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Studies on the Saponins of Ginseng. I. Structures of Ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd

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Ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd, the saponins of Ginseng root (root of *Panax ginseng* C. A. Meyer) have been isolated. The structures of these saponins have been established as I, II, III, IV, and V, respectively. Ginsenoside-Ro is identical with chikusetsusaponin V, isolated from *P. japonicum* C. A. Meyer (=*P. pseudoginseng* var. *japonicum* Hara) and *P. pseudoginseng* subsp. *himalaicus* var. *angustifolius*, while ginsenoside-Rb_1 is identical with saponin-D, isolated from the latter.

As already reported, the thin-layer chromatography (TLC) of the Ginseng extract has demonstrated the presence of numerous saponins designated as ginsenoside-Ro, -Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rf, -Rg_1, -Rg_2, -Rg_3 and -Rh.[2]

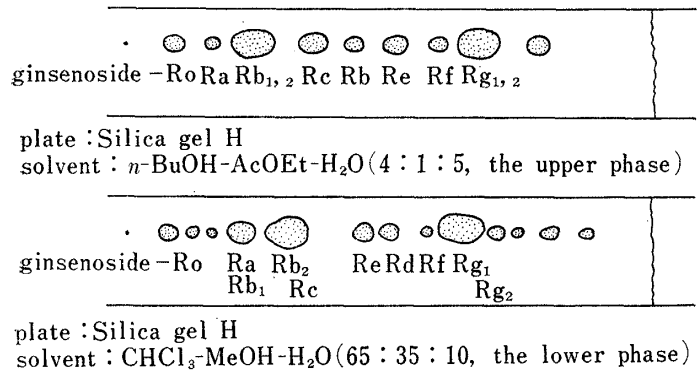

Fig. 1. Thin-Layer Chromatograms of Ginseng Saponins

Of these saponins, ginsenoside-Rg_1 was established by Shibata, *et al.*[3] as being 6,20-di-O-β-D-glucosyl-20*S*-protopanaxatriol. The present paper describes the isolation and the structure elucidation of ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd.

The roots of ginseng cultivated at Nagano prefecture were extracted with hot methanol and the extract was separated as shown in Chart 1. The TLC chromatograms of butanol extract on Silica gel H are shown in Fig. 1.

The general properties of ginsenoside-Ro(I), -Rb_1(II), -Rb_2(III), -Rc(IV) and -Rd(V) are given in Table I.

On acid hydrolysis with HCl-dioxane-water, ginsenoside-Ro gave oleanolic acid (VI) as an aglycone, while ginsenoside-Rb_1, -Rb_2, -Rc and -Rd gave panaxadiol (VII).[4] The monosaccharide components of each saponin are listed in Table II. The aglycones and monosaccharides were identified by TLC and gas-liquid chromatography (GLC), respectively.

The genuine aglycone of ginsenoside-Rb_1, -Rb_2, -Rc and -Rd was established to be 20*S*-protopanaxadiol[5] (VIII) by Smith's oxidative degradation.[6]

1) Location: *a) 1-5-8, Hatanodai Shinagawa-ku, Tokyo; b) 1-2-3, Kasumi Hiroshima; c) Hongo, Tokyo.*
2) S. Shibata, O. Tanaka, T. Ando, M. Sado, S. Tsushima, and T. Ohsawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 595 (1966).
3) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
4) M. Nagai, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **19**, 2349 (1971).
5) M. Nagai, T. Ando, N. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972).
6) F. Smith, G. W. Hay, and B.A. Lewis, "Method in Carbohydrate Chemistry," Vol. 5, Academic Press, New York and London, 1965, p. 361.

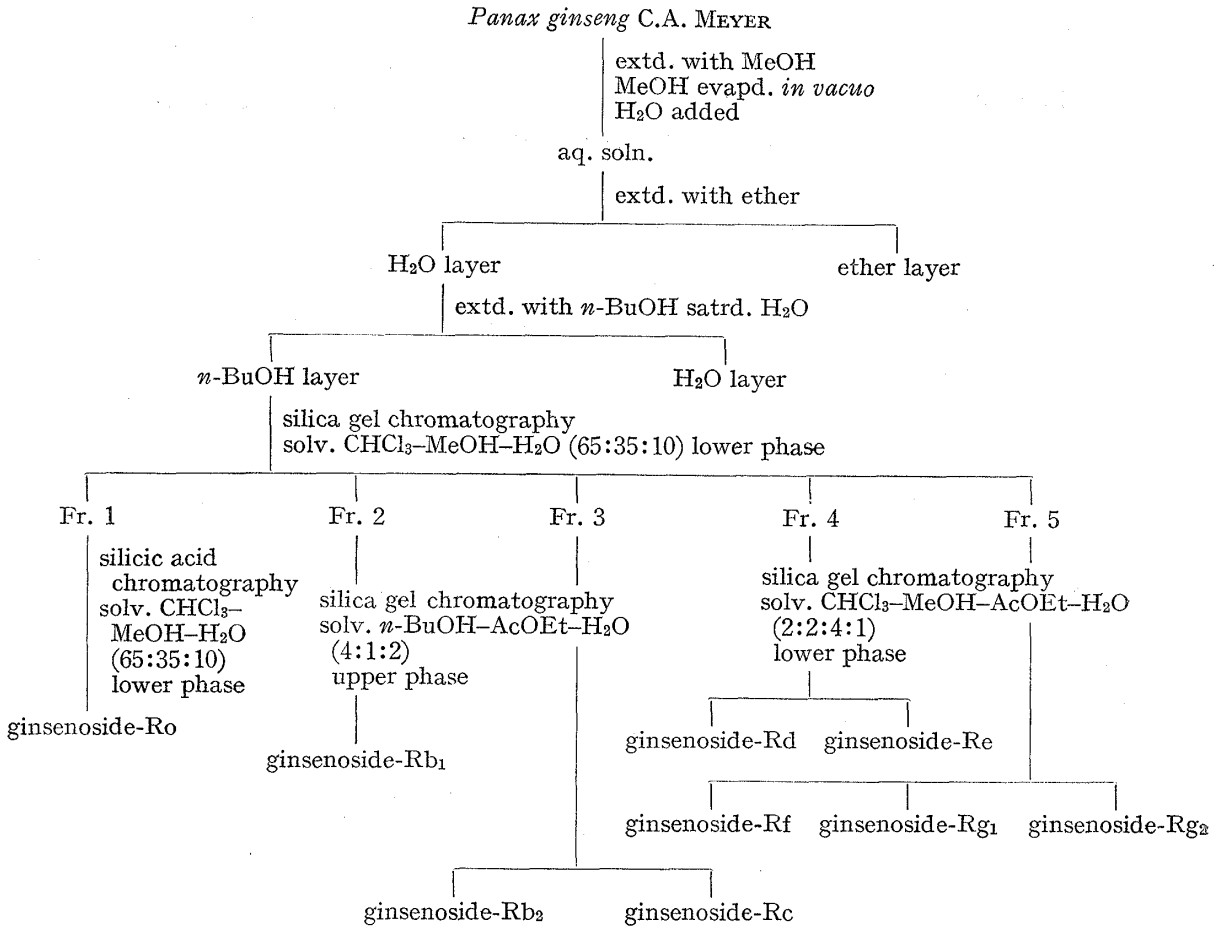

Chart 1. Extraction and Isolation of Ginseng Saponins

TABLE I

Ginsenoside	Properties	mp (°C)	$[\alpha]_D^{22}$ (*c* in MeOH)	Formula	IR (KBr) cm^{-1}
Ro	colorless needles (MeOH)	239—241	+15.33° (0.91)	$C_{48}H_{76}O_{19}$	3400(OH), 1740(COOR), 1728(COOH)
Rb_1	white powder (EtOH–BuOH=1:1)	(197—198)	+12.42° (0.91)	$C_{54}H_{92}O_{23}$	3400(OH), 1620(C=C)
Rb_2	white powder (EtOH–BuOH=1:5)	(200—203)	+3.05° (0.98)	$C_{53}H_{90}O_{22}$	3400(OH), 1620(C=C)
Rc	white powder (EtOH–BuOH=1:5)	(199—201)	+1.93° (1.03)	$C_{53}H_{90}O_{22}$	3400(OH), 1620(C=C)
Rd	white powder (EtOH–AcOEt=1:1)	(206—209)	+19.38° (1.03)	$C_{48}H_{82}O_{18}$	3400(OH), 1620(C=C)

TABLE II

Ginsenoside	Aglycone (genuine aglycone)	Sugar component (mole)
Ro	oleanolic acid	glucose (2), glucuronic acid (1)
Rb_1	panaxadiol (20*S*-protopanaxadiol)	glucose (4)
Rb_2	panaxadiol (20*S*-protopanaxadiol)	arabinose (1), glucose (3)
Rc	panaxadiol (20*S*-protopanaxadiol)	arabinose (1), glucose (3)
Rd	panaxadiol (20*S*-protopanaxadiol)	glucose (3)

On partial hydrolysis with 50% acetic acid refluxing for 2 hr, ginsenoside-Rb_1, -Rb_2, -Rc and -Rd gave a prosapogenin, $C_{42}H_{72}O_{13}\cdot 1/2H_2O$. Based on the physical properties, this prosapogenin was suggested to be protopanaxadiol-3-O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside, (IX), as reported by Shibata, *et al.*,[7] and has finally been identified by mixed fusion and the comparisons of TLC and infrared (IR) spectra with an authentic sample. Consequently the two moles of glucose out of three or four in ginsenoside-Rb_1, -Rb_2, -Rc and -Rd were revealed to link to the $C_{(3)}$ hydroxyl group of 20*S*-protopanaxadiol.

The formations of gentiobiose from ginsenoside-Rb_1, a biose which consists of glucose and arabinose from -Rb_2, glucose and arabinose from -Rc and glucose from -Rd were confirmed by these partial hydrolyses.

As the presence of ester linkage was suggested in IR spectrum, ginsenoside-Ro was methylated repeatedly by the Kuhn method to avoid the degradation under the basic condition, while ginsenoside-Rb_1, -Rb_2, -Rc and -Rd were methylated by the Hakomori method. The properties of these O-methylated saponins are shown in Table III.

Per-O-methylginsenoside-Ro, $C_{59}H_{98}O_{19}$, gave, on reduction with $LiAlH_4$, erythrodiol bioside (X), $C_{48}H_{82}O_{12}$, 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol.

Table III

O-Methylated ginsenoside	Properties	$[\alpha]_D^{22}$ (c in $CHCl_3$)	Formula	IR in CCl_4 (cm^{-1})	NMR Anomeric proton (δ)
Ro	white powder (aq. MeOH)	+12.6° (0.63)	$C_{59}H_{98}O_{19}$	nil. (OH)	4.40(1H(d) J=7 Hz), 4.55(1H(d) J=7 Hz) 5.3—5.45 (1H, overlapped on $\rangle$C=C$\langle$)
Rb_1	white powder (aq. MeOH)	+0.96° (1.03)	$C_{68}H_{120}O_{23}$	3420 (OH)	4.17(1H(d) J=7 Hz), 4.26(1H(d) J=7 Hz) 4.38(1H(d) J=7 Hz), 4.57(1H(d) J=7 Hz)
Rb_2	white powder (aq. MeOH)	+1.03° (0.97)	$C_{66}H_{116}O_{22}$	3420 (OH)	4.23(1H(d) J=7 Hz), 4.30(1H(d) J=7 Hz) 4.40(1H(d) J=7 Hz), 4.65(1H(d) J=7 Hz)
Rc	white powder (aq. MeOH)	+1.24° (0.80)	$C_{66}H_{116}O_{22}$	3400 (OH)	4.27(1H(d) J=8 Hz), 4.47(1H(d) J=7 Hz) 4.67(1H(d) J=7 Hz), 5.00(1H(s))
Rd	white powder (aq. MeOH)	+14.43° (0.97)	$C_{59}H_{104}O_{18}$	3400 (OH)	4.24(1H(d) J=7 Hz), 4.45(1H(d) J=7 Hz) 4.65(1H(d) J=7 Hz)

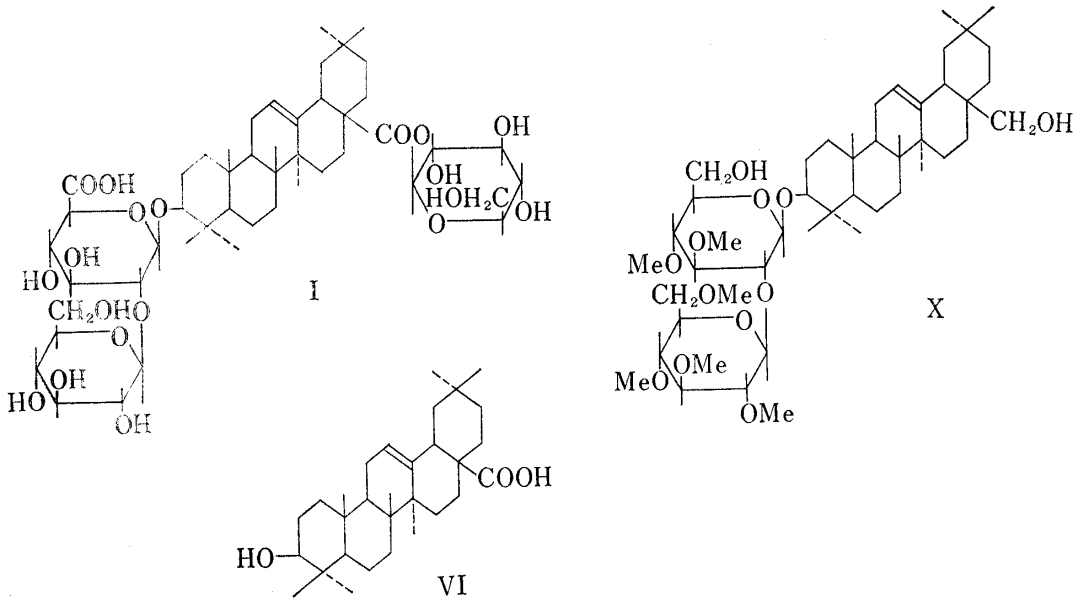

Fig. 2

7) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

Methanolysis of erythrodiol bioside with methanolic 2N HCl gave erythrodiol, mp 234—236°, methyl 2,3,4,6-tetra-O-methyl-D-glucoside and methyl 3,4-di-O-methyl-D-glucoside. All the properties of ginsenoside-Ro and its per-O-methyl ether mentioned above strongly suggest that this saponin must be identical with chikusetsusaponin-V (I), the main saponin of *Panax japonicum* C.A. MEYER(=*P. pseudoginseng* var, *japonicum* HARA)[8] and *P. pseudoginseng* subsp. *himalaicus* var. *angustifolius*.[9] The identity of both compounds has finally been proved by the direct comparison.

Taking account of the presence of one free hydroxyl group in O-methylated ginsenoside-Rb_1, -Rb_2, -Rc and -Rd, methanolysis of these compounds with HCl was carried out to examine O-methylmono-saccharides formed by TLC and GLC (Table IV).

TABLE IV

O-Methylated ginsenoside	O-Methylated monosaccharides	*Rf* values (TLC)[a]	t_R (min)[b]
Rb_1	methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside	0.58, 0.40	3.5, 4.8
	methyl 2,3,4-tri-O-methyl-D-glucopyranoside	0.15	10.0
	methyl 3,4,6-tri-O-methyl-D-glucopyranoside	0.19	8.6
Rb_2	methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside	0.58, 0.40	3.5, 4.8
	methyl 2,3,4-tri-O-methyl-D-glucopyranoside	0.15	10.0
	methyl 3,4,6-tri-O-methyl-D-glucopyranoside	0.19	8.6
	methyl 2,3,4-tri-O-methyl-L-arabinopyranoside	0.50	3.3
Rc	methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside	0.58, 0.40	3.5, 4.8
	methyl 2,3,4-tri-O-methyl-D-glucopyranoside	0.15	10.0
	methyl 3,4,6-tri-O-methyl-D-glucopyranoside	0.19	8.6
	methyl 2,3,5-tri-O-methyl-L-arabinofuranoside	0.82	2.1, 2.6
Rd	methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside	0.58, 0.40	3.5, 4.8
	methyl 3,4,6-tri-O-methyl-D-glucopyranoside	0.19	8.6

a) plate: Silica gel H; solvent: hexane–acetone (7:2)
b) column: 5% NPGS (on Chromosorb W) 3 mm×2m; column temp.: 175°; injection temp.: 230°; carrier gas: N_2 1 kg/cm²

As the sugar linkage to the C_{20}-tertiary hydroxyl group of protopanaxadiol or protopanaxatriol is readily hydrolyzed with 50% acetic acid,[3,9] the sugar moieties of ginsenoside-Rb_1, -Rb_2, -Rc and -Rd are suggested to be attached to the C_{20} hydroxyl group. To confirm the position of the hydroxyl group which resists to the Hakomori permethylation, O-methylated ginsenoside-Rb_1, -Rb_2, -Rc and -Rd were hydrogenated. The dihydro derivatives were oxidized with CrO_3 in pyridine to give the corresponding ketone derivatives, which show IR absorption band of six membered C=O at 1710—1714 cm^{-1}. These ketones were treated with methanolic 7% HCl to afford common aglycone (XI) which shows an absorption maximum at 265 nm (log ε 3.39) in ultraviolet (UV) spectrum.[10] These results suggest that the hydroxyl group which resists to the methylation by the Hakomori method is oxidized to ketone, and the hydrolysis of these ketone derivatives with HCl resulted in dehydration and hydride shift to afford α,β-unsaturated ketones. The location of the free hydroxyl group has therefore been assigned to $C_{(12)}$.

As we reported in the previous paper,[9] ginsenoside-Rb_1 resembles to saponin-D, 20*S*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[O-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside], which was isolated from *P. pseudoginseng* subsp. *himalaicus* var. *angustifolius*, and the identity was established by a direct comparison.

The configuration of each sugar of ginsenoside-Rb_2, -Rc and -Rd was revealed by the coupling constants (J=7—8 Hz) of anomeric proton signals in nuclear magnetic resonance

8) N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).
9) N. Kondo, J. Shoji, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 2702 (1973).
10) S. Shibata, M. Fujita, H. Itokawa, O. Tanaka, and T. Ishii, *Chem. Pharm. Bull.* (Tokyo), **6**, 759 (1963).

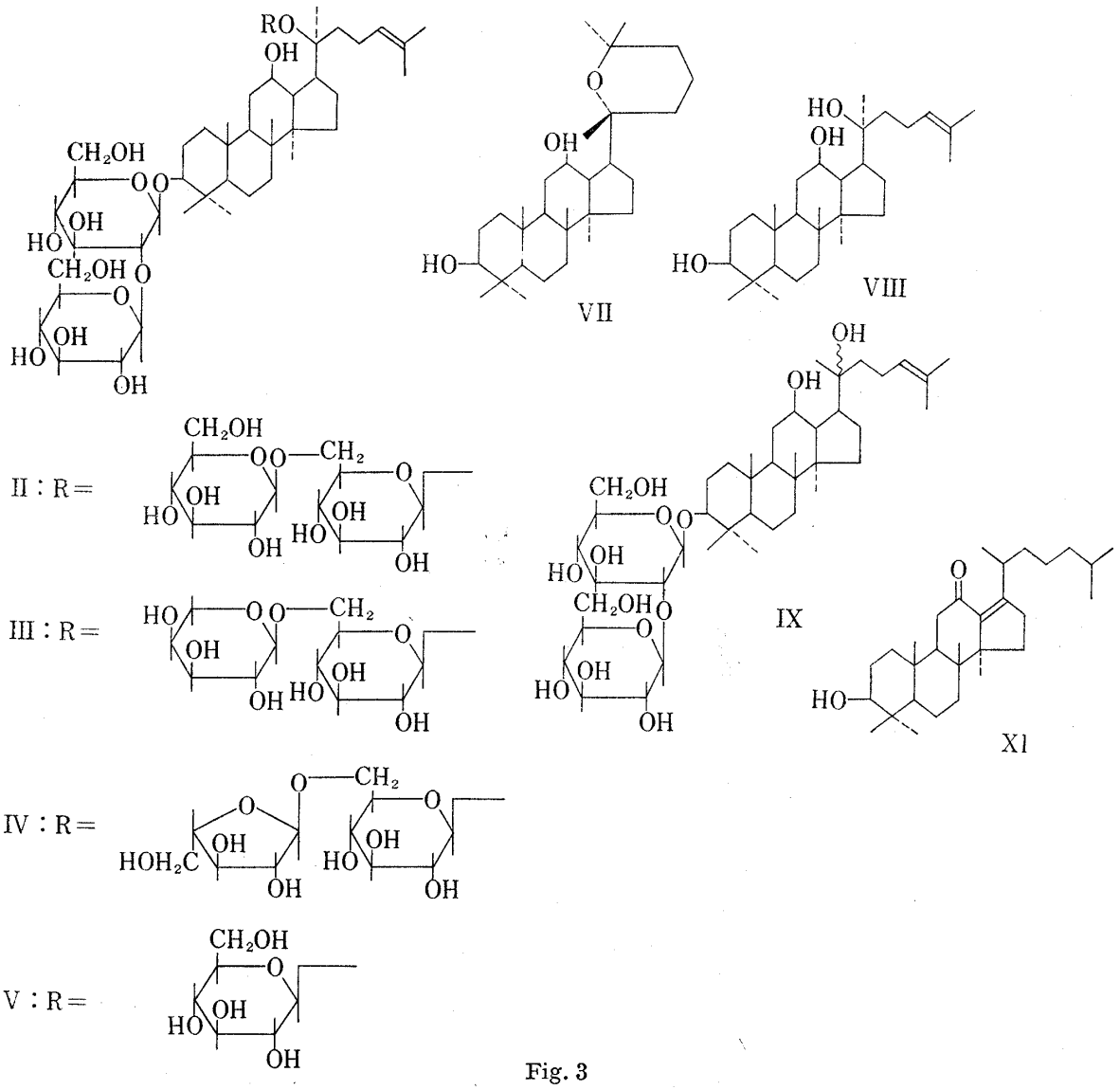

Fig. 3

(NMR) spectra (Table III). The difference of molecular optical rotations between ginsenoside-Rc ($[M]_D = +20.8°$) and ginsenoside-Rd ($[M]_D = +183.3°$) is $-162.5°$, which reveals the α configuration of L-arabinofuranoside in ginsenoside-Rc.[11]

Consequently, the structures of ginsenoside-Rb_2, -Rc and -Rd were established to be 20*S*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[O-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside], 20*S*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[O-α-L-arabinofuranosyl(1→6)-β-D-glucopyranoside] and 20*S*-protopanaxadiol-3-[O-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside]-20-[O-β-D-glucopyranoside], respectively.

It is very interesting that ginsenoside-Rb_2(arabinopyranoside) and ginsenoside-Rc(arabinofuranoside) coexist in the same plant.

Elyakov, *et al.* have reported the isolation of panaxoside A—F and the structure of carbohydrate chains of panaxoside D, E and F from the root of Ginseng cultivated in the Far Eastern region of the USSR.[12,13] The direct comparisons of the physical and chemical

11) Methyl α-L-arabinofuranoside $[M]_D = -226°$, methyl β-L-arabinofuranoside $[M]_D = +208°$.
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.

properties of ginsenoside-Ro, -Rb_1, -Rb_2, -Rc and -Rd with those of panaxoside D, E and F have not been made. The structures of carbohydrate chains of these saponins do not agree with Elyakov's formulae.

The pharmacological and biochemical investigations of ginsenoside-Ro, -Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rf, -Rg_1 and others 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 TLC on Silica gel H using solvent A: $CHCl_3$–MeOH–H_2O (65 : 35 : 10 the lower phase); solvent B: $CHCl_3$–MeOH–H_2O (7 : 3 : 1 the lower phase); solvent C: $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1 the lower phase); solvent D: *n*-BuOH–AcOEt–H_2O (4 : 1 : 2 the upper phase); solvent E: *n*-BuOH–AcOH–H_2O (4 : 1 : 5 the upper phase); solvent F: benzene–AcOEt (1 : 1); solvent G: benzene–acetone (2 : 1); solvent H: benzene–acetone (3 : 1); solvent I: benzene–acetone (4 : 1); solvent J: hexane–acetone (2 : 1); solvent K: hexane–acetone (3 : 1); solvent L: hexane–acetone (7 : 2), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Material——The material, dried roots of *Panax ginseng* C. A. MEYER, which were cultivated at Nagano prefecture, were kindly given us from Takeda Chemical Industries, Ltd.

Extraction——Three kg of crushed material was extracted five times with 2 liter of hot methanol. After evaporation of the solvent under a reduced pressure, 530 g of the brown residue was obtained (yield from the dried material 17.7%). The extract was suspended in water and extracted with *n*-BuOH saturated with water. The *n*-BuOH layer was concentrated *in vacuo* to afford 103 g of crude saponin (yield from the dried material 3.4%). The *n*-BuOH soluble fraction was detected by TLC and revealed to contain ginsenoside-Ro, -Ra, -Rb_1, -Rb_2 -Rc, -Rd, -Re, -Rf, -Rg_1, and -Rg_2 (Fig. 1).

Isolation of Saponins——The crude saponin fraction was submitted to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (65 : 35 : 10 the lower phase) to afford five fractions (Fr. 1—Fr. 5). Fr. 1 was purified by chromatography on silicic acid with $CHCl_3$–MeOH–H_2O (65 : 35 : 10 the lower phase) to give pure ginsenoside-Ro. Fr. 2 and 3 were submitted to column chromatography on silica gel with *n*-BuOH–AcOEt–H_2O (4 : 1 : 2 the upper phase) to afford ginsenoside-Rb_1, from the former and ginsenoside-Rb_2 and -Rc from the latter, respectively. 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 (Table I)——Ginsenoside-Ro (I): The yield from the butanol extract was 0.8%. *Anal.* Calcd. for $C_{48}H_{76}O_{19}\cdot 2H_2O$: C, 57.06; H, 7.75. Found: C, 56.76; H, 8.02.

Ginsenoside-Rb_1 (II): The yield from the butanol extract was 18.8%. *Anal.* Calcd. for $C_{54}H_{92}O_{23}\cdot 3H_2O$: C, 55.75; H, 8.49. Found: C, 56.17; H, 8.19.

Ginsenoside-Rb_2 (III): The yield from the butanol extract was 8.4%. *Anal.* Calcd. for $C_{53}H_{90}O_{22}\cdot 4H_2O$: C, 55.23; H, 8.58. Found: C, 55.13; H, 8.40.

Ginsenoside-Rc (IV): The yield from the butanol extract was 10.4%. *Anal.* Calcd. for $C_{53}H_{90}O_{22}\cdot 3H_2O$: C, 56.15; H, 8.53. Found: C, 56.17; H, 8.53.

Ginsenoside-Rd (V): The yield from the butanol extract was 6%. *Anal.* Calcd. for $C_{48}H_{82}O_{18}\cdot 3H_2O$: C, 57.57; H, 8.87. Found: C, 57.68; H, 8.86.

Hydrolyses of Ginsenoside-Ro, -Rb_1, -Rb_2, -Rc, and -Rd——I, II, III, IV, and V were hydrolyzed with 2 N HCl (4 N 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 authentic sample.

Genins: TLC (*Rf*) oleanolic acid: 0.54 (solvent F); panaxadiol: 0.54 (solvent H).

Sugars: TLC (*Rf*) glucose: 0.10 (solvent A), 0.19 (solvent E); arabinose: 0.23 (solvent A), 0.34 (solvent E); glucoronic acid: 0.17 (solvent E); glucuronolactone: 0.30 (solvent E). GLC (column: 5% SE-52 on chromosorb w 6 mm × 2 m; column temp.: 180°; injection temp.: 240°; carrier gas: N_2 1 kg/cm²; samples: TMS derivatives) t_R (min) glucose: 11.7, 16.8; arabinose: 4.0, 4.5; glucuronolactone: 9.0.

Methylation of Ginsenoside-Ro with CH_2N_2 (Formation of Chikusetsusaponin V Monomethyl Ester)——Ginsenoside-Ro was treated as described in the previous paper[8] to afford a monomethyl ester, a white powder from EtOH–AcOEt, (mp 244—245°). The product was identified with chikusetsusaponin V monomethyl ester by TLC (*Rf* 0.48 (solvent A)) and by comparison of IR spectra.

Per-O-methylation of Ginsenoside-Ro (Formation of Per-O-methylchikusetsusaponin V)——According to the previous paper,[8] ginsenoside-Ro was methylated by the Kuhn method. The product was purified by chromatography on silica gel using benzene–acetone (5: 1) followed by reprecipitation from dilute MeOH, affording per-O-methylginsenoside-Ro as a white powder, (mp 116—118°), *Anal.* Calcd. for $C_{59}H_{98}O_{19}$: C, 63.74; H, 8.88. Found: C, 63.43; H, 8.48. IR ν_{max}^{Nujol} cm^{-1}: OH(nil), 1757(COOR). NMR (in $CDCl_3$) δ: 0.78—1.12 (3H(s)×7 C$\underline{H}_3$), 3.34—3.78 (3H(s)×11 OC$\underline{H}_3$), 4.40 (1H(d) J=7 Hz anomer $\underline{H}$), 4.55 (1H(d) J=7 Hz anomer $\underline{H}$), 5.3—5.45 (2H(broad) >C=C<$^{\underline{H}}$, anomer H). Pre-O-methylginsenoside-Ro was identified with per-O-methylchikusetsusaponin V by comparing TLC (*Rf* 0.70 (solvent K)) and IR spectra.

Reductive Cleavage of Per-O-methylginsenoside-Ro with $LiAlH_4$——Per-O-methylginsenoside-Ro was reduced by the method described in the previous paper.[8] The ether soluble fraction was purified by reprecipitation from dilute MeOH, affording hexa-O-methylerythrodiol bioside as a white powder, (mp 127—129°), *Anal.* Calcd. for $C_{48}H_{62}O_{12}$: C, 67.76; H, 9.76. Found: C, 67.65; H, 9.30. NMR (in $CDCl_3$) δ: 0.82—1.14 (3H(s)×7 C$\underline{H}_3$), 3.34—3.62 (3H(s)×6 OC$\underline{H}_3$), 4.35 (1H(d) J=7 Hz anomer $\underline{H}$), 4.65 (1H(d) J=7 Hz anomer $\underline{H}$), 5.20 (1H(m) >C=C<$^{\underline{H}}$). It was identified with hexa-O-methylerythrodiol bioside derived from per-O-methylchikusetsusaponin V by comparing IR and NMR spectra.

The $CHCl_3$-soluble fraction was examined by TLC to reveal the presence of two methylated monosaccharides, which were identified with authentic samples of 2,3,4,6-tetra-O-methylglucose and 2,3,4,6-tetra-O-methylsorbitol by TLC (solvent G) and GLC (column: 3% SE-30 on chromosorb w, 3 mm×1 m; column temp.: 150°; carrier gas: N_2 flow 0.65 kg/cm²; samples: TMS derivatives). 2,3,4,6-tetra-O-methylglucose: *Rf* 0.35, t_R (min) 3.4; 2,3,4,6-tetra-O-methylsorbitol: *Rf* 0.21, t_R (min) 7.0.

Methanolysis of Hexa-O-methylerythrodiol Bioside——Hexa-O-methylerythrodiol bioside was refluxed with methanolic 2 N HCl for 2 hr and the reaction mixture was treated by the method described in the previous paper.[8] Erythrodiol, methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 3,4-di-O-methyl-D-glucopyranoside were identified with authentic samples by mixed fusion and by TLC (solvent G) and GLC (column: 5% NPGS on chromosorb w 3 mm×2 m; column temp.: 170°; injection temp.: 230°; carrier gas: N_2 1 kg/cm²).

Erythrodiol: colorless needles from acetone, mp 234—236°, *Rf* 0.63.

Methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside: *Rf* 0.58, 0.49; t_R (min) 3.2, 4.3.

Methyl 3,4-di-O-methyl-D-glucopyranoside: *Rf* 0.11; t_R (min) 18.6, 21.5.

Partial Hydrolyses of Ginsenoside-Rb_1, -Rb_2, -Rc, and -Rd 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 product was recrystallized from MeOH to give colorless needles, mp 260—264° (lit. 262—264°), IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1620 (C=C). This product was identified with an authentic sample by comparing TLC (*Rf* 0.50 (solvent B)) and IR spectra and by mixed fusion.

Oxidative Degradation of Ginsenoside-Rb_1, -Rb_2, -Rc, and -Rd with $NaIO_4$——Ginsenoside-Rb_1, -Rb_2, -Rc and -Rd were treated by the method described in the previous paper.[8] The product was purified by chromatography on silica gel eluted with hexane–acetone (3: 1) to afford colorless needles from benzene, mp 199°, which was identified to be 20*S*-protopanaxadiol (VIII) by TLC and GLC. TLC (solvent J) *Rf* 0.31 (*cf.* 20*R*-protopanaxadiol *Rf* 0.30). GLC (column: glass column 1.5% SE-30 DMCS chromosorb w, 3 mm× 2 m; column temp.: 280°; carrier gas: N_2 flow 1.5 kg/cm²; sample: TMS derivatives) t_R (min) 8.3 (*cf.* 20*R*-protopanaxadiol t_R (min) 9.3).

Methylation of Ginsenoside-Rb_1, -Rb_2, -Rc and -Rd by Hakomori Method——Ginsenoside-Rb_1, -Rb_2, -Rc and -Rd were methylated by the method described in the previous paper. The property of each O-methyl derivative was listed in Table III.

O-Methylginsenoside-Rb_1: mp 85—87°. *Anal.* Calcd. for $C_{68}H_{120}O_{23}$: C, 62.55; H, 9.26. Found: C, 62.93; H, 8.95. NMR (in $CDCl_3$) δ: 0.80—1.00 (3H(s)×5 C$\underline{H}_3$), 1.27 (3H(s) C$\underline{H}_3$), 1.60 (3H(s) C$\underline{H}_3$), 1.67 (3H(s) C$\underline{H}_3$), 3.33—3.57 (3H(s) ×14 OC$\underline{H}_3$), 4.17 (1H(d) J=7 Hz anomer $\underline{H}$), 4.26 (1H(d) J=7 Hz anomer $\underline{H}$), 4.38 (1H(d) J=7 Hz anomer $\underline{H}$), 4.75 (1H(d) J=7 Hz anomer $\underline{H}$).

O-Methylginsenoside-Rb_2: mp 88—90°. *Anal.* Calcd. for $C_{66}H_{116}O_{22}$: C, 62.83; H, 9.20. Found: C, 62.73; H, 8.89. NMR (in $CDCl_3$) δ: 0.79—0.98 (3H(s)×5 C$\underline{H}_3$), 1.28 (3H(s) C$\underline{H}_3$), 1.51 (3H(s) C$\underline{H}_3$), 1.65 (3H(s) C$\underline{H}_3$), 3.31—3.60 (3H(s)×13 OC$\underline{H}_3$), 4.23 (1H(d) J=7 Hz anomer $\underline{H}$), 4.30 (1H(d) J=7 Hz anomer $\underline{H}$), 4.40 (1H(d) J=7 Hz anomer $\underline{H}$), 4.65 (1H(d) J=7 Hz anomer $\underline{H}$).

O-Methylginsenoside-Rc: mp 78—79°. *Anal.* Calcd. for $C_{66}H_{116}O_{22}$: C, 62.83; H, 9.20. Found: C, 62.40; H, 9.19. NMR (in $CDCl_3$) δ: 0.80—1.00 (3H(s)×5 C$\underline{H}_3$), 1.29 (3H(s) C$\underline{H}_3$), 1.59 (3H(s) C$\underline{H}_3$), 1.67 (3H(s) C$\underline{H}_3$), 3.31—3.61 (3H(s)×13 OC$\underline{H}_3$), 4.27 (1H(d) J=8 Hz anomer $\underline{H}$), 4.47 (1H(d) J=7 Hz anomer $\underline{H}$), 4.67 (1H(d) J=7 Hz anomer $\underline{H}$), 5.00 (1H(s) anomer $\underline{H}$).

O-Methylginsenoside-Rd: mp 91—93°. NMR (in $CDCl_3$) δ: 0.79—1.00 (3H(s)×5 C$\underline{H}_3$), 1.24 (3H(s) C$\underline{H}_3$), 1.57 (3H(s) C$\underline{H}_3$), 1.67 (3H(s) C$\underline{H}_3$), 3.29—3.58 (3H(s)×11 OC$\underline{H}_3$), 4.21 (1H(d) J=7 Hz anomer $\underline{H}$), 4.45 (1H(d) J=7 Hz anomer $\underline{H}$), 4.65 (1H(d) J=7 Hz anomer $\underline{H}$). *Anal.* Calcd. for $C_{59}H_{104}O_{18}$: C, 64.36; H, 9.45. Found: C, 63.85; H, 9.24.

Methanolyses of O-Methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd with Methanolic 5% HCl——O-Methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd were methanolyzed with methanolic 5% HCl refluxing for 2 hr, respec-

tively. The reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was examined by TLC and GLC. The results were listed in Table IV.

Catalytic Reduction of O-Methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd——O-Methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd 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-Rb_1, -Rb_2, -Rc, and -Rd were examined by NMR. NMR (in $CDCl_3$) δ: 0.8—1.0 (3H (s)$\times$7 C$\underline{H}_3$), 1.3 (3H(s) C$\underline{H}_3$).

Chromium Trioxide Oxidation of Dihydro-O-methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd——To each solution of dihydro-O-methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd (200 mg) in pyridine (5 ml) were added the solution of CrO_3 (500 mg) in pyridine (10 ml) dropwise with stirring and the 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 a white powder which was purified by reprecipitation from MeOH–H_2O.

Monoketo-dihydro-O-methylginsenoside-Rb_1: A white powder, (mp 80—83°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1710 (six membered ketone). *Anal.* Calcd. for $C_{68}H_{120}O_{23}$: C, 62.55; H, 9.26. Found: C, 62.60; H, 8.75. NMR (in $CDCl_3$) δ: 0.72 (3H(s) C$\underline{H}_3$), 0.82 (3H(s)$\times$2 C$\underline{H}_3$), 0.89 (3H(s)$\times$2 C$\underline{H}_3$), 1.01 (3H(s) C$\underline{H}_3$), 1.06 (3H(s) C$\underline{H}_3$), 1.17 (3H(s) C$\underline{H}_3$), 3.33—3.74 (3H(s)$\times$14 OC$\underline{H}_3$), 4.26 (1H(d) J=7 Hz anomer $\underline{H}$), 4.27 (1H(d) J=7 Hz anomer $\underline{H}$), 4.36 (1H(d) J=7 Hz anomer $\underline{H}$), 4.68 (1H (d) J=7 Hz anomer $\underline{H}$).

Monoketo-dihydro-O-methylginsenoside-Rb_2: A white powder, (mp 79—81°), IR ν_{max}^{Nujol} cm^{-1}: OH(nil), 1714 (six membered ketone). *Anal.* Calcd. for $C_{66}H_{116}O_{22}$: C, 62.83; H, 9.20. Found: C, 63.03; H, 8.86. NMR (in $CDCl_3$) δ: 0.72 (3H(s) C$\underline{H}_3$), 0.82 (3H(s)$\times$2 C$\underline{H}_3$), 0.89 (3H(s)$\times$2 C$\underline{H}_3$), 1.01 (3H(s) C$\underline{H}_3$), 1.06 (3H (s) C$\underline{H}_3$), 1.17 (3H(s) C$\underline{H}_3$), 3.33—3.59 (3H(s)$\times$13 OC$\underline{H}_3$), 4.27 (1H(d) J=8 Hz anomer $\underline{H}$), 4.28 (1H(d) J=7 Hz anomer $\underline{H}$), 4.37 (1H(d) J=7 Hz anomer $\underline{H}$), 4.68 (1H(d) J=7 Hz anomer $\underline{H}$).

Monoketo-dihydro-O-methylginsenoside-Rc: A white powder from MeOH–H_2O, (mp 80—82°), IR ν_{max}^{Nujol} cm^{-1}: OH(nil), 1710 (six membered ketone). *Anal.* Calcd. for $C_{66}H_{116}O_{22}$: C, 62.83; H, 9.20. Found: C, 62.42; H, 8.80. NMR (in $CDCl_3$) δ: 0.72 (3H(s) C$\underline{H}_3$), 0.82 (3H(s)$\times$2 C$\underline{H}_3$), 0.89 (3H(s)$\times$2 C$\underline{H}_3$), 1.01 (3H(s) C$\underline{H}_3$), 1.06 (3H(s) C$\underline{H}_3$), 1.17 (3H(s) C$\underline{H}_3$), 3.32—3.61 (3H(s)$\times$13 OC$\underline{H}_3$), 4.27 (1H(d) J=7 Hz anomer $\underline{H}$), 4.37 (1H(d) J=7 Hz anomer $\underline{H}$), 4.68 (1H(d) J=7 Hz anomer $\underline{H}$), 5.0 (1H(s) anomer $\underline{H}$).

Monoketo-dihydro-O-methylginsenoside-Rd: A white powder, (mp 92—95°), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1714 (six membered ketone), *Anal.* Calcd. for $C_{59}H_{104}O_{18}$: C, 64.36; H, 9.45. Found: C, 64.01; H, 9.04. NMR (in $CDCl_3$) δ: 0.72 (3H(s) CH_3), 0.82 (3H(s)$\times$2 C$\underline{H}_3$), 0.89 (3H(s)$\times$2 C$\underline{H}_3$), 1.01 (3H(s) C$\underline{H}_3$), 0.16(3H(s) C$\underline{H}_3$), 1.17 (3H(s) C$\underline{H}_3$), 3.33—3.62 (3H(s) $\times$11 OC$\underline{H}_3$), 4.26 (1H(d) J=7 Hz anomer $\underline{H}$), 4.37 (1H(d) J=7 Hz anomer $\underline{H}$), 4.68 (1H (d) J=7 Hz anomer $\underline{H}$).

Degradation of Monoketo-dihydro-O-methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd with Methanolic 7% HCl——Monoketo-dihydro-O-methylginsenoside-Rb_1, -Rb_2, -Rc, and -Rd were refluxed with methanolic 7% HCl for 4 hr, respectively. 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 (XI). TLC (solvent I) *Rf* 0.22, UV λ_{max}^{EtOH} nm (log ε): 265 (3.39); $\lambda_{max}^{Cyclohexane}$ nm (log ε): 257 (3.42).

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