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STUDIES ON THE ALKALOIDS OF PACHYSANDRA TERMINALIS SIEB. ET ZUCC. : STRUCTURE OF PACHYSANDRINE A AND B.

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Plants of Pachysandra genus have been known to contain alkaloidal constituents[1], but no report on isolation and structure elucidation of their alkaloids has been found in the past literatures. The authors examined systematically the basic fraction of Pachysandra terminalis SIEB. et ZUCC. and succeeded to isolate a number of alkaloids in pure crystalline forms. Two alkaloids among these, for which we proposed the name pachysandrine A and B, were demonstrated to have the structure Ia and If, respectively.

Pachysandrine A, $C_{33}H_{50}O_3N_2$, m.p. 235-6° *, $[\alpha]_D^{13}$ +80° ** showed, IR ν_{max}^{Nujol} 1730, 1245 ($-OCOCH_3$), 1620 cm^{-1} ***(conjugated

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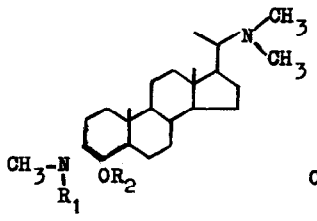
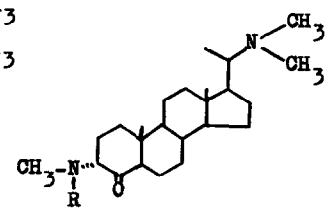
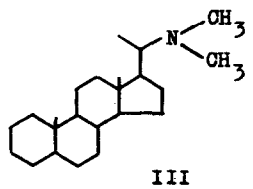
* All the melting points were uncorrected.
** Optical rotations were measured in chloroform solutions.
*** We thank Dr. K. Machida of this Faculty for IR measurements.



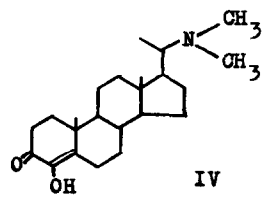
	R_1	R_2
Ia :	C_6H_5CO	CH_3CO
Ib :	C_6H_5CO	H
Ic :	H	H
Id :	CH_3	H
Ie :	CH_3	CH_3CO
If :	$(CH_3)_2C{=}CHCO$	CH_3CO
Ig :	$(CH_3)_2C{=}CHCO$	H



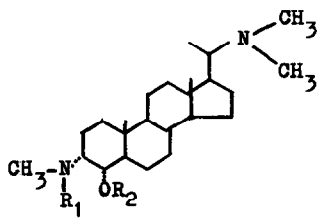
IIa : R=H
IIb : R=CH_3



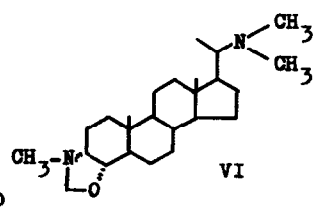
III



IV



	R_1	R_2
Va :	H	H
Vb :	CH_3	H
Vc :	CH_3	CH_3CO
Vd :	H	$(CH_3)_2C{=}CHCO$



VI

Chart 1.

amide CO) and NMR signals* at 2.67 (5H, phenyl), 4.62 (1H, quartet, J's 5, 6 c.p.s.; CH-CHOAc-CH), 5.42 (1H, broad), 7.07 (3H, RCON-CH_3), 7.84 (6H, $N(CH_3)_2$), 7.98 (3H, CH_3COO-), 9.06 and 9.36 (6H, two tert. CH_3) and 9.13 τ (3H, doublet, J 6 c.p.s.; one sec. CH_3).

Hydrolysis of pachysandrine A with 3% NaOH-MeOH gave O-desacyl compound (Ib), $C_{31}H_{48}O_2N_2$, m.p. 195°, $[\alpha]_D^{10}$ +91°, IR $\nu_{max}^{CHCl_3}$ 1610 cm^{-1} (amide CO), which returned to pachysandrine A upon acetylation. When hydrolysed with 20% KOH-EtOH it afforded benzoic acid and O,N-desacyl compound (Ic), $C_{24}H_{44}ON_2$, m.p. 226-227°, $[\alpha]_D^{10}$ +28°. Treatment of this compound with formalin-formic acid or with formalin-sodiumborohydride[2] yielded N-methyl compound (Id), $C_{25}H_{46}ON_2 \cdot \frac{1}{2}H_2O$, m.p. 126-150° **, $[\alpha]_D^{10}$ +21°, NMR signals 6.20 (1H, broad, -CHOH), 7.75 and 7.86 (12 H, two $N(CH_3)_2$), 8.95 and 9.35 (6H, two tert. CH_3) and 9.16 τ (3H, doublet, J 7 c.p.s.; one sec. CH_3); O-acetate (Ie), $C_{27}H_{48}O_2N_2$, m.p. 169-170°, $[\alpha]_D^{15}$ +16°.

Chromic acid oxidation of Ic in acetic acid gave the corresponding oxo compound (IIa), $C_{24}H_{42}ON_2$, m.p. 169-170°, $[\alpha]_D^{10}$ -28°, IR $\nu_{max}^{CHCl_3}$ 1710 cm^{-1} (CO), UV λ_{max}^{MeOH} 312 mμ (ε =105), which was characterized by its reduction back to the starting amino-alcohol (Ic). Also Id yielded the oxo compound (IIb), $C_{25}H_{44}ON_2$, m.p. 187-188°, $[\alpha]_D^{23}$ +24°, IR $\nu_{max}^{CHCl_3}$ 1710 cm^{-1} (CO), UV λ_{max}^{MeOH} 294 mμ (ε =47), upon chromic acid oxidation.

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* NMR spectra were measured in $CDCl_3$ and chemical shifts are reported in τ values. We wish to thank Dr. T. Shingu of this Faculty and Dr. K. Tori, Shionogi & Co., Ltd. for these determinations.

** This substance did not give sharp melting point.

Both IIa and IIb gave negative Zimmermann tests[3] and their ORD curves in MeOH showed negative Cotton effects: viz., for IIa, Trough $[\phi]_{330}$ -8310° and Peak $[\phi]_{285}$ +11500°; for IIb, Trough $[\phi]_{319}$ -4320° and Peak $[\phi]_{275}$ +6040°. These are very similar to that of cholestan-4-one*,[4].

Huang-Minlon reduction of IIa and IIb afforded a compound $C_{23}H_{41}N$, m.p. 141-142°, $[\alpha]_D^{17}$ +25°, which was believed to be III on the basis of its NMR spectrum [7.85 (6H, $N(CH_3)_2$), 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3), 9.23 and 9.36 τ (6H, two tert. CH_3)]. Since the usual Hofmann degradation and von Brown reaction failed to convert this to an N-free substance, we carried out the synthesis of III starting from bisnorallocholanic acid (VII)[5], m.p. 214-215°, $[\alpha]_D^{10}$ +7°. (Chart 2).

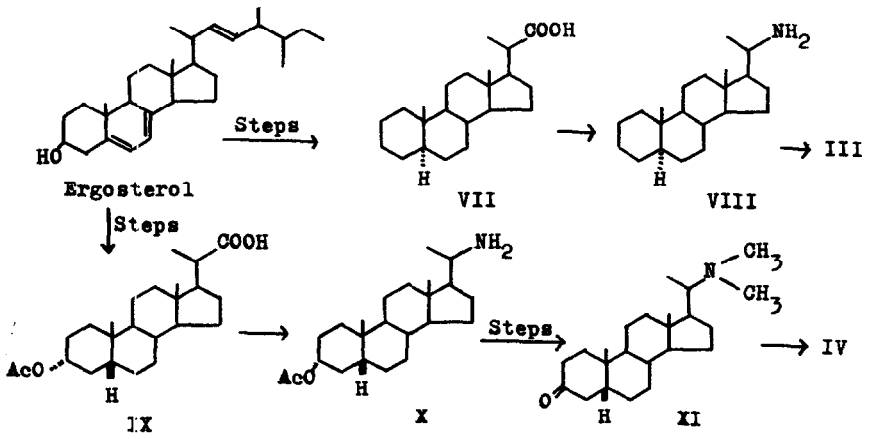

Chart 2.

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* We are indebted to Dr. K. Kuriyama, Shionogi & Co., Ltd., for ORD measurements and helpful disscussions.

The synthesized compound, m.p. 141-142°, $[\alpha]_D^{20}$ +26°, was shown to be idential with the above described compound originated from pachysandrine A by mixed melting point determination and IR comparison (KBr).

When the ketone (IIa) was treated with KOH in EtOH[6], there was obtained a diosphenol (IV), $C_{23}H_{37}O_2N$, m.p. 192-193°, $[\alpha]_D^{17}$ +19°, UV λ_{max}^{MeOH} 279mμ (ε =9100), IR ν_{max}^{KBr} 3420, 1670, 1630, 1380 cm^{-1} (-CO-C(OH)=C-), which, on Huang-Minlon reduction, gave III. The structure of the diosphenol was established by the comparison with the synthesized compound (IV), m.p. 195.5-196.5°, $[\alpha]_D^{17}$ +24°, which was obtained by air oxidation[7] of 20α-dimethylamino-5β-pregnan-3-one (XI), m.p. 103-104°, $[\alpha]_D^{10}$ +34° *. (Chart 2).

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

Turning now to the stereochemistry of pachysandrine A, evidence for the 3α,4β-configuration was advanced. Reaction of O-desacetyl-pachysandrine A (Ib) with $POCl_3$ in pyridine followed by alkaline hydrolysis gave the 4-epi-alcohol (Va) in a good yield, $C_{24}H_{44}ON_2$ m.p. 215-216°, $[\alpha]_D^{10}$ -38°, whose characterization was achieved by its oxidation with chromic acid to IIa. The formation of 4-epimer (Va) is understandable on the basis of Cornforth's acyl migration mechanism[8], which involves an oxazoline intermediate formed by the nucleophyllic backside attack of the N-acyl group and hence can occur in only 1,2-diaxial-

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* VII and XI were derived from ergosterol by essentially the published routes as shown in Chart 2. We express our deep gratitude to Dr. W. Nagata, Shionogi & Co. Ltd., for supply with ergosterol and for valuable advices.

aminocycloalkanol systems: 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, Ic and Id (8.95, 8.95 and 8.95 τ, respectively) occured in about 15-18 c.p.s. lower field than those of 4-epimeric alcohols and 4-oxo compounds (Va, Vb, IIa, and IIb: 9.18, 9.20, 9.25, and 9.27 τ, respectively). Acetylation of the former group caused slight diamagnetic shifts (ie., 9.06 for Ia and 9.00 τ for Ie), while O-acetate (Vc) of the 4-epimer (Vb) showed a slight paramagnetic shift to 9.10 τ. This behavior of the 19-methyl signals indicates[9] that the 4-hydroxy groups of Ib, Ic and Id are in 1,3-diaxial relation to the 19-methyl groups: hence β-configuration.

2) The spin-spin coupling constants between C_4-hydrogen and C_3-,C_5-hydrogens of pachysandrine A (Ia) (J's 5,6 c.p.s.) and of Vc (J's 3,12 c.p.s.) suggest the 3α,4β-configuration for the former and the 3α,4α-configuration for the latter[10].

3) Ic, upon treatment with formalin-formic acid, gave the N-methyl compound (Id), whereas, under the same condition, the 4-epimer (Va) afforded the oxazolidine compound (VI), $C_{25}H_{44}ON_2$, m.p. 201-202°, $[\alpha]_D^{10}$ -58°, NMR 5.32 and 6.27 τ (2H, doublet, J 2.5 c.p.s.; N-CH_2-O), which could be converted to the corresponding N-dimethyl compound (Vb), $C_{25}H_{46}ON_2$, m.p. 173-174°, $[\alpha]_D^{10}$ -34°, by the reduction with $LiAlH_4$.

4) Infrared spectrum of Id in CCl_4 showed a monomeric OH band at 3640 cm^{-1}, while that of the 4-epimer (Vb) occured at 3330 cm^{-1} (internally bonded OH). The lack of intramolecular

hydrogen bonding in the former favors the 3α,4β-configuration.

The second alkaloid, pachysandrine B (If), $C_{31}H_{52}O_3N_2$, m.p. 187-189°, $[\alpha]_D^{19}$ +93.4°, showed IR ν_{max}^{Nujol} 1735, 1235 (CH_3-COO-), 1660, 1630 cm^{-1} (-C=C-CON-), and NMR signals at 4.23 (1H, multiplet; oleffinic proton), 4.75 (1H, quartet, J's 4,6 c.p.s.; -CH-CHOAc-CH-), 5.45 (1H, broad; N-CH-), 7.08 (3H, RCON-CH_3), 7.83(6H, N-$(CH_3)_2$), 7.97 (3H, CH_3COO), 8.15 (6H, two doublets, J's < 2 c.p.s.; $(CH_3)_2C$=C), 8.94 and 9.33 (6H, two tert. CH_3) and 9.14 τ (3H, doublet, J 7 c.p.s.; sec. CH_3).

Upon basic hydrolysis it gave O-desacetyl compound (Ig), $C_{29}H_{50}O_2N_2$, m.p. 184-185°, $[\alpha]_D^{10}$ +127°, IR $\nu_{max}^{CHCl_3}$ 1595, 1650 cm^{-1} (conjugated amide), which, on acetylation, returned to pachysandrine B. Ig resisted further basic hydrolysis under vigorous conditions.

However, on acidic hydrolysis of Ig with conc. HCl or with AcOH-conc. HCl mixture, there was obtained an ester (Vd), m.p. 201-204°, $[\alpha]_D^{10}$ -6°, which might be formed by the migration of the N-acyl group with inversion[11]. Mild alkaline hydrolysis of this ester yielded the aminoalcohol Va, $C_{22}H_{44}ON_2$, m.p. 215-216°, $[\alpha]_D^{10}$ -39°, which was identified by IR comparison with the substance (Va) derived from pachysandrine A. Va was also obtained by the reaction of Ig with $POCl_3$ followed by acidic hydrolysis. Its NMR spectrum was characterized by the remarkable diamagnetic shift (17 c.p.s.) of the 19-methyl signal compared with that of Ig. This provides a support for the epimerization at 4-position during the acidic hydrolysis. Therefore the structure of pachysandrine B must be 3α-methylamino-4β-acetoxy-20α-dimethylamino-5α-pregnane 3-amide.

The acid moiety of the 3-amide group was believed to be β,β-dimethylacrylic acid from the consideration of the IR spectrum (α,β-unsaturated amide band), NMR spectrum (two methyl groups adjacent to double bond and one proton attached to the α-carbon atom), and the result of ozonolysis of pachysandrine B, on which acetone and formalin were obtained as their 2,4-dinitrophenyl hydrazones.

The confirmative proof was provided by the treatment of O,N-desacyl pachysandrine A (Ic) with β,β-dimethylacrylyl chloride in pyridine. Mild alkaline hydrolysis of the product gave O-desacetyl pachysandrine B.

Thus the structure of pachysandrine A and B are unambiguously assigned to Ia and If, respectively*.

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