Gardneria Alkaloids. Part 13. Structure of Gardmultine and Demethoxygardmultine; Bis-type Indole Alkaloids of *Gardneria multiflora* Makino

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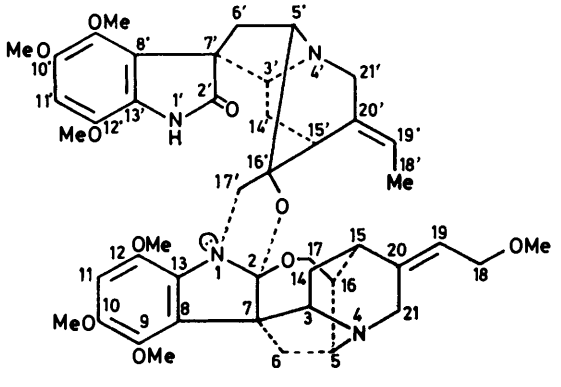
Structures of two bis-type alkaloids, gardmultine (1) and demethoxygardmultine (2), isolated from *Gardneria multiflora* Makino (Loganiaceae), have been elucidated on the basis of the chemical and spectroscopic evidence.

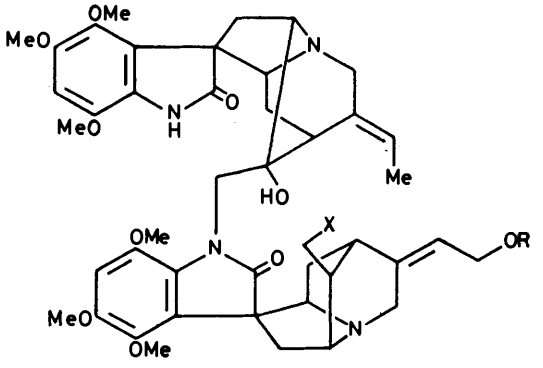
THE isolation procedure and physical properties of gardmultine (1) have been described in the earliest paper of our present series [1] where this compound was referred to as alkaloid E. The structure of (1) was deduced by chemical and spectroscopic means and the result was reported in the form of a short communication.[2] Since then we have carried out further work to obtain more direct proof for this unprecedented type of structure. Concurrently an *X*-ray crystallographic study was undertaken by Silverton and Akiyama.[3] During the course of the latter parallel study, a suspicion arose that there was a contaminant alkaloid in the crude batch of crystalline gardmultine (1).[3] After careful purification a new dimeric alkaloid, demethoxygardmultine (2), was isolated as an accompanying base. The present paper describes the structure elucidation of the two alkaloids. Some of the experiments concerned with the structural elucidation of gardmultine (1) which have been the subject of a preliminary communication [2] are given in the experimental part of this paper; slight corrections to the earlier reported physical data have been made now that pure gardmultine (1) and its derivatives have been obtained.

We proposed structure (1) for gardmultine on the basis of spectroscopic and chemical evidence. In particular the n.m.r. signal of the highly shielded C-18′ olefinic methyl group which appeared at δ 0.88 was the most important clue.[2] Only the stereochemical arrangement of the asymmetric centres and the indicated conformation of the pyramidal N-1 atom shown bring the olefinic methyl into the shielding region of the benzene ring of the lower monomeric half of the molecule.



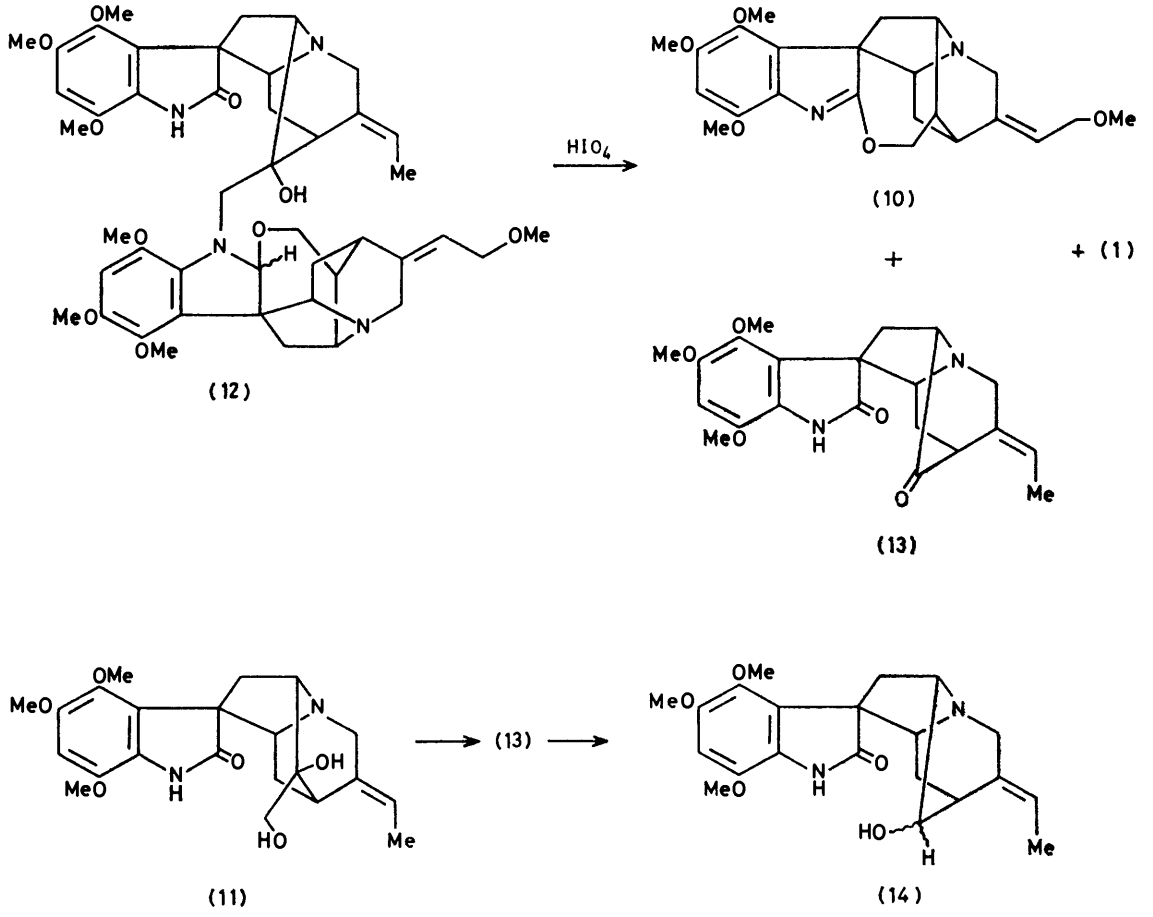
(1)

This novel structure of gardmultine (1) having a lactam acetal function clearly accounts for the formation of the bis-oxoindole derivatives (3)—(9) by the action of various acids. Treatment of compound (9) with potassium t-butoxide regenerated gardmultine (1), thus proving the unchanged skeletal structure of these acid-cleaved products.



	X	R
(3)	OAc	Me
(4)	Cl	Me
(5)	Cl	H
(6)	Br	Me
(7)	Br	H
(8)	OH	Me
(9)	OMs	Me

To obtain more direct evidence that the individual constituent bases were gardneramine (10) [4] and chitosenine (11),[5] cleavage of a dimeric derivative to give the monomeric units was attempted. Compound (12), derived from (1) on reduction with sodium borohydride in acetic acid,[6] was characterized on the basis of the singlet n.m.r. signal due to the aminoacetal proton at δ 4.74. Periodic acid oxidation of (12) in methanol gave a complex mixture of products from which gardneramine (10), chitosenine norketone (13), and gardmultine (1) were isolated. Chitosenine (11) [5] was oxidized with periodic acid to give the same norketone (13). All efforts, however, to crystallize the latter compound were unsuccessful. Further, since its mass spectrum showed a molecular ion peak with only 1% intensity of the base peak (produced by the characteristic loss of CO from the



SCHEME 1

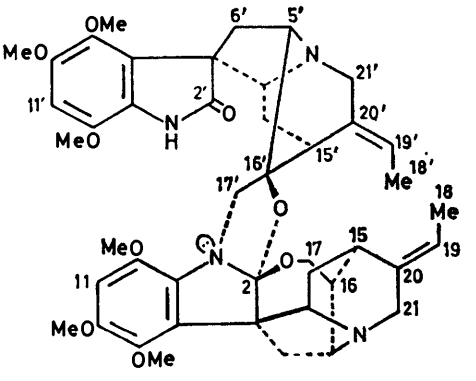
molecular ion), determination of the molecular formula of (13) by high-resolution mass-spectral measurement was impossible. The compound was therefore reduced with sodium borohydride to the alcohol (14) which was proved to have the required molecular formula by the high-resolution mass-spectral measurement (see Scheme 1).

As stated above, another structurally related alkaloid was isolated from a batch of crystals of gardmultine (1) after preparative t.l.c. and recrystallization. This new base, demethoxygardmultine (2), $C_{44}H_{52}N_4O_9$, m.p. 267—271 °C, $[\alpha]_D^{16}$ −81°, had u.v. and i.r. spectra which were similar to those of (1). The structure deduced for (2) is shown below.

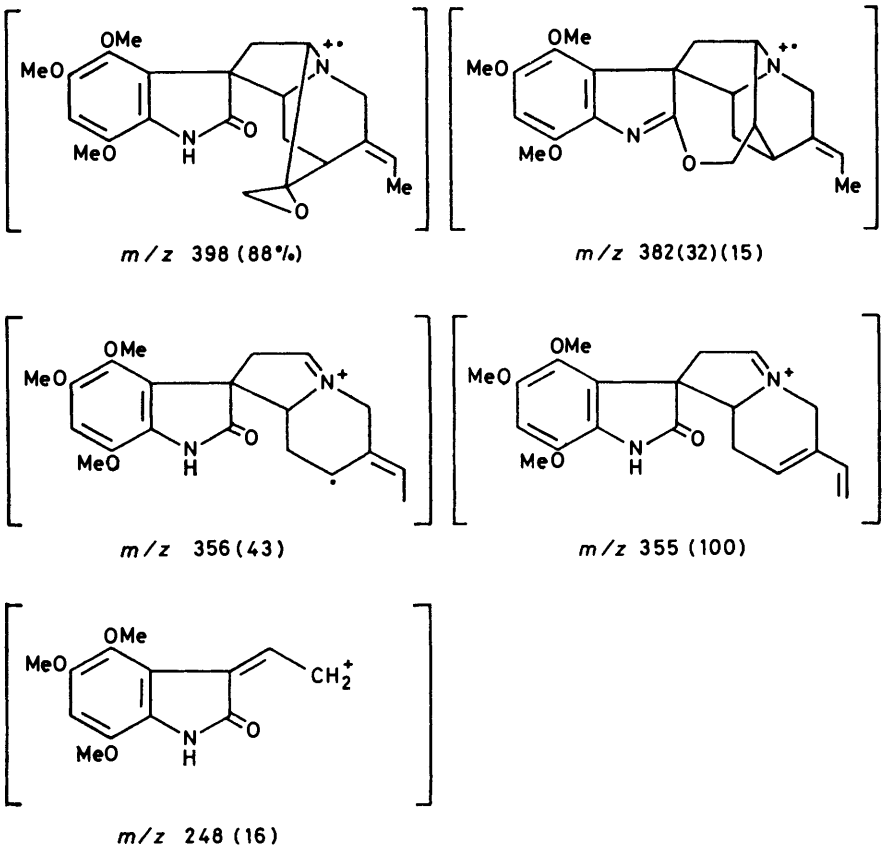
The mass spectrum of demethoxygardmultine (2) showed fragments assignable to the ions illustrated in Scheme 2,[2] suggesting that the constituent components were chitosenine (11) and demethoxygardneramine (15).[7]

The n.m.r. spectrum of demethoxygardmultine (2) revealed a doublet at δ 0.88, characteristic of a highly shielded, olefinic methyl signal; this was taken to indicate the same geometrical configuration for the ethylidene side-chain of the chitosenine part [C(18′)–C(20′)] and the same overall stereochemical construction for the molecule as that in gardmultine (1). The presence of a second ethylidene grouping was demonstrated by an olefinic methyl signal at δ 1.57 (d, J = 6.5 Hz) and an adjacent olefinic proton at δ 5.01 (q, J = 6.5 Hz) in the n.m.r. spectrum; this showed that the 18-methoxy-group in gardmultine (1) was replaced by hydrogen in compound (2).

When demethoxygardmultine was treated with dilute hydrobromic acid or hydrochloric acid, the amideacetal function underwent attack to produce the expected products (16) and (17) respectively.

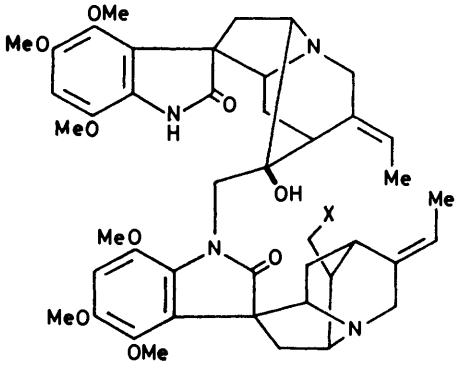


(2)



SCHEME 2 Mass spectral fragments of demethoxygardmultine (2)

The remaining point to be elucidated was the configuration of the ethylidene moiety in the lower half of the molecule. Earlier we reported a method, based on a ^{13}C n.m.r. study, for configurational assignments of



(16) X = Br
(17) X = Cl

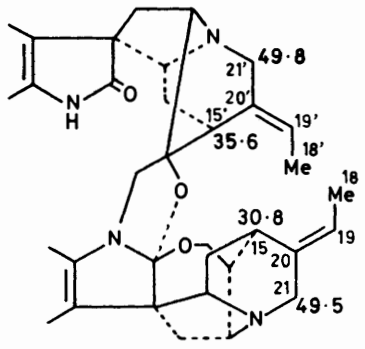
side-chain double-bonds in various Gardneria alkaloids [8] and the method described therein was applied here. The chemical shifts of C-15, C-21, C-15′, and C-21′ of demethoxygardmultine (2) are shown in Scheme 3. For comparison, the corresponding chemical shifts of the carbons of the side-chain moiety of gardmultine (1) are also shown. These results showed that C-15 of demethoxygardmultine (2) is shielded by the olefinic methyl carbon (C-18) by 7.3 p.p.m. compared with the corresponding carbon of gardmultine (1); this indicated an *E* configuration for C(19)=C(20) in the side chain. This assignment was in good accord with our former observations on the ^{13}C n.m.r. chemical shifts of the monomeric Gardneria alkaloids.[8]

EXPERIMENTAL

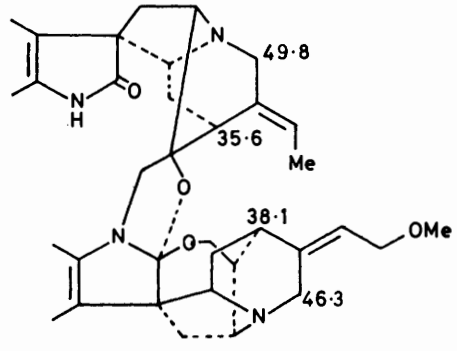
M.p.s are uncorrected. ^1H N.m.r. spectra were obtained on a JEOL MH-100 (100 MHz) spectrometer with $CDCl_3$ as the solvent unless otherwise specified. The chemical shifts are presented in δ (p.p.m.) from the internal standard ($SiMe_4$). ^{13}C N.m.r. spectra were run on a JEOL FX 60 FT NMR spectrometer operating at 15.04 MHz. $CDCl_3$ was used as the solvent and the chemical shifts are presented in δ (p.p.m) from the internal standard ($SiMe_4$). Mass spectra were recorded on Hitachi RMU 6E and 7M spectrometers. I.r. spectra were recorded on a Hitachi 215 instrument. U.v. spectra were obtained on a Hitachi EPS 3T spectrometer for methanol solutions unless otherwise specified.

The analytical t.l.c. work was done by using pre-coated plates, Silica Gel 60 F-254, Merck. Silica Gel 60 (70—230 mesh), Merck, and Al_2O_3 nach Brockmann, activity II—III, Merck, were used for column chromatography.

Physical Properties of Gardmultine (1).—M.p. 293—295 °C



(2)



(1)

SCHEME 3 ^{13}C N.m.r. chemical shifts of C-15, C-15′, C-21, and C-21′ of demethoxygardmultine (2) and gardmultine (1)

(colourless prisms from aqueous MeOH) (Found: C, 66.2; H, 6.85; N, 6.7. $C_{45}H_{54}N_4O_{10}\cdot 1/2H_2O$ requires C, 65.92; H, 6.76; N, 6.83%); $[\alpha]_D^{21}$ −92.5° (*c* 0.2 in $CHCl_3$); $\lambda_{max.}$ 213, 247, and 304 nm (log ε, 4.76, 4.17, and 3.84); $\nu_{max.}$ (KBr) 3 480 (NH) and 1 700 cm^{-1} (C=O); *m/z* 810 (M^+, 8%), 562 (3), 412 (33), 398 (97), 356 (51), 355 (100), and 248 (21); δ, 0.88 (3 H, d, *J* 6.8 Hz, 18′-H_3), 3.28 (3 H, s, aliph. OMe), 3.74 (6 H, s), 3.79 (9 H, s) and 3.84 (3 H, s) (arom. OMe × 6), 4.25 (1 H, d, *J* 12 Hz, 17-H), 3.56 (1 H, d, *J* 12 Hz, 17′-H), 4.62 (1 H, d, *J* 12 Hz, 17′-H), 4.93 (1 H, quart. tripl., $J_{18',19'}$ 6.8 Hz, $J_{19',21'}$ 1 Hz, 19′-H), 5.26 (1 H, td, $J_{18.19}$ 6.8 Hz, $J_{19.21}$ 1 Hz, 19-H), 6.36 and 6.40 (each 1 H, s, 11- and 11′-H), 7.59 (1 H, br s, NH); ^{13}C n.m.r., δ, 11.8 (C-18′), 22.7 (C-14′), 30.7 (C-14), 31.7 (C-6), 33.0 (C-6′), 35.6 (C-15′), 38.1 (C-15), 43.4 (C-16), 46.3 (C-21), 49.8 (C-21′), 53.4 (C-16′), 56.4 (arom. OMe), 57.2 (arom. OMe), 57.8 (aliph. OMe), 68.1 (C-18), 84.3 (C-2), 98.6 (C-11′), 99.5 (C-11), 112.7 (C-19′), 113.4 (C-19), and 179.3 (C-2′); c.d. $\lambda_{min.max.}$ 330 (Δε, +0.09), 315 (−0.23), 299 (+1.18), 265 (+3.01), and 238 nm (−24.3).

Reaction of Gardmultine (1) *with Acetic Acid.*—Gardmultine (1) (501 mg) was refluxed in acetic acid under argon for 3 h. The solvent was removed under reduced pressure and aqueous ammonia was added to the residue. Extraction with methylene chloride afforded a foamy residue which was subjected to column chromatography over silica gel (20 g). The acetate (3) was obtained from the fraction eluted with methylene chloride–methanol (5%) as a foam (517 mg), *m/z* 870 (M^+, 6%), 472 (45), 413 (100), 399 (39), 398 (25), 357 (39), 355 (21), and 248 (35); $\lambda_{max.}$ 260sh and 314 nm (log ε 4.10 and 3.86); $\nu_{max.}$ 3 420, 1 705, and 1 250 cm^{-1}; δ 1.42 (3 H, d, *J* 6.5 Hz, 18′-H_3), 2.03 (3 H, s, OAc), 3.30 (3 H, s, aliph OMe), 3.78 (6 H, s, arom. OMe × 2), 3.83 (9 H, s, arom. OMe × 3), 3.91 (3 H, s, arom. OMe), 4.49 (2 H, d, *J* 7.5 Hz, 17-H_2), 5.14 (2 H, m, 19- and 19′-H), 6.44 (1 H, s) and 6.46 (1 H, s) (11- and 11′-H), and 7.76 (1 H, br s, NH).

Reaction of Gardmultine (1) *with Dilute Hydrochloric Acid.*—Gardmultine (1) (229 mg) was heated in 2M-HCl (4 ml) for 45 min under reflux. The solution was diluted with ice–water, basified with aqueous ammonia, and extracted with chloroform. The crude product (228 mg) was chromatographed over silica gel (25 g). From the fraction eluted with chloroform–methanol (2%), 67 mg of the chloride (4) was obtained in a crystalline state, m.p. 166—172 °C (Found: C, 62.15; H, 6.6; N, 6.2. $C_{45}H_{55}N_4O_{10}Cl\cdot H_2O$ requires C, 62.45; H, 6.64; N, 6.47%); $\lambda_{max.}$ 260sh and 313.5 (log ε 4.14 and 3.92); $\nu_{max.}$ (KBr) 3 470, 1 730, and 1 710 cm^{-1}; *m/z* no M^+, 810 (33%), 796 (17), 413 (61), 412 (50), 399 (83), 398 (89), 384 (33), 357 (100), 356 (61), 355 (78), and 248 (42); δ 1.42 (3 H, dt, $J_{18',19'}$ 6 Hz, $J_{18',21'}$ 1 Hz, 18-H_3) 3.29 (3 H, s, 18-OMe), 3.77 (6 H, s, arom. OMe × 2), 3.82 (9 H, s, arom. OMe × 3), 3.89 (3 H, s, arom.-OMe), 5.2 (2 H, m, 19- and 19′-H), 6.44 (2 H, s, 11- and 11′-H), and 7.37 (1 H, br s, NH); ^{13}C n.m.r. δ 12.3 (C-18′), 29.6 (C-15′), 35.7 (C-15), 46.6 (C-21), 49.5 (C-21′), 56.4, 56.6, 56.9, and 61.8 (arom. OMe), 57.8 (18-OMe), 68.0 (C-18), 70.6 (C-17), 98.4 and 99.1 (C-11 and -11′), 113.1 (C-19′), 114.1 (C-19), 139.0 (C-20′), 147.8 (C-20), 179.6 (C-2 and -2′).

From the fraction eluted with chloroform–methanol (10%), the diol (5) (20 mg) was obtained as colourless needles, m.p. 189—194 °C (Found: C, 61.45; H, 6.4; N, 6.15. $C_{44}H_{53}N_4O_{10}Cl\cdot H_2O$ requires C, 62.07; H, 6.51; N, 6.58%), $\nu_{max.}$ (KBr) 3 480 and 1 710 cm^{-1}; $\lambda_{max.}$ 260sh and 314 (log ε 4.13 and 3.90); *m/z* no M^+, 399 (59%), 385 (63), 358 (63), 357 (100), 356 (44), 355 (50), 343 (72), and 248 (38); δ 1.42 (3 H, dt, $J_{18',19'}$ 7 Hz, $J_{18',21'}$ 2 Hz, 18′-H_3), 3.77 (6 H, s, arom. OMe × 2), 3.82 (9 H, s, arom. OMe × 3), 3.89 (3 H, s, arom. OMe), 4.06 (2 H, dt, $J_{18,19}$ 7 Hz, $J_{18,21}$ 1 Hz, 18-H_2), 5.2 (2 H, m, 19- and 19′-H), 6.43 (1 H, s) and 6.45 (1 H, s) (11- and 11′-H), and 7.42 (1 H, br s, NH); ^{13}C n.m.r. δ 12.3 (C-18′), 29.6 (C-15′), 35.5 (C-15), 46.5 (C-21), 49.6 (C-21′), 56.4, 56.7, 57.0 and 61.8 (arom. OMe), 70.6 (C-17), 98.3 and 99.0 (C-11 and -11′), 113.1 (C-19′), 116.9 (C-19), 139.0 (C-20′), 146.6 (C-20), 179.3 and 179.6 (C-2 and -2′).

Reaction of Gardmultine (1) *with Dilute Hydrobromic Acid.*—Gardmultine (1) (320 mg) was treated with 2M-HBr (10 ml) in a manner similar to that described above to give the two bromine-containing products (6) (147 mg) and (7) (60 mg): (6) formed colourless needles from methanol–water, m.p. 158—162 °C (Found: C, 60.4; H, 6.45; N, 5.95. $C_{45}H_{55}N_4O_{10}Br\cdot 1/2H_2O$ requires C, 60.00; H, 6.27; N, 6.22%), $\lambda_{max.}$ 220, 260, 273sh, and 314 nm (log ε 4.67, 4.12, 3.83, and 3.89); $\nu_{max.}$ 3 470, 1 720, and 1 700 cm^{-1}; *m/z* no M^+, 399 (8%), 398 (17), 383 (32), 237 (20), 223 (33), and 208 (23); δ 1.42 (3 H, dt, $J_{18',19'}$ 7 Hz, $J_{18',21'}$ 2 Hz, 18′-H_3), 3.30 (3 H, s, aliph. OMe), 3.78 (6 H, s, arom. OMe × 2), 3.84 (9 H, s, arom. OMe × 3), 3.90 (3 H, s, arom. OMe), 4.07 (1 H, s, 16′-OH), 5.04—5.40 (2 H, m, 19- and 19′-H), 6.44 (1 H, s) and 6.46 (1 H, s) (11- and 11′-H), and 7.52 (1 H, br s, NH): (7) formed colourless needles from methanol–water, m.p. 188—192 °C, $\lambda_{max.}$ 260sh and 314 nm (log ε 4.14

and 3.89); $\nu_{max.}$ 3 440, 1 720, and 1 700 cm^{-1}; *m/z* no M^+, 385 (6%), 343 (12), 249 (11), 237 (22), 223 (39), and 208 (25); δ 1.44 (3 H, dt, $J_{18',19'}$ 6.5 Hz, $J_{18',21'}$ 2 Hz, 18'-H_3), 3.76 (3 H, s, arom. OMe), 3.78 (3 H, s, arom. OMe), 3.84 (9 H, s, arom. OMe × 3), 3.90 (3 H, s, arom. OMe), 4.09 (2 H, d, *J* 6.5 Hz, 18-H_2), 5.02—5.46 (2 H, m, 19- and 19'-H), 6.44 (1 H, s) and 6.46 (1 H, s) (11- and 11'-H), and 7.44 (1 H, br s, NH).

Hydrolysis of Compound (3) *and Formation of Compound* (8).—A solution of compound (3) (472 mg) in ethanolic KOH (3%; 10 ml) and water (1 ml) was heated under reflux for 3 h. Work-up afforded a foamy residue (436 mg) which was subjected to chromatography on a column of silica gel (20 g). From the fraction eluted with 4—10% methanol in methylene chloride the diol (8) (244 mg) was obtained as an amorphous powder, $\lambda_{max.}$ 257 and 314 nm (log ε 4.12 and 3.88); $\nu_{max.}$ ($CHCl_3$) 3 415 and 1 705 cm^{-1}; *m/z* 828 (M^+, 3%), 430 (67), 415 (54), 399 (62), 398 (23), 385 (38), 357 (21), 355 (25), 249 (49), and 248 (33); δ 1.39 (3 H, dt, $J_{18',19'}$ 6.5 Hz, $J_{18',21'}$ 1 Hz, 18'-H_3), 3.25 (3 H, s, aliph. OMe), 3.80 (15 H, s, arom. OMe × 5), 3.89 (3 H, s, arom. OMe), 3.98 (2 H, d, *J* 6.5 Hz, 18-H_2), 5.14 (1 H, quart. tripl., $J_{18',19'}$ 6.5 Hz, $J_{19',21'}$ 1 Hz, 19-H), 5.25 (1 H, tt, $J_{18,19}$ 6.5 Hz, $J_{19,21}$ 1 Hz, 19-H), 6.41 (1 H, s) and 6.45 (1 H, s) (11- and 11'-H), and 7.88 (1 H, br s, NH).

The Mesylate (9).—Methanesulphonyl chloride (66 mg) was added to a solution of the diol (8) (248 mg) in dry pyridine (7 ml). Work-up followed by purification with column chromatography over silica gel gave compound (9) (219 mg) as a homogeneous powder, $\lambda_{max.}$ 261sh and 314 nm; $\nu_{max.}$ ($CHCl_3$) 3 415, 1 700, 1 334, and 1 173 cm^{-1}; δ 1.21 (3 H, dt, $J_{18',19'}$ 6 Hz, $J_{18',21'}$ 1 Hz, 18'-H_3), 3.07 (3 H, s, OSO_2CH_3), 3.30 (3 H, s, aliph. OMe), 3.74 (3 H, s, arom. OMe), 3.78 (3 H, s, arom. OMe), 3.82 (9 H, s, arom. OMe × 3), 3.90 (3 H, s, arom. OMe), 4.73 (2 H, d, *J* 9 Hz, 17-H_2), 5.18 (2 H, m, 19- and 19'-H), 6.44 (1 H, s) and 6.46 (1 H, s) (11- and 11'-H), and 7.62 (1 H, br s, NH).

Gardmultine (1) *from the Mesylate* (9).—Potassium t-butoxide (32 mg) was added to a solution of the mesylate (9) (170 mg) in t-butyl alcohol (10 ml), and the reaction mixture was heated for 1.5 h under reflux. The solvent was removed under reduced pressure and the residue was diluted with ice-water and extracted with methylene chloride; removal of solvent from the extract left a residue (135 mg). From a column of silica gel (10 g), 20 mg of gardmultine (1) was eluted with 4—10% $CHCl_3$–MeOH. Recrystallization of this from methanol gave pure crystalline material (11 mg), m.p. 278—280 °C, which was shown to be identical with an authentic specimen of gardmultine (1) by comparison of the i.r. spectra and mixed fusion.

Reduction of Gardmultine (1) *with* $NaBH_4$ *in Acetic Acid; Formation of Dihydrogardmultine* (12).—Gardmultine (1) (441 mg) was dissolved in acetic acid (8 ml) and $NaBH_4$ was added to the water-cooled solution in small portions during 1.5 h. Basification of the reaction solution with ammonia, extraction of the product with chloroform, and removal of the solvent afforded an amorphous residue (481 mg). Chromatography over silica gel gave compound (12) as an amorphous powder (346 mg), $\lambda_{max.}$ 251 and 311 nm; $\nu_{max.}$ ($CHCl_3$), 3 420 (NH, OH), and 1 710 cm^{-1} (C=O); *m/z* 812 (M^+, 7%), 752 (30), 427 (21), 399 (97), 367 (100), 357 (32), and 248 (20); δ 1.59 (3 H, d, *J* 6 Hz, 18'-H_3), 3.31 (3 H, s, 18-OMe), 3.79 (9 H, s), 3.82 (6 H, s) and 3.86 (3 H, s) (arom. OMe × 6), 2.44 (1 H, s, OH), 4.74 (1 H, s, 2-H), 5.16 (2 H, m, 19- and 19'-H), 6.40 (2 H, s, 11- and 11'-H), and 7.45 (1 H, br s, NH); ^{13}C n.m.r. δ 12.3 (C-18'), 43.4 (C-16), 73.9 (C-16'), 107.5 (C-2), and 180.4 (C-2').

Periodic Acid Oxidation of Compound (12).—To a solution of dihydrogardmultine (12) (50 mg) in methanol (6 ml), 1N-HIO_4 aqueous solution (0.3 ml, 4.8 equiv.) was added and the reaction mixture was stirred at 30—35 °C for 7 d. Repetition of the same reaction on a similar scale gave 489 mg of a mixture of the reaction products. Column chromatographic separation of the mixture on silica gel with chloroform containing 0.5—1% methanol as eluant afforded chitosenine norketone (13) (47 mg), which was further purified using a column of Al_2O_3 (10 g) to give 30 mg of the pure material as an amorphous powder: (13) *m/z* 384 (M^+, 1%) and 356 (M^+ − CO, 100); $\lambda_{max.}$ 218, 260sh and 314 nm (log ε 4.22, 3.65, and 3.47); $\nu_{max.}$ 3 425 (NH) and 1 725 cm^{-1} (C=O); δ 1.64 (3 H, dt, $J_{18,19}$ 7 Hz, $J_{18,21}$ 2 Hz, 18-H_3), 3.81 (3 H, s, arom. OMe), 3.84 (6 H, s, arom. OMe × 2), 5.37 (1 H, quart. tripl., $J_{18,19}$ 7 Hz, $J_{19,21}$ 2 Hz, 19-H), 6.47 (1 H, s, 11-H), and 7.92 (1 H, br s, NH); c.d. $\lambda_{max.min.}$ (*c* 0.01, MeOH) 331 (Δε +1.22), 294 (−0.12), 260 (+2.04), and 233 nm (−15.9). The sample of the norketone (13) obtained here was identical with that prepared from chitosenine (11) as described below on the basis of chromatographic behaviour and i.r. spectral evidence.

From the fraction eluted with chloroform containing 1—3% methanol, crude gardneramine (10) (51 mg) was obtained. Further purification on a column of Al_2O_3 (5 g) gave gardneramine (10) (20 mg), m.p. 129—132 °C, $\lambda_{max.}$ 264, 272sh, 281sh, and 323 nm (log ε 3.67, 3.65, 3.42, and 3.59), $\nu_{max.}$ (KBr) 1 580 cm^{-1} (C=N); *m/z* 412 (M^+, 100%), 397 (10), 381 (23), 367 (11), and 259 (12); c.d. (*c* 0.01, MeOH); $\lambda_{max.min.}$ 321, 265, 243, and 214 nm (Δε −1.75, −0.91, +2.10, and −16.9). Mixed fusion and comparison of the i.r. spectra proved the obtained material to be gardneramine (10).

From the fraction eluted with 3—5% MeOH–$CHCl_3$, 209 mg of gardmultine (1) was obtained. Recrystallization from methanol gave 121 mg of the pure material, m.p. 293—295 °C, $\nu_{max.}$ (KBr) 3 490 (NH) and 1 700 cm^{-1} (C=O); *m/z* 810 (M^+, 10%), 412 (12), 398 (100), 356 (88), and 248 (12). Identification of this material as gardmultine (1) was made by mixed fusion and comparison of their i.r. spectra.

Preparation of the Norketone (13) *from Chitosenine* (11).—To a solution of chitosenine (11) (49 mg) in methanol (10 ml), 1N-HIO_4 (0.9 ml) was added and the mixture was stirred at room temperature. Work-up and column chromatography purification of the product over Al_2O_3 gave the norketone (13) (33 mg) as an amorphous powder, $\lambda_{max.}$ 218, 260sh, and 315 nm (log ε 4.23, 3.68, and 3.47); $\nu_{max.}$ ($CHCl_3$) 3 425 (NH) and 1 725 cm^{-1} (C=O); *m/z* 384 (M^+, 0.5%), 356 (M^+ − CO, 100), 341 (15), 325 (7), 313 (10), and 248 (5); δ 1.63 (3 H, dt, $J_{18,19}$ 7 Hz, $J_{18,21}$ 2 Hz, 18-H_3), 3.79 (3 H, s, arom. OMe), 3.82 (6 H, s, arom. OMe × 2), 5.34 (1 H, quart. tripl., $J_{18,19}$ 7 Hz, $J_{19,21}$ 2 Hz, 19-H), 6.43 (1 H, s, 11-H), and 7.72 (1 H, br s, NH); c.d. (*c* 0.01, MeOH) $\lambda_{max.min.}$ (Δε) 331 (+1.16), 296 (−0.12), 260 (+1.63), and 234 nm (−16.3).

Reduction of the Norketone (13) *with* $NaBH_4$.—To a solution of chitosenine norketone (13) (26 mg) in methanol (4 ml), $NaBH_4$ (26 mg) was added and the mixture was stirred at room temperature. Work-up gave the alcohol (14) (23 mg) as an amorphous powder, $\lambda_{max.}$ 255 and 314 nm; $\nu_{max.}$ ($CHCl_3$) 3 300 and 1 680 cm^{-1}; *m/z* 386 (M^+, 100%), 357 (62), and 248 (11); *m/z* 386.184. $C_{21}H_{26}H_2O_5$ requires 386.184; δ 1.63 (3 H, dt, $J_{18,19}$ 7 Hz, $J_{18,21}$ 1 Hz,

18-H_3), 3.76 (3 H, s, arom. OMe), 3.85 (6 H, s, arom. OMe × 2), 5.21 (1 H, m, 19-H), 6.47 (1 H, s, 11-H), and 8.33 (1 H, br s, NH).

Demethoxygardmultine (2).—A batch of a crude preparation of gardmultine (1) (570 mg) from the natural source (*Gardneria multiflora* Makino) was submitted to silica-gel layer chromatography using the solvent system MeOH–$CHCl_3$ (15 : 85); 150 mg of demethoxygardmultine (2) was obtained. Recrystallization from methanol gave an analytical sample, m.p. 267—271 °C, $[\alpha]_D^{16}$ −81° (*c* 0.2, $CHCl_3$) (Found: C, 67.6; H, 7.0; N, 6.95. $C_{44}H_{52}N_4O_9$ requires C, 67.67; H, 6.71; N, 7.17%), $\lambda_{max.}$ 213, 247, and 304 nm (log ε 4.75, 4.17, and 3.82); $\nu_{max.}$ (KBr) 3 430 and 1 700 cm^{-1}; *m/z* 780 (M^+, 8%), 532 (2), 398 (88), 382 (32), 356 (43), 355 (100), and 248 (16); δ 0.88 (3 H, d, *J* 6.5 Hz, 18′-H_3), 1.57 (3 H, d, *J* 6.5 Hz, 18-H_3), 3.77 (9 H, s, arom. OMe × 3), 3.83 (6 H, s, arom. OMe × 2), 3.87 (3 H, s, arom. OMe), 4.27 (1 H, d, *J* 13 Hz, 17-H), 4.65 (1 H, d, *J* 13 Hz, 17′-H), 4.94 (1 H, q, *J* 6.5 Hz, 19′-H), 5.01 (1 H, q, *J* 6.5 Hz, 19-H), 6.37 (1 H, s) and 6.43 (1 H, s) 11- and (11′-H), and 8.01 (1 H, s, NH); ^{13}C n.m.r. δ 11.8 (C-18′), 12.6 (C-18), 22.7 (C-14′), 29.8 (C-14), 30.8 (C-15), 31.7 (C-6), 33.0 (C-6′), 35.6 (C-15′), 43.1 (C-16), 49.5 and 49.8 (C-21 and -21′), 54.3 (C-16′), 56.4 and 57.2 (arom. OMe), 84.2 (C-2), 98.6 and 99.5 (C-11 and -11′), 110.5 (C-19), 112.7 (C-19′), and 179.3 (C-2′); c.d. (*c* 0.02, $CHCl_3$) $\lambda_{max,min.}$ (Δε) 330 (+0.09), 315 (−0.23), 299 (+1.18), 265 (+3.01), and 238 nm (−24.3).

Reaction of Demethoxygardmultine (2) *with Dilute Hydrobromic Acid.*—Demethoxygardmultine (2) (80 mg) was dissolved in 2M-HBr (3 ml) and the solution was heated under reflux for 45 min. Ammoniacal ice–water was added to the solution which was then extracted with chloroform. Removal of the solvent afforded a residue (96 mg) which was chromatographed on silica gel (6 g). The eluate with benzene–ethyl acetate (1 : 1) was crystallized from methanol to give the bromide (16) (47 mg) as colourless needles, m.p. 210—216 °C (Found: C, 60.1; H, 6.2; N, 6.45. $C_{44}H_{53}N_4O_9Br\cdot H_2O$ requires C, 60.07; H, 6.30; N, 6.37%), $\lambda_{max.}$ 220, 260, 273sh, and 314 nm (log ε 4.69, 4.13, 3.85, and 3.88); $\nu_{max.}$ (KBr) 3 400, 1 725, and 1 700 cm^{-1}; *m/z* no M^+, 766 (5%), 752 (4), 621 (5), 607 (5), 399 (9), 398 (8), 385 (15), 368 (24), 343 (32), and 253 (31); δ 1.42 (3 H, dt, $J_{18',19'}$ 6.5 Hz, $J_{18',21'}$ 2 Hz, 18′-H_3), 1.59 (3 H, dt, $J_{18,19}$ 6.5 Hz, $J_{18,21}$ 2 Hz, 18-H_3), 3.76 (3 H, s), 3.78 (3 H, s), 3.84 (9 H, s) and 3.90 (3 H, s) (arom. OMe × 6), 4.11 (1 H, s, 16′-OH), 4.92—5.32 (2 H, m, 19- and 19′-H), 6.45 (2 H, s, 11- and 11′-H), and 7.48 (1 H, br s, NH).

Reaction of Demethoxygardmultine (2) *with Dilute Hydrochloric Acid.*—Demethoxygardmultine (2) (61 mg) was dissolved in 2M-HCl (3 ml) and the solution was heated under reflux for 45 min. Work-up afforded the reaction products (64 mg) from which crystalline (17) (30 mg) was obtained after column chromatography on silica gel (6 g) and recrystallization from methanol; it had m.p. 201—205 °C (decomp.) (Found: C, 63.0; H, 6.5; N, 6.45. $C_{44}H_{53}ClN_4O_9\cdot H_2O$ requires C, 63.26; H, 6.39; N, 6.71%); $\lambda_{max.}$ 260sh and 314 nm (log ε 4.16 and 3.92); $\nu_{max.}$ (KBr) 3 510, 1 740, and 1 720 cm^{-1}; *m/z* 816 (M^+, 6%), 780 (23), 399 (70), 398 (52), 384 (20), 383 (25), 382 (29), 357 (100), 356 (43), 355 (59), and 248 (23); δ 1.43 (3 H, dt, $J_{18',19'}$ 6.5 Hz, $J_{18',21'}$ 2 Hz, 18′-H_3), 1.57 (3 H, dt, $J_{18,19}$ 6.5 Hz, $J_{18,21}$ 2 Hz, 18-H_3), 3.74 (3 H, s), 3.77 (3 H, s), 3.82 (9 H, s) and 3.89 (3 H, s) (arom. OMe × 6), 4.08 (1 H, s, 16-OH), 4.92—5.28 (2 H, m, 19- and 19′-H), 6.44 (2 H, s, 11- and 11′-H) and 7.62 (1 H, br s, NH); ^{13}C n.m.r. δ 12.2 (C-18′), 12.5 (C-18), 28.1 (C-15), 29.6 (C-15′), 49.4 (C-21 and -21′), 56.7, 57.2, and 61.7 (arom. OMe), 98.4 and 98.8 (C-11 and -11′), 110.7 (C-19), 113.1 (C-19′), 139.0 (C-20′), 142.3 (C-20), 179.6 (C-2 and -2′).

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