Tautomerism, Protonation, and Methylation in Methylthiopurines; Factors determining Electrophilic Attack on Purines

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

The predominant tautomeric forms, the position of protonation in aqueous solution, and the course of methylation in aprotic solvents have been determined for all possible mono- and bis-methylthiopurines and for 2,6,8-trismethylthiopurine. As a rule, protonation creates resonating cations in which the charge is distributed over both rings. The site of methylation varies. Like purine itself, the 8-methylthio- and the 2,8-bismethylthio-derivatives are attacked by methyl iodide at N-1. In 6-methylthio-, 6,8-bismethylthio- and 2,6,8-trismethylthiopurine, N-3 undergoes alkylation. In 2-methylthio- and in 2,6-bismethylthio-purine, methylation takes place at both positions 7 and 9. These results are explained in terms of the combined influence of electronic and steric factors.

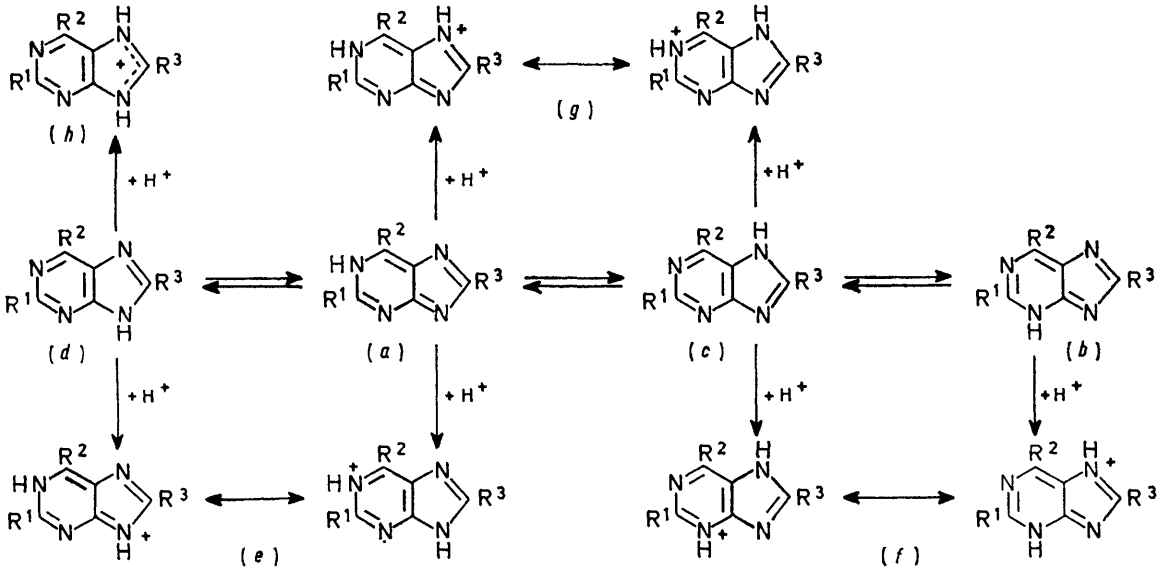
In order to understand the behaviour of purines in chemical and biochemical systems, it is necessary (*a*) to define the tautomeric forms present in aqueous solution; (*b*) to localise ionisation processes; and (*c*) to determine the point of attack of chemical reagents. For xanthine-type compounds, the problems of tautomerism and ionisation have been satisfactorily solved by a combination of u.v. and n.m.r. spectroscopy.[1,2] The tautomerism of guanine has recently been elucidated by Lee and Chan.[3] However the same methods have proved less satisfactory for compounds of the hypoxanthine type.[4]

While these studies have contributed much to our knowledge of the fine structure of purines in aqueous solution, general rules for the reactions of purines with nucleophilic and electrophilic reagents have not yet been established. We report experiments designed to discover whether any relation exists between the sites of protonation and methylation. The former, as a reversible

[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 II*. 1972. 1676.
[3] G. C. Lee and S. I. Chan, *J. Amer. Chem. Soc.*, 1972, **94**, 3218.
[4] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, 1972, **10**. 805.

reaction, is thermodynamically controlled. On the other hand, under the experimental conditions used, methylations are essentially irreversible and thus are kinetically controlled. However in both reactions, the *direction* of the electrophilic attack is determined by similar electronic factors, unless steric interference becomes important. Indeed, our results show that in many cases both hydrogen ion and methyl iodide attack the same positions.

Tautomerism in Purine and its Methylthio-derivatives.— In aqueous solution, purine essentially consists of a mixture of 7- and 9-NH tautomers. This statement is based on comparisons with the u.v. spectra of *N*-methylpurines. Whereas $\lambda_{max.}$ values of the neutral molecules of the 7- and 9-methyl derivatives differ only by 4—6 nm materially. This is concluded from the fact that the u.v. absorption maxima of the neutral forms of 6-methylthiopurine (3) and its 7- and 9-methyl derivatives form one group, the absorption maxima of which differ from those of the 1- and 3-methyl isomers, a situation similar to that for the *N*-methyl derivatives of purine. For compound (3) itself, attempts have been made to determine whether the predominant tautomeric form is (c) or (d). The dipole moment of (3) in dioxan solution is much closer to that of the 9-methyl derivative than of the 7-methyl isomer,[9] indicating the preponderance of the 9-NH tautomer (3d). This is further supported by the mass spectrum.[10] Similarly the u.v. data (Table 1) indicate that the neutral molecule of 2,6-bismethylthiopurine prefers the 9-NH form (5d). We do not have a



SCHEME 1

(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = R^2 = H$, $R^3 = SMe$
(3) $R^1 = R^3 = H$, $R^2 = SMe$
(4) $R^1 = SMe$, $R^2 = R^3 = H$
(5) $R^1 = R^2 = SMe$, $R^3 = H$
(6) $R^1 = H$, $R^2 = R^3 = SMe$
(7) $R^1 = R^3 = SMe$, $R^2 = H$
(8) $R^1 = R^2 = R^3 = SMe$

from that of purine itself (1), the values for the 1-methyl[5] and 3-methyl[6] derivatives are *ca.* 15 nm to higher wavelength (see Table 1). Likewise, the ^{13}C n.m.r. spectrum of purine shows the presence of 7- and 9-NH tautomers.[7] According to the MO calculations of Pullman *et al.*[8] the imidazole tautomers of purine [(1c) and (1d) in Scheme 1] are more stable than the 1- or the 3-NH form (1a) or (1b).

In methylthiopurines, the 'aromatic' structure of purine is preserved. Although the u.v. maxima are shifted to longer wavelengths by 31—68 nm (see Table 1), the predominance of the imidazole tautomers (c) and (d) in the uncharged molecules (Scheme 1) is not altered sufficient number of methyl derivatives to make definite statements about the tautomerism in the uncharged forms of 6,8- (6) and 2,8-bismethylthiopurine (7) on the basis of their u.v. spectra.

The $\lambda_{max.}$ values of the neutral molecule of the trismethylthiopurine (8) are close to those of the 9-methyl derivative and markedly different from those of the 3-methyl isomer (11), indicating the presence of the tautomeric structures (8c) and (8d) in aqueous solution.

Additional information on purine tautomerism can be obtained from the p*K* values for cation formation (Table 1). All derivatives which are alkylated in the

[5] L. B. Townsend and R. K. Robins, *J. Org. Chem.*, 1962, **27**, 990.
[6] L. B. Townsend and R. K. Robins, *J. Heterocyclic Chem.*, 1966, **3**, 241.
[7] R. J. Pugmire and D. M. Grant, *J. Amer. Chem. Soc.*, 1971, **93**, 1880.
[8] B. Pullman, H. Berthod, E. D. Bergmann, F. Bergmann, Z. Neiman, and H. Weiler-Feilchenfeld, *Compt. rend.*, 1968, **267C**, 1461.
[9] B. Pullman, H. Berthod, F. Bergmann, Z. Neiman, H. Weiler-Feilchenfeld, and E. D. Bergmann, *Tetrahedron*, 1970, **26**, 1483.
[10] J. Deutsch, Z. Neiman, and F. Bergmann, *Org. Mass Spectrometry*, 1971, **5**, 279.

TABLE 1

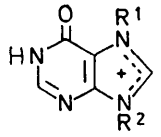
U.v. absorption maxima and pK values of purines [a]

No.	Compound	$\lambda_{max.}$/nm N(a)	C(b)	Δ(a − b)	pK for cation formation: 'aromatic' [b] system	'quinonoid' [b] system
(1)	*Purine*	260	263	+3	2·4	
(12)	1-Methyl-	275	268	−7		5·2
	3-Methyl-	276	275	−1		
	7-Methyl-	266·5	277·5	+11	2·3	
	9-Methyl-	264	262·5	−1·5	2·4	
(2)	8-*Methylthio-purine*	220	231	+11	2·95	
		293	306	+13		
(13)	1-Methyl-	227	227	0		3·9
		309	309	0		
(3)	6-*Methylthio-purine*	291	294	+3	1·3	
	1-Methyl-	308	309	+1		5·0
	3-Methyl-	312	314	+2		4·3
	7-Methyl-	293	300	+7	1·9	
	9-Methyl-	288	293	+5	1·8	
	7,9-Dimethyl-		297			
	3,7-Dimethyl-		320			
(4)	2-*Methylthio-purine*	230	240	+10	1·9	
		304	313	+9		
(5)	2,6-*Bismethylthio-purine*	258	261	+3	1·8	
		308	313	+5		
	1-Methyl-	340	333	−7		5·9
(17)	3-Methyl-	323	330	+7		5·2
(14)	7-Methyl-	240	220	−20	1·9	
		258	263	+5		
		316	318	+2		
(15)	9-Methyl-	234	221	−13	1·4	
		259	264	+5		
		308	318	+10		
(16)	7,9-Dimethyl-		229			
			267			
			323			
(18)	3,7-Dimethyl-		332			
(6)	6,8-*Bismethylthio-purine*	246	252	+6	1·25	
		311	335	+24		
(10)	3-Methyl-	259	259	0		3·9
			285			
		340	340	0		
(7)	2,8-*Bismethylthio-purine*	224	240	+16	2·2	
		248	264	+16		
		319	326	+7		
(19)	1-Methyl-	262	262	0		4·1
		323	327	+4		
(8)	2,6,8-*Trismethyl-thiopurine*	256	263	+7	1·1	
		328	339	+11		
(11)	3-Methyl-	264	259	−5		4·1
			290			
		355	355	0		
	9-Methyl-	256	261	+5	1·1	
		326	335	+9		
(21)	3,7-Dimethyl-		227			
			294			
			358			

[a] All measurements in aqueous buffer solutions, at room temperature; N = neutral form; C = cation; pK values were determined by spectrophotometric methods. [b] 'Aromatic' refers to structures like (c) and (d) in Scheme 1; 'quinonoid' to structures like (a) and (b).

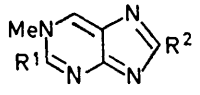
pyrimidine ring exhibit pK values in the range 4—5. On the other hand, the parent compound of each series and the corresponding 7- and 9-methyl derivatives show pK 1—3. Thus in general, the basicity of the quinonoid 1- and 3-methyl derivatives is much higher than that of the purines with an NH or NMe group in the imidazole ring. Hence the pK values of compounds (2)—(8) (Table 1) support the assumption that their neutral forms can all be represented essentially as imidazole-NH tautomers (c) and (d) (Scheme 1).

Introduction of methylthio-groups causes shielding of the aromatic protons in the purine ring.[11] The effect



(9) a; $R^1 = R^2 = H$
b; $R^1 = R^2 = Me$

(10) R = H
(11) R = SMe



(12) $R^1 = R^2 = H$
(13) $R^1 = H$, $R^2 = SMe$
(19) $R^1 = R^2 = SMe$

depends on the position of the SMe groups and increases with their number (Table 2).

Protonation of Methylthiopurines.—The four tautomers (a)—(d) of the neutral form of purine (1) (Scheme 1) can produce six tautomeric cations: 1,9-di-NH (e); 3,7-di-NH (f); 1,7-di-NH (g); 7,9-di-NH (h); and the 1,3- and 3,9-di-NH forms (not shown). Tautomer (h) and the 1,3-di-NH form represent amidinium-like 'fixed' * cations, whereas the other four tautomers permit charge distribution between the pyrimidine and imidazole systems, as indicated in Scheme 1 for structures (e)—(g) ('resonating cations'). The 3,9-di-NH tautomer would also fall into the latter category; however its contribution is probably very small, because of steric interference between the two NH groups.[1,2,12]

The question arises as to whether resonance distribution of the charge over both rings is preferred to 'fixation'. Calculations by Neiman, using the CNDO/2 method, show that protonation of the neutral forms (1c) and (1d) of purine in the pyrimidine system is energetically preferred over formation of the amidinium-like cation, resulting from attachment of the proton to the 7-position (1d) or to N-9 (1c).[12]

That purine is protonated at N-1 is confirmed by splitting of the 2-H and 6-H n.m.r. signals in the cation.[11] We have found the same for the cations of 9-methylpurine and of 8-methylthiopurine; the latter is thus represented by (2e) and (2g).

The two different modes of cation formation can be distinguished experimentally by the magnitude of the downfield shifts of the n.m.r. signals for the protons in

* This term indicates cations in which resonance distribution of the charge is confined to one ring of the purine system.

[11] W. C. Coburn, M. C. Thorpe, J. A. Montgomery, and K. Hewson, *J. Org. Chem.*, 1965, **30**, 1110; 1965, **30**, 1114.
[12] Z. Neiman, *Israel J. Chem.*, 1972, **10**, 819.

the purine ring. We have reported previously that in hypoxanthine and purine-6-thione, protonation takes place in the imidazole ring to produce 'fixed' cations like (9a).[4] This is shown in the value of $\Delta\delta(N - C)_{8\text{-}H}$ * (*ca.* 1 p.p.m.). Thus the 8-H signal of the 'fixed' cations mentioned (δ 9·1—9·2) is in a similar position in the n.m.r. spectra of the 7,9-dimethylhypoxanthinium ion (9b) and the 7,9-dimethylxanthinium ion ($\delta_{8\text{-}H}$ in both cases 9·11 p.p.m.). Similarly the $\delta_{2\text{-}H}$ value for the corresponding, positively charged 3,7-dimethyl derivatives, show deshielding of 8-H by not more than 0·7 p.p.m.

Thus we may assume that a $\Delta\delta(N - C)$ value of about 1 p.p.m. is diagnostic for the formation of 'fixed' cations, such as tautomer (h) in Scheme 1.

Combination of deductions from n.m.r. and u.v. spectra and p*K* values (Tables 1 and 2) leads to the conclusions about protonation summarised in Table 3.

TABLE 2

N.m.r. spectra of purines [a]

		δ (p.p.m.)														
		2-H			6-H			8-H			2-S·CH_3		6-S·CH_3		8-S·CH_3	
No.	Compound	N [b]	C [b]	Δ [b]	N	C	Δ	N	C	Δ	N	C	N	C	N	C
(1)	*Purine*	9·02	9·30 [c]	0·28	9·22	9·51 [c]	0·29	8·63	9·01	0·38						
(12)	1-Methyl-	8·98 [c]	9·50 [c]	0·52	9·13 [c]	9·80 [c]	0·67	8·65	9·13	0·48						
(2)	8-*Methylthio-purine*	8·74	9·24 [c]	0·50	8·68	9·21 [c]	0·53								2 79	2·88
(13)	1-Methyl-	8·74 [c]	9·23 [c]	0·49	8·66 [c]	9·31 [c]	0·65								2·73	2·87
(3)	6-*Methylthio-purine*	8·78	8·89	0·11				8·46	8·78	0·32			2·76	2·78		
	1-Methyl-	8·79	9·20	0·41				8·40	8·77	0·37			3·41	3·48		
	3-Methyl-	8·81	9·29	0·48				8·20	8·97	0·77			2·84	2·93		
	7-Methyl-	8·84	8·91	0·07				8·50	8·84	0·34			2·79	2·82		
	9-Methyl-	8·79	8·94	0·15				8·41	8·76	0·35			2·77	2·79		
	3,7-Dimethyl-		9·24						8·87					2·92		
	7,9-Dimethyl-		9·05						9·44					2·99		
(4)	2-*Methylthio-purine*				8·90	9·20	0·30	8·36	8·98	0·62	2·61	2·67				
(5)	2,6-*Bismethylthio-purine*							8·22	8·69	0·47	2·66	2·69	2·77	2·79		
	1-Methyl-							8·00	8·64	0·64	2·72	2·76	3·57	3·65		
(17)	3-Methyl-							8·03	8·75	0·72	2·87	2·92	2·83	2·92		
(14)	7-Methyl-							8·31	8·78	0·47	2·62	2·63	2·74	2·75		
(15)	9-Methyl-							8·23	8·53	0·30	2·68	2·68	2·74	2·75		
(16)	7,9-Dimethyl- (cation)								9·56			2·72			2·83	
(18)	3,7-Dimethyl- (cation)								8·74			2·86		2·86		
(6)	6,8-*Bismethylthio-purine*	8·52	8·73	0·21									2·73	2·80	2·69	2·82
(10)	3-Methyl-	8·51	9·07	0·56									2·81	2·89	2·73	2·87
(7)	2,8-*Bismethylthio-purine*				8·44	8·77	0·33				2·60	2·65			2·66	2·80
(19)	1-Methyl-				8·65	9·26	0·61				2·81	2·88			2·73	2·87
(8)	2,6,8-*Trismethyl-thiopurine*										2·63	2·66	2·71	2·74	2·71	2·79
(11)	3-Methyl-										2·81	2·90	2·85	2·90	2·73	2·86
	9-Methyl-										2·72	2·73	2·79	2·79	2·68	2·67
(21)	3,7-Dimethyl- (cation)											2·91		2·91		2·91

[a] All measurements in $(CD_3)_2SO$–D_2O (9 : 1 v/v) at 70°. The methods used to assign individual signals to aromatic protons or to methylthio-groups will be described in a separate paper. [b] N = Neutral form; C = cation; Δ = difference (N − C). [c] Doublet, *J ca.* 1·1 Hz.

cation of 3-methylhypoxanthine (9·24 p.p.m.) is close to that of the 'fixed' 1,3-dimethylhypoxanthinium ion ($\delta_{2\text{-}H}$ 9·50 p.p.m.). The same large shift of the 8-H signal of 1 p.p.m. or more is also observed when one proceeds from the neutral forms of 6-methylthio- or 2,6-bismethylthiopurine and their 7- and 9-methyl derivatives to the corresponding 'fixed' 7,9-dimethyl cations (Table 2). On the other hand, in the 'resonating' cations of purine (1e)—(1g) both the 2- and 8-H bands show deshielding by only 0·2—0·4 p.p.m. (Table 2).

In comparison with the uncharged forms of 3-methyl-6-methylthio- and 3-methyl-2,6-bismethylthiopurine, the Assignment of structure (6f) to the cation of 6,8-bismethylthiopurine is based on the observation that the $\lambda_{max.}$ values of this cation are close to those of the 3-methyl derivative (10). Protonation of 2,8-dimethylthiopurine presumably involves N-1 (7e) and (7g): the spectrum of this cation is similar to that of its 1-methyl derivative (19).

The cation of 2,6-bismethylthiopurine (5) has a u.v. absorption curve closely resembling those of the cations of the 7- (14) and 9-methyl derivatives (15) and of the

* This symbol represents the difference between the δ values of the neutral form and the cation.

7,9-dimethyl-2,6-bismethylthiopurinium cation (16). Thus the tautomer (5h) probably makes some contribution to the cation. On the other hand, if compounds (5), (14), and (15) were to undergo protonation exclusively in the imidazole ring, one would expected $\Delta\delta$(N — C) values near 1 to bring the chemical shifts of the 8-H signals close to the value for compound (16) ($\delta_{8\text{-H}}$ 9·56). However, the 8-H signals in the cations of (5), (14), and (15) are in the range δ 8·53—8·78, *i.e.* the $\Delta\delta$ values are only 0·3—0·47 p.p.m. (Table 2). Therefore it appears that the cations of (5), (14), and (15) are mixtures of tautomers (e), (g), and (h).

Methylation of Methylthiopurines in Polar, Aprotic Solvents.—In the irreversible methylation reactions of the neutral molecules, the ambiguities which were encountered in the attempts to localise the protonation processes are eliminated. On the other hand, methylation may be expected to be more sensitive to steric interference than protonation. Therefore the rules establishing the preferred sites of cation formation in methylthiopurines may not apply to the alkylation reactions.

Purine is attacked at N-1. The product (12) is known;[5] it shows doublets for the 2- and 6-H n.m.r. signals, regardless of the state of ionisation (Table 2). Likewise, 8-methylthiopurine (2) forms the 1-methyl derivative (13), which again shows doublets (J 1·1 Hz) for the signals of its aromatic protons.

The course of methylation of compound (4) was not established unequivocally, because the product was not stable and no well characterised crystalline derivative was isolated. However, n.m.r. measurements on the crude product suggest that positions 7 and 9 are attacked (see Experimental section).

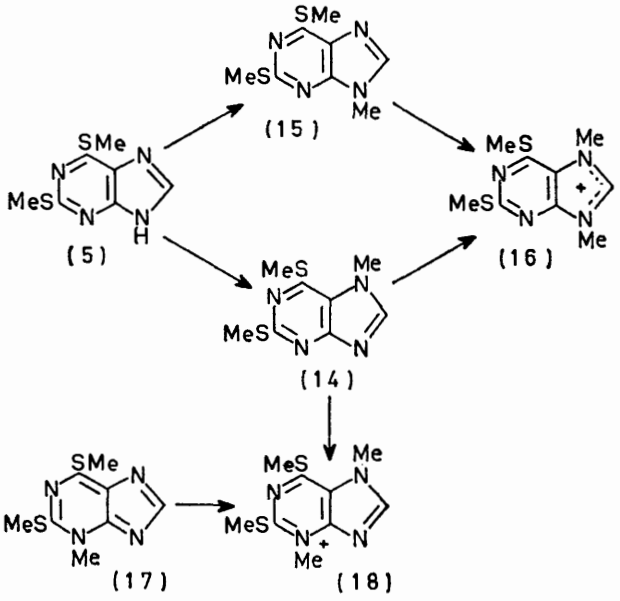
Alkylation of compound (3) by methyl iodide at N-3 has been described previously.[13]

2,6-Bismethylthiopurine (5) is attacked simultaneously at N-7 and N-9, the end-product being the 7,9-dimethyl-2,6-bismethylthiopurinium cation (16) (Scheme 2). Chromatographic analysis of samples removed at various times from the reaction mixture revealed the presence of both intermediates (14) and (15), the latter predominating. Both (14) and (15) were identified by comparisons with samples produced by independent syntheses (see Experimental section), which were also converted into compound (16) by treatment with methyl iodide. Assignment of structure (16) to the end-product follows from this fact. The compound shows properties characteristic of a ' fixed ' cation of this form, *e.g.* the 8-proton is exchanged instantaneously in D_2O.[14,15]

When the free base of compound (14) was treated with methyl iodide in dimethylformamide at 90°, then, in addition to (16), small amounts of the 3,7-dimethyl derivative (18) were isolated. The latter was the only product obtained from alkylation of 3-methyl-2,6-bismethylthiopurine (17) (see Scheme 2). Structure of (18) was established by its formation from both (14) and (17).

[13] Z. Neiman and F. Bergmann, *Israel J. Chem.*, 1965, **3**, 161.
[14] Z. Neiman, *J. Chem. Soc.* (*C*), 1970, 91.

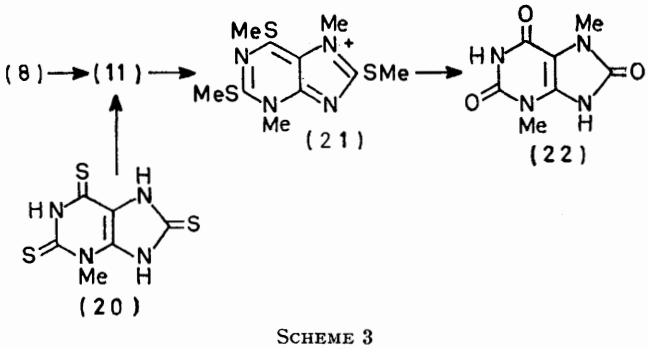
6,8-Bismethylthiopurine (6) yielded the 3-methyl derivative (10). The latter was also obtained from the known 3-methyl-6,8-dithioxanthine [16] by *S*-methylation.



SCHEME 2

2,8-Bismethylthiopurine (7) is attacked at N-1. Assignment of structure (19) to the product is based on nuclear Overhauser effect (NOE) studies, *i.e.* the area of the 6-H n.m.r. signal was increased by 20% upon irradiation at the frequency of the *N*-methyl signal. The 6-H band of compound (19) is shifted by 0·61 p.p.m. upon cation formation; this figure is similar to the value of $\Delta\delta$(N — C)$_{6\text{-H}}$ for compound (13). In fact the absolute values of $\delta_{6\text{-H}}$ for structures (13) and (19) are very close to one another.

The 2,6,8-trismethylthio-compound (8) undergoes a two-step alkylation reaction. The first product is the



SCHEME 3

3-methyl derivative (11), which was identified by comparison with the product of *S*-methylation of 3-methyl-2,6,8-trithiouric acid (20). Upon extended exposure to

[15] D. Lichtenberg, F. Bergmann, and Z. Neiman, preceding paper.
[16] G. Elion, *J. Org. Chem.*, 1962, **27**, 2478.

methyl iodide, (11) was transformed into the 3,7-dimethyl derivative (21) (Scheme 3), identified by conversion into the known 3,7-dimethyluric acid (22) [17] (see Experimental section).

A summary of all methylation reactions is included in Table 3.

TABLE 3

Protonation and methylation of methylthiopurines

No.	Compound	Protonation at position	Methylation at position
(1)	Purine	1	1
(2)	8-Methylthiopurine	1	1
(3)	6-Methylthiopurine	(Pyrimidine) [a]	3
(4)	2-Methylthiopurine	(Pyrimidine + imidazole) [a]	(Imidazole)
(5)	2,6-Bismethylthiopurine	(Pyrimidine + imidazole) [a]	7 and 9
(6)	6,8-Bismethylthiopurine	3	3
(7)	2,8-Bismethylthiopurine	1	1
(8)	2,6,8-Trismethylthiopurine	(Pyrimidine + imidazole) [a]	3

[a] These cations are probably mixtures of tautomers. [b] Because of decomposition of the methylation product, the exact site of substitution was not established.

protonation. However, steric interference may change the course of alkylation. Thus in 2,6-bismethylthiopurine (5), attack is directed towards positions 7 and 9, although protonation probably involves both the pyrimidine and imidazole rings. This we ascribe to hindrance by the bulky methylthio-substituents to attack at the pyrimidine ring. This conclusion is supported by the observation that the trismethylthio-derivative (8) is methylated exclusively at N-3, *i.e.* when steric factors in the two ring systems of purine are equal, the formation of type (f) transition states (Scheme 1; 3-H replaced by 3-Me) is favoured over those of type (h). In compound (8) substitution at N-1 is hindered by the presence of methylthio-substituents at both positions 2 and 6.

EXPERIMENTAL

M.p.s were determined with a Fisher–Johns apparatus. For u.v. spectra, a Hitachi–Perkin-Elmer model 124 spectrophotometer was used. N.m.r. spectra were measured with a JEOL MH-100 instrument, at 70°, with TSP (sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate; Merck, Sharp, and Dohme) as internal standard. For NOE experiments, the

TABLE 4

Methylation of methylthiopurines in aprotic solvents

Purine used	No.	Reaction conditions: Solvent	Temp. (°C)	Time (h)	Product	No.	Yield (%)	M.p. or decomp. (°C)	R_F [a] (A) (B) (Fluorescence)	Cryst. solvent	Crystals
Purine	(1)	MeCN	70	1	1-Methylpurine	(12)	35	235		EtOH	Pale yellow plates
8-Methylthiopurine	(2)	MeCN–Me_2N·CHO	70	0·7 [b]	1-Methyl-8-methylthiopurinium iodide	(13)	22	210—215 (decomp.)	0·60 0·68 (blue)	EtOH	Yellow microcrystals
6,8-Bismethylthiopurine	(6)	MeCN	70	2 [c]	3-Methyl-6,8-bismethylthiopurine	(10)	66	236—237	0·78 0·72 (violet)	EtOAc	Pale yellow needles
2,8-Bismethylthiopurine	(7)	MeCN	70	3 [c]	1-Methyl-2,8-bismethylthiopurine	(19)	56	220—222	0·81 0·71 (blue)	EtOAc	Colourless needles
2,6-Bismethylthiopurine	(5)	MeCN	70	5 [b]	7,9-Dimethyl-2,6-bismethylthiopurinium iodide	(16)	58	192—194	0·76 0·70 (greenish)	EtOH	Pale yellow needles
9-Methyl-2,6-bismethylthiopurine	(15)	Me_2N·CHO	90	4 [b]	7,9-Dimethyl-2,6-bismethylthiopurinium iodide [d]	(16)	90				
3-Methyl-2,6-bismethylthiopurine	(17)	Me_2N·CHO	90	4 [b]	3,7-Dimethyl-2,6-bismethylthiopurinium iodide	(18)	95	188—189	0·57 0·60 (greenish)	EtOH	Yellow needles
2,6,8-Trismethylthiopurine	(8)	MeCN	70	4 [e]	3-Methyl-2,6,8-trismethylthiopurinium iodide	(11)	16	168—170	0·86 0·76 (violet)	Petroleum (b.p. 60—80°)	Yellow needles
					and 3,7-Dimethyl-2,6,8-trismethylthiopurinium iodide	(21)	42	201—203	0·77 0·66 (yellow)	EtOH	Yellow plates
3-Methyl-2,6,8-trismethylthiopurine	(11)	MeCN	70	4	3,7-Dimethyl-2,6,8-trismethylthiopurinium iodide	(21)	49				

[a] For solvents (A) and (B), see Experimental section. Spots were located by their fluorescence under a Mineralight u.v. lamp (λ *ca.* 255 nm). [b] After evaporation of the solvent, the residue was triturated with ethyl acetate and then recrystallised from a minimal volume of ethanol. [c] After evaporation of the solvent, the residue was triturated with 25% ammonia and the insoluble portion recrystallised. [d] The same product, together with (18), was obtained by treating 7-methyl-2,6-bismethylthiopurine (14) with methyl iodide in dimethylformamide at 90°; separation of (16) and (18) by paper chromatography. [e] After evaporation of the solvent, the residue was dried in a vacuum desiccator and then extracted with boiling petroleum (b.p. 60—68°). After cooling this solution, compound (11) crystallised as the free base. The insoluble portion (21) was recrystallised from ethanol.

TABLE 5

Analyses for products in Table 4

No.	Formula	Found (%) C	H	N	S	Required (%) C	H	N	S
(13)	$C_7H_9N_4SI$	27·2	3·0	18·0	10·4	27·3	2·9	18·2	10·4
(10)	$C_8H_{10}N_4S_2$	42·4	4·8	25·0	28·6	42·5	4·4	24·8	28·3
(19)		42·95	4·6		28·9				
(11)	$C_9H_{12}N_4S_3$	39·6	4·4	20·4	35·1	39·7	4·4	20·6	35·3
(16)	$C_9H_{13}N_4S_2I$	29·1	3·8	15·3	17·0 [a]	29·3	3·5	15·2	17·4 [c]
(18)		29·1	3·5	15·2	17·3 [b]				
(21)	$C_{10}H_{15}N_4S_3I$	28·9	3·5	13·2	22·9 [d]	29·0	3·6	13·5	23·2 [e]

[a] I, 35·0%. [b] I, 34·75%. [c] I, 34·5%. [d] I, 30·3%. [e] I, 30·7%.

Conclusions.—In all protonation reactions, formation of 'resonating' cation is preferred to that of 'fixed' cations. In the absence of influence by steric factors, methylations are directed to the same positions as

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built-in oscillator of the JEOL instrument was used for irradiation.

For paper chromatography on Whatman No. 1 sheets, the following solvents were used: (A) n-butanol–acetic acid–water (12 : 3 : 5 v/v); (B) ethanol–dimethylformamide–water (3 : 1 : 1 v/v).

Purines.—The following purines were synthesised by known methods: 2-methylthiopurine (4);[18] 6-methylthiopurine (3) and its *N*-methyl derivatives;[13] 8-methylthiopurine (2);[18] 2,6-bismethylthiopurine (5)[19] and its 7-methyl derivative (14);[20] 6,8-bismethylthiopurine (6);[21] 2,8-bismethylthiopurine (7);[22,23] 2,6,8-trismethylthiopurine (8);[23] 1,3-dimethylhypoxanthinium ion;[15] 7,9-dimethylhypoxanthinium ion;[24] 9-methylxanthine;[25] 7,9-dimethylxanthinium ion.[26]

Pyrimidines.—4,5-Diamino-2-thiouracil was a product of Aldrich Chemical Co. 4,5-Diamino-3-methyl-2-thiouracil was prepared according to Levin *et al.*[27]

Methylation of 2-Methylthiopurine (4).—A solution of 2-methylthiopurine (4) (0·5 g) in acetonitrile (100 ml) was refluxed with methyl iodide (2 ml) for 1 h. The solvent was removed *in vacuo* and the crude, oily product dissolved in trifluoroacetic acid. The n.m.r. spectrum showed three S·CH_3 signals (δ 2·87, 2·91, and 2·92 p.p.m.), in the same range as the methylthio-bands for the cations shown in Table 2. Furthermore, four N·CH_3 signals were visible: δ 4·23 (corresponding to the 9-CH_3 bands for the cations of Table 2), and 4·38 and 4·41 p.p.m. (in the region of the 7-methyl signals for the cations of Table 2). In addition, aromatic proton signals were observed at δ 9·50, 9·54, and 9·73 p.p.m. It is thus probable that the crude alkylation product contained a mixture of 7- and 9-methyl derivatives (and perhaps also the 7,9-dimethyl derivative). All attempts to isolate pure products failed.

1-*Methyl*-2,6-*bismethylthiopurine Picrate.*—A solution of 1-methyl-2-methylthiopurine-6-thione[16] (2 g) and methyl iodide (5 ml) in dimethylformamide (200 ml) was kept at room temp. for 2 h. The crude product was precipitated by addition of ether (500 ml) and was converted directly into the picrate by treatment with ethanolic 4% picric acid (50 ml), to give yellow prismatic *plates* (0·5 g, 12%), m.p. 206—210° (from ethanol), R_F (A) 0·62, (B) 0·68; greenish-blue fluorescence (Found: C, 36·8; H, 2·85; N, 21·4; S, 14·0. $C_{14}H_{13}N_7O_7S_2$ requires C, 36·9; H, 2·9; N, 21·5; S, 14·1%).

3-*Methyl*-2,6-*bismethylthiopurine* (17).—A solution of 3-methyl-2,6-dithioxanthine[16] (4 g) in 2N-sodium hydroxide (250 ml) was stirred at room temp. with methyl iodide (10 ml) for 15 min. The precipitate formed *rods* (3·5 g, 77%), m.p. 177—178° (from ethanol) (Found: C, 42·15; H, 4·1; N, 25·1; S, 27·9. $C_8H_{10}N_4S_2$ requires C, 42·5; H, 4·4; N, 24·8; S, 28·3%).

9-*Methyl*-2,6-*bismethylthiopurine* (15).—(*a*) A mixture of 9-methylxanthine (3 g), phosphorous pentasulphide (9 g), and β-picoline (200 ml) was stirred and refluxed for 6 h. The solvent was removed *in vacuo* and the residue stirred and refluxed with water (200 ml) for 15 min. The insoluble portion was dissolved in 2N-sodium hydroxide; the solution was decolourised with charcoal and acidified with glacial acetic acid. 9-Methyl-2,6-dithioxanthine (2 g, 56%) showed $\lambda_{max.}$ (pH 7) 345 nm. The crude product was used directly for the next step.

(*b*) A solution of the foregoing product (2 g) in 2N-sodium hydroxide (100 ml) was stirred at room temp. with methyl iodide (2 ml) for 10 min. The precipitate (15) was filtered off, dried (P_2O_5), and recrystallised from light petroleum (b.p. 60—80°) to give *needles* (1 g, 43%), m.p. 161—162° (Found: C, 42·85; H, 4·5; N, 25·1; S, 28·9. $C_8H_{10}N_4S_2$ requires C, 42·5; H, 4·4; N, 24·8; S, 28·3%).

3-*Methyl*-6,8-*bismethylthiopurine* (10).—A solution of 3-methyl-6,8-dithioxanthine[16] (140 mg) in 2N-sodium hydroxide (5 ml) was treated at room temperature with dimethyl sulphate (500 mg). The precipitate was crystallised from ethyl acetate to give needles (80 mg, 50%), m.p. 236—237°, identical with the product described in Table 4.

3-*Methyl*-2,6,8-*trismethylthiopurine* (11).—(a) 3-*Methyl*-2,8-*dithiouric acid.* A solution of 4,5-diamino-3-methyl-2-thiouracil (20 g) in pyridine (200 ml) was stirred with solid sodium hydroxide (2 g) and carbon disulphide (18 ml) and refluxed for 5 h. The volatile components were removed *in vacuo* and the residue was dissolved in 2N-sodium hydroxide; the solution was decolourised with charcoal and acidified with conc. hydrochloric acid. The product was purified by reprecipitation (yield 20 g, 80%) to give *microcrystals*, decomp. >300°; $\lambda_{max.}$ (pH 7) 249 and 321 nm (Found: C, 33·1; H, 2·4; N, 25·7; S, 29·5. $C_6H_6N_4OS_2$ requires C, 33·6; H, 2·8; N, 26·2; S, 29·9%).

(b) 3-*Methyl*-2,6,8-*trithiouric acid* (20). A mixture of 3-methyl-2,8-dithiouric acid (30 g), phosphorous pentasulphide (100 g) and pyridine (1500 ml) was stirred and refluxed for 5 h. The mixture was evaporated to dryness *in vacuo* and the residue treated with boiling water (500 ml) for 30 min. The insoluble portion was dissolved in 2N-sodium hydroxide (charcoal) and the product (20) precipitated with conc. hydrochloric acid as *microcrystals*, decomp. >300° (17 g, 53%); $\lambda_{max.}$ (pH 6) 279 and 393 nm (Found: N, 24·8; S, 42·1. $C_6H_6N_4S_3$ requires N, 24·3; S, 41·7%).

(c) 3-*Methyl*-2,6,8-*trismethylthiopurine* (11). A solution of compound (20) (5 g) in 2N-sodium hydroxide (200 ml) was stirred with methyl iodide (10 ml) at room temp. for 15 min. The precipitate crystallised from light petroleum (b.p. 60—80°) as needles (3 g, 51%), m.p. 168—170°, identical with compound (11) resulting from methylation of (8) (see Table 4).

9-*Methyl*-2,6,8-*trismethylthiopurine.*—(a) 9-*Methyl*-2,8-*dithiouric acid.* A solution of 4,5-diamino-2-thiouracil (16 g) and methyl thiocyanate (10 g) in pyridine (150 ml) was refluxed for 3 h. The precipitate obtained on cooling was washed with acetone and then heated for 5 h with conc. hydrochloric acid (200 ml) with stirring. The hot solution was filtered through sintered glass. The solid separating from the filtrate upon cooling was purified by dissolution in ammonia and reprecipitation with glacial acetic acid to give *microcrystals*, m.p. >300° (10 g, 46%); $\lambda_{max.}$ (pH 8)

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244 and 300 nm; (pH 1) 245 and 318 nm (Found: C, 33·3; H, 3·0; N, 26·2; S, 29·7. $C_6H_6N_4OS_2$ requires C, 33·6; H, 2·8; N, 26·2; S, 29·9%).

(b) 9-*Methyl*-2,6,8-*trithiouric acid*. A mixture of the foregoing compound (5 g), phosphorous pentasulphide (10 g), and pyridine (150 ml) was stirred and refluxed for 3 h. The solvent was evaporated off and the residue decomposed with hot water; the insoluble portion was washed with acetone and purified from ammonia–acetic acid to give yellow *microcrystals* (2·5 g, 47%), m.p. >300°; $\lambda_{max.}$ (pH 8) 266, 305sh, and 374 nm (Found: C, 31·1; H, 2·6; N, 24·3; S, 42·4. $C_6H_6N_4S_3$ requires C, 31·3; H, 2·6; N, 24·3; S, 41·7%).

(c) 9-*Methyl*-2,6,8-*trismethylthiopurine*. A solution of the foregoing compound (1 g) in 6% sodium hydroxide (50 ml) was stirred at room temp. with dimethyl sulphate (4 ml), the solution being kept alkaline. The precipitate (0·5 g, 42%) crystallised from ethanol in *needles*, m.p. 155—156° (Found: C, 39·6; H, 4·8; N, 20·4; S, 35·0. $C_9H_{12}N_4S_3$ requires C, 39·7; H, 4·4; N, 20·6; S, 35·3%).

Two-step Conversion of 3,7-*Dimethyl*-2,6,8-*trismethylthiopurinium cation* (21) *into* 3,7-*Dimethyluric Acid* (22).—(*a*) A solution of compound (21) (200 mg) in 2N-sodium hydroxide (40 ml) was stirred and refluxed for 2 h, the starting material had then all dissolved. Treatment with glacial acetic acid precipitated 3,7-dimethyl-8-methylthioxanthine, needles (100 mg), m.p. 268° (from ethanol) (lit.,[28] 263°).

(*b*) A suspension of the foregoing product in 6N-hydrochloric acid was stirred and refluxed for 6 h. The product, isolated after cooling, was identical with authentic 3,7-dimethyluric acid.[17]

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