New Cytotoxic Cembranolides from the Soft Coral *Lobophytum michaelae*

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Eleven new cytotoxic cembranolides, michaolides A—K (1—11), and crassolide (12) were isolated from the CH_2Cl_2 extract of the Formosan soft coral *Lobophytum michaelae*. Their structures were established by extensive spectral analysis. The cytotoxicity of the isolates against selected cancer cells was measured *in vitro*.

Key words *Lobophytum michaelae*; cembranolide; cytotoxicity

Soft corals of the genus *Lobophytum* are rich sources of bioactive terpenoids and steroids.[1—10] In our search for bioactive substances from marine organisms, the Formosan soft coral *Lobophytum michaelae* TIXIER-DURIVAULT (Alcyoniidae) was studied because the CH_2Cl_2 extract showed significant cytotoxity against HT-29 (human colon adenocarcinoma) and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[11,12] Bioassay-guided fractionations of extract resulted in the isolation of eleven new cytotoxic cembranolides, michaolides A—K (**1**—**11**), and crassolide (**12**).

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25}$ −29° (c=0.1, $CHCl_3$). HR-ESI-MS, ^{13}C-NMR, and DEPT spectroscopic data established the molecular formula of **1** as $C_{24}H_{32}O_8$. The IR spectrum of **1** indicated the presence of the functionalities of ester group(s) (ν_{max} 1735 cm^{-1}) and an α-methylene-γ-lactone (ν_{max} 1765, 1665 cm^{-1}). The presence of the α-methylene-γ-lactone system in **1** was also demonstrated by UV absorption at 221 (log ε 3.66) nm and signals at δ 5.73 (H-16) and 6.40 (H-16) in the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum of **1** also showed signals for two olefinic protons at δ 5.11 (H-11), and 5.24 (H-7) ppm; three oxymethine protons at δ 3.94 (dd, J=9.9, 3.0 Hz, H-9), 4.49 (dd, J=11.1, 3.6 Hz, H-5), 4.98 (t, J=3.6 Hz, H-2), and 5.25 (d, J=11.7 Hz, H-14); one methine proton at δ 3.18 (br s, H-1), two olefinic methyl groups at δ 1.72 (H_3-19) and 1.81 (H_3-20); and two methyl groups in acetate esters at δ 2.00 and 2.11. HMBC spectrum exhibited a methyl-bearing trisubstituted epoxy [δ_H 2.82 (d, J=3.6 Hz, H-3), 1.45 (H_3-18); δ_C 61.4 (CH), 61.9 (qC), 13.2 (CH_3)]. The spectral data of **1** indicated some similarities to those of crassolide (**12**),[13] except for the data due to C-9. The ^{1}H–^{1}H COSY spectrum exhibited correlations from H-13 to H-3, H-5 to H-7, H-9 to H-11. ^{1}H–^{1}H long-range correlations were also observed between H-1 to H_2-16, H-7 to H_3-19, and H-11 to H_3-20. These spectroscopic findings and the nine degrees of unsaturations indicated that **1** was a 14-membered cembrane-type diterpene skeleton with an α-methylene-γ-lactone. After assignments between all the C–H bondings were made based on HSQC experiment, the plane structure was determined by HMBC analysis. The correlations according to HMBC are shown in Fig. 1. The stereochemistry for the trisubstituted olefins of **1** was determined by NOESY analysis. The NOESY correlations between H-7 and H-9, and H-11 and H-13 disclosed the *E* configurations for the trisubstituted olefins. The chemical shift values at δ_C 10.8 and 15.9 (for C-19, and C-20 respectively) also supported the *E* configurations.[13] The NOESY correlations (Fig. 2) observed between H-3 and H-1/H-5/H-11/H-13α, H-14 and H-1/H_3-20, H-7

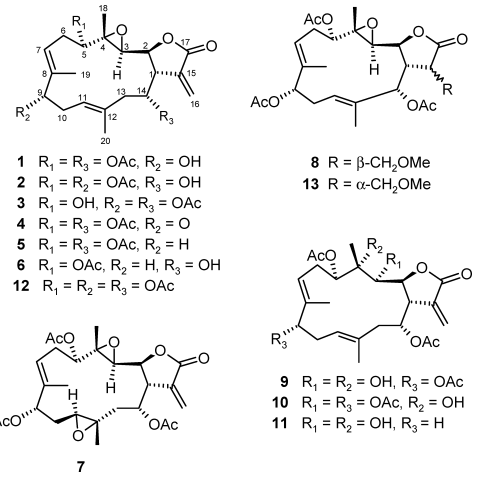

Chart 1. Structures of **1**—**13**

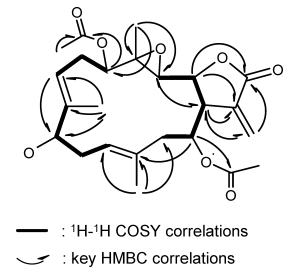

Fig. 1. ^{1}H–^{1}H COSY and Key HMBC Correlations of **1**

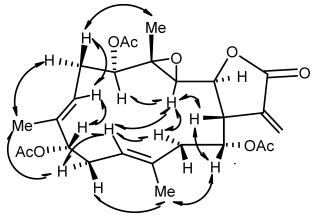

Fig. 2. Selected NOESY Correlations of **1**

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and H-9/H-6β, H_3-19 and H-6α/H-10α, H_3-18 and H-5β indicated the relative configurations for the 14-membered ring carbons, which were identical to the X-ray diffraction data of the methanolysis product (**13**) of crassolide (**12**).[14]

Compound **2** was shown to have the same molecular formula as **1** by HR-ESI-MS and from its ^{13}C-NMR data. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **2** closely resembled those of **1** except for the signals at C-9 and C-14. ^{1}H–^{1}H COSY cross peak between H-9 and H-10 as well as HMBC correlations between H-9 and C-10/C-11/C-19 revealed that the secondary hydroxyl attached to C-9 in **1** was replaced by an acetoxyl [δ_H 4.94 (dd, J=3.3, 11.2 Hz,

Table 1. ^{1}H-NMR Data[a] (300 MHz) of **1**—**6**

H	1	2	3	4	5	6
1	3.18 br s	3.04 br s	3.14 br s	2.97 br s	3.30 br s	3.16 br s
2	4.98 t (3.6)[b]	5.08 br s	5.11 t (3.0)	5.11 t (3.3)	4.99 m	5.04 t (3.0)
3	2.82 d (3.6)	2.80 d (3.3)	2.80 d (3.0)	2.88 d (3.3)	2.87 d (3.6)	2.83 d (3.0)
5	4.49 dd (3.6, 11.1)	4.38 dd (3.6, 11.1)	3.11 br s	4.56 dd (2.7, 10.4)	4.46 dd (3.9, 11.4)	4.36 dd (3.6, 10.4)
6	2.17 m	2.17 m	2.12 m	2.54 m	2.18 m	2.08 m
	2.50 m	2.54 m	2.53 m	2.66 m	2.45 m	2.48 m
7	5.24 m	5.27 t (7.5)	5.29 d (7.2)	6.36 m	4.97 m	4.94 t (7.2)
9	3.94 dd (3.0, 9.9)	4.94 dd (3.3, 11.2)	4.92 dd (3.6, 11.1)		2.00 m	2.28 m
					2.27 m	
10	2.33 m	2.38 m	2.28 m	3.27 m	2.12 m	2.25 m
	2.50 m	2.52 m	2.54 m	3.55 m	2.30 m	1.09 m
11	5.11 m	5.09 m	5.00 t (6.9)	5.41 t (7.8)	5.25 m	5.24 t (7.2)
13	2.22 m	2.25 m	2.17 m	2.19 m	2.26 m	2.23 m
	2.42 m	2.40 m	2.45 m	2.53 m	2.43 m	2.41 m
14	5.25 br d (11.7)	4.14 br d (11.1)	5.21 br d (11.7)	5.15 m	5.26 m	4.09 br d (10.5)
16	5.73 br s	5.74 d (1.8)	5.77 br s	5.63 br s	5.73 d (2.7)	5.68 br s
	6.40 br s	6.46 d (1.8)	6.41 br s	6.36 br s	6.40 d (2.1)	6.39 br s
18	1.45 s	1.44 s	1.39 s	1.42 s	1.45 s	1.41 s
19	1.72 s	1.74 s	1.68 s	1.76 s	1.67 s	1.66 s
20	1.81 s	1.78 s	1.85 s	1.78 s	1.76 s	1.68 s
5-OAc	2.11 s	2.11 s		2.00 s	2.11 s	2.08 s
9-OAc		2.06 s	2.05 s			
14-OAc	2.00 s		1.99 s	2.14 s	2.01 s	

a) Recorded in $CDCl_3$ (assigned by COSY, HSQC, and HMBC experiments). *b*) *J* values (in Hz) in parentheses.

Table 2. ^{13}C-NMR Spectral Data[a] (75 MHz) of **1**—**12** in $CDCl_3$

	1	2	3	4	5	6	7	8	9	10	11	12
1	42.2	43.8	41.6	41.6	42.4	43.8	43.7	39.9	42.1	42.8	43.8	42.2
2	73.6	72.4	72.8	72.4	73.8	72.9	72.8	74.6	80.6	79.4	80.8	73.2
3	61.4	62.2	63.1	61.6	61.7	62.1	62.3	62.3	74.7	74.5	74.9	61.9
4	61.9	61.1	63.5	60.9	61.6	61.3	61.2	62.6	77.1	76.1	76.7	61.3
5	77.4	77.9	77.1	78.0	77.8	78.4	77.3	77.8	76.0	75.3	77.4	77.6
6	29.5	29.5	31.3	31.7	30.1	30.1	29.8	30.3	29.4	29.1	29.2	29.4
7	119.9	122.2	123.4	136.4	118.1	118.1	123.6	122.0	125.0	123.3	121.5	122.0
8	141.0	137.2	136.6	138.8	138.9	138.9	136.4	135.9	134.0	135.0	137.1	137.1
9	77.3	78.9	79.2	197.9	24.5	24.6	75.7	78.5	78.4	78.5	38.9	78.6
10	32.8	30.3	30.4	39.6	40.2	40.3	30.9	29.8	30.0	30.1	24.6	30.3
11	124.9	123.3	123.8	122.6	127.6	128.8	59.3	123.6	122.1	123.3	128.6	123.9
12	132.5	134.5	134.1	135.3	130.1	130.9	57.3	133.0	136.2	133.1	130.7	133.8
13	42.2	45.5	42.4	42.3	42.1	45.4	41.6	41.9	39.9	41.1	40.4	42.2
14	73.2	72.6	73.7	73.9	73.3	72.4	73.0	70.9	75.1	75.6	74.2	73.3
15	135.2	136.9	135.1	135.3	135.5	137.4	134.8	44.0	136.2	137.3	136.7	135.0
16	124.3	123.8	124.9	122.6	124.1	123.3	125.5	69.3	123.4	122.1	122.5	124.7
17	169.0	169.5	169.1	168.9	169.9	170.3	169.8	175.4	170.4	169.9	170.2	168.9
18	13.2	12.8	10.9	12.0	13.1	12.7	12.6	13.7	21.1	23.1	21.1	13.0
19	10.8	11.3	11.1	12.0	15.1	15.2	12.0	11.2	11.4	11.3	15.3	11.3
20	15.9	16.2	16.0	16.3	15.8	16.1	16.8	15.7	16.5	15.9	16.3	15.8
3-OAc										170.2		
										21.4		
5-OAc	170.1	170.3		170.2	170.2	169.9	170.2	170.1	170.3	170.2	170.4	170.2
	21.2	21.3		20.9	21.3	21.3	20.8	21.2	21.3	21.2	21.4	21.2
9-OAc		170.3	170.2				170.0	170.3	170.5	170.5		170.2
		21.4	21.3				21.3	21.3	21.3	21.2		21.4
14-OAc	170.2		170.1	169.9	170.2		170.0	170.5	170.5	170.5	170.5	170.1
	21.0		20.9	21.2	21.0		20.8	21.4	21.4	21.2	21.1	20.9
OMe								59.2				

a) Assigned by DEPT, COSY, HSQC, and HMBC experiments.

H-9), δ_C 78.9 (CH, C-9)] in **2**. ^{1}H–^{1}H COSY cross peaks between H-14 and H-1/H_2-13 as well as HMBC correlations between H-14 and C-13/C-12/C-1 suggested the secondary acetoxyl attached to C-14 in **1** to be replaced by a hydroxyl [δ_H 4.14 (br d, J=11.1 Hz, H-14), δ_C 72.6 (CH, C-14)] in **2**. NOESY data for **2** were comparable to those of **1**. Both compounds thus had to possess identical stereochemistry.

Compound **3** analyzed for $C_{24}H_{32}O_8$ from its HR-ESI-MS and NMR spectroscopic data. The NMR features of compound **3** were analogous to those of **1** with exception that the secondary acetoxyl attached to C-5 was replaced by a hydroxyl [δ_H 3.11 (br s, H-5), δ_C 77.1 (CH, C-5)] and the secondary hydroxyl attached to C-9 was replaced by an acetoxyl [δ_H 4.92 (dd, J=3.6, 11.1 Hz, H-9), δ_C 79.2 (CH, C-9)]. ^{1}H–^{1}H COSY cross peaks between H-6 and H-5 and between H-9 and H-10 as well as HMBC correlations between H-6 and C-5 and between H-9 and C-10/C-11/C-19 helped asertain these assignments. The relative stereochemistry of **3** was identical to **1** from NOESY analysis.

Compound **4** had the molecular formula, $C_{24}H_{30}O_8$, 2 mass units lower than that of **1**. Detailed comparison of the ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **4** and **1** revealed that **4** differed from **1** at C-9. HMBC correlations from H_3-19 to C-7/C-8/C-9 revealed that the secondary hydroxyl attached to C-9 in **1** was replaced by a ketone (δ_C 197.9) in **4**. The relative stereochemistry of **4** was established from a NOESY spectrum as shown in Fig. 3.

Compound **5** had the molecular formula, $C_{24}H_{32}O_7$, 16 mass units lower than that of **1**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) resembled those of **1** except that the absence of secondary hydroxyl at C-9. HMBC correlations from H_3-19 to H-7/H-8/H-9 suggested these assignments The relative stereochemistry of **5** was similar to **1** from NOESY analysis.

Compound **6** had the molecular formula, $C_{22}H_{30}O_6$, 58 mass units lower than that of **2**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) were similar to those of **2** except for the missing of the secondary acetoxyl at C-9. HMBC correlations from H_3-19 to H-7/H-8/H-9 confirmed the missing of the secondary acetoxyl at C-9. The relative stereochemistry of **5** was similar to **2** from NOESY analysis.

To determine the absolute configuration, compound **6** 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 (**6a**, **6b**), respectively.[15] The MTPA esters formed at C-14 were elucidated from the ^{1}H-NMR chemical shifts and coupling constants of H-14 in **6a** and **6b** (**6a**, δ 5.50, 1H, d, J=9.9 Hz, H-14; **6b**, δ 5.42, 1H, d, J=10.5 Hz, H-14). Comparison of the ^{1}H-NMR chemical shifts for **6a** and **6b** ($\Delta\delta$ values shown in Fig. 4) led to the assignment of the *R*-configuration at C-14. Therefore, the absolute structure of **6** was determined as shown in formula **6**.

Compound **7** had the molecular formula, $C_{26}H_{34}O_{10}$, 16 mass units higher than that of **12**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 2, 3) of **7** showed some similarity to those of **12** except that a trisubstituted olefin was replaced by a trisubstituted epoxide [δ_H 2.58 (m, H-11), δ_C 59.3 (CH, C-11), 57.3 (qC, C-12)]. This trisubstituted epoxy was located at C-11/C-12 upon observation of COSY correlations from H-10 to H-11/H-9 and HMBC correlations from H_3-20 to C-

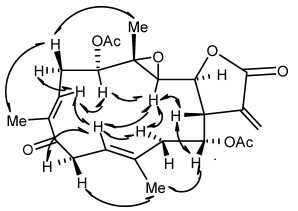
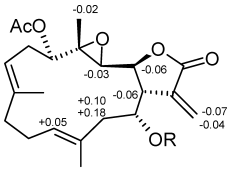


Fig. 3. Selected NOESY Correlations of **4**



6a R = (*S*)-MTPA
6b R = (*R*)-MTPA

Fig. 4. Absolute Stereochemistry of **6**: $\Delta\delta$ Values (δ_S–δ_R) in ppm for the Two MTPA Esters **6a** and **6b**

11/C-12/C-13. The relative stereochemistry of **7** was established by a NOESY spectrum as shown in Fig. 5. NOESY correlations from H_3-20 to H-10/H-14, from H-14 to H-1, from H-7 to H-9/H-6β, from H_3-18 to H-6β positioned H-7, H-9, H-14, H_3-18, H_3-20 on the β-face of the 14-membered ring and help asertain the *E* configuration for the trisubstituted the epoxy at C-11/C-12.

Compound **8** had the molecular formula, $C_{27}H_{39}O_{10}$, 16 mass units higher than that of **12**. Analysis of the ^{1}H- and ^{13}C-NMR spectral data (Tables 2, 3) of **7** revealed that the exomethylene in **12** was substituted by a methoxyl [δ_H 3.56 (dd, J=3.0, 9.6 Hz, H-16), 3.78 (dd, J=3.6, 9.6 Hz, H-16), 3.41 (s, OMe); δ_C 44.0 (CH, C-15), 69.3 (CH_2, C-16), 59.2 (OMe)]. HMBC correlations from O$\underline{C}H_3$ to C-16 confirmed the methoxyl at C-16. NOESY correlations from H-1 to H_2-16 and from H_3-18 to OCH_3 at C-16 permitted the placement of the methoxyl on the β-face of the molecule.

Compound **9** had the molecular formula, $C_{26}H_{36}O_{10}$, 18 mass units higher than that of **12**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 2, 3) of **9** exhibited some similarity those of **12** except that the epoxy at C-3/C-4 in **12** was replaced by two hydroxyls [δ_H 3.80, br s, H-3], δ_C 74.7 (CH, C-3), 77.1 (qC, C-4)] in **9**. HMBC correlations from H_3-18/H-2 to C-4/C-3 confirmed the hydroxyls at C-4 and C-3. The relative stereochemistry of **9** was established from a NOESY spectrum as shown in Fig. 6. NOESY correlations from H_3-18 to H-3/H-6β, from H-7 to H-9/H-6β, from H_3-20 to H-10/H-14, from H-14 to H-1 demontstrted that H-3, H_3-18, H-7, H-9, H_3-20, and H-14 were on the β-face of the 14-membered ring.

Compound **10** had the molecular formula, $C_{28}H_{38}O_{11}$, 42 mass units higher than that of **9**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **10** were analogous to those of **9** except that the hydroxy at C-3 in **9** was replaced by an acetoxy [δ_H 4.97, br s, H-3], δ_C 74.5 (CH, C-3), 76.1 (qC, C-4)] in **10**. HMBC correlations from H_3-18/H-2 to C-4/C-3 enabled the correct poisoning of the acetoxy at C-3. The relative stereochemistry of **10** was identical to **9**, as established from NOESY analysis.

Compound **11** had the molecular formula, $C_{24}H_{34}O_8$, 58

Table 3. ^{1}H-NMR Data[a] (300 MHz) of **7**—**12**

H	7	8	9	10	11	12
1	3.08 br s	2.93 t (7.5)	3.60 br s	3.43 br s	2.30 br s	3.13 br s
2	4.98 t (3.0)[b]	4.89 dd (3.0, 7.5)	4.65 br s	4.80 br s	4.79 br s	4.98 t (3.6)
3	2.63 d (3.0)	2.80 d (3.0)	3.80 br s	4.97 br s	3.84 br s	2.81 d (3.6)
5	4.37 dd (4.2, 11.7)	4.42 dd (3.3, 11.1)	4.81 br d (6.3)	4.80 br s	4.94 br d (8.4)	4.40 dd (3.0, 11.1)
6	2.20 m	2.23 m	2.29 m	2.29 m	2.18 m	2.13 m
	2.66 m	2.49 m	2.69 m	2.69 m	2.86 m	2.53 m
7	5.42 t (7.5)	5.43 t (7.2)	5.74 m	5.86 t (7.2)	5.30 m	5.25 t (7.2)
9	5.14 d (10.8)	4.97 dd (3.0, 9.6)	5.13 dd (9.3, 4.8)	5.12 dd (9.9, 4.8)	5.13 dd (9.3, 4.8)	4.93 dd (3.5, 10.5)
10	2.41 m	2.51 m	2.52 m	2.52 m	2.45 m	2.30 m
	1.66 m	2.28 m	2.29 m	2.29 m	2.22 m	2.50 m
11	2.58 m	5.10 t (6.9)	5.08 t (6.9)	5.30 t (6.6)	5.13 t (6.3)	5.08 m
13	1.38 m	2.20 m	2.23 m	2.23 m	2.36 m	2.24 m
	2.45 m	2.50 m	2.31 m	2.31 m		2.41 m
14	5.19 br d (12.0)	5.19 br d (9.0)	5.30 m	5.18 br d (9.0)	5.30 m	5.22 m
16	5.89 br s	3.56 dd (3.0, 9.6)	5.74 br s	5.62 br s	5.67 br s	5.74 br s
	6.48 br s	3.78 dd (3.3, 9.6)	6.29 br s	6.25 br s	6.28 br s	6.39 br s
18	1.46 s	1.54 s	1.34 s	1.36 s	1.25 s	1.41 s
19	1.79 s	1.71 s	1.62 s	1.64 s	1.58 s	1.70 s
20	1.53 s	1.74 s	1.71 s	1.78 s	1.71 s	1.81 s
3-OAc				2.16 s		
5-OAc	2.12 s	2.06 s	2.10 s	2.09 s	2.03 s	2.08 s
9-OAc	2.06 s	2.10 s	2.07 s	2.05 s		2.02 s
14-OAc	2.01 s	2.12 s	2.06 s	1.96 s	2.10 s	1.96 s
OMe		3.41 s				

a) Recorded in $CDCl_3$ (assigned by COSY, HSQC, and HMBC experiments). *b*) *J* values (in Hz) in parentheses.

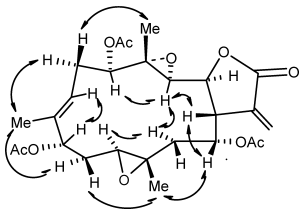


Fig. 5. Selected NOESY Correlations of **7**

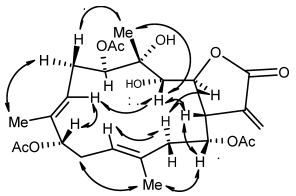


Fig. 6. Selected NOESY Correlations of **9**

Table 4. Cytotoxicity[a] of **1**—**12**

Compounds	Cell lines ED_{50} (μg/ml)	
	HT-29	P-388
1	0.7	0.1
2	0.03	0.001
3	0.8	0.4
4	1.5	0.2
5	0.05	0.007
6	0.001	0.004
7	0.8	0.3
8	3.1	0.4
9	1.4	0.8
10	0.8	0.6
11	9.9	0.6
12	0.05	0.04

a) For significant activity of pure compounds, an ED_{50} of $\leqq$4.0 μg/ml is required.

mass units lower than that of **9**. The ^{1}H- and ^{13}C-NMR spectral data (Tables 1, 2) of **10** were similar to those of **9** except that the acetoxy at C-9 was missing. HMBC correlations from H_3-19 to H-7/H-8/H-9 confirmed the missing of the secondary acetoxyl at C-9. The NOESY analysis suggested **11** to have similar stereochemistry as **9**.

The cytotoxicity of compounds **1**—**12** is shown in Table 4. Compounds **2** and **6** showed potent cytotoxicity against HT-29 and P-388 cell lines. Compounds **9**—**11** showed moderate cytotoxicity against HT-29 and P-388 cell lines. Hydroxylation at C-14 together with an α-exo-methylene-γ-lactone and a 3,4-trisubstituted epoxy may be important for potent cytotoxicity.

Experimental

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 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. ESI-MS spectra were obtained with a Bruker APX II mass spectrometer. Si gel 60 (Merck, 230—400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material The soft coral *Lobophytum michaelae* Tixier-Durivault (Alcyoniidae) was collected at Ken-tin (southern Taiwan), in July 1992, at a depth of 2—3 m and was stored for 1 week in a freezer until extraction. A voucher specimen, NSUMR-12, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation The bodies of the soft coral *L. michaelae* were freeze dried to give 1.90 kg of a solid, which was extracted with CH_2Cl_2 (5.01×3). After removal of solvent *in vacuo*, the residue (40 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane–EtOAc mixtures of increasing polarity. Elution with *n*-hexane/EtOAc (19 : 1) gave fractions containing **5** and **12**, with *n*-hexane/EtOAc (9 : 1) gave fractions containing **1** and **2**, with *n*-hexane/EtOAc (8 : 1) gave fractions containing **3** and

7, with *n*-hexane/EtOAc (7 : 1) gave fractions containing **4**, **6**, and **8**, with *n*-hexane/EtOAc (6 : 1) gave fractions containing **9**, **10**, and **11**. Compounds **1** and **2** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (78 : 22). Compounds **3** and **7** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (76 : 24). Compounds **5** and **12** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (82 : 18). Compounds **4**, **6**, and **8** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (70 : 30). Compounds **9**—**11** were further purified by RP-C_{18} HPLC separation, by eluting with MeOH/H_2O (65 : 35).

Michaolide A (**1**): (3 mg); $[\alpha]_D^{25}$ −29° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 221 (3.66). IR (neat) cm^{-1}: 3410, 1765, 1735, 1665. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=471.1993 (Calcd for $C_{24}H_{32}O_8Na$=471.1995).

Michaolide B (**2**): (4 mg); $[\alpha]_D^{25}$ −37° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 222 (3.68). IR (neat) cm^{-1}: 3420, 1768, 1732, 1665. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=471.1995 (Calcd for $C_{24}H_{32}O_8Na$=471.1995).

Michaolide C (**3**): (8 mg); $[\alpha]_D^{25}$ −33° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 220 (3.56). IR (neat) cm^{-1}: 3426, 1763, 1736, 1669. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2; HR-ESI-MS m/z=471.1997 (Calcd for $C_{24}H_{32}O_8Na$=471.1995).

Michaolide D (**4**): (2 mg); $[\alpha]_D^{25}$ −7° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 226 (3.82). IR (neat) cm^{-1}: 1769, 1739, 1670. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=469.1836 (Calcd for $C_{24}H_{30}O_8Na$=469.1838).

Michaolide E (**5**): (4 mg); $[\alpha]_D^{25}$ −97° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 224 (3.49). IR (neat) cm^{-1}: 1763, 1736, 1666. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=455.2043 (Calcd for $C_{24}H_{32}O_7Na$=455.2046).

Michaolide F (**6**): (6 mg); $[\alpha]_D^{25}$ −88° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 225 (3.55). IR (neat) cm^{-1}: 3440, 1765, 1733, 1660. ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=413.1942 (Calcd for $C_{22}H_{30}O_6Na$=413.1940).

Michaolide G (**7**): (4 mg); $[\alpha]_D^{25}$ −10° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 225 (3.65). IR (neat) cm^{-1}: 1769, 1738, 1666. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=529.2048 (Calcd for $C_{26}H_{34}O_{10}Na$=529.2050).

Michaolide H (**8**): (2 mg); $[\alpha]_D^{25}$ −13° (c=0.1, $CHCl_3$). IR (neat) cm^{-1}: 1786, 1732. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=523.2545 (Calcd for $C_{27}H_{39}O_{10}$=523.2543).

Michaolide I (**9**): (3 mg); $[\alpha]_D^{25}$ +10° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 226 (3.41). IR (neat) cm^{-1}: 3426, 1766, 1732, 1665. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=531.2204 (Calcd for $C_{26}H_{36}O_{10}Na$=531.2206).

Michaolide J (**10**): (3 mg); $[\alpha]_D^{25}$ +19° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 223 (3.55). IR (neat) cm^{-1}: 3460, 1765, 1733, 1661. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=573.2314 (Calcd for $C_{28}H_{38}O_{11}Na$=573.2312).

Michaolide K (**11**): (2 mg); $[\alpha]_D^{25}$ +61° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 224 (3.62). IR (neat) cm^{-1}: 3450, 1765, 1735, 1666. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=473.2150 (Calcd for $C_{24}H_{34}O_8Na$=473.2152).

Crassolide (**12**): (60 mg); $[\alpha]_D^{25}$ −19° (c=0.1, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 220 (3.68). IR (neat) cm^{-1}: 1769, 1738, 1668. ^{1}H-NMR: see Table 3. ^{13}C-NMR: see Table 2. HR-ESI-MS m/z=491.2280 (Calcd for $C_{26}H_{35}O_9$=491.2281).

Preparation of (*R*)- and (*S*)-MTPA Esters (**6a**, **6b**) of **6**: To a solution of compound **6** (1.1 mg, 2.5 mmol) in pyridine (0.5 ml) at room temperature were 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 water to give the corresponding (*S*)-MTPA ester **6a** (0.5 mg): ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.37 (3H, s, H_3-18), 1.64 (3H, s, H_3-19), 1.77 (3H, s, H_3-20), 2.12 (3H, s, 5-OAc), 2.11 (H, m, H-6), 2.34 (1H, m, H-13), 2.54 (1H, d, J=12.3 Hz, H-13), 2.83 (1H, J=4.5 Hz, H-3), 3.29 (1H, br s, H-1), 3.45 (3H, s, OMe), 4.53 (1H, dd, J=10.5, 3.9 Hz, H-5), 4.69 (1H, t, J=4.5 Hz, H-2), 4.96 (1H, t, J=7.2 Hz, H-7), 5.30 (1H, t, J=7.2 Hz, H-11), 5.50 (1H, br d, J=9.9 Hz, H-14), 5.75 (1H, d, J=2.1 Hz, H-16), 6.37 (1H, d, J=2.7 Hz, H-16), 7.40—7.65 (5H, aromatic H). Treatment of **6** (1.2 mg) in the same manner with (*S*)-MTPA chloride in pyridine gave the corresponding (*R*)-MTPA ester **6b** (0.6 mg): ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.39 (3H, s, H_3-18), 1.65 (3H, s, H_3-19), 1.78 (3H, s, H_3-20), 2.10 (3H, s, 5-OAc), 2.12 (H, m, H-6), 2.16 (1H, m, H-13), 2.44 (1H, d, J=12.6 Hz, H-13), 2.80 (1H, J=4.5 Hz, H-3), 3.35 (1H, br s, H-1), 3.42 (3H, s, OMe), 4.41 (1H, dd, J=10.8, 3.6 Hz, H-5), 4.75 (1H, t, J=4.5 Hz, H-2), 4.96 (1H, t, J=6.9 Hz, H-7), 5.25 (1H, m, H-11), 5.42 (1H, br d, J=10.5 Hz, H-14), 5.79 (1H, d, J=2.1 Hz, H-16), 6.44 (1H, d, J=2.1 Hz, H-16), 7.41—7.66 (5H, aromatic H).

Cytotoxicity Testing P-388 cells were kindly supplied by Prof. J. M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago; HT-29 were purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously.[16]

Acknowledgment This work was supported by grants from the National Science Council and Ministry of Education (96C030311) of Taiwan awarded to C.-Y.D.

References and Notes

1) Krishnaraju A. V., Rao T. V., Vanisree M., Subbaraju G. V., *Asian J. Chem.*, **16**, 1311—1314 (2004).
2) Vanisree M., Subbaraju G. V., *Asian J. Chem.*, **14**, 957—960 (2002).
3) Radhika P., Rao P. R., Archana J., Rao N. K., *Biol. Pharm. Bull.*, **28**, 1311—1313 (2005).
4) Li G. Q., Zhang Y. L., Deng Z. W., van Ofwegen L., Proksch P., Lin W. H., *J. Nat. Prod.*, **68**, 649—652 (2005).
5) Chai M. C., Wang S. K., Dai C. F., Duh C. Y., *J. Nat. Prod.*, **63**, 843—844 (2000).
6) Duh C. Y., Wang S. K., Huang B. T., Dai C. F., *J. Nat. Prod.*, **63**, 884—885 (2000).
7) Rashid M. A., Gustafson K. R., Boyd M. R., *J. Nat. Prod.*, **63**, 531—533 (2000).
8) Morris L. A., Christie E. M., Jaspars M., van Ofwegen L. P., *J. Nat. Prod.*, **61**, 538—541 (1998).
9) Edrada R. A., Proksch P., Wray V., Witte L., van Ofwegen L., *J. Nat. Prod.*, **61**, 358—361 (1998).
10) Yamada K., Ryu K., Miyamoto T., Higuchi R., *J. Nat. Prod.*, **60**, 798—801 (1997).
11) Geran R. I., Greenberg N. H., MacDonald M. M., Schumacher A. M., Abbott B. J., *Cancer Chemother. Rep.*, **3**, 1—91 (1972).
12) Hou R. S., Duh C. Y., Chiang M. Y., Lin C. N., *J. Nat. Prod.*, **58**, 1126—1130 (1995).
13) Wang S. K., Duh C. Y., Wu Y. C., Wang Y., Cheng M. C., Soong K., Fang L. S., *J. Nat. Prod.*, **55**, 1430—1435 (1992).
14) Tursch B., Braekman J. C., Daloze D., Dedeurwaerder H., Karlsson R., *Bull. Soc. Chim. Belg.*, **87**, 75—81 (1978).
15) Dale J. A., Dull D. L., Mosher H. S., *J. Org. Chem.*, **34**, 2543 (1969).
16) Duh C. Y., Chia M. C., Wang S. K., Chen H.-J., El-Gamal A. A. H., Dai C. F., *J. Nat. Prod.*, **64**, 1028—1031 (2001).