[Chem. Pharm. Bull. 31(6)2120—2125(1983)]

Saponins of Red Ginseng

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(Received November 18, 1982)

From Red Ginseng, all of the known saponins of White Ginseng were isolated in yields similar to those obtained from White Ginseng. Two additional minor saponins, notoginsenoside-R_1 (**19**) (the saponin of roots of *Panax notoginseng*) and quinquenoside-R_1 (**20**) (the saponin of roots of *P. quinquefolium*), were also isolated and identified. Besides these known saponins, two new minor saponins named ginsenosides-Rs_1 (**17**) and -Rs_2 (**18**) were also isolated. Compounds **17** and **18** were established to be monoacetates at the 6-hydroxyl group of the terminal glucosyl moiety of the sophorosyl unit of ginsenosides-Rb_2 (**5**) and -Rc (**7**), respectively.

Keywords——*Panax ginseng*; Red Ginseng; ginsenoside-Rs_1; ginsenoside-Rs_2; notoginsenoside-R_1; quinquenoside-R_1; dammarane-saponin; ^{13}C-NMR; EI-MS; FD-MS

Two kinds of Ginseng preparation have generally been used in oriental medicine, White Ginseng and Red Ginseng; the former is prepared by drying the roots after removal of the epidermis, while the latter is produced by drying the roots after steaming them without removal of the epidermis. Most of the previous studies on isolation and structure determination of Ginseng saponins, ginsenosides-Ro[1] (=chikusetsusaponin-V) (**1**),[2] -Ra_1(**2**),[3,4] -Ra_2(**3**),[3] -Rb_1(**4**), -Rb_2(**5**),[1] -Rb_3(**6**),[5] -Rc(**7**), -Rd(**8**),[1] -Re(**9**), -Rf(**10**),[6] -Rg_1(**11**),[7] -Rg_2(**12**),[6] -Rg_3(**13**),[8] -Rh_1(**14**)[9] and 20-gluco-ginsenoside-Rf(**15**),[5] were carried out not on Red Ginseng but on White Ginseng or the dried lateral roots. Since Red Ginseng has been more commonly used in oriental countries, a comparison of the saponins of Red Ginseng with those of White Ginseng would be desirable from a pharmaceutical point of view. In order to elucidate whether or not the relatively unstable saponins remain unchanged after the steaming, we have isolated and identified the saponins of Red Ginseng.

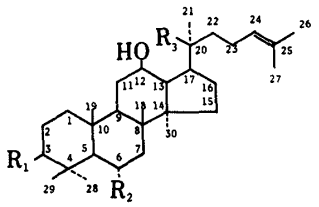
A crude saponin fraction of a methanolic extract of Red Ginseng produced by the Office of Monopoly, Korea, was subjected to a combination of column chromatographies on silica gel, silanized silica gel and reversed-phase highly porous polymer to give the following known saponins (yield); **1** (0.04%), **2** (0.02%), **3** (0.03%), **4** (0.38%), **5** (0.15%), **6** (0.01%), **7** (0.14%), **8** (0.04%), **9** (0.17%), **10** (0.07%), **11** (0.29%) and **15** (0.008%). The identifications of these saponins were substantiated by comparison of the mobilities on thin layer chromatography (TLC) on a variety of plates, the mass spectra of their acetates or trimethylsilyl (TMSi) ethers and the ^{13}C nuclear magnetic resonance ^{13}C (NMR) spectra with those of authentic samples.

Very recently, Kitagawa *et al.* reported the isolation of very small amounts of **12**, **13** and **14** and their corresponding 20-epimers, **12**R, **13**S and **14**R, along with a new saponin named ginsenoside-Rh_2 (**16**), from the less polar fraction of the methanolic extract of Red Ginseng.[10]

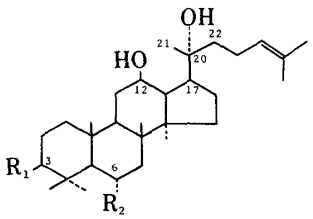
We also isolated two compounds (in yields of 0.02 and 0.01%, respectively) which showed single spots corresponding to **12** and **13** on TLC. It was reported that the 20-epimers of 12-β-hydroxy-dammarane type triterpenes can be distinguished from each other by the difference of the chemical shifts due to C-17, -21 and -22.[11] Comparison of the ^{13}C NMR spectra of both the compounds with those of **12** and **13** (Table I) revealed that the compounds are mixtures

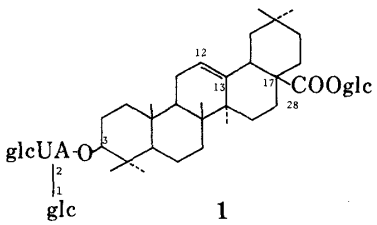
of **12** and **12R**, and **13** and **13S**, respectively. Since it has been reported that a glycosyl linkage at the C-20-*tert*-hydroxyl group of dammarane saponins is rather unstable and is hydrolyzed even under mild acidic conditions, affording a 20-epimeric mixture of the prosapogenin or sapogenin,[12] these epimeric pairs of the minor saponins, **12R**, **13S** and **14R** and some of **12**, **13** and **14** in Red Ginseng may be formed from the corresponding parent saponins during the process of production of Red Ginseng. In any case, the contents of these partially hydrolyzed saponins are very low and the present isolation of the major saponins from Red Ginseng in yields similar to those from White Ginseng indicates that most of the saponins remain undegraded even after the steaming.

Besides the above saponins, several minor saponins were isolated. New saponins named



	R_1	R_2	R_3
2:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}ara(p)^{4}—^{1}xyl
3:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}ara(f)^{2}—^{1}xyl
4:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}glc
5:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}ara(p)
6:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}xyl
7:	–O–glc^{2}—^{1}glc	H	–O–glc^{6}—^{1}ara(f)
8:	–O–glc^{2}—^{1}glc	H	–O–glc
9:	OH	–O–glc^{2}—^{1}rha	–O–glc
10:	OH	–O–glc^{2}—^{1}glc	OH
11:	OH	–O–glc	–O–glc
12:	OH	–O–glc^{2}—^{1}rha	OH
13S:	–O–glc^{2}—^{1}glc	H	OH
14:	OH	–O–glc	OH
15:	OH	–O–glc^{2}—^{1}glc	–O–glc
16:	–O–glc	H	OH
17:	–O–glc^{2}—^{1}glc^{6}—Ac	H	–O–glc^{6}—^{1}ara(p)
18:	–O–glc^{2}—^{1}glc^{6}—Ac	H	–O–glc^{6}—^{1}ara(f)
19:	OH	–O–glc^{2}—^{1}xyl	–O–glc
20:	–O–glc^{2}—^{1}glc^{6}—Ac	H	–O–glc^{6}—^{1}glc





1

	R_1	R_2
12R:	OH	–O–glc^{2}—^{1}rha
13:	–O–glc^{2}—^{1}glc	H

glc: β-D-glucopyranosyl ara(p): α-L-arabinopyranosyl
ara(f): α-L-arabinofuranosyl xyl: β-D-xylopyranosyl
rha: α-L-rhamnopyranosyl glcUA: β-D-glucuronic acid

Chart 1

ginsenosides-Rs_1 (**17**) and -Rs_2 (**18**) were isolated in yields of 0.008 and 0.01%, respectively. An infrared (IR) band at 1735 cm^{-1} and carbon signals at δ 20.9 (q) and 170.8 (s) in both compounds indicated the presence of an acetoxyl group. On alkaline hydrolysis, **17** and **18** yielded **5** and **7**, respectively. Since all of the carbon signals due to the aglycone moiety of both compounds appeared at almost the same positions as those of **5** and **7**, the acetoxyl group must be located on the sugar moieties. The electron impact mass spectra (EI-MS) of the TMSi ethers of **17** and **18** exhibited fragment ions at *m/z* 709 (glucose$(TMSi)_3$-glucose$(TMSi)_3$-Ac^+—TMSiOH), 421 (glucose$(TMSi)_3Ac^+$), 349 (arabinose$(TMSi)_3^+$) and 481 (arabinose$(TMSi)_3$-$OCH_2CH{=}O^+TMSi$, characteristic of a 1,6-linked TMSi ether of arabinosyl glucose[13]), demonstrating that the acetoxyl group of **17** and **18** must be present at the terminal glucosyl moiety of the 3-*O*-β-sophorosyl group of **5** and **7**. This conclusion was also supported by the field desorption mass spectra (FD-MS), which showed ions at *m/z* 1143 $(M+Na)^+$, 1101 $(M+Na-CH_2CO)^+$, 939 (M+Na−glucoseAc)$^+$, 849 (M+Na−arabinose−glucose)$^+$ and 777 (M+Na−glucose−glucoseAc)$^+$. In the ^{13}C NMR spectra (Table II), on going from **5** to **17** and from 7 to **18**, a signal due to C-6 of one of the glucosyl moieties was displaced downfield by 2.0 ppm and a signal due to C-5 of one of the glucosyl moieties was shielded by 3.5 ppm, while the other carbon signals remained almost unshifted.[14] It follows that the acetyl group of **17** and **18** must be located at the 6-hydroxyl group of the terminal glucosyl moiety of the β-sophorosyl unit of **5** and **7**, respectively.

Further, two additional minor saponins, **19** and **20** were also isolated in yields of 0.007 and 0.015%, respectively. The saponin (**19**) was identical with notoginsenoside-R_1, previously isolated from Sanchi-Ginseng, roots of *Panax notoginseng*.[15] Another saponin (**20**) was

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

	5	**17**	**7**	**18**	**12**	**12R**	**13S**	**13**
C- 1	39.4	39.2	39.0	39.1	39.7		39.1	
C- 2	26.6	26.6	26.6	26.6	27.8		26.8	
C- 3	89.1	89.2	89.0	89.2	78.4		88.9	
C- 4	39.6	39.6	39.6	39.6	40.1		39.5	
C- 5	56.4	56.4	56.3	56.3	60.9		56.3	
C- 6	18.3	18.4	18.3	18.4	74.3		18.2	
C- 7	35.1	35.1	35.1	35.1	46.2		35.2	
C- 8	39.9	39.9	39.9	39.9	41.2		39.8	
C- 9	50.1	50.2	50.1	50.1	49.9		50.2	
C-10	36.8	36.8	36.8	36.8	39.7		36.8	
C-11	30.7	30.6	30.7	30.7	32.2		31.8	
C-12	70.1	70.1	70.2	70.2	71.1		70.8	
C-13	49.4	49.3	49.5	49.3	48.3		48.4	(49.2)
C-14	51.3	51.3	51.4	51.3	51.7		51.6	
C-15	30.7	30.6	30.8	30.7	31.4		31.3	
C-16	26.4	26.6	26.6	26.6	27.1		26.8	
C-17	51.6	51.6	51.6	51.6	54.8	(50.5)	54.5	(50.4)
C-18	16.2[a]	16.2[a]	16.2[a]	16.3[a]	17.2[a]		16.2[a]	
C-19	15.9[a]	15.9[a]	15.9[a]	15.9[a]	17.7[a]		15.8[a]	
C-20	83.5	83.4	83.1	83.3	73.0		72.9	
C-21	22.2	22.2	22.2	22.2	27.0	(22.7)	26.8	(22.7)
C-22	36.3	36.0	36.0	36.0	35.9	(45.0)	35.7	(43.2)
C-23	23.1	23.1	23.1	23.1	23.1		22.8	
C-24	125.8	125.8	125.9	125.8	126.4		126.0	
C-25	131.0	131.0	130.9	130.9	130.7		130.5	
C-26	25.8	25.7	25.7	25.7	26.0		25.8	
C-27	17.9[a]	17.8[a]	17.8[a]	17.8[a]	17.7[a]		17.6[a]	
C-28	28.0	27.9	28.0	27.9	32.2		28.0	
C-29	16.5[a]	16.2[a]	16.5[a]	16.3[a]	17.0[a]		16.4[a]	
C-30	17.3[a]	17.3[a]	17.3[a]	17.2[a]	17.0[a]		16.9[a]	

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

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

		5	17	7	18
3-glc	1	105.0	104.8	104.9	104.8
(inner)	2	83.0	84.1	83.1	84.0
	3	78.1[a]	77.9[a]	77.8[a]	77.8[a]
	4	71.5	71.6[b]	71.5	71.8[b]
	5	78.1[a]	77.9[a]	77.8[a]	77.8[a]
	6	62.7	62.7	62.6	62.5
3-glc	1	105.7	106.1	105.6	105.9
(terminal)	2	76.9	76.6	76.8	76.5
	3	79.0[a]	79.0[a]	78.7[a]	78.7[a]
	4	71.5	70.9	71.5	70.8
	5	78.7[a]	75.2	78.0[a]	75.2
	6	62.7	64.7	62.6	64.5
20-glc	1	97.9	97.9	97.9	98.0
	2	74.8	74.8	74.9	74.9
	3	78.7[a]	78.4[a]	78.0[a]	78.3[a]
	4	71.5	71.3[b]	71.5	71.2[b]
	5	76.6	76.6	76.3	76.5
	6	69.0	69.1	68.3	68.3
20-ara	1	104.5	104.5	109.9	109.9
	2	72.0	72.0	83.3	83.3
	3	73.9	73.9	78.9	79.0
	4	68.5	68.4	85.8	85.8
	5	65.5	65.4	62.6	62.5
$\underline{C}H_3CO$			20.8		20.8
$C\underline{H}_3CO$			170.8		170.8

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

identical with quinquenoside-R_1, monoacetyl ginsenoside-Rb_1, which was recently isolated from American Ginseng, roots of *P. quinquefolium*.[16]

It is curious that neither of the minor saponins, **17** and **18**, could be detected in White Ginseng or dried Ginseng (roots dried without removal of the epidermis; Japanese name, Shoboshi Ninjin), though **19** and **20** were detected in White Ginseng by TLC and isolated from dried Ginseng.

Experimental

The ^{13}C NMR spectra were taken on a JEOL PFT-100 spectrometer (25.15 MHz) in pyridine-d_5 and chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard. EI mass spectra were recorded on JEOL O1-SG-2 and JEOL JMS-DX300 mass spectrometers at 75 and 70 eV, respectively. FD mass spectra were taken on a JEOL JMS-DX300 mass spectrometer with an emitter heating current of 24—28 mA. The IR spectra were obtained with a Hitachi Model 215 spectrophotometer (KBr disk method). TLC was performed on Silica gel $60F_{254}$ (Merck), HPTLC Rp-18 F_{254s} (Merck), and HPTLC Rp-8 F_{254s} (Merck) and spots were visualized by spraying of 5% H_2SO_4 followed by heating.

Identification of the Known Saponins——Each saponin isolated in this work was proved to be identical with a corresponding authentic sample by TLC comparisons on Silica gel $60F_{254}$ (solvent A, $CHCl_3$–MeOH–H_2O=7: 3: 0.5 (homogeneous); solvent B, $CHCl_3$–EtOAc–MeOH–H_2O=2: 4: 2: 1 (lower phase); solvent C, 1-BuOH–EtOAc–H_2O=4: 1: 2 (upper phase)), HPTLC Rp-18 F_{254s} (solvent, 80% aqueous MeOH), and HPTLC Rp-8 F_{254s} (solvent, 60% aqueous MeOH), MS of the acetates or TMSi ethers and ^{13}C NMR spectroscopy.

Isolation of Saponins from Red Ginseng——Powdered Red Ginseng (2.3 kg) produced by the Office of Monopoly, Korea, was extracted five times with boiling MeOH (1 l) for 2 h each. The MeOH extract (590 g), suspended in H_2O (0.5 l), was extracted with EtOAc and then with 1-BuOH (saturated with H_2O). The combined BuOH layer was concentrated to dryness *in vacuo* and the residue (124 g) was applied to a column of silanized silica gel (Merck) and eluted with 15% aqueous MeOH and then MeOH. The MeOH-eluted fraction (63 g), which mainly consisted of saponins, was chromatographed on silica gel (gradient elution

with $CHCl_3$–MeOH–H_2O (50: 10: 1 (homogeneous)→7: 3: 0.5→13: 7: 2 (lower phase))) to give fractions (Fr.) I—VIII.

Fr. III was subjected to silica gel column chromatography using solvent B to yield two fractions, IIIa and IIIb. Fr. IIIa was rechromatographed on reversed-phase highly porous polymer (MCI CHP20P, Mitsubishi Chemical Ind., Ltd.) (solvents, 60% aqueous MeOH and then 90% aqueous MeOH) to afford epimeric mixtures of **12** and **12R** (yield: 0.02%) and of **13** and **13S** (yield: 0.01%), respectively. Further chromatography of Fr. IIIb on silanized silica gel (gradient elution with 35—80% aqueous MeOH) afforded **10** (yield: 0.07%) and **11** (yield: 0.29%).

Fr. IV was chromatographed on silanized silica gel (solvents, 40% aqueous MeOH and then MeOH). The MeOH-eluted fraction was further subjected to chromatography on silica gel (solvents, $CHCl_3$–MeOH–H_2O=7: 2: 0.2 (homogeneous) and solvent A, successively) to provide three fractions, IVa—c (in increasing order of polarity). Fr. IVa was separated by column chromatography on ODS silica gel (Waters Assoc.) (solvent, 70% aqueous MeOH) to give **8** (yield: 0.04%) and a saponin mixture. This saponin mixture was purified by silica gel chromatography using solvent A to give **18** (yield: 0.01%). Fr. IVb was subjected to repeated column chromatography; first on highly porous polymer (solvent, 75% aqueous MeOH), then on silica gel (solvent C), affording **17** (yield: 0.008%). Fr. IVc was purified on a column of silica gel (solvents A and C), affording **20** (yield: 0.015%). The fraction eluted with 40% aqueous MeOH was chromatographed on highly porous polymer (solvent, 55% aqueous MeOH) to afford **9** (yield: 0.17%) and a saponin mixture. The latter was further purified on a column of silica gel (solvent C), giving **19** (yield: 0.007%).

Fr. V was chromatographed on a column of silanized silica gel (solvents, 40% aqueous MeOH and then MeOH). The fraction eluted with 40% aqueous MeOH was subjected to repeated column chromatography: first on silica gel (solvent A), then on highly porous polymer (solvent, 55% aqueous MeOH) to give **15** (yield: 0.008%).

A mixture of Fr. VI and the MeOH fraction of Fr. V was chromatographed on silica gel (solvent C), affording **5** (yield: 0.15%), **7** (yield: 0.14%), and a saponin mixture. This saponin mixture was further purified by silica gel chromatography (solvent A) to give **6** (yield: 0.01%).

Fr. VII was subjected to repeated column chromatography on silica gel (solvent A), yielding **4** (yield: 0.38%) and ginsenoside-Ra fraction. The ginsenoside-Ra fraction was separated according to the previous paper,[3] affording **2** (yield: 0.02%) and **3** (yield: 0.03%).

Fr. VIII was purified by column chromatography on silanized silica gel using 50% aqueous MeOH and finally **1** was obtained from MeOH (yield: 0.04%).

Ginsenoside-Rs_1 (**17**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{16}$ +19.0° (c=1.0, MeOH). *Anal.* Calcd for $C_{55}H_{92}O_{23}\cdot 2H_2O$: C, 57.08; H, 8.36. Found: C, 57.09; H, 8.15.

Ginsenoside-Rs_2 (**18**): White powder (reprecipitated from EtOH–EtOAc), $[\alpha]_D^{16}$ +2.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{55}H_{92}O_{23}\cdot 2H_2O$: C, 57.08; H, 8.36. Found: C, 57.16; H, 8.14.

Isolation of 19 and 20 from Dried Ginseng——Powdered roots (cultivated in Nagano prefecture, Japan, 2.3 kg) were extracted with boiling MeOH. The MeOH extract was suspended in H_2O and the suspension was extracted with EtOAc and then with 1-BuOH (saturated with H_2O). The combined BuOH layer was concentrated to dryness *in vacuo* and the residue (59 g) was chromatographed on a column of silanized silica gel (solvents, 15% aqueous MeOH and then MeOH). The MeOH-eluted fraction (40.5 g) was separated in the manner described above, affording **19** (yield: 0.002%) and **20** (yield: 0.002%).

Saponification of 17 and 18——Each saponin, **17** (25 mg) or **18** (25 mg), was heated with 5% methanolic KOH (5 ml) for 2 h. The reaction mixture was poured into H_2O and then extracted with 1-BuOH (saturated with H_2O). The combined BuOH layer was washed with H_2O and concentrated to dryness, affording **5** (12 mg); $[\alpha]_D^{21}$ +4.1° (c=0.72, MeOH), and **7** (14 mg); $[\alpha]_D^{21}$ +1.3° (c=0.92, MeOH).

Acknowledgement We are grateful to the Office of Monopoly, Korea, and to the Korea Ginseng and Tobacco Research Institute for the supply of authentic Red Ginseng Powder.

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