Proton Transfer from Heterocyclic Compounds. Part II.[1] Purine, 9-Alkylpurines, and Imidazo[4,5-b]pyridine

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The rates of detritiation of [8-³H]purine, 9-isopropyl[8-³H]purine, 9-t-butyl[8-³H]purine, and [2-³H]imidazo-[4,5-b]pyridine have been measured over a pH range at 85°. The bell-shaped rate–pH profiles for purine and imidazo[4,5-b]pyridine are interpreted in terms of rate-determining attack by hydroxide ion on the conjugate acid, giving rise to an ylide intermediate. The rate–pH profiles for the 9-alkylpurines are also consistent with this pathway, with an additional hydroxide-catalysed exchange of the neutral species becoming increasingly important at high pH. The first-mentioned mechanism requires protonation (or partial protonation) of purine and 9-substituted purines at N-7 rather than at N-1, the generally accepted position.

THE purines possess an imidazole ring system similar to that in the previously studied[1] benzimidazoles. However, they are of more interest in that they are precursors of the nucleic acids, and several derivatives exhibit important enzymic activity in the body. Furthermore, simple structural modifications of naturally occurring purines have made available a variety of purine analogues which are potent antagonists of many biological systems.

Isotopic hydrogen exchange at C-8 of purine, first reported by Ts'o and his co-workers,[2] was induced merely by heating in D_2O at 105° for 4 h. The position of exchange was established from the finding that desulphurisation of 8-mercaptopurine with deuteriated Raney nickel gave the same product. Further confirmation came from the unambiguous synthesis[3] of [8-²H]-purine by ring closure of 4,5-diaminopyrimidine with [²H₂]formic acid.

The known lability of the C-8 proton in purine suggests important applications, such as the preparation of labelled nucleosides[4] and the *in vitro* synthesis of tritiated DNA,[5,6] RNA, and polynucleotides.[6,7] Despite the many observations of isotopic hydrogen exchange in purine-containing compounds only the recent work of Tomasz and his co-workers[8] on the rates of detritiation from C-8 of guanosine and adenosine has been concerned with the mechanistic aspects; base-catalysed hydrogen exchange from related heterocyclic systems has recently been reviewed.[9] We report a study of mechanism for isotopic hydrogen exchange from purine and some 9-alkylpurines; imidazo[4,5-b]pyridine was also studied because of its intermediate position between benzimidazole and purine.

EXPERIMENTAL

Materials.—Purine (1a) was obtained commercially. 9-Isopropyl- (1b) and 9-t-butyl- (1c) purines were provided by Professor G. K. Helmkamp. Imidazo[4,5-b]pyridine (2)

was a gift from Dr. Nitya Anand. After purification by vacuum sublimation the compounds had the following m.p.s: (1a) 219°, (1b) 97°, (1c) 118—119°, (2) 143°. Both deuterium oxide (99·8%) and tritiated water (5 Ci ml⁻¹) were obtained commercially and sodium deuterioxide was prepared by a standard procedure.[10]

(1)   (2)
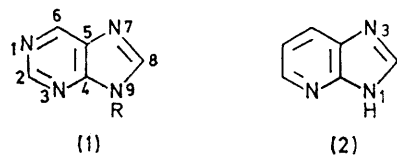
a; R = H
b; R = Pr^i
c; R = Bu^t

All the tritiated compounds were prepared by homogeneous exchange. Thus [8-³H]purine was prepared by maintaining a solution of *ca.* 30 mg of substrate in 0·1 ml of the tritiated water at 85° for 24 h. The solvent was removed by freeze drying, a small amount of water was added to exchange labile hydrogen atoms and the solution was lyophilised again.

[8-²H]Purine was prepared by maintaining a solution of purine (0·5 g) in deuterium oxide (2 ml) at 85° for 18 h. The solvent was removed by freeze-drying and the procedure was repeated. The [8,9-²H₂]purine obtained was dissolved in water (2 ml) in order to remove the nitrogen-bound deuterium and the solution was freeze-dried. The proton n.m.r. spectrum of this compound in deuterium oxide confirmed the absence of a hydrogen atom at C-8.

Kinetics.—For reactions with half-lives of less than 6 h the procedure for following the rates of detritiation at 85° was similar to that used in the benzimidazole study.[1] Samples were withdrawn at intervals, the solutions were freeze-dried, and the collected water was analysed for its tritium content (C_t). The first-order rate constant k_{obs} was then obtained from the slope of the plot of $\log_{10}(C_\infty - C_t)$ against time (t), C_∞ being the tritium content of the water on completion of the reaction.

[1] Part I, J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and J. C. Turner, *J.C.S. Perkin II*, 1973, 432.

[2] M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P.O.P.Ts'o, *J. Amer. Chem. Soc.*, 1964, **86**, 696.

[3] F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, 1964, **29**, 1988.

[4] K. R. Shelton and J. M. Clark, *Biochemistry*, 1967, **6**, 2735.

[5] L. A. Osterman, V. V. Adler, R. Bibilashvily, and Ya. M. Varshavsky, *Biokhimiya*, 1966, **31**, 398; K. R. Shelton and J. M. Clark, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 850.

[6] D. G. Searcy, *Biochim. Biophys. Acta*, 1968, **166**, 360.

[7] R. N. Maslova, E. A. Lesnik, and Ya. M. Varshavsky, *Biochem. Biophys. Res. Comm.*, 1969, **34**, 260.

[8] M. Tomasz, J. Olson, and C. M. Mercado, *Biochemistry*, 1972, **11**, 1235.

[9] J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *Adv. Heterocyclic Chem.*, 1973, **16**, in the press.

[10] E. A. Halevi and F. A. Long, *J. Amer. Chem. Soc.*, 1961, **83**, 2809.

For still slower reactions an initial rate method [11] was employed. Instead of following the increase in the tritium content of the water over the duration of the reaction, we used a higher concentration of labelled substrate and followed the tritium content of the water over only the first 3% of the reaction. The familiar equation (i) then reduces

$$k_{obs} = \{\ln [C_\infty/(C_\infty - C_t)]\}/t \qquad \text{(i)}$$

to equation (ii). The tritium content of the water in this

$$k_{obs} = C_t/C_\infty t = k'/C_\infty \qquad \text{(ii)}$$

case increases linearly with time and the zero-order rate constant (k') obtained from the plot of C_t against t was converted into a first-order rate constant by dividing by the total radioactivity of the substrate (C_∞) in an un-separated sample. At the low concentrations of substrate employed ($<10^{-5}$M) the efficiency of counting such a sample was the same as that for the same volume of tritiated water. The constancy of the radioactive content of unseparated samples over the duration of the experiment showed that losses due to evaporation were negligible. As the initial rate method is very sensitive to trace impurities it was customary to check occasionally that the rate constants obtained by this method agreed with those obtained by the conventional method; this was always found to be the case.

The dedeuteriation of a 0·2M-solution of [8-²H]purine at 85° in H_2O was followed by n.m.r. spectroscopy. The rate of appearance of the 8-H peak was measured and the first-order rate constant obtained from a plot of log{[8-H/(6-H + 2-H)]$_\infty$ − [8-H/(6-H + 2-H)]$_t$} vs. t, the two terms in square brackets representing the ratio of the 8-H peak height to the sum of the 2-H and 6-H peak heights (used as non-exchanging internal standards) at equilibrium and at time t, respectively. Peak heights were preferred to integrated peak areas because of the relatively low signal-to-noise ratio encountered in this work. The validity of this procedure was checked by measuring the relative peak heights of the 8-H of purine solutions of various concentrations (0·02—0·2M).

The deuteriation of a 0·2M-solution of purine at 85° in deuterium oxide was followed by measuring the rate of disappearance of the 8-H peak. Plots of log[8-H/(6-H + 2-H)] against t were strictly linear over two reaction half-lives and the observed first-order rate constant was obtained from the slope ($-k_{obs}/2·303$).

For kinetic measurements, acetate and formate buffers were used and it was assumed that the pH of these solutions did not change appreciably [12] on going from 20 to 85 °C. Hydrochloric acid and sodium hydroxide were used to prepare solutions of low and high pH, respectively. The value of K_w at 85° is 12·50 (ref. 13) and any volume change in going from 20 to 85° was taken as being insignificant.

RESULTS AND DISCUSSION

In the pH range 2—12 both purine and imidazo[4,5-b]-pyridine, like benzimidazole, can exist in three forms, the monoprotonated (BH$_2$$^+$), the neutral (BH), and the monoanionic (B$^-$). If the mechanism of isotopic exchange is the same as for benzimidazole, namely rate-determining hydroxide ion attack on BH$_2$$^+$, then a bell-shaped rate–pH profile would be expected for both

[11] A. J. Kresge and Y. Chiang, J. Amer. Chem. Soc., 1961, 83, 2877; 1967, 89, 4411.

these compounds. This is borne out by the experimental findings (Table 1 and Figure 1).

TABLE 1

Rate–pH data for [8-³H]purine (1a), 9-isopropyl[8-³H]-purine (1b), 9-t-butyl[8-³H]purine (1c), and [2-³H]-imidazo[4,5-b]pyridine (2)

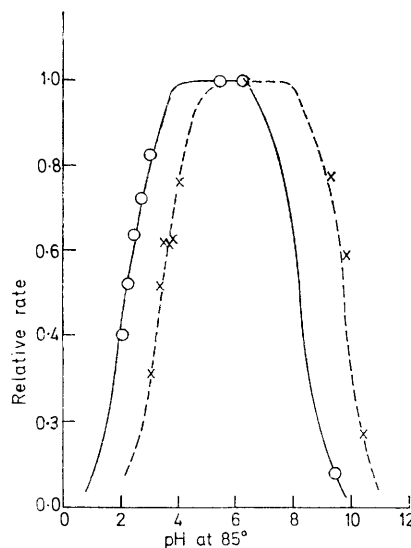| pH (at 85°) | $10^6 k_{obs}/s^{-1}$ | | | |
	(1a)	(1b)	(1c)	(2)
2·08			9·25	
2·13	13·1	34·5		
2·31	16·9	43·5	18·8	
2·51	20·6	54·9	21·4	
2·72	23·4	70·0	25·9	
2·84				28·4
3·00		34·0		
3·10				49·4
3·12	26·6	82·5		
3·38				77·5
3·50			42·5	
3·61				100
3·64				99·5
3·91				99·9
4·18				124
5·45	31·8			
6·25	32·0	104	55·3	162
9·47				126
9·50	2·71			
10·01				75·0
10·50		256	87·5	27·0
10·80		369	107	
11·20		735	176	
11·32		835	244	
11·40		973	272	
11·50		1170	304	

FIGURE 1 Rate–pH profile for [8-³H]purine (○), and [2-³H]-imidazo[4,5-b]pyridine (×) at 85°; the calculated lines (——, - - -) are drawn from values given in the text

The relative rate is defined as a fraction of the observed rate in the region where it is pH-independent and the calculated curves were constructed from the previously derived [1] equation (iii), using a trial and error procedure.

Relative rate $= K_a/\{K_a + (K_a K_a'/[H^+]) + [H^+]\}$ (iii)

[12] R. P. Bell, ' The Proton in Chemistry,' Methuen, London, 1959, p. 65.
[13] H. L. Clever, J. Chem. Educ., 1968, 45, 231.

The relatively small number of experimental points does not justify a more sophisticated analysis. Values of pK_a (protonation on nitrogen) and pK_a' (ionisation of N-H) at 25° for purine are 2·60 and 8·94, respectively,[14] whereas only a pK_a value of 3·92 at 25° for imidazo-[4,5-b]pyridine is known.[15] Use of the semi-empirical Perrin equation [15] gives values at 85° of 2·3 and 7·6 for purine and 3·5 for imidazo[4,5-b]pyridine, close to the values which were found to give the best fit to the experimental results: 2·3 and 8·1 for purine, and 3·5 for imidazo[4,5-b]pyridine. The calculated pK_a' value for the latter compound, giving the best fit, was 9·95.

The 9-alkylpurines are analogous to the 1-alkyl-benzimidazoles, but whereas the rate of detritiation of 1-methyl[2-³H]benzimidazole[1] is pH-independent over the range 6·25—11·50, the corresponding rates for the 9-isopropyl- and 9-t-butyl-purines show a dramatic increase. The values of k_{obs} for these two compounds at pH 11·50 are greater by factors of eleven and nearly six, respectively, than at pH 6·25 (Figure 2). This suggests that an additional mechanism, most probably
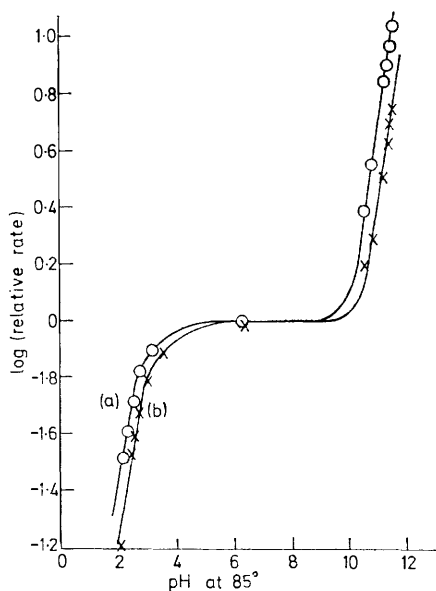
FIGURE 2 Rate-pH profiles for (a) 9-isopropyl[8-³H]purine, and (b) 9-t-butyl[8-³H]purine at 85°; the calculated lines (———) are drawn from values given in the text

involving rate-determining attack by the hydroxide ion on the neutral molecule, comes into operation at high pH [equation (iv)]. With $[B]_T = [BH^+] + [B]$, and

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

$K_a = [B][H^+]/[BH^+]$, equation (iv) leads to equations (v) and (vi). For $[H^+] \ll K_a$, equation (vii) follows,

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

$$k_{obs} = \frac{kK_w}{K_a + [H^+]} + \frac{k'K_a[OH^-]}{K_a + [H^+]} \quad (vi)$$

$$k_{obs} = kK_w/K_a + k'[OH^-] \quad (vii)$$

so that a plot of k_{obs} against $[OH^-]$ at high pH should give a straight line of slope k' and intercept kK_w/K_a. This is shown to be the case in Figure 3, the values of k'
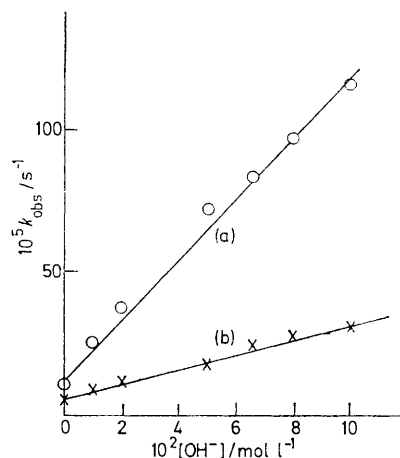
FIGURE 3 Rates of detritiation of (a) 9-isopropyl[8-³H]purine, and (b) 9-t-butyl[8-³H]purine at 85° in aqueous sodium hydroxide

being $1·07 \times 10^{-2}$ and $2·54 \times 10^{-3}$ l mol⁻¹ s⁻¹ for the 9-isopropyl- and 9-t-butyl-purine, respectively. The calculated curves in the rate-pH profiles of these two compounds (Figure 2) were constructed by use of equation (vi) and these two values of k'. The pK_a values found to give the best fit were 2·5 (9-isopropyl) and 2·8 (9-t-butyl); although no experimental values are available these are, in comparison with those for purine, reasonable and consistent with the acid-weakening effects of adjacent alkyl groups.

The difference in behaviour of the 1-alkylbenz-imidazoles and the 9-alkylpurines in the region of high pH can be ascribed to the lower pK_a and pK_a' values of the latter. Extension of the studies on 1-alkylbenz-imidazoles to highly basic media should make it possible to observe isotopic hydrogen exchange via the second pathway.

Further support for the incursion of this second pathway at high pH for both alkylpurines comes from

TABLE 2

Rate constants for detritiation of 9-isopropyl[8-³H]purine in sodium deuterioxide–deuterium oxide

[OD⁻]/mol l⁻¹	k_{obs}/s⁻¹	$10^2 k_{OD}^T$/l mol⁻¹ s⁻¹
0·110	$1·60 \times 10^{-3}$	1·45
0·074	$1·11 \times 10^{-3}$	1·50
0·052	$8·10 \times 10^{-4}$	1·55

a study of secondary isotope effects. The rates of detritiation of 9-isopropyl[8-³H]purine were measured in sodium deuterioxide–deuterium oxide (Table 2) as well

[14] T. M. Marshall and E. Grunwald, J. Amer. Chem. Soc., 1969, 91, 4541.
[15] D. D. Perrin, J. Chem. Soc., 1965, 5590.

as in sodium hydroxide–water, and the ratio of the second-order rate constants ($k_{OD^-}^T/k_{OH^-}^T$) was found to be $1·50 \times 10^{-2}/1·07 \times 10^{-2} = 1·40$. Similar values have been obtained for other uncharged carbon acids where the ionisation of a carbon–hydrogen bond is known to be rate-determining, *e.g.* acetone [16] (1·47), phenylacetylene [10] (1·34), nitroethane [17] (1·39), and 1,4-dicyanobut-2-ene [18] (1·38).

The primary hydrogen isotope effects obtained for purine (Table 3), even if we allow for the rather high

TABLE 3

Primary kinetic isotope effects for hydrogen exchange at the 8-position of purine at 85°

| Substrate	Solvent	$10^5 k_{obs}/s^{-1}$	k_H/k_T [a]	k_H/k_D [a,b]
[8-¹H]Purine	D₂O	10·7		
[8-²H]Purine	H₂O	6·13		$2·0 \pm 0·3$
[8-³H]Purine	H₂O	3·20		
[8-³H]Purine	D₂O	2·76	$3·9 \pm 0·3$	

[a] Measured in D₂O. [b] k_D Is calculated from the solvent isotope effect (obtained from the rates of exchange of [8-³H]-purine in H₂O and D₂O) and the rate of dedeuteriation in H₂O.

temperature used, are considerably lower than the maximum permitted values. Although relatively few investigations have been made this seems a common feature of proton-transfer reactions involving hetero-cycles: k_H/k_T values of 2·7 and 5·8 at 30° for hydroxide-catalysed isotope exchange at C-2 of 3-benzyl-4,5-dimethylthiazolium bromide and 3-benzylbenzothia-zolium bromide,[19] respectively, have been reported, and k_H/k_T is 5·2 at 28° for 3-methylthiazolium iodide.[20] These isotope effects, like the reported Brönsted β values [21] of close to unity, suggest that the transition states for these reactions resemble the products more closely than the reactants.

Finally, there are other instances of reactions of this kind which proceed by two mechanisms, one involving an equilibrium protonation on nitrogen followed by a rate-determining carbon–hydrogen ionisation and oper-ative at intermediate pH, and another a base-induced proton abstraction from the neutral molecule which only becomes important at high pH. This is so for the deuteriation of the 4(5)-position of imidazole [22] and the 2-position of thiazole [23] as well as for the same position in both pyridine [24] and 4-alkylaminopyridines.[25]

The single remaining factor concerns the site of

protonation. In the Scheme it is assumed that proton-ation occurs at the adjacent basic nitrogen atom giving rise to an ylide intermediate which is then reprotonated in a fast step. Although the site of protonation in
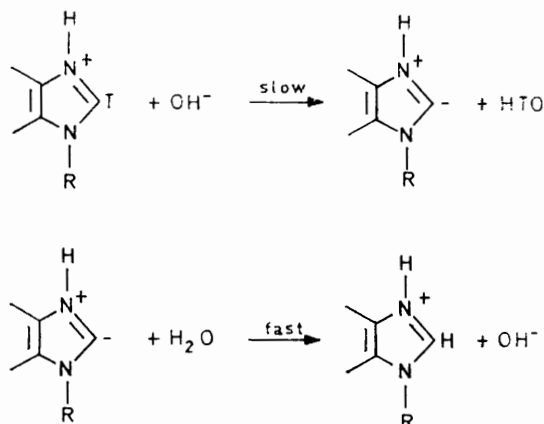
SCHEME

benzimidazole is firmly established as N-3 it is not known for imidazo[4,5-*b*]pyridine. As far as purine is concerned, several studies identify N-1 as the site of protonation. Albert,[26] for example, has argued in favour of this site from a study of the basic pK_a data for 2- and 6-methylthiopurines. In support of this con-clusion can be cited the differences in the u.v. spectra of the cations of 7- and 9-methylpurines [27] and the much greater base-weakening effect evident in 6-trifluoro-methylpurine (pK_a <0) as compared with 8-trifluoro-methylpurine [28] (pK_a 1·0).

On the other hand, Read and Goldstein,[29] from a study of the variation of ^{13}C–H coupling constants with pH, which probably provides the least ambiguous basis for a postulated pattern of protonation, conclude that purine is partially protonated at N-1, N-3, and N-7, the percentage protonations being 47, 24, and 29, re-spectively. Similarly Pugmire and Grant [30] found that the change in ^{13}C chemical shift on going from neutral to protonated purine was consistent with the presence of an equilibrium mixture of the protonated forms. *X*-Ray studies [31] on purine show that it is the N-7 position that is protonated in the crystalline salt. We

[16] J. R. Jones, *Trans. Faraday Soc.*, 1965, **61**, 95.

[17] S. H. Maron and V. K. La Mer, *J. Amer. Chem. Soc.*, 1938, **60**, 2558.

[18] E. A. Walters and F. A. Long, *J. Phys. Chem.*, 1971, **93**, 2836.

[19] D. S. Kemp and J. T. O'Brien, *J. Amer. Chem. Soc.*, 1970, **92**, 2554.

[20] W. Hafferl, R. Lundin, and L. L. Ingraham, *Biochemistry*, 1963, **2**, 1298.

[21] P. Haake, L. B. Bauscher, and W. B. Miller, *J. Amer. Chem. Soc.*, 1969, **91**, 1113.

[22] J. D. Vaughan, Z. Mughrabi, and E. Chung Wu, *J. Org. Chem.*, 1970, **35**, 1141.

[23] R. A. Coburn, J. M. Landesberg, D. S. Kemp, and R. A. Olofson, *Tetrahedron*, 1970, **26**, 685.

[24] J. A. Zoltewicz and C. L. Smith, *J. Amer. Chem. Soc.*, 1967, **89**, 3358.

[25] J. A. Zoltewicz and J. D. Meyer, *Tetrahedron Letters*, 1968, 421.

[26] A. Albert, 'Physical Methods in Heterocyclic Chemistry,' ed. A. R. Katritzky, Academic Press, New York, 1963, vol. 1, p. 50.

[27] A. Bendich, P. J. Russell, jun., and J. J. Fox, *J. Amer. Chem. Soc.*, 1954, **76**, 6073.

[28] A. Bendich, A. Giner-Sorolla, and J. J. Fox, 'The Chemistry and Biology of Purines,' ed. G. E. W. Wolstenholme and C. M. O'Connor, J. and A. Churchill, London, 1957, p. 3.

[29] J. M. Read, jun., and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1965, **87**, 3440.

[30] R. J. Pugmire and D. M. Grant, *J. Amer. Chem. Soc.*, 1971, **93**, 1880.

[31] D. G. Watson, R. M. Sweet, and R. Marsh, *Acta Cryst.*, 1965, **19**, 573.

therefore assume that isotopic exchange in the purines takes place *via* the 7-protonated form. It seems in-

(3)
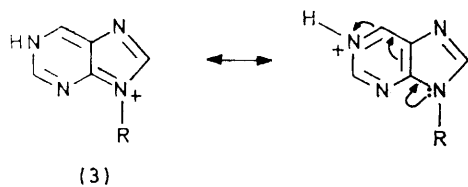
trinsically unlikely that the protonation step which has to precede the exchange steps should involve a basic centre remote from the exchange site; resonance forms such as (3), derived from protonation at N-1 and which could share in the stabilisation of the ylide, are not thought to be major contributors.