Tetrahedron. Vol 27, pp 5969–5978 Pergamon Press 1971. Printed in Great Britain

PURINE N-OXIDES—XL
THE 3-ACYLOXYPURINE 8-SUBSTITUTION REACTION: SCOPE: SYNTHESES OF 8-SUBSTITUTED XANTHINES AND GUANINES*

N. J. M. BIRDSALL, U. WÖLCKE, T.-C. LEE and G. B. BROWN
Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University, New York, New York 10021

(*Received in the USA* 23 *July* 1971; *Received in the UK for publication* 17 *August* 1971)

Abstract—The 3-acyloxypurine 8-substitution reaction is a substitution-elimination reaction involving attack at C-8 by inorganic or organic nucleophiles and departure of an acyloxy group from N-3. It has been studied with 3-acetoxyxanthine, 3-hydroxyguanine and a number of related derivatives and is the method of choice for the preparation of many 8-substituted xanthines or guanines. It proceeds extremely rapidly in neutral aqueous solutions at room temperature. With water alone an 8-hydroxypurine results, and water always competes to some degree with other nucleophiles. The reaction can be carried out in dipolar aprotic solvents, in which it is also possible to prepare the acyloxy derivative *in situ* and to choose more effective leaving groups such as mesyloxy or tosyloxy. The reaction has been demonstrated with chloride, bromide, nitrite, and azide ions; with the thioether of methionine; a variety of pyridine derivatives, and with primary and secondary alcohols. This reaction is apparently restricted to 3-acyloxypurines which are also substituted at position-2. The behaviour of 3-acetoxy-1-methylxanthine is similar to that of 3-acetoxyxanthine, but 3-acetoxy-7-methylxanthine fails to undergo the reaction.

IN HOT ACETIC ANHYDRIDE 3-hydroxyxanthine (**1**) forms uric acid (**3**) and with trifluoroacetic anhydride 3-hydroxyguanine (**7**) leads to 8-hydroxyguanine (**9**: R = OH).[1] Preliminary communications[2,3] reported some reactions of 3-acetoxyxanthine (**2**), an intermediate in the first apparent rearrangement. The scope of this unique substitution at position-8, including reactions with several inorganic and organic nucleophiles, has been further explored in water, alcohols and in aprotic solvents. The reactivities of several N-alkyl-3-acetoxyxanthines,[4] of 1-acetoxyxanthine[4] and of hypoxanthine 3-oxide[5] have been compared.

RESULTS

Reactions in water and alcohols

The reaction of 3-acetoxyxanthine (**2**) in water at room temp is complex. At pH's of about 5 uric acid (**3**) is produced in about 12% yield. The yield of uric acid is increased at higher temps. That reaction is accompanied by some hydrolytic reversion to 3-hydroxyxanthine (**1**), some production of a highly insoluble blue compound (**5**); and some reduction to xanthine (**4**). The last was previously noted occasionally,[2] but material balance studies of many reaction mixtures now show it to be a usual product. The overall reaction with water is rapid. with a half-time of 50 min as followed by changes of the UV absorption of about 10^{-4} M solutions of 3-acetoxyxanthine in water. In 0·9% NaCl the half-time is about 15 min and 8-chloroxanthine (**6**; R = Cl) is a major product.

* This work was supported in part by funds from the National Cancer Institute (Grant No. CA 08748) and from the Atomic Energy Commission (Contract No. AT[30–1]910). T.-C. L. was a Fellow of the Damon Runyon Memorial Fund, 1969–70.

The products of the reaction of 3-acetoxyanthine in the presence of nucleophiles were examined at times when the reactions had gone to completion (Table 1). Aliquots of the mixtures were assayed for the various products by chromatography with Dowex-50. Water is always a competitive reactant, both in the hydrolysis to 3-hydroxyxanthine. and as a competitive nucleophile. The reaction with chloride ion gave a 42%

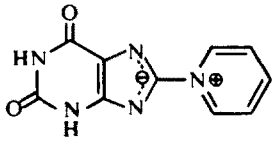
TABLE 1. REACTIONS OF 3-ACYLOXYXANTHINES IN WATER AND ALCOHOLS

Reaction conditions: Conc. nm	Nucleophile (equiv.)	T°	Hr	Products and Yields: 3-OH-Xan.	Xan.	Uric Acid	8-R Xan	Ref.
		Reactions of 3-acetoxyxanthine:						
2	H_2O	23	24	75	5	12		[a]
2	H_2O	100	1	17[b]	13[b]	70[b]		[a]
2	80% Dioxane	80	24	10	4	75		[a]
2	F^-(5)	23	24	50	22	18	—	
2	Cl^-(5)	23	24	27	7	5	42[c]	11[a]
2	Br^-(5)	23	24	47	13	16	15[c]	12[a]
2	I^-(5)	23	24	—	100	—	—	[d]
16	NO_2^-(8)	23	24	9	20	<1	40[c]	13[a]
16	N_3^-(4)	23	24	—	—	—	87[e]	
16	Methionine(4)	23	24	54	—	—	40[c]	14[a]
2	Pyridine(5)	23	24	24	5	4	44[e]	
28	Nicotinamide(10)	23	24	—	—	—	70[e]	
20	Guanosine(2)	23	48	56	10	6	—	[f]
20	Methanol[g]	70	2	—	[h]	—	90	
20	Ethanol[g]	70	3	—	[h]	—	84	
20	isoPropanol[g]	70	2	—	[h]	—	85	
		Reactions of 3-acetoxy-1-methylxanthine:						
4·4	H_2O	100	1	5	71	24		15[a]
14	H_2O	23	48	64	26		10[b]	[a]
14	Cl^-(16)	23	48	48	20	5	21[c]	
28	NO_2^-(1·5)	23	6	—	—	—	38[c]	
14	Methionine(4)	23	24	20	<5	—	28[c]	
12	Methanol[g]	70	4	—	[h]	—	~100	
12	Ethanol[g]	78	4	—	[h]	—	~100	
		Reaction of 3-acetoxy-1-benzylxanthine:						
7	Methanol[g]	70	1·5	—	[h]	—	~100	
		Reaction of 3-acetoxy-7-methylxanthine:						
2	H_2O	23	24	~100[e]	0	0	0	16
		Reaction of 3-acetoxy-8-azaxanthine:						
2	H_2O	23	24	~100[e]	0	0	0	17
		Reaction of 1-acetoxyxanthine:						
2	H_2O	23	24	~100[e]	0	0	0	7

[a] Blue compound detected. [b] Ratios of products other than blue compound. [c] Isolated after chromatography. [d] One equivalent of I_2 titrated by thiosulfate. [e] Precipitated from the solution. [f] Blue compound precipitated in quantity. see experimental. [g] As solvent and nucleophile. [h] Traces of xanthine were found upon chromatography of the products on Dowex-50 columns.

yield of 8-chloroxanthine and decreased the extents of hydrolysis and uric acid formation. Other inorganic nucleophiles such as bromide, nitrite or azide ions gave 8-bromo-, 8-nitro- and 8-azidoxanthines in good yields. Fluoride ion is apparently not a sufficiently strong nucleophile to compete with water and no 8-fluoroderivative was obtained. With iodide ion the reaction takes a completely different course and the 3-acetoxyxanthine is reduced to xanthine with quantitative oxidation of the iodide ion to iodine.*

Many organic nucleophiles react under the same gentle conditions. Methionine reacts very rapidly, undoubtedly via a sulfonium intermediate which eliminates homoserine lactone to yield 8-methylmercaptoxanthine (**6**; R = SMe). Pyridine reacts to give a bright yellow compound to which we assign the structure of 8-(N-pyridinium)xanthine betaine:



In base this is hydrolyzed quantitatively to 8-aminoxanthine, as are other N-substituted pyridinium compounds.[6] Although xanthine ionizes at position 3, the inductive effect of the pyridinium moiety should be sufficient to bring about ionization of the imidazole proton, as depicted. Nicotinamide reacts to yield 8-(N-pyridinium-3-carboxamide)xanthine betaine in excellent yield.

In alcohols 3-acetoxyxanthine reacts surprisingly rapidly with the very weakly nucleophilic solvent. The reaction with MeOH, followed spectrophotometrically at low concentrations, is complete in 30 min at room temp. With primary or secondary alcohols 8-alkoxyxanthines are formed in high yields, accompanied by a little xanthine. The structures were confirmed by the similarity of their UV and NMR spectra to that of 8-methylxanthine and by their hydrolyses to uric acid.

Among the other acetoxyxanthine derivatives which are available, only 3-acetoxy-1-methylxanthine exhibits a reactivity in aqueous solutions which is comparable to that of **2**. With water, chloride, nitrite or methionine, it yields the corresponding 1-methyl-8-substituted xanthines. In alcohols it and the 1-benzyl analog yield 8-alkoxyxanthines. Obviously substitution at the 1-position has little influence upon the reaction. Under similar conditions the isomeric 1-acetoxyxanthine[4] undergoes only hydrolysis to 1-hydroxyxanthine,[7] and the 3-acetoxy-7-methylxanthine and 3-acetoxy-8-azaxanthine are hydrolyzed to the parent 3-hydroxy derivatives.

Reactions in aprotic solvents

The 8-substitution of 3-acetoxyxanthine can be carried out in dipolar aprotic solvents such as DMF, DMAc and DMSO, in which the competitive reaction with water is avoided (Table 2). Such solvents also permit the conversion of the parent 3-hydroxypurine to its acyloxy derivative which can then be allowed to react *in situ* with the desired nucleophile (Table 2). The acylation *in situ* by an acid chloride does add

* Aqueous KI does not reduce 3-hydroxyxanthine.

TABLE 2. REACTIONS OF 3-ACYLOXYPURINES IN APROTIC SOLVENTS

Wt. g	Acylating agent ml	Nucleophile	T°	Hr	Solvent ml	8-Nu purine, yield
		Reactions of 3-acetoxyxanthine:				
0·01	none	pyridine 7·5 mg	24	24	DMF(0·5)	60[a]
0·01	none	6 N HCl 10 μl	24	1	DMF(0·5)	90[b]
		Reactions of 3-hydroxyxanthine:				
0·10	AcCl(0·35)	Cl^-	100	1	DMF(1·5)	40[a]
0·10	MsCl(0·40)	Cl^-	23	2	DMF(1·5)	35[a]
0·10	TsCl(0·5)	Cl^-	23	18	DMF(1·5)	50[a]
0·10	$ClSO_3H$(0·4)	Cl^-	23	1	DMF(1·5)	65[b,c]
2·5	MsCl(7·5)	pyridine 7·5 ml	0	1	DMF(50)	65[a]
1·0	MsCl(2·0)	γ-picoline 2·0 ml	0	1	DMF(25)	23[a]
1·0	MsCl(2·0)	nicotinic acid (2·0 g)	0	1	DMF(25)	20[a]
1·0	MsCl(2·0)	nicotinamide (2·0 g)	0	1	DMF(25)	25[a]
0·10	MsCl(0·3)	quinoline (0·3 ml)	0	1	DMF(1)	28[a]
		Reactions of 3-hydroxy-7-methylxanthine:				
0·10	MsCl(0·2)	Cl^-	50	2	DMF(1)	48[a]
0·04	MsCl(0·1)	pyridine (0·1 ml)	23	1	DMF(1)	47[a]
		Reactions of 3-hydroxyguanine:				
0·60	MsCl(1·2)	Cl^-	23	1	DMF(6)	75[b]
1·0	AcCl(2·0)	pyridine (5 ml)	23	20	DMF(20)	30[a]
		Reaction of 3-acetoxyxanthine:				
0·10	—	HMPA[d] (10 ml)	100	0·1	HMPA(10)	27[b,e]

[a] Precipitated from solution. [b] Isolated by chromatography. [c] Also 12% as uric acid. [d] As solvent and nucleophile. [e] 8-Dimethylaminoxanthine; also 17% uric acid. 11% 3-hydroxyxanthine and about 1% xanthine.

chloride ion as a competing nucleophile, but this procedure also permits the introduction of better leaving groups, such as mesyloxy or tosyloxy. With chlorosulfonic acid as the acylating agent a sulfate ester is the presumed intermediate. This general method also permits the use of 3-hydroxypurines, notably 3-hydroxyguanine,[8] from which no 3-acyloxy derivative has been isolated.

The addition of pyridine to the reaction product of 3-hydroxyxanthine and $MeSO_2Cl$ at 0° gives the 8-(N-pyridinium) xanthine betaine in excellent yield. Analogous pyridinium derivatives are obtained from γ-picoline, quinoline, nicotinamide, and

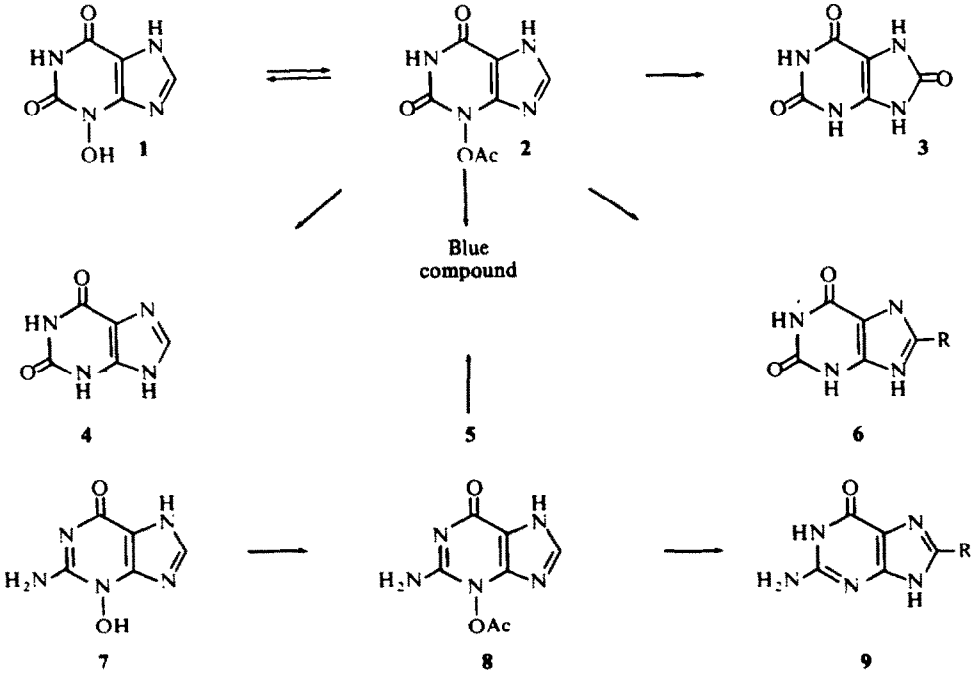
nicotinic acid. No products were obtained from s-collidine, acridine or phenanthridine, probably because of steric effects.

Although the acetoxy group of 3-acetoxy-7-methylxanthine is merely hydrolyzed in water, this 7-Me derivative can be converted *via* the mesyloxy derivative in DMF to either 8-chloro- or 8-pyridinium-7-methylxanthines. We have previously noted that 3-hydroxy-9-methylxanthine gives 9-methyluric acid in hot Ac_2O, but that its 3-acetoxy derivative has not been isolated.[4]

As part of the proof of structure of 3-hydroxyxanthine, we noted that dimethylsulfate in DMF leads to 3-hydroxy-7,9-dimethylxanthine which, in turn, goes to 7,9-dimethyluric acid,[9] presumably *via* a 3-methylsulfate derivative. A by-product, $C_7H_8N_4O_6S$, isolated[10] from such a mixture, has now been shown to be the 3-O-sulfate ester of 7,9-dimethyl-3-hydroxyxanthine. Heating it in DMF, or thermal decomposition of the solid at 240°,[10] yields 7,9-dimethyluric acid.[1,9] This provides another indication that a sulfate ester can undergo the 8-substitution reaction.

Spectral evidence has shown[8] that the guanine 3-N-oxide derivative exists at pH 5 to 7 largely as 3-hydroxyguanine or its anion. Although we have not isolated 3-acetoxyguanine, a 3-acyloxyguanine must be involved in the formation of 8-hydroxyguanine in $(F_3CCO)_2O$.[1] A 3-acetoxyguanine derivative, prepared *in situ* in DMF, reacts with added pyridine to give N^2-acetyl-8-(N-pyridinium)guanine betaine. The N^2-acetyl group can be removed in acid to give 8-(N-pyridinium)guanine chloride. With $MeSO_2Cl$ in DMF 8-chloroguanine is obtained in good yield from 3-hydroxyguanine.

SCHEME 1



Hexamethylphosphoric triamide (HMPA) was tried as an aprotic solvent but it reacted as a nucleophile. Considerable 8-dimethylaminoxanthine as well as some 3-hydroxyxanthine and uric acid were obtained.

DISCUSSION

Many heterocyclic N-oxides react with acylating agents in the presence of nucleophiles to yield C-substitution products, usually at the adjacent position and under vigorous conditions.[18, 19] The reactions described here are characterized by the rapidity with which very weak nucleophiles react under extremely mild, essentially physiological. conditions. These 8-substitutions of purine derivatives are accompanied by elimination of an acyl group from N-3. From our present experience it appears that the reaction is limited to 3-acyloxypurines which are also substituted in the 2-position, and perhaps to only those which can exist as 3-hydroxy tautomers. For brevity, and to differentiate it from reactions involving adjacent positions, we refer to it as the 3-acyloxypurine 8-substitution reaction.

Kawashima and Kumashiro have recently reported[20] the formation of 8-chlorohypoxanthine from hypoxanthine 3-oxide in hot HCl. That reaction must involve a different mechanism since it is acid catalyzed while the 3-acyloxypurines are stabilized in acid[4] and undergo this facile 8-substitution only at or near neutrality. Upon acetylation hypoxanthine 3-oxide is attacked at the adjacent 2-position and leads to xanthine,[1] while reaction with Ac_2O and pyridine in DMF yields 2-(N-pyridinium)hypoxanthine.[21] These are in contrast to the attacks at the 8-positions which occur with 3-hydroxyxanthine or 3-hydroxyguanine under similar conditions.

The isolation of 8-substituted xanthines[22] and guanines[23] from the urine of rats to which 3-hydroxyxanthine or 3-hydroxyguanine has been administered provides evidence that. *in vivo.* both esterification, presumably enzymatic,[23] and spontaneous chemical reaction of that ester with nucleophiles can take place. These reactions could be involved in the initiation of the tumors which these purine N-oxide derivatives can induce.[24] Esters of N-hydroxyarylamines and their chemical reaction with nucleophilic groups, including a similar reaction with methione. have been implicated in oncogenesis by N-hydroxyacetylaminofluorene,[25] dimethylaminoazobenzene[26, 27] and 4-hydroxyaminoquinoline N-oxide.[28]

The reasons for the ready reactivity of 3-acetoxy-1-methylxanthine and of the failure of 3-acetoxy-7-methylxanthine to undergo the reaction in water are clarified in the following manuscript[29] on the mechanism of this reaction. The utility of this 3-acyloxypurine 8-substitution reaction as a preparative method for 8-substituted xanthines and guanines, some otherwise unavailable, is to be emphasized.

EXPERIMENTAL

Reactions in water or alcohols. The nucleophile was dissolved in 20 ml of distilled water, 8·4 mg of 3-acetoxyxanthine[4] was added and the mixture stirred at the temp and time indicated (Table 1). For known products the reference to the authentic sample is given in the table, and for new products characterizations are described below. A 0·1 to 1·0 ml aliquot of the mixture was chromatographed over a column of Dowex-50, 8-X (H^+), 200–400 mesh and monitored with ISCO-UV Analyzer. The amounts of each product were determined from the OD and volume of each fraction. The column sizes varied. but for a 9 × 150 mm column eluted at 60 ml per hr several of the products show the following retention volumes:

Compounds:	Eluants 0·05 N HCl	1·0 N HCl
	ml	ml
Uric acid	13	13
3-Acetoxyxanthine	33	dec.
3-Hydroxyxanthine	85	38
8-Methylmercaptoxanthine	175	75
Xanthine	340	60
8-Pyridinium xanthine	—	204

Water or dilute acid was often used to elute the first three, followed by 1 or 2 N HCl to elute the more basic compounds. The chloro-, bromo-, nitro- and azidoxanthines appear at 13–16 ml and are but partially separated from uric acid except on longer columns. Their yields are based upon the isolated yields. Traces of a blue precipitate were often noted.

8-*Azidoxanthine.* This precipitated from the aqueous mixture and was recrystallized from water. UV (max): 289·5 nm at pH 5 and 299 at pH 11. The IR spectrum (KBr), with a peak at 2.175 cm^{-1} suggests it to be an azide.[30] rather than a tetrazole as found[33] for crystals of 6-azidopurine.[32,33] (Calc. for $C_5H_3N_7O_2 \cdot 0{\cdot}5H_2O$: C, 29·70; H, 1·98; N. 48·51. Found: C, 30·01; H, 1·89; N, 48·33%).

Preparation of the blue compound. To guanosine (2·0 g) in 40 ml of water at 80° was added 3-acetoxyxanthine (840 mg) and the solution stirred at 80° for 10 min. A flocculent deep blue precipitate formed and the solution was added to 450 ml H_2O at 90° and stirred for a further 10 min. The solution was centrifuged, the precipitate resuspended in 250 ml H_2O at 90° and again centrifuged. This process was repeated three times and each deep blue colloidal suspension was acidified with 2 ml of conc HCl to initiate precipitation. The deep blue powder, 115 mg, is stable in air. A blue product is also obtained in lesser yield if the guanosine is omitted. The analysis reported is that of a sample prepared in the presence of guanosine. It corresponds to that for uric acid or 3-hydroxyxanthine. The guanosine does not appear to be incorporated into the product, but may serve a catalytic or solubilizing function. (Calc. for $(C_5H_4N_4O_3)_x$: C, 35·72; H, 2·40; N, 33·33. Found: C, 35·98; H. 2·35; N, 33·68%). The blue compound decomposes in boiling water or in solution in DMF or DMSO. In 2 N NaOH it gives a pale yellow solution which upon chromatography over a Dowex-50 column yields about 5% of the total OD as 3-hydroxyxanthine, which may well be an impurity, variable amounts of 8-aminoxanthine,[34] and at least five unidentified products.

8-*Chloro*-1-*methylxanthine.* This was eluted with water, concentrated and recrystallized from water. UV (max): 275 nm at pH 4 and 286 at pH 10. (Calc. for $C_6H_5N_4O_2Cl \cdot H_2O$: C, 32·96; H, 3·22; N, 25·63. Found: C, 33·02; H. 2·50; N, 25·47%).

1-*Methyl*-8-*nitroxanthine.* This was eluted with water, concentrated and recrystallized from water. UV (max): 231 and 363 nm at pH 2; 383 at pH 5; and 260, 305 and 430 at pH 8. (Calc. for $C_6H_5N_5O_4$: C, 34·13; H, 2·39; N, 33·17. Found: C, 34·20; H 2·50; N, 32·95%).

1-*Methyl*-8-*methylmercaptoxanthine.* This was eluted with 1 N HCl, concentrated and recrystallized from water. UV (max): 291 nm at pH 4 and 297 at pH 11. (Calc. for $C_7H_8N_4O_2S$: C, 39·63; H, 3·80; N, 26·40; Found: C, 39·74; H, 3·71; N, 26·24%).

8-*Alkoxyxanthines.* These were isolated by evaporation of the alcohol and trituration with H_2O. Hydrolyses in HCl yield uric acid or 1-methyluric acid.[15] The NMR spectra of the 8-alkoxyxanthines show the absence of a hydrogen at the 8-position and are otherwise similar to that of 8-methylxanthine. They each show maxima in the UV at 277 nm, with shoulders at 230 nm at pH 2, and maxima at 261 and 288·5 at pH 11.

8-*Methoxyxanthine.* (Calc. for $C_6H_6N_4O_3$: C, 39·57; H, 3·32; N, 30·76. Found: C, 39·37; H, 3·27; N, 30·52%).

8-*Ethoxyxanthine.* (Calc. for $C_7H_8N_4O_3$: C, 42·86; H, 4·11; N, 28·56. Found: C, 43·19; H, 4·15; N, 28·66%).

8-*Isopropoxyxanthine.* (Calc. for $C_8H_{10}N_4O_3 \cdot O{\cdot}5H_2O$: C, 43·84; H, 5·06; N, 25·56. Found: C. 44·23; H, 4·90; N, 26·01%). The 8-alkoxy-1-methylxanthines show maxima at 277 at pH 4·5 and 287 with shoulders at 245 at pH 10.

1-*Methyl*-8-*methoxyxanthine.* (Calc. for $C_7H_8N_4O_3$: C, 42·86; H, 4·11; N, 28·56. Found: C, 42·70; H, 4·07; N, 28·43%).

1-*Methyl*-8-*ethoxyxanthine*. (Calc. for $C_8H_{10}N_4O_3$: C, 45·71; H, 4·80; N, 26·65. Found: C, 45·64; H, 4·66; N, 26·73%).

1-*Benzyl*-8-*methoxyxanthine*. (Calc. for $C_{13}N_{12}N_4O_3$: C, 57·35; H, 4·42; N, 20·58. Found: C, 57·62; H, 4·54; N, 20·40%).

Reactions in dipolar aprotic solvents (Table 2). (a) 3-Acetoxyxanthine in DMF is stable for many hr, but some 3-hydroxyxanthine and uric acid are formed from the unavoidable traces of water. When pyridine is added a precipitate appears within minutes. The insolubility of NaCl prevents a satisfactory reaction but reaction with HCl is rapid.

(b) The 3-hydroxypurine dissolved in DMF was cooled to 0° and the acylating agent added. After 10 min the nucleophile was added and the stirring continued at the temp indicated. In those reactions examined chromatographically, there was always some 8-chloroxanthine, the amount of which increased with delay in the addition of the nucleophile or at higher temps. Similar results, with chloride or pyridine as nucleophiles, were obtained with DMAc or DMSO as solvents. Without addition of the acylating agent 3-hydroxyxanthine remains unchanged under such conditions.

8-(N-*Pyridinium*)*xanthine betaine*. The 2·4 g of yellow solids collected from the dark red DMF after the reaction of 2·5 g of 3-hydroxyxanthine contained traces of sulfur. It was recrystallized twice from 2 N HCl, and was obtained as the betaine.

It and related substituted pyridinium xanthines showed the characteristics detailed in Table 3.

TABLE 3

Recrystn. solvent	Absorption maxima, nm [ ] = shoulder	Analyses (%) C H N
8-(N-*Pyridinium*)*xanthine betaine* ($C_{10}H_7N_5O_2 \cdot O \cdot 5H_2O$):		
2 N HCl	pH 2: 242, 266, 372, pH 11: 250, 275, 414,	Calc.: 50·42; 3·38; 29·53 Found: 50·62; 3·02; 29·26
8-(N-γ-*Picolinium*)*xanthine betaine* ($C_{10}H_9N_5O_2 \cdot 1 \cdot 25H_2O$):		
1 N HCl	pH 0: 228,[255],337, pH 5:[226],266, 362, pH 11: 274, 400,	Calc.: 49;30; 4·30 26·15 Found: 49;32; 3·93 26·38
8-(N-*Pyridinium*-3-*carboxylic acid*)*xanthine betaine* ($C_{11}H_7N_5O_4 \cdot H_2O$): Decomp. upon heating, may decarboxylate.		
2 N HCl	pH 0: 255, 362, pH 5: 253,[270],380, pH 11: 263,[280],420,	Calc.: 45·37; 3·12; 24·05 Found: 45·72; 2·48; 24·03
8-(N-*Pyridinium*-3-*carboxamide*)*xanthine chloride* ($C_{11}H_8N_6O_3 \cdot HCl \cdot H_2O$):		
2 N HCl	pH 0: 254, 364, pH 5: 251,[270],380, pH 11: 262,[280],420,	Calc.: 40·44; 3·39; 25·72 Found: 40·36; 3·10; 25·62
8-(N-*Quinolinium*)*xanthine betaine* ($C_{14}H_9N_5O_2 \cdot H_2O$):		
2 N HCl	pH 0: 265, 319, 350, pH 5: 238, 269, 318, 390, pH 11: 239, 285,[316],434,	Calc.: 56·57; 3·73; 23·51 Found: 56·17; 3·22; 23·56

7-*Methyl*-8-(N-*pyridinium*)*xanthine methylbisulfate* ($C_{11}H_{10}H_5O_2 \cdot CH_3SO_3$): The precipitate from the DMF was washed with alcohol and ether. It contained sulfur and the analysis corresponded to that for the methylbisulfate. pH 0:[220], 250, 331, pH 11: 240, 288, 454, (Calc. for C, 42·47; H, 3·86; N, 20·64. Found: C. 42·35; H, 3·87; N, 20·35%).

2-(N-Pyridinium)benzimidazole and related compounds also show an absorption band near 400 nm[35]

8-Aminoxanthine from 8-(N-pyridinium)xanthine. A sample (28 mg) in 2 N NaOH (1 ml)[6] was warmed at 75° for 30 min. The clear red-brown solution, which showed a UV peak at 364 nm, the reported value for glutaconic dialdehyde anion,[36, 37] was cooled, acidified with glacial AcOH and evaporated to dryness. The residue was dissolved in H_2O (12 ml) and chromatographed over a 10 × 100 mm Dowex-50 [H^+] column. Three minor peaks were eluted with water and then 8-aminoxanthine (18 mg. 95%) with 1 N HCl. Recrystallization from 2 N H_2SO_4 gave a sample which was identical, on chromatography in three solvents, in UV spectra at 3 pH's and IR spectrum with an authentic sample.[34]

N^2-Acetyl-8-(N-pyridinium)guanine betaine. The product which precipitated from the DMF was recrystallized twice from H_2O. Analysis showed it to be an acetyl derivative. UV (max) 243, 275 and 347 nm at pH 0; 248, 270 sh. and 370 at pH 5; and 250 and 394 nm at pH 11. The NMR spectrum shows the same pyridinium peaks as 8-(N-pyridinium)xanthine, and also a peak at 7·85 τ, comparable to that of 7·81 τ for N^2-acetylguanine.[38] (Calc. for $C_{12}H_{10}N_6O_2 \cdot H_2O$: C, 49·85; H. 4·46: N, 29·07. Found: C, 50·05; H, 3·91; N 29;28%).

8-(N-Pyridinium)guanine chloride. The acetyl derivative (510 mg) was heated in N HCl (10 ml) at 80° for 90 min. When cooled to 0° the chloride (440 mg, 95%) precipitated. This was recrystallized twice from N HCl. UV (max): 233, 347 nm at pH 0; 214, 267·5 and 386 at pH 5; 250·5 and 420 at pH 11. (Calc. for $C_{10}H_9N_6OCl$: C, 45·38; H, 3·43; N, 31·75; Cl. 13·39. Found: C, 45·37; H, 3·32; N, 31·90; Cl, 13·60%).

8-Chloro-7-methylxanthine. This was precipitated from the DMF by addition of dioxane and recrystallized from H_2O. UV (max): 273 at pH 0 and 291 at pH 11. (Calc. for $C_6H_5N_4O_2Cl$: C, 35·93; H, 2·51; N, 27·93; Cl. 17·67. Found: C, 35·84: H, 2·48; N, 27·89; Cl, 17·57%).

8-Chloroguanine hydrochloride. The red DMF mixture was added to 25 ml N HCl, evaporated to dryness, redissolved in HCl and chromatographed over a 4 × 7·5 cm Dowex-50 column. A little 8-hydroxyguanine was eluted by 1 N HCl, and the chloro derivative with 2 N HCl. The latter fraction was evaporated to yield 565 mg, which was recrystallized from 2 N HCl. UV (max): pH 0, 270 nm with a shoulder at 250; pH 11, 277 with shoulder at 260. (Calc. for $C_5H_4N_5OCl \cdot HCl$: C, 25·01; H, 2·94; N. 29·17; Cl, 29·54. Found: C. 25·36; H, 2·87; N, 29·28; Cl, 29·34%). The free base was precipitated from NaOH by the addition of AcOH. (Calc. for $C_5H_4N_5OCl \cdot H_2O$: N, 34·57: Cl, 17·49. Found: N, 34·56; Cl. 17·70%).

8-Dimethylaminoxanthine. The product from the reaction of 3-acetoxyxanthine in commercial HMPA[39] was precipitated by the addition of 40 ml of ether. By chromatography fractions were obtained at the positions of uric acid, 17%, 3-hydroxyxanthine, 11% and xanthine less than 1%. and a fourth peak, about 27%. The latter was concentrated and the residue recrystallized from water. It showed maxima in the UV 238. 286 at pH 0· 217 [245], 301 at pH 5: 227 [255] and 301 at pH 11. (Calc. for $C_7H_9N_5O_2 \cdot O \cdot 5H_2O$: C, 41·18; H, 4·93; N, 34·30. Found: C, 41·34; H, 5·07; N, 34·54%). The analysis, the similarity of the UV spectrum to to that of 8-aminoxanthine, and the absence of an 8-proton in the NMR spectrum indicates it to be 8-dimethylaminoxanthine. A transient purple colour during the reaction suggests a complex intermediate.

The 3-O-sulfate of 3-hydroxy-7,9-dimethylxanthine. Evidence for this structure for the $C_7H_8N_4O_6S$ compound previously reported[10] as a by-product of the action of Me_2SO_4 in DMF on 3-hydroxyxanthine was obtained through its hydrolytic products. A 50 mg sample in 2 ml of 6 N HCl at 23° for 20 hr gave a clear solution. which was evaporated. redissolved and amberlite IR 45 added until the pH was 6 to 7. Chromatography showed no starting material. The filtrate was concentrated and the residue recrystallized from EtOH-H_2O to yield 22 mg of 7,9-dimethyl-3-hydroxyxanthine, identical with an authentic sample[9] in its UV spectrum at 3 pH's and R_f's in three solvents.[9]

From an alkaline hydrolysis of 60 mg of the $C_7H_8N_4O_6S$ compound in 0·5 ml N NaOH for 10 min the addition of 9 ml of EtOH precipitated 45 mg of a crude Na salt which was washed with EtOH and ether. This 45 mg of material showed only one broad absorption maximum, at 274·5 nm at pH 2, and 275 at pH 5 and 275·5 at pH 11, and was probably largely the disodium salt of the sulfate ester of the 1-hydroxy-6-methylamino-5-N-methylformamidouracil. which resulted from opening of the imidazole ring. (Calc. for $C_7H_8N_4O_7SNa$: C, 24·8; H, 2·4; N, 16·15; S, 9·5; Na, 13·6. Found: C, 24·9; H, 3·1: N, 14·4; S, 9·3; Na, 13·3%).

A sample of this salt was dissolved in 6 N HCl. After 4 hr the solution was evaporated to dryness, water added and again evaporated. The solid had identical R_f's in three solvents and identical spectra at 3 pH's with those of an authentic sample of 1-hydroxy-6-methylamino-5-N-methylformamidouracil.[9]

Acknowledgement—We thank Dr. Gerhard Stöhrer for useful discussions.

REFERENCES

[1] U. Wölcke, W. Pfleiderer, T. J. Delia and G. B. Brown, *J. Org. Chem.* **34**, 981 (1969)
[2] U. Wölcke, N. J. M. Birdsall and G. B. Brown, *Tetrahedron Letters* 785 (1969)
[3] N. J. M. Birdsall, U. Wölcke and G. B. Brown, *157th Meeting American Chemical Society*, Minneapolis. Abstract 0-172. April (1969)
[4] N. J. M. Birdsall, T. C. Lee and U. Wölcke, *Tetrahedron* **27**, 5961 (1971)
[5] I. Scheinfeld, J. C. Parham, S. Murphy and G. B. Brown, *J. Org. Chem.* **34**, 2153 (1969)
[6] E. N. Shaw, *Pyridine and its Derivatives* Part 2, (Edited by E. Klingsberg): p. 1. Interscience, New York (1961)
[7] J. C. Parham, J. Fissekis and G. B. Brown, *J. Org. Chem.* **32**, 1151 (1967)
[8] J. C. Parham, T. G. Winn and G. B. Brown *Ibid.* **36**, 2639 (1971)
[9] U. Wölcke and G. B. Brown, *Ibid.* **34**, 978 (1969)
[10] T. J. Delia and G. B. Brown, *Ibid.* **31**, 178 (1966)
[11] R. K. Robins, *Ibid.* **26**, 447 (1961); J. B. Lloyd, *Chem. Ind.* 953 (1963)
[12] A. G. Beaman, J. F. Gerster and R. K. Robins, *J. Org. Chem.* **27**, 986 (1962)
[13] J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.* **82**, 3773 (1960)
[14] F. Bergmann, H. Kwienty-Govrin, H. Unger-Waron A. Kalmus and M. Tamari, *Biochem. J.* **86**, 567 (1963)
[15] E. Fischer and H. Clemm, *Ber. Dtsch. Chem. Ges.* **30**, 3089 (1897)
[16] N. J. M. Birdsall, T.-C. Lee, T. J. Delia and J. C. Parham, *J. Org. Chem.* **36**, 2635 (1971)
[17] R. M. Cresswell, H. K. Maurer, T. Strauss and G. B. Brown, *Ibid.* **30**. 408 (1965)
[18] E. Ochiai, *Aromatic Amine Oxides*, Elsevier, New York (1967)
[19] V. F. Traynellis, *Mechanism of Molecular Migrations* (Edited by B. S. Thyagarajan): p. 1. Interscience, New York (1969)
[20] H. Kawashima and I. Kumashiro, *Bull. Chem. Soc. Jap.* **42**, 750 (1969)
[21] H. Kawashima and I. Kumashiro, Jap. Pat. 6929658, *Chem. Abstr.* **72**, 79099 (1970)
[22] G. Stöhrer and G. B. Brown, *Science* **167**, 1622 (1970)
[23] G. B. Brown, G. Stöhrer. K. Sugiura and M. N. Teller, Abstracts, *10th International Cancer Congress*, Medical Arts Publishing Co., Austin, Texas, p. 8 (1970)
[24] K. Sugiura, M. N. Teller, J. C. Parham and G. B. Brown, *Cancer Res.* **30**, 184 (1970)
[25] J. A. Miller and E. C. Miller, *Progr. Exp. Tumor Res.* **11**, 273 (1969)
[26] P. D. Lotlikar, J. D. Scribner, J. A. Miller and E. C. Miller, *Life Sciences* **5**, 1263 (1966)
[27] L. A. Poirier, J. A. Miller, E. C. Miller and K. Sato, *Cancer Res.* **27**, 1600 (1967)
[28] M. Enomoto, K. Sato, E. C. Miller and J. A. Miller, *Life Sciences* **5**, 1052 (1968)
[29] N. J. M. Birdsall, U. Wölcke, J. C. Parham and G. B. Brown, *Tetrahedron* in press
[30] J. A. Johnson, Jr., H. J. Thomas and H. J. Schaeffer, *J. Am. Chem. Soc.* **80**, 699 (1958)
[31] J. P. Glusker, D. van der Helm, W. E. Love, J. A. Minkin and A. L. Patterson, *Acta Crystallogr.* **B24**, 359 (1968)
[32] A. Bendich, A. Giner-Sorolla and J. J. Fox, *Ciba Fndn. Symp. Chem. and Biol. of Purines*, p. 3. Churchill Ltd., London (1957)
[33] A. Giner-Sorolla, *J. Heterocycl. Chem.* **7**, 75 (1970)
[34] L. F. Cavalieri and A. Bendich, *J. Am. Chem. Soc.* **72**, 2587 (1950)
[35] G. V. Boyd, *Tetrahedron Letters* 3369 (1966)
[36] G. Schwarzenbach, K. Lutz and E. Felder, *Helv. Chim. Acta* **27**, 576 (1944)
[37] J. C. Riviere, *Trans. Faraday Soc.* **55**, 1673 (1959)
[38] R. Shapiro, B. I. Cohen S. J. Shiney and H. Maurer, *Biochemistry* **8**, 238 (1969)
[39] H. Normat, *Angew. Chem. Internat. Edition* **6**, 1046 (1967)