Tautomerism and Ionisation Processes in 6-Methylthio-2-oxopurines

By **Dov Lichtenberg, Felix Bergmann,*** and **Zohar Neiman,** Department of Pharmacology, The Hebrew University–Hadassah Medical School, Jerusalem, Israel

6-Methylthio-2-oxopurine (1) is present in aqueous solution mainly as the 3,7-di-NH tautomer. This supports the general assumption that purines avoid 3,9-disubstituted structures. Anion formation in structure (1) takes place first at the 7- and then at the 3-position. Cations bearing a 1-methyl group are unstable, undergoing hydrolysis, even at pH 4·5, to the corresponding xanthines. In the n.m.r. spectra of the 1-methyl derivatives of (1), steric interference causes a marked downfield shift of the 6-SMe signal.

A COMBINATION of u.v. and n.m.r. spectroscopy can often lead to important conclusions about the fine structure of purines in aqueous solution.[1-4] We have now applied these methods to 6-methylthio-2-oxopurines, in order to obtain information about their tautomerism and ionisation processes.

TABLE 1

Physical properties of 6-methylthio-2-oxopurines

No.	Me at position	$\lambda_{max.}$/nm (log ε)					pK Values for formation of		δ(8-H)				
		N	A	Δ(A − N)	C	Δ(C − N)	anion	cation	N	A	Δ(N − A)	C	Δ(C − N)
(1)		316(4·09)	318[a](4·10)	+2	338(4·18)	+22	7·7[b]	1·4	7·86	7·54[c]	+0·32	8·43	+0·57
		268(3·94)	256(3·92)	−12	258(3·81)	−10							
(2)	1	335(4·09)	342(3·97)	+7	335(3·86)	0	8·8	2·5	8·02	7·86	+0·16	8·28	+0·26
		246(3·85)	280(3·73)	+34	264(3·95)	+18							
(3)	3	317(4·19)	320(4·21)	+3	341(4·28)	+24	7·7	1·4	7·90	7·50	+0·40	8·36	+0·46
		268(4·06)	260(4·03)	−8	256(3·85)	−12							
(4)	7	317(4·15)	322(4·07)	+5	339(4·06)	+22	8·7	1·3	8·03	7·93	+0·10	8·56	+0·53
		270(4·05)	255(3·91)	−15	259(3·96)	−11							
(5)	9	332(4·07)	314(4·12)	−18	331(4·14)	−1	6·3	1·7	7·99	7·89	+0·10	8·50	+0·51
		252(3·88)	242(4·05)	−10	258(3·89)	+6							
(6)	1,3	336(4·13)			338(4·06)	+2		4·5	7·98			8·29	+0·31
		258(3·91)			267(4·02)	+9							
(7)	3,7	319(4·20)			343(4·26)	+24		1·1	7·93			8·38	+0·45
		272(4·07)			258(3·95)	−14							

N = neutral form; A = anion; C = cation

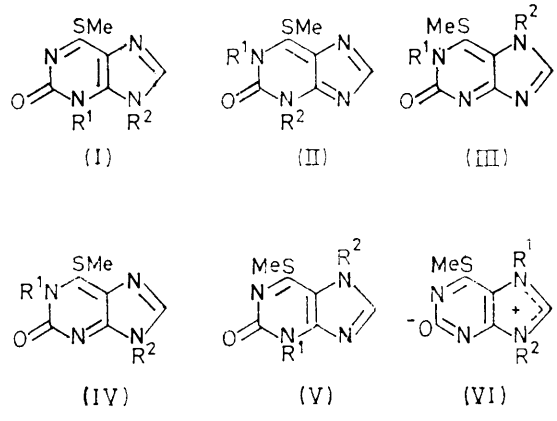
[a] The dianion of (1) shows $\lambda_{max.}$ 319 and 240 nm (log ε 4·08 and 3·97). [b] Dianion formation is characterised by pK_{A_2} = 11·7. [c] The dianion of (1) shows δ 7·49 (8-H).

Tautomerism.—Apart from lactim forms, six tautomeric structures can be formulated for 6-methylthio-2-oxopurine (1) (I—VI; $R^1 = R^2 = H$).

3,7-Dimethyl-6-methylthio-2-oxopurine (7) must have structure (V; $R^1 = R^2 = Me$). Table 1 shows the close similarity of the u.v. spectra of compounds (1), (3), (4), and (7) ($\lambda_{max.}$ 317 ± 2 and 270 ± 2 nm), to which we therefore ascribe the common structure (V). Further support for this is adduced in section on protonation.

For the 9-methyl derivative (5), we have to choose between tautomers (I), (IV), and (VI) ($R^1 = H$, $R^2 =$ Me). We have previously suggested that, wherever possible, purines avoid structures in which both positions 3 and 9 bear substituents (H or alkyl).[1-3] For this reason, we may exclude the 3-NH tautomer (I). Tautomer (VI) possesses a 'fixed' positive charge † in the imidazole ring. We have observed repeatedly that in purines with an imidazolinium ring, the 8-H n.m.r. signal is shifted downfield by about 1 p.p.m. relative to derivatives with an uncharged imidazole system.[1-3] However the chemical shift of 8-H in (5) does not reveal any marked deshielding (Table 1). Therefore we ascribe to (5) the tautomeric structure (IV; $R^1 = H$, $R^2 = Me$).

For 1-methyl-6-methylthio-2-oxopurine (2), tautomers (II)—(IV) ($R^1 = Me$, $R^2 = H$) have to be considered.



Strong interference between the *N*- and *S*-alkyl substituents is indicated by the marked downfield shift of the

† This term designates amidinium-like structures in which resonance distribution of the positive charge is confined to one ring of the purine system.

[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.*, 1972, **10**, 805.

[4] U. Reichman, F. Bergmann, D. Lichtenberg, and Z. Neiman, *J.C.S. Perkin I*, 1973, 793.

SMe n.m.r. signal (Table 2). A similar effect is manifest in the n.m.r. spectrum of (6). In these two derivatives the SMe group is forced into a position near to N-7, preventing formation of tautomer (III). Since the u.v. spectrum of (2) resembles those of both (5) and (6), it is not possible at present to make a choice between tautomers (II) and (IV).

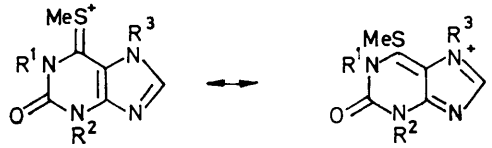
At first sight, the predominance of tautomer (V) for compound (1) is surprising, because in 6-methylthiopurine preference for the 9-NH form has been demonstrated.[5,6] However, formal introduction of a carbonyl group into position 2 is accompanied by insertion of a new NH group, either in the imidazole ring to form the betaine (VI; $R^1 = R^2 = H$), or at position 1 or 3. As mentioned before, the lack of any marked deshielding of the 8-proton excludes the first possibility. The simultaneous presence of 1-NH and 6-SMe would cause steric interference, as in the case of compound (2). Therefore position 3 is preferred for the new proton. Insertion of a 3-NH group can be regarded as causing a prototropic shift from N-9 to N-7 and formation of tautomer (V; $R^1 = R^2 = H$), to avoid the less stable 3,9-di-NH tautomer (I).

Protonation.—If protonation were to create 'fixed' cations like (VII) or (VIII), the 8-H n.m.r. signal should shift downfield by about 1 p.p.m. However, all such shifts are in the range 0·26—0·57 (Table 1). Therefore structures like (VII) or (VIII) can make only small

(VII)

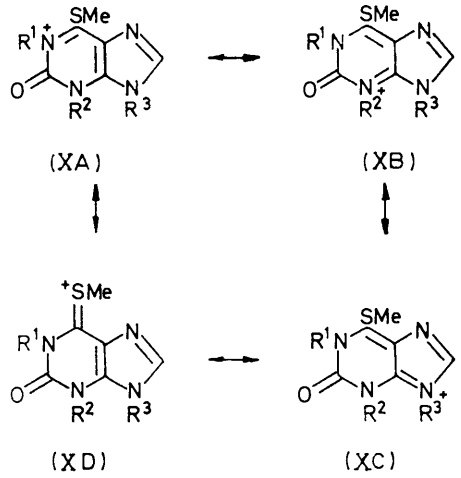
(VIII)

(IXA) (IXB)



(IXD) (IXC)

contributions, and the protonated forms of the present series must be represented essentially by (IX) and (X). For example the cation of 7-methyl-6-methylthio-2-oxopurine (4) is represented by (IXA—D; $R^1 = R^2 = H$, $R^3 = Me$), and that of the 3,7-dimethyl derivative (7) by (IX; $R^1 = H$, $R^2 = R^3 = Me$). In the latter case, mesomer (IXB) must make a major contribution to the cation, since the shift of the 3-methyl n.m.r. signal on



(XA) (XB)

(XD) (XC)

protonation (0·19 p.p.m.) is much larger than that (0·06) for the 7-methyl signal (see Table 2).

The u.v. spectral changes of compounds (1), (3), (4), and (7) are very similar. Upon cation formation, the higher $\lambda_{max.}$ value undergoes a large bathochromic shift and the lower maximum a considerable hypsochromic displacement (Table 1). Therefore we assume also for compounds (1) and (3) that protonation of structure (V) leads to structure (IX).

In the cation of (5), represented by (X), the shift of the 9-methyl n.m.r. signal on protonation is 0·13 p.p.m., indicating the importance of the resonance form C. The low pK value of (5) (1·7) is ascribed to steric interference between the 3-NH and 9-NMe groups. Nevertheless (X) is preferred to (VII; $R^1 = R^2 = H$, $R^3 = Me$), because only the former structure permits spreading of the charge over both rings.

N.m.r. data do not give a clear indication as to the tautomeric form of the cations of (2) and (6). For these two derivatives, the shifts of the 8-proton n.m.r. signals on protonation are 0·26 and 0·31 p.p.m., respectively, whereas for the 7-methyl derivative (4) this shift is 0·53 and in the 9-methyl isomer (5) 0·51. Thus the resonance forms (IXC) and (XC), which are important for the cations of (4) and (5), must make only a small contribution to the cations of (2) and (6).

However the u.v. spectra of compounds (2) and (6) provide decisive evidence. Cation formation in these derivatives is characterised by a negligible shift of the long-wave maximum and a considerable bathochromic displacement of the short-wave maximum. These features are shared only by compound (5) (see Table 1) and thus support the suggestion that the protonated forms of (2) and (6) can be represented largely by structures (X).

If these conclusions are correct, then compound (5)

[5] B. Pullman, H. Berthod, F. Bergmann, Z. Neiman, H. Weiler-Feilchenfeld, and E. D. Bergmann, *Tetrahedron*, 1970, **26**, 1483.

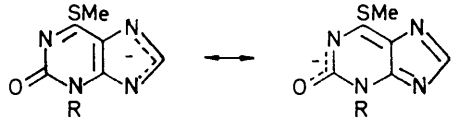
[6] J. Deutsch, Z. Neiman, and F. Bergmann, *Org. Mass Spectrometry*, 1971, **5**, 279.

suffers protonation at N-3, (2) at N-3 and/or N-9, and (6) at N-9. This may explain the large differences in pK values for protonation of these compounds (Table 1). On the other hand, in the group comprising compounds (1), (3), (4), and (7), the differences between the pK values are small, in agreement with our suggestion that these four derivatives are all represented by structure (V) and their cations by (IX).

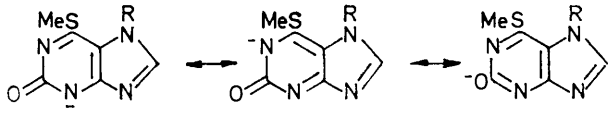
The cations of the 1-methyl derivatives are characterised by their instability in acidic solution: even at pH 4·5 (acetic acid), the methylthio-group undergoes rapid hydrolysis. Thus at 90°, compound (2) is 40% degraded to 1-methylxanthine within 40 min, and compound (6) is 80% hydrolysed to theophylline within 20 min. In contrast, in 2 h compound (3) is less than 20% hydrolysed and (4) and (7) react to a negligible degree.

Anion Formation.—Anion formation in compounds (1), (3), and (4) is characterised by a small bathochromic shift of the long-wave u.v. absorption maximum and a much larger, hypsochromic shift of the short-wave maximum (Table 1). However in (3), the 7-NH group undergoes dissociation, whereas in (4) the 3-NH is involved. Therefore on this basis alone it is difficult to determine the sequence of ionisation in (1). However, the pK value of (1) (7·7) for monoanion formation is identical with that of (3), and both are one unit lower than the pK of (4). We conclude that the 7-NH group is mainly responsible for the first ionisation step (see XI; R = H). The ratio $K_{7\text{-NH}} : K_{3\text{-NH}}$ (10 : 1) indicates a *ca.* 10% contribution of the tautomer (XII; R = H) to the monoanion of (1).

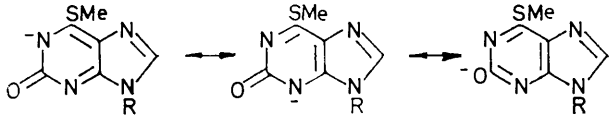
These conclusions are supported by n.m.r. data (Table 1). Thus the upfield shift of the 8-proton



(XIA) (XIB)



(XIIA) (XIIB) (XIIC)



(XIIIA) (XIIIB) (XIIIC)

n.m.r. signal on monoanion formation is 0·32 p.p.m. for compound (1), *i.e.* close to the value (0·40) for (3), whereas the corresponding signal of (4) undergoes only a small upfield shift (0·1 p.p.m.). Furthermore in the dianion of (1), we observe only a small diamagnetic shift of 0·05 p.p.m. for the 8-proton. Thus we can derive the main sequence of ionisation in (1) as involving first the 7- and then the 3-position.

Anion formation in (5), which takes place at position 1, is accompanied by a marked hypsochromic shift of both u.v. absorption maxima. In the anion (XIII; R = Me) the lone pair of electrons can create steric interference either with the 6-methylthio-group [as in (XIIIA)] or with the 9-Me group [as in (XIIIB)],[7,8] thus leaving form (XIIIC) as the main contributor. The aromatic structure of the pyrimidine ring in (XIIIC) is held responsible for the low value of $\lambda_{max.}$. This follows from the observation that the u.v. absorption maximum is 315 nm for the neutral form of 2-oxopurine, whereas the corresponding value for 2-methoxypurine is 283 nm.[9] In the anions of all other members of the present series, the canonical forms (XIA and B) and (XIIA) participate significantly. Compound (5) (pK_a 6·3) is the strongest acid of the series. This we may ascribe to relief of steric strain when a proton is removed from position 1 in (IV).

Anion formation in (2) represents a special case, being characterised by a bathochromic shift of both absorption maxima (Table 1). Regardless whether it is derived from (II) or (IV) (R^1 = Me, R^2 = H) this anion can be represented by structures (XIVA and B).

(XIVA) (XIVB)

Chemical shifts of the S- *and* N-*Methyl Groups.*—The signals of the SMe groups in the neutral molecules and

TABLE 2

Chemical shifts of *S*- and *N*-methyl groups in 6-methylthio-2-oxopurines

No.	*N*-Me at position	δ(SMe) N	A	C	Δ(C−N)	δ(NMe) [a] N	A	C
(1)		2·65	2·63 [b]	2·84	+0·19			
(2)	1	3·24	3·19	3·36	+0·12	(1)3·69	3·70	3·78 [c]
(3)	3	2·65	2·61	2·90	+0·25	(3)3·59	3·59	3·74
(4)	7	2·70	2·65	2·81	+0·11	(7)4·04	3·96	4·14
(5)	9	2·68	2·67	2·91	+0·23	(9)3·73	3·70	3·86
(6)	1,3	3·31		3·35	+0·04	(1)3·72		3·80 [c]
						(3)3·68		3·77
(7)	3,7	2·69		2·85	+0·16	(3)3·56		3·75
						(7)4·06		4·12

N = neutral form; A = anion; C = cation.

[a] Figures in parentheses indicate position of *N*-methyl group. Assignment of *N*-methyl signals in (6) and (7) is based on comparison with the monomethyl derivatives. The individual assignments in compound (6) are uncertain. [b] In the dianion of (1), δ(SMe) = 2·61. [c] Compounds (2) and (6) decompose rapidly in acidic solution.

in the anions are all in the range 2·65 ± 0·05 p.p.m. However, the presence of a 1-methyl substituent causes

[7] E. L. Eliel, *Kem. Tidskr.*, 1969, **81**, 22.
[8] J. A. Zoltewicz and L. W. Deady, *J. Amer. Chem. Soc.*, 1972, **94**, 2765.
[9] S. F. Mason, *J. Chem. Soc.*, 1954, 2071.

a marked downfield shift of about 0·6 p.p.m. in compounds (2) and (6) (Table 2). In the cations of compounds (1), (3), (7), and (5) the SMe group is deshielded (shift 0·16—0·25 p.p.m.). These values are large in comparison with those for other series of methylthiopurines,[4] suggesting a contribution of resonance forms (IXD) and (XD) to the cations. This explanation is supported by the small downfield shift of the SMe signal in (2) and (6), where R^1 = Me, thus greatly reducing the contribution of (IXD). Similarly, the rather low value of the shift for the methylthio-signal in (4) indicates interference between the substituents at positions 6 and 7.

The *N*-methyl signals follow the sequence (upfield ⟶ downfield) 3-Me, 1-Me, 9-Me, 7-Me. The 1- and 3-methyl groups are shielded by the 2-carbonyl group, but the 1-methyl substituent is also influenced by the neighbouring 6-methylthio-group.

EXPERIMENTAL

U.v. spectra were measured on a Cary 14 spectrophotometer and n.m.r. spectra on a JEOL MH-100 instrument, with as internal standard sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate (Merck, Sharp, and Dohme). All n.m.r. measurements were performed on solutions in 9 : 1 (v/v) $(CD_3)_2SO$–D_2O being adjusted by the use of $CF_3{\cdot}CO_2H$, $CD_3{\cdot}CO_2D$, Na_2CO_3, and NaOD.

Purines.—The 6-methylthio-2-oxopurines (1)—(7) have been prepared previously.[2] Attempts to synthesise the other dimethyl derivatives of the series led to formation of trimethyl-6-thioxanthines.

Methylation of 1,7-*Dimethyl*-6-*thioxanthine.*—A solution of this compound (0·5 g) in 0·1N-sodium hydroxide (40 ml) was mixed with dimethyl sulphate (0·27 ml) at 5°. The precipitate formed after 15 min was filtered off. The product (0·4 g, 76%) proved identical with 1,3,7-trimethyl-6-thioxanthine.[2]

Methylation of 1,9-*Dimethyl*-6-*thioxanthine.*—Methylation in alkaline solution, under the same conditions as before, did not produce a precipitate. However, if more concentrated solutions were used (*e.g.* 4 ml of N-NaOH and 0·5 g of substrate), 1,7,9-*trimethyl*-2-*oxopurinium*-6-*thiolate* precipitated in 40% yield, forming needles, decomp. >300° (from ethanol), $\lambda_{max.}$ (pH 8·5) 260 and 347 nm (log ε 4·72 and 5·16), δ ($CF_3{\cdot}CO_2H$) 8·74 (8-H), 4·34 (7-Me), 3·93 (9-Me), and 3·79 (1-Me) (Found: C, 46·0; H, 5·0; N, 26·8. $C_8H_{10}N_4OS$ requires C, 45·7; H, 4·8; N, 26·7%), decomposing at pH 10 (Na_2CO_3 solution). This assignment is based on comparison with the 1,7-, 1,9- and 7,9-dimethyl derivatives of 6-thioxanthine. The compound differs from the known 1,3,7- and 1,3,9-trimethyl-6-thioxanthines.[2]

[2/2347 *Received*, 16*th October*, 1972]