Cytotoxic Triterpenoid Glycosides from the Roots of *Gordonia chrysandra*

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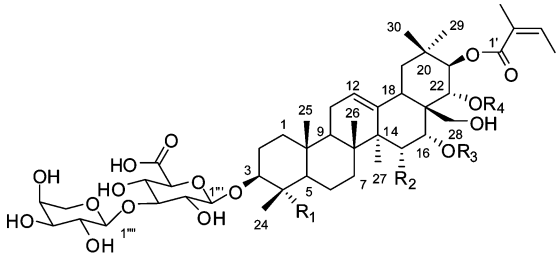
Eight new oleanane triterpenoid glycosides, gordonosides A−H (**1**−**8**), were isolated from a 50% EtOH extract of the roots of *Gordonia chrysandra*. Their structures were determined by spectroscopic analysis, including 1D and 2D NMR and ESIMS, and by chemical methods. Among these substances, compounds **1**, **3**, **5**, and **6** exhibited cytotoxic activity against several human cancer cell lines, with **3** being the most potent.

The genus *Gordonia* (Theaceae) consists of 40 species, with six species found in mainland China. *Gordonia chrysandra* Cowan is widely distributed in Sichuan, Guizhou, and Yunnan Provinces of the People's Republic of China.[1] Previous phytochemical studies on *Gordonia* species have led to the identification of triterpenoids,[2−6] steroids,[3] tannins,[7] and several other components.[8] Some of these substances have exhibited antifungal[8] and apoptosis-inducing activities.[7] In previous work, *G. chrysandra* roots were extracted successively with 95% EtOH and 50% EtOH in H_2O. The 95% EtOH extract exhibited hepatoprotective activity, and two active flavanonol glycosides were isolated.[9] The 50% EtOH extract showed cytotoxic activity against several human cancer cell lines, and bioassay-guided fractionation of this extract led to the isolation of eight cytotoxic acylated triterpenoid saponins (**1**−**8**). In this paper, we report the structural elucidation and cytotoxic activities of these new compounds.



1 R_1 = CH_2OH, R_2 = R_3 = H, R_4 = angeloyl
2 R_1 = CH_2OH, R_2 = H, R_3 = Ac, R_4 = angeloyl
3 R_1 = Me, R_2 = R_3 = H, R_4 = angeloyl
4 R_1 = Me, R_2 = H, R_3 = Ac, R_4 = angeloyl
5 R_1 = CHO, R_2 = R_3 = H, R_4 = angeloyl
6 R_1 = COOMe, R_2 = R_3 = H, R_4 = angeloyl
7 R_1 = COOMe, R_2 = OH, R_3 = H, R_4 = angeloyl
8 R_1 = COOMe, R_2 = OH, R_3 = H, R_4 = 2-methylbutanoyl

Results and Discussion

Compound **1** was isolated as a white, amorphous powder, and its IR spectrum displayed absorptions for hydroxy (3391 cm^{-1}) and conjugated carbonyl (1676 cm^{-1}) groups. The positive-ion HRESIMS of **1** showed a quasimolecular ion peak at *m/z* 1001.5056 $[M + Na]^+$ and indicated a molecular formula of $C_{51}H_{78}O_{18}$ (calcd for $C_{51}H_{78}O_{18}Na$, *m/z* 1001.5086). The ^1H NMR spectrum of **1** showed signals attributable to six tertiary methyl groups at δ 0.85 (H_3-26), 0.88 (H_3-25), 0.92 (H_3-24), 1.01 (H_3-29), 1.27 (H_3-30), and 1.75 (H_3-27), two isolated oxymethylenes at δ 3.36 and 3.62 (1H each, d, J = 10.5 Hz, H_2-28) and 3.69 and 4.28 (1H each, d, J = 10.5 Hz, H_2-23), and five oxymethine and/or olefinic protons at δ 4.30 (1H, m, H-3), 4.42 (1H, m, H-16), 6.26 (1H, d, J = 10.0 Hz, H-22), 6.62 (1H, d, J = 10.0 Hz, H-21), and 5.37 (1H, brs, H-12). Signals were also observed assignable to two anomeric protons of two sugar units at δ 5.19 (1H, d, J = 8.0 Hz, H-1‴) and 5.26 (1H, d, J = 7.5 Hz, H-1⁗), as well as partially overlapped signals due to the oxymethylenes and oxymethines of the sugar units between δ 4.01 and 4.51. Acid hydrolysis of **1** yielded D-glucuronic acid and L-arabinose, which were confirmed by measuring their specific rotations. The coupling constants of the anomeric protons (8.0 and 7.5 Hz, respectively) indicated a β- and an α-configuration at the anomeric carbon of the glucuronic acid and arabinose moiety, respectively.[10] In addition, the ^1H NMR spectrum exhibited three pairs of characteristic signals due to two angeloyloxy groups at δ 1.87 (3H, s, H_3-5″) and 1.96 (3H, s, H_3-5′), 2.01 (3H, d, J = 6.5 Hz, H_3-4″) and 2.03 (3H, d, J = 7.0 Hz, H_3-4′), and 5.88 (1H, q, J = 6.5 Hz, H-3″) and 5.93 (1H, q, J = 7.0 Hz, H-3′).[11] These data suggested that **1** is a highly oxygenated 23,28-dihydroxyolean-12-ene diglycoside derivative with β-D-glucuronyl and α-L-arabinosyl sugar units and two angeloyloxy groups as additional substituents. This was supported by the ^{13}C NMR spectroscopic data of **1** (Table 2).

The structure of **1** was finalized by a comprehensive analysis of its 2D NMR spectroscopic data. The proton and protonated carbon signals in the NMR spectra of **1** were assigned unequivocally (Tables 1 and 2) on the basis of TCOSY and HSQC spectroscopic analysis. In the HMBC spectrum of **1**, two- and three-bond correlations from protons to carbons of the aglycon moiety, together with the chemical shifts of these protons and carbons, confirmed that **1** possesses a 23,28-dihydroxyolean-12-ene nucleus with four oxygenated substituents at C-3, C-16, C-21, and C-22, respectively. HMBC correlations from H-21 to C-1′ and from H-22 to C-1″, in combination with chemical shifts of these protons and carbons, indicated unambiguously that the two angeloyloxy ester groups are attached to C-21 and C-22 of the aglycon, respectively. In addition, HMBC correlations from H-1‴ to C-3 confirmed that the β-D-glucuronopyranosyloxy unit is located at C-3 of the aglycon. A long-range correlation from H-1⁗ to C-3‴ (δ 85.7) suggested that the α-L-arabinopyranosyl unit is linked to C-3 of the β-D-glucuronopyranosyl unit. On taking account of the molecular composition of **1**, its planar structure was elucidated as 3-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-21,22-diangeloyloxyolean-12-en-16,23,28-triol.

The relative configuration of **1** was established by a NOE difference experiment and from the vicinal coupling constants of related protons. In the NOE difference spectrum of **1**, irradiation of H-23b enhanced the H-3 and H-5 signals, suggesting that the diglycosyl moiety at C-3 has a β-orientation. Irradiation of H-28b gave enhancements of H-16, H-22, and H-26, indicating that both the hydroxy at C-16 and the angeloyloxy at C-22 possess an

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Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**−**8**[a]

position	1	2	3	4	5	6	7	8
3	4.30, m	4.26, m	3.38, dd (12.0, 4.2)	3.30, dd (11.5, 3.0)	4.18, m	4.47, m	4.50, m	4.48, m
5	1.62, m	1.60, m	0.79, s	0.73, m	1.38, m	1.68, m	1.76, m	1.76, m
9	1.73, m	1.74, m	1.72, m	1.36, m	1.78, m	1.80, m	1.82, m	1.80, m
12	5.37, brs	5.38, brs	5.42, brs	5.41, brs	5.40, brs	5.39, brs	5.48, brs	5.47, brs
15	1.52, m	1.60, m	1.62, m	1.65, m	1.55, m	1.58, m	4.18, d (3.5)	4.17, d (4.2)
	1.80, m	1.80, m	1.88, m	1.83, m	1.83, m	1.80, m		
16	4.42, m	5.49, brs	4.49, brs	5.53, brs	4.48, brs	4.46, m	4.41, d (3.5)	4.37, d (4.2)
18	3.05, m	3.03, dd (13.5, 4.5)	3.11, s	3.06, dd (11.5, 3.0)	3.06, m	3.08, m	3.05, s	3.06, m
19a	3.05, m	2.62, t (14.0)	3.11, s	2.67, t (9.0)	3.08, m	3.06, m	3.05, s	3.06, m
19b	1.34, m	1.42, m	1.41, m	1.47, m	1.40, m	1.39, m	1.40, m	1.40, m
21	6.62, d (10.0)	5.96, d (10.0)	6.70, d (10.2)	5.99, d (10.5)	6.70, d (10.5)	6.67, d (10.5)	6.69, d (10.5)	6.64, d (10.2)
22	6.26, d (10.0)	6.26, d (10.0)	6.32, d (10.2)	6.27, d (10.5)	6.32, d (10.5)	6.29, d (10.5)	6.31, d (10.5)	6.24, d (10.2)
23	3.69, d (10.5)	3.71, d (10.5)	1.31, s	1.29, s	9.74, s			
	4.28, d (10.5)	4.35, d (10.5)						
24	0.92, s	0.92, s	0.99, s	0.97, s	1.30, s	1.43, s	1.43, s	1.44, s
25	0.88, s	0.86, s	0.82, s	0.78, s	0.81, s	0.83, s	0.84, s	0.85, s
26	0.85, s	0.76, s	0.86, s	0.76, s	0.81, s	0.83, s	0.98, s	0.99, s
27	1.75, s	1.38, s	1.87, s	1.51, s	1.83, s	1.81, s	1.82, s	1.82, s
28a	3.62, d (10.5)	3.60, d (10.5)	3.66, d (10.2)	3.61, d (10.5)	3.62, d (10.5)	3.62, d (10.5)	3.74, d (10.5)	3.73, d (10.2)
28b	3.36, d (10.5)	3.43, d (10.5)	3.41, d (10.2)	3.45, d (10.5)	3.39, d (10.5)	3.39, d (10.5)	3.49, d (10.5)	3.47, d (10.2)
29	1.01, s	1.04, s	1.09, s	1.08, s	1.09, s	1.06, s	1.07, s	1.06, s
30	1.27, s	1.26, s	1.33, s	1.29, s	1.32, s	1.31, s	1.31, s	1.29, s
3′	5.93, q (7.0)	5.94, m	5.95, q (7.2)	5.93, m	5.95, q (7.0)	5.95, q (7.5)	5.96, q (7.0)	6.06, q (7.2)
4′	2.03, d (7.0)	2.01, m	2.07, d (7.2)	2.02, m	2.07, d (7.0)	2.07, d (7.5)	2.08, d (7.0)	2.15, d (7.2)
5′	1.96, s	1.94, s	2.00, s	1.95, s	2.00, s	1.99, s	2.00, s	2.03, s
2″								2.07, q (7.2)
3″	5.88, q (6.5)	5.90, m	5.90, q (7.2)	5.92, m	5.89, q (7.0)	5.90, q (7.0)	5.78, q (7.0)	1.21, m 1.58, m
4″	2.01, d (6.5)	2.03, m	2.04, d (7.2)	2.04, m	2.03, d (7.0)	2.03, d (7.0)	1.95, d (7.0)	0.66, t (7.2)
5″	1.87, s	2.00, s	1.89, s	2.02, m	1.88, s	1.88, s	1.73, s	1.01, d (7.2)
1‴	5.19, d (8.0)	5.20, d (7.5)	5.01, d (7.2)	4.96, d (7.0)	4.90, d (8.0)	4.95, d (8.0)	4.95, d (7.5)	4.95, d (7.8)
2‴	4.01, m	4.12, t (8.0)	4.14, t-like (9.0, 7.8)	4.13, t (8.0)	4.02, t (8.5)	4.02, t-like (9.0, 8.0)	4.02, t (8.5)	4.02, t (8.4)
3‴	4.21, t (9.0)	4.22, m	4.40, d (9.6)	4.38, d (9.0)	4.31, m	4.29, m	4.29, m	4.30, m
4‴	4.44, m	4.48, m	4.55, d (9.0)	4.53, m	4.50, m	4.47, m	4.47, m	4.51, m
5‴	4.51, d (9.5)	4.54, m	4.68, d (9.6)	4.66, d (9.0)	4.63, d (9.5)	4.62, d (10.0)	4.63, d (9.0)	4.62, d (9.0)
1⁗	5.26, d (7.5)	5.31, d (7.0)	5.36, d (7.2)	5.35, d (7.5)	5.33, d (6.5)	5.33, d (7.5)	5.34, d (6.5)	5.33, d (6.6)
2⁗	4.47, d (8.0)	4.50, m	4.52, t-like (8.4, 7.2)	4.51, m	4.52, m	4.50, m	4.47, m	4.50, m
3⁗	4.15, m	4.21, m	4.19, dd (8.4, 3.0)	4.19, dd (8.5, 2.5)	4.18, m	4.17, m	4.16, m	4.17, m
4⁗	4.30, m	4.33, m	4.30, brs	4.30, brs	4.34, m	4.30, m	4.33, m	4.30, m
5⁗	3.78, d (11.5)	3.82, d (11.5)	3.81, d (11.4)	3.80, d (11.5)	3.78, d (11.5)	3.78, d (12.0)	3.76, m	3.75, m
	4.33, m	4.39, m	4.38, dd (11.4, 3.0)	4.36, m	4.37, m	4.34, m	4.37, m	4.35, m
Ac		2.48, s		2.52, s				
OMe						3.81, s	3.77, s	3.78, s

[a] ^{1}H NMR data (δ) were measured in C_5D_5N at 500 MHz for **1**, **2**, and **4**−**7**, and at 600 MHz for **3** and **8**. Coupling constants (J) in Hz are given in parentheses. The assignments are based on DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC experiments.

α-orientation. In the ^{1}H NMR spectrum of **1**, the vicinal coupling constant between H-21 and H-22 (10.0 Hz) suggested that these two protons adopt a *trans* diaxial orientation. This indicated that the angeloyloxy group at C-21 is β-oriented, which was confirmed by an enhancement of H_3-27 caused by irradiation of H-21. Thus, the structure of **1** was determined as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-21β,22α-diangeloyloxyolean-12-ene-16α,23,28-triol. This compound has been assigned the trivial name gordonoside A.

Compound **2** was obtained as a white, amorphous powder, and its positive-ion HRESIMS gave a quasimolecular ion peak at *m*/*z* 1043.5206 $[M + Na]^+$, which indicated the molecular formula to be $C_{53}H_{80}O_{19}$ (calcd for $C_{53}H_{80}O_{19}Na$, *m*/*z* 1043.5192). The IR and NMR spectroscopic features of **2** were similar to those of **1** (see Experimental Section and Tables 1 and 2), except for additional signals [δ_H 2.48(3H, s), δ_C 169.9 and 22.0] assignable to an acetyl group in the NMR spectrum of **2**. These data suggested that **2** is an acetyl derivative of **1**, which was confirmed by appropriate 2D NMR experiments on **2**. In the ^{1}H NMR spectrum, H-16 was deshielded by $\Delta\delta$ 1.07 ppm as compared to **1**, indicating that the acetyl group is located at C-16 in **2**. In the HMBC spectrum of **2**, a long-range correlation from H-16 to the carbonyl carbon of the acetyl unit confirmed the location of the acetyl unit. In the NOESY spectrum of **2**, correlations between H-16 and H_2-28 and H_3-26 proved that the acetyloxy at C-16 possesses an α-orientation. Therefore, compound **2** (gordonoside B) was determined as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-16α-acetoxy-21β,22α-diangeloyloxyolean-12-ene-23,28-diol.

Compound **3** was obtained as a white, amorphous powder. The positive-ion HRESIMS of **3** showed a quasimolecular ion peak at *m*/*z* 985.5156 $[M + Na]^+$, indicating a molecular formula of $C_{51}H_{78}O_{17}$ (calcd for $C_{51}H_{78}O_{17}Na$, *m*/*z* 985.5137), one oxygen atom less than that of **1**. Comparison of the NMR data of **3** and **1** (see Experimental Section and Tables 1 and 2) indicated that signals of the oxymethylene group of **1** (CH_2-23) were replaced by those of a methyl group of **3** (δ_H 1.31, δ_C 28.1; CH_3-23). These data suggested that **3** is a 23-deoxygenated derivative of **1**, which was confirmed by 2D NMR experiments on **3**. Therefore, compound **3** (gordonoside C) was determined as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-21β,22α-diangeloyloxyolean-12-ene-16α,28-diol.

Compound **4** was obtained as a white, amorphous powder. The positive-ion HRFABMS of **4** showed a quasimolecular ion peak at *m*/*z* 1027.5258 $[M + Na]^+$, indicating the molecular formula to be $C_{53}H_{80}O_{18}$ (calcd for $C_{53}H_{80}O_{18}Na$, *m*/*z* 1027.5242). The IR and NMR data of **4** resembled those of **3** (see Experimental Section and Tables 1 and 2). However, the NMR spectrum of **4** showed additional signals attributed to an acetyl unit (δ_H 2.52, and δ_C 169.9 and 22.0) and a deshielded shift of H-16 ($\Delta\delta$ 1.04 ppm) as compared to that of **3**. These data suggested that **4** is a 16-acetyl derivative of **3**. Therefore, compound **4** (gordonoside D) was elucidated as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-16α-acetoxy-21β,22α-diangeloyloxyolean-12-en-28-ol.

Compound **5** was isolated as a white, amorphous powder. Its molecular formula, $C_{51}H_{76}O_{18}$ (calcd for $C_{51}H_{76}O_{18}Na$, *m*/*z* 999.4929),

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

position	1	2	3	4	5	6	7	8
1	38.7	38.6	38.8	38.6	38.1	38.5	38.7	38.8
2	25.9	26.1	26.7	26.5	25.2	26.0	26.0	26.1
3	82.1	81.9	89.2	89.0	82.2	85.7	85.4	85.6
4	43.4	43.5	39.6	39.5	55.5	53.6	53.5	53.6
5	47.4	47.2	55.7	55.5	47.5	52.3	52.1	52.1
6	18.0	17.8	18.4	18.2	20.3	21.1	21.4	21.5
7	32.7	32.7	33.1	33.0	32.3	32.7	36.3	36.4
8	40.0	40.0	40.1	40.0	40.3	40.3	41.6	41.7
9	46.9	46.9	46.9	46.8	46.8	47.1	47.2	47.3
10	36.6	36.5	36.8	36.7	35.9	36.5	36.6	36.6
11	23.7	23.7	23.9	23.7	23.7	23.7	23.9	24.0
12	123.5[b]	125.1	123.5[b]	125.1	123.5[b]	123.5[b]	125.0	125.1
13	142.6	140.9	142.8	140.9	142.8	142.8	143.7	143.8
14	41.5	41.1	41.7	41.1	41.7	41.6	47.6	47.7
15	34.7	30.7	34.9	30.8	34.7	34.8	67.4	67.5
16	68.6	72.0	68.7	72.1	68.5	68.6	73.4	73.0
17	47.9	46.9	48.1	46.9	48.0	48.0	48.3	48.5
18	40.0	39.4	40.1	39.5	40.0	40.0	40.9	40.8
19	47.0	47.0	47.2	47.1	47.1	47.2	46.8	46.8
20	36.2	36.0	36.4	36.1	36.4	36.3	36.3	36.4
21	78.6	78.0	78.8	78.0	78.7	78.7	78.5	78.6
22	73.5	72.4	73.7	72.4	73.5	73.7	73.3	73.2
23	64.1	64.0	28.1	27.9	206.6	178.0	178.0	178.0
24	13.4	13.6	17.0	16.9	10.3	12.2	12.3	12.3
25	16.1	16.1	15.7	15.6	15.7	16.1	16.2	16.2
26	16.8	16.7	16.9	16.7	16.7	16.7	17.3	17.4
27	27.4	27.0	27.6	27.0	27.5	27.5	21.2	21.2
28	63.5	63.3	63.7	63.4	63.5	63.6	63.1	63.0
29	29.3	29.4	29.6	29.4	29.5	29.4	29.4	29.4
30	20.1	19.7	20.3	19.7	20.3	20.2	20.2	20.2
1′	167.5	167.7	167.7	167.7	167.7	167.7	167.7	167.6
2′	128.8	128.5	129.1	128.5	128.9	129.0	128.9	128.7
3′	136.9	138.1	137.2	138.1	137.2	137.1	137.4	138.6
4′	15.6	15.9	15.94	15.9	15.9	15.8	15.9	16.1
5′	20.8	20.9	21.1	20.9	21.0	20.9	21.0	21.1
1″	168.1	167.2	168.2	167.2	168.1	168.2	168.0	176.6
2″	128.8	128.3	129.1	128.4	128.9	129.0	129.1	41.5
3″	137.0	138.4	137.1	138.4	137.1	137.1	136.5	26.9
4″	15.6	15.7	15.85	15.7	15.8	15.7	15.7	11.9
5″	20.6	20.8	20.9	20.8	20.8	20.7	20.6	16.7
1‴	105.6	106.0	106.9	106.8	104.9	105.9	105.9	105.9
2‴	74.5	74.7	74.8	74.7	74.4	74.2	74.2	74.3
3‴	85.7	85.6	85.9	85.8	85.4	85.6	85.6	85.6
4‴	71.3	71.4	71.5	71.5	71.3	71.3	71.3	71.4
5‴	77.5	77.5	77.6	77.5	77.5	77.4	77.5	77.6
6‴	172.1	172.3	172.3	172.3	172.1	172.0	172.2	172.2
1⁗	105.6	105.8	106.0	105.9	105.8	105.7	105.8	105.8
2⁗	72.6	72.8	72.9	72.8	72.8	72.8	72.9	72.9
3⁗	74.2	74.4	74.5	74.4	74.4	74.4	74.4	74.5
4⁗	69.1	69.2	69.3	69.3	69.2	69.2	69.2	69.3
5⁗	67.0	67.1	67.2	67.1	67.0	67.0	67.0	67.1
Ac		169.9		169.9				
		22.0		22.0				
OMe						52.1	52.1	52.2

[a] ^{13}C NMR data (δ) were measured in C_5D_5N at 125 MHz for **1**, **2**, and **4**−**7**, and at 150 MHz for **3** and **8**. The assignments are based on DEPT, $^1H-^1H$ COSY, HSQC, and HMBC experiments. [b] Signal overlapped by solvent peaks.

was proposed by the positive-ion HRESIMS at *m/z* 999.4945 [M + Na]$^+$. The IR and NMR data of **5** (see Experimental Section and Tables 1 and 2) were similar to those of **1** except that the resonances of the oxymethylene of **1** (CH_2-23) were replaced by signals due to an aldehyde unit in **5** (δ_H 9.74 and δ_C 206.6). These spectroscopic data revealed that **5** is a C-23 aldehyde form of **1**. This was proved by 2D NMR experiments on **5**, especially by HMBC correlations from the aldehyde proton to both C-5 and C-24 and from H-3 to both C-1‴ and the aldehyde carbonyl carbon. This was further supported by a correlation between H_3-24 (δ 1.30) and H_3-25 (δ 0.81) in the NOESY spectrum of **5**. Therefore, compound **5** (gordonoside E) was determined as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-D-glucuronopyranosyl]-21β,22α-diangeloyloxy-23-oxoolean-12-ene-16α,28-diol.

Compound **6** was obtained as a white, amorphous powder. Its molecular formula, $C_{52}H_{78}O_{19}$ (calcd for $C_{52}H_{78}O_{19}Na$, *m/z* 1029.5035), was assigned by HRFABMS at *m/z* 1029.5100 [M + Na]$^+$. The IR and NMR spectroscopic data of **6** were similar to those of **1** (see Experimental Section, and Tables 1 and 2). However, the resonances of the oxymethylene (C-23) of **1** were replaced by resonances of a methoxycarbonyl of **6** (δ_H 3.81, and δ_C 178.0 and 52.1). Meanwhile, C-4 of **6** (δ_C 53.6) was significantly deshielded as compared to that of **1**. These spectroscopic data indicated that **6** is a methyl 23-oic acid ester form of **1**.[12] This was supported by correlations from H-3, H-5, H_3-24, and the methoxyl protons to the carbonyl carbon (δ_C 178.0) in the HMBC spectrum of **6**. It was also confirmed by a NOESY experiment on **6** showing cross-peaks between H-3 and H-5 and between H_3-24 (δ 1.43) and H_3-25 (δ 0.83). Consequently, compound **6** (gordonoside F), was determined as 3β-*O*-[α-L-arabinopyranosyl(1→3)-β-Dglucuronopyranosyl]-21β,22α-diangeloyloxy-23-methoxycarbonylolean-12-ene-16α,28-diol.

Table 3. Evaluation of the Cytotoxic Potential of Compounds **1**−**7**

	cell line IC_{50} (μM)				
compound	HCT-8	Bel-7402	BGC-823	A-549	A2780
1	>10	4.7	>10	>10	5.5
3	1.2	0.7	2.5	1.8	0.4
5	2.7	1.1	>10	>10	2.0
6	>10	1.3	>10	>10	2.0
paclitaxel[b]	3.6	6.3	0.04	1.0×10^{-3}	0.9

[a] Compounds **2**, **4**, and **7** were inactive against all cell lines tested (IC_{50} >10 μM). [b] Positive control.

Compound **7** was obtained as a white, amorphous powder. Its molecular formula, $C_{52}H_{78}O_{20}$ (calcd for $C_{52}H_{78}O_{20}Na$, *m/z* 1045.4984), was determined by HRFABMS at *m/z* 1045.4934 [M + Na]$^+$. The IR and NMR spectroscopic features of **7** were almost identical to those of **6**. However, detailed comparison of the NMR data of **7** and **6** (Experimental Section, and Tables 1 and 2) indicated that signals of an oxymethine (CH-15) of **7** replaced those of the methylene (CH_2-15) of **6** and that H-16 of **7** was shielded by $\Delta\delta_H$ 0.05 ppm. In turn, C-14 and C-16 of **7** were deshielded by $\Delta\delta_C$ 6.00 and 4.80 ppm as compared to those of **6**, respectively. These spectroscopic data suggested that **7** is a derivative of **6** with an additional hydroxy group at C-15. This was proved unambiguously by 2D NMR experiments on **7**. In the HMBC spectrum of **7**, correlations from both H_3-27 and H-16 to C-15 (δ_C 67.4) confirmed that the additional hydroxy group is located at C-15. In the NOESY spectrum of **7**, correlations between H-15 with H_3-26 and H_2-28, together with correlations between H-16 and H_2-28, were used to show that both hydroxy groups at C-15 and C-16 have an α-orientation.[13] Therefore, compound **7** (gordonoside G) was determined as 3*β*-*O*-[α-L-arabinopyranosyl(1→3)-*β*-D-glucuronopyranosyl]-21*β*,22α-diangeloyloxy-23-methoxycarbonylolean-12-ene-15α,16α,28-triol.

Compound **8** was obtained as a white, amorphous powder. Its molecular formula, $C_{52}H_{80}O_{20}$ (calcd for $C_{52}H_{80}O_{20}Na$, *m/z* 1047.5141), as determined by HRESIMS at *m/z* 1047.5125 [M + Na]$^+$, showed two hydrogen atoms more than that of **7**. The IR and NMR spectroscopic data of **8** were similar to those of **7** (see Experimental Section, and Tables 1 and 2). However, in the NMR spectra of **8**, signals ascribed to a 2-methylbutanoyl unit replaced those of an angeloyl unit in **7**. The NMR data of **8** were assigned unambiguously by HSQC experiments. In the HMBC spectrum, long-range correlations of C-1″ with H-22, H-2″, H-3″, and H-5″ revealed the 2-methylbutanoyl unit to be located at C-22. Thus, compound **8** (gordonoside H) was determined as 3*β*-*O*-[α-L-arabinopyranosyl-(1→3)-*β*-D-glucuronopyranosyl]-21*β*-angeloyloxy-22α-(2-methylbutanoyloxy)-23-methoxycarbonylolean-12-ene-15α,16α,28-triol.

The cytotoxic activities of compounds **1**−**7** (purity of each compound >90%) were evaluated against several human cancer cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) with paclitaxel as a positive control (see Table 3). Compound **3** exhibited significant cytotoxicity for all the tested human cancer cell lines. Compounds **1**, **5**, and **6** showed selective cytotoxicities for the Bel-7402 and A-2780 cell lines. These results indicate that the free hydroxy group at C-16 may play an important role in mediating cytotoxicity. Acetylation of the hydroxy group at C-16 (**2** and **4**) and the presence of a hydroxyl group at C-15 (**7**) decreased the resultant cytotoxic activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 automatic digital polarimeter. UV spectra were recorded on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer by a microscope transmission method. 1D and 2D NMR spectra were obtained at 500 and 125 MHz or 600 and 150 MHz for ^{1}H and ^{13}C, respectively, on a Bruker AVANCE DRX 500 spectrometer or a SYSTEM-600 FT spectrometer, with solvent (pyridine-d_5) peaks as references. HRESIMS and HRFABMS were performed on an Autospec-Ultima ETOF mass spectrometer. ESIMS data were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Preparative HPLC was carried out on a Shimadzu LC-6AD instrument with a SPD-10A detector, using a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography was performed with macroporous resin D101 (26−60 mesh, Tianjin Haiguang Chemistry Company, Tianjin, People's Republic of China), silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), ODS (50 μm; YMC, Kyoto, Japan), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), respectively. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

Plant Material. The roots of *Gordonia chrysandra* were collected in Yunnan Province, People's Republic of China, in May 2005. The plant material was identified by Prof. Cui Jingyun (Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences). A voucher specimen (No. 20050512) was deposited at the Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, People's Republic of China.

Extraction and Isolation. The dried roots (6.4 kg) were extracted with 95% EtOH in H_2O, and then the residues were extracted with 50% EtOH in H_2O. After removing the solvent, a brown residue (424 g) was obtained from the 50% EtOH extract. The residue was partitioned between *n*-BuOH and H_2O. The *n*-BuOH phase was concentrated under reduced pressure to yield a *n*-BuOH-soluble portion (160 g), which was fractionated by column chromatography over silica gel, using a solvent system of CH_3Cl−MeOH−H_2O (7:3:0.5), to yield 12 fractions. The saponin-enriched fraction V (5.70 g) was subjected to reversed-phase MPLC (YMC-ODS-A 50 μm, 500 mm × 50 mm, flow rate 20.0 mL/min), eluting with a gradient of increasing methanol (25%−100%) in H_2O, to obtain 10 fractions (Fr. 1−10). Fr. 1 was subjected to column chromatography over C_{18} silica gel eluting with MeOH−H_2O (7:3) to afford an amorphous powder, and reversed-phase HPLC (YMC-ODS-A 5 μm, 250 mm × 20 mm, detection at 210 nm, flow rate 10.0 mL/min) purification, using MeOH−H_2O (4:3) containing 0.05% TFA as mobile phase, yielded compounds **3** (15 mg) and **4** (9 mg). Fr. 2 was separated by reversed-phase HPLC with CH_3CN−H_2O (47:53) containing 0.05% TFA as mobile phase to afford compounds **1** (48 mg), **2** (12 mg), **5** (12 mg), and **6** (80 mg). Fr. 3 was subjected to reversed-phase HPLC separation with CH_3CN−H_2O (4:6) containing 0.05% TFA as mobile phase, to yield compounds **7** (15 mg) and **8** (6 mg).

Gordonoside A (1): white, amorphous powder; $[\alpha]^{20}_D$ −3.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.29), 254 (4.05) nm; IR ν_{max} 3391, 2926, 1676, 1437, 1387, 1240, 1202, 1143, 1080, 1044 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1001 [M + Na]$^+$; negative-ion ESIMS *m/z* 977 [M − H]$^-$; positive-ion HRESIMS *m/z* 1001.5056 [M + Na]$^+$ (calcd for $C_{51}H_{78}O_{18}Na$, 1001.5086).

Gordonoside B (2): white, amorphous powder; $[\alpha]^{20}_D$ −3.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.23), 254 (3.56) nm; IR ν_{max} 3399, 2956, 2929, 1683, 1441, 1381, 1203, 1148, 1081, 1044 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1043 [M + Na]$^+$; negative-ion ESIMS *m/z* 1019 [M − H]$^-$; positive-ion HRESIMS *m/z* 1043.5206 [M + Na]$^+$ (calcd for $C_{53}H_{80}O_{19}Na$, 1043.5192).

Gordonoside C (3): amorphous, white powder; $[\alpha]^{20}_D$ −5.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.34) nm; IR ν_{max} 3397, 2950, 2922, 1700, 1649, 1455, 1384, 1357, 1243, 1164, 1081, 1044, 1021 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 985 [M + Na]$^+$; negative-ion ESIMS *m/z* 961 [M − H]$^-$; positive-ion HRESIMS *m/z* 985.5156 [M + Na]$^+$ (calcd for $C_{51}H_{78}O_{17}Na$, 985.5137).

Gordonoside D (4): amorphous, white powder; $[\alpha]^{20}_D$ −23.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.30) nm; IR ν_{max} 3413, 2952, 2924, 1743, 1717, 1646, 1455, 1378, 1236, 1151, 1081, 1043, 1025 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion HRFABMS *m/z* 1027.5258 [M + Na]$^+$ (calcd for $C_{53}H_{80}O_{18}Na$, 1027.5242).

Gordonoside E (5): white, amorphous powder; $[\alpha]^{20}_D$ +1.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.26), 254 (3.46) nm; IR ν_{max} 3388, 2959, 2923, 1698, 1442, 1387, 1204, 1151, 1082, 1044 cm^{-1};

^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 999 [M + Na]$^+$; negative-ion ESIMS *m/z* 975 [M − H]$^-$; positive-ion HRESIMS *m/z* 999.4945 [M + Na]$^+$ (calcd for $C_{51}H_{76}O_{18}Na$, 999.4929).

Gordonoside F (6): white, amorphous powder; $[\alpha]^{20}_D$ −5.9 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.29), 254 (3.20) nm; IR ν_{max} 3355, 2954, 2923, 1673, 1435, 1388, 1243, 1201, 1143, 1080, 1044 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1029 [M + Na]$^+$; negative-ion ESIMS *m/z* 1005 [M − H]$^-$; positive-ion HRFABMS *m/z* 1029.5100 [M + Na]$^+$ (calcd for $C_{52}H_{78}O_{19}Na$, 1029.5035).

Gordonoside G (7): white, amorphous powder; $[\alpha]^{20}_D$ −1.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.19) nm; IR ν_{max} 3263, 2962, 1669, 1435, 1390, 1243, 1185, 1135, 1083, 1045 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1045 [M + Na]$^+$; negative-ion ESIMS *m/z* 1021 [M − H]$^-$; positive-ion HRFABMS *m/z* 1045.4934 [M + Na]$^+$ (calcd for $C_{52}H_{78}O_{20}Na$, 1045.4984).

Gordonoside H (8): white, amorphous powder; $[\alpha]^{20}_D$ −2.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.30) nm; IR ν_{max} 3405, 2961, 2931, 1710, 1680, 1436, 1388, 1243, 1196, 1147, 1078, 1044 cm^{-1}; ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2, respectively; positive-ion ESIMS *m/z* 1047 [M + Na]$^+$, negative-ion ESIMS *m/z* 1023 [M − H]$^-$; positive-ion HRESIMS *m/z* 1047.5125 [M + Na]$^+$ (calcd for $C_{52}H_{80}O_{20}Na$, 1047.5141).

Acid Hydrolysis of Compound 1 and Determination of Sugar Configurations. A solution of compound **1** (25 mg) was hydrolyzed with 5% aqueous H_2SO_4−1,4-dioxane (1:1, v/v, 2 mL) under reflux for 4 h. On cooling, the reaction mixture was extracted with $CHCl_3$ (3 × 1 mL) to yield a $CHCl_3$ extract and a H_2O phase.

The H_2O phase of **1** was subjected to passage through an anion-exchange resin column, eluting with H_2O and then with 0.1 N aqueous HCl. The H_2O and aqueous HCl solutions were separately concentrated to dryness. The residue from the H_2O solution was subjected to normal-phase preparative TLC with $CHCl_3$−MeOH−H_2O−AcOH (7:3:0.5:0.5) as solvent system to yield arabinose (2.14 mg), with $[\alpha]^{23}_D$ +99.2 (*c* 0.21, H_2O), and glucuronic acid (1.50 mg), with $[\alpha]^{23}_D$ +30.5 (*c* 0.19, H_2O), respectively. L-Arabinose (R_f 0.40) and D-glucuronic acid (R_f 0.12−0.25) were identified by comparison of $[\alpha]_D$ and R_f values with authentic sugar samples.

Cells, Culture Conditions, and Cell Proliferation 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 cancer cell line), and A2780 (human ovarian cancer cell line) were maintained in the RPMI 1640 medium containing 10% fetal bovine serum supplemented with L-glutamine, 100 units/mL of penicillin, and 100 μg/mL of streptomycin. Cultures were incubated at 37 °C in 5% CO_2 in air.

HCT-8, Bel-7402, BGC-823, A549, and A2780 cells (1.5×10^3) were seeded in 96-well tissue culture plates, and 100 μL of cell suspension was placed in each well. After 24 h, 100 μL of DMSO solution containing the test compounds 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. A 100 μL aliquot of 0.5 mg/mL MTT in RPMI 1640 was added to every well, and the plate was reincubated in 5% CO_2 in 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 150 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 (see Table 3).

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

References and Notes

(1) Institute of Botany, Chinese Academy of Sciences. *Iconographia Cormophytorum Sinicorum Supplementum II*; Science Press: Beijing, 1983; p 468.
(2) Herath, H. M. T. B.; Athukoralage, P. S. *Nat. Prod. Sci.* **1998**, *4*, 253–256.
(3) Herath, H. M. T. B.; Athukoralage, P. S.; Jamie, J. F. *ACGC Chem. Res. Commun.* **1999**, *9*, 3–8.
(4) Herath, H. M. T. B.; Athukoralage, P. S. *Nat. Prod. Sci.* **2000**, *6*, 102–105.
(5) Herath, H. M. T. B.; Athukoralage, P. S.; Jamie, J. F. *Phytochemistry* **2000**, *54*, 823–827.
(6) Herath, H. M. T. B.; Athukoralage, P. S.; Jamie, J. F. *Nat. Prod. Lett.* **2001**, *15*, 339–344.
(7) Wang, C. C.; Chen, L. G.; Yang, L. L. *Toxicology* **2001**, *168*, 231–240.
(8) Athukoralage, P. S.; Herath, H. M. T. B.; Deraniyagala, S. A.; Wijesundera, R. L. C.; Weerasinghe, P. A. *Fitoterapia* **2001**, *72*, 565–567.
(9) Wang, K.; Yang, J. Z.; Zuo, L.; Zhang, D. M. *Chin. Chem. Lett.* **2008**, *1*, 61–64.
(10) Diome, C.; Mitaine-Offer, A. C.; Miyamoto, T.; Delaude, C.; Mirjolet, J. F.; Duchamp, O.; Lacaille-Dubois, M. A. *J. Nat. Prod.* **2007**, *70*, 1680–1682.
(11) Yoshikawa, M.; Morikawa, T.; Li, N.; Nagatomo, A.; Li, X.; Matsuda, H. *Chem. Pharm. Bull.* **2005**, *53*, 1559–1564.
(12) Morikawa, T.; Li, N.; Nagatomo, A.; Matsuda, H.; Li, X.; Yoshikawa, M. *J. Nat. Prod.* **2006**, *69*, 185–190.
(13) Lu, Y.; Umeda, T.; Yagi, A.; Sakata, K.; Chaudhuri, T.; Ganguly, D. K.; Sarma, S. *Phytochemistry* **2000**, *53*, 941–946.

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