Tetrahedron Vol 23, pp. 4563 to 4586. Pergamon Press Ltd. 1967 Printed in Great Britain

BUXUS ALKALOIDS—XII[1]

BENZAMIDE ALKALOIDS FROM *BUXUS SEMPERVIRENS* L.

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(*Received in USA* 18 *January* 1967; *accepted for publication* 10 *April* 1967)

Abstract- The isolation and structural elucidation of ten new alkaloids from the "additional weak bases" fraction of *Buxus sempervirens* L. are reported. A structural feature common to eight of the new alkaloids is the presence of a secondary benzamide function at the C-3 position. Evidence is presented for assignment of structures for N-benzoylcycloxobuxine-F (I), N-benzoylcycloxobuxidine-F (Xa), N-benzoyldihydrocyclomicrophylline-F (XII), N-benzoylcycloxobuxoline-F (XV), N-benzoyl-O-acetylcycloxobuxoline-F (XVI), N-benzoylbuxidienine-F (XIX), N-benzoylcycloprotobuxoline-D (XXIIIa), N-benzoylcycloprotobuxoline-C (XXXIIIb), tigloylcyclovirobuxeine-B (XXIXb), and N-acetylcycloprotobuxine-D (XXXc).

IN 1962, we reported the elucidation of structure[2] and configuration[3] of cyclobuxine-D, an alkaloid isolated from *Buxus sempervirens* L.[4] Cyclobuxine-D was shown to be the prototype of a new class of steroidal alkaloids which contain a cyclopropane ring and which have a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme, between lanosterol and cholesterol-type steroids. Subsequent studies have characterized many structurally related alkaloids, from a variety of *Buxus* species.[5-7] The present report describes the isolation and structural elucidation of ten new *Buxus* alkaloids, eight of which possess a secondary benzamide function at the C-3 position.

The new alkaloids were isolated from the "additional weak bases" fraction of the alkaloids of *Buxus sempervirens L.* obtained by the procedure described earlier.[2b] Absorption chromatography of the 1:1 benzene-Skellysolve B soluble material on basic alumina yielded six fractions. Each chromatographic fraction was further separated by partition chromatography.[8] The second fraction yielded N-*benzoylcycloprotobuxoline*-C, $C_{34}H_{52}N_2O_2$. The third fraction gave *tigloylcyclovirobuxeine-B*, $C_{32}H_{52}N_2O_2$. The fourth fraction gave tigloylcyclovirobuxeine-B, cyclovirobuxeine-B,[5,9,10] irehine,[11] and N-*benzoylcycloxobuxine-F*, $C_{33}H_{48}N_2O_2$. The

[1] Part XI: R. T. Puckett, G. A. Sim, E. Abushanab, and S. M. Kupchan, *Tetrahedron Letters* 3815 (1966).
[2] K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, [a] **84**, 4590 (1962); [b] **86**, 4414 (1964).
[3] K. S. Brown, Jr., and S. M. Kupchan, *J. Am. Chem. Soc.*, [a] **84**, 4592 (1962); [b] **86**, 4424 (1964).
[4] K. Heusler and E. Schlittler, *Helv. Chim. Acta* **32**, 2226 (1949).
[5] S. M. Kupchan and G. Ohta, *J. Org. Chem* **31**, 608 (1966).
[6] F. Khuong-Huu, D. Herlem-Gaulier, Q. Khuong-Huu, E. Stanislas, and R. Goutarel, *Tetrahedron* **22**, 3321 (1966).
[7] T. Nakano, S. Terao, and Y. Saeki, *J. Chem. Soc.* 1412 (1966).
[8] K. S. Brown, Jr., and S. M. Kupchan, *J. Chromatography* **9**, 71 (1962).
[9] F. Khuong-Huu-Laine, M. Magdelaine, N. Bisset, and R. Goutarel, *Bull. Soc. Chim. Fr.* 758 (1966).
[10] J. P. Calame and D. Arigoni, private communication.
[11] J. Tomko, Z. Votický, V. Paulick, A. Vassova, and O. Bauerova, *Chemicki Zvesti*, **18**, 721 (1964).

fifth fraction yielded cyclobuxoxine.[12] N-*benzoyl*-O-*acetylcycloxobuxoline-F*, $C_{35}H_{50}N_2O_4$, N-*benzoylbuxidienine-F*,[13-15] $C_{33}H_{48}N_2O_3$, *N-benzoyldihydrocyclomicrophylline-F*, $C_{33}H_{50}N_2O_3$, N-*benzoylcycloxobuxidine-F*, $C_{33}H_{48}N_2O_4$, and N-*acetylcycloprotobuxine-D*, $C_{28}H_{48}N_2O$. The sixth fraction gave N-*benzoylcycloprotobuxoline-D*, $C_{33}H_{50}N_2O_2$. N-*Benzoylcycloxobuxoline-F*, $C_{33}H_{48}N_2O_3$, was obtained by meticulous alumina chromatography of the "additional weak bases" fraction.

Evidence for assignment of structure I for N-benzoylcycloxobuxine-F was adduced from the observations which follow. The IR spectrum showed characteristic bands at 2·92 μ (NH), 3·30 μ (cyclopropyl), 6·02 μ (CO group conjugated with cyclopropyl; also "amide I" band of a secondary amide), 6·60 and 7·61 μ ("amide II and III" bands respectively- characteristic of a secondary amide), 7·02 μ (α-carbonyl methylene) and 12·00 μ. The latter band (12·00 μ) was present in the spectra of only those compounds which possess the conjugated CO group and may be characteristic for a C-11 ketone in this series of alkaloids. The UV spectrum showed λ_{max}^{EtOH} 225 mμ (ε 13,500), attributable to the additive absorption of the two isolated chromophores, Ph-CO-NH- and cyclopropyl-CO-. The NMR spectrum (Table 1) of N-benzoylcycloxobuxine-F (I) showed the presence of five aromatic protons, one amido hydrogen with hindered rotation about the C-N bond, an α-carbonyl methylene, two N-methyls, four tertiary C-Me's, and one secondary C-Me. Signals for a cyclopropane ring were not apparent in the NMR spectrum. The cited physical data compared closely with those reported for N-isobutyrylcycloxobuxine-F ("baleabuxine");[15,16] the major differences indicated the presence of a benzamide at C-3 instead of an isobutyramide in compound I. The IR and UV data suggested the presence of a CO function in conjugation with a point of unsaturation, probably the cyclopropane ring. The presence of an electron-withdrawing group (CO) adjacent to the cyclopropane ring (position 1 or 11) would be expected to deshield the cyclopropyl methylene protons from their normal position downfield into the Me region of the spectrum. The CO was assigned to the C-11 position rather than C-1 since the signal in the NMR spectrum for the α-carbonyl methylene appeared as a singlet. This finding is compatible with location of the active methylene at position C-12, flanked on one side by the CO and on the other by a quaternary carbon. If the CO were at position C-1, the signal for the methylene protons at position C-2 would be expected to be split to a doublet or multiplet, depending on their degree of equivalence, by the lone proton at C-3.[cf 15,29]

Reduction of I with LAH in ether for 3 hr at room temperature afforded the C-11

[12] S. M. Kupchan and E. Abushanab, *J. Org. Chem.* 30, 3931 (1965).

[13] After this work had been completed, we were kindly informed by Dr R. Goutarel of his independent isolation and structural elucidation of N-benzoylcycloxobuxidine-F and N-benzoylbuxidienine-F from *Buxus bealearica* Willd. We thank Dr. Goutarel cordially for sending us a copy of reference 6 prior to publication.

[14] During a visit to Gif-sur-Yvette during June 1966, S.M.K. discussed the problem of nomenclature of the newer *Buxus* alkaloids with Dr. Goutarel. It was agreed that the prefix "cycloxo" be adopted for all those dibasic *Buxus* alkaloids which possess a 9β,19-cyclo-11-oxo system, and that the "buxidienine" name be adopted for the parent alkamine of N-benzoylbuxidienine-F.[cf 15]

[15] O. Herlem-Gaulier, F. Khuong-Huu-Laine, and R. Goutarel, *Bull. Soc. Chim. Fr.* 3478 (1966).

[16] O. Herlem-Gaulier, F. Khuong-Huu-Laine, F. Stanislas, and R. Goutarel, *Bull. Soc. Chim. Fr.* 657 (1965).

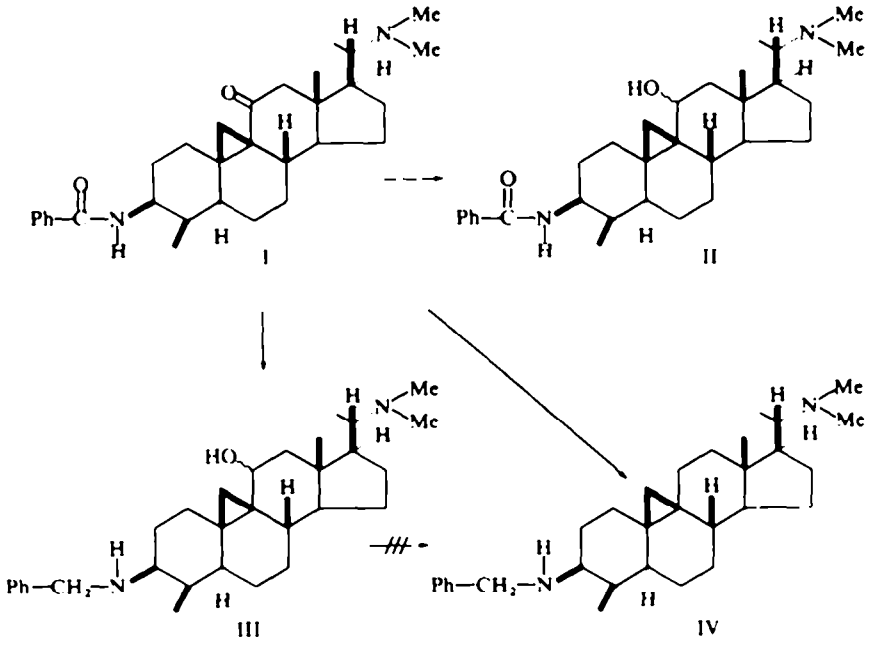


TABLE 1.* NMR SPECTRAL DATA OF THE ALKALOIDS AND THEIR DERIVATIVES

Group	I	II	III	IV	V
Cyclopropane		9·50 (1H), d *J* 4	9·50 (1H), d *J* 4·5	9·46, d 9·71, d *J* 4	9·47, d 9·72, d *J* 4
CH_3 C	8·95 (3H) 9·00 (3H) 9·14 (6H)	9·00 (3H) 9·04 (3H) 9·11 (3H) 9·12 (3H)	9·02 (6H) 9·17 (6H)	9·05 (6H) 9·10 (3H) 9·23 (3H)	8·95 (3H) 9·05 (3H) 9·08 (3H) 9·15 (3H)
CH_3–CH	9·16, d *J* 7	9·14, d *J* 6	9·15, d *J* 6·5	9·18, d *J* 6·5	9·18, d *J* 6
CH_3 N	7·79 (6H)	7·80 (6H)	7·81 (6H)	7·81 (6H)	7·82 (9H)
CH_2OR CHOR		6·08, m	6·09, m		
C 3H	6·00, m	6·08, m			
CO NH -**	3·96	4·00			
CH_3 CO					
Olefin					
H Ar	2·10 2·65 (5H), m	2·10 2·66 (5H), m	2·68(5H)	2·68 (5H)	2·52 2·75 (5H), r
Ar CH_2 N			6·17, q, *J* 13 (6·01 and 6·33)	6·18, q, *J* 13 (6·02 and 6·35)	6·37, q, *J* 13·5 (6·16 and 6·59)

TABLE 1.* *-continued*

Group	VII	Xa	Xb	XI	XII
Cyclopropane	9·44, d 9·68, d *J* 4·5				9·43, d 9·65, d *J* 4·5
CH_3- C<	8·99 (3H) 9·05 (6H) 9·13 (3H)	8·76 (3H) 9·16 (3H) 9·35 (3H)	8·81 (3H) 9·14 (3H) 9·17 (3H)	8·79 (3H) 9·07 (3H) 9·15 (3H)	8·85 (3H) 9·02 (3H) 9·32 (3H)
CH_3 -CH<	9·18, d *J* 6	9·12, d *J* 6	9·20, d *J* 6	9·12, d *J* 6	9·12, d *J* 7
CH_3 -N	7·08 (3H)** 7·82 (6H)	7·74 (6H)	7·85(6H)	7·72 (6H)	7·75 (6H)
-CH_2OR		6·71, q, *J* 12 (6·52 and 6·90)	6·14	6·49, m	6·75, q, *J* 12·5 (6·57 and 6·94)
-CHOR		5·85, m	4·75, m	5·90, m	5·91, m
C-3H	5·30	5·85, m	5·61, m		5·91, m
-CO-NH-**		3·75	4·08, m		4·00
CH_3-CO			7·90 (3H) 8·00 (3H)		
Olefin					
H-Ar	2·62 (5H), m	2·10-2·67 (5H), m	2·19-2·67 (5H), m		2·05-2·62 (5H), m
Ar-CH_2-N					

Group	XIII	XV	XVI	XVII	XIX
Cyclopropane	9·40, d 9·68, d *J* 4				
CH_3- C<	8·88 (3H) 9·03 (6H)	8·95 (3H) 9·13 (3H) 9·33 (3H)	8·96 (3H) 9·17 (6H)	9·00 (3H) 9·15 (3H) 9·20 (3H)	9·08 (3H) 9·24 (3H) 9·42 (3H)
CH_3 -CH<	9·12, d *J* 7	9·17, d *J* 6	9·15, d *J* 6	9·18, d *J* 6	9·11, d *J* 6
CH_3-N	7·74 (6H)	7·80 (6H)	7·79 (6H)	7·81 (6H)	7·74 (6H)
-CH_2OR	6·44, q, *J* 10·5 (6·32 and 6·56)	6·74, q, *J* 13 (6·57 and 6·91)	6·12	5·71, q, *J* 11·5 (5·50 and 5·92)	6·71, q, *J* 12·5 (6·56 and 6·86)
-CHOR	5·95, m				5·92, m
C-3H		5·78, m	5·62, m		5·92, m
-CO-NH**		3·74	4·19		3·82, m
CH_3CO			7·90		
Olefin					3·97 (1H) 4·46 (1H), m
H-Ar		2·10-2·67 (5H), m	2·17-2·67 (5H), m	1·85-2·62 (5H), m	2·07-2·60 (5H), m
Ar-CH_2-N					

Group	XXIIIa	XXIIIb	XXIXb	XXXc	XXIVa
Cyclopropane	9·45, d 9·60, d *J* 4	9·45, d 9·60, d *J* 4	10·18, (1H) d *J* 4	9·42, d 9·67, d *J* 4	9·42, d 9·64, d *J* 4
CH_3 C<	9·02-9·25 (12H)	8·87-9·10 (12H)	8·88 (3H) 8·97 (3H) 9·00 (3H) 9·22 (3H)	8·90 (3H) 9·04 (6H) 9·23 (3H)	8·90 (3H) 9·01 (3H) 9·07 (3H) 9·22 (3H)

TABLE 1.*—*continued*

Group	XXIIIa	XXIIIb	XXIXb	XXXc	XXIVa
CH_3 -CH<	8·96, d *J* 6	9·16, d *J* 7	8·93, d *J* 6	8·98, d *J* 6	8·97, d *J* 6
CH_3 -N	7·00 (3H)** 7·62 (3H)	7·02 (3H)** 7·81 (6H)	7·66 (3H) 7·71 (6H)	7·20 (3H)** 7·53 (3H)	7·35 (3H) 7·62 (3H)
-CH_2OR -CHOR C 3H CO -NH-** CH_3-CO	5·33 5·88, m	5·40–5·90, m	4·72 5·05, m	7·87 7·94 (3H)	6·34–6·88, m
Olefin			2·95 3·40 (1H), m 4·39–4·67 (2H), m		
H-Ar Ar CH_2-N	2·59 (5H)	2·62 (5H)			

Group	XXIVb	XXVI	XXVII	XXIIId	XXVIIIb	XXVIIIa
Cyclopropane	9·43, d 9·66, d *J* 4	9·56, d 9·72, d *J* 4	9·43, d 9·58, d *J* 4	9·40 (2H)	9·28, d 9·43, d *J* 4	9·44 (2H)
CH_3-C<	9·02 (6H) 9·12 (3H) 9·23 (3H)	8·84 (9H) 9·05 (3H)	8·84 (3H) 9·02 (6H) 9·12 (3H)	9·02 (6H) 9·12 (3H) 9·23 (3H)	8·88 (6H) 9·03 (3H) 9·07 (3H)	8·87 (3H) 9·05 (6H) 9·12 (3H)
CH_3-CH<	9·15, d *J* 6	9·16, d *J* 6	8·93, d *J* 6	8·98, d *J* 6	9·17, d *J* 6	9·17, d *J* 6
CH_3 N	7·37 (3H) 7·78 (6H)	7·06 (3H) 7·78 (6H)	6·91 (3H)** 7·60 (3H)	7·10 (3H)** 7·79 (6H)	7·57 (3H) 7·80 (6H)	7·69 (3H) 7·81 (6H)
-CH_2-OR CHOR C 3H -CO -NH -** CH_3-CO	6·30 6·90, m	5·65 6·15, m		4·50–5·03, m 7·87 7·94 (3H)	4·50 4·92, m 7·85 7·92 (3H)	4·59–4·99, m 7·92 (3H)
Olefin H Ar Ar-CH_2 N			2·58 (5H)	2·53 2·71 (5H), m	2·69 (5H), m 6·04, g *J* 14 (5·69 and 6·40)	2·67 (5H), m 5·81 6·47, m

* Chemical shifts are given in ppm on the τ scale. Coupling constants (*J*) are expressed as c/s, d = doublet, m = multip
q = quadruplet; all other resonances are singlets, except where stated otherwise.

** Showing restricted internal rotation.

OH-derivative II, with IR bands indicative of an OH group (2·80 μ) and a secondary amido function (6·03, 6·60 and 7·60 μ). The 6·03 μ band was less intense than in the precursor, in accord with expectation based upon reduction of the 11-CO function. Similarly, the bands at 7·02 and 12·00 μ were absent from the IR spectrum of II. The NMR spectrum (Table 1) showed signals for one proton adjacent to an OH

group (6·08 τ, m) and a proton corresponding to one of the cyclopropyl methylene protons (9·50 τ, part A of the AB quartet, J 4 c/s).

In an effort to reduce both the amide function at C-3 and the CO at C-11, N-benzoylcycloxobuxine-F was treated with LAH in dioxan under reflux conditions for 48 hr. The major product of the reaction was the C-11 desoxy derivative N-benzylcycloprotobuxine-F (IV). The IR spectrum of IV showed no bands attributable to an OH group or an amide function. A weak broad band was present at 3·0 μ which was attributable to the absorption of the secondary amine at C-3. The NMR spectrum (Table 1) showed signals corresponding to a methylene adjacent to a phenyl ring in an electronically unsymmetrical environment (AB quartet centered at 6·18 τ with J 13 c/s); however, no signal was present for a proton adjacent to an OH group.

Reduction at both centers, with very little hydrogenolysis at C-11, was achieved by treatment of I with LAH in ether for 14 hr at room temperature. The major product III showed, in the IR, a sharp band at 2·79 μ (unassociated OH). The NMR spectrum (Table 1) showed signals for one proton adjacent to an OH group, a methylene adjacent to a phenyl ring in an unsymmetrical environment (AB quartet centered at 6·17 τ with J 13 c/s), and a proton corresponding to one of the cyclopropyl methylene protons (9·50 τ, doublet with J 4·5 c/s). This series of spectra demonstrated the effect of an oxygenated function at C-11 on the chemical shift of the neighboring cyclopropyl methylene protons in the NMR spectrum. In the parent compound I both protons were deshielded into the Me region of the spectrum by the CO group at C-11. When the CO group was reduced to an OH function, as in compounds II and III, the degree of deshielding was reduced to the extent that one-half of the AB quartet (part A) for the cyclopropyl methylene protons was clearly visible. In the spectrum of the hydrogenolysis product IV, the signals for these protons appeared at the normal position.[cf 6]

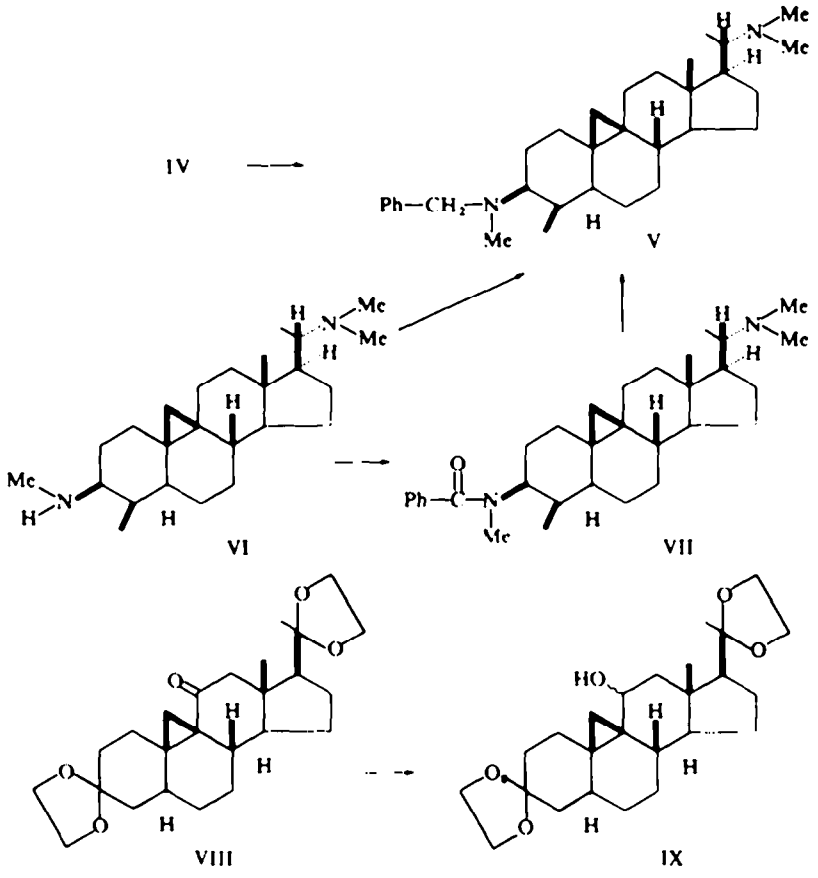
Strong support for assignment of structure I for N-benzoylcycloxobuxine-F was adduced by interrelation with cycloprotobuxine-C (VI).[11, 17] Treatment of IV with formic acid-formalin gave N-*benzylcycloprotobuxine*-C (V), m.p. 142–143°. A signal for an additional N-Me group appeared in the NMR spectrum of V at 7·82 τ. The cyclopropyl methylene signals occurred at 9·47 and 9·72 τ, as an AB system with J 4 c/s. Cycloprotobuxine-C (VI) was treated with benzoyl chloride in pyridine to give N-benzoylcycloprotobuxine-C (VII). The NMR spectrum showed the presence of five aromatic protons, one N-Me group with restricted rotation, and a cyclopropyl methylene (9·44 and 9·68 τ, AB system with J 4·5 c/s). Reduction of N-benzoylcycloprotobuxine-C (VII) with LAH in dioxan at reflux temperature afforded N-benzylcycloprotobuxine-C (V). Treatment of cycloprotobuxine-C with benzyl chloride and potassium carbonate in benzene also led to N-benzylcycloprotobuxine-C.[cf 15]

The hydrogenolysis of the alicyclic C-11 ketone function of I (and related compounds[6, 15]) with LAH is apparently unprecedented, although the hydrogenolysis of several aromatic ketones has been reported.[18] In an attempt to determine if the hydrogenolysis reaction proceeded in step-wise fashion through an OH intermediate, compound III was treated with LAH in dioxan at reflux temperature for 48 hr. The reaction did not lead to the desoxy compound, and starting material was recovered

[17] S. M. Kupchan and E. Kurosawa, *J. Org. Chem.* **30**, 2046 (1965)

[18] Cf. N. Gaylord, *Reduction with Complex Metal Hydrides*, Chap. 16, Interscience, New York (1956)

unchanged. The hydrogenolysis reaction appeared to be of potential value for the synthesis of 9β,19-cyclosteroid analogs of *Buxus* alkaloids, *via* the available intermediate 9β,19-cyclo-5α-pregnane-3,11,20-trione-3,20-diethylene ketal (VIII).[19] However, treatment of VIII with LAH in dioxan for 48 hr at reflux temperature yielded



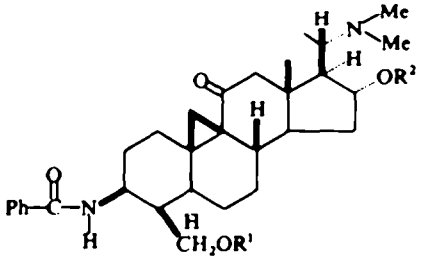
the 11-OH compound IX, in 53% yield. The difference in reactivity of the 11-ketone in I and VIII is noteworthy, and is probably attributable to differences in the conformations of the respective molecules. In support of the latter view, the ORD curves for I and VIII were both positive, but showed a wide difference in amplitude. The curve for I showed $[\theta]_{316}$ +3610° and $[\theta]_{290}$ +2293°, whereas the curve for VIII showed $[\theta]_{312}$ +5460° and $[\theta]_{271}$ −3460°.[20]

N-Benzoylcycloxobuxidine-F (Xa) also showed a UV spectrum which indicated the presence of a secondary benzamide and a CO group conjugated with a cyclopropane ring (λ_{max}^{EtOH} 225 mμ, ε 13,500). The IR spectrum closely resembled that of I, except for the presence of additional bands at 9·52 and 9·60 μ, attributable to the C–O

[19] [a] H. Wehrli, M. S. Heller, K. Schaffner, and O. Jeger, *Helv. Chim. Acta* **44**, 2162 (1961); [b] Attempted Wolff-Kishner reduction of VIII led, *via* carbocyclic ring cleavage to 9(10→19)*abeo*-pregnane derivatives; S. M. Kupchan and E. Abushanab, *Tetrahedron Letters* 3075 (1965).

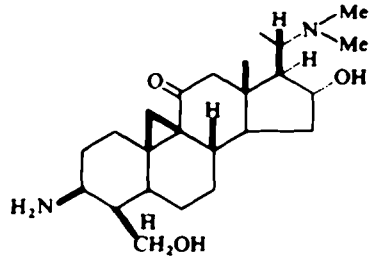
[20] The amplitude of the curve for VIII resembled that recorded for an 11-oxo-9β,10β-steroid; cf. C. Djerassi and W. Klyne, *J. Chem. Soc.* 4929 (1962).

stretching vibration of the alcohol grouping. The NMR spectrum (Table 1) showed the presence of five aromatic protons, one amido proton (—CO—NH—) with hindered rotation about the C—N bond, two N-Me's, three tertiary C-methyls, one secondary C-Me, one proton adjacent to a secondary OH group (5·85 τ, m), and a signal centered at 6·71 τ for two protons adjacent to a primary OH group in an electronically unsymmetrical environment (AB quartet, 6·52 and 6·90 τ, J 12 c/s, centered at 6·71 τ). The foregoing data led to the proposal of structure Xa for the compound. Comparing the spectrum of Xa with that of N-benzoylcycloxobuxine-F (I), two major differences were evident, attributable to the presence of the two OH functions in Xa. The signal for one of the tertiary C-Me groups of compound Xa was shifted to 8·76 τ. This shift downfield was probably the result of deshielding of the protons of the angular Me at C-14 by the neighboring α-hydroxyl function at C-16. The cyclomicrophylline *Buxus* alkaloids (4-hydroxymethyl-16-hydroxy compounds) generally show a Me signal in this region of the spectrum.[21] However, the spectrum of N-benzoylcycloxobuxoline-F, an alkaloid possessing only the C-4 hydroxymethyl grouping, did not show a Me signal downfield (Table 1). A second difference in the two spectra was attributable to a shielding effect of the C-4 hydroxymethyl on the C-4 Me protons in compound Xa. A tertiary Me signal appeared up-

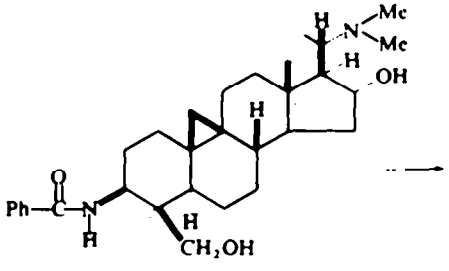


(a) $R^1 = R^2 = H$ X

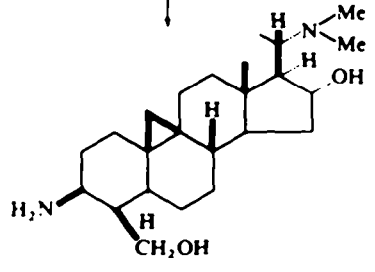
(b) $R^1 = R^2 = Ac$



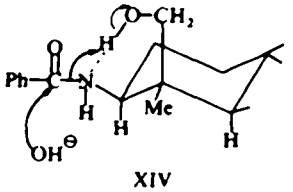
XI



XII



XIII



XIV

[21] T. Nakano and S. Terao, *J. Chem. Soc.* 4512 (1965).

field at 9·35 τ, indicating a shift of at least 0·21 τ from the signal in the spectrum of I, since the farthest upfield tertiary Me signal for I appeared at 9·14 τ. The C-4 hydroxymethyl compound XV, as well as the cyclomicrophyllines,[21] showed a tertiary Me signal in the 9·35 τ region of the spectrum.

Acetylation of N-benzoylcycloxobuxidine-F (Xa) with acetic anhydride in pyridine afforded the diacetate Xb with IR bands indicative of acetate esters (5·79 and 8·05 μ). The NMR spectrum showed two α-carbonyl methyl signals (7·90 and 8·00 τ) corresponding to acetate esters and confirmed the presence of two acylable hydroxyl groups in Xa.

When N-benzoylcycloxobuxidine-F was treated with an ethanolic solution of sodium hydroxide (6% w/v) at reflux temperature for 24 hr, cycloxobuxidine-F (XI) was obtained, in addition to an equimolar amount of benzoic acid. The relatively facile hydrolysis of the benzamide contrasted with the previously-observed resistance to hydrolysis of benzamides of *Buxus* alkaloid derivatives such as XXV.[2b, 16] On the other hand, several cases of amide hydrolysis facilitated by neighboring OH groups among derivatives of *Buxus* alkaloids such as XXI have been observed.[3b, 9, 21] The hydrolysis of N-benzoylcycloxobuxidine-F (Xa) was apparently facilitated by hydrogen bonding of the OH hydrogen of the hydroxymethyl at C-4 with the lone pair of electrons of the amide nitrogen at C-3, making the amide CO more electro-positive and susceptible to nucleophilic attack by the base (perhaps as shown in partial structure XIV).[22] The IR spectrum of cycloxobuxidine (XI) was characterized by the absence of bands corresponding to an aryl ring and an amide carbonyl. A band was present in the spectrum at 6·00 μ; however, this was attributed to the CO at C-11 since the "amide II and III" bands were absent. The spectrum also showed bands at 6·30 μ (—NH_2) and 12·00 μ (C-11 CO deformation). The NMR spectrum showed a downfield shift in the signal of the hydroxymethyl protons at C-4 to 6·49 τ (from 6·71 τ in the parent compound Xa). This lower signal position is characteristic of a β-oriented (axial) hydroxymethyl at C-4.[21] The signal for these two protons in the spectrum of the parent alkaloid Xa occurred upfield (6·71 τ), due to the shielding effect of the amide CO at C-3.

Treatment of cycloxobuxidine-F (XI) with an excess of LAH in dioxan at reflux temperature for 4 days afforded the known C-11 desoxy compound dihydrocyclomicrophylline-F (XIII)[21] in 60% yield. The interrelation with the known alkaloid constitutes strong support for assignment of structure Xa for N-benzoylcycloxobuxidine-F.

Structure XII was assigned for N-benzoyldihydrocyclomicrophylline-F on the basis of the following evidence. The IR spectrum indicated a secondary benzamide (6·10, 6·61 and 7·59 μ, "amide I, II and III" bands) and at least one OH function (2·90, 2·98, 9·55 and 9·65 μ). No bands were present in the 6·0 or 12·0 μ regions, indicating the absence of a C-11 keto function. The UV spectrum showed λ_{max}^{EtOH} 227 mμ (ε 9,335), attributable to a secondary benzamide. The low intensity of the band, compared with that for the 11-keto compound (Xa, λ_{max}^{EtOH} 225 mμ, ε 13,500), indicated the absence of a ketone at C-11 in N-benzoyldihydrocyclomicrophylline-F (XII). The NMR spectrum showed five aromatic protons, one amido proton with hindered rotation about the

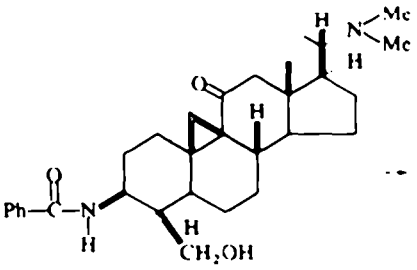
[22] Other mechanisms are readily conceivable; for a review of the mechanisms of neighboring hydroxyl group participation in ester and amide hydrolysis, see T. C. Bruice and S. J. Benkovic, *Bioorganic Mechanisms* Vol. 1, p. 146. Benjamin, New York, N.Y. (1966).

C–N bond, one proton adjacent to a secondary OH function (5·91 τ, m), two hydroxymethyl protons (AB quartet, 6·57 and 6·94 τ, J 12·5 c/s, centered at 6·75 τ), two N-Me's, three tertiary C-Me's, one secondary C-Me, and two cyclopropyl methylene protons (9·43 and 9·65 τ, AB quartet with J 4·5 c/s). The position of the C-4 hydroxymethyl signals (6·75 τ) indicated the proximity of the protons to the amide CO, supporting location of the benzamide at the C-3 position. The appearance of signals attributable to the cyclopropyl methylene protons further substantiated the absence of an 11-keto function, since, as noted above, a CO at C-11 will cause a paramagnetic shift of the cyclopropyl methylene protons into the Me or methylene envelope of the spectrum. On the basis of the latter data, structure XII was proposed for N-benzoyldihydrocyclomicrophylline-F. Confirmation was achieved by hydrolysis of XII using 6% sodium hydroxide in ethanol, whereupon dihydrocyclomicrophylline-F (XIII) was obtained.

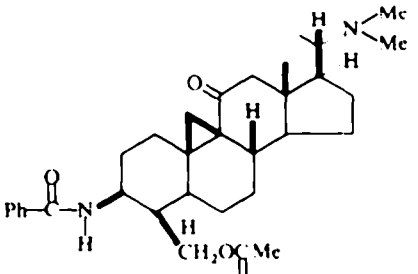
Structures XV for N-benzoylcycloxobuxoline-F and XVI for N-benzoyl-O-acetylcycloxobuxoline-F were proposed on the basis of the observations which follow. The IR spectrum of N-benzoylcycloxobuxoline-F indicated the alkaloid to be a secondary benzamide (6·09, 6·60 and 7·59 μ, "amide I, II and III" bands) which possesses the 9β,19-cyclo-11-keto partial structure (3·27 μ, cyclopropyl; 6·02 and 12·00 μ, CO at C-11; 7·04 μ, methylene at C-12). The IR spectrum of XV was very similar to that of N-benzoylcycloxobuxidine-F (Xa), with slight differences in the OH and fingerprint regions of the spectrum. Whereas Xa showed a strong band at 2·98 μ and bands at 9·52 and 9·60 μ (OH), XV showed a weaker band at 2·97 μ and a single band at 9·55 μ (OH), supporting the view that XV was a monohydroxy compound. This was indicated further by the results of microanalysis, which indicated the presence of only three oxygen functions. The UV spectrum showed λ_{max}^{EtOH} 226 mμ (ε 14,300), in good accord with the presence of a secondary benzamide at C-3 and a CO at C-11. The NMR spectrum showed the presence of five aromatic protons, one amido proton, two hydroxymethyl protons (6·57 and 6·91 τ, AB quartet, J 13 c/s, centered at 6·74 τ), an α-carbonyl methylene (7·57 τ, 2H, s), two N-Me's, three tertiary C-Me's, and one secondary C-Me. The C-16 hydroxyl function present in N-benzoylcycloxobuxidine-F (Xa) is absent from N-benzoylcycloxobuxoline-F (XV) as shown in the NMR spectrum by the absence of a signal attributable to the proton of the

-CHOH grouping in the latter compound.

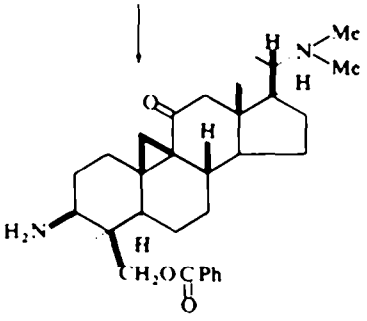
N-Benzoyl-O-acetylcycloxobuxoline-F showed a UV spectrum with λ_{max}^{EtOH} 223·5 mμ (ε 12,300) and an IR spectrum with bands at 2·93 μ (NH), 5·80 μ (acetate carbonyl), 6·02 μ (C-11 CO, "amide I" band), 6·62 and 7·60 μ ("amide II and III") 7·90 to 8·25 μ (C-O-C stretch of ester) and 12·00 μ (C-11 CO). The NMR spectrum showed signals characteristic for five aromatic protons, one amide proton, an α-carbonyl methylene (7·57 τ, s), two N-Me's, three tertiary C-Me's, one secondary C-Me, an α-carbonyl methyl (CH_3CO_2-, 7·90 τ, s) and an acetoxymethyl group (CH_3–CO_2CH_2–, 6·12 τ, s). The acetoxymethyl signal showed $\Delta\tau = 0{\cdot}62$ with respect to that for the hydroxymethyl of the parent alkaloid, in good agreement with that expected upon acetylation of a primary OH function. The foregoing data led to formulation of N-benzoylcycloxobuxoline-F as XV and N-benzoyl-O-acetylcycloxobuxoline-F as XVI. Support for the assignments was achieved by acetylation of XV with acetic anhydride in pyridine, whereupon XVI was obtained.



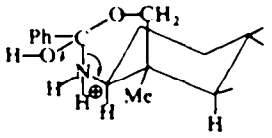
XV



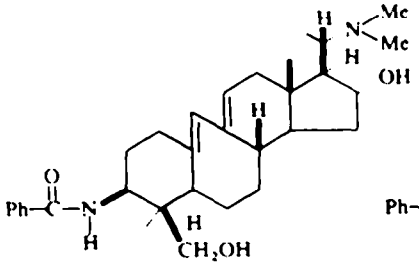
XVI



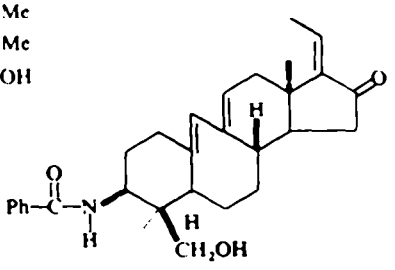
XVII



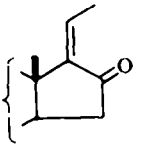
XVIII



XIX



XXa (*cis*)



XXb (*trans*)

Treatment of N-benzoylcycloxobuxoline-F (XV) with 3% aqueous sulfuric acid at 90° for 1 hr afforded O-benzoylcycloxobuxoline-F (XVII). The IR spectrum suggested the presence of primary amine (2·99 and 6·30 μ) and ester (5·84 and 7·80–7·90 μ) functions. The aromatic bands were still present, indicating the ester to be a benzoate. The 9β,19-cyclo-11-keto system was still intact, as evidenced by the presence of bands at 6·02 and 12·00 μ (C-11 CO). The NMR spectrum showed an AB quartet centered at 5·71 τ (5·50 and 5·92 τ, J 11·5 c/s), attributable to the Ar $-CO_2-C\underline{H}_2-$ grouping. N → O Acyl migrations such as XV → XVII, probably proceeding *via* a 6-membered cyclic intermediate like XVIII, are not uncommon.[23]

Structure XIX was proposed for N-benzoylbuxidienine-F on the basis of the evidence which follows. The UV spectrum showed λ_{max}^{EtOH} 238 mμ (ε 39,320), 246 mμ (ε 41,520)

[23] Cf. e.g., T. Kikuchi, S. Uyeo, and T. Nishinaga, *Tetrahedron Letters* 1749 (1966).

and 255 mμ (ε 25,970), with shoulders at 229 mμ, 280 mμ, and 293 mμ. The spectrum of buxenine G showed λ_{max}^{EtOH} 238 mμ (ε 27,200), 247 mμ (ε 29,100), and 255 mμ (ε 18,650), with shoulders at 215, 230, and 290 mμ.[24] The position and intensity of each peak relative to the others corresponded very closely in the respective compounds. The higher intensity of each peak in N-benzoylbuxidienine-F was attributable to the presence in the molecule of a secondary benzamide with overlapping absorption in that area. The IR spectrum showed bands attributable to an OH function, a secondary benzamide, and a heteroannular diene (i.e., 6·06 (m), 6·21 and 10·33 μ).[25] The latter spectral data were suggestive that N-benzoylbuxidienine-F contains the 9(10 → 19)*abeo*-steroidal diene system present in the group of *Buxus* alkaloids exemplified by buxenine-G.[1 24] The supposition was strengthened by the NMR spectrum, which showed the presence of five aromatic protons, a secondary amido hydrogen with internal hindered rotation about the C - N bond, two N-Me's, three tertiary C-Me's, one secondary C-Me, one proton adjacent to a secondary OH ($-\underset{|}{C}\underline{H}OH$, 5·82 τ, m), two protons adjacent to a primary OH ($-C\underline{H}_2OH$, 6·71 τ, AB quartet, J 12·5 c/s, respective doublets at 6·86 and 6·56 τ) and two vinyl protons (3·97 τ, 1H, broad singlet and 4·46 τ, 1H, multiplet). The splitting pattern of the vinyl protons was characteristic of the diene partial structure $-CH_2-CH=C-CH=C-CH_2-$, which had been shown to make up the heteroannular diene system of buxenine-G and related alkaloids.[23 26 28] The latter data substantiated the presence of the 9(10 → 19)*abeo*-diene system in N-benzoylbuxidienine-F (XIX). The positions of the signals for the hydroxymethyl protons in the NMR spectrum of XIX indicated their close proximity to the amide CO, in support of location of the amide at the C-3 position, as in other amido-*Buxus* alkaloids.[7 16 29]

To interrelate the C-16 secondary OH function of XIX with the dimethyl amino function at C-20, the compound was treated with 1·2 molar equivalents of chromium trioxide in acetic acid for 6 hr at room temperature, after which the crude product was refluxed in methanol.[7] Alumina chromatography afforded a non-basic product, yellow in color and oily in nature, but apparently homogeneous as shown by TLC. The IR spectrum of the oil showed the presence of an OH function and a secondary benzamide, the peak intensity of which was not diminished when compared with the parent alkaloid. A medium intensity band was present at 5·83 μ which could be attributed to the CO of a *cis*- and/or a *trans-cisoid* cyclopentenone (XXa and b respectively). A band at 5·84 μ has been reported for cyclopentenone derivatives of the cyclomicrophylline class of *Buxus* alkaloids.[7] The NMR spectrum of the oil showed it to be inhomogeneous; however, the spectrum did show the absence of the signal for the N-dimethyl protons and the presence of new peaks at 7·90 τ (d, J 7·5 c/s) and 8·20 τ (d, J 7·5 c/s). The signal at 7·90 τ is characteristic for the C-21 methyl protons of the *trans*-isomer (reported 7·92 τ, J 7·5 c/s for a similar compound[7]) while that at 8·20 τ is characteristic for the corresponding protons of the *cis*-isomer (reported 8·19 τ, d,

[24] S. M. Kupchan and W. L. Asbun, *Tetrahedron Letters* 3145 (1964).
[25] K. Nakanishi, *Infrared Absorption Spectroscopy*, p. 46. Holden Day, San Francisco (1962).
[26] D. Stauffacher, *Helv. Chim. Acta* **47**, 968 (1964).
[27] J. P. Calame, Doctoral Dissertation, E.T.H., Zurich, 1965.
[28] J. Tomko, O. Bauerova, Z. Voticky, R. Goutarel, and P. Longevialle, *Tetrahedron Letters* 915 (1966).
[29] F. Khuong-Huu-Laine, D. Herlem-Gaulier, and R. Goutarel, *C. R. Acad. Sci. Paris* **261**, 4139 (1965).

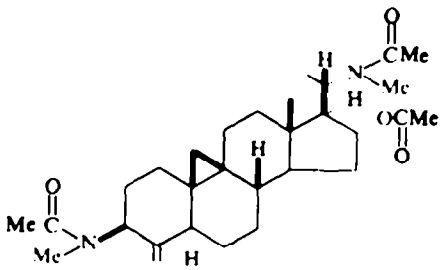
J 7·5 c/s for a similar compound[7]). Although the oxidation-elimination reaction yielded an oily mixture, the IR and NMR spectra indicated that oxidation with subsequent elimination of the amine function did indeed take place, and the nature of the spectra supported the view that the secondary OH function in the parent alkaloid is at the C-16 position. On the basis of the foregoing data, structure XIX is favored for N-benzoylbuxidienine-F.

Support for assignment of structure XXIIIa for N-benzoylcycloprotobuxoline-D and XXIIIb for N-benzoylcycloprotobuxoline-C was adduced from the observations which follow.

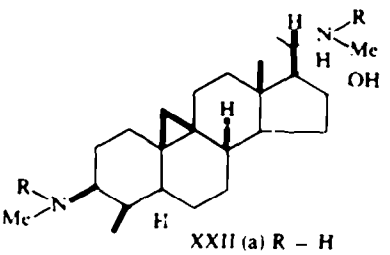
The IR spectrum of N-benzoylcycloprotobuxoline-D showed a strong band at 6·20 μ, attributable to an amide function. The NMR spectrum indicated the presence of one N-Me group with restricted internal rotation,[30] one N-Me group, one proton adjacent to an OH, five aromatic protons and a cyclopropyl methylene. The cited data were compatible with the view that N-benzoylcycloprotobuxoline-D might be a benzamide derivative of cyclovirobuxine-D (XXIIa). However, benzoylation of the naturally-occurring monobenzamide gave a dibenzamide XXIIIc isomeric with, but not identical to, N,N'-dibenzoylcyclovirobuxine-D (XXIIb). N-Benzoylcycloprotobuxoline-C showed spectral properties which closely resembled those of the C_{33}benzamide. However, the NMR spectrum of the C_{34} benzamide showed a signal (7·81 τ, 6H) indicative of the presence of an N-dimethyl group. That the two benzamides differed only in the substitution pattern of one of the nitrogen atoms was demonstrated by methylation of N-benzoylcycloprotobuxoline-D with formaldehyde-formic acid, whereupon N-benzoylcycloprotobuxoline-C was obtained. Basic hydrolysis of N-benzoylcycloprotobuxoline-D (XXIIIa) by treatment with methanolic potassium hydroxide under reflux for 2·5 hr yielded *cycloprotobuxoline-D* (*XXIVa*), $C_{26}H_{46}N_2O$. The NMR spectrum showed the presence of two N-Me groups and cyclopropyl methylene. Acidification of the alkaline reaction mixture yielded benzoic acid. Similar hydrolysis of N-benzoylcycloprotobuxoline-C (XXIIIb) yielded *cycloprotobuxoline-C* (XXIVb), $C_{27}H_{48}N_2O$. It was postulated that the ease of hydrolysis of the N-benzoylcycloprotobuxolines might be attributable to facilitation of amide hydrolysis by a neighboring OH group. Strong support for the presence of a neighboring OH group was obtained by treatment of XXIVb in benzene solution with 12·5% phosgene in benzene, whereupon the oxazolidone XXVI was formed. The presence in cycloprotobuxoline-D of the grouping · –CHOH –CH(NHCH$_3$) – was indicated by its periodic acid consumption (1 molar equiv after ½ hr, 1·2 molar after 1 hr, 1·2 molar equiv after 3 hr). Oxidation of N-benzoylcycloprotobuxoline-D (XXIIIa) with 1·2 molar equiv of chromic acid yielded ketone XXVII. The IR spectrum of XXVII showed a band at 5·82 μ, which corresponds to the absorption expected for a 6-membered ring ketone. Consequently, the OH group was assigned to C-2.

Acetylation of N-benzoylcycloprotobuxoline-C (XXIIIb) yielded the O-acetate XXIIId. The NMR spectrum of XXIIId showed a signal, corresponding to the C-2 proton, at 4·77 τ as a broad multiplet with a half-width ca. 20 c/s. An earlier study has shown that a similar signal was ascribable to an axial proton coupled with four

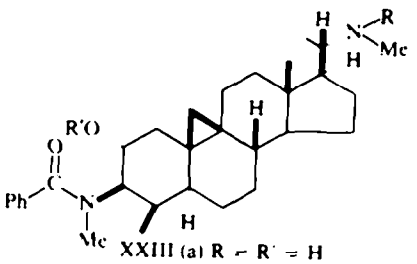
[30] J. M. Pople, W. G. Schneider, and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance*, pp. 336–371. McGraw Hill, New York, N.Y. (1959).



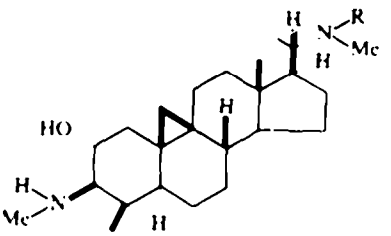
XXI



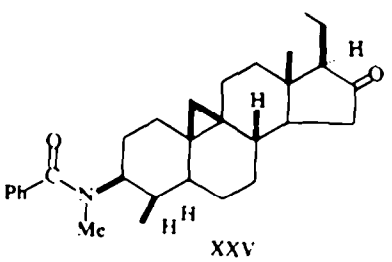
XXII (a) R = H
(b) R = COPh



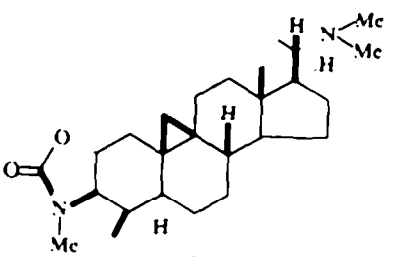
XXIII (a) R = R' = H
(b) R = Me; R' = H
(c) R = COPh; R' = H
(d) R = Me; R' = Ac



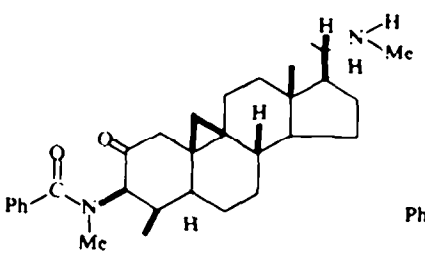
XXIV (a) R = H
(b) R = Me



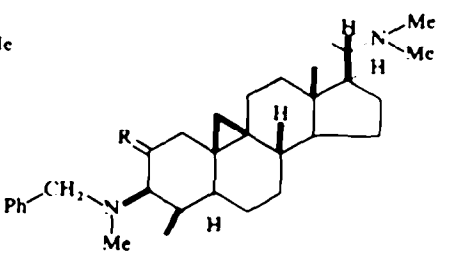
XXV



XXVI



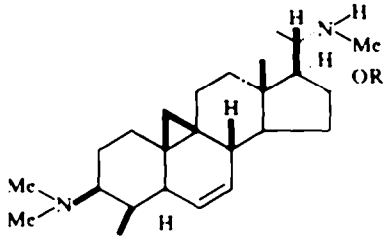
XXVII



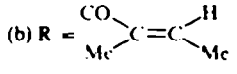
XXVIII (a) R = H, AcO

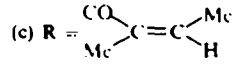
(b) R = AcO, H

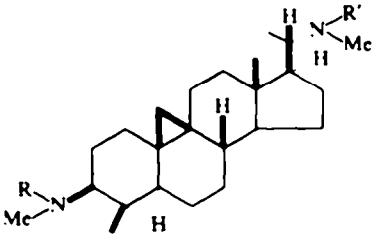
protons on adjacent carbons.[31] The present case involves spin-spin coupling of the C-2 proton with three protons on adjacent carbons. However, since axial-axial interactions result in a larger splitting constant than do either axial-equatorial or equatorial-equatorial interactions,[32] the large half-width for the C-2 proton was deemed consistent with that expected for an axial proton. To seek support for the latter assignment, the epimeric acetates XXVIIIa and XXVIIIb were prepared with a view toward comparing the half-widths of the respective C-2 proton signals. LAH reduction of the ketone XXVII yielded a mixture of the epimeric alcohols, which were separated by partition chromatography. The isomer having the same relative configuration as the naturally-occurring material was identified by comparison with an authentic sample obtained from the LAH reduction of XXIIIa. N-Methylation of the two epimeric alcohols, followed by acetylation, gave the epimeric acetates XXVIIIa and XXVIIIb. The resonance of the C-2 proton of acetate XXVIIIa (4·79 τ) appeared as an unresolved multiplet with half-width ca. 20 c/s. The resonance of the C-2 proton of acetate XVIIIb (4·71 τ) appeared as an unresolved multiplet of half-width ca. 10 c/s. Since acetate XXVIIIa was prepared from the alcohol possessing the same relative configuration as the naturally-occurring compound, the foregoing observations support assignment of α-(equatorial)-configuration for the C-2 OH group in XXIIIa and XXIIIb.



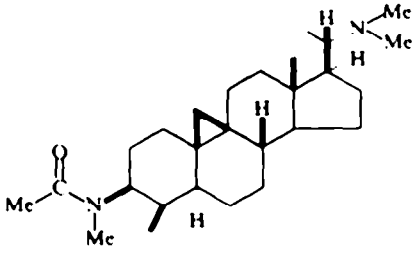
XXIX (a) R = H

(b) R = 

(c) R = 



XXX (a) R = R' = H
(b) R = R' = Ac
(c) R = H; R' = Ac
(d) R = Ac; R' = H
(e) R = Me; R' = Ac



XXXI

[31] R. V. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.* **80**, 6098 (1958).
[32] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry*, pp. 80–81. Holden-Day, San Francisco (1964).

The NMR spectrum of tigloylcyclovirobuxeine-B indicated the presence of 2 vinyl protons (4·39–4·67 τ) and a cyclopropyl methylene (10·18 τ, ½ of the ABq, J 4 c/s). The high upfield shift of half of the AB quartet of the cyclopropyl methylene has been observed with other *Buxus* alkaloids containing a $\Delta^{6,7}$ double bond.[5,21] Other NMR and IR characteristics (see Experimental) suggested that the compound might be an O-acyl derivative of cyclovirobuxeine-B (XXIXa). Saponification of the ester with methanolic potassium hydroxide yielded cyclovirobuxeine-B and tiglic acid, in support of assignment of the 16-tigloylcyclovirobuxeine-B structure XXIXb for the compound. At this point in the argument, a 16-angelate ester structure XXIXc could not be precluded, because earlier studies in this Laboratory had shown that naturally-occurring angelate ester alkaloids, such as germanitrine[33] and cevadine,[34] yield tiglic acid upon alkaline hydrolysis. However, the chemical shift of the vinyl proton of XXIXb (3·18 τ) was characteristic of a tiglate (approx. 3·28 τ)[35] rather than an angelate (approx. 4·02 τ).[35,36] Hence the tiglate configuration is favored for the ester at C-16 in XXIXb.

Acetylcycloprotobuxine-D could be tentatively formulated as an acetamide of cycloprotobuxine-D (XXXa) on the basis of spectral and analytical data. Acetylation of the naturally-occurring compound yielded N,N'-diacetylcycloprotobuxine-D (XXXb), and the monoacetamide could be presumed to possess structure XXXc or XXXd. N-Methylation of the new monoacetamide gave a product (XXXe) *isomeric with* N-acetylcycloprotobuxine-C (XXXI). The non-identity of the methylation product with XXXI indicated that the newly isolated compound is 20-N-acetylcycloprotobuxine-D (XXXc).

EXPERIMENTAL

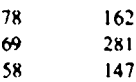
Mps were determined with a Thomas-Hoover Unimelt apparatus. IR spectra were measured, unless otherwise specified, in $CHCl_3$ solns (10%) on a Beckman double beam recording spectrophotometer model IR-5A. NMR spectra were determined in $CDCl_3$ at 60 Mc on Varian Associates A-60 and A-60A recording spectrometers using TMS as the internal standard, and were electronically integrated. UV spectra were determined in 95% EtOH on a Beckman recording spectrophotometer model DK2A. Optical rotations were determined in $CHCl_3$ solns with a Zeiss-Winkel polarimeter and are approximated to the nearest degree. ORD were determined on a Cary Model 60 Recording Spectropolarimeter, in EtOH (c 1·0 mg/ml), in a 0·1 dm tube, at 25°. Skellysolve A refers to petroleum ether, fraction distilling at 30–60°; Skellysolve B refers to that fraction which distills at 60–68°. Ether refers to Et_2O, U.S.P. All dioxan used was stored over KOH and distilled in the presence of LAH. TLC was performed on silica gel "G" in all cases. Thin layer chromatograms, unless otherwise specified, were developed using the upper phase of the system n-butanol–AcOH–water (4:1:5) and sprayed with Dragendorff's reagent. Solvent systems used for partition chromatography consisted of Skellysolve B–ethylene dichloride–MeOH–water (10:2·3:0·2; solvent system A, 10:1·3:0·2; solvent system B, and 10:5·2:0·3; solvent system C). Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. We are grateful to Ciba Pharmaceutical Company for procurement and large-scale extraction of plant material.

Isolation of alkaloids. The "additional weak bases" fraction (80 g), obtained from *Buxus sempervirens* L. by a fractionation procedure described earlier,[28] was dissolved in benzene (200 ml). Skellysolve B (200 ml) was added and the resulting ppt (18·2 g) was removed by filtration. The filtrate was evaporated to dryness and the resulting yellow-brown foam (61·8 g) was redissolved in Skellysolve B–benzene (1:1, 400 ml) and added to a column of basic Woelm grade III alumina (2400 g). The following fractions were collected:

[33] S. M. Kupchan and A. Afonso, *J. Am. Pharm. Assoc.* **48**, 731 (1959).
[34] Idem., *J. Am. Pharm. Assoc.* **49**, 242 (1960).
[35] R. R. Frazer, *Canad. J. Chem.* **38**, 549 (1960).
[36] W. Herz and M. V. Lakshmikantham, *Tetrahedron* **21**, 1711 (1965).

Fraction	Eluant	Wt (g)
1	Skellysolve B-benzene (1:1, 7,200 ml)	1·071
2	Benzene (4,800 ml)	7·637
3	Benzene (4,800 ml)	4·295
4	Benzene ether (9:1, 4,800 ml)	7·710
5	Benzene ether (1:1, 9,600 ml)	31·987
6	Ether (4,800 ml)	3·221

Partition chromatography[8] of the second fraction (2·652 g) on a Celite 545 partition column (330 g) using solvent system B gave 4 bands. Re-chromatography of the first band (3·238 g; from several columns), R_f 0·95, on Woelm basic alumina, activity III (98 g), yielded 6 sub-fractions. Treatment of the sixth sub-fraction (494 mg; eluted with ether) with Skellysolve B gave a solid (344 mg) shown to be a mixture by TLC. Recrystallization twice from acetone gave XXIIIb (19·5 mg), m.p. 252–255° dec. (Found: C, 78·28; H, 9·92; N, 5·35; $C_{34}H_{52}N_2O_2$ requires: C, 78·41; H, 10·07; N, 5·38%); $[\alpha]_D^{27}$ +43° (*c* 0·85); $\lambda_{max}^{CHCl_3}$ 6·20 μ (s).

Partition chromatography of the third fraction (2·287 g) on a Celite 545 partition column (330 g) using solvent system B gave 5 bands. Treatment of the first band (783 mg), R_f 0·96, with Skellysolve B gave a colorless solid (423 mg). Recrystallization from Skellysolve B gave XXIXb (209 mg), m.p. 178–183°. (Found: C, 77·41; H, 10·33; N, 5·58; $C_{32}H_{52}N_2O_2$ requires: C, 77·37; H, 10·55; N, 5·64%); $[\alpha]_D^{27}$ −150° (*c* 1·03); $\lambda_{max}^{CHCl_3}$ 5·90 (s), 6·09 (m), 7·90 (s), 8·75 μ (s); NMR signal at 8·19 τ (6H, ).

Partition chromatography of the fourth fraction (1·25 g) on a Celite 545 partition column (150 g) using solvent system B yielded 7 bands:

Band	R_f	Wt (mg)
1	0·95	150
2	0·78	162
3	0·69	281
4	0·58	147
5	0·46	151
6	0·38	88
7	0·30	333

Band 7 (R_f 0·30, 333 mg), upon treatment with ether–Skellysolve B, yielded I (158 mg), m.p. 213–216° dec. Recrystallization from the same solvent gave crystals with m.p. 214–216° dec. (Found: C, 78·17; H, 9·31; N, 5·57; $C_{33}H_{48}N_2O_2$ requires: C, 78·52; H, 9·59; N, 5·55%); $[\alpha]_D^{28}$ +90° (*c* 1·01); $\lambda_{max}^{CHCl_3}$ 2·92, 3·30, 6·02, 6·32, 6·60, 6·72, 7·02, 7·61, 12·0 μ; λ_{max}^{EtOH} 225 (ε 13,500) mμ; NMR signal at 7·56 τ (2H, C-12 methylene).

Rechromatography of the fifth fraction (57·7 g; eluted with benzene–ether, (1:1)) on basic Woelm grade III alumina (1750 g) gave 14 sub-fractions:

Fraction	Eluant	Wt (g)
1	Skellysolve B-benzene [(1:1), 5·51.]	
2	Benzene (3 l.)	2·306
3	Benzene (3 l.)	4·667
4	Benzene (3 l.)	4·707
5	Benzene–ether [(9:1), 3 l.]	6·497
6	Benzene–ether [(9:1), 3 l.]	8·355
7	Benzene–ether [(9:1), 3 l.]	7·564
8	Benzene–ether [(9:1), 3 l.]	4·951
9	Benzene–ether [(8:2), 3 l.]	4·103
10	Benzene–ether [(8:2), 3 l.]	3·201
11	Benzene–ether [(7:3), 3 l.]	2·964
12	Benzene–ether [(7:3), 3 l.]	1·967
13	Benzene–ether [(7:3), 3 l.]	1·618
14	Ether	2·459

Sub-fraction 2 (2·30 g), upon treatment with Skellysolve B, yielded crystals (69 mg). Recrystallization from ether gave XVI (46 mg), m.p. 216–218° (Found: C, 74·66; H, 8·92; N, 4·96; $C_{35}H_{50}N_2O_4$ requires: C, 74·69; H, 8·96; N, 4·98%); $[\alpha]_D^{29}$ +114° (*c* 1·00); $\lambda_{max}^{CHCl_3}$ 2·93, 5·80 (s), 6·02 (s), 6·62 (s), 7·60, 6·24, 6·33, 6·73, 8·05 (s), 12·0 μ (m); λ_{max}^{EtOH} 223·5 (ε 12,300) mμ.

Partition chromatography of a portion of sub-fraction 5 (2·141 g) on a Celite 545 partition column (330 g) using solvent system B gave 6 bands. Treatment of the third band (168 mg), R_f 0·44, with Skellysolve B gave a colorless solid (94·5 mg). Recrystallization from acetone gave XXXc (52·8 mg), m.p. 221–224° (Found: C, 77·96; H, 11·27; N, 6·57; $C_{28}H_{48}N_2O$ requires: C, 78·45; H, 11·29; N, 6·54%); $[\alpha]_D^{29}$ +53° (*c* 0·50); $\lambda_{max}^{CHCl_3}$ 6·15 μ (s).

Treatment of sub-fraction 6 (8·355 g) with ether–Skellysolve B gave a white crystalline material (550 mg), shown to be a mixture by TLC. Recrystallization from acetone yielded XIX (205 mg), m.p. 286–288° dec. (Found: C, 75·92; H, 9·39; N, 5·42; $C_{33}H_{48}N_2O_3$ requires: C, 76·11; H, 9·29; N, 5·38%); $[\alpha]_D^{28}$ −36° (*c* 1·02); λ_{max}^{KBr} 2·97 (s), 6·06 (m), 6·21, 10·33, 6·13 (s), 6·58 (s), 7·64, 6·27, 6·36, 6·75, 9·73 (s), 9·78 μ (s); λ_{max}^{EtOH} 238·4 (ε 39,300), 246 (ε 41,500), 255 (ε 26,000), with shoulders at 229, 280, 293 mμ.

Treatment of sub-fraction 7 (7·564 g) with ether–Skellysolve B gave an amorphous solid (1·622 g) which was shown by TLC to be a mixture. The mother liquor was evaporated to dryness to give a yellow foam (5·94 g), which, when treated with AcOEt, gave a crystalline material (146 mg), m.p. 282–290° dec. Recrystallization twice from acetone gave XII (56 mg), m.p. 292–294° dec. (Found: C, 75·05; H, 9·60; N, 5·27; $C_{33}H_{50}N_2O_3$ requires: C, 75·82; H, 9·64; N, 5·36%); $[\alpha]_D^{28}$ +19° (*c* 0·36); $\lambda_{max}^{CHCl_3}$ 2·91, 2·97 (s), 3·28, 6·10 (s), 6·61 (s), 7·59, 6·23, 6·33, 6·72, 9·55, 9·65 μ; λ_{max}^{EtOH} 227 (ε 9,350) mμ.

Sub-fraction 10 (3·201 g), upon treatment with AcOEt yielded a crystalline mixture (400 mg) as shown by TLC. Recrystallization from acetone gave pure Xa (180 mg), m.p. 274–276° dec. (Found: C, 73·70; H, 9·30; N, 5·26; $C_{33}H_{48}N_2O_4$ requires: C, 73·84; H, 9·01; N, 5·22%); $[\alpha]_D^{26}$ +56° (*c* 0·88); $\lambda_{max}^{CHCl_3}$ 2·90, 2·98 (s), 3·28, 6·02 (s), 6·08 (s), 6·60 (s), 7·60, 6·22, 6·32, 6·73, 7·02, 9·52, 9·60, 12·0 μ (m); λ_{max}^{EtOH} 225 (ε 13,500) mμ.

Partition chromatography of the sixth fraction (2·592 g; eluted with ether) on a Celite 545 partition column (330 g) using solvent system C gave 4 bands. Treatment of the second band (1·370 g), R_f 0·63, with benzene–Skellysolve B gave colorless crystals (618 mg), m.p. 235–238° dec. A sample (344 mg) was recrystallized from benzene to give XXIIIa (186 mg), m.p. 236–238° dec. (Found: C, 78·60; H, 9·90; N, 5·48; $C_{33}H_{50}N_2O_2$ requires: C, 78·21; H, 9·95; N, 5·53%); $[\alpha]_D^{26}$ +42° (*c* 1·05); $\lambda_{max}^{CHCl_3}$ 6·20 μ (s).

In a second isolation procedure, the "additional weak bases" fraction (80 g) was triturated with benzene–Skellysolve B [(1:1), 400 ml] and the insoluble material (21·5 g) removed by filtration. The soluble fraction (58·5 g) was added to a basic Woelm grade III alumina column (1700 g) in Skellysolve B–benzene [(1:1), 400 ml] and 16 fractions were collected.

Fraction	Fluant	Wt (g)
1	Skellysolve B–benzene [(1:1), 8 l.]	1·002
2	Benzene (5·5 l.)	9·063
3	Benzene (5·5 l.)	1·491
4	Benzene–ether [(9:1), 5·5 l.]	5·078
5	Benzene–ether [(1:1), 1 l.]	1·693
6	Benzene–ether [(1:1), 1 l.]	3·449
7	Benzene–ether [(1:1), 1 l.]	5·473
8	Benzene–ether [(1:1), 1 l.]	3·823
9	Benzene–ether [(1:1), 1·5 l.]	2·520
10	Benzene–ether [(1:1), 1 l.]	1·170
11	Benzene–ether [(1:1), 1 l.]	1·266
12	Benzene–ether [(1:1), 1 l.]	0·878
13	Benzene–ether [(1:1), 1 l.]	0·801
14	Benzene–ether [(1:1), 1 l.]	0·646
15	Ether (6 l.)	0·400
16	Methanol (4 l.)	6·601

Fraction 5 (1·693 g) was crystallized from an ethereal soln to give XV (227 mg), m.p. 248–250° dec. Recrystallization from acetone afforded an analytical sample (157 mg), m.p. 255–256° dec. (Found: C, 75·91; H, 9·13; N, 5·42; $C_{33}H_{48}N_2O_3$ requires: C, 76·11; H, 9·29; N, 5·38%); $[\alpha]_D^{27}$ +76° (*c* 0·84),

$\lambda_{max}^{CHCl_3}$ 2·90, 2·97, 3·27, 6·02 (s), 6·09 (s), 6·60 (s), 7·59, 6·24, 6·33, 6·72, 7·04, 9·55 (m), 12·0 μ (m); λ_{max}^{EtOH} 226 (ε 14,300) mμ.

Reduction of N-*benzoylcycloxobuxine-F* (I) *with lithium aluminium hydride*

(a) *Under mild conditions.* N-Benzoylcycloxobuxine-F (240 mg) in ether (20 ml) was added to a suspension of LAH (210 mg) in anhyd ether (5 ml). After being stirred 3 hr at room temp the reaction mixture was cooled in an ice bath and ether saturated with water was added cautiously to decompose the excess reagent. The suspension was filtered and the inorganic ppt washed with CH_2Cl_2. The filtrate was dried over anhyd Na_2SO_4 and concentrated. The residue was purified by chromatography on a Celite 545 partition column (solvent system B) to give a solid material (210 mg), m.p. 242–244° dec which was shown by TLC to be homogeneous. Recrystallization from acetone afforded the alcohol (II) (186 mg), m.p. 244–247° dec. (Found: C, 78·04; H, 9·91; N, 5·58; $C_{33}H_{50}N_2O_2$ requires: C, 78·21; H, 9·95; N, 5·53%); $[\alpha]_D^{27}$ +78° (*c* 0·92); $\lambda_{max}^{CHCl_3}$ 2·80, 2·92, 6·03, 6·60 (s), 7·60 μ; λ_{max}^{EtOH} 224·5 (ε 11,650) mμ.

(b) *Under moderate conditions.* N-Benzoylcycloxobuxine-F (300 mg) was treated with LAH (400 mg) in anhyd ether (70 ml) as before. The reaction mixture was allowed to stand at room temp. with stirring for 14 hr. The product (283 mg, oil) was fractionated by partition chromatography (solvent system B) which yielded 2 major bands. Band 1 (least polar) gave a crystalline material (36 mg), m.p. 153–155°, which was shown to be identical with IV by mixture m.p. (no depression observed) and IR analysis (spectra superimposable). Band 2 gave a colorless solid (201 mg), m.p. 154–156°, which was shown to be homogeneous by TLC. Recrystallization from Skellysolve B afforded III (148 mg), m.p. 165–167°. (Found: C, 80·23; H, 10·69; N, 5·61; $C_{33}H_{52}N_2O$ requires: C, 80·43; H, 10·64; N, 5·69%); $[\alpha]_D^{28}$ +112° (*c* 0·85); $\lambda_{max}^{CHCl_3}$ 2·79, 3·05, 6·24, 6·74 μ.

(c) *Under rigorous conditions.* N-Benzoylcycloxobuxine-F (100 mg) was dissolved in dioxan (15 ml) and added to a suspension of LAH (155 mg) in dioxan (10 ml). The reaction mixture was heated at reflux temp. with stirring for 48 hr. The reaction vessel was cooled in an ice bath and excess reagent decomposed by the addition of ether saturated with water. The suspension was filtered and the inorganic precipitate washed with CH_2Cl_2. The filtrate was dried over anhyd Na_2SO_4 and concentrated to give a yellow oil (102 mg), which was fractionated by adsorption chromatography on basic Woelm grade III alumina (8 g). Elution with benzene (50 ml) gave a solid material (57·4 mg) which was recrystallized from acetone–Skellysolve B to yield IV (44 mg), m.p. 155–156°. (Found: C, 82·98; H, 10·93; N, 6·04; $C_{33}H_{54}N_2$ requires: C, 83·13; H, 10·99; N, 5·88%); $[\alpha]^{25}$ +86° (*c* 1·00); $\lambda_{max}^{CHCl_3}$ 6·25, 6·70 μ; λ_{max}^{EtOH} 247 (ε 2,500), with shoulders at 243, 269, 279, 282 mμ. Additional fractions from the column (39·8 mg total) did not crystallize and were shown by TLC to be mixtures.

Reaction of alcohol III *with lithium aluminium hydride*

Alcohol III (60 mg) in dioxan (20 ml) was added to a suspension of LAH (60 mg) in dioxan (5 ml) and the mixture was heated to reflux temp. with stirring for 48 hr. Treatment of the reaction mixture in the usual manner gave a semi-crystalline material (51 mg) which was purified by partition chromatography (solvent system B) to afford a crystalline substance (47 mg), m.p. 165–166°, shown to be identical with the starting alcohol III by TLC, mixture m.p. (no depression) and IR analysis (identical spectra).

Methylation of N-*benzylcycloprotobuxine-F* (IV) *to* N-*benzylcycloprotobuxine-C* (V)

N-Benzylcycloprotobuxine-F (52 mg) was dissolved in a mixture of formaldehyde (37%; 0·5 ml) and formic acid (100%; 0·5 ml). The soln was heated on a boiling water bath for 6 hr, poured into water (20 ml) and treated with solid Na_2CO_3 until alkaline. The soln was extracted with CH_2Cl_2, the extracts dried over anhyd Na_2SO_4 and concentrated to give a solid material (54·5 mg). Recrystallization from acetone afforded V (37·5 mg), m.p. 142–143°. The m.p. was not depressed by admixture of a sample of N-benzylcycloprotobuxine-C obtained synthetically from cycloprotobuxine-C and the IR (KBr) spectra of the respective samples were identical.

Benzoylation of cycloprotobuxine-C (VI)

Cycloprotobuxine-C (150 mg) was dissolved in pyridine (anhyd, 4 ml) and the soln was cooled in an ice bath. Benzoyl chloride (84 mg) was added to pyridine (1 ml) which had been cooled in an ice bath. The soln of benzoyl chloride was added to the soln of cycloprotobuxine-C and the reaction mixture was allowed to stand at room temp for 12 hr. The mixture was poured into water (50 ml) and was extracted with

benzene (3 × 50 ml), the extracts washed with water (3 × 200 ml), dried over anhyd Na_2SO_4 and concentrated. The residue was dissolved in ether (35 ml), warmed on a steam bath in the presence of Norit A, and the mixture filtered. The filtrate (colorless) was concentrated to give a white crystalline material (166 mg). Recrystallization from acetone afforded VII (149 mg), m.p. 218–220° dec. (Found: C, 80·79; H, 10·40; N, 5·56; $C_{34}H_{52}N_2O$ requires: C, 80·90; H, 10·38; N, 5·55%); $[\alpha]_D^{26}$ +32° (*c* 1·00); $\lambda_{max}^{CHCl_3}$ 6·18 μ.

Reduction of N-benzoylcycloprotobuxine-C (VII) *to N-benzylcycloprotobuxine-C* (V)

N-Benzoylcycloprotobuxine-C (80 mg) was dissolved in dioxan (10 ml) and added to a suspension of LAH (100 mg) in dioxan (5 ml). The reaction mixture was heated at reflux temp, with stirring, for 22 hr. Treatment of the reaction mixture in the usual manner gave a white solid (74 mg). Recrystallization twice from acetone afforded N-benzylcycloprotobuxine-C (52·7 mg), m.p. 142·5–144·5°. (Found: C, 83·23; H, 11·09; N, 5·69; $C_{34}H_{54}N_2$ requires: C, 83·20; H, 11·09; N, 5·70%); $[\alpha]_D^{24}$ +57° (*c* 1·01); λ_{max}^{KBr} 6·70, 13·63, 14·33 μ.

Benzylation of cycloprotobuxine-C (VI) *to N-benzylcycloprotobuxine-C* (V)

A mixture of cycloprotobuxine-C (100 mg) and anhyd K_2CO_3 (35 mg) in dry benzene (3 ml) was treated with benzyl chloride (100 mg) and heated to reflux temp for 12 hr, at which time additional benzyl chloride (100 mg), K_2CO_3 (65 mg) and benzene (2 ml) were added to the reaction mixture. The mixture was heated at reflux temp for an additional 24 hr (36 hr total). Ice was added and the benzene layer separated. The aqueous soln was extracted with benzene (3 × 50 ml) and the benzene solns were combined, concentrated to 50 ml and extracted with 5% HCl aq. The aqueous acidic extract was made alkaline by the addition of 28% NH_4OH and extracted with $CHCl_3$ (3 × 50 ml), the extracts dried over anhyd Na_2SO_4 and concentrated. The residue was recrystallized from acetone to give N-benzylcycloprotobuxine-C (56 mg).

Lithium aluminium hydride reduction of ketone VIII

A soln of ketone VIII[19a] (200 mg) in dioxan (15 ml) was added to a suspension of LAH (300 mg) in dioxan (10 ml). The mixture was heated at reflux temp, with stirring, for 48 hr. Treatment of the reaction mixture in the usual manner gave a white solid (205 mg) which was shown to be a mixture by TLC. The reaction product was applied, in $CHCl_3$, to a plate (400 × 200 × 0·5 mm) of silica gel $HF_{254+366}$ (Brinkmann). The plate was developed using benzene–AcOEt (1:2) and 5 bands were visually detected with the use of UV light. Each band was scraped from the plate and extracted with $CHCl_3$. Concentration of the $CHCl_3$ extracts afforded:

Band	Yield (mg)	(%)	Purity (No. spots by TLC)*	R_f (TLC)
1 (most polar)	54·4	27·2	4	0–0·8
2	106·0	53·0	1	0·21
3	10·0	5·0	2	0·40–0·43
4	12·4	6·2	1	0·55
5	10·3	5·1	1	0·75
Total	193·1	96·5		

* Thin layer chromatograms were developed using benzene–AcOEt (1:2). Plates were sprayed with 3% ceric sulfate solution in 3N H_2SO_4.

Band 1 gave an oil (54·4 mg). No attempt was made to purify the oil from band 1 because it was evident from the TLC analysis that it was comprised of at least 4 compounds, none of which was the C-11 alcohol IX or a product of hydrogenolysis. Band 2 gave a solid (106·0 mg), m.p. 179–182°, which was shown by TLC to be homogeneous. Recrystallization from acetone–Skellysolve B afforded IX (57 mg), m.p. 181–183°. (Found: C, 71·50; H, 9·03; $C_{25}H_{38}O_3$ requires: C, 71·74; H, 9·15%); $[\alpha]_D^{28}$ +9° (*c* 0·77); NMR signals at 6·02 (8H, $-O(C\underline{H}_2)_2O-$); 6·38 (1H, m, $-C\underline{H}OH$); 8·69 (3H, s, C-21 Me); 9·12 (3H, s, C-18 Me); 9·50 (1H, cyclopropyl C$\underline{H}$).

Band 3 gave an oil (10·0 mg) which was shown to be a mixture of two different compounds by TLC. Band 4 gave an oil (12·4 mg) which failed to crystallize but appeared to be homogeneous by TLC. The product showed $\lambda_{max}^{CHCl_3}$ 10·55 μ (ketal); bands attributable to OH or CO functions were absent. Band 5 gave an oil (10·3 mg) which could not be crystallized; it appeared to be homogeneous by TLC. The product showed $\lambda_{max}^{CHCl_3}$ 5·80 μ (m); bands attributable to OH or ketal functions were absent.

Acetylation of N-*benzoylcycloxobuxidine-F* (Xa)

N-Benzoylcycloxobuxidine-F (50 mg) was dissolved in a mixture of Ac_2O (1 ml) in dry pyridine (3 ml) and was allowed to stand at room temp for 40 hr. MeOH (5 ml) was added and the soln concentrated under reduced press. Water (5 ml) was added and the soln made alkaline by the addition of 28% NH_4OH, and extracted with $CHCl_3$. The $CHCl_3$ extracts were washed several times with water, dried over anhyd Na_2SO_4 and concentrated. The residue was crystallized from Skellysolve B and recrystallized from acetone to afford Xb (42 mg), m.p. 230° dec. (Found: C, 71·44; H, 8·40; N, 4·47; $C_{37}H_{52}N_2O_6$ requires: C, 71·58; H, 8·44; N, 4·51%); $[\alpha]_D^{28}$ +58° (*c* 0·91); $\lambda_{max}^{CHCl_3}$ 2·91 (sharp, -NH), 5·79 (s), 6·01 (s), 6·61 (s), 7·60, 8·05 (s), 12·0 (m); λ_{max}^{EtOH} 223·5 (ε 16,400) mμ.

Hydrolysis of N-*benzoylcycloxobuxidine-F* (Xa)

N-Benzoylcycloxobuxidine-F (75 mg) was dissolved in a 6% ethanolic NaOH (30 ml) and heated to reflux temp for 24 hr. The soln was concentrated, diluted with water and extracted with CH_2Cl_2, the CH_2Cl_2 extract dried over anhyd Na_2SO_4 and concentrated to give an oil (59 mg), which solidified after standing. Recrystallization from AcOEt afforded XI (38·1 mg), m.p. 224–225° dec. (Found: C, 71·86; H, 9·98; N, 6·35; $C_{26}H_{44}N_2O_3$ requires: C, 72·18; H, 10·25; N, 6·48%); $[\alpha]_D^{28}$ +101° (*c* 0·64); $\lambda_{max}^{CHCl_3}$ 2·98, 3·13, 6·00 (s), 6·31 (-NH_2), 12·0 μ; λ_{max}^{EtOH} 218 (ε 6,350) mμ.

The aqueous soln was made acidic by the addition of 5% HCl aq, extracted with $CHCl_3$, and the $CHCl_3$ extracts were dried over anhyd Na_2SO_4 and concentrated to give a white solid (6·5 mg), m.p. 119–121°. The m.p. was not depressed upon admixture with an authentic sample of benzoic acid.

Lithium aluminium hydride hydrogenolysis of cycloxobuxidine-F (XI) *to dihydrocyclomicrophylline-F* (XIII)

Cycloxobuxidine-F (45 mg) was dissolved in dioxan (10 ml) and added to a suspension of LAH (100 mg) in dioxan (5 ml). The mixture was heated at reflux temp, with stirring, for 96 hr. Treatment of the reaction mixture in the usual manner gave an amorphous solid (42 mg), which was purified by partition chromatography (solvent system A) to give XIII (25·5 mg). Recrystallization from MeOH gave plates (14·2 mg), m.p. 255–258° dec., $[\alpha]_D^{27}$ +18° (*c* 0·68); $\lambda_{max}^{CHCl_3}$ 2·97 (OH), 3·05 (NH), 6·02, 6·32 μ (-NH_2). The physical data correspond closely to those reported for dihydrocyclomicrophylline-F.[21]

Hydrolysis of N-*benzoyldihydrocyclomicrophylline-F* (XII) *to dihydrocyclomicrophylline-F* (XIII)

N-Benzoyldihydrocyclomicrophylline-F (25 mg) was dissolved in an 95% ethanolic NaOH (20 ml) and heated at reflux temp for 24 hr. Treatment of the reaction mixture in the usual manner gave an amorphous solid (18·2 mg) which was purified by partition chromatography (solvent system A) to give dihydrocyclomicrophylline-F (14·5 mg), m.p. 250–256° dec. Recrystallization from MeOH gave plates, m.p. 255–258° dec. The m.p. was not depressed upon admixture with a sample obtained by hydrogenolysis of cycloxobuxidine-F, and the IR spectra of the respective samples were identical.

Acetylation of N-*benzoylcycloxobuxoline-F* (XV) *to* N-*benzoyl-O-acetylcycloxobuxoline-F* (XVI)

N-Benzoylcycloxobuxoline-F (100 mg) was dissolved in dry pyridine (4 ml). Ac_2O (1 ml) was added and the soln was allowed to stand at room temp for 48 hr. MeOH was added and, after standing at room temp for 20 min, the mixture was concentrated under reduced press, diluted with water (10 ml), made alkaline by the addition of solid K_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed several times with water, dried over anhyd Na_2SO_4 and concentrated to give an oil (110·2 mg) which crystallized from an ether soln. Recrystallization from ether afforded XVI (90 mg), m.p. 216–218°. The m.p. was not depressed upon admixture with a sample of naturally-occurring XVI and the IR spectra of the two samples were identical.

N → O-*Acyl migration in* N-*benzoylcycloxobuxoline-F* (XV)

N-Benzoylcycloxobuxoline-F (160 mg) was dissolved in 3% H_2SO_4 aq (15 ml) and heated at 90° for 1 hr. The reaction mixture was cooled in an ice bath, diluted with water (250 ml), made alkaline by the addition

of solid Na_2CO_3 (in the cold), and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhyd Na_2SO_4 and concentrated. Partition chromatography (solvent system B) of the residue gave a crystalline material (90·4 mg), m.p. 227–229° dec, shown to be homogeneous by TLC. Recrystallization from ether–Skellysolve B afforded XVII (66·8 mg), m.p. 228–230° dec. (Found: C, 75·89; H, 9·11; N, 5·29; $C_{33}H_{48}N_2O_3$ requires: C, 76·11; H, 9·29; N, 5·38%); $[\alpha]_D^{28}$ +79° (*c* 1·17); $\lambda_{max}^{CHCl_3}$ 2·99, 6·30 (—NH_2), 5·84 (s), 7·85 (s), 6·0 (s), 6·23 and 6·75 (Ar), 12·0 μ (m); λ_{max}^{EtOH} 227 (ε 14,300) mμ.

Oxidation of N-*benzoylbuxidienine-F* (XIX) *with subsequent elimination at* C-20

N-Benzoylbuxidienine-F (70 mg) was dissolved in glacial AcOH (5 ml) and a soln of CrO_3 (16 mg) in water (0·7 ml) was added. The soln was allowed to stand at room temp for 6 hr after which water was added, the soln basified by the addition of solid K_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried over anhyd Na_2SO_4 and concentrated. The residue was dissolved in MeOH (5 ml) and heated at reflux temp for 1 hr. The MeOH soln was concentrated to give a yellow oil (55 mg) which was chromatographed on neutral Woelm grade I alumina (15 g), eluting with CH_2Cl_2. The first fraction (500 ml) yielded a yellow oil (32 mg) which appeared to be homogenous as shown by TLC. The compound did not stain with Dragendorff's reagent and was insoluble in 1N HCl (10 ml). $\lambda_{max}^{CHCl_3}$ 2·95, 5·83 (m), 6·02, 6·09 (s), 6·62 (s), 6·34, 6·73 μ; NMR signals at 7·90 (d, *J* 7·5 c/s) and 8·20 τ (d, *J* 7·5 c/s); signal for $(CH_3)_2N$ was absent.

Methylation of N-*benzoylcycloprotobuxoline-D* (XXIIIa) *to* N-*benzoylcycloprotobuxoline-C* (XXIIIb)

N-Benzoylcycloprotobuxoline-D (89·3 mg) was dissolved in formic acid (97%; 0·89 ml) and formaldehyde (40%; 0·89 ml). The mixture was heated for 6 hr in a boiling water bath, then poured into water (10 ml) and treated with solid Na_2CO_3 until alkaline. The soln was extracted with CH_2Cl_2, the extracts dried over anhyd Na_2SO_4 and concentrated. Recrystallization of the residue 3 times from acetone gave colorless needles (22·9 mg), m.p. 251–256° (dec). The m.p. was not depressed by admixture of a sample of N-benzoylcycloprotobuxoline-C, and the IR spectra of the respective samples were identical.

Benzoylation of N-*benzoylcycloprotobuxoline-D* (XXIIIa)

A mixture of N-benzoylcycloprotobuxoline-D (87·6 mg) and anhyd K_2CO_3 (1·0 g) in anhyd benzene (20 ml) was treated with benzoyl chloride (113 mg) and stirred for 1·5 hr. The mixture was concentrated and the residue treated with water (10 ml) and $CHCl_3$ (10 ml). After stirring 1 hr, the $CHCl_3$ soln was separated and the aqueous portion extracted again with $CHCl_3$. The $CHCl_3$ solutions were combined, dried over anhyd Na_2SO_4 and concentrated. The residue was crystallized from acetone to give colorless needles (69·5 mg), m.p. 260–264° dec (softens at 220°). Recrystallization from acetone gave XXIIIc (38·8 mg), m.p. 262–264° dec. (Found: C, 78·70; H, 8·87; N, 4·62; $C_{40}H_{54}N_2O_3$ requires: C, 78·64; H, 8·91; N, 4·58%); $[\alpha]_D^{27}$ +15° (*c* 0·97); λ_{max}^{KBr} 6·15 μ (s).

Benzolation of cyclovirobuxine-D (XXIIa)

A mixture of cyclovirobuxine-D (99·0 mg) and anhyd K_2CO_3 (2·0 g) in anhyd benzene (20 ml) was treated with benzoyl chloride (196 mg). Treatment of the reaction mixture in the usual manner gave colorless needles (70·9 mg), m.p. 302–305° dec. Recrystallization from acetone gave XXIIb (24·7 mg), m.p. 302–304° dec. (Found: C, 78·56; H, 8·84; N, 4·70; $C_{40}H_{54}N_2O_3$ requires: C, 78·64; H, 8·91; N, 4·58%); $[\alpha]_D^{30}$ −21° (*c* 1·12); λ_{max}^{KBr} 6·15 μ (s).

Alkaline hydrolysis of N-*benzoylcycloprotobuxoline-D* (XXIIIa)

N-Benzoylcycloprotobuxoline-D (57·2 mg) was dissolved in 97% MeOH (17 ml) containing KOH (0·5 g). The mixture was refluxed for 2·5 hr. The reaction mixture was concentrated, the residue diluted with water and extracted with $CHCl_3$. The extracts were dried over anhyd Na_2SO_4 and concentrated. The residue was chromatographed on a Celite 545 partition column (5 g) using solvent system B. The band of R_f 0·20 gave a colorless solid (32·2 mg), m.p. 132–138°. Recrystallization from Skellysolve B gave XXIVa (22·5 mg), m.p. 139–141°. (Found: C, 77·71; H, 11·34; N, 7·00; $C_{26}H_{46}N_2O$ requires: C, 77·55; H, 11·52; N, 6·96%); $[\alpha]_D^{24}$ +19° (*c* 0·91).

The aqueous alkaline soln was acidified with conc HCl and extracted with CH_2Cl_2. The extracts were dried over anhyd Na_2SO_4 and concentrated to give a colorless solid (5 mg), m.p. 118–121°. The m.p. was not depressed by admixture with an authentic sample of benzoic acid, and the IR spectra of the respective samples were identical.

Alkaline hydrolysis of N-*benzoylcycloprotobuxoline-C* (XXIIIb)

Similar treatment of N-benzoylcycloprotobuxoline-C (250 mg) gave a colorless solid (156·3 mg), m.p. 152–156°. A portion of this product (82·9 mg) was recrystallized from Skellysolve B to give XXIVb (26·1 mg), m.p. 160–163° (softens at 154°). (Found: C, 77·58; H, 11·53; N, 6·80; $C_{32}H_{48}N_2O$ requires: C, 77·82; H, 11·61; N, 6·72%); $[\alpha]_D^{29}$ +20° (*c*, 0·95).

Reaction of cycloprotobuxoline-C (XXIVb) *with phosgene*

Cycloprotobuxoline-C (89·5 mg) was dissolved in a mixture of benzene (10 ml) and anhyd pyridine (2 ml) and cooled in an ice bath. A benzene soln of phosgene (12·5%; 3·5 ml) was added. After 5 min, the ice bath was removed and the mixture stirred 12 hr at room temp. Ice was added and the benzene layer separated. The water soln was extracted with $CHCl_3$ and the extract and benzene soln were combined, washed with water, dried over anhyd Na_2SO_4 and concentrated. The residue was chromatographed on a Celite 545 partition column (5 g) using solvent system B. The band of R_f 0·60 gave a light yellow solid (39·1 mg), m.p. 188–192°. Recrystallization from Skellysolve B gave the XXVI (23·9 mg), m.p. 192–195° (Found: C, 75·94; H, 10·44; N, 6·30; $C_{28}H_{46}N_2O_2$ requires: C, 75·97; H, 10·47; N, 6·33%); $[\alpha]_D^{29}$ +21° (*c*, 0·60); λ_{max}^{KBr} 6·20 μ (s).

Periodic acid titration of cycloprotobuxoline-D (XXIVa)

Cycloprotobuxoline-D (32·0 mg) was dissolved in 5% AcOH (5·0 ml) and water (15·0 ml) and 0·14 N HIO_4 (3·0 ml) was added. Aliquots (5·0 ml) were withdrawn at 30 min, 60 min, and 180 min and titrated.* Blank solns (prepared as above but with no compound added) were titrated at the same time intervals.

Molar equivalents of HIO_4 consumed (min): 1 (30); 1·2 (60); 1·2 (180).

Oxidation of N-*benzoylcycloprotobuxoline-D* (XXIIIa)

N-Benzoylcycloprotobuxoline-D (60·0 mg) was dissolved in glacial AcOH (5 ml) and a soln of CrO_3 (14·3 mg) in water (0·6 ml) was added. The mixture was stirred at room temp for 18 hr, then diluted with water and treated with solid K_2CO_3 until alkaline. The alkaline soln was extracted with $CHCl_3$, the extracts dried over anhyd Na_2SO_4 and concentrated. The residue was chromatographed on a Celite 545 partition column (5 g) using solvent system B. The band of R_f 0·28 gave a solid (42·6 mg), m.p. 205–209° dec. Crystallization from a conc acetone soln gave the ketone XXVII (17·7 mg), m.p. 210–213° dec (Found: C, 78·42; H, 9·66; N, 5·62; $C_{33}H_{48}N_2O_2$ requires: C, 78·52; H, 9·59; N, 5·55%). $[\alpha]_D^{28}$ +19° (*c* 0·98), $\lambda_{max}^{CHCl_3}$ 5·82 (s), 6·14 μ (s).

Acetylation of N-*benzoylcycloprotobuxoline-C* (XXIIIb)

N-Benzoylcycloprotobuxoline-C (110 mg) was dissolved in a mixture of Ac_2O (1·0 ml) and anhyd pyridine (3·0 ml) and allowed to stand overnight at room temp. MeOH was added and after standing for 30 min, the mixture was concentrated. The residue was diluted with water, treated with solid K_2CO_3 until alkaline and extracted with CH_2Cl_2. The extracts were dried over anhyd Na_2SO_4 and concentrated. The residue was chromatographed on Woelm basic alumina, activity III (2·0 g). The fraction eluted with benzene was recrystallized from acetone to yield XXIIId (22·7 mg), m.p. 260–262° dec. (Found: C, 76·85; H, 9·76; N, 5·15. $C_{36}H_{54}N_2O_3$ requires: C, 76·82; H, 9·67; N, 4·98%); $[\alpha]_D^{26}$ +60° (*c* 1·10); $\lambda_{max}^{CHCl_3}$ 5·79 (s), 6·18 μ (s).

Lithium aluminium hydride reduction of ketone XXVII

Ketone XXVII (104 mg) was reduced in 2 equal portions. The ketone (52·0 mg) was dissolved in anhyd ether (30 ml) and LAH (85·5 mg) was added while cooling the reaction flask in an ice bath. After 1 hr, the ice bath was removed and stirring was continued for 3 hr at room temp followed by refluxing for 0·5 hr. A few drops of water, 20% NaOH, and finally water were added and the granular ppt was removed by filtration and washed thoroughly with ether. The ether soln was concentrated and the residue purified by chromatography on a Celite 545 partition column (5 g) using solvent system B to give 4 bands. The band of R_f 0·65 gave a colorless solid (29·9 mg total from 104 mg of ketone) and the band of R_f 0·48 gave a colorless solid (47·2 mg total). Both were homogeneous solids (TLC). The two remaining bands of lower R_f gave less than 3 mg of material combined.

Both solids were compared (TLC, partition chromatography, IR spectra) with the LAH reduction

* The procedure used was that of Jackson, *Organic Reactions* Vol. II, p. 361.

product of XXIIIa. The isolated material gave a reduction product identical to the material obtained from XXVII, band of R_f 0·48.

The material obtained from the band of R_f 0·65 (117 mg, from several reductions) was methylated by the usual method and the product was acetylated without further purification by stirring 12·5 hr in a mixture of Ac_2O (1·0 ml) and anhyd pyridine (3·0 ml). The reaction mixture was treated in the usual manner to give fine colorless needles (71·3 mg) from a concentrated acetone soln. TLC indicated a mixture of starting material and product. Several recrystallizations from acetone gave XXVIIIb (16·6 mg), m.p. 154–157°. (Found: C, 78·89; H, 10·46; N, 5·15; $C_{36}H_{56}N_2O_2$ requires: C, 78·78; H, 10·29; N, 5·10%); $[\alpha]_D^{26}$ +80° (*c* 0·41); λ_{max}^{KBr} 5·75 μ (s).

The material obtained from the band of R_f 0·48 (107 mg) was treated in an identical manner to give, after several recrystallizations from acetone, XXVIIIa (14·5 mg), m.p. 188–191° (Found: C, 78·83; H, 10·18; N, 5·16; $C_{36}H_{56}N_2O_2$ requires: C, 78·78; H, 10·29; N, 5·10%); $[\alpha]_D^{27}$ +22° (*c* 0·99); λ_{max}^{KBr} 5·76 μ (s).

Hydrolysis of tigloylcyclovirobuxeine-B (XXIXb)

Tigloylcyclovirobuxeine-B (211 mg) was dissolved in 3% methanolic KOH (20 ml) and the mixture refluxed 1 hr. The reaction mixture was concentrated, the residue diluted with water and extracted with ether. The extracts were dried over anhyd Na_2SO_4 and concentrated. The residue (183 mg), m.p. 192–196°, was recrystallized from Skellysolve B several times to give colorless needles (25·1 mg), m.p. 200–202°. The m.p. was not depressed by admixture with an authentic sample of cyclovirobuxeine-B and the IR spectra of the respective samples were identical.

The aqueous alkaline soln was acidified with conc HCl and extracted with CH_2Cl_2. The extracts were dried over anhyd Na_2SO_4 and concentrated. The residue was chromatographed on a silicic acid column (2 g). The fraction eluted with $CHCl_3$ crystallized on standing to yield a colorless solid (8·2 mg), m.p. 61–63°. The m.p. was not depressed by admixture with an authentic sample of tiglic acid, and the IR spectra of the respective samples were identical.

Acetylation of N-*acetylcycloprotobuxine-D* (XXXc)

A mixture of N-acetylcycloprotobuxine-D (30·0 mg) and anhyd K_2CO_3 (0·5 g) in anhyd benzene (10 ml) was treated with acetyl chloride (0·10 ml) and stirred for 24 hr. Treatment of the reaction mixture in the usual manner gave, after recrystallization from acetone, XXXb (20·1 mg), m.p. 275–279°. The m.p. was not depressed by admixture with an authentic sample (reported[17] m.p. 276–278°) and the IR spectra of the respective samples were identical.

Methylation of N-*acetylcycloprotobuxine-D* (XXXc)

N-Acetylcycloprotobuxine-D (34·8 mg) was methylated in the usual manner to give, after recrystallization from acetone, XXXe (14·4 mg), m.p. 241–243° dec. (Found: C, 78·84; H, 11·36; N, 6·39; $C_{29}H_{50}N_2O$ requires: C, 78·67; H, 11·38; N, 6·33%); $[\alpha]_D^{27}$ +57° (*c* 0·36); λ_{max}^{KBr} 6·15 μ (s).

Acetylation of cycloprotobuxine-C

A mixture of cycloprotobuxine-C (74·3 mg) and anhyd K_2CO_3 (1·0 g) in anhyd benzene (20 ml) was treated with acetyl chloride (0·20 ml) and stirred for 3 hr. Treatment of the reaction mixture in the usual manner gave, after two recrystallizations from acetone, XXXI (27·2 mg), m.p. 227–230° dec. (Found: C, 78·76; H, 11·58; N, 6·11; $C_{29}H_{50}N_2O$ requires: C, 78·67; H, 11·38; N, 6·33%); $[\alpha]_D^{27}$ +15° (*c* 1·03); λ_{max}^{KBr} 6·10 μ (s).

Acknowledgement- This work was supported, in part, by a research grant (HE-02275) from the National Institutes of Health, U.S. Public Health Service. R.M.K. was National Institutes of Health Predoctoral Fellow, 1962–1966.