and C were identical with those of authentic 6-methylamino-5-N-methylformamidouracil prepared according to Biltz, *et al.*[9]

7,9-Dimethyl-1-hydroxyxanthine Methyl Bisulfate.—1-Hydroxyxanthine[10] (350 mg) in 10 ml of DMF was treated with 2 ml of Me_2SO_4 as specified above. To the solution, 20 ml of *i*-PrOH and then 20 ml of Et_2O were added. The colorless reaction product (400 mg, 65%) was collected and washed thoroughly with ether. An analytical sample was obtained from MeOH–EtOAc and dried overnight at 110°: mp 197–198° dec. R_f values follow: A, 0.33; B, 0.17; C, 0.87.

Anal. Calcd for $C_8H_{12}N_4O_7S$ (308.3): C, 31.17; H, 3.90; N, 18.18. Found: C, 31.15; H, 3.95; N, 18.22.

The nmr spectrum shows three CH_3 peaks at 3.40, 3.82, and 4.08 ppm. The C-8 H is found at 9.24 and a broad peak centered at 10.6 ppm integrates for two protons (NH and OH).

Hydrogenation of 7,9-Dimethyl-1-hydroxyxanthine Methyl Bisulfate.—7,9-Dimethyl-1-hydroxyxanthine methyl bisulfate (100 mg) was reduced with H_2 for 18 hr as specified above using 3 ml of a Raney nickel suspension in EtOH. After treatment with charcoal, the filtrate was evaporated *in vacuo* to give *ca.* 50 mg, 50%, of slightly gray material. The uv spectrum, and the R_f values were identical with those of authentic 7,9-dimethylxanthine.[8]

3-Hydroxyxanthine from 6-Amino-5-formamido-1-hydroxyuracil in Hexamethyldisilazane.—6-Amino-5-formamido-1-hydroxyuracil[11] (190 mg) was suspended in 7 ml of hexamethyldisilazane. When refluxed it dissolved within 30 min and after 4 hr the reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated with EtOH. The remaining solids in 10 ml of dilute NH_4OH were absorbed on a Dowex 50W-X8, 200–400 mesh, 4.4 cm × 10 cm column and developed with 0.1 *N* HCl. The 3-hydroxyxanthine (50 mg, 22%) was eluted first, followed by traces of xanthine, and finally some starting material. The first fraction was evaporated and the 3-hydroxyxanthine was recovered as the hydrate hydrochloride.[29] Its ir and uv spectra were identical with those of the xanthine N-oxide derivative obtained by hydrolysis of the guanine N-oxide prepared by oxidation of guanine.[2]

When allowed to proceed for 17 hr, the reaction was still incomplete, and, as indicated by paper chromatography, there was more deoxygenation of 3-hydroxyxanthine to xanthine.

Registry No.—**1**, 18905-29-8; **3**, 13479-29-3; **4**, 69-93-2; **5**, 19039-38-4; **6**, 19039-39-5; **6** (methyl bisulfate), 19039-40-8; **10**, 12321-47-0; **10** (methyl bisulfate), 12321-48-1; 7,9-dimethyluric acid, 19039-41-9.

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Purine N-Oxides. XXIII. Rearrangements of Purine 3-N-Oxides on Acylation and Methylation[1]

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Reactions of several purine 3-N-oxides with acid anhydrides are described. The two types of rearrangements observed are dependent upon the structure of the N-oxides. Each reaction involves the loss of an oxygen atom from N-3 and the introduction of an oxygen atom at either position 2 or position 8 of the purine system. The latter rearrangement is also shown to occur in an analogous methylation reaction. Plausible mechanisms are discussed. The reaction of purine 3-N-oxides in acid has also been examined.

An unusual rearrangement occurred when 6-amino-5-formamido-1-hydroxyuracil was treated with formic acid and acetic anhydride.[4] Uric acid instead of the expected 3-hydroxyxanthine was formed.[5] This result prompted us to study the behavior of purine 3-N-oxides in acid anhydrides and in acids. Reactions of some purine 1-N-oxides with acetic anhydride have been investigated,[6,7] and only rearrangements already common with pyridine N-oxides had been encountered.

The low solubility of some of the purine N-oxides in formic acid and in acetic anhydride sometimes necessitated the use of trifluoroacetic acid (TFA), its anhydride, or mixtures of trifluoroacetic acid with acetic anhydride. Acetic anhydride, rather than trifluoroacetic acid, seems to be the agent required to induce the rearrangement of 3-hydroxyxanthine since the latter

(1) This investigation 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).

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TABLE I

Purine N-oxide (mg)	Solvent (ml)	Total reaction time, hr (temp)	Reaction product	Yield, %
3-Hydroxyxanthine[a,b] (170)	TFA (5) Ac_2O (5)	0.5 (reflux)	Uric acid	50
7,9-Dimethyl-3-hydroxyxanthine hemihydrate[a] (50)	Ac_2O (4)	0.5 (reflux)	7,9-Dimethyluric acid	20
Guanine 3-oxide hemihydrochloride[a,b] (185)	F_3Ac_2O (5)	36 (20°)	8-Hydroxyguanine[c]	60
Adenine 3-oxide[d] (50)	Ac_2O (3)	0.5 (reflux)	Isoguanine[e]	40
6-Methoxypurine 3-oxide[d] (100)	Ac_2O (5)	½ (reflux)	2-Hydroxy-6-methoxypurine[f]	30
Hypoxanthine 3-oxide[d,g]	Ac_2O	3.5 (reflux)	Xanthine	62[g]
Hypoxanthine 3-oxide	HCOOH	3.5 (reflux)	6,8-Dioxypurine	60[g]

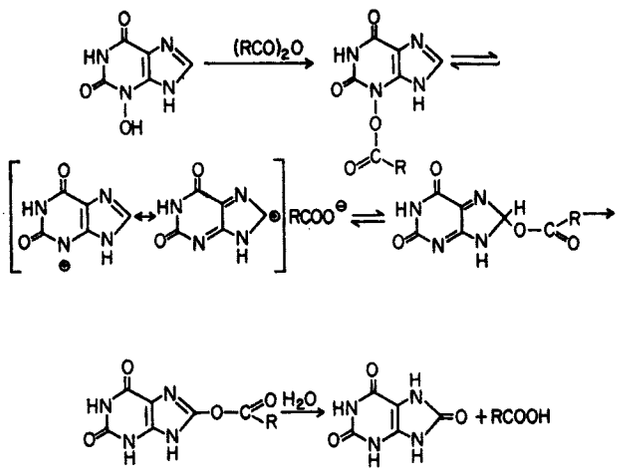
[a] See ref 5. [b] See ref 11. [c] Recrystallization from 54% HI gave 8-hydroxyguanine·HI. [d] See ref 9. [e] The crude product in 3 ml of H_2O and a little NH_4OH was absorbed on a Dowex 50W-X8 column (20 × 1 cm) and eluted with 2 *N* HCl. Recrystallization of the evaporated eluate from 5% H_2SO_4 gave isoguanine·$0.5H_2SO_4$·$0.5H_2O$ [A. Bendich, J. F. Tinker, and G. B. Brown, *J. Amer. Chem. Soc.*, 70, 3109 (1948)], identified by its infrared (ir) spectrum. [f] The crude product was kept in 5 ml of dilute NH_4OH for 30 min. The repeatedly evaporated filtrate was dissolved in 15 ml of hot H_2O, some amorphous material was removed, and the solution was concentrated to 2–3 ml. After separation of more amorphous material the pure compound[12] crystallized. [g] See ref 10.

failed to react in trifluoroacetic acid. 6-Mercaptopurine 3-oxide[8] decomposed under any of the conditions used for attempted rearrangement. All of the purine 3-N-oxides listed in Table I were examined for rearrangement in either formic acid or TFA, but only hypoxanthine 3-oxide rearranged in acid alone. Hypoxanthine 3-oxide,[9] refluxed in 98% formic acid for 4 hr, was transformed into 6,8-dioxypurine. During our investigation this rearrangement was reported, in a patent,[10] to also occur in hydrochloric acid, or in acetic acid, as we were able to confirm.

In acetic and trifluoroacetic anhydride two types of rearrangenmet were observed (Table I). 3-Hydroxyxanthine,[5,11] 7,9-dimethyl-3-hydroxyxanthine,[5] and guanine 3-oxide[5,11] were converted into uric acid, 7,9-dimethyluric acid, and 8-hydroxyguanine, respectively. In contrast, adenine 3-oxide[9] and 6-methoxypurine 3-oxide[9] were converted into isoguanine (2-hydroxyadenine) and 2-hydroxy-6-methoxypurine,[12] respectively. Similarly, hypoxanthine 3-oxide reacts with acetic anhydride to yield xanthine, as is also mentioned in the patent cited.[10]

When heterocyclic N-oxides react with acid anhydrides, it is generally accepted that the O-acyl derivative is formed[13–16] as an initial step. Similar O acylations also occur with hydroxamic acids and certain other N-hydroxy compounds.[17–19] O-Acyl derivatives of 3-hydroxyxanthine and related compounds would, in effect, be O-acylated hydroxamic acids or their derivatives. In the Lossen rearrangement of O-acylated hydroxamic acids, the heterolytic cleavage of the N–O bond to form the acylate ion is the rate-determining step.[17,18,20] For 3-hydroxyxanthine, its 7,9-dimethyl derivative and guanine 3-oxide, we suggest the mechanism shown in Scheme I as a plausible

SCHEME I



explanation for the rearrangement observed in the presence of acid anhydrides. The acylate ion and the purine cation could react either as an ion pair or as free ions.[21] Alternatively the attack of a nucleophile at position 8 of the purine system might occur with concerted cleavage of the N–O bond.

When the methyl bisulfate of 7,9-dimethyl-3-hydroxyxanthine[5] was stirred with dimethyl sulfate in dimethylformamide at 80°, 7,9-dimethyluric acid precipitated slowly from the solution, but, in dimethylformamide (DMF) alone at 80°, it was stable. This suggests that alkylation of the N-hydroxy group induces the transformation, and it correlates with the fact that the methylation of 3-hydroxyxanthine at elevated temperatures leads directly to the formation of 7,9-dimethyluric acid (Scheme II). Originally the latter was tentatively assigned[22] the structure of the betaine of

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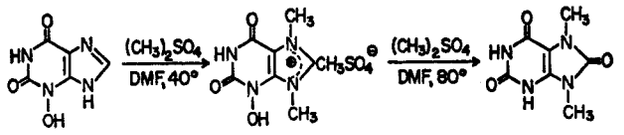
SCHEME II



7-methoxy-9-methylxanthine based upon the thought that the parent compound was a 7-N-oxide derivative, the analogy of the conditions for its formation to those for the betaine of 7,9-dimethylxanthine,[23] and the absence of properties characteristic of a N-oxide.[22] The synthesis of an authentic sample of 7,9-dimethyluric acid[24] has now been accomplished by cyclization of ethyl N-methyl(4-methylaminouracil-5-yl)carbamate. It has proven to be identical in all respects with that obtained from 3-hydroxyxanthine.

Adenine 3-oxide, 6-methoxypurine 3-oxide, and hypoxanthine 3-oxide, or their O-acyl derivatives, do not have the structural elements of hydroxamic acids, and position 2 of their ring system is unsubstituted. Their reaction in acetic anhydride resembles that of pyridine N-oxide which is transformed into pyridone-2.[13,25,26]

The unique behavior of hypoxanthine 3-oxide in acids cannot be explained at the present time. The closely related 6-methoxypurine 3-oxide fails to rearrange similarly under the same conditions.

The unusual reactivity of 3-hydroxyxanthine and guanine 3-oxide should be considered when seeking an explanation for their oncogenicity.[11,27] This reactivity is reminiscent of that of N-acyloxyarylamine oncogens, which react *in vivo* and *in vitro* with proteins[28] and with nuclei acids.[29]

Experimental Section

Paper chromatograms were developed, ascending, on Whatman No. 1 paper and viewed under ultraviolet (uv) light. The solvent systems used were (A) CH_3CN–H_2O (3:1, v/v), (B) *n*-BuOH–H_2O–HOAc (4:1:1), (C) NH_4Cl (3%). For thin layer chromatography (tlc), Eastman chromagram sheets with a silica gel layer containing a fluorescent indicator were used. The uv spectra were determined with a Unicam SP 800 spectrophotometer, ir spectra (KBr) with a Perkin-Elmer Model 221 spectrophotometer, and nmr spectra with a Varian A-6. Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as a solvent with tetramethylsilane (TMS).

General Procedure for the Qualitative Study of the Rearrangement of Some Purine 3-N-Oxides in Acid Anhydrides and/or in Acids.—Several milligrams of a purine N-oxide were refluxed in 2 ml of a solvent for 4 hr as indicated in Table I. Samples were taken from the reaction mixture after 10, 30, 60, 120, 240 min. When the solvent was Ac_2O, they were treated with H_2O and then NH_4OH (pH 8–9). When acid was used as a solvent, the samples were used directly for chromatographic analysis. All samples, with controls, were chromatographed in solvent systems A and C. Unless otherwise stated, rearrangement products obtained from quantitative studies were identified by their uv spectra at several pH values and by paper chromatography in solvent systems A, B, and C.

Uric Acid from 3-Hydroxyxanthine.—As a typical example for the quantitative study of the rearrangement of some purine 3-N-oxides in acid anhydrides 3-hydroxyxanthine (170 mg) was suspended in 5 ml of TFA and 5 ml of Ac_2O. The mixture was refluxed for 30 min and then repeatedly evaporated *in vacuo* with added H_2O and finally with EtOH. The residue was recrystallized from H_2O with a little charcoal, to yield 85 mg, 50%, of crystalline material, which was identified as uric acid.

Reaction of 7,9-Dimethyl-3-hydroxyxanthine Methyl Bisulfate with Me_2SO_4 in DMF at 80°.—7,9-Dimethyl-3-hydroxyxanthine methyl bisulfate[5] (30 mg) was dissolved in 1 ml of DMF. Three drops of Me_2SO_4 was added and the mixture was stirred at 80° ± 2°. The reaction was monitored by tlc (solvent system: EtOH–H_2O, 8:2). After 66 hr the reaction was still not complete. A white precipitate had formed, which was collected and washed with a little absolute EtOH and Et_2O to yield 10 mg, 50%. Without further treatment, the ir spectrum of the reaction product was identical with that of pure 7,9-dimethyluric acid.

7,9-Dimethyl-3-hydroxyxanthine methyl bisulfate in DMF did not react in the absence of Me_2SO_4.

7,9-Dimethyluric Acid.[24]—Ethyl N-methyl(4-methylaminouracil-5-yl)carbamate[24] (1 g) was refluxed with 20 ml of 5 *N* NaOH for 2 hr. It was acidified with concentrated HCl to pH 1 and when cooled the precipitate was collected. Recrystallization from 200 ml of H_2O yielded 0.5 g of colorless crystals, mp 375–380°.

Anal. Calcd for $C_7H_8N_4O_3$ (196.7): C, 42.86; H, 4.11; N, 28.56. Found: C, 43.12; H, 4.26; N, 28.50.

A sample prepared in 75% yield from 3-hydroxyxanthine[22] had the following R_f values—A, 0.29; B, 0.41; C, 0.67—and analysis.

Anal. Found: C, 43.01; H, 4.14; N, 28.60.

The nmr spectrum showed two methyl peaks at 3.34 and 3.22 ppm and exchangeable protons at 10.80 and 12.00 ppm. The uv spectrum[5] is quite similar to that of uric acid and of some of its methyl derivatives, but is not identical with those of five of the six possible dimethyluric acids.

Registry No.—3-Hydroxyxanthine, 13479-29-3; 7,9-dimethyl-3-hydroxyxanthine, 19039-39-5; guanine 3-oxide, 19039-44-2; adenine 3-oxide, 19039-45-3; 6-methoxypurine 3-oxide, 19039-46-4; hypoxanthine 3-oxide, 19039-47-5.

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