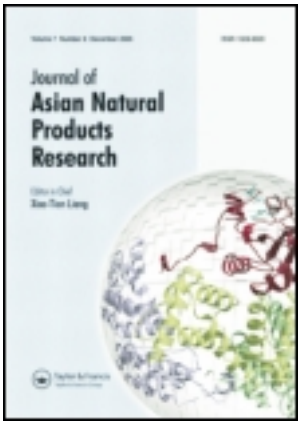

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A new steroidal saponin from *Dioscorea panthaica*

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A new steroidal saponin and three known saponins were isolated from the rhizomes of *Dioscorea panthaica*. Their structures were elucidated as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20,22-seco-25(*R*)-furosta-5-ene-20,22-dione-3β,26-diol (**1**), pregnadienolone-3-*O*-β-chacotriside (**2**), pseudoprotodioscin (**3**), and dioscoreside D (**4**) mainly by NMR techniques and chemical methods. The inhibitory activities of the saponins against α-glucosidase were investigated, and compound **2** was found to exhibit potent activity with IC_{50} values of 0.04 ± 0.01 mM.

Keywords: steroidal saponin; *Dioscorea panthaica*; α-glucosidase inhibitory activity

1. Introduction

The rhizomes of *Dioscorea panthaica* Prain et Burk are used as a traditional Chinese medicine for the treatment of gastric diseases, bone injuries, and rheumatic arthritis [1]. It is also the essential herbal resource of Diaoxinxuekang for the treatment of cardiovascular diseases [2]. Previous phytochemical studies on this plant led to the isolation of several saponins such as progenin II, progenin III, dioscin, gracillin, and pseudoprotodioscin [3]. In the course of our investigation for bioactive constituents of this plant, a new steroidal saponin 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20,22-seco-25(*R*)-furosta-5-ene-20,22-dione-3β,26-diol (**1**), along with three known steroidal saponins pregnadienolone 3-*O*-β-chacotriside (**2**), pseudoprotodioscin (**3**), and dioscoreside D (**4**), was isolated. Herein, we report their isolation and characterization.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder, which gave positive Liebermann–Burchard and Molisch reagent tests. The results suggested that **1** was a furostanol saponin. The molecular formula was determined as $C_{45}H_{72}O_{19}$ by HR-ESI-MS at *m/z* 934.5046 $[M + NH_4]^+$. The ^1H NMR spectrum of **1** showed three tertiary methyl proton groups at δ 1.23 (3H, s, CH_3-18), 2.14 (3H, s, CH_3-21), and 1.07 (3H, s, CH_3-19), and a secondary methyl proton at δ 0.93 (3H, d, $J = 6.6$ Hz, CH_3-27), which were recognized as typical signals of steroidal saponin [4]. Moreover, one trisubstituted olefinic protons at δ 5.32 (1H, br s, H-6) could be readily assigned in the ^1H NMR spectrum (Table 1). These data, combined with the ^{13}C NMR spectral

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Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectral data for compound **1** in pyridine-d_5 (δ in ppm and J in Hz).

Position	δC	δH	Position	δC	δH
1	37.8	0.92, 1.69 (each 1H, overlapped)	Glc (inner)		
2	30.8	1.84, 2.06 (each 1H, m)	1	100.7	5.05 (1H, d, 7.2 Hz)
3	78.1	3.90 (1H, m)	2	80.0	4.31 (1H, m)
4	39.3	2.82, 2.75 (each 1H, m)	3	78.3	4.23 (1H, m)
5	141.3		4	72.0	4.18 (1H, m)
6	121.8	5.32 (1H, br s)	5	78.6	4.02 (1H, m)
7	32.2	1.83 (2H, overlapped)	6	63.0	4.17m, 4.25 overlapped (each 1H)
8	31.3	1.53 (1H, overlapped)	Rha (1 → 2)		
9	50.7	0.89 (1H, overlapped)	1	102.4	6.39 (1H, br s)
10	37.4		2	72.9	4.80 (1H, m)
11	20.9	1.48 (2H, overlapped)	3	73.2	4.63 (1H, m)
12	38.6	1.08, 2.12 (each 1H, overlapped)	4	74.5	4.39 (1H, m)
13	42.6		5	69.8	5.01 (1H, m)
14	54.4	0.83 (1H, m)	6	19.0	1.78 (1H, d, 6.6 Hz)
15	32.6	1.53, 2.42 (each 1H, overlapped)	26-*O*-Glc		
16	74.5	5.69 (1H, m)	1	105.2	4.81 (1H, d, 7.2 Hz)
17	67.0	2.50 (1H, d, 7.8 Hz)	2	75.0	4.03 (1H, m)
18	14.5	1.23 (3H, s)	3	78.9	4.23 (1H, m)
19	19.9	1.07 (3H, s)	4	72.0	4.31 (1H, m)
20	205.8		5	78.8	4.02 (1H, m)
21	30.5	2.14 (3H, s)	6	63.2	4.5, 4.38 (each 1H, overlapped)
22	173.6				
23	35.8	1.33, 2.43 (each 1H, overlapped)			
24	30.3	1.56, 1.97 (each 1H, overlapped)			
25	33.7	1.84 (1H, m)			
26	75.5	3.52, 3.90 (each 1H, m)			
27	17.2	0.93 (3H, d, 6.6 Hz)			

data [three angular methyl groups at δ 14.5, 30.5, and 19.9, one secondary methyl group at δ 17.2, one trisubstituted C=C double bond at δ 141.3 and 121.8, and a methylene group linked to an oxygen atom at δ 75.5 (Table 1)], indicated an aglycone with a furost-5-ene skeleton.

In the HMBC spectrum, both H-21 at δ 2.14 (3H, s) and H-17 at δ 2.50 (1H, d, $J = 7.8$ Hz) showed long-range correlations with the carbon at δ 205.8 (C-20), indicating that a carbonyl group was at C-20. H-23 at δ 1.33 and 2.43 (each 1H, overlapped) showed long-range correlations with the quaternary carbon at δ 173.6 (C-22), indicating that a carbonyl group was at C-22. Comparison of the ^{13}C NMR spectroscopic signals of the aglycone moiety of **1** with those in the literature [5], coupled with an extensive analysis of HMBC spectrum (Figure 1), showed that the aglycone of **1** was 25(*R*)-furosta-5-ene-20,22-dione-3β,26-diol. The absolute configuration of C-25 was

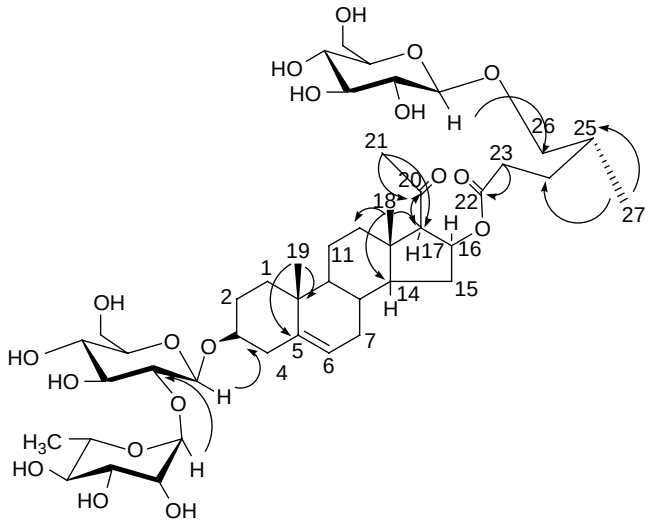

Figure 1. Selected HMBC correlations for compound **1**.

deduced to be *R* on the basis of the difference in chemical shifts of the germinal protons H_2-26 ($\delta a - \delta b = 0.27$ ppm) [6]. The NOESY spectrum of **1** showed NOESY correlations of H-14 at δ 0.83 with H-16 at δ 5.69 and H-17 (2.50, d, $J = 7.5$ Hz), which indicated the α-configurations of H-16 and H-17. By comparing with compound **3**, the absolute stereochemistry of the aglycone of **1** is, therefore, assumed to be the same as that of the glycone of **3** with *R*-configuration of C-17 and *S*-configuration of C-16 [7].

Of the 45 carbon signals observed in the ^{13}C NMR spectrum of **1**, 27 were assigned to the aglycone part and the remaining 18 to the oligosaccharide moiety. The ^{1}H NMR and the ^{13}C NMR spectra of **1** showed three anomeric protons at δ 6.39 (1H, br s), 5.05 (1H, d, $J = 7.2$ Hz), and 4.81 (1H, d, $J = 7.2$ Hz), and corresponding carbons at δ 102.4, 100.7, and 105.2 (Table 1), respectively. And the downfield shifts of C-3 (δ 78.1) and C-26 (δ 75.5) suggested the glycosylation sites. On acidic hydrolysis, **1** afforded L-rhamnose and D-glucose which were confirmed by direct HPLC analysis using a combination of refractive index and optical rotation (OR) detectors. The sequence of the oligosaccharide chains in **1** was determined by combined analysis of HMQC, HMBC, and NOESY experiments. The HMBC correlations of H-1 (δ 5.05) of Glc-I with C-3 (δ 78.1), H-1 (δ 6.39) of Rha-I with C-2 (δ 80.0) of Glc-I indicated the linkage of sugar moiety at C-3 position. The presence of glucose connected to C-26 was proven by the HMBC correlation between H-1 (δ 4.81) of Glc-II and C-26 (δ 75.5). The *J* values (7.2 and 7.2 Hz) of two anomeric protons of glucoses indicated the β-configuration [8,9], and the chemical shift of C-5 (δ 69.8) of the rhamnose indicated the α-configuration [7]. The NOESY analysis also confirmed the above conclusions. Therefore, the structure of **1** was characterized as 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-*O*-β-D-glucopyranosyl-20,22-seco-25(*R*)-furosta-5-ene-20,22-dione-3β,26-diol.

In addition, three known compounds were isolated. By comparing their NMR spectroscopic data with those in the literature, their structures were determined to be pregnadienolone 3-*O*-β-chacotriside (**2**) [10], pseudoprotodioscin (**3**) [7], and dioscoreside D (**4**) [5] (Figure 2).

Four isolated compounds were tested for inhibitory activity against α-glucosidase.

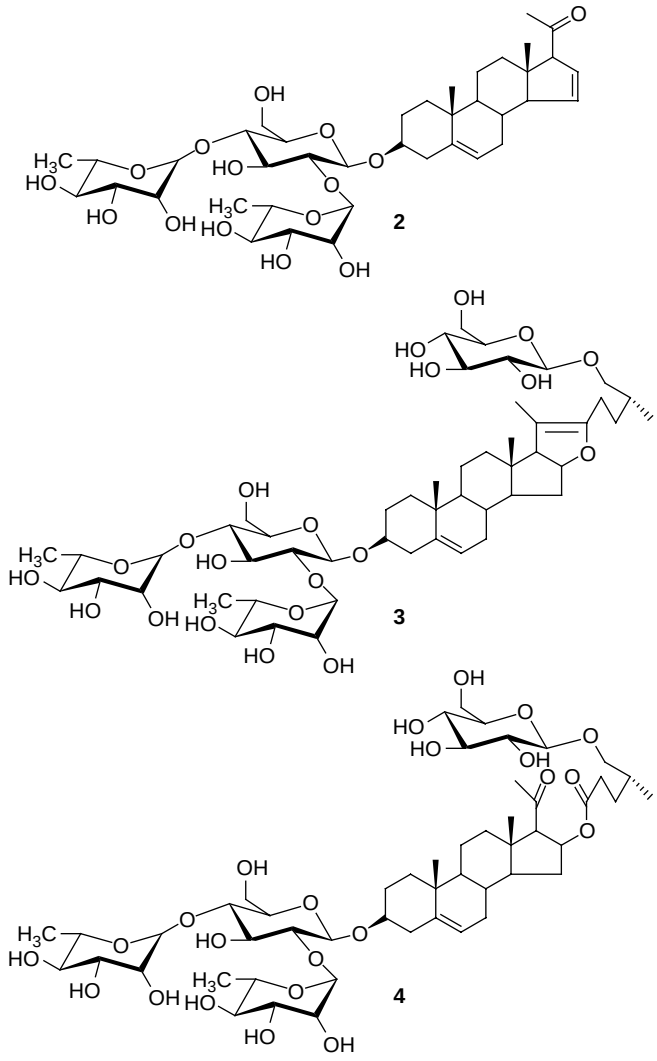

Figure 2. Structures of compounds **2**–**4**.

Pregnadienolone 3-*O*-β-chacotriside (**2**) was found to exhibit potent activity with IC_{50} value of 0.04 ± 0.01 mM, whereas the positive control acarbose showed an IC_{50} value of 0.07 ± 0.01 mM.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a micro melting point apparatus taike-X6. ORs were measured with a PE-341 polarimeter. IR spectra were recorded in KBr with a SHIMADZU FTIR-8400S spectrophotometer. The ^{1}H, ^{13}C, DEPT, HMBC, HMQC, and NOESY spectra were recorded on a Bruker-AV-600 spectrometer (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR). Measurements were done in pyridine-d_5 at ambient temperature with TMS as the internal standard. The HR-ESI-MS experiments were done on a JMS-T100CS mass spectrometer. The silica gel (200–300 mesh) used for column

chromatography (CC) was purchased from Qingdao Marine Chemical Factory, Qingdao, China. The TLC plates and silica gel used for ODS CC were purchased from Merck, Darmstadt, Germany. Spots were detected on the TLC plates under UV light at 254 and 365 nm, or by heating after spraying with 10% H_2SO_4 in C_2H_5OH. The D101 macropore resin was from Nan-kai University, Tianjin, China. All chemical solvents used for isolation were of analytical grade or higher.

3.2 Plant material

The plant of *D. panthaica* was collected from Xichang of Sichuan province of China in December 2008 and identified as rhizomes of *D. panthaica* by Prof. Gui-Fang Fu of China Academy of Chinese Medical Science. A voucher specimen (No. 200806) has been preserved in the Herbarium of China Academy of Chinese Medical Science.

3.3 Extraction and isolation

The dried rhizomes of *D. panthaica* were powdered and extracted with boiled water four times. After filtration, the water suspension was passed through a D_{101} macroporous resin column eluted successively with H_2O, 10% EtOH, and 75% EtOH. The 75% EtOH fraction was evaporated under reduced vacuum to produce the extract (55 g). Then the extract was chromatographed over a silica gel column and eluted with a gradient $CHCl_3$–MeOH from 5:1 to 0:1. Three fractions (A–C) were collected at last. Fraction B (14.5 g) was further subjected to chromatography on a silica gel column and eluted with a gradient $CHCl_3$–MeOH–H_2O from 50:10:0 to 50:10:1 to obtain four sub-fractions (Fr. B1–Fr. B4). The sub-fraction Fr. B4 (4.2 g) was isolated by silica gel CC with $CHCl_3$–MeOH–H_2O (30:10:1) as eluent to obtain two sub-fractions (Fr. B4.1 and Fr. B4.2). The sub-fraction Fr. B4.1 (100 mg) was further purified by an ODS CC with MeOH–H_2O (4:1) as eluent to obtain compounds **2** (55 mg) and **3** (28 mg). Fraction C (23.5 g) was further subjected to silica gel CC with a gradient $CHCl_3$–MeOH–H_2O (28:10:1-10:10:1) as eluent to obtain four fractions (Fr. C1–Fr. C4). The sub-fraction Fr. C3 (2.6 g) was isolated by repeated silica gel CC with $CHCl_3$–MeOH–H_2O (14:6:1) as eluent and further purified by ODS eluted with H_2O–MeOH (13:7) to yield compounds **1** (25 mg) and **4** (38 mg).

3.3.1 *3-O-[α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl]-26-O-β-D-glucopyranosyl-20,22-seco-25(R)-furosta-5-ene-20,22-dione-3β,26-diol*

White amorphous powder, mp 178–180°C, $[\alpha]_D^{20}$ −47.0 ($c = 0.095$, MeOH), IR (KBr) ν_{max}: 3413, 2926, 1736, 1710, 1042 cm^{-1}. For ^1H NMR and ^{13}C NMR spectral data see Table 1. HR-ESI-MS *m/z*: 934.5046 $[M + NH_4]^+$ (calcd for $C_{45}H_{76}O_{19}N$, 934.5011).

3.4 Acid hydrolysis of compound 1

Compound **1** (1.5 mg) was hydrolyzed with 2 M CF_3COOH (5 ml) at 100°C for 9 h in a sealed tube. After cooling, the reaction mixture was extracted with $CHCl_3$ (3.5 ml). The aqueous layer was evaporated with N_2. After evaporation, H_2O (1 ml) was added to the residue and the solution was analyzed by HPLC carried out on an Agilent 1200 system equipped with a quaternary solvent delivery system under the following conditions: column, Agilent ZORBAX NH_2 (5 μm, 250 mm × 4.6 mm i.d.); solvent, MeCN–H_2O (17:3); flow rate, 0.9 ml/min; and detection, Alltech ELSD 2000ES. HPLC analysis of the sugar fraction under the same conditions as in the case of **1** showed the presence of D-glucose and L-rhamnose. t_R (min): 27.81 (D-glucose, positive OR), 12.54 (L-rhamnose, negative OR).

3.5 Evaluation of α-glucosidase inhibitory activity

The assessment of α-glucosidase inhibitory activity was performed later. Briefly, 100 mg of rat intestinal acetone powder was homogenized in 3 ml of 0.1 M phosphate buffer (pH 6.8) at 4°C. After centrifugation (BECKMAN X-22R) for 20 min, 10 μl of the supernatant was incubated with 100 μl of the substrate solution (0.25 M maltose) and 100 μl of the sample at various concentrations in 0.1 M phosphate buffer (pH 6.8) at 37°C for 20 min. The reaction was stopped by adding 1 M sodium carbonate (dissolved in deionized water). The released glucose was measured spectrophotometrically at 505 nm on a microplate reader (Model 3550 Microplate Reader, Bio-Rad, Hercules, CA, USA) at ambient temperature. Acarbose was used as the positive control for this assay. The α-glucosidase inhibitory activity was expressed as an IC_{50} value.

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