Purine N-Oxides. XXV. 3-N-Oxides of Adenine and Hypoxanthine[1]

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Received December 3, 1968

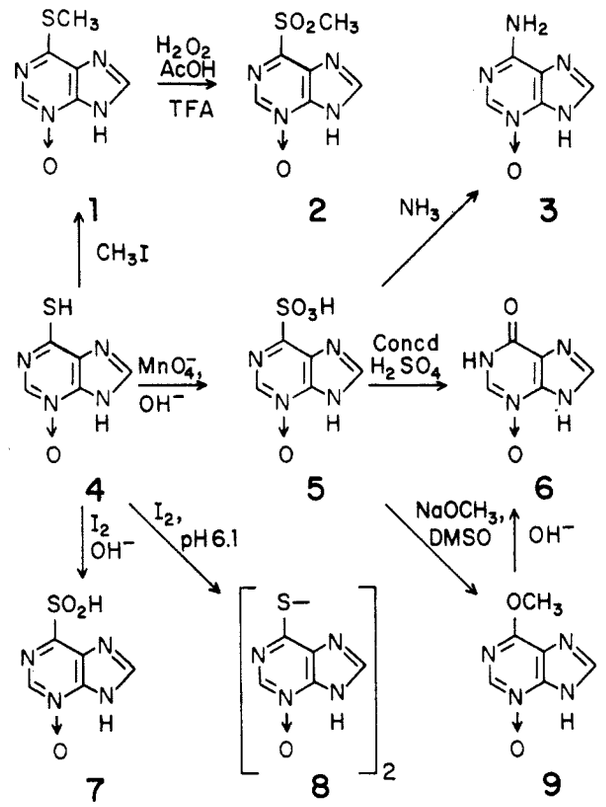
The 3-N-oxides of adenine, hypoxanthine, and 6-methoxypurine are synthesized by displacements of the sulfonyl group of 6-sulfonylpurine 3-oxides. The latter is obtained from 6-mercaptopurine 3-oxide, as are the corresponding sulfinyl-, methylsulfonyl-, and disulfide derivatives. An oxidation product of 6-methoxypurine is shown to be the 3-N-oxide.

Adenine 1-oxide,[2,3] and what is now known to be guanine 3-oxide,[4-6] each obtained by direct oxidation, and 3-hydroxyxanthine prepared from the latter, have been shown[5,7,8] to be chemical oncogens.[9] The isomeric 1-hydroxyxanthine, obtained by chemical modification of adenine 1-oxide,[10] is a far weaker oncogenic agent[8] than 3-hydroxyxanthine; that pair of isomers offered the first major indication of considerable structural specificity with respect to oncogenicity.

A variety of purine N-oxides must be evaluated before a correlation can be established between structure and oncogenicity. Most will be obtainable only by total synthesis since direct oxidation fails to introduce oxygen into positions where it is desired. We now report total syntheses of adenine 3-oxide, (**3**) and hypoxanthine 3-oxide (**6**).

Oxidation of 7-aminothiazolo[5,4-*d*]pyrimidine to its 6-oxide, and rearrangement, had provided a synthesis[11,12] of 6-mercaptopurine 3-oxide (**4**). A series of oxidation products of **4**, the sulfinate **7**, sulfonate **5**, and disulfide **8**, and the methylsulfonyl **2** from its S-methyl derivative **1** were prepared (Scheme I) for studies of the ease of displacement of the 6 substituents. The methods were analogous to those used for the corresponding derivatives from 6-mercaptopurine;[13] there was no undue influence of the 3-N-oxide function on the oxidation of the 6 substituents.

The methylmercapto group of 6-methylmercaptopurines can be readily displaced by amines.[14] Attempted displacement of the methylmercapto group of 6-methylmercaptopurine 3-oxide (**1**) with liquid ammonia at room temperature or in ethanolic ammonia at elevated temperatures produced adenine. Similar



treatment of 6-chloropurine 3-oxide[15] also resulted in deoxygenation as well as displacement of the chloro group.

Successful displacement with retention of the N-oxide function was accomplished, on a preparative scale, by the reaction of the potassium salt of purine-6-sulfonate 3-oxide (**5**) with concentrated aqueous ammonia at 100° for 18 hr. The adenine 3-oxide (**3**) was isolated either as the hemihydrate or as a complex with 1 mole of ammonium sulfate.

Purine-6-sulfonate 3-oxide, like purine-6-sulfonate, is alkali stable and acid labile. Although purine-6-sulfonate is readily hydrolyzed to hypoxanthine by hydrochloric acid,[13] no analogous product could be isolated from the hydrolysis of purine-6-sulfonate 3-oxide (**5**) under similar conditions. However, when **5** was dissolved in concentrated sulfuric acid, immediate evolution of sulfur dioxide occurred, and a single product could be isolated. It was hypoxanthine 3-oxide (**6**) which is stable in the solid state and in alkaline solution, but somewhat unstable in aqueous acid.[4]

(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

CHEMICAL SHIFTS (τ)

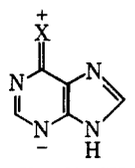
	6-XCH_3			H-2			H-8		
	X			X			X		
Protons	S	SO	SO_2	S	SO	SO_2	S	SO	SO_2
Purine 6-XCH_3[a,c]	7.29	6.88	6.43	1.23	0.95	0.81	1.52	1.25	1.05
Purine 3-oxide 6-XCH_3[a]	7.30		6.58	1.12		0.81	1.52		1.20
9-Methylpurine XCH_3[b,d]	7.29	6.86	6.52	1.26	0.91	0.91	2.08	1.85	1.65

[a] Solvent DMSO-d_6 (TMS). [b] Solvent $DCCl_3$ (TMS): P. W. Ford, Thesis, Australian National University, Canberra, 1968. [c] Registry numbers: X = S, 50-66-8; X = SO, 19769-31-4; X = SO_2, 19769-32-5. [d] Registry numbers: X = S, 1127-75-9; X = SO, 19769-34-7; X = SO_2, 19769-35-8.

A synthesis of hypoxanthine 3-oxide recently reported in the patent literature[16] was accomplished by oxidation of 6-methoxypurine to an N-oxide **9**, followed by alkaline hydrolysis of the methoxyl group. We have long had an N-oxidation product from 6-methoxypurine,[17] but until now have been unable to assign the position of oxidation. The 3-N-oxide of 6-methoxypurine (**9**) has now been synthesized by heating purine-6-sulfonate 3-oxide (**5**) with sodium methoxide in dimethyl sulfoxide. A comparison of the 6-methoxypurine 3-oxide prepared by oxidation with that prepared by the displacement reaction showed them to be identical, and demonstrated that oxidation had occurred at the 3 position. We, too, have obtained hypoxanthine 3-N-oxide from 6-methoxypurine 3-oxide,[16] and find it identical with that obtained by the hydrolysis of purine 6-sulfonate 3-oxide and different from 1-hydroxyhypoxanthine,[18] a substantiation of the assignment of 3-N-oxide structures to **6** and **9**. Further confirmation is afforded by the conversion, in this laboratory, of 6-chloropurine 3-oxide to the 6-methoxy and 6-hydroxy derivatives.[15]

Conversions of **4** to the methyl sulfoxide and methyl sulfone were also undertaken but only the latter was obtained. 6-Methylmercaptopurine 3-oxide (**1**) was inert to oxidation by peroxyacetic acid even at 60°; oxidation in peroxytrifluoroacetic acid did not yield reproducible results and numerous by-products were detectable by paper chromatography. The addition of acetic acid to trifluoroacetic acid and the use of limited amounts of hydrogen peroxide moderated the reaction; a product precipitated and no further oxidation occurred. The product was shown to be the sulfone, 6-methylsulfonylpurine 3-oxide (**2**) by its strong absorption bands in the regions of 1120–1160 and 1310–1350 cm^{-1} in the infrared, which are characteristic of sulfones.[19] The absence of an absorption band in the region 1040–1060 cm^{-1} eliminated the possibility that it was a sulfoxide.[19] The assignment of the sulfone structure was further supported by a comparison of the nmr methyl chemical shifts of the purine N-oxides to those of 6-SCH_3, 6-$SOCH_3$, and 6-SO_2CH_3 purines. Table I shows that as the oxidation state of the sulfur increases there is a progressive downfield shift of the position of the methyl signal. The value of τ 6.58 is in agreement with that of other sulfone methyl groups at τ 6.43 and 6.53, and distinctly different from the sulfoxide methyl values found at about τ 6.9. The proximity of τ values for the methyl groups of 6-methylmercaptopurine and its 3-oxide indicates that the N-oxide function in the 3 position exerts little influence on the S-methyl shift.

Of the 6-substituted purines, the amino-[2,3] and methylpurines[20] are oxidized on the nitrogen adjacent to the substituent and yield 1-oxides, while the methoxy- and chloropurines[15] are oxidized at the nitrogen "*para*" to the substituent and yield 3-oxides. These offer the first assessment of directive influence by these groups on N-oxidation in the purine ring. The 4-alkoxyquinazolines are oxidized on the nitrogen "*para*" to the substituent.[21] The oxidation of 4-alkoxypyrimidines[22] and 4-phenylpyrimidines[23] is also reported to occur on the "*para*" nitrogen. Oxidation of 4-methylpyrimidine, however, produces a mixture of N-oxides in which the N-oxide adjacent to the substituent predominates by a factor of 3.5:1.[24] The effect of a substituent on the electron density at the ring nitrogens should be the primary influence that determines the position of oxidation, although steric influence, solvent effects, or both, may contribute. The amino and methyl groups appear to provide strong activation for N-oxidation at the adjacent position. The chloro and methoxy groups, however, would tend to deactivate the adjacent position by inductive electron withdrawal, but could provide activation elsewhere in the ring by resonance. Thus, a mesomeric contribution by **10** could explain N-oxidation "*para*" to the substituent in 6-chloro- and 6-methoxypurines.



10, X = Cl, CH_3O

The ultraviolet spectra for several pH's, and the pK's, are given in Table II. From them it is deduced that the neutral molecules of **3**, **6**, and **9** exist primarily as the N-oxide tautomers, since the strong absorption at 220–230 mμ is associated with the neutral molecules

(16) Ajinomoto Co., Brevet d'Invention P. V. no. 83,853, French Patent 1,500,662 (1967).

(17) 6-Methoxypurine was first oxidized by R. M. Cresswell. The method described here is a modification of that of T. J. Delia.

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TABLE II

SPECTRAL DATA AND pK_aS

pH	Charge	λ_{max}, mμ ($\epsilon \times 10^{-3}$)	pK_a
		Adenine 3-Oxide (**3**)	
0	(+)	224[a] (5.8), 277 (8.5)	
5	(0)	229 (9.0), 293 (7.0)	2.87 (±0.06)
10	(−)	231 (11.7), 278[a] (5.7), 290 (6.3)	E[b] 6.91 (±0.07)
		Hypoxanthine 3-Oxide (**6**)	
−1	(+)	212 (14.4), 275 (8.0)	
3	(0)	223 (17.2), 271 (9.4)	1.2 (±0.1)
8	(−)	218 (19.5), 286 (12.1)	5.08 (±0.1)
12	(−2)	224 (22.0), 275[a] (10.1), 285 (10.3)	9.3 (±0.1)
		6-Methoxypurine 3-Oxide (**9**)	
0	(+)	263 (6.9)	
4	(0)	224 (24.1), 283 (10.1)	1.47 (±0.05)
9	(−)	227 (28.2), 276 (9.1)	E[b] 6.75 (±0.02)
		Purine-6-sulfonate 3-Oxide (**5**)	
3	(−)	229 (22.8), 311 (12.2)	E[b] 6.60 (±0.05)
12	(−2)	230 (28.6), 316 (8.5)	
		6-Methylmercaptopurine 3-Oxide (**1**)	
−1	(+)	236 (8.1), 317 (21)	
3	(0)	236 (7.3), 254 (8.2), 319 (18.9)	1.00 (±0.02)
12	(−)	214 (16.0), 245 (16.1), 312 (17.0), 322[a] (15.4)	6.02 (±0.02)
		Disulfide of 6-Mercaptopurine 3-Oxide (**8**)	
6	(0)	238 (26.2), 323 (20.5)	
		6-Methylsulfonylpurine 3-Oxide (**2**)	
3	(0)	232 (36), 299[a] (14), 322 (23)	
9	(−)	233 (43), 258[a] (9.0), 302 (13), 332 (15)	E[b] 5.10 (0.08)

[a] Shoulder. [b] Determined electrometrically with 0.01 M solutions.

and is suppressed in the protonated species.[25] With **1**, the spectrum is shifted bathochromically, as is usual with sulfur derivatives, and no well-defined change can definitely be associated with the N-oxide function. By analogy to the sulfur-containing derivatives **2** and **5** and to the O-methyl derivative **9**, the S-methyl derivative **1** is named as the N-oxide.

Experimental Section

Analyses were performed by the Spang Microanalytical Laboratories, Ann Arbor, Mich., and Galbraith Laboratories, Inc., Knoxville, Tenn. Melting points were obtained on a Mel-Temp apparatus and are corrected. Chromatograms were developed, ascending, on Whatman No. 1 paper; R_f values are given in Table III.

TABLE III

R_f VALUES

Compd	Solvents[a] A	B	C
6-Methoxypurine 3-oxide (**9**)	0.50	0.63	0.52
Purine-6-sulfonate 3-oxide (**5**)	0.07	0.80	0.26[b]
Purine-6-sulfinate 3-oxide (**7**)	0.07	0.80	
Disulfide of 6-mercaptopurine 3-oxide (**8**)	0.32	0.44	0.40[b]
6-Methylsulfonylpurine 3-oxide (**2**)	0.36	0.70	0.50
Adenine 3-oxide (**3**)	0.36[b]	0.40	0.40
Hypoxanthine 3-oxide (**6**)	0.30[b]	0.68	0.18
6-Mercaptopurine 3-oxide (**4**)	0.30	0.52	0.17

[a] A, BuOH–HAc–H_2O (60:15:25 v/v); B, 5% disodium phosphate–isoamyl alcohol (3:2 v/v) [C. E. Carter, *J. Am. Chem. Soc.*, **72**, 1466 (1950)]; C, *i*-PrOH–NH_4OH–H_2O (7:1:2 v/v). [b] Trailing.

The pK values were determined spectrophotometrically by methods described,[26] with 0.01 M buffers[27] at 20–22° or electrometrically with 0.01 M solutions. The infrared data were obtained using a Perkin-Elmer Model 137B Infracord spectrophotometer (KBr pellet). The uv spectra were determined with Beckman DU and Unicam SP 800A spectrophotometers. The nmr data were obtained on a Varian A-60 spectrometer.

Purine-6-sulfinate 3-Oxide (**7**).—To a stirred solution of 6-mercaptopurine 3-oxide (0.56 g, 3 mmol), dissolved in 30 ml of 1 N NaOH, was added 22.5 ml (5.4 mmol) of 0.5 N I_2 dropwise over a period of 15 min. The pH was adjusted to 5 by addition of AcOH, and the volume was reduced to *ca.* 20 ml under reduced pressure. EtOH (70 ml) was added to precipitate the product, the flask was chilled, and the product was collected and dried over P_2O_5 to yield 0.54 g (80%). The sample was recrystallized from H_2O and EtOH to yield a fine yellow powder, 400 mg. The analytical sample was dried at 110°.

Anal. Calcd for $C_5H_3N_4SO_3Na_2$: C, 24.50; H, 1.22; N, 22.90; S, 13.10; Na, 18.76. Found: C, 24.51; H, 1.00; N, 23.20; S, 13.21; Na, 18.47.

Purine-6-sulfonate 3-Oxide (**5**).—A solution of 6-mercaptopurine 3-oxide (7.81 g, 0.042 mol) in 130 ml 0.05 N KOH was cooled in an ice–water bath and stirred. A solution of 13.3 g of potassium permanganate in 250 ml of H_2O was added slowly. The addition completed, the resulting suspension was stirred cold for an additional 15 min and at room temperature for 4 hr. The suspension was filtered through a Celite pad, and *i*-PrOH (100 ml) was added to ensure complete reduction of the permanganate. The pH was adjusted to *ca.* 7 with glacial AcOH, and, after evaporation *in vacuo* to *ca.* 200 ml, was brought to pH ≈4 with glacial AcOH, and kept overnight at *ca.* 5°. The product was collected, washed with anhydrous EtOH followed by Et_2O, and air dried to yield 8.30 g (90.5%). For analysis it was recrystallized from 70% EtOH, mp >400°.

Anal. Calcd for $C_5H_3N_4SO_4K$: C, 23.62; H, 1.19; N, 22.03; S, 12.61. Found: C, 23.54; H, 1.27; N, 21.84; S, 12.79.

This product is identical with that prepared from 6-chloropurine 3-oxide, which had in turn been prepared from 6-mercaptopurine 3-oxide.[15]

Electrophoretic Separation.—The 6-sulfonate and 6-sulfinate 3-oxides showed very similar R_f values by paper chromatography, but could be separated by ionophoretic migration on Whatman 3 MM paper in a buffer of pH 7.7 (0.032 M Na_2HPO_4 and 0.004 NaH_2PO_4), 2 hr, at about 750 V and 30 mA. A single spot, anodic migration 15.2 cm, was shown by **7**, and a single one at 17.0 cm by **5**.

Disulfide of 6-Mercaptopurine 3-Oxide (**8**).—A sample of 6-mercaptopurine 3-oxide (0.93 g, 5 mmol) was dissolved in 300

(25) G. B. Brown, *Progr. Nucleic Acid Res. Mol. Biol.*, **8**, 209 (1968).

(26) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," John Wiley & Sons, Inc., New York, N. Y., 1962.

(27) D. D. Perrin, *Australian J. Chem.*, **16**, 572 (1963).

ml of NaH_2PO_4 buffer (pH 6.1) by warming to 40°. After the solution had cooled to room temperature, 10 ml of 0.5 *N* iodine solution was added dropwise, which reacted rapidly. The product precipitating during addition of I_2 was collected after the mixture had been chilled. It was washed thoroughly with H_2O, EtOH, and finally Et_2O, to yield 635 mg (68%). The analytical sample was dried overnight at 80° over P_2O_5 and was found to darken above 250°.

Anal. Calcd for $C_{10}H_6N_8S_2O_2 \cdot 0.5H_2O$: C, 34.98; H, 2.05; N, 32.63; S, 18.67. Found: C, 35.20; H, 2.27; N, 32.72; S, 18.20.

It was identical spectrally and chromatographically with an anhydrous sample, prepared by oxidation with butyl nitrite, and for which an explosion point is described.[15]

6-Methoxypurine 3-Oxide (9).[17]—A solution of 6-methoxypurine (2 g, 13 mmol), dissolved in 10 ml of trifluoroacetic acid and 4 ml of 30% H_2O_2, was stirred at room temperature for *ca.* 20 hr; from it, a yellow oil separated upon addition of Et_2O. The reaction flask was chilled, and the Et_2O layer was decanted and promptly discarded. Crystallization of the oily residue was induced by warming on a steam bath with 20 ml of MeOH. The suspension was cooled, 50 ml of Et_2O was added, and the solvents were decanted and discarded. The remaining white granular solid, after being warmed on a water bath with 20 ml of *n*-PrOH to remove unreacted 6-methoxypurine, was washed with Et_2O and air dried; yield 1.43 g (66%). Paper chromatography proved the sample to be homogeneous. The analytical sample was obtained as colorless needles from H_2O and *n*-PrOH and dried overnight at 110°, mp 216–218°.

Anal. Calcd for $C_6H_6N_4O_2$: C, 43.38; H, 3.64; N, 33.72. Found: C, 43.59; H, 3.89; N, 33.43.

It was identical chromatographically and by uv spectra with material prepared from 6-chloropurine 3-oxide.[15]

6-Methylsulfonylpurine 3-Oxide (2).—6-Methylmercaptopurine 3-oxide (**1**)[12] (220 mg, 1.2 mmol) was suspended in a mixture composed of 0.35 ml (9.5 mmol) of trifluoroacetic acid, 2.1 ml of AcOH, and 0.4 ml of H_2O_2. After a few minutes of stirring, a clear solution resulted from which, about 1 hr later, precipitation of a white solid, began; stirring was continued at room temperature for an additional 1 hr. The product was collected, washed with anhydrous EtOH and Et_2O, and air dried to yield 115 mg (44%) of a light yellow chromatographically pure material, mp 192–193° dec.

Anal. Calcd for $C_6H_6N_4SO_3$: C, 33.64; H, 2.82; N, 26.15; S, 14.96. Found: C, 33.78; H, 2.85; N, 26.07; S, 14.94.

The infrared spectrum shows strong absorption in two band groups in the two regions characteristic of sulfone absorption.[19] In the region between 1310 and 1350 cm^{-1}, the bands are found at 1300, 1315, and 1330 cm^{-1}; in the 1120–1160-cm^{-1} region they are observed at 1125, 1140, and 1150 cm^{-1}.

Adenine 3-Oxide (3) Hemihydrate.—Purine-6-sulfonate 3-oxide (2.0 g, 9.25 mmol) was dissolved in *ca.* 40 ml of concentrated NH_4OH and placed in a glass-lined, high-pressure reaction vessel. The reaction vessel was heated at 100° for 18 hr, and cooled to 0°. The solution was evaporated on a rotary evaporator to *ca.* 15 ml. Concentrated NH_4OH was added to a pH of *ca.* 11, and water to a final volume of about 35 ml, when all the solids had dissolved. The solution was neutralized with glacial AcOH and cooled at 5° overnight. The precipitate was collected, redissolved in dilute NH_4OH, reprecipitated by the addition of glacial AcOH, and again cooled overnight at *ca.* 5°. The product was collected, washed with anhydrous EtOH and then Et_2O, and air dried, yielding 0.920 g of a white material (62%), dec pt >350°.

Anal. Calcd for $C_5H_5N_5O \cdot 0.5H_2O$: C, 37.50; H, 3.77; N, 43.73. Found: C, 37.77; H, 3.39; N, 43.72.

Adenine 3-Oxide–Ammonium Sulfate Complex.—If after evaporation and before the reprecipitation, 6 *N* H_2SO_4 is utilized instead of glacial AcOH, the resulting product contains 1 mol of $(NH_4)_2SO_4$.

Anal. Calcd for $C_5H_5N_5O \cdot (NH_4)_2SO_4$: C, 21.20; H, 4.63; N, 34.61; S, 11.31. Found: C, 21.41; H, 4.53; N, 34.32; S, 10.90.

Hypoxanthine 3-Oxide (6).—Purine-6-sulfonate 3-oxide (**5**) (3.44 g, 16 mmol) in 50 ml of concentrated H_2SO_4 was stirred until solution was complete, when it was added dropwise to 1 lb of anhydrous Et_2O and the mixture was refrigerated overnight. The Et_2O was decanted, and the solid was dissolved in 100 ml H_2O, stirred with Darco at room temperature, and filtered through a Celite pad. EtOH was then added until a permanent cloudiness resulted and the solution was again refrigerated overnight. The precipitate was collected and washed with anhydrous EtOH and then Et_2O to yield 3.32 g (undried). The filtrate, after evaporation *in vacuo* to *ca.* 50 ml and the addition of 50 ml of EtOH, was cooled at *ca.* −10° overnight. The additional precipitate was collected and washed with EtOH and Et_2O. The 2.20 g obtained was combined with that previously obtained and suspended in 25 ml of H_2O, and 1 *N* NaOH was added slowly with cooling in an ice bath until it dissolved (the pH was *ca.* 7), when it was stirred with Darco and filtered through a Celite pad. Glacial AcOH was then added until precipitation commenced (pH *ca.* 4); the reaction mixture was cooled at 5° overnight, and the precipitate was collected and washed with anhydrous EtOH and Et_2O to yield 1.54 g (62.5%). For analysis it was dried at 80° *in vacuo*.

Anal. Calcd for $C_5H_4O_2$: C, 39.48; H, 2.65; N, 36.83. Found: C, 39.28; H, 2.72; N, 36.81.

Column chromatography with Dowex-50, 200–400 mesh, convex gradient of 0.05 *N* plus 3 *N* HCl, which separates hypoxanthine 3-oxide, 1-hydroxyhypoxanthine, and hypoxanthine in that sequence, was used to demonstrate homogeneity of the product.

Reduction of Hypoxanthine 3-Oxide.—A mixture of Raney nickel (*ca.* 100 mg), 10 ml of 5% NH_4OH, and 4.79 mg (3.15×10^{-2} mmol) of **6** was heated under reflux for 2 hr, filtered, and found, spectrophotometrically, to contain 47.3% of the theoretical amount of hypoxanthine. The solid residue, dissolved in 2 *N* HCl, contained 57.8% of the calculated amount of hypoxanthine, a total recovery of 105%. Column chromatography on Dowex-50, 200–400 mesh, with a convex gradient starting with 0.05 *N* HCl showed only hypoxanthine.

Reduction of Adenine 3-Oxide.—Adenine 3-oxide (2.3 mg, 1.48×10^{-2} mmol) was treated as above and refluxed for 1.5 hr. The filtrate contained 28.5% of the calculated amount of adenine, whereas the solution obtained by dissolving the residue with 2 *N* HCl contained 74.1%, a total recovery of 102.7%.

Registry No.—**1**, 2846-86-8; **2**, 19769-23-4; **3**, 19769-24-5; **5**, 19765-63-0; **6**, 19769-26-7; **7**, 19769-27-8; **8**, 19765-66-3; **9**, 19765-64-1.

Acknowledgments.—We wish to acknowledge the capable assistance of Mr. Marvin Olsen with the nmr spectra and of Mr. Thomas Winn and Mr. John Schwarz with the p*K* determinations, and helpful discussions with Dr. A. Giner-Sorolla and Dr. J. D. Fissekis. A sample of 6-methylsulfinylpurine was kindly furnished by Dr. G. H. Hitchings of the Wellcome Research Laboratories, Tuckahoe, N. Y.