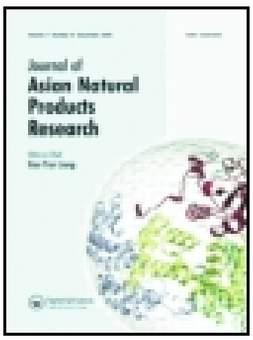

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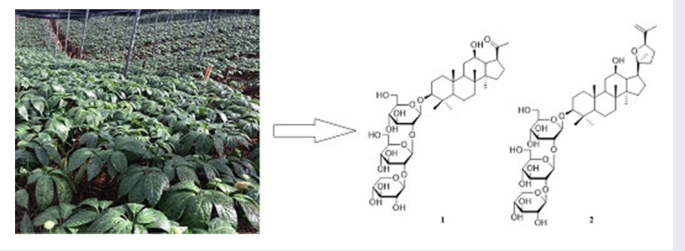
Two new dammarane-type triterpenoids from the stems and leaves of *Panax notoginseng*

Chong-Kang Yang[a,b,c], Jiao Xiong[a,b,c] and Yong Shen[a,b,c]

[a]College of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, China; [b]Research & Development center for Functional products, Yunnan Agricultural University, Kunming 650201, China; [c]Kunming Kangren Biotechnology CO., Ltd, Kunming 650033, China

ABSTRACT

Two new dammarane-type triterpenoids, notoginsenoside SY1 (**1**) and notoginsenoside SY2 (**2**), were isolated from the stems and leaves of *Panax notoginseng*. Their structures were elucidated to be 3β, 12β-dihydroxy-22, 23, 24, 25, 26, 27-hexanordammarane-20-one 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**1**), 3β, 12β-dihydroxy-20*S*, 24 *R*-epoxydammar-25-ene 3-*O*-β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranoside (**2**) by IR, HRESIMS and NMR experiments.



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Panax notoginseng; dammarane-type triterpenes; notoginsenoside SY1; notoginsenoside SY2

1. Introduction

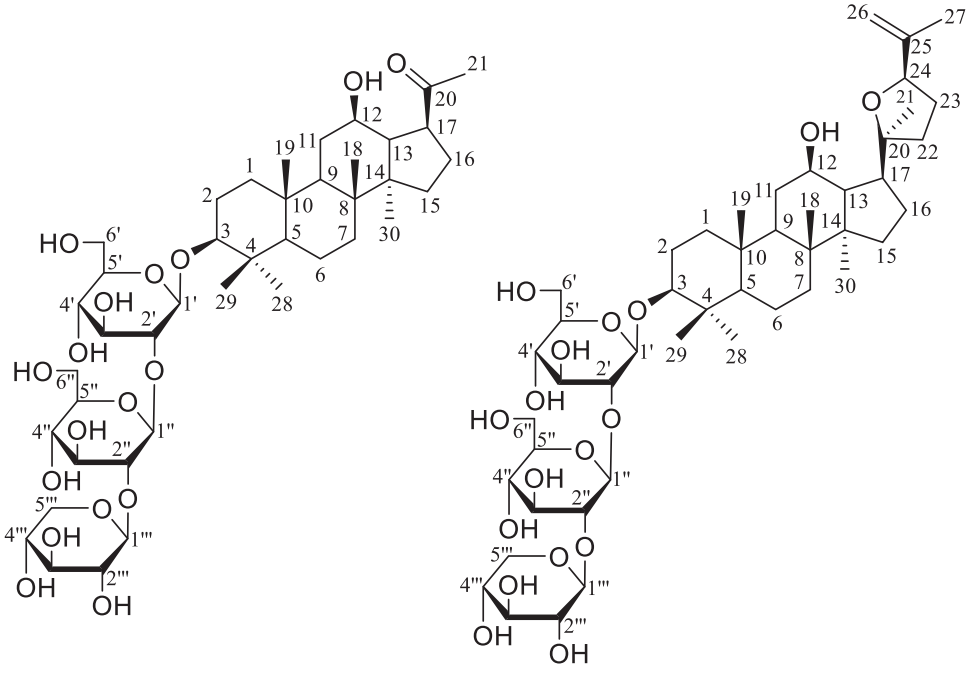
Panax notoginseng (Burk.) F. H. Chen, a traditional Chinese medicine known as "Sanqi" in Chinese, is mainly distributed in Yunnan and Guangxi Provinces in China [1]. The stems and leaves of *P. notoginseng* were traditionally used to treat bone fractures, eliminate swelling, and stop bleeding [2]. Previous phytochemical investigations on the stems and leaves of this plant revealed that protopanaxadiol type saponins, such as ginsenosides Rb_1, Rc, Rb_2, and Rb_3, were the primary constituents, which had been found to possess some beneficial pharmacological effects with anti-oxidative, antihyperlipidemic and hepatoprotective activities [3–6]. Their total saponins and ginsenoside Rb_1 are clinically employed for the treatment of various insomnia and forgetfulness in China [7]. To find more biologically active substances, the stems and leaves of *P. notoginseng* were phytochemically investigated to afford two new

CONTACT Jiao Xiong 543499252@qq.com; Yong Shen pharmsy@sina.com College of Agriculture and Biotechnology, Yunnan Agricultural University, Kunming 650201, China

saponins, notoginsenoside SY1 (**1**) and notoginsenoside SY2 (**2**) (Figure 1). This paper described their isolation and structural elucidation.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, and its molecular formula was assigned as $C_{41}H_{68}O_{17}$ based on the HRESIMS ion peak at *m/z* 855.4348 $[M+Na]^+$. The IR spectrum showed absorption bands for hydroxyl (3413 cm^{-1}) and carbonyl (1697 cm^{-1}) groups. Compound **1** revealed the presence of 41 resonances in the ^{13}C NMR (DEPT) spectrum (Table 1) attributing to six methyls, ten methylenes, twenty methines and five quaternary C-atoms, including three anomeric carbons (δ_C 103.2, 105.2, and 106.6) of sugar moieties and one carbonyl carbon (δ_C 217.1). The ^1H NMR spectrum data (Table 1) of **1** displayed six singlet methyls [δ_H 0.86, 0.90, 0.92, 1.05, 1.06, and 2.22 (each 3 H, s)] and three anomeric protons at δ_H 4.42 (1 H, d, $J=7.5$ Hz, H-1′), 4.78 (d, $J=7.7$ Hz, H-1″), 4.52 (d, $J=7.4$ Hz, H-1‴). The large coupling constants of the anomeric protons were compatible with the β-configuration for three sugar moieties [8], which were determined to be D configurations based on acidic hydrolysis experiment. The ^1H and ^{13}C NMR spectral data (Table 1) of compound **1** were identical to those of ginsenoside R_{10} [9], except that **1** was shown to possess one more glucopyranosyl and xylopyranosyl units in sugar chain. In the HMBC spectrum of **1**, the long-range correlations between δ_H 4.42 (H-1′) and δ_C 91.5 (C-3), δ_H 4.78 (H-1″) and δ_C 81.5 (C-2′), δ_H 4.52 (H-1‴) and δ_C 85.1 (C-2″) revealed the



1 **2**

Figure 1. Structures of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C NMR (500 MHz, 125 MHz in MeOD) spectral data for compounds **1** and **2**.

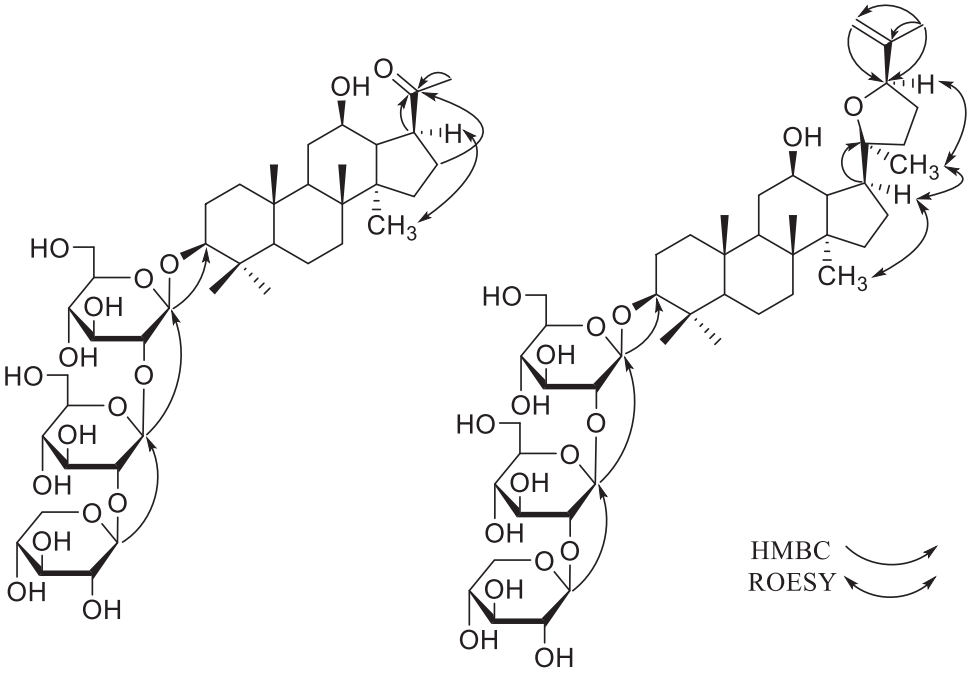
	1		2	
Position	δ_C	δ_H	δ_C	δ_H
1	40.4	1.04-1.06 m, 1.73*	40.3	1.00-1.04 m, 1.70*
2	27.3	1.75*, 1.98*	27.2	1.72*, 1.98*
3	91.5	3.20-3.23 m	91.5	3.18-3.20 m
4	40.7		40.7	
5	57.5	0.80 d (11.7)	57.6	0.79 d (11.6)
6	19.3	1.28-1.30 m, 1.50*	19.3	1.49*, 1.57*
7	36.4	1.33-1.36 m, 1.51*	35.9	1.29-1.32 m, 1.49*
8	41.1		41.0	
9	52.0	1.47*	51.5	1.48*
10	38.1		37.9	
11	32.9	1.46*, 1.79*	29.4	1.36-1.38 m, 1.86*
12	72.5	3.50*	71.8	3.46*
13	54.7	2.08*	51.1	1.65*
14	52.5		53.1	
15	33.6	1.19-1.23 m, 1.58*	33.3	1.10-1.12 m, 1.59*
16	28.5	1.60-1.62 m, 2.05*	27.1	1.16-1.19 m, 1.98*
17	53.6	2.91 td (10.7, 5.8)	50.0	2.18*
18	16.2	1.05 s	16.0	1.01 s
19	16.7	0.86 s	16.7	0.86 s
20	217.1		87.5	
21	30.4	2.22 s	27.2	1.30 s
22			32.2	1.74*, 1.88*
23			30.1	1.77*, 2.11-2.13 m
24			82.1	4.44 d (7.4)
25			144.8	
26			113.5	4.87 s, 4.97 s
27			19.1	1.78 s
28	28.3	1.06 s	28.2	1.06 s
29	17.0	0.90 s	16.9	0.91 s
30	17.2	0.92 s	18.3	0.92 s
3-O-Glc				
1′	105.2	4.42 d (7.5)	105.2	4.43 d (7.5)
2′	81.5	3.52*	81.5	3.49*
3′	78.0	3.28*	78.0	3.25*
4′	70.8	3.52*	71.9	3.28*
5′	77.2	3.26*	77.2	3.29*
6′	63.3	3.65 dd (11.8, 5.3), 3.84*	63.3	3.64 dd (12.0, 5.3), 3.82*
Glc				
1"	103.2	4.78 d (7.7)	103.1	4.78 d (7.7)
2"	85.1	3.37*	85.1	3.34*
3"	78.2	3.28*	78.2	3.26*
4"	71.0	3.31*	71.0	3.33*
5"	77.7	3.55*	77.7	3.53*
6"	62.9	3.58*, 3.84*	62.8	3.56*, 3.82*
Xyl				
1"′	106.6	4.52 d (7.4)	106.6	4.52 d (7.5)
2"′	75.8	3.28*	75.8	3.25*
3′"	78.5	3.53*	78.5	3.50*
4′"	70.8	3.52*	70.8	3.53*
5′"	67.3	3.25*, 3.97 dd (11.5, 5.3)	67.3	3.23*, 3.95 dd (11.5, 5.3)

*Overlapped.

linkage sequence of sugar units (Figure 2). The α-orientation of H-17 was demonstrated by the ROESY correlations between H-17 (δ_H 2.91, 1 H, td, $J = 10.7$, 5.8 Hz) and H-30 (δ_H 0.91, 3 H, s) (Figure 2). Other key 2 D NMR correlations confirmed the structure of **1** as shown in Figure 2. Therefore, the structure of compound **1** was characterized as 3*β*, 12*β*-dihydroxy-22, 23, 24, 25, 26, 27-hexanordammarane-20-one

3-*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside, named notoginsenoside SY1.

Compound **2** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{47}H_{78}O_{17}$ based on the *m/z* 937.5131 $[M+Na]^+$. The IR spectrum displayed strong absorption bands at 3418 and 1637 cm^{-1} due to hydroxyl and olefinic carbonyl functional groups. In the ^{13}C NMR (DEPT) spectrum, compound **2** exhibited 47 resonances, bearing 7 methyls, 13 methylenes, 21 methines, and 6 quaternary C-atoms. The signals at δ_C 144.8 and 113.5 in the ^{13}C NMR spectrum showed the existence of two olefinic carbons. In the ^1H NMR spectrum (Table 1), seven singlet methyls [δ_H 0.86, 0.91, 0.92, 1.01, 1.06, 1.30, 1.78 (each 3 H, s)], a pair of olefinic protons [δ_H 4.87 (1 H, s) and δ_H 4.97 (1 H, s)], and three anomeric protons signals [(δ_H 4.43 (d, $J=7.5$ Hz), 4.78 (d, $J=7.7$ Hz), 4.52 (d, $J=7.5$ Hz)] were observed. The glucose and the xylose were determined to be *β* configuration via the coupling constants of their anomeric protons [8]. Acid hydrolysis of compound **2** provided D-glucose and D-xylose by GC. The ^1H and ^{13}C NMR spectra (Table 1) of compound **2** were identical with those of compound **1** except for the signals caused by the side chain part (C-20–C-27) in **2**, which were similar to those of 3*β*,6*α*,12*β*-trihydroxy-20*S*, 24 *R*-epoxydammar-25-ene [10]. This speculation was further confirmed by the following key HMBC correlations: from δ_H 2.18 (H-17) to δ_C 87.5 (C-20), from δ_H 1.30 (H-21) to δ_C 50.0 (C-17), δ_C 87.5 (C-20) and δ_C 32.2 (C-22), from δ_H 4.97 (H-26) to δ_C 82.1 (C-24) and δ_C 144.8 (C-25), and from δ_H 1.78 (H-27) to δ_C 82.1 (C-24), δ_C 144.8 (C-25) and δ_C 113.5 (C-26) (Figure 2). H-24 and CH_3-21 were



1 **2**

Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

elucidated to be α-oriented based on the ROESY correlations between H-24 (δ_H 4.44) and CH_3-21 (δ_H 1.30), CH_3-21 and H-17 (δ_H 2.18, α-orientation) (Figure 2). From the above data, the structure of compound **2** was established to be 3*β*, 12*β*-dihydroxy-20*S*, 24 *R*-epoxydammar-25-ene 3-*O*-*β*-D-xylopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside, named notoginsenoside SY2.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a Jasco model 1020 polarimeter (Horiba, Tokyo, Japan). IR (KBr discs, cm^{-1}) spectra were recorded on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA). 1 D and 2 D NMR were recorded on Bruker DRX-500 (Bruker, Bremerhaven, Germany). High resolution electrospray ionization mass spectrum (HRESIMS) were run on a VG Spec3000 spectrometer (VG, Manchester, UK) and Waters AutoSpec Premier P776 (Waters, Milford, MA). D101 macroreticular resin (Tianjin Haiguang Chemical Co., Ltd, Haiguang, Tianjing, China), silica gel (200–300 mesh, Qingdao Makall Chemical Company, Makall, Qingdao, China), and RP C18 silica gel (300–400 mesh, Merck KGaA, Merck, Darmstadt, Germany) were used in the open column chromatography. Gas chromatography (GC) was performed on the Agilent technologies 6890 N apparatus (Agilent, Santa Clara, USA). TLC was carried on the GF254 plates (Makall, Qingdao, China). Analytical-grade reagents of chloroform ($CHCl_3$), ethanol (EtOH), and methanol (MeOH) were purchased from Kemiou Pure Chemical Co. Ltd. (Kemiou Tianjin, China). Fractions were visualized by silica gel plates sprayed with 10% H_2SO_4 ethanol solution followed by heating.

3.2. Plant material

The air-dried stems and leaves of *Panax notoginseng* (Burk.) F. H. Chen were collected from WenShan County, Yunnan province, China, in August 2015, and authenticated by Prof. Qing-Er Yang from South China Botanical Garden, Chinese Academy of Sciences. The voucher specimen (No. YAU 2015-8-1) has been deposited in the College of Agriculture and Biotechnology, Yunnan Agricultural University.

3.3. Extraction and isolation

The air-dried stems and leaves of *P. notoginseng* (5 kg) were extracted with 70% EtOH for three times (1.5 h × 3) under reflux. After removal of the ethanol under reduced pressure, the ethanolic extract was subjected to D101 resin column, eluting with H_2O and 90% EtOH, successively. The EtOH eluent was combined and concentrated under reduced pressure to give the total saponin fraction (416 g), which was subjected to column chromatography (CC) on silica gel (2 kg, 200 − 300 mesh) eluted with a gradient $CHCl_3$-MeOH-H_2O (9:1:0.1→6.5:3.5:0.5) to provide five fractions (Frs.A − E). Fr.C (8 g) was carried out on silica gel CC ($CHCl_3$-MeOH-H_2O, 8.5:1.5:0.15) to give five fractions (Frs.C1 − C5). Fr.C3 (2.3 g) was repeatedly

chromatographed over silica gel ($CHCl_3$-MeOH-H_2O, 8.5:1.5:0.15), Rp-18 columns (MeOH-H_2O, 8:2) to yield **1** (16 mg). Fr.D (12 g) was subjected to a silica gel column and eluted with $CHCl_3$-MeOH-H_2O (8:2:0.2) to afford six fractions (Frs.D1 – D6). Fr.D2 (2.7 g) was applied to silica gel CC with an eluent of $CHCl_3$-MeOH-H_2O (8:2:0.2), and further purified through Rp-18 columns (MeOH-H_2O, 8.5:2.5) to obtain **2** (18 mg).

3.3.1. Notoginsenoside SY1 (1)

White amorphous powder; Libermann-Burchard and Molish reactions were positive; $[\alpha]_D^{22}$ +10.34 (*c* 0.16, MeOH); IRν_{max} 3413, 2943, 2879, 1697, 1631, 1389, 1360, 1173, 1075, 1044, and 895 cm^{-1}; ^1H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) spectral data are shown in Table 1; HRESIMS: *m/z* 855.4348 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{17}Na$, 855.4349).

3.3.2. Notoginsenoside SY2 (2)

White amorphous powder; Libermann-Burchard and Molish reactions were positive; $[\alpha]_D^{22}$ −1.75 (*c* 0.22, MeOH); IRν_{max} 3418, 2967, 2945, 2878, 1720, 1637, 1453, 1388, 1200, 1077, 1045, and 896 cm^{-1}; ^1H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) spectral data are shown in Table 1; HRESIMS: *m/z* 93s7.5131 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{17}Na$, 937.5131).

3.4. Acid hydrolysis

The acid hydrolysis of new compounds and sugar determination were based on Gu's method by GC analysis [11]. Compounds **1** and **2** (each 5 mg) were hydrolyzed in 2 M HCl (5 ml) at 65 °C for 6 h, respectively. The reaction mixture was extracted with $CHCl_3$ three times (3 × 5 ml). The aqueous layer was neutralized with 2 M NaOH and dried to produce a monosaccharide mixture. Then, a solution of the sugar mixture in pyridine (2 ml) was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h. After this, trimethylsilylimidazole (1.5 ml) was added to the reaction mixture in an ice – water bath and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, run on an Agilent 6890 N gas chromatograph, equipped with a 30 m × 0.32 mm HP-5 column and a H_2 flame ionization detector with the following conditions: column temperature, 200 – 260 °C; programmed increase, 10 °C/min; carrier gas, N_2 (1 ml/min); injector and detector temperature, 260 °C; injection volume, 1 μl; and split ratio 1/50. The retention times for the authentic samples (Sigma), after being treated in the similar manner, were 19.82 min (D-glucose) and 14.12 min (D-xylose). The peaks of the hydrolysate of two compounds were detected at t_R 19.82 and 14.12 min for D-glucose and D-xylose, respectively.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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