reaction product was virtually identical with that of the protonated (uncharged) form of N-propionyl-O-cinnamoylhydroxylamine. This spectrum was independent of pH in the pH range 4–9.

Preparation of N-Propionyl-O-cinnamoylhydroxylamine.—Two methods of preparation of this compound were employed—the reaction of N-propionylhydroxamic acid with cinnamoyl chloride in anhydrous pyridine, and the reaction of the same hydroxamic acid with cinnamoylimidazole in anhydrous dioxane. Following reaction (which appeared to be nearly instantaneous in both instances) the organic solvent was removed by a flash evaporation *in vacuo*. The residual solid was extracted with acetate buffer, pH 5.5, and with H_2O. The crude sticky solid was extracted with ethyl acetate, and the ethyl acetate extract was evaporated to dryness. The resultant solid was recrystallized from hot water–dioxane; m.p. 119° (cor.); neut. equiv. calcd. 219, found 221.

Methods. pH Measurements.—All potentiometric titrations reported herein were carried out in 0.1 *M* KCl as solvent, employing KOH as titrant. A Radiometer precision pH meter (Model 25SE) was used throughout. All buffers were calibrated against National Bureau of Standards pH standards employing a Radiometer Model pH m-4 precision pH meter. All pH measurements were carried out in thermostated water-jacketed vessels maintained at 25.0 ± 0.1°.

Spectral Measurements.—A Cary Model 14 recording spectrophotometer was used in all measurements reported herein. Inner and outer cell compartments were thermostated at 25.0 ± 0.1°.

Kinetics of acylation and deacylation of cinnamoylimidazole were followed essentially according to the methods of Bender, Schonbaum, and Zerner.[3] The hydroxamates were all transparent above 270 mμ at pH 6.86 or below at the concentration levels employed, obviating the need for highly precise matching of reference and sample. At high pH (near or above the pK_A') significant absorption was noted below 280 mμ (although no new absorption peaks were detected above 230 mμ) and precise blanks were prepared in such experiments as well as in all experiments involving furoyl- or acetyl-acylating agents.

D_2O Experiments.—Reactions in D_2O were compared to reactions in H_2O by dilution of concentrated aqueous phosphate buffer with either D_2O or H_2O. The final concentration of D_2O was 97% in every D_2O experiment. The phosphate buffers maintained the pH (or pD) such that all measured rates were within 5% of the optimal rates (in the respective solvents) at this temperature.

$FeCl_3$ Test for Hydroxamates.—A stock solution containing 1.33% $FeCl_3$, 0.013 *M* HCl, and 0.53 *M* monochloracetic acid was used in all hydroxamate tests. This solution has the advantages of yielding stable (nonfading) ferric–hydroxamic acid complexes which are insensitive to dilution over the dilution range employed. In typical experiments, 10–500 × 10^{-3} ml. of sample was mixed with 3 ml. of stock solution and the optical density read at 520 mμ with a Beckman Model DU spectrophotometer. Aliphatic acylhydroxamates all gave the same extinction, *viz.*, 1.09 × 10^3 O.D./cm. *M*. Formohydroxamate gave a considerably lower extinction (0.85 × 10^3).

Acknowledgment.—This work was supported by research grants from the U. S. Public Health Service and the National Science Foundation.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN, MADISON 6, WIS.]

Buxus Alkaloids. III.[1] The Structure of Cyclobuxine

BY KEITH S. BROWN, JR.,[2] AND S. MORRIS KUPCHAN[3]

RECEIVED APRIL 6, 1964

Physical and chemical evidence have led to the assignment of structure Ia (3,20-bis(methylamino)-4-methylene-14-methyl-9,19-cyclopregnan-16-ol) to cyclobuxine, the major alkaloid of the acetone-insoluble portion of the bases of *Buxus sempervirens* L. The gross skeletal structure for cyclobuxine was indicated by the structures suggested for the products of selenium dehydrogenation (naphthalenes III and VI, phenanthrene V, and anthracene IV); dehydrogenation of decyclized cyclobuxine (XIa) gave only phenanthrene-related material. Key degradation products in the structure assignment were a conjugated diene (VIIIa) produced by Hofmann degradation; a mixture of *cis*- and *trans-cisoid* cyclopentenones (XVIa and b) arising from oxidation followed by facile elimination of methylamine; and the product from the ozonolysis of cyclobuxine followed by alkali treatment of the thus resultant α-aminoketone, the diosphenol XIIIa, which showed additional conjugation in the ultraviolet spectrum.

Extracts of *Buxus sempervirens* L. have been used since ancient times in the treatment of a wide variety of diseases, including malaria and venereal disease.[4] More recently an alkaloidal extract of the plant has been reported to possess antitubercular properties.[5] Previous chemical studies have indicated the multicomponent nature of the alkaloidal extract.[4,6–8] We have isolated four alkaloids from the acetone-insoluble portion of the strong bases of *B. sempervirens*, and have elucidated the structures of the three major bases. This paper reports the structure of the major alkaloid, cyclobuxine (Ia), the first steroidal alkaloid recognized to contain a cyclopropane ring and the first having a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme between lanosterol- and cholesterol-type steroids.[9] The configuration of cyclobuxine[10] and the constitutions of the remaining two alkaloids[11,12] will be presented in future communications.

Cyclobuxine (Ia), $C_{25}H_{42}ON_2$, demonstrated infrared bands for a terminal methylene (6.09 and 11.20 μ) and n.m.r.[13] peaks for the terminal methylene in an elec-

(1) Parts I and II: Keith S. Brown, Jr., and S. Morris Kupchan, *J. Am. Chem. Soc.*, **84**, 4590, 4592 (1962). The material presented in this paper was first outlined in Part I.

(2) National Science Foundation Cooperative Predoctoral Fellow in Chemistry, 1960–1962.

(3) To whom inquiries concerning this paper should be directed. This investigation was supported in part by research grants from the National Institutes of Health (H-2952 and CY-4500).

(4) E. Schlittler, K. Heusler, and W. Friedrich, *Helv. Chim. Acta*, **32**, 2209 (1949).

(5) L. E. Weller, C. T. Redemann, R. V. Gottshall, J. M. Roberts, E. H. Lucas, and H. M. Sell, *Antibiot. Chemotherapy*, **3**, 603 (1953); Merck and Co., Inc., British Patent 782,469 (1957).

(6) (a) K. Heusler and E. Schlittler, *Helv. Chim. Acta*, **32**, 2226 (1949); (b) W. Friedrich and E. Schlittler, *ibid.*, **33**, 873 (1950); (c) E. Schlittler and W. Friedrich, *ibid.*, **33**, 878 (1950).

(7) L. Laurent, Doctoral Dissertation, University of Toulouse, 1947, pp. 17–34. These investigations were published in (a) D. Vincent and T. Mathou, *Compt. rend.*, **220**, 474 (1945); (b) D. Vincent, I. Séro, and R. Laurent, *Thérapie*, **3**, 29 (1948); (c) D. Vincent and M. Parant, *Compt. rend. soc. biol.*, **148**, 1878 (1954).

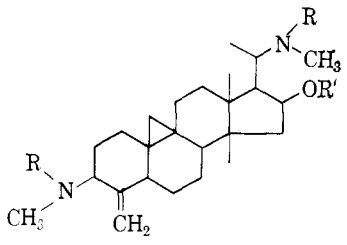
(8) K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatog.*, **9**, 71 (1962).

(9) Cyclobuxine is band II, the alkaloid of R_f 0.68 in column 1 of Table II, ref. 8. It is most probably the same as "alkaloid A" of ref. 6a; although no comparison sample of "A" is available, the physical constants of cyclobuxine and its derivatives correspond closely to those of "A" and the respective derivatives.

(10) K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4424 (1964).

(11) Cyclobuxamine; K. S. Brown, Jr., and S. M. Kupchan, *ibid.*, **86**, 4430 (1964).

(12) Cyclovirobuxine; K. S. Brown, Jr., and S. M. Kupchan, *Tetrahedron Letters*, in press.

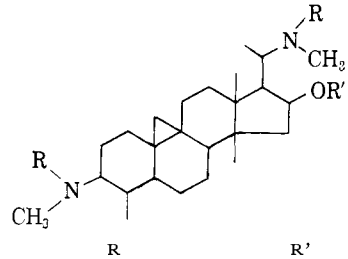


	R	R′
Ia,	H	H
b,	CH_3	H
c,	CH_3	$COCH_3$
d,	$COCH_3$	$COCH_3$
e,	$COCH_3$	H
f,	CHO	CHO
g,	p-$NO_2C_6H_4CH_2OCO$	H

tronically unsymmetrical environment (5.20 and 5.43 τ),[14] the grouping CH_2–C*H*OH–CH (5.29 τ, octet, J 3, 7, and 9.5 c./sec.), two N-methyls (7.53 and 7.57 τ), two tertiary C-methyls (8.87 and 9.03 τ), one secondary C-methyl (8.92 τ), and a cyclopropyl methylene (9.72 and 9.95 τ).[15] A number of disalts of cyclobuxine could be prepared by standard methods,[6a] showing both nitrogens to be present as basic amino groups. Formaldehyde–formic acid methylation gave an N,N′-dimethyl derivative Ib,[6a] with a characteristic strong sharp band for the dimethylamino group at 3.60 μ in the infrared spectrum, in addition to the strong terminal methylene bands at 6.09 and 11.10 μ. The n.m.r. spectrum of Ib showed a marked shift in the signal of the secondary methyl group from that of the parent alkaloid Ia (to 9.13 τ), indicating its proximity to one of the amino functions. O-Acetyl-N,N-dimethylcyclobuxine (Ic)[6a] showed the expected infrared band at 5.81 μ, and n.m.r. signals for the grouping CH_2–C*H*(OAc)–CH (4.95 τ) and O-acetate (8.03 τ).

Acetic anhydride–pyridine acetylation of cyclobuxine gave the O,N,N′-triacetyl derivative Id,[6a] showing strong bands in the infrared for an ester (5.78 μ) and two tertiary amide (6.14 μ, very strong) functions, as well as the terminal methylene (11.08 μ). The n.m.r. spectrum of the triacetyl derivative Id showed restricted internal rotation[16] of both N-methyl groups and of one N-acetyl group, which varied with the solvent. Milder acetylation of cyclobuxine (Ia) with acetyl chloride and potassium carbonate in benzene,[17] or mild hydrolysis of triacetylcyclobuxine Id gave the N,N′-diacetyl derivative Ie, lacking the 5.78 μ band in the infrared spectrum and showing in the n.m.r. spectrum restricted rotation of the N-acetyl group and of only one N-methyl group. An O,N,N′-triformyl derivative If (λ_{max} 5.78, 6.00, and 11.05 μ) was also prepared from cyclobuxine.

Hydrogenation of cyclobuxine with platinum in ethanolic acetic acid gave in 97% yield the single dihydro compound IIa,[6a,11] lacking the terminal methylene peaks in the infrared and n.m.r. spectra, and showing in the latter a signal for a new secondary methyl group (9.22 τ) as well as considerable deshielding of the cyclopropyl methylene (9.43 and 9.73 τ).



	R	R′
IIa,	H	H
b,	C_6H_5CO	H
c,	C_6H_5CO	$COCH_3$
d,	p-$NO_2C_6H_4CH_2OCO$	H
e,	p-$NO_2C_6H_4CH_2OCO$	$COCH_3$

Selenium dehydrogenation of cyclobuxine[6a,c] at 350–370° gave in 36% yield an exceedingly complex mixture of hydrocarbons, which could be separated by chromatography on silica gel and acetylated cellulose[18] into fractions roughly consisting of tetrahydroanthracenes, tetrahydrophenanthrenes, trisubstituted anthracenes, and 1,2,8-trisubstituted phenanthrenes[19] (in order of decreasing percentage of the mixture). These were then further purified through the formation of crystalline complexes with 1,3,5-trinitrobenzene[20]; only one hydrocarbon was obtained in fully purified form, an 8-methyltetrahydrophenanthrene derivative, for which structure III is suggested on the basis of elemental analysis ($C_{21}H_{28}$) and the spectral properties: λ_{max} 12.21 μ (two adjacent aromatic protons[21]); λ_{max}^{EtOH} 233.5, 280.5, 285 sh, 290.5, and 325.5 mμ; n.m.r. spectrum showed the presence of: (1) two α- and three β-aromatic protons,[22] supporting the 1,2,5-trisubstituted naphthalene structure; (2) an aromatic methyl group, the grouping Ar–C*H*$_2$–CH_2, and no additional protons on carbons directly attached to the aromatic nucleus; and (3) two tertiary methyl groups, and two primary (ethyl) methyl groups, one strikingly deshielded (8.08 τ).[23]

In an effort to isolate and characterize members of the other hydrocarbon classes detected in the dehydrogenation mixture from cyclobuxine, a mother-liquor fraction in which cyclobuxine was at least 30% of the weight was dehydrogenated on a larger scale. Similar work-up gave a mixture nearly identical with that obtained from pure cyclobuxine, from which were isolated the pure tetrahydrophenanthrene derivative III and three additional pure hydrocarbons (one

(13) We thank Mr. R. Matsuo and Mr. A. Krubsack for the n.m.r. determinations. All chemical shifts are reported in τ-values (p.p.m.).

(14) *Cf.* M. D. Nair and R. Adams, *J. Am. Chem. Soc.*, **83**, 922 (1961).

(15) R. McCrindle and C. Djerassi, *J. Chem. Soc.*, 4034 (1962); this assignment was made in this work independently on the basis of the coupling constant of the AB doublets (4 c./sec.); *cf.* H. S. Gutowsky, M. Karplus, and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).

(16) *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.

(17) *Cf.* W. J. Hickinbottom, "The Reactions of Organic Compounds," 3rd. Ed., Longmans, Green and Co., New York, N. Y., 1957, pp. 404–405.

(18) T. M. Spotswood, *J. Chromatog.*, **3**, 101 (1960).

(19) F. A. Askew, *J. Chem. Soc.*, 509 (1935); E. Heilbronner, H. U. Daniker, and P. A. Plattner, *Helv. Chim. Acta*, **32**, 1723 (1949); H. Dannenberg and W. Steidle, *Z. Naturforsch.*, **9b**, 294 (1954).

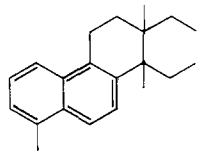
(20) Picrate complexes of these aromatic hydrocarbons were highly unstable, possibly because of steric interaction of the picric acid with the aliphatic portions of the aromatic donors.

(21) *Cf.* L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 75–79.

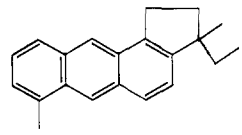
(22) See ref. 16, pp. 247–254.

(23) Examination of a Dreiding model of III (assuming the configuration[10] unchanged from that of cyclobuxine) reveals that the methyl of the ethyl group nearest the aromatic nucleus, owing to interaction with the other ethyl and two methyl groups, may most favorably occupy a position near the plane of the aromatic nucleus adjacent to the C-3 proton, in accord with the observed anomalous n.m.r. spectral signal position (ref. 16, pp. 180–183; J. S. Waugh and R. W. Fessenden, *J. Am. Chem. Soc.*, **79**, 846 (1957); H. Conroy in "Advances in Organic Chemistry—Methods and Results," Vol. II, R. A. Raphael, E. C. Taylor, and H. Wynberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1960, pp. 282–284.

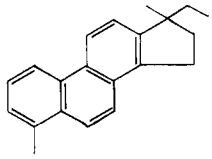
representing each of the additional classes of compound produced in the reaction), for which structures IV, V, and VI are suggested on the basis of elemental analyses and spectral properties. The 5-methyl-



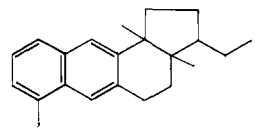
III



IV



V

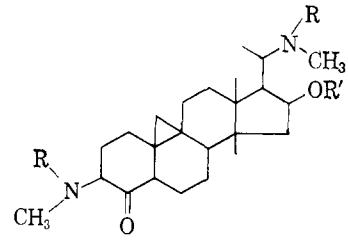


VI

cyclopentenoanthracene derivative IV ($C_{21}H_{22}$) showed λ_{max} 11.36 (lone aromatic protons at C-9 and C-10[21]), 12.53 (two adjacent aromatic protons[21]), and 13.50 (in cyclohexane; three adjacent aromatic protons[21]) μ; λ_{max}^{EtOH} 259.5, 334, 350, 367, and 385.5 mμ; n.m.r. spectrum showing the presence of (1) the 9- and 10-protons, and 2α- and 3β-aromatic protons,[22] supporting 1,2,5-trisubstitution; (2) an aromatic methyl group, the grouping Ar–CH_2–CH_2, and no additional protons on carbons bound directly to the aromatic nucleus; (3) a tertiary C–ethyl group, a tertiary C–methyl group, and the grouping CH_2–CH_2–C. The other trinuclear aromatic hydrocarbon, the 8-methyl-1,2-cyclopentenophenanthrene derivative V, was highly crystalline: $C_{21}H_{22}$, λ_{max} 12.22 μ (two adjacent aromatic protons[21]); λ_{max}^{EtOH} 261, 284, 294.5, 307 mμ; n.m.r. spectrum showing the presence of (1) the C-4,5,9, and 10 protons, and three additional aromatic protons, supporting 1,2,8-trisubstitution[22]; and (2) aliphatic protons essentially identical with those seen in the spectrum of the anthracene IV, indicating the aliphatic portions of the two molecules to be identical. The crystalline representative of the tetrahydroanthracenes, VI (C_{22}-H_{28}), showed λ_{max} 11.35 μ (lone aromatic protons at C-9 and C-10[21]), λ_{max}^{EtOH} 233.5 (weaker than in the tetrahydrophenanthrene derivative III), 277.5, 287, and 324.5 mμ; n.m.r. spectrum showing the presence of (1) 3-α- and 2β-aromatic protons,[22] supporting the 2,3,5-trisubstituted naphthalene structure; (2) an aromatic methyl, the grouping Ar–CH_2–CH_2, and no additional protons on carbons bound directly to the aromatic nucleus; and (3) a primary methyl group, and two tertiary methyl groups, one strongly shielded (9.53 τ).[24]

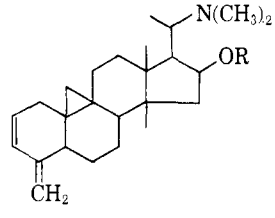
In an effort to bring some clarity into the confusing picture presented by these products, a small sample of decyclized cyclobuxine (XIa) was dehydrogenated at 330°. Similar work-up gave a total binuclear aromatic fraction (9%) with infrared and ultraviolet spectral characteristics identical with those of III (λ_{max} 12.21 μ, no trace of 11.35 μ; λ_{max}^{EtOH} 233.5, 280.5, 285, 290.5, and 325.5 mμ, no trace of 277.5 or 287 mμ), and a fluorescent trinuclear aromatic fraction (7%) whose ultraviolet spectrum was typical for an 8-methyl-1,2-cyclopentenophenanthrene derivative[19] (λ_{max}^{EtOH} 262, 283, 294, and 307 mμ) and showed no trace whatsoever of anthracene absorption above 340 mμ. Therefore, the complexity of the hydrocarbon mixture from cyclobuxine is related to the presence of the cyclopropane ring, anthracenoid material (IV, VI) possibly resulting from initial cleavage of the 1,-10- or 9,11-bond, followed by recyclization onto C-6 or C-7 and elimination of the cyclopropyl methylene.

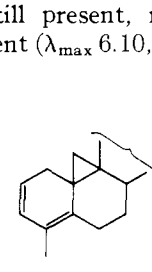
Ozonolysis of O,N,N′-triacetylcyclobuxine (Id) gave the ketone VIIa (λ_{max} 5.79 μ, stronger than in the starting material), $C_{30}H_{46}O_5N_2$ (showing replacement of C=CH_2 by C=O), n.m.r. confirming the loss of the methylene grouping and showing the N-acetyl group and one N-methyl group with freed rotation[25]; this compound developed a deep red color under the conditions of the Zimmermann test,[26] but the spectrum of the product showed only slowly rising absorption in the 400–600 mμ region (no maximum), so the test was assumed to be negative.



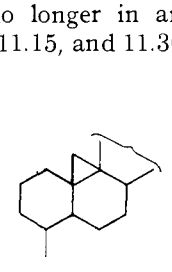
VIIa, R = $COCH_3$, R′ = $COCH_3$
b, R = p-$NO_2C_6H_4CH_2OCO$, R′ = H
c, R = H, R′ = H

Hofmann degradation[6a] of a monomethiodide prepared from N,N′-dimethylcyclobuxine (Ib) led to a des-N-base VIIIa[6a] in addition to an appreciable recovery of N,N′-dimethylcyclobuxine (10–20%) and a small amount of a homoannular diene isomer (presumably IX) which was also produced by acid or chloranil treatment of the major product VIIIa. The major product ($C_{25}H_{39}ON$) possessed an acylable hydroxyl group and a basic dimethylamino function (also evident in the infrared and n.m.r. spectra); the C-methyl groups showed n.m.r. signals unchanged from those in the starting material Ib. The terminal methylene group was still present, no longer in an unsymmetrical environment (λ_{max} 6.10, 11.15, and 11.30





IX



X

VIIIa, R = H
b, R = $COCH_3$

μ; n.m.r. 5.34 τ (2H)), and *conjugated with a new double bond* (λ_{max}^{EtOH} 229.5 mμ (log ϵ 4.22); 2 moles uptake

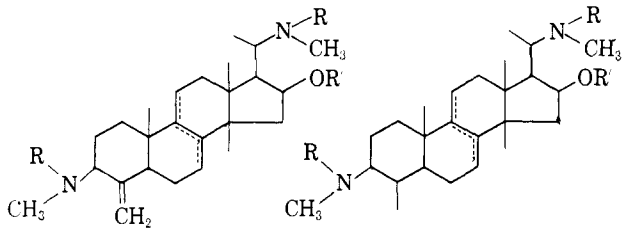
(24) Examination of a Dreiding model of VI (assuming the configuration[10] unchanged from that of cyclobuxine) reveals that the strain imposed by the cyclopentane ring forces the tertiary methyl group on the second carbon removed from the 2-position to lie nearly under the 2,3-bond of the aromatic system, in accord with the observed shielding of the n.m.r. spectral signal (see ref. 23, and also M. Gorman, N. Neuss, and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962)).

(25) Examination of a Dreiding model of VIIa shows that the 3-acetylmethylamino group suffers much less hindrance to rotation in the case of a ketone at C-4 than when there is a bulky methylene group at the same position.

(26) W. Zimmermann, *Z. physiol. Chem.*, **300**, 141 (1955).

upon hydrogenation, n.m.r. 3.6–4.6 τ (ABX pattern)). The degradation product VIIIa would not add maleic anhydride under forcing conditions, and was not dehydrogenated to aromatic material by chloranil.[27]

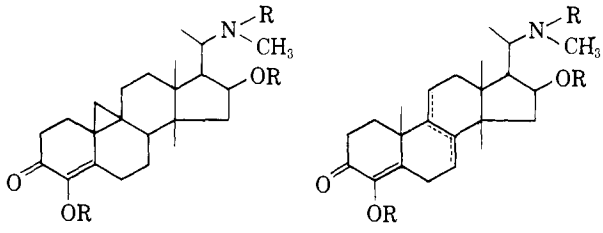
Decyclization[28] of various derivatives of cyclobuxine led to mixtures (presumably XI and XII) exhibiting



XIa, R = H, R′ = H
b, R = $COCH_3$, R′ = $COCH_3$
c, R = p-$NO_2C_6H_4CH_2OCO$, R′ = H

XIIa, R = H, R′ = H
b, R = $COCH_3$, R′ = $COCH_3$
c, R = $COCH_3$, R′ = H

a new tertiary methyl group in the n.m.r. spectra, in accord with the presence in each precursor of a cyclopropyl methylene; the double bonds thus formed could not be hydrogenated to any appreciable extent (typical of steroid 7,8- and 8,9-double bonds, less so for 9,11-), and showed only a partial new vinyl hydrogen in the n.m.r. spectra. Ozonolysis of cyclobuxine di-p-nitrobenzylcarbamate (Ig) and hydrogenolysis of the product gave an unstable α-aminoketone (VIIc) which upon treatment with base was oxidized and hydrolyzed to the diosphenol XIIIa (analyzed as the triacetyl derivative XIIIb). The ultraviolet spectra of the diosphenol XIIIa (λ_{max}^{EtOH} 296.5 mμ (log ϵ 3.95), $\lambda_{max}^{EtOH-NaOH}$ 343.5 mμ (log ϵ 3.81)) and of its triacetyl derivative XIIIb (λ_{max}^{EtOH} 277 mμ (log ϵ 4.10)) showed additional conjugation over those of known steroidal 3,4-diosphenols (*e.g.*, from cholesterol, λ_{max}^{EtOH} 278 mμ, acetate λ_{max}^{EtOH} 247 mμ (log ϵ 4.18)[29]; from cevagenine, λ_{max}^{EtOH} 278 mμ,

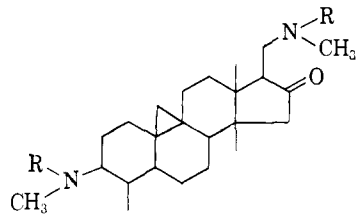


XIIIa, R = H
b, R = $COCH_3$

XIVa, R = H
b, R = $COCH_3$

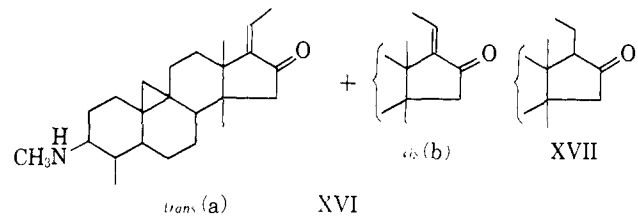
$\lambda_{max}^{EtOH-NaOH}$ 320 mμ[30]). The corresponding decyclized diosphenol (probably XIV) had normal spectral characteristics: λ_{max}^{EtOH} 277 mμ, $\lambda_{max}^{EtOH-NaOH}$ 322 mμ, triacetyl derivative λ_{max}^{EtOH} 247 mμ (log ϵ 4.10). These observations lent strong support to the presence of a 9β,19-cyclopropane ring in cyclobuxine.

Chromic acid oxidation of a series of nitrogen-protected derivatives of dihydrocyclobuxine gave ketones (XV) showing λ_{max} 5.78–5.80 μ (five-membered-ring carbonyl) and strong Zimmermann tests.[26] It was not possible to move the double bond of decyclized materials into conjugation with these ketones (in accord with a skeletal structure possessing an intermediate quaternary carbon, C-14). The ketones could also be obtained by oxidation with manganese dioxide, which ordinarily attacks only allylic alcohols.[31] The parent ketone XVd, with the nitrogens unprotected



XVa, R = CH_3CO
b, R = C_6H_5CO
c, R = p-$NO_2C_6H_4CH_2OCO$
d, R = H

(prepared through N,N′-di-p-nitrobenzyloxycarbonyldihydrocyclobuxine, IId), rapidly eliminated methylamine in basic solution, giving a mixture of *cis*- and *trans-cisoid* cyclopentenones (XVIa and b) ,λ_{max} 5.85, and 6.09 μ, λ_{max}^{EtOH} 244 mμ (log ϵ 3.89), which were interconvertible in basic solution and separable by partition chromatography, and gave positive Zimmermann tests[26] (confirming the environment of the original alcohol as CH_2–CHOH–CH). Configurations were assigned to the two isomers by their n.m.r. spectra; in the less polar compound XVIa, the vinyl methyl group signal (doublet, J 7 c./sec. at 7.92 τ) was relatively deshielded (δ from more polar −0.27 p.p.m.), hence it was the *trans* isomer as shown[32]; in the more



trans (a) XVI *cis* (b) XVII

polar (*cis*) isomer XVIb, the vinyl proton group signal (quadruplet, J 7.5 c./sec. at 3.53 τ) was similarly deshielded (δ from less polar −0.82 p.p.m.).[33] Equilibrium mixtures consisted of 50–70% of the *cis* isomer XVIb.[34]

From these data, structure Ia (3,20-bis(methylamino)-4-methylene-14-methyl-9,19-cyclo-pregnan-16-ol) was assigned to cyclobuxine. Further substantiation of this structure, and evidence relating to the configuration of cyclobuxine, is in the following paper.[10]

(27) *Cf.* E. A. Braude, L. P. Jackman, R. P. Linstead, and G. Lowe, *J. Chem. Soc.*, 3123 (1960).
(28) *Cf.* D. H. R. Barton and P. deMayo, *ibid.*, 2178 (1953).
(29) L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.*, **76**, 1728 (1954).
(30) E. Sundt, O. Jeger, and V. Prelog, *Chem. Ind.* (London), 1365 (1953).
(31) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J. Chem. Soc.*, 1094 (1952); see, however, I. T. Harrison, *Proc. Chem. Soc.*, 110 (1964).
(32) Named by the previous convention (L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948)).
(33) *Cf.* L. M. Jackman and R. H. Wiley, *J. Chem. Soc.*, 2881 (1960), and ref. 14.
(34) This is not in accord with earlier suggestions on the relative stability of 17-ethylidene steroids (but note that the 16-ketone complicates the picture); ref. 32, and L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, pp. 615–617.

Experimental[35]

Isolation of the Total Alkaloids.[4,36]—Dried leaves of *Buxus sempervirens* L. (7.85 kg.), ground to a fine powder, were macerated 10 hr. with 0.5% methanolic acetic acid (30 l.), transferred to a percolation apparatus, and percolated slowly with an additional 30 l. of the same solvent. The total extract was evaporated to 7 l. at 35° under reduced pressure, water (7 l.) was added, and the resulting suspension was kept at 0° for 2 weeks, filtered, concentrated to 7.0 l., and refiltered through a bed of Celite; the total alkaloid mixture (189 g., 2.4%) was recovered from the filtrate with ammonium hydroxide and chloroform.

The large-scale extraction was performed at Ciba Pharmaceutical Co. in Summit, N. J.[36] The dried leaves, collected in Basle, Switzerland (135 kg.), were ground to 20 mesh and defatted with petroleum ether (b.p. 60–90°) (400 and 300 l.), recycling each volume 3 hr.; these extracts were discarded. The leaves were then extracted in the cold with 0.5% methanolic acetic acid (2 × 400 l.) and methanol (2 × 400 l.). The total extracts were combined and evaporated to 135 l., and water (135 l.) was added; the suspension was filtered with the aid of Supercel, washed with water, evaporated to 100 l., and (with cooling) made basic to pH 9.5 with concentrated ammonium hydroxide. The basic solution was extracted with chloroform (3 × 100 l.) to give the crude mixed alkaloids (including tars and some nonalkaloidal material); 4.0 kg. (2.66%).

Preliminary Fractionation.[4]—The crude total alkaloid mixture from 7.85 kg. of leaves (189 g.) was dissolved in 2 *N* acetic acid (1500 ml.) and extracted with chloroform (3 × 600 ml.); the chloroform extracts were combined, back-extracted with 2 *N* ammonium hydroxide (1000 ml.), evaporated to give 95 g., and dissolved in 2 *N* hydrochloric acid (200 ml.) and chloroform (200 ml.). After agitation, the chloroform layer was separated and further extracted with 2 *N* hydrochloric acid (250 ml.); the acidic solutions were combined, yielding upon basification and extraction with chloroform the "weak bases" (4.8 g.). The original acetic acid solution above was neutralized to pH 7 (until the white flocculent bases barely started to precipitate) with concentrated ammonium hydroxide; extraction with chloroform (3 × 600 ml.) gave the "moderate bases" (21.3 g.). The remaining aqueous solution was then made basic to pH 9.5 with ammonium hydroxide and extracted with chloroform (2 × 600 ml.) to give the "strong bases" (71.7 g.); the aqueous layer then showed a negative Mayer test for alkaloids.

A large-scale preliminary fractionation was performed at Ciba[36]: crude total alkaloid (4.0 kg.) was dissolved in 2 *N* acetic acid (33 l.), and the solution extracted with chloroform (3 × 22 l.); the combined extracts were evaporated to 6.6 l., and extracted with 2 *N* hydrochloric acid (3 × 11 l.) and with water (1 × 5, then 4 × 2.1 l.) to give the "weak bases" (210 g.) and the "additional weak bases" (287 g.), respectively (after basification and chloroform extraction of the combined aqueous extracts). Both of the latter fractions were discovered to be mixtures of all three classes of bases, with the "weak" predominating. The "strong bases" and "moderate bases" (about 2.0 kg.) were then recovered as a mixture by basification and chloroform extraction; from this mixture (1200 g.), pure "strong base" fraction (1020 g.) was isolated in this laboratory by the above procedure.

Acetone-Insoluble Strong Bases.[4]—Total crude "strong base" fraction (330.6 g.) from 30 kg. of leaves was triturated with the upper phase of system A below (3300 ml.); this left a dark tarry material (not investigated further) and gave a clear yellow solution yielding upon evaporation the purified "strong base" fraction (281.6 g.), which was triturated with alcohol-free acetone (1400 ml.), leaving a white residue (17.98 g.). The supernatant was concentrated to 300 ml. and cooled to −20° for 1 week, giving another 11.89 g. of solid. The combined solids (29.97 g.) were further fractionated as below; the soluble portion was retained for further separations.

Partition chromatography was performed on dry-packed columns[8] having characteristics as designated below.

System A: hexane–ethylene dichloride–methanol–water (100:50:20:3)[8]
- column A-1: 1.8 cm. i.d., 20 ml. lower phase + 5 mg. phenol red on 30 g. of Celite 545, run until fully wet, retention volume 45 ml.
- column A-2: 4.5 cm. i.d. 200 ml. lower phase + 80 mg. phenol red on 330 g. of Celite 545, run until fully wet, retention volume 500 ml.

System B: hexane–ethylene dichloride–methanol–water (40:12:8:1)
- column B: 1.8 cm. i.d. 20 ml. lower phase + 5 mg. phenol red on 30 g. of Celite 545, run until fully wet, retention volume 45 ml.

System C: hexane–ethylene dichloride–methanol–water (250:25:50:4)
- column C: 1.8 cm. i.d.; 20 ml. lower phase + 5 mg. brom thymol blue on 30 g. of Celite 545, run until fully wet, retention volume 45 ml.

Separation of the Acetone-Insoluble Strong Bases by Partition Chromatography.[8]—The acetone-insoluble strong base fraction (89.46 g.) was dissolved in the upper phase of system A (900 ml.) and chromatographed in 10-ml. (1 g.) portions in a continuous fashion (adding a new portion after passage of 550 ml. from the previous portion) on a series of columns A-2. Four red bands were visible on the column, at R_f 0.76, 0.68, 0.59, and 0.48, with a minor band at 0.18; the first three of these (**cyclovirobuxine, cyclobuxine, and cyclobuxamine**, respectively) were collected separately, while the remaining two bands, and areas between the earlier bands, were collected together as a mixed fraction for rechromatography and selenium dehydrogenation experiments.

The cyclovirobuxine band[12] (26.8 g., R_f 0.76) crystallized from acetone to give 16.45 g., m.p. near 215°; the mother liquor was added to the above mixed fraction.

The cyclobuxine band (32.0 g., R_f 0.68) crystallized from methanol to give 20.08 g., m.p. near 244° dec.; the mother liquor was added to the above mixed fraction.

The cyclobuxamine band[11] (13.6 g., R_f 0.59) crystallized from acetone to give cyclobuxamine isopropylidenimine[11] (6.57 g.), m.p. near 240° dec.; the mother liquor was added to the above mixed fraction.

Purification of Cyclobuxine.—Crude cyclobuxine as above (1.0 g., m.p. 244–245° dec., about 5% cyclovirobuxine by the n.m.r. peak at 9.27 τ) was treated with dilute methanolic hydrobromic acid to neutrality; isopropyl ether was added, and the solution was allowed to evaporate slowly at room temperature. This gave cyclobuxine dihydrobromide (1125 mg. in two crops), transformed back to the base (715 mg.) by partition between dilute ammonium hydroxide and chloroform; crystallization from benzene gave pure cyclobuxine (Ia, 580 mg.), m.p. 245–247° dec., $[\alpha]^{23}D$ +98° (*c* 4.40); λ_{max} 6.09 and 11.20 μ; n.m.r.[13] 5.20, 5.43 (2H, two singlets; terminal methylene[14]), 5.92 (1H, octet, *J* 3, 7, and 9.5 c./sec.; $CH_2CHOCHC$), 7.53, 7.57 (6H, two sharp peaks; 2 N-methyl), 8.87, 9.03 (6H, two sharp peaks; 2-tertiary C-methyl), 8.92 (3H, doublet *J* 6 c./sec.; secondary C-methyl), 9.72, and 9.95 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene[15]).

Anal. Calcd. for $C_{25}H_{42}ON_2$: C, 77.66; H, 10.95; N, 7.25. Found: C, 78.09; H, 10.61; N, 7.19.

Salts[6a] were prepared with the appropriate acids in ethanol–ethyl ether of methanol–isopropyl ether:

The dihydriodide[6a] showed m.p. 276–278° dec.

Anal. Calcd. for $C_{25}H_{44}ON_2I_2$: C, 46.73; H, 6.90; I, 39.50. Found: C, 46.86; H, 7.04; I, 38.88.

The dihydrobromide showed m.p. 288–292° dec. (rapid heating).

Anal. Calcd. for $C_{25}H_{44}ON_2Br_2$: C, 53.50; H, 8.09; Br, 29.14. Found: C, 53.66; H, 8.12; Br, 27.73.

The diperchlorate[6a] showed m.p. 244–245° dec.

The dioxalate[6a] (amorphous, insoluble in ethanol) showed m.p. 264–267° dec.

The O,N,N'-triacetyl derivative Id[6a] was prepared with acetic anhydride–pyridine, 46 hr. at room temperature; crystallized from ethyl acetate, m.p. 256–258° dec. (if heated slowly from 25°), 246–248° dec. (if placed on the stage at 200°); R_f on column A-1 0.63; $[\alpha]^{24}D$ −12° (*c* 2.40); λ_{max} 5.78 (s) and 6.14 (vs) μ; n.m.r.

(35) Melting points, taken on a Köfler block, are corrected to the nearest degree. Infrared spectra were measured in chloroform solution on a Beckman Model IR-5A spectrophotometer; ultraviolet spectra were measured in ethanol solution on a Cary Model 11MS spectrophotometer. Rotations, determined in chloroform solution, have been approximated to the nearest degree. The authors are indebted to Mr. J. Alicino (Metuchen, N. J.) for the bulk of the microanalyses; a few were performed by Mr. S. Nagy and Associates (Cambridge, Mass.). All samples were dried for at least 12 hr. at 60° and 0.1 mm. pressure; note, however, that some samples were hygroscopic.

(36) We are grateful to Ciba Pharmaceutical Company for procurement and large-scale extraction of plant material, and especially thank Drs. Emil Schlittler, Daniel Dickel, and Karl Heusler for their kind interest and cooperation in this project.

4.70–5.70 (3H, complex; terminal methylene + $CH_2CH(OAc)$-CH), 7.07, 7.20 and 7.10, 7.23 (6H, two split peaks; 2 N-methyl with restricted rotation[16]), 7.85, 7.93 (3H, split peak; N-acetyl with restricted rotation), 7.98 (3H, unsplit; N-acetyl), 8.02 (3H, unsplit; O-acetyl), 8.86, 8.87 (6H, two sharp peaks; 2 tertiary C-methyl), 8.87 (3H, doublet, J 6 c./sec.; secondary C-methyl), 9.62, and 9.83 τ (2H, AB doublets, J 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{31}H_{48}O_4N_2$: C, 72.62; H, 9.44. Found: C, 72.35; H, 9.36.

The O,N,N'-triformyl derivative If was prepared with acetic anhydride–100% formic acid (1:1, preheated 1 hr. at 70°), 40 hr. at room temperature; crystallized from ethyl acetate, m.p. 224–225° dec.; R_f on column A-1 0.56; λ_{max} 5.78 (s), 6.00 (vs), and 11.05 (m) μ.

Anal. Calcd. for $C_{28}H_{42}O_4N_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.17; H, 9.04; N, 5.79.

N,N'-Diacetylcyclobuxine (Ie).[17]—(a) Cyclobuxine (200 mg.) was stirred 1 hr. at room temperature with acetyl chloride (140 mg.) and potassium carbonate (2 g.) in benzene (30 ml.). Chloroform (20 ml.) and water were added, and after additional stirring for 1 hr. the product (295 mg.) was recovered from the organic layer and crystallized from ethanol to give 205 mg., m.p. 272–274° dec. Pure material (obtained by three recrystallizations from ethanol) showed m.p. 278–281° dec., $[\alpha]^{24}D$ +10° (c 1.94); λ_{max} 2.95 (m) and 6.15 (vs) μ; n.m.r. 4.75–6.35 (4H, complex; terminal methylene + $CH_2CHOHCH$ + OH), 7.17 (3H, unsplit; N-methyl), 7.15, 7.20 (3H, split peak; N-methyl with restricted rotation), 7.99 (3H, unsplit; N-actyl), 7.91, 7.99 (3H, split peak; N-acetyl with restricted rotation), 8.90, 8.98 (6H, two sharp peaks; 2 tertiary C-methyl), 8.97 (3H, doublet, J 6 c./sec.; secondary C-methyl), 9.73, and 9.97 τ (2H, broad peaks; cyclopropyl methylene).

Anal. Calcd. for $C_{29}H_{46}O_3N_2 \cdot 2H_2O$: C, 68.74; H, 9.95; N, 5.53. Found: C, 68.47; H, 9.67; N, 5.27.

(b) **O,N,N'-Triacetylcyclobuxine** (Id, 30 mg.) was hydrolyzed 10 min. with refluxing 5% ethanolic potassium hydroxide (4 ml.). The product, recovered with dilute hydrochloric acid and chloroform, was crystallized from ethanol to give 21 mg., m.p. 277–280°, m.m.p. with authentic diacetate Ie 278–281°, infrared spectrum superimposable upon that of authentic material.

N,N'-Dimethylcyclobuxine (Ib).[6a]—Cyclobuxine (300 mg.) was heated at reflux overnight with 88% formic acid (0.45 ml.) and 40% formaldehyde (0.30 ml.); water (20 ml.) and 2 N hydrochloric acid (2 ml.) were added, and the solution was extracted with ether (2 × 20 ml.), basified with ammonium hydroxide, and extracted with chloroform (2 × 30 ml.) to give the product (340 mg.), crystallized from ethanol to give Ib (245 mg.), m.p. 204–205°, $[\alpha]^{25}D$ +99° (c 2.20); λ_{max} 3.00 (m), 3.60 (m), 6.09 (m), 9.95 (s), and 11.10 (m) μ; n.m.r. 5.07, 5.38 (2H, two singlets; terminal methylene), 5.60 (1H, broad singlet; OH), 5.98 (1H, octet, J 3, 7, and 9.5 c./sec.; $CH_2CHOHCH$), 7.68, 7.77 (12H, two sharp peaks; 2 $N(CH_3)_2$), 8.87, 9.03 (6H, two sharp peaks; 2 tertiary C-methyl), 9.13 (3H, doublet, J 6.5 c./sec.; secondary C-methyl), 9.72, and 9.97 τ (2H, AB doublets, J 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{27}H_{46}ON_2$: C, 78.20; H, 11.18; N, 6.76. Found: C, 78.06; H, 11.18; N, 7.02.

The O-acetyl derivative Ic[6a] was prepared with acetic anhydride–pyridine, 33 hr. at room temperature; crystallized from acetone at 0°, m.p. 173–175°, $[\alpha]^{25}D$ +69° (c 2.63), λ_{max} 5.81 μ; n.m.r. 4.95 (1H, octet, J 3, 7, and 9.5 c./sec.; $CH_2CH(OAc)CH$) 5.01, 5.37 (2H, two singlets; terminal methylene), 7.63, 7.75 (12H, two sharp peaks; 2 $N(CH_3)_2$, 8.03 (3H, unsplit; O-acetyl), 8.82, 8.92 (6H, two sharp peaks; 2 tertiary C-methyl), 9.12 (3H, doublet, J 6.5 c./sec.; secondary C-methyl), 9.58, and 9.88 τ (2H, AB doublets, J 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{29}H_{48}O_2N_2$: C, 76.26; H, 10.59. Found: C, 75.98; H, 10.59.

O-Acetyl-N,N'-dimethylcyclobuxine dihydrochloride showed m.p. 273–280° dec.

Dihydrocyclobuxine (IIa).[6a]—Cyclobuxine (1.00 g.) was hydrogenated with reduced platinum oxide (250 mg.) in 10% ethanolic acetic acid (50 ml.); the uptake was 0.85 mole equiv. in 2 hr. The product (1034 mg., semicrystalline) was recovered by filtration, evaporation of the ethanol, treatment with dilute ammonium hydroxide and chloroform, and crystallization from acetone; first crop, 613 mg., m.p. 207–208°; second crop, 274 mg., m.p. 204–207°; third crop, 87 mg., m.p. 206–208°; total, 974 mg. (97%). Pure material showed m.p. 208–209°, R_f on column B 0.65; $[\alpha]^{25}D$ +46° (c 2.42); infrared lacking the 6.09 and 11.20 μ bands of cyclobuxine; n.m.r. 5.88 (1H, octet, J 3, 7, and 9.5 c./sec.; $CH_2CHOHOH$), 7.55, 7.60 (6H, two sharp peaks; 2 N-methyl), 8.86, 9.01 (6H, two sharp peaks; 2 tertiary C-methyl), 8.91 (3H, doublet, J 6 c./sec.; secondary C-methyl), (3H, doublet, J 7 c./sec.; secondary C-methyl), 9.43, and 9.73 τ (2H, AB doublets, J 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{25}H_{44}ON_2$: C, 77.26; H, 11.41; N, 7.21. Found: C, 77.34; H, 11.44; N, 7.09.

Dihydrocyclobuxine dihydroiodide had m.p. 282–286° dec.

Selenium Dehydrogenation Experiments.[6a,c] (1) **Acetone-Insoluble Mixture. Isolation of the Total Hydrocarbon Fraction.**—The solid mixture of crystallization mother liquors from the acetone-insoluble bases, of which cyclobuxine was the major component (at least 30%; 3 g.), was intimately mixed with selenium powder (10 g.) and sealed into a heavy-walled 1 in. Pyrex tube, 8 in. long. The tube, protected by an iron pipe, was heated in a furnace for 23 hr. at 340–350°, and 6 hr. at 370°, cooled, and broken open carefully in an efficient hood; after the hydrogen selenide had dispersed, the product mixture was ground to a fine powder and exhaustively extracted with ether. The combined extracts were concentrated to 50 ml., washed with 2 N hydrochloric acid (2 × 50 ml., filtering through cotton to separate the flocculent tarry precipitate), dilute sodium bicarbonate solution (2 × 50 ml.), and water (2 × 50 ml.). The hydrochloric acid extracts contained 44 mg. of mixed aromatic amines; no pure compounds could be isolated from this fraction. Evaporation of the ether solutions (including ether backwashes of the aqueous extracts) gave an ill-smelling dark oil (1.0 g.).

Separation into Different Hydrocarbon Classes.—The crude oily product (1.0 g.) was dissolved in a small volume of hexane, filtered from insoluble material, and added to a column (1.8 cm. i.d., wrapped with a black cloth) fairly tightly slurry-packed in hexane with silica gel (Davison, through 200 mesh)–Celite 545 (4:1; 60 g.). Elution proceeded with hexane; fractions were cut on the basis of dry weight and fluorescence under long-wave ultraviolet light (best observed in the dark). The first 400 ml. of hexane eluate contained 377 mg. of nonaromatics and mono- and binuclear aromatics (very weak blue fluorescence) (fraction 1); a further 80 ml. eluted 47 mg. showing strong blue fluorescence (fraction 2); 180 ml. eluted 124 mg., strong blue fluorescence (fraction 3); further elution with 400 ml. gave 66 mg. containing considerable ill-smelling selenium-containing material. Fractions 2 and 3 were evaporated under nitrogen and saved at 0° in the dark and under an inert atmosphere.

Fraction 1 was further separated on a column similar to that above, containing 30 g. of silica gel–Celite mixture. Elution with hexane (100 ml.) gave mainly nonfluorescent materials (91 mg.); further elution (100 ml.) yielded the mixed naphthalene fraction (262 mg.), showing λ_{max}^{EtOH} 234 mμ. The total fraction (262 mg.) was treated with trinitrobenzene (250 mg.) in hot ethanol (60 ml.); the resulting deep-yellow solution, concentrated at 80° to 10 ml. and let stand at 0°, yielded a first crop of crude mixed naphthalene–trinitrobenzene complex (217 mg.) from which the purified naphthalene fraction (110 mg.) was regenerated by a rapid pass through Merck alumina (slightly basic, approximately activity II, 20 g.) in hexane–benzene (2:1).

Isolation of the Individual Hydrocarbons. a. Acetylated Cellulose.[18]—Unwashed Whatman standard grade cellulose (100 g.) was stirred gently 17 hr. at room temperature with acetic anhydride (400 ml.) and benzene (900 ml.) containing 98% sulfuric acid (2.4 g.) and 60% perchloric acid (0.8 g.). The acetylation mixture was removed by vacuum filtration, and the powder washed with strong stirring, first using 95% ethanol (1200 ml.), then reagent methanol (1500 ml.). The resulting fluffy white solid was tumble-dried for 16 hr. at 55° and 40 mm. to give the acetylated cellulose (137 g.) used for this work. Hydrolysis of an aliquot with refluxing 0.5 N sodium hydroxide and distillation of the acidified solution from n-amyl alcohol indicated (by titration) a 31% acetyl content for this batch.

For separation of hydrocarbon fractions, this acetylated cellulose (75 g.) was slurried in the system methanol–toluene–water (10:1:1), blended for 30 sec. in a high-speed Waring blendor, and packed with the aid of moderate air pressure into a column of 2.7 cm. i.d.; the final retention volume (as determined with thymol blue) was 160 ml. Hydrocarbon fractions (up to 120 mg.) were added to this column as an emulsion obtained by dissolving in ether, adding the solvent system above, and gently boiling out the ether; the resulting cloudy solutions would stay

emulsified for a minute or more, sufficient time to be added to the top of the column, after which they would demonstrate excellent partition behavior (giving sharp fluorescent bands).

b. Anthracene Derivative IV.—Fraction 2 from the initial separation (47 mg.) was added as described to the above acetylated cellulose column; a broad fluorescent band (R_f 0.76 to 0.48) appeared, and was collected in 20-ml. fractions. Fraction 3 (18 mg., 0.9%, R_f 0.64 to 0.59) gave upon treatment with an equal weight of trinitrobenzene in hot ethanol, followed by concentration and cooling, red crystals (20 mg.), m.p. 160–165°. Three recrystallizations from ethanol gave highly crystalline sparkling red needles, m.p. 158°, which resolidified and melted sharply at 168–169°.

Anal. Calcd. for $C_{27}H_{25}O_6N_3$: C, 66.52; H, 5.17. Found: C, 66.78; H, 5.66.

The trinitrobenzene complex (43 mg., m.p. above 160°) was transformed back to the hydrocarbon with alumina, rapidly filtering in hexane–benzene (2:1); the resulting crystalline solid (26 mg.) was recrystallized twice from methanol to give 3 mg., m.p. 110–112°, $[\alpha]^{25}D$ +3° (*c* 1.50); λ_{max} 11.36 (vs), 12.53 (w), 13.50 (s, in cyclohexane) μ; λ_{max}^{EtOH} 259.5, 319, 334, 350, 367, and 385.5 mμ (log ϵ 5.20, 3.12, 3.42, 3.65, 3.78, and 3.75); n.m.r. 1.83 (2H, unsplit; 9- and 10-protons), 2.28, 2.43 (2H, two sharp peaks; 2 α-protons), 2.87 and 2.97 (3H complex; 3 β-protons), 6.80 (2H, triplet *J* 7 c./sec.; Ar–CH_2–CH_2), 7.35 (3H, unsplit; Ar–CH_3), 8.02 (2H, quartet, *J* 7 c./sec.; C–CH_2–CH_3), 8.52 (triplet, *J* 7 c./sec.; Ar–CH_2CH_2–C), 8.78 (3H, unsplit; tertiary C-methyl), and 9.30 τ (3H, triplet, *J* 7 c./sec.; C–CH_2–CH_3).

Anal. Calcd. for $C_{21}H_{22}$: C, 91.92; H, 8.08. Found: C, 92.4; H, 7.6.

c. Phenanthrene Derivative V.—Fraction 3 from the initial separation (124 mg.) was chromatographed in two portions on the acetylated cellulose column (washed free from material from previous runs); a broad blue fluorescent band appeared (R_f 0.75 to 0.50), collected in 20-ml. fractions. Impure phenanthrene V appeared in fractions 3–6 (R_f 0.67 to 0.50; 45 mg., 2.2%); the purest fractions (near R_f 0.55) gave upon treatment with trinitrobenzene 10 mg. of orange-yellow clumps, m.p. 155–169°. Two recrystallizations from ethanol gave orange-yellow micro crystals, m.p. 165–168°.

Anal. Calcd. for $C_{27}H_{25}O_6N_3$: C, 66.52; H, 5.17. Found: C, 66.27; H, 5.37.

The trinitrobenzene complex (37 mg.) was transformed back to the hydrocarbon as above yielding a highly crystalline solid (20 mg.); two recrystallizations from methanol gave 8 mg., m.p. 145–147°, $[\alpha]^{25}D$ −20° (*c* 1.30); λ_{max} 12.22 (s) μ; λ_{max}^{EtOH} 261, 284, 294.5, 307, 323, 338, and 353.5 mμ; log ϵ 4.79, 4.09, 4.05, 4.15, 2.91, 2.95, and 2.91; n.m.r. 1.50, 1.63 (2H, two sharp peaks; C-4 and C-5 protons), 2.22 and 2.30 (2H, two sharp peaks; C-9 and C-10 protons), 2.68 (3H, complex), 6.80 (2H, triplet, *J* 7 c./sec.; Ar–CH_2–CH_2), 7.32 (3H, unsplit; Ar–CH_3), 7.95 (2H, quartet, *J* 7 c./sec.; C–CH_2–CH_2), 8.42 (2H, triplet, *J* 7 c./sec.; Ar–CH_2–CH_2–C), 8.72 (3H, unsplit; tertiary C-methyl), and 9.20 τ (3H, triplet, *J* 7 c./sec.; C–CH_2–CH_3).

Anal. Calcd. for $C_{21}H_{22}$: C, 91.92; H, 8.08. Found: C, 91.52; H, 8.01.

d. Tetrahydrophenanthrene Derivative III.—This, the most easily obtained of the large number of naphthalenes in the complex dehydrogenation mixture, ran well behind the bulk of the material on the acetylated cellulose column. The purified naphthalene mixture (110 mg.) was added to the column; material appearing at R_f 0.67 to 0.57 (23 mg., 1.1%) gave, upon treatment with trinitrobenzene, good crystals, m.p. 137–142°. Pure material crystallized in flat yellow needles, m.p. 143–145°.

Anal. Calcd. for $C_{27}H_{31}O_6N_3$: C, 65.70; H, 6.33. Found: C, 65.73; H, 6.31.

The trinitrobenzene complex (37 mg.) was transformed back to the hydrocarbon (18.5 mg.) as above; two crystallizations from methanol gave 6 mg., m.p. 134–135°. $[\alpha]^{24}D$ +24° (*c* 1.85); λ_{max} 7.10 (m) and 12.21 (s) μ; λ_{max}^{EtOH} 233.5, 280.5, 285 sh, 290.5, 311, 317.5, and 325.5 mμ; log ϵ 5.05, 3.77, 3.78, 3.82, 3.31, 3.04, and 3.18; n.m.r. 2.07, 2.20 (2H, two broad peaks; 2 α-protons), 2.62, 2.73 (3H, complex; 3 β-protons), 6.83 (2H, triplet, *J* 7 c./sec.; Ar–CH_2–CH_2), 7.33 (3H, unsplit; Ar–CH_3), 8.88, 9.38 (6H, two sharp peaks; 2 tertiary C-methyl), 8.08, and 9.00 τ (6H, two triplets, *J* 7 c./sec.; two primary C-methyl).

Anal. Calcd. for $C_{21}H_{28}$: C, 89.94; H, 10.06. Found: C, 89.88; H, 10.09.

e. Tetrahydroanthracene Derivative VI.—Extensive rechromatography (on acetylated cellulose) and recrystallization of the trinitrobenzene complex of the naphthalene mixture of R_f 0.73 to 0.67 gave a tetrahydroanthracene (total 33 mg. of the trinitrobenzene complex (0.8%) from the purified naphthalene mixture after two chromatographies and one crystallization). Moderately pure trinitrobenzene complex (60 mg.) was recrystallized four times from ethanol to yield fluffy yellow needles (21 mg.), m.p. 140–143°; an analytical sample melted at 139–141°.

Anal. Calcd. for $C_{29}H_{31}O_6N_3$: C, 66.52; H, 6.18. Found: C, 66.60; H, 5.94.

The trinitrobenzene complex (42 mg.) was transformed back to the hydrocarbon (24 mg.) as described above; three recrystallizations from methanol gave 5 mg., m.p. 111–117°, λ_{max} 11.35 (s) μ; λ_{max}^{EtOH} 233.5, 269 sh, 277.5, 287, 296 sh, and 324.5 mμ; log ϵ 4.76, 3.65, 3.77, 3.80, 3.65, and 2.68; n.m.r. 2.60 (3H, complex; 3 α-protons), 2.90, 3.00 (2H, two sharp peaks; 2 β-protons), 6.97 (2H, triplet, *J* 7 c./sec.; Ar–CH_2–CH_2), 7.44 (3H, unsplit; Ar–CH_3), 8.92, 9.53 (6H, two sharp peaks; 2 tertiary C-methyl), and 9.05 τ (3H, triplet, *J* 7 c./sec.; primary C-methyl).

Anal. Calcd. for $C_{22}H_{28}$: C, 90.35; H, 9.65. Found: C, 90.5; H, 9.5.

(2) **Pure Cyclobuxine.**—Cyclobuxine (1.0 g.) was mixed with selenium (3.3 g.) and dehydrogenated at 280 (14 hr.), 350 (10 hr.), and 375° (10 hr.) in a heavy-walled sealed tube as above; the product (465 mg.) was recovered exactly as for the above mixture, and separated on silica gel–Celite (20 g.), eluting with gradually increasing concentrations of benzene in hexane. Hexane (50 ml.) eluted nonfluorescent material (8 mg.); the next 50 ml. gave primarily naphthalenes (169 mg.; infrared analysis at 11.35 and 12.21 μ indicated 100 mg. of tetrahydroanthracenes and 60 mg. of tetrahydrophenanthrenes). A crude anthracene fraction (22 mg.) was eluted with hexane (25 ml.) and hexane–benzene (100:1; 10 ml.); further elution with hexane–benzene (100:1, 40 ml., and 30:1, 25 ml.) gave a crude phenanthrene fraction (32 mg.). Continued elution gave only selenium-containing compounds. Analysis of the crude anthracene and phenanthrene fractions by ultraviolet indicated a total of 25 mg. of 1,2,5-trisubstituted anthracenes and 17 mg. of 1,2,8-trisubstituted phenanthrenes.

The central naphthalene fractions (145 mg.) were combined and treated with trinitrobenzene (125 mg.) in hot ethanol, concentrated, and cooled; the deep yellow solid complex resulting (184 mg.) was transformed as before back to the purified naphthalene fraction (84 mg.), which was added to a fresh acetylated cellulose column (85 g. of carrier, retention volume 180 ml.). Fractions of 10 ml. were collected after 210 ml. (R_f 0.86) and treated with trinitrobenzene. Fractions 8 and 9 (R_f 0.64–0.60, 7 mg., 1%) gave a trinitrobenzene complex (10 mg., m.p. 139–143°); the hydrocarbon (5.8 mg.) was recovered from this and crystallized from methanol to give 4 mg., m.p. 133–135°, $[\alpha]^{24}D$ +24° (*c* 0.58); infrared and ultraviolet spectra identical with those of the naphthalene III from the mixture; m.p. of trinitrobenzene complex (purified) 143–145°.

Fractions 5 and 6 gave, after two recrystallizations from ethanol, a purified complex (20 mg., 1.5%), m.p. 135–142°. The crystalline hydrocarbon (10 mg.) regenerated from this was further purified on the acetylated cellulose column; the fraction of R_f 0.72–0.67 (8 mg.) gave a trinitrobenzene complex which after two crystallizations from ethanol yielded 6 mg., m.p. 139–141°. The hydrocarbon recovered from the mother liquors of the crystallization showed spectral characteristics essentially identical with those of the pure tetrahydroanthracene VI.

The crude anthracene fraction from the original separation (22 mg.) was purified on the acetylated cellulose column; no good crystalline trinitrobenzene complex could be obtained from any fraction so obtained, but the material at R_f 0.66 to 0.59 (7 mg., 1%) showed infrared and ultraviolet spectral characteristics substantially identical with those of the pure anthracene IV from the mixture.

The crude phenanthrene fraction (32 mg.) gave upon chromatography on the acetylated cellulose column only impure phenanthrene fractions, corresponding very closely in spectral properties to the phenanthrene V from the mixture.

(3) **Decyclized Cyclobuxine (XIa).**—Crude decyclized cyclobuxine (100 mg., see below) was mixed with selenium (350 mg.) and dehydrogenated at 325–335° for 26 hr.; work-up in identical fashion with the above gave a crude oily product (26 mg.).

Chromatography of this on silica gel–Celite (3 g.) gave a crude naphthalene fraction (7 mg.) with infrared and ultraviolet spectra essentially identical with those of the tetrahydrophenanthrene III (no trace of 287 or 277.5 mμ absorption in the ultraviolet, high peak at 325.5 mμ; no trace of 11.35 μ absorption in the infrared); and a fluorescent fraction (5 mg.) showing in the ultraviolet only absorption for a 1,2,8-trisubstituted phenanthrene (λ_{max}^{EtOH} 262, 283, 294, and 307 mμ; no trace of anthracene absorption in the 340–400 mμ region).

Ozonolysis of O,O,N′-Triacetylcyclobuxine (Id).—A slow regulated stream of ozonated oxygen from the "sample" valve of a Welsbach ozonator T-23 (0.0075 mmoles of ozone per minute, determined with aqueous iodide, then acetic acid and 0.1 *N* sodium thiosulfate) was passed through a cold (0°) solution of cyclobuxine O,N,N′-triacetate (25.6 mg., 0.050 mmole) in acetic acid (5 ml.) and ethyl acetate (5 ml.) for 8 min. 10 sec. (120%). The solution was allowed to stand 10 min. at 0°, treated with water (5 ml.) and 30% hydrogen peroxide (0.2 ml.), and let stand at room temperature overnight. The ethyl acetate was evaporated, water (15 ml.) was added, and the product (21 mg.) was recovered with chloroform (backwashing the chloroform extracts with water) and crystallized from acetone–isopropyl ether to give 16 mg., m.p. 220–222° dec. Chromatographically pure material showed a variable decomposition–melting point about 230°, R_f on column A-1 0.39, $[\alpha]^{23}D$ −11° (*c* 2.32); λ_{max} 5.79 (vs) and 6.14 (vs) μ; n.m.r. 4.90 (1H, complex; $CH_2CH(OAc)CH$), 7.08 (3H, unsplit; N-methyl), 7.20, 7.25 (3H, split peak; N-methyl with restricted rotation), 7.88, 8.00 (6H, two sharp peaks; 2 N-acetyl), 8.03 (3H, unsplit; O-acetyl), 8.90, 8.92 (6H, two sharp peaks; 2 tertiary C-methyl), 8.93 (3H, doublet, *J* 6 c./sec.; secondary C-methyl), 9.53, and 9.83 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{30}H_{46}O_5N_2$: C, 70.00; H, 9.01; N, 5.44. Found: C, 69.52; H, 8.93; N, 5.44.

This ketone VIIa (2 mg.) in ethanol (1 ml.) was treated with 10% ethanolic potassium hydroxide (1 ml.) and *m*-dinitrobenzene (2 mg.) at room temperature.[26] A red color slowly developed, but the visible spectrum of the solution showed no maximum, merely slowly rising absorption in the 400–600 mμ range.

Hofmann Degradation of N,N′-Dimethylcyclobuxine.[6a]—N,N′-Dimethylcyclobuxine (Ib, 245 mg., free of N,N′-dimethylcyclovirobuxine) was dissolved in chloroform (10 ml.), heated to 65°, and treated with methyl iodide (1.5 and 1.0 ml., 1 hr. each). The solution was cooled, filtered from suspended solid (partly dimethiodide,[6a] 13 mg.), taken to dryness (weight 342 mg.) and crystallized from methanol; first crop, 200 mg., m.p. 221–225° dec. with evolution of trimethylamine; second crop, 50 mg., m.p. 220–223° dec. The melting point of this monomethiodide varied greatly with the rate of heating.

Anal. Calcd. for $C_{28}H_{49}ON_2I$: C, 60.41; H, 8.87; I, 22.80. Found: C, 60.55; H, 8.69; I, 22.69.

Crude monomethiodide (430 mg., prepared from cyclobuxine containing 5% cyclovirobuxine) was dissolved in ethylene glycol (3.3 ml.) and water (0.9 ml.) containing potassium hydroxide (1060 mg.) and heated 8 hr. at 160–165° (bath). Nitrogen was slowly bubbled into the solution and out into a receiver containing 0.093 *N* hydrochloric acid (15.0 ml.). At the end of the reaction period, the solution in the receiver was titrated with 0.50 *N* sodium hydroxide; this indicated an uptake of 0.53 mmole of acid, or an evolution of 69% of the theoretical amount of trimethylamine. The reaction mixture was diluted with water (20 ml.) and extracted with chloroform (4 × 15 ml.); the combined chloroform extracts were concentrated to 20 ml. and extracted with 2 *N* hydrochloric acid (3 × 20 ml.), and the combined hydrochloric acid extracts were backwashed with chloroform (2 × 40 ml.). All of the chloroform extracts (100 ml.) were united, concentrated to 30 ml., washed with dilute ammonium hydroxide, and evaporated (along with three 30-ml. ether backwashes of the ammoniacal solution) to give 266 mg.; after removal of methanol insolubles, 197 mg. (70%) of crystalline des-N-base VIIIa. Recrystallization from methanol afforded 108 mg. in three crops; the first crop (64 mg.) melted at 161–164°. Pure material (from several recrystallizations) showed m.p. 169–170°, $[\alpha]^{22}D$ +170° (*c* 2.17); λ_{max} 3.00 (m), 6.10 (m), 6.25 (m), 11.15 (m), and 11.30 (s) μ; λ_{max}^{EtOH} 229.5 mμ; log ε 4.22, with shoulders at 225 and 238 mμ, log ε 4.21, 4.02; n.m.r. 3.60–4.60 (2H, AB of ABX pattern; C-2,3-vinyl protons), 5.34 (2H, broadened singlet; terminal methylene), 6.06 (1H, octet, *J* 2, 7, and 9.5 c./sec.; $CH_2CHOHCH$), 6.80 (1H, unsplit; OH, 7.73 (6H, unsplit; $N(CH_3)_2$), 8.87, 9.03 (6H, two sharp peaks; 2 tertiary C-methyl), 9.14 (3H, doublet, *J* 7 c./sec.; secondary C-methyl), 9.73, and 9.90 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{25}H_{39}ON$: C, 81.24; H, 10.64; N, 3.79. Found: C, 81.32; H, 10.78; N, 3.81.

The mother liquors from the des-N-base VIIIa contained a trace (5% or less) of a homoannular diene (probably IX), visible in the ultraviolet spectrum (λ_{max}^{EtOH} 282.5 mμ, shoulders at 272, 294).

The combined hydrochloric acid extracts from the work-up were made basic with ammonium hydroxide, and the product (82 mg.) recovered with chloroform and crystallized from methanol to yield 19 mg. (6%) of N,N′-dimethylcyclovirobuxine,[12] m.p. 228–231°, identical with an authentic sample[12] by rotation and infrared and n.m.r. spectra. Purified chloroform-insoluble hydrochloride fraction from three degradations (70 mg.) was separated by partition chromatography, on a dry-packed 1.8-cm. i.d. column containing 20 ml. of the lower phase of the system hexane–ethylene dichloride–methanol–water–nitromethane (500:50:100:8:1) + brom cresol purple (5 mg.) on Celite 545 (30 g.). Although the bands tailed considerably owing to the presence of the acidic nitromethane in the stationary phase, two well-separated bands appeared corresponding to N,N′-dimethylcyclovirobuxine (R_f 0.75, 22 mg., 7%) and N,N′-dimethylcyclobuxine (R_f 0.66, 27 mg., 9%; crystallized from acetone, m.p. and m. m.p. 198–200°).

A similar Hofmann degradation of methiodide from purified cyclobuxine (prepared through N,N′-dimethylcyclobuxine of m.p. 204–205°, free of contaminating N,N′-dimethylcyclovirobuxine) (225 mg.) gave 63 mg. (41%) of the des-N-base VIIIa in four crops, and a ditertiary amine fraction (19 mg., 11%) containing, by chromatography and crystallization, only N,N′-dimethylcyclobuxine (Ib).

The O-acetyl derivative VIIIb of the des-N-base VIIIa, prepared with acetic anhydride–pyridine, 20 hr. at room temperature, could not be crystallized from solvents (although on long standing neat at 0° it solidified); $[\alpha]^{22}D$ +122° (*c* 0.68), λ_{max} 3.60 (m), 5.80 (s), 6.10 (m), 6.24 (m), and 11.25 (s) μ.

Attempted Diels–Alder Reaction with the Des-N-base VIIIa.—The des-N-base (10 mg.) was sealed into a tube containing freshly purified maleic anhydride (3 mg.) and toluene (1 ml.), and heated at 100° for 5 hr., then let sit 14 hr. at room temperature. Basic hydrolysis of the reaction mixture and extraction with chloroform gave 6.3 mg. of material with infrared and ultraviolet spectra identical with those of the starting material VIIIa.

Attempted Aromatization of the Des-N-base VIIIa.[27]—The des-N-base (5 mg.) was dissolved in benzene (1 ml.) containing chloranil (5 mg.), let sit 90 min. at room temperature, and heated 10 min. at 80°. The product was recovered by dilution with hexane (20 ml.) and extraction with 2 *N* hydrochloric acid (2 × 20 ml.); from the basified extracts was obtained 6 mg., infrared showing no benzenoid bands, no 6.10, 6.25, 11.15, and 11.30 μ; ultraviolet showing a sharp triplet, λ_{max}^{EtOH} 282.5 mμ (ε 3.72), shoulders at 273, 292 mμ (compare with the spectrum of the isomer found in the Hofmann reaction mixture, probably IX).

Decyclization of the Des-N-base VIIIa.[28]—The des-N-base (25 mg.) was decyclized in chloroform (3 ml.), acetic acid (1 ml.), and 1 drop of water, then saturated with gaseous hydrogen chloride for 19 hr. at room temperature. The product (recovered with water, ammonium hydroxide, and chloroform) showed in the infrared no bands at 6.10, 6.25, 11.15, and 11.30 μ, and in the ultraviolet two triads of peaks: 241, 248, and 256 mμ (possibly a $\Delta^{2,4(4a),5}$-triene), and 271, 282, and 294 mμ (as in the isomer IX). No evidence could be found for compounds possessing an aromatic ring A in either the infrared or ultraviolet spectra.

Tetrahydro Des-N-base (X).[6a]—The des-N-base VIIIa (100 mg.) was hydrogenated with reduced platinum oxide (25 mg.) in 10% ethanolic acetic acid (20 ml.); the uptake was 1.94 mole equiv. in 30 min. The product (100 mg.), recovered as for dihydrocyclobuxine, was crystallized from methanol; first crop, 51 mg., m.p. 158–163°; second crop, 38 mg., m.p. 152–157°. Recrystallization from methanol gave pure material, m.p. 161–164°, $[\alpha]^{23}D$ +36° (*c* 2.16).

Anal. Calcd. for $C_{25}H_{43}ON$: C, 80.37; H, 11.60; N, 3.75. Found: C, 80.49; H, 11.77; N, 4.18.

Decyclization of Cyclobuxine.[28]—A solution of cyclobuxine (110 mg.) in chloroform (5 ml.) and acetic acid (2 ml.) containing 1 drop of water was saturated with gaseous hydrogen chloride and allowed to stand 80 hr. at room temperature. The total product (113 mg.) was recovered with water, ammonium hydroxide, and chloroform, and immediately hydrolyzed (before any O- to N-acetyl migration could occur) 20 min. with refluxing 5% ethanolic

potassium hydroxide. The product was recovered with water and chloroform and added to column B (considerable difficulty being encountered in dissolution). The decyclized mixture XIa (88 mg., R_f 0.42 *vs.* cyclobuxine 0.52) was crystallized from methanol; material of m.p. 249–252° dec. crystallized first (23 mg.); later crops melted at 246–248° dec., then 235–237° dec. (from acetone), indicating the presence of more than one double bond isomer. Recrystallized first crop material showed m.p. 252–254° dec., $[\alpha]^{24}D$ +141° (*c* 0.91); infrared spectrum superimposable upon that of cyclobuxine, except for a peak of medium intensity at 9.82 μ (cyclobuxine, 9.75 μ); ultraviolet showing only end absorption, higher than that for cyclobuxine; n.m.r. 4.69 (0.5H, broad peak; partial new vinyl proton), 5.10, 5.37 (2H, two singlets; terminal methylene), 5.92 (1H, octet, *J* 3, 7, and 9.5 c./sec.; $CH_2CHOHCH$), 7.52, 7.55 (6H, two sharp peaks; 2 N-methyl), 8.86, 9.02 (6H, two sharp peaks; 2 tertiary C-methyl), 8.90 (3H, doublet, *J* 6 c./sec.; secondary C-methyl), 9.18, and 9.35 τ (3H, split peak; new tertiary C-methyl in two different double bond environments).

Anal. Calcd. for $C_{28}H_{42}ON_2 \cdot 0.5H_2O$: C, 75.89; H, 10.96; N, 7.08. Found: C, 76.24; H, 10.83; N, 6.93.

Decyclization under the above conditions for only 15 min. gave nearly 50% opening of the cyclopropane ring, as determined by partition chromatography on the product.

Decyclization of O,N,N'-Triacetylcyclobuxine (Id).[28]—A solution of O,N,N'-triacetylcyclobuxine (40 mg.) in chloroform (2 ml.) and 1 drop of water was saturated with gaseous hydrogen chloride and let stand 20 hr. at room temperature. The product (40 mg.), recovered with water, ammonium hydroxide, and chloroform, was purified on column B; the band of R_f 0.36 (30 mg.) was crystallized from acetone–isopropyl ether to give 21 mg. m.p. 245–246° dec., $[\alpha]^{26}D$ +22° (*c* 2.13), R_f on column A-1 0.63, infrared spectrum essentially superimposable upon that of O,N,N-triacetylcyclobuxine (Id); n.m.r. 4.80–5.50 (3H+, complex; terminal methylene + $CH_2CH(OAc)CH$ + partial new vinyl proton), 7.07, 7.20, 7.10, 7.23 (6H, two split peaks; 2 N-methyl with restricted rotation), 7.87, 7.97 (3H, split peak; N-acetyl with restricted rotation), 8.01 (3H, unsplit; N-acetyl), 8.02 (3H, unsplit; O-acetyl), 8.88, 8.90 (6H, two sharp peaks; 2 tertiary C-methyl), 8.90 (3H, doublet, *J* 6 c./sec.; secondary C-methyl), and 9.13 τ (3H, unsplit; new tertiary C-methyl).

Anal. Calcd. for $C_{31}H_{48}O_4N_2 \cdot 0.5H_2O$: C, 71.36; H, 9.47; N, 5.37. Found: C, 71.61; H, 9.65; N, 5.37.

Hydrogenation of Decyclized O,N,N'-Triacetylcyclobuxine (XIb).—Decyclized O,N,N'-triacetylcyclobuxine (11 mg., m.p. 245–247° dec.) was hydrogenated in 10% ethanolic acetic acid (20 ml.) with reduced platinum oxide (15 mg.); the uptake of hydrogen was 1.16 mole equiv., essentially immediately. The product, recovered as for dihydrocyclobuxine, was crystallized from acetone–isopropyl ether to give XIIb (or its 4-epimer[11]; 7 mg.), m.p. 215–219°.

Decyclization of Dihydrocyclobuxine (IIa).[28]—Dihydrocyclobuxine (50 mg.) was decyclized for 56 hr. and the product hydrolyzed for 30 min., as for the decyclization of cyclobuxine above. The product (58 mg.) was separated on column B; minor bands of dihydrocyclobuxine (3 mg., R_f 0.62) and decyclized dihydrocyclobuxine 20-N-acetate (4 mg., R_f 0.35) appeared; the major band (33 mg., R_f 0.54) was crystallized from acetone to give 22 mg. of XIIa, m.p. 222–223° dec., $[\alpha]^{23}D$ +66° (*c* 1.87), infrared spectrum essentially identical with that of dihydrocyclobuxine n.m.r. 4.80 (1H, broad peak; almost complete new vinyl proton) 5.92 (1H, octet, *J* 3, 7, and 9.5 c./sec.; $CH_2CHOHCH$), 7.55 (6H, sharp peak; 2 N-methyl), 8.98, 9.03 (6H, two sharp peaks; 2 tertiary C-methyl), 8.90 (3H, doublet, *J* 6 c./sec.; secondary C-methyl), 9.18 (3H, doublet, *J* 7 c./sec.; secondary C-methyl), and 9.36 τ (3H, unsplit; new tertiary C-methyl). Recrystallization of 41 mg. of the crude material (m.p. 221–224° dec.) from acetone gave a purified sample (29 mg.), m.p. 225–228° dec.

Anal. Calcd. for $C_{25}H_{44}ON_2 \cdot 0.5H_2O$: C, 75.51; H, 11.41; N, 7.05. Found: C, 75.76; H, 10.97; N, 7.00.

Hydrogenation of Decyclized Cyclobuxine (XIa).—Decyclized cyclobuxine (5 mg.) was hydrogenated with reduced platinum oxide (15 mg.) in 5% ethanolic acetic acid (10 ml.); the uptake of hydrogen was 1.1 mole equiv. The product (5 mg.) had an infrared spectrum identical with that of decyclized dihydrocyclobuxine (XIIa), and upon crystallization from acetone gave 3 mg., m.p. 224–227° dec., m.m.p. with authentic material 224–226° dec.

Attempted Hydrogenation of Decyclized Dihydrocyclobuxine (XIIa).—Decyclized dihydrocyclobuxine (6 mg.) was hydrogenated with a very large excess of reduced platinum oxide (197 mg.) in acetic acid (10 ml.); although an instantaneous uptake of 0.41 mole equiv. of hydrogen was observed, the product was identical with starting material and homogeneous by partition chromatography.

N,N'-Di-*p*-nitrobenzyloxycarbonylcyclobuxine (Ig).—Cyclobuxine (250 mg.) was treated with *p*-nitrobenzylchloroformate (350 mg., m.p. 33–34°) and potassium carbonate (5 g.) in benzene (20 ml.) with strong stirring for 1 hr. at room temperature. The benzene was removed by evaporation and the residue stirred 1 hr. with chloroform (10 ml.) and water (10 ml.); the product, recovered with chloroform, was crystallized from chloroform–ethanol to give 466 mg., m.p. 205–208°. Pure material showed m.p. 206–207°, $[\alpha]^{24}D$ +21° (*c* 2.30), λ_{max} 5.94 (vs) μ; n.m.r. 1.78, 2.48 (8H, doublets, *J* 9 c./sec.; aromatic protons), 4.75 (4H, unsplit; benzylic protons), 5.38, 5.43 (2H, singlets; terminal methylene), 5.80 (1H, complex; $CH_2CHOHCH$), 7.07 (3H, unsplit N-methyl), 7.06, 7.10 (3H, split peak; N-methyl with restricted rotation), 8.83, 8.95 (6H, two sharp peaks; 2 tertiary C-methyl), 8.87 (3H, doublet *J* 6 c./sec.; secondary C-methyl), 9.67, and 9.88 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{41}H_{52}O_8N_4 \cdot 2H_2O$: C, 63.23; H, 7.23; N, 7.20. Found: C, 62.88; H, 6.82; N, 7.50.

The diamide Ig (50 mg.) was hydrogenated and hydrogenolyzed[37,38] with 10% palladium-on-charcoal (40 mg.) in 20% ethanolic acetic acid (20 ml.), stirring 3 hr. at room temperature. The product, recovered as for dihydrocyclobuxine, was purified on column B; the major band (16 mg., R_f 0.62) was crystallized from acetone to give 10 mg., m.p. 210–211°, m.m.p. with authentic dihydrocyclobuxine (IIa) 209–212°, $[\alpha]^{25}D$ +49° (*c* 0.89); infrared spectrum identical with that of dihydrocyclobuxine.

Ozonolysis of N,N'-Di-*p*-nitrobenzyloxycarbonylcyclobuxine and Hydrogenolysis of the Product.—N,N'-Di-*p*-nitrobenzyloxycarbonylcyclobuxine (Ig, 37 mg.) was dissolved[38] in acetic acid (2 ml.) and ethyl acetate (2 ml.), cooled to 0°, and treated with a stream of ozonated oxygen (0.0028 mmole/min.) for 21 min. The solution was let stand for 35 min. at 0°, then added to water (5 ml.) and 30% hydrogen peroxide (0.1 ml.), and let crystallize at 0° for 2 days. The product VIIb (33 mg.) showed m.p. 220–222° dec., $[\alpha]^{25}D$ +10° (*c* 2.11); λ_{max} 5.80 (s), 5.95 (vs) μ; no 6.06, 11.10 μ.

Anal. Calcd. for $C_{40}H_{50}O_{10}N_4$: C, 64.89; H, 6.64; N, 7.38. Found: C, 64.29; H, 6.96; N, 7.94.

The ozonolysis product VIIb (194 mg.) was hydrogenolyzed[38] with 30% palladium-on-charcoal (50 mg.) in ethanol (30 ml.) containing 1 *N* hydrochloric acid (2 ml.) until hydrogen uptake (owing to reduction of the nitro groups) ceased (about 3 hr.). The product (116 mg.) was recovered as for dihydrocyclobuxine (evaporating with nitrogen) and added to column A-1; the major band (66 mg., R_f 0.60) was highly unstable toward oxygen and heat, m.p. (crystallized neat) 167–173° dec., R_f on column B 0.45, Zimmermann test[6] negative (red color, but no maximum in the 400–600 mμ region). This unstable ketone VIIc (44 mg.) was dissolved in methanol (3 ml.) and treated with 3% hydrochloric acid in methanol to neutrality; the total salt (63 mg.) was precipitated with isopropyl ether and recrystallized twice from methanol–isopropyl ether to give 22 mg., m.p. 225° dec.

Anal. Calcd. for $C_{24}H_{42}O_2N_2Cl_2 \cdot H_2O$: C, 60.11; H, 9.25; N, 5.84; Cl, 14.79. Found: C, 60.17; H, 9.14; N, 6.03; Cl, 15.15.

Diosphenol (XIIIa).—The unstable ozonolysis product of cyclobuxine (VIIc, prepared through the di-*p*-nitrobenzyloxycarbonyl derivative; 5 mg.) was dissolved in 5% ethanolic potassium hydroxide (1 ml.) with brief heating, and let stand at 0° under nitrogen for 15 hr. The crude product XIIIa (5 mg., recovered with water and chloroform) showed λ_{max} 2.91 (s), 6.04 (s), and 6.18 (s) μ; λ_{max}^{EtOH} 296.5 mμ, log ϵ 3.95; $\lambda_{max}^{EtOH-NaOH}$ 343.5 mμ, log ϵ 3.81.

The O,O',N-triacetyl derivative XIIIb was prepared with acetic anhydride–pyridine, 72 hr. at room temperature; crystallized from acetone–isopropyl ether, m.p. 245–250° dec., R_f on column

(37) W. H. Hartung and R. Simonoff, *Org. Reactions*, **7**, 253 (1953).

(38) It was necessary to transform all crystalline N,N,-di-*p*-nitrooenzyloxycarbonyl derivatives to an amorphous form (by dissolution in chloroform and evaporation to dryness) before most reactions, to increase solubility in solvents other than chloroform. For hydrogenolyses, the diamide was generally rendered amorphous as above and added to the suspension of equilibrated catalyst as a solution in a small volume of ethyl acetate.

A-1 0.64; λ_{max} 5.67 (s), 5.78 (s), 5.97 (s), and 6.14 (s) μ; λ_{max}^{EtOH} 277 mμ, log ϵ 4.10.

Anal. Calcd. for $C_{29}H_{41}O_6N$: C, 69.71; H, 8.27; N, 2.80. Found: C, 69.75; H, 8.61; N, 3.09.

Decyclized Diosphenol (XIV).—Decyclized cyclobuxine (23 mg., m.p. 249–252° dec.) was transformed to the di-*p*-nitrobenzyloxycarbonyl derivative in benzene (10 ml.) with potassium carbonate (1 g.) and *p*-nitrobenzyl chloroformate (35 mg.), stirring 90 min. at room temperature. The product, recovered as for N,N′-di-*p*-nitrobenzyloxycarbonylcyclobuxine, was crystallized from ethanol to give 44 mg. of XIc, m.p. 252–255°, λ_{max} 5.93 (vs) μ. This total was ozonized[38] at 0° in acetic acid (3 ml.) and ethyl acetate (3 ml.), using a very slow flow of ozone (0.0006 mmole/min.) for 100 min. (exactly 100%), seeking to achieve selective cleavage of the terminal methylene. The product (47 mg.), obtained and recovered as for the ozonolysis product of O,N,N′-triacetylcyclobuxine, was directly hydrogenolyzed[38] with 30% palladium-on-charcoal (25 mg.) in ethanol (20 ml.) containing 1 *N* hydrochloric acid (0.5 ml.); the total product was purified on column A-1. The desired product (5 mg., 22%, R_f 0.30; λ_{max} 5.80 (s) μ) was dissolved in 5% ethanolic potassium hydroxide (1 ml.) and let stand 16 hr. at 0°. The recovered crude decyclized diosphenol XIVa had λ_{max} 2.91 (s) and 6.00 (s) μ; λ_{max}^{EtOH} 277 mμ, $\lambda_{max}^{EtOH-NaOH}$ 322 mμ. It was directly acetylated with acetic anhydride–pyridine, 74 hr. at room temperature. The triacetyl derivative XIVb was purified by partition chromatography on column A-1 (1 mg., R_f 0.67) and showed λ_{max} 5.68 (s), 5.78 (s), 5.96 (s), and 6.13 (s) μ; λ_{max}^{EtOH} 247 mμ, log ϵ 4.10. Identical material prepared from the decyclization product of the ketone from cyclobuxine VIIc showed m.p. 238–242° dec.

Anal. Calcd. for $C_{29}H_{41}O_6N$: C, 69.71; H, 8.27; N, 2.80. Found: C, 70.40; H, 8.21; N, 3.62.

N,N′-Diacetyl-16-dehydrodihydrocyclobuxine (XVa).—N,N′-Diacetyldihydrocyclobuxine *A*[11] (10 mg.) was stirred 1 hr. at room temperature with chromium trioxide (2 mg.) in 95% acetic acid (4 ml.). The product, recovered with dilute ammonium hydroxide and chloroform, was purified on column A-1; the fraction of R_f 0.75 (6 mg.) was crystallized from acetone–isopropyl ether to give 2 mg., m.p. 215–220°; λ_{max} 5.79 (s), 6.15 (vs) μ; Zimmermann test[26] strongly positive, deep red; $\lambda_{max}^{EtOH-NaOH}$ 495 mμ, shoulder at 540 mμ.

N,N′-Dibenzoyldihydrocyclobuxine (IIb).—Dihydrocyclobuxine (50 mg.) was stirred 75 min. at room temperature with benzoyl chloride (70 mg.) and potassium carbonate (1 g.) in benzene (10 ml.). The benzene was removed, and the residue stirred with water (8 ml.) and chloroform (8 ml.) overnight; the product, recovered from the chloroform layer, was purified on column B. The fraction of R_f 0.55 (78 mg.) was crystallized from acetone to give 31 mg., m.p. 268–276° dec.; recrystallization from acetone gave 10 mg., m.p. 273–275° dec., $[\alpha]^{26}D$ −27° (*c* 2.80), λ_{max} 6.20 (vs) μ.

Anal. Calcd. for $C_{39}H_{52}O_3N_2$: C, 78.48; H, 8.78. Found: C, 78.18; H, 8.91.

The O-acetyl derivative IIc was prepared with acetic anhydride–pyridine, 40 hr. at room temperature; amorphous, $[\alpha]^{22}D$ −54° (*c* 0.58); λ_{max} 5.79 (s) and 6.13 (vs) μ.

N,N′-Dibenzoyl-16-dehydrodihydrocyclobuxine (XVb).—N,N′-Dibenzoyldihydrocyclobuxine (78 mg.) was stirred at room temperature in 95% acetic acid (10 ml.) with chromium trioxide (12 and 8 mg., 1 hr. each). The product, recovered with dilute ammonium hydroxide and chloroform, was purified on column B; the fraction of R_f 0.59 (73 mg.) was crystallized from acetone to give 47 mg., m.p. 281–283° dec., $[\alpha]^{21}D$ −53° (*c* 0.58), λ_{max} 5.78 μ, Zimmermann test[26] strongly positive (wine-magenta), $\lambda_{max}^{EtOH-NaOH}$ 557 mμ.

Anal. Calcd. for $C_{39}H_{50}O_3N_2$: C, 78.75; H, 8.47; N, 4.71. Found: C, 78.75; H, 8.58; N, 4.62.

Activated Manganese Dioxide.[31]—Manganese sulfate monohydrate (1.68 g.) in water (5 ml.) and 40% sodium hydroxide (2.34 ml.) were added dropwise and simultaneously to a hot solution of potassium permanganate (1.91 g.) in water (12 ml.); after 1 hr. at 100°, the suspension was cooled and the product recovered by centrifugation, dried for 4 hr. at 100°, ground to a fine powder (2.23 g.), and stored.

Attempts to Conjugate the Double Bond in Decyclized Materials with the 16-Ketone. 1. With N,N′-Diacylated 16-Dehydrodihydrocyclobuxines.[28]—A solution of N,N′-diacetyl-16-dehydrodihydrocyclobuxine (XVa, 6 mg.) in chloroform (3 ml.) and 1 drop of water was saturated with gaseous hydrogen chloride and let stand 42 hr. at room temperature. The product (6 mg., recovered with water and chloroform) showed no trace of a 5.85 μ band in the infrared and only end absorption in the ultraviolet. Similar treatment of N,N′-dibenzoyl-16-dehydrodihydrocyclobuxine (XVb) for 70 hr. gave a product showing a very slight shoulder at 5.85 μ in the infrared, and a similar faint shoulder (on end absorption) at 231 mμ in the ultraviolet (possibly arising from methyl migration from C-14 to C-8).

2. From Decyclized Dihydrocyclobuxine (XIIa).[31]—Decyclized dihydrocyclobuxine (35 mg.) was acetylated as for N,N′-diacetylcyclobuxine; the product XIIc (48 mg., λ_{max} 6.15 (vs), no 5.80 μ) was dissolved in benzene (5 ml.) and stirred vigorously 2 hr. at room temperature with activated manganese dioxide (250 mg.). The solution was filtered, and the solid washed with chloroform and discarded; the filtrate contained 45 mg., λ_{max} 5.78 (s) and 6.15 (vs) μ; infrared and ultraviolet spectra identical with those of the decyclized N,N′-diacetyl-16-dehydrodihydrocyclobuxine prepared as above. Purification was accomplished on column B; no fractions showed any trace of enone absorption in the ultraviolet. The product (21 mg., R_f 0.32) was crystallized from acetone–isopropyl ether to give poor clumps, m.p. 194–219°.

N,N′-Di-*p*-nitrobenzyloxycarbonyldihydrocyclobuxine (IId).—Dihydrocyclobuxine (100 mg.) was stirred 90 min. at room temperature with *p*-nitrobenzylchloroformate (150 mg.) and potassium carbonate (3 g.) in benzene (14 ml.); the benzene was removed, and the residue was stirred overnight with water (30 ml.) and chloroform (30 ml.). The layers were separated and the aqueous part extracted with a further 30 ml. of chloroform; the combined chloroform extracts were concentrated to 20 ml., backwashed with 2 *N* hydrochloric acid (2 × 15 ml.) and water (20 ml.), and evaporated to give the product (244 mg.), crystallized from chloroform–ethanol to give 176 mg., m.p. 226–228°, $[\alpha]^{22}D$ −17° (*c* 1.59); λ_{max} 5.93 (vs) μ.

Anal. Calcd. for $C_{41}H_{54}O_9N_4 \cdot 0.5H_2O$: C, 65.13; H, 7.34; N, 7.43. Found: C, 65.14; H, 7.35; N, 7.86.

The O-acetyl derivative IIe was formed with acetic anhydride–pyridine,[38] 41 hr. at room temperature; crystallized poorly from ethanol, m.p. 221–223°, $[\alpha]^{26}D$ −50° (*c* 1.74); λ_{max} 5.79 (s) and 5.93 (vs) μ.

N,N′-Di-*p*-nitrobenzyloxycarbonyl-16-dehydrodihydrocyclobuxine (XVc).—**N,N′-Di-*p*-nitrobenzyloxycarbonyldihydrocyclobuxine** (IId, 105 mg.) was stirred at room temperature in 95% acetic acid[38] (10 ml.) with chromium trioxide (15 and 10 mg., 1 hr. each). The product (113 mg.), recovered with dilute ammonium hydroxide and chloroform, was crystallized from ethanol to give 92 mg., m.p. 185–192°; λ_{max} 5.78 (s) and 5.93 (vs) μ.

16-Dehydrodihydrocyclobuxine (XVd).—Crude N,N′-di-*p*-nitrobenzyloxycarbonyl-16-dehydrodihydrocyclobuxine (145 mg.) was hydrogenolyzed[38] 4 hr. with 30% palladium-on-charcoal (100 mg.) in ethanol (30 ml.) containing 1 *N* hydrochloric acid (1 ml.). The product (91 mg.) was recovered (as for dihydrocyclobuxine) as rapidly as possible, and added to column B; two bands appeared, R_f 0.70 (6 mg.) and 0.58 (42 mg., 56%); the second of these, upon rechromatography on the same column, again gave two bands, R_f 0.75 (22 mg.) and 0.62 (21 mg.). The second band (XVd) showed λ_{max} 3.00 (w) and 5.80 (s) μ, and gave a positive Zimmermann test[26] showing $\lambda_{max}^{EtOH-NaOH}$ 495 mμ, shoulder at 540 mμ (compare the N,N′-diacetyl derivative XVa); stable in acidic solution, but rapidly decomposed in neutral or basic solution to give the first band above (see below). Benzoylation of this material (as for N,N′-dibenzoyldihydrocyclobuxine) and chromatography of the product on column B gave material (R_f 0.52) with an infrared spectrum completely superimposable upon that of N,N′-dibenzoyl-16-dehydrodihydrocyclobuxine (XVb), and m.p. (upon crystallization from acetone) 284–287° dec.; m.m.p. with authentic material of m.p. 278–283° dec., 278–285° dec.

***cis*- and *trans*-Des-N′-16-dehydrodihydrocyclobuxine (XVI).**—Crude N,N′-di-*p*-nitrobenzyloxycarbonyl-16-dehydrodihydrocyclobuxine (from 500 mg. of dihydrocyclobuxine) was hydrogenolyzed[38] as above (6 hr.) with 30% palladium-on-charcoal (150 mg.) in ethanol (70 ml.) containing 2 *N* hydrochloric acid (5 ml.). The product (670 mg., including some *p*-toluidine) was recovered as above, and treated 1 hr. at room temperature with 80% ethanol (10 ml.) containing potassium hydroxide (500 mg.). The total eliminated product (660 mg., recovered with water, evaporation of the ethanol, and extraction with chloroform) was separated on column C (in two portions); two bands appeared, at R_f 0.72 (XVIa, 100 mg., 21%) and 0.55 (XV Ib, 230 mg., 49%). Both of these fractions, although highly crystalline neat, were hygroscopic and very difficult to crystallize from solvent (acetone–isopropyl ether was fair).

The *trans* isomer XVIa showed m.p. 134–137°, $[\alpha]^{21}D$ −65° (*c* 1.60), λ_{max} 5.86 (s), 6.09 (s), and fingerprint bands at 7.43, 8.48, 8.66, 8.77, 9.20, 9.87, and 10.12 μ; n.m.r. 4.35 (1H, quartet, *J* 7 c./sec.; =C*H*CH₃), 7.62 (3H, unsplit; N-methyl), 7.92 (3H, doublet, *J* 7 c./sec.; =CHC*H*₃), 8.77, 9.08 (6H, two sharp peaks; 2 tertiary C-methyl), 9.21 (3H, doublet, *J* 7 c./sec.; secondary C-methyl), 9.33, and 9.69 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

The *cis* isomer XVIb showed m.p. 149–152°, $[\alpha]^{21}D$ −83° (*c* 2.00), λ_{max} 5.86 (s), 6.09 (s, and stronger than for XVIa), and fingerprint bands at 7.82, 8.40, 8.60, 8.76, 9.14, 9.72, 10.17, and 11.61 μ; n.m.r. 3.53 (1H, quadruplet, *J* 7.5 c./sec.; =CHCH₂), 7.60 (3H, unsplit; N-methyl), 8.19 (3H, doublet, *J* 7.5 c./sec.; =CHC*H*₃), 8.68, 9.05 (6H, two sharp peaks; 2 tertiary C-methyl), 9.19 (3H, doublet, *J* 7 c./sec.; secondary C-methyl), 9.34, and 9.67 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

These isomers were readily interconvertible in base; either one gave an equilibrium mixture of 55–70% *cis* isomer. The mixture showed λ_{max}^{EtOH} 244 mμ, log ε 3.89 (in accord with structure XVI), and gave a strongly positive Zimmermann test[26] ($\lambda_{max}^{EtOH-NaOH}$ 540 mμ, hence probably the source of the shoulder in the spectra of the Zimmermann test products from the ketones XVa and XVd).

The N-benzoyl derivative mixture was produced with benzoyl chloride and potassium carbonate in benzene, and separated on column C; the two isomers were crystallized from a small volume of ethyl ether.

The *trans*-N-benzoyl derivative (R_f 0.62) showed m.p. 223–227°, λ_{max} 5.85 (s), 6.09 (s), 6.20 (vs), and fingerprint bands at 7.28, 8.48, 8.64, 8.73, 9.03, 9.20, 9.87, 10.11, and 10.59 μ.

Anal. Calcd. for $C_{31}H_{41}O_2N$: C, 81.00; H, 8.99; N, 3.05. Found: C, 80.77; H, 9.07; N, 3.44.

The *cis*-N-benzoyl derivative (R_f 0.59) showed m.p. 200–202°, $[\alpha]^{22}D$ −93° (*c* 1.63); λ_{max} 5.84 (s), 6.09 (s), 6.20 (vs), and fingerprint bands at 7.26, 7.33, 8.40, 8.60, 8.72, 9.10, and 10.15 μ.

Anal. Calcd. for $C_{31}H_{41}O_2N$: C, 81.00; H, 8.99; N, 3.05. Found: C, 80.62; H, 9.03; N, 3.39.

The isomers of the N-benzoyl derivative were similarly readily interconvertible by base treatment.

N-Benzoyldihydro-des-N′-16-dehydrodihydrocyclobuxine (XVII).—*trans*-N-Benzoyl-des-N′-16-dehydrodihydrocyclobuxine (50 mg.) was hydrogenated with reduced platinum oxide (25 mg.) in 10% ethanolic acetic acid (20 ml.); uptake of hydrogen was 1.03 mole equiv. in 30 min. The product (55 mg.), recovered as for dihydrocyclobuxine, was purified on column C; crystallization of the R_f 0.63 band (27 mg.) from ethanol gave 20 mg., m.p. 220–223°, $[\alpha]^{22}D$ −109° (*c* 0.53); λ_{max} 5.79 (s) and 6.20 (vs) μ.

Anal. Calcd. for $C_{31}H_{43}O_2N$: C, 80.65; H, 9.39. Found: C, 80.62; H, 9.37.

The N-benzoyl group of XVII and similar compounds were unaffected by heating at 180° in ethylene glycol containing 20% potassium hydroxide, or at 150° in ethylene glycol containing 33% phosphoric acid; prolonged hydrolysis at 210° with sodium in diethylene glycol succeeded in removing the group.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN, MADISON 6, WIS.]

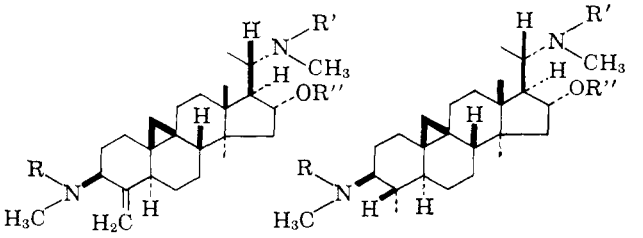
Buxus Alkaloids. IV.[1] The Configuration of Cyclobuxine and Its Interrelation with Cycloeucalenol

BY KEITH S. BROWN, JR.,[2] AND S. MORRIS KUPCHAN[3]

RECEIVED APRIL 6, 1964

Cyclobuxine (Ia) has been interrelated chemically with the known steroid cycloeucalenol (Va), *via* the common degradation product IV (4α,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione), obtained from dihydrocyclobuxine (IIa) by Ruschig degradation followed by selective hydrogenation, and from cycloeucalenol by standard methods of steroid side-chain degradation. This interrelation established the absolute configuration of cyclobuxine at positions 5, 8, 9, 10, 13, and 14. The configurations at 3, 16, 17, and 20 were inferred from physical and chemical evidence, including optical rotatory dispersion measurements on 4- and 16-ketones, nuclear magnetic resonance properties, molecular rotation relationships, Hofmann degradation at the 3-position, and a marked 1,3-*cis* interaction of the 16α-hydroxyl group with the 20α-amino function. Cyclobuxine is thus formulated as 3β,20α-bis(methylamino)-4-methylene-14α-methyl-9β,19-cyclo-5α-pregnan-16α-ol.

Cyclobuxine (Ia), an unusual cyclosteroid alkaloid isolated from the acetone-insoluble portion of the strong bases from *Buxus sempervirens* L., was assigned the structure shown (exclusive of stereochemistry) by a series of chemical degradations described in the previous paper.[1] Structure Ia for cyclobuxine was strongly supported by the interrelation achieved with the known steroid cycloeucalenol (Va).[4] Ruschig degradation[5] of dihydrocyclobuxine (IIa)[1] led through the crystalline dichloramine IIh to the oily one–enone III, characterized spectrally (λ_{max} 5.87, 6.01, and 6.28 μ, λ_{max}^{EtOH} 243 mμ (log ε 3.89)); this was selectively reduced to the diketone IV (4α,14α-dimethyl-9β,19-



	R	R′	R″
Ia,	H	H	H
b,	$COCH_3$	$COCH_3$	$COCH_3$
c,	CH_3	CH_3	H
d,	$COCH_3$	$COCH_3$	H
e,	$COCH_3$	H	H

	R	R′	R″
IIa,	H	H	H
b,	CH_3	CH_3	H
c,	C_6H_5CO	C_6H_5CO	H
d,	*p*-$NO_2C_6H_4CH_2OCO$	*p*-$NO_2C_6H_4CH_2OCO$	H
e,	H	CH_3	H
f,	$COCH_3$	CH_3	$COCH_3$
g,	$COCH_3$	H	H
h,	Cl	Cl	H

(1) Part III is the previous paper (K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4414 (1964)). The material in this paper was originally outlined in part II (K. S. Brown, Jr., and S. M. Kupchan, *ibid.*, **84**, 4592 (1962)).

(2) National Science Foundation Cooperative Predoctoral Fellow in Chemistry, 1960–1962.

(3) To whom inquiries concerning this paper should be directed. This investigation was supported in part by research grants from the National Institutes of Health (H-2952 and CY-4500).

(4) J. S. G. Cox, F. E. King, and T. J. King, *J. Chem. Soc.*, 1384 (1956); 514 (1959). We acknowledge gratefully the kindness of Dr. T. J. King, of the University of Nottingham, in making available to us a generous gift of cycloeucalenol for the described degradation.

(5) H. Ruschig, W. Fritsch, J. Schmidt-Thomé, and W. Haede, *Chem. Ber.*, **88**, 883 (1955); L. Labler and F. Šorm, *Collection Czech. Chem. Commun.*, **24**, 2975 (1960).