Experimental Section[8]

1,4-Di-*t*-butylcyclohexene (II).—To a solution of 41.4 g (0.217 mole) of *p*-di-*t*-butylbenzene (I), mp 75–76.5°, in 760 ml of ethylenediamine (Matheson Coleman and Bell) at 100° in a nitrogen atmosphere was added 12.2 g (1.74 g-atoms) of lithium during 1.5 hr. The blue mixture was then heated under reflux for 2 hr and cooled, and water was added with cooling, dropwise at first, until 1500 ml had been added. A white solid remained which was soluble in ether. The mixture was extracted with eight 200-ml portions of ether. The total ether extract was washed with five 200-ml portions of 5% ammonium chloride, and was dried over anhydrous sodium sulfate. Removal of the ether gave 38.9 g (93%) of product containing 18% of starting material (ultraviolet analysis for *p*-di-*t*-butylbenzene). Recrystallization from methanol gave a first crop of 18.7 g (44%) of needle-like crystals, mp 53.5–54°, containing 12% of I (ultraviolet analysis). The analytical sample, prepared by further recrystallization from methanol, contained an upper limit of 4% of I (ultraviolet analysis) and had mp 54–54.5°.

Anal. Calcd for $C_{14}H_{26}$: C, 86.51; H, 13.49. Found: C, 86.72; H, 13.32.

Gas chromatography on a 300-cm, 0.25-in. o.d., column packed with 20% silicone gum rubber on Chromosorb P at 130° with 70-cc/min helium flow gave retention times as follows (in min): I, 45.5; II, 50.8; *cis* III, 53.4; *trans* III, 53.4.[9] The recrystallized samples of II contained small amounts (3–12%) of I, but no components other than I and II were detected by gas chromatography.[10]

The nmr spectrum of I shows a singlet at 1.29 ppm (18.0 protons) and a second singlet at 7.18 ppm (4.1 protons). The nmr spectrum of a sample of II containing 12% of I showed the two singlets of I with integrated intensity consistent with the presence of 13 ± 2% of I. In addition, the absorption attributable to II gave sharp singlets at 0.86 ppm (9.0 protons) and 1.00 ppm (9.0 protons), assigned to the *t*-butyl groups at C-4 and C-1, respectively, a multiplet at 5.38 ppm (1.0 proton), assigned to the olefinic proton at C-2, and broad absorption in the region 1.0–2.3 ppm (6.3 protons), assigned to the protons at C-3–C-6.

Acknowledgment.—We wish to thank the National Science Foundation for support of this work.

(8) Nmr spectra were recorded by Don C. Wiley. A Varian A-60 spectrometer was used with solutions in carbon tetrachloride containing 3% tetramethylsilane as internal standard. The infrared spectrum of II has been reproduced in ref 3. Microanalysis was performed by Dr. S. M. Nagy.

(9) It is interesting to note that the *cis*- and *trans*-1,4-di-*t*-butylcyclohexanes (*cis* III and *trans* III) were not separated from one another under these conditions or under any other conditions of gas chromatography yet tried in this laboratory.

(10) NOTE ADDED IN PROOF.—Professor Daniel J. Pasto (private communication, Dec 30, 1965) has suggested the following method for purification of II. Alumina coated with silver nitrate, when used in chromatography as described by R. Wolovsky [*J. Am. Chem. Soc.*, **87**, 3638 (1965)] gave II of 98–99% purity.

Purine N-Oxides. XVIII. Deamination of Adenine N-Oxide Derivatives[1]

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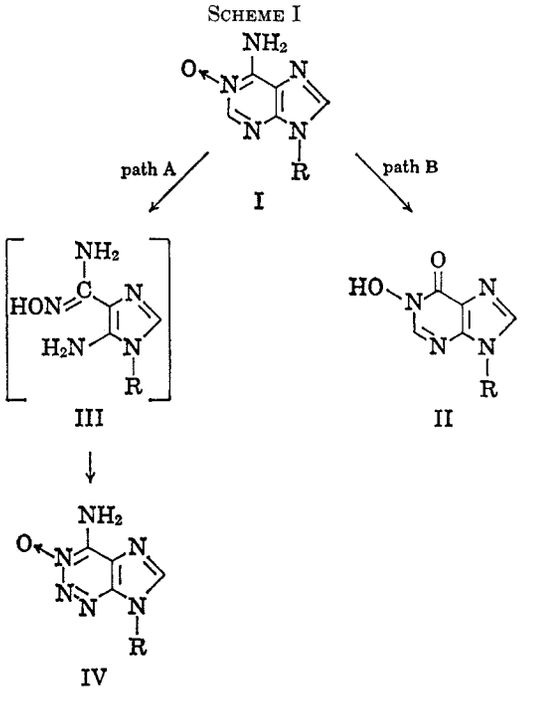
It was recently demonstrated[2] that xanthine 7-N-oxide and guanine 7-N-oxide, which may well exist as the 7-N-hydroxy derivatives,[3] can induce a variety of tumors in rats. This has stimulated interest in evaluating the N-oxides of other naturally occurring purines and their ribosyl derivatives for similar oncogenic behavior. Adenine 1-N-oxide (Ia) (6-aminopurine 1-N-oxide) showed no such activity[2] under the assay conditions employed. In considering the structural requirements for oncogenicity in purine N-oxides, it becomes of particular interest to test the structurally analogous hypoxanthine derivative, the neutral molecule of which should exist largely as 1-hydroxy-6-purinone or 1-hydroxyhypoxanthine (IIa).

The preparation of "hypoxanthine 1-N-oxide" (IIa) from methyl 4-nitroimidazole-5-carboxylate has been reported,[4] but the synthesis is laborious and does not lend itself to the quantities needed for biological studies. Since hypoxanthine is inert to direct oxidation by peroxy acids, the 1-N-oxide must be prepared either by total synthesis or by modification of a related purine 1-N-oxide. We now report procedures for obtaining 1-hydroxyhypoxanthine and its nucleoside, by deamination of the corresponding adenine 1-N-oxide derivatives. Earlier attempts at diazotization of Ia produced 2-azaadenine 1-N-oxide (IVa).[5] Since the starting material is readily hydrolyzed to 4-aminoimidazole-5-carboxamidoxime (IIIa) even in dilute mineral acid,[6] the 2-aza derivative IVa results[5] from diazotization of the imidazole intermediate and ring closure of the diazonium salt (path A, Scheme I). Previous attempts in this laboratory to obtain only deamination (path B) produced mixtures containing material similar to that obtained by Taylor, Cheng, and Vogl,[4] along with 2-azaadenine 1-N-oxide and other

SCHEME I



a series, R = H
b series, R = β-D-ribofuranosyl

(1) This investigation was supported in part by funds from the Atomic Energy Commission (Contract No. AT[30-1],910) and from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-03190-09).

(2) G. B. Brown, K. Sugiura, and R. M. Cresswell, *Cancer Res.*, **25**, 986 (1965).

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

(4) E. C. Taylor, C. C. Cheng, and O. Vogl, *ibid.*, **24**, 2019 (1959).

(5) M. A. Stevens, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **82**, 3189 (1960).

(6) M. A. Stevens and G. B. Brown, *ibid.*, **80**, 2759 (1958).

TABLE I

IONIZATION CONSTANTS AND SPECTRAL DATA

Compd	pH (charge)	λ, mμ (ε × 10⁻³) max	λ, mμ (ε × 10⁻³) min	pK_a
1-Hydroxyhypoxanthine	3 (0)	250 (8.3)	227 (3.9)	5.68 ± 0.06[a]
				5.65 ± 0.04[b]
	8 (−1)	259 (5.6)	246 (4.6)	
		228 (36.0)		10.10 ± 0.05[b]
	13[c] (−2)	264 (6.4)	249 (4.8)	
		229 (44.0)		
1-Hydroxyinosine	3 (0)	251 (9.1)	227 (4.0)	5.46 ± 0.03[a]
	9 (−1)	294 (4.0)	275 (2.8)	
		256 (6.3)	248 (5.7)	
		229 (30.0)		

[a] Determined spectrophotometrically. [b] Determined potentiometrically, with potassium hydroxide. [c] The previously reported values, mμ (ε × 10⁻³), at pH 13 were 261 (6.6) and 229 (18.0); that at pH 1 was 248 (8.1);[4] for pH 1, we find 249 (8.4).

products. To minimize the undesirable products arising by path A, the reaction was carried out at low temperature in the presence of dilute acetic acid. The crude yellow product from the reaction showed ultraviolet absorption properties similar to those for 2-azaadenine 1-N-oxide (IVa), but paper chromatographic examination showed that the crude product contained two components, one ultraviolet absorbing and the other fluorescent, neither of which was IVa. Several recrystallizations of this crude sample led to a pale yellow powder whose properties agreed with those reported[4] for "hypoxanthine 1-N-oxide," although there still were traces of the fluorescent impurity. The two components could be separated best by allowing the ammonia to evaporate from an ammoniacal solution of the product. The fluorescent impurity separated as a dark yellow precipitate. Analytically pure 1-hydroxyhypoxanthine was obtained from the clear filtrate as white crystals in 47% yield.

Pure 1-hydroxyhypoxanthine does not manifest the extreme hygroscopicity encountered previously.[4] In contrast to the extremely sensitive ferric chloride test reported[4] for IIa, the pure sample showed only a weak orange ferric chloride test of no unusual sensitivity. The ultraviolet absorption spectra differed slightly from the reported[4] values (Table I). It is very slowly reduced to hypoxanthine by catalytic hydrogenation with Raney nickel.[7]

The 1-hydroxyhypoxanthine shows pK values of 5.65 and 10.10 which are associated with proton removals. By analogy to the pK of 5.46 in 1-hydroxyinosine, the pK of 5.65 in IIa is associated with the 1-position. In each compound proton loss in this region is accompanied by increasing absorption at 229 mμ. That these derivatives exist predominately as cyclic hydroxamic acids is confirmed by the presence of strong carbonyl absorption in the infrared spectra. The 1-hydroxyhypoxanthine shows an absorption band at 1695 cm^{-1}, which is very near the carbonyl absorption of 1-methylhypoxanthine (1698 cm^{-1})[8] and significantly shifted from that of hypoxanthine (1670 cm^{-1}). The 1-hydroxyinosine shows carbonyl absorption at 1699 cm^{-1}.

Structural studies are in progress on the fluorescent by-product. Certain of its properties now explain the previously puzzling properties of the products obtained in this reaction. The ultraviolet absorption spectrum of the compound exhibits a strong band near 350 mμ at all pH values. This absorption band causes the ultraviolet spectrum of the crude product mixture containing 1-hydroxyhypoxanthine and the by-product to resemble the spectrum of 2-azaadenine 1-N-oxide (230, 270, and 335 mμ).[5] The fluorescent by-product gives a bright red ferric chloride test, and it is apparent that the presence of a small quantity of this material in the partially purified 1-hydroxyhypoxanthine would produce a mixture possessing the properties previously reported[4] for IIa, *viz.*, a pale yellow solid which gives a sensitive ferric chloride test[9] and unreproducible analytical results.

The deamination of adenosine 1-N-oxide (Ib) to the corresponding inosine derivative (IIb) could not be accomplished under the conditions that proved satisfactory for Ia. The diazotization of Ib in dilute acetic acid produced only low yields of 2-azaadenosine 1-N-oxide[5] (IVb) (path A). By carrying out the reaction in aqueous dimethylformamide with dilute acetic acid, the 1-hydroxyinosine (IIb) was obtained as a yellow oil which was crystallized and then desalted by cation-exchange chromatography to produce pure IIb in 21% yield. During the course of our investigation, Sigel and Brintzinger[10] also prepared IIb by diazotization of Ib with nitrosyl chloride in dimethylformamide and designated it as inosine 1-N-oxide. The infrared and ultraviolet data from our sample of IIb are in agreement with their published[10] values. However, our procedure affords a product which is completely free of salt and requires considerably less ion-exchange chromatography for purification. IIb is readily hydrolyzed to IIa.

These procedures provide convenient access to preparative quantities of the 1-hydroxy derivatives of hypoxanthine and inosine from the readily available[11] adenine 1-N-oxide derivatives. In contrast to the adenine 1-N-oxide series,[6] these exist predominately as the 1-hydroxy tautomers.

(7) This is in agreement with the observations of Taylor, Cheng, and Vogl,[4] who found no reduction with Raney nickel and accomplished complete reduction only by hydrogenation for several days at elevated temperature and pressure in the presence of Adams catalyst.

(8) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 682 (1957).

(9) It is of interest to note that the 4-nitroimidazole-5-hydroxamic acid employed in the earlier synthesis was also yellow and "gave a very sensitive purple-red color with ferric chloride."[4]

(10) H. Sigel and H. Brintzinger, *Helv. Chim. Acta*, **48**, 433 (1965).

(11) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958).

Experimental Section

Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. Melting points are corrected. Chromatograms were developed by the ascending technique with Whatman No. 1 paper and were viewed under ultraviolet light primarily of wavelength 253.8 mμ. The solvent systems employed were (A) 3% ammonium chloride; (B) 5% disodium hydrogen phosphate–isoamyl alcohol (3:2); (C) isopropyl alcohol–water–28% ammonium hydroxide (7:2:1); and (D) *t*-butyl alcohol–methyl ethyl ketone–88% formic acid–water (40:30:15:15 v/v). The ultraviolet absorption data are given in Table I and chromatographic data in Table II. The p*K* values were determined by methods described,[12] at 20°, spectrophotometrically with 0.01 *M* buffers,[13] or potentiometrically with 0.01 *M* solutions. The infrared data were obtained using a Perkin-Elmer Model 221 spectrophotometer by the potassium bromide disk method.

TABLE II

Compd	R_f in solvents[a] A	B	C	D
1-Hydroxyhypoxanthine (IIa)	0.64	0.62	0.20	0.44
1-Hydroxyinosine (IIb)	0.82	0.80	0.30	0.30
Adenine 1-N-oxide	0.62	0.48[b]	0.50	0.49
2-Azaadenine 1-N-oxide	0.59	0.49[c]	0.53	0.36
Hypoxanthine	0.58	0.56	0.45	0.41
Adenosine 1-N-oxide	0.75	0.71[b]	0.38	0.38
Unknown by-product	0.15[d]	0.07[d]	0.11[d]	0.08[d]

[a] See Experimental Section for solvents. [b] Reference 6. [c] Reference 5. [d] Fluorescent.

1-Hydroxyhypoxanthine (IIa).—Adenine 1-N-oxide[11] (20.0 g, 0.13 mole) was suspended in a solution containing 36.8 g of $NaNO_2$ (0.52 mole) in 650 ml of water. The mixture was cooled in an ice bath to about 10°, and 140 ml of 50% aqueous acetic acid was added dropwise with stirring over a period of 30–40 min. The temperature of the solution did not rise above 10° during this period. After the addition of acid was complete, the solution was heated at 70–80° for 2 hr, then cooled to 10°. The precipitate was collected and washed with alcohol and ether to afford 13.7 g of a yellow powder. The crude product was dissolved in *ca.* 1 l. of hot dilute ammonia (pH 8–9) and the solution was allowed to cool and stand overnight. The fine yellow powder which precipitated (1.8 g) was collected and washed with ethanol and ether. The filtrate was concentrated under vacuum to about 500 ml, and an additional 2.2 g of yellow product was obtained. Both fractions were identical chromatographically and migrated as a single bright fluorescent spot. The pale yellow filtrate was then treated with charcoal (Darco), and the pH of the colorless solution was adjusted to 5.5 to 6 by addition of glacial acetic acid. The 1-hydroxyhypoxanthine slowly precipitated as a fine white microcrystalline solid, which proved to be chromatographically and analytically pure: yield 9.5 g (47%), mp 356° (decomposed with darkening above 340°).

Anal. Calcd for $C_5H_4N_4O_2$: C, 39.48; H, 2.65; N, 36.84. Found: C, 39.45; H, 2.73; N, 36.98.

A sample of analytically pure IIa, obtained from an aqueous solution and stored in a bottle for 4 months, was dried at 100° for 2 hr. The sample lost 0.06% of its weight. It was redried at 140° for 3 hr and lost an additional 0.04% of its weight. The sample gained no weight whatsoever when allowed to equilibrate with the atmosphere overnight. It is probable that the extreme hygroscopicity (average weight loss 10.1%) encountered by Taylor, Cheng, and Vogel[4] is due to hydration of impurities rather than hydration of IIa.

A solution of 75 mg of IIa dissolved in 10 ml of 0.1 *N* NaOH and containing *ca.* 400 mg of activated Raney nickel was hydrogenated at room temperature and 1 atm for 60 hr. An aliquot was chromatographed in solvent C and contained two spots, one corresponding to hypoxanthine and the other to unchanged IIa. A sample was chromatographed on a cellulose plate in solvent C and the two bands were quantitatively eluted with 0.1 *N* NaOH. Calculation of the molar quantities from the optical densities of the elution solutions indicated 10% of the N-oxide had been reduced to hypoxanthine under these conditions.

1-Hydroxyinosine (IIb).—Adenosine 1-N-oxide[11] (12.04 g, 0.04 mole) was suspended in a solution of 8.28 g (0.12 mole) of $NaNO_2$ dissolved in 80 ml of dimethylformamide. To the reaction mixture 20 ml of 50% aqueous acetic acid was added dropwise with stirring at room temperature over a period of 10–15 min. After the addition of acid was complete, the solution was stirred at room temperature for 1 hr and 50–60° for 2 hr. To this, when cooled, was added 600 ml of ether, which caused the separation of a yellow oil. The ether layer was discarded and the oily residue was washed with two 200-ml portions of ether. The yellow oil was dissolved in 400 ml of hot methanol and induced to crystallize by slow removal of the solvent on a steam bath until crystals began to form and then cooling the solution. A fine white precipitate was obtained which was collected and washed with ether to yield 4.50 g of a white microcrystalline powder. An additional 0.51 g was obtained from the mother liquors by successive reductions in volume and chilling.

The crude product (5.01 g) was dissolved in 15 ml of water and desalted by percolating the solution through a column containing Dowex 50, hydrogen form (W × 4, 20–50 mesh), and eluting with water. The elution was followed by monitoring the optical density of the eluate at 228 mμ.

The solvent was removed under reduced pressure while the temperature of the solution was maintained at 35–40°. The product, which crystallized from solution as large colorless prisms, was collected and washed with ethanol and ether to yield 2.40 g (21%) of analytically pure 1-hydroxyinosine, mp >400°, gradual decomposition and charring above 170°.

Anal. Calcd for $C_{10}H_{12}N_4O_6$: C, 42.14; H, 4.52; N, 19.65. Found: C, 42.24; H, 4.30; N, 19.80.

No hydrolysis of the sugar occurred during the ion-exchange elution (pH >4) nor did it take place in 0.1 *N* hydrochloric acid solution at room temperature. When a solution of 56 ml of IIb in 5 ml of *N* hydrochloric acid was warmed on a steam bath, complete hydrolysis was accomplished in 15 min. The product isolated from the reaction medium was shown to be chromatographically and spectrally identical with an authentic sample of IIa: yield 17.6 mg (58%).

Acknowledgment.—We wish to acknowledge the preliminary studies on this problem by Dr. Alfredo Giner-Sorolla and Dr. Ronald M. Cresswell. Dr. George Bosworth Brown wishes to thank Professor A. Albert and Dr. D. D. Perrin, The John Curtin School of Medical Research, Canberra, Australia, for hospitality and advice on the p*K* determinations.

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

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

The Preparation and Cyclization of Chloroethyl Carbazates. Some Clarifications[1]

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During the course of studies on 2-oxazolidinone chemistry, we were interested in preparing some 3-(substituted amino)-2-oxazolidinones (I). A possible route to these compounds involved the cyclization of (2-chloroethyl) 3-substituted carbazates (II). We have

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