New Dihydroagarofuranoid Sesquiterpenes from *Celastrus paniculatus*

by **Jing-Ru Weng***[a])[b]) and **Ming-Hong Yen**[c])

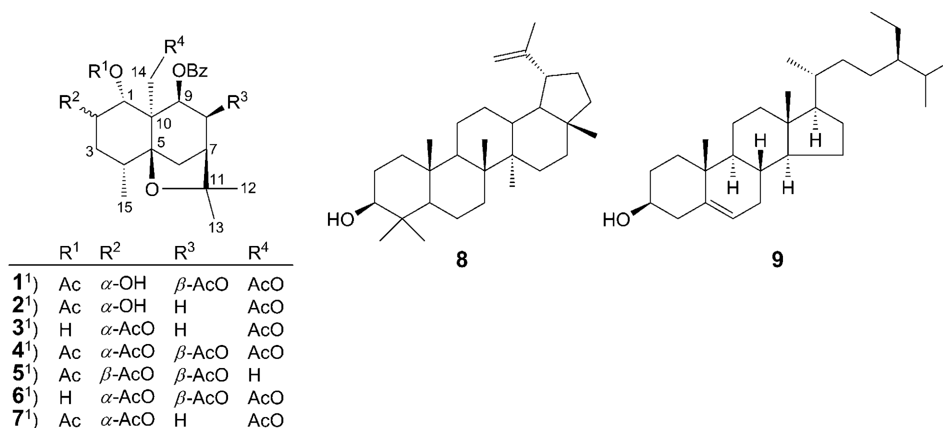
[a]) Department of Biological Science and Technology, China Medical University, Taichung 404, Taiwan (phone: +886-4-22053366 (ext. 2511); fax: +886-4-22071507; e-mail: columnster@gmail.com)

[b]) Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung 404, Taiwan

[c]) School of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan

The six new dihydro-β-agarofuranoid sesquiterpenes **1**–**6** and three known compounds were isolated from the whole plant of *Celastrus paniculatus*. The structures including relative configurations were elucidated by means of spectroscopic analyses. Compounds **1**–**6** were evaluated for cytotoxicity against a panel of three human-tumor cell lines.

Introduction. – *Celastrus paniculatus* (Celastraceae) is an evergreen shrub distributed throughout Hengchun peninsula of Taiwan, India, and Malaysia [1]. The family Celastraceae is well known for producing dihydroagarofuran derivatives and alkaloids [2], some of which exhibit insecticidal [3], antitumor [4][5], anti-inflammatory [6], multidrug-resistance (MDR) reversing [7][8], and immunosuppressive [9] activities. Moreover, seed oil of *C. paniculatus* has been reported to improve memory [10] and intestinal complaints [11][12], and display antioxidant [13], and hypolipidemic [14] effects. In our preliminary cytotoxicity screening for the genus *Celastrus* in Taiwan, the whole plant extract of *C. paniculatus* showed *in vitro* activity. In this article, we report the isolation and structural elucidation of the six new sesquiterpenes **1**–**6**[1]) and of three known compounds, including a dihydro-β-agarofuranoid sesquiterpene,



	R1	R2	R3	R4
1[1])	Ac	α-OH	β-AcO	AcO
2[1])	Ac	α-OH	H	AcO
3[1])	H	α-AcO	H	AcO
4[1])	Ac	α-AcO	β-AcO	AcO
5[1])	Ac	β-AcO	β-AcO	H
6[1])	H	α-AcO	β-AcO	AcO
7[1])	Ac	α-AcO	H	AcO

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

© 2010 Verlag Helvetica Chimica Acta AG, Zürich

triptogelin D 1 (**7**), a triterpenoid, lupeol (**8**), and a steroid, β-sitosterol (**9**), as well as the antitumor activities of **1**–**6** against a panel of human-cancer cell lines.

Results and Discussion. – *Chemistry.* Repeated chromatography of the MeOH extract of the whole plant of *C. paniculatus* (2 kg dry weight) on silica gel afforded compounds **1**–**9**. Compound **1** was isolated as an optically active, white powder. The molecular formula was determined as $C_{28}H_{36}O_{10}$ by its HR-FAB-MS from the $[M+H]^+$ signal at m/z 533.2386. The IR spectra showed absorption bands at 3474, 1749, and 1720 cm^{-1}, characteristic of OH and C=O functions, respectively. The ^{13}C-NMR spectrum of **1** (*Table 1*) revealed six Me, three CH_2, and six CH groups, four quaternary C-atoms, and four ester C=O groups (δ(C) 165.8, 169.7, 170.7, and 170.8). The ^{1}H-NMR spectrum of **1** (*Table 2*) indicated the presence of two tertiary Me groups (δ(H) 1.22 and 1.52), one secondary Me group (δ(H) 1.32), three AcO groups (δ(H) 1.71, 1.88, and 2.25), and one BzO group (δ(H) 8.08 (d, $J=7.8$ Hz), 7.58 (d, $J=7.8$ Hz), and 7.45 (d, $J=7.8$ Hz)). The signals observed at δ(H) 4.65 and 4.78 (2 d, $J=12.6$ Hz, 1 H each), 5.66 (dd, $J=6.6$, 3.0 Hz), 5.57 (d, $J=6.6$ Hz), and 5.59 (d, $J=3.0$ Hz) were assigned to one CH_2 and three CH groups bearing an O-atom function. Taken together, these spectral data suggested that compound **1** contained a dihydro-β-agarofuran

Table 1. *^{13}C-NMR Data* (150 MHz, $CDCl_3$) *of Compounds* **1**–**6**. δ in ppm.

	1	**2**	**3**	**4**[a])	**5**[a])	**6**
C(1)	74.0	74.6	69.9	71.0	72.6	70.5
C(2)	68.8	69.0	74.5	69.7	69.0	75.2
C(3)	32.7	32.7	31.1	30.8	32.8	31.1
C(4)	39.3	39.5	39.2	39.0	40.1	39.0
C(5)	86.6	87.1	86.5	86.0	86.3	86.4
C(6)	36.4	36.3	36.3	36.3	35.7	31.4
C(7)	48.2	43.6	43.4	48.2	48.4	47.5
C(8)	71.8	34.0	32.9	71.6	70.3	74.7
C(9)	68.9	70.1	69.2	68.5	72.4	71.3
C(10)	51.7	50.9	51.5	51.5	49.7	50.1
C(11)	82.1	81.9	82.2	82.3	82.6	82.0
C(12)	31.0	30.0	30.1	30.8	31.1	30.2
C(13)	25.1	24.3	24.3	25.0	25.0	24.2
C(14)	64.8	65.8	66.0	64.3	19.0	67.2
C(15)	19.1	19.1	19.1	18.7	18.2	19.0
*Ac*O–C(1)	169.7, 20.7	169.7, 20.7		169.9, 20.8	169.2, 20.4	
*Ac*O–C(2)			170.4, 21.4	169.3, 20.2	170.6, 21.0	170.3, 21.3
*Ac*O–C(8)	170.7, 20.9			169.9, 21.2	169.7, 20.7	169.3, 21.1
*Ac*O–C(14)	170.8, 21.4	170.7, 21.5	171.0, 21.3	170.6, 21.3		170.3, 21.3
Bz:						
C=O	165.8	165.5	165.7	165.7	166.2	165.1
C(1′)	133.3	133.2	132.9	133.3	132.9	133.1
C(2′,6′)	130.3	130.1	129.6	130.2	130.3	129.7
C(3′,5′)	128.2	128.2	128.5	128.2	128.0	128.5
C(4′)	129.3	129.5	130.5	129.1	129.9	130.0

[a]) At 100 MHz.

(= (3*R*,5a*S*,9*R*,9a*S*)-octahydro-2,2,5a,9-tetramethyl-2*H*-3,9a-methano-1-benzoxepin) skeleton found in Celastraceae sesquiterpene esters [3][15]. The ^{13}C-NMR spectrum of the sesquiterpene moiety of **1** was similar to that of salasol A [16], except for the C(6) and C(8) signals (*Table 1*). Assignments of the H- and C-atom signals of **1** (*Tables 1* and *2*) were made by comparing with the corresponding signals of salasol A (= (3*R*,5*S*,5a*R*,6*R*,7*S*,9*R*,9a*S*,10*R*)-5a-[(acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-5,6,7,10-tetrol 6,10-diacetate 5-benzoate) [16] and confirmed by ^{1}H,^{1}H-COSY and NOESY analyses (*Figs. 1* and *2*). The linkage of the AcO group to C(8) was supported by the HMBCs between both H–C(8) (δ(H) 5.66) and AcO–C(8) (δ(H) 1.88) and the ester C=O resonance (δ(C) 170.7). The positions of the other three ester groups were assigned to be at C(1), C(9), and C(14) based on the following correlations: H–C(1) (δ(H) 5.59) and AcO–C(1) (δ(H) 1.71)/Me*C*=O (δ(C) 169.7), H–C(9) (δ(H) 5.57) and H–C(2′,6′) (δ(H) 8.08)/Ph*C*=O (δ(C) 165.8), and CH$_2$(14) (δ(H) 4.65 and 4.78)/Me*C*=O (δ(C) 170.8). Assignments of the relative configurations at C(1), C(2), C(4), C(8), C(9), and C(10) were based on the splitting patterns, on the coupling constants of H–C(1) (δ(H) 5.59 (*d*, *J* = 3.0 Hz), H–C(2) (δ(H) 4.36 (*dd*, *J* = 5.4, 3.0 Hz), H–C(8) (δ(H) 5.66 (*dd*, *J* = 6.6, 3.0 Hz), and H–C(9) (δ(H) 5.57 (*d*, *J* = 6.6 Hz), and on the selected cross-peaks Me(15) (δ(H) 1.32)/

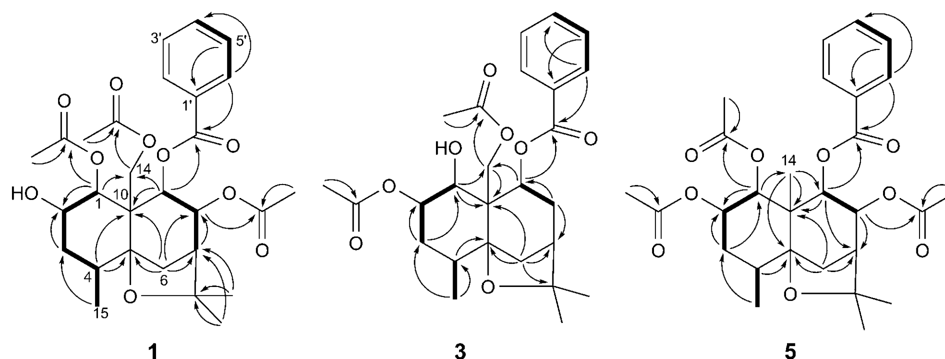


Fig. 1. *Key HMBCs* (H → C) *and ^{1}H,^{1}H-COSYs* (—) *of* **1**, **3**, *and* **5**[1])

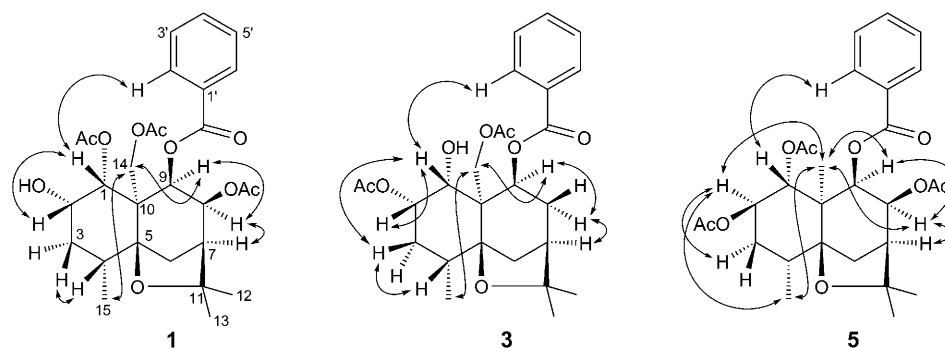


Fig. 2. *Selected NOESY correlations and relative configurations of* **1**, **3**, *and* **5**[1])

$CH_2(14)$ (δ(H) 4.65 and 4.78), and $CH_2(14)$ (δ(H) 4.65 and 4.78)/H–C(9) (δ(H) 5.57) in the NOESY plot and comparison with those of known Celastraceae sesquiterpene esters [3][17][18]. Accordingly, we characterized compound **1** as ($1\alpha,2\alpha,8\beta,9\beta$)-1,8,14-tris(acetyloxy)-9-(benzoyloxy)-2-hydroxydihydro-β-agarofuran.

Compound **2** was isolated as an optically active, white powder. The molecular formula was determined as $C_{26}H_{34}O_8$ by HR-FAB-MS (*m/z* 475.2322 ($[M+H]^+$)). The ^{1}H- and ^{13}C-NMR spectra indicated that **2** contained two AcO groups and one BzO group (*Tables 1* and *2*), one fewer AcO group than compound **1**. Similarity in the spectral data of these two compounds suggested that **2** also contained a dihydro-β-agarofuran skeleton (*Table 2*). However, relative to compound **1**, **2** lacked the AcO group at C(8). Signals for the $CH_2(8)$ group of **2** were observed at δ(H) 2.08–2.11 and 2.26–2.29, and δ(C) 34.0. The structure of **2** was deduced by HMQC and HMBC spectral analyses, and the relative configurations at C(1), C(2), C(9), and C(10) of **2** were determined by comparison with the relative configuration of **1**. Thus, compound **2** was established as ($1\alpha,2\alpha,9\beta$)-1,14-bis(acetyloxy)-9-(benzoyloxy)-2-hydroxydihydro-β-agarofuran.

Compound **3** showed the same molecular formula and IR spectrum as **2**. The ^{13}C-NMR spectrum of **3** (*Table 1*) exhibited a high degree of similarity to that of **2**, however, with differences in the chemical shifts of C(1) and C(2). Comparison of the ^{1}H-NMR spectra of **2** and **3** revealed differences in two H-atom signals showing an extreme upfield shift (δ(H) 5.58 in **2** *vs.* 4.62 in **3**) and a downfield shift (δ(H) 4.38 in **2** *vs.* 5.31 in **3**), respectively. These differences might arise from a shift of the AcO group from C(1) to C(2) in **3**. The relative configurations of **3** were resolved by analysis of the coupling constants and confirmed by a NOESY experiment (*Fig. 2*). Accordingly, we characterized compound **3** as ($1\alpha,2\alpha,9\beta$)-2,14-bis(acetyloxy)-9-(benzoyloxy)-1-hydroxydihydro-β-agarofuran.

The molecular formula of **4** was determined to be $C_{30}H_{38}O_{11}$ by HR-FAB-MS (*m/z* 575.2487 ($[M+H]^+$)). The ^{1}H- and ^{13}C-NMR spectra of **4** resembled those of angulatueoid B (=(3*S*,4*S*,5*S*,5a*S*,6*R*,7*S*,9*R*,9a*S*)-5a-[(acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7-tetrol 4,6,7-triacetate 5-benzoate) [19], except that the H–C(9) signal of **4** was shifted to higher field relative to that of the corresponding H-atom signal of angulatueoid B. The relative configurations at C(1), C(2), C(4), C(8), and C(10) were determined by comparison with the original configuration determined for angulatueoid B [19]. The β-configuration of the BzO group at C(9) was supported by a NOESY experiment, which showed interactions between H_α–C(14) (δ(H) 4.49) and H–C(9) (δ(H) 5.56). Thus, **4** was elucidated as ($1\alpha,2\alpha,8\beta,9\beta$)-1,2,8,14-tetrakis(acetyloxy)-9-(benzoyloxy)dihydro-β-agarofuran.

Compound **5** had a molecular formula $C_{28}H_{36}O_9$, as deduced from its HR-EI-MS and NMR data. The ^{1}H-NMR spectrum of **5** (*Table 2*) was very similar to that of **4**, except for the lack of signals associated with an $AcOCH_2$ moiety and the presence of a signal characteristic of a tertiary Me group. In the HMBC plot, the Me(14) (δ(H) 1.39) showed ^2J correlation with C(10) (δ(C) 49.7), and ^3J coupling with C(9) (δ(C) 72.4) and C(5) (δ(C) 86.3) confirmed the position of the tertiary Me group at C(10). In addition, the NOESY experiment indicated that compound **5** differed from **4** in the configuration at C(2) (*Fig. 2*). NOESY Correlations observed between Me(15) and

Table 2. *^{1}H-NMR Data* (600 MHz, $CDCl_3$) *of Compounds* **1**–**6**. δ in ppm, *J* in Hz.

	1	**2**	**3**	**4**[a])	**5**[a])	**6**
H−C(1)	5.59 (*d*, *J* = 3.0)	5.58 (*d*, *J* = 3.0)	4.62 (*d*, *J* = 3.6)	5.64 (*d*, *J* = 3.0)	5.72 (*d*, *J* = 10.4)	4.49 (br. *s*)
H−C(2)	4.36 (*dd*, *J* = 5.4, 3.0)	4.38 (*dd*, *J* = 5.4, 3.0)	5.31 (*dd*, *J* = 6.6, 3.6)	5.52 (*dd*, *J* = 6.0, 3.0)	5.16 (*dt*, *J* = 10.4, 4.4)	5.30 (*dd*, *J* = 6.6, 3.6)
CH_2(3)	1.81–1.84 (*m*), 2.35–2.37 (*m*)	1.80 (*dd*, *J* = 14.0, 3.0), 2.36–2.39 (*m*)	1.83 (*d*, *J* = 15.0), 2.36 (*ddd*, *J* = 15.0, 6.6, 3.6)	1.76 (*dd*, *J* = 15.0, 3.6), 2.44 (*ddd*, *J* = 15.0, 6.6, 3.6)	1.76–1.81 (*m*), 2.29–2.33 (*m*)	1.89–1.92 (*m*), 2.34–2.37 (*m*)
H−C(4)	1.93 (br. *q*, *J* = 7.2)	1.90 (br. *q*, *J* = 7.8)	1.92 (*q*, *J* = 7.8)	1.96 (br. *q*, *J* = 7.8)	2.02–2.06 (*m*)	1.94–1.96 (*m*)
CH_2(6)	2.26–2.29 (*m*), 2.40 (*d*, *J* = 12.6)	2.08–2.11 (*m*), 2.34 (*d*, *J* = 12.0)	2.10–2.13 (*m*)	2.28–2.37 (*m*), 2.34 (*d*, *J* = 12.0)	2.09–2.12 (*m*), 2.26–2.30 (*m*)	2.03–2.05 (*m*), 2.34–2.37 (*m*)
H−C(7)	2.27–2.29 (*m*)	2.07–2.09 (*m*)	2.10–2.13 (*m*)	2.27–2.29 (*m*)	2.24–2.27 (*m*)	2.29–2.31 (*m*)
H−C(8) or CH_2(8)	5.66 (*dd*, *J* = 6.6, 3.0)	2.08–2.11 (*m*), 2.26–2.29 (*m*)	2.19 (*d*, *J* = 15.0), 2.29 (*ddd*, *J* = 15.0, 6.6, 3.6)	5.65 (*dd*, *J* = 6.0, 3.0)	5.38 (*dd*, *J* = 6.0, 3.0)	5.31 (br. *s*)
H−C(9)	5.57 (*d*, *J* = 6.6)	5.39 (*d*, *J* = 6.6)	5.50 (*d*, *J* = 6.6)	5.56 (*d*, *J* = 6.0)	5.27 (*d*, *J* = 6.0)	5.50 (*s*)
Me(12)	1.22 (s)	1.21 (*s*)	1.23 (*s*)	1.22 (*s*)	1.23 (*s*)	1.26 (*s*)
Me(13)	1.52 (*s*)	1.38 (*s*)	1.46 (*s*)	1.52 (*s*)	1.51 (*s*)	1.57 (*s*)
CH_2(14) or Me(14)	4.65 (*d*, *J* = 12.6), 4.78 (*d*, *J* = 12.6)	4.62 (*d*, *J* = 12.6), 4.86 *d*, *J* = 12.6)	4.54 (*d*, *J* = 12.0), 4.66 (*d*, *J* = 12.0)	4.49 (*d*, *J* = 12.6), 4.69 (*d*, *J* = 12.6)	1.39 (*s*)	4.61 (*d*, *J* = 12.0), 4.95 (*d*, *J* = 12.0)
Me(15)	1.32 (*d*, *J* = 7.8)	1.32 (*d*, *J* = 8.4)	1.24 (*d*, *J* = 7.8)	1.26 (*d*, *J* = 8.4)	1.19 (*s*)	1.21 (*d*, *J* = 8.4)
*Ac*O−C(1)	1.71 (*s*)	1.66 (*s*)		1.87 (*s*)	1.75 (*s*)	
*Ac*O−C(2)			2.15 (*s*)	1.61 (*s*)	1.92 (*s*)	2.01 (*s*)
*Ac*O−C(8)	1.88 (*s*)			2.07 (*s*)	1.87 (*s*)	2.15 (*s*)
*Ac*O−C(14)	2.25 (*s*)	2.16 (*s*)	2.06 (*s*)	2.25 (*s*)		2.17 (*s*)
H−C(2′,6′)	8.08 (*d*, *J* = 7.8)	8.05 (*d*, *J* = 7.2)	8.09 (*d*, *J* = 7.2)	8.05 (*d*, *J* = 7.2)	8.11 (*d*, *J* = 7.2)	8.10 (*d*, *J* = 7.2)
H−C(3′,5′)	7.45 (*d*, *J* = 7.8)	7.43 (*dt*, *J* = 7.2)	7.44 (*d*, *J* = 7.2)	7.42–7.46 (*m*)	7.44–7.47 (*m*)	7.44 (*d*, *J* = 7.2)
H−C(4′)	7.58 (*d*, *J* = 7.8)	7.55 (*dt*, *J* = 7.2)	7.55–7.58 (*m*)	7.54–7.58 (*m*)	7.58 (*d*, *J* = 7.2)	7.56 (*dt*, *J* = 7.2)

[a]) At 400 MHz.

H–C(2) and Me(14), and the large coupling constant ($J_{1,2}$ = 10.4 Hz) between H–C(1) and H–C(2) of **5** suggested that the configurations of AcO–C(2) and Me–C(10) were β and α, respectively. Accordingly, we characterized compound **5** as (1α,2β,8β,9β)-1,2,8-tris(acetyloxy)-9-(benzoyloxy)dihydro-β-agarofuran.

Compound **6** exhibited a molecular formula identical to that of **1** with a similar IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **6** were similar to those of **1**, except for the signals of the CH(1) and CH(2) moieties. This finding suggested a difference in the locality of the AcO group, *i.e.*, C(1) *vs.* C(2), between these two molecules. In the light of the upfield shift of H–C(1) (δ(H) 4.49 in **6** *vs.* 5.59 in **1**) and downfield shift of H–C(2) (δ(H) 5.30 in **6** *vs.* 4.36 in **1**), the OH group and the AcO group in **6** were assigned to C(1) and C(2), respectively. The relative configuration was determined by comparison with the relative configuration of **1**. Therefore, **6** was elucidated as (1α,2α,8β,9β)-2,8,14-tris(acetyloxy)-9-(benzoyloxy)-1-hydroxydihydro-β-agarofuran.

The known compounds triptogelin D 1 (**7**) [15], lupeol (**8**) [20], and β-sitosterol (**9**) [20] were identified by spectroscopic methods and comparison with the reported spectral data or with those of authentic samples.

Biological Studies. To assess the potential anticancer activities of these dihydro-β-agarofuran derivatives, we examined the cytotoxicity of compounds **1**–**6** in a panel of human-cancer cell lines by MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-dimethyl-2*H*-tetrazolium bromide) assays, including MCF-7 breast cancer, PC-3 prostate cancer, and Hep3B hepatocellular carcinoma, with 5-fluorouracil (5-FU) as a positive control. The antiproliferative activitiy of compound **7** was not tested due to insufficient quantities. As shown, compounds **3**–**5** exhibited differential activities against MCF-7 cells, with IC_{50} values ranging from 13–48 μM (*Table 3*), while compounds **1**, **2**, and **6** showed no appreciable effect on suppressing MCF-7 cell viability. However, although compounds **2** and **6** were ineffective in suppressing the viability of MCF-7 cells, they showed cell-line-specific cytotoxicity against PC-3 and Hep3B cells, respectively. This cell-line specificity suggests that each of these derivatives might display a unique mode of antitumor action.

Table 3. *Cytotoxic Activities of* **1**–**6** *against Different Cancer Cell Lines*

	IC_{50} [μg/ml][a])		
	MCF-7[b])	PC-3[b])	Hep3B[b])
1	> 50	> 50	> 50
2	> 50	46.0 ± 0.7	> 50
3	48.3 ± 2.9	> 50	> 50
4	13.4 ± 1.0	> 50	> 50
5	32.4 ± 0.6	> 50	> 50
6	> 50	> 50	22.8 ± 0.5
5-Fu	3.9 ± 0.8	19.5 ± 0.6	7.4 ± 0.2

[a]) Data are presented as mean ± s.e.m. (n = 3–6). 5-Fu (5-fluorouracil) was used as a positive control.
[b]) Key to all cell lines: MCF-7, human-breast adenocarcinoma; PC-3, human-prostate-cancer cell; Hep3B, hepatomacellur carcinoma.

With regard to MCF-7 cells, it seems that the compound with a Me group at C(10) (*i.e.*, **5**) had a slightly decreased cytotoxicity, while compounds with a free OH group at C(1) or C(2) (*i.e.*, **1** and **6**) showed no such activity in suppressing cell viability. This finding suggests that the mode of antitumor action of compounds **3**–**5** might be related to the inhibition of estrogen-receptor signaling in breast cancer cells, which warrants further investigation. Moreover, as compounds **4** and **5** exhibited higher activities than **3**, and, to a greater extent, than **1** in suppressing the viability of MCF-7 cells, the AcO group at both C(1) and C(2) played an integral role in mediating the cytotoxicity.

This work was supported by grants from the *National Science Council of Republic of China* (NSC 94-2314-B-039-033, NSC 95-2320-B-039-041) and China Medical University (CMU95-171, CMU96-103, CMU96-200).

Experimental Part

General. TLC: silica gel (SiO_2) *60 F_{254}* precoated plates (*Merck*). Column chromatography (CC): SiO_2 *60* (70–230 or 230–400 mesh; *Merck*). Optical rotation: *Jasco-DIP-370* polarimeter; in $CHCl_3$. UV Spectra: *Jasco-UV-240* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer-2000* FT-IR, IR *Prestige-21* spectrophotometers; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR and 2D-NMR Spectra: *Varian-Unity-600* and *Bruker-AV-400* spectrometers; δ in ppm rel. to Me_4Si as internal standard, J in Hz. EI- and HR-EI-MS: *MAT-95XL* mass spectrometer; in m/z (rel. %). FAB- and HR-FAB-MS: *JMS-SX/SX102A* mass spectrometer; 3-nitrobenzyl alcohol as matrix; in m/z.

Plant Material. The whole plant of *Celastrus paniculatus* (Celastraceae) was collected in Ping Tung Hsieng, Taiwan, in October, 2005, and a voucher specimen (2005) has been deposited with the School of Pharmacy, Kaohsiung Medical University.

Extraction and Isolation. The whole plant of *C. paniculatus* (2.0 kg) was ground, and extracted with MeOH at r.t., and the extract concentrated to afford a brown residue (90 g). This residue (90 g) was fractioned by CC (SiO_2, hexane/AcOEt 19:1, 9:1, and 2:1, hexane/AcOEt/MeOH 4:1:1 and 1:1:1, and AcOEt/MeOH 1:1): *Fractions A–F*. *Fr. D* was resubjected to CC (SiO_2, CH_2Cl_2/acetone 19:1): **1** (10 mg), **2** (20 mg), and **5** (4 mg). *Fr. E* was further purified by CC (SiO_2, hexane/acetone 1:1): *Frs. E_1* and *E_2*. *Fr. E_1* was further purified by CC (SiO_2, $CHCl_3$/acetone 9:1): **3** (21 mg) and **4** (25 mg). *Fr. E_2* was further purified by CC (SiO_2, $CHCl_3$/acetone 7:1): **6** (4 mg). *Fr. C* was further purified by CC (SiO_2, hexane/acetone 3:1): *Frs. C_1* and *C_2*. *Fr. C_1* was further purified by CC (SiO_2, hexane/acetone 7:3): **7** (2 mg). *Fr. B* was further purified by CC (SiO_2, hexane/AcOEt 5:1): *Frs. B_1* and *B_2*. *Fr. B_1* was further purified by CC (SiO_2, hexane/AcOEt 4:1): **8** (25 mg) and **9** (26 mg).

(1α,2α,8β,9β)-1,8,14-Tris(acetyloxy)-9-(benzoyloxy)-2-hydroxydihydro-β-agarofuran (= rel-*(3*R*,4*R*,5*S*,5a*R*,6*S*,7*R*,9*S*,9a*R*)-5a-[(Acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7-triol 4,6-Diacetate 5-Benzoate*; **1**): White powder. $[\alpha]_D^{22} = +20.2$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 228 (4.08), 272 (2.91). IR (KBr): 3474, 1749, 1720. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 533 (13, $[M+H]^+$). HR-FAB-MS: 533.2386 ($[M+H]^+$, $C_{28}H_{37}O_{10}^+$; calc. 533.2387).

(1α,2α,9β)-1,14-Bis(acetyloxy)-9-(benzoyloxy)-2-hydroxydihydro-β-agarofuran (= rel-*(3*R*,5*S*,5a*R*,6*R*,7*S*,9*R*,9a*S*)-5a-[(Acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-5,6,7-triol 6-Acetate 5-Benzoate*; **2**): White powder. $[\alpha]_D^{22} = +49.8$ ($c = 0.22$, $CHCl_3$). UV (MeOH): 227 (4.03), 272 (2.85). IR (KBr): 3464, 1747, 1723, 1710. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 475 (18, $[M+H]^+$). HR-FAB-MS: 475.2322 ($[M+H]^+$, $C_{26}H_{35}O_8^+$; calc. 475.2332).

(1α,2α,9β)-2,14-Bis(acetyloxy)-9-(benzoyloxy)-1-hydroxydihydro-β-agarofuran (= rel-*(3*R*,5*S*,5a*S*,6*R*,7*S*,9*R*,9a*S*)-5a-[(Acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-5,6,7-triol 7-Acetate 5-Benzoate*; **3**): White powder. $[\alpha]_D^{22} = +18.0$ ($c = 0.21$, $CHCl_3$). UV (MeOH): 228 (4.03), 271 (2.85). IR (KBr): 3509, 1740, 1721. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 474 (1, M^+). HR-EI-MS: 474.2257 (M^+, $C_{26}H_{34}O_8^+$; calc. 474.2254).

(1α,2α,8β,9β)-1,2,8,14-Tetrakis(acetyloxy)-9-(benzoyloxy)dihydro-β-agarofuran (= rel-*(3*R*,4*R*,5*S*,5a*R*,6*S*,7*R*,9*S*,9a*R*)-5a-[(Acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7-tetrol 4,6,7-Triacetate 5-Benzoate*; **4**): White powder. $[\alpha]_D^{22} = +22.5$ ($c = 0.21$, $CHCl_3$). UV (MeOH): 230 (4.05), 274 (2.78). IR (KBr): 1742, 1720, 1602. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 575 (15, $[M+H]^+$). HR-FAB-MS: 575.2487 ($[M+H]^+$, $C_{30}H_{39}O_{11}^+$; calc. 575.2492).

(1α,2β,8β,9β)-1,2,8-Tris(acetyloxy)-9-(benzoyloxy)dihydro-β-agarofuran (= rel-*(3*R*,4*R*,5*S*,5a*R*,6*S*,7*S*,9*S*,9a*R*)-Octahydro-2,2,5a,9-tetramethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7-tetrol 4,6,7-Triacetate 5-Benzoate*; **5**): White powder. $[\alpha]_D^{22} = +44.8$ ($c = 0.25$, $CHCl_3$). UV (MeOH): 229 (4.08), 272 (2.74). IR (KBr): 1745, 1740, 1715. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 516 (10, M^+). HR-EI-MS: 516.2351 (M^+, $C_{28}H_{36}O_9^+$; calc. 516.2359).

(1α,2α,8β,9β)-2,8,14-Tris(acetyloxy)-9-(benzoyloxy)-1-hydroxydihydro-β-agarofuran (= rel-*(3*R*,4*R*,5*S*,5a*R*,6*S*,7*R*,9*S*,9a*R*)-5a-[(Acetyloxy)methyl]octahydro-2,2,9-trimethyl-2*H*-3,9a-methano-1-benzoxepin-4,5,6,7-tetrol 4,7-Diacetate 5-Benzoate*; **6**): White powder. $[\alpha]_D^{22} = +11.5$ ($c = 0.19$, $CHCl_3$). UV (MeOH): 228 (4.03), 272 (2.84). IR (KBr): 3462, 1711. ^1H- and ^{13}C-NMR: *Tables 1* and *2*. FAB-MS: 533 (31, $[M+H]^+$). HR-FAB-MS: 533.2391 ($[M+H]^+$, $C_{28}H_{37}O_{10}^+$; calc. 533.2387).

Cytotoxicity Bioassay. MCF-7 Breast cancer cells, PC-3 prostate cancer cells, and Hep3B hepatocellular carcinoma cells were purchased from the *American Type Culture Collection* (Manassas, VA), and cultured in RPMI-1640 medium or DMEM/Ham's F-12 medium containing 10% of heat-inactivated FBS (fetal bovine serum). The effect of individual test agents on inhibiting cell viability was assessed by using the MTT (2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) assay in six replicates. Cells were seeded and incubated in 96-well, flat-bottomed plates in 10% FBS-supplemented medium for 24 h and were exposed to various concentrations of test agents dissolved in DMSO (final DMSO concentration, 0.1%) in 5% FBS-supplemented medium. Controls received DMSO vehicle at a concentration equal to that of drug-treated cells. The medium was removed and replaced by 200 μl of 0.5 mM MTT in 10% FBS-containing RPMI-1640 medium, and cells were incubated in the 5% CO_2 incubator at 37° for 2 h. Supernatants were removed from the wells, and the reduced MTT dye was solubilized in 200 μl/well of DMSO. Absorbance at 570 nm was determined on a plate reader.

Statistical Analysis. Data are presented as means ± s.d. One-way analysis of variance was used for multiple comparison, and if there was significant variation between the treatment groups and the inhibitor-treated groups, they were then compared with the control group by *Student*'s *t*-test. Values of $P < 0.05$ were considered statistically significant.

Supplemental Information. ^1H- and ^{13}C-NMR, HMQC, HMBC, COSY, and NOESY plots and data of compounds **1**–**6** are available free of charge from *J.-R. Weng*.

REFERENCES

[1] S. U. Lu, Y. P. Yang, 'Celastraceae', in 'Flora of Taiwan', 2nd edn., Editorial Committee of the Flora of Taiwan, Taipe, 1993, Vol. III, p. 640.

[2] X.-H. Su, M.-L. Zhang, W.-H. Zhan, C.-H. Huo, Q.-W. Shi, Y.-C. Gu, H. Kiyota, *Chem. Biodiversity* **2009**, *6*, 146.

[3] W. Wu, M. Wang, J. Zhu, W. Zhou, Z. Hu, Z. Ji, *J. Nat. Prod.* **2001**, *64*, 364.

[4] J.-J. Chen, T.-H. Chou, C.-Y. Duh, I.-S. Chen, *J. Nat. Prod.* **2006**, *69*, 685.

[5] Y. Zhu, Z. Miao, J. Ding, W. Zhao, *J. Nat. Prod.* **2008**, *71*, 1005.

[6] H. Z. Jin, B. Y. Hwang, H. S. Kim, J. H. Lee, Y. H. Kim, J. J. Lee, *J. Nat. Prod.* **2002**, *65*, 89.

[7] S. E. Kim, Y. H. Kim, J. J. Lee, Y. C. Kim, *J. Nat. Prod.* **1998**, *61*, 108.

[8] S. E. Kim, H. S. Kim, Y. S. Hong, Y. C. Kim, J. J. Lee, *J. Nat. Prod.* **1999**, *62*, 697.

[9] X. Wang, W. Gao, Z. Yao, S. Zhang, Y. Zhang, Y. Takaishi, H. Duan, *Chem. Pharm. Bull.* **2005**, *53*, 607.

[10] P. B. Godkar, R. K. Gordon, A. Ravindran, B. P. Doctor, *Phytomedicine* **2006**, *13*, 29.

[11] N. Borbone, F. Borrelli, D. Montesano, A. A. Izzo, S. De Marino, R. Capasso, F. Zollo, *Planta Med.* **2007**, *73*, 792.

[12] F. Borrelli, N. Borbone, R. Capasso, D. Montesano, S. De Marino, G. Aviello, G. Aprea, S. Masone, A. A. Izzo, *J. Ethnopharmacol.* **2009**, *122*, 434.
[13] M. H. V. Kumar, Y. K. Gupta, *Phytomedicine* **2002**, *9*, 302.
[14] D.-K. Patel, K.-S. Amin, D.-D. Nanvatl, *Indian Drugs* **1993**, *32*, 566.
[15] Y. Takaishi, S. Tamai, K. Nakano, K. Murakami, T. Tomimatsu, *Phytochemistry* **1991**, *30*, 3027.
[16] T. Morikawa, A. Kishi, Y. Pongpiriyadacha, H. Matsuda, M. Yoshikawa, *J. Nat. Prod.* **2003**, *66*, 1191.
[17] Y. Takaishi, S. Ohshima, K. Nakano, T. Tomimatsu, H. Tokuda, H. Nishino, A. Iwashima, *J. Nat. Prod.* **1993**, *56*, 815.
[18] T.-H. Chou, I.-S. Chen, P.-J. Sung, C.-F. Peng, P.-C. Shieh, J.-J. Chen, *Chem. Biodiversity* **2007**, *4*, 1594.
[19] C. Cheng, D. Wu, J. Liu, *Phytochemistry* **1992**, *31*, 2777.
[20] J.-R. Weng, H.-J. Su, M.-H. Yen, S.-J. Won, C.-N. Lin, *Chin. Pharm. J.* **2003**, *55*, 267.

Received October 8, 2009