Cytotoxic Constituents from the Formosan Soft Coral *Clavularia inflata* var. *luzoniana*

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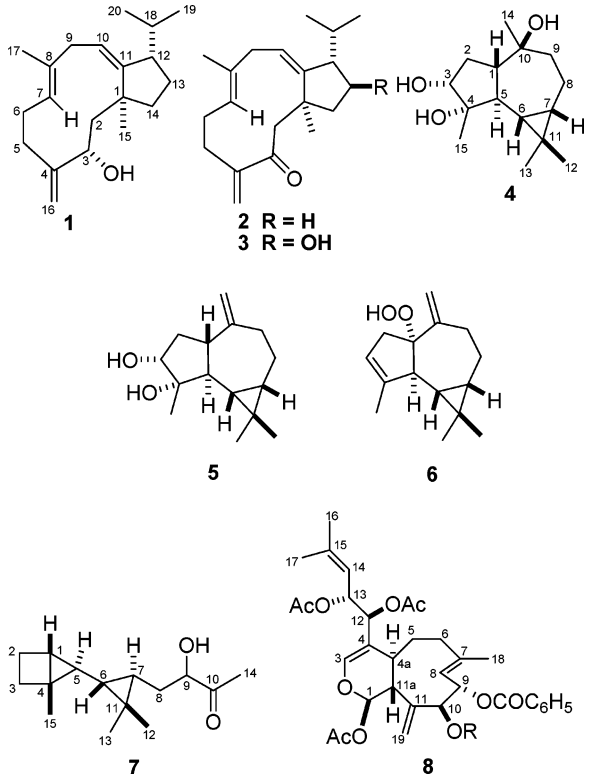
Three new cytotoxic dolabellane diterpenes, **1**−**3**, three new aromandendrane sesquiterpenoids, **4**−**6**, a new sesquiterpene, **7** (having a new carbon skeleton), and a new cytotoxic xenicane diterpene, **8**, were isolated from the methylene chloride solubles of the Formosan soft coral *Clavularia inflata* var. *luzoniana.* The structures were elucidated by extensive 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.[1−12] As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Clavularia inflata* var. *luzoniana* (class Anthozoa, subclass Octocorallia, order Stolonifera) was studied because the CH_2Cl_2 extract showed significant cytotoxicity to HT-29 (human colon adenocarcinoma) and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[13,14] Bioassay-guided fractionations of the methylene chloride solubles from *C. inflata* var. *luzoniana* resulted in the isolation of three new cytotoxic dolabellane diterpenes, **1**−**3**, three new aromandendrane sesquiterpenoids, **4**−**6**, a new sesquiterpene, **7** (having a new carbon skeleton), and a new cytotoxic xenicane diterpene, **8**.



Results and Discussion

The molecular formula of compound **1** was shown to be $C_{20}H_{32}O$ by HRESIMS and NMR data (Tables 1 and 2). The ^{13}C NMR and DEPT spectra showed four methyls, six sp^3 methylenes, three sp^3 methines, one sp^3 quaternary carbon, two sp^2 methines, two sp^2 methylenes, and three sp^2 quaternary carbons. The presence of a secondary hydroxyl group was indicated by an IR absorption (3408 cm^{-1}) and by the ^{13}C NMR signal at δ 73.3 (CH, C-3) (Table 2). The ^1H NMR spectrum disclosed two olefinic protons due to two trisubstituted olefins at δ 5.02 (1H, dd, J = 6.0, 9.3 Hz, H-7) and 4.97 (1H, dd, J = 12.0, 2.1 Hz, H-10) as well as two *exo* methylene protons at δ 4.84 (1H, s) and 4.96 (1H, s). These spectral data, coupled with the degrees of unsaturation (five), suggested that compound **1** was a bicyclic diterpenoid with a secondary hydroxyl group.

Analysis of the $^1H-^1H$ COSY and HSQC of **1** enabled us to distinguish four spin systems (Figure 1). These substructures were connected through HMBC correlations (Figure 1). The connection between C-8 and C-9 was indicated by the HMBC correlation from H-17 [1.57 (3H, s)] to C-9 [39.4 (CH_2)]. The location of the *exo* methylene group between C-3 and C-5 was demonstrated by HMBC correlations from H_2-16 [4.84 (1H, s) and 4.96 (1H, s)] to C-3 [73.3 (CH)], C-4 [155.4 (qC)], and C-5 [35.9 (CH_2)]. HMBC correlations from H-2 [1.58 (2H, m)] to C-1 [46.1 (qC)] and C-11 [150.0 (qC)], from H-15 [1.03 (3H, s)] to C-1, C-14 [37.5 (CH_2)] and C-2 [51.7 (CH_2)], and from H-10 [4.97 (1H, dd)] to C-1, C-11, and C-12 [49.6 (CH)] revealed connectivities around the ring junction.

The relative stereochemistry of **1** was determined by NOESY analysis. As shown in Figure 2, NOESY correlation between H-6 and H-17 indicated a 7*E* configuration. The NOESY correlations from H-10 to H-15/H-2/H-9β/H-17 and from H-9α to H-7/H-12 demonstrated the conformation from C-7 to C-12 and from C-1 to C-2 as depicted in Figure 2. The NOESY correlations between H-3 and H-7, and H-7 and H-9α, thus indicated the relative configuration at C-3.

To determine the absolute configuration, compound **1** was treated with (*R*)- or (*S*)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(*R*)- or (*S*)-MTPA-Cl] in the presence of pyridine to yield the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**), respectively.[15] The MTPA esters formed at C-3 were indicated from the ^1H NMR chemical shifts of H-3 in **1a** and **1b** (**1a**, δ 5.12; **1b**, δ 5.07). Comparison of the ^1H NMR chemical shifts for **1a** and **1b** ($\Delta\delta$ values shown in Figure 2) led to the assignment of the *S* configuration at C-3. Therefore, the absolute structure of **1** was determined as shown.

Compound **2** had the molecular formula $C_{20}H_{30}O$, as determined by HRESIMS and NMR data. The IR spectrum showed the presence of an α,β-unsaturated ketone (1680 cm^{-1}) moiety. The NMR spectra of **2** were similar to those of **1** except that the resonances for the

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Table 1. ^{1}H NMR Data[a] of **1**−**3**

proton	1	2	3
2	1.58 m	1.28 m	1.25 m
3	3.59 d (6.6)[b]		
5α	2.35 m	2.34 m	2.87 m
5β	2.08 m	1.64 m	2.07 m
6	2.18 m	2.20 m	2.15 m
7	5.02 dd (9.3, 6.0)	4.82 t (7.5)	4.82 t (7.2)
9α	2.86 t (12.0)	2.79 t (12.0)	2.72 t (12.0)
9β	2.40 dd (12.0, 2.1)	2.27 d (12.0)	2.29 d (12.0)
10	4.97 dd (12.0, 2.1)	4.98 d (12.0)	4.96 d (12.0)
12	2.66 m	2.68 m	2.56 d (6.3)
13	1.75 m, 1.56 m	1.91 m, 1.57 m	4.10 d (7.5)
14	1.94 m, 1.53 m	2.32 m	2.25 m, 2.15 m
15	1.03 s	1.18 s	1.15 s
16	4.84 s	5.65 s	5.74 s
	4.96 s	5.83 s	5.93 s
17	1.57 s	1.58 s	1.55 s
18	1.76 m	1.89 m	1.59 m
19	0.79 d (6.9)	0.77 d (6.9)	0.84 d (6.9)
20	0.88 d (6.9)	0.89 d (6.9)	0.93 d (6.9)

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [b] J values (in Hz) in parentheses.

Table 2. ^{13}C NMR Data[a] of **1**−**4**, **6**, and **7**

carbon	1	2	3	4	6	7
1	46.1	46.5	46.5	52.1	86.8	22.2
2	51.7	29.6	29.7	31.9	44.5	16.4
3	73.3	202.1	204.1	77.8	120.0	32.5
4	155.4	152.2	151.8	79.7	141.9	19.5
5	35.9	28.8	27.9	45.1	56.6	20.4
6	30.5	28.7	28.9	28.3	28.8	22.1
7	124.9	125.6	125.9	26.8	24.8	28.1
8	136.7	136.8	136.3	20.1	21.2	34.0
9	39.4	38.8	39.6	44.6	33.2	77.5
10	122.7	123.2	124.0	75.0	150.9	210.2
11	150.0	146.1	147.5	19.7	18.4	18.6
12	49.6	48.8	60.5	28.7	28.6	16.0
13	24.6	22.7	74.5	16.4	15.8	28.2
14	37.5	34.5	50.2	20.7	112.9	25.6
15	29.0	29.1	32.1	22.3	15.4	19.7
16	111.4	123.3	125.3			
17	17.2	17.6	17.4			
18	32.1	31.4	32.1			
19	18.5	17.5	19.7			
20	21.7	21.8	21.1			

[a] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. The values are in ppm downfield from TMS, and assignments were made by DEPT, COSY, HMQC, and HMBC experiments.

secondary hydroxy [δ_H 3.59 d; δ_C 73.3 (CH)] adjacent to the exocyclic methylene (δ_H 4.84 s, 4.96 s; δ_C 111.4, CH_2; 155.4, qC) in **1** were replaced by a ketone function (δ_C 202.1, qC) alpha to the exocyclic methylene [δ_H 5.65 (qC), 5.83 (qC); δ_C 123.3 (CH_2)] in **2**. HMBC correlations between H-15 [1.18 (3H, s)] and C-1 [46.5 (qC)], C-2 [29.6 (CH_2)]; H-2 [1.28 (2H, m)] and C-3 [202.1 (qC)], C-1, C-11 [146.1 (qC)], C-15 [29.0 (CH_3)], C-14 [34.5 (CH_2)], C-4 [152.2 (qC)]; and H-16 [5.65 (1H, s) and 5.83 (1H, s)] and C-3, C-4, C-5 [28.8 (CH_2)] clearly positioned the ketone at C-3. NOESY correlation between H-6 and H-17 indicated a 7*E* configuration. The NOESY correlations from H-10 to H-15//H-9β/H-17 and from H-9β to H-7/H-12 demonstrated the conformation from C-7 to C-12 and from C-1 to C-2 as depicted in Figure 3.

The molecular formula of compound **3** was found to be $C_{20}H_{30}O_2$ by HRESIMS and NMR data. The IR spectrum showed an absorption maximum at 3415 cm^{-1} due to a hydroxyl group and exhibited the presence of an α,β-unsaturated ketone (1686 cm^{-1}) moiety. The NMR spectra of **3** were analogous to those of **2** except for the presence of a secondary hydroxyl group at C-13: δ_H 4.10 (1H, d, J = 7.5 Hz), δ_C 74.5 (CH). COSY correlations between H-13 and H-12/H-14 as well as HMBC correlations between H-13 [4.10 (1H, d)] and C-12 [60.5 (CH)], C-14 [50.2 (CH_2)], C-1 [46.5 (qC)], C-11 [147.5 (qC)] helped ascertain the position of the secondary hydroxyl at C-13.

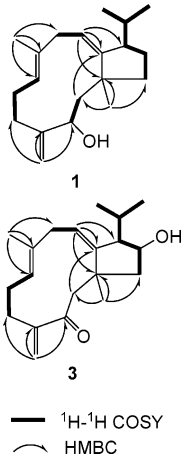


Figure 1. Key COSY and HMBC correlations of **1** and **3**.

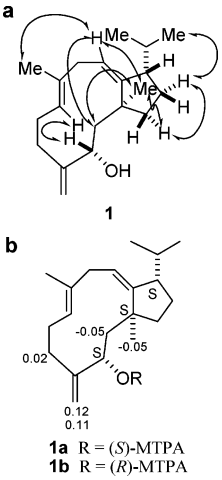


Figure 2. Relative and absolute configuration of **1**: (a) arrows indicate critical NOE correlations used to establish relative configuration and (b) $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **1a** and **1b**.

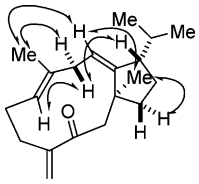
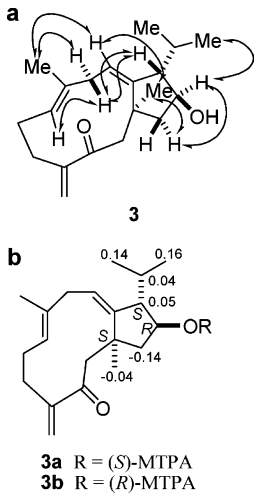


Figure 3. Key NOESY correlations of **2**.

Analysis of the proton chemical shift $\Delta\delta$ values between the (*S*)- and (*R*)-MTPA esters demonstrated that C-13 had the *S* configuration [$\Delta\delta$ values (=$\delta_S - \delta_R$) in ppm are given in Figure 4]. This information, along with the NOESY data (Figure 4), defined the absolute configurations at C-1, C-12, and C-13 in **3** as *S*, *R*, and *S*, respectively. The structure of compound **3** was thus established as (1*S*,12*R*)-dolabella-4(16),7,10-trien-13*S*-ol-3-one.

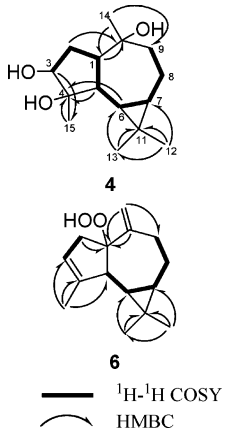
Compound **4** analyzed for $C_{15}H_{26}O_3$ from its HRESIMS and NMR data. The ^{13}C NMR spectrum of **4** (Table 2) exhibited four methyls, three sp^3 methylenes, five sp^3 methines, and three sp^3 quaternary carbons. The presence of two tertiary hydroxyl groups was indicated by the IR absorption at 3360 cm^{-1} and two quaternary carbon signals at δ_C79.7 and 75.0. The ^{1}H NMR spectrum of **4** (Table 3) also suggested a secondary hydroxyl group due to an oxygenated methine at δ 3.60 (1H, t, H-3) and two cyclopropyl methine protons at δ 0.42 (1H, t, J = 9.6 Hz, H-6) and 0.61 (1H, td, J = 9.6, 6.0 Hz, H-7). These spectral data, coupled with three



3

3a R = (*S*)-MTPA
3b R = (*R*)-MTPA

Figure 4. Relative and absolute configuration of **3**: (a) arrows indicate critical NOE correlations used to establish relative configuration and (b) $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **3a** and **3b**.



4

6

^{1}H-^{1}H COSY
HMBC

Figure 5. Key COSY and HMBC correlations of **4** and **6**.

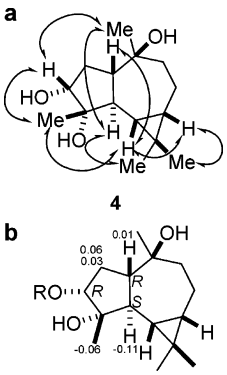
Table 3. ^{1}H NMR Data[a] of **4**, **6**, and **7**

proton	4	6	7
1	1.79 m		0.57 m
2	1.90 m	3.18 br d (16.5)[b]	1.78 m
	1.65 m	2.27 br d (16.5)	0.98 m
3	3.60 t (6.0)	5.23 br s	1.62 m, 0.85 m
5	1.34 t (9.6)	2.42 d (10.5)	0.59 m
6	0.42 t (9.6)	0.12 dd (10.5, 9.0)	0.16 d (5.0)
7	0.61 td (9.6, 6.0)	0.60 ddd (12.5, 9.0, 5.0)	0.43 dt (8.5, 5.0)
8	0.89 m	1.50 m	1.83 ddd (13.0, 8.5, 5.0)
	1.80 m	1.81 m	1.65 m
9	1.51 t (11.7)	2.82 dd (14.0, 8.5)	4.30 dd (6.6, 5.0)
	1.73 m	2.35 ddd (14.0, 11.5, 8.0)	
12	1.01 s	1.01 s	0.88 s
13	1.01 s	1.11 s	1.02 s
14	1.15 s	4.95 s, 4.98 s	2.23 s
15	1.16 s	1.72 s	0.94 s

[a] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [b] *J* values (in Hz) in parentheses.

degrees of unsaturation, indicated that compound **4** was a tricyclic sesquiterpenoid with a secondary hydroxyl group and two tertiary hydroxyl groups.

The planar structure of **4** was elucidated by analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. The ^{1}H−^{1}H COSY experiment revealed sequences for the correlations (Figure 5) from H-3 [3.06 (1H, t)] through H-9 [1.51 (1H, t), 0.73 (1H, m)] as depicted by the bold lines in Figure 4. The connections between C-1 and C-10 bearing the tertiary hydroxyl group, C-10 and C-14, and C-10 and C-9 were indicated by the HMBC correlations from H-14 [1.15 (3H, s)] to C-1 [52.0 (CH)], C-10 [75.0 (qC)], and C-9 [44.6 (CH_2)]. The presence of a dimethylcyclopropyl group at C-6 and C-7 was shown by the HMBC correlations from H-13 [1.01 (3H, s)] to C-6 [28.3 (CH)] and C-11 [19.7 (C)] and from H-12 [1.01 (3H, s)] to C-7 and C-11. Finally, the connections between C-3 and C-4, C-4 and C-15, and C-4 and C-5 were indicated by the HMBC correlations from H-15 [1.16 (3H, s)] to C-4 [79.7 (qC)], C-5 [45.1 (CH)], and C-3 [77.8 (CH)].



4

4a R = (*S*)-MTPA
4b R = (*R*)-MTPA

Figure 6. Relative and absolute configuration of **4**: (a) arrows indicate critical NOE correlations used to establish relative configuration and (b) $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **4a** and **4b**.

The relative stereochemistry of **4** was determined by a NOESY experiment (Figure 6). NOESY correlations between H-1 and H-3, H-15 and H-3, H-6 and H-15, and H-7 and H-6 established that these protons were oriented on the same side. On the other hand, the NOESY correlation between H-5 and H-14 and between H-13 and H-5 indicated that these protons were oriented on the opposite side.

Using Mosher's method, the absolute stereochemistry of **4** was readily defined by analysis of NMR shift data from the corresponding C-3 (*R*)- and (*S*)-MTPA esters. The significant proton chemical shift differences between the (*S*)- and (*R*)-MTPA esters **4a** and **4b** demonstrated that C-3 possessed the *R* configuration (Figure 6). This analysis required that **4** possessed 1*R*, 2*R*, and 5*S* absolute configuration.

The spectroscopic data of **5** were identical to those of (−)-ent-3β-hydroxyspathulenol[16] isolated from a Chilean liverwort, *Lipicolea ochroleuca*. To determine the absolute configuration, compound **5** was treated with (*R*)- or (*S*)-MTPA-Cl in the presence of pyridine to yield the (*S*)- and (*R*)-MTPA esters (**5a** and **5b**), respectively.[15] Comparison of the ^{1}H NMR chemical shifts for **5a** and **5b** ($\Delta\delta$ values shown in Figure 7) led to the assignment of the *R* configuration at C-3. Therefore, the absolute structure of **5** was determined as shown in formula **5**.

The molecular formula of compound **6** proved to be $C_{15}H_{22}O_2$ by HRESIMS and NMR data. The ^{13}C NMR and DEPT spectrum showed three methyls, three sp^3 methylenes, three sp^3 methines, two sp^3 quaternary carbons, one sp^2 methylene, one sp^2 methine, and two sp^2 quaternary carbon. The ^{13}C NMR spectrum of **6** indicated the presence of a tertiary hydroperoxy group at δ 86.8 (qC, C-1). The ^{1}H NMR spectrum of **6** (Table 2) exhibited two *exo* methylene protons at δ 4.95 (1H, s, H-14) and 4.98 (1H, s, H-14), one trisubstituted olefinic proton at δ 5.30 (1H, br s, H-3), and two cyclopropyl methine protons at δ 0.12 (1H, dd, J = 10.5, 9.0 Hz, H-6) and 0.60 (1H, ddd, J = 5.0, 9.0, 12.5 Hz, H-7). These spectral data, coupled with five degrees of unsaturation, suggested that compound **6** was a tricyclic sesquiterpenoid with a tertiary hydroperoxy group.

After direct ^{1}H−^{13}C correlations were established from HMQC, the planar structure of **6** was elucidated on the basis of ^{1}H−^{1}H

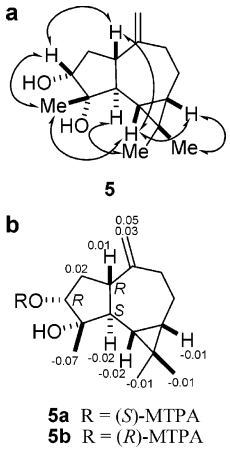


Figure 7. Relative and absolute configuration of **5**: (a) arrows indicate critical NOE correlations used to establish relative configuration and (b) $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **5a** and **5b**.

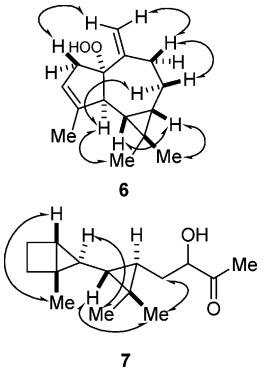


Figure 8. Key NOESY correlations of **6** and **7**.

COSY and HMBC spectra (Figure 5). The $^1H-^1H$ COSY spectrum revealed two substructures, as depicted by the bold lines in Figure 4. The HMBC correlation from H-2 [3.18 (1H, d), 2.27 (1H, d)] to C-1 [86.8 (qC)] indicated the connectivity between C-1 and C-2. The location of the *exo* methylene group between C-1 and C-9 was demonstrated by the HMBC correlations from H-14 [4.95 (1H, s), 4.98 (1H, s)] to C-9 [33.2 (CH_2)] and C-1. The presence of a dimethylcyclopropyl group at C-6 and C-7 was exhibited by the HMBC correlations from H-13 [1.11 (3H, s)] to C-6 [28.8 (CH)] and C-11 [18.4 (qC)] and from H-12 [1.01 (3H, s)] to C-7 [24.8 (CH)] and C-11. Finally, the connections between C-3 and C-4, C-15 and C-4, and C-4 and C-5 were indicated by the HMBC correlations from H-15 [1.72 (3H, s)] to C-3 [120.0 (CH)], C-4 [141.9 (qC)], and C-5 [56.6 (CH)].

The relative configuration of **6** was determined by the NOESY correlations (Figure 8) between H-5 and H-13, H-8α and H-5, H-6 and H-7, H-7 and H-8β, H-8β and H-9β, H-9α and H-14b, and H-2β and H-14a as well as by consideration of a Dreiding model of compound **6**.

Compound **7** gave a molecular formula of $C_{15}H_{24}O_2$, as indicated by its HRESIMS and NMR data. The ^{13}C NMR spectrum of **7** (Table 2) exhibited four methyls, three sp^3 methylenes, five sp^3 methines, two sp^3 quaternary carbons, and one ketone carbon. The presence of the secondary hydroxyl group was indicated by the IR absorption at 3460 cm^{-1} and the methine carbon signal at δ_C 77.5. The ^1H NMR spectrum of **7** (Table 3) also revealed a secondary hydroxyl group due to an oxygenated methine at δ 4.30 (1H, dd, H-9) and four cyclopropyl methine protons at δ 0.16 (1H, d, J = 5.0 Hz, H-6), 0.43 (1H, dt, J = 8.5, 5.0 Hz, H-7), 0.57 (1H, m), and 0.59 (1H, m). These data, coupled with the four degrees of unsaturation, suggested that compound **7** was a tricyclic sesquiterpenoid with a secondary hydroxyl group and a ketone group.

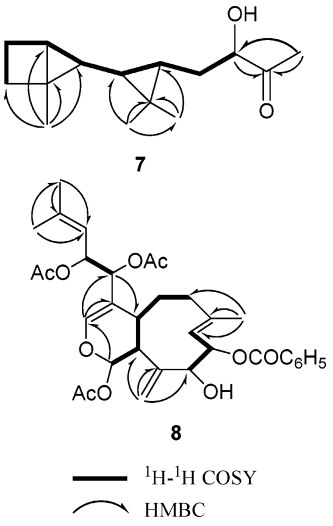


Figure 9. Key COSY and HMBC correlations of **7** and **8**.

The planar structure of **7** was deduced by analysis of $^1H-^1H$ COSY, HSQC, and HMBC spectra. The $^1H-^1H$ COSY spectrum revealed correlations from H-3 [1.62 (1H, m), 0.85 (1H, m)] through H-9 [4.30 (1H, dd)] as depicted by the bold lines in Figure 9. The connections between C-9 and C-10 and between C-10 and C-14 were indicated by the HMBC correlations from H-14 [2.23 (3H, s)] to C-9 [77.5 (CH)] and C-10 [210.2 (qC)] and from H-9 [4.30 (1H, dd)] to C-10. The presence of a dimethylcyclopropyl group at C-6 and C-7 was shown by the HMBC correlations from H-13 [1.02 (3H, s)] to C-6 [22.1 (CH)] and C-11 [18.6 (C)] and from H-12 [0.88 (3H, s)] to C-7 [22.1 (CH)] and C-11. Finally, the connections between C-3 and C-4, C-4 and C-15, and C-4 and C-5 were indicated by the HMBC correlations from H-15 [0.94 (3H, s)] to C-4 [19.5 (qC)], C-5 [20.4 (CH)], and C-3 [32.5 (CH_2)].

The relative configuration of **7** was determined by NOESY correlations between H-1 and H-15, H-5 and H-13, H-8 and H-12 and H-6, and H-12 and H-6 (Figure 8).

HRESIMS and ^{13}C NMR data revealed compound **8** to have a molecular formula of $C_{33}H_{40}O_{10}$. The DEPT spectrum showed six methyls, two sp^3 methylenes, seven sp^3 methines, 10 sp^2 methines, and nine sp^2 quaternary carbons. The ^1H NMR spectrum (Table 4) exhibited the presence of a benzoyl moiety [δ_H 7.99 (2H, d), 7.41 (2H, m), and 7.55 (1H, m)], three olefinic protons [δ_H 6.40 (1H, s), 5.28 (1H, s), and 5.19 (1H, d)] due to three trisubstituted olefins, three secondary acetoxyl groups [δ_H 2.02, 2.04, 2.06 (3H each, s, COC*H*$_3$), δ_H 5.64, 5.68, 5.84 (1H each)], and one *exo*-methylene [δ_H 4.98 (1H, s) and 5.03 (1H, s)].

Three substructures (Figure 9) were deduced by use of $^1H-^1H$ COSY and HMQC of **8**. These substructures were connected through HMBC correlations between H-1 [5.84 (1H, d)] and C-3 [137.4 (CH)], between H-3 [6.40 (1H, s)] and C-12 [71.6 (CH)], C-4 [113.2 (qC)], and C-4a [38.1], between H-12 [5.68 (1H, d)] and C-4a, between CH_3-18 [1.88 (3H, s)] and C-6 [40.8 (CH_2)], C-7 [137.6 (qC)], C-8 [122.4 (CH)], and C-9 [78.2 (CH)], and between the *exo* methylene protons H_2-19 [4.98 (1H, s) and 5.03 (1H, s)] and C-11a [42.0 (CH)] and C-10 [81.7 (CH)]. The relative configuration of the side chain was determined as 12*S**, 13*R** by comparison of the ^1H and ^{13}C NMR chemical shifts and coupling constants of **8** with those of antheliatin isolated from the soft coral *Anthelia glauca*.[17] The relative configuration of the nine-membered ring was deduced from a NOESY experiment (Figure 9). NOESY correlations from Me-18 to H-11a and H-9 showed that these protons occurred on the same face (β) of the ring system. NOESY correlations from H-19a to H-1 and H-4a, from H-4a to H-8, and from H-19b to H-10 showed that these protons occurred on the opposite face (α) of the ring system.

Table 4. NMR Data of **8**

position	δ_C[a]	δ_H[b]	mult	*J* (Hz)	HMBC (H to C)
1	91.9	5.84	d	2.0	3, 4a, 11
3	137.4	6.40	s		1, 4, 4a, 12
4	113.2				
4a	38.1				
5	28.9	1.61	m		
		1.91	m		
6	40.8	2.31	t	12.0, 2.5	5, 7, 8, 18
		2.08	m		
7	137.6				
8	122.4	5.28	d	9.0	6
9	78.2	5.74	d	9.0	7, 8, 10, 11, 1′
10	81.7	4.36	s		8, 9, 11, 11a, 19
11	150.2				
11a	42.0	2.46	br s		1, 4, 4a, 11, 19
12	71.6	5.68	d	4.0	3, 4, 4a, 13, 14
13	70.7	5.64	dd	9.5, 4.0	4, 14, 15
14	117.9	5.19	d	9.5	
15	141.1				
16	25.9	1.74	s		14, 15, 17
17	18.9	1.73	s		14, 15, 16
18	18.3	1.88	s		6, 7, 8
19	115.6	4.98	s		1, 10, 11, 11a
19		5.03	s		1, 10, 11,
1′	166.3				
2′	130.1				
3′, 7′	129.6	7.99	m		4′, 5′, 7′
4′, 6′	128.5	7.41	m		3′, 4′, 6′
5′	133.3	7.55	m		3′, 7′
OAc-1	169.9	2.11	s		
	21.1				
OAc-12	170.0	2.03	s		
	21.1				
OAc-13	170.4	2.00	s		
	21.1				

[a] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C. [b]Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C. The values are ppm downfield from TMS, and assignments were made by DEPT, COSY, HMQC, and HMBC experiments.

The absolute configuration of **8** was assigned by interpretation of chemical shift data derived from the corresponding C-10 (*R*)- and (*S*)-MTPA esters. Significant chemical shift differences between the (*R*)- and (*S*)-MTPA esters **8a** and **8b** defined C-10 as *R* (Figure 10). Considering the relative configuration defined for all chiral centers allowed assignment of 1*R*, 4a*S*, 9*S*, 10*S*, 11a*R*, 12*S*, and 13*R*.

Compounds **1**−**3** and **8** exhibited cytotoxicity against the P-388 cell line with ED_{50}'s of 3.6, 0.6, 1.5, and 0.5 μg/mL, respectively. Compounds **2** and **8** exhibited cytotoxicity against the HT-29 cell line with ED_{50}'s of 2.8 and 1.2 g/mL, respectively. The other isolated compounds were not cytotoxic to P-388 and HT-29 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 ^{1}H and 75 MHz for ^{13}C, respectively, in $CDCl_3$ 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 CC; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *C. inflata* var. *luzoniana* was collected at Green Island, off Taiwan, in May 2003, at a depth of 4−6 m and was stored for 3 weeks in a freezer until extraction. A voucher specimen, NSUGN-056, identified by Dr. C.-F. Dai (Institute of Oceanography, National Taiwan University), was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

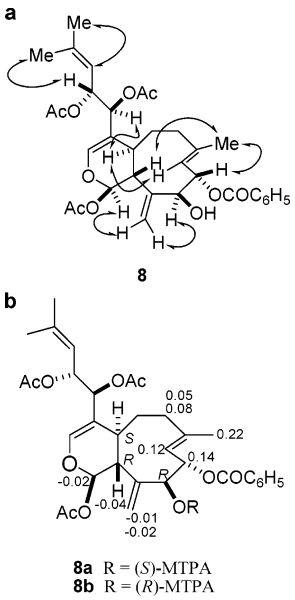


Figure 10. Relative and absolute configuration of **8**: (a) arrows indicate critical NOE correlations used to establish relative configuration and (b) $\Delta\delta$ values ($\delta_S - \delta_R$) in ppm for the two MTPA esters **8a** and **8b**.

Extraction and Isolation. The bodies of the soft coral *C. inflata* var. *luzoniana* were freeze-dried to give 1.20 kg of a solid, which was extracted with CH_2Cl_2 (3.0 L × 3). After removal of solvent in vacuo, the residue (66 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane−EtOAc mixtures of increasing polarity. Elution by *n*-hexane−EtOAc (9:1) afforded fractions containing compounds **1**−**3**. Elution by *n*-hexane−EtOAc (8:2) afforded fractions containing compounds **6** and **7**. Elution by *n*-hexane−EtOAc (7:3) afforded fractions containing compound **8**. Elution by EtOAc afforded fractions containing compounds **4** and **5**. Compounds **1**−**3** were further purified by Si gel CC, by eluting with *n*-hexane−acetone (11:1). Compounds **4** and **5** were further purified by Si gel CC by eluting with MeOH−CH_2Cl_2 (1:19) as solvent system. Compounds **6** and **7** were further purified by Si gel CC by eluting with *n*-hexane−CH_2Cl_2 (7:3) as solvent system. Compound **8** was further purified by Si gel CC by eluting with acetone−CH_2Cl_2 (1:15) as solvent system.

Compound 1: $[\alpha]^{25}_D$ +28 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3408, 1650 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 288.2456 (calcd for $C_{20}H_{32}O$, 288.2458).

Compound 2: $[\alpha]^{25}_D$ −32 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 234 (3.89); IR (KBr) ν_{max} 1680, 1656 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 286.2295 (calcd for $C_{20}H_{30}O$, 286.2299).

Compound 3: $[\alpha]^{25}_D$ −47 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 236 (3.99); IR (KBr) ν_{max} 3415, 1701, 1660 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; HREIMS *m*/*z* 302.2252 (calcd for $C_{20}H_{30}O_2$, 302.2248).

Compound 4: $[\alpha]^{25}_D$ +16 (*c* 0.09, $CHCl_3$); IR (KBr) ν_{max} 3360 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 254.1878 (calcd for $C_{15}H_{26}O_3$, 254.1883).

Compound 5: $[\alpha]^{25}_D$ +9 (*c* 0.1, $CHCl_3$); EIMS *m*/*z* 236 $[M]^+$ (1), 218 (30), 200 (11), 221 (1), 146 (3).

Compound 6: $[\alpha]^{25}_D$ +33 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3360, 1650 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HREIMS *m*/*z* 234.1626 (calcd for $C_{15}H_{22}O_2$, 234.1630).

Compound 7: $[\alpha]^{25}_D$ −51 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3510, 1738 cm^{-1}; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 236.1772 (calcd for $C_{15}H_{24}O_2$, 236.1777).

Compound 8: $[\alpha]^{25}_D$ +16.7 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} nm (log ϵ) 240 (4.3), 278 (3.1); IR (KBr) ν_{max} 1730, 1690, 1660 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 4; EIMS *m*/*z* 416 $[M]^+$ (1), 356 (8), 306 (10), 296 (22), 153 (96), 135 (100); HESIMS *m*/*z* 537.2488 [M − OAc] (calcd for $C_{31}H_{37}O_8$, 537.2478).

Preparation of (*R*)- and (*S*)-MTPA Esters (1a and 1b) of 1. To a solution of compound **1** (1.2 mg, 4.2 μmol) in pyridine (0.5 mL) at

RT was added (*R*)-MTPA-Cl (2.0 μL, 10.6 μmol), and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was worked up by adding 2 mL of H_2O to give the corresponding (*S*)-MTPA ester **1a** (0.6 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.40−7.65 (5H, aromatic H), 5.23 (1H, s, H-16), 5.15 (1H, m, H-7), 5.13 (1H, s, H-16), 5.12 (1H, m, H-3), 5.09 (1H, m, H-10), 5.09 (1H, m, H-10), 3.49 (3H, s, OMe), 2.88 (1H, dd, J = 13.5, 12.6 Hz, H-9), 2.71 (1H, m, H-12), 2.45 (1H, dd, J = 13.5, 3.6 Hz, H-9), 2.27 (2H, m, H_2-5), 2.13 (2H, s, H_2-6), 2.00 (2H, m, H_2-2), 1.82 (1H, m, H-18), 1.64 (3H, s, H_3-17), 1.47 (2H, m, H_2-13), 1.30 (2H, m, H_2-14), 1.01 (3H, s, H_3-15), 0.91 (3H, d, J = 6.6 Hz, H_3-20), 0.78 (3H, d, J = 6.6 Hz, H_3-19). Treatment of **1** (1.1 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **1b** (0.5 mg): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40−7.65 (5H, aromatic H), 5.15 (1H, m, H-7), 5.12 (1H, m, H-10), 5.11 (1H, s, H-16), 5.07 (1H, m, H-3), 5.02 (1H, s, H-16), 3.49 (3H, s, OMe), 2.87 (1H, dd, J = 13.5, 12.6 Hz, H-9), 2.72 (1H, m, H-12), 2.45 (1H, dd, J = 13.5, 3.6 Hz, H-9), 2.10 (2H, s, H_2-6), 2.25 (2H, m, H_2-5), 2.05 (2H, m, H_2-2), 1.85 (1H, m, H-18), 1.64 (3H, s, H_3-17), 1.60 (2H, m, H_2-14), 1.52 (2H, m, H_2-13), 1.06 (3H, s, H_3-15), 0.91 (3H, d, J = 6.6 Hz, H_3-20), 0.78 (3H, d, J = 6.6 Hz, H_3-19).

Preparation of (*R*)- and (*S*)-MTPA Esters (3a and 3b) of 3. Compound **3** (1.4 mg, 4.7 μmol) was treated with (*R*)-MTPA chloride (2.0 μL, 10.6 μmol) following the procedure for the Mosher reaction of compound **1** to give the corresponding (*S*)-MTPA ester **3a** (0.6 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information. Treatment of **3** (1.5 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **3b** (0.7 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information.

Preparation of (*R*)- and (*S*)-MTPA Esters (4a and 4b) of 4. Compound **4** (1.1 mg, 4.3 μmol) was treated with (*R*)-MTPA chloride (2.0 μL, 10.6 μmol) following the procedure for the Mosher reaction of compound **1** to give the corresponding (*S*)-MTPA ester **4a** (0.5 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information. Treatment of **4** (1.2 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **4b** (0.6 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information.

Preparation of (*R*)- and (*S*)-MTPA Esters (5a and 5b) of 5. Compound **5** (1.2 mg, 5.0 μmol) was treated with (*R*)-MTPA chloride (2.0 μL, 10.6 μmol) following the procedure for the Mosher reaction of compound **1** to give the corresponding (*S*)-MTPA ester **5a** (0.6 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information. Treatment of **5** (1.4 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **5b** (0.7 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information.

Preparation of (*R*)- and (*S*)-MTPA Esters (8a and 8b) of 8. Compound **8** (2.0 mg, 3.0 μmol) was treated with (*R*)-MTPA chloride (2.0 μL, 10.6 μmol) following the procedure for the Mosher reaction of compound **1** to give the corresponding (*S*)-MTPA ester **8a** (0.9 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information. Treatment of **8** (2.1 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **8b** (1.0 mg): ^{1}H NMR ($CDCl_3$, 300 MHz), see Supporting Information.

Cytotoxicity Testing. P-388 cells were kindly supplied by J. M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; HT-29 was purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[14]

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Supporting Information Available: ^{1}H NMR data for (*R*)- and (*S*)-MTPA esters of **3**, **4**, **5**, and **8** are available free of charge via the Internet at http://pubs.acs.org.

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