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MINOR DAMMARANE SAPONINS FROM *PANAX NOTOGINSENG*

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Key Word Index—*Panax notoginseng*; Araliaceae; roots; South China Ginseng; dammarane-saponins; notoginsenosides R_8 and R_9.

Abstract—Further investigation of the roots of *Panax notoginseng* yielded two new minor dammarane saponins, notoginsenosides R_8 and R_9, whose structures were established by means of spectral evidence as (20*S*)-dammar-22-ene-3β, 6α, 12β, 20, 25-pentol 6-*O*-β-D-glucopyranoside and its (20*R*)-epimer analogue, respectively.

INTRODUCTION

Although Ginseng (roots of *Panax ginseng* C. A. Meyer) is well known for its tonic value worldwide, South China Ginseng (roots of *Panax notoginseng* (Burk.) F. H. Chen) which has similar medical effects in traditional Chinese medicine appears to be unrecognized in the West. *P. notoginseng* originally grew in Yunnan province of China, and is called San-Qi or Tian-Qi locally. Because it is used for many medicinal preparations in China, this plant has been widely cultivated. Chemical investigations on South China Ginseng have been reported by our research group [1–8]. In a continuation of investigations on the saponin composition of this important medicinal plant, we report here the isolation and structural elucidation of two new minor saponins, notoginsenosides R_8 (**1**) and R_9 (**2**).

RESULTS AND DISCUSSION

Notoginsenosides R_8 (**1**) and R_9 (**2**) were isolated by repeated column chromatography of the saponin fractions in yields of 0.00011 and 0.00003%, respectively. Comparison of their ^{13}C and ^1H NMR signals with those of saponins and related derivatives previously obtained from *P. notoginseng* [2,4] indicated that **1** and **2** were panaxatriol-type dammarane saponins with only one glucose in each saponin. The presence of glucose was also supported by the acid hydrolysis results.

Saponin **1** displayed a quasimolecular ion peak at *m/z* 653 $[M - H]^-$ and fragment ions at *m/z* 635 $[653 - H_2O]^-$ and 473 $[653 - \text{glucose}]^-$ in the negative ion FAB-mass spectrum. This information, together with the data from the ^{13}C NMR spectrum allowed its molecular formula to be assigned as $C_{36}H_{62}O_{10}$. Saponin **1** showed a close resemblance with ginsenoside Rh_1(**3**) in their ^{13}C NMR spectra [2]. The only difference between the two saponins was observed in the side-chain. Saponin **3**, which has a side-chain common in ginsenosides [1–7], showed resonances at δ 126.4 (*d*) and 130.8 (*s*) for C-24 and C-25, respectively, while saponin **1** had double-bond carbons resonating at δ 127.4 (*d*) and 137.7 (*d*). There are two possibilities for the location of the double bond—between C-23 and C-24, and between C-22 and C-23. However, the chemical shifts of the two olefinic carbons were quite different from those seen in compounds with a double bond between C-23 and C-24, as commonly occurs in ginsenosides, such as chikusetsusaponin L9a [9]. The coupling system of the side-chain was established as follows. In the ^1H–^{13}C COSY spectrum of **1**, the carbon signals at δ 127.4 and δ 137.7 correlated with the proton signals at δ 6.25 (*ddd*, J = 15.9, 8.4, 5.6 Hz) and 6.06 (*d*, J = 15.9 Hz), respectively. In the ^1H–^1H COSY spectrum, the proton signal at δ 6.25 correlated not only with δ 6.06 but also with two geminal proton signals at δ 2.78 (*dd*, J = 14.0, 5.3 Hz) and 2.39 (*dd*, J = 14.0, 9.6 Hz). The two geminal protons showed cross-peaks with a carbon signal at δ 40.2 in the ^1H–^{13}C COSY spectrum. Up to this point it could be concluded that the double bond might be located between C-22 and C-23, and that the carbon signal at δ 40.2 arises from C-24 in the side chain. This was confirmed by the COLOC and NOESY experiments. The important correlations are shown in Fig. 1. For example, C-24 showed long-range correlations with two tertiary methyl groups assignable to H-26 and H-27. NOE cross-peaks were observed between H-23 and two methyl groups of H-26 and H-27, and between glycosidic linkage positions, H-6 of the aglycone and the anomeric proton. By comprehensive analyses of all two-dimensional NMR spectra, the carbon and proton signals of **1** were unequivocally assigned as shown in Tables 1 and 2. All spectral data are self-consistent. The structure of notoginsenoside R_8 (**1**) was thus formulated as (20*S*)-

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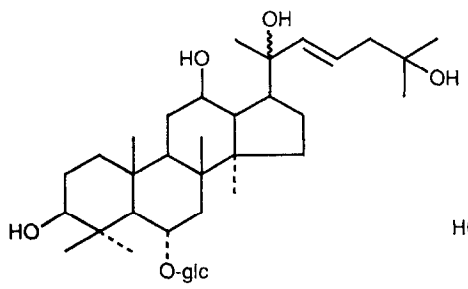
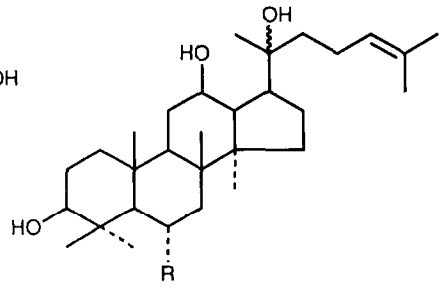


1 (20 *S*)
2 (20 *R*)



	R
3 (20 *S*)	-O-glc
4 (20 *S*)	-H
5 (20 *R*)	-H

glc: β-D-glucopyranosyl

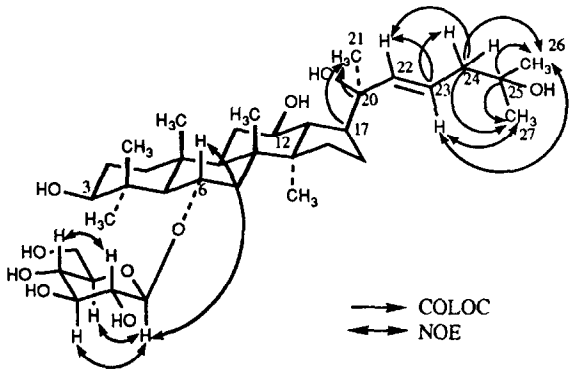


Fig. 1. Important COLOC and NOE correlations of compound **1**.

dammar-22-ene-3β,6α,12β,20,25-pentol 6-*O*-β-D-glucopyranoside.

Saponin **2** had the same molecular formula $C_{36}H_{62}O_{10}$ as saponin **1**, which was determined by a combination of the information from the FAB-mass spectrum and ^{13}C NMR spectrum. The ^{13}C NMR spectra of the two saponins were very similar except for the typical differences at C-17, C-21 and C-24. It is known that the configuration of C-20 may result in a significant difference of the vicinal carbon signal of C-20, such as seen in (20*S*)- and (20*R*)-protopanaxadiol (**4** and **5**) [10]. Table 1 lists the ^{13}C NMR chemical shifts of compounds **4** and **5**. For saponins **1** and **2**, the chemical shift variation at the vicinal carbons of C-20 are in accordance with those of compounds **4** and **5** (Table 1), suggesting that saponin **2** should be the (20*R*)-epimer of **1**. In addition, two-dimensional NMR spectra of ^1H–^1H COSY, ^1H–^{13}C COSY, NOESY and COLOC were employed to confirm the proposed structure. The important COLOC and NOE correlations are shown in Fig. 2. Therefore, the structure of notoginsenoside R_9 (**2**) was established as (20*R*)-dammar-22-ene-3β, 6α, 12β, 20, 25-pentol 6-*O*-β-D-glucopyranoside.

Table 1. ^{13}C NMR spectral data of compounds **1**–**5** in pyridine-d_5 (δ)*

C	1	2	3 [2]	4 [10]	5 [10]
Aglycone					
1	39.6	39.5	39.5	39.5	39.5
2	28.0	28.0	28.0	28.2	28.2
3	78.8	78.7	78.7	77.9	78.0
4	40.4	40.4	40.4	39.5	39.5
5	61.6	61.5	61.5	56.3	56.4
6	80.1	80.1	80.1	18.8	18.7
7	45.4	45.3	45.3	35.2	35.3
8	41.3	41.2	41.2	40.0	40.1
9	50.4	50.3	50.3	50.4	50.6
10	39.8	39.8	39.8	37.3	37.4
11	32.3	32.3	32.2	32.0	32.2
12	71.2	71.0	71.1	70.9	70.8
13	48.7	49.1	48.4	48.5	49.2
14	51.6	51.8	51.7	51.6	51.7
15	31.4	31.4	31.3	31.3	31.5
16	26.7	26.5	26.9	26.8	26.6
17	54.2	51.2	54.8	54.7	50.6
18	17.6	17.5	17.5	16.2	16.3
19	17.8	17.7	17.7	15.8	15.9
20	73.4	73.9	73.1	72.9	72.9
21	27.8	22.5	27.1	26.9	22.7
22	137.7	138.1	35.9	35.8	43.2
23	127.4	126.9	23.1	22.9	22.7
24	40.2	46.4	126.4	126.2	126.0
25	81.4	81.4	130.8	130.6	130.6
26	25.4	25.4	25.9	25.8	25.9
27	25.2	25.3	17.7	17.6	17.7
28	31.8	31.8	31.8	28.6	28.7
29	16.5	16.4	16.4	16.4	16.5
30	17.0	17.1	16.9	17.0	17.3
Glucosyl					
1	106.0	106.0	106.0	—	—
2	75.6	75.5	75.6	—	—
3	79.6	79.6	79.6	—	—
4	72.1	72.1	72.1	—	—
5	78.1	78.1	78.1	—	—
6	63.3	63.3	63.3	—	—

*Assignments of **1** and **2** are based on ^1H–^{13}C COSY, ^1H–^1H COSY, COLOC and NOESY spectra.

Table 2. ^{1}H NMR spectral data of saponins **1** and **2** in pyridine-d_5 (δ)*

^{1}H	1	2
Aglycone		
1	1.71 (1H, *m*)	1.70 (1H, *m*)
	1.03 (1H, *m*)	0.98 (1H, *m*)
2	1.86 (1H, *m*)	1.86 (1H, *m*)
	1.42 (1H, *br s*)	1.42 (1H, *br s*)
3	3.53 (1H, *dd*, 10.8, 5.0)	3.52 (1H, *dd*, 10.8, 5.2)
5	1.44 (1H, *br s*)	1.43 (1H, *br s*)
6	4.45 (1H, *ddd*, 10.2, 10.2, 3.0)	4.43 (1H, *ddd*, 10.2, 10.2, 3.0)
7	2.54 (1H, *dd*, 12.9, 2.7)	2.53 (1H, *dd*, 13.0, 2.9)
	1.94 (1H, *t*, 10.8)	1.96 (1H, *t*, 10.6)
9	1.59 (1H, *m*)	1.61 (1H, *m*)
11	2.14 (1H, *dd*, 9.2, 3.6)	2.13 (1H, *dd*, 8.8, 3.4)
	1.51 (1H, *d*, 4.8)	1.48 (1H, *br s*)
12	3.90 (1H, *m*)	3.91 (1H, *m*)
13	2.03 (1H, *d*, 10.6)	2.01 (1H, *m*)
15	1.66 (1H, *m*)	1.66 (1H, *m*)
	1.11 (1H, *t*, 10.4)	1.15 (1H, *t*, 10.2)
16	1.77 (1H, *ddd*, 12.2, 9.9, 3.0)	1.86†
	1.35 (1H, *d*, 8.0)	1.26 (1H, *m*)
17	2.30 (1H, *dd*, 10.8, 7.2)	2.36 (1H, *dd*, 10.8, 7.0)
18	1.26 (3H, *s*)	1.21 (3H, *s*)
19	1.06 (3H, *s*)	1.06 (3H, *s*)
21	1.39 (3H, *s*)	1.40 (3H, *s*)
22	6.06 (1H, *d*, 15.9)	6.05 (1H, *d*, 15.8)
23	6.25 (1H, *ddd*, 15.9, 8.4, 5.6)	6.27 (1H, *ddd*, 15.8, 8.5, 5.6)
24	2.78 (1H, *dd*, 13.9, 5.3)	2.51†
	2.39 (1H, *dd*, 14.0, 9.6)	2.43†
26	1.56 (3H, *s*)	1.54 (3H, *s*)
27	1.56 (3H, *s*)	1.54 (3H, *s*)
28	2.07 (3H, *s*)	2.06 (3H, *s*)
29	1.62 (3H, *s*)	1.64 (3H, *s*)
30	0.80 (3H, *s*)	0.81 (3H, *s*)
Glucosyl		
1	5.04 (1H, *d*, 7.6)	5.03 (1H, *d*, 7.8)
2	4.10 (1H, *t*, 8.8)	4.10 (1H, *t*, 8.8)
3	4.27 (1H, *t*, 9.0)	4.30 (1H, *t*, 9.4)
4	4.21 (1H, *t*, 9.2)	4.23 (1H, *t*, 9.4)
5	3.95 (1H, *m*)	3.96 (1H, *m*)
6	4.57 (1H, *dd*, 11.6, 3.2)	4.54 (1H, *dd*, 11.6, 3.2)
	4.37 (1H, *dd*, 11.6, 6.0)	4.38 (1H, *dd*, 11.6, 6.0)

*Assignments are based on ^{1}H–^{13}C COSY, ^{1}H–^{1}H COSY, COLOC and NOESY spectra. Coupling constants (*J* values in Hz) are shown in parentheses.

†Coupling patterns overlap.

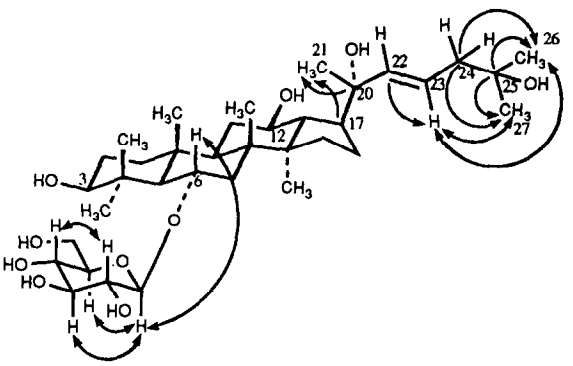


Fig. 2. Important COLOC and NOE correlations of compound **2**.

EXPERIMENTAL

General procedures. NMR spectra were taken in pyridine-d_5 using TMS as int. standard on a Bruker AM-400 spectrometer; ^{13}C NMR (DEPT) at 100 MHz and ^{1}H NMR at 400 MHz. 2D-NMR experiments were carried out with standard pulse sequences. FAB-MS: negative ion mode, glycerol matrix, VG Autospec 3000 system, atom source Cs, 35 kV, emitter current 2 μA. Optical rotations were measured on Horiba SEPA-300 polarimeter. For HPLC (Beckman gold system), YMC-Pack A 312 ODS column (250 × 16 mm i.d.) was used; solvent: 55% MeOH, flow rate: 4 ml min^{-1}; detection: UV at 205 nm. For CC, silica gel H (10–40 μ, Qingdao) was used. Hydrolysis of saponins with mineral acid and identification of the resulting sugar with TLC were performed as described in ref. [8].

Extraction and separation of saponins. Dried roots of *P. notoginseng* were extracted and sepd as described in the ref. [8]. The crude saponin fr. (213 g) was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (50:10:1 → 20:10:1) to yield frs 1–3 in increasing order of polarity. Fr. 1 was subjected to repeated chromatography on silica gel with $CHCl_3$–MeOH (15:1 → 9:1) to yield a mixture of saponins, which was further purified by prep. HPLC with 55% MeOH to give **1** (55 mg) and **2** (15 mg).

Notoginsenoside R_8 (**1**). White powder, $[\alpha]_D^{18}$ + 29° (MeOH; *c* 0.45). FAB-MS (neg.) *m/z*: 653 $[M(C_{36}H_{62}O_{10}) - H]^-$, 635 $[(M - H) - H_2O]^-$, 473 $[(M - H - H_2O) - Glc]^-$. ^{13}C and ^{1}H NMR data: Tables 1 and 2.

Notoginsenoside R_9 (**2**). White powder, $[\alpha]_D^{18}$ + 27° (MeOH; *c* 0.32). FAB-MS (neg.) *m/z*: 653 $[M(C_{36}H_{62}O_{10}) - H]^-$, 635 $[(M - H) - H_2O]^-$, 473 $[(M - H - H_2O) - Glc]^-$. ^{13}C and ^{1}H NMR data: Tables 1 and 2.

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