[Chem. Pharm. Bull. 30(7)2380—2385(1982)]

Ginsenoside-Ra_1 and Ginsenoside-Ra_2, New Dammarane-Saponins of Ginseng Roots

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(Received December 19, 1981)

Two new dammarane-saponins, named ginsenoside-Ra_1 (**1**) and ginsenoside-Ra_2 (**2**) were isolated from the root of *Panax ginseng* C.A. MEYER by means of chromatography on silica gel and reversed phase chromatography on highly porous polymer. The structures of saponins **1** and **2** were established to be 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside-20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside and 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside-20-*O*-*β*-D-xylopyranosyl(1→2)-*α*-L-arabinofuranosyl(1→6)-*β*-D-glucopyranoside, respectively.

Keywords——ginseng roots; Araliaceae; dammarane-saponins; ginsenoside-Ra_1; ginsenoside-Ra_2; ^{13}C NMR of oligoglycosides

Although isolation and structure determination of a number of saponins of ginseng roots have been reported,[1] little work has been done on ginsenoside-Ra, the presence of which in ginseng extract was detected by thin layer chromatography (TLC).[2] The present paper deals with the further separation of this ginsenoside-Ra fraction and with the structure elucidation of the resulting two new dammarane-saponins.[3]

A crude saponin-fraction from the methanolic extract of white ginseng was subjected to column chromatography on silica gel to give the ginsenoside-Ra fraction, which seemed to be homogeneous on silica gel TLC. However, TLC on octadecyldimethylsilyl silica gel (ODS silica) revealed that this fraction is still a mixture of two saponins, as illustrated in Fig. 1. Further reversed phase column chromatography of this fraction on highly porous polymer furnished the two new saponins, named ginsenoside-Ra_1 (**1**) (yield: 0.03%) and ginsenoside-Ra_2 (**2**) (yield: 0.02%).

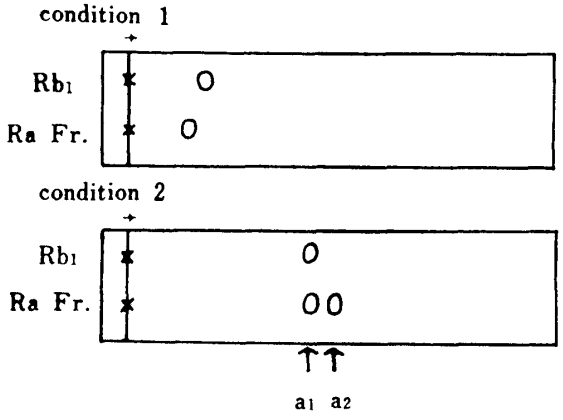
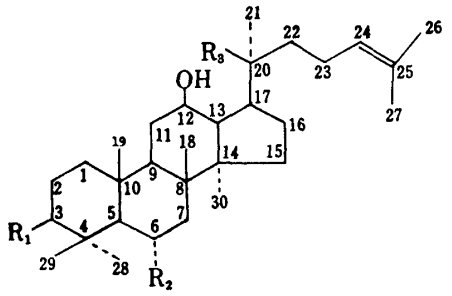


Fig. 1. TLC of Ginsenosides-Rb_1, -Ra_1, and -Ra_2

condition 1: on silica gel $100F_{254}$ (Merck); solvent, $CHCl_3$–MeOH–H_2O (13: 7: 2, lower phase).
condition 2: on RP-18 F_{2545} (Merck); solvent, 80% MeOH.
color reagent: H_2SO_4.

We have reported assignments of the carbon signals of ginseng sapogenins[4] and saponins[5,6] as well as the glycosylation shifts of carbon signals for a variety of glucosides,[6] mannosides, rhamnosides,[7] and arabinosides,[8] and ^{13}C nuclear magnetic resonamce (^{13}C NMR) spectroscopy is now the most powerful tool available for the identification and structure determination of saponins of this type. In the ^{13}C NMR spectra of **1** and **2**, all of the carbon signals due to the aglycone moiety appeared at almost the same positions as those of ginsenoside-Rb_2 (**3**) and -Rc (**4**), indicating that **1** and **2** are glycosides of 20(*S*)-protopanaxadiol (**5**) having sugar units

at both the 3- and 20-hydroxyl groups. The presence of five monosaccharide units in **1** and **2** was demonstrated by the anomeric carbon signals in the ^{13}C NMR spectra. On hydrolysis with mineral acid, both **1** and **2** afforded glucose, arabinose, and xylose. It was reported that the glycosyl linkage at the 20-*tert*-hydroxyl group of dammarane-saponins such as **3** and **4** is readily hydrolyzed even under mild conditions, yielding a C-20-epimeric mixture of the corresponding prosapogenin or sapogenin.[9] Further, in the mass spectra of their acetates or trimethylsilyl ethers, neither M^+ nor fragment ions having an intact *O*-glycosyl group at the C-20 position could be observed.[10] On mild hydrolysis with aqueous acetic acid, **1** and **2** afforded oligosaccharides, **6** and **7**, respectively, along with the common prosapogenin (**8**) which was identical with that previously obtained from **3** and **4** by the same treatment.[9] As shown in Chart 2, the mass spectra of the acetates of **1** and **2** exhibited a pair of ions, *m/z* 1042 and 1043 due to the prosapogenin moiety and ions at *m/z* 259, 331, 475, and 619, characteristic of terminal pentose, terminal hexose, pentose-pentose, and hexose-hexose moieties.



1: R_1=-*O*-glc^{2}—^{1}glc, R_2=H, R_3=-*O*-glc^{6}—^{1}ara(pyr)^{4}—^{1}xyl
2: R_1=-*O*-glc^{2}—^{1}glc, R_2=H, R_3=-*O*-glc^{6}—^{1}ara(fur)^{2}—^{1}xyl
3: R_1=-*O*-glc^{2}—^{1}glc, R_2=H, R_3=-*O*-glc^{6}—^{1}ara(pyr)
4: R_1=-*O*-glc^{2}—^{1}glc, R_2=H, R_3=-*O*-glc^{6}—^{1}ara(fur)
5: R_1=OH, R_2=H, R_3=OH
8(20*S*+20*R*): R_1=-*O*-glc^{2}—^{1}glc, R_2=H, R_3=OH
9: R_1=OH, R_2=OH, R_3=-*O*-glc^{6}—^{1}ara(pyr)^{4}—^{1}xyl

glc: β-D-glucopyranosyl
ara(pyr): α-L-arabinopyranosyl
ara(fur): α-L-arabinofuranosyl
xyl: β-D-xylopyranosyl

Chart 1

m/z 259 xyl(Ac)$_3^+$
m/z 331 glc(Ac)$_4^+$
m/z 475 ara(Ac)$_2$-xyl(Ac)$_3^+$
m/z 619 glc(Ac)$_3$-glc(Ac)$_4^+$

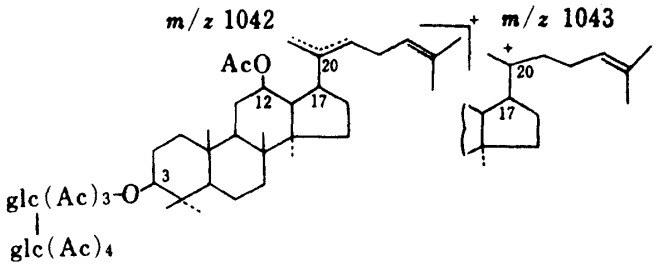


Chart 2. Mass Fragment Ions

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

	5	3	1	4	2
C- 1	39.5	39.4	39.1	39.0	39.1
C- 2	28.2	26.6	26.7	26.6	26.6
C- 3	77.9	89.1	89.1	89.0	89.0
C- 4	39.5	39.6	39.6	39.6	39.6
C- 5	56.3	56.4	56.5	56.3	56.4
C- 6	18.7	18.3	18.5	18.3	18.4
C- 7	35.2	35.1	35.2	35.1	35.1
C- 8	40.0	39.9	40.0	39.9	39.9
C- 9	50.4	50.1	50.2	50.1	50.2
C-10	37.3	36.8	36.8	36.8	36.8
C-11	32.0	30.7	30.6	30.7	30.7
C-12	70.9	70.1	70.1	70.2	70.3
C-13	48.5	49.4	49.4	49.5	49.2
C-14	51.6	51.3	51.3	51.4	51.3
C-15	31.8	30.7	30.6	30.8	30.7
C-16	26.8	26.4	26.7	26.6	26.6
C-17	54.7	51.6	51.3	51.6	51.3
C-18	16.2[a]	16.2[a]	16.2[a]	16.2[a]	16.2[a]
C-19	15.8[a]	15.9[a]	16.0[a]	15.9[a]	16.0[a]
C-20	72.9	83.5	83.5	83.1	83.4
C-21	26.9	22.2	22.2	22.2	22.3
C-22	35.8	36.3	36.1	36.0	36.0
C-23	22.9	23.1	23.1	23.1	23.0
C-24	126.2	125.8	125.8	125.9	126.0
C-25	130.6	131.0	131.0	130.9	131.0
C-26	25.8	25.8	25.7	25.7	25.7
C-27	17.6[a]	17.9[a]	17.9[a]	17.8[a]	17.9[a]
C-28	28.6	28.0	28.0	28.0	28.0
C-29	16.4[a]	16.5[a]	16.5[a]	16.5[a]	16.5[a]
C-30	17.0[a]	17.3[a]	17.4[a]	17.3[a]	17.3[a]

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

A comparison of the ^{13}C NMR spectrum of **1** with those of **3** and chikusetsusaponin-L_5 (**9**) previously isolated from leaves of *Panax japonicus* C.A. Meyer collected in Hiroshima[5a] revealed that the signals due to the sugar moiety of **1** consisted of those due to the 3-*O*-*β*-sophorosyl moiety of **3** and those assigned to the 20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranosyl group of **9**. Further, permethylation of the oligosaccharide (**6**) obtained from **1** by partial hydrolysis (*vide supra*) followed by methanolysis afforded methyl 2,3,4-tri-O-methyl-glucopyranoside (**10**), methyl 2,3,4-tri-*O*-methyl-xylopyranoside (**11**), and methyl 2,3-di-*O*-methyl-arabinopyranoside (**12**). It follows that **1** can be formulated as 20(*S*)-protopanaxadiol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside-20-*O*-*β*-D-xylopyranosyl(1→4)-*α*-L-arabinopyranosyl(1→6)-*β*-D-glucopyranoside, *i.e.*, xylo-ginsenoside-Rb_2. The isolation of the same saponin from ginseng roots and its structure elucidation were independently reported by Shoji *et al.*[11]

The oligosaccharide (**7**) obtained from **2** by partial hydrolysis was subjected to permethylation followed by methanolysis. The gas chromatographic analysis of the products demonstrated the formation of **10**, **11**, and unidentified peaks probably due to partially methylated methyl arabinoside. A comparison of the ^{13}C NMR spectrum of **2** with that of **4** revealed an additional set of signals assignable to a terminal *β*-xylopyranosyl unit in the spectrum of **2**. Further, on going from **4** to **2**, a carbon signal at δ 109.9, which is characteristic of the anomeric carbon of *α*-arabinofuranosides,[12] was shielded by 1.9 ppm and a carbon resonance at δ 83.3 due to C-2 of the *α*-arabinofuranosyl unit[12] was displaced to low-field by 7.3 ppm, while other signals of the sugar moiety of **4** remained almost unshifted. These results coupled with the

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

		3	1	9	4	2
3-glc	1	105.0	104.9		104.9	105.0
(inner)	2	83.0	83.0		83.1	83.1
	3	78.1[a]	78.0[a]		77.8[a]	77.3[a]
	4	71.5	71.5		71.5	71.6
	5	78.1[a]	78.0[a]		77.8[a]	78.0[a]
	6	62.7	62.7		62.6	62.7
3-glc	1	105.7	105.6		105.6	105.7
(terminal)	2	76.9	76.8		76.8	76.9
	3	79.0[a]	79.1[a]		78.9[a]	79.1[a]
	4	71.5	71.5		71.5	71.6
	5	78.7[a]	78.0[a]		78.0[a]	78.0[a]
	6	62.7	62.7		62.6	62.7
20-glc	1	97.9	97.9	97.9	97.9	98.0
	2	74.8	74.7	74.6	74.9	74.8
	3	78.7[a]	79.1[a]	79.1	78.0[a]	78.0[a]
	4	71.5	71.5	71.7	71.5	71.6
	5	76.6	76.8	76.6	76.3	76.9
	6	69.0	69.7	69.7	68.3	68.2
20-ara	1	104.5	104.9	104.8	109.9	108.0
	2	72.0	72.6	72.7	83.3	90.6
	3	73.9	73.7	73.7	78.6	79.1[a]
	4	68.5	78.0	78.4	85.8	85.3
	5	65.5	65.5	65.5	62.6	62.7
20-xyl	1		106.6	106.7		104.3
	2		75.2	75.2		74.8
	3		78.0	78.4		78.0[a]
	4		70.8	70.7		70.9
	5		67.0	67.2		67.2

glc: β-D-glucopyranosyl.
ara: α-L-arabinopyranosyl or α-L-arabinofuranosyl.
xyl: β-D-xylopyranosyl.
a) Assignments in any column may be reversed, though those given here are preferred.

mass spectrum (*vide supra*) led to the formulation of **2** as 20(*S*)-protopanaxadiol 3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside-20-*O*-β-D-xylopyranosyl (1→2)-α-L-arabinofuranosyl (1→6)-β-D-glucopyranoside, *i.e.*, xylo-ginsenoside-Rc.

It is noteworthy that **1** and **2** are the first examples of dammarane-saponins having five monosaccharide units. Compounds **1** and **2** were also isolated and identified from Korean red ginseng.[13] Further studies on several other minor saponins of ginseng roots are in progress.

Experimental

The ^{13}C NMR spectra were taken on a JEOL PFT-100 NMR spectrometer at 25.15 MHz in pyridine-d_5, and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Infrared (IR) absorption spectra were obtained with a Hitachi Model 215 spectrophotometer. Gas liquid chromatography (GLC) was run on Varian 2100 and Shimadzu GC-4A gas chromatographs. Spraying with 5% H_2SO_4 and heating were used for visualization of spots on TLC.

Extraction and Separation of Saponins (1 and 2)——Powdered commercial white ginseng produced in Korea (200 g) was extracted with hot MeOH (0.5 l) five times. After removal of the solvent by evaporation, a suspension of the resulting MeOH-extract (38 g) in H_2O was extracted with EtOAc and then 1-BuOH (saturated with H_2O). The combined BuOH layers were concentrated to dryness *in vacuo*. The residue (8.1 g) was chromatographed on a column of silanized silica gel (Merck) (solvent: 15% aqueous MeOH and MeOH). The fraction (5.0 g) eluted with MeOH (which mainly consisted of saponins) was separated by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7: 3: 0.5)) to give ginsenoside-Ra fraction (0.4 g), and this was further subjected to reversed phase chromatography on highly porous polymer (MCI

CHP20P, Mitsubishi Chemical Ind. Ltd.) (solvent: 70% MeOH) and then on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7: 3: 0.5)), affording **1** and **2** in yields of 0.03% and 0.02%, respectively.

Ginsenoside-Ra_1 (**1**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{25}$ +12.8° (c=1.0, MeOH), *Anal.* Calcd for $C_{58}H_{98}O_{26}\cdot 4H_2O$: C, 54.28; H, 8.32. Found: C, 54.39; H, 8.49.

Ginsenoside-Ra_2 (**2**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{25}$ −2.4° (c=1.0, MeOH), *Anal.* Calcd for $C_{58}H_{98}O_{26}\cdot 4H_2O$: C, 54.28; H, 8.32. Found: C, 54.35; H, 8.23.

Hydrolysis of 1 and 2——A saponin (a few mg) was heated with 4 N HCl–dioxane (1: 1 v/v) (1 ml) in a sealed tube on a boiling water bath for 4 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature. TLC: on silica gel $60F_{254}$ (Merck); solvent, $CHCl_3$–MeOH–H_2O (13:7: 2, lower phase); detection, naphthoresorcinol. *Rf*; glucose (0.11), arabinose (0.23), xylose (0.28). For GLC analysis, the residue was trimethylsilylated by the same procedure as that for mass spectra (MS). GLC: 2% OV-17 on Gas Chrom Q, glass column 2 mm×1.5 m; detector, FID; injection temperature, 250°C; column temperature, 170°C; carrier gas, N_2 (4.5 kg/cm²). t_R (min); glucose (18.2, 26.7), arabinose (5.7, 6.9), xylose (9.0, 11.0).

Partial Hydrolysis of 1 and 2——A solution of the saponin (100 mg) in 50% AcOH was heated at 70°C for 4 h. The reaction mixture was diluted with H_2O and extracted with 1-BuOH (saturated with H_2O). The BuOH layers were concentrated to dryness and the residue was purified by column chromatography on silica gel with $CHCl_3$–EtOAc–MeOH–H_2O (2: 4: 2: 1, lower phase), giving prosapogenin (**8**) as a white powder (20 mg), which was identified by comparison of the ^{13}C NMR spectrum and TLC behavior (on silica gel (solvent: $CHCl_3$–EtOAc–MeOH–H_2O (2: 4: 2: 1, lower phase)) with those of an authentic sample. The aqueous layer was deionized on Amberlite IR-45 and concentrated to dryness *in vacuo* to give oligosaccharide (**6** or **7**).

Permethylation followed by Methanolysis of C-20-Oligosaccharides (6 and 7)——According to Hakomori's method,[14] a mixture of NaH (100 mg) and DMSO (4 ml) was heated at 65°C for 1 h under N_2, and a solution of the oligosaccharide **6** or **7** (from 100 mg of the saponin) in DMSO (4 ml) was added to this mixture. The whole was stirred for 1 h at room temperature, then MeI (3 ml) was added to the solution and the mixture was allowed to stand overnight at room temperature. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and concentrated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel (solvent: $CHCl_3$–MeOH (70: 1)), affording a permethylated oligosaccharide.

Permethylether of **6**; IR ($CHCl_3$): no OH absorption; EI-MS (m/z): 570 (M^+), 469 ($M^+-C_5H_9O_2$), 379 (permethylated pentosyl-hexosyl cation), 219 (permethylated hexosyl cation), 175 (permethylated pentosyl cation).

Permethylether of **7**; IR ($CHCl_3$): no OH absorption; EI-MS (m/z): 525 ($M^+-CH_2OCH_3$), 469 ($M^+-C_5H_9O_2$), 335 (permethylated pentosyl-pentosyl cation), 219 (permethylated hexosyl cation), 175 (permethylated pentosyl cation).

A solution of the resulting permethylated oligosaccharide in 5% methanolic HCl (5 ml) was refluxed for 4 h. The reaction mixture was neutralized with Ag_2CO_3. After removal of the precipitate by filtration, the filtrate was concentrated to dryness, and the resulting hydrolysate was subjected to GLC on a glass column (4 mm×2 m) packed with 5% NPGS on chromosorb WAW, column temperature 170°C, carrier gas N_2 (1.6 kg/cm²). t_R (min): 10 (8.4, 12.0), 11 (1.5, 2.0), 12 (4.5, 5.6), unidentified methyl sugar (3.7, 7.7).

Acetylation for MS——A saponin (a few mg) was heated with Ac_2O/pyridine in a sealed microtube at 80°C for 2—3 h. The reaction mixture was concentrated by blowing N_2 gas over it and the residue was subjected to MS.[10]

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