Mechanism of Hydrogen–Deuterium Exchange in Hypoxanthines

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The first aromatic proton in hypoxanthines to undergo exchange for deuterium is that at position 8, with the exception of 3-methyl derivatives, which undergo deuteriation first at C-2. Thus rapid deuteriation takes place always in that ring to which a proton is attached to form the monocation. The anions react faster than the neutral molecules; only 1-methylhypoxanthine shows the reverse behaviour. The exchange can be conveniently described by a protonation–deprotonation mechanism, which for anions involves formation of a zwitterion as reactive species. If zwitterion formation is impossible, as in the dianion of hypoxanthine or in the monoanion of its 1-methyl derivative, the exchange is very slow.

THE 2- and 8-H signals in the n.m.r. spectrum of hypoxanthines have recently been assigned unequivocally by means of the nuclear Overhauser effect (NOE).[1] It has thus become possible to identify the sites at which 'spontaneous' deuteriation takes place.[2] In the present study, we have measured the kinetics of H–D exchange under various conditions, in order to elucidate its mechanism.

The exchange of one aromatic proton in hypoxanthines is always much faster than that of the second one (Table 1). The area underneath the n.m.r. signal of the latter remains constant for a short time and thus permits measurement of the rate of the first, rapid exchange by determination of R = (decreasing proton area)/(constant proton area) as a function of time. The rate of the second, slow deuteriation step was measured either by comparison with the area of an N-methyl signal or (in the case of hypoxanthine itself) by use of TSP (sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate) as external standard.

Plots of log R *versus* time yield straight lines, indicating first-order kinetics.[3] The k values of these reactions are indeed independent of substrate concentrations; thus the same value was found for 0·02M- and 0·2M-solutions of various hypoxanthines.

Table 1 shows that in all compounds rapid deuteriation takes place at position 8, regardless of the molecular form of the substrate; only in 3-methyl- and in 3,7-dimethylhypoxanthine, is position 2 involved in the rapid reaction.

We recently concluded from measurements of n.m.r. spectra that only the 3-methyl derivatives form monocations by protonation at N-1. In all other members of the series, the proton becomes attached to the imidazole ring.[4] Thus deuteriation takes place first in that ring which is involved in cation formation. This makes it probable that H–D exchange is initiated by protonation; apparently the positive charge guides attack to the preferred CH group by increasing the acidity of the proton to be detached. It is thus likely that the

TABLE 1

Physical constants and rates of deuteriation of hypoxanthines

Substituents in hypoxanthine	First proton bound at position	pK_a	Monoanion formed at position	pK_b	pK_b'[a]	10^4k_{obs}/s^{-1} 2-H N	2-H A[b]	8-H N	8-H A_1	8-H A_2[b]	ΔE_{act}/kcal mol^{-1} N	ΔE_{act}/kcal mol^{-1} A	Rate constants for first H–D exchange 10^7k_1[c]	Rate constants for first H–D exchange 10^5k_1[d]
	7(9)	2·3	9(7) and 1	8·4	4·5			0·5	2·2	0·05		18·3	2·5	7·0
1-Methyl	7(9)	2·5	9(7)	8·6	*e*	0·015		0·75	*f*				2·4	*e*
3-Methyl	1	2·6	7(9)	8·4	5·0	5·50	38·50	0·04			25·4	25·9	13·7	5·5
7-Methyl	9	2·4	1	9·4	5·6	0·02		3·5	16·5			26·5	14·0	4·1
9-Methyl	7	2·5	1	10·3	6·6	<0·09		1·9	28·9		21·5	28·1	6·2	0·7
1,7-Dimethyl	9	2·1						6·8					5·4	
1,9-Dimethyl	7	2·6						2·3					5·8	
3,7-Dimethyl	1	2·8				9·6							15·4	
7,9-Dimethyl	1	6·2[g]											19·0	380
1,3-Dimethyl	7	6·0											62·5	104

[a] Calculated according to equation (v), from pK_b' = pK_b + pK_a − pK_a''. [b] N = Neutral molecule, A = Anion, A_1 = monoanion, A_2 = dianion. [c] For deuteriation of neutral molecule, $k_1 = k_{obs}K_a/K_w$. [d] For deuteriation of anions, $k_1' = k_{obs}'K_b/K_w$. [e] 1-Methylhypoxanthine does not form a true zwitterion (see Scheme 4). [f] Deuteriation of the anion of 1-methylhypoxanthine (pK 8·6) could not be measured because of decomposition of this purine in alkaline solutions. [g] For these compounds, the values shown are pK_a'' (see Schemes 2 and 3).

[1] F. Bergmann, D. Lichtenberg, and I. Ringel, *J. Magnetic Resonance*, 1972, **6**, 600.

[2] F. Bergmann, D. Lichtenberg, and Z. Neiman, *Chem. Comm.*, 1969, 992; in this publication we reported that the position of deuteriation of 1-methylhypoxanthine could not be assigned unequivocally. By means of NOE, it has now been established that position 8 is involved.[1]

[3] F. Bergmann, D. Lichtenberg, and Z. Neiman, Proceedings of the Second Jerusalem Symposium on 'Quantum Aspects of Heterocyclic Compounds in Chemistry and Biochemistry,' 1970, Israel Academy of Science and Humanity, p. 314.

[4] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, 1972, **10**, 805.

protonation–deprotonation mechanism, proposed previously for other heterocycles,[5-9] is also applicable to purines. This mechanism is exemplified in Scheme 1 for 9-methylhypoxanthine (I). Here, hydroxide ion abstracts a proton from the protonated purine (II) in the rate-determining step [(II) ⟶ (III)]. Thus one obtains the rate equation (i), where k_1 = rate constant for CH

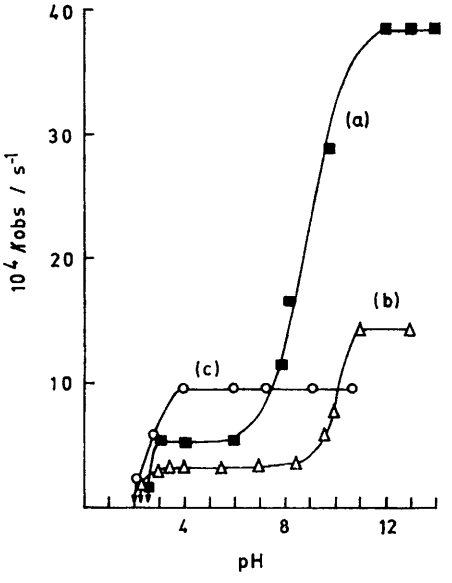
$$v = k_1 K_w[S]/(K_a + [D^+]) \quad \text{(i)}$$

deprotonation (the slow step), K_a = dissociation constant of the conjugate acid, K_w = ion product of water, and [S] = concentration of substrate.

According to equation (i), when $K_a \gg [D^+]$, the rate is directly proportional to [S] and nearly independent of pH. Under these conditions, we obtain equation (ii).

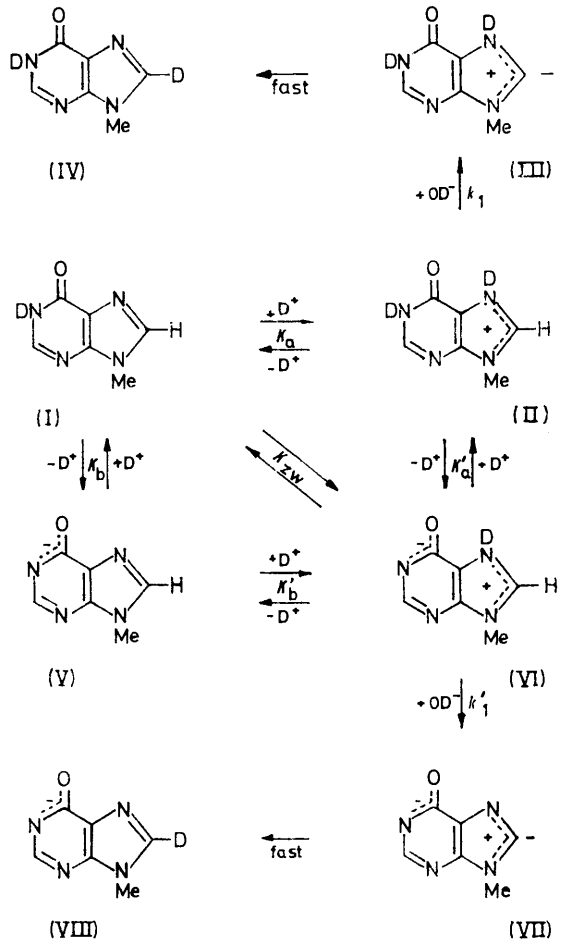
$$k_{obs} = k_1 K_w/K_a \quad \text{(ii)}$$

The Figure shows that the rate of deuteriation is indeed pH-independent over certain pH ranges, in which



Rate constants for H–D exchange as function of pH: (a) 3-methylhypoxanthine (deuteriation at C-2); (b) 7-methylhypoxanthine (deuteriation at C-8); (c) 3,7-dimethylhypoxanthine (deuteriation at C-2)

a given form (neutral molecule or anion) is predominant. However, the reaction velocity changes in those pH regions in which the substrate passes from one form into another (compare Figure with the pK values in Table 1). For hypoxanthine itself, this applies also to the transition monoanion ⟶ dianion (Table 1). Accordingly, in the case of the 1,7-, 1,9-, and 3,7-dimethyl derivatives, which cannot form anions, the rate is constant throughout the pH range 4—10 [Figure, curve (c)]. All rates approach zero for the monocations, *i.e.* when [D$^+$] becomes much larger than the dissociation constant K_a for the process cation ⟶ neutral form.



SCHEME 1

Table 1 shows that the activation energies for the reactions of neutral molecules or anions are 20—30 kcal mol^{-1}. These results support the hypothesis that the same exchange mechanism applies to all molecular forms of hypoxanthines. Furthermore, these values resemble those reported for other heterocycles[6,7] and strengthen the assumption that the same mechanism applies to the latter and to hypoxanthines.

For deuteriation of the anions, the protonation–deprotonation mechanism may be interpreted to mean that, for example, the anion (V) of 9-methylhypoxanthine is converted into the neutral molecule (I) before rupture of the CH bond takes place (Scheme 1). However, this does not explain why in the anions also the rapid H–D exchange always involves the CH group in that ring to which a proton is attached for cation formation. We have recently suggested that the ' active species ' of the anion is not the neutral form (I), but rather the zwitterion (VI) which is in tautomeric equilibrium with (I).[10] Thus

[5] T. M. Harris and J. C. Randall, *Chem. and Ind.*, 1965, 1728.
[6] R. A. Coburn, J. M. Landesberg, D. S. Kemp, and R. A. Olofson, *Tetrahedron*, 1970, **26**, 685.
[7] P. Beak and R. N. Watson, *Tetrahedron*, 1971, **27**, 953.
[8] J. D. Vaughan, Z. Mughrabi, and E. C. Wu, *J. Org. Chem.*, 1970, **35**, 1141.
[9] M. Tomasz, J. Olson, and C. M. Mercado, *Biochemistry*, 1972, **11**, 1235.
[10] F. Bergmann, D. Lichtenberg, and Z. Neiman, *Israel J. Chem.*, 1971, **9**, 199P.

both in the neutral molecule and in the anion, protonation involves the same ring; the path (V) ⟶ (VIII) is mechanistically analogous to (I) ⟶ (IV). In the zwitterion (VI), the 'fixed' positive charge in the imidazole ring facilitates rupture of the C(8)–H bond by hydroxide ion. At pH values where all the substrate is present as anion, we obtain equation (iii), where $k_1' =$

$$k_{obs}' = k_1'K_w/K_b' \qquad \text{(iii)}$$

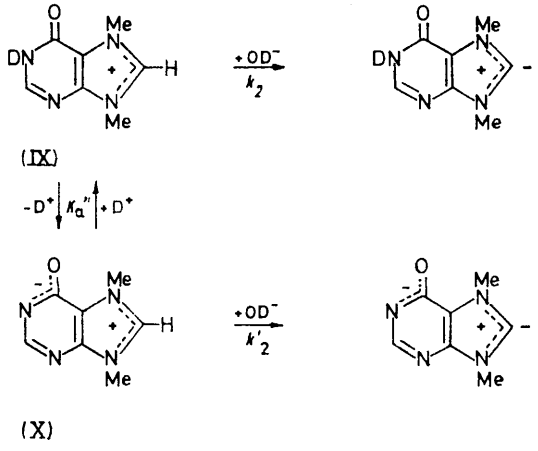
rate constant for deprotonation of the zwitterion (VI) and K_b' = dissociation constant of the latter.

The dissociation constants in Scheme 1 are defined as follows: $K_a = [D^+][(I)]/[(II)]$; $K_a' = [D^+][(VI)]/[(II)]$; $K_b = [D^+][(V)]/[(I)]$; $K_b' = [D^+][(V)]/[(VI)]$. Hence one obtains equations (iv) and (v). K_a and K_b are, to a

$$K_{zw} = [(VI)]/[(I)] = K_b/K_b' = K_a'/K_a \qquad \text{(iv)}$$

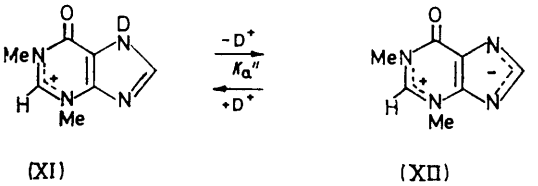
$$K_b' = K_bK_a/K_a' \qquad \text{(v)}$$

good approximation (see later), the basic and acidic dissociation constants of the neutral molecule (Table 1). For K_a' of hypoxanthine and its 7- and 9-methyl derivatives, we may assume close similarity to the dissociation constant K_a'' of the fixed cation 7,9-dimethylhypoxanthinium (IX) (Scheme 2), *i.e.* we can use



SCHEME 2

as a good approximation $K_a'' = [D^+][(X)]/[(IX)]$ for these three compounds. Similarly K_a'' for 1,3-dimethylhypoxanthinium ion [(XI) ⇌ (XII); Scheme 3] serves to replace K_a' of 3-methylhypoxanthine.

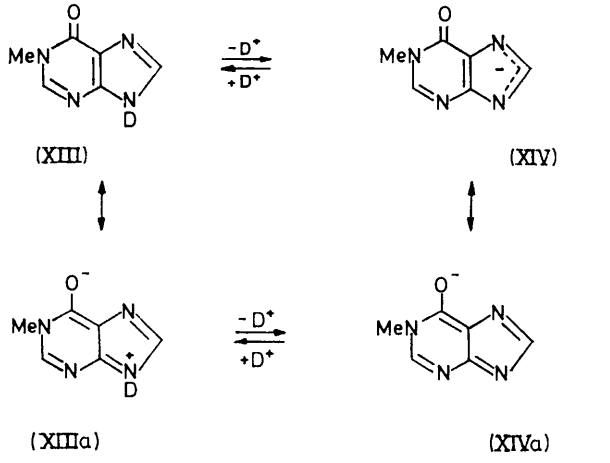


SCHEME 3

These considerations lead to equation (vi) for the observed rate constant of deuteriation for the anions of hypoxanthine and its 7- and 9-methyl derivatives. The values of K_a, K_b, and K_a'' were determined by u.v.

$$k_{obs}' = k_1'K_wK_a''/K_bK_a \qquad \text{(vi)}$$

spectroscopy (see Experimental section) and that of k_{obs}' by n.m.r. measurements. Thus, the values of k_1' could be calculated. Similarly, the values of k_1



SCHEME 4

could be derived from equation (i) (see Table 1). The rates of exchange in neutral molecules and the corresponding anions are connected by equation (vii).

$$k_{obs}/k_{obs}' = k_1K_b/k_1'K_a'' \qquad \text{(vii)}$$

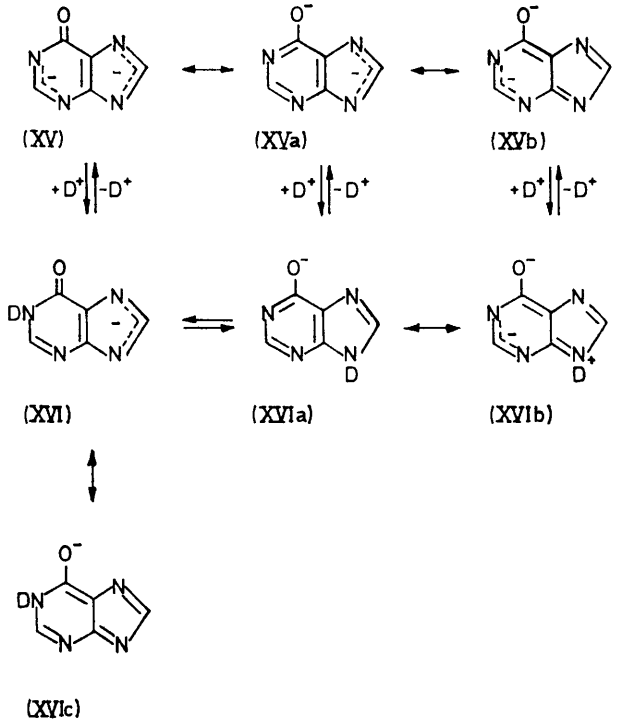
It is now possible to explain the greater H–D exchange rate for the anions. Rupture of a CH bond by hydroxide ion attack would at first sight be expected to be more difficult in the anion than in the neutral molecule, *i.e.* $k_1' < k_1$ (Table 1). However, the Table also shows that $K_a'' \gg K_b$. Therefore, although *N*-(or *O*-)protonations and deprotonations are fast compared with *C*-deprotonation (the 'slow' step), the anions react faster than the neutral forms. Table 1 indicates that all pK_b' values are more than 3 units smaller than the corresponding values of pK_b, *i.e.* the zwitterions contribute less than 0·1% when the anions add a proton to form the neutral molecules.* However, this small concentration of zwitterions represents the 'active species.'

The foregoing considerations lead to a simple explanation of the exceptional behaviour of the 1-methyl derivative. The neutral form (XIII) reacts much faster than the anion [(XIV) ⇌ (XIVa), Scheme 4]. (The exchange in the latter case is very slow and cannot be measured accurately because of decomposition of the substrate at alkaline pH.) In the neutral form (XIII) protonation takes place in the imidazole ring,[4] *i.e.* the ring that is involved also in anion formation. Thus, in contrast to hypoxanthine and its 3-, 7-, and 9-methyl derivatives, where the zwitterion is a tautomer of the

* The small contribution of zwitterions to the tautomeric mixtures of hypoxanthines confirms our previous statement that the pK values in Table 1 faithfully represent the dissociation processes of the neutral molecules.

neutral molecule, the ' zwitterion ' (XIIIa) is a resonance form of the neutral molecule (XIII) and the positive charge near position 8 is too small to facilitate H–D exchange. In fact we were unable to establish unequivocally which position in compound (XIV) is attacked first.

In the dianion of hypoxanthine [(XV) ⟷ (XVa) ⟷ (XVb), Scheme 5], two protonation processes are possible. (*a*) Attachment of a proton to N-1 produces the mono-anion (XVI) ⟷ (XVIc). Here the overall negative charge reduces the rate of attack by OD^-. (*b*) If protonation takes place in the imidazole ring, then the ' zwitterion ' (XVIb) is identical with the mono-anion (XVIa) and thus does not possess a ' fixed '



SCHEME 5

positive charge near position 8; the situation is similar to that in the ' zwitterion ' (XIIIa) of 1-methylhypoxanthine. Thus the adverse effect of the overall negative charge is not eliminated. It is thus understandable that the rate of exchange in the dianion of hypoxanthine is similar to the rate for the slow exchange at C-2 in the anion of 7-methylhypoxanthine or at C-8 in the anion of the 3-methyl isomer (Table 1).

At this stage, it becomes apparent why a reaction mechanism involving only attack by water [11] does not adequately explain the experimental data. (*a*) It would be hard to understand the specific localisation of the exchange processes in each hypoxanthine derivative. (*b*) If the anions in general were to react faster with water than do the neutral molecules, it would be difficult to explain the reverse behaviour of 1-methylhypoxanthine and the very slow exchange in the dianion of hypoxanthine.

In the quaternary compounds (IX) and (XI) the rate of H–D exchange is pH-dependent, as has been demonstrated previously for ' fixed ' cations of other heterocycles.[12,13] Here equation (viii) applies.

$$v = k_2[S][OD^-] \qquad \text{(viii)}$$

For a given pH, $[OD^-]$ is constant. Therefore equation (ix) is obtained where $k_{obs} = k_2[OD^-]$.

$$v = k_{obs}[S] \qquad \text{(ix)}$$

We have determined k_2 at various pH levels; * the values obtained were identical.

Any substitution in the hypoxanthine molecule changes K_a and k_1 in the same direction. The rate, depending on both these factors [see equation (i)], varies less than either of them individually. This is well demonstrated by experiments with 6-mercaptopurines and 6-hydroselenopurines (Table 2). 3-Methylhypoxanthine (pK 2·6) is a stronger base than its thio-analogue (pK 1·8), due to the greater polarisability of the sulphur atom. If this were the only difference between the two derivatives, one would expect the exchange in 3-methylhypoxanthine to be about ten times faster than in the thio-analogue. On the other hand, in the latter, the 2-proton must be more acidic than in the corresponding hypoxanthine because of the greater polarisability of the C(6)=S as compared to the C(6)=O group. This effect should be still more pronounced in the anion. However, Table 2 shows that the rates of 2-H ⟶ 2-D exchange in these two compounds are similar. The exchange in the neutral molecule of 3-methylhypoxanthine is faster than in the thio-analogue [*i.e.* K_a increases more than k_1 when

TABLE 2

2-H ⟶ 2-D exchange rates in some 6-substituted 3-methylpurines

6 Substituent	8 Substituent	pH	Temp. (°C)	Molecular form [a]	$10^4 k_{obs}$/ s^{-1} [b]
OH	H	5	85	N	5·5
SH	H	5	85	N	2·9
OH	H	13	70	A	6·1
SH	H	13	70	A	7·7
OH	H	13	23	A	0·02
SH	H	13	23	A	0·02
SeH	H	13	23	A	0·01
OH	Me	5	85	N	6·8
SH	Me	5	85	N	5·3
OH	Me	13	70	A	16·5
SH	Me	13	70	A	8·9

[a] N = Neutral form; A = anion. [b] In D_2O, at 85°.

* Since the H–D exchange at pH > 3 is too fast to be measured at 85°, we have studied these two reactions at 23° and have obtained the values given in Table 1 by extrapolation to 85° assuming E_{act} = 25 kcal.

[11] G. E. Wright, L. Bauer, and C. L. Bell, *J. Heterocyclic Chem.*, 1966, **3**, 440.
[12] R. A. Olofson, W. R. Thompson, and J. S. Michelman, *J. Amer. Chem. Soc.*, 1964, **86**, 1866.
[13] W. W. Paudler and S. A. Humphrey, *J. Org. Chem.*, 1970, **35**, 3467.

passing from C(6)=O to C(6)=S] whereas the reverse is true for the anions.

Deuteriation in the anion of 6-hydroseleno-3-methylpurine is slower even than in the analogous hypoxanthine, indicating that K_b' increases more than k_1'. This shows that the C(6)=Se group has a large influence on zwitterion formation.

The exchange rates in 3-methylhypoxanthine and its 6-thio-analogue are enhanced by a methyl substituent at position 8, regardless of the molecular form involved (Table 2).

EXPERIMENTAL

The synthesis of most of the hypoxanthines is given in ref. 4. The following compounds were prepared according to known procedures: 3,8-dimethylhypoxanthine,[14] 6-mercapto-3-methylpurine [15] and its 8-methyl derivative,[16] 6-hydroseleno-3-methylpurine,[17] and 7,9-dimethylhypoxanthinium betaine.[18]

1,3-Dimethylhypoxanthinium betaine was prepared according to Bergmann *et al.*[19] In the original publication, the product was erroneously described as 3-methyl-6-methoxypurine, m.p. 233—235°, but it was found later that the latter has entirely different properties and shows m.p. 162—163°.[20] Unequivocal identification of the 1,3-dimethyl derivative (XI) is based on its n.m.r. spectrum: δ (D_2O; 70°; pH 7·0) 9·49 (2-H), 8·34 (8-H), 3·82 (1-Me), and 4·14 (3-Me). Upon dissolution of (XI) in D_2O, the 2-proton is exchanged instantaneously. Therefore the 2-H band was located by dissolving the compound in H_2O. The low-field position of the 2-H signal at neutral pH indicates the presence of a fixed cationic group; a similar value characterises the cation of 3-methylhypoxanthine (δ 8·34 for the uncharged form and 9·24 for the cation). Authentic 3-methoxy-6-methylpurine [20] under the same conditions shows δ 8·60 (2-H), 8·20 (8-H), 4·32 (3-Me), and 4·18 (OMe); assignment of the 3-Me and 2-H signals was based on NOE studies.[1]

pK Values were determined from a plot of $\lambda_{max.}$ as a function of pH, except in the case of compound (XI) where the change in $\varepsilon_{max.}$ was used.

N.m.r. spectra were measured for solutions in D_2O at 85° with a JEOL MH-100 instrument. When needed, TSP was used as standard. pD Values were adjusted either with NaOD or with one of the acids DCl, D_2SO_4, or $CF_3{\cdot}CO_2D$. The concentration of all purine solutions was 0·025M.

We thank Dr. Z. Neiman for discussions and Mr. I. Ringel for carrying out some of the kinetic measurements.

[2/833 *Received*, 13*th April*, 1972]

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[17] F. Bergmann and M. Rashi, *Israel J. Chem.*, 1969, **7**, 63.
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[20] F. Bergmann, Z. Neiman, and M. Kleiner, *J. Chem. Soc.* (*C*), 1966, 10.