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Further Studies on Dammarane-Saponins of American Ginseng, Roots of *Panax quinquefolium* L.

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Several Ginseng saponins, ginsenosides-Ro(**1**), -Rb_1(**2**), -Rb_2(**3**), -Rc(**4**), -Rd(**5**), and -Re(**6**), have already been isolated from American Ginseng, roots of *Panax quinquefolium* L. In the present work, further investigation of minor saponins of this plant drug led to the isolation and identification of ginsenosides-Rg_1(**7**), -Rg_2(**8**), -Rb_3(**9**), and -F_2(**11**), pseudoginsenoside-F_{11}(**10**), and gypenoside-XVII(**13**). In addition to these known saponins, a new saponin named quinquenoside-R_1(**12**) was also isolated and its structure was determined as mono-*O*-acetyl-ginsenoside-Rb_1 with the acetyl group located at the 6-hydroxyl group of the terminal glucosyl moiety of the β-sophorosyl group.

Keywords——American ginseng; *Panax quinquefolium* L.; Araliaceae; dammarane-saponins; ginsenosides-Rg_1, -Rg_2, -Rb_3, and -F_2; pseudoginsenoside-F_{11}; gypenoside-XVII; quinquenoside-R_1; ^{13}C NMR of oligoglycosides

A number of dammarane-saponins have been isolated from the famous oriental plant drug, Ginseng (roots of *Panax ginseng* C.A. Meyer, Araliaceae).[1] In the U.S.A. and Canada, the related plant *P. quinquefolium* L. is cultivated and its roots, known as American Ginseng, are also a well-known plant drug which has been used in the same way as Ginseng. From American Ginseng, Sanada *et al.* isolated several Ginseng saponins,[2] ginsenosides-Ro (**1**), -Rb_1(**2**), -Rb_2(**3**), -Rc(**4**), -Rd(**5**),[1b] and -Re(**6**),[1c] and Lui *et al.* recently reported a comparison of the saponins of American Ginseng and its leaves with those of other *Panax* spp.[3] In our series of studies on Panax saponins, a recent reinvestigation of the leaf-saponins of this plant[4] disclosed the presence of ginsenoside-Rb_3(**9**)[1d] and pseudoginsenoside-F_{11}(**10**), the latter of which had already been isolated from the leaves of Himalayan Panax, *P. pseudo-ginseng* subsp. *himalaicus*.[5] The present paper deals with a further study on the saponins of this plant drug.

A crude saponin fraction of the methanolic extract of American Ginseng was subjected to repeated column chromatography on silica gel and on reversed-phase highly porous polymer to afford six known saponins **1**—**6**, which have already been reported by Sanada *et al.*[2] In addition to these saponins, five minor saponins **9** (yield: 0.03%), **10** (yield: 0.04%), ginsenoside-Rg_1(**7**) (yield: 0.15%), ginsenoside-Rg_2(**8**) (yield: 0.008%), and ginsenoside-F_2(**11**) (yield: 0.018%), previously isolated from leaves of *P. ginseng*,[6] were also isolated. The identification of these saponins was achieved by comparison with authentic samples on the basis of thin layer chromatography (TLC) on silica gel and on octadecyldimethylsilyl or octyldimethylsilyl silica gel (ODS or OS silica gel, reversed phase), ^{13}C nuclear magnetic resonance (NMR)[7] and mass spectra (MS) of the acetates[8] or trimethylsilyl (TMSi) ethers,[9] and the results of complete or partial hydrolysis.

Two additional minor saponins, **12** and **13**, were also isolated in yields of 0.01% and 0.03%, respectively. On hydrolysis with mineral acid, the new saponin (**12**), named quinquenoside-R_1, yielded glucose. An infrared (IR) band at 1735 cm^{-1} (KBr) as well as carbon signals at

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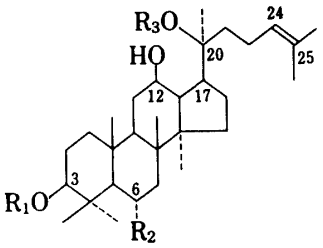
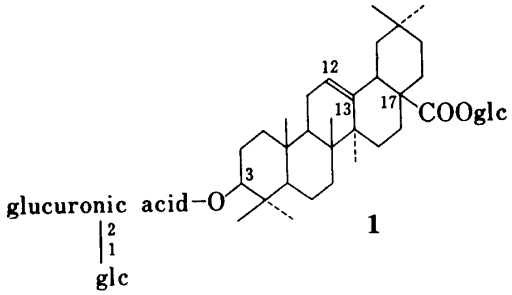
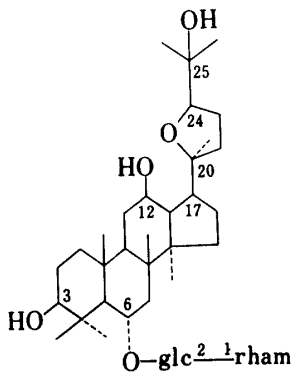




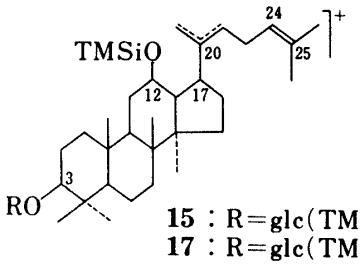
	R_1	R_2	R_3
2:	glc²—¹glc	H	glc⁶—¹glc
3:	glc²—¹glc	H	glc⁶—¹ara(py)
4:	glc²—¹glc	H	glc⁶—¹ara(fu)
5:	glc²—¹glc	H	glc
6:	H	O-glc²—¹rham	glc
7:	H	O-glc	glc
8:	H	O-glc²—¹rham	H
9:	glc²—¹glc	H	glc⁶—¹xyl
11:	glc	H	glc
12:	glc²—¹glc⁶—OAc	H	glc⁶—¹glc
13:	glc	H	glc⁶—¹glc
14:	H	H	H



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

Chart 1

δ 20.9 and 170.8 (Table II) indicated the presence of an *O*-acetyl group in this saponin (**12**). On alkaline hydrolysis with methanolic KOH, **12** afforded a desacetyl saponin which was identified as **2** by comparison (TLC and ^{13}C NMR spectrum) with an authentic sample. Upon comparison of the ^{13}C NMR spectrum of **12** with that of **2** (Table I),[10] all of the carbon signals due to the aglycone moiety (20(*S*)-protopanaxadiol (**14**)) of both saponins appeared at almost the same positions, indicating that the acetyl group (Ac) of **12** must be located not at the 12β-hydroxyl group of the aglycone moiety but in the sugar moiety. In the mass spectrum of the TMSi ether of **12**, ions at *m/z* 421 and 799 can be assigned to glucosyl(TMSi)$_3$Ac and glucosyl(TMSi)$_3$-glucosyl(TMSi)$_3$Ac, respectively, demonstrating that the acetyl group must be present at one of the two terminal moieties. It has been revealed that a glycosyl linkage at the 20-*tert*-hydroxyl group of dammarane-saponins is very unstable, and in the mass spectra of their acetates or TMSi ethers, no fragment ion having an intact *O*-glycosyl group at the C-20 position can be observed.[8,9] Accordingly, a fragment ion at *m/z* 1313 in the spectrum of TMSi-**12** should be formulated as **15** (Chart 2), indicating that the acetyl group must be located at the



15 : R=glc(TMSi)$_3$-glc(TMSi)$_3$Ac
17 : R=glc(TMSi)$_4$

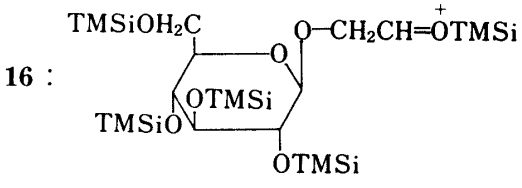


Chart 2

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terminal glucosyl moiety of its 3-*O*-*β*-sophorosyl group. In the ^{13}C NMR spectrum (Table II), on going from **2** to **12**, one of the four C-6 signals of the glucosyl moieties was displaced downfield by 2.1 ppm, while one of the four C-5 signals was shielded by 2.8 ppm.[11] It follows that the *O*-acetyl group of **12** must be located at C-6 of the terminal glucosyl moiety of its 3-*O*-*β*-sophorosyl group. It is noteworthy that the acetylation of the 6-hydroxyl group of the terminal glucosyl moiety of this *β*-sophorosyl group led to a downfield shift of the C-2 signal of the inner glucosyl moiety by 1.4 ppm (Table II).

On acid hydrolysis, the saponin (**13**) yielded glucose. The ^{13}C NMR spectrum of **13** indicated the presence of three glucosyl units, and comparison of the carbon signals due to the aglycone moiety with those of **2** demonstrated that **13** must be a glycoside of **14** having *O*-glycosyl linkages at both the 3- and 20-hydroxyl groups. The mass spectrum of TMSi-**13** exhibited ions at *m/z* 451 (glucosyl$(TMSi)_4$), 829 (glucosyl $(TMSi)_3$—glucosyl $(TMSi)_4$) and 583 (**16**), which reported to be characteristic of the hexosyl(1—6)hexose unit (gentiobiose type) by Kochetkov *et al.*[12] The fragment ion at *m/z* 1052 (**17**) formed by loss of the 20-*O*-glycosyl linkage revealed the presence of a glucosyl unit at the 3-hydroxyl group. Consequently, a gentiobiose unit of **13** must be located at the 20-hydroxyl group. Further, comparison of the carbon signals due to the sugar moieties of **13** with those of **2** and **11** led to the formulation of **13** as 3-*O*-*β*-D-glucopyranosyl-20-*O*-*β*-gentiobiosyl-20(*S*)-protopanaxadiol, which is identical with gypenoside-XVII previously isolated from *Gynostemma pentaphyllum* (Cucurbitaceae) by Takemoto *et al.*[13]

TABLE I. ^{13}C NMR Chemical Shifts: Aglycone Moiety (in C_5D_5N)

	14	**2**	**12**	**13**
C- 1	39.5	39.1	39.2	39.2
C- 2	28.2	26.6	26.6	26.6
C- 3	77.9	89.3	89.2	88.7
C- 4	39.5	39.6	39.7	39.6
C- 5	56.3	56.3	56.4	56.3
C- 6	18.7	18.6	18.4	18.4
C- 7	35.2	35.1	35.2	35.1
C- 8	40.0	39.9	40.0	40.0
C- 9	50.4	50.1	50.2	50.1
C-10	37.3	36.8	36.9	36.9
C-11	32.0	30.8	30.8	30.6
C-12	70.9	70.1	70.1	70.1
C-13	48.5	49.3	49.5	49.4
C-14	51.6	51.3	51.3	51.3
C-15	31.8	30.8	30.8	30.6
C-16	26.8	26.6	26.6	26.6
C-17	54.7	51.6	51.6	51.5
C-18	16.2[a]	16.2[a]	16.4[a]	16.2[a]
C-19	15.8[a]	15.9[a]	16.0[a]	16.0[a]
C-20	72.9	83.5	83.4	83.3
C-21	26.9	22.6	22.4	22.3
C-22	35.8	36.1	36.2	36.1
C-23	22.9	23.1	23.2	23.1
C-24	126.2	125.8	125.9	125.8
C-25	130.6	131.0	131.0	130.8
C-26	25.8	25.8	25.8	25.7
C-27	17.6[a]	17.9[a]	17.9[a]	17.8[a]
C-28	28.6	28.0	28.0	28.0
C-29	16.4[a]	16.5[a]	16.4[a]	16.7[a]
C-30	17.0[a]	17.3[a]	17.4[a]	17.5[a]

a) Values in any column may be reversed, though those given here are preferred.

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Table II. ^{13}C NMR Chemical Shifts: Sugar Moiety

		2	12	11	13
3-glc	1	105.0	104.9[b]	106.9	106.7
(inner)	2	82.9	84.3	75.7	75.6
	3	77.2[a]	77.0[a]	79.2[a]	79.0[a]
	4	71.5	71.5	71.6	71.6
	5	78.0[a]	78.2[a]	78.2[a]	78.6[a]
	6	62.6	62.7	63.1	62.9
3-glc	1	105.6	106.1		
(terminal)	2	76.7	76.6		
	3	78.8[a]	79.1[a]		
	4	71.5	71.5		
	5	78.0[a]	75.2		
	6	62.6	64.7		
20-glc	1	97.9	98.0	98.2	97.9
(inner)	2	74.9	74.9	75.1	75.1
	3	78.0[a]	78.2[a]	78.7[a]	78.1[a]
	4	71.5	71.5	71.8	71.6
	5	76.7	76.6	78.2[a]	76.8
	6	71.5	71.5	62.8	71.6
20-glc	1	105.0	105.2[b]		105.1
(terminal)	2	74·9	74.9		74.6
	3	78.0[a]	78.2[a]		78.1[a]
	4	71.5	71.5		71.6
	5	78.0[a]	78.2[a]		78.1[a]
	6	62.6	62.7		62.7
$\underline{C}H_3CO$			20.9		
$CH_3\underline{C}O$			170.8		

glc: β-D-glucopyranosyl.
a, *b*) Values in any column may be reversed, though those given here are preferred.

Experimental

The ^{13}C NMR spectra were taken in C_5D_5N on a JEOL PFT-100 spectrometer (25.15 MHz) and the chemical shifts are expressed on the δ scale from an internal standard, tetramethylsilane (TMS). Mass spectra were recorded on JEOL 01-SG-2 and JEOL JMS-DX300 mass spectrometers at 75 and 70 eV, respectively. IR spectra were obtained with a Hitachi Model 215 spectrophotometer. Gas liquid chromatography (GLC) was run on a Varian 2100 gas chromatograph. Identification of the resulting monosaccharides after hydrolysis and acetylation, and trimethylsilylation for MS were carried out as described in previous papers.[1f,7]

Identification of the Known Saponins——Each saponin isolated in this paper was identified by comparison with a corresponding authentic sample. The methods used were TLC on silica gel $60F_{254}$ (Merck), using solvent A ($CHCl_3$–MeOH–H_2O=7: 3: 0.5, homogeneous) and solvent B (1-BuOH–AcOEt–H_2O=4: 1: 2, upper phase), HPTLC Rp-18 F_{254s} (Merck; solvent, 80% aqueous MeOH) and HPTLC Rp-8 F_{254s} (Merck; solvent, 60% aqueous MeOH) (detection with H_2SO_4), ^{13}C NMR spectroscopy and mass spectroscopy of the acetates or TMSi ethers.

Extraction and Separation of Saponins——Powdered American Ginseng imported from the U.S.A. (0.5 kg) was extracted five times with hot MeOH (1 l). After evaporation of the solvent, a suspension of the resulting MeOH extract in H_2O was extracted with AcOEt (0.2 l) and then with 1-BuOH (saturated with H_2O) (0.3 l×5). The combined 1-BuOH layers were concentrated to dryness *in vacuo*, and the residue (34 g) was chromatographed on a column of silanized silica gel (Merck) (solvent: 15% aqueous MeOH and then MeOH). The fraction (28 g) eluted with MeOH, which mainly consisted of saponins, was chromatographed on silica gel (solvent A) to give fractions (Fr.) 1—6. Further chromatography of Fr. 2 on reversed-phase highly porous polymer (MCI CHP20P, Mitsubishi Chemical Ind. Ltd.) by gradient elution with 55—85% aqueous MeOH afforded **7** (yield: 0.15%), **8** (yield: 0.008%), **10** (yield: 0.04%), and crude **11**; the last of these was further chromatographed on ODS silica gel (Waters) (solvent: 80% aqueous MeOH), affording **11** (yield: 0.018%). Fr. 3 was chromatographed on silanized silica gel, affording **6** (yield: 1.0%) (eluted with 40% aqueous MeOH) and a saponin mixture (eluted with MeOH). This saponin mixture was subjected to repeated column chromatography first on silica gel (solvent A), then on ODS silica gel (solvent: 70% aqueous MeOH),

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affording **5** (yield: 0.45%) and **13** (yield: 0.03%). Fr. 4 was chromatographed on silica gel (solvents A and B), affording **3** (yield: 0.03%), **4** (yield: 0.31%), **9** (yield: 0.03%), and **12** (yield: 0.01%). Fr. 5 was purified by silica gel chromatography using $CHCl_3$–MeOH–H_2O (13: 7: 2, lower phase) to afford **2** (yield: 1.84%). Fr. 6 was separated by column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (13: 7: 2, lower phase) to give **1** (yield: 0.07%).

Quinquenoside-R_1(**12**): white powder (reprecipitated from EtOH–AcOEt), $[\alpha]_D^{16}$ +12.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{56}H_{94}O_{24}\cdot 3H_2O$: C, 55.80; H, 8.36. Found: C, 55.52; H, 8.19.

Gypenoside-XVII (**13**): white powder (reprecipitated from EtOH–AcOEt), $[\alpha]_D^{16}$ +17.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{48}H_{82}O_{18}\cdot 2/5H_2O$: C, 58.11; H, 8.84. Found: C, 58.20; H, 8.65.

Saponification of 12——A saponin (35 mg) was heated with 5% methanolic KOH (5 ml) for 2 h. The reaction mixture was poured into H_2O and extracted with 1-BuOH (saturated with H_2O). The combined BuOH layer was washed with H_2O and concentrated to dryness, affording **2** (21 mg).

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