straight line in Fig. 5, have a substituent of negative π-values, that is, of lowering the lipid solubility of the compound, suggesting that the change in the lipid solubility has also a minor effect on the rate of the cell penetration.

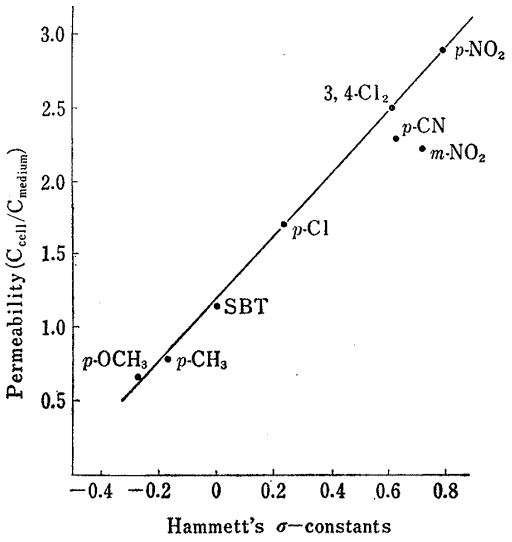


Fig. 5. Relation between the Red Cell Permeability to Substituted S-Benzoylthiamines and Hammett's σ-Constants of the Substituents

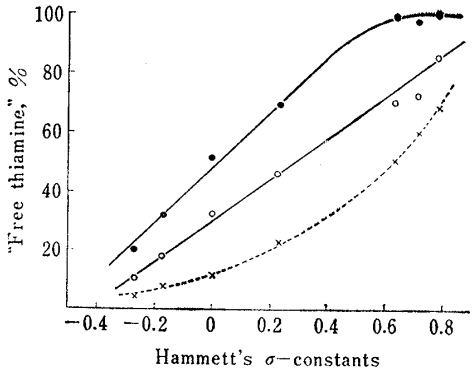


Fig. 6. Relation between the Rate of Decomposition of Substituted S-Benzoylthiamines and Hammett's σ-Constants of the Substituents

—●— In 80% red cell homogenate, 0.3 m*M*/L. SBT derivatives, incubation at 37° for 30 minutes
—○— In 40%red cell homogenate
------×------ In phosphate buffer, pH 7.4

The authors express their deep gratitudes to Dr. G. Sunagawa, director of this laboratories, for his helpful advices and kind encouragement throughout the course of this work, and to Drs. I. Iwai and A. Ito of this laboratories for their helpful discussions and encouragements. Thanks are also due to Dr. M. Nagawa and Mr. M. Kataoka of this laboratories for the preparation of substituted S-benzoylthiamines.

[Chem. Pharm. Bull. 15(3) 302~306 (1967)]

UDC 547.94.02 : 582.761

37. Tohru Kikuchi and Shoichiro Uyeo : Pachysandra Alkaloids. Ⅳ.*1 Structure of Pachysamine-A and -B.*2

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

Pachysamine-A and -B are minor alkaloids of *Pachysandra terminalis* Sieb. et Zucc., a Buxaceous plant. The structures of both alkaloids were discussed herewith and assigned to the formulas Ⅲ and Ⅷ, respectively.

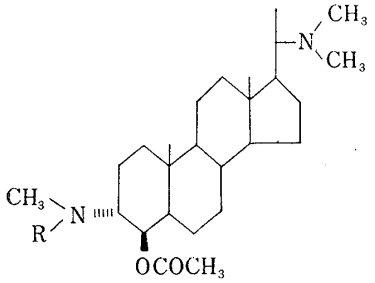
(Received May 27, 1966)

In Part Ⅱ[1] and Ⅲ*1 of this series, we reported the structures and stereochemistry of pachysandrine-A (Ia), -B (Ib), -C (Ⅱa), and -D (Ⅱb), which had been isolated from *Pachysandra terminalis* Sieb. et Zucc. (Japanese name : Fukki-so) and belong to the

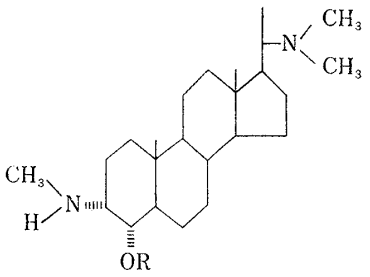
*1 Part Ⅲ. T. Kikuchi, S. Uyeo, Jr. : This Bulletin, **15**, 207 (1967).
*2 Preliminary communication of this work appeared in Tetrahedron Letters, No. 25, 1641 (1964).
*3 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto (菊池 徹, 上尾庄一郎).
1) M. Tomita, S. Uyeo, Jr., T. Kikuchi : This Bulletin, **15**, 193 (1967).

NII-Electronic Library Service

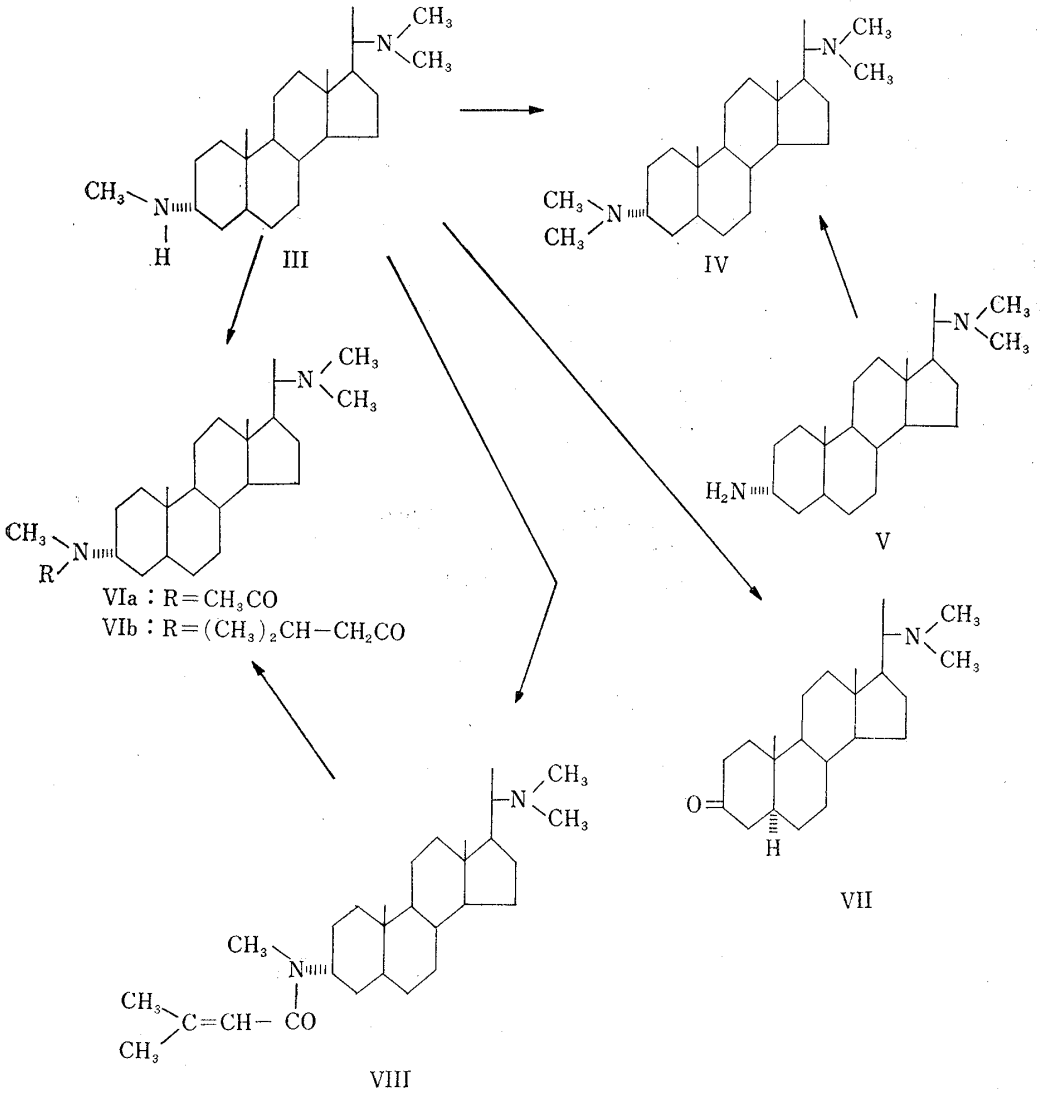
new type of pregnane alkaloid having an oxygen function at the 4-position. The present paper covers the full details of structure determination of pachysamine-A and -B, new alkaloids isolated from the same plant,[2] whose structures are now assigned



Ia : R=C_6H_5CO
Ib : R=$(CH_3)_2C=CHCO$



IIa : R=H
IIb : R=$(CH_3)_2C=CHCO$



VIa : R=CH_3CO
VIb : R=$(CH_3)_2CH-CH_2CO$

Chart 1.

2) M. Tomita, T. Kikuchi, S. Uyeo, Jr., T. Nishinaga, M. Yasunishi (née Ando), A Yamamoto : Yakugaku Zasshi, **87**, 215 (1967).

NII-Electronic Library Service

to III and VIII, respectively. They present the first examples of 3α,20α-bisamino-5α-pregnane type alkaloid discovered from the natural sources, although the syntheses[3,4] of their derivatives had already been achieved and the Δ_5-analogues had been isolated from Apocynaceous plants.[5]

Pachysamine-A (III), m.p. 167~168°, $[\alpha]_D$ +20° ($CHCl_3$), was analyzed for $C_{24}H_{44}N_2$ and showed NMR signals*4 for one N-methyl group (7.61τ), one N-dimethyl group (7.84τ), one secondary C-methyl group (9.15τ, doublet, J 6 c.p.s.), and two tertiary C-methyl groups (9.20 and 9.35τ).

On treatment with acetic anhydride in pyridine, it gave an N-acetate (IVa), m.p. 150~152.5°, showing a tertiary amide band at 1623 cm^{-1} in the infrared spectrum.*5 When methylated by the formalin-sodium borohydride procedure,[6] it afforded an N-methyl compound (IV), $C_{25}H_{46}N_2$, m.p. 165.5~167°, $[\alpha]_D$ +16° ($CHCl_3$). The formation of a new N-methyl group was indicated by the NMR signals for two N-dimethyl groups (7.78 and 7.84τ, 12H).

Properties of this compound (IV) are in agreement with the reported values of synthesized 3α,20α-bisdimethylamino-5α-pregnane (IV)[3] and the identity was established by direct comparison (mixed melting point, infrared (KBr), and NMR spectra) with the N,N-dimethyl compound, m.p. 164~166°, $[\alpha]_D$ +20° ($CHCl_3$), derived from the synthesized sample of 3α-amino-20α-dimethylamino-5α-pregnane (V).[4] This provides a confirmatory proof for the fundamental skeleton and stereochemistry of pachysamine-A (III).

In order to determine the position of the methylamino group, Ruschig degradation[7] of pachysamine-A was performed with the formation of a keto-amine (VII), m.p. 174~176°, $[\alpha]_D$ +43° ($CHCl_3$), which showed a carbonyl absorption band (1710 cm^{-1}) in the infrared spectrum, but no signal for methyl-ketone in the NMR spectrum. Its optical rotatory dispersion (ORD) curve in methanol demonstrated a positive Cotton effect (peak, $[\phi]_{307}$ +2880°; trough, $[\phi]_{268}$ −2590°) which is the characteristic of 3-keto-5α-steroids.[8] This compound (VII) was found to be identical with funtumafrine-C (VII),[9] isolated by Goutarel, *et al.* from an Apocynaceae plant, by mixed melting point determination and infrared comparison ($CHCl_3$).

Accordingly, pachysamine-A must be 3α-methylamino-20α-dimethylamino-5α-pregnane (III). An independent support for the structure of pachysamine-A (III) was also provided by mass spectrometric study. The mass spectrum*6 of the alkaloid (III) exhibited a very intense peak at m/e 72 (fragment a) and moderately intense peaks at m/e 70 and 96 (b and c, respectively). This is the characteristic cracking pattern of

*4 All nuclear magnetic resonance (NMR) spectra were taken on a Varian Associate A-60 High-Resolution Spectrometer at 60 Mc. in deuterated chloroform and chemical shifts are reported in τ values using tetramethylsilane as the internal reference.

*5 All infrared (IR) spectra were measured in chloroform solutions unless otherwise specified.

*6 The mass spectrum was measured on a Hitachi Mass Spectrometer Model RMU-6D.

3) M. M. Janot, F. Laine, Q. Khuong-Huu, R. Goutarel : Bull. soc. chim. France, 111 (1962); V. Cerny, L. Labler, F. Sorm : Collection Czechoslov. Chem. Communs., **22**, 76 (1957).
4) P. Chien, W. E. McEwen, A. W. Burgstahler, N. T. Iyer : J. Org. Chem., **29**, 315 (1964).
5) R. Tschesche, P. Otto : Chem. Ber., **95**, 1144 (1962); L. Labler, F. Sorm : Collection Czechoslov. Chem. Communs., **28**, 2345 (1963).
6) J. H. Biemann : "Mass Spectrometry, Organic Chemical Applications," 358 (1962), McGraw-Hill, New York; K. A. Schellenberg : J. Org. Chem., **28**, 3259 (1963).
7) H. 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); *Ibid.*, **86**, 4424 (1964).
8) C. Djerassi : "Optical Rotatory Dispersion," 42 (1960), McGraw-Hill, New York.
9) M. M. Janot, Q. Khoung-Huu, R. Goutarel : Compt. rend., **250**, 2445 (1960); R. Goutarel : "Les alkaloides steroidiques des Apocynacees," 58 (1964), Hermann, Paris.

NII-Electronic Library Service

3-methylamino-20-dimethylamino-5α-pregnane, whose fragmentation can be visualized as shown below.[10]

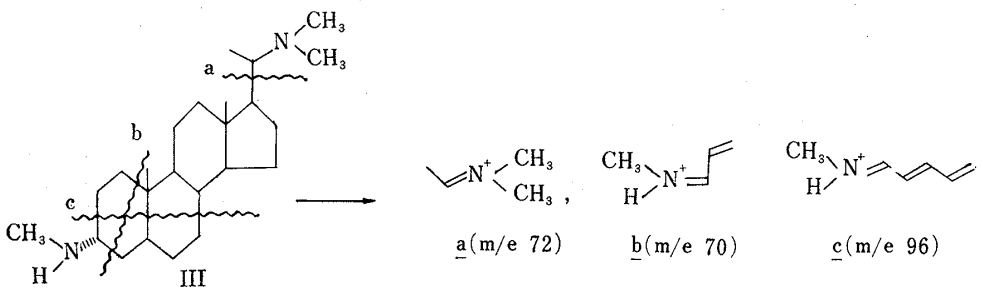


Chart 2.

The second alkaloid, pachysamine-B (Ⅷ), $C_{29}H_{50}ON_2$, showed m.p. 171~173° and $[\alpha]_D$ +67° ($CHCl_3$). Its infrared spectrum in chloroform exhibited absorption bands for an α,β-unsaturated tertiary amide (1655 and 1600 cm^{-1}) and the NMR spectrum revealed signals which could be attributed to the $(CH_3)_2C=CHCO$ grouping (one olefinic proton at 4.22τ and two allylic C-methyl groups at 8.15 and 8.18τ (two doublets, J's 1 c.p.s.)) along with two tertiary C-methyl, one secondary C-methyl, one N-dimethyl, and one amide N-methyl signals.

On catalytic hydrogenation over platinum oxide, pachysamine-B (Ⅷ) gave a dihydro compound (Ⅵb), $C_{29}H_{52}ON_2$, m.p. 138.5~139.5°, showing a saturated tertiary amide band at 1620 cm^{-1}.

Although attempts to hydrolyze both pachysamine-B (Ⅷ) and its dihydro compound (Ⅵb) under drastic acidic and basic conditions resulted in failure, it was considered to be β,β-dimethylacrylylamide of pachysamine-A on the basis of the NMR spectrum and by analogy with pachysandrine-B (Ib).*7

After all, we prepared the compound (Ⅷ) by treating pachysamine-A (Ⅲ) with β,β-dimethylacrylyl chloride according to the Schotten-Baumann method. The product (Ⅷ), $C_{29}H_{50}ON_2$, m.p. 173~174°, $[\alpha]_D$ +55°, was proved to be quite identical in all respects with pachysamine-B.

From the evidences so far described, the structures of pachysamine-A and -B are unambiguously assigned to Ⅲ and Ⅷ, respectively.

Experimental*8

N-Acetylpachysamine-A (VIa)——A solution of pachysamine-A (Ⅲ) (10 mg.) and acetic anhydride (0.1 ml.) in pyridine (0.5 ml.) was allowed to stand overnight at room temperature. After dilution with water, the mixture was basified with Na_2CO_3 and extracted with ether. Washing of the extract with water, drying over K_2CO_3, and evaporation left a crystalline residue which was chromatographed over alumina (0.7×3 cm.). Elution with benzene and with benzene-ether mixture afforded crude N-acetylpachysamine-A (Ⅵa) (10 mg.). Recrystallizations from acetone gave colorless leaves, m.p. 150~152.5°, $[\alpha]_D^{25}$ +64° (c=1.0). *Anal.* Calcd. for $C_{26}H_{45}ON_2$: C, 77.55; H, 11.52. Found: C, 77.27; H, 11.51. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1623 (*tert.* amide).

N-Methylpachysamine-A (IV)——To a solution of pachysamine-A (Ⅲ) (45 mg.) in MeOH (10 ml.) was added 37% formalin (0.2 ml.) and left stand with occasional warming for 3 hr. and then at room temperature overnight. Sodium borohydride (0.4 g.) was added to this mixture and stirred for one hour at room

*7 At this point of structural argument, the possibility of the 3-epimer could not be precluded.

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

10) H. Budzikiewicz, C. Djerassi, D. H. Williams: "Interpretation of Mass Spectra of Organic Compounds," 75~80 (1964), Holden-Day, Inc., San Francisco; L. Dolejs, V. Hanus, V. Cerny, F. Sorm: Collection Czechoslov. Chem. Communs., 28, 1584 (1963); W. Vetter, P. Longevialle, F. Khuong-Huu-Laine, Q. Khuong-Huu, R. Goutarel: Bull. soc. chim. France, 1324 (1963).

NII-Electronic Library Service

temperature. After the solvent was removed under reduced pressure, the residue was diluted with aqueous NaOH and extracted with CH_2Cl_2. The extract was dried over K_2CO_3 and evaporated to give the crystalline N-methyl compound (IV) (40 mg.), m.p. 160~168°. Recrystallizations from acetone afforded colorless long plates, m.p. 165.5~167°, which were identified with the synthesized specimen of 3α,20α-bisdimethylamino-5α-pregnane (IV) by mixed m.p. and IR (KBr) and NMR comparison. $[\alpha]_D^{19}$ +16° (c=1.05). *Anal.* Calcd. for $C_{25}H_{46}N_2$: C, 80.15; H, 12.38; N, 7.48. Found: C, 80.30; H, 12.38; N, 7.53. NMR τ: 7.78, 7.84 (12H, two N-$(CH_3)_2$), 9.15 (3H, doublet, J 6 c.p.s.; *sec.* CH_3), 9.19, and 9.37 (6H, two *tert.* CH_3).

3α,20α-Bisdimethylamino-5α-pregnane (IV)——3α-Amino-20α-dimethylamino-5α-pregnane (V) (19 mg.) was dissolved in formic acid (0.5 ml.) and 37% formalin (0.5 ml.) and the mixture was heated for 4 hr. on a boiling water bath. The product, isolated by the usual treatment, was recrystallized from acetone-CH_2Cl_2 to give the N-dimethyl compound (IV) (10 mg.) as long plates, m.p. 164~166°. $[\alpha]_D^{25}$ +20° (c=1.0). This compound is identical in every respect with N-methylpachysamine-A.

Ruschig Reaction of Pachysamine-A (III)——A solution of the alkaloid (III) (120 mg.) and N-chlorosuccinimide (100 mg.) in CH_2Cl_2 (5 ml.) was kept at room temperature for 1 hr. The solution was then washed with water, dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo* at room temperature to leave the crystalline N-chloro compound. This was dissolved in an ethanolic sodium ethoxide solution, prepared from metallic sodium (100 mg.) and absolute EtOH (8 ml.), and the solution was refluxed for 1.5 hr. After removal of the solvent *in vacuo*, the residue was dissolved in 10% H_2SO_4 and was allowed to stand overnight at room temperature. The acidic solution was then made basic with Na_2CO_3 and extracted with CH_2Cl_2. The extract was washed successively with 3% HCl, dil. NH_4OH, and water, dried over K_2CO_3, and evaporated. The residue (40 mg.) was dissolved in benzene and chromatographed on alumina (2 g.). Elution with benzene (30 ml.), 5% ether-benzene (10 ml.), and 10% ether-benzene (10 ml.) gave the crude amino-ketone (VII) (20 mg.) which was recrystallized from acetone to afford colorless plates, m.p. 174~176°. The melting point of this substance showed no depression upon admixture with an authentic sample of funtumafrine-C (VII) (m.p. 171~177°) and their IR spectra ($CHCl_3$) are identical.*9 $[\alpha]_D^{19}$ +43° (c=1.15). ORD (in MeOH, 23°, c=0.215%): positive Cotton effect; peak, $[\phi]_{307}$ +2880°; trough, $[\phi]_{268}$ −2590°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (ketone). NMR τ: 7.83 (6H, N-$(CH_3)_2$), 9.14 (3H, doublet, J 6 c.p.s.; *sec.* CH_3), 8.99, and 9.33 (6H, two *tert.* CH_3).

Dihydropachysamine-B (VIb)——A solution of pachysamine-B (VIII) (100 mg.) in MeOH (20 ml.) was shaken with hydrogen in the presence of $PtO_2 \cdot 2H_2O$ (50 mg.) at room temperature and at atmospheric pressure until the uptake of hydrogen ceased. After removal of the catalyst and evaporation of the solvent *in vacuo*, the residue was dissolved in CH_2Cl_2, washed successively with 3% HCl and dil. NH_4OH, dried over K_2CO_3, and evaporated. The crystalline residue was recrystallized from acetone to give the dihydro compound (VIb) (75 mg.), m.p. 138~139°. Further recrystallization raised the melting point to 138.5~139.5°. $[\alpha]_D^{19}$ +54° (c=1.34). *Anal.* Calcd. for $C_{29}H_{52}ON_2$: C, 78.32; H, 11.79; N, 6.30. Found: C, 78.16; H, 11.70; N, 6.60. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1620 (saturated amide). NMR τ: 6.96 (3H, RCON-CH_3), 7.83 (6H, N-$(CH_3)_2$), 9.05 (6H, doublet, J 6 c.p.s.; two *sec.* CH_3), 9.14 (3H, doublet, J 6 c.p.s.; *sec.* CH_3), 9.18, and 9.35 (6H, two *tert.* CH_3).

Synthesis of Pachysamine-B (VIII) from Pachysamine-A (III)——A solution of pachysamine-A (III) (85 mg.) in ether-CH_2Cl_2 (1:1, 5 ml.) was placed on an aqueous 10% NaOH solution (5 ml.) and stirred vigorously. To this mixture was added dropwise β,β-dimethylacrylyl chloride (0.1 ml.) and stirring was continued for 5 hr. at room temperature. The organic solvent phase was then separated and evaporated. The residue was dissolved in CH_2Cl_2, washed successively with 3% HCl and dil. NH_4OH, dried over K_2CO_3, and evaporated to give a crystalline mass (50 mg.) which was recrystallized from acetone. There was obtained the N-acyl compound (VIII) as colorless crystals (35 mg.), m.p. 173~174°, $[\alpha]_D^{19}$ +55° (c=1.34). IR spectrum (KBr) of this compound was superimposable upon that of pachysamine-B and the mixed melting point did not depress. *Anal.* Calcd. for $C_{29}H_{50}ON_2$: C, 78.69; H, 11.38. Found: C, 78.39; H, 11.43.

In another experiment, the alkaloid (III) was treated with β,β-dimethylacrylyl chloride in pyridine. The usual working up afforded the same product as above.

The authors express their deep gratitude to Professor M. Tomita of this Faculty for his guidance and hearty encouragement. The authors also wish to thank Dr. A. W. Burgstahler of the University of Kansas, U. S. A., for a gift of the synthetic 3α-amino-20α-dimethylamino-5α-pregnane and Dr. R. Goutarel of the Institute for Chemistry of Natural Products, France, for the sample of funtumafrine-C. Also the authors are indebted to Dr. K. Kuriyama of Shionogi & Co., Ltd. for the ORD measurement, Dr. T. Shingu for determining NMR spectra, Dr. K. Miyajima and collaborators for IR determinations in KBr disc, Mr. A. Kato for taking the mass spectrum, and Dr. K. Konobu and Miss Y. Mano and their collaborators for elemental analyses.

*9 However, their infrared spectra in KBr showed small differences which may probably be due to the difference in crystal forms.

NII-Electronic Library Service