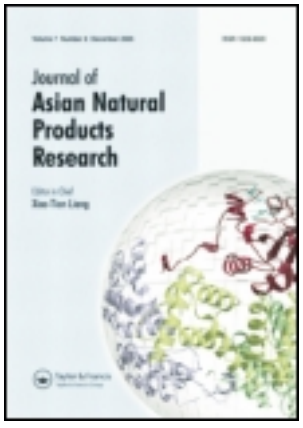

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Minor dammarane saponins from the hongshen extract of Shenmai injection

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A new dammarane-type triterpenoid saponin, (20*R*)-ginsenoside ST_2 (**1**), along with five known saponins was isolated from the hongshen extract of Shenmai injection. The structure of **1** was elucidated to be (20*R*)-dammar-23(*E*)-ene-3β,6α,12β,20,25-pentol 6-*O*-β-D-glucopyranoside by means of spectroscopic methods.

Keywords: Shenmai injection; hongshen extract; dammarane saponins; (20*R*)-ginsenoside ST_2

1. Introduction

Shenmai injection (SMI) is a formula derived from the traditional Chinese medicine recipe *Shen Mai San* [1] and made of the extracts of hongshen (red ginseng, *Radix Ginseng Rubra*) and maidong [roots of *Ophiopogon japonica* (Thunb.) Ker-Gawl., *Radix Ophiopogonis*], which has been used to treat cardiac emergencies, such as coronary atherosclerotic cardiopathy, viral myocarditis, and myocardial infarction, and also to raise tumor patient's immunity. HPLC/MS analysis of SMI showed that its main ingredients were water-soluble ginsenosides from hongshen and a few from maidong [2,3]. In order to improve the quality control level of SMI, we conducted a chemical investigation to search the minor ingredients of the hongshen extract of SMI, which led us to the isolation of a new dammarane saponin, (20*R*)-ginsenoside ST_2 (**1**), along with five known triterpenoid glycosides, (20*S*)-ginsenoside ST_2 (**2**) [4], notoginsenosides R_8 (**3**) and R_9 (**4**) [5], notopanaxoside A (**5**) [6], and 25-hydroxy-(20*S*)-ginsenoside Rh_1 (**6**) [7] (Figure 1). The above ginsenosides are reported from hongshen for the first time.

2. Results and discussion

Compound **1**, a white amorphous powder, has the molecular formula of $C_{36}H_{62}O_{10}$ as deduced from the HR-ESI-MS (found $[M - H]^-$ *m/z* 653.4267, calcd 653.4265). The sugar moiety of **1** was elucidated to be a β-D-glucopyranosyl on the basis of the ^1H and ^{13}C NMR signals at δ_H 5.06 (d, $J = 7.6$ Hz) and δ_C 106.1 (d), 79.8 (d), 78.3 (d), 75.6 (d), 72.0 (d), and 63.0 (t), as well as acid hydrolysis experiment and GC analysis. The ^1H and ^{13}C NMR spectra (Table 1) of **1** for aglycone showed signals for a pair of *E*-double bond (δ_H 6.48 and 6.03, $J_{gemical} = 15.5$ Hz; δ_C 123.0 d and 142.6 d), three oxygenated methines (δ_C

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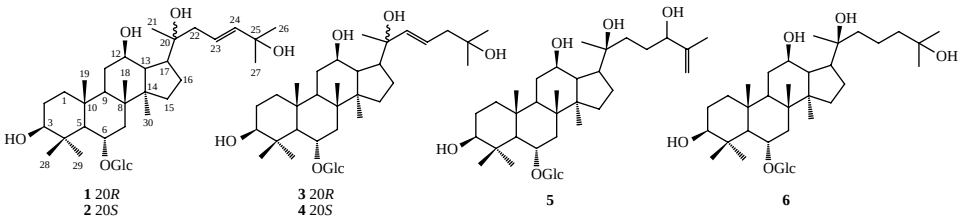

Figure 1. Structures of compounds **1**–**6**.

80.2, 78.7, and 71.0) and two oxygen-bearing quaternary carbons (δ_C 74.0 and 70.0), as well as 23 saturated carbons (8 CH_3, 7 CH_2, 4 CH, and 4 C), indicating a dammarane-type triterpenoid [7]. The ^{13}C NMR spectral data of the aglycone of **1** were nearly superposed with those of (20*R*)-protopanaxatriol derivatives, such as **3** and (20*R*)-ginsenoside Rh_1 [7], except for the signals due to the side chain. In the side chain, two hydroxyls at C-20 and C-25, and the *E*-double bond between C-23 and C-24 were elucidated by the HMBC correlations (Figure 2). The stereochemistry of C-20 of **1** was determined to be *R* according to the ^{13}C chemical shift of C-17 at δ 51.0, while that of 20*S*-epimer is usually observed at δ 54–55 as indicated by Teng *et al.* [7]. The above evidences demonstrated the structure of **1** to be (20*R*)-dammar-23(*E*)-ene-3β,6α,12β,20,25-pentol 6-*O*-β-D-glucopyranoside.

Compound **2** was identified to be (20*S*)-epimer of **1**, i.e. ginsenoside ST_2, by comparison of its NMR, MS, and physical properties with those reported in the literature [4]. The major difference of the ^{13}C NMR spectral data between (20*R*)- and (20*S*)-ginsenoside ST_2 (**1** and **2**) was observed at C-17, C-21, and C-22 ($\Delta\delta_{1-2} = -3.3$, -5.1, and $+6.3$ ppm, respectively).

Previous studies have disclosed that red ginseng (steamed ginseng) had many less polar dammarane saponins with various side chains that were not present in white ginseng due to the high temperature during the preparation procedure [8]. Compounds **1**–**6** might be related with the special process of the hongshen extract.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Perkin-Elmer 341 polarimeter. The IR spectra were recorded on a Nicolet-Magna-750-FTIR spectrometer. The NMR spectra were taken on a Bruker AV-400 spectrometer with TMS as internal standard. ESI-MS and HR-ESI-MS were obtained on an Esquire 3000plus and a Q-TOF-Ultima mass spectrometers, respectively. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., Qingdao, China), D-1400 macroporous resin (Yangzhou Pharmaceutical Factory, Yangzhou, China), and C_{18}-reversed phase silica gel (150–200 mesh, Fuji Silysia Chemical Ltd, Aichi, Japan) were used for column chromatography (CC). Silica gel $HSGF_{254}$ (Yantai Jiangyou Guijiao Kaifa Co., Yantai, China) was used for TLC. GC analysis was performed on a Perkin-Elmer Sigma-115 gas chromatograph. Analytical and prepared HPLC isolation were conducted on a Varian HPLC system (pump: Prepstar SD-1, detector: UV–vis 320, column: Merck, 5 μm, I.D. 4.6 × 250 mm, and Merck 12 μm, I.D. 25 × 250 mm, respectively).

3.2 Plant material

The hongshen extract was prepared as per the manufacture procedure of SMI, provided by the Chiatai Qingchunbao Phar-

Table 1. ^{1}H and ^{13}C NMR spectral data of **1** and **2** (400 and 100 MHz, C_5D_5N).

Site	δ_C of **1**	δ_H of **1**	δ_C of **2**	δ_H of **2**
1	39.6 t	1.69 (dd, 12.7, 6.8), 1.03 (m)	39.6 t	1.76 (dd, 11.4, 4.8), 1.06 (m)
2	28.1 t	1.89 (m), 1.83 (m)	28.1 t	1.94 (m), 1.87 (m)
3	78.7 d	3.53 (dd, 11.0, 5.2)	78.8 d	3.56 (dd, 11.0, 5.3)
4	40.5 s	–	40.5 s	–
5	61.5 d	1.46 (d, 10.7)	61.6 d	1.47 (d, 10.5)
6	80.2 d	4.47 (td, 10.8, 2.0)	80.2 d	4.47 (td, 10.5, 2.5)
7	45.3 t	2.56 (dd, 12.3, 2.0), 1.90 (dd, 12.3, 10.8)	45.4 t	2.56 (dd, 12.5, 2.5), 1.98 (dd, 12.5, 10.5)
8	41.2 s	–	41.3 s	–
9	50.3 d	1.59 (m)	50.4 d	1.59 (m)
10	39.8 s	–	39.8 s	–
11	32.3 t	2.18 (m), 1.59 (m)	32.3 t	2.17 (m), 1.59 (m)
12	71.0 d	3.90 (m)	71.2 d	3.91 (m)
13	49.2 d	2.04 (m)	48.7 d	2.12 (m)
14	51.8 s	–	51.8 s	–
15	31.4 t	1.70 (dd, 10.8, 3.0), 1.16 (br t, 10.1)	31.4 t	1.70 (dd, 12.0, 3.8), 1.16 (t, 12.0)
16	26.5 t	1.97 (m), 1.33 (m)	26.9 t	1.81 (m), 1.40 (m)
17	51.0 d	2.38 (m)	54.3 d	2.32 (m)
18	17.6 q	1.26 (s, 3H)	17.6	1.29 (s, 3H)
19	17.8 q	1.08 (s, 3H)	17.8	1.10 (s, 3H)
20	74.0 s	–	73.5	–
21	22.6 q	1.43 (s, 3H)	27.7 q	1.42 (s, 3H)
22	46.3 t	2.54 (dd, 13.9, 6.9), 2.44 (dd, 13.9, 7.8)	40.0 t	2.83 (dd, 13.7, 5.3), 2.48 (dd, 13.7, 8.9)
23	123.0 d	6.48 (ddd, 15.5, 7.8, 6.9)	123.4 d	6.34 (ddd, 15.6, 8.9, 5.3)
24	142.6 d	6.03 (d, 15.5)	142.2 d	6.06 (d, 15.6)
25	70.0 s	–	70.0 s	–
26	30.9 q	1.55 (s, 3H)	30.9 q	1.57 (s, 3H)
27	30.9 q	1.55 (s, 3H)	30.9 q	1.56 (s, 3H)
28	31.9 q	2.08 (s, 3H)	31.9 q	2.09 (s, 3H)
29	16.5 q	1.63 (s, 3H)	16.5 q	1.63 (s, 3H)
30	17.1 q	0.84 (s, 3H)	17.0 q	0.86 (s, 3H)
Glu-1′	106.1 d	5.06 (d, 7.6)	106.1 d	5.06 (d, 7.5)
2′	75.6 d	4.12 (dd, 8.1, 7.8)	75.6 d	4.12 (t, 7.8)
3′	79.8 d	4.29 (t, 8.7)	79.8 d	4.28 (t, 8.7)
4′	72.0 d	4.24 (t, 8.8)	72.0 d	4.23 (t, 9.0)
5′	78.3 d	4.00 (m)	78.3 d	3.95 (m)
6′	63.0 t	4.57 (dd, 11.3, 1.4), 4.40 (dd, 11.3, 5.4)	63.2 t	4.55 (dd, 11.2, 1.4), 4.37 (dd, 11.2, 5.4)

maceutical Co. In brief, 10 kg of dried red ginseng (purchased from the ginseng market in Jilin Province) was refluxed 2 h in 30 liters of 80% EtOH. The extract was concentrated to 5 liters under reduced pressure, following that placed into refrigerator overnight, then the solid residue was filtered out. To the solution, 30 g of active carbon was added, stirred fully, and filtered after 30 min. The percolate was adjusted to pH 7.5–7.7 using 1% NaOH, then heated and kept boiling for 40 min, and decolorised again using 25 g of active carbon as in the above method. To the filtrate, distilled water was added to 5 liters to yield the hongshen extract.

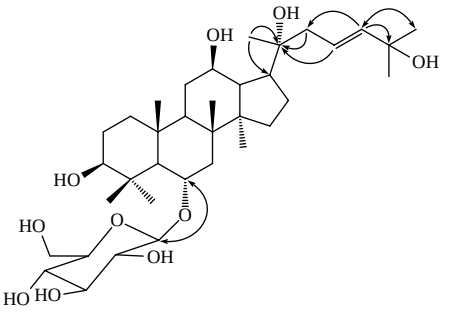

Figure 2. Selected HMBC correlations of **1**.

3.3 Extraction and isolation

The concentrated hongshen extract (1 liters) was subjected to the CC of macroporous resin eluted with gradient EtOH in H_2O (0, 20%, 40%, 70%, 95%). The combined 40% and 70% EtOH fractions (about 13 g) were subjected to CC of silica gel with gradient $CHCl_3$–MeOH as eluant. The $CHCl_3$–MeOH (6:1) fraction (about 1 g) was separated into subfractions 1–6 by CC of Rp-18 silica gel (increasing MeOH in H_2O, 25–60%). Fraction 3 (40% MeOH–H_2O, 150 mg) was further isolated by prepared HPLC system (CH_3CN–H_2O, 19%, UV, 202 nm) to obtain **1** (8 mg), **2** (14 mg), **3** (7 mg), **4** (15 mg), **5** (10 mg), and **6** (35 mg).

3.3.1 (20R)-Ginsenoside ST_2 (*1*)

White amorphous powder. $[\alpha]_D^{14}$ +34 (*c* 0.085, MeOH). IR (KBr) ν_{max}: 3421, 2926, 1630, 1462, 1385, 1155, 1078, 1028 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z* 653 $[M - H]^-$, 699 $[M + HCOO]^-$ (negative), 677 $[M + Na]^+$ (positive); HR-ESI-MS (negative) *m/z* 653.4267 $[M - H]^-$ (calcd for $C_{36}H_{61}O_{10}$, 653.4265).

3.3.2 Acid hydrolysis of *1*

Compound **1** (1 mg) was refluxed in 2 N HCl–dioxane (1:1 v/v, 2 ml) for 2 h. On cooling, the mixture was neutralized with $NaHCO_3$. After extraction with AcOEt, the aqueous layer was concentrated by blowing with N_2. The residue was purified by CC of Sephadex LH-20 (MeOH–H_2O 1:1, v/v) to give the sugar moiety. The purified sugar and standard D-glucose (Sigma-Aldrich, St Louis, USA) were treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (1 ml) at 60°C for 1 h. Then, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.02 ml) at 60°C for 1 h. The supernatant was subjected to GC analysis to identify the sugars. Conditions for GC were: capillary column, DB5-MS (30 m × 0.25 mm × 0.25 μm), oven temperature program, 180–300°C at 6°C/min; injection temperature 350°C; carrier gas, He at 1 ml/min. D-Glucose was detected by comparing its retention time with that of the authentic sample (t_R = 12.28 min).

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