Two New Steroidal Saponins from the Fresh Leaves of *Agave sisalana*

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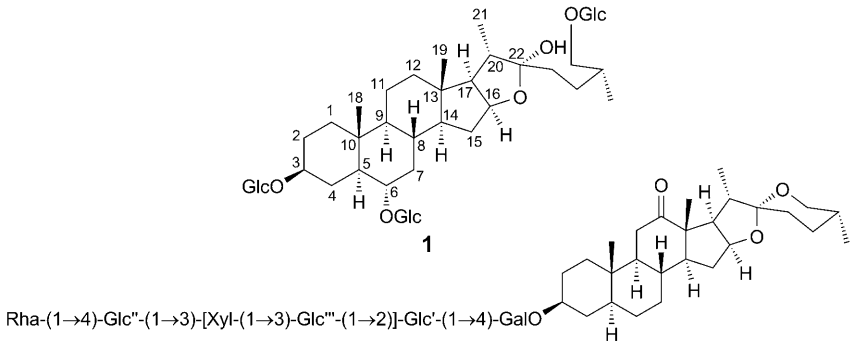
A new furostanol saponin, sisalasaponin C (**1**), and a new spirostanol saponin, sisalasaponin D (**2**), were isolated from the fresh leaves of *Agave sisalana*, along with three other known steroidal saponins and two stilbenes. Their structures were identified as (3*β*,5*α*,6*α*,22*α*,25*R*)-3,26-bis[(*β*-D-glucopyranosyl)oxy]-22-hydroxyfurostan-6-yl *β*-D-glucopyranoside (**1**), (3*β*,5*α*,25*R*)-12-oxospirostan-3-yl 6-deoxy-*α*-L-mannopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**2**), (3*β*,5*α*,6*α*,22*α*,25*R*)-22-methoxyfurostane-3,6,26-triyl tris-*β*-D-glucopyranoside, cantalasaponin-1, polianthoside D, (*E*)- and (*Z*)-2,3,4′,5-tetrahydroxystilbene 2-*O*-*β*-D-glucopyranosides. The last three known compounds were isolated from the fresh leaves of *Agavaceae* for the first time. The structures of the new compounds were elucidated by detailed spectroscopic analysis, including 1D- and 2D-NMR experiments, and chemical techniques.

Introduction. – Plants belonging to the family of Agavaceae with more than 300 species occur natively in the arid and tropical regions of the Western Hemisphere, particularly Mexico and Central America. In China, several species of the 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. Agavaceae is known to be a rich source for steroidal saponins. It is also a source of fiber and steroidal sapogenins. Several species of genus *Agave* are used in the treatment of scabies, tumors, syphilis, and dysentery and also as insecticides [1]. The chemical constituents of leaves of several species have been studied, and several steroidal sapogenins and saponins were found by several groups [2–8]. Previously, we reported two new furostanol saponins from the fresh leaves of *A. sisalana* [5]. In our continuing search for new furostanol saponins, we isolated three furostanol saponins and two spirostanol saponins, including a new furostanol saponin, **1**, and a new spirostanol saponin, **2**. Further, polianthoside D, and (*E*)- and (*Z*)-2,3,4′,5-tetrahydroxystilbene 2-*O*-*β*-D-glucopyranosides are reported from *Agavaceae* for the first time. Here, we describe the isolation and structure elucidation of the new steroidal saponins on the basis of extensive spectral analysis, including ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HSQC, HMBC, and HSQC-TOCSY experiments, as well as chemical evidences.



Results and Discussion. – The crude extract of the fresh leaves of *A. sisalana* PERR. was chromatographed on macroporous resin, SiO_2, *Sephadex LH-20*, and octadecylsilanized (*ODS*) SiO_2, and further purified by semi-preparative HPLC to afford the new steroidal saponins **1** and **2**, together with (3*β*,5*α*,6*α*,22*α*,25*R*)-22-methoxyfurostane-3,6,26-triyl tris-*β*-D-glucopyranoside, cantalansaporin-1, polianthoside, and (*E*)- and (*Z*)-2,3,4′,5-tetrahydroxystilbene 2-*O*-*β*-D-glucopyranosides. The structures were elucidated by 1D- and 2D-NMR in combination with MS studies.

Sisalasaponin C (**1**) was obtained as a white amorphous powder, which showed positive *Libermann–Burchard* and *Ehrlich* reactions, indicating a furostanol saponin. The molecular formula was assigned as $C_{45}H_{76}O_{20}$ on the basis of the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS; *m*/*z* 959.4861 ($[C_{45}H_{76}O_{20}+Na]^+$); calc. 959.4828), and the ^{1}H- and ^{13}C-NMR data. The ESI-QTOF-MS also showed a *quasi*-molecular-ion peak at *m*/*z* 935.4857 ($[M-H]^-$), and some characteristic fragment-ion peaks at *m*/*z* 773.4351 ($[M-H-162]^-$), 611.3807 ($[M-H-162-162]^-$), and 449.3316 ($[M-H-162-162-162]^-$), attributed to the sequential loss of three hexose residues. Compound **1** was hydrolyzed with acid to afford D-glucose as the only sugar component. This was identified by comparison with authentic samples by TLC and GC analysis. Based on ^{1}H- and ^{13}C-NMR (*Table*) and 2D-NMR analyses, the structure of **1** was elucidated as (3*β*,5*α*,6*α*,22*α*,25*R*)-3,26-bis(*β*-D-glucopyranosyloxy)-22-hydroxyfurostan-6-yl *β*-D-glucopyranoside.

The ^{1}H-NMR spectrum showed signals for four Me groups at δ(H) 1.30 (*d*, $J = 6.9$, Me(21)), 0.99 (*d*, $J = 6.6$, Me(27)), 0.81 (*s*, Me(18)), and 0.70 (*s*, Me(19)). Moreover, signals for three anomeric H-atom signals at δ(H) 4.81 (*d*, $J = 7.8$), 4.89 (*d*, $J = 7.8$), and 5.13 (*d*, $J = 7.8$) could be readily assigned. The *J* values (> 7 Hz) of three anomeric H-atoms indicated the *β*-orientation at the anomeric centers for these sugars. The ^{13}C-NMR spectrum of **1** showed three anomeric C-atom signals at δ(C) 101.7, 105.0, and 106.2. Analysis of the ^{1}H- and ^{13}C-NMR data of **1** in comparison with those in the literature [9] implied that the aglycone of **1** was the same as that of (3*β*,5*α*,6*α*,25*R*)-5-spirostane-3,6-diyl bis-*β*-D-glucopyranoside. Furthermore, the ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra allowed the assignment of the glucose residue at C(3), at C(6), and at C(26). The chemical shifts of glycosyloxy CH_2 group (H_a–C(26) and H_b–C(26)) (Δ_{ab} ($\delta_a - \delta_b$) < 0.48 for (25*R*); $\Delta_{ab} > 0.57$ for (25*S*)) were proposed to ascertain the

Table. *[1]H- and [13]C-NMR Data* ((D$_5$)Pyridine) of Compounds **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	0.79–0.83 (*m*), 1.46–1.53 (*m*)	37.6	0.69–0.74 (*m*), 1.26–1.30 (*m*)	36.6
CH_2(2)	1.64–1.68 (*m*), 2.02–2.08 (*m*)	29.9	1.55[a]), 1.98–2.01 (*m*)	29.7
H–C(3)	3.91–3.98 (*m*)	77.0	3.85–3.90 (*m*)	77.1
CH_2(4)	1.43–1.50 (*m*), 3.40 (*d*, *J* = 5.4)	28.6	1.30–1.35 (*m*), 1.75–1.80 (*m*)	34.6
H–C(5)	1.21–1.27 (*m*)	51.0	0.80–0.85 (*m*)	44.5
H–C(6) or CH_2(6)	3.60–3.64 (*m*)	79.7	1.04–1.10 (*m*), 1.04–1.10 (*m*)	28.6
CH_2(7)	1.21–1.27 (*m*), 2.53–2.57(*m*)	41.1	1.55[a]), 2.06–2.10 (*m*)	31.5
CH_2(8)	1.46–1.53 (*m*)	34.0	1.72–1.76 (*m*)	34.3
H–C(9)	0.50–0.54 (*m*)	53.9	0.85–0.90 (*m*)	55.5
C(10)		36.7		36.3
CH_2(11)	1.21–1.27 (*m*), 1.29–1.38 (*m*)	21.2	2.20–2.25 (*m*), 2.33–2.37 (*m*)	38.0
CH_2(12) or C(12)	1.02–1.09 (*m*), 1.65–1.69 (*m*)	40.1		212.8
C(13)		41.5		55.4
H–C(14)	1.02–1.09 (*m*)	56.3	1.35–1.39 (*m*)	55.9
CH_2(15)	1.29–1.35 (*m*), 1.90–1.92 (*m*)	32.3	1.54[a]), 1.60[a])	31.7
H–C(16)	4.75–4.80 (*m*)	81.0	4.45[a])	79.7
H–C(17)	1.85–1.89 (*m*)	63.9	2.75 (*dd*, *J* = 7.4, 7.2)	54.3
Me(18)	0.81 (*s*)	16.8	1.07 (*s*)	16.1
Me(19)	0.70 (*s*)	13.4	0.66 (*s*)	11.7
H–C(20)	2.04–2.08 (*m*)	40.1	1.89–1.95 (*m*)	42.6
Me(21)	1.30 (*d*, *J* = 6.6)	16.4	1.34 (*d*, *J* = 7.2)	13.9
C(22)		110.6		109.3
CH_2(23)	1.98[a]), 2.01[a])	37.2	1.91 (*t*, *J* = 6.6)	31.8
CH_2(24)	1.67[a]), 2.02[a])	28.4	1.54[a]), 1.55[a])	29.2
H–C(25)	1.90–1.92 (*m*)	34.3	1.57[a])	30.6
CH_2(26)	3.60–3.64 (*m*), 3.91–3.98 (*m*)	75.3	3.46–3.49 (*m*), 3.54–3.58 (*m*)	67.0
Me(27)	0.99 (*d*, *J* = 6.6)	17.5	0.68 (*d*, *J* = 6.0)	17.3
	Glc		Gal	
H–C(1)	5.13 (*d*, *J* = 7.8)	101.7	4.84 (*d*, *J* = 7.8)	102.5
H–C(2)	4.00–4.06 (*m*)	75.5	4.38–4.41 (*m*)	73.1
H–C(3)	4.25[a])	78.1	4.08–4.13 (*m*)	75.4
H–C(4)	4.20–4.22 (*m*)	71.8	4.52–4.62 (*m*)	79.9
H–C(5)	3.91–3.98 (*m*)	78.7	3.97–4.01 (*m*)	75.6
CH_2(6)	4.32–4.38 (*m*), 4.42–4.55 (*m*)	62.7	4.21[a]), 4.62[a])	60.7
	Glc		Glc′	
H–C(1)	4.81 (*d*, *J* = 7.8)	105.0	5.12 (*d*, *J* = 7.8)	104.8
H–C(2)	4.00–4.06 (*m*)	75.2	4.30–4.34 (*m*)	80.8
H–C(3)	4.27[a])	78.7	4.08–4.13 (*m*)	88.1
H–C(4)	4.23[a])	71.8	3.70–3.73 (*m*)	70.6
H–C(5)	3.91–3.98 (*m*)	78.1	3.79[a])	77.5
CH_2(6)	4.23[a]), 4.45[a])	62.9	3.97[a]), 4.41[a])	63.0
	Glc		Glc″	
H–C(1)	4.89 (*d*, *J* = 7.8)	106.2	5.17 (*d*, *J* = 7.8)	104.2
H–C(2)	4.00–4.06 (*m*)	78.0	3.98–4.02 (*m*)	75.6
H–C(3)	4.23[a])	78.1	4.08[a])	76.5
H–C(4)	4.20–4.22 (*m*)	71.8	4.30–4.34 (*m*)	78.0
H–C(5)	3.85–3.90 (*m*)	78.7	3.79[a])	77.3
CH_2(6)	4.33[a]), 4.43[a])	62.7	4.04[a]), 4.22[a])	61.1

Table (cont.)

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
Rha				
H–C(1)			5.78 (br. *s*)	102.7
H–C(2)			4.63[a])	72.6
H–C(3)			4.51[a])	72.7
H–C(4)			4.30–4.34 (*m*)	74.0
H–C(5)			4.91–4.95 (*m*)	70.4
Me(6)			1.68 (*d*, *J* = 6.0)	18.5
Glc‴				
H–C(1)			5.57 (*d*, *J* = 7.2)	104.0
H–C(2)			4.06[a])	75.1
H–C(3)			4.05[a])	87.0
H–C(4)			4.06[a])	69.1
H–C(5)			3.80[a])	78.2
CH_2(6)			4.29[a]), 4.47[a])	62.1
Xyl				
H–C(1)			5.09 (*d*, *J* = 7.2)	106.2
H–C(2)			3.91–3.95 (*m*)	75.4
H–C(3)			4.06[a])	77.7
H–C(4)			4.08–4.13 (*m*)	70.8
CH_2(5)			3.52[a]), 4.19[a])	67.1

[a]) Overlapped with other signals.

orientation of the Me(27) group of furostane-type steroidal saponin [10][11]. In the ^{1}H,^{1}H-COSY and HSQC spectrum of **1**, the signals of the H-atoms at C(26) were observed at δ(H) 3.63 (H_a–C(26)) and 3.96 (H_b–C(26)), with a chemical-shift difference (Δ_{ab}) of 0.33 (Δ_{ab} ($\delta_a - \delta_b$) < 0.48), suggesting (25*R*)-configuration of **1**. The complete assignment of the glycosidic NMR signals was performed by analyses of ^{1}H,^{1}H-COSY, HSQC, and HMBC data. In the HMBC spectrum of **1** (*Fig.*), the HMBC cross-peaks δ(H) 4.81 (Glc H–C(1))/δ(C) 75.3 (C(26)), δ(H) 5.13 (Glc H–C(1))/δ(C) 75.3 (C(3)), and δ(H) 4.89 (Glc H–C(1))/δ(C) 79.7 (Glc H–C(6)) were observed, which confirmed the sugar sequence and the glycosylation position.

Sisalasaponin D (**2**) was obtained as a white amorphous powder, which gave positive *Liebermann–Burchard* and negative *Ehrlich* reagent tests, suggesting a spirostanol saponin. The HR-ESI-MS showed a peak at *m*/*z* 1379.6148, which indicated a formula $C_{62}H_{100}O_{32}$ ([*M* + Na]$^+$; calc. 1379.6090). The ESI-QTOF-MS showed a peak at *m*/*z* 1355.6251 ([*M* – H]$^-$), and significant fragment-ion peaks at *m*/*z* 1223.5770 ([*M* – H – 132]$^-$), 1061.5205 ([*M* – H – 132 – 162]$^-$), 915.4227 ([*M* – H – 132 – 162 – 146]$^-$), 753.3995 ([*M* – H – 132 – 162 – 146 – 162]$^-$), 591.3532 ([*M* – H – 132 – 162 – 146 – 162 – 162]$^-$), and 429.2926 ([*M* – H – 132 – 162 – 146 – 162 – 162 – 162]$^-$), which were attributed to loss of xylose, glucose, rhamnose, and galactose present in the molecule, in agreement with the results of the acid hydrolysis of **2**, which afforded D-xylose, D-galactose, D-glucose, and L-rhamnose as the sugar components. On the basis of extensive ^{1}H- and ^{13}C-NMR (*Table*), and 2D-NMR analyses, the

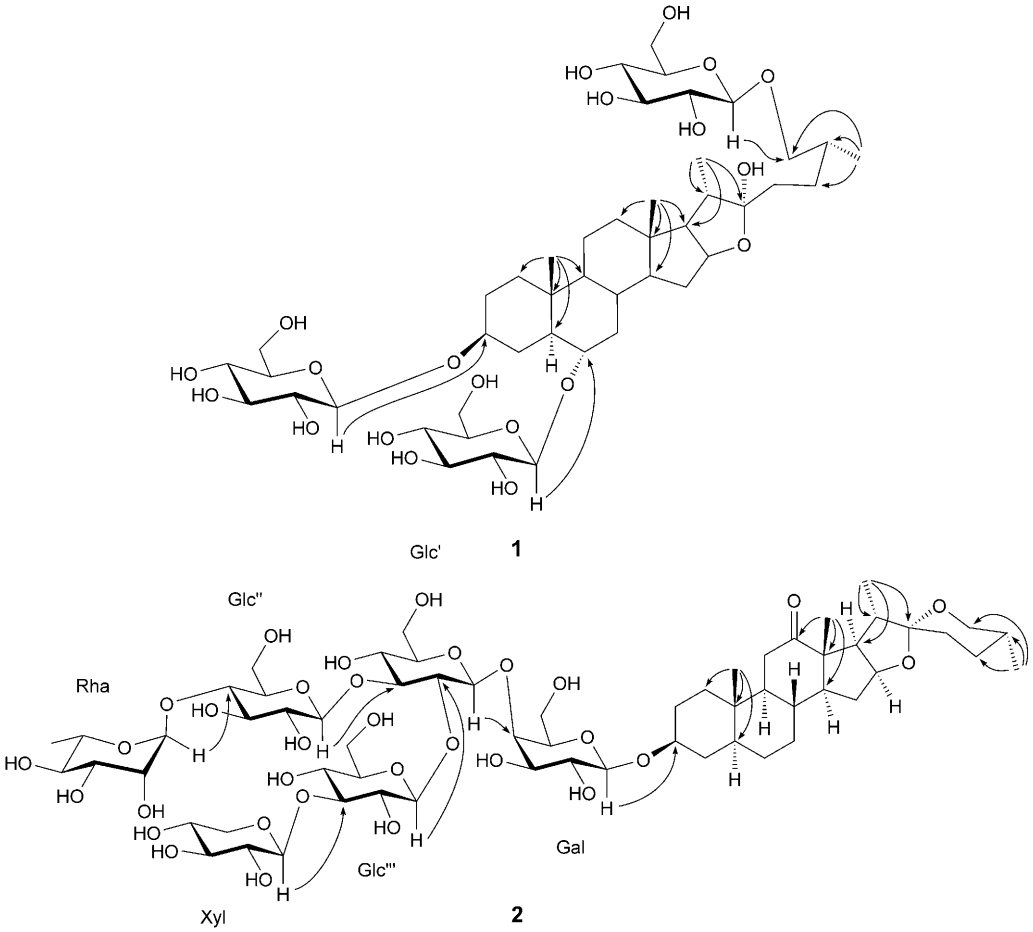


Figure. *Key HMBCs for compounds* **1** *and* **2**

structure of **2** was elucidated as (3β,5α,25R)-12-oxospirostan-3-yl 6-deoxy-α-L-mannopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 3)-[β-D-xylopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside.

The ^{1}H-NMR spectrum of **2** exhibited two *singlet* Me signals at δ(H) 0.66 (*s*) and 1.07 (*s*), and three *doublet* Me signals at δ(H) 0.68 (*d*, J = 6.0), 1.34 (*d*, J = 7.2), and 1.68 (*d*, J = 6.0), corresponding to the typical spirostanol Me(19), Me(18), Me(27), and Me(21) Me H-atoms, and rhamnose Me H-atoms. Moreover, signals for six anomeric H-atoms at δ(H) 4.84 (*d*, J = 7.2), 5.12 (*d*, J = 7.8), 5.57 (*d*, J = 7.2), 5.17 (*d*, J = 7.8), 5.78 (*s*), and 5.09 (*d*, J = 7.2) could be readily assigned. The ^{13}C-NMR spectrum of **2** showed six anomeric C-atom signals at δ(C) 102.5, 104.8, 104.0, 104.2, 102.7, and 106.2. Other characteristic features were the hemiketalic function at C(22) (δ(C) 109.3) and the C(12)=O group (δ(C) 213.0). The above ^{1}H-NMR data and a

comparison of the ^{13}C-NMR signals of the aglycone moiety of **2** with those described in the literature [12] indicated the structure of the aglycone to be hecogenin.

The ^{1}H,^{1}H-COSY, HSQC, HMBC, and HSQC-TOCSY spectra allowed the assignment of the saccharide moiety at C(3). HSQC-TOCSY was used to solve the severe overlapping of the H-atom signals for the six sugar sequences of **2**. On the H–C(1) track through the anomeric ^{1}H,^{13}C correlations at δ(H/C) 5.17/104.2, four cross-peaks were observed; with δ(C) 78.0 (CH), 77.3 (CH), 76.5 (CH), and 75.6 (CH), these ^{13}C chemical shifts showed correlations in HSQC with the signals at δ(H) 4.34 (*m*), 3.79 (*m*), 4.08 (*m*), and 3.99 (*m*), respectively. The signal at δ(H) 3.99 (*m*) exhibited correlation with the signal at δ(C) 61.1 in HSQC-TOCSY, and the signal at δ(C) 61.1, which showed correlations with signals at δ(H) 3.52 (*m*) and 4.19 (*m*) in the HSQC spectrum, was assigned to C(6) of a glucose. The anomeric ^{1}H/^{13}C correlations at δ(H/C) 5.09/106.2 led to four cross-peaks, with ^{13}C chemical shifts of δ(C) 75.4 (CH), 77.7 (CH), 70.8 (CH), and 67.1 (CH_2). The signal at δ(C) 67.1, which showed ^{1}J(C,H) correlations with δ(H) 3.52 (*m*) and 4.19 (*m*) in the HSQC spectrum, was assigned to C(5) of a xylose. The anomeric H-atom signal at δ(H) 5.78 showed correlation peaks with the ^{13}C signals at δ(C) 102.7 and 72.7; Me H-atom signal at δ(H) 1.68 exhibited correlation peaks with the ^{13}C signals at δ(C) 74.0, 72.6, 70.4, and 18.5; and the signal at δ(H) 4.63 (*m*) showed correlation peaks with the signal at δ(H) 4.51 (*m*) in ^{1}H,^{1}H-COSY, indicating the presence of one terminal rhamnose. The anomeric H-atom signal at δ(H) 4.84 displayed correlation peaks with the ^{13}C signals at δ(C) 102.5, 73.1, 75.6; other signals of the galactose were assigned by ^{1}H,^{1}H-COSY and HSQC. The cross-peaks of other anomeric H-atoms at δ(H) 5.12 and 5.57 were also observed in HSQC and TOCSY, respectively. In the HMBC spectrum (*Fig.*), the anomeric H-atom signal of galactose (δ(H) 4.84, H–C(1) of Gal) showed correlations with C(3) of the aglycone (δ(C) 77.1), whereas other anomeric H-atom signals, *i.e.*, at δ(H) 5.12 (Glc′), 5.17 (Glc″), 5.57 (Glc‴), 5.78 (Rha), and 5.09 (Xyl), showed correlations with C(4) of Gal (δ(C) 79.9), C(3) of (Glc′) (δ(C) 88.1), C(2) of (Glc′) (δ(C) 80.8), C(4) of (Glc″) (δ(C) 78.0), and C(3) of (Glc‴) (δ(C) 87.0), respectively. The full assignments of these sugar signals were confirmed by ^{1}H,^{1}H-COSY, HSQC, HMBC, and HSQC-TOCSY experiments.

Comparison of the physicochemical properties and NMR data with those reported in the literature allowed us to identify (3β,5α,6α,22α,25*R*)-methoxy-5-furostane-3,6,26-triyl tris-β-D-glucopyranoside [6], cantalasaponin-1 [13], polianthoside D [14], and (*E*)- and (*Z*)-2,3,4′,5-tetrahydroxystilbene 2-*O*-β-D-glucopyranosides [15]. The last three compounds are reported from the *Agavaceae* for the first time.

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Experimental Part

General. Column chromatography (CC): macroporous resin *SP825* (*Mitsubishi Chemical*, Japan), silica gel (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China), and *ODS* SiO_2 (120 Å, 50 μm, *YMC*, Japan). HPLC: *Waters2695 Alliance* system (*Waters*, USA) equipped with a *YMC-Pack ODS-A* C_{18} column (*YMC*, 4.6 mm i.d. × 250 mm, ODS, 5 μm, Japan). GC: *Agilent* 6890 gas chromatograph

equipped with an H_2 flame ionization detector; *HP-5* cap. column (30 m × 0.25 mm × 0.25 μm; *Agilent*, USA). Optical rotations: *Perkin-Elmer* 343 polarimeter. HR-ESI-MS: 9.4 T *Q-FT-MS Apex Qe* (*Bruker Co.*). NMR spectra: *Varian* UNITY*INOVA* 600 (599.8 MHz for ^{1}H and 150.8 MHz for ^{13}C); the chemical shifts in δ [ppm] scale with Me_4Si as an internal standard. ESI-QTOF-MS: *Synapt MS* system (*Waters*, USA), mass accuracy was maintained using a lock spray with leucine enkephalin for negative-ion mode ($[M-H]^-$ 554.2615) at a concentration of 200 pg/μl and a flow rate of 50 μl/min as reference. The full scan data acquisition range was 100 to 1500 Da.

Plant Material. The material was collected from Sanya County of Hainan Province, P. R. China, in August 2005, and was identified as leaves of *Agave sisalana* PERR. by Prof. *L.-J. Z.*, Tianjing University of Traditional Chinese Medicine. A voucher specimen (No. 040123) has been deposited with the Herbarium of the Beijing Institute of Radiation Medicine, Beijing.

Extraction and Isolation. The fresh leaves of *Agave sisalana* PERR. (74.0 kg) were extracted three times with 50% aq. EtOH at 120° for 1 h each time (3 × 200 l). The combined extract was concentrated under reduced pressure. CC of the extract was performed on 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, *Frs. A–C*. *Fr. B* (1120 g) was chromatographed on macroporous resin (*SP825*) and eluted with a gradient mixture of Me_2CO/H_2O (3:20, 5:20, 7:20, and 20:20) to give four fractions, *Frs.* B_1 (182 g), B_2 (208 g), B_3 (202 g), and B_4 (330 g). A part of *Fr.* B_2 (208 g) was chromatographed on *ODS* SiO_2 (50 μm) and eluted with MeOH/H_2O (38:62, 45:55, and 70:30), the part eluted with MeOH/H_2O (70:30) was chromatographed on SiO_2 and eluted with $CHCl_3$/MeOH/H_2O (60:35:10; lower phase); combination of *Frs. 8–11* gave compounds **1** (147 mg) and (3*β*,5*α*,6*α*,22*α*,25*R*)-22-methoxyfurostane-3,6,26-triyl tris-*β*-D-glucopyranoside (23 mg). A part of *Fr.* B_4 (310 g) was chromatographed on SiO_2 and eluted with $CHCl_3$/MeOH/H_2O (70:25:10, lower phase); recrystallization yielded cantalasaponin-1 (235 mg). A part of *Fr.* B_2 (200 g) was chromatographed on *ODS* SiO_2 (50 μm) and eluted with MeOH/H_2O (38:62, 45:55, and 70:30). The part eluted with MeOH/H_2O 45:55 was chromatographed on *ODS* SiO_2 and eluted with MeOH/H_2O (35:65, 38:62, 40:60, 44:56, and 70:30), and polianthoside (46 mg) was obtained from the part that eluted with MeOH/H_2O 44:56 by semi-prep. HPLC with MeCN/H_2O 18:82. A part of *Fr.* B_3 (180 g) was chromatographed on *ODS* SiO_2 (50 μm) and eluted with Me_2CO/H_2O (20:80, 24:76, and 50:50); the part eluted with Me_2CO/H_2O 20:80 was chromatographed on *Sephadex LH-20* and eluted with Me_2CO/H_2O 50:50 to afford (*E*)- and (*Z*)-2,3,4′,5-tetrahydrostilbene 2-*O*-*β*-D-glucopyranosides (46.7 and 16.3 mg, resp.) were obtained by semi-prep. HPLC with Me_2CO/H_2O 23:77. *Fr. C* (20 g) was chromatographed on SiO_2 with a gradient mixture of $CHCl_3$/MeOH/H_2O (100:20:5, 70:30:10, 65:35:10, lower phase) to give five fractions, *Frs.* C_1–C_5. A part of *Fr.* C_1 (2 g) was chromatographed on *ODS* SiO_2 (50 μm) and eluted with MeOH/H_2O (50:50, 80:20, and 100:0); the second fraction was recrystallized to yield compound **2** (13 mg).

Sisalasaponin C (= *(3β,5α,6α,22α,25*R*)-3,26-Bis[(β-*D*-glucopyranosyl)oxy]-22-hydroxyfurostan-6-yl β-*D*-Glucopyranoside*; **1**). White amorphous power. $[\alpha]_D^{20} = -40.3$ ($c = 0.064$, pyridine). ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-QTOF-MS (neg.): 935.4857 ($[M-H]^-$), 773.4351 ($[M-H-162]^-$), 611.3807 ($[M-H-162-162]^-$), 449.3316 ($[M-H-162-162-162]^-$). HR-ESI-MS (pos.): 959.4861 ($[M+Na]^+$, $C_{45}H_{76}NaO_{20}^+$; calc. 959.4828).

Sisalasaponin D (= *(3β,5α,25*R*)-12-Oxospirostan-3-yl 6-Deoxy-α-L-mannopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside*; **2**). White amorphous power. $[\alpha]_D^{20} = -60.4$ ($c = 0.086$, pyridine). ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-QTOF-MS (neg.): 1355.6251 ($[M-H]^-$), 1223.5770 ($[M-H-132]^-$), 1061.5205 ($[M-H-132-162]^-$), 915.4227 ($[M-H-132-162-146]^-$), 753.3995 ($[M-H-132-162-146-162]^-$), 591.3532 ($[M-H-132-162-146-162-162]^-$), 429.2926 ($[M-H-132-162-146-162-162-162]^-$). HR-ESI-MS (pos.): 1379.6148 ($[M+Na]^+$, $C_{62}H_{100}NaO_{32}^+$; calc. 1379.6090).

Acid Hydrolysis. Compound **1** (2.0 mg) was treated with 1M HCl (dioxane/H_2O, 1:1, 2 ml) at 100° for 1.5 h. The mixture was neutralized with Ag_2CO_3, and the solvent was thoroughly driven out under N_2 overnight. The residue was extracted with $CHCl_3$ and H_2O. Then, in the monosaccharide mixture, glucose from **1** was detected by TLC analysis on a cellulose plate with BuOH/AcOEt/C_5H_5N/H_2O 6:1:5:4 and aniline-*o*-phthalic acid for detection, comparing with the authentic sample (glucose: R_f 0.46).

Furthermore, the sugar residue in pyridine (1 ml) was added to L-cysteine methyl ester hydrochloride (3.0 mg), and the mixture was kept at 60° for 1 h. Then, HMDS/TMCS (hexamethyldisilazane/trimethylchlorosilane; 0.6 ml) was added to the mixture and kept at 60° for 0.5 h. The supernatant (1.0 ml) was analyzed by GC (*Agilent Technologies 6890 GC* was the equipment carrying H_2 flame ionization detector and *HP-5* cap. column (30 m × 0.25 mm × 0.25 μm; column temp., 180°/250°; programmed increase, 3°/min, carrier gas, N_2 (1 ml/min); injection and detector temp., 250°; injection volume, 4.0 μl; split ratio, 1:50). The retention times (t_R [min]) of the derivatives of the authentic standards were: t_R 17.95 (D-glucose derivative), t_R 18.39 (L-glucose derivative), t_R 19.57 (D-galactose derivative), and t_R 19.18 (L-galactose derivative), t_R 14.53 (L-rhamnose derivative), t_R 13.00 (D-xylose derivative), and t_R 13.66 (L-xylose derivative). The t_R value of the derivative of D-glucose (D-glucose derivative) for compound **1** was 17.93. The same procedures carried out for **2** (*ca.* 2.0 mg) provided the t_R values of 17.94, 14.53, 19.57, and 13.00 for D-glucose, L-rhamnose, D-galactose, and D-xylose, resp.

REFERENCES

[1] G. M. Hocking, 'A Dictionary of Natural Products', Plexus Publishing Inc., New Jersey, 1977, p. 22.
[2] A. T. Peana, M. D. L. Moretti, V. Manconi, G. Desole, P. Pippia, *Planta Med.* **1997**, *63*, 199.
[3] A. Yokosuka, Y. Mimaki, M. Kuroda, Y. Sashida, *Planta Med.* **2000**, *66*, 393.
[4] J.-M. Jin, Y.-J. Zhang, C.-R. Yang, *Chem. Pharm. Bull.* **2004**, *52*, 654.
[5] P. Zou, J. Fu, H.-S. Yu, J. Zhang, L.-P. Kang, B.-P. Ma, X.-Z. Yan, *Magn. Reson. Chem.* **2006**, *44*, 1090.
[6] A. Yokosuka, Y. Mimaki, *Chem. Pharm. Bull.* **2007**, *55*, 145.
[7] A. Yokosuka, Y. Mimaki, *Phytochemistry* **2009**, *70*, 807.
[8] J. Eskander, C. Lavaud, D. Harakat, *Fitoterapia* **2010**, *81*, 371.
[9] S. C. Sharma, O. P. Sati, *Phytochemistry* **1982**, *21*, 1820.
[10] P.-K. Agrawal, *Magn. Reson. Chem.* **2004**, *42*, 990.
[11] P.-K. Agrawal, *Steroids* **2005**, *70*, 715.
[12] J. Zhang, B.-P. Ma, L.-P. Kang, H.-S. Yu, Y. Yang, X.-Z. Yan, *Chin. J. Magn. Reson.* **2006**, *23*, 31.
[13] O. P. Sati, G. Pant, K. Miyahara, T. Kawasaki, *J. Nat. Prod.* **1985**, *48*, 395.
[14] J.-M. Jin, Y.-J. Zhang, C.-R. Yang, *J. Nat. Prod.* **2004**, *67*, 5.
[15] G.-I. Nonaka, N. Miwa, I. Nishioka, *Phytochemistry* **1982**, *21*, 429.

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