[Chem. Pharm. Bull. 21(12)2702—2711(1973)]

UDC 547.918.02.05 : 581.192 : 615.322.011.5

Studies on the Constituents of Himalayan Ginseng, *Panax pseudoginseng*. I. The Structures of the Saponins. (1)

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(Received May 2, 1973)

The chemical structures of saponin-A (I) (=chikusetsusaponin V), $C_{48}H_{76}O_{19}$, $[\alpha]_D^{20}$ +14.5° (MeOH) and saponin-D (V) (=ginsenoside Rb_1), $C_{54}H_{92}O_{23}$, $[\alpha]_D^{20}$ +15.78° (MeOH), which were isolated from *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius* (Araliaceae), were established on the basis of physical data and chemical investigations.

One of the authors, Tanaka, was a member of botanical expedition to Eastern Himalaya organized by the University of Tokyo in 1967 and collected the rhizoma of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius* (araliaceae). The morphological similarity of this plant to *Panax japonicum* C.A. Meyer,[2] which grows in Japan and has been investigated chemically by us,[3] has urged us to study the constituents of both plants, comparatively. The present paper deals mainly with the study on the structures of two main saponins of *Panax pseudoginseng*, which leads to the assignments of the structure I and V, respectively.

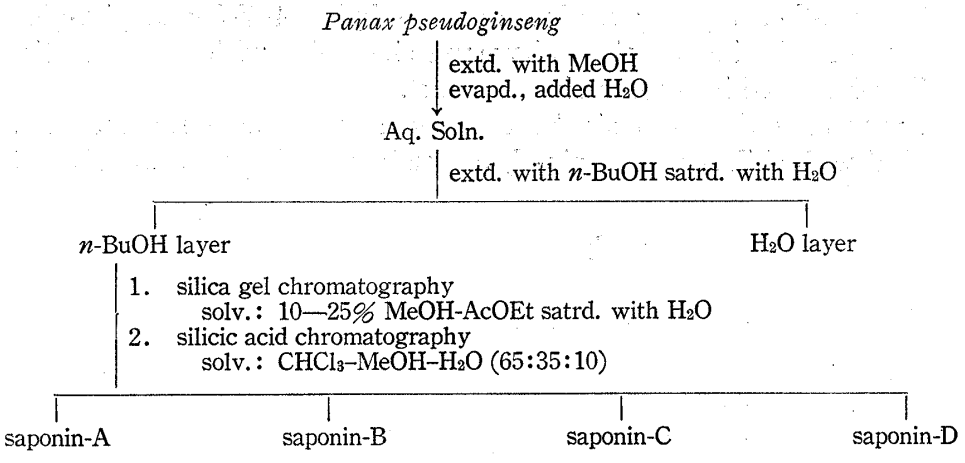


Chart 1. Extraction and Separation of *Panax pseudoginseng* Constituents

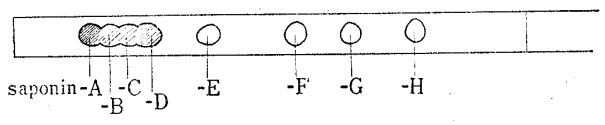


Fig. 1. Thin-Layer Chromatogram of *Panax pseudoginseng* Glycosides on Kiesel gel H
solvent: the lower phase of $CHCl_3$–MeOH–H_2O (65:35:10)
color reagent: 10% H_2SO_4

The dried material which has been collected at Western Bhutan was crushed and extracted twice with hot ether and the residue was extracted with hot methanol. The yield of methanol extract from the dried material was 37.5%. The methanol extract was suspended in water and extracted with *n*-butanol saturated

1) Location: *a) Hatanodai, Shinagawa-ku, Tokyo; b) Kasumi 1-2-3, Hiroshima.*
2) H. Hara, *Shokubutsu Kenkyu Zasshi*, **45**, 197 (1970).
3) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968); N. Kondo, N. Nagumo, and N, Komatsu, *ibid*, **89**, 846 (1969); N. Kondo, K. Aoki, H. Ogawa, R. Kasai, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **18**, 1558 (1970), N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).

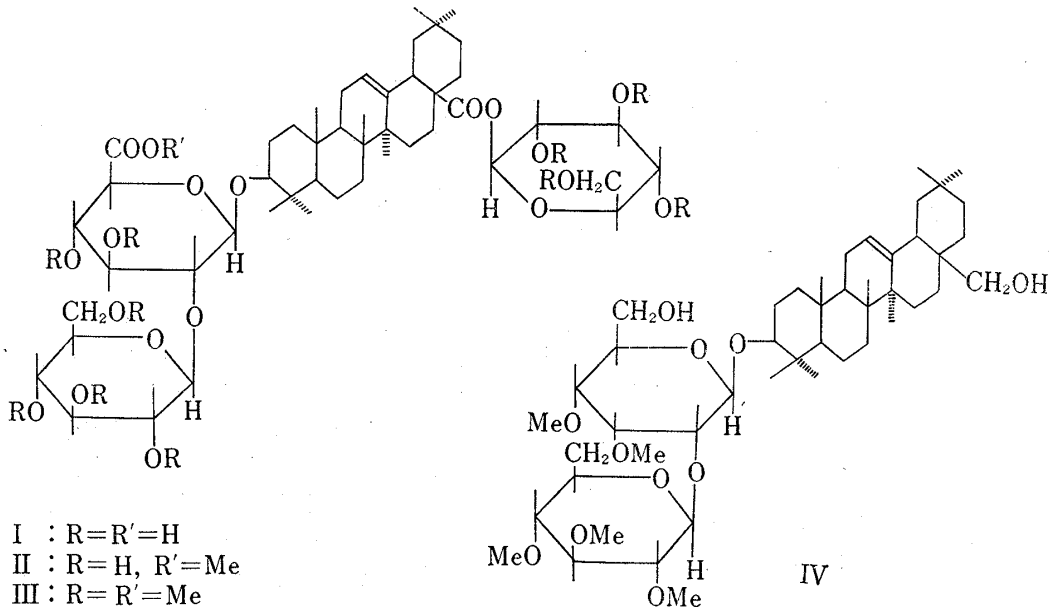
with water. The yield of *n*-butanol extract from the dried material was 18.1%. The thin-layer chromatogram (TLC) of butanol extract on silica gel H developed with chloroform–methanol–water (65: 35: 10, the lower phase) revealed the presence of more than eight saponins (Fig. 1). The isolation of each saponin was carried out by chromatography on silica gel using ethyl acetate–methanol (20: 1—3: 1) and the each fraction was purified repeatedly by column chromatography on silica gel eluted with chloroform–methanol–water (65: 35: 10, the lower phase). Finally, the saponin A (*Rf* 0.054) and the saponin D (*Rf* 0.17) were isolated in pure state.

The saponin A (I), $C_{48}H_{76}O_{19}\cdot 2H_2O$, $[\alpha]_D^{20}$ +14.5° (in methanol) was obtained as a white powder reprecipitated from methanol–ethyl acetate. The infrared (IR) spectrum of I shows the presence of hydroxyl group (3400 cm^{-1}), ester group (1740 cm^{-1}) and carboxyl group (1728 cm^{-1}).

On methylation with diazomethane in methanol, I gave a monomethyl ester (II), $C_{49}H_{78}O_{19}$, $[\alpha]_D^{20}$ +192.3° (in methanol), which shows the ester absorption band at 1738 cm^{-1} in IR spectrum and O-methyl signal at 3.65 ppm (3H(s)) in nuclear magnetic resonance (NMR) spectrum. Hydrolysis of I with diluted hydrogen chloride gave oleanolic acid, glucose and glucuronic acid which were identified by direct comparison with the authentic samples by TLC and gas liquid chromatography (GLC), respectively.

Per-O-methylsaponin-A (III), $C_{59}H_{98}O_{19}$, $[\alpha]_D^{20}$ +12.6° [4] (in chloroform), prepared from I by repeated methylation by the Kuhn's method,[5] gave, on reduction with lithium aluminium hydride, erythrodiol bioside (IV), $C_{48}H_{82}O_{12}$, $[\alpha]_D^{20}$ +37.4° (in chloroform), 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol. Methanolysis of erythrodiol bioside with 2N hydrogen chloride in methanol gave erythrodiol, colorless needles, mp 234—236°, methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 3,4-di-O-methyl-D-glucoside. The O-methylated monosaccharides obtained above were identified with the authentic samples by TLC and GLC as described in the experimental part.

All of the properties of the saponin-A mentioned above strongly suggest that this saponin must be identical with chikusetsusaponin-V, the main saponin of *P. japonicum* C.A. MEYER,



I : R=R'=H
II : R=H, R'=Me
III : R=R'=Me

IV

Chart 2

4) The optical rotation, $[\alpha]_D^{26}$ −74.05°, reported in the previous paper[3] is erroneous and it was revised in this paper.

5) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).

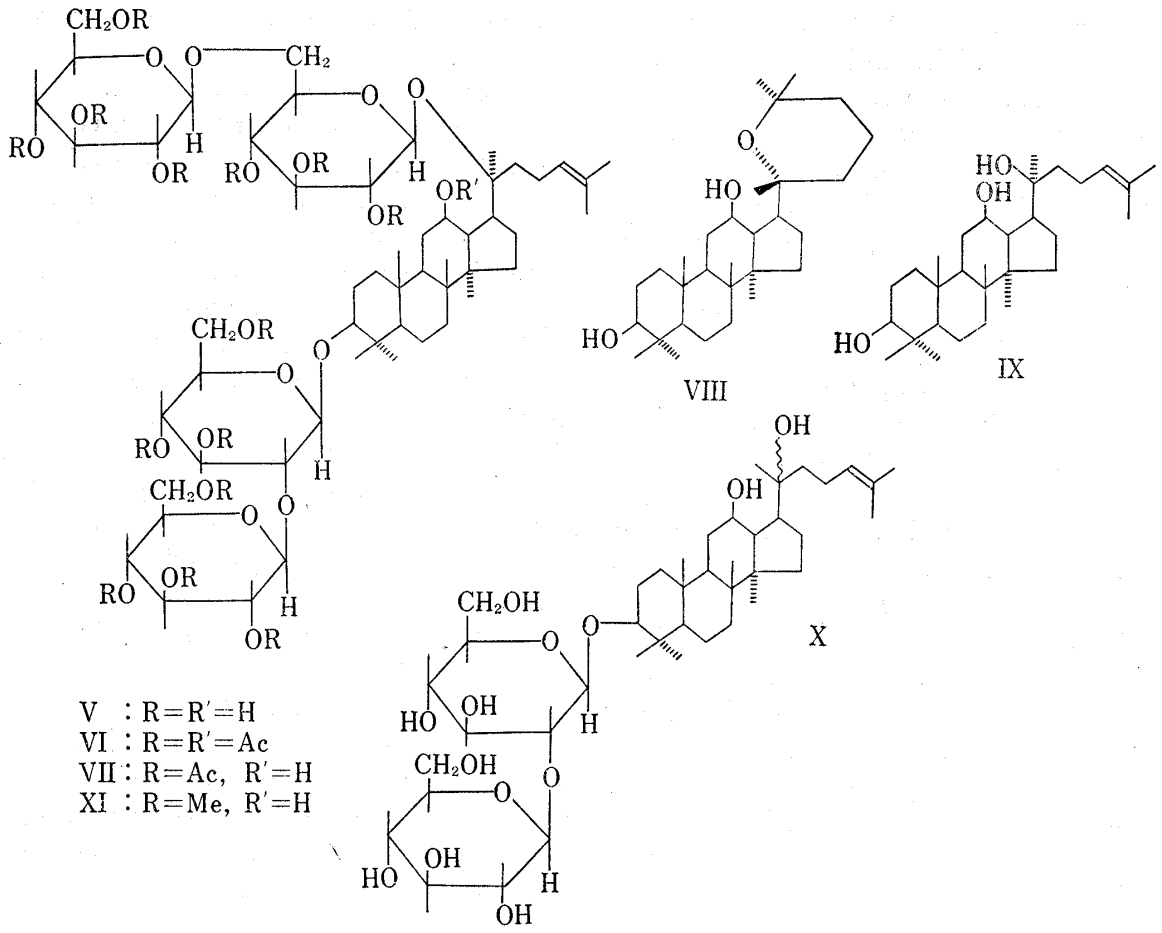


Chart 3

the structure of which has been established as I.[3] The identity of both compounds finally proved by the direct comparison.

The saponin-D (V), was obtained by repeated precipitation from ethanol as a white powder, $C_{54}H_{92}O_{23}\cdot 3H_2O$, $[\alpha]_D^{20}$ +15.78° (in methanol). The IR spectrum of V shows the presence of hydroxyl (3400 cm^{-1} broad) and olefinic bonds (1620 cm^{-1}). On acetylation with acetic anhydride and pyridine for 2 days, V gave a pentadecaacetate (VI), $C_{84}H_{122}O_{38}$, $[\alpha]_D^{20}$ −9.72° (in chloroform), which shows no hydroxyl absorption band in the IR spectrum, and a tetradecaacetate (VII), $C_{82}H_{120}O_{37}$, which shows the hydroxyl absorption band at 3466 cm^{-1} in the IR spectrum. The formation of two kinds of acetylation products from V suggests that one of the hydroxyl groups of this glycoside is sterically hindered.

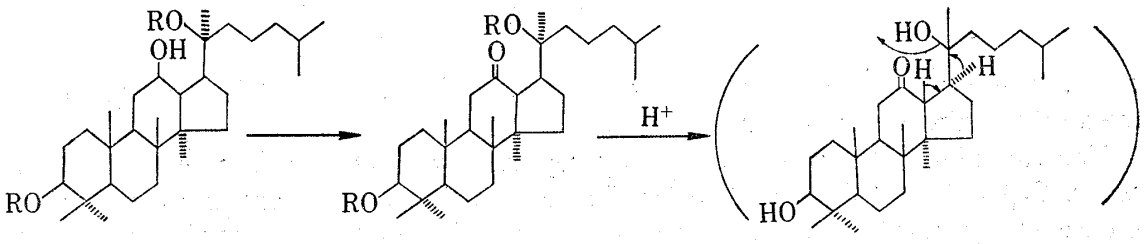
The acid hydrolysis of V with hydrogen chloride-dioxane-water gave panaxadiol (VIII)[6] and glucose which were identified by TLC, paper partition chromatography (PPC) and GLC. Furthermore, the genuine aglycone of V was established to be 20S-protopanaxadiol (IX)[7] by oxidative degradation of V according to the Smith's procedure.[8] Consequently, V was deduced to be a glycoside which consists of 20S-protopanaxadiol and four moles of glucose.

On partial hydrolysis with 50% acetic acid refluxing for 2 hr, V gave a prosapogenin (X), $C_{42}H_{72}O_{13}\cdot 1/2H_2O$, colorless needles from methanol, mp 279°. Based on the physical properties, X was suggested to be the prosapogenin, protopanaxadiol (3)-[β-D-glucopyranosyl ($1_{glu}\rightarrow 2_{glu}$)-β-D-glucopyranoside], which was obtained from ginsenoside Rb, one of the saponins

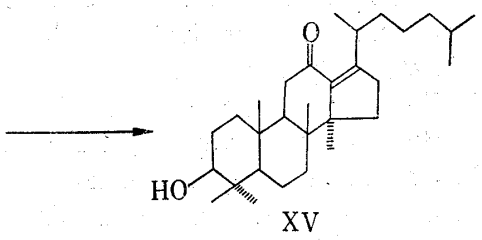
6) M. Nagai, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **19**, 2349 (1971).
7) M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972).
8) F. Smith, G.W. Hay, and B.A. Lewis, "Method in Carbohydrate Chemistry," Vol. 5, Academic Press, New York and London, 1965, p. 361.

from *Panax ginseng* C.A. Meyer, reported by Shibata, *et al.*,[9] and the identity was established by mixed fusion, TLC and the comparison of IR spectra.

Consequently the two moles of glucose out of four in V were confirmed to link to the C_3 hydroxyl group of protopanaxadiol moiety, while the location of the other two moles of glucose was decided as follows. According to the Hakomori's method,[10] V was methylated repeatedly, but the resulted O-methyl ether (XI), $C_{68}H_{120}O_{23}$, a white powder from aqueous methanol, $[\alpha]_D^{20}$ +38.6° (in chloroform), still showed the hydroxyl absorption band at 3451 cm^{-1}. Taking account of the presence of one free hydroxyl group in XI, further experiments were carried out. After hydrogenation of XI the formed dihydro-O-methyl ether (XII) was methanolyzed with 2N hydrogen chloride in methanol to afford methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside, methyl 2,3,4-tri-O-methyl-D-glucopyranoside and methyl 3,4,6-tri-O-methyl-D-glucopyranoside. The formation of methyl 2,3,4-tri-O-methyl-D-glucopyranoside from XII suggests that two moles of glucose in V form a gentiobiose type linkage. Furthermore, acid hydrolysis of XII with concentrated hydrogen chloride gave dihydroprotopanaxadiol (XIII). Based on these experimental data the structure of saponin-D was suggested to be 20S-protopanaxadiol-(3)-[β-D-glucopyranosyl($1_{glu}\rightarrow 2_{glu}$)-$\beta$-D-glucopyranoside]-(20)-[D-glucopyranosyl($1_{glu}\rightarrow 6_{glu}$)-D-glucopyranoside]. To confirm the location of the gentiobioside type sugar moiety, compound XII was oxidized with chromium trioxide in pyridine to give a ketone, (XIV), $C_{68}H_{120}O_{23}$. Compound XIV was treated with 6N hydrogen chloride in ethanol to afford an aglycone (XV) which shows the absorption maximum at 265 mμ(log ε 3.39) in ultraviolet (UV) spectrum.[11] This result suggested that the hydroxyl group which resisted to the methylation by the Hakomori's method was oxidized to a ketone, and the hydrolysis of this ketone with hydrogen chloride was followed by dehydration and hydride shift to afford the α,β-unsaturated ketone. (see Chart) It seems to be very reasonable that the possible location of the free hydroxyl group in XI may be assigned to C-12.



XII : R=methylated sugar XIV : R=methylated sugar
XIII : R=H



XV

Chart 4

The configurations of four glucose are assumed to be all β-form from the coupling constants (6—7 Hz) and chemical shifts (δ: 4.27—4.67) in NMR spectrum of XI. Consequently, the structure of V was established to be 20S-protopanaxadiol-(3)-[β-D-glucopyranosyl($1_{glu}\rightarrow 2_{glu}$)-$\beta$-D-glucopyranoside]-(20)-[β-D-glucopyranosyl($1_{glu}\rightarrow 6_{glu}$)-$\beta$-D-glucopyranoside].

9) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).
10) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
11) S. Shibata, M. Fujita, H. Itokawa, O. Tanaka, and T. Ishii, *Chem. Pharm. Bull.* (Tokyo), **6**, 759 (1963).

The identity of saponin-D with ginsenoside-Rb_1, one of the saponins from *Panax ginseng* C.A. MEYER, will be reported in the next paper, so that it is noted that the structure of ginsenoside-Rb_1 has been established by this study. The study on the other saponins are now being in progress.

Experimental

All melting points were taken on a Yanagimoto micro melting points apparatus and uncorrected. IR absorption spectra were obtained with a Hitachi Model 215. NMR spectra were measured with a Hitachi Model R-20 High Resolution NMR spectrometer and a Hitachi Model R-22 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvents used are indicated. Gas chromatography used was a Hitachi Model K-53 with hydrogen flame ionization detector. The *Rf* values were determined by thin-layer chromatography on silica gel H using solvent A: chloroform–methanol–water (65: 35: 10 the lower phase); solvent B: phenol saturated with water; solvent C: AcOEt; solvent D: benzene–acetone (2: 1); solvent E: benzene–acetone (3: 1); solvent F: benzene–acetone (4: 1); solvent G: hexane–acetone (4: 3); solvent H: hexane–acetone (2: 1), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Material——The material, dried rhizoma of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolia* (Araliaceae), was collected at Khosa (1800 m), Western Bhutan May 10, 1967 by Tanaka, a member of Botanical Expedition of Eastern Himalaya organized by the University of Tokyo (Chief Prof. H. Hara).

Extraction——Two hundreds g of crushed material was extracted twice with ether. The residual material was extracted repeatedly with 1 liter of hot methanol. After evaporation of the solvent under a reduced pressure, 75 g of the syrupy brown residue was obtained (yield from the dried material 37.5%) and dissolved in water. The aqueous solution was extracted with *n*-BuOH saturated with water. The *n*-BuOH layer was concentrated *in vacuo* to afford 36.3 g of powder (yield from the dried material 18.1%). The *n*-BuOH soluble fraction was detected by TLC (solvent A) and revealed to contain more than eight saponins (Fig. 1).

Isolation of Glycosides——The crude glycoside fraction was submitted to column chromatography on silica gel with ethyl acetate saturated with water and eluted with the same solvent containing 5—25% MeOH. The crude saponin-A (*Rf* 0.054), -B (*Rf* 0.1), -C (*Rf* 0.13) and -D (*Rf* 0.17) were obtained. The crude saponin-A and -D were purified by chromatography on silica gel using $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase) and chromatographically pure saponin-A (14.5 g) and saponin-D (2.1 g) were obtained.

Saponin-A (I)——The pure saponin-A (I) was obtained as a white powder by repeated precipitation from MeOH–AcOEt, (mp 241°), $[\alpha]_D^{20}$ +14.5° (c=0.76, MeOH), *Anal.* Calcd. for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 57.06; H, 8.02. Found: C, 57.32; H, 7.97. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH broad), 1740 (COOR), 1728 (COOH).

Hydrolysis of Saponin-A (I)——I (50 mg) was hydrolyzed with 5 ml of 2N HCl in dioxane–H_2O (1: 7, v/v) with refluxing on a water bath for 4 hr. The reaction mixture was treated as described in the previous paper and oleanolic acid, glucose and glucuronic acid were identified.

Methylation of I with CH_2N_2 (Formation of Chikusetsusaponin V Monomethyl Ester (II))——I (130 mg) was dissolved in MeOH and excess CH_2N_2 in ether was added and allowed to stand for 4 hr. The reaction mixture was treated as described in the previous paper to afford a monomethyl ester, a white powder from EtOH–AcOEt, $[\alpha]_D^{20}$ +192.3° (c=0.56, MeOH). The product was identified with chikusetsusaponin V monomethyl ester by TLC and by comparison of IR spectra.

Per-O-methylation of I (Formation of Per-O-methylchikusetsusaponin V (III))——According to the previous paper, I (523 mg) was methylated. The product was submitted to chromatography on silica gel using benzene–acetone (4: 1) as a solvent followed by reprecipitation from dilute MeOH, affording per-O-methylsaponin-A as a white powder (278 mg), $C_{59}H_{98}O_{19}$, $[\alpha]_D^{20}$ +12.6° (c=0.63, $CHCl_3$). *Anal.* Calcd. for $C_{59}H_{98}O_{19}$: C, 63.74; H, 8.88. Found: C, 63.52; H, 8.77. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1757 (COOR). NMR (in $CDCl_3$) δ: 0.78—1.12 (3H (s)×7, CH_3), 3.34—3.78 (3H (s)×11, OCH_3), 4.40 (1H (d), J=7 Hz, anomer H), 4.55 (1H (d), J=7 Hz, anomer H), 5.3—5.45 (2H (broad), $>C=C<^{H}$, anomer H). III was identified with per-O-methylchikusetsusaponin V by comparing TLC (solvent H: *Rf* 0.57; solvent G: *Rf* 0.77) and IR spectra.

Reductive Cleavage of Per-O-methylsaponin-A (III) with $LiAlH_4$——Per-O-methylsaponin-A (200 mg) in absolute ether was reduced with $LiAlH_4$ (70 mg) under refluxing for 2 hr and the reaction mixture was treated by the same method as described in per-O-methylchikusetsusaponin V. The ether soluble fraction was purified by reprecipitation from dilute MeOH, affording the hexa-O-methylerythrodiol bioside (IV) as a white powder, $[\alpha]_D^{20}$ +37.4° (c=0.80, $CHCl_3$). *Anal.* Calcd. for $C_{48}H_{82}O_{12}$: C, 67.76; H, 9.76. Found: C, 67.58; H, 9.70. NMR (in $CDCl_3$) δ: 0.82—1.10 (3H (s)×7, CH_3), 3.32—3.60 (3H (s)×6, OCH_3), 4.35 (1H (d), J=7 Hz, anomer H), 4.70 (1H (d), J=7 Hz, anomer H), 5.22 (1H (m), $>C=C<^{H}$. IV was identified with hexa-O-methylerythrodiol bioside derived from per-O-methylchikusetsusaponin V by comparing IR and NMR spectra.

The chloroform soluble fraction was examined by TLC (solvent D) to reveal the presence of two methylated monosaccharides, which were identified with the authentic samples of 2,3,4,6-tetra-O-methylglucose

and 2,3,4,6-tetra-O-methylsorbitol by TLC (solvent D) and GLC (column 3% SE-30 on chromosorb W 3 mm × 1 m, column temp. 150°, N_2 flow 0.65 kg/cm²). 2,3,4-Tetra-O-methylglucose: TLC *Rf* 0.22, t_R (min) 3.4 (TMS derivatives). 2,3,4,6-tetra-O-methylsorbitol: TLC *Rf* 0.05, t_R (min) 7.0 (TMS derivatives).

Methanolysis of Hexa-O-methylerythrodiol Bioside (IV)——Compound IV was refluxed with methanolic 2N HCl for 2 hr, the solution was treated as described in the previous paper. Erythrodiol, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside were identified by comparing with the authentic samples. Erythrodiol: colorless needles from acetone, mp 234—236°. Methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside: *Rf* 0.34, 0.46 (solvent C), t_R (min) 4.0, 4.8 (column 3% SE-30, column temp. 110°, N_2 flow 1.0 kg/cm²). Methyl 3,4-di-O-methyl-D-glucopyranoside: *Rf* 0.10 (solvent C), t_R (min) 12.5.

Saponin-D (V)——The pure saponin-D (V) was obtained as a white powder by repeated precipitation from EtOH, (mp 189°), $[\alpha]_D^{20}$ +15.78° (c=1.13, MeOH). *Anal.* Calcd. for $C_{58}H_{92}O_{23}\cdot 3H_2O$: C, 55.75; H, 8.49. Found: C, 55.89; H, 8.21. IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1620 (>C=C<).

Acetylation of V with Acetic Anhydride and Pyridine——Compound V (145 mg) was acetylated with acetic anhydride (3 ml) and pyridine (3 ml) for 48 hr at room temperature. The reaction mixture was worked up as usual and the products (180 mg) were purified by chromatography on silica gel eluted with benzene-acetone (5: 1) to give two kinds of acetates (VI and VII). Pentadecaacetate (VI): A white powder from MeOH–H_2O, (mp 135°), $[\alpha]_D^{20}$ −9.72° (c=0.72, $CHCl_3$). *Anal.* Calcd. for $C_{84}H_{122}O_{38}$: C, 57.96; H, 7.08. Found: C, 58.12; H, 7.16. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1750 (-OCOCH$_3$), 1230 (C-O-C). Tetradecaacetate (VII): A white powder from MeOH–H_2O, (mp 132°). *Anal.* Calcd. for $C_{82}H_{120}O_{37}$: C, 57.99; H, 7.07. Found: C, 58.09; H, 7.23. IR ν_{max}^{Nujol} cm^{-1}: 3466 (OH), 1753 (-OCOCH$_3$), 1230 (C-O-C).

Hydrolysis of V with 2N HCl in Aqueous Dioxane——Compound V (30 mg) was hydrolyzed with 2N HCl in dioxane–H_2O (1: 7) (3 ml) by refluxing on the water bath for 4 hr. After cooling the formed precipitate was filtered and dried. The product was identified to be panaxadiol (VIII) by comparing with the authentic sample by TLC. *Rf* 0.69 (solvent C), 0.29 (solvent E).

The filtrate was neutralized with Ag_2CO_3 and evaporated *in vacuo*. The residue was identified to be glucose by comparing with a authentic sample by PPC, TLC and GLC. PPC paper: Toyo-Roshi No. 51. solvent: BuOH–AcOH–H_2O (4: 1: 5 the upper layer). *Rf* 0.16, TLC *Rf* 0.27 (solvent B) GLC column: SE-52, 3 mm × 2 m, column temperature: 175°, carrier gas. N_2 18 ml/min, sample: TMS derivatives. t_R (min) 11.6 (α-glucose), 16.7 (β-glucose).

Oxidative Degradation of V with $NaIO_4$——To the solution of V (500 mg) in MeOH–EtOH–H_2O (20 ml–33.3 ml–100 ml) $NaIO_4$ (1.69 g) was added. The solution was stirred for 120 hr at 2°. The formed precipitate was filtered off and the filtrate was added water (50 ml) and concentrated *in vacuo* to 150 ml. The solution was extracted with BuOH saturated with water. The BuOH solution was evaporated *in vacuo* and the residue was dissolved in EtOH (2.5 ml)–MeOH (5 ml). To this solution a solution of KOH (1.25 g) in H_2O (17.5 ml) was added and the mixture was heated at 95° for 4 hr under N_2 gas flow. The reaction mixture was neutralized with 2N H_2SO_4 and extracted with ether. The ether solution was washed with water and dried over Na_2SO_4. The ether solution was evaporated and the residue was purified by chromatography on silica gel eluted with benzene–acetone (3: 1) to afford colorless needles (14.2 mg) from benzene, mp 199°, which was identified to be 20S-protopanaxadiol (IX) by comparing IR spectra, TLC and GLC. TLC *Rf* 0.20 (solvent F, 20R-protopanaxadiol *Rf* 0.23). GLC column: 1.5% SE-30 on chromosorb W DMCS, 3 mm × 2 m, column temperature: 280°, carrier gas: N_2 flow 1.5 kg/cm², sample: TMS derivatives. t_R (min) 5.2 (20S-protopanaxadiol).

Partial Hydrolysis of Saponin-D (V)——Compound V (200 mg) was heated with 50% AcOH for 2 hr on the water bath. The formed precipitate was filtered, washed with water and dried. The product was recrystallized from MeOH to give colorless needles, mp 279°. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1620 (C=C). *Anal.* Calcd. for $C_{42}H_{72}O_{13}\cdot 1/2H_2O$: C, 63.53; H, 9.04. Found: C, 63.54; H, 9.04. This product was identified to be prosapogenin of ginsenoside Rb[9] by comparing TLC and IR spectra and by mixed fusion.

Methylation of V by the Hakomori's Method——According to the Hakomori's method, NaH (500 mg) was warmed with dimethylsulfoxide (DMSO 25 ml) at 70° for 1 hr with stirring under N_2 gas flow. To this reagent compound V (510 mg) in DMSO (5 ml) was added and the mixture was kept at 70° for 10 min with stirring under N_2 gas flow. CH_3I (5 ml) was added and the reaction mixture was allowed to stand at room temperature for 6 hr with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated to dryness. The residue was chromatographed on silica gel eluted with benzene–acetone (4: 1) to give tetradeca-O-methyl ether of V (XI, 239 mg), a white powder, (mp 87°), $[\alpha]_D^{20}$ +38.60° (c=0.57, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 3451 (OH), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3436 (OH). *Anal.* Calcd. for $C_{68}H_{120}O_{23}$: C, 62.53; H, 9.26. Found: C, 62.22; H, 9.04. NMR (in $CDCl_3$) δ: 0.8—1.0 (3H (s) × 5, CH_3), 1.3 (3H (s), CH_3), 1.6, 1.7 (3H (s) × 2, CH_3), 3.33—3.67 (3H (s) × 14, OCH_3), 4.27 (1H (d), J=6 Hz, anomer H), 4.34 (1H (d), J=7 Hz, anomer H), 4.46 (1H (d), J=7 Hz, anomer H), 4.67 (1H (d), J=7 Hz, anomer H), 5.12 (1H (broad), >C=C<^{H}).

Catalytic Reduction of XI and Hydrolysis of Dihydrosaponin-D Tetradeca-O-methyl Ether (XII)——Compound XI (300 mg) was catalytically reduced on PtO_2 (100 mg) in EtOH (20 ml) and the reaction mixture

was filtered. The filtrate was evaporated *in vacuo* and the residue was preparatively purified by TLC (solvent H) to give dihydrosaponin-D tetradeca-O-methyl ether (XII). NMR (in $CDCl_3$) δ: 0.8—1.0 (3H (s) $\times$ 7, CH_3), 1.3 (3H (s), CH_3).

Compound XII (26.8 mg) was hydrolyzed with methanolic 2N HCl refluxing for 4 hr. The reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was examined by TLC and GLC. TLC (solvent G): *Rf* 0.80, 0.88 (α- and β-methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside); 0.49, 0.61 (α- and β-methyl 2,3,4-tri-O-methyl-D-glucopyranoside); 0.54 (α- and β-methyl 3,4,6-tri-O-methyl-D-glucopyranoside).

GLC (column: 5% NPGS on microsorb W; column temperature: 170°; carrier gas: N_2 0.7 kg/cm^2) t_R (min) 5.7, 4.4 (α-and β-methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside); 8.5, 11.2 (α- and β-methyl 2,3,4-tri-O-methyl-D-glucopyranoside); 9.6, 10.8 (trace) (α- and β-methyl 3,4,6-tri-O-methyl-D-glucopyranoside).

Furthermore, compound XII (20 mg) was hydrolyzed with conc. HCl (1 ml) for 8 hr at room temperature. The reaction mixture was diluted with water and extracted with ether. The ether extract was examined by TLC (solvent D) and the product was identified with an authentic sample of dihydroprotopanaxadiol (XIII, *Rf* 0.32).

Chromium Trioxide Oxidation of XII——To a solution of compound XII (278 mg) in pyridine (5 ml) was added the solution of CrO_3 (500 mg) in pyridine (10 ml) dropwise with stirring and the mixture was kept at room temperature overnight. The reaction mixture was poured into water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave white powder which was purified by reprecipitation from aqueous ethanol. Compound XIV was obtained as a white powder, (mp 73°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1711 (six membered ketone). *Anal.* Calcd. for $C_{68}H_{120}O_{23}$: C, 62.53; H, 9.26. Found: C, 62.83; H, 9.13.

Degradation of XIV with Methanolic 7% HCl——Compound XIV (150 mg) was refluxed with methanolic 7% HCl for 4 hr and the reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was purified by chromatography on silica gel eluted with benzene-acetone (8: 1—6: 1) to give a trace of aglycone (XV). TLC (solvent F) *Rf* 0.22, UV λ_{max}^{EtOH} mμ (log ε): 265 (3.39); $\lambda_{max}^{cyclohexane}$ mμ (log ε): 257 (3.42).

Acknowledgement The authors express their gratitude to Prof. S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo, Prof. H. Hara, Faculty of Sciences, University of Tokyo and other members of the expedition for their encouragement throughout the course of this work. Thanks are also due to the members of Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analyses and NMR spectral measurement.