250 ml. of *N* sodium hydroxide and the solution refluxed with Raney nickel (40 g.) for 3 hr. The solution was filtered and an additional 40 g. of wet Raney nickel added and the solution refluxed for an additional 3 hr. The solution was cooled and filtered and the filtrate acidified with acetic acid to yield 5.1 g. of 2-amino-6-hydroxy-9-isobutylpurine (XI, R = *i*-C_4H_9). The product was dissolved in dilute hydrochloric acid and the hot solution neutralized with ammonium hydroxide. The white product was filtered and washed with water and a small amount recrystallized from N,N-dimethylformamide for analysis.

Anal. Calcd. for $C_9H_{13}N_5O$: C, 52.5; H, 6.3. Found: C, 52.2; H, 6.7.

Preparation of 2-Amino-9-ethyl-6-hydroxypurine (XI, R = C_2H_5).—Twenty-five grams of 2,5-diamino-4,6-dihydroxypyrimidine (IX) was treated with 17 g. of ethyl isothiocyanate. The resulting product was isolated and cyclized with hydrochloric acid to give 14 g. of 2-amino-6-hydroxy-9-ethyl-8-purinethiol (XI, R = C_2H_5). The procedure followed in this preparation and in converting XI, R = C_2H_5, to 2-amino-9-ethyl-6-hydroxypurine with Raney nickel was essentially the same as for the preparation of 2-amino-9-methyl-6-hydroxypurine (XII, R = CH_3). The yield of 2-amino-9-ethyl-6-hydroxypurine (XI, R = C_2H_5) thus obtained was 6.0 g.

Anal. Calcd. for $C_7H_9N_5O$: C, 46.9; H, 5.0. Found: C, 46.7; H, 5.4.

Preparation of 2-Amino-9-methyl-6-purinethiol (XIII, R = CH_3).—Eight grams of 2-amino-6-hydroxy-9-methylpurine (XI) and 32 g. of phosphorus pentasulfide were ground together in a mortar and then transferred to a flask containing 500 ml. of dry pyridine. The mixture was refluxed for 8 hr. The excess pyridine was distilled under reduced pressure and 500 ml. of ice-water carefully added to the residue in the flask. The solution was then heated 3 hr. on the steam-bath and finally chilled overnight. The yield of crude product was 5.0 g. For purification the compound was reprecipitated twice from hot, dilute sodium hydroxide with acetic acid and finally recrystallized from dilute acetic acid to yield light-yellow crystals, m.p. >300°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.8; H, 3.9. Found: C, 39.9; H, 3.9.

Preparation of 9-*p*-Chlorophenyluric Acid (XII).—Seventy-one grams of uramil was dissolved in 1.5 l. of *N* sodium hydroxide and the solution heated to 60–70°; 75 g. of *p*-chlorophenyl isocyanate was added dropwise to the solution with constant stirring. The addition of the isocyanate required approximately 1.5 hr. After all the isocyanate had been added, the solution was stirred at the same temperature for an additional 2 hr. The solution was cooled and acidified with glacial acetic acid, and the pale-yellow product was filtered and washed with a little cold water. The crude product was then added to 1 liter of concentrated hydrochloric acid and the solution refluxed for a period of 6 hr. The mixture was then diluted with water to 1500 ml., and the white crystals of 9-*p*-chlorophenyluric acid were filtered immediately and washed with a little water. The yield of product was 70 g. For analysis the product was recrystallized from acetic acid to give white needles, m.p. >300°. The crystals were dried at 180° for 24 hr. for analysis.

Anal. Calcd. for $C_{11}H_7N_4O_3Cl$: C, 47.4; H, 2.51; N, 20.1. Found: C, 47.2; H, 3.0; N, 19.9.

Similarly, 54 g. of uramil and 50 g. of *o*-chlorophenyl isocyanate gave 49.0 g. of 9-*o*-chlorophenyluric acid. For analysis the product was recrystallized from a dilute acetic acid solution to give white needles, m.p. >300°.

Anal. Calcd. for $C_{11}H_7N_4O_3Cl \cdot H_2O$: C, 44.5; H, 3.04; N, 18.9. Found: C, 44.8; H, 3.34; N, 18.7.

TEMPE, ARIZONA

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Purine N-Oxides. I. Mono-oxides of Aminopurines[1]

BY MARCUS A. STEVENS,[2] DAVID I. MAGRATH,[3] HERMAN W. SMITH AND GEORGE BOSWORTH BROWN

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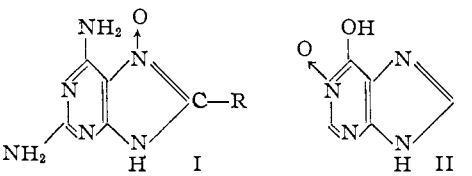
Mono-N-oxides have been isolated from the mixtures resulting from the oxidation of adenine (6-aminopurine), adenosine, 2',3'-isopropylideneadenosine or 2,6-diaminopurine with hydrogen peroxide–acetic acid.

Direct oxidation of purines to N-oxides has not previously been reported, although numerous examples of the oxidation of pyridines[4] and a few examples of the oxidation of pyrimidines[5–7] to N-oxides are known. The interesting influences of the N-oxide grouping on the chemical behavior of the total molecule, the possibility that such derivatives might be significant in the metabolic roles of purines in those co-enzymes which function in oxidation–reduction systems, and their possible significance in the enzymatic hydroxylation of purines *in vivo*, make purine N-oxides of interest. The possibility that N-oxides of natural purine derivatives might serve as antimetabolites is also to be considered.

Recently Timmis[8] reported that a purine 7-N-oxide (I) could be made by treating an aromatic aldehyde anil with 2,4,6-triamino-5-nitrosopyrimi-



dine, and Taylor[9] reported the synthesis of hypoxanthine 1-N-oxide (II) by orthoformate ring closure of 4-aminoimidazole-5-hydroxamic acid or of aminomalonamidine-hydroxamic acid. At the same time[10] an initial report was made that an oxide of adenine could be obtained by direct oxidation with hydrogen peroxide in glacial acetic acid.

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(2) Fellow of Sloan-Kettering Institute.

(3) Fellow of Sloan-Kettering Institute.

(4) J. Meisenheimer and E. Stotz, *Ber.*, **58**, 2334 (1925).

(5) E. Ochiai, H. Ishikawa and S. Zai-Ren, *J. Pharm. Soc. Japan*, **67**, 34 (1957).

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(7) R. H. Wiley and S. C. Slaymaker, THIS JOURNAL, **79**, 2233 (1957).

(8) G. M. Timmis, I. Cooke and R. G. W. Spickett, in "Ciba Foundation Symposium on the Chemistry and Biology of Purines," Little, Brown and Co., Boston, Mass. 1957, p. 139.

(9) E. C. Taylor, T. S. Osdene, E. Richter and O. Vogl, *ibid.*, p. 23,

(10) G. B. Brown, *ibid.*, p. 143.

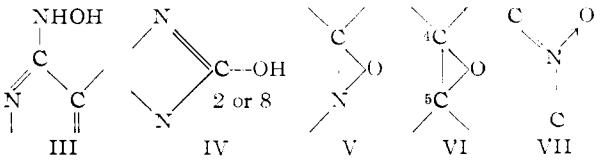
TABLE I
COMPARISONS OF PROPERTIES OF PURINE N-OXIDES WITH RELATED COMPOUNDS

Purine	M.p. or d.p., °C.	Sol. in H_2O 1 part in	t, °C.	R_f A[a]	R_f B[a]	pK's	Maxima pH	Maxima mμ	$A_M \times 10^{-3}$
6-Hydroxylaminopurine	254 d.	1,660	20	0.47	0.41	1.23 and 6.73	1.23 6.73	271 268	13.3 11.8[b]
2-Hydroxyadenine	Dec.	16,000	20	.28	.41	...	6.5	240, 286	8.0 9.8[c]
8-Hydroxyadenine	Dec.	....		.61	.35	...	6.5	270	12.8[d]
Adenine	360–365 d.	1,086	20	.61	.38	4.15 and 9.80	1.0 6.47	262 261	13.1 12.6
Adenine N-oxide	297–307 d.	1,250	25	.48	.48	2.6 9.0 and *ca.* 13	1.0 7.0 13.0	258.5 231, 262.5 233, 275	11.5 41.5 8.1 46.2 7.4
Adenosine	229	....		.61	.50	...	2.0 6.4	257 260	14.6 14.9
Adenosine N-oxide	155 d.	144	25	.44	.71	2.14 and 12.5	2.0 5.3 13.0 	258 232.5, 260 230 272.5 312	11.9 40.8 9.2 23.7 8.4 4.5
2′,3′-Isopropylideneadenosine	200–204	....		.87	.43	...	...		
2′,3′-Isopropylideneadenosine N-oxide	176–178 d.	....		.79	.69	...	11.0 5.0	237, 268 232.5, 261	... ...
2,6-Diaminopurine		420	20	.19	.23	...	6.49	247, 280	7.0 8.3
2,6-Diaminopurine N-oxide	Dec. >280	....		.38	.37	1.0 3.7 9.7 and 12.0	1.0 5.5 13.0	248, 290 230, 290 228, 295	8.6 8.7 31.3 7.0 26.8 7.9
2,6-Diamino-8-hydroxypurine	...	....		..	.16	...	6.5	246, 287	8.1 9.6[d]

[a] For solvents see Experimental. [b] A. Bendich and A. Giner-Sorolla, THIS JOURNAL, in press. [c] E. Shaw, *J. Biol. Chem.*, 185, 439 (1950). [d] L. F. Cavalieri and A. Bendich, THIS JOURNAL, 72, 2592 (1950).

In preliminary experiments carried out to study the action of hydrogen peroxide–acetic acid on various purines, notably adenine, 2,6-diaminopurine, guanine, uric acid, hypoxanthine and various purine derivatives such as adenosine, the formation of oxidation products was followed by chromatographic methods. All the materials mentioned yielded ultraviolet-absorbing products. In the case of hypoxanthine, guanine and uric acid, oxidation at room temperature over a three-month period produced only small yields of oxidation products. In the case of purine, oxidation products were formed rapidly (two days), but decomposed when an attempt was made to isolate them. As a rule, therefore, most purines are difficult to oxidize using hydrogen peroxide–acetic acid mixtures, and attempts made to speed oxidation by raising the temperature result in breakdown of the intermediate oxidation products to non-ultraviolet-absorbing materials. Adenine, its 9-substituted derivatives and 2,6-diaminopurine are exceptions in that they readily give good yields of their respective oxidation products.

Adenine is oxidized slowly at room temperature by a mixture of 30% hydrogen peroxide and acetic acid. After a period of oxidation of 3 to 5 days, a crystalline product is obtained in yields as high as 80 to 90%. From the composition of this primary oxidation product, $C_5H_5N_5O$, and the fact that it can be reduced readily with hydrogen and Raney nickel to adenine, it is concluded that the primary oxidation product of adenine is a simple mono-oxy derivative. Such a simple mono-oxy compound could have oxygen bound into the molecule in any of these five ways



III IV V VI VII

Possibilities III and IV are excluded since the oxidation product of adenine is different in spectral and chromatographic behavior from 2-hydroadenine, 8-hydroxyadenine and 6-hydroxylaminopurine[11] (Table I). In addition, compounds IV would not be readily reducible with hydrogen and nickel. Possibility V is discounted on the basis that the primary oxidation product of adenine, unlike an oxazirane,[12] does not liberate iodine from potassium iodide solution. Possibility VI is

(11) A. Bendich, A. Giner-Sorolla and J. J. Fox, *ibid.*, p. 13.
(12) W. D. Emmons, THIS JOURNAL, 78, 6208 (1956).

ruled out on the basis that the 4,5-epoxide of adenine would not have the necessary structural characteristics[13] to absorb in the ultraviolet. The primary oxidation product of adenine must therefore be an N-oxide, *i.e.*, a 1-, 3-, 7- or 9-oxy-derivative of adenine.

Adenosine is oxidized at about the same rate as is adenine by hydrogen peroxide–acetic acid. In 6 days it yields a primary oxidation product in a yield of 95%. Careful hydrolysis of this oxidation product yields adenine N-oxide, showing that the oxidation product of adenosine is the 9-β-D-ribofuranosyl derivative of adenine N-oxide.

This hydrogen peroxide–acetic acid oxidation technique also seems to be applicable to derivatives of adenosine, although yields are somewhat lower because of difficulty in crystallizing the products. Thus 2',3'-isopropylideneadenosine is oxidized by hydrogen peroxide–acetic acid to a mono-N-oxide in a yield of 43.5%. This oxide, like adenosine N-oxide, yields adenine N-oxide upon hydrolysis and thus must be the 9-β-D-(2',3'-isopropylidene)-ribofuranosyl derivative of adenine N-oxide.

2,6-Diaminopurine is oxidized by hydrogen peroxide–acetic acid in 3 days to give an oxidation product which is chromatographically relatively pure. When attempts are made to isolate the oxidation product by crystallization, problems of co-crystallization and decomposition are encountered. To solve this difficulty, the 2,6-diaminopurine oxide is separated by selective elution with ammonium hydroxide–ammonium chloride solution from Dowex-1 chloride. The oxidation product separated by this means has a composition represented by the formula $C_5H_6N_6O$. Since this oxidation product is readily reduced by hydrogen to 2,6-diaminopurine, is ultraviolet absorbing (eliminating structure type VI), does not oxidize potassium iodide solution (eliminating structure type V), is not a hydroxylamino compound by test with ferric ion[14] (eliminating structure type III) and is not spectrally similar to 2,6-diamino-8-hydroxypurine, it must be a mono-N-oxide.

The detection, estimation and isolation of the N-oxides of adenine and its derivatives are greatly facilitated by the unusual ultraviolet spectrum of these compounds. They possess a high absorption maximum in very weakly acid, neutral and alkaline solution at about 233 mμ. In the case of adenine N-oxide, this absorption at about 233 mμ can be as much as 6.2 times as great as the 260–270 mμ absorption which is noted in adenine, and also in adenine N-oxide itself in acid. The ultraviolet absorption spectra of adenine N-oxide, adenosine N-oxide and 2,6-diaminopurine N-oxide are given in Figs. 1, 2 and 3, respectively, of Part II.[15] The spectrophotometrically determined pK's for these oxides are shown in Table I.

Adenine N-oxide is quite stable in neutral aqueous solutions over long periods. There is no tendency for the oxide to lose oxygen and revert to adenine. It also has been shown that, in aqueous solution, there is no tendency for a transfer of oxygen between adenine N-oxide and adenine molecules. This has been demonstrated by chromatographing mixtures of adenine-8-C^{14} and adenine N-oxide which have been standing many days in aqueous solution and assaying for radioactivity in the adenine N-oxide spot. In hot acetic acid, however, adenine N-oxide is partially converted into an unidentified material resembling adenine in R_f and ultraviolet spectrum. A maximum of about 0.45% conversion to this adenine-like material has been effected in this way.

(13) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3875 (1948).

(14) A. Hantzsch and C. H. Besch, *Ann.*, **323**, 23 (1902).

(15) M. A. Stevens and G. B. Brown, THIS JOURNAL, **80**, 2759 (1958).

Experimental

All chromatographic analyses were performed ascending on Whatman No. 1 paper at 25° with the following developing solvents: A, (1% ammonium sulfate–isopropyl alcohol, 1:2 vol./vol.), paper previously soaked in 1% ammonium sulfate and dried,[16] and B, (5% disodium phosphate–isoamyl alcohol, 3:2 vol./vol.).[17] R_f values are given in Table I. Measurements of ultraviolet absorption were performed on a Beckman DK-2 spectrophotometer, except in the case of accurate determinations of extinction coefficients, etc., which were carried out on a Beckman DU spectrophotometer.

Preparation of Adenine N-Oxide.—Adenine (10 g.) was dissolved in hot acetic acid (60 ml.). The solution was then cooled to 20°, 30% hydrogen peroxide (37 ml.) was added and the solution was allowed to stand at room temperature for 2.5 days. At the end of this time a substantial proportion of the product had crystallized. This material (7.0 g.) was collected and washed with a little acetic acid. Further batches (1.6 and 0.2 g.) were collected 3.5 and 4.5 days after the start of the oxidation. These crops each showed single spots on chromatography. Further oxide (0.6 g.) could be obtained from the final filtrate after destroying the hydrogen peroxide by the addition of palladium-on-charcoal and removing some of the acetic acid *in vacuo*. The total yield was 84%. Crystallization of adenine oxide from boiling water yielded white filamentous crystals, dec. point 297–307°.

Anal. Calcd. for $C_5H_5N_5O$: C, 39.73; H, 3.33; N, 46.35. Found: C, 39.81; H, 3.16; N, 46.32.

Hydrogenation of Adenine N-Oxide.—Adenine N-oxide (250 mg.) was dissolved in a mixture of water (100 ml.) and concentrated ammonium hydroxide (1 ml.). Raney nickel (3 ml.) was added, and the mixture was shaken at 30° with hydrogen at atmospheric pressure for 6 hours. After this time, the solution no longer showed the characteristic spectrum of adenine N-oxide. One mole proportion of hydrogen was absorbed. The catalyst was removed and the filtrate was evaporated to dryness. The residual adenine weighed 220 mg. (94%). Its identity was established by mixed melting point (m.p. product 350°, mixed m.p. with adenine 348–350°), R_f in solvents A and B, and by ultraviolet spectrum. The hydrogenation of adenine N-oxide also can be carried out very readily with Raney nickel in acetic acid, but the adenine produced in this case is contaminated with nickel salts.

Stability of Adenine N-Oxide.—Adenine N-oxide (adenine free) (100 mg.) was heated in acetic acid (10 ml.) for 20 minutes on a steam-bath. At the end of this time 100 λ of this solution was placed on a chromatogram and developed with solvent A. One main spot of adenine N-oxide, and a smaller spot of R_f 0.48, corresponding to adenine, were obtained. Elution of the adenine N-oxide spot with water (80 ml.) gave a solution of O.D. 0.257 at 260 mμ and the elution of the spot corresponding in R_f to adenine with water (5 ml.) gave a solution of O.D. 0.03 at 260 mμ. Since this product of R_f 0.48 was only formed in minute amounts (*ca.* 0.45% yield), it has not been possible to isolate and identify it.

Preparation of Adenosine N-Oxide.—Anhydrous adenosine (10.0 g.) was suspended in a mixture of acetic acid (500 ml.) and 30% aqueous hydrogen peroxide (50 ml.) and kept at room temperature for 6 days. The mixture was shaken

(16) N. Anand, V. M. Clark, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1952).

(17) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

occasionally during the first two days, after which time it had become homogeneous. On the 6th day it was cooled in an ice-bath and stirred with 5% palladium-on-charcoal (4 g.) to decompose the excess hydrogen peroxide. When a test with potassium iodide–starch paper indicated the complete decomposition of hydrogen peroxide, the solution was filtered and the filtrate evaporated to 250 ml. *in vacuo* and allowed to crystallize; 6.4 g. of material was obtained. Further evaporation *in vacuo* to 20 ml. yielded an additional 4.4 g. The material so obtained (yield 95%) was substantially pure hydrated adenosine N-oxide. A batch of 2.30 g. of the product was crystallized from ethanol (450 ml.) to give 1.30 g. of adenosine N-oxide. One further crystallization yielded fine needles which on drying *in vacuo* for a day gave the anhydrous adenosine N-oxide, m.p. 155°, dec. point 160°.

Anal. Calcd. for $C_{10}H_{13}N_5O_5$: C, 42.40; H, 4.62; N, 24.72. Found: C, 42.56; H, 4.78; N, 24.81.

Hydrolysis of Adenosine N-Oxide.—Adenosine oxide (30 mg.) was dissolved in 1 *N* HCl (5 ml.) and the solution was boiled under reflux for 15 minutes. The solution was then cooled, and two drops were evaporated at the origin of each of two paper chromatograms. The chromatograms were developed with controls of unhydrolyzed adenosine N-oxide, and adenine N-oxide in solvents A and B. The major hydrolysis product of adenosine N-oxide had the same R_f as adenine N-oxide on each chromatogram. Confirmation that this was adenine N-oxide was afforded by eluting the spot and determining its ultraviolet spectrum. Characteristic absorption maxima at 231 and 262 mμ were observed.

Preparation of 2′,3′-Isopropylideneadenosine N-Oxide.—2′,3′-Isopropylideneadenosine (2.0 g.) was dissolved in a mixture of acetic acid (100 ml.) and 30% aqueous hydrogen peroxide (10 ml.), and the solution was kept at room temperature. After 3 days, the major component in the oxidizing solution was the desired N-oxide, but there were also traces of two other products and some starting material. After 5 days it was stirred for one day with 10% palladium-on-charcoal (0.5 g.) at 20°. The solution was then filtered and evaporated *in vacuo* at room temperature to remove most of the solvent. The viscous brown residue (3.5 g.) was dissolved in hot ethanol (15 ml.), treated with Norite, and cooled in the refrigerator. The gel that resulted was broken up by warming it with an additional 10 ml. of ethanol. The clear solution was cooled slowly and deposited a microcrystalline (radiating needles) powder (845 mg.). Upon again evaporating the filtrate to dryness *in vacuo* and crystallizing the residue from ethanol (10 ml.), a further 300 mg. of product crystallized. The first batch was shown to be chromatographically pure, while the second contained traces of another oxidation product. The yield was 43.5% (assuming the product to be a dihydrate, as determined by weight loss on drying). Recrystallization of this oxidation product and drying at 110° over phosphorus pentoxide for 3 hours yielded 2′,3′-isopropylideneadenosine N-oxide as a white powder, m.p. 176–178° dec.

Anal. Calcd. for $C_{13}H_{17}N_5O_5$: C, 48.29; H, 5.30; N, 21.66. Found: C, 48.77; H, 5.27; N, 21.30.

Hydrolysis of 2′,3′-Isopropylideneadenosine N-Oxide.—2′,3′-Isopropylidene adenosine N-oxide (5 mg.) was dissolved in 1 *N* hydrochloric acid (2 ml.). The solution was brought rapidly to boiling and kept there for 2 minutes. At times of 30 seconds and 1 and 2 minutes after the start of the hydrolysis, portions of the hydrolyzing solution were withdrawn and chromatographed using solvent B. After one-half minute no hydrolysis was detectable. After one minute about 30% had hydrolyzed to a material R_f 0.48 identical with adenine N-oxide in R_f and ultraviolet spectrum, and different in R_f from adenosine N-oxide and 2′,3′-isopropylideneadenosine N-oxide. After 2 minutes about 60% of the starting material had been hydrolyzed to adenine N-oxide.

Preparation of 2,6-Diaminopurine N-Oxide.—2,6-Diaminopurine (410 mg.) was dissolved in a mixture of acetic acid (23 ml.) and 30% aqueous hydrogen peroxide (1.8 ml.). The solution quickly deposited a white slurry of the acetate. This slurry was stirred at 25–30° for 3 days, by which time it had dissolved. A chromatogram (solvent B) run at this point on the crude oxidation mixture showed it to contain predominantly one oxidation product plus a considerable amount of starting material. The oxidation was stopped at this point to avoid further oxidation of the desired primary oxidation product. To terminate the oxidation the solution was cooled to 0°, 10% palladium-on-charcoal (125 mg.) was added and the mixture was stirred at room temperature for one day. The solution was filtered and the filtrate taken to dryness at 25–30°, *in vacuo*. The residue was suspended in water (10 ml.) and sufficient concentrated ammonia solution was added to cause solid material to dissolve. The homogeneous solution was diluted to two liters, the *p*H adjusted to 10.8 and the resulting solution, which represented 6.2×10^3 O.D. units, (an O.D. unit is the optical density of the solution at 260 mμ times the volume in ml.) was fed to a 250-ml., 200–400 mesh, Dowex-1 chloride column (3.5 × 29 cm.); 44 O.D. units appeared in the effluent. The column was eluted with ammonium chloride solution of progressively increasing concentration obtained by running 0.01 *M* ammonium chloride solution (made to *p*H 10.4 with ammonia) from a reservoir into a three-liter mixer containing 0.001 *M* ammonium chloride solution (made to *p*H 11.0 with ammonia) and thence through the column at 5.8 ml./min. When 5,310 ml. of 0.01 *M* ammonium chloride solution had been run from the reservoir, it was filled with 690 ml. of 0.02 *M* ammonium chloride solution (*p*H 10.4) and the elution was continued. During the passage of the 0.02 *M* ammonium chloride solution into the mixer about 70 O.D. units of a minor oxidation product of 2,6-diaminopurine was eluted from the column. The reservoir was filled with 6,325 ml. of 0.04 *M* ammonium chloride solution (*p*H 10.4) and the elution continued. During the passage of the 0.04 *M* ammonium chloride solution through the mixer, 2,6-diaminopurine N-oxide (representing 2829 O.D. units) was eluted from the column. Passage of ammonium chloride solution (0.04 *M*) into the mixer was continued until the O.D. of the column eluate had fallen to 0.2, at which point the concentration of the feed to the mixer was changed to 0.08 *M* in order to elute the 2,6-diaminopurine remaining on the column. Passage of 2760 ml. of 0.08 *M* ammonium chloride caused 1882 O.D. units of 2,6-diaminopurine to be eluted. The fraction containing the diaminopurine N-oxide was evaporated at room temperature to a volume of 300 ml. at which point the 2,6-diaminopurine N-oxide (60 mg. 13.5%) crystallized.

Anal. Calcd. for $C_5H_6N_6O$: C, 36.14; H, 3.64; N, 50.59. Found: C, 36.36; H, 3.75; N, 50.50.

Hydrogenation of 2,6-Diaminopurine N-Oxide.—2,6-Diaminopurine N-oxide (7.5 mg.) was dissolved in hot water (15 ml.). Raney nickel (*ca.* 3 mg.) was added and the resulting solution was shaken at 30° with hydrogen at atmospheric pressure for 140 min. The uptake of hydrogen leveled off after this period at 0.9 ml. (0.9-mole proportions). The catalyst was separated and the solution was found to be chromatographically homogeneous and free of oxide. The R_f of the hydrogenation product in solvent B was 0.22, identical with that of 2,6-diaminopurine. The ultraviolet spectrum of the hydrogenation product was also identical with that of 2,6-diaminopurine.

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