1242. *Buxus Alkaloids. Part VI.* The Constitutions of Cyclomicrobuxine, Cyclomicrobuxinine, and Alkaloid-L*

By T. Nakano and M. Hasegawa

The structure of cyclomicrobuxine (Ia) has been confirmed by the interrelation achieved with the known alkaloid cyclobuxine-D (IV). The isolation and structure of another new alkaloid, cyclomicrobuxinine (Ib), from *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino, is also described.

Alkaloid-L (VIa), isolated from *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino, has been correlated chemically with the known alkaloid cyclomicrophylline-A (VIII).

In Part IV [1] of this Series, the isolation of eight new alkaloids from *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino was described and the structures and absolute stereochemistry of seven of them were unequivocally established. The remaining alkaloid, cyclomicrobuxine, $C_{25}H_{39}NO_2$, is unique in that, unlike the other groups [2] of alkaloids, it contains only one nitrogen atom, and we tentatively assigned the structure (Ia) to it on the basis of spectroscopic data as well as from a biogenetic consideration. An additional amount of this alkaloid being available, chemical work on it has been continued and this structural assignment is now confirmed.

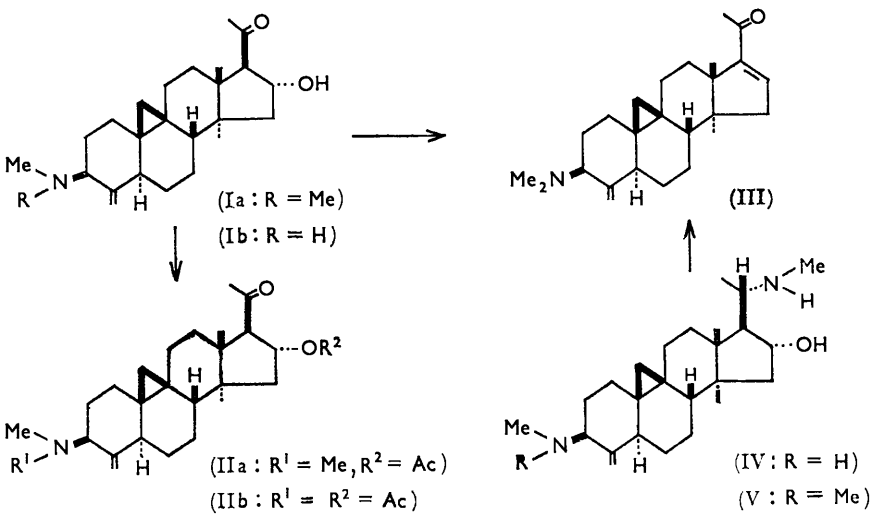
Chemical proof for the β-hydroxy-ketone system involved in cyclomicrobuxine was provided by the dehydration experiments. On being heated either with 2N-hydrochloric acid at 60° for 16 hr. or with 1% methanolic sodium hydroxide under reflux for 1·5 hr., the alkaloid furnished a ketone, $C_{25}H_{37}NO$. Its ultraviolet absorption spectrum [$\lambda_{max.}$ 243 mμ (log ε 3·99)] and also its infrared bands in potassium bromide disc at 6·01 (conjugated ketonic carbonyl) and 6·27 μ (double bond) demonstrated the presence of the αβ-unsaturated ketone system. The presence of one proton on the ethylenic linkage of the αβ-unsaturated ketone grouping was shown by a nuclear magnetic resonance signal at 3·32 τ ($J = 2·7$ c./sec.). This ketone must be formulated as (III) and attempts were

* Part V, T. Nakano and S. Terao, *J.*, 1965, 4537.

[1] T. Nakano and S. Terao, *J.*, 1965, 4512.

[2] See Part III, *Tetrahedron Letters*, 1964, 3679; and Parts IV and V, *J.*, 1965, 4512, 4537.

made to synthesise it from a related alkaloid, cyclobuxine-D.[3] We expected that, if partial methylation of cyclobuxine-D (IV) took place selectively at the nitrogen function at C-3, then Ruschig degradation [4] of the resulting *N*-methyl derivative (V) would lead to the desired ketone (III). Attempted partial methylation using a limited amount of methyl iodide in chloroform below 10° led to an *N*-methyl derivative, $C_{26}H_{44}N_2O$, the nuclear



magnetic resonance spectrum of which showed peaks at 7·57 (3H, *N*-CH_3) and 7·66 τ (6H, NMe_2). Since, in cyclobuxine-D, the *N*-methyl protons at the positions 3 and 20 occur at 7·53 and 7·57 τ, respectively, the signal at 7·66 τ corresponding to six protons is due to the *N*-methyl protons at C-3. This indicated that the methylation took a completely favourable course and the product is compound (V). Ruschig degradation [4] of this compound accompanied dehydration and the expected unsaturated ketone (III) was obtained. Direct comparison of this ketone with the corresponding derivative obtained from cyclomicrobuxine showed the complete identity of the two specimens (m. p., mixed m. p., optical rotations, and infrared spectra). Since the structure and stereochemistry [3a] of cyclobuxine-D has been proved rigorously, the above interconversion establishes not only the nuclear skeleton but also the stereochemistry of all the asymmetric centres in cyclomicrobuxine, except those at C-16 and C-17.

Since cyclomicrobuxine exhibited a positive Cotton effect {$[\alpha]_{306}$ +2470° (peak) and $[\alpha]_{266}$ −1050° (trough)}, the 17-methyl ketone substituent must have the β-configuration.[5] The negative molecular rotation increment ($\Delta M_D = -39°$) encountered upon acetylation of cyclomicrobuxine confirmed the α-orientation [6] of the 16-hydroxyl group. Thus, structure (Ia) (3β-dimethylamino-16α-hydroxy-14α-methyl-4-methylene-9β, 19-cyclo-5α-pregnan-20-one) may be considered as established for cyclomicrobuxine.

During the course of the isolation of cyclomicrobuxine, there was obtained another new alkaloid, named cyclomicrobuxinine, $C_{24}H_{37}NO_2$. This alkaloid, upon chromatography on silicic acid, is somewhat more polar than cyclomicrobuxine and was eluted with chloroform–methanol (9 : 1). It showed an infrared spectrum similar to that of

[3] (*a*) K. S. Brown, jun., and S. M. Kupchan, *J. Amer. Chem. Soc.*, 1964, **86**, 4424; (*b*) See also Part IV.
[4] L. Lábler and F. Šorm, *Coll. Czech. Chem. Comm.*, 1960, **24**, 2975; see also ref. 3(*a*).
[5] (*a*) R. Neher, P. Desaulles, E. Vischer, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, 1958, **41**, 1667; (*b*) C. Djerassi, " Optical Rotatory Dispersion," McGraw-Hill, New York, 1960, p. 128.
[6] D. K. Fukushima and T. F. Gallagher, *J. Amer. Chem. Soc.*, 1951, **73**, 196; L. F. Fieser and M. Fieser, " Steroids," Reinhold Publ. Corpn., New York, 1959, p. 179.

cyclomicrobuxine with the bands at 2·81 (hydroxyl), 5·88 (methyl ketone), and 6·06 and 11·12 μ (terminal methylene). Its nuclear magnetic resonance spectrum also showed a close similarity to that of cyclomicrobuxine with similar peaks assignable to the terminal methylene (5·18 and 5·40 τ), the 16β-proton (4·9—5·5 τ), the 17α-proton (6·95 τ, doublet, $J = 6·7$ c./sec.), an *N*-methyl (7·52 τ), a methyl ketone (7·86 τ), two quaternary *C*-methyls (8·78 and 9·09 τ), and a cyclopropyl methylene (9·70 and 9·93 τ, AB doublets, $J =$ 4 c./sec.). Apart from the signal position and intensity of an *N*-methyl group, the spectra of the two alkaloids are superimposable, and we therefore assumed that cyclomicrobuxinine is the *N*-demethylcyclomicrobuxine (Ib). On acetylation with acetic anhydride–pyridine, cyclomicrobuxinine yielded the ON-diacetate (IIb), $C_{28}H_{41}NO_4$, $\lambda_{max.}$ 5·78 (ester carbonyl), 5·86 (saturated ketone), and 6·10 μ (amide carbonyl).

Attempted *N*-methylation of cyclomicrobuxinine with formic acid–formalin resulted in the formation of a product, the infrared spectrum of which showed, besides the original carbonyl absorption at 5·87 μ, a band at 6·01 μ due to the conjugated carbonyl grouping. This indicated that during this *N*-methylation dehydration also took place and some of the product corresponding to the ketone (III) was formed. In order to complete dehydration this mixture, without further separation, was heated at 60° with 2N-aqueous hydrochloric acid, whereby a conjugated ketone was obtained pure. This compound was shown to be identical with the ketone (III) obtained earlier from cyclomicrobuxine.

The optical rotatory dispersion curve of cyclomicrobuxinine showed a very strong positive Cotton effect {$[\alpha]_{306}$ +2160° (peak) and $[\alpha]_{266}$ −1090° (trough)}, similar to that observed for cyclomicrobuxine, and this allows the assignment of the β-configuration [5] to the 17-methyl ketone grouping. The α-orientation of the 16-hydroxyl group was suggested by the nuclear magnetic resonance signal of the 16-proton of cyclomicrobuxinine diacetate (IIb) which exhibited the same pattern of splitting at 4·39 τ as octuplet ($J_{16\beta-15\alpha} =$ 2 c./sec., $J_{16\beta-17\alpha} = 6·7$ c./sec., and $J_{16\beta-15\beta} = 8·7$ c./sec.) as observed for that of cyclomicrobuxine acetate (IIa). Cyclomicrobuxinine is therefore formulated as 16α-hydroxy-14α-methyl-3β-methylamino-4-methylene-9β,19-cyclo-5α-pregnan-20-one.

Alkaloid-L,* $C_{27}H_{48}N_2$, is one of the seven alkaloids† which Schlittler *et al.*[7] isolated in 1949 from the leaves of *Buxus sempervirens* L. The most characteristic feature of this alkaloid is that it contains no oxygen function, which makes it easy to distinguish this alkaloid from the other six which contain one or two oxygen atoms. In the course of an investigation of the alkaloidal constituents of *Buxus microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino,‡ we have isolated an alkaloid which possesses the same molecular formula and other physical constants as Schlitter *et al.* recorded for alkaloid-L. The nuclear magnetic resonance spectrum of this alkaloid in pyridine solution showed the presence of one methylamino (7·49 τ, 3H), one dimethylamino (7·80 τ, 6H), one tertiary *C*-methyl (9·15 τ, 3H, doublet, $J = 6$ c./sec.), and a cyclopropylmethylene group (9·51 and 9·72 τ, 2H, AB doublets, $J = 4$ c./sec.). In addition, *four* quaternary *C*-methyl groups were observed as a sharp singlet at 8·87, 8·98, 9·02, and 9·10 τ. This fact, when coupled with the biogenetic relationship between these related alkaloids, suggested that this alkaloid has structure (VIa) or (VIb).

On *N*-methylation with formic acid–formalin the alkaloid furnished the *NN'*-dimethyl derivative, $C_{28}H_{50}N_2$, the nuclear magnetic resonance spectrum of which showed the presence of four *N*-methyl groups. This compound must correspond to (VII), and we planned to synthesise it from cylcomicrophylline-A [1] (VIII), the constitution of which has been

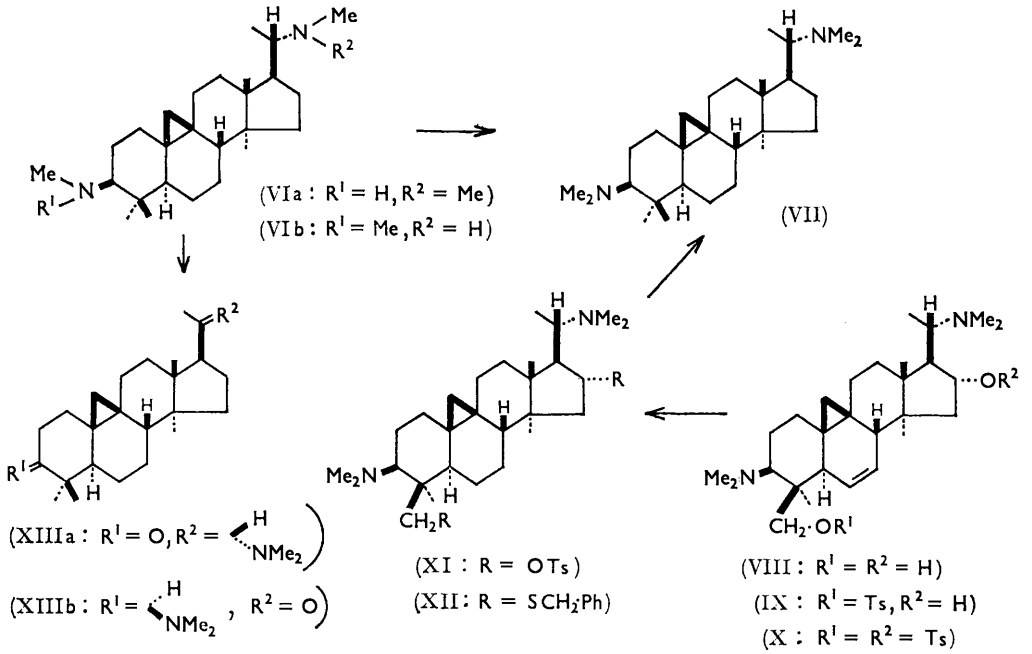
* For a preliminary communication, see T. Nakano and M. Hasegawa, *Tetrahedron Letters*, 1964, 3679.

† They were named Alkaloid-A, -B, -C, -D, -L, -M, and -N. See ref. 7.

‡ This plant is a variety of *B. microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino.

[7] E. Schlittler, K. Heusler, and W. Friedrich, *Helv. Chim. Acta*, 1949, **32**, 2209; K. Heusler and E. Schlittler, *ibid.*, 1949, **32**, 2226; W. Friedrich and E. Schlittler, *ibid.*, 1950, **33**, 873; E. Schlittler and W. Friedrich, *ibid.*, p. 878.

unambiguously established, by eliminating its hydroxyl groups reductively. A straightforward approach seemed to involve reduction of cyclomicrophylline-A ditoluene-*p*-sulphonate with lithium aluminum hydride. However, when the mono-ester (IX) was subjected to this reduction, only the parent compound (VIII) was recovered. Therefore, the following alternative route [8] had to be chosen. The di-ester (X), after hydrogenation to the dihydro-derivative (XI), was converted with sodium benzylmercaptide in dimethylformamide into the bisbenzyl thioether (XII), and the latter was desulphurised with Raney



nickel, whereby the objective hydroxyl-free derivative (VII) was obtained. Direct comparison of this compound with *NN'*-dimethyl alkaloid-L, obtained above, showed that they are identical.

The remaining problem is thus to determine whether the methylamino-group is located at C-3 or C-20, namely, by which of the structures (VIa) and (VIb) alkaloid-L should be represented. This differentiation can be made possible by subjecting the alkaloid to the Ruschig degradation,[4] since in this case either the six-membered ketone (XIIIa) or the methyl ketone (XIIIb) should be obtained. Treatment of this alkaloid with *N*-chlorosuccinimide and subsequent dehydrohalogenation of the resultant chloramine, followed by acid hydrolysis, gave the amino-ketone, $C_{26}H_{43}NO$, $\lambda_{max.}$ 5·84 μ (ketonic carbonyl). Its nuclear magnetic resonance spectrum showed no signal of a methyl ketone grouping but a signal corresponding to six protons at 7·78 τ. This signal apparently arises from a dimethylamino-group since it shifts downfield upon addition of trichloroacetic acid. The negative Cotton-effect curve [9] {$[\alpha]_{313}$ −250° (trough) and $[\alpha]_{269}$ +480° (peak)} of this amino-ketone confirmed that the ketone is not the 20-oxo- (XIIIb) but the 4,4'-dimethyl-3-oxo-derivative (XIIIa). This result established that alkaloid-L has structure (VIa) (4-dimethyl-20α-dimethylamino-14-methyl-3β-methylamino-9β, 19-cyclo-5α-pregnane).

Calame and Arigoni [10] have recently published the isolation and stucture of alkaloid-L

[8] C. A. Henrick and P. R. Jefferies, *Tetrahedron Letters*, 1964, 1507; A. S. Hussey, H. P. Liao, and R. H. Baker, *J. Amer. Chem. Soc.*, 1953, **75**, 4727.
[9] Ref. 5*b*, p. 90.
[10] J. P. Calame and D. Arigoni, *Chimia (Switz.)*, 1964, **18**, 185.

from *Buxus sempervirens* L. in the preliminary form. We note that concerning the structure of this alkaloid they have independently reached the same conclusion through a different sequence of reactions.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were obtained on a Hitachi EPI-S spectrometer with potassium bromide discs unless otherwise specified, and ultraviolet spectra of 95% ethanol solutions on a Shimadzu SV-50 spectrometer. Rotations were measured on a Rex NEP-2 photoelectric polarimeter for chloroform solutions and optical rotatory dispersion curves on a Jasco ORD/UV-5 spectrometer for methanol solutions at room temperature. Nuclear magnetic resonance spectra were measured for deuterochloroform solutions on a Varian A-60 spectrometer and had tetramethylsilane as an internal standard. Identity of products was established by mixed m. p.s, rotations, infrared spectra (potassium bromide discs), and ultraviolet spectra, where necessary. Brockmann's standardised alumina (Merck) or Mallinckrodt silicic acid was used for chromatography. All extracts were dried over anhydrous magnesium sulphate before evaporation under reduced pressure.

Isolation of Cyclomicrobuxine (Ia) *and Cyclomicrobuxinine* (Ib).—The crude alkaloid extract (150 g.) [1] was chromatographed in benzene on alumina (1·2 kg.) which had been treated with a solution of 70% aqueous ethylamine (120 ml.) and set aside at room temperature overnight. Elution was effected with benzene. Fractions 1—4 (9·7 g.) were combined, dissolved in ether, and the ether solution was extracted successively with Sörensen buffer solutions of pH 6·3, 5·5, and 4·9 (sodium citrate–sodium hydroxide system). The pH 5·5 and 4·9 extracts were combined, basified with ammonia, and extracted with chloroform. Washing of the chloroform extract with water, drying, and evaporation left a residue (7·4 g.). This was chromatographed in chloroform on silicic acid (100 g.) and elution with chloroform–methanol (9 : 1) gave two peaks. The first peak consisted of cyclomicrobuxine (225 mg.) and the second one of cyclomicrobuxinine (200 mg.).

Cyclomicrobuxine, after recrystallisation from acetone, showed m. p. 178—180°, $[\alpha]_D$ +172° (*c* 1·98) (Found: C, 77·6; H, 10·4; N, 3·75. $C_{25}H_{39}NO_2$ requires C, 77·85; H, 10·2; N, 3·65%), $\lambda_{max.}$ ($CHCl_3$) 2·77 (OH), 5·88 (C=O), and 6·07 and 11·08 μ (C=CH_2); see ref. 1 for other physical data.

Cyclomicrobuxinine was recrystallised from acetone to show m. p. 178—181°, $[\alpha]_D$ +152° (*c* 0·52) (Found: C, 73·95; H, 10·3; N, 3·65. $C_{24}H_{37}NO_2,H_2O$ requires C, 74·0; H, 10·1; N, 3·6%), $\lambda_{max.}$ ($CHCl_3$) 2·81 (OH), 5·88 (C=O), and 6·06 and 11·12 μ (C=CH_2); r.d. (*c* 0·32): $[\alpha]_{700}$ +94°, $[\alpha]_{589}$ +125°, $[\alpha]_{306}$ +2160° (peak), $[\alpha]_{266}$ −1090° (trough), $[\alpha]_{240}$ −156°.

Acetylation of Cyclomicrobuxine.—The compound (50 mg.) was acetylated with pyridine–acetic anhydride as described before.[1] Upon crystallisation from acetone *cyclomicrobuxine* O-*acetate* (IIa) (35 mg.) was obtained; m. p. 232—233°, $[\alpha]_D$ +146° (*c* 2·2) (Found: C, 76·1; H, 9·75; N, 3·75. $C_{27}H_{41}NO_3$ requires C, 75·85; H, 9·65; N, 3·3%), $\lambda_{max.}$ 5·76 (OAc), 5·85 (C=O), and 6·07 and 11·08 μ (C=CH_2), τ 9·95 and 9·67 (2H, AB doublets, J = 4 c./sec., cyclopropyl methylene), 9·05 and 8·87 (6H, quaternary C–Me), 8·01 (3H, OAc), 7·85 (3H, COMe), 7·65 (6H, NMe_2), 6·82 (1H, doublet, J = 6·7 c./sec., 17-H), 5·33 and 5·02 (2H, C=CH_2), and 4·93 (1H, octuplet, J = 8·7, 6·7, and 2 c./sec., CHOAc).

Acetylation of Cyclomicrobuxinine.—The compound (50 mg.) was treated with acetic anhydride–pyridine (1—5 ml.) at room temperature for 2 days. The solution was diluted with water, acidified with 10% hydrochloric acid, and extracted with chloroform. Crystallisation of the product from hexane–acetone yielded *cyclomicrobuxinine* ON-*diacetate* (IIb) (30 mg.), m. p. 213—215°, $[\alpha]_D$ +87° (*c* 0·67) (Found: C, 74·0; H, 9·2; N, 3·0. $C_{28}H_{41}NO_4$ requires C, 73·8; H, 9·05; N, 3·05%), $\lambda_{max.}$ 5·78 (OAc), 5·86 (C=O), 6·10 (NAc), and 11·21 μ (C=CH_2), τ 9·87 and 9·63 (2H, cyclopropyl methylene), 9·03 and 8·83 (6H, quaternary C–Me), 8·05—7·87 (6H, OAc and NAc), 7·83 (3H, COMe), 7·09 and 7·05 (3H, NMe with restricted rotation),[3a] 6·79 (1H, doublet, J = 6·7 c./sec., 17-H), 5·6—5·2 (2H, C=CH_2), and 4·39 (1H, octuplet, J = 8·7, 6·7, and 2 c./sec., CHOAc).

Dehydration of Cyclomicrobuxine.—The compound (40 mg.) in a mixture of 2N-hydrochloric acid (10 ml.) and methanol (5 ml.) was heated at 60° for 16 hr. After dilution with water, followed by basification with ammonia, the product was extracted with chloroform. The extract was washed with water, dried, and evaporated. The residue was chromatographed

in benzene on alumina (1·5 g.). The benzene eluate gave the *conjugated ketone* (III) (30 mg.), m. p. 138—139°, after recrystallisation from acetone, $[\alpha]_D$ +188° (*c* 0·16) (Found: C, 82·0; H, 10·25; N, 3·65. $C_{25}H_{37}NO$ requires C, 81·7; H, 10·15; N, 3·8%), $\lambda_{max.}$ 243 mμ (log ε 3·99), $\lambda_{max.}$ 6·01 (conjugated C=O), 6·27 (olefin), and 11·20 μ (C=CH_2), τ 9·92 and 9·60 (2H, AB doublets, J = 4 c./sec., cyclopropyl methylene), 9·00 and 8·78 (6H, quaternary C–Me), 7·72 (3H, COMe), 7·63 (6H, NMe_2), 5·31 and 5·01 (2H, C=CH_2), and 3·32 (1H, triplet, J = 2·7 c./sec., 16-H).

N-*Methylation of Cyclomicrobuxinine*.—The compound (60 mg.) was heated with formic acid (0·9 ml.) and formalin (0·6 ml.) on a boiling-water bath for 8 hr. After dilution with water, the solution was washed with ether, basified with ammonia, and extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated to afford the crude product (60 mg.) the infrared spectrum of which showed two bands, at 5·87 (methyl ketone) and 6·01 μ (conjugated ketone). This suggested that the *N*-methylation accompanied dehydration. In order to complete dehydration this product was heated with 2N-hydrochloric acid (10 ml.) and methanol (5 ml.) at 60° for 12 hr. The solution was diluted with water, basified with ammonia, and extracted with chloroform. The product obtained was purified by chromatography on alumina (3 g.) and subsequent crystallisation from acetone furnished a conjugated ketone (40 mg.), identical with the ketone (III) obtained by dehydration of cyclomicrobuxine.

Partial Methylation of Cyclobuxine-D (IV).—The compound (200 mg.) in chloroform (10 ml.) was treated with methyl iodide (0·1 ml.) and kept below 10° for 4 days. The resulting precipitate was collected (130 mg.), washed with a small amount of chloroform, and dissolved in 10% methanolic sodium hydroxide solution. The solution was diluted with water and extracted with chloroform. Washing of the extract with water, drying, and evaporation left the product (120 mg.) which was chromatographed in benzene–chloroform (1 : 1) on alumina (5 g.). Elution with the same solvent mixture and crystallisation from acetone furnished the N-*methyl derivative* (V) (30 mg.), m. p. 233—234°, $[\alpha]_D$ +104° (*c* 0·14) (Found: C, 77·0; H, 11·3. $C_{26}H_{44}N_2O$ requires C, 77·95; H, 11·05), τ 9·95 and 9·70 (2H, AB doublets, J = 4 c./sec., cyclopropyl methylene), 9·02 and 8·87 (6H, quaternary C-Me), 8·92 (3H, doublet, J = 6 c./sec., tertiary C–Me), 7·66 (6H, NMe_2), 7·57 (3H, NMe), 5·90 (1H, octuplet, J = 9·5, 7 and 3 c./sec., CHOH), and 5·35 and 5·05 (2H, C=CH_2).

Ruschig Degradation of the N-*Methyl Derivative* (V).—To a solution of the compound (80 mg.) in methylene chloride (5 ml.) was added *N*-chlorosuccinimide (30 mg.) in methylene chloride (5 ml.) and the solution was kept below 10° overnight. The solution was evaporated at room temperature, the residue dissolved in a solution of sodium (80 mg.) in dry methanol (8 ml.), and the solution refluxed for 2 hr. with exclusion of moisture. After removal of the solvent *in vacuo*, water was added, and the product was extracted with chloroform. The chloroform extract was washed with water, dried, and evaporated. The residue (80 mg.) was dissolved in methanol (5 ml.), 2N-hydrochloric acid (10 ml.) added, and the solution heated at 60° for 16 hr. Dilution with water, extraction into chloroform, and washing with water gave a product (40 mg.). Chromatography of this on alumina (1·5 g.) and elution with benzene, followed by crystallisation from acetone, yielded the conjugated ketone (III) (22 mg.), identical with the corresponding ketone obtained from cyclomicrobuxine.

Isolation of Alkaloid-L (VIa).—The air-dried leaves and twigs of *B. microphylla* Sieb. *et* Zucc. *var. suffruticosa* Makino *forma major* Makino, collected in Mikurajima, Tokyo, were combined (70 kg.), finely ground, and extracted twice with boiling ethanol (120 l.). The extracts were combined and concentrated *in vacuo* to 10 l. Aqueous citric acid solution (5%; 20 l.) was then added and the solution extracted with chloroform. The aqueous layer was basified with ammonia, extracted with chloroform, and the chloroform extract evaporated, yielding a sticky basic residue (200 g.). This material, dissolved in benzene, was chromatographed on alumina (2 kg.) which had been deactivated with a solution of 70% aqueous ethylamine (400 ml.) in benzene (2 l.). Elution was effected with benzene and each 500-ml. fraction was collected. Fractions 1—3 (52 g.) were combined and rechromatographed in benzene on alumina (500 g.), and elution with benzene gave a material (1·7 g.). Crystallisation of this from acetone furnished crude *alkaloid-L* (500 mg.). It showed m. p. 200—202°, after recrystallisation from methylene chloride–acetone, $[\alpha]_D$ +76° (*c* 1·09) (Found: C, 80·8; H, 12·1; N, 6·7. $C_{27}H_{48}N_2$ requires C, 80·95; H, 12·05; N, 7·0%).

Methylation of Alkaloid-L.—The compound (80 mg.) was heated with formic acid (1·5 ml.) and formalin (1·0 ml.) on a boiling-water bath for 2 hr. The solution was diluted with water,

basified with ammonia, and extracted with chloroform. The extract was washed with water, dried, and evaporated, yielding a crude product (80 mg.). Recrystallisation from acetone yielded the NN′-*dimethyl derivative* (VII) (50 mg.), m. p. 207—208°, $[\alpha]_D$ +31° (*c* 0·54) (Found: C, 81·4; H, 11·9; N, 6·9. $C_{28}H_{50}N_2$ requires C, 81·1; H, 12·15; N, 6·75%), τ 9·71 and 9·45 (2H, AB doublets, J = 4 c./sec., cyclopropyl methylene), 9·20, 9·07, and 9·02 (12H, quaternary C–Me), 9·17 (3H, doublet, J = 6 c./sec., tertiary C–Me), and 7·80 and 7·70 (12H, NMe_2).

*Conversion of Dihydrocyclomicrophylline-A Ditoluene-*p*-sulphonate* (XI) *into* NN′-*Dimethyl-Alkaloid-L* (VII).—Reduction of the mono-ester (IX) (160 mg.) with lithium aluminum hydride (800 mg.) in ether yielded cyclomicrophylline-A (VIII) (60 mg.) and a somewhat more polar substance (42 mg.) which was not further examined.

The di-ester (XI) was prepared by hydrogenation of cyclomicrophylline-A ditoluene-*p*-sulphonate (X) with platinum dioxide in acetic acid (see ref. 1). The diester (320 mg.) and toluene-ω-thiol (200 mg.) in dimethylformamide (4 ml.) was treated with sodium (55 mg.) and heated at 100° for 4 hr. Chloroform was added, and the solution was extracted with 10% hydrochloric acid. The aqueous extract was basified with ammonia and extracted with chloroform. The product (140 mg.), isolated in the usual way, was chromatographed in benzene on alumina (5 g.) and elution with benzene gave the bisbenzyl thioether (XII) (100 mg.) which, without further purification, was used for the next desulphurisation.

The bisbenzyl thioether (100 mg.) in 95% ethanol (15 ml.) was refluxed with W-7 Raney nickel [11] for 8 hr. The nickel was filtered and washed with chloroform. The filtrates were combined and evaporated to yield a product (35 mg.) which was chromatographed in benzene on alumina (3 g.). The product from the benzene eluate, after recrystallisation from acetone, gave the compound (VII) (15 mg.), identical with the *NN′*-dimethyl-alkaloid-L, obtained as described above.

Ruschig Degradation of Alkaloid-L.—The compound (150 mg.) and *N*-chlorosuccinimide (150 mg.) in methylene chloride (20 ml.) were set aside at room temperature overnight. The solvent was removed *in vacuo* at room temperature, and the residue was dissolved in dry methanol (40 ml.) and treated with sodium (400 mg.). The solution was refluxed for 1·5 hr., and, after removal of the solvent, the product was taken up in 2N-aqueous sulphuric acid and kept for 2 days. After dilution with water and extraction with chloroform, the product (100 mg.) was obtained, which was chromatographed in benzene on alumina (5 g.). Elution with benzene–ether (9 : 1) gave the *ketone* (XIIIa) (25 mg.), m. p. 197° (from 95% ethanol), $[\alpha]_D$ +14° (*c* 0·82) (Found: C, 80·15; H, 11·35; N, 3·75. $C_{26}H_{43}NO$ requires C, 81·0; H, 11·25; N, 3·65%), $\lambda_{max.}$ 5·84 μ (C=O), τ 9·97 and 9·44 (2H, AB doublets, J = 4 c./sec., cyclopropyl methylene), 9·13 (3H, doublet, J = 6 c./sec., tertiary C–Me), 9·07, 9·00, 8·95, and 8·90 (12H, quaternary C–Me), and 7·78 (6H, NMe_2); r.d. (*c* 0·15): $[\alpha]_{700}$ +3°, $[\alpha]_{589}$ +3°, $[\alpha]_{313}$ −250° (trough), $[\alpha]_{269}$ +480° (peak), $[\alpha]_{250}$ +370°.

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