Cytotoxic Triterpenoid Saponins from *Symplocos chinensis*

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Six new triterpenoid saponins were isolated as methyl or butyl esters from an *n*-BuOH extract of the roots of *Symplocos chinensis*. Their structures were established as symplocososides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), and F (**6**), by extensive 1D and 2D NMR as well as HR-MS analysis and chemical methods. Compounds **1**, **3**, and **6** were cytotoxic against one or more cell lines, and the derivative from **1** (**1d**) showed significant selectivities between KB cells and normal cells.

Symplocos chinensis (Lour.) Druce (Symplocaceae) is a toxic herb distributed in Guangxi Province, China. It has been used as a folk medicine to treat tumefaction, enteritis, nephritis, and snake bite. In our investigations, the BuOH-soluble extract exhibited significant cytotoxic activity, and six new triterpenoid saponins (**1**−**6**) were isolated from this extract. Herein, we report the structural elucidation of these triterpenoid saponins and their cytotoxic activities against several tumor cell lines.

Results and Discussion

Symplocososide A (**1**) was obtained as a white amorphous powder and had a molecular formula of $C_{63}H_{100}O_{23}$, which was determined from its positive ion HRESIMS and confirmed by ^{13}C NMR and DEPT analysis. The 1D NMR spectra revealed the presence of seven tertiary methyl groups resonating between δ 0.87 and 1.84 and a double bond with typical ^{13}C NMR resonances (δ 125.3 and 143.8), indicating an olean-12-ene triterpene derivative.[1] The ^1H NMR spectrum exhibited three tertiary methyl signals (δ 1.68, 1.82, and 1.65) and two olefinic protons (δ 5.93 and 5.32), which were attributed to a (2*Z*)-3,7-dimethyl-2,6-octadienoyl,[2] monoterpenoid unit (MT_1), one methyl triplet [δ 0.68 (3H, t, J = 7.5 Hz, H-4)], and one methyl doublet [δ 1.05 (3H, d, J = 7.5 Hz, H-5)] attributed to a 2-methylbutanoyl moiety (MB).[6] The ^1H NMR also exhibited two doublets at δ 6.64 (d, J = 10.5 Hz) and 6.25 (d, J = 10.5 Hz), suggesting the presence of acylated oxymethine units at C-21 and C-22. As observed in the HMBC spectrum, the correlations of H-21 of the aglycon with C-1 (δ 166.5) of MT_1 and H-22 of the aglycon with the C-1 (δ 176.7) of MB established that MT_1 and MB were attached to C-21 and C-22 of the aglycon, respectively. Acid hydrolysis of **1** afforded the 21,22-disubstituted aglycon (**1a**), and three sugars were detected: glucuronic acid, glucose, and arabinose. The upfield shift of C-3 ($\Delta\delta$ −11.3 ppm) suggested that the sugar moiety was attached to C-3 of the aglycon. The ^1H NMR and ^{13}C NMR signals of the two acyl groups (MT_1 and MB) were further assigned by a combination of HMQC, HMBC, and $^1H-^1H$ COSY experiments. Alkaline hydrolysis of **1a** yielded the aglycon R_1-barrigenol (**1b**), which was elucidated on the basis of ^1H NMR and ^{13}C NMR spectra and from observation of connectivities in COSY, HMQC, and HMBC experiments of **1a**. The set of data of **1b** was in full agreement with those reported in the literature.[3] The upfield shifts of H-21 (δ 4.81) and H-22 (δ 4.60) of **1b** also indicated they were the positions of the acylation. The stereochemistry of the aglycon was established by a NOESY experiment and the information from the vicinal coupling constants of the key protons. The sugar moiety at C-3 was assigned as having β-equatorial orientation because of the cross-peak between H-3 and H-5 as well as the methyl protons at C-23. H-15 was correlated with methyl protons at C-26 (δ 1.03, s) in the NOESY spectrum, indicating that the 15-OH group is α-equatorial. The small coupling constant ($J_{15,16}$ = 4.0 Hz, observed from the ^1H NMR spectrum of **1a**) between H-15 and H-16 suggested that H-16 is β-equatorial. In the NOESY experiment, the cross-peaks between H-21 (δ 6.64) and H-19 (δ 3.09), as well as H-22 (δ 6.25) and H-18 (δ 3.08), suggested that H-21 and H-22 are α- and β-axial, respectively, which means that the acyl groups at C-21 and C-22 are β- and α-equatorial, respectively. Thus, the structure of **1a** was elucidated as 21β-*O*-[(2*Z*)3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

The identification of the sugar moiety was established by chemical degradation, HPLC analysis, and extensive NMR experiments. The sugar moiety of **1** consisted of three residues, with anomeric signals at δ 105.1, 105.5, and 108.8 in the ^{13}C NMR spectrum, attached to proton signals at δ 4.92 (d, J = 7.5 Hz), 5.39 (d, J = 7.5 Hz), and 5.78 (br s), respectively (HSQC expt). On enzymatic hydrolysis with cellulase, which is used to hydrolyze 1→4 glycosidic linkages, **1** provided prosapogenin **1c** and arabinose (Ara). The carbon signals at δ 108.8, 82.8, 78.4, 87.2, and 62.4 of **1**, which did not emerge in the ^{13}C NMR spectrum of **1c**, suggested the presence of a terminal arabinose of **1**. The furanose nature of this sugar was determined by a long-range correlation between C-4 (δ 87.2) and H-1 (d 5.78). Identification of glucose (Glc) was based on HSQC, $^1H-^1H$ COSY, and TOCSY experiments. The ^{13}C signals at δ 106.0, 76.8, 77.8, 71.7, 78.3, and 62.7 of **1c** were attributed to glucose. The glucuronic acid carbonyl of **1c** resonated at δ 170.5 and showed a correlation with the methyl protons of an *O*-methyl group; the carbonyl also coupled with H-4 (δ 4.22) of the same sugar in HMBC. The ^{13}C NMR signals of the glucuronic acid of **1c** were fully determined in the $^1H-^1H$ COSY and $^{13}C-^1H$ COSY experiments. In the HMBC spectrum of **1**, a correlation between H-1 (δ 5.39) of glucose and C-2 (δ 81.6) of glucuronic acid indicated that glucose was attached to C-2 of the glucuronic acid. Similarly, the linkage of arabinose to C-4 of glucuronic acid was indicated by the resonance between H-1 (δ 5.78) of arabinose and C-4 (δ 78.0) of glucuronic acid; the cleavage of arabinose on enzymatic hydrolysis also proved the 1→4 connection.

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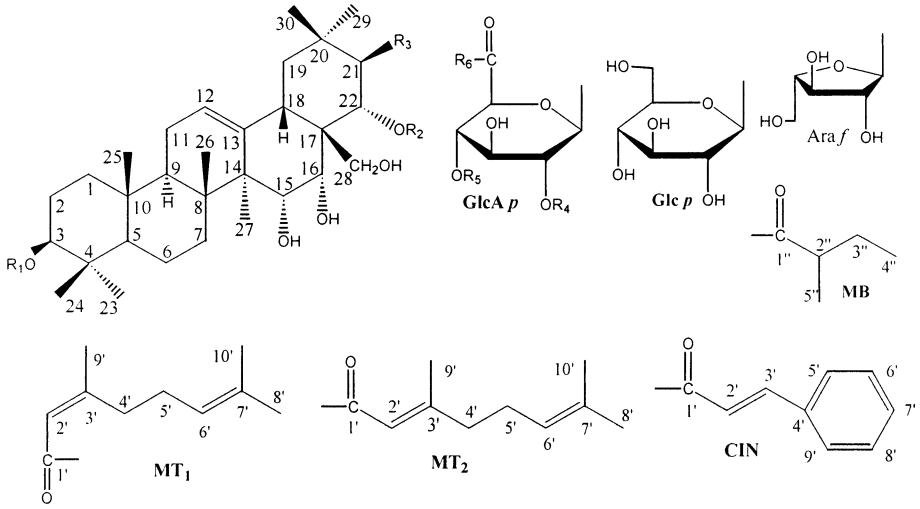
Chart 1



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1	GlcA *p*	MB	OMT_1	Glc *p*	Ara *f*	OCH_3
1a	H	MB	OMT_1			
1b	H	H	OH			
1c	GlcA *p*	MB	OMT_1	Glc *p*	H	OCH_3
1d	GlcA *p*	H	OH	Glc *p*	Ara *f*	OCH_3
2	GlcA *p*	MB	OMT_2	Glc *p*	Ara *f*	OCH_3
3	GlcA *p*	MB	OMT_1	Glc *p*	Ara *f*	$O(CH_2)_3CH_3$
4	GlcA *p*	MB	OMT_2	Glc *p*	Ara *f*	$O(CH_2)_3CH_3$
4a	H	MB	OMT_2			
5	GlcA *p*	MB	OCIN	Glc *p*	Ara *f*	$O(CH_2)_3CH_3$
6	GlcA *p*	MT_1	H	Glc *p*	Ara *f*	$O(CH_2)_3CH_3$

H-1 (δ 4.92) of glucuronic acid showed a correlation with the carbon atom that resonated at δ 89.3 (C-3) of the aglycon. Absolute configurations of the three sugars were determined by an HPLC method. [4] HPLC retention times of each sugar derivative were compared with those of the authentic samples prepared in the same manner. Thus the sugar components of **1** were defined as β-D-glucuronic acid (GlcA *p*), β-D-glucose (Glc *p*), and α-L-arabinose (Ara *f*), and compound **1** was elucidated as 3β-*O*-{[β-D-glucopyranosyl-(1→2)][α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside methyl ester}-21β-*O*-[(2*Z*)3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **2** was isolated as a white amorphous powder, and the molecular formula was $C_{63}H_{100}O_{23}$ as determined by combined HRESIMS and ^{13}C NMR. Comparison of the NMR data (see Tables 1−4) indicated that **2** had the same aglycon and the same sugar moiety at C-3 as **1**, and the only difference was the chemical shifts of the monoterpenoid unit. In the ^{13}C NMR spectrum, the carbon resonances at δ 33.8 of MT_1-4 and δ 25.0 of MT_1-9 of **1** changed into δ 41.1 and 18.9, respectively, and the chemical shifts of the other carbons of MT_1 differed slightly. In the ^1H NMR spectrum, the resonance of MT_1-9 at δ 1.82 changed to δ 2.30. By comparison with ref 2, the monoterpenoid unit was characterized as (2*E*)-3,7-dimethyl-2,6-octadienoic acid (MT_2). Sugar analysis was carried out using the same method described above for **1**. Thus, compound **2** was defined as 3β-*O*-{[β-D-glucopyranosyl(1→2)][α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside methyl ester}-21β-*O*-[(2*E*)3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **3** was isolated as a white amorphous powder, and its molecular formula was deduced as $C_{66}H_{106}O_{23}$ on the basis of HRESIMS and ^{13}C NMR analysis. The NMR data of this compound revealed that **3** had the same aglycon, the same two acyl groups (MT_1 and MB), and the same three sugars as those obtained for **1**. However, proton (δ 3.72) and carbon (δ 52.0) signals of the *O*-methyl group were absent and four carbon signals [δ 65.4 (C-1), 30.7 (C-2), 19.2 (C-3), 13.7 (C-4)] appeared, which were assigned to an *O*-butyl group. HMBC correlations were observed between H-1 (δ 4.22, m) of the butyl group and the carbonyl (δ 169.6) of glucuronic acid. These data suggested the replacement of the methyl group of glucuronic acid with a butyl group in **1**, which is consistent with the molecular formula. On the basis of the above findings and information from $^1H-^1H$ COSY, HSQC, HMBC, NOESY, and TOCSY experiments, compound **3** was elucidated to be 3β-*O*-{[β-D-glucopyranosyl(1→2)][α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside butyl ester}-21β-*O*-[(2*Z*)3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **4** was isolated as a white amorphous powder, and its positive HRESIMS showed a molecular formula of $C_{66}H_{106}O_{23}$. Acid hydrolysis of **4** afforded **4a**, 21,22-disubstituted R_1-barrigenol. The NMR data of compound **4a** were similar to those obtained for **1a**, and the only difference

Table 1. ^{13}C NMR Data (δ) for the Triterpenoid Moieties of Compounds **1**−**6**, **1a**, **1b**, **1c**, **1d**, and **4a**[a]

C	1	1a	1b	1c	1d	2	3	4	4a	5	6
1	39.0	39.3	39.4	39.0	39.0	39.0	39.0	39.0	39.3	39.0	39.0
2	26.6	28.2	28.2	26.7	26.7	26.6	26.6	26.6	27.8	26.6	26.6
3	89.3	78.0	78.1	89.2	89.2	89.4	89.3	89.3	78.1	89.3	89.3
4	39.5	39.4	39.4	39.5	39.5	39.5	39.5	39.5	39.2	39.5	39.5
5	55.5	55.6	55.6	55.6	55.6	55.6	55.5	55.5	55.6	55.5	55.5
6	18.8	19.2	19.1	18.8	18.8	18.8	18.8	18.9	18.9	18.8	18.7
7	36.7	37.4	36.8	36.7	36.7	36.8	36.7	36.7	36.7	36.7	36.6
8	41.4	41.6	41.5	41.4	42.0	41.5	41.5	41.5	41.5	41.5	41.4
9	47.2	47.3	47.5	47.2	47.5	47.2	47.2	47.2	47.3	47.2	47.2
10	37.0	36.9	37.5	37.0	37.0	37.0	37.0	37.0	37.3	37.0	37.0
11	24.0	24.1	24.1	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
12	125.3	125.5	124.5	125.4	124.7	125.4	125.3	125.4	125.7	125.4	124.7
13	143.8	143.7	144.8	143.8	144.8	143.8	143.8	143.8	143.4	143.8	144.6
14	47.8	47.9	47.4	47.8	47.8	47.5	47.8	47.8	47.8	47.8	47.8
15	67.5	67.6	67.4	67.5	67.3	67.5	67.5	67.5	67.6	67.5	67.5
16	73.1	73.1	72.4	73.1	74.0	73.1	73.1	73.1	73.1	73.1	74.4
17	48.4	48.5	48.1	48.4	48.1	48.5	48.5	48.4	48.4	48.5	45.2
18	40.9	41.0	42.0	40.9	41.4	41.0	40.9	40.9	40.9	40.9	41.6
19	46.9	47.0	47.8	46.9	47.3	47.0	46.9	46.9	46.9	46.9	47.1
20	36.4	36.4	36.4	36.4	36.4	36.4	36.4	36.3	36.3	36.5	32.0
21	78.0	78.0	78.4	78.0	77.5	78.0	78.0	78.0	78.2	78.0	41.8
22	73.3	73.3	77.2	73.3	77.1	73.3	73.3	73.3	73.3	73.1	71.6
23	27.9	28.7	28.7	28.0	28.0	28.0	28.0	28.0	28.7	28.0	28.0
24	16.8	16.8	16.6	16.8	16.8	16.9	16.8	16.9	16.6	16.9	16.8
25	15.8	15.8	16.0	15.8	15.9	15.8	15.8	15.8	15.9	15.8	15.8
26	17.6	17.7	17.6	17.6	17.6	17.6	17.6	17.6	17.7	17.6	17.5
27	21.2	21.1	21.1	21.2	21.2	21.2	21.2	21.2	21.1	21.2	21.2
28	63.0	63.1	67.8	63.1	63.5	63.1	63.1	63.0	62.9	63.0	62.9
29	29.5	29.5	30.6	29.5	30.0	29.5	29.5	29.5	29.4	29.5	33.5
30	20.0	20.0	19.4	20.2	19.4	20.0	20.0	20.1	20.1	20.0	24.9

[a] 125 MHz in pyridine-d_5.

Table 2. ^{13}C NMR Data (δ) for the Acyl Moieties of Compounds **1**−**6**, **1a**, **1c**, and **4a** (125 MHz in pyridine-d_5)

C	1	1a	1c	2	3	4	4a	5	6
	MT_1	MT_1	MT_1	MT_2	MT_1	MT_2	MT_2		MT_1
1	166.5	166.5	166.5	167.0	166.5	167.0	167.2		166.4
2	117.2	117.2	117.2	116.9	117.2	116.9	116.7		117.4
3	160.2	160.2	160.2	159.4	160.2	159.4	160.0		159.2
4	33.8	33.8	33.8	41.1	33.8	41.0	41.0		33.5
5	27.2	27.2	27.2	26.4	27.2	26.4	26.3		27.2
6	124.5	124.5	124.5	123.1	124.5	124.0	124.0		124.4
7	132.1	132.1	132.1	132.3	132.1	132.3	132.4		132.0
8	25.7	25.7	25.8	25.8	25.7	25.7	25.7		25.7
9	25.0	25.0	25.1	18.9	25.0	18.9	19.0		25.1
10	17.8	17.8	17.8	17.7	17.8	17.6	17.6		17.7
	MB	MB	MB	MB	MB	MB	MB	MB	
1	176.7	176.8	176.7	176.7	176.7	176.7	177.1	176.7	
2	41.6	41.6	41.6	41.6	41.6	41.6	41.6	41.6	
3	26.9	26.9	27.0	27.0	27.0	26.9	26.9	26.9	
4	11.9	11.9	11.9	11.9	11.9	11.9	11.9	11.9	
5	16.8	16.6	16.8	16.8	16.8	16.8	16.9	16.8	
								CIN	
1								167.1	
2								119.7	
3								144.9	
1′								135.1	
2′6′								128.6	
3′5′								129.3	
4′								130.6	

Table 3. ^{13}C NMR Data (δ) for the Sugar Moieties of Compounds **1**−**6**, **1c**, and **1d** (125 MHz in pyridine-d_5)

C	1	1c	1d	2	3	4	5	6
GlcA								
1	105.1	105.4	105.0	105.1	105.1	105.1	105.1	105.1
2	81.6	82.1	81.5	81.6	81.7	81.7	81.7	81.6
3	74.9	77.1	74.8	74.8	75.0	75.0	75.0	75.0
4	78.0	72.9	78.0	78.0	78.0	78.0	78.0	78.0
5	75.8	78.0	76.0	75.7	75.8	75.8	75.8	75.8
6	169.6	170.5	170.5	170.0	169.6	169.6	169.6	169.6
OMe	52.0	52.1	52.1	52.4				
butyl[a]-1					65.4	65.4	65.4	65.4
butyl-2					30.7	30.7	30.7	30.7
butyl-3					19.2	19.2	19.2	19.2
butyl-4					13.7	13.7	13.7	13.7
Glc								
1	105.5	106.0	105.4	105.4	105.5	105.5	105.5	105.4
2	76.9	76.8	76.8	76.9	76.9	76.9	76.9	76.9
3	78.4	77.8	78.6	78.4	78.5	78.4	78.4	78.5
4	71.7	71.7	71.6	71.8	71.7	71.7	71.7	71.8
5	78.4	78.3	78.6	78.4	78.5	78.5	78.5	78.5
6	62.8	62.7	62.7	62.8	62.8	62.8	62.8	62.8
Ara								
1	108.8		108.0	108.9	108.8	108.9	108.9	108.8
2	82.8		82.7	82.8	82.8	82.8	82.8	82.8
3	78.4		78.4	78.3	78.4	78.5	78.5	78.3
4	87.2		86.6	87.2	87.3	87.3	87.3	87.3
5	62.4		62.3	62.4	62.4	62.4	62.4	62.4

[a] Butyl = butyl group.

was the chemical shifts of the monoterpenoid unit, which was identified as MT_2. Through complete analysis of the 1D and 2D NMR spectra, compound **4** was defined as 3β-*O*-{[β-D-glucopyranosyl(1→2)][α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside butyl ester}-21β-*O*-[(2*E*)3,7-dimethyl-2,6-octadienoyl]-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **5** was isolated as a white amorphous powder, and its molecular formula was deduced as $C_{65}H_{98}O_{23}$ by HRESIMS and ^{13}C NMR. Careful examination of the ^{1}H and ^{13}C NMR data revealed that **5** was structurally identical to **3** with respect to the aglycon, the 2-methylbutanoyl group, and the sugar moiety. However, the ^{1}H and ^{13}C NMR data for **5** displayed signals for two coupled doublets of trans olefinic protons (δ 6.88 and 8.06), and five aromatic protons and carbons, which were attributed to a cinnamoyl moiety (CIN). In the HMBC experiments, the correlations of H-21 of the aglycon with C-1 of CIN and H-22 of the aglycon with C-1 of the 2-methylbutanoyl group clearly indicated that the monoterpenoid unit in **3** was substituted with a cinnamoyl moiety. Hence, **5** is 3β-*O*-{[β-

Table 4. ^{1}H NMR Data (δ) for the Sugar Moieties of Compounds **1**−**6**, **1c**, and **1d** (500 MHz in pyridine-d_5)

position	**1**	**1c**	**1d**	**2**	**3**	**4**	**5**	**6**
GlcA								
1	4.92 (J = 7.5 Hz)	4.97 (J = 7.5 Hz)	4.93 (J = 7.5 Hz)	4.91 (J = 7.5 Hz)	4.90 (J = 8.0 Hz)	4.90 (J = 7.5 Hz)	4.91 (J = 6.5 Hz)	4.84 (J = 8.0 Hz)
2	4.30 m	4.33 m	4.30 m	4.30 m	4.31 m	4.30 m	4.30 m	4.27 m
3	4.51 m	4.53 m	4.51 m	4.51 m	4.53 m	4.53 m	4.53 m	4.44 m
4	4.22 m	3.80 m	4.23 m	4.22 m	4.24 m	4.24 m	4.23 m	4.21 m
5	4.29 m	4.22 m	4.29 m	4.28 m	4.30 m	4.30 m	4.29 m	4.28 m
OMe	3.72 s	3.71 s	3.71 s	3.72 s				
butyl[a]-1					4.22 m	4.29 m	4.24 m	4.28 m
butyl-2					1.58 m	1.59 m	1.59 m	1.55 m
butyl-3					1.30 m	1.31 m	1.32 m	1.26 m
butyl-4					0.78 (t, J = 7.0 Hz)	0.78 (t, J = 7.0 Hz)	0.78 (t, J = 7.0 Hz)	0.73 (t, J = 7.0 Hz)
Glc								
1	5.39 (J = 7.5 Hz)	5.40 (J = 7.5 Hz)	5.39 (J = 7.5 Hz)	5.41 (J = 7.5 Hz)	5.40 (J = 7.5 Hz)	5.40 (J = 7.5 Hz)	5.40 (J = 7.5 Hz)	5.34 (J = 8.0 Hz)
2	4.08 m	4.20 m	4.11 m	4.08 m	4.09 m	4.09 m	4.07 m	3.98 m
3	4.20 m	4.13 m	4.21 m	4.18 m	4.78 m	4.81 m	4.80 m	4.73 m
4	4.29 m	4.31 m	4.32 m	4.29 m	4.31 m	4.30 m	4.29 m	4.17 m
5	3.93 m	3.91 m	3.93 m	3.92 m	3.91 m	3.92 m	3.92 m	3.87 m
6	4.38 m	4.41 m	4.38 m	4.39 m	4.41 m	4.44 m	4.42 m	4.44 m
	4.49 m	4.50 m	4.47 m	4.49 m	4.51 m	4.48 m	4.53 m	4.27 m
Ara								
1	5.78 (br s)		5.77 (br s)	5.70 (br s)	5.79 (br s)	5.79 (br s)	5.79 (br s)	5.69 (br s)
2	4.79 m		4.70 m	4.79 m	4.79 m	4.78 m	4.78 m	4.73 m
3	3.93 m		3.91 m	3.93 m	3.94 m	3.96 m	3.93 m	3.87 m
4	4.85 m		4.82 m	4.88 m	4.88 m	4.89 m	4.89 m	4.84 m
5	4.13 m		4.15 m	4.13 m	4.12 m	4.13 m	4.14 m	4.09 m
	4.24 m		4.28 m	4.25 m	4.25 m	4.23 m	4.27 m	4.18 m

[a] Butyl = butyl group.

D-glucopyranosyl(1→2)][α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside butyl ester}-21β-*O*-cinnamoyl-22α-*O*-(2-methylbutanoyl)-R_1-barrigenol.

Compound **6** was isolated as a white amorphous powder, and its molecular formula was deduced as $C_{61}H_{98}O_{21}$. Analysis of the ^{13}C NMR suggested that **6** had the same sugar moiety and the same substituent, MT_1, as compound **3**. No MB was observed in the ^{13}C NMR of **6**. The aglycon was elucidated as 22-substituted A_1-barrigenol[5] by analysis of the ^{1}H and ^{13}C NMR (see Tables 1 and 2) and from the observation of connectivities in the ^{1}H−^{1}HCOSY, HSQC, and HMBC spectra. The ^{13}C NMR signal for C-21 (δ 41.8) clearly indicated that no substituent was connected to C-21. The^{1}H NMR spectrum showed H-22 (δ 6.05, dd, J = 5.5, 12.0 Hz) coupled to two protons at δ 1.93 (dd, J = 13.5, 3.0 Hz, H-21ax) and 2.98 (dd, J = 13.5, 3.0 Hz, H-21eq). In the HMBC spectrum, a correlation between H-22 of A_1-barrigenol and a carbonyl (δ 166.4) of MT_1 showed that MT_1 was attached to C-22. The stereochemistries of H-3, H-15, and H-16 of the triterpene moiety were established by a NOESY experiment. H-22 was correlated with H-18 (δ 2.70, m) in the NOESY spectrum, indicating that the H-22 is β-axial, which suggested that MT_1 at C-22 is α-equatorial. The sugar analysis was carried out using the same method for **1**. From the above evidence, the structure of **6** was deduced to be 3β-*O*-{[β-D-glucopyranosyl(1→2)]-[α-L-arabinofuranosyl(1→4)]-β-D-glucuronopyranoside butyl ester}-22α-*O*-[(2*Z*)3,7-dimethyl-2,6-octadienoyl]-A_1-barrigenol.

The cytotoxic activities of compounds **1**, **1d**, **3**, **4**, **5**, and **6** were evaluated against a panel of cancer cell lines (Table 5). After the cells were treated for 120 h, the cell growth was measured with an MTT assay procedure, and the IC_{50} values were calculated from a dose-dependent curve on KB, HCT-8, A549, MCF-7, and BGC-823 cells. Compound **3** exhibited cytotoxic activity against the HCT-8 cells (IC_{50} = 2.86 μg/mL), **6** showed cytotoxic activities against the HCT-8 cells (IC_{50} = 4.04 μg/mL) and BGC-823 cells (IC_{50} = 7.29 μg/mL), and **1d**, the derivative from alkaline hydrolysis of **1**, showed good activity against KB cells (IC_{50} = 0.3 μg/mL) and weak activity against normal human cells (HELF) (IC_{50} > 10 μg/mL). Thus, **1d** was selectively cytotoxic against the KB cells with over 33 times the potency of normal cells.

Table 5. Evaluation of the Cytotoxic Potential of Compounds **1**, **1d**, **3**, and **6**

	cell line[a] IC_{50} (μg/mL)				
sample	KB	HCT-8	A549	BGC-823	HELF
1	1.72	4.31	0.67		4.62
1d	0.30	6.80	0.66		>10
3		2.86		7.29	
6		4.04			

[a] KB = human epidermoid carcinoma; HCT-8 = human colon carcinoma; A549 = human lung carcinoma; BGC-823 = human stomach carcinoma; HELF = human embryo lung fibroblast.

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micromelting point apparatus (uncorrected). Optical rotations were measured on a Perkin-Elmer digital polarimeter. UV spectra were recorded on a Shimadzu UV-260 spectrometer. IR spectra were determined on a Perkin-Elmer 683 infrared spectrometer as KBr pellets. NMR spectra were taken with TMS as internal standard on an Inova 500 FT-NMR spectrometer. HRESIMS were measured on a Bruker FTMS APEXIII 7.0T spectrometer. Silica GF254 for TLC and silica gel (200−300 mesh) for CC were produced by Qingdao Marine Chemical Company, Qingdao, China. Solvents and chemicals were of analytical grade and purchased from Beijing Chemical Company, Beijing, China. Preparative HPLC was performed with a Shimadzu SPD-6AD pump connected with a Shimadzu SPD-10A VP detector. A reversed-phase C_{18} column (YMC-Pack ODS-A Φ 20 × 250 mm, 5 μm) was employed.

Plant Material. The roots of *Symplocos chinenesis* (Lour.) Druce were collected in November 1998 from Guangxi Prov-

ince, China. It was identified by Prof. Liu Shouyang, Department of Pharmacognosy, Guangxi College of Chinese Traditional Medicine. The roots were harvested and air-dried at room temperature in darkness. A voucher specimen was deposited in the Institute of Materia Medica, Chinese Academy of Medical Sciences.

Extraction and Isolation. The dried roots (22 kg) were ground and extracted with EtOH, and 2710 g of extract was obtained. The extract was partitioned with EtOAc, BuOH, and H_2O. A part of the BuOH extract (180 g) was chromatographed to give 15 fractions on a silica gel column using gradient solvents of CH_2Cl_2−MeOH−H_2O as eluents. The crude saponin fraction III (1170 mg) was then repeatedly subjected over Rp-18 silica gel with MeOH−H_2O (79:21) to afford an amorphous powder, following by HPLC purification with MeCN−H_2O (78:22) to yield **1** (110 mg) and **2** (30 mg). The crude saponin fraction IV (1820 mg) yielded compounds **3** (78 mg), **4** (66 mg), **5** (51 mg), and **6** (17 mg) on preparative HPLC with MeOH−H_2O (86:14).

Symplocososide A (1): amorphous white powder; mp 189−191 °C; $[\alpha]^{18}_D$ −29° (*c* 0.99, MeOH); IR (KBr) ν_{max} 3413, 2929, 1716, 1645, 1074 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.22 (1H, dd, J = 4.0, 11.0 Hz, H-3), 5.52 (1H, br s, H-12), 4.16 (1H, H-15), 4.41 (1H, H-16), 6.64 (1H, d, J = 10.5 Hz, H-21), 6.25 (1H, d, J = 10.5 Hz, H-22), 1.19 (3H, s, H-23), 1.07 (3H, s, H-24), 0.87 (3H, s, H-25), 1.03 (3H, s, H-26), 1.84 (3H, s, H-27), 3.49 (1H, d, J = 11.0 Hz, H-28), 3.76 (1H, d, J = 11.0 Hz, H-28), 1.13 (3H, s, H-29), 1.33 (3H, s, H-30); MT$_1$: 5.93 (1H, s, H-2′), 5.32 (1H, br s, H-6′), 1.68 (3H, s, H-8′), 1.82 (3H, s, H-9′), 1.65 (3H, s, H-10′); MB: 0.68 (3H, t, J = 7.5 Hz, H-4″), 1.05 (3H, d, J = 7.5 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; ESIMS (negative ion mode)-*m/z* 1223 [M − H]$^-$; HRESIMS (positive ion mode) *m/z* 1247.6552 [M + Na]$^+$ (calcd for $C_{63}H_{100}O_{23}Na$, 1247.6547).

Symplocososide B (2): amorphous white powder; mp 189−191 °C; $[\alpha]^{18}_D$ −23° (*c* 1.02, MeOH); IR(KBr) ν_{max} 3415, 2962, 1710, 1635, 1075 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.18 (1H, dd, 4.0, J = 11.5 Hz, H-3), 5.52 (1H, br s, H-12), 4.20 (1H, H-15), 5.85 (1H, H-16), 6.64 (1H, d, J = 10.5 Hz, H-21), 6.25 (1H, d, J = 10.5 Hz, H-22), 1.12 (3H, s, H-23), 1.08 (3H, s, H-24), 0.85 (3H, s, H-25), 1.02 (3H, s, H-26), 1.83 (3H, s, H-27), 3.49 (1H, d, J = 11.0 Hz, H-28), 3.80 (1H, d, J = 11.0 Hz, H-28), 1.13 (3H, s, H-29), 1.34 (3H, s, H-30); MT$_2$: 5.93 (1H, s, H-2′), 5.33 (1H, br s, H-6′), 1.68 (3H, s, H-8′), 2.30 (3H, s, H-9′), 1.65 (3H, s, H-10′); MB: 0.67 (3H, t, J = 7.5 Hz, H-4″), 1.05 (3H, d, J = 7.5 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; ESIMS (negative ion mode)-*m/z* 1223 [M − H]$^-$; HRESIMS (positive ion mode) *m/z* 1247.6554 [M + Na]$^+$ (calcd for $C_{63}H_{100}O_{23}Na$, 1247.6547).

Symplocososide C (3): amorphous white powder; mp 217−219 °C; $[\alpha]^{24}_D$ −23.3° (*c* 1.03, MeOH); UV (MeOH) λ_{max} 210.8 nm; IR (KBr) ν_{max} 3413, 2962, 2877, 1716, 1645, 1464, 1388, 1225, 1074, 889, 658 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.22 (1H, dd, J = 4.0, 11.0 Hz, H-3), 5.52 (1H, br s, H-12), 4.19 (1H, H-15), 4.39 (1H, d, J = 4.5 Hz, H-16), 6.63 (1H, d, J = 10.0 Hz, H-21), 6.25 (1H, d, J = 10.0 Hz, H-22), 1.19 (3H, s, H-23), 1.07 (3H, s, H-24), 0.87 (3H, s, H-25), 1.03 (3H, s, H-26), 1.84 (3H, s, H-27), 3.50 (1H, d, J = 7.0 Hz, H-28), 3.77 (1H, d, J = 7.0 Hz, H-28), 1.13 (3H, s, H-29), 1.33 (3H, s, H-30); MT$_1$: 5.93 (1H, s, H-2′), 5.32 (1H, br s, H-6′), 1.68 (3H, s, H-8′), 1.82 (3H, s, H-9′), 1.65 (3H, s, H-10′); MB: 0.69 (3H, t, J = 7.5 Hz, H-4″), 1.05 (3H, d, J = 7.5 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; HRESIMS (positive ion mode) *m/z* 1289.6992 [M + Na]$^+$ (calcd for $C_{66}H_{106}O_{23}Na$, 1289.7017).

Symplocososide D (4): amorphous white powder; mp 213−215 °C; $[\alpha]^{24}_D$ −15.8° (0.70, MeOH); UV (MeOH) λ_{max} 211.8 nm; IR (KBr) ν_{max} 3417, 2964, 2877, 1716, 1645, 1456, 1377, 1159, 1074, 1022 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.22 (1H, dd, J = 4.0, 11.0 Hz, H-3), 5.52 (1H, br s, H-12), 4.20 (1H, H-15), 4.39 (1H, d, J = 4.5 Hz, H-16), 6.65 (1H, d, J = 10.0 Hz, H-21), 6.26 (1H, d, J = 10.0 Hz, H-22), 1.20 (3H, s, H-23), 1.06 (3H, s, H-24), 0.88 (3H, s, H-25), 1.03 (3H, s, H-26), 1.85 (3H, s, H-27), 3.50 (1H, d, J = 9.0 Hz, H-28), 3.77 (1H, d, J = 9.0 Hz, H-28), 1.14 (3H, s, H-29), 1.33 (3H, s, H-30); MT$_2$: 5.79 (1H, s, H-2′), 5.39 (1H, br s, H-6′), 1.65 (3H, s, H-8′), 2.35 (3H, s, H-9′), 1.55 (3H, s, H-10′); MB: 0.71 (3H, t, J = 7.2 Hz, H-4″), 1.08 (3H, t, J = 7.2 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; HRESIMS (positive ion mode) *m/z* 1289.6990 [M + Na]$^+$ (calcd for $C_{66}H_{106}O_{23}Na$, 1289.7017).

Symplocososide E (5): amorphous white powder; mp 211−213 °C; $[\alpha]^{24}_D$ −21.4° (*c* 1.02, MeOH); UV (MeOH) λ_{max} 205.4, 275.8 nm; IR (KBr) ν_{max} 3410, 2962, 2877, 1716, 1635, 1464, 1388, 1282, 1203, 1074, 891, 864, 768, 660 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.24 (1H, dd, J = 3.5, 12.0 Hz, H-3), 5.54 (1H, br s, H-12), 4.21 (1H, H-15), 4.41 (1H, d, J = 5.0 Hz, H-16), 6.73 (1H, d, J = 10.0 Hz, H-21), 6.37 (1H, d, J = 10.0 Hz, H-22), 1.20 (3H, s, H-23), 1.04 (3H, s, H-24), 0.88 (3H, s, H-25), 1.08 (3H, s, H-26), 1.86 (3H, s, H-27), 3.52 (1H, d, J = 7.0 Hz, H-28), 3.86 (1H, d, J = 7.0 Hz, H-28), 1.16 (3H, s, H-29), 1.38 (3H, s, H-30); CIN: 6.88 (1H, d, J = 16.0 Hz, H-2′), 8.06 (1H, d, J = 16.0 Hz, H-3′), 7.61(2H, m, H-5′, 9′), 7.34 (2H, m, H-6′, 8′), 7.34 (1H, m, H-7′); MB: 0.66 (3H, t, J = 7.5 Hz, H-4″), 0.99 (3H, d, J = 7.5 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; HRESIMS (positive ion mode) *m/z* 1269.6389 [M + Na]$^+$ (calcd for $C_{63}H_{98}O_{23}Na$, 1269.6391).

Symplocososide F (6): amorphous white powder; mp 234−236 °C; $[\alpha]^{24}_D$ −24.3° (*c* 0.70, MeOH); UV (MeOH) λ_{max} 205.6 nm; IR (KBr) υ_{max} 3398, 2927, 1734, 1684, 1645, 1456, 1385, 1163, 1070, 1041, 650 cm^{-1}; ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.17 (1H, dd, J = 4.0, 12.0 Hz, H-3), 5.49 (1H, m, H-12), 4.23 (1H, H-15), 4.48 (1H, d, J = 4.0 Hz, H-16), 2.98 (1H, dd, H-21), 1.93 (1H, dd, H-21), 6.05 (1H, dd, J = 5.5, 12.0 Hz, H-22), 1.26 (3H, s, H-23), 1.06 (3H, s, H-24), 0.86 (3H, s, H-25), 1.07 (3H, s, H-26), 1.85 (3H, s, H-27), 3.53 (1H, d, J = 10.5 Hz, H-28), 3.70 (1H, d, J = 10.5 Hz, H-28), 1.19 (3H, s, H-29), 1.03 (3H, s, H-30); MT$_1$: 5.58 (1H, s, H-2′), 5.18 (1H, br s, H-6′), 1.58 (3H, s, H-8′), 1.69 (3H, s, H-9′), 1.52 (3H, s, H-10′); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3; HRESIMS (positive ion mode) *m/z* 1189.6489 [M + Na]$^+$ (calcd for $C_{61}H_{98}O_{23}Na$, 1189.6492).

Acid Hydrolysis[7] of 1. Compound **1** (35 mg) was refluxed with 7% HCl at 70 °C for 3 h. The reaction mixture was extracted with CH_2Cl_2, and the organic phase was washed with H_2O and evaporated to yield **1a** (15 mg). The aqueous phase was retained to study the sugar components by HPLC and checked by HPTLC (silica gel), together with the authentic sugar samples (CH_2Cl_2−MeOH−H_2O). The monosaccharides were identified as arabinose, glucose, and glucuronic acid.

Compound 1a: amorphous white powder; mp 235−237 °C; $[\alpha]^{18}_D$ +12° (*c* 0.97, MeOH); ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.48 (1H, dd, J = 4.5,10.0 Hz, H-3), 5.56 (1H, br s, H-12), 4.22 (1H, d, J = 4.0 Hz, H-15), 4.38 (1H, d, J = 4.0 Hz, H-16), 6.57 (1H, d, J = 10.5 Hz, H-21), 6.21 (1H, d, J = 10.5 Hz, H-22), 1.24 (3H, s, H-23), 1.10 (3H, s, H-24), 0.98 (3H, s, H-25), 1.06 (3H, s, H-26), 1.85 (3H, s, H-27), 3.51 (1H, d, J = 10.5 Hz, H-28), 3.79 (1H, d, J = 10.5 Hz, H-28), 1.14 (3H, s, H-29), 1.35 (3H, s, H-30); MT$_1$: 5.90 (1H, s, H-2′), 5.32 (1H, br s, H-6′), 1.68 (3H, s, H-8′), 1.82 (3H, s, H-9′), 1.65 (3H, s, H-10′); MB: 0.69 (3H, t, J = 7.0 Hz, H-4″), 1.05 (3H, d, J = 7.0 Hz, H-5″); ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m/z* 763 [M + Na]$^+$, 781 [M + Na + H_2O]$^+$; HRESIMS (positive ion mode) *m/z* 763.5115 [M + Na]$^+$ (calcd for $C_{45}H_{72}O_8Na$, 763.5119).

Alkaline Hydrolysis[8] of 1a. Compound **1a** (12 mg) was refluxed for 3 h in a solution of 3% KOH (5 mL). The reaction mixture was extracted with CH_2Cl_2 (5 mL), and the organic phase was washed with H_2O and evaporated to yield **1b** (3 mg).

Compound 1b: amorphous white powder; mp > 300 °C; $[\alpha]^{18}_D$ +36° (*c* 0.98, MeOH); ^{1}H NMR (pyridine-d_5, 500 MHz) δ 0.98 (3H, s, H-25), 1.05 (3H, s, H-26), 1.10 (3H, s, H-24), 1.22 (3H, s, H-29), 1.34 (3H, s, H-23), 1.38 (3H, s, H-30), 1.85 (3H, s, H-27), 3.48 (1H, dd, J = 4.0, 11.0 Hz, H-3), 4.10 (1H, d, J = 5.0 Hz, H-15), 4.43 (1H, d, J = 5.0 Hz, H-16), 4.60 (1H, d, J = 9.5 Hz, H-22), 4.81 (1H, d, J = 9.5 Hz, H-21); ^{13}C NMR, see Table 1; ESIMS (positive ion mode) *m/z* 529 [M + Na]$^+$.

Enzymic Hydrolysis[9] of 1. Cellulase (Shanghai boao Bio. Co., Ltd.) was added to a solution of **1** (20 mg) in 20% EtOH (2 mL), and the mixture was incubated at 37 °C for 4 days, then extracted with BuOH. Arabinose in the aqueous phase was detected by HPTLC. The BuOH extract was purified on semipreparative HPLC (MeOH−H_2O, 82:18) to give **1c** (5 mg).

Compound 1c: amorphous white powder; mp 256−261 °C; $[\alpha]^{18}_D$ −18° (*c* 1.03, MeOH); ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.28 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.52 (1H, br s, H-12), 4.24 (1H, H-15), 4.40 (1H, H-16), 6.63 (1H, d, J = 10.0 Hz, H-21), 6.25 (1H, d, J = 10.0 Hz, H-22), 1.25 (3H, s, H-23), 1.11 (3H, s, H-24), 0.87 (3H, s, H-25), 1.03 (3H, s, H-26), 1.84 (3H, s, H-27), 3.49 (1H, d, J = 11.0 Hz, H-28), 3.77 (1H, d, J = 11.0 Hz, H-28), 1.13 (3H, s, H-29), 1.34 (3H, s, H-30); MT_1: 5.93 (1H, s, H-2′), 5.33 (1H, br s, H-6′), 1.68 (3H, s, H-8′), 1.82 (3H, s, H-9′), 1.65 (3H, s, H-10′); MB: 0.68 (3H, t, J = 6.5 Hz, H-4″), 1.05 (3H, d, J = 6.5 Hz, H-5″); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1−3.

Alkaline Hydrolysis[8] of 1. Compound **1** (35 mg) was refluxed for 3 h in a solution of 5% KOH (5 mL) and EtOH (5 mL). After acidification with 2 N HCl until pH 4, the reaction mixture was extracted with BuOH. The BuOH extract was purified on silica gel ($CHCl_3$−MeOH−H_2O, 7:3:0.2) to give **1d** (11 mg).

Compound 1d: amorphous white powder; mp 203−205 °C; $[\alpha]^{18}_D$ −24° (*c* 0.95, MeOH); ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.22 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.52 (1H, br s, H-12), 4.16 (1H, H-15), 4.41 (1H, H-16), 4.64 (1H, d, J = 10.0 Hz, H-21), 4.24 (1H, d, J = 10.0 Hz, H-22), 1.20 (3H, s, H-23), 1.07 (3H, s, H-24), 0.85 (3H, s, H-25), 1.03 (3H, s, H-26), 1.83 (3H, s, H-27), 3.50 (1H, d, J = 11.5 Hz, H-28), 3.76 (1H, d, J = 11.5 Hz, H-28), 1.13 (3H, s, H-29), 1.33 (3H, s, H-30); ^{1}H NMR of sugar moieties, see Table 4; ^{13}C NMR, see Tables 1 and 3.

Acid Hydrolysis[7] of 4. Compound **4** (25 mg) was treated in the same manner as **1** to afford **4a** (6 mg).

Compound 4a: amorphous white powder; mp 235−237 °C; $[\alpha]^{24}_D$ +14° (*c* 0.95, MeOH); ^{1}H NMR (500 MHz in pyridine-d_5) δ 3.39 (1H, dd, J = 4.0, 11.0 Hz, H-3), 5.55 (1H, br s, H-12), 4.18 (1H, d, J = 4.0 Hz, H-15), 4.36 (1H, d, J = 4.0 Hz, H-16), 6.49 (1H, d, J = 10.5 Hz, H-21), 6.15 (1H, d, J = 10.5 Hz, H-22), 1.12 (3H, s, H-23), 0.96 (3H, s, H-24), 0.86 (3H, s, H-25), 1.04 (3H, s, H-26), 1.75 (3H, s, H-27), 3.47 (1H, d, J = 10.5 Hz, H-28), 3.72 (1H, d, J = 10.5 Hz, H-28), 1.03 (3H, s, H-29), 1.26 (3H, s, H-30), MT_2: 5.89 (1H, s, H-2′), 5.04 (1H, br s, H-6′), 1.59 (3H, s, H-8′), 2.28 (3H, H-9′), 1.49 (3H, s, H-10′); MB: 0.6 9 (3H, t, J = 7.0 Hz, H-4″), 1.02 (3H, d, J = 7.0 Hz, H-5″); ^{1}H NMR (500 MHz in pyridine-d_5) 3.22 (1H, dd, J = 4.0, 11.5 Hz, H-3), 5.52 (1H, br s, H-12), 4.16 (1H, H-15), 4.41 (1H, H-16), 4.64 (1H, d, J = 10.0 Hz, H-21), 4.24 (1H, d, J = 10.0 Hz, H-22), 1.20 (3H, s, H-23), 1.07 (3H, s, H-24), 0.85 (3H, s, H-25), 1.03 (3H, s, H-26), 1.83 (3H, s, H-27), 3.50 (1H, d, J = 11.5 Hz, H-28), 3.76 (1H, d, J = 11.5 Hz, H-28), 1.13 (3H, s, H-29), 1.33 (3H, s, H-30); ^{13}C NMR, see Tables 1 and 2; ESIMS (positive ion mode) *m/z* 763 $[M + Na]^+$, 781 $[M + Na + H_2O]^+$; HRESIMS (positive ion mode) *m/z* 763.5112 $[M + Na]^+$ (calcd for $C_{45}H_{72}O_8Na$, 763.5119).

Determination of Absolute Configuration of Sugars.[4] A solution of L-(−)-MBA (α-methylbenzylamine) (10 mg) and $NaBH_3CN$ (2 mg) in 0.2 mL of CH_3OH was added to a solution of a sugar (10 mg) in 0.2 mL of H_2O. The mixture was allowed to stand overnight, acidified to pH 3−4 by addition of glacial acetic acid, and evaporated to dryness. The resultant oily material was acetylated by acetic anhydride−dry pyridine (1:1) (1 mL) at 100 °C for 1 h in a sealed tube. After codistillation of the acetic anhydride with toluene, H_2O (1 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (1 mL). The $CHCl_3$ layer was evaporated to dryness to give an oily residue, which was purified by preparative TLC and normal HPLC in turn and subjected to HPLC (Inertsil SIL-100A, 5 μm, Φ 4.6 × 250 mm; eluant, *n*-hexane−ethanol (95:5); flow rate, 1.2 mL/min; detection at 230 nm, 0.04 aufs). The retention times of the derivatives of each sugar were determined: t_R (min): D-glucose (39.9), L-glucose (38.0), d-arabinose (30.0), L-arabinose (34.5), D-glucuronic acid (22.2), L-glucuronic acid was not found to test. The aqueous phase obtained from the acid hydrolysis of each symplocososide (**1**−**6**) was treated as the authentic sample in the same manner described above, and HPLC retention times of the hydrolysate derivatives were compared with those of the authentic samples.

Cell Culture and Assay for Cytotoxic Activity. The cells 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. The cells were washed and resuspended in the above medium to 3×10^4 cells/mL, and 196 μL of this cell suspension was placed in each well of a 96-well plate. The cells were incubated in 5% CO_2 air for 24 h at 37 °C. After incubation, 4 μL of EtOH−H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.001−10 μg/mL; 4 μL of EtOH−H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by an MTT assay procedure. [15] After termination of the cell culture, 10 μL of 5 mg/mL MTT in phosphate-buffered saline was added to every well and the plate was further reincubated in 5% CO_2 air for 4 h at 37 °C. The plate was then centrifuged to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from every well, and 175 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 550 nm. A dose−response curve was plotted for each compound, and the concentration giving 50% inhibition (IC_{50}) was calculated (Table 5).

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