Purine N-Oxides. XXXVI. The Tautomeric Structures of the 3-N-Oxides of Xanthine and Guanine[1]

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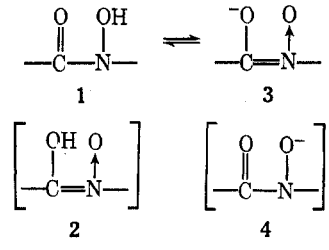
The predominant tautomeric structures present in each of the various ionic species of the title compounds have been deduced from their ultraviolet spectra and those of their several *N*-methyl and *O*-methyl derivatives. The *N*-hydroxy tautomer predominates in the neutral species of 3-hydroxyxanthine. The neutral form of guanine 3-*N*-oxide is a mixture, mostly the 3-hydroxy tautomer with some 1-H, 3-oxide tautomer. The ionization sequences have been determined as 3,9,1 for 3-hydroxyxanthine and 3,7 for 3-hydroxyguanine. The influence of the *N*-oxide group on the ionization constants and on the ultraviolet spectra is examined in detail.

The availability of all of the mono-*N*-methyl and of two dimethyl derivatives of 3-hydroxyxanthine and guanine 3-*N*-oxide[2] has permitted the determination, from the characteristics of their ultraviolet spectra, of the ionization sequences and of the tautomeric structures of their parent *N*-oxide derivatives. This knowledge should contribute to a greater understanding of the nucleophilic substitution with rearrangement at C-8 of the 3-*O*-acyl derivatives[2,3] of these oncogenic compounds.[4]

The neutral species of 3-hydroxyxanthine and its *N*-methyl derivatives, as well as those of 1-hydroxyhypoxanthine[5] and 1-hydroxyxanthine,[6] all show ultraviolet absorption bands similar to those of the neutral species of the parent purines[7-9] (Table I). The ultraviolet spectra of the *N*-acetoxy derivatives of 1- and 3-hydroxyxanthine[10] and that of 3-methoxyxanthine (Table I) show a close resemblance to those of the corresponding 1- and 3-methylxanthine.[9] It is accepted that the neutral species of xanthine and hypoxanthine exist mainly in the carbonyl (oxo) form in solution,[11-13] and from ultraviolet evidence the predominant tautomers of the *N*-oxide derivatives in solution must be the oxo-*N*-hydroxy **1**, rather than hydroxy *N*-oxide **2** forms.[14] Such tautomers are further supported by the observation of strong carbonyl absorptions in the ir spectra of the solids, particularly the presence of two distinct bands in the spectrum of 3-hydroxy-1-methylxanthine.

The 3-*N*-oxide group[15] in both the guanine and the xanthine 3-*N*-oxide[16] series exerts a significant acid strengthening influence on the molecule as a whole and decreases all pK_a's of ionization by 1 to 4 units, relative to the corresponding parent purine,[19] but has little effect on the pK_a's of protonation (Table I).



The first ionizations of 3-hydroxyxanthine (**5**) and of its mono-*N*-methyl derivatives occur at pK_a's between 6.2 and 7.0. This ionization is, in each case, accompanied by the appearance of an intense ultraviolet absorption band near 220 nm and of another new band at 300 to 310 nm (Figure 1). The 1,7-dimethyl-3-hydroxyxanthine, with only one ionizable proton, that of the 3-hydroxyl, pK = 7.08, exhibits the same spectral behavior. In contrast only a slight shift of the 270-nm band (Table I) is associated with the first pK_a of 3-methoxyxanthine (**5**, OH = OCH_3). Therefore the first ionization in the 3-hydroxyxanthine series can be definitively assigned to the 3-hydroxyl group.

The spectra of the monoanions of **5** and its 1- and 9-methyl derivatives are similar (Figure 1). From this and the differences from the 7-methyl and 1,7-dimethyl monoanion spectra, it may be inferred that the 9-H imidazole tautomer, **6**, predominates.

It has been noted that the *N*-oxide group causes the appearance of one very prominent ultraviolet absorption band at 220 to 230 nm in the neutral species of 6-

(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).

(2) N. J. M. Birdsall, T.-C. Lee, T. J. Delia, and J. C. Parham, *J. Org. Chem.*, **36**, 2635 (1971).

(3) U. Wölcke, N. J. M. Birdsall, and G. B. Brown, *Tetrahedron Lett.*, 785 (1969).

(4) K. Sugiura, M. N. Teller, J. C. Parham, and G. B. Brown, *Cancer Res.*, **30**, 184 (1970).

(5) J. C. Parham, J. Fissekis, and G. B. Brown, *J. Org. Chem.*, **31**, 966 (1966).

(6) J. C. Parham, J. Fissekis, and G. B. Brown, *ibid.*, **32**, 1151 (1967).

(7) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).

(8) L. F. Cavalieri, J. J. Fox, A. Stone, and N. Chang, *J. Amer. Chem. Soc.*, **76**, 1119 (1954).

(9) W. Pfleiderer and G. Nübel, *Justus Liebigs Ann. Chem.*, **647**, 155 (1961).

(10) N. J. M. Birdsall, T. C. Lee, and U. Wölcke, unpublished data.

(11) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience, New York, N. Y., 1963, p 206.

(12) A. R. Katritzky and J. M. Lagowski, *Advan. Heterocycl. Chem.*, **2**, 56 (1963).

(13) J. H. Lister, *ibid.*, **6**, 1 (1966).

(14) For a review of cyclic hydroxamic acids, see J. B. Bapat, D. St. C. Black, and R. F. C. Brown, *ibid.*, **10**, 199 (1969).

(15) The designation "*N*-oxide" has been used for a compound or series of compounds in this paper to denote only that an oxygen is on N-3 (or other nitrogen), without regard to its tautomeric structure. The presence of the specific tautomer containing the *N*-oxide functional group has been indicated by the use of "3-oxide" in a specific name, as in *Chemical Abstracts* usage.

(16) These were first reported to be 7-*N*-oxide derivatives,[17] but were later shown to be 3-*N*-oxides.[18]

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

(18) U. Wölcke and G. B. Brown, *ibid.*, **34**, 978 (1969).

(19) The single exception is the compound assigned the structure 3-hydroxy-9-methylxanthine. It has a slightly higher first ionization pK_a than 9-methylxanthine. The latter shows an unusually low first ionization pK_a for a methylxanthine. This suggests that steric crowding by the peri 3-H and 9-methyl, which can be relieved by ionization of the 3-H, may be responsible for the lower pK_a. Ionization of 3-hydroxy-9-methylxanthine does not produce a similar relaxation of steric hindrance, since the 3-oxygen is still present in the monoanion. The pK_a of this compound thus falls in the same range as the other 3-hydroxyxanthines and supports the assigned structure.

TABLE I
SPECTRAL DATA AND pK_a'S

pH	Charge[a,b]	λ_{max}, nm ($\epsilon \times 10^{-3}$)			Apparent pK_a values[c,d] (±)	Parent purines
		3-Hydroxyxanthine[18]				
−2	(+1)	238 (7.0)	268 (8.9)			
					0.35 (0.02)	0.89[e]
3	(0)	205 (24)	230[e] (3.7)	272 (10.1)		
					6.71 (0.06)[d]	7.70[e]
8.17	[−1]	218 (22)	257 (5.6)	299 (6.8)		
					9.65 (0.06)	11.94[g]
11.4	[−2]	225 (28)		297 (8.6)		
					13.2 (0.2)	...
15	[−3]	224 (29)		292 (8.8)		
		3-Hydroxy-7-methylxanthine				
4	(0)	235[e] (4.7)	274 (9.4)			
					6.93 (0.02)[d]	8.42[g]
9	(−1)	222 (26)	253[e] (3.9)	309 (6.7)		
					10.92 (0.05)	>13[g]
13	(−2)	223 (27.3)	248[e] (5.3)	304 (6.8)		
		3-Hydroxy-9-methylxanthine				
4	(0)	239 (8.0)	271 (9.2)			
					6.28 (0.04)[d]	6.12[g]
9	(−1)	218 (20.3)	262 (7.9)	297 (7.7)		
					11.24 (0.05)	>13[g]
13	(−2)	221 (24.3)	265[e] (6.7)	290 (8.0)		
		3-Hydroxy-1-methylxanthine				
4	(0)	230[e] (3.9)	272 (9.0)			
					6.83 (0.02)[d]	7.90[g]
8.4	[−1]	221 (23.6)	258 (4.8)	298 (5.9)		
					9.73 (0.04)	12.23[g]
13	(−2)	226 (20.9)		297 (5.7)		
		1,7-Dimethyl-3-hydroxyxanthine				
4	(0)	210 (20.2)	230[e] (4.8)	274 (8.1)		
					7.08 (0.01)[d]	8.65[g]
10	(−1)	223 (25.7)	244[e] (4.1)	258[e] (2.9)		
			309 (5.8)			
		3-Methoxyxanthine				
5	(0)		270.5 (10.8)			
					7.74 (0.05)	
9.4	(−)		274 (11.8)			
					11[e,h]	
13	(−2)	235[e] (5.4)	275 (11.9)			
		3-Hydroxyguanine[18]				
−2	[+2]		244 (10.6)	260[e]		
					−1.14 (0.1)	−1.33[c,i]
1	[+1]	213 (12.5)	245 (7.8)	267 (9.5)		
					3.45 (0.05)[i]	3.34[i]
4.8	[0]	217 (23)	270 (8.8)	300[e] (2.3)		
					5.97 (0.03)	9.32[26]
8.0	(−1)	224 (31)	254 (5.2)	292 (6.6)		
					10.67 (0.05)	12.62[26]
12–15	(−2)	226 (31)	283 (9.7)			
		3-Hydroxy-8-methylguanine				
−2.7	(+2)		245 (13.0)	259[e] (10.7)		
					−0.53 (0.05)	−1.10[k]
1.46	[+1]	213 (16.6)	248 (8.4)	271 (10.6)		
					3.39 (0.05)	4.03[27]
4.75	(0)	218 (21.5)	271 (9.6)	303[e] (2.4)		
					6.02 (0.06)	9.70[27]
9	(−1)	226 (29)	252 (6.7)	291 (7.5)		
					11.24 (0.01)	13.0[27]
14	(−2)	227 (28)	283 (9.9)			

TABLE I
(*Continued*)

pH	Charge[a,b]	λ_{max}, nm ($\epsilon \times 10^{-3}$)			Apparent pK_a values[c,d] (±)	Parent purines
			3-Hydroxy-7-methylguanine			
−3	(+2)		245 (11.2)	265[e] (8.5)		
					−1.44 (0.1)	
1	(+1)	215 (17.6)	250[e] (7.5)	268 (9.0)		
					3.33 (0.05)	3.50[26]
4.5	[0]	221 (23.0)	272 (8.5)	303[e] (1.9)		
					5.92 (0.04)[d]	9.95[26]
10	(−1)	226 (28.0)	254 (5.3)	296 (6.8)		
			3-Hydroxy-9-methylguanine			
					−0.3[g] (0.2)	
1	[+1]		249 (9.4)	265[e] (8.0)		
					2.80 (0.07)	2.83[26]
4.3	[0]	219 (17.2)	268 (8.7)	297[e] (4.4)		
					5.70 (0.03)[d]	9.80[26]
9	(−1)	224 (23.6)	280 (8.0)			
			1-Methylguanine 3-Oxide			
1	(+1)	211 (21)	249[e] (6.7)	268 (8.7)		
					3.59 (0.05)	3.13[26]
6	(0)	224 (25.6)	266 (6.8)	298 (5.6)		
					8.24 (0.03)	10.54[26]
11	(−1)	229 (29.7)	275 (7.7)	288 (7.6)		
					14[g]	
15	[−2]	232 (36)	265 (7.1)	316 (6.4)		
			1,7-Dimethylguanine 3-Oxide			
2	(+1)	217 (20.2)	250[e] (6.2)	269 (7.9)		
					3.71 (0.03)	3.40[26]
7	(0)	227 (27.5)	268 (5.2)	302 (5.1)		
					11.2 (0.08)	
13	(−1)	231 (24.2)	260[e] (5.8)	327 (4.3)		
			2-Amino-6-methoxypurine 3-Oxide			
1	(+)	213 (27)	236[e] (5.2)	286 (12.5)		
					3.57 (0.03)	
6	(0)	225.5 (36)	255.5 (5.4)	301 (7.4)		
					8.18 (0.04)	
11	(−)	228.5 (35)	265[e] (5.6)	294.5 (8.7)		

[a] Parentheses indicate pure species. [b] Brackets indicate that pure species are not available. [c] Determined spectrophotometrically. [d] Determined electrometrically. [e] Shoulder. [f] No spectral change to pH 15. [g] Estimated from isosbestic spectra. [h] 3-Methoxyxanthine is unstable in base: $t_{1/2}$ 3 to 4 days at pH 7, 1 day at pH 9, 6–8 hr at pH 13. It is stable at pH 5. [i] In H_2SO_4. This agrees with values of −1.33 and −1.26 in $HClO_4$: J. A. Zoltewicz, D. F. Clark, T. W. Sharpless, and G. Grahe, *J. Amer. Chem. Soc.*, **92**, 1741 (1970). [j] The determination of the pK_a for the first protonation of guanine suffers from small spectral differences between the neutral and protonated species. Recorded values vary from 3.37 to 2.95.[26] From replicate determinations in 4-cm cells, we find pK_a = 3.34 (±0.03). The differentials between the pK_a's of protonations of guanine and 3-hydroxyguanine were confirmed in parallel determinations with a single set of buffer solutions and a single wavelength setting of the spectrophotometer. [k] ±0.1.

aminopurine 1-oxides[20] and of 6-substituted purine 3-oxides.[21] This band, attributed to the influence of the *N*-oxide group on the purine chromophore, is not observed in the parent purines.[7] The occurrence of a similar band in the ultraviolet spectra of the monoanions, but not in those of the neutral species, of 1-hydroxyhypoxanthine and 1-hydroxyinosine, has been cited[22] as evidence for an analogous *N*-oxide structure, as in **3**, for their monoanions. The assignment of such an enolate structure parallels the enolate forms proposed for the anions of hypoxanthine[23] and xanthine.[8]

In the ultraviolet spectra of the monoanions of the *N*-methyl-3-hydroxyxanthines, the band near 220 nm (Figure 1) is similar in intensity and position to that reported for other *N*-hydroxypurine monoanions.[5] This corroborates the above assignment of the 3-hydroxyl as the site of first ionization and indicates that an enolate, as in **6**, is also the major resonance form of these monoanions. The absence of 220- or 300-nm absorption bands in the spectra of the anions of 3-methoxyxanthine (**5**, OH = OCH_3) confirms that such bands must be associated with a conjugated *N*-oxide group in the anions of *N*-hydroxypurines. The ultra-

(20) M. A. Stevens and G. B. Brown, *J. Amer. Chem. Soc.*, **80**, 2759 (1958).

(21) (a) E. C. Taylor and R. K. Loeffler, *J. Org. Chem.*, **24**, 2035 (1959); (b) I. Scheinfeld, J. C. Parham, S. Murphy, and G. B. Brown, *ibid.*, **34**, 2153 (1969); (c) A. Giner-Sorolla, C. Gryte, A. Bendich, and G. B. Brown, *ibid.*, **34**, 2157 (1969).

(22) (a) H. Sigel and H. Brintzinger, *Helv. Chim. Acta*, **48**, 433 (1965); (b) G. B. Brown, *Progr. Nucl. Acid Res. Mol. Biol.*, **8**, 209 (1968).

(23) L. B. Clark and I. Tinoco, *J. Amer. Chem. Soc.*, **87**, 11 (1965).

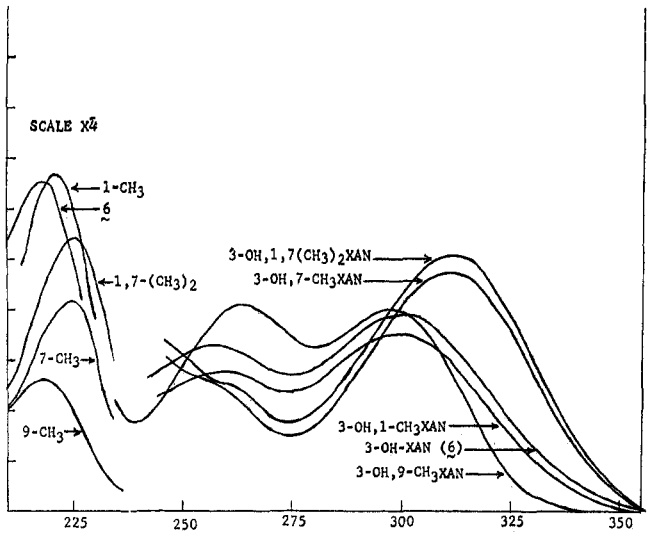


Figure 1.—3-Hydroxyxanthine series: monoanions. To facilitate convenient comparisons and minimize coincidences, the spectra are not plotted at equimolar concentrations. The extinction coefficients for each ionic species are given in Table I.

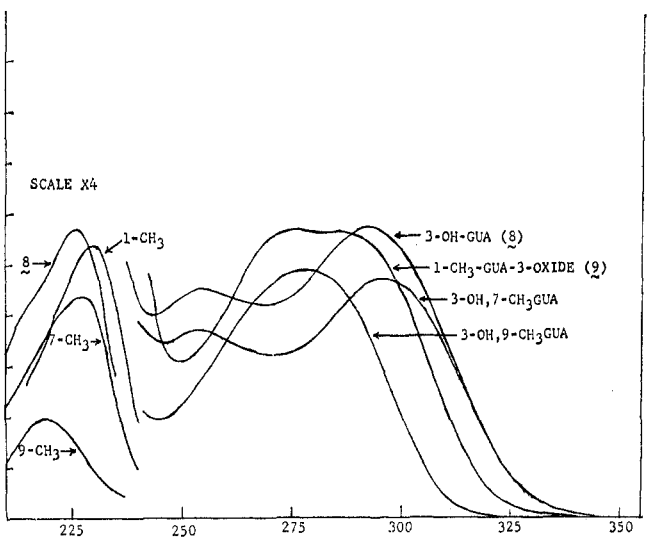


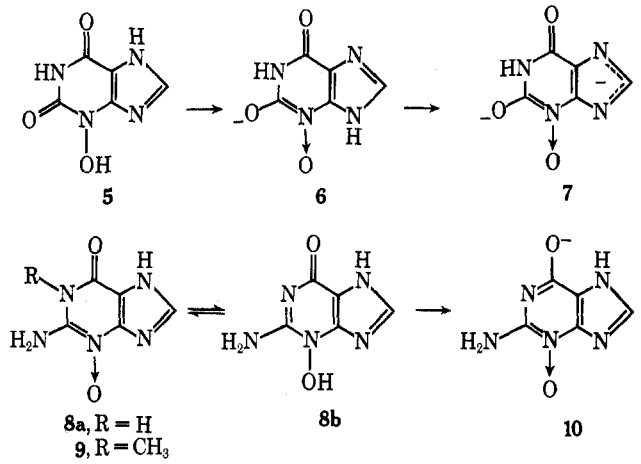
Figure 2.—Guanine 3-*N*-oxide series: anions. See footnote to Figure 1.

violet spectra of the ionic species of 3-methoxyxanthine (Table I) are remarkably close to those of 3-methylxanthine.[24]

The close values for the second ionization pK_a's of **5** (9.65) and 3-hydroxy-1-methylxanthine (9.73) and the nearly identical changes in the ultraviolet spectra associated with these pK_a's (Table I) indicate that this ionization takes place from the imidazole ring, as indicated by **7**. Supporting this deduction, the second ionizations, at N-1, of the 7- and 9-methyl derivatives of **5**, are much higher (10.9 and 11.2).

Xanthine shows no third ionization to pH 15, but the acid-strengthening effect of the 3-*N*-oxide induces a third ionization of **5** with a pK of 13.2. This ionization causes a hypsochromic shift (Table I) of the 300-nm band in **7**, which is similar to those observed during the second ionizations of the 7- and 9-methyl derivatives of **5**. These similar shifts, all associated with an ionization at N-1, provide confirmation that the ionization sequence of 3-hydroxyxanthine is 3,9,1, parallel to that of xanthine.[25]

Guanine 3-*N*-oxide (**8**) shows two ionizations with pK_a's of 5.97 and 10.67. The similarity of its first pK_a to those of its 7- and 9-methyl derivatives (5.92 and 5.70, respectively) and the much higher pK_a (8.24) of 1-methylguanine 3-oxide (**9**) establish that the first ionization of **8** occurs from the pyrimidine rather than the imidazole moiety. This is also the position deduced for the first ionization of guanine[26] and for 8-methylguanine.[27]



In the guanine 3-*N*-oxide series, distinct changes in two absorption bands are associated with transformations not only from the cations to the neutral species but also from the latter to the monoanions. Those are the bands near 225 and 300 nm, and changes in either band can be informative. Although the monoanions of both guanine 3-*N*-oxide (**8**) and 1-methylguanine 3-oxide (**9**) absorb strongly near 300 nm (Figure 2), there is a notable difference in the intensity of the 300-nm band in the neutral species (Figure 3). The 1-methyl derivative, **9**, must exist as the 1-R-6-oxo 3-oxide form, which is analogous to the predominant tautomer of guanine.[28] The difference between the spectra of **8** and **9** indicates that **8a** cannot be the predominant tautomer in the neutral species of **8**. This suggests an alternative tautomer, **8b**, with the pyrimidine hydrogen on the more electronegative oxygen at N-3. This alternative receives support when coupled with the deductions that the pyrimidine hydrogen is the first to ionize and that this ionization is accompanied by an increase in intensity of absorption bands similar to those associated with ionization of the N–OH in the 3-hydroxyxanthine series. In further support the neutral species of **8** and of its 7- and 9-methyl derivatives exhibit similar ultraviolet spectra, with maxima near 225 and 270 nm and a weaker absorption band near 300 nm (Figure 3). This demonstrates that the tautomeric structures in the neutral species of the 7- and 9-methyl derivatives of **8**,

(24) 3-Methylxanthine shows pK_a's at 8.45 and 11.92 and absorbs at 270 nm (0); 274 (−) and [232], 274 (−2).[9]

(25) The ionization sequence of xanthine and alkylxanthines is 3,7, with 1 ionizable only when the 3 or 7 nitrogen is alkylated.[8] It has been suggested that a 7-H is shifted to the 9 position in the monoanion of xanthine.[9] The position of the imidazole hydrogen in the neutral species of **5** is indeterminate, but also becomes stabilized at position 9 in the monoanion, **6**.

(26) W. Pfleiderer, *Justus Liebigs Ann. Chem.*, **647**, 167 (1961).

(27) W. Pfleiderer and M. Shanshal, *ibid.*, **726**, 201 (1969).

(28) The neutral species of guanine and 1-methylguanine show nearly identical ultraviolet absorption, and guanine is thus the 1-H-6-oxo tautomer.[26,29]

(29) For a review of the structure, properties, and reactions of guanine, see R. Shapiro, *Progr. Nucl. Acid Res. Mol. Biol.*, **8**, 73 (1968).

in which only the pyrimidine hydrogen is mobile, do not differ greatly from that of the neutral species of **8**. On the other hand, the 1-methyl and 1,7-dimethyl derivatives have well-defined maxima near 300 nm in addition to those near 225 and 270 nm. These N-1 methyl derivatives can exist only as tautomers with an *N*-oxide structure at N-3, as in **8a**. This is the species characterized by strong absorption at both 225–230 and near 300 nm, in addition to that at 266 to 268 nm. This last band, which appears as the main band of the neutral species of the guanine 3-*N*-oxides (Figure 3), is directly comparable to the single absorption band near 270 nm in the spectra of all of the 3-hydroxyxanthine neutral species (Table I).

Comparison of the ultraviolet spectrum of the neutral species of 1-methylguanine 3-oxide (**9**) with that of the monoanion of 3-hydroxyguanine (**10**) shows the resemblance in the 225- and 300-nm peaks (Figure 4). This suggests that similar *N*-oxide structures at N-3 are predominant in both, and implies that **10** is mainly a 6-enolate anion.[30] This deduction is confirmed by the close resemblance (Figure 4) of the spectrum of the anion **10** to that of the neutral species of 2-amino-6-methoxypurine 3-oxide, a derivative of **10** constrained in the 6-enol form. The similar ultraviolet spectra of **10**, its 6-OCH_3 derivative, and the 3-hydroxyxanthine monoanion (**6**) (Figure 4) provide mutual support for the *N*-3-oxide and enolate assignments, **6** and **10**.[32] The close correspondence between the ultraviolet spectra of the 3-hydroxyguanine and 3-hydroxy-7-methylguanine monoanions and the differences of both from that of the 9-methyl isomer (Figure 2) suggest that the monoanion of **8** can be further defined as the 7-H imidazole tautomer, as in **10**.

The spectra in Figure 4 depict the association of the *N*-oxide function at N-3 of guanine or xanthine with bands at 220–225 nm and near 300 nm.[33] The 300-nm band is not absent from the spectra of the neutral species of **8** and its 7- and 9-methyl derivatives (Figure 3), but is represented by pronounced shoulders. The spectra in Figure 3, taken at pH's half-way between the pK_a's of protonation and ionization are at least 1.2 pH units from each pK_a and therefore are of mixtures with 90–93% of the neutral molecules and 4–6% of each ionized species. The shoulders are thus too prominent to be due to absorption by the small amounts of monoanions. This suggests that the 300-nm shoulder evident in the spectrum of **8** is due to the presence of some tautomer of the type **8a** in equilibrium with the major tautomer **8b**.[34]

A change of solvent can significantly alter a tautomeric equilibrium,[35] and methanol, with a lower dielectric constant (ϵ = 33) than water (80), should favor the less polar 3-hydroxy tautomer. The spectra of the guanine 3-*N*-oxide series in methanol (Figure 5) indicate that the 1-methyl derivatives, which are restrained in the more polar 3-oxide form, still show distinct bands near 300 nm. In the spectra of **8** and its 7-methyl derivative, this band is greatly depressed in methanol (Figure 5), in comparison to that in water (Figure 3). This depression of the 300-nm band in a solvent of low polarity confirms that two tautomeric forms are in equilibrium in the neutral form of **8**.[36] The distinct shoulder near 300 nm in the spectrum of 3-hydroxy-9-methylguanine indicates that a significant amount of the 3-oxide tautomer is also present in methanol. This must reflect an interaction of the 3-oxide and 9-methyl groups.[37]

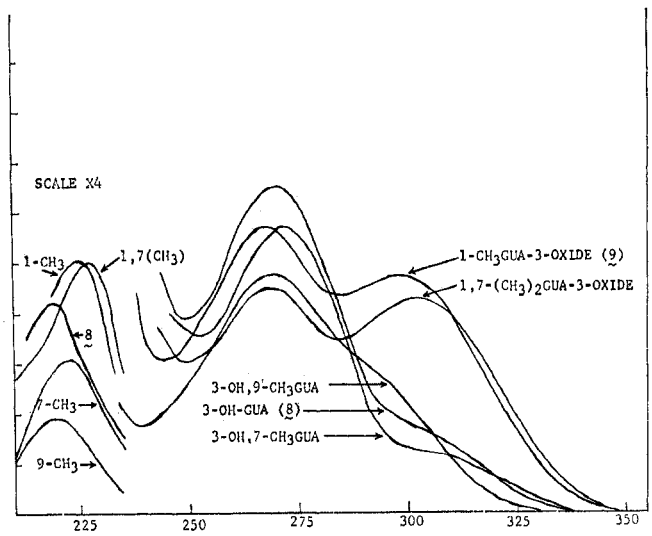


Figure 3.—Guanine 3-*N*-oxide series: neutral molecules. See footnote to Figure 1.

From inspection of Figures 2, 3, and 5 and comparison of the extinction coefficients (Table I) of the 300-nm shoulder of the neutral species of guanine 3-*N*-oxide, **8** (ϵ 2300), with the 300-nm bands in the neutral species of 1-methyl- (ϵ 5600) and 1,7-dimethylguanine 3-oxides (ϵ 5100), it is calculated that over 50% of **8** is the 3-hydroxyguanine tautomer, **8b**. This is a minimum proportion since calculations from the ϵ values of its anion, **10** (ϵ 6600), or the neutral species of 2-amino-6-methoxypurine 3-oxide (ϵ 7400) indicate more nearly two-thirds of **8b**.

(30) A comparable assignment of an enolate form for the guanosine anion is based on ir studies in D_2O.[31]

(31) H. T. Miles, F. B. Howard, and J. Frazier, *Science*, **142**, 1458 (1963).

(32) The similarity in spectra of **6** and **9** demonstrates that these compounds follow the Jones rule [R. N. Jones, *J. Amer. Chem. Soc.*, **67**, 2127 (1945)], *i.e.*, that the contribution of the 2-oxo enolate group of **6** is spectrally equivalent to that of the 2-amino group of **9**. The agreement of these purine derivatives with this rule adds to the several examples reported in the pyrimidine series: D. J. Brown and J. M. Lyall, *Aust. J. Chem.*, **15**, 851 (1962).

(33) A further example of association of two absorption bands with a 3-hydroxy enolate anion is furnished by 7,9-dimethyl-3-hydroxyxanthine.[18] The neutral species is a zwitterion, with the cation in the imidazole and the negative charge at the 3-*N*-hydroxy anion, and both it and its anion show strong absorptions near 220 and 300 nm, the former at 218 and 313, and the latter at 227 and 308 nm. These bands are not present in the cation.

(34) The closer resemblance of the spectrum of **8** to that of the neutral molecule of 7-methylguanine 3-oxide (Figure 3) suggests a greater contribution of the 7-H than the 9-H imidazole tautomer in **8**.

(35) A recent illustration of this is a study of 2-ethoxy-4-pyrimidone: J. Pitha, *J. Org. Chem.*, **35**, 903 (1970).

(36) A similar solvent effect has been observed on the tautomerism of benzimidazole *N*-oxide: S. Takahashi and H. Kano, *Chem. Pharm. Bull.*, **11**, 1375 (1963)).

(37) Examination of a Leybold molecular model (La Pine Scientific Co.) of 3-hydroxy-9-methylguanine indicates that steric interference between the hydrogen of the 3-hydroxyl and those of the 9-methyl is sufficient to inhibit free rotation of these groups. The 3-oxide tautomer model (using either carbonyl or hydroxyl oxygen) shows the 9-methyl hydrogens to be in close proximity to the 3-oxygen, but free rotation of the methyl is not restricted. This suggests that steric interactions in the 3-hydroxy tautomer may be sufficient to alter the tautomeric equilibrium to favor a larger contribution of the 3-oxide tautomer, even in methanol. Hydrogen bonding between the methyl hydrogens and oxygen has been suggested for 2-methylpyridine 1-oxide.[38] The model indicates that this should be possible in 9-methylguanine 3-oxide and that intramolecular H bonding could also contribute to the stability of the 3-oxide tautomer.

(38) E. Ochiai, "Aromatic Amine Oxides," Elsevier Publishing Co., New York, N. Y., 1967, Chapter 4.

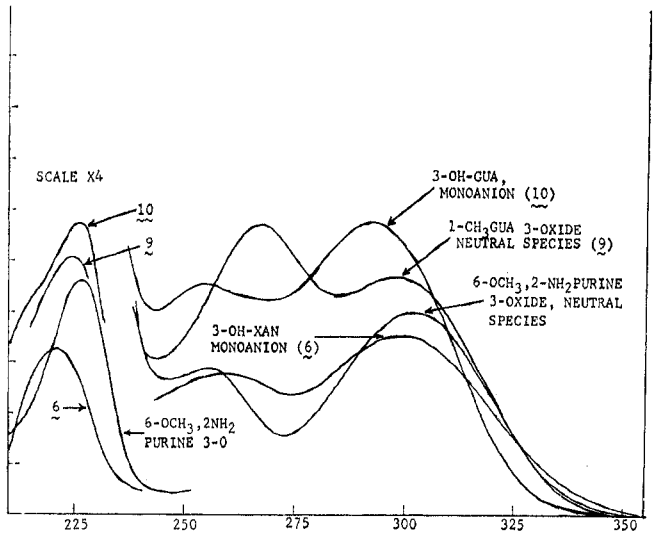


Figure 4.—Comparison of selected species containing the 3-oxide group. See footnote to Figure 1.

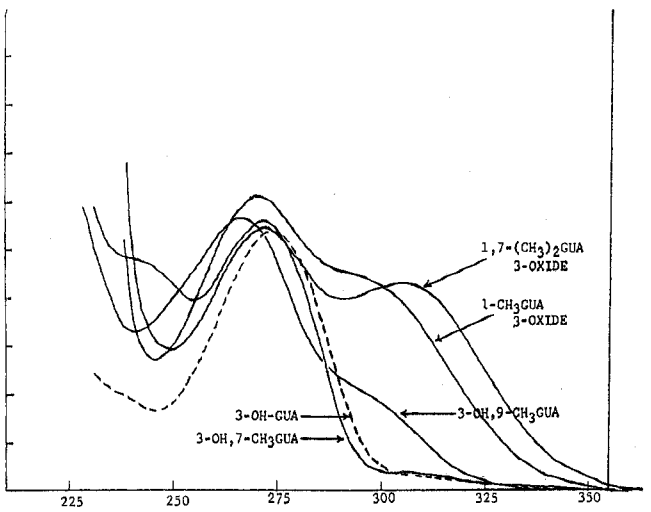
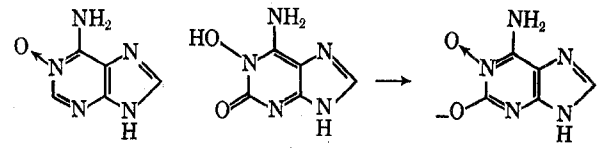


Figure 5.—Guanine 3-*N*-oxide series in methanol. The spectra were determined on nearly saturated solutions in 4-cm cuvettes and are not at equimolar concentrations.

The predominance of 3-hydroxyguanine rather than guanine 3-oxide in the neutral species of **8** was not expected.[18] Aminopurine oxides, notably the 1-oxides of adenine, **11**, adenosine, and 2,6-diaminopurine, exist predominantly as the *N*-oxide tautomers, as deduced from the behavior of the 230-nm band.[20] The *N*-hydroxy tautomer was, however, known to be preferred over the *N*-oxide form when an oxo group is present on an adjacent carbon. The 2-oxo derivative of adenine 1-*N*-oxide (**12**) does not exhibit the high extinction 230-nm band shown by the neutral species of **11** until after the first ionization (pK_a = 5.0).[6] This was interpreted[6] to mean that the *N*-hydroxy tautomer is favored in the neutral species of **12**. Absorption at 228 and 295 nm in the monoanion, **13**, is similar to that of **6** and **10**, and indicates that an enolate anion comparable to **3** is also predominant in **13**.

The absorption band at 230 nm in the adenine 1-oxide and adenosine 1-oxide spectra is lost upon protonation,[20] and protonation must involve the *N*-oxide function to explain the accompanying loss of that absorption. In the guanine 3-*N*-oxide series comparable bands of the neutral species at 217 to 227 nm do not



completely disappear but decrease in intensity and shift to 211 to 217 nm upon protonation.

The nearly identical spectra of the cations of **8**, and of its 1- and 7-methyl and 1,7-dimethyl derivatives (Figure 6), suggest that these cations are similar in structure. These spectra closely resemble that of the 3-methylguanine cation[39] and are distinctly different from those of the 3-hydroxy-9-methylguanine and 9-methylguanine cations. The latter has the additional proton at N-7,[26,29,31] and 3-hydroxy-9-methylguanine must protonate at the same position. The differences between the spectra of the cations of **8**, its 1,7-dimethyl derivative, and 3-methylguanine from that of the 3-hydroxy-9-methylguanine cation suggest that the site of first protonation in **8** is the pyrimidine ring (**14**, R = H). This protonation of the pyrimidine moiety of 3-hydroxyguanine contrasts with that of guanine and its 1-, 7-, 8-, or 9-methyl derivatives, for all of which protonation of the imidazole ring is deduced.[27,29] A second pK_a of protonation is found at −1.33 for **8**, and the spectrum of the dication is then quite similar to that of the monocation of 3-hydroxy-9-methylguanine (Figure 5). Such evidence for the second protonation of **8** at N-9 to yield **15** corroborates the assignment of the first protonation to the pyrimidine ring. The spectrum of **14** resembles those of the cations of 8-trifluoromethylguanine and its 1- and 7-methyl derivatives, which are deduced to protonate at N-3.[27] Further evidence for the positions and order of protonation is provided by a comparison of the influence of methyl substituents on the p*K*'s of **8** and of guanine (Table II). Data indi-

TABLE II

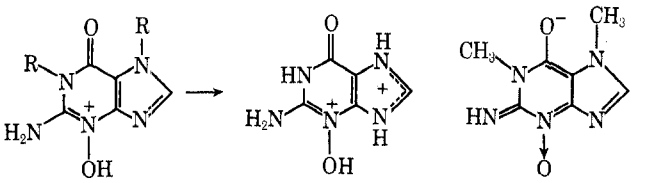
Δp*K*'s RELATIVE TO THE CORRESPONDING 8-H DERIVATIVE

	3-OH-8-CH_3-Gua	8-CH_3-Gua
Second protonation	+0.6	+0.2
First protonation	−0.06	+0.7
First ionization	+0.1	+0.4
Second ionization	+0.6	+0.4

cate that the sequence of protonation[29] and ionization[26] for guanine and its 8-methyl derivative[27] is the same, and the similar spectral changes indicate that this is also true for **8** and its 8-methyl derivative (Table I). There is an increase of 0.2–0.7 pH unit for all p*K*'s of 8-methylguanine (Table II). The largest increase is in the first protonation pK_a which occurs[27,29] in the methyl-substituted imidazole ring. In contrast, an 8-methyl substituent on 3-hydroxyguanine induces almost no change in either the first ionization or the first protonation p*K*'s (Table II), both of which were deduced independently from other evidence to occur on the pyrimidine ring, as in **10** and **14**. The second protonation and second ionization p*K*'s, both associated with the imidazole ring of **8**, are increased by 0.6 pH unit, which is comparable to the effect of an 8-methyl group on all of

(39) L. B. Townsend and R. K. Robins, *J. Amer. Chem. Soc.*, **84**, 3008 (1962).

the pK's of guanine. The only derivatives of **8** which possess a higher first protonation pK than **8** are those bearing a 1-methyl substituent (Table I). This and the selective increase by an 8-methyl substituent of only the pK's deduced for the imidazole ring thus support the assigned positions of protonation and ionization. Even though there is a hydroxyl group on N-3 in the cations of the guanine 3-*N*-oxide series, bands are still observable at 211–217 nm, and these bands must result from the influence of the 3-oxygen.



14, R = H, CH_3 **15** **16**

The fact that the increases in intensity of the bands near 224 and near 300 nm are associated with two pK_a's, both the deprotonation of the cation and the first ionization, is consistent with the presence of **8b** and some **8a** in the neutral species of **8**. The full effect of the *N*-oxide function on both the 224- and 300-nm bands of **8** is not realized until after ionization to **10**.

Tautomers of **8** that require a 6-enol rather than a 6-keto, or a 2-imino rather than the 2-amino, form are conceivable, but would not be expected.[13] The difference between the ultraviolet spectra of the neutral species of **8** and of 2-amino-6-methoxypurine 3-oxide (Table I) is evidence against a 6-enol structure for **8** in solution. In addition, strong carbonyl absorption is manifest at 1700 cm^{-1} in the ir spectrum of **8** (solid state, KBr). Available data indicate that both 2-aminopyridine[40] and guanine[29,41] exist in the 2-amino form; recent MO calculations agree.[42] The amino tautomer is retained in the *N*-oxide of 2-aminopyridine[43] and, by analogy, an *N*-oxide group at N-3 of guanine should not induce a tautomeric change of the 2-amino to a 2-imino structure.

Although 1,7-dimethylguanine does not ionize in strong base, its 3-oxide does, pK_a = 11.20. Since the only available hydrogens are on the 2-amino group, ionization must take place from there. A large bathochromic shift of the 300-nm band to 327 nm accompanies this pK_a, thereby implying additional conjugation and suggesting that the C-6 enolate anion and the imino tautomer at C-2, **16**, may predominate. With 1-methylguanine 3-oxide a second pK_a of ~14 is also associated with a bathochromic shift of the 300-nm band and must also represent ionization of the 2-amino group, an ionization not shown by 1-methylguanine.[44]

The knowledge that **8** exists mainly as the 3-hydroxy tautomer indicates that it should be able to form an ester comparable to 3-acetoxyxanthine,[3] which in turn should also undergo substitution at position 8. This facilitates interpretation of its rearrangement to 8-hydroxyguanine with trifluoroacetic anhydride *in vitro*[45] and the formation of 8-methylmercaptoguanine *in vivo*.[46,47]

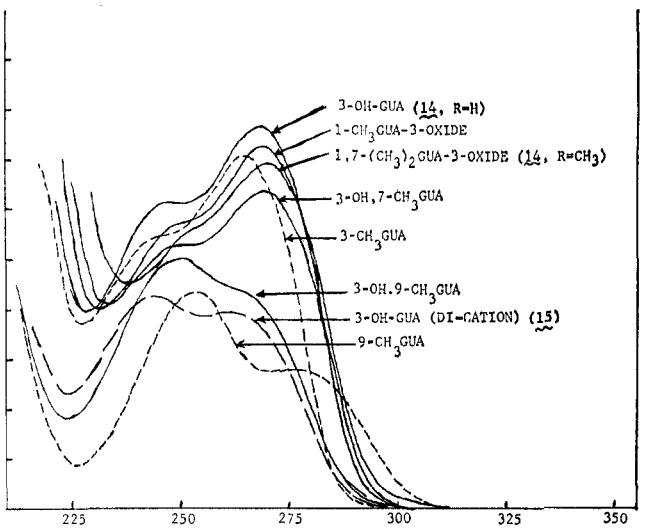


Figure 6.—Guanine 3-*N*-oxide series: cations. See footnote to Figure 1.

Experimental Section

The extinction coefficients at selected pH's for the various molecular species (Table I) were determined with a Beckman DU or Cary Model 15 spectrophotometer. Isosbestic uv spectra and those in the figures were determined with a Unicam SP800 recording spectrophotometer. The latter are plotted for convenient comparisons and are not at equimolar concentration and no OD scale is given. Values above ~240 nm were determined in 4-cm cells and those below 240 in 1-cm cells because of the great differences in extinction coefficients (Table I). The pK_a's were determined by methods described,[48] at 23 ± 1°, spectrophotometrically with 0.01 *M* buffers[49] or electrometrically with 0.001 *M* solutions, with the use of a Beckman DU spectrophotometer and a Beckman Research Model pH meter.

Ir spectra were determined with an Infracord spectrophotometer (KBr disk) on samples dried at 110° over P_2O_5 for 24 hr. The carbonyl absorption of 1-hydroxyhypoxanthine appears at 1695 cm^{-1},[5] while that of 1-hydroxyxanthine shows a broad, partially resolved band with peaks at 1665 and 1680 cm^{-1}. The carbonyl absorption of 3-hydroxyxanthine appears as a single broad band centered at 1655 cm^{-1}, while two carbonyl bands of 3-hydroxy-1-methylxanthine are well resolved, appearing at 1635 and 1710 cm^{-1}. This parallels results obtained both in KBr[50] and in dioxane solution[51] from xanthine and its 1,3-di-, 3,7-di-, and 1,3,7-trimethyl derivatives, in which the two carbonyl absorption bands were not resolved unless a 1-methyl substituent was present. These carbonyl absorption bands differ from those attributed to the N–O group in heterocyclic *N*-oxides, which appear at 1200–1300 cm^{-1}.[38]

Registry No.—**5**, 13479-29-3; **5** (OH = OCH_3), 30345-91-6; **8b**, 30345-22-3; **9**, 30345-23-4; 3-hydroxy-7-methylxanthine, 30409-21-3; 3-hydroxy-9-methylxanthine, 30345-24-5; 3-hydroxy-1-methylxanthine, 14002-16-5; 1,7-dimethyl-3-hydroxyxanthine, 30345-

(40) A. R. Katritzky and J. M. Lagowski, *Advan. Heterocycl. Chem.*, **1**, 404 (1963).

(41) Part of the evidence for predominance of the 2-amino tautomer in guanine is based on nmr data on guanosine in DMSO-d_6.[31] Attempts to obtain similar nmr data on the even less soluble **8** and **9** were unsuccessful.

(42) N. Bodor, M. J. S. Dewar, and A. J. Harget, *J. Amer. Chem. Soc.*, **92**, 2929 (1970).

(43) A. R. Katritzky, *J. Chem. Soc.*, 191 (1957).

(44) Neither 3-hydroxyguanine nor guanine shows, to pH 15, any spectral change attributable to ionization of the 2-amino group.

(45) U. Wölcke, W. Pfleiderer, T. J. Delia, and G. B. Brown, *J. Org. Chem.*, **34**, 981 (1969).

(46) G. B. Brown, G. Stöhrer, K. Sugiura and M. N. Teller, Abstracts, 10th International Cancer Congress, Houston, Texas, 1970, Medical Arts Publishing Co., Austin, Tex., 1970, p 8.

(47) G. Stöhrer and G. B. Brown, *Science*, **167**, 1622 (1970).

(48) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Wiley, New York, N. Y., 1962.

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

(50) E. R. Blout and M. Fields, *J. Amer. Chem. Soc.*, **72**, 479 (1950).

(51) M. Horák and J. Gut, *Collect. Czech. Chem. Commun.*, **26**, 1680 (1961).

26-7; 3-hydroxy-8-methylguanine, 30409-22-4; 3-hydroxy-7-methylguanine, 30345-27-8; 3-hydroxy-9-methylguanine, 30345-28-9; 1,7-dimethylguanine 3-oxide, 30345-29-0; 2-amino-6-methoxypurine 3-oxide, 30345-92-7.

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Synthesis of 2-Thio-D-ribose and 2′-Thioadenosine Derivatives[1]

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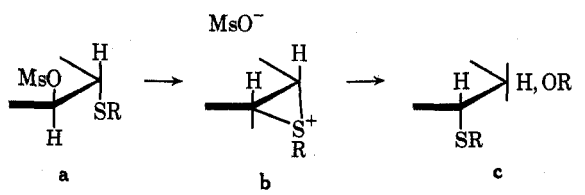
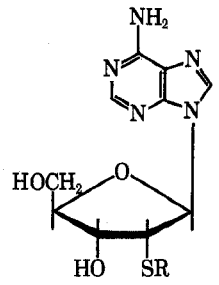
2-Thio-D-ribose derivatives, both furanose and pyranose, have been synthesized from the corresponding *S*-alkyl 1-thio-α-D-arabinoside 2-*O*-mesylates. The alkylthio group underwent stereospecific migration to C-2 with ejection of the *trans*-2-*O*-mesyl group. Depending on the medium, the 2-thio-D-ribose derivatives were obtained as methyl glycosides or as 1-*O*-acetates. In a deblocking sequence, methyl 2-thio-D-ribofuranoside was obtained as the free thiol. The *S*-methyl, *S*-benzyl, and tetrabenzoyl derivatives of 2′-thioadenosine were obtained from the furanose 1-*O*-acetates or their chloro sugars in reactions with purine bases. In some of the nucleoside condensations, 7-nucleosides were obtained as by-products and were identified by infrared and ultraviolet spectral properties, not previously reported, and characteristic of 7-substituted 6-benzamidopurines.

A number of new thio sugars have been synthesized in recent years, often for biological interest in their nucleoside derivatives. Of the positional isomers of thio-D-ribose, only 2-thio-D-ribose has not been synthesized previously. The requisite *cis*-3-OH,2-SH arrangement has not been achieved synthetically in any sugar, although numerous 2-thio sugars have been prepared with a trans mercapto–alcohol system. The *cis*-3-SH,-2-OH system of 3-thio-D-ribofuranose derivatives and 3′-thioadenosine was attained[2] only recently by the technique of internal displacement at C-3 with a *trans*-2-*O*-thionobenzoate. A related internal displacement at C-3 with a *trans*-2-*S*-thiolbenzoate was attempted[3] as a synthesis of 2-thio-D-ribofuranose but was unsuccessful; only the 2,3-episulfide was formed by neighboring participation of sulfur rather than oxygen.

The synthesis of 2-thio-D-ribose derivatives has now been accomplished in a related process by generating a 1,2-episulfonium ion (**b**) as intermediate. Starting from an *S*-alkyl 1-thio-α-D-arabinoside (**a**), with a readily displaced *trans*-*O*-mesylate at C-2 (and stable blocking groups at C-3 and at C-4 or C-5), the 1-alkylthio group underwent stereospecific migration to C-2. Ejection of the mesylate occurred with inversion at C-2 giving the D-ribo configuration, and the intermediate episulfonium ion (**b**) was opened by regiospecific attack at C-1 by a nucleophile provided by the medium. The 2-thio-D-ribose derivatives obtained by this means have been converted to the *S*-methyl, *S*-benzyl, and tetrabenzoyl derivatives (β-9-**33**, β-9-**31**, β-9-**29**) of 2′-thio-adenosine. 2′-Thioadenosine could not be obtained on deblocking. There was evidence for cleavage of the nucleoside link when the free thiol was liberated. Alternatively, 2′-*S*-methyl-2′-thioadenosine was selected as the target compound for study of biological properties.[4]





2′-thioadenosine, R = H
β-9-**33**, R = Me
β-9-**31**, R = CH_2Ph

The synthesis was studied first with pyranose sugars as models (Scheme I), owing to the ready availability of *S*-benzyl 1-thio-α-D-arabinopyranoside[5] (**3**) as starting material. The 3,4-*O*-cyclohexylidene acetal (**4**) was obtained as a crystalline substance and was used in preference to the isopropylidene analog, an oil. A 2-*O*-tosylate could not be formed from **4**, perhaps because of steric restrictions in this fused ring system. The 2-*O*-mesylate (**5**) was readily obtained; it could be stored at 5° for 1 week without deterioration but decomposed upon prolonged storage or on heating. Treatment of **5** with refluxing methanol containing sodium bicarbonate as acid acceptor caused nearly quantitative rearrangement to the methyl pyranoside **6** of 2-*S*-benzyl-2-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, **33**, 1783 (1968).

(3) K. J. Ryan, E. M. Acton, and L. Goodman, *ibid.*, **33**, 3727 (1968).

(4) 2′-*O*-Methyl ribonucleosides have been found in RNA from a wide variety of sources. Although the biological function is unknown, it has been suggested that these may play an important role in protein biosynthesis: T. A. Khwaja and R. K. Robins, *J. Amer. Chem. Soc.*, **88**, 3640 (1966), and leading references.

(5) H. Zinner, A. Koine, and H. Nimz, *Chem. Ber.*, **93**, 2705 (1960).