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 0.50) 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.]

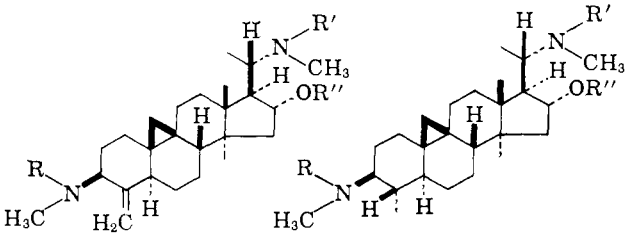
Buxus Alkaloids. IV.[1] The Configuration of Cyclobuxine and Its Interrelation with Cycloeucalenol

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)).

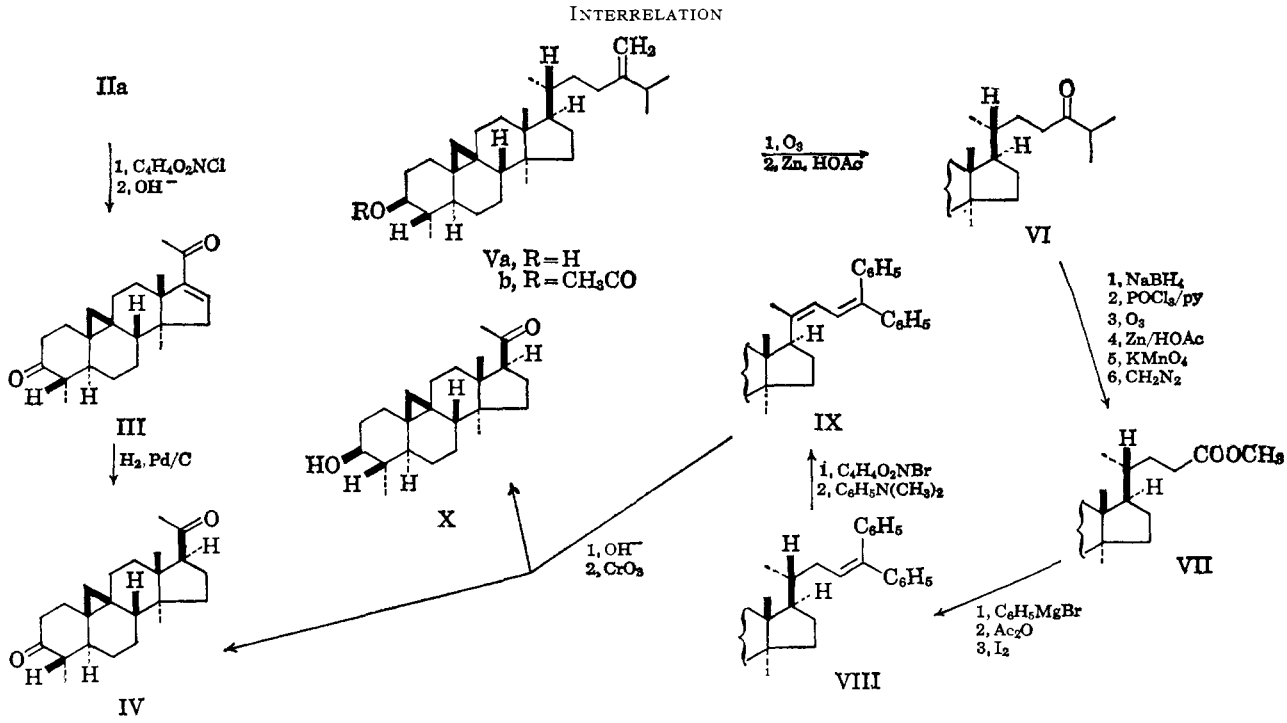
(2) National Science Foundation Cooperative Predoctoral Fellow in Chemistry, 1960–1962.

(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.

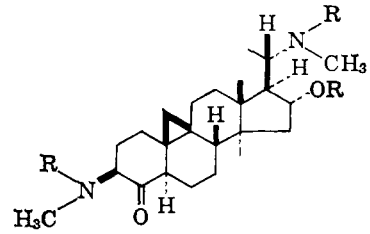
(5) H. Ruschig, W. Fritsch, J. Schmidt-Thomé, and W. Haede, *Chem. Ber.*, **88**, 883 (1955); L. Labler and F. Šorm, *Collection Czech. Chem. Commun.*, **24**, 2975 (1960).

INTERRELATION



cyclo-5α-pregnane-3,20-dione), having infrared and n.m.r. spectral characteristics in accord with the assigned structure. Degradation of cycloeucalenol (Va)[4] through 28-nor-24-oxocycloeucalanyl acetate (VI)[6] to the tetranoracid methyl ester acetate VII proceeded by essentially the published method[4]; Meystre–Miescher degradation then proceeded in standard fashion[7] through the diphenylethylene acetate VIII to the diphenylbutadiene acetate IX, which (in crude form) was hydrolyzed and oxidized with chromic acid to give the ketoalcohol X, and the diketone IV, which was identical with the product from Ruschig degradation of dihydrocyclobuxine (IIa) by melting point and mixture melting point, infrared spectrum, and chromatographic behavior. The ketoalcohol X was also oxidized with chromic acid–pyridine[8] to the diketone IV; the over-all yield of the diketone from cycloeucalenol was 3%, and from the known tetranor acid methyl ester acetate VII, 17%. As cycloeucalenol (Va) has been related to cycloartenol[4] and thence to lanosterol,[9] this interrelation not only supports the assigned structure Ia for cyclobuxine but also establishes the absolute configuration at six of its ten asymmetric centers (5α, 8β, 9β, 10β, 13β, and 14α).

The ozonolysis product (XIa)[1] of O,N,N′-triacetylcyclobuxine (Ib)[1] demonstrated in the O.R.D. a weak negative Cotton effect (M_{316} −2740°) comparable to that of cholestan-4-one ($M_{307.5}$ −3000°).[10] If the 3-acetylmethylamino group were α(axial), it would be expected to contribute a large negative increment to the first extremum of the Cotton effect[11]; the fact that such is not observed supports assignment of β-(equatorial) configuration at the 3-position. The dihydrochloride of the ozonolysis product (XIb)[1] of



XIa, R=$COCH_3$
b, R=H

cyclobuxine (obtained through the N,N′-di-*p*-nitrobenzyloxycarbonyl derivative, and unstable as the free base) had a similarly weak negative Cotton effect in the O.R.D. as far down as measurements could be taken (340 mμ). Furthermore, the 3-monomethiodide of N,N′-dimethyldihydrocyclobuxine (IIb) melted at 245–255° without evolution of trimethylamine, and under vigorous Hofmann degradation conditions gave a 56% recovery of N,N′-dimethyldihydrocyclobuxine and no detectable olefin. Haworth[12] has pointed out that 3β(*i.e.*, equatorial)-trimethylammonium-5α-steroids give primarily the dimethylamino compounds back upon Hofmann degradation, owing to the impossibility of the *trans*-diaxial (coplanar) elimination which allows facile degradation of the corresponding 3α-epimers.

Further support for the assignment of 5α-configuration was provided by the above O.R.D. and Hofmann results, as well as the difference in molecular rotation (−496°) between the Hofmann degradation product

(6) This is "24-demethyloxocycloeucalanyl acetate" (ref. 4); the alternate nomenclature is used here to reduce ambiguity in the naming of later compounds in the degradation sequence.

(7) Ch. Meystre, H. Frey, A. Wettstein, and K. Miescher, *Helv. Chim. Acta*, **27**, 1815 (1944); Ch. Meystre, H. Frey, R. Neher, A. Wettstein, and K. Miescher, *ibid.*, **29**, 627 (1946); Ch. Meystre, A. Wettstein, and K. Miescher, *ibid.*, **30**, 1022 (1947); C. S. Barnes, *Australian J. Chem.*, **9**, 228 (1956).

(8) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

(9) H. R. Bentley, J. A. Henry, D. S. Irvine, and F. S. Spring, *J. Chem. Soc.*, 3673 (1953); D. S. Irvine, J. A. Henry, and F. S. Spring, *ibid.*, 1316 (1955).

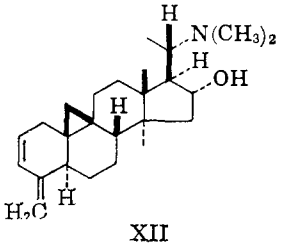
(10) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, pp. 43, 50–51.

(11) C. Djerassi, *ibid.*, pp. 178–190.

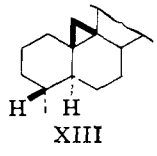
(12) R. D. Haworth, J. McKenna, and R. G. Powell, *J. Chem. Soc.*, 1110 (1953).

of N,N′-dimethylcyclobuxine[1] (XII) and its tetrahydro derivative XIII.[1,13]

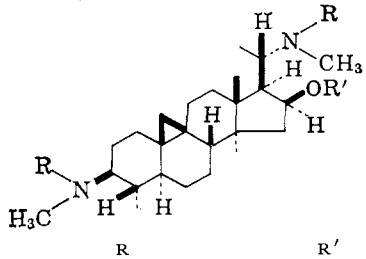
The α-orientation of the 16-hydroxyl group was suggested by the marked negative molecular rotation increments encountered upon acetylation of a variety of nitrogen-protected derivatives of cyclobuxine and dihydrocyclobuxine (see Table I).[14] Support for this assignment came in the sodium borohydride reduction of two nitrogen-protected 16-ketones, which led to alcohols (XIV) isomeric with the starting analogs (prepared from natural material), relatively unstable, and giving strong positive molecular rotation increments upon acetylation (see Table I).[14,15] The 16β-



XII



XIII



	R	R′
XIVa,	C_6H_5CO	H
b,	C_6H_5CO	$COCH_3$
c,	p-$NO_2C_6H_5CH_2OCO$	H
d,	p-$NO_2C_6H_4CH_2OCO$	$COCH_3$

epimers produced by reduction had more negative rotations than their 16α-counterparts, suggesting a vicinal side-chain interaction (which has been observed previously at this position[15]). This interaction in both epimers is also in accord with the relative instability of the 16β-alcohols, and the ease of oxidation (with manganese dioxide) and acetyl hydrolysis observed at the 16-position.

The O.R.D. curves of N,N′-diacylated derivatives of 16-dehydrodihydrocyclobuxine showed the very strong negative Cotton effect, with a trough far out and a shoulder about 5 mμ below it, observed previously for 16-ketosteroids with a 17β-side chain[16] (*e.g.*, the N,N′-dibenzoyl derivative[1] XV, M_{331} −6300°, $M_{325-320}$ −6000°). Furthermore, the n.m.r. signal of the 16β-proton in all (except acetylated) derivatives of cyclobuxine examined was split by one nearly opposing proton (J 9.5 c./sec.)[17]; examination of models of cyclobuxine

(13) The change in molecular rotation for the transformation cyclobuxine → dihydrocyclobuxine is −205°; thus the increment for the saturation of the 2,3-double bond is −291°. The listed values are, for 5α-steroids, −152°; for 5β-steroids, +24° (W. Klyne, in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N. Y., 1955, p. 111).

(14) Average reported for 16α-hydroxy to 16α-acetoxy, −240°; for 16β-hydroxy to 16β-acetoxy, +65° (D. A. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951); ref. 13, pp. 108–118; ref. 21).

(15) Stereospecific rear attack at C-16 ketones, to yield 16β-alcohols, has been previously noted for a variety of reducing agents, including lithium aluminum hydride (S. Bernstein, M. Heller, and S. M. Stolar, *J. Am. Chem. Soc.*, **77**, 5327 (1955)).

(16) Reference 10, pp. 44–45, 57–58; C. Djerassi, R. Riniker, and B. Riniker, *J. Am. Chem. Soc.*, **78**, 6362 (1956).

(17) H. Conroy, in "Advances of 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. 310–311.

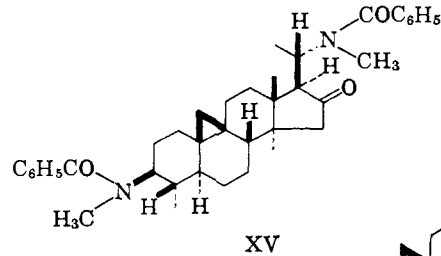
TABLE I

CHANGES IN MOLECULAR ROTATION AT THE 16-POSITION

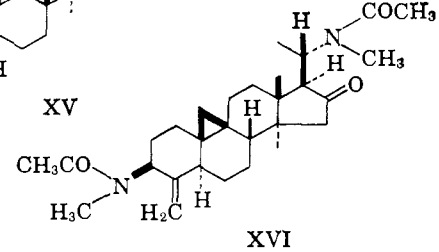
	Compound	Molecular rotations: Free 16-OH	Acetate	ΔM_D for acetylation
A.	N,N′-Dimethylcyclobuxine[1] (Ic)	+411°	+315°	−96°
	N,N′-Diacetyldihydrocyclobuxine (*B*)[18]	−83	−180	−97
	N,N′-Diacetyldihydrocyclobuxine (*A*)[18]	−208	−319	−111
	N,N′-Diacetylcyclobuxine[1] (Id)	+51	−62	−113
	Hofmann degradation product of N,N′-dimethylcyclobuxine[1] (XII)	+630	+503	−127
	N,N′-Dibenzoyldihydrocyclobuxine[1] (IIc)	−161	−346	−185
	N,N′-Di-*p*-nitrobenzyloxycarbonyldihydrocyclobuxine[1] (IId)	−127	−394	−267
	Average (seven pairs)			−142°
B.	N,N′-Dibenzoyl-16-epidihydrocyclobuxine (XIVa)	−185°	+25°	+210°
	N,N′-Di-*p*-nitrobenzyloxycarbonyl-16-epidihydrocyclobuxine (XIVc)	−239°	+16°	+255°

or of the steroid ring system in general shows that this proton could only occupy the 17α-position.

Biogenetic precedent strongly favored the 20α-configuration for cyclobuxine (all known natural 20-aminosteroids possess the α-configuration at that center[19]). Chemical support for this assignment was seen in the results of strong basic hydrolysis of N,N′-diacetylcyclobuxine[1] (Id), which led in nearly quantitative yield to the 3-N-monoacetyl derivative Ie, basic to phenol red, homogeneous upon partition chromatography, and showing a strong band at 6.20 μ in the infrared spectrum. The remaining N-acetyl group could be removed only under vigorous acidic conditions. The monoacetyl derivative Ie, which could be converted back to the diacetyl derivative Id with acetyl chloride, gave upon oxidation a ketone which rapidly lost methylamine in basic solution to give material possessing the typical spectral characteristics of the cyclopentenone mixture encountered before[1] (λ_{max} 5.85 and 6.08 μ); thus, the 20-N-acetyl group was hydrolyzed under the strongly basic conditions, presumably assisted by a *cis* interaction with the 16α-hydroxyl



XV



XVI

(18) See part V in this series (K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4430 (1964)).

(19) See R. Goutarel, *Tetrahedron*, **14**, 126 (1961); O. Jeger and V. Prelog in "The Alkaloids," Vol. VII, R. H. F. Manske, Ed., Academic Press, Inc., New York, N. Y., 1960, pp. 319–341.

group.[20] Facilitation of hydrolysis in a steroid 16α,-20α-diol diacetate has been observed and attributed to the interaction between the 18- and 21-methyl groups, forcing the 16α- and 20α-substituents to adopt parallel conformations.[21] Thus, mild hydrolysis of 3β,16α,20α-pregnanetriol triacetate gave the 16,20-diacetate and the free triol, but no monoacetate; in contrast, mild hydrolysis of 3β,16α,20β-pregnanetriol triacetate gave a good yield of 20-monoacetate.[21] The presence of a similar facilitation of hydrolysis between the 16α-hydroxyl group and the 20-N-acetyl group in N,N'-diacetylcyclobuxine was supported by the 62% recovery of N,N'-diacetyl-16-dehydrocyclobuxine (XVI) after hydrolysis under the strong basic conditions above (which transformed N,N'-diacetylcyclobuxine quantitatively into the monoacetyl derivative Ie).

This reaction was used to provide confirmation that the N-methyldihydrocyclobuxine (IIe) produced by partial reductive methylation of dihydrocyclobuxine (IIa) was selectively methylated at the 20-position. The O,N-diacetyl derivative IIf of IIe (which was hydrolyzed only to an N-monoacetyl derivative under strongly basic conditions) proved to be identical with the product of N-methylation and O-acetylation of the 3-N-monoacetyl derivative (IIg) produced by strong basic hydrolysis of N,N'-diacetyldihydrocyclobuxine (*A*).[18]

Experimental[22]

Partition Chromatography.—Three dry-packed columns[23] were employed, having characteristics as designated below.

System A: hexane–ethylene dichloride–methanol–water (100:50:20:3)[23]
column A: 1.8 cm. i.d.; 20 ml. of lower phase + 5 mg. of phenol red on 30 g. of Celite 545; run until fully wet, retention volume 45 ml.
System B: hexane–ethylene dichloride–methanol–water (40:12:8:1)
column B: same characteristics as column A
System C: hexane–ethylene dichloride–methanol–water (250:25:50:4)
column C: same as columns A and B, except brom thymol blue as indicator

Dihydrocyclobuxine Dichloramine (IIh).[5]—Dihydrocyclobuxine (500 mg.) was dissolved in chloroform (5 ml.) and ether (25 ml.), cooled to 0°, and treated with N-chlorosuccinimide powder (360 mg., 2.1 mole equiv.) with strong stirring until the solution was just neutral; after 15 min. further stirring, the solution was extracted with water (3 × 30 ml.), dried, evaporated, and crystallized from chloroform–methanol to give 448 mg., dec. 150° and up.

Anal. Calcd. for $C_{25}H_{42}ON_2Cl_2 \cdot 0.5H_2O$: C, 64.36; H, 9.29; N, 6.01; Cl, 15.20. Found: C, 64.65; H, 9.21; N, 5.18; Cl, 14.04.

Two more crops of crude dichloramine, totaling 148 mg., could be obtained; for most degradations, no effort was made to crystallize the somewhat unstable dichloramine, which was essentially pure as prepared.

The N-chlorosuccinimide could also be added advantageously as a dilute solution in cold chloroform.

Ruschig One-enone III.[5]—The dichloramine IIh (121 mg.) was dissolved in a trace of chloroform and methanol (1 ml.), cooled to 0°, and treated with a solution of sodium (100 mg.) in methanol (5 ml.); the mixture was let warm slowly to room temperature, then heated very slowly to boiling and refluxed 2 hr. (with moisture not excluded). Then 0.5 *N* hydrochloric acid (10 ml.) was added, the methanol was evaporated at 60°, and the total product (95 mg.) recovered with chloroform. This reaction, entailing three eliminations and two hydrolyses, was somewhat erratic; when imine hydrolysis was incomplete at this stage, the reaction mixture was heated at 80° in 5% ethanolic sodium hydroxide, or at 160° in aqueous ethylene glycol containing 10% potassium hydroxide, until the maximum yield of the one-enone XIX was obtained (determined by the intensity of the 5.87 μ band in the infrared spectrum relative to that at 6.01 μ; maximum yield near 50%). Further base treatment led to more degradation than imine hydrolysis, and hydrolysis with 2 *N* sulfuric acid gave no better results.

The above crude one-enone (95 mg.) was purified on Merck neutral alumina (5 g.). Benzene eluted no material; the product (39 mg., 44%) was eluted with chloroform–benzene (1:3, 70 ml., and 1:1, 75 ml.), and was directly followed by the 3-imine, necessitating careful fraction collection and infrared analysis. Rigorous purification of the one-enone III on column C gave pure material (R_f 0.75), faint yellow oil; λ_{max} 5.87 (s), 6.01 (s), and 6.28 (m) μ; λ_{max}^{EtOH} 243 mμ, log ε 3.89.

4α,14α-Dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione (IV).—The one-enone (III (15 mg.) was hydrogenated with 30% palladium-on-charcoal (15 mg.) in 10% ethanolic acetic acid (20 ml.), and the total product (14 mg.) recovered with water, evaporation of the ethanol, ammonium hydroxide, and chloroform, and purified on column C. The band of R_f 0.72 (10 mg.) crystallized from isopropyl ether to give 5 mg., m.p. 180–183°; pure material showed m.p. 182–184°, $[\alpha]^{23}D$ +114° (*c* 0.91); λ_{max} 5.87 (vs), 7.03 (m), and 7.37 (m) μ; n.m.r. 7.13 (1H, doublet, *J* 9.5 c./sec.; 4β-proton, split by the 5α), 7.95 (3H, $COCH_3$), 9.08 (3H, doublet, *J* 6 c./sec.; secondary C-methyl), 9.09, 9.13 (6H, two sharp peaks; 2 tertiary C-methyl), 9.39, and 9.68 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.07. Found: C, 80.15; H, 10.21.

Cycloeucalenyl Acetate (Vb).[4]—Cycloeucalenol[4] (Va, 4.10 g., crude, m.p. 131–133°) was acetylated with acetic anhydride (20 ml.) and pyridine (20 ml.) 80 min. at 100°. The excess anhydride was decomposed with water (200 ml.) and hydrochloric acid (35 ml.) at 60°; after 20 min., the solution was cooled and extracted with chloroform (2 × 100 ml.). The combined chloroform extracts were backwashed with dilute ammonium hydroxide, dried, evaporated, and crystallized from chloroform–methanol to give Vb (3.73 g., 83%), m.p. 109–111° (lit.[4] m.p. 109–110°).

28-Nor-24-oxocycloeucalanyl Acetate (VI).[4,6]—Cycloeucalenyl acetate (5.125 g., 11.0 mmoles), dissolved in methylene chloride (100 ml.), was treated at −80° with a stream of ozonated oxygen (about 0.8 mmole/min, delivered through a fritted glass diffuser) until the solution just lost its yellowish color and began turning blue (15 min.). The solution was stoppered and let warm to room temperature; acetic acid (30 ml.) was added, followed by zinc dust (10 g.) in small portions with strong stirring over 40 min. Finally, the solution was stirred 2 hr. at room temperature and filtered. A solution of sodium carbonate (30 g.) in water (200 ml.) was added to the filtrate with vigorous stirring under a stream of air. The lower layer was separated, washed with very dilute hydrochloric acid, dried, evaporated, and crystallized from methanol (first removing a sparingly soluble flocculent material); first crop, 3.980 g., m.p. 93–96° (lit.[4] 96–98°; other samples prepared in this laboratory had the higher m.p.), λ_{max} 5.85 (s) μ. The mother liquor wax partitioned between the phases of system A (25 ml. lower, 2 × 25 ml. upper, backwashed combined upper with 10 ml. lower) and the material in the upper phase (845 mg.) purified on Merck alumina (20 g.). Elution with chloroform–benzene (1:4, 150 ml.) gave 364 mg., m.p. (crystallized neat) 82–94°, infrared identical with that of the first crop; total product, 4.344 g. (85%). Over-ozonization by as little as 10% resulted in a marked decrease in the yield of this product (to 60–75%).

(20) *Cf.* S. M. Kupchan, S. P. Eriksen, and M. Friedman, *J. Am. Chem Soc.*, **84**, 4159 (1962), S. M. Kupchan, S. P. Eriksen and Y.-T. Shen, *ibid.*, **85**, 350 (1963).

(21) H. Hirshmann and F. B. Hirshmann, *J. Biol. Chem.*, **184**, 259 (1950).

(22) 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; optical rotatory dispersion curves were measured down to approximately 310 mμ on a Rudolph model 200AS spectropolarimeter. The authors are indebted to Mr. J. Alicino (Metuchen, N. J.) for microanalyses; all samples were dried for at least 12 hr. at 60° and 0.1 mm. pressure; note, however, that some samples were hygroscopic.

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

3β-Acetoxy-25,26,27,28-tetranor-24-cycloeucalanoic Acid Methyl Ester (VII).[4]—28-Nor-24-oxocycloeucalanyl acetate (VI, 1024 mg., m.p. 93–98°) was dissolved in absolute ethanol (40 ml.) and stirred 12 hr. at room temperature with a solution of sodium borohydride (330 mg.) in absolute ethanol (10 ml.). The reaction mixture was poured into 0.5 *N* hydrochloric acid (100 ml.) and the product (1098 mg., λ_{max} 2.90 (m), 5.85 (s), and 7.54 (m) μ) recovered with chloroform (2 × 100 ml.).

This total was heated on a steam bath for 90 min. with phosphorus oxychloride (2.6 ml.) in pyridine (10 ml.). The reaction mixture was cooled and poured slowly onto ice (150 g.) containing hydrochloric acid (15 ml.). The slurry was let warm to room temperature and extracted with chloroform (2 × 50 ml.), giving crude product (1.0 g.), purified by a rapid filtration in benzene through alumina (20 g.); yield of crude ester–olefin mixture, 823 mg., λ_{max} 5.81 (s), 6.13 (w), no 2.90 μ. If the total product was removed from the cold aqueous suspension with ether instead of chloroform, the chromatographic purification was not necessary, but great difficulties were encountered in the separation of the layers during the extraction.

The total 823 mg. (1.88 mmoles) was dissolved in methylene chloride (40 ml.), cooled to −80°, and treated with a stream of ozonated oxygen (about 0.8 mmole/min.) until the yellow color just faded and the solution began to turn blue; this took 1 min. 20 sec., indicating about 470 mg. of olefin present in the starting material. The ozonide was cleaved at room temperature with acetic acid (6 ml.) and zinc dust (2 g., in small portions), and the product recovered after 2 hr. stirring, exactly as for 28-nor-24-oxocycloeucalanyl acetate (using only 8 g. of sodium carbonate in the neutralization). The total product was dissolved in acetone (20 ml.) and 2 *N* sulfuric acid (2 ml.), cooled to 0°, and treated dropwise with stirring with a solution of potassium permanganate (450 mg.) in water (10 ml.) until the magenta color persisted for 15 min. (total addition time about 1 hr.). The solution was then stirred 10 min. at room temperature, the excess permanganate and manganese dioxide were reduced with aqueous sodium bisulfite, the acetone was removed by evaporation, and the total crude product (786 mg.) was recovered with chloroform. This was dissolved in the lower phase of system A (25 ml.), treated dropwise with 10% methanolic sodium hydroxide until basic, and extracted with the upper phase (3 × 25 ml.). The combined upper phases were backwashed with the lower phase (10 ml.), and the combined lower phases acidified with dilute hydrochloric acid, evaporated to remove methanol, and extracted with chloroform to give purified tetranor acid acetate (395 mg.), λ_{max} 3.0 and 5.8 μ (both broad).

The total amount of acid was methylated at 0° in chloroform (5 ml.) and ether (5 ml.) with a solution of diazomethane in ether (prepared from N-methyl-N-nitroso-*p*-toluenesulfonamide), until a distinct excess of diazomethane was present; the total product (398 mg.), recovered by evaporation in the hood, was added in benzene to alumina (20 g.). The first 25 ml. of eluate which contained 24 mg., with a poor infrared spectrum, was discarded (this was better separated in other runs by preliminary elution with hexane–benzene (1:1)). The crude product (320 mg.) was eluted with benzene (100 ml.) and chloroform–benzene (1:9, 50 ml.) and crystallized from methanol–water to give 252 mg. of VII (25% over-all), m.p. 124–126° (lit.[4] 96–98°); λ_{max} 5.80 (s), 7.96 (vs), and 8.55 (s) μ; n.m.r. 5.52 (1H, broad; 3-proton), 6.38 (3H, unsplit; OCH_3), 8.02 (3H, unsplit; O-acetyl), 9.10, 9.17 (6H, two sharp peaks; 2 tertiary C-methyl), 9.22 (6H, two superimposed doublets, *J* about 6 c./sec.; 2 secondary C-methyl), 9.67, and 9.92 τ (2H, AB doublets, *J* 4 c./sec.; cyclopropyl methylene).

Anal. Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.91; H, 10.22.

25,26,27,28-Tetranor-24,24-diphenyl-Δ^{23}-cycloeucalen-3β-yl Acetate (VIII).[7]—The Grignard reagent was prepared slowly, with strong stirring, from bromobenzene (3.5 ml.) and magnesium (800 mg.) in ether (29 ml.), refluxing 20 min. at the end to complete reaction. To this was added the tetranor acid methyl ester acetate VII (300 mg.) in benzene (20 ml.) at 0°, with strong stirring. The solution was brought slowly to reflux, 15 ml. of ether was removed, and the reaction mixture was refluxed gently overnight (temperature of refluxing vapors, 60–65°). The cloudy mixture was then poured onto ice (100 g.) and ammonium chloride (8 g.), and the product (1280 mg.) recovered with ether (2 × 100 ml.) and steam distilled for 25 min. (until no more water-insoluble material appeared in the distillate). The crude product (561 mg.) was recovered with chloroform and heated 1 hr. at reflux in acetic anhydride (15 ml.). The excess anhydride was decomposed with water; the product was recovered with ammonium hydroxide and chloroform (2 × 50 ml.), dissolved in benzene (5 ml.), heated 20 min. at reflux with iodine (20 mg.), cooled and treated with hexane (7 ml.) and Norit (500 mg.), heated 5 min., and added to alumina (20 g.), eluting with hexane–benzene (2:1). The first 75 ml. of eluate was discarded; the acetylated and dehydrated product (104 mg.) was eluted in the next 25 ml., with hexane–benzene (1:2, 50 ml.). Benzene (100 ml.) eluted 80 mg. (24%) of the phenyl-24-ketone derived from XXII (infrared showing only one phenyl group, two carbonyl groups; λ_{max}^{EtOH} 246 mμ, log ε 3.40). Elution with chloroform–benzene (1:1, 50 ml.) and chloroform (100 ml.) gave 245 mg. of nondehydrated product (λ_{max} 2.92 (m), 5.85 (s), and 8.00 (vs) μ) which (now free of contaminants) was smoothly dehydrated with iodine (40 mg.) in refluxing benzene (10 ml.) for 1 hr.; then treated with Norit, heated 15 min., and passed through alumina (15 g.) in hexane–benzene (1:2). Elution with 75 ml. gave the dehydrated product (191 mg.), which was combined with the 104 mg. above and crystallized from chloroform–methanol to give the diphenylethylene acetate VIII (243 mg., 66%), m.p. 156–162°. Pure material showed m.p. 160–162°, λ_{max} 5.80 (s), 6.25 (m), 6.69 (m), 7.96 (vs), and 14.36 (s) μ; λ_{max}^{EtOH} 252 mμ, log ε 4.14 (lit.[7] for this system, λ_{max}^{EtOH} 250 mμ, log ε 4.11).

Anal. Calcd. for $C_{40}H_{52}O_2$: C, 85.05; H, 9.28. Found: C, 85.13; H, 9.38.

Transformation of the Diphenylethylene Acetate VIII to 4α,14α-Dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione (IV).[7]—The diphenylethylene acetate VIII (300 mg.) was dissolved in carbon tetrachloride (20 ml.) and treated at reflux with N-bromosuccinimide (100 mg., 110%) under a 275-watt Westinghouse sun lamp until the solid on the bottom was completely transformed to succinimide on the surface of the solution (about 12 min.; hydrogen bromide gas evolution started after 10 min.). The suspension was cooled and filtered, dimethylaniline (3 ml.) was added *immediately* to the filtrate, the carbon tetrachloride was removed, and the dark solution was refluxed at 190° for 10 min., evaporated to 1 ml., and cooled. The resulting gummy oil was taken up in ether (20 ml.), and extracted with 2 *N* hydrochloric acid (3 × 20 ml.) and water (20 ml.); the aqueous solutions were combined and backwashed with ether. The combined ether extracts were evaporated, and the resulting 324 mg. (crude IX) dissolved in isopropyl alcohol (7 ml.), treated with potassium hydroxide (1.0 g.) in isopropyl alcohol (8 ml.) and water (0.5 ml.), and refluxed 110 min. on a steam bath. The product (331 mg.), recovered with dilute hydrochloric acid and chloroform, was purified on alumina (10 g.); elution with chloroform–benzene (1:4) gave 25 mg., discarded. The product (267 mg.) was removed with chloroform (100 ml.), and showed λ_{max}^{EtOH} 308 mμ, log ε 4.09 (lit.[17] for this system, λ_{max}^{EtOH} 305 mμ, log ε 4.44; hence about 120 mg. of product was present (40%).

The total crude product was dissolved in chloroform (4 ml.) and 90% acetic acid (4 ml.), cooled to 0°, treated dropwise with stirring with chromium trioxide (130 mg.) in 90% acetic acid (4 ml.), and let stand 9 hr. at 5°. Water and a small amount of sodium bisulfite were added, and the product (274 mg.) removed with chloroform (backwashing with dilute sodium bicarbonate solution) and added to alumina (10 g.). Elution with benzene (40 ml.), and chloroform–benzene (1:9, 40 ml., and 1:3, 40 ml.) gave 75 mg. (25% from the diphenylethylene acetate VIII), mostly the 3-ketone derived from VIII, which was crystallized from chloroform–methanol to give material of m.p. 188–190°, λ_{max} 5.87 (s), 6.25 (m), 6.69 (m), 14.35 (s), no 8.00 μ; λ_{max}^{EtOH} 251.5 mμ, log ε 4.12. Elution of the column with chloroform (50 ml.) gave 139 mg., which was further purified on a freshly packed column C. Much material (32 mg.) ran with the front; the expected diketone IV (22 mg., 12%, highly crystalline) appeared at the expected R_f 0.74, and the 3-alcohol-20-ketone X (26 mg., 14%, highly crystalline) at R_f 0.38.

The diketone IV was rechromatographed on the same column to give purified product (14 mg. in a narrow cut), which was crystallized from isopropyl ether to give 4α,14α-dimethyl-9β,19-cyclo-5α-pregnane-3,20-dione, m.p. 180–183°, m.m.p. with IV from degradation of dihydrocyclobuxine 183–185°; the infrared spectra of the two materials were completely superimposable, and the two were indistinguishable on paper chromatography (using heptane–methanol–water, 5:4:1[24]; detection by Zimmermann's test[25]).

(24) I. E. Bush, *Biochem. J.*, **50**, 370 (1952).

The keto alcohol X crystallized well from isopropyl ether to give 4α,14α-dimethyl-9β,19-cyclo-5α-pregnan-3β-ol-20-one, m.p. 178–181°, $[\alpha]^{20}D$ +110° (*c* 0.81); λ_{max} 2.75 (m), 2.90 (m), 5.88 (s), 7.35 (m), and 9.93 (m) μ; infrared otherwise very similar to that of the diketone IV. The total crude alcohol (26 mg.) was oxidized with chromium trioxide (30 mg.) in pyridine (1.0 ml.)[8] overnight at room temperature; the product (31 mg., recovered with dilute hydrochloric acid and chloroform) gave upon chromatography on column C the diketone IV (17 mg.), R_f 0.73, infrared superimposable upon that of the authentic diketone, m.p. upon crystallization from isopropyl ether 180–183°.

Optical Rotatory Dispersion.—The characteristics of the curves were:

Ozonolysis Product (XIa) of O,N,N′-triacetylcyclobuxine (Ib) (*c* 0.23 in methanol): M_{700} −226°, M_{589} −246°, M_{400} − 704°, M_{316} (min.) −2740°.

Ozonolysis product (XIb) of cyclobuxine (*c* 0.20 in methanol): M_{589} +76°, M_{400} −150°, M_{350} −500°, M_{340} − 620°.

N,N′-Dibenzoyl-16-dehydrodihydrocyclobuxine (XV).—(*c* 0.145 in methanol): M_{700} −286°, M_{589} −535°, M_{400} −1720°, M_{331} (min.) −6300°, $M_{325-320}$ (shoulder) −6000°.

N,N′-Dimethyldihydrocyclobuxine (IIb).—Dihydrocyclobuxine (100 mg.) was heated under reflux for 7 hr. with 40% formaldehyde (0.20 ml.) and 88% formic acid (0.26 ml.); the reaction mixture was worked up as for N,N′-dimethylcyclobuxine. Crystallization of the product (128 mg.) from acetone, nitromethane, or methanol–water gave crystals including the solvent of crystallization and melting near 160–170°; crystallization from methanol–isopropyl ether gave poor crystals melting near 205°; $[\alpha]^{22}D$ on a dried sample, +29° (*c* 1.14).

Attempted Hofmann Degradation of N,N′-Dimethyldihydrocyclobuxine.—N,N′-Dimethyldihydrocyclobuxine (68 mg.) was dissolved in chloroform (3 ml.), heated to 65°, and treated with methyl iodide (0.5 ml., 75 min., and 0.3 ml., 60 min.). The solution was cooled and filtered; from the filtrate was obtained the product (87 mg.), crystallized from methanol–methyl ethyl ketone to give 45 mg., m.p. 245–250° dec. without evolution of trimethylamine.

Anal. Calcd. for $C_{28}H_{51}ON_2I \cdot H_2O$: C, 58.32; H, 9.27; N, 4.86; I, 22.01. Found: C, 57.89; H, 9.44; N, 4.71; I, 22.15.

The crude monomethiodide (72 mg.) was heated in ethylene glycol (1.0 ml.), water (0.25 ml.), and potassium hydroxide (0.20 g.) at 180° (bath) for 8 hr. Nitrogen was passed through the reaction mixture and out into a receiver containing 0.100 *N* hydrochloric acid (5.0 ml.); titration of the receiver at the end of the reaction indicated 38% production of a volatile base. However, when the reaction mixture was worked up with water and chloroform and the product partitioned between chloroform and 2 *N* hydrochloric acid, there was obtained: (1) a chloroform-soluble hydrochloride fraction (17 mg.), whose infrared indicated N,N′-dimethyldihydrocyclobuxine (5 mg.) and no detectable olefin in the (at most) 8 mg. of organic material present; and (2) a chloroform-insoluble hydrochloride fraction (25 mg.), pure N-N′-dimethyldihydrocyclobuxine by infrared spectrum. Therefore, the recovery of starting material was about 30 mg. (56%), and no degraded material could be detected.

N,N′-Dibenzoyl-16-epidihydrocyclobuxine (XIVa).—N,N′-Dibenzoyl-16-dehydrodihydrocyclobuxine (39 mg.) was dissolved in absolute ethanol (10 ml.) and stirred 14 hr. at room temperature with sodium borohydride (50 mg.). The product (32 mg.), recovered by pouring the reaction mixture into very dilute hydrochloric acid and extracting with chloroform (evaporating the extracts under nitrogen and a minimum amount of heat), was purified on column B to give a single band (R_f 0.53), 30 mg., crystallized from acetone–isopropyl ether to give 22 mg., m.p. 261–262° dec., $[\alpha]^{26}D$ −31° (*c* 0.83); infrared very similar to that of N,N′-dibenzoyldihydrocyclobuxine (IIc); m.m.p. with the latter, 230–250°.

Anal. Calcd. for $C_{39}H_{52}O_3N_2$: C, 78.48; H, 8.78. Found: C, 78.22; H, 8.97.

The O-acetate XIVb was prepared with acetic anhydride–pyridine, 37 hr. at room temperature; amorphous, R_f on column C 0.30, $[\alpha]^{21}D$ +4° (*c* 1.20); λ_{max} 5.79 (s) and 6.18 (vs) μ.

The 16-epi-dibenzoyl derivative XIVa (13 mg.) was stirred at room temperature in 95% acetic acid (5 ml.) with chromium trioxide (5 and 3 mg., 1 hr. each); the product, recovered with dilute ammonium hydroxide and chloroform, was purified on column B. The band at R_f 0.55 (12 mg.) was crystallized from acetone to give N,N′-dibenzoyl-16-dehydrodihydrocyclobuxine[1] (XV), m.p. 283–285° dec., m.m.p. with authentic material of m.p. 281–283° dec., 282–285° dec.; infrared spectrum superimposable upon that of authentic material.

N,N′-Di-*p*-nitrobenzyloxycarbonyl-16-epidihydrocyclobuxine (XIVc).—N,N′-Di-*p*-nitrobenzyloxycarbonyl-16-dehydrodihydrocyclobuxine[1] (75 mg.) was stirred 14 hr. at 30° in absolute ethanol (10 ml.) with sodium borohydride (40 mg.) (the material was first rendered amorphous with chloroform, then dissolved in ethyl acetate (0.2 ml.); then the ethanol was added, with stirring). The product (which crystallized from the reaction mixture) was recovered with very dilute hydrochloric acid and chloroform and purified on column B; the fraction of R_f 0.66 (53 mg.) was crystallized from chloroform–ethanol to give 31 mg., m.p. 185° and up; $[\alpha]^{22}D$ −32° (*c* 2.07); infrared very similar to that of N,N′-di-*p*-nitrobenzyloxycarbonyldihydrocyclobuxine (IId).

The O-acetate XIVd was prepared with acetic anhydride–pyridine, 40 hr. at room temperature; crystallized from ethanol, m.p. 194–198°, $[\alpha]^{23}D$ +2° (*c* 1.47): λ_{max} 5.79 (s) and 5.92 (vs) μ.

3-N-Acetylcyclobuxine (Ie).—N,N′-Diacetylcyclobuxine (Id, 25 mg.) was dissolved in ethanol (2 ml.) and water (0.5 ml.) containing potassium hydroxide (1.0 g.) and heated 3 hr. at 80°. Water was added, the ethanol was evaporated, and the product (24 mg.) recovered with chloroform; chromatography on a column A gave a single sharp red band, R_f 0.80, 19 mg. (90%), which was crystallized with great difficulty from acetone–isopropyl ether; m.p. for neat-crystallized material, 187–192°; $[\alpha]^{22}D$ +41° (*c* 1.90); λ_{max} 6.20 (s) μ.

Anal. Calcd. for $C_{27}H_{44}O_2N_2$: C, 75.65; H, 10.35. Found: C, 75.35; H, 10.45.

The monoacetyl derivative Ie (10 mg.) was stirred 1 hr. at room temperature with acetyl chloride (10 mg.) and potassium carbonate (500 mg.) in benzene (5 ml.); the product, recovered by evaporation and partition of the residue between water and chloroform, gave upon crystallization from ethanol N,N′-diacetylcyclobuxine (Id, 5 mg.), m.p. 281–284° dec., infrared spectrum superimposable upon that of authentic material.

Transformation of 3-N-Acetylcyclobuxine (Ie) into a Cyclopentenone Mixture.—3-N-Acetylcyclobuxine (36 mg.) was converted to the 20-N-*p*-nitrobenzyloxycarbonyl derivative with *p*-nitrobenzyl chloroformate (25 mg.) and potassium carbonate (500 mg.), stirring 90 min. at room temperature in benzene (10 ml.). The benzene was removed, and the total product recovered with water and chloroform and purified on column B; the fraction of R_f 0.49 (40 mg.) was crystallized with difficulty from acetone–isopropyl ether to give 8 mg., m.p. 205–206°. This was combined with the mother liquor, dissolved in 95% acetic acid (10 ml.), and stirred at room temperature with chromium trioxide (7 and 5 mg., 1 hr. each). The infrared spectrum of the product (40 mg., recovered with dilute ammonium hydroxide and chloroform) showed some double bond attack; it was purified on column B. The fraction of R_f 0.64 (24 mg., 60%) was hydrogenated and hydrogenolyzed with 30% palladium-on-charcoal (20 mg.) in ethanol (20 ml.) containing 1 *N* hydrochloric acid (0.5 ml.); the total product was treated with 80% ethanol (4 ml.) containing potassium hydroxide (0.2 g.) for 1 hr. The product (12 mg., 96%) had the typical cyclopentenone bands in the infrared spectrum at 5.84 (s) and 6.08 (s) μ.

The total was hydrogenated with reduced platinum oxide (40 mg.) in 10% ethanolic acetic acid (20 ml.); the product (12 mg.) was purified on column B. The fraction of R_f 0.82 (11 mg.) showed λ_{max} 5.80 (s) and 6.20 (s) μ, the spectrum being essentially identical with that of material prepared by hydrogenation and N-acetylation of *cis*-des-N′-16-dehydrodihydrocyclobuxine[1] (possibly differing in the configuration at the 4-position[18]).

N,N′-Diacetyl-16-dehydrocyclobuxine (XVI).—N,N′-Diacetylcyclobuxine (25 ml.) was dissolved in benzene (10 ml.) and stirred with activated manganese dioxide[1] (125 mg.) for 2 hr. at room temperature. The reaction mixture was filtered and the product (27 mg.) recovered by evaporation of the filtrate and crystallized from acetone–isopropyl ether to give 18 mg., m.p. 222–225°, $[\alpha]^{22}D$ −39° (*c* 1.78); λ_{max} 5.77 (s), 6.14 (vs), and 11.08 (s) μ.

Anal. Calcd. for $C_{29}H_{44}O_3N_2$: C, 74.32; H, 9.46. Found: C, 74.21; H, 9.68.

Attempted Hydrolysis of N,N′-Diacetyl-16-dehydrocyclobuxine.—N,N′-Diacetyl-16-dehydrocyclobuxine (24 mg.) was heated 2 hr. in refluxing 80% ethanol (2.5 ml.) containing potassium hydroxide (1.0 g.); the mixture turned very dark. The product (20 mg., recovered with water, evaporation of the ethanol, and

(25) W. Zimmermann, *J. physiol. Chem.*, **300**, 141 (1955).

chloroform) gave upon chromatography on column B a single band, nonbasic, R_f 0.83 (15 mg., 62%), with infrared spectrum superimposable upon that of starting material XVI.

20-N-Methyldihydrocyclobuxine (IIe).—Dihydrocyclobuxine (55 mg.) was dissolved in ethanol (4 ml.) containing 40% formaldehyde (0.05 ml.), and the solution added to reduced platinum oxide (50 mg.) in acetic acid (10 ml.), stirred under hydrogen. One mole equivalent of hydrogen was taken up rapidly (15 min.), the next very slowly (many hours). The hydrogenation was halted when the uptake was about 1.5 mole equiv., and the product (recovered with water, evaporation of the ethanol, ammonium hydroxide, and chloroform) purified on column C. The slower-running major band, R_f 0.75 (22 mg., 38%), crystallized from acetone–water as needles (12 mg.), m.p. 123–125°, $[\alpha]^{22}D$ +21° (*c* 0.29); infrared spectrum similar to that of N,N′-dimethyldihydrocyclobuxine, but stronger at 2.9 μ.

Anal. Calcd. for $C_{28}H_{45}ON_2$: C, 77.55; H, 11.52. Found: C, 78.05; H, 11.55.

The faster-running major band, R_f 0.95 (24 mg., 41%), had an infrared spectrum superimposable upon that of N,N′-dimethyldihydrocyclobuxine (IIb), and demonstrated similarly poor crystallization behavior.

O,N-Diacetyl-20-N-methyldihydrocyclobuxine (IIf).—20-N-Methyldihydrocyclobuxine (IIe, 8 mg.) was acetylated with acetic anhydride–pyridine, 42 hr. at room temperature; the product was purified on column C, and the band of R_f 0.65 (8 mg.) crystallized from acetone–isopropyl ether to give 3 mg., m.p. 202–205°; λ_{max} 3.60 (m), 5.82 (s), and 6.16 (s) μ.

Anal. Calcd. for $C_{30}H_{50}O_3N_2$: C, 74.03; H, 10.39. Found: C, 74.31; H, 10.34.

Hydrolysis of the O,N-diacetyl derivative IIf (20 mg.) in refluxing 80% ethanol (2.5 ml.) containing potassium hydroxide (1.0 g.) for 2 hr. gave a product, purified on column C (R_f 0.25, 7 mg.) showing λ_{max} 6.16 (s) μ; there was no trace of a band above R_f 0.5 corresponding to fully hydrolyzed 20-N-methyldihydrocyclobuxine.

N-Acetyldihydrocyclobuxine (IIg).—N,N′-Diacetyldihydrocyclobuxine *A*[18] (from 50 mg. of dihydrocyclobuxine) was hydrolyzed as above, and the product purified on column B to give a single sharp red band, R_f 0.48 (31 mg.), crystallized with difficulty from acetone to give 13 mg., m.p. 237–240°, λ_{max} 6.18 (s) μ.

Anal. Calcd. for $C_{27}H_{46}O_2N_2$: C, 75.30; H, 10.77. Found: C, 75.38; H, 10.53.

The monoacetyl derivative IIg (25 mg.) was methylated in ethanol (4 ml.) and acetic acid (12 ml.), using platinum oxide (50 mg.) and 40% formaldehyde (0.05 ml.). One mole equivalent of hydrogen was taken up, *rapidly*. The total product (recovered as for 20-N-methyldihydrocyclobuxine) was acetylated with acetic anhydride–pyridine, 10 hr. at room temperature, and the product purified on column C. The major band, R_f 0.59 (20 mg.), had an infrared spectrum superimposable upon that of O,N-diacetyl-20-N-methyldihydrocyclobuxine prepared as above; crystallization from acetone–isopropyl ether gave material, m.p. 198–201°; m.m.p. with IIf of m.p. 200–203°, 198–203°.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN, AND THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIFORNIA]

Buxus Alkaloids. V.[1] The Constitution of Cyclobuxamine, a 4β-Monomethyl Cyclosteroid Alkaloid[2]

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The physical and chemical properties of cyclobuxamine (Ia, 3β-amino-20α-methylamino-4β,14α-dimethyl-9β,19-cyclo-5α-pregnan-16α-ol), an alkaloid isolated from the acetone-insoluble portion of the bases of *Buxus sempervirens* L., suggested that it was a 3-N-demethyldihydrocyclobuxine. However, trimethylcyclobuxamine (Ig) was found to be different from dimethyldihydrocyclobuxine. Molecular rotation comparisons indicated that, while dihydrocyclobuxine and its tri- and diacetyl derivatives IIIa–c possess a 4α-methyl group, the hydrogenation products Ih and Ii from tri- and diacetylcyclobuxine (IIb and IIc) have the 4β-methyl configuration. The triacetyl derivative Ih was synthesized from cyclobuxamine (Ia) *via* the 3-N-monoformyl derivative Im, confirming the structure and configuration of cyclobuxamine, which appears to be the first 4β-monomethyl steroid to be isolated from natural sources.

Cyclobuxamine (Ia), isolated by partition chromatography[1,5] from the acetone-insoluble portion of the strong bases from *Buxus sempervirens* L.,[2] is the most polar of the three major components of this fraction[6] and may be distinguished by its crystallization from acetone as the very stable isopropylideneimine Ib.[7] The imine Ib ($C_{27}H_{46}ON_2$) showed an infrared spectrum very close to that of dihydrocyclobuxine (IIIa)[1] with the addition of a strong sharp band at 6.01 μ for the C=N linkage. The n.m.r. spectrum[8] of Ib also showed its close similarity to IIIa, with similar peaks in the spectra of the two assignable to the 16β-proton (5.92 τ, octet, *J* 3, 7, and 9.5 c./sec.), an N-methyl (7.57 τ), two tertiary C-methyls (8.87 and 9.03 τ), two secondary C-methyls (8.92 and 9.32 τ), and a cyclopropyl methylene (9.58 and 9.83 τ, AB doublets of *J* 4 c./sec.); the spectrum of Ib also showed signals for the two methyl groups of the isopropylidene moiety (8.02 and 8.19 τ).

The imine could be hydrogenated with platinum in acetic acid, and the resulting isopropylamine Ic gave a triacetyl derivative Id showing typical infrared bands for an O-acetyl (5.77 μ) and two tertiary N-acetyl (6.15 μ) groups, and n.m.r. signals as observed before[1] for two N-acetyl and one N-methyl groups with restricted internal rotation. In the n.m.r. spectrum of Ic, the signals for the isopropylidene group of Ib are shifted upfield to appear as an isopropyl doublet (8.97 τ), while the signal for the 4β-methyl group is shifted downfield (to 9.00 τ).

(1) Parts III and IV: K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, **86**, 4414, 4424 (1964).

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(3) National Science Foundation Cooperative Predoctoral Fellow in Chemistry (University of Wisconsin), 1960–1962; National Institutes of Health Postdoctoral Fellow (Stanford University), 1963.

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(5) K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatog.*, **9**, 71 (1962). Cyclobuxamine is band III, the alkaloid of R_f 0.59 in column 1 of Table II.

(6) The other two are cyclobuxine (IIa) (see ref. 1) and cyclovirobuxine (K. S. Brown, Jr., and S. M. Kupchan, *Tetrahedron Letters*, in press).

(7) For further examples of this behavior in 3- and 20-aminosteroids see S.A. Oletta, Belgian Patent 627,935 (May 5, 1963).

(8) 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.).