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Steroidal saponins from the rhizomes and roots of *Smilax scobinicaulis*

Cun-Li Zhang [a,b], Jin-Ming Gao [a,c,*], Wei Zhu [a]

[a] College of Science, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China
[b] College of Life Science, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China
[c] Shaanxi Engineering Center of Bioresource Chemistry & Sustainable Utilization, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China

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ABSTRACT

Two new spirostane-type steroidal saponins, named smilscobinosides A (**1**) and B (**2**), together with a known congener (**3**), have been isolated from the EtOH extract of the rhizomes and roots of *Smilax scobinicaulis*. The structures of the new compounds were determined by means of chemical evidence and 1D- and 2D-NMR spectroscopic analysis, FABMS and HRESIMS.

1. Introduction

The genus *Smilax* (family Liliaceae) comprises about 300 species mainly distributed in the tropical and subtropical areas of the world, of which 79 species are native to China. Many of them have long been used as herbal medicines (Ju and Jia, 1992, 1993; Nikaido et al., 1992a; Woo et al., 1992; Bernardo et al., 1996; Satou et al., 1996; Li et al., 2006; Shao et al., 2007; Belhouchet et al., 2008). Phytochemical investigations of this genus have revealed that it is a rich source of steroidal saponins (Liu et al., 2001; Belhouchet et al., 2008), some of which showed antimicrobial, cytotoxic, as well as cAMP phosphodiesterase inhibitory bioactivities (Nikaido et al., 1992b; Liu et al., 2001; Shu et al., 2006; Sautour et al., 2005, 2006). The roots of *Smilax scobinicaulis* C.H. Wright, known as "Hei Ci Ba Qia" in Chinese, are used in Chinese traditional medicine for the treatment of rheumatic arthritis, lumbago, gout, tumors and inflammatory diseases (Zhang et al., 2003a). Previous chemical investigations of this plant led to the isolation of some steroidal saponins (Zhang et al., 2003a,b).

In continuation of our ongoing search for new biologically bioactive metabolites from traditional Chinese herbal medicines grown in Qinling mountainous region, Shaanxi province, China, we reinvestigated chemical constituents of the 70% EtOH extract of the rhizomes and roots of *S. scobinicaulis*, resulting in the isolation of two new spirostane-type steroidal saponins, named smilscobinosides A (**1**) and B (**2**), and a known relative **3**. Herein, we report the isolation, structure determination, and cytotoxic activities of these compounds.

2. Results and discussion

The 70% EtOH extracts of the air-dried rhizomes and roots of *S. scobinicaulis* were suspended in water and extracted successively with petroleum ether, EtOAc and *n*-BuOH. The *n*-BuOH portion was subjected to column chromatography on Diaion HP-20, RP-C18 and silica gel followed by preparative HPLC, affording two new steroidal saponins **1** and **2** along with a known analogue **3** (Fig. 1). Compound **3** was identified as (25*S*)-5α-spirostan-3β,17α, 27-triol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside by comparison of its NMR and MS data with those reported in the literature (Zhang et al., 2003b).

Compound (**1**) was obtained as a white amorphous powder and showed a positive reaction in the Liebermann–Burchard test. It exhibited the molecular formula $C_{44}H_{70}O_{18}$, as deduced from the quasi-molecular ion peak $[M-H]^-$ at *m/z* 885.4481 (calcd. for $C_{44}H_{69}O_{18}$, 885.4483) in the negative HRESI-MS and ^{13}C NMR data (Tables 1 and 2). Its IR spectrum showed strong absorptions for hydroxy groups (3473 and 1085 cm^{-1}). The ^1H NMR spectrum (Table 2) showed signals for three anomeric protons at δ_H 4.92 (d, J = 7.8 Hz, H-1′), 5.59 (d, J = 7.8 Hz, H-1″), and 5.10 (d, J = 7.5 Hz,

* Corresponding author at: College of Science, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China. Tel.: +86 29 87092335; fax: +86 29 87092335.
E-mail address: jinminggao@nwsuaf.edu.cn (J.-M. Gao).

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Table 1
NMR data of the aglycone moieties of **1** and **2** in pyridine-d_5 (δ in ppm, J in Hz).[a]

Aglycone moiety	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	37.5	1.76 m, 1.00 m	37.2	1.49 m, 0.75 m
2	30.3	2.12 m, 1.74 m	30.0	1.95 m, 1.68 m
3	78.6	3.81 m	77.6	3.83 m
4	39.6	2.83 m, 2.72 m	34.8	1.83 m, 1.56 m
5	141.0		44.6	0.84 m
6	121.7	5.29 m	28.9	1.10 m
7	32.2	2.02 m, 1.66 m	32.4	1.93 m, 1.32 m
8	31.7	1.63 m	35.2	1.33 m
9	50.3	1.02 m	54.4	0.45 m
10	37.1		35.2	
11	21.1	1.64 m, 1.61 m	21.3	1.40 m, 1.32 m
12	39.9	2.33 m, 1.62 m	40.1	1.60 m, 0.97 m
13	40.5		40.8	
14	56.7	2.17 m	56.4	0.95 m
15	32.3	2.24 m, 1.65 m	32.1	1.45 m, 0.73 m
16	81.0	4.45 m	81.2	4.44 m
17	62.6	1.73 m	62.6	1.72 m
18	16.4	0.89 s	16.6	0.81 s
19	19.4	0.83 s	12.3	0.64 s
20	42.1	1.84 m	42.1	1.83 m
21	15.1	1.16 d (6.9)	15.0	1.14 d (6.8)
22	109.8		109.7	
23	31.6	2.57 m, 1.73 m	31.6	2.58 m, 1.73 m
24	24.1	2.18 m, 1.95 m	24.1	2.18 m, 1.96 m
25	39.2	2.03 m	39.2	2.02 m
26	64.1	3.89 dd (3.5, 11) 3.64 dd (11, 10.5)	64.1	3.88 dd (3.5,10.5) 3.64 dd (10.5, 11)
27	64.5	3.73 dd (5.3, 11) 3.66 dd (7.2, 11)	64.4	3.74 dd (5.2, 11) 3.64 dd (7.2, 11)

[a] The assignments were based on the DEPT, HSQC, H-H COSY and HMBC experiments.

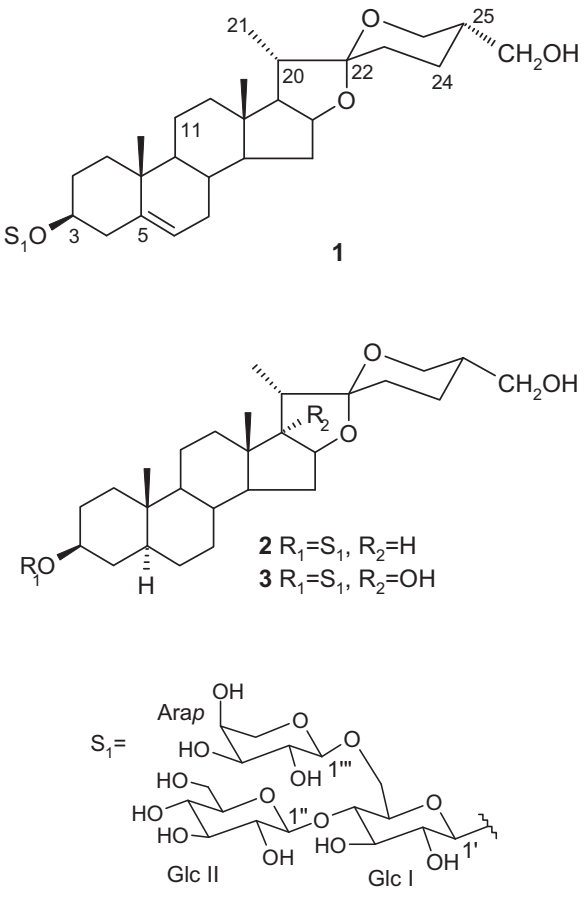

Fig. 1. Chemical structures of compounds **1**–**3**.

H-1‴), which showed correlations in the HSQC spectrum with three anomeric carbon signals at δ_C 102.6, 104.9, and 105.7, respectively. Acid hydrolysis of **1** gave D-glucose and L-arabinose, which were confirmed by co-TLC comparison with the authentic samples and the reaction with L-cysteine methyl ester hydrochloride followed by GC analysis (Hara et al., 1987). These sugar units were consistent with the negative FABMS spectrum of **1**, which showed its quasi-molecular ion peak $[M-H]^-$ at m/z 885, and two fragment ion peaks $[M-pentose-H]^-$ at m/z 753 and

Table 2
NMR data of the sugar portion of **1** and **2** in pyridine-d_5 (δ in ppm, J in Hz).[a]

Sugar moiety	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
3-Glc I-1′	102.6	4.92 d (7.8)	102.6	4.92 d (7.5)
2′	74.6	3.88 dd (9.3, 7.8)	74.6	3.88 dd (9.3, 7.8)
3′	76.5	4.24 overlapped	76.5	4.24 overlapped
4′	81.2	4.42 dd (9.2, 8.8)	81.2	4.42 dd (9.2, 8.8)
5′	74.8	3.96 m	74.8	3.96 m
6′	68.3	4.84 dd (13.2, 8.0) 4.57 dd (8.0, 5.6)	68.4	4.84 dd (13.2, 8.0) 4.57 dd (8.0, 5.6)
4′-Glc II-1″	104.9	5.59 d (7.8)	104.9	5.57 d (7.8)
2″	75.3	4.10 dd (9.0, 7.8)	75.2	4.10 dd (9.0, 7.8)
3″	78.6	4.31 dd (9.0, 9.2)	78.5	4.31 dd (9.0, 9.2)
4″	71.9	4.25 overlapped	71.8	4.25 overlapped
5″	78.5	4.17 dd (3.5, 10)	78.5	4.17 dd (3.5, 10)
6″	62.6	4.30 dd (5.5, 11.5) 4.20 t (10.5, 9.0)	62.5	4.30 dd (5.5, 11.5) 4.20 t (10.5, 9.0)
6′-Ara-1‴	105.7	5.10 d (7.5)	105.6	5.27 d (7.4)
2‴	72.6	4.53 dd (8.7, 7.5)	72.6	4.53 dd (8.7, 7.5)
3‴	74.8	4.06 dd (8.7, 3.6)	74.8	4.06 dd (8.6, 3.6)
4‴	69.9	4.23 overlapped	69.8	4.23 overlapped
5‴	67.3	4.24 overlapped 3.72 br d (11.5)	67.3	4.24 overlapped 3.72 br d (11.5)

[a] The assignments were based on the DEPT, HSQC, H-H COSY and HMBC experiments.