Manaarenolides A−I, Diterpenoids from the Soft Coral *Sinularia manaarensis*

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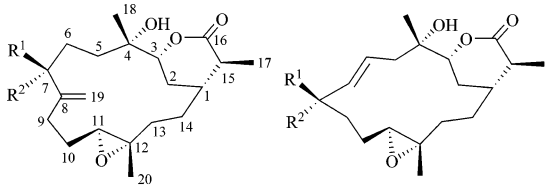
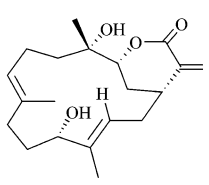
Nine cembrane-type diterpenoids, manaarenolides A−I (**1**−**9**), along with two known cembranolides, **10** and **11**, have been isolated from the ethyl acetate extract of the Taiwanese soft coral *Sinularia manaarensis*. Among these metabolites, diterpenes (**1**, **2**, **5**, and **6**) were discovered for the first time as the hydroperoxycembranolides possessing a δ-lactone ring. The 13-epimeric metabolites **7** and **8** have been shown to exhibit moderate cytotoxic activity against Hepa59T/VGH, KB, Hela, and Med cancer cell lines. The biosynthetic relationship between these new metabolites and **10** and **11** is also discussed.

Previous chemical investigations on soft corals of the genus *Sinularia* have led to the isolation and identification of a variety of oxygenated cembrane-type diterpenoids.[1−12] Some of these were found to exhibit cytotoxic activity against the growth of various cancer cell lines.[1−4,6,9,12] In our continuing search for bioactive metabolites from Taiwanese soft corals,[13−17] we have chemically investigated *Sinularia manaarensis* Versveldt and have succeeded in the isolation of nine new cembranolides (**1**−**9**), along with two related known metabolites (**10** and **11**) from its EtOAc extract. The structures of **1**−**9**, including their stereochemistries, have been established by detailed spectroscopic analyses, particularly mass and 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) spectroscopy. Cytotoxicities of metabolites **1**−**9** against Hepa59T/VGH (human liver carcinoma), KB (human oral epitheloid carcinoma), Hela (human cervical epitheloid carcinoma), and Med (human medulloblastoma) cancer cells are also reported. The biosynthetic relationship of the above 11 metabolites is also discussed.



1 R^1 = H R^2 = OOH
2 R^1 = OOH R^2 = H
3 R^1 = H R^2 = OH
4 R^1 = OH R^2 = H

5 R^1 = OOH R^2 = CH_3
6 R^1 = CH_3 R^2 = OOH



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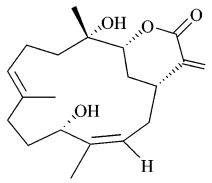

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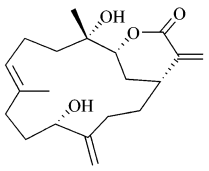

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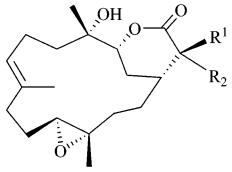

10 $R^1 + R^2$ = CH_2
11 R^1 = CH_3, R^2 = H

Results and Discussion

The EtOAc extract of the freeze-dried animal was fractionated by silica gel column chromatography, and the eluted fractions were further separated utilizing normal-phase HPLC to yield cembranolides **1**−**11** as white solids. Compounds **10** and **11** were found to be identical with the known cembranoids sinularin and dihydrosinularin, respectively, on the basis of the comparison of their physical and spectroscopic data with those reported previously.[12] Since the new metabolites **1**−**9** were isolated together with **10** and **11** from the same organism and possess very similar molecular skeletons, it was proposed that these 11 compounds should all have the same absolute configurations at C-1, C-3, and C-4 from biogenetic considerations.

The HRFABMS spectrum of **1** exhibited a molecular ion peak at *m*/*z* 369.2274 $[M + H]^+$, consistent with the molecular formula $C_{20}H_{32}O_6$ and implying five degrees of unsaturation. The IR spectrum revealed the presence of hydroxy (ν_{max} 3356 cm^{-1}) and ester (ν_{max} 1720 cm^{-1}) groups. The ^{13}C NMR data of **1** (Table 1, $CDCl_3$) showed the presence of 20 carbon signals of a diterpenoid, which were assigned by the assistance of a DEPT spectrum to three methyls, seven sp^3 methylenes, one sp^2 methylene, five sp^3 methines (including three oxymethines), two sp^3 quaternary carbons, and two sp^2 quaternary carbons. The NMR signals appearing at δ_C 174.5 (qC), 83.2 (CH), 43.0 (CH), 38.0 (CH), 27.2 (CH_2), and 17.1 (CH_3) and at δ_H 3.98 (1H, d, J = 11.0 Hz), 1.50 (1H, m), and 1.37 (3H, d, J = 7.0 Hz) and the IR absorption at 1722 cm^{-1} were assigned to an α-methyl−δ-lactone functionality by comparison with similar metabolites[3,12] as in **11**.[12] Furthermore, carbon signals of two methyls (δ 15.7, CH_3; 24.5, CH_3), one trisubstituted epoxide (δ 59.2, C; 63.6, CH), one 1,1-disubstituted carbon−carbon double bond (δ 114.2, CH_2; 142.5, qC), one oxygen-bearing methine (δ 85.3), and one oxygenated quaternary carbon (δ 73.0) were observed. From the ^{1}H NMR (Table 2) spectrum of **1**, the presence of one hydroperoxy proton resonating as a broad singlet at δ_H 8.19 was observed.[18−20] Moreover, the ^{1}H NMR spectrum revealed the presence of two olefinic methylene protons as two singlets at δ 5.29 and 5.33. A proton signal appearing at δ 2.88 (1H, t, J = 6.0

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Table 1. ^{13}C NMR Spectral Data for Compounds **1**−**6**

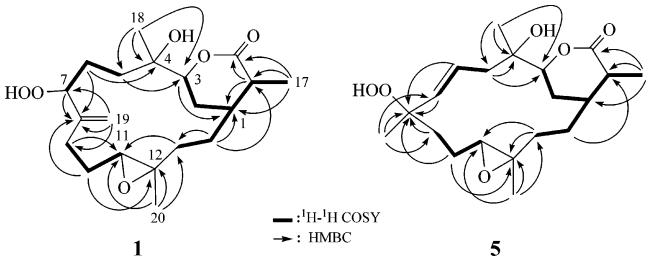
	1[a]		**2**[a]		**3**[b]		**4**[b]		**5**[a]		**6**[a]	
1	38.0	(CH)[c]	37.7	(CH)	37.8	(CH)	37.7	(CH)	37.4	(CH)	37.4	((CH)
2	27.2	(CH_2)	27.3	(CH_2)	27.2	(CH_2)	27.3	(CH_2)	26.2	(CH_2)	26.2	(CH_2)
3	83.2	(CH)	82.6	(CH)	83.1	(CH)	82.7	(CH)	84.3	(CH)	84.4	(CH)
4	73.0	(C)	72.9	(C)	73.1	(C)	73.0	(C)	72.7	(C)	72.7	(C)
5	30.7	(CH_2)	33.9	(CH_2)	30.1	(CH_2)	34.2	(CH_2)	42.3	(CH_2)	42.2	(CH_2)
6	26.7	(CH_2)	25.4	(CH_2)	29.0	(CH_2)	31.0	(CH_2)	122.7	(CH)	127.6	(CH)
7	85.3	(CH)	90.4	(CH)	72.4	(CH)	77.6	(CH)	135.3	(CH)	135.2	((CH)
8	142.5	(C)	143.1	(C)	147.1	(C)	147.7	(C)	83.9	(C)	85.5	(C)
9	31.5	(CH_2)	24.8	(CH_2)	29.7	(CH_2)	24.2	(CH_2)	32.8	(CH_2)	33.8	(CH_2)
10	24.9	(CH_2)	23.5	(CH_2)	24.0	(CH_2)	23.4	(CH_2)	22.6	(CH_2)	23.8	(CH_2)
11	63.6	(CH)	64.2	(CH)	64.1	(CH)	64.4	(CH)	66.1	(CH)	65.9	(CH)
12	59.2	(C)	59.4	(C)	59.3	(C)	59.4	(C)	59.7	(C)	59.7	(C)
13	34.4	(CH_2)	34.3	(CH_2)	34.3	(CH_2)	34.3	(CH_2)	34.7	(CH_2)	34.7	(CH_2)
14	30.3	(CH_2)	30.9	(CH_2)	30.7	(CH_2)	30.1	(CH_2)	29.8	(CH_2)	29.6	(CH_2)
15	43.0	(CH)	43.0	(CH)	43.0	(CH)	43.0	(CH)	42.4	(CH)	42.5	(CH)
16	174.5	(C)	174.5	(C)	174.6	(C)	174.6	(C)	174.4	(C)	174.1	(C)
17	17.1	(CH_3)	17.4	(CH_3)	17.3	(CH_3)	17.6	(CH_3)	16.2	(CH_3)	16.0	(CH_3)
18	24.5	(CH_3)	24.4	(CH_3)	24.5	(CH_3)	24.4	(CH_3)	25.9	(CH_3)	25.0	(CH_3)
19	114.2	(CH_2)	117.2	(CH_2)	111.2	(CH_2)	114.7	(CH_2)	25.0	(CH_3)	19.6	(CH_3)
20	15.7	(CH_3)	15.6	(CH_3)	15.6	(CH_3)	15.6	(CH_3)	15.1	(CH_3)	15.1	(CH_3)

[a]Spectra recorded at 500 MHz in $CDCl_3$. [b] Spectra recorded at 400 MHz in $CDCl_3$. [c] Attached protons were deduced by DEPT experiments.

Table 2. ^1H NMR Spectral Data for Compounds **1**−**6**

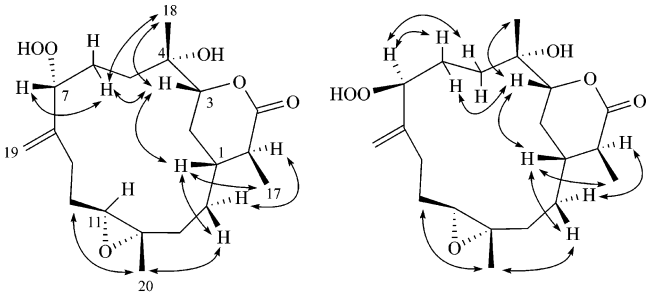
	1[a]	**2**[a]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
1	1.50 m	1.51 m	1.51 m	1.51 m	1.40 m	1.37 m
2	1.91 m; 1.40 m	1.91 m; 1.40 m	1.91 m; 1.40 m	1.91 m; 1.40 m	1.91 m; 1.32 m	1.92 m; 1.36 m
3	3.98 d (11.0)[c]	4.02 d (10.0)	4.01 d (10.8)	4.06 d (10.8)	3.98 d (10.5)	3.87 d (10.2)
5	1.68 m; 1.58 m	1.58 m; 1.55 m	1.67 m; 1.58 m	1.58 m; 1.53 m	2.55 dt (15.0, 2.5); 2.49 dd (15.0, 10.5)	2.54 dt (15.0, 2.5); 2.48 dd (15.0, 10.5)
6	2.08 m; 1.34 m	1.76 m; 1.20 m	1.89 m; 1.33 m	1.72 m; 1.32 m	5.46 ddd (16.0, 10.5, 2.5)	5.38 ddd (16.0, 10.5, 2.5)
7	4.52 t (3.5)	4.40 dd (11.5, 3.5)	4.33 br s	4.20 dd (11.3, 2.8)	5.59 dd (16.0, 2.5)	5.81 ddd (16.0, 2.5)
9	2.43 m; 2.11 m	2.40 m; 2.06 m	2.30 m; 2.19 m	2.45 m; 2.05 m	2.23 m; 1.67 m	2.25 m; 1.67 m
10	1.77 m; 1.70 m	2.00 m; 1.70 m	1.93 m; 1.65 m	1.95 m; 1.68 m	1.84 m; 1.27 m	1.86 m; 1.28 m
11	2.88 t (6.0)	2.85 dd (7.0, 3.5)	2.91 t (6.7)	2.85 t (5.5)	2.81 d (9.0)	2.81 d (9.0)
13	2.09 m; 1.28 m	2.10 m; 1.28 m	2.08 m; 1.29 m	2.10 m; 1.28 m	2.08 m; 1.23 m	2.08 m; 1.22 m
14	1.90 m; 1.26 m	1.91 m; 1.27 m	1.90 m; 1.27 m	1.90 m; 1.26 m	1.83 m; 1.25 m	1.84 m; 1.25 m
15	2.19 m	2.19 m	2.19 m	2.19 m	2.11 m	2.11 m
17	1.37 d (7.0)	1.38 d (7.0)	1.37 d (7.2)	1.39 d (7.2)	1.36 d (7.0)	1.36 d (7.0)
18	1.38 s	1.39 s	1.38 s	1.40 s	1.51 s	1.53 s
19	5.33 s; 5.29 s	5.32 s; 5.23 s	5.35 s; 5.23 s	5.15 s; 5.13 s	1.38 s	1.42 s
20	1.27 s	1.27 s	1.27 s	1.28 s	1.21 s	1.21 s
8-OOH	8.19 br s	7.84 br s			7.48 br s	7.60 br s

[a]Spectra recorded at 500 MHz in $CDCl_3$. [b] Spectra recorded at 400 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses.



1 **5**

Figure 1. ^1H−^1H COSY and HMBC correlations for **1** and **5**.



1 **2**

Figure 2. Key NOESY correlations for **1** and **2**.

Hz) and correlating with a carbon signal at δ 63.6 in the HMQC spectrum was due to the proton of the trisubstituted epoxide. On the basis of the above results and by the assistance of ^1H−^1H COSY and HMBC experiments (Figure 1), the molecular framework of **1** could be established.

The relative configurations of the seven chiral centers at C-1, C-3, C-4, C-7, C-11, C-12, and C-15 in **1** were elucidated by the following NOE analysis, as shown in Figure 2. It was found that H-3 (δ 3.90, d, J = 11.0 Hz) showed NOE interactions with H-1 and H_3-18 (δ 1.38, s). Thus, assuming the β-orientation of H-3,[12] H-1 and H_3-18 should be positioned on the β-face, as well. One of the methylene protons at C-14 (δ 1.90, m) exhibited NOE correlations with H-1 and was assigned as H-14β, while the other (δ 1.26, m) was denoted as H-14α. The NOE correlations observed between H_3-17 and H-1, H-15 and H-14α, and H_3-20 and H-14β reflected the β-orientations of both methyls at C-15 and C-12. Also, H_3-20 was found to interact with H_2-10, but not with H-11, revealing the *trans* geometry of the trisubstituted epoxide. Furthermore, the NOE interactions found between the hydroperoxymethine proton H-7 and H-6β and between H-6β and H_3-18 determined the α-orientation of the hydroperoxy group. On the basis of the above observations and as the absolute configurations of **10** and **11** have been determined as shown,[12] the structure of compound **1** could be fully established as (1*R*,3*R*,4*S*,7*R*,11*S*,12*S*,15*S*)-4-hydroxy-7-hydroperoxy-11,12-epoxycembr-8(19)-en-16,3-olide.

Manaarenolide B (**2**) was found to be more polar than **1** and was isolated as a white solid. It possessed the same molecular

formula ($C_{20}H_{32}O_6$) as that of **1**, as revealed from HRFABMS. Furthermore, it was found that the NMR spectral data of **2** were very similar to those of **1** (Tables 1 and 2) except for the significant downfield shift observed at C-7 ($\Delta\delta_C$ +5.1 ppm) and the upfield shift at C-9 ($\Delta\delta_C$ −6.7 ppm), relative to those of **1**, suggesting that **2** could be the C-7 epimer of **1**. By 2D NMR ($^1H-^1H$ COSY, HMQC, and HMBC), compound **2** was shown to possess the same molecular framework as that of **1**, while its stereochemistry, particularly at C-7, was resolved by NOESY experiments. It was found that one proton (δ 1.20, m) of CH_2-6 showed an NOE interaction with H-3 (δ 4.02, d, J = 10.0 Hz) and was assigned as H-6β. H-6α (δ 1.76, m) was found to correlate with H-7 (δ 4.40, dd, J = 11.5, 3.5 Hz), revealing the α-orientation of H-7 and thus the *S*-configuration at C-7 (Figure 2). Further analysis of other NOE interactions established **2** as (1*R*,3*R*,4*S*,7*S*,11*S*,12*S*,15*S*)-4-hydroxy-7-hydroperoxy-11,12-epoxycembr-8(19)-en-16,3-olide.

Manaarenolide C (**3**) was isolated as a white solid and exhibited a pseudomolecular ion peak at *m*/*z* 353.2325 by HRFABMS, appropriate for a molecular formula of $C_{20}H_{32}O_5$, with one oxygen atom less than those of **1** and **2**. The IR spectrum also revealed the presence of lactone (ν_{max} 1722 cm^{-1}) and hydroxy (ν_{max} 3404 cm^{-1}) moieties. The FABMS showed ion peaks at *m*/*z* 335 [M − H_2O + H]$^+$ and 317 [M − 2 H_2O + H]$^+$, suggesting the presence of two hydroxy groups in **3**. The NMR spectral data were found to be very similar to those of **1** (Tables 1 and 2), except at position 7, which become upfield shifted (δ_C 72.7 and δ_H 4.33) relative to that of **1** (δ_C 85.3 and δ_H 4.52). Therefore, the hydroperoxyl group attached at C-7 in **1** was assumed to be converted to a hydroxy group in **3**. This was further supported by reduction of **1** with triphenylphosphine[19,20] to yield **3**, as evidenced from the identical NMR spectroscopic data of **3** and the reduced product. Therfore, **3** was established as (1*R*,3*R*,4*S*,7*R*,11*S*,12*S*,15*S*)-4,7-dihydroxy-11,-12-epoxycembr-8(19)-en-16,3-olide.

Manaarenolide D (**4**) was isolated as a white solid and showed a similar [M + H]$^+$ ion peak in the HRFABMS corresponding to the molecular formula $C_{20}H_{32}O_5$, the same as that of **3**. The FABMS of **4** also showed peaks at *m*/*z* 335 [M − H_2O + H]$^+$ and 317 [M − 2 H_2O + H]$^+$, due to the presence of two hydroxy groups. Comparison of NMR data of **4** with those of **2** (Tables 1 and 2) suggested the structural difference to be confined to C-7. It was found that C-7 and H-7 of **4** were upfield shifted ($\Delta\delta_C$ −12.8 and $\Delta\delta_H$ −0.20 ppm) relative to those of **2**, a shift that was similar to that shown in **3** relative to **1** ($\Delta\delta_C$ −12.9 and $\Delta\delta_H$ −0.20 ppm). Therefore, **4** was deduced as the 7-hydroxy derivative of **2**, the C-7 epimer of **3**. This conclusion was also supported by the similar splitting pattern observed for H-7 in **4** and **2**. Thus, the structure of manaarenolide D was established as (1*R*,3*R*,4*S*,7*S*,11*S*,12*S*,15*S*)-4,7-dihydroxy-11,12-epoxycembr-8(19)-en-16,3-olide.

The new metabolite manaarenolide E (**5**) was obtained as a white solid and possessed the molecular formula $C_{20}H_{32}O_6$, as established from the HRFABMS and NMR data, implying five degrees of unsaturation. Similar to **1**−**4**, the IR spectrum of **5** indicated the presence of hydroxy (ν_{max} 3422 cm^{-1}) and lactone (ν_{max} 1711 cm^{-1}) functionalities. The FABMS showed ion peaks at *m*/*z* 351 [M + H − H_2O]$^+$ and 317 [M + H − H_2O − H_2O_2]$^+$, suggesting the presence of one hydroxyl and one hydroperoxy group in **5**. The latter was supported by the ^1H NMR signal appearing at δ 7.48 (1H, br s).[18−20] Comparison of the NMR data of **5** with those of **1**−**4** (Tables 1 and 2) revealed that **5** had the same structural unit extending from C-5 to C-1 and further from C-1 to C-9 and C-16, including the attached methyls at C-4, C-12, and C-15. However, it was found that **5** possessed a C6−C8 moiety containing a 1,2-disubstituted double bond (δ 122.7 and 135.3, each CH), a methyl (δ 25.0), and a quaternary oxycarbon (δ 83.9, qC), instead of the 1,1-disubstituted double bond, methylene, and oxymethine found in **1**−**4**. HMBC correlations (Figure 1) observed from H_3-18 to both C-4 and the allylic methylene carbon (δ 42.3, C-5) indicated

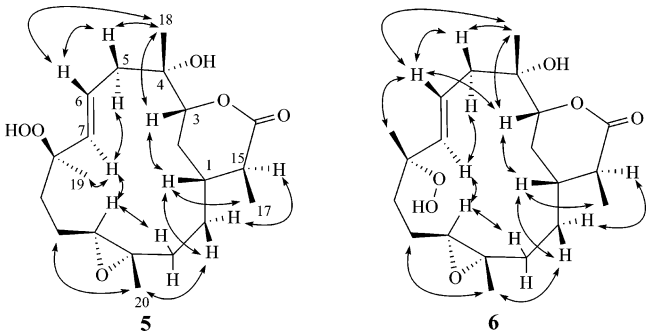


Figure 3. Key NOESY correlations for **5** and **6**.

the C-6 and C-7 position of the double bond, while those found from H-6 (δ 5.46), H-7 (δ 5.59), and the protons of a tertiary methyl (δ 1.38) to the oxycarbon C-8 revealed that the hydroperoxy group should be positioned at C-8. On the basis of the above observations, and by the assistance of additional 2D NMR ($^1H-^1H$ COSY, HMQC and HMBC) experiments, it was possible to establish the planar structure of **5** as illustrated in Figure 1.

The relative configurations of the seven chiral centers at C-1, C-3, C-4, C-8, C-11, C-12, and C-15 in **5** were determined on the basis of NOE correlations observed by NOESY (Figure 3). It was found that H_3-18 (δ 1.51, s) showed NOE interactions with both H-3 (δ 3.98, d, J = 10.5 Hz) and H-6 (δ 5.46, ddd, J = 16.0, 10.5, 2.5 Hz), while H-7 (δ 5.59, dd, J = 16.0, 2.5 Hz) was NOE correlated with H_3-19 (δ 1.38, s). Therefore, H-3, H-6, and H_3-18 are situated on the same β-face, and in contrast, H-7 and H_3-19 should be positioned on the α-face. The above finding, together with J values for both H-6 and H-7 (16.0 Hz), confirmed the *E*-configuration of the 6,7-double bond. Further NOE analysis revealed that **5** possessed the same configurations at C-1, C-3, C-4, C-11, C-12, and C-15 as in compound **1** (Figure 3). On the basis of the above results, the structure of **5** was established as (1*R*,3*R*,4*S*,8*R*,11*S*,12*S*,15*S*,6*E*)-4-hydroxy-8-hydroperoxy-11,12-epoxycembr-6-en-16,3-olide.

Manaarenolide F (**6**) was isolated as a white solid. Its HRFABMS exhibited a [M + H]$^+$ ion peak at 369.2278 *m*/*z*, establishing a molecular formula of $C_{20}H_{32}O_6$. By 2D NMR spectra, including $^1H-^1H$ COSY, HMQC, and HMBC, compound **6** was shown to possess the same molecular framework as that of **5**. Furthermore, it was found that the NMR data of **6** were very similar to those of **5** (Tables 1 and 2), suggesting that **6** might be an isomer of **5**. However, the significant downfield shift at C-6 ($\Delta\delta_C$ +4.9 ppm) and the upfield shift at C-19 ($\Delta\delta_C$ −5.4 ppm) relative to those of **5** (Table 2) suggested that **6** might be the C-8 epimer of **5**. By NOESY (Figure 3), it was found that the β-oriented H-3 (δ 3.87, d, J = 10.2 Hz) showed NOE interactions with H-1 (δ 1.37, m), H-6 (δ 5.38, ddd, J = 16.0, 10.5, 2.5 Hz), and H_3-18 (δ 1.53, s), while H-6 showed NOE interactions with both H_3-18 and H_3-19 (δ 1.42, s), indicating the β-orientation of H_3-19. This inferred the *S*-configuration at C-8. Further analysis of other NOE interactions revealed that **6** possessed the same relative configurations at C-1, C-3, C-4, C-11, C-12, and C-15 as those of **5** (Figure 3). Therefore, **6** was found to be the C-8 epimer of **5** and assigned as (1*R*,3*R*,4*S*,8*S*,11*S*,12*S*,15*S*,6*E*)-4-hydroxy-8-hydroperoxy-11,12-epoxycembr-6-en-16,3-olide.

Manaarenolide G (**7**) was obtained as a white solid. It exhibited a pseudomolecular ion peak at *m*/*z* 335.2226, consistent with the molecular formula $C_{20}H_{30}O_4$. The IR spectrum of **7** indicated the presence of hydroxy (ν_{max} 3422 cm^{-1}) and α,β-unsaturated lactone (ν_{max} 1714 cm^{-1}) moieties. Among the 20 signals appearing in the ^{13}C NMR spectrum of **7** (Table 3), the signals at δ 38.2 (CH), 85.1 (CH), 125.9 (CH_2), 138.8 (qC), and 165.8 (qC) revealed the presence of an α-exomethylenic−δ-lactone ring.[2,3,12] This was further supported by the two sp^2 methylene protons that appeared at δ 5.69 and 6.55 (each 1H, d, J = 2.6 Hz). By comparison of the

Table 3. ^{1}H and ^{13}C NMR Spectral Data for Compounds **7**−**9**

	7		**8**		**9**	
C/H	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[c]	^{13}C[d]
1	2.37 m	38.2 (CH)[f]	2.15 m	41.2 (CH)	2.60 m	33.0 (CH)
2	2.24 m; 1.44 m	27.7 (CH_2)	2.06 m; 1.67 m	31.2 (CH_2)	2.01 m; 1.38 m	28.5 (CH_2)
3	4.17 dd (11.6, 2.4)[e]	85.1 (CH)	3.91 d (10.8)	84.2 (CH)	4.00 dd (10.5, 2.0)	83.7 (CH)
4		73.8 (C)		73.7(C)		74.2 (C)
5	1.79 m; 1.60 m	38.4 (CH_2)	1.79 m	37.3 (CH_2)	1.83 m; 1.60 m	38.8 (CH_2)
6	2.35 m; 1.77 m	22.1 (CH_2)	2.26 m; 2.06 m	22.9 (CH_2)	2.32 m; 1.82 m	22.3 (CH_2)
7	4.99 t (7.2)	124.7 (CH)	5.22 t (7.2)	126.6 (CH)	5.08 t (7.2)	124.4 (CH)
8		133.5 (C)		135.0 (C)		136.2 (C)
9	2.18 m; 2.01 m	34.1 (CH_2)	2.28 m; 2.06 m	35.0 (CH_2)	2.13 m; 1.98 m	32.9 (CH_2)
10	1.94 m	29.5 (CH_2)	1.92 m; 1.49 m	32.0 (CH_2)	2.00 m; 1.69 m	30.5 (CH_2)
11	4.11 dd (9.2, 4.4)	77.0 (CH)	4.42 dd (9.2, 4.0)	67.9 (CH)	4.09 br t (6.0)	73.0 (CH)
12		138.8 (C)		140.4 (C)		147.9 (C)
13	5.55 t (8.4)	124.5 (CH)	5.61 t (7.2)	126.3 (CH)	2.31 m; 2.03 m	29.7 (CH_2)
14	2.67 m; 1.96 m	29.7 (CH_2)	2.39 m; 2.12 m	33.0 (CH_2)	2.22 m; 1.54 m	31.4 (CH_2)
15		138.8 (C)		139.7 (C)		139.8 (C)
16		165.8 (C)		166.0 (C)		166.6 (C)
17	6.55 d (2.6)	125.9 (CH_2)	6.53 d (2.6)	128.2 (CH_2)	6.54 d (2.5)	126.8 (CH_2)
	5.69 d (2.6)		5.70 d (2.6)		5.74 d (2.5)	
18	1.39 s	24.7 (CH_3)	1.41 s	25.1 (CH_3)	1.40 s	25.0 (CH_3)
19	1.61 s	14.5 (CH_3)	1.62 s	17.0 (CH_3)	1.63 s	15.7 (CH_3)
20	1.64 s	11.5 (CH_3)	1.73 s	16.5 (CH_3)	5.28 s; 5.07 s	109.1 (CH_2)

[a]Spectra recorded at 400 MHz in $CDCl_3$. [b] Spectra recorded at 100 MHz in $CDCl_3$. [c] Spectra recorded at 500 MHz in $CDCl_3$. [d] Spectra recorded at 125 MHz in $CDCl_3$. [e] *J* values (in Hz) in parentheses. [f] Attached protons were deduced by DEPT experiments.

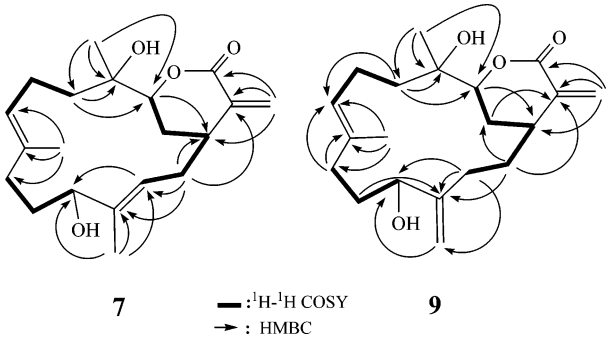


Figure 4. ^{1}H−^{1}H COSY and HMBC correlations for **7** and **9**.

^{13}C NMR data of **7** with those of sinularin (**10**),[12] it was found that the 11,12-trisubstituted epoxide and C-13 methylene in **10** were replaced by a trisubstituted double bond (δ_H 5.55, 1H, t, J = 8.4 Hz; δ_C 124.5, CH and 138.8, qC) and a hydroxy-bearing methine (δ_H 4.11, 1H, dd, J = 9.2, 4.4 Hz; δ_C 77.0, CH) in **7**. The planar structure of **7**, including the positions of the above double bond and hydroxyl, was determined by ^{1}H−^{1}H COSY and HMBC correlations (Figure 4). Accordingly, HMBC correlations observed from the vinylic methyl at δ 1.64 to the olefinic carbons at δ 138.8 (qC) and 124.5 (CH), and the oxymethine carbon at δ 77.0, together with HMBC correlations found from H_2-14 to these olefinic carbons, revealed the hydroxyl at C-11 and the presence of a double bond at C-12 and C-13.

The 1*R*,3*R*,4*S* configurations of **7** were revealed from the NOE interactions (Figure 5) and chemical shift values of the relevant carbons similar to those in **1**−**6**. Moreover, the NOE correlations observed by the β-oriented H-3 with the olefinic proton H-7, and H-7 with H_2-9 but not with H_3-19, established the methyl group at C-8 to be on the α-face and, hence, an *E*-configuration for the 7,8-trisubstituted double bond. This was further supported by NOE interactions found between H_3-19 and one of the H_2-6 protons (δ 2.35). Furthermore, NOE correlations displayed by the β-oriented H-3 with H-2β (δ 2.24, m), H-2β with H-13, and H-13 with H-11 but not with H_3-20 indicated the α-orientation of hydroxyl at C-11 and the *E*-configuration of the 12,13-trisubstituted double bond, respectively. The latter finding was further supported from NOEs found between the methyl group at C-12 and one of the H_2-14 protons (δ 2.67). On this basis along with other NOESY correlations (Figure 5), the structure of metabolite **7** was identified as (1*R*,3*R*,4*S*,-11*S*,7*E*,13*E*)-4,11-dihydroxycembra-7,12(13),15(17)-trien-16,3-olide.

Manaarenolide H (**8**) was found to possess the same molecular formula, $C_{20}H_{30}O_4$, as that of **7** on the basis of the HRFABMS (*m*/*z* 335.2226 $[M + H]^+$) and NMR data (Table 3). An α,β-unsaturated lactone and a hydroxyl were also revealed in **7** from the IR absorption bands at ν_{max} 1714 and 3439 cm^{-1}, respectively. Comparison of the ^{1}H and ^{13}C NMR data of **8** with those of **7** (Table 3) showed that both compounds possess similar structures. This was further supported by the planar structure established by 2D NMR analysis of **8**. However, it was found that the chemical

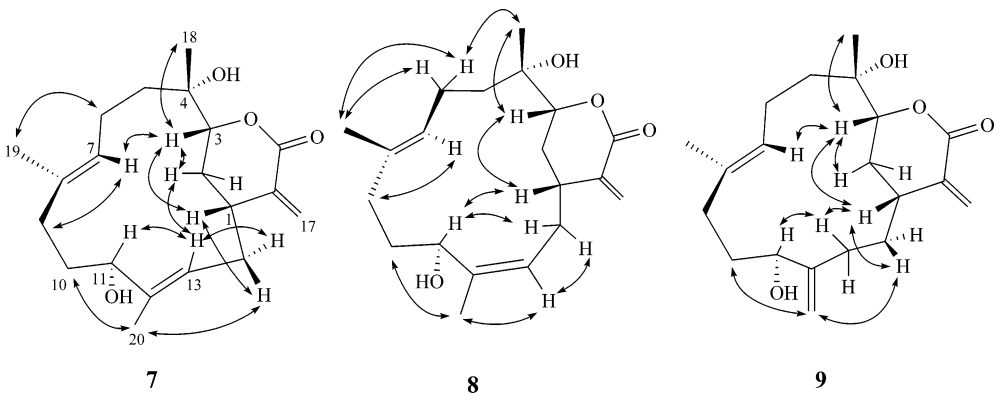


Figure 5. Key NOESY correlations for **7**, **8**, and **9**.

shifts of C-11 (δ 67.9) and C-20 (δ 16.5) in **8** were markedly different from those of **7** (δ 77.0 and 11.5, respectively). These data revealed the *Z*-geometry of the 12,13-double bond in **8**, as C-20 could not be shielded by the γ-effect[13] exerted by C-14. Moreover, the similar splitting patterns and *J*-values of both protons at C-11 in **7** and **8**, combined with the NOE interactions (Figure 5) observed between the β-oriented H-1 (δ 2.15, m) and H-11 (δ 4.42, dd, J = 9.2, 4.0 Hz), revealed the α-orientation of the 11-OH in **8**. Furthermore, the NOE correlations displayed between the olefinic proton at C-13 (δ 5.61, t, J = 7.2 Hz) and H_3-20 (δ 1.73, s) assigned the *Z*-configuration of the double bond between C-12 and C-13. On this basis, the structure of manaarenolide H was determined as (1*R*,3*R*,4*S*,11*S*,7*E*,13*Z*)-4,11-dihydroxycembra-7,12(13),15(17)-trien-16,3-olide.

Manaarenolide I (**9**) was isolated as a white solid. Its HRFABMS (*m*/*z* 335.2227 $[M + H]^+$) and NMR data (Table 3) established a molecular formula of $C_{20}H_{30}O_4$. Thus, **9** was an isomer of both **7** and **8**. The IR spectrum of **9** revealed the presence of hydroxy (ν_{max} 3437 cm^{-1}) and α,β-unsaturated lactone (ν_{max} 1714 cm^{-1}) functionalities. By comparison of NMR data of **9** with those of **7** and **8** (Table 3), it was found that the vinylic methyl at C-12 and the sp^2 methine at C-13 in **7** and **8** were replaced by an exomethylene (δ_H 5.28 and 5.07, each 1H, s; δ_C 109.1) and an sp^3 methylene (δ_H 2.31 and 2.03, each 1H, m; δ_C 29.7) in **9**, respectively. Analyses of $^1H-^1H$ COSY and HMBC correlations established the planar structure of **9** as shown in Figure 4, which showed the C-12 positioning of the exocyclic methylene. Careful analysis of the NOESY spectrum of **9**, in comparison with that of **7**, allowed determination of the relative stereochemistry of manaarenalide I as shown in Figure 5. Thus, the structure of **9** was established as (1*R*,3*R*,4*S*,11*S*,7*E*,13*Z*)-4,11-dihydroxycembra-7,12(20),15(17)-trien-16,3-olide.

The cytotoxicity of diterpenoids **1**−**11** against the growth of Hepa59T/VGH, KB, Hela, and Med cancer cells was studied. The results showed that only compounds **7** and **8** exhibited moderate cytotoxicities against the tested cell lines (ED_{50}'s 7.2, 8.7, 10.9, and 13.4 μg/mL for **7**, and 7.4, 7.6, 9.3, and 5.8 μg/mL for **8**, against the growth of Hepa59T/VGH, KB, Hela, and Med cells, respectively). It seemed that the C-11 to C-13 hydroxy olefin moiety in these two compounds is critical for the cytotoxic activity of cembranolides.

Biogenetically, hydroperoxycembranolides (**1**, **2**, **5**, and **6**) could be considered as the products of the ene reaction formed by addition of singlet oxygen[21,22] to the allylic 7,8-double bond in **11**. Metabolites **7**−**9** are assumed to be derived from acid-catalyzed reactions of the 12,13-epoxide. To the best of our knowledge, the hydroperoxycembranolides possessing a δ-lactone ring are reported herein for the first time.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Hitachi I-2001 infrared spectrophotometer. FABMS were obtained with a VG Quattro GC/MS spectrometer. HRFABMS spectra were recorded on a JEOL-SX/SX 102A mass spectrometer. The NMR spectra were recorded on a Bruker AMX-400 FT-NMR at 400 MHz for ^1H and 100 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^1H and 125 MHz for ^{13}C, respectively, in $CDCl_3$ using TMS as internal standard. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.2 mm) were used for analytical TLC analyses.

Animal Material. *S. manaarensis* (Alcyonidae) was collected by hand via scuba at the coast of Pingtung, southern Taiwan, in July 2001, at a depth of 10 to 15 m, and stored in a freezer until extraction. A voucher sample is deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-Sen University.

Extraction and Separation. The lyophilized bodies of *S. manaarensis* (2.2 kg, wet wt) were minced and exhaustively extracted with EtOAc (1 L × 5). The solvent-free EtOAc extract (20.2 g) was subjected to Si gel CC and eluted with *n*-hexane in EtOAc (0−100%, gradient) to yield 28 fractions. Fraction 16 eluted with *n*-hexane−EtOAc (10:1) and was chromatographed by normal-phase HPLC using *n*-hexane−acetone (8:1) to yield **10** (25 mg) and **11** (28.2 mg), respectively. Fraction 17 eluted with *n*-hexane−EtOAc (5:1) and was separated by normal-phase HPLC using *n*-hexane−acetone (6:1) to yield **1** (4.2 mg), **2** (2.5 mg), **5** (3.0 mg), and **6** (3.2 mg), respectively. Finally, fraction 18 eluted with *n*-hexane−EtOAc (2:1) and was further purified by normal-phase HPLC using *n*-hexane−acetone (5:1 to 4:1) to afford **7** (2.5 mg), **8** (2.9 mg), **9** (2.6 mg), **3** (2.5 mg), and **4** (1.9 mg), respectively.

Manaarenolide A (1): white solid; mp 81−83 °C; $[\alpha]_D^{27}$ −23 (*c* 0.38, $CHCl_3$); IR (neat) ν_{max} 3356, 1720, 1381, and 1232 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 369 [0.4, $(M + H)^+$], 351 [0.4, $(M - H_2O + H)^+$], and 317 [0.3, $(M - H_2O - H_2O_2 + H)^+$]; HRFABMS *m*/*z* 369.2274 (calcd for $C_{20}H_{33}O_6$ 369.2278).

Manaarenolide B (2): white solid; mp 85−87 °C; $[\alpha]_D^{28}$ −21 (*c* 1.08, $CHCl_3$); IR (neat) ν_{max} 3373, 1722, 1381, and 1219 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 369 [0.3, $(M + H)^+$], 351 [0.2, $(M - H_2O + H)^+$], and 317 [0.2, $(M - H_2O - H_2O_2 + H)^+$]; HRFABMS *m*/*z* 369.2275 (calcd for $C_{20}H_{33}O_6$ 369.2278).

Manaarenolide C (3): white solid; mp 78−80 °C; $[\alpha]_D^{28}$ −26 (*c* 0.28, $CHCl_3$); IR (neat) ν_{max} 3404, 1722, 1381, and 1217 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 353 [0.2, $(M + H)^+$], 335 [0.2, $(M - H_2O + H)^+$], and 317 [0.2, $(M - 2\ H_2O + H)^+$]; HRFABMS *m*/*z* 353.2325 (calcd for $C_{20}H_{33}O_5$ 353.2329).

Manaarenolide D (4): white solid; mp 83−85 °C; $[\alpha]_D^{25}$ −13 (*c* 0.60; IR (neat) ν_{max} 3400, 1722, and 1379 cm^{-1};^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 353 [0.3, $(M + H)^+$], 335 [0.2, $(M - H_2O + H)^+$], and 317 [1.1, $(M - 2\ H_2O + H)^+$]; HRFABMS 353.2325 *m*/*z* (calcd for $C_{20}H_{33}O_5$ 353.2329).

Manaarenolide E (5): white solid; mp 115−118 °C; $[\alpha]_D^{28}$ −33 (*c* 0.38, $CHCl_3$); IR (neat) ν_{max} 3422, 1711, 1631, 1381, and 1236 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 369 [0.5, $(M + H)^+$], 351 [0.2, $(M - H_2O + H)^+$], and 317 [0.3, $(M - H_2O - H_2O_2 + H)^+$]; HRFABMS *m*/*z* 369.2278 (calcd for $C_{20}H_{33}O_6$ 369.2278).

Manaarenolide F (6): white solid; mp 103−105 °C; $[\alpha]_D^{29}$ −13 (*c* 0.75, $CHCl_3$); IR (neat) ν_{max} 3383, 1722, 1379, and 1236 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 1, respectively; FABMS *m*/*z* 369 [0.3, $(M + H)^+$]; HRFABMS *m*/*z* 369.2278 (calcd for $C_{20}H_{33}O_6$ 369.2278).

Manaarenolide G (7): white solid; mp 85−87 °C; $[\alpha]_D^{28}$ −41 (*c* 0.72, $CHCl_3$); IR (neat) ν_{max} 3422, 1714, 1651, 1622, and 1385 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 335 [0.5, $(M + H)^+$], 317 [1.0, $(M - H_2O + H)^+$], and 299 [1.0, $(M - 2\ H_2O + H)^+$]; HRFABMS *m*/*z* 335.2226 (calcd for $C_{20}H_{31}O_4$ 335.2223).

Manaarenolide H (8): white solid; mp 70−72 °C; $[\alpha]_D^{28}$ −28 (*c* 0.56, $CHCl_3$); IR (neat) ν_{max} 3439, 1714, and 1635 cm^{-1};^1H and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 335 [0.2, $(M + H)^+$], 317 [0.3, $(M - H_2O + H)^+$], and 299 [0.4, $(M - 2\ H_2O + H)^+$]; HRFABMS *m*/*z* 335.2226 (calcd for $C_{20}H_{31}O_4$ 335.2223).

Manaarenolide I (9): white solid; mp 83−85 °C; $[\alpha]_D^{28}$ −46 (*c* 0.78, $CHCl_3$); IR (neat) ν_{max} 3437, 1714, 1622, and 1265 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; ^1H and ^{13}C NMR data, see Table 3; FABMS *m*/*z* 335 [1.0, $(M + H)^+$], 317 [1.5, $(M - H_2O + H)^+$], and 299 [1.5, $(M - 2\ H_2O + H)^+$]; HRFABMS *m*/*z* 335.2227 (calcd for $C_{20}H_{31}O_4$ 335.2223).

Reduction of Manaarenolide A (1). Manaarenolide A (**1**) (2.2 mg) was stirred with 8 mg of triphenylphosphine in 5 mL of ether for 4 h at room temperature. After evaporation of excess reagent, the residue was separated by short column chromatography on silica gel (EtOAc−*n*-hexanes = 1:2) to give a reduced product of **1** (1.9 mg, 90%). Physical and spectral data of this product were found to be in full agreement with those of the natural product **3**.

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1**−**9** were performed using the MTT [3-(4,5-dimethylthiazole-2-yl)- 2,5-diphenyltetrazolium bromide] colorimetric method.[23,24]

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