JOURNAL OF NATURAL PRODUCTS

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Cytotoxic Triterpenoid Saponins from *Lysimachia clethroides*

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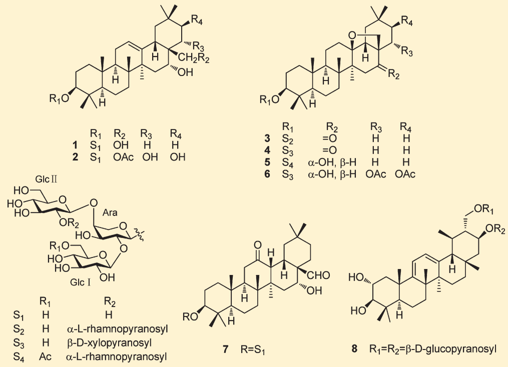
Supporting Information

ABSTRACT: Seven oleanane-type triterpenoid saponins, named clethroidosides A−G (**1**−**7**), an ursane-type triterpenoid saponin, clethroidoside H (**8**), and six known saponins were isolated from the aerial parts of *Lysimachia clethroides*. The structures of the saponins were elucidated on the basis of physical data analysis (1D and 2D NMR, HR-ESIMS) and chemical evidence. The cytotoxic activities of compounds **1**−**14** were evaluated against five human tumor cell lines (HT-29, HePG2, BGC-823, A549, and A375). Compounds **3**, **4**, **6**, and **11**−**13** exhibited moderate cytotoxic activity, with IC_{50} values of 0.75−2.62 μM, while compound **5** showed selective cytotoxic activity.



The genus *Lysimachia* (Primulaceae) consists of about 180 species, 132 of which can be found in China.[1] *Lysimachia clethroides* Duby. is a traditional Chinese folk medicine, distributed widely in many provinces of China. The aerial parts of this plant have been used for the treatment of throatache, edema, menoschesis, etc.[2] Previous phytochemical studies on *L. clethroides* have led to the isolation and identification of triterpenoid saponins, flavonoids, and several other components.[3] As part of an ongoing search for bioactive compounds from plants, we selected the 70% EtOH extract of the aerial parts of *L. clethroides* for investigation. Compounds isolated in the present study included eight new triterpenoid saponins, clethroidosides A−H (**1**−**8**), and six known saponins (**9**−**14**). In this paper, we report the isolation and structural elucidation of the new triterpenoid saponins, along with the evaluation of the cytotoxic activities of the 14 compounds against five human tumor cell lines.

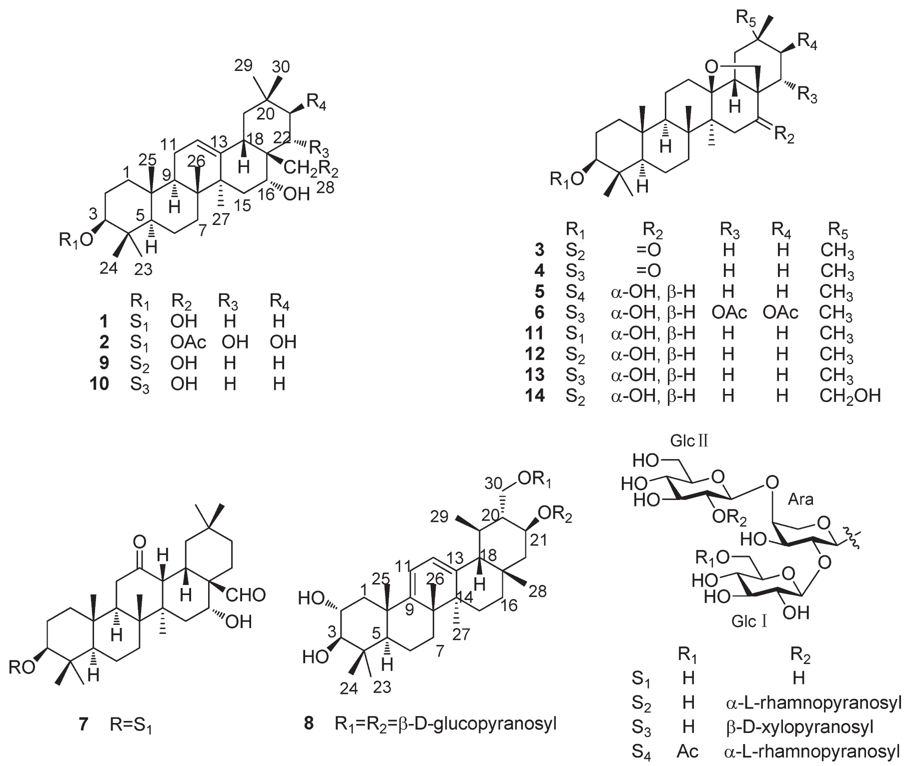
RESULTS AND DISCUSSION

The 70% EtOH extract from dried aerial parts of *L. clethroides* was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The *n*-BuOH-soluble extract was subjected to column chromatography and purified by preparative HPLC, to afford eight new triterpenoid saponins, clethroidosides A−H (**1**−**8**), as well as six known saponins (**9**−**14**).

Clethroidoside A (**1**) was obtained as an amorphous powder, $[\alpha]^{20}_{D}$ +2.6 (*c* 0.19, MeOH). Its molecular formula was determined as $C_{47}H_{78}O_{17}$ on the basis of the positive-ion HR-ESIMS (937.5152 $[M + Na]^+$, calcd 937.5131) and supported by the NMR spectroscopic data. The 1D NMR data (Tables 1 and 2) revealed the presence of seven tertiary methyl groups at δ_H 1.80 (H_3-27), 1.15 (H_3-23), 1.12 (H_3-30), 1.05 (H_3-29), 1.02 (H_3-24), 0.94 (H_3-26), and 0.89 (H_3-25), one olefinic proton at δ_H 5.38 (br s) with two typical olefinic carbon signals at δ_C 122.3 and 145.2, and a pair of oxygenated methylene protons at δ 3.60 and 3.73 (1H each, d, J = 13.1 Hz, H_2-28), indicative of an olean-12-ene skeleton. Two oxymethine proton signals assignable to H-3 and H-16 of the aglycone moiety were observed at δ 3.14 (dd, J = 11.6, 4.2 Hz) and 4.62 (br s), respectively. NOESY correlations between H-3 and H-5 (δ 0.71, d, J = 11.6 Hz) and between H-16 and H_2-28 indicated the α-orientation of H-3 and β-orientation of H-16. Thus, the aglycone was identified as 3β,16α,28-trihydroxyolean-12-ene (primulagenin A).[4] After acid hydrolysis, the sugar units were confirmed to be L-arabinose and D-glucose in a ratio of 1:2, which were identified by gas chromatographic (GC) analysis of their trimethylsilyl L-cysteine derivatives. The ^{1}H NMR spectrum showed three anomeric protons for three sugar moieties that resonated at δ_H 5.16 (d, J = 7.8 Hz, GlcII-H-1), 5.13 (d, J = 7.7 Hz, GlcI-H-1), and 4.92 (d, J = 5.1 Hz, Ara-H-1). The coupling constants confirmed the β-glycosidic linkages for two glucose units. The arabinose unit was determined to be the α-anomer on the basis of the $^3J_{H1,H2}$ value (5.1 Hz) and the correlations between H-1 and H-3 and between H-1 and H-5 in the NOESY experiment.[5] The arabinose was connected to C-3 of the aglycone, which was deduced from the HMBC correlation to be between Ara-H-1 (δ_H 4.92) and C-3 (δ_C 88.9). The sequence of the sugar chain at C-3 was further

Received: May 11, 2011
Published: September 19, 2011

Chart 1



determined by analysis of the HMBC and NOESY spectra. Thus, HMBC correlations were observed between GlcI-H-1 (δ_H 5.13) and Ara-C-2 (δ_C 80.7) and between GlcII-H-1 (δ_H 5.16) and Ara-C-4 (δ_C 77.1). NOESY correlations were observed between GlcI-H-1 (δ_H 5.13) and Ara-H-2 (δ 4.55) and between GlcII-H-1 (δ_H 5.16) and Ara-H-4 (δ 4.49). On the basis of the above data, the structure of clethroidoside A (**1**) was elucidated as 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl-3*β*,16*α*,28-trihydroxyolean-12-ene.

Clethroidoside B (**2**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −37.2 (*c* 0.11, MeOH). Its molecular formula, $C_{49}H_{80}O_{20}$, was determined from the positive-ion HR-ESIMS (1011.5140 $[M + Na]^+$, calcd 1011.5135) and ^{13}C NMR data. The structure of the sugar chain was determined to be the same as that of **1** by comparison of their ^1H and ^{13}C NMR data (Tables 1 and 2). The ^{13}C NMR of **2** showed 49 carbon signals, 30 of which were assigned to the aglycone, 17 to the sugar moieties, and the remaining two to an acetyl group. NMR analysis indicated that the aglycone of **2** contained two additional oxymethine signals resonating at δ_C 78.6 (δ_H 4.78) and 73.7 (δ_H 4.39) compared to **1**. The locations of the two oxymethines were assigned to C-21 and C-22 by HMBC correlations between H-21 (δ 4.78) and C-29 (δ 30.5) and C-30 (δ 19.4) together with COSY correlation between H-21 and H-22 (δ 4.39). On the basis of literature data, the NMR data of its aglycone were similar to those of barringtogenol C,[6] except for an additional acetyl group [δ_C 170.8, 20.7 and δ_H 1.96 (3H, s)]. HMBC correlation between H-28b (δ_H 4.24) and the carbonyl carbon at δ 170.8 indicated that the acetyl group was located at C-28. NOESY correlation between H-16 and H_2-28 implied that H-16 was *β*-orientated. The coupling constant (9.8 Hz) between H-21 and H-22 indicated their *trans*-diaxial orientation, which was confirmed by NOESY cross-peaks of H-22/H_3-30 and H-21/H-19*α*/H_3-27. Thus, the structure of clethroidoside B (**2**) was determined as 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl-28-acetyl-3*β*,16*α*,21*β*,22*α*,28-pentahydroxyolean-12-ene.

Clethroidoside C (**3**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −30.5 (*c* 0.45, MeOH). Its molecular formula was determined as $C_{53}H_{86}O_{21}$ on the basis of the positive-ion HR-ESIMS (1081.5600 $[M + Na]^+$, calcd 1081.5554) and ^{13}C NMR spectrum. The ^1H NMR data (Table 1) showed signals corresponding to seven tertiary methyls at δ 1.27 (H_3-26), 1.15 (H_3-23), 1.05 (H_3-27), 1.01 (H_3-24), 0.89 (H_3-29), 0.81 (H_3-30), and 0.80 (H_3-25). A quaternary carbon signal at δ_C 86.1 due to C-13 together with a pair of oxygenated methylene protons at δ 3.49 and 3.88 (1H each, d, J = 8.1 Hz, H_2-28) showed that the aglycone of **3** was based on a 13,28-epoxyoleanane skeleton. HMBC correlations between H_2-28 and H_2-15 [δ_H 2.80 and 1.95 (1H each, d, J = 16.0 Hz)] and the ketocarbonyl at δ_C 212.5 confirmed that the carbonyl group was located at C-16. Further, *α*-orientation of H-3 was deduced by the ROESY correlation between H-3 and H-5. The above analysis revealed that the aglycone of **3** was 13,28-epoxy-3*β*-hydroxyolean-16-one.[7] Acid hydrolysis of **3** afforded L-arabinose, D-glucose, and L-rhamnose in a ratio of 1:2:1 through GC analysis. Compound **3** has the same sugar sequence as that of 3-*O*-*β*-D-glucopyranosyl-(1→2)-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)]-*α*-L-arabinopyranosyl-13*β*,28-epoxy-3*β*,16*α*-dihydroxyoleanane-(**12**)[8] because of the identical ^1H and ^{13}C NMR data in the sugar moieties. This conclusion was confirmed by HMBC correlations between Ara-H-1 (δ_H 4.92) and C-3 (δ_C 89.0), between GlcI-H-1 (δ_H 5.36) and Ara-C-2 (δ_C 80.8), between GlcII-H-1 (δ_H 5.24) and Ara-C-4 (δ_C 74.6), and between Rha-H-1 (δ_H 6.39) and GlcII-C-2 (δ_C 77.3). Accordingly, the structure of clethroidoside C (**3**) was

Table 1. ^{1}H NMR Data of Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
1a	1.52, br d (13.5)	1.51, m	1.58, br d (13.0)	1.59, m	1.64, m	1.64, m	1.36, m	2.64 (overlapped)
1b	0.88 (overlapped)	0.88 (overlapped)	0.74, br d (13.0)	0.79, m	0.85, m	0.86, m	0.71, br d (13.1)	1.68, m
2a	1.99, dd (13.6, 3.6)	1.99, m	1.98 (overlapped)	1.98, m	2.01, m	2.00 (overlapped)	1.96, br d (14.0)	4.18 (overlapped)
2b	1.79 (overlapped)	1.79, m	1.82, m	1.78 (overlapped)	1.82, m	1.80, m	1.72, m	
3	3.14, dd (11.6, 4.2)	3.15, dd (11.3, 4.4)	3.11, dd (11.6, 4.0)	3.14, dd (11.8, 4.3)	3.19, dd (11.8, 4.4)	3.16, m	3.09, br d (11.1)	3.39, d (9.1)
5	0.71, d (11.6)	0.71, d (10.8)	0.58, d (9.5)	0.63, d (10.8)	0.70, dd (11.5, 1.3)	0.69, d (11.9)	0.64, d (12.4)	1.10 (overlapped)
6a	1.43 (overlapped)	1.44, m	1.37, m	1.37, br d (15.0)	1.45 (overlapped)	1.46, m	1.45, br d (14.8)	1.62 (overlapped)
6b	1.27, m	1.29 (overlapped)	1.29 (overlapped)	1.33 (overlapped)	1.40, m	1.40 (overlapped)	1.29, m	1.51, m
7a	1.56, m	1.55, m	1.29 (overlapped)	1.32 (overlapped)	1.54, m	1.53 (overlapped)	1.38, m	1.65 (overlapped)
7b	1.30 (overlapped)	1.28 (overlapped)	0.97, m	1.01, m	1.21, m	1.22 (overlapped)	1.21, m	1.24, m
9	1.71, m	1.71, m	1.07, m	1.11, dd (12.3, 2.0)	1.27, m	1.26, m	1.69, m	
11a	1.88, m	1.90, m	1.73, dd (13.1, 3.5)	1.74, dd (12.6, 4.2)	1.77, m	1.73, m	2.39, dd (16.0, 5.6)	5.78, d (5.6)
11b			1.43, m	1.44, m	1.45 (overlapped)	1.44 (overlapped)	2.24, dd (16.0, 14.2)	
12a	5.38, br s	5.48, br s	1.85, m	1.86, m	2.05, dd (14.1, 5.5)	2.00 (overlapped)		5.56, d (5.6)
12b			1.49, br d (12.6)	1.49, m	1.44 (overlapped)	1.44 (overlapped)		
13							2.63, br s	
15a	2.19, dd (13.7, 2.3)	1.94, m	2.80, d (16.0)	2.81, d (16.0)	2.23, dd (14.4, 5.1)	2.13, m	2.05, br d (14.1)	1.83, dd (13.1, 11.5)
15b	1.61, br d (13.7)	1.64, d (14.5)	1.95, d (16.0)	1.96, d (16.0)	1.46 (overlapped)	1.54, m	1.65, d (14.5)	0.97 (overlapped)
16a	4.62, br s	4.79, br s			4.18 (overlapped)	4.42 (overlapped)	4.77, br s	2.05, dd (15.4, 12.3)
16b								1.32, m
18	2.49, dd (13.7, 2.6)	2.87, dd (13.4, 2.6)	1.99 (overlapped)	2.00, m	1.67, m	1.90, m	2.97, m	1.63 (overlapped)
19a	2.73, dd (13.4, 13.1)	3.02, t (13.4)	1.40, m	1.42, m	2.76, dd (14.1, 12.3)	3.05, t (13.4)	2.32, d (8.9)	2.22, m
19b	1.31 (overlapped)	1.42, dd (13.4, 3.2)			1.32, m	1.39 (overlapped)		
20								1.28, m
21a	2.41, td (12.5, 4.7)	4.78, d (9.8)	1.79, m	1.80 (overlapped)	2.54, td (13.5, 4.8)	6.55, d (9.9)	2.37, m	4.51 (overlapped)
21b	1.43 (overlapped)		1.18, m	1.19 (overlapped)	1.23 (overlapped)		1.32 (overlapped)	
22a	2.28, m	4.39 (overlapped)	2.24, br d (12.8)	2.25, m	1.88, dd (13.8, 3.6)	5.35, d (9.9)	1.74, m	2.64 (overlapped)
22b	2.22, m		1.16, m	1.17 (overlapped)	1.58, m		1.32 (overlapped)	1.74, dd (12.6, 12.1)
23	1.15, s	1.16, s	1.15, s	1.21, s	1.22, s	1.22, s	1.15, s	1.29, s
24	1.02, s	1.02, s	1.01, s	1.07, s	1.04, s	1.10, s	0.99, s	1.12, s
25	0.89, s	0.88, s	0.80, s	0.809, s	0.87, s	0.84, s	0.75, s	1.31, s
26	0.94, s	1.00, s	1.27, s	1.28, s	1.34, s	1.29, s	0.90, s	1.18, s
27	1.80, s	1.81, s	1.05, s	1.06, s	1.52, s	1.53, s	1.59, s	0.95, s
28a	3.73, d (13.1)	4.39 (overlapped)	3.88, d (8.1)	3.89, d (8.2)	3.59, d (7.4)	3.84, d (7.7)	9.44, s	0.99, s
28b	3.60, d (13.1)	4.24, d (11.0)	3.49, d (8.1)	3.49, d (8.2)	3.31, d (7.4)	3.61, d (7.7)		
29	1.05, s	1.33, s	0.89, s	0.89, s	1.06, s	1.11, s	1.03, s	1.08, d (6.4)
30a	1.12, s	1.38, s	0.81, s	0.813, s	0.96, s	1.10, s	1.16, s	4.84, m
30b								4.39 (overlapped)
OAc		1.96, s				2.08, s (C-21)		

dx.doi.org/10.1021/np2004038 |J. Nat. Prod. 2011, 74, 2128–2136

Table 1. Continued

position	1	2	3	4	5	6	7	8
OAc						1.94, s (C-22)		
	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	
1	4.92, d (5.1)	4.92, d (4.7)	4.92, br s	4.79, d (5.8)	4.89, d (4.9)	4.79, d (4.6)	4.90[b]	
2	4.55, dd (7.0, 5.5)	4.55, m	4.57 (overlapped)	4.57 (overlapped)	4.55, dd (7.2, 5.5)	4.57 (overlapped)	4.54, dd (7.2, 6.0)	
3	4.45, m	4.44, m	4.47, m	4.28, m	4.44 (overlapped)	4.27 (overlapped)	4.43, m	
4	4.49 (overlapped)	4.49 (overlapped)	4.58 (overlapped)	4.24 (overlapped)	4.49, m	4.23 (overlapped)	4.49 (overlapped)	
5a	4.40 (overlapped)	4.40 (overlapped)	4.39, m	4.61, dd (12.1, 4.1)	4.46, m	4.62, br d (12.0)	4.37 (overlapped)	
5b	3.81, br d (11.7)	3.81, d (11.3)	3.78, m	3.65, br d (12.1)	3.75, m	3.65, br d (12.0)	3.79, br d (12.4)	
	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc at C-21
1	5.13, d (7.7)	5.12, d (7.3)	5.36, d (7.5)	5.48, d (7.7)	5.40, d (7.6)	5.50, d (7.3)	5.12, d (7.7)	5.21, d (7.6)
2	4.05, m	4.05, m	4.07 (overlapped)	4.08, t (8.0)	4.06, dd (8.5, 8.0)	4.09, m	4.05, m	4.05 (overlapped)
3	4.15, dd (9.3, 9.0)	4.15, dd (9.2, 8.8)	4.29 (overlapped)	4.25 (overlapped)	4.31, dd (9.2, 9.0)	4.26 (overlapped)	4.15, dd (9.5, 8.9)	4.19, m
4	4.28, dd (9.5, 9.1)	4.28, t (9.1)	4.22, m	4.24 (overlapped)	4.09, dd (9.7, 8.5)	4.25 (overlapped)	4.29, t (9.2)	4.17, m
5	3.77, m	3.77, m	4.06 (overlapped)	4.01 (overlapped)	4.19 (overlapped)	4.02 (overlapped)	3.76, m	4.15, m
6a	4.40 (overlapped)	4.40 (overlapped)	4.49, m	4.56 (overlapped)	4.92, dd (11.9, 1.8)	4.56 (overlapped)	4.40 (overlapped)	4.82, m
6b	4.39 (overlapped)	4.39 (overlapped)	4.37, m	4.42 (overlapped)	4.77, dd (11.7, 5.0)	4.42, m	4.40 (overlapped)	4.06 (overlapped)
OAc					2.02, s			
	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc at C-30
1	5.16, d (7.8)	5.17, d (7.4)	5.24, d (7.2)	5.01, d (7.8)	5.14, d (7.7)	5.01, d (7.8)	5.19, d (7.9)	5.11, d (7.6)
2	4.03, m	4.03, m	4.27 (overlapped)	3.94, t (8.3)	4.26 (overlapped)	3.94, t (7.8)	4.04, m	4.08, m
3	4.19, dd (9.2, 8.8)	4.19, t (8.8)	4.19, m	4.20 (overlapped)	4.18 (overlapped)	4.21 (overlapped)	4.20, t (8.7)	4.25 (overlapped)
4	4.24, t (9.1)	4.24 (overlapped)	4.11, t (8.9)	4.21 (overlapped)	4.13, t (9.3)	4.22 (overlapped)	4.25, t (9.1)	4.26 (overlapped)
5	3.87, m	3.87, m	3.79, m	3.79, m	3.77, m	3.79, m	3.87, m	3.92, m
6a	4.48, dd (11.8, 1.2)	4.48, d (11.6)	4.45, m	4.43 (overlapped)	4.43, m	4.44, m	4.49 (overlapped)	4.51 (overlapped)
6b	4.35, dd (11.8, 4.9)	4.36, m	4.28 (overlapped)	4.30, m	4.28 (overlapped)	4.30, m	4.37 (overlapped)	4.39 (overlapped)
			Rha	Xyl	Rha	Xyl		
1			6.39, br s	4.92[b]	6.38, br s	4.92[b]		
2			4.71, br s	4.02 (overlapped)	4.71, br s	4.03 (overlapped)		
3			4.67, br d (9.1)	4.03 (overlapped)	4.68, dd (9.4, 3.1)	4.03 (overlapped)		
4			4.27 (overlapped)	4.13, m	4.26 (overlapped)	4.13, m		
5a			5.03, m	4.55 (overlapped)	5.02, m	4.55 (overlapped)		
5b				3.70, t (10.8)		3.71, dd (11.1, 10.4)		
6			1.79, d (6.0)		1.79, d (6.2)			

[a] ^{1}H NMR data (δ) were measured in pyridine-d_5 at 500 MHz for **1**, **3**, and **6**−**8** and at 600 MHz for **2**, **4**, and **5**. Coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, COSY, TOCSY, NOESY(ROESY), HSQC(HMQC), and HMBC experiments. [b] Signal overlapped by solvent peaks.

dx.doi.org/10.1021/np2004038 |J. Nat. Prod. 2011, 74, 2128–2136

Table 2. ^{13}C NMR Data of Compounds 1−8[a]

position	1	2	3	4	5	6	7	8
1	38.9, CH$_2$	38.9, CH$_2$	39.0, CH$_2$	39.1, CH$_2$	39.2, CH$_2$	39.2, CH$_2$	38.1, CH$_2$	46.8, CH$_2$
2	26.4, CH$_2$	26.4, CH$_2$	26.5, CH$_2$	26.6, CH$_2$	26.6, CH$_2$	26.6, CH$_2$	26.3, CH$_2$	69.1, CH
3	88.9, CH	88.8, CH	89.0, CH	88.9, CH	89.2, CH	89.0, CH	88.5, CH	83.5, CH
4	39.5, C	39.5, C	39.6, C	39.7, C	39.7, C	39.8, C	39.5, C	39.7, C
5	55.8, CH	55.8, CH	55.5, CH	55.6, CH	55.7, CH	55.7, CH	55.4, CH	51.8, CH
6	18.5, CH$_2$	18.4, CH$_2$	17.7, CH$_2$	17.8, CH$_2$	18.0, CH$_2$	18.0, CH$_2$	18.4, CH$_2$	18.7, CH$_2$
7	33.2, CH$_2$	33.1, CH$_2$	33.8, CH$_2$	33.8, CH$_2$	34.5, CH$_2$	34.4, CH$_2$	32.2, CH$_2$	32.2, CH$_2$
8	40.0, C	40.0, C	42.9, C	43.0, C	42.5, C	42.6, C	41.6, C	43.4, C
9	47.1, CH	47.0, CH	50.1, CH	50.2, CH	50.5, CH	50.4, CH	48.8, CH	155.0, C
10	36.9, C	36.8, C	36.7, C	36.8, C	36.9, C	36.9, C	36.7, C	40.2, C
11	23.9, CH$_2$	23.9, CH$_2$	18.88, CH$_2$	18.9, CH$_2$	19.3, CH$_2$	19.2, CH$_2$	38.7, CH$_2$	115.6, CH
12	122.3, CH	123.7[b], CH	31.8, CH$_2$	31.8, CH$_2$	32.9, CH$_2$	32.8, CH$_2$	210.8, C	123.8[b], CH
13	145.2, C	143.1, C	86.1, C	86.2, C	86.4, C	86.1, C	52.8, CH	141.5, C
14	42.0, C	41.9, C	49.8, C	49.9, C	44.60, C	44.9, C	41.8, C	40.9, C
15	34.8, CH$_2$	34.6, CH$_2$	45.7, CH$_2$	45.8, CH$_2$	37.0, CH$_2$	36.1, CH$_2$	35.7, CH$_2$	26.4, CH$_2$
16	74.2, CH	68.1, CH	212.5, C	212.5, C	77.2, CH	69.3, CH	71.6, CH	29.4, CH$_2$
17	41.0, C	46.5, C	56.1, C	56.2, C	44.64, C	51.1, C	52.4, C	35.3, C
18	42.5, CH	40.8, CH	54.6, CH	54.6, CH	51.6, CH	50.1, CH	31.2, CH	57.0, CH
19	48.4, CH$_2$	47.8, CH$_2$	40.0, CH$_2$	40.0, CH$_2$	39.0, CH$_2$	38.0, CH$_2$	36.2, CH$_2$	32.1, CH
20	31.3, C	36.4, C	31.8, C	31.8, C	31.9, C	37.2, C	30.9, C	53.4, CH
21	37.2, CH$_2$	78.6, CH	35.6, CH$_2$	35.7, CH$_2$	36.8, CH$_2$	78.43, CH	35.8, CH$_2$	76.4, CH
22	30.6, CH$_2$	73.7, CH	25.0, CH$_2$	25.1, CH$_2$	31.9, CH$_2$	78.1, CH	27.3, CH$_2$	49.3, CH$_2$
23	28.2, CH$_3$	28.1, CH$_3$	28.0, CH$_3$	28.1, CH$_3$	28.0, CH$_3$	28.1, CH$_3$	28.0, CH$_3$	29.5, CH$_3$
24	16.8, CH$_3$	16.8, CH$_3$	16.4, CH$_3$	16.6, CH$_3$	16.5, CH$_3$	16.7, CH$_3$	16.5, CH$_3$	17.7, CH$_3$
25	15.8, CH$_3$	15.7, CH$_3$	16.1, CH$_3$	16.1, CH$_3$	16.4, CH$_3$	16.4, CH$_3$	15.3, CH$_3$	26.7, CH$_3$
26	17.0, CH$_3$	17.1, CH$_3$	18.8, CH$_3$	18.8, CH$_3$	18.6, CH$_3$	18.5, CH$_3$	15.9, CH$_3$	22.3, CH$_3$
27	27.3, CH$_3$	27.4, CH$_3$	21.8, CH$_3$	21.8, CH$_3$	19.6, CH$_3$	19.6, CH$_3$	20.8, CH$_3$	17.7, CH$_3$
28	70.2, CH$_2$	67.0, CH$_2$	75.1, CH$_2$	75.1, CH$_2$	78.0, CH$_2$	75.9, CH$_2$	205.3, CH	28.5, CH$_3$
29	33.5, CH$_3$	30.5, CH$_3$	33.4, CH$_3$	33.4, CH$_3$	33.8, CH$_3$	29.8, CH$_3$	33.7, CH$_3$	17.4, CH$_3$
30	24.8, CH$_3$	19.4, CH$_3$	23.5, CH$_3$	23.5, CH$_3$	24.8, CH$_3$	20.4, CH$_3$	23.8, CH$_3$	70.4, CH$_2$
OAc		170.8, C				170.7, C (C-21)		
		20.7, CH$_3$				20.9, CH$_3$		
OAc						170.6, C (C-22)		
						20.6, CH$_3$		
	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	Ara at C-3	
1	104.3, CH	104.4, CH	104.5, CH	104.7, CH	104.4, CH	104.7, CH	104.4, CH	
2	80.7, CH	80.8, CH	80.8, CH	79.8, CH	80.9, CH	79.7, CH	81.0, CH	
3	72.4, CH	72.4, CH	72.4, CH	73.2, CH	72.1, CH	73.3, CH	72.5, CH	
4	77.1, CH	77.2, CH	74.6, CH	78.5, CH	75.5, CH	78.6, CH	77.1, CH	
5	63.5, CH$_2$	63.6, CH$_2$	63.7, CH$_2$	64.2, CH$_2$	64.8, CH$_2$	64.2, CH$_2$	63.6, CH$_2$	
	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc-I	Glc at C-21
1	105.8, CH	105.8, CH	105.4, CH	105.0, CH	105.1, CH	104.9, CH	105.9, CH	105.5, CH
2	76.1, CH	76.2, CH	76.4, CH	76.3, CH	76.3, CH	76.3, CH	76.2, CH	75.8, CH
3	78.2, CH	78.2, CH	78.0, CH	78.4, CH	77.9, CH	78.39, CH	78.2, CH	78.5, CH
4	71.6, CH	71.6, CH	71.8, CH	71.8, CH	71.2, CH	71.9, CH	71.6, CH	71.8, CH
5	78.1, CH	78.1, CH	78.1, CH	78.0, CH	75.0, CH	78.0, CH	78.1, CH	77.1, CH
6	62.6, CH$_2$	62.6, CH$_2$	62.9, CH$_2$	63.0, CH$_2$	64.7, CH$_2$	63.0, CH$_2$	62.6, CH$_2$	58.9, CH$_2$
OAc					170.9, C			
					20.9, CH$_3$			
	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc-II	Glc at C-30
1	105.6, CH	105.6, CH	103.1, CH	104.2, CH	103.6, CH	104.2, CH	105.6, CH	105.4, CH
2	75.7, CH	75.7, CH	77.3, CH	85.5, CH	77.4, CH	85.5, CH	75.7, CH	75.3, CH
3	78.4, CH	78.4, CH	79.5, CH	77.6, CH	79.6, CH	77.6, CH	78.4, CH	78.4, CH
4	71.4, CH	71.4, CH	71.9, CH	71.1, CH	71.8, CH	71.1, CH	71.4, CH	71.6, CH

Table 2. Continued

position	1	2	3	4	5	6	7	8
5	78.7, CH	78.7, CH	78.4, CH	78.3, CH	78.4, CH	78.3, CH	78.8, CH	78.6, CH
6	62.6, CH_2	62.6, CH_2	62.6, CH_2	62.4, CH_2	62.6, CH_2	62.4, CH_2	62.6, CH_2	62.7, CH_2
			Rha	Ara	Rha	Ara		
1			101.5, CH	107.7, CH	101.7, CH	107.7, CH		
2			72.4, CH	76.2, CH	72.5, CH	76.2, CH		
3			72.7, CH	77.9, CH	72.7, CH	77.9, CH		
4			74.8, CH	70.7, CH	74.6, CH	70.7, CH		
5			69.4, CH	67.5, CH_2	69.6, CH	67.5, CH_2		
6			18.93, CH_3		18.9, CH_3			

[a] ^{13}C NMR data (δ) were measured in pyridine-d_5 at 125 MHz for **1**, **3**, and **6**−**8** and at 150 MHz for **2**, **4**, and **5**. The assignments were based on DEPT, COSY, TOCSY, NOESY(ROESY), HSQC(HMQC), and HMBC experiments. [b] Signal overlapped by solvent peaks.

assigned as 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-3β-hydroxyolean-16-one.

Clethroidoside D (**4**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −55.4 (*c* 0.07, MeOH). Its molecular formula was determined as $C_{52}H_{84}O_{21}$ on the basis of the positive-ion HR-ESIMS (1067.5421 $[M + Na]^+$, calcd 1067.5397). Acid hydrolysis of **4** afforded L-arabinose, D-glucose, and D-xylose in a ratio of 1:2:1 through GC analysis. Its ^{1}H and ^{13}C NMR data were similar to those of **3** except that the α-L-rhamnose moiety in **3** was replaced by a β-D-xylose unit in **4**. This conclusion was confirmed by the HMBC correlation between Xyl-H-1 (δ_H 4.92) and GlcII-C-2 (δ_C 85.5). Thus, the structure of clethroidoside D (**4**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-3β-hydroxyolean-16-one.

Clethroidoside E (**5**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −23.0 (*c* 0.11, MeOH). Its molecular formula was determined as $C_{55}H_{90}O_{22}$ on the basis of the positive-ion HR-ESIMS (1125.5805 $[M + Na]^+$, calcd 1125.5816). Comparison of the ^{1}H and ^{13}C NMR data of **5** (Tables 1 and 2) with those of **3** indicated that the ketocarbonyl in **3** (δ_C 212.5) was replaced by an oxymethine group in **5** [δ_C 77.2 (δ_H 4.18)]. NOESY correlation between H-16 and H-28a (δ_H 3.59) implied that H-16 was β-orientated. Therefore, the aglycone of **5** was established as 13,28-epoxy-3β,16α-dihydroxyoleanane (protoprimulagenin A).[9] Acid hydrolysis afforded L-arabinose, D-glucose, and L-rhamnose in a ratio of 1:2:1 through GC analysis. For the sugar moieties of **5**, the ^{13}C NMR signals were similar to those of **3** except that GlcI-C-6 exhibited a downfield shift by 1.8 ppm and additional acetyl carbon signals at δ 20.9 and 170.9, which indicated the presence of an acetoxy group at GlcI-C-6. Moreover, in the HMBC experiment, a cross-peak observed between GlcI-H-6b (δ_H 4.77) and the carbon at δ 170.9 supported this conclusion. On the basis of the above analysis, the structure of clethroidoside E (**5**) was elucidated as 3-*O*-β-D-6-acetylglucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-3β,16α-dihydroxyoleanane.

Clethroidoside F (**6**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −12.5 (*c* 0.09, MeOH). Its molecular formula was determined as $C_{56}H_{90}O_{25}$ on the basis of the positive-ion HR-ESIMS (1185.5705 $[M + Na]^+$, calcd 1185.5663) and ^{13}C NMR spectrum. Comparison of the ^{1}H and ^{13}C NMR data of **6** (Tables 1 and 2) with those of **4** revealed that the signals of their sugar moieties were superimposable, suggesting the sugar structure at C-3 was the same as that in **4**. NMR analysis indicated that the aglycone of **6** contained two additional oxymethine signals resonating at δ_C 78.43 (δ_H 6.55) and δ_C 78.1 (δ_H 5.35) compared to **5**. The ^{13}C NMR data of **6** showed 56 carbons, of which 30 were assigned to the aglycone part, 22 to the sugar moieties, and the remaining four to two acetyl groups. In the HMBC spectrum, cross-peaks between H-21 (δ 6.55) and the carbonyl carbon (δ_C 170.7) of one acetyl group, C-29 (δ 29.8), and C-30 (δ 20.4) and between H-22 (δ 5.33) and the carbonyl carbon (δ_C 170.6) of the other acetyl group, C-16 (δ 69.3), and C-28 (δ 75.9) were observed. Thus, the two acetyl groups must be linked to C-21 and C-22. The coupling constant (9.9 Hz) between H-21 and H-22 indicated their *trans*-diaxial orientation, which was confirmed by NOESY cross-peaks of H-22/H_2-28/H_3-30 and H-21/H-19α/H_3-27. Consequently, the structure of clethroidoside F (**6**) was concluded to be 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-21,22-diacetyl-3β,16α,21β,22α-tetrahydroxyoleanane.

Table 3. Cytotoxic Activity of Compounds 1−14 by the MTT Method

	IC$_{50}$ (μM)[a]				
sample	HT-29	HePG2	BGC-823	A549	A375
3	1.99	1.48	1.93	2.62	1.14
4	1.98	1.73	1.36	2.36	1.91
5	1.40	6.26	8.05	>10	>10
6	2.04	1.26	1.95	2.28	2.19
11	1.81	0.75	1.14	2.47	1.71
12	1.96	0.99	2.07	1.95	2.13
13	1.96	1.00	1.75	1.91	1.94
paclitaxel[b]	3.94×10^{-3}	4.4×10^{-3}	3.29×10^{-3}	4.49×10^{-2}	4.9×10^{-3}

[a] Compounds **1**, **2**, **7**−**10**, and **14** were inactive against all cell lines tested (IC$_{50}$ > 10 μM). [b] Positive control.

Clethroidoside G (**7**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ −40.3 (*c* 0.04, MeOH). Its molecular formula was determined as $C_{47}H_{76}O_{18}$ on the basis of the positive-ion HR-ESIMS (951.4907 $[M + Na]^+$, calcd 951.4924). Acid hydrolysis of **7** yielded L-arabinose and D-glucose in a ratio of 1:2 through GC analysis. The structure of the sugar chain was determined to be the same as that of **1** by comparison of their ^{1}H and ^{13}C NMR data (Tables 1 and 2). The ^{1}H and ^{13}C NMR data of the aglycone moiety in **7** were also similar to those of **1**, except for the absence of the signals due to the double bond at C-12 and the hydroxymethylene group at C-28 in **1**, which were replaced by a ketocarbonyl (δ_C 210.8) and a formyl group (δ_H 9.44, δ_C 205.3), respectively. These assignments could be confirmed by the HMBC correlations between H-13 (δ_H 2.63) and H_2-11 (δ_H 2.39, 2.24) and C-12 (δ_C 210.8) and between H-28 (δ_H 9.44) and C-17 (δ_C 52.4). NOESY correlations between H-13 and H-18 (δ_H 2.97) and H_3-26 (δ_H 0.90) implied that H-13 was β-orientated. Thus, the structure of clethroidoside G (**7**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-3β,16α-dihydroxy-12-oxoolean-30-al.

Clethroidoside H (**8**) was obtained as an amorphous powder, $[\alpha]^{20}_D$ +30.8 (*c* 0.07, MeOH). Its molecular formula was determined as $C_{42}H_{68}O_{14}$ on the basis of the positive-ion HR-ESIMS (819.4515 $[M + Na]^+$, calcd 819.4501). The ^{13}C NMR spectrum displayed 42 carbons, of which 30 were assigned to the aglycone part and 12 to the sugar moieties. The ^1H NMR spectrum (Table 1) exhibited signals due to six tertiary methyls at δ_H 1.31 (H_3-25), 1.29 (H_3-23), 1.18 (H_3-26), 1.12 (H_3-24), 0.99 (H_3-28), and 0.95 (H_3-27), a secondary methyl at δ_H 1.08 (d, *J* = 6.4 Hz, H_3-29), and an oxygenated methylene at δ_H 4.39 and 4.84, which suggested an ursane-type skeleton. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed two olefinic protons at δ_H 5.56 (1H, d, *J* = 5.6 Hz) and 5.78 (1H, d, *J* = 5.6 Hz), with four typical olefinic carbon signals at δ_C 115.6, 123.8, 141.5, and 155.0. The coupling constant (5.6 Hz) of the two double bonds suggested that they comprised as a homoannular diene,[10] which was confirmed by the absorption maximum at 279 nm in the UV spectrum. After appropriate 2D NMR experiments, the aglycone of **8** was identified as 2α,3β,21β,30-tetrahydroxyurs-9(11), 12-diene. Acid hydrolysis of **8** yielded D-glucose. The ^1H NMR spectrum showed two anomeric protons resonating at δ_H 5.21 (d, *J* = 7.6 Hz) and 5.11 (d, *J* = 7.6 Hz), which were correlated with carbons at δ_C 105.5 and 105.4 from the HMQC spectrum. The coupling constants confirmed the β-glycosidic linkages for two glucose units. In the HMBC spectrum, correlations between δ_H 5.21 and C-21 (δ_C 76.4) and between δ_H 5.11 and C-30 (δ_C 70.4) indicated that **8** was a bidesmosidic glycoside, in which the two glucose units resided at C-21 and C-30. ROESY correlations between δ_H 5.21 and H-21 (δ_H 4.51) and between δ_H 5.11 and H_2-30 (δ_H 4.84, 4.39) confirmed this conclusion. The relative configuration of **8** was determined by the coupling constants and the ROESY spectrum. The coupling constant (9.1 Hz) between H-2 and H-3 indicated their *trans*-diaxial orientation, which was confirmed by key ROESY cross-peaks of H-2/H_3-25 and H-3/H_3-23/H-5. ROESY correlation between H-21 (δ_H 4.51) and H-19 (δ_H 2.22) indicated the α-orientation of H-21. On the basis of the above data, the structure of clethroidoside H (**8**) was elucidated as 21,30-di-*O*-β-D-glucopyranosyl-2α,3β,21β,30-tetrahydroxyurs-9(11),12-diene.

The known saponins were identified as 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-3β,16α,28-trihydroxyolean-12-ene (**9**),[11] candidoside (**10**),[12] ardisianoside E (**11**),[5] 3-*O*-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranosyl-13β,28-epoxy-3β,16α-dihydroxyoleanane (**12**),[8] lysikokianoside 1 (**13**),[13] and ardisimamiloside E (**14**)[3d,14] by NMR analysis and comparison with literature data.

The structures of the triterpenoid saponins could be classified into four types: olean-12-ene (**1**, **2**, **9**, **10**); 13β,28-epoxyoleanane (**3**−**6**, **11**−**14**); 12-oxoolean-30-al (**7**), and ursane (**8**). The first two types are common in the genus *Lysimachia*.[15] The third type of 12-oxoolean-30-al is rare in natural products. Compound **8** is the first triterpenoid saponin of ursane type isolated from this genus.

Saponins **1**−**14** were evaluated for their cytotoxic activities against five human cancer cell lines (HT-29, HePG2, BGC-823, A549, and A375) (Table 3) with paclitaxel as a positive control. Compounds **3**, **4**, **6**, and **11**−**13** exhibited moderate cytotoxicity against all the tested human cancer cell lines, whereas compound **5** showed selective cytotoxicity against HT-29, HePG2, and BGC-823 cell lines. Compounds **1**, **2**, and **7**−**10** were inactive (>10 μM). Among the 13β,28-epoxyoleanane-type triterpenoid saponins, compounds **3**−**6** and **11**−**13** were active, but compound **14** was inactive (>10 μM), which suggested that the hydroxy group at C-29 decreased the resultant cytotoxic activity.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter, and UV spectra with a JASCO V-650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 spectrometer by an FT-IR microscope transmission method. NMR measurements were performed on VNS-600, INOVA-500, and Bruker AV500-III spectrometers in pyridine-d_5. HR-ESIMS were obtained using an Agilent 1100 series LC/MSD ion trap mass spectrometer. Preparative HPLC was performed on a Lumtech instrument equipped with an Alltech 500 ELSD detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (GE), and ODS (50 μm, YMC, Japan) were used for column chromatography. TLC was carried out with GF254 plates (Qingdao Marine Chemical Factory). Spots were visualized by spraying with 10% H_2SO_4 acid in EtOH followed by heating. GC was conducted on an Agilent 7890A instrument.

Plant Material. *L. clethroides* was collected in Mount Lushan, Jiangxi Province, People's Republic of China, in September 2009, and was identified by Professor Ce-Ming Tan (Jiujiang Institute of Forestry). A voucher specimen (No. 21787) was deposited at the Herbarium of the Department of Medicinal Plants, the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. Air-dried and powdered aerial parts of *L. clethroides* (10 kg) were exhaustively extracted with 70% aqueous EtOH (3 × 50 L) at reflux. The combined extracts were concentrated under reduced pressure to dryness. The residue was suspended in H_2O and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The *n*-BuOH-soluble residue (500 g) was subjected to silica gel CC and eluted with a gradient of CH_2Cl_2−MeOH−H_2O (5:1:0.1; 3:1:0.1; 2:1:0.1; 1:1:0.1; 100% MeOH). The fractions were combined according to TLC profiles into five main fractions. Fraction D (66 g) was subjected to repeated silica gel CC ($CHCl_3$−MeOH−H_2O, 3:1:0.1; 2:1:0.2; 1:1:0.25; 100% MeOH) to afford seven subfractions. The saponin-containing fraction D-e (15 g) was further separated by MPLC (ODS, 50 μm, YMC), eluted with 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 100% MeOH−H_2O, to afford 30 subfractions. Fraction D-e-17 (110 mg) was purified by preparative HPLC using 62% MeOH−H_2O (5 mL/min) as mobile phase to yield compound **2** (6 mg, t_R 64.5 min). Fraction D-e-20 (130 mg) was subjected to preparative HPLC using 64% MeOH−H_2O (5 mL/min) to give compounds **8** (5 mg, t_R 56.3 min), **7** (10 mg, t_R 65.8 min), and **6** (6 mg, t_R 69.9 min). Fraction D-e-21 (150 mg) was purified by preparative HPLC using 72% MeOH−H_2O (5 mL/min) to give compound **1** (50 mg, t_R 42.3 min). Fraction D-e-24 (120 mg) was also purified by preparative HPLC using 79% MeOH−H_2O (5 mL/min) to afford compounds **11** (10 mg, t_R 53.4 min), **5** (8 mg, t_R 57.7 min), **4** (5 mg, t_R 71.0 min), and **3** (30 mg,

t_R 79.6 min). Fraction D-f (30 g) was separated on Sephadex LH-20 eluted with MeOH to afford three subfractions. The saponin-enriched fraction D-f-a (3.66 g) was further separated by repeated MPLC and preparative HPLC purification with aqueous MeOH to afford compounds **14** (8 mg, t_R 60.2 min, 64% MeOH), **10** (80 mg, t_R 60.5 min, 69% MeOH), **9** (210 mg, t_R 68.5 min, 69% MeOH), **13** (150 mg, t_R 57.7 min, 75% MeOH), and **12** (500 mg, t_R 64.2 min, 75% MeOH).

*Clethroidoside A (**1**):* amorphous powder, $[\alpha]^{20}_D$ +2.6 (*c* 0.19, MeOH); IR ν_{max} 3423, 2945, 1650, 1445, 1372, 1163, 1065 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 937 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 937.5152 [M + Na]$^+$ (calcd for $C_{47}H_{78}O_{17}Na$, 937.5131).

*Clethroidoside B (**2**):* amorphous powder, $[\alpha]^{20}_D$ −37.2 (*c* 0.11, MeOH); IR ν_{max} 3406, 2926, 1720, 1649, 1374, 1255, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 1011 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 1011.5140 [M + Na]$^+$ (calcd for $C_{49}H_{80}O_{20}Na$, 1011.5135).

*Clethroidoside C (**3**):* amorphous powder, $[\alpha]^{20}_D$ −30.5 (*c* 0.45, MeOH); IR ν_{max} 3346, 2946, 1701, 1454, 1388, 1365, 1041 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 1081 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 1081.5600 [M + Na]$^+$ (calcd for $C_{53}H_{86}O_{21}Na$, 1081.5554).

*Clethroidoside D (**4**):* amorphous powder, $[\alpha]^{20}_D$ −55.4 (*c* 0.07, MeOH); IR ν_{max} 3374, 2943, 1702, 1448, 1387, 1364, 1075, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 1067 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 1067.5421 [M + Na]$^+$ (calcd for $C_{52}H_{84}O_{21}Na$, 1067.5397).

*Clethroidoside E (**5**):* amorphous powder, $[\alpha]^{20}_D$ −23.0 (*c* 0.11, MeOH); IR ν_{max} 3386, 2949, 1723, 1449, 1362, 1054 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 1125 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 1125.5805 [M + Na]$^+$ (calcd for $C_{55}H_{90}O_{22}Na$, 1125.5816).

*Clethroidoside F (**6**):* amorphous powder, $[\alpha]^{20}_D$ −12.5 (*c* 0.09, MeOH); IR ν_{max} 3416, 2927, 1727, 1366, 1241, 1041 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 1185 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 1185.5705 [M + Na]$^+$ (calcd for $C_{56}H_{90}O_{25}Na$, 1185.5663).

*Clethroidoside G (**7**):* amorphous powder, $[\alpha]^{20}_D$ −40.3 (*c* 0.04, MeOH); IR ν_{max} 3384, 2926, 1715, 1686, 1365, 1076 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 951 [M + Na]$^+$; positive-ion HR-ESIMS *m/z* 951.4907 [M + Na]$^+$ (calcd for $C_{47}H_{76}O_{18}Na$, 951.4924).

*Clethroidoside H (**8**):* amorphous powder, $[\alpha]^{20}_D$ +30.8 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.57), 279 (4.30) nm; IR ν_{max} 3355, 2952, 2920, 1673, 1458, 1376 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; positive-ion ESIMS *m/z* 797 [M + H]$^+$; positive-ion HR-ESIMS *m/z* 819.4515 [M + Na]$^+$ (calcd for $C_{42}H_{68}O_{14}Na$, 819.4501).

Acid Hydrolysis of the Saponins and Determination of the Absolute Configuration of the Monosaccharides. Compound **1** (2 mg) was dissolved in 2 M HCl−H_2O (2 mL) and heated at 85 °C for 15 h. The reaction mixture was extracted with EtOAc. 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, HP-5 (30 m × 0.25 mm, with a 0.25 μm film, Dikma); detection, FID; 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, N_2 gas. From the acid hydrolysate of **1**, D-glucose and L-arabinose 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 19.01 and 15.63 min, respectively. The constituent sugars of compounds **2**−**8** were identified by the same method as **1**. Retention times of authentic samples were detected at 16.77 min (L-rhamnose) for compounds **3** and **5** and 15.73 min (D-xylose) for compounds **4** and **6**.

Cytotoxicity Assay. Compounds **1**−**14** were tested for cytotoxicity against HT-29 (human colon cancer cell line), HePG2 (human hepatoma cancer cell line), BGC-823 (human gastric cancer cell line), A549 (human lung epithelial cell line), and A375 (human amelanotic melanoma cell line) by means of the MTT method as described in the literature.[16]

ASSOCIATED CONTENT

S Supporting Information. MS and NMR spectra of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

The research work was supported by the National Science and Technology Project of China (No. 2009ZX09311-004) .We thank the Department of Medicinal Analysis, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, for the measurements of IR, NMR, and HR-ESIMS spectra.

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