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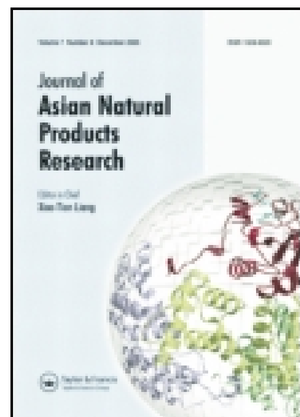

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Two new dammarane-type triterpene saponins from red American ginseng

Jin-Ping Liu [a], Dan Lu [a] & Ping-Ya Li [a]
[a] Institute of Frontier Medical Science, Jilin University, Changchun, 130021, China

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Two new dammarane-type triterpene saponins from red American ginseng

Jin-Ping Liu, Dan Lu and Ping-Ya Li*

Institute of Frontier Medical Science, Jilin University, Changchun 130021, China

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Two new dammarane-type triterpene saponins were isolated from the red American ginseng. The new saponins were named as pseudoginsenoside G_1 (**1**) and pseudoginsenoside G_2 (**2**). Their structures were elucidated by the combined analysis of NMR and mass spectrometry as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*R*-epoxy-3β,25-diol (pseudoginsenoside G_1) (**1**) and 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*S*-epoxy-3β,25-diol (pseudoginsenoside G_2) (**2**).

Keywords: red American ginseng; pseudoginsenoside G_1; pseudoginsenoside G_2

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1. Introduction

In some countries, ginseng (*Panax ginseng* C. A. Meyer) root is air dried into white ginseng or steamed at 100°C to red ginseng [1]. Compared with Asian white ginseng, steamed ginseng has stronger anticancer activities [2,3]. American ginseng (*Panax quinquefolium* L.) has the benefiting and nourishing effect [4]. It was reported that the possessing of red American ginseng was as follows: selecting, brushing, drying in the sun, steaming at 100°C, shearing, and drying naturally. The steamed American ginseng has a stiff texture, which can not only protect the effective ingredients but also produce new constituents [5]. Recently, there have been reports that the red American ginseng had antiproliferative activities [6] and protective effect on V79-4 cells induced by oxidative stress [7]. In this paper, two new dammarane-type saponins from the steamed American ginseng (*Panax quinquefolius* L.) were reported. This paper describes the isolation and the structural elucidation of the new constituents, pseudoginsenoside G_1 (**1**) and pseudoginsenoside G_2 (**2**), by the chemical and spectroscopic methods (1D and 2D NMR, MS). The structure of **1** was determined as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*R*-epoxy-3β,25-diol, and the structure of **2** was determined as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*S*-epoxy-3β,25-diol. Compound **2** was characterized as a C_{24}-epimer of compound **1**.

2. Results and discussion

Repeated column chromatography (CC) of the EtOH extract of the red *P. quinquefolium* L. led to the isolation of new dammarane-type glycosides **1** and **2** (Figure 1).

Compound **1** was obtained as a white amorphous solid (MeOH). The molecular formula was determined as $C_{42}H_{70}O_{14}$ by NMR spectra and HR-ESI-MS at *m*/*z*

*Corresponding author. Email: lipy@jlu.edu.cn

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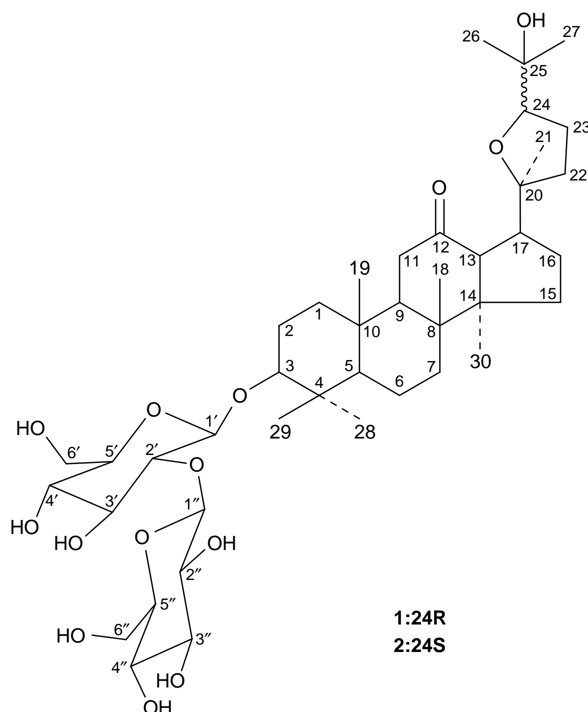

Figure 1. The structures of compounds **1** and **2**.

797.4705 $[M - H]^-$. Acid hydrolysis of **1** yielded D-glucose. The ^{1}H NMR spectrum of **1** displayed eight methyl signals at δ 1.40 (3H, s), 1.37 (3H, s), 1.23 (3H, s), 1.20 (3H, s), 1.10 (3H, s), 1.06 (3H, s), 0.80 (3H, s), and 0.76 (3H, s). The sugars were determined as two β-D-glucopyranosyl moieties by the protons at δ 4.89 (1H, d, $J = 7.5$ Hz), 5.35 (1H, d, $J = 7.5$ Hz) and two anomeric carbon signals at δ 106.0 and δ 105.1, respectively. Compared with neoalsoside G_1 [8], the ^{1}H NMR spectrum also displayed the disappearance of H-12 at δ 3.75 and the appearance of H-13 at δ 3.11 (1H, d, $J = 9.5$ Hz) and H-17 at δ 2.73 (1H, m) in compound **1**. The ^{13}C NMR spectrum of **1** revealed 42 signals (Table 1). Except for the signals of sugar units, the aglycone of compound **1** revealed nine methylenes, six methines [two of them bearing an oxygen atom (δ 88.6 and 84.7)], six quaternary [two of them bearing an oxygen atoms (δ 85.4 and 71.2)] and eight methyl carbons and one carbonyl (δ 210.7). The chemical shifts of **1** showed resemblance with those of chikusetsusaponin LT_8 [9] except for the signals of the side chain. Furthermore, compared with neoalsoside G_1 [8], whose aglycone is dammar-12-one-20*S*,24*R*-epoxy-3β,25-diol, a 20*S*,24*R*-epoxy group was deduced to exist in compound **1**. In the HMBC spectrum of **1**, the correlations between H-11, H-13, and H-17 with C-12 were observed. Combined with ^{1}H NMR spectrum, the location of the carbonyl was determined to be at C-12. HMQC and HMBC experiments also showed correlations between H-21 with C-17, C-20, and C-22; H-17 with C-12, C-13, C-20, C-21, and C-22; H-13 with C-12, C-14, C-17, C-20, and C-30.

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** (500 and 125.8 MHz, pyridine-d_5).

No.	δ_C	δ_H (J in Hz)	HMBC ($^1H \rightarrow {}^{13}C$)
1	38.7	1.20 (m), 0.63 (overlapped)	C-5, 19
2	26.6	2.14 (1H, m), 1.75 (1H, m)	
3	88.6	3.22 (1H, dd, $J = 11.5, 4.5$ Hz)	C-4, 28, 29, 1′
4	39.7		
5	56.1	0.63 (overlapped)	C-4, 6, 7
6	18.5	1.44 (1H, m), 1.34 (1H, m)	
7	34.6	1.35 (1H, m), 1.25 (1H, m)	C-5, 9, 14
8	40.6		
9	54.6	1.62 (1H, dd, $J = 13.5, 4.0$ Hz)	C-5, 8, 10, 14, 18, 19
10	37.4		
11	39.9	2.25 (1H, m), 2.20 (1H, m)	C-8, 9, 12, 13
12	210.7		
13	57.3	3.11 (1H, d, $J = 9.5$ Hz)	C-12, 14, 17, 20, 30
14	55.9		
15	32.3	1.73 (1H, m), 1.08 (1H, m)	C-13, 17, 30
16	27.0	2.01 (1H, m), 1.91 (1H, m)	C-20
17	43.1	2.73 (1H, m)	C-12, 13, 20, 21, 22
18	15.6	1.10 (3H, s)	C-7, 8, 9, 14
19	16.1	0.76 (3H, s)	C-1, 9, 10
20	85.4		
21	25.2	1.20 (3H, s)	C-17, 20, 22
22	35.8	1.91 (1H, m), 1.53 (1H, m)	C-17, 20, 21, 24
23	25.1	1.80 (1H, m), 1.22 (overlapped)	C-20, 22
24	84.7	3.94 (overlapped)	C-26, 27
25	71.2		
26	26.4	1.40 (3H, s)	C-24, 25, 27
27	27.0	1.37 (3H, s)	C-24, 25, 26
28	28.0	1.23 (3H, s)	C-3, 4, 5, 29
29	16.5	1.06 (3H, s)	C-3, 4, 5, 28
30	16.8	0.80 (3H, s)	C-8, 13, 14, 15
3-glc-1′	105.1	4.89 (1H, d, $J = 7.5$ Hz)	C-3, 3′
2′	83.4	4.23 (overlapped)	C-1′, 3′,4′
3′	77.9	4.22 (overlapped)	C- 4′, 5′
4′	71.7	4.12 (overlapped)	C-5′, 3′, 6′
5′	78.0	3.90 (overlapped)	C-3′, 4′, 6′
6′	62.8	4.57 (1H, m), 4.34 (overlapped)	C-5′
2′-glc-1″	106.0	5.35 (1H, d, $J = 7.5$ Hz)	C-2′, 2″
2″	77.1	4.10 (overlapped)	C-1″, 3″
3″	78.3	4.30 (overlapped)	C-2″, 4″
4″	71.6	4.31 (overlapped)	C-3″, 6″
5″	78.2	3.91 (overlapped)	C-3″, 4″, 6″
6″	62.7	4.47 (overlapped), 4.44 (overlapped)	C-4″, 5″

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The location of the β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl unit was determined to be at C-3 of the aglycone by 2D NMR spectra (Figure 2). By the analysis of 2D NMR spectra, the proton and carbon signals of **1** were assigned as shown in Table 1. The configuration at C-24 was assigned on the basis of ^{13}C NMR spectrum. It could be summarized that in the ^{13}C NMR spectrum of ocotillol-type triterpenes having dammar-20, 24-epoxy-12-one group, the major difference between the 20*S*,24*R* and 20*S* was 24*S*-epimers observed in the chemical

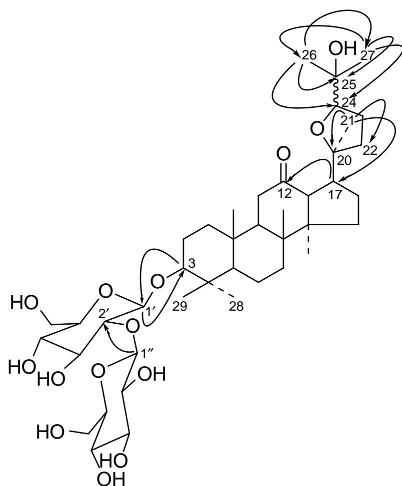

Figure 2. Key HMBC correlations of glycosides **1** and **2**.

shifts of C-24 and C-25. According to the literature, the difference of 20(*S*) and 20(*R*) in ocotillol-type saponin could be observed from the carbon signal of C-21 (*S*: δ 27; *R*: δ 20) [10]. In the ^{13}C NMR spectrum of compound **1**, the chemical shift of C-20 at δ 25.2 showed that the configuration of C-20 was *S*-form. In the case of dammar-20, 24-epoxy-12-one triterpene with C-20(*S*), C-24(*R*) configuration, C-24 appeared at δc 84.7, C-25 at δc 71.2 [8]. In the case of dammar-20, 24-epoxy-12-one triterpene with C-20(*S*), C-24(*S*) configuration, C-24 appeared at δc 87.6, C-25 at δc 70.3 [10]. The chemical shifts of C-24 and C-25 in compound **1** located at δ 84.7 and 71.2, respectively, and thus the configuration of C-24 was identified as *R*-form. The important and diagnostic NOEs observed in the NOE differential spectrum of **1** are illustrated in Figure 3. The β-configurations of C-3, C-17, C-19, and C-18 were affirmed by the NOEs between (i) H-3 and H-5, H-1, H-2, (ii) H-17 and H-21, H-30, (iii) H-19 and H-29 and (iv) H-18 and H-19 respectively. Furthermore, the *S*-configuration of C-21 and the *R*-configuration of C-24 were confirmed by observation of the NOE between (i) H-21 and H-17, (θ) H-24 and H-21.

Based on the above evidence, the structure of **1** could be characterized as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*R*-epoxy-3β,25-diol (Figure 1). Compound **1** is a minor glycoside in the red *P. quinquefolium* L.

Compound **2** was obtained as a white amorphous solid. The acid hydrolysis of **2** provided D-glucose. The molecular formula was determined as $C_{42}H_{70}O_{14}$ by NMR spectra and HR-ESI-MS at *m*/*z* 797.4682. The spectral data of compound

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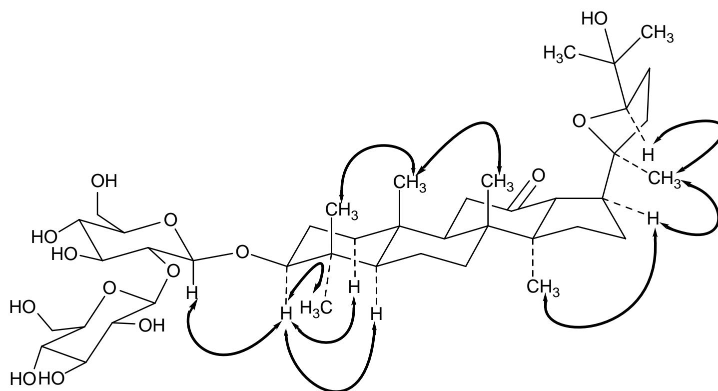

Figure 3. NOE correlations of glycoside **1**.

2 showed close resemblance with those of compound **1** except that the C-24 and C-25 chemical shifts of compound **2** appeared at δc 88.3 and 70.4, respectively, and so its C-24 configuration is *S*. By the analysis of 2D NMR spectra, the proton and carbon signals of **2** were assigned as shown in Table 2. Based on the NMR evidence, the structure of **2** could be characterized as 3-*O*-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-dammar-12-one-20*S*,24*S*-epoxy-3β,25-diol, the epimer of **1**. Compound **2** is also a minor glycoside in the red *P. quinquefolium* L.

Table 2. ^{1}H and ^{13}C NMR spectral data of compound **2** (500 and 125.8 MHz, pyridine-d_5).

No.	δ_C	δ_H (*J* in Hz)	HMBC ($^{1}H \rightarrow ^{13}C$)
1	38.8	1.21 (m), 0.64 (overlapped)	C-2, 5, 9
2	26.6	2.15 (1H, m), 1.76 (1H, m)	C-4, 10
3	88.6	3.22 (1H, dd, *J* = 11.5, 3.0 Hz)	C-4, 28, 29, 1′
4	39.7		
5	56.1	0.64 (overlapped)	C-4, 6, 7, 28, 29
6	18.5	1.48 (1H, m), 1.36 (overlapped)	C-8, 10
7	34.6	1.36 (overlapped), 1.27 (overlapped)	C-5, 14
8	40.6		
9	54.6	1.62 (1H, m)	C-5, 8, 10, 14, 18, 19
10	37.4		
11	39.9	2.19 (1H, m),1.64 (1H, m)	C-8, 9, 10
12	210.5		
13	57.5	3.07 (1H, d, *J* = 9.0 Hz)	C-8, 12, 14, 17, 20, 30
14	56.0		
15	32.2	1.74 (1H, m), 1.09 (overlapped)	C-14, 17, 30
16	26.8	2.01 (1H, m), 1.89 (1H, m)	C-20
17	43.3	2.70 (1H, m)	C-12, 13, 20
18	15.6	1.09 (3H, s)	C-7, 14
19	16.2	0.79 (3H, s)	C-1, 5, 9
20	85.5		
21	26.4	1.19 (3H, s)	C-17, 20, 22
22	37.0	1.97 (1H, m), 1.60 (1H, m)	C-17, 21, 24
23	25.2	1.73 (1H, m), 1.38 (1H, m)	C-20, 25
24	88.3	3.90 (overlapped)	C-26, 27
25	70.4		
26	26.8	1.39 (3H, s)	C-24, 25, 27
27	26.3	1.31 (3H, s)	C-24, 25, 26
28	28.0	1.24 (3H, s)	C-3, 4, 5, 29
29	16.5	1.08 (3H, s)	C-3, 4, 5, 28
30	16.8	0.82 (3H, s)	C-8, 14, 15
3-glc-1′	105.1	4.89 (1H, d, *J* = 6.5 Hz)	C-3, 3′
2′	83.4	4.23 (overlapped)	C-1′, 3′, 4′
3′	78.0	4.22 (overlapped)	C-4′, 5′
4′	71.7	4.11 (overlapped)	C-5′, 3′, 6′
5′	78.2	3.90 (overlapped)	C-3′, 4′, 6′
6′	62.9	4.57 (1H, m), 4.34 (overlapped)	–
2′-glc-1″	106.0	5.35 (1H, d, *J* = 6.0 Hz)	C-2′, 2″
2″	77.1	4.10 (overlapped)	C-1″, 3″
3″	78.4	4.30 (overlapped)	C-2″, 4″
4″	71.6	4.31 (overlapped)	C-3″, 6″
5″	78.3	3.91 (overlapped)	C-3″, 4″, 6″
6″	62.7	4.47 (overlapped), 4.45 (overlapped)	–

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3. Experimental

3.1 General experimental procedures

IR spectra were taken on an AVATAR 330 FT infrared spectrophotometer. NMR spectra were measured at 500 MHz for ^{1}H NMR, 125.8 MHz for ^{13}C NMR, and 500 MHz for HMBC and HMQC on a Bruker Avance-500 spectrometer (Karlsruhe, Germany). NMR spectra were measured in pyridine-d_5 using TMS as internal standard (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). HR-ESI-MS spectra were recorded using Ionspec 7.0 TFT-ICR-MS (IonSpec Corporation, Lake Forest, CA, USA). Chemical shifts (δ) are expressed in ppm. Preparative HPLC was carried out on a 2998 Photodiode Array Detector and SunFire Prep C18 Column (10 μm, 10 × 150 mm), 1525 BINARY HPLC PUMP (Waters). Silica gel H (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China) was used in CC. Also, silica gel G plates (Qingdao Marine Chemical Inc., Qingdao, China) were used in thin layer chromatography.

3.2 Plant material

The red American ginseng was provided by the Jilin TongBao Chinese Traditional Medicine Science & Technology Development Co., Ltd. A voucher specimen (No. 20080118) has been deposited at the Institute of Frontier Medical Science, Jilin University, China.

3.3 Extraction and isolation

The red Amercican ginseng (1.0 kg) was extracted with 80% EtOH (5 L × 3) and the EtOH-soluble fraction was concentrated. The residue (59 g) was subjected to macro-reticular absorption resin (D101) and eluted with H_2O (10 L) and 95% EtOH (10 L). The EtOH fraction (48 g) was then subjected to silica gel CC eluting with $CHCl_3$–MeOH mixture to give 138 fractions. Fractions 88–93 (56 mg) were combined and then subjected to preparative RP-HPLC with MeOH–H_2O (62:38) as mobile phase to obtain compounds **1** (11 mg, 0.001%) and **2** (9 mg, 0.001%).

3.3.1 Compound 1 (11 mg)

White amorphous power (MeOH); IR (KBr)ν_{max}: 3400, 2964, 1703, 1381, 1075 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125.8 MHz, pyridine-d_5) spectral data, see Table 1. Negative HR-ESI-MS: *m/z* 797. 4705 [M − H]$^-$ (calcd for $C_{42}H_{69}O_{14}$, 797.4693).

3.3.2 Compound 2 (9 mg)

White amorphous power (MeOH); IR (KBr) ν_{max}: 3401, 2964, 1704, 1380, 1075 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125.8 MHz, pyridine-d_5) spectral data, see Table 2. Negative HR-ESI-MS: *m/z* 797.4682 [M − H]$^-$ (calcd for $C_{42}H_{69}O_{14}$, 797.4693).

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