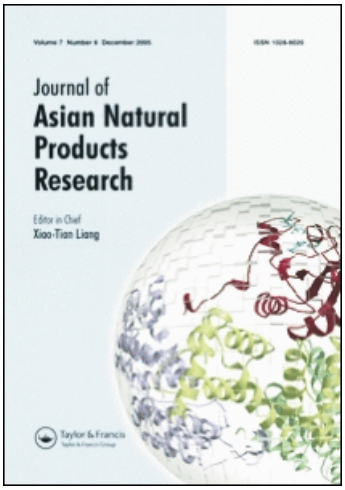

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Steroidal glycosides from tubers of *Ophiopogon japonicus*

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Note

Steroidal glycosides from tubers of *Ophiopogon japonicus*

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A new steroidal saponin, named *ophiopogonin E* (**1**), has been isolated from the tubers of *Ophiopogon japonicus*, along with five known steroidal saponins (**2**–**6**). The structure of the new steroidal glycoside was characterized by spectroscopic analysis and acid-catalyzed hydrolysis as pennogenin 3-*O*-β-D-xylopyranosyl (1 → 4)-β-D-glucopyranoside (**1**).

Keywords: *Ophiopogon japonicus*; Liliaceae; Steroidal saponin; Pennogenin

1. Introduction

Ophiopogon japonicus (Thunb.) Ker-Gawl. (Liliaceae) is widely distributed in South China, especially in Hangzhou city, Zhejiang Province, and Mianyang city, Sichuan Province, known as "Zhemaidong" and "Chuanmaidong", respectively. Its rhizomes have been employed in traditional Chinese medicine as expectorant, anti-cough and tonic agent as well as showing pharmacological effects on the cardiovascular system [1]. As a part of our contribution to the study of on *Ophiopogon* species [2,3], we have established phytochemical screening of the tubers of *O. japonicus*, resulting in the isolation of a new pennogenin diglycoside, together with five known steroidal saponins, pennogenin 3-*O*-[2′-*O*-acetyl-α-L-rhamnopyranosyl (1 → 2)]-β-D-xylopyranosyl(1 → 3)-β-D-glucopyranoside (**2**) [4], 25(*R*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside (**3**) [5], 25-(*R*)-ruscogenin (**4**) [6], diosgenin 3-*O*-[2-acetyl-α-L-rhamnopyranosyl (1 → 2)][β-D-xylopyranosyl(1 → 3)-β-D-glucopyranoside (**5**) [7] and diosgenin 3-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-glucopyranoside (**6**) [8]. Compound **4** was isolated from the plant for the first time. This paper deals with the structural elucidation of the new saponin on the basis of analysis, including 2D NMR spectroscopic data and the result of acid hydrolysis.

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2. Results and discussion

Ophiopogonin E (**1**) was obtained as a white amorphous powder (MeOH); positive-ion high-resolution ESIMS gave a molecular formula of $C_{38}H_{60}O_{13}$, which was also deduced by analysis of the ^{13}C NMR and DEPT spectral data. The IR spectrum exhibits characteristic absorption bands at 976, 920, 901 and 868 cm^{-1} for the spirostanol type of steroidal sapogenin – the weaker intensity of the band at 920 cm^{-1} than at 901 cm^{-1} shows that **1** belongs to the 25(*R*) series of spirostane [9].

The ^1H NMR spectrum of **1** exhibits signals for two three-proton singlets of angular methyl groups at δ0.91 (3H, s, H-18), 0.94 (3H, s, H-19), two three-proton doublets at δ0.67 (3H, d, $J = 5.4$ Hz, H-27) and 1.22 (3H, d, $J = 7.2$ Hz, H-21) and an olefinic proton at δ5.27 (1H, br. s, H-6), indicative of the steroidal character of **1**. The ^1H NMR also displayed signals for two anomeric protons at δ4.98 (d, $J = 7.8$ Hz, H-1′) and 5.14 (d, $J = 7.7$ Hz, H-1″), which gave correlations in the HSQC spectrum with carbon signals at δ103.1 (C-1′) and 106.2 (C-1″) (table 1). The diagnostic quaternary carbon signal at δ110.5 (C-22) and the superimposed signals at 90.8 (C-16, 17) in the ^{13}C NMR spectrum suggest that **1** is a steroidal saponin with a spirostanol skeleton of pennogenin [10]. Comparison of carbon chemical shifts of **1** with those of pennogenin shows that the structure of the A-F ring of **1** is identical to that of pennogenin, including the orientation of the C-3 oxygen atom, except for a significantly different signal in the A ring due to a C-3 glycoside group. Correlation signals based on the combined detailed analysis of the HSQC and HMBC spectra provided good support for the structure of the aglycone (figure 1).

On acid hydrolysis, **1** afforded a mixture of glucose and xylose, which were identified by HPTLC with authentic samples. Sequential assignments of the two sugars were established by $ESIMS^2$ analysis of **1**. The glucose is attached to the aglycone because the daughter ion *m/z* 615 $[(M - 132) + Na]^+$ of the parent ion *m/z* 747 $[M + Na]^+$ appears in the tandem mass. Comparison of the carbon chemical shifts with those of the reference methyl glycosides [11], taking into account the known effects of *O*-glycosylation, indicate that **1** contains a terminal β-xylopyranosyl and a C-4 substituted β-glucopyranosyl. The positions of the two sugars were further confirmed by the correlations between H-1″ (δ5.14) of the xylose with C-4′ (δ81.7) of the glucose, and H-4′ (δ4.37) of the glucose and C-1″ (δ106.2) of the xylose. Consequently, by comparing the chemical shift and coupling constants of the anomeric protons of the glucose (δ4.98, 7.8 Hz) and the xylose (δ5.14, 7.7 Hz) with literature data, the structure of **1** was elucidated as pennogenin 3-*O*-β-D-xylopyranosyl(1 → 4)-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

Mps were obtained on an X4 micro-melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°C. IR spectra were recorded on an Impact-410 (Nicolet) instrument. ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AM-500 spectrometer and chemical shifts are reported in ppm using the solvent (pyridine-d_5) as reference. ESIMS and HR-ESIMS spectra were obtained on an Agilent 1100 Series LC/MSD Trap and a Bruker instrument, respectively.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of **1** in pyrindine-d_5.

	1			
C	^{13}C	^{1}H	*HMBC (H → C)*	*DEPT*
1	38.2	1.71 m		CH_2
		0.94 overlap		
2	32.4			CH_2
3	79.0	4.12 m	C-1′	CH
4	40.0	2.65 dd like		CH_2
		2.45 m		
5	141.6			C
6	122.4	5.27 br. s	C-7, 10	CH
7	32.7			CH_2
8	31.1			CH
9	50.9	0.94 overlap		CH
10	37.7			C
11	21.6			CH_2
12	32.4	1.58 m		CH_2
13	45.7			C
14	53.7	2.08 m		CH
15	30.9	2.20 m	C-13, 16, 17	CH_2
16	90.8	4.43 m	C-13, 17, 20	CH
17	90.8			C
18	17.8	0.94 s	C-12, 13, 14, 17	CH_3
19	20.1	0.92 s	C-5, 9, 10	CH_3
20	45.4	2.26 d (7.2 Hz)	C-13, 16, 17, 21, 22	CH
21	10.2	1.22 d (7.2 Hz)	C-17, 20, 22	CH_3
22	110.5			C
23	33.0			CH_2
24	29.4	1.54 m		CH_2
25	33.0	2.07 m		CH
26	67.4	3.49 d like	C-25	CH_2
27	17.9	0.67 d (5.4 Hz)	C-25, 26	CH_3
Sugars	Glc-(inner)			
1′	103.1	4.98 d (7.8 Hz)	C-3, C-2′	CH
2′	75.7	4.05 m	C-1′,3′	CH
3′	77.1	4.31 t (8.8 Hz)	C-2′,4′	CH
4′	81.7	4.37 t (9.2 Hz)	C-3′,5′,1″	CH
5′	77.1	3.90 m		CH
6′	62.6	4.59 dd (3.8, 12.1 Hz)		CH_2
		4.45 m		
	Xyl-			
1″	106.2	5.14 d (7.7 Hz)	C-4′, C-2″	CH
2″	75.6	4.02 m	C-1″, 3″	CH
3″	79.0	3.86 m	C-2″, 4″	CH
4″	71.5	4.18 m	C-5″, C-3″	CH
5″	68.0	4.25 dd (5.0, 11.1 Hz)	C-4″	CH

Column chromatography was carried out on silica gel (200-300 mesh, Qingdao Marine Chemistry Company, China) and Sephadex LH-20 (MeOH, Pharmacia, Sweden). HPTLC was conducted on silica-gel 60 F_{254} plates (Merck, Germany).

3.2 Plant material

Tubers of *O. japonicus* were collected from Hangzhou city, Zhejiang Province, China in August 2001, and were identified by Dr Bo-yang Yu. A voucher specimen (Herbarium No. 1109) has been deposited at the Herbarium of Department of Traditional Chinese Medicine, China Pharmaceutical University.

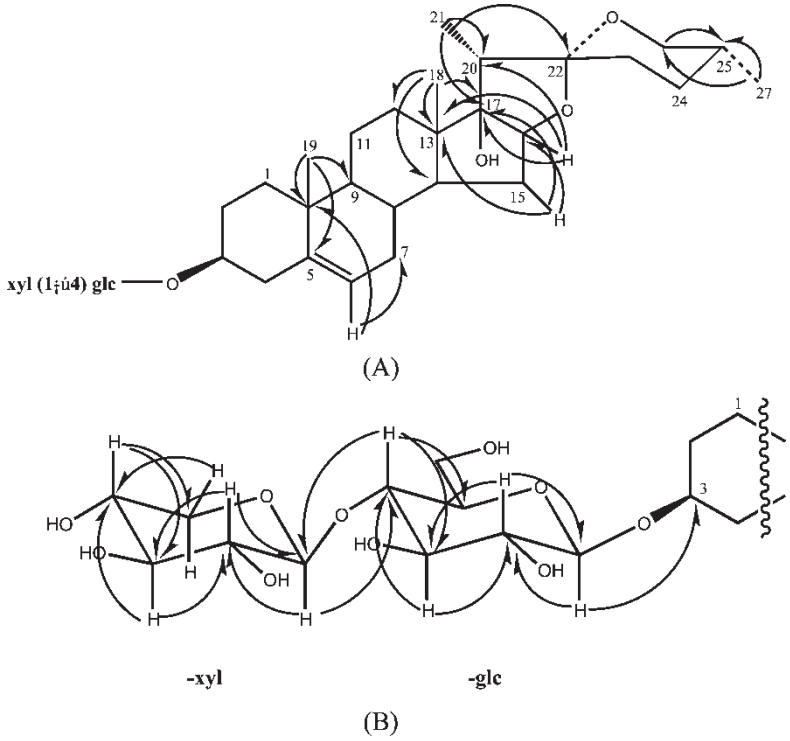

Figure 1. Key HMBC correlations for the aglycone (A) and sugar moieties (B) of **1**.

3.3 Extraction and isolation

The dried tubers (50 kg) of *Ophiopogon japonicus* were extracted with hot methanol (250 L × 3). The extract was then evaporated to dryness *in vacuo*. The residue (1.0 kg) was dissolved in water and extracted with diethyl ether to afford an ether-soluble fraction that was chromatographed on a silica-gel column eluted with light petroleum-EtOAc (20: 1) to give **4** (50 mg). The aqueous layer was extracted with *n*-BuOH saturated with water, and the *n*-BuOH-soluble fraction was concentrated *in vacuo* to afford a brown powder (40.5 g), which was subjected to silica-gel column chromatography eluted with $CHCl_3$-CH_3OH (25:1, 10:1, and 5:1) to afford three fractions (Fraction I–III). Fraction I was subjected to further column chromatography on silica gel with $CHCl_3$–MeOH (10: 1) to afford **1** (12 mg). Fraction II was purified by Sephadex LH-20 with MeOH to afford **5** (5 mg). Fraction III was also separated by repeated column chromatography on silica gel with $CHCl_3$–CH_3OH (5:1) to afford **2** (10 mg), **3** (350 mg) and **6** (21 mg).

3.4 Acid hydrolysis of 1

Compound **1** (3 mg) was heated with 5% H_2SO_4 (EtOH–H_2O, 1:1) at 100°C for 4 h. After cooling, the reaction mixture was neutralized with 8% NaOH and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$-soluble phase was concentrated and revealed a steroidal aglycone through TLC (solvents: $CHCl_3$–MeOH, 10:1) giving a dark green spot by spraying with 10% H_2SO_4 followed by heating. The H_2O-soluble phase was concentrated and examined by

comparison with authentic samples through high-performance TLC ($CHCl_3$–MeOH–H_2O, 65:35:10, lower layer) to detect glucose and xylose.

3.5 Ophiopogonin E: Pennogenin 3-O-β-D-xylopyranosyl(1 → 4)-β-D-glucopyranoside (1)

A white amorphous powder (MeOH), mp 300–302°C $[\alpha]_D^{20}$ − 76.0 (*c* 0.68, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3437 (OH), 2933 (CH), 1632 (C═C), 1449, 1382, 1157, 1047 (C—O), 976, 920, 901, 868 [intensity 920 < 901, (25*R*) spiroketal]; ESIMS *m/z* 747 $[M + Na]^+$; $ESIMS^2$ of *m/z* 747 $[M + Na]^+$: 615 $[(M - 132) + Na]^+$; HRESIMS *m/z* 747.3926 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_{13}Na$, 747.3937); ^1H and ^{13}C NMR (pyridine-d_5, 500/100 MHz) spectral data are shown in table 1.

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