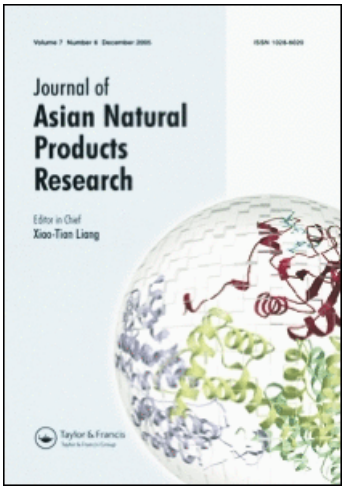

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TRITERPENOID SAPONINS FROM LEAVES AND STEMS OF *PANAX QUINQUEFOLIUM* L.

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In the chemical investigation on the saponin composition of leaves and stems of *Panax quinquefolium* L., two new minor dammarane saponins, quinquenoside L_1 (**1**) and L_2 (**2**) have been isolated. By means of physico-chemical evidences and spectral analysis their structures were established as 3-O-[β-*D*-glucopyranosyl-(1-2)-β-*D*-glucopyranosyl]-20-O-β-*D*-glucopyranosyl-dammara-23,25-diene-3β, 12β, 20(S)-triol (**1**) and 3-O-[β-*D*-glucopyranosyl-(1-2)-β-*D*-glucopyranosyl]-20-O-β-*D*-glucopyranosyl-(24Z)-dammar-24-ene-3β, 12β, 20(S), 26-tetraol (**2**).

Keywords: *Panax quinquefolium* L.; *Araliaceae*; Triterpenoid saponin; Quinquenoside L_1 and L_2

INTRODUCTION

American ginseng (roots of *Panax quinquefolium* L.) is well known for its tonic value worldwide; leaves and stems of *Panax quinquefolium* L. also show similar medical effects in recent research. Chemical investigations on the latter have been reported by us [1, 2]. In a continuation of investigations on saponin composition of leaves and stems of *Panax quinquefolium* L. we

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report here the isolation and structural elucidation of two new minor saponins, quinquenoside L_1 (**1**) and L_2 (**2**).

RESULTS AND DISCUSSION

Quinquenoside L_1 (**1**) and L_2 (**2**) were isolated by silica gel column chromatography and HPLC from the saponin fractions in yields of 0.00008% and 0.00003%, respectively.

Quinquenoside L_1 (1) was obtained as a white amorphous solid, and a quasimolecular ion peak at *m/z* 967.5248 (M+Na) in the HR-MS allowed its molecular formula to be $C_{48}H_{80}O_{18}$. The IR spectrum showed absorption at 3428 (OH) and 1652 (C=C) cm^{-1}. **1** showed a close resemblance with ginsenoside Rd [3] in their ^{13}CNMR spectra (75.4 MHz, C_5D_5N). The only difference between the two saponins was observed in the side-chain. Ginsenoside Rd (**3**) with a common ginsenoside side-chain, showed resonances at δ126.01 and δ131.02 for C-24 and C-25, respectively, while **1** had four olefenic carbon signals at δ142.30, 135.37, 127.19 and 114.65. A maximum absorption peak at 230.8 nm in the UV spectrum of **1**, indicated the presence of two conjugated double-bonds. In the HMQC spectrum of **1**, two geminal olefenic proton signals at δ5.01 (*d*, J = 2.0 Hz) and δ4.95 (*d*, J = 2.0 Hz) correlated with the carbon signals at δ114.65, and the proton signals at δ6.06 (*ddd*, J = 15.6, 8.4, 6.0 Hz) and δ6.37 (*d*, J = 15.6 Hz) correlated with the carbon signals at δ127.19 and δ135.37. In the ^{1}H-^{1}H COSY spectrum, the two geminal olefin proton signals at δ5.01 and δ4.95 showed long-range correlation with a methyl proton signal at δ1.91 (*s*), and the proton signal at δ6.06 correlated not only with δ6.37 but also with two geminal proton signals at δ3.07 (*dd*, J = 14.1, 6.0 Hz) and δ2.83 (*dd*, J = 14.1, 8.4 Hz).

The two geminal proton signals at δ3.07 and δ2.83 showed cross-peaks with a carbon signal at δ39.97 in the HMQC spectrum. Up to this point it could be concluded that the double bonds might be located at C-23/C-24 and C-25/C-26. This was confirmed by HMBC experiment (Fig. 1). H-26 showed long-range correlation with a methyl group assignable to C-27, and H-24 showed long-range correlation with two olefin carbon signals of C-22 and C-26. By comprehensive analyses of all 2D-NMR spectra, the carbon and proton signals of **1** were unequivocally assigned as shown in Tables I and II. The structure of quinquenoside L_1 (**1**) was thus formulated as 3-O-[β-*D*-glucopyranosyl-(1-2)-β-*D*-glucopyranosyl]-20-O-β-*D*-glucopyranosyl-dammara-23,25-diene-3β,12β,20(S)-triol.

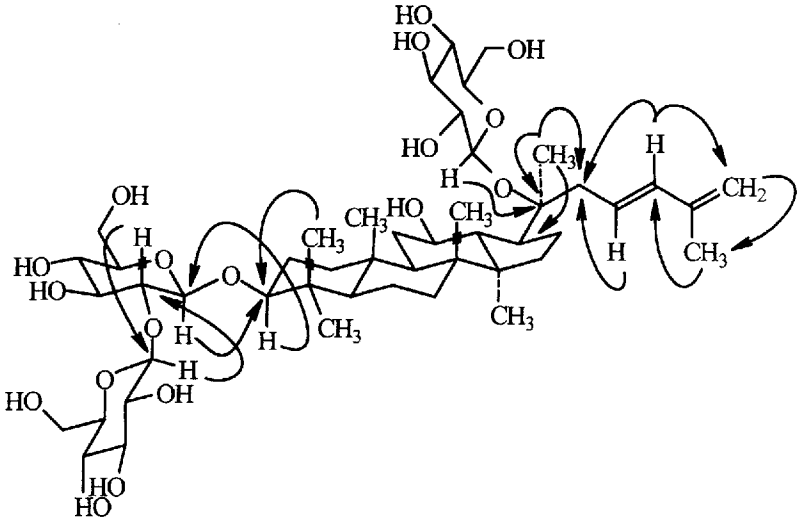

FIGURE 1 HMBC correlations for quinquenoside L_1 (**1**).

TABLE I NMR data for the aglycon parts of quinquenoside L_1 (**1**)

Carbon		*Correlated proton*		^1H-^1H
No.	*δ C*	*One-bond*[1]	*Long-range*[2]	*COSY*[3]
1	39.89	1.51 (1e), 0.76 (1a)	19	1e, 2a
2	26.28	2.19 (*dd*, $J = 12.6$, 2 Hz, 2e), 1.74 (2a)		1a, 3, 2e
3	88.69	3.26 (*dd*, $J = 11.4$, 4.2 Hz)	3-glc-1′	2a, 2e
4	38.43		28, 29	
5	56.11	0.66 (*d*, $J = 10.5$ Hz)	28, 29, 19	6a
6	18.16	1.49 (6e), 1.38 (6a)		5, 7a
7	34.78	1.45 (7e), 1.17 (7a)	18	6e, 6a
8	39.74		18, 30	
9	49.80	1.35	18, 19	11e
10	36.62		19	
11	30.72	2.02 (11e), 1.49 (11a)		9, 13, 12
12	70.22	3.95		11a, 13
13	49.21	1.99 (*t*)	30	11, 12, 17
14	51.27		18, 30	
15	30.34	1.49 (15e), 0.94 (15a)	30	15, 16
16	26.47	1.81 (16e), 1.45 (16a)	17	16, 17, 15a
17	51.92	2.37 (*m*)	21	13, 16
18	15.56	0.97 (*s*)	7	
19	16.03	0.83 (*s*)		
20	83.16		22, 17, 20-1‴	
21	23.19	1.58 (*s*)	22	
22	39.97	3.07 (*dd*, $J = 14.1$, 6.0 Hz)		23
		2.83 (*dd*, $J = 14.1$, 8.4 Hz)		
23	127.19	6.06 (*ddd*, $J = 15.6$, 8.4, 6.0 Hz)	22	22, 24
24	135.37	6.37 (*d*, $J = 15.6$ Hz)	26, 22	23

TABLE I (Continued)

Carbon		*Correlated proton*		^1H-^1H
No.	δ *C*	*One-bond*[1]	*Long-range*[2]	*COSY*[3]
25	142.30		23, 27	
26	114.65	5.01 (*d*, $J=2$ Hz), 4.95 (*d*, $J=2$ Hz)	27	27
27	18.65	1.91 (*s*)	26, 24	26
28	27.85	1.28 (*s*)	29, 3	
29	16.33	1.11 (*s*)	28, 5	
30	16.78	0.86 (*s*)		

[1] According to the HMQC spectrum (C_5D_5N), chemical shifts.
[2] According to the HMBC spectrum (C_5D_5N), numbers of correlated protons.
[3] Numbers of correlated protons.

TABLE II NMR Data for the sugar moieties of quinquenoside L_1 (**1**)

Carbon		*Correlated proton*		^1H-^1H
No.	δ *C*	*One-bond*[1]	*Long-range*[2]	*COSY*[3]
3-glc-1′	104.85	4.94 (*d*, J = 7.5 Hz)	3	3-glc-2′
2′	82.95	4.27	2′-glc-1″	3-glc-1′
3′	77.67[a]	4.35		
4′	71.37[b]	4.38		
5′	78.55[a]	3.91 ~ 3.96		
6′	62.43[c]	4.32 ~ 4.58		
2′-glc-1″	105.77	5.39 (*d*, J = 7.5 Hz)	3-glc-2′	2′-glc-2″
2″	76.87	4.16		2′-glc-1″
3″	77.99[a]	4.25		
4″	71.28[b]	4.14		
5″	77.84[a]	3.91 ~ 3.96		
6″	62.61[c]	4.32 ~ 4.58		
20-glc-1‴	98.08	5.19 (*d*, J = 7.8 Hz)	20	20-glc-2‴
2‴	75.02	4.02 (*t*, J = 7.8 Hz)		20-glc-1‴
3‴	78.22[a]	4.22		
4‴	71.37[b]	4.14		
5‴	78.07[a]	3.91 ~ 3.96		
6‴	62.61[c]	4.32 ~ 4.58		

[1] According to the HMQC spectrum (C_5D_5N), chemical shifts.
[2] According to the HMBC spectrum (C_5D_5N), numbers of correlated protons.
[3] Numbers of correlated protons.
[a,b,c] Assignments may be interchanged within the same column.

Quinquenoside L_2 (2) was also obtained as a white amorphous solid, m.p. 165 ~ 168°C (MeOH). **2** displayed a quasimolecular ion peak at m/z 985.5 (M + Na) in the LSI-MS. This information, together with the data from the ^{13}CNMR spectrum allowed its molecular formula to be assigned as $C_{48}H_{82}O_{19}$. **2** showed a close resemblance with ginsenoside Rd [3] in their ^{13}CNMR spectra (75.4 MHz, C_5D_5N). The only difference between

the two saponins was observed in the side-chain. The double-bond carbons resonating at δ136.21 and 127.81 in the ^{13}CNMR spectrum, along with its ^{1}HNMR data, indicated that saponin **2** had (24Z)-24-ene-26-hydroxyl moiety as actinostemnosides A,B,C,D [4]. In the ^{1}H-^{1}H COSY spectrum, the olefen proton signal at δ5.37 (1H, *t*, $J=7.0$ Hz, H-24) correlated not only with two geminal proton signals at δ2.41 and δ2.60 (H-23), but also with a methyl proton signal at δ1.92 (H-27). In the NOESY spectrum, NOE cross-peaks were observed between the olefenic proton signal at δ5.37 (H-24) and the methyl signal at δ1.92 (H-27), and between two geminal proton signals at δ2.41, 2.60 (H-23) and other two geminal proton signals at δ4.44 (H-26). Up to this point it could be concluded that the double-bond might be located between C-24 and C-25, and a hydroxyl group in C-26.

Acid hydrolysis of saponin **2** yielded *D*-glucose. The ^{1}H and ^{13}C NMR spectra demonstrated that **2** had three β-*D*-glucopyranosyl moieties. The chemical shifts of carbon signals assignable to the sugar moieties and the carbon C-3 and C-20 of **2** closely corresponded to those of ginsenoside Rd (Tab. III). The NOE cross-peaks were observed between H-3 (δ3.30) and an anomeric proton signal at δ4.91 (*d*, $J=7.2$ Hz, 3-glc-1′), and between the signal of H-21 at δ1.57 (3H, *s*) and an other anomeric proton signal at δ5.16 (*d*, $J=7.8$ Hz, 20-glc-1‴). The NOESY correlation was also observed between another anomeric proton signal at δ5.36 (*d*, $J=7.5$ Hz, 2′-glc-1″) and 3-glc-2′H signal at δ4.25 (Fig. 2). Thus, the structure of **2** was

TABLE III The ^{13}CNMR data of quinquenoside L_2 (**2**) and ginsenoside Rd

No.	*Q-L*$_2$	*Rd*	*No.*	*Q-L*$_2$	*Rd*	*No.*	*Q-L*$_2$	*Rd*
1	39.77	39.78	18	16.33	17.43	4′	71.79	71.74
2	26.82	26.72	19	16.63	16.68	5′	79.21	78.16
3	89.07	89.09	20	83.41	83.42	6′	62.94	62.81
4	39.26	39.27	21	22.89	22.52	2′-glc		
5	56.46	56.47	22	36.38	36.16	1″	106.04	106.02
6	18.50	18.52	23	22.50	23.34	2″	77.15	77.19
7	35.19	35.23	24	127.81	126.01	3″	78.43	79.31
8	40.08	40.11	25	136.21	131.02	4″	71.79	71.74
9	50.25	50.27	26	60.98	25.83	5″	78.43	78.04
10	36.96	36.98	27	21.85	16.04	6″	62.83	62.81
11	30.80	30.90	28	28.17	28.18	20-glc		
12	70.36	70.33	29	16.67	16.35	1‴	98.34	98.34
13	49.46	49.50	30	16.39	17.86	2‴	75.24	75.21
14	51.50	51.52	3-glc			3‴	78.33	78.31
15	30.94	30.90	1′	105.05	105.15	4‴	71.79	71.74
16	26.82	26.84	2′	83.41	83.42	5‴	78.16	78.16
17	51.91	51.81	3′	78.04	78.32	6‴	62.83	62.93

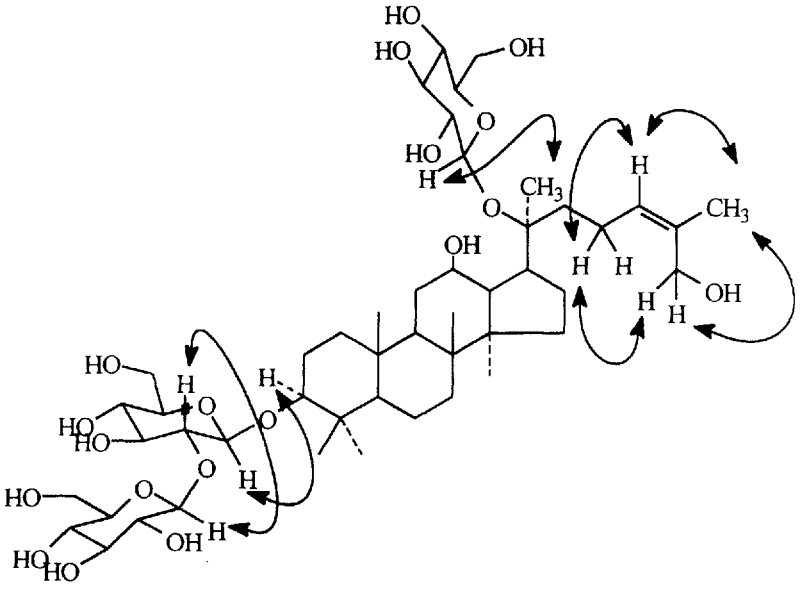

FIGURE 2 The NOESY correlations for quinquenoside L_2 (**2**).

established as 3-O-[β-*D*-glucopyranosyl-(1-2)-β-*D*-glucopyranosyl]-20-O-β-*D*-glucopyranosyl-(24Z)-dammar-24-ene-3β,12β, 20(S), 26-tetraol, named quinquenoside L_2.

EXPERIMENTAL SECTION

General Experimental Procedures

All melting points were determined on Yanaco MP-S3 Micro-hot stage and are uncorrected. HR-MS data were taken on a JEOL JMS-700T spectrometer, LSI-MS (Liquid Secondary Ion-MS) was taken on Concept LSI-MS. NMR spectra were taken in pyridine-d_5 on a Bruker ARX-300 spectrometer, 2D-NMR experiments were carried out with standard pulse sequences. For HPLC (Shimadzu-6A system), ODS column was used: solvent: 45% ~ 85% MeOH, flow rate: 2 ml min^{-1}; detection UV at 203 nm. For column chromatography, silica gel H (10 – 40 μ, Qingdao) and highly porous polymer D101 (Qingdao) were used. Hydrolysis of saponins with mineral acid and identification of the resulting sugar with TLC were performed as described by Zhao [5].

Plant Material

The leaves and stems of *Panax quinquefolium* L. were collected from Canada by Dalian Tianma Pharmacy Co. LTD.

Extraction and Isolation

Dried leaves and stems of *Panax quinquefolium* L. (2.0 Kg) were extracted with hot water (20 *l* × 3) and the water soluble fraction was extracted with $CHCl_3$ and *n*-BuOH. The *n*-BuOH fraction was subjected to column chromatography on reversed-phase highly porous polymer, column D101 (2.0 Kg), eluted with H_2O (40 *l*) and 95% EtOH (40 *l*), affording a H_2O fraction and an EtOH fraction (312 g). A part of the EtOH fraction (100 g) was chromatographed over silica gel {gradient elution with $CHCl_3$ – MeOH [100 : 1 (I); 100 : 2 (II); 100 : 8 (III); 100 : 9 (IV); 100 : 12 (V); 100 : 15 (VI, VII); 100 : 18 (VIII); 100 : 20 (IX); 100 : 30 (X); 100 : 40 (XI)]} to provide eleven fractions in increasing order of polarity.

Fraction VIII was separated into seven fractions, frs. VIII *a* – *h*, by HPLC (ODS, 75% MeOH). From frs. VIII *f*, saponin I was obtained as white amorphous solid (0.00008% yield). Fraction VIII *b* was further purified by HPLC (ODS, 65% MeOH) to afford saponin 2 (0.00003% yield).

Quinquenoside L_1(1), m.p. 208 ~ 210°C (MeOH), IR (KBr) cm^{-1}: 3428, 2933, 1652, 1635, 1080, 571. Liebermann – Burchard and Molish reactions were positive. LR-MS: 967 (M + Na), HR-MS: 967.5248 ($C_{48}H_{80}O_{18}Na$, cal cd. 967.5242), ^{1}H-NMR (300 MHz, C_5D_5N): δ6.06 (1H, *dt*, J = 15.3, 6.0 Hz), 6.37 (1H, *d*, J = 15.3 Hz), 5.01 (1H, br.s), 4.94 (1H, br.s), 4.94 (1H, *d*, J = 7.5 Hz, 3-glc-1′), 5.39 (1H, *d*, J = 7.5 Hz, 2′-glc-1″), 5.19 (1H, *d*, J = 7.5 Hz, 20-glc-1‴), 1.91, 1.58, 1.28, 1.11, 0.97, 0.86, 0.83 (each 3H, *s*). ^{13}C-NMR (75.4 MHz, C_5D_5N) data see Tables I and II.

Quinquenoside L_2 (19), m.p. 165 ~ 168°C (MeOH), Liebermann – Burchard and Molish reactions were positive. LSI-MS: 985.5 (M + Na), 603.6 (M-2glc-2H_2O), ^{1}H-NMR (300 MHz, C_5D_5N): δ5.37 (1H, *t*, J = 7.0 Hz), 4.91 (1H, *d*, J = 7.2 Hz, 3-glc-1′), 5.36 (1H, *d*, J = 7.5 Hz, 2′-glc-1″), 5.16 (1H, *d*, J = 7.8 Hz, 20-glc-1‴), 3.30 (1H, *m*, 3-H), 1.92, 1.57, 1.26, 1.09, 0.91, 0.91, 0.76 (each 3H, *s*). ^{13}C-NMR (75.4 MHz, C_5D_5N) data see Table III.

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