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The NMR studies on two new furostanol saponins from *Agave sisalana* leaves

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The detailed NMR studies and full assignments of the ^{1}H and ^{13}C spectral data for two new furostanol saponins isolated from *Agave sisalana* leaves are described. Their structures were established using a combination of 1D and 2D NMR techniques including ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, TOCSY, HSQC, HMBC and HSQC-TOCSY, and also FAB-MS spectrometry and chemical methods. The structures were established as (25*S*)-26-(*β*-D-glucopyranosyl)-22 ξ-hydroxyfurost-12-one-3*β*-yl-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (1) and (25*S*)-26-(*β*-D-glucopyranosyl)-22ξ-hydroxyfurost-5-en-12-one-3*β*-yl-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→2)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (2). Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: NMR; 2D NMR; furostanol saponins; *Agave sisalana*

INTRODUCTION

The genus *Agave* belongs to the *Agaveceae* family with more than 300 species and occurs natively in the arid and tropical regions of the Western Hemisphere, particularly Mexico and Central America. In China, several species of genus *Agave*, such as *A. americana* and *A. sisalana*, are widely cultivated in the southern parts for the fiber industry and as important horticultural plants. Several *Agave spp*. have been used in the treatment of scabies, tumors, syphilis and dysentery, and as insecticides. They are also a source of fiber, and produce steroidal sapogenins and saponins, the raw materials for steroid hormone synthesis.[1]

The chemical constituents of the leaves of several species have been studied, and the isolation and identification of steroidal saponins have also been reported by several groups.[2–4] Further phytochemical analyses of the leaves of *Agave sisalana* with attention to the steroidal glycoside constituents led to the isolation of two new furostanol saponins. This paper reports the isolation and structural determination of two new furostanol saponins, compound **1** and **2** (Fig. 1). The results of the extensive application of 1D (^{1}H NMR, ^{13}C NMR) and 2D (COSY, TOCSY, HSQC, HMBC, HSQC-TOCSY) techniques were used to characterize the structures and to establish the ^{1}H and ^{13}C resonance assignments of these two new furostanol saponins.

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RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{25} - 42.2^\circ$ ($c = 0.021$, pyridine), which gave positive Liebermann–Burchard and Ehrlish reagent tests. It suggested that **1** was a furostanol saponin. High-resolution FAB-MS showed a peak at m/z 1409.4878 (calcd. 1409.4898, $[M(C_{63}H_{86}O_{34})Na]^+$) and also showed a quasi-molecular ion peak at m/z 1409.5 ($[M + Na]^+$), consistent with a molecular formula $C_{63}H_{86}O_{34}$, which was also confirmed by its ^{1}H and ^{13}C NMR spectral data (Table 1). Other significant peaks corresponding to the positive ions were observed at m/z 1225.5 ($[M - 162 + H]^+$), 1079.4 ($[M - 162 - 146]^+$), 917.4 ($[M - (162 \times 2) - 146 + H]^+$), 755.4 ($[M - (162 \times 3) - 146 + H]^+$), 593.4 ($[M - (162 \times 4) - 146 + H]^+$) and 431.3 ($[M - (162 \times 5) - 146 + H]^+$), which were attributed to the sequential losses of glucose → rhamnose → glucose → glucose → glucose → galactose present in the molecule, in agreement with the results of the acid hydrolysis of **1,** which afforded D-galactose, D-glucose and L-rhamnose as the only sugar components, after comparison of the high-performance liquid chromatographic (HPLC) retention times of the hydrolysis products with those of authentic samples of those monomers.

The ^{1}H NMR spectrum of **1** in pyridine-d_5 showed two singlet methyl signals at δ 0.65 (s) and 1.11 (s) and three doublet methyl signals at δ 1.02 (d, $J = 6.6$ Hz), 1.53(d, $J = 7.2$ Hz) and 1.70(d, $J = 6.0$ Hz). Moreover, the signals for six anomeric protons at δ 4.85 (1H, d, $J = 7.2$ Hz), 5.12 (1H, d, $J = 7.8$ Hz), 5.58 (1H, d, $J = 7.8$ Hz), 5.27 (1H, d, $J = 8.4$ Hz), 5.76 (s) and 4.81 (1H, d, $J = 7.8$ Hz) could be

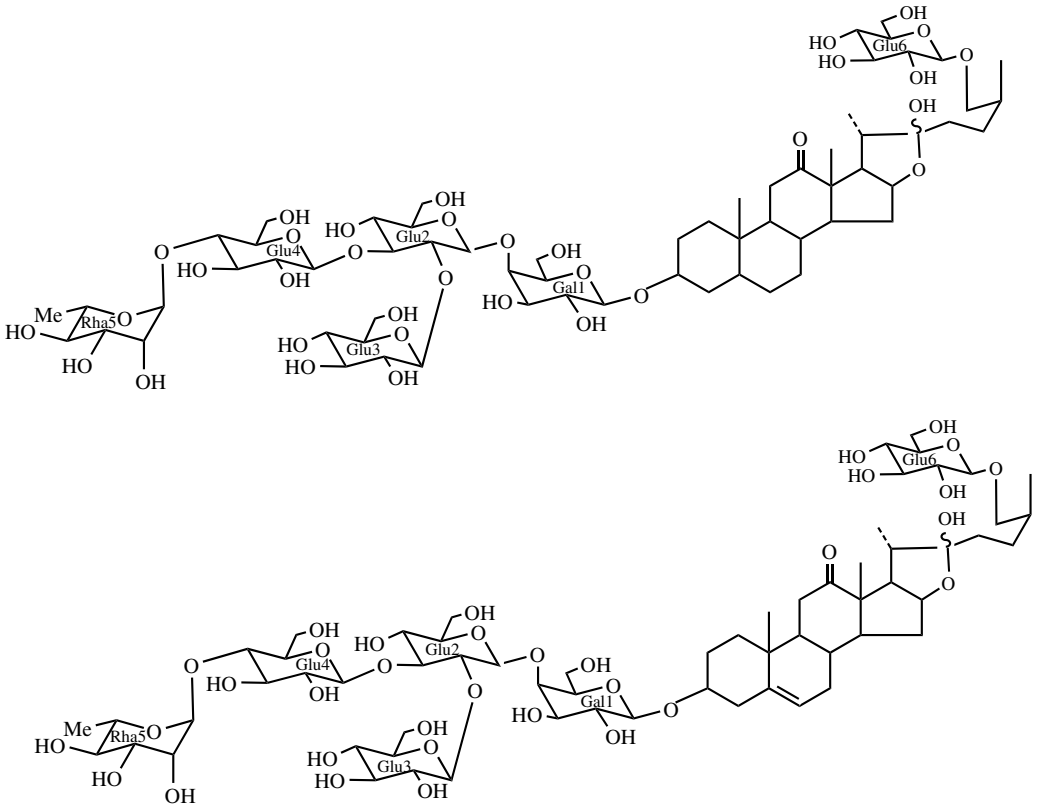

Figure 1. Structures of compound **1** and **2**.

Table 1. ^{1}H NMR (599.8 MHz) and ^{13}C NMR (150.8 MHz) NMR data for **1** and **2** in pyridine-d_5

Position		**1**		**2**	
		δ_C	δ_H J(Hz)	δ_C	δ_H J(Hz)
1	CH_2	36.65	0.69 m 1.29 m	37.00	0.86 m 1.48 m
2	CH_2	29.69	1.55 m 1.98 m	29.90	2.06 m 1.67 m
3	CH	77.11	4.42 m	77.89	3.84 m
4	CH_2	34.66	1.34 m 1.77 d 13.2	39.08	2.40 t 11.4 2.66 d 12.6
5	CH	44.49	0.84 m	140.84	–
6	CH_2	28.62	1.11s	121.42	5.25 br s
7	CH_2	31.70	1.55 m 2.08 m	31.78	1.42 m 1.85 m
8	CH	34.42	1.73 br s	30.88	1.83 m
9	CH	55.56	0.90 m	52.32	1.29 m
10	C	36.29	–	37.57	
11	CH_2	38.00	2.37 t 13.8 2.23 d 4.2	37.57	2.28 dd 5.4, 14.4 2.51 m
12	C	212.95	–	212.80	–
13	C	55.74	–	55.34	–
14	CH	55.84	1.35 m	55.92	1.42 m
15	CH_2	31.70	1.56 m 2.20 m	31.83	1.59 m 2.06 m
16	CH	79.70	4.84 m	79.74	4.84 m
17	CH	55.07	2.88 dd 7.2, 7.8	54.78	2.92 dd 7.8, 7.2
18	CH_3	16.25	1.11 s	16.04	1.13 s
19	CH_3	11.73	0.65 s	18.79	0.90 s
20	CH	41.31	2.19 m	41.33	2.19 m
21	CH_3	15.26	1.53 d 7.2	15.22	1.51 d 6.6

(*Continued*)

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Table 1. (*Continued*)

Position		1		2	
		δ_C	δ_H *J*(Hz)	δ_C	δ_H *J*(Hz)
22	C	110.76	–	110.79	–
23	CH_2	37.10	1.96 m 2.08 m	37.08	1.95 m 2.05 m
24	CH_2	28.34	1.67 m 2.06 br s	28.32	1.67 m 2.05 m
25	CH	34.33	1.92 m	34.41	1.93 m
26	CH_2	75.26	4.08 m 3.49 dd 7.2, 7.2	75.26	4.07 m 3.48 dd 6.6, 9.6
27	CH3	17.48	1.02 d 6.6	17.47	1.01 d 6.6
Gal 1-(1→3)-hecogenin					
1		102.45	4.85 d 7.8	102.44	4.84 d 7.8
2		73.20	4.42 m	73.18	4.42 m
3		75.61	4.09 m	75.57	4.09 m
4		80.28	4.57 br s	80.23	4.56 m
5		75.36	3.99 m	75.35	3.99 m
6		60.63	4.66 m 4.21 m	60.53	4.65m 4.21 m
Glc 2-(1→4)-Gal 1					
1		105.07	5.12 d 7.8	105.07	5.12 d 7.8
2		81.48	4.35 m	81.47	4.35 m
3		88.13	4.15 m	88.13	4.15 d 9.0
4		70.69	3.74 m	70.69	3.74 m
5		77.49	3.85 m	77.48	3.85 m
6		63.01	3.98 m 4.44 m	63.01	3.98 m 4.44 m
Glc 3-(1 → 2)-Glc 2					
1		104.86	5.58 d 7.8	104.86	5.58 d 7.8
2		76.21	4.06 m	76.21	4.06 m
3		77.99	4.14 m	77.98	4.14 m
4		70.97	4.25 m	70.98	4.25 m
5		78.66	3.86 m	78.65	3.86 m
6		62.38	4.37 m 4.51 m	62.40	4.37 m 4.51 m
Glc 4-(1 → 3)-Glc 2					
1		104.27	5.27 d 8.4	104.26	5.27 d 8.4
2		75.42	3.98 m	75.42	3.98 m
3		76.72	4.10 m	76.72	4.10 m
4		78.52	4.34 m	78.52	4.34 m
5		77.30	3.80 m	77.29	3.80 m
6		61.15	4.22 m 4.05 m	61.14	4.22 m 4.05 m
Rha 5-(1 → 4)-Glc 4					
1		102.84	5.76 s	102.84	5.76 s
2		72.57	4.62 br s	72.57	4.62 br s
3		72.74	4.52 m	72.74	4.52 m
4		73.92	4.32 m	73.92	4.32 m
5		70.49	4.85 m	70.48	4.85 m
6		18.54	1.70 d, 6.0	18.53	1.70 d, 6.0
26-O-Glc					
1		105.16	4.81 d 7.8	105.16	4.81 d 7.8
2		75.26	4.00 m	75.29	4.02 m
3		78.72	4.23 m	78.27	4.23 m
4		71.74	4.23 m	71.74	4.23 m
5		78.43	3.94 m	78.72	3.95 m
6		62.86	4.54 m 4.39 m	62.86	4.55 m 4.39

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readily assigned. The ^{13}C NMR spectrum of **1** in pyridine-d_5 showed six anomeric carbons at δ 102.45, 105.07, 104.86, 104.27, 102.84 and 105.16. The $^1H-^1H$ COSY, HSQC HMBC and HSQC-TOCSY spectra enabled the glucose residue at C-26 and the saccharide moiety at C-3 to be assigned. HSQC-TOCSY was used to solve the severe overlapping of the protons for the six saccharide sequences of **1**. On the 1-H track through the anomeric $^1H/^{13}C$ correlations at $\delta_{H/C} = 5.58/104.86$, five relayed cross-peaks were observed, with ^{13}C chemical shifts of δ 78.66(CH), 77.99(CH), 76.21(CH), 70.97(CH), and 62.38(CH_2). The signal at δ 62.38, which showed $^1J_{C,H}$ correlations with δ 4.37(m) and 4.51(m) in the HSQC spectrum, was assigned to C-6 of a glucose. The anomeric proton at δ 5.76 showed relayed correlation peaks with the ^{13}C signals at δ 102.84 and 73.92, the methyl proton at δ 1.70 showed relayed correlation peaks with the ^{13}C signals at δ 72.74, 72.57, 70.49 and 18.54, and the signal at 4.62(m) showed relayed correlation peaks with the signal at 4.52(m) in $^1H-^1H$ COSY, indicating the presence of one terminal rhamnose. The anomeric proton at δ 4.85 showed relayed correlation peaks with the ^{13}C signals at δ 102.45, 73.20, 75.61 and 80.28, and the C-5 and C-6 signals were assigned by $^1H-^1H$ COSY and HSQC. The cross-peaks of the other anomeric protons at δ 5.12, 5.27 and 4.81 were also observed in HSQC-TOCSY. In the HMBC spectrum, the anomeric proton signals of galactose (δ 4.85, Gal1, H-1) showed correlations with C3 of the aglycone (δ 77.11), whereas other anomeric signals, δ 5.12 of Glc2, δ 5.58 of Glc3, 5.27 of Glc4, 5.76 of rhamnose and δ 4.81 of 26-*O*-Glc, showed correlations with C-4 of galactose (δ 80.28), C-2 of glucose2 (δ 81.48), C-3 of glucose3 (δ 77.99), C-4 of glucose4 (δ 78.52) and C-26 of the aglycone (δ 75.26), respectively (Fig. 2). A comparison of the data for **1** with those reported for a known compound[5] indicated that both compounds have similar aglycone (A, B, C and D rings) and the pentaglycoside sequences at C-3.

In the studies on the steroid saponin, a pair of stereoisomeric furostanol saponins, kingianoside D and (25*S*)-kingianoside D, were isolated from the fresh rhizomes of *Polyginatum kingianum*,[6] and the protons of glycosyloxy methylene (H_2-26) (Δ ab $<= 0.48$ for 25*R*; Δ ab $>= 0.57$ for 25*S*) have been assigned, which was proposed for ascertaining 25*R*/*S* orientation of the 27-methyl group of furostane-type steroidal saponin.[7,8] In the $^1H-^1H$ COSY spectrum of **1** (Fig. 3), the proton signals at C-26 [δ 3.49 (1H, 26-Ha) and δ 4.08 (1H, 26-Hb)] of **1** were observed at the same positions as those of (25*S*)-kingianoside D, with a Δ ab of 0.59 (>0.57), suggesting an *S*-configuration of the 27-methyl group of **1.** Consequently, the structure of **1** is assigned as (25*R*)-26-(β-D-glucopyranosyl)-22 ξ-hydroxyfurost-12-one-3β-yl-*O*-α-L-rhamnopyranosyl-(1→4) -β-D-glucopyranosyl-(1→3)-*O*-[*O*-β-D-glucopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, which is a new furostanol saponin.

Compound **2** was also obtained as a white amorphous powder, $[\alpha]_D^{25} - 36.7°$ ($c = 0.015$, pyridine), and gave positive Liebermann–Burchard and Ehrlish reagent tests. Its molecular formula was assigned as $C_{63}H_{84}O_{34}$ on the basis of the ^{13}C NMR data (Table 1), high-resolution FAB-MS (m/z 1407.4731 calcd. 1407.4742, [M ($C_{63}H_{84}O_{34}$)Na]$^+$) and FAB-MS (m/z 1407.4 [M + Na]$^+$). Important positive ions were observed at m/z 1223.5 ([M − 162 + H]$^+$), 1077.5 ([M − 162 − 146]$^+$), 915.5 ([M − (162 × 2) − 146 + H]$^+$), 753.5 ([M − (162 × 3) − 146 + H]$^+$), 591.5 ([M − (162 × 4) − 146 + H]$^+$) and 429.5 ([M − (162 × 5) − 146 + H]$^+$), consistent with the sequential loss of six monosaccharide units. Similarly, acid hydrolysis of **2** afforded D-galactose, D-glucose and L-rhamnose, and the aglycone was gentrogenin, which were identified by the same procedure as described for **1**. The major difference observed between ^1H and ^{13}C spectral data for **1** and **2** (Table 1) was justified by the double bond between C-5 and C-6, as could be explained by the observed changes in the ^{13}C chemical shifts of C-5 (δ 140.84) and C-6 (δ 121.42). The presence of a double bond was also suggested by the peak at m/z 429. In the $^1H-^1H$ COSY spectrum of **2**, the proton signals of C-26 were observed at δ 4.07 (1H, 26-Ha) and δ 3.48 (1H, 26-Hb), with a Δab of 0.59 (>0.57), for an *S*-configuration of the 27-methyl group.

All these data, in combination with the results revealed by the COSY, TOCSY, HMBC and HSQC spectra, were used to elucidate the structure of this novel saponin as (25*S*)-26-(β-D-glucopyranosyl)-22ξ-hydroxyfurost-5-en-12-one-3β-yl-*O*-α-L-rhamnopyranosyl-(1→4)–β-D-glucopyranosyl-(1→3)-*O*-[*O*-β-D-glucopyranosyl-(1→2)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

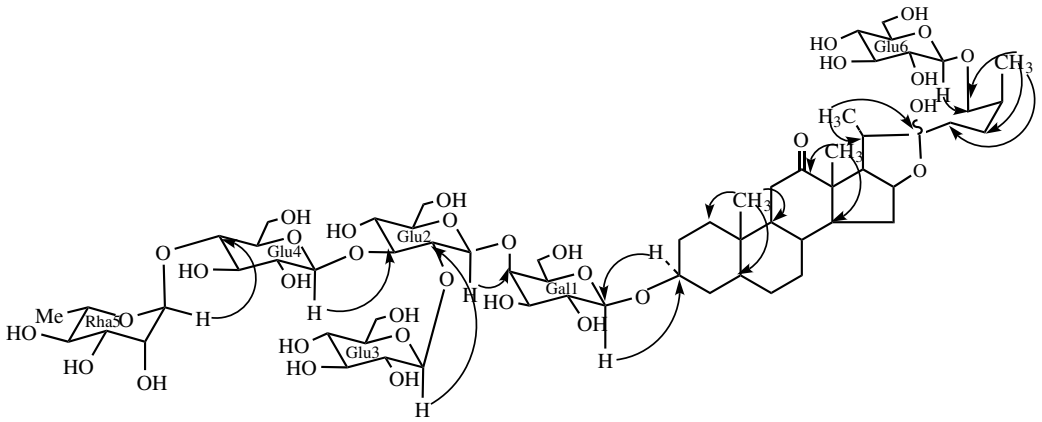

Figure 2. Key HMBC correlations for the compound **1**.

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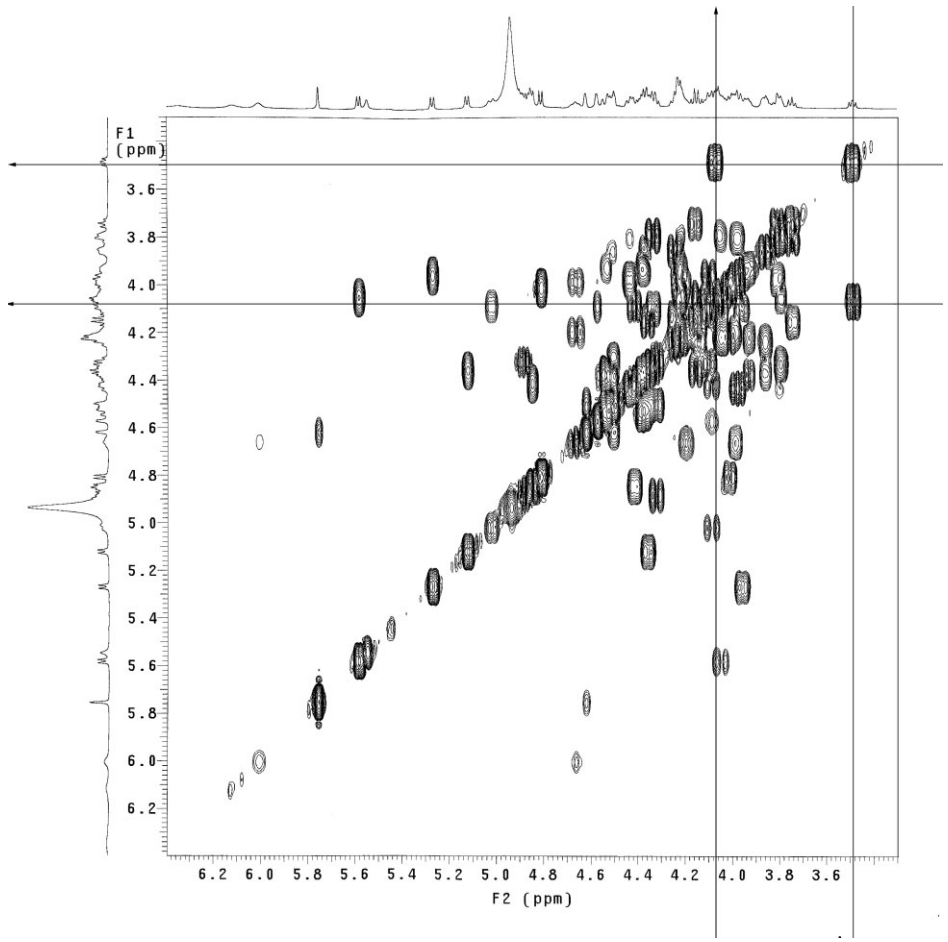

Figure 3. $^{1}H-^{1}H$ COSY spectrum of the compound **1**.

EXPERIMENTAL

Plant material

The fresh leaves of *Agave sisalana* were collected from the Sanya county of the Hainan Province, People's Republic of China, in August 2005. The plant was identified by Prof. Jian-mei Huang, and a voucher specimen (No. 040123) has been deposited in the Herbarium of the Beijing Institute of Radiation Medicine, Beijing.

Extraction and isolation of compounds 1 and 2

The fresh leaves of *Agave sisalana* (74.0 kg) were extracted three times with 50% aqueous EtOH. The combined extract was concentrated under reduced pressure. Column chromatography of the extraction was performed on the macroporous resin AB-8 and eluted with a gradient mixture of Me_2CO-H_2O (1:9, 1:1 and 8:2) to give three fractions (Fr. A–C). Fraction B (1120 g) was chromatographed on the macroporous resin SP825 and eluted with a gradient mixture Me_2CO-H_2O (3:20, 1:4, 7:20 and 1:1) to give four fractions, fraction B_1 (182 g), B_2 (208 g), B_3 (202 g) and B_4 (330 g). A part of fraction B_2 (500 mg) was chromatographed on ODS silica gel (50 μm) and with preparative HPLC to yield compounds **1** (194 mg) and **2** (20 mg).

Acid hydrolysis

Compounds **1** and **2** (10 mg) were refluxed with 1 M HCl–dioxane (1:1, v/v, 4 ml) in a water bath for 6 h. After cooling, the reaction mixture was extracted with EtOAc (20 ml, twice). The solvent extract was purified by preparative TLC on silica gel plates (20:1 $CHCl_3-CH_3OH$) to afford the aglycone. The aglycones were determined as gentrogenin and hecogenin by comparison to authentic samples by co-TLC and HPLC. The configurations were confirmed *S* by their ^{13}C NMR.

The solution was concentrated by blowing with N_2, and then analyzed by HPLC under the following conditions: column, Hypersil APS-2 (4.6 mm i.d. ×250 mm, 5 μm); solvent, $MeCN-H_2O$ (17:3); flow rate, 1.0 ml/min; detection, ELSD (temp. 105.0 °C, gas 2.4 l/min). Identification of D-galactose, D-glucose and L-rhamnose present in the sugar fraction was carried out by comparison of their retention times with those of authentic samples; t_R (min): 7.80 (L-rhamnose), 14.65 (D-galactose), 15.37 (D-glucose).

General experimental procedures

HPLC was performed using Agilent 1100 series. Apollo C_{18} (10 μm, 250 × 8 mm); Detector: Agilent RID; Macroporous resins SP825 (Mitsubishi-Chemical, Japan) and AB-8 (NanKai Chemical Co., Ltd., China) and ODS-A silica gel (120 Å, 50 μm, YMC) were used for chromatography. Optical rotations were measured with a Perkin-Elmer 343 polarimeter. FAB-MS was carried out by Micromass Zabspec. The NMR spectra were recorded on a Varian ^{UNITY}INOVA 600 (599.8 MHz for ^{1}H NMR and 150.8 MHz for ^{13}C NMR), and

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the chemical shifts were given with tetramethylsilane as an internal standard. The NMR experiments were carried out at 300 K with the following parameters:

^{1}H-NMR spectrum: spectral width 12 000 Hz, acquisition time 2.67 s, relaxation delay 3.0 s;

^{13}C-NMR spectrum: spectral width 40 000 Hz, acquisition time 0.80 s, relaxation delay 1.0 s;

COSY spectrum: spectral width 5800 Hz, acquisition time 0.18 s, FT size 4096 × 4096, relaxation delay 1.0 s;

TOCSY spectrum: spectral width 5800 Hz, mixture time 0.10 s, FT size 4096 × 4096, relaxation delay 1.0 s.

HSQC spectrum: spectral width of the proton dimension 5800 Hz, spectral width of the carbon dimension 28 000 Hz, relaxation delay 1.0 s, data points 1024 × 128 complex points, data matrix for FT 2048 × 1024 complex point, before FT, the data points of F1 linear predicted to 256.

HMBC spectrum: spectral width of the proton dimension 5800 Hz, spectral width of the carbon dimension 34 000 Hz, relaxation delay 1.0 s, data points for proton and carbon set as 1024 × 256 complex points, data for carbon linear prediction to 512 before FT, FT data matrix 2048 × 1024 complex points.

HSQC-TOCSY spectrum: spectral width of the proton dimension 5800 Hz, spectral width of the carbon dimension 28 000 Hz, data points for proton and carbon set as 1024 × 256 complex points, data points of F1 linear predicted to 512.

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