Reactions of Some 8-(3-Pyridyl)-6-thioxanthines with Methyl Iodide

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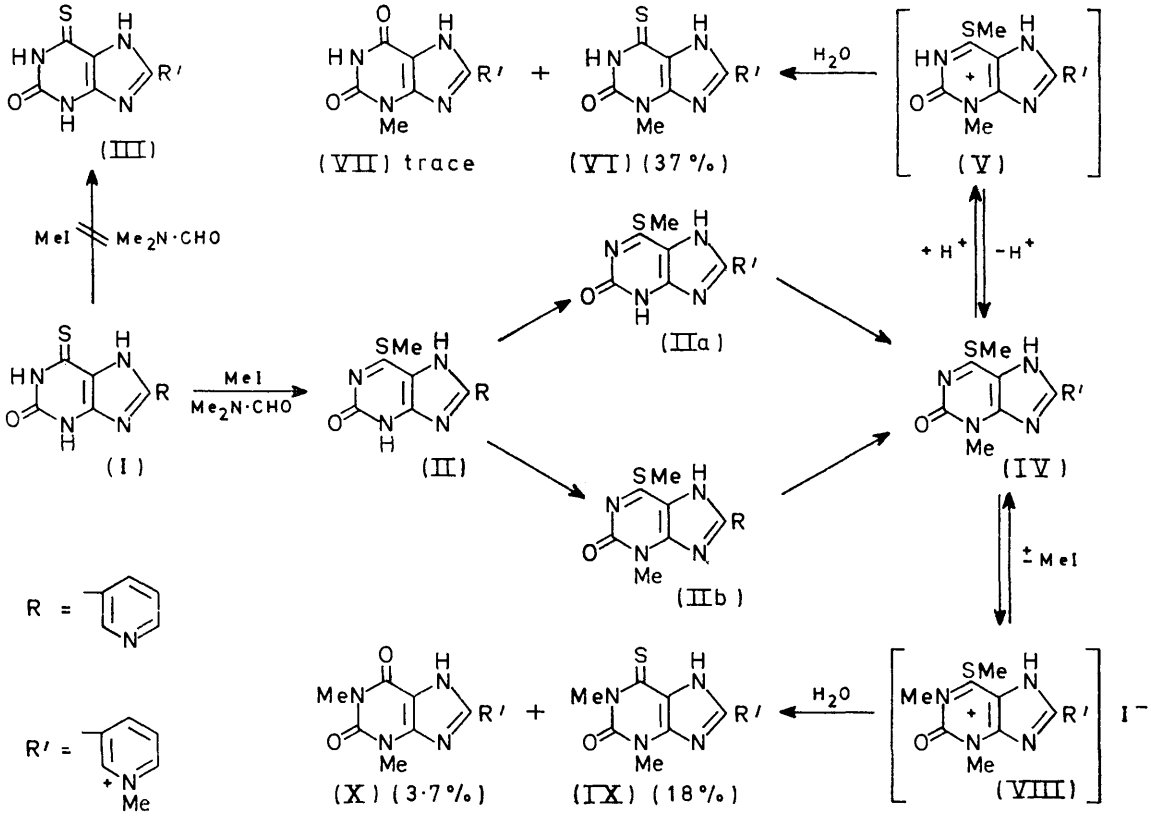
8-(3-Pyridyl)-6-thioxanthine (I) reacts with methyl iodide in dimethylformamide to produce the corresponding thioether (II). In the presence of an excess of methyl iodide the reaction further proceeds to quaternise the pyridine nitrogen atom, and to methylate N-3 and N-1 in the purine nucleus. Simultaneously, *S*-demethylation occurs, so that the end products are the *N*-methyl-thioxanthines (VI) and (IX).

ALKYLATION of thiopurines in dipolar aprotic solvents has received much recent attention, particularly since it was first observed that 6-mercaptopurine underwent a two-step methylation reaction to yield ultimately 3-methyl-6-methylthiopurine.[1a,2] The two steps were shown to be (a) 6-*S*-methylation; (b) 3-methylation.

RESULTS

When compound (I) was treated with an excess of methyl iodide in dimethylformamide at steam-bath temperature, a sequence of consecutive reactions took place, as evidenced by chromatography. The *S*-methyl derivative (II) was formed immediately, and at this



SCHEME 1

This pathway was valid also for the initial steps of the corresponding reaction with 6-thioxanthine.[2] Whereas in the thiopurine series these reactions are well understood, the understanding of the behaviour of 8-(3-pyridyl)thioxanthines towards methylating agents in aprotic solvents cannot be guided by previous experience.[3]

Since 8-(3-pyridyl)-6-thioxanthine (I) and several of its methylated derivatives were required in connection with some studies involving the enzyme xanthine oxidase, we have investigated the reaction of compound (I) with methyl iodide in dimethylformamide in detail.

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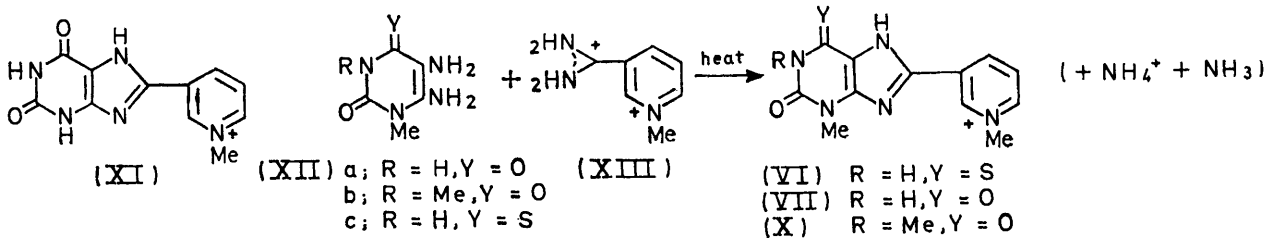
stage there was no competing reaction. Later, the *NS*-dimethyl compound (IV) and the *N*-methylated thioxanthines (VI) and (IX) and xanthines (VII) and (X) were observed. After 4 h, eight distinct components were identified. Four of these were isolated and identified as the thioxanthines (VI) and (IX) (37 and 18% yield, respectively) and the xanthines (VII) and (X). The last two were obtained in trace amounts only.

[1] (*a*) J. W. Jones and R. K. Robins, *J. Amer. Chem. Soc.*, 1963, **85**, 193; (*b*) H. Bredereck, O. Christmann, W. Koser, P. Schellenberg, and R. Nast, *Chem. Ber.*, 1962, **95**, 1812.

[2] F. Bergmann and M. Kleiner, *Israel J. Chem.*, 1963, **1**, 477.

[3] M. Kleiner, unpublished results.



*Identification of Products and Intermediates.**—Since the intermediate (IIa) could not be isolated in crystalline form from the reaction mixture, its resolved spot was extracted with water from the chromatogram. It was identified as the quaternary analogue of (II) by its u.v. spectra [similar to (IV); see Table 1] and by acidic hydrolysis to give the quaternary 8-(3-pyridyl)xanthine (XI).[4]

the assignment of structure (VI) was obtained through the sequence of degradation reactions: (VI) $\xrightarrow{\text{MeI, NH}_4\text{OH}}$ (IV) $\xrightarrow{\text{6N-HCl}}$ (VII). The blue shift of 18 nm during the transformation (VI) → (IV) indicated that *S*-methylation had occurred. The identity of compound (VII) thus obtained was established through comparison (chromatography and u.v. spectra) with an authentic sample

TABLE 1

Physical properties of purines *

Purine	$\lambda_{max.}$/nm (pH 8)	log $\varepsilon_{max.}$	R_F in solvent (A)	(B)	(C)	Fluorescence (*ca.* 255 nm)	M.p. (°C)	Cryst. from
(I)	247, 366	4·29, 4·45	0·29	0·35		Grey-blue	>310	NaOH–NH_4Cl
(II)	228, 263, 351	3·85, 3·89, 4·12	0·38	0·32		Blue	305 (decomp.)	H_2O
(IIa) †	271, 365		0·2	0·27		Orange-yellow		
(IIb)	229, 263, 351	4·19, 4·19, 4·44	0·65	0·67		Blue	270—290 (slow) (decomp.)	MeOH
(III)	226, 265, 382	4·21, 4·25, 4·31	0·17	0·31	0·15	Red-brown	296 (decomp.)	H_2O
(IV)	225, 272, 365	4·48, 4·18, 4·39	0·44	0·54	0·36	Orange	252 (iodide) 268 (picrate)	90% EtOH
(VI)	266, 383	4·28, 4·39	0·22	0·42		Grey-brown	>300 (hydrated betaine) 266 (picrate)	1·6N-HCl 2·0N-NH_4OH H_2O
(IX)	227, 271, 384	4·35, 4·18, 4·27	0·57			Brown-green	282 (decomp.)	H_2O
(VII)	250, 330	4·06, 4·18	0·25	0·27	0·16	Orange	>310 (hydrated betaine) 285 (picrate)	H_2O
(VIIa) ‡	234, 315	4·20, 4·30	0·48	0·58	0·41	Blue	>310	NaOH–NH_4Cl
(X)	225, 333	4·44, 4·22	0·51	0·37	0·35	Orange-brown	No clear m.p. (iodide) 292 (hydrated betaine) 277 (picrate)	H_2O
(Xa) ‡	233, 315	4·26, 4·67	0·73	0·67		Grey-blue	>310	NaOH–NH_4Cl
(XI)	251, 330	4·02, 4·19	0·11	0·24	0·12	Orange-brown	>310 (betaine)	H_2O
(XIa) ‡	225, 315	4·21, 4·31	0·4	0·3		Blue	>310	NaOH–NH_4Cl
(XIV)	262, 366	4·31, 4·45	0·7	0·73		Dark blue	304	Me_2N·CHO
(XV)	248, 362 §	4·34, 4·31	0·67	0·72		White-blue	219	PrⁱOH
(XVI)	228, 268, 370	4·43, 4·18, 4·35	0·56	0·52		White-green	220—260 (decomp.)	MeCN–H_2O (20 : 1)

* Physical properties such as $\lambda_{max.}$ and R_F for quaternary compounds are independent of the conjugate anion under the conditions of measurement used here (aqueous medium). This can be seen in paper chromatograms of quaternary purines associated with different anions. In particular, for picrates it is clearly seen that the purine and picrate anion migrate independently. † Extracted from paper chromatogram. ‡ (VIIa), (Xa), and (XIa) refer here, as in Table 2, to the unmethylated pyridyl analogues of (VII), (X), and (XI), respectively. § In PrⁱOH.

Elemental analysis of compound (VI) indicated that it was a dimethyl derivative. Its u.v. spectrum (Table I) excluded the presence of a thioether group,† and analogy with the methylation of 6-thioxanthine [2] suggested the presence of a 3-methyl group. Support for prepared by fusion of 1-methyl-5,6-diaminouracil and the quaternary nicotinamidine (XIII),[4] according to the general procedure of Bergmann and Tamari.‡ [5] The same route was chosen to synthesise compound (VI), from the pyrimidine (XIIc).

Reasoning along the lines described for the identification of compound (VI) indicated that (IX) must be 8-(1-methyl-3-pyridinio)-6-thiotheophylline. This product was degraded by chlorine in methanol to the theophylline (X), which was prepared independently by fusion of the diaminopyrimidine (XIIb) and the quatern-

* Structures not explicity considered were identified by comparison with authentic samples.

† Table 1 shows that in the present series of compounds *S*-alkylation causes a blue shift of *ca.* 15 nm, quaternisation causes a red shift of *ca.* 15 nm, and purine skeletal alkylation hardly affects the maximum of u.v. absorption.

‡ The remote possibility that a Dimroth rearrangement took place during the fusion was eliminated by showing that the diaminopyrimidines (XIIa—c) do not rearrange under the fusion conditions employed.

[4] F. Bergmann, M. Rashi, M. Kleiner, and R. Knafo, *J. Chem. Soc.*, 1967, 1254.

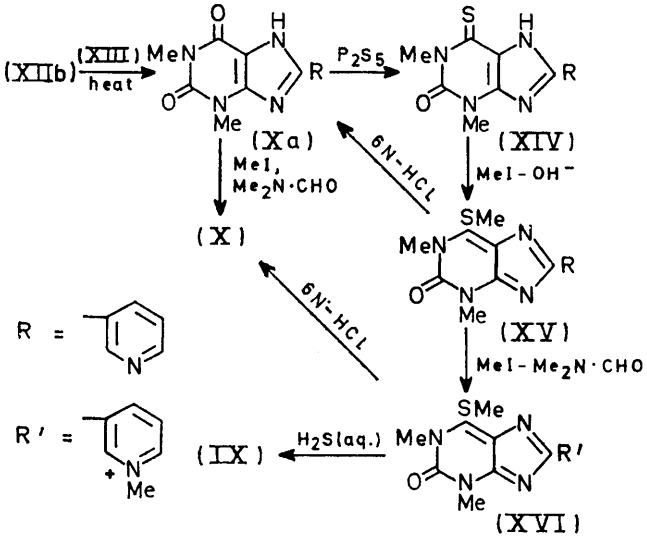
[5] F. Bergmann and M. Tamari, *J. Chem. Soc.*, 1961, 4468.

TABLE 2

Methods of preparation and analyses

Purine	Method	Yield (%)	Formula	Found (%) C	H	N	S	Calc. (%) C	H	N	S
(I)	A	94	$C_{10}H_7N_5OS,0{\cdot}5H_2O$	46·6	2·7	27·4	12·5	47·2	3·1	27·55	12·6
(II)	C	69	$C_{11}H_9N_5OS$	50·9	3·3		11·7	51·0	3·5		12·3
(IIb)	C	24	$C_{12}H_{11}N_5OS,0{\cdot}5H_2O$	51·8	4·3	24·35	11·4	51·1	4·25	24·8	11·3
(III) (hydrated betaine)	A	20	$C_{11}H_9N_5OS,H_2O$	47·6	3·96	25·30		47·87	3·99	24·92	
(IV) (iodide)	C	38	$C_{13}H_{14}IN_5OS$	37·9	3·4	16·95		37·6	3·9	16·95	
(IV) (picrate)			$C_{19}H_{16}N_8O_8S$	44·0	2·9	22·2	6·0	44·2	3·1	21·7	6·2
(VI) (hydrated betaine)			$C_{12}H_{11}N_5OS,H_2O$	51·9	4·2	24·9	11·4	51·9	4·2	24·8	11·3
(VI) (picrate)			$C_{18}H_{14}N_8O_8S$	43·5	3·0	22·45	6·6	43·0	2·8	22·3	6·4
(IX) (iodide)			$C_{13}H_{14}IN_5OS,0{\cdot}5H_2O$	36·8	4·0	16·55	8·0	36·8	3·5	16·5	7·5
(VII) (hydrated betaine)	A	13	$C_{12}H_{11}N_5O_4,H_2O$	51·7	5·0	25·65		52·4	4·7	25·45	
(VII) (picrate)	B	76	$C_{18}H_{14}N_8O_9,H_2O$	43·0	3·6	22·4		42·85	3·2	22·2	
(X) (hydrated betaine)			$C_{13}H_{13}N_5O_2,H_2O$	53·1	5·1	24·0		54·0	5·2	24·2	
(X) (iodide)	B	62	$C_{13}H_{14}IN_5O_2$	38·6	4·3	17·5		39·1	3·5	17·5	
(X) (picrate)			$C_{19}H_{16}N_8O_9$	45·8	3·2	22·6		45·6	3·2	22·4	
(VIIa)	A	63	$C_{11}H_9N_5O_2$	54·4	3·7	29·0		54·3	3·7	28·8	
(Xa)	A	95	$C_{12}H_{11}N_5O_2$	55·9	4·5	27·1		56·0	4·3	27·2	
(XIV)		92	$C_{12}H_{11}N_5OS$	52·6	4·3	25·9	11·9	52·7	4·0	25·6	11·7
(XV)	C	54	$C_{13}H_{13}N_5OS$	54·5	4·8	24·5	11·2	54·35	4·5	24·4	11·1
(XVI) (iodide)		30	$C_{14}H_{16}N_5IOS$	39·3	3·8	16·4		39·2	3·7	16·3	

ary nicotinamidine (XIII). Unambiguous synthesis of compound (IX) was achieved as depicted in Scheme 2.



SCHEME 2

DISCUSSION

The first step in alkylation of thiopurines occurs invariably at the sulphur atom.[2,6] Chromatographic study of the reaction between compound (I) and methyl iodide showed that this rule was obeyed, even though the strongly nucleophilic pyridine nitrogen atom was present. It is therefore remarkable that the substituted 6-thioxanthines (VI) and (IX) were ultimately isolated, rather than methylated derivatives of purine thioethers. A possible mechanism is depicted in Scheme 1. Thus compound (I) undergoes *S*-methylation, and the product (II) is then converted into the intermediates (IIa and b), which were detected on the chromatograms. Further methylation then gives a common intermediate (IV).* It is important to establish the likelihood that some protonated form of (IV), say (V), is an intermediate on the way to the end products (VI) and (VII). For each equivalent of (IV) produced from (I), two equivalents of HI are created. In the aprotic dimethylformamide this acid will tend to bind to a nucleophilic centre, *e.g.* a lone pair on a purine nitrogen atom. This would obstruct further substitution. Indeed, under similar reaction conditions, 6-mercaptopurine produces a 3-methyl-6-methylthiopurine salt,[1,2] in which the bound acid is effective in preventing further substitution. On the other hand, when the purine salt is released from the acid, further methylation occurs to form the trimethyl product.[6a] These arguments indicate that (in an excess of methyl iodide) isolation of (VI) as the main end product (37%) rather than (IX) (18%) is due to an equilibrium of the type (IV) $\rightleftharpoons$ (V) which bars much of (IV) from further reaction along the alternative route (IV) $\longrightarrow$ (VIII) $\longrightarrow$ (IX). Since I^- is a powerful nucleophile in dimethylformamide[7] it can form a tight ion pair with (V) which decomposes mostly to (VI) but also to a small quantity of (VII) on addition of water. The breaking of an *S*-alkyl bond in ' strained ' *S*-alkyl thiopurines by a strong nucleophile has been previously observed by Balsiger *et al.*;[8] this suggests that ' demethylation ' can be successfully achieved by I^- in dimethylformamide.

The present observations are similar to those made in the uracil nucleoside series.[9] In particular the statement that uracil *N*(3),*O*(6)-bisglucoside will cleave at O-6 in high yield under the influence of $Hg(CN)_2$–MeCN (a strong Lewis acid in an aprotic polar medium) supports the foregoing arguments. In order to rationalise the isolation of compounds (IX) and (X) we invoke slightly modified arguments. The fact that authentic (IV) iodide is stable to heating in dimethylformamide under the conditions employed here suggests that the

* This statement is imprecise in view of the postulated equilibrium (IV) $\rightleftharpoons$ (V), but is retained for simplicity.

[6] Z. Neiman and F. Bergmann, *Israel J. Chem.*, (*a*) 1965, **3**, 161; (*b*) 1967, **5**, 243.

[7] A. J. Parker, *Quart. Rev.*, 1962, **16**, 163.

[8] R. W. Balsiger, A. L. Fikes, T. P. Johnston, and J. A. Montgomery, *J. Org. Chem.*, 1961, **26**, 3447.

[9] G. T. Rogers and T. L. V. Ulbricht, *J. Chem. Soc.* (*C*), 1969, 2450.

formation of (IX) from (IV) does not occur by way of the formally possible intramolecular rearrangement but is better understood in terms of the Hilbert–Johnson reaction,[10] although reports of its occurrence in heterocyclic thioethers are scarce. Thus, according to this argument, compound (IV) adds methyl iodide to form the salt (VIII), which produces (IX) and (X) on transfer to a protic environment.

The present results suggest that *S*-dealkylation might be a common phenomenon in alkylation reactions of thiopurines, particularly in the presence of a base. In this case, the equilibrium (IV) $\rightleftarrows$ (V) would be shifted to the left and the end product should be an exhaustively methylated mercaptopurine. Indeed the curious methylation of 6-thioxanthine in the presence of aqueous base to yield 6-thiocaffeine,[11] which must proceed through the intermediate thioether, can be rationalised along these lines. Another apparent anomaly, the methylation of 6-mercapto-1-methylpurine to give 1,7-dimethyl-6-thiopurine,[12] is also explicable in these terms.

EXPERIMENTAL

Microanalyses were performed by Weiler and Strauss, Oxford. U.v. absorption maxima were measured with a Beckman DU spectrophotometer. For measuring molar absorptions, solutions were prepared in 0·1M-phosphate buffer (pH 8). Chromatograms (descending) were developed on Whatman No. 1 paper with solvents (v/v) (A) 95% ethanol–acetic acid–water (17 : 1 : 2), (B) 95% ethanol–dimethylformamide–water (3 : 1 : 1), (C) propan-2-ol–dimethylformamide–25% ammonia (13 : 5 : 2). Spots were located by their fluorescence under a mineralight lamp (λ *ca.* 255 nm). R_F Values are expressed relative to the standard value of 0·68 for theophylline in all solvents.

The syntheses of nicotinamidine hydrochloride and nicotinamidine methiodide hydriodide (XIII) used for the unambiguous preparation of tertiary and quaternary 8-(3-pyridyl)xanthines have been described before.[4] For the following pyrimidines, known synthetic procedures were employed: 4,5-diamino-6-thiouracil;[13] 5,6-diaminouracil;[14] 5,6-diamino-1-methyluracil;[15] 5,6-di-amino-1,3-dimethyluracil.[16] Purines (XI) and (XIa) (Table 1) have been described before.[4]

General Procedures. (A) *Condensation reactions of 4,5-diaminopyrimidines.* An intimate mixture of the pyrimidine with the amidine salt (2 equiv.) and anhydrous sodium acetate (2 equiv.) was heated slowly till an inner temperature of 200 °C was reached and a homogeneous melt resulted. The solid formed on cooling was further treated in a way depending on whether nicotinamidine or its quaternary analogue (XIII) was employed. (*a*) For tertiary compounds, the fusion product was extracted with hot N-sodium hydroxide; the extract was decolourised with charcoal, and filtered and the product was slowly precipitated by gradual addition of solid ammonium chloride. For purification the same treatment was repeated, followed by washing with hot water. (*b*) When the quaternary amidine was employed, the product was recrystallised from water. In general, fusion with (XIII) was not satisfactory as a preparative method, but served for the unambiguous characterisation of products. Usually direct methylation was used for preparative ends.

(B) *Quaternisation of xanthines.* The methylated tertiary xanthine (VIIa) or (Xa) (see Table 1) was dissolved in dimethylformamide and methyl iodide (100 equiv.) was added. After 4 h at water-bath temperature, the solvent was evaporated off under reduced pressure and the product was crystallised from water. This furnished the quaternary purine as an iodide, which was converted into the betaine by addition of concentrated ammonia or into the picrate by addition of picric acid.

(C) *Selective methylation at Sulphur.* To a solution of the mercaptopurine (1 equiv.) in 2N-sodium hydroxide (1·3 equiv.), methyl iodide (1·2 equiv.) was added, with enough ethanol to make the system homogeneous. After 2 h stirring at room temperature, the solution was neutralised with acetic acid and cooled overnight. This procedure produced the end-product in crystalline form. For quaternary compounds, where the presence of alkali was undesirable, the method was modified as exemplified by the preparation of compound (IV). To a solution of compound (VI) (1 g) in cold concentrated ammonia (0·7 l) were added methyl iodide (0·5 ml) and ethanol till a homogeneous solution resulted. After 48 h at room temperature, the solvent was evaporated off under reduced pressure, and the crude product was recrystallised from 90% ethanol.

(D) *Hydrolysis of thioethers to xanthines.* Hydrolyses of this type were performed for identification purposes. A solution of the thioether in 6N-hydrochloric acid (1 mg per ml) was boiled until evolution of methanethiol ceased. The solution was then neutralised with a few drops of concentrated ammonia solution and subjected to paper chromatography in various solvents, with authentic samples of starting material and end products as markers. Usually complete hydrolysis demanded no less than 10 h boiling. For identification, however, 4 h sufficed to produce a high enough concentration of product to be detected on the chromatograms.

(E) *Chromatographic study of the methylation of compound* (I). To a solution of compound (I) in dimethylformamide (2·8 mg per ml), methyl iodide (10 equiv.) was added. For convenience of sampling and detection of all possible intermediates, the reaction was conducted at room temperature. Samples (0·02 ml) were taken for chromatography every 2 min for the first 15 min, then every 5 min. Chromatograms were developed in solvents (A) and (B), and spots were detected by fluorescence under a mineralight lamp (λ *ca.* 255 nm). Identity of products and intermediates was established through comparison with authentic samples as markers.

Reaction of Compound (I) *with Methyl Iodide in Dimethyl-*

[10] (*a*) G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, 1930, **52**, 2001; (*b*) J. Pliml and M. Pristas, *Adv. Heterocyclic Chem.*, 1967, **8**, 115.

[11] K. R. H. Wooldridge and R. Slack, *J. Chem. Soc.*, 1962, 1863.

[12] (*a*) L. B. Townsend and R. K. Robins, *J. Org. Chem.*, 1962, **27**, 990; (*b*) J. A. Montgomery and H. J. Thomas, *ibid.*, 1963, **28**, 2304.

[13] G. Levine, A. Kalmus, and F. Bergmann, *J. Org. Chem.*, 1960, **25**, 1752.

[14] M. T. Bogert and D. Davidson, *J. Amer. Chem. Soc.*, 1933, **55**, 1668.

[15] (*a*) M. Polonovski, R. Vieillefosse, S. Guinand, and H. Jerome, *Bull. Soc. chim. France*, 1946, 80 (*Chem. Abs.*, 1946, **40**, 6080); (*b*) T. Ukai, Y. Yamamoto, and S. Kanetomo, *J. Pharm. Soc. Japan*, 1954, **74**, 674 (*Chem. Abs.*, 1954, **48**, 10743).

[16] (*a*) F. F. Blicke and M. C. Godt, *J. Amer. Chem. Soc.*, 1954, **76**, 2798; (*b*) H. Bredereck, G. Kupsch, and H. Wielland, *Chem. Ber.*, 1959, **92**, 583.

formamide.—Methyl iodide (30 ml) was added to compound (I) (5 g) in dimethylformamide (250 ml); the mixture was heated on a water-bath for 4 h, then evaporated under reduced pressure. Water (200 ml) was added to the residue and the suspension was boiled for 3 min. The insoluble crude product (VI) was filtered off (yield 3 g, 37%) and crystallised from 6N-hydrochloric acid to give needles. Cooling the aqueous extract gave the crude product (IX) (1·6 g, 18%). This was dissolved in 0·1N-hydrochloric acid and neutralised to pH 4·5; cooling precipitated pure (IX). Since paper chromatography indicated the presence of compounds (VII) and (X) in crude (IX), separation on neutral alumina was attempted. The crude material (0·5 g) dissolved in a little water was loaded on a column of neutral alumina (30 × 3 cm). Elution was performed with 80% methanol (7 ml fractions). Fractions 5—23 were combined and rechromatographed (elution with 50% methanol; 30 fractions of 5 ml each). Fractions 2—4 yielded compound (X) (0·2 g); fractions 9—17 yielded a little pure (VII). Under these conditions (IX) was retained by the column. While this procedure is practical for the preparation of compounds (VI) and (IX), it is not recommended for the xanthines (VII) and (X).

3-*Methyl*-6-*methylthio*-8-(3-*pyridyl*)*xanthine* (IIb).—Selective *S*-methylation of 3-methyl-8-(3-pyridyl)-6-thioxanthine [obtained (60%) from fusion by procedure (A) of 5,6-diamino-3-methyl-4-thiouracil [13] and nicotinamidine hydrochloride] gave *compound* (IIb), $\lambda_{max.}$ (pH 8) 258 (log ε 4·35) and 365 (4·51) nm; R_F (B) 0·64 (blue), m.p. >320° (from NaOH–NH_4Cl) (Found: C, 47·65; H, 4·0; N, 25·3; S, 11·55. $C_{11}H_9N_5O,H_2O$ requires C, 48·0; H, 3·7; N, 25·2; S, 11·4%).

8-(3-*Pyridyl*)-6-*thiotheophylline* (XIV).—A suspension of compound (Xa) (10 g) and phosphorus pentasulphide (50 g) in dry β-picoline (900 ml) was refluxed with stirring for 3·5 h. The β-picoline was distilled off under reduced pressure and the residue was decomposed with water (200 ml) and filtered. The crude (XIV) thus obtained was precipitated from 0·25N-sodium hydroxide (400 ml) with glacial acetic acid.

1,3-*Dimethyl*-8-(1-*methyl*-3-*pyridino*)-6-*methylthio*-3H-*purin*-2(1H)-*one* (XVI) *Iodide*.—Compound (XV) (320 mg) and methyl iodide (3 ml) in 50% aqueous dimethylformamide (80 ml) were kept at room temperature for 3 days. The dimethylformamide was distilled off under reduced pressure and the residue was crystallised [from MeCN–H_2O (20 : 1)]. When the product (XVI) was heated briefly in 6N-hydrochloric acid, total hydrolysis to (X) took place. Likewise, heating briefly in aqueous hydrogen sulphide resulted in quantitative conversion into (IX).

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