Cytotoxic Flavonoids from the Leaves of *Cryptocarya chinensis*

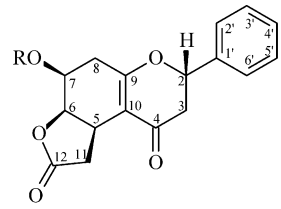
Tsung-Hsien Chou,[†] Jih-Jung Chen,[‡] Shiow-Ju Lee,[§] Michael Y. Chiang,[#] Cheng-Wei Yang,[§] and Ih-Sheng Chen*[,†]

Graduate Institute of Pharmaceutical Sciences, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China, Graduate Institute of Pharmaceutical Technology, Tajen University, Pingtung, Taiwan 907, Republic of China, Division of Biotechnology, Pharmaceutical Research, National Health Research Institute, Miaoli, Taiwan 350, Republic of China, and Department of Chemistry, National Sun Yat-sen University, Kaohsiung, Taiwan 804, Republic of China

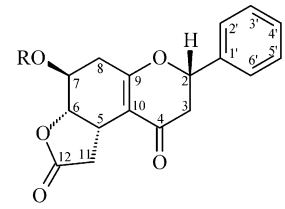
Received January 7, 2010

Bioassay-guided fractionation led to the isolation of six new tetrahydroflavanones, cryptochinones A−F (**1**−**6**), from the neutral $CHCl_3$ fraction of *Cryptocarya chinensis* leaves, together with 14 known compounds (**7**−**20**). The structures of these new compounds were determined through spectroscopic analyses, including 2D-NMR, MS, CD, and X-ray crystallographic analysis. Among the isolates, infectocaryone (**7**) showed cytotoxic activities with IC_{50} values of 11.0 and 3.7 μM against NCI-H460 and SF-268 cell lines, respectively, and cryptocaryanone A (**9**) showed cytotoxic activities with IC_{50} values of 5.1, 4.3, and 5.0 μM against MCF-7, NCI-H460, and SF-268 cell lines, respectively.

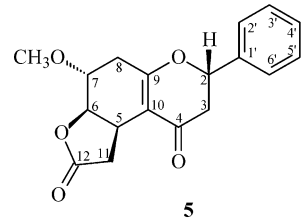
Cryptocarya chinensis (Hance) Hemsl. (Lauraceae) is a medium-sized evergreen tree distributed throughout southern China, Japan, and Taiwan.[1] Flavonoids,[2−4] pyrones,[5−7] pavines,[8−10] aporphines,[9−11] benzylisoquinolines,[11,12] lignans,[13] and their derivatives are widely distributed in plants of the genus *Cryptocarya*, and many of these compounds exhibit cytotoxic[2−4,6,7,13] and antioxidant activities.[5] In our studies on the cytotoxic constituents of Formosan plants,[14−20] about 1000 species have been screened for in vitro cytotoxic activity against MCF-7, NCI-H460, and SF-268 cell lines. *C. chinensis* has been found to be one of the active species. The methanolic extract of leaves was subjected to liquid−liquid partition to afford a cytotoxic neutral $CHCl_3$ fraction with IC_{50} values of 8.1, 4.6, and 6.5 μg/mL against MCF-7, NCI-H460, and SF-268 cell lines, respectively. Bioassay-guided fractionation of the neutral $CHCl_3$ fraction of leaves from this species led to the isolation of six new tetrahydroflavanones, cryptochinones A−F (**1**−**6**), together with 14 known compounds (**7**−**20**). This paper describes the structural elucidation of **1**−**6** and the cytotoxic activities of the isolates.



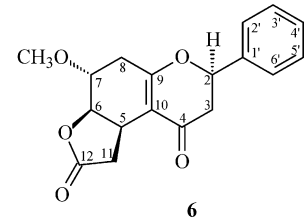
1 R = H
3 R = CH_3



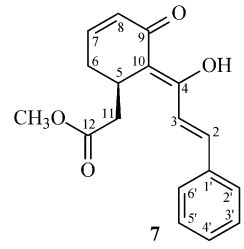
2 R = H
4 R = CH_3



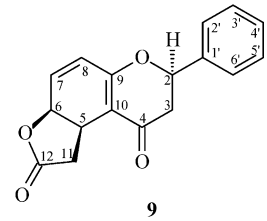
5



6



7



9

Results and Discussion

Cryptochinone A (**1**) was isolated as colorless needles, $[\alpha]^{25}_D$ +168. Its molecular formula, $C_{17}H_{16}O_5$, was determined on the basis of the positive HRESIMS ion at *m/z* 323.0893 $[M + Na]^+$ (calcd 323.0895) and supported by the ^1H, ^{13}C, and DEPT NMR data. The IR spectrum showed the presence of hydroxy (3368 cm^{-1}) and carbonyl (1770 and 1660 cm^{-1}) groups. The ^1H (Table 1) and ^{13}C NMR data of **1** were similar to those of cryptocaryanone B,[3] except that a single bond between C-7 and C-8 and a β-hydroxy group at C-7 of **1** replaced the double bond at C-7, 8 of cryptocaryanone B.[3] This was supported by the following $^1H-^1H$ COSY, NOESY, and HMBC correlations: (a) $^1H-^1H$ COSY correlations were observed between H_α-7 (δ 4.16) and both H_α-6 (δ 4.81) and H_α-8 (δ 2.66), (b) NOESY correlations were observed between H_α-7 (δ 4.16) and H_α-5 (δ 3.55), H_α-6 (δ 4.81), and H_α-8 (δ 2.66), (c) HMBC correlations were observed between H_α-6 (δ 4.81) and both C-8 (δ 32.3) and C-10 (δ 110.6) and between H-8 (δ 2.66 and 2.72) and C-6 (δ 79.5), C-7 (δ 66.8), C-9 (δ 168.2), and C-10 (δ 110.6). The relative configuration of **1** was evidenced by X-ray crystallographic analysis (Figure 1), and the relative configuration of **1** was determined to be *rel*-2*S*,5*R*,6*R*,7*S*. The 2*S* configuration of **1** was evidenced by a positive Cotton effect at 328 nm (Figure 2) due to the n → π^* transition, in analogy with those of cryptocaryanone B,[3] a flavanone with a reduced A-ring. Thus, the structure of cryptochinone A was elucidated as **1** and was further confirmed by ^{13}C, COSY, DEPT, HSQC, HMBC (Figure 3), and NOESY (Figure 3) experiments.

Cryptochinone B (**2**) was obtained as a yellowish oil, $[\alpha]^{25}_D$ +71. The sodium adduct ion $[M + Na]^+$ (*m/z* 323.0897) in the HRESIMS was consistent with the formula $C_{17}H_{16}O_5Na$. The IR spectrum showed the presence of hydroxy (3458 cm^{-1}) and carbonyl (1778 and 1664 cm^{-1}) groups. The ^1H NMR data (Table 2) of **2** were similar to those of **1**, except that the 5α,6α-configurations of **2** replaced the 5β,6β-configurations of **1**. The relative configuration of **2** was supported by NOESY correlations between H_β-5 (δ 3.61)/H_β-6 (δ 4.71), H_β-6 (δ 4.71)/H_β-8 (δ 2.71), and H_α-7 (δ 4.31)/H_α-8 (δ 2.64). However, no NOESY correlations were observed

* To whom correspondence should be addressed. Tel: +886-7-3121101, ext. 2191. Fax: +886-7-3210683. E-mail: m635013@kmu.edu.tw.
[†] Kaohsiung Medical University.
[‡] Tajen University.
[§] National Health Research Institute.
[#] National Sun Yat-sen University.

10.1021/np100014j
Published on Web 08/12/2010

Table 1. ^{1}H NMR Data for **1** and **3**[a]

	1	**3**		
position	δ_H (J in Hz)	δ_H (J in Hz)	NOESY	HMBC
2	5.40 dd (14.9, 3.3)	5.40 dd (14.6, 3.3)	3, 2′, 6′	3, 4, 1′, 2′, 6′
3	2.89 dd (17.7, 14.9) (α)	2.87 dd (17.1, 14.6) (α)	2	2, 4, 10, 1′
	2.69 dd (17.7, 3.3) (β)	2.68 dd (17.1, 3.3) (β)	2	2, 4, 10, 1′
5	3.55 ddd (9.0, 6.6, 3.6)	3.49 ddd (8.6, 6.6, 4.8)	6, 7, 11	6, 7, 9, 10, 11, 12
6	4.81 dd (6.6, 2.4)	4.81 dd (6.6, 2.6)	5, 7, 8, 11	5, 7, 8, 10, 11
7	4.16 br s	3.72 ddd (7.6, 5.2, 2.6)	5, 6, 8	5, 6, 8, 9, OCH_3
8	2.72 dd (18.0, 5.7) (β)	2.72 ddd (17.6, 7.6, 1.2) (α)	6, 7, 8	6, 7, 9, 10
	2.66 ddd (18.0, 9.0, 1.2) (α)	2.66 br dd (17.6, 5.2) (β)	7, 8	6, 7, 9, 10
11	3.03 dd (18.0, 9.0) (α)	2.97 dd (17.5, 8.6) (α)	5, 11	5, 6, 10, 11
	2.57 dd (18.0, 3.6) (β)	2.53 dd (17.5, 4.8) (β)	5, 11	5, 6, 10, 11
2′	7.43 m	7.43 m	2, 3′	2, 1′, 3′, 4′, 6′
3′	7.41 m	7.41 m	2′, 4′	1′, 2′, 4′, 5′
4′	7.41 m	7.41 m	2′, 6′	2′, 3′, 5′, 6′
5′	7.41 m	7.41 m	4′, 6′	1′, 2′, 3′, 4′, 6′
6′	7.43 m	7.43 m	2, 5′	2, 1′, 2′, 4′, 5′
OCH_3		3.45 s	7	7

[a] Recorded in $CDCl_3$ at 600 (**1**) and 400 (**3**) MHz. Values in ppm (δ). J (in Hz) in parentheses.

between H_β-5 (δ 3.61) and H_α-7 (δ 4.31), and the relative configuration of **2** was determined to be *rel*-2*S*,5*S*,6*S*,7*S*. The absolute configuration at C-2 of **2** was also assigned as 2*S* by a positive Cotton effect at 332 nm (Figure 2), as in the case of **1**.[3] Thus, the structure of cryptochinone B was elucidated as **2**, which was further confirmed by ^{13}C, COSY, NOESY (Figure 4), DEPT, HSQC, and HMBC (Figure 4) techniques.

Compound **3** was obtained as an amorphous powder, $[\alpha]^{25}_D$ +171. HRESIMS gave an $[M + Na]^+$ ion at *m*/*z* 337.1051 (calcd for $C_{17}H_{16}O_5Na$, 337.1051), consistent with a molecular formula of $C_{18}H_{18}O_5Na$. Carbonyl groups were indicated by the bands at 1777 and 1664 cm^{-1} in the IR spectrum and were confirmed by resonances at δ 190.6 and 176.5 in the ^{13}C NMR spectrum. The ^{1}H (Table 1) and ^{13}C NMR data of **3** were similar to those of **1**, except that the 7β-methoxy group of **3** replaced the 7β-hydroxy group of **1**. This was supported by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between MeO-7 (δ 3.45) and H_β-8 (δ 2.66) and (b) HMBC correlations were observed between MeO-7 (δ 3.45) and C-7 (δ 75.3). The relative configuration of **3** as *rel*-2*S*,5*R*,6*R*,7*S* was supported by NOESY correlations between H_α-5 (δ 3.49)/H_α-6 (δ 4.81), H_α-6 (δ 4.81)/H_α-7 (δ 3.72), H_α-6 (δ 4.81)/H_α-8 (δ 2.72), H_α-7 (δ 3.72)/H_α-8 (δ 2.72), and 7β-MeO (δ 3.45)/H_β-8 (δ 2.66). Compound **3** showed a similar CD curve when compared to **1** (Figure 2), and the relative configuration of **3** must be (2*S**,5*R**,6*R**,7*S**). The absolute configuration at C-2 of **3** was also assigned as 2*S* by CD (Figure 2) with a positive Cotton effect at 329 nm, as in the cases of **1** and **2**.[3] On the basis of the above data, the structure of compound **3** was identified as 7-*O*-methylcryptochinone A, which was further confirmed by ^{13}C, COSY, NOESY (Table 1), DEPT, HSQC, and HMBC (Table 1) experiments.

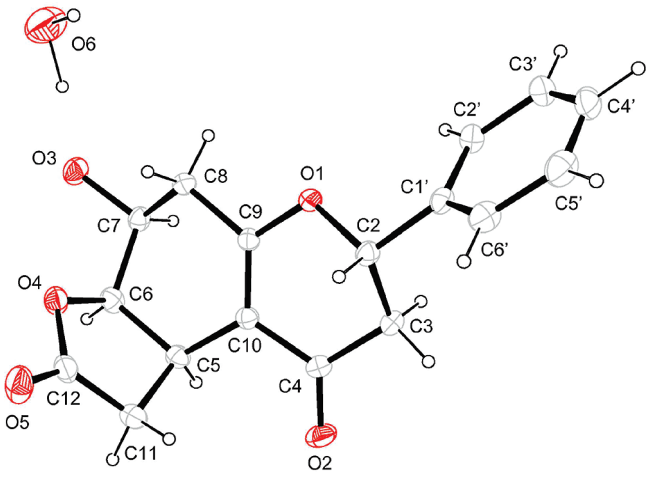


Figure 1. X-ray crystallographic analysis of **1**.

Compound **4** was isolated as a yellowish oil, $[\alpha]^{25}_D$ +68. The ESIMS of **4** afforded an $[M + Na]^+$ ion at *m*/*z* 337, implying a molecular formula of $C_{18}H_{18}O_5$, which was confirmed by HRESIMS. The presence of carbonyl groups was revealed by the bands at 1778 and 1666 cm^{-1} in the IR spectrum and was confirmed by signals at δ 190.8 and 176.9 in the ^{13}C NMR spectrum. Comparison of the ^{1}H NMR data (Table 2) of **4** with those of **2** suggested that their structures were closely related, except that the 7β-methoxy group of **4** replaced the 7β-hydroxy group of **2**. This was supported by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between MeO-7 (δ 3.42) and both H_β-6 (δ 4.68) and H_β-8 (δ 2.54) and (b) HMBC correlations were observed between MeO-7 (δ 3.42) and C-7 (δ 75.5). The relative configuration of **4** as *rel*-2*S*,5*S*,6*S*,7*S* was supported by NOESY correlations between H_β-5 (δ 3.56)/H_β-6 (δ 4.68), H_β-6 (δ 4.68)/H_β-8 (δ 2.54), 7β-MeO (δ 3.42)/H_β-6 (δ 4.68), 7β-MeO/H_β-8 (δ

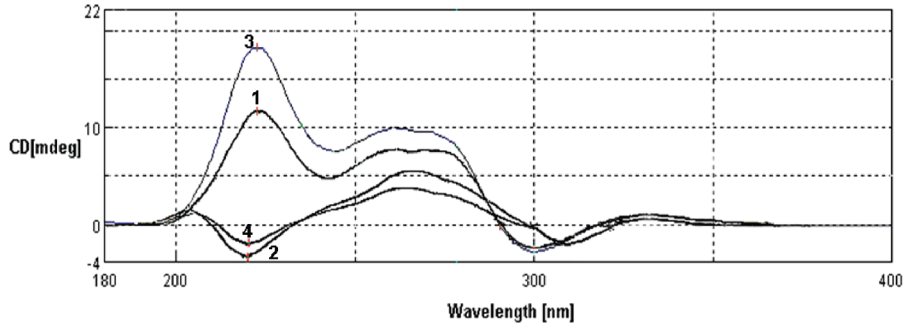


Figure 2. CD spectra of **1**−**4**.

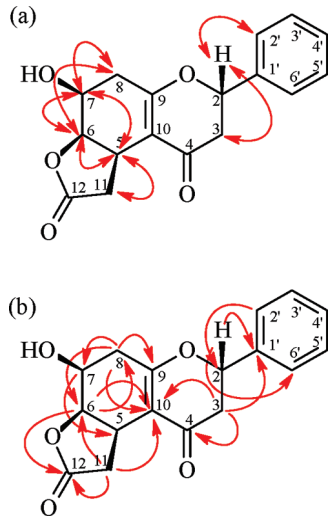


Figure 3. (a) NOESY (↔) and (b) HMBC (↷) correlations of **1**.

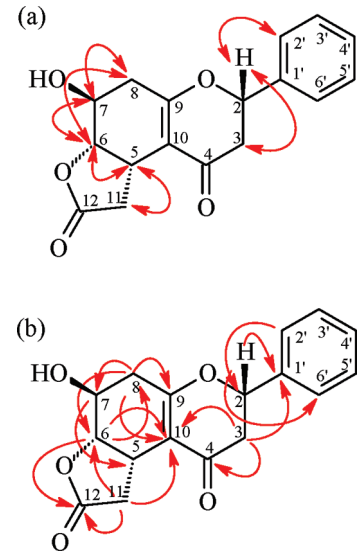


Figure 4. (a) NOESY (↔) and (b) HMBC (↷) correlations of **2**.

2.54), and H_α-7 (δ 3.81)/H_α-8 (δ 2.74). However, NOESY correlations were not observed between H_β-5 (δ 3.56) and H_α-7 (δ 3.81). Compound **4** showed a similar CD curve when compared to **2** (Figure 2), and the relative configuration of **4** must be inferred to be (2*S**,5*S**,6*S**,7*S**). The absolute configuration at C-2 of **4** was also assigned as 2*S* by CD (Figure 2) with a similar positive Cotton effect at 333 nm when compared to **1**−**3**.[3] Compound **4** was thus defined as 7-*O*-methylcryptochinone B. This structure was further supported by ^{13}C, COSY, NOESY (Table 2), DEPT, HSQC, and HMBC (Table 2) experiments.

7-*epi*-7-*O*-Methylcryptochinone A (**5**) and 2,7-di-*epi*-7-*O*-methylcryptochinone A (**6**) were obtained in the mixture as colorless needles, $[\alpha]^{25}_D$ +96. The HRESIMS gave a $[M + Na]^+$ ion at *m*/*z* 337.1050, corresponding to the formula $C_{18}H_{18}O_5Na$. Analysis of the ^{1}H NMR spectrum of the two compounds indicated that a pair of epimers were present in a ratio of 0.92:1 and could not be separated. The presence of carbonyl groups was revealed by the bands at 1779 and 1661 cm^{-1} in the IR spectrum and was confirmed by the resonances at δ 190.6/190.7 and 176.0/176.2 in the ^{13}C NMR spectrum of **5/6**. The ^{1}H (Table 3) and ^{13}C NMR data of **5** were similar to those of **3**, except that the 7β-methoxy group of **5** replaced the 7α-methoxy group of **3**. This was supported by the following NOESY correlations: (a) NOESY correlations were observed between OMe-7α (δ 3.47) and both H_α-6 (δ 4.67) and H_α-8 (δ 2.50), (b) NOESY correlations were also observed between H_β-7 (δ 3.83) and H_β-8 (δ 2.76), but (c) NOESY correlations were not observed between H_β-7 (δ 3.83) and H_α-5 (δ 3.61). Comparison of the ^{1}H NMR data of **6** with those of **5** suggested that their structures were closely related, except that the 2*R* configuration of **6** replaced the 2*S* configuration of **5**. This was supported by the following data: (a) NOESY correlations were marked between H_α-2 (δ 5.48) and H_α-3 (δ 2.71), and (b) the coupling constant (J = 13.3, 3.3 Hz) of H-2 of **6** was similar to that of crytocaryanone A with a 2*R* configuration.[3] According to the evidence above, the structures of 7-*epi*-7-*O*-methylcryptochinone A and 2,7-di-*epi*-7-*O*-methylcryptochinone A were elucidated as **5** and **6**, respectively. This conclusion was further confirmed by ^{13}C, COSY, NOESY (Table 3), DEPT, HSQC, and HMBC (Table 3) (Figure 5) experiments.

The known isolates were readily identified by comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values, and these included two dihydroxychalcones, infectocaryone (**7**)[3] and larrein (**8**),[21] three flavanones, cryptocaryanone A (**9**),[3] (2*S*)-7-hydroxyflavanone (**10**),[22] and (2*S*)-5-hydroxy-7-methoxyflavanone (**11**),[23] three flavones, 3,7-dimethoxy-5-hydroxyflavone (**12**),[24] 3,7,8-trimethoxy-5-hydroxyflavone (**13**),[25] and 6,7-dimethoxy-5-hydroxyflavone (**14**),[26] three steroids, β-sitostenone (**15**)[27] and a mixture of β-sitosterol (**16**)[28] and stigmasterol (**17**),[28] a benzopyran, α-tocopheryl quinone (**18**),[29] a benzenoid, vanillin (**19**),[30] and a triterpene, squalene (**20**).[31]

These isolates were tested for cytotoxicity against MCF-7, NCI-H460, and SF-268 cancer cell lines in vitro. IC_{50} values of these

Table 2. ^{1}H NMR Data for **2** and **4**[a]

	2	**4**		
position	δ_H (J in Hz)	δ_H (J in Hz)	NOESY	HMBC
2	5.42 dd (14.0, 3.3)	5.39 dd (14.3, 3.4)	3, 2′, 6′	3, 4, 1′, 2′, 6′
3	2.97 dd (16.7, 14.0) (α)	2.98 dd (16.7, 14.3) (α)	2	4
	2.69 dd (16.7, 3.3) (β)	2.67 dd (16.7, 3.4) (β)	2	2, 4, 10
5	3.61 ddd (9.4, 8.2, 7.6)	3.56 ddd (9.6, 9.4, 7.8)	6, 11	6, 7, 9, 10, 11, 12
6	4.71 dd (7.6, 2.8)	4.68 dd (7.8, 3.0)	5, 7, 8	10, 12
7	4.31 br s	3.81 ddd (4.4, 3.6, 3.0)	6, 8	
8	2.71 ddd (18.1, 4.8, 1.0) (β)	2.74 dd (18.4, 4.4) (β)	6, 7, 8	6, 7, 9, 10
	2.64 ddd (18.1, 4.2, 1.4) (α)	2.54 ddd (18.4, 3.6, 1.2) (α)	7, 8	6, 9
11	3.02 dd (17.8, 9.4) (α)	2.96 dd (17.7, 9.6) (α)	5	5, 6, 10, 12
	2.48 dd (17.8, 8.2) (β)	2.43 dd (17.7, 9.4) (β)	5	5, 10, 12
2′	7.43 m	7.43 m	2, 3′	2, 1′, 3′, 4′, 6′
3′	7.41 m	7.41 m	2′, 4′	1′, 2′, 4′, 5′
4′	7.41 m	7.41 m	2′, 6′	2′, 3′, 5′, 6′
5′	7.41 m	7.41 m	4′, 6′	1′, 2′, 3′, 4′, 6′
6′	7.43 m	7.43 m	2, 5′	2, 1′, 2′, 4′, 5′
OCH_3		3.42 s	6, 7, 8	7

[a] Recorded in $CDCl_3$ at 400 MHz. Values in ppm (δ). J (in Hz) in parentheses.

Table 3. ^{1}H NMR Data for **5** and **6**[a]

	5	**6**		
position	δ_H (J in Hz)	δ_H (J in Hz)	NOESY	HMBC
2	5.35 dd (14.5, 3.3)	5.48 dd (13.3, 3.3)	3, 2′, 6′	3, 4, 1′, 2′, 6′
3	2.94 dd (16.9, 14.5) (α)	2.92 dd (16.5, 13.3) (β)	2	4
	2.65 dd (16.9, 3.3) (β)	2.71 dd (16.5, 3.3) (α)	2	2, 4, 10
5	3.61 ddd (7.8, 7.0, 5.5)	3.51 br dd (8.0, 7.0)	6, 11	6, 7, 9, 10, 11, 12
6	4.67 dd (7.0, 6.0)	4.74 dd (7.0, 6.0)	5, 7	10, 12
7	3.83 ddd (6.0, 5.5, 4.5)	3.96 ddd (6.0, 3.5, 2.8)	6, 8	
8	2.76 dd (18.0, 4.5) (β)	2.67 dd (18.6, 3.5) (α)	6, 7, 8	6, 7, 9, 10
	2.50 dd (18.0, 5.5) (α)	2.57 ddd (18.6, 2.8) (β)	6, 7, 8	6, 9
11	3.03 dd (17.9, 7.8) (α)	3.02 dd (17.8, 8.0) (α)	5	5, 6, 10, 12
	2.35 dd (17.9, 5.5) (β)	2.55 dd (17.8, 1.5) (β)	5	5, 10, 12
OCH_3	3.47	3.46 s	7	7

[a] Recorded in $CDCl_3$ at 500 MHz. Values in ppm (δ). J (in Hz) in parentheses.

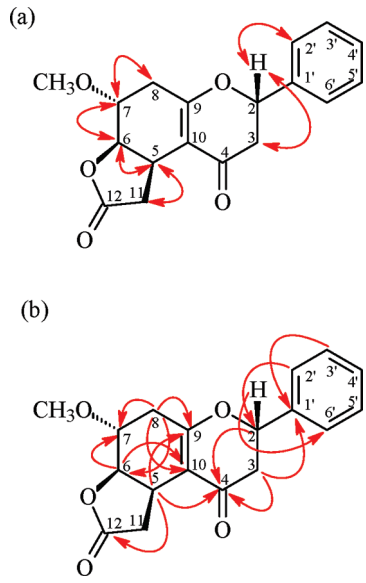


Figure 5. (a) NOESY (↔) and (b) HMBC (↷) correlations of **5**.

compounds were determined and are shown in Table 4. The clinically applied anticancer agent, actinomycin D, was used as positive control for cytotoxicity assays at concentrations of 10 nM and 10 μM in each 96-well plate. The values represent averages of three independent experiments, each with duplicate samples. Infectocaryone (**7**) showed cytotoxic activities ($IC_{50} \leq 13.4$ μM) against NCI-H460 and SF-268 cell lines, whereas the other compound, cryptocaryanone A (**9**), showed cytotoxic activities ($IC_{50} \leq 14.2$ μM) against MCF-7, NCI-H460, and SF-268 cell lines. As can be seen from the cytotoxicity test results in comparison with the inactive tetrahydroflavanones (**1**−**6**), the presence of a C-7−C-8 double bond in cryptocaryanone A (**9**) seems to play an important role in cytotoxicity. It is interesting that compared to the other six analogues (**1**−**6**), only compound **9** exerts potent cytotoxic activity. Demethoxylation at the C-7 position of compound **9** and further unsaturation by introduction of a C-7−C-8 double bond increases the potency greater than 40-fold when compared to that of compound **6**. Further exploration of related analogues might lead to finding more potent analogues.

The various phytochemical properties of *Cryptocarya* species have been described,[8,10,32,33] but the cytotoxicity of compounds isolated from *Cryptocarya* species has not yet been thoroughly tested, especially with regard to the neutral fractions of Formosan *Cryptocarya* species. In this study, bioassay-guided fractionation of the neutral $CHCl_3$ fraction of *C. chinensis* leaves led to the isolation of six new compounds, belonging to a type of tetrahydroflavanone that has not previously been reported. In a past study,[3] compounds **7** and **9** exhibited cytotoxicities with IC_{50} values of 1.7 and 2.5 μM against KB cells, respectively. In our study,

Table 4. IC_{50} Values of **1**−**20** against MCF-7, NCI-H460, and SF-268 Cell Lines

	IC_{50} (μM)[a]		
compound	MCF-7	NCI-H460	SF-268
cryptochinone A (**1**)	>50.0	>50.0	>50.0
cryptochinone B (**2**)	>50.0	>50.0	>50.0
7-*O*-methylcryptochinone A (**3**)	>50.0	>50.0	>50.0
7-*O*-methylcryptochinone B (**4**)	>50.0	>50.0	>50.0
mixture of 7-*epi*-7-*O*-methylcryptochinone A (**5**) and 2,7-di-*epi*-7-*O*-methylcryptochinone A (**6**)	>50.0	>50.0	>50.0
infectocaryone (**7**)	24.3	11.0	3.7
larrein (**8**)	>50.0	>50.0	>50.0
cryptocaryanone A (**9**)	5.1	4.3	5.0
(2*S*)-7-hydroxyflavanone (**10**)	>50.0	>50.0	>50.0
(2*S*)-5-hydroxy-7-methoxyflavanone (**11**)	>50.0	>50.0	>50.0
3,7-dimethoxy-5-hydroxyflavone (**12**)	>50.0	>50.0	>50.0
3,7,8-trimethoxy-5-hydroxyflavone (**13**)	>50.0	>50.0	>50.0
6,7-dimethoxy-5-hydroxyflavone (**14**)	>50.0	>50.0	>50.0
β-sitostenone (**15**)	>50.0	>50.0	>50.0
a mixture of β-sitosterol (**16**) and stigmasterol (**17**)	>50.0	>50.0	>50.0
α-tocopherylquinone (**18**)	>50.0	37.3	>50.0
vanillin (**19**)	>50.0	>50.0	>50.0
squalene (**20**)	>50.0	>50.0	>50.0
actinomycin D[b]	0.103	0.008	0.016

[a] The concentration inhibiting 50% of tumor cell growth after 72 h at 37°. [b] Positive control.

dihydrochalcone **7** and dihydroflavanone **9** also showed cytotoxicities against MCF-7, NCI-H460, and SF-268 cell lines, respectively. Thus, the detailed action mechanisms of **7** and **9** seem to be worth further exploration.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1020 digital polarimeter, UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeOH, and IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Gemini 200 spectrometer operating at 200 MHz (^{1}H) and 50 MHz (^{13}C) and Varian Unity 400, 500, and 600 spectrometers operated at 400, 500, and 600 MHz (^{1}H) and 100, 125, and 150 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. Chemical shifts were internally referenced to the solvent signals in $CDCl_3$ (^{1}H, δ 7.26; ^{13}C, δ 77.0) with TMS as the internal standard. Low-resolution ESIMS spectra were obtained on an API 3000 (Applied Biosystems), and high-resolution ESIMS spectra on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EIMS spectra were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70−230, 230−400 mesh) (Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used

for TLC and PTLC. Further purification was performed by medium-performance liquid chromatography (MPLC) (EYELA; ceramic pump: VSP-3050).

Plant Material. The leaves of *C. chinensis* were collected in Lai-I, Pingtung County, Taiwan, in May 2005, and identified by one of the authors (I.-S.C.). A voucher specimen (Chen 5470) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

Extraction and Separation. The dried leaves of *C. chinensis* (14 kg) were sliced and extracted with MeOH (30 L, ×3 for 3 days). The MeOH solution was concentrated under reduced pressure to afford a MeOH extract (1920 g). The extract (850 g) was partitioned between $CHCl_3$ and H_2O (1:1). The $CHCl_3$ solution was then extracted with 2% aqueous H_2SO_4 to afford a neutral $CHCl_3$-soluble layer and acid-soluble layer. The neutral $CHCl_3$ layer was concentrated to yield a residue (fraction A, 220 g), which showed cytotoxic activity against MCF-7, NCI-H460, and SF-268 cell lines, respectively. The acid-soluble layer was basified with NH_4OH and extracted with $CHCl_3$, then dried with $MgSO_4$ and evaporated under vacuum to afford tertiary bases (fraction B, 60 g). The water layer was further extracted with *n*-BuOH and afforded an *n*-BuOH-soluble fraction (fraction C, 200 g) and a water-soluble fraction (fraction D, 220 g). Part of fraction A (150 g) was chromatographed on silica gel (70−230 mesh, 6.0 kg), eluting with *n*-hexane, gradually increasing the polarity with EtOAc and MeOH to give 24 fractions: A1 (3.5 L, *n*-hexane), A2 (6 L, *n*-hexane/EtOAc, 99:1), A3 (5 L, *n*-hexane/EtOAc, 95:1), A4 (8 L, *n*-hexane/EtOAc, 90:1), A5 (7 L, *n*-hexane/EtOAc, 80:20), A6 (5 L, *n*-hexane/EtOAc, 70:30), A7 (8 L, *n*-hexane/EtOAc, 60:40), A8 (6 L, *n*-hexane/EtOAc, 50:50), A9 (7 L, *n*-hexane/EtOAc, 50:50), A10 (8 L, *n*-hexane/EtOAc, 50:50), A11 (5 L, *n*-hexane/EtOAc, 50:50), A12 (4 L, *n*-hexane/EtOAc, 40:60), A13 (5 L, *n*-hexane/EtOAc, 40:60), A14 (6 L, *n*-hexane/EtOAc, 40:60), A15 (4 L, *n*-hexane/EtOAc, 40:60), A16 (5 L, *n*-hexane/EtOAc, 40:60), A17 (7 L, *n*-hexane/EtOAc, 20:80), A18 (5 L, *n*-hexane/EtOAc, 20:80), A19 (5 L, *n*-hexane/EtOAc, 20:80), A20 (5 L, *n*-hexane/EtOAc, 20:80), A21 (10 L, EtOAc), A22 (7 L, EtOAc/MeOH, 85:15), A23 (9 L, EtOAc/MeOH, 50:50), A24 (10 L, MeOH). Fraction A2 (9.2 g) was chromatographed on silica gel (70−230 mesh, 320 g) eluting with *n*-hexane/acetone (10:1) to give eight fractions (each 1.1 L, A2-1−A2-8). Fraction A2-4 (230 mg) was further purified by preparative TLC (silica gel, *n*-hexane/acetone, 3:1) to obtain **20** (4.8 mg). Fraction A2-6 (900 mg) was purified by MPLC (*n*-hexane/EtOAc, 10:1) to obtain nine fractions: A2-6-1−A2-6-9. Fraction A2-6-6 (88 mg) was further purified by preparative TLC (silica gel, *n*-hexane/EtOAc, 4:1) to afford **11** (6.0 mg). Fraction A3 (11.5 g) was chromatographed further on silica gel (70−230 mesh, 390 g) eluting with *n*-hexane/acetone (8:1) to give 10 fractions (each 750 mL, A3-1−A3-10). Fraction A3-3 (318 mg) was purified by MPLC (*n*-hexane/EtOAc, 10:1) to obtain 10 fractions: A3-3-1−A3-3-10. Fraction A3-3-3 (39 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/EtOAc, 30:1) to obtain **18** (6.4 mg). Fraction A3-3-6 (22 mg) was further purified by preparative TLC (silica gel, *n*-hexane/acetone, 3:1) to obtain **7** (5.1 mg) and **19** (5.4 mg). Fraction A8 (8.7 g) was chromatographed on silica gel (70−230 mesh, 350 g) eluting with $CHCl_3$/EtOAc (10:1) to give 12 fractions (each 1.3 L, A8-1−A8-12). Fraction A8-6 (800 mg) was purified by MPLC (*n*-hexane/EtOAc, 3:1) to obtain 12 fractions: A8-6-1−A8-6-12. Fraction A8-6-11 (75 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/EtOAc, 1:1) to obtain **1** (5.5 mg) and **2** (4.2 mg). Fraction A9 (12.7 g) was chromatographed on silica gel (70−230 mesh, 380 g) eluting with $CHCl_3$/EtOAc (15:1) to give 15 fractions (each 1.2 L, A9-1−A9-15). Fraction A9-5 (474 mg) was washed with MeOH to yield **9** (378 mg) after recrystallization from acetone. Fraction A9-10 (230 mg) was chromatographed on silica gel (70−230 mesh, 900 g) eluting with $CHCl_3$/acetone (10:1) to give 18 fractions (each 900 mL, A9-10-1−A9-10-18). Fraction A9-10-12 (26 mg) was purified by MPLC (CH_2Cl_2/acetone, 15:1) to obtain six fractions: A9-10-12-1−A9-10-12-6. Fraction A9-10-12-2 (5.8 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/MeOH, 50:1) to yield **4** (3.8 mg). Fraction A9-10-12-3 (7.1 mg) was further purified by preparative TLC (silica gel, CH_2Cl_2/acetone, 15:1) to yield **3** (3.4 mg). Fraction A10 (5.6 g) was chromatographed on silica gel (70−230 mesh, 240 g) eluting with *n*-hexane/EtOAc (8:1) to give 10 fractions (each 1.5 L, A10-1−A10-10). Fraction A10-2 (215 mg) was purified by MPLC ($CHCl_3$/EtOAc, 30:1) to obtain eight fractions: A10-2-1−A10-2-8. Fraction A10-2-3 (35 mg) was further purified by preparative TLC (silica gel, *n*-hexane/EtOAc, 7:1) to obtain **12** (6.3 mg) and **15** (11.8 mg). Fraction A10-2-7 (24 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/MeOH, 45:1) to obtain **8** (7.1 mg). Fraction A11 (6.5 g) was chromatographed on silica gel (70−230 mesh, 260 g) eluting with *n*-hexane/EtOAc (6:1) to give eight fractions (each 1.3 L, A11-1−A11-8). Fraction A11-5 (175 mg) was purified by MPLC ($CHCl_3$/acetone, 50:1) to obtain nine fractions: A11-5-1−A11-5-9. Fraction A11-5-5 (38 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/EtOAc, 20:1) to obtain **13** (3.9 mg). Fraction A11-5-8 (74 mg) was washed with MeOH to yield a mixture of **16** and **17** (56.8 mg) after recrystallization from acetone. Fraction A12 (7.2 g) was chromatographed on silica gel (70−230 mesh, 310 g) eluting with *n*-hexane/acetone (8:1) to give seven fractions (each 1.5 L, A12-1−A12-7). Fraction A12-6 (76 mg) was purified by MPLC ($CHCl_3$/EtOAc, 50:1) to obtain eight fractions: A12-6-1−A12-6-8. Fraction A12-6-7 (29 mg) was further purified by preparative TLC (silica gel, $CHCl_3$/acetone, 40:1) to obtain **14** (4.8 mg). Fraction A13 (6.6 g) was chromatographed on silica gel (70−230 mesh, 270 g) eluting with CH_2Cl_2/EtOAc (100:1) to give 15 fractions (each 700 mL, A13-1−A13-15). Fraction A13-7 (520 mg) was purified by MPLC ($CHCl_3$/EtOAc, 50:1) to obtain seven fractions: A13-7-1−A13-7-7. Fraction A13-7-2 (21 mg) was further purified by preparative TLC (silica gel, *n*-hexane/acetone, 2:1) to obtain **10** (11.5 mg). Fraction A14 (4.7 g) was chromatographed on silica gel (70−230 mesh, 190 g) eluting with *n*-hexane/EtOAc (3:1) to give 16 fractions (each 500 mL, A14-1−A14-16). Fraction A14-3 (330 mg) was purified by MPLC (*n*-hexane/acetone, 3:2) to obtain 13 fractions: A14-3-1−A14-3-13. Fraction A14-3-3 (17 mg) was further purified by preparative TLC (silica gel, *n*-hexane/EtOAc, 1:1) to obtain a mixture of **5** and **6** (11.5 mg).

Biological Assay. MCF-7 (human breast adenocarcinoma), NCI-H460 (non-small-cell lung cancer), and SF-268 (glioblastoma) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid (Life Technologies, Inc.) and maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2. Human cancer cells were seeded in 96-well microtiter plates in 100 μL of culture medium at cell number/well of 6500, 2500, and 7500 for MCF-7, NCI-H460, and SF-268, respectively. After an overnight adaptation period, the cells were treated with at least eight different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl)tetrazolium salt (MTS) reduction assay,[34] and the experiment was performed in accordance with the manufacturer's recommendations (Promega, Madison, WI). DMSO 0.1% (final concentration) was used as vehicle control, and results were expressed as a percentage of DMSO control. These assays were used to obtain the dose−response curves from which IC_{50} values were determined. The values shown represent averages of three independent experiments, each with duplicate samples. The clinically applied anticancer agent actinomycin D was used as the reference compound. A value of $IC_{50} \leq 13.3$ μM is considered to be indicative of significant cytotoxicity. Compounds **1**−**6** were all inactive at concentrations up to 50.0 μM, whereas **7** and **9** showed cytotoxic activities (≤24.3 μM) against MCF-7, NCI-H460, and SF-268 cell lines, respectively (Table 4).

Cryptochinone A (1): colorless needles from acetone (Figure 1, Table 5); mp 194−196 °C; $[\alpha]^{25}_D$ +168 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 (3.66) nm; CD (MeOH) $[\theta]_{328}$ +914, $[\theta]_{318}$ 0, $[\theta]_{300}$ −2823, $[\theta]_{290}$ 0, $[\theta]_{269}$ +8500, $[\theta]_{261}$ +8527, $[\theta]_{223}$ +12 953, $[\theta]_{197}$ 0; IR (KBr) ν_{max} 3368 (OH), 1770 (C=O), 1660 (C=O) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz) δ 32.3 (C-8), 32.7 (C-5), 36.6 (C-11), 42.5 (C-3), 66.8 (C-7), 79.5 (C-6), 80.6 (C-2), 110.6 (C-10), 126.3 (C-2′,6′), 128.9 (C-3′,5′), 129.1 (C-4′), 137.4 (C-1′), 168.2 (C-9), 176.3 (C-12), 190.6 (C-4); ESIMS *m/z* 323 $[M + Na]^+$; HRESIMS *m/z* 323.0893 $[M + Na]^+$ (calcd for $C_{17}H_{16}O_5Na$, 323.0895).

Cryptochinone B (2): yellowish oil; $[\alpha]^{25}_D$ +71 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 (3.47) nm; CD (MeOH): $[\theta]_{356}$ 0, $[\theta]_{332}$ +827, $[\theta]_{320}$ −1538, $[\theta]_{303}$ 0, $[\theta]_{276}$ +6101, $[\theta]_{255}$ +2888, $[\theta]_{239}$ 0, $[\theta]_{223}$ −4108, $[\theta]_{213}$ 0, $[\theta]_{208}$ +2192; IR (KBr) ν_{max} 3458 (OH), 1778 (C=O), 1664 (C=O) cm^{-1}; ^1H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.0 (C-5), 33.4 (C-8), 34.9 (C-11), 42.8 (C-3), 66.4 (C-7), 78.4 (C-6), 81.0 (C-2), 110.8 (C-10), 126.3 (C-2′,6′), 128.9 (C-3′,5′), 129.1 (C-4′), 137.6 (C-1′), 167.3 (C-9), 177.0 (C-12), 190.8 (C-4); ESIMS *m/z* 323 $[M + Na]^+$; HRESIMS *m/z* 323.0897 $[M + Na]^+$ (calcd for $C_{17}H_{16}O_5Na$, 323.0895).

7-*O*-Methylcryptochinone A (3): amorphous powder; $[\alpha]^{25}_D$ +171 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 (3.68) nm; CD (MeOH)

Table 5. Crystal Data and Structural Refinement of Cryptochinone A (**1**)

empirical formula	$C_{17}H_{18}O_6$
fw	318.31
temperature (K)	298(2)
cryst syst	monoclinic
space group	$P2_1$
a (Å)	8.032(3)
b (Å)	8.278(3)
c (Å)	11.532(4)
α (deg)	90
β (deg)	90
γ (deg)	90
V (Å^3)	756.9(5)
Z	2
d_x (Mg/m^3)	1.397
μ (mm^{-1})	0.106
F(000)	336
cryst size (mm)	0.6 × 0.4 × 0.2
range (deg)	1.79−26.00
indices ranges	$-9 \le h \le 9$
	$0 \le k \le 10$
	$0 \le l \le 14$
reflns collected	1672
unique reflns	1594 [R(int) = 0.0217]
R_1 [$I > 2\sigma(I)$]	0.0335
wR_2	0.0926

$[\theta]_{370}$ 0, $[\theta]_{329}$ +1044, $[\theta]_{319}$ 0, $[\theta]_{300}$ −3202, $[\theta]_{291}$ 0, $[\theta]_{261}$ +11 373, $[\theta]_{223}$ +20 818, $[\theta]_{195}$ 0; IR (KBr) ν_{max} 1777 (C═O), 1664 (C═O) cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.5 (C-8), 32.8 (C-5), 36.2 (C-11), 42.5 (C-3), 57.5 (MeO-7), 75.3 (C-7), 77.1 (C-6), 80.5 (C-2), 110.9 (C-10), 126.2 (C-2′,6′), 128.9 (C-3′,5′), 129.1 (C-4′), 137.5 (C-1′), 168.3 (C-9), 176.5 (C-12), 190.6 (C-4); ESIMS m/z 337 [M + Na]$^+$; HRESIMS m/z 337.1051 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1052).

7-*O*-Methylcryptochinone B (4): yellowish oil; $[\alpha]^{25}_D$ +68 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 271 (3.45) nm; CD (MeOH) $[\theta]_{355}$ 0, $[\theta]_{333}$ +836, $[\theta]_{322}$ −1461, $[\theta]_{303}$ 0, $[\theta]_{276}$ +6164, $[\theta]_{239}$ 0, $[\theta]_{224}$ −3582, $[\theta]_{213}$ 0, $[\theta]_{206}$ +1560; IR (KBr) ν_{max} 1778 (C═O), 1666 (C═O) cm^{-1}; ^{1}H NMR data, see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.8 (C-8), 32.3 (C-5), 34.5 (C-11), 42.7 (C-3), 58.4 (MeO-7), 75.5 (C-7), 77.2 (C-6), 81.0 (C-2), 111.2 (C-10), 126.1 (C-2′,6′), 128.9 (C-3′,5′), 129.1 (C-4′), 137.7 (C-1′), 167.3 (C-9), 176.9 (C-12), 190.8 (C-4); EIMS m/z 314 [M]$^+$; HRESIMS m/z 337.1052 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1052).

7-*epi*-7-*O*-Methylcryptochinone A (5) and 2,7-di-*epi*-7-*O*-methylcryptochinone A (6): $[\alpha]^{25}_D$ +96 (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 270 (3.57) nm; IR (KBr) ν_{max} 1779 (C═O), 1661 (C═O) cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 30.0/28.5 (C-8), 31.8/31.2 (C-5), 34.7/36.1 (C-11), 42.8/42.8 (C-3), 58.0/57.8 (MeO-7), 74.5/73.3 (C-7), 78.1/76.5 (C-6), 80.5/80.7 (C-2), 110.5/110.4 (C-10), 126.2/126.3 (C-2′,6′), 129.1/128.9 (C-3′,5′), 129.1/128.9 (C-4′), 137.7/137.6 (C-1′), 167.7/168.4 (C-9), 176.0/176.2 (C-12), 190.6/190.7 (C-4); EIMS m/z (%) 314 ([M]$^+$, 12), 237 (12), 131 (12), 104 (100), 78 (13), 58 (12); HRESIMS m/z 337.1050 [M + Na]$^+$ (calcd for $C_{18}H_{18}O_5Na$, 337.1052).

Acknowledgment. This work was supported by a grant from the National Science Council of the Republic of China (NSC 96-2628-B-037-003-MY3).

Supporting Information Available: NMR spectra of compounds **1**−**6**, CIF of the X-ray data for compound **1**, physical and spectroscopic data of compounds **7**−**14**, and cytotoxic effects of extracts (Table 1S). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Liao, J. C. *Lauraceae in Flora of Taiwan*, 2nd ed.; Editorial Committee of the Flora of Taiwan: Taipei, Taiwan, 1996; Vol. 2, pp 448−451.
(2) Dumontet, V.; Van Hung, N.; Adeline, M. T.; Riche, C.; Chiaroni, A.; Sévenet, T.; Guéritte, F. *J. Nat. Prod.* **2004**, *67*, 858–862.
(3) Dumontet, V.; Gaspard, C.; Van Hung, N.; Fahy, J.; Tchertanov, L.; Sévenet, T.; Guéritte, F. *Tetrahedron* **2001**, *57*, 6189–6196.
(4) Fu, X.; Sévenet, T.; Remy, F.; Païs, M.; Hamid, A.; Hadi, A.; Zeng, L. M. *J. Nat. Prod.* **1993**, *56*, 1153–1163.
(5) Ricardo, M. A. G.; Andreo, M. A.; Cavalheiro, A. J.; Gamboa, I. C.; Bolzani, V. S.; Silva, D. H. S. *ARKIVOC* **2004**, *6*, 127–136.
(6) Fu, X.; Sévenet, T.; Hamid, A.; Hadi, A.; Remy, F.; Païs, M. *Phytochemistry* **1993**, *33*, 1272–1274.
(7) Sturgeon, C. M.; Cinel, B.; Díaz-Marrero, A. R.; McHardy, L. M.; Ngo, M.; Andersen, R. J.; Roberge, M. *Cancer Chemother. Pharmacol.* **2008**, *61*, 407–413.
(8) Chang, W. T.; Lee, S. S.; Chueh, F. S.; Liu Karin, C. S. *Phytochemistry* **1998**, *48*, 119–124.
(9) Wu, T. S.; Lin, F. W. *J. Nat. Prod.* **2001**, *64*, 1404–1407.
(10) Lin, F. W.; Wu, P. L.; Wu, T. S. *Chem. Pharm. Bull.* **2001**, *49*, 1292–1294.
(11) Lee, S. S.; Lin, Y. J.; Chen, C. K.; Liu Karin, C. S.; Chen, C. H. *J. Nat. Prod.* **1993**, *56*, 1971–1976.
(12) Lee, S. S.; Chen, C. H. *J. Nat. Prod.* **1993**, *56*, 227–232.
(13) Saad, J. M.; Soepadamo, E.; Fang, X. P. J.; McLaughlin, L.; Fanwick, P. E. *J. Nat. Prod.* **1991**, *54*, 1681–1683.
(14) Chang, H. S.; Lin, Y. J.; Lee, S. J.; Lin, W. Y.; Tsai, I. L.; Chen, I. S. *Phytochemistry* **2009**, *70*, 2064–2071.
(15) Yang, C. H.; Cheng, M. J.; Lee, S. J.; Yang, C. W.; Chang, H. S.; Chen, I. S. *Chem. Biodiversity* **2009**, *6*, 846–857.
(16) Chang, S. Y.; Cheng, M. J.; Kuo, Y. H.; Lee, S. J.; Chang, H. S.; Wang, C. J.; Chen, I. S. *Helv. Chim. Acta* **2008**, *91*, 1156–1165.
(17) Chen, J. J.; Chou, T. H.; Duh, C. Y.; Chen, I. S. *J. Nat. Prod.* **2006**, *69*, 685–688.
(18) Chen, J. J.; Huang, S. Y.; Duh, C. Y.; Chen, I. S.; Wang, T. C.; Fang, H. Y. *Planta Med.* **2006**, *72*, 935–938.
(19) Chen, J. J.; Chou, E. T.; Duh, C. Y.; Yang, S. Z.; Chen, I. S. *Planta Med.* **2006**, *72*, 351–357.
(20) Chen, J. J.; Fang, H. Y.; Duh, C. Y.; Chen, I. S. *Planta Med.* **2005**, *71*, 470–475.
(21) Furlong, J. J. P.; Nudelman, N. S. *J. Chem. Soc., Perkin Trans. 2* **1985**, *2*, 633–640.
(22) Tanrisever, N.; Fronczek, F. R.; Fischer, N. H.; Williamson, G. B. *Phytochemistry* **1987**, *26*, 175–179.
(23) Hodgetts, K. J. *Tetrahedron* **2005**, *61*, 6860–6870.
(24) Jaipetch, T.; Reutrakul, V.; Tuntiwachwuttikul, P.; Santisuk, T. *Phytochemistry* **1983**, *22*, 625–626.
(25) Proksch, M.; Proksch, P.; Weissenboeck, G.; Rodriguez, E. *Phytochemistry* **1982**, *21*, 1835–1836.
(26) Morimoto, M.; Tanimoto, K.; Nakano, S.; Ozaki, T.; Nakano, A.; Komai, K. *J. Agric. Food Chem.* **2003**, *51*, 389–393.
(27) Kuo, Y. H.; Li, Y. C. *J. Chin. Chem. Soc.* **1997**, *44*, 321–325.
(28) Kojima, H.; Sato, N.; Hatano, A.; Ogura, H. *Phytochemistry* **1990**, *29*, 2351–2355.
(29) Pascual Teresa, J. de; Urones, J. G.; Marcos Sánchez, I.; Ferreras Fernández, J.; Lithgow Bertelloni, A. M.; Basabe Barcala, P. *Phytochemistry* **1990**, *26*, 1481–1485.
(30) Sun, R.; Sacalis, J. N.; Chin, C. K.; Still, C. C. *J. Agric. Food Chem.* **2001**, *49*, 5161–5164.
(31) Chang, M. H.; Wang, G. J.; Kuo, Y. H.; Lee, C. K. *J. Chin. Chem. Soc.* **2000**, *47*, 1131–1136.
(32) Lin, F. W.; Wang, J. J.; Wu, T. S. *Chem. Pharm. Bull.* **2002**, *50*, 157–159.
(33) Chen, C. H.; Lee, S. S.; Lai, C. F. *J. Nat. Prod.* **1979**, *42*, 163–167.
(34) Gieni, R. S.; Li, Y.; HayGlass, K. T. *J. Immunol. Methods* **1995**, *187*, 85–93.

NP100014J