835. *Buxus Alkaloids. Part IV.*[1] *Isolation and Structure Elucidation of Eight New Alkaloids, Cyclomicrophylline-A, -B, and -C, Dihydrocyclomicrophylline-A and -F, Cyclomicrophyllidine-A, Dihydrocyclomicrophyllidine-A, and Cyclomicrobuxine, from* B. microphylla *Sieb. et Zucc.* var. suffruticosa *Makino*

By T. Nakano and S. Terao

The alkaloid extracts of *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino have yielded eight new alkaloids whose structures and absolute stereochemistry, (Ia—d), (IIa—c), and (III), are described. Apparently, they belong to a new class of triterpene alkaloids and are divided into two series; the first two groups, (I)—(II), possess a fundamental skeleton of the cycloartane type, and the last, (III), one of the cycloeucalane type.

The extracts of Buxus plants have long been used in the treatment of disease, including malaria and skin disease,[2] and they have been investigated chemically for some time.[3] Schlittler *et al.*[4] isolated seven new alkaloids, A, B, C, D, L, M, and N, from the leaves of *Buxus sempervirens* L., but, in spite of extensive investigation, none of the structures of these alkaloids has been elucidated. Very recently, however, the structure of one of the alkaloids from this same plant, cyclobuxine,[5] was reported in preliminary form. Our interest in *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino was stimulated by earlier work[6] which showed that this plant contained more than eight unidentified alkaloids. Preliminary accounts of some of our results have been presented,[7] and the present Paper covers the structures and the stereochemistry of all eight of the new alkaloids which we have isolated from our plant source. These alkaloids have been named * cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic), dihydrocyclomicrophylline-A (IIa) and -F (IIb),[8] cyclomicrophyllidine-A (Id), dihydrocyclomicrophyllidine-A (IIc), and cyclomicrobuxine (III). For convenience we have at the outset given the correct formulæ and stereochemistry, and will summarise later the relevant evidence. The molecular formulæ, physical constants, and nuclear magnetic resonance (n.m.r.) spectral data are listed in the Table. These compounds belong to a novel class of tetracyclic triterpenoid alkaloids, having a cyclopropane ring and with a variation of the substitution pattern at the 4-position.

The common structural relationship of these eight alkaloids is evident from their n.m.r. spectra, which indicate a cyclopropane ring bearing two non-equivalent hydrogen

* The letter suffix of the trivial name of each alkaloid does not designate a chronological sequence but the substitution pattern of the C-3 and C-20 nitrogen functions. The mode of its designation follows the convention which has been adopted after discussion with Drs. D. Arigoni, R. Goutarel, and S. M. Kupchan, at the I.U.P.A.C. meeting in Kyoto in April 1964. It is illustrated below.

	C-3 N		C-20 N			C-3 N		C-20 N			C-3 N		C-20 N	
A	Me	Me	Me	Me	D	H	Me	H	Me	G	H	Me	H	H
B	Me	Me	H	Me	E	Me	Me	H	H	H	H	H	H	Me
C	H	Me	Me	Me	F	H	H	Me	Me	I	H	H	H	H

[1] Part III, T. Nakano and M. Hasegawa, *Tetrahedron Letters*, 1964, 3679.
[2] E. Schlittler, K. Heusler, and W. Friedrich, *Helv. Chim. Acta*, 1949, **32**, 2209.
[3] M. Fauré, *J. Pharm. Chim.*, 1830, **16**, 428; G. F. Walz, *Neues Jahrbuch für Pharmazie*, 1860, **14**, 428; M. Scholz, *Arch. Pharm.*, 1898, **236**, 542.
[4] E. Schlittler, K. Heusler, and W. Friedrich, *Helv. Chim. Acta*, 1949, **32**, 2209; K. Heusler and E. Schlittler, *ibid.*, p. 2226; W. Friedrich and E. Schlittler, *ibid.*, 1950, **33**, 873; E. Schlittler and W. Friedrich, *ibid.*, p. 878.
[5] (*a*) K. S. Brown, jun., and S. M. Kupchan, *J. Amer. Chem. Soc.*, 1962, **84**, 4590, 4592; (*b*) D. Stauffacher, *Helv. Chim. Acta*, 1964, **47**, 968.
[6] K. Sawa, K. Okabe, and H. Tada, unpublished results (personal communication from Dr. Sawa, of Shionogi and Co.).
[7] T. Nakano and S. Terao, presented at the meeting of the Japanese Pharmaceutical Society, Kyoto, January 1964; T. Nakano and S. Terao, *Tetrahedron Letters*, 1964, 1035, 1045.
[8] T. Nakano and S. Terao, presented at the meeting of the Japanese Pharmaceutical Society, Kyoto, March 1964.

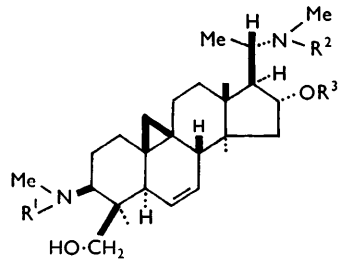
Nuclear magnetic resonance spectral data *

Alkaloid	M. p. $[\alpha]_D$	Cyclopropane	$-C-CH_3$	$>CH-CH_3$	$-N-CH_3$ †	CH_2OH ‡	$>CHOH$	$>CHOBz$	Olefinic protons	Aromatic H $>C=CH_2$ $CO-CH_3$
Cyclomicrophylline-A (Ia)	232—233°	10·16, d.	9·07 (6H)	9·12, d.	7·75 (6H)	6·32, q., J = 10·5	5·90, m.	—	4·52—4·56	—
$C_{28}H_{48}N_2O_2$	−92	9·22, d.	8·88 (3H)	J = 7	7·66 (6H)	(6·48 and 6·15)	(1H)		(2H)	
		J = 4								
Cyclomicrophylline-B (Ib)	251—252	10·16, d.	9·08 (6H)	9·02, d.	7·56 (3H)	6·32, q., J = 10·5	5·90, m.	—	4·51—4·58	—
$C_{27}H_{46}N_2O_2$	−65	9·22, d.	8·90 (3H)	J = 6	7·69 (6H)	(6·49 and 6·15)	(1H)		(2H)	
		J = 4								
Cyclomicrophylline-C (Ic)	282—283	10·16, d.	9·08 (3H)	9·12, d.	7·75 (6H)	6·32, q., J = 10·5	5·89, m.	—	4·50—4·58	—
$C_{27}H_{46}N_2O_2$	−40	9·22, d.	9·06 (6H)	J = 7	7·56 (3H)	(6·49 and 6·15)	(1H)		(2H)	
		J = 4								
Cyclomicrophyllidine-A (Id)	—	10·14, d.	9·00 (6H)	8·96, d.	7·92 (6H)	6·31, q., J = 10·5	—	4·59, m.	4·52—4·62	2·40—2·90 (3H)
$C_{34}H_{52}N_2O_3$	−160	—	8·73 (3H)	J = 7	7·67 (6H)	(6·47 and 6·14)		(1H)	(2H)	1·85—2·16 (2H)
		J = 4								
Dihydrocyclomicrophylline-A (IIa)	271—272	9·69, d.	9·03 (3H)	9·12, d.	7·75 (6H)	6·39, q., J = 10·5	5·94, m.	—	—	—
	+37	9·40, d.	8·88 (6H)	J = 7	7·69 (6H)	(6·51 and 6·26)	(1H)			
$C_{28}H_{50}N_2O_2$		J = 4								
Dihydrocyclomicrophylline-F (IIb)	260	9·69, d.	9·03 (6H)	9·12, d.	7·75 (6H)	6·41, q., J = 10·5	5·95, m.	—	—	—
	+4·6	9·40, d.	8·87 (3H)	J = 7	—	(6·54 and 6·29)	(1H)			
$C_{26}H_{46}N_2O_2$		J = 4								
Dihydrocyclomicrophyllidine-A (IIc)	—	9·64, d.	9·04 (3H)	8·96, d.	7·91 (6H)	6·40, q., J = 10·5	—	4·59, m.	—	2·40—2·90 (3H)
	−33	9·36, d.	8·89 (6H)	J = 7	7·70 (6H)	(6·52 and 6·28)		(1H)		1·85—2·16 (2H)
$C_{34}H_{54}N_2O_3$		J = 4								
Cyclomicrobuxine (III)	178—180	9·93, d.	9·08 (3H)	—	—	—	5·20, m.	—	—	7·86 (3H)
$C_{25}H_{39}NO_2$	+172	9·67, d.	8·79 (3H)		7·66 (6H)		(1H)			5·35 and 5·04 (2H)

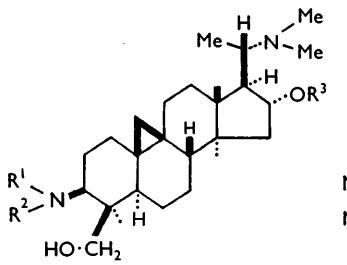
* Chemical shifts are given in p.p.m. on the τ scale. Coupling constants (J) are expressed as c./sec. d = doublet, m = multiplet, q = quadruplet. All other resonances are singlets, except where stated otherwise. † Upper and lower signal values correspond to the *N*-methyl groups at C-3 and C-20, respectively. ‡ These hydroxymethylene proton resonances constitute an AB quadruplet. The centre of gravity of each resonance has been calculated by the method of H. J. Bernstein, J. A. Pople, and W. G. Schneider, *Canad. J. Chem.*, 1957, **35**, 65.

atoms (see Table), and also from an infrared band at 3·32 μ due to the cyclopropyl methylene stretching vibration.[9] The seven alkaloids (I)—(II), except cyclomicrobuxine (III), contain one hydroxymethylene, one tertiary and three quaternary methyl groups, as is also apparent from their n.m.r. spectra. The presence of the double bond in cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) was indicated by the n.m.r. spectra and also by a strong infrared band near 14·3 μ (in potassium bromide discs), which is characteristic of a *cis*-disubstituted ethylenic linkage.[10a] The infrared spectra of (Ia—c) contain a broad OH band at 2·91 μ, but those of (Ib) and (Ic) had an additional sharp band at 3·04 μ (NH). The Feigl test [11] for a secondary amino-group was negative for alkaloid (Ia), but positive for (Ib) and (Ic).

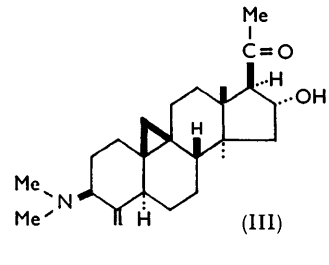
On acetylation with acetic anhydride–pyridine, cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) yielded the *O*-diacetate (IVa), the *N'*-acetyl *O*-diacetate (IVb), and the *N*-acetyl *O*-diacetate (IVc), respectively. Refluxing of the *O*-diacetate (IVa) with aqueous methanol in the absence of added alkali gave back the parent alkaloid (Ia). It seems that in the methanolysis of this *O*-diacetate the intramolecular amino-groups participate by acting as bases,[12] as illustrated in partial formula (A). On the other hand, neither (IVb) nor (IVc) underwent hydrolysis under these conditions, but on treatment with 3% aqueous methanolic alkali they were hydrolysed to the parent compounds (Ib) and (Ic). Facilitation of the hydrolysis at the *N*-acetyl groups in both of these alkaloids under such mild alkaline conditions * is striking. The first step in this reaction seems to be the hydrolysis of the *O*-acetate groups which would involve nucleophilic participation by the neighbouring amide groups.[13] The resultant hydroxyl groups would then provide



(Ia: $R^1 = R^2 = Me$, $R^3 = H$)
(Ib: $R^1 = Me$, $R^2 = R^3 = H$)
(Ic: $R^1 = R^3 = H$, $R^2 = Me$)
(Id: $R^1 = R^2 = Me$, $R^3 = COPh$)



(IIa: $R^1 = R^2 = Me$, $R^3 = H$)
(IIb: $R^1 = R^2 = R^3 = H$)
(IIc: $R^1 = R^2 = Me$, $R^3 = COPh$)



(III)

anchimeric assistance in subsequent hydrolysis of the *N*-acetyl functions as shown in partial formula (B). In all these cases, the interference between the 18- and 21-methyl groups forces the 16α- and 20α-substituents to adopt parallel conformations.

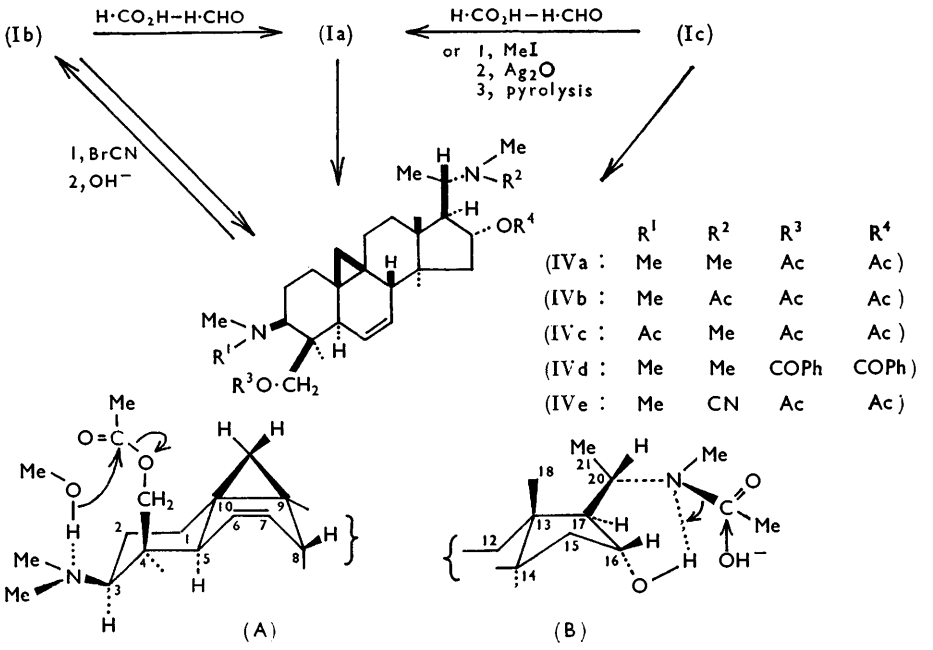
The inter-relationship of cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic), and cyclomicrophyllidine-A (Id), was established as follows. As is seen from the Table, alkaloids (Ib) and (Ic) differ from (Ia) only in that the former possess three instead of four *N*-methyl groups. When heated with formic acid–formalin,[14] (Ib) and (Ic) yielded (Ia). Methylation of (Ic) with methyl iodide in acetone at room temperature led to the monometh-

* Hydrolysis of the acetates of 3-amino-steroids with no adjacent hydroxyl groups requires very strong basic or acidic conditions (see R. D. Haworth, J. McKenna, and G. H. Whitfield, *J.*, 1953, 1102).

[9] A. R. H. Cole, *Chem. and Ind.*, 1953, 946; 1954, 3807, 3810.
[10] L. J. Bellamy, " The Infra-red Spectra of Complex Molecules," Wiley, New York, 1958, (*a*) p. 48; (*b*) p. 240.
[11] F. Feigl and V. Anger, *Mikrochim. Acta*, 1937, **1**, 138.
[12] For a similar reference, see S. M. Kupchan, S. P. Eriksen, and Y. T. Shen, *J. Amer. Chem. Soc.*, 1963, **85**, 350.
[13] B. Capon, *Quart. Rev.*, 1964, **18**, 71.
[14] H. T. Clarke, H. B. Gillespie, and S. Z. Weisshaus, *J. Amer. Chem. Soc.*, 1933, **55**, 4571.

iodide, which, on treatment with silver oxide followed by pyrolysis under reduced pressure, gave (Ia). Attempted decomposition of (Ia) with cyanogen bromide in either anhydrous benzene or chloroform resulted in the recovery of the starting compound. However, its *O*-diacetate (IVa), under the same reaction conditions, readily afforded the cyanamide (IVd), which was then hydrolysed with alkali to (Ib). The failure of von Braun decomposition in the former case may be the result of the decreased nucleophilic reactivity of the basic nitrogen atom owing to its hydrogen bonding with the adjacent hydroxyl group. That cyclomicrophyllidine-A (Id) is a monobenzoate of cyclomicrophylline-A (Ia) was readily deduced from its n.m.r., infrared, and ultraviolet spectra. This was proved by the alkaline hydrolysis of this alkaloid, from which cyclomicrophylline-A and benzoic acid were obtained. Evidence for the assignment of the methylamino-group to C-3 in cyclomicrophylline-B (Ib) and to C-20 in cyclomicrophilline-C (Ic), and the *O*-benzoyl group to C-16 in cylomicrophyllidine-A (Id), follows from later experiments.

On hydrogenation with platinum oxide in acetic acid, cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) absorbed one molar equivalent of hydrogen to yield the dihydro-derivatives (Va), (Vb), and (Vc). The dihydro-compound (Va) is identical with natural dihydrocyclomicrophylline-A (IIa). These dihydro-derivatives do not give the n.m.r. signals of two olefinic protons, or the infrared band near 14·3 μ (*cis*-disubstituted ethylenic linkage). Whilst cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) exhibit strong ultraviolet absorption at 204 mμ (ε 5800), their dihydro-derivatives do not absorb above 190 mμ. The steroidal Δ^6-compounds are known to show an ultraviolet band at 204 mμ, but its molar extinction coefficient (ε) is usually ~1500.[15] The enhancement of the intensity of this band for these alkaloids may be ascribable to the transannular interaction between the π-orbitals of the double bond and the π-like overlapping *p*-orbitals [16] of a cyclopropane ring in the distorted boat form of ring B. In the n.m.r. spectra of cyclomicrophilline-A



	R¹	R²	R³	R⁴
(IVa :	Me	Me	Ac	Ac)
(IVb :	Me	Ac	Ac	Ac)
(IVc :	Ac	Me	Ac	Ac)
(IVd :	Me	Me	COPh	COPh)
(IVe :	Me	CN	Ac	Ac)

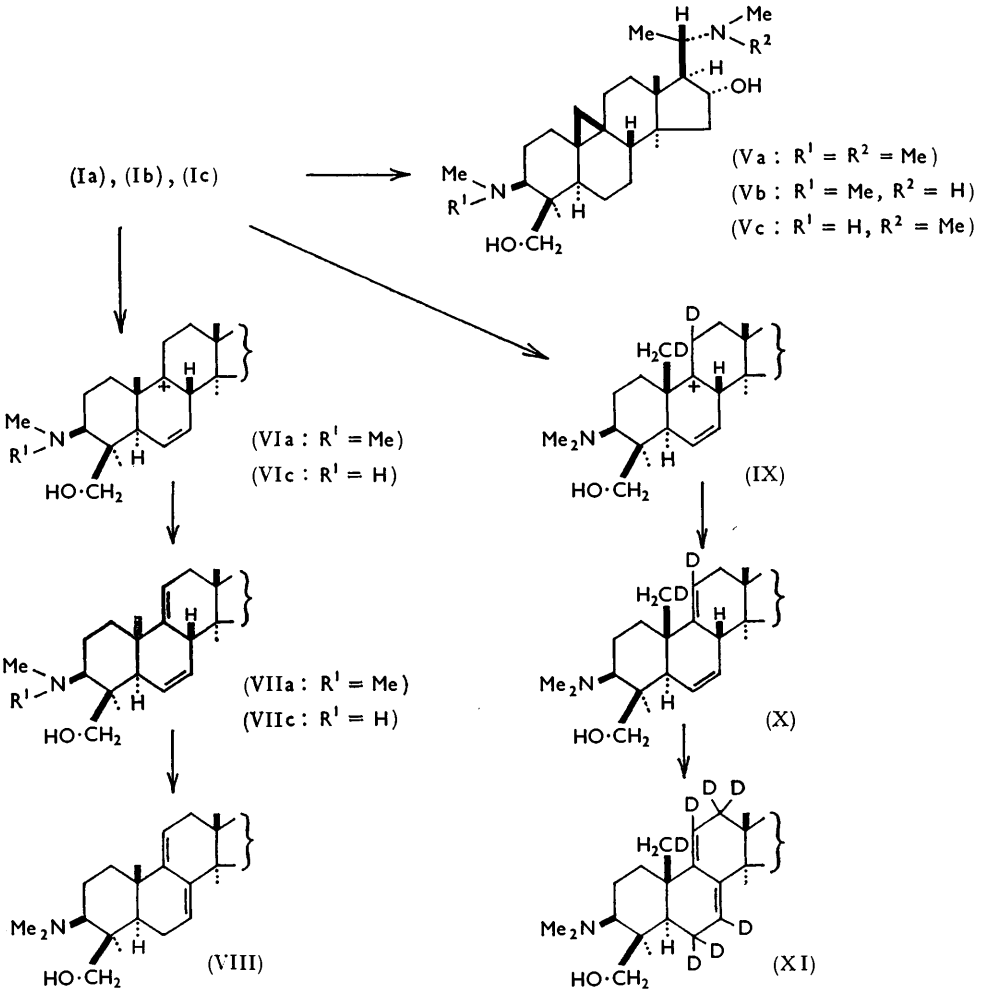
(Ia), -B (Ib), and -C (Ic), the signal of one of the cyclopropyl methylene protons occurs unusually upfield (τ 10·16), but in those of their dihydro-derivatives it moves back to the

[15] L. Dorfman, *Chem. Rev.*, 1953, **53**, 47; P. Bladon, H. B. Henbest, and G. W. Wood, *Chem. and Ind.*, 1951, 866; P. Bladon, H. B. Henbest, and G. W. Wood, *J.*, 1952, 2737.

[16] D. J. Patel, M. E. H. Howden, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1963, **85**, 3218.

normal position (see Table). This great shielding must be attributed to long-range effects [17] arising from the diamagnetic anisotropy of the double bond in ring B [see formula (A)].

The acid-catalysed scission of cyclopropane rings followed the equivalent of Markownikoff's rule.[18] Cleavage occurred between the most- and least-substituted carbon atoms in such a way as to produce the most stable carbonium ion (VI).[19] Heating of cyclomicrophylline-A (Ia) and -C (Ic) with either hydrogen chloride in chloroform or hydrochloric acid for 5—10 min. furnished the 6,9(11)-dienes (VIIa) and (VIIb) accompanied by small amounts (5—6%) of the conjugated 7,9(11)-dienes (VIIIa) and (VIIIb), respectively. In this case, formation of the corresponding 6,8-isomers was excluded by



the absence of the appropriate ultraviolet absorption bands. The n.m.r. spectra of the non-conjugated dienes (VIIa) and (VIIb) showed the presence of a new vinyl proton (τ 4·84) and a new methyl group whose signal position (τ 8·94) was confirmed by the

[17] E. Mueller, H. Kessler, H. Fricke, and H. Suhr, *Tetrahedron Letters*, 1963, 1047; E. Vogel, W. Wiedemann, W. F. Harrison, and H. Kiefer, *ibid.*, p. 673; E. J. Corey and R. L. Dawson, *J. Amer. Chem. Soc.*, 1963, **85**, 1782; R. R. Sauers and P. E. Sonnet, *Chem. and Ind.*, 1963, 786.

[18] R. A. Raphael, " Chemistry of Carbon Compounds," ed. E. H. Rodd, Elsevier, New York, 1953, vol. II, Part A, p. 26; J. F. King and P. de Mayo, " Molecular Rearrangements," ed. P. de Mayo, Interscience, New York, 1964, Part 2, p. 804.

[19] D. H. R. Barton, J. E. Page, and E. W. Warnhoff, *J.*, 1954, 2715; G. Büchi and D. M. White, *J. Amer. Chem. Soc.*, 1957, **79**, 750.

following deuteration experiments. Similar treatment of cyclomicrophylline-A (Ia) with deuterium chloride in deuterium oxide yielded the deuterated product. In its n.m.r. spectrum, two protons corresponding to a CH_2D group appear as a broad triplet peak [20] at τ 8·94, but, contrary to expectations, no absorption associated with one olefinic proton at C-11 appeared. This compound also showed the C–D stretching infrared bands [21] due to the $>C{=}CH-$ and the CH_2–D groupings at 4·41 and 4·53 μ, respectively. These spectroscopic data are in agreement with the formulation (X).* Since treatment of the diene (VIIa) with deuterium chloride in deuterium oxide under the same conditions as above did not effect such deuteration, this deuterated derivative must have been formed through the intermediate carbonium ion (IX), whose exchange of the C-11 hydrogens for deuterium was made feasible by virtue of the adjacent positively charged carbon atom.

Prolonged heating of either cyclomicrophylline-A (Ia) or the diene (VIIa) with hydrochloric acid led to the formation of a high-melting compound (VIIIa) (see also above). It showed strong ultraviolet bands at 236, 243·5, and 252 mμ (ε 14,100, 16,200, and 10,600). These triplet peaks are characteristic of those of lanosta-7,9(11)-diene-3β-ol. The n.m.r. spectrum indicated two olefinic protons at τ 4·53 and 4·79.

Decyclisation of cyclomicrophylline-A with deuterium chloride in deuterium oxide for 6 hr. produced a polydeuterated conjugated diene which we now formulate as (XI).† This compound showed the C–D stretching infrared bands at 4·42, 4·58, and 4·76 μ. N.m.r. measurements confirmed the absence of olefinic protons.

Oxidation of cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) with 1·2 molar equivalents of chromium trioxide in acetic acid yielded the unstable cyclopentanones (XIIa), (XIIb), and (XIIc), respectively. The cyclopentanone (XIIa) was also obtained by oxidation of cyclomicrophylline-A (Ia) with activated manganese dioxide in chloroform. These compounds showed a five-membered carbonyl band in the infrared spectrum. The cyclopentanones (XIIa), (XIIb), and (XIIc), on treatment with 0·1% methanolic potassium hydroxide, rapidly lost dimethylamine [in (XIIa) and (XIIc)] or methylamine [in (XIIb)] to afford a 1 : 1 mixture of *cis*- and *trans*-cyclopentenones [(XIIIa) and (XIIIa′) from (XIIa) and (XIIb); (XIIIb) and (XIIIb′) from (XIIc)]. Treatment of the cyclopentanones (XIIa), (XIIb), and (XIIc) with neutral alumina in methylene chloride solutions, furnished exclusively *cis*-isomers [(XIIIa) from (XIIa) and (XIIb); (XIIIb) from (XIIc)]. These cyclopentenones showed, besides the appropriate infrared bands, the $\alpha\beta$-unsaturated carbonyl chromophore ($\lambda_{max.}$ 244 mμ) in their ultraviolet spectra. These elimination reactions of the C-20 amino-function established the assignment of the location of the methylamino-group in cyclomicrophylline-B (Ib) and -C (Ic). The distinction between these isomeric $\alpha\beta$-unsaturated cyclopentenones was made on the basis of the n.m.r. spectra.[22] The vinyl proton signal in the *trans*-isomer (XIIIa′) occurs as a quartet at τ 4·35, whereas that of the *cis*-isomer (XIIIa) is at τ 3·51. This greater deshielding of the vinyl proton in the *cis*-isomer is due to its close proximity to the carbonyl group. Similarly, examination of the doublet due to the C-21 methyl hydrogen atoms shows that, in the *trans*-isomer, absorption occurs farther downfield (τ 7·92) relative to the *cis*-isomer (τ 8·19).

Ozonolysis of a mixture of the cyclopentenones (XIIIa) and (XIIIa′) in acetic acid furnished acetaldehyde, characterised as the 2,4-dinitrophenylhydrazone derivative. Hydrogenation of the same mixture yielded the saturated cyclopentanone (XIV).

Oxidation of cyclomicrophylline-A (Ia) with 2·4 molar equivalents of chromium trioxide in acetic acid and subsequent chromatography of the reaction product afforded, together

* The mass spectrum also supported this formulation, showing a parent peak at *m/e* 446.

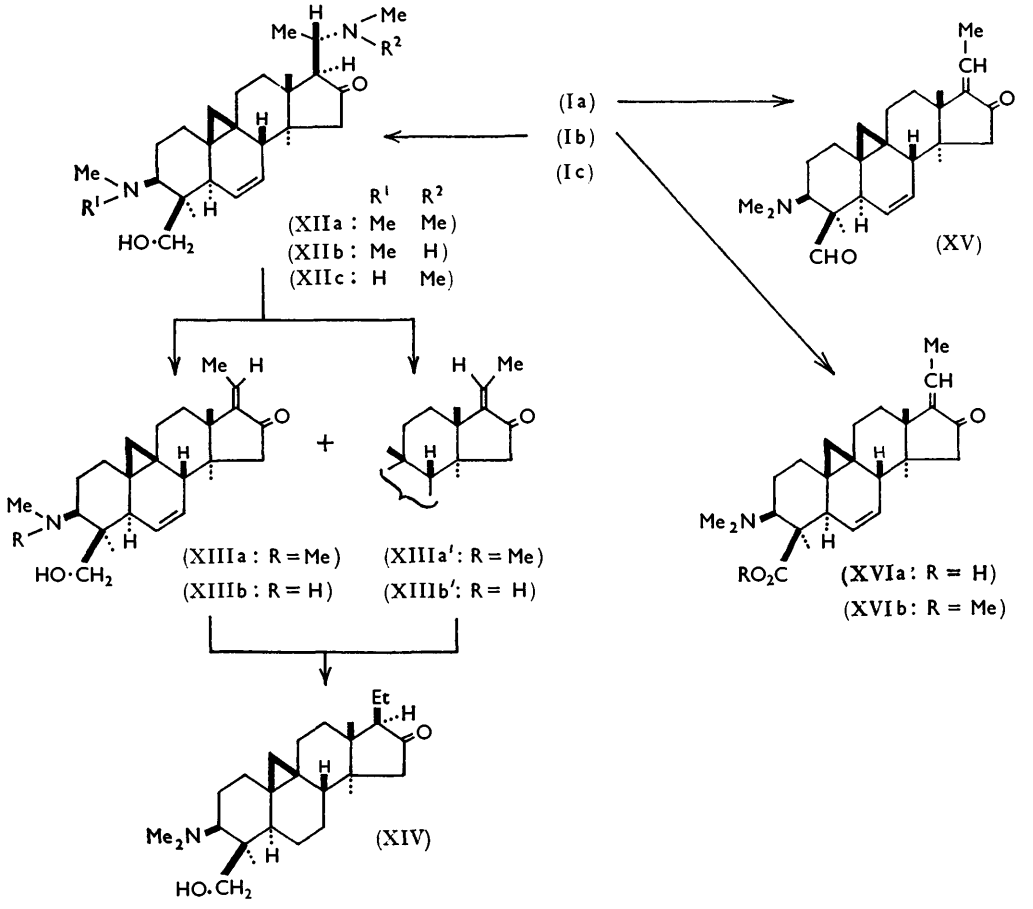
† The mass spectrum is consistent with this formulation, showing an intense parent peak at *m/e* 451.

[20] J. D. Roberts, "An Introduction to the Analysis of Spin-Spin Splitting in High-Resolution Nuclear Magnetic Resonance Spectra," Benjamin, New York, 1961, p. 9.

[21] E. G. Hoffmann, *Annalen*, 1959, **618**, 276.

[22] L. M. Jackman and R. H. Wiley, *J.*, 1960, 2881; G. Albers-Schönberg and H. Schmid, *Helv. Chim. Acta*, 1961, **44**, 1447; W. R. Benn and R. M. Dodson, *J. Amer. Chem. Soc.*, 1964, **29**, 1142.

with a mixture of compounds (XIIIa) and (XIIIa'), a mixture of the *cis*- and *trans*-keto-amino-aldehydes (XV). The presence of the aldehyde group in the latter mixture was clearly detected by its n.m.r. absorption at τ 0·54. On the other hand, the use of 3·2 molar

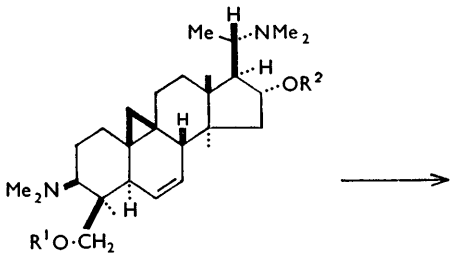


equivalents of the same reagent, followed by treatment with alkali, led to a mixture of the isomeric keto-amino-acids (XVIa). The infrared spectrum of this amino-acid mixture showed a band at 6·26 μ for the ionised carboxyl group [10b] owing to its intramolecular salt formation with the adjacent dimethylamino-group. On the same grounds, the n.m.r. signal of the 3-dimethylamino-group underwent a downfield shift to τ 7·24. On methylation with ethereal diazomethane, the keto-amino-acids (XVIa) gave the methyl esters (XVIb).

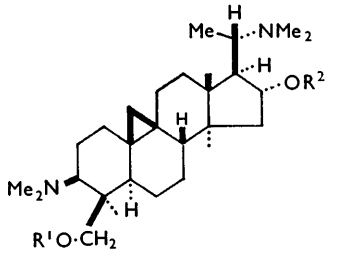
Treatment of cyclomicrophylline-A with 1·2 molar equivalents of toluene-*p*-sulphonyl chloride in pyridine afforded the mono-toluene-*p*-sulphonyl compound (XVII), whereas the use of 2·4 molar equivalents of the reagent, and heating, led to the di-toluene-*p*-sulphonyl derivative (XVIII). The compound (XVII), on hydrogenation with platinum oxide in acetic acid, yielded the dihydro-derivative (XIX), which was oxidised with chromium trioxide to the cyclopentanone (XX). The latter, on passing through a column of neutral alumina in methylene chloride solution, readily underwent elimination of dimethylamine to give the *cis*-cyclopentenone (XXI). Similar oxidation and deamination of the derivative (XVII) led to the unsaturated *cis*-cyclopentenone (XXII). Hydrogenation of both compounds yielded the same cyclopentanone (XXIII).

Benzoylation of the mono-toluene-*p*-sulphonyl derivative (XVII) with benzoyl chloride

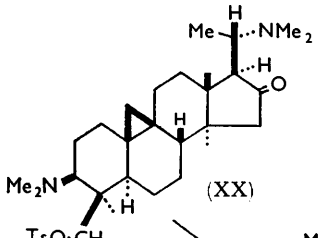
in pyridine gave the benzoate (XXIV), which is identical with that obtained by toluene-*p*-sulphonation of cyclomicrophyllidine-A (Id). This confirmed that cyclomicrophyllidine-A (Id) is the 16-*O*-benzoate of cyclomicrophylline-A (Ia). Hydrogenation of the toluene-*p*-sulphonyl benzoate (XXIV) with platinum oxide in acetic acid yielded the



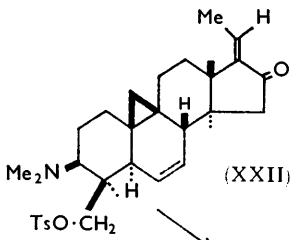
(XVII : $R^1 = SO_2 \cdot C_6H_4 \cdot Me$, $R^2 = H$)
(XVIII : $R^1 = R^2 = SO_2 \cdot C_6H_4 \cdot Me$)
(XXIV : $R^1 = SO_2 \cdot C_6H_4 \cdot Me$, $R^2 = COPh$)



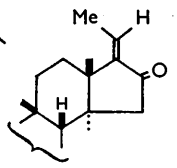
(XIX : $R^1 = SO_2 \cdot C_6H_4 \cdot Me$, $R^2 = H$)
(XXV : $R^1 = SO_2 \cdot C_6H_4 \cdot Me$, $R^2 = COPh$)



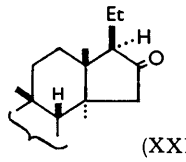
(XX)



(XXII)



(XXI)



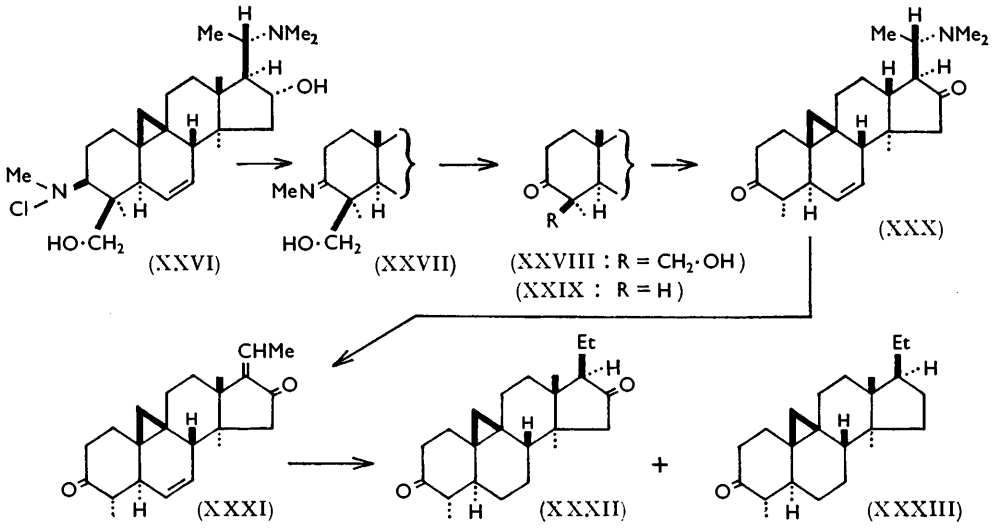
(XXIII)

dihydro-derivative (XXV), which is identical with the toluene-*p*-sulphonate of dihydrocyclomicrophyllidine-A (IIc). Thus, dihydrocyclomicrophyllidine-A (IIc) is the 16-*O*-benzoate of dihydrocyclomicrophylline-A (IIa).

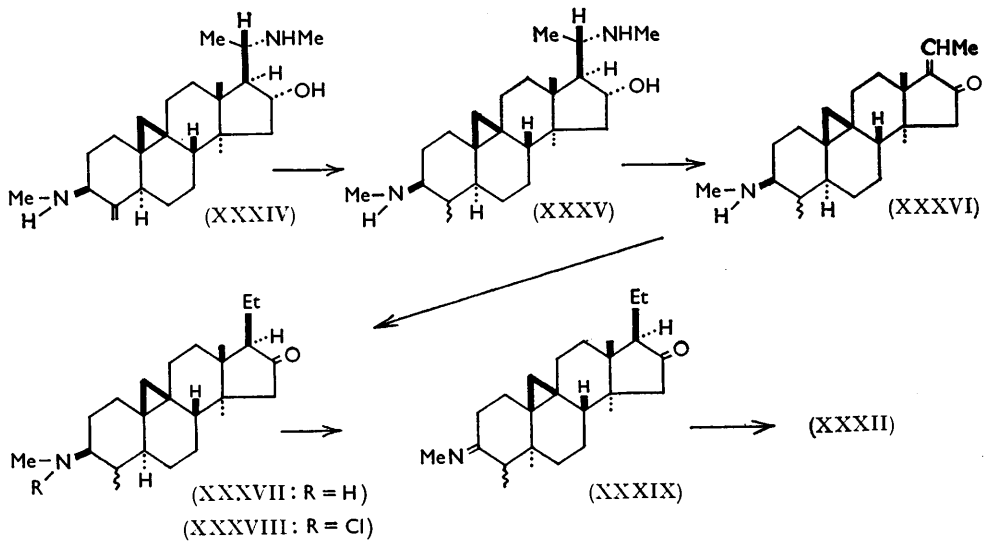
Subsequent experiments constitute a sequence of degradation reactions leading to the diketone (XXXII) which turned out to be the key intermediate for the successful structure proof. Ruschig degradation [23] of cyclomicrophylline-C (Ic) proceeded smoothly. Action of *N*-chlorosuccinimide in dry methylene chloride furnished a crystalline chloramine (XXVI). This, on dehydrohalogenation with potassium methoxide in anhydrous methanol, followed by hydrolysis with dilute sulphuric acid, led, through the intermediate (XXVII), to the ketone (XXVIII). This compound showed a six-membered-ring carbonyl band in the infrared region, and, on mild alkaline treatment, underwent a retro-aldol type reaction [24] to afford formaldehyde and the nor-ketone (XXIX). The n.m.r. spectrum of the latter indicated the absence of the protons of a hydroxymethylene group. Chromium trioxide oxidised the nor-ketone (XXIX) to the amino-diketone (XXX). The latter, without isolation, was directly treated with mild alkali, to furnish a mixture of the isomeric nitrogen-free cyclopentenones (XXXI). Hydrogenation of this with platinum oxide in acetic acid caused the uptake of a little more than two molar equivalents of hydrogen, to yield a saturated diketone (XXXII) and a monoketone (XXXIII) in a ratio of 9 : 1. The former had the infrared bands of both five- and six-membered-ring carbonyl groups at

[23] H. Ruschig, W. Fritsch, J. Schmidt-Thomé, and W. Haede, *Chem. Ber.*, 1955, **88**, 883; L. Labler and F. Sorm, *Coll. Czech. Chem. Comm.*, 1960, **24**, 2975.
[24] D. H. R. Barton and P. de Mayo, *J.*, 1953, 887.

5·77 and 5·84 μ, respectively. The absence of a five-membered-ring ketone band in the infrared spectrum of the latter indicates the reduction of the carbonyl group at C-16.



The diketone (XXXII) is a 4,14-methylated pregnane derivative containing a cyclopropane ring between C-9 and C-10, and was expected to be derivable from cyclobuxin-D.*



As an extension of our present work, we have also examined the alkaloidal constituents of *B. microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino. This variety has provided us with a reasonable amount of cyclobuxine-D,† together with some other alkaloids, which enabled us to carry out the following degradation experiments. Hydrogenation of cyclobuxine-D (XXXIV) gave the dihydro-derivative (XXXV), and the latter was oxidised with chromium trioxide followed by mild alkali to a mixture of the

* According to the convention of the nomenclature of Buxus alkaloids, cyclobuxine (see ref. 5) corresponds to cyclobuxine-D.

† The isolation of cyclobuxine-D was carried out by Mr. M. Hasegawa of this laboratory. We are indebted to Dr. Kupchan for his kind donation of a sample of cyclobuxine-D for direct comparison.

cis- and *trans*-isomeric cyclopentenones (XXXVI), which on hydrogenation yielded the cyclopentanone (XXXVII). This was converted into the chloramine (XXXVIII), and the latter, on dehydrohalogenation followed by mild acid hydrolysis, led, through the methylimine (XXXIX), to the diketone which was identical with the diketone (XXXII).

Since cyclobuxine-D has been related to a known triterpenoid, cycloeucalenol,[5a] the foregoing result not only strongly supports a skeletal structure for this group of alkaloids, but also establishes the absolute configuration at six of the eleven asymmetric centres (5α-H, 8β-H, 9β,10β-cyclopropyl, 13β-Me, and 14α-Me) in their molecule. The position of the double bond in these alkaloids is already clear from the degradative reactions and from the ultraviolet and n.m.r. data so far presented. Additional evidence came from the rotatory dispersion of cyclomicrophylline-A (Ia), for which a strong negative plain curve [25] was obtained. Furthermore, the changes of molecular rotation for conversion of cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) into their dihydro-derivatives were found to be −574°, −384°, and −397°, respectively. These extremely large negative molecular rotation increments are typical of those (average −506°) observed with the steroidal 5α-H-6-enes.[26]

The β-orientation of the 4-hydroxymethylene group in cyclomicrophylline-A (Ia), -B (Ib), and -C (Ic) is based on their n.m.r. spectra. In dihydrocyclomicrophylline-A (IIa) and its *O*-diacetate, two methylene protons of the CH_2·OH and CH_2·OAc groupings occur as a quadruplet (AB system) centred at τ 6·39 and 5·93, respectively. These signal positions are characteristic of the axial hydroxymethylene group.[27]

Comparison of the infrared spectrum of cyclomicrophylline-A (Ia) with those of compounds (XIIIa) and (XVII) in carbon tetrachloride showed that the absorptions due to the 4-primary hydroxyl and the 16-secondary hydroxyl groups appear as broad intense bands at 3·15 and at 2·90 μ, respectively. These bands do not vary with concentration, and therefore they must arise from intramolecular bonded hydroxyl. Hydrogen bonding between hydroxyl and amino-groups has been demonstrated in a number of amino-alcohols.[28] Burford *et al.*[29] observed that, in 3-aminocyclohexanols, only *cis*-isomers are involved in intramolecular hydrogen bonding. Thus, the 3-dimethylamino-group in these compounds must be *cis* to the β-oriented primary hydroxyl at C-4 and have the equatorial conformation. This assumption was further confirmed by the following Hofmann degradation reactions.

Haworth *et al.*[30] reported that, in the steroidal amines, the 3α-(axial) trimethyl-ammonium salts undergo smooth *E*2 type deamination on Hofmann degradation, whereas the corresponding 3β-(equatorial) epimers resist this type of elimination to recover the parent tertiary amines through the scheme $HO \rightarrow Me\overset{+}{N}Me_2$. Action of methyl iodide on cyclomicrophylline-A (Ia) in acetone at room temperature resulted in the methylation of the 3-dimethylamino-group, and the monomethiodide (XL) was obtained. This methiodide, under vigorous conditions as employed by Corey *et al.*,[31] failed to undergo Hofmann degradation, and gave a 90% recovery of the parent compound (Ia) and no detectable olefin. This behaviour upon Hofmann decomposition favours the assignment of the β-configuration (equatorial conformation) to the 3-dimethylamino-group. Heating

[25] C. Djerassi, W. Closson, and A. E. Lippman, *J. Amer. Chem. Soc.*, 1956, **78**, 3163; G. G. Lyle and R. E. Lyle, "Determination of Organic Structures by Physical Methods," ed. F. C. Nachod and W. D. Phillips, Academic Press, New York, 1962, vol. 2, p. 13.

[26] (*a*) L. F. Fieser and M. Fieser, "Steroids," Reinhold, New York, 1959, p. 177; (*b*) W. Klyne, "Determination of Organic Structures by Physical Methods," ed. E. A. Braude and F. C. Machod, Academic Press, New York, 1955, vol. 1, p. 111.

[27] E. Wenkert and P. Beak, *Tetrahedron Letters*, 1961, 358; R. C. Cambie and L. N. Mander, *Tetrahedron*, 1962, **18**, 465; A. Gaudemer, J. Polonsky, and E. Wenkert, *Bull. Soc. chim. France*, 1964, 407.

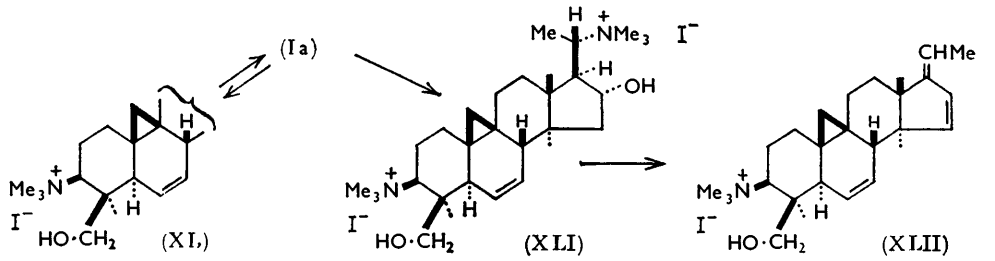
[28] E. D. Bergman, E. Gil-Av, and S. Pinchas, *J. Amer. Chem. Soc.*, 1953, **75**, 68; T. Kanzawa, *Bull. Chem. Soc. Japan*, 1956, **29**, 398, 479, 604.

[29] R. R. Burford, F. R. Hewgill, and P. R. Jefferies, *J.*, 1957, 2937.

[30] R. D. Haworth, J. McKenna, and R. G. Powell, *J.*, 1953, 1110; R. Ledger, J. McKenna, and P. B. Smith, *Tetrahedron Letters*, 1963, 1433.

[31] E. J. Corey and E. W. Cantrall, *J. Amer. Chem. Soc.*, 1959, **81**, 1745.

of cyclomicrophylline-A (Ia) with methyl iodide in methanol furnished, in small amount, the dimethiodide (XLI), which under the same conditions as above was decomposed to trimethylamine and a non-volatile fraction. The latter, after chromatography on alumina and subsequent methylation, led to the methiodide (XLII).



Evidence for the β-configuration of the 17-side-chain in the 16-ketones (XIIa) and (XXIII) was secured from their optical rotatory dispersion curves. Both compounds showed strong negative Cotton effects which are typical of the 16-keto-steroids with 13β,14α,17β-configuration.[32] Provided that, in the chromium trioxide–acetic acid or manganese dioxide–chloroform oxidation of cyclomicrophylline-A (Ia) to the 16-ketone (XIIa), epimerisation * at C-17 did not occur, the same configuration (β) may be assigned to the 17-substituent of the parent 16-hydroxy compound.†

The α-orientation of the 16-hydroxy-group was suggested by the changes in molecular rotation observed upon toluene-*p*-sulphonation and benzoylation of cyclomicrophylline-A (Ia) and its dihydro-compound (IIa). The M_D increment for toluene-*p*-sulphonation of compound (XVII) to compound (XVIII) is −166°, and those for benzoylation of compound (Ia) to compound (Id), compound (XVII) to compound (XXIV), compound (IIa) to compound (IIc), and compound (XIX) to compound (XXV), are −452°, −473°, −347°, and −102°, respectively. These strong negative molecular rotational changes upon esterification are in accord with a 16α-configuration.[33]

The configuration of the remaining asymmetric centre at C-20 was established by the pyrolytic *cis*-elimination reaction [34] of the amino-ketone (XIIa). Heating of this amino-ketone at 140—150° under reduced pressure caused the elimination of dimethylamine to afford the *cis*-αβ-unsaturated cyclopentenone (XIIIa), which had also been obtained by treatment of this same amino-ketone with neutral alumina in methylene chloride solution. The mechanism for these *cis*-eliminations is outlined in the Figure. These results allow the assignment of the α-configuration to the 20-dimethylamino-group, which is in accord with biogenetic precedent.[35] It is known that the presence of electron-attracting

* It is pertinent to mention that in this oxidation no trace of the corresponding 16-ketone with a 17α-side-chain was formed.

† Kupchan *et al.*, reported that the n.m.r. signal for the 16β-proton in cyclobuxine was split by one nearly opposing proton (J = 9·5 c./sec.), and examination of its molecular models shows that this proton could only occupy the 17α-position (see ref. 5*a*). This argument is weak since coupling constants for protons on adjacent carbon atoms can vary considerably according to the nature of the other substituents borne by the carbon atoms, and furthermore it is not known to what extent the strain inherent in ring D affects coupling constant magnitude (see A. D. Cross and P. Crabbé, *J. Amer. Chem. Soc.*, 1964, **86**, 1221). It should be noted, however, that the n.m.r. spectrum of cyclomicrophylline-A at 100 Mc, with decoupling experiments, found the 16β-proton at τ 5·92 as a septet ($J_{16\beta-15\alpha}$ = 2·5 c./sec., $J_{16\beta-15\beta}$ = 6·8 c./sec., and $J_{16\beta-17\alpha}$ = 9·6 c./sec.) and these coupling constants are in good agreement with those described for cyclobuxine. We are indebted to Dr. L. J. Durham of Stanford University for this information.

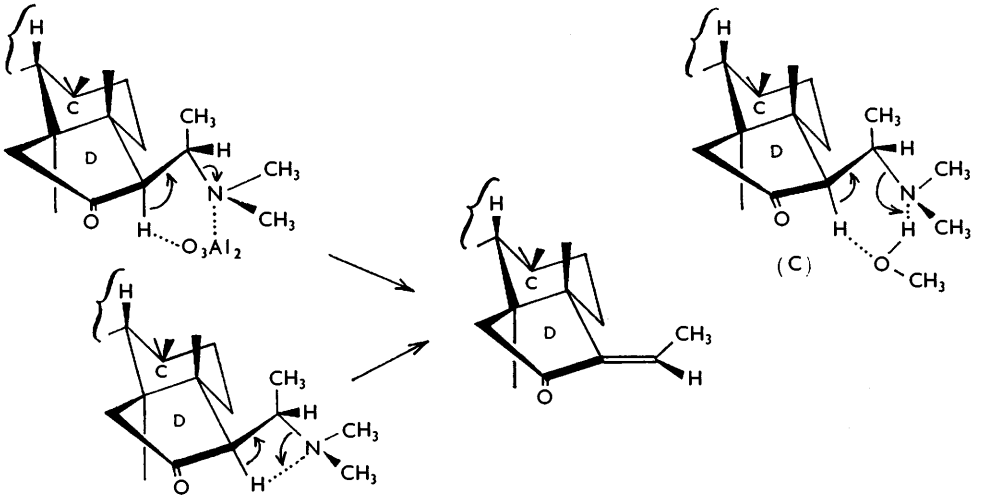
[32] (*a*) C. Djerassi, " Optical Rotatory Dispersion," McGraw-Hill, New York, 1960, pp. 44, 57; (*b*) C. Djerassi, R. Riniker, and B. Riniker, *J. Amer. Chem. Soc.*, 1956, **78**, 6362.

[33] D. K. Fukushima and T. F. Gallagher, *J. Amer. Chem. Soc.*, 1951, **73**, 196; ref. 26*a*, p. 179.

[34] D. J. Cram, " Steric Effects in Organic Chemistry," ed. M. S. Newman, Wiley, New York, 1956, p. 304.

[35] R. Goutarel, *Tetrahedron*, 1961, **14**, 126; O. Jeger and V. Prelog, " The Alkaloids," ed. R. H. F. Manske, Academic Press, New York, 1960, vol. VII, pp. 319—342.

groups has a profound influence on the rate and course of elimination reactions in either open-chain [36] or cyclic compounds.[37] The amino-ketone (XIIa), when heated either with methanol alone or with mild alkali, underwent an *E*2 type of *cis*-elimination in competition with the *trans*-bimolecular elimination, to yield a mixture of *cis*- and *trans*-cyclopentenones in a ratio of 3 : 1 or 1 : 1,* respectively. In the absence of added alkali, methanol seems to act as a base, as illustrated in formula (C). In the above experiments, formation of the *trans*-isomer (XIIIa′) through the base-catalysed equilibration from the initially formed



cis-isomer (XIIIa) was excluded [38] since it was demonstrated that the *cis*-compound, under the same conditions of alkalinity, did not isomerise to the *trans*-compound. Refluxing of the *cis*-isomer (XIIIa) with 10% methanolic alkali, however, gave a 1 : 1 equilibrium mixture [39] of both isomers. The preponderance [37] of *cis*-elimination in the above base-catalysed bimolecular elimination reactions probably results from the increased acidity of the hydrogen (C-17) α to the carbonyl group at C-16.

Dihydrocyclomicrophylline-F (IIb) is an alkaloid which is very difficultly soluble in the usual organic solvents and differs from dihydrocyclomicrophylline-A (IIa) only in that it contains two instead of four *N*-methyl groups (see Table). The presence of a primary amino-group was readily demonstrated by the formation of an *N*-isopropylidene derivative. Acetylation of this alkaloid with acetic anhydride and pyridine gave the *N*-acetyl *O*-diacetate (XLIII), which had the infrared bands at 6·00 and 6·61 μ, characteristic of the NHAc grouping. This compound was hydrolysed by alkali to the *N*-acetyl derivative (XLIV). When heated with acetone, dihydrocyclomicrophylline-F (IIb) formed the *N*-isopropylidene derivative (XLV), which was hydrolysed by acetic acid to the parent compound. Acetylation of the *N*-isopropylidene derivative (XLV), and subsequent alkaline hydrolysis, furnished the *N*-acetyl derivative (XLIV), whereas hydrogenation with platinum oxide in acetic acid gave the *N*-isopropyl derivative (XLVI). On methylation with methyl iodide in acetone at room temperature, the *N*-isopropylidene derivative (XLV) yielded the monomethiodide (XLVII), and the latter, on treatment with silver oxide followed by pyrolysis, furnished dihydrocyclomicrophylline-C (Vc). On the other

* This ratio was determined on the basis of the signal areas of the vinyl proton resonances of *cis*- and *trans*-isomers.

[36] C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell Univ. Press, Ithaca, 1953, p. 443.

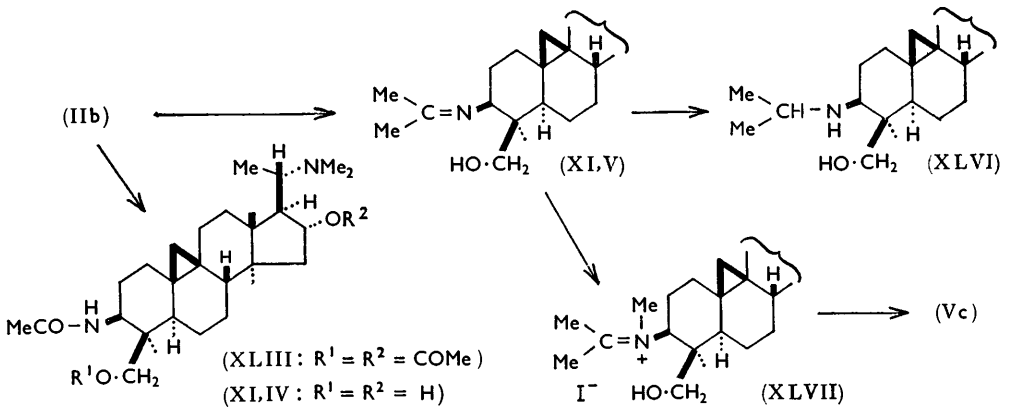
[37] F. G. Bordwell and R. J. Kern, *J.*, 1955, 1141; F. G. Bordwell and M. L. Peterson, *J.*, 1955, 1145; J. Weinstock, R. G. Pearson, and F. G. Bordwell, *J. Amer. Chem. Soc.*, 1954, **76**, 4748.

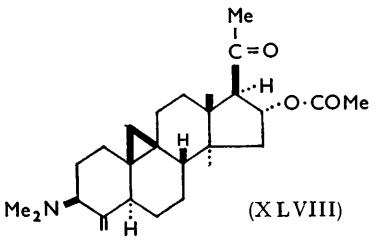
[38] Cf. T. Nakano and S. Terao, *Tetrahedron Letters*, 1964, 1050.

[39] C. Beard, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Amer. Chem. Soc.*, 1964, **86**, 270; G. Büchi and N. C. Yang, *Helv. Chim. Acta*, 1955, **28**, 1338.

hand, heating of either (XLV) or (XLVII) with methyl iodide in methanol, followed by similar treatment of the methylation product, led to dihydrocyclomicrophylline-A (IIa). These transformation reactions provided unambiguous proof for the assignment of the primary amino-group in dihydrocyclomicrophylline-F.

Cyclomicrobuxine, unlike the other seven alkaloids of this group, contains only one nitrogen atom which is present as a dimethylamino-group, and no primary hydroxyl function, as is evident from its molecular formula and its n.m.r. spectrum (see Table). The signals which occur at τ 5·35 and 5·04, at τ 7·86, and at τ 5·20, showed the presence of one terminal methylene, one methyl ketone, and one secondary hydroxyl group. The assign-





ment of these functional groups was also supported by the infrared absorption bands at 2·92 (OH), 5·89 (methyl ketone), 6·07 and 11·12 μ (terminal methylene). By analogy with cyclobuxine-D,[5] and on biogenetic grounds, we assumed that this alkaloid has structure (III). Acetylation of cyclomicrobuxine gave the *O*-acetate (XLVIII), whose n.m.r. spectrum showed a broad triplet centred at τ 4·40 due to a proton adjacent to the secondary acetate grouping. The n.m.r. signal of the 17-hydrogen either in cyclomicrobuxine (III) or in its *O*-acetate (XLVIII) appears as a doublet (J = 7 c./sec.) centred at τ 6·92 or at τ 6·82, respectively. This is attributed to the coupling with one adjacent proton, possibly at C-17. The optical rotatory dispersion curve of cyclomicrobuxine (III) exhibited a positive Cotton effect,[40] suggesting the β-configuration for the 17-methyl ketone grouping. The α-orientation of the 16-hydroxyl group was assigned on the basis of the difference (ΔM_D −39°)[33] in molecular rotation between cyclomicrobuxine (III) and its *O*-acetate (XLVIII). The establishment of the structure of this last alkaloid will be presented elsewhere if more material is available.

The above eight compounds apparently belong to a new class of alkaloids, which have a 4,14-methylated pregnane skeleton containing a cyclopropane ring. The first two groups, (I)—(II), are fully methylated at the 4-position, which is typical of triterpenoids, and the last alkaloid, (III), possesses a substitution pattern which is intermediate in the

[40] R. Neher, P. Desaulles, E. Vischer, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, 1958, **41**, 1667; ref. 32*a*, p. 128.

biogenetic scheme between lanosterol- and cholesterol-type steroids. The unsaturation pattern of the alkaloids of series (I) is particularly interesting since no examples of naturally occurring triterpenes or steroids with the double bond between C-6 and C-7 have been hitherto identified.

Experimental

Melting points were taken on a Kofler hot-stage apparatus. Infrared spectra were recorded on a Hitachi model EPI-S spectrometer for potassium bromide discs unless otherwise specified. Ultraviolet spectra were determined on a Shimazu model SV-50 spectrophotometer for 95% ethanol solutions. Rotations were measured on a Kreis polarimeter " 0·01° " for chloroform solutions (0·8—2·5%, 10—25°). N.m.r. spectra were obtained for deuterochloroform solutions in a Varian A-60 spectrometer, with tetramethylsilane as internal standard. Optical rotatory dispersion curves were taken on a Rudorf automatically recording spectropolarimeter for methanol solutions. Alumina and silica gel for chromatography refer to Woelm neutral alumina, activity grade I, and Mallinckrodt silicic acid, respectively, unless otherwise stated. Thin-layer chromatograms were prepared on silica gel G and developed with methylene chloride–ethylamine (usually, 100 : 2—5, v/v); the spots were observed by spraying either with Dragendorff's reagent (*Chem. Abs.*, 1953, 47, 4552) or with 10% sulphuric acid and heating at 150—200°. All extracts were dried over anhydrous sodium sulphate before evaporation. Identity of samples was established by mixed melting points, infrared (KBr) and n.m.r. spectra, and optical rotations, except where stated.

Isolation of Cyclomicrophylline-A (Ia), *-B* (Ib), *-C* (Ic), *and Dihydrocyclomicrophylline-A* (IIa).—The air-dried leaves and twigs (68 kg.) of *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino, collected in Kagoshima, were ground and extracted thrice with boiling 95% ethanol (250 l.) for 6 hr. The ethanolic extracts were combined and concentrated *in vacuo* to 10 l. 3% Aqueous citric acid solution (40 l.) was added, and the mixture was shaken with methylene chloride to remove acidic and neutral substances. The aqueous layer was made alkaline with ammonia and extracted exhaustively with methylene chloride. Washing of the methylene chloride extracts with water, drying, and evaporation *in vacuo* yielded a viscous basic material (150 g.). Part (45·2 g.) of this material, dissolved in benzene, was chromatographed on 10% deactivated alumina (1·2 kg.) which had been treated with a solution of 70% aqueous ethylamine (120 ml.) in benzene (1·2 l.) and allowed to stand at room temperature overnight. Elution was effected with benzene, and the following results were obtained (volume of eluate and weight of product): fractions 1—4, 3·2 l., 9·7 g.; fractions 5—10, 4·8 l., 11·2 g.; fractions 11—16, 4·8 l., 3·6 g.; fractions 17—24, 6·4 l., 2·5 g. Elution with methylene chloride–methanol (6 : 1) yielded 16·9 g. of material.

Fractions 5—10 were combined and crystallised from acetone to yield *cyclomicrophylline-A* (Ia) (1·64 g.), m. p. 232—233°, $[\alpha]_D$ −92°, $\lambda_{max.}$ 2·90 (OH), 6·04 (C=C), and 14·28 μ (out-of-plane deformation vibration of *cis*-disubstituted ethylene), $\lambda_{max.}$ 204 mμ (ε 5800). Rotatory dispersion: $[\phi]_{700}$ −365°, $[\phi]_{589}$ −408°, $[\phi]_{350}$ −1780°, and $[\phi]_{250}$ −7260° (Found: C, 75·8; H, 10·9; N, 6·3; O, 7·3. $C_{28}H_{48}N_2O_2$ requires C, 75·6; H, 10·9; N, 6·3; O, 7·2%).

Dihydrocyclomicrophylline-A (IIa) (240 mg.), isolated from the mother-liquor of the alkaloid (Ia), had m. p. 271—272° (from acetone), $[\alpha]_D$ +37°, $\lambda_{max.}$ (Nujol) 2·95 μ (OH), no selective ultraviolet absorption down to 190 mμ (Found: C, 75·25; H, 11·35; N, 6·35; O, 7·2. $C_{28}H_{50}N_2O_2$ requires C, 75·3; H, 11·3; N, 6·25; O, 7·15%).

Acetic anhydride–pyridine acetylation of dihydrocyclomicrophylline-A, and chromatography of the product on alumina, gave the O-*diacetate*, m. p. 156—157° (from hexane), $[\alpha]_D$ +8·3°, $\lambda_{max.}$ 5·80 μ (acetate), τ 9·45 and 9·67 (2H, doublets, *J* 4 c./sec., cyclopropane), 9·23, 9·01, and 8·90 (9H, quaternary methyl), 8·02 and 7·93 (6H, acetyl), 7·85 and 7·75 (12H, *N*-methyl), 6·13 and 5·72 (2H, quadruplet centred at 5·93, *J* 11 c./sec., CH_2OAc), and 4·88 (1H, multiplet, >C*H*OAc) (Found: C, 72·35; H, 10·35; N, 5·2. $C_{32}H_{54}N_2O_4$ requires C, 72·4; H, 10·25; N, 5·3%).

Cyclomicrophylline-B (Ib) (608 mg.), obtained from fractions 12—16, had m. p. 251—252° (from acetone), $[\alpha]_D$ −65°, $\lambda_{max.}$ 2·97 (OH), 3·05(NH), and 6·04 and 14·40 μ (*cis*-disubstituted ethylene), $\lambda_{max.}$ 204 mμ (ε 5780) (Found: C, 75·55; H, 10·95; N, 6·45; O, 7·35. $C_{27}H_{46}N_2O_2$ requires C, 75·3; H, 10·75; N, 6·5; O, 7·45%).

Fractions 19—24 were combined and crystallised from acetone, to afford *cyclomicrophylline-C* (Ic) (870 mg.), m. p. 282—283°, $[\alpha]_D$ −40°, $\lambda_{max.}$ 2·90 (OH), 3·06 (NH), and 6·06 and

14·32 μ (*cis*-disubstituted ethylene), $\lambda_{max.}$ 204 mμ (ε 5680) (Found: C, 75·55; H, 10·75; N, 6·55; O, 7·45. $C_{27}H_{46}N_2O_2$ requires C, 75·3; H, 10·75; N, 6·5; O, 7·45%).

Isolation of Cyclomicrophyllidine-A (Id), *Dihydrocyclomicrophyllidine-A* (IIc), *and Dihydrocyclomicrophylline-F* (IIb).—Fractions 1—4 (see above) were combined, dissolved in benzene, and chromatographed on 5% deactivated alumina (300 g.) (5 ml. of 70% aqueous ethylamine per 100 g. of alumina). Elution with benzene gave the following fractions: 1—5, 1·5 l., 6·73 g.; 6—11, 1·8 l., 2·48 g. Further elution with methylene chloride afforded a material (0·60 g.).

Thin-layer chromatography revealed that fractions 1—5 were a mixture of cyclomicrophyllidine-A (Id) and dihydrocyclomicrophyllidine-A (IIc) in a ratio of about 2 : 1. The separation of each individual alkaloid was made possible by careful chromatography of this mixture on silica gel G (Merck) using methylene chloride as a solvent.

Cyclomicrophyllidine-A (Id), amorphous, $[\alpha]_D$ −160°, $\lambda_{max.}$ ($CHCl_3$) 2·97 (OH), and 5·82 and 6·24 μ (benzoate), $\lambda_{max.}$ 265, 273, and 280 mμ (ε 220, 240, and 200) (Found: C, 76·5; H, 9·65; N, 5·2. $C_{35}H_{52}N_2O_3$ requires C, 76·6; H, 9·55; N, 5·1%).

Dihydrocyclomicrophyllidine-A (IIc), amorphous, $[\alpha]_D$ −33°, $\lambda_{max.}$ ($CHCl_3$) 2·97 (OH), and 5·82 and 6·24 μ (benzoate), $\lambda_{max.}$ 265, 273, and 280 mμ (ε 220, 240, and 200) (Found: C, 76·4; H, 9·75; N, 5·2. $C_{35}H_{54}N_2O_3$ requires C, 76·3; H, 9·9; N, 5·1%).

Dihydrocyclomicrophylline-F (IIb) (1·8 g.) was obtained from fractions 6—11. It was very sparingly soluble in the usual organic solvents except acetone. Recrystallisation was effected from methanol, m. p. 260°, $[\alpha]_D$ +4·6°, $\lambda_{max.}$ 2·98 (OH), 3·04 (NH stretching), and 6·00 and 6·30 μ (NH deformation) (Found: C, 74·65; H, 11·25; N, 6·7; O, 7·75. $C_{26}H_{46}N_2O_2$ requires C, 74·6; H, 11·1; N, 6·7; O, 7·65%).

Feigl's test for a secondary amine: cyclomicrophylline-A (Ia), negative; cyclomicrophylline-B (Ib) and -C (Ic), positive (blue-violet colour).

All seven of the alkaloids isolated above were homogeneous on thin-layer chromatography under a variety of conditions.

Acetylation of Cyclomicrophilline-A, -B, and -C.—(*a*) The compound (Ia) (100 mg.) in anhydrous pyridine (3 ml.) was treated with acetic anhydride (1 ml.) and left at room temperature overnight. The excess of reagent was decomposed with methanol and the solvent was removed *in vacuo*. The residue was taken up in methylene chloride, and the organic layer was washed with 3% aqueous sodium carbonate and water, dried, and evaporated *in vacuo*. The product, on chromatography in benzene on alumina (2 g.) deactivated with 5% (w/w) of water, furnished the O-*diacetate* (IVa) (125 mg.) as an amorphous compound, $[\alpha]_D$ −100°, $\lambda_{max.}$ ($CHCl_3$) 5·81 μ (acetate), τ 10·14 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·28 (3 H) and 9·11 (6H) (quaternary, methyl), 8·06 and 8·00 (6H, acetyl), 7·85 and 7·79 (12H, *N*-methyl), 6·00 and 5·72 (2H, quadruplet centred at 5·86, *J* 11 c./sec., CH_2OAc), 4·93 (1H, multiplet, >*CH*OAc), and 4·58 (2H, olefinic protons) (Found: C, 72·35; H, 10·15; N, 5·15. $C_{32}H_{52}N_2O_4$ requires C, 72·7; H, 9·9; N, 5·3%).

(*b*) The compound (Ib) (100 mg.) was acetylated with acetic anhydride–pyridine (1 : 4; 5 ml.) at room temperature overnight. After removal of the solvent *in vacuo*, the residue was diluted with 3% hydrochloric acid and the product was extracted with methylene chloride. The extract was washed with 3% aqueous sodium carbonate and water, dried, and evaporated *in vacuo*, to afford the N′-*acetyl* O-*diacetate* (IVb) as prisms (124 mg.), m. p. 202—203° (from acetone–hexane), $[\alpha]_D$ −151°, $\lambda_{max.}$ 5·77 (*O*-acetate) and 6·11 μ (*N*-acetyl), τ 10·12 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·25, 9·10, and 8·90 (9H, quaternary methyl), 8·80 (3H, doublet, *J* 7 c./sec., tertiary methyl), 8·01, 8·00, and 7·96 (9H, *N*- and *O*-acetyl), 7·75 (6H) and 7·20 (3H) (*N*-methyl), 5·98 and 5·66 (2H, quadruplet centred at 5·82, *J* 11 c./sec., CH_2OAc), 5·00 (1H, multiplet, >*CH*OAc), and 4·52 (2H, olefinic protons) (Found: C, 71·3; H, 9·65; N, 5·0. $C_{33}H_{52}N_2O_5$ requires C, 71·2; H, 9·4; N, 5·05%).

(*c*) The compound (Ic) (100 mg.) was treated with acetic anhydride–pyridine (1 : 4; 5 ml.) at room temperature overnight. The product, isolated as above, was purified by filtration through an alumina column in benzene solution. The N-*acetyl* O-*diacetate* (IVc) (116 mg.) was obtained as an amorphous compound, $[\alpha]_D$ −132°, $\lambda_{max.}$ ($CHCl_3$) 5·78 (*O*-acetate) and 6·11 μ (*N*-acetyl,) τ 10·12 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·12 (3H) and 9·06 (6H) (quaternary methyl), 9·18 (3H, doublet, *J* 7 c./sec., tertiary methyl), 8·05, 8·04, and 7·92 (9H, *N*- and *O*-acetyl), 7·87 (6H) and 7·12 (3H) (*N*-methyl), 6·20 (2H, singlet, CH_2OAc), 4·92 (1H, multiplet, >*CH*OAc), and 4·50 (2H, olefinic protons) (Found: C, 71·25; H, 9·5; N, 5·3. $C_{33}H_{52}N_2O_2$ requires C, 71·2; H, 9·4; N, 5·05%).

Benzoylation of Cyclomicrophylline-A.—The compound (Ia) (100 mg.) in anhydrous pyridine (3 ml.) was treated with benzoyl chloride (0·5 ml.) and left at room temperature overnight. After dilution with water, the mixture was made alkaline with ammonia and the product was extracted with methylene chloride. Washing of the extract with water, drying, and evaporation *in vacuo*, left a viscous residue which was chromatographed in benzene on alumina (4 g.). The O-*dibenzoate* (IVd) (136 mg.) was obtained as an amorphous compound, m. p. 105—113°, $[\alpha]_D$ −94°, $\lambda_{max.}$ ($CHCl_3$) 5·80, 6·20, and 6·27 μ (benzoate), τ 10·09 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·16 (3H) and 8·99 (6H) (quaternary methyl), 8·96 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·91 (6H) and 7·71 (6H) (*N*-methyl), 5·86 and 5·29 (2H, quadruplet centred at 5·58, *J* 12 c./sec., CH_2OBz), 4·59 (1H, multiplet, >$CHOBz$), 4·45 (2H, olefinic protons), and 2·40—2·90 (6H) and 1·85—2·16 (4H) (benzenoid protons) (Found: C, 77·5; H, 8·75; N, 4·5. $C_{42}H_{56}N_2O_4$ requires C, 77·25; H, 8·65; N, 4·3%).

The *O*-dibenzoate (IVd) formed a *dipicrate*, m. p. 253—254° (decomp.) (from acetone) (Found: C, 56·65; H, 6·0; N, 10·15. $C_{54}H_{62}N_8O_{18},2H_2O$ requires C, 56·55; H, 5·8; N, 9·75%).

Hydrolysis of Cyclomicrophylline-A O-*Diacetate* (IVa).—(a) *With aqueous methanol.* The diacetate (100 mg.) was heated under reflux with 5% aqueous methanol (10 ml.) for 1 hr. The mixture was poured into water, made alkaline with solid sodium carbonate (100 mg.), and the product was extracted with methylene chloride. Washing of the organic layer with water, drying, and evaporation *in vacuo* furnished the parent compound (Ia) (78 mg.).

(b) *With aqueous methanolic potassium hydroxide or sodium acetate.* In one experiment, the diacetate (100 mg.) in 1% methanolic potassium hydroxide (20 ml.) was set aside at room temperature overnight. In another experiment, the diacetate (100 mg.) was refluxed with a solution of sodium acetate (100 mg.) in methanol (10 ml.) containing a minimum amount of water for 1 hr. In either case, working up of the product in the usual way afforded the parent compound (Ia) (80—84 mg.).

Hydrolysis of Cyclomicrophylline-B N′-*Acetyl* O-*Diacetate* (IVb) *and Cyclomicrophylline-C* N-*Acetyl* O-*Diacetate* (IVc).—(a) *With aqueous methanolic potassium hydroxide.* Samples (100 mg.) of each of the triacetates (IVb) and (IVc) were hydrolysed with 3% methanolic potassium hydroxide (30 ml.) by heating on a boiling-water bath for 0·5 hr. Usual working up gave the parent compound (Id) or (Ic) quantitatively.

(b) *With aqueous methanol.* Refluxing of samples (100 mg.) of each of the triacetates (IVb) and (IVc) with 5% aqueous methanol and working up of the products in the usual way gave starting materials.

Methylation of Cyclomicrophylline-B and -C with Formic Acid–Formalin.—Samples (100 mg.) of each of the compounds (Ib) and (Ic) were heated with a mixture (1 : 1; 2 ml.) of 100% formic acid and 37% formalin on a boiling-water bath for 6 hr. The mixture was poured into water, basified with solid sodium carbonate, and extracted with methylene chloride. The organic layer was washed with water, dried, and evaporated *in vacuo*, and the product was crystallised from acetone to afford cyclomicrophylline-A (Ia) (90 mg.).

Methylation of Cyclomicrophilline-C with Methyl Iodide.—The compound (Ic) (100 mg.) in acetone (10 ml.) was treated with methyl iodide (1 ml.) and the mixture was left at room temperature overnight. The deposited crystals were filtered off and recrystallised from acetone, to furnish a *monomethiodide* (122 mg.), m. p. >320° (Found: C, 59·7; H, 8·85; N, 4·65; I, 21·9. $C_{28}H_{49}IN_2O_2$ requires C, 59·4; H, 8·7; N, 4·95; I, 22·3%).

This methiodide (108 mg.) in methanol (20 ml.) was shaken with moistened silver oxide (1 g.) at room temperature for 10 min. The precipitate was filtered off and the solvent evaporated *in vacuo*. Pyrolysis of the residue at 210—220°/0·04 mm. afforded a crystalline mass (70 mg.). Recrystallisation of this from acetone yielded cyclomicrophylline-A (Ia).

Conversion of Cyclomicrophylline-A into -B.—The *O*-diacetate (IVa) (260 mg.) in dry benzene (10 ml.) was added dropwise with stirring to a solution of cyanogen bromide (200 mg.) (distilled before use) in dry benzene (10 ml.). After the addition, the mixture was refluxed for 6 hr. and the solvent was distilled off. Water was added to the residue, and the solution was made acid with dilute hydrochloric acid and extracted with methylene chloride. Washing of the organic layer with 3% aqueous sodium carbonate and water, drying, and evaporation *in vacuo* left an amorphous material (239 mg.). Chromatography of this on alumina (6 g.) and elution with benzene gave an unidentified product (51 mg.). Elution with methylene chloride yielded an amorphous O-*diacetyl-cyanamide* (IVe) (129 mg.), $\lambda_{max.}$ 4·51 μ (C≡N), τ 7·16 (three protons) due

to the CH_3–N–CN group. The cyanamide (129 mg.) was refluxed with 25% aqueous methanolic sodium hydroxide (30 ml.) for 20 hr., water was added, and the precipitate was extracted with ether. The product (90 mg.) was crystallised from acetone to yield cyclomicrophylline-B (Ib) (50 mg.).

Saponification of Cyclomicrophyllidine-A.—The compound (Id) (200 mg.) was heated under reflux with 3% methanolic sodium hydroxide (20 ml.) for 30 min. After removal of the solvent, the mixture was diluted with water, and the product was extracted with ether. The ether extract was dried and evaporated *in vacuo*, and the product was crystallised from acetone to furnish cyclomicrophylline-A (Ia) (14; mg.).

Acidification of the alkaline aqueous layer with dilute hydrochloric acid, extraction with methylene chloride, drying, and evaporation yielded benzoic acid (30 mg.).

Catalytic Hydrogenation of Cyclomicrophylline-A, -B, and -C.—Samples (100 mg.) of each of the compounds (Ia), (Ib), and (Ic) in glacial acetic acid (20 ml.) were hydrogenated over prehydrogenated platinum oxide (30 mg.) at room temperature and atmospheric pressure. After 20 hr., one molar equivalent of hydrogen had been absorbed. The catalyst was filtered off, and the solution diluted with water, made alkaline with solid potassium carbonate, and extracted with methylene chloride. After washing of the extract with water, drying, and evaporation, the following dihydro-derivatives were obtained.

The dihydro-compound (Va) is identical with dihydrocyclomicrophylline-A (IIa) isolated from the mother-liquor of crystallisation of cyclomicrophylline-A.

Dihydrocyclomicrophylline-B (Vb) was crystallised from acetone, m. p. 247—248°, $[\alpha]_D$ +26°, $\lambda_{max.}$ 2·92 (OH) and 3·04 μ (NH), τ 9·69 and 9·40 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·04, 8·90, and 8·87 (9H, quaternary methyl), 8·90 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·69 (6H) and 7·54 (3H) (*N*-methyl), 6·50 and 6·25 (2H, quadruplet centred at 6·38, *J* 10·5 c./sec., CH_2OH), and 5·89 (1H, multiplet, >$CHOH$) (Found: C, 74·9; H, 11·25; N, 6·25. $C_{27}H_{48}N_2O_2$ requires C, 74·95; H, 11·2; N, 6·5%).

Dihydrocyclomicrophylline-C (Vc), after crystallisation from acetone, had m. p. 265°, $[\alpha]_D$ +52°, $\lambda_{max.}$ 2·95 (OH) and 3·04 μ (NH), τ 9·66 and 9·39 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·02 (6H) and 8·88 (3H) (quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·76 (6H) and 7·56 (3H) (*N*-methyl), 6·51 and 6·26 (2H, quadruplet centred at 6·39, *J* 10·5 c./sec., CH_2OH), and 5·94 (1H, multiplet, >$CHOH$) (Found: C, 74·8; H, 11·2; N, 6·15. $C_{27}H_{48}N_2O_2$ requires C, 74·95; H, 11·2; N, 6·5%).

Cyclopropane Ring Fission.—(*a*) An excess of hydrogen chloride was bubbled through a solution of samples (100 mg.) of each of the compounds (Ia) and (Ic) in chloroform (10 ml.) at room temperature for 10 min. The mixture was diluted with water, basified with ammonia, and the product extracted with methylene chloride. Washing of the extract with water, drying, and evaporation *in vacuo* gave the following products.

The *product* (VIIa) (93 mg.) from compound (Ia) had m. p. 306° (from methanol), $[\alpha]_D$ −78°, $\lambda_{max.}$ 2·92 (OH), 6·05 (C=C), 11·66 (trisubstituted double bond), and 14·10 μ (*cis*-disubstituted double bond), τ 9·30, 9·11, 8·94 (new absorption), and 8·83 (12H, quaternary methyl), 9·09 (3H, doublet, *J* 6·5 c./sec., tertiary methyl), 7·76 and 7·69 (12H, *N*-methyl), 6·65 and 6·26 (2H, quadruplet centred at 6·46, *J* 11 c./sec., CH_2OH), 5·88 (1H, mutliplet, >$CHOH$), 4·85 (1H, multiplet, new olefinic proton), and 4·45 (2H, singlet, olefinic protons) (Found: C, 75·4; H, 10·85; N, 6·2. $C_{28}H_{48}N_2O_2$ requires C, 75·6; H, 10·9; N, 6·3%).

The *product* (VIIb) (95 mg.) from compound (Ic) had m. p. 285° (from methanol), $[\alpha]_D$ −42°, $\lambda_{max.}$ 2·98 (OH), 3·08 (NH), 6·08 (C=C), and 14·16 μ (*cis*-disubstituted double bond), τ 9·30, 9·12, 8·99, and 8·94 (new absorption) (12H, quaternary methyl), 9·09 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·75 (6H) and 7·56 (3H) (*N*-methyl), 6·63 and 6·25 (2H, quadruplet centred at 6·44, *J* 10 c./sec., CH_2OH), 5·88 (1H, multiplet, >$CHOH$), 4·85 (1H, multiplet, new olefinic proton), and 4·43 (2H, singlet, olefinic protons) (Found: C, 75·45; H, 10·8; N, 6·6. $C_{27}H_{46}N_2O_2$ requires C, 75·3; H, 10·75; N, 6·5%).

(*b*) *With concentrated hydrochloric acid.* The compound (Ia) (100 mg.) was heated with concentrated hydrochloric acid (37%; 5 ml.) on a boiling-water bath for 5 min. Working up as above gave the product (VIIa) (92 mg.).

This product (50 mg.) in 20% deuterium chloride in deuterium oxide (1 ml.) (Merck) was set aside at room temperature overnight. Isolation of the product in the usual way, and crystallisation from methanol gave starting material (43 mg.).

(*c*) *With deuterium chloride in deuterium oxide.* The compound (Ia) (100 mg.) was treated

with 20% deuterium chloride in deuterium oxide (1 ml.) (Merck) on a boiling-water bath for 5 min. Working up of the product in the usual way and crystallisation from methanol yielded a *compound* (X) (94 mg.) as cubes, m. p. 293—294°, $[\alpha]_D$ −87°, $\lambda_{max.}$ 2·92 (OH) and 4·41 and 4·53 (>C=CD– and CH_2D groups due to the C–D stretch), τ 9·30, 9·11, and 8·83 (9H, quaternary methyl), 8·94 (2H, diffuse triplet, CH_2D), 9·09 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·76 and 7·69 (12H, *N*-methyl), 6·65 and 6·26 (2H, quadruplet centred at 6·46, *J* 11 c./sec., CH_2OH), 5·88 (1H, multiplet, >C*H*OH), and 4·45 (2H, singlet, olefinic protons) (Found: C, 75·2; H, 11·2; N, 6·15. $C_{28}H_{46}D_2N_2O_2$ requires C, 75·3; H, 11·3; N, 6·25%).

In the above acid-catalysed cleavage reactions, the products (VIIa), (VIIb), and (X) were contaminated with a small amount (5—6%) of the corresponding 7,9(11)-dienes, as detected from their ultraviolet spectra.

Isomerisation of Cyclomicrophylline-A and Compound (VIIa).—(a) *With concentrated hydrochloric acid.* Samples (100 mg.) of each of the compounds (Ia) and (VIIa) in concentrated hydrochloric acid (37%; 10 ml.) were heated on a boiling-water bath for 6 hr. Crystallisation of the product from methanol gave the *conjugated diene* (VIIIa) (90 mg.), m. p. 320°, $[\alpha]_D$ +50°, $\lambda_{max.}$ 2·94 (OH), 6·00—6·40 (C=C), and 10·04 μ (*trans*-conjugated diene), $\lambda_{max.}$ 236, 243·5, and 252 mμ (ε 14,100, 16,200, and 10,600), τ 9·42, 8·95, 8·85, and 8·75 (12H, quaternary methyl), 9·08 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·70 and 7·64 (12H, *N*-methyl), 6·55 and 6·20 (2H, quadruplet centred at 6·38, *J* 11 c./sec., CH_2OH), 5·86 (1H, multiplet, >C*H*OH), and 4·79 and 4·53 (2H, multiplet, conjugated diene protons) (Found: C, 75·4; H, 10·8; N, 6·15. $C_{28}H_{48}N_2O_2$ requires C, 75·6; H, 10·9; N, 6·3%).

(b) *With deuterium chloride in deuterium oxide.* The compound (Ia) (50 mg.) was treated with 20% deuterium chloride in deuterium oxide (2 ml.) (Merck) under the same condition as above. The product was crystallised from methanol to furnish the deuterated *conjugated diene* (XI) as prisms (43 mg.), m. p. 315°, $[\alpha]_D$ +48°, $\lambda_{max.}$ 2·93 (OH), 4·42, 4·58, and 4·76 μ (C–D stretch), $\lambda_{max.}$ 236, 243, and 252 mμ (ε 14,300, 16,300, and 10,800), τ 9·42, 8·95, and 8·75 (9H, quaternary methyl), 8·85 (2H, diffuse peak, CH_2D), 9·08 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·70 and 7·64 (12H, N-methyl), 6·55 and 6·20 (2H, quadruplet centred at 6·38, J 11 c./sec., CH_2OH), 5·86 (1H, multiplet, >C*H*OH), and no absorption of olefinic protons (Found: C, 74·7; H, 11·9; N, 6·15. $C_{28}H_{41}D_7N_2O_2$ requires C, 74·4; H, 12·25; N, 6·2%).

Oxidation of Cyclomicrophylline-A, -B, and -C with Chromium Trioxide.—Samples (200 mg.) of each of the compounds (Ia), (Ib), and (Ic) in acetic acid (10 ml.) were treated with 1·2 molar equivalents of chromium trioxide in water (1 ml.) and left at room temperature overnight. The mixture was diluted with water, made alkaline with solid potassium carbonate, and the product was extracted with methylene chloride. The *amino-ketone* (XIIa) (140 mg.) obtained from compound (Ia) had m. p. 156—157° (slight decomp.) (from acetone), $[\alpha]_D$ −170°, $\lambda_{max.}$ ($CHCl_3$) 2·92 (OH) and 5·77 μ (cyclopentanone), τ 10·09 and 9·15 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·03, 8·89, and 8·86 (9H, quaternary methyl), 9·02 (3H, doublet, *J* 6 c./sec., tertiary methyl), 7·74 and 7·68 (12H, *N*-methyl), 6·49 and 6·14 (2H, quadruplet centred at 6·32, *J* 11 c./sec., CH_2OH), and 4·50—4·61 (2H, olefinic protons). Rotatory dispersion: $[\phi]_{700}$ −728°, $[\phi]_{589}$ −753°, $[\phi]_{320}$ −12,000° (trough), $[\phi]_{280}$ +10,600° (peak), and $[\phi]_{260}$ +7800° (Found: C, 75·8; H, 10·2; N, 6·1. $C_{28}H_{46}N_2O_2$ requires C, 75·95; H, 10·45; N, 6·35%).

The *amino-ketone* (XIIb) (135 mg.) obtained from compound (Ib) had m. p. 162—164° (slight decomp.) (from acetone), $[\alpha]_D$ −156°, $\lambda_{max.}$ ($CHCl_3$) 2·99 (NH and OH) and 5·77 μ (C=O), τ 7·69 (6H) and 7·58 (3H) (*N*-methyl) (Found: C, 75·4; H, 11·2; N, 6·35. $C_{27}H_{44}N_2O_2$ requires C, 75·65; H, 10·35; N, 6·55%).

The *amino-ketone* (XIIc) (140 mg.) obtained from compound (Ic) had m. p. 160—164° (slight decomp.) (from acetone), $[\alpha]_D$ −140°, $\lambda_{max.}$ ($CHCl_3$) 3·00 (NH and OH) and 5·77 μ (C=O), τ 7·78 (6H) and 7·52 (3H) (*N*-methyl) (Found: C, 75·4; H, 10·5; N, 6·2. $C_{27}H_{44}N_2O_2$ requires C, 75·65; H, 10·35; N, 6·55%).

These amino-ketones are unstable, and drying for analysis was effected with caution over phosphorus pentoxide at room temperature.

Deamination of C-20 *Amino-function.*—(a) *With mild alkali.* The amino-ketone (XIIa) (100 mg.) was treated with 0·1% methanolic potassium hydroxide (20 ml.) at room temperature overnight. After removal of the solvent *in vacuo*, water was added, and the precipitate was extracted with methylene chloride. Washing of the extract with water, drying, and evaporation *in vacuo* left a residue which was crystallised from methanol to give a mixture of the isomeric αβ-*unsaturated cyclopentenones* (XIIIa) and (XIIIa′) (75 mg.), m. p. 191—194°, $[\alpha]_D$

$-171°$, $\lambda_{max.}$ ($CHCl_3$) 2·98 (OH), 5·84 (cyclopentenone), 6·07 (double bond conjugated with carbonyl), and 9·60 μ (C–O), $\lambda_{max.}$ 244 mμ (ε 8300) (Found: C, 78·6; H, 9·7; N, 3·45. $C_{26}H_{39}NO_2$ requires C, 78·55; H, 9·9; N, 3·5%). The n.m.r. spectrum showed that the mixture consisted of *cis*-isomer (XIIIa) and *trans*-isomer (XIIIa′) in a ratio of 1 : 1. For *cis*-isomer, τ 10·09 and 9·15 (2H, two doublets, *J* 4·5 c./sec., cyclopropane), 9·25 (broad), 8·89, and 8·75 (9H, quaternary methyl), 8·19 (3H, doublet, *J* 7·5 c./sec., vinyl methyl coupled with vinyl proton), 7·69 (6H, *N*-methyl), 6·50 and 6·15 (2H, quadruplet centred at 6·33, *J* 11 c./sec., CH_2OH), 4·53 (2H, olefinic protons), and 3·51 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton coupled with vinyl methyl); for *trans*-isomer, τ 7·92 (3H, doublet, *J* 7·5 c./sec., vinyl methyl coupled with vinyl proton) and 4·35 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton coupled with vinyl methyl).

Under the same conditions as above, the amino-ketone (XIIb) (100 mg.) gave a 1 : 1 mixture of the cyclopentenones (XIIIa) and (XIIIa′) (72 mg.). Its infrared spectrum in chloroform was identical with that of a product obtained from the amino-ketone (XIIa) (see above).

Similarly, the amino-ketone (XIIc) (100 mg.) yielded a product (74 mg.) which was a mixture of the isomeric *cyclopentenones* (XIIIb) and (XIIIb′) in a ratio of 1 : 1. It was crystallised from acetone to show m. p. 182—186°, $[\alpha]_D$ $-164°$, $\lambda_{max.}$ ($CHCl_3$) 3·00 (OH and NH), 5·84 (cyclopentenone), and 6·07 μ (C=C), $\lambda_{max.}$ 244 mμ (ε 8900) (Found: C, 78·1; H, 9·85; N, 3·35. $C_{25}H_{37}NO_2$ requires C, 78·3; H, 9·7; N, 3·65%). The n.m.r. spectra showed: for *cis*-isomer (XIIIb), τ 10·05 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·20 (broad), 9·02, and 8·73 (9H, quaternary methyl), 8·19 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·52 (3H, *N*-methyl), 6·50 and 6·13 (2H, quadruplet centred at 6·32, *J* 10 c./sec., CH_2OH), 4·50 (2H, olefinic protons), and 3·52 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton); for *trans* isomer (XIIIb′), τ 7·92 (3H, doublet, *J* 7·5 c./sec., vinyl methyl) and 4·35 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton).

(b) *With alumina.* Samples (100 mg.) of each of the amino-ketones (XIIa) and (XIIb), dissolved in benzene, were adsorbed on a column of alumina (20 g.) and kept at room temperature for 1 hr. Elution with methylene chloride–methanol (98 : 2) afforded exclusively the *cis*-isomer (XIIIa) as needles (60—65 mg.), m. p. 197—198° (from methanol), $[\alpha]_D$ $-178°$. Peak positions of the n.m.r. signals of this *cis*-isomer were given above.

Similarly, the amino-ketone (XIIc) (70 mg.) gave the *cis*-isomer (XIIIb) as needles (51 mg.), m. p. 192—193° (from acetone–hexane), $[\alpha]_D$ $-162°$. For the n.m.r. signals of this *cis*-isomer, see above.

(c) *With methanol.* The amino-ketone (XIIa) (100 mg.) was refluxed with methanol (10 ml.) for 1 hr. The n.m.r. spectrum showed that the product is a mixture of the isomeric cyclopentenones (XIIIa) and (XIIIa′) in a ratio of 3 : 1.

Pyrolytic Deamination of Amino-ketone (XIIa).—The amino-ketone (130 mg.) was heated at 140—150°/0·04 mm. for 30 min. The crude product formed remained in the reaction flask, and the n.m.r. spectrum showed that it is the *cis*-αβ-unsaturated cyclopentenone (XIIIa) and no *trans*-isomer was present. After one recrystallisation from methanol, this product showed m. p. 197—198° (114 mg.). Identity was confirmed by mixed m. p. and infrared spectrum.

Oxidation of Cyclomicrophylline-A with Manganese Dioxide.—The compound (Ia) (150 mg.) in dry chloroform (20 ml.) was left with activated manganese dioxide (1·5 g.), with stirring, at room temperature for 5 days. Filtration and evaporation of the filtrate *in vacuo* left a residue (140 mg.). The infrared spectrum showed that it is a *ca.* 3 : 1 mixture of the amino-ketone (XIIa) and an unsaturated ketone. Chromatography of this crude product on alumina and elution with methylene chloride furnished the *cis*-αβ-unsaturated cyclopentenone (XIIIa) (120 mg.).

Ozonolysis of αβ-Unsaturated Cyclopentenones (XIIIa) *and* (XIIIa′).—A mixture of the ketones (120 mg.) in glacial acetic acid (30 ml.) was ozonised with 3—6% ozonised oxygen at room temperature (19°) for 30 min. The solution was diluted with water (39 ml.), treated with ferrous sulphate (1 g.), and steam-distilled. A volatile fraction was collected in a solution of 2,4-dinitrophenylhydrazine in sulphuric acid, to afford acetaldehyde 2,4-dinitrophenylhydrazone, identified by m. p., mixed m. p., and infrared spectrum. A non-volatile fraction was resinous and was not examined further.

Catalytic Hydrogenation of αβ-Unsaturated Cyclopentenones (XIIIa) *and* (XIIIa′).—A mixture of the ketones (70 mg.) in glacial acetic acid–ethanol (1 : 1; 20 ml.) was hydrogenated in the presence of pre-reduced platinum oxide (20 mg.) at room temperature and atmospheric pressure. After 6 hr., two molar equivalents of hydrogen had been absorbed. Working up of the product

in the usual way afforded the saturated *ketone* (XIV) (50 mg.), m. p. 168° (from acetone–hexane), $[\alpha]_D$ −132°, $\lambda_{max.}$ 2·97 (OH) and 5·77 μ (cyclopentanone) (Found: C, 77·65; H, 10·85; N, 3·35. $C_{26}H_{43}NO_2$ requires C, 77·75; H, 10·8; N, 3·5%).

Oxidation of Cyclomicrophylline-A with Chromium Trioxide.—(a) *Formation of keto-amino-aldehyde* (XV). The compound (Ia) (200 mg.) in acetic acid (10 ml.) was treated with chromium trioxide (70 mg., 0·7 mmole) in water (1 ml.) at room temperature for 18 hr. The mixture was worked up in the usual way, and a crude material (167 mg.) isolated was treated with 0·1% methanolic potassium hydroxide (30 ml.) at room temperature overnight. The product was chromatographed on alumina (2 g.), and elution with benzene gave a 1 : 1 mixture of the cis- and trans-*keto-amino-aldehydes* (XV) (86 mg.) as needles, m. p. 198—205° (from acetone–hexane), $[\alpha]_D$ −155°, $\lambda_{max.}$ 3·70 (C–H stretching vibration due to the aldehyde group), 5·82 (aldehyde and cyclopentenone), and 6·06 μ (C=C), $\lambda_{max.}$ 244 mμ (ε 9300) (Found: C, 79·1; H, 9·45; N, 3·2. $C_{26}H_{37}NO_2$ requires C, 78·95; H, 9·45; N, 3·55%). The n.m.r. spectrum for *cis*-isomer, τ 10·03 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·20 (broad), 8·88, and 8·74 (9H, quaternary methyl), 8·18 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·72 (6H, *N*-methyl), 4·50—5·00 (2H, olefinic protons), 3·48 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton), and 0·54 (1H, aldehyde proton); for *trans*-isomer, τ 7·90 (3H, doublet, *J* 7·5 c./sec., vinyl methyl) and 4·36 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton).

Elution with methylene chloride gave a 1 : 1 mixture of the isomeric keto-alcohols (XIIIa) and (XIIIa′) (42 mg.).

(b) *Formation of keto-amino-acid* (XVIa). The compound (Ia) (200 mg.) in acetic acid (5 ml.) was treated with chromium trioxide (96 mg., 0·96 mmole), and the mixture was kept at room temperature until the colour of the solution turned green (3 days). After evaporation of the solvent *in vacuo*, the residue was heated with 1% aqueous sodium hydroxide (30 ml.) on a water-bath for 10 min. After cooling, the solution was made acid with solid citric acid, and the product was extracted with methylene chloride. The extract was washed with water, dried, and evaporated *in vacuo* to afford a product (127 mg.) which was a 1 : 1 mixture of the *cis*- and *trans*-keto-amino-acids (XVIa). Recrystallisation of this mixture from ethyl acetate furnished a cis-*keto-amino-acid* as needles (46 mg.), m. p. 210—214°, $[\alpha]_D$ −192°, $\lambda_{max.}$ 5·80 (cyclopentenone) 6·06 (C=C), and 6·25 μ (carboxylate ion), $\lambda_{max.}$ 244 mμ (ε 9200), τ 10·06 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·20 (3H) (broad) and 8·72 (6H) (quaternary methyl), 8·20 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·24 (6H, *N*-methyl), 4·90—4·00 (2H, olefinic protons), and 3·50 (1H, quadruplet, *J* 7·5 c./sec., vinyl protons) (Found: C, 75·65; H, 9·2; N, 3·25. $C_{26}H_{37}NO_3$ requires C, 75·85; H, 9·05; N, 3·4%).

The trans-*keto-amino-acid* (23 mg.) was obtained from the mother-liquor of the above *cis*-isomer, and, after recrystallisation from acetone–hexane, had m. p. 193—198°, τ 7·92 (3H, doublet, *J* 7·5 c./sec., vinyl methyl) and 4·00—5·00 (3H, two olefinic and one vinyl protons).

Methylation of Keto-amino-acid (XVIa).—The *cis*-keto-amino-acid (30 mg.) in ether was treated with ethereal diazomethane. Usual working up of the product and crystallisation from hexane yielded the *methyl ester* (XVIb) as needles (25 mg.), m. p. 123—126°, $[\alpha]_D$ −145°, $\lambda_{max.}$ ($CHCl_3$) 5·84 (methyl ester and cyclopentenone) and 6·06 μ (C=C), $\lambda_{max.}$ 244 mμ (ε 9300), τ 10·05 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·20 (broad), 8·84, and 8·74 (9H, quaternary methyl), 8·19 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·72 (6H, *N*-methyl), 6·29 (3H, *O*-methyl), 4·75 (2H, olefinic protons), and 3·49 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton) (Found: C, 76·25; H, 9·15; N, 3·2. $C_{27}H_{39}NO_3$ requires C, 76·2; H, 9·25; N, 3·3%).

Toluene-p-*sulphonation of Cyclomicrophylline-A.*—(*a*) The compound (Ia) (200 mg.) in anhydrous pyridine (10 ml.) was treated with toluene-*p*-sulphonyl chloride (103 mg., 0·54 mmole) and kept at room temperature overnight. The solution was evaporated *in vacuo*, made alkaline with 3% aqueous potassium carbonate, and the product was extracted with methylene chloride. The *mono-toluene*-p-*sulphonate* (XVII) (252 mg.) was obtained after crystallisation from methanol, m. p. 176—177°, $[\alpha]_D$ +12°, $\lambda_{max.}$ 2·93 (OH), 6·26 (toluene-*p*-sulphonyl benzene), and 7·35, 8·42, and 8·48 μ ($-SO_2-$), τ 10·22 and 9·37 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·34, 9·10, and 9·09 (9H, quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·55 (3H, toluene-*p*-sulphonyl methyl), 7·76 (12H, *N*-methyl), 6·32 and 5·50 (2H, quadruplet centred at 5·91, *J* 9 c./sec., CH_2OTs), 5·88 (1H, multiplet, $>CHOH$), 4·75—5·08 (2H, olefinic protons), and 2·67 and 2·22 (4H, two doublets, *J* 8 c./sec., toluene-*p*-sulphonyl benzene protons) (Found: C, 70·3; H, 9·3; N, 4·45. $C_{35}H_{54}N_2O_4S$ requires C, 70·2; H, 9·1; N, 4·7%).

(*b*) The compound (Ia) (100 mg.) and toluene-*p*-sulphonyl chloride (103 mg., 0·54 mmole) in

anhydrous pyridine (10 ml.) were warmed at 60—70° for 5 hr. The crude product (170 mg.), obtained as above, was chromatographed on alumina (3 g.). Elution with benzene gave the *di-toluene*-p-*sulphonate* (XVIII) (120 mg.), m. p. 201—202° (from methylene chloride–methanol), $[\alpha]_D$ −12°, $\lambda_{max.}$ 6·24 (toluene-*p*-sulphonyl benzene) and 7·38, 8·42, and 8·50 μ (−SO_2−), τ 10·22 and 9·38 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·36, 9·12, and 9·10 (9H, quaternary methyl), 9·24 (3H, doublet, *J* 7 c./sec., tertiary methyl), 8·25 and 7·75 (12H, *N*-methyl), 7·56 and 7·49 (6H, toluene-*p*-sulphonyl methyl), 6·33 and 5·48 (2H, quadruplet centred at 5·91, *J* 9 c./sec., CH_2OTs), 5·02 (1H, multiplet, >CHOTs), 5·14 (2H, olefinic protons), and 2·58 and 2·20 (8H, two doublets, *J* 8 c./sec., toluene-*p*-sulphonyl benzene protons) (Found: C, 67·5; H, 8·0; N, 3·65. $C_{42}H_{60}N_2O_6S_2$ requires C, 67·45; H, 8·1; N, 3·75%).

Catalytic Hydrogenation of Mono-toluene-p-*sulphonate* (XVII).—The mono-toluene-p-sulphonate (150 mg.) in glacial acetic acid was hydrogenated over pre-reduced platinum oxide (50 mg.) in the usual way. The mixture was stirred until one molar equivalent of hydrogen had been absorbed. The *dihydromono-toluene*-p-*sulphonate* (XIX) (150 mg.) had m. p. 188—189° (from methanol), $[\alpha]_D$ +37°, $\lambda_{max.}$ 2·94 (OH), 6·24 (toluene-*p*-sulphonyl benzene), and 7·33, 8·40, and 8·49 μ (−SO_2−), τ 9·73 and 9·54 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·32, 9·06, and 8·98 (9H, quaternary methyl), 9·11 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·82 and 7·75 (12H, *N*-methyl), 7·55 (3H, toluene-*p*-sulphonyl methyl), 6·40 and 5·64 (2H, quadruplet centred at 6·02, *J* 9 c./sec., CH_2OTs), 5·98 (1H, multiplet, >CHOH), and 2·68 and 2·20 (4H, quadruplet centred at 2·44, *J* 8 c./sec., toluene-*p*-sulphonyl benzene protons) (Found: C, 70·1; H, 9·4; N, 4·45. $C_{35}H_{56}N_2O_2S$ requires C, 69·95; H, 9·4; N, 4·65%).

Oxidation of the Dihydromono-toluene-p-*sulphonate* (XIX) *with Chromium Trioxide*.—The dihydromono-toluene-*p*-sulphonate (120 mg.) in acetic acid (10 ml.) was treated with chromium trioxide (24 mg., 0·24 mmole) in the minimum of water and kept at room temperature for 2 days. The mixture was diluted with water, basified with solid sodium carbonate, and extracted with methylene chloride. Washing of the organic layer with water, drying, and evaporation *in vacuo* gave a crystalline mass (115 mg.). Careful recrystallisation from acetone furnished the *dihydro-toluene*-p-*sulphonyl-ketone* (XX) as plates, m. p. 152°, $[\alpha]_D$ −23°, $\lambda_{max.}$ 5·77 μ (cyclopentanone) (Found: C, 70·1; H, 9·25; N, 4·45. $C_{35}H_{54}N_2O_4S$ requires C, 70·2; H, 9·1; N, 4·7%).

Deamination of the Dihydro-toluene-p-*sulphonyl-ketone* (XX).—The dihydro-toluene-*p*-sulphonyl-ketone (83 mg.) in methylene chloride was adsorbed on a column of alumina (30 g.). Elution with the same solvent afforded a cis-αβ-*unsaturated ketone* (XXI) (72 mg.) as needles, m. p. 206—207° (from methanol), $[\alpha]_D$ −31°, $\lambda_{max.}$ 5·82 (cyclopentenone) and 6·06 μ (C=C), $\lambda_{max.}$ 243 mμ (ε 9800), τ 9·63 and 9·43 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·31 (broad), 9·06 and 8·69 (9H, quaternary methyl), 8·16 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·84 (6H, *N*-methyl), 7·55 (3H, toluene-*p*-sulphonyl methyl), 6·38 and 5·64 (2H, quadruplet centred at 6·01, *J* 10 c./sec., CH_2OTs), 3·45 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton), and 2·68 and 2·21 (4H, quadruplet centred at 2·45, *J* 8 c./sec., toluene-*p*-sulphonyl benzene protons) (Found: C, 71·55; H, 8·45; N, 2·44. $C_{33}H_{47}NO_4S$ requires C, 71·6; H, 8·55; N, 2·55%).

Catalytic Hydrogenation of the Toluene-p-*sulphonyl*-cis-αβ-*unsaturated Ketone* (XXI).—The ketone (60 mg.) in 95% ethanol–glacial acetic acid (1 : 1; 10 ml.) was hydrogenated over pre-reduced platinum oxide (10 mg.). After absorption of one molar equivalent of hydrogen, the product was isolated in the usual way. After crystallisation from methanol, the *dihydro-toluene*-p-*sulphonyl-cyclopentanone* (XXIII) was obtained as needles (54 mg.), m. p. 186—187°, $[\alpha]_D$ −20°, $\lambda_{max.}$ 5·77 μ (cyclopentanone), τ 9·64 and 9·44 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·32, 8·97, and 8·93 (9H, quaternary methyl), 9·01 (3H, triplet, *J* 5 c./sec., secondary methyl), 7·84 (6H, *N*-methyl), and 6·39 and 5·67 (2H, quadruplet centred at 6·03, *J* 8 c./sec., CH_2OTs). Rotatory dispersion: $[\phi]_{700}$ −96°, $[\phi]_{589}$ −111°, $[\phi]_{320}$ −8200° (trough), $[\phi]_{280}$ +17,000° (peak), and $[\phi]_{260}$ +12,500° (Found: C, 71·25; H, 8·95; N, 2·65. $C_{33}H_{49}NO_4S$ requires C, 71·3; H, 8·9; N, 2·5%).

Formation of the Unsaturated Toluene-p-*sulphonyl*-cis-αβ-*unsaturated Ketone* (XXII).—The mono-toluene-*p*-sulphonate (XVII) (100 mg.) in acetic acid (10 ml.) was oxidised with chromium trioxide (20 mg., 0·20 mmole) in the minimum of water at room temperature for 2 days. The product obtained in the usual way was dissolved in benzene and passed through a column of alumina to afford the cis-αβ-*unsaturated ketone* (XXII) (80 mg.) as needles, m. p. 143—144° (from methanol), $[\alpha]_D$ −134°, $\lambda_{max.}$ 5·80 (cyclopentenone), 6·06 μ (C=C), $\lambda_{max.}$ 243 mμ (ε 9800), τ 10·11 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·33, 9·25 (broad), and 8·76 (9H, quaternary

methyl), 8·19 (3H, doublet, *J* 7·5 c./sec., vinyl methyl), 7·80 (6H, *N*-methyl), 7·56 (3H, toluene-*p*-sulphonyl methyl), 6·25 and 5·53 (2H, quadruplet centred at 5·89, *J* 9 c./sec., CH_2OTs), 4·74 (2H, olefinic protons), and 3·49 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton) (Found: C, 71·65; H, 8·45; N, 2·65. $C_{33}H_{45}NO_4S$ requires C, 71·85; H, 8·2; N, 2·55%).

Catalytic Hydrogenation of the Unsaturated Toluene-p-*sulphonyl*-cis-αβ-*unsaturated Ketone* (XXII).—The unsaturated ketone (50 mg.) in glacial acetic acid—95% ethanol (1 : 1; 10 ml.) was hydrogenated with platinum oxide (20 mg.) in the usual way. Working up of the product and crystallisation from methanol afforded the dihydro-toluene-*p*-sulphonyl-cyclopentanone (XXIII) (43 mg.) (see above).

Benzoylation of the Mono-toluene-p-*sulphonate* (XVII).—The mono-toluene-*p*-sulphonate (70 mg.) in anhydrous pyridine (5 ml.) was treated with benzoyl chloride (0·2 ml.) and left at room temperature overnight. Working up in the usual way afforded the *toluene*-p-*sulphonyl benzoate* (XXIV) (73 mg.), m. p. 173—175° (from methanol), $[\alpha]_D$ −57°, $\lambda_{max.}$ 5·84 and 6·24 μ (benzoate), τ 10·18 (1H, doublet, *J* 5 c./sec., cyclopropane), 9·36 (3H) and 9·03 (6H) (quaternary methyl), 9·14 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·90 and 7·79 (12H, *N*-methyl), 7·72 (3H, toluene-*p*-sulphonyl methyl), 6·33 and 5·52 (2H, quadruplet centred at 5·93, *J* 9 c./sec., CH_2OTs), and 4·50—5·00 (3H, two olefinic and one >$CHOBz$ protons) (Found: C, 71·9; H, 8·15; N, 3·65. $C_{42}H_{58}N_2O_5S$ requires C, 71·75; H, 8·3; N, 4·0%). This compound is identical with the toluene-*p*-sulphonate obtained from cyclomicrophyllidine-A (Id) by treatment with pyridine–toluene-*p*-sulphonyl chloride (see above).

Catalytic Hydrogenation of the Toluene-p-*sulphonyl Benzoate* (XXIV).—The toluene-*p*-sulphonyl benzoate (100 mg.) in glacial acetic acid (10 ml.) was hydrogenated with platinum oxide (30 mg.) in the usual way to afford the *dihydro-derivative* (XXV) (90 mg.), m. p. 202—203° (from methanol–acetone), $[\alpha]_D$ +17°, $\lambda_{max.}$ 5·84 and 6·24 μ (benzoate) (Found: C, 71·7; H, 8·45; N, 3·7. $C_{42}H_{60}N_2O_5S$ requires C, 71·55; H, 8·6; N, 3·95%).

This compound is identical with the toluene-*p*-sulphonate of dihydrocyclomicrophyllidine-A (IIc) (see above).

Ruschig Degradation of Cyclomicrophylline-C.—The compound (Ic) (1 g.) in dry methylene chloride (100 ml.) was treated with *N*-chlorosuccinimide (500 mg.) and left at room temperature for 16 hr. The solution was washed with water, dried, and evaporated *in vacuo* to yield a crude product (1·12 g.). Careful recrystallisation of this from acetone–methanol gave the pure N-*chloro-amine* (XXVI), m. p. 255—260° (decomp.), $[\alpha]_D$ −38°, $\lambda_{max.}$ (Nujol) 2·89 (OH) and no NH absorption, 6·04 (C=C), and 14·42 μ (*cis*-disubstituted ethylene), τ 10·12 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·08 (6H) and 8·97 (3H) (quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·75 (6H) and 7·02 (3H) (*N*-methyl), 6·53 and 6·15 (2H, quadruplet centred at 6·34, *J* 10·5 c./sec., CH_2OH), 5·88 (1H, multiplet, >$CHOH$), and 4·45—4·56 (2H, olefinic protons). This compound is unstable, and the analytical sample was dried over phosphorus pentoxide at room temperature and atmospheric pressure for 3 days (Found: C, 67·0; H, 10·2; N, 5·65. $C_{27}H_{45}ClN_2O_2,H_2O$ requires C, 67·1; H, 9·8; N, 5·8%).

The crude *N*-chloro-amine (1 g.) was heated under reflux with a solution of potassium methoxide (200 mg.) in absolute methanol (50 ml.) for 1 hr. Evaporation of the solution *in vacuo* left a *residue* (XXVII) which showed an infrared band at 6·08 μ (>C=N−). This material was treated with 2N-sulphuric acid (100 ml.) and kept at room temperature overnight. Extraction of the product with methylene chloride, washing with 3% aqueous sodium carbonate and water, drying, and evaporation gave a crystalline mass (312 mg.). This was recrystallised from acetone to afford the *ketone* (XXVIII), m. p. 216—217°, $[\alpha]_D$ −52°, $\lambda_{max.}$ ($CHCl_3$) 2·95 (OH) and 5·84 μ (six-membered-ring ketone), τ 10·11 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·06 (6H) and 8·90 (3H) (quaternary methyl), 9·11 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·76 (6H) (*N*-methyl), 6·32 and 5·53 (2H, quadruplet centred at 5·93, *J* 8 c./sec., CH_2OH), 6·00 (1H, multiplet, >$CHOH$), and 4·62 (2H, olefinic protons). This compound is unstable, and drying for analysis was effected over phosphorus pentoxide at room temperature and 0·05 mm. for 1 hr. (Found: C, 75·05; H, 10·15; N, 3·25. $C_{26}H_{41}NO_3$ requires C, 75·15; H, 9·95; N, 3·35%).

The acid aqueous solution left after extraction with methylene chloride was made alkaline with ammonia, and the deposited mass was extracted with methylene chloride. Usual working up recovered the starting material (Ic) (280 mg.).

Retro-aldol Reaction of the Ketone (XXVIII).—The crude ketone (300 mg.) in a solution of 10% aqueous ethanol (25 ml.) containing sodium hydroxide (242 mg.) was set aside at room

temperature overnight. The mixture was made acid with acetic acid and then steam-distilled. The distillate was collected in a solution of 2,4-dinitrophenylhydrazine in sulphuric acid, to afford formaldehyde 2,4-dinitrophenylhydrazone (30 mg.), identified by m. p., mixed m. p., and infrared spectrum.

The non-volatile fraction after steam-distillation was made alkaline with ammonia, and the product was extracted with methylene chloride. The extract was washed with water, dried, and evaporated *in vacuo* to dryness. Chromatography of the residue on alumina (10 g.) and elution with ether furnished the *nor-ketone* (XXIX) (150 mg.), m. p. 188—190° (from acetone), $[\alpha]_D$ −32°, $\lambda_{max.}$ ($CHCl_3$) 2·95 (OH) and 5·84 μ (six-membered-ring ketone), τ 10·04 (1H, doublet, *J* 4 c./sec., cyclopropane), 9·05 (6H) and 8·74 (3H) (quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·75 (6H, *N*-methyl), 6·00 (1H, multiplet, >C*H*OH), 4·47 (2H, olefinic protons), and no signal for a CH_2OH group (Found: C, 77·65; H, 10·1; N, 3·4. $C_{25}H_{39}NO_2$ requires C, 77·85; H, 10·2; N, 3·65%).

Oxidation of the Ketone (XXIX) *with Chromium Trioxide.*—The ketone (140 mg.) in acetic acid (10 ml.) was oxidised with chromium trioxide (29 mg., 0·29 mmole) in the minimum of water. After standing at room temperature overnight, the mixture was made alkaline with 10% aqueous sodium hydroxide and kept at room temperature overnight. Extraction of the product with ether, washing of the extract with water, drying, and evaporation left a residue (128 mg.) which was chromatographed on silicic cid (10 g.). Elution with methylene chloride gave a neutral product (93 mg.). It is a 1 : 1 mixture of the isomeric αβ-*unsaturated ketones* (XXXI), m. p. 140—144° (from acetone–hexane), $[\alpha]_D$ −164°, $\lambda_{max.}$ ($CHCl_3$) 5·84 (cyclopentenone) and 6·06 μ (double bond conjugated with carbonyl), $\lambda_{max.}$ 244 mμ (ε 8900). The n.m.r. spectra: *cis-isomer*, τ 9·96 (1H, doublet, *J* 4 c./sec., cyclopropane), 8·20 (3H, doublet, *J* 7·5 c./sec., vinyl methyl) and 3·63 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton); for *trans*-isomer, τ 7·93 (3H, doublet, *J* 7·5 c./sec., vinyl methyl) and 4·38 (1H, quadruplet, *J* 7·5 c./sec., vinyl proton) (Found: C, 81·5; H, 8·85. $C_{23}H_{30}O_2$ requires C, 81·6; H, 8·95%).

Catalytic Hydrogenation of the Neutral Unsaturated Ketone (XXXI).—A mixture of the isomeric unsaturated ketones (80 mg.) in glacial acetic acid (10 ml.) was hydrogenated over pre-reduced platinum oxide (30 mg.) in the usual way. The uptake of hydrogen ceased after slightly more than two molar equivalents of hydrogen had been absorbed. The product (82 mg.) isolated in the usual manner was chromatographed on silicic acid (10 g.), and elution with methylene chloride afforded the *monoketone* (XXXIII) (8 mg.), m. p. 82° (from hexane–light petroleum), $[\alpha]_D$ +70°, $\lambda_{max.}$ ($CHCl_3$) 5·86 μ (six-membered-ring ketone). Rotatory dispersion: $[\phi]_{700}$ +207°, $[\phi]_{589}$ +230°, $[\phi]_{305}$ +2370° (peak), $[\phi]_{266}$ −1480° (trough), and $[\phi]_{250}$ −886° (Found: C, 84·15; H, 11·2. $C_{23}H_{36}O$ requires C, 84·1; H, 11·05%).

Further elution with the same solvent gave the *diketone* (XXXII) (60 mg.), m. p. 134—135° (from hexane), $[\alpha]_D$ −97°, $\lambda_{max.}$ ($CHCl_3$) 5·78 (cyclopentanone) and 5·86 μ (six-membered-ring ketone), τ 9·52 and 9·27 (2H, two doublets, *J* 4 c./sec., cyclopropane), 8·90 (6H, quaternary methyl), and 8·98 (3H, doublet, *J* 6 c./sec., tertiary methyl). Rotatory dispersion: $[\phi]_{700}$ −295°, $[\phi]_{589}$ −332°, $[\phi]_{320}$ −8200° (trough), $[\phi]_{280}$ +17,000° (peak), and $[\phi]_{260}$ +11,200° (Found: C, 80·8; H, 10·25. $C_{23}H_{34}O_2$ requires C, 80·65; H, 10·0%).

Catalytic Hydrogenation of Cyclobuxine-D (XXXIV).—Cyclobuxine-D, m. p. 248—249°, $[\alpha]_D$ +96°, was isolated in 0·01% yield (4 g.) from the leaves and twigs (42 kg.) of *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino, collected at Mikurajima island in Tokyo. Identity was established by direct comparison with the authentic sample kindly supplied by Professor S. M. Kupchan.

The compound (850 mg.) in glacial acetic acid was hydrogenated with platinum oxide in the usual way. Crystallisation of the product from acetone yielded the dihydro-compound (XXXV) (800 mg.), m. p. 212—213°, $[\alpha]_D$ +52° (lit.,[5a] m. p. 208—209°, $[\alpha]_D$ +46°).

Formation of the αβ-*Unsaturated Ketone* (XXXVI).—The dihydro-compound (XXXV) (750 mg.) in acetic acid (50 ml.) was treated with chromium trioxide (150 mg., 1·50 mmoles) in the minimum of water. After standing at room temperature overnight, the mixture was concentrated *in vacuo* and treated with 1% aqueous methanolic potassium hydroxide solution for 1 hr. The product was extracted with methylene chloride and isolated in the usual way. Crystallisation from acetone yielded a mixture of the isomeric unsaturated ketones (XXXVI) (680 mg.), whose ratio (1 : 1) was determined from the n.m.r. spectrum. It had m. p. 132—140°, $[\alpha]_D$ −142°, $\lambda_{max.}$ ($CHCl_3$) 3·00 (NH), 5·82 (cyclopentenone), and 6·06 μ (C=C) (Found: C, 80·95; H, 10·6; N, 3·85. $C_{24}H_{37}NO$ requires C, 81·05; H, 10·5; N, 3·95%).

Catalytic Hydrogenation of the αβ-Unsaturated Ketone (XXXVI).—A mixture of the unsaturated ketones (650 mg.) in glacial acetic acid (20 ml.) was hydrogenated with platinum oxide (60 mg.) in the usual way. The product was crystallised from acetone to yield the saturated *ketone* (XXXVII) (600 mg.), m. p. 137°, $[\alpha]_D$ −120°, $\lambda_{max.}$ ($CHCl_3$) 3·00 (NH) and 5·78 μ (cyclopentanone) (Found: C, 80·8; H, 11·25; N, 3·75. $C_{24}H_{39}NO$ requires C, 80·6; H, 11·0; N, 3·9%).

N-*Chlorination of the Ketone* (XXXVII).—The ketone (580 mg.) and *N*-chlorosuccinimide (266 mg., 1·96 mmoles) in dry methylene chloride (50 ml.) were left at room temperature overnight. The solution was washed with water, dried, and evaporated *in vacuo* to give the N-*chloro-amine* (XXXVIII) (627 mg.). A portion of this chloro-amine was carefully crystallised from acetone to furnish prisms, decomp. *ca.* 140°, $[\alpha]_D$ −60°, $\lambda_{max.}$ 5·77 μ (cyclopentanone) and no NH absorption. The analytical sample was dried over phosphorus pentoxide at room temperature for 3 days (Found: C, 68·65; H, 9·9; N, 3·1. $C_{24}H_{37}ClNO,\frac{2}{3}H_2O$ requires C, 68·95; H, 9·65; N, 3·35%).

Conversion of the N-*Chloro-amine* (XXXVIII) *to the Diketone* (XXXII).—The *N*-chloro-amine (500 mg.) was heated under reflux with a solution of potassium methoxide (200 mg.) in absolute methanol (50 ml.) for 1 hr. After removal of the solvent *in vacuo*, the residue was treated with 2N-sulphuric acid (50 ml.) and the solution left at room temperature overnight. Extraction of the solution with ether gave a neutral product (230 mg.) which was chromatographed on silicic acid (20 g.). Elution with methylene chloride furnished the diketone (XXXII) (194 mg.), identical with that derived from cyclomicrophilline-C (Ic), described above.

Cyclomicrophylline-A Monomethiodide.—The compound (Ia) (200 mg.) in acetone (30 ml.) was treated with methyl iodide (1 ml.) and kept at room temperature overnight. After removal of the solvent *in vacuo*, the product was recrystallised from acetone to afford the *monomethiodide* (XL) (250 mg.) as needles, m. p. 265—270° (decomp.) (Found: C, 59·6; H, 8·85; I, 21·95; N. 4·4. $C_{29}H_{51}IN_2O_2$ requires C, 59·35; H, 8·75; I, 21·65; N, 4·8%).

Attempted Hofmann Degradation of the Monomethiodide (XL).—The monomethiodide (230 mg.) was heated under reflux with t-butyl alcohol (20 ml.) containing potassium t-butoxide (3 g.) for 8 hr. Concentration of the solution *in vacuo*, dilution with water, and extraction with methylene chloride gave the parent compound (Ia) (220 mg.).

Cyclomicrophylline-A Dimethiodide.—The compound (Ia) (200 mg.) in absolute methanol (10 ml.) was refluxed with methyl iodide (2 ml.) for 8 hr. Removal of the solvent left a residue (315 mg.) which was divided into a methanol-soluble fraction (monomethiodide) and a methanol-insoluble fraction (dimethiodide). The latter was recrystallised from 95% ethanol to furnish the *dimethiodide* (XLI) (170 mg.), m. p. >320° (Found: C, 46·85; H, 7·8; I, 33·25; N, 3·4. $C_{30}H_{54}I_2N_2O_2,2H_2O$ requires C, 47·1; H, 7·65; I, 33·2; N, 3·65%).

Hofmann Degradation of the Dimethiodide (XLI).—Hofmann degradation of the dimethiodide (150 mg.) was carried out under the same conditions as described above. A volatile amine was evolved, which was collected into 3% aqueous hydrochloric acid solution. Evaporation of this acid solution *in vacuo* to dryness yielded an amine hydrochloride (17 mg.) which, on treatment with sodium picrate (20 mg.) in water (2 ml.), was converted into a picrate, m. p. 213—214° (23 mg.), identified as trimethylamine picrate by m. p., mixed m. p., and infrared spectrum.

The non-volatile fraction was worked up in the usual way, and the product was chromatographed in benzene on alumina. Elution with benzene–ether (1 : 1) gave an amorphous material (73 mg.) which was converted with methyl iodide into the *methiodide* (XLII) (85 mg.), m. p. >325° (sublime) (from methanol), $\lambda_{max.}$ (Nujol) 3·00 (OH) and 6·13 μ (C=C), $\lambda_{max.}$ 219·5 mμ (ε 20,300) (Found: C, 61·65; H, 8·1; I, 24·65; N, 2·35. $C_{27}H_{42}INO$ requires C, 61·95; H, 8·1; I, 24·2; N, 2·7%).

Attempted Base-catalysed Equilibration of the cis-*Isomer* (XIIIa).—(a) *With* 0·1% *methanolic potassium hydroxide.* The *cis*-isomer (100 mg.) in 0·1% methanolic potassium hydroxide (30 ml.) was set aside at room temperature overnight. Concentration of the solution *in vacuo* at room temperature, dilution with water, and extraction with methylene chloride gave the unchanged material whose n.m.r. spectrum showed no formation of the *trans*-isomer.

(b) *With* 10% *aqueous methanolic potassium hydroxide.* The *cis*-isomer (100 mg.) was refluxed with a solution of potassium hydroxide (3 g.) in methanol (30 ml.) containing 5% of water for 3 hr. Usual working up gave a crude product whose n.m.r. spectrum showed that it is a mixture of *cis*- (XIIIa) and *trans*- (XIIIa′) isomers in a ratio of 1 : 1.

Acetylation of Dihydrocyclomicrophylline-F.—The compound (IIb) (100 mg.) was acetylated

with pyridine–acetic anhydride (4 : 1; 5 ml.) at room temperature overnight. The product was isolated in the usual way to afford the NO-*triacetate* (XLIII) (120 mg.), m. p. 298—300° (from acetone), $[\alpha]_D$ +18°, $\lambda_{max.}$ 3·00 (NH), 5·81 (*O*-acetate), and 6·05 and 6·51 μ (NH·$COCH_3$), τ 9·65 and 9·45 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·22, 9·02, and 8·85 (9H, quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 8·06 (3H), 7·91 (3H), and 7·76 (9H) (three acetyl and two *N*-methyl), 6·24 (2H, singlet, CH_2OAc), and 5·92 (1H, multiplet, >*CH*OAc) (Found: C, 70·25; H, 9·8; N, 5·0. $C_{32}H_{52}N_2O_5$ requires C, 70·55; H, 9·6; N, 5·15%).

Hydrolysis of the NO-*Triacetate* (XLIII).—The triacetate (60 mg.) was boiled with 3% methanolic potassium hydroxide solution (20 ml.) for 10 min. The N-*monoacetate* (XLIV) (46 mg.) had m. p. 278—279° (from ethyl acetate), $[\alpha]_D$ +3·6°, $\lambda_{max.}$ 2·92 (OH), 3·05 (NH), and 6·10 and 6·40 μ (NH·$COCH_3$), τ 9·65 and 9·45 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·38, 9·02, and 8·86 (9H, quaternary methyl), 9·10 (3H, doublet, *J* 7 c./sec., tertiary methyl), 7·97 (3H, *N*-acetyl), and 7·73 (6H, *N*-methyl) (Found: C, 73·25; H, 10·25; N, 6·1. $C_{28}H_{48}N_2O_3$ requires C, 73·0; H, 10·5; N, 6·1%).

Formation of the N-*Isopropylidene Derivative* (XLV) *of Dihydrocyclomicrophylline-F*.—The compound (IIb) (100 mg.) was heated under reflux with acetone (30 ml.) for 1 hr. After removal of the solvent, the product was crystallised from acetone to yield the N-*isopropylidene derivative* (XLV) as needles (97 mg.), m. p. 229—230°, $[\alpha]_D$ +75°, $\lambda_{max.}$ 2·82 (OH), 3·00 (OH), and 6·11 μ (>C=N–), τ 9·68 and 9·38 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·03 (6H) and 8·86 (3H) (quaternary methyl), 9·12 (3H, doublet, *J* 7 c./sec., tertiary methyl), 8·62 and 8·59 (6H, *N*-isopropylidene methyl), 7·75 (6H, *N*-methyl), 6·47 (2H, singlet, CH_2OH), and 5·94 (1H, multiplet, >*CH*OH) (Found: C, 75·65; H, 11·1; N, 6·25. $C_{29}H_{50}N_2O_2$ requires C, 75·95; H, 11·0; N, 6·1%).

The *N*-isopropylidene derivative was readily hydrolysed by warming on a water-bath with 50% aqueous acetic acid for 1 hr. to the parent compound.

Formation of the N-*Monoacetate* (XLIV) via *Acetylation of the* N-*Isopropylidene Derivative* (XLV).—The *N*-isopropylidene derivative (100 mg.) in pyridine (5 ml.) was treated with acetic anhydride (1 ml.) at room temperature overnight. After usual working up, the product was obtained as a non-crystalline mass, $\lambda_{max.}$ ($CHCl_3$) 2·86 (OH), 5·80 (*O*-acetate), and 6·02 μ (*N*-acetyl). This material (124 mg.) was hydrolysed with boiling 3% methanolic potassium hydroxide (30 ml.) for 0·5 hr. The mixture was worked up in the usual way to afford a crystalline product (60 mg.), identical with the *N*-monoacetate (XLIV) described above.

Catalytic Hydrogenation of the N-*Isopropylidene Derivative* (XLV).—The *N*-isopropylidene derivative (100 mg.) in glacial acetic acid (10 ml.) was hydrogenated with platinum oxide (10 mg.) in the usual manner. Crystallisation of the product from acetone gave the N-*isopropyl derivative* (XLVI) (83 mg.) as needles, m. p. 234—235°, [α] +65°, $\lambda_{max.}$ 2·92 (OH) and 3·13 μ (NH), τ 9·68 and 9·49 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·03 (3H) and 9·00 (6H) (quaternary methyl), 8·89 (6H, *N*-isopropyl methyl), 7·75 (6H, *N*-methyl), 6·50 and 6·25 (2H, quadruplet centred at 6·38, *J* 10 c./sec., CH_2OH), and 5·92 (1H, multiplet, >*CH*OH) (Found: C, 75·45; H, 11·2; N, 5·95. $C_{29}H_{52}N_2O_2$ requires C, 75·6; H, 11·4; N, 6·1%).

The N-*Isopropylidene Monomethiodide* (XLVII).—The *N*-isopropylidene derivative (100 mg.) was treated with methyl iodide (1 ml.) in acetone (30 ml.) and the mixture kept at room temperature overnight. The deposited crystals were filtered and recrystallised from acetone to give the *monomethiodide* (XLVII) (94 mg.) as needles, m. p. 282—284° (decomp.), $\lambda_{max.}$ 2·99 μ (OH, sharp) (Found: C, 56·5; H, 8·85; I, 20·54; N, 4·3. $C_{30}H_{53}IN_2O_2,2H_2O$ requires C, 56·6; H, 9·0; I, 20·3; N, 4·4%).

Conversion of the Monomethiodide (XLVII) *into Dihydrocyclomicrophylline-C*.—The monomethiodide (100 mg.) in methanol (30 ml.) was shaken with moist silver oxide (0·5 g.) at room temperature for 10 min. After filtration, the filtrate was concentrated *in vacuo* and the residue was distilled at 200—210°/0·04 mm., to furnish a crystalline product (65 mg.) identical with dihydrocyclomicrophylline-C (Vc).

Conversion of the N-*Isopropylidene Derivative* (XLV) *into Dihydrocyclomicrophylline-A*.—The *N*-isopropylidene derivative (150 mg.) in absolute methanol (20 ml.) was refluxed with methyl iodide (2 ml.) for 3 hr. Evaporation of the solution gave an amorphous methiodide (180 mg.), which was treated with moist silver oxide (1 g.) in methanol for 10 min. Pyrolysis of the product as described above furnished a crystalline distillate (106 mg.) identical with dihydrocyclomicrophylline-A (IIa).

Cyclomicrobuxine (III). This compound was isolated in about 0·0005—0·001% yield from the weak-base fractions of both *B. microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino and *B. microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino. It had m. p. 178—180° (from acetone), $[\alpha]_D$ +172°, $\lambda_{max.}$ 2·98 (OH), 5·89 (methyl ketone), and 6·07 and 11·12 μ (terminal methylene), τ 9·93 and 9·67 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·08 and 8·79 (6H, quaternary methyl), 7·86 (3H, methyl ketone), 7·66 (6H, *N*-methyl), 6·96 (1H, doublet, *J* 7 c./sec., 17α-H), 5·20 (1H, multiplet, >C*H*OH), and 5·35 and 5·04 (2H, doublets, *J* <1 c./sec., terminal methylene). Rotatory dispersion: $[\phi]_{700}$ +405°, $[\phi]_{589}$ +675°, $[\phi]_{400}$ +2030°, $[\phi]_{350}$ +3510°, $[\phi]_{306}$ +9590° (peak), $[\phi]_{265}$ −3920° (trough), and $[\phi]_{250}$ −1760° (Found: C, 77·95; H, 10·15; N, 3·8. $C_{25}H_{39}NO_2$ requires C, 77·85; H, 10·2; N, 3·65%).

Acetylation of Cyclomicrobuxine.—The compound (III) (50 mg.) was treated with pyridine–acetic anhydride (3 : 1; 4 ml.) and left at room temperature overnight. The mixture was poured into water, made alkaline with ammonia, and the product was extracted with methylene chloride. Washing of the extract with water, drying, and evaporation *in vacuo* left a residue (57 mg.) which was dissolved in benzene and chromatographed on alumina (2 g.). Elution with benzene afforded the O-*acetate* (XLVIII) (50 mg.), m. p. 232—233° (from acetone), $[\alpha]_D$ +146°, $\lambda_{max.}$ 5·80 (acetate), 5·88 (methyl ketone), and 6·07 and 11·12 μ (terminal methylene), τ 9·95 and 9·68 (2H, two doublets, *J* 4 c./sec., cyclopropane), 9·05 and 8·85 (6H, quaternary methyl), 8·01 (3H, acetyl methyl), 7·85 (3H, methyl ketone), 7·66 (6H, *N*-methyl), 6·82 (1H, doublet, *J* 10 c./sec., 17α-H), 5·36 and 5·02 (2H, doublets, *J* <1 c./sec., terminal methylene), and 4·40 (1H, multiplet, >C*H*OAc) (Found: C, 75·7; H, 9·7; N, 3·35. $C_{27}H_{41}NO_3$ requires C, 75·85; H, 9·65; N, 3·3%).

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