CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 15 No. 5 May 1967

[Chem. Pharm. Bull. 15(5) 549~570 (1967)]

UDC 547.94 : 582 : 761

66. Tohru Kikuchi and Shoichiro Uyeo : Pachysandra Alkaloids. Ⅷ.*1 Structures of Pachystermine-A and -B, Novel Type Alkaloids Having a β-Lactam Ring.*2

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

Pachystermine-A (I) and -B (II), weakly basic alkaloids isolated from *Pachysandra terminalis* SIEB. et ZUCC. (Buxaceae), were investigated. The structures and stereochemistry of these alkaloids are now assigned to the formula I and II, respectively. They represent a novel type of 3,20-diamino-5α-pregnane alkaloid carrying a four-membered β-lactam ring system.

(Received July 26, 1966)

Pachystermine-A is one of the major alkaloids of *Pachysandra terminalis* SIEB. et ZUCC. (Japanese name : Fukki-so) and it was isolated from the weakly basic alkaloid fraction along with pachystermine-B, a minor alkaloid obtained by careful alumina chromatography.[1] The interrelationship of both alkaloids was indicated as follows : the reduction of pachystermine-A with sodium borohydride gave rise to pachystermine-B and the latter alkaloid, in turn, reformed the former upon chromium trioxide oxidation in acetic acid. We have proposed the complete structures (I and II) for pachystermine-A and -B,*2 respectively, on the basis of a series of degradative evidences and the partial syntheses. They represent a novel type of steroidal alkaloid carrying a β-lactam ring.*4 This paper deals with the full detail of structure elucidation of these two alkaloids, and their syntheses will be presented in future communication.

Pachystermine-A (I), m.p. 220~224°, $[\alpha]_D$ +24° ($CHCl_3$), was analyzed for $C_{29}H_{48}O_2N_2$, which is supported by the appearance of molecular ion peak at m/e 456 in the mass spectrum.*5 It showed no characteristic absorption peak in the ultraviolet spectrum, but two carbonyl bands at 1735 and 1715 cm^{-1} in the infrared spectrum*6 (Fig. 1). As

*1 Part Ⅶ. T. Kikuchi, S. Uyeo, Jr., T. Nishinaga : Yakugaku Zasshi, in press.
*2 Preliminary report of this work appeared in Tetrahedron Letters, No. 39, 3473 (1965).
*3 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto (菊池 徹, 上尾庄一郎).
*4 It should be mentioned that only penicillins and a few other antibiotics are known to have a fused β-lactam ring system.
*5 Mass spectra were determined on a Hitachi Mass Spectrometer Model RMU-6D using an all glass inlet system.
*6 IR spectra were taken in chloroform solutions unless otherwise specified.
1) Part I. M. Tomita, T. Kikuchi, S. Uyeo, Jr., T. Nishinaga, M. Yasunishi (née Ando), A. Yamamoto: Yakugaku Zasshi, 87, 215 (1967).

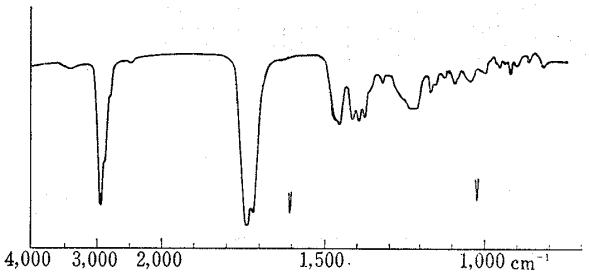

Fig. 1. Infrared Spectrum of Pachystermine-A (I)

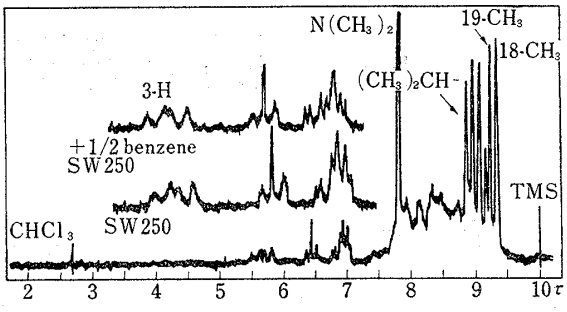

Fig. 2. Nuclear Magnetic Resonance Spectrum of Pachystermine-A (I)

shown in Fig. 2, its NMR (nuclear magnetic resonance) spectrum*7 indicated clearly the presence of one N-dimethyl (7.85τ), one secondary C-methyl (9.15τ, doublet, J=6 c.p.s.), and two tertiary C-methyls (9.26 and 9.36τ). Other characteristic NMR signals occurred at 8.90, 9.00, and 9.10τ would be attributable to a hindered isopropyl group, showing two doublets with a coupling constant of 6 c.p.s. The ORD (optical rotatory dispersion) curve of the alkaloid (I) in methanol demonstrated a negative Cotton effect (Fig. 5) which is comparable to that of cholestan-4-one.[2]

On the other hand, pachystermine-B (II), $C_{29}H_{50}O_2N_2$ (mol. wt. 458.71, molecular ion peak at m/e 458 in the mass spectrum), m.p. 257~258°, $[\alpha]_D$ −16° ($CHCl_3$), showed an infrared band at 1718 cm^{-1} (Fig. 3) and its NMR spectrum had a similar pattern to that of pachystermine-A (I), but it was characterized by the appearance of a new signal for a proton adjacent to the hydroxyl group (5.96τ, broad) and the appreciable paramagnetic shift (25 c.p.s.) of one of the tertiary C-methyl groups (19-methyl) (Fig. 4). The ORD curve of pachystermine-B in methanol exhibited a negative plane curve, suggesting the carbonyl group is not contained in the ring system.

The mass spectra of both pachystermine-A (I) and -B (II) had a very strong base peak at m/e 72 (Fig. 6), which is the characteristic of 20-dimethylaminopregnane derivatives.[3]

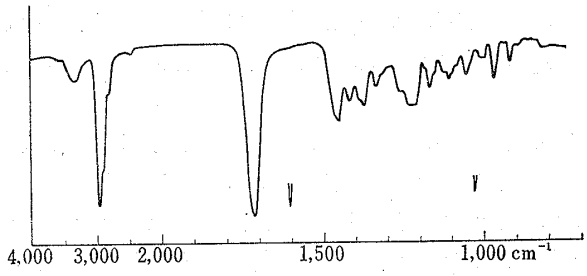

Fig. 3. Infrared Spectrum of Pachystermine-B (II)

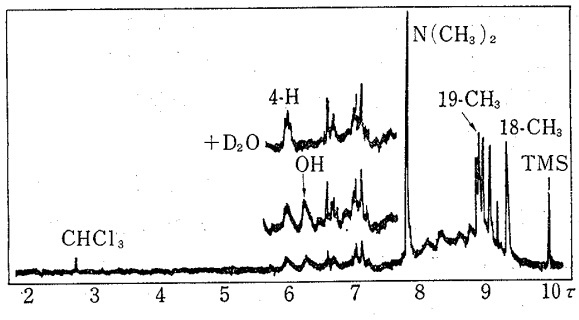

Fig. 4. Nuclear Magnetic Resonance Spectrum of Pachystermine-B (II)

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

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

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.

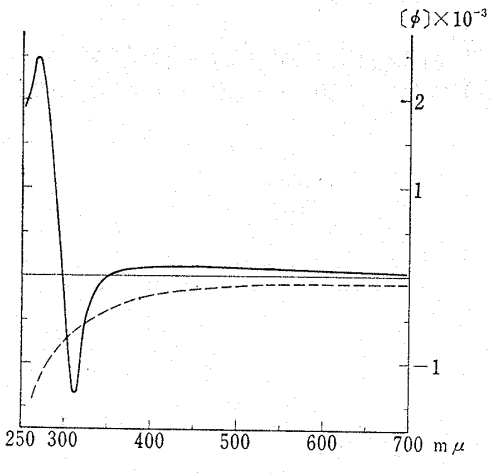

Fig. 5. Optical Rotatory Dispersion Curves (in Methanol)

——— Pachystermine-A (I)
------- Pachystermine-B (II)

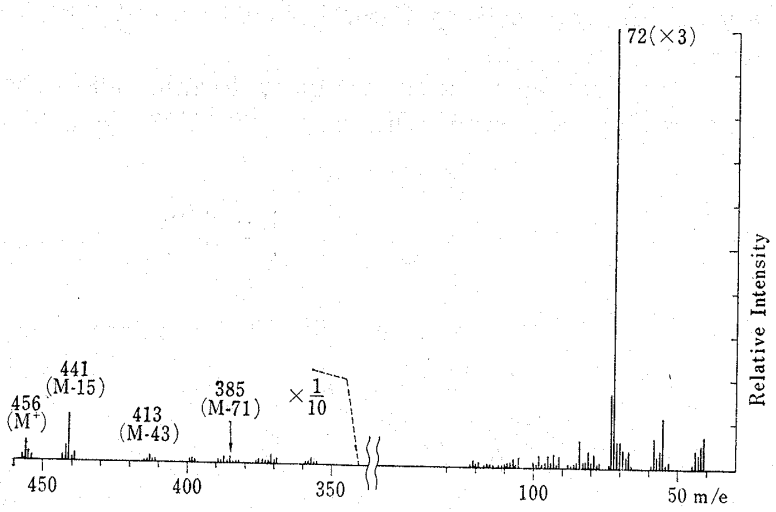

Fig. 6. Mass Spectrum of Pachystermine-A (I)

A proof for the suggested skeletal structure of the alkaloids was provided by the treatment of pachystermine-A (I) with 5% ethanolic potassium hydroxide,[4] whereupon the diosphenol (III), $C_{23}H_{37}O_2N$, m.p. 188~191°, was obtained. The identity was established by direct comparison with the synthesized specimen reported in the previous paper[5] (mixed melting point determination and IR (KBr) comparison).

Furthermore, when pachystermine-A (I) was subjected to the modified Wolff-Kishner reduction (Huang-Minlon modification and Nagata modification),[6] there were obtained 20α-dimethylamino-5α-pregnane (IV),[5] $C_{23}H_{41}N$, m.p. 137~139°, $[\alpha]_D$ +22° ($CHCl_3$), and the isopropylidenehydrazone derivative (Vb) of an amino-ketone, $C_{26}H_{45}N_3$, m.p. 164~167°. Acid hydrolysis of the latter gave rise to the amino-ketone (Va), m.p. 164~166°, whose infrared spectrum showed a carbonyl band at 1705 cm^{-1}. Its ORD curve in methanol gave a negative Cotton effect (peak $[\phi]_{272}$ +5400°, trough $[\phi]_{308}$ −3930°) which is very similar to those of 4-keto-5α-steroids[2] as well as pachystermine-A (I) and the NMR spectrum revealed evidently the presence of an N-dimethyl (7.83τ), a secondary C-methyl (9.13τ, doublet, J=6 c.p.s.), and two tertiary C-methyl groups (9.25 and 9.34τ). On the basis of the foregoing observations, the amino-ketone was considered to be 20α-dimethylamino-5α-pregnan-4-one (Va). Then, Va was synthesized from the diosphenol (III) by reduction with hydroiodic acid in acetic acid.[7] The product (Va), m.p. 165~167°, $[\alpha]_D$ +42° ($CHCl_3$), was found to be identical with the above amino-ketone (Va) by mixed melting point determination and infrared (KBr) comparison.

Another evidence for the skeletal structure was advanced as follows.

Alkali degradation of pachystermine-B (II) under the condition of Wolff-Kishner reduction, afforded a diamino-alcohol (VIa),*8 which was characterized as its O,N-diacetate (VIb), $C_{27}H_{46}O_3N_2 \cdot 1/2H_2O$, m.p. 237~241°. The O,N-diacetate (VIb) demonstrated infrared bands for a secondary N-acetyl (3400, 1670, and 1510 cm^{-1}) and an O-acetyl group (1735 and 1240 cm^{-1}), and NMR signals for one proton geminal to O-acetyl grouping (4.83τ), one N-dimethyl(7.83τ), one N-acetyl and one O-acetyl (7.90

*8 Initial step of this degradation might be the formation of amino-acid (XIV), described later, which may undergo β-elimination to give the amino-alcohol (VIa).

4) K. S. Brown, Jr., S. M. Kupchan: J. Am. Chem. Soc., **86**, 4417 (1964).
5) Part II. M. Tomita, S. Uyeo, Jr., T. Kikuchi: This Bulletin, **15**, 193 (1967).
6) W. Nagata, H. Itazaki: Chem. & Ind. (London), **1964**, 1194.
7) W. Reusch, R. LeMahieu: J. Am. Chem. Soc., **85**, 1669 (1963).

and 8.09τ), two tertiary C-methyls (9.03 and 9.38τ), and one secondary C-methyl (9.15τ, doublet, J=6 c.p.s.).

Upon treatment with formalin-formic acid, the diamino-alcohol (Ⅵa) yielded an N-dimethyl compound (Ⅶa), m.p. 180~181°, $[\alpha]_D$ +50° ($CHCl_3$). Elemental analyses of

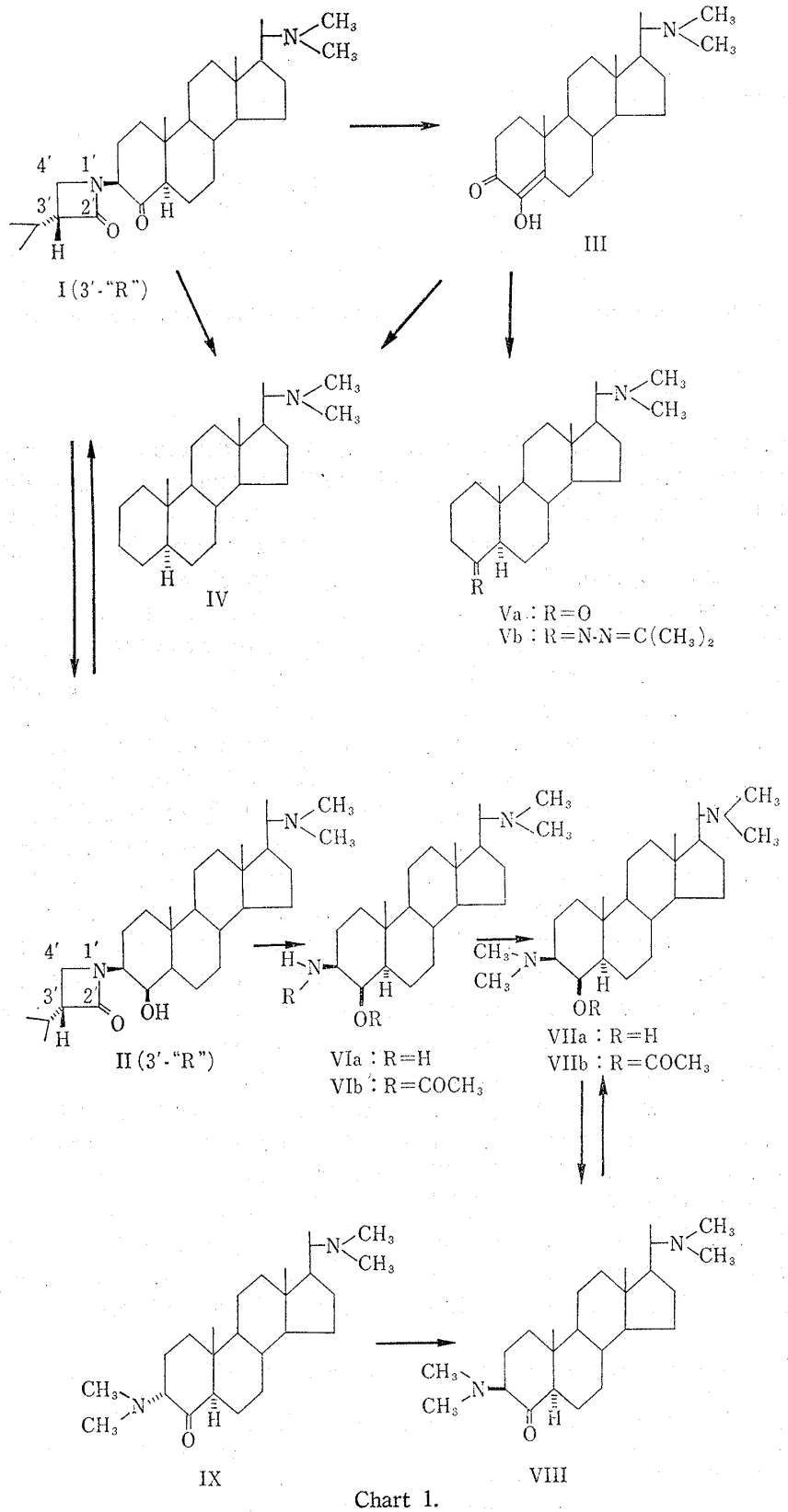

Chart 1.

this compound (VIIa) supported the empirical formula $C_{25}H_{46}ON_2$, and the NMR spectrum demonstrated signals for two N-dimethyl groups (7.72 and 7.85τ) together with signals of one proton geminal to the hydroxyl group (6.22τ, triplet, J=3 c.p.s.), two tertiary C-methyls (8.97 and 9.35τ), and one secondary C-methyl (9.15τ, doublet, J=6 c.p.s.) (Fig. 7).

Chromic acid oxidation of VIIa in acetic acid afforded a diamino-ketone (VIII), $C_{25}H_{44}ON_2$, m.p. 120~125°. $[\alpha]_D$ +47° ($CHCl_3$), which, on subsequent Wolff-Kishner reduction (Nagata modification), gave rise to 20α-dimethylamino-5α-pregnane (IV) and its 4-ketone (Va).

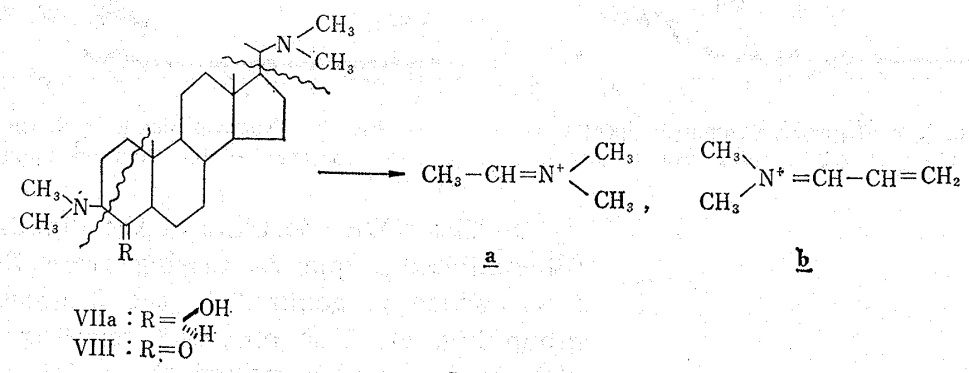

Chart 2.

As reported in the preceding paper,*1 the mass spectra of the N-methylated diamino-alcohol (VIIa) and the diamino-ketone (VIII) exhibited a moderately intense peak at m/e 84 (b) and a very intense peak at m/e 72 (a) along with other characteristic peaks (M^+, M^+-15, M^+-71, and M^+-86). This suggested strongly the 4-oxygenated 3,20-bisdimethyl-aminopregnane structure for the bases.

The stereochemistry of the above diamino-alcohol (VIa) and of its derivatives, VIIa and VIII, was determined on the basis of the following evidences :

1) The 19-methyl signals in the NMR spectra of the N-dimethyl compounds (VIIa) and its O-acetate (VIIb), m.p. 215~218°, $[\alpha]_D$ +18° ($CHCl_3$), occurred in lower field (8.97 and 9.02τ, respectively) than the standard region (9.1~9.2τ), indicating the 4-hydroxy grouping placed in the 1,3-diaxial relation with respect to the 19-methyl group.[8] This permits the 4β,5α-assignment to VIIa and VIIb. Also the spin-spin coupling constants (3 c.p.s.) between C_4-hydrogen and C_3-, C_5-hydrogens in the compound (VIIa) supported the 3β,4β,5α-assignment[9] (Fig. 7).

2) The β-orientation of the 3-amino grouping in diamino-ketone (VIII) was first suggested by the non-identity with 3α,20α-bisdimethylamino-5α-pregnan-4-one (IX), reported already in Part II[5] of this series, since the former ketone, as well as the latter, demonstrated a negative Cotton effect which could be ascribed to 4-keto-5α-steroid, as shown in Fig. 9. In accordance with the octant rule, the smaller amplitude with the diamino-ketone (VIII) than IX supported the equatorial orientation for the 3-amino grouping.*9 Moreover, the ultraviolet spectrum of VIII showed a carbonyl absorption band at 284 mμ (ε 96), whereas it occurred at 294 mμ (ε 47)[5] in the spectrum

*9 It may be pertinent in this connection to note that a few steroidal 2- and 3-ketones carrying a methoxyl or an acetoxyl group at α-position have been reported to violate seemingly the "octant rule." This unusual observation has been ascribed to a distortion of ring-A from the normal chair form to a twist conformation. See K. L. Williamson, W. S. Johnson : J. Am. Chem. Soc., **83**, 4623 (1961); S. S. Stradling, D. S. Tarbell : J. Org. Chem., **29**, 1170 (1964).

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

9) M. Karplus : J. Chem. Phys., **30**, 11 (1959); *Idem* : J. Am. Chem. Soc., **85**, 2870 (1963).

of Ⅸ. This observation is also consistent with the proposed β-configuration for the former.*[10]

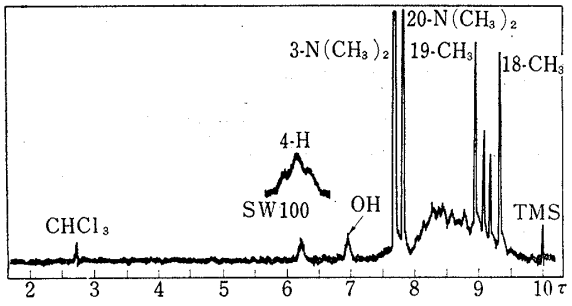

Fig. 7. Nuclear Magnetic Resonance Spectrum of the N-Methylated Diamino-alcohol (Ⅶa)

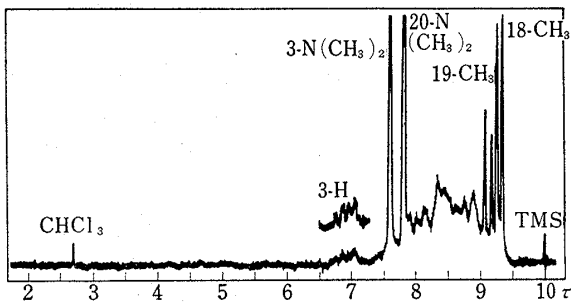

Fig. 8. Nuclear Magnetic Resonance Spectrum of the Diamino-ketone (Ⅷ)

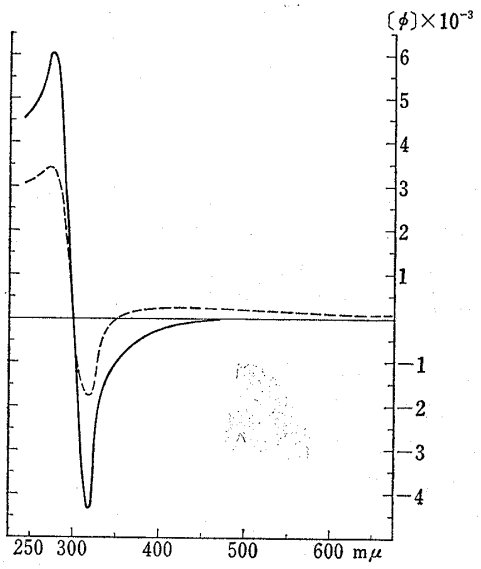

Fig. 9. Optical Rotatory Dispersion Curves (in Methanol)

------- the diamino-ketone (Ⅷ)
——— 3α,20α-bisdimethylamino-5α-pregnan-4-one (Ⅸ)

3) The NMR spectrum of the diamino-ketone (Ⅷ) exhibited a quartet arising from the proton (6.90τ) which is geminal to the 3-dimethylamino group (Fig. 8). The observed coupling constants (6.5 and 11 c.p.s.) suggested the axial orientation of the 3-hydrogen,[10] hence β-configuration of the 3-amino grouping.

Eventually, we attempted to prepare the dimethylamino compound (Ⅶa) from 3α,20α-bis dimethylamino-5α-pregnan-4-one (Ⅸ). Base or acid treatment of Ⅸ caused the epimerization and afforded the 3β-isomer (Ⅷ), whose formation was checked by thin-layer chromatography.*[11] Subsequent reduction of the product with sodium borohydride*[12] led to 3β,20α-bisdimethylamino-4β-hydroxy-5α-pregnane (Ⅶa), $C_{25}H_{46}ON_2$, m.p. 180~181°, $[\alpha]_D$ +40° ($CHCl_3$). This compound was found to be quite identical in every respect with the above N-methylated diamino-alcohol (Ⅶa) obtained from pachystermine-B.

Therefore, the structure of pachystermine-A and -B are now represented by the expanded formulas (Ⅰ′) and (Ⅱ′), respectively. The configuration at 3, 4, and 5-positions in Ⅱ′ must be the same as the above amino-alcohol (Ⅶa), since no configurational change was expected to occur during the alkali degradation. This was supported by the chemical shift of the 19-methyl signal (8.93τ) and the half-band width (about 7 c.p.s.) of the C_4-hydrogen signal in the NMR spectrum of pachystermine-B (Fig. 4), indicating

*10 As to effects of polar α-substituents on the carbonyl $n\rightarrow\pi^*$ absorption, see J.C.D. Brand, A.I. Scott: "Elucidation of Structures by Physical and Chemical Methods" (ed. K.W. Bentley), Part I, 78 (1963), Interscience Publishers, New York; E.M. Kosower, G.S. Wu, T.S. Sorensen: J. Am. Chem. Soc., **83**, 3150 (1961).

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

*12 In this connection it should be mentioned that 3α,20α-bisdimethylamino-5α-pregnan-4-one (Ⅸ) is not practically reduced with sodium borohydride. See ref. 5).

10) N.S. Bhacca, D.H. Williams: "Applications of NMR Spectroscopy in Org. Chem.," 145 (1964), Holden-Day, Inc., San Francisco.

1,3-diaxial correlation between the 19-methyl and the 4-hydroxyl group. With pachystermine-A (Fig. 2), the NMR signal attributable to C_3-hydrogen (5.67τ) splitted into a quartet with the observed coupling constants of 8 and 11 c.p.s., in accord with the 3β-assignment for the amino grouping.[10]

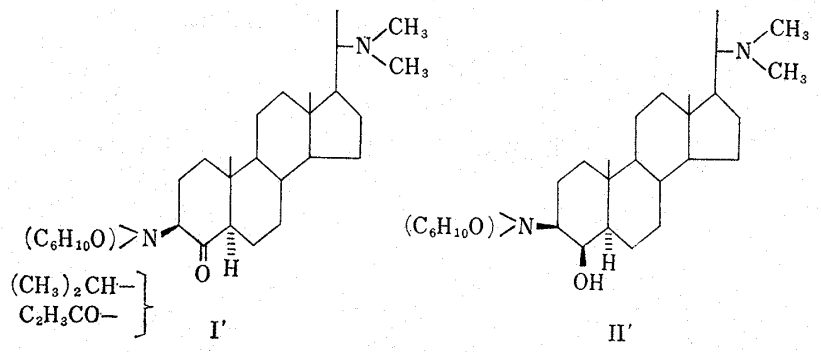

Chart 3.

We are now in a position to discuss the constitution of the side chain moiety ($C_6H_{10}O$) of the alkaloids. As mentioned previously, the presence of isopropyl group was deduced from the inspection of NMR spectra. The remaining $-C_3H_3O-$moiety, containing a carbonyl group, appeared to form a ring system since no olefinic proton was observed in the NMR spectra.

When oxidized with chromium trioxide in pyridine, pachystermine-A (I) afforded a neutral N-formyl compound (X), $C_{29}H_{46}O_3N_2 \cdot 1/2H_2O$, m.p. 225~226°, showing an infrared amide band at 1660 cm^{-1} along with two carbonyl bands (1740 and 1715 cm^{-1}) which were found in the spectrum of the original pachystermine-A. The formation of the new N-formyl group was evidently demonstrated by the NMR signal at 1.92τ associated with the proton of formyl group and the amide N-methyl signal at 7.25τ (3H). Also these spectral data and the empirical formula of the compound (X) indicated that the oxidation had taken place only at the 20-dimethylamino group and no chemical change had resulted at the other molecular part of pachystermine-A. Neutrality of the N-formyl compound (X), then, means the nitrogen at 3-position is also forming a neutral amide grouping.

These observations led us to assume that the C_3H_3O ($=C_2H_3CO$) portion and the nitrogen at the 3-position form a four-membered lactam ring (β-lactam). The infrared bands at 1735 cm^{-1} of pachystermine-A (I) and O-acetylpachystermine-B, and at 1740 cm^{-1} of the N-formyl compound (X) also support the assumption.[11] With pachystermine-B (II), the infrared band at 1715 cm^{-1} may be explainable in cosnideration of the bathochromic shift owing to the hydrogen bonding between the β-lactam carbonyl and the 4-hydroxyl group.

The existence of β-lactam ring system in pachystermine-A and -B was confirmed by the following reactions.

The reaction of lithium aluminum hydride on the N-substituted β-lactams has been known to cause the ring opening with the formation of amino-carbinols.[12] In accord with this generalization, lithium aluminum hydride reduction of pachystermine-A (I), -B (II), and the N-formyl compound (X) afforded the same product, pachystermine-diol (XIa), m.p. 197~198°, $[\alpha]_D$ −3° ($CHCl_3$), which gave analytical results in agreement with the formula $C_{29}H_{54}O_2N_2$. On subsequent acetylation, it gave an O,N-diacetate

11) L. J. Bellamy: "The Infrared Spectra of Complex Molecules," 214 (1958), John Wiley & Sons, Inc., New York.

12) M. E. Speeter, W. H. Maroney: J. Am. Chem. Soc., **76**, 5810 (1954).

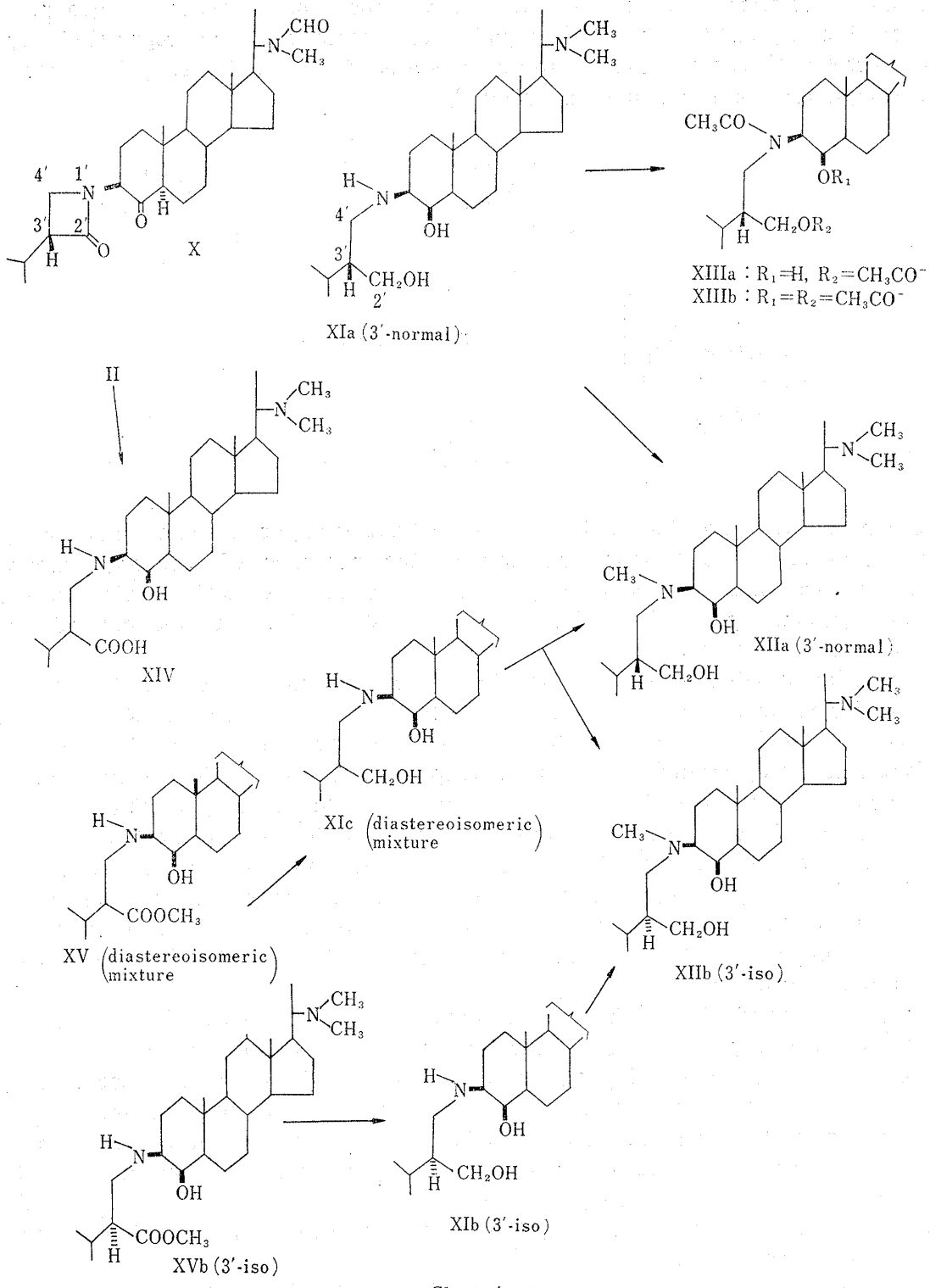

Chart 4.

(XIIIa), $C_{33}H_{58}O_4N_2$, m.p. 187~190°, $[\alpha]_D$ +22° ($CHCl_3$), and an O,O,N-triacetate (XIIIb), $C_{35}H_{60}O_5N_2$, m.p. 136~141°. The infrared spectrum of the O,N-diacetate (XIIIa) demonstrated clearly a hydroxyl band at 3250cm^{-1} along with absorption bands for an O-acetyl (1735 and 1240 cm^{-1}) and a tertiary N-acetyl group (1620 cm^{-1}), while the O,O,N-triacetate (XIIIb) very strong ester bands (1735 and 1240 cm^{-1}) and a tertiary amide band (1635 cm^{-1}). The NMR spectrum of the former (XIIIa) exhibited a broad signal at 6.10τ for a proton adjacent to the hydroxyl group and a doublet at 5.91τ (2H, J=5 c.p.s.) which was

assigned to two protons geminal to the O-acetyl group. This finding was indicative of that the acetylated alcohol group in the O,N-diacetate (XIIIa) is the primary one.

N-methylation of pachystermine-diol (XIa) by the formalin-sodium borohydride procedure yielded an N-methyl compound (XIIa), $C_{30}H_{56}O_2N_2$, m.p. 196~197°, $[\alpha]_D$ −11° ($CHCl_3$), showing an NMR signal at 7.70τ for the newly introduced N-methyl group.

On the other hand, of interest was the observation that pachystermine-B (II) underwent readily base hydrolysis to give amino-acid (XIV), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 and 1570, which was found to be a mixture of the diastereoisomeric pair formed probably by partial racemization at the carbon atom adjacent to the carboxyl group. Treatment of the amino-acid hydrochloride with diazomethane in ether-methanol gave rise to the corresponding methyl ester (XV, mixture of diastereoisomers), infrared spectrum of which demonstrated the expected absorption bands at 1725 and 1165 cm^{-1} for the $-COOCH_3$ grouping.

Lithium aluminum hydride reduction of the amino-acid (XIV) and of the ester (XV) afforded a crystalline compound (XIc), whose infrared spectrum in chloroform solution was superimposable on that of pachystermine-diol (XIa), derived directry from pachystermine-A and -B. Upon subsequent N-methylation, the diol compound (XIc) gave an N-methylated mixture, which showed two spots on thin-layer chromatogram in an approximate ratio of one to one. This could be separated by repeated alumina chromatography into each diastereoisomer: *i.e.* (a) m.p. 196~197° (XIIa), $C_{30}H_{56}O_2N_2$, $[\alpha]_D$ −9° ($CHCl_3$), and (b) m.p. 171~172° (XIIb), $C_{30}H_{56}O_2N_2$, $[\alpha]_D$ +40° ($CHCl_3$).*[13] The former was found to be identical with N-methylpachystermine-diol (XIIa), described previously, by mixed melting point determination, thin-layer chromatography, and infrared (KBr) comparison.

When the above methyl ester mixture (XV) was recrystallized repeatedly from aqueous acetone, a fairly pure ester (XVb), m.p. 133~137°, was obtained. This was subjected to lithium aluminum hydride reduction to yield a diol (XIb), $C_{29}H_{54}O_2N_2$, m.p. 223~224°, $[\alpha]_D$ +19° ($CHCl_3$), which, on N-methylation, led to the N-methyl compound (XIIb), $C_{30}H_{56}O_2N_2$, m.p. 171~174°, $[\alpha]_D$ +40° ($CHCl_3$), identified in all respects with the above N-methylated diol (XIIb) melting at 171~172°. Therefore, these compounds (XVb, XIb, XIIb) would represent 3′-iso series. It must be noted herewith that the infrared spectra of XIb and XIIb in chloroform were superimposable upon those of XIa and XIIa, respectively, in support of that these are the diastereoisomeric pairs, although they showed distinct difference in potassium bromide discs.

The occurrence of racemization in the amino-acid during alkaline hydroysis suggested the location of the isopropyl group at the 3′-position of the β-lactam ring in pachystermine-B (II), not at the 4′-position.

An independent support for the proposed structures, XIIa and XIIb, for diastereoisomeric N-methyl-diols was provided by mass spectrometric investigation. In the preceding paper,*[1] we reported that one of the characteristic peaks of 4-oxygenated 3,20-diamino-5α-pregnane type alkaloids carrying a long-chain alkyl group at the 3-amino group is associated with the fragment ion (corresponding to d) generated by the α-cleavage respective to the charged nitrogen in 3-alkylamino grouping. In consistency with this observation, mass spectra of the N-methyl-diols (XIIa and XIIb) exhibited intense peaks at m/e 72 (a, base peak), 170 (c) and 389 (d) along with other characteristic peaks (M^+, M^+-15, and M^+-71)*[1] (Fig. 10) and their patterns were mutually very similar in support of that both are diastereoisomeric.

*[13] N-methylation of the amino-acid (XIV) with formalin-sodium borohydride and the subsequent methylation with diazomethane gave an N-methylated methyl ester as a viscous oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1725 and 1165 ($-COOCH_3$). Upon lithium aluminum hydride reduction and careful alumina chromatography, this substance also gave rise to the N-methylated diols, XIIa and XIIb.

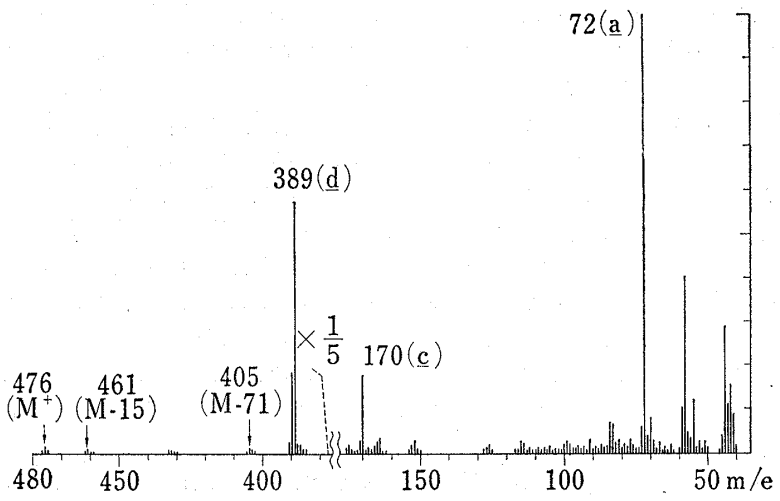

Fig. 10. Mass Spectrum of N-Methylpachystermine-diol (XIIa)

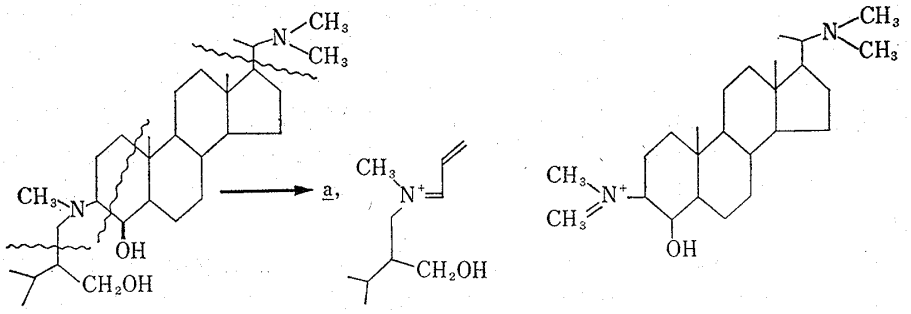

Chart 5.

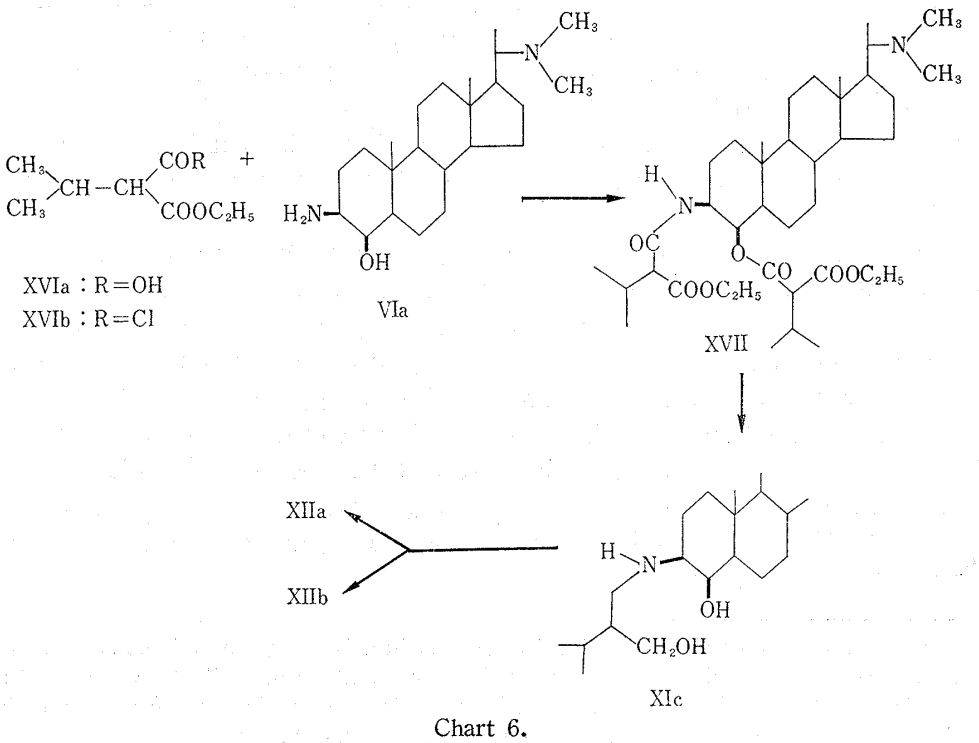

Chart 6.

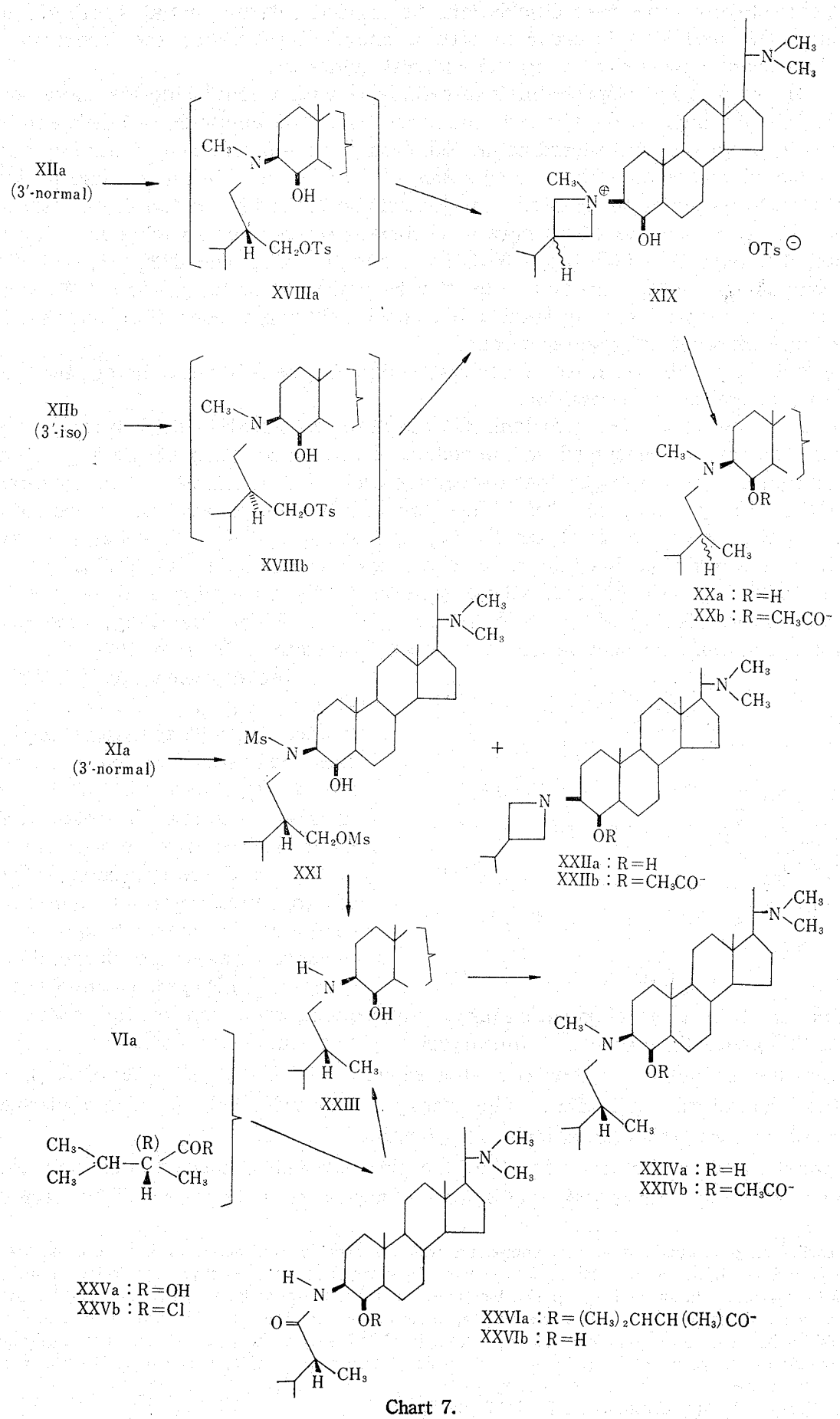

Chart 7.

At this stage of structure elucidation, we carried out the partial synthesis of the compounds (XIIa and XIIb), in order to give a chemical proof for the isopropyl group which had been suggested only by the spectral evidences.

Treatment of ethyl isopropylmalonate (XVIa)[13] with thionyl chloride gave an acid chloride (XVIb) which was allowed to react with 3β-amino-20α-dimethylamino-4β-hydroxy-5α-pregnane (Va) according to Schotten-Baumann method, whereby obtained an O,N-diacyl compound (XVII). Reduction of XVII with lithium aluminum hydride followed by N-methylation afforded a mixture of the desired N-methyl-diols. Separation of this mixture was achieved by careful chromatography over alumina and there were obtained XIIa, m.p. 193~194°, $[\alpha]_D$ −3° ($CHCl_3$), and XIIb, m.p. 171~172°, $[\alpha]_D$ +40° ($CHCl_3$). Both compounds were identified with the N-methylpachystermine-diol (XIIa) and its 3′-epimer (XIIb), respectively, by mixed melting point determinations, thin-layer chromatography, and infrared (KBr) comparisons.

Consequently, the structure of pachystermine-A and -B were determined except for the configuration at 3′-position.

Turning now to the last problem, the absolute configuration of 3′-isopropyl group, our attention was concentrated on the reductive dehydroxylation at the 2′-position.

Treatment of the N-methylpachystermine-diol (XIIa, 3′-normal) with *p*-toluenesulfonyl chloride in pyridine resulted in the formation of a crystalline compound (XIX), m.p. 250~253°, which was analyzed for $C_{30}H_{55}ON_2 \cdot C_7H_7SO_3 \cdot H_2O$. This was then reduced with lithium aluminum hydride to give a deoxy compound (XXa), $C_{30}H_{56}ON_2 \cdot 1/4H_2O$, m.p. 170~173°, $[\alpha]_D$ +36° ($CHCl_3$), whose structure (XXa) was supported by the occurrence of characteristic peaks at m/e 460 (M^+), 389 (d), 154 (e), and 72 (a, base peak) in its mass spectrum. On acetylation, it gave an O-acetate (XXb), m.p. 164~168.°

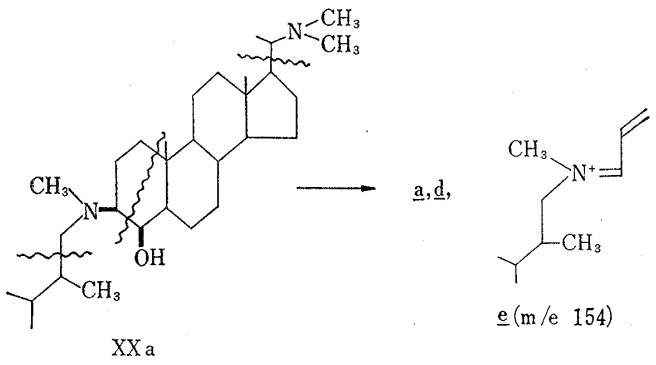

Chart 8.

However, when the 3′-iso N-methyl diol compound (XIIb) was similarly treated with *p*-toluenesulfonyl chloride, there was obtained a mono-tosylate (XIX), m.p. 248~251°, whose infrared spectrum in potassium bromide was found to be superimposable upon that of the specimen (XIX) derived from the 3′-normal N-methyl-diol (XIIa) and the melting point of their mixture showed no depression. In addition, both demonstrated the same Rf value on thin-layer chromatography. Subsequent reduction of the above mono-tosylate (XIX) with lithium aluminum hydride yielded a deoxy compound (XXa), m.p. 155~173° (the product in another run showed m.p. 168~172°), $[\alpha]_D$ +38° ($CHCl_3$), whose O-acetate showed m.p. 169~173°. This deoxy compound (XXa) was also indistinguishable in all respects from the sample (XXa) obtained from XIIa.

It must therefore be concluded that the mono-tosylates, derived from both XIIa and XIIb, may be the same and they would be the azetidinium tosylate (XIX)*14 formed by

*14 At earlier stage of this study, this compound was erroneously considered to be the mono-O-tosylate (XVIIIa and XVIIIb) in view of that it was isolated by extraction with methylene chloride from aqueous Na_2CO_3-alkaline solution and was purified by alumina chromatography from methylene chloride. Although there is no direct proof for the quarternary azetidinium salt structure of this compound since the low solubility in most solvents make unable to take its NMR spectrum, present structural assignment is supported by the result of its reduction with lithium aluminum hydride, which precludes the alternative ether structure.

13) E. J. Corey : J. Am. Chem. Soc., **74**, 5897 (1952).

cyclization of the O-tosylate intermediates (XVIIIa and XVIIIb). This was supported by the infrared (KBr) absorption bands at 1230, 1180, and 1035 cm^{-1}, attributable to a tosylate anion.

Then, the deoxy compound (XXa) produced by lithium aluminum hydride reduction of the mono-tosylate (XIX) is most likely a mixture of the epimeric pair at 3′-position, because both the N-$C_{2'}$ and N-$C_{3'}$ linkage in the azetidinium compound (XIX) may equally undergo the reductive cleavage.[14] In any event, the configuration at 3′-position of the alkaloids (I and II) could not be determined on the basis of the above experiments.

It was then expected that, if the 3-nitrogen atom is protected as a suitable acid amide, formation of the undesired azetidinium compound (XIX) may be prohibited. This was shown to be the case by a sequence of reactions outlined below.

Treatment of pachystermine-diol (XIa) with methanesulfonyl chloride*[15] in pyridine afforded an weakly basic O,N-dimesylate (XXI) together with an azetidine compound (XXIIa), $C_{29}H_{52}ON_2 \cdot 1/2H_2O$, m.p. 212~213°, $[\alpha]_D$ +35° ($CHCl_3$). The reductive elimination of OSO_2CH_3 grouping of the O,N-dimesylate (XXI), with concomitant N-demesylation,[15] was successfully performed with lithium aluminum hydride and the resulting deoxy compound (XXIII) was then N-methylated by formalin-sodium borohydride procedure to give an N-methylated deoxy compound (XXIVa), m.p. 171~172°, $[\alpha]_D$ +28° ($CHCl_3$). The O-acetate (XXIVb) of this compound showed m.p. 165~167° and $[\alpha]_D$ −11° ($CHCl_3$). There was no doubt that the compounds (XXIVa and XXIVb) hold the same absolute configuration at 3′-position as the starting compound (XIa), because, in this case, the presumable intermediate towards the apparent racemization at 3′-position is the azetidine compound (XXIIa), which was found to be unchanged by lithium aluminum hydride reduction.

The infrared spectrum (KBr) of the above N-methylated deoxy compound (XXIVa) demonstrated small but distinct differences in the finger-print region when compared with that of XXa, described previously, although no discrimination could be recognized by mixed melting point determination nor by infrared spectra in chloroform. In contrast, the O-acetate (XXIVa) could not be distinguished in all respects from XXb derived from N-methylpachystermine-diol (XIIa).

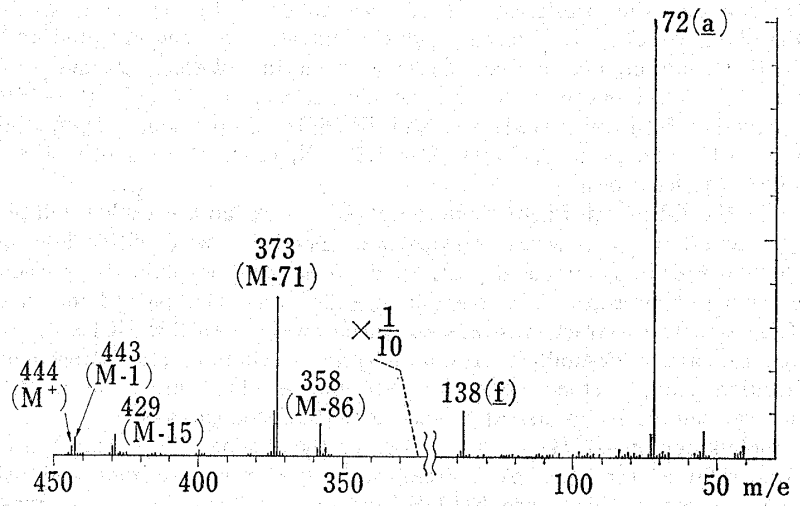

Fig. 11. Mass Spectrum of the Azetidine Compound (XXIIa)

*15 Attempted reaction of XIa with *p*-toluenesulfonyl chloride or *p*-bromobenzenesulfonyl chloride in pyridine resulted in recovery of the starting material. The reason for this failure is not clear at present.

14) A. C. Cope, E. Ciganek, L. J. Fleckenstein, M. A. P. Meisinger: J. Am. Chem. Soc., **82**, 4651 (1960).
15) D. Klamann: Monatsh., **84**, 651 (1953); L. Field, F. A. Grunwald: J. Org. Chem., **16**, 946 (1951).

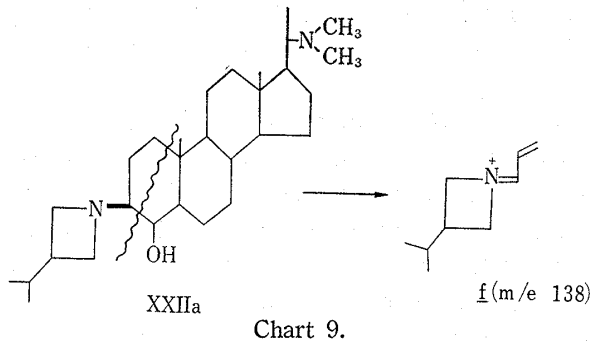

Chart 9.

Finally we synthesized the compound (XXIVa) starting from 3β-amino-20α-dimethylamino-4β-hydroxy-5α-pregnane (Ⅵa) and (−)*R*-2,3-dimethylbutylic acid (XXVa) which had been prepared by Tsuda, *et al.*[16]

The Schotten-Baumann condensation of Ⅵa and (−)*R*-2,3-dimethylbutylyl chloride yielded an O,N-diacyl compound (XXVIa), which, on lithium aluminum hydride reduction followed by N-methylation, gave rise to XXIVa, m.p. 169~172°, $[\alpha]_D$ +27° ($CHCl_3$), whose O-acetate (XXIVb) showed m.p. 167~168°, $[\alpha]_D$ −10° ($CHCl_3$). The infrared spectrum in potassium bromide disc of the synthesized XXIVa was found to be quite identical with that of the sample obtained from the O,N-dimesylate (XXI) and also their mixture gave no melting point depression.

As to the structure of the azetidine compound (XXIIa), first suggestion was provided by its resistance to N-methylation and by the formation of O-acetate, m.p. 216~217°, implying that the amino group at 3-position is most likely a cyclic tertiary one. The NMR spectrum of the compound (XXIIa) exhibited signals arising from five C-methyl groups : *i.e.* two tertiary C-methyls at 8.99 and 9.35τ, one secondary C-methyl at 9.15τ (doublet, J=6 c.p.s.), and two secondary C-methyls at 9.19τ (6H, doublet, J=6 c.p.s.).

A strong support for the proposed structure (XXIIa) was forthcoming when the mass spectrum demonstrated no peak due to a fragment ion (d) associated with the α-cleavage in the 3-N-alkyl grouping, but the expected intence peak at m/e 138 which was visualized in the formula f (Fig. 11).

In conclusion, the complete structure of pachystermine-A and -B are unequivocally assigned to Ⅰ and Ⅱ, respectively, based on evidences so far presented.

Experimental*16

Sodium Borohydride Reduction of Pachystermine-A (Ⅰ)——To a solution of pachystermine-A (Ⅰ) (1.10 g.) in MeOH (40 ml.) and CH_2Cl_2 (20 ml.) was added sodium borohydride (1.0 g.) in small portions and the mixture was stirred at room temperature for 3 hr. After evaporation of the solvent under reduced pressure, water was added to the residue and the precipitated solid was collected by filtration, washed with water, and dried. This was dissolved in CH_2Cl_2, filtered, and the filtrate was concentrated to small volume. On addition of acetone to this solution, the product (Ⅱ) crystallized in colorless prisms (1.06 g.), m.p. 253~260°. Recrystallizations from CH_2Cl_2–acetone gave a pure sample, m.p. 256~259°, $[\alpha]_D^{20}$ −18°(c=1.0), which was identified with pachystermine-B (Ⅱ) by mixed m.p. and IR (KBr) comparison. *Anal.* Calcd. for $C_{29}H_{50}O_2N_2$: C, 75.93; H, 10.99; N, 6.11. Found : C, 75.98; H, 11.08; N, 6.09. MS (m/e) : 458 (M^+), 443 (M^+-CH_3), 387 ($M^+-C_4H_9N$), and 72 (a, base peak).

Chromium Trioxide Oxidation of Pachystermine-B (Ⅱ)——To an ice-cooled solution of pachystermine-B (Ⅱ) (50 mg.) in acetic acid (2 ml.) and water (2 drops) was added dropwise with vigorous stirring a solution of chromium trioxide (100 mg.) in acetic acid (1 ml.) and water (2 drops), and the stirring was continued for additional 40 min. at room temperature. The reaction mixture was then poured into a dil. aqueous Na_2CO_3 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 crystalline residue (40 mg.). Alumina chromatography (0.7×2.0 cm.) from ether and recrystallization from acetone gave pachystermine-A (Ⅰ) (25 mg.), m.p. 218~224°, which was identified with the natural sample by mixed m.p. and IR ($CHCl_3$) comparison.

Acetylation of Pachystermine-B (Ⅱ)——A mixture of the alkaloid (Ⅱ) (100 mg.), acetic anhydride (2 ml.), and pyridine (2 ml.) was refluxed for 5 hr. After removal of the excess reagent and solvent *in vacuo*, the residue was diluted with water, basified with NH_4OH, and extracted with CH_2Cl_2. Washing of the extract with 3% HCl and dil. Na_2CO_3, drying, and evaporation gave a crystalline residue (90 mg.) which was recrystallized from acetone to give the O-acetate (Ⅱ, 4-OAc) (50 mg.), m.p. 246~247°, $[\alpha]_D^{15}$ −10°(c=1.0).

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

16) K. Sakai, K. Tsuda : This Bulletin, **11**, 650 (1963).

Anal. Calcd. for $C_{31}H_{52}O_3N_2 \cdot \frac{1}{4}H_2O$: C, 73.69; H, 10.47; N, 5.55. Found: C, 73.79, 73.77, 73.38; H, 10.50, 10.56, 10.77; N, 5.50. NMR (τ): 4.78 (1H, broad, CH-OAc), 7.85 (6H, N-$(CH_3)_2$), 7.93 (3H, $OCOCH_3$), 8.90~9.15 (6H, three peaks, isopropyl), 9.15 (3H, doublet, J=6.5 c.p.s.; *sec* CH_3), 9.00, and 9.37 (6H, two *tert* CH_3). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (β-lactam and $OCOCH_3$), and 1240 ($OCOCH_3$).

Treatment of Pachystermine-A (I) with 5% KOH-EtOH——To a solution of the alkaloid (I) (150 mg.) in 95% ethanol (5 ml.) was added 10% KOH-EtOH (5 ml.) and the mixture was stirred at room temperature for 10 hr. and then allowed to stand for 3 days. The reaction mixture was diluted with large quantity of water and the product was taken up in CH_2Cl_2. After washing with 3% HCl and dil. Na_2CO_3, the solution was dried over K_2CO_3 and evaporated to give a weakly basic product (45 mg.) which was crystallized from acetone in colorless leaves (25 mg.), m.p. 178~185°. Further recrystallizations from acetone-CH_2Cl_2 gave a pure diosphenol (III), m.p. 188~191°. The identity was confirmed by mixed m.p. and IR (KBr) comparison with a synthesized specimen (III). *Anal.* Calcd. for $C_{23}H_{37}O_2N$: C, 76.83; H, 10.37; N, 3.90. Found: C, 77.13; H, 10.62; N, 3.82. UV λ_{max}^{EtOH} mμ (ε): 279 (11,500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1670, 1640, 1390, and 1170 (-CO-C(OH)=C).

Wolff-Kishner Reduction (Huang-Minlon Modification) of Pachystermine-A (I)——A mixture of pachystermine-A (I) (380 mg.), 80% hydrazine hydrate (3 ml.), diethylene glycol (5 ml.), and abs. EtOH (15 ml.) was heated on a boiling water bath until the most of crystals dissolved. To this mixture was added potassium hydroxide pellets (2 g.) and refluxed for 30 min. The majority of EtOH was then evaporated by heating the mixture in an oil bath (~150°) and the residual mixture was heated for 2.5 hr. at 180~190° (bath temperature) (striking foaming, due to the insoluble substance deposited during the reaction, made unable to raise the bath temperature above 190°). After cooling, the mixture was diluted with water and extracted thoroughly with $CHCl_3$. The $CHCl_3$ extract was shaken with 3% HCl in order to separate the weakly basic and the strongly basic products.

The weak base fraction (ca. 60 mg.), obtained from the $CHCl_3$ phase, was chromatographed over alumina (4 g.) in benzene. Elution with benzene followed by crystallization from acetone afforded a crystalline substance (8 mg.), m.p. 110~130°, which was again chromatographed on alumina (1 g.) and recrystallized from acetone to give colorless prisms (3 mg.), m.p. 134~138°. This compound was identified with 20α-dimethylamino-5α-pregnane (IV), synthesized previously, by mixed m.p. and IR (KBr) comparison. The next fraction eluted with ether-benzene (1:4) showed single spot on TLC.*11 Trituration with acetone of this fraction afforded a small amount of 20α-dimethylamino-5α-pregnan-4-one (Va).

The strong basic fraction (ca. 230 mg.), obtained from the acidic phase by usual working up, was treated with acetone to give crystals (63 mg.), m.p. 140~168°, which were also chromatographed on alumina (3 g.). Elution with benzene and ether-benzene, followed by recrystallization, gave the hydrazoneisopropylidene compound (Vb) (42 mg.) as colorless prisms, m.p. 164~167°. The mother liquor was also purified by way of alumina chromatography (7 g.) and additional crop of Vb (28 mg.), m.p. 161~164°, was obtained. *Anal.* Calcd. for $C_{26}H_{45}N_3$: C, 78.14; H, 11.35; N, 10.51. Found: C, 78.44; H, 11.06; N, 10.40. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640 (C=N). NMR (τ): 7.82 (6H, $N(CH_3)_2$), 8.00, 8.18 (6H, $(CH_3)_2C=C$), 9.12 (3H, doublet, J=6 c.p.s.; *sec* CH_3), 9.22, and 9.35 (6H, two *tert* CH_3).

Acid Hydrolysis of the Hydrazoneisopropylidene Compound (Vb)——The compound (Vb) (28 mg.) was dissolved in 10% sulfuric acid (5 ml.) and left at room temperature with occasional warming for 3 hr. After basification by addition of saturated aqueous Na_2CO_3, the product was taken up in CH_2Cl_2, dried, and evaporated. The residue was again dissolved in 3% HCl, washed with ether, and then extracted with CH_2Cl_2. The extract was dried over K_2CO_3 and evaporated to afford a crystalline residue (26 mg.), which was chromatographed over alumina (2 g.). The eluates with benzene and benzene-ether (up to 50%) were combined (21 mg.) and recrystallized from *n*-hexane and then from aqueous acetone to yield the aminoketone (Va) (9 mg.), m.p. 164~166°. This was identified with the synthetic sample of 20α-dimethylamino-5α-pregnan-4-one (Va) by mixed m.p. and IR (KBr) comparison. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (ketone). NMR (τ): 7.83 (6H, N-$(CH_3)_2$), 9.13 (3H, doublet, J=6 c.p.s.; *sec* CH_3), 9.25 and 9.34 (6H, two *tert* CH_3). ORD (MeOH, c=0.5): peak, $[\phi]_{272}$ +5400°; trough, $[\phi]_{308}$ −3930°.

Wolff-Kishner Reduction (Nagata modification) of Pachystermine-A (I)——The alkaloid (I) (310 mg.) was dissolved in abs. EtOH (10 ml.) containing acetic acid (0.05 ml.). To this solution were added diethylene glycol (10 ml.), hydrazine hydrochloride (0.47 g.), and 80% hydrazine hydrate (2.5 ml.) and the mixture was heated in an oil bath (130~140°) for 30 min. to give a homogeneous solution, to which KOH pellets (2 g.) were added. The temperature of oil bath was then raised gradually to 210° with concomitant evaporation of low boiling solvents and the reaction was continued at this temperature for 2 hr. After cooling, the reaction mixture was treated in the usual manner and the products were separated into a weakly basic and a strongly basic fraction.

The weak base fraction (180 mg.) was purified by alumina chromatography (1 g.) from benzene and crystallized from acetone to afford 20α-dimethylamino-5α-pregnane (IV) (145 mg.), m.p. 131~137°. Two recrystallizations from acetone gave a pure sample in colorless plates (120 mg.), m.p. 137~139°, $[\alpha]_D^{22}$ +22° (c=1.0), identified by mixed m.p. determination and IR (KBr) comparison with an authetic specimen. *Anal.* Calcd. for $C_{23}H_{41}N$: C, 83.31; H, 12.41; N, 4.22. Found: C, 83.02; H, 12.47; N, 4.22. MS (m/e): 331 (M^+), 316 (M^+-15), and 72 (a, base peak).

The strong base fraction (52 mg.), which hardly crystallized, was treated with warm 10% H_2SO_4 for 5 hr. The product, isolated with CH_2Cl_2 and NH_4OH, was partitioned between CH_2Cl_2 and 2% HCl. Evaporation of the CH_2Cl_2 phase afforded a weakly basic product (20 mg.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (ketone), which was chromatographed on alumina (1 g.). The eluates (9 mg.) with benzene (10 ml.) and ether–benzene (1:9, 10 ml.) were then combined and submitted to vaccum sublimation (0.07 mm. Hg) at 145~160° (bath temperature). Crystallization of the sublimate from aqueous acetone gave a samll amount of amino-ketone (Va), m.p. 140~150°, identified with the synthetic 20α-dimethylamino-5α-pregnan-4-one (Va) by IR ($CHCl_3$) comparison and TLC.

On the other hand, attempts to purify the strongly basic product (27 mg.), obtained from the 2% HCl layer, failed to give satisfactory result.

Synthesis of 20α-Dimethylamino-5α-pregnan-4-one (Va)——A solution of the diosphenol (Ⅲ) (120 mg.) in acetic acid (10 ml.) and conc. HI (0.3 ml.) was refluxed for 30 min. After the reaction mixture was diluted with 3% HCl and washed thoroughly with ether, it was made basic with NH_4OH, extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated to leave a pale yellow residue (100 mg.). Crystallization from acetone gave the amino-ketone (Va) (50 mg.), m.p. 160~164°. Further recrystallizations from the same solvent afforded a pure sample, m.p. 165~167°, $[\alpha]_D^{15}$ +42° (c=1.0). *Anal.* Calcd. for $C_{23}H_{39}ON$: C, 79.74; H, 11.36; N, 4.05. Found: C, 79.77; H, 11.35; N, 3.90. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1705 (ketone).

Alkali Degradation of Pachystermine-B (II) under the Condition of Wolff-Kishner Reaction (Formation of 3β-Amino-20α-dimethylamino-4β-hydroxy-5α-pregnane (VIa))——To a solution of the alkaloid (Ⅱ) (320 mg.), acetic acid (0.05 ml.), abs. EtOH (5 ml.), and hydrazine hydrochloride (450 mg.) in diethylene glycol (10 ml.) was added 80% hydrazine hydrate (2.5 ml.) and the mixture was heated on an oil bath at 130~140° (bath temperature) for 1 hr. in order to dissolve the majority of deposited solid. Potassium hydroxide pellets (2 g.) were then added to this mixture and heated at 180~200° for 1 hr. and at 210~215° for 2 hr. After cooling, the reaction mixture was diluted with water and extracted thoroughly with $CHCl_3$. The combined extracts were concentrated (ca. 100 ml.) and shaken with 5% acetic acid. The $CHCl_3$ phase, on evaporation, gave only a trace of substance (5 mg.). The acidic phase was made alkaline with NH_4OH, extracted with much $CHCl_3$, dried over K_2CO_3, and evaporated to give a crystalline residue (200 mg.). This was again dissolved in $CHCl_3$–CH_2Cl_2 and the insoluble material was removed by filtration, and the filtered solution was concentrated to a small volume. On standing, the amino-alcohol (Ⅵa) crystallized in needles (160 mg.), which were collected by suction and washed with ether–CH_2Cl_2, m.p. 199~204°.

O,N-Diacetate (VIb): The above amino-alcohol (crude, 39 mg.) was acetylated by heating with acetic anhydride (0.7 ml.) and pyridine (0.7 ml.) on a boiling water bath for 1.5 hr. The product (45 mg.), obtained by the usual working up, was recrystallized from acetone–CH_2Cl_2 to give the O,N-diacetate (Ⅵa) as colorless plates (30 mg.), m.p. 237~241°. *Anal.* Calcd. for $C_{27}H_{46}O_3N_2 \cdot \frac{1}{2}2H_2O$: C, 71.17; H, 10.41; N, 6.15. Found: C, 71.59, 71.21; H, 10.25, 10.28; N, 6.31, 6.04. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (NH), 1670, 1510 (NH–Ac), 1735, and 1240 (O–Ac). NMR (τ): 4.47 (1H, doublet, J=9 c.p.s.; NH–CH), 4.83 (1H, broad doublet, CH–OAc), 7.83 (6H, N–$(CH_3)_2$), 7.90, 8.09 (6H, N–$COCH_3$ and O–$COCH_3$), 9.03, 9.38 (6H, two *tert* CH_3), and 9.15 (3H, doublet, J=6 c.p.s.; *sec* CH_3).

N-Methylation of VIa (3β, 20α-Bisdimethylamino-4β-hydroxy-5α-pregnane (VIIa))——The compound (Ⅵa) (crude, 380 mg.) was heated with formic acid (2 ml.) and 37% formalin (2 ml.) for 5 hr. on a boiling water bath. The products (415 mg.), obtained in the usual way, was crystallized from acetone–CH_2Cl_2 to give the N-dimethyl compounds (Ⅶa) (285 mg.), needles, m.p. 162~169°. Further recrystallizations from the same solvents yielded a pure sample as colorless prisms, m.p. 180~181°, $[\alpha]_D^{18}$ +50° (c=1.2). Additional crop (65 mg.), m.p. 168~172°, was obtained from the mother liquor. *Anal.* Calcd. for $C_{25}H_{46}ON_2$: C, 76.86; H, 11.87; N, 7.17. Found: C, 76.72; H, 11.87; N, 7.11. NMR (τ): 6.22 (1H, triplet, J=3 c.p.s.; CH–OH), 7.72, 7.85 (12H, two N–$(CH_3)_2$), 9.15 (3H, doublet, J=6 c.p.s., *sec* CH_3), 8.97, and 9.35 (6H, two *tert* CH_3).

O-Acetate (VIIb): The compound (Ⅶa) (47 mg.) was treated with acetic anhydride (1 ml.) and pyridine (1 ml.) at room temperature for 5 days. The products was purified by alumina chromatography followed by recrystallization from acetone–CH_2Cl_2 to afford the O-acetate (Ⅶb) in prisms (45 mg.), m.p. 215~218°, $[\alpha]_D^{18}$ +18° (c=1.02). *Anal.* Calcd. for $C_{27}H_{48}O_2N_2$: C, 74.95; H, 11.18; N, 6.48. Found: C, 74.93; H, 11.27; N, 6.37. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1250 (O–Ac). NMR (τ): 4.64 (1H, broad, CH–OAc), 7.73, 7.85 (12H, two N–$(CH_3)_2$), 7.95 (3H, O–Ac), 9.02, 9.36 (6H, two *tert* CH_3), 9.15 (3H, doublet, J=6 c.p.s.; *sec* CH_3).

Chromium Trioxide Oxidation of the N-Dimethyl Compound (VIIa) (3β,20α-Bisdimethylamino-5α-pregnan-4-one (VIII))——To a solution of the compound (Ⅶa) (216 mg.) in acetic acid (4 ml.) and water (0.7 ml.) was added dropwise with mechanical agitation a solution of chromium trioxide (300 mg.) in acetic acid (3 ml.) and water (0.5 ml.) at room temperature and the reaction continued for 3.5 hr. The reaction mixture was poured into an ice-cooled Na_2CO_3 solution and the product was taken up in CH_2Cl_2, dried over K_2CO_3, and evaporated to leave a crystalline residue (200 mg.), which was chromatographed over alumina (5 g.). Elution with benzene and ether–benzene (up to 3:7), followed by recrystallization from aqueous acetone, gave the ketone (Ⅷ) (105 mg.) in colorless prisms, m.p. 118~123°. After further recrystallization from acetone, the pure sample showed m.p. 120~125°, $[\alpha]_D^{18}$ +47° (c=1.4). *Anal.* Calcd. for $C_{25}H_{44}ON_2$: C,

77.26; H, 11.41; N, 7.21. Found: C, 76.96; H, 11.43; N, 7.31. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (ketone). UV λ_{max}^{EtOH} mμ (ε): 284 (96). NMR (τ): 6.90 (1H, quartet, J=6.5 and 11 c.p.s.; N-CH-CO), 7.61, 7.83 (12H, two N-$(CH_3)_2$), 9.29, 9.35 (6H, two *tert* CH_3), and 9.15 (3H, doublet, J=6 c.p.s.; *sec* CH_3). ORD (MeOH, c=0.5): peak, $[\phi]_{274}$ +3440°; trough, $[\phi]_{318}$ −1790°.

Wolff-Kishner Reduction (Nagata Modification) of the Ketone (VIII)——A mixture of the ketone (Ⅷ) (92 mg.), hydrazine dihydrochloride (200 mg.), diethylene glycol (3 ml.), abs. EtOH (1 ml.), and 80% hydrazine hydrate (1 ml.) was refluxed on a water bath until most of the crystals were dissolved (30 min.). Potassium hydroxide pellets (0.5 g.) were then added to the mixture and it was heated on an oil bath in a flask fitted with an air condensor. The bath temperature was raised gradually and was kept at 210~220° (bath temp.) for 2 hr. After cooling, the reaction mixture was worked up as usual and the product was partitioned between CH_2Cl_2 and 3% HCl solution to give a weakly basic and a strongly basic fraction. Crystallization of the former fraction (64 mg.) from acetone afforded crystals (45 mg.) melting at 115~125°, which were chromatographed over alumina (1 g.) and eluted with benzene and benzene-ether (9:1). Recrystallization of the eluate from acetone-CH_2Cl_2 gave 20α-dimethylamino-5α-pregnane (Ⅳ) (27 mg.), m.p. 135~137°, identified with the synthetic sample (Ⅳ) by mixed m.p. and IR (KBr) and NMR comparisons. MS (m/e): 331 (M^+), 316 (M^+-15), and 72 (a, base peak).

The strongly basic fraction (16 mg.), on the other hand was hydrolyzed with 10% sulfuric acid at room temperature for 4 days. The product, isolated in the usual way, was dissolved in CH_2Cl_2, washed with 3% HCl solution, dried over K_2CO_3, and evaporated to leave a crystalline residue (8 mg.), which was sublimed at 160°/0.07 mm. Hg. Recrystallization of the sublimate (6 mg.) from aqueous acetone gave the amino-ketone (Va), m.p. 145~150°, whose IR ($CHCl_3$) spectrum was almost identical with that of the synthetic sample (Va).

Preparation of 3β,20α-Bisdimethylamino-4β-hydroxy-5α-pregnane (VIIa) from 3α,20α-Bisdimethylamino-5α-pregnan-4-one (IX)——A solution of the 3α-dimethylamino-4-keto compound (Ⅸ) (135 mg.), derived from pachysandrine-A, in 1% KOH-MeOH (20 ml.) was refluxed for 2 hr. On TLC,*11 the reaction mixture showed two spots which were corresponding to the starting material (Ⅸ) and its 3-epimer (Ⅷ), respectively, in the approximate ratio of 1:2. After cooling, sodium borohydride (30 mg.) was added to the reaction mixture at room temperature and let it stand overnight. The mixture was again refluxed for 2 hr. and thereafter reduced with sodium borohydride (20 mg.) as above. Usual working up gave a crystalline product (130 mg.) which was recrystallized from acetone to give the 3β-dimethylamino-4β-hydroxy compound (VIIa) (75 mg.), m.p. 180~181°, as colorless prisms. This was shown to be identical with the dimethyl compound (VIIa), derived from pachystermine-B, by mixed m.p. and IR (KBr) comparison. $[\alpha]_D^{15}$ +40° (c=1.0). *Anal.* Calcd. for $C_{25}H_{46}ON_2$: C, 76.86; H, 11.87; N, 7.17. Found: C, 76.97; H, 11.78; N, 6.97.

In another run, the compound (Ⅸ) (100 mg.) was refluxed in 95% EtOH (6 ml.) containing conc. HCl (6 drops) for 3 hr. The product isolated was chromatographed on alumina (6 g.) and eluted with benzene (120 ml.) and ether-benzene mixture (5:95, 20 ml.; 1:9, 40 ml.; 1:1, 50 ml.). The eluate (45 mg.) with ether-benzene mixture was then reduced with sodium borohydride as usual. Vacuum sublimation of the product (35 mg.), followed by recrystallization from acetone, gave the amino-alcohol (VIIa) (7 mg.), m.p. 173~176°, identified by mixed m.p. and IR ($CHCl_3$) comparison.

Chromium Trioxide-Pyridine Oxidation of Pachystermine-A (I)——A solution of the alkaloid (I) (150 mg.) in pyridine (3 ml.) was added to a mixture of chromium trioxide (300 mg.) and pyridine (5 ml.), and the mixture was allowed to stand overnight at room temperature. The reaction mixture was poured into an aqueous Na_2CO_3 solution and extracted with ether. The etherial extract was washed successively with 3% HCl and dil. Na_2CO_3, dried over K_2CO_3, and evaporated. Recrystallization of the crystalline residue afforded the N-formyl compound (X) (65 mg.) melting at about 200°. Further recrystallizations from the same solvent gave a pure sample, m.p. 225~226°, $[\alpha]_D^{22}$ +12° (c=1.0). *Anal.* Calcd. for $C_{29}H_{46}O_3N_2 \cdot \frac{1}{2}H_2O$: C, 72.60; H, 9.87; N, 5.85. Found: C, 73.23; H, 10.10; N, 5.67. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740 (β-lactam), 1715 (ketone), 1660 (N-CHO). NMR (τ): 1.92 (1H, N-CHO), 7.19, 7.25 (3H, HCON-CH_3).

Lithium Aluminum Hydride Reduction of Pachystermine-A (I)——To a suspension of lithium aluminum hydride (500 mg.) in dry ether (30 ml.) was added dropwise with mechanical agitation a solution of pachystermine-A (200 mg.) in tetrahydrofuran (20 ml.) and the stirred mixture was heated for 2 hr. under gentle refluxing. Excess reagent was then decomposed with water and the inorganic precipitate was filtered off and washed thoroughly with $CHCl_3$. The filtrate and the washings were combined and evaporated to give a crystalline residue which was recrystallized from acetone-CH_2Cl_2 to give pachystermine-diol (Ⅺa) (133 mg.) in needles, m.p. 191~193°. After further recrystallizations, the pure sample showed m.p. 197~198°, $[\alpha]_D^{30}$ −3° (c=1.0). *Anal.* Calcd. for $C_{29}H_{54}O_2N_2$: C, 75.60; H, 11.38. Found: C, 75.42; H, 11.66. NMR (τ): ca. 6.25 (3H, CH-OH and CH-OH), 7.85 (6H, N-$(CH_3)_2$), 9.00, 9.35 (6H, two *tert* CH_3), 9.10 (6H, doublet, J=6 c.p.s.; isopropyl), and 9.13 (3H, doublet, J=6 c.p.s.; *sec* CH_3).

Lithium Aluminum Hydride Reduction of Pachystermine-B (II)——A solution of pachystermine-B (Ⅱ) (50 mg.) in tetrahydrofuran (10 ml.) was added with stirring to a suspension of lithium aluminum hydride (100 mg.) in ether (20 ml.) at room temperature and the mixture was refluxed for 2 hr. Usual working up as for pachystermine-A and crystallization from acetone gave the diol (Ⅺa) (36 mg.) in colorless needles, m.p.

190～197°, identified with pachystermine-diol (XIa) obtained from pachystermine-A (I) by mixed m.p. and IR ($CHCl_3$).

Lithium Aluminum Hydride Reduction of the N-Formyl Compound (X)——The N-formyl compound (X) (38 mg.) was treated with lithium aluminum hydride (50 mg.) in ether (3 ml.) and tetrahydrofuran (3 ml.) in the same manner as above. Thereupon was obtained pachystermine-diol (XIa) (16 mg.), m.p. 197～198°. Identity was confirmed by mixed m.p. and IR ($CHCl_3$) comparison with an authentic sample.

Acetylation of Pachystermine-diol (XIa). i) O,N-Diacetate (XIIIa)——The diol (XIa) (225 mg.) was treated with acetic anhydride (1.5 ml.) in pyridine (3 ml.) for 15 hr. at room temperature. The product (300 mg.), isolated as usual, was chromatographed over alumina (6 g.) in benzene. Fractions eluted with benzene (100 ml.) and ether-benzene (1:9, 3:7, and 5:5; each 50 ml.) were combined and evaporated to give a crystalline residue (230 mg.) which was recrystallized twice from *n*-hexane to afford the O,N-diacetate (XIIIa) (130 mg.) as colorless needles, m.p. 185～190°. Further recrystallizations raise the melting point to 187～190°. $[\alpha]_D^{18}$ +22°(c=1.06). *Anal.* Calcd. for $C_{33}H_{58}O_4N_2$: C, 72.48; H, 10.69; N, 5.12. Found: C, 72.42, 72.19; H, 10.71, 10.42; N, 5.01. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3250 (OH), 1735, 1240 (O-Ac), 1620 (N-Ac). NMR (τ): 5.91 (2H, doublet, J=5 c.p.s.; CH-C<u>H</u>$_2$-OAc), 6.10 (1H, broad, C<u>H</u>-OH), 6.5～7.0 (3H, C<u>H</u>$_2$-N(Ac)-C<u>H</u>), 7.83 (6H, N-$(CH_3)_2$), 7.90, 7.95 (6H, N-$COCH_3$ and O-$COCH_3$), 8.90, 9.35 (6H, two *tert* CH_3), 9.03, 9.06, and 9.15 (9H, three doublets, J=6 c.p.s.; three *sec* CH_3).

ii) O,O,N-Triacetate (XIIIb)——A solution of pachystermine-diol (XIa) (50 mg.) in pyridine (0.7 ml.) and acetic anhydride (0.5 ml.) was allowed to stand at room temperature for 7 days. The product was chromatographed over alumina (1.5 g.) and eluted with benzene and ether-benzene (up to 1:1) to give a crystalline mass (68 mg.), which, upon recrystallizations from *n*-hexane, afforded the O,N-diacetate (XIIIa), m.p. 187～190°, as colorless needles. The mother liquor containing an easily soluble substance was concentrated to small volume, whereupon crystals (ca. 35 mg.) melting at 130～140° were obtained. This substance was again chromatographed on alumina (1 g.). Earlier fractions eluted with ether-benzene (1:9, 30 ml.; and 2:8, 100 ml.) were combined (28 mg.) and recrystallized from *n*-hexane to yield the O,O,N-triacetate (XIIIb) (20 mg.), prisms, m.p. 136～141°. *Anal.* Calcd. for $C_{35}H_{60}O_5N_2$: C, 71.39; H, 10.27; N, 4.76. Found: C, 71.06; H, 10.23; N, 4.78. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1240 (O-Ac), 1635 (N-Ac).

N-Methylpachystermine-diol (XIIa)——The diol (XIa) (310 mg.) was treated with 37% formalin (0.7 ml.) in MeOH (30 ml.) for 15 hr. with occasional warming. Thereafter sodium borohydride (1.2 g.) was added to this solution and stirred for 1 hr. After removal of the solvent *in vacuo* and dilution with aqueous NaOH solution, the product was taken up in CH_2Cl_2, dried, and evaporated. Chromatography of the residual solid over alumina (1.2×8 cm.) from $CHCl_3$ and crystallization from acetone furnished the N-methyldiol (XIIa) (240 mg.) as colorless long plates, m.p. 193～196°. Further recrystallization from acetone-CH_2Cl_2 gave a pure sample, m.p. 196～197°, $[\alpha]_D^{25}$ −11°(c=1.0). Occasionally, this compound crystallized in needles, whose IR spectrum in KBr gave slight difference from that of the above long plates, although both gave no discrimination in IR spectra in chloroform and the same melting point. *Anal.* Calcd. for $C_{30}H_{56}O_2N_2$: C, 75.57; H, 11.84; N, 5.88. Found: C, 75.64, 75.68; H, 11.72, 11.69; N, 5.90, 5.72. NMR (τ): 6.10 (1H, broad, C<u>H</u>-OH), 6.31 (2H, broad, C<u>H</u>$_2$-OH), 7.70 (3H, N-CH_3), 7.86 (6H, N-$(CH_3)_2$), 8.98, 9.37 (6H, two *tert* CH_3), 9.05～9.20 (9H, four peaks, three *sec* CH_3). MS (m/e): 476 (M^+, weak), 461 (M^+-CH_3, weak), 405 (M^+-C_4H_9N, weak), 389 (<u>d</u>, 11%), 170 (<u>c</u>, 17%), 72 (<u>a</u>, base peak).

Alkaline Hydrolysis of Pachystermine-B (II)——The alkaloid (II) (620 mg.) was heated under refluxing with 10% NaOH-MeOH for 2 hr. and the mixture was left stand at room temperature overnight. Thereupon were deposited fine crystals (ca. 500 mg.), which were separated from the solution and washed with MeOH. Several recrystallizations of the crystals from MeOH-CH_2Cl_2 mixture gave the amino-acid (XIV: mixture of 3′-epimeric pair) (400 mg.), m.p. 244～245°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH, NH), 1570 (-COO^-). (This substance may not be the sodium salt because it is soluble in $CHCl_3$).

The alkaline, methanolic mother liquor was concentrated under reduced pressure, diluted with water, and extracted with CH_2Cl_2. Drying the extract over anhydrous $MgSO_4$ and evaporation gave an additional crop of the amino-acid (XIV) (180 mg.).

Lithium Aluminum Hydride Reduction of the Amino-acid (XIV) and the Subsequent N-Methylation (Formation of N-Methylpachystermine-diol (XIIa) and 3′-Iso-N-Methylpachystermine-diol (XIIb))——The above amino-acid (XIV, m.p. 244～245°) (135 mg.) was treated with excess lithium aluminum hydride in boiling tetrahydrofuran (20 ml.) for 5 hr. Working up in the usual way afforded a crystalline product (130 mg.) (XIc: mixture of 3′-epimeric pair). A small portion of this product was recrystallized from acetone to give colorless crystals melting at 212～215°, whose IR spectrum in $CHCl_3$ was identical with that of pachystermine-diol (XIa), but distinct differences were observed in their spectra in KBr.

The above, whole product (XIc) was then N-methylated by treating with formalin followed by sodium borohydride reduction. The product (130 mg.), isolated in the usual manner, showed two spots in a ratio of nearly 1:1 on TLC. On standing the acetone solution of this product, there was deposited a small amount of plates, which was collected by filtration. Four recrystallizations of the crystals from acetone gave N-methylpachystermine-diol (XIIa, 3′-normal) (16 mg.), m.p. 196～197°, as long plates. $[\alpha]_D^{20}$ −9°(c=1.0). The identity was confirmed by mixed m.p. and IR (KBr) comparison with a sample (XIIa), derived directly from

pachystermine-A. *Anal.* Calcd. for $C_{30}H_{56}O_2N_2$: C, 75.57; H, 11.84; N, 5.88. Found: C, 75.37; H, 12.14; N, 5.73.

The mother liquor, separated from the above crystals, was evaporated to dryness. The residue was chromatographed on alumina (1×5 cm.) and eluted with benzene, ether-benzene mixture (5:95, 1:9, 2:8, and 5:5), and ether. The later fractions showing single spot on TLC. (from 1:9 ether-benzene to ether elution) were combined and recrystallized from acetone to give 3′-iso N-methyl compound (XIIb) in colorless needles (7 mg.), m.p. 171~172°, $[\alpha]_D^{25}$ +40°(c=1.0). This compound identified with the 3′-iso-N-methylpachystermine-diol (XIIb), described later, by mixed m.p., IR (KBr) comparison, and TLC. On the other hand, the compound (XIIb) gave a remarkable melting point depression when admixed with N-methylpachystermine-diol (XIIa, 3′-normal) and also showed distinct differences in IR (KBr) spectra.

Methylation of the Amino-acid (XIV) with Diazomethane——A solution of pachystermine-B (II) (0.5 g.) in 10% KOH-EtOH (60 ml.) was refluxed for 3 hr. Then the mixture was made slightly acidic with acetic acid, and EtOH was removed under reduced pressure. The residue was diluted with 3% acetic acid and washed with CH_2Cl_2 in order to remove the weakly basic impurity. This acidic solution was basified with Na_2CO_3 and the precipitated solid (amino-acid (XIV)) was collected by filtration, washed with water, and dried. The crude amino-acid thus obtained was dissolved in MeOH-$CHCl_3$ and purified by filtration through an alumina column (1.5×5 cm.) and eluted with MeOH-$CHCl_3$ (2:8). The filtered solution was concentrated to 100 ml. and the amino-acid was converted to its hydrochloride by addition of conc. HCl (ca. 0.2 ml.). To this solution was added an etherial solution of diazomethane and left stand at room tenperature for several hours. Thereafter solvents were removed *in vacuo* and the residue was partitioned between CH_2Cl_2 and 3% HCl solution. Basification of the aqueous acidic phase with Na_2CO_3, extraction with ether, drying, and evaporation gave the crude methyl ester (XV) (0.5 g.). Repeated recrystallizations of this from aqueous acetone afforded the fairly pure 3′-iso methyl ester (XVb) as colorless leaves (0.20 g.), m.p. 133~137°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1725, 1165 (-$COOCH_3$). NMR (τ): 6.32 (3H, -$COOCH_3$), ca. 6.4 (1H, C<u>H</u>-OH), 7.23 (1H, broad, C<u>H</u>-$COOCH_3$), 7.85 (6H, N-$(CH_3)_2$), 9.00, 9.37 (6H, two *tert* CH_3), 9.05, 9.08, 9.16 (9H, three doublets, J=6 c.p.s.: three *sec* CH_3).

The mother liquor (0.29 g.) showed the identical IR($CHCl_3$) spectrum as that of the above crystalline methyl ester (XVb),

Lithium Aluminum Hydride Reduction of the Amino-acid Methyl Ester (XV) and the Subsequent N-Methylation. i) Reduction of the 3′-Iso Methyl Ester (XVb) (Formation of 3′-Iso-pachystermine-diol (XIb))——The methyl ester (XVb, m.p. 133~137°) (150 mg.) was refluxed with lithium aluminum hydride in ether (10 ml.) for 3 hr. The product (130 mg.), recovered in the usual way, was recrystallized from acetone-CH_2Cl_2 to give the 3′-iso-pachystermine-diol (XIb) (87 mg.), needles, m.p. 223~224°, $[\alpha]_D^{30}$ +19°(c=1.0). The IR spectrum in KBr differed from that of pachystermine-diol (XIa, 3′-normal), but identical in chloroform solution. *Anal.* Calcd. for $C_{29}H_{54}O_2N_2$: C, 75.60; H, 11.38; N, 6.05. Found: C, 75.59; H, 11.67; N, 6.02.

ii) 3′-Iso-N-methylpachystermine-diol (XIIb)——The above 3′-iso-diol (XIb) (75 mg.) was N-methylated by formalin-sodium borohydride procedure in the usual manner. The crude product (75 mg.), obtained, crystallized from acetone to afford the 3′-iso-N-methyldiol (XIIb) (50 mg.), m.p. 168~172°. Further recrystallizations from the same solvent gave a pure sample as colorless plates, m.p. 171~174°. $[\alpha]_D^{24}$ +40°(c=1.0). IR (KBr) spectrum of this compound showed distinct differences from that of XIIa and their mixture also gave melting point depression. *Anal.* Calcd. for $C_{30}H_{56}O_2N_2$: C, 75.57; H, 11.84. Found: C, 75.37; H, 11.84. NMR (τ): ca. 6.2 (3H, broad, C<u>H</u>-OH and C<u>H</u>$_2$-OH), 7.65 (3H, N-CH_3), 7.86 (6H, N-$(CH_3)_2$), 8.98, 9.37 (6H, two *tert* CH_3), 9.05~9.25 (9H, diffuse doublet, three *sec* CH_3). MS (m/e): 476 (M^+, weak), 461 (M^+-CH_3, weak), 405 ($M^+-C_4H_9N$, weak), 389 (d, 9%), 170 (c, 13%), 72 (a, base peak).

iii) Reduction of the Mother Liquor of the Methyl Ester (XV) and the Subsequent N-Methylation——The mother liquor fraction (290 mg.) of the amino-acid methyl ester (XV), described previously, was reduced with excess lithium aluminum hydride in the same manner as above. The product (230 mg.) was crystallized from acetone to give a crystalline substance (XIc) melting at 210~215° (90 mg.) and the mother liquor weighed 140 mg. upon evaporation. N-Methylation of the crystalline product by formalin sodium borohydride procedure gave a crude, crystalline N-methyl compound (90 mg.), which was chromatographed on alumina (4 g.) in benzene. The eluate (28 mg.) with benzene was recrystallized several times from acetone to afford N-methylpachystermine-diol (XIIa, 3′-normal) (10 mg.) as colorless plates, m.p. 190~197°, identified by TLC and mixed m.p. determination with an authentic sample. Further elution with ether-benzene (5:95, 1:9, and 2:8) gave fractions exhibiting single spot on TLC which were combined and evaporated. Recrystallization of the residual solid (ca. 50 mg.) from acetone yielded 3′-iso-N-methylpachystermine-diol (XIIb) as prisms (30 mg.), m.p. 171~174°. Identity was established by TLC and mixed m.p. determination.

The mother liquor fraction (140 mg.) of the diol compound (XIc) was also N-methylated and the product (117 mg.) was examined by alumina chromatography (3 g.), whereupon N-methylpachystermine-diol (XIIa, 3′-normal) (26 mg.), m.p. 196~197°, and 3′-iso-N-methylpachystermine-diol (XIIb) (10 mg.), m.p. 168~174°, were obtained.

Synthesis of N-Methylpachystermine-diol (XIIa) and 3′-Isomer (XIIb). i) Diethyl Isopropylmalonate——Diethyl malonate (63.5 g.) was added slowly to a stirred solution of sodium (9.0 g.) in abs. EtOH (200 ml.)

at 50°. To this solution was added dropwise isopropyl bromide (45.5 g.) over a period of 30 min. and the mixture was refluxed for 1.5 hr.[17] After the mixture was kept at room temperature overnight, EtOH was removed and the residue was diluted with water, extracted with ether, dried over anhydrous $MgSO_4$, and evaporated. Distillation of the oily residue gave diethyl isopropylmalonate (57.5 g.), b.p. 113~116°/27 mm. Hg.

ii) Ethyl Isopropylmalonate (XVIa)——The above diethyl ester (39.0 g.) was dissolved in a solution of KOH (10.8 g.) in abs. EtOH (200 ml.) and the solution was stirred for 2 hr. at room temperature and then refluxed for 3 hr. After EtOH was removed by evaporation, the residue was diluted with water, acidified with HCl, and extracted with CH_2Cl_2. The extract was washed with water, dried over anhydrous $MgSO_4$, and evaporated to yield ethyl isopropylmalonate (XVIa) (25 g.), oil. The nice purity of this compound was confirmed by its NMR spectrum. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : ca. 1720 (broad, ester and -COOH). NMR (τ) : 1.42 (1H, singlet, COOH). 5.80 (2H, quartet, J=7 c.p.s.; $COOC\underline{H}_2$-CH_3), 6.88 (1H, doublet, J=8 c.p.s., -$C\underline{H}\langle^{COOH}_{COOR}$), 7.62(1H, sextet, $(CH_3)_2C\underline{H}$-), 8.71 (2H, triplet, J=7 c.p.s.; CH_2-$C\underline{H}_3$), and 8.97 (6H, broad doublet, J=6.5 c.p.s.; $(C\underline{H}_3)_2$-CH).

iii) Synthesis of the 3′-Epimeric N-Methyl-diols (XIIa and XIIb)——On a solution of 3β-amino-20α-dimethylamino-4β-hydroxy-5α-pregnane (VIa) (130 mg.) in $CHCl_3$ (20 ml.) and tetrahydrofuran (10 ml.) was placed 10% aqueous KOH solution (10 ml.) and the mixture was cooled in an ice-bath. To this mixture was added dropwise with vigorous stirring the acid chloride (XVIIb) which had been prepared by treating ethyl isopropylmalonate (XVIa) (1.0 g.) with thionyl chloride (0.4 ml.) at room temperature, After the stirring continued for additional 2 hr. at room temperature, the organic phase was separated from the aqueous phase which was further extracted with CH_2Cl_2. The combined solution was washed successively with 3% HCl and dil. Na_2CO_3, dried over K_2CO_3, and evaporated to give a crystalline residue (O,N-diacyl compound, XVII) (300 mg.).

This substance, without further purification, was treated with excess lithium aluminum hydride in tetrahydrofuran (20 ml.) for 6 hr. The strongly basic product (XIc) (100 mg.), obtained by usual working up, was crystallized from acetone-CH_2Cl_2 and separated into two fractions : *i.e.*, a crystal fraction melting at 210~220° (45 mg.) and a mother liquor fraction (ca. 50 mg.).

The crystal fraction was N-methylated by formalin (37%, 3 drops)-sodium borohydride (30 mg.) procedure in the usual manner and the product (45 mg). was chromatographed on alumina (0.5 × 10 cm.) in benzene. Elution with benzene and ether-benzene (5:95) gave a small quantity of crystals, m.p. 180~190°. Subsequent elution with ether-benzene (1:9) and ether gave a homogeneous substance, which was recrystallized from acetone to afford the N-methyl-diol compound (XIIb) (12 mg.), m.p. 171~172°, $[\alpha]_D^{30}$ +40° (c=1.0). This compound was identified with the 3′-iso-N-methylpachystermine-diol (XIIb) by TLC, mixed m.p., and IR (KBr) comparison.

The mother liquor fraction was also N-methylated in the similar manner. The product (35 mg.) was chromatographed on alumina (0.5 × 10 cm.) and eluted with benzene and ether-benzene (5:95). Earlier fractions showing single spot on TLC were combined and recrystallized from acetone to give the N-methyl-diol compound (XIIa) as colorless plates (5 mg.), m.p. 193~194°, $[\alpha]_D^{30}$ −3° (c=1.0). This compound was proved to be identical with N-methylpachystermine-diol (XIIa) by TLC, mixed m.p., and IR (KBr) comparison.

Treatment of N-Methylpachystermine-diol (XIIa) with *p*-Toluenesulfonyl Chloride——To a solution of the compound (XIIa) (200 mg.) in pyridine (2 ml.) was added a solution of *p*-toluenesulfonyl chloride (135 mg.) in pyridine (1 ml.) at room temperature and the mixture was allowed to stand overnight. Dilution of the reaction mixture with dil. Na_2CO_3 and extraction with CH_2Cl_2 yield a crystalline product, which was chromatographed on alumina (1 × 5 cm.) in benzene. Elution with benzene and ether gave a mixture of the starting material and the mono-tosylate. Further elution with CH_2Cl_2 gave the mono-tosylate (XIX) (120 mg.) which was recrystallized from acetone-CH_2Cl_2 to afford the pure material, m.p. 250~253°. This compound is difficult-soluble in most solvents including $CHCl_3$. *Anal.* Calcd. for $C_{37}H_{62}O_4N_2S \cdot H_2O$: C, 68.48; H, 9.94. Found : C, 68.76; H, 10.05. IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1230, 1180, 1120, 1035, 830 (tosylate anion).

Lithium Aluminum Hydride Reduction of the Mono-tosylate (XIX)——A suspension of the mono-tosylate (XIX) (70 mg.) in tetrahydrofuran (10 ml.) and ether (10 ml.) was refluxed with lithium aluminum hydride (200 mg.) for 2 hr. The crystalline product (58 mg.), isolated in the usual way, was recrystallized from acetone to afford the deoxy compound (XXa) (30 mg.), m.p. 164~166°, in colorless plates. Further recrystallization from the same solvent raised the melting point to 170~173°, $[\alpha]_D^{18}$ +36° (c=1.0). The IR spectrum (KBr) of this compound showed distinct differences in finger-print region from that of synthetic specimen (XXIVa) described later. *Anal.* Calcd. for $C_{30}H_{56}ON_2 \cdot \frac{1}{4}H_2O$: C, 77.44; H, 12.24. Found : C, 77.66; H, 12.59. IR $\nu_{max}^{CHCl_3}$ cm^{-1} : 3350 (OH). NMR (τ) : 6.25 (1H, broad, $C\underline{H}$-OH), 7.78 (3H, N-CH_3), 7.83 (6H, N-$(CH_3)_2$), 8.95 (3H, *tert* CH_3), 9.05~9.25 (12H, four *sec* CH_3), 9.34 (3H, *tert* CH_3). MS (m/e) : 460 (M^+, weak), 445 (M^+-15, weak), 389 (d, 3%), 154 (e, 14%), 72 (a, base peak).

O-Acetate (XXb) : The above product (XXa) (20 mg.) was acetylated by treatment with acetic anhydride (1 ml.) in pyridine (1 ml.) at room temperature overnight. The product was chromatographed on alumina (0.5 × 10 cm.) in benzene. Elution with the same solvent followed by recrystallization from acetone furnished

17) R. Adams, R. M. Kamm : "Organic Syntheses," Col. Vol. 1, 250 (1948).

the O-acetate (XXb) (15 mg.), m.p. 164~168°, as colorless leaves. $[\alpha]_D^{30}$ −1°(c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1250 (O-Ac).

Treatment of 3′-Iso-N-methylpachystermine-diol (XIIb) with *p*-Toluenesulfonyl Chloride and Subsequent Reduction with Lithium Aluminum Hydride. i) The Mono-Tosylate (XIX)——A mixture of the 3′-iso-N-methyl-diol (XIIb) (75 mg.), pyridine (1 ml.), and *p*-toluenesulfonyl chloride (70 mg.) was kept in a refrigerator overnight. The product, isolated with CH_2Cl_2 and dil. Na_2CO_3, was recrystallized from acetone-CH_2Cl_2 to give the mono-tosylate (XIX) (40 mg.), m.p. 248~251°, as colorless needles. Further treatment of the mother liquor gave an additional crop of the material (19 mg.), m.p. 243~249°. This compound (XIX) gave the identical IR (KBr) spectrum with that of the sample obtained from XIIa and no melting point depression could be recognized upon admixture.

ii) Deoxy Compound (XXa)——The above mono-tosylate (XIX) (35 mg.) was reduced in the usual manner with lithium aluminum hydride (200 mg.) in tetrahydrofuran (15 mg.) and thereby obtained the deoxy compound (XXa) (17 mg.), m.p. 150~174°. After further recrystallization from acetone, it showed m.p. 155~173°, $[\alpha]_D^{30}$ +38° (c=1.0). In another experiment, the product (XXa) showed m.p. 168~172°. This compound was estimated to be identical with the deoxy compound (XXa) derived from XIIa by mixed m.p., TLC, and IR (KBr) comparison. However, the IR (KBr) spectrum was not identical with that of the synthesized compound (XXIVa) in finger-print region.

O-Acetate (XXb): colorless leaves, m.p. 169~173°. This was also indistinguishable from the sample (XXb) derived from XIIa in every respect.

Treatment of Pachystermine-diol (XIa) with Methanesulfonyl Chloride. i) Azetidine Compound (XXIIa)——To a chilled solution of pachystermine-diol (XIa) (120 mg.) in pyridine (2 ml.) was added with stirring methanesulfonyl chloride (0.5 ml.) and the mixture was kept in a refrigerator for 20 hr. The reaction mixture was diluted with dil. Na_2CO_3, extracted with CH_2Cl_2, and the CH_2Cl_2 solution was again extracted with 3% HCl. The acidic extract was basified with NH_4OH, extracted with CH_2Cl_2, dried over K_2CO_3, and evaporated. Crystallization of the residue from acetone gave a crystalline substance (35 mg.). The mother liquor was chromatographed on alumina (1×4 cm.) in CH_2Cl_2 to give an additional crop of crystals (35 mg.). The combined crystals were recrystallized from acetone to give the azetidine compound (XXIIa) (50 mg.), m.p. 212~213°, $[\alpha]_D^{30}$ +35° (c=1.0). *Anal.* Calcd. for $C_{29}H_{52}ON_2 \cdot 1/2\ H_2O$: C, 76.78; H, 11.77. Found: C, 76.85; H, 11.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH). NMR (τ): 6.35~6.75 (3H), 7.00~7.40 (2H), 7.83 (6H, N-$(CH_3)_2$), 8.99, 9.35 (6H, two *tert* CH_3), 9.15 (3H, doublet, J=6 c.p.s.; *sec* CH_3), 9.19 (6H, doublet, J=6 c.p.s.; isopropyl). MS (m/e): 444 (M^+), 443 (M^+ −1), 429 (M^+ −CH_3), 373 (M^+ −C_4H_9N, 3%), 358 (M^+−86, 0.7%), 138 (f, 10%), 72 (a, base peak).

O-Acetate (XXIIb): Acetylation of XXIIa with acetic anhydride-pyridine gave rise to the O-acetate (XXIIb), m.p. 216~217°, $[\alpha]_D^{31}$−1° (c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1250 (O-Ac).

ii) O,N-Dimesyl Compound (XXI) and the Azetidine Compound (XXIIa)——To a solution of pachystermine-diol (XIa) (120 mg.) in pyridine (2 ml.) was added methanesulfonyl chloride (0.5 ml.) and the mixture was allowed to stand overnight at room temperature. The reaction mixture was diluted with dil. Na_2CO_3, extracted with CH_2Cl_2, dried, and evaporated. Trituration of the oily residue gave crystals (XXIIa) (20 mg.), whose IR ($CHCl_3$) spectrum was identical with that of the azetidine compound (XXIIa). The mother liquor, on TLC demonstrated a big spot just below the spot corresponding to the azetidine (XXIIa). The former was considered to correspond to the O,N-dimesylate (XXI). The IR spectrum also showed absorption bands which may be ascribed to sulfonate groupings (1360, 1335, and 1170 cm^{-1}), This material was then submitted to lithium aluminum hydride reduction without further purification.

Lithium Aluminum Hydride Reduction of the O,N-Dimesyl Compound (XXI) and the Subsequent N-Methylation——The above crude O,N-dimesylate (XXI) was reduced by refluxing with excess of lithium aluminum hydride in tetrahydrofuran (5 ml.) for 5 hr. The strongly basic product, isolated in the usual way, was crystallized from acetone to afford the azetidine compound (XXIIa) (20 mg.), m.p. 205~208°. The mother liquor (ca. 30 mg.) was then N-methylated by formalin-sodium borohydride procedure as usual. The product, obtained by the usual working up, was chromatographed on alumina (1×5 cm.) in benzene. Elution with the same solvent and repeated recrystallization from acetone gave the N-methylated des-mesyl compound (XXIVa) (6 mg.), m.p. 171~172°, $[\alpha]_D^{30}$ +28° (c=1.0), which was identified in all respects with the synthesized specimen (XXIa), described below. However, IR spectrum in KBr was not identical in finger-print region with that of XXa obtained by de-tosylation of XIX, though mixed m.p. did not depress.

The O-Acetate (XXIVb): The mother liquor of XXIVa was acetylated as usual. Recrystallization of the product from acetone gave the O-acetate (XXIVb) (9 mg.), m.p. 165~167°, which was identified in every respect with the synthetic sample (XXIVb). $[\alpha]_D^{30}$ −11° (c=1.0).

Synthesis of 3β-Methyl,(2′*R*-2′,3′-Dimethyl)butylamino-20α-dimethylamino-4β-hydroxy-5α-pregnane (XXIVa). i) O,N-Diacyl Compound (XXVIa)——(−)-2*R*-2,3-dimethylbutylic acid (XXVa) (ca. 300 mg.), prepared from its quinine salt (1.20 g.), was treated with thionyl chloride (0.3 ml.) at room temperature for 30 min. A solution of the resulting acid chloride (XXVb) in benzene (5 ml.) was added dropwise with stirring to a chilled mixture of 10% aqueous NaOH solution (15 ml.) and a solution of 3β-amino-20α-dimethylamino-4β-hydroxy-5α-pregnane (VIa) (200 mg.) in $CHCl_3$ (15 ml.) and the stirring was continued for 3 hr. at room temperature. Thereafter, the $CHCl_3$ phase was separated and the aqueous phase was extracted

with CH_2Cl_2. The combined $CHCl_3$ and CH_2Cl_2 solutions were washed successively with 3% HCl and dil. Na_2CO_3, dried over K_2CO_3, and evaporated to yield the crystalline O,N-diacyl compound (XXVIa) (ca. 400 mg.), which was reduced with lithium aluminum hydride without further purification. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (OCOR), 1655, 1500 (NHCOR).

ii) Lithium Aluminum Hydride Reduction of the O,N-Diacyl Compound (XXVIa)——The above, crude O,N-diacyl compound (XXVIa) was refluxed with lithium aluminum hydride (300 mg.) in tetrahydrofuran (10 ml.) for 1.5 hr. The product, obtained in the usual way, was dissolved in CH_2Cl_2 and shaken with 3% HCl. Thereupon was deposited a difficult-soluble precipitate (hydrochloride of the N-acyl compound (XXVIb)), which was separated by filtration.

The acidic, aqueous phase of the filtrate was made alkaline with NH_4OH and extracted with CH_2Cl_2. Washing of the extract with water, drying, and evaporation gave the crude NH compound (XXIII) (30 mg.).

The above precipitate, on the other hand, was suspended in dil. NH_4OH and extracted with CH_2Cl_2 to give the N-acyl compound (XXVIb) (60 mg.), which showed m.p. 264~267° upon recrystallization from acetone. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1655, and 1510 (NHCOR).

The CH_2Cl_2 phase of the above filtrate was evaporated *in vacuo* to dryness. The residue was combined with the N-acyl compound (XXVIb) and again reduced by refluxing with excess lithium aluminum hydride in tetrahydrofuran for 25 hr. Usual working up gave the NH compound (XXIII) (40 mg.).

iii) N-Methylation of the NH Compound (XXIII)——The above, crude NH compound (XXIII) (70 mg.) was N-methylated as usual with formalin and sodium borohydride in MeOH. The product, isolated in the usual way, was chromatographed on alumina (1×10 cm.) from benzene. Elution with benzene gave a crystalline compound (70 mg.) which was recrystallized from acetone to furnish XXIVa (30 mg.), m.p. 166~168°. Further recrystallization from the same solvent gave a pure sample, m.p. 169~172°, $[\alpha]_D^{15}$ +27° (c=1.0). This compound was shown to be identical with XXIVa derived from the O,N-dimesylate (XXI) by TLC, mixed m.p., and IR (KBr) comparison.

O-Acetate (XXIVb): The above product (XXIVa) (60 mg.) was acetylated heating with acetic anhydride (2 ml.) and pyridine (0.5 ml.) for 2 hr. Recrystallizations of the product from acetone afforded the O-acetate (XXIVb) (40 mg.), m.p. 167~168°, $[\alpha]_D^{15}$ −10° (c=1.0). This was identified in all respects with XXIVb derived from the O,N-dimesylate (XXI).

The authors express their deep gratitude to Professor M. Tomita of this Faculty for his guidance and hearty encouragement. Also the authors wish to thank Professor K. Tsuda of the University of Tokyo for a gift of the quinine salt of (−)-2*R*-2,3-dimethyl-butylic acid, Dr. K. Kuriyama of Shionogi & Co., Ltd. for the ORD determinations, Dr. T. Shingu and Mr. M. Yamamoto for NMR measurements, Dr. K. Miyajima and Mr. M. Yamamoto for taking IR (KBr) spectra, Dr. T. Ibuka for taking mass spectra, and Dr. K. Konobu and Miss Y. Mano and collaborators for micro-analyses.