Capnellenes from the Formosan Soft Coral *Capnella imbricata*

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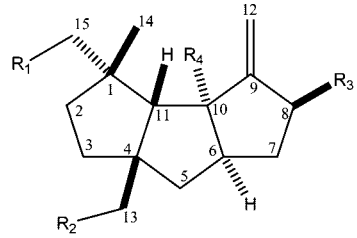
Received October 30, 2007

Three anti-inflammatory sesquiterpenes, $\Delta^{9(12)}$-capnellene-8β,10α-diol (**1**), 8α-acetoxy-$\Delta^{9(12)}$-capnellene-10α-ol (**2**), and $\Delta^{9(12)}$-capnellene-10α-ol-8-one (**3**), five new sesquiterpenes, $\Delta^{9(12)}$-capnellene-8β,15-diol (**4**), $\Delta^{9(12)}$-capnellene-8β,10α,13-triol (**5**), $\Delta^{9(10)}$-capnellene-12-ol-8-one (**6**), 8β,10α-diacetoxy-$\Delta^{9(12)}$-capnellene (**7**), and 8β-acetoxy-$\Delta^{9(12)}$-capnellene (**8**), and a known sesquiterpene, $\Delta^{9(12)}$-capnellene-8β-ol (**9**), were isolated from the acetone/methylene chloride extracts of the Formosan soft coral *Capnella imbricata*. The structures of these compounds were elucidated by extensive spectroscopic analysis.

Soft corals of the genus *Capnella* are rich sources of sesquiterpenes with the capnellane skeleton[1–6] and are reported to also contain precapnellane sesquiterpenes,[7] xenicane diterpenes,[8] and steroids.[8,9] As part of our search for bioactive substances from marine resources, three anti-inflammatory sesquiterpenes, $\Delta^{9(12)}$-capnellene-8β,10α-diol (**1**),[1] 8α-acetoxy-$\Delta^{9(12)}$-capnellene-10α-ol (**2**),[5] and $\Delta^{9(12)}$-capnellene-10α-ol-8-one (**3**),[3] five new sesquiterpenes, $\Delta^{9(12)}$-capnellene-8β,15-diol (**4**), $\Delta^{9(12)}$-capnellene-8β,10α,13-triol (**5**), $\Delta^{9(10)}$-capnellene-12-ol-8-one (**6**), 8β,10α-diacetoxy-$\Delta^{9(12)}$-capnellene (**7**), and 8β-acetoxy-$\Delta^{9(12)}$-capnellene (**8**), and a known sesquiterpene, $\Delta^{9(12)}$-capnellene-8β-ol (**9**),[6] were isolated from the Formosan soft coral *Capnella imbricata* (Quoy and Gaimard, 1983) (family Nephtheidae).



1 R_1 = H, R_2 = H, R_3 = OH, R_4 = OH
2 R_1 = H, R_2 = H, R_3 = OAc, R_4 = OH
3 R_1 = H, R_2 = H, R_3 = O, R_4 = OH
4 R_1 = OH, R_2 = H, R_3 = OH, R_4 = H
5 R_1 = H, R_2 = OH, R_3 = OH, R_4 = OH
7 R_1 = H, R_2 = H, R_3 = OAc, R_4 = OAc
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9 R_1 = H, R_2 = H, R_3 = OH, R_4 = H

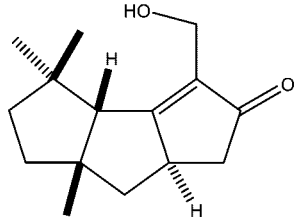

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Table 1. ^{13}C NMR Data for Compounds **4–8**

	4[a]	**5**[a]	**6**[b]	**7**[a]	**8**[a]
1	47.2	44.5	44.7	44.3	43.9
2	36.2	43.9	42.3	43.6	41.7
3	39.8	37.5	40.4	41.7	40.9
4	53.6	55.5	55.2	50.2	53.6
5	48.8	41.5	46.2	45.3	48.4
6	41.6	49.5	44.1	48.1	42.6
7	39.7	38.2	42.2	35.6	36.3
8	75.5	74.2	211.3	75.4	76.2
9	160.2	164.3	135.5	151.5	156.3
10	49.1	90.6	186.7	95.7	49.5
11	65.2	62.5	61.9	65.3	67.7
12	105.9	111.8	56.8	116.4	108.6
13	31.6	71.4	30.1	31.9	31.6
14	25.7	30.7	31.1	31.4	30.2
15	69.9	23.8	26.0	24.4	25.5
OAc-8				170.9 21.3	171.9 21.4
OAc-10				169.6 22.0	

[a] Recorded at 75 MHz in $CDCl_3$. [b] Recorded at 125 MHz in $CDCl_3$. The values are in ppm downfield from TMS and assignments were made by DEPT, COSY, HMQC, and HMBC experiments.

Results and Discussion

The bodies of the soft coral *C. imbricata*, collected at Green Island off Taiwan, were freeze-dried and then extracted with CH_2Cl_2/acetone. After removal of solvent *in vacuo*, the residue was chromatographed over a column containing silica gel 60 using *n*-hexane/EtOAc and EtOAc/MeOH mixtures as eluting solvents. Compounds **1–9** were further purified by RP-18 HPLC. A combination of DEPT and ^{13}C NMR spectra of **1–3** and **9** revealed that these compounds were similar to capnellane alcohols. The spectroscopic data obtained were consistent with a tricyclic skeleton which contained a *gem* dimethyl, a tertiary methyl, and an exocyclic methylene group. Through comparison of the spectroscopic and physical data with previously reported capnellenes, compounds **1–3** and **9** were identified as $\Delta^{9(12)}$-capnellene-8β,10α-diol,[1] 8α-acetoxy-$\Delta^{9(12)}$-capnellene-10α-ol,[5] $\Delta^{9(12)}$-capnellene-10α-ol-8-one,[3] and $\Delta^{9(12)}$-capnellene-8β-ol,[6] respectively.

$\Delta^{9(12)}$-Capnellene-8β,15-diol (**4**) was assigned a molecular formula of $C_{15}H_{24}O_2$ by HRESIMS, ^{13}C NMR, and DEPT, indicating the presence of four degrees of unsaturation. The carbon resonances (Table 1) at δ_C 160.2 (qC) and 105.9 (CH_2) in the ^{13}C NMR and DEPT spectra indicatd the presence of an exo-methylene group. In addition, the presence of two oxygenated carbons was inferred from the carbon signals at δ_C 69.9 (CH_2) and 75.5 (CH). Four methylene groups were deduced from DEPT signals at δ_C 36.2, 39.7, 39.8, and 48.8, three methine signals at δ_C 41.6, 49.1,

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10.1021/np0706116 CCC: $40.75
Published on Web 02/27/2008

Table 2. ^{1}H NMR Data for Compounds **4**–**8**

	4[a]	**5**[a]	**6**[b]	**7**[a]	**8**[a]
2	1.41 m 1.58 m	1.46 m	1.56 m 1.66 m	1.45 m	1.44 m
3	1.42 m	1.69 m	1.78 m	1.54 m 1.64 m	1.53 m
5	1.49 m	1.60 m	1.03 dd (4.5, 12.3)	1.26 dd (9.5, 13.8)	1.45 m
	1.80 dd (8.1, 12.8)[c]	1.88 dd (10.1, 14.2)	2.09 dd (8.0, 12.3)	1.83 dd (9.5, 13.8)	1.77 m
6	2.43 m	2.67 m	3.02 m	2.77 m	2.48 m
7	1.39 m	1.59 m	2.05 dd (2.0, 18.4)	1.47 m	1.54 m
	2.25 m	2.28 m	2.64 dd (6.5, 18.4)	2.51 m	2.25 m
8	4.51 t(5.2)	4.78 m		5.72 m	5.53 t (3.4)
10	2.86 m				2.71 m
11	1.89 d (3.2)	2.02 s	2.47 s	2.34 s	1.78 m
12	5.00 s	5.41 s	4.32 dd (1.7, 13.4)	5.45 d (2.2)	4.99 s
	5.14 s	5.43 s	4.37 dd (1.7, 13.4)	5.51 d (2.2)	5.08 s
13	1.23 s	3.35 m	1.24 s	1.09 s	1.19 s
14	1.15 s	1.21 s	1.21 s	1.11 s	1.06 s
15	3.48 d (10.8) 3.58 d (10.8)	1.29 s	0.87 s	1.12 s	0.98 s
	3.58 d (10.8)				
OAc-8				2.07 s	2.09 s
OAc-10				1.95 s	

[a] Recorded at 300 MHz in $CDCl_3$. [b] Recorded at 500 MHz in $CDCl_3$. The values are ppm downfield from TMS, and assignments were made by COSY, HMQC, and HMBC experiments. [c] *J* values (in Hz) in parentheses.

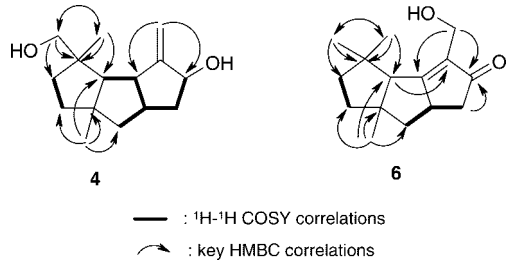

Figure 1. COSY and HMBC correlations of compounds **4** and **6**.

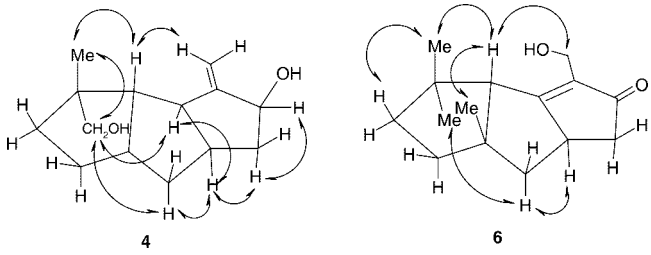

Figure 2. Selected NOESY correlations of compounds **4** and **6**.

and 65.2, two quarternary carbon signals at δ_C 47.2 and 53.6, and two methyl signals at δ_C 25.7 and 31.6. The ^{1}H NMR spectra (Table 2) confirmed the presence of an exo double bond by the fact that two signals were observed at δ_H 5.00 (s) and 5.14 (s). One oxygenated methine at δ_H 4.51 (t, 5.2) and one oxygenated methylene at δ_H 3.48 (d, J = 10.8 Hz) and 3.58 (d, J = 10.8 Hz) were also observed. In addition, two singlet methyl signals were also observed at δ_H 1.15 and 1.23. These NMR data were analogous to those of **9** except that a tertiary methyl was oxygenated to a primary alcohol (δ_H 3.48, 3.58 and δ_C 69.9).[6] The positioning of the primary hydroxyl group at C-15 was suggested by HMBC correlations (Figure 1) from H_2-15 to C-1/C-2/C-11/C-14 and NOESY correlations from H_2-15 to H-6/H-10. In order to determine the relative stereochemistry of **4**, a NOESY experiment was performed. These data are summarized in Figure 2, from which it can be seen that there are *cis* ring junctions at C4/C11 and C6/C10. The NOE correlations from H-11 to H-7β/H-12/Me-l3/Me-14 confirmed these protons are on the same face of the molecule. In addition, further correlations from H-10 to H_2-15/H-6, from H-6 to H_2-15, from H-5α to H_2-15, and from H_2-l5 to H-3α indicated that all of these must be on the opposite face of the molecule from H-11. This stereochemistry is identical to that found previously in other capnellenes.[1–6]

$\Delta^{9(12)}$-Capnellene-8β,10α,13-triol (**5**) had a molecular formula of $C_{15}H_{24}O_3$ as determined by HRESIMS and ^{13}C NMR data. Its ^{13}C and DEPT NMR spectra showed the presence of 15 carbon atoms, two sp^3 quaternary, one oxygenated sp^3 quaternary, one sp^2 quaternary, two methines, one oxygenated methine, four sp^3 methylenes, one oxygenated sp^3 methylene, one sp^2 methylene, and two methyls. These NMR data were similar to those of **1** except that hydroxylation of one of the tertiary methyl groups must have occurred (AB system centered at 3.35 ppm). This was further supported by the ^{13}C NMR spectrum of **5**, wherein one of the three tertiary methyl group signals of the capnellane skeleton was lacking and replaced by a methylene at 71.4 ppm. The presence of the primary hydroxyl at C-13 was indicated by HMBC correlations from H_2-13 to C-3/C-4/C-5/C-11. The NOE correlations from H-11 to H-7β/H-12/Me-l3/Me-14 confirmed these protons were on the same face of the molecule. In addition, further correlations from H-6 to Me-15, from H-5α to Me-15, and from Me-l5 to H-3α indicated that all these must be on the opposite face of the molecule from H-11.

$\Delta^{9(10)}$-Capnellene-12-ol-8-one (**6**) proved to have a molecular formula of $C_{15}H_{22}O_2$ as indicated by HRESIMS and ^{13}C NMR data. The IR spectrum of **6** showed an absorption due to hydroxyl (3500 cm^{-1}) and α,β-unsaturated carbonyl (1708 cm^{-1}) functionalities. ^{13}C and DEPT NMR spectra exhibited 15 carbon atoms, two sp^3 quaternary, three sp^2 quaternary, two methines, four sp^3 methylenes, one oxygenated sp^3 methylene, and three methyls. These NMR data resembled those of **3** except that an α-exo double bond and a tertiary hydroxyl were replaced by a tetrasubstituted enone [δ_C 211.3 (qC), 135.5 (qC), and 186.7 (qC)] with a hydroxymethyl [δ_C 56.8 (CH_2), δ_H 4.32 d, 4.35 d] at the α-position.[3] This was supported by HMBC correlations from H_2-12 to C-8/C-9/C-10, from H-11 to C-9/C-10, and from H-7 to C-8/C-9, and from COSY correlations (Figure 1). The NOE correlations (Figure 2) from H-11 to H-7β/H_2-12/Me-l3/Me-14 confirmed these protons were on the same face of the molecule. In addition, NOE correlations from H-6 to H-5α, from H-5α to Me-15, and from Me-l5 to H-3α indicated that all these must be on the opposite face of the molecule from H-11.

8β,10α-Diacetoxy-$\Delta^{9(12)}$-capnellene (**7**), isolated as a diacetate of **1**, was found to have a molecular formula of $C_{19}H_{28}O_4$ by HRESIMS and ^{13}C NMR data. The IR spectrum of **7** showed absorption due to an acetate ester (1739 cm^{-1}). The NMR data indicated that it had the same carbon skeleton as **1**,[1] but had a further four carbon atoms, two quaternary and two primary, giving an attached proton formula of $C_{19}H_{28}$. The ^{1}H NMR spectrum showed

the presence of two acetate methyls at δ 1.95 and 2.07, and the presence of two acetate moieties were inferred from the carbon signals at δ_C 170.9 (qC), 21.3 (CH_3), 169.6 (qC), and 22.0 (CH_3). HMBC correlations from H_2-12 to C-8/C-9/C-10, from H-6 to C-8/C-10, and from H-11 to C-1/C-9/C-10/C-14/C-15 helped ascertain the positions of the acetoxyls at C-8 and C-10. The relative stereochemistry of **7**, which was identical to that of **1**, was determined by a NOESY experiment.

8β-Acetoxy-$\Delta^{9(12)}$-capnellene (**8**) was isolated as a monoacetate of **9**. High-resolution mass spectrometry gave a molecular formula of $C_{17}H_{26}O_2$. The ^{1}H NMR spectrum showed the presence of an acetate methyl at δ 2.09, and the presence of an acetate moiety was confirmed from the ^{13}C NMR data. The location of the acetoxyl group was assigned at C-8 by HMBC correlations from H_2-12 to C-8/C-9/C-10 and by COSY correlations from H-7 to H-8/H-6 and from H-6 to H-10.

The anti-inflammatory activities of compounds **1**–**9** were tested using LPS-stimulated cells. Stimulation of RAW 264.7 cell with LPS resulted in up-regulation of the pro-inflammatory iNOS and COX-2 proteins. Compounds **1**–**3** at concentrations of 10 μM significantly reduced the levels of the iNOS protein (1.2 $\pm$ 0.1, 54.4 $\pm$ 12.0, and 34.8 $\pm$ 10.2, respectively) compared with control cells stimulated with LPS. Compounds **1** and **2** significantly reduced the levels of the COX-2 protein (24.8 $\pm$ 7.5 and 62.9 $\pm$ 13.7, respectively) at a concentration of 10 μM compared with control cells stimulated with LPS. At the same concentrations, the other isolated capnellenes did not inhibit iNOS and COX-2 protein expression.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P1020 polarimeter. UV spectra were obtained on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 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 or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using TMS as internal standard. Chemical shifts are given in δ (ppm) and coupling constants in Hz. ESIMS were recorded on a Bruker APEX II mass spectrometer. EIMS were obtained with a JEOL JMSSX/SX 102A mass spectrometer at 70 eV. Si gel 60 (Merck, 230–400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis. High-performance liquid chromatography (HPLC) was performed on a Hitachi L-7420 UV detector L-7100 pump apparatus equipped with a Merck Hibar Lichrospher RP-18 column (250 $\times$ 25 mm, 5 μm).

Animal Material. The soft coral *C. imbricata* was collected at Green Island off Taiwan. A voucher specimen, NSUGN-075, 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 *C. imbricata* were freeze-dried to give 0.3 kg of a solid, which was extracted with CH_2Cl_2/acetone (2.0 L $\times$ 3). After removal of solvent *in vacuo*, the residue (15 g) was chromatographed over a column containing silica gel 60 using *n*-hexane/EtOAc and EtOAc/MeOH mixtures as eluting solvents. Elution with *n*-hexane/EtOAc (98:2) gave fractions containing **8**, that with *n*-hexane/EtOAc (88:12) gave fractions containing **9**, that with *n*-hexane/EtOAc (82:18) gave fractions containing **1**, **3**, **5**, **6**, and **7**, and that with MeOH/EtOAc (60:40) gave fractions containing **2** and **4**. Compounds **1** (200 mg), **3** (15 mg), **5** (3.0 mg), **6** (3.0 mg), and **7** (2.0 mg) were further purified by passage over a RP-18 HPLC column, using MeOH/H_2O (80:20) as the solvent system. Compounds **2** (25 mg) and **4** (15.5 mg) were further purified by RP-18 HPLC using MeOH/H_2O (75:25) as the solvent system. Compounds **8** (2.0 mg) and **9** (56 mg) were further purified by RP-18 HPLC using MeOH/H_2O (88:12) as the solvent system.

$\Delta^{9(12)}$-Capnellene-8β,15-diol (4): $[\alpha]_D^{22}$ +56.4 (*c* 0.1, MeOH); IR ν_{max} 3416, 907 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRESIMS *m/z* 259.1673 (calcd for $C_{15}H_{24}O_2Na$ 259.1674).

$\Delta^{9(12)}$-Capnellene-8β,10α,13-triol (5): $[\alpha]_D^{22}$ +29.6 (*c* 0.1, MeOH); IR ν_{max} 3364, 910 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRESIMS *m/z* 275.1625 (calcd for $C_{15}H_{24}O_3Na$ 275.1623).

$\Delta^{9(10)}$-Capnellene-12-ol-8-one (6): $[\alpha]_D^{22}$ +35.9 (*c* 0.1, MeOH); UV ν_{max} (MeOH) nm (log ϵ) 239.0 (3.75); IR ν_{max} 3500, 1708 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRESIMS *m/z* 285.3643 (calcd for $C_{15}H_{22}ONa$ 285.3643).

8β,10α-Diacetoxy-$\Delta^{9(12)}$-capnellene (7): $[\alpha]_D^{22}$ +86.2 (*c* 0.1, MeOH); IR ν_{max} 1739, 917 cm^{-1}, ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRESIMS *m/z* 343.1883 (calcd for $C_{19}H_{28}O_4Na$ 343.1885).

8β-Acetoxy-$\Delta^{9(12)}$-capnellene (8): $[\alpha]_D^{22}$ +25.9 (*c* 0.1, MeOH); IR ν_{max} 1739, 917 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 1; HRESIMS *m/z* 285.3643 (calcd for $C_{17}H_{26}O_2Na$ 285.3645).

***In Vitro* Anti-inflammatory Assay.** The anti-inflammatory assay was modified from Ho et al.[10] and Park et al.[11] Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC, No. TIB-71). The cells were activated by incubation in medium containing *Escherichia coli* LPS (0.01 μg/mL; Sigma) for 16 h in the presence or absence of various compounds. Then, cells were washed with ice-cold PBS, lysed in ice cold lysis buffer, and then centrifuged at 20000*g* for 30 min at 4 °C. The supernatant was decanted from the pellet and retained for Western blot analysis. Protein concentrations were determined by the DC protein assay kit (Bio-Rad) modified by the method of Lowry et al.[12] Samples containing equal quantities of protein were subjected to SDS-polyacrylamide gel electrophoresis, and the separated proteins were electrophoretically transferred to polyvinylidene difluoride membranes (PVDF; Immobilon-P, Millipore, 0.45 μm pore size). The resultant PVDF membranes were incubated with blocking solution and incubated for 180 min with antibody against inducible nitric oxide synthase (iNOS; 1:1000 dilution; Transduction Laboratories) and cyclooxygenase-2 (COX-2; 1:1000 dilution; Cayman Chemical). The blots were detected using ECL detection reagents (Perkin-Elmer, Western Blot Chemiluminescence Reagent Plus) according to the manufacturer's instructions.

Acknowledgment. Financial support was provided by the Ministry of Education (95C030313) and National Science Council of Taiwan (NSC 96-2320-B-110-MY3) awarded to C.-Y.D. We thank C. F. Dai, Institute of Oceanography, National Taiwan University, for the identification of the soft coral.

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NP0706116