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Studies of Purine *N*-Oxides. III. The Synthesis of Purine 3-*N*-Oxides[1]

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6-Methoxy- and 6-ethoxypurine 3-*N*-oxides were obtained by the direct oxidation of the corresponding 6-alkoxypurines with hydrogen peroxide in aqueous acetic acid. 6-Chloropurine was oxidized with monoperphthalic acid in ether to give 6-chloropurine 3-*N*-oxide. These purine 3-*N*-oxides were then converted to hypoxanthine 3-*N*-oxide by hydrolysis with a sodium hydroxide solution. 6-Methoxypurine 3-*N*-oxide and hypoxanthine 3-*N*-oxide were identified with authentic samples unambiguously synthesized from 6-methylsulfonylpurine 3-*N*-oxide, which had itself been obtained by the oxidation of 6-methylthiopurine 3-*N*-oxide. The treatment of hypoxanthine 3-*N*-oxide with hydrochloric acid gave 6,8-dihydroxypurine, 8-chlorohypoxanthine, hypoxanthine, and 2-chloro-4-amino-5-imidazolecarboxamide.

The synthesis of 3-*N*-oxides of purine derivatives by direct oxidation has never been described. Adenine,[2,3] 2,6-diaminopurine,[2] 6-methylpurine,[4] and 6-methyl-8-hydroxypurine[4] are, however, known to be converted to the corresponding 1-*N*-oxides by direct oxidation. Purine[4] and 8-hydroxypurine[4] have been oxidized to mono-*N*-oxides, in which the positions of the *N*-oxide groups are still unknown. Guanine[5,6] was oxidized to guanine 7-*N*-oxide with trifluoroperacetic acid.

Generally, purine 3-*N*-oxides have been synthesized by a cyclization process (*e.g.*, 7-methyladenine 3-*N*-oxide[7] and xanthine 3-*N*-oxide[8]). 6-Mercaptopurine 3-*N*-oxide was obtained from 7-aminothiazolo[5,4-d]pyrimidine 6-*N*-oxide by treating it with a sodium hydroxide solution.[9,10]

In the present paper the synthesis of purine 3-*N*-oxides by direct oxidation will be described. 6-Methoxy purine (Ia) was treated with a mixture of a 30% hydrogen peroxide solution and acetic acid at room temperature. After fifteen days the reaction mixture was evaporated to dryness, and the resulting residue was washed with ethanol and crystallized from water to give fine, colorless crystals. Elementary analysis revealed the composition of the oxidation product (IIa) to be $C_6H_6N_4O_2$, corresponding to that of the expected 6-methoxypurine *N*-oxide. The reduction of this oxidation product (IIa) with hydrogen in the presence of Raney nickel gave 6-methoxypurine (Ia). In addition, IIa possesses a strong absorption maximum in neutral and alkaline solutions near 230 mμ; this is characteristic of purine *N*-oxides in general.[2,11] These properties of this product (IIa) suggest its mono-*N*-oxide structure. 6-Ethoxypurine (Ib) was then similarly oxidized to give a mono-oxidized product (IIb); it had the composition of $C_7H_8N_4O_2$, and showed the properties of purine *N*-oxides.

The direct oxidation of 6-chloropurine has been reported to yield the nonoxidizable hypoxanthine.[9] Since this failure was considered to be due to the presence of water, the oxidation of 6-chloropurine was attempted under an anhydrous condition. 6-Chloropurine (III) partially suspended in an ether solution of monoperphthalic acid was stirred at room temperature for a few days. The resulting precipitation was washed with ether to remove the unchanged 6-chloropurine and the phthalic acid. The ether-insoluble residue showed a new single spot on a paper chromatogram. Since this product was decomposed upon heating in water or some organic solvents, it was reprecipitated from an ammonia-methanol solution by the addition of ether to give pure crystals. The crystals had the

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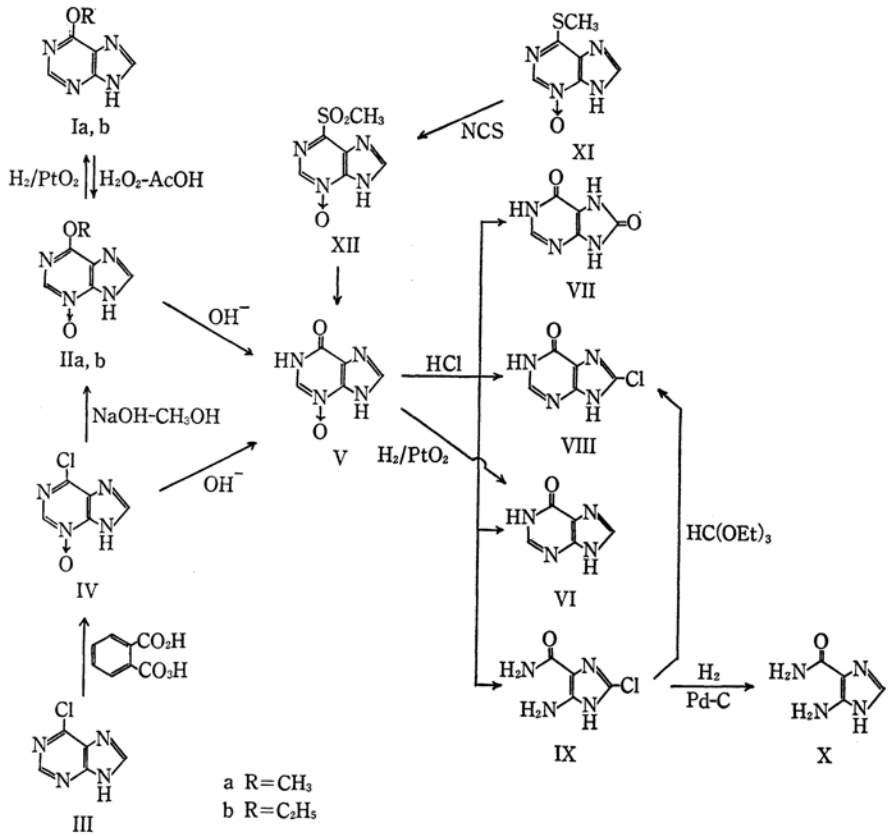

Fig. 1

composition of $C_5H_5N_4OCl\cdot NH_3$ and were convertible to 6-methoxypurine *N*-oxide (IIa) when treated with sodium methoxide. From these properties, the structure of this product was considered to be 6-chloropurine mono-*N*-oxide (IV).

Upon being heated in a 1 N sodium hydroxide solution under refluxing, these 6-alkoxypurine *N*-oxides (IIa, b) and 6-chloropurine *N*-oxide (IV) were both converted to the same new product (V). This new product (V) possessed the composition of $C_5H_4N_4O_2$ and a strong absorption maximum in an alkaline solution at about 230 mμ. Though V was reduced with hydrogen in the presence of Raney nickel to yield hypoxanthine (VI), it was not identified with hypoxanthine 1-*N*-oxide.[11-13] Consequently, V is likely to be hypoxanthine 3-, 7-, or 9-*N*-oxide.

In order to elucidate the structure of V, we attempted the hydrolysis of V with 3 N hydrochloric acid. The hydrolysis products were separated through a Dowex 50 (H^+ type) column to give 6,8-dihydroxypurine (VII), 8-chlorohypoxanthine (VIII), hypoxanthine (VI) and 2-chloro-4-amino-5-imidazolecarboxamide (IX). Compounds VII and VIII were identified with the respective authentic samples.[14]

The structure of IX was determined from the following reactions of IX. The catalytic reduction of IX with hydrogen in the presence of 10% palladium-on-charcoal gave 4-amino-5-imidazolecarboxamide. A cyclization of IX with ethyl orthoformate resulted in the formation of 8-chlorohypoxanthine (VIII).[14]

Purine *N*-oxides have been known, upon hydrolyis in an acid, to give hydroxylaminopyrimidine or -imidazole derivatives by the loss of the carbons adjacent to the *N*-oxide groups. For instance, 2,6-diamino-8-phenylpurine 7-*N*-oxide has been hydrolyzed to give 2,4,6-triamino-5-hydroxylaminopyrimidine,[15] and adenine 1-*N*-oxide has similarly been converted to 4-amino-5-imidazolecarboxamidoxime.[3]

Contrary to expectations, however, the hydrolysis

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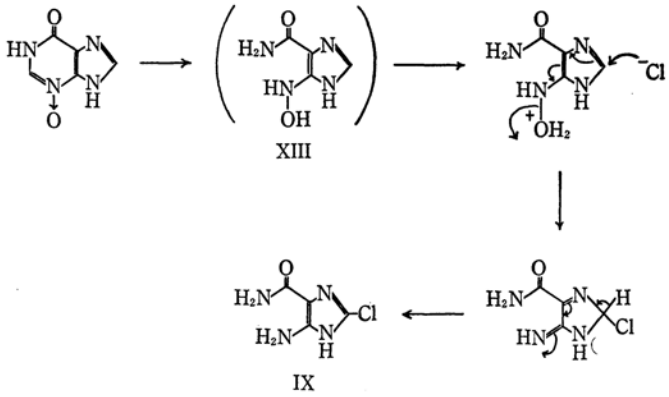
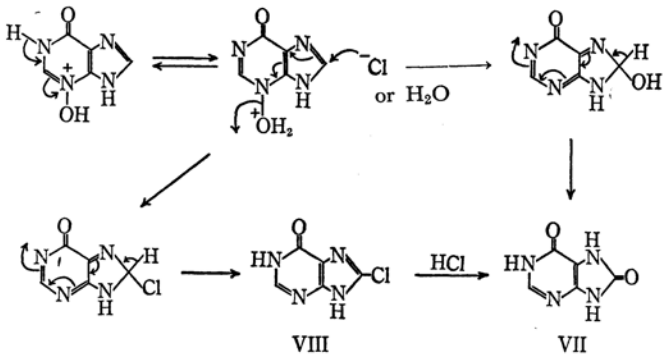


XIII

IX

Fig. 2



VIII VII

Fig. 3

of hypoxanthine 3-*N*-oxide (V) produced 2-chloro-4-amino-5-imidazolecarboxamide (IX), but not 4-hydroxylamino-5-imidazolecarboxamide (XIII). The opening of the pyrimidine ring of V, with an accompanying loss of 2-carbon, suggests the 3-*N*-oxide structure of V. However, more evidence is desirable.

The identification of V with an authentic hypoxanthine 3-*N*-oxide unambiguously synthesized from 6-mercaptopurine 3-*N*-oxide[9,10] was attempted.

6-Methylthiopurine is known to be oxidized with *N*-chlorosuccinimide to yield 6-methylsulfonylpurine.[16] This method was applied to the synthesis of 6-methylsulfonylpurine 3-*N*-oxide (XII) from 6-methylthiopurine 3-*N*-oxide (XI). XII was treated with a 1 N sodium hydroxide solution to give hypoxanthine 3-*N*-oxide, which was identified with V. Consequently, the 3-*N*-oxide structures of IIa, IIb, IV, and V were confirmed. XII was converted to 6-methoxypurine 3-*N*-oxide (IIa) upon being treated with a solution of sodium hydroxide in methanol.

The route to 2-chloro-4-amino-5-imidazolecarboxamide (IX) from hypoxanthine 3-*N*-oxide (V) upon hydrolysis was estimated to be as follows.

8-Chlorohypoxanthine (VIII), one of the hydrolysis products of hypoxanthine 3-*N*-oxide (V), is not certainly a direct precursor of IX, since VIII is converted to 6,8-dihydroxypurine (VII) upon being refluxed in 3 N hydrochloric acid. At first, prior to the halogenation, V may be hydrolyzed to the presumed ring-opening intermediate (XIII). Then the latter may be attacked by the chloride anion at 2-position to give IX, accompanied by N–O bond breaking (Fig. 2).

Further, the mechanism of the formation of 6,8-dihydroxypurine (VII) and 8-chlorohypoxanthine (VIII) from V upon hydrochloric acid treatment was suggested to be as follows (Fig. 3).

Experimental

6-Methoxypurine 3-*N*-Oxide (IIa). A 30% aqueous hydrogen peroxide solution (150 m*l*) was added to a solution of 6-methoxypurine hemihydrate (15 g) in glacial acetic acid (375 m*l*), and then left to stand at room temperature for 17 days. The volatile materials were removed from the reaction mixture at 40°C under reduced pressure. The yellow residue was washed with ethanol and then twice with hot ethanol to give a white powder of IIa (11.2 g, 72%). The paper chromatogram (solvents A and B) of the

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TABLE 1. PROPERTIES OF PURINE 3-*N*-OXIDES AND THE RELATED COMPOUNDS

Compound	Mp (°C)	R_f[a]		Spectral deta		
		A	B	pH	λ_{max} mμ	ε
6-Methoxypurine 3-*N*-oxide	215 d[b]	0.63	0.40	1	268	11400
				6	226	24800
					285	10200
				13	228	31600
					282	9300
6-Ethoxypurine 3-*N*-oxide	213 d	0.73	0.59	1	268	12600
				6	226	25000
					285	10800
				13	228	31800
					282	9800
6-Chloropurine 3-*N*-oxide	165 d[c]	0.70	0.45	1	230	20600
					304	7900
				6	231	29000
					308	6700
				13	232	27200
					308	7100
Hypoxanthine 3-*N*-oxide	210—300 d	0.35	0.17	1	261	6500
				6	280	10200
				13	226	25000
					286	10400
6-Methylsulfonylpurine 3-*N*-oxide	197—198 d	0.71	0.36	1	234	10900
					300	3100
					326	4600
				7	234	16100
					305	5100
					330	6500
				13	228	19100
					290	7100
					336	300
2-Chloro-4-aminoimidazole-5-carboxamide	above 150 d[d]	0.57	0.68	1	274	6300
				6	273	9700
				13	284	9700

a) Solvent system, A: *n*-PrOH - aq.NH_4OH - H_2O (20 : 12 : 3 vol/vol); B: *n*-BuOH - AcOH-H_2O (4 : 1 : 1 vol/vol) b) d: decompose c) Ammonium salt d) Hydrochloride

resultant white powder showed a single spot. A portion of this product was crystallized from water to give colorless crystals, mp 215°C (d).

Found: C, 43.30; H, 3.49; N, 33.57%. Calcd for $C_6H_6N_4O_2$: C, 43.37; H, 3.63; N, 33.73%.

Hydrogenation of 6-Methoxypurine 3-*N*-Oxide (IIa). IIa (1 g) was dissolved in a 2 N ammonium hydroxide solution (100 m*l*), and Raney nickel (2 m*l*) was added. The mixture was shaken at room temperature for 35 hr until 145 m*l* of hydrogen had been absorbed. The catalyst was then removed and the filtrate was evaporated to dryness. The residue (600 mg) was crystallized from water; and it was identified with authentic 6-methoxypurine by R_f in solvents A and B, and by a study of the ultraviolet and infrared spectra.

6-Ethoxypurine 3-*N*-Oxide (IIb). A 30% aqueous hydrogen peroxide solution (40 m*l*) was added to a solution of 6-ethoxypurine hemihydrate (4 g) in glacial acetic acid (100 m*l*), and then left to stand at room temperature for 11 days. The reaction mixture was treated in the same manner as the case of 6-methoxypurine 3-*N*-oxide (IIa) to yield IIb (1.5 g, 36%). The resulting powder was crystallized from water to give light-yellow crystals, mp 213°C (d).

Found: C, 46.74; H, 4.80; N, 31.19%. Calcd for $C_7H_8N_4O_2$: C, 46.66; H, 4.48; N, 31.10%.

6-Chloropurine 3-*N*-Oxide (IV). 6-Chloropurine (5 g) was suspended in 1500 m*l* of an ether solution containing 105 g of monoperphthalic acid, and then stirred at room temperature for 12 days. After the reaction, a solid material was collected. From this soild 6-chloropurine and phthalic acid were continuously extracted with ether for 50 hr. The resulting residual light-yellow powder (2.5 g, 45%) showed a single spot on a paper chromatogram (solvents A and B). Methanolic ammonia was then added to the powder, and an insoluble solid was removed by filtration. To the filtrate ether was added to precipitate ammonium salt of 6-chloropurine 3-*N*-oxide (IV). There precipitation

TABLE 2. THE RESULTS OF ION-EXCHANGE COLUMN CHROMATOGRAPHY OF THE HYDROLYSIS PRODUCTS OF HYPOXANTHINE 3-*N*-OXIDE (V) WITH 3 N HCl

Eluting Solvent	Effluent No.	Effluent ml	Compound	Yield g	Yield %	Molecular formura		Anal. % C	H	N	Cl
H_2O	1	1000	none								
	2	1000	6,8-Dihydroxypurine (VII)[a]	1.3	13						
	3	2300	none								
	4	2300	8-Chlorohypoxanthine (VIII)[a]	1.0	8.9	$C_5H_3N_4OCl$	Found	35.01	1.96	32.65	
							Calcd	35.21	1.77	32.85	
	5	1000	none								
1.2 N HCl	6	2450	none								
	7	1850	Hypoxanthine (VI)[a]	0.8	8.9						
	8	500	none								
	9	2400	2-Chloro-4-amino-5-imidazolecarboxamide hydrochloride (IX)[b]	1.0	7.7	$C_4H_5N_4OCl \cdot HCl$	Found	23.81	3.54	28.30	37.0
							Calcd	24.38	3.07	28.44	36.0
	10	1600	none								

a) The effluent was evaporated to dryness. The residue was recrystallized from water, and identified with the authentic sample by R_f (solvents A and B) and ultraviolet and infrared spectra.

b) The effluent was evaporated under reduced pressure at 40°C to dryness. The residue was precipitated from methanol by addition of ether.

was performed once more to yield a colorless powder, mp 165°C (d).

Found: C, 31.87; H, 3.58; N, 36.94; Cl, 18.50%. Calcd for $C_5H_3N_4OCl \cdot NH_3$: C, 32.01; H, 3.22; N, 37.33; Cl, 18.90%.

The Reaction of 6-Chloropurine 3-*N*-Oxide (IV) with Sodium Methoxide. 6-Chloropurine 3-*N*-oxide (1 g) was dissolved in a sodium methoxide solution prepared from 300 mg of sodium and 30 m*l* of methanol, and then heated to 100°C for 4 hr in a sealed tube. The reaction mixture was evaporated to dryness, and water (20 m*l*) was added to the residue. The solution was filtered and acidified to pH 4 to precipitate light-yellow crystals. These crystals showed a single spot on a paper chromatogram (solvent A), and the R_f, ultraviolet, and infrared spectra agreed with those of 6-methoxypurine 3-*N*-oxide (IIa).

Hypoxanthine 3-*N*-Oxide (V) from 6-Methoxypurine 3-*N*-Oxide (IIa). 6-Methoxypurine 3-*N*-oxide (8 g) was dissolved in a 1 N sodium hydroxide solution (400 m*l*), and the mixture was refluxed for 1.5 hr. After the reaction mixture had cooled, concentrated hydrochloric acid was added to adjust the pH to 9, and the mixture was decolorized with charcoal. The solution was acidified to pH 1 to precipitate a light yellow powder of V (6.1 g, 83%). This product was reprecipitated twice with a sodium hydroxide solution-hydrochloric acid, and washed well with water, to yield a fine, colorless powder. The product darkened slowly from 210°C to 300°C.

Found: C, 39.71; H, 2.75; N, 36.68%. Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.84%.

Hypoxanthine 3-*N*-Oxide (V) from 6-Chloropurine 3-*N*-Oxide (IV). 6-Chloropurine 3-*N*-oxide (0.1 g) was dissolved in a 1 N sodium hydroxide solution (5 m*l*), and the mixture heated under refluxing for 1 hr. The reaction mixture showed a single spot on a paper chromatogram (solvents A and B), and its R_f and ultraviolet spectra agreed with those of hypoxanthine 3-*N*-oxide (V).

Hydrogenation of Hypoxanthine 3-*N*-Oxide (V). Hypoxanthine 3-*N*-oxide (1 g) was dissolved in a 2 N ammonium hydroxide solution (100 m*l*). Raney nickel (5 m*l*) was then added, and the mixture was shaken at room temperature with hydrogen at an atmospheric pressure for 22 hr, by which time 160 m*l* of hydrogen had been absorbed. The catalyst was then removed, and the filtrate was evaporated to dryness. The residue (500 mg) was crystallized from water and identified with hypoxanthine by R_f (solvents A and B), and by a study of the ultraviolet and infrared spectra.

Hydrolysis of Hypoxanthine 3-*N*-Oxide (V) with Hydrochloric Acid. Hypoxanthine 3-*N*-oxide (10 g) was divided into five aliquots. Each of them was dissolved in 3 N hydrochloric acid (100 m*l*), refluxed for five minutes, and cooled immediately. After being cooled, the five reaction mixtures were combined and passed through a column packed with 1200 m*l* of Dowex 50WX4 (H^+ type), and then eluted with water and 1.2 N hydrochloric acid. The eluting procedure and the results are presented on Table 2.

Reduction of 2-Chloro-4-amino-5-imidazolecarboxamide (IX). 2-Chloro-4-amino-5-imidazolecarboxamide hydrochloride (50 mg) was dissolved in water (20 m*l*). To this solution 10% palladium on charcoal (100 mg) was added, and the mixture was shaken for 3 hr at room temperature with hydrogen at atmospheric pressure. The catalyst was then removed by filtration. The filtrate showed a single spot on a paper chromatogram, and its R_f (solvents A and B) and ultraviolet spectrum were identical with those of 4-amino-5-imidazolecarboxamide (X).

Cyclization of 2-Chloro-4-amino-5-imidazolecarboxamide (IX). 2-Chloro-4-amino-5-imidazolecarboxamide hydrochloride (350 mg) was dissolved in a mixture of ethyl orthoformate (10 m*l*) and acetic anhydride (1 m*l*), and the resultant mixture was refluxed for 30 min. After being cooled, the reaction mixture

was evaporated to dryness under reduced pressure. The residue was dissolved in water (50 m*l*) passed, through a column packed with 100 m*l* of Dowex 50W X4 (H^+ type), and eluted with water. The first 600-m*l* portion of the effluent was discarded; the next 350-m*l* portion of the effluent was evaporated to dryness under reduced pressure to give 8-chlorohypoxanthine (0.1 g). Its R_f (solvents A and B) and ultraviolet and infrared spectra agreed with those of the authentic sample.

6-Methylsulfonylpurine 3-*N*-Oxide (XII). 6-Methylmercaptopurine 3-*N*-oxide (300 mg) was suspended in 50% methanol (30 m*l*). *N*-Chlorosuccinimide (870 mg) was then added, and the mixture was stirred for 6 hr at room temperature. After the reaction mixture had been left to stand overnight, the precipitated product was filtered and washed with methanol. The crystals obtained (0.2 g, 57%) were recrystallized from water. Mp 197—198°C (d).

Found: C, 33.55; H, 2.76; N, 26.25%. Calcd for $C_6H_6N_4O_3S$: C, 33.64; H, 2.82; N, 26.16%.

Hypoxanthine 3-*N*-Oxide from Methylsulfonylpurine 3-*N*-Oxide (XII). 6-Methylsulfonylpurine 3-*N*-oxide (500 mg) was dissolved in a 1 N sodium hydroxide solution (25 m*l*), and the mixture was refluxed for 2 hr. After being cooled, the reaction mixture was decolorized by adding charcoal and acidified to pH 3 with concentrated hydrochloric acid; this precipitated a light-yellow powder of hypoxanthine 3-*N*-oxide (230 mg, 65%). This product was reprecipitated with a sodium hydroxide solution - hydrochloric acid, and washed well with water, to yield a fine colorless powder. The product darkened slowly above 230°C.

Found: C, 39.62; H, 2.81; N, 36.77%. Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.84%.

6-Methoxypurine 3-*N*-Oxide from 6-Methylsulfonylpurine 3-*N*-Oxide. 6-Methylsulfonylpurine 3-*N*-oxide (0.5 g) was dissolved in a methanol solution (20 m*l*) which contained sodium hydroxide (0.8 g), and the mixture was refluxed for 1 hr. After being cooled, the reaction mixture was neutralized by adding glacial acetic acid and evaporated to dryness. The residue was dissolved in water adsorbed on a column packed with 100 m*l* of Amberlite IR-120 (H^+ type), washed well with water, and eluted with a 1 N ammonium hydroxide solution. The effluent was evaporated to dryness to give 6-methoxypurine 3-*N*-oxide (IIa, 250 mg, 64%), which was then recrystallized from water. This product darkened from 200 to 215°C.

Found: C, 41.93; H, 4.06; N, 33.06%. Calcd for $C_6H_6N_4O_2 \cdot \frac{1}{4}H_2O$: C, 42.23; H, 3.84; N, 32.83%.

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