Tautomerism, Ionisation, and Methylation of 2-(Methylthio)- and 2,8-Bis-(methylthio)-hypoxanthines

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Anion formation in 2-(methylthio)- and 2,8-bis(methylthio)-hypoxanthine occurs first at the 1-NH group and then at 7(9)-NH. Protonation involves the imidazole ring, with the exception of the 3-methyl derivatives, which attach the proton to N-1.

3-Methylhypoxanthine is methylated to form the 1,3-dimethylhypoxanthinium cation, but in the 3-methyl derivatives of 2-(methylthio)- and 2,8-bis(methylthio)-hypoxanthine attack at N-7 is predominant over that at N-1. Formation of the cations $[RN{=}C(SMe){-}NMe]^+$ is followed by *S*-demethylation.

In previous studies we have attempted to determine the predominant tautomeric structure and to localise the ionisation processes of hypoxanthine [purin-6(1*H*)-one] and its *N*-methyl derivatives in aqueous solutions.[1,2] 2-(Methylthio)- and 2,8-bis(methylthio)-derivatives of hypoxanthine are now available,[3,4] and we describe here our observations on these two new series of purines.

TABLE 1

U.v. absorption and p*K* values of 2-(methylthio)hypoxanthines

Compound	Position of *N*-methyl groups	$\lambda_{max.}$/nm (log ε) Neutral form	Monoanion	Cation	p*K* Anion formation at N-1	Anion formation at N-7(9)	Cation formation at N-1	Cation formation at N-9(7)
Class (a)								
(1)		262(4·19)	271*(4·16)	264·5(4·21)	7·6	12		2·4
(2)	1	262(4·06)	272(4·06)	265·5(4·14)		9·5		3·1
(3)	7	265(4·22)	274·5(4·15)	266(4·27)	8·0			2·5
(4)	9	263(4·20)	272·5(4·20)	266(4·32)	9·5			1·5
Class (b)								
(5)	3	237·5(4·14)	234(4·31)	232·5(4·06)		8·7	0	
		279(4·20)	283(4·13)	278(4·04)				
(6)	3, 7	232(4·12)		228(4·06)			−0·2	
		279(4·16)		272(4·10)				

* For the dianion of (1), $\lambda_{max.}$ 275 nm (log ε 4·17).

TABLE 2

N.m.r. spectra {δ in [$^2H_6$]dimethyl sulphoxide–[^{2}H]water (9 : 1)} of 2-(methylthio)hypoxanthines *

Compound	Position of methyl groups	8-H N	A_1	Δ(N − A_1)	A_2	Δ(A_1 − A_2)	C	Δ(N − C)	2-SMe N	A_1	Δ(N − A_1)	A_2	C	Δ(N − C)	NMe N	A	Δ(N − A)	C	Δ(N − C)
Class (a)																			
(1)		7·84	7·64	0·20	7·24	0·40	8·99	−1·15	2·50	2·40	0·10	2·36	2·59	−0·09					
(2)	1	7·84	7·35	0·49			8·92	−1·08	2·57	2·54	0·03		2·59	−0·02	3·46	3·44	0·02	3·48	−0·02
(3)	7	7·84	7·64	0·20			8·90	−1·06	2·44	2·35	0·09		2·54	−0·10	3·88	3·88	0	4·04	−0·16
(4)	9	7·78	7·58	0·20			8·86	−1·08	2·45	2·35	0·10		2·54	−0·09	3·65	3·63	0·02	3·83	−0·18
Class (b)																			
(5)	3	7·69	7·26	0·43			8·24	−0·55	2·53	2·53	0		2·73	−0·20	3·70	3·66	0·04	3·86	−0·16
(6)	{3	7·90					8·42	−0·52	2·70				2·99	−0·29	3·87			4·16	−0·29
	{7														4·11			4·20	−0·09

* N = Neutral form, A_1 = monoanion, A_2 = dianion, C = cation.

(I) 2-(*Methylthio*)*hypoxanthines.*—(*a*) *Tautomerism of neutral molecules.* It was shown previously [2] that the predominant tautomer of hypoxanthine is the 1-NH-9-NH-form. The u.v. spectra, recorded in Table 1, reveal the similarity of 2-(methylthio)hypoxanthine (1) and its 1- (2), 7- (3), and 9-methyl derivatives (4). These compounds thus form class (a). The long-wave absorption maxima of (5) and (6) are *ca.* 16 nm higher; these two derivatives form class (b). The identical value of $\lambda_{max.}$ for (5) and (6) supports the 7-NH-structure for the neutral molecule of the former (see Scheme 2).

The parent compound (1) contains a 1-NH-group, because its absorption maximum is identical with that of the 1-methyl derivative (2). On the other hand, the u.v. spectra do not permit us to assign a definite tautomeric form to the imidazole ring of (1) and (2). The chemical shift of the 8-hydrogen is identical in (1)—(3), while the value of δ_{8H} in (4) is 0·06 p.p.m. at higher field (Table 2). This difference is too small to conclude that (1) and (2)

[1] F. Bergmann, M. Kleiner, Z. Neiman, and M. Rashi, *Israel J. Chem.*, 1964, **2**, 185.

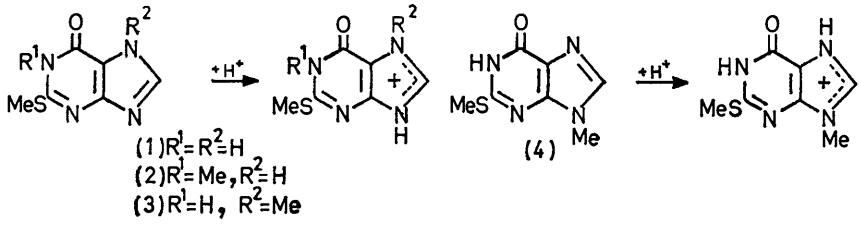
[2] D. Lichtenberg, F. Bergmann, and Z. Neiman, *Israel J. Chem.*, 1972, **10**, 805.

[3] U. Reichman, F. Bergmann, D. Lichtenberg, and Z. Neiman, *J. Org. Chem.*, 1973, **32**, 2066.

[4] U. Reichman, F. Bergmann, and Z. Neiman, *J. Org. Chem.*, 1973, in the press.

are the 7-NH-tautomers, but some support for this assumption will be given below.

(*b*) *Anion formation.* Only the parent compound (1) can form a mono- and a di-anion. If in (1), the 7(9)-NH-group were to lose a proton first, we should expect a pK value similar to that of (2). However, Table 1 shows a large difference of 1·9 units between the pK values of these two compounds, suggesting for (1) proton loss from 1-NH, then 7(9)-NH [written as 1 ⟶ 7(9)]. This is supported by the fact that the pK of (1) (7·6) is closest to that of the 7-methyl derivative (4). We thus have another indication that (1) may be present in aqueous solution mainly as the 1-NH-7-NH-tautomer.



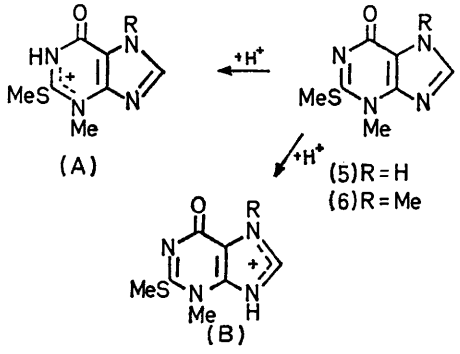
SCHEME 1 Cation formation in class (a)

In hypoxanthine, the sequence of anion formation has been established as 9(7) ⟶ 1.[1,2] Therefore the reversal of the sequence in (1) is surprising and requires further evidence. Table 2 gives important information about the upfield shift of the 8-H signals upon anion formation. In (3) and (4), $\Delta\delta_{(N-A)}$ * is 0·20 p.p.m.; this value is thus characteristic for dissociation of the 1-NH-group. On the other hand, dissociation of the imidazole NH-group in compounds (2) and (5) leads to a $\Delta\delta_{(N-A)}$ value of 0·46 ± 0·03 p.p.m. In compound (1), δ_{8H} shifts upfield by 0·20 p.p.m. for the first and by 0·40 p.p.m. for the second ionisation step, again indicating the sequence 1 ⟶ 7(9).

The chemical shifts of the 2-methylthio-groups support this conclusion (Table 2). Dissociation of the 1-NH-group in (3) and (4) displaces this signal by 0·09—0·10 p.p.m. to higher field. On the other hand, anion formation in (2) and (5), involving dissociation of the imidazole NH-group, is accompanied by a very small shielding of the SMe-signal [$\Delta\delta_{(N-A)}$ 0 and 0·03 p.p.m., respectively]. For compound (1) we find a difference of 0·10 for the first and of 0·04 p.p.m. for the second ionisation step and can thus again derive the sequence 1 ⟶ 7(9).

(*c*) *Cation formation.* All members of class (a) are protonated in the imidazole ring (Scheme 1). This follows from the large downfield shift of their 8-H signals, $\Delta\delta_{(N-C)}$ 1·10 ± 0·05 p.p.m. (Table 2). It has been demonstrated previously that formation of 'fixed', amidinium-like cations in hypoxanthines is accompanied by a deshielding effect of *ca.* 1 p.p.m.[2]

In contrast, in the cations of class (b), the downfield displacement of the 8-H signal is only 0·52—0·55 p.p.m. Thus the proton is predominantly attached to position 1, as shown in tautomer A of Scheme 2. This conclusion is supported by the data on the chemical shifts of the SMe groups (Table 2). The value of $\Delta\delta_{(N-C)}$ for 2-SMe is 0·20 in (5) and (0·29) p.p.m. in (6), while for the members of class (a) this value is 0·10 or less. Analogous observations can be made on the NMe signals (Table 2). When protonation takes place in the ring bearing the N-methyl substituent, the signal of the latter is displaced to lower field by 0·16—0·29 p.p.m. [compounds (3)—(6) in Table 2]. On the other hand, if protonation occurs in the non-methylated ring, the downfield shift is much smaller



SCHEME 2 Cation formation in class (b)

[0·02 and 0·09 p.p.m., respectively, in (2) and for the 7-methyl group in (6)].

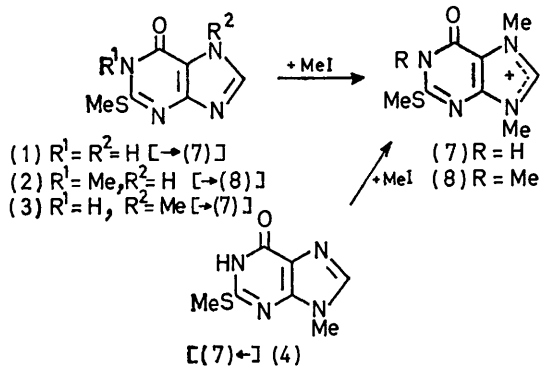
The value of $\Delta\delta_{(N-C)}$ for 8-H (0·54 ± 0·02 p.p.m.) in the 3-methyl derivatives (5) and (6) is much higher than the corresponding changes reported for 3-methyl- and 3,7-dimethyl-hypoxanthine (0·20 and 0·16 p.p.m.).[2] It is thus suggested that protonation of (5) and (6) may also partially involve the imidazole ring, *i.e.* the cations of these derivatives may be a mixture of tautomers A and B, form A predominating (Scheme 2).

In contrast to the hypoxanthine series,[1,2] the lowest pK values for cation formation are found in the 3-methyl derivatives of 2-(methylthio)hypoxanthine (Table 1). The 2-methylthio-substituent decreases the basic strength of a neighbouring nitrogen atom; conversely, it increases the acidity of an adjacent NH-group, as seen by comparing the pK for anion formation in (3) and (4) with the corresponding values of 7- (9·4) and 9-methylhypoxanthine (10·3).[2]

The formation of the tautomeric cations (5B) and (6B) and the low pK values of (5) and (6) may be ascribed to steric hindrance; approach of a proton to position 1 is made difficult by the bulky 2-SMe substituent.

* This symbol indicates the difference in δ-values between the neutral form (N) and the anion (A) or cation (C).

(*d*) *Methylation of the neutral molecules of 2-(methylthio)hypoxanthines.* In accordance with the direction of protonation, discussed in the previous section, all members of class (a) are attacked by methyl iodide in the imidazole ring to give as products (7) [5] and (8), respectively (Scheme 3).



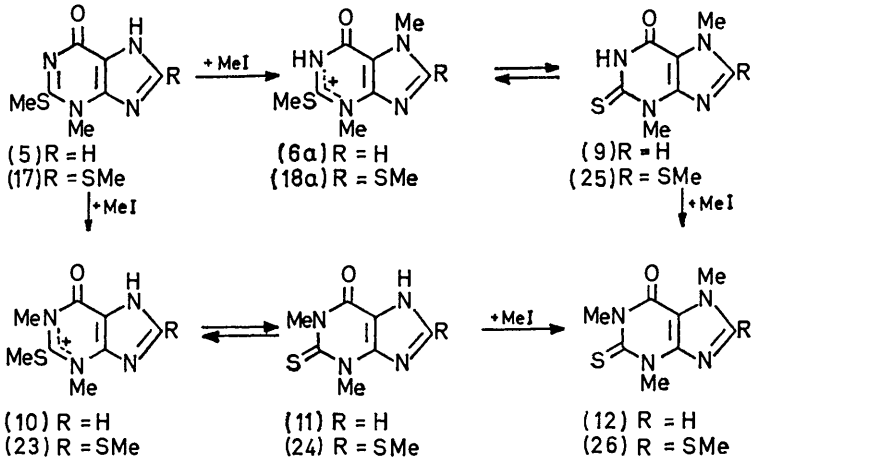
SCHEME 3 Methylation of neutral molecules of class (a)

In accordance with the formation of tautomeric cations (5A and B), we should expect (5) to undergo alkylation at N-1 and to a lesser degree at N-7. However at room temperature, (5) was attacked exclusively at N-7 with formation of the cation of (6) (see Scheme 4). When the solution of the latter was heated, the compound underwent *S*-demethylation to 3,7-dimethyl-2-thioxanthine (9). At room temperature, (9) reacted with methyl iodide to regenerate the cation of (6). However at 95° (in DMF), (9) was converted into 1,3,7-trimethyl-2-thioxanthine (12).[6]

When (5) was methylated under drastic conditions (95° for 5 hours), it was attacked both at N-1 and N-7 to give a mixture of (9), (11), and (12). In analogy to the observations on the cation of (6), we assume that (5) is first converted into the 'fixed' cation (10), the latter undergoing *S*-demethylation to 1,3-dimethyl-2-thioxanthine (11).[7] Both (9) and (11) are ultimately transformed into (12). It should be noted that (10) has not been identified as a reaction intermediate.

In Table 5 we compare the direction of protonation and methylation of 2-(methylthio)hypoxanthines. For evaluation of the parallelism of these two reactions, we have included the data on the corresponding hypoxanthines to show that in both series protonation and alkylation take identical courses, with the exception of compound (5). While 3-methylhypoxanthine is converted quantitatively into 1,3-dimethylhypoxanthinium ion,[8] (5) yields exclusively (6) under mild conditions. When the temperature is raised, alkylation at N-1 and formation of (11), presumably *via* (10) also occur. This we ascribe to steric interference of the 2-methylthio-substituent with the approach of a methyl group to position 1. Therefore methylation at N-7 is facilitated relative to reaction at position 1, while protonation of (5) proceeds predominantly at N-1 to give (5A) (see Scheme 2).

(II) 2,8-*Bis*(*methylthio*)*hypoxanthines.*—(*a*) *Tautomerism of neutral molecules.* Table 3 shows that the u.v.



SCHEME 4 Methylation of 3-methyl-2-(methylthio)- and 3-methyl-2,8-bis(methylthio)-hypoxanthine

absorption maxima of compounds (13)—(16) are identical [class (c)], suggesting that the parent compound (13) of this series is present in aqueous solution as the 1-NH-9-NH-tautomer. However, since the 7-methyl derivative of (13) is unknown, a final decision about the predominant tautomeric structure of the imidazole ring in (13) and (14) cannot be made at present.

The absorption maxima of the 3-methyl derivatives (17) and (18), which represent class (d), are 17·5 nm higher than those of class (c). Again we describe (17) as the 7-NH-tautomer.

(*b*) *Anion formation.* The p*K* value for the first dissociation step in (13) is identical with the p*K* of (15)

[5] J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, 1962, **84**, 1914.
[6] A. M. Khaletskii and M. S. Eshman, *Zhur. obshchei Khim.*, 1948, **18**, 2116.
[7] K. R. H. Wooldridge and R. Slack, *J. Chem. Soc.*, 1962, 1863.
[8] D. Lichtenberg and F. Bergmann, *J.C.S. Perkin I*, 1973, 789.

TABLE 3

U.v. absorption and pK values of 2,8-bis(methylthio)hypoxanthines

Compound	Position of N-methyl groups	$\lambda_{max.}$/nm (log ε) Neutral form	Monoanion	Cation	pK Anion formation at N-1	Anion formation at N9(7)	Cation formation at N-1	Cation formation at N-7(9)
Class (c)								
(13)		280(4·30)	289 [a](4·26)	283(4·36)	7·5	10·75		1·4
(14)	1	280(4·39)	286(4·36)	283(4·40)		8·2		1·7
(15)	9	280(4·21)	286(4·24)	283(4·20)	7·5			2·5
(16)	1, 9	280(4·32)		283(4·42)				1·6
(19) [b]	1	263(4·22) [b] 350(4·15)						
Class (d)								
(17)	3	297·5(4·30)	303(4·30)	310(4·16)		7·9	0·5	
(18)	3, 7	297·5(4·30)		310(4·18)			0·5	

[a] For the dianion of (13), $\lambda_{max.}$ 291·5 nm (log ε 4·26). [b] Measured in methanol.

TABLE 4

N.m.r. signals (δ) of 2,8-bis(methylthio)hypoxanthines *

Compound	Position of methyl groups	2-SMe N	2-SMe C	2-SMe Δ(N − C)	8-SMe N	8-SMe C	8-SMe Δ(N − C)	NMe N	NMe C	NMe Δ(N − C)
Class (c)										
(13)		2·64	2·65	−0·01	2·74	2·90	−0·16			
(14)	1	2·65	2·65	0	2·75	2·90	−0·15	3·65	3·70	−0·05
(15)	9	2·62	2·71	−0·09	2·76	2·98	−0·22	3·55	3·80	−0·25
(16)	1	2·60	2·72	−0·12	2·75	2·97	−0·22	3·60	3·70	−0·10
	9							3·57	3·82	−0·25
(19)	1	2·76	2·80	−0·04	2·84	3·00	−0·16	4·16	4·20	−0·04
Class (d)										
(17)	3	2·67	2·85	−0·18	2·69	2·75	−0·06	3·84	4·00	−0·16
(18)	3	2·62	2·85	−0·23	2·67	2·77	−0·10	3·72	4·00	−0·28
	7							3·87	3·87	0

* The signals of the anions could not be measured, because chloroform was the only solvent suitable for all derivatives of this series. All measurements were made in $CDCl_3$ at room temperature; for cations, CF_3CO_2H was added.

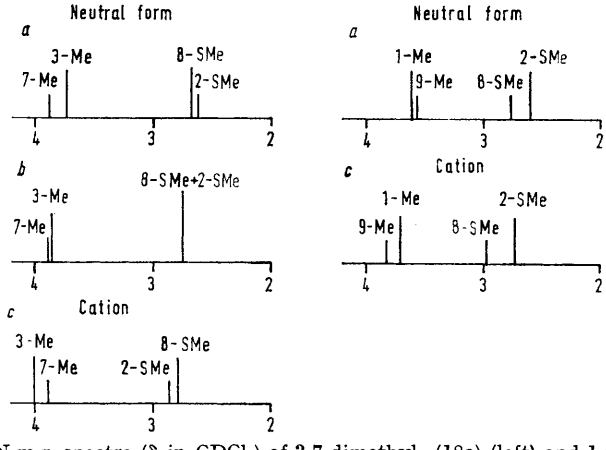
(Table 3), and is 0·7 units lower than the value of the 1-methyl derivative (14). It is thus suggested that the sequence of ionisation of (13) is 1 ⟶ 9(7), as in the case of compound (1).

Introduction of the 8-methylthio-substituent increases the acid strength of the imidazole NH-group. Thus the pK value of (14) is 0·4 units lower than that of 1-methylhypoxanthine and 1·3 units below that of (2). A similar increase of acidity is observed for the 3-methyl derivative (*cf.* Tables 1 and 3).

(*c*) *Cation formation.* Because of the lack of CH-groups in the ring, only the relative deshielding of the SMe- and NMe-signals can assist in the localisation of proton attachment in 2,8-bis(methylthio)hypoxanthines.

In the neutral molecules of both class (c) and (d), the 8-SMe group is deshielded relative to the 2-SMe substituent (Table 4). For the members of class (c), $\Delta\delta_{(N-C)}$ for 8-SMe is 0·15—0·22 p.p.m., while for the 2-SMe signals this difference is always much smaller. Therefore the distance of the two SMe-bands increases on going from the neutral form to the cation [see Figure, compound (16a)]. The same is true for (19), the 6-thio-analogue of (14). Thus all these derivatives are protonated in the imidazole ring.

In contrast, in class (d) $\Delta\delta_{(N-C)}$ is much larger for the 2- than for the 8-SMe group. A 'crossing-over' of the



N.m.r. spectra (δ in $CDCl_3$) of 3,7-dimethyl- (18a) (left) and 1,9-dimethyl-2,8-bis(methylthio)hypoxanthine (16a) (right); *a* compound only, *b* compound + 1 drop trifluoracetic acid. *c* compound + excess of trifluoracetic acid.

2- and 8-SMe-bands takes place during the transformation of the neutral molecules into their cations [Figure,

compound (18a)]. It is concluded that in (17) and (18) the proton is attached predominantly to N-1.

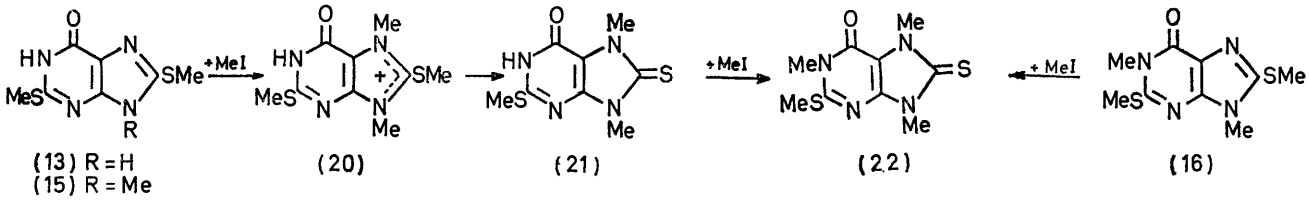
The above assignments of cationic structures are further supported by the data on NMe-signals (Table 4). Whenever protonation takes place in the ring bearing an *N*-methyl substituent, the signal of the latter moves downfield by a much larger value than is the case for protonation in the other ring. Thus the 1-methyl derivative (14), which attaches the proton to the imidazole ring, shows $\Delta\delta_{(N-C)}$ for 1-Me = 0·05 p.p.m. In contrast, in (15) and (16), $\Delta\delta_{(N-C)}$ for 9-Me is 0·25 p.p.m., indicating that cation formation again involves the imidazole ring; the corresponding difference of the 1-Me signal in (16) is only 0·10 p.p.m. In (17), $\Delta\delta_{(N-C)}$ for 3-Me = 0·16 and in (18), 0·28 p.p.m., but for the 7-methyl signal in (18) the difference is zero. Thus, (17) and (18) are protonated at N-1.

The greater shift of the 9-Me signal in (16a) and of the 3-Me signal in (18a) is responsible for ' crossing-over ' of the N-methyl bands in these two compounds upon cation formation (Figure).

The p*K* values for cation formation are lowest in class (d), *i.e.* N-1 in class (d) exhibits lower basicity than the imidazole nitrogens in class (c).

The 3-methyl derivative (17) behaves similarly to (5) (Scheme 4). At room temperature, only position 7 is attacked to give the cation of (18), but at elevated temperatures N-1 also participates in alkylation. The intermediate (23) undergoes 2-*S*-demethylation to (24); the analogous process converts the cation of (18) into (25). Both (24) and (25) are ultimately transformed into 1,3,7-trimethyl-8-methylthio-2-thioxanthine (26) (see Scheme 4).

The results of protonation and methylation reactions of this series are included in Table 5.



SCHEME 5 Methylation of neutral molecules of class (c)

The observation that (17) can be attacked either at N-1 or at N-7 suggests that protonation of (17) may also involve both these positions; however the physical data of Tables 2—4 are insufficient to support such a conclusion.

(III) *Mechanism of* S-*Demethylation.*—In the present study, *S*-demethylation has been observed for cations with the structure $[RN{=}C(SMe){-}NMe]^+$. When R = H, as in the formation of (6) from (5) and of (18) from (17), this intermediate could be identified on paper chromatograms or could sometimes be isolated, because methylation at N-7 proceeded at room temperature, while removal of methyl iodide from (6) and (18) required application of heat. On the other hand, if R = Me, *i.e.* in the conversions (5) ⟶ (10) or (17) ⟶ (23), only the demethylated products (11) and (24) were detected, since alkylation at N-1 took place only at elevated temperatures.

Similar reactions were observed in other series of purines. Thus 1-methyl-2,8-bis(methylthio)purine-6-thione (19) gave 1,7-dimethyl-2-(methylthio)purine-6,8-dithione (27), and 9-methyl-2,6,8-tris(methylthio)purine

TABLE 5

Comparison of positions of protonation and methylation

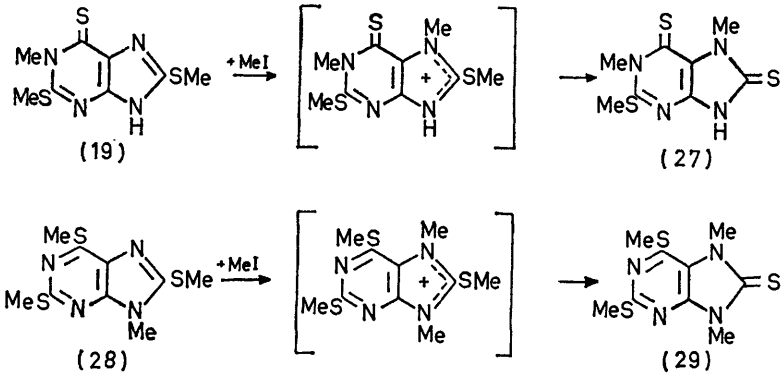
Position of methyl	Hypoxanthines		2-(Methylthio)hypoxanthines		2,8-Bis(methylthio)hypoxanthines	
	Protonation	Methylation	Protonation	Methylation	Protonation	Methylation
	7(9)	7,9 [a,b]	9(7)	7,9 [a]	7(9)	7,9 [a]
1	7(9)	7, 9	9(7)	7,9	7(9)	7,9
3	1	1 [c]	1 and 7	7 and 1	1 (and 7?)	7 and 1
7	9	9 [b]	9	9		
9	7	7 [b]	7	7	7	7
3,7			1	1	1	1
1,9					7	7

[a] The product formed is the 7,9-dimethyl derivative. The pathway (*via* 7-, 9-, or both) has not been determined. [b] Reference 5. [c] Reference 8.

(*d*) *Methylation of the neutral molecules of* 2,8-*bis*-(*methylthio*)*hypoxanthines.* Both (13) and (15) are converted by methyl iodide in DMF into the 7,9-dimethyl derivative (20) (Scheme 5). The latter loses the methyl group of the 8-methylthio-substituent to give (21), which again reacts with excess of methyl iodide to yield 1,7,9-trimethyl-2-(methylthio)hypoxanthine-8-thione (22). The same product results from the reaction of (14) and (16) with methyl iodide.

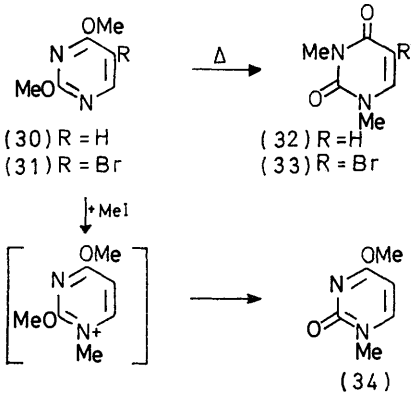
(28) yielded 7,9-dimethyl-2,6-bismethylthio)purine-8-thione (29) (Scheme 6).

Analogous reactions have been reported for diazaheterocycles. Thus on heating, 1,3-dimethyl-2-methylthiobenzimidazolinium salt undergoes *S*-demethylation to 1,3-dimethylbenzimidazole-2-thione.[9] Hilbert and Johnson observed thermal rearrangement of 2,4-dimethoxypyrimidine (30) to 1,3-dimethyluracil (32).[10]



SCHEME 6

Similarly, 5-bromo-2,4-dimethoxypyrimidine (31), in the presence of sodium iodide, rearranges to 5-bromo-1,3-dimethyluracil (33).[11] On the other hand, (30) reacts with methyl iodide at room temperature to yield 4-methoxy-1-methylpyrimidine-2-one (34) (Scheme 7).[10]



SCHEME 7

From these observations it is apparent that two different mechanisms may be operative in *N*-alkylation of such heterocycles: (i) intramolecular transalkylation from sulphur or oxygen to nitrogen; (ii) *N*-alkylation and formation of a ' fixed ' cation, followed by spontaneous *O*- or *S*-demethylation. Since in the conversion (5) ⟶ (11) and (17) ⟶ (24), the intermediates (10) and (23) (Scheme 4) could not be isolated, we have sought evidence for mechanism (ii) by the use of CD_3-labelling. Thus (14a) reacts with methyl iodide to give (22), while (16a) yields (22a). Similarly, (18a) is converted into (26a) (Scheme 8). Conversely, (14) reacted with [$^2H_3$]methyl iodide to give (22b), (16) yielded with the same reagent (22c), and (18) gave (26b) (Scheme 9). These reactions not only excluded intramolecular rearrangements, but also permitted selective replacement of NMe-substituents by the NCD_3-group so that the various NMe-signals could be assigned individually (see Tables 2, 4, and 6). Similarly, the reactions described in Scheme 8 served to identify the methylthio-group suffering demethylation [8-SMe in (14) and (16), and 2-SMe in (18)].

In the same way, demethylation of the 8-SMe group during the reaction (19) ⟶ (27) was proved by the use of (19a), bearing an 8-SCD_3-substituent.

(IV) *Evidence for the Structure of the Methylation Products.*—Compounds (7),[5] (11),[7] (12),[6] and (24) [12] were identified by comparison with authentic samples (u.v., i.r., and n.m.r. spectra, and R_F values).

Conversion of 1-methylhypoxanthine into 1,7,9-trimethylhypoxanthinium ion (35) is a new reaction. The u.v. absorption of (35) ($\lambda_{max.}$ 253 nm at pH 1) is close to that of the known 7,9-dimethylhypoxanthinium ion ($\lambda_{max.}$ 251 nm at pH 1).[5] Upon dissolution in D_2O, one of the two aromatic protons in (35) exchanges instantaneously. This was shown to be 8-H: 1-methylhypoxanthine was first deuteriated at position 8.[8] After conversion into the [$^2H_8$]derivative of (35) and dissolution of the latter in D_2O, the same proton signal survived as before. Thus exchange must have involved position 8, indicating the presence of the 7,9-dimethylimidazolinium group in (35).

The n.m.r. spectrum of (8) in [^2H]chloroform shows a single aromatic proton (8-H). This signal disappeared instantaneously when the compound was dissolved in [$^2H_6$]dimethyl sulphoxide–D_2O, indicating the presence of a ' fixed ' imidazolinium cation, as in (35).

The structure of (9) was established by its transformation into 3,7-dimethylxanthine upon treatment with methyl iodide in alkali, followed by hydrolysis of the 2-SMe-group.

When the ammoniacal solution of compound (20) was treated with Raney nickel, the betaine (7) was obtained.

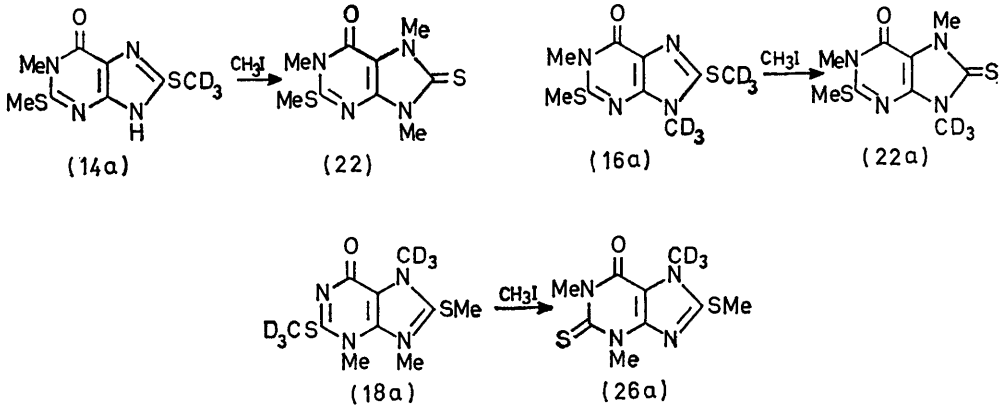
[9] B. H. Klanderman, *J. Org. Chem.*, 1965, **30**, 2469.
[10] G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2001.
[11] T. L. V. Ulbricht, *J. Chem. Soc.*, 1961, 3345.
[12] A. J. Dietz, jun., and R. M. Burgison, *J. Med. Chem.*, 1966, **9**, 160.

The structure of (22) follows from the fact that it is formed by methylation of both (16) and (21).

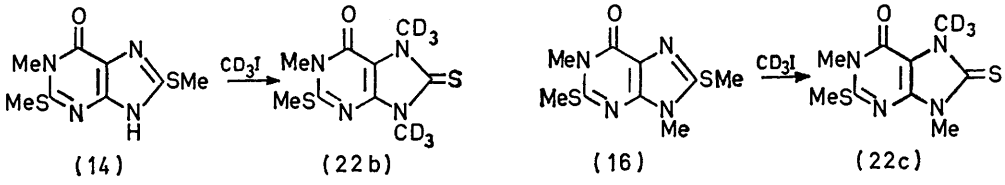
The structure of (25) is based on its reconversion into (18) by treatment with methyl iodide at room temperature and on its transformation into 3,7-dimethyl-8-(methylthio)xanthine [4] (see Experimental section).

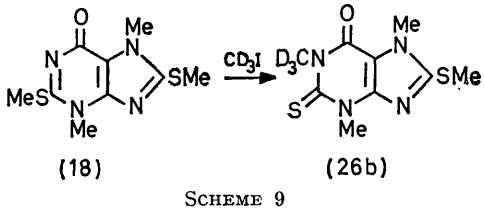
Compound (26) is formed both from (24) and (25); these reactions establish its structure.

Compound (27) was converted into the known 1,7-dimethyluric acid [13] (see Experimental section) to locate the two *N*-methyl substituents.



SCHEME 8





SCHEME 9

Compound (29) was transformed by boiling hydrochloric acid into the known 7,9-dimethyl-8-thiouric acid.[14]

EXPERIMENTAL

M.p.s were determined with a Fisher–Jones apparatus. For paper chromatography by the descending method, Whatman paper No. 1 was used with the following solvents: (A) n-butanol–acetic acid–water (12 : 3 : 5, v/v); (B) ethanol–dimethylformamide–water (3 : 1 : 1, v/v). Spots were located by their fluorescence under a u.v. lamp ($\lambda \sim$ 254 nm); theophylline (R_F 0·68) served as standard. U.v. spectra were measured on a Hitachi–Perkin-Elmer spectrophotometer, model 124, and chemical shifts with a JEOL MH-100 instrument. As internal standards, both tetramethylsilane and sodium 3-trimethylsilyl[$^2H_4$]propionate of Merck, Sharp, and Dohme, Canada, were used.

General Procedure for Methylation of Neutral Molecules.—A sample of the purine (2 g) was dissolved in DMF (*ca.* 50 ml) and treated with methyl iodide (4 ml) at room temperature. Where indicated, the solution was heated to 95° for 3—5 h. The solvent was removed *in vacuo* and the residue treated with a small volume of acetone until it solidified. Yields varied between 70 and 80%. Methods of purification and physical properties of the products are shown in Table 6.

In the case of (5) and (17), extension of the reaction time to 12 h gave (12) and (26) as the only products, instead of the mixtures obtained after 3—5 h.

Purines. The following compounds were synthesised by known procedures: 1-methylhypoxanthine; [15] 2-(methylthio)hypoxanthine (1) [16] and its 1- (2),[15] 3- (5),[15] 7- (3),[3] and 9-methyl (4) [3] derivatives; 2,8-bis(methylthio)hypoxanthine (13) [17] and its 1- (14),[3] 3- (17),[4] and 9-methyl (15) [3]

[13] C. W. Bills, S. E. Gebura, J. S. Meek, and O. J. Sweeting, *J. Org. Chem.*, 1962, **27**, 4633.
[14] H. Bredereck, G. Kupsch, and H. Wieland, *Chem. Ber.*, 1959, **92**, 566.
[15] G. B. Elion, *J. Org. Chem.*, 1962, **27**, 2478.
[16] G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Amer. Chem. Soc.*, 1956, **78**, 217.
[17] C. W. Noell and R. K. Robins, *J. Amer. Chem. Soc.*, 1959, **81**, 5997.

TABLE 6

Preparation and properties of purines

	Method(s) of preparation)	M.p. or decomposition /°C	Solvent [a] for crystallisation	Crystalline form	N.m.r. (δ) (solvent)	$\lambda_{max.}$(MeOH)/ nm (log ε)	R_F (solvent) (A)	(B)	Formula	Analyses Required (%) C	H	N	S	Found (%) C	H	N	S
2-Thioxanthines																	
(11)	(i) Ref. 7 (ii) From (5)	>300			8H 8·91 1-Me 3·87 3-Me 4·00 (CF_3CO_2H)	286(4·25)	0·85	0·68									
(9) [b]	From (5)	>300	E	Prisms	8H 8·94 3-Me 3·94 7-Me 4·21 (CF_3CO_2H)	286(4·24)	0·72	0·68	$C_7H_8N_4OS$	42·9	4·1	28·6	16·3	42·6	4·2	28·5	16·1
(12)	(i) Ref. 11 (ii) From (5), (6), (9) and (11)	234	E	Needles	8H 8·74 1-Me 3·83 3-Me 3·97 7-Me 4·25 (CF_3CO_2H)	286(4·25)	0·98	0·70									
(24)	(i) Ref. 12 (ii) From (17)	>300	E	Prismatic plates	1-Me 3·92 3-Me 4·03 8-SMe 3·01 (CF_3CO_2H)	301(4·24)	0·94	0·59									
(25)	From (17)	265	E	Needles	3-Me 4·00 7-Me 4·13 8-SMe 2·89 (CF_3CO_2H)	301(4·24)	0·90	0·65	$C_8H_{10}N_4OS_2$	39·7	4·1	23·1	26·4	39·6	4·0	22·9	26·2
(26) [c]	From (17), (18), (24) and (25)	218	EA	Needles	1-Me 3·90 3-Me 4·05 7-Me 4·22 8-SMe 2·92 (CF_3CO_2H)	241(4·08) 301(4·26)	0·97	0·57	$C_9H_{12}N_4OS_2$	42·2	4·7	21·9	25·0	42·2	4·9	22·1	24·7
Hypoxanthines																	
(35)	From 1-methylhypoxanthine	211—214	E	Pale yellow plates	2H 8·64 8H 9·30 1-Me 3·77 7-Me 4·34 9-Me 4·10 (D_2O)	253(4·02) [d]	0·78	0·80	$C_8H_{11}IN_4O$ [e]	31·4	3·6	18·3		31·1	3·5	17·9	
(8)	From (2)	194	Chloroform	Needles	8H 9·34 1-Me 3·64 7-Me 4·20 9-Me 3·97 2-SMe 2·72 (D_2O)	275(4·20)	0·75	0·78	$C_9H_{13}IN_4OS$ [f]	30·7	3·7	15·9	9·1	30·5	3·5	15·9	9·1
(6) [g]	From (9)	215—220	E	Yellow needles			0·90	0·80	$C_8H_{10}N_4OS$	45·7	4·8	26·7	15·2	45·5	4·5	26·3	14·7
18) [h]	(i) Ref. 4 (ii) From (25)	190	EA	Needles			1·0	0·90	$C_9H_{12}N_4OS_2$	42·2	4·7	21·9	25·0	42·1	4·8	22·1	24·9
Purinethiones																	
(21)	From (13) and (15)	>300	B	Prisms	7-Me 4·24 9-Me 4·02 2-SMe 2·83 (CF_3CO_2H)	298(4·23)	0·76	0·83	$C_8H_{10}N_4OS_2$	39·7	4·1	23·1	26·4	39·5	4·2	23·0	26·1
(22)	From (13), (14), (15), (16), and (21)	257	EA	Needles	1-Me 3·97 7-Me 3·72 9-Me 3·60 2-SMe 2·60 ($CDCl_3$)	248(4·10) 296(4·30)	0·81	0·87	$C_9H_{12}N_4OS_2$	42·2	4·7	21·9	25·0	41·8	4·9	21·7	25·3
(27)	From (19)	280	EA	Yellow needles	1-Me 4·06 7-Me 3·68 2-SMe 2·70 ($CDCl_3$)	261(4·12) 328(4·10) 373(4·32)	0·73	0·72	$C_8H_{10}N_4S_3$	37·2	3·9	21·7	37·2	36·8	3·5	21·3	36·7
(29)	From (28) and 2,6,8,-tris(methylthio)purine	214—216	P	Needles	7-Me 4·01 9-Me 3·71 2-SMe 2·61 6-SMe 2·69 ($CDCl_3$)	340(4·28)	0·95	0·76	$C_9H_{12}N_4S_3$	39·7	4·4	20·6	35·3	39·5	4·3	20·3	34·9
Miscellaneous																	
(28) [i]					9-Me 3·62 2-SMe 2·63 6-SMe 2·69 8-SMe 2·78 ($CDCl_3$)												

[a] E = ethanol, EA = ethyl acetate, B = n-butanol, P = petroleum (b.p. 60—80°). [b] This compound has been described previously.[19] However, the structure of the product, from the debenzylation and demethylation of 2-benzylthio-3,7,9-trimethylhypoxanthinium cation, was not proven. [c] A claimed synthesis of this compound (H. Biltz and H. Rackett, *Ber.*, 1928, **61**, 1418) has recently been shown (R. Walentowski and H. W. Wanzlick, *Chem. Ber.*, 1969, **102**, 3000) to be incorrect. [d] Measured in N-HCl. [e] Found I, 41·1. Formula requires 41·5%. [f] Found I, 35·9. Formula requires 36·1%. [g] For spectral characteristics, see Tables 1 and 2. [h] For spectral characteristics, see Tables 3 and 4. [i] Reference 18.

derivatives; 1,9- (16) [4] and 3,7-dimethyl-2,8-bis(methylthio)hypoxanthine (18); [4] 1-methyl-2,8-bis(methylthio)-thiohypoxanthine (19); [3] 1,3-dimethyl-2-thioxanthine (11) [8] and 1,7-dimethyl-2-thioxanthine (12); [7] 1-methyl-2-(methylthio)hypoxanthine-8-thione [3] and 1-methyl-2-(methylthio)purine-6,8-dithione; [3] 2,6,8-tris(methylthio)purine [17] and its 9-methyl derivative (28).[18]

Separation of Mixtures of Methylation Products.—(*a*) *Methylation of* 3-*methyl*-2-(*methylthio*)*hypoxanthine* (5). The mixture, from the reaction of (5) with methyl iodide at 95° for 5 h, was evaporated to dryness and the residue was shaken with 2N-NaOH. The insoluble portion was 2-thiocaffeine (12), identical with an authentic sample.[6] The alkaline solution was neutralised with acetic acid to precipitate a mixture of (9) and (11). Boiling water dissolved only 3,7-*dimethyl*-2-*thioxanthine* (9), which was recrystallised from ethanol (see Table 6). The water-insoluble material was identified as 1,3-dimethyl-2-thioxanthine (11).[7]

(*b*) *Methylation of* 3-*methyl*-2,8-*bis*(*methylthio*)*hypoxanthine* (17). Using the same method as above, 1,3,7-*trimethyl*-8-*methylthio*-2-*thioxanthine* (26), which is insoluble in cold 2N-NaOH, was separated. From the alkaline solution, a mixture of (24) and (25) was precipitated by neutralisation with acetic acid. The precipitate was dissolved in hot ethyl acetate. Upon slow cooling to room temperature, 3,7-*dimethyl*-8-*methylthio*-2-*thioxanthine* (25) crystallised first. This substance was obtained pure (by t.l.c.) by recrystallisation from ethanol (see Table 6). The filtrate from (25) was evaporated and the residue was extracted with boiling water. The insoluble portion was recrystallised from ethanol to give pure 1,3-*dimethyl*-8-*methylthio*-2-*thioxanthine* (24).

(*c*) *Methylation of* 2,8-*bis*(*methylthio*)*hypoxanthine* (13). The reaction mixture was evaporated and the residue shaken with N-NaOH. The insoluble portion was 1,7,9-*trimethyl*-2-(*methylthio*)*hypoxanthine*-8-*thione* (22). From the alkaline filtrate, acetic acid precipitated 7,9-*dimethyl*-2-(*methylthio*)-*hypoxanthine*-8-*thione* (21).

(*d*) *Methylation of* 2,6,8-*tris*(*methylthio*)*purine and its* 9-*methyl derivative* (28). The mixture resulting from methylation of 2,6,8-tris(methylthio)purine in dimethylformamide (DMF) at 95°,[18] was evaporated, and the residue was extracted with boiling petroleum (b.p. 60—80°). The insoluble portion was a mixture of 3-methyl-2,6,8-tris(methylthio)purine and 3,7-dimethyl-2,6,8-tris(methylthio)purinium iodide.[18]

From the petroleum solution, compound (29) crystallised in needles. The same purine was obtained as sole reaction product by methylation of the 9-methyl derivative (28).

Derivatives of Uric Acid.—(*a*) *Conversion of* 1,7-*dimethyl*-2-(*methylthio*)*purine*-6,8-*dithione* (27) *into* 1,7-*dimethyluric acid*. To a solution of (27) (100 mg) in 2N-NaOH (10 ml) was added dropwise 30% hydrogen peroxide (6 ml) at room temperature during 10 min. The solution was kept at room temperature for 2 h and then heated to 80° for 1 h. After neutralisation with acetic acid, 1,7-dimethyluric acid precipitated, identical with authentic material.[13]

(*b*) *Conversion of* 7,9-*dimethyl*-2,6-*bis*(*methylthio*)*purine*-8-*thione* (29) *into* 7,9-*dimethyl*-8-*thiouric acid*. A suspension of (29) (100 mg) in 6N-HCl (50 ml) was refluxed for 2 h. The solution was concentrated to a small volume and then neutralised with 25% ammonia. The precipitate was identical with an authentic sample of 7,9-dimethyl-8-thiouric acid.[14]

Conversion of Compounds (9) *and* (25) *into the Corresponding* 3,7-*Dimethylxanthines*.—A solution of the 2-thione derivative (9) or (25) (0·5 g) in 2N-NaOH was treated at room temperature with methyl iodide (2 ml) for 30 min. Upon neutralising with acetic acid, the corresponding 2-oxo-derivatives, *viz.* 3,7-dimethylxanthine and 3,7-dimethyl-8-(methylthio)xanthine,[4] were isolated. The corresponding 1,3-dimethyl derivatives (11) and (24) did not react with methyl iodide under these conditions.

Synthesis of Purines Bearing SCD_3 *or* NCD_3 *Groups*.—The reactions were carried out with pure CD_3I (E. Merck) or with a 1 : 1 mixture of CD_3I and CH_3I (see Figure).

(*a*) *One-step preparation*. A solution of the substrate (100 mg) in DMF (5 ml) was treated with CD_3I (0·2 ml). The solvent was removed *in vacuo* and the residue recrystallised as shown in Table 6. Compounds were prepared as follows: (14a), methylation of 1-methyl-2-(methylthio)hypoxanthine-8-thione [3] at room temperature for 12 h; (19a), methylation of 1-methyl-2-(methylthio)purine-6,8-dithione [3] under the same conditions; (16a), a solution of 1-methyl-2-(methylthio)hypoxanthine-8-thione [3] (100 mg) in 2N-NaOH (10 ml) was stirred at room temperature with CD_3I (0·2 ml) for 15 min. The product (16a) precipitated directly. In this case both the 9-methyl- and the 8-SMe-groups are labelled.

(*b*) *Two-step preparation*. Compounds were prepared as follows: (6a), a solution of (5) (200 mg) in DMF (5 ml) was treated with CD_3I at 95° for 5 h. The residue remaining after removal of the solvent {3-methyl-7-[$^2H_3$]methyl-2-thioxanthine (9a)} was dissolved in acetonitrile and treated at room temperature with CD_3I for 6 h to yield (6a); (18a), a solution of (27) (300 mg) in DMF (5 ml) was stirred at 95° with CD_3I (0·2 ml) for 3 h, to yield (25a) (labelling of the 7-methyl group). This compound was dissolved in acetonitrile and treated at room temperature with CD_3I for 6 h, to label the 2-SMe group.

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[3/1097 *Received*, 30*th May*, 1973]

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[19] H. Bredereck, P. Schellenberg, R. Nast, H. Heise, and O. Christmann, *Chem. Ber.*, 1966, **99**, 944.