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158. Osamu Tanaka, Masahiro Nagai, and Shoji Shibata : Chemical Studies on the Oriental Plant Drugs. XVI.[*1] The Stereochemistry of Protopanaxadiol, a Genuine Sapogenin of Ginseng.[*2]

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As reported in our preceding paper1), protopanaxadiol (I) was shown as being the genuine sapogenin of ginsenosides-Rb_1, -Rb_2, and -Rc, the neutral saponins of Ginseng roots. Panaxadiol (II) was proved to be an artifact derived from protopanaxadiol (I) during the acid hydrolysis of these saponins.

Prior to the study on the genuine sapogenin, the configurations of A, B, and C rings of panaxadiol (II) were elucidated by the correlation of II to isotirucallenol.2) The configuration of the hydroxyl at C-12 was proved to be β (equatorial) by the chemical and spectroscopical studies comparing with its 12-epimer (III).

Regarding to the stability of the skeletal configuration of 3-O-acetylpanaxanolone (IV) to alkali, two alternative stereochemical structure (13-H/17-H *trans*), C/D *trans* 17α-H and C/D *cis* 17β-H, were proposed for panaxadiol (II). Regeneration of panaxadiol (II) from IV by reduction with sodium and isopropanol (see experimental part of this paper) excluded the possibility of the inversion of the skeletal configuration during the process of the oxidation of 3-O-acetylpanaxadiol to IV. As the presence of

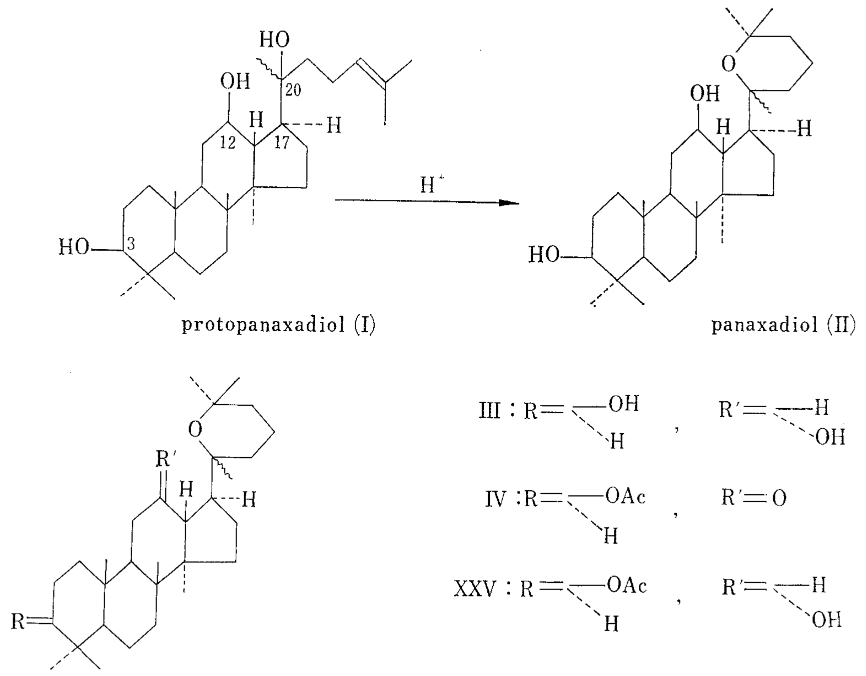

Chart 1.

[*1] Part XV. S. Shibata, I. Kitagawa, H. Fujimoto : This Bulletin, **14**, 1023 (1966).

[*2] The preliminary report of this work appeared in Tetrahedron Letters, **1964**, 2291.

[*3] Hongo, Tokyo (田中 治, 永井正博, 柴田承二).

1) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, T. Ohsawa : This Bulletin, **14**, 595 (1966) (preliminary report : Tetrahedron Letters, **1963**, 795).

2) S. Shibata, M. Fujita, H. Itokawa, O. Tanaka, T. Ishii : This Bulletin, **11**, 759 (1963) (preliminary report : Tetrahedron Letters, **1962**, 419).

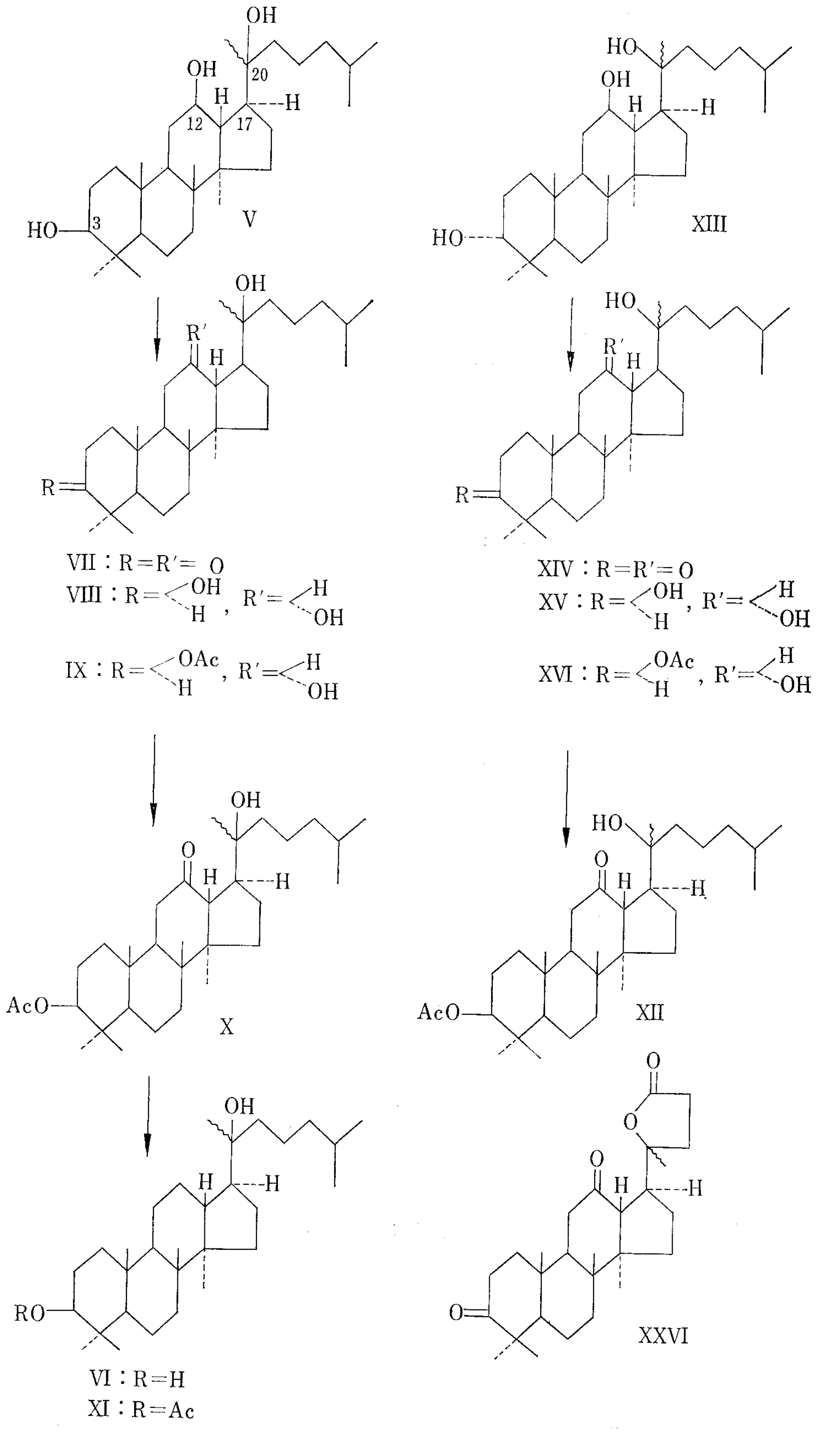

Chart 2.

intramolecular hydrogen bonding between 12-hydroxyl and the oxygen of the tetrahydropyrane ring in 12-epi-panaxadiol (Ⅲ) was observed, the C/D *cis* 17β-H structure was seemed to be more probable for panaxadiol (Ⅱ).[3]

However, the stereochemical study of dihydroprotopanaxadiol (V), with which the present paper mostly concerns, has led us to amend the former proposal for the stereochemistry of C/D ring juncture, and protopanaxadiol and panaxadiol have now been formulated as I and Ⅱ (C/D trans 17α-H), respectively.*2

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3) S. Shibata, O. Tanaka, M. Nagai, T. Ishii: This Bulletin, **11**, 763 (1963) (preliminary report: Tetrahedron Letters, **1962**, 1239).

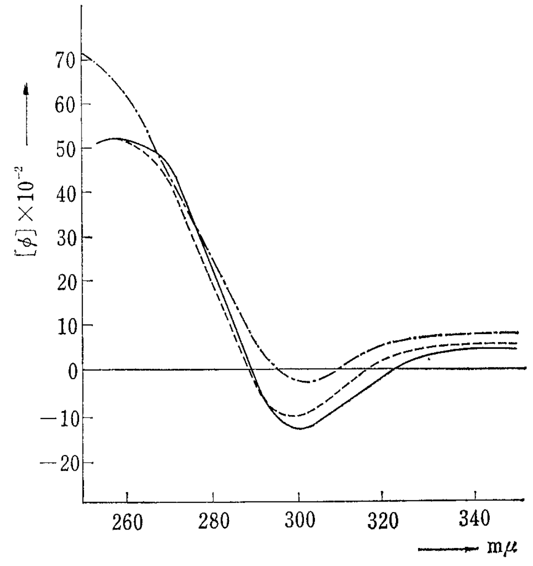

Fig. 1. Optical Rotatory Dispersion Curves (in methanol)

——— : X - - - - - : XII —·—·— : IV

The stereochemical correlation of dihydroprotopanaxadiol (V) with dammarane-diol-I (VI)$^{4)}$ has been established as follows: The chromic acid oxidation of V afforded a diketone (VII), which was reduced with lithium aluminum hydride to give 12-epi-dihydroprotopanaxadiol (VIII, 12α-OH, axial). The selective acetylation of 3-hydroxyl (equatorial) of VIII was achieved by the action of acetic anhydride and pyridine at 4°C to yield the monoacetate (IX), which on oxidation with chromic acid afforded 3-O-acetyl-12-keto derivative (X). Since dihydroprotopanaxadiol (V) was reproduced from X by the reduction with sodium and isopropanol, it is evident that no inversion of the skeletal configuration is involved in the above reaction process. As the optical rotatory dispersion curve of X resembled to that of 3-O-acetylpanaxanolone (IV), X should have the same stable skeletal configuration as that of IV (C/D *trans* 17α-H or C/D *cis* 17β-H) and no change of the skeletal configuration took place during the process of the acid catalyzed cyclization of the side chain of protopanaxadiol (I) converting into panaxadiol (II). On the modified Wolff-Kishner reduction$^{5)}$ followed by acetylation, X yielded a compound, m.p. 146°, $[\alpha]^{20}_{D}$+37.6° ($CHCl_3$), which was proved to be identical with 3-O-acetyldammaranediol-I (XI)$^{4)}$ by a mixed fusion and comparison of the IR spectra, and thin layer

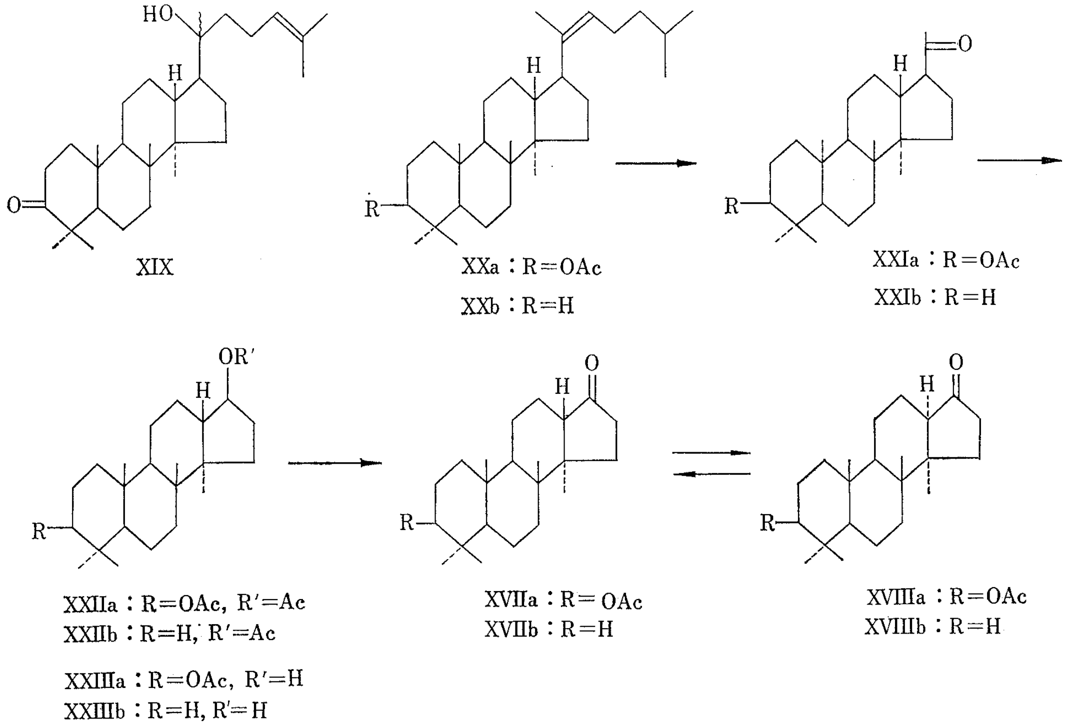

Chart 3.

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4) J. S. Mills, *et al.*: J. Chem. Soc., **1955**, 3132; *Ibid.*, **1956**, 2196.
5) M. G. F. Grundon, H. B. Henbest, M. D. Scott: *Ibid.*, **1963**, 1855.

chromatograms with authentic sample. The optical rotatory dispersion curve of X was almost superimposable with that of the 3β-O-acetyl-12-keto derivative (XII), prepared from betulafolianetriol (XIII)[6a,b] (3-epi-12β-hydroxydammaranedilol-II[*4]) via XIV, XV, and XVI (Fig. 1). Consequently, now it can be concluded that dihydroprotopanaxadiol (V) is formulated as 12β-hydroxydammarane-diol-I which differs from betulafolianetriol (XIII) in the configurations at C-3 and C-20.

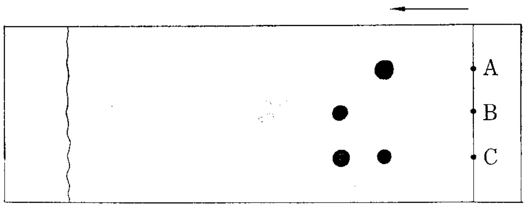


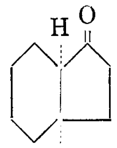
Fig. 2. Thin-Layer Chromatograms

on silica gel G, solvent: benzene
A: XVIIb B: XVIIIb
C: an equilibrated mixture of XVIIb and XVIIIb

Ourisson, *et al.*[7] have already proposed a C/D *trans* 17α-H structure for dammarane nucleus. The C/D *trans* system was based on the study of the optical rotatory dispersion curves of 17-keto derivative (XVIIa) (positive Cotton effect) and its C-13 isomer (XVIIIa) (negative Cotton efffect)[8] prepared from dipterocarpol (XIX) (3-keto derivative of dammarenediol-II) via XXa, XXIa, XXIIa, and XXIIIa.

We prepared the 3-deacetoxy derivative of XVIIa (XVIIb) from betulafolianetriol (XIII) via XXb, XXIb,[6b] XXIIb, and XXIIIb. On treatment with alkali or acid,[9] or even by the process of column chromatography on neutral alumina, this 17-keto compound (XVIIb) (positive Cotton effect) was partly converted into its C-13 isomer (XVIIIb) to form an equilibrated mixture. On recrystallization of this mixture from ethanol, the isomer (XVIIIb) was isolated which showed a negative Cotton effect.

The configuration of the C/D ring fusion of dammarane nucleus has also been proved by the study on the methyl signals of the NMR spectra of XVIIb and XVIIIb. Recently, Lehn, *et al.* have studied on the NMR spectra of the various types of triterpenes including dammarane type compounds[10,11] to discuss the chemical shifts of methyl signals. Referring their works, the methyl signals of XVIIb have been assigned as follows: τ 8.96 (singlet, 1-C$\underline{\mathrm{H}}_3$) C-8 CH$_3$; 9.12 (singlet, 3H) C-14 CH$_3$; 9.15 (singlet, 6H) and 9.20 (singlet, 3H) methyls on A ring. The shifting of the methyl signals by the isomerization of XVIIb into XVIIIb are consistent with the formulation of XVIIIb as a C/D *cis* fused structure (consequently XVIIb *trans*). It has been observed that the angular methyl signal at 3a-position of the *cis* hexahydro-1-hydrindanone derivative (XXIV) appears in the markedly lower field than that of the *trans* isomer.[10~12] The lowest methyl signal (τ 8.75) in the NMR spectrum of XVIIIb can be assigned for the angular methyl at C-14. Two (or three) out of four other methyl signals of XVIIIb are observed in the higher field than those of XVIIb. This can be explained by the anisotropic effect of



XXIV

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*4 Fischer and Seiler reported that the diketo-trisnorlactone (XXVI) derived from betulafolienetriol underwent the inversion of the configuration of C-13 by alkaline treatment.[6b] However, such an inversion has not been observed in our re-examination of their experiment. Dammaranediol-II is the C-20 epimer of dammaranediol-I.[4]

6) *a*) F.G. Fischer, N. Seiler: Ann., **626**, 185 (1959). *b*) F.G. Fischer, N. Seiler: *Ibid.*, **644**, 146, 162 (1961). The isolation of betulafolienetriol from Japanese white birch leaves (***Betula platyphylla*** Sukatchev var. ***joponica*** (Miq.) Hara, S. Shibata, M. Nagai, O. Tanaka: Shôyakugaku Zasshi (Jap. J. Pharmacog.), **18**, 27 (1964).

7) J.F. Bielmann, C. Crabbé, G. Ourisson: Tetrahedron, **3**, 303 (1958).

8) Ref. W. Klyne: Tetrahedron, **13**, 29 (1961).

9) J.F. Bielmann, D. Francetić, G. Ourisson: Tetrahedron Letters, **1960**, 4.

10) J.-M. Lehn: Bull. soc. chim. France, **1963**, 1832.

11) J.-M. Lehn, G. Ourisson: *ibid.*, **1962**, 1137; J.-M. Lehn: *Ibid.*, **1963**, 1702; Tetrahedron, **19**, 2255 (1963).

12) R.F. Zürcher: Helv. Chim. Acta, **46**, 2054 (1963); A.I. Cohen, S. Rock Jr.: Steroids, **3**, 245 (1964).

17-carbonyl group to C-8 and C-10 methyls (or to the axial methyl at C-4) in XVIIIb. Accordingly it can be now concluded that protopanaxadiol (I) as well as panaxadiol (II) should have a C/D *trans* 17α-H structure.

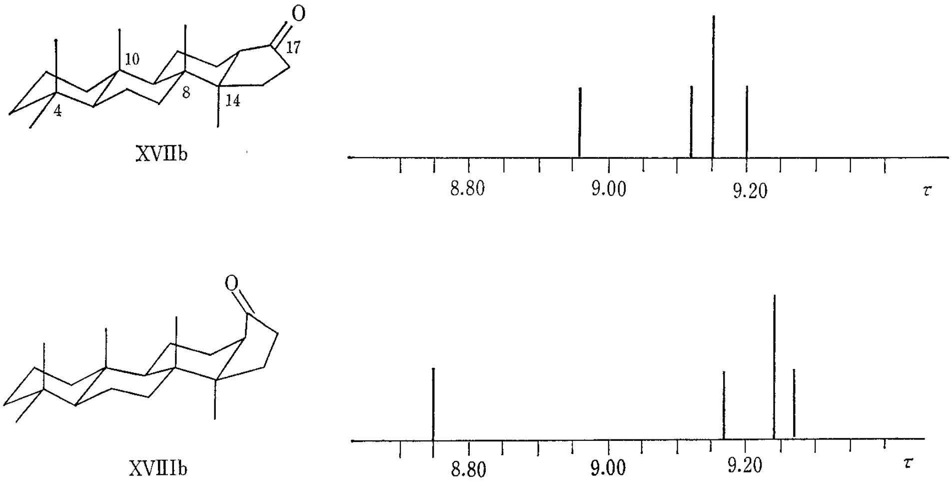

Fig. 3. Nuclear Magnetic Resonance Spectra of XVIIb and XVIIIb (in $CDCl_3$, 60 Mc.)

On oxidation with chromic acid, 3-O-acetyl-12-epi-panaxadiol (XXV) afforded IV. Therefore, it is evident that no stereochemical change of the nucleus is involved in the process of the formation of 12-epi-panaxadiol (III) from panaxadiol (II). Consequently, 12-epi-panaxadiol (III) also should have the C/D *trans* 17α-H structure. The intramolecular hydrogen bonding observed in III would be due to an anomalous distortion of molecule.

Experimental[*5]

Oxidation of Dihydroprotopanaxadiol (V)——To a solution of dihydroprotopanaxadiol (V) (1.0 g.) in acetone (200 ml.) was added Jones reagent[13] (4.9 ml.) and the reaction mixture was allowed to stand at room temperature for 3 hrs. After working up in the usual way, the product was crystallized from ether to give the diketone (VII), colorless crystals, m.p. 124~126°, $[\alpha]_D^{17}$ +54.4°(c=1.28, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ 3490 (intramolecularly hydrogen bonded OH), 1698 cm^{-1}(intramolecularly hydrogen bonded six membered C=O), *Anal.* Calcd. for $C_{30}H_{50}O_3$: C, 78.55 : H, 10.99. Found : C, 78.32; H, 10.79.

3-O-Acetyl-12-epi-dihydroprotopanaxadiol (IX)——A solution of the diketone (VII) (1.6 g.) in absolute ether (100 ml.) was added gradually to suspension of $LiAlH_4$ in absolute ether (100 ml.) and the reaction mixture was refluxed for 40 min. The product was crystallized from ethyl acetate to give 12-epi dihydroprotopanaxadiol (VIII), colorless crystals, m.p. 212~214°, which was subjected to acetylation without further purification.

A solution of VIII in a mixture of pyridine (50 ml.) and acetic anhydride (25 ml.) was allowed to stand at 4° for 22 hr. Working up in the usual way, the product was recrystallized from ethyl acetate to afford 3-O-acetyl-12-epi-dihydroprotopanaxadiol (IX), colorless crystals, m.p. 216~217°, $[\alpha]_D^{17}$ + 43.0° (c = 1.15, $CHCl_3$), *Anal.* Calcd. for $C_{32}H_{56}O_4$: C, 76.14; H, 11.18. Found : C, 76.04; H, 11.18.

Oxidation of IX to 3-O-Acetyl-12-keto-Derivative (X)——3-O-Acetyl-12-epi-dihydroprotopanaxadiol (IX) (0.5 g.) was oxidized with Jones reagent[13] in acetone (in the usual way). The product was crystallized from 90% aqueous acetone to give X, colorless crystals, m.p. 160~161°, $[\alpha]_D^{17}$ +38.9°(c=1.09, $CHCl_3$). IR $\nu_{max}^{CS_2}$ 3523 (OH), 1743 $\left(CH_3-\underset{\underset{O}{\|}}{C}-O-\right)$ and 1702 cm^{-1}(six-membered C=O), *Anal.* Calcd. for $C_{32}H_{54}O_4$: C, 76.44; H, 10.83. Found : C, 76.79; H, 10.73.

[*5] All melting points were measured on a Kopfler block and uncorrected. NMR spectra were obtained in $CDCl_3$ solution by a Japan Optics Lab. 3H-60 NMR spectrometer (60 Mc). Optical activities were measured with a Yanagimoto Photo magnetic Direct Reading Polarimeter, Model OR-20, and optical rotatory dispersion curves were obtained by a spectrophotometer model ORD/UV-5, Japan Spectroscopic Co., Ltd.

13) K. Bowden, I. M. Heilbron, E. R. H. Jones, B. C. L. Weedon : J. Chem. Soc., **1946**, 39.

3-O-Acetyldammaranediol-I (XI) from X——3-O-Acetyl-12-keto derivative (X) (0.2 g.) was refluxed with hydrazine hydrate (80%, 3 ml.) in ethanol (8 ml.) for 2 hr. The cooled reaction mixture was diluted with water and the resulted precipitate was collected by filtration and dried to give the hydrazone of X, which was subjected to the reduction without further purification. A mixture of the above hydrazone and potassium *tert*-butoxide (freshly sublimed, 0.2 g.) in absolute toluene (5 ml.) was refluxed for 4 hr. After cooling, 2*N* HCl was added and the mixture was extracted with ether. The etheral solution was washed with 2*N* HCl and water successively, dried, and evaporated to dryness. The residue was acetylated with pyridine (4 ml.) and acetic anhydride (2 ml.) at room temperature by standing overnight. The product was purified by chromatography on Florisil and crystallized from methanol to give colorless crystals, m.p. 146~147°, $[\alpha]_D^{20}$ +37.6° (c=1.02, $CHCl_3$), which was comfirmed to be identical with 3-O-acetyldammaranediol-I (XI) by mixed fusion and comparison of optical rotation, IR spectra, and thin layer chromatograms*6 with an authentic sample.

Reduction of 3-O-Acetyl-12-keto Derivative (X) to Dihydroprotopanaxadiol (V)——A mixture of X (0.3 g.) and sodium (1,7 g.) in isopropanol was refluxed for 40 min. After working up in the usual way, the product was crystallized from acetone to give colorless crystals, m.p. 246~248°, which was proved to be identical with dihydroprotopanaxadiol (V) by mixed fusion, and comparison of IR spectra and thin layer chromatograms with an authentic sample.

3-O-Acetyl-12-keto Derivative (XII) from Betulafolianetriol (XIII)——The diketone (XIV) (1.5 g.), prepared from betulafolianetriol (XIII) according to the procedure of Fischer, *et al.*,[6] was reduced by refluxing with $LiAlH_4$ (2.9 g.) in ether (100 ml.) for 40 min. The product (XV) was subjected to acetylation under the same condition as the acetylation of VIII to IX. The crude acetate was purified by chromatography on neutral alumina to give the monoacetate (XVI), whith was oxidized with Jones reagent under the same condition as that of the oxidation of IX to X, yielding 3β-O-acetyl-12-keto derivative (XII) of betulafolianetriol, m.p. 167~169° (from *n*-hexane), $[\alpha]_D^{17}$ +42.8° (c=1.06, $CHCl_3$), $C_{32}H_{54}O_4$: C, 76.44; H, 10.83. Found : C, 76.64; H, 10.83.

Treatment of the Diketo-trisnorlactone (XXVI) with Alkali (Reexamination of Fischer's Experiment)[6]——The diketo-trisnorlactone (XXVI) (m.p. 235~237°, $[\alpha]_D^{15}$ +117° (c=0.72, $CHCl_3$), lit.[6b] m.p. 229~232°, $[\alpha]_D$ +122° ($CHCl_3$)) was prepared from betulafolienetriol by chromic acid oxidation in acetic acid according to the procedure of Fischer and Seiler.[6b] This diketo-trisnorlactone (XXVI) (200 mg,) was heated under reflux with 0.1*N* KOH in methanol (66 ml.) for 30 min. The reaction mixture was acidified with dil. HCl and the resulted precipitate was collected by filtration and crystallized from methanol to give the starting material. Treatment of XXVI even with 0.25*N* KOH in 50% aqueous methanol by refluxing for 1.5 hr. also gave the unchanged starting material, m.p. 236~238°, $[\alpha]_D^{17}$ +112° (c=1.25, $CHCl_3$) in a fair yield and the formation of the compound (m.p. 225°, $[\alpha]_D$ +71° ($CHCl_3$)) reported by Fischer and Seiler was not observed.

Baeyer-Villiger Oxidation of 3,12-Bisdeoxy-hexanor-20-Keto Derivative (XXIb)——3,12-Bisdeoxy-hexanor-20-keto derivative (XXIb) was prepared from betulafolianetriol by the procedure reported by Fischer and Seiler.[6b] A solution of trifluoroperacetic acid in CH_2Cl_2, prepared from trifluoroacetic anhydride (4.2 ml.) and 90% H_2O_2 (0.72 ml.). was added dropwise during 25 min. to a mixture of XXIb (1.2 g.) and Na_2HPO_4 (9.0 g.) in CH_2Cl_2 under ice cooling and stirring. After stirring for additional 50 min. at room temperature, the reaction mixture was diluted with water and extracted with ether. The ether layer was washed with water, 2*N* Na_2CO_3 solution and water, dried and concentrated to dryness. The residue was recrystallized from methanol to give colorless crystals (XXIIb) (700 mg.), m.p. 135~137°, $[\alpha]_D^{15}$ +58.8° (c=0.99, $CHCl_3$). IR 1742, 1255 cm^{-1} in CCl_4 (acetate). *Anal.* Calcd. for $C_{24}H_{40}O_2$: C, 79.94; H; 11.18. Found : C, 79.84; H, 10.92.

The Octanor-17-keto Derivative (XVIIb)——To a solution of the acetate (XXIIb) (700 mg.) in ethanol (50 ml.) was added 1*N* KOH (10 ml.) and the solution was allowed to stand at room temperature for 16 hr. The reaction mixture was diluted with water and extracted with ether. The ether layer was washed with water, dried, and evaporated to dryness to give a crystaline residue (XXIIIb) which was subjected to oxidation without further purification. To a solution of XXIIIb (100 mg.) in acetic acid (4 ml.) was added a solution of $Na_2Cr_2O_7$ (350 mg.) in acetic acid (0.5 ml.) and the mixture was allowed to stand at room temperature for 19 hr. Dilution of the mixture with water afforded a crystaline precipitate, which was collected, washed with water, and recrystallized from methanol to give colorless crystals, XVIIb, m.p. 149~151°, IR $\nu_{max}^{CCl_4}$ 1748 cm^{-1} (five membered C=O). *Anal.* Calcd. for $C_{22}H_{36}O$: C, 83.48; H, 11.47. Found : C, 83.41; H, 11.38. ORD : (c=0.118, MeOH) $[\alpha]_{590}^{16}$ +106°, $[\alpha]_{440}$ 212°, $[\alpha]_{350}$ +742°, $[\alpha]_{330}$ +1460°, $[\alpha]_{320}$ +2420°, $[\alpha]_{309}$ +3280° (peak), $[\alpha]_{292}$ ±0, $[\alpha]_{272}$ −3370° (trough), [A]=+211.

The same compound (XVIIb) was obtained by the Jones oxidation of XXIIIb. To a solution of XXIIIb (900 mg.) in acetone (30 ml.) was added dropwise Jones reagent[13] during 30 min. until constant orange color was obtained. The reaction mixture was diluted with water and the resulted precipitate was recrystallized from methanol to give XVIIb, m.p. 149~151°. The thin layer chromatogram of this compound was shown in Fig. 2.

[*6] Thin layer chromatography; on Silica gel G, coloring reagent H_2SO_4 (heated at 100° after spraying 10% H_2SO_4), solvent-$CHCl_3$ for XI and a mixture of $CHCl_3$-ether-MeOH (1:1:trace) for V.

The Isomerization of the Octanor-17-keto Derivative (XVIIb)——The 17-keto derivative was dissolved in 0.4*N* ethanolic KOH and the solution was heated on a water bath for 5 min. The solution was diluted with water and the precipitate was collected, washed with water and dried. The thin layer chromatograms of this precipitate showed the presence of XVIIb and its isomer (XVIIIb) (see Fig. 2). The isolation of XVIIIb from this mixture was furnished by recrystallization from ethanol. Recrystallization of this mixture from ethanol gave colorless crystals (XVIIIb), m.p. 178~181°, IR $\nu_{\max}^{\mathrm{CCl_4}}$ 1744 cm^{-1} (five membered C=O). *Anal.* Calcd. for $C_{22}H_{36}O$: C, 83.48; H, 11.47. Found : C, 83.38; H, 11.53. ORD : (c=0.110, MeOH) $[\alpha]_{590}^{20}$ −72.6°, $[\alpha]_{440}$ −145°, $[\alpha]_{350}$ −472°, $[\alpha]_{330}$ −835°, $[\alpha]_{320}$ −1310°, $[\alpha]_{305}$ −1910° (trough), $[\alpha]_{289}$ ±0°, $[\alpha]_{268}$ +1870° (peak), $[A] = -120$.

The compound (XVIIb) was also easily isomerized to give the equilibrated mixture. The separation of this mixture by chromatography was failed due to this rapid equilibration of both isomers on neutral alumina.

Reductin of 3-O-Acetylpanaxanolone (IV)——3-O-Acetylpanaxanolone (IV) (500 mg.) was reduced by refluxing with sodium (4 g.) in isopropanol (35 ml.). After working up in the usual way, the crude product was acetylated with acetic anhydride (8.2 ml.) and pyridine (16.4 ml.) (100° for 4.5 hr.). The crude acetate was chromatographed on Florisil (25 g.) to give 3,12-di-O-acetyl-12-epi-panaxadiol, m.p. 181~184° (from methanol) (90 mg.) and 3-O-acetylpanaxadiol, m.p. 217~219° (from methanol) (50 mg.). The identifications of both compounds were established by mixed melting points, and comparison of IR spectra, and thin layer chromatograms (on Silice gel G, solvent; $CHCl_3$) with authentic samples, respectively.

Oxidation of 3-O-Acetyl-12-epi-panaxadiol (XXV) to 3-O-Acetylpanaxanolone (IV)——3-O-Acetyl-12-epi-panaxadiol (XXV)[3] (200 mg.) was subjected to Jones oxidation[13] in the usual way to give 3-O-acetylpanaxanolone (IV), m.p. 187~189° (from methanol) (130 mg.). The identification was confirmed by mixed melting point, and comparison of IR spectra, and thin layer chromatograms (on Silica gel G, solvent; $CHCl_3$).

We are grateful to Dr. J. S. Mills, National Gallery, London, for kindly supply of the samples, to Miss M. Ohnishi and Dr. Y. Kawazoe, National Cancer Research Center Institute, Tokyo, for NMR spectral measurements, and to Prof. G. Ourisson, University of Strasbourg, France, for his communication. Thanks are also due to the members of Tokyo Kyoiku University Forest, Nobeyama, for supplying fresh leaves of Japanese white birch, to the members of Analytical Laboratory of this Faculty for elemental analysis, IR and ORD measurements, and to the Research Laboratory of Takeda Chemical Industry Co., Ltd., for the extraction of materials. We thank Ministry of Education of Japan and Yakurikenkyu-kai for grants.

Summary

The stereochemical correlation of dihydroprotopanaxadiol (V) with dammaranediol-I (VI) has been established. It has been concluded that protopanaxadiol and panaxadiol should have C/D trans 17β-H configuration and can be formulated as I and II respectively.

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