Triterpenoid Saponins from *Ardisia gigantifolia*

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Four new triterpenoid saponins (1—4) were isolated from the rhizome of *Ardisia gigantifolia* Stapf. The structures of new saponins were established as 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane (1), 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-30-acetoxy-oleanane (2), 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane (3) and 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-6-*O*-acetylglucopranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane (4) were isolated from *Ardisia gigantifolia* Stapf. Their structures were elucidated by means of ^{1}H- and ^{13}C-NMR spectroscopic studies, including 2D-NMR technique. The cytotoxic activities of saponins 1—4 are reported against three human cancer cell lines, namely, Hela human cervical carcinoma cells, EJ human bladder tumor cells, and BCG-823 human gastric carcinoma cells.

Key words *Ardisia gigantifolia*; Myrsinaceae; triterpenoid saponin; cytotoxic activity

The dried rhizome of *Ardisia gigantifolia* Stapf is mainly used as Chinese folk medicine in south of China for the treatment of rheumatism, pain of muscles and bones and traumatic injury. It distributes in the provinces of Guangxi, Jiangxi and Fujian in China, which is a kind of evergreen dwarf shrub.[1)] Previous chemical studies showed that triterpenoid saponins were the main components from this genus. 32 Triterpenoid saponins have been isolated from the rhizome of *Ardisia gigantifolia* Stapf over the last 20 years.[2)] In this paper, the isolation and characterization of four new triterpenoid saponins from 60% ethanol percolation of the dried rhizome of the plant were reported.

Results and Discussion

Compound **1**, obtained as a white powder, showed positive Libermann–Burchard and Molish reactions, suggesting that **1** might be a triterpenoid saponin or steroidal saponin. high resolution-electrospray ionization-time-of-flight (HR-ESI-TOF)-MS of **1** showed the quasi-molecular ion at *m*/*z* 1191.6279 $[M-H]^-$, establishing the molecular formula of $C_{58}H_{96}O_{25}$. The ^{13}C-NMR spectral data of the sapogenin part of **1** were similar to those of the known oleananetype triterpene cyclamiretin A.[3)] In cyclamiretin A, one aldehyde carbon correspond to ^{13}C-NMR resonances at δ 207.5 (C30, CH in distortionless enhancement by polarization transfer (DEPT)), However, in compound **1**, there was a lack ofany resonance due to C30 at δ 207.5 ppm, instead, a signal was observed at δ 24.8 ppm (CH_3 by DEPT). The ^{1}H-NMR spectrum displayed signals for seven methyl groups at δ 1.53 (3H, s), 1.34 (3H, s), 1.16 (3H, s), 1.08 (3H, s), 1.02 (3H, s), 0.98 (3H, s) and 0.83 (3H, s), attributable to aglycone moiety, one more methyl group than those of cyclamiretin A. This signal indicated that a CH_3 group should be at C-30. This assignment was confirmed by the long-range coupling of H-30 with C-29, C-19, C-20 and C-21 in heteronuclear multiple bond connectivity (HMBC). Based on this evidence, the structure of the sapogenin of **1** was established as 3β,16α-dihydroxy-13,28-epoxy-oleanane (protoprimulagenin A).[4)]

On acid hydrolysis with 2 M HCl, **1** afforded sugar moieties that identified as L-rhamnose, L-arabinose, D-xylose, and D-glucose in the relative proportions of 1 : 1 : 1 : 2 based on the GC-MS analysis of their chiral derivatives.[5)] In ^{1}H-NMR, five anomericsingals appearing at δ: 5.98 (1H, br s, Rha-1), 5.41 (1H, d, J=7.6 Hz, GlcI-1), 5.02 (1H, d, J=6.8 Hz, Xyl-1), 4.87 (1H, d, J=7.2 Hz, GlcII-1), and 4.84 (1H, d, J=5.2 Hz, Ara-1). The attachment points of the sugar chain and inter glycosidic linkage were established by HMBC and nuclear Overhauser effect spectroscopy (NOESY) correlations. HMBC correlations were observed between H-1 (δ 4.84) of arabinosyl and C-3 (δ 88.9) of the aglycone, H-1 (δ 5.41) of glucosyl-I and C-2 (δ 79.4) of arabinosyl, H-1 (δ 4.87) of glucosyl-II and C-4 (δ 78.1) of arabinosyl, H-1 (δ 5.02) of xylosyl and C-2 (δ 81.1) of glucosyl-II, and H-1 (δ 5.98) of rhamnosyl and C-3 (δ 85.7) of glucosyl-II. NOESY correlations were observed between δ_H 5.41 (GlcI-H-1) and δ_H 4.53 (Ara-H-2), δ_H 4.87 (GlcII-H-1) and δ_H 4.26 (Ara-H-4), δ_H 5.02 (Xyl-H-1) and δ_H 4.01 (GlcII-H-2), δ_H 5.98 (Rha-H-1) and δ_H 4.13 (GlcII-H-3). In the ^{1}H-NMR spectrum of **1**, the relatively large $^3J_{H\text{-}1,H\text{-}2}$ coupling constant of the anomeric protons for the Glc, Xyl, and Ara (between 6.0, 8.0 Hz) moieties indicated a β-configuration for D-Glc and D-Xyl and an α-configuration for L-Ara. The broad singlet of the anomeric proton of the Rha unit indicated an α-orientation.[6)] On the basis of the above data, the structure of **1** was established as 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane.

Compound **2** was also obtained as a white powder, showed positive Libermann–Burchard and Molish reactions, suggesting that **2** might be a triterpenoid saponin or steroidal saponin. HR-ESI-TOF-MS of **2** showed the quasi-molecular ion at *m*/*z* 1249.6344 $[M-H]^-$, establishing the molecular formula of $C_{60}H_{98}O_{27}$. The ^{13}C-NMR data of the aglycone of **2** were similar to that of **1**, but the methyl group signal for C-30 was replaced by δ_C 67.9 and an acetyl group at δ_C 20.6

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Table 1. The ^{1}H-NMR Data for the Aglycone Moieties of Compounds **1**—**4** (400 MHz, Pyridine-d_5)[a,b]

	1	**2**	**3**	**4**
1	0.81, 1.60	0.83, 1.64	0.81, 1.56	0.84, 1.61
2	1.76 (m), 1.95 (br s)	1.72, 1.86 (m)	1.71 (m), 1.85 (m)	1.76, 1.94
3	3.11 (dd, 11.6, 4.4)	3.05 (dd, 11.4 ,4.0)	3.03 (dd, 11.2, 4.0)	3.15 (dd, 11.2 4.0)
4	—	—	—	—
5	0.65 (d, 10.0)	0.59 (d, 10.8)	0.58 (d, 10.4)	0.71 (d, 10.8)
6	1.40	1.34	1.36	1.39
7	1.24, 1.55	1.15, 1.14	1.17, 1.51	1.22, 1.57
8	—	—	—	—
9	1.28	1.19	1.20	1.25
10	—	—	—	—
11	1.43, 1.78 (m)	1.41, 1.64 (m)	1.36, 1.71 (m)	1.42, 1.76 (m)
12	1.45, 2.05 (m)	1.40, 1.99 (m)	1.36, 1.97 (m)	1.42, 1.96 (m)
13	—	—	—	—
14	—	—	—	—
15	1.45, 2.22 (m)	1.36, 2.15 (dd, 14.0, 4.8)	1.39, 2.17 (dd, 14.4, 4.4)	1.45, 2.22 (m)
16	4.01	4.16	4.13	4.20
17	—	—	—	—
18	1.65	1.64	1.56	1.58
19	1.32, 2.77 (t, 13.5)	1.67, 2.70 (t, 14.0)	1.25, 2.71 (t, 12.4)	1.29, 2.76 (t, 14.0)
20	—	—	—	—
21	1.25, 2.56 (m)	2.06, 2.52 (dt, 13.4, 5.6)	1.63, 2.49 (dt, 13.6, 4.8)	1.25, 2.54 (m)
22	1.60, 1.92	1.52, 1.82	1.53, 1.87	1.57, 1.91
23	1.16 (s)	1.10 (s)	1.10 (s)	1.22 (s)
24	1.02 (s)	0.96 (s)	0.97 (s)	1.06 (s)
25	0.83 (s)	0.78 (s)	0.77 (s)	0.87 (s)
26	1.34 (s)	1.25 (s)	1.26 (s)	1.34 (s)
27	1.53 (s)	1.49 (s)	1.47 (s)	1.52 (s)
28	3.32 (d, 7.2), 3.61	3.22 (d, 7.6), 3.47	3.25 (d, 7.2), 3.55	3.31(d, 7.6), 3.59
29	1.08 (s)	1.07 (s)	1.04 (s)	1.07 (s)
30	0.98 (s)	4.05, 4.35	0.91 (s)	0.96 (s)
$CO\underline{CH_3}$		2.02 (s)		

1D and 2D NMR spectra were taken on a JNM-ctrometer in pyridine-d_5. Chemical shifts were expressed in δ downfield from internal TMS; coupling constants (J) were reported in Hz. Assignments based on TOCSY, HMQC and HMBC experiments. Overlapped signals are reported without designating multiplicity. *a*) Assignments based on TOCSY, HMQC and HMBC experiments. *b*) Overlapped signals are reported without designating multiplicity.

and δ_C 170.8. This assignment was confirmed by the HMBC correlation between δ_H 1.07 (H-29) and δ_C 67.9 (C-30), δ_H 4.05 and 4.35 (H_2-30) and δ_C 170.8 (C=O). The NOESY correlations between δ_H 1.64 (H-18) and δ_H 4.05 and 4.35 (H_2-30) were observed. Therefore, the aglycone of **2** was established as 16α-hydroxy-13,28-epoxy-30-acetoxyoleane. On acid hydrolysis, **2** afforded L-rhamnose, L-arabinose, D-xylose, and D-glucose in a ratio of 1 : 1 : 1 : 2. The ^{1}H- and ^{13}C-NMR data assignable to the sugars in **2** were identical to those in **1**. The sequence of the sugar chains was further confirmed from HMBC correlations. Hence, HMBC correlations were observed between δ_H 5.24 (GlcI-H-1) and δ_C 79.2 (Ara-C-2),δ_H 4.82 (GlcII-H-1) and δ_C 77.9 (Ara-C-4), δ_H 4.97 (Xyl-H-1) and δ_C 80.9 (GlcII-C-2), δ_H 5.94 (Rha-H-1) and δ_C 85.4 (GlcII-C-3). Thus, the structure of **2** was elucidated as 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-30-acetoxyoleanane.

Compound **3** was also obtained as a white powder, showed positive Libermann–Burchard and Molish reactions, suggesting that **3** might be a triterpenoid saponin or steroidal saponin. HR-ESI-TOF-MS of **3** showed the quasi-molecular ion at *m/z* 1353.6787 $[M-H]^-$, establishing the molecular formula of $C_{64}H_{106}O_{30}$. The ^{1}H- and ^{13}C-NMR data assignable to the aglycon moiety of **3** were identical to those of **1** (Tables 1, 3), suggesting the aglycon also to be protoprimulagenin A. Further comparison of the ^{1}H- and ^{13}C-NMR data assignable to the sugar chain between **1** and **3** (Tables 2, 4) suggested one more set of terminal β-D-glucopyranose moiety signals (Glc-III) at Xyl-C-3 in **3** than **1**. The sequence of the sugar chain was confirmed using HMBC and NOESY correlations. Thus, HMBC correlations were observed between δ_H 5.31 (GlcI-H-1) and δ_C 79.4 (Ara-C-2), δ_H 4.80 (GlcII-H-1) and δ_C 78.0 (Ara-C-4), δ_H 4.95 (Xyl-H-1) and δ_C 80.9 (GlcII-C-2), δ_H 5.87 (Rha-H-1) and δ_C 85.2 (GlcII-C-3), and δ_H 5.12 (GlcIII-H-1) and δ_C 72.5 (Xyl-C-3). NOESY correlations were observed between δ_H 5.31 (GlcI-H-1) and δ_H 4.45 (Ara-H-2), δ_H 4.80 (GlcII-H-1) and δ_H 3.95 (Ara-H-4), δ_H 4.95 (Xyl-H-1) and δ_H 3.95 (GlcII-H-2), δ_H 5.87 (Rha-H-1) and δ_H 4.04 (GlcII-H-3), and δ_H 5.12 (GlcIII-H-1) and δ_H 3.95 (Xyl-H-3). On the basis of the above results, the structure of compound **3** was elucidated as 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)-β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane.

Compound **4** was also obtained as a white powder, showed positive Libermann–Burchard and Molish reactions, suggesting that **4** might be a triterpenoid saponin or steroidal saponin. HR-ESI-TOF-MS of **4** showed the quasi-molecular ion at *m/z* 1233.6385 $[M-H]^-$, establishing the molecular formula of $C_{60}H_{98}O_{26}$. The ^{1}H- and ^{13}C-NMR data assignable to the aglycon moiety of **4** were identical to those of **1** (Tables 1, 3), suggesting the aglycon also to be protoprimulagenin A. Further comparison of the ^{1}H- and ^{13}C-NMR data assignable to the sugar chain between **1** and **4** (Tables 2, 4) suggested the presence of acetyl group combining with the

Table 2. The ^{1}H-NMR Data for Sugar Moieties of Compounds **1**—**4** (400 MHz, Pyridine-d_5)[a,b]

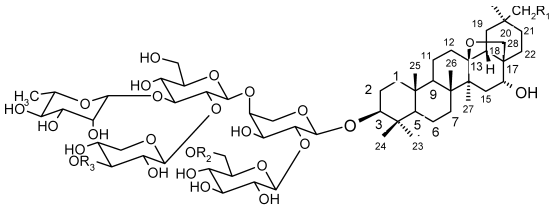
	1	**2**	**3**	**4**
Ara-1	4.84 (d, 5.2)	4.78 (d, 7.2)	4.77	4.72 (d, 5.2)
2	4.53	4.47	4.45	4.50
3	4.32	4.26	4.23	4.24
4	4.26	3.96	3.95	4.02
5	3.64, 4.53	3.59, 4.47	3.58, 4.47	3.66, 4.52
GluI-1	5.41 (d, 7.6)	5.24 (d, 7.2)	5.31 (d, 7.6)	5.36 (d, 7.6)
2	4.03	3.96	3.95	4.02
3	4.23	4.16	4.15	4.02
4	4.25	4.16	4.15	4.09
5	4.01	3.96	3.95	4.09
6	4.39, 4.53	4.39 (m), 4.47	4.31(m), 4.48	4.77 (br d, 11.6), 4.91 (br d, 11.6)
$CO\underline{CH_3}$				2.04 (s)
GlcII-1	4.87 (d, 7.2)	4.82 (d, 7.6)	4.80 (d, 7.6)	4.86 (d, 7.2)
2	4.01	3.92	3.95	4.02
3	4.13	4.04	4.04	4.09
4	4.26	4.20	4.23	4.24
5	3.66	3.59	3.61	3.66
6	4.23, 4.32	4.21, 4.28	4.21, 4.28	4.29, 4.33
Xyl-1	5.02 (d, 6.8)	4.97 (d, 7.2)	4.95 (d, 7.2)	5.09 (d, 6.8)
2	4.01	3.96	3.95	4.02
3	4.23	4.16	3.95	4.02
4	4.26	4.20	4.01	4.22
5	3.56, 4.45	3.49, 4.39	3.53, 4.29	3.57, 4.46
Rha-1	5.98 (br s)	5.94 (br s)	5.87 (br s)	5.95 (s)
2	4.87	4.96	4.81	4.94
3	4.34	4.29	4.45	4.49
4	4.03	4.26	4.23	4.30
5	4.54	4.77	4.77	4.50
6	1.65 (d, 6.0)	1.60 (d, 6.4)	1.60 (d, 6.0)	1.64 (d, 6.0)
GlcIII-1			5.12 (d, 7.6)	
2			3.95	
3			3.95	
4			4.23	
5			3.97	
6			3.99, 4.31	

a) Assignments based on TOCSY, HMQC and HMBC experiments. *b*) Overlapped signals are reported without designating multiplicity.

Table 3. The ^{13}C-NMR Data for the Aglycone Moieties of Compounds **1**—**4** (400 MHz, Pyridine-d_5)[a,b]

Carbon	**1**	**2**	**3**	**4**
1	39.2	39.0	38.9	39.1
2	26.5	26.3	26.3	26.5
3	88.9	88.7	88.8	89.1
4	39.6	39.5	39.4	39.7
5	55.6	55.4	55.4	55.7
6	17.9	17.7	17.7	17.9
7	34.4	34.1	34.2	34.4
8	42.4	42.3	42.2	42.4
9	50.5	50.2	50.3	50.5
10	36.6	36.7	36.7	36.9
11	19.3	19.0	19.1	19.2
12	32.9	32.5	32.6	32.8
13	86.4	86.2	86.2	86.3
14	44.6	44.3	44.4	44.5
15	36.9	36.7	36.7	36.9
16	77.1	76.8	76.9	77.1
17	44.6	44.2	44.4	44.5
18	51.5	50.7	51.3	51.5
19	39.0	33.5	38.8	38.9
20	31.8	35.1	31.7	31.8
21	36.8	32.4	36.7	36.9
22	31.8	31.3	31.7	31.8
23	28.1	27.8	27.8	28.0
24	16.6	16.4	16.4	16.5
25	16.4	16.2	16.2	16.4
26	18.6	18.4	18.4	18.4
27	19.6	19.5	19.4	19.5
28	78.0	77.5	77.9	78.0
29	33.8	28.4	33.6	33.7
30	24.8	67.9	24.6	24.7
$CO\underline{CH_3}$		20.6		
$\underline{C}OCH_3$		170.8		

a) Assignments based on TOCSY, HMQC and HMBC experiments. *b*) Overlapped signals are reported without designating multiplicity.



	R_1	R_2	R_3
1	H	H	H
2	OAc	H	H
3	H	H	β-D-glucopyranosyl
4	H	Ac	H

Fig. 1. The Structures of New Compounds **1**—**4**

information of the δ_C 20.9, 171.0 and δ_H 2.04. The downfield signals of GlcI-6 at δ_H 4.91 (br d, J=11.6 Hz), 4.77 (br d, J=11.6 Hz) indicated that the location of the acetyl group was at this position, which was further confirmed by the correlations between these two protons and the carbonyl carbon (δ_C 171.0) of acetyl group in the HMBC spectrum, respectively. From the above evidences, the structure of **4** was elucidated as 3β-*o*-α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-[β-D-6-*o*-acetylglucopranosyl-(1→2)]-α-L-arabinopyranosyl-16α-hydroxy-13,28-epoxy-oleanane.

To the best of our knowledge compounds **1**—**4** are new triterpenoid saponins and compounds **1**—**4** were evaluated for their cytotoxic activities against Hela human cervical carcinoma cells, EJ human bladder tumor cells, and BCG-823 human gastric carcinoma cells. Compouds **1** and **4** showed moderate cytotoxic activity against Hela cells, with IC_{50} values of 14.1 and 54.7 μM, respectively, while the other saponins (**2**, **3**) were inactive. Compounds **1**—**4** showed cytotoxic activity against EJ cells, with IC_{50} values of 6.8—28.2 μM, respectively. Compound **1** showed cytotoxic activity against BCG-823 cells, with an IC_{50} values of 6.9 μM.

Experimental

General Experimental Procedures Optical rotations were determined on a PolAAr-3005 digital polarimeter (OA Co., Ltd.). HR-ESI spectra were recorded using Waters SY-NATT and ESI-Q-TOF mass spectrometer. 1D and 2D NMR spectra were taken on a JNM-ECA-400 spectrometer in pyridine-d_5. Chemical shifts were expressed in δ down field from internal TMS; coupling constants were reported in Hz. TLC was carried out on silicagel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China.), and the spot were visualized by spraying with 10% H_2SO_4 and heating. Purification of the extract was carried out using macroporous resin D101 and column chromatography with silica gel (Qindao Haiyang Chemical Co., Ltd.). Compounds were finally isolated with the help of a Alltech LC-626 preparative HPLC system equipped with Alltech UVIS-200 detector using an ODS column (YMC-ODS, 20×250 mm, 5 μm).

Plant Material The dry rhizome of *A. gigantifolia* Stapf was obtained from Guangdong, China in 2007 and was authenticated by Prof. Ping Liu. The voucher specimen has been deposited in traditional Chinese medicine pharmacy of General Hospital of PLA.

Table 4. The ^{13}C-NMR Data for Sugar Moieties of Compounds **1**—**4** (400 MHz, Pyridine-d_5)[a,b]

Carbon	1	2	3	4
Ara-1	104.3	104.1	104.1	104.4
2	79.4	79.2	79.4	80.0
3	72.5	72.5	72.5	72.8
4	78.1	77.9	78.0	78.3
5	63.1	62.7	63.0	63.7
GluI-1	104.9	104.7	104.8	105.0
2	76.1	75.9	75.9	76.0
3	78.1	77.9	78.0	78.1
4	71.7	70.3	71.4	71.0
5	78.0	77.8	77.8	75.0
6	62.9	62.7	62.6	64.8
$CO\underline{C}H_3$				20.9
$\underline{C}OCH_3$				171.0
GlcII-1	103.8	103.6	103.6	103.8
2	81.1	80.9	80.9	81.0
3	85.7	85.4	85.2	85.8
4	69.5	69.3	69.3	69.5
5	78.0	77.8	77.8	78.0
6	62.0	61.8	61.8	62.0
Xyl-1	105.5	105.4	104.9	105.4
2	75.2	75.0	72.0	75.0
3	78.0	77.9	72.5	78.0
4	70.7	70.5	73.6	70.4
5	66.9	66.7	70.2	66.8
Rha-1	103.6	103.5	103.4	103.6
2	72.3	72.1	72.0	72.2
3	72.6	72.5	72.5	72.6
4	73.8	73.7	73.6	73.8
5	70.5	70.3	73.8	70.6
6	18.5	18.4	18.4	18.5
GlcIII-1			104.6	
2			73.8	
3			77.9	
4			71.3	
5			78.2	
6			62.2	

a) Assignments based on TOCSY, HMQC and HMBC experiments. *b*) Overlapped signals are reported without designating multiplicity.

Extraction and Isolation The dried and cut rhizome parts of *A. gigantifolia* (13.5 kg) was percolated with 60% ethanol. The 60% ethanol extract (1.54 kg) was partitioned successively between water and petroleum, ethyl acetate, *n*-butanol, respectively. After removing the solvent, the *n*-butanol layer (600 g) was subjected to a macroporous resin D101 column and eluted with H_2O and 30%, 50%, 70%, and 95% EtOH. The 70% EtOH eluate (52 g) was chromatographed over a silica gel column with $CHCl_3$–MeOH (3 : 1) to give six fractions. The fraction 5 (22 g) was further separated by preparative HPLC at a flow rate of 15.2 ml/min to afford **2** (58 mg, t_R=49.6 min, 68% MeOH, 200 nm) and **3** (82 mg, t_R=54.9 min, 68% MeOH, 200 nm). The fraction 6 (10 g) was subjected to a silica gel column with $CHCl_3$–MeOH–H_2O (65 : 35 : 10), which gave rise to **1** (745 mg). The 50% EtOH eluate (50 g) was chromatographed over a silica gel column with a gradient of $CHCl_3$–MeOH (3 : 1, 2 : 1, 1 : 1) to give three fractions 5-1, 5-2 and 5-3. The fraction 5-2 was further subjected to a silica gel H column with $CHCl_3$–MeOH–H_2O (5 : 2 : 0.1) to afford **4** (395 mg).

Compound **1**: White amorphous powder; $[\alpha]_D^{26}$ −21.5 (c=0.10, MeOH); ESI-MS m/z 1191 $[M-H]^-$. HR-ESI-TOF-MS m/z 1191.6279 $[M-H]^-$ (Calcd for $C_{58}H_{95}O_{25}$). ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz) are given in Tables 1—4.

Compound **2**: White amorphous powder; $[\alpha]_D^{26}$ −19.8 (c=0.10, MeOH); ESI-MS m/z 1249 $[M-H]^-$. HR-ESI-TOF-MS m/z 1249.6344 $[M-H]^-$ (Calcd for $C_{60}H_{97}O_{27}$). ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz) are given in Tables 1—4.

Compound **3**: White amorphous powder; $[\alpha]_D^{26}$ −30.8 (c=0.10, MeOH); ESI-MS m/z 1353 $[M-H]^-$. HR-ESI-TOF-MS m/z 1353.6787 $[M-H]^-$ (Calcd for $C_{64}H_{105}O_{30}$). ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz) are given in Tables 1—4.66

Compound **4**: White amorphous powder; $[\alpha]_D^{26}$ −21.1 (c=0.09, MeOH); ESI-MS m/z 1233 $[M-H]^-$. HR-ESI-TOF-MS m/z 1233.6385 $[M-H]^-$ (Calcd for $C_{60}H_{97}O_{26}$). ^{1}H-NMR (C_5D_5N, 400 MHz) and ^{13}C-NMR (C_5D_5N, 100 MHz) are given in Tables 1—4.

Table 5. Cytotoxic Activity of Compounds **1**—**4** against Three Human Cancer Cell Lines[a]

Sample	IC_{50} (μM)		
	Hela	EJ	BCG-823
1	14.1±1.5	28.2±4.1	6.9±2.1
2	>100	6.8±1.2	>100
3	>100	8.2±3.7	>100
4	54.7±6.8	15.4±4.4	>100

a) Values were expressed as mean±S.D. of three independent determinations.

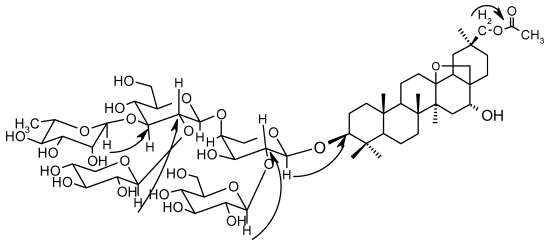


Fig. 2. Selected HMBC Correlations for Compound **2**

Acid Hydrolysis of Compounds 1—4 and Determination of Absolute Configuration of Monosaccharides Each compound (5 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). Each remaining aqueous layer was concentrated to dryness to give a residue. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M D-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h. The mixture was partitioned between hexane and H_2O (0.3 ml each), and the hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-17 MS (30 m×0.25 mm i.d., 0.25 μm film), ion source temperature, 250 °C; interface temperature, 300 °C, Carrier, Helium gas (1.16 ml/min). In the acid hydrolysate of **1**, L-rhamnose, D-glucose, L-arabinose and D-xylose were confirmed by comparison of the retention times of their derivatives with those of the derivatives of L-rhamnose, D-glucose, L-arabinose and D-xylose prepared in a similar way, which showed retention times of 5.29, 7.33, 5.22 and 6.10 min, respectively. The constituent sugars of compounds **2**—**4** were also identified by the same method.

Cytotoxicity Assay Hela, EJ and BCG-823 cells were seeded in 96-well plates at adensity of 1×10^4 cells/well and incubated for 24 h. Test samples were dissolved in dimethyl sulfoxide (DMSO) and added to the medium. Following a 48 h incubation, the wells were incubated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (100 μl/well concentrated at 5 mg/ml) at 37 °C for 4 h. The supernatant was aspired, and the 200 μl of DMSO was added to redissolve the formazan crystals. The optical density was measured by an enzyme-linked immunosorbent assay plate reader at 570 nm (PerkinElmer 1420-012, China). The experiments were repeated three times and cytotoxicity was expressed as the IC_{50} value, which reduces the number of viable cells by 50%.

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