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Cytotoxic Dihydroagarofuranoid Sesquiterpenes from the Seeds of *Celastrus orbiculatus*

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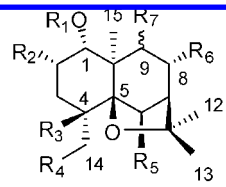
A chemical study on the seeds of *Celastrus orbiculatus* has led to the isolation of nine new (**1**−**9**) and 13 known dihydro-β-agarofuran derivatives. The identification and structural elucidation of the new compounds were based on spectroscopic data analysis, and the absolute configurations of compounds **1**−**6**, **8**−**10**, and **16**, as well as derivatives **2a** and **6a**, were determined by CD studies or by chemical methods. All compounds isolated were evaluated for cytotoxic activity against HL-60 human leukemia cells.

Celastrus orbiculatus Thunb. (Celastraceae) is a perennial shrub that has been used in Chinese folk medicine as a treatment for rheumatoid arthritis and bacterial infections.[1] The family Celastraceae is well known for producing various dihydro-β-agarofuran derivatives, which have attracted much interest due to their broad range of biological activities such as insecticidal,[2] reversal of the multidrug resistance (MDR) phenotype,[3,4] cytotoxic,[5] antitumor-promoting,[6] antitubercular,[7] immunosuppressive,[8] and anti-inflammatory effects.[9] As part of an ongoing search for new bioactive metabolites from plants used in traditional Chinese medicine, a chemical investigation has been undertaken on the seeds of *C. orbiculatus*. Herein, we report the isolation and structural elucidation of nine new sesquiterpenes (**1**−**9**) and 13 known secondary metabolites, along with their cytotoxic activity against HL-60 human leukemia cells.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	Bz	H	H	H	OAc	OBut	α-OBz
2	H	H	H	H	OAc	OAc	α-OBz
3	H	H	H	H	OAc	OH	α-OBz
4	Bz	H	H	H	OH	OBz	α-OBz
5	Cin	H	H	H	OAc	OH	α-OBz
6	Ac	OH	H	H	OAc	H	β-OCin
7	Ac	OAc	OH	H	H	H	β-OCir
8	Ac	H	H	OH	OBz	H	β-OBz
9	Ac	H	H	H	OH	H	β-OBz
10	Bz	H	H	H	OAc	OBz	α-OBz
11	Ac	H	H	H	OAc	OAc	β-OBz
12	Bz	H	H	H	OBz	H	β-OAc
13	Ac	H	H	H	H	H	β-OCin
14	Cin	OAc	H	H	H	H	β-OAc
15	H	OAc	H	H	H	H	β-OCin
16	Ac	H	H	H	OCin	H	β-OBz
2a	Bz	H	H	H	OAc	OAc	α-OBz
6a	Ac	OBz	H	H	OAc	H	β-OCin

Results and Discussion

Powdered, air-dried seeds of *C. orbiculatus* (10.0 kg) were extracted with 95% EtOH at room temperature (3 × 72 h). After removal of solvent, the aqueous residue was partitioned in sequence with petroleum ether and EtOAc, yielding petroleum ether and EtOAc fractions. The two fractions were subjected to a series of chromatographic steps to afford nine new dihydro-β-agarofuran derivatives (**1**−**9**) and 13 known metabolites.

Compound **1**, isolated as a white, amorphous powder, showed an accurate $[M + Na]^+$ ion at *m/z* 629.2727 in the HRESIMS, corresponding to the molecular formula $C_{35}H_{42}O_9Na$. It displayed IR absorptions indicative of the presence of ester groups at 1743 and 1727 cm^{-1}. The UV spectrum exhibited an absorption maximum at 274 nm, which suggested the existence of aromatic moieties.[7] The ^1H NMR spectrum of **1** (Table 1) indicated the presence of signals due to one acetyl group at δ 2.12 (3H, s), two benzoyl groups at δ 7.61 (2H, d, $J = 7.4$ Hz), 6.92 (2H, t, $J = 7.4$ Hz), 7.18 (1H, t, $J = 7.4$ Hz), and 7.59 (2H, d, $J = 7.4$ Hz), 7.10 (2H, t, $J = 7.4$ Hz), and 7.32 (1H, t, $J = 7.4$ Hz), respectively, and also signals of a butyrate group at δ 0.88 (3H, t, $J = 7.6$ Hz), 1.62 (2H, m), and 2.34 (2H, t, $J = 7.6$ Hz), with those assignments supported by $^1H-^1H$ COSY and HMBC correlations (Figure 1). In addition, resonances belonging to acylated oxymethine protons at δ 5.96 (1H, s), 5.67 (1H, d, $J = 4.6$ Hz), 5.54 (1H, dd, $J = 11.2$, 4.0 Hz), and 5.52 (1H, brs), two sets of typical methylene protons at δ 1.80 and 1.78 (both 1H, m), and δ 2.20 and 1.48 (1H, m, each), and four characteristic methyl groups appearing as a doublet at δ 1.10 (3H, d, $J = 7.2$ Hz) and three singlets at δ 1.44, 1.59, and 1.61 (3H, s, each) were also observed. The ^{13}C NMR spectrum of **1** (Table 2) indicated 35 carbon signals separated by DEPT experiments into four carbonyls at δ 172.3, 169.9, 165.5, and 164.8, three quaternary sp^3 carbons with two linked to an oxygen atom, two quaternary sp^2 carbons, 16 tertiary carbons comprising six sp^3 carbons with four linked to an oxygen atom and 10 sp^2 carbons, four secondary sp^3 carbons, and six methyl carbons. The complete assignments of the protonated carbons were made from the HSQC spectrum, while a detailed analysis of the $^1H-^1H$ COSY and HMBC spectra of **1** led to the establishment of a tetrasubstituted dihydroagarofuran sesquiterpene for the structure of **1** (Figure 1). The regiosubstitution of the ester functions was determined by HMBC correlations of the carbonyl signals of the benzoate groups at δ 164.8 and 165.5 with signals at δ 5.67 (H-9) and 5.54 (H-1) and of the carbonyl signal of the acetate group at δ 169.9 with the signal at δ 5.96 (H-6), while the carbonyl signal of the butyrate group at δ 172.3 correlated with the signal at δ 5.52 (H-8). The relative configuration of **1** was established on the basis of a ROESY experiment (Figure 2), in which NOE effects were found between Me-14 and H-6 and Me-15, between H-2″ (δ 1.62) of the C-8 butyrate group and H-6, between Me-12 and H-8 and H-9, and between H-9 and H-1. The absolute configuration of **1** was confirmed by the dibenzoate chirality method, an extension of the circular dichroism exciton chirality method,[10] which showed a Davidoff-type split curve with a first Cotton effect at 239.7 nm

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Table 1. ^{1}H NMR Spectroscopic Data of **1**−**9** (400 MHz, $CDCl_3$)[a]

position	1	2	3	4	5	6	7	8	9
1	5.54 dd (11.2, 4.0)	4.08 dd (11.4, 3.9)	4.08 dd (11.0, 4.4)	5.58 dd (10.3, 5.7)	5.36 dd (11.0, 4.8)	5.50 d (3.3)	5.49 d (3.5)	5.52 dd (12.0, 4.3)	5.46 dd (12.0, 4.3)
2α	1.80 m	1.61 m	1.61 m	1.82 m	1.68 m	4.39 brd (2.7)	5.55 dd (3.5, 3.1)	1.96 m	1.62 m
2*β*	1.78 m	1.50 m	1.47 m	1.80 m	1.64 m			1.48 m	1.50 m
3α	1.48 m	1.39 m	1.39 m	1.52 m	1.42 m	1.82 m	2.00 m	1.89 m	1.42 m
3*β*	2.20 m	2.00 m	2.00 m	2.24 m	2.12 m	2.26 m	2.10 m	2.20 m	2.12 m
4	2.26 m	2.15 m	2.16 m	2.32 m	2.19 m	2.25 m		2.45 m	2.28 m
6	5.96 s	5.88 s	6.16 s	5.16 s	6.15 s	5.39 s	2.42 m 1.85 m	5.49 s	4.41 s
7	2.50 d (4.2)	2.55 d (4.5)	2.52 d (4.5)	2.58 brs	2.45 d (4.4)	2.17 brs	2.06 m	2.43 brs	2.11 brs
8	5.52 brs	5.50 dd (4.5, 5.0)	4.39 t (4.5)	5.78 brs	4.38 t (4.4)	2.36 m 2.08 m	2.18 m 2.04 m	2.50 m 2.22 m	2.24 m 2.14 m
9	5.67 d (4.6)	5.63 d (5.0)	5.58 d (4.5)	5.78 brs	5.50 d (4.4)	4.78 d (6.9)	4.81 d (6.2)	5.10 d (6.9)	4.98 d (6.4)
12	1.59 s	1.54 s	1.47 s	1.65 s	1.46 s	1.38 s	1.45 s	1.47 s	1.40 s
13	1.44 s	1.38 s	1.40 s	1.59 s	1.39 s	1.36 s	1.32 s	1.47 s	1.52 s
14	1.10 d (7.2)	1.01 d (7.5)	1.01 d (7.3)	1.31 d (7.3)	1.02 d (7.1)	1.24 d (7.3)	1.43 s	3.55 m 3.61 m	1.19 d (7.2)
15	1.61 s	1.38 s	1.39 s	1.70 s	1.56 s	1.49 s	1.38 s	1.15 s	1.32 s
OAc-1						1.87 s	1.82 s	1.62 s	1.60 s
OAc-2							2.05 s		
OAc-6	2.12 s	2.07 s	2.10 s		2.15 s	2.07 s			
OAc-8		2.08 s							

[a] Data for additional ester groups are provided in the Experimental Section.

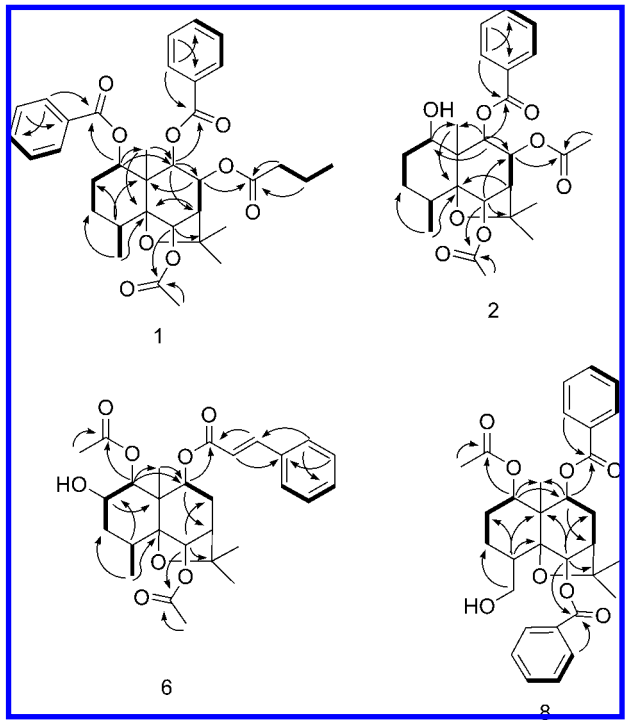

Figure 1. Main ^{1}H−^{13}C long-range correlation (^{1}H⤳^{13}C) and ^{1}H−^{1}H correlation (—) signals in the HMBC and COSY spectra of **1**, **2**, **6**, and **8**.

and a second one at 223.3 nm, due to the couplings of the two benzoate chromophores at C-1α and C-9α. Thus, the structure and absolute configuration of **1** were assigned as (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-acetoxy-1,9-dibenzoyloxy-8-butyryloxydihydro-*β*-agarofuran.

Compound **2**, purified as a white, amorphous powder, gave the molecular formula $C_{26}H_{34}O_8$, as deduced from the HRESIMS and NMR analysis. The NMR data (Tables 1 and 2) of **2** revealed **2** was very similar to those of **1** except that one benzoate group at C-1 in **1** was displaced by one free hydroxyl group in **2**, and one additional acetate group at C-8 in **2** appeared, instead of one butyrate group in **1**. The HMBC experiment (Figure 1) established the regiosubstitution in the molecule of **2**, and the relative configuration was resolved by analysis of a ROESY experiment (Figure 2). Thus compound **2** was assigned as the 8-acetoxy-1-debenzoyl derivative of **1**. To determine the absolute configuration of **2**, it was necessary to introduce another chromophoric group. Benzoylation of **2** yielded the benzoate derivative, **2a**, which was suitable for applying the dibenzoate chirality method.[10] Its CD spectrum showed a split curve with a first negative Cotton effect at 241.4 nm and a second positive effect at 221.7 nm. Therefore, **2** was established as (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8-diacetoxy-9-benzoyloxy-1-hydroxydihydro-*β*-agarofuran.

Compounds **3** and **4** were assigned the molecular formulas $C_{24}H_{32}O_7$ and $C_{36}H_{38}O_8$, respectively, as deduced from their HRESIMS and NMR data. The NMR spectroscopic data (Tables 1 and 2) revealed that compounds **3** and **4** both possessed an identical dihydro-*β*-agarofuran skeleton to that of **2**. A difference in the ^{1}H NMR spectrum of **3** was due to an additional hydroxyl group at C-8 instead of an acetate group in **2**. Thus, **3** was determined as the 8-deacetyl derivative of **2**. Similarly, the ^{1}H NMR and ^{13}C NMR spectroscopic data of **4** corresponded to those of **2** except that two additional benzoate groups signals appeared in **4**, and no acetate group signals were present. An analysis of the NMR spectra of **4** revealed that **4** is the 1,8-dibenzoyloxy-6-hydroxy derivative of **2**. The relative configurations of compounds **3** and **4** were resolved by analysis of the coupling constants and confirmed by ROESY experiments.

Benzoylation of **3** yielded the known derivative **10**. The absolute configuration of **10** was determined by CD studies, with the curve showing a first negative Cotton effect at 236.5 nm and a second positive one at 221.9 nm. As a result, the structure and absolute configuration of **3** were proposed as (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-acetoxy-9-benzoyloxy-1,8-dihydroxydihydro-*β*-agarofuran. The CD spectrum of **4** showed a very close curve to that of **10**, supporting the structure and absolute configuration assignment as (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-1,8,9-tribenzoyloxy-6-hydroxydihydro-*β*-agarofuran.

Compound **5** gave a molecular formula of $C_{33}H_{38}O_8$, as deduced from its HRESIMS and NMR data. Examination of the NMR spectra (Tables 1 and 2) revealed that this compound was a trisubstituted dihydro-*β*-agarofuran sesquiterpene with the presence of a free tertiary hydroxyl and a cinnamyl group [δ 6.92 (2H, d, J = 7.5 Hz), 7.16 (2H, t, J = 7.5 Hz), and 7.25 (1H, t, J = 7.5 Hz), and δ 7.22 (1H, d, J = 15.9 Hz) and 5.68 (1H, d, J = 15.9 Hz)]. The HMBC experiment established the regiosubstitution in the molecule of **5**, and the relative stereochemistry was resolved by analysis of coupling constants and a ROESY experiment, which showed **5** to be the 1-cinnamyloxy derivative of **3**. The CD spectrum of **5** showed a split curve very similar to that of **1**, and its absolute configuration was accordingly established as (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-acetoxy-9-benzoyloxy-1-cinnamyloxy-8-hydroxydihydro-*β*-agarofuran.

Compounds **6** and **7** were both assigned the molecular formula $C_{28}H_{36}O_8$ by HRESIMS. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **6** and **7** indicated that these two compounds were triesterified dihydro-*β*-agarofuran sesquiterpenes with the presence of free hydroxy groups. The HMBC experiments (Figure 1) established

Table 2. ^{13}C NMR Spectroscopic Data of **1**−**9** (100 MHz, $CDCl_3$)[a]

position	1	2	3	4	5	6	7	8	9
1	79.0 d	76.2 d	76.4 d	79.5 d	79.0 d	73.6 d	70.0 d	73.3 d	74.1 d
2	22.3 t	25.6 t	25.7 t	22.4 t	21.9 t	69.0 d	69.2 d	22.1 t	21.6 t
3	26.5 t	26.7 t	26.9 t	26.7 t	26.5 t	32.5 t	40.6 t	22.7 t	26.9 t
4	33.8 d	33.9 d	34.0 d	33.6 d	33.7 d	33.7 d	69.5 s	44.9 d	33.7 d
5	91.1 s	91.1 s	91.5 s	92.5 s	91.1 s	89.8 s	90.3 s	88.8 s	91.4 s
6	75.1 d	75.3 d	75.0 d	73.1 d	74.6 d	79.1 d	30.9 t	80.2 d	78.1 d
7	52.5 d	52.4 d	54.3 d	54.5 d	54.2 d	48.6 s	43.4 d	48.8 d	50.8 d
8	71.0 d	71.6 d	70.4 d	72.4 d	69.8 d	31.4 t	30.5 t	32.3 t	32.4 t
9	74.4 d	75.2 d	77.7 d	74.6 d	76.4 d	72.9 d	73.1 d	72.6 d	73.8 d
10	49.1 s	49.6 s	49.5 s	48.6 s	48.7 s	49.6 s	47.5 s	50.2 s	50.1 s
11	81.7 s	81.3 s	81.1 s	82.0 s	81.2 s	82.4 s	83.8 s	82.6 s	82.6 s
12	24.1 q	24.0 q	24.1 q	24.5 q	24.0 q	25.9 q	24.3 q	26.0 q	26.3 q
13	30.6 q	30.6 q	30.8 q	31.1 q	30.6 q	30.6 q	30.0 q	30.7 q	31.0 q
14	16.8 q	16.7 q	16.8 q	17.4 q	16.7 q	18.9 q	25.5 q	62.6 t	18.1 q
15	12.2 q	10.7 q	11.1 q	12.6 q	12.3 q	20.7 q	20.6 q	17.9 q	19.0 q
OAc-1						20.9 q 170.0 s	20.6 q 170.0 s	20.8 q 169.9 s	20.8 q 170.0 s
OAc-2							21.2 q 169.8 s		
OAc-6	21.3 q 169.9 s	21.2 q 169.9 s	21.4 q 169.9 s		21.2 q 169.8 s	21.3 q 170.0 s			
OAc-8		20.9 q 169.9 s							

[a] Data for additional ester groups are provided in the Experimental Section.

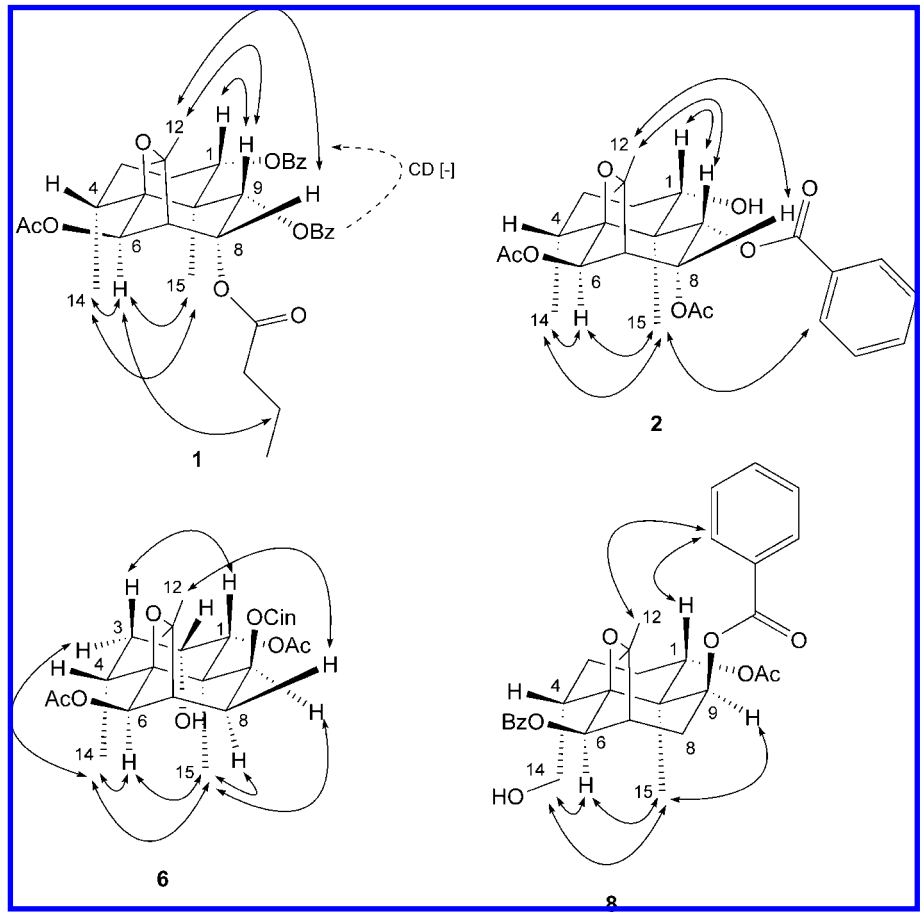

Figure 2. Main NOE correlation signals (↔) in the ROESY spectra of **1**, **2**, **6**, and **8**, and CD exciton coupling (dashed arrow) for **1**.

the regiosubstitution in the molecules of **6** and **7**, and their relative stereochemistry was resolved by analysis of coupling constants and ROESY experiments (Figure 2). The hydroxyl group in **6** was located at C-2 based on the HMBC cross-peak between C-2 at δ 69.0 and H-4 at δ 2.25 and between H-2 at δ 4.39 and C-10 at δ 49.6. The hydroxy group in **7** was attached to C-4 on the basis of the HMBC correlations between the hydroxyl proton at δ 2.76 (OH-4) and carbons at δ 69.5 (C-4) and 25.5 (C-14). Accordingly, the structure **7** was deduced as 1α,2α-diacetoxy-9β-cinnamyloxy-4β-hydroxydihydro-β-agarofuran.

To determine the absolute configuration of **6**, it was necessary to introduce another chromophoric group. Benzoylation of **6** yielded the benzoate derivative, **6a**. The CD spectrum of **6a** showed a broad positive Cotton effect at 276.3 nm, while the second maximum could not be observed due possibly to the strong positive absorption overlaying background ellipticity.[10] Thus, the structure and absolute configuration of **6** were proposed as (1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*R*)-1,6-diacetoxy-9-cinnamyloxy-2-hydroxydihydro-β-agarofuran.

Compounds **8** and **9** were assigned the molecular formulas $C_{31}H_{36}O_8$ and $C_{24}H_{32}O_6$, respectively, as deduced from their HRESIMS and NMR data. The ^1H and ^{13}C NMR data (Tables 1 and 2) indicated that compound **8** was a triesterified dihydro-β-agarofuran sesquiterpene with one acetate, two benzoyl, and one secondary hydroxyl group, and **9** was a diesterified dihydro-β-agarofuran sesquiterpene with one acetate,

Table 3. Cytotoxic Activity of Compounds **1**, **5**, and **11**−**16** against the HL-60 Human Leukemia Cell Line

compound	IC_{50} (μM)
1	5.3
5	8.3
11	6.8
12	2.8
13	6.8
14	3.3
15	7.2
16	1.9
etoposide[a]	0.2

[a] Etoposide was used as a positive control.

one benzoyl, and one tertiary hydroxyl group. The HMBC experiments (Figure 1) established the regiosubstitution in the molecules of **8** and **9**, and their relative stereochemistry was resolved by analysis of coupling constants and ROESY experiments (Figure 2). The hydroxyl group in **8** was sited at C-14 on the basis of the HMBC cross-peaks between the carbon at δ 62.6 (C-14) and the proton at δ 2.45 (H-4) and between protons at δ 3.61, 3.55 (H_2-14) and the carbon at δ 22.7 (C-3). The hydroxyl group in **9** was established at C-6 on the basis of the HMBC correlations between the carbon at δ 78.1 (C-6) and the proton at δ 2.24, 2.14 (H-8) and between the proton at δ 4.41 (H-6) and carbons at δ 82.6 (C-11) and 32.4 (C-8). The CD spectrum of **8** displayed a weak split curve with a first positive Cotton effect at 242.7 nm and a second negative one at 221.4 nm ascribable to the homobenzoate interaction at C-6β and C-9β, providing its structure and absolute configuration as (1*S*,4*S*,5*S*,6*R*,7*R*,9*S*,10*S*)-1-acetoxy-6,9-dibenzoyloxy-14-hydroxydihydro-β-agarofuran. Cinnamylation of **9** gave the known compound **16**, which was suitable for applying the dibenzoate chirality method. The CD spectrum of **16** showed a split curve with a first positive Cotton effect at 279.1 nm and a second negative one at 235.0 nm. Thus, the structure and the absolute configuration of **9** were accordingly deduced as (1*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1-acetoxy-9-benzoyloxy-6-hydroxydihydro-β-agarofuran.

In addition to the nine new dihydro-β-agarofuran derivatives (**1**−**9**), 13 known metabolites were also isolated and characterized by comparison with literature data as 6β-acetoxy-1α,8α,9α-tribenzoyloxydihydro-β-agarofuran (**10**),[11] celafolin C-1 (**11**),[12] 9α-acetoxy-1β,6α-dibenzoyloxydihydro-β-agarofuran (**12**),[13] 1β-acetoxy-9α-cinnamyloxydihydro-β-agarofuran (**13**),[13] 2α,9β-diacetoxy-1α-cinnamyloxydihydro-β-agarofuran (**14**),[14] 6β-acetoxy-1α,8α-dibenzoyloxy-9α-hydroxydihydro-β-agarofuran (**15**),[15] celafolin A-1 (**16**),[12] celafolin B-3,[12] 6β,9β-diacetoxy-1α-benzoyloxydihydro-β-agarofuran,[14] 1α,6α,14-triacetoxy-9β-benzoyloxydihydro-β-agarofuran,[16] triptogelin B-1,[17] celafolin B-1,[12] and 6β-acetoxy-8α,9α-dibenzoyloxy-1α,2α-dihydroxydihydro-β-agarofuran.[18]

To test the potential anticancer activities of all the isolates obtained, we used a standard in vitro cytotoxicity evaluation system, the HL-60 cell line, to analyze their cytotoxic activities by MTT assays. As a result, the new compounds **1** and **5** and known sesquiterpene derivatives **11**−**16** were observed to exhibit cytotoxic activities with IC_{50} values ranging from 1.9 to 8.3 μM (Table 3), while the others exhibited less than 50% of cell growth inhibition at a concentration of up to 10 μM. Preliminary analysis of the structure−activity relationship from these natural sesquiterpenes revealed that compounds with a hydroxy group at C-6, C-8, or C-9 (**4**, **5**, **9**, and **15**) had a slightly decreased cytotoxicity, while compounds with a free hydroxy group at C-1, C-2, or C-14 showed no such activity, as deduced from **2**, **6**, **8**, triptogelin B-1, celafolin B-1, celafolin B-3, and 6β-acetoxy-8α,9α-dibenzoyloxy-1α,2α-dihydroxydihydro-β-agarofuran.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241MC instrument. CD spectra were recorded on a JASCO J-810 spectrometer. UV spectra were obtained on a Beckman DU-7 spectrometer. IR spectra were recorded using a Perkin-Elmer 577 spectrometer. LRESIMS were measured using a Finnigan LCQ-Deca instrument, and HRESIMS data were obtained on a Mariner mass spectrometer. NMR experiments were run on a Bruker AM 400 spectrometer with TMS as internal standard. Preparative HPLC was carried out using a Varian SD-1 instrument equipped with a Merck NW25 C_{18} column (12 μM, 20 mm × 250 mm) and ProStar 320 UV/vis detector. Column chromatographic (CC) separations were performed using silica gel H60 (300−400 mesh), zcx-II (100−200 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China), ODS (40−63 μM) (Merck), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

Plant Material. The seeds of *C. orbiculatus* were collected in a suburb of Liaoyuan, Jilin Province, People's Republic of China, in January 2007, and identified by Professor Jingui Shen of Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen (no. 20061202) is deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Powdered and air-dried seeds of *C. orbiculatus* (10.0 kg) were percolated with 95% EtOH at room temperature (3 × 72 h). The solvents were evaporated in vacuo, and the residue was suspended in H_2O and then partitioned with petroleum ether and EtOAc (2 L × 3 each), successively, yielding petroleum ether (1.1 kg) and EtOAc (20.5 g) extracts. The petroleum ether-soluble fraction (1.1 kg) was subjected to silica gel CC eluting with a gradient of petroleum ether and acetone (100:1 to 0:1), and six fractions (F_1−F_6) were obtained. F_2 (135.2 g) was chromatographed on silica gel eluting with petroleum ether−acetone (P−A) (40:1) to give **12** (100.1 mg) and **13** (1.0 g). Then, F_3 (120.1 g) was separated into four subfractions (F_{31}−F_{34}) by CC eluting with P−A (40:1). F_{33} (50.5 g) and F_{34} (10.5 g) were subjected to CC over silica gel eluting with P−A (40:1), followed by preparative HPLC using a gradient of MeOH−H_2O (70% to 100% over 80 min, 10 mL·min^{-1}) to afford 6β,9β-diacetoxy-1α-benzoyloxydihydro-β-agarofuran (200.1 mg) and **16** (150.2 mg), and **11** (1.3 g), respectively. F_4 (80.2 g) was purified by a combination of silica gel CC eluting with P−A (40:1) and preparative HPLC, using a gradient of MeOH−H_2O (70% to 100% over 80 min, 10 mL·min^{-1}), as well as by preparative TLC ($CHCl_3$−acetone, 100:1), to yield **1** (41.2 mg), **10** (80.2 mg), 1α,6α,14-triacetoxy-9β-benzoyloxydihydro-β-agarofuran (160.3 mg), and **14** (630.2 mg). F_6 (60.2 g) was separated by ODS CC eluting with a gradient of MeOH−H_2O (1:1, 7:3, 9:1, and 1:0) to give three subfractions (F_{61}−F_{63}). F_{61} (1.5 g) was chromatographed on silica gel eluting with P−A (10:1) and then purified by preparative TLC ($CHCl_3$−acetone, 20:1) to obtain **2** (26.9 mg). F_{62} (15.3 g) was subjected to silica gel CC eluting with $CHCl_3$−acetone (100:1), and four fractions (F_{621}−F_{624}) were obtained. F_{621} (3.2 g) was separated by a combination of silica gel CC eluting with P−A (10:1) and preparative HPLC using a gradient of MeOH−H_2O (60% to 100% over 80 min, 10 mL·min^{-1}) and further by preparative TLC ($CHCl_3$−acetone, 40:1) to give triptogelin B-1 (144.2 mg). Compounds **4** (7.0 mg), **5** (113.2 mg), **7** (15.8 mg), **9** (3.0 mg), and **15** (49.5 mg) were obtained from F_{623} (1.4 g) by using the same steps as described for F_{621}. F_{624} (1.0 g) was purified by preparative HPLC using a gradient of MeOH−H_2O (60% to 100% over 70 min, 10 mL·min^{-1}) followed by CC eluting with P−A (8:1) and then passed through a Sephadex LH-20 column with ethanol as eluent, to afford **6** (90.2 mg), celafolin B-1 (30.1 mg), and celafolin B-3 (11.2 mg). The EtOAc extract (20.5 g) was subjected to silica gel CC eluting with P−A (10:1), and four fractions (F_1−F_4) were obtained. Purification of F_4 (9.6 g) by repeated preparative HPLC using a gradient of MeOH−H_2O (40% to 100% over 80 min, 10 mL·min^{-1}) and further by preparative TLC ($CHCl_3$−MeOH, 50:1) resulted in the isolation of compounds **3** (10.2 mg), **8** (6.0 mg), and 6β-acetoxy-8α,9α-dibenzoyloxy-1α,2α-dihydroxydihydro-β-agarofuran (15.2 mg).

(1*S*,4R,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-Acetoxy-1,9-dibenzoyloxy-8-butyryloxydihydro-β-agarofuran (1): white, amorphous powder; $[\alpha]_D^{20}$ −35.0 (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 227 (4.34), 274 (3.46) nm; CD (MeOH) λ_{ext} ($\Delta\epsilon$) 239.7 (−7.71), 223.3 (+14.56) nm; IR (KBr) ν_{max} 2968, 1743, 1727, 1452, 1382, 1280, 1226, 1112, 1093, 962, 707 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OBz-1 [7.61 (2H, d, J = 7.4 Hz, H-2′/6′), 6.92 (2H, t, J = 7.4 Hz, H-3′/5′), and 7.18 (1H, t, J = 7.4 Hz,

H-4′)], OBut-8 [2.34 (2H, t, J = 7.6 Hz, H-1″), 1.62 (2H, m, H-2″), and 0.88 (3H, t, J = 7.6 Hz, H-3″)], and OBz-9 [7.59 (2H, d, J = 7.4 Hz, H-2‴/6‴), 7.10 (2H, t, J = 7.4 Hz, H-3‴/5‴), and 7.32 (1H, t, J = 7.4 Hz, H-4‴)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-1 [129.8 (s, C-1′), 129.2 (d, C-2′/6′), 127.5 (d, C-3′/5′), 132.2 (d, C-4′), and 165.5 (s, CO_2-1)], OBut-8 [36.4 (t, C-1″), 18.3 (t, C-2″), 13.7 (q, C-3″), and 172.3 (s, CO_2-8)], and OBz-9 [129.6 (s, C-1‴), 129.1 (d, C-2‴/6‴), 127.8 (d, C-3‴/5‴), 132.4 (d, C-4‴), and 164.8 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 629 [M + Na]$^+$; HRESIMS m/z 629.2727 [M + Na]$^+$ (calcd for $C_{35}H_{42}O_9Na$, 629.2727).

(1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8-Diacetoxy-9-benzoyloxy-1-hydroxydihydro-*β*-agarofuran (2): white, amorphous powder; $[\alpha]_D^{20}$ −59.0 (c 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.18), 273 (3.11) nm; IR (KBr) ν_{max} 3533, 2925, 1747, 1708, 1452, 1369, 1282, 1236, 1097, 1035, 962, 711 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OBz-9 [7.98 (2H, d, J = 7.7 Hz, H-2′/6′), 7.40 (2H, t, J = 7.7 Hz, H-3′/5′), and 7.55 (1H, t, J = 7.7 Hz, H-4′)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-9 [130.1 (s, C-1′), 129.5 (d, C-2′/6′), 128.4 (d, C-3′/5′), 133.0 (d, C-4′), and 165.8 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 497 [M + Na]$^+$; HRESIMS m/z 497.2128 [M + Na]$^+$ (calcd for $C_{26}H_{34}O_8Na$, 497.2151).

Benzoylation of 2. Compound **2** (5.0 mg) was dissolved in dry pyridine (0.5 mL), and benzoyl chloride (6 drops) and a catalytic amount of 4-(dimethylamino)pyridine were added. Then, the mixture was stirred at room temperature for 48 h, poured over H_2O, extracted with EtOAc, and purified by preparative TLC with a solvent of petroleum ether−EtOAc (5:1), to give compound **2a** (4.0 mg, R_f 0.28).

(1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6,8-Diacetoxy-1,9-dibenzoyloxydihydro-*β*-agarofuran (2a): white, amorphous powder; $[\alpha]_D^{20}$ −32.0 (c 0.10, $CHCl_3$); CD (MeOH) λ_{ext} ($\Delta\epsilon$) 241.4 (−13.58), 221.7 (+18.47) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.54 (1H, m, H-1), 1.80 (2H, m, H-2), 2.20 (2H, m, H-3), 2.26 (1H, m, H-4), 5.95 (1H, s, H-6), 2.51 (1H, d, J = 4.4 Hz, H-7), 5.52 (1H, m, H-8), 5.65 (1H, d, J = 5.0 Hz, H-9), 1.42 (3H, s, H-12), 1.58 (3H, s, H-13), 1.09 (3H, d, J = 7.1 Hz, H-14), 1.61 (3H, s, H-15), OAc-6 [2.12 (3H, s)], OAc-8 [2.10 (3H, s)], OBz-1 [7.60 (2H, d, J = 7.6 Hz, H-2′/6′), 6.91 (2H, t, J = 7.6 Hz, H-3′/5′), and 7.14 (1H, t, J = 7.6 Hz, H-4′)], and OBz-9 [7.60 (2H, d, J = 7.6 Hz, H-2″/6″), 7.11 (2H, t, J = 7.6 Hz, H-3″/5″), and 7.33 (1H, t, J = 7.6 Hz, H-4″)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 79.0 (d, C-1), 22.3 (t, C-2), 26.6 (t, C-3), 33.9 (d, C-4), 91.1 (s, C-5), 75.1 (d, C-6), 52.4 (d, C-7), 71.4 (d, C-8), 74.3 (d, C-9), 49.2 (s, C-10), 81.7 (s, C-11), 30.6 (q, C-12), 24.1 (q, C-13), 16.7 (q, C-14), 12.2 (q, C-15), OAc-6 [21.3 (q), 169.9 (s, CO_2-6)], OAc-8 [20.9 (q), 169.9 (s, CO_2-8)], OBz-1 [129.9 (s, C-1′), 129.2 (d, C-2′/6′), 127.5 (d, C-3′/5′), 132.1 (d, C-4′), and 165.5 (s, CO_2-1)], and OBz-9 [129.6 (s, C-1″), 129.1 (d, C-2″/6″), 127.9 (d, C-3″/5″), 132.4 (d, C-4″), and 164.8 (s, CO_2-9)].

(1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-Acetoxy-9-benzoyloxy-1,8-dihydroxydihydro-*β*-agarofuran (3): white, amorphous powder; $[\alpha]_D^{20}$ −42.0 (c 0.19, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (4.13), 273 (2.99) nm; IR (KBr) ν_{max} 3540, 2817, 1731, 1708, 1450, 1384, 1282, 1255, 1099, 1027, 962, 713 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OBz-9 [8.03 (2H, d, J = 7.9 Hz, H-2′/6′), 7.45 (2H, t, J = 7.9 Hz, H-3′/5′), and 7.56 (1H, t, J = 7.9 Hz, H-4′)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-9 [130.2 (s, C-1′), 129.7 (d, C-2′/6′), 128.6 (d, C-3′/5′), 133.3 (d, C-4′), and 166.1 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 455 [M + Na]$^+$; HRESIMS m/z 455.2042 [M + Na]$^+$ (calcd for $C_{24}H_{32}O_7Na$, 455.2046).

Benzoylation of 3. Compound **3** (5.0 mg) was benzoylated under the same conditions described above for **2**, to yield the known compound **10** (3.7 mg, R_f 0.35), whose absolute configuration was determined by CD study to be (1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-acetoxy-1,8,9-tribenzoyloxydihydro-*β*-agarofuran.

(1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-1,8,9-Tribenzoyloxy-6-hydroxydihydro-*β*-agarofuran (4): white, amorphous powder; $[\alpha]_D^{20}$ −134.0 (c 0.19, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 227 (4.57), 273 (3.49) nm; CD (MeOH) λ_{ext} ($\Delta\epsilon$) 235.0 (−49.36), 220.9 (+21.26) nm; IR (KBr) ν_{max} 2929, 1727, 1602, 1452, 1319, 1282, 1107, 1068, 956, 703 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OBz-1 [7.58 (2H, d, J = 7.8 Hz, H-2′/6′), 6.87 (2H, t, J = 7.8 Hz, H-3′/5′), and 7.14 (1H, t, J = 7.8 Hz, H-4′)], OBz-8 [8.00 (2H, d, J = 8.1 Hz, H-2″/6″), 7.46 (2H, t, J = 8.1 Hz, H-3″/5″), and 7.58 (1H, t, J = 8.1 Hz, H-4″)], and OBz-9 [7.44 (2H, d, J = 7.7 Hz, H-2‴/6‴), 6.98 (2H, t, J = 7.7 Hz, H-3‴/5‴), and 7.24 (1H, t, J = 7.7 Hz, H-4‴)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-1 [129.9 (s, C-1′), 129.1 (d, C-2′/6′), 127.5 (d, C-3′/5′), 132.1 (d, C-4′), and 165.5 (s, CO_2-1)], OBz-8 [130.0 (s, C-1″), 129.6 (d, C-2″/6″), 128.5 (d, C-3″/5″), 133.2 (d, C-4″), and 165.3 (s, CO_2-8)], and OBz-9 [129.6 (s, C-1‴), 129.1 (d, C-2‴/6‴), 127.7 (d, C-3‴/5‴), 132.2 (d, C-4‴), and 164.8 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 621 [M + Na]$^+$; HRESIMS m/z 621.2455 [M + Na]$^+$ (calcd for $C_{36}H_{38}O_8Na$, 621.2464).

(1*S*,4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,10*S*)-6-Acetoxy-9-benzoyloxy-1-cinnamyloxy-8-hydroxydihydro-*β*-agarofuran (5): white, amorphous powder; $[\alpha]_D^{20}$ −12.0 (c 0.27, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 223 (4.40), 281 (4.32) nm; CD (MeOH) λ_{ext} ($\Delta\epsilon$) 251.5 (−8.11), 227.8 (+26.96) nm; IR (KBr) ν_{max} 2931, 1718, 1637, 1450, 1328, 1282, 1251, 1091, 1027, 979, 711 cm^{-1};^{1}H NMR (400 MHz, $CDCl_3$) δ OCin-1 [6.92 (2H, d, J = 7.5 Hz, H-2′/6′), 7.16 (2H, t, J = 7.5 Hz, H-3′/5′), 7.25 (1H, t, J = 7.5 Hz, H-4′), 5.68 (1H, d, J = 15.9 Hz, H-α), and 7.22 (1H, d, J = 15.9 Hz, H-*β*)], and OBz-9 [7.93 (2H, d, J = 7.8 Hz, H-2″/6″), 7.19 (2H, t, J = 7.8 Hz, H-3″/5″), and 7.27 (1H, t, J = 7.8 Hz, H-4″)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OCin-1 [133.9 (s, C-1′), 127.7 (d, C-2′/6′), 128.2 (d, C-3′/5′), 129.7 (d, C-4′), 117.9 (d, C-α), 143.9 (d, C-*β*), and 166.0 (s, CO_2-1)], and OBz-9 [129.8 (s, C-1″), 129.5 (d, C-2″/6″), 128.2 (d, C-3″/5″), 132.6 (d, C-4″), and 165.0 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 585 [M + Na]$^+$; HRESIMS m/z 585.2460 [M + Na]$^+$ (calcd for $C_{33}H_{38}O_8Na$, 585.2464).

(1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*R*)-1,6-Diacetoxy-9-cinnamyloxy-2-hydroxydihydro-*β*-agarofuran (6): white, amorphous powder; $[\alpha]_D^{20}$ +36.0 (c 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 279 (4.27) nm; IR (KBr) ν_{max} 3505, 2919, 1731, 1693, 1639, 1450, 1369, 1240, 1093, 1024, 979, 771 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OCin-9 [7.55 (2H, m, H-2′/6′), 7.38 (2H, m, H-3′/5′), 7.38 (1H, m, H-4′), 6.38 (1H, d, J = 16.0 Hz, H-α), and 7.70 (1H, d, J = 16.0 Hz, H-*β*)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OCin-9 [134.2 (s, C-1′), 128.1 (d, C-2′/6′), 128.7 (d, C-3′/5′), 130.2 (d, C-4′), 117.9 (d, C-α), 145.2 (d, C-*β*), and 165.9 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 523 [M + Na]$^+$; HRESIMS m/z 523.2297 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_8Na$, 523.2308).

Benzoylation of 6. Compound **6** (5.0 mg) was benzoylated under the same conditions described above for compound **2**, to give compound **6a** (4.7 mg, R_f 0.31).

(1*R*,2*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*R*)-1,6-Diacetoxy-2-benzoyloxy-9-cinnamyloxydihydro-*β*-agarofuran (6a): white, amorphous powder; $[\alpha]_D^{20}$ +31.0 (c 0.18, $CHCl_3$); CD (MeOH) λ_{ext} ($\Delta\epsilon$) 276.3 (+14.15) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.71 (1H, d, J = 3.7 Hz, H-1), 5.85 (1H, brd, J = 3.0 Hz, H-2), 1.95/2.40 (both 1H, m, H-3), 2.39 (1H, m, H-4), 5.44 (1H, s, H-6), 2.23 (1H, brs, H-7), 2.18/2.55 (each 1H, m, H-8), 4.78 (1H, d, J = 7.1 Hz, H-9), 1.41 (3H, s, H-12), 1.42 (3H, s, H-13), 1.28 (3H, d, J = 7.2 Hz, H-14), 1.58 (3H, s, H-15), OAc-1 [1.80 (3H, s)], OAc-6 [2.12 (3H, s)], OBz-2 [7.98 (2H, d, J = 8.0 Hz, H-2′/6′), 7.45 (2H, t, J = 8.0 Hz, H-3′/5′), and 7.56 (1H, t, J = 8.0 Hz, H-4′)], and OCin-9 [7.55 (2H, m, H-2″/6″), 7.38 (2H, m, H-3″/5″), 7.38 (1H, m, H-4″), 6.38 (1H, d, J = 16.0 Hz, H-α), 7.70 (1H, d, J = 16.0 Hz, H-*β*)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.0 (d, C-1), 70.5 (d, C-2), 30.9 (t, C-3), 33.0 (d, C-4), 88.9 (s, C-5), 78.4 (d, C-6), 48.2 (d, C-7), 30.7 (t, C-8), 72.0 (d, C-9), 49.0 (s, C-10), 82.3 (s, C-11), 30.0 (q, C-12), 25.2 (q, C-13), 18.2 (q, C-14), 19.9 (q, C-15), OAc-1 [19.9 (q), 169.3 (s, CO_2-1)], OAc-6 [20.6 (q), 169.2 (s, CO_2-6)], OBz-2 [129.8 (s, C-1′), 128.8 (d, C-2′/6′), 128.0 (d, C-3′/5′), 132.4 (d, C-4′), and 165.1 (s, CO_2-2)], and OCin-9 [133.8 (s, C-1″), 127.6 (d, C-2″/6″), 128.3 (d, C-3″/5″), 129.7 (d, C-4″), 117.4 (d, C-α), 144.7 (d, C-*β*), and 165.4 (s, CO_2-9)].

1α,2α-Diacetoxy-9*β*-cinnamyloxy-4*β*-hydroxydihydro-*β*-agarofuran (7): white, amorphous powder; $[\alpha]_D^{20}$ +83.0 (c 0.06, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 217 (4.19), 279 (4.32) nm; IR (KBr) ν_{max} 3504, 2929, 1745, 1697, 1637, 1450, 1384, 1367, 1282, 1253, 1143, 1027, 771 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ OCin-9 [7.58 (2H, m, H-2′/6′), 7.40 (2H, m, H-3′/5′), 7.40 (1H, m, H-4′), 6.38 (1H, d, J = 15.9 Hz, H-α), and 7.71 (1H, d, J = 15.9 Hz, H-*β*)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OCin-9 [134.3 (s, C-1′), 128.3 (d, C-2′/6′), 128.8 (d, C-3′/5′), 130.4 (d, C-4′), 118.0 (d, C-α), 145.4 (d, C-*β*), and 166.1 (s, CO_2-9)], for other signals, see Table 2; ESIMS m/z 523 [M + Na]$^+$; HRESIMS m/z 523.2317 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_8Na$, 523.2308).

(1*S*,4*S*,5*S*,6*R*,7R,9*S*,10*S*)-1-Acetoxy-6,9-dibenzoyloxy-14-hydroxydihydro-*β*-agarofuran (8): white, amorphous powder; $[\alpha]_D^{20}$ +20.0

(*c* 0.03, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 (4.32), 275 (3.79) nm; CD (MeOH) λ_{ext} ($\Delta\epsilon$) 242.7 (+2.04), 221.4 (−1.38) nm; IR (KBr) ν_{max} 2925, 1716, 1452, 1384, 1276, 1240, 1107, 1026, 713 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ OBz-6 [8.12 (2H, d, J = 7.5 Hz, H-2′/6′), 7.50 (2H, m, H-3′/5′), and 7.61 (1H, m, H-4′)], and OBz-9 [8.10 (2H, d, J = 8.3, H-2″/6″), 7.46 (2H, m, H-3″/5″), and 7.58 (1H, m, H-4″)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-6 [129.7 (s, C-1′), 129.7 (d, C-2′/6′), 128.8 (d, C-3′/5′), 133.5 (d, C-4′), and 165.8 (s, CO_2-6)], OBz-9 [129.6 (s, C-1″), 130.0 (d, C-2″/6″), 128.3 (d, C-3″/5″), 133.3 (d, C-4″), and 165.5 (s, CO_2-9)], for other signals, see Table 2; ESIMS *m*/*z* 559 $[M + Na]^+$; HRESIMS *m*/*z* 559.2300 $[M + Na]^+$ (calcd for $C_{31}H_{36}O_8Na$, 559.2308).

(1*S*,4*R*,5*S*,6*R*,7*R*,9*S*,10*S*)-1-Acetoxy-9-benzoyloxy-6-hydroxydihydro-β-agarofuran (9): white, amorphous powder; $[\alpha]_D^{20}$ +55.0 (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 230 (3.99), 273 (2.98) nm; IR (KBr) ν_{max} 2925, 1715, 1450, 1384, 1276, 1107, 714 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ OBz-9 [8.10 (2H, d, J = 7.5 Hz, H-2′/6′), 7.42 (2H, t, J = 7.5 Hz, H-3′/5′), and 7.58 (1H, t, J = 7.5 Hz, H-4′)], for other signals, see Table 1; ^{13}C NMR (100 MHz, $CDCl_3$) δ OBz-9 [129.8 (s, C-1′), 130.1 (d, C-2′/6′), 128.3 (d, C-3′/5′), 133.2 (d, C-4′), and 165.7 (s, CO_2-9)], for other signals, see Table 2; ESIMS *m*/*z* 439 $[M + Na]^+$; HRESIMS *m*/*z* 439.2085 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_6Na$, 439.2097).

Cinnamoylation of 9. Compound **9** (2.5 mg) was dissolved in dry pyridine (0.5 mL), and cinnamoyl chloride (3 drops) and a catalytic amount of 4-(dimethylamino)pyridine were added. Then, the mixture was stirred at rt for 48 h, poured into H_2O, extracted with EtOAc, and purified on preparative TLC developed with a solvent of petroleum ether−EtOAc (5:1), to give compound **16** [1.5 mg, R_f 0.45; CD (MeOH) λ_{ext} ($\Delta\epsilon$) 279.1 (+5.22), 235.0 (−4.01) nm].

Cytotoxicity Assays. HL-60 human leukemia cells were plated into 96-well plates containing 90 μL of medium. Cells were treated in triplicate with gradient concentrations of the tested compounds for 72 h. Thereafter, 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO) solution was added to each well. After 4 h of incubation at 37 °C, 50 μL of extraction buffer (10% SDS, 5% isobutanol, and 0.01 M hydrochloric acid) was added, the cells were incubated overnight at 37 °C, and the absorbance was then measured at 570 nm using a 96-well multiscanner (Molecular Devices, Mississauga, Ontario, Canada). The concentrations giving 50% growth inhibition (IC_{50}) were calculated with the Logit method. The anticancer drug etoposide was used as a positive control.

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