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67. Tohru Kikuchi and Shoichiro Uyeo: Pachysandra Alkaloids. IX.*1 Structure of Pachysantermine-A, a Novel Intramolecular Ester Alkaloid.*2

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Pachysantermine-A is one of the minor alkaloids of *Pachysandra terminalis* SIEB. et ZUCC., a Buxaceous plant, and the structure including absolute configuration of pachysantermine-A is now assigned to the formula Ia. It is unique in that it has a seven-membered ring ester grouping containing a nitrogen atom. Also a biogenetic pathway of pachysantermine-A (Ia) in the living plant and of pachystermine-A (VIIa) and -B (VIIb), which are the closely related alkaloids, was presumed.

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Pachysantermine-A is one of the minor alkaloids of *Pachysandra terminalis* SIEB. et ZUCC. (Japanese name: Fukki-so), a Buxaceous plant, and it was isolated from the strong base fraction together with a number of pregnane type alkaloids.[1] The particular feature of this alkaloid is that it has a seven-membered ring ester grouping involving a nitrogen atom and represents a novel type of pregnane alkaloid. The structure of pachysantermine-A is now assigned to the formula (Ia) based on a series of degradative evidences described below. As will be clear in the sequel, the alkaloid (Ia) is closely related to pachystermine-A (VIIa) and -B (VIIb), reported in the preceding paper.*1

Pachysantermine-A (Ia), m.p. 260~263°, $[\alpha]_D$ +43° ($CHCl_3$), was analyzed for $C_{29}H_{48}O_2N_2$, which was supported by the molecular ion peak at m/e 456 in the mass spectrum.*4 The infrared spectrum*5 had a carbonyl absorption band at 1710 cm^{-1} and a weak NH band at 3300 cm^{-1}, whereas the ultraviolet spectrum in ethanol exhibited an absorption maximum at about 214 mμ (ε 10,000) which could be attributed to an α,β-unsaturated ester grouping.[2] As shown in Fig. 1, its NMR (nuclear magnetic resonance) spectrum*6 revealed evidently the presence of no olefinic proton in the molecule, but one proton geminal to O-acyl grouping (5.70τ, triplet, J=3 c.p.s.), one N-dimethyl (7.85τ), two allylic methyls (8.18 and 8.32τ, broad), two tertiary C-methyls (8.96 and 9.35τ), and one secondary C-methyl (9.15τ, doublet, J=6 c.p.s.). Another characteristic signal at 6.48τ (broad), corresponding with two protons, was later assigned to the methylene standing between the nitrogen atom and the double bond on the basis of the chemical shift and by comparison with the spectrum of dihydropachysantermine-A (IIa). These spectral data coupled with the empirical formula suggested that pachysantermine-A, like other Pachysandra alkaloids already determined, might have the 3,20-diamino-5α-pregnane structure.

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*1 Part VIII. T. Kikuchi, S. Uyeo, Jr.: This Bulletin, **15**, 549 (1967).

*2 Preliminary accounts of this work appeared in Tetrahedron Letters, No. 39, 3487 (1965).

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*4 Mass spectra were taken on a Hitachi Mass Spectrometer Model RMU-6D equipped with a direct inlet system (Model MG-150).

*5 Infrared spectra were determined in chloroform solutions unless otherwise specified.

*6 All the NMR spectra were measured on a Varian Associates A-60 NMR Spectrometer (60 Mc.) in deuterated chloroform solutions and chemical shifts are reported in τ values using tetramethylsilane as the internal reference.

1) Part I. M. Tomita, T. Kikuchi, S. Uyeo, Jr., T. Nishinaga, M. Yasunishi (nèe Ando), A. Yamamoto: Yakugaku Zasshi, **87**, 215 (1967).

2) J. C. D. Brand, A. I. Scott: "Elucidation of Structures by Physical and Chemical Methods" (K. W. Bentley, Ed.), Part I, 84 (1963), Interscience Publishers, New York.

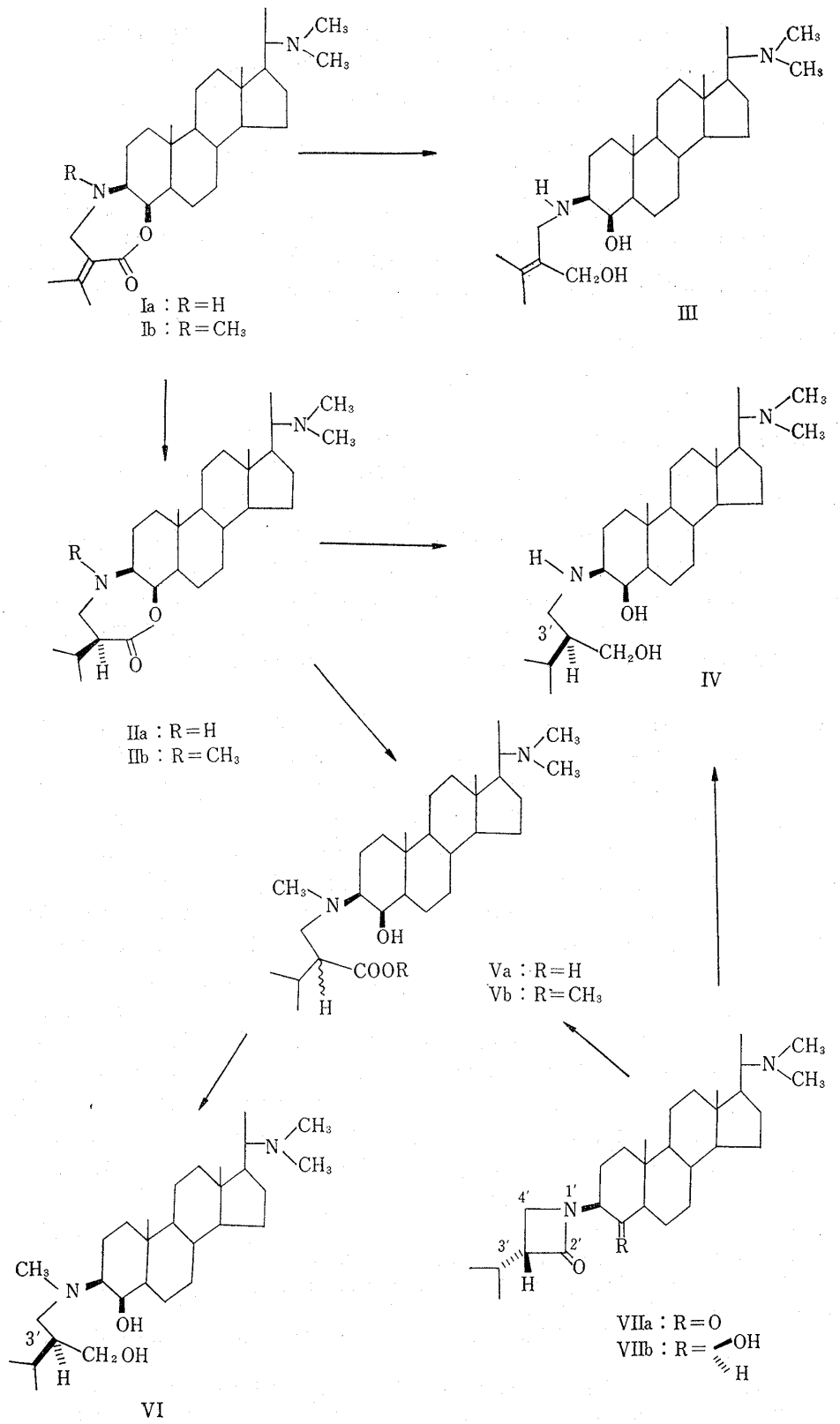

Chart 1.

In consistency with the above supposition, the mass spectrum of the alkaloid (Ia) exhibited a very strong base peak at m/e 72 (CH_3-CH=N^+(CH_3)$_2$) (Fig. 2),*7 which is the characteristic of 20-dimethylaminopregnane alkaloids.[3]

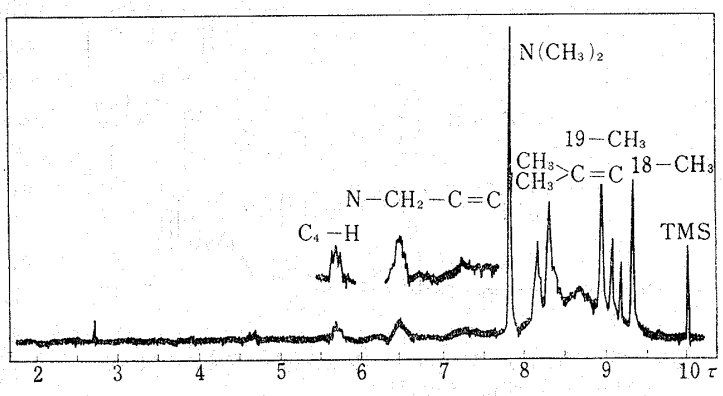

Fig. 1. Nuclear Magnetic Resonance Spectrum of Pachysantermine-A (Ia)

In addition, the ORD (optical rotatory dispersion) curve of the alkaloid (Ia) in methanol demonstrated a positive plane curve in the range of 300~700 mμ, indicating that no carbonyl group is associated with the skeletal ring system.

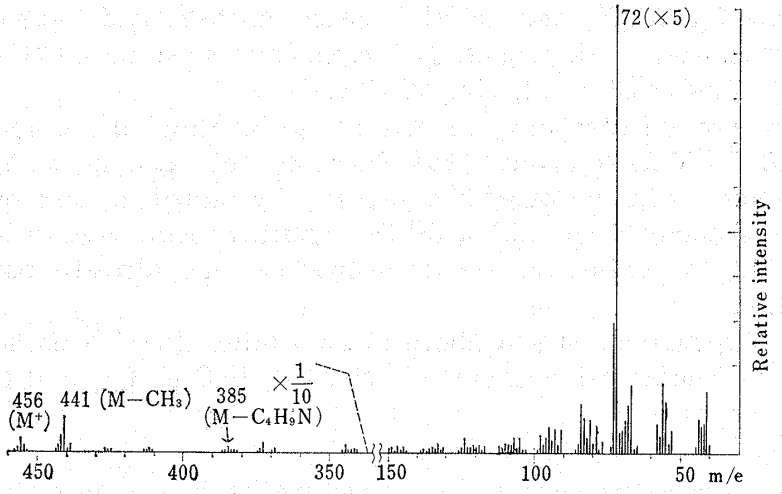

Fig. 2. Mass Spectrum of Pachysantermine-A (Ia)

Treatment of pachysantermine-A (Ia) with formalin-formic acid gave an N-methyl compound (Ib), $C_{30}H_{50}O_2N_2$, m.p. 258~261°, showing an NMR signal for one N-methyl group (7.62τ) along with the N-dimethyl signal (7.85τ) which appeared in the spectrum of original pachysantermine-A (Ia). The formation of a new N-methyl group indicated that one of the amino groups, probably at 3-position, in the alkaloid (Ia) is secondary. In addition, the chemical shift of a broad signal (5.73τ), attributable to a proton geminal to the oxygen function, coupled with the occurrence of no hydroxyl absorption in the infrared spectrum suggetsed that the oxygen and the carbonyl group may be forming an ester grouping.

Upon hydrogenation of pachysantermine-A (Ia) over platinum oxide in methanol containing acetic acid,*8 there was obtained a dihydro compound (IIa), m.p. 260~265°, $[\alpha]_D$ +30° ($CHCl_3$). Treatment of this compound (IIa) with formalin followed by sodium borohydride reduction gave rise to a dihydro-N-methyl compound (IIb), m.p. 230~235°.

*7 Another significant feature concerning the mass spectrum is that no fragment ion associated with the cleavage of the A-ring occurred. This provides a support for the proposed structure (Ia).

*8 No hydrogen uptake was observed when palladized charcoal and methanol were used.

3) L. Dolejs, V. Hanus, V. Cerny, F. Sorm: Collection Czech. Chem. Communs., **28**, 1584 (1963); W. Vetter, P. Longevialle, F. Khuong-Huu-Laine, Q. Khuong-Huu, R. Goutarel: Bull. soc. chim. France, **1963**, 1324; H. Budzikiewicz, C. Djerassi, D. H. Williams: "Interpretation of Mass Spectra of Organic Compounds," 74 (1964), Holden-Day, Inc., San Francisco. See also Part VII of this series.

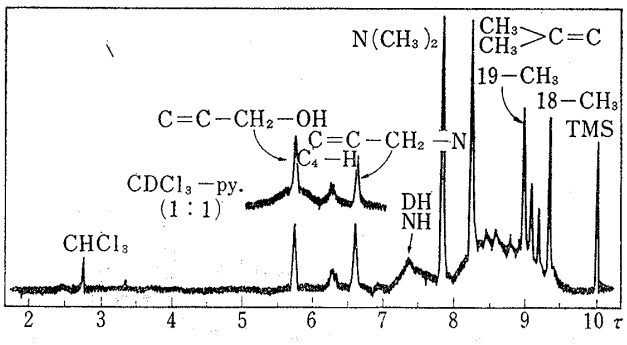

Fig. 3. Nuclear Magnetic Resonance Spectrum of Pachysantermine-diol (Ⅲ)

Each of the above two compounds, Ⅱa and Ⅱb, showed an infrared sbsorption band at 1725 cm^{-1} which could be ascribed to a saturated ester grouping. Their NMR spectra were characterized by the disappearance of two allylic hydrogen and two allylic methyl signals and the appearance of signals corresponding to five C-methyl groups in the range of 8.97~9.35τ, the two of which were later assigned to an isopropyl group (9.03τ, doublet, J=7 c.p.s.).

Although pachysantermine-A (Ia) was not affected with sodium borohydride, but it was reduced with lithium aluminum hydride to give a diol (Ⅲ), m.p. 222~223°, $[\alpha]_D$ +11° ($CHCl_3$). Elemental analyses of this compound (Ⅲ) gave results in agreement with the empirical formula $C_{29}H_{52}O_2N_2$ and its NMR spectrum exhibited the signals arising from a secondary alcohol (6.27τ, 1H, triplet, J=3 c.p.s.) and groupings, C=C-C<u>H</u>$_2$OH (5.73τ, 2H, singlet) and C=C-C<u>H</u>$_2$-N (6.61τ, 2H, singlet) (Fig. 3).

In the same way, dihydropachysantermine-A (Ⅱa) afforded a dihydro-diol compound (Ⅳ), m.p. 215~220°. This compound was shown to be identical with 3′-iso-pachystermine-diol (Ⅳ), reported in the preceding paper,*1 by mixed melting point determination and infrared (KBr) comparison, although the melting point was a little low.*9 The stereochemistry at 3′-position in the compound (Ⅳ) has already been decided to be "*S*"-configuration.*1

From these observations stated above, pachysantermine-A was believed to have a conjugated intramolecular ester grouping, -NH-CH_2-C(COO-)=C-$(CH_3)_2$, and the structure may be assigned to Ia.

The confirmative evidence for the structure (Ia) was provided by the following sequence of reactions.

Base treatment of N-methyldihydropachysantermine-A (Ⅱb) led to an amino-acid (Va), whose infrared spectrum in chloroform showed a broad band at 1590 cm^{-1} for a carboxylate ion. The amino-acid (Va) was then led to the methyl ester (Vb) by treatment with diazomethane. This compound (Vb) demonstrated ester bands at 1725 and 1165 cm^{-1} in the infrared spectrum which was almost superimposable upon that of the amino-acid methyl ester (Vb) (mixture of the diastereoisomers at 3′-position) obtained from pachystermine-B (Ⅶb).*1

Upon reduction with lithium aluminum hydride, followed by careful alumina chromatography, the above methyl ester gave a diol (Ⅵ), m.p. 170~172°, $[\alpha]_D$ +36° ($CHCl_3$). This was found to be quite identical with 3′-iso(*S*)-N-methylpachystermine-diol (Ⅵ), m.p. 170~172°, $[\alpha]_D$ +40° ($CHCl_3$), by direct comparison (mixed melting point and IR (KBr)).

On the basis of chemical evidences so far presented, the structure of pachysantermine-A is now represented by the formula (Ia).

In the end of this report, we would like to refer to a biogenetic pathway for pachysantermine-A (Ia) and the closely related alkaloids, pachystermine-A (Ⅶa) and -B

*9 This may be due to the contamination of a trace of 3′-normal (*R*) epimer. The homogeneity of dihydropachysantermine-A (Ⅱa) must also be fairly good. This indicated that the catalytic hydrogenation of Ia had taken place stereospecifically.

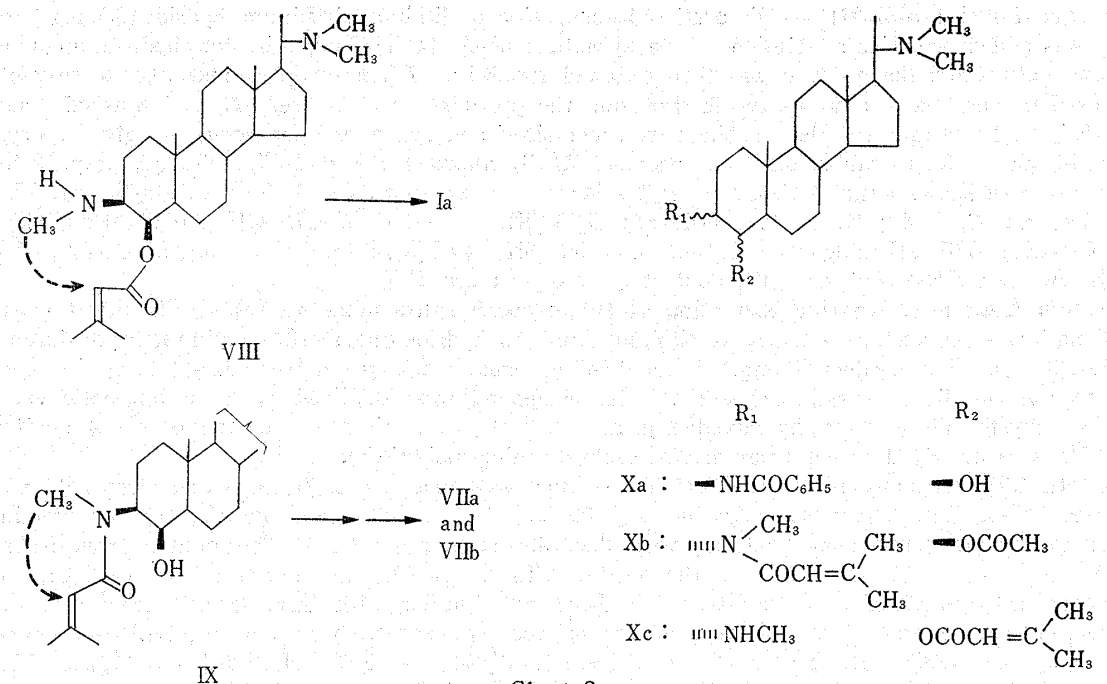

Chart 2.

(VIIb), in the living plant. Probable precursors might be VIII and IX or their analogues, respectively. An intramolecular ring closure between the N-methyl group and the α-carbon atom respective to the carbonyl group may occur to afford the alkaloids. This participation of the N-methyl group in biogenetic cyclization is comparable with the so-called "berberine bridge" formation reaction, in which the bridge methylene is known to originate from the N-methyl group of a benzylisoquinoline precursor.[4]

As a matter of course, this argument is only a speculation, but the presence of such precursors as VIII and IX is acceptable since alkaloids with very similar structure, namely epipachysandrine-A (Xa),[5] pachysandrine-B (Xb) and pachysandrine-D (Xc), have actually been isolated from the same plant.

Experimental*10

N-Methylpachysantermine-A (Ib)——A solution of pachysantermine-A (Ia) (50 mg.) in formic acid (2 ml.) and 37% formalin (2 ml.) was heated on a water bath for 4 hr. After cooling, the mixture was made alkaline with dil. Na_2CO_3, extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated. The crystalline residue (50 mg.) was recrystallized to show m.p. 258~261°. *Anal.* Calcd. for $C_{30}H_{50}O_2N_2$: C, 76.54; H, 10.71; N, 5.95. Found: C, 76.41; H, 10.51; N, 6.16. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715 (conj. ester). NMR τ: 5.73 (1H, broad, C<u>H</u>-OCOR), 6.78 (2H, broad, N-C<u>H</u>$_2$-C=C), 7.62 (3H, N-CH_3), 7.85 (6H, N-$(CH_3)_2$), 8.17, 8.31 (6H, $(CH_3)_2$-C=C), 8.95, 9.35 (6H, two *tert* CH_3), and 9.13 (3H, doublet, J=6 c.p.s.; *sec* CH_3). MS (m/e): 470 (M^+), 455 ($M^+ - CH_3$), 399 ($M^+ - C_4H_9N$), 384 ($M^+ - C_4H_9N \cdot CH_3$), and 72 (base peak, CH_3-CH=$N^+(CH_3)_2$).

Catalytic Hydrogenation of Pachysantermine-A (Ia)——The alkaloid (Ia) (63 mg.) was hydrogenated over pre-reduced platinum oxide (50 mg.) in MeOH (10 ml.) containing acetic acid (2 drops) at room temperature and atmospheric pressure until hydrogen uptake ceased. Catalyst was filtered off and the filtrate was evaporated under reduced pressure. The residue was taken up in CH_2Cl_2, washed with dil. Na_2CO_3, dried over K_2CO_3, and the solvent was removed by evaporation. Recrystallizations of the crystalline residue (60 mg.) from acetone-CH_2Cl_2 gave dihydropachysantermine-A (IIa), m.p. 260~265°, $[\alpha]_D^{25}$ 30° (c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (saturated ester). NMR (τ): 5.55 (1H, broad, C<u>H</u>-OCOR), 7.83 (6H, N-$(CH_3)_2$), 8.98, 9.33 (6H, two *tert* CH_3), 9.03 (6H, doublet, J=7 c.p.s.; $(CH_3)_2$CH-), and 9.15 (3H, doublet, J=6 c.p.s.; *sec* CH_3).

*10 All the melting points were determined on a Yanagimoto Micro Melting Point Apparatus and not corrected. All the specific rotations were measured in chloroform solutions.

4) D. H. R. Barton, R. H. Hesse, G. W. Kirby: J. Chem. Soc., **1965**, 6379.
5) T. Kikuchi, S. Uyeo, Jr., T. Nishinaga: Tetrahedron Letters, No. 16, 1749 (1966).

Pachysantermine-diol (III)——To a stirred suspension of lithium aluminum hydride (350 mg.) in ether (20 ml.) was added dropwise a solution of pachysantermine-A (Ia) (185 mg.) in tetrahydrofuran (15 ml.) at room temperature and the mixture was then refluxed for 2 hr. After complex and excess reagent were decomposed by addition of aqueous tetrahydrofuran, the precipitate was filtered off and washed thoroughly with $CHCl_3$. The filtrate and the washings were combined and evaporated *in vacuo* to give a crystalline product (160 mg.). Recrystallization from acetone-CH_2Cl_2 afforded the diol (III) (138 mg.), m.p. 219~222°. The pure sample showed m.p. 222~223°, $[\alpha]_D^{30}$ +11° (c =1.0). *Anal.* Calcd. for $C_{29}H_{52}O_2N_2$: C, 75.60; H, 11.38. Found: C, 75.88; H, 11.59. NMR (τ): 5.73 (2H, singlet, C=C-C<u>H</u>$_2$-OH-), 6.27 (1H, triplet, J =3 c.p.s.; C<u>H</u>-OH), 6.61 (2H, singlet, N-C<u>H</u>$_2$-C=C), 7.86 (6H, N-$(CH_3)_2$), 8.27 (6H, singlet, $(CH_3)_2$C=C), 9.00, 9.37 (6H, two *tert* CH_3), and 9.15 (3H, doublet, J=7 c.p.s.; *sec* CH_3).

Lithium Aluminum Hydride Reduction of Dihydropachysantermine-A (IIa)——The dihydro compound (IIa) (15 mg.) was reduced with excess of lithium aluminun hydride under refluxing in tetrahydrofuran (3 ml.) and ether (3 ml.). The product (10 mg.), isolated in the usual working up, was recrystallized from acetone-CH_2Cl_2 to give the diol (IV), m.p. 215~220°. This compound was identified with an authentic sample of 3′-iso(*S*)-pachystermine-diol (IV) by mixed m.p. and IR (KBr) comparison. But, the IR (KBr) spectrum of the diol (IV) was clearly different from that of pachystermine-diol (3′-*R*).

N-Methyldihydropachysantermine-A (IIb)——Dihydro compound (IIa) (55 mg.) was dissolved in MeOH (5 ml.) and CH_2Cl_2 (5 ml.), and treated with 37% formalin (0.1 ml.) at room temperature for 30 min. To this mixture, sodium borohydride (200 mg.) was added and stirred for 2 hr. The product was isolated by removal of solvents *in vacuo*, dilution of the residue with dil. Na_2CO_3, and extraction with CH_2Cl_2. It was chromatographed over alumina (0.5 × 10 cm.) in benzene. Elution with benzene and ether-benzene (1:4) gave the N-methyl compound (IIb) (40 mg.) which was recrystallized from acetone to afford colorless crystals (23 mg.), m.p. 230~235°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (ester). NMR (τ): 5.65 (1H, broad, C<u>H</u>-OCOR), 7.61 (3H, N-CH_3), 7.85 (6H, N-$(CH_3)_2$), 8.97, 9.35 (6H, two *tert* CH_3), 9.04 (6H, doublet, J=7 c.p.s.; isopropyl), and 9.17 (3H, doublet, J=6 c.p.s.; *sec* CH_3).

Alkaline Hydrolysis of N-Methyldihydropachysantermine-A (IIb) and the Subsequent Methylation with Diazomethane——A solution of the compound (IIb) (40 mg.) in 5% NaOH-MeOH (4 ml.) was refluxed for 3 hr. The solvent was removed by evaporation under reduced pressure and the residue was diluted with water, neutralized with dil. HCl, and then made basic with NH_4OH. This mixture was extracted with CH_2Cl_2 and the organic phase was washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated to afford the oily amino-acid (Va) (49 mg.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1590 (-COO^-). This compound was methylated without further purification.

The above amino-acid (Va) was dissolved in CH_2Cl_2-MeOH (1:1, 5 ml.) and treated with etherial diazomethane overnight. After removal of the solvents under reduced pressure, the residue was chromatographed on alumina (0.5 × 10 cm.) and eluted with benzene to give the oily methyl ester (Vb) (32 mg.), giving a single spot on thin-layer chromatography.*11 IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1725, and 1165 (-$COOCH_3$). The IR spectrum ($CHCl_3$) of this substance was found to be identical with the amino-acid methyl ester (Vb) obtained from pachystermine-B (VIIb).

Lithium Aluminum Hydride Reduction of the Methyl Ester (Vb)——The above methyl ester (Vb) (32 mg.) was reduced by refluxing with lithium aluminum hydride (50 mg.) in ether for 3 hr. The product obtained by the usual working up was dissolved in CH_2Cl_2 and extracted with 3% HCl. The acidic extract was made basic with NH_4OH and again extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated. Crystallization of the residue (30 mg.) from acetone gave the diol compound (VI) (15 mg.), m.p. 163~167°, which was chromatographed over alumina (0.5 × 10 cm.) from benzene. Subsequent to the benzene elution, the column was eluted with ether-benzene mixture and with ether to give fractions showing single spot on thin-layer chromatography. These were combined and evaporated. Recrystallization of the eluate from acetone gave the diol compound (VI) as colorless plates (6 mg.), m.p. 170~172°, $[\alpha]_D^{30}$ +36° (c=1.0). The IR (KBr) spectrum was superimposable upon that of an authentic sample of 3′-iso(*S*)-N-methylpachystermine-diol (VI) and their mixture showed no melting point depression.

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*11 Merck, aluminium oxide G acc. to Stahl. Developing solvent: chloroform or chloroform-acetone (2:1). Coloring reagent: iodine vapor or Dragendorff reagent.