Cytotoxic Dihydroagarofuranoid Sesquiterpenes from the Seeds of *Celastrus orbiculatus*

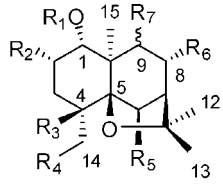
Yingdong Zhu, Zehong Miao, Jian Ding, and Weimin Zhao*

Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

Received January 23, 2008

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-OCin
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

* Corresponding author. Tel & Fax: 86-21-50806052. E-mail: wmzhao@mail.shcnc.ac.cn.

10.1021/np800052d CCC: $40.75
Published on Web 05/29/2008

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
2β	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
3β	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.

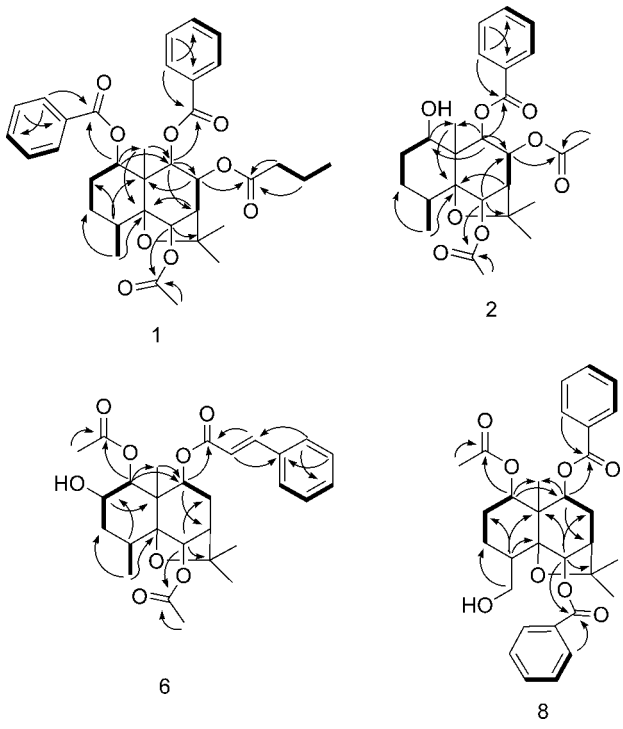


Figure 1. Main ^{1}H−^{13}C long-range correlation (^{1}H⤳^{13}C) and ^{1}H−^{1}H correlation (—) signals in the HMBC and COSY spectra of **1**, **2**, **6**, and **8**.

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.

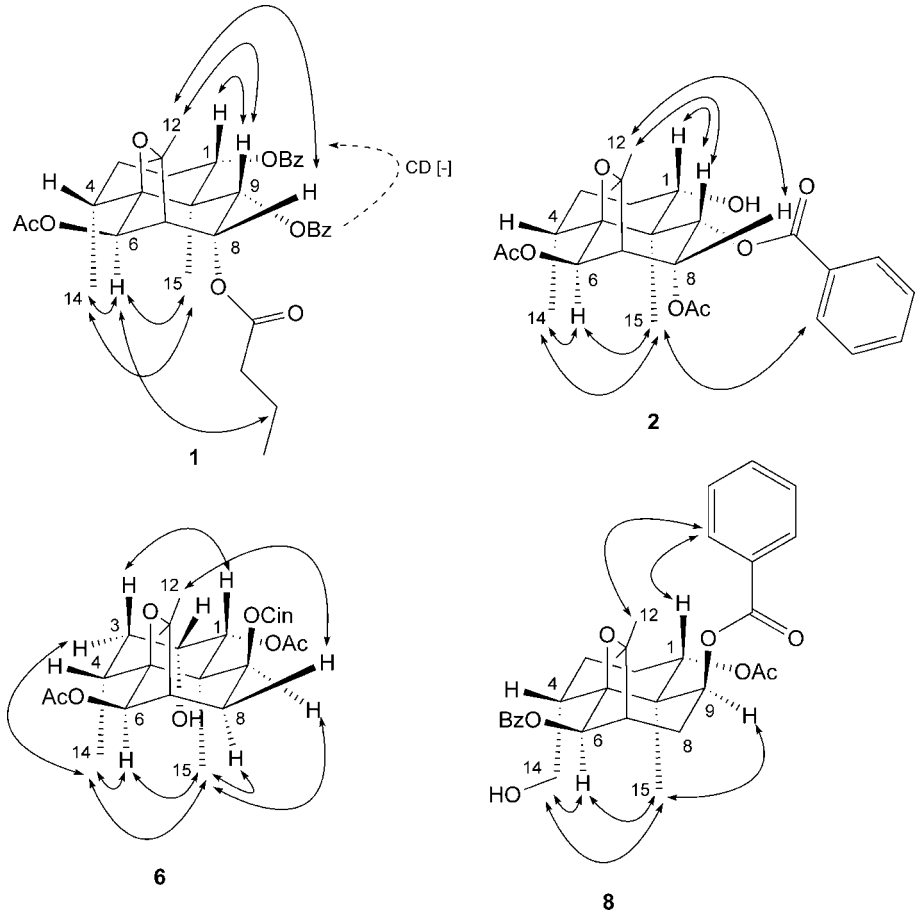


Figure 2. Main NOE correlation signals (↔) in the ROESY spectra of **1**, **2**, **6**, and **8**, and CD exciton coupling (dashed arrow) for **1**.

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.

Acknowledgment. The authors are grateful for partial financial support from the Science and Technology Commission of Shanghai Municipality (06DZ22028).

References and Notes

(1) Jiangsu New Medical College. Dictionary of Chinese Herb Medicines; Shanghai Scientific and Technologic Press: Shanghai, 1975; p 1563.
(2) Wu, W.; Wang, M.; Zhu, J.; Zhou, W.; Hu, Z.; Ji, Z. *J. Nat. Prod.* **2001**, *64*, 364–367.
(3) Muñoz-Martínez, F.; Mendoza, C. R.; Bazzocchi, I. L.; Castanys, S.; Jiménez, I. A.; Gamarro, F. *J. Med. Chem.* **2005**, *48*, 4266–4275.
(4) Kennedy, M. L.; Cortés-Selva, F.; Pérez-Victoria, J. M.; Jiménez, I. A.; González, A. G.; Muñoz, O. M.; Gamarro, F.; Castanys, S.; Ravelo, A. G. *J. Med. Chem.* **2001**, *44*, 4668–4676.
(5) Chen, J. J.; Chou, T. H.; Duh, C. Y.; Chen, I. S. *J. Nat. Prod.* **2006**, *69*, 685–688.
(6) Jiménez, I. A.; Bazzocchi, I. L.; Núñez, M. J.; Mukainaka, T.; Tokuda, H.; Nishino, H.; Konoshima, T.; Ravelo, A. G. *J. Nat. Prod.* **2003**, *66*, 1047–1050.
(7) Chen, J. J.; Chou, T. H.; Peng, C. F.; Chen, I. S.; Yang, S. Z. *J. Nat. Prod.* **2007**, *70*, 202–205.
(8) Wang, X.; Gao, W.; Yao, Z.; Zhang, S.; Zhang, Y.; Takaishi, Y.; Duan, H. *Chem. Pharm. Bull.* **2005**, *53*, 607–610.
(9) Jin, H. Z.; Hwang, B. Y.; Kim, H. S.; Lee, J. H.; Kim, Y. H.; Lee, J. J. *J. Nat. Prod.* **2002**, *65*, 89–91.
(10) Harada, H.; Nakanishi, K. *Circular Dichroism Spectroscopy: Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
(11) Tu, Y. Q.; Hu, Y. J.; Wu, W. J.; Chen, N. Y.; Pan, X. F. *Phytochemistry* **1992**, *31*, 3633–3634.
(12) Takaishi, Y.; Ohshima, S.; Nakano, K.; Tomimatsu, T.; Tokuda, H.; Nishino, H.; Iwashima, A. *J. Nat. Prod.* **1993**, *56*, 815–824.
(13) Tu, Y. Q.; Wang, D. Z.; Zhang, H. J.; Zhou, L. *Phytochemistry* **1991**, *30*, 271–273.
(14) Smith, C. R.; Miller, R. W.; Weisleder, D.; Rohwedder, W. K. *J. Org. Chem.* **1976**, *41*, 3264–3269.
(15) Guo, Y. Q.; Li, X.; Xu, J.; Li, N.; Meng, D. L.; Wang, J. H. *Chem. Pharm. Bull.* **2004**, *52*, 1134–1136.
(16) Munoz, O. M.; Gonzalez, A. G.; Ravelo, A. G.; Luis, J. G.; Vazquez, J. T.; Nunez, M. P.; Jimenez, I. A. *Phytochemistry* **1990**, *29*, 3225–3228.
(17) Takaishi, Y.; Noguchi, H.; Murakami, K.; Nakano, K.; Tomimatsu, T. *Phytochemistry* **1990**, *29*, 3869–3873.
(18) Wu, D.; Liu, J.; Cheng, C. *Phytochemistry* **1992**, *31*, 4219–4222.

NP800052D