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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 $R_9$.

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**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-3$\beta$, 6$\alpha$, 12$\beta$, 20, 25-pentol 6-*O*-$\beta$-D-glucopyranoside and its (20*R*)-epimer analogue, respectively.

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## 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 $^{1}H$ 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 $\delta$ 126.4 (*d*) and 130.8 (*s*) for C-24 and C-25, respectively, while saponin **1** had double-bond carbons resonating at $\delta$ 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 $^{1}H$–$^{13}C$ COSY spectrum of **1**, the carbon signals at $\delta$ 127.4 and $\delta$ 137.7 correlated with the proton signals at $\delta$ 6.25 (*ddd*, $J = 15.9, 8.4, 5.6$ Hz) and 6.06 (*d*, $J = 15.9$ Hz), respectively. In the $^{1}H$–$^{1}H$ COSY spectrum, the proton signal at $\delta$ 6.25 correlated not only with $\delta$ 6.06 but also with two geminal proton signals at $\delta$ 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 $\delta$ 40.2 in the $^{1}H$–$^{13}C$ 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 $\delta$ 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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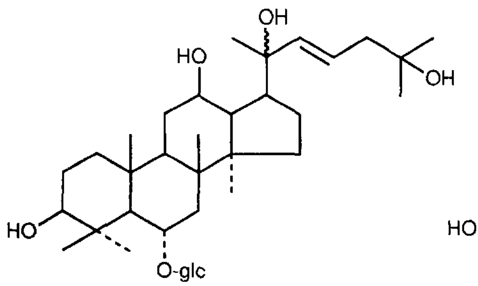
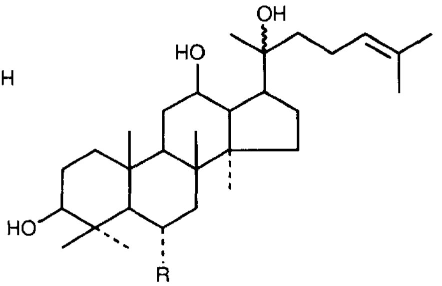


**1** (20*S*)
**2** (20*R*)



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

glc: β-D-glucopyranosyl

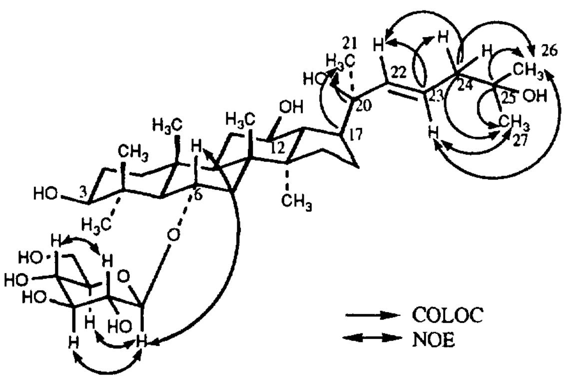


Fig. 1. Important COLOC and NOE correlations of compound **1**.

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

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

| 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-$d_5$ ($\delta$)*

| $^{1}H$ | **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.

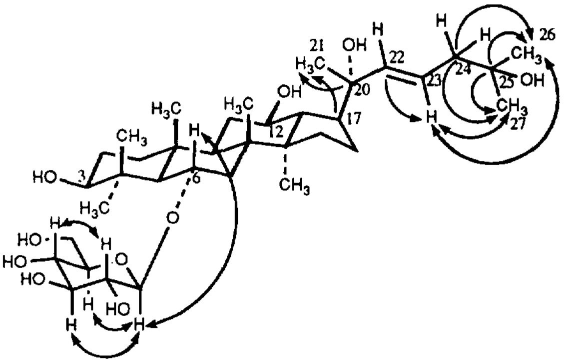


Fig. 2. Important COLOC and NOE correlations of compound **2**.

## 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 $\mu$A. 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 $\mu$, 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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