Cytotoxic Triterpenoid Saponins Acylated with Monoterpenic Acid from *Pithecellobium lucidum*

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Three new oleanane-type triterpene saponins, named pithelucosides A−C (**1**–**3**), together with two known saponins (**4**, **5**) were isolated from the roots of *Pithecellobium lucidum*. The structures of the new saponins were established on the basis of extensive 1D and 2D NMR experiments and mass spectrometry and confirmed by acid and alkaline hydrolysis. Compounds **1**–**5** and **7** (pro-sapogenin obtained from the mild alkaline hydrolysate of **1**) were evaluated for cytotoxic activity on five human tumoral cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) and for hemolytic property against rabbit erythrocytes. Compounds **2**–**5** showed significant cytotoxic activities with IC_{50} values of 0.61–7.56 μM. All tested compounds did not exhibit any hemolytic activity in the concentration range 0.01–100 μM.

Pithecellobium is a genus of Mimosaceae consisting of 120 species, four of which are found in China. *Pithecellobium lucidum* Benth. is widely distributed in the southwestern and southeastern parts of China and is also found in Vietnam and India. The leaves and stems have been used as Chinese folk medicine for the treatment of rheumatalgia and wounds.[1] Previous phytochemistry studies on other species of *Pithecellobium* plants led to the identification of flavonoids,[2] steroid glycosides,[3] and triterpenoid saponins,[4] along with several other components.[5] Three triterpenoid saponins isolated from *P. dulce* and julibrosides (julibrosides I, J_2, and J_7) isolated from *Albizia julibrissin* (Mimosaceae) share the following structural feature: they possess the same aglycone unit (acacic acid) substituted by anoligosaccharide moieties at C-28 and C-3 and acylated at C-21.[6] This class of triterpenoid saponins from *Albizia* species exhibited cytotoxicity against various cancer cell lines *in vitro*.[6,7] As part of an ongoing program to screen toxic herbs for cytotoxic compounds, the EtOH extract of dried roots of *P. lucidum* was examined and it exhibited cytotoxicity in three cultured human tumoral cell lines (HCT-8, Bel-7402, and A2780) with IC_{50} values of 17.48–37.26 μg/mL. Bioassay-guided fractionation led to the isolation of five acylated triterpenoid saponins (**1**–**5**), including three new triterpenoid saponins, pithelucosides A−C (**1**–**3**). In this paper, we report the isolation and structure elucidation of new triterpenoid saponins and the evaluation of the cytotoxic activities of saponins **1**–**5** against five human tumor cell lines and their hemolytic activity on rabbit erythrocytes.

Results and Discussion

The 95% EtOH extract from dried roots of *P. lucidum* was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to polyamide column chromatography to give a crude saponins fraction, which showed cytotoxicity against three cultured human tumoral cell lines (HCT-8, BGC-823, and A2780) with IC_{50} values of 4.76–18.31 μg/mL. The crude saponins fraction, on chromatographic purification over D101 resin and normal Si gel, followed by repeated HPLC purification, afforded five acylated triterpenoid saponins, pithelucosides A−C (**1**–**3**) and two known saponins (**4** and **5**), which were identified as 21-*O*-[2*E*-hydroxymethyl-6*S*-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(l→4)-[β-D-glucopyranosyl-(l→3)]-α-L-rhamnopyranosyl-(l→2)-β-D- glucopyranosyl ester (prosapogenin-10)[6a] and 21-*O*-[2*E*-hydroxymethyl-6*S*-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl]-3-*O*-β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(l→4)-[β-D-glucopyranosyl-(l→3)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranosyl ester (julibroside J_{29}),[7d] by comparison of their NMR data with literature values. Although known in *Albizia*, this is the first report of the isolation of these two known compounds from the genus *Pithecellobium*.

Pithelucoside A (**1**) was obtained as an amorphous powder, $[\alpha]^{25}_{D}$ −32.4 (MeOH). The positive-ion high-resolution (HR) ESIMS of **1** showed an accurate $[M + Na]^+$ ion peak at *m/z* 1865.8541, in accordance with an empirical molecular formula of $C_{86}H_{138}O_{42}Na$, which was supported by the ^{13}C NMR spectrum and various DEPT data. The IR spectrum showed carbonyl group (1734 cm^{-1}) and α,β-unsaturated carbonyl group (1688 cm^{-1}) absorption. Upon acid hydrolysis with 2 M HCl at 95 °C, **1** afforded the aglycone **6**, which was identified as acacic acid lactone by comparison of its NMR data with literature data,[4d] and monosaccharides L-rhamnose, L-arabinose, D-fucose, D-xylose, D-quinovose, and D-glucose in a ratio of 1:1:1:1:2:2, which was identified by gas–liquid chromatographic (GLC) analysis of their trimethylsilyl L-cysteine derivatives.[8] The ^1H and ^{13}C NMR spectra of **1** showed eight anomeric protons at δ_H 4.84 (1H, d, J = 8.0 Hz, Qui), 4.89 (1H, d, J = 7.5 Hz, Glc), 4.98 (1H, overlapped, Fuc), 5.08 (1H, d, J = 6.5 Hz, Xyl), 5.26 (1H, d, J = 7.5 Hz, Qui′), 6.11 (1H, d, J = 7.0 Hz, Glc′), 6.25 [1H, br s, Ara(*f*)], and 6.27 (1H, br s, Rha) and the corresponding carbon resonances at δ 99.4, 106.8, 103.4, 107.0, 105.6, 95.6, 111.3, and 101.4, respectively. On the basis of the coupling constants of anomeric protons and the chemical shifts of anomeric carbons, the anomeric configuration of the sugar moieties was determined as β for glucose, xylose, fucose, and quinovose moieties and α for rhamnose and arabinose (in furanose form) moieties.[4d] The ^{13}C NMR spectrum of **1** showed 86 carbon resonances, 30 attributable to acacic acid and 46 to the sugar moieties. The remaining 10 resonances were consistent with the presence of a monoterpene carboxylic acid. Alkaline hydrolysis of **1** with 0.5 M NaOH in aqueous MeOH at room temperature gave prosapogenin (**7**) and monoterpene glycoside (**8**) as major components. Compound **7** was found to be identical to the known saponin 3-*O*-β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)-β-D-glucopyranosylacacic acid (prosapogenin-1), which was obtained by alkaline hydrolysis of the crude saponin fraction extracted from *Albizia julibrissin*.[6a] The above data suggested that **1** was a 21-acyl-3,28-bidesmoside of acacic acid. This was confirmed by the observation of glycosylation- and acylation-induced shifts in the

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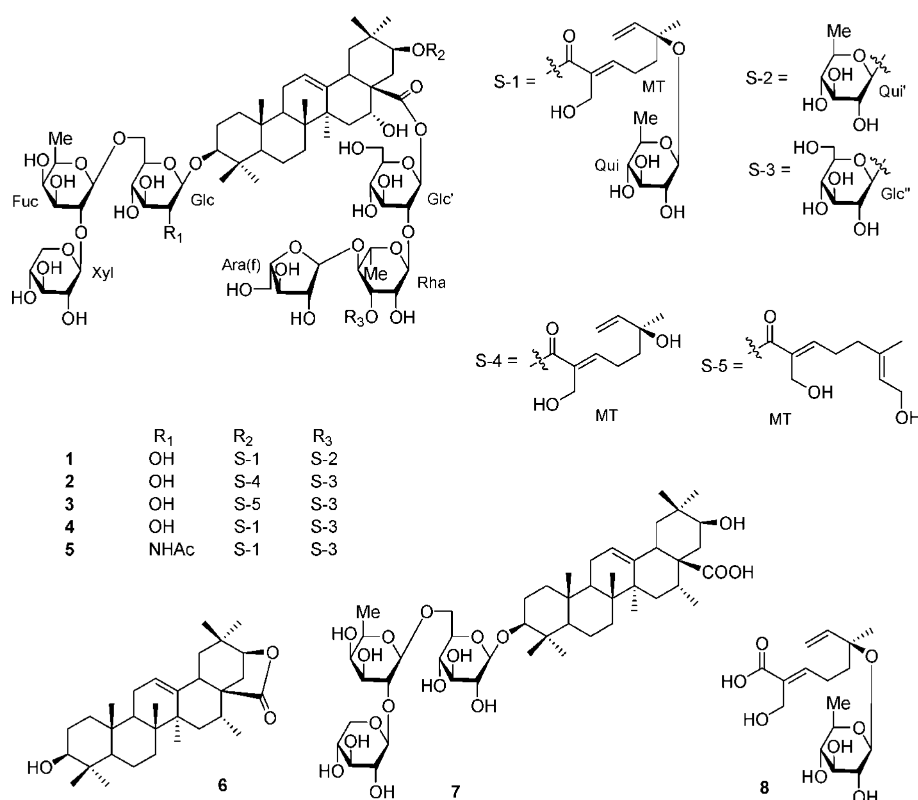
Chart 1



^{13}C NMR spectrum at δ_C 88.5 (downfield shift of C-3), δ_C 77.2 (downfield shift of C-21), and δ_C 174.6 (upfield shift of C-28). By analysis of the NMR data, and literature rotation data for its methyl ester, the monoterpenoid **8** was determined as the previously known 6*S*-hydroxy-2*E*-hydroxymethyl-6-methyl-2,7-octadienoic acid 6-*O*-β-D-quinovopyranoside.[9] Comparison of the ^{13}C NMR chemical shifts of **7** and **1** permitted definition of the position of the linkage of the monoterpene quinovoside to the aglycone moiety. When compared to **7**, the resonances for C-20, C-21, and C-22 in **1** underwent an upfield shift of 1.6 ppm, a downfield shift of 3.6 ppm, and an upfield shift of 5.6 ppm, respectively, as a consequence of the acylation at C-21. Further, the HMBC spectrum exhibited significant correlation between H-21 (δ_H 6.24) of the aglycone and the carbonyl carbon (δ_C 167.6) of the monoterpene quinovoside unit. Thus, a 6*S*-hydroxy-2*E*-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7-octadienoyl residue was located at C-21 of the aglycone.

The assignments of the ^{1}H and ^{13}C NMR resonances of **1** from the ^{1}H−^{1}H COSY, HSQC, and HMBC spectra showed that the four remaining sugars were a terminal Qui (Qui′), a disubstituted Glc (Glc-1,2)(Glc′), a terminal Ara(*f*), and a trisubstituted Rha (Rha-1,3,4). The long-range correlation in the HMBC spectrum at δ_C/δ_H 174.6/6.11 (d, J = 7.0 Hz) showed that the Glc′ residue was linked to the carboxylic group of the aglycone by an ester linkage. This conclusion was supported by the upfield shift of C-28 at δ_C 174.6, in comparison with the free carboxylic acid observed in **7** at δ_C 179.6 ppm. The long-range correlations observed in the HMBC spectrum between the ^{1}H NMR resonances at δ_H 6.27 (H-Rha-1) and the ^{13}C NMR resonances at δ_C 76.0 (C-Glc′-2), between δ_H 5.26 (H-Qui′-1) and δ_C 82.0 (C-Rha-3), and between δ_H 6.25 [H-Ara(*f*)-1] and δ_C 79.1 (C-Rha-4) showed that the tetrasaccharide residue *O*-α-L-arabinofuranosyl-(1→4)-[β-D- quinovopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl was linked to the acacic acid unit at C-28. The same conclusion with regard to the sugar sequence was also drawn from the NOESY experiment. On the basis of the above data, the structure of pithelucoside A (**1**) was elucidated as 21-*O*-[6*S*-hydroxy-2*E*-hydroxymethyl-6-methyl-6-*O*-β-D-quinovopyranosyl-2,7- octadienoyl]-3-*O*-β-D-xylopyranosyl-(1→2)- β-D-fucopyranosyl-(1→6)-β-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-quinovopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester.

Pithelucoside B (**2**), amorphous powder, possessed the molecular formula $C_{80}H_{128}O_{39}$, as determined by HRESIMS in the positive-ion mode (HRESIMS *m/z* [M + Na]$^+$ 1735.7966, calcd for $C_{80}H_{128}O_{39}Na$, 1735.7930) and supported by the ^{13}C NMR spectrum and various DEPT data. Acid hydrolysis of **2** afforded L-rhamnose, L-arabinose, D-fucose, D-xylose, and D-glucose in a ratio of 1:1:1:1:3. The ^{13}C NMR chemical shifts due to the aglycone moiety and the sugar moieties attached at C-3 and C-28 of **2** were superimposable on those of the known compound **4**. In contrast, the acyl moiety at C-21 in **2**, the only monoterpenoid unit (MT) in the molecule, was not glycosylated at the C-6 position (δ_C 72.2) (Table 1). Furthermore, the ^{13}C NMR chemical shifts of C-MT-5, C-MT-6, C-MT-7, and C-MT-10 of compound **2** were similar to those of C-MT-5 (δ_C 41.6), C-MT-6 (δ_C 72.2), C-MT-7 (δ_C 146.6), and C-MT-10 (δ_C 28.6) of the related compound pitheduloside J, which showed an *S* configuration at the C-MT-6 position.[4d] The data indicated that compound **2** possessed an *S* configuration at the C-6 of the monoterpenoid moiety. The trisubstituted double bond in the monoterpenoid moiety was assigned an *E* configuration, as evidenced by a NOESY correlation between H_2-MT-9 (δ_H 4.74) and H_2-MT-4 (δ_H 2.65). Therefore, the structure of **2** was derived as 21-*O*-[6*S*-hydroxy-2*E*-hydroxymethyl-6-methyl-2,7-octadienoyl]-3-*O*-β-D-xylopyranosyl-(1→2)-β-D-fucopyranosyl-(1→6)- β-D-glucopyranosyl acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D- glucopyranosyl ester.

Pithelucoside C (**3**) was isolated as an amorphous powder, with the molecular formula $C_{80}H_{128}O_{39}$, as determined from the positive-ion HRESIMS (*m/z* [M + Na]$^+$ 1735.7981), ^{13}C NMR, and various DEPT spectra. On acid hydrolysis, **3** afforded the same sugars in the same ratio as those of compound **2**. The ^{13}C NMR chemical shifts due to the aglycone moiety and the sugar moieties attached at C-3 and C-28 of **3** were superimposable on those of **2**. The

Table 1. ^{13}C NMR Spectroscopic Data of **1**−**4** and **7** (125 MHz, in pyridine-d_5)[a,b]

position	1	2	3	4	7	position	1	2	3	4
aglycon						sugar (C-28)				
C-1	39.0	38.9	38.9	38.9	38.8	Glc′ 1	95.6	95.7	95.7	95.6
2	26.9	26.9	26.8	26.8	26.8	2	76.0	77.1	77.0	77.4
3	88.5	88.4	88.4	88.3	88.3	3	79.0	78.0	78.0	78.0
4	39.8	39.7	39.6	39.6	39.6	4	71.3	71.8	71.8	71.7
5	56.1	56.0	56.0	56.0	55.9	5	79.1	79.0	79.0	78.9
6	18.9	18.7	18.7	18.8	18.5	6	62.1	62.0	62.0	61.9
7	33.9	33.6	33.6	33.6	33.5	Rha (1→2) Glc′				
8	40.2	40.2	40.1	40.1	39.9	1	101.4	101.9	101.8	101.7
9	47.2	47.2	47.2	47.1	47.2	2	71.3	70.5	70.5	70.5
10	37.2	37.1	37.1	37.1	36.7	3	82.0	82.0	82.0	82.0
11	24.0	23.9	23.9	23.8	23.9	4	79.1	79.1	79.1	79.0
12	123.2	123.1	123.1	123	122.6	5	68.7	69.2	69.2	69.1
13	143.5	143.4	143.4	143.3	144.6	6	18.8	18.9	18.9	18.8
14	42.1	42.0	42.0	42.0	42.0	Qui′ (1→3)Rha				
15	36.0	35.9	35.9	35.8	35.9	1	105.6			
16	73.8	73.9	73.9	73.8	74.5	2	75.6			
17	51.8	51.6	51.6	51.6	51.8	3	78.38			
18	41.2	41.0	40.94	40.9	41.0	4	76.8			
19	48.0	47.9	47.9	47.8	48.4	5	73.1			
20	35.5	35.5	35.5	35.4	37.1	6	18.6			
21	77.2	77.0	77.1	77.0	73.6	Glc″ (1→3)Rha				
22	36.4	36.4	36.4	36.4	42.0	1		105.8	105.8	105.7
23	28.3	28.2	28.2	28.2	28.2	2		75.4	75.4	75.3
24	16.0	15.9	15.9	15.8	15.7	3		78.35	78.3	78.3
25	17.3	17.2	17.16	17.1	17.1	4		71.4	71.34	71.6
26	17.5	17.4	17.4	17.3	17.2	5		78.2	78.2	78.1
17	27.4	27.3	27.3	27.2	27.3	6		62.8	62.8	62.7
28	174.6	174.5	174.4	174.4	179.6	Ara (1→4) Rha				
29	29.2	29.2	29.2	29.1	30.1	1	111.3	111.0	111.1	111.0
30	19.2	19.2	19.1	19.0	18.4	2	84.4	84.5	84.6	84.4
sugar (C-3)						3	78.6	78.43	78.4	78.4
Glc 1	106.8	106.7	106.7	106.6	106.7	4	85.8	85.4	85.5	85.4
2	75.8	75.8	75.8	75.7	75.8	5	62.7	62.6	62.5	62.5
3	78.44	78.43	78.4	78.35	78.4	MT				
4	71.7	71.7	71.7	71.7	71.3	1	167.6	167.6	167.5	167.5
5	76.8	76.8	76.7	76.7	76.9	2	133.8	133.7	134.1	133.6
6	70.1	70.0	70.0	70.0	70.0	3	145.4	145.7	144.4	145.3
Fuc (1→6) Glc						4	23.7	24.0	27.2	23.6
1	103.4	103.4	103.3	103.3	103.4	5	40.9	41.96	38.7	40.8
2	82.2	82.2	82.1	82.0	82.3	6	79.6	72.2	135.1	79.5
3	75.3	75.2	75.2	75.1	75.2	7	144.1	146.6	127.0	144.0
4	72.3	72.2	72.2	72.2	72.2	8	114.9	111.7	58.8	114.8
5	71.4	71.2	71.2	71.2	71.7	9	56.4	56.3	56.4	56.3
6	17.2	17.23	17.2	17.14	17.6	10	23.9	28.6	16.1	23.7
Xyl (1→2) Fuc						Qui 1	99.4			99.3
1	107.0	106.9	106.9	106.8	106.9	2	75.7			75.7
2	75.8	75.8	75.8	75.7	75.8	3	78.44			78.4
3	77.6	77.5	77.5	77.5	77.5	4	76.9			76.8
4	70.8	70.8	70.8	70.7	70.8	5	72.6			72.5
5	67.3	67.2	67.2	67.1	67.2	6	19.0			19.1

[a] Assignments based on the DEPT, HSQC, and HMBC experiments. [b] MT = Monoterpenoid acid moiety.

structure of the remaining monoterpenoid moiety (MT) linked at C-21 was established by comparison with those in **2**. The most critical differences were the chemical shifts of the carbons of C-MT-6, C-MT-7, and C-MT-8. In the ^{13}C NMR spectrum, the carbon resonances at δ_C 72.2 (C-MT-6), 146.6 (C-MT-7), and 111.7 (C-MT-8) in **2** were substituted by resonances at δ_C 135.1, 127.0, and 58.8 in **3**, respectively, and the chemical shifts of the other carbons of MT differed slightly. These data indicated the presence of one trisubstituted double bond ($\Delta^{6,7}$) in the monoterpenoid moiety, which was supported by the ^{1}H−^{1}H COSY correlation between H_2-MT-8 [δ_H 4.39 (2H, d, J = 6.0 Hz)] and H-MT-7 [δ_H 5.72 (1H, t, J = 6.0 Hz)]. Further, the configuration of the trisubstituted $\Delta^{2,3}$ double bond in the monoterpenoid moiety was determined as *E* by a NOESY correlation between H_2-MT-9 (δ_H 4.71) and H_2-MT-4 (δ_H 2.52), while the trisubstituted $\Delta^{6,7}$ double bond was assigned an *E* configuration by NOE difference experiments. In the NOE spectrum, NOEs were observed between δ_H 4.39 (H_2- MT-8) and δ_H 1.60 (H_3-MT-10). Thus, the ester residue at C-21 in **3** was determined to be 21-*O*-8-hydroxy-2*E*-hydroxymethyl-6*E*-methyl-2,6-octadienoyl ester. From the above evidence, the structure of **3** was determined as 21-*O*-8-hydroxy-2*E*-hydroxymethyl-6*E*-methyl-2,6-octadienoyl-3-*O*-β-D-xylopyranosyl-(l→2)-β-D-fucopyranosyl-(1→6)- β-D-glucopyranosylacacic acid 28-*O*-α-L-arabinofuranosyl-(l→4)-[β-D-glucopyranosyl-(l→3)]-α-L-rhamnopyranosyl-(l→2)-β-D-glucopyranosyl ester.

The cytotoxic activities of compounds **1**–**5** and **7** were evaluated against human cancer cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) with camptothecin as positive control. Compound **5** exhibited significant cytotoxicity against all human tumoral cell lines tested, while compound **1**, showing structural similarity with **5**, was inactive against all cell lines tested (IC_{50} > 10 μM) (Table 3). By comparing the cytotoxicity of the genuine saponins **2**–**5** with prosapogenin **7**, the tetrasaccharide at C-28 and the ester moiety at C-21 of the aglycone may be considered to be important for the mediation of their cytotoxicity, as reported in the case of julibrosides I-III.[6a] Further, comparing the structures of compounds **1** and **5** with **4**, the difference in the activities among them seems to suggest

Table 2. ^{1}H NMR Data of Compounds **1**, **2**, and **3** (500 MHz, in pyridine-d_5)[a,b]

position	1	2	3
aglycon			
1	1.62	1.64	1.62
	1.22	1.21	1.20
2	2.28	2.30	2.28
	1.93	1.92	1.93
3	3.58	3.60	3.60
5	0.97	0.96	0.96
6	1.82	1.80	1.81
	1.58	1.58	1.58
7	1.81	1.76	1.78
9	1.92	1.91	1.94
11	2.09	2.04	2.05
12	5.62 (br s)	5.60 (br s)	5.60 (br s)
15	2.25	2.24	2.22
	2.10	2.02	2.04
16	5.26 (br s)	5.20 (br s)	5.20 (br s)
18	3.41 (dd, 14.0, 3.0)	3.42 (dd, 14.0, 3.5)	3.42 (dd, 13.5, 3.5)
19	2.92 (t, 13.5)	2.94 (t, 13.5)	2.95 (t, 13.5)
	1.37 (dd, 13.5, 4.0)	1.38 (m)	1.38 (dd, 13.5, 4.5)
21	6.24 (m)	6.28 (dd, 10.5, 5.0)	6.27 (dd, 10.5, 5.0)
22	2.80 (dd, 12.5, 4.5)	2.71 (m)	2.71 (dd, 13.0, 4.5)
	2.20	2.19 (m)	2.18
23	1.31 (s)	1.30 (s)	1.30 (s)
24	1.058 (s)	1.02 (s)	1.02 (s)
25	0.99 (s)	0.95 (s)	0.96 (s)
26	1.21 (s)	1.15 (s)	1.15 (s)
27	1.89 (s)	1.89 (s)	1.88 (s)
29	0.99 (s)	1.01 (s)	1.02 (s)
30	1.062 (s)	1.07 (s)	1.08 (s)
sugar (C-3)			
Glc 1	4.89 (d, 7.5)	4.90 (d, 7.5)	4.91 (d, 7.5)
2	4.00	3.98	3.98
3	4.15	4.15	4.16
4	4.15	4.13	4.14
5	4.07	3.98	3.96
6	4.74	4.74	4.74
	4.37	4.36	4.36
Fuc (1→6) Glc			
1	4.98	4.98	4.99
2	4.42	4.43	4.44
3	4.13	4.12	4.14
4	3.99	3.99	4.00
5	3.74 (m)	3.75 (m)	3.75 (m)
6	1.47 (d, 7.0)	1.47 (d, 6.5)	1.47 (d, 6.5)
Xyl (1→2) Fuc			
1	5.08 (d, 6.5)	5.08 (d, 7.0)	5.08 (d, 7.0)
2	4.02	4.02	4.01
3	4.07	4.05	4.04
4	4.09	4.08	4.08
5	4.45	4.48	4.48
	3.56	3.58	3.58

position	1	2	3
sugar (C-28)			
Glc′ 1	6.11 (d, 7.0)	6.04 (d, 8.0)	6.05 (d, 8.0)
2	4.23	3.99	3.98
3	4.22	4.15	4.17
4	4.21	4.15	4.14
5	3.96	3.92	3.92
6	4.35	4.30	4.30
	4.24	4.20	4.20
Rha (1→2) Glc′			
1	6.27 (br s)	5.86 (br s)	5.87 (br s)
2	4.99	5.17	5.17
3	4.75	4.92	4.92
4	4.47	4.46	4.46
5	4.62 (m)	4.53	4.53
6	1.76 (d, 6.5)	1.75 (d, 6.5)	1.74 (d, 5.5)
Qui′ (1→3) Rha			
1	5.26 (d, 7.5)		
2	3.94 (t, 8.3)[c]		
3	4.07 (t, 8.7)[c]		
4	3.62 (t, 8.8)[c]		
5	3.82 (t, 7.8)[c]		
6	1.48 (d, 5.0)		
Glc″ (1→3) Rha			
1		5.31 (d, 8.0)	5.32 (d, 7.5)
2		3.96	3.96
3		4.16	4.16
4		4.13	4.14
5		3.99	4.00
6		4.50	4.50
		4.25	4.25
Ara (f) (1→4) Rha			
1	6.25 (br s)	6.25 (br s)	6.26 (br s)
2	4.99	4.95	4.96
3	4.79	4.82	4.82
4	4.76	4.71	4.71
5	4.22	4.20	4.20
	4.14	4.16	4.16
MT −3	7.02 (t, 7.0)	7.14 (t, 7.5)	7.06
4	2.65 (m)	2.65	2.52 (m)
5	1.79	1.84	2.12 (t, 7.5)
7	6.17 (dd, 18.0, 11.0)	6.08 (m)	5.72 (t, 6.0)
8	5.36 (d, 17.5)	5.52 (dd, 17.0, 1.5)	4.39 (d, 6.0)
	5.15 (d, 11.0)	5.10 (dd, 11.0, 1.5)	
9	4.68 (m)	4.74	4.71
10	1.49 (s)	1.41 (s)	1.60 (s)
Qui 1	4.84 (d, 8.0)		
2	3.95 (t, 7.8)[c]		
3	4.08 (t, 8.3)[c]		
4	3.66 (m)		
5	3.65 (m)		
6	1.58 (d, 5.5)		

[a] Overlapped signals are reported without designated multiplicities. [b] MT = Monoterpenoid acid moiety. [c] $^3J_{H1,H2}$ coupling value was obtained by 1D TOCSY experiment.

Table 3. Cytotoxicity of Compounds **1–5** and **7** against Five Human Cancer Cell Lines[a]

	IC$_{50}$ (μM)				
compound	HCT-8	Bel-7402	BGC-823	A549	A2780
2	>10	2.54	>10	>10	1.23
3	4.24	7.56	>10	>10	1.66
4	1.50	>10	>10	>10	1.35
5	1.50	1.65	1.90	1.80	0.61
camptothecin[b]	3.15	12.5	9.67	3.12	0.28

[a] Compounds **1** and **7** were inactive against all cell lines tested (IC$_{50}$ > 10 μM). [b] Positive control.

that the *N*-acetylglucosamine moiety at C-3 may intensify the cytotoxicity, while terminal quinovose at C-28 may weaken the cytotoxicity.

Hemolysis is probably the most general activity shared by many structurally disparate saponins (triterpene or steroid glycosides). Therefore, the hemolytic activity of compounds **1–5** and **7** was evaluated against rabbit erythrocytes according to a procedure adapted from the literature.[10] No hemolytic activity was observed for all compounds tested in the concentration range 0.01–100 μM. The concentrations where the compounds **2–5** were observed to be cytotoxic on the human cancer cell lines are far below the concentration of 100 μM. These results indicate that mechanisms other than hemolytic effects are responsible for the cell cytotoxicity. Although the triterpenoid saponin of adianthithifolioside D (closely related to **5**) was shown to induce inhibition of the Jurkat cell proliferation by induction of apoptosis,[11] the mechanism involved in the cytotoxic activity of **5** is not clear.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 automatic digital polarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a microscope transmission method. NMR spectra were obtained on an INOVA-500, MP-400, or SX-600 spectrometer for ^{1}H, ^{13}C, TOCSY, HSQC, HMBC, ^{1}H−^{1}H COSY, and NOESY. The carbon type (CH_3, CH_2, CH) was

determined by DEPT experiments. Chemical shifts are given in δ (ppm) with solvent (pyridine-d_5 or methanol-d_4) peaks as references. ESIMS were measured on an Agilent 1100 Series LC/MSD trap mass spectrometer. HRESIMS data were recorded using a micromass Autospec-Ultima ETOF spectrometer. Preparative HPLC was performed on a Shimadazu 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, China), macroporous resin D101 (26–60 mesh, Tianjin Haiguang Chemistry Company, Tianjin, China), Si gel (160–200, 200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, 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. GLC was carried out on a TSQ7000 (Finnigan) GC-MS instrument.

Plant Material. The roots of *Pithecellobium lucidum* Benth. were collected from Guangxi Province, China, and identified by Prof. Songji Wei (Guangxi College of Chinese Traditional Medicine) in September 2004. A voucher specimen (no. 90211) was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation. Air-dried, powdered roots of *P. lucidum* (4.5 kg) were macerated for 3 h with 15 L of 95% EtOH and further refluxed for 9 h (15 L × 3). The filtrate was concentrated under reduced pressure, and the residue (490 g) was suspended in H_2O, then successively partitioned with EtOAc and *n*-BuOH. The *n*-BuOH layer was evaporated under vacuum to yield a residue (300 g), which was subjected to a column of polyamide eluted with 40 and 95% EtOH. The 40% EtOH fraction (71 g) was further passed through a D101 macroporous resin column eluted with 80 and 95% EtOH. The 80% EtOH fraction (60 g) was separated by Si gel (1.5 kg) using $CHCl_3$−MeOH−H_2O gradient mixtures (15:1:0–6:4:1) to afford five fractions (A−E). Fraction B (2.5 g) was subjected to an ODS column (45–70 μm, 200 g) eluted with a gradient of MeOH−H_2O (50:50–85:15) to provide six subfractions. Subfraction 2 (430 mg) was first purified by HPLC using 72% MeOH−H_2O and then using 30% CH_3CN−H_2O (4 mL/min) to yield compound **1** (210 mg, t_R = 38 min). Fraction D (4.7 g) was subjected to an ODS column eluted with a gradient of MeOH−H_2O (45:55–85:15) to provide 12 subfractions. Subfraction 4 (300 mg) was purified by HPLC using 30% CH_3CN−H_2O (4 mL/min) to yield compound **4** (110 mg, t_R = 35 min). Fraction E (12 g) was purified on an ODS column using a gradient of MeOH−H_2O (40:60–85:15) to give 20 subfractions. Subfraction 3 (150 mg) was further purified by HPLC using 28% CH_3CN−H_2O (5 mL/min) to yield compound **3** (19 mg, t_R = 40 min), and subfraction 7 (140 mg) was further purified by HPLC using 28% CH_3CN−H_2O (5 mL/min) to afford compound **5** (21 mg, t_R = 48 min) and compound **2** (25 mg, t_R = 65 min).

Pithelucoside A (1): white, amorphous powder, $[\alpha]^{25}_D$ −32.4 (*c* 0.92, MeOH); IR υ_{max} 3400, 2924, 1734, 1688, 1640, 1364, 1056, 1037, 1004, 635 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) data, see Table 2; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1; ESIMS (in positive-ion mode) *m/z* 1866 $[M + Na + 1]^+$; HRESIMS (in positive-ion mode) *m/z* 1865.8541 $[M + Na]^+$ (calcd for $C_{86}H_{138}O_{42}Na$, 1865.8554).

Pithelucoside B (2): white, amorphous powder, $[\alpha]^{25}_D$ −23.7 (*c* 0.76, MeOH); IR υ_{max} 3398, 2931, 1737, 1690, 1641, 1368, 1075, 1045, 642 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) data, see Table 2; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1; ESIMS (in positive-ion mode) *m/z* 1736 $[M + Na + 1]^+$; HRESIMS (in positive-ion mode) *m/z* 1735.7966 $[M + Na]^+$ (calcd for $C_{80}H_{128}O_{39}Na$, 1735.7930).

Pithelucoside C (3): white, amorphous powder, $[\alpha]^{25}_D$ −13.5 (*c* 0.58, MeOH); IR υ_{max} 3391, 2928, 1737, 1691, 1644, 1384, 1073, 1045, 645 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) data, see Table 2; ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1; ESIMS (in positive-ion mode) *m/z* 1736 $[M + Na + 1]^+$; HRESIMS (in positive-ion mode) *m/z* 1735.7981 $[M + Na]^+$ (calcd for $C_{80}H_{128}O_{39}Na$, 1735.7930).

Prosapogenin-10 (4): white, amorphous powder, $[\alpha]^{25}_D$ −21.5 (*c* 0.89, MeOH); IR υ_{max} 3405, 2932, 1735, 1693, 1645, 1378, 1073, 1045, 646 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data were in full agreement with reference data for this compound;[6a] ^{13}C NMR data, see Table 1; ESIMS (in positive-ion mode) *m/z* 1882 $[M + Na + 1]^+$.

Julibroside J$_{29}$ (5): white, amorphous powder, $[\alpha]^{25}_D$ −21.7 (*c* 0.87, MeOH); IR υ_{max} 3382, 2931, 1735, 1691, 1644, 1377, 1050, 640 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) data were in full agreement with reference data for this compound;[7d] ESIMS (in positive-ion mode) *m/z* 1923 $[M + Na + 1]^+$.

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides.[8] Compound **1** (20 mg) was dissolved in 2 M HCl−H_2O (30 mL) and heated at 95 °C for 10 h. After filtration of the reaction mixture, the precipitate was subjected to Si gel column chromatography eluted with $CHCl_3$−MeOH (25:1) to afford acacic acid lactone (**6**, 3 mg), while the filtrate was evaporated under vacuum. After addition of H_2O, the acidic solution was evaporated again to remove HCl. This procedure was repeated until a neutral solution was obtained, which was finally evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (1 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60 °C for 2 h and evaporated under N_2 stream and dried *in vacuo*. The residue was trimethylsilylated with *N*-trimethylsilylimidazole (0.2 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (2 mL each), and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, DB-5 (30 m × 0.25 mm × 0.25 μm); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature was maintained at 100 °C for 2 min and then raised to 280 °C at the rate of 10 °C/min, and final temperature was maintained for 5 min; carrier, N_2 gas. In 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 L-arabinose, L-rhamnose, D-fucose, D-quinovose, D-xylose, and D-glucose derivatives prepared in a similar way, which showed retention times of 17.79, 18.38, 18.52, 18.24, 17.65, and 19.55 min, respectively. The constituent sugars of compounds **2** and **3** were also identified by the same method.

Acacic acid lactone (6): white, amorphous powder; IR υ_{max} 3415, 2937, 1765, 1464, 1368, 1171, 1035, 995 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 0.83, 0.88, 0.94, 1.04, 1.06, 1.22, 1.34 (each, 3H, s, Me), 3.43 (1H, br s, H-3), 4.25 (1H, d, *J* = 5.5 Hz, H-21), 4.55 (1H, m, H-16), 5.38 (1H, br s, H-12); ^{13}C NMR (125 MHz, pyridine-d_5) δ_C 15.7 (C-25), 16.3 (C-26), 16.5 (C-24), 18.7 (C-6), 23.8 (C-11), 24.3 (C-30), 27.2 (C-22), 28.1 (C-2), 28.5 (C-23), 28.66 (C-29), 28.75 (C-27), 32.6 (C-7), 34.2 (C-20), 37.3 (C-10), 38.2 (C-15), 38.9 (C-1), 39.4 (C-4), 40.4 (C-8), 41.8 (C-18), 42.9 (C-19), 43.3 (C-14), 47.4 (C-9), 50.0 (C-17), 55.9 (C-5), 66.7 (C-16), 78.0 (C-3), 83.4 (C-21), 124.5 (C-12), 140.2 (C-13), 181.2 (C-28); ^{1}H and ^{13}C NMR data were in full agreement with reference data for this compound.[4d]

Alkaline Hydrolysis of 1. Compound **1** (52 mg) was hydrolyzed with 0.5 M NaOH (20 mL) and MeOH (4 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 HPLC using 30% CH_3CN−0.05% aqueous HOAc (4 mL/min) to afford compound **7** (8 mg, t_R = 30.5 min). The *n*-BuOH extract was separated by HPLC using 29% CH_3CN−0.05% aqueous HOAc (4 mL/min) to afford compound **8** (4 mg, t_R = 16.8 min).

Prosapogenin-1 (7): white, amorphous powder; IR υ_{max} 3379, 2942, 1686, 1447, 1372, 1162, 1039 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_H 0.89, 1.01 × 2, 1.34 × 2, 1.43, 1.95 (each, 3H, s, Me), 1.49 (3H, d, *J* = 6.5 Hz, H-Fuc-6), 4.94 (1H, d, *J* = 8.0 Hz, H-Xyl-1), 5.03 (1H, d, *J* = 7.5 Hz, H-Glc-1), 5.09 (1H, d, *J* = 6.0 Hz, H-Fuc-1), 5.30 (1H, br s, H-16), 5.63 (1H, br s, H-12); ^{1}H and ^{13}C NMR data were in full agreement with reference data for this compound;[6a] ^{13}C NMR (125 MHz, pyridine-d_5) data, see Table 1; ESIMS (in positive-ion mode) *m/z* 927 $[M - H]^-$.

Monoterpene glycoside (8): colorless syrup, $[\alpha]^{25}_D$ −23.1 (*c* 0.55, MeOH); IR υ_{max} 3394, 2979, 2932, 1696, 1650, 1413, 1069, 1007 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ_H 1.18 (3H, d, *J* = 6.0 Hz, H_3-Qui-6), 1.32 (3H, s, H_3-10), 1.66 (2H, dd, *J* = 9.5, 5.5 Hz, H_2-5), 2.32 (2H, m, H_2-4), 2.92 (1H, t, *J* = 9.0 Hz, H-Qui-4), 3.11 (1H, t, *J* = 8.5 Hz, H-Qui-2), 3.17 (1H, dd, *J* = 6.5, 3.5 Hz, H-Qui-5), 3.21 (1H, t, *J* = 9.0 Hz, H-Qui-3), 4.25 (2H, br d, H_2-9), 4.29 (1H, d, *J* = 7.5 Hz, H-Qui-1), 5.15 (1H, d, *J* = 11.0 Hz, Hb-8), 5.22 (1H, d, *J* = 17.5 Hz, Ha-8), 5.89 (1H, dd, *J* = 18.0, 11.0 Hz, H-7), 6.76 (1H, br t, H-3); ^{13}C NMR (100 MHz, CD_3OD) δ_C 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). The above carbon resonances were further confirmed by 2D NMR (600 MHz) experiments. However the only

carbon resonance of free carboxylic acid (C-1) was not detected in the ^{13}C NMR spectrum and could not be assigned by 2D NMR (600 MHz) experiments (see Supporting Information); ESIMS (in negative-ion mode) *m/z* 345 $[M - H]^-$, 691 $[2M - H]^-$ and in positive-ion mode *m/z* 347 $[M + H]^+$, 369 $[M + Na]^+$, 385 $[M + K]^+$, 715 $[2M + Na]^+$.

Cytotoxicity Assay. HCT-8 (human colon cancer cell line), Bel-7402 (human hepatoma cancer cell line), BGC-823 (human gastric cancer cell line), A549 (human lung epithelial cell line), and A2780 (human epithelial carcinoma cell line) were maintained in RPMI 1640 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.

HCT-8, Bel-7402, BGC-823, A549, and A2780 cells (1.5×10^3) were seeded in 96-well tissue culture plates. After 24 h, 100 μL of DMSO solution containing the test compounds was added to give the final concentration 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.[12] Two hundred microliters of 0.2% MTT in RPMI 1640 was added to every well, and the plate was further reincubated in 5% CO_2 air for 4 h at 37 °C. The plate was then centrifuged to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from every 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.[13] The hemolytic activity of **1**–**5** and **7** 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.[10]

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Supporting Information Available: IR, MS, and 1D and 2D NMR spectra of compounds **1**–**3** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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