3-Cycloethylenedithio-5α-cholestane and 7-cycloethylenedithio-5α-cholestane were recovered unchanged from the hydroboration and subsequent oxidation procedures.

3-Cycloethylenedithiocholest-4-ene (I).—The product from the borohydration of this compound (920 mg., 2 mmoles) and subsequent oxidation of the resulting organoborane was chromatographed on alumina (60 g.). Elution with pentane gave starting material (400 mg.), while elution with pentane–benzene (2:1) gave 5β-cholestan-3α-ol (III, 250 mg., 0.64 mmole, 32%), m.p. 116–117° (from methanol), $[\alpha]_D$ +30°, lit.[9] m.p. 110.5–111.5°, $[\alpha]_D$ +31°. Oxidation with 8 *N* chromic acid in acetone at 0° gave 5β-cholestan-3-one, m.p. 62–63° (from methanol), $[\alpha]_D$ +36°, lit.[9] m.p. 61–62°, $[\alpha]_D$ +36°.

Elution with pentane–benzene (1:1) gave 5α-cholestan-4α-ol (II, 100 mg., 0.26 mmole, 13%), m.p. 188–189° (from methanol–ether), $[\alpha]_D$ +4°, lit.[4] m.p. 188–189°, $[\alpha]_D$ + 3°. Oxidation with 8 *N* chromic acid in acetone at 0° gave 5α-cholestan-4-one, m.p. 97–98° (from methanol), $[\alpha]_D$ +33°, lit.[4] m.p. 99–100°, $[\alpha]_D$ +30°.

7-Cycloethylenedithiocholest-5-ene.—The product from the borohydration of this compound (920 mg., 2 mmoles) and subsequent oxidation of the organoborane was chromatographed on alumina (70 g.). Elution with pentane gave starting material (500 mg.), while elution with pentane–benzene (3:1) gave 5α-cholestan-7β-ol (290 mg., 0.75 mmole, 38%), m.p. 113–114° (from methanol–ether), $[\alpha]_D$ +50°, lit.[10] m.p. 112–113°, $[\alpha]_D$ +52°. Oxidation with 8 *N* chromic acid in acetone at 0° gave 5α-cholestan-7-one, m.p. 116–117° (from methanol), $[\alpha]_D$ −45°, lit.[10] m.p. 116–118°, $[\alpha]_D$ −42°.

Elution then with pentane–benzene (1:1) gave 5α-cholestan-6α-ol (120 mg., 0.31 mmole, 16%), m.p. 126–128° (from ether–methanol), $[\alpha]_D$ +36°, lit.[4] m.p. 128–129°, $[\alpha]_D$ +35°. Oxidation with 8 *N* chromic acid in acetone at 0° gave 5α-cholestan-6-one, m.p. 95–97° (from methanol), $[\alpha]_D$ +7°, lit.[11] m.p. 96–98°, $[\alpha]_D$ +5°.

Acknowledgment.—The author would like to thank Dr. M. Green of this university for useful discussions.

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(10) R. J. W. Cremlyn and C. W. Shoppee, *J. Chem. Soc.*, 3515 (1954).
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Buxus Alkaloids. IX.[1] The Isolation and Constitution of Cyclobuxoxine[2]

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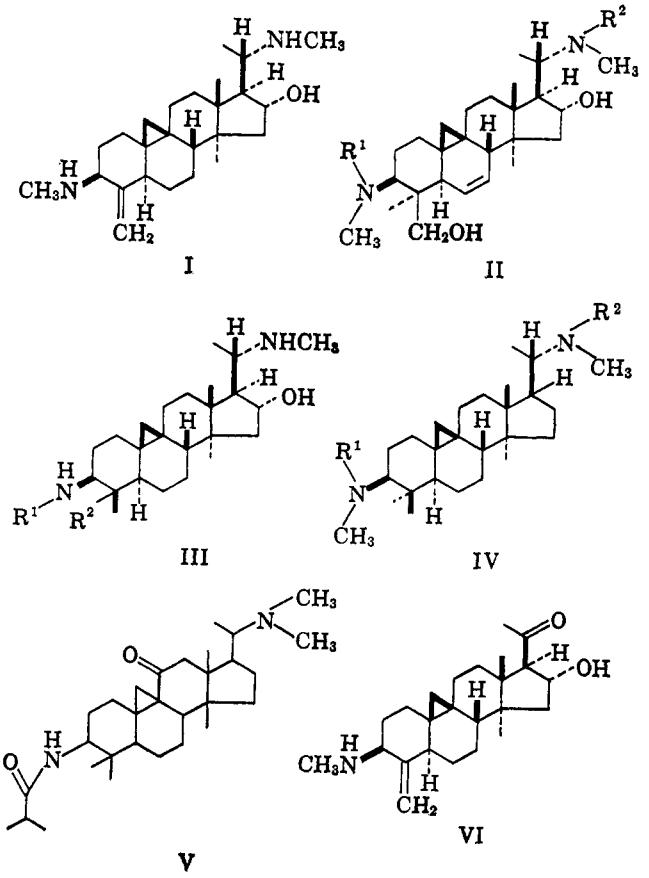
Received June 1, 1965

In 1962, we reported the elucidation of structure[3] and configuration[4] of cyclobuxine-D (I), an alkaloid isolated from *Buxus sempervirens* L.[5] Cyclobuxine-D was shown to be the prototype of a new class of steroidal alkaloids which contain a cyclopropane ring and which have a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme, between lanosterol- and cholesterol-type steroids. Subsequent studies have characterized the following structurally related alkaloids (see Chart I): cyclomicrophylline-A

CHART I



(II, $R^1 = R^2 = CH_3$),[6] cyclomicrophylline B (II, $R^1 = CH_3$; R^2 = H),[6,7] cyclomicrophylline-C (II, R^1 = H; $R^2 = CH_3$),[6] cyclobuxamine-H (III, $R^1 = R^2$ = H),[8] cyclovirobuxine-D (III, $R^1 = R^2 = CH_3$),[9] cycloprotobuxine-C (IV, R^1 = H; $R^2 = CH_3$),[10] cycloprotobuxine-D (IV, $R^1 = R^2$ = H),[1] and baleabuxine (V).[7] In addition, several new alkaloids containing a novel 9(10→19)-*abeo*-steroidal diene system have recently been isolated from *Buxus sempervirens* L.[11,12] The isolation from *Buxus sempervirens* L. and elucidation of the structure of an additional new alkaloid, cyclobuxoxine (VI), are described in the present report.

Cyclobuxoxine was isolated from the "weak bases" fraction obtained by the fractionation procedure described earlier.[3b] Adsorption chromatography on basic Woelm grade III alumina yielded cyclobuxoxine, $C_{24}H_{37}NO_2$, m.p. 181–183°, $[\alpha]^{27}_D$ +169° (*c* 0.63, chloroform). The n.m.r. data for cyclobuxoxine and its derivatives are shown in Table I. Its infrared spectrum, in Nujol, showed bands attributable to hydroxy and amino functions (2.82 and 2.97 μ), a carbonyl group (5.91 μ), and a terminal methylene group (6.18 and 11.18 μ).

(1) Part VIII: S. M. Kupchan and E. Kurosawa, *J. Org. Chem.*, **30**, 2046 (1965).
(2) This investigation was supported in part by Public Health Service Research Grants HE-02275 and HE-02952, from the National Heart Institute.
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(5) K. Heusler and E. Schlittler, *Helv. Chim. Acta*, **32**, 2226 (1949).
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(8) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4430 (1964).
(9) K. S. Brown, Jr., and S. M. Kupchan, *Tetrahedron Letters*, 2895 (1964). The convention on use of letter suffixes to designate substitution pattern at C-3 and C-20 nitrogen functions is described in this paper.
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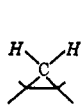
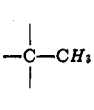
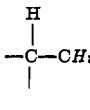
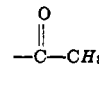
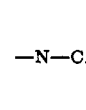
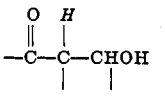
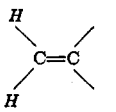
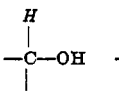
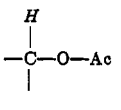
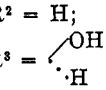
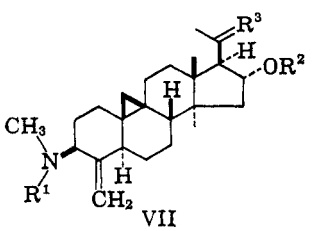
(12) D. Stauffacher, *Helv. Chim. Acta*, **47**, 968 (1964).

TABLE I
N.M.R. DATA[a]

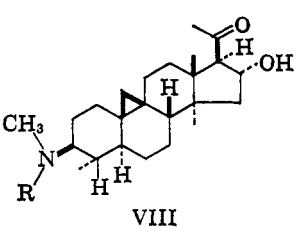
Compd.									
Cyclobuxoxine (VI)	9.93, 9.69 (2H) d, J = 4.5	9.08 (3H) s, 8.89 (3H) s	...	7.87 (3H) s	7.52 (3H) s	6.98 (1H) d, J = 7	5.42 (1H) s, 5.18 (1H) m	5.18 (1H) m	...
Dihydrocyclobuxoxine-a (VIII, R = H)	9.72, 9.40 (2H) d, J = 4	9.08 (3H) s, 8.89 (3H) s	9.21 (3H) d, J = 7	7.86 (3H) s	7.6 (3H) s	6.98 (1H) d, J = 7	...	5.13 (1H) m	...
O,N-Diacetylcyclobuxoxine (VII, R^1 = R^2 = $COCH_3$; R^3 = O)	9.85, 9.67 (2H)	9.05 (3H) s, 8.85 (3H) s	...	7.86 (3H) s, 8.05 (6H) s	7.1 (3H) d, J = 2	6.83 (1H) d, J = 7	5.55, 5.30 (2H) m	...	4.4 (1H) m
Dihydrocyclobuxoxine-b (VII, R^1 = R^2 = H; R^3 = )	9.93, 9.7 (2H)	8.9 (3H) s, 8.83 (3H) s	8.66 (3H) d, J = 6	...	7.52 (3H) s	...	5.40 (1H) s, 5.18 (1H) s	6.09 (2H) m	...

[a] All chemical shifts are reported in τ values (parts per million). The coupling constants, J, are expressed in cycles per second.

Acetylation with acetic anhydride in pyridine yielded O,N-diacetylcyclobuxoxine (VII, R^1 = R^2 = $CO-CH_3$; R^3 = O). The shift of the signal for the N-CH_3 group in the n.m.r. is probably due to restricted rotation.[3b,13] The infrared spectrum showed bands indicative of an acetate ester (5.74 and 8.05 μ) as well as a tertiary amide group (6.08 μ). Catalytic hydrogenation in ethanolic acetic acid furnished confirmatory evidence for the presence of exocyclic methylene in cyclobuxoxine, for the disappearance of the n.m.r. signal for exocyclic methylene was accompanied by the appearance of a signal for a secondary C-methyl group in dihydrocyclobuxoxine-a (VIII, R = H). Sodium borohydride reduction of cyclobuxoxine yielded dihydrocyclobuxoxine-b (VII, R^1 = R^2 = H; R^3 =), which showed the expected shift of the C-21 methyl group (τ 8.66, 3H, doublet of 6 c.p.s.), along with absence of the carbonyl band in the infrared. Thin layer chromatographic analysis of the sodium borohydride reduction product indicated that dihydrocyclobuxoxine-b had been produced in over 80% yield. The C-20 hydroxyl group in dihydrocyclobuxoxine-b is consequently assigned the β configuration.[14]



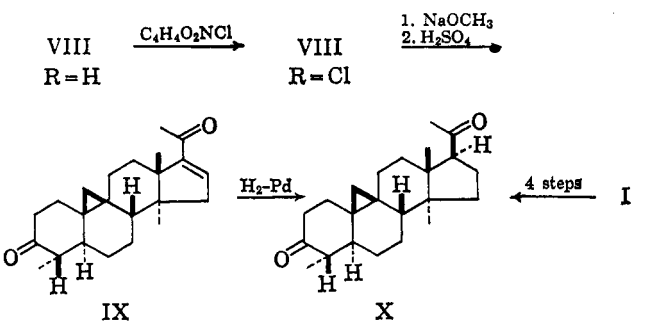
VII



VIII

Strong support for assignment of constitution VI to cyclobuxoxine was adduced by interrelation of the alkaloid with cyclobuxine-D. Ruschig degradation of dihydrocyclobuxoxine-a proceeded, *via* the crystalline monochloramine VIII, R = Cl, to the diketone X (4,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione) previously obtained from cyclobuxine-D (I).

Configurations at C-3 and C-16 were assigned on the basis of biogenetic analogy to the companion alkaloids cyclobuxine-D (I), cyclovirobuxine-D (III, R^1 = R^2 = CH_3), and cyclobuxamine-H (III, R^1 = R^2 = H).



Experimental Section[15,16]

Adsorption Chromatography of the Weak Bases. Isolation of Cyclobuxoxine.—The crude alkaloid mixture (10 g.) was dissolved in benzene (100 ml.) and filtered (residue weighed 0.7 g.). The solution was added to a column of basic Woelm grade III alumina (300 g.). Elution with benzene (4 l.) gave a noncrystalline mixture of alkaloids (4.6 g.). Ether (650 ml.) elution yielded

(13) *Cf.* J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959, pp. 366–371.

(14) *Cf., e.g.*, J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955); S. A. Szpilfogel, P. A. Van Hempert, and M. S. DeWinter, *Rec. trav. chim.*, **75**, 1227 (1956).

(15) Melting points were determined on a Kofler block. Infrared spectra were measured, unless otherwise specified, in chloroform solution on a Beckman Model IR-5A spectrophotometer. N.m.r. spectra were determined on a Varian A-60 spectrometer in deuteriochloroform. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(16) We are grateful to CIBA Pharmaceutical Co., for procurement and large-scale extraction of plant material, and especially thank Dr. E. Schlittler, Dr. D. Dickel, and Dr. K. Heusler for their kind interest and cooperation in this project.

crystalline cyclobuxoxine (329 mg.). Recrystallization from methanol–ether gave colorless plates: m.p. 181–183°; $[\alpha]^{27}D$ +169° (*c* 0.63, chloroform); λ_{max}^{Nujol} 2.82, 2.97, 5.91, 6.18, 11.18 μ; mass spectral peaks *m/e* 353 (M − 18), 338 (M − 18 − 15), 310 (M − 18 − 15 − 28).

Anal. Calcd. for $C_{24}H_{37}NO_2 \cdot 0.5CH_3OH$: C, 75.92; H, 10.14; N, 3.61. Found: C, 75.85; H, 10.28; N, 3.64.

O,N-Diacetylcyclobuxoxine (VII, $R^1 = R^2 = COCH_3$; R^3 = O).—A solution of cyclobuxoxine (20 mg.) in dry pyridine (2 ml.) and acetic anhydride (1 ml.) was allowed to stand at room temperature for 24 hr. Dilution with water (20 ml.) gave, after 4 hr. of standing, needles (19.5 mg.): m.p. 211–213°; $[\alpha]^{30}D$ +92° (*c* 0.25, chloroform); $\lambda_{max}^{CHCl_3}$ 5.74, 6.08, 8.05 μ.

Anal. Calcd. for $C_{28}H_{41}NO_4$: C, 73.81; H, 9.07; N, 3.07. Found: C, 73.55; H, 9.30; N, 3.21.

Dihydrocyclobuxoxine-a (VIII, R = H).—A solution of cyclobuxoxine (214 mg.) in 10% acetic acid in ethanol (20 ml.) was added to reduced platinum oxide (150 mg.) in the same solvent mixture (20 ml.). Hydrogenation was carried out at atmospheric pressure and room temperature for 3 hr. Filtration, evaporation of the solvent under reduced pressure, neutralization with 2 *N* ammonium hydroxide solution, extraction with chloroform, drying, and evaporation under reduced pressure yielded 210 mg. of colorless solid residue. Crystallization from acetone gave colorless needles (114 mg.), m.p. 198–200°, $[\alpha]^{30}D$ +100° (*c* 0.38, chloroform). Second and third crops totaled 81 mg.

Anal. Calcd. for $C_{24}H_{39}NO_2$: C, 77.16; H, 10.52; N, 3.75. Found: C, 77.01; H, 10.49; N, 3.87.

Dihydrocyclobuxoxine-b (VII, $R^1 = R^2 = H$; R^3 = ).—A solution of sodium borohydride (20 mg.) in methanol (2 ml.) was added dropwise at room temperature to a stirred solution of cyclobuxoxine (25 mg.) in methanol (2 ml.). The reaction mixture was refluxed on a steam bath for 2 hr. Treatment with 5% aqueous sodium bicarbonate, followed by extraction with chloroform, drying, and evaporation under reduced pressure yielded 19.5 mg. of colorless solid. Thin layer chromatographic analysis on silica gel G, using the upper layer of the system acetic acid–1-butanol–water (1:4:5) and spraying with Dragendorf reagent, showed a predominance of over 80% of the major product. Crystallization from methanol–ether gave colorless needles (10 mg.), m.p. 192–194°, $[\alpha]^{30}D$ +60° (*c* 0.05, chloroform).

Anal. Calcd. for $C_{24}H_{39}NO_2$: C, 77.16; H, 10.52; N, 3.75. Found: C, 76.90; H, 10.72; N, 3.65.

N-Chlorodihydrocyclobuxoxine-a (VIII, R = Cl).—A solution of dihydrocyclobuxoxine-a (50 mg.) in methylene chloride (2 ml.) was cooled to 0° and treated dropwise with stirring with a solution of N-chlorosuccinimide (20 mg.) in chloroform (1 ml.). After stirring for 10 min. at 0°, the solution was washed with water, dried, and evaporated to dryness under reduced pressure. A crystalline residue was obtained (55 mg.), m.p. 232° dec.

Ruschig Degradation of N-Chlorodihydrocyclobuxoxine-a.—The chloramine VIII (R = Cl, 55 mg.) was treated with a solution of sodium methoxide (100 mg.) in methanol (3 ml.), and the mixture was refluxed for 2 hr. After evaporation to dryness, water (5 ml.) was added. Extraction with chloroform, with subsequent drying and evaporation under reduced pressure, yielded a yellowish oil. The oil was dissolved in ethanol (6 ml.) and 6 *N* sulfuric acid (3 ml.), and the solution was allowed to stand at room temperature for 6 hr. The mixture was diluted with water and extracted with chloroform, and the chloroform extract was evaporated to dryness. The residue was filtered on Woelm neutral alumina, grade I (3 g.), using 5% ether in benzene (150 ml.). The crystalline residue (one–enone IX, 17.3 mg.) showed an infrared spectrum containing bands for a carbonyl group at 5.87 μ and for an α,β-unsaturated carbonyl group at 6.00 and 6.16 μ. A solution of the one–enone (17.3 mg.) in 10% acetic acid and ethanol (5 ml.) was added to 10% palladium on carbon (35 mg.) in the same solvent mixture (5 ml.). The hydrogen consumption under atmospheric pressure and at room temperature was complete in 30 min. Evaporation of the solvent under reduced pressure, followed by the addition of water (25 ml.), gave a crystalline solid, which was filtered and dried (16.8 mg.). Recrystallization from acetone–Skellysolve B gave colorless needles, m.p. 185–187°. The infrared spectrum was superimposable upon that of an authentic sample of 4,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione (X) and the melting point was not depressed upon admixture with the authentic sample.

Tumor Inhibitors. IX.[1] Synthesis of 10-Nitro-1-phenanthroic Acid[2,3]

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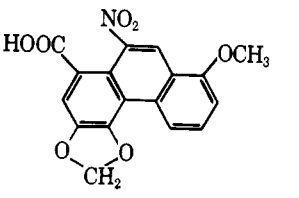
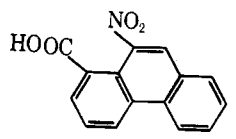
Received June 11, 1965

As part of a program on the synthesis of compounds structurally related to the naturally occurring tumor inhibitor, aristolochic acid (I),[5] we undertook the preparation of an analog with no oxygen–ether functions, namely, 10-nitro-1-phenanthroic acid (II). There were two principal methods available for the introduc-



I



II

tion of a nitro group into the phenanthrene nucleus.[6] The first involved direct nitration; the reaction affords a variety of isomers and is generally unsuited for the synthesis of specifically substituted compounds.[7] The second approach involved substitution of nitro for amino group in a Sandmeyer-type reaction. The latter method has been utilized effectively only for substituted nitrophenanthrenes and the yields for the majority of isomers seldom exceeded 10%.[8] Nevertheless, this method (Scheme I) was chosen in preference to the direct nitration method because of its specificity.

A Perkin condensation between the sodium salt of 2-bromophenylacetic acid (III)[9] and *o*-nitrobenzaldehyde (IV) afforded 2-bromo-2′-nitro-*cis*-stilbene-α-carboxylic acid (V). The nitro group was reduced using an ammoniacal solution of ferrous sulfate; a 65% yield of amino acid VI was obtained. 1-Bromo-10-phenanthroic acid (VIII) was then prepared by Pschorr cyclization of diazonium chloride VII. The method of Rutherford and Newman served to convert the acid into amine Xa *via* a variant of the Schmidt reaction.[10] A lithium–halogen exchange reaction, followed by a

(1) Part VIII: S. M. Kupchan, J. R. Knox, and M. S. Udayamurthy, *J. Pharm. Sci.*, **54**, 929 (1965).

(2) This investigation was supported in part by research grants from the National Cancer Institute (CA-04500) and the American Cancer Society (T-275).

(3) Abstracted from a part of the dissertation submitted by H. C. Wormser to the University of Wisconsin Graduate School, June 1965, in partial fulfillment of the requirements of the Ph.D. degree.

(4) American Foundation for Pharmaceutical Education Fellow, 1961–1963; National Institutes of Health Predoctoral Fellow, 1963–1965.

(5) S. M. Kupchan and R. W. Doskotch, *J. Med. Pharm. Chem.*, **5**, 657 (1962).

(6) A new approach to nitrophenanthrenes has subsequently been described: see S. M. Kupchan and H. C. Wormser, *Tetrahedron Letters*, 359 (1965); *J. Org. Chem.*, **30**, 3792 (1965).

(7) M. J. S. Dewar and E. W. T. Warford, *J. Chem. Soc.*, 3570 (1956).

(8) P. M. G. Bavin and M. J. S. Dewar, *ibid.*, 4477 (1955).

(9) G. S. Misra and J. S. Shukla, *J. Indian Chem. Soc.*, **28**, 480 (1951).

(10) K. G. Rutherford and M. S. Newman, *J. Am. Chem. Soc.*, **79**, 213 (1957).