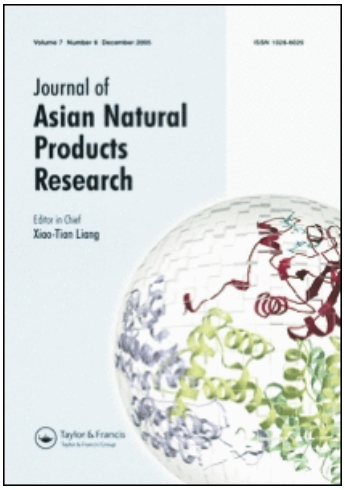

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Two new dammarane triterpene glycosides from the rhizomes of *Panax notoginseng*

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Two new dammarane triterpene glycosides named notoginsenosides Rw 1 (**1**) and Rw 2 (**2**) were isolated from the rhizomes of *Panax notoginseng,* together with 20 known compounds including protopanaxadiol (**3**), protopanaxatriol (**4**), ginsenosides Rb_1 (**5**), Rd (**6**), Re (**7**), Rg_1 (**8**), Rg_2 (**9**), 20-(*S*)-Rg_3 (**10**), 20-(*R*)-Rg_3 (**11**), Rh_1 (**12**), Rh_4 (**13**), Rf (**14**), 20-*O*-glucopyranosyl Rf (**15**), notoginsenosides R_1 (**16**), R_2 (**17**), T_5 (**18**), S (**19**), T (**20**), and Fa (**21**), and koryoginsenoside R_1 (**22**). Based on FABMS, HRFABMS, IR, ^{1}H, ^{13}C, and 2D-NMR (HSQC, HMBC, and COSY) spectral data, the structures of the new compounds were elucidated as 6-*O*-β-D-xylopyranosyl-20-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-dammar-24-ene-3β,6α,12β,20(*S*)tetraol (**1**) and 6-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyldammar-22-ene-(*trans*)-3β,6α,12β,20(*S*), 25-pentaol (**2**). Compounds **3**, **4**, **13**, and **22** were isolated from *P. notoginseng* for the first time.

Keywords: *Panax notoginseng*; dammarane; triterpene glycosides; notoginsenoside Rw 1; notoginsenoside Rw 2

1. Introduction

Panax notoginseng (Burk.) F.H. Chen, which belongs to the family Araliaceae, is a well-known traditional Chinese medicine. Its rhizomes and roots were reported to possess various therapeutic uses, such as promotion of blood circulation, removal of blood stasis, hemostasis, relieving swelling, alleviating pain, trauma, and bleeding caused by internal and external injuries [1]. More than 50 dammarane-type triterpenes have been isolated from the underground roots, rhizomes, seeds, and flowers [2–4]. As a part of our project, the rhizomes of *P. notoginseng* were phytochemically investigated to afford two new dammarane triterpenes, along with 20 known compounds. Based on spectral analyses including FABMS, HRFABMS, IR, ^{1}H, ^{13}C NMR, and 2D-NMR (HMQC, HMBC, and COSY), the structures of the two new compounds were identified as 6-*O*-β-D-xylopyranosyl-20-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyldammar-24-ene-3β,6α,12β,20(*S*)tetraol (**1**), and 6-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyldammar-22-ene-(*trans*)-3β,6α,12β,20(*S*), 25-pentaol (**2**). This paper deals with the isolation and structural elucidation of two new dammarane-type triterpenoid glycosides.

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2. Results and discussion

Compound **1** was obtained as white amorphous powder and positive to Liebermann–Burchard and Molish reactions. The IR spectrum exhibited absorptions ascribable to —OH (3440 cm^{-1}) and olefinic (1633 cm^{-1}) groups. The negative FABMS gave a quasi-molecular ion peak at *m/z* 901 $[M - H]^-$, in agreement with a molecular formula $C_{46}H_{78}O_{17}$ revealed by the negative HRFABMS experiment. After hydrolysis of compound **1** with 5% H_2SO_4, glucose, xylose, and protopanaxatriol were detected by TLC and PC comparison with the authentic samples. In the ^1H NMR spectrum of compound **1** (Table 1), eight methyl singlets and an olefinic proton signal at δ_H 5.30 (H-24) assignable to a protopanaxatriol-type aglycone, along with three anomeric proton signals at δ_H 5.09 (2H, d, $J = 7.6$ Hz) and 4.90 (1H, d, $J = 6.6$ Hz), were observed, suggesting the presence of three β-linked sugar moieties. The ^{13}C NMR spectrum of compound **1** exhibited 46 carbon signals, of which the anomeric carbons of three sugar moieties at δ_C 97.8, 104.8, and 106.7 were observed. The signals at δ_C 125.7 (C-24) and 130.9 (C-25) showed the existence of two olefinic carbon signals. The ^{13}C NMR spectral data for the aglycone part of compound **1** were similar to those of protopanaxatriol except that C-6 and C-20 in compound **1** were shifted downfield to δ_C 74.6 (C-6) and 83.2 (C-20), indicating that the sugar moieties were presented at C-6 and C-20, respectively. In the HMBC experiment, the long-range correlations between H-1″ and C-20, H-1‴ and C-6″, H-1′ and C-6 were displayed, establishing that the inner glucose was located at C-20, and xylose was linked to C-6″ of the inner glucose, while another xylose was at C-6. Thus, compound **1** was characterized as 6-*O*-β-D-xylopyranosyl-20-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyldammar-24-ene-3β,6α,12β,20(*S*)tetraol (**1**) (Figure 1).

Compound **2** was obtained as white amorphous powder and reacted positively to Liebermann–Burchard and Molish reactions. Its molecular formula was determined as $C_{41}H_{70}O_{14}$ by negative HRFABMS (*m/z* 785.4704 $[M - H]^-$). The IR spectrum of compound **2** showed absorptions for —OH groups at 3424 cm^{-1} and an olefin function at 1633 cm^{-1}. Acidic hydrolysis of compound **2** liberated glucose and xylose, which were identified by TLC and PC comparison with the authentic samples. In the ^1H NMR spectrum (Table 1), eight methyl singlets were observed, together with two olefinic protons at δ_H 6.29 (1H, m) and 6.10 (1H, d, $J = 15.8$ Hz) ascribable to a *trans*-disubstituted C=C bond. The two anomeric proton signals at δ_H 5.79 (1H, d, $J = 6.8$ Hz) and 4.98 (1H, d, $J = 7.2$ Hz) suggested the existence of two β-linked sugar moieties. The ^{13}C NMR spectrum of compound **2** indicated the presence of a disubstituted C=C bond at δ_C 127.1 (C-23, d) and 137.3 (C-22, d), and two sugar moieties at δ_C 103.2 (C-1′) and 104.6 (C-1″). Analysis of the NMR spectral data of compound **2** suggested that the structure of compound **2** was very similar to that of notoginsenoside [5] R_8, except that there was an additional xylopyranose unit in compound **2**. The HMBC experiment showed correlation between H-1″ (δ_H 5.79) and C-2′ (δ_C 79.8), thus the additional xylopyranose unit was attached at the C-2′ of the inner glucopyranose. The cross peak of H-1′ (δ_H 4.98) and C-6 (δ_C 79.2) indicated that the inner glucose was linked at C-6. The other HMBC correlations in Figure 2 confirmed the structure of compound **2**. Consequently, compound **2** was deduced as 6-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyldammar-22-ene-(*trans*)-3β,6α,12β,20(*S*),25-pentaol (**2**).

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XRC-1 apparatus and are uncorrected. Optical rotations were carried out on a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were recorded on a Bio-Rad

Table 1. ^{13}C NMR (100 MHz) spectral data of compounds **1** and **2** in C_5D_5N (δ in ppm, J in Hz).

	1	2		1	2
1	39.2 (t)	39.1 (t)	22	36.0 (t)	137.3 (d)
2	27.9 (t)	27.5 (t)	23	22.9 (t)	127.1 (d)
3	78.3 (d)	78.5 (d)	24	125.7 (d)	40.0 (t)
4	40.1 (s)	39.8 (s)	25	130.9 (s)	81.0 (s)
5	61.5 (d)	61.1 (d)	26	25.5 (q)	24.8 (q)
6	74.6 (d)	79.2 (d)	27	17.2 (q)	25.0 (q)
7	47.3 (t)	44.7 (t)	28	31.7 (q)	31.5 (q)
8	41.0 (s)	40.8 (s)	29	16.2 (q)	16.5 (q)
9	49.7 (d)	49.9 (d)	30	17.2 (q)	16.5 (q)
10	39.1 (s)	39.3 (s)	6-Glc		
11	30.6 (t)	31.9 (t)	1′	97.8	103.2
12	70.8 (d)	71.4 (d)	2′	73.6	79.8
13	49.0 (d)	48.2 (d)	3′	78.4	78.5
14	51.1 (s)	51.5 (s)	4′	71.7	70.8
15	30.5 (t)	30.9 (t)	5′	76.6	79.6
16	26.4 (t)	26.5 (t)	6′	69.6	62.6
17	51.3 (d)	53.8 (d)	Xyl		
18	17.4 (q)	17.1 (q)	1″	104.8	104.6
19	17.6 (q)	17.4 (q)	2″	75.6	75.6
20	83.2 (s)	73.0 (s)	3″	78.1	77.8
21	22.0 (q)	27.4 (q)	4″	69.9	71.4
			5″	66.4	67.0
			20-Xyl		
			1″	106.7	
			2″	75.2	
			3″	79.0	
			4″	70.5	
			5″	67.1	

FTS-135 spectrometer with KBr pellets, ν in reciprocal centimetre. 1D- and 2D-NMR experiments were performed on a Bruker-AM-400 (^1H and ^{13}C at 400 and 100 MHz, respectively) spectrometer, with TMS as internal reference, J in Hertz. Mass spectra were recorded on a VG-Auto-Spec-3000 instrument. Silica gel (200–300 mesh) for column chromatography was obtained from Qingdao Marine Chemical Factory, Qingdao, China; D_{101} macroreticular resins were obtained from Tianjing Pesticide Chemical Company (Tianjing, China); ODS, MCI gel CHP-20P (70–150 μm) was purchased from Mitsubishi Chemical Corporation, Tokyo, Japan; Lichrospher Rp-8 gel (40–63 μm) was purchased from Merck & Co. Inc., Germany. Detection was performed by silica gel TLC with 10% H_2SO_4 in EtOH sprayed, followed by heating.

3.2 Plant material

The rhizomes of *P. notoginseng* used in this experiment were collected in October 2002 in Wenshan prefecture, where they are cultivated on a large scale, Yunnan Province, China, and were identified as *P. notoginseng* (Burch.) F.H. Chen by Professor Luo-Shan Xu. A voucher specimen (No. 2002-10-18) is deposited in Wenshan Prefecture Sanqi Research Institute.

3.3 Extraction and isolation

The air-dried rhizomes (48 kg) were extracted three times with 80% and 40% ethanol for 2 h under reflux, respectively. The combined ethanolic extract was concentrated under vacuum to give a residue (18.6 kg). The residue, after suspending in 20 l of water, was submitted to a column chromatography (CC,

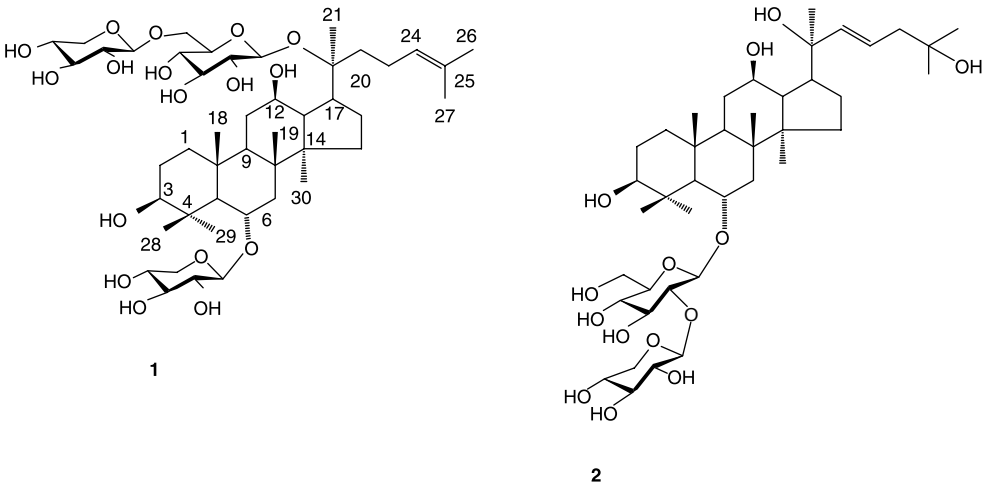

Figure 1. The structures of compounds **1** and **2**.

D_{101} macroreticular resins) and eluted with H_2O and 80% EtOH/H_2O successively. The 80% ethanolic eluent was concentrated *in vacuo* to supply powder (8.6 kg), which was chromatographed on silica gel column (40 kg, $CHCl_3$/MeOH/H_2O, 90:10:1 → 60:40:5) to give 10 fractions: A1 (500 g), A2 (230 g, mainly ginsenoside Rh4), A3 (339 g, a mixture of notoginsenoside Rx-1, ginsenoside Rh4, and Rh1), A4 (80 g, a mixture of notoginsenoside Rx-1 and ginsenoside Rh1), A5 (685 g, a mixture of ginsenoside Rg1, Rg2, Rg3, and notoginsenoside R2, and others), A6 (938 g), A7 (604 g, mainly notoginsenoside R1 and ginsenoside Re), A8 (609 g, mainly ginsenoside Rd), A9 (1630 g, mainly ginsenoside Rb1), and A10 (600 g).

Twenty grams of fraction A2 was subjected to CC (MCI gel, CHP-20P, MeOH/H_2O, 70:30 → 90:10) to provide four fractions: A2.1–5. Fraction A2.3 was submitted to CC (silica gel $CHCl_3$/MeOH, 90:10) and further purified by CC (Rp-8 gel, MeOH/H_2O, 85:15) successively to obtain compound **1** (120 mg). The fraction A4 (20 g) was carried on CC (MCI gel, CHP-20P, MeOH/H_2O, 70:30 → 90:10) to afford six fractions (Fractions A4.1–6). Fraction A4.3 was purified on CC (silica gel: $CHCl_3$/MeOH/H_2O, 90:10:1; Rp-18 gel: MeOH/H_2O, 80:20) successively to yield compound **2** (38 mg).

*3.3.1 6-O-β-D-xylopyranosyl-20-O-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-dammar-24-ene-3β,6α,12β,20(S)tetraol (**1**)*

White amorphous power, mp 205–208; $[\alpha]_D^{23.2}$ + 26.5 (*c* 0.4, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3440, 2929, 1633, 1078, and 1041; ^1H NMR (pyridine-d_5, 400 MHz) δ_H: 5.30 (1H, m, H-24), 5.09 (2H, d, J = 7.6 Hz, H-1″, H-1‴), 4.90 (1H, d, J = 6.6 Hz, H-1′), 4.38–4.40 (1H, m, H-6), 3.90 (1H, m, H-12), 3.48 (1H, m, H-3), 2.10 (3H, s, H-28), 1.97 (3H, s, H-28), 1.64 (3H, s, H-26), 1.62 (3H, s, H-21), 1.60 (3H, s, H-27), 1.44 (3H, s, H-29), 1.08 (3H, s, H-18), 0.99 (3H, s, H-19), and 0.97 (3H, s, H-30). ^{13}C NMR (pyridine-d_5, 100 MHz) spectral data, see Table 1. FABMS (−) *m/z*: 901 $[M - H]^-$(100), 769 $[M - H\text{-}132]^-$ (5); 637 $[M - H\text{-}2 \times 132]^-$ (5). HRFABMS (−): 901.5171 $[M - H]^-$ (calcd for $C_{46}H_{77}O_{17}^-$, 901.5160).

*3.3.2 6-O-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranosyldammar-22-ene-(*trans*)-3β, 6α,12β,20(S), 25-pentaol (**2**)*

White amorphous power, mp 180–183; $[\alpha]_D^{16.8}$ + 16.6 (*c* 0.4, MeOH); IR(KBr) ν_{max} (cm^{-1}): 3424, 2933, 1633, 1077, 1046; ^1H

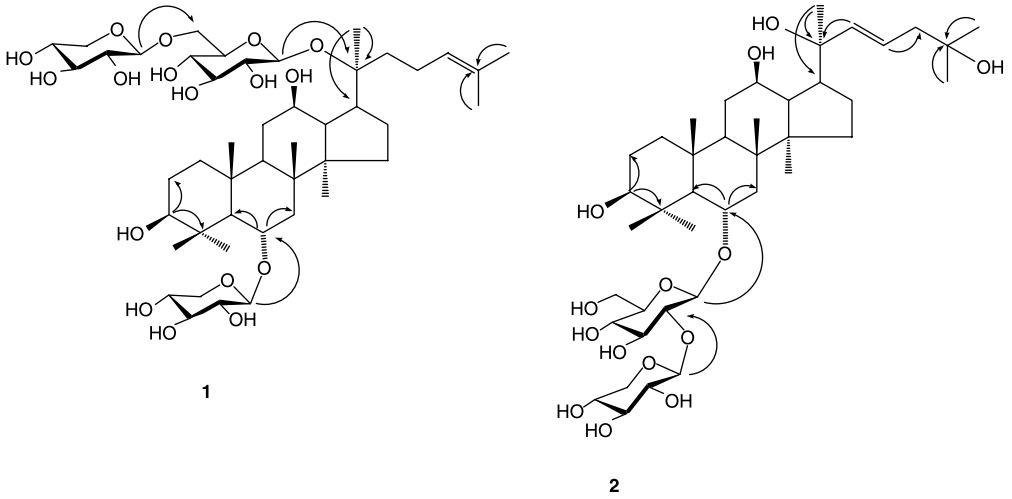

Figure 2. Selected HMBC correlations of compounds **1** and **2**.

NMR (pyridine-d_5, 400 MHz) δ_H: 6.29 (1H, m, H-23), 6.10 (1H, d, $J = 15.8$ Hz, H-22), 5.79 (1H, d, $J = 6.8$ Hz, H-1″), 4.98 (1H, d, $J = 7.2$ Hz, H-1′), 4.40–4.42 (1H, m, H-6), 3.91 (1H, m, H-12), 3.51 (1H, m, H-3), 2.10 (3H, s, H-28), 1.58 (3H, s, H-27), 1.59 (3H, s, H-26), 1.49 (3H, s, H-29), 1.42 (1H, br s, H-5), 1.41 (3H, s, H-21), 1.25 (3H, s, H-18), 1.02 (3H, s, H-19), and 0.81 (3H, s, H-30). For ^{13}C NMR (pyridine-d_5, 100 MHz) spectral data, see Table 1. FABMS (−) *m/z*: 785 $[M - H]^-$ (100), 653 $[M - H\text{-}132]^-$ (10). HRFABMS: 785.4704 $[M - H]^-$ (calcd for $C_{41}H_{69}O_{14}^-$, 785.4687).

3.4 Acidic hydrolysis

Each solution of compounds **1** and **2** (each 3 mg) in a mixture of MeOH (2 ml) and 5% H_2SO_4 (2 ml) was refluxed for 2 h. The hydrolysate was allowed to cool, diluted two fold with H_2O, and extracted with EtOAc. The aqueous layer was neutralized with aq. $Ba(OH)_2$ and filtered, and the filtrate was concentrated *in vacuo* to give a residue, in which glucose and xylose were identified by comparison with authentic samples (BuOH/EtOAc/H_2O, 4:1:5, upper layer; PhOH/H_2O, 4:1) on paper chromatography.

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