Three New Triterpenoid Saponins from *Dianthus superbus*

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Three new triterpenoid saponins (1—3) were isolated from the dried aerial parts of *Dianthus superbus* L. (Caryophyllaceae). Their structures were established as 3-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-[β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl(1→6)]-β-D-glucopyranoside (1), 3-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-[β-D-glucopyranosyl(1→3)][β-D-6-*O*-((3*S*)-hydroxyl-3-methylglutaryl)glucopyranosyl(1→6)]-β-D-glucopyranoside (2), 3-*O*-α-L-arabinopyranosyl-3β,16α-dihydroxyolean-12-en-23,28-dioic acid 28-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (3), on the basis of various spectroscopic analyses and chemical degradations.

Key words *Dianthus superbus*; Caryophyllaceae; triterpenoid saponin

Dianthus superbus L. (Caryophyllaceae) is a small herb distributed in Shandong province and elsewhere in the northeast of China. This plant, known as "Qumai," is an important traditional Chinese medicine (TCM) used as a diuretic and an anti-inflammatory agent for the treatment of urinary infections, carbuncles, and carcinomas.[1] Previous chemical investigation on this species and its variety *D. superbus* L. var. *longicalycinus* Williams led to the isolation of saponins, flavones, and cyclopeptides.[2—5] As part of our search for bioactive saponins from TCMs, we previously reported the structure elucidation of two new triterpenoid saponins.[6] In our continued research, further chemical investigation of this plant led to the isolation of three novel triterpenoid saponins. We now report the isolation and structural elucidation of three new saponins (**1**—**3**, see Fig. 1).

Results and Discussion

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −1.2 (c=0.10, MeOH). The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) showed a pseudo molecular ion peak at m/z 1139.5236 $[M+Na]^+$ (Cacld for $C_{54}H_{84}O_{24}Na$, 1139.5245) corresponding to the molecular formula of $C_{54}H_{84}O_{24}$. Its IR spectrum revealed absorption bands at 3430 cm^{-1} (OH), 1721 cm^{-1} (C=O), 1650 cm^{-1} (C=C), and 1077 cm^{-1} (C–O–C). The NMR data showed the presence of six tertiary methyl protons at δ_H 0.86 (s, H-29), 0.88 (s, H-30), 0.94 (s, H-25), 1.07 (s, H-26), 1.19 (s, H-27), and 1.57 (s, H-24), and an olefinic proton at δ_H 5.38 (br s, H-12), connected to carbons at δ_C 33.1, 23.7, 16.0, 17.4, 26.1, 12.7 and 122.7 (C-12) according to the heteronuclear single quantum coherence (HSQC) spectrum, respectively, and thereby were indicative of an olean-12-ene skeleton. In addition, the heteronuclear multiple bond correlation (HMBC) of H-24 (δ_H 1.57), H-3 (δ_H 4.65) with δ_C 180.5 showed that the carboxyl carbon at δ_C 180.5 was assigned to C-23, the other carboxyl carbon at δ_C 176.5 was attributed to C-28. In the nuclear Overhauser effect spectroscopy (NOESY) spectrum, observation of an NOE between H-3 and H-5α and the appearance of H-3 (δ_H 4.65) as a doublet (J=12.0, 4.4 Hz) supported β-orientation of 3-OH. An NOE correlation between signals of an axial methyl at C-10 (H_3-25) and a methyl at C-4 indicated that the COOH group was assigned to be α-oriented at C-4 (Fig. 2). Therefore, the aglycone was determined to be 3β-hydroxyolean-12-en-23,28-dioic acid, gypsogenic acid.[7] The downfield ^{13}C-NMR chemical shift at δ_C 85.1 (C-3) and the upfield ^{13}C-NMR chemical shift at δ_C 176.5 (C-28) suggested that **1** was a bisdesmosidic saponin with glycosidic linkages at C-3 through an *O*-heterosidic bond and at C-28 through an ester bond.[8] The sugar unit was confirmed to be glucose by co-TLC with standard sugars after hydrolysis, and the D-configuration was proved by GC-MS results of a derivatizated sample. The three sugar anomeric carbons were detected at δ_C 105.4, 105.1, and 95.6 in ^{13}C-NMR spectrum, attached to protons at δ_H 5.05 (d,

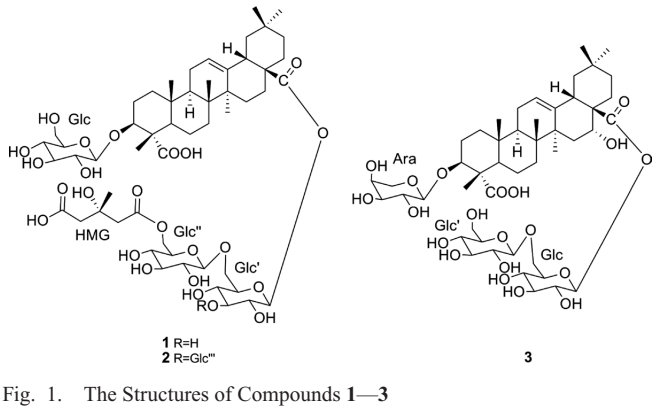

Fig. 1. The Structures of Compounds **1**—**3**

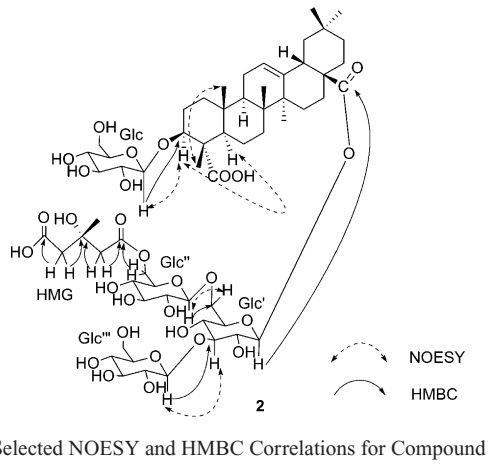

Fig. 2. Selected NOESY and HMBC Correlations for Compound **2**

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J=7.6 Hz) and 4.99 (d, J=7.8 Hz) and 6.19 (d, J=8.2 Hz), respectively, in the HSQC experiment. The β-anomeric configurations of the D-glucopyranose units were determined from their $^3J_{H1,H2}$ coupling constants (7—8 Hz).[9] The sequence of the sugar residue was subsequently determined by the HMBC experiment. The HMBC correlations of δ_H 5.05 (Glc-H-1)/δ_C 85.1 (C-3), δ_H 6.19 (Glc′-H-1)/δ_C 176.5 (C-28), δ_H 4.99 (Glc″-H-1)/δ_C 69.4 (Glc′-C-6) were used to determine the sugar moieties as 3-*O*-β-D-glucopyranosyl and 28-*O*-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl ester units, respectively. Besides those of sugar moieties (Table 2) and aglycone (Table 1), there were other signals in ^{1}H- and ^{13}C-NMR spectra of **1**, which suggested the presence of 3-hydroxyl-3-methylglutaryl (HMG) group: a *tert*-methyl group at δ_H 1.72 (3H, s) and δ_C 28.2, two methylenes [δ_H 3.09 and 3.14 (each 1H, d, 14.4 Hz)], δ_C 46.3 and δ_H 3.13 (2H, br s), δ_C 46.6 and two carbonyl groups at δ_C 171.8, 174.0, one quaternary carbon at δ_C 70.0. The esterification position of the HMG unit at Glc″-C-6 was suggested by the HMBC correlation of HMG-C-1 (δ_C 171.8) and Glc″-H-6 (δ_H 4.97, 4.73), which was also confirmed by the presence of a deshielded signal at C-6 of the terminal glucose unit (+δ_C 2.0). The absolute configuration of HMG was established to be 3*S* by a modified method of Fujimoto *et al.*[10,11] On basis of above information, the structure of **1** was elucidated as 3-*O*-β-D-glucopyranosyl gypsogenic acid 28-*O*-[β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **2** was obtained as a white amorphous powder, its HR-ESI-MS positive ion at *m/z* 1301.5751 $[M+Na]^+$ (Calcd for $C_{60}H_{94}O_{29}Na$, 1301.5773) indicated that the compound has the molecular formula of $C_{60}H_{94}O_{29}$. Comparing the proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra (Tables 1, 2) of **2** with those of **1** indicated that **2** had the same aglycone as **1** but differed in saccharide moieties. Thus the aglycone of **2** was also determined to be gypsogenic acid. The ^{1}H- and ^{13}C-NMR data of the monosaccaride residues were assigned starting from the readily identifiable anomeric protons by means of the total correlation spectroscopy (TOCSY), HSQC, and HMBC spectra obtained for this compound. Compared the NMR data (Table 2) of the sugar chain attached to C-28 of **1**, it was observed that there is one more glucose residue and an additional glycosylation shift at Glc′-C-3 (δ_C 88.4) in **2**. These suggested that the sugar moieties in **2** are 3-*O*-β-D-glucopyranosyl and 28-*O*-[β-D-glucopyranosyl-(1→3)][β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl, which was confirmed by the HMBC correlations between δ_H 5.02 (Glc-H-1) and δ_C 84.9 (C-3), δ_H 6.12 (Glc′-H-1) and δ_C 176.2 (C-28), Glc″-H-1(δ_H 4.93) and Glc′-C-6 (δ_C 68.7), Glc‴-H-1 (δ_H 5.19) and Glc′-C-3 (δ_C 88.4). Moreover, the location of the HMG group at the Glc″-C-6 was also determined by the HMBC cross-peak from the Glc″-H-6 (δ_H 4.67, 4.93) to the HMG-C-1 (δ_C 171.7). The absolute configuration of HMG was also established to be 3*S* by the same method as used for **1**. On the basis of the above results, the structure of **2** was determined to be 3-*O*-β-D-glucopyranosyl

Table 1. ^{1}H-, ^{13}C-NMR Data for the Aglycone Moieties of Compounds **1**—**3** (Pyridine-d_5)[a]

No.	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	38.7	1.48 (m), 1.02 (m)	38.7	1.45 (m), 1.00 (m)	38.9	1.63(m), 1.17(m)
2	26.0	2.24 (m), 1.86 (m)	26.1	1.84 (m), 2.22 (m)	26.2	2.21(m), 1.94(m)
3	85.1	4.65 (dd, 12.0, 4.4)	84.9	4.67 (dd, 11.5, 5.9)	85.1	4.60 (dd, 12.5, 4.0)
4	53.3		53.2		53.3	
5	52.1	1.91 (m)	52.0	1.85 (m)	52.2	1.96 (m)
6	23.2	2.02 (m), 1.89 (m)	21.3	1.59 (m), 1.44 (m)	21.3	1.51 (m), 1.40 (m)
7	32.9	1.96 (m), 1.73 (m)	32.4	1.82 (m), 1.68 (m)	33.1	1.35 (m), 1.71 (m)
8	40.2		40.1		40.4	
9	48.3	1.74 (m)	48.2	1.70 (m)	47.1	2.73 (m)
10	36.7		36.6		36.7	
11	23.8	1.87 (m), 1.92 (m)	23.7	1.88 (m), 1.93(m)	23.8	2.01 (m), 1.93 (m)
12	122.7	5.38 (br s)	123.3	5.36 (br s)	122.5	5.58 (br s)
13	144.2		144.0		144.4	
14	42.1		41.6		42.0	
15	28.3	2.24 (m), 1.05 (m)	28.2	2.12 (m), 1.03 (m)	36.1	2.47 (m), 1.68 (m)
16	23.3	2.01 (m), 1.88 (m)	23.2	1.97 (m), 1.82 (m)	74.1	5.22 (br s)
17	47.0		46.9		49.1	
18	41.7	3.14 (m)	41.6	3.12 (m)	41.2	3.49 (m)
19	46.2	1.70 (m), 1.20 (m)	46.1	1.68 (m), 1.18 (m)	47.4	1.94 (m), 1.34 (m)
20	30.8		30.7		30.8	
21	34.0	1.31 (m), 1.13 (m)	33.9	1.26 (m), 1.08 (m)	35.9	2.36 (m), 1.25 (m)
22	32.6	1.92 (m), 1.75 (m)	32.8	1.54 (m), 1.20 (m)	32.1	2.37 (m), 2.16 (m)
23	180.5		180.5		180.5	
24	12.7	1.57 (s)	12.6	1.55 (s)	12.6	1.53 (s)
25	16.0	0.94 (s)	15.9	0.92 (s)	16.2	0.99 (s)
26	17.4	1.07 (s)	17.3	1.03 (s)	17.5	1.12 (s)
27	26.1	1.19 (s)	26.0	1.15 (s)	27.1	1.75 (s)
28	176.5		176.2		175.9	
29	33.1	0.86 (s)	33.0	0.83 (s)	33.2	0.94 (s)
30	23.7	0.88 (s)	23.6	0.85 (s)	24.7	1.03 (s)

a) The assignments were based upon ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, NOESY and TOCSY spectra.

Table 2. ^{13}C- and ^{1}H-NMR Data for Sugar Moieties of Compounds **1**—**3** (Pyridine-d_5)[a,b]

	1			**2**			**3**	
	δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)		δ_C	δ_H (J in Hz)
3-*O*-Glc			3-*O*-Glc			3-*O*-Ara		
1	105.4	5.05 (d, 7.6)	1	105.3	5.02 (d, 7.6)	1	105.9	4.93 (d, 6.6)
2	75.6	4.01	2	75.5	3.95	2	74.2	4.04
3	78.4	3.88	3	78.3	3.92	3	72.7	4.35
4	71	4.28	4	71.5	3.95	4	69.1	4.25
5	78.8	4.17	5	78.3	3.92	5	66.4	3.73, 4.28
6	62.9	4.37, 4.51	6	62.4	4.46, 4.20			
28-*O*-Glc′			28-*O*-Glc′			28-*O*-Glc		
1	95.6	6.19 (d, 8.2)	1	94.9	6.12 (d, 8.0)	1	95.8	6.21 (d, 8.1)
2	73.9	4.08	2	75.3	4.1	2	73.9	4.04
3	78.2	4.11	3	<u>88.4</u>	4.15	3	78.7	4.15
4	71.6	4.19	4	72.6	4.06	4	71.5	4.18
5	78.2	4.11	5	78.2	4.14	5	77.9	4.07
6	<u>69.4</u>	4.36, 4.73	6	<u>68.7</u>	4.24, 4.59	6	<u>69.5</u>	4.67, 4.30
Glc″			Glc″			Glc′		
1	105.1	4.99 (d, 7.8)	1	105.1	4.93 (d, 7.7)	1	105.3	4.99 (d, 8.1)
2	75.2	3.99	2	74.9	3.96	2	75.1	3.96
3	78.0	4.08	3	78.5	3.92	3	78.4	4.16
4	71.0	3.98	4	71.6	4.12	4	71.0	4.25
5	78.8	4.17	5	78.1	3.99	5	78.3	3.85
6	<u>64.7</u>	4.97, 4.73	6	<u>64.7</u>	4.93, 4.67	6	62.6	4.44, 4.32
			Glc‴					
			1	105.6	5.19 (d, 7.8)			
			2	75.1	3.96			
			3	77.5	3.97			
			4	71.6	4.12			
			5	78.1	3.99			
			6	62.8	4.48, 4.35			
HMG			HMG					
1	171.8		1	171.7				
2	46.3	3.14 (1H, d, 14.4) 3.09 (1H, d, 14.4)	2	46.3	3.14 (1H, d, 14.4) 3.07 (1H, d, 14.4)			
3	70.0		3	70.0				
4	46.6	3.13 (2H, br s)	4	46.5	3.12 (2H, br s)			
5	174.0		5	174.7				
6	28.2	1.72 (3H, s)	6	28.2	1.70 (3H, s)			

a) The assignments were based upon ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, NOESY and TOCSY spectra. *b*) ^{13}C chemical shifts of substituted residues are underlined.

gypsogenic acid 28-*O*-[β-D-glucopyranosyl(1→3)][β-D-6-*O*-((3*S*)-3-hydroxyl-3-methylglutaryl)glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder, its HR-ESI-MS positive ion at *m/z* 981.4659 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{20}Na$, 981.4666), consistent with the molecular formula of $C_{47}H_{74}O_{20}$. The spectral features and physicochemical properties suggested **3** also to be a triterpenoid saponin. The aglycone of **3** was similar to **1** except for an additional 16-hydroxy. This was supported by the downfield shifts of C-15 (δ_C 36.1) and C-16 (δ_C 74.1) in the ^{13}C-NMR spectrum of **3** with respect to the corresponding value of δ 28.3 and 23.3 in **1**. The appearance of H-16 as a broad singlet at δ_H 5.22 indicated that 16-OH was α-oriented.[11] Two carbonyls at δ_C 180.5 and 175.9 were assigned to be C-23 and C-28 by analysis of the HMBC experiment, respectively. The above detailed NMR (Table 1) analysis identified the aglycone as 3β,16α-dihydroxyolean-12-en-23,28-dioic acid.[11] The downfield ^{13}C-NMR chemical shift at δ_C 85.1 (C-3) and the upfield ^{13}C-NMR chemical shift at δ_C 175.9 (C-28) suggested that **3** was also a bidesmosidic saponin. The sugar unit was confirmed to be glucose and arabinose by co-TLC with standard sugars after hydrolysis, and the D-glucose and L-arabinose was proved by GC-MS analysis of their chiral derivatives. Inspection of its NMR spectral data of **3** showed three anomeric carbons at δ_C 105.9, 105.3 and 95.8, that correlated to the protons at δ_H 4.93 (d, J=6.6 Hz), 4.99 (d, J=8.1 Hz) and 6.21 (d, J=8.1 Hz), in the HSQC experiment, respectively, indicating the presence of one arabinosyl in the α-form and two glucosyl units in the β-form. The positions of the sugars were determined by HMBC correlations between δ_H 4.93 (Ara-H-1) and δ_C 85.1 (C-3), δ_H 6.21 (Glc-H-1) and C-28 (δ_C 175.9), δ_H 4.99 (Glc′-H-1) and δ_C 69.5 (Glc-C-6). Therefore, the structure of **3** was determined to be 3-*O*-α-L-arabinopyranosyl 3β,16α-dihydroxyolean-12-en-23,28-dioic acid 28-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO P-1020 polarimeter (cell length: 1.0 dm). IR (KBr-disks) spectra were recorded by Brucker Tensor 27 spectrometer. Mass spectra were obtained on a MS Agilent 1100 Series LC/MSD Trap mass spectrometer (ESI-MS) and a G1969A TOF MS (HR-ESI-MS), respectively. 1D- and 2D-NMR spectra were measured in pyridine-d_5 at 300 K on a Bruker ACF-500 NMR (^{1}H: 500 MHz, ^{13}C: 125 MHz) spectrometer, with tetramethylsilane (TMS) as an internal standard, in which coupling constants were given in Hz. Gas chromatography was done on Varian CP-3800 Gas Chromato-

graph equipped with a Saturn 2200 Mass detector (detection temperature 220 °C). Column: CP-sil 5 CB capillary column (30 m, 0.25 mm i.d., 0.25 μm). Column temperature: 150—260 °C with the rate of 8 °C/min, and the carrier gas was He (0.8 ml/min), split ratio 1/10, injection temperature: 250 °C. Injection volume: 0.5 μl. TLC was performed on precoated silica gel 60 F_{254} plates (Qingdao Haiyang Chemical Co., Ltd., China), and detection was achieved by 10% H_2SO_4–EtOH for saponins. Sephadex LH-20 (20×100 mm, Pharmacia, U.S.A.), macroporous resin D101 (pore size B 13—14 nm, 26—60 mesh), and ODS-C_{18} (40—63 μm, Fuji, Japan) were used for column chromatography. Preparative HPLC was carried out using Agilent 1100 Series with Shim-park RP-C_{18} column (200×20 mm i.d.) and 1100 Series Multiple Wavelength detector. L-Cysteine methyl ester hydrochloride, trimethylchlorosilane, and the authentic standard compounds L- and D-arabinose, L- and D-glucose, and (3*R*)- and (3*S*)-mevalonolactone were purchased from Sigma-Aldrich (Shanghai). Tetrahydrofuran (THF) was distilled from sodium-benzophenone ketyl. LiEt3BH and Ar were purchased from Beijing Sanshengtengda Co., Ltd., and Nanjing Special Gases Factory Co., Ltd., China, respectively.

Plant Material The aerial parts of *D. superbus* were collected in Shandong province, China, in June 2008, and identified by Prof. Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 20080901) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation The air-dried aerial parts of *D. superbus* (5 kg) were powdered and refluxed three times with 95% EtOH. After concentration *in vacuo*, the residue was suspended in 50% EtOH, cold preservation and standing, partitioned with supernatant and precipitation (chlorophyll) successively. The solution was concentrated under reduced pressure to give a residue (84 g), which was further chromatographed over a macroporous resin D101 column eluted initially with water, and then with 50% and 70% EtOH to give fractions 1 and 2. Fraction 1 was subjected to MCI (MeOH/H_2O, 5 : 5, v/v), repeated ODS-C_{18} column (MeOH/H_2O, 5 : 5, v/v), silica gel column using $CHCl_3$/MeOH/H_2O gradiently, followed by Sephadex LH-20 chromatographic purification (MeOH as eluent) and *prep*-HPLC (MeCN–H_2O, 70 : 30), UV detection at 210 nm (t_{R1}=9.5 min, t_{R2}=10.7 min, t_{R3}=8.1 min), affording **1** (9 mg), **2** (8 mg), **3** (7 mg), respectively.

Compound **1**: White amorphous powder; $[\alpha]_D^{25}$ −1.2 (c=0.10, MeOH); IR (KBr) cm^{-1}: 3430, 2948, 1721, 1650, 1454, 1388, 1260, 1077, 1023; ESI-MS *m/z*: 1115.6 $[M-H]^-$; HR-ESI-MS *m/z*: 1139.5236 $[M+Na]^+$ (Calcd for $C_{54}H_{84}O_{24}Na$, 1139.5245); The data of ^{1}H-NMR (pyridine-d_5, 500 MHz) and ^{13}C-NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2.

Compound **2**: White amorphous powder; $[\alpha]_D^{25}$ +3.0 (c=0.02, MeOH); IR (KBr) cm^{-1}: 3443, 2925, 1721, 1650, 1454, 1388, 1260, 1077, 1023; ESI-MS *m/z*: 1277.7 $[M-H]^-$; HR-ESI-MS *m/z*: 1301.5751 $[M+Na]^+$ (Cacld for $C_{60}H_{94}O_{29}Na$, 1301.5773); The data of ^{1}H-NMR (pyridine-d_5, 500 MHz) and ^{13}C-NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2.

Compound **3**: White amorphous powder; $[\alpha]_D^{25}$ +0.4 (c=0.05, MeOH); IR (KBr) cm^{-1}: 3414, 1718, 1653, 1556, 1460, 1389, 1248, 1080; ESI-MS *m/z*: 993.5 $[M+Cl]^-$, HR-ESI-MS *m/z*: 981.4659 $[M+Na]^+$ (Cacld for $C_{47}H_{74}O_{20}Na$, 981.4666); The data of ^{1}H-NMR (pyridine-d_5, 500 MHz) and ^{13}C-NMR (pyridine-d_5, 125 MHz) are given in Tables 1 and 2.

Acid Hydrolysis and Determination of the Absolute Configuration of Monosaccharides Each compound (3 mg) was heated in 2 M HCl (5 ml) at 90 °C for 4 h. The reaction mixture was extracted with $CHCl_3$ (5 ml×3). The $CHCl_3$ extract was purified by chromatography on Sephadex LH-20 (2.0×100 cm). Comparing TLC with authentic samples, the aglycone was determined to be gypsogenic acid in the samples of compounds **1** and **2**, while 16α-hydroxygypsogenic acid was confirmed in **3**. Each remaining aqueous layer containing monosaccharides was neutralized and concentrated to dryness to give a residue and dissolved in pyridine (2 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution.[12] The mixture was heated at 60 °C for 1 h, and trimethylchlorosilane (0.5 ml) was added, followed by heating at 60 °C for 30 min. Then, the solution was concentrated to dryness and dissolved in water (1 ml×3), followed by extraction with *n*-hexane (1 ml×3). The hexane extract was subjected to GC/MS analysis. The absolute configurations of the monosaccharides in compounds **1** and **2** were confirmed as D-glucose by comparison of the retention time with standard glucose sample (14.51 min). Similarly, the absolute configurations of the sugar units in compound **3** were determined as L-arabinose and D-glucose by comparison of the retention times with standard samples: L-arabinose (12.72 min) and D-glucose (14.51 min), respectively.

Determination of the Absolute Configuration of HMG in Compounds 1 and 2 The protocols applied to determinate the stereochemistry of HMG were the same as our previous research,[11] modified according to the method of Fujimoto *et al.*[10] Using about 4 mg of each compound, the ester functional group was selectively reduced by $LiEt_3BH$ in dry THF (100 μl) an ice bath with an inflow of high-purity Ar. The reduced product was then hydrolyzed by 0.1 M HCl. The reaction mixtures were then stirred at room temperature under Ar gas for 48 h to allow the lactones to form. Each reaction mixture was portioned with EtOAc (1.0 ml×3), and the EtOAc layer containing a mevalonolactone was analyzed by chiral HPLC with a column of CHIRALPAK AS-H (46×150 mm; Daicel, Japan) using a mobile phase of hexane/2-propanol (9 : 1); wavelength, 220 nm; flow rate, 1.0 ml/min, column temperature, 35 °C. Authentic (3*S*)- and (3*R*)-mevalonolactones had retention times of 18.6 and 22.6 min, respectively. The retention times of two samples were at 22.6 min, thus the HMG group was elucidated as (3*S*)-3-hydroxy-3-methylglutaryate.

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References

1) Jiangsu New Medical College "Zhong Yao Da Ci Dian (The Dictionary of Chinese Crude Drugs)," Shanghai Science and Technology Press, Shanghai, 1977, p. 2702.
2) Shimizu M., Hayashi T., Shimizu K., Morita N., *Phytochemistry*, **21**, 245—247 (1982).
3) Oshima Y., Ohsawa Y., Oikawa K., Konno C., Hikino H., *Planta Med.*, **50**, 40—43 (1984).
4) Oshima Y., Ohsawa T., Hikino H., *Planta Med.*, **50**, 43—47 (1984).
5) Wang Y. C., Tan N. H., Zhou J., Wu H. M., *Phytochemistry*, **49**, 1453—1456 (1998).
6) Chen X., Luo J. G., Kong L. Y., *J. Asian Nat. Prod. Res.*, **12**, 458—463 (2010).
7) Oshima Y., Ohsawa T., Oikawa K., Konno C., Hikino H., *Planta Med.*, **50**, 40—43 (1984).
8) Mahato S. B., Kundu A. P., *Phytochemistry*, **37**, 1517—1575 (1994).
9) Glensk M., Wray V., Nimtz M., Schopke T., *J. Nat. Prod.*, **62**, 717—721 (1999).
10) Fujimoto H., Nakamura E., Kim Y. P., Okuyama E., Ishibashi M., Sassa T., *J. Nat. Prod.*, **64**, 1234—1237 (2001).
11) Ma L., Gu Y. C., Luo J. G., Wang J. S., Huang X. F., Kong L. Y., *J. Nat. Prod.*, **72**, 640—644 (2009).
12) Luo J. G., Ma L., Kong L. Y., *Bioorg. Med. Chem.*, **16**, 2912—2920 (2008).