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Further Study on Dammarane-Type Saponins of Roots, Leaves, Flower-Buds, and Fruits of *Panax ginseng* C.A. Meyer

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New saponins, named ginsenosides-Rh_1 (**11**) and -M_{7cd} (**17**) were isolated from roots and flower-buds of Ginseng, repectively. Structures of **11** and **17** were established as 6-O-β-D-glucopyranoside of 20(*S*)-protopanaxatriol and 20-O-β-D-glucopyranoside of dammar-25-ene-3β,6α,12β,20(*S*),24ξ-pentaol, respectively by means of carbon-13 nuclear magnetic resonance spectroscopy as well as their preparations from the known saponins.

Further isolation and identification of the following dammarane-type saponins from the aerial parts of Ginseng were also reported: ginsenosides-Rb_1 (**1**), -Rb_2 (**2**), -Rc (**4**) from the leaves; **1**, **2**, **4**, and ginsenoside-F_3 (**15**) from the flower-buds, and **2**, **4**, ginsenosides-Rd (**5**), -Re (**6**), and -Rg_1 (**8**) from the fruits. As shown in Table II, isolation of these biologically active saponins in relatively high yields indicated the significance of the aerial parts of Ginseng as the good medicinal sources.

Keywords——dammarane-type saponins; ginsenosides-Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rg_1, -Rh_1; ginsenosides-F_3, -M_{7cd}; ^{13}C-NMR of triterpene-glucosides; *Panax ginseng* C.A. Meyer; Ginseng; Araliaceae

The structures of dammarane-type saponins of Ginseng roots, ginsenosides-Rb_1 (**1**), —Rb_2(**2**),[2a] —Rb_3(**3**),[2b] —Rc(**4**), —Rd(**5**),[2a] —Re(**6**), —Rf(**7**),[2c] —Rg_1(**8**),[2d] —Rg_2(**9**),[2c] and 20-O-glucoginsenoside-Rf(**10**)[2b] were already elucidated. In our previous studies for searching better sources of biologically active saponins of this type, isolation of several saponins from leaves[3] and flower-buds[4] of Ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) has been reported. In continuation of this series of studies, the present paper reports further isolation and identification of the known and new saponins from the roots, the leaves, the flower-buds, and the fruits.

During the course of our study on biologically active principles of aqueous extract of Ginseng roots (white Ginseng),[5] we encountered isolation of a new saponin named ginsenoside-Rh_1(**11**) in a yield of 0.0015%. In the enzymatic hydrolysate of **11**, 20(*S*)-protopanaxatriol (**12**)[2d] and glucose were detected by thin layer chromatography (TLC) and gas chromatography, respectively. Assignments of carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra of Ginseng sapogenins[6] as well as the glycosylation shifts of their carbon signals[7] have been reported, being promising for structure determination of dammarane-type saponins and sapogenins. Comparison of the ^{13}C NMR spectrum of **11** with those of **8** and **12** is shown in Table I. On going from **12** to **11**, signal due to 6C was deshielded by +10.4 ppm and signals due to 5C and 7C were displaced upfield by —0.3 and —2.2 ppm, respectively, while other signals of the aglycone moiety of **11** were observed at almost the same positions as those of

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2) *a*) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974); *b*) S. Sanada and J. Shoji, *ibid.*, **26**, 1694 (1978); *c*) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *ibid.*, **22**, 2407 (1974); *d*) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
3) S. Yahara, O. Tanaka, and T. Komori, *Chem. Pharm. Bull.* (Tokyo), **24**, 2204 (1976).
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6) J. Asakawa, R. Kasai, K. Yamasaki, and O. Tanaka, *Tetrahedron*, **33**, 1935 (1977).
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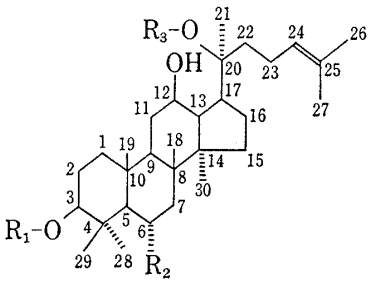
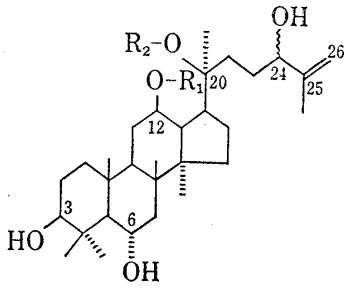
12. With regard to the sugar carbon signals, a set of carbon signals assignable to 6-O-β-D-glucopyranosyl unit of **8** was also found in the spectrum of **11** at almost the same positions. It follows that **11** must be formulated as 6-O-β-D-glucopyranoside of 20(*S*)-protopanaxatriol. The structure of **11** was further confirmed by its preparation from **6** which can be isolated from the flower-buds in a high yield (surprisingly *ca.* 2.5%) without the aid of column chromatography.[4] Partial hydrolysis of **6** with Takadiastase-Y (crude mixture of glycosidases produced by fungi, Sankyo Co. Ltd.) yielded a glucoside which was identical with **11**.

From the leaves, the root-saponins, **5**, **6**, and **8**, and three new saponins ginsenosides-F_1 (**13**), $-F_2$(**14**), and $-F_3$(**15**) have been already isolated.[3] In addition to these saponins, root-saponins **1**, **2**, and **4** were now isolated from the glycoside-fraction of the leaves and identified.

Isolation of saponins **5**, **6**, and **8** from the flower-buds have already published.[4] Further study on the glycoside-fraction of this part led to the isolation of saponins **1**, **2**, **4** and **15** and several new saponins named ginsenosides-M_{3-7} which have an aglycone with an oxygenated side chain like chikusetsusaponins-L_{9a} and $-L_{9bc}$ (**16**), saponins of leaves of *P. japonicus* C.A. MEYER.[8] Of these new saponins, ginsenosides-M_7 was further separated, through its acetate, into three saponins, ginsenosides-M_{7a}, $-M_{7b}$, and $-M_{7cd}$(**17**). Comparison of the ^{13}C NMR spectrum of **17** with those of **13** and **16** are shown in Table I. Signals due to both the sugar and aglycone moieties of **17** other than those of 22C—27C of the side chain were found at almost the same positions as those of **13**, while the signals due to 22C—27C of 17 appeared at almost the same positions as those of **16**, proposing the structure for **17** as shown in Chart 1. The structure of **17** was finally confirmed by its preparation from **13** by the photosensitized oxidation followed by reduction of the resulting peroxide.[8] As in the case of **16**,[8] occurence of a sub-peak for each signal of 17C, 20C and 22C—27C indicated that **17**





	R_1	R_2	R_3
1:	glc^{2}—^{1}glc	H	glc^{6}—^{1}glc
2:	glc^{2}—^{1}glc	H	glc^{6}—^{1}ara(py)
3:	glc^{2}—^{1}glc	H	glc^{6}—^{1}xyl(py)
4:	glc^{2}—^{1}glc	H	glc^{6}—^{1}ara(fu)
5:	glc^{2}—^{1}glc	H	glc
6:	H	O-glc^{2}—^{1}rham	glc
7:	H	O-glc^{2}—^{1}glc	H
8:	H	O-glc	glc
9:	H	O-glc^{2}—^{1}rham	H
10:	H	O-glc^{2}—^{1}glc	glc
11:	H	O-glc	H
12:	H	OH	H
13:	H	OH	glc
14:	glc	H	glc
15:	H	OH	glc^{6}—^{1}ara(py)

	R_1	R_2
16	glc	H
17	H	glc

glc=β-D-glucopyranosyl
rham=α-L-rhamnopyranosyl
xyl=β-D-xylopyranosyl
ara(py)=α-L-arabinopyranosyl
ara(fu)=α-L-arabinofuranosyl

Chart 1

8) S. Yahara, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **25**, 2041 (1977).

(natural and synthesized) must be an epimeric mixture at C-24, the separation of which has remained unsuccessful. The structure study of the other new saponins are under progress.

Saponins of fruits of this plant have been left unidentified. A suspension of methanolic extract of the dried fruits in water was washed with ether and then extracted with *n*-butanol (saturated with water). The resulting butanolic extract (crude glycoside-fraction) was

Table I. ^{13}C Chemical Shifts

	Aglycone moieties							Aglycone moieties					
	11	**8**	**12**	**17**	**13**	**16**		**11**	**8**	**12**	**17**	**13**	**16**
C-1	39.4	39.5	39.3	39.3	39.2	39.0	C-19	17.6[a]	17.4[a]	17.4[a]	17.4[a]	17.3[a]	17.4[a]
2	27.9	27.6	28.0	28.0	27.9	28.0	20	73.0	83.3	72.9	83.2	83.2	73.2
3	78.6	78.6	78.3	78.4[b]	78.4[b]	78.4[b]					(83.4)		(72.9)
4	40.3	40.1	40.2	40.2	40.1	40.3	21	26.8	22.3	26.9	22.8	22.3	27.0
5	61.4	61.3	61.7	61.7	61.6	61.7	22	35.8	35.9	35.7	32.3	35.9	32.2
6	78.0	77.8	67.6	67.6	67.6	67.6					(32.5)		(32.5)
7	45.2	44.9	47.4	47.3	47.3	47.2	23	23.0	23.2	22.9	30.8	23.1	30.0
8	41.1	41.0	41.1	41.1	41.1	41.0							(30.4)
9	50.2	49.9	50.1	49.8	49.8	49.9	24	126.3	125.8	126.2	75.6	125.8	75.9
10	39.6	39.5	39.3	39.3	39.2	39.4					(76.1)		(76.2)
11	32.0	30.8[b]	31.9	30.8	30.8	28.0	25	130.6	130.9	130.6	149.7	130.8	150.1
12	71.0	70.3	70.9	70.4	70.2	78.6[b]	26	25.8	25.7	25.8	109.9	25.7	109.7
13	48.2	48.9	48.1	48.8	48.9	46.4					(110.2)		(110.0)
14	51.6	51.3	51.6	51.4	51.3	52.0	27	17.6[a]	17.7[a]	17.7	18.5	17.7	18.6
15	31.1	30.6[b]	31.3	30.8	30.5	31.3					(18.2)		(18.2)
16	27.2	26.4	26.8	26.6	26.6	26.9	28	31.7	31.6	31.9	31.8	31.8	31.9
17	54.7	51.6	54.6	52.0	51.6	54.2	29	16.4[a]	16.2[a]	16.4[a]	16.4[a]	16.4[a]	16.4[a]
				(52.3)			30	16.8[a]	17.0[a]	17.0	17.4[a]	17.3	17.4
18	17.4[a]	17.4[a]	17.5[a]	17.4[a]	17.3[a]	17.4[a]							

( ): sub-peaks.

		Sugar moieties				
		11	**8**	**17**	**13**	**16**
6-O-β-D-Glucopyranosyl	C-1	105.9	105.7			
	2	75.4	75.3			
	3	80.0[b]	80.0[c]			
	4	71.8	71.6[d]			
	5	79.5[b]	79.3[c]			
	6	63.1	62.9			
20-O-β-D-Glucopyranosyl	C-1		98.1	98.2	98.0	
	2		74.9	75.1	74.9	
	3		78.8[c]	78.7[b]	78.9[b]	
	4		71.3[d]	71.4	71.4	
	5		77.8[c]	78.2[b]	78.0[b]	
	6		62.6	62.8	62.9	
12-O-β-D-Glucopyranosyl	C-1					100.1
	2					75.1
	3					78.3[b]
	4					71.1
	5					77.3[b]
	6					62.4

δ ppm from internal TMS in C_5D_5N; JEOL JNM-PFT-100NMR spectrometer at 25.15 MHz; concentration: 0.1—0.4 M; temperature: 25°; using 10 mm tubes; FT NMR conditions; spectral width: 4 KHz; pulse flipping angle: 45° or 90°; acquistion time: 0.5 sec; number of data points: 4096; recycle time: 1—2 sec: number of recycle: 1000—30000.

a, b, c, and d: These assignments may be reversed in each vertical column.

subjected to separation by droplet counter current chromatography (DCC) followed by column chromatography, affording several known saponins, which were identified with **2**, **4**, **5**, **6** and **8**, respectively. It is notable that **6** was obtained from the fruits in a significantly high yield, 6.0%.

Table II. Yields of Common Saponins (%)

	Ginsenosides					
	1	**2**	**4**	**5**	**6**	**8**
Roots (white Ginseng)	0.5	0.2	0.3	0.2	0.2	0.2
Leaves	0.1	0.4	0.2	1.5	1.5	1.5
Flower-buds	0.2	0.2	0.2	0.5	2.8	0.2
Fruits		0.2	0.1	0.1	6.0	0.04

Comparison of yields of saponins from various parts of this plant are listed in Table II. The relatively high yields of dammarane-type saponins from the aerial parts revealed the utility of the leaves, the flower-buds, and the fruits as valuable medicinal sources.

Experimental

Identification of the Known Saponins (General Procedures)——Each saponin isolated in this paper was identified with a corresponding authentic sample by comparison of thin layer chromatography (TLC) on silica gel, using solvent systems; $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer) and *n*-BuOH–AcOEt–H_2O (15: 1: 4, upper layer) (detection with H_2SO_4), ^{13}C NMR spectrum (in C_5D_5N, 25°, at 25.15 MHz), optical rotation, and mass spectrum as acetate[9] or trimethylsilyl ether.[10] Comparison of an aglycone (or a prosapogenin) and monosaccharides formed by the enzymatic hydrolysis with crude hesperidinase[11] was also employed for the identification; condition of the hydrolysis and the identification: see previous paper.[3,4]

Isolation of 11 from the Roots——The powdered roots (commercial white Ginseng, 1.5 kg) was extracted with hot H_2O and the aqueous extract was concentrated to a small volume (*ca.* 950 ml) under reduced pressure. The solution was then extracted with *n*-BuOH (saturated with H_2O) and the resulting BuOH-solution was evaporated to dryness under reduced pressure, yielding a residue (20 g). An aqueous solution of this residue was dialyzed and the non-dialyzed fraction (5 g) was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (3000: 110: 17→3000: 120: 17), affording **11** (23 mg) along with other known ginsenosides; **11**: white powder, $[\alpha]_D^{22}$ +20° (c=0.88, MeOH), ^1H NMR in C_5D_5N δ 4.98 (1H, d. J=7 Hz, anomeric H). *Anal.* Calcd. for $C_{36}H_{62}O_9 \cdot 1\frac{1}{2}H_2O$: C, 64.93; H, 9.84. Found: C, 65.38; H, 9.49. TLC of the dialyzed fraction of the aqueous extract as well as the MeOH extract of white Ginseng demonstrated also the presence of **11** therein.

Micro-Scale Hydrolysis of 11 with Crude Hesperidinase——A solution of **11** (5 mg) and crude hesperidinase (Tanabe Pharm. Ind., Co. Ltd., 5 mg)[11] in phosphate buffer (pH 4.0, 5 ml) (added a few drops of toluene) was incubated at 40° for 3 days and the reaction mixture was extracted with ether. In the ether layer, **12** was detected by TLC (on silica gel, solvent: $CHCl_3$–MeOH (5: 1)), detection with H_2SO_4. The aqueous layer was dialyzed and the dialyzed fraction was deionized by passing through a column of Amberlite-MB3 and then concentrated to dryness. In the resulting residue, glucose was identified by gas liquid chromatography (condition: see previous paper[3,4]).

Preparation of 11 (Partial Hydrolysis of 6)——A solution of **6** (2.5 g) and Takadiastase Y (Sankyo Co. Ltd., Tokyo) in phosphate buffer (pH 4.0, 1 l) (added a few drops of toluene to avoid fungal contamination) was incubated at 40° for 26 days. The reaction mixture was extracted with *n*-BuOH (saturated with H_2O) and the BuOH layer was concentrated to dryness. The residue was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (150: 35: 3)) to give **11** (75 mg), $[\alpha]_D^{22}$ +27° (c=1.30, MeOH), which was identical with a sample of natural **11** by comparison of TLC and ^{13}C NMR spectra.

Isolation of 1, 2, and 4 from the Leaves——The nondialyzed fraction of the crude glycoside fraction reported previously[3] was chromatographed on silica gel repeatedly; solvent: firstly $CHCl_3$–MeOH–H_2O (300: 70: 7) and next $CHCl_3$–MeOH–H_2O (300: 140: 17) to give **1** (white powder, $[\alpha]_D^{15}$ +11.3° (c=0.98, MeOH)), **2** (white powder, $[\alpha]_D^{15}$ +15.1° (c=1.59, MeOH)), and **4** (white powder, $[\alpha]_D^{15}$ +1.2° (c=1.63, MeOH)), yields: see Table II.

9) T. Komori, O. Tanaka, and Y. Nagai, *Org. Mass Spectr.*, **9**, 744 (1974).
10) R. Kasai, K. Matsuura, O. Tanaka, S. Sanada, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **25**, 3277 (1977).
11) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).

Isolation of Saponins from the Flower-Buds——The separation of the non-dialyzed fraction of the crude glycoside fraction of the dried flower-buds (see previous paper[4]) by DCC (solvent system: $CHCl_3$–MeOH–H_2O–*n*-PrOH (5: 6: 4: 1), stationary phase: lower layer and mobile phase: upper layer) afforded several fractions, Fr. 1—6. Fr. 2 was subjected to column chromatography on polyamide (solvent: H_2O–MeOH (50: 1)) and then on silica gel (solvent $CHCl_3$–MeOH–H_2O (30: 16: 3), affording **1** (white powder, $[\alpha]_D^{24}$ +13.9° (c=0.49, MeOH), and new saponins ginsenosides-M_{3-5}. Fr. 3 was also subjected to repeated column chromatography on silica gel by eluting firstly with $CHCl_3$–MeOH–H_2O (30: 15: 2), and next with *n*-BuOH–AcOEt–H_2O (4: 1: 1), affording **2** (white powder, $[\alpha]_D^{24}$ +13.6° (c=0.49, MeOH)), and **4** (white powder, $[\alpha]_D^{24}$ +2.0° (c=0.46, MeOH)) along with a new saponin, ginsenoside-M_6. Further purification of these new saponins (ginsenosides-M_{3-6}) and their structure study will be published in the near future. Yields of the known saponins are summarized in Table II.

Since it was suspected that new saponins, ginsenosides-M_{3-6} which seemed to have an aglycone with the oxygenated side chain might be formed during the storage of the flower-buds, extraction of the fresh flower-buds was conducted, demonstrating that these saponins were not artifacts. This experiment also led to isolate an additional new saponin, ginsenoside-M_7. Fresh flower-buds (3.7 kg) collected at Daikon-jima, Shimane-ken (May, 16, 1977) were extracted with hot MeOH. The resulting MeOH-extract was suspended with H_2O (700 ml) and the suspension was washed with ether and then extracted with *n*-BuOH (saturated with H_2O). The BuOH layer, after evaporation to dryness, was dissolved in H_2O and the aqueous solution was subjected to graduent dialysis to give a non-dialyzed fraction and three dialyzed fractions, tentatively named D-F-1, D-F-2, and D-F-3 in the order of dialysis. In the non-dialyzed fraction, ginsenoside-M_{3-6} and saponins, **1**, **2**, **4**, and **5** were detected by TLC. The dialyzed fraction, D-F-3 was subjected to repeated column chromatography: firstly on silica gel, solvent $CHCl_3$–MeOH–H_2O (300: 110: 17), next on polyamide, solvent H_2O–MeOH (50: 1 or 10: 1), and finally on silica gel, solvent $CHCl_3$–MeOH–H_2O (5: 1: 0.1), affording **15** (white powder, $[\alpha]_D^{20}$ +20.2° (c=1.58, MeOH), yield 0.03%) and ginsenoside-M_7 latter of which was also isolated from the non-dialyzed fraction by the similar repeated chromatography (total yield 0.03%).

Ginsenoside-M_7 (300 mg)[12] was acetylated with $(AcO)_2O$ (1.5 ml) and C_5H_5N (anhydrous, 3 ml) on standing at room temperature for 5 days. After working up in the usual way, the resulting crude acetate was chromatographed on silica gel (solvent: C_6H_6–acetone (23: 2)) to furnish the separation into ginsenosides-M_{7a} acetate (46 mg), -M_{7b} acetate (31 mg), and -M_{7cd} (**17**) acetate (124 mg). Saponification of the acetate (120 mg) of **17** by refluxing with 5% KOH in 50% aqueous EtOH for 3 hr followed by column chromatography of the resulting free saponin on silica gel (solvent: $CHCl_3$–MeOH–H_2O (5: 1: 0.1)) afforded **17**, white powder, $[\alpha]_D^{21}$ +29.0° (c=0.33, MeOH), yield 38 mg. *Anal.* Calcd. for $C_{36}H_{62}O_{10}\cdot 1\frac{1}{2}H_2O$: C, 63.41; H, 9.61. Found: C, 63.38; H, 9.44.

Preparation of 17 from 13——A solution of **13** (130 mg), Rose Bengal (20 mg) in iso-PrOH (50 ml) was irradiated by two of 100W-tungsten lamps and a 20W-fluorescent lamp for 185 hr under the stream of O_2. After decolorized with charcoal, the reaction mixture was concentrated to dryness and the residue was dissolved in MeOH (15 ml) and reduced with $NaBH_4$ (70 mg) at room temperature for 1 hr. After working up in the usual way, the products were separated by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (60: 12: 1)), yielding **17** (45 mg).[13] The identification was confirmed by comparison of TLC, optical rotation, and the ^{13}C NMR spectrum.

Isolation of Saponins from the Fruits[14]——The dried fruits (8 g) collected at Maruco-machi, Nagano-ken in September, 1975 were extracted with hot MeOH. The MeOH-extract was suspended in H_2O and the suspension was washed with ether and then extracted with *n*-BuOH (saturated with H_2O). The BuOH-layer was concentrated to dryness to give a crude glycoside-fraction (1.9 g). This fraction was subjected to DCC (solvent system: $CHCl_3$–MeOH–H_2O–*n*-PrOH (10: 12: 9: 2), stationary phase: lower layer and mobile phase: upper layer) followed by repeated column chromatography on silica gel (solvent: firstly $CHCl_3$–MeOH–H_2O (25: 10: 1), and next $CHCl_3$–MeOH–H_2O (100: 30: 3)), affording **2** (white powder, $[\alpha]_D^{15}$+10.0° (c=4.19, MeOH)), **4** (white powder, $[\alpha]_D^{15}$ +1.5° (c=2.60, MeOH)), **5** (white powder, $[\alpha]_D^{15}$ +18.4° (c=1.25, MeOH)), **6** (colorless needles, mp 191—194°, $[\alpha]_D^{15}$ −2.1° (c=2.40, MeOH)), and **8** (white powder, $[\alpha]_D^{15}$ +24.8°, (c=0.93, MeOH). Yields of saponins: see Table II.

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12) The acetylation was carried out after confirmation of the absence of an acetyl group in this saponin by ^{13}C NMR.

13) The ^{13}C NMR spectrum of the synthesized **17** indicated that the synthesized sample was also a mixture of C-24 epimers.

14) Extracted after removing the seeds.