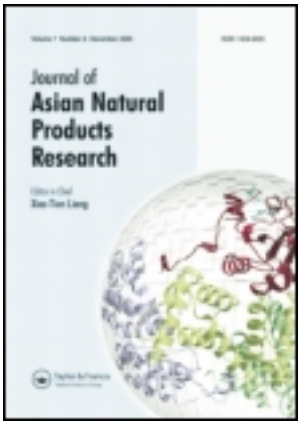

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A new triterpenoid saponin from Gymnema sylvestre

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NOTE

A new triterpenoid saponin from *Gymnema sylvestre*

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Besides four known compounds, a new triterpenoid saponin was isolated from the stems of *Gymnema sylvestre*. The structure of the new triterpenoid saponin was established as 3β,16β,22α-trihydroxy-olean-12-ene 3-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside (**1**) on the basis of 1D and 2D NMR techniques, including COSY, HMBC, HMQC, and NOESY correlations. Four known compounds **2**, **3**, **4**, and **5** were identified on the basis of spectroscopic data.

Keywords: *Gymnema sylvestre*; triterpenoid saponin; Asclepiadaceae

1. Introduction

The plant *Gymnema sylvestre* R. Br. (Asclepiadaceae) is a vine which grows in the southwestern region of China, including the Guangdong, Guangxi, and Fujian provinces. This plant species is also distributed in India where it is used as a stomachic, diuretic, and anti-diabetic remedy. The total saponin fraction of the leaves had an anti-sweetening effect and was shown to be able to inhibit glucose absorption in the small intestine and to suppress elevated glucose levels in blood following the administration of sucrose in rats [1,2]. The antisweet properties of the extracts as well as some of the saponin components have also been reported [3–7]. In this study, we report the structure elucidation of a new triterpenoid saponin, which was isolated from the stems of *G. sylvestre*, by using 1D, 2D NMR techniques, MALDI/TOF-MS, ESI-MS analysis as well as chemical methods.

2. Results and discussion

Compound **1** was obtained as an amorphous white powder. The molecular formula, $C_{47}H_{78}O_{17}$, was determined on the basis of positive MALDI/TOF-MS (*m/z* 937 $[M + Na]^+$, *m/z* 953 $[M + K]^+$) and HR-MS data (*m/z* 937.5131 $[M + Na]^+$). Further fragment ion peaks in the ESI-MS/MS were observed at *m/z* 805 $[M\text{-}132 + Na]^+$ and 481 $[M\text{-}132\text{-}162\text{-}162 + Na]^+$ due to the loss of terminal pentose and two hexose units. Among the 47 carbons in the ^{13}C NMR spectrum, 30 were assigned to the triterpenoid aglycone, and 17 to the oligosaccharide moiety. Eight signals of the triterpenoid part were assigned to methyl carbons at δ 15.8, 17.0, 17.1, 24.0, 26.3, 26.8, 28.4, and 33.6, and the corresponding protons were identified by a HSQC experiment (Table 1). Three oxygen-bearing methine carbons were found at δ 66.8, 75.8, and 89.1 and a pair of olefinic carbons was observed at δ 122.8

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Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** (recorded at 500/125 MHz in pyridine-d_5; δ in ppm, J in Hz).

No.	δ_c	δ_H (J in Hz)	No.	δ_c	δ_H (J in Hz)
1	38.9	0.90 (m), 1.48 (m)	C-3		
2	26.8	1.22 (m), 2.30 (m)	Glc 1	107.0	4.74 (d, 8.4)
3	89.1	3.22 (dd, 11.7, 4.0)	2	75.1	3.85 (m)
4	39.7	–	3	78.5	4.04 (m)
5	55.9	0.63 (d, 11.2)	4	71.2	4.16 (m)
6	18.6	1.20 (m), 1.38 (m)	5	77.1	3.99 (m)
7	33.1	1.38 (m)	6	70.0	4.80 (overlapped)
8	40.3	–			4.66 (d, 11.2)
9	47.2	1.76 (m)	Glc′ 1	105.5	4.92 (d, 7.6)
10	36.1	–	2	75.7	3.85 (m)
11	24.2	1.72 (m), 0.89 (m)	3	78.7	4.04 (m)
12	122.8	5.11 (m)	4	71.7	4.12 (m)
13	144.0	–	5	76.9	4.04 (m)
14	41.2	–	6	69.1	4.31 (d, 10.4)
15	36.9	1.65 (m), 2.13 (m)			4.19 (overlapped)
16	66.8	4.53 (br s)	Xyl 1	106.1	4.83 (d, 7.2)
17	43.9	–	2	74.9	3.85 (m)
18	44.6	2.28 (dd, 13.5, 4.0)	3	78.2	4.04 (m)
19	47.2	1.76 (m), 1.25 (m)	4	70.5	4.04 (m)
20	31.2	–	5	67.1	3.54 (d, 10.0)
21	42.9	1.65 (m), 2.13 (m)			4.53 (overlapped)
22	75.8	3.48 (m)			
23	28.4	1.12 (3H, s)			
24	17.0	0.85 (3H, s)			
25	15.8	0.75 (3H, s)			
26	17.1	0.94 (3H, s)			
27	26.3	1.71 (3H, s)			
28	26.8	2.30 (3H, s)			
29	33.6	0.85 (3H, s)			
30	24.0	0.90 (3H, s)			

and 144.0. These data, coupled with information from the ^{1}H NMR spectrum: eight methyl singlets at δ 0.75, 0.85, 0.88, 0.90, 0.94, 1.12, 1.71, and 2.30; three oxygen-bearing methine protons at δ 3.22 (dd, $J = 11.7$, 4.0 Hz), 3.48 (m), and 4.53 (br s), and an olefinic proton at δ 5.11 (m), were characteristic signals of a polyhydroxyolean-12-ene triterpene derivative. The carbon signal at δ 66.8, which correlated with H-15 (δ 1.65), H-18 (δ 2.28), and H-28 (δ 2.30) in the HMBC spectrum (Figure 1), could be assigned to C-16, which was also supported by the splitting pattern of H-16 (δ 4.53, br s). The proton signal at δ 3.48 could be assigned to H-22 on the basis of its correlations with C-16 (δ 66.8), C-18 (δ 44.6), C-21 (δ 42.9), and C-28 (δ 26.8), in the HMBC spectrum (Figure 1). The correlations between H-16 (δ 4.53), H-27 (δ 1.71), H-7 (δ 1.38), and H-5 (δ 0.63) in the NOESY spectrum indicated a β-configuration of the hydroxyl group at C-16. The correlation between H-22 (δ 3.48) and H-30 (δ 0.90) in the NOESY spectrum indicated an α-configuration of the hydroxyl group at C-22, which was corroborated by the splitting pattern of H-22 (m). Correlations of H-3 (δ 3.22) with H-5 (δ 0.63) and H-23 (δ 1.12) in the NOESY spectrum indicated a β-configuration of the hydroxyl group at C-3. Thus, the aglycon of **1** could be identified as 3β,16β,22α-trihydroxyolean-12-ene.

The sugar part of **1** consisted of three residues as evidenced by ^{1}H and ^{13}C NMR

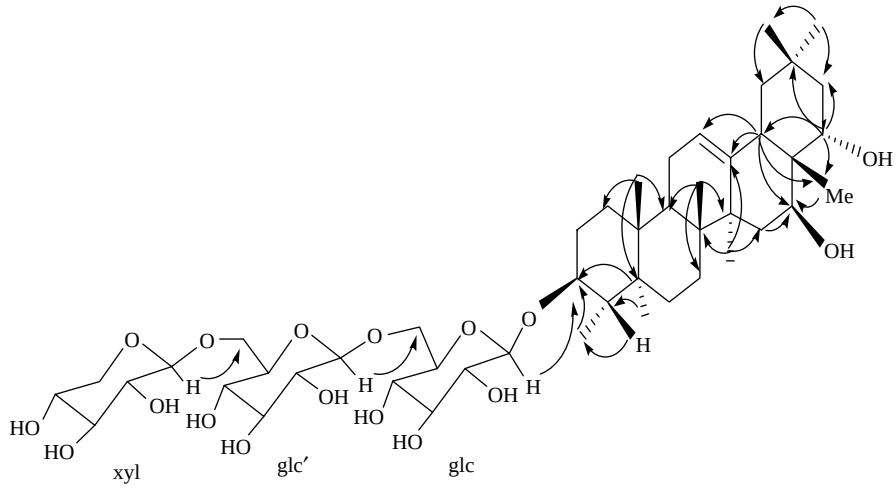

Figure 1. Key HMBC correlations for **1**.

spectra which displayed three anomeric protons at δ 4.74 (d, $J = 8.4$ Hz), 4.92 (d, $J = 7.6$ Hz), and 4.83 (d, $J = 7.2$ Hz), attached to anomeric carbons at δ 107.0, 105.5, and 106.1, respectively, in the HSQC spectrum. Acid hydrolysis and analysis of COSY, HMQC, and NOESY experiments allowed the full identification of the sugar residues as one β-D-xylopyranosyl(xyl) and two β-D-glucopyranosyl (glc and glc′) groups. Their anomeric configurations were determined by coupling constants and by the comparison of ^{13}C NMR spectral data with those in the literature [8]. The coupling constants of anomeric protons of glc (8.4 Hz), glc′ (7.6 Hz), and xyl (7.2 Hz) groups, along with the correlations between H-1 (δ 4.74) and H-5 (δ 4.14) of glc group, H-1 (δ 4.92) and H-5 (δ 4.14) of glc′ group, in the NOESY experiment, indicated β-configuration for the glc and glc′ groups, and the correlations between H-1 (δ 4.83), H-3 (δ 4.15), and H-5 (δ 4.18) of the xylose unit in the NOESY experiment confirmed the α-axial orientation of these protons and the β-anomeric configuration. In addition, the long-range correlations were observed between H-1 of glc at δ 4.74 and C-3 of the aglycone at δ 89.1, between H-1 of glc′ group at δ 4.92 and C-6 of glc group at δ 70.0, and between H-1 of xyl group at δ 4.83 and C-6 of glc′ group at δ 69.1 (Figure 1). Consequently, the structure of compound **1** was concluded to be 3β,16β,22α-trihydroxy-olean-12-ene 3-*O*-β-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranoside.

Four known compounds **2**–**5** were identified as 3-*O*-β-D-glucopyranosyl (1 → 3)-α-L-rhamnopyranosyl chichipegenin 28-α-L-rhamnopyranoside (**2**) [9], oleanolic acid 3-*O*-β-D-glucopyranosyl (1 → 6)-β-D-glucopyranoside (**3**) [10], oleanolic acid 3-*O*-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**4**) [3], and 3-*O*-β-D-xylopyranosyl(1 → 6)-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl ester (**5**) [3] by comparison of their physical and spectroscopic data with those reported in the literature, respectively.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on WZZ-2S auto-polarimeter (Shanghai, China). Melting points were determined on an X-6 microscope apparatus and are

uncorrected (Beijing, China). IR spectra were measured as films on KBr pellets using a Bruker VERTEX70 spectrometer (Bruker Corporation, Ettlingen, Germany). NMR spectra were obtained on a Bruker AV600 instrument (Bruker Company, Rheinstetten, Switzerland), using tetramethylsilane as internal standard. The HR-MS and MS/MS were recorded on autoflex III MALDI/TOF/TOF-MS instrument (Bruker Company, Ettlingen, Germany). HPLC (Shimadzu Company, Kyoto, Japan) was carried out using an octadecylsilane bonded silica column (HC-ODS, 250 × 4.6 mm). Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Oceanic Chemical Industry, Qingdao, China), reversed silica gel (20 × 20 cm, MERCK KGaA, Darmstadt, Germany), and macroporous resin D_{21} made in Shandong Lu Neng Gel Co. (Shandong, China). Spots were detected after spraying with 10% H_2SO_4.

3.2 Plant material

The stems of *G. sylvestre* were purchased from the Company of Chinese Medicinal Materials in Nanning, Guangxi Province, China, in September 2009, and were identified by Professor Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (No. 201008) has been deposited in the phytochemistry laboratory of Jilin Academy of Chinese Medicine Sciences.

3.3 Extraction and isolation

The dried and powdered stems (9.5 kg) of *G. sylvestre* were exhaustively extracted with 75% EtOH, and then heated on steam bath to remove EtOH. The extract (105 g) was partitioned between EtOAc and H_2O. The water solution was chromatographed on 1.5 kg D_{21} macroporous resin, eluting with water until the elute was colorless, and then with 50% EtOH (6 liters). The 50% EtOH solution was further subjected to neutral resin to remove most of color material and then evaporated to dryness to give crude saponins (27 g). Part of the crude saponins (27 g) was chromatographed on silica gel (200–300 mesh) with $CHCl_3$–MeOH gradients 20:1 to 10:15 and finally with MeOH (500 ml per part), to give Frs 1–7. Fr. 4 (5.0 g) was subjected to repeated column chromatography on silica gel (200 mesh, 520 g) with $CHCl_3$–*n*-BuOH–H_2O gradients (8:2:0.2 to 7:3:0.5, 250 ml per part) to afford Frs 4-1–4-6. Fr. 4-2 (330 mg) was subjected to HPLC eluting with 45% MeOH (flow velocity 5 ml/min, evaporative light-scattering detection, retention time 18 min) to give compounds **1** (43 mg) and **2** (28 mg). Fr. 4-3 (380 mg) was further purified on an ODS column using MeOH–H_2O (30:70 to 70:30) as eluent to afford compounds **3** (45 mg), **4** (30 mg), and **5** (35 mg).

3.3.1 Compound *1*

Amorphous powder, mp 200–201°C; $[\alpha]_D^{25}$ + 6.3 (*c* 3.2, MeOH); IR (KBr) ν_{max} 3326, 2940, 1051, and 1168 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. ESI-MS: *m/z* 937 $[M + Na]^+$, 953 $[M + K]^+$, 805 $[M + Na\text{-}132]^+$, and 481 $[M + Na\text{-}132\text{-}162\text{-}162]^+$. HR-TOF-MS: *m/z* 937.5131 $[M + Na]^+$ (calcd for $C_{47}H_{78}O_{17}Na$, 937.5137).

3.4 Acid hydrolysis

Compound **1** (10 mg) was dissolved in 1 mol/l HCl in MeOH–H_2O (1:1) and refluxed for 2 h. The reaction mixture (10 μl) was analyzed by analytical HPLC using gradient elution of 15–85% aqueous CH_3CN containing 0.1% acetic acid, at a flow rate of 1 ml/min, over a 35 min run. D-Glucose (rt 17.15 min) and D-xylose (rt 17.90 min) were identified by comparing their retention times with those of authentic samples, while L-glucose and L-xylose

showed different retention times of 17.09 and 17.86 min, respectively.

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