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Studies on the Saponins of Ginseng. II.[1] Structures of Ginsenoside-Re, -Rf and -Rg_2

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Ginsenoside-Re, -Rf and -Rg_2, the saponins of Ginseng root (root of *Panax ginseng* C.A. Meyer) were isolated. The structures of these saponins were established as being 20*S*-protopanaxatriol-6-[O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside]-20-O-β-D-glucopyranoside(II), 20*S*-protopanaxatriol-6-O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside(III) and 20*S*-protopanaxatriol-6-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside-(IV), respectively.

As we reported in the previous paper[1] the structures of five saponins, ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd, which were obtained from Ginseng root (root of *Panax ginseng* C.A. Meyer), have been established. Furthermore, the structure of ginsenoside-Rg_1 (I), one of the saponins composed of 20*S*-protopanaxatriol, was established by Shibata, *et al.*[3] as being 6,20-di-O-β-D-glucosyl-20*S*-protopanaxatriol.

The present paper describes the structure elucidation of ginsenoside-Re (II), -Rf (III) and -Rg_2 (IV), which possess 20*S*-protopanaxatriol as the aglycone.

The isolation of ginsenoside-Re, -Rf and -Rg_2 has been reported in the previous paper,[1] whose general properties are given in Table I.

On acid hydrolysis with HCl-dioxane-water, ginsenoside-Re, -Rf and -Rg_2 gave panaxatriol (V)[4] which was identified by thin-layer chromatography (TLC). The water soluble fraction of each hydrolysate was examined by TLC and gas-liquid chromatography (GLC) and the results are given in Table II.

Furthermore, the genuine aglycones of ginsenoside-Re, -Rf and -Rg_2 were established to be 20*S*-protopanaxatriol (VI)[4] by Smith's oxidative degradation.[5]

It has been reported that on the treatment of ginsenoside-Rb_1, -Rb_2 and Rc with aqueous acetic acid, the 20-O-glycosyl moieties are readily hydrolyzed being accompanied by the equilibrated epimerization of the C-20-*tert*-hydroxyl groups.[6,7] On the partial hydrolysis with 50% acetic acid, ginsenoside-Re gave a prosapogenin $C_{42}H_{72}O_{13}$, mp 187—189° and glucose, while no hydrolysis of the glycosyl linkage was observed in case of ginsenoside-Rf and -Rg_2. The further treatment of the prosapogenin of ginsenoside-Re with dil. mineral acid yielded panaxatriol, glucose, and rhamnose. The comparison of TLC (plate: silica gel H; solvent:

1) Part I: S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974).
2) Location: *a) Hatanodai 1-5-8, Shinagawa-ku, Tokyo; b) Kasumi 1-2-3, Hiroshima; c) Hongo, Bunkyo-ku, Tokyo.*
3) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
4) S. Shibata, O. Tanaka, K. Soma, and Y. Iida, *Tetrahedron Letters*, **1967**, 391.
5) F. Smith, G.W. Hay, and B.A. Lewis, "Method in Carbohydrate Chemistry," Vol. 5, Academic Press, New York and London, 1965, p. 361.
6) S. Sbibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).
7) M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972) and the references cited therein.

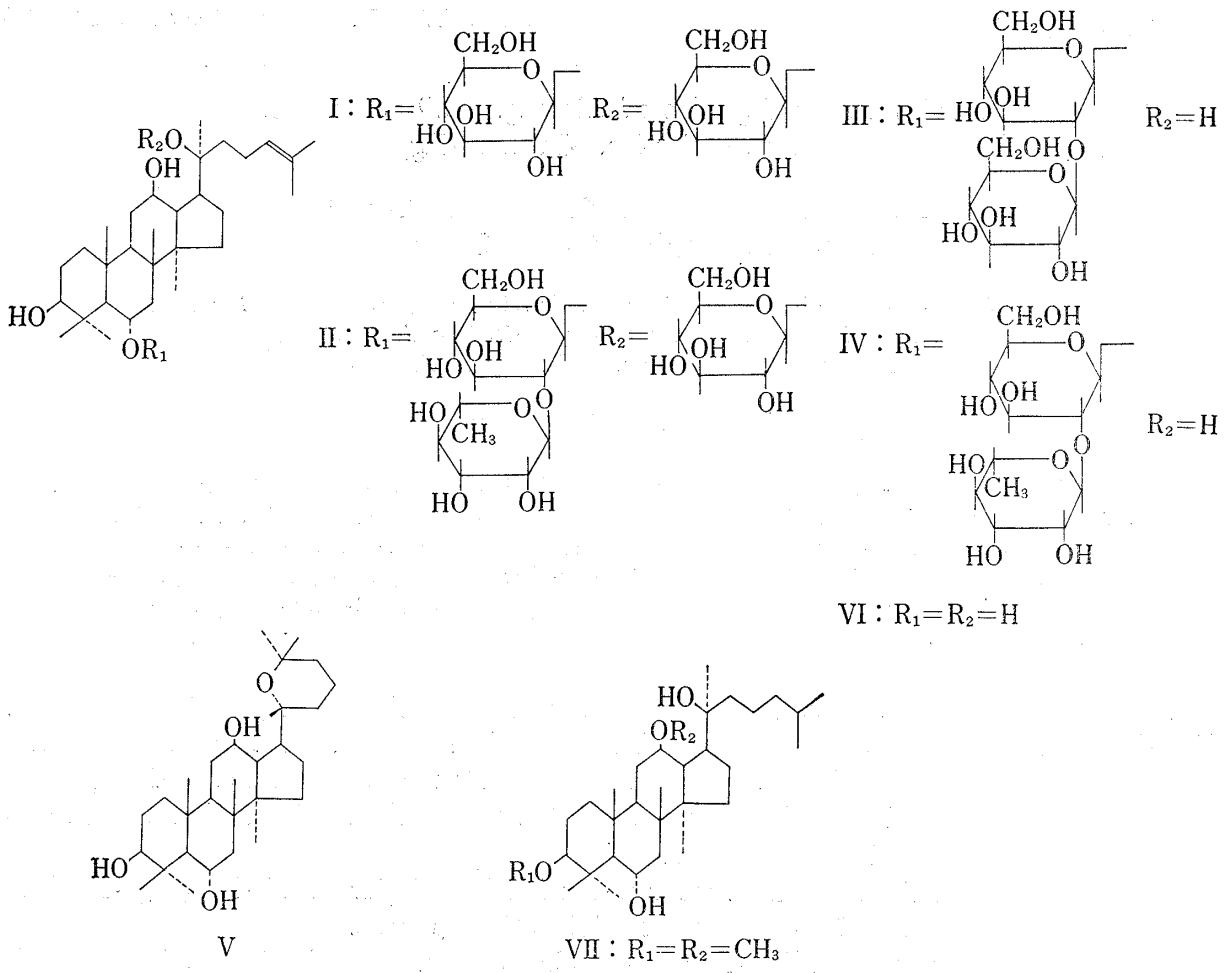

Fig. 1

TABLE I

Ginsenoside	Properties	mp (°C)	$[\alpha]_D^{30}$ (c in MeOH)	Formula	IR (KBr) cm^{-1}
Re	colorless needles (50% EtOH)	201—203	0—−1.00° (1.00)	$C_{48}H_{82}O_{18}$	3380(OH), 1620(C=C)
Rf	white powder (acetone)	(197—198)	+6.99° (1.00)	$C_{42}H_{72}O_{14}$	3380(OH), 1620(C=C)
Rg_2	colorless needles (EtOH)	187—189	+5.00—6.00° (1.00)	$C_{42}H_{72}O_{13}$	3400(OH), 1620(C=C)

TABLE II

Ginsenoside	Aglycone (Genuine aglycone)	Sugar component (mole)
Re	panaxatriol (20*S*-protopanaxatriol)	glucose (2), rhamnose (1)
Rf	panaxatriol (20*S*-protopanaxatriol)	glucose (2)
Rg_2	panaxatriol (20*S*-protopanaxatriol)	glucose (1), rhamnose (1)

chloroform: methanol: ethyl acetate: water=2: 2: 4: 1, *Rf* 0.25), infrared (IR) and nuclear magnetic resonance (NMR) spectra indicated that this prosapogenin must be identical with ginsenoside-Rg_2 (or with either its C-20 epimer or the mixture of both isomers). This was further confirmed by the comparison of O-methyl derivatives of both compounds. These observation suggests that ginsenoside-Rf and -Rg_2 have no glycosyl linkage at the C-20 hydroxyl group of their aglycones and ginsenoside-Re would be a 20-O-glucoside of ginsenoside-Rg_2.

Ginsenoside-Re, -Rf and -Rg_2 were methylated by the Hakomori method. The properties of these O-methylated saponins are shown in Table III. The IR spectra of O-methylated ginsenoside-Rf and -Rg_2 show the hydroxyl absorption band at 3360 cm^{-1} and 3400 cm^{-1}, respectively. The IR spectra of derivatives of protopanaxadiol and protopanaxatriol have been investigated and the absorption maxima of hydrogen bonded hydroxyl groups at $C_{(12)}$ and/or $C_{(20)}$ are summarized in Fig. 2.

Observations on the IR spectra of O-methylated ginsenoside-Rf and -Rg_2 suggest that the hydroxyl groups resisted to the Hakomori methylation are $C_{(20)}$-OH of ginsenoside-Rf

Table III

O-Methylated ginsenoside	Properties	$[\alpha]_D^{15}$ (c in $CHCl_3$)	Formula	IR in CCl_4 (cm^{-1}) OH band	NMR Anomeric proton (δ)
Re	crystalline powder (MeOH)	+0.58° (1.02)	$C_{60}H_{106}O_{18}$	—	4.40(1H(d) J=7Hz) 4.60(1H(d) J=7Hz) 5.31(1H(broad s))
Rf	crystalline bundles (hexane)	+0.30° (0.99)	$C_{51}H_{90}O_{14}$	3360	4.39(1H(d) J=7Hz) 4.77(1H(d) J=7Hz)
Rg_2	colorless plates (EtOH)	±0.00° (1.00)	$C_{50}H_{88}O_{13}$	3400	4.60(1H(d) J=7Hz) 5.31(1H(broad s))

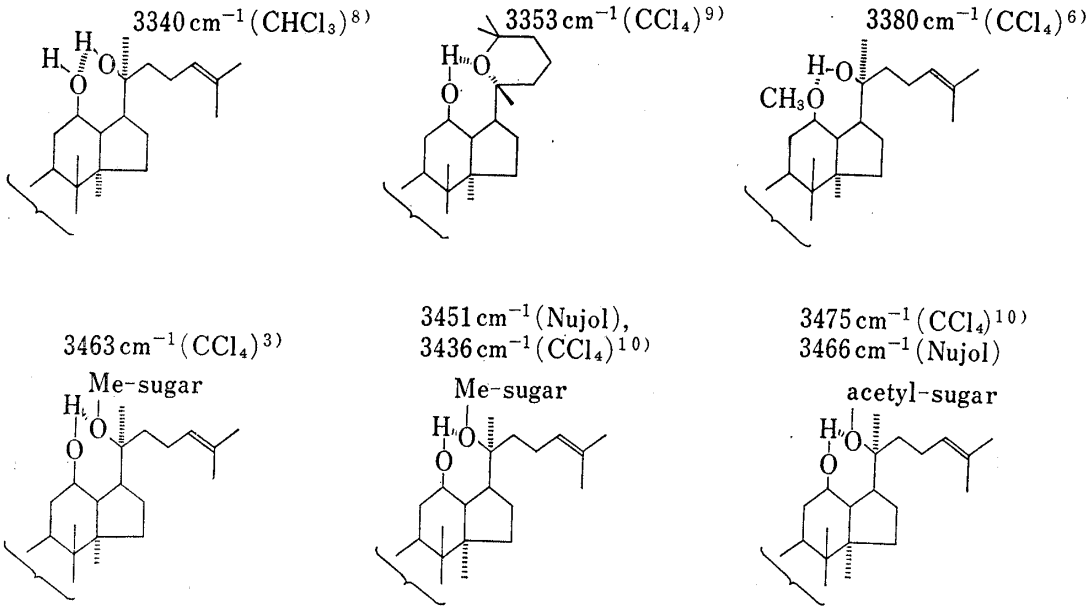

Fig. 2

8) S. Shibata, O. Tanaka, T Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966).
9) S. Shibata, O. Tanaka, M. Nagai, and T. Ishii, *Chem. Pharm. Bull* (Tokyo), **11**, 762 (1963); O. Tanaka, M. Nagai, and S. Shibata, *ibid.*, **14**, 1150 (1966).
10) N. Kondo, J. Shoji, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 2702 (1973).

and -Rg_2, respectively. It revealed that ginsenoside-Re has a sugar moiety at $C_{(20)}$ hydroxyl group, while ginsenoside-Rf and -Rg_2 have no glycoside linkage at $C_{(20)}$.

Taking account of the presence of one free hydroxyl group in O-methylated ginsenoside-Rf and -Rg_2, methanolyses of O-methylated ginsenoside-Re, Rf and -Rg_2 with hydrogen chloride were carried out to examine O-methylmonosaccharides by TLC and GLC. The formation of methyl 2,3,4,6-tetra-O-methylglucoside, methyl 3,4,6-tri-O-methylglucoside and methyl 2,3,4-tri-O-methylrhamnoside from O-methylginsenoside-Re, methyl 2,3,4,6-tetra-O-methylglucoside and methyl 3,4,6-tri-O-methylglucoside from O-methylginsenoside-Rf, methyl 3,4,6-tri-O-methylglucoside and methyl 2,3,4-tri-O-methylrhamnoside from O-methylginsenoside-Rg_2 was proved.

On catalytic reduction O-methylginsenoside-Re, -Rf and -Rg_2 gave dihydro derivatives which were hydrolyzed to reveal the formation of 3,12-di-O-methyldihydroprotopanaxatriol (VII) from O-methyldihydroginsenoside-Re, -Rf and -Rg_2.

The configuration of glucose of ginsenoside-Re, -Rf and -Rg_2 was revealed by the coupling constant (J=7 Hz) of anomeric proton signal in NMR spectra. On the other hand, the difference of molecular optical rotations between ginsenoside-Rg_1 ($[M]_D$=+240.3°) and ginsenoside-Re ($[M]_D$=0°——9.47°) is −240.3°——249.77° which shows the α configuration of L-rhamnopyranoside in ginsenoside-Re.[11] Accordingly, the α-configuration of L-rhamnopyranoside of ginsenoside-Rg_2 was deduced.

The structures of ginsenoside-Re, -Rf and -Rg_2 were established to be 20*S*-protopanaxatriol-6-[O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside]-20-O-β-D-glucopyranoside, 20*S*-protopanaxatriol-6-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside and 20*S*-protopanaxatriol-6-O-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside, respectively.

Elyakov, *et al.*[12,13] reported the isolation of panaxoside A—F from the root of Ginseng cultivated in the Far Eastern region of the USSR. Although the direct comparisons of the physical and chemical properties of ginsenoside-Ro—-Rg_2 with those of panaxoside A—F have not been made, the structures of carbohydrate chains of these saponins do not agree with Elyakov's formula.

The pharmacological and biochemical investigations of ginsenosides are now 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 a hydrogen flame ionization detector. The *Rf* values were determined by thin-layer chromatography on silica gel H using solvent A: $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase); solvent B: benzene–acetone (3: 1); solvent C: hexane–acetone (2: 1); solvent D: hexane–acetone (3: 1); solvent E: hexane–acetone (7: 2); solvent F: hexane–acetone (4: 1); solvent G: $CHCl_3$–MeOH–AcOEt–H_2O (2: 2: 4: 1 the lower phase); solvent H: BuOH–AcOH–H_2O (4: 1: 5 the upper phase), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Isolation of Saponins——As we reported in the previous paper[1] the crude saponin fraction was submitted to column chromatography on silica gel to afford five fractions (Fr. 1—Fr. 5). Fr. 4 and 5 were purified on silica gel with $CHCl_3$–MeOH–AcOEt–H_2O (2: 2: 4: 1 the lower phase) to afford ginsenoside-Rd and -Re from the former and ginsenoside-Rf, -Rg_1 and -Rg_2 from the latter.

Properties of Saponins——Ginsenoside-Re(II): The yield from the butanol extract was 6.0%. *Anal.* Calcd. for $C_{48}H_{82}O_{18}\cdot 2H_2O$: C, 58.64; H, 8.83. Found: C, 58.92; H, 8.66. Ginsenoside-Rf(III): The yield from the butanol extract was 2.0%. *Anal.* Calcd. for $C_{42}H_{72}O_{14}\cdot 2H_2O$: C, 60.52; H, 9.16. Found: C, 60.40;

11) methyl α-L-rhamnopyranoside $[M]_D=-111°$, methyl β-L-rhamnopyranoside $[M]_D=+170°$.
12) G.B. Elyakov, L.I. Strigina, N.I. Uvarova, V.E. Vaskovsky, A.K. Dzizenko, and N.K. Kochetkov, *Tetrahedron Letters*, **1964**, 3591.
13) G.B. Elyakov, N.I. Uvarova, and R.P. Gorshkova, *Tetrahedron Letters*, **1965**, 4669.

H, 8.95. Ginsenoside-Rg_2(IV): The yield from the butanol extract was 0.4%. *Anal.* Calcd. for $C_{42}H_{72}O_{13}\cdot 2H_2O$: C, 61.43; H, 9.35. Found: C, 61.57; H, 9.32.

Hydrolyses of Ginsenoside-Re, -Rf and -Rg_2——II, III and IV were hydrolyzed with 2N HCl (4N HCl–50% dioxane=1: 1 v/v) with refluxing on a water bath for 4 hr, respectively. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried on anhyd. Na_2SO_4 and evaporated. The aqueous layer was neutralized with Amberlite IR-4B and evaporated *in vacuo* to dryness. The $CHCl_3$ soluble fraction (genins) and water soluble fraction (sugars) were identified by TLC and GLC by comparing with an authentic sample. Genins: TLC (solvent B) *Rf* 0.32 (panaxatriol). Sugars: TLC (solvent A) *Rf* 0.10 (glucose), 0.36 (rhamnose). GLC (column: 5% SE-52 on chromosorb W 3 mm×2 m; column temp.: 165°; injection temp.: 240°; carrier gas: N_2 1 kg/cm²; samples: trimethylsilane (TMS) derivatives) t_R (min) 10.0, 14.0 (glucose), 3.7, 4.8 (rhamnose).

Oxidative Degradation of Ginsenoside-Re, -Rf and -Rg_2 with $NaIO_4$——II, III and IV were treated by the method described in the previous paper.[10] The product was purified by chromatography on silica gel eluted with hexane–acetone (2: 1) to give a white powder from benzene, which was identified to be 20*S*-protopanaxatriol (VI) by TLC and GLC. TLC (solvent C) *Rf* 0.24 (*cf.* 20*R*-protopanaxatriol *Rf* 0.23). GLC (column: glass column 1.5% SE-30 DMCS chromosorb W, 3 mm×2 m; column temp.: 270°; injection temp.: 360°; carrier gas: N_2 flow 1.5 kg/cm², sample: TMS derivatives) t_R (min) 7.4 (*cf.* 20*R*-protopanaxatriol t_R (min) 9.3).

Partial Hydrolyses of Ginsenoside-Re, -Rf and -Rg_2 with 50% AcOH——Each glycoside was heated with 50% AcOH for 4 hr at 70°. The formed precipitate was filtered, washed with water and dried. The aqueous solution was extracted with *n*-BuOH saturated with water and the aqueous layer was evaporated *in vacuo*. In case of ginsenoside-Rf and -Rg_2, no significant change of the *Rf* value in TLC was observed revealing that no cleavage of the glycoside linkage occurred. Ginsenoside-Re afforded a prosapogenin, colorless needles from EtOH, mp 187—189°. The mixed fusion and the comparison of TLC (solvent G), IR and NMR spectra indicated that this prosapogenin was identical with ginsenoside-Rg_2 (or with either its C-20 epimer or the mixture of both isomers). The aqueous layer of hydrolysate of ginsenoside-Re was examined by TLC and GLC. TLC: *Rf* 0.10 (solvent A) glucose, *Rf* 0.19 (solvent H) glucose. GLC (column: 5% SE-52 on chromosorb W 3 mm×2 m; column temp.: 165°; injection temp.: 240°; carrier gas: N_2 1 kg/cm²; samples: TMS derivatives) t_R (min) glucose: 9.7, 13.5.

Methylation of Ginsenoside-Re, -Rf and -Rg_2 by Hakomori Method——II, III and IV were methylated by the method described in the previous papers.[1,11] The property of each O-methyl derivative was listed in Table III. O-Methylginsenoside-Re: mp 115°. *Anal.* Calcd. for $C_{60}H_{106}O_{18}$: C, 64.60; H, 9.58. Found: C, 64.62; H, 9.35. NMR (in $CDCl_3$) δ: 0.85 (3H(s) C$\underline{H}_3$), 0.88 (6H(s) 2×C$\underline{H}_3$), 0.98 (3H(s) C$\underline{H}_3$), 1.16 (3H(s) C$\underline{H}_3$), 1.22 (3H(d) J=6 Hz C$\underline{H}_3$), 1.24 (3H(s) C$\underline{H}_3$), 1.60 (3H(s) C$\underline{H}_3$), 1.64 (3H(s) C$\underline{H}_3$), 3.33—3.60 (3H(s) 12×OC$\underline{H}_3$), 4.40 (1H(d) J=7 Hz, anomer $\underline{H}$), 4.60 (1H(d) J=7 Hz, anomer $\underline{H}$), 5.10 (1H(broad) $\underline{H}$>C=C<), 5.31 (1H(broad s), anomer $\underline{H}$). O-Methylginsenoside-Rf: mp 171°. *Anal.* Calcd. for $C_{51}H_{90}O_{14}$: C, 66.05; H, 9.78. Found: C, 66.30; H, 9.59. NMR (in $CDCl_3$) δ: 0.87 (3H(s) C$\underline{H}_3$), 0.90 (3H(s) C$\underline{H}_3$), 0.94 (3H(s) C$\underline{H}_3$), 1.02 (3H(s) C$\underline{H}_3$), 1.07 (3H(s) C$\underline{H}_3$), 1.30 (3H(s) C$\underline{H}_3$), 1.60 (3H(s) C$\underline{H}_3$), 1.67 (3H(s) C$\underline{H}_3$), 3.32—3.60 (3H(s) 9×OC$\underline{H}_3$), 4.39 (1H(d) J=7 Hz, anomer $\underline{H}$), 4.77 (1H(d) J=7 Hz, anomer $\underline{H}$), 5.14 (1H(broad) $\underline{H}$>C=C<). O-Methylginsenoside-Rg_2: mp 126°. *Anal.* Calcd. for $C_{50}H_{88}O_{13}$: C, 66.93; H, 9.88. Found: C, 67.10; H, 9.79. NMR (in $CDCl_3$) δ: 0.88 (3H(s) 3×C$\underline{H}_3$), 1.00 (3H(s) C$\underline{H}_3$), 1.07 (3H(s) C$\underline{H}_3$), 1.22 (3H(d) J=6 Hz, C$\underline{H}_3$), 1.26 (3H(s) C$\underline{H}_3$), 1.60 (3H(s) C$\underline{H}_3$), 1.65 (3H(s) C$\underline{H}_3$), 3.33—3.57 (3H(s) 8×OC$\underline{H}_3$), 4.60 (1H(d) J=7 Hz, anomer $\underline{H}$), 5.15 (1H(broad) $\underline{H}$>C=C<), 5.31 (1H(broad s) anomer $\underline{H}$).

Methanolyses of O-Methylginsenoside-Re, -Rf and -Rg_2 with Methanolic 5% HCl——O-Methylginsenoside-Re, -Rf and -Rg_2 were methanolyzed with methanolic 5% HCl refluxing for 2 hr, respectively. The reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was examined by TLC and GLC to identify methyl 2,3,4,6-tetra-O-methylglucopyranoside, methyl 3,4,6-tri-O-methylglucopyranoside and methyl 2,3,4-tri-O-methylrhamnopyranoside from O-methylginsenoside-Re, methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 3,4,6-tri-O-methylglucopyranoside from O-methylginsenoside-Rf and methyl 3,4,6-tri-O-methylglucopyranoside and methyl 2,3,4-tri-O-methylrhamnopyranoside from O-methylginsenoside-Rg_2, respectively. TLC (solvent E) *Rf* 0.58, 0.40 (methyl 2,3,4,6-tetra-O-methylgucopyranoside), 0.19 (methyl 3,4,6-tri-O-methylglucopyranoside), 0.71 (methyl 2,3,4-tri-O-methylrhamnopyranoside). GLC (column: 5% NPGS on chromosorb W 3 mm×2 m; column temp.: 165°; injection temp.: 230°; carrier gas: N_2 1 kg/cm²). t_R (min) 4.7, 6.6 (methyl 2,3,4,6-tetra-O-methylglucopyranoside), 12.2 (methyl 3,4,6-tri-O-methylglucopyranoside), 2.3 (methyl 2,3,4-tri-O-methylrhamnopyranoside).

Catalytic Reduction of O-Methylginsenoside-Re, -Rf and -Rg_2——O-Methylginsenoside-Re, -Rf and -Rg_2 were catalytically reduced on PtO_2 in EtOH and the reaction mixture was filtered. The filtrate was evaporated *in vacuo* and the residue was purified by reprecipitation from MeOH–H_2O. Dihydro-O-methylginsenoside-Re, -Rf and -Rg_2 were examined by NMR. NMR (in $CDCl_3$) δ: 0.81—1.25 (3H(s)×8, 3H(d)×1) (dihydro-O-methylginsenoside-Re and -Rg_2). δ: 0.81—1.30 (3H(s)×8) (dihydro-O-methylginsenoside-Rf).

Hydrolyses of Dihydro-O-methylginsenoside-Re, -Rf and -Rg_2 with Conc. HCl——Dihydro-O-methylginsenoside-Re, -Rf and -Rg_2 were hydrolyzed with conc. HCl at room temperature with stirring for 7 hr,

respectively. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and dried. After evaporation of the solvent *in vacuo* the residue was purified by chromatography on silica gel using hexane–acetone (8: 1) to give 3,12-di-O-methyldihydroprotopanaxatriol, colorless needles from hexane–acetone=20: 1, mp 180—182°, which was identified with an authentic sample by comparing TLC (solvent F, *Rf* 0.19) and IR spectra and mixed fusion.