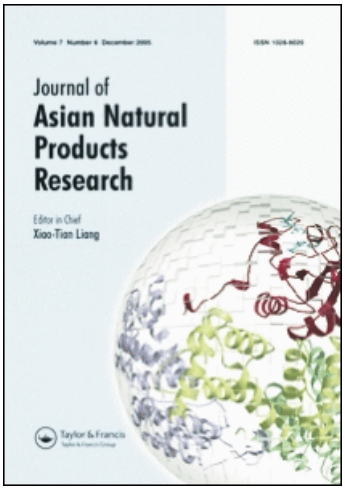

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A PAIR OF 24-HYDROPEROXYL EPIMERIC DAMMARANE SAPONINS FROM FLOWER-BUDS OF *PANAX GINSENG*

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Further investigation on the saponins of the flower-buds of *Panax ginseng* C. A. Meyer has resulted in the isolation and structural elucidation of a pair of new 24-epimers of dammarane type saponins named ginsenoside I and II. The structures of the epimers were characterized on the basis of chemical and spectral evidence as 3-O-[β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]-20-S-O-β-D-glucopyranosyl-3β,12β,20(S)-trihydroxy-24ξ-hydroperoxydammar-25-ene, except for their C-24 configurations. Ginsenoside I is a new triterpene glycoside, and ginsenoside II is a known compound first isolated from a natural plant.

Keywords: *Panax ginseng*; Flower-buds; Triterpene saponins; Ginsenoside I; Ginsenoside II

INTRODUCTION

Panax ginseng C. A. Meyer (Araliaceae) is a famous traditional Chinese medicinal herb, and ginsenosides are generally considered to be its main bioactive constituents. Our study showed that the flower-buds of *panax ginseng* also contain ginsenosides including ginsenoside Rb_2, Rc, Rd, Re, 20(S)-Rg_2, 20(R)-Rg_2, 20(R)-Rh_1, gypenoside XVII, notoginsenoside E,

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ginsenoside III [1]. Further investigation on the saponins of the flower-buds of *Panax ginseng* afforded a pair of 24-hydroperoxy dammarane glycoside epimers, named ginsenoside I (**1**) and II (**2**). The present paper deals with the structural determination of the epimers.

RESULTS AND DISCUSSION

The 70% ethanolic extract from the flower-buds of *Panax ginseng* was fractionated over D101 macroporous resin column, the 50% EtOH eluted fraction was further subjected to silica gel column chromatography and high performance liquid chromatography to afford compound **1** and **2**.

Compound **1**, obtained as a white powder m.p. 193–195°C, has a hydroperoxyl residue as shown by its positive response to the N,N-dimethyl-p-phenylene diammonium dichloride reagent and the ferrous thiocyanate reagent [2]. The empirical molecular formula $C_{48}H_{82}O_{20}$ was in accordance with HRFABMS (negative mode), $[M\text{-}H]^{-1}$ ion at m/z 977.5321 and the ^{13}C-NMR data. Its infrared spectrum displayed a strong absorption band at 3400 cm^{-1} assignable to hydroxyl group. Acid hydrolysis with 2% H_2SO_4 gave glucose as the only sugar constituents. Three doublet signals at δ 4.91 (J = 7.8 Hz), 5.17 (J = 7.8 Hz) and 5.36 (J = 7.3 Hz) in the ^{1}H-NMR spectrum indicated the presence of three glucose units in **1**, this was also confirmed by the ^{13}C-NMR spectrum showing signals at δ105.1, 98.3, 106.1 as the corresponding anomeric carbons. From the coupling constants of the anomeric protons and the ^{13}C-NMR chemical shifts, all sugar moieties must be β-glucopyranosyl units. A comparison of the ^{13}NMR spectrum of **1** with that of ginsenoside Rd [3], showed good agreement in the sugar moiety and the aglycone except for signals due to the side-chain carbons, suggesting that one glucosyl unit attached to the 20-hydroxyl group and the other sugar chain was a glucosyl–glucosyl unit located at C-3 of the aglycone. The sites of glycosidic and interglucosidic linkages were confirmed by the ^{1}H–^{1}H COSY and NOE spectroscopy. In the NOESY spectrum of **1**, cross-peaks were observed between the anomeric proton of a glucosyl unit (4.91) and H-3 of the aglycone (3.26), and between that of a terminal glucosyl unit (5.36) and the above inner glucose H-2′ (4.22), indicating that the sugar chain was a β-sophorosyl unit linked to the aglycone at C-3. The remaining glucosyl unit whose anomeric proton and carbon signals appeared at 5.17 and 98.3 must therefore be located at C-20.

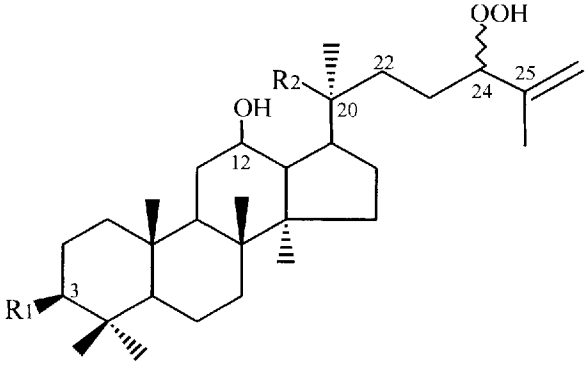
For the side-chain of **1**, the ^{13}C-NMR spectrum showed the presence of two methylene signals at δ 32.8 and 26.6, one methane signal at δ 90.0, two

olefinic carbon signals at δ146.1 and 113.4, and one methyl signal at 17.7, which could be assigned as C-22 ~ C-27, respectively by comparison with those in the literatures [4–6] (only a little difference in C-22, C-23, C-25, C-26 and C-27 due to C-24 configuration). These assignments were further supported by long-range correlations in the HMBC spectrum of **1**, which were observed between the following protons and carbons: H-22 and C-23, 24; H-24 and C-25; H-26 and C-24, 25, 27; H_3-27 and C-24, 25, 26.

S-configuration of C-20 was determined on the basis of coincidence of chemical shifts in C-17, C-20, C-21, C-22 with those of ginsenoside Rd [3].

From the above evidence, the structure of **1** was characterized as 3-O-(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)-20-S-O-β-D-glucopyranosyl-3β,12β,20(S)-trihydroxy-24ξ-hydroperoxy-dammar-25-ene, with uncertain C-24 configuration (Fig. 1).

Compound **2**, obtained as a white powder of mp 197–199°C, displayed a bit longer retention time than **1** on HPLC (Waters RP-18 column: 10 × 250 mm, 70% CH_3OH as an eluent). It has a hydroperoxyl residue as shown by its positive response to the N,N-dimethyl-p-phenylene diammonium dichloride reagent and the ferrous thiocyanate reagent [2]. The molecular formula $C_{48}H_{82}O_{20}$ was determined by the HRFABMS



	R1	R2	
1	OGlc^{2}-^{1}Glc	OGlc	24(S)or(R)
2	OGlc^{2}-^{1}Glc	OGlc	24(R)or(S)

FIGURE 1 Structures of ginsenoside I (**1**) and II (**2**).

(negative mode) showing $(M-H)^{-1}$ ion at m/z 977.5343 (calculated: 977.5321), and the ^{13}C-NMR data. A comparison of ^{13}C-NMR spectrum of **2** with that of **1** indicated that there was very good agreement in the part of structural skeleton and sugar moieties except for a little difference in C-22, C-23, C-25, C-26 and C-27 signals, further comparison with that of the photosensitized oxygenated product [4] showed that **2** and the oxygenated product seem to be the same triterpene saponin(coincident ^{13}C-NMR), this deduction was also supported by the ^{1}H-NMR and HMBC spectra. Thus, **2** was identified as 3-O-(β-D-glucopyranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranosyl)-20-S-O-β-D-glucopyranosyl-3β, 12β, 20(S)-trihydroxy-24ξ-hydroperoxy-dammar-25-ene, with uncertain C-24 configuration (Fig. 1). It is a known compound first isolated from a natural plant.

Yoshikawa *et al.*, reported that **2** is one of oxygenated products of ginsenoside Rd by photosensitized reaction [4]. Owing to no selectivity of reacting reagent in the reaction, Compound **1** (the 24-epimer of **2**) should be another product. Consequently, the two glycosides **1** and **2** reported in this paper might be artifacts.

EXPERIMENTAL SECTION

General Experimental Procedure

Melting points were measured on a X6 micro-melting point apparatus (hot-stage type) and are uncorrected; IR spectra were taken on a Perkin Elmer 983 spectrometer; NMR spectra were recorded on a G8X-400 spectrometer in C_5D_5N using tetramethylsilane (TMS) as an internal standard, including ^{1}H-NMR, ^{13}C-NMR, DEPT, ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, NOESY and HMBC; HPLC was performed on an ODS column (20 $\times$ 250 mm) and a UV detector at 205 nm.

Plant Material

The dried flower-buds of *Panax ginseng* C. A. Meyer were collected from Huairen of Liaoning Province, China in 1993 and identified by Prof. Z. R. Jiang of Shenyang Pharmaceutical University, where a voucher specimen is deposited.

Extraction and Isolation

Dried and powdered flower-buds of *Panax ginseng* (5 kg) were extracted with 70% EtOH (25l $\times$ 3) under reflux. The extract was concentrated in

vacuo to yield a residue (350 g) which was subjected to D101 macroporous resin chromatography eluting with H_2O, 25%, 50% and 95% EtOH. The 50% EtOH fraction (200 g) was further chromatographed on silica gel column (200–300 mesh) using $CHCl_3$-MeOH (5:1, 3:1, 2:1, 1:1.) as eluent. Each 250 ml of eluate was one fraction. The fractions 43–50 containing **1** and **2** were combined and subjected to reversed phase HPLC (column: Waters Rp-18, 20 × 250 mm; flow rate: 2 ml/min; detection: 205 nm) eluted with MeOH-H_2O (7:3) to afford **1** (t_R = 43 min, 16 mg) and **2** (t_R = 49 min, 7 mg).

Ginsenoside I (**1**) white powder; m.p. 193–195°C. $[\alpha]_D^{15}$: 20 (MeOH; c = 0.05). Molecular formula $C_{48}H_{82}O_{20}$ (negative HRFABMS 977.5321 calcd. 977.5321); IR(KBr)ν_{max}: 3400, 2945, 1384, 1077 cm^{-1}. ^{1}H-NMR(C_5D_5N)δppm: 0.78(3H, s, H_3-18), 0.92(3H, s, H_3-30), 0.93(3H, s, H_3-19), 1.09(3H, s, H_3-29), 1.26(3H, s, H_3-28), 1.60(3H, s, H_3-21),

TABLE I ^{13}C-NMR data of ginsenoside I (**1**) and II (**2**) (δppm, C_5D_5N)

	Aglycone			*Sugars*	
	1	**2**		**1**	**2**
1	39.2	39.2	(inner)3-glc-		
2	26.8	26.8	1′	105.1	105.1
3	89.0	89.0	2′	83.5	83.5
4	39.7	39.7	3′	78.3[a]	78.3[a]
5	56.4	56.4	4′	71.7	71.7
6	18.5	18.5	5′	78.1[a]	78.1[a]
7	35.1	35.1	6′	62.9[b]	63.0[b]
8	40.0	40.1	(outer)glc-		
9	50.2	50.2	1″	106.1	106.1
10	36.9	36.9	2″	77.2	77.2
11	30.8	30.8	3″	78.4[a]	78.4[a]
12	70.3	70.2	4″	71.7	71.7
13	49.5	49.5	5″	78.3[a]	78.4[a]
14	51.4	51.5	6″	62.9[b]	63.0[b]
15	31.0	31.0	20-glc-		
16	26.6	26.7	1‴	98.3	98.3
17	51.5	51.7	2‴	75.1	75.1
18	16.3	16.3	3‴	79.2	79.2
19	16.0	15.9	4‴	71.7	71.7
20	83.3	83.1	5‴	78.0[a]	78.0[a]
21	22.6	22.6	6‴	62.7[b]	62.7[b]
22	32.8	32.6			
23	26.6	26.3			
24	90.0	90.0			
25	146.1	145.9			
26	113.4	113.6			
27	17.7	17.5			
28	28.1	28.1			
29	16.6	16.6			
30	17.4	17.3			

[a,b] These assignments may be interchangeable in each column.

1.90(3H, s, H_3-27), 3.01(1H, m, H_a-22), 3.26(1H, dd, J = 4.4 Hz, 11.7 Hz, H-3), 3.29(1H, m, H-22_b), 4.15(1H, m, H-12), 4.71(1H, dd-like, H-24), 4.91(1H, d, J = 7.8 Hz, H-1′), 5.04(1H, d-like, H_a-26), 5.17(1H, s, J = 7.8 Hz, H-1‴), 5.22(1H, d-like, H_b-26), 5.36(1H, d, J = 4.4 Hz, H-1″). The data of ^{13}C-NMR (C_5D_5N) are shown in Table I.

Ginsenoside II (**2**) white powder, m.p. 197–199°C. $[\alpha]_D^{15}$: 12 (MeOH; $c = 0.05$). Molecular formula $C_{48}H_{82}O_{20}$ (negative HRFABMS 977.5343 calcd. 977.5321). IR(KBr)ν_{max}: 3400, 2941, 1641, 1380, 1077 cm^{-1}. ^{1}H-NMR(C_5D_5N)δppm: 0.81(3H, s, H_3-18), 0.92(3H, s, H_3-30), 0.97(3H, s, H_3-19), 1.11(3H, s, H_3-29), 1.28(3H, s, H_3-28), 1.61(3H, s, H_3-21), 1.93(3H, s, H_3-27), 3.08(1H, m, H_a-22), 3.27(1H, dd, J = 3.9 Hz, 11.7 Hz, H-3), 3.34(1H, m, H-22_b), 4.15(1H, m, H-12), 4.76(1H, dd, J = 6.4 Hz, H-24), 4.93(1H, d, J = 7.3 Hz, H-1′), 5.09(1H, d-like, H_a-26), 5.19(1H, d, J = 7.8 Hz, H-1‴), 5.25 (1H, d-like, H_b-26), 5.39(1H, d, J = 7.8 Hz, H-1″). The data of ^{13}C-NMR (C_5D_5N) are shown in Table I.

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