Bioactive Terpenoids from Octocorallia. 3. A New Eunicellin-Based Diterpenoid from the Soft Coral *Cladiella sphaeroides*

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A new diterpenoid, cladiellaperoxide (**2**), has been isolated from the soft coral *Cladiella sphaeroides*, together with the known diterpenoid cladiellisin (**1**). Their structures were characterized by NMR studies and chemical conversion. The absolute configurations of **1** and **2** have been elucidated by the modified Mosher method. Compound **2** showed lethality toward brine shrimp.

Recently, we reported the structures of two hemolytic diterpenoids, litophynols A and B, isolated from the mucus of the soft coral *Litophyton* sp.,[1] and of an ichthyotoxic diterpenoid, deoxyxeniolide B, isolated from the soft coral *Xenia elongata*.[2] In a continuing search for the biologically active constituents of other soft corals, we found that the *n*-hexane-soluble part of the MeOH extracts of the soft coral *Cladiella sphaeroides* "Nou−Tosaka" in Japanese; genus: *Cladiella*, family: *Alcyoniidae*, order: *Alcyonacea*) collected in Miyazaki Prefecture showed lethality toward brine shrimp. Bioassay-directed fractionation of the extract led to the isolation of a known compound (**1**)[3] and a new bioactive compound **2**. In this paper, we describe the structures of these compounds based on spectroscopic data and chemical transformation. The absolute configurations of **1** and **2** were determined by the modified Mosher method.[4] The biological activity of these compounds is also discussed.

The MeOH extract of *C. sphaeroides* (wet wt 170 g) was partitioned between *n*-hexane and H_2O. Si gel chromatography of the *n*-hexane extract (4.5 g) followed by purification by HPLC afforded compound **1** and cladiellaperoxide (**2**).

Compound **1** was isolated as an amorphous solid, $[\alpha]^{23}_D$ −21.3° (*c* 0.51, $CHCl_3$). The molecular formula of $C_{20}H_{32}O_3$ was established by HRMS. Its IR spectrum indicated the presence of a hydroxyl (3600, 3450 cm^{-1}) and exocyclic methylene groups (3070, 1640, 900 cm^{-1}). The ^{1}H-NMR, ^{13}C-NMR, and ^{1}H−^{13}C COSY spectra of **1** showed the presence of one isopropyl and two exocyclic methylenes. These data suggested that compound **1** was an eunicellin-type diterpenoid and had the same relative stereostructure as that of the known diterpenoid cladiellisin obtained from the soft coral *Cladiella similis*. Compound **1** was identified as cladiellisin by comparison of MS and ^{1}H- and ^{13}C-NMR spectra.

Cladiellaperoxide (**2**) was isolated as an amorphous solid, $[\alpha]^{23}_D$ −27.8° (*c* 0.42, $CHCl_3$). The molecular formula of **2**, $C_{20}H_{33}O_4$, was established by a high resolution positive ion FABMS. Its IR spectrum indicated the presence of a hydroperoxyl (3530 cm^{-1}),[5] hydroxyl (3600, 3300 cm^{-1}), and exocyclic methylene groups (3070, 1645, 900 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **2** resembled those of **1** (Table 1). These data and the molecular formula of **2** suggested that **2** was the peroxylated analogue of **1**, which was verified from the fact that **2** was converted to **1** by means of $NaBH_4$ reduction.[6] The position of the hydroperoxyl group in **2** was suggested by comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** (Table 1), because the signals due to C-6 and H-6 in **2** shifted to a lower field, indicating that the 6-hydroxy group was peroxylated. The relative stereochemistry of **2** was confirmed on the basis of a NOESY experiment as shown in Figure 1 and the *J* values of each proton in the ^{1}H-NMR spectrum (Table 1). Thus, the relative stereochemistry of **2** was characterized on the basis of correlation with the known compound **1** and NMR studies.

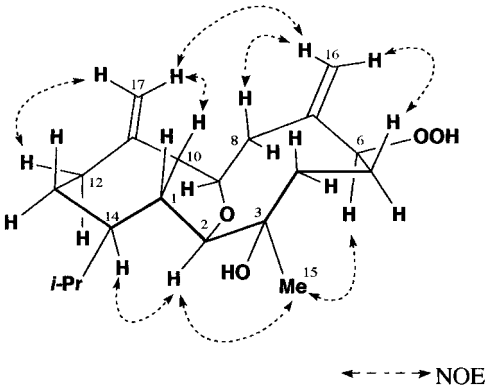

Figure 1. NOESY correlations for **2**.

Because the absolute configuration of cladiellisin (**1**) has not yet been determined, we examined this using the modified Mosher method of Kusumi *et al.*[4] The α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters **3** and **4** were prepared from **2** by reduction of the peroxide followed by treatment with (*R*)-(+)-MTPA and (*S*)-(−)-MTPA, respectively (Figure 2).

The ^{1}H-NMR and NOESY spectra for each compound were measured. The $\Delta\delta$ values (δ of the (*S*)-(−)-MTPA ester **4** − δ of the (*R*)-(+)-MTPA ester **3**) are shown in Figure 3, and the NOESY correlations for these compounds are shown in Table 2. The $\Delta\delta$ values are positive for protons on the right-hand side of the O−C bond, and negative for those on the left-hand side. The only exception is for H-8α , and this can be explained by a deshielding effect of the benzene ring of the MTPA group in **3** on this proton, which is approximately the same plane as the benzene ring. This fact was confirmed by an energy-minimized structure of **3** calculated

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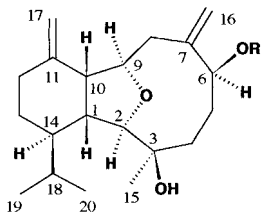
Table 1. ^{13}C-NMR[a] and ^{1}H-NMR[b] Data for Compound **1** and Cladiellaperoxide (**2**) in $CDCl_3$

	1		**2**	
position	δC	δH(*J*, Hz)	δC	δH(*J*, Hz)
1	44.42 (d)	2.21 (1H, m)	44.42 (d)	2.24 (1H, m)
2	91.87 (d)	3.66 (1H, s)	91.77 (d)	3.67 (1H, s)
3	74.09 (s)		74.09 (s)	
4	35.58 (t)	2.07 (1H, m), 2.28 (1H, m)	34.76 (t)	2.28 (1H, m), 2.03 (1H, m)
5	31.81 (t)	1.87 (1H, m), 2.08 (1H, m)	29.68 (t)	1.70 (1H, dd, 3.2, 8.9), 2.12 (1H, m)
6	72.86 (d)	4.40 (1H, dd, 4.0, 10.9)	86.34 (d)	4.74 (1H, dd, 3.2, 11.5)
7	152.20 (s)		147.68 (s)	
8	39.27 (t)	2.30 (1H, d, 13.5), 2.79 (1H, ddd, 1.3, 4.8, 13.5)	40.04 (t)	2.34 (1H, d, 13.9), 2.78 (1H, dd, 5.6, 13.9)
9	79.72 (d)	4.13 (1H, dd, 4.8, 10.7)	79.56 (d)	4.12 (1H, dd, 5.6, 10.6)
10	47.71 (d)	2.96 (1H, dd, 7.8, 10.7)	47.98 (d)	2.93 (1H, dd, 7.9, 10.6)
11	146.29 (s)		146.16 (s)	
12	35.12 (t)	2.08 (1H, m), 2.10 (1H, m)	31.78 (t)	2.15 (1H, m), 2.22 (1H, m)
13	25.30 (t)	1.07 (1H, td, 3.4, 10.1), 1.75 (1H, m)	25.28 (t)	1.03 (1H, td, 3.4, 13.5), 1.76 (1H, m)
14	44.12 (d)	1.30 (1H, dt, 3.4, 12.8)	44.01 (d)	1.30 (1H, dt, 3.4, 11.8)
15	27.03 (q)	1.23 (3H, s)	27.09 (q)	1.23 (3H, s)
16	116.62 (t)	5.14 (1H, s), 5.53 (1H, s)	117.95 (t)	5.26 (1H, d, 1.2), 5.52 (1H, d, 1.2)
17	111.18 (t)	4.67 (1H, t, 2.0), 4.80 (1H, t, 2.0)	111.25 (t)	4.68 (1H, t, 2.0), 4.81 (1H, t, 2.0)
18	27.92 (d)	2.00 (1H, m)	27.91 (d)	1.80 (1H, m)
19	15.17 (q)	0.79 (3H, d, 6.8)	15.14 (q)	0.74 (3H, d, 7.1)
20	21.88 (q)	0.96 (3H, d, 6.8)	21.97 (q)	0.96 (3H, d, 7.1)
−OOH				7.86 (1H, s)

[a] Spectra were aquired at 23 °C at 67.8 MHz. Chemical shifts were given in ppm. Multiplicity was given in DEPT. [b] Spectra were aquired at 23 °C at 270 MHz. Chemical shifts were given in ppm. *J* values were given in hertz.



1 R = H
2 R = OH
3 R = (*R*)-(+)-MTPA
4 R = (*S*)-(-)-MTPA

Figure 2.

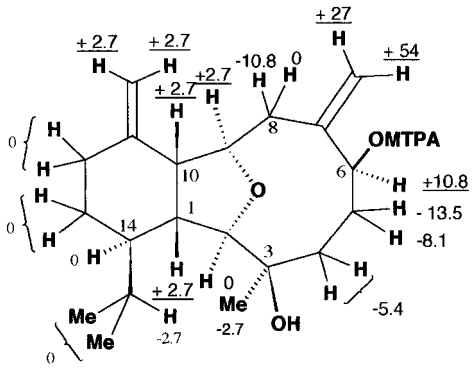


Figure 3. $\Delta\delta$ values ($\Delta\delta = \delta_S - \delta_R$ at 270 MHz) obtained for MTPA esters **3** and **4**. The values are given in Hz.

by MM2[7] as shown in Figure 4.[8] Thus, the 6*S* configuration of compound **1** was demonstrated, and therefore, the absolute configuration of **1** as 1*R*,2*R*,3*R*,6*S*,9*R*,10*R*, 14*R* was established. Because the absolute configuration of cladiellisin, native **1**, and derivative **1** from **2** must be the same on the basis of their optical rotations (cladiellisin; $[\alpha]^{20}_D$ −8.6°, native **1**; $[\alpha]^{23}_D$ −21.3°, and derivative **1**; $[\alpha]^{28}_D$ −23.0°), the absolute configurations of cladiellisin (**1**) and cladiellaperoxide (**2**) are as shown in Figure 2.[9]

Cladiellaperoxide (**2**) displayed toxicity in the brine shrimp lethality bioassay[10] at a 30-ppm concentration, but cladiellisin (**1**) was inactive.

Table 2. NOESY Correlations for (*R*)-(+)-MTPA Ester **3** and (*S*)-(−)-MTPA Ester **4**

	NOESY correlated protons	
H	**3**	**4**
1	19Me	19Me
2	15Me, 18H	15Me, 18H
6	15Me	15Me
8β	16H, 17H	16H, 17H
10	17H	17H
16	17H	17H

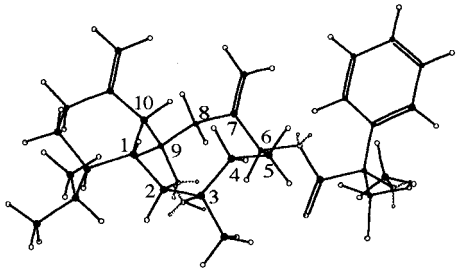


Figure 4. Configuration of **3** obtained by MM2 calculations.

Experimental Section

General Details. IR spectra were obtained by using a JASCO IR-700 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured at 270 and 67.8 MHz, respectively, with a JEOL GX-270 spectrometer. Chemical shifts were given on a δ (ppm) scale with TMS as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Positive ion FABMS were recorded by using a JEOL DX-300 (6 kV, xenon atom beam), and the spectra were measured in $CHCl_3$−*m*-NBA (*m*-nitrobenzoic acid) solution. EI mass spectra were recorded by using a JEOL JMS-DX-300 data system, accelerating potential of 3 kV, ionizing potential of 30 eV, sample temperature of 200−250 °C. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Column chromatography was performed with Sephadex LH-20 (Pharmacia), Kieselgel 60 (no. 7734, Merck), and Cosmosil 5C18 (Nacalai Tesque). HPLC was conducted with a JASCO BIP-I model and a

RID-300 RI detector with a Wakosil 5C18 (ODS) column (Wako). TLC was performed with Kieselgel 60 F_{254} (no. 5715, Merck).

Animal Material. The soft coral *Cladiella sphaeroides* was collected on the coral reef off Nangou-cho (Miyazaki Prefecture, Japan) in November 1989, at a depth of 2−3 m and identified as *Cladiella sphaeroides* Utinomi, 1953, by Mr. Yukimitsu Imahara. A voucher specimen (no. WMNH-94-INV-3) is on deposit at the Wakayama Prefectural Museum of Natural History (Wakayama, Japan).

Extraction and Isolation. Wet specimens (170 g) were homogenized with MeOH (1 L) and left at room temperature for a few hours. After filtration, the MeOH solution was concentrated *in vacuo* to an aqueous suspension (200 mL) and extracted with *n*-hexane (100 mL × 3). The *n*-hexane layer was subsequently dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue (4.5 g), which showed lethality toward brine shrimp, was permeated through a Sephadex LH-20 column with MeOH−$CHCl_3$ (1:1); four fractions were collected. The lethal third fraction (0.54 g) was subjected to chromatography on a Si gel column; elution with *n*-hexane−acetone mixtures of increasing polarity (98:2−10:90) gave 15 fractions, A−O. Rechromatography of fraction K (52.2 mg) on Cosmosil 5C18 using MeOH−H_2O (75%−100%) as the eluent, followed by reversed-phase HPLC (75% MeOH−H_2O), yielded cladiellaperoxide (**2**) (3.8 mg). Fraction L (39.5 mg) was rechromatographed on Cosmosil 5C18 with 75% MeOH−H_2O and subsequently purified by reversed-phase HPLC (75% MeOH−H_2O) to obtain **1** (cladiellisin) (5.1 mg).

Cladiellisin (1): amorphous solid; $[\alpha]^{23}_D$ −21.3° (*c* 0.51, $CHCl_3$); EIMS *m/z* (M^+) 320 (1.3), 302 (71), 179 (97), 177 (37), 107 (56), 95 (74), 81 (54), 69 (39), 43 (100); HR positive ion FABMS *m/z* 321.2415; calcd for $C_{20}H_{33}O_4$ $[M + H]^+$ 321.2431; IR ($CHCl_3$) ν_{max} 3600, 3530, 1640, 920 cm^{-1} ; ^{1}H-NMR and ^{13}C-NMR, see Table 1. Compound **1** was shown to be identical with cladiellisin (**1**) by MS and ^{1}H-NMR comparisons, although its $[\alpha]_D$ value differed from that reported.[9]

Cladiellaperoxide (2): amorphous solid; $[\alpha]^{23}_D$ −27.8° (*c* 0.42, $CHCl_3$); EIMS *m/z* (M^+) 336 (1.0), 318 (11), 302 (22), 277 (29), 179 (65), 177 (35), 107 (46), 95 (69), 81 (52), 69 (44), 43 (100); HR positive ion FABMS *m/z* 337.2380; calcd for $C_{20}H_{33}O_4$ $[M + H]^+$ 337.2380; IR ($CHCl_3$) ν_{max} 3600, 3530, 3300, 3070, 1645, 900 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Table 1.

Conversion of Cladiellaperoxide (2) into Cladiellisin (1).[6] $NaBH_4$ (3.0 mg, 0.079 mmol) was added to a solution of **2** (7.4 mg, 0.022 mmol) in EtOH (1.5 mL). The mixture was stirred at room temperature for 80 min. The reaction mixture was neutralized with 0.2 N HCl and concentrated *in vacuo*. The residue was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was washed with aqueous saturated NaCl and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure from the $CHCl_3$ extract gave a compound that was identified as cladiellisin (**1**) by $[\alpha]^{28}_D$ −23.0° (*c* 0.28, $CHCl_3$), MS, and ^{1}H-NMR comparisons.

Conversion of 1 into its MTPA Esters. (*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (6.1 mg, 0.026 mmol), dicyclohexylcarbodiimide (6.3 mg, 0.031 mmol), and 4-(dimethylamino)pyridine (3.2 mg, 0.026 mmol) were added to a solution of **1** (3.5 mg, 0.011 mmol) in dry CH_2Cl_2 (1.5 mL). The mixture was stirred at room temperature for 55 h. The reaction mixture was concentrated *in vacuo* to dryness. The residue was purified by column chromatography (SiO_2, 3 g, *n*-hexane−EtOAc 90:10 to 85:15) to furnish **3** (2.6 mg, amorphous solid).

A similar reaction of **1** (3.5 mg, 0.011 mmol) with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid furnished **4** (2.6 mg, amorphous solid).

6-*O*-[(*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetyl]cladiellisin (3): $[\alpha]^{23}_D$ −44.2° (*c* 0.22, $CHCl_3$); MS *m/z* 536; HR positive ion FABMS 537.2806, calcd for $C_{30}H_{40}F_3O_5$ $[M + H]^+$ 537.2829; ^{1}H NMR ($CDCl_3$) δ 0.74 (3H, d, J = 6.9 Hz, H-19), 0.97 (3H, d, J = 6.9 Hz, H-20), 1.06 (1H, m, H-13), 1.28 (3H, s, H-15), 1.29 (1H, m, H-14), 1.60 (1H, m, H-5), 1.63 (1H, m, H-4), 1.74 (1H, m, H-13), 1.82 (1H, m, H-18), 1.88 (1H, m, H-4), 2.02 (1H, m, H-5), 2.07 (1H, m, H-12), 2.21 (1H, m, H-1), 2.26 (1H, m, H-12), 2.28 (1H, d, J = 13.4 Hz, H-8), 2.90 (1H, dd, J = 8.1, 10.4 Hz, H-10), 3.13 (1H, ddd, J = 1.3, 5.0, 13.4 Hz, H-8), 3.54 (3H, s, OMe), 3.67 (1H, s, H-2), 4.13 (1H, dd, J = 5.0, 10.4 Hz, H-9), 4.64 (1H, t, J = 1.9 Hz, H-17), 4.80 (1H, t, J = 1.9 Hz, H-17), 5.12 (1H, s, H-16), 5.27 (1H, s, H-16), 5.48 (1H, dd, J = 4.8, 11.0 Hz, H-6), 7.38 (3H, m, phenyl), 7.46 (2H, m, phenyl).

6-*O*-[(*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetyl]cladiellisin (4): $[\alpha]^{23}_D$ −85.7° (*c* 0.16, $CHCl_3$); MS *m/z* 536; HR positive ion FABMS 536.2744, calcd for $C_{30}H_{39}F_3O_5$ $[M]^+$ 536.2751; ^{1}H-NMR ($CDCl_3$) δ 0.74 (3H, d, J = 7.1 Hz, H-19), 0.97 (3H, d, J = 7.1 Hz, H-20), 1.06 (1H, m, H-13), 1.27 (3H, s, H-15), 1.29 (1H, m, H-14), 1.57 (1H, m, H-5), 1.61 (1H, m, H-4), 1.74 (1H, m, H-13), 1.81 (1H, m, H-18), 1.86 (1H, m, H-4), 1.97 (1H, m, H-5), 2.07 (1H, m, H-12), 2.22 (1H, m, H-1), 2.26 (1H, m, H-12), 2.28 (1H, d, J = 13.5 Hz, H-8), 2.91 (1H, dd, J = 7.9, 10.7 Hz, H-10), 3.09 (1H, ddd, J = 1.3, 6.5, 13.5 Hz, H-8), 3.52 (3H, s, OMe), 3.67 (1H, s, H-2), 4.14 (1H, dd, J = 6.5, 10.7 Hz, H-9), 4.65 (1H, t, J = 1.9 Hz, H-17), 4.81 (1H, t, J = 1.9 Hz, H-17), 5.22 (1H, d, J = 1.0 Hz, H-16), 5.47 (1H, s, H-16), 5.52 (1H, dd, J = 5.6, 10.2 Hz, H-6), 7.46 (3H, m, phenyl), 7.50 (2H, m, phenyl).

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(8) The initial geometry of the molecule for energy minimization was based on the results of the NOESY experiment. Energy minimization by MM2 method was performed using the CAChe system on a Macintosh linked to an IBM RS /6000 server.
(9) We cannot explain the large numerical difference between the rotation of cladiellisin reported by Liu *et al.*[3] and those obtained on our samples. However, the fact that the rotations are all negative strongly suggests that the sample described in ref 3 and our samples have the same absolute configurations.
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