Cytotoxic Steroidal Saponins from *Polygonatum zanlanscianense*

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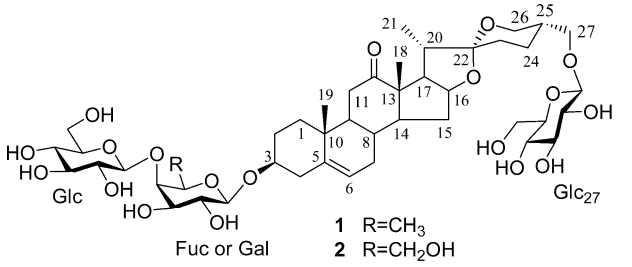
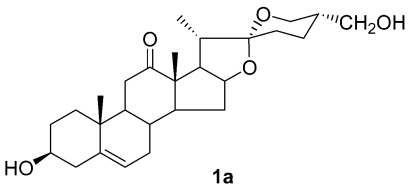
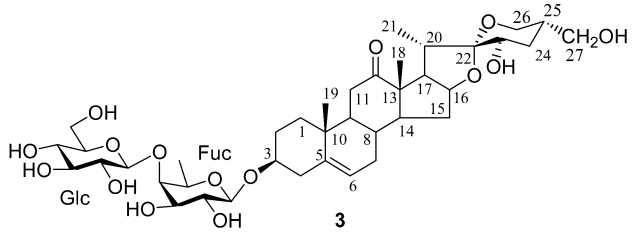
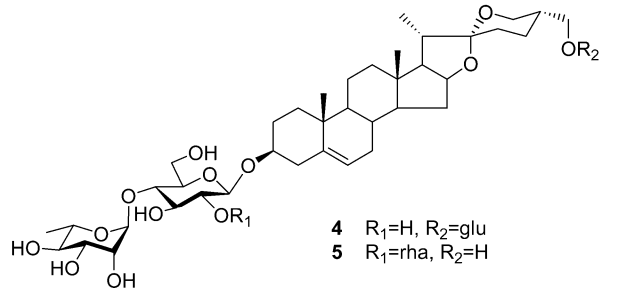
Four new steroidal saponins, polygonatosides A−D (**1**−**4**), were isolated from the rhizomes of *Polygonatum zanlanscianense*, together with six known spirostanols (**5**−**10**) and a known megastigmane glycoside (**11**). Their structures were elucidated on the basis of spectroscopic analysis and the results of acidic and enzymatic hydrolysis. The cytotoxic activities of **1**−**11** against HeLa cells are described.

Plants of the genus *Polygonatum*, comprising about 40 species, grow mainly in the north temperate zone of the world. Of these, 31 species are found in the People's Republic of China, especially in the southwest. The underground parts of some species of this genus, known as "Huangjing" and "Yuzhu", are famous traditional Chinese medicines.[1,2] *P. zanlanscianense* Pamp. (Liliaceae) is widely distributed in the central and southwest areas of China, and its rhizomes constitute one of the crude materials of "Huangjing", which is used as a tonic and a remedy for lung troubles and ringworm.[2] Various steroidal saponins have been isolated from species of this genus,[3−12] but no chemical studies have been performed on *P. zanlanscianense*. As a part of our continuing work to discover novel steroidal saponins from *Polygonatum* plants,[8,9] we have chemically investigated the rhizomes of *P. zanlanscianense*. This study has resulted in the isolation of four new steroidal saponins, polygonatosides A−D (**1**−**4**), together with six known saponins (**5**−**10**) and a known megastigamane glycoside (**11**). We describe herein the structure determination of **1**−**4**. The cytotoxic activities of **1**−**11** against HeLa cells are also reported.



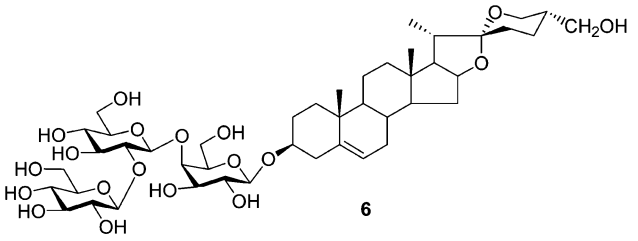


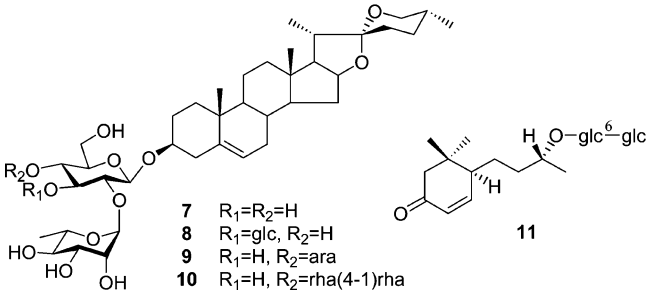




Results and Discussion

The air-dried rhizomes of *P. zanlanscianense* (8.4 kg) were extracted with MeOH under reflux. The MeOH extract was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was concentrated and then chromatographed repeatedly over silica gel and RP-8 columns to give compounds **1**−**4** and seven known glycosides, identified as steroidal saponins isonarthogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*β*-D-glucopyranoside (**5**),[13] isonarthogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**6**),[9] prosapogenin A of dioscin (**7**),[14] gracillin (**8**),[14] Pa (**9**),[15] and parissaponin P_b (**10**),[16] and a megastimane glycoside, (6*R*,9*R*)-9-hydroxy-4-megastigmen-3-one 9-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (**11**),[17] respectively.





Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{45}H_{70}O_{19}$ on the basis of ^{13}C DEPT data and negative ion HRFABMS ([M − H]$^-$, *m/z* 913.4379). The negative ion FABMS also

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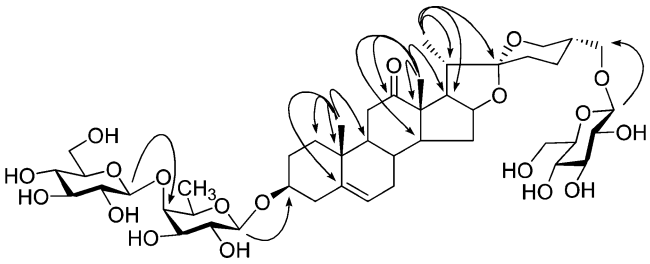

Figure 1. Important HMBC correlations of **1**.

showed fragment ion peaks at *m/z* 975 $[M - H - 162]^-$, suggesting the existence of a hexosyl unit in the molecule. The ^1H NMR spectrum of **1** showed four methyl signals at δ 1.08 (s), 0.93 (s), 1.30 (d, J = 6.3 Hz), and 1.61 (d, J = 5.8 Hz), as well as three anomeric proton signals at δ 4.79 (d, J = 7.3 Hz), 5.20 (d, J = 7.6 Hz), and 4.76 (d, J = 7.9 Hz). The DEPT spectrum also showed a characteristic quaternary carbon signal at δ 109.7. These observations suggested that **1** was a spirostanol glycoside. The DEPT spectrum of **1** also exhibited olefinic carbon signals at δ 141.0 (C) and 121.5 (CH), characteristic of Δ^5, and a carbonyl carbon signal at δ 212.8, suggesting the existence of a carbonyl group at C-12.[8] Acid hydrolysis of **1** produced a new steroidal sapogenin, **1a**, and D-glucose and D-fucose as sugar residues determined by GLC analysis. The J values (>7 Hz) of the anomeric proton signals indicated the β-orientation at the anomeric centers of D-glucopyranose and D-fucopyranose. The methyl proton signal at δ 1.61 was attributable to the methyl in the fucopyranosyl unit. The appearance of an additional carbon signal at δ 72.0 (CH_2) in the ^{13}C NMR spectrum suggested that C-27 was oxygenated and attached to a sugar unit.[9]

Sapogenin **1a** was a white amorphous powder and exhibited typical fragment ion peaks at *m/z* 155 (base peak), 142, and 131 in the FABMS, suggesting a hydroxyl group associated with ring F. The presence of three characteristic methyl signals at δ 1.13 (s, H-18), 1.05 (s, H-19), and 1.36 (d, J = 6.7 Hz, H-21) in the ^1H NMR spectrum indicated that C-27 was hydroxylated. The ^{13}C NMR data arising from rings A−E of **1a** were in good agreement with those of gentrogenin,[8] suggesting the same partial structure of rings A−E and the β-orientation of the hydroxyl group at C-3 as in gentrogenin. The same ^{13}C chemical shifts observed for ring F in **1a** as for isonarthogenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**6**)[9] allowed us to assign the 25*S* configuration in **1a**. Consequently, **1a** was deduced to be (25*S*)-3β,27-dihydroxyspirost-5-en-12-one.

The sequence of the sugars and binding sites to the aglycone of **1** were determined by 2D NMR experiments. The HMQC-TOCSY spectrum assigned the ^{13}C NMR chemical shifts for each sugar unit. In the HMBC spectrum of **1** (Figure 1), correlations of δ_H 4.76 (H-Glc$_{27}$-1) with δ_C 72.0 (C-27), δ_H 4.79 (H-Fuc-1) with δ_C 77.7 (C-3), and δ_H 5.20 (H-Glc-1) with δ_C 83.3 (C-Fuc-4) were observed. Therefore, **1** was determined to be (25*S*)-3β,27-dihydroxyspirost-5-en-12-one 27-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside, and named polygonatoside A.

The molecular formula of **2** was assigned to be $C_{45}H_{70}O_{20}$ on the basis of its negative ion HRFABMS ($[M - H]^-$ *m/z* 929.4425). The negative ion FABMS also showed a fragment ion peak at *m/z* 767 $[M - H - 162(\text{hexosyl})]^-$, suggesting the existence of a hexosyl unit in the molecule. The ^1H and ^{13}C NMR spectral features of the aglycone moiety of **2** were very similar to those of **1**, which showed a quaternary carbon signal at δ 109.7 (C-22), olefinic carbon signals at δ 140.9 (C-5) and 121.5 (C-6), a carbonyl carbon signal at δ 212.9, and three methyl proton signals at δ 1.07 (s, H-18), 0.89 (s, H-19), and 1.30 (d, J = 6.5 Hz, H-21), in addition to three anomeric proton signals at δ 4.88 (d, J = 7.3 Hz), 5.27 (d, J = 8.6 Hz), and 4.78 (d, J = 7.9 Hz). These observations suggested that **2** had the same aglycone as **1**, but different sugar moieties. Acid hydrolysis of **2** yielded (25*S*)-3β,27-dihydroxyspirost-5-en-12-one (**1a**), D-glucose, and D-galactose. The J values (>7 Hz) of the anomeric proton signals indicated β-orientation at the anomeric centers. In the HMBC spectrum of **2**, correlations of δ 4.78 (H-Glc$_{27}$-1) with 72.0 (C-27), δ 4.87 (H-Gal-1) with 77.9 (C-3 of the aglycone), and δ 5.20 (H-Glc-1) with 80.0 (C-4 of galactose) confirmed the sugar locations and sequence of **2**. Therefore, polygonatoside B (**2**) was determined to be (25*S*)-3β,27-dihydroxyspirost-5-en-12-one 27-*O*-β-D-glucopyranosyl-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **3** had the molecular formula $C_{39}H_{59}O_{15}$ on the basis of the negative ion HRFABMS ($[M - H]^-$ *m/z* 767.3870). Acid hydrolysis of **3** yielded D-glucose and D-fucose as sugar residues. Comparing the ^1H and ^{13}C NMR spectral data of **3** with those of **1**, it was evident that their structures were similar, except for the F ring. In addition, **3** showed only two anomeric proton signals at δ 4.79 (d, J = 7.8 Hz) and 5.20 (d, J = 7.6 Hz). These observations indicated that **3** had one more hydroxyl group (attached at C-23) and no glucopyranosyl unit linked at C-27, which led to the downfield shifts of C-23 and C-22 to δ 67.7 and 112.3 and the upfield shift of C-27 to δ 64.0 in **3**. The ^{13}C NMR data of the F ring were in good agreement with those of (23*S*, 25*S*)-5α-spirostan-3β,23,27-triol (agavegenin B),[18] suggesting the 23α orientation of the hydroxyl group and the 25*S* configuration in **3**. NOE correlations (Figure 2) of δ 3.89 (H-3) with 0.90 (H-1α), 2.08 (H-2α), and 2.71(H-4α), and δ 3.98 (H-23) with 3.03 (H-20) and 2.29 (H-25) in the ROESY spectrum confirmed these configurations of **3**. In the HMBC spectrum of **3** (Figure 2), correlations of δ 4.79 (H-Fuc-1) with 77.7 (C-3 of the aglycone) and δ 5.20 (H-Glc-1) with 83.3 (C-4-Fucose) determined the sugar linkages. Thus, **3** was deduced to be (23*S*,25*S*)-3β,23,27-trihydroxyspirost-5-en-12-one 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside and was named polygonatoside C. As far as we can determine, **3** is the first example of a saponin having a new aglycone, (23*S*,25*S*)-3β,23,27-trihydroxyspirost-5-en-2-one.

Compound **4** was determined to have the molecular formula $C_{45}H_{72}O_{18}$ on the basis of its negative ion HRFABMS ($[M - H]^-$ *m/z* 899.4728). The ^1H NMR spectrum of **4** showed the existence of three sugar units [anomeric H: δ 4.77 (d, J = 7.6 Hz), 4.96 (d, J = 7.0 Hz), and 5.91 (br s)]. Acid hydrolysis of **4** with 1 M HCl gave (25*S*)-spirost-5-ene-3β,27-diol (isonarthogenin)[19] as the aglycone, and L-rhamnose and D-glucose as sugar residues. In the HMBC spectrum of **4**, correlations of δ 4.77 (H-Glc$_{27}$-1) with 72.0 (C-27 of the aglycone), δ 4.96 (H-Glc$_3$-1) with 78.3 (C-3), and δ 5.91 (H-Rha-1) with 78.6 (C-Glc$_3$-4) were observed. Therefore, polygonatoside D (**4**) was assigned to be (25*S*)-spirost-5-ene-3β,27-diol 27-*O*-β-D-glucopyranosyl-3-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside.

Although some spirostane sapogenins and their glycosides with hydroxylation on C-27 have been isolated from liliaceous plants,[19−23] C-27 glycosides are still very few.[9] In this work, 10 steroidal saponins were isolated from the rhizome of *P. zanlanscianense*, among which **1**−**6** were hydroxylated at C-27. Another four compounds (**7**−**10**) were

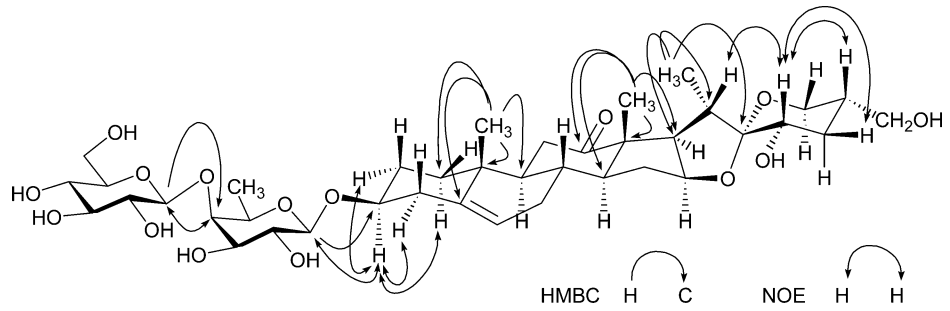

Figure 2. Significant HMBC and NOE correlations of **3**.

Table 1. ^{13}C NMR Data (δ) of Compounds **1**, **1a**, and **2**−**4**[a]

aglycone	**1**	**1a**	**2**	**3**	**4**	sugar	**1**	**2**	**3**	**4**
1	37.1 (t)	37.4 (t)	37.0 (t)	37.1 (t)	37.5 (t)	Fuc-1	102.7 (d)		102.7 (d)	
2	30.1 (t)	31.7 (t)	30.0 (t)	30.0 (t)	30.2 (t)	2	73.0 (d)		73.0 (d)	
3	77.7 (d)	71.1 (d)	77.9 (d)	77.7 (d)	78.3 (d)	3	76.3 (d)		76.3 (d)	
4	39.1 (t)	43.2 (t)	39.1 (t)	39.1 (t)	39.3 (t)	4	83.3 (d)		83.3 (d)	
5	141.0 (s)	141.8 (s)	140.9 (s)	140.9 (s)	141.0 (s)	5	70.6 (d)		70.6 (d)	
6	121.5 (d)	120.9 (d)	121.5 (d)	121.5 (d)	121.8 (d)	6	17.7 (q)		17.7 (q)	
7	31.6 (t)	32.3 (t)	31.6 (t)	31.6 (t)	32.2 (t)	Gal 1		103.0 (d)		
8	31.0 (d)	31.0 (d)	30.9 (d)[b]	30.9 (d)[c]	31.7 (d)	2		73.5 (d)		
9	52.4 (d)	52.5 (d)	52.3 (d)	52.4 (d)	50.3 (d)	3		75.8 (d)		
10	37.7 (s)	37.6 (s)	37.6 (s)	37.6 (s)	37.1 (s)	4		80.0 (d)		
11	37.7 (t)	37.7 (t)	37.6 (t)	37.6 (t)	21.2 (t)	5		76.0 (d)		
12	212.8 (s)	213.1 (s)	212.9 (s)	213.0 (s)	39.9 (t)	6		61.0 (t)		
13	55.0 (s)	55.1 (s)	55.0 (s)	55.6 (s)	40.5 (s)	Glc 1	107.0 (d)	107.1 (d)	107.0 (d)	105.5 (d)
14	56.0 (d)	56.2 (d)	56.0 (d)	56.0 (d)	56.7 (d)	2	75.6 (d)	75.3 (d)	75.6 (d)	75.6 (d)
15	31.8 (t)	31.7 (t)	31.8 (t)[b]	31.8 (t)[c]	32.3 (t)	3	78.6 (d)	78.8 (d)	78.6 (d)	76.7 (d)
16	79.8 (d)	79.9 (d)	79.8 (d)	80.4 (d)	81.2 (d)	4	71.7 (d)	72.3 (d)	71.7 (d)	78.6 (d)
17	54.0 (d)	54.1 (d)	54.0 (d)	53.7 (d)	62.8 (d)	5	78.6 (d)	78.5 (d)	78.7 (d)	77.2 (d)
18	16.0 (q)	16.0 (q)	15.9 (q)	16.1 (q)	16.4 (q)	6	62.9 (t)	63.2 (t)	62.9 (t)	61.6 (t)
19	18.9 (q)	19.1 (q)	18.9 (q)	18.8 (q)	19.5 (q)	Rha 1				102.8 (d)
20	42.7 (d)	42.8 (d)	42.7 (d)	36.5 (d)	42.0 (d)	2				72.7 (d)
21	13.9 (q)	14.0 (q)	13.9 (q)	13.7 (q)	15.0 (q)	3				72.9 (d)
22	109.7 (s)	109.9 (s)	109.7 (s)	112.3 (s)	109.6 (s)	4				74.0 (d)
23	31.3 (t)	34.8 (t)	31.3 (t)	67.7 (d)	31.3 (t)	5				70.4 (d)
24	24.0 (t)	24.1 (t)	24.0 (t)	39.1 (t)	24.0 (t)	6				18.6 (q)
25	36.7 (d)	39.2 (d)	36.7 (d)	40.5 (d)	36.7 (d)	Glc_{27} 1	105.1 (d)	105.1 (d)		105.1 (d)
26	63.8 (t)	64.1 (t)	63.8 (t)	63.4 (t)	63.7 (t)	2	75.2 (d)	75.3 (d)		75.2 (d)
27	72.0 (t)	64.4 (t)	72.0 (t)	64.0 (t)	72.0 (t)	3	78.6 (d)	78.6 (d)		78.6 (d)
						4	71.7 (d)	71.7 (d)		71.7 (d)
						5	78.6 (d)	78.6 (d)		78.6 (d)
						6	62.9 (t)	62.9 (t)		62.9 (t)

[a] Spectra were measured at 125 MHz for ^{13}C and 500 MHz for ^{1}H in pyridine-d_5. [b,c] Values may be interchanged in the same column.

Table 2. Cytotoxic Activities of **1**−**11** against HeLa Cells

compound	IC_{50} (μg/mL)	compound	IC_{50} (μg/mL)
1	5.06	**7**	6.23
2	5.13	**8**	12.74
3	7.45	**9**	14.57
4	5.83	**10**	3.14
5	3.62	**11**	20.05
6	5.24	cisplatin	0.75

saponins with diosgenin as the sapogenin. Steroidal saponins **1**−**10** as well as a known megastigamane glycoside (**11**) obtained from this plant were tested for in vitro cytotoxicity against HeLa cells. The IC_{50} values of **1**−**11** are listed in Table 2. All of them showed weak cytotoxicity (IC_{50} > 3 μg/mL) against HeLa cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR (KBr) spectra were measured on a Bio-Rad FTS-135 spectrophotometer. NMR spectra were recorded on a Bruker DRX-500 instrument (500 MHz for ^{1}H NMR, and 125 MHz for ^{13}C NMR) at 25 °C, using TMS as an internal standard. The negative ion and high-resolution FAB mass spectra were recorded on a VG AutoSpec-3000 mass spectrometer using glycerol as matrix. Precoated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done by spraying the plates with 5% anisaldehyde-sulfric acid, followed by heating.

Plant Material. The rhizomes of *Polygonatum zanlanscianense* were obtained from Wufeng County of Hubei Province, People's Republic of China, and identified by Prof. Da-Chang Liu (Senior Pharmaceutist of Yunnan Institute for Drug Control, Kunming City, Yunnan Province, People's Republic of China).

Extraction and Isolation. The air-dried rhizomes of *P. zanlanscianense* (8.4 kg) were extracted three times with hot 80% EtOH. The combined extract was concentrated and then partitioned between *n*-butanol and H_2O. The *n*-butanol residue (109 g) was applied to a silica gel column and chromatographed with $CHCl_3$/MeOH/H_2O (7:2.5:0.4) to give four fractions (I−IV). Fractions 2 and 3 were rich in steroidal saponins. Fraction 2 (12 g) was repeatedly chromatographed over silica gel ($CHCl_3$/MeOH/H_2O) and RP-8 (MeOH/H_2O) to afford **3** (15 mg), **7** (21 mg), **8** (30 mg), **9** (215 mg), and **10** (45 mg). Fraction 3 (13.5 g) was separated by repeated CC over silica gel ($CHCl_3$/MeOH/H_2O) and RP-8 (MeOH/H_2O) to yield **1** (46 mg), **2** (12 mg), **4** (5 mg), **5** (12 mg), **6** (5 mg), **8** (8 mg), **10** (4 mg), and **11** (15 mg).

GLC Analysis for Sugar Residues.[24] After hydrolysis of the saponins with 1 M HCl and partitioning between $CHCl_3$ and H_2O four times for each of the saponins, the H_2O layers were neutralized with Amberlite MB-3 and concentrated to dryness to yield mixtures of sugars. According to the Oshima method, a solution of the sugar mixture in H_2O (40 μL) and a solution of L-(−)-MBA (7 mg) and $NaBH_3CN$ (1.6 mg) in EtOH (40 μL) was kept at 40 °C for 3.5 h. Several drops of Ac_2O were added, and the mixture was evaporated to dryness with a N_2 stream and further dried over P_2O_5 in a vacuum oven. The residue was trimethylsilylated with TMS-HT (50 μL) at room temperature for 20 min and then directly subjected to GLC analysis. Identification of peaks was made by comparing with those of the reaction product of an authentic sugar and L-(−)-MBA. GLC conditions: AC-5 capillary column (30 m × ϕ 0.25 mm); detection, FID; column temperature, 180 °C; carrier gas, He (2 kg cm^{-2}). t_R (min): L-Rha (5.41), D-Fuc (6.21), D-Gal (7.24), D-Glu (8.41).

Compound 1: white amorphous powder; $[\alpha]^{20}_D$ −24.51° (*c* 0.1489, pyridine); IR (KBr) ν_{max} 3406, 2933, 1707, 1653, 1457, 1376, 1227, 1165, 1073, 1043, 950, 910, 893, 839 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.31 (1H, br d, J = 5.2 Hz, H-6), 5.20 (1H, d, J = 7.7 Hz, H-Glc-1), 4.80 (1H, d, J = 7.3 Hz, H-Fuc-1), 4.76 (1H, d, J = 7.7 Hz, H-Glc$_{27}$-1), 4.42 (1H, q-like, J = 7.2 Hz, H-16), 3.84 (1H, m, H-3), 1.61 (3H, d, J = 6.5 Hz, H-Fuc-6), 1.08 (3H, s, H-18), 0.92 (3H, s, H-19), 1.30 (3H, d, J = 6.4 Hz, H-21); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m/z* 913 $[M - H]^-$, 751 $[M - H - 162(hexosyl)]^-$; HRFABMS *m/z* 913.4379 $[M - H]^-$ (calcd for $C_{45}H_{69}O_{19}$, 913.4433).

Acid Hydrolysis of 1. Compound **1** (7 mg) was refluxed with 1 M HCl/dioxane (1:1, v/v, 2 mL) on a H_2O bath for 6 h. The reaction mixture was evaporated to dryness. The dry reaction mixture was partitioned four times between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated and subjected to silica gel CC ($CHCl_3$/EtOAc) to provide **1a** (2 mg): white amorphous powder; ^1H NMR (pyridine-d_5) δ 3.87 (1H, m, H-3), 5.34 (1H, br d, J = 5.6 Hz, H-6), 4.49 (1H, q-like, J = 6.7 Hz, H-16), 1.13 (3H, s, H-18), 1.05 (3H, s, H-19), 1.36 (3H, d, J = 6.7 Hz, H-21); ^{13}C NMR (pyridine-d_5), see Table 1; EI-MS *m/z* 444, 416, 386, 369, 356, 314, 306, 271, 155 (base peak), 142, 131, 119, 105, 91, 69, 57; HREIMS *m/z* 444.2865 (calcd for $C_{27}H_{40}O_5$, 444.2876). The H_2O layer was subjected to GLC analysis to give D-glucose and D-fucose as sugar moieties.

Compound 2: white amorphous powder; $[\alpha]^{20}_D$ −19.19° (*c* 0.0521, pyridine); IR (KBr) ν_{max} 3439, 2933, 1704, 1633, 1454, 1043, 981, 908 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.32 (1H, br d, J = 5.1 Hz, H-6), 5.27 (1H, d, J = 8.6 Hz, H-Glc-1), 4.88 (1H, d, J = 7.3 Hz, H-Gal-1), 4.78 (1H, d, J = 7.9 Hz, H-Glc$_{27}$-1), 4.40 (1H, q-like, J = 6.8 Hz, H-16), 3.84 (1H, m, H-3), 1.30 (3H, d, J = 6.5 Hz, H-21), 1.07 (3H, s, H-18), 0.89 (3H, s, H-19); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m/z* 929 $[M - H]^-$, 767 $[M - H - 162(hexosyl)]^-$; HRFABMS *m/z* 929.4425 $[M - H]^-$ (calcd for $C_{45}H_{69}O_{20}$, 929.4382).

Acid Hydrolysis of 2. Compound of **2** (3 mg) was subjected to acid hydrolysis as described for **1** to give D-glucose and D-galactose as sugar moieties by GLC analysis.

Compound 3: white solid; $[\alpha]^{20}_D$ −48.43° (*c* 0.0351, pyridine); IR (KBr) ν_{max} 3428, 2932, 1707, 1643, 1457, 1375, 1265, 1165, 1072, 1043, 957, 900 cm^{-1}; 23.21 (*c* 0.0474, pyridine); IR (KBr) ν_{max} 3407, 2927,1704, 1373, 1160, 1071, 1040, 894 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.32 (1H, br d, J = 5.9 Hz, H-6), 5.20 (1H, d, J = 7.6 Hz, H-Glc-1), 4.79 (1H, d, J = 7.8 Hz, H-Fuc-1), 4.58 (1H, q-like, J = 6.0 Hz, H-16), 3.89 (1H, m, H-3), 3.85 (1H, dd, J = 8.6, 6.8 Hz, H-23), 1.60 (3H, d, J = 6.3 Hz, H-Fuc-6), 1.42 (3H, d, J = 6.8 Hz, H-21), 1.28 (3H, s, H-18), 0.85 (3H, s, H-19); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m/z* 767 $[M - H]^-$; HRFABMS *m/z* 767.3870 $[M - H]^-$ (calcd for $C_{39}H_{59}O_{15}$, 767.3854).

Acid Hydrolysis of 3. Compound of **3** (3 mg) was subjected to acid hydrolysis as described for **1** to give D-glucose and D-fucose as sugar moieties by GLC analysis.

Compound 4: white amorphous powder; $[\alpha]^{20}_D$ −50.31° (*c* 0.0141, pyridine); IR (KBr) ν_{max} 3416, 2933, 1642, 1456, 1375, 1155, 1068, 1050, 910 cm^{-1}; ^1H NMR (pyridine-d_5) δ 5.91 (1H, br s, H-Rha-1), 5.31 (1H, br d, J = 5.4 Hz, H-6), 4.96 (1H, d, J = 7.0 Hz, H-Glc-1), 4.77 (1H, d, J = 7.6 Hz, H-Glc$_{27}$-1), 4.57 (1H, q-like, J = 5.8 Hz, H-16), 3.92 (1H, m, H-3), 1.72 (3H, d, J = 7.6 Hz, H-Rha-6), 1.08 (3H, d, J = 6.8 Hz, H-21), 1.05 (3H, s, H-19), 0.80 (3H, s, H-18); ^{13}C NMR (pyridine-d_5), see Table 1; FABMS (negative mode) *m/z* 899 $[M - H]^-$, 753 $[M - H - 146(hexosyl)]^-$; HRFABMS *m/z* 899.4781 $[M - H]^-$ (calcd for $C_{45}H_{71}O_{18}$, 899.4640).

Acid Hydrolysis of 4. Compound of **4** (2 mg) was subjected to acid hydrolysis as described for **1** to give D-glucose and L-rhamnose as sugar moieties by GLC analysis.

Cytotoxicity against HeLa Cells. The cytotoxicity against HeLa cells was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay on 96-well microplates as described in a previous paper.[25] The OD value was read on a plate reader at a wavelength of 570 nm. The cytotoxicity was expressed as IC_{50} value (μg/mL), which was the mean of three determinations and reduced the viable cell number by 50%.

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