Ginsenoside Rg_8, a New Dammarane-Type Triterpenoid Saponin from Roots of *Panax quinquefolium*

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A new dammarane-type triterpenoid saponin, ginsenoside Rg_8 (1), was isolated from the roots of *Panax quinquefolium*, along with five known saponins, (20*E*)-ginsenoside F_4 (2), ginsenosides Rh_1 (3), Rg_2 (4), F_1 (5), and (20*R*)-ginsenoside Rh_1 (6). The structure of ginsenoside Rg_8 (1) was determined to be (3β,6α,12β,20*E*)-24,25-epoxy-3,12,23-trihydroxydammar-20(22)-en-6-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside by various spectroscopic analyses. Among the known saponins, (20*E*)-ginsenoside F_4 (2) and ginsenoside F_1 (5) were first reported from the title plant.

Key words *Panax quinquefolium*; Araliaceae; dammarane; saponin; ginsenoside Rg_8

The roots of the herbaceous *Panax* species (Araliaceae), *e.g. P. ginseng* (Asian ginseng), *P. quinquefolium* (American ginseng), and *P. notoginseng* (Sanchi ginseng), have been widely used throughout the world for the treatment of a number of diseases, including anaemia, diabetes, gastritis, insomnia, and sexual impotence.[1)] Triterpenoid saponins are considered the main principal constituents for the clinical effects of the *Panax* species, and various bioactivities have been reported.[2)] Previously, we have investigated the saponins of *P. ginseng*[3—5)] and their effects on type 1 and type 2 cytokines production[6)] and protein tyrosine kinase activation.[7)] In *P. quinquefolium*, another important *Panax* species, mainly growing in North America, difference in the content and type of ginsenosides from those of *P. ginseng* has been found.[8)] As our continuous interest in the chemistry and bioactivity of saponins from the *Panax* species, we carried out a chemical investigation on American ginseng, the roots of *P. quinquefolium* cultivated in Quebec, Canada, and resulted in the isolation of six saponins **1**—**6**. The known saponins **2**—**6** were identified to be (20*E*)-ginsenoside F_4 (**2**),[9)] ginsenosides Rh_1 (**3**),[10)] Rg_2 (**4**),[10)] F_1 (**5**),[11)] and (20*R*)-ginsenoside Rh_1 (**6**),[10)] by comparison of their spectral data with those in the references. Although (20*E*)-ginsenoside F_4 (**2**)[9)] and ginsenoside F_1 (**5**)[11)] were known in other *Panax* species, it is the first report from *P. quinquefolium*. This paper deals with the structural elucidation of the new saponin **1**, named ginsenoside Rg_8, on the basis of spectroscopic analyses.

Ginsenoside Rg_8 (**1**) was obtained as an amorphous solid. The molecular formula, $C_{42}H_{70}O_{12}$, was established on the basis of the quasi-molecular ion peak in the high-resolution (HR)-FAB-MS at *m*/*z* 821.4695 $[M+Na]^+$. On enzymatic hydrolysis, **1** afforded D-glucose and L-rhamnose as component sugars, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives. Inspection of the anomeric protons in the ^{1}H-NMR spectrum disclosed the presence of two glycosyl units with the resonances for the anomeric protons at δ 5.27 (1H, d, J=6.7 Hz) and 6.48 (1H, br s). All proton and carbon signals due to the sugars were assigned by careful analyses of the double quantum filter correlation spectroscopy (DQF-COSY), the total correlation spectroscopy (TOCSY) and the heteronuclear multiple quantum coherence (HMQC) spectra, revealing the presence of the β-glucopyranosyl and the α-rhamnopyranosyl moieties (Table 1). The ^{13}C-NMR spectrum of **1** exhibited 30 carbon signals for the aglycon, of which the signal of a methine carbon at δ 60.9 is characteristic of C-5 of the protopanaxatriol-type skeleton.[3)] Comparison of the ^{1}H- and ^{13}C-NMR data between **1** and ginsenoside Rg_2 (**4**) revealed identical signals, except for those at the C-17 side chain. The structure of the C-17 side-chain in **1** was elucidated by interpretation of the DQF-COSY, HMQC, and the heteronuclear multiple bond connectivity (HMBC) data. In the DQF-COSY spectrum, the

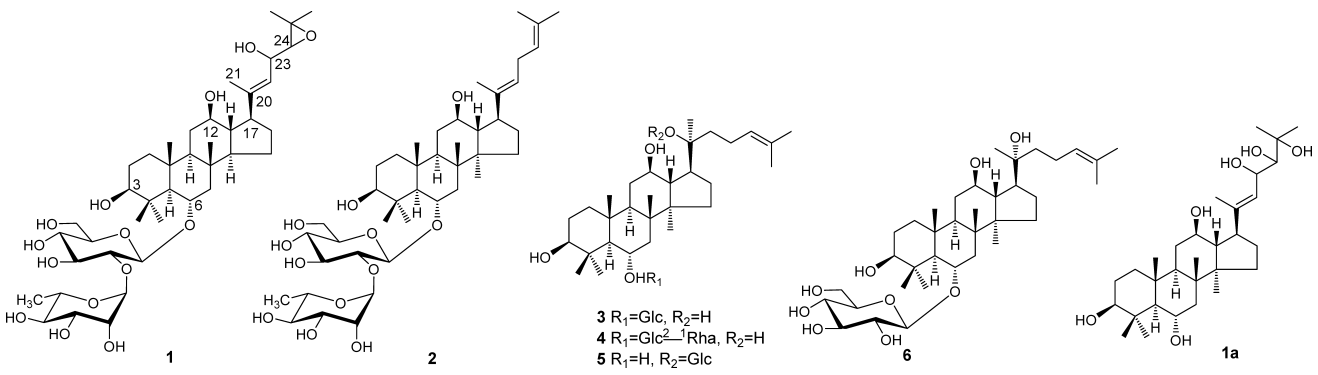

Chart 1

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Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data of **1** and **1a** in Pyridine-d_5

Position	**1**		**1a**	Position	**1**		**1a**
	δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)		δ_H (mult, J in Hz)	δ_C	δ_H (mult, J in Hz)
1	0.94[a]	39.7	1.05[a]	20		143.1	
	1.64 (br d, 13.0)		1.71 (dt, 13.0, 3.6)	21	1.88 (s)	13.8	1.93 (s)
2	1.78[a] (2H)	28.7	1.86 (m)	22	5.89 (d, 9.4)	124.7	6.10 (d, 8.7)
			1.95 (m)	23	4.67[a]	68.5	5.06 (dd, 8.7, 5.2)
3	3.48 (m)	78.4	3.53 (m)	24	3.24 (d, 8.0)	68.8	3.84 (d, 4.8)
4		40.0		25		58.5	
5	1.41 (d, 10.5)	60.9	1.23 (d, 10.5)	26	1.31 (s)[d]	25.2[e]	1.65 (s)[c]
6	4.70 (td, 10.1, 3.9)	74.5	4.42 (m)	27	1.49 (s)[d]	20.1[e]	1.57 (s)[c]
7	2.01 (dd, 12.0, 10.7)	46.2	1.90 (dd, 12.4, 3.9)	28	2.12 (s)	32.2	2.00 (s)
	2.28 (dd, 12.0, 3.5)		1.98 (t, 11.0)	29	1.37 (s)	17.6	1.46 (s)
8		41.5		30	0.97 (s)	17.0	0.93 (s)[b]
9	1.53 (dd, 11.0, 3.9)	50.2	1.59 (dd, 13.0, 3.6)	Glc-1′	5.27 (d, 6.7)	101.9	
10		39.5		2′	4.36 (t, 7.2)	78.6	
11	1.42 (m)	32.5	1.50 (br d, 12.8)	3′	4.34[a]	79.4	
	1.98 (m)		2.06 (br d, 11.9)	4′	4.20 (t, 8.8)	72.7	
12	3.89 (br t, 11.6)	72.5	3.89 (m)	5′	3.98 (ddd, 9.6, 5.1, 2.8)	78.4	
13	1.97 (t, 11.0)	50.8	1.99[a]	6′	4.38[a]	63.2	
14		51.0			4.53 (dd, 11.0, 2.6)		
15	0.95[a]	32.8	1.08 (br d, 10.1)	Rha-1″	6.48 (br s)	101.9	
	1.57 (m)		1.69 (m)	2″	4.79 (br d, 1.8)	72.4	
16	1.78[a]	27.8	1.53 (m)	3″	4.67 (dd, 9.1, 3.5)	72.3	
	1.85[a]		1.88[a]	4″	4.33 (t, 9.1)	74.2	
17	2.83 (td, 10.5, 6.2)	50.7	2.84 (td, 10.7, 6.5)	5″	4.93[a]	69.5	
18	1.25 (s)	17.2	1.16 (s)[b]	6″	1.79 (d, 6.8)	18.8	
19	0.99 (s)	17.8	1.03 (s)[b]				

a) Overlapped signals. Signals marked with superscript *b*), *c*), *d*) and *e*) may be exchangeable with those with the same superscript.

carbinylic proton signal at δ 4.67 (H-23) was observed to correlate with both the olefinic proton signal at δ 5.89 (H-22) and the epoxyl proton signal at δ 3.24 (H-24). The methyl at C-20 was assigned by the HMBC correlations between δ_H 1.88 (H_3-21) and δ_C 50.7 (C-17) and 124.7 (C-22). The two methyls at C-25 were assigned by the HMBC correlations between δ_H 1.31, 1.49 (H_3-26 or H_3-27) and δ_C 68.8 (C-24). The stereochemistry of the double bond at C-20(22) was determined to be *E* from the fact that the signal of C-21 was observed at δ 13.8 in the ^{13}C-NMR spectrum, while in the case of the *Z*-type structure, it was usually observed at δ 25—30.[3] When comparing the ^{1}H- and ^{13}C-NMR data of **1** with those of notoginsenoside T_1,[12] a known saponin from *P. notoginseng* with the structure differing from **1** by lacking an α-L-rhamopyranosyl moiety, the data for the aglycon was in good agreement with each other. The β-D-glucopyranosyl moiety bonding to C-6 of the aglycon and the α-L-rhamnopyranosyl moiety linking to C-2 of the β-D-glucopyranosyl moiety were determined by the HMBC correlations between δ_H 5.27 (Glc-H-1′) and δ_C 74.5 (C-6), and δ_H 6.48 (Rha-H-1″) and δ_C 78.6 (Glc-C-2′). On enzymatic hydrolysis, **1** afforded an artificial genin **1a**, which was determined as a hydrolyte formed by opening of the 24,25-epoxy ring of the aglycon of **1**. The stereochemistry of the asymmetric centers at C-23 and C-24 in **1** and **1a** was not determined because of the very small amount of the compounds available. Thus, the structure of ginsenoside-Rg_8 (**1**) was determined as (3β,6α,12β,20*E*)-24,25-epoxy-3,12,23-trihydroxydammar-20(22)-en-6-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside.

Experimental

General Experiment Procedures The IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrophotometer. FAB-MS and HR-FAB-MS were taken on a JEOL JMS-700 Mstation spectrometer. The ESI-MS was taken on an LCQ mass spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). Column chromatography was carried out on macro-adsorption reticular resin D_{101} (Tianjin Pesticide Manufacturer, P. R. China), silica gel H (Qingdao Factory of Marine Chemical Industry, P. R. China) and ODS (Chromatorex, 100—200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan). TLC was taken on precoated Merck Kieselgel 60 plates with the developing solvents of $CHCl_3$–MeOH–H_2O (69 : 27 : 4) and $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1, lower layer). Preparative HPLC was performed using an ODS column (YMC-Pack Pro C18, 10 mm i.d.×250 mm, YMC Co., Ltd., Kyoto, Japan, detector: UV 203 nm), GLC was carried out on a PerkinElmer Clarus 500 GC-MS instrument.

Extraction and Isolation The roots of *P. quinquefolium* were collected in September, 1997, in Quebec, Canada and identified by Dr. Michael ZC Li (Chai-Na-Ta Company, Canada) and Professor Yingjie Chen, (Shenyang Pharmaceutical University). The air-dried roots (3 kg) were extracted repeatedly with 70% EtOH (30 l×3). The extracts were combined and evaporated to give the residue, which was then suspended in H_2O and extracted with $CHCl_3$ three times with the same volume of H_2O. The resulting H_2O layer was subjected to a D_{101} column and eluted with H_2O, 70% EtOH and 95% EtOH successively. The 70% EtOH eluate was collected and evaporated under vacuum to afford the crude saponin fraction (120 g). The crude saponin fraction (100 g) was chromatographed on a silica gel column (1.5 kg) and eluted with $CHCl_3$, followed by a gradient of $CHCl_3$–MeOH to 1 : 1 to give 10 fractions. Further separation of Fr. 3 was achieved by HPLC using 70% MeOH to give **3** (210 mg, ginsenoside Rh_1), **6** (100 mg, (20*R*)-ginsenoside Rh_1) and **5** (40 mg, ginsenoside F_1). Column chromatography of Fr. 4 on ODS eluting with 60% and 80% MeOH gave 2 fractions (Fr. 4.1, 4.2). Fr. 4.1 was recrystallized in MeOH to give **4** (30 mg, ginsenoside Rg_2). Fr. 4.2 was separated by HPLC with 80% MeOH to give **1** (5 mg, ginsenoside Rg_8) and **2** (8 mg, (20*E*)-ginsenoside-F_4).

Ginsenoside Rg_8 (**1**): Amorphous solid. $[\alpha]_D^{25}$ +1.9° (c=0.4, MeOH). IR (KBr) ν_{max}: 3421, 2928, 1633, 1382, 1049 cm^{-1}. ^{1}H- (500 MHz, pyridine-d_5)

and ^{13}C-NMR (125 MHz, pyridine-d_5): see Table 1. FAB-MS (positive) *m/z* 821 $[M+Na]^+$. HR-FAB-MS (positive) *m/z* 821.4695 $[M+Na]^+$ (Calcd for $C_{42}H_{70}O_{14}Na$, 821.4663).

Enzymatic Hydrolysis of 1 A solution of **1** (2.5 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (Sigma Chemical Co., 2 units) and stirred at 40 °C for 12 h. The reaction mixture was extracted with $CHCl_3$ (1 ml×3). The $CHCl_3$ extract (1.4 mg) was purified by RP-HPLC with 40% CH_3CN to give an artificial genin **1a** (0.8 mg). ESI-MS (positive): 531 $[M+Na]^+$. ^{1}H-NMR (500 MHz, pyridine-d_5): see Table 1. The aqueous layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (1 ml), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.2 ml) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 ml each) and the hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY™-1 (30 m×0.25 mm×0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas; detection in EI mode, ionization potential, 70 eV; ion-source temperature, 280 °C. D-Glucose and L-rhamnose were confirmed by comparison of the retention times of their derivatives with those of D-glucose, L-glucose and L-rhamnose derivatives prepared in a similar way.

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