Tetrahedron Letters No.27, pp. 1817-1823, 1964. Pergamon Press Ltd.
Printed in Great Britain.

STUDIES ON THE ALKALOIDS OF PACHYSANDRA TERMINALIS SIEB. ET ZUCC. (3). : SYSTEMATIC SEPARATION AND CHARACTERIZATION OF ALKALOIDS.

T. Kikuchi, S. Uyeo, Jr., M. Ando, and A. Yamamoto
Faculty of Pharmaceutical Sciences, Kyoto University
Yoshida-Konoe-cho, Sakyo-ku, Kyoto, Japan

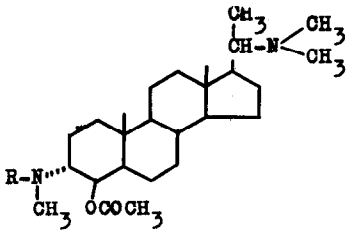
(Received 15 May 1964)

In the previous communications[1] it was reported that pachysandrine-A, pachysandrine-B, pachysamine-A, and pachysamine -B, new alkaloids isolated from Pachysandra terminalis SIEB. et ZUCC., have the structure Ia, Ib, IIa, and IIb, respectively. Present communication deals with the systematic separation and characterization of alkaloidal constituents of the same plant.

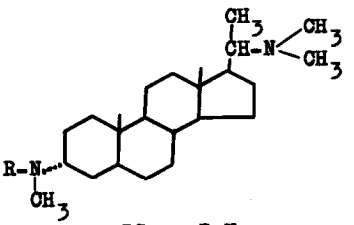
Alcoholic extract of the plant material was treated in the usual manner and the crude alkaloid mixture obtained was distributed between chloroform and 3% HCl solution to give a weak base fraction and a strong base fraction. Multi-buffer extractions[2] of these fractions, followed by alumina chromatography, afforded several crystalline alkaloids. The mother liquor of the weakly basic alkaloid fraction was then hydrolyzed with 20% KOH - EtOH, and the resulting hydrolysate was separated into a weak base and a strong base fraction, the latter of which was N-methylated by treatment with formalin - formic acid.

TABLE I.

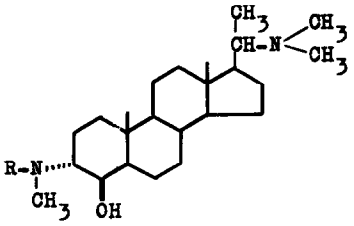
Name	Formula[3]		M.P.(°C)[4]	$[\alpha]_D$(°)[5]
(A). Weakly basic alkaloids.				
Pachysandrine-A	$C_{33}H_{50}O_3N_2$	(Ia)	235 - 236	+80
Pachysandrine-B	$C_{31}H_{52}O_3N_2$	(Ib)	187 - 189	+93
Pachysterine	$C_{28-29}H_{48-50}O_2N_2$		220 - 224	+24
Base-IV			210 - 215	
Base-V			218 - 221	
Base-VI			290 - 295	
Epipachysamine-A	$C_{26}H_{46}ON_2$	(VIa)	203 - 205	-17
(B). Hydrolysis products of the mother liquor of (A) — Weak bases.				
Pachysamine-B	$C_{29}H_{50}ON_2$	(IIb)	171 - 173	+67
Base-IX (O-desacetyl-pachysandrine-B)	$C_{29}H_{50}O_2N_2$	(IIIa)	184 - 185	+127
(C). Hydrolysis products of the mother liquor of (A) — N-methylated strong bases.				
Base-X (N-methyl-pachysamine-A)	$C_{25}H_{46}N_2$	(IIc)	165 - 166	+16
Base-XI	$C_{25}H_{44}ON_2$	(V)	201 - 202	-60
Pachysandrine-C	$C_{24}H_{44}ON_2$	(IVa)	212 - 214	
Base-XIII (O,N-desacyl-N-methylpachysandrine-A)		(IIIb)	126 - 150	
(D). Strongly basic alkaloids.				
Epipachysamine-B			260 - 262	
Base-XV			260 - 263	
Base-XVI			272 - 276	
Pachysandrine-D	$C_{29}H_{50}O_2N_2$	(IVb)	184 - 185	+2
Pachysamine-A	$C_{24}H_{44}N_2$	(IIa)	167 - 168	+20
Base-XIX	$C_{23}H_{41}O_2N$		243 - 244	



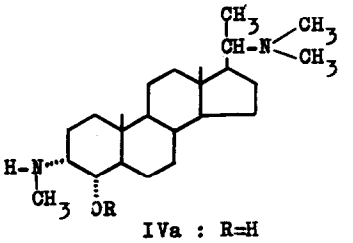
Ia : R=C_6H_5CO
Ib : R=$(CH_3)_2C=CHCO$



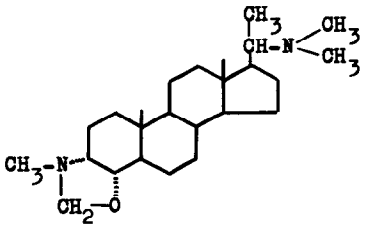
IIa : R=H
IIb : R=$(CH_3)_2C=CHCO$
IIc : R=CH_3



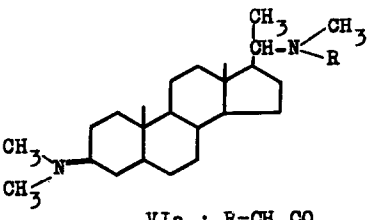
IIIa : R=$(CH_3)_2C=CHCO$
IIIb : R=CH_3



IVa : R=H
IVb : R=$(CH_3)_2C=CHCO$
IVc : R=$(CH_3)_2CHCH_2CO$



V



VIa : R=CH_3CO
VIb : R=H
VIc : R=CH_3

Working up of both fractions in the same manner as above gave additional crystalline alkaloids.

The alkaloids isolated are listed in TABLE I.

Pachysandrine-C, obtained in small amount from the N-methylated strong base fraction of alkaline hydrolysis product of the mother liquor of weakly basic alkaloids, was shown to be identical with 3α-methylamino-4α-hydroxy-20α-dimethylamino-5α-pregnane (IVa), prepared from pachysandrine-A (Ia)[1], by IR comparison, mixed melting point determination, and thin layer chromatography. Also, base-XI was demonstrated to be the oxazolidine derivative (V) of pachysandrine-C by direct comparison with the authentic sample[1](mixed melting point, thin layer chromatography, and IR and NMR spectra).

Pachysandrine-D (IVb), isolated from the strongly basic alkaloid fraction, showed IR ν_{max}^{KBr} 3370 (NH), 1710 and 1655 cm^{-1} (α,β-unsaturated ester), and NMR signals[6] at 4.30 (1H, broad, oleffinic proton), 5.04 (1H, quartet, J's 12, 4 c.p.s.; CH-C<u>H</u>(OCOR)-CH), 7.62 (3H, broad, N-CH_3), 7.85 (9H, $N(CH_3)_2$ and one CH_3-C=C), 8.10 (3H, doublet, J 2 c.p.s.; one CH_3C=C), 9.11 (3H, tert. CH_3), 9.15 (3H, doublet, J 6 c.p.s.; sec. CH_3), and 9.38 τ(3H, tert. CH_3). Upon catalytic hydrogenation over platinum oxide it gave a dihydro compound (IVc), $C_{29}H_{52}O_2N_2$, m.p. 181 - 182°, $[\alpha]_D$ -8°, IR ν_{max}^{KBr} 3340 (NH) and 1730 cm^{-1}(saturated ester), whose NMR spectrum was characterized by the disappearance of the oleffinic proton and two allylic methyl signals and

appearance of new signals due to two methyl groups (9.00 and 9.10 τ). Mild alkaline hydrolysis of pachysandrine-D yielded pachysandrine-C (IVa), $C_{24}H_{44}ON_2$, m.p. 214 - 215°, $[\alpha]_D$ -40°, whose identity was established by IR comparison and mixed melting point determination with the authentic sample.

These observations together with the relation to pachysandrine-B (Ib) led us to suppose that pachysandrine-D should be pachysandrine-C O-β,β-dimethylacrylate (IVb). An evidence was provided by the acyl migration reaction of O-desacetyl-dihydro-pachysandrine-B with conc. HCl in acetic acid[7], yielding the ester IVc, $C_{29}H_{52}O_2N_2$, m.p. 181 - 182°, $[\alpha]_D$ -6°. The IR spectrum in KBr of this substance was found to be superimposable with that of dihydro-pachysandrine-D (IVc) and also mixed melting point did not depress.

Epipachysamine-A (VIa) exhibited a tertiary amide band (1625 cm^{-1}) in the IR spectrum ($CHCl_3$). Its NMR spectrum gave a rather complicated pattern[8] : i. e. 7.21, 7.26 (3H, two peaks; amide N-CH_3), 7.72 (6H, $N(CH_3)_2$), 7.90, 7.96 (3H, two peaks; CH_3CO-), 8.71, 8.88, 8.97 (3H ?, three peaks; sec. CH_3), 9.23 (3H, tert. CH_3), and 9.23 and 9.27 τ(3H, two peaks; tert. CH_3). It remained unchanged upon alkaline or acidic hydrolysis under various conditions, but the treatment with phenyllithium in ether - benzene led to a desacyl compound (VIb), $C_{24}H_{44}N_2 \cdot \frac{1}{2}H_2O$, m.p. 96 - 98°, $[\alpha]_D$ +20°, NMR signals at 7.65 (3H, N-CH_3), 7.74 (6H, $N(CH_3)_2$), 8.93 (3H, doublet, J 6 c.p.s.; sec. CH_3), 9.24 (3H, tert. CH_3), and 9.33 τ(3H, tert. CH_3), which, on acetylation,

returned to epipachysamine-A, m.p. 203 - 205°, $[\alpha]_D$ -14°.

Treatment of this desacyl compound (VIb) with formalin - formic acid gave an N-methyl compound (VIc), m.p. 107 - 109°, $[\alpha]_D$ +12°, NMR signals at 7.73 and 7.85 τ(12H, two $N(CH_3)_2$).

Physical properties of the desacyl-epipachysamine-A and its N-methyl derivative are very close to those of 3β-dimethylamino-20α-methylamino-5α-pregnane (VIb)[9] and the 20α-dimethylamino analogue (VIc)[10], respectively.

From these experimental results, the structure of epipachysamine-A is believed to be VIa.

Acknowledgement — The authors express their deep gratitude to Prof. M. Tomita of this Faculty for his guidance and hearty encouragement.

REFERENCES

1). Part (1)., M. Tomita, S. Uyeo, Jr., and T. Kikuchi, Tetrahedron Letters, No. 18, 1053 (1964).
Part (2)., M. Tomita, S. Uteo, Jr., and T. Kikuchi, ibid, in press.
2). Y. Watanabe, M. Uchiyama, and K. Yasuda, Yakugaku-Zasshi, 77, 807 (1957) ; M. Tomita and T. Kikuchi, ibid, 77, 997 (1957).
3). All the compounds with cited empirical formulas gave satisfactory elemental analyses.
4). All the melting points are uncorrected.
5). All the optical rotations reported in this communication were taken in chloform solutions at 10 - 25°C.

6). All NMR spectra were determined on a Varian Associates recording spectrometer (A - 60) at 60 Mc. in deuterated chloroform or chloroform. Chemical shifts are reported in τ values, using tetramethylsilane as the internal reference.

7). In Part (1) of this series, the same reaction of O-desacetyl-pachysandrine-B (IIIa) was described. The resulting ester, m.p. 201 - 204°, which was first supposed to be IVb, showed a saturated ester band at 1710 cm^{-1} in its IR spectrum and hence it might be a hydrated ester. However, we did not examine further because of its poor yield and difficulty in purification.

8). This behavior may be due to the steric inhibition of free rotation of the 17β -CH(N-CH_3)CH_3 grouping.
COCH$_3$

9). E. J. Corey and W. R. Hertler, J. Am. Chem. Soc., 81, 5209 (1959).

10). M. M. Janot, F. Laine, Q. Khuong-Huu, and R. Goutarel, Bull. soc. chim. France, 111 (1962) ; V. Cerny, L. Labler, and F. Sorm, Coll. Czech. Chem. Comm., 22, 76 (1957).