

## New Cytotoxic Constituents from the Formosan Soft Corals *Clavularia viridis* and *Clavularia violacea*

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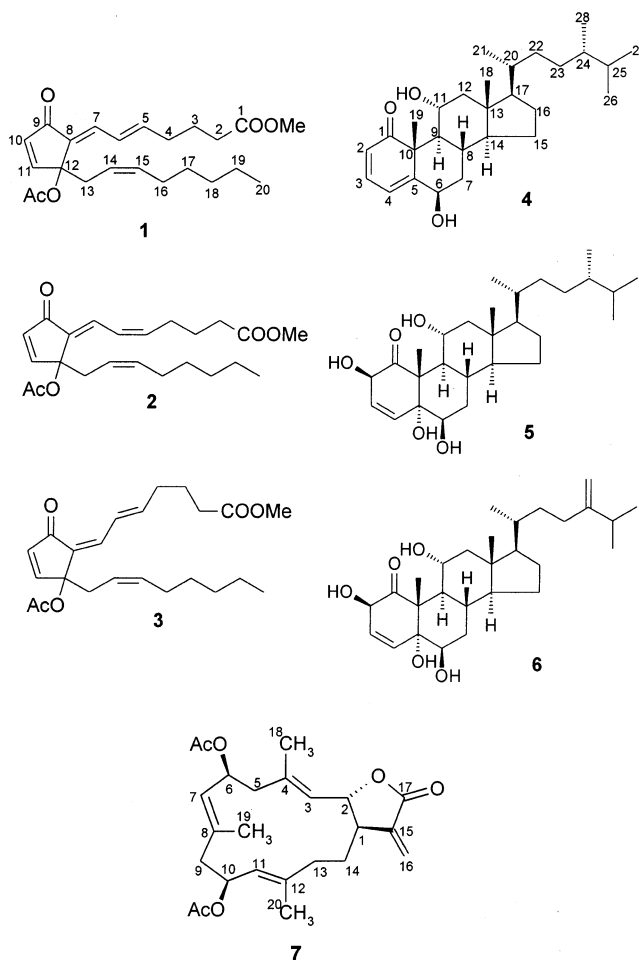
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Three new cytotoxic prostanoids, claviridenone E–G (**1–3**), and three new cytotoxic steroids, stoloniferone E–G (**4–6**), were isolated from the methylene chloride solubles of the Formosan soft coral *Clavularia viridis*. A cytotoxic cembranoid, claviolide (**7**), was isolated from the methylene chloride solubles of the Formosan soft coral *Clavularia violacea*. The structures were elucidated by 1D and 2D NMR spectral analysis, and their cytotoxicity against selected cancer cells was measured in vitro.

The genus *Clavularia* has afforded many types of bioactive prostanoids, terpenoids, and steroids.<sup>1</sup> As part of our search for bioactive substances from marine organisms, the Formosan soft corals *Clavularia viridis* Quoy and Gaimard (class Anthozoa, subclass Octocorallia, order Stolonifera) as well as *C. violacea* Quoy and Gaimard were studied because their CH<sub>2</sub>Cl<sub>2</sub> extracts showed significant cytotoxicity against A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.<sup>2,3</sup> Bioassay-guided fractionations resulted in the isolation of three new cytotoxic prostanoids, claviridenone E–G (**1–3**), and three new cytotoxic steroids, stoloniferone E–G (**4–6**), from *C. viridis* as well as a new cytotoxic cembranoid, claviolide (**7**), from *C. violacea*.

### Results and Discussion

Compound **1** was shown to have a molecular formula of C<sub>23</sub>H<sub>32</sub>O<sub>5</sub> as indicated by HREIMS and NMR data. The IR spectrum of **1** showed absorption due to acetate ester (1735, 1235 cm<sup>-1</sup>) and  $\alpha,\beta$ -unsaturated cyclopentenone (1705 cm<sup>-1</sup>) functionalities. The presence of a cross-conjugated system in **1**, corresponding to that of the clavulones,<sup>4</sup> was demonstrated by UV absorption at 226 (log  $\epsilon$  3.88) and 290 (log  $\epsilon$  4.04) nm. The <sup>13</sup>C NMR and DEPT spectrum exhibited 23 carbon resonances which were attributable to two methyls ( $\delta$  21.4 q and 14.1 q), one methoxyl ( $\delta$  51.6 q), one ketone carbonyl ( $\delta$  193.9 s), two ester carbonyls ( $\delta$  173.6 and 169.4 s), eight sp<sup>3</sup> methylene ( $\delta$  33.3 t, 23.9 t, 32.7 t, 35.7 t, 27.5 t, 29.1 t, 31.6 t, 22.6 t), seven sp<sup>2</sup> methines ( $\delta$  146.5 d, 125.4 d, 131.4 d, 134.8 d, 157.5 d, 121.3 d, 135.3 d), one sp<sup>2</sup> quaternary carbon ( $\delta$  134.0 s), and one sp<sup>3</sup> quaternary carbon ( $\delta$  85.6 s) (Table 1). The <sup>1</sup>H NMR spectrum of **1** disclosed five olefinic protons in the cross-conjugated system at  $\delta$  6.22 (1H, dt,  $J$  = 7.2, 15.0 Hz, H-5), 6.41 (1H, d,  $J$  = 6.0 Hz, H-10), 6.54 (1H, dd,  $J$  = 11.7, 15.0 Hz, H-6), 6.92 (1H, d,  $J$  = 11.7 Hz, H-7), 7.48 (1H, d,  $J$  = 6.0 Hz, H-11); two olefinic protons on a carbon–carbon double bond at  $\delta$  5.17 and 5.50 m; and a terminal methyl at  $\delta$  0.88 (3H, t,  $J$  = 6.9 Hz, H<sub>3</sub>-20). The analysis of the <sup>1</sup>H–<sup>1</sup>H COSY spectrum (Figure 1) revealed a sequence of



the correlations starting from a doublet at  $\delta_{\text{H}}$  6.92 (1H, d,  $J$  = 11.7 Hz, H-7) and carried through to a triplet at  $\delta_{\text{H}}$  2.35 (2H, t,  $J$  = 7.5 Hz, H-2), indicating the partial structure of H-7 through H-2 on the  $\alpha$ -side chain shown as a bold line in Figure 1. The connectivity from H-13 to H-20 on the  $\omega$ -side chain was indicated by the correlations in the <sup>1</sup>H–<sup>1</sup>H COSY spectrum starting from two doublets of doublets at  $\delta_{\text{H}}$  2.70 (1H, dd,  $J$  = 14.4, 8.1 Hz, H-13) and 2.96 (1H, dd,  $J$  = 14.4, 7.2 Hz, H-13) and ending with the methyl protons at  $\delta_{\text{H}}$  0.88 (3H, t,  $J$  = 6.9 Hz, H-20). These spectroscopic findings showed **1** to have a structure similar to that of clavulone II,<sup>4</sup> except for C-4 (CH<sub>2</sub> in **1**; CHOAc

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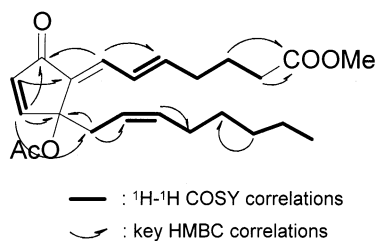
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<sup>‡</sup> National Taiwan University.

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectral Data of **1–3** in  $\text{CDCl}_3$ 

position	<b>1</b>		$^{13}\text{C}^a$	<b>2</b>		$^{13}\text{C}^a$	<b>3</b>	
	$^{13}\text{C}^a$	$^1\text{H}^b$		$^1\text{H}^b$	$^1\text{H}^b$			
1	173.6 s		173.7 s		174.6 s			
2	33.3 t	2.35 (t, 7.5)	33.4 t	2.34 m	33.5 t	2.36 (t, 7.5)		
3	23.9 t	1.81 (t, 8.1)	24.5 t	1.63 (ddd, 13.5, 8.0, 7.5)	24.1 t	1.81 (t, 7.2)		
	32.7 t	2.33 m	27.3 t	2.36 m	32.5 t	2.29 (dd, 14.1, 6.9)		
5	146.5 d	6.22 (dt, 15.0, 7.2)	146.5 d	6.04 (dt, 10.8, 8.1)	145.6 d	6.11 (dt, 15.2, 7.2)		
6	125.4 d	6.54 (dd, 15.0, 11.7)	123.2 d	6.54 (dd, 12.3, 10.8)	126.7 d	7.61 (dd, 15.2, 11.4)		
7	131.4 d	6.92 (d, 11.7)	125.6 d	7.25 (d, 12.6)	134.7 d	6.54 (d, 11.4)		
8	134.0 s		135.5 s		133.3 s			
9	193.9 s		194.0 s		194.4 s			
10	134.8 d	6.41 (d, 6.0)	135.3 d	6.43 (d, 6.0)	136.9 d	6.36 (d, 6.3)		
11	157.5 d	7.48 (d, 6.0)	157.8 d	7.48 (d, 6.0)	155.8 d	7.51 (d, 6.3)		
	85.6 s		85.5 s		85.7 s			
13	35.7 t	2.96 (dd, 14.4, 7.2) 2.70 (dd, 14.4, 8.1)	35.6 t	2.97 (dd, 14.0, 6.6) 2.70 (dd, 14.0, 8.4)	35.6 t	2.88 (dd, 14.5, 7.5) 2.65 (dd, 14.5, 7.5)		
14	121.3 d	5.17 m	121.3 d	5.17 m	120.6 d	5.23 m		
15	135.3 d	5.50 m	134.9 d	5.51 m	135.4 d	5.53 m		
16	27.5 t	1.97 (dd, 6.9, 5.1)	27.5 t	1.95 m	27.5 t	1.95 m		
17	29.1 t	1.29 m	29.7 t	1.28 m	29.1 t	1.29 m		
18	31.6 t	1.30 m	31.6 t	1.26 m	31.6 t	1.27 m		
19	22.6 t	1.32 m	22.6 t	1.31 m	22.6 t	1.31 m		
20	14.1 q	0.88 (t, 6.9)	14.1 q	0.88 (t, 6.9)	14.1 q	0.88 (t, 6.6)		
OCH <sub>3</sub>	51.6 q	3.68 s	51.7 q	3.69 s	51.6 q	3.68 s		
CH <sub>3</sub> CO	169.4 s		169.4 s		169.8 s			
CH <sub>3</sub> CO	21.4 q	2.04 s	21.4 q	2.04 s	21.4 q	1.99 s		

<sup>a</sup> Multiplicities of resonances were deduced by DEPT experiments. <sup>b</sup> Multiplicities and  $J$  (Hz) values are presented in parentheses.

**Figure 1.**  $^1\text{H}$ - $^1\text{H}$  COSY and key HMBC correlations of **1**.

in clavulone II) on the  $\alpha$ -side chain. Assignments between the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were made on the basis of HSQC correlations. The data from the HMBC spectrum fully supported the assigned structure, and key HMBC correlations are shown in Figure 1.

The molecular formula of compound **2** was assigned as  $\text{C}_{23}\text{H}_{32}\text{O}_5$  by HREIMS and NMR data. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **2** were very similar to those of **1** except for the  $^1\text{H}$  coupling constant between the two olefinic protons at H-5 and H-6 (10.8 Hz in **2**; 15.0 Hz in **1**) and the  $^{13}\text{C}$  chemical shifts at C-4 ( $\delta_{\text{C}}$  27.3 in **2**; 32.7 in **1**) and C-7 ( $\delta_{\text{C}}$  125.6 in **2**; 131.4 in **1**). Compound **2** was thus assigned as a *5Z* isomer of **1** on the basis of the comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with those of clavulone II.<sup>4</sup> NOESY correlations from H-5 to H-6 and from H-4 to H-7 confirmed this assignment. The assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were accomplished by COSY, HSQC, HMBC, and NOESY experiments.

The molecular formula of compound **3** was shown to be  $\text{C}_{23}\text{H}_{32}\text{O}_5$  by HREIMS and NMR data. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **3** were also very similar to those of **1** except for the  $^1\text{H}$  chemical shift values at H-6 ( $\delta_{\text{H}}$  7.61 in **3**; 6.54 in **1**) and H-7 ( $\delta_{\text{H}}$  6.54 in **3**; 6.92 in **1**) and the  $^{13}\text{C}$  chemical shifts at C-7 ( $\delta_{\text{C}}$  134.7 in **3**; 131.4 in **1**). Compound **3** was thus assigned as a *7Z* isomer of **1** on the basis of the comparison of  $^1\text{H}$  and  $^{13}\text{C}$  NMR data with those of clavulone II.<sup>4</sup> The assignments of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals were confirmed by COSY, HSQC, HMBC, and NOESY experiments.

Compound **4** had a molecular formula of  $\text{C}_{28}\text{H}_{44}\text{O}_3$  as indicated by HREIMS.  $^{13}\text{C}$  NMR and DEPT spectra of **4**

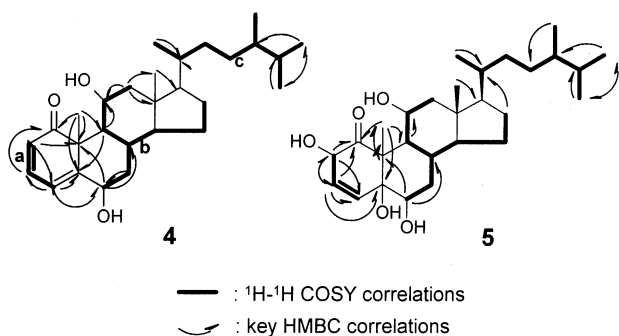
exhibited the presence of six methyls, six  $\text{sp}^3$  methylenes, nine  $\text{sp}^3$  methines, three  $\text{sp}^2$  methines, two  $\text{sp}^3$  quaternary carbons, and two  $\text{sp}^2$  quaternary carbons. The IR spectrum of **4** showed absorption due to an  $\alpha,\beta$ -unsaturated enone system (1676  $\text{cm}^{-1}$ ). The presence of a conjugated enone system in **4** was also indicated by UV absorptions at 222 nm ( $\log \epsilon$  3.79) and 280 ( $\log \epsilon$  4.01) nm as well as  $^1\text{H}$  NMR [ $\delta$  6.18 (1H, d,  $J$  = 9.6 Hz), 6.19 (1H, d,  $J$  = 6.0 Hz), 6.99 (1H, dd,  $J$  = 9.6, 6.0 Hz)] and  $^{13}\text{C}$  NMR [ $\delta$  118.9 (CH), 126.7 (CH), 140.8 (CH), 157.8 (C)] spectra (Table 2). IR absorption at 3300  $\text{cm}^{-1}$  and NMR signals at  $\delta_{\text{H}}$  4.58 (1H, br s) and 4.04 (1H, dt,  $J$  = 3.7, 10.5 Hz) as well as at  $\delta_{\text{C}}$  73.6 (CH) and 66.9 (CH) indicated the presence of two secondary hydroxyl groups. The spectral data of **4** exhibited some similarity to those of yonasterol E,<sup>5</sup> except for the presence of a trisubstituted double bond and lacking the epoxide. All C-H correlations of **4** were detected in the HSQC experiment. The  $^1\text{H}$ - $^1\text{H}$  COSY spectrum exhibited partial structures **a**, **b**, and **c** (Figure 2). In the HMBC spectrum, partial structure **a** could be connected to **b** through two quaternary carbons (C-5 and C-10) and H<sub>3</sub>-19 (Figure 2). Partial structure **b** could be connected to **c** through the remaining quaternary carbons (C-13) and H<sub>3</sub>-18. On the basis of these findings, the gross structure of **4** was concluded as in Figure 2. The NOESY correlations (Figure 3) observed between H-11 and H-8, H-11 and H<sub>3</sub>-18, H-11 and H<sub>3</sub>-19, H-4 and H-6, H-9 and H-14, H<sub>3</sub>-18 and H-8, H<sub>3</sub>-19 and H-8, H<sub>3</sub>-18 and H-20, H<sub>3</sub>-21 and H-12 $\beta$ , and H-9 and H-12 $\alpha$  indicated the relative configurations for each ring junction and chiral center. Stereochemistry at C-20 and C-24 was determined by comparison of  $^{13}\text{C}$  NMR data with those of yonasterol E and stoniferone-c.<sup>5,6</sup>

HREIMS and  $^{13}\text{C}$  NMR data revealed **5** to have a molecular formula of  $\text{C}_{28}\text{H}_{46}\text{O}_5$ .  $^{13}\text{C}$  and  $^1\text{H}$  NMR data (Table 2) showed some similarity to **4**, except for the presence of two additional hydroxyls and the absence of the trisubstituted double bond. The location of the hydroxyls on C-2 and C-4 was made on the basis of  $^1\text{H}$ - $^1\text{H}$  COSY correlations from H-2 to H-3 and H-3 to H-4 and HMBC correlations (Figure 2) from H-2 to C-1, C-3, C-4; H-3 to C-1, C-2, C-4, C-5; and H-19 to C-1, C-5, C-9, C-10.

**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectral Data of **4–6** in  $\text{CDCl}_3$ 

position	<b>4</b>		<b>5</b>		<b>6</b>	
	$^{13}\text{C}^a$	$^1\text{H}^b$	$^{13}\text{C}^a$	$^1\text{H}^b$	$^{13}\text{C}^a$	$^1\text{H}^b$
1	212.4 (C)		212.5 (C)		212.8 (C)	
2	126.7 (CH)	6.18 (d, 9.6)	78.6 (CH)	7.02 (dd, 8.1, 1.2)	78.5 (CH)	7.01 (dd, 8.0, 1.5)
3	140.8 (CH)	6.99 (dd, 9.6, 6.0)	126.5 (CH)	6.70 (dd, 8.1, 6.3)	126.4 (CH)	6.70 (dd, 8.0, 6.5)
4	118.9 (CH)	6.19 (d, 6.0)	141.8 (CH)	4.63 (dd, 6.3, 1.2)	141.7 (CH)	4.62 (dd, 6.5, 1.5)
5	157.8 (C)		84.0 (C)		83.9 (C)	
6	73.6 (CH)	4.58 br s	67.0 (CH)	4.08 m	66.9 (CH)	4.07 m
7	40.3 (CH <sub>2</sub> )	1.28 m 2.01 m	34.9 (CH <sub>2</sub> )	1.69 m	34.8 (CH <sub>2</sub> )	
8	29.7 (CH)	2.08 m	27.8 (CH)	1.85 m	27.7 (CH)	
9	58.1 (CH)	1.40 m	50.1 (CH)	2.00 (t, 9.9)	49.9 (CH)	2.00 (t, 10.5)
10	55.4 (C)		49.3 (C)		49.2 (C)	
11	66.9 (CH)	4.04 m	67.1 (CH)	4.10 m	67.0 (CH)	4.10 m
12	49.5 (CH <sub>2</sub> )	2.39 (dd, 9.6, 6.0) 1.23 m	48.6 (CH <sub>2</sub> )	2.37 (dd, 12.6, 4.8) 1.31 m	48.5 (CH <sub>2</sub> )	2.37 (dd, 12.0, 4.5) 1.30 m
13	42.8 (C)		42.8 (C)		42.7 (C)	
14	54.8 (CH)	1.12 m	54.4 (CH)	1.29 m	54.3 (CH)	
15	24.5 (CH <sub>2</sub> )	1.13 m 1.57 m	24.4 (CH <sub>2</sub> )	1.12 m 1.63 m	24.3 (CH <sub>2</sub> )	
16	28.2 (CH <sub>2</sub> )	1.28 m 1.87 m	28.2 (CH <sub>2</sub> )	1.32 m 1.88 m	28.1 (CH <sub>2</sub> )	
17	55.9 (CH)	1.13 m	56.1 (CH)	1.24 m	56.0 (CH)	
18	13.0 (CH <sub>3</sub> )	0.79 s	13.2 (CH <sub>3</sub> )	0.75 s	13.1 (CH <sub>3</sub> )	0.75 s
19	18.7 (CH <sub>3</sub> )	1.71 s	20.2 (CH <sub>3</sub> )	1.42 s	20.1 (CH <sub>3</sub> )	1.41 s
20	36.1 (CH)	1.38 m	36.3 (CH)	1.37 m	35.7 (CH)	
21	19.9 (CH <sub>3</sub> )	0.96 (d, 6.3)	18.8 (CH <sub>3</sub> )	0.97 (d, 6.6)	18.5 (CH <sub>3</sub> )	0.98 (d, 6.6)
22	33.5 (CH <sub>2</sub> )	0.95 m 1.40 m	33.6 (CH <sub>2</sub> )	0.96 m 1.40 m	34.5 (CH <sub>2</sub> )	
23	30.6 (CH <sub>2</sub> )	1.39 m	30.7 (CH <sub>2</sub> )	0.97 m 1.38 m	31.0 (CH <sub>2</sub> )	
24	39.0 (CH)	1.20 m	39.1 (CH)	1.22 m	156.6 (C)	
25	31.4 (CH)	1.55 m	32.0 (CH)	1.57 m	33.8 (CH)	
26	20.5 (CH <sub>3</sub> )	0.86 (d, 6.6)	20.6 (CH <sub>3</sub> )	0.86 (d, 6.9)	22.1 (CH <sub>3</sub> )	1.03 (d, 6.5)
27	17.6 (CH <sub>3</sub> )	0.78 (d, 6.6)	17.7 (CH <sub>3</sub> )	0.79 (d, 6.7)	22.0 (CH <sub>3</sub> )	1.04 (d, 6.5)
28	15.4 (CH <sub>3</sub> )	0.79 (d, 6.3)	15.5 (CH <sub>3</sub> )	0.80 (d, 6.7)	106.0 (CH <sub>2</sub> )	4.66 br s 4.72 br s

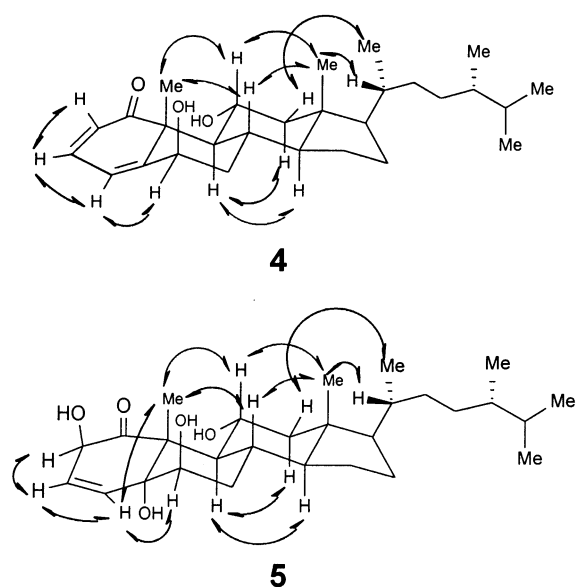
<sup>a</sup> Multiplicities of resonances were deduced by DEPT experiments. <sup>b</sup> Multiplicities and *J* (Hz) values are presented in parentheses.

**Figure 2.**  $^1\text{H}$ - $^1\text{H}$  COSY and key HMBC correlations of **4** and **5**.

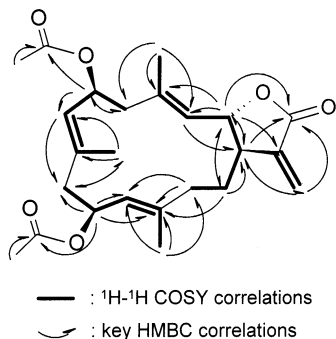
The NOESY correlations (Figure 3) observed between H-4 and H<sub>3</sub>-19, H-4 and H-6, H-11 and H-8, H-11 and H<sub>3</sub>-18, H-11 and H<sub>3</sub>-19, H-9 and H-14, H<sub>3</sub>-18 and H-8, H<sub>3</sub>-19 and H-8, H<sub>3</sub>-18 and H-20, H<sub>3</sub>-21 and H-12 $\beta$ , and H-9 and H-12 $\alpha$  indicated the relative configurations for each ring junction and chiral center.

The molecular formula of compound **6** was assigned as  $\text{C}_{28}\text{H}_{44}\text{O}_5$  by HREIMS and NMR data. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **6** were very similar to those of **5** except for NMR signals due to the side chain. Stereochemistry at C-20 was determined by comparison of  $^{13}\text{C}$  NMR data with those of stoniferone-a.<sup>6</sup>

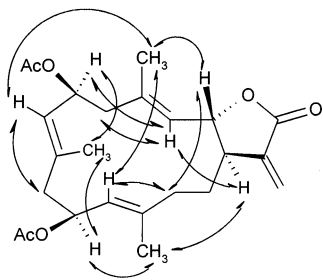
Compound **7** was isolated as a colorless oil,  $[\alpha]_{\text{D}}^{25} -33.8^\circ$  (*c* 0.05,  $\text{CHCl}_3$ ). HREIMS,  $^{13}\text{C}$  NMR, and DEPT spectra established the molecular formula of **7** as  $\text{C}_{24}\text{H}_{32}\text{O}_6$ . The IR spectrum of **7** indicated the presence of the functionalities of ester group(s) ( $\nu_{\text{max}}$  1730, 1240  $\text{cm}^{-1}$ ) and  $\alpha$ -methylene- $\gamma$ -lactone ( $\nu_{\text{max}}$  1760, 1660  $\text{cm}^{-1}$ ). The presence of the

**Figure 3.** Selective NOESY correlations of **4** and **5**.

$\alpha$ -methylene- $\gamma$ -lactone system in **7** was also demonstrated by UV absorption at 210 ( $\log \epsilon$  4.12) nm and signals at  $\delta$  5.57 (H-16<sub>a</sub>) and 6.27 (H-16<sub>b</sub>) in the  $^1\text{H}$  NMR spectrum. The  $^1\text{H}$  NMR spectrum of **7** also showed signals for three olefinic protons at  $\delta$  5.02 (H-3), 5.09 (H-11), and 5.15 (H-7) ppm; three oxymethine protons either bearing three acetates or in the  $\gamma$ -lactone group at  $\delta$  4.84 (H-2), 5.57 (H-6), and 5.71 (H-10); three olefinic methyl groups at  $\delta$  1.69 (H<sub>3</sub>-19), 1.73 (H<sub>3</sub>-20), and 1.83 (H<sub>3</sub>-18); and two methyl groups



**Figure 4.** <sup>1</sup>H-<sup>1</sup>H COSY and key HMBC correlations of **7**.



**Figure 5.** Selective NOESY correlations of **7**.

**Table 3.** Cytotoxicity<sup>a</sup> of **1-7**

compound	cell lines ED <sub>50</sub> (μg/mL)		
	A549	HT-29	P-388
<b>1</b>	4.1 × 10 <sup>-1</sup>	1.02	1.1 × 10 <sup>-1</sup>
<b>2</b>	5.0 × 10 <sup>-3</sup>	5.1 × 10 <sup>-2</sup>	5.2 × 10 <sup>-7</sup>
<b>3</b>	5.1 × 10 <sup>-2</sup>	1.22	2.6 × 10 <sup>-1</sup>
<b>4</b>	3.2 × 10 <sup>-4</sup>	9.1 × 10 <sup>-3</sup>	1.2 × 10 <sup>-4</sup>
<b>5</b>	3.69	6.46	2.36
<b>6</b>	3.58	5.86	2.12
<b>7</b>	4.91	8.4 × 10 <sup>-1</sup>	3.8 × 10 <sup>-1</sup>

<sup>a</sup> For significant activity of pure compounds, an ED<sub>50</sub> of ≤4.0 μg/mL is required.

in acetate esters at  $\delta$  2.01 and 2.03. The <sup>1</sup>H-<sup>1</sup>H COSY spectrum exhibited correlations from H-13 to H-3, H-5 to H-6, and H-9 to H-11. <sup>1</sup>H-<sup>1</sup>H long-range correlations were also observed from H-1 to H<sub>2</sub>-16, H-3 to H<sub>3</sub>-18, H-7 to H<sub>3</sub>-19, and H-11 to H<sub>3</sub>-20. These spectroscopic findings and the nine degrees of unsaturations indicated that **7** was a 14-membered cembrane-type diterpene skeleton with an  $\alpha$ -methylene- $\gamma$ -lactone. After assignments between all the C-H bondings were made on the basis of HSQC experiment, the planar structure was determined by HMBC analysis. The correlations according to HMBC are shown in Figure 4. The stereochemistry for the three trisubstituted olefins of **7** was determined by NOESY analysis. The NOESY correlations between H-3 and H-5, H-7 and H-9, and H-11 and H-13 disclosed the all-*E* configurations for the three trisubstituted olefins. The chemical shift values at  $\delta_C$  19.9, 16.3, and 15.5 (for C-18, C-19, and C-20, respectively) also supported the all-*E* configurations.<sup>7</sup> The relative configurations at C-1 and C-2 were determined by the coupling constant observed for the H-1 and H-2 proton signals and NOESY correlations (Figure 5) between H-1 and H-3 and H-2 and H-13. The relative configurations of the remaining two chiral centers at C-6 and C-10 were deduced from the following NOE analysis. NOESY correlations (Figure 5) between H-2 and H-18, H-18 and H-7, and H-18 and H-11 indicated that these protons (H-2, H-7, H-11, and H-18) were oriented on the same side, while NOESY correlations between H-1 and H-3 and H-1 and H-20 demonstrated that these protons (H-1, H-3, and H-20)

were oriented on the opposite face of the molecule. According to the relationships of these protons, the relative configurations at C-6 and C-10 were determined by NOESY correlations (Figure 5) between H-6 and H-3, H-6 and H-19, H-19 and H-10, and H-10 and H-20.

The cytotoxicity of compounds **1-7** is shown in Table 3. Compounds **2** and **4** exhibited potent cytotoxicity against P-388, HT-29, and A549 cells. Compound **3** showed exceptionally potent cytotoxicity against A549 cells.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for <sup>1</sup>H and 75 MHz for <sup>13</sup>C, respectively, in CDCl<sub>3</sub> using TMS as internal standard. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230-400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F<sub>254</sub>, 0.25 mm) were used for TLC analysis.

**Animal Material.** The soft coral *C. viridis* was collected at Green Island, off Taiwan, in May 2001, at a depth of 1-2 m and was stored for 1 month in a freezer until extraction. A voucher specimen, NSUGN-052, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

The soft coral *C. violacea* was collected at Green Island, off Taiwan, in October 2000, at a depth of 5-6 m and was stored for 1 week in a freezer until extraction. A voucher specimen, NSUGN-033, was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

**Extraction and Isolation.** The bodies of the soft coral *C. viridis* were freeze-dried to give 1.60 kg of a solid, which was extracted with CH<sub>2</sub>Cl<sub>2</sub> (4.0 L × 3). After removal of solvent in vacuo, the residue (70 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane-EtOAc mixtures of increasing polarity. Elution by *n*-hexane-EtOAc (8:2) afforded fractions containing compounds **1-3**. Elution by *n*-hexane-EtOAc (6:4) afforded fractions containing compounds **4-6**. Compounds **1-3** were further purified by Si gel column chromatography, by eluting with *n*-hexane-acetone (11:1). Compounds **4-6** were further purified by Si gel column chromatography by eluting with CH<sub>2</sub>Cl<sub>2</sub>-EtOAc (7:3) and C<sub>18</sub> HPLC column by using MeOH-H<sub>2</sub>O (85:15) as solvent system.

The bodies of the soft coral *C. violacea* were freeze-dried to give 240 g of a solid, which was extracted with CH<sub>2</sub>Cl<sub>2</sub> (2.0 L × 3). After removal of solvent in vacuo, the residue (20 g) was chromatographed over Si gel 60 using CH<sub>2</sub>Cl<sub>2</sub> and CH<sub>2</sub>Cl<sub>2</sub>-acetone mixtures of increasing polarity. Elution by CH<sub>2</sub>Cl<sub>2</sub> afforded a fraction containing compound **7**. Compound **7** was further purified by Si gel column chromatography by eluting with *n*-hexane-EtOAc (1:1).

**Claviridenone E (1):** oil (25 mg); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +8.6° (*c* 0.30, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 226 (3.88), 290 (4.04); IR (KBr)  $\nu_{max}$  1735, 1705, 1235 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS *m/z* 388 [M]<sup>+</sup> (1), 345 (2), 331 (4), 257 (3), 231 (10), 201 (12), 173 (18), 146 (28), 131 (42), 109 (36), 55 (100); HREIMS *m/z* 388.2246 (calcd for C<sub>23</sub>H<sub>32</sub>O<sub>5</sub>, 388.2251).

**Claviridenone F (2):** amorphous solid (17 mg); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +6.7° (*c* 0.31, CHCl<sub>3</sub>); IR (KBr)  $\nu_{max}$  1734, 1708, 1240 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 224 (3.89), 288 (4.06); <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS *m/z* 388 [M]<sup>+</sup> (1), 345 (2), 287 (2), 235 (8), 203 (9), 147 (15), 129 (26), 103 (26), 55 (100); HREIMS *m/z* 388.2248 (calcd for C<sub>23</sub>H<sub>32</sub>O<sub>5</sub>, 388.2251).

**Claviridenone G (3):** oil (7 mg); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +5.4° (*c* 0.10, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ) 223 (3.89), 286 (4.06); IR (KBr)  $\nu_{max}$  1738, 1710, 1230 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 1; EIMS *m/z* 388 [M]<sup>+</sup> (1), 346 (3), 329 (5), 235 (18), 203 (34), 109 (20), 55 (100); HREIMS *m/z* 388.2244 (calcd for C<sub>23</sub>H<sub>32</sub>O<sub>5</sub>, 388.2251).

**Stoloniferone E (4):** amorphous solid (6 mg);  $[\alpha]_D^{25} +10.0^\circ$  (*c* 0.05, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 222 (3.79), 280 (4.01); IR (KBr)  $\nu_{\max}$  3300, 1676 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS *m/z* 428 [M]<sup>+</sup> (1), 410 (2), 362 (1), 340 (2), 283 (2), 255 (5), 221 (5), 150 (100), 55 (86); HREIMS *m/z* 428.3299 (calcd for C<sub>28</sub>H<sub>44</sub>O<sub>3</sub>, 428.3292).

**Stoloniferone F (5):** amorphous solid (4 mg);  $[\alpha]_D^{25} -30.6^\circ$  (*c* 0.11, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 208 (3.68); IR (KBr)  $\nu_{\max}$  3460, 1680 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS *m/z* 462 [M]<sup>+</sup> (1), 270 (1), 256 (1), 221 (1), 192 (3), 176 (5), 154 (45), 137 (100), 107 (66); HREIMS *m/z* 462.3342 (calcd for C<sub>28</sub>H<sub>46</sub>O<sub>5</sub>, 462.3347).

**Stoloniferone G (6):** amorphous solid (3 mg);  $[\alpha]_D^{25} -21.7^\circ$  (*c* 0.12, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3510, 1678 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR, see Table 2; EIMS *m/z* 460 [M]<sup>+</sup> (1), 268 (1), 254 (1), 220 (1), 190 (4), 175 (6), 152 (40), 137 (100), 107 (76); HREIMS *m/z* 460.3186 (calcd for C<sub>28</sub>H<sub>44</sub>O<sub>5</sub>, 460.3190).

**Claviolide (7):** oil (80 mg);  $[\alpha]_D^{25} -33.8^\circ$  (*c* 0.05, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ) 210 (4.12); IR (KBr)  $\nu_{\max}$  1760, 1730, 1660, 1240 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\delta$  1.69 (3H, br s, H-19), 1.73 (3H, br s, H-20), 1.83 (3H, br s, H-18), 1.86 (2H, m, H-14), 2.01 (3H, s, OCOCH<sub>3</sub>), 2.03 (3H, s, OCOCH<sub>3</sub>), 2.09 (1H, m, H-13 $\beta$ ), 2.16 (1H, m, H-5 $\beta$ ), 2.18 (1H, m, H-9 $\beta$ ), 2.31 (1H, m, H-13 $\alpha$ ), 2.40 (1H, m, H-1), 2.60 (1H, m, H-5 $\alpha$ ), 2.62 (1H, m, H-9 $\alpha$ ), 4.84 (1H, dd, *J* = 4.0, 9.0 Hz, H-2), 5.02 (1H, br d, *J* = 9.0 Hz, H-3), 5.09 (1H, br d, *J* = 8.5 Hz, H-11), 5.15 (1H, br d, *J* = 9.5 Hz, H-7), 5.57 (1H, m, H-6), 5.57 (1H, d, *J* = 2.0 Hz, H-16), 5.71 (1H, m, H-10), 6.27 (1H, d, *J* = 2.6 Hz, H-16); <sup>13</sup>C NMR  $\delta$  15.5 (q, C-20), 16.3 (q, C-19), 19.9 (q, C-18), 21.3 (q, 2  $\times$  COCH<sub>3</sub>), 32.6 (t, C-14), 36.0 (t, C-13), 42.3 (t, C-5), 43.0 (d, C-1), 44.6 (t, C-9), 67.4 (d, C-10), 69.1 (d, C-6), 78.9 (d, C-2), 122.3 (t, C-16), 124.2 (d, C-3), 125.1 (d, C-11), 126.8 (d, C-7), 137.7 (s, C-8), 139.1 (s, C-15), 139.9 (s, C-4), 141.0 (s, C-12), 170.0 (s, C-17), 170.3 (s, 2  $\times$  COCH<sub>3</sub>); EIMS *m/z* 416 [M]<sup>+</sup> (1), 356 (8), 306 (10), 296 (22), 153 (96), 135 (100); HEIMS *m/z* 416.2195 (calcd for C<sub>24</sub>H<sub>32</sub>O<sub>6</sub>, 416.2199).

**Cytotoxicity Testing.** P-388 cells were kindly supplied by Prof. J. M. Pezzuto, Department of Medicinal Chemistry and

Pharmacognosy, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.<sup>3</sup>

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