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159. Shoji Shibata, Toshio Ando,*1 and Osamu Tanaka : Chemical Studies on the Oriental Plant Drugs. XVII.*2 The Prosapogenin of the Ginseng Saponins (Ginsenosides-Rb_1, -Rb_2, and -Rc)

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In the preceding papers,*2,1) the structure of protopanaxadiol (I), the genuine sapogenin of ginseng saponins, ginsenosides-Rb_1, -Rb_2, and -Rc, has been elucidated. The present report deals with the study on the prosapogenin obtained from these saponins by the partial hydrolysis.

Previously, Kotake described α-panaxin, a prosapogenin, which was prepared from the neutral saponin mixture of ginseng roots by the hydrolysis with boiling 0.5% sulfuric acid in 50% aqueous methanol.[1,2] However, his method is not suitable to obtain a pure prosapogenin with constant physical properties. On treating ginsenoside-Rb_1, -Rb_2, -Rc, or a mixture of these saponins[1] with 50% aqueous acetic acid at 70°, a prosapogenin (II), $C_{42}H_{72}O_{13}$, m.p. 260~262°, was yielded; octa-acetate, (III), $C_{58}H_{88}O_{21}$, m.p. 175~177.°

This prosapogenin (II) was hydrolyzed by refluxing with dil. mineral acid to give D-glucose and panaxadiol (IV). Chromic acid oxidation of the octa-acetate (III) followed by alkaline saponification and acid hydrolysis, gave a lactonic compound (V), $C_{27}H_{44}O_4$, m.p. 262~263°, IR $\nu_{max}^{CHCl_3}$ 1772 cm^{-1} (five membered lactone), and 3550 cm^{-1} (intramolecularly hydrogen bonded OH band, concentration independent), for which the formula V has been assigned by the analogy of the formation of the trisnorlactones from dammaranediol[3] and betulafolienetriol.[4] Appearance of signals at τ 8.31 (broad singlet, 3H) and 8.38 (broad singlet, 3H) in the NMR (nuclear magnetic resonance) spectrum of the octa-acetate (III) in deuterochroloform proved the presence of an isopropylidene type double bond in the side chain.[1] As already reported,[1] the acetate of Kotake's α-panaxin, a prosapogenin mixture which includes mainly our prosapogenin (II), afforded dihydroprotopanaxadiol (VI) on catalytic hydrogenation followed by alkaline saponification and subsequent acid hydrolysis. Consequently, it is evident that no structural change in the sapogenin moiety takes place during the process of the prosapogenin formation from the saponins, and the genuine sapogenin of this prosapogenin should be represented by protopanaxadiol (I).

Methylation of dihydroprotopanaxadiol (VI) by Hakomori's method[5] using a sodium hydride-dimethylsulfoxide-chloroform system afforded 3,12-dimethyl ether (VII), IR $\nu_{max}^{CCl_4}$ 3380 cm^{-1} (interamoleclularly hydrogen bond OH band, concentration independent). The resistance of this dimethyl ether (VII) against acetylation and oxidation indicates that a tertiary hydroxyl at C-20 was remained unmethylated. Methylation of dihydroprotopanaxadiol (VI) with methyl iodide and silver oxide in dimethylformamide

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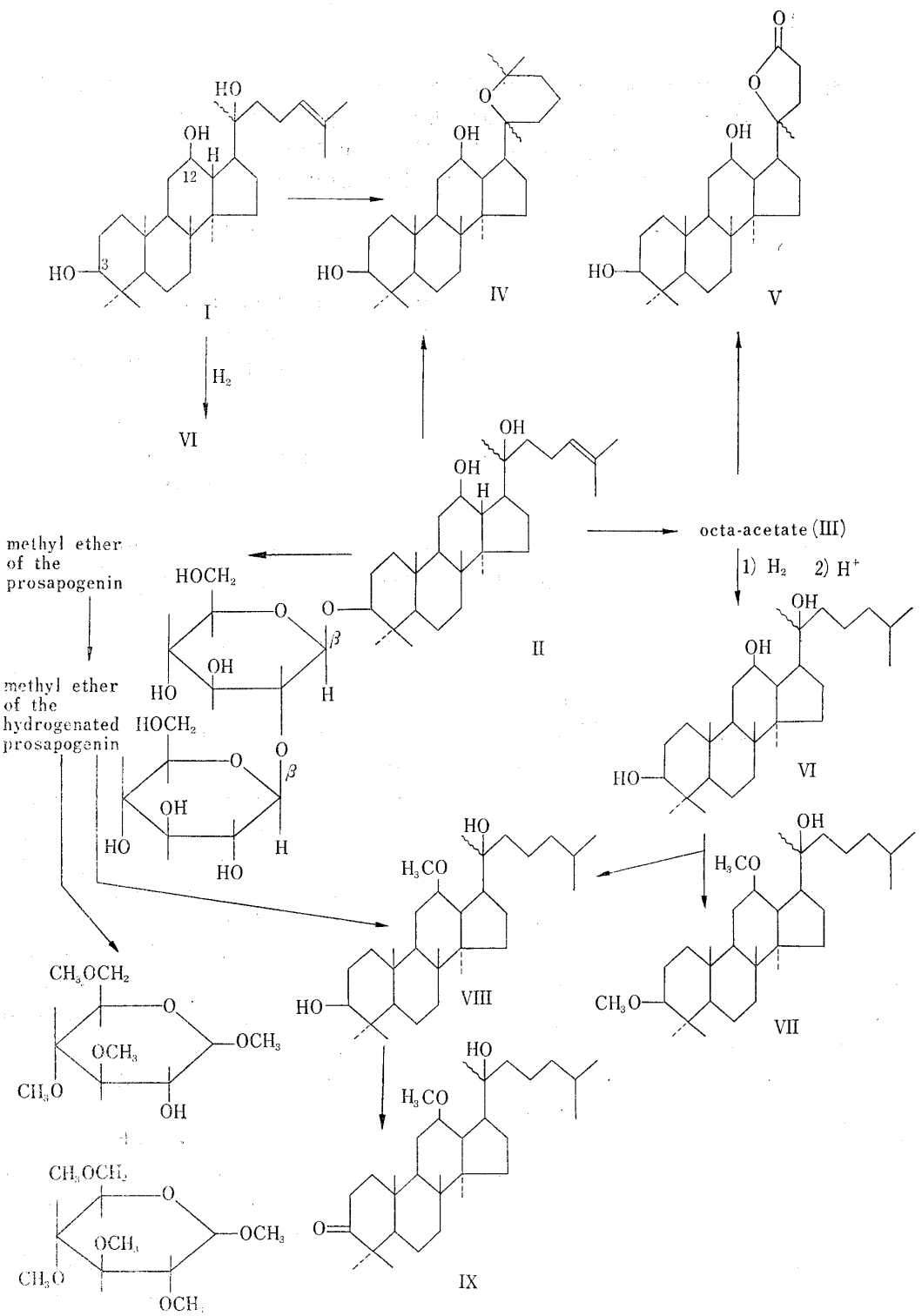
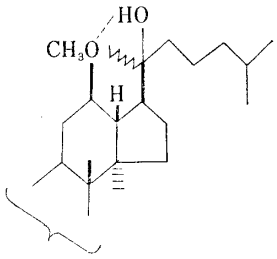
1) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, T. Ohsawa : This Bulletin, **14**, 595 (1966) (preliminary communication : Tetrahedron Letters, **1963**, 795).
2) M. Kotake : Nippon-Kagaku-Kaishi (J. Chem. Soc. Japan), **51**, 357 (1930).
3) J. S. Mills : J. Chem. Soc., **1965**, 2196.
4) F. G. Fisher, N. Seiler : Ann., **626**, 185 (1959); *Ibid.*, **644**, 146 (1961).
5) S. Hakomori : J. Biochem., **55**, 205 (1964).



yielded monomethyl ether (Ⅷ), IR $\nu_{max}^{CCl_4}$ 3620, 3380 cm^{-1}, along with a small amount of impure dimethyl ether (Ⅶ). On oxidation with chromic acid, the monomethyl ether (Ⅷ) gave a ketone (Ⅸ), IR $\nu_{max}^{CCl_4}$ 3380, 1712 cm^{-1}, whose carbonyl group must be located in the C-3 position, since it showed a positive Cotton effect in the optical rotatory dispersion curve. It has been demonstrated that in the dammarane series, the 12-keto derivatives exhibit negative Cotton effect*[2] and the 3-keto derivatives showed positive one.[6] Accordingly, the monomethyl ether (Ⅷ) should be formulated as 12-O-methyldihydroprotopanaxadiol.

6) C. Djerassi, J. Osiecki, W. Closson : J. Am. Chem. Soc., **81**, 4587 (1959).

The prosapogenin (II) was methylated by Hakomori's method to yield a methyl ether, which still showed an intramolecularly hydrogen bonded OH band in its IR spectrum in carbon tetrachloride at the almost same position as that given by the dimethyl ether (VII) to suggest the presence of system (X). Catalytic hydrogenation of this methyl ether and the subsequent hydrolysis with conc. hydrochloric acid at room temperature afforded the above mentioned monomethyl ether (VIII). The IR spectrum of the octa-acetate of the prosapogenin (III) exhibits an intramolecularly hydrogen bonded OH band at the same position as observed in the IR spectrum of 3,12-di-O-acetylprotopanaxadiol.[1] These evidences indicate that the hydroxyl groups at C-12 and C-20 of the prosapogenin are free and the sugar moiety is combined with 3-hydroxyl group of protopanaxadiol (I) in the molecule of the prosapogenin (II).



X

The methyl ether of the hydrogenated prosapogenin was subjected to methanolysis, and the sugar part of the product was examined by the gas-liquid chromatography,[7] which indicated the formation of equimolecular α- and β-methyl 2,3,4,6-tetra-O-methyl-, and α- and β-methyl 3,4,6-tri-O-methyl-glucopyranoside (Fig. 2). The NMR spectrum of the methyl ether of the hydrogenated prosapogenin showed the signals at τ 5.83 (doublet, J=8 c.p.s. 1H) and 5.43 (doublet J=7.7 c.p.s. 1H) corresponding to the protons at C-1 of the methyl ethers of two D-glucopyranose moieties. The coupling constants of these signals indicate the β-linkage[8] of the both glucosyl bonds in the prosapogenin (II).

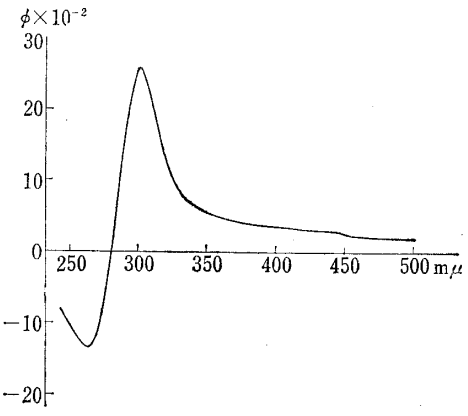


Fig. 1. Optical Rotatory Dispersion Curve of IX (in methanol)

On the basis of these finding, it can be now concluded that the prosapogenin (II) is represented by 3-O-(2-β-D-glucopyranosyl-β-D-glucopyranosyl)protopanaxadiol.

Very recently, Elyakov, *et al.*[9] reported the structure of carbohydrate chain of panaxosides D, E, and F, which would be corresponded to our ginsenosides-Rb~d. They obtained a prosapogenin by the partial hydrolysis of these saponins with dil. sulfuric acid in aquous methanol, for which the structure "D-gl-1-3-D-gl-1-3-D-gl-1-genin" (gl=glucose) was proposed. The discrepancy between their finding and our present result will be discussed in the near future after the identification of our saponins with their compounds is completed.

Experimental*4

Preperation of the Prosapogenin (II)——A solution of the mixture[1] of ginsenosides-Rb_1, -Rb_2, and -Rc (4 g.) in 50% aqueous acetic acid (100 ml.) was heated on a water bath at 70° for 6 hr. During the process of the reaction, colorless precipitate was resulted. The reaction mixture was diluted with water and the precipitate was collected, washed with water, dried and dissolved in boiling methanol. Concentration of this

*4 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.p.s.). 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.

7) T. Yamakawa, N. Ueda: Jap. J. Exp. Med., **34**, 37 (1964). According to their procedure, penta- and all of tetra-O-methyl-D-glucopyranoses can readily be distinguished each other.
8) J. M. van der Veen: J. Org. Chem., **28**, 564 (1963).
9) G. B. Elyakov, N. I. Uvarova, R. P. Gorshkova: Tetrahedron Letters, **1965**, 4669.

methanolic solution afforded the prosapogenin (II), colorless crystals, m.p. 260~262°, (1 g.), $[\alpha]_D^{25}$ −20.0° (c=0.64, pyridine). *Anal.* Calcd. for $C_{42}H_{72}O_{13}$: C, 64.26; H, 9.21. Found: C, 64.58; H, 9.25.

The same prosrpogenin was obtained from ginsenosides-Rb_1, -Rb_2, or -Rc by the same treatment.

The Octa-acetate (III) of the Prosapogenin——The prosapogenin (II) was acetylated with acetic anhydride and pyridine at room temperature for 20 hrs. After working up in the usual way, the octa-acetate (III) was obtained as colorless crystals (from petr. ether), m.p. 175~177°, $[\alpha]_D^{25}$ −17.4 (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{58}H_{88}O_{22}$: C, 62.13; H, 7.91, mol. wt. 1121.68. Found: C, 62.08; H, 7.67, mol. wt. 1077 (Osmotic pressure method, in ethyl acetete). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3542 (intramolecularly hydrogen bonded OH, concentration independent).

Hydrolysis of the Prosapogenin (II)——The prosapogenin (II) (200 mg.) was refluxed with 7%H_2SO_4 in 50% aquous ethanol (10 ml.) for 6 hr. The reaction mixture was diluted with water, and extrcted with ether. The ether extract was chromatographed on slica gel and crystallized from ethyl acetate affording panaxadiol (IV), m.p. 250°.

The water layer was neutralized by passing through the column of ion exchange resin (IR 4B) and concentrated to dryness to give syrupy residue. The paper chromatography of this residue (solvent: *n*-BuOH-AcOH-H_2O=4:1:5, coloring reagent; aniline hydrogenphthalate) showed the presence of D-glucose, which was further confirmed by the formation of phenyl D-glucosazone.

The Trisnorlactone (V)——To a solution of the octa-acetate (III) (0.6 g.) in acetic acid (4 ml.) was added a solution of CrO_3 (0.3 g.) in 80% aqueous acetic acid. After heating at 80° for 30 min., the reaction mixture was diluted with water and the resulting precipitate was taken up in ether. The etheral solution was washed with dil. HCl, dil. Na_2CO_3 solution, and water successively, dried over anhyd. Na_2SO_4, and concentrated to dryness. The residue was saponified by refluxing with 3% ethanolic KOH solution for 1.5 hr. After dilution with water, the reaction mixture was concentrated to remove most of ethanol, acidified, and extracted with *n*-butanol (saturated with water). The butanolic layer was washed with water (saturated with *n*-butanol) and evaporated to dryness to give an oily residue, which was hydrolyzed by refluxing with 7% H_2SO_4 in 50% aqueous ethanol (6 ml.) for 6 hrs. After working up in the usual way, the product was crystallized from ethyl acetate affording the trisnorlactone (V), as colorless crystals, m.p. 262~263°. *Anal.* Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.94; H, 10.27. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (free OH), 3542 (intramolecularly hydrogen bonded OH, concentration independent), and 1772 (five membered lactone).

Methylation of Dihydroprotopanaxadiol (VI)——1) Commercial NaH (50%) (1 g.) was washed with petr. ether and warmed with dimethylsulfoxide (50 ml.) at 75° for 1 hr. To this reagent thus prepared was added a solution of dihydroprotopanxadiol (VI) (0.5 g.) in dimethylsulfoxide (35 ml.) and the mixture was kept at 75° for 1 hr. CH_3I (6 g.) was added under ice cooling and the reaction mixture was allowed to stand at room temperature for 3 hr. After dilution with water, the mixture was extracted with ether and the organic layer was washed with water, dried, and concentrated to dryness. The residue was methylated again under the same condition as above and the product was chromatographed on silica gel (graduent elution: petr. ether → benzene → $CHCl_3$) to give 3,12-di-O-methyldihydroprotopanaxadiol (VII), colorless crystals, m.p. 142~143°(from methanol), (0.22 g.), $[\alpha]_D^{27}$ +4.1 (1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{58}O_3$: C, 78.31; H, 11.91, Found: C, 78.28; H, 11.78. NMR τ 6.67 (singlet, 6H, -OC$\underline{H}_3$). A small amount of 12-O-methyldihydroprotopanaxadiol (VIII) (40 mg.) was also yielded as colorless crystals, m.p. 150~151°(from 90% aqueous acetone), $[\alpha]_D^{27}$ −44.3 (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{56}O_3 \cdot \frac{1}{2}H_2O$: C, 76.67; H, 11.73. Found: C, 76.54; H, 11.86. NMR τ 6.70 (singlet, 3H, -OC$\underline{H}_3$).

2) A mixture of dihydroprotopanaxadiol (VI) (0.5 g.), Ag_2O (3.3 g.), and CH_3I (3 ml.) in dimethylformamide (25 ml.) was stirred at room temperature. After 44 hrs., the mixture was filtered and to the filtrate were added Ag_2O (3.3 g.) and CH_3I (3 ml.). The methylation was continued for additional 44 hrs. The mixture was filtered and a solution of KCN was added to the filtrate. The solution was extracted with $CHCl_3$ and the organic layer was washed with water, dried, evaporated to dryness to give the residue, which was purified by chromatography on silica gel, affording dimethyl ether (VII) (crude, 100 mg., m.p. 138~139°) and monomethyl ether (VIII) (200 mg., m.p. 150~151°).

Oxidation of 12-O-Methyldihydroprotopanaxadiol (VIII)——To a solution of monomethyl ether (VIII) (100 mg.) in acetone (30 ml.) was added Jone's reagent[10] (0.5 ml.) and the solution was allowed to stand at room temperature for 3 hr. After working up in the usual way, the product was crystallized from methanol to give the ketone (IX) (64 mg.) as colorless crystals, m.p. 181~183, $[\alpha]_D^{27}$ +17.5 (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{54}O_3$: C, 78.42; H, 11.47. Found: C, 78.30; H, 11.40.

12-O-Methyldihydroprotopanaxadiol (VIII) from Methyl Ether of the Hydrogenated Prosapogenin (II)——The prosapogenin (II) (1.5 g.) was methylated with NaH (50%) (1.5 g.) and CH_3I (18 g.) in dimethylsulfoxide in the same way as the methylation of dihydroprotopanaxadiol (VI). The product was purified by passing through a column of neutral alumina (grade II) and catalitically hydrogenated with Adams catalyst (0.15 g.) in a mixture of methanol and acetic acid yielding the methyl ether of the hydrogenated prosapogenin (0.8 g.) as colorless powder. This hydrogenated methyl ether (0.52 g.) was treated with conc. HCl (20 ml.) at room

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

temperature for 7.5 hr. After dilution with water, the reaction mixture was extracted with ether and ether layer washed with water, dried, and concentrated to dryness to give an oily residue, which was purified by chromatography on silica gel affording 12-monomethyl ether (Ⅷ) (40 mg.) (mixed melting point, comparison of IR spectra and thin-layer chromatograms with the authentic samle).

Methanolisis of the Methyl Ether of the Hydrogenated Prosapogenin——The above methyl ether of the hydrogenated prosapogenin (30 mg.) was heated 5% methanolic HCl in a sealed tube on a boiling water bath for 6 hrs. The solution was neutralized by passing through a column of IR-4B and concentrated. The presence of equimolecule of 1,2,3,4,6-tetra-O-methyl-D-glucopyranose and 1,3,4,6-tetra-O-methyl-D-glucopyranose in this residue was demonstrated by gas liquid chromatography: Condition: Column; 5% neopentylglycol succinate on Gaschrom C. L. H (2 m. × 3 mm.); Sample heater temperature, 295°. Column temperature, 200°; Carrier gas, N_2(1 kg./cm^2). On Hitachi-Perkin Elmer F-6 type with flame ionization detector (Fig. 2).

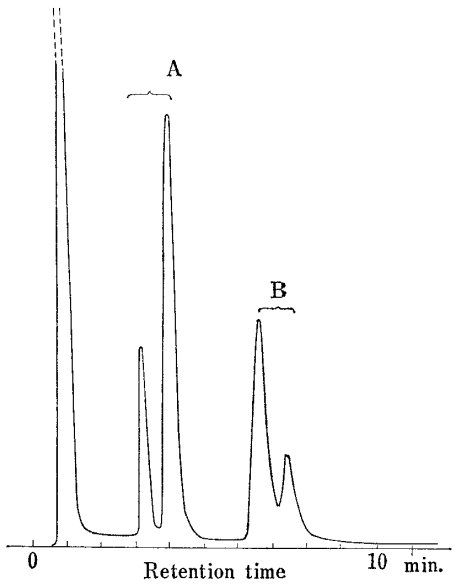


Fig. 2. Gas Chromatogram of the Methanolysis Product of the Methyl Ether of the Hydrogenated Prosapogenin

A: α- and β-methyl 2,3,4,6-tetra-O-methylglucosides
B: α- and β-methyl 3,4,6-tri-O-methylglucosides

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Summary

It has been proved that the prosapogenin obtained from ginsenosides-Rb_1, -Rb_2, and-Rc (ginseng saponins) by the partial hydrolysis with hot aqueous acetic acid is formulated as Ⅱ.

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