fluxing at this temperature for about 2 hr. the reaction mixture was cooled, diluted with water (3 l.), and the separated potassium salt of 3α-hydroxycholanic acid was centrifuged. The free acid (92 g.), obtained after adding concentrated hydrochloric acid to a solution of the potassium salt in hot water, was practically pure. Recrystallization from methanol yielded 90 g. of crystals melting at 184° α_D^{16} +36.5° ± 0.5° (0.68% in EtOH) (reported[10] m.p. 187–188° α_D^{11} +32.1°). The yield based upon methyl cholate used is 95%.

When the ordinary Huang-Minlon procedure was adopted for the reduction of methyl 3α-succinoxy-7,12-diketocholanate, as described previously,[11] the yield of 3α-hydroxycholanic acid was less than 40% and at the same time the free acid was obtained in an impure state requiring many recrystallizations for its purification.

Methyl 3α-hydroxycholanate (I). The esterification of 3α-hydroxycholanic acid (80 g.) was effected in quantitative yield on treatment with boiling methanolic hydrochloric acid (2.5%) (1500 ml.) for 2 hr. Recrystallization from petroleum-ether (40–60°), after chromatography over alumina, yielded the labile form of m.p. 90–92°,[12] transforming into the stable form, m.p. 125–126° (reported[12] m.p. 126–127°), after two days standing in a vacuum desiccator.

3α-Acetoxy-24,24-diphenylchol-23-ene (II). For the preparation of this compound, the usual Barbier-Wieland procedure was employed. I (0.2 mole) was treated with an excess of phenyl magnesium bromide (3.0 mole) in boiling benzene for 24 hr. The resulting carbinol (not isolated) was acetylated by means of acetic anhydride (60 ml.) and dry pyridine (100 ml.). After removal of solvents by vacuum distillation it was then dehydrated by boiling with glacial acetic acid (200 ml.) for 20 hr. The acetylated diphenylethylene (II), which crystallized out on cooling, was practically pure. Recrystallization from acetone afforded white needles melting at 160° (reported[4] m.p. 160–167°); α_D^{20} +67° (1% in $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 255–257 mμ, (log ε 4.18).

Anal. Calcd. for $C_{38}H_{50}O_2$: C, 84.7; H, 9.35. Found: C, 84.7; H, 9.39.

The infrared spectrum (KBr) showed bands (cm.$^{-1}$) at 3077, 2963, 2899 (CH), 1733 (acetate CO), 1650, 1595, 1495 (double bonds), 1246 [acetate (C—O)], 757–760, 696–700 (CH aromatic).

24,24-Diphenyl-chol-23-en-3α-ol. II was readily deacetylated by means of ethanolic potassium hydroxide. Recrystallization from ethanol gave crystals melting at 140–141° (reported[4] m.p. 110–140°); $\lambda_{max}^{CHCl_3}$ 255–258 mμ (log ε 4.23); α_D^{17} +52° ± 2° (0.2% in $CHCl_3$).

Anal. Calcd. for $C_{36}H_{48}O$: C, 87.0; H, 9.74. Found: C, 86.5; H, 9.70.

The infrared spectrum shows bands (cm.$^{-1}$) at 3460, 3413 (OH), 2950, 2878 (CH), 1653, 1600, 1493 (double bonds), 1499, 1445, 1418, 1375 (CH deformation), 757–760, 695 (phenyl).

3α-Acetoxynorcholanic acid (III). (a) *Ruthenium oxide-catalyzed periodate oxidation of* II. When the solutions of II (2 g.) in acetone (200 ml.) and ruthenium tetroxide[7] (120 mg.) in aqueous sodium periodate (5%) (10 ml.) were mixed at room temperature, an immediate black precipitate of ruthenium oxide was obtained. While the temperature of the stirred mixture was maintained at 20–25°, a total of 4.5 g. of finely powdered sodium metaperiodate was added in portions over a period of 4 hr. To the mixture (now dark brown) a few ml. of isopropanol was added to reduce the catalyst (now black), which was then removed by filtration. After removal of solvent, water was added and the mixture was extracted with ether. The extract proved to contain a mixture of benzophenone and III. They were easily separated on treatment of the ethereal extract with aqueous sodium carbonate (10%). The benzophenone, left in the ether extract, was identified by its 2,4-dinitrophenylhydrazone derivative. III was first chromatographed over silica gel (7.5 g.) using benzene as eluting solvent, and then recrystallized from aqueous acetone, giving 1.20–1.25 g. (80–83%) of pure 3α-acetoxynorcholanic acid (III) of m.p. 177–178° (reported[7] m.p. 175–176°), α_D^{20} +51° (1% in $CHCl_3$).

(b) *Chromium trioxide oxidation of* II. A suspension of II (3 g.) in glacial acetic acid (10 ml.) was mixed with a solution of chromium trioxide (3 g.) in glacial acid (80 ml.) and then left to stand in a thermostat at 40–45° for 12 hr. Excess of reagent was destroyed by the addition of dry methanol, followed by removal of solvent in vacuum and then ether extraction. The desired acid was isolated and purified in a fashion here reported, giving 1.5 g. (65%) of crystalline product of m.p. 177–178°. II was oxidized only partially at 35–36°, whereas at 100°, even after a short time, the required acid could not be obtained. This apparently led to the formation of a complex mixture of oxidation products.

3α-Hydroxynorcholanic acid. Deacetylation of III in a manner here reported, afforded in almost quantitative yield the pure 3α-hydroxynorcholanic acid after recrystallization from methanol, m.p. 185–186° (reported[12] m.p. 181–182°), α_D^{20} +32° ± 3° (0.4% in EtOH).

Anal. Calcd. for $C_{23}H_{38}O_3$: C, 76.55; H, 10.71. Found: C, 76.59; H, 10.82.

The infrared spectrum of this hydroxyacid shows bands (cm.$^{-1}$) at 3410–3390, 3096 (OH), 2920, 2857 (CH), 1716, 1688 (CO), 1468, 1455–1449, 1414, 1377 (CH deformation).

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Studies in Purine Chemistry. IV. Hypoxanthine-1-*N*-oxide[1]

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Received May 6, 1959

Purine-*N*-oxides are receiving current attention,[2–9] not only because of their potential as possible purine antimetabolites, but also because of the possibility that they may function as intermediates in biological interconversions of purines.

(1) This investigation was supported by a grant (C-2551-PET) to Princeton University from the National Cancer Institute of the National Institutes of Health.

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The only purine-*N*-oxides known so far are the 1-*N*-oxides of adenine,[2–5,7] adenosine,[2,3,8] 2′,3′-isopropylideneadenosine,[2] and various adenine nucleotides,[6] mono-*N*-oxides of 2,6-diaminopurine[2] and ATP[8] and several 8-phenylpurine-7-*N*-oxides;[9] all but the latter were prepared by direct oxidation with hydrogen peroxide. This method suffers from the limitation that the position of oxidation cannot be assumed and must be determined independently, and that some purines are either degraded (*i.e.*, purine[2]) or are essentially unaffected (*i.e.*, hypoxanthine, guanine, 7-methyladenine, and uric acid[2]) by hydrogen peroxide.

We wish to report in the present paper an unambiguous synthesis of hypoxanthine-1-*N*-oxide by a route not involving peroxide oxidation. Methyl 4-nitroimidazole-5-carboxylate was prepared by known procedures and converted into the hydroxamic acid by reaction with hydroxylamine. Catalytic reduction in dimethylformamide solution, in the presence of Adams' catalyst, yielded 4-aminoimidazole-5-hydroxamic acid, which was then cyclized to hypoxanthine-1-*N*-oxide with ethyl orthoformate.[10] This cyclization is thus analogous to the previously described conversion of 2-aminopyrazine-3-hydroxamic acid to 4-hydroxypteridine - 3 - *N* - oxide (3 - hydroxy - 4(3*H*)-pteridinone).[11]

Hypoxanthine-1-*N*-oxide is moderately soluble in water, insoluble in most organic solvents, and very difficult to purify. Moreover, considerable difficulty was encountered in obtaining correct and reproducible microanalytical results because of its hygroscopicity. Samples prepared and dried in the usual manner were found to contain variable amounts of water by the time they reached the microanalyst's hand. Satisfactory microanalytical results were obtained only when the sample was allowed to equilibrate with the atmosphere, dried to constant weight at 137° (thus losing, on the average, 10.1% of its weight) and then immediately subjected to microcombustion.

Table I gives the paper chromatographic behavior and the ultraviolet absorption spectra of hypoxanthine-1-*N*-oxide. As one would expect, the R_f values for the *N*-oxide are generally higher than for hypoxanthine itself. The ultraviolet absorption spectra of hypoxanthine-1-*N*-oxide in alkaline and neutral solution exhibit two absorption maxima, the one at the shorter wave length being the more intense. The spectrum of adenine-1-*N*-oxide is similar,[3] and an intense absorption band at ~230 mμ (in neutral and in alkaline solution) may prove to be characteristic of purine-*N*-oxides in general. The spectrum of hypoxanthine-1-*N*-oxide in acid solution is similar to the spectrum of hypoxanthine itself.[12]

Catalytic reduction of hypoxanthine-1-*N*-oxide in the presence of Adams' catalyst yielded hypoxanthine, but the use of Raney nickel under conditions which sufficed for the reduction of adenine-1-*N*-oxide to adenine[3] was without effect.

TABLE I

Chromatographic Behavior Descending, 22°		Ultraviolet Absorption Spectra		
Solvent	R_f	Solvent	λ max	log ε
n-BuOH/5*N* HOAc	0.360 (0.335)[a]	0.1*N* NaOH	229	4.25
			261	3.82
3% NH_4Cl	0.603 (0.564)	H_2O	225 (s)	3.95
4% Sodium citrate	0.537 (0.537)		249	3.88
		0.1*N* HCl	248	3.91

[a] The values in parentheses are for hypoxanthine.

EXPERIMENTAL[13]

4-Nitroimidazole-5-hydroxamic acid. To a solution of 4.2 g. (0.061) mol. of hydroxylamine hydrochloride and 6.0 g. (0.15 mol.) of sodium hydroxide in 120 ml. of water was added portion-wise and with stirring 7.0 g. (0.04 mol.) of methyl 4-nitroimidazole-5-carboxylate.[14] After addition was complete, the deep yellow solution was stirred at room temperature for 2 hr. and then allowed to stand for 3 days. It was then acidified to *p*H 6 with dilute hydrochloric acid and the light yellow crystalline solid which separated was collected by filtration, washed with ice water, and dried at 100° *in vacuo* to give 6.0 g. (85.4%), m.p. 206° dec. Recrystallization from water raised the decomposition point to 220°. The material gave a very sensitive purple-red color with ferric chloride.

Anal. Calcd. for $C_4H_4N_4O_4$: C, 27.9; H, 2.34; N, 32.6. Found: C, 28.3, 28.4; H, 2.1, 2.6; N, 32.3.

Hypoxanthine-1-N-oxide. A solution of 3 g. of 4-nitroimidazole-5-hydroxamic acid in 40 ml. of dimethylformamide containing 0.3 g. of platinum oxide was hydrogenated at room temperature at 60 p.s.i. Hydrogen uptake was complete after 5 min. The reaction mixture was filtered from the catalyst and added quickly to 20 ml. of ethyl orthoformate. The resulting mixture, which now contained a light-brown solid, was heated under reflux at 155° (oil bath temperature) for 20 min. and was then poured into 100 ml. of ice water. The resulting solution containing a light-brown solid suspension was concentrated under reduced pressure to dryness and the residual solid recrystallized from methanol in the presence of a small amount of water and several drops of benzene. After three recrystallizations, 1.8 g. of a light yellow microcrystalline solid was obtained; m.p. >360°. Hypoxanthine-1-*N*-oxide gives an extremely sensitive ferric chloride test.

Anal. Calcd. for $C_5H_4N_4O_2$: C, 39.5; H, 2.7; N, 36.8. Found: C, 39.3; H, 3.3; N, 37.0, 36.6.

Reduction of hypoxanthine-1-N-oxide to hypoxanthine. A solution of 0.5 g. of hypoxanthine-1-*N*-oxide in 120 ml. of water containing 0.2 g. of platinum oxide was hydrogenated at 60 p.s.i. at 60° for 24 hr. The hot reduction mixture was

(10) A preliminary report of this synthesis was given by E. C. Taylor, T. S. Osdene, E. Richter, and O. Vogl in *The Chemistry and Biology of Purines*, ed. by G. E. W. Wolstenholme and C. M. O'Connor, J. and A. Churchill, Ltd., London, 1957, p. 23.

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filtered from the catalyst and spotted on Whatman No. 1 paper using 3% ammonium chloride as solvent. Two spots were obtained, one corresponding to hypoxanthine and the other corresponding to unchanged hypoxanthine-1-*N*-oxide, thus indicating that reduction was incomplete. Fresh catalyst (0.29 g.) was therefore added to this solution, which was again hydrogenated at 60 p.s.i. at 80° for 2.5 days. After removal of the catalyst from the hot reduction mixture, the filtrate was again spotted on Whatman No. 1 paper, using 3% ammonium chloride as developing solvent. Only one absorption spot, corresponding to hypoxanthine, was obtained. This was confirmed by a simultaneous run with authentic hypoxanthine. Evaporation of this solution to dryness followed by recrystallization of the residue from water yielded a light tan solid which, although it still gave a positive ferric chloride test (indicating the presence of a small amount of unreduced hypoxanthine-1-*N*-oxide) apparently was essentially hypoxanthine, since it gave only one spot on paper chromatography (corresponding precisely in R_f value with authentic hypoxanthine) and its ultraviolet absorption spectrum in 0.1*N* NaOH was identical with the spectrum given by authentic hypoxanthine. It is interesting to note that the ferric chloride test in this instance is considerably more sensitive in detecting a small amount of hypoxanthine-1-*N*-oxide in the product than is paper chromatography.

Attempted reduction of hypoxanthine-1-*N*-oxide using Raney nickel under the conditions previously described for the reduction of adenine-1-*N*-oxide to adenine[8] was completely unsuccessful. No hypoxanthine could be detected in the reduction product.

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3-Substituted 1,8,8-Trimethyl-3-azabicyclo-[3.2.1]octanes

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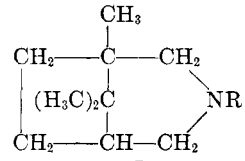
Received May 11, 1959

Recent publications[2–6] lead us to report certain aspects of our work on 3-substituted 1,8,8-trimethyl-3-azabicyclo[3.2.1]octanes, I. The 1,8,8-trimethyl-3-azabicyclo[3.2.1]octanes have been known as camphidines because of the mode of preparation from imides of camphoric acid.[7] We have made the several compounds from camphidine rather than through the imides; the method of Schmidt and Klavehn[8] provided a convenient route from camphene to camphidine.

The compounds showed only moderate activities as depressants on automatic ganglia and the central nervous system.



I

R

a. $-CH_2CH_2N(CH_3)_2$
b. $-(CH_2)_6-$

EXPERIMENTAL[9]

1,8,8-Trimethyl-3-azabicyclo[3.2.1]octane (Camphidine). The procedure was essentially that outlined by Schmidt and Klavehn,[8] wherein a ring expansion of camphene with hydrazoic acid[10] gave a mixture of 1,8,8-trimethyl-3-azabicyclo[3.2.1]octene-2 with the related octene-3 (α- and β-dehydrocamphidine) which was reduced catalytically to camphidine.

Camphene (260 g., 1.9 mol.) was dissolved in 2.8 l. of benzene which contained hydrazoic acid (160 g., 3.7 mol.).[10] The stirred solution was kept at 5 to 12° during the addition of tin (IV) chloride (1075 g., 4.12 mol.) over a 2 hr. period. It was warmed to room temperature for 0.5 hr. and allowed to stir for an hour longer before cooling to 15° and basified (*p*H 9–10) with sodium carbonate solution. Stirring was rendered difficult by the separation of white solid. The tin salt was collected after chilling and extracted well with benzene, giving a total of *ca.* 7 l. benzene solution which was then extracted with 2*N* hydrochloric acid. The dehydrocamphidines were liberated from the acidic extracts with 35% sodium hydroxide and taken up in benzene. Concentration of the dried extracts *in vacuo* was done with use of a column and a Dry Ice trap. The mixed bases were obtained in total yield of 80% (240 g.) by reworking the distillates.

A solution of 240 g. (1.58 mol.) of the mixed 1,8,8-trimethyl-3-azabicyclo[3.2.1]octenes in 500 cc. of methanol was treated with 1 g. of Adams' catalyst and 5 g. of charcoal for reduction at 25° under 1500 p.s.i. The temperature rose to 43° during the reduction, which was completed in 3 hr. Careful removal of solvent left a quantitative yield of camphidine (243 g.). The camphoraceous base was distilled with some difficulty because of its volatility b.p. *ca.* 150° (200 mm.); small quantities were purified by sublimation, m.p. 168–170°.

Anal. Calcd. for $C_{10}H_{19}N$: N,[11] 9.14. Found: N,[11] 8.84.

3-(2-Dimethylaminoethyl)-1,8,8-trimethyl-3-azabicyclo-[3.2.1]octane. A mixture of 7.7 g. (0.05 mol.) of camphidine and 5.5 g. (0.05 mol.) of 2-dimethylaminoethyl chloride was heated on the steam bath for 0.5 hr., an additional 7.7 g. of camphidine was added (because some camphidine had deposited in the condenser), and the mixture was heated for 3 hr. The cooled material was diluted with ether and a quantitative recovery (9.5 g.) of camphidine hydrochloride was obtained. The product was left as a golden oil (9.4 g., 84%) when the filtrates were concentrated. It passed over as a colorless oil at 64–65° (0.3 mm.).

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