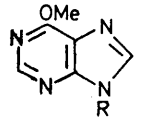
Reactivities and Electronic Aspects of Nucleic Acid Heterocycles. Part III.[1] Hydrolytic Behaviour of 6-Methoxypurines

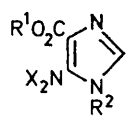
By **John L. Wong** * and **David S. Fuchs**, Department of Chemistry, University of Louisville, Louisville, Kentucky, U.S.A. 40208

Contrary to common belief, aqueous acidic hydrolysis of 6-methoxypurines yields a complex mixture of products. At 0·1M concentration, 6-methoxy-9-methylpurine (2) yielded methyl 5-amino-1-methylimidazole-4-carboxylate (5) and 9-methylhypoxanthine (4) in the ratio 7 : 3. Similar reactions were observed for both the 9-unsubstituted purine (3) and the 9-riboside (1). At concentrations of the methoxypurines >0·2M, O→N methyl migration became competitive. The quaternary intermediates 6-methoxy-7,9-dimethylpurinium chloride (6) and 6-methoxy-3-methylpurinium chloride (12) were isolated. A purine dication is proposed as the key intermediate in the hydrolytic reactions. The relevance of these results to the isolation of nucleic acid bases is discussed.

If alkylation of nucleic acids is germane to the process of chemical carcinogenesis, then O(6)- rather than N(7)-alkylation of the guanine base is most likely to be the causal event. This hypothesis, first advanced by Loveless,[2] is supported by Lawley and his co-workers,[3,4] who showed a correlation between the appearance of *O*(6)-methylguanine residues in DNA and RNA after *in vitro* and *in vivo* methylation by S_N1 but not S_N2 methylating carcinogens. A similar observation was made by Frei.[5] Since the prevalent procedure used for the isolation of *N*- and *O*-alkylated bases in studying the molecular mechanism of mutagenesis and carcinogenesis involves acidic hydrolysis of the nucleic acid polymers,[3-5] it has become essential to elucidate the hydrolytic behaviour of the acid-labile *O*-alkylated heterocycles. We reported earlier that the reaction of diazomethane with uracil and thymine yielded methoxypyrimidines [1] and that these underwent competitive demethylation and O ⟶ N methyl migration in aqueous acids of pH 1—5.[6] We now describe the complex hydrolytic behaviour of three *O*-methylhypoxanthine derivatives, *viz.* 6-methoxypurine 9-riboside (1),[7] the isoelectronic 9-methyl analogue (2),[8] and the parent *O*-methylhypoxanthine (3).[9] Since purine nucleosides are known to lose the ribosyl group

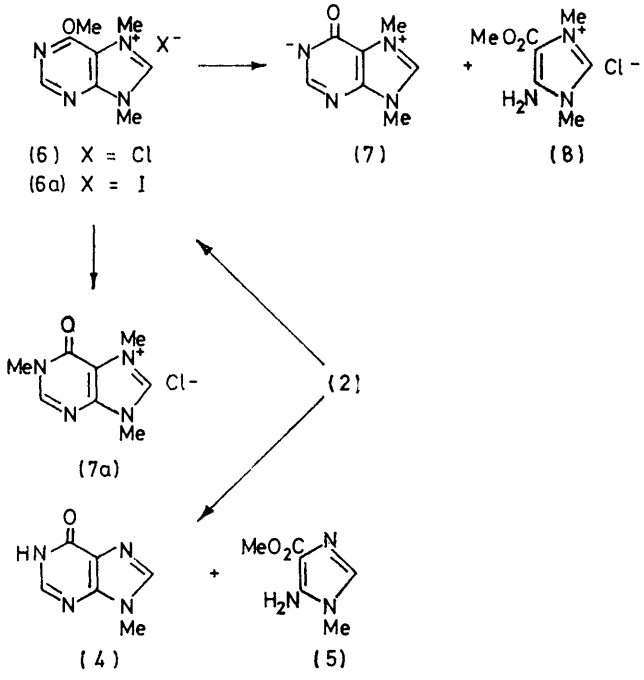


(1) R = β-D-ribofuranosyl
(2) R = Me
(3) R = H



(5) $R^1 = R^2 = Me$, X = H
(5a) $R^1 = Et$, $R^2 = Me$, X = H
(5b) $R^1 = Me$, $R^2 = X = H$
(5c) $R^1 = Me$, $R^2 = H$, X = O

readily in aqueous acid,[10] the latter two purines were examined in detail (Schemes 1 and 2, respectively) to provide an overall view of the reactivity of the ribonucleoside (1). The results indicate that the alkylation sites in transformed nucleic acids may sometimes be



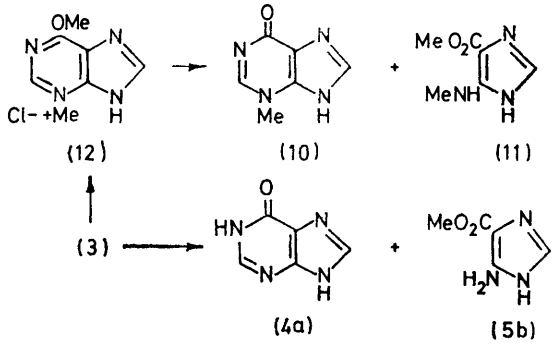
SCHEME 1

misidentified on account of the acidic conditions used in the isolation procedure.

Experiments at Low Methoxypurine Concentration.—A 0·1M-solution of 6-methoxy-9-methylpurine (2) in 0·1N-hydrochloric acid at pH 1·5, when refluxed overnight, gave 9-methylhypoxanthine (4) (28%) and methyl 5-amino-1-methylimidazole-4-carboxylate (5) (65%). The u.v. spectrum of the latter [$\lambda_{max.}$ (pH 7) 267 nm (ε 13,000)] is similar to those of other 1-alkyl-5-aminoimidazole-4-carboxylic esters [*ca.* 270 nm (ε 16,000)],[11] and the compound was identical with a sample pre-

[1] Part II, J. L. Wong and D. S. Fuchs, *J. Org. Chem.*, 1971, **36**, 848.
[2] A. Loveless, *Nature*, 1969, **233**, 206.
[3] (*a*) P. D. Lawley and C. J. Thatcher, *Biochem. J.*, 1970, **116**, 693; (*b*) P. D. Lawley and M. Jarman, *ibid.*, 1972, **126**, 893; (*c*) P. J. O'Connor, M. J. Capps, A. W. Craig, P. D. Lawley, and S. A. Shah, *ibid.*, 1972, **129**, 519; (*d*) P. D. Lawley and S. A. Shah, *ibid.*, 1972, **128**, 117.
[4] P. J. O'Connor, M. J. Capps, and A. W. Craig, *Brit. J. Cancer*, 1973, **27**, 153.
[5] J. F. Frei, *Internat. J. Cancer*, 1971, **7**, 436.
[6] J. L. Wong and D. S. Fuchs, *J. Org. Chem.*, 1970, **35**, 3786.
[7] J. A. Johnson, jun., H. J. Thomas, and H. J. Schaeffer, *J. Amer. Chem. Soc.*, 1958, **80**, 699.
[8] R. K. Robins and H. H. Lin, *J. Amer. Chem. Soc.*, 1957, **79**, 2190.
[9] Z. F. Waldhof and M. Waldhof, *Chem. Ber.*, 1957, **90**, 698.
[10] E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, 1972, **94**, 8532.
[11] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.

pared independently by transesterifying the imidazole ethyl ester (5a) [12] with sodium methoxide.



SCHEME 2

Similar treatment of 6-methoxypurine (3) either in refluxing 0·1N-hydrochloric acid or in 6N-acid at room temperature yielded the hypoxanthine (4a) as the minor product and the methyl 5-aminoimidazole-4-carboxylate (5b) as the major one. The latter was also generated independently by hydrogenation of a commercial sample of 4-methoxycarbonyl-5-nitroimidazole (5c) over 5% palladium–charcoal.[13] The same two products in similar ratio were obtained when 6-methoxypurine 9-riboside (1) was refluxed in 0·1N-acid for 24 h. The results are summarized in Table 1.

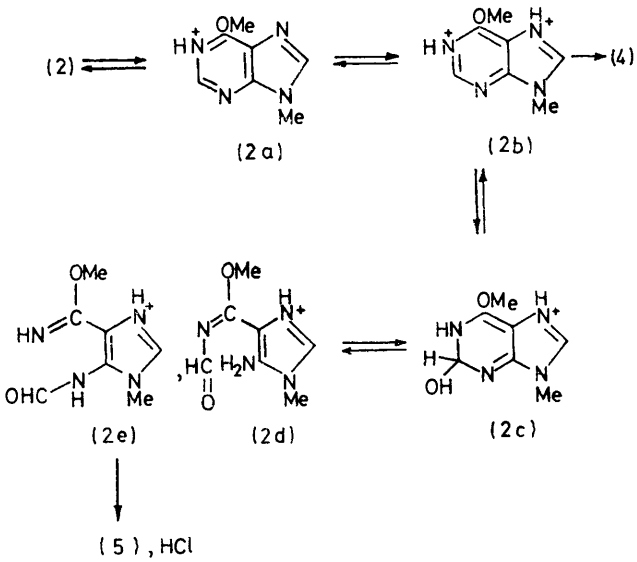
TABLE 1

Reactions of 6-methoxypurines (0·1M) in aqueous acid

Compound	Conditions	Overall yield (%)	Products (mol %) (4a)	(4)	(5)	(5b)
(1)	*a*	99	39			61
(2)	*a*	93		30	70	
	b	40—50		100		
(3)	*a*	79	30			70
	c	76	38			

[a] 0·1N-HCl at 100° for 24 h. [b] 1—12N-HCl at 100°. [c] 6N-HCl at 25° for 1 h; yield based on consumption of (3).

The acid-catalysed hydrolysis of alkoxypurines to the oxo-derivatives has long been accepted as a standard preparative reaction; [14] the oxopurines normally precipitate out of the aqueous solutions or are otherwise readily isolable. It is therefore surprising that the major product of the acidic hydrolysis of the 6-methoxypurines (1)—(3) is the water-soluble imidazole derivative. Although the stability of the aromatic nucleus of 6-monosubstituted purines such as hypoxanthine [15] and adenine [16] towards aqueous acids is well documented, several recent reports have illustrated the facility of pyrimidine ring opening in certain polysubstituted purines. Thus, Garrett *et al.*[10] observed that 1-methyladenine was cleaved upon heating at 80° in 0·1—1N-hydrochloric acid to yield 5-aminoimidazole-4-*N'*-methylcarboxamidine; Brown *et al.*[17] reported that 1-hydroxyisoguanine in refluxing 3N-hydrochloric acid generated 5-amino-4-imidazolecarboxamide oxime; Albert [18] found that the 6-methylthio-derivatives of 8-azapurine and purine were degraded by boiling 1N-hydrochloric acid to 4-amino-5-(methylthio)carbonyl-1,2,3-triazole and the corresponding imidazole; and Robins *et al.*[19] discovered that the pyrimidine ring of a 3,5'-anhydroinosine opened even in neutral water at room temperature to form an imidazole cyclonucleoside. The first three ring cleavages were catalysed by acid, and, in each case, it was suggested that a purine dication underwent covalent hydration at C-2 followed by rupture of the six-membered ring. In this context, the hydrolytic behaviour of the 6-methoxypurines can be interpreted in terms of the mechanism depicted in Scheme 3 for compound (2).



SCHEME 3

Measurements of the first and second basic pK_a values of compound (2) have provided us with a basis for estimating the availability of the dication (2b). Since Read and Goldstein [20] had established the interrelationship between chemical shifts (ν_i), pK_a, and pH for purine itself [equations (i) and (ii)], we recorded

$$f = [1 + \text{antilog}\,(\text{pH} - pK_a)]^{-1} \quad \text{(i)}$$

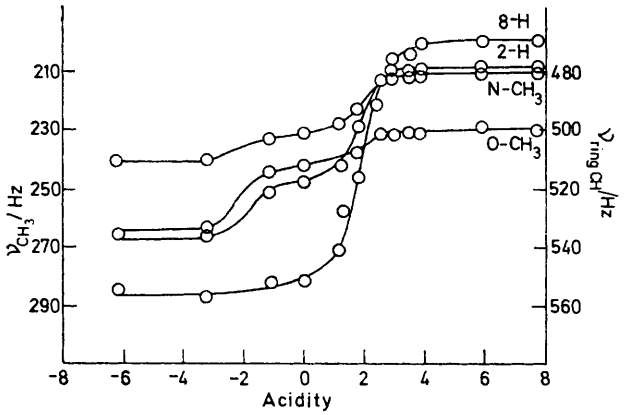
$$\nu_i = f\nu_i + (1 - f)\nu^0 \quad \text{(ii)}$$

the chemical shifts for 2-H, 8-H, N–CH_3, and O–CH_3 of compound (2) as a function of acidity (see Figure), and fitted these data into the expressions (i) and (ii) by a least-squares computer routine. The first and

[12] A. H. Cook, J. D. Downer, and I. Heilbron, *J. Chem. Soc.*, 1948, 2028.
[13] N. P. Shen and P. L. McGeer, *J. Chromatog.*, 1965, **20**, 147.
[14] J. H. Lister, 'Fused Pyrimidines. II. Purines,' ed. D. J. Brown, Interscience, New York, 1971, pp. 236—237, and references cited.
[15] A. Albert and D. J. Brown, *J. Chem. Soc.*, 1954, 2060.
[16] L. F. Cavalieri, J. F. Tinker, and G. B. Brown, *J. Amer. Chem. Soc.*, 1949, **71**, 3973.
[17] J. C. Parham, J. Fissekis, and G. B. Brown, *J. Org. Chem.*, 1967, **32**, 1151.
[18] A. Albert, *J. Chem. Soc.* (*C*), 1969, 2379.
[19] J. T. Witkowski, G. P. Kreishman, M. P. Schweizer, and R. K. Robins, *J. Org. Chem.*, 1973, **38**, 180.
[20] J. M. Read, jun., and J. H. Goldstein, *J. Amer. Chem. Soc.*, 1965, **87**, 3440.

second pK_a values were thus found to be 2·2 and −2·1, respectively, the former value being in good agreement with that of 6-methoxypurine [15] (3) (2·21). The sharp decline of the 8-H profile relative to that of 2-H in the pH region also agrees well with the large, long-range perturbation effect due to $N(1)$-protonation of the purine nucleus recently established by Grant *et al.*[21] The overall shift of 88 Hz for 8-H as opposed to 56 Hz for 2-H in the acidity range +4 to −4 indicates the formation of the 1,7-diprotonated species (2b). This dication can, by mesomerism sustain more than unit positive charge on the imidazole ring, whereas 1,3-diprotonation would tend to concentrate charge on the pyrimidine system. The assignment of structure (2b) is therefore uniquely compatible with the shift data, and also agrees with the structure of the purine and adenine dications proposed by Wagner and von Philipsborn.[22]

Since compound (2) showed negligible reactivity in a boiling aqueous solution at pH 3·1, where 10% of (2)



ν_{C-H} of compound (2) (0·2M in D_2O–D_2SO_4) *vs.* acidity; pD = pH (meter) + 0·4;[20] $-D_0$ region uncorrected for isotope effect

should exist in the monoprotonated form (2a), the bulk of the hydrolytic products obtained at lower pH values must have been derived from the dication (2b). Thus, the hydrolytic reaction of (2) at pH ≤2 containing at least 0·01% of (2) as the dication (2b) in dynamic equilibrium, upon hydration at C-6 and C-2 of (2b), would lead to 9-methylhypoxanthine (4) and the aminoimidazolecarboxylate (5), respectively. Preference for the latter route may be explained on the basis of Albert's observation [23] that covalent hydration of an *N*-heteroaromatic imino-group can be considerably hindered by a methyl group at the site of nucleophilic attack. The presence of the 6-methoxy-group in (2) stabilizes the imine system both sterically as well as by lone pair conjugation. By contrast, the unblocked 2-position of (2) is susceptible to nucleophilic attack by water, yielding the tetrahedral adduct (2c), which can follow a general acid–base-catalysed breakdown to form either one or both of the two ring-opened intermediates (2d) and (2e) in the same way as in the hydrolysis of a formamidinium compound.[24] The highly aromatic imidazole system in (2d) and (2e) should help in driving the reaction forward.

This mechanistic scheme should also function under more strongly acidic conditions. However, when compound (2) was refluxed in 1—12N-hydrochloric acid, only 9-methylhypoxanthine (4) was found (40—50%). The greater yield of the hypoxanthine product in stronger acid may be attributable to the increasing operation of the S_N2 lactim ether cleavage mechanism, as evidenced by the increasing formation of methyl chloride (detectable by ^1H n.m.r.). Disappearance of the aminoimidazole ester (5) under these stringent hydrolytic conditions was ascertained by subjecting (5) to refluxing 1N-acid overnight, which resulted in an intractable tar. This type of acid-catalysed decarboxylation followed by decomposition of the resulting 5-aminoimidazole has been noted previously.[25]

Experiments at Methoxypurine Concentration >0·1M.—At a concentration ≥0·2M, the hydrolysis products of 6-methoxy-9-methylpurine (2) are much more complicated. Illustrative is the n.m.r. spectrum of a 1M-solution of (2) in D_2O at pD 1·7 after 60 h under reflux, which reveals eleven methyl resonances in the region δ 3·5—4·6. Use of a shorter reaction time led to a more manageable mixture of products. Thus, preparative t.l.c. after 6 h under reflux showed compounds (4) (25 mol %) and (5) (63 mol %) and 6-methoxy-7,9-dimethylpurinium chloride (6) (12 mol %). The salt (6) was identical with a sample prepared independently by stirring (2) in an excess of methyl iodide in acetonitrile at 25° [to yield the purinium iodide (6a)] followed by halide exchange in aqueous mercury(II) chloride. Both samples yielded 7,9-dimethylhypoxanthine (7) as the only product upon heating with anhydrous hydrogen chloride in chloroform. In refluxing aqueous 0·1M-acid and at 0·1M concentration, the salt (6) was degraded to a 1 : 3 mixture of the hypoxanthine (7) and the $N(3)$-methyl derivative (8) of the imidazole (5), separated on a Dowex 50 cation-exchange column. Heating the purinium chloride (6) alone in dry methanol gave a good yield of 1,7,9-trimethylhypoxanthinium chloride (7a), identified on the basis of the resemblance of its u.v. spectrum [$\lambda_{max.}$ (pH 7) 250 nm (ε 9010)] to that of 1,7,9-tribenzylhypoxanthinium bromide [(pH 7) 256 nm (ε 9200)],[26] and the proven difficulty of alkylating at the 3-position of a 9-methylpurine.[27] Compound (7a) was also

[21] (*a*) R. J. Pugmire and D. M. Grant, *J. Amer. Chem. Soc.*, 1971, **93**, 1880; (*b*) R. J. Pugmire, D. M. Grant, L. B. Townsend, and R. K. Robins, *ibid.*, 1973, **95**, 2791.

[22] R. Wagner and W. von Philipsborn, *Helv. Chim. Acta*, 1971, **54**, 1543.

[23] A. Albert and W. L. F. Armarego, *Adv. Heterocyclic Chem.*, 1965, **4**, 1.

[24] D. R. Robinson and W. P. Jencks, *J. Amer. Chem. Soc.*, 1967, **89**, 7088.

[25] G. T. Litchfield and G. Shaw, *Chem. Comm.*, 1965, 563, and references cited.

[26] J. A. Montgomery, K. Hewson, S. J. Clayton, and H. J. Thomas, *J. Org. Chem.*, 1966, **31**, 2202.

[27] Z. Neiman and F. Bergmann, *Israel J. Chem.*, 1967, **5**, 243, and references cited.

obtained by methylation of 1,9-dimethylhypoxanthine (9) in neat methyl iodide at 25° followed by treatment with mercury(II) chloride. A trial-and-error admixture of the hydrolysates of 6-methoxy-9-methylpurine (2) [containing the major components (4) and (5), as well as the minor products (6), (7), (7a), and (8)] provided a solution with an n.m.r. spectrum closely resembling that of the mixture obtained by prolonged acidic treatment of (2) (see Scheme 1). The methyl migration pathway was still noticeable down to the 0·2M level, but none was apparent at 0·1M.

Likewise, a 0·5M-solution of 6-methoxypurine (3) in 0·1N-hydrochloric acid under reflux for 2 days gave four major products: hypoxanthine (4a) (31%), 3-methylhypoxanthine (10) (19%), methyl 5-aminoimidazole-4-carboxylate (5b) (33%), and methyl 5-methylaminoimidazole-4-carboxylate (11) (17%). From the presence of the last three components, which were separated by preparative t.l.c., it can be deduced that 6-methoxy-3-methylpurinium chloride (12) [28] was the key intermediate. When heated under reflux in 0·1N-acid overnight, compound (12) gave the 5-methylaminoimidazole (11) and 3-methylhypoxanthine (10) in a ratio of 7 : 3, in 92% overall yield. The latter reaction constitutes a proof of the structure of compound (11) derived from (3).

The methyl rearrangement at higher concentration of the 6-methoxypurines in aqueous acid can be seen as a Hilbert–Johnson-type reaction induced by protonation. Although this is unprecedented in the purine literature, we have shown [6] previously that several 2,4-dialkoxypyrimidines undergo such isomerization *via* intermolecular alkylation catalysed by a 1-alkyl-2,4-dialkoxypyrimidinium salt formed *in situ*. Although this postulated pyrimidine intermediate proved elusive, the purine counterparts are relatively stable; hence the successful trapping of the quaternary 7- and 3-methyl derivatives of 9-methyl-6-methoxypurine (2) and the 9-unsubstituted analogue (3), respectively, from the acidic reaction mixtures. The isolation of these methylmethoxypurinium salts confirmed that the ionic mechanism of intermolecular methyl transfer proposed for the alkoxypyrimidines also operates in the hydrolysis of the 6-methoxypurines. In the same manner that 6-methoxypurine (3) in acetonitrile was benzylated at N-3 by benzyl bromide,[26] the 6-methoxy-3-methylpurinium salt (12) is the result of methylation at N-3 of (3), probably by the methoxy-group of a dicationic species of (3). That the 9-methyl group in (2) redirects methylation to N-7 to form 6-methoxy-7,9-dimethylpurinium chloride (6) can be attributed to steric hindrance at N-3 due to the 9-methyl group and, concurrently, its inductive stabilization of the quaternized nitrogen atom at position 7. These methoxypurinium salts are capable of catalysing further methyl migrations among the purine molecules present, as shown by Montgomery and his co-workers [20] in their study of the benzylation of 6-methoxypurine (3) in dipolar aprotic solvents, which offers further insights into the complexity of multiple alkylations as a function of reaction time and temperature. On another front, the independently prepared methylmethoxypurinium salts (6) and (12) undergo ring cleavage to the same extent as the parent methoxypurine bases in aqueous solutions of pH <3 but not at lower acidities, thus substantiating the role of the dicationic species and the generality of the pyrimidine ring opening depicted in Scheme 3.

Relevance to Isolation of Methylated Bases.—We conclude that no *O*-methylated pyrimidines or purines can survive the acidic conditions used prevalently in the chemical hydrolysis of nucleic acids.[29,30] This may be the main reason for the apparent rarity of these lactim bases. Even reports of the commonly isolated *N*-methyl heterocycles are not completely free of the suspicion that these compounds may in part originate from methyl rearrangement of the methoxy-bases during acidic work-up. The concentration of the liberated heterocycles is seldom a matter of concern in isolation practice; however, even if the requirement of high dilution is attended to, the intermolecular methyl transfer remains a likely event in certain oligonucleotides, for an oligonucleotide sequence can be considered as a concentrated solution of the bases. In this context, we can appreciate the wisdom of using a multitude of isolation methods (*e.g.* neutral, mildly acidic, strongly acidic, and enzymic hydrolysis of the nucleic acids) for base identification, as reported by Lawley and his co-workers.[3,4] The cross-checking of results in such an elaborate routine minimizes the possibility of misidentification of modified bases.

EXPERIMENTAL

Instrumentation and general conditions for g.l.c. and t.l.c. analyses have been described in Part I.[6] T.l.c. plates were also developed by use of iodine vapour or Ehrlich reagent (*p*-dimethylaminobenzaldehyde in ethanolic hydrogen chloride). Other conditions of chromatographic analyses are specified in this paper. ^{1}H N.m.r. spectra of the hydrolysis products and their sources are listed in Table 2. Microanalyses were performed by M.H.W. Laboratories, Garden City, Michigan 48135.

Hydrolysis Study.—The procedure for 6-methoxy-9-methylpurine (2) is described as an example.

Concentration 0·1M. Compound (2) (164 mg, 1·0 mmol) in aqueous 0·10N-hydrochloric acid (10 ml; pH 1·5) was refluxed for 24 h. The reaction was stopped by neutralising with aqueous 2N-ammonia. T.l.c. revealed the presence of the starting material (2) (faint), the imidazole product (5) (intense), and the hypoxanthine (4) (medium) by comparison with authentic samples [relative R_F values 3·0, 1·8, and 1·0, respectively, with chloroform–methanol

[28] F. Bergmann and M. Kliener, *J. Chem. Soc.* (*C*), 1966, 10.

[29] (*a*) V. M. Craddock, *Biochim. Biophys. Acta*, 1972, **272**, 288; 1970, **240**, 376; 1969, **195**, 351; (*b*) A. E. Pegg, *ibid.*, 1972, **262**, 283; 1971, **232**, 630; (*c*) P. Gippo, M. Iaccarino, E. Parisi, and E. Scarano, *J. Mol. Biol.*, 1968, **36**, 195.

[30] (*a*) R. Sussmuth, R. Haerlin, and F. Lingens, *Biochim. Biophys. Acta*, 1972, **269**, 276; (*b*) M. Inose, S. Miyata, and Y. Iwanami, *ibid.*, 1972, **259**, 96; (*c*) E. D. Whittle, *ibid.*, 1969, **195**, 381.

(5 : 1)]. The aqueous solution was extracted continuously with chloroform overnight; the extract was dried and evaporated to yield a brown solid (110·7 mg). A sample (1 mg) was treated with 100 μl each of *NO*-bis(trimethylsilyl)trifluoroacetamide and pyridine.[31] The silylated product was chromatographed on a column packed with 1·5% SE 30 on Anachrom AS (60—70 mesh) (T_I 280°, T_O 190°, T_D 250°; nitrogen flow rate 60 cm³ min⁻¹). The chromatogram showed one peak for (5) t_R 7 min. The extracted product was recrystallized from ethyl acetate

TABLE 2

¹H N.m.r. spectra of the hydrolysis products of 6-methoxypurines

No.	Compd.	δ(D₂O) [a] Ring CH	OCH₃	N(1)CH₃ N(3)CH₃	N(7)CH₃, N(9)CH₃ N(5)CH₃
	(A) Hypoxanthines [b]				
	1-Me	8·20, 8·20		3·72	
(10)	3-Me	8·45, 8·37		4·00	
(3)	*O*(6)-Me	8·31, 8·28	4·20		
	7-Me	8·13, 8·13			4·05
(4)	9-Me	8·13, 7·80			3·65
(12)	3,*O*(6)-Me₂	9·22, 8·92	4·50	4·43	
(9)	1,9-Me₂	8·20, 7·97		3·77	3·63
(2)	*O*(6),9-Me₂	8·18, 8·08	4·10		3·77
(7)	7,9-Me₂	9·30, 8·50			4·23, 4·03
(7a)	1,7,9-Me₃ [c]	9·50, 8·80		3·77	4·33, 4·13
(6)	*O*(6),7,9-Me₃ [c]	11·03, 8·97	4·38		4·37, 4·17
	(B) Methyl 5-aminoimidazole-4-carboxylates [c]				
(5b)	Unsubst.[d]	7·60	3·97		
(5)	1-Me	7·33	3·90	3·53	
(11)	*N*(5)-Me	7·50	3·90		2·97
(8)	1,3-Me₂	8·47	3·99	3·97, 3·72	

Solutions in D_2O with sodium 3-(trimethylsilyl)propane-1-sulphonate as internal standard. [b] Prepared in this laboratory according to reported procedures. See pp. 481—482 in ref. 14 for references and other physical properties of these methylated hypoxanthines. [c] Preparations given in the Experimental section. [d] Ref. 13.

to yield *methyl 5-amino-1-methylimidazole-4-carboxylate* (5) (65%), m.p. 231°, $\lambda_{max.}$ (pH 7) 267 nm (ε 13,000); δ 3·85 (3H, s), 3·48 (3H, s), and 9·37 (1H, s) (Found: C, 46·65; H, 6·0; N, 26·8. $C_6H_9N_3O_2$ requires C, 46·45; H, 5·85; N, 27·1%). The aqueous layer was shown by t.l.c. and u.v. to contain only 9-methylhypoxanthine (4) (42 mg, 28%), which was assayed spectrophotometrically (5 l of solution showed OD_{250} 0·599).

Ethyl 5-amino-1-methylimidazole-4-carboxylate (5a) in anhydrous methanol (5 ml) containing sodium methoxide [from sodium (50 mg)] was refluxed for 2 h. Water (10 ml) was added, and the solution was continuously extracted with chloroform for 15 h. The extract was dried and evaporated, and the residue recrystallized from ethyl acetate to give (5) (20 mg, 36%), identical with the sample isolated from the hydrolysis of (2).

Concentration 1·0M. Compound (2) (164 mg, 1·0 mmol) in aqueous 0·11N-hydrochloric acid (1 ml; pH 1·7) was refluxed for 6 h. The mixture was neutralized with concentrated sodium hydroxide to pH 7. The solvent was evaporated off and the residue triturated with 95% ethanol (10 ml). The solid which did not dissolve was filtered off and washed with water (2 ml) to give 9-methylhypoxanthine (4) (15 mg, 0·1 mmol). The ethanolic extract was concentrated and subjected to preparative t.l.c. [chloroform and methanol (6 : 1)] on silica gel. Three bands at relative R_F values 76, 40, and 1·0, corresponding to the starting material (2), the imidazole ester (5), and 6-methoxy-7,9-dimethoxypurinium chloride (6) were detected. The last two bands were eluted with methanol and assayed by quantitative u.v. spectroscopy: (5), 39·4 mg (0·254 mmol); (6), 11·0 mg (0·051 mmol). Thus, the mole % distribution of (4), (5), and (6) is 25, 62·5, 12·5%, respectively. These products were identified spectrophotometrically as well as by t.l.c. comparison with authentic samples.

6-Methoxy-7,9-dimethylpurinium Salts (6) *and* (6a).—A mixture of 6-methoxy-9-methylpurine (2) (65 mg, 0·4 mmol) and methyl iodide (5 ml) was stirred at room temperature for 3 days. The methyl iodide was evaporated off and the residue was recrystallized from propan-2-ol–methanol (1 : 1) to yield the *iodide* (6a) (110 mg, 91%), m.p. 207°, $\lambda_{max.}$ (pH 1—7) 228 (ε 12,800) and 250sh nm (6900) (Found: C, 31·35; H, 3·7; N, 18·15. $C_8H_{11}IN_4O$ requires C, 31·4; H, 3·6; N, 18·3%). The iodide (6a) (15 mg, 0·05 mmol) and mercury(II) chloride (14 mg, 0·05 mmol) were stirred in water (1 ml) for 1 h. The red precipitate was removed and the filtrate concentrated to yield the purinium chloride (6) (10 mg, 94%), $\lambda_{max.}$ (pH 6) 254 nm (ε 6500), identical with that isolated from the 1M hydrolysis of (2).

1,7,9-Trimethylhypoxanthinium Chloride (7a).—1,9-Dimethylhypoxanthine (9) (75 mg, 0·5 mmol) was added to methyl iodide (1 ml) and the mixture was stirred at room temperature for 3 days. The methyl iodide was evaporated off and the residue recrystallized from isopropyl alcohol to give *1,7,9-trimethylhypoxanthinium iodide* (120 mg, 80%), m.p. 210° (Found: C, 31·1; H, 3·9; N, 18·35. $C_8H_{11}IN_4O$ requires C, 31·4; H, 3·6; N, 18·3%). This was subjected to the mercury chloride treatment to yield compound (7a), $\lambda_{max.}$ (pH 7) 250 nm (ε 9010). The same 1,7,9-trimethylhypoxanthinium chloride (7a) was obtained when 6-methoxy-7,9-dimethylpurinium chloride (6) (10 mg) in anhydrous methanol (2 ml) was refluxed for 24 h. Upon cooling, compound (7a) precipitated (7 mg).

5-Amino-4-methoxycarbonyl-1,3-dimethylimidazolium Chloride (8).—6-Methoxy-7,9-dimethylpurinium iodide (6a) (30·6 mg, 0·1 mmol) in 0·1N-[²H]hydrochloric acid (1 ml) was refluxed for 15 h. Integration of the methyl n.m.r. singlets at the end of the reflux period revealed the presence of 7,9-dimethylhypoxanthine (7) (δ 4·23 and 4·03) and the 5-amino-4-methoxycarbonyl-1,3-dimethylimidazolium salt (8) (δ 3·99, 3·97, and 3·72) in a ratio of 1 : 3. No starting purinium salt remained. The mixture was applied to a Dowex AG-50 column and chromatographed with 1N-hydrochloric acid. The first component [$\lambda_{max.}$ (pH 1) 255 nm; (pH 7) 267] eluted was (7), which was followed by the *salt* (8) (12·2 mg), $\lambda_{max.}$ (pH 1—10) 267 nm (ε 13,500); the latter was precipitated five times from acetone–water (Found: C, 37·5; H, 6·35; N, 18·7. $C_7H_{12}ClN_3O_2,H_2O$ requires C, 37·7; H, 6·35; N, 18·8%).

Methyl 5-Methylaminoimidazole-4-carboxylate (11).—6-Methoxy-3-methylpurinium chloride [28] (12) (32 mg, 0·2 mmol) in 0·10N-hydrochloric acid (2 ml) was refluxed for 24 h. The reaction was stopped by neutralization with aqueous 2N-ammonia and the mixture was continuously

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extracted with chloroform. The aqueous layer was shown by u.v. assay to contain 3-methylhypoxanthine (10) (8·24 mg, 27·5%). The organic extracts were dried and evaporated, and the residue was recrystallized from ethyl acetate to yield the *imidazole ester* (11) (20 mg, 0·13 mmol, 65%), m.p. 147°, $\lambda_{max.}$ (pH 7) 285 nm (ε 14,200), analysed as the hydrochloride (Found: C, 37·75; H, 5·2; N, 21·8. $C_6H_{10}ClN_3O_2$ requires C, 37·6; H, 5·25; N, 21·95%).

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