the solvent and crystallization from ether-MeOH mixture afforded the diosphenol (XVIII, 125 mg.), which was identical with the diosphenol obtained above.

The authors are indebted to Prof. Y. Urushibara (Sophia University), Dr. H. Ando, Dr. S. Wada and Mr. K. Yasuda (this Laboratory) for their kind guidances and encouragements throughout the course of this work. They are also grateful to Dr. Y. Kondo (Tohoku University) for NMR spectral measurement.

[Chem. Pharm. Bull. 15(2) 193～207 (1967)]

UDC 581.19 : 582.761 : 547.94

27. Masao Tomita, Shoichiro Uyeo, and Tohru Kikuchi : Pachysandra Alkaloids. II.*1 Structure of Pachysandrine-A, a New Pregnane Type Alkaloid.*2

(*Faculty of Pharmaceutical Sciences, Kyoto University**3)

Structure and stereochemistry of pachysandrine-A, one of the major alkaloids isolated from the weakly basic alkaloid fraction of *Pachysandra terminalis* SIEB. et ZUCC. (Buxaceae), was discussed and assigned to the formula Ia. This presents the first example of 4-oxygenated 3,20-diamino-5α-pregnane alkaloid discovered in natural source.

(Received May 27, 1966)

In the preceding paper,*1 we reported the isolation and characterization of a number of alkaloids of *Pachysandra terminalis* SIEB. et ZUCC. (Japanese name : Fukkiso), a Buxaceous plant. This paper deals with the full detail of the structural elucidation of pachysandrine-A (Ia), one of the major alkaloids obtained from the weakly basic alkaloid fraction. As will be clear in the sequel, this alkaloid, together with pachysandrine-B which will be discussed in the following paper, is the first pregnane type alkaloid discovered from a plant family other than Apocynaceae and also the first example of the pregnane alkaloid having an oxygen function at the 4-position.

Pachysandrine-A (Ia), m.p. 234～236°, $[\alpha]_D$ +80° ($CHCl_3$), was analyzed for $C_{33}H_{50}O_3N_2$ and demonstrated infrared bands*4 for an O-acetyl (1730 and 1245 cm^{-1}) and a conjugated tertiary amide (1620 cm^{-1}). Its NMR spectrum*5 revealed the presence of a phenyl group (2.67τ, 5H, singlet), a CH-CH-(O-Ac)-CH grouping (4.62τ, 1H, quartet, J 5 and 6 c.p.s.), an amide N-methyl (7.07τ,3H), an N-dimethyl (7.84τ,6H), an O-acetyl (7.98τ, 3H), two tertiary C-methyls (9.06 and 9.36τ, 6H), and a secondary C-methyl group (9.13τ, 3H, doublet, J 6 c.p.s.) (Fig. 3).

Hydrolysis of the alkaloid (Ia) with 5% methanolic sodium hydroxide gave an O-desacyl compound (Ib), $C_{31}H_{48}O_2N_2$, m.p. 194～195°, showing still an amide band (1610 cm^{-1}), but no O-acetyl band in the infrared spectrum. Upon acetylation, the O-desacyl compound (Ib) returned to pachysandrine-A (Ia).

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*1 Part I. M. Tomita, T. Kikuchi, S. Uyeo, Jr., T. Nishinaga, M. Yasunishi (née Ando), A. Yamamoto : Yakugaku Zasshi, **87**, 198 (1967).

*2 Preliminary report of this work appeared in Tetrahedron Letters, No. 18, 1053 (1964).

*3 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto (富田真雄, 上尾庄一郎, 菊池 徹).

*4 All the IR spectra were determined in chloroform solutions unless otherwise stated. For identification of compounds, spectra were measured in KBr discs using a Koken DS-301 Spectrometer.

*5 All the NMR spectra were measured on a Varian Associates A-60 High-Resolution NMR Spectrometer in deuterated chloroform using tetramethylsilane as the internal reference and chemical shifts are reported in τ values.

When hydrolyzed with 20% ethanolic potassium hydroxide, pachysandrine-A (Ia) afforded benzoic acid and the O,N-desacyl compound (IIa), $C_{24}H_{44}ON_2$, m.p. 226~227°, which, on acetylation, gave an O,N-diacetate (IVa), m.p. 192~193°. Therefore, pachysandrine-A has an O-acetyl and an N-benzoyl group.

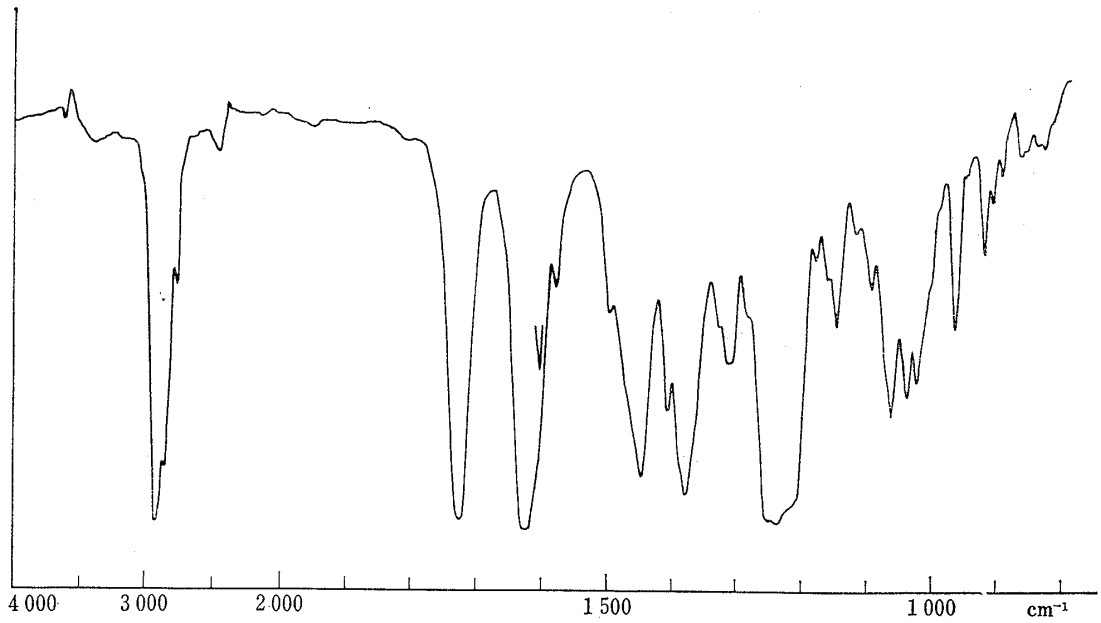


Fig. 1. Infrared Spectrum of Pachysandrine-A (Ia)

Treatment of O,N-desacylpachysandrine-A (IIa) with formalin-formic acid or with formalin-sodium borohydride[1] yielded an N-methyl compound (IIb), m.p. 126~150°,*6 $[\alpha]_D$ +21° ($CHCl_3$). Elemental analyses of this compound gave a result supporting the empirical formula $C_{25}H_{46}ON_2 \cdot 1/2\ H_2O$, and the NMR spectrum exhibited signals attributable to a geminal hydrogen with respect to the OH group (6.20τ, 1H, broad), two N-dimethyls (7.75 and 7.86τ, 12H), two tertiary C-methyls (8.95 and 9.35τ, 6H), and a secondary C-methyl (9.15τ, 3H, doublet, J 6 c.p.s.). Acetylation of this compound gave an O-acetate (IVb), m.p. 169~170°.

From the foregoing rerults, pachysandrine-A could be represented by the expanded molecular formula $C_{18}H_{24}(OCOCH_3)(N\text{-}(CH_3)COC_6H_5)(N\text{-}(CH_3)_2)$(tert. $CH_3)_2$(sec. CH_3).

Chromic acid oxidation of O,N-desacylpachysandrine-A (IIa) and its N-methyl compound (IIb) in acetic acid afforded the corresponding oxo compounds (IIIa), $C_{24}H_{42}ON_2$, m.p. 169~170°, and (IIIb), $C_{25}H_{44}ON_2$, m.p. 187~188°, respectively. Both the oxo compounds showed the six-membered ketone band at 1710 cm^{-1} in the infrared spectra and their ORD curves exhibited negative Cotton effects which are very similar to that of cholestane-4-one[2] (Fig. 2).

Although both ketones (IIIa and IIIb) were not affected by sodium borohydride reduction, the ketone IIIa was reduced effectively with lithium aluminum hydride to reform O,N-desacylpachysandrine-A (IIa).

Of interest in this connection was the observation that the reduction of IIIb with the same reagent resulted in the formation of a mixture of two possible epimeric isomers.*7

*6 This compound did not give sharp melting point.

*7 Major product in this case seemed to be the 4-epimeric alcohol (XXIVb), since the IR spectrum of the crude product obtained was very close to that of XXIVb.

1) J. H. Biemann : "Mass Spectrometry, Organic Chemical Applications," 358 (1962), McGraw-Hill, New York; K. A. Schellenberg : J. Org. Chem., **28**, 3259 (1963).

2) C. Djerassi : "Optical Rotatory Dispersion," 43 (1960), McGraw-Hill, New York.

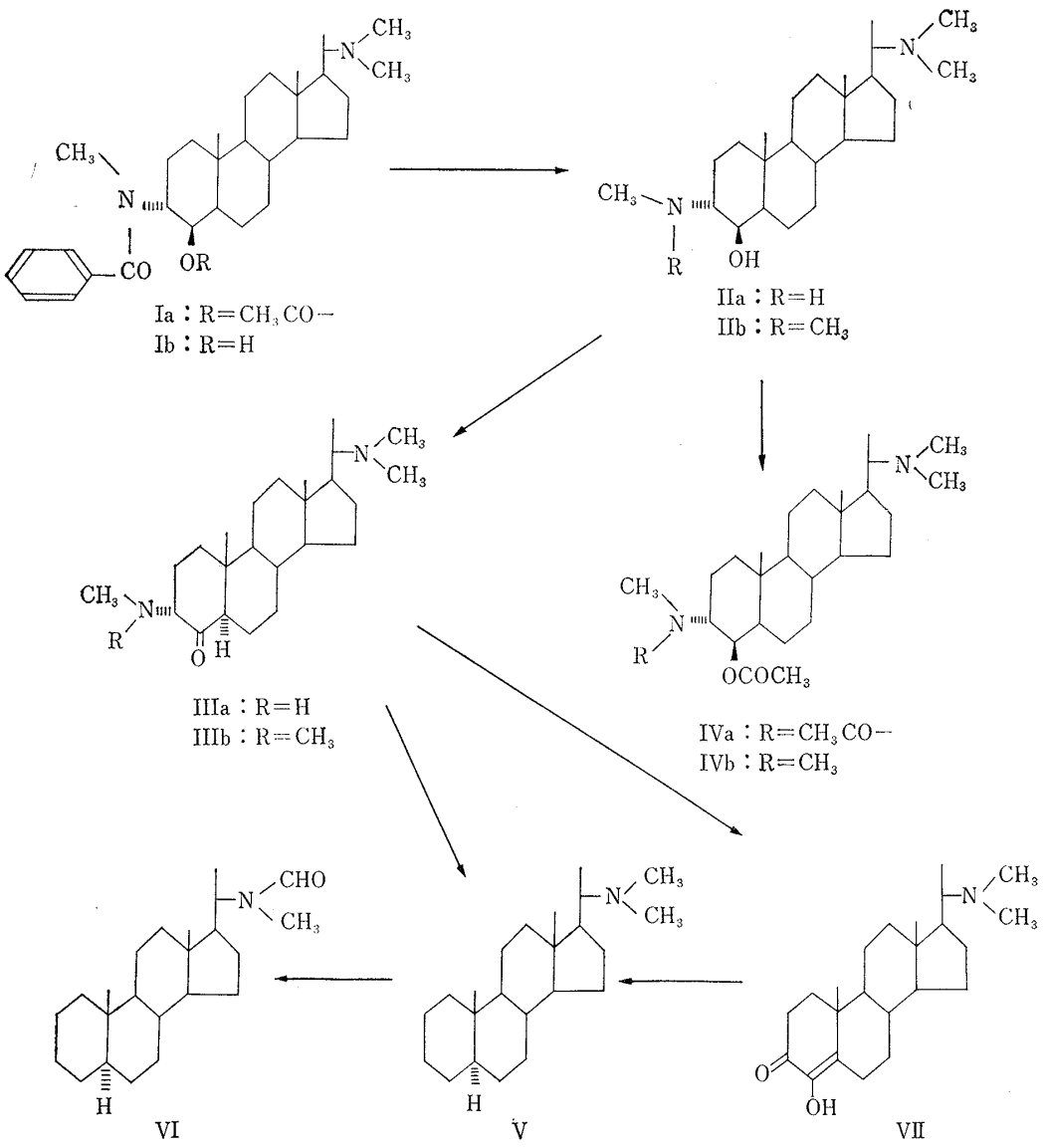


Chart 1.

The above ketone Ⅲa was then subjected to the Huang-Minlon modification of Wolff-Kishner reduction, whereupon, rather surprisingly, was obtained a saturated monoamino compound (V),[*8] $C_{23}H_{41}N$, m.p. 141~142°, $[\alpha]_D$ +25° ($CHCl_3$). This observation is suggestive of that the methylamino group is placed in proximity of the carbonyl group. The same product (V) was also obtained by the modified Wolff-Kishner reduction of the N-methyl ketone (Ⅲb).

The NMR spectrum of this product showed signals arising from an N-dimethyl (7.85τ, 6H), a secondary C-methyl (9.15τ, 3H, doublet, J 6 c.p.s.), and two tertiary C-methyls (9.23 and 9.36τ, 6H) and clearly indicated that the elimination of the methylamino group, originally forming the benzoylamino grouping, was effected during the Wolff-Kishner reduction (Fig. 5). The structure of this substance was believed to be 20-dimethylamino-5α-pregnane on the basis of its NMR spectrum and the

*8 The initial step of this reduction might be either the formation of a Δ_3-ene compound, which could be hydrogenated by hydrazine in the presence of an oxidizing agent (see E. J. Corey, *et al.*: Tetrahedron Letters, 347 (1961); J. Am. Chem. Soc., **83**, 2957 (1961)), or the diosphenol formation by air oxidation in alkaline solution. However, any conclusive indication of the reaction mechanism is not available at present.

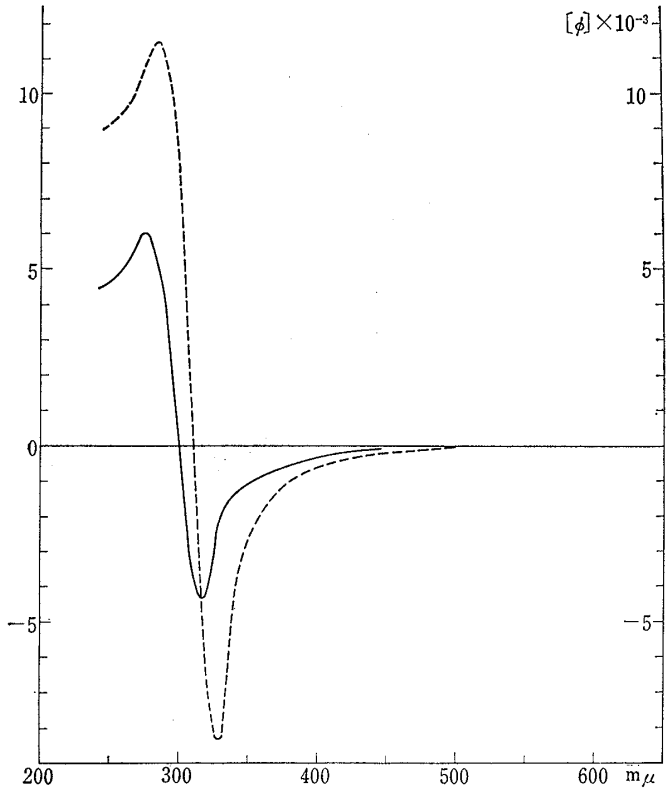


Fig. 2. Optical Rotatory Dispersion Curves (in methanol) of :

- - - - - 4-oxo compound (IIIa) from O,N-desacylpachysandrine-A

——— 4-oxo compound (IIIb) from N-methyl-O,N-desacylpachysandrine-A

molecular formula. Furthermore, biogenetic precedents favored strongly the 20α-configuration for this compound.[3]

On this supposition, a most promising procedure for attack might be the von Braun reaction. It was expected that the resulting NH compound may be transformed to the corresponding ketone by Ruschig degradation[4] which has been widely employed in the field of steroidal alkaloids. However, the reaction of the monoamine (V) with cyanogen bromide in boiling chloroform proved to be unsatisfactory due to incomplete reaction. Alternative attempt was then made without success to hydrolyze the N-formyl compound (VI), m.p. 172~174°, which had been obtained by chromic acid-pyridine oxidation. Since the usual Hofmann degradation also failed to convert this to an N-free substance,*9 we carried out the synthesis of V starting from bisnorallocholanic acid (XIII).[5] Bisnorallocholanic acid which we used for the synthesis was derived from ergosterol as follows:

Oppenauer oxidation and acid isomerization of ergosterol (VIII) gave isoergosterone (IX) which was then partially hydrogenated to ergosta-4,22-dien-3-one (X).[6,7] Ozonolysis of X followed by chromic acid oxidation led to 3-keto-bisnor-4-cholenic acid (XIb)[8] which was subsequently reduced with sodium borohydride into the allylic alcohol (XII). Hydrogenolysis[9] of the latter in the presence of platinum oxide and perchloric acid gave rise to bisnorallocholanic acid (XIII), m.p. 213~214°.[5]

Curtius reaction of XIII was performed in the analogous manner as described by Julian, *et al.*[10] and subsequent N-methylation led to 20α-dimethylamino-5α-pregnane (V), m.p. 141~142°, $[\alpha]_D$ +26° ($CHCl_3$). This compound was shown to be identical with the

*9 At the time of this investigation, no sufficient amount of the material was available to permit other experiments.

3) R. Goutarel : Tetrahedron, **14**, 126 (1961); O. Jeger, V. Prelog : "The Alkaloids" (R. H. F. Manske, Ed.) Vol. VII, 319 (1960), Academic Press, New York.
4) R. Ruschig, W. Fritsch, J. Schmidt-thome, W. Haede : Chem. Ber., **88**, 883 (1955); K. S. Brown, Jr., S. M. Kupchan : J. Am. Chem. Soc., **84**, 4592 (1962).
5) E. Fernholz : Ann., **507**, 128 (1933); "Elsevier's Encyclopaedia of Organic Chemistry," Vol. 14, 167, 2996s (1962).
6) D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, Jr., J. E. Stafford, R. L. Pederson, A. C. Ott : J. Am. Chem. Soc., **77**, 1212 (1955).
7) A. F. Daglish, J. Green, V. D. Poole : J. Chem. Soc., 2627 (1954).
8) M. E. Herr, F. W. Heyl : J. Am. Chem. Soc., **74**, 3627 (1952).
9) C. W. Shoppee, B. D. Agashe, G. H. R. Summers : J. Chem. Soc., 3107 (1957).
10) P. L. Julian, E. W. Mayer, H. C. Printy : J. Am. Chem. Soc., **70**, 887 (1948).

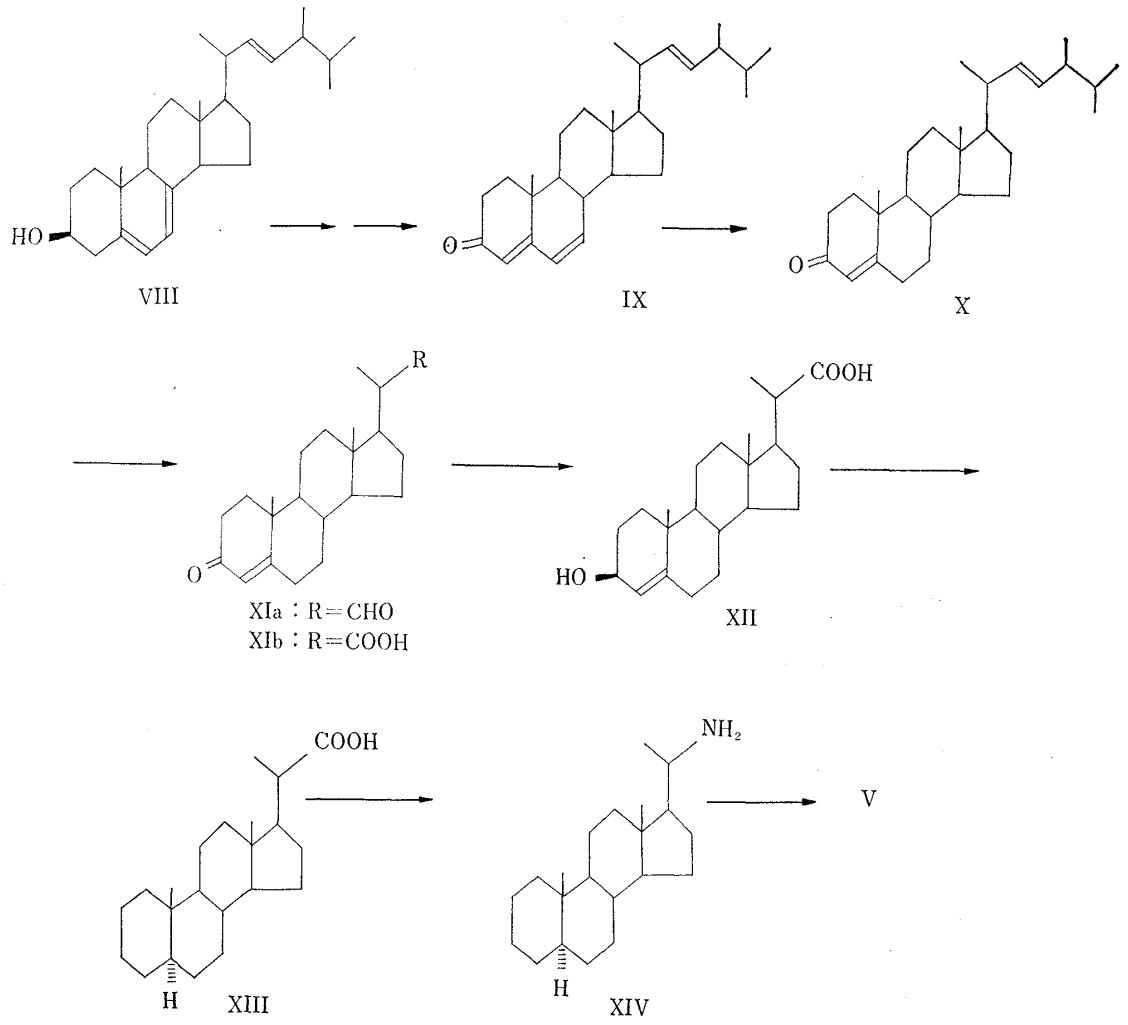


Chart 2.

above described compound (V) originated from pachysandrine-A by mixed melting point determination and infrared comparison (KBr), and thus verified that pachysandrine-A (Ia) is a member of pregnane type alkaloid.

The other amino grouping, which was eliminated during the Wolff-Kishner reactions of the two oxo compounds (IIIa) and (IIIb), must be located at 3-position from the biogenetic point of view. Position of the oxygen function was suggested by optical rotatory dispersion (ORD) curves of the oxo compounds (IIIa, IIIb) to be 4-position, as mentioned previously. In confirmation of this supposition, a degradative pathway was advanced as follows :

When the ketone (IIIa) was treated with 5% potassium hydroxide in ethanol,[11] there was obtained a diosphenol (VII), $C_{23}H_{37}O_2N$, m.p. 192~193°, $[\alpha]_D$ +19° ($CHCl_3$), whose ultraviolet spectrum (λ_{max}^{MeOH} 279 mμ (ε=9100)) and infrared spectrum (3400, 1670, 1640, 1390, and 1170 cm^{-1}) were very similar to those of the known steroidal 3,4-diosphenols.[12] Lack of olefinic proton signal and N-methyl signal in the NMR spectrum also supported the same conclusion. Wolff-Kishner reduction of this diosphenol (VII) afforded readily the already determined 20α-dimethylamino-5α-pregnane (V).

11) K. S. Brown, Jr., S. M. Kupchan : *Ibid.*, **86**, 4417 (1964).
12) L. F. Fieser, R. Stevenson : *Ibid.*, **76**, 1728 (1954).

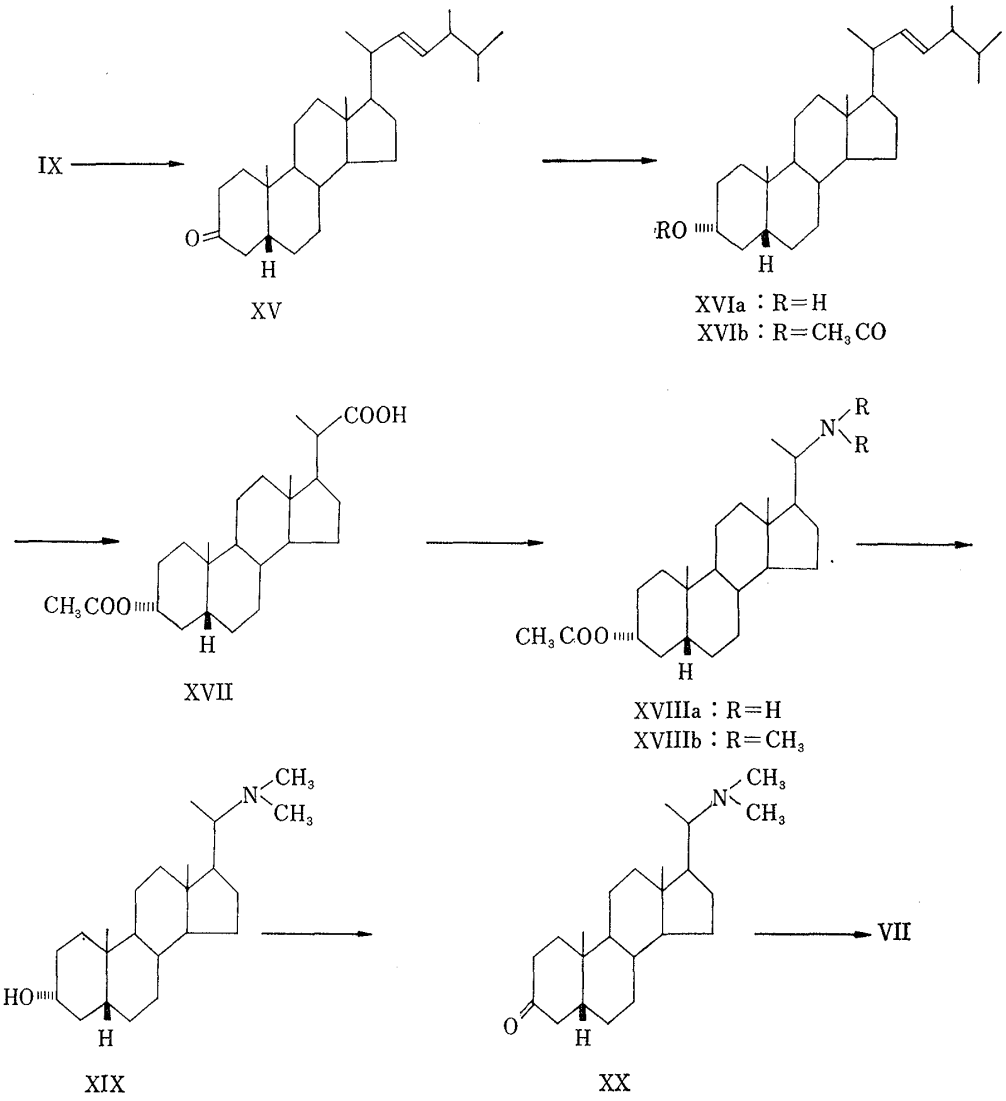


Chart 3.

The structure of the diosphenol was established by direct comparison with the synthetic compound (Ⅶ) derived from isoergosterone according to the scheme as shown in Chart 3.

5β-ergost-22-en-3-one (XV),[7] which was obtained by hydrogenation of isoergosterone (Ⅸ) over palladized charcoal, was reduced with sodium borohydride to 3α-hydroxy compound (XVIa). Acetylation of XVIa followed by ozonolysis[6] and chromic acid oxidation,[8] gave 3α-acetoxy-bisnorcholanic acid (XVII).[13] Curtius reaction[10] of the latter, followed by N-methylation and alkaline hydrolysis, led to 3α-hydroxy-20α-dimethylamino-5β-pregnane (XIX), $C_{23}H_{41}ON$, m.p. 150~151°, which was then oxidized with chromic acid in acetic acid to 3-keto compound (XX), $C_{23}H_{39}ON$, m.p. 103~104°.

Air oxidation of the ketone (XX) in the presence of potassium *t*-butoxide in *t*-butanol[14] afforded the desired diosphenol (Ⅶ), $C_{23}H_{37}O_2N$, m.p. 195~196.5°, $[\alpha]_D$, +24° ($CHCl_3$), which was shown to be identical in every respect with the specimen obtained from pachysandrine-A.

Thus the structure of pachysandrine-A should be 3-methyl, benzoylamino-4-acetoxy-20α-dimethylamino-5α-pregnane.

13) "Elsevier's Encyclopaedia of Organic Chemistry," Vol. 14, 174, 3068s (1962).
14) B. Camerino, B. Patelli, R. Sciaky : Tetrahedron Letters, No. 16, 554 (1961).

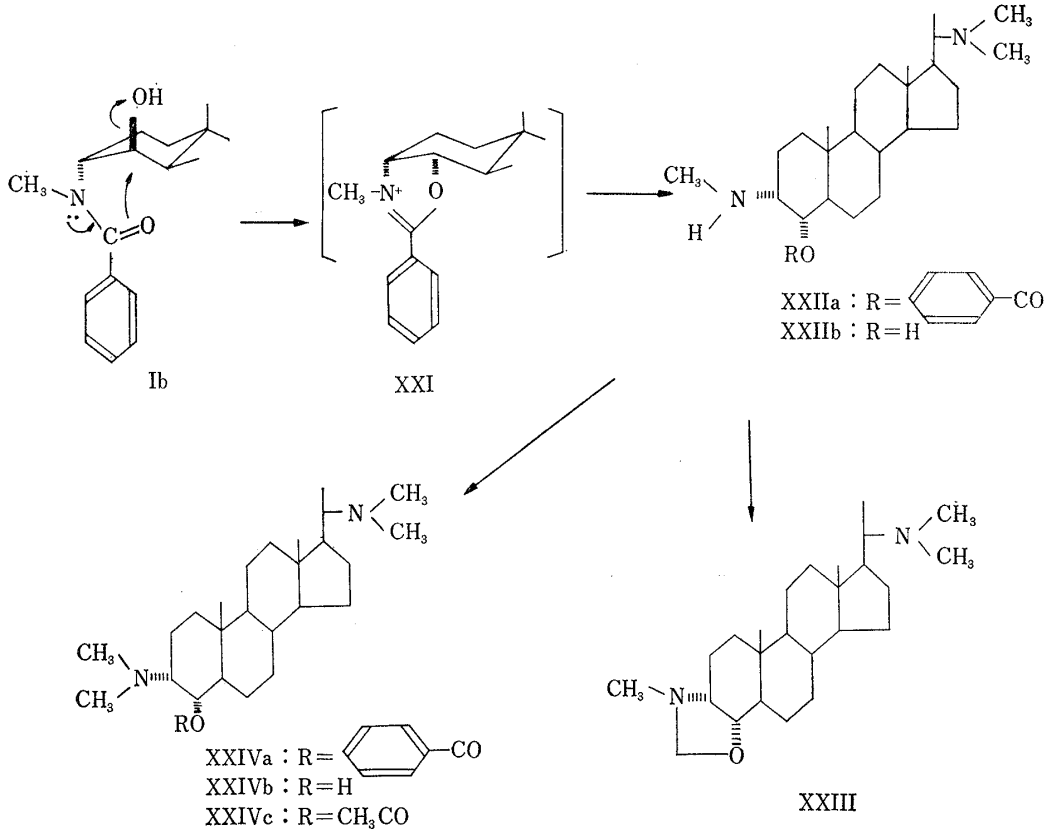


Chart 4.

Turning now to the stereochemistry of pachysandrine-A, evidence for the 3α,4β-configuration was advanced.

Reaction of O-desacylpachysandrine-A (Ib) with phosphorous oxychloride gave in good yield an O-benzoate (XXIIa), m.p. 205~206°, $[\alpha]_D$ −18° ($CHCl_3$), showing distinctly an ester band at 1715 cm^{-1} in the infrared spectrum. Alkaline hydrolysis of this O-benzoate (XXIIa) and of its N-methylated compound (XXIVa), m.p. 156~157°, afforded the 4-epi-alcohols, (XXIIb), $C_{24}H_{44}ON_2$, m.p. 215~216°, $[\alpha]_D$ −38° ($CHCl_3$), and (XXIVb), $C_{25}H_{46}ON_2$, m.p. 172~173°, $[\alpha]_D$ −28° ($CHCl_3$), respectively. Characterization of both compounds was achieved by oxidation with chromic acid to the corresponding ketones, (IIIa) and (IIIb). This observation indicated that, during the reaction between Ia and phosphorous oxychloride, a rearrangement of the acyl group to C_4-position had actually taken place with the inversion of configuration, because alkaline hydrolysis of ester do not usually cause the epimerization of alcohol.

The formation of the 4-epi-O-benzoate (XXIIa) is understandable on the basis of Cornforth's acyl migration mechanism,[15] which involves an oxazoline intermediate (XXI) formed by the nucleophyllic backside attack of the N-acyl group and hence can occur only in 1,2-diaxial-aminocycloalkanol system : *viz.* 3α-acylamino-4β-hydroxy-5α-pregnane orientation.

Further supports for the 3α,4β-assignment are as follows :

1) In NMR studies of these compounds, it was found that the 19-methyl signals of Ib, IIa and IIb (8.95, 8.96, and 8.95τ, respectively) occurred at about 15~18 c.p.s. lower field than those of 4-epimeric alcohols and 4-oxo compounds (XXIIb, XXIVb, IIIa, and IIIb:

15) J. Attenburrow, D. F. Elliott, G. F. Penny : J. Chem. Soc., 310 (1948), footnote 1 ; C. Pfister, C. A. Robinson, A. C. Shabica, M. Tishler : J. Am. Chem. Soc., **71**, 1101 (1949); W. S. Johnson, E. N. Schubert : *Ibid.*, **72**, 2187 (1950).

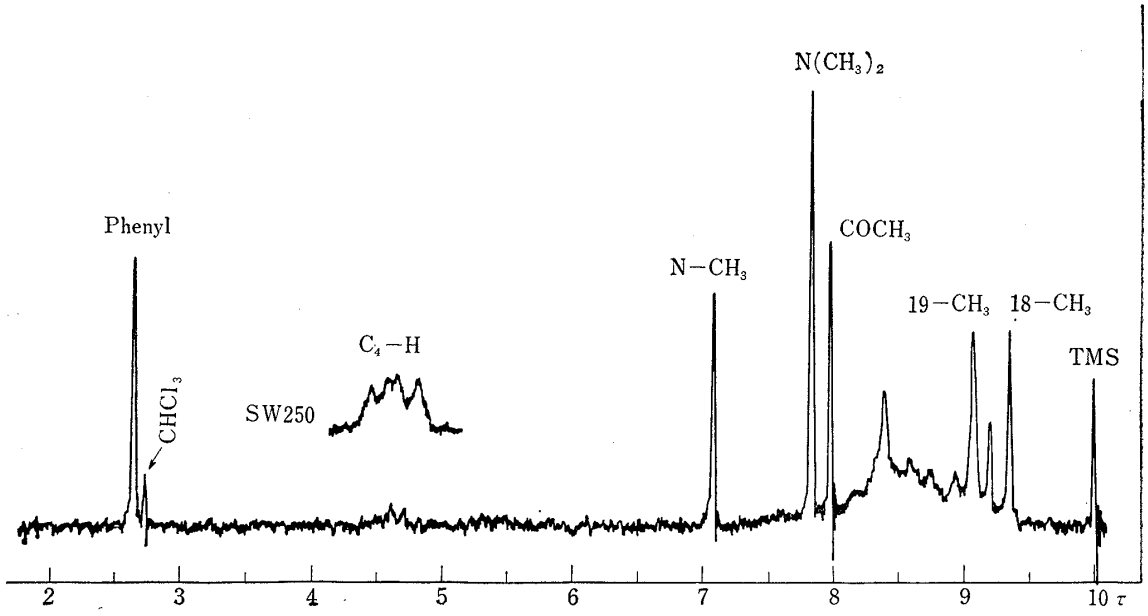


Fig. 3. NMR Spectrum of Pachysandrine-A (Ia)

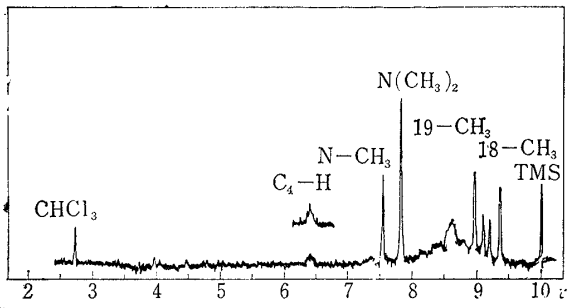


Fig. 4. NMR Spectrum of O,N-Desacyl-pachysandrine-A (IIa)

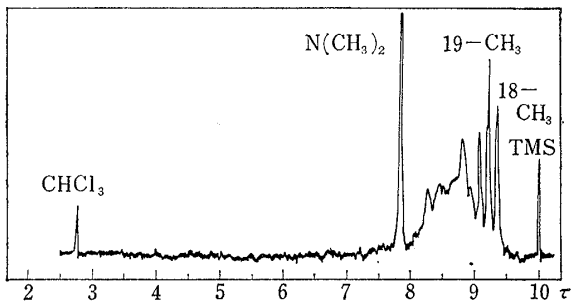


Fig. 5. NMR Spectrum of 20α-Dimethylamino-5α-pregnane (V)

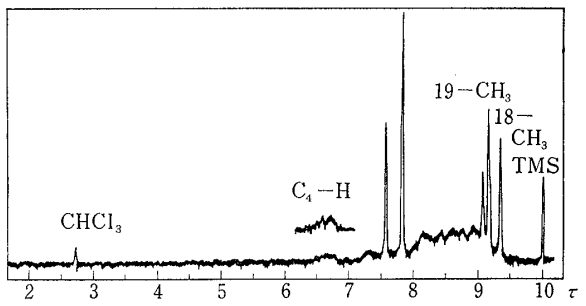


Fig. 6. NMR Spectrum of 3α-Methylamino-20α-dimethylamino-4α-hydroxy-5α-pregnane (XXIIb)

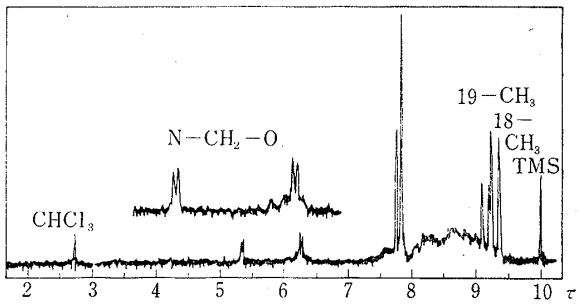


Fig. 7. NMR Spectrum of the Oxazolidine Compound (XXIII)

9.18, 9.20, 9.25, and 9.27τ, respectively). Acetylation of the former group caused slight diamagnetic shifts (*i.e.*, 9.06τ for Ia and 8.99τ for IVb), while O-acetate (XXIVc) of the 4-epimer (XXIVb) showed a slight paramagnetic shift to 9.10τ. This behavior of the 19-methyl signals indicates that the 4-hydroxyl groups of Ib, IIa, and IIb are in 1,3-diaxial relation to the 19-methyl groups[16] : hence, β-configuration (Table I).

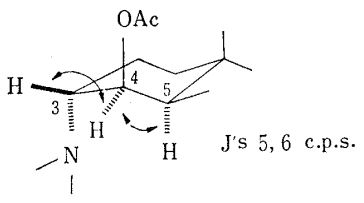
16) Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, K. Tsuda : This Bulletin, **10**, 338 (1962).

Table I.

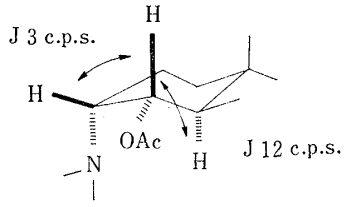
Compound	3	4	19-CH_3 (τ)	C_4-H (τ)	$J_{C_4-C_3}$ (c.p.s.)	$J_{C_4-C_5}$ (c.p.s.)
Ib	α-N$\langle^{CH_3}_{COC_6H_5}$	β-OH	8.95	6.11(t.)	5	5
IIa	α-N$\langle^{CH_3}_{H}$	〃	8.96	6.41(broad)		
IIb	α-N$\langle^{CH_3}_{CH_3}$	〃	8.95	6.20(〃)		
Ia	α-N$\langle^{CH_3}_{COC_6H_5}$	β-$OCOCH_3$	9.06	4.62(q.)	5	6
IVb	α-N$\langle^{CH_3}_{CH_3}$	〃	8.99	4.99(broad)		
IIIa	α-N$\langle^{CH_3}_{H}$	C=O	9.25			
IIIb	α-N$\langle^{CH_3}_{CH_3}$	〃	9.27			
XXIIIb	α-N$\langle^{CH_3}_{H}$	α-OH	9.18	6.63(q.)	5	11
XXIVb	α-N$\langle^{CH_3}_{CH_3}$	〃	9.20	6.65(〃)	6	10
XXIVc	〃	α-$OCOCH_3$	9.10	5.05(〃)	3	12

2) The above conclusion is also consistent with the fact that the C_4-hydrogens of XXIIIb, XXIVb, and XXIVc resonate at the higher region than those of corresponding epimers, IIa, IIb, and IVb, respectively, because the axial hydrogens are well known to resonate at the higher field than the equatorial hydrogens[17] (Table I).

3) The spin-spin coupling constants between C_4-hydrogen and C_3-, C_5-hydrogens of pachysandrine-A (Ia) (J 5 and 6 c.p.s.) and of XXIVc (J 3 and 12 c.p.s.) suggest the 3α,4β-configuration for the former and the 3α,4α-configuration for the latter[18] (Table I). The situations are illustrated below.



Ia



XXIVc

4) O,N-Desacylpachysandrine-A (IIa), upon treatment with formalin-formic acid, gave the N-methyl compound (IIb), whereas, under the same condition, the 4-epimer (XXIIb) afforded the oxazolidine compound (XXIII),*[10] $C_{25}H_{44}ON_2$, m.p. 201~202°, whose NMR spectrum demonstrated two doublets (2H) at 5.33 and 6.28τ with the coupling constant of 2.5 c.p.s. (Fig. 7). This compound could be converted to the corresponding N-dimethyl compound (XXIVb), $C_{25}H_{46}ON_2$, m.p. 173~174°, by the reduction with lithium

*[10] Recently Goutarel, *et al.* reported the same cyclyzation in synthesized pregnane alkaloids. (Bull. soc. chim. France, 2158 (1964); *Ibid.*, 3225 (196.)).

17) *a*) L.M. Jackman : "Application of NMR Spectrometry in Organic Chemistry," 116 (1959), Pergamon Press, London.; *b*) N. S. Bhacca, D. H. Williams : "Application of NMR Spectroscopy in Organic Chemistry," 47 (1964), Holden-Day, Inc., San Francisco.

18) M. Karplus : J. Chem. Phys., **30**, 11 (1959); *Idem* : J. Am. Chem. Soc., **85**, 2870 (1963); M. Karplus, D.H. Anderson : J. Chem. Phys., **30**, 6 (1959).

aluminum hydride. Such a cleavage of the ether linkage and the small NMR coupling constant*11 provided a proof for an N-CH_2-O grouping. The oxazolidine ring-closure is suggestive of the 3,4-*cis* orientation in XXIIIb and therefore 3,4-*trans* orientation for IIa.

5) The infrared spectrum of O,N-desacyl-N-methylpachysandrine-A (IIb) in tetrachloromethane showed a monomeric OH band at 3640 cm^{-1}, while that of the 4-epimer (XXIVb) occurred at 3330 cm^{-1} (internally bonded OH). The lack of intramolecular hydrogen bonding in the former favors the 3α,4β-configuration.

On the basis of foregoing evidences, we herewith propose the complete structure Ia for pachysandrine-A.

Experimental*12

O-Desacylpachysandrine-A (Ib)——A solution of pachysandrine-A (Ia) (105 mg.) in 5% NaOH-MeOH (10 ml.) was refluxed for 2 hr. After evaporation of the solvent under reduced pressure, water was added to the residue and extracted with CH_2Cl_2. The extract was washed successively with 3% HCl and dil. Na_2CO_3, dried over K_2CO_3, and evaporated to leave a colorless oil (100 mg.). Trituration with acetone gave crystals which were recrystallized from acetone to give O-desacylpachysandrine-A (Ib) in colorless needles, m.p. 194~195°, $[\alpha]_D^{10}$ +91°(c=1.8). *Anal.* Calcd. for $C_{31}H_{48}O_2N_2$: C, 77.45; H, 10.07; N, 6.03. Found : C, 77.46; H, 10.24; N, 5.77. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3350 (OH), 1610 (conjugated amide), 1580, and 1500 (phenyl). NMR τ : 2.60 (5H, phenyl), 5.56 (1H, broad, RCON-CH-), 6.11 (1H, triplet, J 5 c.p.s.; CH-OH), 7.05 (3H, N(COR)-CH_3), 7.84 (6H, N-$(CH_3)_2$), 8.95 (3H, tert. CH_3), 9.13 (3H, doublet, J 6 c.p.s.; sec. CH_3), and 9.34 (3H, tert. CH_3).

Acetylation of O-Desacylpachysandrine-A (Ib)——O-Desacylpachysandrine-A (Ib) (25 mg.) was treated with acetic anhydride (2 ml.) in pyridine (2 ml.) at room temperature for 12 hr. Usual working up and recrystallization from acetone gave colorless crystals, m.p. 227~230°, which were identified with pachysandrine-A by mixed m.p. determination and IR (KBr) comparison.

O,N-Desacylpachysandrine-A (IIa)——A solution of pachysandrine-A (Ia) (1.03 g.) in 20% KOH-EtOH (40 ml.) was refluxed for 5 hr. and the solvent was removed by evaporation under reduced pressure. To the residue was added water and extracted with CH_2Cl_2, and the organic phase was again extracted with 3% HCl. The product obtained from the acidic aqueous phase in the usual treatment was recrystallized from acetone-CH_2Cl_2 to give IIa (655 mg.) in colorless needles, m.p. 226~227°, $[\alpha]_D^{10}$ +28°(c=1.1). *Anal.* Calcd. for $C_{24}H_{44}ON_2$: C, 76.54; H, 11.78; N, 7.44. Found : C, 76.67; H, 11.68; N, 7.51. NMR τ : 6.41 (1H, broad, CH-OH), 7.55 (3H, N-CH_3), 7.83 (6H, N-$(CH_3)_2$), 8.96 (3H, tert. CH_3), 9.15 (3H, doublet, J 7 c.p.s.; sec. CH_3), and 9.37 (3H, tert. CH_3).

The alkaline aqueous solution, on the other hand, was acidified by addition of conc. HCl and extracted with CH_2Cl_2. Evaporation and recrystallization from water gave benzoic acid (118 mg.), m.p. 201~202°. Identity was established by direct comparison with an authentic sample (mixed m.p. and IR).

Acetylation of O,N-desacylpachysandrine-A (IIa)——The compound (IIa) (60 mg.) in dry pyridine (4 ml.) was treated with acetic anhydride (2 ml.) and left at room temperature for 24 hr. After dilution with water, the mixture was made alkaline with NH_4OH and extracted with CH_2Cl_2. Washing of the extract with water, drying, and evaporation *in vacuo*, left a crystalline residue (60 mg.), which was recrystallized from acetone to give colorless needles (IVa), m.p. 192~193°. *Anal.* Calcd. for $C_{28}H_{48}O_3N_2 \cdot \frac{1}{2}H_2O$: C, 71.55; H, 10.51. Found : C, 71.59, 71.87; H, 10.76, 10.72. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1725, 1240 (OCOCH$_3$), 1630 (N-COCH$_3$).

N-Methyl-O,N-desacylpachysandrine-A (IIb)——A solution of O,N-desacyl compound (IIa) (120 mg.) in formic acid (1 ml.) and 37% formalin (1 ml.) was heated on a water bath for 3 hr. After dilution with 3% HCl, the mixture was washed with CH_2Cl_2 and then basified with NH_4OH, extracted with ether. The etherial extract was dried over K_2CO_3 and evaporated to give a crystalline residue (125 mg.). Recrystallizations from acetone-CH_2Cl_2 afforded big prisms (IIb) melting at about 126~150°. (This substance did not give sharp melting point.) $[\alpha]_D^{10}$ +21°(c=1.34). *Anal.* Calcd. for $C_{25}H_{46}ON_2 \cdot \frac{1}{2}H_2O$: C, 75.25; H, 11.89; N, 7.02. Found : C, 75.31, 75.59; H, 11.80, 11.88; N, 7.20, 7.24. NMR τ : 6.20 (1H, broad, CH-OH), 7.75, 7.86 (12H, two N-$(CH_3)_2$), 8.95, 9.35 (6H, two tert. CH_3), and 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3).

O-Acetate (IVb)——A mixture of the N-methyl compound (IIb) (138 mg.), acetic anhydride (2 ml.) and dry pyridine (2 ml.) was kept at room temperature overnight and then worked up in the usual manner. Recrystallization of the product from acetone gave the O-acetate (IVb) (113 mg.) in colorless needles, m.p.

*11 The coupling constants of geminal hydrogens are known to be negative and the strong contribution of two electronegative atoms (oxygen and nitrogen) towards increasing the coupling constant gave an apparent small J-value (see ref. 17), b), p. 54, 104).

*12 All the melting points were taken on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. All the optical rotations were measured in chloroform solutions.

166～168°. Further recrystallizations furnished an analytical sample, m.p. 169～170°. $[\alpha]_D^{15}$ +16°(c=1.26). *Anal.* Calcd. for $C_{27}H_{48}O_2N_2$: C, 74.98; H, 11.18. Found : C, 74.82; H, 11.06. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1730, 1250 ($OCOCH_3$). NMR τ : 4.99 (1H, broad, CH-OAc), 7.72, 7.84 (12H, two tert. CH_3), 7.97 (3H, OAc), 8.99, 9.37 (6H, two tert. CH_3), and 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3).

Chromium Trioxide Oxidation of O,N-Desacylpachysandrine-A (IIa)——A solution of chromium trioxide (200 mg.) in acetic acid (2 ml.) containing a few drops of water was added dropwise under vigorous stirring to a solution of the compound (IIa) (153 mg.) in acetic acid (2 ml.) and water (2 drops) cooled in an ice-water bath. After the stirring was continued for additional 2 hr. under cooling, the reaction mixture was diluted with water, made alkaline with saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2. The extract was washed with water, dried over anhydrous $MgSO_4$, and evaporated to yield a crystalline residue (130 mg.). Recrystallizations from acetone gave the pure amino ketone (IIIa) as colorless needles, m.p. 169～170°. $[\alpha]_D^{10}$ −28° (c=1.13). ORD (in MeOH, c=0.1305%) : trough, $[\phi]_{330}$ −8310°; peak, $[\phi]_{285}$ +11500°. ORD (in MeOH containing one drop of conc. HCl, c=0.1305%) : trough, $[\phi]_{320}$ −4080°; peak, $[\phi]_{275}$ +6400°. *Anal.* Calcd. for $C_{24}H_{42}ON_2$: C, 76.95; H, 11.30; N, 7.48. Found : C, 77.22; H, 11.58; N, 7.60. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3330 (NH), 1710 (ketone). UV λ_{max}^{MeOH} : 312 mμ (ε 105). NMR τ : 7.03 (1H, broad, N-CH-CO), 7.70 (3H, $N-CH_3$), 7.83 (6H, $N-(CH_3)_2$), 9.13 (3H, doublet, J 7 c.p.s.; sec. CH_3), 9.25, and 9.35 (6H, two tert. CH_3). **N-Acetate** : The compound (IIIa) (50 mg.) was treated with acetic anhydride (1 ml.) in pyridine (1 ml.) as usual. Recrystallizations of the crude N-acetate (57 mg.) from *n*-hexane gave an analytical sample, m.p. 151～153°. $[\alpha]_D^{15}$ −5°(c=1.0). ORD (in MeOH, c=0.204%) : negative Cotton effect; trough, $[\phi]_{313.5}$ −3710°; peak, $[\phi]_{263}$ +6400°. *Anal.* Calcd. for $C_{26}H_{44}O_2N_2$: C, 74.95; H, 10.65. Found : C, 75.07; H, 10.63. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1710 (ketone), 1635 ($N-COCH_3$).

Chromium Trioxide Oxidation of N-Methyl-O,N-desacylpachysandrine-A (IIb)——The compound (IIb) (440 mg.) was oxidized with chromium trioxide (600 mg.) in the same manner as described above. The crude product (410 mg.) obtained was recrystallized from acetone to give the amino-ketone (IIIb) as colorless needles (330 mg.), m.p. 187～188°. $[\alpha]_D^{23}$ +24°(c=1.06). ORD (in MeOH, c=1.203%) : trough, $[\phi]_{319}$ −4320°; peak, $[\phi]_{275}$ +6040°. ORD (in MeOH containing one drop of conc. HCl, c=1.203%) : trough, $[\phi]_{320}$ −5000°; peak, $[\phi]_{276}$ +6590°. *Anal.* Calcd. for $C_{25}H_{44}ON_2$: C, 77.26; H, 11.41; N, 7.21. Found : C, 77.17; H, 11.71; N, 7.18. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1710 (ketone). UV λ_{max}^{MeOH} : 294 mμ (ε 47). NMR τ : 7.85 (12H, two $N-(CH_3)_2$), 9.15 (3H, doublet, J 7 c.p.s.; sec. CH_3), 9.27, and 9.37 (6H, two tert. CH_3).

Lithium Aluminum Hydride Reduction of the Methylamino-ketone (IIIa)——The compound (IIIa) (50 mg.) was refluxed with $LiAlH_4$ (120 mg.) in tetrahydrofuran (15 ml.) for 2 hr. After the excess reagent was decomposed by careful addition of aqueous tetrahydrofuran, the inorganic precipitate was removed by filtration and washed thoroughly with CH_2Cl_2. The combined filtrates were evaporated *in vacuo* and the crystalline residue was recrystallized from acetone-CH_2Cl_2 to give needles (34 mg.), m.p. 225～226°. This compound was identified with O,N-desacylpachysandrine-A (IIa) by mixed m.p. determination and infrared comparison (in $CHCl_3$).

Lithium Aluminum Hydride Reduction of the Dimethylamino-ketone (IIIb)——The compound (IIIb) (105 mg.) was treated with $LiAlH_4$ as usual. The product obtained appeared to be a mixture of two epimeric amino-alcohols (IIb and XXIVb). Repeated recrystallization gave a small amount of crystalline substance, m.p. 168～169°, whose IR spectrum ($CHCl_3$) was identical with that of XXIVb. Further purification, however, could not be achieved.

Wolff-Kishner Reduction (Huang-Minlon Modification) of the Aminoketones, IIIa and IIIb——Samples (420 mg.) of each of the compounds, (IIIa) and (IIIb), were mixed with 80% hydrazine hydrate (2 ml.), KOH pellets (1.5 g.), abs. EtOH (2 ml.), and diethylene glycol (10 ml.) and heated in an oil bath, the temperature of which raised gradually to 150～160°. At this temperature the reaction started with vigorous foaming. After the foaming subsided, the bath temperature was slowly raised to 210° and the mixture was heated for 1.5 hr. to complete the reaction. After cooling, water was added to the reaction mixture and the product was extracted with CH_2Cl_2, washed with water, dried, and evaporated to leave a viscous oil (320 mg.), which was chromatographed on alumina (20 g.). Elution with benzene and recrystallizations from acetone-CH_2Cl_2 gave the deoxo-deamino compound (V) (79 mg.) as colorless plates, m.p. 141～142°. $[\alpha]_D^{17}$ +25°(c=1.97). *Anal.* Calcd. for $C_{23}H_{41}N$: C, 83.31; H, 12.46; N, 4.22. Found : C, 83.42; H, 12.27; N, 3.92. NMR τ : 7.85 (6H, $N-(CH_3)_2$), 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3), 9.23, and 9.36 (6H, two tert. CH_3).

Subsequent elution of the alumina column with ether gave a crystalline substance (163 mg.), which showed m.p. 178～179° on recrystallization from acetone. However, characterization of this substance has not been achieved yet.

Oxidation of the Deoxo-deamino Compound (V) with Chromium Trioxide-pyridine Complex——A mixture of the compound (V) (118 mg.), chromium trioxide (220 mg.), and pyridine (5 ml.) was allowed to stand overnight at room temperature. To this mixture was added dil. NH_4OH and extracted with CH_2Cl_2 to give a viscous oil which was dissolved in ether, washed successively with 3% HCl and water, dried over K_2CO_3, and evaporated. Recrystallizations of the residue (108 mg.) from ether gave the N-formyl compound (VI), m.p. 172～174°. $[\alpha]_D^{15}$ −4°(c=1.12). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1660 (N-CHO). NMR τ : 1.95～2.14 (1H, N-CHO), 7.21～7.26 (3H, $N-CH_3$), 8.8～9.3 (9H, three $C-CH_3$).

Attempted hydrolyses of this compound under alkaline and acidic conditions resulted in recovery of the starting material.

Synthesis of 20α-Dimethylamino-5α-pregnane (V). i) 4,22-Ergostadien-3-one (X)——Isoergosterone (IX), prepared from ergosterol, was subjected to Birch reduction[7] or catalytic hydrogenation over palladized charcoal in 4% KOH-MeOH.[6] The product (X) showed m.p. 124~127°. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1665, 1615 (C=C-C=O). (lit.[6,7] : m.p. 129~130°).

ii) 3-Keto-bisnor-4-cholenic Acid (XIb)——A solution of 4,22-ergostadien-3-one (X) (3.25 g.) in CH_2Cl_2 (150 ml.) and pyridine (0.9 ml.) was treated with the excess of ozone at −70°(acetone-dry ice bath). Acetic acid (20 ml.) and zinc dust (6 g.) were added to the reaction mixture and stirred for 1.5 hr. under ice cooling. Zinc dust was then removed and CH_2Cl_2 was distilled off *in vacuo* to leave an acetic acid solution of the aldehyde (XIa).[6] To this solution were added acetic acid (60 ml.), benzene (60 ml.), and a solution of chromium trioxide (2.0 g.) in acetic acid-water (9:1; 50 ml.) and the mixture was stirred for 1 hr. at ice-bath temperature.[8] After the excess reagent was decomposed with MeOH, the reaction mixture was diluted with water, extracted with ether and the etherial extract was shaken with 10% NaOH. The aqueous phase deposited a crystalline solid (sodium salt of the keto-acid (XIb)), from which the free acid (1.0 g.), m.p. 260~264°, was recovered. After recrystallization from MeOH-CH_2Cl_2, the compound (XIb) showed m.p. 269~273°. $[\alpha]_D^{15}$ +55°(c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3400~2400, 1710 (-COOH), 1665, and 1615 (C=C-C=O). (lit.[8] : m.p. 268~270°, $[\alpha]_D^{15}$ +60°).

iii) 3β-Hydroxy-bisnor-4-cholenic Acid (XII)——To a stirred solution of the keto-acid (XIb) (185 mg.) in MeOH (10 ml.) and CH_2Cl_2 (5 ml.) was added sodium borohydride (183 mg.) in small portions and the mixture was refluxed for 2 hr. The excess reagent was decomposed by addition of a small quantity of acetone and the solution was evaporated to dryness under reduced pressure. To the residue was added 3% HCl and the product was extracted once with ethyl acetate and twice with CH_2Cl_2. The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to leave a crystalline residue (166 mg.), melting at 235~237°. Recrystallization from ethyl acetate-CH_2Cl_2 gave colorless needles (XII), m.p. 237°.

iv) Bisnorallocholanic Acid (XIII)——The hydroxy-acid (XII) (78 mg.) was hydrogenated in ethyl acetate (30 ml.) over $PtO_2 \cdot 2H_2O$ (30 mg.) in the presence of $HClO_4$ (60%) (3 drops) at room temperature.[9] After 1 hr., hydrogen uptake stopped. Filtering the catalyst off and evaporating the solvent under reduced pressure left a crystalline mass (65 mg.) which was recrystallized from MeOH-CH_2Cl_2 to afford bisnorallocholanic acid (XIII) (40 mg.) melting at 210~213°. Further recrystallizations from the same solvent mixture raised the m.p. to 213~214°. $[\alpha]_D^{19}$ +7°(c=1.4). (lit.[5] : m.p. 213~214°, $[\alpha]_D^{17}$ ±0.00°). *Anal.* Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.91. Found : C, 79.76; H, 10.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3300~2400 and 1707 (-COOH). **Methyl ester** was prepared by treating the acid (XIII) with etherial diazomethane as usual and recrystallized from MeOH. Needles, m.p. 95° and 110~112° (double melting point), $[\alpha]_D^{19}$ 0°(c=1.9). (lit.[5] : m.p. 93~94° and 100°, $[\alpha]_D^{16}$ +8°). *Anal.* Calcd. for $C_{23}H_{38}O_2$: C, 79.68; H, 11.03. Found : C, 79.42; H, 10.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1730 and 1170 (-$COOCH_3$).

v) 20α-dimethylamino-5α-pregnane (V)——The acid (XIII) (90 mg.) was allowed to react with thionyl chloride (0.5 ml.) under ice cooling. After the mixture became a homogeneous solution, excess thionyl chloride was removed by distillation *in vacuo*. The residue was dissolved successively in several portions of dry benzene and this solvent was evaporated *in vacuo* to remove the last trace of thionyl chloride. The resulting acid chloride was dissolved in acetone (3 ml.) and chilled in an ice-water bath. To this solution was added dropwise, with mechanical agitation, a solution of sodium azide (90 mg.) in water (1 ml.) and the agitation continued for additional 20 minutes at ice-bath temperature. Then the reaction mixture was diluted with water (10 ml.) and the deposited crystalline azide was collected by suction, which was again dissolved in acetic acid-water (9:1, 5 ml.). After this solution was stirred at 50° for 30 minutes and at 100° for additional 10 minutes, 20% HCl (2 ml.) was added and the mixture was heated in a boiling water bath for 3 hr. Thereafter, the reaction mixture was made alkaline with NH_4OH and extracted with ether. The etherial extract was shaken with 3% HCl solution, whereupon precipitated the crystalline hydrochloride (68 mg.) of amine (XIV), which was collected by suction. The hydrochloride was then methylated by heating with formic acid (3 ml.) and 37% formalin (4 ml.) and worked up in the usual manner. Chromatography of the crude product over alumina (2 g.) from benzene and recrystallization from acetone gave 20α-dimethylamino-5α-pregnane (V) (42 mg.), colorless prisms, m.p. 141~142°. $[\alpha]_D^{21}$ +26°(c=1.67). This was identified with the natural degradation product (V) by mixed m.p. and IR (KBr) comparison. *Anal.* Calcd. for $C_{23}H_{41}N$: C, 83.31; H, 12.46; N, 4.22. Found : C, 83.35; H, 12.58; N, 4.46.

Alkali Treatment of the Amino-ketone (IIIa). Formation of the Diosphenol (VII)——A suspension of the amino-ketone (IIIa) (235 mg.) in 5% KOH-EtOH (40 ml.) was stirred at room temperature for 2 hr., during which all the compounds dissolved. After standing the solution overnight at room temperature, it was made acidic with acetic acid and then basic with NH_4OH. The product was taken up in CH_2Cl_2, washed successively with 3% HCl and dil. Na_2CO_3, and then dried over K_2CO_3. Evaporation of the solution left colorless crystals (163 mg.), which were recrystallized from MeOH-CH_2Cl_2 to afford the diosphenol (VII) (82 mg.), needles, m.p. 188~190°. A pure sample showed m.p. 192~193°. $[\alpha]_D^{17}$ +19°(c=1.44). *Anal.* Calcd. for $C_{23}H_{37}O_2N$: C, 76.73; H, 10.37; N, 3.98. Found : C, 76.87; H, 10.20; N, 4.08. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1670, 1640, 1390, 1170 (-CO-C(OH)=C-). UV λ_{max}^{MeOH} : 279 mμ (ε 9100). NMR τ : 7.84 (6H, N-(CH_3)$_2$),

8.83, 9.30 (6H, two tert. CH_3), and 9.13 (3H, doublet, J 6 c.p.s.; sec. CH_3).

Wolff-Kishner Reduction (Huang-Minlon Modification) of the Diosphenol (VII)——The diosphenol (VII) (62 mg.) was treated with 80% hydrazine hydrate (2 ml.) and KOH (0.5 g.) in EtOH-diethylene glycol (1:1, 4 ml.) in the analogous way as for the reduction of amino-ketones (IIIa and IIIb). The product obtained was chromatographed over alumina (1×10 cm.) and the benzene eluate (31 mg.) was recrystallized from acetone to yield colorless plates, m.p. 141~142°. This compound showed no melting point depression on admixture with the synthesized sample of 20α-dimethylamino-5α-pregnane (V) and also their IR spectra (KBr) are superimposable.

Synthesis of the Diosphenol (3-Keto-4-hydroxy-20α-dimethylamino-Δ_4-pregnene) (VII). i) 5β-Ergost-22-en-3-one (XV)——According to the literature,[7] isoergosterol (2.0 g.) was hydrogenated over palladium-carbon in 2% KOH-EtOH (80 ml.) to afford XV (1.1 g.), m.p. 107~110° (lit.: m.p. 114~115°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (ketone).

ii) 3α-Acetoxy-5β-ergost-22-ene (XVIb)——The compound (XV) (3.3 g.) was reduced with sodium borohydride (2.0 g.) in MeOH (150 ml.) and CH_2Cl_2 (30 ml.) and worked up as usual. Subsequent acetylation of the resulting 3α-hydroxy compound (XVIa) with acetic anhydride (20 ml.) and pyridine (4 ml.) was performed in the usual manner to yield the crystalline O-acetate (XVIb) (3.0 g.), which was used without further purification for the following reaction. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725, 1250 (O-Ac).

iii) 3α-Acetoxybisnorcholanic Acid (XVII)——Ozonized oxygen was passed through a solution of XVIb (3.0 g.) in CH_2Cl_2 (200 ml.) at −70° (dry ice-acetone bath) until blue color appeared (30 minutes). To this solution were added acetic acid (50 ml.) and zinc dust (10 g.) and stirred for 5 hr. at room temperature. Filtration and subsequent evaporation of low-boiling CH_2Cl_2 *in vacuo* (room temperature) left an acetic acid solution of the aldehyde, to which was added a solution of chromium trioxide (2.0 g.) in water (3 ml.) and stirred overnight at room temperature. A small portion of MeOH was added in order to decompose the excess reagent and the reaction mixture was then diluted with water, extracted with ether. The etherial extract was washed with water and shaken with 10% NaOH solution in which the insoluble sodium salt of the acid (XVII) deposited. This salt was collected by filtrarion and suspended in 3% HCl, and the acid regenerated was taken up in CH_2Cl_2, dried over anhydrous $MgSO_4$, and evaporated. There remained the crystalline free acid (XVII) (1.9 g.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (shoulder), 1250 (O-Ac), 3300~2400, and 1700 (-COOH). This acid was used for next Curtius reaction without further purification.

iv) 3α-Hydroxy-20α-dimethylamino-5β-pregnane (XIX)——The above crude acid (XVII) (1.9 g.) was treated with thionyl chloride (5 ml.) at room temperature until all the acid dissolved. After the removal of excess thionyl chloride under reduced pressure, the residue was dissolved in dry benzene and again evaporated to remove the trace of thionyl chloride. The residue was dissolved in acetone (3 ml.) and to this solution was added dropwise a solution of sodium azide (1.0 g.) in water (3 ml.) under ice cooling. After stirring for 30 minutes at ice-water bath temperature, the reaction mixture was diluted with water (80 ml.) and the precipitated solid (azide) was collected by filtration. The azide thus obtained was suspended in 90% acetic acid (33 ml.) and warmed to 50°, at this temperature the azide dissolved with bubbling into a homogeneous solution. After the solution was kept there for 30 minutes and then at 100° for 10 minutes, 20% HCl (12 ml.) was added to the solution and heated on a steam bath for 1 hr. After cooling, the solution was washed with ether and then made alkaline with NH_4OH. Extraction with CH_2Cl_2, drying over K_2CO_3, and evaporation gave the crystalline acetoxy-amine (XVIIIa). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3330, 3150, 1580 (NH_2), 1720, and 1250 ($OCOCH_3$). This was then heated with formic acid (10 ml.) and formalin (37%) (15 ml.) on a boiling water bath for 5 hr. The reaction mixture was made basic by addition of dil. NH_4OH and the product was taken up in CH_2Cl_2. Evaporation of the solvent afforded the 3α-acetoxy-20α-dimethylamino compound (XVIIIb), which was hydrolyzed without further purification.

Refluxing of the compound (XVIIIb) with 5% KOH-EtOH (50 ml.) for 3 hr. and the usual working up yielded 20α-dimethylamino-3α-hydroxy-5β-pregnane (XIX) (1.2 g.). Recrystallization from acetone gave colorless crystals (620 mg.), m.p. 150~151°, $[\alpha]_D^{15}$ +24° (c=1.0). *Anal.* Calcd. for $C_{23}H_{41}ON$: C, 79.47; H, 11.89; N, 4.03. Found: C, 79.47; H, 11.98; N, 3.97.

v) 3-Keto-20α-dimethylamino-5β-pregnane (XX)——To a stirred solution of the amino-alcohol (XIX) (700 mg.) in acetic acid (20 ml.) was added dropwise a solution of chromium trioxide (1.0 g.) in acetic acid (4 ml.) and water (1 ml.) at room temperature and the reaction mixture was left overnight. The mixture was poured into ice-cooled dil. NH_4OH and extracted with CH_2Cl_2. The extract was dried over K_2CO_3 and evaporated to give the crude amino-ketone (XX) (615 mg.). Recrystallization from aqueous acetone gave colorless crystals, m.p. 103~104°, $[\alpha]_D^{10}$ +34° (c=1.43). *Anal.* Calcd. for $C_{23}H_{39}ON$: C, 79.94; H, 11.38; N, 4.05. Found: C, 79.81; H, 11.35; N, 3.75. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (ketone). NMR τ: 7.83 (6H, N-$(CH_3)_2$), 9.12 (3H, doublet, J 6 c.p.s.; sec. CH_3), 8.97, and 9.32 (6H, two tert. CH_3).

vi) 20α-Dimethylamino-3-keto-4-hydroxy-Δ_4-pregnene (Diosphenol) (VII)——A solution of the above amino-ketone (XX) (400 mg.) and potassium *t*-butoxide (900 mg.) in *t*-butanol (10 ml.) was stirred in an open flask at 30° for 24 hr. The reaction mixture was diluted with water, extracted with CH_2Cl_2, dried over K_2CO_3, and the solvent was removed under reduced pressure. Recrystallization of the residual solid from acetone gave the diosphenol (VII) (100 mg.), colorless needles, m.p. 195.5~196.5°, $[\alpha]_D^{17}$ +24° (c=1.58). This compound was found to be identical with VII derived from pachysandrine-A by mixed m.p. and IR (KBr) comparison.

Anal. Calcd. for $C_{23}H_{37}O_2N$: C, 76.83; H, 10.37; N, 3.90. Found : C, 76.59; H, 10.20; N, 3.68.

Treatment of O-Desacylpachysandrine-A (Ib) with Phosphorous Oxychloride——O-Desacylpachysandrine-A (Ib) (130 mg.) in pyridine (3 ml.) was allowed to react with $POCl_3$ (1 ml.) at room temperature for 20 hr. The reaction mixture was poured into ice water and made basic with dil. NH_4OH, extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated. Recrystallization of the crystalline residue (138 mg.) from MeOH gave the O-benzoate (XXIIa), colorless plates, m.p. 205~206°, $[\alpha]_D^{10}$ −18°(c=1.3). *Anal.* Calcd. for $C_{31}H_{48}O_2N_2$: C, 77.45; H, 10.07. Found : C, 77.74; H, 10.12. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1715, 1280, 1118 ($OCOC_6H_5$), 1605, and 1585 (phenyl).

N-Methylation of the O-Benzoate (XXIIa)——A solution of the O-benzoate (XXIIa) (100 mg.) in formic acid (2 ml.) and 37% formalin (2 ml.) was heated on a steam bath for 6 hr. The product, obtained by the usual working up, was then chromatographed over alumina (1×5 cm.) from benzene. Recrystallization of the benzene eluate (100 mg.) from acetone yielded N-methyl-O-benzoate (XXIVa) as needles melting at 156~157°. $[\alpha]_D^{15}$ +14°(c=1.16). *Anal.* Calcd. for $C_{32}H_{50}O_2N_2$: C, 77.64; H, 10.19. Found : C, 77.37; H, 10.13. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1705, 1285, 1115 (-OCOR), 1600, and 1585 (phenyl).

Hydrolysis of the O-Benzoate (XXIIa)——A solution of the O-benzoate (XXIIa) (138 mg.) in 5% NaOH-MeOH (15 ml.) was refluxed for 1 hr. After evaporation of MeOH *in vacuo* and dilution with water, the product was extracted with CH_2Cl_2 and then re-extracted with 3% HCl. The acidic phase was made basic with NH_4OH and again extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated to give the amino-alcohol (XXIIb) (108 mg.). Recrystallization from acetone-CH_2Cl_2 gave colorless needles, m.p. 215~216°, $[\alpha]_D^{10}$ −38° (c=1.06). *Anal.* Calcd. for $C_{24}H_{44}ON_2$: C, 76.54; H, 11.78; N, 7.44. Found : C, 76.45; H, 11.58; N, 7.61. NMR τ : 6.63 (1H, quartet, J 5 and 6 c.p.s.; C<u>H</u>-OH), 7.58 (3H, N-CH_3), 7.84 (6H, N-$(CH_3)_2$), 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3), 9.18, and 9.36 (6H, two tert. CH_3).

Hydrolysis of the N-Methylated O-Benzoate (XXIVa)——Alkaline hydrolysis of the compound (XXIVa) (80 mg.) was performed in the same manner as described above. The product (XXIVb) (50 mg.) crystallized in needles from MeOH and showed m.p. 172~173°. $[\alpha]_D^{15}$ −28°(c=1.14). *Anal.* Calcd. for $C_{25}H_{46}ON_2$: C, 76.86; H, 11.86. Found : C, 76.93; H, 11.75. IR $\nu_{max}^{CCl_4}$ cm^{-1} : 3330 (in 10 mm. cell, bonded OH). NMR τ : 6.65 (1H, quartet, J 6 and 10 c.p.s.; C<u>H</u>-OH), 7.63, 7.85 (12H, two N-$(CH_3)_2$), 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3), 9.20, and 9.38 (6H, two tert. CH_3).

O-Acetate (XXIVc)——Of this compound was prepared by treatment with acetic anhydride and pyridine for 12 hr. at room temperature. After recrystallizations from acetone, the acetate (XXIVc) showed m.p. 165~167°, $[\alpha]_D^{10}$ +32°(c=1.2). *Anal.* Calcd. for $C_{27}H_{48}O_2N_2$: C, 74.98; H, 11.18. Found : C, 75.28; H, 11.08. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 1725, 1250 (O-Ac). NMR τ : 5.05 (1H, quartet, J 3 and 12 c.p.s.; C<u>H</u>-OAc), 7.70, 7.83 (12H, two N-$(CH_3)_2$), 7.95 (3H, O-Ac), 9.10, 9.37 (6H, two tert. CH_3), and 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3).

Chromium Trioxide Oxidation of the Amino-alcohol (XXIIb)——Chromium trioxide (200 mg.) in acetic acid (1 ml.) containing 4 drops of water was added dropwise to a stirred solution of the amino-alcohol (XXIIb) (45 mg.) in acetic acid (1 ml.) and water (2 drops) at room temperature. After continued the stirring for 1 hr., a few drops of MeOH was added to the reaction mixture, and it was poured into ice-cooled aqueous $NaHCO_3$ solution, extracted with CH_2Cl_2. Evaporation of the dried extract and recrystallization of the residue (35 mg.) from acetone-CH_2Cl_2 gave the amino-ketone (IIIa) as needles, m.p. 172~173°, which was identified with the amino-ketone (IIIa) obtained from O,N-desacylpachysandrine-A (IIa) by mixed m.p. determination and IR ($CHCl_3$) comparison.

Chromium Trioxide Oxidation of the N-Methylated Amino-alcohol (XXIVb)——The compound (XXIVb) (190 mg.) was oxidized with chromium trioxide (160 mg.) in the analogous manner as described above. On crystallization from acetone-CH_2Cl_2, the product (110 mg.) showed m.p. 186~187°. This was found to be identical in every respect with the amino-ketone (IIIb) derived from N-methyl-O,N-desacylpachysandrine-A (IIb).

Formation of the Oxazolidine Compound (XXIII) from the Amino-alcohol (XXIIb)——A solution of XXIIb (20 mg.) in formic acid (1 ml.) and 37% formalin (1 ml.) was heated on a steam bath for 5 hr. The crystalline product (20 mg.) was isolated by dilution with water, basification with NH_4OH, and extraction with CH_2Cl_2. Recrystallizations from acetone gave the pure oxazolidine compound (XXIII), needles, m.p. 201~202°, $[\alpha]_D^{10}$ −58°(c=1.16). *Anal.* Calcd. for $C_{25}H_{44}ON_2$: C, 77.26; H, 11.41; N, 7.21. Found : C, 77.53; H, 11.46; N, 7.33. NMR τ : 5.33, 6.28 (2H, two doublets, J 2.5 c.p.s.; N-CH_2-O), 6.20 (1H, quartet, J 6 and 10 c.p.s.; C<u>H</u>-OH), 7.78 (3H, N-CH_3),7.85 (6H, N-$(CH_3)_2$), 9.17 (3H, doublet, J 7 c.p.s.; sec. CH_3), 9.25, and 9.38 (6H, two tert. CH_3).

Lithium Aluminum Hydride Reduction of the Oxazolidine Compound (XXIII)——The compound (XXIII) (113 mg.) in dry tetrahydrofuran (15 ml.) containing $LiAlH_4$ (230 mg.) was refluxed with agitation for 6 hr. Usual working up gave the amino-alcohol (XXIVb) (110 mg.), needles, m.p. 165~170°, which, on recrystallizations from MeOH-CH_2Cl_2, showed m.p. 173~174°, $[\alpha]_D^{10}$ −34°(c=1.02). This was identified with the 3α-dimethylamino-4α-hydroxy compound (XXIVb), obtained from the N-methylated O-benzoate (XXIVa), by mixed m.p. determination and IR comparison ($CHCl_3$). *Anal.* Calcd. for $C_{25}H_{46}ON_2$: C, 76.86; H, 11.86; N, 7.17. Found : C, 77.27; H, 11.64; N, 7.37.

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28. **Tohru Kikuchi and Shoichiro Uyeo**: Pachysandra Alkaloids. Ⅲ.*1 Structures of Pachysandrine-B, -C, and -D.*2

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Structures of pachysandrine-B, -C, and -D isolated from *Pachysandra terminalis* Sieb. et Zucc., a Buxaceous plant, were investigated and their structures were proved to be Ⅲa, Ⅱ, and Ⅴa, respectively.

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In the preceding paper,*1 we discussed the constitution of pachysandrine-A, one of the major alkaloids of *Pachysandra terminalis* Sieb. et Zucc. (Japanese name: Fukkiso), and gave the complete structure (Ⅰ) to this alkaloid.

In the present paper, structure elucidation of three closely related alkaloids isolated from the same plant, for which we proposed the name pachysandrine-B, -C, and -D, will be discussed herewith.

Of these alkaloids, pachysandrine-C (Ⅱ), m.p. 214～215°, $[\alpha]_D$ −38° ($CHCl_3$), was first isolated in a very poor yield*4 from the N-methylated product of strong base fraction obtained by alkaline hydrolysis of the mother liquor of weakly basic alkaloid fraction. Later, this alkaloid was discovered in the strongly basic alkaloid fraction of the plant.[1] This alkaloid was analysed for $C_{24}H_{44}ON_2$ and was found to be identical with 3α-methylamino-4α-hydroxy-20α-dimethylamino-5α-pregnane (Ⅱ),*1 which had been derived from pachysandrine-A (Ⅰ), by direct comparison (mixed m.p. and IR in KBr).

Pachysandrine-B (Ⅲa), m.p. 187～189°, $[\alpha]_D$ +93° ($CHCl_3$), was analysed for $C_{31}H_{52}O_3N_2$ and revealed the infrared bands*5 for an O-acetyl (1730 and 1240 cm^{-1}) and a conjugated tertiary amide (1650 and 1610 cm^{-1}) (Fig. 1). As shown in Fig. 2, the NMR spectrum*6 of this alkaloid revealed the presence of one olefinic proton (4.23τ, 1H, multiplet), the grouping CH-CH(O-Ac)-CH (4.75τ, quartet, J 4 and 6 c.p.s.), one amide N-methyl (7.08τ, 3H), one N-dimethyl (7.83τ, 6H), one O-acetyl (7.97τ, 3H), one isopropylidene (8.12 and 8.17τ, 6H, two doublets, J about 1 c.p.s.),*7 two tertiary C-methyls (8.94 and 9.33τ, 6H), and one secondary C-methyl (9.14τ, 3H, doublet, J 7 c.p.s.) in the molecule.

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*1 Part Ⅱ. M. Tomita, S. Uyeo, Jr., T. Kikuchi: This Bulletin, **15**, 193 (1967).

*2 Preliminary communications of this work appeared in Tetrahedron Letters, No. 18, 1053 (1964); *Ibid.*, No. 27, 1817 (1964).

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*4 This was because major part of pachysandrine-C had been transformed to the oxazolidine compound by formalin-formic acid procedure and only small amount of the unreacted material was obtained.

*5 Infrared spectra were taken in chloroform solutions unless otherwise specified. For identification of compounds, spectra were measured on a Koken DS-301 Spectrometer in KBr discs.

*6 All the NMR spectra were determined on a Varian Associates A-60 High-Resolution NMR Spectrometer in deuterated chloroform and chemical shifts are reported in τ scale using tetramethylsilane as the internal reference.

1) Part Ⅰ. M. Tomita, T. Kikuchi, S. Uyeo, Jr., T. Nishinaga, M. Yasunishi (née Ando), A. Yamamoto: Yakugaku Zasshi, **87**, 198 (1967).