MRC Letters

Magnetic Resonance in Chemistry

Received: 23 July 2012 Revised: 10 September 2012 Accepted: 11 September 2012 Published online in Wiley Online Library: 8 October 2012

(wileyonlinelibrary.com) DOI 10.1002/mrc.3883

Steroidal saponins and pregnane glycosides from *Smilax microphylla*

Tao Lin,[a] Hui-Lian Huang,[a]* Rong-Hua Liu,[a] Ji-Cheng Shu,[a] Gang Ren,[a] Feng Shao[a] and Li-sha Liu[a]

Six steroidal saponins and two pregnane glycosides were isolated from the BuOH subfraction of 70% EtOH extract of *Smilax microphylla* C.H.Wright, among them two were new compounds (1 and 7). Pregnane glycosides were firstly isolated from the genus *Smilax* (Smilacaceae). Structures of the new compounds were determined on the basis of HR-ESI-MS, 1D and 2D NMR spectroscopic analysis. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: steroidal saponins; pregnane glycosides; ^{1}H NMR; ^{13}C NMR; *Smilax microphylla*

Introduction

The genus *Smilax* (Smilacaceae) comprises about 370 species, which are mainly distributed in the tropical and temperate zones throughout the world, especially in East Asia and North America.[1] Many steroidal saponins have been isolated and identified from the family of Smilacaceae.[2–10]

Smilax microphylla C.H.Wright is a perennial plant distributed in Southeast of China and indigenous to Hunan, Hubei, Jiangxi, Zhejiang, Jiangsu and Guangxi Provinces of China. It is an evergreen shrub or semi-shrub with climbing branches and stapler tendrils. Its roots are used in traditional Chinese medicine to dispell wind evil, eliminate dampness and detoxify.[11] *S. macrophylla*, administered at doses of 1 or 2 g/kg in normal rats or in hyperuricemic or hyperuricosuric rats, increases the excretion of uric acid and allantoin.[12] Previous investigations have demonstrated that the rhizomes and roots of *Smilax microphylla* are rich in steroidal saponins.[13]

In previous papers, we reported the isolation and structural determination of new steroidal saponins, phenylpropanoid-substituted catechins, and epicatechins from *Smilax china*.[14,15] Until now, a chemical study of the species of *Smilax microphylla* has not been made, and there has not been any report on the constituents of this plant. In the continuation of our ongoing search on the phytochemistry of plants of the Smilacaceae family, we investigated chemical constituents of the BuOH subfraction of 70% EtOH extract of *Smilax microphylla*, resulting in the isolation of two new compounds, named 23-oxo-pseudoprotodioscin and pregna-5,16-diene-3β-ol-20-one 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosid. Herein, we report the isolation and structure determination of these compounds.

Results and Discussion

Compound **1** (Fig. 1) was isolated as a colorless amorphous solid with a molecular formula of $C_{51}H_{80}O_{22}$ as determined by its positive ion at *m/z* 1067.6741 $[M+Na]^+$ in the HR-ESI-MS. The structure of **1** was identified by comparison of its ^{1}H, ^{13}C NMR (Tables 1 and 2), HSQC, DEPT, and HMBC data with those of congeners from *Smilax menispermoidea*[9] and *Asparagus cochinchinensis*.[16]

The ^{1}H NMR spectrum of **1** showed tertiary methyl groups observed at δ_H 0.68 (s, H-19), δ_H 1.02 (s, H-18), δ_H 2.10 (s, H-21), while one secondary methyl group resonated as a doublet at δ_H 1.01 (d, J = 5.6 Hz, H-27); a trisubstituted olenfinic proton at δ_H 5.31 (br d, H-6); and two oxymethylene protons at δ_H 3.74 (dd, J = 9.6 and 6.0 Hz, H-26a), δ_H 4.08 (dd, J = 9.6 and 6.4 Hz, H-26b). The DEPT and ^{13}C NMR spectra showed 51 signals comprising 6 methyls, 11 methylenes, 28 methines, and 6 quaternary carbons. Three signals at δ_C 19.4 (C-18), δ_C 13.9 (C-19), and δ_C 13.3 (C-21) were assigned to angular methyl groups. Four signals at δ_C 140.8 (C-5), δ_C 121.7 (C-6), δ_C 123.9 (C-20), and δ_C 148.8 (C-22) were assigned to olefinic carbons. Moreover, in the HSQC and HMBC spectrum of **1**, the signals of the H-atoms at C-26 were assigned to H-26a (δ_H 3.74) and H-26b (δ_H 4.08). With a difference (Δ_{ab}) in chemical-shift of 0.34 (Δ_{ab} (δ_a–δ_b) < 0.48), we can deduce a 25*R* configuration. So, **1** can be easily recognized to be a $\Delta^{5,20}$ and 25*R* furostanol derivative.[17–19] The carbonyl at C-23 was confirmed by the deshielding δ_c 195.8 (C-23). In addition, HMBC experiments showed the correlations between H-21 and C-17, C-20 and C-22; between H-25 and C-23, C-24, C-26 and C-27; between H-24 and C-23, C-25. The correlations of the chain bound to δ 195.8 (C-23) were thus identified (Fig. 2).

The sugar moieties were also determined similarly. HSQC spectrum showed signals for four sugar anomeric protons at δ_H 4.94 (H-1′), δ_H 6.40 (H-1″), δ_H 5.86 (H-1‴), and δ_H 4.81 (H-1⁗), which have correlations with four anomeric carbon signals at δ_C 100.3 (C-1′), δ_C 102.1 (C-1″), δ_C 102.9 (C-1‴), and δ_C 105.1 (C-1⁗), respectively (Table 3). With an acid hydrolysis experiment, HPLC analysis revealed that compound **1** has two L-rhamnose and D-glucose sugars, which were confirmed by the chemical shifts of two methyl

* *Correspondence to: Hui-Lian Huang, Key Laboratory of Modern Preparation of TCM, Jiangxi University of Traditional Chinese Medicine, Ministry of Education, Nanchang 330004, China. E-mail: huilianh@gmail.com*

a *Key Laboratory of Modern Preparation of TCM, Jiangxi University of Traditional Chinese Medicine, Ministry of Education, Nanchang, China*

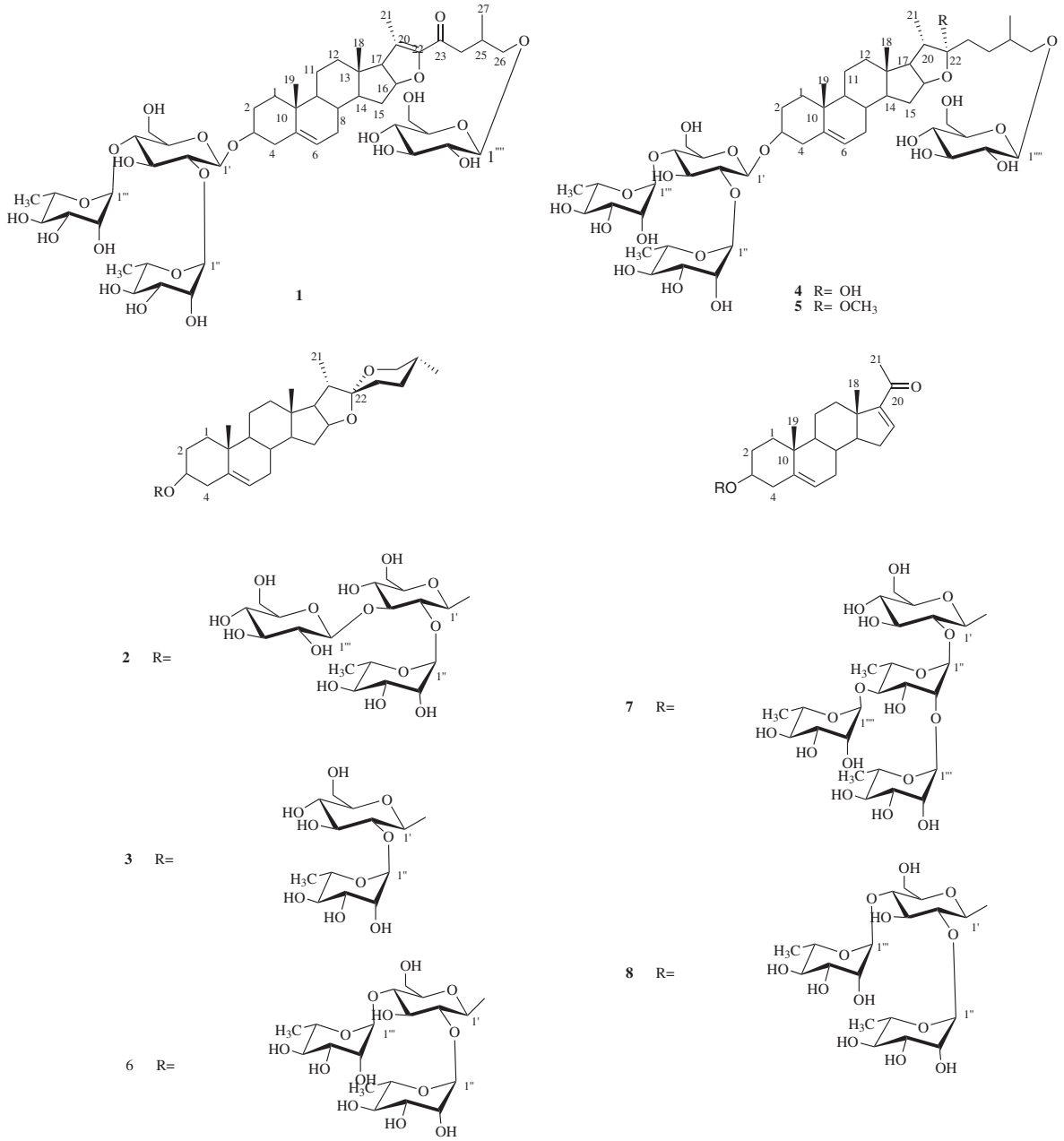

Figure 1. Structures of compounds **1–8**.

groups at δ_C 18.6 (C-6″) and δ_C 18.5 (C-6‴). Moreover, the connectivity of each sugar units from C-1 to C-6 was determined from HSQC and HMBC. Supported by the following HMBC experiments correlations: H-1′ (δ 4.94)/C-3 (δ 78.0); H-1″ (δ 6.40)/C-2′ (δ 77.69); H-1‴ (δ 5.86)/C-4′ (δ 78.54), and H-1⁗ (δ 4.81)/C-26 (δ 74.5) (Fig. 2). Therefore, **1** showed the presence of 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranosyl and 26-O-β-D-glucopyranosyl groups (Fig. 2).

On the basis of the above results, the structure of **1** was elucidated as (25*R*) 26-O-β-D-glucopyranosyl-5,20-diene-23-oxo-3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside and named 23-oxo-pseudoprotodioscin.

Compound **7** (Fig. 1), a white amorphous powder, was deduced as $C_{45}H_{70}O_{19}$ from its positive ion at *m/z* 937.5051 $[M + Na]^+$ in the HR-ESI-MS. The ^{1}H-NMR spectrum of **7** showed signals ascribable to three tertiary methyls δ_H 0.92 (s, H-18), δ_H 1.07 (s, H-19), and δ_H 2.26 (s, H-21), two olefinic protons δ_H 5.42 (br d, H-6), δ_H 6.81 (m, H-16) (Table 4). Observation of its ^{13}C NMR spectrum showed 45 carbon signals including 6 methyls, 8 methylenes, 26 methines, and 5 quaternary carbons. With the help of COSY, HSQC spectrum and the comparison of congeners from *Dioscorea collettii var. hypoglauca*[20] and *Dioscorea spongionsa*,[21] the pregnane skeleton of **7** was determined, supported by the following HMBC correlations: H-2/C-3; H-6/C-7, C-8; H-9/C-5, C-8, C-10, C-11; H-14/C-13, C-17; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9; H-21/C-17, C-20.

With the help of an acid hydrolysis experiment and HPLC analysis, it was revealed that compound **7** has one D-glucose and three L-rhamnose sugars, which were supported by the presence of three methyl groups at δ_C 16.47 (C-6″), δ_C 16.59 (C-6‴), and δ_C 17.18 (C-6⁗). Otherwise, the ^{1}H-NMR spectrum showed four sugar anomeric protons at δ_H H-1′ (d, δ_H 4.85), H-1″ (br s, δ_H 5.197), H-1‴ (br s, δ_H 4.51), and H-1⁗ (br s, δ_H 5.206) and three methyl

Table 1. ^{1}H NMR data of compounds **1–6**

Position	**1**	**2**	**3**	**4**	**5**	**6**
	δ_H (mult,*J*,Hz)	δ_H (mult,*J*,Hz)	δ_H (mult,*J*,Hz)	δ_H (mult,*J*,Hz)	δ_H (mult,*J*,Hz)	δ_H (mult,*J*,Hz)
1a	0.96 (m)	0.92 (m)	0.92 (m)	0.94 (m)	0.92 (m)	0.90 (m)
1b	1.72 (m)	1.73 (m)	1.72 (m)	1.73 (m)	1.75 (m)	1.75 (m)
2a	1.83 (dd,1.6,6.4)	1.84 (dd,2.4,6.4)	1.83 (dd,2.0,6.4)	1.84 (dd,1.6,6.4)	1.80 (dd,2.0,6.4)	1.75 (dd,2.4,6.4)
2b	2.05 (dd,1.6,6.4)	2.01 (dd,2.0,6.4)	2.12 (dd,2.0,6.4)	2.0 (dd,2.0,6.4)	2.15 (dd,1.6,6.4)	2.01 (dd,2.0,6.4)
3	3.87 (m)	3.81 (m)	3.89 (m)	3.85 (m)	3.84 (m)	3.80 (m)
4a	2.72 (dd,1.6,8.4)	2.75 (dd,2.0,8.0)	2.72 (dd,2.4,8.0)	2.78 (dd,2.4,7.6)	2.76 (dd,1.6,7.6)	2.44 (dd,2.4,8.0)
4b	2.81 (dd,2.4,8.4)	2.82 (dd,2.0,8.0)	2.80 (m)	2.85 (dd,2.0,7.6)	2.82 (dd,2.0,7.6)	2.80 (dd,2.0,8.0)
6	5.31 (br d)	5.36 (br d)	5.35 (br d)	5.37 (br d)	5.38 (br d)	5.37 (br d)
7a	1.48 (m)	1.42 (m)	1.42 (m)	1.42 (m)	1.45 (m)	1.46 (m)
7b	1.86 (dd,2.0,6.8)	1.75 (dd,2.4,6.8)	1.83 (dd,2.4,6.4)	1.92 (dd,2.0,6.8)	1.92 (dd,1.6,6.8)	1.90 (dd,2.0,6.8)
8	1.47 (m)	1.53 (m)	1.49 (m)	1.52 (m)	1.54 (m)	1.54 (m)
9	0.88 (m)	0.97 (m)	0.89 (m)	0.91 (m)	0.89 (m)	0.89 (m)
11a	1.37 (m)	1.42 (m)	1.41 (m)	1.41 (m)	1.49 (m)	1.40 (m)
11b	1.42 (m)	1.44 (m)	1.44 (m)	1.43 (m)	1.41 (m)	1.41 (m)
12a	1.16 (d,7.2)	1.15 (d,7.2)	1.13 (d,7.2)	1.15 (d,7.6)	1.17 (d,7.6)	1.13 (d,7.2)
12b	1.71 (d,7.2)	1.73 (d,7.2)	1.72 (m)	1.73 (m)	1.78 (m)	1.73 (m)
14	0.86 (m)	0.88 (m)	0.88 (m)	0.82 (m)	0.88 (m)	0.80 (m)
15a	1.53 (m)	1.48 (m)	1.51 (m)	1.52 (m)	1.54 (m)	1.49 (m)
15b	2.15 (m)	2.04 (m)	2.05 (m)	2.13 (m)	2.16 (m)	2.01 (m)
16	4.81 (m)	4.57 (m)	4.94 (m)	4.83 (m)	4.83 (m)	4.58 (m)
17	2.52 (d, 6.4)	2.43 (d, 6.4)	2.46 (d, 6.4)	2.30 (m)	2.43 (m)	2.43 (d, 6.4)
18	1.02 (s)	1.03 (s)	0.95 (s)	1.04 (s)	1.04 (s)	1.04 (s)
19	0.68 (s)	0.79 (s)	0.86 (s)	0.71 (s)	0.83 (s)	0.82 (s)
20		2.27 (m)	2.10 (m)	2.0 (m)	2.02 (m)	2.20 (m)
21	2.10 (s)	1.72 (d, 6.4)	1.73 (d, 6.4)	1.63 (d, 6.4)	1.64 (d, 6.4)	1.59 (d, 6.4)
23a		1.40 (m)	1.43 (m)	1.48 (m0	1.31 (m)	1.40 (m)
23b		1.85 (m)	1.83 (m)	2.15 (m)	2.02 (m)	1.76 (m)
24a	1.53 (m)	1.51 (m)	1.51 (m)	1.55 (m)	1.55 (m)	1.50 (m)
24b	2.01 (m)	1.70 (m)	1.69 (m)	2.30 (m)	2.29 (m)	1.68 (m)
25	2.68 (m)	1.65 (m)	1.61 (m)	1.41 (m)	1.50 (m)	1.66 (m)
26a	3.74 (dd,9.6,6.0)	3.44 (m)	3.52 (m)	3.72 (dd,9.6,6.8)	3.72 (m)	3.57 (m)
26b	4.08 (dd,9.6,6.4)	3.55 (m)	3.60 (m)	4.12 (dd,9.6,6.4)	4.11 (m)	3.43 (m)
27	1.01 (d, 5.6)	0.80 (d, 6.4)	0.74 (d, 6.4)	0.94 (d, 6.0)	1.01 (d, 6.0)	0.78 (d, 6.0)
OCH_3					3.30 (s)	

groups at H-6″ (d, δ_H 1.26), H-6‴ (d, δ_H 1.27), and H-6⁗ (d, δ_H 1.32) (Table 3). A combination of COSY and HMBC spectra concluded the connectivities from C-1 to C-6 of the sugar units. An HMBC experiment showed correlations between H-1′ (d, δ_H 4.85)/C-3 (δ_C 78.2); H-1″ (br s, δ_H 5.197)/C-2′ (δ_C 77.92); H-1‴ (br s, δ_H 4.51)/C-2″ (δ_C 75.30); and H-1⁗ (br s, δ_H 5.206)/C-4″ (δ_C 77.66) (Fig. 3). Therefore, connectivities of the saccharide moieties were determined to be 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl (Fig. 3).

From all the above data, the structure of **7** was identified as pregna-5,16-diene-3β-ol-20-one 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

Experimental Part

General experimental procedures:

NMR Spectra: a Bruker AM-400 spectrometer (^{1}H 400-MHz,^{13}C 400-MHz) in MeOH-d_4 at room temperature (25°C); HR–ESI–MS: Finnigan-LC-QDECA instrument, in *m/z*. Chemical shifts are in ppm (δ), relative to tetramethylsilane as internal standard, and coupling constants are in hertz; ^{1}H, HSQC, HMBC, and COSY by using the standard pulse sequences. Column chromatography: Sephadex LH-20 (Amersham Bio-sciences AB, Uppsala, Sweden); Silica gel (200–300 mesh); $HSGF_{254}$ for TLC were produced by Qing-dao Ocean Chemical Group Co. of China and C_{18} SPE Bulk Sorbent (Grace Davison Discovery Sciences). All solvents used for the isolation were of anal grade or higher.

Plant material

The tubers of *Smilax microphylla C.H.Wright* (Smilacaceae) were collected from Hunan province, China, in October 2010. The plant was identified by Xue-wen Lai, associate professor at Jiangxi University of Traditional Chinese Medicine, and a voucher specimen (No. 20101008) had been deposited at the Key Laboratory of Modern Preparation of TCM, Jiangxi University of TCM, China.

Extraction and isolation

A crude extract (535.5 g),refluxed with 70% aqueous EtOH three times (each for 1.5 h), which was suspended in water and

Table 2. ^{13}C NMB data for compound **1–6**

Position	**1**	**2**	**3**	**4**	**5**	**6**
	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	37.5	37.18	38.3	37.68	38.06	36.66
2	30.2	30.61	30.5	30.03	30.77	29.37
3	78.0	77.9	77.9	77.85	77.91	77.92
4	39.0	39.5	38.7	38.17	39.52	37.18
5	140.8	140.0	142.1	140.57	141.9	140.5
6	121.7	121.0	123.0	121.22	122.6	121.3
7	32.3	31.8	32.9	32.8	32.80	31.42
8	31.3	31.4	31.5	31.4	31.41	31.36
9	50.2	50.4	51.6	50.4	51.73	50.32
10	37.1	37.2	38.0	37.2	38.59	31.18
11	21.2	20.6	20.7	20.78	21.96	20.6
12	39.4	40.06	40.6	39.79	40.85	39.55
13	44.3	41.6	41.1	40.47	41.18	40.04
14	54.8	56.4	57.9	56.38	57.76	56.42
15	34.6	31.4	31.8	32.8	33.20	31.81
16	84.8	80.9	82.4	81.1	82.5	80.8
17	65.4	69.04	64.1	64.13	65.06	62.34
18	19.4	16.58	19.7	18.49	19.88	16.49
19	13.9	16.48	17.6	16.1	16.84	18.48
20	123.9	41.6	40.6	40.52	41.85	41.53
21	13.3	15.35	16.3	15.9	16.17	13.51
22	148.8	109.7	110.7	112.0	111.0	109.2
23	195.8	31.36	31.4	31.2	31.40	31.05
24	44.9	22.9	30.5	28.4	28.99	28.50
25	30.0	38.16	33.6	33.8	35.02	30.05
26	74.5	75.9	68.1	75.19	75.2	66.49
27	17.4	18.4	17.5	16.6	17.32	16.614
OCH_3					47.68	

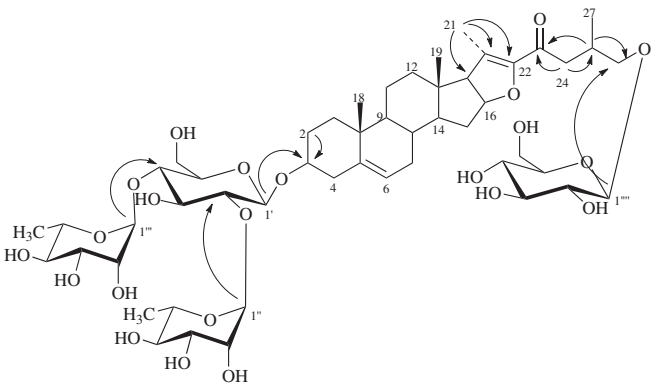

Figure 2. Key HMBC correlations (H → C) of compound **1**.

successively partitioned with petroleum ether, EtoAc, and n-butanol. The *n*-butanol soluble part was concentrated under reduced pressure, and the residue (236.1 g) was first chromatographed on the macroporous resin HP-20 and successively eluted with gradient mixture of EtOH-H_2O (3:7, 1:1, 7:3, 9:1) to get four fractions (A, B, C, and D).

Fr. A (30.5g) was separated by CC (silica gel; $CHCl_3$-MeOH-H_2O 4:1:0.1 to 6:4:0.2), then further purified by CC on Sephadex LH-20 (MeOH-H_2O, 1:1) to give **4** (12 mg), **5** (14 mg), and **3** (11 mg). Fr. C (13.1 g) was applied to a silica gel column using a mixture of

Table 3. ^{1}H and ^{13}C NMR data of the sugar portion of compoud 1 and 7

Position	1		7	
	δ_H (mult,J,Hz)	δ_C	δ_H (mult,J,Hz)	δ_C
1′	4.94 (d, 7.6)	100.3	4.85 (d, 8.0)	99.12
2′	3.87 (m)	77.69	3.41 (m)	77.92
3′	4.29 (m)	76.98	3.57 (m)	78.17
4′	3.64 (m)	78.54	3.76 (m)	79.46
5′	4.22 (m)	77.98	3.54 (m)	78.09
6′	4.09 (m),4.20 (m)	61.03	3.56 (m)	60.55
1″	6.40 (br s)	102.1	5.197 (br s)	101.8
2″	4.34 (m)	71.69	3.79 (m)	75.30
3″	4.01 (m)	72.59	3.64 (m)	71.53
4″	4.54 (m)	73.92	3.74 (m)	77.66
5″	4.58 (m)	65.3	3.90 (m)	68.40
6″	1.76 (d, 6.0)	18.6	1.26 (d, 6.0)	16.47
1‴	5.86 (br s)	102.9	4.51 (br s)	101.3
2‴	4.83 (m)	72.55	3.91 (m)	71.0
3‴	5.54 (m)	72.77	3.63 (m)	71.56
4‴	4.34 (m)	74.15	3.43 (m)	72.56
5‴	4.96 (m)	69.57	3.94 (m)	69.06
6‴	1.62 (d, 6.0)	18.5	1.27 (d, 6.0)	16.59
1⁗	4.81 (d, 7.6)	105.1	5.206 (br s)	100.9
2⁗	4.01 (m)	75.29	3.95 (m)	70.76
3⁗	3.84 (m)	77.26	3.58 (m)	76.59
4⁗	4.93 (m)	70.4	3.53 (m)	72.48
5⁗	4.62 (m)	72.86	4.06 (m)	67.72
6⁗	4.39 (m),4.55 (m)	62.87	1.32 (d, 6.0)	17.18

Table 4. Spectral data for compounds 7 and 8

Position	**7**		**8**	
	δ_H (mult)	δ_C	δ_H (mult)	δ_C
1	0.93(o), 1.64(o)	36.7	1.06 (m),1.69(m)	37.09
2	1.61(o), 1.92(o)	29.5	1.88 (m),2.07(m)	29.34
3	3.84(m)	78.2	3.56 (m)	77.89
4	2.38(m), 2.36(m)	38.1	2.32 (m),2.36(m)	38.13
5		140.9		140.87
6	5.42 (br d)	121.2	5.42 (br d)	121.06
7	1.84(m), 1.82(m)	31.3	2.02 (m),2.08 (m)	31.25
8	1.54 (m)	30.1	1.68 (m)	30.12
9	1.20 (m)	50.7	1.05 (m)	50.74
10		37.1		36.69
11	1.51 (m)	20.4	1.62 (m)	20.42
12	1.33(m), 2.36(m)	34.6	1.37(m),2.34(m)	34.59
13		46.0		45.9
14	1.44 (m)	56.4	1.30 (m)	56.43
15	2.35 (m)	31.9	2.34(m),2.40(m)	31.89
16	6.81 (m)	145.9	6.91 (m)	145.99
17		155.0		154.95
18	0.92 (s)	14.8	0.94 (s)	14.76
19	1.07 (s)	18.4	1.09 (s)	18.38
20		198.0		198.01
21	2.26 (s)	25.8	2.27 (s)	25.78

$CHCl_3$-MeOH (10:1 to 1:1) to give six fractions (1–6). Fr.C.2 was separated by column chromatography on silica gel with a step-wise gradient mixture of $CHCl_3$-MeOH-H_2O (8:3:0.1 to 6:4:0.2),

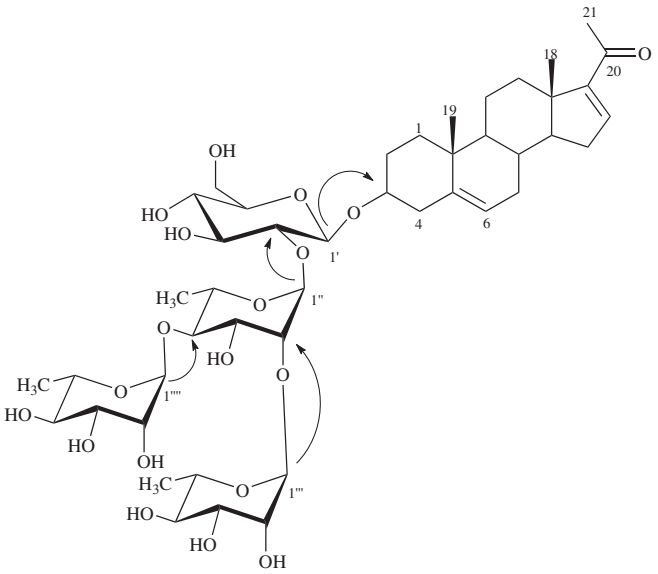

Figure 3. Key HMBC correlations (H → C) of compound **7**.

purified by Sephadex LH-20 column, and eluted with MeOH to afford **7** (10.2 mg) and **8** (50 mg). Fr.C.4 was subjected to Sephadex LH-20 column, eluted with MeOH, and then further purified by C_{18} reverse-phase chromatographic column with stepwise gradient mixture of MeOH-H_2O (3:2, 1:1, 7:3) to get **1** (12 mg). Fr. D (17.3 g) was applied to CC (silica gel; $CHCl_3$-MeOH-H_2O 8:3:0.1 to 6:4:0.2), followed by Sephadex LH-20 (MeOH-H_2O, 1:1) to afford **2** (8.2 mg) and **6** (12 mg).

23-oxo-pseudoprotodioscin, (25*R*) 26-O-β-D-glucopyranosyl-5,20-diene-23-oxo-3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside; **1**: colorless amorphous solid; IR ν_{max} KBr cm^{-1}: 3422, 1710, 1041; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

pregna-5,16-diene-3β-ol-20-one 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside; **7**: white amorphous powder; IRν_{max} KBr cm^{-1}: 3417, 1713, 1040; ^1H and ^{13}C NMR spectral data (Table 4).

Gracillin (**2**),[22] white amorphous powder; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

Prosapogenin A (**3**),[23] white amorphous powder; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

Protodioscin (**4**),[24] white amorphous powder; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

Methylprotodioscin (**5**),[9] white amorphous powder; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

Dioscin (**6**),[25] white amorphous powder; ^1H and ^{13}C NMR spectral data (Tables 1 and 2).

Pregna - 5,16- diene-3β-ol-20-one 3-O-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside (**8**),[20] white amorphous powder; ^1H and ^{13}C NMR spectral data (Table 4).

Acid hydrolysis of 1 and 7

Compounds **1** and **7** (2 mg, each) in 2 M CF_3COOH (5 mL) were hydrolyzed on a water bath for 4 h. After extraction with $CHCl_3$ (3 × 5 mL), the aqueous layer was evaporated to dryness with MeOH. The residue was dissolved in 1 mL H_2O, to which L-(−)-α-methyl-benzylamine (5 mg) and $NaBH_3CN$ (8 mg) in EtOH (1 mL) were added. After being stirred at 40 °C for 4 h followed by addition of glacial HOAc (0.2 mL) and evaporated to dryness, the reaction mixture was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) for 24 h. After evaporation, 1 mL H_2O was added to the residue, and the solution was passed through an SPE cartridge washed with H_2O, H_2O/MeCN (4:1, 1:1, v/v each 5 mL), successively. The H_2O/MeCN (1:1) eluate was analyzed, and the acetate derivatives were identified by HPLC analysis with the derivative of a standard sugar.[3,26] HPLC conditions: Inertsil ODS-3, 4.6 × 250 mm; solvent, MeCN /H_2O (3:3, v/v); flow rate: 1.0 mL/min; detection, UV absorbance at 230 nm. The derivatives of D-glucose and L-rhamnose were detected at t_R of 15.23 and 24.44 min, respectively.

Acknowledgements

The authors acknowledge the financial support of the National Natural Science Foundation of China (Grant Nos. 30960494 and 81160507) and Jiangxi Province NSFC (Grant No. 2009 GZY0099).

References

[1] Y. Tsukamoto. *The grand Dictionary of Horticulture* **1988**, *2*, 507. Shogakukan, Tokyo.
[2] M. Sautour, T. Miyamoto, M. A. Lacaille-Dubois. *J. Nat. Prod.* **2005**, *68*, 1489.
[3] B. Shao, H.-Z. Guo, Y.-J. Cui, M. Ye, J. Han, D.-A. Guo. *Phytochemistry* **2007**, *68*, 623.
[4] Y. Sashida, S. Kubo, Y. Mimaki, T. Nikaido, T. Ohmoto. *Phytochemistry* **1992**, *31*, 439.
[5] S. Kubo, Y. Mimaki, Y. Sashida, T. Nikaido, T. Ohmoto. *Phytochemistry* **1992**, *31*, 445.
[6] S. W. Kim, K. C. Chung, K. H. Son, S. S. Kang. *Saengyak Hakhoechi* **1989**, *20a*, 76.
[7] S. W. Kim, K. C. Chung, K. H. Son, S. S. Kang. *Saengyak Hakhoechi* **1989**, *20b*, 145.
[8] R. R. Bernardo, A. V. Pinto, J. P. Parente. *Phytochemistry* **1992**, *43*, 465.
[9] Y. Ju, Z.-J. Jia. *Phytochemistry* **1992**, *31*, 1349.
[10] Z. Belhouchet, M. Sautour, T. Miyamoto, M.-A. Lacaille-Dubois. *Chem. Pharm. Bull.* **2008**, *56*, 1324.
[11] Y.-H. Chen, Z.-Y. Liu, X. Liu, Z.-W. Zhang, J. Shen. *Resource Develepment Market* **2008**, *24*, 997.
[12] D. Giachetti, I. Taddei, E. Taddei. *Pharmacol. Res. Commun.* **1988**, *20*, 59–62.
[13] H.-L. Huang, H.-P. Huang, F. Shao, G. Ren, C. Wang, K.-L. Liu, F. Duan, R.-H. Liu. *J. Jiangxi University of TCM.* **2010**, *5*, 58.
[14] H.-L. Huang, Z.-Q. Lu, G.-T. Chen, J.-Q. Zhang, W. Wang, M. Yang, D.-A. Guo. *Helv. Chim. Acta* **2007**, *90*, 1751.
[15] H.-L. Huang, R.-H. Liu, F. Shao. *Magn. Reson. Chem.* **2009**, *47*, 741.
[16] Z.-Z. Liang, R. Aquino, F. De Simone, A. Dini, O. Schettino, C. Pizza. *Planta Med.* **1988**, 54,344.
[17] L.-P. Kang, Z.-J. Liu, L. Zhang, D.-W. Tan, Y. Zhao, Y. Zhao, H.-B. Chen, B.-P. Ma. *Magn. Reson. Chem.* **2007**, *45*, 725.
[18] P. K. Agrawal. *Magn. Reson. Chem.* **2004**, *42*, 990.
[19] J. Zhang, B.-P. Ma, L.-P. Kang, H.-S. Yu, Y. Yang, X.-Z. Yan, F.-T. Dong. *Chem. Pharm. Bull.* **2006**, *54*, 931.
[20] K. Hu, A.-J. Dong, H. Kobayashi, S. Iwasaki, Y.-K. Jing, X.-S. Yao. *J. Nat. Prod.* **1999**, *62*, 299.
[21] J. Yin, K. Kouda, Y. Tezuka, Q. L. Tran, T. Miyahara, Y. Chen, S. Kadota. *J. Nat. Prod.* **2003**, *66*, 646.
[22] S. H. Shim, S. Y. Lee, J. S. Kim, K. H. Son, S. S. Kang. *Arch. Pharm. Res.* **2005**, *28*, 1239.
[23] X.-W. Han, H. Yu, X.-M. Liu, X.-H. Bao. *Chin. J. Magn. Reson.* **1999**, *6*, 16.
[24] K. Hu, I. Dong, X. Yao, H. Kobayashi, S. Iwasaki. *Planta Med.* **1997**, *63*, 161.
[25] Y. Hirai, S. Sanada, Y. Ida, J. Shoji. *Chem. Pharm. Bull.* **1984**, *32*, 295.
[26] H.-Z. Guo, K. Koike, W. Li, D.-A. Guo, T. Nikaido. *Phytochemistry* **2004**, *65*, 481.