Purine N-Oxides. XIV. A Total Synthesis of a Pyrimidine N-Oxide, a Pteridine 1-N-Oxide, and Xanthine 3-N-Oxide[1]

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Cyclization of β-carboxyureidoacetamidoxime resulted in a pyrimidine 1-N-oxide, which could be converted to a pteridine 1-N-oxide and to 8-azaxanthine and xanthine 3-N-oxides. The pteridine and purine N-oxides proved difficult to reduce.

Since the first reports[2,3] of the production of purine N-oxides by direct oxidation of the parent bases, several authors[4-7] have given details of other routes to such compounds. In all those syntheses the N-oxide function was introduced on the 1- or 3-nitrogen of the purine ring by ring closure of a hydroxylamine derivative. A synthesis of purine 7-N-oxides in which the N-oxide was again introduced in the final ring closure of the imidazole ring, has also been mentioned.[8] It was attractive to attempt a synthesis of a pyrimidine N-oxide, and thence a purine N-oxide, by a Traube-type[9] ring closure of an aliphatic hydroxylamine derivative.

Cyanoacetylurea (I) reacts readily with hydroxylamine in aqueous solution and gives a good yield of β-carboxyureidoacetamidoxime (II). This amidoxime on brief treatment with acetic anhydride gives a monoacetate and not an oxadiazole.[10,11] Since the acetate no longer gives a color with ferric chloride, the oxime probably bears the acetate group.

When β-carboxyureidoacetamidoxime (II) is heated to reflux in dimethyl formamide, a rapid conversion, with the evolution of ammonia, to 6-amino-2,4-dihydroxypyrimidine 1-N-oxide (III) takes place. The yield in this ring closure is low, of the order of 10 to 12%, although about one-third of the starting material is recovered. The selection of reaction conditions is complicated by the fact that the pyrimidine N-oxide decomposes in hot dimethylformamide. The N-oxide structure, rather than the alternative 2,4-dihydroxy-6-hydroxylaminopyrimidine, is supported by the fact that III was unchanged after treatment with alkaline sodium dithionite, a procedure which reduces hydroxylamino substituents in purines[12] and pyrimidines.[13] This ring closure is another case of selective closure[5-7] of an amidoxime to an N-oxide. An alternative synthesis of this pyrimidine N-oxide is recently reported by W. Klotzer.[14]

6-Amino-2,4-dihydroxypyrimidine 1-N-oxide is readily nitrosated by nitrous acid in either hydrochloric or acetic acid solution to give a red 5-nitroso derivative (IV). This nitroso group was reduced to an amino group with sodium dithionite in dilute alkali, and the product, 5,6-diamino-2,4-dihydroxypyrimidine 1-N-oxide (V), was used for subsequent syntheses without further purification. Its characterization as an N-oxide depends upon its positive color reaction in the ferric chloride test and its nonidentity with the known 4,5-diaminouracil.[15]

5,6-Diamino-2,4-dihydroxypyrimidine 1-N-oxide reacts with nitrous acid at room temperature and yields 8-aza-2,6-dihydroxypurine 3-N-oxide (VI, 8-azaxanthine 3-N-oxide). Catalytic reduction of the N-oxide function in VI proceeded with difficulty but gave 8-aza-2,6-dihydroxypurine (VII) identical with an authentic specimen.[16] When 8-azaxanthine 3-N-oxide (VI) is refluxed with phosphorus pentasulfide in pyridine solution, the product no longer has an N-oxide grouping and is identical with the product of the same reaction on 8-azaxanthine. On the basis of its analysis and ultraviolet absorption spectrum in alkali, this product was assigned the structure 8-aza-2-hydroxy-6-mercaptopurine (XII). The reduction proceeds more readily than the thiation, since in short runs some VII was produced. We also demonstrated that adenine can be obtained from the reaction of P_2S_5 or PCl_3 with adenine 1-N-oxide.

When V was treated with biacetyl, a good yield of 2,4-dihydroxy-6,7-dimethylpteridine 1-N-oxide (VIII) was obtained. The N-oxide function of VIII was quite resistant to hydrogenation. Before all traces of a compound giving a color with ferric chloride were removed, 3 moles of hydrogen had been absorbed. The reduction was stopped and the tetrahydropteridine was oxidized by atmospheric oxygen until a solid precipitated. This product was identical with authentic 2,4-dihydroxy-6,7-dimethylpteridine (IX).[17]

Attempts to react V with carbon disulfide failed because of its insolubility in the reagents in two[18,19] of the modifications of this ring closure, and its already men-

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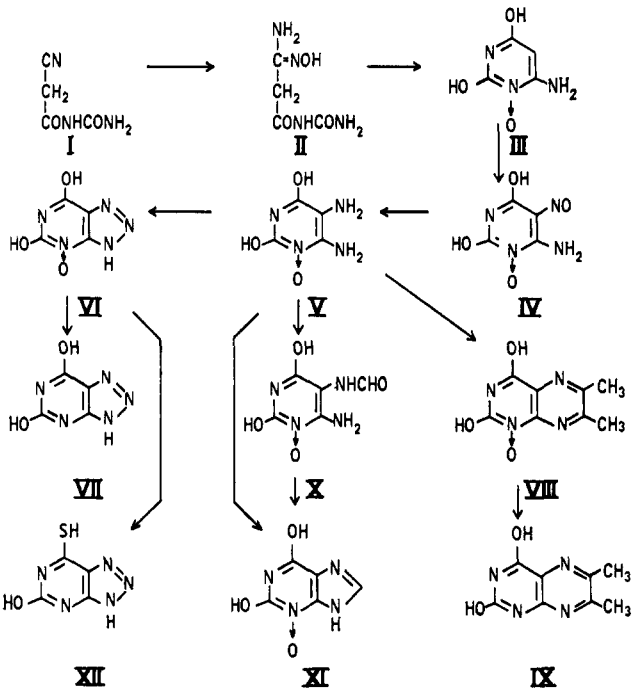
TABLE I

Compd.	R_f values: Solvent A	Solvent B	λ_{max} ($A_m \times 10^{-3}$)	pH
6-Amino-2,4-dihydroxypyrimidine 1-N-oxide (III)	0.29	0.58	266 (18.0)	1.1
			276 (8.9)	12.3
6-Amino-2,4-dihydroxy-5-nitrosopyrimidine 1-N-oxide (IV)	0.21	0.57	318 (9.2)	1.2
			314 (15.5)	12.5
6-Amino-2,4-dihydroxy-5-formylaminopyrimidine 1-N-oxide (X)	0.13	0.68	266 (16.9)	1.0
			276 (8.5)	12.5
8-Aza-2,6-dihydroxypurine 3-N-oxide (VI)	0.37	0.51	269 (5.2)	1.2
			302 (5.5)	12.5
2,4-Dihydroxy-6,7-dimethylpteridine 1-N-oxide (VIII)	0.39	0.63	337 (9.8)	1.2
			272 (24.6), 394 (8.6)	12.5
8-Aza-2-hydroxy-6-mercaptopurine (XII)	0.0	0.0	272 (9.9)	1.0
			246 (11.1), 313 (9.7)	12.5
Xanthine 3-N-oxide (XI)	0.30	0.42	230 (16.2), 284 (10.3)	1.2
			216 (22.0), 295 (12.0)	12.5

tioned self-condensation in dimethylformamide in a third.[20]

Refluxing V in 98% formic acid, readily gave 6-amino-5-formylamino-2,4-dihydroxypyrimidine 1-N-oxide (X). The formyl derivative X could not be closed to 2,6-dihydroxypurine 3-N-oxide (XI, xanthine 3-N-oxide) by heating it in formamide,[21] because at 100° a crystalline ammonium salt of X precipitates and at 170° or higher reduction of the N-oxide function takes place (at high temperature formamide also removes the N-oxide from adenine oxide).



The ring closure was accomplished by dissolving either the 6-amino-5-formamido-2,4-dihydroxypyrimidine 1-N-oxide (X) or the 5,6-diamine V in hot formic acid and adding acetic anhydride. The xanthine 3-N-oxide (XI) obtained was slowly reduced to xanthine in the presence of very active Raney nickel but unreduced N-oxide was still present.

In addition to those mentioned here, we have other examples of N-oxides which are difficult to reduce. Several methods of reduction have been tested, and, among them, the addition of sodium borohydride to a solution of the compound in the presence of Pd–C is quite satisfactory for many purine N-oxides, but is not effective with xanthine 3-N-oxide.

Observations by Dr. George S. Tarnowski, to whom we are indebted, indicate no toxicity of xanthine 3-N-oxide in Swiss mice at 500 mg./kg./day for 1 week, and no influence on the growth of sarcoma 180.[22]

Experimental

Melting points are corrected. Yields of substances that have no definite melting point refer to the stage when they appeared homogeneous on paper chromatograms. Chromatograms were developed by the ascending technique, and the solvents were (A) *n*-butyl alcohol–acetic acid–water (4:1:1) and (B) 3% aqueous ammonium chloride with Whatman No. 1 paper. They were viewed in ultraviolet light principally of wave length 253.8 mμ. R_f values and ultraviolet absorption spectra of the products are given in Table I.

β-Carboxyureidoacetamidoxime (II).—Cyanoacetylurea (20 g.) was dissolved in hot water (800 ml.) and was treated with a solution of hydroxylamine hydrochloride (11 g.) in water containing sodium hydroxide (6.3 g.). The reaction mixture, which developed a reddish tinge almost immediately, was stirred for 5 min. and then cooled in an ice bath. An off-white solid that rapidly separated was collected, washed with ethanol, and recrystallized from dimethylformamide to white needles (20.5 g., 82%), m.p. 196–198° dec. It turned brown with ferric chloride.

Anal. Calcd. for $C_4H_8N_4O_3$: C, 30.0; H, 5.0; N, 35.0. Found: C, 30.2; H, 5.1; N, 34.8.

β-Carboxyureidoacetamidoxime (0.5 g.) was suspended in acetic anhydride (3 ml.) and heated at 90° for 3 min. A product separated as the solution cooled. Recrystallization from water gave white needles, the analysis of which was closer to the acetate than to an oxadiazole of β-carboxylureidoacetamidoxime (0.18 g.), m.p. 205°, and which gave no color with ferric chloride.

Anal. Calcd. for $C_6H_{10}N_4O_4$: C, 35.6; H, 5.0; N, 27.7. Found: C, 36.1; H, 5.3; N, 28.4.

6-Amino-2,4-dihydroxypyrimidine 1-N-Oxide (III).—A solution of β-carboxyureidoacetamidoxime (40 g.) was refluxed in dimethylformamide (100 ml.) in a 500-ml. flask for exactly 5 min. (oil bath, 185–190°). The reaction mixture was then allowed to cool undisturbed for 10 min. and filtered rapidly through a large Büchner funnel. The precipitate which formed immediately in the filtrate was a mixture of starting material and product. This mixture was collected and refluxed for another 5 min. in dimethylformamide. The solution was allowed to cool undisturbed for 10 min. and then was filtered very quickly, *i.e.*, before crystallization of the unchanged starting material began. The solid product (6 g.) was washed with ethanol and ether and recrystallized from water (charcoal) to give fine white needles m.p. >300° (4.1 g., 11.5% yield). From the dimethylformamide filtrates, about 30% of the starting material could be recovered

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by cooling the solution. The pyrimidine 1-N-oxide gives a purple color with ferric chloride.

Anal. Calcd. for $C_4H_5N_3O_3 \cdot 0.5H_2O$; C, 31.6; H, 4.0; N, 27.6. Found: C, 31.4; H, 4.4; N, 27.5.

6-Amino-2,4-dihydroxy-5-nitrosopyrimidine 1-N-Oxide (IV).—6-Amino-2,4-dihydroxypyrimidine 1-N-oxide (0.72 g.) was dissolved in hot water (25 ml.) and as the solution was cooled to 0°, fine crystals precipitated. Sodium nitrite (0.35 g.) and 1 *N* HCl (5 ml.) were added dropwise to the stirred suspension. The reaction mixture quickly became red and after 30 min. at room temperature a deep red product (0.77 g.) had precipitated. Recrystallization from water, in which it is quite soluble, gave small, bright red crystals, m.p. 224° (yield 89%).

Anal. Calcd. for $C_4H_4N_4O_4$: C, 27.9; H, 2.3; N, 32.6. Found: C, 27.5; H, 2.1; N, 32.4.

5,6-Diamino-2,4-dihydroxypyrimidine 1-N-Oxide (V).—6-Amino-2,4-dihydroxy-5-nitrosopyrimidine 1-N-oxide (0.7 g.) was dissolved in 1 *N* sodium hydroxide (25 ml.) and the red solution was treated slowly, with stirring, with just enough sodium dithionite to render the solution colorless. Immediately a pale yellow precipitate formed. By acidifying the filtrate with a few drops of glacial acetic acid and cooling, additional material could be obtained. The product (0.53 g., 82.5%, m.p. >300°) was washed with a little cold water. Recrystallization was not possible because of the tendency of this compound to self-condense in solution.

8-Aza-2,6-dihydroxypurine 3-N-Oxide (VI).—A suspension of 5,6-diamino-2,4-dihydroxypyrimidine 1-N-oxide (1.65 g.) in 2 *N* acetic acid (25 ml.) was cooled to 0°, and a solution of sodium nitrite (1.8 g.) in cold water (40 ml.) was slowly added. The mixture was stirred at 0° for 10 min. and then left at room temperature for another 30 min. The crystalline precipitate (1.4 g.; 78%) was collected and recrystallized, with charcoal, to give light yellow needles, m.p. >300°, that show a purple color with ferric chloride.

Anal. Calcd. for $C_4H_3N_5O_3 \cdot H_2O$: C, 25.7; H, 2.7; N, 37.4. Found: C, 25.3; H, 2.5; N, 36.7.

Another analysis was performed after drying under vacuum at 130°.

Anal. Calcd. for $C_4H_3N_5O_3$: C, 28.4; H, 1.8; N, 41.4. Found: C, 27.7; H, 1.7; N, 41.3.

8-Aza-2,6-dihydroxypurine (VII).—A solution of 8-aza-2,6-dihydroxypurine 3-N-oxide (0.5 g.) in water (75 ml.) was reduced with hydrogen at atmospheric pressure over a prereduced platinum oxide catalyst (0.25 g.). Uptake of hydrogen proceeded very slowly and, after 3 days, the supernatant, when sampled, showed two ultraviolet light absorbing compounds on paper chromatography that corresponded to 8-aza-2,6-dihydroxypurine and starting material. Hydrogenation was resumed for 2 more days after which the catalyst was removed by filtration, and the volume of the filtrate was reduced under vacuum until solids precipitated. The solids (0.27 g.), which gave no color with ferric chloride, were collected and recrystallized from water to yield fine white needles; they proved to be identical with 8-aza-2,6-dihydroxypurine on paper chromatograms and in ultraviolet and infrared absorption spectra. The mother liquor of the hydrogenation when examined by means of paper chromatography was found to contain more 8-aza-2,6-dihydroxypurine and a small quantity of starting material.

2,4-Dihydroxy-6,7-dimethylpteridine 1-N-Oxide (VIII).—5,6-Diamino-2,4-dihydroxypyrimidine 1-N-oxide (0.3 g.) was suspended in water (5 ml.) and an excess of biacetyl in ethanol (3 ml.) was added. The mixture was heated at 90° until all the starting material had gone into solution. The solution was cooled at room temperature until crystallization was complete. The product (0.3 g., 76%) was collected and recrystallized from water with charcoal treatment to give off-white needles, m.p. >300°, that give a purple with ferric chloride.

Anal. Calcd. for $C_8H_8N_4O_3$: C, 46.2; H, 3.9. Found: C, 46.4; H, 4.1.

2,4-Dihydroxy-6,7-dimethylpteridine (IX).—2,4-Dihydroxy-6,7-dimethylpteridine 1-N-oxide (0.5 g.) was dissolved, with heating, in water (500 ml.) and reduced with platinum oxide (0.5 g.) and hydrogen. Hydrogenation was continued until uptake became negligible, and the supernatant no longer gave a color with ferric chloride (about 4–5 days). The ultraviolet absorption spectrum of the solution was typical of that of a tetrahydropteridine (λ_{max} 267 mμ) and changed steadily on exposure to air. After removal of the catalyst, air was bubbled through the filtrate for 5 days, by which time the volume was greatly reduced and a solid had precipitated. The solid (0.15 g.) was collected and shown to be identical with an authentic specimen[17] of 2,4-dihydroxy-6,7-dimethylpteridine on paper chromatography and in its ultraviolet absorption spectrum.

6-Amino-5-formamido-2,4-dihydroxypyrimidine 1-N-Oxide (X).—5,6-Diamino-2,4-dihydroxypyrimidine 1-N-oxide (0.85 g.) was refluxed in 98% formic acid (5 ml.) until a crystalline deposit formed. The crystals were collected and recrystallized from water, with charcoal treatment to give white needles (0.75 g., 75%), m.p. 295–300° dec., that give a purple with ferric chloride.

Anal. Calcd. for $C_5H_6N_4O_4$: C, 32.3; H, 3.3; N, 30.1. Found: C, 32.2; H, 3.5; N, 29.9.

The foregoing 5-formamidopyrimidine (0.5 g.) was suspended in formamide (3 ml.) and slowly heated in an oil bath. At 75–80° all starting material had dissolved, and at 95–100° crystallization of a solid began. The temperature was held at 100° for 5 min., and the reaction mixture was then cooled. The off-white crystalline precipitate (0.43 g.) when collected and washed with ethanol, m.p. 300°, proved to be identical with the starting 5-formamidopyrimidine in its ultraviolet absorption spectrum at several pH values and analyzed for an ammonium salt of the same.

Anal. Calcd. for $C_5H_9N_5O_4$: C, 29.6; H, 4.5; N, 34.5. Found: C, 30.2; H, 4.7; N, 34.5.

Xanthine 3-N-Oxide (XI). A.—5,6-Diamino-2,4-dihydroxypyrimidine 1-N-oxide (500 mg.) was dissolved in 5 ml. of hot formic acid, and acetic anhydride (5 ml.) was then added. The mixture was refluxed for 0.5 hr. and a solid was collected. Additional solid separated when ethanol was added. The combined solids were partly purified by treatment with 25 ml. of hot water to remove the soluble impurities, and the residue was recrystallized from 500 ml. of water (charcoal) to yield 200 mg., 44%, m.p. >300°.

Anal. Calcd. for $C_5H_4N_4O_3$: C, 35.7; H, 2.4; N, 33.3. Found: C, 35.8; H, 2.6; N, 33.4.

B.—The procedure, as in A, was repeated on 6-amino-5-formamido-2,4-dihydroxypyrimidine 1-N-oxide (0.37 g.) with 98% formic acid (20 ml.) and acetic anhydride (20 ml.). The product (0.21 g., 63% yield) was identical with xanthine 3-N-oxide from procedure A.

Anal. Calcd. for $C_5H_4N_4O_3$: C, 35.7; H, 2.4; N, 33.3. Found: C, 35.5; H, 2.2; N, 33.5.

Hydrogenation of Xanthine 3-N-Oxide.—Xanthine 3-N-oxide (*ca.* 3 mg.) was suspended in 20 ml. of water and very active Raney nickel (*ca.* 20 mg.) was added. The mixture was shaken with hydrogen at room temperature and atmospheric pressure for 14 hr. Chromatographic analysis showed the presence of starting material and xanthine. Confirmation that reduction occurred was afforded by elution of the spots and determination of the ultraviolet spectrum, which was also identical with xanthine.

Reduction of Adenine 1-N-Oxide with P_2S_5 or with PCl_3.—Adenine 1-N-oxide (1 g.) was suspended in pyridine (25 ml.) and treated with P_2S_5 (1.5 g.). The mixture was refluxed for 4 hr., cooled, poured into water (100 ml.), and heated at 60 to 80° until evolution of H_2S ceased. A small quantity of sulfur was collected, and the filtrate was taken to dryness by evaporation under vacuum. The solid residue on recrystallization from water gave a white solid (0.83 g.), m.p. >300°, which was identical both on paper chromatography and spectrally with adenine.

Suspension of adenine 1-N-oxide in PCl_3 at room temperature overnight also resulted in extensive reduction to adenine, but insolubilities of many purine N-oxides prevent significant reduction under these conditions.

8-Aza-2-hydroxy-6-mercaptopurine (XII). A.—The P_2S_5 treatment of 8-aza-2,6-dihydroxypurine 3-N-oxide (0.25 g.) with pyridine (10 ml.), at a reflux time of 2.5 hr., and concentration of the filtrate to 10 ml. yielded a precipitate that was reprecipitated from sodium hydroxide by hydrochloric acid to yield 0.11 g., m.p. >300°.

Anal. Calcd. for $C_4H_3N_5OS$: C, 28.4; H, 1.8; N, 41.4; S, 19.0. Found: C, 28.3; H, 2.0; N, 41.3; S, 18.9.

B.—The procedure was repeated with the ammonium salt of 8-aza-2,6-dihydroxypurine (3 g.), pyridine (100-ml.), and P_2S_5 (4.8 g.). The product (1.05 g.) was identical with 8-aza-2-hydroxy-6-mercaptopurine from procedure A.

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