

Article

New Cytotoxic Cembranolides from the Soft Coral *Lobophytum michaelae*

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Abstract: Six new cembranolides, michaelolides L–Q (1–6), and a known cembranolide, lobomichaelolide (7) were isolated from the CH₂Cl₂ extract of the soft coral *Lobophytum michaelae*. Their structures were established by extensive spectral analysis. The anti-HCMV (human cytomegalovirus) activity of 1–7 and their cytotoxicity against selected cell lines were evaluated.

Keywords: *Lobophytum michaelae*; cembranolides; cytotoxicity

1. Introduction

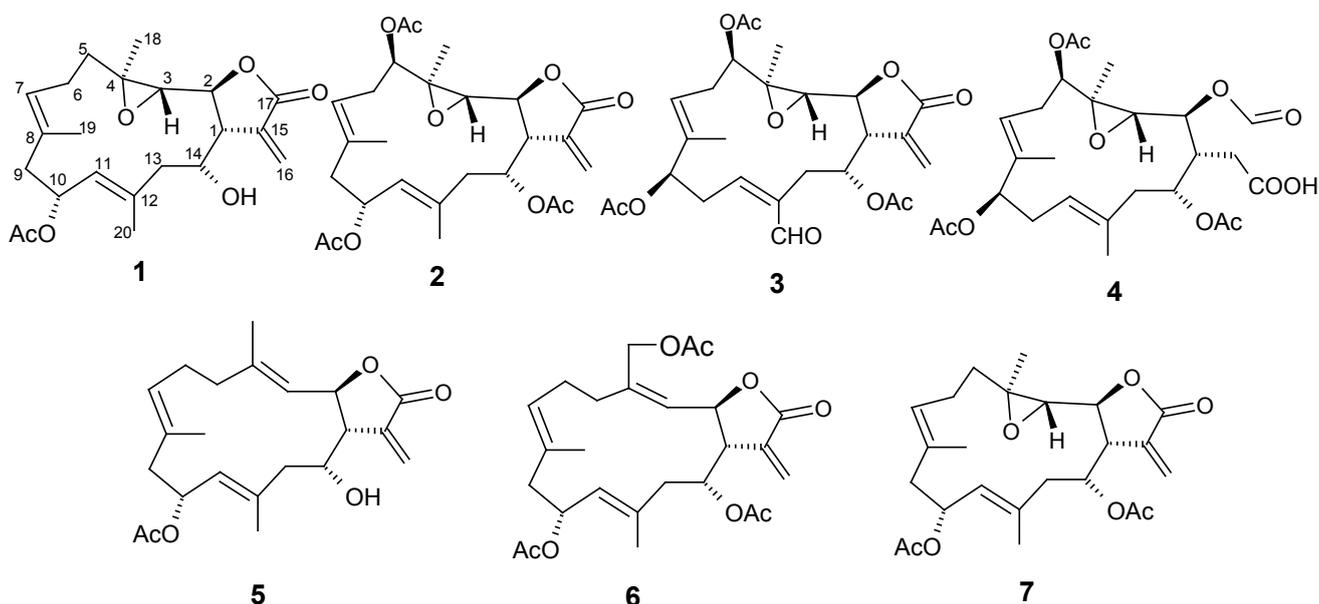
Soft corals of the genus *Lobophytum* (Alcyoniidae) have been reported as a rich source of secondary metabolites endowed with a range of structural diversity and various biological activities [1–24]. Previous bioassay results of some cembranoids and their analogues have demonstrated remarkable pharmacological potential such as cytotoxicity against various cancer cell lines [2–9], anti-inflammatory properties [11–13], antimicrobial activities [11], and HIV-inhibitory activity [14]. In previous papers [2–4], we reported the isolation of several cytotoxic cembranolides, lobomichaelolide, crassolide, and michaelolides A–K from samples of the soft coral *Lobophytum michaelae* Tixier-Durivault (Alcyoniidae) (Figure 1). In this report, a new specimen of the soft coral *L. michaelae* was studied since the CH₂Cl₂ solubles exhibited significant cytotoxicity against HT-29

(human colon adenocarcinoma) and P-388 (mouse lymphocytic leukemia) cell lines as determined by standard procedures. [25,26] Bioassay-guided fractionation of the extract resulted in the isolation of six new cembranolides, michaolides L–Q (1–6), together with the known cembranolide, lobomichaolide (7) [2,3] (Figure 2).

Figure 1. Soft coral *Lobophytum michaelae*.



Figure 2. Structures of compounds 1–7.



2. Results and Discussion

Michaolide L (1) was isolated as a colorless oil, $[\alpha]_D^{25} +13.3$ (c 0.1, CHCl_3). HRESIMS, ^{13}C NMR, and DEPT spectroscopic data established the molecular formula of 1 as $\text{C}_{22}\text{H}_{30}\text{O}_6$. The IR spectrum of 1 indicated the presence of the functionalities of ester group(s) (ν_{max} 1734 cm^{-1}) and an α -methylene- γ -lactone (ν_{max} $1766, 1668\text{ cm}^{-1}$). The presence of the α -methylene- γ -lactone system in 1 was also demonstrated by UV absorption at 222 ($\log \epsilon$ 3.68) nm and signals at δ 5.67 (H-16) and 6.44 (H-16) in the ^1H NMR spectrum (Table 1). The ^1H NMR spectrum of 1 also showed signals for two

olefinic protons at δ 5.65 (H-11), and 5.19 (H-7) ppm; four oxymethine protons at δ 4.46 (t, $J = 6.8$ Hz, H-2), 2.70 (d, $J = 6.8$ Hz, H-3), 5.64 (m, H-10), and 4.38 (m, H-14); one methine proton at δ 2.90 (m, H-1), two olefinic methyl groups at δ 1.55 (H₃-19) and 1.72 (H₃-20); and a methyl group in acetate ester at δ 2.05. HMBC spectrum exhibited a methyl-bearing trisubstituted epoxide [δ_{H} 2.70 (d, $J = 6.8$ Hz, H-3), 1.42 (H₃-18); δ_{C} 59.6 (CH), 64.0 (qC), 20.4 (CH₃)] (Table 1). The spectral data of **1** indicated some similarities to those of lobomichaolide (**7**) [2,3], except for the data due to C-14. The H¹-H¹ COSY spectrum exhibited correlations from H-13 to H-3, H-5 to H-7, and H-9 to H-11. ¹H-¹H long-range correlations were also observed between H-1 to H₂-16, H-7 to H₃-19, and H-11 to H₃-20. 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 an HSQC experiment, the planar structure was determined by HMBC analysis. The correlations according to HMBC are shown in Figure 3. 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} 15.6 and 15.9 (for C-19 and C-20, respectively) also supported the *E* configurations [2,3]. The NOESY correlations (Figure 4) observed between H-3 and H-1/H-11/H₃-19, H-14 and H-1/H₃-20, H-7 and H-9/H-11, H-10 and H₃-20/H₃-19, and H₃-18 and H-2 indicated the relative configurations for the 14-membered ring carbons, which were identical to those of lobomichaolide (**7**). Analysis of the $\Delta\delta_{S-R}$ values (Figure 5) according to the Mosher model pointed to an *R* configuration for C-14 of **1**, because H₂-13, H-11, and Me-20 of (*S*)-MTPA ester **1a** were less shielded by the phenyl ring of MTPA products. Therefore, the absolute stereochemistry of Michaelide L (**1**) was established as (1*R*,2*S*,3*S*,4*R*,10*S*,14*R*,7*E*,11*E*)-10-acetoxy-14-hydroxy-3,4-epoxycembra-7,11-dien-17,2-olide ambiguously.

Figure 3. COSY and HMBC correlations of compounds **1** and **2**.

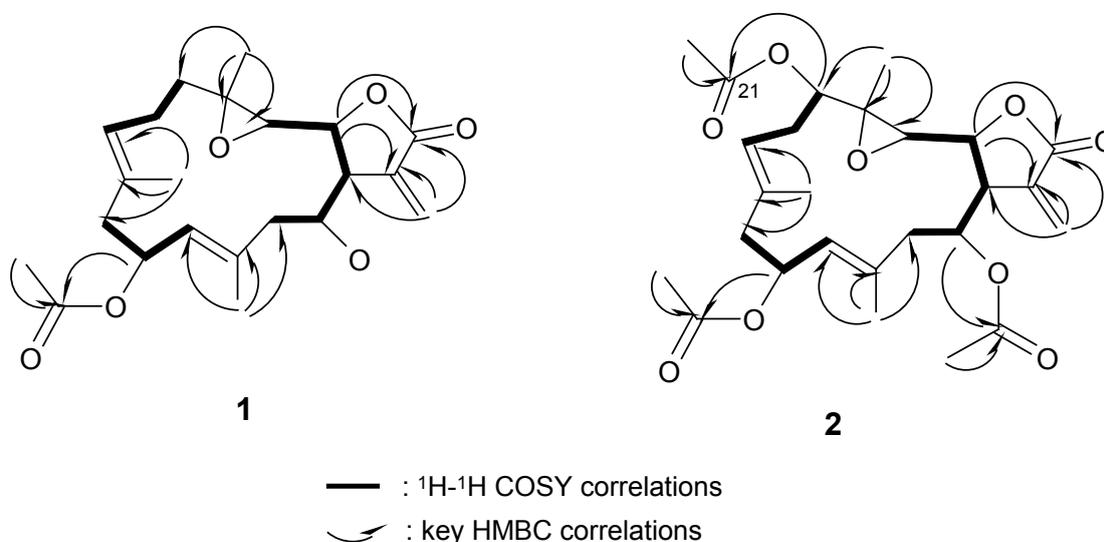
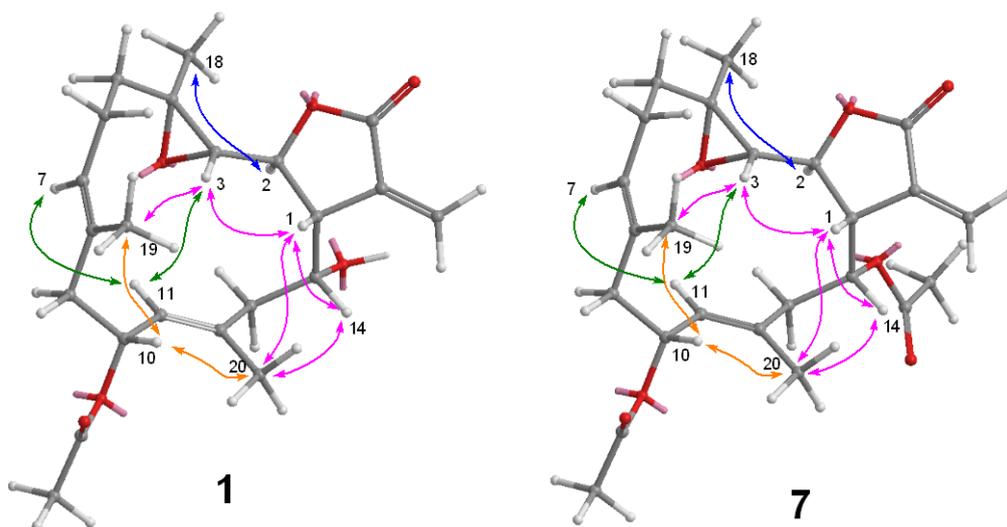
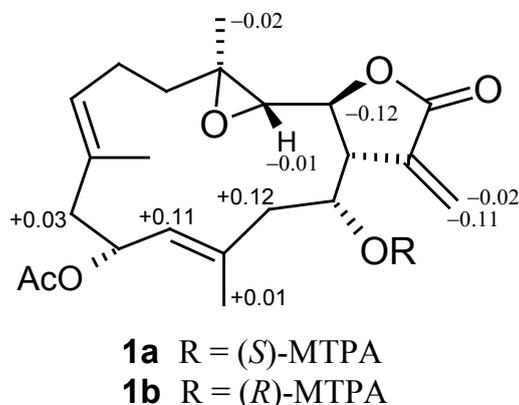


Table 1. ^1H and ^{13}C NMR data for compounds 1–3.

Position	1		2		3	
	δ_{H} (<i>J</i> in Hz) ^a	δ_{C} ^b	δ_{H} (<i>J</i> in Hz) ^a	δ_{C} ^b	δ_{H} (<i>J</i> in Hz) ^a	δ_{C} ^b
1	2.90 m	48.1	3.07 m	44.1	2.97 m	45.2
2	4.46 t (6.8) ^c	76.7	4.68 t (6.8)	75.2	4.52 t (6.8)	75.3
3	2.70 d (6.8)	59.6	2.83 d (6.8)	60.3	2.76 d (6.8)	59.9
4		64.0		62.7		63.4
5	1.91 m	23.8	4.83 dd (10.8, 3.2)	75.5	5.05 dd (11.2, 3.6)	74.3
6	2.03 m, 2.18 m	33.5	2.21 m, 2.42 m	30.3	2.33 m, 2.54 m	29.1
7	5.19 br d (8.0)	129.5	5.10 t (5.6)	122.5	5.46 t (6.4)	121.7
8		127.5		132.3		131.3
9	2.18 m, 2.45 m	44.7	2.38 m	44.5	5.25 br s	75.3
10	5.64 m	67.8	5.67 m	68.4	2.82 m, 3.27 m	29.4
11	5.65 m	127.8	5.45 m	128.1	6.73 dd (9.6, 4.4)	147.0
12		137.5		135.8		137.8
13	2.48 m, 2.91 m	44.2	2.37 m, 2.51 m	41.8	2.28 m, 2.89 m	34.7
14	4.38 m	68.6	5.40 m	71.1	5.77 m	69.3
15		136.7		134.9		136.1
16	5.67 m, 6.44 d (2.8)	122.3	5.72 s, 6.40 s	124.0	5.75 d (2.8), 6.39 d (2.8)	124.7
17		169.4		168.9		168.4
18	1.42 s	20.4	1.25 s	14.7	1.49 s	15.7
19	1.55 s	15.6	1.66 s	16.5	1.67 s	12.9
20	1.72 s	15.9	1.83 s	16.0	10.14 s	190.3
5-OAc			2.01 s	170.1	2.13 s	170.1
				21.1		21.2
9-OAc					2.13 s	169.8
						20.8
10-OAc	2.05 s	170.5	2.05 s	170.2		
		21.4		21.2		
14-OAc			2.11 s	170.3	2.02 s	169.8
				21.3		20.5

^a 400 MHz in CDCl_3 (assigned by COSY, HSQC, and HMBC experiments); ^b 100 MHz in CDCl_3 (assigned by DEPT, COSY, HSQC, and HMBC experiments); ^c *J* values (Hz) in parentheses.

Figure 4. NOESY correlations of compounds **1** and **7**.**Figure 5.** Absolute stereochemistry of **1**: $\Delta\delta_{S-R}$ values in ppm for MTPA esters **1a** and **1b**.

Michaelide M (**2**) was shown to have the molecular formula of $C_{24}H_{34}O_9$ by HRESIMS and from its ^{13}C NMR data. The 1H and ^{13}C NMR spectral data (Table 1) of **2** closely resembled those of **7** except for the signals at C-5. 1H - 1H COSY cross peak (Figure 3) between H-5 and H-6/H-7 as well as HMBC correlations (Figure 3) between H-5 and C-6/C-4/C-21 revealed the presence of an additional acetoxy [δ_H 4.83 (dd, $J = 10.8, 3.2$, H-5), δ_C 75.5 (CH, C-5), 170.1 (qC), 21.2 (CH₃)] at C-5 in **2**. NOESY correlations (Figure 6) between H-5 and H-7, H-3 and H-1/ H-11/H₃-19, H-14 and H-1/H₃-20, H-7 and H-9/H-11, H-10 and H₃-20/H₃-19, and H₃-18 and H-2 indicated the relative configurations for **2** resembled those of **7** except for the additional C-5 (*R*) acetoxy.

Michaelide N (**3**) analyzed for $C_{26}H_{32}O_{10}$ from its HRESIMS and NMR spectroscopic data. The NMR features of compound **3** were analogous to those of **2** with exception that the secondary acetoxy attached to C-10 was shifted to C-9 and the methyl attached to C-12 was replaced by an aldehyde [δ_H 10.14, δ_C 190.3] (Table 1). 1H - 1H COSY cross peaks (Figure 6) between H-9 and H-10/H-11 as well as HMBC correlations (Figure 7) between H-20 and C-11/C-12/C-13 as well as between H₃-19 and C-7/C-8/C-9 helped to ascertain these assignments. The relative stereochemistry of **3** was

determined by NOESY correlations (Figure 6) between H-7 and H-5/H-9/H-11, H-3 and H-1/H-11, H-14 and H-1/H-20, H-10 and H-20, H₃-18 and H-2, and H-11 and H₂-13.

Figure 6. NOESY correlations of compounds **2** and **3**.

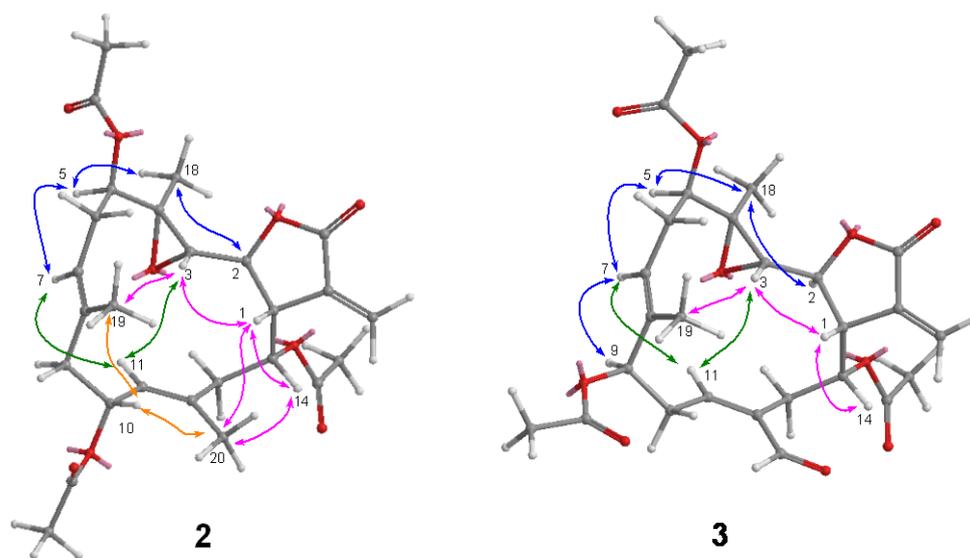
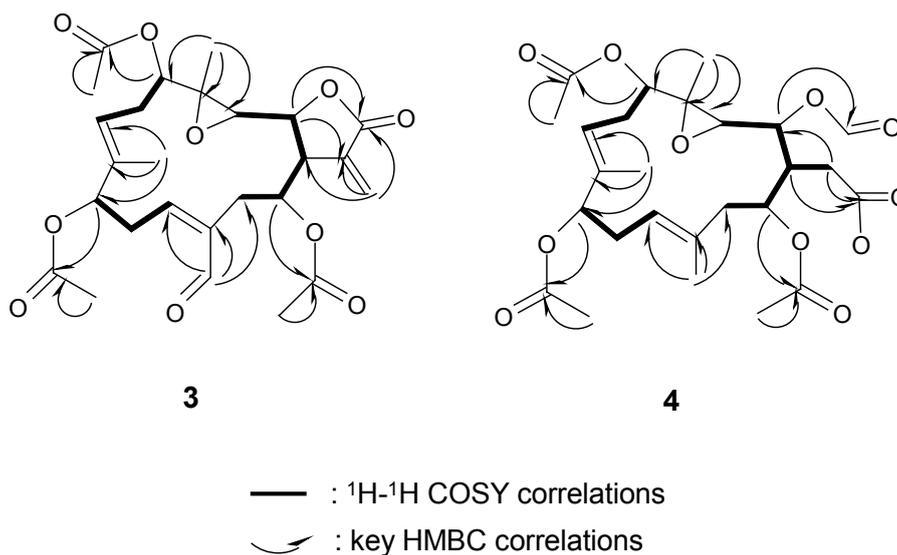


Figure 7. COSY and HMBC correlations of compounds **3** and **4**.



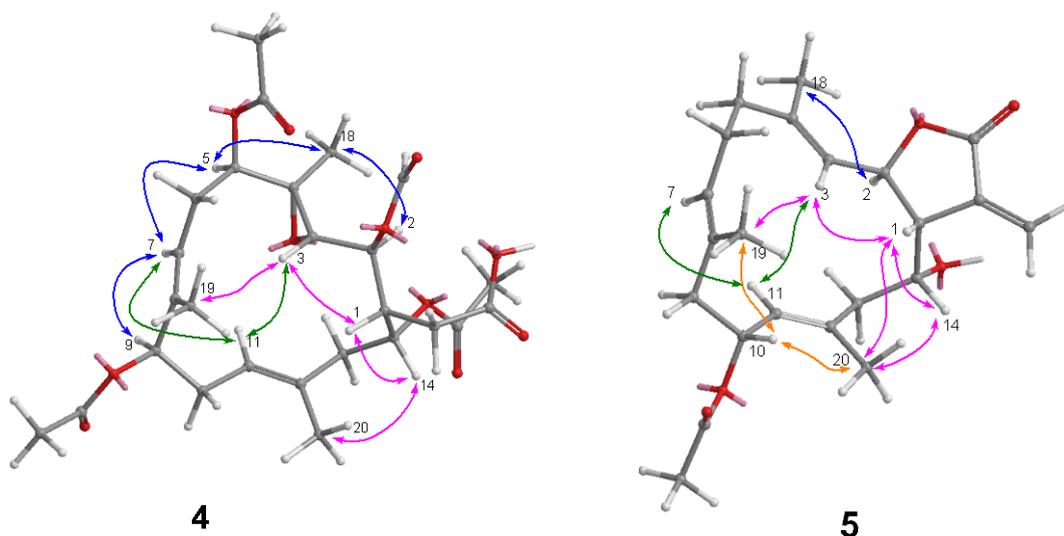
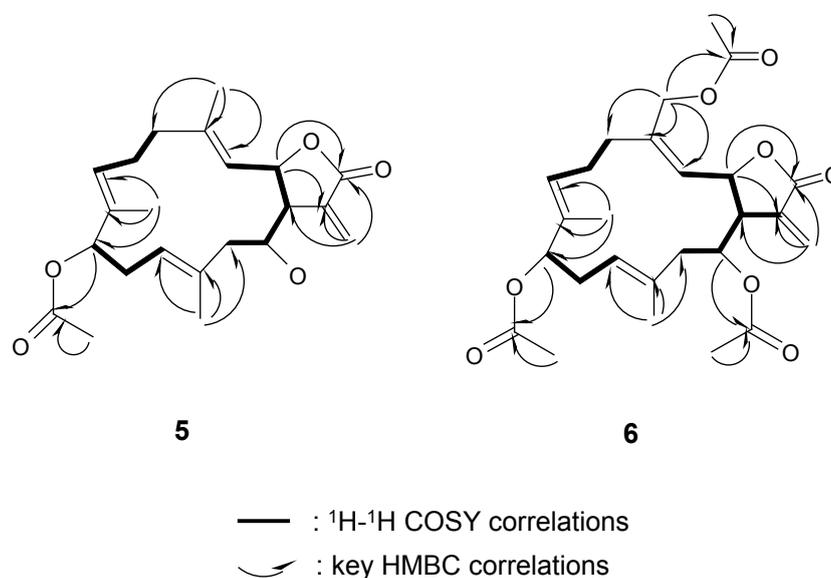
Michaelide O (**4**) had the molecular formula, C₂₆H₃₆O₁₁. Detailed comparison of the ¹H and ¹³C NMR spectral data (Table 2) of **4** and **3** revealed that **4** differed from **3** at C-20 and the α -exo-methylene- γ -lactone moiety. A COSY correlation (Figure 7) from H-1 to H₂-15 and HMBC correlations (Figure 7) from H₂-15 to C-16/C-2 and from H-2 to C-17 revealed that the α -exo-methylene- γ -lactone moiety in **3** was oxidized to a formylxyl (δ_{H} 8.27 s, δ_{C} 161.6) at C-2 and carboxymethyl at C-1 in **4**. The relative stereochemistry of **4** was determined by NOESY correlations (Figure 8) between H-7 and H-5/H-9/H-11, H-3 and H-1/H-11/H₃-19, H-14 and H-1/H₃-20, H₃-18 and H-2, and H-11 and H₂-13.

Table 2. ^1H and ^{13}C NMR data for compounds 4–6.

Position	4		5		6	
	δ_{H} (J in Hz) ^a	δ_{C} ^b	δ_{H} (J in Hz) ^a	δ_{C} ^b	δ_{H} (J in Hz) ^a	δ_{C} ^b
1	2.61 m	43.2	2.68 br s	48.0	2.79 m	46.1
2	4.52 t (6.8) ^c	76.4	5.38 dd (8.8, 2.7)	73.5	5.53 dd (8.7, 6.8)	72.5
3	2.82 d (6.8)	60.2	5.03 d (8.8)	123.3	5.18 m	127.2
4		63.4		140.0		140.6
5	4.89 dd (10.8, 3.6)	75.1	2.30 m	24.1	2.33 m	24.1
6	2.82 m, 2.55 m	29.8	2.21 m	37.9	2.36m, 2.40 m	33.1
7	5.34 m	120.1	4.96 m	129.7	4.93 m	128.8
8		133.5		130.6		131.1
9	5.16 m	76.6	2.32 m, 2.39 m	44.5	2.29 m, 2.40 m	44.5
10	2.37 m, 2.48 m	41.8	5.65 m	68.5	5.66 m	68.0
11	5.30 m	123.4	5.16 d (9.1)	126.6	5.19 m	127.7
12		131.7		134.5		136.8
13	2.31 m, 2.68 m	43.8	2.21 m, 2.43 m	45.5	2.28 m, 2.53 m	41.8
14	5.32 m	69.2	4.15 m	72.9	5.28 m	74.0
15	3.75m, 3.85 m	35.2		136.8		136.6
16		175.6	5.65 s, 6.41 s	122.7	5.70 s, 6.37 s	124.4
17	8.26 s	161.6		167.8		167.3
18	1.48 s	15.7	1.80 s	17.3	4.55 d (12.6), 4.91 d (12.6)	62.3
19	1.60 s	12.3	1.64 s	16.7	1.61 s	16.6
20	1.74 s	15.6	1.75 s	16.3	1.81 s	16.0
5-OAc	2.11 s	170.1				
		21.1				
9-OAc	2.11 s	170.3				
		21.2				
10-OAc			2.04 s	170.0	2.01 s	170.2
				21.4		21.0
14-OAc	2.12 s	170.3			2.02 s	170.8
		21.3				21.3
18-OAc					2.03 s	169.3
						20.9
16-OH	6.18 brs					

^a 400 MHz in CDCl_3 (assigned by COSY, HSQC, and HMBC experiments); ^b 100 MHz in CDCl_3 (assigned by DEPT, COSY, HSQC, and HMBC experiments); ^c *J* values (Hz) in parentheses.

Michaelide P (**5**) was shown to have the molecular formula of $\text{C}_{22}\text{H}_{30}\text{O}_5$ by HRESIMS and from its ^{13}C NMR data. The ^1H and ^{13}C NMR spectral data (Table 2) of **5** closely resembled those of **1** except for the replacement of the trisubstituted epoxy by a trisubstituted olefin at Δ^3 . HMBC correlations (Figure 9) between H₃-18 and C-3/C-4/C-5 confirmed the presence of a trisubstituted olefin at C-3. The relative stereochemistry was determined by NOESY correlations (Figure 8) between H-3 and H-1/H-11/H₃-19, H-14 and H-1/H₃-20, H-7 and H-9/H-11, H-10 and H₃-20/H₃-19, and H₃-18 and H-2.

Figure 8. NOESY correlations of compounds **4** and **5**.**Figure 9.** COSY and HMBC correlations of compounds **5** and **6**.

Michaelide Q (**6**) had the molecular formula of $\text{C}_{26}\text{H}_{34}\text{O}_8$ by HRESIMS and from its ^{13}C NMR data. The ^1H and ^{13}C NMR spectroscopic data (Table 2) of **6** closely resembled those of **5** except for the signals at C-18 and C-14. The low field chemical shift and HMBC correlations (Figure 9) between H₂-18 and C-3/C-4/C-5/C-21 confirmed the presence of an acetoxy group at C-18. HMBC correlations (Figure 8) between H-14 and C-22 revealed the presence of a second acetoxy group at C-14. The relative stereochemistry was determined by NOESY correlations between H-3 and H-1/H-11/H₃-19, H-14 and H-1/H₃-20, H-7 and H-9/H-11, H-10 and H₃-20/H₃-19, and H₃-18 and H-2.

The cytotoxicity toward P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), A-549 (human lung epithelial carcinoma) tumor cells, and human embryonic lung (HEL) cells of michaelides L–Q (**1–6**) and lobomichaelide (**7**) were shown in Table 3. Non-cytotoxic cembranoid, michaelide O (**4**) was tested for anti-HCMV activity and showed a negative result ($\text{IC}_{50} > 200 \mu\text{M/mL}$). The α -exo-methylene- γ -lactone moiety is important for cytotoxicity by

comparing the cytotoxicity of **4** with those of **1–3**, **5**, and **6** [4]. The absolute stereochemistry of the known cembranolide, lobomichaolide (**7**) [2,3] should be drawn as in Figure 2 since cembranolides **1** and **7** both exhibited positive optical rotations.

Table 3. Cytotoxicity of **1–7**.

Compounds	Cell Lines ED ₅₀ (μM/mL)			
	A549	HT-29	P-388	HEL
1	1.2	0.8	0.3	1.0
2	2.0	4.9	1.5	3.2
3	2.1	1.6	0.4	2.0
4	61.3	61.5	39.6	60.2
5	3.2	2.8	2.0	2.9
6	2.0	1.5	1.0	1.8
7	1.9	1.4	0.4	1.7

3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were determined with a JASCO P1020 digital polarimeter. Ultraviolet (UV) and infrared (IR) spectra were obtained on JASCO V-650 and JASCO FT/IR-4100 spectrophotometers, respectively. NMR spectra were recorded on a Varian MR 400 NMR spectrometer at 400 MHz for ¹H and 100 MHz for ¹³C. ¹H NMR chemical shifts are expressed in δ (ppm) referred to the solvent peaks δ_H 7.27 for CDCl₃, and coupling constants are expressed in Hz. ¹³C NMR chemical shifts are expressed in δ (ppm) referred to the solvent peaks δ_C 77.0 for CDCl₃. ESI-MS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, Germany, 230–400 mesh) and LiChroprep RP-18 (Merck, 40–63 μm) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F₂₅₄, 0.25 mm) and precoated RP-18 F_{254s} plates (Merck) were used for thin-layer chromatography (TLC) analysis. High-performance liquid chromatography (HPLC) was carried out using a Hitachi L-7100 pump equipped with a Hitachi L-7400 UV detector at 220 nm together with a semi-preparative reversed-phase column (Merck, Hibar LiChrospher RP-18e, 5 μm, 250 × 25 mm).

3.2. Biological Material

The soft coral *L. michaelae* Tixier-Durivault (Alcyoniidae) was collected at Ken-Ting, Ping-Tong County, Taiwan, in June 2002, at a depth of 3–4 m and was stored for 2 weeks in a freezer until extraction. Identification was kindly verified by Prof. Keryea Soong, Institute of Marine Biology, National Sun Yat-sen, Taiwan. A voucher specimen, MR-004, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

3.3. Extraction and Isolation

The bodies of the soft coral *L. michaelae* were freeze dried to give 1.10 kg of a solid, which was extracted with CH₂Cl₂ (3.0 L × 3). After removal of solvent in vacuo, the residue (20 g) was chromatographed over Si gel 60 using *n*-hexane and *n*-hexane/EtOAc mixtures of increasing polarity. Elution with *n*-hexane/EtOAc (49:1) gave fractions containing **7**, with *n*-hexane/EtOAc (9:2) gave fractions containing **2** and **6**, with *n*-hexane/EtOAc (5:2) gave fractions containing **3**, with *n*-hexane/EtOAc (3:2) gave fractions containing **4**, with *n*-hexane/EtOAc (1:1) gave fractions containing **1** and **5**. Compounds **1–7** were further purified by RP-18 HPLC eluting with MeOH/H₂O (50:50), MeOH/H₂O (76:24), MeOH/H₂O (70:30), MeOH/H₂O (66:34), MeOH/H₂O (50:50), MeOH/H₂O (76:24), and MeOH/H₂O (78:22), respectively.

Michaelide L (**1**): White amorphous powder (5 mg); $[\alpha]_D^{25} +13.3$ (*c* 0.1, CHCl₃); UV λ_{\max} (MeOH) nm (log ϵ): 222 (3.68); IR (neat) ν_{\max} 3412, 1766, 1734, 1668 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 1; HRESIMS *m/z* 413.1916 [M + Na]⁺ (calcd. for C₂₂H₃₀O₆Na, 413.1914).

Preparation of Mosher's Esters of **1**. In separate NMR tubes, duplicate (1.0 mg) samples of **5** were dissolved in 0.6 mL of pyridine-*d*₅ and allowed to react for 3 h at room temperature with (*R*)- and (*S*)-MTPA chloride (one drop) to yield (*S*)-MTPA ester **1a** and (*R*)-MTPA ester **1b**, respectively. Selected ¹H NMR (pyridine-*d*₅, 300 MHz) of **1a**: ¹H NMR (CDCl₃, 300 MHz): δ 1.35 (3H, s, H₃-18), 1.51 (3H, s, H₃-19), 1.98 (3H, s, H₃-20), 2.08 (3H, s, 10-OAc), 2.32 (1H, dd, *J* = 12.1, 8.9 Hz, H-9), 2.56 (1H, d, *J* = 12.1 Hz, H-9), 2.86 (2H, m, H-13), 3.02 (1H, d, *J* = 6.1 Hz, H-3), 3.54 (3H, s, OMe), 4.57 (1H, t, *J* = 6.9 Hz, H-2), 5.22 (1H, br d, *J* = 8.2 Hz, H-7), 5.93 (1H, d, *J* = 8.4 Hz, H-11), 6.05 (1H, d, *J* = 2.8 Hz, H-16), 6.12 (1H, ddd, *J* = 10.8, 6.0, 1.5 Hz, H-14), 6.47 (1H, d, *J* = 3.2 Hz, H-16), 7.41–7.61 (5H, m, Ph). Selected ¹H-NMR (pyridine-*d*₅, 300 MHz) of **1b**: ¹H NMR (CDCl₃, 300 MHz): δ 1.37 (3H, s, H₃-18), 1.52 (3H, s, H₃-19), 1.97 (3H, s, H₃-20), 2.08 (3H, s, 10-OAc), 2.32 (1H, dd, *J* = 12.3, 9.4 Hz, H-9), 2.53 (1H, d, *J* = 12.3 Hz, H-9), 2.74 (2H, m, H-13), 3.03 (1H, d, *J* = 6.8 Hz, H-3), 3.53 (3H, s, OMe), 4.69 (1H, t, *J* = 6.6 Hz, H-2), 5.20 (1H, br d, *J* = 6.9 Hz, H-7), 5.82 (1H, d, *J* = 8.8 Hz, H-11), 6.14 (1H, d, *J* = 2.2 Hz, H-16), 6.58 (1H, dd, *J* = 11.4, 3.8 Hz, H-14), 6.58 (1H, d, *J* = 3.2 Hz, H-16), 7.42–7.62 (5H, m, Ph).

Michaelide M (**2**): White amorphous powder (3 mg); $[\alpha]_D^{25} +11.2$ (*c* 0.1, CHCl₃); UV λ_{\max} (MeOH) nm (log ϵ): 221 (3.67); IR (neat) ν_{\max} 1765, 1736, 1728, 1669 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 1; HRESIMS *m/z* 489.2102 [M + Na]⁺ (calcd. for C₂₄H₃₄O₉Na, 489.2101).

Michaelide N (**3**): White amorphous powder (1 mg); $[\alpha]_D^{25} +7.6$ (*c* 0.1, CHCl₃); UV λ_{\max} (MeOH) nm (log ϵ): 220 (3.76); IR (neat) ν_{\max} 2820, 2730, 1765, 1736, 1726, 1669 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 1; HRESIMS *m/z* 527.1885 [M + Na]⁺ (calcd. for C₂₆H₃₂O₁₀Na, 527.1884).

Michaelide O (**4**): White amorphous powder (2 mg); $[\alpha]_D^{25} +3.1$ (*c* 0.1, CHCl₃); IR (neat) ν_{\max} 3420, 1740, 1731, 1712, 1675 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 2; HRESIMS *m/z* 547.2155 [M + Na]⁺ (calcd. for C₂₆H₃₆O₁₁Na, 547.2156).

Michaolide P (**5**): White amorphous powder (1 mg); $[\alpha]_D^{25} +122.0$ (*c* 0.1, CHCl₃); UV λ_{\max} (MeOH) nm (log ϵ): 221 (3.96); IR (neat) ν_{\max} 3450, 1765, 1735, 1666 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 2; HRESIMS *m/z* 397.1993 [M + Na]⁺ (calcd. for C₂₂H₃₀O₅Na, 397.1992).

Michaolide Q (**6**): White amorphous powder (1 mg); $[\alpha]_D^{25} +81.6$ (*c* 0.1, CHCl₃); IR (neat) ν_{\max} 1762, 1731, 1675 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) data in Table 2; HRESIMS *m/z* 497.2154 [M + Na]⁺ (calcd. for C₂₆H₃₄O₈Na, 497.2152).

Lobomichaolide (**7**): Colorless prism (25 mg); m.p. 180–181; $[\alpha]_D^{25} +55.6$ (*c* 0.1, CHCl₃).

3.4. Cytotoxicity Assay

Cytotoxicity was determined on P-388 (mouse lymphocytic leukemia), HT-29 (human colon adenocarcinoma), and A-549 (human lung epithelial carcinoma) tumor cells using a modification of the MTT colorimetric method according to a previously described procedure [25,26]. The provision of the P-388 cell line was supported by J.M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago. HT-29 and A-549 cell lines were purchased from the American Type Culture Collection.

3.5. Anti-HCMV Assay

To determine the effects of natural products upon HCMV cytopathic effect (CPE), confluent human embryonic lung (HEL) cells grown in 24-well plates were incubated for 1 h in the presence or absence of various concentrations of tested natural products. Then, cells were infected with HCMV at an input of 1000 pfu (plaque forming units) per well of 24-well dish. Antiviral activity was expressed as IC₅₀ (50% inhibitory concentration), or compound concentration required to reduce virus induced CPE by 50% after 7 days as compared with the untreated control. To monitor the cell growth upon treating with natural products, an MTT-colorimetric assay was employed [27].

4. Conclusion

The α -exo-methylene- γ -lactone moiety is important for cytotoxicity by comparing the cytotoxicity of **4** with those of **1–3**, **5**, and **6** [4]. The absolute stereochemistry of the known cembranolide, lobomichaolide (**7**) [2,3] should be drawn as in Figure 2 since cembranolides **1** and **7** both exhibited positive optical rotations.

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References

1. Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Prinsep, M.R. Marine natural products. *Nat. Prod. Rep.* **2010**, *27*, 165–237.
2. Wang, S.-K.; Duh, C.-Y.; Wu, Y.-C.; Wang, Y.; Cheng, M.-C.; Soong, K.; Fang, L.-S. Cytotoxic cembranolidides from the soft coral *Lobophytum michaelae*. *J. Nat. Prod.* **1992**, *55*, 1430–1435.
3. Wang, S.-K.; Duh, C.-Y.; Wu, Y.-C.; Wang, Y.; Cheng, M.-C.; Soong, K.; Fang, L.-S. Cytotoxic cembranolidides from the soft coral *Lobophytum michaelae*. *J. Nat. Prod.* **2009**, *72*, 324.
4. Wang, L.-T.; Wang, S.-K.; Soong, K.; Duh, C.-Y. New Cytotoxic Cembranolidides from the Soft Coral *Lobophytum michaelae*. *Chem. Pharm. Bull.* **2007**, *55*, 766–770.
5. Coval, S.J.; Patton, R.W.; Petrin, J.M.; James, L.; Rothofsky, M.L.; Lin, S.L.; Patel, M.; Reed, J.K.; McPhil, A.T.; Bishop, W.R. A Cembranolide Diterpene Farnesyl Protein Transferase Inhibitor from the Marine Soft Coral *Lobophytum cristagalli*. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 909–912.
6. Higuchi, R.; Miyamoto, T.; Yamada, K.; Komori, T. Cytotoxic and ichthyotoxic compounds from marine opisthobranchia and soft coral. *Toxicon* **1998**, *36*, 1703–1705.
7. Matthee, G.F.; Konig, G.M.; Wright, A.D. Three new diterpenes from the marine soft coral *Lobophytum crassum*. *J. Nat. Prod.* **1998**, *61*, 237–240.
8. Duh, C.-Y.; Wang, S.-K.; Huang, B.-T.; Dai, C.-F. Cytotoxic cembrenolide diterpenes from the Formosan soft coral *Lobophytum crassum*. *J. Nat. Prod.* **2000**, *63*, 884–885.
9. Chao, C.-H.; Hang, H.-C.; Wu, Y.-C.; Lu, C.-K.; Dai, C.-F.; Sheu, J.-H. Glycolipids from the Formosan Soft Coral *Lobophytum crassum*. *Chem. Pharm. Bull.* **2007**, *55*, 1720–1723.
10. Zhang, W.; Krohn, K.; Ding, J.; Miao, Z.-H.; Zhou, X.-H.; Chen, S.-H.; Pescitelli, G.; Salvadori, P.; Kurtan, T.; Guo, Y.-W. Structural and stereochemical studies of α -methylene- γ -lactone-bearing cembrane diterpenoids from a south China sea soft coral *Lobophytum crassum*. *J. Nat. Prod.* **2008**, *71*, 961–966.
11. Lin, S.-T.; Wang, S.-K.; Cheng, S.-Y.; Duh, C.-Y. Lobocrasol, a new diterpenoid from the soft coral *Lobophytum crassum*. *Org. Lett.* **2009**, *14*, 3012–3014.
12. Cheng, S.-Y.; Wen, Z.-H.; Chiou, S.-F.; Wang, S.-K.; Hsu, C.-H.; Dai, C.-F.; Chiang, M.Y.; Duh, C.-Y. Durumolidides A–E, Anti-inflammatory and antibacterial cembranolidides from the soft coral *Lobophytum durum*. *Tetrahedron* **2008**, *64*, 9698–9704.
13. Cheng, S.-Y.; Wen, Z.-H.; Wang, S.-K.; Chiou, S.-F.; Hsu, C.-H.; Dai, C.-F.; Duh, C.-Y. Anti-inflammatory cembranolidides from the soft coral *Lobophytum durum*. *Bioorg. Med. Chem.* **2009**, *17*, 3763–3769.
14. Cheng, S.-Y.; Wen, Z.-H.; Chiou, S.-F.; Wang, S.-K.; Hsu, C.-H.; Dai, C.-F.; Chiang, M.Y.; Duh, C.-Y. Unprecedented hemiketal cembranolidides with anti-inflammatory activity from the soft coral *Lobophytum durum*. *J. Nat. Prod.* **2009**, *72*, 152–155.
15. Rashid, M.A.; Gustafson, K.R.; Boyd, M.R. HIV-inhibitory cembrane derivatives from a Philippines collection of the soft coral *Lobophytum* species. *J. Nat. Prod.* **2000**, *63*, 531–533.
16. Kashman, Y.; Groweiss, A. Lobolide: A new epoxy cembranolide from marine origin. *Tetrahedron Lett.* **1977**, *13*, 1159–1160.

17. Kashman, Y.; Carmely, S.; Groweiss, A. Further cembranoid derivatives from the Red Sea soft corals *Alcyonium flaccidum* and *Lobophytum crassum*. *J. Org. Chem.* **1981**, *46*, 3592–3596.
18. Kinamoni, Z.; Groweiss, A.; Carmely, S.; Kashman, Y. Several new cembranoid diterpenes from three soft corals of the Red Sea. *Tetrahedron* **1983**, *39*, 1643–1648.
19. Bowden, B.F.; Coll, J.C.; Tapiolas, D.M. Studies of Australian Soft Corals. XXXIII. New cembranoid diterpenes from a *Lobophytum* species. *Aust. J. Chem.* **1983**, *36*, 2289–2295.
20. Bowden, B.F.; Coll, J.C.; Decosta, M.S.L.; Desilva, E.D.; Mackay, M.F.; Mahendran, M.; Willis, R.H. The structure determination of a new cembranolide diterpene from the soft coral *Lobophytum cristigalli* (Coelenterata, Octocorallia, Alcyonacea). *Aust. J. Chem.* **1984**, *34*, 545–552.
21. Bowdon, B.F.; Coll, J.C.; Heaton, A.; Konig, G.; Bruck, M.A.; Cramer, R.E.; Klein, D.M.; Scheuer, P.J. The structures of four isomeric dihydrofuran-containing cembranoid diterpenes from several species of soft coral. *J. Nat. Prod.* **1987**, *50*, 650–659.
22. Subrahmanyam, C.; Rao, C.V.; Anjaneyulu, V.; Satyanarayana, P.; Rao, P.V.S. New diterpenes from a new species of *Lobophytum* soft coral of the South Andaman coast. *Tetrahedron* **1992**, *48*, 3111–3120.
23. Yamada, K.; Ryu, K.; Miyamoto, T.; Higuchi, R. Bioactive terpenoids from octocorallia. 4. Three new cembrane-type diterpenoids from the soft coral *Lobophytum schoedei*. *J. Nat. Prod.* **1997**, *60*, 798–801.
24. Cheng, S.-Y.; Chen, P.-W.; Chen, H.-P.; Wang, S.-K.; Duh, C.-Y. New Cembranolides from the Dongsha Atoll Soft Coral *Lobophytum durum*. *Mar. Drugs* **2011**, *9*, 1307–1318.
25. Geran, R.I.; Greenberg, N.H.; MacDonald, M.M.; Schumacher, A.M.; Abbott, B.J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother. Rep.* **1972**, *3*, 1–91.
26. Hou, R.-S.; Duh, C.-Y.; Chiang, M.Y.; Lin, C.-N. Sinugibberol, a new cytotoxic cembranoid diterpene from the soft coral *Sinularia gibberosa*. *J. Nat. Prod.* **1995**, *58*, 1126–1130.
27. Stevens, M.; Balzarini, J.; Tabarrini, O.; Andrei, G.; Snoeck, R.; Cecchetti, V.; Fravolini, A.; De Clercq, E.; Pannecouque, C. Cell-dependent interference of a series of new 6-aminoquinolone derivatives with viral (HIV/CMV) transactivation. *J. Antimicrob. Chemother.* **2005**, *56*, 847–855.

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