.45; N, 11.41; C₉H₄N₂Br. H₂O requires C, 44.1; H, 5.31; N, 11.4).

Imidazo. [4,5-b] pyridine, 9-isopropyl- and 9-tertiarybutylpurine, 5, 6-dichlorobenzimidazole, 4, 5, 6-trichlorobenzimidazole and 4,5,6,7-tetrachlorobenzimidazole were obtained as gifts from various sources.

These compounds were purified by recrystallisation, vacuum distillation or vacuum sublimation. The 1-alkylbenzimidazoles were characterized by the preparation of
picrates. The melting points of the compounds are presented in Table 1.2.

Table 1.2 Melting Points of the Purified Compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>M. P.</th>
<th>M. P. (lit.)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazole</td>
<td>171-2</td>
<td>170</td>
<td>44</td>
</tr>
<tr>
<td>1-Methylbenzimidazole</td>
<td>247(Picrate)</td>
<td>246-7,250,244</td>
<td>45, 46, 47</td>
</tr>
<tr>
<td>1-Ethylbenzimidazole</td>
<td>220(Picrate)</td>
<td>219</td>
<td>48</td>
</tr>
<tr>
<td>1-Isopropylbenzimidazole</td>
<td>196-8(Picrate)</td>
<td>197-9</td>
<td>49</td>
</tr>
<tr>
<td>1,3-Dimethylbenzimidazolium bromide</td>
<td>244-6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Purine</td>
<td>219</td>
<td>217</td>
<td>50, 51</td>
</tr>
<tr>
<td>9-Isopropylpurine</td>
<td>97-99</td>
<td>96</td>
<td>52</td>
</tr>
<tr>
<td>9-Tertiarybutylpurine</td>
<td>118-119</td>
<td>118</td>
<td>52</td>
</tr>
<tr>
<td>Imidazo [4,5-b]Pyridine</td>
<td>143</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Preparation of tritiated compounds

All the labelled compounds were prepared by homogeneous exchange using tritiated water (5Ci/ml or 0.2 Ci/ml). A typical procedure involved dissolving the compound (10-30 mg) in sufficient dioxan to give a homogenous solution with ca. 0.1 ml. of THO at 85° and the resulting solution was kept at 85° for 18 hrs. The THO and solvent were freeze-dried off, a small amount of H₂O added to exchange labile hydrogen and the H₂O once again removed. In some cases, the compounds were sufficiently soluble to dissolve directly in THO (e.g. purine), while in others, dimethylosulphoxide was needed to.
achieve homogeneity (e.g. 9-methylhypoxanthine). Specific exchange at the 8-position of purines and at the 2-position of benzimidazoles and of imidazo [4,5-b] pyridine was checked by following the deuteriation of these compounds by NMR. The conditions for the preparation of the tritiated compounds are given in Table 1.3.

Table 1.3. Preparation of the labelled compounds at 85°C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Weight (g)</th>
<th>Vol. of Solvent (ml)</th>
<th>Vol. THO (ml)</th>
<th>Time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purine-8-T</td>
<td>0.028</td>
<td>-</td>
<td>0.02 (5Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>9-Isopropylpurine-8-T</td>
<td>0.029</td>
<td>-</td>
<td>0.20 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>9-Tertiarybutylpurine-8-T</td>
<td>0.028</td>
<td>-</td>
<td>0.20 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>Benzimidazole-2-T</td>
<td>0.182</td>
<td>1.0 (Dioxan)</td>
<td>0.30 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>1-Methylbenzimidazole-2-T</td>
<td>0.063</td>
<td>0.5 (Dioxan)</td>
<td>0.50 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>1-Ethylbenzimidazole-2-T</td>
<td>0.112</td>
<td>0.5 (Dioxan)</td>
<td>0.50 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>1-Isopropylbenzimidazole-2-T</td>
<td>0.062</td>
<td>0.1 (Dioxan)</td>
<td>0.10 (5Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>1,3-Dimethylbenzimidazole-2-T-bromide</td>
<td>0.028</td>
<td>-</td>
<td>0.10 (5Ci/ml)</td>
<td>6</td>
</tr>
<tr>
<td>5,6-Dichlorobenzimidazole-2-T</td>
<td>0.010</td>
<td>0.1 (Dioxan)</td>
<td>0.05 (5Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>4,5,6-Trichlorobenzimidazole-2-T</td>
<td>0.009</td>
<td>0.2 (Dioxan)</td>
<td>0.05 (5Ci/ml)</td>
<td>36</td>
</tr>
<tr>
<td>4,5,6,7-Tetrachlorobenzimidazole-2-T</td>
<td>0.014</td>
<td>0.5 (Dioxan)</td>
<td>0.10 (5Ci/ml)</td>
<td>36</td>
</tr>
<tr>
<td>Imidazo[4,5-b]pyridine-2-T</td>
<td>0.010</td>
<td>-</td>
<td>0.20 (0.2Ci/ml)</td>
<td>18</td>
</tr>
<tr>
<td>9-Methylhypoxanthine-8-T</td>
<td>0.017</td>
<td>1.0 (DMSO)</td>
<td>0.09 (5Ci/ml)</td>
<td>36</td>
</tr>
</tbody>
</table>
Preparation of Reaction Solutions

The reaction solutions were made up using boiled-out deionized water. Carbon dioxide-free solutions were prepared using 'analar' grade reagents and titrating with potassium hydrogen phthalate, using phenolphthalein as indicator. Hydrochloric acid solutions were standardised against sodium hydroxide solutions. Buffer solutions were prepared by partially neutralising formic and acetic acid solutions with standardised sodium hydroxide solutions. 'Analar' sodium chloride was used to keep the ionic strength of these buffers at 0.10. pH measurements were made at 20° on a Radiometer 26pH-meter, standardised at pH = 4.00 with 0.05M potassium hydrogen phthalate and at pH = 9.22 with 0.01 M sodium tetraborate.

Ionisation Constants

The ionisation constants of most of the compounds have been measured previously at 25° and are collected in Table 1.4. Ionisation constants of organic bases vary appreciably with temperature, the usual change for the reaction

\[ BH^+ \rightleftharpoons B + H^+ \]  

being a decrease in pKa with increasing temperature. Because the kinetic work was carried out at 85°, a knowledge of pKa's at this temperature was necessary. Perrin [53] has proposed a semi-empirical equation which predicts the temperature coefficient of pKa's of nitrogen bases with considerable accuracy.
The equation is

\[ \frac{d\text{pK}_a}{dT} = \frac{\text{pK}_a - 0.9}{T} \]  

(1.2)

where T is the temperature at which the pKa is measured.

Thus for 1-methylbenzimidazole (pKa = 5.57 at 25°), the temperature coefficient is

\[ \frac{5.57 - 0.9}{298} = -0.0157 \]

Hence at 85°,

\[ \text{pK}_a = 5.57 + 60(-0.0157) = 4.63. \]

The calculated temperature coefficients and pKa values at 85° are also presented in Table 1.4. The experimental pKa values were chosen to give best fit with the kinetic results at 85° and are in good agreement with those calculated by the Perrin equation. This equation was formulated to give temperature coefficients for the ionisation constants of monovalent nitrogen cations but is frequently used, as in the present work, to estimate temperature coefficients of neutral and monoanion nitrogen acids.

The pKa values of acetic acid are known to be temperature independent [76] and it was assumed that the pH of formate and acetate buffers did not change on going from 25 to 85°.

The variation of Kw with temperature is well known [77] and the value of pKw at 85° is 12.50. Hence a 0.10M solution of NaOH (at 25°) has a pH of 12.50 - 1.00 = 11.50 at 85° (neglecting the change in volume from 25 to 85°).
Table 1.4 Temperature coefficients and $pK_a$ values of the heterocyclic compounds studied.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$pK_a(25^\circ)$</th>
<th>$pK'_a(25^\circ)$</th>
<th>$pK''_a(25^\circ)$</th>
<th>$\frac{dpK_a}{dT}$</th>
<th>$pK_{calc}(85^\circ)$</th>
<th>$pK_{expt}(85^\circ)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Methylbenzimidazole</td>
<td>5.57[54]</td>
<td>-</td>
<td>-</td>
<td>-0.0157</td>
<td>4.63</td>
<td>4.60</td>
</tr>
<tr>
<td>1-Ethylbenzimidazole</td>
<td>5.62[54]</td>
<td>-</td>
<td>-</td>
<td>-0.0158</td>
<td>4.67</td>
<td>4.60</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>5.53[55]</td>
<td>13.2[56]</td>
<td>-</td>
<td>-0.0155</td>
<td>4.40</td>
<td>4.50</td>
</tr>
<tr>
<td>Purine</td>
<td>2.60[51]</td>
<td>8.94[51]</td>
<td>-</td>
<td>-0.0054</td>
<td>2.30</td>
<td>2.30</td>
</tr>
<tr>
<td>9-iso-Propylpurine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.0101</td>
<td>3.54</td>
<td>3.50</td>
</tr>
<tr>
<td>9-t-Butylpurine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.0101</td>
<td>3.54</td>
<td>3.50</td>
</tr>
<tr>
<td>Adenine</td>
<td>4.20[57]</td>
<td>9.87[57]</td>
<td>-</td>
<td>-0.0087</td>
<td>2.98</td>
<td>2.95</td>
</tr>
<tr>
<td>Adenosine</td>
<td>3.50[58]</td>
<td>12.35[58]</td>
<td>-</td>
<td>-0.0087</td>
<td>2.98</td>
<td>2.95</td>
</tr>
<tr>
<td>Guanine</td>
<td>2.95[59]</td>
<td>9.32[59]</td>
<td>12.62[59]</td>
<td>-0.0069</td>
<td>2.54</td>
<td>2.60</td>
</tr>
<tr>
<td>Guanosine</td>
<td>1.90[58]</td>
<td>9.25[58]</td>
<td>12.33[58]</td>
<td>-0.0033</td>
<td>1.70</td>
<td>1.70</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>1.70[58]</td>
<td>8.91[58]</td>
<td>12.64[58]</td>
<td>-0.0033</td>
<td>1.70</td>
<td>1.90</td>
</tr>
<tr>
<td>Inosine</td>
<td>1.5[60]</td>
<td>8.96[58]</td>
<td>12.36[58]</td>
<td>-0.0006</td>
<td>1.47</td>
<td>1.50</td>
</tr>
<tr>
<td>Imidazo[4,5-b] pyridine</td>
<td>3.92[55]</td>
<td>-</td>
<td>-</td>
<td>-0.0101</td>
<td>3.32</td>
<td>3.50</td>
</tr>
</tbody>
</table>

Measurement of the rate of detritiation.

(A) Separation Technique.

The rate of detritiation of the heterocyclic compounds was measured by separating the compound from the reaction solution and measuring the increase in radioactivity in the aqueous portion. The method of separation was that of freeze-drying [61-64]. This consists essentially of freezing the reaction solution containing the heterocyclic compound and subliming the ice at low temperature and pressure. The principle of the method can be seen by
referring to the phase diagram for the water system (Fig. 1.1). AB denotes the situation in which vapour and liquid water are in equilibrium, AC that in which ice and liquid are in equilibrium and AD that in which vapour and ice are in equilibrium. A is the water triple point which occurs at $T_c = 0.0098^\circ C$ and $P_c = 4.58 \text{ mm Hg}$.

When ice at a pressure $P_2$ (which is less than $P_c$) is heated from temperature $T_2$ (less than $T_c$) to $T_1$, it is converted directly into vapour. In practice the vapour can be removed by (1) direct pumping to the atmosphere, (2) by use of chemical desiccant or (3) as was done in the present work, by condensing it as ice on a cold surface. The condenser is kept at a lower temperature than the subliming ice and a degree of dehydration, corresponding to the vapour pressure of the ice at the condenser temperature, is ultimately reached. The heat necessary to convert ice to vapour (latent heat of sublimation) is obtained by conduction through the frozen material and the walls of the supporting container. Because of the low thermal conductivity of ice, a sufficiently thin ice layer ensures that the rate of sublimation is maximized.

A detailed procedure of a typical freeze-drying separation is now outlined. An aliquot (0.60 ml) of the reaction solution was withdrawn and immediately frozen in flask A (containing ca. 0.1 g of sodium chloride) of the freeze-drying apparatus (Fig. 1.2), by rotating it in a dewar vessel containing liquid nitrogen. The sodium chloride was found necessary to prevent solid particles
FIGURE 1.1 PHASE DIAGRAM FOR THE WATER SYSTEM.
Figure 1.2 Freeze-Drying Apparatus.

TO ROTARY VACUUM PUMP

STOPCOCK

FLASK CONTAINING FROZEN MATERIAL

CONDENSER

LIQUID N₂

DEWAR FLASK
being drawn over with the water vapour during the freeze-drying process. Rotation of the flask during freezing ensured a thin film of ice of large area. The apparatus was assembled and connected to a rotary vacuum pump, and then evacuated with flask A immersed in liquid nitrogen. When the apparatus was fully evacuated, the stopcock B was closed and the condenser tube C immersed in liquid nitrogen, the flask being suspended in air at room temperature. The contents of the flask remained frozen during the separation because the amount of heat available to sublime the ice, was always less than that available by conduction through the flask from outside. When the drying was completed, the vacuum was released cautiously and the melted ice was assayed for tritium.

(B) Liquid Scintillation Counting.

The average energy of tritium beta-particles is only 6 Kev and consequently its low penetration of matter, amounting to a maximum range in air of 0.425 cm and 0.0007 cm in water, presents some detection difficulties. These have been overcome by the development of the liquid scintillation method of counting [2]. The principle of the method is based on the fact that when a tritiated compound is dissolved in a liquid scintillator consisting of a solvent (usually toluene) and a solute (2,5-diphenyloxazole is most common), the energy of the beta-particles is sufficient to excite the solute. The solute emits this extra energy as photons of light (scintillations) which are detected and counted electronically. A secondary solute is sometimes necessary to shift the wavelengths of the photons so that they coincide
with the optimum detecting wavelength of the photomultiplier tubes. To reduce background noise from the photomultiplier tubes a coincidence arrangement of photomultiplier tubes is used to discriminate between true scintillation pulses (occurring simultaneously in both channels) and random noise.

To count aqueous solutions a scintillator system of the following composition is frequently used [65]: dioxan (880 ml), methanol (100 ml), ethylene glycol (20 ml), naphthalene (60 g), 2,5-diphenyloxazole (4 g) and 1,4-di-2-(5-phenyloxazolyl)-benzene (200 mg). In the present work a commercially available liquid scintillator (NE 250, Nuclear Enterprises Ltd.) capable of accepting 20% W/V of water with a reported efficiency of 18% was employed. The liquid scintillation counter used was a Beckmann LS 100 instrument with a print-out of the radioactivity in counts per minute (CPM).

(C) Conventional Method.

Two methods were used to follow the rate of detritiation, a conventional method was used for the faster reactions and an initial rate method for the very slow reactions. The procedure followed for the faster reactions is now given. To 10 ml of the reaction solution, which had been equilibrated in a thermostat bath at 85± 0.1°C, a small amount of labelled compound was added; the concentration of substrate was always less than 10⁻⁴ M. A 0.6 ml sample was quickly withdrawn after the contents had been thoroughly shaken, and frozen in liquid nitrogen. The compound was separated from the tritiated water by freeze-drying. Further samples
were withdrawn at appropriate time intervals and the same procedure followed. 0.50 ml samples of the tritiated water were then added to 2.50 ml of the liquid scintillator (NE 250) in a counting vial and assayed for tritium; sufficient counts were taken to give statistical accuracy of better than \( \pm 0.2\% \).

The infinity samples were taken after more than 10 reaction half-lives had elapsed and the tritium content determined in the same manner as previously. It could also be found by taking advantage of the fact that as the compound was present in such low concentrations, the efficiency of counting an unseparated aliquot was the same as that for a tritiated water aliquot of the same volume. The constancy of the tritium count of unseparated aliquots over the duration of the experiment showed that evaporation was negligible.

Because the concentration of tritiated compound \([X]\) is so low, the reaction kinetics will follow first-order behaviour and

\[
\text{Rate} = - \frac{d[X]}{dt} = k[X], \quad (1.3)
\]

\(k\) being the first order rate constant. Integrating this equation gives

\[
\ln \left( \frac{[X_0]}{[X_t]} \right) = kt \quad (1.4)
\]

where \([X_0]\) and \([X_t]\) represent the initial concentration of tritiated substance in solution and the concentration of substance in solution at time \(t\), respectively. In the particular case where one follows the increase in radio-
activity of the solvent, then

\[ [X_0] \propto a_\infty \]  \hspace{2cm} (1.5)

where \( a_\infty \) = radioactivity of THO when the exchange reaction is completed, and

\[ [X_t] \propto (a_\infty - a_t) \]  \hspace{2cm} (1.6)

where \( a_\infty - a_t \) = radioactivity of THO at time \( t \).

Therefore the first order rate equation becomes

\[ \ln \left( \frac{a_\infty}{(a_\infty - a_t)} \right) = kt \]  \hspace{2cm} (1.7)

A plot of \( \log_{10} (a_\infty - a_t) \) VS \( t \) gives a straight line with slope \( \frac{k}{2.303} \).

Reactions were normally followed over at least two half-lives, 10 samples being taken during each kinetic run. A typical set of results obtained for 1-isopropylbenzimidazole-2-T is given in Table 1.5 and plotted in Figure 1.3. The experimental accuracy was \( \pm 3-5\% \).
FIGURE 1.3 KINETIC PLOT FOR THE DETRITIATION OF 1-iso-PROPYLBENZIMIDAZOLE-2-T IN H₂O AT 85°.
Table 1.5  Detritiation of 1-iso-Propylbenzimidazole-2-T in H₂O at 85°.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Radioactivity of THO, aₜ (CPM)</th>
<th>aₜ - a₀ (CPM)</th>
<th>Log₁₀(aₜ/a₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13,100</td>
<td>103,900</td>
<td>5.017</td>
</tr>
<tr>
<td>1</td>
<td>23,200</td>
<td>93,800</td>
<td>4.973</td>
</tr>
<tr>
<td>2</td>
<td>30,400</td>
<td>86,600</td>
<td>4.939</td>
</tr>
<tr>
<td>3.1</td>
<td>37,800</td>
<td>79,200</td>
<td>4.898</td>
</tr>
<tr>
<td>4</td>
<td>46,100</td>
<td>70,900</td>
<td>4.851</td>
</tr>
<tr>
<td>5</td>
<td>52,900</td>
<td>64,100</td>
<td>4.806</td>
</tr>
<tr>
<td>6</td>
<td>58,200</td>
<td>58,800</td>
<td>4.771</td>
</tr>
<tr>
<td>7</td>
<td>63,300</td>
<td>53,700</td>
<td>4.732</td>
</tr>
<tr>
<td>8</td>
<td>68,900</td>
<td>48,100</td>
<td>4.681</td>
</tr>
<tr>
<td>9</td>
<td>72,800</td>
<td>44,200</td>
<td>4.643</td>
</tr>
</tbody>
</table>

0
10
10
aₜ = 117,700

Slope = -k/2.303 = -0.380/540 sec⁻¹.

k = 1.62 x 10⁻³ sec⁻¹.

(D) Initial Rate Method.

Kinetic runs with reaction half-lives of greater than six hours at 85° were too slow to follow conveniently by the conventional method and an initial rate method [66, 67] was used in such instances. Equation (1.7) can be rearranged to give

\[
\ln \left( \frac{1}{1 - \frac{a_t}{a_\infty}} \right) = kt \quad (1.8)
\]
For a small fraction of the reaction $\frac{n_t}{n_\infty}$, equation (1.8) reduces to

$$\frac{n_t}{n_\infty} = kt$$

as $\ln(1 - X) = X$ for small $X$. A plot of $n_t$ against $t$ should give a slope of $k n_\infty$ and hence $k$ can be calculated.

Experimentally the only difference from the conventional method is that a higher concentration of tritiated compound is employed ($n_\infty$ is usually of the order of $10^6$ CPM). The results of a typical run (the detritiation of adenine-8-T in $H_2O$ at $85^\circ$) are summarised in Table 1.6 and plotted in Figure 1.4.

The initial rate method is very sensitive to the presence of trace impurities and is also experimentally more demanding. It was therefore customary to check that the results obtained by this method agreed with those obtained by the conventional method. Results of the two kinetic techniques are compared in Table 1.7.
FIGURE 1.4 KINETIC PLOT FOR THE DETRITIATION OF ADENINE-8-T (INITIAL RATE METHOD).

RADIOACTIVITY OF THO
(CPM x 10^{-3})

TIME (MINS.)
Table 1.6. Detritiation of Adenine-8-T in H₂O at 85°C (Initial Rate Method).

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Radioactivity of THO, αₜ (CPM)</th>
<th>Radioactivity of reaction solution, α∞ (CPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3,600</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8,800</td>
<td>810,000</td>
</tr>
<tr>
<td>5</td>
<td>11,900</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>15,600</td>
<td>825,000</td>
</tr>
<tr>
<td>9</td>
<td>18,800</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>21,200</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>25,700</td>
<td>818,000</td>
</tr>
<tr>
<td>15</td>
<td>28,200</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>30,600</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>34,400</td>
<td>819,000</td>
</tr>
</tbody>
</table>

α∞ = 819,000 ± 9000
Slope = k α∞ = 32,000/20 x 60 sec⁻¹
k = 3.26 x 10⁻⁵ sec⁻¹

Table 1.7. Comparison of the two kinetic techniques used for measuring the rates of detritiation (H₂O at 85°C).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Initial Rate Method, k (sec⁻¹)</th>
<th>Conventional Method, k (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine-8-T</td>
<td>3.26 ± 0.10 x 10⁻⁵</td>
<td>3.30 ± 0.15 x 10⁻⁵</td>
</tr>
<tr>
<td>Purine-8-T</td>
<td>3.16 ± 0.11 x 10⁻⁵</td>
<td>3.20 ± 0.10 x 10⁻⁵</td>
</tr>
</tbody>
</table>

(E) Stability of purines and benzimidazoles in acid and alkali.

Purines and benzimidazoles are known to decompose under strongly acidic or basic conditions at high temperature. Albert and Brown [68] studied the stability of some purines...
in 10 N NaOH (1 hr. at 100°) and found that 9-methylpurine was largely destroyed whereas purine and hypoxanthine did not decompose. All these compounds were stable in 1N H₂SO₄ (1 hr. at 100°) [68]. Hurst and Kurkiss [69] found that adenine, guanine and hypoxanthine were stable in 1N NaOH at 100°. Adenosine was hydrolysed in 0.1N KOH (1 hr. at 100°) giving 7.5% adenine and 3.5% inosine [70], whereas adenine, guanine, guanosine, hypoxanthine, inosine were stable in 1N KOH (1 hr. at 100°) [70]. 9-Methylhypoxanthine was also stable under the same conditions [71].

Much is known qualitatively about the acid-catalysed hydrolysis of purine nucleosides [72], - the ribose group is split off at the 9-position leaving the parent purine intact. In a kinetic study of the hydrolysis of guanosine at 100°, Zoltewicz [73] found that \( t_\frac{1}{2} = 11 \) mins. in 0.1M perchloric acid. The kinetic runs in the present work were carried out at 85° over a wide range of pH varying from 0.1 M acid to 0.1 M alkali. No departure from first order kinetics was observed in all cases over approximately two half-lives, and therefore decomposition of the substrate was negligible over the period studied.

1,3-dialkylbenzimidazolium salts react with hydroxide in basic solution even at room temperature giving a ring-opened product [74]. However, the rate of exchange of 1,3-dimethylbenzimidazolium-2-T bromide was studied at pH 2-5 where it is stable even at 85°.
Measurement of the rate of hydrogen-deuterium exchange of purine

(A) Measurement of the rate of deuteriation of purine.

A solution of purine was prepared by dissolving about 0.024 g in 0.5 ml D_2O (0.2 M). The solution was transferred to an NMR tube and placed in the spectrometer probe. The probe temperature (85.0±0.2°C) was measured at intervals during each kinetic run by means of a thermocouple. The integrated areas measured for the 8-hydrogen signal were compared with the 2- and 6-hydrogen signals but reproducible results were not obtained because of instability of the integrator and somewhat low signal/noise ratio. By comparing the peak-height of the 8-H [75] with the 2- and 6-H peak-heights (as non-exchanging internal standards) as a function of time, good reproducibility was obtained.

The validity of using peak-height, rather than the area under the peak, as a measure of concentration was checked by measuring the relative peak-heights of the 8-H of a number of solutions of purine in H_2O from 0.02 M to 0.20 M. An average of ten measurements was made for each kinetic point and each reaction was followed over about two half-lives. Plots of log (8-H/2-H + 6-H) versus time gave good straight lines and pseudo-first order rate constants were obtained from Slope = −k/2.303. A typical plot is shown in Figure 1.5.

(B) Measurement of the rate of dedeuteriation of purine-8-D.

Purine-8-D was prepared by heating 0.5 g. of purine in 2.0 ml of D_2O (99.7%) at 85°C for 18 hours. The D_2O
FIGURE 1.5 DEUTERIATION OF THE 8-POSITION OF PURINE IN D₂O AT 85°.
was removed by freeze-drying and the procedure was repeated. The Purine-8,9-D$_2$ was then dissolved in 2.0 ml of H$_2$O to remove deuterium bound to nitrogen and the HDO was removed by freeze drying. The rate of appearance of the 8-H peak of 0.2M solution was measured at 85º by comparing the peak-height of the 8-H signal with the 2-H and 6-H peak-heights as a function of time. Pseudo-first order rate constants were obtained by plotting $\text{Log } \left[ (8-H/6-H+2-H)_\infty -(8-H/6-H+2-H)_t \right]$ versus time $t$, where $(8H/6H+2H)_\infty$ and $(8H/6H+2H)_t$ are the ratio of the 8-H peak-height to the sum of the 2-H and 6-H peak-heights at equilibrium and at time $t$, respectively. This plot gave good straight lines over at least two half-lives and the observed slope = $-k/2.303$. 


1.3 RESULTS

Benzimidazoles

The rate of detritiation of 1-methylbenzimidazole-2-T was determined as a function of pH at 85°. The results are presented in Table 1.8 and are plotted in the form of a pH-rate profile in Figure 1.6. The rate increases with increasing pH and then levels off in a pH independent region. By analogy with the dependence of the rate of exchange of the 2- position of 1-methylimidazole [10] and imidazole [12], such behaviour is consistent with a mechanism in which the conjugate acid of the substrate is being attacked by hydroxide ion in a rate-determining step.

If $[BH^+]$ and $[B]$ represent the concentration of protonated (at N-3) and neutral 1-methylbenzimidazole-2-T respectively, then

$$Rate = k [BH^+][OH^-] \tag{1.10}$$

where $k$ is the second order rate constant. Let $[B]_t$ be the total concentration of 1-methylbenzimidazole-2-T, then

$$[B]_t = [B] + [BH^+] \tag{1.11}$$

If $K_a$ is the acid dissociation constant of protonated 1-methylbenzimidazole, then

$$K_a = [B][H^+] / [BH^+] \tag{1.12}$$

Substituting (1.11) into (1.12) and solving for $[BH^+]$ gives

$$[BH^+] = \frac{[B]_t [H^+]}{K_a + [H^+] + 1} = \frac{[B]_t [H^+]}{K_a + [H^+]} \tag{1.13}$$

Hence combining (1.13) and (1.10) gives

$$Rate = \frac{k [B]_t [H^+][OH^-]}{K_a + [H^+] + 1} = \frac{k Kw [B]_t}{K_a + [H^+]} \tag{1.14}$$
FIGURE 1.6 pH-RATE PROFILE FOR THE DETRITIATION OF 1-ALKYLBENZIMIDAZOLE-2-T AND BENZIMIDAZOLE-2-T AT 85°.
For \([H^+]\) remaining constant during the reaction then

\[
\text{Rate} = k_{\text{obs}} [B]_t \quad \text{where} \quad k_{\text{obs}} = \frac{k \cdot k_w}{K_a + [H^+]},
\]

Equation (1.15) predicts that the rate decreases with increasing \([H^+]\) and for \(K_a \gg [H^+]\), then

\[
k_{\text{obs}} = \frac{k \cdot k_w}{K_a},
\]

i.e. the exchange rate should be constant at high pH.

The relative rate of exchange can be defined as a fraction of \(\frac{k \cdot k_w}{K_a}\),

\[
\text{Relative Rate} = \frac{k \cdot k_w}{K_a + [H^+]} / \frac{k \cdot k_w}{K_a} = \frac{K_a}{K_a + [H^+]} \quad (1.16)
\]

The calculated solid line is plotted using \(pK_a = 4.60\) and the experimental points are plotted as fractions of \(\frac{k \cdot k_w}{K_a} = 246 \times 10^5 \text{ sec}^{-1}\) (Fig. 1.6). The \(pK_a\) value of 4.60 was chosen to give best fit with experimental results and was calculated in the following manner:

\[
\text{Let } R = \text{relative rate} = \frac{K_a}{K_a + [H^+]}
\]

\[
\therefore R \cdot K_a + R[H^+] = K_a
\]

i.e. \(K_a = \frac{R[H^+]}{1 - R}\)

or \(pH = pK_a + \log \frac{R}{1 - R} \quad (1.17)\)
- 49 -

A plot of pH against \( \log \frac{R}{1 - R} \) should give a straight line of slope 1.00 and an intercept at pH = 0 of
\[
\log \frac{R}{1 - R} = -pK_a.
\]
This is plotted in Figure 1.7 and gives \( pK_a = 4.60 \) and slope = 1.01.

Table 1.8 Pseudo first-order rate constants for the detritiation of benzimidazole-2-T, 1-methyl- and 1-ethyl-
benzimidazole-2-T at 85°.

<table>
<thead>
<tr>
<th>pH(20°)</th>
<th>pH(85°)</th>
<th>(10^5k_{obs}) (sec(^{-1}))</th>
<th>Rel. Rate</th>
<th>(10^5k_{obs}) (sec(^{-1}))</th>
<th>Rel. Rate</th>
<th>(10^5k_{obs}) (sec(^{-1}))</th>
<th>Rel. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>2.00</td>
<td>0.65</td>
<td>0.003</td>
<td>8.2</td>
<td>0.034</td>
<td>35</td>
<td>0.14</td>
</tr>
<tr>
<td>3.10</td>
<td>3.10</td>
<td>12.5</td>
<td>0.16</td>
<td>35</td>
<td>0.14</td>
<td>36</td>
<td>0.17</td>
</tr>
<tr>
<td>3.90</td>
<td>3.90</td>
<td>22.2</td>
<td>0.28</td>
<td>35</td>
<td>0.14</td>
<td>36</td>
<td>0.17</td>
</tr>
<tr>
<td>4.18</td>
<td>4.18</td>
<td>38.7</td>
<td>0.49</td>
<td>35</td>
<td>0.14</td>
<td>36</td>
<td>0.17</td>
</tr>
<tr>
<td>4.59</td>
<td>4.59</td>
<td>113</td>
<td>0.46</td>
<td>105</td>
<td>0.49</td>
<td>145</td>
<td>0.67</td>
</tr>
<tr>
<td>4.90</td>
<td>4.90</td>
<td>54.8</td>
<td>0.70</td>
<td>171</td>
<td>0.70</td>
<td>145</td>
<td>0.67</td>
</tr>
<tr>
<td>5.45</td>
<td>5.45</td>
<td>70.7</td>
<td>0.90</td>
<td>213</td>
<td>0.87</td>
<td>200</td>
<td>0.93</td>
</tr>
<tr>
<td>5.55</td>
<td>5.55</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>7.00</td>
<td>6.25</td>
<td>78.7</td>
<td>1.00</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>11.00</td>
<td>9.50</td>
<td>76.8</td>
<td>0.98</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>12.00</td>
<td>10.50</td>
<td>70.4</td>
<td>0.89</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>12.30</td>
<td>10.80</td>
<td>63.3</td>
<td>0.80</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>12.70</td>
<td>11.20</td>
<td>45.6</td>
<td>0.58</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
<tr>
<td>13.00</td>
<td>11.50</td>
<td>33.2</td>
<td>0.42</td>
<td>243</td>
<td>1.00</td>
<td>215</td>
<td>1.00</td>
</tr>
</tbody>
</table>
FIGURE I.7 PLOT FOR THE DETERMINATION
OF THE pKₐ OF 1-METHYLBENZIMIDAZOLE-2-T AT
85° FROM KINETIC DATA.

SLOPE = 1.01

INTERCEPT AT pH = 0, -4.6 = -pKₐ.
The close agreement between the experimental points and the calculated line suggests that the reaction is first order in hydroxide ion and first order in 1-methylbenzimidazole cation. However, because of the equilibrium represented in equation (1.18), another

\[ \text{BH}^+ + \text{OH}^- \xrightarrow{\text{K}} \text{B} + \text{H}_2\text{O} \]  

(1.18)

kinetically indistinguishable possibility exists, i.e. Rate = \( k \left[ \text{BH}^+ \right] \left[ \text{OH}^- \right] \) = \( k \left[ \text{B} \right] \left[ \text{H}_2\text{O} \right] \) = \( k' \left[ \text{B} \right] \left[ \text{H}_2\text{O} \right] \)

where \( K = \frac{K_a \left[ \text{H}_2\text{O} \right]}{K_w} \), and \( k' = \frac{k}{K} \).

The pH-rate profile for the deuteriation of 1-ethylbenzimidazole is expected to closely parallel that of the 1-methyl derivative. This is found to be so experimentally and the measured pseudo-first order rate constants are presented in Table 1.8 and plotted in Figure 1.6, with \( k_{\text{a}} = 4.60 \) and \( \frac{kK_w}{K_a} = 215 \times 10^{-5} \).

In the pH range studied benzimidazole can exist in three forms, namely neutral benzimidazole, benzimidazole cation (formed by protonation on N-3) and benzimidazole anion (formed by ionisation of the N-1 hydrogen). These can be represented by BH, BH\(^+\) and B\(^-\), respectively.

Now \( K_a = \frac{[\text{BH}][\text{H}^+]}{[\text{BH}^+]} \) and \( K_a' = \frac{[\text{B}^-][\text{H}^+]}{[\text{BH}]} \)

(1.19)

and \( [\text{B}]_t = [\text{BH}^+] + [\text{BH}] + [\text{B}^-] \)

(1.20)

Substituting (1.19) and (1.20) and solving for \([\text{BH}^+]\) gives

\[ [\text{BH}^+] = \frac{[\text{B}]_t}{1 + \frac{K_a}{[\text{H}^+]} + \frac{K_a'}{[\text{H}^+]^2}} \]  

(1.21)
Assuming that the rate equation is of the form

\[
\text{Rate} = k \left[ \text{BH}_2^+ \right] \left[ \text{OH}^- \right]
\]

then \[
\text{Rate} = k \frac{\left[ B \right] \left[ \text{OH}^- \right] \left[ \text{H}^+ \right]}{K_a + \frac{K_a K'_a}{[\text{H}^+]} + [\text{H}^+]}
\]

Hence \[
k_{\text{obs}} = \frac{k K_w \left[ B \right]_{t}}{K_a + \frac{K_a K'_a}{[\text{H}^+]} + [\text{H}^+]}
\]

The calculated curve, represented by the solid line, was obtained using \(pK_a = 4.50\) and \(pK'_a = 11.50\) (Fig. 1.6). The reported \(pK_a\) values at \(25^\circ\) are 5.53 and 13.2 which when corrected by Perrin's equation [53] to \(85^\circ\) become 4.40 and 11.70, in good agreement with the calculated values.

The close agreement between the experimental points and the calculated line suggests the correctness of the postulated rate equation or its kinetically indistinguishable equivalent.

To allow a choice between the two kinetically equivalent possibilities, 1,3-dimethylbenzimidazolium bromide was used as a model compound for the benzimidazolium cation. The C-2 hydrogen of the 1,3-dimethylbenzimidazolium ion was found to be extremely labile as is also the case for the corresponding imidazolium ion [7,11]. The rate of detritiation of 1,3-dimethylbenzimidazolium-2-T bromide was determined in acetate and formate buffers at \(85^\circ\) over the pH range 3.35 - 4.56 where measurable rates of exchange were obtained. The rates of detritiation are presented in Table 1.9, and a plot of \(k_{\text{obs}}\) against \([\text{OH}^-]\) is made in Figure 1.8.
FIGURE 1.8 RATE OF DETRITIATION OF 1,3-DIMETHYL BENZIMIDAZOLIUM -2-T BROMIDE IN NaOH SOLUTIONS AT 85°.
The slope gives the second order rate constant \( k_{\text{OH}^-} = 3.14 \times 10^5 \text{ l.mole}^{-1} \text{ sec}^{-1} \) and the intercept at zero indicates the absence of buffer base catalysis or \( \text{H}_2\text{O} \) catalysis. This is supported by the fact that \( k_{\text{OH}^-} \) is virtually constant when the \( \text{CH}_3\text{COO}^-/\text{OH}^- \) ratio was changed by a factor of 5 (Table 1.10).

### Table 1.9 Rates of Detritiation of 1,3-dimethylbenzimidazolium-2-T bromide in aqueous buffers at 85°. \( \mu = 0.1 \text{ M} \).

<table>
<thead>
<tr>
<th>pH at 20°</th>
<th>( 10^5 \text{ kobs (sec}^{-1} )</th>
<th>( 10^{-5} k_{\text{OH}^-} (\text{M}^{-1} \text{sec}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.35</td>
<td>25.4</td>
<td>3.58</td>
</tr>
<tr>
<td>3.70</td>
<td>47.8</td>
<td>3.02</td>
</tr>
<tr>
<td>4.07</td>
<td>113</td>
<td>3.05</td>
</tr>
<tr>
<td>4.25</td>
<td>163</td>
<td>2.90</td>
</tr>
<tr>
<td>4.43</td>
<td>267</td>
<td>3.14</td>
</tr>
<tr>
<td>4.43</td>
<td>261</td>
<td>3.07</td>
</tr>
<tr>
<td>4.43</td>
<td>268</td>
<td>3.15</td>
</tr>
<tr>
<td>4.56</td>
<td>358</td>
<td>3.58</td>
</tr>
</tbody>
</table>

### Table 1.10 The effect of [CH\(_3\)COONa] on the rate of detritiation of 1,3-dimethylbenzimidazolium-2-T bromide at 85°. \( \mu = 0.1 \text{ M (NaCl)} \)

<table>
<thead>
<tr>
<th>pH at 20°</th>
<th>( 10^5 \text{ kobs (sec}^{-1} )</th>
<th>[CH(_3)COONa]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.43</td>
<td>243</td>
<td>.0039</td>
</tr>
<tr>
<td>4.43</td>
<td>281</td>
<td>.0117</td>
</tr>
<tr>
<td>4.43</td>
<td>268</td>
<td>.0195</td>
</tr>
</tbody>
</table>

Assuming that the detritiation of 1-methylbenzimidazole-2-T involves attack of hydroxide on the protonated form, then \( \text{kobs} \) in water at 85° is given by
\[ k_{\text{obs}} = \frac{k_{\text{OH}} K_w}{K_a} \]  

and so \( k_{\text{OH}}^{-} = \frac{k_{\text{obs}} K_a}{K_W} = \frac{2.46 \times 10^{-3} \times 2.52 \times 10^{-5}}{3.10 \times 10^{-13}} \)

\[ = 2.0 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}. \]

Hence the close agreement between the \( k_{\text{OH}}^{-} \) values (1-methylbenzimidazole-2-T, \( 3.1 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1} \); 1,3-dimethylbenzimidazolium-2-T bromide, \( 2 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1} \)) proves conclusively that exchange takes place via the protonated form, as shown in Scheme 1.3.

The effect of various substituents on the rate of exchange also provides support for the exchange mechanism in Scheme 1.3. The introduction of an alkyl group (inductive electron releasing) in the 1-position should make the C-2 hydrogen slightly less acidic and result in a lowering of \( k_{\text{obs}} \) if the mechanism involves deprotonation of the neutral substrate by a water molecule. Similarly the introduction of several chlorine atoms in the benzene ring should increase \( k_{\text{obs}} \) if this mechanism is operative. The results in Table 1.11 lend support to a mechanism involving rate determining attack by hydroxide ion on the protonated substrate.
Table 1.11. Rates of Detritiation at 85° in H₂O.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$10^5 k_{obs}$ (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzimidazole-2-T</td>
<td>78.7</td>
</tr>
<tr>
<td>1-Methylbenzimidazole-2-T</td>
<td>243</td>
</tr>
<tr>
<td>1-Ethylbenzimidazole-2-T</td>
<td>215</td>
</tr>
<tr>
<td>1-i-Propylbenzimidazole-2-T</td>
<td>163</td>
</tr>
<tr>
<td>5,6-Dichlorobenzimidazole-2-T</td>
<td>19.1</td>
</tr>
<tr>
<td>4,5,6-Trichlorobenzimidazole-2-T</td>
<td>7.50</td>
</tr>
<tr>
<td>4,5,6,7-Tetrachlorobenzimidazole-2-T</td>
<td>2.93</td>
</tr>
</tbody>
</table>
Purine and 9-substituted purines.

Like benzimidazole, purine (2) can exist in three forms in the pH range studied, protonated purine, neutral purine and purine anion (formed by ionisation of the N-9 hydrogen) and can be represented as $\text{BH}_2^+$, BH and $\text{B}^-$ respectively. Purine is exactly analogous to benzimidazole and one can predict that the detritiation of purine-8-T should also have a symmetrical bell shaped pH-rate profile if the same mechanism is operative. The pseudo first-order rate constants are presented in Table 1.12 and plotted in Figure 1.9 as a fraction of the rate in $\text{H}_2\text{O}$. Assuming the rate equation is of the form

$$\text{Rate} = k [\text{BH}_2^+] [\text{OH}^-],$$  \hspace{1cm} (1.25)

then a calculated curve, represented by the solid line was obtained from eqn. (1.23) using $pK_a = 2.30$ and $pK_a' = 8.20$. The reported $pK_a'$s at 25° are 2.60 and 8.94. The deviation of the observed points from the calculated line does not exceed the experimental error. In the pH range 4 to 7 the change in hydroxide concentration is compensated by a change in the purine cation concentration. However at pH higher than 7, more and more purine is in the form of the monoanion and the concentration of the purine cation decreases faster than the increase in hydroxide concentration, with the net result of reduction in rate.

9-i-propylpurine and 9-t-butylpurine are analogues of 1-alkylbenzimidazoles. The variation of the rates of detritiation of 9-alkylpurine-8-T with pH would be expected to parallel that of the 1-alkylbenzimidazoles, with
Table 1.12. Pseudo first-order rate constants for the detritiation of Purine-8-T, 9-i-Propyl- and 9-t-Butylpurine-8-T at 85°.

<table>
<thead>
<tr>
<th>pH_{20}</th>
<th>pH_{85}</th>
<th>10^6 k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
<th>10^6 k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
<th>10^6 k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.08</td>
<td>2.08</td>
<td>13.1</td>
<td>0.41</td>
<td>34.5</td>
<td>0.33</td>
<td>9.25</td>
<td>0.16</td>
</tr>
<tr>
<td>2.13</td>
<td>2.13</td>
<td>16.9</td>
<td>0.53</td>
<td>43.5</td>
<td>0.41</td>
<td>18.8</td>
<td>0.34</td>
</tr>
<tr>
<td>2.31</td>
<td>2.31</td>
<td>20.6</td>
<td>0.64</td>
<td>54.9</td>
<td>0.52</td>
<td>21.4</td>
<td>0.39</td>
</tr>
<tr>
<td>2.72</td>
<td>2.72</td>
<td>23.4</td>
<td>0.73</td>
<td>70.0</td>
<td>0.665</td>
<td>25.9</td>
<td>0.47</td>
</tr>
<tr>
<td>3.00</td>
<td>3.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34.0</td>
<td>0.61</td>
</tr>
<tr>
<td>3.12</td>
<td>3.12</td>
<td>26.6</td>
<td>0.83</td>
<td>82.5</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.50</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.5</td>
<td>0.78</td>
</tr>
<tr>
<td>5.45</td>
<td>5.45</td>
<td>31.8</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.00</td>
<td>6.25</td>
<td>32.0</td>
<td>1.00</td>
<td>104.5</td>
<td>1.00</td>
<td>55.3</td>
<td>1.00</td>
</tr>
<tr>
<td>8.87</td>
<td>4.70</td>
<td>0.147</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.00</td>
<td>9.50</td>
<td>2.71</td>
<td>.086</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.00</td>
<td>10.50</td>
<td>256</td>
<td>2.44</td>
<td>87.5</td>
<td>1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.30</td>
<td>10.80</td>
<td>369</td>
<td>3.51</td>
<td>107</td>
<td>1.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.70</td>
<td>11.20</td>
<td>735</td>
<td>7.00</td>
<td>176</td>
<td>3.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.82</td>
<td>11.32</td>
<td>835</td>
<td>7.95</td>
<td>244</td>
<td>4.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.90</td>
<td>11.40</td>
<td>973</td>
<td>9.27</td>
<td>272</td>
<td>4.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.00</td>
<td>11.50</td>
<td>1160</td>
<td>11.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.00</td>
<td>11.50</td>
<td>1170</td>
<td>11.10</td>
<td>304</td>
<td>5.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 1.9  pH - RATE PROFILE FOR THE DETRITIATION OF PURINE-8-T AND IMIDAZO [4,5-b] PYRIDINE-2-T AT 85°.

\[
\text{RATE} = k(BH_2^+)[OH^-] 
\]

\[
\text{RATE} = k(BH^+)[OH^-] + k'(BH)[OH^-] 
\]
\[
\text{k}_\text{obs} = \frac{\text{k} \text{K}_\text{w}}{\text{K}_\text{a} + [\text{H}^+]} \quad (1.26)
\]

This predicts that the rate should increase with increasing pH and for pH greater than pK\(_a\) ([H\(^+\)] \ll K\(_a\)), the exchange rate should be independent of pH. The experimental rates of exchange (Table 1.12, Figure 1.10) however increase very rapidly with pH at pH greater than the pK\(_a\), suggesting the incursion of a second exchange mechanism. In addition to proton abstraction by OH\(^-\) from the conjugate acid, the proposed mechanism also involves attack of OH\(^-\) on the neutral purine.

\[
\text{Rate} = k [\text{BH}^+][\text{OH}^-] + k'[\text{B}][\text{OH}^-] \quad (1.27)
\]

where BH\(^+\) and B represent the 9-alkylpurine cation and the neutral 9-alkylpurine respectively, and k and k\(') are second order rate constants. Then

\[
[B]_T = [B] + [BH^+],
\]

and \(K_a = [B][H^+]/[BH^+]\)

gives \([BH^+] = \frac{[B]_T}{\frac{K_a}{[H^+]} + 1}
\quad (1.28)

and \([B] = \frac{[B]_T}{\frac{[H^+]}{K_a} + 1}
\quad (1.29)

Substituting (1.28) and (1.29) in (1.27) gives

\[
\text{Rate} = \frac{k K_w [B]_T}{K_a + [H^+]} + \frac{k' K_a [B]_T [\text{OH}^-]}{[H^+] + K_a} \quad (1.30)
\]

Thus \(k_{\text{obs}} = \frac{k K_w}{K_a + [H^+]} + \frac{k' K_a [\text{OH}^-]}{[H^+] + K_a}
\quad (1.31)
FIGURE 1.10 PH-RATE PROFILE FOR THE DETRITIATION OF 9-iso-PROPYL-\textsuperscript{(o)} AND 9-tertiary-BUTYLPURINE-B-T (o) AT 85\textdegree.
For $[H^+] \ll K_a$ then

\[
k_{obs} = \frac{k_{Kw}}{K_a} + k' [OH^-].
\]  

(1.32)

Hence a plot of $k_{obs}$ VS $[OH^-]$ should give a slope of $k'$ and intercept $\frac{k_{Kw}}{K_a}$. This is shown in Figure 1.11 for 9-t-propyl- and 9-t-butylpurine-8-T, with $k'$ values of $1.07 \times 10^{-1}$ M sec and $2.54 \times 10^{-1}$ M sec respectively.

The calculated solid line in Figure 1.10 was drawn using eqn. (131) and defining the relative rate as fraction of $\frac{k_{Kw}}{K_a}$ (which corresponds to the plateau in the pH-rate profile) and $pK_a$ values for 9-t-propyl- and 9-t-butylpurine of 2.50 and 2.80, respectively. The experimental points at very high pH are omitted. The closeness of experimental data with the calculated curve indicates the correctness of the postulated rate equation, (1.27).

The rate of detrinitation of 9-t-propylpurine-8-T was also obtained in NaOD - D₂O at 85°C (Table 1.13, Fig. 1.11). The second order rate constant for OD⁻ catalysed exchange $k'_{OD}$ was greater than the OH⁻ catalysed rate constant $k'_{OH}$, $\frac{k'_{OD}}{k'_{OH}} = 1.40$. OD⁻ in D₂O is known to be a stronger base than OH⁻ in H₂O. In compounds where the ionisation of a C-H bond is known to be rate determining, the $k_{OD}/k_{OH}$ ratio falls in the range 1.3 - 1.6, e.g. acetone (1.34) [78], chloroform (1.74) [78], phenylacetylene (1.34) [79], nitroethane (1.39) [80]. Hence the observed ratio for 9-t-propylpurine supports rate determining attack on the neutral molecule by the lyate anion.
FIGURE 1.11 RATE OF DETRITIATION OF 9-i-PROPYLPURINE-8-T IN NaOH-H₂O (O) AND NaOD-DO (Q)
AND 9-t-BUTYLpurine-8-T IN NaOH (O) AT 85°.
Table 1.13. Pseudo first-order rate constants for detritiation of 9-i-propylpurine in NaOD - D$_2$O solutions at 85°.

<table>
<thead>
<tr>
<th>[OD$^-$]</th>
<th>$k_{obs.}$ (sec$^{-1}$)</th>
<th>$k'_{OD^-}$ (M$^{-1}$sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.110</td>
<td>1.60 x 10$^{-3}$</td>
<td>1.45 x 10$^{-2}$</td>
</tr>
<tr>
<td>0.074</td>
<td>1.11 x 10$^{-3}$</td>
<td>1.50 x 10$^{-2}$</td>
</tr>
<tr>
<td>0.052</td>
<td>8.10 x 10$^{-4}$</td>
<td>1.55 x 10$^{-2}$</td>
</tr>
</tbody>
</table>

Table 1.14.

<table>
<thead>
<tr>
<th>Rate of deuteriation of purine in D$_2$O at 85° (sec$^{-1}$)</th>
<th>Rate of dedeuteriation of purine-8-D in H$_2$O at 85°</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.023 x 10$^{-4}$</td>
<td>5.53 x 10$^{-5}$</td>
</tr>
<tr>
<td>1.093 x 10$^{-4}$</td>
<td>6.74 x 10$^{-5}$</td>
</tr>
<tr>
<td>1.087 x 10$^{-4}$</td>
<td>Average 1.07 ± 0.05 x 10$^{-4}$</td>
</tr>
<tr>
<td>Average 1.07 ± 0.05 x 10$^{-4}$</td>
<td>Average 6.13 ± 0.6 x 10$^{-5}$</td>
</tr>
</tbody>
</table>

Table 1.15 Rates of detritiation in D$_2$O at 85° of some tritiated purines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k^T_{D_2O}$ (sec$^{-1}$)</th>
<th>$k^T_{H_2O}/k^T_{D_2O}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purine-8-T</td>
<td>2.76 x 10$^{-5}$</td>
<td>1.18 ± 0.10</td>
</tr>
<tr>
<td>Adenine-8-T</td>
<td>2.70 x 10$^{-5}$</td>
<td>1.22 ± 0.12</td>
</tr>
<tr>
<td>Guanine-8-T</td>
<td>4.10 x 10$^{-5}$</td>
<td>1.10 ± 0.08</td>
</tr>
</tbody>
</table>
A rate equation with one term was sufficient to explain the pH-rate profile for purine itself. It is of interest to determine the effect of the additional term $k'[BH][OH^-]$ on its pH-rate profile.

Assuming $\text{Rate} = k[BH]^+[OH^-] + k'[BH][OH^-]$. (1.33)

and $[B_T] = [BH]^2 + [BH] + [B^-]$ (1.34)

as well as $K_a = [BH][H^+]/[BH]^2$, $K_a' = [B^-][H^+]/[BH]$ we find that $[BH]^+ = \frac{[B_T]}{1 + \frac{K_a K_a'}{[H^+]^2}}$, and $[BH] = \frac{[B_T]}{K_a + 1 + \frac{K_a'}{[H^+]}}$.

Substituting in eqn. (1.33) gives

$$\text{Rate} = k Kw \frac{[B_T]^+}{[H^+] + K_a + \frac{K_a K_a'}{[H^+]^2}} + k'[OH^-][B_T].$$ (1.35)

Hence $k_{obs} = \frac{k Kw}{K_a + [H^+] + \frac{K_a K_a'}{[H^+]^2}} + k'[OH^-]$. (1.36)

For $[H^+] \ll K_a'$ (and so $[H^+] \ll K_a$),

$$k_{obs} = \frac{k Kw}{K_a + K_a K_a'} + \frac{k'[Kw]}{K_a}.$$ (1.37)

For purine at sufficiently high pH the first term becomes negligible and so

$$k_{obs} = \frac{k'[Kw]}{K_a'}. \quad (1.38)$$
Hence if there is a contribution to the observed rate from attack of hydroxide on neutral purine one can predict that the observed rate will not drop to zero at high pH, but will give a non-vanishing pH-independent rate. From Table 1.12 it is possible to calculate a maximum value for $k'$ of $\approx 5 \times 10^2 \text{M}^{-1} \text{sec}^{-1}$ which would be at the limit of detection. The modified pH-rate profile is shown in Figure 1.9. At sufficiently high pH to make the concentration of OH\(^-\) significant, only a minute fraction of purine remains in the reactive neutral form because of ionisation of the N-9 hydrogen. Hence only 9-subsituted purines will allow detection of a contribution to the observed rate by deprotonation of the neutral substrate.

The measured rates of deuteriation of purine and the dedeuteriation of purine-8-D at 85° are given in Table 1.14. The rate of detritiation of purine-8-T in D\(_2\)O at 85° was also measured to allow a direct estimation of $k^H/k^T$ and is shown in Table 1.15. The rate of detritiation of adenine-8-T and guanine-8-T were also measured in D\(_2\)O at 85° and the observed pseudo first-order rate constants are also presented in Table 1.15. The solvent isotope effect on the rate of reaction of all three compounds is seen to be similar.
Imidazo[4,5-b] pyridine

Imidazo[4,5-b] pyridine (29) is analogous to purine and benzimidazole and the rate of deuteriation of this compound labelled in the 2-position should vary with pH in the same manner as its analogues. The measured rates are presented in Table 1.16 and plotted as a fraction of the rate in H$_2$O in Figure 1.9. The symmetrical bell-shaped pH-rate profile again suggests that exchange is brought about by rate determining attack of hydroxide on the protonated substrate. The solid line is drawn in Figure 1.9. using equation (1.23), with the values of $pK_a = 3.50$ and $pK'_a = 9.95$. The close agreement between the experimental points and the calculated line justify the assumed rate equation.

Table 1.16. Pseudo first-order rate constants for deuteriation of imidazo[4,5-b] pyridine-2-T at 85$^\circ$

<table>
<thead>
<tr>
<th>pH 85$^\circ$</th>
<th>$10^5 k_{obs}$ (sec$^{-1}$)</th>
<th>Relative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.84</td>
<td>2.84</td>
<td>0.175</td>
</tr>
<tr>
<td>3.10</td>
<td>4.94</td>
<td>0.310</td>
</tr>
<tr>
<td>3.38</td>
<td>7.75</td>
<td>0.480</td>
</tr>
<tr>
<td>3.61</td>
<td>10.00</td>
<td>0.620</td>
</tr>
<tr>
<td>3.64</td>
<td>9.95</td>
<td>0.615</td>
</tr>
<tr>
<td>3.91</td>
<td>9.99</td>
<td>0.620</td>
</tr>
<tr>
<td>4.18</td>
<td>12.4</td>
<td>0.765</td>
</tr>
<tr>
<td>6.25</td>
<td>16.2</td>
<td>1.000</td>
</tr>
<tr>
<td>9.47</td>
<td>12.6</td>
<td>0.780</td>
</tr>
<tr>
<td>10.01</td>
<td>7.50</td>
<td>0.595</td>
</tr>
<tr>
<td>10.50</td>
<td>2.70</td>
<td>0.166</td>
</tr>
</tbody>
</table>
\((29)\) 

\((30)\) \( R = H \) 

\((31)\) \( R = \text{RIBOSE} \)

\((32)\) \( R = H \) 

\((33)\) \( R = \text{RIBOSE} \)

\((34)\) \( R = H \) 

\((35)\) \( R = \text{RIBOSE} \)

\((36)\) \( R = \text{CH}_3 \)
Adenine and Adenosine

The rate of detritiation of adenine-8-T (30) with pH was investigated and the observed rate constants are presented in Table 1.17 and plotted as fractions of the rate of exchange in H₂O in Figure 1.12. Adenine differs from purine by having an amino group in the 6-position and again a bell-shaped pH-rate profile is predicted. The solid line in Figure 1.12 was calculated using equation (1.23) and pKₐ = 3.50, pKₐ' = 9.00 (at 25°C the literature values are 4.20 and 9.87) [58]. The agreement between the experimental points and the calculated line suggests that the assumed rate equation (first order in protonated adenine and first-order in hydroxide) is correct.

Adenosine (31) is a 9-substituted purine with pKₐ values at 25°C of 3.50 and 12.35 (ionisation of a ribose OH) [58]. If we assume that the rate of exchange is independent of the ribose group ionisation, then adenosine is exactly analogous to 9-i-propyl- and 9-t-butylpurine. Thus if the same rate equation is postulated,

\[ \text{Rate} = k[\text{BH}^+][\text{OH}^-] + k'[\text{B}][\text{OH}^-], \quad (1.39) \]

then the rate should increase with increasing pH and then become independent of pH; at very high pH the rate of exchange is expected to increase very rapidly with increasing pH. The observed pseudo first-order rate constants are presented in Table 1.17 and plotted as fraction of the rate of exchange in H₂O in Figure 1.12. The calculated solid line was drawn using equation (1.31) and pKₐ = 2.90, k' = 1.9 x 10⁻² M⁻¹ sec⁻¹. The value of k' can be calculated
Table 1.17. Pseudo first-order rate constants for the detritiation of adenine-8-T and adenosine-8-T at 85°.

<table>
<thead>
<tr>
<th>pH25</th>
<th>pH85</th>
<th>$10^6 k_{obs}$ (sec(^{-1}))</th>
<th>Rel. Rate</th>
<th>$10^6 k_{obs}$ (sec(^{-1}))</th>
<th>Rel. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.05</td>
<td>2.05</td>
<td>2.26</td>
<td>0.062</td>
<td>7.40</td>
<td>0.110</td>
</tr>
<tr>
<td>2.55</td>
<td>2.55</td>
<td>4.50</td>
<td>0.136</td>
<td>19.10</td>
<td>0.290</td>
</tr>
<tr>
<td>3.00</td>
<td>3.00</td>
<td>10.00</td>
<td>0.303</td>
<td>34.5</td>
<td>0.524</td>
</tr>
<tr>
<td>3.12</td>
<td>3.12</td>
<td>13.6</td>
<td>0.41</td>
<td>57.0</td>
<td>0.864</td>
</tr>
<tr>
<td>3.53</td>
<td>3.53</td>
<td>18.5</td>
<td>0.56</td>
<td>63.0</td>
<td>0.955</td>
</tr>
<tr>
<td>3.75</td>
<td>3.75</td>
<td>20.4</td>
<td>0.62</td>
<td>32.4</td>
<td>0.98</td>
</tr>
<tr>
<td>3.90</td>
<td>3.90</td>
<td>24.1</td>
<td>0.73</td>
<td>26.4</td>
<td>0.80</td>
</tr>
<tr>
<td>4.10</td>
<td>4.10</td>
<td>30.0</td>
<td>1.00</td>
<td>66.6</td>
<td>1.000</td>
</tr>
<tr>
<td>4.65</td>
<td>4.65</td>
<td>29.2</td>
<td>0.88</td>
<td>33.0</td>
<td>1.000</td>
</tr>
<tr>
<td>6.25</td>
<td>9.05</td>
<td>20.4</td>
<td>0.62</td>
<td>29.2</td>
<td>0.88</td>
</tr>
<tr>
<td>10.55</td>
<td>9.17</td>
<td>9.85</td>
<td>0.30</td>
<td>8.10</td>
<td>0.24</td>
</tr>
<tr>
<td>10.67</td>
<td>9.42</td>
<td>4.00</td>
<td>0.121</td>
<td>9.30</td>
<td>0.24</td>
</tr>
<tr>
<td>10.92</td>
<td>9.50</td>
<td>86.3</td>
<td>1.29</td>
<td>11.00</td>
<td>8.10</td>
</tr>
<tr>
<td>11.00</td>
<td>3.06</td>
<td>0.093</td>
<td>3.02</td>
<td>0.061</td>
<td>2.02</td>
</tr>
<tr>
<td>11.99</td>
<td>10.49</td>
<td>277</td>
<td>4.15</td>
<td>435</td>
<td>6.50</td>
</tr>
<tr>
<td>12.30</td>
<td>10.98</td>
<td>625</td>
<td>9.36</td>
<td>9.86</td>
<td>14.80</td>
</tr>
<tr>
<td>12.48</td>
<td>11.19</td>
<td>1440</td>
<td>21.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 1.12 pH-RATE PROFILE FOR THE DETRITIATION OF ADENINE-8-T (○) AND ADENOSINE-8-T (○) AT 85°.
FIGURE 1.13 RATE OF DETRITIATION OF ADENOSINE-8-T IN NaOH-H₂O AT 85°C.

$k_{obs} \times 10^5 \text{ (sec}^{-1})$

slope = $k' = 1.9 \times 10^{-2} \text{ (M}^{-1} \text{ sec}^{-1})$

$10^2 [OH^-] \text{ (Moles litre}^{-1})$
by plotting $k_{obs.}$ VS $[\text{OH}^-]$ as shown in Figure 1.13. The slope = $k$' and the intercept at $[\text{OH}^-] = 0$ is given by $\frac{k K_w}{K_a}$ (the rate of exchange in $\text{H}_2\text{O}$).

Hence the agreement between experimental and calculated values justifies the assumption that ionisation of a ribose OH group does not affect the rate of exchange, and supports the validity of the rate equation (1.39).

The tritiated adenosine used in the kinetic runs had tritium in the 2- position as well as in the 8- position. The exact distribution of tritium in both positions was determined by a kinetic method (assuming that the rate of exchange from the 2- position is negligibly slow compared to that of the 8- position) which is outlined below for the case of hypoxanthine-T(G) and inosine-T(G). An average of five determinations gave the percentage of tritium in the 8-position as $81 \pm 3\%$. The assumption that the rate of exchange from the 2-position is much slower than that of the 8-position was checked in the case of adenine-2-T. This was found to exchange 2,000 times slower in $\text{H}_2\text{O}$ at $85^\circ$ than adenine-8-T.
Guanine and Guanosine

Guanine (32) has three \( pK_a \) values in the pH range 0-14, the values at 20\(^\circ\) being 2.95 (protonation at N-7), 9.32 (ionisation of the N-1 hydrogen) and 12.62 (ionisation of the N-9 hydrogen)[59]. The rate of detritiation of guanine-8-T was obtained as a function of pH at 85\(^\circ\) and the measured rate constants are collected in Table 1.18 and plotted as a fraction of the rate in \( H_2O \) in Figure 1.14. A rate equation of the form

\[
\text{Rate} = k [BH_3^+] [OH^-] + k'[BH_2^-] [OH^-] \tag{1.40}
\]

is postulated where \([BH_3^+]\), \([BH_2^-]\), \([BH^-]\) and \([B^{2-}\]) represent the concentrations of tritiated guanine monocation, neutral guanine, guanine monoanion and guanine dianion, respectively. Then

\[
[B_T] = [BH_3^+] + [BH_2^-] + [BH^-] + [B^{2-}] \tag{1.41}
\]

and

\[
K_a = \frac{[BH_2^-][H^+]}{[BH_3^+]}, \quad K_a' = \frac{[BH^-][H^+]}{[BH_2^-]}, \quad K_a'' = \frac{[B^{2-}][H^+]}{[BH^-]}
\]

We need the rate equation in terms of \([B_T]\) and so (a) \([BH_3^+]\) must be found as a function of \([B_T]\) and (b) \([BH_2^-]\) must be found as a function of \([B_T]\).

(a) Equation (1.41) gives

\[
[B_T] = [BH_3^+] + K_a \frac{[BH_3^+]}{[H^+]} + K_a' \frac{[BH_3^+]}{[H^+]^2} + K_a'' \frac{[BH_3^+]}{[H^+]^3}
\]

Hence

\[
[BH_3^+] = \frac{[B_T]}{1 + K_a \frac{[H^+]}{[H^+]^2} + K_a' \frac{[H^+]}{[H^+]^3} + K_a'' \frac{[H^+]}{[H^+]^4}} \tag{1.43}
\]
Table 1.18. Pseudo first-order rate constants for the
detritiation of guanine-8-T and guanosine-8-T at 85°.

<table>
<thead>
<tr>
<th>pH20°</th>
<th>pH85°</th>
<th>$10^5 k_{obs}$ (sec$^{-1}$)</th>
<th>Rel. Rate</th>
<th>$10^5 k_{obs}$ (sec$^{-1}$)</th>
<th>Rel. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.51</td>
<td>0.51</td>
<td>0.025</td>
<td>0.0055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>0.095</td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>1.50</td>
<td>0.23, 0.24</td>
<td>0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>2.00</td>
<td>0.739</td>
<td>0.162</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.05</td>
<td>2.05</td>
<td></td>
<td></td>
<td>6.43</td>
<td>0.41</td>
</tr>
<tr>
<td>2.50</td>
<td>2.50</td>
<td>1.86</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.75</td>
<td>2.75</td>
<td>2.71</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.83</td>
<td>2.83</td>
<td>2.65</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00</td>
<td>3.00</td>
<td>3.14</td>
<td>0.69</td>
<td>13.5</td>
<td>0.87</td>
</tr>
<tr>
<td>3.50</td>
<td>3.50</td>
<td>4.02</td>
<td>0.88</td>
<td>13.2</td>
<td>0.85</td>
</tr>
<tr>
<td>7.00</td>
<td>6.25</td>
<td>4.45</td>
<td>1.00</td>
<td>15.6</td>
<td>1.00</td>
</tr>
<tr>
<td>9.88</td>
<td>8.38</td>
<td>7.70</td>
<td>1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.15</td>
<td>8.65</td>
<td></td>
<td></td>
<td>74.2</td>
<td>4.75</td>
</tr>
<tr>
<td>10.27</td>
<td>8.77</td>
<td>8.90</td>
<td>2.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.55</td>
<td>9.06</td>
<td>18.4</td>
<td>4.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.67</td>
<td>9.17</td>
<td></td>
<td></td>
<td>118</td>
<td>7.55</td>
</tr>
<tr>
<td>10.80</td>
<td>9.30</td>
<td>22.2</td>
<td>4.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.52</td>
<td>10.02</td>
<td>26.8</td>
<td>5.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.60</td>
<td>10.10</td>
<td></td>
<td></td>
<td>151</td>
<td>9.68</td>
</tr>
<tr>
<td>12.00</td>
<td>10.50</td>
<td>22.6</td>
<td>5.00</td>
<td>152</td>
<td>9.72</td>
</tr>
<tr>
<td>12.48</td>
<td>10.98</td>
<td>18.8</td>
<td>4.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.70</td>
<td>11.20</td>
<td></td>
<td></td>
<td>158</td>
<td>10.10</td>
</tr>
<tr>
<td>13.00</td>
<td>11.50</td>
<td>12.0, 12.2</td>
<td>2.6, 2.7</td>
<td>14.6</td>
<td>9.35</td>
</tr>
</tbody>
</table>
FIGURE 1.14 pH-RATE PROFILE FOR THE DETRITIATION OF GUANINE-8-T AT 85°C.
(b) Equation (1.41) also gives
\[ [B_T] = \frac{[BH_2][H^+]}{K_a [H^+] + [BH_2]} + \frac{K_a' [BH_2]}{[H^+]^2} + \frac{K_a'' [BH_2]}{[H^+]^2} \]  
(1.44)
\[ [BH_2]= \frac{[B_T] + [BH_2]}{[H^+] + 1 + \frac{K_a'}{[H^+]^2} + \frac{K_a''}{[H^+]^2}} \]  
(1.45)

Substituting equations (1.43) and (1.45) in (1.41) gives
\[ \text{Rate} = \frac{k \text{ Kw} [B_T]}{[H^+] + K_a + \frac{K_a' K_a}{[H^+]^2} + \frac{K_a'' K_a}{[H^+]^2}} + \frac{k' \text{ Kw} [B_T]}{K_a [H^+] + \frac{K_a'}{[H^+]^2} + \frac{K_a''}{[H^+]^2}} \]  
(1.46)

Hence \( k \text{ obs} = \frac{k \text{ Kw}}{[H^+] + K_a + \frac{K_a' K_a}{[H^+]^2} + \frac{K_a'' K_a}{[H^+]^2}} + \frac{k' \text{ Kw}}{K_a [H^+] + \frac{K_a'}{[H^+]^2} + \frac{K_a''}{[H^+]^2}} \)  
(1.47)

Relative rate (defined as \( k \text{ obs} /k \text{ Kw} \)) is plotted against pH in Figure 1.14 (solid line) using \( pK_a = 2.60, pK_a' = 8.60, pK_a'' = 11.20 \) and \( k' = 1.85 \times 10^1 \text{ M}^{-1} \text{ sec}^{-1} \).

Guanosine (21) also has three \( pK_a \) values, [58] namely 1.90 (protonation at N-7), 9.25 (ionisation of the N-1 hydrogen) and 12.33 (ionisation of a ribose OH) at 25°C. As found for adenosine, the ionisation of the ribose OH was assumed not to affect the rate of exchange at the 8-position. The rate of detritiation of guanosine-8-T was obtained as a function of pH at 85°C and the measured rate constants are collected in Table 1.18 and plotted as a fraction of the rate in \( H_2O \) in Figure 1.15. The rate
FIGURE 1.15  

**pH-RATE PROFILE FOR THE DETRITIATION OF GUANOSINE-8-T AT 85°.**

![Graph showing the pH-rate profile for the detritution of guanosine-8-T at 85°. The x-axis represents pH, ranging from 0 to 13, and the y-axis represents relative rate, ranging from 0 to 10. The graph shows a curve that rises steeply as pH increases from 7 to 10.](image-url)
increases with increasing pH and then levels off to a constant pH independent rate between pH3 to 7. The rate then increases with increasing pH until it reaches a constant maximum. Guanosine differs from guanine only by having a substituent at the 9-position, i.e. $K_a'' = 0$. Assuming rate equation (1.40) holds, then from equation (1.47) setting $K_a'' = 0$,

$$k_{obs} = \frac{k_{Kw}}{[H^+] + K_a + K_a K_a'} + \frac{k'_{Kw}}{[H^+]^2 + [H^+] + K_a'}$$

For $K_a > [H^+] > K_a'$ and $k > k'$,

then $k_{obs} = \frac{k_{Kw}}{K_a}$, which corresponds to the region pH3-7.

For $[H^+] << K_a'$ (and hence $[H^+] << K_a$) and $k >> k'$,

then $k_{obs} = \frac{k'_{Kw}}{K_a'}$ which corresponds to the region pH greater than 11. The calculated line is plotted using equation (1.48) and $pK_a = 1.70$, $pK_a' = 8.70$ and $k' = 9.50 \times 10^{-1} M^{-1} sec^{-1}$ (calculated from the average $k_{obs}$ at pH greater than 10, $\frac{k'_{Kw}}{K_a} = 1.50 \times 10^{-3}$).
Hypoxanthine, Inosine and 9-methylhypoxanthine

Hypoxanthine (34) has three $pK_a$ values [58] in the pH range, their values at 25° being 1.79 (protonation at N-7), 8.91 (ionisation of the N-1 hydrogen) and 12.64 (ionisation of the N-9 hydrogen). This molecule is analogous to guanine and so a similar dependence of rate of exchange of the 8-position on pH is expected. The measured rate constants are presented in Table 1.19 and plotted in Figure 1.16 as a fraction of the rate in $H_2O$. The calculated line is drawn using equation (1.47) and $pK_a = 1.90$, $pK_a' = 8.50$, $pK_a'' = 11.00$, $k' = 1.30 \text{ M}^{-1} \text{ sec}^{-1}$.

The hypoxanthine-T(G) also had tritium in the 2-position, making it necessary to determine the exact percentage of tritium in the 8-position. This was achieved by following the detrinitiation over a sufficient period until equilibrium was reached. Exchange at the 2-position was found to be at least 50 times slower in $H_2O$ and in 0.1N NaOH, than exchange of the 8-T and was assumed to be negligible during the 24 hours necessary to remove all tritium from the 8-position. An aqueous solution of hypoxantnine-T(G) was assayed for tritium. This was heated for 24 hours at 85° and the separated THO was assayed for tritium in exactly the same manner. The ratio of these two determinations gave the fraction of tritium in the 8-position. This procedure was also used to determine the fraction of tritium in the 8-position of inosine-T(G) and the results are shown in Table 1.20.
Table 1.19. Pseudo first-order rate constants for the detritiation from the 8-position of Hypoxanthine-T(G), Inosine-T(G) and 9-methylhypoxanthine-8-T at 85°.

<table>
<thead>
<tr>
<th>pH_{20}</th>
<th>pH_{85}</th>
<th>10^5k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
<th>10^5k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
<th>10^5k_{obs} (sec^{-1})</th>
<th>Rel. Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>1.00</td>
<td>2.92</td>
<td>0.325</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>2.00</td>
<td></td>
<td></td>
<td>3.14</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.15</td>
<td>2.15</td>
<td>1.80</td>
<td>0.64</td>
<td>7.25</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.12</td>
<td>3.12</td>
<td>2.88</td>
<td>1.02</td>
<td>6.80</td>
<td>0.88</td>
<td>5.50</td>
<td>0.98</td>
</tr>
<tr>
<td>4.07</td>
<td>4.07</td>
<td>2.95</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.70</td>
<td>4.70</td>
<td></td>
<td></td>
<td>1.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.00</td>
<td>6.25</td>
<td>2.82</td>
<td>1.00</td>
<td>7.79</td>
<td>1.00</td>
<td>5.70</td>
<td>1.00</td>
</tr>
<tr>
<td>7.89</td>
<td></td>
<td></td>
<td></td>
<td>14.6</td>
<td>1.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.46</td>
<td></td>
<td></td>
<td></td>
<td>36.9</td>
<td>4.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.70</td>
<td>8.46, 8.05</td>
<td>3.00</td>
<td></td>
<td>8.05</td>
<td>2.85</td>
<td>18.10</td>
<td>3.18</td>
</tr>
<tr>
<td>8.82</td>
<td></td>
<td></td>
<td></td>
<td>49.8</td>
<td>6.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.15</td>
<td></td>
<td></td>
<td></td>
<td>73.6</td>
<td>9.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.20</td>
<td></td>
<td></td>
<td></td>
<td>63.5</td>
<td>8.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37.8</td>
<td>6.63</td>
</tr>
<tr>
<td>9.52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45.5</td>
<td>8.00</td>
</tr>
<tr>
<td>10.98</td>
<td>9.48</td>
<td>11.70</td>
<td>4.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.70</td>
<td>10.20</td>
<td>10.50</td>
<td>3.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.98</td>
<td>10.48</td>
<td>10.60</td>
<td>3.76</td>
<td>81.5</td>
<td>10.5</td>
<td>91.0</td>
<td>15.90</td>
</tr>
<tr>
<td>12.69</td>
<td>11.19</td>
<td>6.86</td>
<td>2.40</td>
<td></td>
<td></td>
<td>93.4</td>
<td>16.1</td>
</tr>
<tr>
<td>12.90</td>
<td></td>
<td></td>
<td></td>
<td>88.5</td>
<td>15.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.98</td>
<td>11.48</td>
<td>3.52, 3.61</td>
<td>1.25, 1.28</td>
<td>84.0</td>
<td></td>
<td>89.0</td>
<td>15.6</td>
</tr>
</tbody>
</table>
FIGURE 1.16 pH-RATE PROFILE FOR THE DETRITIATION OF HYPOXANTHINE-\textsuperscript{T}(G), \( [8\text{-POSITION } ] \), AT 85\textdegree.
Table 1.20. The percentage of Tritium in the 8-position of hypoxanthine-T(G) and inosine-T(G).

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Tritium in the 8-position</th>
<th>Average (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxanthine-T(G)</td>
<td>50.4, 49.0, 48.0, 48.0, 47.5</td>
<td>48.5 ± 1.50</td>
</tr>
<tr>
<td>Inosine-T(G)</td>
<td>77.0, 79.9, 79.9, 78.5, 82.0</td>
<td>80.0 ± 3.0</td>
</tr>
</tbody>
</table>

Inosine (35) and 9-methylhypoxanthine (36) differ from hypoxanthine only by replacing its N-9 hydrogen by β-D-ribofuranosyl and methyl groups, respectively. Because of the close similarity between these molecules and guanosine, the variation of the rate of exchange of the 8-position with pH should closely parallel that of guanosine. The measured rate constants are collected in Table 1.19 and plotted as a pH-rate profile in Figures 1.17 and 1.18. The calculated solid line was obtained by using equation (1.48), (derived for guanosine) and for inosine, $pK_a = 1.50$, $pK_a' = 8.80$ and $k' = 4.52 M^{-1} sec^{-1}$. and $k' = 8.40 M^{-1} sec^{-1}$. 
FIGURE 1.17 PH-RATE PROFILE FOR THE DETRITIATION OF INOSINE-T(G) AT 85°,
(8-POSITION)
FIGURE 1.18  pH-RATE PROFILE FOR THE DETRITIATION OF 9-METHYLMETHYLPXANTHINE-8-T AT 85°.
Effect of temperature on the rate of detritiation of Adenine-8-T and Guanine-8-T.

The rates of detritiation of guanine-8-T and adenine-8-T were measured in H₂O at a number of temperatures and are given in Table 1.21. The values obtained for the activation energies and pre-exponential factors are also given.

Table 1.21. Pseudo first-order rate constants, activation energies and pre-exponential factors for the detritiation of adenine-8-T and guanine-8-T in H₂O.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature (°C)</th>
<th>Rate constant (sec⁻¹)</th>
<th>E act(kcal/mole)</th>
<th>A (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine-8-T</td>
<td>50</td>
<td>1.13±0.03x10⁻⁶</td>
<td>22.3</td>
<td>1.2x10⁹</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>4.87±0.09x10⁻⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>1.39±0.04x10⁻⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>3.30±0.10x10⁻⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanine-8-T</td>
<td>65</td>
<td>6.60±0.30x10⁻⁶</td>
<td>23.1</td>
<td>1.4x10⁹</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>1.85±0.05x10⁻⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>85</td>
<td>4.45±0.20x10⁻⁵</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The variation of the rates of detritiation of the purines and benzimidazoles with pH made it possible to determine the reactive species. The results presented in the previous section show that exchange is brought about by two pathways, rate determining detritiation of the protonated substrate and of the neutral substrate by hydroxide ion. In the case of 1-methylbenzimidazole, benzimidazole, adenine, imidazo [4,5-b] pyridine and purine, the latter pathway was not observed. It is interesting to note that in these cases there is either the absence of rate accelerating ring nitrogens in the 6-membered ring (e.g. alkylbenzimidazoles) or the molecule dissociates to the anionic (unreactive) form in the basic pH region.

Exchange of the protonated substrate

A possible mechanism for exchange of the protonated substrate involves the formation of a ylide intermediate in a rate determining step (Scheme 1.4). The ylide

\[
\begin{align*}
\text{H}^+ & \quad \text{OH}^- & \quad \text{THO} \\
N^+ & \quad N^- & \quad N^+ \\
R & \quad R & \quad R
\end{align*}
\]

\[
\begin{align*}
\text{H}^+ & \quad \text{H}_2\text{O} & \quad \text{OH}^- \\
N^+ & \quad N^- & \quad N^+ \\
R & \quad R & \quad R
\end{align*}
\]

Scheme 1.4
then reacts in a fast step with the solvent $\text{H}_2\text{O}$ regenerating the catalytic hydroxide ion.

Another mechanism involves rate determining addition of hydroxide ion to the protonated substrate producing a carbinol intermediate (Scheme 1.5).

Scheme 1.5

The carbinol intermediate would then exchange the tritium in a fast step with $\text{H}_2\text{O}$ as the base and then revert to exchanged starting material by a succession of fast steps. Even though such carbinol intermediates are known (e.g. 2-hydroxy-1,3-dimethylbenzimidazoline [74,81]), the fast $\text{H}_2\text{O}$ catalysed exchange required by the last step is unlikely.
A mechanism involving a ylide intermediate (Scheme 1.4) has considerable precedence in the literature. Studies of hydrogen exchange of both five-membered and six-membered heterocycles have been rationalised in terms of such intermediates. In the analogous thiazolium salts, Breslow [82] found that the 2-H disappeared in neutral D₂O with t₁/₂ = 20 min., the reaction was accelerated by base and slowed by acid. The exchange mechanism proposed involved formation of a ylide (Scheme 1.6) and the relative stability of this species was used to propose a mechanism for the action of the vitamin thiamine (which requires prior ionisation of the 2-H of the thiazolium moiety). The kinetics of ionisation of

\[
\begin{array}{c}
\text{R}^+ \quad \text{S}^+ \\
\text{N}^+ \quad \text{H} \\
\end{array}
\quad \text{slow}
\quad \begin{array}{c}
\text{R}^+ \\
\text{S} \\
\text{N}^+ \\
\end{array}
\quad \text{HOD}
\]

The kinetics of ionisation of

\[
\begin{array}{c}
\text{R}^+ \quad \text{S}^+ \\
\text{N}^+ \\
\end{array}
\quad \text{fast}
\quad \begin{array}{c}
\text{R}^+ \\
\text{S} \\
\text{N}^+ \\
\text{D} \\
\end{array}
\quad \text{OD}^-
\]

Scheme 1.6

the 2-H of other thiazolium salts [11, 83-87] and the parent compound thiazole [88] all support the mechanism outlined in Scheme 1.6. Similarly, ylide intermediates have
been postulated for the H-D exchange reaction from the 2-
position of 1,3-dimethylimidazolium ion [7], 1-methylimid-
azole [10] and the parent compound imidazole [12]. Other
examples include the pyrazolium ylide [7, 89] (37) and the
tetrazolium ylide [7, 90] (38).

6-membered heterocyclic nitrogen ylides have also been
postulated as reaction intermediates in base-catalysed
hydrogen exchange reactions: These include the N-methyl-2-
pyridone anion (39), [91], 1,3-dimethyl-4-pyrimidonium
ylide (40) [92] and N-substituted pyridinium ylides (41)
[93-95].

Direct evidence for ylide intermediates in base-
catalysed hydrogen exchange reactions of benzimidazoles (and
purines) comes from the fact that their quaternary salts
catalyse the benzoin condensation. Ugai et al. [96] found
that 1,3-dimethylbenzimidazolium iodide catalysed the self-
condensation of benzaldehyde to give benzoin as a product.
By analogy with the classical Lapworth mechanism [97] for
the cyanide-catalysed reaction, the imidazolium ylide added
onto the benzaldehyde carbonyl in a key step, as shown in
Scheme 1.7. Breslow also found that 1,3-dimethylbenzimid-
azolium salts catalysed the acetoin as well as the benzoin
condensation [32]. The addition of the ylide to a carbonyl
group shows unambiguously that there is a large amount of
carbanion character in the reactive position. Tomasz [39]
has recently found that 7-methylguanosine (25) also catalyses
the benzoin condensation as well as exchanging its 8-H
immeasurably fast in D2O at 28°C, again implicating a
ylide intermediate for both the condensation and the exchange
reactions.
(37)  

(38)  

(39)  

(39')

(40)  

(40')

(41) \( R = \mathrm{H, O}^-, \mathrm{CH}_3 \cdot \)

(41')

(42)  

(43)  

(44)  

(45)  

(46)  

(47)  

(48)  

(49)
Purine-8-carboxylic acid (42) is completely decarboxylated to purine by boiling in water for 5 minutes. The anion is stable under the same conditions as is the 6-isomer [20]. Similarly, caffeine-8-carboxylic acid (43) decarboxylates in boiling water [99] and benzimidazole-2-carboxylic acid is much less stable than the isomers with carboxyl groups in the benzene ring portion of the molecule [100]. A generally accepted mechanism [101] for the decarboxylation of heterocyclic acids involves a zwitterion intermediate which loses carbon dioxide to produce a ylide intermediate (Scheme 1.8). Thus the ease of
decarboxylation gives an indication of the stability of
the ylide intermediate for benzimidazoles and purines.

Vaughan et al. [12] have assumed that the rate of
exchange of imidazolium and pyridinium cations is governed
only by the energy difference between the ylide intermediate
and the substrate. Molecular orbital calculations by the
CNDO/2 method gave values for the energy difference which in
turn gave a predicted stability order of ylides in good
agreement with the observed rate of deprotonation. Thus
the assumption that the transition state for hydrogen
exchange closely resembled the ylide intermediate was
justified.

Olofson [90] has shown that 1,3-dialkyltetrazolium
salts undergo base catalysed H-D exchange at the 2-position.
The tetrazolium salts also undergo ring scission to
carbodiimides in a slower reaction, demonstrating the
presence of a ylide as a real intermediate (Scheme 1.9).

\[
\begin{array}{c}
\text{Et} \\
N \quad N + \\
| \\
| \\
N \quad H \\
| \\
| \\
N \quad R \\
\end{array} 
\rightarrow
\begin{array}{c}
\text{Et} \\
N \quad N + \\
| \\
| \\
N \quad R \\
\end{array} 
\rightarrow
\begin{array}{c}
\text{EtN} = C = NR
\end{array}
\]

Scheme 1.9

Measurement of kinetic isotope effects provides a
direct means of ascertaining whether the breaking of a bond
is involved in the rate-limiting step of the overall
reaction. The ratio of the rates of deprotonation and
detritiation of purine at 85° in D₂O, \( k_H/k_T \), was found to be
3.8 ± 0.3, as shown in Table 1.22. From a knowledge of the rate of deuteriation of purine-8-D in H\textsubscript{2}O at 85°, k\textsubscript{D\textsubscript{2}O}, and of the solvent isotope effect on the rate of deuteriation of purine-8-T, k\textsuperscript{T}\textsubscript{H\textsubscript{2}O}/k\textsuperscript{T}\textsubscript{D\textsubscript{2}O}, it is possible to estimate k\textsuperscript{D\textsubscript{2}H\textsubscript{2}O} = (6.13/1.18)10^{-5} = 5.15 \times 10^{-5} \text{ sec}^{-1}.

Thus the ratio of the rates of deprotonation and dedeuteration, k\textsubscript{H\textsubscript{2}O}/k\textsubscript{D\textsubscript{2}O}, in D\textsubscript{2}O at 85° was found to be 2.1 ± 0.3. The approximate maximum values for kinetic isotope effects at 25° are k\textsubscript{CH}/k\textsubscript{CD} = 6.9 and k\textsubscript{CH}/k\textsubscript{CT} = 16 [102] and at 85° these become 5.0 and 10, respectively. The primary kinetic isotope effects for purine are consistent with a rate-determining proton transfer even though they are much smaller than the maximum permitted values.

Variation of the magnitude of primary kinetic isotope effects has been the subject of much recent attention [103] and there are definite indications that the effect passes through a maximum in the vicinity of ΔpK = 0, where ΔpK is the difference in acidity between substrate and the conjugate acid of the catalyst. An estimate of the pK\textsubscript{a} of weak carbon acids is often possible if the water catalysed rate of hydrogen exchange is known. This rate of exchange however was not detectable in the case of the purine and benzimidazole cations studied and so it must be less than 10^{-7} \text{ sec}^{-1} at 85°. The extrapolated rate-constant at 25° (E\textsubscript{act} = 22 k \text{ cal/mole}) is 2 \times 10^{-10} \text{ sec}^{-1} which suggests a pK\textsubscript{a} greater than 21 assuming diffusion controlled reaction between H\textsubscript{3}O\textsuperscript{+} and the ylide formed. Hence ΔpK\textsubscript{a} is greater than 5 (pK\textsubscript{a}H\textsubscript{2}O = 15.7) and so on the basis of this analysis a relatively small kinetic isotope is expected. The only
reported isotope effects are for exchange of the thiazolium system (Table 1.23) and again are small. Various trihalomethanes which undergo base catalysed hydrogen exchange, also have very low isotope effects (Table 1.24) and the carbanion intermediate produced cannot be delocalized by resonance in just the same way as the carbanion in the heterocyclic ylides is localized to one carbon.

Table 1.22. Rate of Isotopic hydrogen exchange of the 8-position of purine at 85°

<table>
<thead>
<tr>
<th>$10^5 k_{H_2O}$(sec$^{-1}$)</th>
<th>$10^5 k_{D_2O}$(sec$^{-1}$)</th>
<th>$10^5 k_{H_2O}$(sec$^{-1}$)</th>
<th>$10^5 k_{D_2O}$(sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.25 ± 0.15</td>
<td>2.76 ± 0.10</td>
<td>10.74 ± 0.50</td>
<td>6.13 ± 0.50</td>
</tr>
</tbody>
</table>

Table 1.23. Primary kinetic isotope effects for hydroxide catalysed hydrogen exchange from the 2-position of thiazoles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature(°C)</th>
<th>$k_H/k_T$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-methylthiazolium iodide</td>
<td>28</td>
<td>5 ± 1</td>
<td>83</td>
</tr>
<tr>
<td>N-benzylthiazolium bromide</td>
<td>30</td>
<td>2.7</td>
<td>87</td>
</tr>
<tr>
<td>N-benzylbenzothiazolium bromide</td>
<td>30</td>
<td>4.8</td>
<td>87</td>
</tr>
</tbody>
</table>
Table 1.24. Primary kinetic isotope effects for hydroxide catalysed exchange of trihalomethanes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(k_H/k_D)</th>
<th>Temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>1.48</td>
<td>20</td>
<td>104</td>
</tr>
<tr>
<td>CHCl₂F</td>
<td>1.52</td>
<td>20</td>
<td>105</td>
</tr>
<tr>
<td>CHBrClF</td>
<td>1.70</td>
<td>15</td>
<td>106</td>
</tr>
</tbody>
</table>

The mechanism for exchange outlined in Scheme 1.4 requires the protonation or partial protonation of purines at N-7. The position of protonation of purine and adenine however is reported to be at N-1. Giner-Sorolla and Bendich [107] varied the electron withdrawing power of the 6-substituent (Table 1.25) and found that the first \(pK_a\) (but not the second) decreased with increasing electron withdrawing power. This was interpreted as preferred protonation on the pyrimidine ring with N-1 as the likely site, whereas the second \(pK_a\) referred to the ionisation at the more removed imidazole ring N-9 hydrogen. On the basis of molecular orbital calculations, Pullman [108] also concluded that the N-1 position was the most likely site for protonation in adenine. Recent calorimetric work [109] also provides evidence of the N-1 protonation site in adenine and adenosine.
Table 1.25. The effect of substituents in the 6-position on the pKₐ of purines [109]

<table>
<thead>
<tr>
<th>pKₐ</th>
<th>pKₐ'</th>
<th>Purine</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>9.8</td>
<td>6-NH₂</td>
</tr>
<tr>
<td>3.8</td>
<td>9.8</td>
<td>6-NHOH</td>
</tr>
<tr>
<td>2.3</td>
<td>9.7</td>
<td>6-NHCO₂CH₃</td>
</tr>
<tr>
<td>2.3</td>
<td>9.9</td>
<td>6-NHCONH₂</td>
</tr>
</tbody>
</table>

X-ray crystallography of protonated adenine derivatives such as adenine hydrochloride [110], adenosine-5'-phosphate [111], adenosine-3'-phosphate [112] and adenosine-2',-uridine-5'-phosphate [113] all show that adenine is protonated at the N-1 position in the solid state.

The position of protonation of purine and substituted purines has been also ascribed to the N-1 position. The basic pKₐ values for 2-methylmercapto- and 6-methylmercapto-purines (1.91, ~0 respectively) were determined by Albert [114] who concluded that the lower basic strength of the 6-substituted derivatives indicated N-1 protonation (the methylmercapto group being inductive electron withdrawing). The cations of 7-methyl- and 9-methylpurine have different UV spectra [115] which would not arise if the protonation site was in the imidazole ring. Further evidence [116] that protonation involved the pyrimidine ring was provided by a much greater base weakening evident in 6-trifluoromethylpurine (pKₐ <0) as compared with the 8-trifluoro-
methylpurine (pK$_a$ = 1.0). Lynch, Robins and Cheng [117] have used resonance stabilisation arguments to establish N-1 over N-3 as the predominant site of protonation. The p-quinoid type of resonance (44) is preferred to the o-quinoid (45) as, for example, the pK$_a$ values of 2-aminopyridine (o-quinoid) and 4-aminopyridine (p-quinoid) are 6.86 and 9.17.

Bullock and Jardetzky [17] found that the change in proton chemical shifts between neutral purine and purine cation of the C-2, C-6 and C-8 protons were comparable, which is not in accord with N-1 as the only site of protonation. A number of authors [118-120] have reported that the NMR peaks due to H-2 and H-6 exhibit a splitting, ascribable to protonation at N-1 which would decrease the quadrupolar line broadening effect of the intervening nitrogen. Read and Goldstein [120] measured the dependence of the proton chemical shifts of the C-protons of purine and the $^{13}$C-H coupling constants as a function of pH. All these spectral parameters exhibit a linear dependence on the fraction of protonated base present which again is not in accord with a single protonation site. To account for the variation of $^{13}$C-H couplings, it was proposed that protonated purine is an equilibrium mixture of tautomeric isomers with one proton bonded largely at N-9 and the other distributed over N-1, N-3 and N-7 with an approximate per cent protonation of 47, 24 and 29% respectively.

The change in the carbon-13 chemical shifts [121] on
going from neutral to protonated purine indicates that N-1 is the most likely site of protonation. The magnitude of the protonation parameters were less than expected and it was proposed that this may be the result of the proton spending a small fraction of its time on the other ring nitrogens. The existence of protonated N-7 and N-3 purine cannot be ruled out on the basis of substituent effects on the observed $pK_a$ or the occurrence of splitting ($J_{2,6}$), as these observations could represent only the contribution of the predominant N-1-H species. A similar situation is likely for adenine and recent work by Chan and Nelson [122] on the effect of pH on the proton NMR parameters of adenyl-(3',5')-adenosine admits the possibility of several monoprotonated adenine species which are in multiple equilibrium with one another. It is noteworthy that when adenine is alkylated in neutral solution with ethyl methanesulphonate [123], 3-alkyl, 9-alkyl and 1-alkyl isomers are produced (yields 25%, 9% and 8%, respectively). Similarly when adenosine is alkylated with dimethylsulphate the 7-methyl- as well as the predominant 1-methyladenine is produced [124].

The sites of protonation of benzimidazole (N-3) [121], guanine and its derivatives, (N-7) [135], and hypoxanthine and its derivatives (N-7) [176], are well established and are in accord with the mechanism outlined in Scheme 1.4.

For purines and substituted purines, adenine and adenosine, exchange at low pH takes place via the 7-protonated purine which is assumed to be present to a small extent. Then the concentration of protonated purine $[BH^+] = [B_1H^+] + [B_3H^+] + [B_7H^+]$ where $[B_1H^+]$ represents the concentration of the protonated N-1-H species etc.
Assume \[ \text{Rate} = k_7[B_7H^+]\text{[OH]}^- \].

If \([B_T] = \text{concentration of tritiated purine in solution}, \]
then \([B_T] = [B] + [B_1H^+] + [B_3H^+] + [B_7H^+] \).

\[ t = K_a \frac{[BH^+/][H^+]}{[H^+] + [B_1H^+] + [B_3H^+] + [B_7H^+]}. \]

\[ = K_a \frac{[B_1H^+]}{[H^+] + K_a[B_3H^+]/[H^+] + K_a[B_7H^+]/[H^+] + [B_1H^+] + [B_3H^+] + [B_7H^+]}. \]

\[ = \frac{K_a}{a} \frac{[B_7H^+]}{[H^+] + b\frac{[B_1H^+]}{[H^+] + c\frac{[B_3H^+]}{H^+] + d\frac{[B_7H^+]}{[H^+]}}} \]

\[ = \frac{K_a}{b} \frac{[B_7H^+]}{[H^+] + c\frac{[B_3H^+]}{[H^+] + d\frac{[B_7H^+]}} \]

\[ = \frac{K_a}{c} \frac{[B_7H^+]}{[H^+] + d\frac{[B_7H^+]}{[H^+]}} \]

\[ \text{where } a = \frac{[B_1H^+]}{[BH^+]}, \quad b = \frac{[B_3H^+][BH^+]}{[BH^+]}, \quad c = \frac{[B_7H^+][BH^+]}{[BH^+]}. \]

\[ \therefore [B_7H^+] = \frac{[B_T]}{(a + b + c + 1) (K_a/[H^+] + 1)} \]

\[ \therefore \text{Rate} = \frac{k_7\text{Kw}[B_T]}{(a + b + c + 1)(K_a + [H^+]}, \]

which gives \[ k_{\text{obs}} = \frac{k_7\text{Kw}}{(a + b + c + 1)(K_a + [H^+])} \times \]

\[ \left( \frac{\frac{K_a}{c} \frac{[B_7H^+]}{[H^+] + d\frac{[B_7H^+]}{[H^+]}}}{\frac{K_a}{c} \frac{[B_7H^+]}{[H^+] + d\frac{[B_7H^+]}{[H^+]}}} \right) \]

where \[ \frac{c}{a + b + c} \]

is the fractional amount of protonated

purine in the N-7-H form. This gives exactly the same pH-rate profile as before except that the \[ k \]
value used previously now becomes

\[ k = \frac{c k_7}{a + b + c} \]
The second order rate constants ($k$) for the hydroxide catalysed detrinitiation from protonated purines are given in Table 1.26. Comparison of the rate of exchange of protonated adenine and purine with protonated hypoxanthine gives an estimate of the fractional amount of the N-7-H protonated form of adenine and purine of approximately 4%. Similar comparison between adenosine and guanosine or inosine, gives about 3% in the N-7-H protonated form.

Table 1.26 also provides other interesting correlations between the value of $k$ and the structure of the compound. The value for the hydroxide catalysed rate of exchange of protonated benzimidazole and 1-substituted benzimidazoles ($10^{-20} \times 10^{4} M^{-1} \text{sec}^{-1}$) agrees well with that of 1,3-dimethylbenzimidazolium ion ($3.4 \times 10^{4} M^{-1} \text{sec}^{-1}$). Comparison of the rates of exchange of adenine and adenosine shows that the latter is five times faster which can be ascribed to the inductive electron withdrawing power of the ribose group (assuming that the fractional amount of the N-7-H protonated form is similar for both compounds). Similarly the exchange of inosine is seven times faster than hypoxanthine and guanosine is 28 times faster than guanine. The irregularity in the $k$ values for purine and 9-substituted purines may reflect different fractions of the N-7-H protonated form for each compound. The position of protonation of imidazo [4,5-b] pyridine has not been established and the small $k$ value may be a consequence of predominant protonation of the nitrogen in the pyridine ring with a small fraction of the reactive N-3-H protonated form present.
Table 1.26. Second order rate constants for the hydroxide catalysed detritiation of protonated \((k)\) and neutral \((k')\) benimidazoles and purines at 85°.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(pK_{85}) (_a) (proton-gained)</th>
<th>(pK_{85}') (_a) (proton lost)</th>
<th>(10^5k) obs in (H_2O) (\text{sec}^{-1})</th>
<th>(10^{-4}k) ((M^{-1} \text{ sec}^{-1}))</th>
<th>(10^3k') ((M^{-1} \text{ sec}^{-1}))</th>
<th>(k/k')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Methyl-benzimidazole-2-T</td>
<td>4.60</td>
<td>-</td>
<td>243</td>
<td>19.2</td>
<td>&lt;0.02</td>
<td>9</td>
</tr>
<tr>
<td>1-Ethyl-benzimidazole-2-T</td>
<td>4.60</td>
<td>-</td>
<td>215</td>
<td>17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzimidazole-2-T</td>
<td>4.50</td>
<td>11.70</td>
<td>78.7</td>
<td>7.8</td>
<td>&lt;5</td>
<td>1.6x10^9</td>
</tr>
<tr>
<td>1,3-dimethylbenzimidazolium-2-T</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>31.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-i-Propylpurine-8-T</td>
<td>2.50</td>
<td>-</td>
<td>10.45</td>
<td>104</td>
<td>10.7</td>
<td>9.8x10^7</td>
</tr>
<tr>
<td>9-t-Butylpurine-8-T</td>
<td>2.80</td>
<td>-</td>
<td>5.53</td>
<td>28</td>
<td>2.54</td>
<td>1.1x10^8</td>
</tr>
<tr>
<td>Purine-8-T</td>
<td>2.30</td>
<td>8.20</td>
<td>3.20</td>
<td>5.07</td>
<td>&lt;50</td>
<td>&gt;10^6</td>
</tr>
<tr>
<td>Imidazo[4,5-b]pyridine-2-T</td>
<td>3.50</td>
<td>9.95</td>
<td>16.2</td>
<td>16.2</td>
<td>&lt;2</td>
<td>8x10^7</td>
</tr>
<tr>
<td>Adenine-8-T</td>
<td>3.50</td>
<td>9.00</td>
<td>3.30</td>
<td>5.2</td>
<td>&lt;2</td>
<td>2x10^7</td>
</tr>
<tr>
<td>Adenosine-8-T</td>
<td>2.90</td>
<td>-</td>
<td>6.66</td>
<td>26.6</td>
<td>19.2</td>
<td>1.4x10^7</td>
</tr>
<tr>
<td>Guanine-8-T</td>
<td>2.60</td>
<td>8.60</td>
<td>4.43</td>
<td>35.4</td>
<td>18.5</td>
<td>1.9x10^6</td>
</tr>
<tr>
<td>Guanosine-8-T</td>
<td>1.70</td>
<td>8.70</td>
<td>15.6</td>
<td>990</td>
<td>950</td>
<td>1.0x10^7</td>
</tr>
<tr>
<td>Hypoxanthine-8-T</td>
<td>1.90</td>
<td>8.50</td>
<td>2.82</td>
<td>112</td>
<td>130</td>
<td>8.6x10^6</td>
</tr>
</tbody>
</table>
Table 1.26 (Cont'd)

<table>
<thead>
<tr>
<th>Compound</th>
<th>pKₐ 85° (proton gained)</th>
<th>pKₐ' 85° (proton lost)</th>
<th>10⁻⁵k obs in H₂O (sec⁻¹)</th>
<th>10⁻⁴k (M⁻¹ sec⁻¹)</th>
<th>10⁻³k' (M⁻¹ sec⁻¹)</th>
<th>k/k'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inosine-β-T</td>
<td>1.50</td>
<td>8.50</td>
<td>7.79</td>
<td>779</td>
<td>840</td>
<td>9.3x10⁶</td>
</tr>
<tr>
<td>9-Methylhypoxanthine-β-T</td>
<td>1.91</td>
<td>8.80</td>
<td>5.70</td>
<td>220</td>
<td>452</td>
<td>4.9x10⁶</td>
</tr>
</tbody>
</table>

(a) From N-H

(b) Largest value which would be experimentally detectable is given for cases in which a term involving k' was not contributing.
The lability of the 8-H of protonated purine and the 2-H of protonated benzimidazole can be ascribed to a number of factors including the high S-character of the C-H bond, an inductive effect of the positive N and other ring nitrogens and stabilization of the ylidic species by resonance contributions.

(1) S-character of the C-H bond [127]: Rates of carbanion formation are influenced by the amount of S-character in the bond being broken. The acidity difference between acetylene ($pK_a \approx 25$) and ethylene ($pK_a \approx 36$) have been ascribed mainly to the % S-character (50% and 33%, respectively). The increase in acidity with increasing S-character is caused by the stronger attraction by the positively charged nucleus on the spherosymmetrically arranged S electrons, than on the p electrons. An approximate measure of the state of hybridisation of an atom may be obtained from bond angles. Zoltewicz [128] has explained the difference in reactivity to H-D exchange of pyridine and pyridine-N-oxide to the fact that the endo angles at C-2 are 124° and less than 120°, respectively. Hence base catalysed exchange of the C-2H of pyridine is several powers of ten times slower than the N-oxide. Also the endo ring angles for pyridine at C-2, C-3 and C-4 are 124°, 118.6° and 118.1° and the relative rates of H-D exchange in CH$_3$OD - CH$_3$ON$_a$ are 1.0: 9.3:12, respectively [128]. The bond angles for some purines are presented in Table 1.27. For purine itself
the angle at C-8 is less than that at C-6 which is in line with the observed order of hydrogen exchange - brief heating (10 mins.) of purine in D₂O at 100° exchanges the 8-H whereas partial exchange was observed at the 6-position after 72 hrs. at 100° [19]. In adenine and adenosine the 6-position is blocked by the amino substituent and the angle at C-8 is less than at C-2. Experimentally, the rate of detritiation at 85° in H₂O is 2,000 times faster at the 8- than at the 2- position. Comparison of the C-8 angle between 9-ethylguanine and Guanine hydrochloride shows that the protonated guanine has a much smaller C-8 angle, and so a larger percentage S-character. Experimentally, neutral guanine exchanges a factor of 10⁶ times slower than protonated guanine (Table 1.26).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Angle:1-2-3</th>
<th>5-6-1</th>
<th>7-8-9</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purine</td>
<td>128.5</td>
<td>119.1</td>
<td>114.1</td>
<td>129</td>
</tr>
<tr>
<td>9-Methyladenine</td>
<td>126.5</td>
<td>(117.4)</td>
<td>112.0</td>
<td>130</td>
</tr>
<tr>
<td>Adenosine</td>
<td>128.8</td>
<td>(118.1)</td>
<td>113.2</td>
<td>131,132</td>
</tr>
<tr>
<td>9-Ethylguanine</td>
<td>(123.3)</td>
<td>(117.7)</td>
<td>114.0</td>
<td>133</td>
</tr>
<tr>
<td>Guanine Hydrochloride</td>
<td>(123.4)</td>
<td>(110.8)</td>
<td>109.6</td>
<td>134</td>
</tr>
</tbody>
</table>
(2) **Inductive Effect:**

Olofson [7] has investigated the effect on the rate of H-D exchange of five membered heterocycles of the positioning of the nitrogen atoms. Addition of two nitrogen atoms to 1,3-dimethylimidazolium ion increased the rate of the tetrazolium salt (46) by a factor of $2 \times 10^9$. With a positively charged nitrogen in a position $\beta$ to the exchanging C-H, thus preventing the formation of a ylide, the rate of exchange of (47) was found to be a factor of about $10^9$ times slower than (46). These effects were mainly assumed to be due to a pure inductive effect [7] with the contribution from the positive nitrogen predominating over that of the other nitrogens. The difference in rate between adenosine, inosine and guanosine and the parent bases (Table 1.26) can be interpreted by the inductive effect of the electron withdrawing ribose group.

(3) **Resonance Stabilisation**

It has been suggested that the ylidic species could be stabilized by resonance contribution from the carbene structure (48). This was first proposed by Breslow [82] by analogy with the resonance forms for isonitriles:

\[
R - N = C \quad \text{or} \quad R - N = C;
\]

Wanzlick [135] succeeded in trapping such a carbene intermediate by reaction with tetracyanoethylene as shown in Scheme 1.10. The carbene was produced by the splitting off of chloroform from 1,3-diphenyl-2-trichloromethylimidazoline or by the reaction of dianilinoethane with orthoformate ester (Scheme 1.10).
Benzothiazolium salts, which exchanges its 2-H in a base catalysed reaction, also produces a dimer (49) when deprotonated in aprotic solvents [136,137], thus showing the existence of a carbene intermediate. Haake [11], however, suggested that stabilization due to the carbene resonance form would be small because the electron deficient carbon might be of considerably higher energy than the ylide form.

The detritiation reactions of protonated purines and benzimidazoles are catalysed by hydroxide ion. They are not significantly catalysed by buffer bases or by \( \text{H}_2\text{O} \) as shown in Table 1.10 for 1,3-dimethylbenzimidazolium-2-T bromide.
A mechanism involving internal return for the H-D exchange reactions of N-substituted pyridinium ions was proposed by Zoltewicz [95] to account for the lack of buffer base catalysis and low kinetic isotope effects of these reactions. Rates of deprotonation of carbon acids in solvents which are considerably more acidic than the acids themselves are subject to the effects of internal return [138]. That is, the rate of back-protonation of the hydrogen bonded carbanion (k-1) may compete with the rate of replacement of tritium by hydrogen from solvent at the carbanionic site (k2) (Scheme 1.11).

\[
\text{C} - \text{T} + \text{B} \xrightarrow{k^{-1}} \text{C}^{-}...\text{TB}^{+} \xrightarrow{k_{2}} \text{C}^{-}...\text{HB}^{+} \xrightarrow{\text{R}} \text{CH} + \text{B}
\]

Scheme 1.11

Rates of exchange are then given by eqn. 1.56 and do not solely reflect rates of detritiation. If the exchange reaction is buffer base catalysed and the Bronsted \( \beta \) has a value between 0 and 1, then proton transfer takes place in a rate-determining step \( k_{-1} \ll k_{2} \) and there is no internal return. This gives \( k_{\text{obs}} = k_{1}[B] \) where \( B \) may be a variety of bases present in the reaction solution. The rate of deprotonation for such general base catalysed reactions then is related to the basicity of \( B \) by the Bronsted equation,

\[
\log k = \beta pK_{a} + G
\]

where \( k \) is the second order rate constant.
for deprotonation by B and $K_a$ is the acidity constant of the conjugate acid of B. When $\beta = 1$ catalysis by buffer bases is small and conclusions regarding the reaction mechanism are less definite. In this case internal return may be present, as when $k_{-1} \gg k_2$, the rate-determining step is separation of the hydrogen bonded complex and this slow step is preceded by an equilibrium. The carbanion intermediate reacts faster with the triton which has just left it than the triton can diffuse away or rotate so that it is replaced by a hydrogen. In this situation, the observed isotope effect consists of the isotope effect on the equilibrium and on the separation of the hydrogen-bonded complex and the magnitude of the isotope effect is expected to be small. The observed isotope effects for the detritiation of purine-8-T and the absence of general base catalysis suggests that internal return is operative in this case. Internal return has also been suggested for deprotonation reactions from annular positions of other heterocyclic compounds [11, 86, 95, 139] in which the negative charge is localized on one carbon and buffer base catalysis has not been detected. In the case of nitroalkanes and ketones, internal return is not expected for the hydrogen exchange reactions as $k_{-1} < k_2$ because back-protonation of the resonance delocalized carbanion is known [138] to be much slower than for largely localized carbanions. It is likely, however, that internal return is also operating in the hydrogen exchange reactions of acetylenes and haloforms.
Exchange of the Neutral Substrate. The second pathway for exchange involves reaction of hydroxide ion and the neutral substrate. This second term of the rate equation was found for exchange of 9-alkylpurines, adenosine, guanine, guanosine, hypoxanthine, 9-methylhypoxanthine and inosine. The simplest mechanism for exchange of the neutral substrate involves hydroxide catalysed formation of a carbanion in a rate determining step (Scheme 1.12). The carbanion then reacts in a fast step with the solvent H$_2$O regenerating the catalyst. A mechanism of this type accompanied by

\[
\text{N}^+ \text{R}^- + \text{OH}^- \stackrel{\text{slow}}{\longrightarrow} \text{N}^- \text{R}^- + \text{THO}
\]

\[
\text{N}^- \text{R}^- + \text{H}_2\text{C} \stackrel{\text{fast}}{\longrightarrow} \text{N}^- \text{R}^- + \text{OH}^-
\]

Scheme 1.12

hydroxide catalysed exchange of the protonated substrate has been proposed for the deuteriation at the 4(5)-position of imidazole [12], the 2-position of thiazole [88] and also the 2-position of pyridine [93] and 4-alkylaminopyridines [140]. Evidence supporting the mechanism in Scheme 1.12.
includes (1) 1-alkylbenzimidazoles are readily metalated in the 2-position by phenyllithium [141] and phenylsodium [142]. This suggests that the carbanion at the 2-position of the imidazole system, as required by the proposed mechanism, has a real existence. (2) The rate of detritiation of 9-i-propylpurine-8-T was obtained in NaOH-H2O and NaOD - D2O and the ratio of the second order rate constants k'OD- / k'OH- = 1.40. OD- in D2O is known to be a stronger base than OH- and H2O+ and in compounds in which ionisation of the C-H is known to be rate determining, the ratio k'OD- / k'OH- falls in the range 1.3 - 1.7 (acetone 1.34 [78], phenylacetylene 1.34 [79]). Hence the observed ratio is consistent with simple hydroxide catalysed deprotonation.

Tomasz et al. [42] in a very recent study of the kinetics of tritium exchange from the 8-position of guanosine, have also found a rate equation of the form:
Rate = k [BH2+][OH-] + k' [BH][OH-].

However, the last term is interpreted as involving not the neutral species (20) but its kinetically equivalent zwitterion (28), which has a positive charge on N-7, and gives a ylide type intermediate. 1-methylguanosine cannot exist as a zwitterion and was found to exchange only via the protonated species, thus supporting the zwitterion as an intermediate. However, these workers also failed to detect the large increase in the rate of exchange of the 8-H of adenosine at higher pH [37], which casts some doubt on their observations on the exchange of 1-methylguanosine-8-T. The rate acceleration in basic pH for 9-i-propylpurine-8-T, 9-t-butylpurine-8-T and adenosine-8-T found in this
work, cannot be ascribable to hydroxide catalysed exchange of a zwitterionic species as these compounds cannot exist as zwitterions. The kinetics of exchange of 2-H of thiazole, the 4,(5)-H of imidazole and the 2-H of pyridine all involve lyate anion catalysed exchange of the neutral (as well as the protonated species) again suggesting that a mechanism outlined in Scheme 1,12 is general.

The measured second order rate constants for hydroxide catalysed detritiation of neutral benximidazoles and purines \(k'\) are presented in Table 1.26. In cases where \(k'\) was too small to be measured, an estimate is made of the largest value which would not be experimentally detectable. The rate constants for adenosine, guanosine and inosine are all larger than their respective parent compounds (adenine, guanine and hypoxanthine), which suggests that the inductive effect of the electron withdrawing ribose group is responsible. Similarly, the rate constant for 9-t-butylpurine is smaller than for 9-i-propylpurine in line with the greater inductive electron-releasing power of the former.

The ratio of the second order rate constants for hydroxide catalysed detritiation of protonated and neutral compounds \(k/k'\) is also shown in Table 1.26. In all cases, this ratio is greater than \(10^6\). It is tempting to ascribe this factor to the inductive effect of a positively charged nitrogen compared with an uncharged nitrogen. However, other effects may be contributing such as resonance stabilization of the ylide or greater S-character of the C-H of the protonated species making it more acidic than in the neutral species (see Table 1.27).
PART 2

TRITON MAGNETIC RESONANCE SPECTRA OF SOME HETEROCYCLIC COMPOUNDS
Proton nuclear magnetic resonance spectroscopy has been applied to a large range of chemical uses and has achieved enormous importance as an analytical technique, especially in organic chemistry. Deuterium NMR spectroscopy has not aroused anything like as much attention because of the inherently lower sensitivity and inferior resolution. Tritium, however, has superior nuclear properties even to the proton in respect of NMR detection. The nuclear properties of the isotopes of hydrogen are shown in Table 2.1. The triton has a spin of \( \frac{1}{2} \) and its relative sensitivity at constant field is higher than that of the proton or of any other nucleus. Its resonance frequency is also higher than that of any other nucleus and is sufficiently different from that of the proton to give first order splittings in triton spectra, allowing the spectra to be readily interpreted. The radioactivity of tritium has discouraged the development of triton magnetic resonance - a compound with one hydrogen completely replaced with tritium has a specific activity of 29,120 Ci/mole [1]. However, use of compounds with low isotopic abundance coupled with instrumentation which gives enhanced signal/noise ratio largely decreases radiological hazards.

Tiers, Brown, Jackson and Lahr [145] obtained a high resolution triton NMR spectra of neat ethylbenzene-1,2-T₂ (ca. 1 atom per cent tritium abundance) by operating at ca. 8800 gauss and 40,000 MHz. The sample contained 10
curies of activity in 300 μl and so was undergoing considerable self-radiolysis. The possibility of pressure building up and bursting the sealed sample tube was then very real. The potential contamination arising in this way or from accidental breakage was emphasized. The technique appeared highly hazardous. Bloxsidge, Elvidge, Jones and Evans [146] used a microbulb sample tube assembly to reduce the amount of radioactive sample to 30 μl and by computerised spectrum accumulation succeeded in improving the limit of detection. A spectrum was obtained with as little as 8 m Ci of radioactivity in the microbulb. Spectra were obtained for a selection of compounds including sodium acetate, glucose, thymidine and uridine. The use of triton magnetic resonance spectroscopy in the study of in situ self-radiolysis was illustrated for uridine.

Other advantages of this technique included the determination of the position and distribution of the label in tritiated compounds by a non-destructive analytical method, especially useful in biogenetic and mechanistic studies. The distribution of deuterium in deuteriated organic material by proton magnetic resonance requires a very high isotopic abundance. A similar determination of the distribution of tritium in a molecule by proton magnetic resonance is possible in principle but because of difficulties caused by the higher cost and by self-radiolysis of compounds with very high tritium isotopic content, triton magnetic resonance is preferred.
The aim of this work was to synthesize tritiated heterocyclic compounds of sufficient isotopic abundance to give triton magnetic resonance spectra and to compile accurate chemical shifts and coupling constants for comparison with proton magnetic resonance data, obtained under comparable conditions.

Table 2.1. Nuclear properties of the isotopes of hydrogen [143,144].

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Spin</th>
<th>NMR frequency (MHz) for 10 kgauss field</th>
<th>% Natural abundance</th>
<th>Rel. Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (1H)</td>
<td>½</td>
<td>42.577</td>
<td>99.9844</td>
<td>1.000</td>
</tr>
<tr>
<td>D (2H)</td>
<td>1</td>
<td>6.536</td>
<td>1.56 x 10^2</td>
<td>9.64 x 10^3</td>
</tr>
<tr>
<td>T (3H)</td>
<td>¼</td>
<td>45.414</td>
<td>&lt; 10^-16</td>
<td>1.21</td>
</tr>
</tbody>
</table>
Equipment

A Perkin-Elmer R.10 spectrometer, operating at 14,100 gauss, with a 64.0 MHz oscillator, and equipped with a Digico computer was used to obtain triton magnetic resonance spectra. The sample temperature was 33.5°.

The micro-cell assembly has been described previously [146] and contains 30-35 μl of sample solution which is filled using a 'Hamilton' 100 μl syringe. The microbulb is then sealed, care being taken that the long stem is not bent during sealing so that it fits the 'Teflon' holder (Figure 2.1) and will slide easily into the standard NMR tube. The microbulb is supported by its stem in a friction grip 'Teflon' chuck. Air bubbles introduced during the loading of the microbulb may be removed to the stem by shaking the bulb. The microbulb with its 'Teflon' holder can be positioned in a standard NMR tube with the aid of a removable rod which screws into the 'Teflon' holder. Carbon tetrachloride is added to the NMR tube so that the space around and below the bulb is filled, thus minimizing wobble when the NMR tube is spun in the spectrometer probe. The microbulb is positioned at a predetermined optimum distance from the bottom of the NMR tube (26 mm) and the positioning rod is unscrewed and removed.

Air in the vicinity of the NMR tube was sucked through liquid scintillator (NE 250, Nuclear Enterprises Ltd.) which was assayed for tritium at intervals as a safety precaution.
FIGURE 2.1 MICRO-CELL ASSEMBLY

- INSERTING ROD
- TEFGLON HOLDER
- MICRO-BULB
- NMR TUBE
- CCl₄
Materials

Imidazole, Benzoxazole, Benzothiazole, Benzoselenazole and Purine were purchased commercially and purified by recrystallization, vacuum distillation or vacuum sublimation. 1-methylbenzimidazole \([43]\), Quinoline-N-oxide, isoquinoline-N-oxide, pyridine-N-oxide and 4-methylpyridine-N-oxide \([147]\) were synthesized by known methods. 1,3-dimethylbenzimidazolium bromide was synthesized as outlined in Part 1. THO (50 Ci/ml) was obtained from the Radiochemical Centre.

Synthesis of Labelled Compounds

All compounds were labelled by simple base catalysed exchange in the microbulb using THO (50 Ci/ml) as the tritium source. The reaction conditions were determined in advance using \(\text{D}_2\text{O}\) and following the disappearance of the exchanging C-H by proton magnetic resonance, and are presented in Table 2.2.
Table 2.2. Preparation of the labelled heterocyclic compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mole Fraction of Compound</th>
<th>Vol. of THO(μl)</th>
<th>Solvent</th>
<th>Time (hrs)</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidazole-2-T</td>
<td>0.21</td>
<td>25</td>
<td>THO</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>1-methyl-benzimidazole-2-T</td>
<td>0.15</td>
<td>15</td>
<td>THO-DMSO</td>
<td>1</td>
<td>85</td>
</tr>
<tr>
<td>1,3-dimethylbenzimidiazolium-2-T Bromide</td>
<td>0.05</td>
<td>35</td>
<td>THO</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Benzoxazole-2-T</td>
<td>0.14</td>
<td>12</td>
<td>THO-DMSO-NaOH</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Benzothiazole-2-T</td>
<td>0.14</td>
<td>10</td>
<td>THO-DMSO-NaOH</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Benzoselenazazole-2-T</td>
<td>0.10</td>
<td>10</td>
<td>THO-DMSO-NaOH</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Quinoline-N-oxide-2-T</td>
<td></td>
<td>25</td>
<td>THC-NaOH</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>Pyridine,N-oxide-3,4,5,6-D4-2-T</td>
<td>0.19</td>
<td>15</td>
<td>THO-NaOH</td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>iso-Quinoline-N-oxide-1,3-T2</td>
<td>0.05</td>
<td>25</td>
<td>THO-NaOH</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>4-methyl-pyridine-N-oxide-2-T</td>
<td>0.10</td>
<td>15</td>
<td>THO-NaOH</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>Purine-8-T</td>
<td>0.07</td>
<td>20</td>
<td>THO</td>
<td>24</td>
<td>50</td>
</tr>
</tbody>
</table>
2.3 RESULTS

Referencing

Tritiated water was used as a reference standard for the compounds considered here. It has been pointed out [146] that water is not an ideal standard because of the temperature and solute concentration - dependence of its chemical shift. Hence the accumulated tritium peak was first referenced to the internal THO peak from the tritiated water which was the source of label, and in turn this was referenced to an internal THO sample (50 Ci/ml) in another microbulb assembly. The external THO and internal THO were scanned successively a number of times by interchanging of samples and consistently reproducible results were obtained. The triton magnetic resonance signal from THO is a sharp singlet, exchange being sufficiently fast to decouple triton-proton splitting. The external THO peak broadened considerably over a period of 12 months caused presumably by the various products of self-radioysis of THO. Triton and proton chemical shifts reported here are give on the $\delta$ scale in ppm, measured from THO at $\delta = 0$.

Spectra of Tritiated heterocyclic compounds

The compounds studied (1-11) are listed in Table 2.3 along with specific activity, mole fraction, triton and proton chemical shift, and isotopic abundance at the position considered. The reported values [153] of other compounds (12-22) are also given in Table 2.3. The proton chemical shift was obtained by scanning the tritiated sample again
using external THO, as a reference. The specific activity (and isotopic abundance) were obtained by assuming that the tritium present was statistically distributed between all exchangeable hydrogens in the compound (usually one or two) and in the tritiated water (two exchanging sites). A typical $^3$H spectrum of benzoeselenazole-2-T is shown in Figure 2.2.
FIGURE 2.2  $^1H$ AND $^3H$ SPECTRA OF BENZOSELENAZOLE-2-T.
Table 2.3. Triton and proton chemical shifts for some heterocyclic compounds (1-11) and other compounds (12-22) [153].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Specific Activity (Ci/mM)</th>
<th>Isotopic Abundance (%)</th>
<th>δ (T) (PPM)</th>
<th>δ (H) (PPM)</th>
<th>δ(T)/δ(H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1). Benzose-nlenazole-2-T</td>
<td>0.343</td>
<td>1.18</td>
<td>5.35</td>
<td>5.34</td>
<td>1.001</td>
</tr>
<tr>
<td>(2). Benzothiazole-2-T</td>
<td>0.319</td>
<td>1.10</td>
<td>4.39</td>
<td>4.36</td>
<td>1.005</td>
</tr>
<tr>
<td>(3). Benzoxazole-2-T</td>
<td>0.324</td>
<td>1.11</td>
<td>3.62</td>
<td>3.67</td>
<td>1.012</td>
</tr>
<tr>
<td>(4). Quinoline-N-oxide-2-T</td>
<td>0.439</td>
<td>1.51</td>
<td>3.47</td>
<td>3.43</td>
<td>1.012</td>
</tr>
<tr>
<td>(5). Pyridine-N-oxide-3,4,5-6-D₄-2-T</td>
<td>0.402</td>
<td>1.38</td>
<td>3.44</td>
<td>3.40</td>
<td>1.011</td>
</tr>
<tr>
<td>(6). Isoquinoline-N-oxide-1,3-T₂</td>
<td>0.866</td>
<td>1.49</td>
<td>3.33</td>
<td>3.30</td>
<td>1.009</td>
</tr>
<tr>
<td>(7). 4-methylpyridine-N-oxide-2-T</td>
<td>0.426</td>
<td>1.46</td>
<td>3.32</td>
<td>3.29</td>
<td>1.009</td>
</tr>
<tr>
<td>(8). Purine-8,9-T₂</td>
<td>0.837</td>
<td>1.44</td>
<td>3.09</td>
<td>3.07</td>
<td>1.006</td>
</tr>
<tr>
<td>(9). 1-Methylbenzimidazole-2-T</td>
<td>0.404</td>
<td>1.39</td>
<td>3.06</td>
<td>2.98</td>
<td>1.025</td>
</tr>
<tr>
<td>(10). 1,3-dimethylbenzimidazolium-2-T-bromide</td>
<td>0.438</td>
<td>1.50</td>
<td>4.31</td>
<td>4.36</td>
<td>0.990</td>
</tr>
<tr>
<td>(11). Imidazole-1,2-T₂</td>
<td>0.710</td>
<td>1.22</td>
<td>2.58</td>
<td>2.60</td>
<td>0.992</td>
</tr>
<tr>
<td>Compound</td>
<td>δ (T) (PPM)</td>
<td>δ (H) (PPM)</td>
<td>δ (T)/δ (H)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------------------------------------------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone-(methyl-T)</td>
<td>-2.63</td>
<td>-2.54</td>
<td>1.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Picoline-(methyl-T)</td>
<td>-2.58</td>
<td>-2.54</td>
<td>0.996</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid-(methyl-T)</td>
<td>-2.77</td>
<td>-2.72</td>
<td>1.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetonitrile-(methyl-T)</td>
<td>-2.25</td>
<td>-2.22</td>
<td>1.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl cyanide-1-T</td>
<td>-2.20</td>
<td>-2.22</td>
<td>0.990</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethylsulphoxide-(methyl-T)</td>
<td>-1.70</td>
<td>1.61</td>
<td>1.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Propyn-1-ol-3-T</td>
<td>-1.68</td>
<td>-167</td>
<td>1.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium malonate-(methylene-T)</td>
<td>-1.39</td>
<td>-1.42</td>
<td>1.079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitromethane-(methyl-T)</td>
<td>-0.01</td>
<td>0.00</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Propen-1-ol-3-T</td>
<td>0.44</td>
<td>0.43</td>
<td>1.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylresorcinol-4-T</td>
<td>1.45</td>
<td>1.53</td>
<td>0.948</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malonitrile-(methylene-T)</td>
<td>-0.75</td>
<td>-0.76</td>
<td>0.986</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4. DISCUSSION

The resonant frequencies at a given field strength of any nucleus depend directly on the magnetogyric ratio of that nucleus. The frequency of radiation $\gamma_x$ necessary to induce transitions of nucleus $x$ from one spin state to another is given by

$$\gamma_x = \gamma_x \frac{B_0}{2\pi}$$

(2.1)

where $\gamma_x$ is the gyromagnetic ratio of nucleus $x$, and is a constant for each particular nucleus; $B_0$ is the magnetic field strength. The gyromagnetic ratio can be written

$$\gamma_x = \frac{g \beta_n}{\hbar/2\pi}$$

(2.2)

where $g_x$ is a constant characteristic of each nucleus; it is not predictable and must be measured experimentally, $g$ for $^1H = 5.585$; $\beta_n$ is the nuclear magneton and is a constant for all nuclei; $\hbar$ is Planck's constant. The gyromagnetic ratio for each magnetic nucleus has been measured and so allows prediction of the resonance frequencies for a given magnetic field strength.

Experimentally

$$\gamma_T/\gamma_H = 1.066636 \pm 0.00001 \ [148,149,150].$$

The resonance frequency of a triton in a particular molecule is given by

$$\gamma_T = \gamma_T \frac{B_0 (1 - \sigma_T)}{2\pi}$$

(2.3)

where $\sigma_T =$ screening constant of tritium.
and the chemical shift $\delta(T)$ is defined as

$$
\delta(T) = \frac{\nu_T - \nu_T^\text{ref}}{\nu_T^\text{ref}} \times 10^6 \text{ ppm} \quad (2.4)
$$

where $\nu_T^\text{ref}$ is the triton resonance frequency of external THO. Similarly, the resonance frequency and chemical shift of a hydrogen in the same molecule is given by

$$
\nu_H = \gamma_H B_0 \left(1 - \sigma_H\right) \quad (2.5)
$$

and

$$
\delta(H) = \nu_H - \nu_H^\text{ref} \times 10^6 \text{ ppm} \quad (2.6)
$$

where $\sigma_H$ is the screening constant of hydrogen in the particular molecular environment and $\nu_H^\text{ref}$ is the proton resonance frequency of external THO.

Substituting (2.3) in (2.4) gives

$$
\delta(T) = \frac{\gamma_T B_0 (1 - \sigma_T) - \gamma_T B_0 (1 - \sigma_T')}{2\pi} \quad (2.7)
$$

where $\sigma_T'$ is the screening constant of T in the reference THO. Substituting (2.5) in (2.6) gives

$$
\delta(H) = \frac{(\sigma_H' - \sigma_H)}{1 - \sigma_H'} \quad (2.9)
$$

where $\sigma_H'$ is the screening constant of H in the reference THO.
Thus \( \frac{\sigma(T)}{\sigma(H)} = \frac{(\sigma_T' - \sigma_T)(1 - \sigma_H')}{(1 - \sigma_T')(\sigma_H' - \sigma_H)} \) (2.10)

If \( \sigma_T = \sigma_H \) and \( \sigma_T' = \sigma_H' \), then

\[
\frac{\sigma(T)}{\sigma(H)} = 1
\] (2.11)

The condition imposed involves the assumption that in a given compound the triton nucleus and the proton nucleus in the same position would be equally shielded. A plot of \( \sigma(T) \) versus \( \sigma(H) \) should give a straight line of slope unity and zero intercept and this is shown in Figure (2.3). (Compounds are numbered as in Table 2.3). The slope of 1.00 observed and the zero intercept justify the assumption that \( \sigma_T = \sigma_H \) and \( \sigma_T' = \sigma_H' \). A similar correlation has been found by Price [151] between \(^{14}\text{N}\) and \(^{15}\text{N}\) chemical shifts (reproduced in Figure 2.4) and by Russian workers between \(^{117}\text{Sn}\) and \(^{119}\text{Sn}\) chemical shifts [152] (reproduced in Figure 2.5). The importance of the simple correlation between triton and proton chemical shifts lies in the fact that one can use the enormous compilation of proton chemical shift data in the literature for the prediction and assignment of triton magnetic resonance spectra.

Quantum mechanical expressions for the spin-spin coupling constants between two nuclei show a direct proportionality between this and the gyromagnetic ratios of the nuclei [144]. Thus

\[
\frac{J_{HT}}{J_{HH}} = \frac{\gamma_H\gamma_T'/\gamma_H\gamma_H} = \frac{\gamma_T/\gamma_H} = 1.066636
\]
FIGURE 2.3 $^3\text{H}$ AND $^1\text{H}$ CHEMICAL SHIFTS (PPM RELATIVE TO THO).
FIGURE 2.4 PLOT OF $^{14}\text{N}$ VERSUS $^{15}\text{N}$ CHEMICAL SHIFTS $\times 100$ DOWNFIELD FROM AMMONIA.
FIGURE 2.5 PLOT OF $^{117}\text{Sn}$ VERSUS $^{119}\text{Sn}$ CHEMICAL SHIFTS. (REFERENCE $\text{Me}_4\text{Sn}$.)
where $J_{HT}$ and $J_{HH}$ are the spin-spin coupling constants between proton and triton and proton and proton, respectively. Of the compounds studied, the triton was not spin-coupled with any adjacent hydrogen, except in the case of quinoline-N-oxide-2-T (doublet), isoquinoline-N-oxide 1,3-T$_2$ (broad multiplets) and 4-methylpyridine-N-oxide-2-T (doublet). The measured H-T coupling constants are presented in Table 2.4 along with the analogous H-H coupling constants (where available), and these are compared with other literature [153] values in Table 2.5 for coupling constants of other compounds. As expected, the ratio of $J_{HT}$ to $J_{HH}$ is near 1.066.
### Table 2.4. H-T coupling constants (Hz)

| Compound                        | \( |J(H_T)| \) | \( |J(H_H)| \) | \( \frac{|J(H_T)|}{|J(H_H)|} \) |
|--------------------------------|---------|---------|----------------|
| Quinoline-N-oxide-2-T          | (2,3)\( \approx \) 5.6 | 5.4     | 1.04           |
| 4-Methylpyridine-N-oxide-2-T   | (2,3)\( \approx \) 6.5 | 6.0     | 1.08           |
| Isoquinoline-N-oxide-1,3-T\(_2\) | (3,4)Broad | Multiplet |                |

### Table 2.5. Literature [153] H-T coupling constants (Hz)

**Geminal:**

| Compound                        | \( |J(H_T)| \) | \( |J(H_H)| \) | \( \frac{|J(H_T)|}{|J(H_H)|} \) |
|--------------------------------|---------|---------|----------------|
| Acetone-(methyl-T)              | 15.4    | 14.9[154] | 1.03           |
| Acetic Acid-(methyl-T)          | 15.7    | 14.5[154] | 1.08           |
| Acetonitrile-(methyl-T)         | 17.1    | 15.2[155] | 1.03           |
| Malonitrile-(methyl-T)          | 20.5    | 20.3[156] | 1.01           |

**Vicinal:**

| Compound                        | \( |J(H_T)| \) | \( |J(H_H)| \) | \( \frac{|J(H_T)|}{|J(H_H)|} \) |
|--------------------------------|---------|---------|----------------|
| 2-methylresorcino1-4-T          | \((4,3)=8.0\) | 7.6     | 1.05           |
| 2-Propyl-ol-3-T                 | \((3,1)=2.5\) | 2.4     | 1.04           |
| Ethyl cyanide-1-T               | \((1,2)=8.0\) | 7.5     | 1.07           |
PART 3

REFERENCES


47. O. Fischer, Ber., 38, 370 (1905).
62. R. J. C. Harris, Vacuum, 1, 11 (1951).
81. Ref. 45, p. 283.
100. Ref. 45, p. 314.


123. B. C. Pal, Biochemistry, 1, 558 (1962).


126. Ref. 99, p. 446.


