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STUDIES ON THE ALKALOIDS OF PACHYSANDRA TERMINALIS SIEB. ET ZUCC. (6). : STRUCTURE OF PACHYSTERMINE - A AND -B, NOVEL TYPE ALKALOIDS HAVING β-LACTAM RING SYSTEM(1).

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Pachystermine - A*) is one of the major alkaloids of Pachysandra terminalis, and it was isolated from the weakly basic alkaloid fraction along with pachystermine - B, a minor alkaloid. The interrelationship of both alkaloids was shown as follows : $NaBH_4$ reduction of pachystermine - A gave pachystermine - B, which, in turn, on CrO_3 oxidation in AcOH gave rise to the former. Herewith we propose the structures I and II for pachystermine - A and - B, respectively, on the basis of following evidences. They represent a novel type of steroid alkaloid carrying a β-lactam ring.

Pachystermine - A (I), m.p. 220 - 224°(2), $[\alpha]_D + 24°$(3), analysed for $C_{29}H_{48}O_2N_2$(4) (molecular ion peak at m/e 456 in mass spectrum(5))

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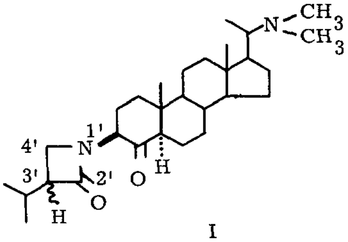
*) In Table I. of Part (3) of this series(7) this alkaloid was reported in the name of "pachysterine". Here we would like to correct it as "pachystermine".

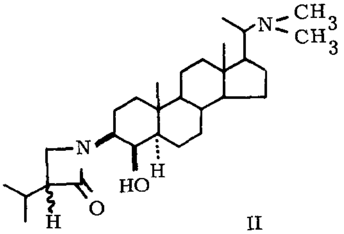
and showed two CO bands at 1735 (β-lactam) and 1715 cm^{-1} (six-membered ketone) in the IR spectrum ($CHCl_3$), though no characteristic absorption was observed in the UV spectrum. Its NMR spectrum(6) revealed the presence of one $N(CH_3)_2$ (7.85τ), one $CH(CH_3)_2$ (8.95 and 9.05τ, two doublets (d.), J 6 c.p.s.), one sec. CH_3 (9.15τ, d., J 6 c.p.s.), and two tert. CH_3 groups (9.26 and 9.36τ). Its ORD curve in methanol demonstrated a negative Cotton effect (peak : $[\phi]_{269}$ + 2480° ; trough : $[\phi]_{312}$ - 1350°) comparable to that of cholestane-4-one(8).

On the other hand, pachystermine - B (II), $C_{29}H_{50}O_2N_2$ (molecular ion peak at m/e 458 in mass spectrum), m.p. 256 - 258°, $[\alpha]_D$ - 18°, showed IR $\nu_{max}^{CHCl_3}$ 1715 cm^{-1} (bonded β-lactam) and NMR pattern very similar to that of pachystermine - A except for peaks at 8.94 (19-CH_3) and 5.96τ (-C$\underline{H}$OH-). The ORD of II exhibited a negative plane curve, suggesting the carbonyl group is contained in the side chain.

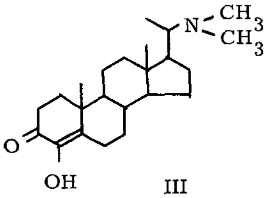
The skeletal structure of the bases was indicated by the treatment of I with 5 % KOH in EtOH, upon which the diosphenol (III), $C_{23}H_{37}O_2N$, m.p. 188 - 191°, was obtained. The identity was established by direct comparison (IR in KBr, mixed melting point) with the synthesized specimen (III) reported in Part (1)(9). Furthermore, Huang-Minlon reduction of I gave 20α-dimethylamino-5α-pregnane (IV), $C_{23}H_{41}N$, m.p. 137 - 139°, $[\alpha]_D$ +22°, and 20α-dimethylamino-5α-pregnane-4-one (V), m.p. 164 - 166°, ORD : negative Cotton effect (peak : $[\phi]_{272}$ + 5400°, trough : $[\phi]_{308}$ - 3930°),



I



II



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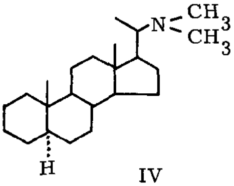

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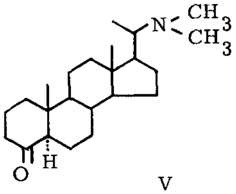
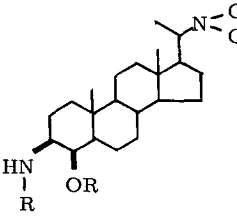
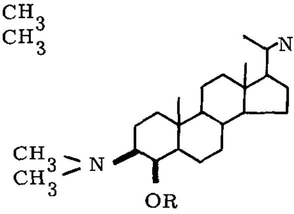
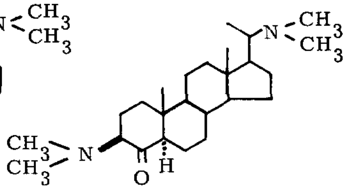


V







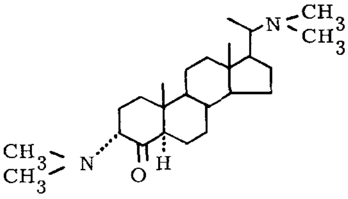
VIa R = H

VIb R = CH_3CO

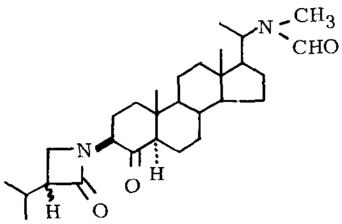
VIIa R = H

VIIb R = CH_3CO

VIII



IX



X

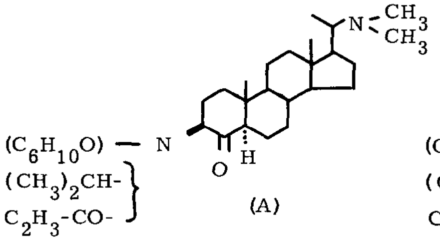
the latter of which was identified with V, $C_{23}H_{39}ON$, m.p. 165 - 167°, $[\alpha]_D + 42°$, synthesized from the diosphenol (III) by HI reduction(10).

Another evidence for the skeletal structure was advanced as follows : Alkali degradation of II under the condition of Huang-Minlon reduction afforded an aminoalkohol (VIa), which was characterized as its O, N-diacetate (VIb), $C_{27}H_{46}O_3N_2 \cdot 1/2H_2O$, m.p. 237 - 241°, IR $\nu_{max}^{CHCl_3}$ 3400, 1670, 1510 (sec. amide), 1735 and 1230 cm^{-1} ($OCOCH_3$); NMR signals at 4.83 (-C$\underline{H}$OCOCH$_3$-), 7.83 ($N(CH_3)_2$), 7.90, 8.09 ($NCOCH_3$ and $OCOCH_3$), 9.03, 9.38 (two tert. CH_3), and 9.15 τ (sec. CH_3). On treatment with HCHO - HCOOH, the aminoalkohol (Va) yielded an N-dimethyl compound (VIIa), $C_{25}H_{46}ON_2$, m.p. 168 - 172°, $[\alpha]_D + 50°$, NMR signals : 6.22 (1H, t., J 3 c.p.s.; -C$\underline{H}$OH-), 7.72, 7.85 (two $N(CH_3)_2$), 9.15 (sec. CH_3), 8.97 and 9.35τ (two tert. CH_3). Chromic acid oxidation of VIIa in AcOH afforded an ami no-ketone (VIII), $C_{25}H_{44}ON_2$, m.p. 120 - 125°, $[\alpha]_D + 47°$, NMR signals : 7.61, 7.83 (two $N(CH_3)_2$), 9.15 (sec. CH_3), 9.29 and 9.35τ (two tert. CH_3), which on subsequent Huang-Minlon reduction gave rise to IV and V.

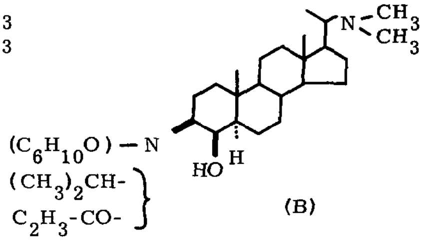
The stereochemistry of VIa, VIIa, and VIII was determined on the bases of the following evidences. The 19-CH_3 signals in the NMR spectra of VIIa and its O-acetate (VIIb), $C_{27}H_{48}O_2N_2$, m.p. 216 - 218°, $[\alpha]_D + 18°$, occured in lower field (8.97 and 9.02τ) than the standard region (9.1 - 9.2τ), indicating the 4-hydroxyl group placed in the 1, 3-diaxial relation to the 19-CH_3 group. This permits the 4β, 5α - assignment to VIa and VIIa.(11) Moreover, the ORD curve of the amino-ketone (VIII) demonstrated a weak negative Cotton effect (peak : $[\Phi]_{274}$ + 3440; trough : $[\Phi]_{318}$ - 1790°), corresponding with that of 4-keto-5α -steroids.(8) The smaller amplitude compared with that of 3α, 20α -dimethylamino-5α-pregnane-4-one (IX)(9) suggested the 3β-configuration for the amino-ketone (VIII).

Finally, we attempted to prepare VIIa from IX. Base or acid treatment of IX followed by $NaBH_4$ reduction led to an aminoalkohol, m.p. 165 - 168°, whose IR spectrum (KBr) was superimposable with that of the above aminoalkohol (VIIa).

Therefore, the structures of pachystermine - A and - B are now represented as (A) and (B), respectively.



(A)



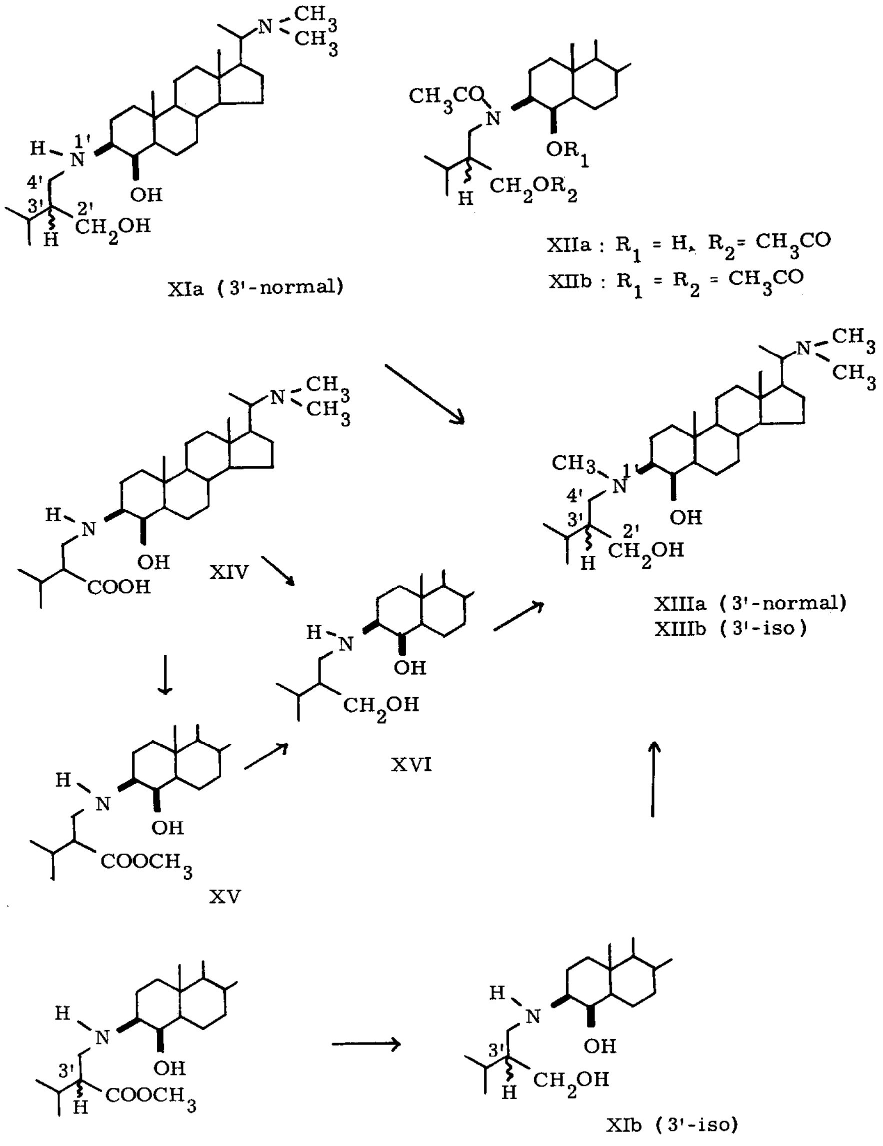
(B)

Turning now to the constitution of the side chain moiety ($C_6H_{10}O$) of the alkaloids, the presence of isopropyl group was deduced from the inspection of NMR spectra. The remaining -C_2H_3-CO- moiety appeared to form a ring system since no oleffinic proton was observed in NMR spectra. When oxidized with CrO_3 in pyridine, pachystermine - A afforded a neutral N-formyl compound (X), $C_{29}H_{46}O_3N_2$, m.p. 225 - 226°, $[\alpha]_D$ + 12° ; IR $\nu_{max}^{CHCl_3}$ 1740 (β-lactam), 1715 (ketone), and 1665 cm^{-1} (N-CHO); NMR signals : 1.92 (C$\underline{H}$O) and 7.25τ (amide N-CH_3), suggesting that the carbonyl group in the side chain might be forming an amide.

These observations led us to assume that the C_2H_3CO portion and the nitrogen form a four-membered β-lactam ring. The IR band at 1735 cm^{-1} of pachystermine - A and O-acetyl-pachystermine - B also supports the assumption.(12)

Upon $LiAlH_4$ reduction,(13) pachystermine - A and - B afforded the same diol (XIa), $C_{29}H_{54}O_2N_2$, m.p. 197 - 198°, $[\alpha]_D$- 3°, which on subsequent acetylation gave an O, N-diacetate (XIIa), $C_{33}H_{58}O_4N_2$, m.p. 187 - 190°, $[\alpha]_D$+ 22°; IR $\nu_{max}^{CHCl_3}$ 3250 (OH), 1735, 1240 ($OCOCH_3$), 1620 cm^{-1} ($NCOCH_3$); NMR signals : 5.91 (2H, d., J 5 c.p.s.; -CH-C$\underline{H}_2$-OAc), 6.10 (1H, -C$\underline{H}$OH-), 7.90 and 7.95τ ($OCOCH_3$, $NCOCH_3$), and an O,O,N-triacetate (XIIb), $C_{35}H_{60}O_5N_2$, m.p. 136 - 141°; IR $\nu_{max}^{CHCl_3}$ 1735, 1240 ($OCOCH_3$), 1635 cm^{-1} ($NCOCH_3$). N-methylation of the diol (XIa) with HCHO - $NaBH_4$ yielded an N-methyl compound (XIIIa), $C_{30}H_{56}O_2N_2$, m.p. 196 - 197°, $[\alpha]_D$ - 11° ; NMR signals : 6.10 (1H, -C$\underline{H}$OH-), 6.31 (2H, -C$\underline{H}_2$OH), 7.70 (NCH_3), 7.86 ($N(CH_3)_2$).

On the other hand, pachystermine - B (II) readily underwent base



XIa (3'-normal)

XIIa : $R_1 = H,\ R_2 = CH_3CO$

XIIb : $R_1 = R_2 = CH_3CO$

XIV

XV

XVI

XIIIa (3'-normal)

XIIIb (3'-iso)

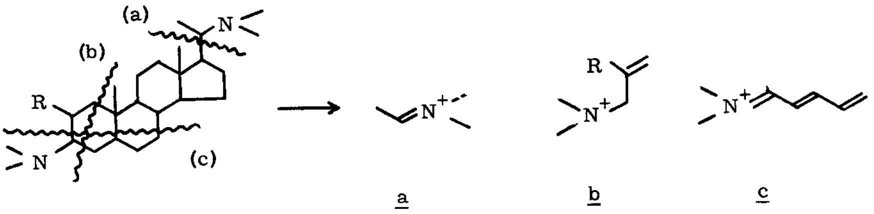
XVb (3'-iso)

XIb (3'-iso)

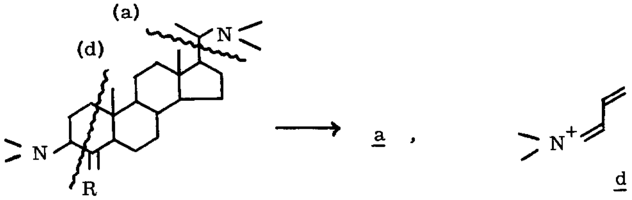
hydrolysis to give an amino-acid (XIV), IR $\nu_{max}^{CHCl_3}$ 3300 and 1570 cm^{-1}, which is a mixture of diastereomers formed probably by partial racemization at the carbon atom adjacent to the carboxyl group. Upon methylation with diazomethane, XIV-hydrochloride gave a methyl ester (XV, diastereoisomeric mixture), IR $\nu_{max}^{CHCl_3}$ 3400 (OH, NH), 1725, 1165 cm^{-1} (-COOCH$_3$). Reduction of this ester with LiAlH$_4$ afforded diol compound (XVI),(14) which, on subsequent N-methylation, gave rise to N-methyl compound. This could be separated by repeated almina chromatography into each diastereomer : i. e. (a), m.p. 196 - 197°, $[\alpha]_D$- 9° ; and (b), m.p. 170 - 172°, $[\alpha]_D$+ 40° (XIIIb). The former was found to be identical with the N-methyl-diol (XIIIa) described above (IR in KBr, mixed melting point).

When the above methyl ester mixture was recrystallized repeatedly from aqueous acetone, a fairly pure diastereomer of ester (XVb), m.p. 133 - 136°, was obtained. This was subjected to LiAlH$_4$ reduction to yield a diol (XIb), m.p. 224 - 225°, $[\alpha]_D$+ 19°, which on N-methylation let to the N-methyl compound XIIIb, m.p. 170 - 172°. Therefore, these compounds would represent 3'-iso series. The IR spectra of XIb and XIIIb in chloroform were almost superimposable with those of XIa and XIIIa, respectively.

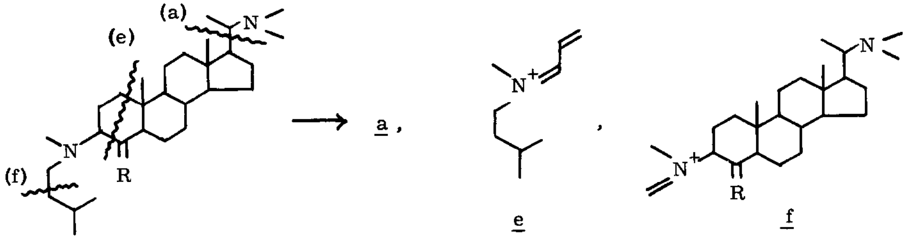
An independent support for the proposed structures, XIIIa and XIIIb, for diastereoisomeric N-methyl-diols was provided by mass



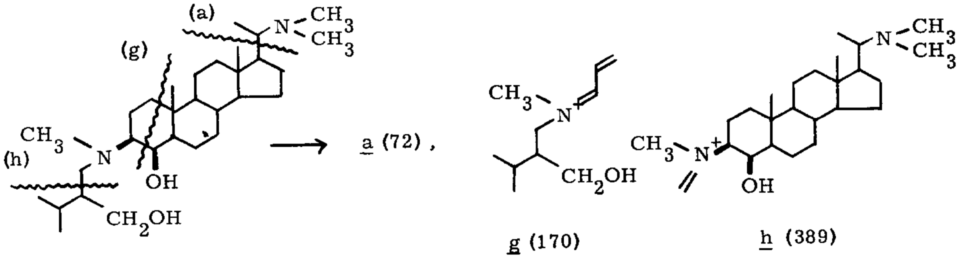
XVII R = H, OH



XVIII R = <H OH , <H OAc , = O



XIX R = <H OH , = O

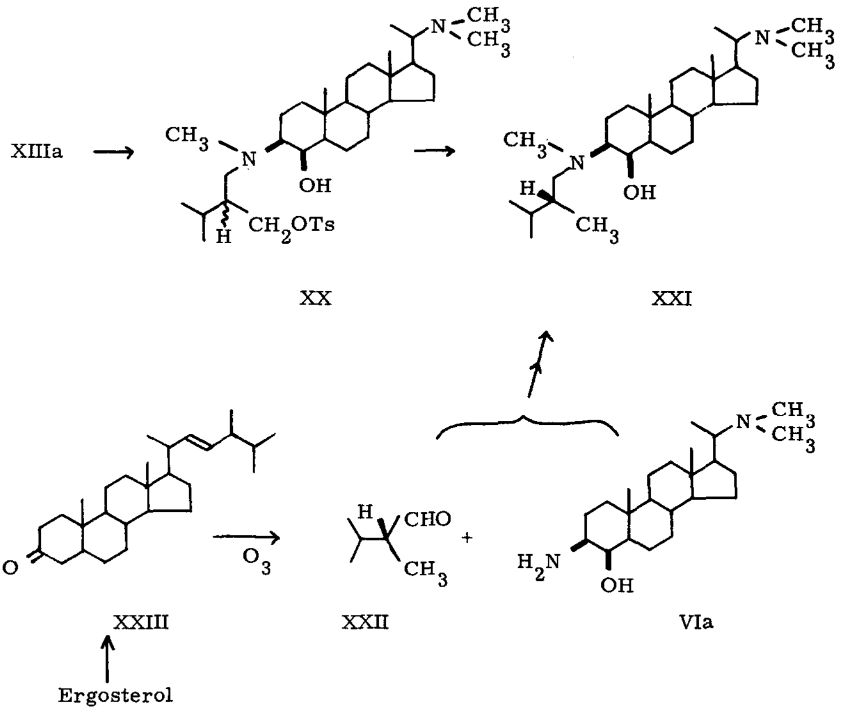


XIIIa and XIIIb

spectrometric investigation. The mass spectra of some pregnane alkaloids have been studied by several groups of workers and reported that alkaloids of the type XVII demonstrate peaks associated with the fragment ions a, b, and c.(15) We also examined the mass spectra of already established Pachysandra alkaloids having an oxygen function at 4-position and found that XVIII gave characteristic ions of m/e 72 (a, base peak) and m/e 84 (d) along with M^+ ion and M^+ - 15 ion. Compounds of type XIX showed characteristic peaks corresponding to a, e, and f.(16)

In accordance with the above observation, the N-methyl-diols, XIIIa and XIIIb, exhibited intense peaks at m/e 72 (a, base peak), 170 (g), and 389 (h) in mass spectra and their patterns were mutually very similar in support of that both are diastereoisomeric.

The confirmatory proof for the structure of pachystermine - A and - B was provided by the sequence of reactions shown below. Treatment of XIIIa with p-tosyl chloride in pyridine afforded a monotosylate (XX), $C_{37}H_{62}O_4N_2S \cdot H_2O$, m.p. 250 - 253°, which was then reduced with $LiAlH_4$ to give a desoxy compound (XXI), $C_{30}H_{56}ON_2$ (molecular ion peak at m/e 460 in mass spectrum), m.p. 170 - 173°, $[\alpha]_D + 36°$. The synthesis of this compound was achieved in the following manner : reaction of the aminoalkohol (VIa) with the aldehyde XXII obtained by ozonolysis of XXIII afforded a Schiff-base, which on successive $NaBH_4$ reduction and N-methylation as usual yielded XXI, m.p. 163 - 168°. Although the melting point was a



little low, the synthesized compound was shown to be identical with the above desoxy compound (XXI) by IR comparison (KBr) and mixed melting point determination, and also the mass spectra of both compounds are almost superimposable.

On the basis of the chemical evidences so far presented, the structures of pachystermine - A and - B could be represented by the formulas I and II, respectively.

The configuration at 3'-position of the alkaloids is considered to be

"R"(17) from the stereochemistry of the synthesized XXI. In the reaction XX ⟶ XXI, however, a possibility of apparent racemization or inversion at 3'-carbon through an azetidinium intermediate can not be excluded. Therefore, we would like to reserve the conclusion until the synthesis of 3'-iso compound of XXI is completed, which is now in progress.

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