Purine N-Oxides. XXII. On the Structures of 3-Hydroxyxanthine and Guanine 3-Oxide[1]

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A xanthine N-oxide derivative obtained from the peroxy acid oxidation product of guanine is shown to bear the oxygen atom on N-3, and the previous suggestion that guanine is oxidized at the 7 position is withdrawn. The evidence included methylation to 7,9-dimethyl-3-hydroxyxanthine, which was opened to 1-hydroxy-6-methylamino-5-N-methylformamidouracil. The 7,9-dimethyl-1-hydroxyxanthine was obtained for comparison. A synthesis designed to lead to 3-hydroxyxanthine is shown to have yielded uric acid; this synthesis is now modified to result in the formation of the desired compound.

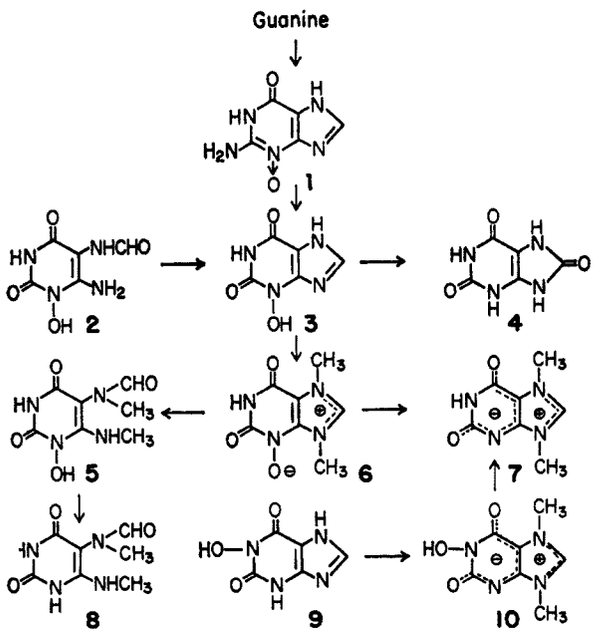
An N-oxide derivative of guanine obtained by peroxy acid oxidation and one of xanthine derived from the former by hydrolysis were referred to as *x*-N-oxides, when their activity as oncogenic agents was first reported,[2] and were later erroneously designated as 7-hydroxy derivatives.[3,4] That assignment was based primarily upon infrared (ir) spectra purported to be those of N-hydroxyglycine, which was believed to have been formed as a degradation product from the purine N-oxides by acid hydrolysis.[3] In the course of studies of α-N-hydroxyamino acids,[5] it was found that the ir spectra mentioned above[3] were those of *p*-tosylglycine. The confusion of those spectra and of the melting points recorded is inexplicable. We have since sought other evidence for the structure of the guanine and xanthine *x*-N-oxide derivatives both by synthetic and degradative methods.

Syntheses of 7-hydroxy derivatives of purines have been successful with methyl groups at N-1 and N-3,[6] but we have failed to obtain such derivatives without methyl groups on the pyrimidine nitrogens.

The first evidence that the *x*-N-oxides are actually 3-N-oxides was obtained from a methylation product. When xanthine *x*-N-oxide in dimethylformamide is treated with an excess of dimethyl sulfate at low temperature, a product could be obtained in 70% yield.[7] A purple ferric chloride test implies that the N-oxide function is still present. The nmr spectrum and analysis correspond to those of a methyl bisulfate salt of a dimethylxanthine *x*-N-oxide.· The salt was converted into a dimethylxanthine N-oxide (**6**) and the free base was reduced to the known 7,9-dimethylxanthine[8] (**7**). This eliminated the possibility that the xanthine *x*-N-oxide is the 7-hydroxy isomer. Additional proof that the N-oxide function is on a pyrimidine nitrogen was provided by the opening of the quaternized imidazole ring in alkali, to yield an N-hydroxy-6-methylamino-5-N-methylformamidouracil (**5**), which can be reduced to known 6-methylamino-5-N-methylformamidouracil (**8**).[9]

From these data, and the nonidentity of the xanthine *x*-N-oxide with the known 1-hydroxyxanthine (**9**),[10] it is now concluded that the oxidation of guanine with peroxy acid[2,3] yields its 3-oxide (**1**), which is hydrolyzed to give 3-hydroxyxanthine (**3**). In addition, the isomeric 1-hydroxyxanthine (**9**) was methylated under conditions similar to those used for 3-hydroxyxanthine to yield 7,9-dimethyl-1-hydroxyxanthine (**10**), which could also be reduced to 7,9-dimethylxanthine (**7**), and compound **10** differs from **6** (Scheme I).

SCHEME I



Further proof for the assignment of structure is derived from a synthesis[11] originally designed to yield **3** from 6-amino-5-formamido-1-hydroxyuracil (**2**). At that time[11] formic acid and acetic anhydride were used for the final closure of the imidazole ring on **2**. We have since found that, in acetic anhydride, the 3-

(1) 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).

(2) G. B. Brown, K. Sugiura, and R. M. Cresswell, *Cancer Res.*, **25**, 986 (1965).

(3) T. J. Delia and G. B. Brown, *J. Org. Chem.*, **31**, 178 (1966).

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(7) This compound is not identical with the previously reported[3] by-product $C_7H_8N_4O_6S$, which decomposes to 7,9-dimethyluric acid when heated.

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(10) J. C. Parham, J. Fissekis, and G. B. Brown, *J. Org. Chem.*, **32**, 1151 (1967).

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TABLE I

IONIZATION CONSTANTS AND SPECTRAL DATA

pH	Charge[a,b]	λ_{max}, mμ ($\epsilon \times 10^{-3}$)	Apparent pK_a values ($\pm$)
		Guanine 3-Oxide[c] (1)	
−2	[+2]	244 (10.6), sh 260	
			−1.14 (0.1)
1.0	[+1]	213 (12.5), 245 (7.8), 267 (9.5)	
			3.45 (0.05)
4.8	[0]	217 (23), 270 (8.8)	
			5.98 (0.06)
8.0	(−1)	224 (31), 254 (5.2), 292 (6.6)	
			10.67 (0.05)
12–15	(−2)	226 (31), 283 (9.7)	
		3-Hydroxyxanthine[c] (3)	
−2	(+1)	238 (7.0), 268 (8.9)	
			0.35 (0.02)
3.0	(0)	205 (24), 272 (10.1)	
			6.71 (0.06)
8.17	[−1]	218 (22), 257 (5.6), 299 (6.8)	
			9.65 (0.06)
11.4	[−2]	225 (28), 297 (8.6)	
			13.2 (0.2)
15	[−3]	224 (29), 292 (8.8)	
		7,9-Dimethyl-3-hydroxyxanthine[d] (6)	
2	(+1)	245 (5.4), 270 (6.8)	
			4.77 (0.03)
6.5	[0]	218 (18.3), 276 (6.6), 313 (5.3)	
			8.48 (0.1)
11	(−1)	227 (19.8), 285 (4.4), 308 (3.8)	
		7,9-Dimethyl-1-hydroxyxanthine[d] (10)	
0	(+1)	262 (15.6)	
			2.01 (0.05)
4	(0)	247 (3.9), 283 (4.8)	
			8.05 (0.1)
10	(−1)	204 (10.9), 238 (9.1), 291 (4.1)	
		Uric Acid[e] (4)	
−6.5	[?][f]	228 (7.1), sh. 278 (5.4), 300 (7.0)	
			−5.60 (0.2)
−4.9	[+2]	229 (7.6), 283 (7.7)	
			−4.04 (0.02)
−3.0	[+1]	231 (7.9), 284 (10.3)	
			−2.44 (0.2)
3.0	(0)	231 (8.3), 285 (12.1)	
			5.45 (0.05)
8.0	(−1)	207 (14.2), 236 (9.8), 292 (12.6)	
			10.6 (0.05)
12.3	(−2)	219 (25), 296 (13.6)	
		7,9-Dimethyluric Acid	
−7.0	[?][f]	215 (12.8), 307 (7.3)	
			−5.6 (0.2)
−5.0	[+2]	235 (8.7), 289 (9.6)	
			−4.05 (0.14)
−2.7	[+1]	235 (7.4), 288 (11.5)	
			−1.66 (0.1)
2.0	0	236 (8.0), 285 (12.1)	
			5.10 (0.05)
10.0	−1	242 (9.8), 294 (11.4)	
			13.9 (0.3)
14.0	−2	242 (6.5), 288 (6.9)	
		1-Hydroxy-6-methylamino-5-N-methylformamidouracil (5)	
3	(0)	270 (9.4)	
			5.97 (0.02)
9	(−1)	231 (22.3), 286 (10.2)	

[a] Parentheses indicate pure species. [b] Brackets indicate that pure species are not available. [c] Spectra at two pH's were previously recorded.[3] [d] At pH 11, 6 and 10 decompose gradually. [e] A variety of ϵ values are recorded for uric acid,[19–23] many at inappropriate pH values and some without recognition of the instability in alkali in the presence of air.[24,25] Johnson's values[19] in the 290-mμ region are in excellent agreement with the current ones, and his plot is satisfactory for the usual pH range. Johnson[19] records pK values of 5.4 ($\pm$0.1) and 10.6 ($\pm$0.1) determined by titration. Bergmann and Dickstein[26] record 5.75 and 10.3, in buffers of various concentrations. Each read the pK values from plots of optical density *vs.* pH. [f] The marked change of spectrum may represent reaction with the 8 to 14 N H_2SO_4.

hydroxyxanthine (**3**) undergoes an unexpected rearrangement to uric acid (**4**)[12,13] and the product previously assumed[11] to be 3-hydroxyxanthine is actually uric acid. Closure of the imidazole ring of **3** has now been accomplished and the rearrangement avoided by refluxing the 6-amino-5-formamido-1-hydroxyuracil (**2**) in hexamethyldisilazane.[16a] The 3-hydroxyxanthine obtained by this total synthesis and that obtained by the hydrolysis of the guanine 3-oxide are identical. When 6-amino-5-formamido-1-hydroxy-3-methylurcil was treated with acetic anhydride and formic acid,[16b] a rearrangement analogous to that of **3** to **4** also occurred, and the product was not 3-hydroxy-1-methylxanthine, but 1-methyluric acid.

This unusual rearrangement, involving the formal transfer of an oxygen atom from N-3 to C-8, is dealt with in greater detail in the accompanying paper.[12]

Interpretation of Ultraviolet Spectra (Table I).—It is generally found with purine N-oxides or their N-hydroxy tautomers, that the N→O or N–O⁻ form has strong absorption in the 220–230-mμ region, which is suppressed by protonation of those species.[17] Based on this observation, the compounds are designated in terms of the tautomer predominant in their neutral molecule, *i.e.*, guanine 3-oxide (**1**) and 3-hydroxyxanthine (**3**).

When forming the monoanion, **3** loses the proton from the 3-hydroxy group. In the neutral forms of **6** and **10**, there is only one removable proton. In the case of **6** which exhibits an absorption of high extinction coefficient at 218 mμ, the proton is assigned to N-1. In **10** the proton is assigned to the oxygen atom attached to N-1. This assignment is supported by the similarity, respectively, of the spectra of the cation and neutral molecule of **10** to those of the neutral molecule and anion of 1-hydroxyxanthine.[11] The dianion of the latter shows a typical strong absorption at 225 mμ, associated with the ionization of the N–OH at the 1 position. However, the anion of more complex **10** shows no maximum readily associated with the corresponding ionization.

The spectrum of uric acid (**4**)[18–26] has been carefully redetermined over a wide pH range and compared with that of 7,9-dimethyluric acid; in strong acid each shows two protonations and a reaction with the solvent. The strong maximum of the dianion of uric acid at 219 mμ is deceptively similar to the strong short wave absorption common to purine N-oxides. With 1-methyluric acid[16b,19] a similar maximum is observed at 217 mμ but none is found with 7,9-dimethyluric acid.

Experimental Section

Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Melting points are corrected. Chromatograms were developed, ascending, on Whatman No. 1 paper and viewed under ultraviolet (uv) light. The solvent systems were (A) CH_3CN–H_2O (3:1, v/v), (B) *n*-BuOH–H_2O–AcOH (4:1:1), (C) aqueous NH_4Cl (3%). The p*K* values were determined by methods described,[27] at 20°, spectrophotometrically in 0.01 *M* buffers,[28] or potentiometrically with 0.01 *M* solutions. ε values were determined with a Beckman DU uv spectrophotometer, complete spectra on a Unicam SP800. The ir spectra were determined with a Perkin-Elmer Model 221 spectrophotometer (KBr), and nmr spectra with a Varian A-60. Dimethyl sulfoxide-d_6 (DMSO-d_6) was used as a solvent with tetramethylsilane (TMS).

7,9-Dimethyl-3-hydroxyxanthine Methyl Bisulfate.—3-Hydroxyxanthine[2] (*ca.* $1.5H_2O$, 3.7 g) was suspended in 100 ml of dimethylformamide (DMF) and 20 ml of Me_2SO_4. The mixture was stirred at 42 ± 2° for 18 hr. The solution was concentrated at 40° *in vacuo* to one-half of its original volume, 200 ml of *i*-PrOH was added, the flask was scratched, and the mixture was cooled overnight. The white product was collected and washed with *i*-PrOH and with Et_2O to yield 4.4 g, 71%. The product gave a purple $FeCl_3$ test. For analysis it was recrystallized from EtOH, dec pt 143–145°. R_f values follow: A, 0.58; B, 0.27; C, 0.87.

Anal. Calcd for $C_8H_{12}N_4O_7S$ (308.3): C, 31.17; H, 3.90; N, 18.18; S, 10.39. Found: C, 31.24; H, 3.80; N, 18.18; S, 10.50.

The nmr shows three CH_3 peaks at 3.40, 4.02, and 4.00 ppm. The C-8 H is found at 9.25 and a broad peak integrating for two protons (NH and OH) centers at 11.90 ppm.

At room temperature the reaction requires 3 days for completion.

7,9-Dimethyl-3-hydroxyxanthine Hemihydrate.—7,9-Dimethyl-3-hydroxyxanthine methyl bisulfate (2 g) was dissolved in 20 ml of H_2O and the solution was brought to pH 6.5 with Amberlite IR-45. The filtrate from the resin was evaporated *in vacuo* at 30° to yield 1 g, 72%, of crystalline material. For analysis, a sample was recrystallized from EtOH–H_2O. When dried overnight at 60°, the compound lost water and turned yellow. It gave a purple color with $FeCl_3$ and decomposed from 180°. R_f values follow: A, 0.08; B, 0.27; C, 0.81.

Anal. Calcd for $C_7H_8N_4O_3 \cdot 0.5H_2O$ (205.2): C, 40.98; H, 4.39; N, 27.30. Found: C, 41.09; H, 4.39; N, 26.97.

Hydrogenation of 7,9-Dimethyl-3-hydroxyxanthine Hemihydrate.—7,9-Dimethyl-3-hydroxyxanthine hemihydrate (100 mg in 10 ml of H_2O) and 0.2 ml of a Raney nickel suspension in EtOH were stirred with H_2 at room temperature and atmospheric pressure for 35 min. The filtrate was evaporated *in vacuo* and the residue recrystallized from EtOH–H_2O with charcoal to yield 60 mg, 66%, of crystalline material. The uv and ir spectra were identical with those of authentic 7,9-dimethylxanthine prepared by the method of Jones and Robins.[8]

1-Hydroxy-6-methylamino-5-N-methylformamidouracil.—7,9-Dimethyl-3-hydroxyxanthine hemihydrate (700 mg) was allowed to stand in 35 ml of 30% NH_4OH for 2.5 hr at room temperature. The solution was then twice evaporated *in vacuo* at 30° with the addition of H_2O. The glassy residue was dissolved in hot EtOH. One equivalent of HCl (3.5 ml of 1 *N*) was added and the solution was filtered and cooled to yield 400 mg, 51%, of analytically pure white crystals, dec pt 242–249°, that gave a purple color with $FeCl_3$. R_f values follow: A, 0.45; B, 0.34; C, 0.82.

Anal. Calcd for $C_7H_{10}N_4O_4$ (214.2): C, 39.26; H, 4.71; N, 26.16. Found: C, 39.14; H, 4.79; N, 26.34.

Hydrogenation of 1-Hydroxy-6-methylamino-5-N-methylformamidouracil.—1-Hydroxy-6-methylamino-5-N-methylformamidouracil (100 mg) was reduced with H_2 for 60 min as specified above. The filtrate was evaporated *in vacuo* to yield 80 mg, 87%, of crystalline material. The uv spectrum and R_f values in A, B,

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(13) When the 7-hydroxypurine structures were being considered, this formation of uric acid was not surprising, since it then appeared to involve a rearrangement of an oxygen atom from N-7 to the adjacent C-8 atom, a type of rearrangement which is commonly observed in pyridine N-oxides[14] and in other purine N-oxides.[15]

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(18) See footnote *e*, Table I.

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(28) D. D. Perrin, *Aust. J. Chem.*, **16**, 572 (1963).

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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(29) G. Stöhrer, private communication.

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

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(2) Alexander von Humboldt and Australian National University Scholar, Australia, 1965.

(3) Fulbright and Australian National University Scholar, Australia, 1965.

(4) R. M. Cresswell, H. K. Maurer, T. Strauss, and G. B. Brown, *J. Org. Chem.*, **30**, 408 (1965).

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