Cytotoxic Oleanane Triterpene Saponins from *Albizia chinensis*

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Three new oleanane-type triterpene saponins, albizosides A−C (**1**−**3**), were isolated from the stem bark of *Albizia chinensis*. Their structures were established by 1D and 2D NMR experiments and chemical methods. Compounds **1**−**3** showed cytotoxic activity against a small panel of human tumor cell lines as well as hemolytic activity against rabbit erythrocytes.

Albizia chinensis (Osb.) Merr., belonging to the family Leguminosae, is distributed in the south and southeast of mainland China.[1] In previous work, a class of oleanane triterpenoid saponins isolated from *Albizia* species was found to possess a common aglycon unit (acacic acid), having various oligosaccharide moieties at C-3 and C-28 and an acyl group at C-21. These triterpenoid saponins are known to exhibit cytotoxicity against various tumor cell lines.[2−6] As part of an ongoing search for bioactive compounds from plants, the EtOH extract of the stem bark of *A. chinensis* was examined. This extract displayed cytotoxicity against five cultured human tumor cell lines. Chromatographic purification led to the isolation of three new oleanane-type triterpene saponins, albizosides A−C (**1**−**3**). This paper describes the isolation and structure elucidation of these new triterpenoid saponins, the evaluation of the cytotoxic activities of saponins **1**−**3** and **4** (a prosapogenin obtained from the mild alkaline hydrolysate of **1**) against five human tumor cell lines, and their hemolytic activity on rabbit erythrocytes.

Results and Discussion

The total EtOH extract from the stem bark of *A. chinensis* was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to passage over a polyamide column and an ODS column to give a crude saponin mixture, which again showed cytotoxicity against the five cultured human tumor cell lines used (Experimental Section). Purification of the crude saponin mixture by ODS column chromatography, followed by repeated preparative HPLC, afforded three complex triterpenoid saponins, albizosides A−C (**1**−**3**).

Albizoside A (**1**) was obtained as a white, amorphous powder. The MALDITOFMS showed a quasimolecular ion peak at *m/z* 2522 $[M + Na]^+$, and QFTMS gave a $[M + Na]^+$ peak at *m/z* 2522.1579, consistent with a molecular formula of $C_{118}H_{186}O_{56}$. The IR spectrum showed carbonyl (1734 cm^{-1}) and α,β-unsaturated carbonyl group (1700 cm^{-1}) absorption. Upon acid hydrolysis with 2 M HCl at 95 °C, **1** afforded an acacic acid lactone unit, which was identified with an authentic sample, and the monosaccharides L-rhamnose, L-arabinose, D-fucose, D-xylose, D-glucose, and D-quinovose, which were identified by GC analysis of their trimethylsilyl thiazolidine derivatives.[7] Mild alkaline hydrolysis of **1** with saturated $NaHCO_3$ in MeOH gave a prosapogenin (**4**) and two monoterpenyl glycosides (**5** and **6**) as major components (Scheme 1). By NMR data analysis, **5** and **6** were found to be identical with two known compounds, the methyl ester of (2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside and the methyl ester of (2*E*,6*S*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside, respectively.[8] The stereochemistry of **5** and **6** was proven as follows. Compound **5** was hydrolyzed with β-glucosidase to give the corresponding aglycon, compound **7**, and comparison of its optical rotation data, $[\alpha]^{20}_D$ +13.3 (*c* 0.15, $CHCl_3$), with a literature value, $[\alpha]^{25}_D$ +19.3 (*c* 0.15, $CHCl_3$),[9] suggested that the absolute configuration at C-6 is *S*. The ^1H NMR chemical shift of H-3 of compound **7** appeared at δ 6.75, in comparison to a *trans*-isomer, for which this signal appeared at δ 6.72,[10] indicating the *E* configuration of the trisubstituted double bond ($\Delta^{2,3}$). This was further evidenced by NOE difference experiments, in which NOEs were observed between H_3-9 (δ 1.83) and H_2-4 (δ 2.22). From a combination of the NMR data obtained, **7** was identified as a known compound, the methyl ester of (2*E*,6*S*)-2,6-dimethyl-6-hydroxy-2,7-octadienoic acid.[8] Thus, the stereochemistry of **5** was concluded to be the same as its aglycon, **7**. The configuration of C-6 for **6** was determined as *S* because the ^{13}C NMR chemical shifts of C-5 (δ 41.3), C-7 (δ 144.1), C-8 (δ 115.9), and C-10 (δ 23.7) were in good agreement with those of **5**. The trisubstituted double bond ($\Delta^{2,3}$) of **6** was assigned with the *E* configuration from the chemical shift of H-3 (δ 6.85), since the *Z*-isomer would appear at higher field.[10,11] This was confirmed by NOEs between H_2-9 (δ 4.25) and H_2-4 (δ 2.37).

The ^1H and ^{13}C NMR signals for **4** showed eight anomeric protons at δ 4.85 (1H, d, J = 8.0 Hz, Qui), 4.92 (1H, overlapped, Glc_1), 5.00 (1H, d, J = 8.0 Hz, Fuc), 5.09 (1H, d, J = 7.0 Hz, Xyl), 5.34 (1H, d, J = 8.0 Hz, Glc″), 5.88 (1H, brs, Rha), 6.05 (1 H, d, J = 8.0 Hz, Glc′), and 6.26 [1H, brs, Ara(*f*)], and the corresponding carbon resonances at δ 99.3, 106.7, 103.4, 106.9, 105.7, 101.8, 95.7, and 111.0, respectively, according to the HSQC spectrum. On the basis of the coupling constants of the anomeric protons and the chemical shifts of the anomeric carbons, the anomeric configurations of the sugar moieties were determined as β for the glucose, xylose, fucose, and quinovose moieties and α for the rhamnose and arabinose (in furanose form)[12] moieties. More aggressive alkaline hydrolysis of **4** with 3% NaOH in aqueous MeOH at room temperature produced compound **8**, which was identified as the known saponin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl acacic acid,[2] and the inner monoterpenyl glycoside (**9**). The NMR spectroscopic data of compound **9** were quite similar to those of compound **6** except for a missing methyl ester signal (δ 3.68; 52.2); thus the relative configuration of **9** was the same as **6**, and it was identified as the known compound (2*E*,6*S*)-6-hydroxy-2-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside.[8]

Comparison of the ^{13}C NMR data of **4** and **8** led to the observation of a glycosylation shift at C-28 (−5.0 ppm) and acylation shifts at C-20 (−1.2 ppm), C-21 (+3.6 ppm), and C-22

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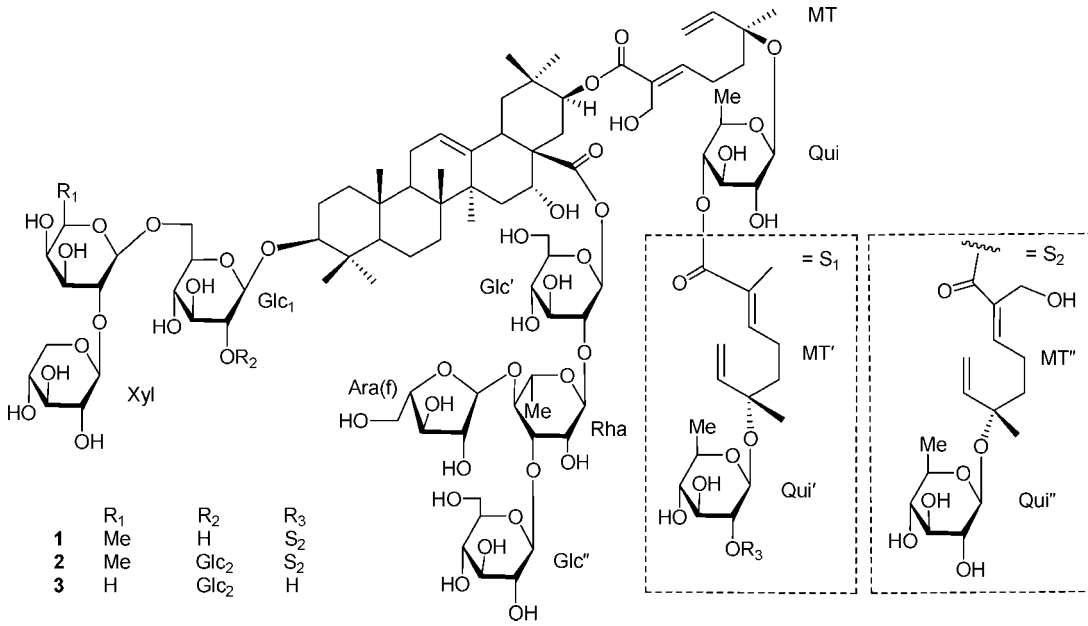
Chart 1



(−5.6 ppm). These data suggested that **4** is a 21-acyl-3,28-bidesmoside of acacic acid. Furthermore, the HMBC correlation in **4** between H-21 (δ 6.30) of the aglycon and the carbonyl carbon (δ 167.5) of the inner monoterpene-quinovopyranosyl unit indicated that the (2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl residue is located at C-21 of the aglycon. The remaining residues to be placed were two β-glucopyranoses, an α-arabinofuranose, and an α-rhamnopyranose. The characteristic signals for the anomeric proton (δ 6.05) and carbon (δ 95.7) of the β-glucopyranose (Glc′) suggested that this glucose should be attached directly to C-28 through an ester bond, which was confirmed by the HMBC correlation between Glc′-1 (δ 6.05) and C-28 (δ 174.4) of the aglycon. The long-range correlations observed between the ^{1}H NMR resonance at δ 5.88 (H-Rha-1) and the ^{13}C NMR resonance at δ 76.9 (C-Glc′-2), between δ 5.34 (H-Glc″-1) and δ 82.0 (C-Rha-3), and between δ 6.26 [H-Ara(*f*)-1] and δ 79.0 (C-Rha-4) indicated that the tetrasaccharide residue at C-28 of the aglycon could be assigned as β-D-glucopyranosyl-(l→3)-[α-L-arabinofuranosyl-(l→4)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranoside.

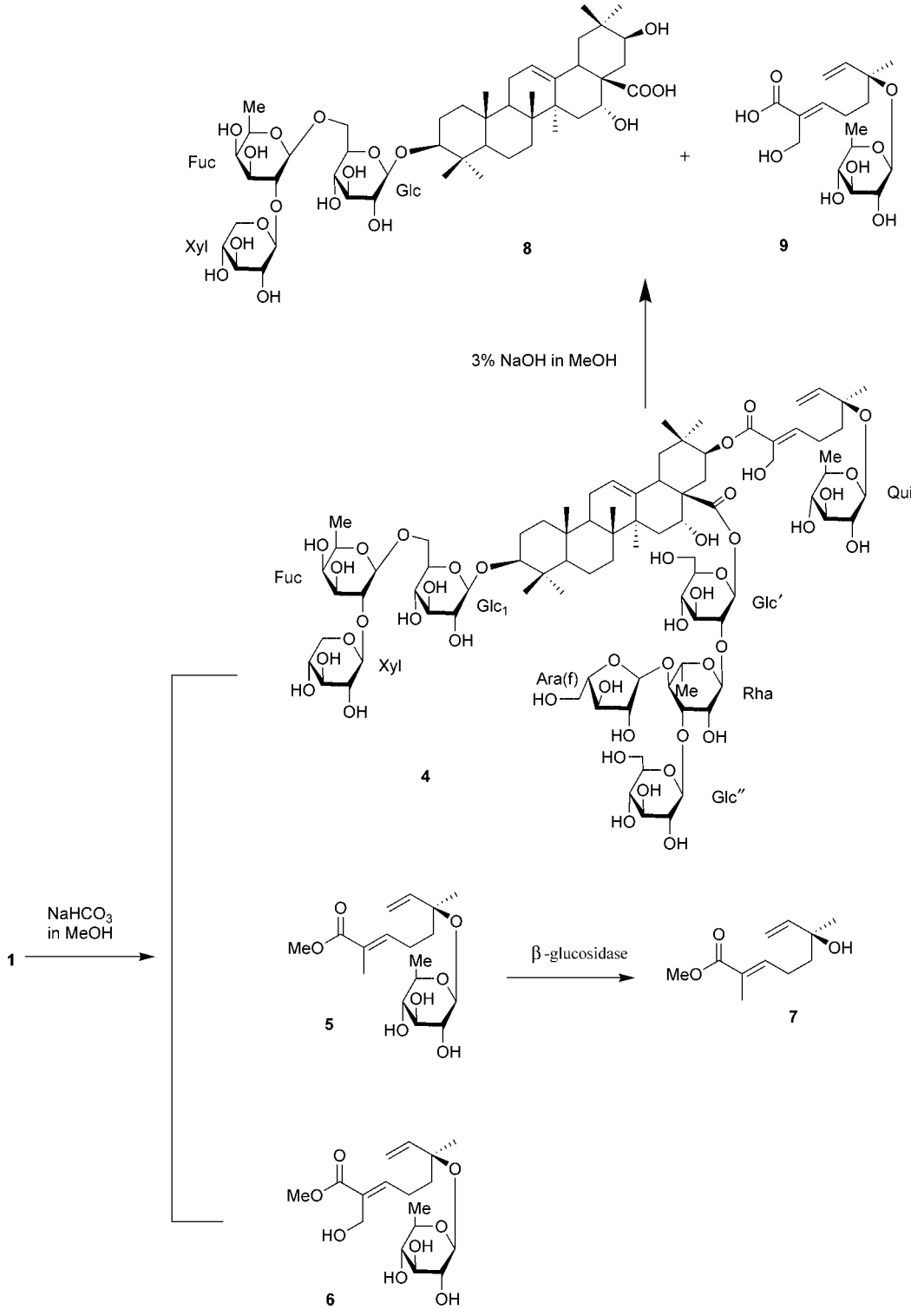
Consequently, the structure of **4** was concluded to be 21-*O*-[(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-[β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl]acacic acid 28-*O*-β-D-glucopyranosyl-(l→3)-[α-L-arabinofuranosyl-(l→4)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranosyl ester. This was found to be identical to a known saponin isolated from the alkaline hydrolysate of the crude saponin fraction of *Albizia julibrissin*.[2]

The linkage between compound **4** and the two monoterpenyl quinovopyranosides **5** and **6** (that is, the methyl ester of the S_1 and S_2 units in **1**) was defined by the acylation shifts. One monoterpene-quinovopyranosyl unit (MT′ and Qui′) could be attached to C-4 of the inner monoterpene-quinovopyranosyl unit (MT and Qui) of **4**, since acylation shifts were present at C-Qui-3 (**4** δ 78.4; **1** δ 75.5), C-Qui-4 (**4** δ 76.8; **1** δ 77.2), and C-Qui-5 (**4** δ 72.6; **1** δ 70.1). The long-range correlations observed in the HMBC spectrum between δ 167.7 (C-MT′-1) and δ 6.94 (H-MT′-3), 1.91 (H-MT′-9), and 5.32 (H-Qui-4) confirmed the connectivity from C-1 of the second monoterpenyl moiety (MT′ in S_1) to C-4 of the inner quinovose (Qui). Therefore, the S_1 unit was linked first to compound **4**. Similarly, the acylation shifts at C-Qui′-1 (**5** δ 99.3; **1** δ 97.0), C-Qui′-2 (**5** δ 75.5; **1** δ 75.6), and C-Qui′-3 (**5** δ 77.9; **1** δ 76.0) indicated that the other monoterpene-quinovopyranosyl unit (MT″ and Qui″) could be placed at C-2 of the second quinovose (Qui′ in S_1). The HMBC spectrum exhibited correlations between δ 167.0 (C-MT″-1) and δ 7.20 (H-MT″-3), 4.74 (H-MT″-9), and 5.67 (H-Qui′-2), confirming the connectivity from C-1 of the terminal monoterpenyl moiety (MT″ in S_2) to C-2 of the second quinovose (Qui′) (Figure 1). On the basis of the above evidence, the structure of **1** was determined as 21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-(2′*E*,6′*S*)-2′,6′-dimethyl-6′-*O*-[2′-*O*-(2″*E*,6″*S*)-2″-hydroxymethyl-6″-methyl-6″-*O*-β-D-quinovopyranosyl-2″,7″-octadienoyl-β-D-quinovopyranosyl]-2′,7′-octadienoyl-β-D-quinovopyranosyl}-2,7-octadienoyl}-3-*O*-[β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosyl]acacic acid 28-*O*-β-D-glucopyranosyl-(l→3)-[α-L-arabinofuranosyl-(l→4)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranosyl ester.

Albizoside B (**2**) was isolated as an amorphous powder. Its molecular formula was deduced as $C_{124}H_{196}O_{61}$ from the MALDITOFMS (*m*/*z* 2684 [M + Na]$^+$) and QFTMS (*m*/*z* [M + Na]$^+$ 2684.2137, calcd for $C_{124}H_{196}O_{61}Na$ 2684.2127). Acid hydrolysis of **2** also gave an acacic acid lactone identified by co-TLC with an authentic sample, and the L-rhamnose, L-arabinose, D-fucose, D-xylose, D-glucose, and D-quinovose units were determined by GC analysis of their trimethylsilyl thiazolidine derivatives.[7] The ^{1}H and ^{13}C NMR chemical shifts of **2**, assigned by 2D NMR experiments, were superimposable with data for **1** except for those of the oligosaccharide moiety linked at C-3 of the aglycon, since **2** possessed an additional glucose attached to C-2 of the inner glucose, according to the HMBC spectrum. The ^{13}C NMR data of the tetrasaccharide unit at C-3 in **2** matched well with those of a known saponin (julibroside A_1) obtained from *Albizia julibrissin* (Tables 1−3).[7] Therefore, the structure of **2** was elucidated as 21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-(2′*E*,6′*S*)-2′,6′-dimethyl-6′-*O*-[2′-*O*-(2″*E*,6″*S*)-2″-hydroxymethyl-6″-methyl-6″-*O*-β-D-quinovopyranosyl-2″,7″-octadienoyl-β-D-quinovopyranosyl]-2′,7′-octadienoyl-β-D-quinovopyranosyl}-2,7-octadienoyl}-3-*O*-{β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}acacic acid 28-*O*-β-D-glucopyranosyl-(l→3)-[α-L-arabinofuranosyl-(l→4)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranosyl ester.

Albizoside C (**3**), an amorphous powder, gave in the MALDITOFMS a quasimolecular ion peak at *m*/*z* 2342 [M + Na]$^+$, and in the QFTMS a [M + Na]$^+$ peak at *m*/*z* 2342.0436, in accordance with the composition, $C_{107}H_{170}O_{54}Na$. Acid hydrolysis of **3** afforded an acacic acid lactone confirmed by co-TLC with an authentic sample,

Scheme 1



and monosaccharides were identified by GC analysis of their trimethylsilyl thiazolidine derivatives[7] as L-rhamnose, L-arabinose, D-xylose, D-glucose, and D-quinovose. By comparing the ^{1}H and ^{13}C NMR signals of **3** with those of **2**, all signals due to the aglycon moiety and the oligosaccharide ester attached at C-28 were in agreement with those of **2**. However, the monoterpene-quinovopyranosyl groups acylated at C-21 no longer were found to have a terminal monoterpenyl-quinovopyranoside, and the oligosaccharide chain at C-3 in **3** was similar to those of **2** except for the appearance of arabinopyranosyl signals instead of fucopyranosyl signals. The spectroscopic data of the tetrasaccharide moiety at C-3 were identical to those of albiziasaponin C obtained from *Albizia lebbeck* (Tables 1−3).[13] Thus, the structure of **3** was elucidated as 21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2′*E*,6′*S*)-2′,6′-dimethyl-6′-*O*-*β*-D-quinovopyranosyl-2′,7′-octadienoyl-*β*-D-quinovopyranosyl]-2,7-octadienoyl}-3-*O*-{*β*-D-xylopyranosyl-(l→2)-*β*-D-arabinopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}acacic acid 28-*O*-*β*-D-glucopyranosyl-(l→3)-[α-L-arabinofuranosyl-(l→4)]-α-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

The cytotoxic activities of compounds **1**−**4** were evaluated against five human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) using the MTT method. Compounds **1**−**3**

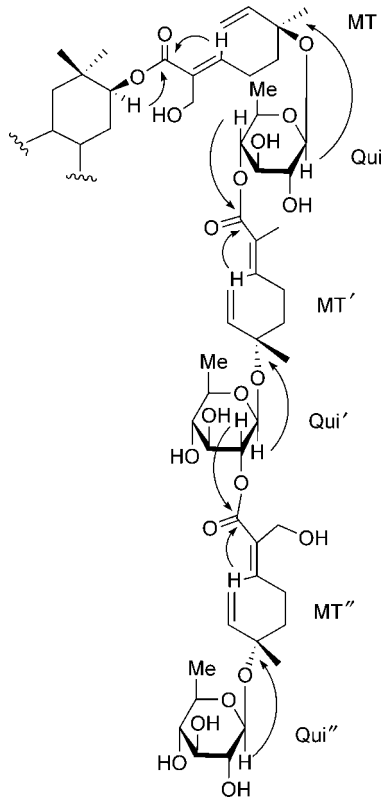


Figure 1. Key HMBC correlations of the acyl moiety at C-21 of **1**.

exhibited cytotoxicity against all human tumor cell lines tested, while prosapogenin **4** was inactive (IC_{50} > 10 μM) (Table 4).

By comparing the structures of compound **1** and **4** with **3**, the difference in the activities among these substituents suggests that two monoterpene-quinovopyranosyl groups acylated at C-21 of the aglycon are needed to exhibit the most potent cytotoxic activity.

Since hemolysis is a typical characteristic of saponins, the hemolytic activity of compounds **1**−**4** was evaluated on rabbit erythrocytes in the concentration range 0.01−100 μM according to a standard procedure.[14] Compounds **1**−**3** exhibited hemolytic activity with HC_{50} values of 0.4, 0.7, and 0.7 μM, respectively, while compound **4** was much less active, with a HC_{50} value of 127.9 μM. The above results indicate that the second monoterpene-quinovopyranosyl moiety is involved in mediating the hemolytic activity by saponins **1**−**3**.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Perkin-Elmer 241 automatic digital polarimeter. UV spectra were measured on a JASCO V650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by the microscope transmission method. NMR spectra were obtained on an INOVA-500 or SX-600 spectrometer. Chemical shifts are given in δ (ppm) with solvent (pyridine-d_5 or methanol-d_4) peaks used as reference. ESIMS were measured on an Agilent 1100 Series LC/MSD ion trap mass spectrometer. MALDITOFMS data were recorded on a AutoflexIII mass spectrometer. QFTMS were carried out on a 9.4 T Q-FT-ICR-MS instrument (Bruker). GC analyses were obtained using a GC-2010 instrument (Shimadzu). Preparative HPLC was performed on a Shimadzu LC-6AD instrument with an SPD-10A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Polyamide (30−60 mesh, Jiangsu Linjiang Chemical Reagents Factory, People's Republic of China) and ODS (45−70 μm, Merck) were used for column chromatography. Si gel 60 F-254 (Qingdao Marine Chemical Factory) was used for TLC.

Plant Material. The stem bark of *Albizia chinensis* was collected in April 2006 at Nanning, Guangxi Province, People's Republic of China, and identified by Prof. Songji Wei (Guangxi College of Traditional Chinese Medicine). A voucher specimen (No. 90269) was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. The air-dried, powdered stem bark of *A. chinensis* (5.5 kg) was refluxed three times with 15 L of 95% EtOH (3 × 15 L), and the residue was refluxed twice with 70% EtOH (2 × 10 L). The combined filtrate was concentrated under reduced pressure to yield a dark brown syrup (1.045 kg). This total EtOH extract displayed

Table 1. ^{13}C NMR Spectroscopic Data of **1**−**4** (in pyridine-d_5)[a]

position	1	2	3	4	position	1	2	3	4
aglycon					29	29.1	29.2	29.2	29.2
1	38.9	39.0	38.9	38.9	30	19.1	19.2	19.1	19.2
2	26.8	26.9	26.7	26.8	sugar (C-28)				
3	88.4	88.6	88.9	88.4	Glc′1	95.7	95.8	95.7	95.7
4	39.6	39.7	39.6	39.66	2	77.0	77.1	76.9	76.9
5	56.0	56.1	56.0	56.0	3	78.0	78.0	78.0	78.0
6	18.7	18.7	18.5	18.7	4	71.2	71.3	71.2	71.2
7	33.6	33.7	33.6	33.6	5	79.1	79.2	79.1	79.1
8	40.1	40.2	40.1	40.2	6	62.0	62.0	61.9	62.0
9	47.1	47.2	47.1	47.2	Rha (1→2) Glc′				
10	37.1	37.1	37.0	37.1	1	101.8	101.9	101.8	101.8
11	23.9	24.0	23.9	23.9	2	70.5	70.5	70.4	70.5
12	123.1	123.2	123.1	123.1	3	82.1	82.1	82.0	82.0
13	143.4	143.5	143.4	143.4	4	79.0	79.1	79.0	79.0
14	42.0	42.1	42.0	42.0	5	69.2	69.3	69.2	69.2
15	35.9	36.0	35.9	35.9	6	18.7	18.97	18.8	18.9
16	73.9	73.96	73.8	73.9	Ara (1→4) Rha				
17	51.6	51.7	51.6	51.6	1	111.0	111.1	111.0	111.0
18	41.1	41.0	40.8	41.0	2	84.4	84.6	84.4	84.4
19	47.9	47.9	47.9	47.9	3	78.4	78.5	78.4	78.4
20	35.5	35.5	35.4	35.5	4	85.4	85.4	85.4	85.4
21	77.0	77.1	77.0	77.05	5	62.5	62.6	62.5	62.5
22	36.4	36.5	36.4	36.4	Glc″ (1→3)Rha				
23	28.2	28.2	28.1	28.2	1	105.7	105.8	105.7	105.7
24	17.2	17.0	16.9	17.2	2	75.4	75.6	75.5	75.4
25	15.9	16.0	15.8	15.9	3	78.0	78.0	78.2	78.0
26	17.3	17.3	17.3	17.4	4	71.8	71.8	71.8	71.7
27	27.3	27.3	27.2	27.3	5	78.2	78.2	78.4	78.2
28	174.4	174.5	174.4	174.4	6	62.7	62.8	62.68	62.8

[a] Assignments based on HSQC and HMBC experiments. Measured at 125 MHz for **1**, **3**, and **4** and 150 MHz for **2**.

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**4** (in pyridine-d_5)[a]

position	1	2	3	4	position	1	2	3	4
sugar (C-3)					Qui				
Glc_1 1	106.7	105.0	104.9	106.7	1	99.3	99.37	99.3	99.3
2	75.68	83.0	82.86	75.8	2	75.4	75.46	75.56	75.5
3	78.4	78.06	77.9	78.4	3	75.5	75.6	75.5	78.4
4	71.67	71.86	71.7	71.8	4	77.2	77.2	77.1	76.8
5	76.8	76.5	76.5	76.8	5	70.1	70.2	70.1	72.6
6	70.0	69.9	69.2	70.1	6	18.4	18.5	18.4	18.9
Fuc (1→6) Glc_1					MT′				
1	103.3	103.5		103.4	1	167.7	167.8	167.7	
2	82.1	82.3		82.08	2	128.1	128.2	127.9	
3	75.2	75.25		75.2	3	143.4	143.2	143.4	
4	72.2	72.2		72.2	4	23.55	23.62	23.7	
5	71.3	71.4		71.4	5	41.1	41.1	40.4	
6	17.2	17.4		17.2	6	79.65	79.71	79.4	
Ara (1→6) Glc_1					7	143.1	143.2	144.1	
1			102.2		8	115.5	115.5	114.8	
2			80.4		9	12.7	12.8	12.7	
3			72.5		10	23.67	23.74	23.6	
4			67.3		Qui′				
5			64.2		1	97.0	97.05	99.3	
Xyl (1→2)					2	75.56	75.7	75.4	
1	106.9	107.1	106.2	106.9	3	76.0	76.06	78.4	
2	75.76	76.0	75.8	75.77	4	76.8	77.0	76.8	
3	77.5	77.6	77.6	77.5	5	72.8	72.9	72.6	
4	70.7	70.8	70.8	70.7	6	18.9	18.8	18.8	
5	67.2	67.3	67.2	67.2	MT″				
Glc_2(1→2) Glc_1					1	167.0	167.1		
1		105.9	105.8		2	133.4	133.5		
2		75.5	75.5		3	146.1	146.2		
3		78.3	78.0		4	23.67	23.7		
4		71.7	71.7		5	40.8	40.89		
5		78.2	78.2		6	79.45	79.52		
6		62.8	62.7		7	143.9	144.0		
MT (C-21)					8	114.9	114.9		
1	167.5	167.6	167.5	167.5	9	56.3	56.3		
2	133.8	133.9	133.8	133.8	10	23.9	23.9		
3	145.2	145.2	145.2	145.3	Qui″				
4	23.6	23.68	23.6	23.6	1	99.3	99.37		
5	40.8	40.9	40.8	40.86	2	75.4	75.56		
6	79.74	79.81	79.7	79.5	3	78.4	78.4		
7	143.9	144.0	143.9	144.1	4	76.7	76.9		
8	115.1	115.2	115.1	114.9	5	72.6	72.7		
9	56.3	56.35	56.2	56.3	6	18.9	18.9		
10	23.6	23.7	23.7	23.8					

[a] Assignments based on HSQC and HMBC experiments. Measured at 125 MHz for **1**, **3**, and **4** and 150 MHz for **2**. MT = monoterpenoid acid moiety.

weak cytotoxicity against five cultured human tumor cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) with IC_{50} values of 22.8−45.0 μg/mL. The EtOH extract was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH extract (200 g), which was the most active fraction, exhibited cytotoxicity against the human tumor cell lines used (HCT-8, Bel-7402, BGC-823, A549, and A2780), with IC_{50} values of 8.1−21.5 μg/mL. Then, this fraction was passed through a column of polyamide, eluted with a gradient solvent system (0 → 95% EtOH−H_2O). The 30% EtOH fraction (22.0 g; 5.5 g quantities each, four times) was subjected to separation over an ODS column (45−70 μm, 170 g), eluted with a gradient solvent system (8%, 25%, 40%, 65%, 85%, 100% MeOH−H_2O). The 85% MeOH fraction was obtained as a crude saponin mixture (10.2 g), which showed cytotoxicity against the human tumor cell lines HCT-8, Bel-7402, BGC-823, A549, and A2780 with IC_{50} values of 0.5−11.5 μg/mL. This extract (2.5 g each, four times) was rechromatographed over an ODS column (45−70 μm, 70 → 85% MeOH−H_2O) to give five fractions (fraction A 0.5 g; fraction B 1.3 g; fraction C 5.3 g; fraction D 1.6 g; fraction E 0.3 g). Fraction C (4.0 g) was separated initially by preparative HPLC using 74% MeOH−H_2O, to afford six subfractions. Subfraction 3 (585 mg) was purified further by preparative HPLC using 39% CH_3CN−H_2O (4 mL/min) to yield compound **1** (100 mg, t_R 40 min). Subfraction 2 (200 mg) was purified by preparative HPLC using 40% CH_3CN−H_2O (4 mL/min) to yield compound **2** (25 mg, t_R 46 min). Fraction B (1.3 g) was separated initially on an ODS column (72 → 74% MeOH−H_2O) to give three subfractions. Subfraction 1 (400 mg) was further purified by preparative HPLC using 37.5% CH_3CN−H_2O (4 mL/min) to afford compound **3** (24 mg, t_R 43 min).

Albizoside A (1): white, amorphous powder; $[\alpha]^{20}_D$ −34.4 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.7) nm; IR ν_{max} 3383, 2932, 1734, 1700, 1648, 1376, 1071, 1015, 642 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) data, see Table 3; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Tables 1 and 2; MALDITOFMS *m/z* 2522 $[M + Na]^+$; QFTMS *m/z* 2522.1579 $[M + Na]^+$ (calcd for $C_{118}H_{186}O_{56}Na$, 2522.1599).

Albizoside B (2): white, amorphous powder; $[\alpha]^{20}_D$ −22.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.6) nm; IR ν_{max} 3365, 2928, 1737, 1675, 1376, 1071, 723, 643 cm^{-1}; ^1H NMR (600 MHz, pyridine-d_5) data, see Table 3; ^{13}C NMR (150 MHz, pyridine-d_5) data, see Tables 1 and 2; MALDITOFMS *m/z* 2684 $[M + Na]^+$; QFTMS *m/z* 2684.2137 $[M + Na]^+$ (calcd for $C_{124}H_{196}O_{61}Na$, 2684.2127).

Albizoside C (3): white, amorphous powder; $[\alpha]^{20}_D$ −16.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.6) nm; IR ν_{max} 3367, 2933, 1737, 1677, 1376, 1074, 1046, 723, 640 cm^{-1}; ^1H NMR (500 MHz, pyridine-d_5) data, see Table 3; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Tables 1 and 2; MALDITOFMS *m/z* 2342 $[M + Na]^+$; QFTMS *m/z* 2342.0436 $[M + Na]^+$ (calcd for $C_{107}H_{170}O_{54}Na$, 2342.0449).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides. Compound **1** (3 mg) was dissolved in 2 M HCl−H_2O (2 mL) and heated at 95 °C for 10 h. The reaction mixture was extracted with EtOAc, and this EtOAc extract was found to contain an acacic acid lactone unit, as identified by co-TLC with an authentic sample [R_f 0.54 $CHCl_3$−MeOH (30:1), 0.41 *n*-hexane−

Table 3. ^{1}H NMR Data of Compounds **1**−**3** (in pyridine-d_5)[a]

position	1	2	3	position	1	2	3
1	1.58	1.58	1.58[c]	−6	4.33	4.32	4.33
	1.27[c]	1.26[c]	1.24[c]		4.20	4.20	4.20
2	1.91[c]	1.89[c]	1.90[c]	Rha-1	5.88, brs	5.85, brs	5.87, brs
	2.28[c]	2.24[c]	2.27[c]	2	5.17	5.17	5.18
3	3.60	3.50	3.40	3	4.94	4.94	4.94
5	0.97	0.89	0.90	4	4.48	4.47	4.48
6	1.48[c]	1.48[c]	1.48[c]	5	4.50	4.50	4.54
	1.32[c]	1.33[c]	1.30[c]	6	1.76, d (5.5)	1.76, d (6.0)	1.76, d (5.5)
7	1.62[c]	1.63 [c]	1.62[c]	Ara(*f*)−1	6.26, brs	6.26, brs	6.26, brs
	1.34[c]	1.34 [c]	1.34[c]	2	4.98	4.98	4.98
9	1.93[c]	1.92 [c]	1.92[c]	3	4.80	4.79	4.80
11	2.10[c]	2.08	2.10[c]	4	4.74	4.74	4.74
12	5.61, brs	5.60, brs	5.61, brs	5	4.27	4.25	4.27
15	2.26	2.24	2.26		4.12	4.12	4.13
	2.02	1.98	2.04	Glc″-1	5.32	5.32	5.32
16	5.21, brs	5.21, brs	5.21, brs	2	3.98	3.98	3.98
18	3.43, dd	3.43, dd	3.43	3	4.18[c]	4.18[c]	4.17[c]
19	2.94, t (13.5)	2.94, t (13.8)	2.96, t (13.5)	4	4.06[c]	4.07[c]	4.06[c]
	1.39	1.39	1.41	5	3.96[c]	3.96	3.96
21	6.30	6.29, dd (6.0, 10.8)	6.30	6	4.48	4.50	4.50
22	2.72, m	2.72, m	2.72, m		4.22	4.22	4.22
	2.18, m	2.18, m	2.18, m	MT(C-21)			
23	1.31, s	1.26, s	1.26, s	3	7.02, t (7.5)	7.02, t (7.2)	7.03
24	1.03, s	1.14, s	1.12, s	4	2.68, m	2.70, m	2.68, m
25	0.96, s	0.96, s	0.95, s	5	1.80, m	1.80, m	1.80, m
26	1.16, s	1.15, s	1.15, s	7	6.20, dd (9.5, 17.0)	6.20, dd (10.2, 17.4)	6.20, dd (9.5, 17.0)
27	1.89, s	1.87, s	1.85, s	8	5.41, d (17.0)	5.41, d (17.4)	5.42, d (18.0)
29	1.03, s	1.02, s	1.05, s		5.21	5.22, d (10.2)	5.21
30	1.07, s	1.07, s	1.08, s	9	4.71, brs	4.71, brs	4.72, brs
Sugar				10	1.50, s	1.50, s	1.50, s
Glc_1-1	4.92	4.87, d (7.2)	4.86	Qui-1	4.83, d (8.5)	4.83, d (7.2) [b]	4.83
2	4.04	4.26	4.22	2	3.98	3.98, t (9.0) [b]	3.98
3	4.19	4.22	4.20	3	4.15	4.16, t (9.0) [b]	4.20
4	4.18[c]	4.27	4.29	4	5.32	5.31, t (9.6) [b]	5.33
5	4.12[c]	4.12[c]	4.10[c]	5	3.64	3.64	3.65
6	4.76	4.71	4.56	6	1.33	1.31, d (6.6) [b]	1.34, d (5.5)
	4.38	4.32	4.20	MT′			
Fuc-1	5.00	4.95, d (7.8)		3	6.94, t (7.0)	6.93, t (7.2)	7.03
2	4.45	4.42		4	2.48, m	2.48, m	2.41, m
3	4.15	4.12		5	1.72, m	1.72, m	1.72, m
4	4.02	4.02		7	6.10, dd (11.0, 17.5)	6.10, dd (10.8, 17.4)	6.21, dd (9.5, 18.0)
5	3.75	3.75		8	5.35, d (17.0)	5.35, d (17.4)	5.40, d (17.0)
6	1.48	1.48, d (7.2)			5.26, d (11.0)	5.26, d (10.8)	5.18
Ara-1			5.12	9	1.91, s	1.90, s	1.87, s
2			4.50	10	1.37, s	1.37, s	1.53, s
3			4.39	Qui′-1	4.98	4.97, d (7.8) [b]	4.86
4			4.40	2	5.67	5.66, t (9.0) [b]	3.97
5			4.31	3	4.21	4.20, t (9.0) [b]	4.08
			3.74	4	3.72	3.71	3.68
Xyl-1	5.08, d (6.5)	5.04, d (7.2)	4.98	5	3.72	3.71	3.68
2	4.08	4.06	4.08	6	1.58	1.57, d (5.4) [b]	1.58, d (4.5)
3	4.02	4.02	4.02	MT″			
4	4.08	4.08	4.08	3	7.20	7.20	
5	4.48	4.45	4.40	4	2.70, m	2.68, m	
	3.58	3.58	3.58	5	1.78, m	1.80, m	
Glc_2-1		5.40	5.35	7	6.18, dd (10.5, 17.0)	6.18, dd (10.8, 17.4)	
2		4.10	4.10	8	5.38, d (17.0)	5.38, d (17.4)	
3		4.17	4.16		5.16	5.17, d (10.8)	
4		4.04	4.04	9	4.74, brs	4.74, brs	
5		3.90	3.90	10	1.52, s	1.52, s	
6		4.44	4.44	Qui″-1	4.85, d (8.0)	4.85, d (7.8)	
		4.26	4.26	2	3.97	3.97	
Glc′-1	6.04, d (7.5)	6.04, d (7.8) [b]	6.05, d (7.0)	3	4.08	4.08	
2	4.01	3.98, t (7.8) [b]	4.00	4	3.68	3.68	
3	4.14[c]	4.14	4.13[c]	5	3.68	3.68	
4	4.17[c]	4.17	4.17[c]	6	1.58	1.58, d (5.4)	
5	3.94	3.94	3.94				

[a] Overlapped signals are reported without designated multiplicities. Measured at 500 MHz for **1** and **3**and 600 MHz for **2**. MT = monoterpenoid acid moiety. [b] $^3J_{H1,H2}$ coupling value was obtained by a 1D TOCSY experiment. [c] Signals were assigned by comparing prosapogenins (**4** and **8**).

acetone (3:1)]. The aqueous layer was evaporated under vacuum, diluted repeatedly with H_2O, and evaporated in vacuo to furnish a neutral residue. The residue was dissolved in anhydrous pyridine (1 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h, and after evaporation in vacuo to dryness, 0.2 mL of *N*-trimethylsilylimidazole was added; the mixture was kept at 60 °C for another 2 h. The reaction mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract analyzed by GC under the following conditions: capillary column, DM-5 (30 m × 0.25 mm, with a 0.25 μm film, Dikma); detection, FID;

Table 4. Cytotoxicity of Compounds **1**−**4** against Five Human Cancer Cell Lines[a]

	IC_{50} (μM)				
compound	HCT-8	Bel-7402	BGC-823	A549	A2780
1	5.6	2.6	1.6	1.9	1.3
2	1.3	1.3	3.8	0.3	1.2
3	0.4	0.4	1.7	0.01	0.3
camptothecin[b]	3.2	12.5	9.7	3.1	0.3

[a] For key to cell lines used, see Experimental Section. Compound **4** was inactive (IC_{50} > 10 μM) for all cell lines. [b] Positive control.

detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 160 °C, then raised to 280 at 5 °C/min, final temperature maintained for 10 min; carrier, He gas. From the acid hydrolysate of **1**, L-arabinose, L-rhamnose, D-fucose, D-quinovose, D-xylose, and D-glucose were confirmed by comparison of the retention times of their derivatives with those of authentic sugars derivatized in a similar way, which showed retention times of 15.28, 15.72, 16.58, 17.05, 19.27, and 19.65 min, respectively. The constituent sugars of compounds **2** and **3** were also identified by the same method.

Mild Alkaline Hydrolysis of 1. A solution of compound **1** (62 mg) in saturated $NaHCO_3$ in MeOH (6 mL) was refluxed for 1 h. After adjusting the pH to 3.0 with 1 N HCl, the reaction mixture was concentrated in vacuo to dryness. Then, the residue was dissolved in water and partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH extract was separated by preparative HPLC using 31% CH_3CN (4 mL/min) to afford compound **4** (6 mg, t_R 37 min). The EtOAc extract was purified by preparative HPLC using 35% CH_3CN (4 mL/min), to yield compounds **6** (3 mg, t_R 18 min) and **5** (3 mg, t_R 47 min).

Mild Alkaline Hydrolysis of the Saponin Fraction. Fraction C (1.0 g) was hydrolyzed in the same manner as described for **1** to afford compound **4** (30 mg) and compound **5** (15 mg).

Compound 4: white, amorphous powder; $[\alpha]^{20}_D$ −34.5 (*c* 0.055, MeOH); ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.97, 1.03, 1.04, 1.07, 1.16, 1.31, 1.90 (3H, each, s, Me), 3.60 (1H, m, H-3), 4.85 (1H, d, J = 8.0 Hz, H-Qui-1), 4.92 (1H, overlapped, H-Glc$_1$-1), 5.00 (1H, d, J = 8.0 Hz, H-Fuc-1), 5.09 (1H, d, J = 7.0 Hz, H-Xyl-1), 5.21 (1H, brs, H-16), 5.34 (1H, d, J = 8.0 Hz, H-Glc″-1), 5.61 (1H, brs, H-12), 5.88 (1H, brs, H-Rha-1), 6.05 (1H, d, J = 8.0 Hz, H-Glc′-1), 6.26 [1H, brs, H-Ara(*f*)-1], 6.30 (1H, dd, J = 5.5, 11.0 Hz, H-21); ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m*/*z* 1883 [M + Na +1]$^+$ ($C_{86}H_{138}O_{43}Na$).

Compound 5: colorless oil; $[\alpha]^{20}_D$ −18.5 (*c* 0.13, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.17 (3H, d, J = 6.5 Hz, H$_3$-Qui-6), 1.31 (3H, s, H$_3$-10), 1.65 (2H, m, H$_2$-5), 1.75 (3H, s, H$_3$-9), 2.22 (2H, m, H$_2$-4), 2.91 (1H, t, J = 9.0 Hz, H-Qui-4), 3.10 (1H, t, J = 8.5 Hz, H-Qui-5), 3.17 (1H, dd, J = 9.0, 6.0 Hz, H-Qui-2), 3.21 (1H, t, J = 9.0 Hz, H-Qui-3), 3.65 (3H, OMe), 4.29 (1H, d, J = 8.0 Hz, H-Qui-1), 5.15 (1H, d, J = 11.0 Hz, H-8b), 5.22 (1H, d, J = 18.0 Hz, H-8a), 5.87 (1H, dd, J = 18.0, 11.0 Hz, H-7), 6.70 (1H, t, J = 7.5 Hz, H-3); ^{13}C NMR (125 MHz, CD_3OD) δ 12.4 (C-9), 18.3 (C-Qui-6), 23.5 (C-10), 24.4 (C-4), 41.0 (C-5), 52.5 (C-OMe), 72.9 (C-Qui-5), 75.5 (C-Qui-2), 77.1 (C-Qui-4), 77.9 (C-Qui-3), 81.0 (C-6), 99.3 (C-Qui-1), 115.8 (C-8), 128.4 (C-2), 144.1 (C-7), 144.3 (C-3), 170.3 (C-1); ESIMS (positive-ion mode) *m*/*z* 367 [M + Na]$^+$, 711 [2 M + Na]$^+$ ($C_{17}H_{28}O_7Na$).

Compound 6: colorless oil; $[\alpha]^{20}_D$ −18.3 (*c* 0.12, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.17 (3H, d, J = 6.0 Hz, H$_3$-Qui-6), 1.32 (3H, s, H$_3$-10), 1.66 (2H, m, H$_2$-5), 2.37 (2H, m, H$_2$-4), 2.91 (1H, t, J = 9.0 Hz, H-Qui-4), 3.10 (1H, t, J = 8.5 Hz, H-Qui-5), 3.17 (1H, dd, J = 9.0, 6.0 Hz, H-Qui-2), 3.21 (1H, t, J = 9.0 Hz, H-Qui-3), 3.68 (3H, OMe), 4.25 (2H, brs, H$_2$-9), 4.29 (1H, d, J = 8.0 Hz, H-Qui-1), 5.15 (1H, d, J = 11.0 Hz, H-8b), 5.22 (1H, d, J = 17.5 Hz, H-8a), 5.88 (1H, dd, J = 17.5, 11.0 Hz, H-7), 6.85 (1H, t, J = 8.0 Hz, H-3); ^{13}C NMR (125 MHz, CD_3OD) δ 18.3 (C-Qui-6), 23.7 (C-10), 24.2 (C-4), 41.3 (C-5), 52.2 (C-OMe), 56.5 (C-9), 72.9 (C-Qui-5), 75.5 (C-Qui-2), 77.1 (C-Qui-4), 77.9 (C-Qui-3), 80.9 (C-6), 99.3 (C-Qui-1), 115.9 (C-8), 132.4 (C-2), 144.1 (C-7), 148.3 (C-3), 169.4 (C-1); ESIMS (positive-ion mode) *m*/*z* 383 [M + Na]$^+$, 743 [2 M + Na]$^+$ ($C_{17}H_{28}O_8Na$).

Enzymatic Hydrolysis of 5. Compound **5** (6 mg) and β-glucosidase (106 units from Fluka) were dissolved in acetate buffer (0.2 M, pH 5.0, 1 mL) and incubated at 37 °C for 72 h. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ solution evaporated to give compound **7** (3 mg).

Compound 7: colorless oil; $[\alpha]^{20}_D$ +13.3 (*c* 0.15, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 (3H, s, H$_3$-10), 1.64 (2H, m, H$_2$-5), 1.83 (3H, s, H$_3$-9), 2.22 (2H, m, H$_2$-4), 3.72 (3H, OMe), 5.09 (1H, d, J = 11.0 Hz, H-8b), 5.23 (1H, d, J = 17.5 Hz, H-8a), 5.91 (1H, dd, J = 17.5, 11.0 Hz, H-7), 6.75 (1H, t, J = 8.0 Hz, H-3); ESIMS (positive-ion mode) *m*/*z* 221 [M + Na]$^+$ ($C_{11}H_{18}O_3Na$).

Strong Alkaline Hydrolysis of 4. Compound **4** (30 mg) was hydrolyzed with 3% NaOH (4 mL) and MeOH (0.8 mL) for 10 h at room temperature. After adjusting the pH to 5.0 with 2 M HCl, the reaction mixture was extracted successively with EtOAc and *n*-BuOH. The EtOAc extract was separated by preparative HPLC using 30% CH_3CN−0.05% aqueous HOAc (4 mL/min) to afford compound **8** (5 mg, t_R 29 min). The *n*-BuOH extract was separated by preparative HPLC using 28% CH_3CN−0.05% aqueous HOAc (4 mL/min) to yield compound **9** (3 mg, t_R 19 min).

Compound 8: white, amorphous powder; ^{1}H NMR (500 MHz, pyridine-d_5) δ 0.90, 1.02, 1.03, 1.34 × 2, 1.43, 1.94 (3H, each, s, Me), 1.49 (3H, d, J = 6.0 Hz, H-Fuc-6), 3.65 (1H, dd, J = 11.5, 4.5 Hz, H-3), 4.95 (1H, d, J = 8.0 Hz, H-Glc-1), 4.99 (1H, m, H-21), 5.02 (1H, d, J = 7.5 Hz, H-Fuc-1), 5.10 (1H, d, J = 7.0 Hz, H-Xyl-1), 5.29 (1H, brs, H-16), 5.64 (1H, brs, H-12); ^{1}H and ^{13}C NMR data were in full agreement with reference data for this compound.[2] ESIMS (positive-ion mode) *m*/*z* 951 [M + Na]$^+$ ($C_{47}H_{76}O_{18}Na$), (negative-ion mode) *m*/*z* 927 [M − H]$^-$.

Compound 9: colorless oil; $[\alpha]^{20}_D$ −22.0 (*c* 0.10, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.18 (3H, d, J = 6.0 Hz, H$_3$-Qui-6), 1.31 (3H, s, H$_3$-10), 1.65 (2H, m, H$_2$-5), 2.27 (2H, m, H$_2$-4), 2.91 (1H, t, J = 9.0 Hz, H-Qui-4), 3.10 (1H, t, J = 8.5 Hz, H-qui-5), 3.16 (1H, dd, J = 6.0, 9.0 Hz, H-Qui-2), 3.21 (1H, t, J = 9.0 Hz, H-Qui-3), 4.25 (2H, brs, H$_2$-9), 4.28 (1H, d, J = 8.0 Hz, H-Qui-1), 5.15 (1H, d, J = 11.0 Hz, H-8b), 5.21 (1H, d, J = 17.5 Hz, H-8a), 5.88 (1H, dd, J = 11.0, 18.0 Hz, H-7), 6.64 (1H, t, H-3); ^{13}C NMR (125 MHz, CD_3OD) δ 18.3 (C-Qui-6), 23.6 (C-10), 24.1 (C-4), 41.5 (C-5), 57.2 (C-9), 72.9 (C-Qui-5), 75.5 (C-Qui-2), 77.1 (C-qui-4), 77.9 (C-Qui-3), 81.0 (C-6), 99.3 (C-Qui-1), 115.9 (C-8), 133.5 (C-2), 144.1 (C-7), 146.2 (C-3); ESIMS (positive-ion mode) *m*/*z* 369 [M + Na]$^+$, 715 [2 M + Na]$^+$ ($C_{16}H_{26}O_8Na$), (negative-ion mode) *m*/*z* 345 [M − H]$^-$, 691 [2 M − H]$^-$.

Cytotoxicity Assay. HCT-8 (human colon cancer), Bel-7402 (human hepatoma cancer), BGC-823 (human gastric cancer), A549 (human lung epithelial cancer), and A2780 (human ovarian cancer) cell lines were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin sulfate. Cultures were incubated at 37 °C in 5% CO_2 air. The cells (1.5×10^3) were seeded in 96-well tissue culture plates. After 24 h, 100 μL of DMSO solution containing each test compound was added to give final concentrations of 0.01−10 μmol/mL; 100 μL of DMSO was added into control wells. The cells were treated with various concentrations of the test compounds for 96 h, and then cell growth was evaluated by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay procedure.[15] Then 200 μL of 0.2% MTT in RPMI 1640 medium was added to each well, and the plate was further incubated in 5% CO_2 air for 4 h at 37 °C. The plate was then centrifuged to precipitate cells and formazan. An aliquot (150 μL) of the supernatant was removed from each well, and 200 μL of DMSO was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader at 570 nm. All compounds were tested at five concentrations, and each concentration of the compounds was tested in three parallel wells. A dose−response curve was plotted for each compound, and the IC_{50} value was calculated as the concentration of the test compound resulting in 50% reduction of optical density compared with the control.

Hemolysis Assay. Rabbit erythrocytes were prepared according to the method described in the *Pharmacopoeia of the People's Republic of China*.[16] The hemolytic activity of **1**−**4** was evaluated on rabbit erythrocytes in the concentration range 0.01−100 μM (0.01, 0.05, 0.1, 0.5, 2.5, 10, 100 μM), according to a procedure adapted from the literature.[14]

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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