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The Structures of Gardnerine, Gardnutine and Hydroxygardnutine and the Absolute Configuration of Gardnerine[1]

Shin-ichiro Sakai, Akinori Kubo, Takenori Hamamoto, Mikio Wakabayashi, Katsuhiro Takahashi, Hiroyuki Ohtani and Joju Haginiwa

Faculty of Pharmaceutical Sciences, Chiba University[2]

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Four kinds of indole alkaloids, gardneramine, gardnerine, gardnutine and hydroxygardnutine, were isolated from *Gardneria nutans* Sieb. et Zucc. (Hōraikazura). Among them, gardnerine, gardnutine and hydroxygardnutine were examined and the structures, I, II and III were assigned respectively to gardnerine, gardnutine and hydroxygardnutine on the basis of chemical and physical data.

The main alkaloid gardnerine (I) was interrelated with ajmaline (IV) (of established absolute configuration) by two different routes (A and B). In the route A, I was transformed to 1-demethyl-Δ^1-17,21-dideoxyajmaline (XXVIII) which in turn was derived from ajmaline (IV). On the other hand, in the route B, I was transformed to XXXII which in turn was derived from isoajmaline (XXXV) (C_{20}-ethyl epimer of ajmaline). The stereochemistry of the ethylidene side chain of II was determined by NOE measurement. These correlations as well as the nuclear overhauser effect (NOE) experiment established the absolute stereochemistry of I, II and III as shown in Chart 15.

In the previous papers,[3,4] we reported on the isolation of four kinds of indole alkaloids, gardneramine, gardnerine, gardnutine and hydroxygardnutine from the dry roots and stems of *Gardneria nutans* Sieb. et Zucc. (Loganiaceae, Japanese name: Hōraikazura).

Subsequently, we have reported in preliminary form[1] on the structural elucidation of gardnerine (I), gardnutine (II) and hydroxygardnutine (III) on the basis of the chemical and spectroscopical data and on the absolute stereochemistry of gardnerine (I) by chemical correlation with ajmaline (IV) whose absolute configuration has been established.[5] In the present paper we wish to report the details of the structural elucidation of gardnerine (I), gardnutine (II) and hydroxygardnutine (III) and of the absolute stereochemistry of gardnerine (I) (Chart 1).

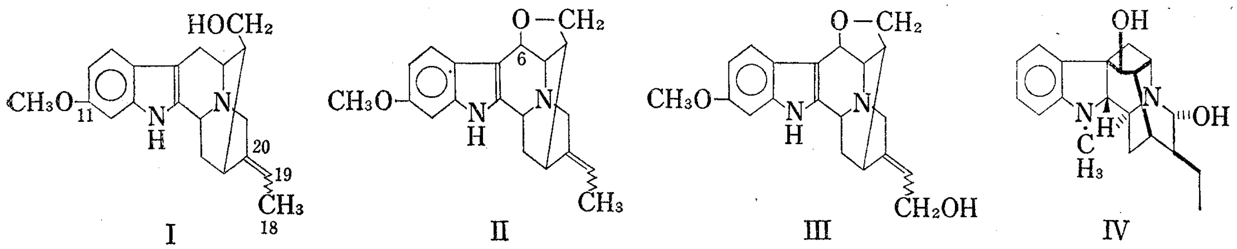

Chart 1

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[1] For a preliminary communication, see *Tetrahedron Letters*, **1969**, 1485 and *ibid.*, **1969**, 1489. This work was presented at the 10th Symposium on the Chemistry of Natural Products, Tokyo, 1966 (symposium papers p. 17) and 12th Symposium on the Chemistry of Natural Products, Sendai, 1968 (symposium papers p. 74).

[2] Location: *1-33, Yayoi, Chiba.*

[3] J. Haginiwa, S. Sakai, A. Kubo and T. Hamamoto, *Yakugaku Zasshi*, **87**, 1484 (1967).

[4] For a preliminary communication on the structure of gardneramine, see *Tetrahedron Letters*, **1971**, 2057.

[5] M.F. Bartlett, R. Sklar, W.I. Taylor, E. Schlittler, R.L.S. Amai, Peter Beak, N.V. Bringi and E. Wenkert *J. Am. Chem. Soc.*, **84**, 622 (1962).

Structure of Gardnerine (I)

Gardnerine (I), colorless prisms, mp 243—244°, $[\alpha]_D -29.4$, was shown to have the formula, $C_{20}H_{24}O_2N_2 \cdot H_2O$, on the basis of analytical and mass spectral data (M^+, m/e 324). Its ultraviolet (UV) spectrum [UV λ_{max}^{MeOH} 228.5 mμ (log ε 4.56), 268.5 mμ (log ε 3.70) and 298 mμ (log ε 3.77)] showed a striking resemblance to that of 11-methoxy-3,4-tetramethylenetetrahydro-β-carboline (V) as shown in Fig. 1. This fact suggested the presence of 2,3-disubstituted 6-methoxyindole chromophore in gardnerine (I).

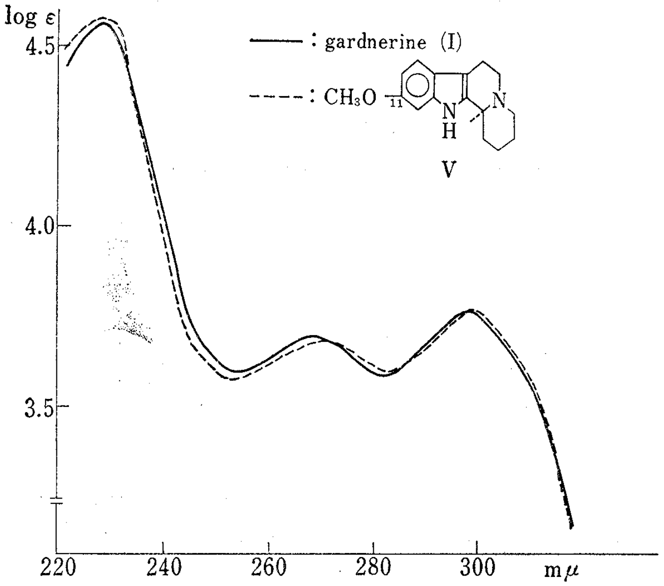


Fig. 1. UV spectra of I and V

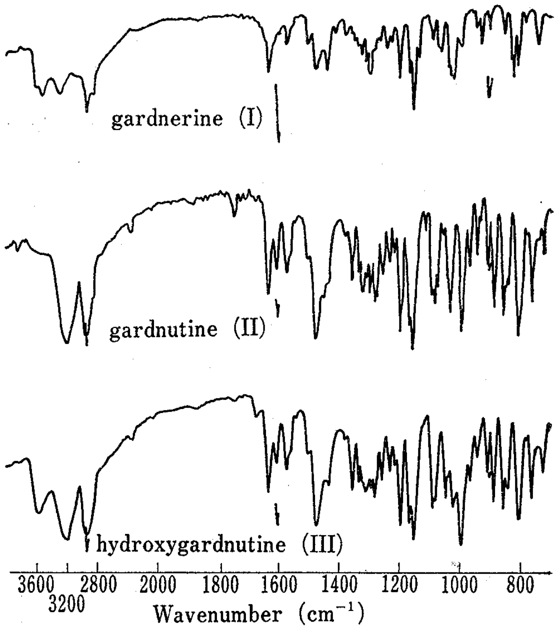
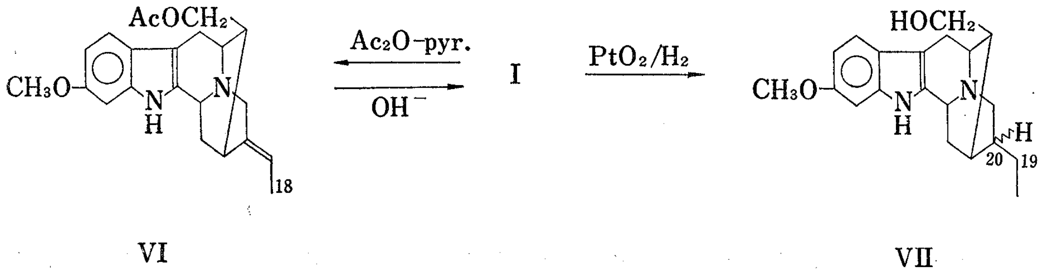


Fig. 2. IR spectra of I, II, III (KBr)

The infrared (IR) spectrum (KBr) showed the presence of an indolic N-H (ν_{max} 3520 cm^{-1}) and a hydroxy group (ν_{max} 3290 cm^{-1}) as shown in Fig. 2. Its nuclear magnetic resonance (NMR) spectrum in CF_3COOH showed a three-proton doublet ($J=7$ Hz) at δ 1.79, which could be assigned to a methyl group attached to a double bond. Acetylation of gardnerine (I) with acetic anhydride–pyridine at room temperature afforded the monoacetate (VI), mp 218—219°, $C_{22}H_{26}O_3N_2$ (M^+, m/e 366) (IR $\nu_{max}^{CHCl_3}$ 1736 cm^{-1}) with no N-acetyl group, indicating that the hydroxyl group was present as an alcoholic function and that the second nitrogen in the molecule was tertiary (Chart 2).



pyr. = pyridine

Chart 2

The monoacetylgardnerine (VI) was hydrolyzed to furnish the parent gardnerine (I). Catalytic hydrogenation of gardnerine (I) over Adams catalyst in glacial acetic acid afforded the corresponding 19,20-dihydrogardnerine (VII) as an isomeric mixture, mp 230—240°, $C_{20}H_{26}O_2N_2$ (M^+, m/e 326). The dihydrogardnerine (VII) yielded both acetic and propionic acids by Kuhn-Roth method,[6] while gardnerine (I) gave only acetic acid by this method. The methoxyl analysis indicated that gardnerine (I) contained one OCH_3 group (Found 8.81%, Calcd. 9.06%). These observations indicated the presence of functional groups (1×double bond, 1×C-OH, 1×OCH_3 and 1×C-CH_3) in the gardnerine (I) molecule. NMR spectrum ($CDCl_3$) of the monoacetylgardnerine (VI) confirmed the presence of an ethylidene group (δ 1.6, 3H, d. and δ 5.21, 1H, q. with $J=7$ Hz) and an aromatic methoxyl group (δ 3.7, 3H, s.).

Furthermore, it showed a resemblance to that of 7-methoxy-1,2,3,4-tetrahydrocarbazole in the region of aromatic ring protons.[7,8] Striking evidence of the skeleton of gardnerine (I) was obtained from its mass spectrum. Its mass spectrum, measured with heated inlet system, showed strong peaks at *m/e* 324 (M^+, 56%), 323 (M−1,57%), 306 (M−H_2O, 59%), 293 (M−CH_3OH, 36%), 212 (16%), 199 (84%) and 198 (100%) as shown in Fig. 3.

Two characteristic peaks at *m/e* 199 and 198 were diagnostically important for the sarpagine skeleton having a methoxyl group on the indole aromatic ring.

These data suggested that gardnerine (I) was closely related to polyneuridine (VIII). Moreover, the dehydration peak at *m/e* 306 (M−H_2O, 59%) indicated that the relative stereochemistry of 16-hydroxymethylene group of gardnerine (I) was same as that of polyneuridine (VIII), whose mass spectrum showed the strong M−H_2O peak (50%), whereas in the spectrum of akuammidine (IX), C_{16}-epimer of polyneuridine (VIII), the M−H_2O peak was absent.[9] These spectral data indicated that the skeletal structure of gardnerine (I) was represented by 11-methoxy-16-desmethoxycarbonylpolyneuridine. Therefore, structure I could tentatively be postulated to gardnerine. The correctness of this tentative assignment was proved by the following experiments.

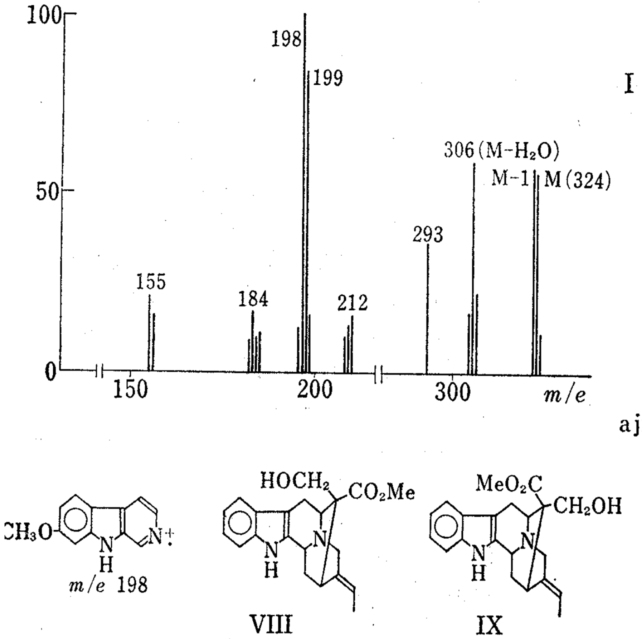
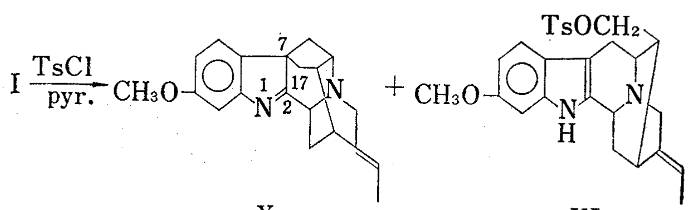
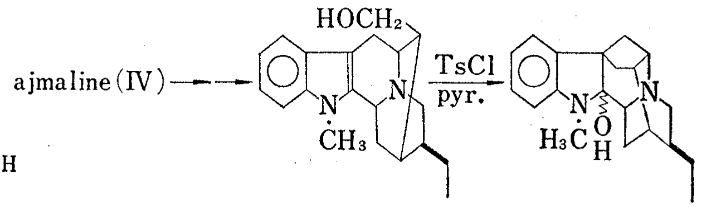


Fig. 3. Mass Spectrum of Gardnerine (I)



X XI

Chart 3



XII 21-deoxyajmalol-A

XIII 2-hydroxy-17,21-dideoxyajmaline

Chart 4

Upon treatment with *p*-toluenesulfonyl chloride in pyridine at room temperature for 5 days, gardnerine (I) afforded the indolenine derivative (X), mp 141—142°, $C_{20}H_{22}ON_2$ (M^+, *m/e* 306) in 80% yield, which was accompanied by a small quantity of gardnerine O-tosylate (XI), mp 194—195°, $C_{27}H_{30}O_4N_2S$ (Chart 3).

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[6] C.F. Garbers, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **37**, 1336 (1954).

[7] S. Sakai, M. Wakabayashi and M. Nishina, *Yakugaku Zasshi*, **89**, 1061 (1969).

[8] G. Van Binst, *Tetrahedron Letters*, **1964**, 973.

[9] H. Budzikiewicz, C. Djerassi and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 1, Holden-Day. Inc., San Francisco, 1964, pp. 81—86.

The UV spectrum of the indolenine derivative (X) showed absorption bands at 230, 275 and 300 mμ, which were slightly different from those of gardnerine (I).

Its IR spectrum (KBr) showed strong absorption at 1583 cm^{-1} but no indolic N-H band. On the other hand, the IR spectrum ($CHCl_3$) of gardnerine O-tosylate (XI) exhibited an indolic N-H band at 3505 cm^{-1} and tosylate bands at 1360 and 1173 cm^{-1}. This type of ring closure reaction was first described by Taylor and coworkers[10] in the study of the chemistry of ajmaline (IV) as shown in Chart 4.

Reduction of the indolenine derivative (X) with lithium aluminum hydride afforded solely an indoline derivative (XIV), mp 79—81°, $C_{20}H_{24}ON_2$ (M^+, *m/e* 308), whose UV spectrum showed absorption at 295 mμ (log ε 3.66). Furthermore, lead tetraacetate oxidation of indoline derivative (XIV) gave an indolenine in 50% yield, which was proved to be identical with X by mixture melting point determination and direct comparison of IR spectra (KBr). A similar interconversion between XVI and XVII was carried out as a model experiment[7] for these transformation (X⇄XIV) as shown in Chart 5.

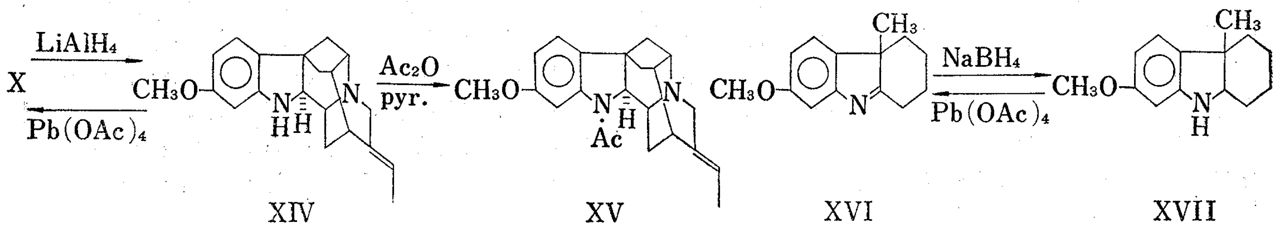

Chart 5

Acetylation of indoline derivative (XIV) with acetic anhydride in pyridine provided a noncrystalline N_a-acetyl derivative (XV) (M^+, *m/e* 350), whose IR spectrum ($CHCl_3$) showed absorption at 1660 cm^{-1} and whose NMR spectrum showed a three-proton singlet at δ 2.4 due to an acetyl group. On the other hand, mesylation of indoline derivative (XIV) with methanesulfonyl chloride in pyridine furnished a crystalline N_a-mesyl derivative (XXIX) as described later. Moreover, oxidation of gardnerine (I) with chromium trioxide-sulfuric acid in acetone at room temperature gave rise to two compounds which were separated by chromatography over alumina. The first compound to be eluted was found to be indolenine ether (XVIII), mp 151—152°, $C_{20}H_{22}O_2N_2$ (17% yield). Further elution provided the indole ether (XIX), mp 319—320°, $C_{20}H_{22}O_2N_2$, in 16% yield (Chart 6).

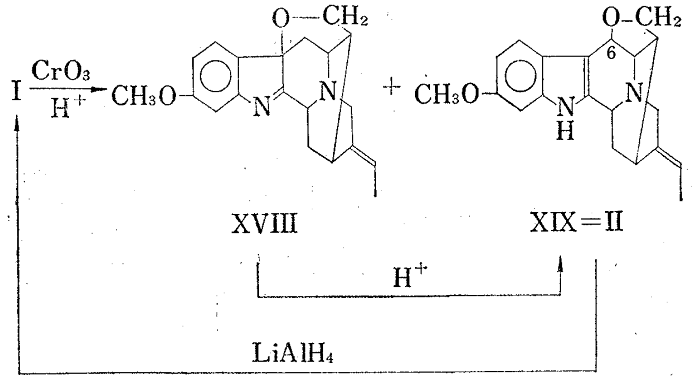

Chart 6

The UV spectrum of XVIII showed absorption at 285 mμ (log ε 3.42) and its IR spectrum (KBr) showed an indolenine band at 1600 cm^{-1} and no indolic N-H band.

On the other hand, the UV spectrum of XIX was similar to that of gardnerine (I). Treatment of the former product (XVIII) with strong acid gave the rearranged indole ether in 60% yield, which was shown to be identical with the oxidation product (XIX) by direct comparison of IR spectra (KBr) and mixture melting point determination. This experiment

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10) M.F. Bartlett, B.F. Lambert, H.M. Werblood and W.I. Taylor, *J. Am. Chem. Soc.*, 85, 475 (1963).

established that the primary oxidation product is the indolenine ether (XVIII). An analogous transformation was observed by Taylor and coworkers[11] in the lead tetraacetate oxidation of 21-deoxyajmalol-A (XII) as shown in Chart 7. Leete has observed that $LiAlH_4$ reduction of 3-hydroxy methyl indole leads to skatole[12] (Chart 7).

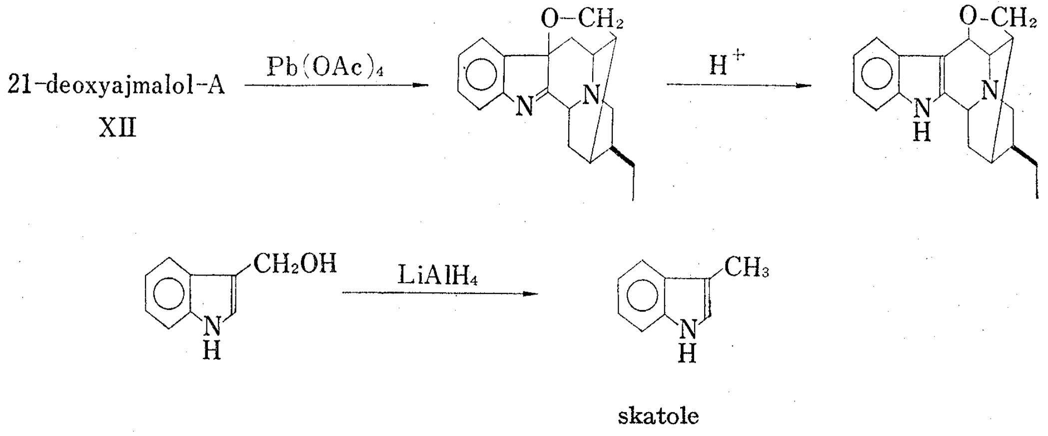

Chart 7

By analogy, we found that lithium aluminum hydride reduction of the rearranged indole ether (XIX) regenerated gardnerine (I) in quantitative yield as shown by comparison of IR spectra (KBr), mixture melting point determination and optical rotation measurement (Chart 6). This result made clear the presence of an oxygen or its equivalent at C-6 in the indole ether (XIX). On the basis of these experiments, structure I may be assigned to gardnerine without definition of the stereochemistry of the ethylidene group. The determination of the stereochemistry as well as the absolute configuration of gardnerine (I) is described later.

Structure of Gardnutine (II)

Gardnutine (II) was obtained as prisms, mp 319—320° and analyzed for $C_{20}H_{22}O_2N_2$ (M^+, *m/e* 322), $[\alpha]_D$ +30.3. II was shown to have $1\times OCH_3$, $1\times C\text{-}CH_3$ and 1×double bond. Its UV spectrum showed absorption bands at 223.5 mμ (log ε 4.63), 260 mμ (log ε 3.64), 295 mμ (log ε 3.73) and 302 mμ (log ε 3.63), which were similar to those of gardnerine (I). The mass spectrum of II showed two characteristic fragment ions at *m/e* 199 (86%) and 198 (100%) which indicated that II should possess the same carbon skeleton as gardnerine (I). Its IR spectrum (KBr) showed only one absorption band at 3200 cm^{-1} (N-H) in the region of O-H and N-H stretching absorptions as shown in Fig. 2. Therefore, the remaining oxygen atom should be present as an ether group. Moreover, its IR spectrum was superimposable to that of the rearranged oxidation product (XIX) of gardnerine (I) obtained as above. The identity of II and XIX was further confirmed by mixture melting point determination and optical rotation measurement. Reduction of gardnutine (II) with lithium aluminum hydride gave a compound which was shown to be identical with natural gardnerine (I). Therefore, gardnutine had structure represented by II. It should be stressed at this stage that gardnutine (II) is a rare example of a 6-oxygenated indole alkaloid.[13]

Structure of Hydroxygardnutine (III)

Hydroxygardnutine (III), $C_{20}H_{22}O_3N_2$ (M^+, *m/e* 338), was obtained as prisms, mp 311—312°, $[\alpha]_D$ +36.2. III was shown to have $1\times OCH_3$, but no $C\text{-}CH_3$ group. Its UV spectrum showed absorption bands at 223 mμ (log ε 4.61), 260.5 mμ (log ε 3.69), 295 mμ (log ε 3.79)

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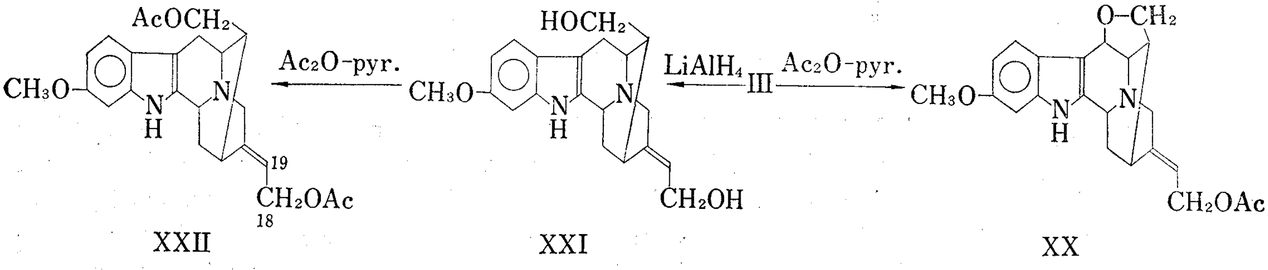
[11] M.F. Bartlett, B.F. Lambert and W.I. Taylor, *J. Am. Chem. Soc.*, **86**, 729 (1964).

[12] E. Leete, *J. Am. Chem. Soc.*, **81**, 6023 (1959).

[13] G. Tirions, M. Kaisin, J.C. Breaekman, J. Pecher and R.H. Martin, *Chimia*, **22**, 87 (1968).

and 301 mμ (log ε 3.70 sh.), which were similar to those of gardnerine (I) and gardnutine (II). Its mass spectrum showed two characteristic fragment ions at m/e 199 (100%) and 198 (87%) which indicated that III possesses the same carbon skeleton as gardnerine (I) and gardnutine (II). Moreover, its IR spectrum (KBr) showed the presence of N-H (3200 cm^{-1}) and O-H (3580 cm^{-1}) as shown in Fig. 2. Acetylation of III with acetic anhydride–pyridine afforded a monoacetate (XX), mp 278—280°, $C_{22}H_{24}O_4N_2$ (M^+, m/e 380) whose IR spectrum exhibited a single absorption band (3160 cm^{-1} (N-H)) in the O-H and N-H stretching region.

Therefore, the remaining oxygen atom must be present as an ether group in hydroxygardnutine (III) as already established for gardnutine (II). Reduction of III with lithium aluminum hydride afforded the corresponding dihydroxy derivative (XXI), mp 162—164°, $C_{20}H_{24}O_3N_2$ (M^+, m/e 340) in 52% yield, which gave the diacetate (XXII), mp 182—183°, $C_{24}H_{28}O_5N_2$ (M^+, m/e 424) upon treatment with acetic anhydride–pyridine. The NMR spectrum of the diacetate (XXII) showed signals at δ 4.59 (2H, d.) and δ 5.43 (1H, t.) with J= 7 Hz, indicating the presence of an acetoxy ethylidene group ($AcO-CH_2-CH=C$) which led to the conclusion that III must have an allylic alcohol group (Chart 8).



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The presence of an allylic alcohol group in III was confirmed chemically by correlation with gardnutine (II). Catalytic hydrogenation of III over Adams catalyst in glacial acetic acid led to absorption of nearly 2 mole equivalents of hydrogen to afford 19,20-dihydrogardnutine (XXIII), mp 294—296°, $C_{20}H_{24}O_2N_2$ (M^+, m/e 324), $[\alpha]_D$ +41.4, in 34% yield. The same dihydro compound was also obtained in 60% yield by direct catalytic hydrogenation of II over Adams catalyst. The identity of both dihydro compounds was confirmed by mixture melting point determination, optical rotation measurement and direct comparison of IR and mass spectra. Moreover, oxidation of III with active MnO_2[14] in pyridine at room temperature for 5 days furnished the corresponding α,β-unsaturated aldehyde (XXIV), mp> 310° (dec.), $C_{20}H_{20}O_3N_2 \cdot 1/2H_2O$ (M^+, m/e 336) in 38% yield. Its IR spectrum (KBr) showed the unsaturated ketonic band at 1673 cm^{-1} and its NMR spectrum in DMSO-d_6 at 100° exhibited a doublet (1H, J=7 Hz) at δ 5.98 due to a proton of the type $=C\underline{H}-CHO$ and a doublet (1H, J=7 Hz) at δ 9.83 due to the aldehyde proton, $=CH-C\underline{H}O$. The α,β-unsaturated aldehyde (XXIV), in turn, regenerated natural hydroxygardnutine (III) upon sodium borohydride reduction.

Therefore, the structure of hydroxygardnutine could be represented as III (Chart 9).

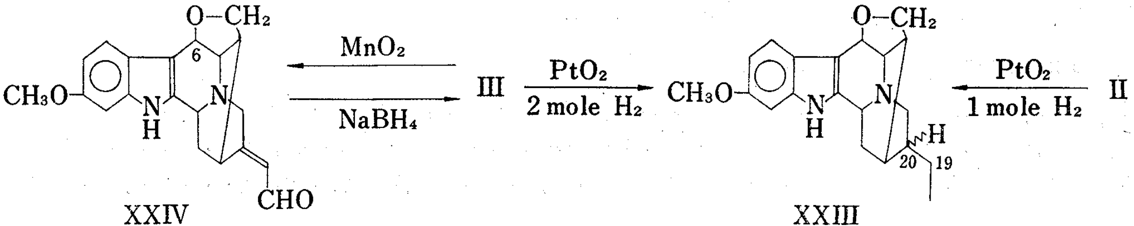

Chart 9

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[14] W. Acklin, T. Fehr and D. Arigoni, *Chem. Commun.*, **1966**, 799.

Since the structures of gardnerine, gardnutine and hydroxygardnutine were established by chemical correlation of the three alkaloids, it is concluded that all three alkaloids (I—III) must have the same carbon skeleton. There appeared to be a way of converting gardnerine (I) into a compound of known absolute configuration which would afford not only a solution to its stereochemistry but also a simple structure proof. The interrelationship of gardnerine (I) to ajmaline (IV) (of established absolute configuration) was accomplished by two different routes A and B.

Route A

As described in the above structural studies, gardnerine (I) gave in good yield the cyclized compound (X) by treatment with *p*-toluenesulfonyl chloride-pyridine. In order to correlate with ajmaline (IV), the indolenine derivative (X) had to be converted into the corresponding desmethoxyl compound. Treatment of X with 10—12 molar equivalent borontrichloride in dry dichloromethane at dry ice/acetone temperature furnished the expected phenol (XXV), mp 261—263°, $C_{19}H_{20}ON_2 \cdot 1/2H_2O$ (M^+, *m/e* 292) in 80% yield. Its UV spectrum showed absorption bands at 232, 276 and 304 mμ. Elimination of the phenolic group from compound XXV was accomplished by Musliner's method[15] which involved the hydrogenolysis of the tetrazolyl ether. Thus, XXV was refluxed with one molar equivalent 1-phenyl-2-chlorotetrazole in acetone solution in the presence of potassium carbonate for 44 hr. From the reaction mixture, the tetrazolyl ether (XXVI), mp 232—233°, $C_{26}H_{24}ON_6$ (M^+, *m/e* 436), was isolated in 91—95% yield after silicagel chromatography. Its IR spectrum (KBr) showed an absorption band at 1535 cm^{-1} (tetrazolyl ether) and its mass spectrum showed a base peak at *m/e* 291 (M−145). In order to obtain the deoxy compound, catalytic reduction of the tetrazolyl ether (XXVI) over Pd/charcoal was attempted but under these conditions[15] the desired product was not obtained. However, under stronger hydrogenolysis condition using Adams catalyst in glacial acetic acid solution at 50—60°, XXVI furnished the deoxy compound (XXVII) in 31% yield after alumina chromatography. This compound (XXVII), mp 76—83°, (M^+, *m/e* 280), however, was found to be contaminated with an epimer at C-2 or/and C-20 position. Therefore, the impure deoxy compound (XXVII) was oxidized with 1.2 molar equivalent lead tetraacetate in dry benzene solution for 2 hr at room temperature to afford the pure 1,2-dehydro derivative (XXVIII), mp 188—189.5°, $C_{19}H_{22}N_2$ (M^+, *m/e* 278) in 40% yield after alumina chromatography. Compound XXVIII was found to be identical with 1-demethyl-Δ^1-17,21-dideoxyajmaline,[5] which was directly obtained in 25% yield by the

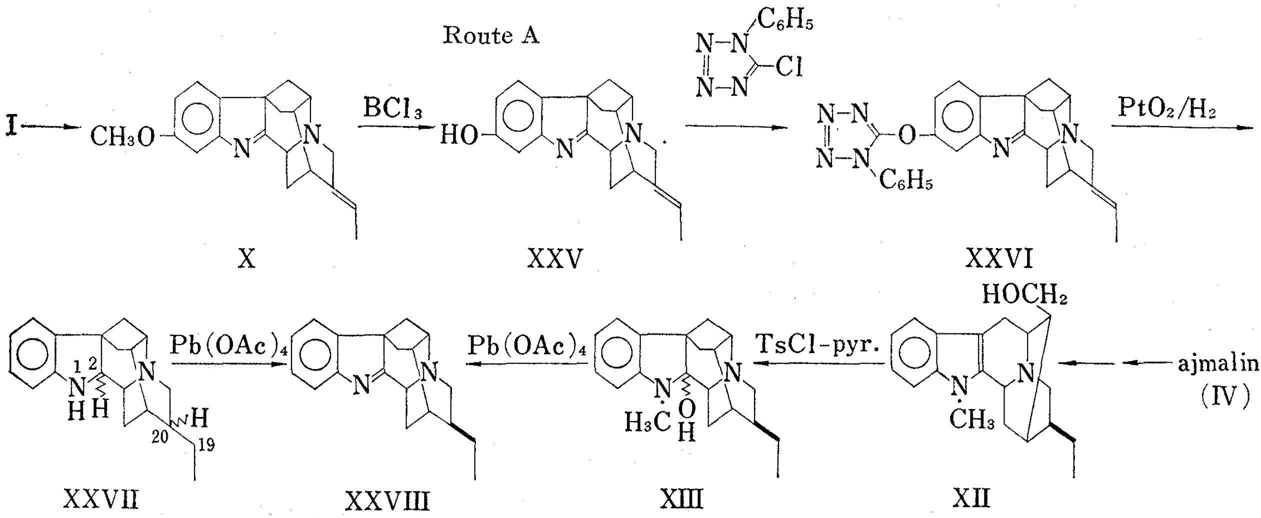

Chart 10

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[15] W.J. Musliner and J.W. Gates, Jr., *J. Am. Chem. Soc.*, **88**, 4271 (1966).

oxidation of 2-hydroxy-17, 21-dideoxyajmaline (XIII) with excess lead tetraacetate. The identity of the two samples was established by mixture melting point determination as well as by direct comparison of their UV, IR, mass and optical rotatory dispersion (ORD) spectra (Chart 10).

Route B

Reduction of the ring closed compound (X) with lithium aluminum hydride gave rise to a single indoline derivative (XIV) in high yield and subsequent treatment of XIV with methanesulfonyl chloride–pyridine resulted in the formation of the corresponding crystalline N_a-mesyl derivative (XXIX), mp 215—216°, $C_{21}H_{26}O_3N_2S$ (M^+, *m/e* 386) in 83% yield as described in the above structural studies. The IR spectrum (KBr) of XXIX showed absorption bands at 1348 and 1168 cm^{-1} (=N-SO_2-) and its mass spectrum showed a base peak at *m/e* 307 (M$-SO_2CH_3$). Treatment of XXIX with 10 molar equivalent BCl_3 in dry dichloromethane provided the demethylated phenolic compound (XXX), mp 175—185°, $C_{20}H_{24}O_3N_2S\cdot 1/2C_6H_6$ (M^+, *m/e* 372) in nearly quantitative yield. Its mass spectrum showed a fragment ion at *m/e* 293 (M$-SO_2CH_3$, 100%) and its IR spectrum (KBr) showed absorption bands at 1354, 1163 cm^{-1} (=N-SO_2-) and 3600 cm^{-1}. Moreover, its NMR spectrum confirmed the presence of 1/2 mole of benzene of crystallization which showed a three-proton singlet at δ 7.34. The action of diazomethane on the phenol derivative (XXX) regenerated the parent XXIX. The phenol (XXX) was converted to the corresponding tetrazolyl ether derivative (XXXI), mp 124—126°, $C_{27}H_{28}O_3N_6S$ (M^+, *m/e* 516) in 62% yield by means of Musliner's method.

Its IR spectrum exhibited absorption bands at 1539 cm^{-1} (tetrazolyl ether) and 1360, 1166 cm^{-1} (=N-SO_2-). The tetrazolylether (XXXI) was catalytically reduced over Adams catalyst at 60° to furnish the expected desmethoxy-19,20-dihydro derivative (XXXII), mp 83—102°, $C_{20}H_{26}O_2N_2S\cdot H_2O$ (M^+, *m/e* 358) in 61% yield after alumina chromatography. Its UV spectrum showed absorption bands at 231, 277 and 283 mμ and its IR spectrum ($CHCl_3$) exhibited N_a-mesyl bands at 1357 and 1165 cm^{-1}.

The mass spectrum of XXXII showed a base peak at *m/e* 279 (M$-SO_2CH_3$). Furthermore, its NMR spectrum showed a three-proton singlet at δ 2.88 due to $SO_2C\underline{H}_3$ and a three-proton triplet ($J=7$ Hz) at δ 0.92 due to C-18 methyl group. These spectral data established the structure of XXXII (Chart 11).

Route B

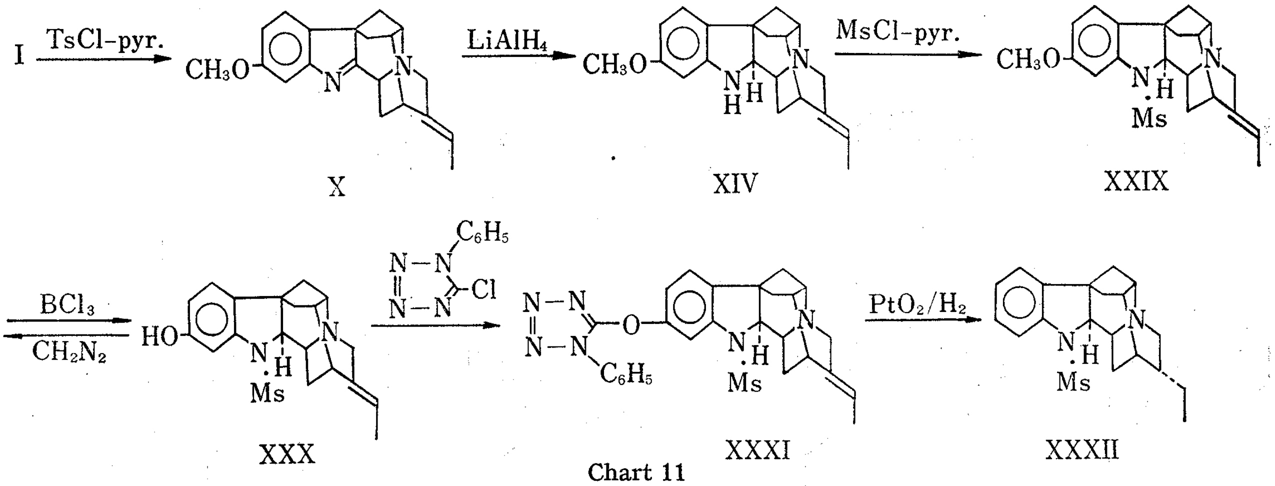
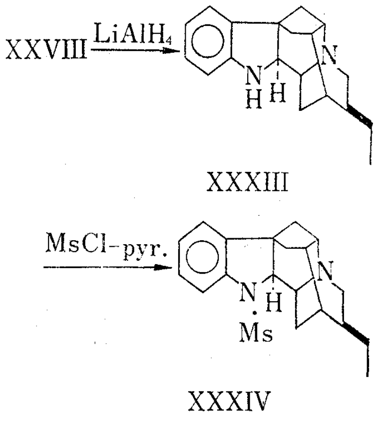


Chart 11

For correlation purposes, ajmaline (IV) was first transformed into 1-demethyl-Δ^1-17,21-dideoxyajmaline (XXVIII) and then into the corresponding N_a-mesyl derivative (XXXIV). Reduction of XXVIII with lithium aluminum hydride in refluxing THF furnished the corresponding indoline derivative (XXXIII), mp 89°, $C_{19}H_{24}N_2\cdot H_2O$ (M^+, *m/e* 280) in 69%

yield. Treatment of XXXIII with mesyl chloride–pyridine afforded an amorphous Na-mesyl derivative (XXXIV), which was characterized as its hydrochloride, mp 290—293°. This compound (XXXIV) was not identical with the corresponding Na-mesyl derivative (XXXII) by direct comparison of IR ($CHCl_3$) and NMR spectra as shown in Fig. 4. However, their mass spectra were found to be identical which led us to the conclusion that compound XXXIV was the C-20 diastereoisomer of the corresponding XXXII (Chart 12).



pyr.=pyridine

Chart 12

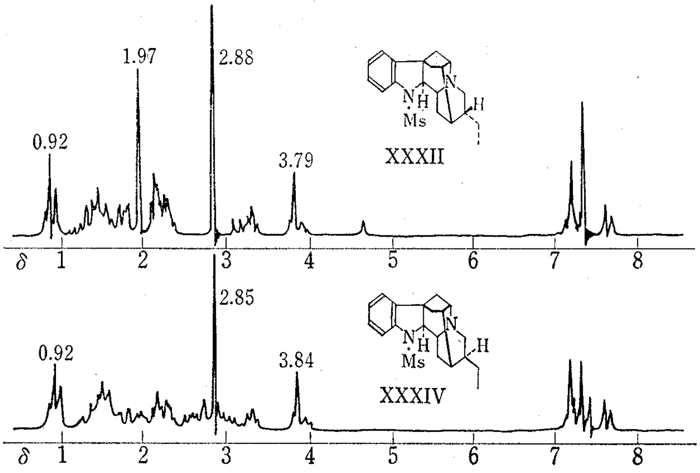


Fig. 4. NMR Spectra of XXXII and XXXIV

Therefore by an analogous series of reactions isoajmaline (XXXV) was transformed into the corresponding Na-mesyl compound (XXXII) through 21-deoxyisoajmalol-A (XXXVI) without isolation of crystalline intermediates. The Na-mesyl compound (XXXII) was found to be identical with the degradation product (Chart 11) by mixture melting point determination as well as by the direct comparison of their UV, IR, NMR, mass and ORD spectra (Chart 13).

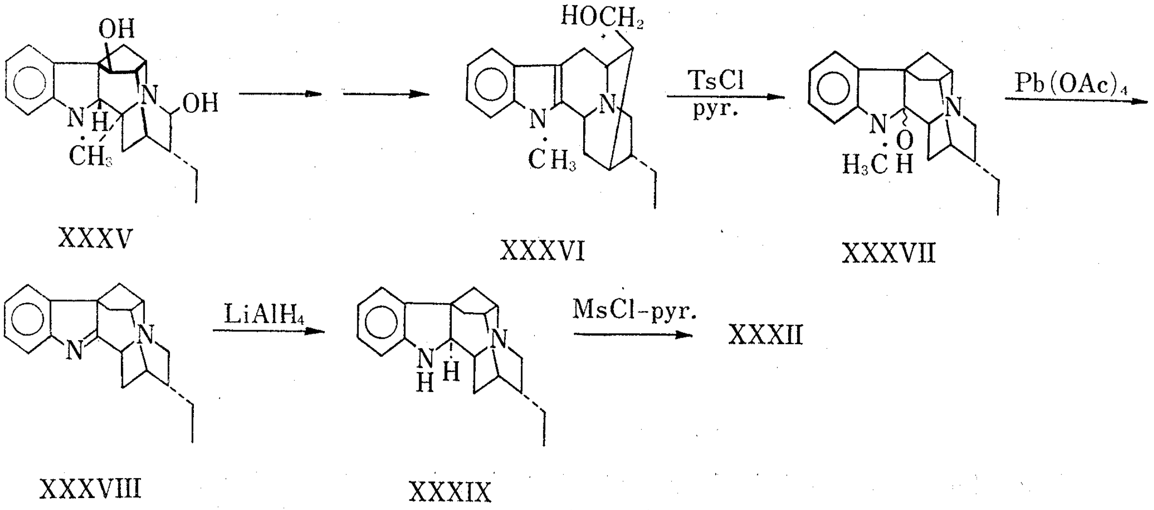


Chart 13

Concerning the configuration of C-2 hydrogen in the indoline derivatives (XIV, XXXIII and XXXIX), reduction of the indolenine derivatives (X, XXVIII and XXXVIII) with lithium aluminum hydride should take place from the less hindered topside leading to the stereochemistry at C-2 as depicted in XIV, XXXIII and XXXIX, *i.e.* C-2 hydrogen is α-oriented (Chart 14).

On the other hand, the mode of the catalytic reduction of the ethylidene group in XXVI and XXXI cannot be easily rationalized. Another case in which the course of a catalytic reduction cannot be readily predicted is that of a sarpagine derivative.[5]

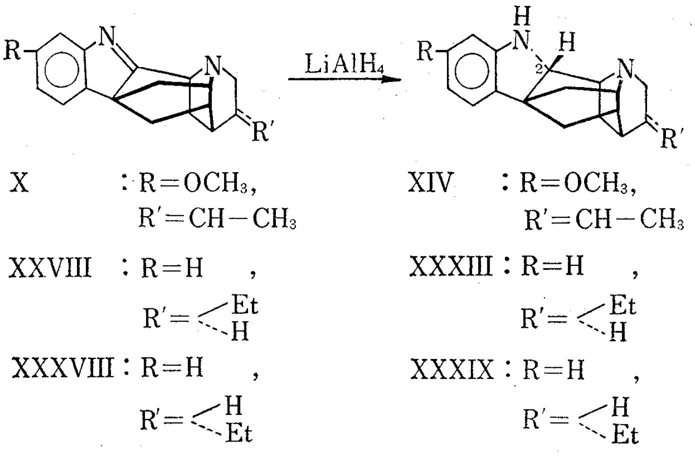


Chart 14

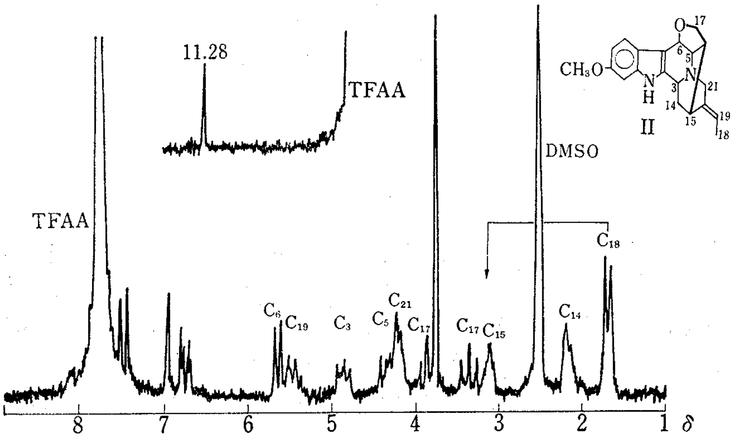


Fig. 5. NMR spectrum of Gardnutine (II) (DMSO-d_6+CF_3COOH)

Thus the stereochemistry of gardnerine (I) was established with the exception of the geometry of the ethylidene group. The geometry of the C-18 methyl attached to the ethylidene group of other sarpagine alkaloid was demonstrated by a nuclear overhauser effect (NOE) experiment.[16] Thus, the NOE technique was applied to gardnutine (II), which had a very rigid skeleton.

The NMR spectrum of II in DMSO-d_6+CF_3COOH showed two signals corresponding to an ethylidene side chain (δ 1.69, 3H, d. and δ 5.50, 1H, q. with J=7 Hz).

Furthermore, it allowed the assignment of the following protons: C_{14}-2H (δ 2.17, 2H, br. s.), C_{15}-H (δ 3.10, 1H, m.), C_{17}-2H (δ 3.37, 1H, t., J=10 Hz and δ 3.81, 1H, m.), Ar. OCH_3 (δ 3.77, 3H, s.), C_{21}-2H (δ 4.23, 2H, br. s.), C_3-H (δ 4.87, 1H, m.), C_5-H and C_6-H (δ 4.36, 1H, d. and δ 5.65, 1H, d. with J=7.5 Hz) and indolic N-H (δ 11.28, 1H, s.). The correctness of this assignment was supported by double resonance experiments (Fig. 5).

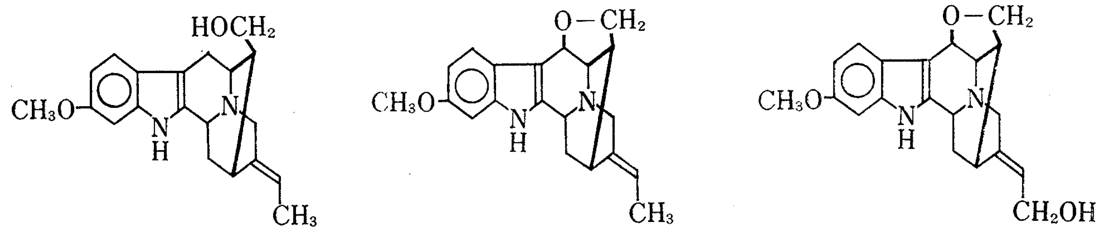


Chart 15

The signal due to the C-15 hydrogen (δ 3.10) of II in NMR spectrum (DMSO-d_6+CF_3COOH) showed an increase in the integration value by 13% when irradiated at the C-18 methyl group (δ 1.69). This result confirmed that the CH_3 of the ethylidene side chain of II was *cis* with respect to the bridgehead C-15 hydrogen atom. Thus the structures and the absolute configurations of gardnerine (I), gardnutine (II) and hydroxygardnutine (III)[17] are firmly established as shown in Chart 15.

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16) *a)* J.C. Nouls, P. Wollast, J.C. Braekman, G. Van Binst, J. Pecher and R.H. Martin, *Tetrahedron Letters*, **1968**, 2731; *b)* G.E. Bachers and T. Schaefer, *Chem. Rev.*, **71**, 617 (1971).

17) Though this compound (III) has not been subjected to the above NOE experiment, biogenetic considerations require that it has the same ethylidene geometry as I and II.

Experimental[18]

Monoacetylgardnerine (VI)——Gardnerine (I) was allowed to stand in pyridine and acetic anhydride for 3 days at room temperature. The solution was evaporated to dryness *in vacuo*. The residue was diluted with ice-water, was made basic with Na_2CO_3 and extracted with $CHCl_3$. After drying, the solvent was removed *in vacuo*.

Crystallization of the residue from MeOH–H_2O furnished colorless prisms of VI, mp 218—219°. *Anal*. Calcd. for $C_{22}H_{26}O_3N_2$: C, 72.10; H, 7.15; N, 7.65. Found: C, 71.90; H, 7.36; N, 7.70. IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1736. Mass Spectrum *m/e*: 366 (M^+). NMR ($CDCl_3$, δ); 1.6 (3H, d. $J=7$ Hz) (C_{18}-$(H)_3$), 1.95 (3H, s.) ($COCH_3$), 3.7 (3H, s.) (Ar. OCH_3), 5.21 (1H, q. $J=7$ Hz) (C_{19}-H).

Hydrolysis of Monoacetylgardnerine (VI)——A solution of VI (50 mg) in 3% KOH–MeOH–H_2O (5 ml) was refluxed for 30 min. Upon cooling, the product crystallized out of the solution was collected and washed with water to furnish I (26 mg), mp 243—245°. The IR spectrum of this compound was identical in all respects with I and their mixed mp showed no depression.

19,20-Dihydrogardnerine (VII)——Gardnerine (I) (200 mg) in AcOH (36 ml) was hydrogenated in the presence of PtO_2 (50 mg). After filtration, the solvent was removed *in vacuo*. The residue was made basic with 10% NH_4OH and extracted with $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. Then the residue (200 mg) was chromatographed on alumina (20 g). The material (50 mg) eluted with 1% MeOH–$CHCl_3$ crystallized from MeOH, yielding VII-1 (32 mg), mp 229—232°.

The material (90 mg) eluted with 3% MeOH–$CHCl_3$ crystallized from MeOH, yielding VII-2 (43 mg), mp 234—238°. Moreover, the material (75 mg) eluted with 5% MeOH–$CHCl_3$ crystallized from MeOH, yielding VII-3 (20 mg), mp 236—239°. These three crystalline components (VII-1,2,3) showed the same *Rf* value and a singlet spot on TLC. *Anal*. Calcd. for $C_{20}H_{26}O_2N_2$: C, 73.59; H, 8.03; N, 8.58. Found(VII-1): C, 73.08; H, 7.78; N, 8.59: Found(VII-3): C, 73.68; H, 7.75; N, 8.46.

1-Demethyl-$\Delta^{1,19}$-11-methoxy-17,21-dideoxyajmaline (X) (from Gardnerine (I))——I (1.8 g, 5.26 mM) was dissolved in pyridine (20 ml) containing tosyl chloride (1.8 g, 9.42 mM) and was allowed to stand at room temperature for 2 days. Additional tosyl chloride (1.8 g) was added, continuing the reaction for 3 more days. The solvent was removed *in vacuo* followed by extraction of the residue with aq. $NaHCO_3$ and $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (2.36 g) was chromatographed on alumina (30 g). The material (1.62 g) eluted with benzene–$CHCl_3$ (1: 1) crystallized from ether, yielding X (1.29 g, 80%), mp 138—139°.

Further elution with $CHCl_3$ gave O-Tosylate (XI) (73 mg, 3%), mp 194—195°.

1-Demethyl-$\Delta^{1,19}$-11-methoxy-17,21-dideoxyajmaline (X): *Anal*. Calcd. for $C_{20}H_{22}ON_2$: C, 78.40; H, 7.24; N, 9.14. Found: C, 78.72; H, 7.05; N, 9.26. UV λ_{max}^{MeOH} mμ (log ε): 230 (4.37), 275 (3.51), 300 (3.34). λ_{min}^{MeOH} mμ (log ε): 259.5 (3.45), 295 (3.33). IR ν_{max}^{KBr} cm^{-1}: 1618, 1583. Mass Spectrum *m/e* (%): 306 (M^+, 100), 199 (13), 198 (36). NMR ($CDCl_3$, δ): 1.65 (3H, d. $J=7$ Hz) (C_{18}-$(H)_3$), 1.91 (2H, br. s.) (C_{14}-2H), 2.97 (1H, br. s.) (C_{15}-H), 3.36 (1H, t. $J=5$ Hz) (C_5-H), 3.52 (2H, br. s.) (C_{21}-2H), 3.82 (3H, s.) (Ar. OCH_3), 4.16 (1H, d.d. $J=2.5$, 7.5 Hz) (C_3-H), 5.28 (1H, q. $J=7$ Hz) (C_{19}-H).

Gardnerine O-Tosylate (XI): *Anal*. Calcd. for $C_{27}H_{30}O_4N_2S$: C, 67.76; H, 6.32; N, 5.85. Found: C, 67.32; H, 6.30.; N, 5.95. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3505; 1360, 1173 (–OSO_2–). UV λ_{max}^{MeOH} mμ: 227, 265, 273 (sh.), 297; λ_{min}^{MeOH} mμ: 251, 281.

1-Demethyl-Δ^{19}-11-methoxy-2-epi-17,21-dideoxyajmaline (XIV) (from Gardnerine (I))——To a stirred solution of X (2.16 g, 7.06 mM) in dry ether (140 ml), $LiAlH_4$ (5.4 g, 141.2 mM) was added directly under ice cooling and the mixture was refluxed for 24 hr. After decomposing the excess hydride with wet ether, the mixture was filtered and the residue was washed with ether. The ether layer was dried and removed *in vacuo* to afford an oil (2.4 g), which was crystallized from ether–hexane to furnish XIV (1.693 g, 86%), mp 79—81°. *Anal*. Calcd. for $C_{20}H_{24}ON_2 \cdot 1/4H_2O$: C, 76.76; H, 7.89; N, 8.95. Found: C, 77.03; H, 7.77; N, 8.47. $[\alpha]_D^{24}$ +40.4 ($c=0.76$, MeOH). UV λ_{max}^{MeOH} mμ (log ε): 237 (3.68 sh.), 295 (3.66); λ_{min}^{MeOH} mμ (log ε): 266 (2.85). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420. Mass Spectrum *m/e*(%): 308 (M^+, 100), 173 (70), 160 (40), 121 (40). NMR ($CDCl_3$, δ): 1.6 (3H, d. $J=7$ Hz) (C_{18}-$(H)_3$), 3.16 (1H, t. $J=6$ Hz) (C_5-H), 3.4 (2H, br. s.) (C_{21}-2H), 3.73 (3H, s.) (Ar, OCH_3), 3.8 (1H, d. $J=5$ Hz) (C_2-H), 5.19 (1H, q. $J=7$ Hz) (C_{19}-H). ORD ($c=0.00311$, MeOH): $[\phi]$(mμ): +225(400), 0(356), −1158(320), −2975(307, trough), 0(296), +3696(280, peak), +2091(260, trough), +9967(245, peak): CD ($c=0.00332$, MeOH) $\Delta\varepsilon$ (mμ): −1.42(295), −0.292(268), −1.09(252), 0(243.5), +0.585(237), 0(231), −0.512(227), 0(222).

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[18] All melting points are uncorrected. NMR spectra were recorded on the JNM-4H 100 and Varian HA100 spectrometer operated at 100 Hz with tetramethylsilane as internal standard. Mass spectra were determined by a Hitachi Double-focussing mass spectrometer RMU-6E using a direct inlet system.

ORD and CD curves were measured on a JASCO ORD/UV-5 and J-20. Thin-layer chromatography (TLC) was performed on Silica Gel G (Merck). The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad; sh., shoulder.

Na-Acetyl-1-demethyl-Δ^{19}-11-methoxy-2-epi-17,21-dideoxyajmaline (XV) (from Gardnerine (I))——XIV (531 mg, 1.72 mM) in pyridine (5 ml) was treated with Ac_2O (3 ml) for 2 days at room temperature. The solution was evaporated to dryness *in vacuo*. The residue was made alkaline with NH_4OH and extracted with $CHCl_3$. After drying, the solvent was removed to afford a brown oil (578 mg), which was chromatographed on alumina (40 g), eluting an amorphous XV (520 mg) with benzene–$CHCl_3$ (2: 1—1: 1) and $CHCl_3$. UV λ_{max}^{MeOH} mμ: 251, 295, 300 (sh.); λ_{min}^{MeOH} mμ: 237, 272. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660. Mass Spectrum *m/e* (%): 350 (M^+, 92), 121(100). NMR ($CDCl_3$, δ): 1.6(3H,d. J=7 Hz) (C_{18}-$(H)_3$), 2.35 (3H, s.) ($COCH_3$), 3.20 (1H,t. J=5 Hz) (C_5-H), 3.40 (2H, br. s.) (C_{21}-2H), 3.75 (3H, s.) (Ar. OCH_3), 4.06 (1H, d. J=5 Hz) (C_2-H), 4.32 (1H, d.d. J=5, 10 Hz) (C_3-H), 5.17 (1H, q. J= 7Hz) (C_{19}-H).

Lead Tetraacetate Oxidation of 1-Demethyl-Δ^{19}-11-methoxy-2-epi-17,21-dideoxyajmaline (XIV) (from Gardnerine (I))——XIV (50 mg, 0.16 mM) in dry benzene was treated with lead tetraacetate (86 mg, 0.19 mM) under N_2. After 2 hr of stirring at room temperature, the reaction mixture was filtered through a a column of alumina (10 g). The material (70 mg) eluted with benzene–$CHCl_3$ crystallized from ether to furnish pure X (25 mg, 50%), mp 133—138°.

This compound was identified as X by mixed mp determination and comparison of IR spectra (KBr).

Jones Oxidation of Gardnerine (I)——To a stirred solution of I (400 mg, 1.23 mM) in acetone (50 ml), 6N CrO_3–30% H_2SO_4 solution was added dropwise until no more generating precipitate. Then the reaction mixture was made basic with K_2CO_3 and extracted with $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (349 mg) was chromatographed on alumina (30 g). The material (J-1, 49 mg, 17%) eluted with $CHCl_3$ (fr. 14—16) crystallized from ether, yielding prisms of the indolenine ether XVIII (21 mg), mp 151—152°. The material (J-2, 43 mg, 16%) eluted with 1% MeOH–$CHCl_3$ (fr. 21—24) crystallized from MeOH, yielding needles of the indole ether XIX (7 mg), mp 319—320°. This compound was identified as gardnutine (II) by mixed mp determination and comparison of IR spectra (KBr).

33 mg of the starting material (I) was recovered from the 1% MeOH–$CHCl_3$ eluate (fr. 27—29).

Indolenine Ether (XVIII): *Anal.* Calcd. for $C_{20}H_{22}O_2N_2$: C, 74.51; H, 6.88; N. 8.69. Found: C, 74.47; H, 6.83; N, 8.47. UV λ_{max}^{MeOH} mμ (log ε): 285 (3.42). IR ν_{max}^{KBr} cm^{-1}: 1600.

Acid-Catalyzed Rearrangement of the Indolenine Ether (XVIII)——XVIII (10 mg) was dissolved in 1N-H_2SO_4 (1 ml). After heating for 45 min on a steam bath, the solution was made basic with NH_4OH and extracted with $CHCl_3$ containing 10% MeOH.

After dryimg, the solvent was evaporated to dryness *in vacuo*. The residue (10 mg) was crystallized from MeOH, yielding the indole ether XIX (6 mg) in 60% yield. The identity was established by mixed mp determination and comparison of IR spectra (KBr).

$LiAlH_4$ Reduction of Gardnutine (II)——To a stirred suspension of $LiAlH_4$ (360 mg, 9.3 mM) in THF (10 ml), II (100 mg, 0.31 mM) in THF (50 ml) was added slowly under ice cooling and the mixture was refluxed for 14 hr. After decomposing the excess hydride with wet THF, the mixture was filtered and the residue was washed with $CHCl_3$. The filtrates were evaporated to dryness and the residue (212 mg) was subjected to chromatography on alumina (15 g). The material eluted with 3% MeOH–$CHCl_3$ crystallized from acetone, yielding prisms of gardnerine (I) (96 mg, 95%), mp 242—243°. This compound was identified as gardnerine (I) by mixed mp determination, $[\alpha]_D$ and comparison of IR spectra (KBr).

19,20-Dihydrogardnutine (XXIII)——Gardnutine (II) (300 mg) in AcOH (40 ml) was hydrogenated in the presence of PtO_2 (90 mg). It took up 1 mole hydrogen. After filtration, the solvent was removed *in vacuo*. The residue was made basic with NH_4OH and extracted with $CHCl_3$ containing MeOH. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (298 mg) was chromatographed on alumina. Elution with $CHCl_3$ gave XXIII (181 mg, 60%), mp 295—297°. *Anal.* Calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 73.71; H, 7.04; N, 8.54. $[\alpha]_D$ +40.1. Mass Spectrum *m/e* (%): 324 (M^+, 60), 295 (33), 267 (50), 237 (67), 199 (33), 198 (100).

Monoacetylhydroxygardnutine (XX)——A solution of hydroxygardnutine (III) (100 mg) in pyridine (2 ml) was treated with Ac_2O (1 ml) for 3 days at room temperature.

The solution was evaporated to dryness *in vacuo*. The residue was chromatographed on alumina (5 g). The material eluted with benzene–$CHCl_3$ crystallized from MeOH, yielding XX, mp 278—280° (decomp.). *Anal.* Calcd. for $C_{22}H_{24}O_4N_2$: C, 69.45; H, 6.36; N, 7.36. Found: C, 69.27; H, 6.12; N, 7.17. IR ν_{max}^{KBr} cm^{-1}: 3160, 1730. Mass Spectrum *m/e*(%): 380 (M^+, 61), 337 (25), 322 (25), 321 (100), 199 (53), 198 (53).

$LiAlH_4$ Reduction of Hydroxygardnutine (III)——To a stirred suspension of $LiAlH_4$ (640 mg, 16.8 mM) in THF (15 ml), III (190 mg, 0.56 mM) in THF (90 ml) was added slowly under ice cooling and the mixture was refluxed for 15 hr. After decomposing the excess hydride with wet THF, the mixture was filtered and the residue was washed with 10% MeOH–$CHCl_3$. The combined extracts were concentrated *in vacuo*. The residue (211 mg) was chromatographed on alumina (18 g). The material (201 mg) eluted with 3% MeOH–$CHCl_3$ crystallized from acetone, yielding dihydroxy derivative (XXI) (98.5 mg, 52%), mp 162—164°. *Anal.* Calcd. for $C_{20}H_{24}O_3N_2 \cdot H_2O$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.44, 67.50; H, 6.93, 7.21; N, 7.85, 7.81. Mass Spectrum *m/e* (%): 340 (M^+, 70), 295 (45), 267 (65), 237 (100), 199 (40), 198 (100).

Diacetyl Derivative (XXII) of Dihydroxy Compound (XXI)——A solution of XXI (70 mg) in pyridine was treated with Ac_2O. Evaporation of the solution gave a crystalline residue. Recrystallization from MeOH–H_2O afforded needles of XXII, mp 182—183°. *Anal.* Calcd. for $C_{24}H_{28}O_5N_2$: C, 67.90; H, 6.65;

N, 6.60. Found: C, 67.54, 67.63; H, 6.34, 6.24; N, 6.29. Mass Spectrum *m/e* (%): 424 (M^+, 37), 423 (32), 363(89), 362 (100), 304 (58), 199 (63), 198 (100): NMR ($CDCl_3$, δ): 2.0 (3H, s.) and 2.1 (3H, s.) ($COCH_3 \times 2$), 3.8 (3H, s.) (Ar. OCH_3), 4.6 (2H, d. $J=7$ Hz) (C_{18}-2H), 5.43 (1H, t. $J=7$ Hz) (C_{19}-H).

Hydrogenolysis of Hydroxygardnutine (III)——III (300 mg) in AcOH (40 ml) was hydrogenated over PtO_2 (90 mg) at room temperature. It took up about 2 mole hydrogen. After filtration. the solvent was removed *in vacuo*. The residue was made basic with NH_4OH and extracted with $CHCl_3$ containing MeOH. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (300 mg) was chromatographed on alumina. The material (110 mg) eluted with $CHCl_3$ crystallized from MeOH, yielding XXIII (98 mg, 34%), mp 291—296°. *Anal.* Calcd. for $C_{20}H_{24}O_2N_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 73.52; H, 7.67; N, 8.29. $[\alpha]_D^{29}$ +41.4: Mass Spectrum *m/e*: 324 (M^+). This compound was identified as dihydrogardnutine (XXIII) by mixed mp determination, $[\alpha]_D$ and comparison of IR (KBr) and mass spectra.

Manganese Dioxide Oxidation of Hydroxygardnutine (III)——To a solution of III (260 mg, 0.77 mM) in pyridine (20 ml), freshly prepared MnO_2 (900 mg) was added and the mixture was stirred for 5 days at room temperature. After filtration, MnO_2 was washed repeatedly with $CHCl_3$. The combined extracts were evaporated to dryness. The residue (202 mg) was chromatographed on alumina (10 g). The material eluted with $CHCl_3$ crystallized from MeOH, yielding XXIV (104 mg, 38%), mp>310° (dec.). *Anal.* Calcd. for $C_{20}H_{20}O_3N_2 \cdot 1/2H_2O$: C, 69.55; H, 6.13. Found: C, 69.57; H, 6.35. UV λ_{max}^{MeOH} mμ (log ε): 224 (4.84), 256 (3.47, sh.), 294 (3.44), 310 (3.35, sh.): λ_{min}^{MeOH} mμ (log ε): 276 (3.19). IR ν_{max}^{KBr} cm^{-1}: 1673. Mass Spectrum *m/e* (%): 336 (M^+, 57), 199 (100), 198 (93). NMR (DMSO-d_6, δ at 100°): 3.75 (3H, s.) (Ar. OCH_3), 5.40 (1H, d. $J=6.5$ Hz) (C_6-H), 5.98 (1H, d. $J=7$ Hz) (=C$\underline{H}$-CHO), 9.83 (1H, d. $J=7$ Hz) (=CH-C$\underline{H}$O), 10.6 (1H, s.) (N-H).

$NaBH_4$ Reduction of α,β-Unsaturated Aldehyde Derivative (XXIV)——XXIV (26 mg) was reduced with $NaBH_4$ (7 mg) in 80% aqueous MeOH. After 3 hr of stirring at room temperature, the solvent was removed *in vacuo*. The residue was made basic with NH_4OH and extracted with $CHCl_3$ containing MeOH. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (19 mg) was recrystallized from MeOH to furnish III (10 mg), mp 312—313°. The identity was established by mixed mp determination and comparison of IR spectra (KBr).

1-Demethyl-$\Delta^{1,19}$-11-hydroxy-17,21-dideoxyajmaline (XXV) (from Gardnerine (I))——BCl_3 (8 ml, 68 mM) was added dropwise to a solution of X (1.608 g, 5.26 mM) in dry CH_2Cl_2 (40 ml) under dry ice-acetone cooling and the mixture was allowed to stand for 16 hr at the same temperature and 2 more days at room temperature. The solvent was evaporated to dryness. The residue was made basic with 5% aq. $NaHCO_3$ and extracted with $CHCl_3$. The phenolic compound was re-extracted with 3% aq. NaOH (×3), which was treated with NH_4Cl and extracted with $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (1.809 g) was crystallized from MeOH–ether to furnish prisms of XXV (1.361 g, 89%), mp 261—263°. *Anal.* Calcd. for $C_{19}H_{20}ON_2 \cdot 1/2H_2O$: C, 75.70; H, 7.02; N, 9.29. Found: C, 75.52; H, 7.33; N, 9.02. $[\alpha]_D^{26}$ +67.1 ($c=0.45$, MeOH). UV λ_{max}^{MeOH} mμ (log ε): 231.6 (4.40), 276 (3.47), 304 (3.30); λ_{min}^{MoOH} mμ (log ε): 259 (3.41), 296 (3.29): $\lambda_{max}^{5\%NaOH-MeOH}$ mμ: 246.

1-Demethyl-$\Delta^{1,19}$-11-O-tetrazolyl-17,21-dideoxyajmaline (XXVI) (from Gardnerine (I))——To a solution of XXV (1.21 g, 4.18 mM) in acetone (40 ml) was added powdered K_2CO_3 (1.154 g, 8.36 mM) and 1-phenyl-5-chlorotetrazole (0.797 g, 4.18 mM). The mixture was refluxed for 44 hr. After cooling and filtration, the filtrate was concentrated *in vacuo* yielding crude product, which was crystallized from acetone to afford prisms of XXVI (1.663 g, 91%), mp 232—233° (dec.). *Anal.* Calcd. for $C_{26}H_{24}ON_6$: C, 71.54; H, 5.54; N, 19.25. Found: C, 71.35; H, 5.33; N, 19.08. $[\alpha]_D^{26.5}$ +6.5 ($c=0.74$, $CHCl_3$): UV λ_{min}^{MoOH} mμ (log ε): 223 (4.60). IR ν_{max}^{KBr} cm^{-1}: 1535 (tetrazolyl ether). Mass Spectrum *m/e* (%): 436 (M^+, 59), 408 (16), 366 (21), 292 (43), 291 (M−145, 100).

Hydrogenolysis of 1-Demethyl-$\Delta^{1,19}$-11-O-tetrazolyl-17,21-dideoxyajmaline (XXVI) (from Gardnerine (I))——A solution of XXVI (1.15 g, 2.63 mM) in AcOH (40 ml) was hydrogenated over Adams catalyst at 60° for 4 hr. After filtration, the solvent was evaporated to dryness *in vacuo*. The residue was made basic with 10% NH_4OH and extracted with $CHCl_3$. After drying, the solvent was removed and the concentrate was dissolved into benzene, which was washed with 3% aq. NaOH and water. Evaporation of the dried solvent afforded a resinous compound (1.159 g), which was chromatographed on alumina (60 g). The crude XXVII (317 mg) was obtained from the benzene–$CHCl_3$ (2: 1) eluate. This was rechromatographed on a column of alumina (25 g) and the substance eluted with benzene–$CHCl_3$ (2: 1) crystallized from hexane, yielding XXVII (229 mg, 31%), mp 76—83°. UV λ_{max}^{MeOH} mμ: 242, 292; λ_{min}^{MeOH} mμ: 266. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380. Mass Spectrum *m/e* (%): 280 (M^+, 100), 279 (20), 182 (28), 168 (27), 150 (55), 143 (50), 130 (55).

Lead Tetraacetate Oxidation of XXVII; 1-Demethyl-Δ^1-17,21-dideoxyajmaline (XXVIII) (from Gardnerine (I))——To a stirred solution of XXVII (200 mg, 0.71 mM) in dry benzene (6 ml), $Pb(OAc)_4$ (382 mg, 0.86 mM) was added portionwise and the reaction mixture was stirred for 2 hr at room temperature. The solution was filtered through a column of alumina (25 g). The material eluted with benzene–$CHCl_3$ (1: 1) crystallized from hexane, yielding crude XXVIII (131 mg, 67%), mp 149—163°. This was purified by recrystallization from ether, yielding the pure XXVIII (78 mg, 40%), mp 188—189.5°. *Anal.* Calcd. for $C_{19}H_{22}N_2$: C, 81.97; H, 7.97; N, 10.06. Found: C, 81.79; H, 8.16; N, 9.79. UV λ_{max}^{MeOH} mμ (log ε): 227 (4.15, sh.), 259 (3.68); λ_{min}^{MeOH} mμ (log ε): 236.5 (3.54). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1590. Mass Spectrum *m/e* (%): 278 (M^+, 100), 263 (22), 249

(28), 182 (25) 168 (25): ORD (c=0.00269, MeOH), $[\phi]^{29}$ (mμ): +445 (400), +2100 (300, peak), +1400 (291, trough), +1900 (287, peak), +1300 (284, trough), +8900 (264, peak), 0 (260), −35200 (241, trough), 0 (230).

This compound was identified as 1-demethyl-Δ^1-17,21-dideoxyajmaline prepared from ajmaline (IV) by mixed mp determination, TLC and comparison of UV, IR, mass and ORD spectra.

Lead Tetraacetate Oxidation of 2-Hydroxy-17,21-dideoxyajmaline (XIII); 1-Demethyl-Δ^1-17,21-dideoxyajmaline (XXVIII) (from Ajmaline (IV))——$Pb(OAc)_4$ (6.0 g, 6 molar equiv.) was added portionwise to XIII (1.0 g) derived from 21-deoxyajmalol-A (XII) (1.0 g), in dry benzene (50 ml) and the mixture was stirred at room temperature for 2.5 hr. The reaction mixture was filtered through a column of alumina (100 g). The material eluted with benzene–$CHCl_3$ (1: 1) crystallized from ether–hexane, yielding 220 mg (25%) of XXVIII, mp 187°. ORD (c=0.00273, MeOH) $[\phi]^{29}$ (mμ): +438 (400), +2100 (300, peak), +1400 (291, trough), +2000 (287, peak), +1300 (284, trough), +8000 (264, peak), 0 (260), −35500 (241, trough).

1-Demethyl-Na-mesyl-Δ^{19}-11-methoxy-2-epi-17,21-dideoxyajmaline (XXIX) (from Gardnerine (I))——XIV (1.86 g, 6.04 mM) in dry pyridine (20 ml) was treated with mesyl chloride (1.04 g, 9.06 mM) for 2 days at room temperature. The solution was removed *in vacuo*. The residue was treated with 10% $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the dried solvent afforded the desired material (2.85 g). Crystallization from benzene yielded XXIX (1.789 g), mp 215—216°. Rechromatography of the mother liquors (0.323 g) on alumina (35 g) furnished an additional mesylate (XXIX) (0.153 g). Total mesylate (XXIX) weighed 1.942 g (83%). *Anal.* Calcd. for $C_{21}H_{26}O_3N_2S$: C, 65.27; H, 6.28; N, 7.25. Found: C, 65.49; H, 6.68; N, 7.26. IR ν_{max}^{KBr} cm^{-1}: 1348, 1168 (=N–SO_2–): Mass Spectrum m/e (%): 386 (M^+, 34), 307 (100), 121 (58). NMR ($CDCl_3$, δ): 1.64 (3H, d. J=7 Hz) (C_{18}-$(H)_3$), 2.91 (3H, s.) (SO_2CH_3), 3.24 (1H, t. J=4.5 Hz) (C_5-H), 3.46 (2H, br. s.) (C_{21}-2H), 3.79 (3H, s.) (Ar. OCH_3), 3.98 (1H, d. d. J=6.5, 11 Hz) (C_3-H), 5.25 (1H, q. J=7 Hz) (C_{19}-H).

1-Demethyl-Na-mesyl-Δ^{19}-11-hydroxy-2-epi-17,21-dideoxyajmaline (XXX) (from Gardnerine (I))——BCl_3 (3.96 g, 34.32 mM) was added dropwise to a solution of XXIX (1.32 g, 3.43 mM) in dry CH_2Cl_2 (25 ml) under dry ice-acetone cooling and the reaction mixture was allowed to stand at the same temperature for 1 hr and 2 more days at room temperature. CH_2Cl_2 and excess BCl_3 were removed *in vacuo* at room temperature. The residue was made basic with 5% aq. $NaHCO_3$ and extracted with $CHCl_3$.

The phenolic compound was re-extracted with 3% aq. NaOH (×3), which was treated with NH_4Cl and extracted with $CHCl_3$. After drying, the solvent was evaporated *in vacuo*. Crystallization of the residue from benzene furnished needles of XXX (1.02 g, quantitative), mp 175—185°. *Anal.* Calcd. for $C_{20}H_{24}O_3N_2S \cdot 1/2C_6H_6$: C, 67.14; H, 6.67; N, 6.81. Found: C, 66.86; H, 6.52; N, 6.59. $[\alpha]_D^{26.5}$ +29.3 (c=0.43, MeOH): UV λ_{max}^{MeOH} mμ: 230, 255, 270—280. IR ν_{max}^{KBr} cm^{-1}: 1354, 1163 (=N–SO_2–). Mass Spectrum m/e (%): 372 (M^+, 66), 293 (100), 121 (38), 78: NMR ($CDCl_3$, δ): 1.64 (3H, s.) (C_{18}-$(H)_3$), 2.83 (3H, s.) (SO_2CH_3), 3.35 (1H, t. J=5 Hz) (C_5-H), 3.55 (2H, br. s.) (C_{21}-2H), 3.87 (1H, d. J=4.5 Hz) (C_2-H), 4.05 (1H, d. d. J=4.5, 9 Hz) (C_3-H), 5.27 (1H, q. J=7 Hz) (C_{19}-H), 7.34 (3H, s.) (benzene of crystallization).

0.23 g (mp 211—215°) of the starting material (XXIX) was recovered.

Methylation of 1-Demethyl-Na-mesyl-Δ^{19}-11-hydroxy-2-epi-17,21-dideoxyajmaline (XXX) (from Gardnerine (I)) with CH_2N_2——XXX (40 mg) in 20% MeOH–ether (10 ml) was treated with CH_2N_2. Crystallization from acetone furnished prisms (15 mg, mp 205—208°).

This compound was identified as XXIX by mixture mp determination and comparison of IR spectra.

1-Demethyl-Na-mesyl-Δ^{19}-11-O-tetrazolyl-2-epi-17,21-dideoxyajmaline (XXXI) (from Gardnerine (I))——To a solution of XXX (1.0 g, 2.69 mM) in acetone (50 ml) was added powdered K_2CO_3 (0.742 g, 5.38 mM) and 1-phenyl-5-chlorotetrazole (0.453 g, 2.51 mM). The mixture was refluxed for 40 hr. After cooling and filtration, the solvent was removed *in vacuo* to afford a yellow solid (1.308 g), which was chromatographed on a column of silica gel (40 g). Elution with 0.5—2% MeOH–$CHCl_3$ gave XXXI (0.966 g, 62.4%), mp 124—126°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1539 (tetrazolyl ether); 1360, 1166 (=N–SO_2–). Mass Spectrum m/e: 516 (M^+), 437 (M−SO_2CH_3).

1-Demethyl-Na-mesyl-2-epi-17,21-dideoxyisoajmaline (XXXII) (from Gardnerine (I))——A solution of XXXI (0.676 g, 1.305 mM) in AcOH (26 ml) was hydrogenat over Adams catalyst (0.2 g) at 60° for 20 hr. After filtration, the solvent was removed *in vacuo*. The residue was made basic with 10% NH_4OH and extracted with 10% MeOH–$CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (0.685 g) was chromatographed on alumina (30 g). The material (0.283 g, 61%) eluted with benzene–$CHCl_3$ (1: 2) crystallized from ether–hexane, yielding XXXII (85 mg), mp 85—105°. *Anal.* Calcd. for $C_{20}H_{26}O_2N_2S \cdot H_2O$: C, 63.81; H, 7.50; N, 7.44. Found: C, 63.89; 63.84, H. 7,50, 7.53; N, 7.11, 7.34 .UV λ_{max}^{MeOH} mμ (log ε): 231.2 (3.80), 276.8 (3.26), 282.5 (3.21): λ_{min}^{MeOH} mμ (log ε): 251.5 (2.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1357, 1165 (=N–SO_2–). Mass Spectrum m/e (%): 358 (M^+, 15), 280 (21), 279 (M−SO_2CH_3, 100), 168 (10): NMR ($CDCl_3$, δ) 0.92 (3H, t. J=7 Hz) (C_{18}-$(H)_3$), 2.88 (3H, s.) (SO_2CH_3), 3.28 (1H, t. J=5 Hz) (C_5-H), 3.79 (1H, d. J=4 Hz) (C_2-H), 3.93 (1H d. J=5 Hz) (C_3-H), 1.97 (H_2O of crystallization): ORD (c=0.00291, MeOH) $[\phi]^{20}$ (mμ): +324 (400), +1790 (288, peak), 0 (284), −21000 (240, trough), 0 (233), +46900 (220, sh.), +66100 (213).

XXXII (12 mg) gave a crystalline hydrochloride, which was recrystallized from EtOH to afford prisms (6 mg), mp>350° (decomp.). *Anal.* Calcd. for $C_{20}H_{26}O_2N_2S \cdot HCl$: C, 60.82; H, 6.69; N, 7.09. Found: C, 60.69; H, 6.95; N, 6.85.

$LiAlH_4$ Reduction of 1-Demethyl-Δ^1-17,21-dideoxyajamline (XXVIII) (from Ajmaline (IV)); 1-Demethyl-2-epi-17,21-dideoxyajmaline (XXXIII)——To a stirred solution of XXVIII (220 mg, 0.79 mM) derived from ajmaline (IV), in dry ether (50 ml), $LiAlH_4$ (650 mg) was added directly under ice cooling and the mixture was refluxed for 30 hr. After decomposing the excess hydride with wet ether, the mixture was filtered and the residue was washed with ether. After drying, the solvent was evaporated to dryness *in vacuo*. The residue was chromatographed on alumina.

The material eluted with benzene–$CHCl_3$ (1: 1) crystallized from ether–hexane, yielding XXXIII (153 mg, 69%), mp 89°. *Anal.* Calcd. for $C_{19}H_{24}N_2 \cdot H_2O$: C, 76.47; H, 8.78. Found: C, 76.69; H, 8.28. UV $\lambda_{\max}^{\text{MeOH}}$ mμ (log ε): 242 (3.78), 292 (3.45): $\lambda_{\min}^{\text{MeOH}}$ mμ (log ε): 222 (3.47), 266 (2.89). IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 3400, 1613. Mass Spectrum *m/e* (%): 280 (M^+, 100).

1-Demethyl-Na-mesyl-2-epi-17,21-dideoxyajmaline (XXXIV) (from Ajmaline (IV))——XXXIII (153 mg, 0.547 mM) in pyridine was treated with mesyl chloride (320 mg, 2.8 mM) for 2 days at room temperature. The solution was evaporated to dryness *in vacuo*. The residue was treated with 10% aq. $NaHCO_3$ and extracted with $CHCl_3$.

After drying, the solvent was evaporated to dryness *in vacuo* and the residue was chromatographed on alumina. Elution with benzene–$CHCl_3$ (1: 2) gave a noncrystalline XXXIV (120 mg, 61%). UV $\lambda_{\max}^{\text{MeOH}}$ mμ (log ε): 230.5 (3.88), 276.5 (3.26), 282.5 (3.23); $\lambda_{\min}^{\text{MeOH}}$ mμ (log ε): 251 (2.41). IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 1357, 1165 (=N–SO_2–). Mass Spectrum *m/e* (%): 358 (M^+, 9), 280 (22), 279 (M-SO_2CH_3, 100). NMR ($CDCl_3$, δ): 0.92 (3H, t. $J=7$ Hz) (C_{18}-$(H)_3$), 2.85 (3H, s.)) SO_2CH_3), 3.30 (1H, t. $J=5.3$ Hz) (C_5-H), 3.84 (1H, d. $J=4$ Hz) (C_2-H), 3.89 (1H, d. $J=5.5$ Hz) (C_3-H). ORD ($c=0.00223$, MeOH) $[\phi]^{20}$ (mμ): +107 (400), +1970 (288, peak), 0 (284), −23900 (240, trough), 0 (233), +69800 (213).

1-Demethyl-Δ^1-17,21-dideoxyisoajmaline (XXXVIII) (from Isoajmaline (XXXV))——21-Deoxyisoajmalol-A(XXXVI) (0.777 g, 2.56 mM) was dissolved in pyridine (15 ml) containing tosyl chloride (1.17 g, 6.1 mM) and was allowed to stand at room temperature for 2 days. Additional tosyl chloride (0.8 g, 4.2 mM) was added, continuing the reaction for 3 more days. The solvent was evaporated to dryness *in vacuo*. The residue was treated with aq. $NaHCO_3$ and extracted with CH_2Cl_2.

After drying, the solvent was removed to furnish a yellow foam of 2-hydroxy-17,21-dideoxyisoajmaline (XXXVII) (1.5 g). Then, to solution of XXXVII (1.5 g) in dry benzene (50 ml), $Pb(OAc)_4$ (7.167 g, 16.1 mM) was added gradually. After 2 hr of stirring at room temperature, the solvent was evaporated to dryness *in vacuo*.

The residue was chromatographed on alumina (70 g), eluting crude product (0.79 g) with $CHCl_3$ (400 ml). This was purified by chromatography on alumina (80 g) to furnish an oil (0.167 g), which was further chromatographed on alumina (25 g), eluting a pure oil (XXXVIII) (0.14 g, 20% yield from XXXVI) with benzene–$CHCl_3$ and $CHCl_3$.

XXXVIII: IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 1595. Mass Spectrum *m/e* (%): 278 (M^+, 100), 249 (23), 182 (43), 168 (13).

$LiAlH_4$ Reduction of 1-Demethyl-Δ^1-17,21-dideoxyisoajmaline (XXXVIII) (from Isoajmaline (XXXV)); 1-Demethyl-2-epi-17,21-dideoxyisoajmaline (XXXIX)——To a stirred solution of XXXVIII (0.14 g, 0.5 mM) in dry ether (30 ml), $LiAlH_4$ (0.57 g, 15 mM) was added directly under ice cooling and the mixture was refluxed for 15 hr. After decomposing the excess hydride with wet ether, the mixture was filtered and the residue was washed with ether and $CHCl_3$. After drying, the solvent was evaporated to dryness *in vacuo*. The residue (0.13 g) was chromatographed on alumina (10 g). Elution with benzene–$CHCl_3$ (1: 1—1: 2) gave a partially crystalline XXXIX (72 mg, 52%), mp 89—91.5°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 3380. Mass Spectrum *m/e* (%): 280 (M^+, 100), 279 (28), 172 (28), 168 (28), 150 (100), 143 (94), 130 (94).

1-Demethyl-Na-mesyl-2-epi-17,21-dideoxyisoajmaline (XXXII) (from Isoajmaline (XXXV))——XXXIX (72 mg, 0.26 mM) in dry pyridine (3 ml) was treated with mesyl chloride (200 mg, 1.74 mM) for 2 days at room temperature. The solution was evaporated to dryness *in vacuo*. The residue was treated with 10% aq. $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the dried solvent afforded a brown oil (0.102 g), which was chromatographed on a column of alumina (10 g). The material (62 mg, 67%) eluted with benzene–$CHCl_3$ (1: 1) crystallized from ether–hexane, yielding prisms of XXXII (11 mg), mp 85—91.5°. This compound was identified as XXXII prepared from gardnerine (I) by mixed mp determination, TLC and comparison of UV, IR, NMR, mass and ORD spectra.

Table I. NOE Experimental Results of II

Irradiation	Area of the signals				Increase in %
	H (17) + H (15)	H (15)	H (17)	H (15)	
None	100	55.7	100	125	13.6(H 15)
at CH_3(18)	100	58.7	100	142	

NOE Experiment——This NOE experiment was carried out on JNM-4H 100 spectrometer that was calibrated using dimethylformamide[19] as a standard. For NOE experiment, a 10% W/V DMSO-d_6 +CF_3-COOH solution of pure gardnutine (II) was momentarily warmed and sealed with N_2. This solution was studied on JNM-4H 100 spectrometer operated at 100 MHz by a frequency sweep method using TMS as an internal filed frequency lock. The results were summarized in Table I.

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