Cytotoxic Oxygenated Triterpenoid Saponins from *Symplocos chinensis*

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A *n*-butanol-soluble fraction of an ethanolic extract from the roots of *Symplocos chinensis* showed cytotoxic activity against several cancer cell lines. Bioassay-guided purification led to the isolation and characterization of eight new triterpenoid saponins, symplocososides L−S (**1**−**8**). The structures of **1**−**8** were elucidated as glycosides based on oxygenated aglycons by spectroscopic and chemical methods. These compounds and their hydrolytic products, along with some additional analogues obtained earlier from *S. chinensis* roots, were evaluated for cytotoxicity in a small cancer cell panel.

Symplocos chinensis (Lour.) Druce (Symplocaceae) is a toxic herb widely distributed in the south of the People's Republic of China and has been used as a folk medicine for the treatment of colds, fevers, malaria, the relief of cough, and detoxification.[1] In the course of previous work, new triterpenoids[2] and triterpenoid saponins,[3−5] showing cytotoxic activities against several cancer cell lines, were isolated from the roots of *S. chinensis*. Additional studies on this plant have now led to the isolation of eight new cytotoxic triterpenoid saponins, symplocososides L−S (**1**−**8**), from the *n*-BuOH-soluble portion of the ethanol extract. The isolation and identification of **1**−**8** and the cytotoxic evaluation of these compounds and some structural analogues are the subject of the present paper.

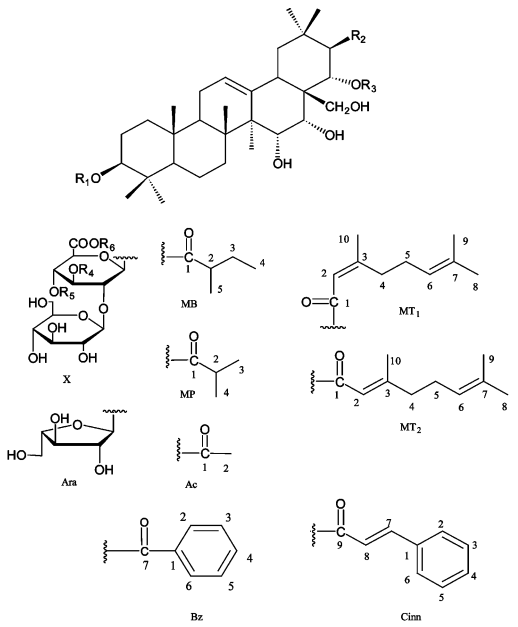
Results and Discussion

The *n*-BuOH-soluble part of the 95% ethanolic extract of the dried roots of *S. chinenesis* was separated by column chromatography over silica gel, Sephadex LH-20, and octadecyl silica gel (ODS), and finally by preparative HPLC, and led to the purification of symplocososides L−S (**1**−**8**). Acid hydrolysis of compounds **1** and **4** afforded **1a** and **4a**, respectively. Alkaline hydrolysis of **1a** and **4a** gave the previously known compounds R_1-barrigenol (**1b**) and A_1-barrigenol (**4b**),[6] respectively.

Compound **1** was obtained as a white, amorphous powder. The molecular formula was determined as $C_{63}H_{98}O_{24}$ by HRESIMS, in which a sodiated molecular ion $[M + Na]^+$ was detected at *m*/*z* 1261.6333. Its IR spectrum displayed absorptions for hydroxyl (3436 cm^{-1}) and conjugated carbonyl (1718 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) revealed the presence of seven tertiary methyl groups between δ 0.82 and 1.84 and a double bond with typical ^{13}C NMR resonances (Table 2) at δ 125.3 and 143.8, indicating an olean-12-ene triterpene derivative.[7] In addition, the ^1H NMR spectrum exhibited signals of three tertiary methyl groups at δ 1.64 (3H, s), 1.67 (3H, s), and 1.80 (3H, s) and two olefinic proton signals at δ 5.90 (1H, s) and 5.30 (1H, br s), which were attributed to a monoterpenoid unit (MT_1) of (2*Z*)-3,7-dimethyl-2,6-octadienoate.[8] Two methyl signals at δ 0.99 (3H, d, J = 7.0 Hz) and 1.05 (3H, d, J = 7.0 Hz) and one methine proton at δ 0.68 (1H, m) were assigned to a 2-methylpropanoyl moiety (MP).[9] Two oxymethine proton signals at δ 6.62 (d, J = 10.0 Hz) and 6.22 (d, J = 10.0 Hz), which correlated with the carbon signals at δ 78.0 and 73.6, respectively, in the HSQC spectrum, were diagnostic for H-21 and H-22. As observed in the HMBC spectrum, the correlations of H-21 of the aglycon with C-1 (δ 166.5) of the MT_1 unit and H-22 of the aglycon with C-1 (δ 177.0) of the MP unit established that the MT_1 and MP moieties are attached to C-21 and C-22 of the aglycon, respectively. Acid hydrolysis of **1** afforded a 21,22-disubstituted aglycon (**1a**). Compound **1a** was hydrolyzed with alkaline to afford needle crystals of R_1-barrigenol (**1b**).[6] Thus, the structure of **1a** was established as 21β-*O*-[(2*Z*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylpropanoyl)-R_1-barrigenol.

The sugar portion of **1** showed two oxymethylene signals (δ 62.6 and 63.6), one carboxylic acid carbonyl signal (δ 171.8), and three

Chart 1



	R_1	R_2	R_3	R_4	R_5	R_6
1	X	OMT_1	MP	Ac	Ara	H
1a	H	OMT_1	MP			
1b	H	OH	H			
2	X	OMT_2	MP	Ac	Ara	H
3	X	OCinn	MB	H	H	H
4	X	H	MT_1	Ac	Ara	H
4a	H	H	MT_1			
4b	H	H	H			
5	X	OBz	MB	Ac	Ara	H
6	X	OMT_1	MB	H	Ara	CH_3CH_2
7	X	OMT_1	MB	H	H	H
8	X	OMT_2	MB	H	H	H

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Table 1. ^{1}H NMR Spectroscopic Data (500 MHz, C_5D_5N) for Compounds **1**−**8**, **1a**, and **4a**

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1a**	**4a**
1	0.85 m	0.86 m	0.88 m	0.86 m	0.89 m	0.83 m	0.86 m	0.88 m	0.94 m	0.92 m
	1.40 m	1.42 m	1.46 m	1.38 m	1.46 m	1.42 m	1.44 m	1.40 m	1.62 m	1.52 m
2	2.10 m	2.12 m	2.20 m	2.08 m	2.10 m	1.98 m	2.15 m	2.18 m	2.22 m	2.12 m
	1.78 m	1.68 m	1.65 m	1.60 m	1.75 m	1.69 m	1.68 m	1.66 m	1.90 m	1.76 m
3	3.20 dd (11.0, 4.0)	3.18 dd (11.0, 4.0)	3.30 dd (12.0, 4.0)	3.18 dd (11.5, 3.5)	3.19 dd (11.5, 4.0)	3.16 dd (11.0, 3.5)	3.29 dd (11.0, 4.0)	3.29 dd (10.5, 5.0)	3.48 dd (5.5, 10.5)	3.47 dd (10.5, 5.0)
5	0.78 m	0.77 m	0.81 m	0.75 m	0.76 m	0.70 m	0.78 m	0.80 m	0.90 m	0.78 m
6	1.58 m	1.62 m	1.55 m	1.55 m	1.58 m	1.40 m	1.56 m	1.52 m	1.64 m	1.60 m
	1.34 m	1.36 m	1.35 m	1.40 m	1.35 m	1.25 m	1.36 m	1.38 m	1.34 m	1.32 m
7	2.14 m	2.12 m	2.11 m	2.14 m	2.16 m	1.96 m	2.09 m	2.12 m	2.16 m	2.14 m
	2.06 m	2.06 m	2.04 m	2.10 m	2.04 m	2.07 m	2.04 m	2.06 m	2.08 m	2.08 m
9	1.70 m	1.68 m	1.68 m	1.69 m	1.70 m	1.35 m	1.67 m	1.70 m	1.78 m	1.76 m
11	1.88 m	1.90 m	1.95 m	1.79 m	1.84 m	1.80 m	1.88 m	1.92 m	1.94 m	1.89 m
12	5.48 br s	5.52 br s	5.52 br s	5.47 s	5.49 br s	5.53 br s	5.55 br s	5.50 br s	5.53 br s	5.50 s
15	4.22 m	4.22 m	4.18 m	4.19 m	4.24 m	4.15 m	4.22 m	4.20 m	4.24 d (3.5)	4.26 d (4.5)
16	4.42 m	4.40 m	4.40 m	4.51 m	4.54 m	4.39 m	4.38 m	4.40 m	4.43 d (3.5)	4.56 d (4.5)
18	3.03 m	3.05 m	3.12 m	3.02 m	3.08 m	2.98 m	3.08 m	3.06 m	2.88 m	3.05 m
19	1.41 m	1.40 m	1.44 m	1.31 m	1.51 m	1.59 m	1.40 m	1.42 m	1.42 m	1.32 m
	3.06 m	3.08 m	3.14 m	2.87 m	3.12 m	3.00 m	3.08 m	3.03 m	3.09 m	2.85 m
21	6.62 d (10.0)	6.66 d (10.0)	6.73 d (10.0)	2.74 m	6.80 d (10.0)	6.47 d (10.0)	6.61 d (10.0)	6.61 d (10.0)	6.62 d (10.5)	2.76 m
				1.98 m						2.00 m
22	6.22 d (10.0)	6.22 d (10.0)	6.37 d (10.0)	6.13 dd (11.0, 6.0)	6.41 d (10.0)	6.14 d (10.0)	6.24 d (10.0)	6.23 d (10.0)	6.23 d (10.5)	6.15 dd (12.0, 5.5)
23	1.12 s	1.13 s	1.22 s	1.10 s	1.12 s	1.09 s	1.24 s	1.22 s	1.23 s	1.22 s
24	1.09 s	1.07 s	1.11 s	1.08 s	1.02 s	1.01 s	1.10 s	1.11 s	1.10 s	1.05 s
25	0.82 s	0.83 s	0.86 s	0.81 s	0.82 s	0.77 s	0.85 s	0.83 s	0.98 s	0.96 s
26	1.00 s	1.00 s	1.03 s	1.00 s	1.10 s	0.97 s	1.02 s	1.00 s	1.06 s	1.10 s
27	1.84 s	1.85 s	1.87 s	1.84 s	1.84 s	1.75 s	1.84 s	1.83 s	1.84 s	1.85 s
28	3.74 d (11.0)	3.73 d (11.5)	3.79 d (10.5)	3.75 d (10.0)	3.78 d (10.0)	3.69 d (10.5)	3.76 d (10.5)	3.75 d (10.0)	3.76 d (10.5)	3.78 d (11.0)
	3.47 d (11.0)	3.46 d (12.5)	3.51 d (10.5)	3.60 d (10.0)	3.51 d (10.0)	3.43 d (10.5)	3.48 d (10.5)	3.47 d (10.0)	3.48 d (10.5)	3.61 d (11.0)
29	1.12 s	1.13 s	1.16 s	1.04 s	1.12 s	1.03 s	1.11 s	1.09 s	1.13 s	1.05 s
30	1.33 s	1.33 s	1.37 s	1.25 s	1.37 s	1.25 s	1.32 s	1.31 s	1.34 s	1.27 s
	MT_1	MT_2	Cinn	MT_1	Bz	MT_1	MT_1	MT_2	MT_1	MT_1
2	5.90 s	5.98 s	7.61brd (7.0)	5.54 s	8.33 d (8.0)	5.88 s	5.92 s	5.96 s	5.92 s	5.54 s
3			7.43 m		7.44 m					
4	2.85 m	2.08 m	7.43 m	2.72 m	7.54 m	2.78 m	2.86 m	2.07 m	2.86 m	2.70 m
5	2.30 m	2.28 m	7.43 m	2.22 m	7.44 m	2.13 m	2.34 m	2.26 m	2.32 m	2.22 m
6	5.30 t-like (7.0)	5.08 br s	7.61brd (7.0)	5.23 t-like (7.5)	8.33 d (8.0)	5.32 t-like (7.5)	5.31 t-like (7.5)	5.10 br s	5.32 t-like (7.0)	5.23 t-like (7.0)
7			8.06 d (16.0)							
8	1.67 s	1.65 s	6.87 d (16.0)	1.62 s		1.61 s	1.67 s	1.64 s	1.69 s	1.63 s
9	1.80 s	2.14 s		1.70 s		1.79 s	1.81 s	2.33 s	1.81 s	1.70 s
10	1.64 s	1.54 s		1.56 s		1.58 s	1.64 s	1.53 s	1.67 s	1.57 s
	MP	MP	MB		MB	MB	MB	MB	MP	
2	2.36 m	2.35 m	2.18 m		2.04 m	2.09 m	2.12 m	2.08 m	2.34 m	
3	1.05 d (7.0)	1.06 d (7.0)	1.63 m		1.49 m	1.55 m	1.56 m	1.52 m	1.05 d (7.0)	
			1.25 m		1.15 m	1.22 m	1.22 m	1.20 m		
4	0.99 d (7.0)	1.00 t (6.0)	0.66 t (7.5)		0.57 t (7.5)	0.66 t (7.5)	0.68 t (7.0)	0.69 t (7.5)	1.02 d (7.0)	
5			0.98 d (7.0)		0.95 d (6.5)	1.00 d (6.5)	1.05 d (6.0)	1.04 d (6.5)		
GlcA										
1	4.89 d (7.5)	4.82 d (7.5)	5.00 d (7.5)	4.88 d (7.5)	4.90 d (7.5)	4.85 d (8.0)	4.98 d (7.5)	4.98 d (7.0)		
2	4.58 m	4.55 m	4.33 m	4.56 m	4.55 m	4.26 m	4.34 m	4.35 m		
3	5.85 m	5.86 m	4.12 m	5.91 m	5.91 m	4.42 m	4.10 m	4.10 m		
4	4.36 m	4.38 m	4.50 m	4.35 m	4.38 m	4.41 m	4.50 m	4.52 m		
5	4.62 m	4.60 m	4.38 m	4.58 m	4.60 m	4.25 m	4.42 m	4.40 m		

Table 1 (Continued)

position	1	2	3	4	5	6	7	8	1a	4a
Ac-3 or CH_3CH_2-	2.38 s	2.34 s		2.37 s	2.37 s	4.21 m				
						1.16 t (7.0)				
Glc										
1	5.10 d (7.5)	5.11 d (8.0)	5.39 d (7.5)	5.08 d (8.0)	5.09 d (7.5)	5.32 d (7.5)	5.37 d (7.5)	5.37 d (7.5)		
2	3.93 m	3.94 m	4.10 m	3.93 m	3.94 m	4.00 m	4.10 m	4.08 m		
3	4.18 m	4.20 m	4.25 m	4.17 m	4.18 m	4.17 m	4.25 m	4.22 m		
4	4.10 m	4.14 m	4.32 m	4.11 m	4.11 m	4.14 m	4.32 m	4.35 m		
5	3.98 m	3.96 m	3.93 m	3.97 m	3.98 m	3.86 m	3.91 m	3.90 m		
6	4.36 m	4.34 m	4.22 m	4.33 m	4.34 m	4.30 m	4.20 m	4.18 m		
	4.60 m	4.58 m	4.48 m	4.55 m	4.54 m	4.42 m	4.46 m	4.42 m		
Ara										
1	5.88 br s	5.89 br s		5.88 br s	5.88 br s	5.63 br s				
2	4.82 m	4.82 m		4.81 m	4.81 m	4.82 m				
3	4.70 m	4.70 m		4.71 m	4.70 m	4.74 m				
4	4.67 m	4.65 m		4.66 m	4.65 m	4.72 m				
5	4.32 m	4.28 m		4.24 m	4.30 m	4.16 m				
	4.18 m	4.16 m		4.18 m	4.20 m	4.08 m				

anomeric signals (δ 110.6, 104.9, and 104.8) in the ^{13}C NMR spectrum and three anomeric proton signals (δ 4.89, 5.10, and 5.88) in the ^{1}H NMR spectrum. These observations and TLC analysis of the acid hydrolysate revealed the presence of glucuronic acid (GlcA), glucose (Glc), and arabinose (Ara) units. The sequence of this trisaccharide was determined by the analyses of DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC NMR data. The significant glycosidation shift of the C-3 signal to δ 89.8 and the cross-peak between H-1 of GlcA (δ 4.89) and C-3 of the aglycon (δ 89.8) in the HMBC spectrum indicated that the GlcA unit is connected to C-3 of the aglycon. Similarly, the linkages of Glc at C-2 of GlcA and Ara at C-4 of GlcA were indicated by the correlations between H-1 of Glc (δ 5.10) and C-2 of GlcA (δ 77.8) and between H-1 of Ara (δ 5.88) and C-4 of GlcA (δ 78.0). Furthermore, the linkage of the acetyl group at C-3 of GlcA was suggested by a cross-peak between H-3 of GlcA (δ 5.85) and C-1 of the acetyl group (δ 170.8). The coupling constants of the anomeric protons of GlcA (7.5 Hz), Glc (7.5 Hz), and Ara (br s) indicated β-configurations for the GlcA and Glc units and an α-configuration for the Ara unit. According to a reported procedure,[10] the absolute configurations of the three sugars were determined as D-glucuronic acid, D-glucose, and L-arabinose, respectively. On the basis of the above observations, the structure of **1** was elucidated as 3β-*O*-{[β-D-glucopyranosyl-(1→2)]-[α-L-arabinofuranosyl-(1→4)]-β-D-(3-*O*-acetyl)-glucuronopyranosyl}-21β-*O*-[(2*Z*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylpropanoyl)-R_1-barrigenol.

Compound **2** was obtained as a white, amorphous powder. The molecular formula was established as $C_{63}H_{98}O_{24}$ from HRESIMS at *m*/*z* 1261.6348 $[M + Na]^+$. The NMR data and TLC analysis of the hydrolysate of **2** indicated that it has the same aglycon, and the same sugar moieties were evident as in compound **1**. The only difference was the chemical shifts of the C-21 substituent group in the ^{1}H NMR and ^{13}C NMR spectra. The chemical shifts for C-4 and C-9 of the MT_1 moiety of **1** changed from δ 33.8 and 25.1 to δ 41.0 and 18.9 in **2**, respectively, and the chemical shifts of other carbons of MT_1 differed slightly between these two compounds. Furthermore, the chemical shift of H-9 of MT_1 of **1** changed from δ 1.81 to δ 2.14 in the ^{1}H NMR spectrum. By comparison with the literature,[8] the monoterpenoid unit was characterized as (2*E*)-3,7-dimethyl-2,6-octadienoic acid (MT_2) in **2**. Thus, the structure of **2** was defined as 3β-*O*-{[β-D-glucopyranosyl-(1→2)]-[α-L-arabinofuranosyl-(1→4)]-β-D-(3-*O*-acetyl)-glucuronopyranosyl}-21β-*O*-[(2*E*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylpropanoyl)-R_1-barrigenol.

Compound **3** was obtained as a white, amorphous powder. The molecular formula was deduced as $C_{56}H_{82}O_{19}$ by HRESIMS at *m*/*z* 1081.5340 $[M + Na]^+$. The NMR data and TLC analysis of the hydrolysate of **3** indicated that it has the same aglycon, GlcA, and Glc moieties as compound **1**. However, the carbon signals of the Ara moiety and the acetyl group of **1** were not observed in the ^{13}C NMR spectrum of compound **3**. The ^{1}H and ^{13}C NMR data for **3** displayed signals for two coupled doublets of *trans*-olefinic protons at δ 6.87 (1H, d, J = 16.0 Hz, H-8) and 8.06 (1H, d, J = 16.0 Hz, H-7) and five aromatic protons at δ 7.61 (2H, br d, J = 7.0 Hz, H-2, 6) and 7.43 (3H, m, H-3, 4, 5) and carbons at δ 135.0 (C-1), 128.5 (C-2, 6), 129.3 (C-3, 5), 130.5 (C-4), 144.8 (C-7), 119.6 (C-8), and 167.1 (C-9), respectively, which were attributed to a cinnamoyl moiety (Cinn). In addition, compound **3** showed carbon signals for a 2-methylbutanoyl moiety at δ 176.7 (C-1), 41.5 (C-2), 26.9 (C-3), 11.9 (C-4), and 16.9 (C-5) in the ^{13}C NMR spectrum. In the HMBC spectrum of **3**, the correlation of H-21 (δ 6.73, d, J = 10.0 Hz) of the aglycon with C-9 (δ 167.1) of the cinnamoyl unit indicated the linkage of the latter at C-21 of the aglycon. Similarly, the linkages of the 2-methylbutanoyl moiety at C-22 of the aglycon and Glc at C-2 of GlcA were determined. The structure of **3** was confirmed by analysis of the HSQC, HMBC, and ^{1}H−^{1}H COSY NMR spectra. Accordingly, the structure of **3** was elucidated

Table 2. ^{13}C NMR Spectroscopic Data (125 MHz, C_5D_5N) for Compounds **1**−**8**, **1a**, and **4a**

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1a**	**4a**
1	38.9	38.9	38.9	39.2	38.9	39.4	38.9	38.9	39.3	39.3
2	26.9	26.6	26.7	26.9	26.6	26.9	26.9	26.9	28.2	28.2
3	89.8	89.8	89.1	90.1	89.8	90.3	89.1	89.1	78.0	78.0
4	39.6	39.6	39.5	39.9	39.6	39.9	39.5	39.5	39.4	39.4
5	55.5	55.5	55.5	55.8	55.5	56.0	55.5	55.5	55.6	55.6
6	18.8	18.9	18.8	19.1	18.8	19.2	18.8	18.8	19.1	19.1
7	36.7	36.7	36.7	37.0	36.7	37.1	36.7	36.7	36.8	36.8
8	41.5	41.5	41.4	41.8	41.5	41.9	41.5	41.4	41.5	41.5
9	47.1	47.1	47.1	47.5	47.1	47.4	47.1	47.1	47.3	47.1
10	37.0	37.0	36.9	37.3	36.9	37.4	36.9	36.9	37.4	37.4
11	24.0	24.0	24.0	24.3	24.0	24.5	24.0	23.9	24.1	24.1
12	125.3	125.3	125.4	125.1	125.5	126.1	125.4	125.4	125.4	124.9
13	143.8	143.8	143.7	144.9	143.7	143.9	143.7	143.7	143.8	144.5
14	47.8	47.8	47.7	48.2	47.8	48.2	47.7	47.7	47.8	48.0
15	67.5	67.5	67.5	67.8	67.6	68.0	67.5	67.5	67.6	67.6
16	72.9	72.9	73.1	74.7	73.2	73.5	73.1	73.1	72.9	74.4
17	48.4	48.4	48.4	45.5	48.5	48.8	48.4	48.4	48.4	45.2
18	41.0	41.0	40.9	41.8	40.9	41.3	40.9	41.0	41.1	41.8
19	46.9	46.9	46.9	47.4	46.9	47.6	46.9	46.9	47.0	47.4
20	36.3	36.3	36.5	32.3	36.6	36.8	36.3	36.3	36.3	32.4
21	78.0	78.1	79.5	42.1	80.2	78.6	78.0	78.0	78.0	41.7
22	73.6	73.6	73.1	71.9	73.0	73.7	73.3	73.3	73.5	71.6
23	27.8	27.8	28.0	28.1	27.8	28.4	28.0	28.0	28.2	28.7
24	16.7	16.7	16.8	17.1	16.6	17.4	16.8	16.7	16.6	16.6
25	15.8	15.8	15.8	16.1	15.8	16.2	15.8	15.7	16.0	16.0
26	17.6	17.6	17.6	17.9	17.6	18.0	17.5	17.6	17.8	17.6
27	21.2	21.2	21.2	21.6	21.2	21.7	21.2	21.1	21.1	21.2
28	63.4	63.4	62.9	63.2	62.8	63.1	63.0	63.0	63.4	62.9
29	29.5	29.5	29.5	33.8	29.5	29.9	29.4	29.4	30.0	33.6
30	20.1	20.1	20.0	25.4	20.0	20.5	20.0	20.0	20.0	25.1
	MT_1	MT_2	Cinn	MT_1	Bz	MT_1	MT_1	MT_2	MT_1	MT_1
1	166.5	167.0	135.0	166.7	133.2	167.2	166.5	166.9	166.5	166.4
2	117.2	116.9	128.5	117.8	130.2	117.5	117.2	116.8	117.2	117.4
3	160.2	159.3	129.3	159.6	128.9	161.3	160.2	159.4	160.2	159.2
4	33.8	41.0	130.5	33.8	131.5	34.3	33.8	40.9	33.8	33.7
5	27.2	26.4	129.3	27.5	128.9	27.7	27.2	26.3	27.2	27.2
6	124.5	123.9	128.5	124.8	130.2	124.9	124.5	123.7	124.5	124.5
7	132.1	132.3	144.8	132.3	166.8	132.8	132.1	132.2	132.1	132.0
8	25.8	25.7	119.6	26.1		26.3	25.7	25.6	25.8	25.7
9	25.1	18.9	167.1	25.3		25.7	25.0	18.7	25.0	25.0
10	17.8	17.7		18.0		18.3	17.1	17.5	17.8	17.7
	MP	MP	MB		MB	MB	MB	MB	MP	
1	177.0	177.0	176.7		176.6	177.7	176.7	176.7	177.0	
2	34.5	34.5	41.5		41.5	42.1	41.4	41.6	34.5	
3	19.2	19.1	26.9		26.8	27.4	26.6	26.6	19.1	
4	19.3	19.2	11.9		11.8	12.4	11.9	11.8	19.3	
5			16.9		16.6	17.2	16.8	16.8		
6										
7										
GlcA										
1	104.9	104.7	105.3	105.2	104.9	105.5	105.2	105.2		
2	77.8	78.1	82.8	78.3	78.0	80.8	82.7	82.7		
3	75.4	75.5	77.1	75.7	75.4	75.3	77.0	77.0		
4	78.0	78.2	73.3	78.1	77.8	77.8	73.2	73.2		
5	76.2	76.1	77.7	76.5	76.2	76.1	77.7	77.7		
6	171.8	171. 8	172.8	172.2	171.9	170.3	173.2	172.8		
Ac-3	170.9	170.8		171.1	170.9	62.5				
	22.0	21.9		22.3	22.0	14.6				
Glc										
1	104.8	104.7	105.9	105.1	104.7	105.1	105.9	105.9		
2	75.4	75.4	75.0	75.7	75.4	76.8	75.2	75.4		
3	78.4	78.4	77.9	78.6	78.4	78.1	77.9	77.8		
4	72.4	72.4	71.6	72.7	72.4	72.0	71.6	71.7		
5	78.2	78.3	78.3	78.6	78.3	78.8	78.3	78.2		
6	63.6	63.7	62.6	63.9	63.6	63.4	62.7	62.6		
Ara										
1	110.6	110.5		110.9	110.6	109.3				
2	83.4	83.3		83.7	83.4	83.2				
3	78.3	78.4		78.7	78.0	78.7				
4	86.1	86.1		86.4	86.1	87.0				
5	62.6	62.7		63.0	62.7	62.8				

as 3β-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-21β-*O*-cinnamoyl-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **4** was obtained as a white, amorphous powder. The molecular formula was established as $C_{59}H_{92}O_{22}$ from the HRESIMS at *m*/*z* 1175.5977 $[M + Na]^+$. By careful analysis of the NMR data, it could be determined that **4** has the same sugar moiety and the same substituent group, MT_1, as compound **1**. Furthermore, signals for a 2-methylpropanoyl moiety were not observed in the

^{13}C NMR spectrum of compound **4**. Acid hydrolysis of **4** afforded **4a**, which then hydrolyzed with alkaline to afford needle crystals (**4b**). The ^{1}H and ^{13}C NMR spectroscopic and optical rotation data of **4b** were in agreement with those of A_1-barrigenol.[6] The ^{1}H−^{1}H COSY spectrum showed the coupling of H-22 (δ 6.13, dd, J = 6.0, 11.0 Hz) to two protons at δ 1.98 (1H, m) and 2.74 (1H, m). The signal for C-21 at δ 42.1 in the ^{13}C NMR spectrum clearly indicated that no substituent was connected to C-21. In the HMBC spectrum, the correlation between H-22 of A_1-barrigenol and C-1 (δ 166.7) of the MT_1 unit showed that the latter group was attached to C-22. The structure of **4** was supported by HSQC, HMBC, and ^{1}H−^{1}H COSY NMR experiments. Thus, the structure of **4** was elucidated as 3β-*O*-{[β-D-glucopyranosyl-(1→2)]-[α-l-arabinofuranosyl-(1→4)]-β-D-(3-*O*-acetyl)-glucuronopyranosyl}-22α-*O*-[(2*Z*)-3,7-dimethyl-2,6-octadienoyl]-A_1-barrigenol.

Compound **5** was obtained as a white, amorphous powder with a molecular formula of $C_{61}H_{90}O_{24}$, based on a sodiated molecular ion peak at *m*/*z* 1229.5713 $[M + Na]^+$ in its HRESIMS. The NMR data and TLC analysis of the hydrolysate of **5** indicated that it has the same R_1-barrigenol, GlcA, Ara, and Glc moieties as in compound **1**. Compound **5** did not show any signals for either a MT_1 (monoterpenoid) moiety or a 2-methylpropanoyl group in its NMR spectra. The ^{1}H and ^{13}C NMR data for **5** displayed signals for five aromatic protons at δ 8.33 (2H, brd, J = 8.0 Hz, H-2, 6), 7.44 (2H, m, H-3, 5), and 7.54 (1H, m, H-4) and carbons at δ 133.2 (C-1), 130.2 (C-2, 6), 128.9 (C-3, 5), 131.5 (C-4), and 166.8, respectively, which were attributed to a benzoyl moiety (Bz). In addition, the ^{1}H NMR and ^{13}C NMR spectra showed a doublet methyl signal [δ 0.95 (d, J = 6.5 Hz)] and a triplet methyl signal [δ 0.57 (t, J = 7.5 Hz)] and carbon signals at δ 176.6, 41.5, 26.8, 11.8, and 16.6, which were attributed to a 2-methylbutanoyl group. In the HMBC spectrum of **5**, the correlation of H-21 (δ 6.80, d, J = 10.0 Hz) of the aglycon with C-7 (δ 166.8) of the benzoate unit indicated the linkage of the latter group at C-21 of the aglycon. Similarly, the linkage of the 2-methylbutanoyl group was determined at C-22 of the aglycon. The structure of **5** was confirmed from its HSQC, HMBC, and ^{1}H−^{1}H COSY NMR data. Accordingly, the structure of **5** was elucidated as 3β-*O*-{[β-D-glucopyranosyl-(1→2)]-[α-l-arabinofuranosyl-(1→4)]-β-D-(3-*O*-acetyl)-glucuronopyranosyl}-21β-*O*-benzoyl-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **6** was obtained as a white, amorphous powder, and its molecular formula was determined as $C_{64}H_{102}O_{23}$ on the basis of the molecular ion peak at *m*/*z* 1261.6736 $[M + Na]^+$ in the HRESIMS. The NMR data and TLC analysis of the hydrolysate of **6** indicated that it has the same R_1-barrigenol, MT_1, GlcA, Ara, and Glc moieties as in compound **1**. Compound **6** did not show the signals of either an acetyl or a 2-methylpropanoyl group in its NMR spectra. Instead, proton signals for an ethyl group [δ 4.21 (2H, m) and 1.16 (3H, t, J = 7.0 Hz), δ 62.5, 14.6] were apparent. In addition, the ^{1}H NMR and ^{13}C NMR spectra showed a doublet methyl signal [δ 0.95 (d, J = 6.5 Hz)] and a triplet methyl signal [δ 0.66 (t, J = 7.5 Hz)] and carbon signals at δ 177.7, 42.1, 27.4, 12.4, and 17.2, which were attributed to a 2-methylbutanoyl group. In the HMBC spectrum of **6**, the correlation of H-1 (δ 4.21, m) of the ethyl group with C-6 (δ 170.3) of the GlcA moiety indicated the linkage of the ethyl group at C-6 of GlcA. Similarly, the linkage of a 2-methylbutanol group at C-22 of the aglycon was determined. The structure of this isolate was confirmed from the HSQC, HMBC, and ^{1}H−^{1}H COSY NMR spectra. Accordingly, the structure of **6** was elucidated as 3β-*O*-{[β-D-glucopyranosyl-(1→2)]-[α-l-arabinofuranosyl-(1→4)]-β-D-(6-*O*-ethyl)-glucuronopyranosyl}-21β-*O*-[(2*Z*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol,

Compound **7** was obtained as a white, amorphous powder with a molecular formula of $C_{57}H_{90}O_{19}$, based on the sodiated molecular ion peak at *m*/*z* 1101.5992 in its HRESIMS. The NMR data and TLC analysis of the hydrolysate of **7** indicated that it has the same R_1-barrigenol, 2-methylbutanoyl, MT_1, GlcA, and Glc moieties as in compound **6**. Compound **7** did not show any signals for either an Ara moiety or an ethyl group in its ^{1}H and ^{13}C NMR spectra. The structure of **7** was confirmed by interpretation of its HSQC, HMBC, and ^{1}H−^{1}H COSY NMR data. Thus, the structure of **7** was elucidated as 3β-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-21β-*O*-[(2*Z*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **8** was obtained as a white, amorphous powder. Its molecular formula was determined as $C_{57}H_{90}O_{19}$ on the basis of the sodiated molecular ion peak at *m*/*z* 1101.6033 in its HRESIMS. The NMR data and TLC analysis of the hydrolysate of **8** indicated that it has the same R_1-barrigenol, 2-methylbutanoyl, GlcA, and Glc moieties as compound **7**, with the only difference being the NMR chemical shifts of the C-21 substituent group, which indicated the presence of a MT_2 moiety instead of a MT_1 moiety. The structure of **8** was confirmed from its HSQC, HMBC, and ^{1}H−^{1}H COSY NMR data. Accordingly, the structure of **8** was determined as 3β-*O*-[β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-21β-*O*-[(2*E*)-3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

In our previous studies,[3−5] 11 triterpenoid saponins, symplocososides C−K (**9**−**17**) and symplocososides X, Y (**18**, **19**), were isolated from the roots of *S. chinensis*. The results of the tests of cytotoxicity against five cancer cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) of these compounds (**1**−**19**) and seven chemical derivatives are presented in Table 3. Most saponins showed cytotoxicity against several of the cancer cell lines used except for compounds **1a**, **4a**, **10a**, **13a**, **1b**, **4b**, and **15b**. The aglycons (**1a**, **4a**, **10a**, and **13a**) and the alkaline hydrolysates (**1b**, **4b**, and **15b**) possessed no activity at all, indicating that both the sugar and the ester parts of compounds in this structural group are necessary for activity. By comparing differences in the influence of different substituted groups at C-21 or C-22 on cytotoxicity, some preliminary conclusions can be made. An activity ranking showed compound **1** > compound **2**, compound **7** > compound **8**, compound **9** > compound **10**, and compound **13** > compound **14**, in order of potency against the HCT-8 and Bel-7402 cancer cell lines, which indicates that the MT_1 group may have a greater contribution toward the mediation of cytotoxicity than the MT_2 group. Compound **13** was more active than compound **1** against

Table 3. Cytotoxic Activities of Compounds **1**−**19**, **1a**, **1b**, **4a**, **4b**, **10a**, **13a**, and **15b**[a,b]

compound	HCT-8 (IC_{50} μM)	Bel-7402 (IC_{50} μM)	BGC-823 (IC_{50} μM)	A549 (IC_{50} μM)	A2780 (IC_{50} μM)
1	1.7	2.7	>10.0	>10.0	2.4
2	2.7	3.8	>10.0	>10.0	2.3
3	1.8	1.7	>10.0	>10.0	3.2
4	1.7	2.4	>10.0	>10.0	>10.0
5	>10.0	>10.0	>10.0	>10.0	3.3
6	>10.0	>10.0	>10.0	>10.0	5.5
7	4.4	4.1	>10.0	>10.0	1.9
8	>10.0	>10.0	>10.0	>10.0	1.9
9	2.9	1.5	4.3	>10.0	1.3
10	>10.0	1.89	>10.0	>10.0	1.74
11	>10.0	1.7	>10.0	>10.0	1.7
12	4.0	>10.0	>10.0	>10.0	>10.0
13	2.0	1.8	1.9	2.1	1.4
14	2.1	2.6	2.5	2.7	1.4
15	1.7	2.7	>10.0	>10.0	3.2
16	2.1	1.7	1.8	3.0	1.4
17	2.4	5.1	>10.0	4.0	0.8
18	1.7	1.8	>10.0	2.1	1.6
19	>10.0	>10.0	>10.0	>10.0	3.4
adriamycin	0.8	1.0	1.0	0.2	0.1

[a] Values are means of three experiments. [b] Compounds **1a**, **1b**, **4a**, **4b**, **10a**, **13a**, and **15b** were inactive (IC_{50} > 10.0 μM) for all cell lines.

four cancer cell lines (except for HCT-8), and compound **14** was more active than compound **2** against all five cancer cell lines, indicating that the 2-methylbutanoyl moiety has a greater influence on the enhancement of activity than the 2-methylpropanoyl moiety. Compounds **3** and **6**−**12** exhibited cytotoxicity against the HCT-8, Bel-7402, and A549 cancer cell lines. However, the replacement of the OH group at C-3 of the glucuronopyranosyl acid moiety by an acetoxy group resulted in reduced selectivity for the cancer cells investigated.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4 micromelting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 241 automatic digital polarimeter. IR spectra were recorded using a Nicolet-Impact 400 IR spectrometer with KBr disks. UV spectra were obtained on a Shimadzu UV-260 spectrometer. ^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC NMR experiments were performed on an INOVA 500 FT-NMR spectrometer. TMS was used as internal standard. HRESIMS were measured on a Bruker FT-MS APEXIII 7.0T spectrometer. HRFABMS were recorded on an Auto spec Ultima-TOF spectrometer. Silica GF_{254} for TLC and silica gel (200−300 mesh) for column chromatography were obtained from Qingdao Marine Chemical Company (Qingdao, People's Republic of China). RP-18 (25−40 μm) silica gel was purchased from Fuji Silysica Chemical Ltd. The authentic sugars, α-methylbenzylamine, and $NaBH_3CN$ were obtained from Aldrich. HPLC was carried out on a Shimadazu LC-6AD instrument, and the detector was a SPD-10A model. Solvents were analytical and chromatographic grades and purchased from Beijing Chemical Company, Beijing, People's Republic of China. A reversed-phase C_{18} column (YMC-Pack ODS-A ϕ 20 × 250 mm, 5 μm) was also employed.

Plant Material. The roots of *Symplocos chinenesis* were collected in January 1997 from Nanning City, Guangxi Province, People's Republic of China, and the plant was identified by Prof. Shouyang Liu, Department of Pharmacognosy, Guangxi College of Chinese Traditional Medicine. The roots were harvested and air-dried at room temperature in darkness. A voucher specimen (002771) was deposited in the herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

Extraction and Isolation. The dried roots (22 kg) of *S. chinenesis* were ground and extracted with 95% EtOH, and 2.7 kg of extract was obtained, which was partitioned with EtOAc, *n*-BuOH, and H_2O, respectively. The *n*-BuOH-soluble extract (1090 g) exhibited cytotoxicity against the KB and A549 cancer cell lines, with IC_{50} values of 2.7 and 4.9 μg/mL, respectively. A part of the *n*-BuOH extract (180 g) was chromatographed on a silica gel column, eluting with CH_2Cl_2−MeOH, to afford seven fractions (I−VII). These fractions were evaluated for cytotoxic activity. Fraction III showed cytotoxicity against the HCT-8, Bel-7402, A549, and KB cancer cell lines, with IC_{50} values of 38.3, 18.0, 34.4, and 24.1 μg/mL, respectively. Fraction III was separated over a Sephadex LH-20 column eluting with MeOH to yield four subfractions, Fr_1−Fr_4. Compounds **5** (t_R 39.7 min, 76 mg) and **6** (t_R 42.5 min, 22 mg) from Fr_2 and compounds **7** (t_R 52.4 min, 32 mg) and **8** (t_R 53.2 min, 66 mg) from Fr_4 were obtained by a preparative HPLC column with MeOH−H_2O (82:18). Fr_3 was subjected to RP-18 silica gel column chromatography using a gradient mixture of MeOH−H_2O to give fractions A−F. Compound **1** (t_R 36.8 min, 72 mg) from fraction B, compound **2** (t_R 37.4 min, 38 mg) from fraction C, compound **4** (t_R 47.4 min, 168 mg) from fraction D, and compound **3** (t_R 45.6 min, 56 mg) from fraction F were obtained by preparative HPLC with MeOH−H_2O (80:20).

Compound 1: white, amorphous powder; mp 248−250 °C; $[\alpha]_D^{25}$ −34.5 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ϵ) 235 (3.52) nm; IR ν_{max} (KBr) 3436, 2966, 1718, 1647, 1444, 1377, 1244, 1163, 1074, 1043, 802 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1261 [M + Na]$^+$; ESIMS^2 *m/z* 1129 [M + Na − Ara]$^+$; HRESIMS *m/z* 1261.6333 [M + Na]$^+$ (calcd for $C_{63}H_{98}O_{24}Na$, 1261.6340).

Compound 2: white, amorphous powder; mp 267−269 °C; $[\alpha]_D^{25}$ −25.7 (*c* 1.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (3.28) nm; IR ν_{max} (KBr) 3429, 2968, 1678, 1435, 1390, 1205, 1142, 1074, 1045, 800 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1261 [M + Na]$^+$, ESIMS^2 *m/z* 1129 [M + Na − Ara]$^+$; HRESIMS *m/z* 1261.6348 [M + Na]$^+$ (calcd for $C_{63}H_{98}O_{24}Na$, 1261.6340).

Compound 3: white, amorphous powder; mp 268−270 °C; $[\alpha]_D^{25}$ −18.6 (*c* 0.70, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.71) nm; IR ν_{max} (KBr) 3429, 2966, 2885, 1680, 1639, 1450, 1390, 1205, 1074, 1045, 802, 723 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1081 [M + Na]$^+$; HRESIMS *m/z* 1081.5340 [M + Na]$^+$ (calcd for $C_{56}H_{82}O_{19}Na$, 1081.5342).

Compound 4: white, amorphous powder; mp 258−260 °C; $[\alpha]_D^{25}$ −40 (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ϵ) 233 (3.81) nm; IR ν_{max} (KBr) 3408, 2952, 1678, 1437, 1377, 1244, 1205, 1074, 1041, 800, 723 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1175 [M + Na]$^+$, 1043 [M + Na − Ara]$^+$; HRESIMS *m/z* 1175.5977 [M + Na]$^+$ (calcd for $C_{59}H_{92}O_{22}$-Na, 1175.5972).

Compound 5: white, amorphous powder; mp 236−238 °C; $[\alpha]_D^{25}$ −36.8 (*c* 1.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 229 (2.44) nm; IR ν_{max} (KBr) 3440, 2966, 1682, 1452, 1279, 1203, 1074, 1045, 947, 714 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1229 [M + Na]$^+$, ESIMS^2 *m/z* 1097 [M + Na − Ara]$^+$; HRESIMS *m/z* 1229.5713 [M + Na]$^+$ (calcd for $C_{61}H_{90}O_{24}Na$, 1229.5720).

Compound 6: white, amorphous powder; mp 216−218 °C; $[\alpha]_D^{25}$ −18.5 (*c* 1.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (1.68) nm; IR ν_{max} (KBr) 3428, 2972, 1688, 1645, 1458, 1385, 1235, 1156, 1076, 802 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1261 [M + Na]$^+$; HRESIMS *m/z* 1261.6736 [M + Na]$^+$ (calcd for $C_{64}H_{102}O_{23}Na$, 1261.6710).

Compound 7: white, amorphous powder; mp 226−228 °C; $[\alpha]_D^{25}$ −26.8 (*c* 1.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (2.60) nm; IR ν_{max} (KBr) 3408, 2968, 1682, 1458, 1390, 1205, 1140, 1076, 839, 802, 723 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1101 [M + Na]$^+$; HRESIMS *m/z* 1101.5992 [M + Na]$^+$ (calcd for $C_{57}H_{90}O_{19}Na$, 1101.5974).

Compound 8: white, amorphous powder; mp 235−236 °C; $[\alpha]_D^{25}$ −19.8 (*c* 0.97, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (2.99) nm; IR ν_{max} (KBr) 3406, 2968, 1684, 1458, 1390, 1205, 1140, 1076, 839, 802, 723 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 1101 [M + Na]$^+$; HRESIMS *m/z* 1101.6033 [M + Na]$^+$ (calcd for $C_{57}H_{90}O_{19}Na$, 1101.5974).

Acid Hydrolysis of Compounds 1 and 4. Compounds **1** (27 mg) and **4** (25 mg) were refluxed with 10% HCl (10 mL) at 75 °C for 3 h. Each reaction mixture was extracted with CH_2Cl_2, and the organic phase was washed with H_2O and evaporated to yield **1a** (16 mg) and **4a** (14 mg), respectively. The water layer was neutralized with 1 N NaOH and then concentrated and analyzed by TLC (CH_2Cl_2−MeOH−H_2O, 7:3:0.4) and paper chromatography (*n*-BuOH−HOAc−H_2O, 4:1:2) with authentic sugars.

21β-*O*-[(2Z)-3,7-Dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylpropanoyl)-R$_1$-barrigenol (1a): colorless needles; mp 171−173 °C; $[\alpha]_D^{25}$ +4.5 (*c* 0.90, MeOH); UV (MeOH) λ_{max} (log ϵ) 250 (3.64) nm; IR ν_{max} (KBr) 3437, 2966, 2929, 1714, 1645, 1464, 1388, 1248, 1163, 1074, 1018, 856 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 749 [M + Na]$^+$; HRFABMS *m/z* 727.5169 [M + H]$^+$ (calcd for $C_{44}H_{71}O_8$, 727.5149).

22α-*O*-[(2Z)-3,7-Dimethyl-2,6-octadienoyl]-A$_1$-barrigenol (4a): colorless needles; mp 166−168 °C; $[\alpha]_D^{25}$ +12 (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.63) nm; IR ν_{max} (KBr) 3410, 2958, 2920, 2850, 1684, 1645, 1464, 1388, 1377, 1254, 1161, 1041, 860, 802 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 125 MHz), see Table 2; ESIMS *m/z* 663 [M + Na]$^+$; HRFABMS *m/z* 641.4798 [M + H]$^+$ (calcd for $C_{40}H_{65}O_6$, 641.4781).

Alkaline Hydrolysis of Compounds 1a and 4a. Compounds **1a** (14 mg) and **4a** (12 mg) were each refluxed for 4 h in a solution of 5% KOH (5 mL). The reaction mixtures were extracted with CH_2Cl_2 (5 mL × 3), and the organic phase was washed with H_2O and evaporated to yield **1b** (7 mg) and **4b** (6 mg), respectively.

Determination of Absolute Configuration of the Sugar Units of 1−8. The absolute configuration of the sugars present in **1**−**8** was confirmed according to a reported procedure.[10]

Cytotoxicity Assay. The cytotoxic activities of compounds **1**−**19**, **1a**, **1b**, **4a**, **4b**, **10a**, **13a**, and **15b** against several cancer cell lines were evaluated by the MTT method, as described previously.[4]

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