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68. Tohru Kikuchi, Shoichiro Uyeo, and Toshinari Nishinaga : Pachysandra Alkaloids. X.*1 Structure of Epipachysandrine-A.*2

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In succession to the structure determination of a number of 3,20-diamino-5α-pregnane type alkaloids of *Pachysandra terminalis* Sieb. et Zucc., another new alkaloid, epipachysandrine-A, which was obtained from the weakly basic alkaloid fraction was investigated. The structure and stereochemistry of the alkaloid was proved to be represented by the formula Ia.

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In the previous papers, we reported the structure determination of a number of new steroidal alkaloids of pregnane type isolated from *Pachysandra terminalis* Sieb. et Zucc. (Japanese name : Fukki-so), a Buxaceae plant. Present paper deals with the structure elucidation of another new alkaloid, for which we proposed the name epipachysandrine-A.

Epipachysandrine-A (Ia),*4 m.p. 290~292°, $[\alpha]_D$ +12° (50 v% methanol-chloroform), is a weakly basic alkaloid which has been isolated in a small quantity from a difficult-soluble fraction in methylene chloride.[1] Elemental analyses of the alkaloid gave results supporting the empirical formula $C_{30}H_{46}O_2N_2$ and its infrared spectrum*5 in Nujol demonstrated a strong absorption band at 3400 cm^{-1} for an OH and/or an NH group and characteristic bands at 1635 and 1520 cm^{-1} for a secondary conjugated amide grouping along with phenyl absorptions (1600, 1580, and 1490 cm^{-1}).

Upon acetylation by heating with acetic anhydride in pyridine, epipachysandrine-A (Ia) gave rise to an O-acetate (Ib), m.p. 280~282°, $[\alpha]_D$ +21° ($CHCl_3$), showing characteristic absorptions at 1735 and 1230 cm^{-1} for the newly formed acetoxyl group in the infrared spectrum together with the amide bands (1660 and 1515 cm^{-1}). The O-acetate (Ib) was analyzed for $C_{32}H_{48}O_3N_2$, which was confirmed by the occurrence of molecular ion peak at m/e 508 in the mass spectrum.*6 As shown in Fig. 1, its NMR (nuclear magnetic resonance) spectrum*7 indicated evidently the presence of a geminal hydrogen with respect to the acetoxyl group (4.75τ, broad), a phenyl group (2.2~2.7τ, 5H), an N-dimethyl (7.83τ), an acetyl (7.89τ), a secondary C-methyl (9.12τ, doublet, J=6 c.p.s.), and two tertiary C-methyl groups (8.98 and 9.34τ) in the molecule.

In addition, the mass spectrum of O-acetylepipachysandrine-A (Ib) exhibited a very intense base peak at m/e 72 (a) which is the characteristic of 20-dimethylamino-pregnane alkaloids.[2] Other moderately intense peaks at m/e 122, 105, and 77 in the

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*1 Part IX. T. Kikuchi, S. Uyeo, Jr. : This Bulletin, **15**, 571 (1967).

*2 Preliminary account of this work appeared in Tetrahedron Letters, No. 16, 1749 (1966).

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*4 The alkaloid does not dissolve in chloroform in sufficient concentration to permit the NMR measurement.

*5 Infrared spectra were determined in chloroform solutions unless otherwise specified.

*6 The mass spectrum was measured on a Hitachi Mass Spectrometer RMU-6D equipped with an all-glass inlet system.

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

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

2) 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. See also Part VII of this series.

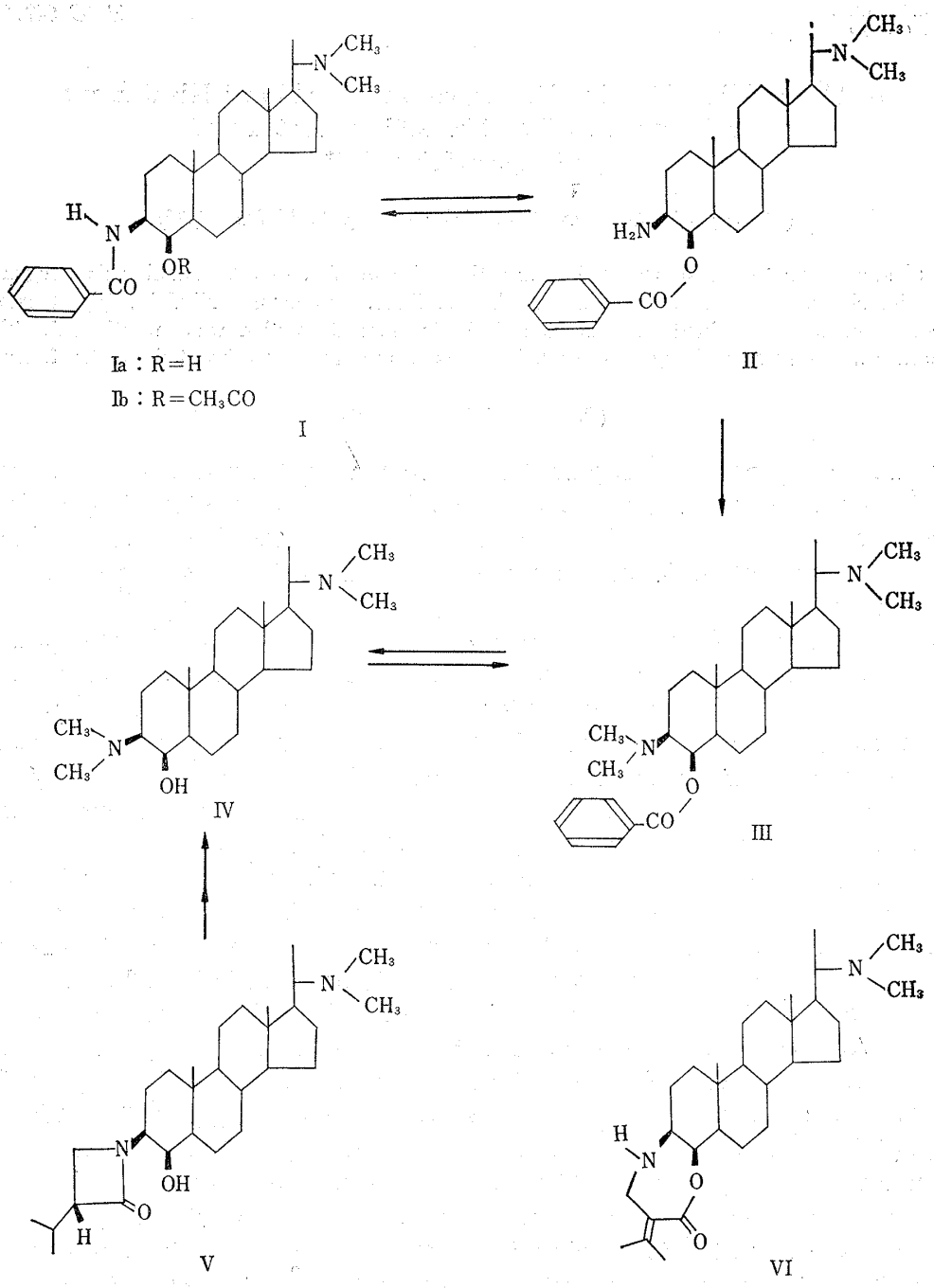

Chart 1.

spectrum would be reasonably attributed to the fragment ions b,[3] c, and phenyl cation, respectively.

The foregoing spectral data suggested strongly that epipachysandrine-A is also a member of 3-amido-20α-dimethylaminopregnane type alkaloids, like many other Pachysandra alkaloids so far established. Then the location of the oxygen function is most likely at the 4-position on the view-point of biogenetic analogy.

On this supposition, the 4-oxygen function should have β-orientation standing in the 1,3-diaxial relation with respect to the 19-methyl group and the A,B-ring juncture

3) H. Budzikiewicz, C. Djerassi D. H. Williams : "Interpretation of mass spectra of Org. Compds.," 89 (1964), Holden-Day, Inc., San Francisco.

trans, since the NMR signal (8.98τ) attributable to the 19-methyl group in O-acetylepipachysandrine-A (Ib) occurred in considerably lower field than the standard region (9.1~9.2τ).[4]

Treatment of epipachysandrine-A (Ia) with hydrochloric acid in acetic acid caused N→O acyl migration to afford an O-acyl-compound (Ⅱ), whose IR spectrum clearly demonstrated characteristic aromatic ester bands at 1715, 1280, and 1115 cm^{-1}. This compound (Ⅱ), in turn, reformed the parent alkaloid (Ia) upon base treatment. Therefore, the amido grouping at the 3-position must be in *cis*-orientation respective to the 4-hydroxyl group, because otherwise the 4-*epi* alcohol must be produced.

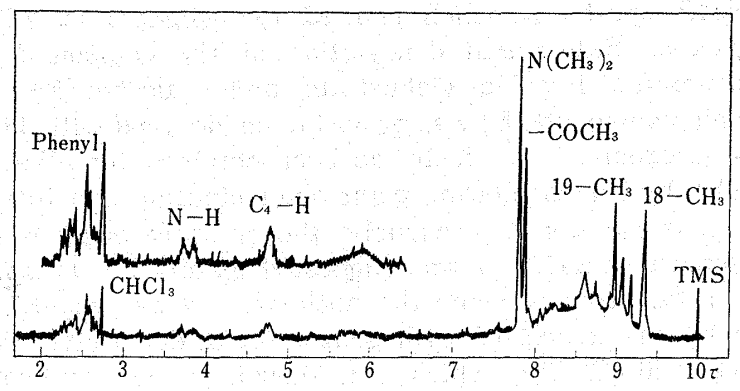

Fig. 1. Nuclear Magnetic Resonance Spectrum of O-Acetylepipachysandrine-A (Ib)

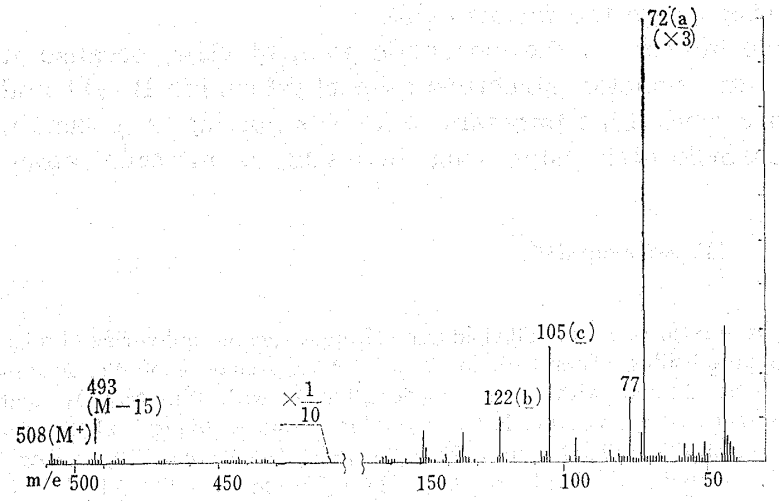

Fig. 2. Mass Spectrum of O-Acetylepipachysandrine-A (Ib)

This was proved by the following series of reactions : N-methylation of the above O-acyl compound (Ⅱ) with formalin-formic acid led to an N-dimethyl compound (Ⅲ), m.p. 165~168°, $[\alpha]_D$ +45° ($CHCl_3$). The formation of a new N-dimethyl group was evidently indicated by its

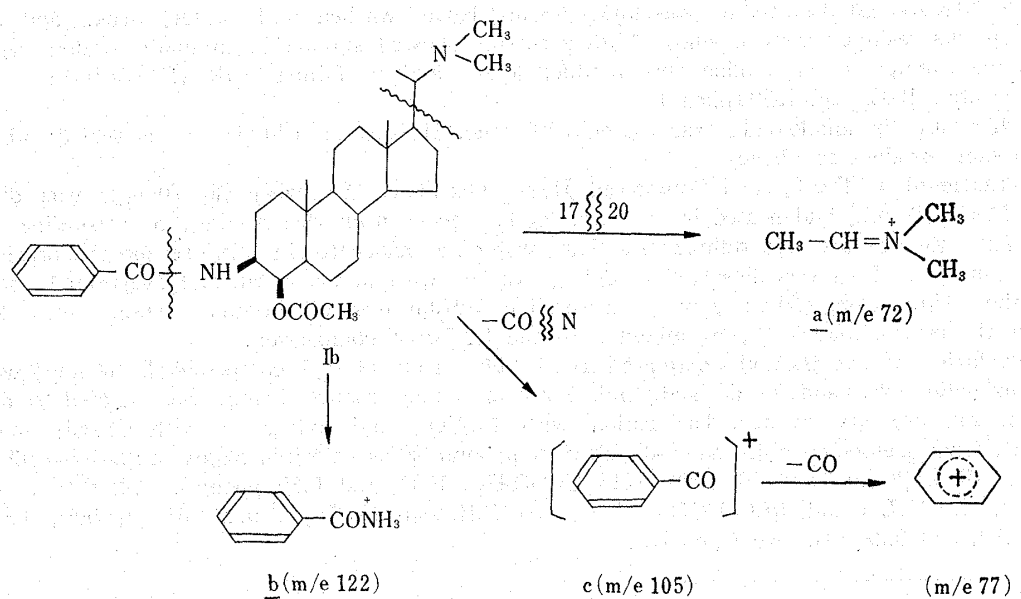

Chart 2.

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

NMR spectrum which showed two singlets at 7.70 and 7.83τ for two N-dimethyl groups. Subsequent deacylation of the compound (III) by the reduction with lithium aluminum hydride yielded an amino-alcohol (IV), m.p. 178~180°, $[\alpha]_D$ +35° ($CHCl_3$). This compound (IV) was proved to be identical with 4β-hydroxy-3β,20α-bisdimethylamino-5α-pregnane (IV), which had been obtained by alkali degradation of pachystermine-B (V),[5] by mixed melting point determination and infrared (KBr) comparison.

As mentioned previously, the presence of an N-benzoyl group at the 3-position of epipachysandrine-A was suggested mainly by the spectral evidences. The confirmation was forthcoming when the authentic 4β-hydroxy-3β,20α-bisdimethylamino-5α-pregnane (IV)[5] was condensed with benzoyl chloride to give the O-benzoate (III), m.p. 163~166°, $[\alpha]_D$ +36° ($CHCl_3$), which was shown to be identical in all respects with the above N-dimethyl-O-acyl compound (III).

On the basis of a series of evidences so far presented, the structure of epipachysandrine-A was unambiguously assigned to the formula (Ia).

This alkaloid is of considerable interest in the biogenetic point of view, because it has the same stereochemistry in the skeletal structure as pachystermine-B (V)[5] and pachysantermine-A (VI)*1 which are novel type pregnane alkaloids having a β-lactam ring and a seven-membered intramolecular ester ring involving a nitrogen atom, respectively.

Experimental*8

O-Acetylepipachysandrine-A (Ib)——A mixture of the alkaloid (Ia) (17 mg.), acetic anhydride (1 ml.), and anhydrous pyridine (1 ml.) was heated in a boiling water bath for 2 hr. After removal of the excess reagent and solvent *in vacuo*, the residue was diluted with water, made alkaline with dil. Na_2CO_3, and extracted with CH_2Cl_2. Drying of the extract and evaporation left a crystalline residue (15 mg.) which was recrystallized from acetone to give the O-acetate (Ib) (10 mg.), m.p. 280~282°, $[\alpha]_D^{25}$ +21° (c= 1.0). *Anal.* Calcd. for $C_{32}H_{48}O_3N_2$: C, 75.55; H, 9.51. Found: C, 75.64; H, 9.75. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (NH), 1735, 1230 ($OCOCH_3$), 1660, 1515 (secondary conj. amide), 1600 and 1580 (phenyl). NMR (τ): 2.2~2.7 (5H, phenyl), 3.76 (1H, doublet, J=8 c.p.s.; NH–CH), 4.75 (1H, broad, RCOO–CH), 5.83 (1H, broad, RCON–CH–), 7.83 (6H, $N(CH_3)_2$), 7.89 (3H, $COCH_3$), 9.12 (3H, doublet, J=6 c.p.s.; *sec* CH_3), 8.98, and 9.34 (6H, two *tert* CH_3).

Acid Treatment of Epipachysandrine-A (Ia)——A solution of the alkaloid (Ia) (70 mg.) in acetic acid (3 ml.) and 15% HCl (20 ml.) was heated in a boiling water bath for 6 hr. After cooling, the mixture was basified with NH_4OH and the product was taken up in CH_2Cl_2, washed with water, dried, and evaporated to leave a viscous residue (70 mg.), whose NMR spectrum showed signals in aromatic proton region (1.8~2.7τ). All the attempts to crystallize this product (II) resulted in failure. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1280, 1115 (O-benzoate), 1600, and 1580 (phenyl).

In another run, the alkaloid (Ia) was heated with conc. HCl-acetic acid (1:1) in a sealed tube at 100° to give the same product as above.

Base Treatment of the O-Acyl Compound (II)——The above O-benzoate (II) (70 mg.) was dissolved in 5% NaOH-MeOH (20 ml.) and heated in a water bath. Soon after the heating, a crystalline solid was deposited. After the mixture was refluxed for 5 hr., water was added to the mixture and the precipitate was collected by suction. This was dissolved in $CHCl_3$, washed with water, dried, and evaporated. Recrystallizations of the residue from CH_2Cl_2 gave a crystalline substance (small prisms, 60 mg.), m.p. 288~290°, identified as the parent alkaloid (Ia) by mixed m.p. and IR (Nujol) comparison.

N-Methylation of the O-Acyl Compound (II)——The crude O-acyl compound (II) (60 mg.) was heated with 37% formalin (1 ml.) and formic acid (1 ml.) for 4 hr. The product (40 mg.) was isolated by dilution of the reaction mixture with water, basification with Na_2CO_3, and extraction with CH_2Cl_2 and it was recrystallized from acetone to give the N-dimethyl compound (III) as colorless plates, m.p. 165~168°, $[\alpha]_D^{27}$ + 45° (c=1.0). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1280, 1115 ($OCOC_6H_5$), 1600, and 1580 (phenyl). NMR (τ): 1.82~2.70 (5H, phenyl), 4.32 (1H, broad, RCOO–CH–), 7.70, 7.83 (12H, two $N(CH_3)_2$), 9.13 (3H, doublet, J=6 c.p.s.; *sec* CH_3), 8.90, and 9.38 (6H, two *tert* CH_3).

*8 All the melting points were determined on a Yanagimoto Micro Melting Point Apparatus and are uncorrected. All the specific rotations were measured in chloroform solution unless otherwise specified. For drying the solutions of bases, anhydrous potassium carbonate was used.

5) Part VIII. T. Kikuchi, S. Uyeo, Jr.: This Bulletin, **15**, 549 (1967).

Deacylation of the N-Dimethyl-O-acyl Compound (III)——The compound (Ⅲ) (20 mg.) was refluxed with lithium aluminum hydride (50 mg.) in tetrahydrofuran (20 ml.) for 4 hr. The product, isolated by the usual working up, was recrystallized from acetone to give the O-desacyl compound (Ⅳ) in colorless prisms (10 mg.), m.p. 178～180°, $[\alpha]_D^{25}$ +35° (c=1.0). This was identified with an authentic sample of 4β-hydroxy-3β,20α-bisdimethylamino-5α-pregnane (Ⅳ), derived from pachystermine-B (Ⅴ), by mixed m.p. and IR (KBr) comparison.

4β-Benzoyloxy-3β,20α-bisdimethylamino-5α-pregnane (III)——To a solution of 4β-hydroxy-3β,20α-bisdimethylamino-5α-pregnane (Ⅳ) (27 mg.) in pyridine (0.6 ml.) was added benzoyl chloride (1 ml.) and the mixture was heated for 10 min. on a water bath and then kept at room temperature overnight. Thereafter, the mixture was poured into ice-water, basified with NH_4OH, and extracted with CH_2Cl_2. Washing of the extract with water, drying, and evaporation afforded a residue (35 mg.) which was purified by filtration through alumina column (2 g. : 0.6 ×5 cm.) in benzene-ether. The filtrate was evaporated (33 mg.) and recrystallized from acetone to give the pure O-benzoate (Ⅲ) as colorless plates, m.p. 163～166°, $[\alpha]_D^{25}$ +36° (c=0.5). The IR spectrum (KBr) was shown to be superimposable upon that of N-dimethyl-O-acyl compound (Ⅲ) derived from epipachysandrine-A (Ia) and also the melting point of their mixture showed no depression.

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