Tautomerism and Ionisation of Purin-8-one and its *N*-Methyl Derivatives

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U.v. and n.m.r. spectra show that in aqueous solutions purin-8-one is present predominantly as the 7*H*,9*H*-tautomer. Anion formation takes place preferentially at N-9. Protonation of purin-8-one and its 7- and 9-methyl derivatives occurs at N-1 and causes splitting of the 6-H and 2-H n.m.r. signals. In purin-8-one and its *N*-methyl derivatives, the 2-H is more deshielded than the 6-H; the reverse is the case for purine itself.

RECENTLY, we have shown that a combination of information from u.v. and n.m.r. spectra of purines and their *N*-methyl derivatives can provide information on the structures of these compounds in aqueous solution.[1-3] We now describe an application of these methods to purin-8-one and its *N*-methyl derivatives.

The i.r. spectrum of purin-8-one shows a carbonyl stretching vibration at 1740 cm^{-1}, but no hydroxy-absorption.[4] Therefore it is assumed that this compound does not enolise to any appreciable degree. For the lactam form of purin-8-one, three tautomeric structures can be formulated, all of which have a 7-NH-group [(1), (1a), and (1b)]. Purin-8-one and its 7- (2) and 9-methyl (3) derivatives have closely related u.v. absorption maxima (Table 1), as noted previously by Brown and Mason.[4] Therefore these compounds can be grouped together as class (a). The remaining *N*-monomethyl derivatives (4) and (5), having higher values of $\lambda_{max.}$ and different structures, form class (b).

CNDO/2 calculations for purin-8-one have shown that the total energy of tautomer (1) is about 45 kcal mol^{-1} lower than that of (1a) and 41 kcal mol^{-1} below that of (1b);[5] therefore structure (1) is the most stable. The foregoing conclusions are strongly supported by n.m.r. spectra.

For interpretation of these spectra, it was first necessary to assign the individual signals to 2-H or 6-H. This was achieved as follows. In the spectrum of compound (5), only the 2-H signal increased its area (by about 15%), when the solution was irradiated at the frequency of the 3-methyl substituent (nuclear Overhauser effect). Table 2 shows that introduction of a *C*-methyl group into the pyrimidine ring in compound (1) shields the remaining aromatic proton, causing in the neutral molecules upfield shifts of 0·11 and 0·20 p.p.m. Thus the 2-H and 6-H signals could be assigned; the former is always at lower field than the latter, in contrast to the situation for purine.[6] The n.m.r. spectra of the neutral molecules of all members of class (a) (Table 1) are closely related; thus the 2-H and 6-H signals for

[1] D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc.* (*C*), 1971, 1676.
[2] D. Lichtenberg, F. Bergmann, and Z. Neiman, *J.C.S. Perkin I*, 1972, 1676.
[3] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, in the press.
[4] D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 1957, 682.
[5] B. Pullman and H. Berthod, *Theoret. Chim. Acta*, 1969, **15**, 205.
[6] W. C. Coburn, M. C. Thorpe, J. A. Montgomery, and K. Hewson, *J. Org. Chem.*, 1965, **30**, 1110.

compounds (2) and (3) may be assigned by analogy with the results for compound (1).

These conclusions are further supported by observations on the cations of class (a) (Table 3). In acid media, but not in neutral solutions, both the 2-H and 6-H signals appear as doublets (J 1·1 Hz). It has been observed previously for purine [6] that protonation at N-1, expected, in the case of the 1-methyl derivative (4), the 2-H and 6-H signals appear as doublets at any pH. In the cations of compounds (1)—(4), the 2-H signal shows less pronounced splitting than the 6-H band, thus permitting identification of either. This phenomenon is probably due to the effect of the N-3 quadrupole on the 2-H signal.†

TABLE 1

Physical constants of purin-8-one and its N-methyl derivatives

		$\lambda_{max.}$/nm *					pK			δ (p.p.m.) (neutral forms)		
		N	A_1	ΔA_1	C	ΔC	Anion		Cation			
Compd.	Me at posn.	(a)	(b)	(b) − (a)	(c)	(c) − (a)	7-NH	9-NH		2-H	6-H	NMe
	Class (a)											
(1)		275	284	+9 [a]	280	+5	13·5 [b]	7·9	2·9	8·71	8·45	
(2)	7	280	288	+8	285	+5		8·0	2·4	8·76	8·49	3·51
(3)	9	278	290	+12	282	+4	9·2		2·0	8·75	8·46	3·48
	Class (b)											
(4)	1	292	302	+10	287	−5	9·8		4·5	8·72 [c]	8·09 [c]	4·19
(5)	3	299	308	+9	294	−5	10·5		1·9	8·56	8·14	4·05

* N = Neutral, A_1 = monoanion, C = cation.

[a] This compound forms also a dianion; $\lambda_{max.}$ 292 nm. [b] This is the pK of the dianion. [c] These signals appear as doublets (J 1·1 Hz).

TABLE 2

N.m.r. spectra [δ (p.p.m.)] of purin-8-one and its C-methyl derivatives (in D_2O; 70 °C)

		2-H			6-H			C-Me		
Compd.	C-Me at posn.	N	A	C	N	A	C	N	A	C
(1)		8·71	8·42	9·16	8·45	8·07	8·67			
(6)	2				8·25	7·87	8·43	2·64	2·55	2·87
(7)	6	8·60	8·35	8·97				2·55	2·51	2·78

TABLE 3

N.m.r. spectra [δ (p.p.m.)]* of ionised forms of purin-8-one and its N-methyl derivatives

		Anions						Cations					
		2-H		6-H		N-Me		2-H		6-H		N-Me	
Compd.	Me at posn.	δ	ΔA [a]	δ	ΔA [a]	δ	ΔA [a]	δ [e]	ΔC [a]	δ [e]	ΔC [a]	δ	ΔC [a]
	Class (a)												
(1)		8·42 [b]	0·29	8·07	0·38			9·16	−0·45	8·67	−0·22		
		8·35 [c]	0·07	8·06 [c]	0·01								
(2)	7	8·53	0·23	8·11	0·38	3·45	0·06	9·13	−0·37	8·66	−0·17	3·57	−0·06
(3)	9	8·50	0·25	8·21	0·25	3·45	0·03	9·15	−0·40	8·62	−0·16	3·62	−0·14
	Class (b)												
(4)	1	8·36 [d]	0·36	7·75 [d]	0·34	4·06	0·13	8·93	−0·12	8·51	−0·42	4·27	−0·08
(5)	3	8·18	0·38	7·87	0·27	3·97	0·08	8·87	−0·31	8·58	−0·44	4·22	−0·17

* Measured in D_2O at 70 °C. Monoanions were measured at pH 12 and the dianion of purin-8-one at pH > 14. Cations were measured at pH 0.

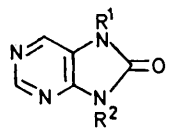
[a] ΔA = Difference between the signals of the neutral and anionic forms; ΔC = difference between the signals of neutral and cationic form. [b] Mono-anion formation at N-9. [c] This value refers to the dianion. [d] Doublets, J 1·1 Hz [e] The 2-H and 6-H signals of compounds (1)—(4) appear as doublets.

by reducing the asymmetry of the electric field around N-1, allows spin–spin coupling to take place between 2-H and 6-H without nitrogen quadrupole relaxation. The analogous phenomenon is observed for the cations of purin-8-ones of class (a) (Table 3), which are thus represented by structures (6)—(8). The n.m.r. signals of all cations of class (a) are again very similar. As

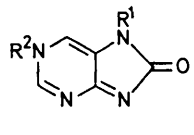
† As will be shown elsewhere, in all purin-8-ones one of the aromatic protons exchanges in D_2O much faster than the other. Therefore once the assignment is known for one form, those for the others follow.[7]

Anion Formation in Purin-8-ones.—All transitions from neutral molecules to anions are characterised by bathochromic displacements of $\lambda_{max.}$ of 8—12 nm (Table 1). In compounds (2)—(5), only a single NH group is available for anion formation [position 9 in compound (2) and position 7 in (3)—(5)]. In the anion of (2), resonance distributes the negative charge over the 8-carbonyl group, N-9 and the two pyrimidine

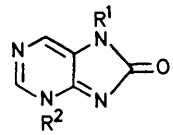
[7] D. Lichtenberg, F. Bergmann, and I. Ringel, *J. Magnetic Resonance*, 1972, **6**, 600.



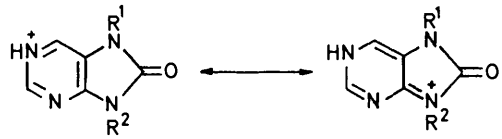
(1) $R^1 = R^2 = H$
(2) $R^1 = Me, R^2 = H$
(3) $R^1 = H, R^2 = Me$



(1a) $R^1 = R^2 = H$
(4) $R^1 = H, R^2 = Me$



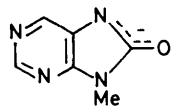
(1b) $R^1 = R^2 = H$
(5) $R^1 = H, R^2 = Me$



(6) $R^1 = R^2 = H$
(7) $R^1 = Me, R^2 = H$
(8) $R^1 = H, R^2 = Me$

nitrogen atoms (9). On the other hand, in the anions of (3)—(5) the charge can be shared only between N-7 and the 8-carbonyl group [*e.g.* (10)].

(9)



(10)

For compound (1) special criteria are needed to distinguish between the participation of the 7- or 9-NH group in mono- and di-anion formation. Table 3 shows that monoanion formation is accompanied by an upfield shift of the 6-H signal, identical with that for compound (2) (0·38 p.p.m.) and different from that (0·25 p.p.m.) for compound (3). It is therefore assumed that anion formation occurs first at position 9. This is supported by comparison of the pK values (Table 1). The lowest values (7·9 and 8·0) are shown for the 9-NH groups [compounds (1) and (2)]; ionisation at N-7 leads to much higher pK values [9·2—10·5 in compounds (3)—(5)].* The higher acidity of the 9-proton may be due to distribution of the negative charge over both rings [*cf.* structures (9) and (10)].

Cation Formation.—As already mentioned, all members of class (a) are protonated at N-1. Energy calculations by the CNDO/2 algorithm, using the parametrisation of Pople and Beveridge [8] and a hydrogen 1s-exponent of 1·2, show that the 1-NH$^+$ form (6) is more stable than the 3-NH$^+$ tautomer by 9 kcal mol^{-1} (Table 4; total energy E_T). The CNDO/2 formalism

TABLE 4

Energy values (in eV) of purin-8-one and its protonated forms

Derivative	Electronic energy	Nuclear repulsion energy	Total energy	MBN [a]
Free base	−9722·672	6897·109	−2825·563	10·7416
1-NH$^+$ form	−10,003·789	7165·510	−2838·279	11·4829
3-NH$^+$ form	−10,019·106	7181·217	−2837·889	11·4410

[a] MBN = Molecular binding number.[10]

also allows dissection of the total energy into its components, the electronic (E_E) and nuclear repulsion (E_N) energies. If one may rely on energy profiles, then the data in Table 4 lead to the following conclusions.

(1) The extra stabilisation of the 1-protonated form can be attributed to the large difference between the nuclear repulsion terms (ΔE_N *ca.* 362·2 kcal mol^{-1}). This is probably due to steric interaction between the hydrogen atoms at N-3 and N-9 in the 3-protonated tautomer.

(2) The E_E values show that the 3-protonated form is stabilised by about 353 kcal mol^{-1} compared to the 1-NH$^+$ tautomer. A similar relationship has been found for purine itself.[9] The difference in E_E between the two cationic forms of purin-8-one may be rationalised by assuming that in the conjugated chain C(4)=C(5)–C(6)=N(1)–C(2)=N(3) protonation at the terminal N(3) would perturb the system to a lesser degree than attachment of the proton to N(1) (in the middle).

(3) The molecular binding number (MBN) has recently been defined [10] on the basis of Mulliken's population analysis [11] and has been used for estimation of bond stabilities of various isomers.[12] Table 4 shows that the 1-NH$^+$ form has the larger MBN value, *i.e.* it represents the preferred, more stable structure.

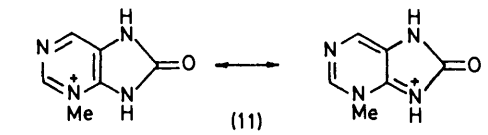
Cation formation in class (a) is accompanied by a bathochromic displacement of λ_{max} (Table 1). In contrast, in class (b), protonation leads to a hypsochromic shift of the u.v. maxima. Consequently, the differences between the maxima of class (a) and (b) are reduced in the cations. Furthermore, in the n.m.r. spectra of the cations, the 6-H signals of compounds (4) and (5) undergo a much larger downfield shift than those of class (a).

The fact that analogous changes are observed in the u.v. and n.m.r. spectra of compounds (4) and (5) indicates that in both cation formation takes place in a similar manner. In compound (5) only position 9 can be involved (11), because here splitting of the 2-H and 6-H signals is not observed. Therefore an analogous proton-

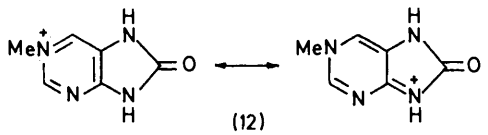
* From the difference between the pK values of compounds (1) and (2) and that of compound (3) (ΔpK *ca.* 1·3), one may infer that the anion of (1) contains about 5% of the tautomer in which dissociation has occurred at N-7. However, our conclusion would not be influenced materially by this finding.

[8] J. A. Pople and D. L. Beveridge, 'Approximate Molecular Orbital Theory,' McGraw-Hill, New York, 1970.
[9] Z. Neiman, *Israel J. Chem.*, 1972, **10**, 819.
[10] M. L. Unland, J. R. Van Wazer, and J. H. Letcher, *J. Amer. Chem. Soc.*, 1969, **91**, 1045,
[11] R. S. Mulliken, *J. Chem. Phys.*, 1955, **23**, 1883.
[12] K. G. R. Pachler and J. P. Tollenaere, *J. Mol. Structure*, 1971, **8**, 83.

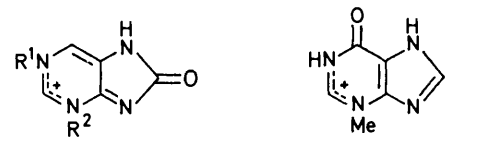
ation process is assumed for compound (4) [see (12)]. The similar spreading of charge in systems (11) and (12)



(11)



(12)



(13) R^1 = Me, R^2 = H
(14) R^1 = H, R^2 = Me

(15)

is reflected in the similar downfield shift of the n.m.r. signals ($\Delta\delta$ for 2-H, 0·21 and 0·31 p.p.m.; for 6-H, 0·42 and 0·44, respectively; see Table 3).

Protonation of compounds (4) and (5) in the pyrimidine ring would give the ' fixed ' cations (13) and (14). Such a situation is in fact encountered with 3-methylhypoxanthine; here formation of the fixed cation (15) is accompanied by a large downfield shift of the 2-H signal (0·9 p.p.m.).[3] The observation that the corresponding shift for the cations of (4) and (5) is much smaller (Table 3), supports the conclusion that protonation does not take place in the pyrimidine ring.

Disturbance of the aromatic structure of the pyrimidine system in structures (4) and (5) should lead to an increase in basicity. Indeed compound (4) shows the highest pK value (4·5) for cation formation (Table 1). A similar value would be expected for (5); here, however, protonation at N-9 introduces steric interference with the 3-methyl group.[1,2] This may explain the low pK value (1·9).

Conclusions.—Owing to the anisotropy of the 8-carbonyl group, the 7- and 9-methyl groups are shielded relative to methyl groups at N-1 and N-3 (Tables 1 and 3).

In all molecular forms of the purin-8-ones, the 2-H signal is at lower field than the 6-H band, in contrast to the behaviour of purine.[6] When passing from purine to its 8-oxo-derivative, both signals move to higher field, but the change is larger for the 6-H band, causing a reversal of the sequence. This is probably due to changes in ring current and in the direction of the dipole moment.

Both anion and cation formation in purin-8-ones proceed in such a way as to create maximal spreading of the charge [*e.g.* dissociation at N-9 in (1), protonation of N-9 in (4) and (5)]. However, whenever N-9 bears a substituent [H or Me in class (a)], protonation at N-3 is avoided. This is due both to a steric factor and to the higher stability of the 1-protonated form, as shown by MO calculations. The only exception to this rule is encountered with the cation of (5), because here only protonation at N-9 can prevent formation of a ' fixed positive charge ' (14).

EXPERIMENTAL

Solvents for paper chromatography were (A) dimethylformamide–propan-2-ol–25% ammonia (13 : 5 : 2 v/v), and (B) ethanol–dimethylformamide–water (3 : 1 : 1 v/v).

U.v. spectra were measured on a Cary model 14 spectrophotometer. N.m.r. spectra were obtained for solutions in deuterium oxide with a JEOL MH-100 instrument at 70° (sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate as internal standard).

Alkaline solutions were obtained by addition of sodium deuteroxide; for acidic solutions deuterium chloride, [$^2H_2$]sulphuric acid, or trifluoroacetic acid was added.

Purin-8-one and its 3-,[13] 7-,[4] and 9-methyl[4] derivatives were prepared according to described procedures.

1-*Methyl*-6-*mercaptopurin*-8-*one*.—A mixture of 1-methylpurine-6,8-dione[14] (5 g) and phosphorous pentasulphide (15 g) in dry β-picoline (300 ml) was stirred and refluxed for 2·5 h. The solvent was removed *in vacuo* and the residue treated with warm water (70°) for 30 min. A solution of the insoluble portion in dilute ammonia was decolourised with charcoal, filtered, and acidified with glacial acetic acid. The *product* formed rectangular plates (50%), decomp. >300° (from water); $\lambda_{max.}$ (pH 1) 240 and 330 nm (ε 10,750 and 13,570), δ 8·40 (2-H) and 3·96 (Me) p.p.m. (at pH 10); R_F (A) 0·38; R_F (B) 0·65 (violet fluorescence) (Found: C, 39·2; H, 3·3; N, 30·2; S, 17·3. $C_6H_6N_4OS$ requires C, 39·6; H, 3·3; N, 30·8; S, 17·6%).

1-*Methylpurin*-8-*one* (4).—A solution of 1-methyl-6-mercaptopurin-8-one (10 g) in 1% ammonia (100 ml) was refluxed with Raney nickel (4 g) for 1·5 h, filtered, and evaporated *in vacuo*. The residue was extracted with boiling dimethylformamide (100 ml). Upon cooling, compound (4) crystallised in fine *needles* (48%), decomp. >300°; $\lambda_{max.}$ (pH 1) 287 nm (ε 15,000); R_F (A) 0·33; R_F (B) 0·54 (Found: C, 47·6; H, 4·3; N, 37·1. $C_6H_6N_4O$ requires C, 48·0; H, 4·0; N, 37·3%).

CNDO/2 Calculations.—The CNDO/2 Fortran program of the QCPE (No. 100) was slightly modified by Dr. A. Y. Meyer (Department of Organic Chemistry, Hebrew University). Since no data on the exact geometry of purin-8-one are reported, the skeleton was constructed by combination of the pyrimidine system of purine[15] and the imidazolone portion of uric acid.[16] The new NH bond in the protonated forms was assigned a length of 0·9 Å; preservation of all angles was assumed. The results (Table 4) are thus of a tentative character.

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