Cladieunicellins A—E, New Eunicellins from an Indonesian Soft Coral *Cladiella* sp.

Yung-Husan Chen,[a,b] Chia-Ying Tai,[a,b] Yueh-Hsiung Kuo,[c] Chia-Ying Kao,[a,b] Jan-Jung Li,[a] Tsong-Long Hwang,[d] Lee-Shing Fang,[e] Wei-Hsien Wang,[a,f,g] Jyh-Horng Sheu,*[,f,g] and Ping-Jyun Sung*[,a,b,f,g,h]

[a] *National Museum of Marine Biology and Aquarium; Pingtung 944, Taiwan:* [b] *Graduate Institute of Marine Biotechnology, National Dong Hwa University; Pingtung 944, Taiwan:* [c] *Tsuzuki Institute for Traditional Medicine, College of Pharmacy, China Medical University; Taichung 404, Taiwan:* [d] *Graduate Institute of Natural Products, Chang Gung University; Taoyuan 333, Taiwan:* [e] *Department of Sport, Health, and Leisure, Cheng Shiu University; Kaohsiung 833, Taiwan:* [f] *Department of Marine Biotechnology and Resources, National Sun Yat-sen University;* [g] *Asia-Pacific Ocean Research Center, National Sun Yat-sen University; Kaohsiung 804, Taiwan: and* [h] *Department of Life Science and the Institute of Biotechnology, National Dong Hwa University; Hualien 974, Taiwan.*

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Five new eunicellin-type diterpenoids, cladieunicellins A—E (1—5), were isolated from an Indonesian soft coral identified as *Cladiella* sp. The structures of diterpenoids 1—5 were established using spectroscopic methods. Eunicellins 2 and 5 were found to be cytotoxic against DLD-1 and HL-60 tumor cells, respectively, and 3 displayed inhibitory effects against superoxide anion generation by human neutrophils.

Key words cladieunicellin; eunicellin; soft coral; *Cladiella*; cytotoxicity; superoxide anion

Octocorals belonging to the genus *Cladiella* (Alcyoniidae) were shown to be a rich source of marine natural products,[1] particularly with eunicellin-type metabolites,[2—7] and most compounds of this class were found to possess complex structures and interesting biological activities. In a continuation of our search for bioactive substances from the marine invertebrates distributed in the tropical West Pacific Ocean, a specimen of octocoral identified as *Cladiella* sp., collected off the Indonesian coast, was studied. Its organic extract exhibited cytotoxicity against DLD-1 (human colorectal adenocarcinoma), HL-60 (human promyelocytic leukemia), and P388D1 (murine macrophage-like cells) (IC_{50}=2.7, 8.9, 7.2 μg/ml, respectively) cell lines. Five new eunicellins, cladieunicellins A—E (**1**—**5**), were isolated from this organism. In this paper, we report the isolation, structural determination, and bioactivity of the new diterpenoids.

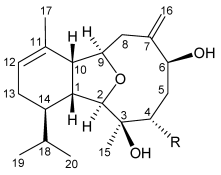
Results and Discussion

Cladieunicellin A (**1**) was isolated as a colorless oil, and the molecular formula of this compound was determined to be $C_{20}H_{32}O_3$ (five degrees of unsaturation) from a sodiated molecule at *m*/*z* 343 in the electronspray ionization (ESI)-MS spectrum, which was further supported by high resolution (HR)-ESI-MS (*m*/*z* 343.2246, Calcd 343.2249, $[C_{20}H_{32}O_3Na]^+$). Comparison of the ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data with the molecular formula indicated that there must be two exchangeable protons, requiring the presence of two hydroxy groups, and this deduction was supported by a broad absorption in the IR spectrum at 3427 cm^{-1}. From the ^{1}H- and ^{13}C-NMR spectra (Table 1), **1** was found to possess a trisubstituted olefin (δ_H 5.49, 1H, m, H-12; δ_C 131.4, s, C-11; 122.9, d, C-12) and an exocyclic carbon–carbon double bond (δ_H 5.15, 1H, s, H-16a; 5.46, 1H, s, H-16b; δ_C 150.6, s, C-7; 115.6, t, C-16). From the above data, two degrees of unsaturation were accounted for and **1** must be tricyclic. In the ^{1}H-NMR spectrum of **1**, two doublets at δ_H 0.93 and 0.78 (each 3H, d, *J*=6.8 Hz, H_3-19, H_3-20) were assigned from two methyls of an isopropyl group. A singlet of the tertiary methyl bonded to an oxygenated carbon was due to the resonance of a signal at δ_H 1.30 (3H, s, H_3-15). In addition, a suite of resonances of proton signals at δ_H 2.37 (1H, ddd, *J*=10.0, 8.0, 3.6 Hz, H-1), 2.65 (1H, brt, *J*=8.0 Hz, H-10), 3.78 (1H, d, *J*=3.6 Hz, H-2), and 4.06 (1H, ddd, *J*=8.0, 4.8, 2.0 Hz, H-9), and carbon signals at δ_C 41.0 (d, C-1), 44.9 (d, C-10), 89.2 (d, C-2), and 82.5 (d, C-9), indicated the presence of a tetrahydrofuran structural unit. Thus, from the above data, the proposed skeleton of **1** was suggested to be a eunicellin-based metabolite.

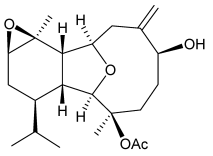
From the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1**, it was possible to identify the separate spin systems among H-1/H-2; H_2-4/H_2-5/H-6; H_2-8/H-9/H-10; and H-10/H-1 (Table 1). These data, together with the assistance of heteronuclear multiple-bond coherence (HMBC) correlations between H-2/C-1, -4, -9; H_2-4/C-2, -3, -5, -6; H_2-5/C-3, -4, -6, -7; H-6/C-7; H_2-8/C-6, -7, -9, -10; H-9/C-7; and H-10/C-1, -8, -9, established the connectivity from C-1 to C-10 within the 10-membered ring (Table 1). An exocyclic carbon–carbon double bond attached at C-7 was confirmed by the HMBC correlations between H_2-16/C-6, -7, -8; H-6/C-16; and H_2-8/C-16. The placements of hydroxy groups at C-3 and C-6 were confirmed from the signals of two oxygenated carbons at δ_C 74.1 (s, C-3) and 73.6 (d, C-6), respectively. The C-15 tertiary methyl bonded to the C-3 oxygenated quaternary carbon was established by the HMBC correlations between H-2/C-15 and H_3-15/C-2, -3, -4. The ether bridge between C-2 and C-9 was supported by an HMBC correlation between H-2/C-9. The 1-isopropyl-4-methylcyclohexene ring, which is fused to the 10-membered ring at C-1 and C-10, was elucidated based on the ^{1}H–^{1}H COSY correlations between H-12/H_2-13/H-14; H-14/H-1; H-14/H-18; and H-18/H_3-19(H_3-20) and further supported by the HMBC correlations between H-2/C-14; H-9/C-11; H-14/C-1, -2; H_2-13/C-1; H-18/C-1; and H_3-17/C-10. The vinyl methyl attached at

* To whom correspondence should be addressed. e-mail: pjsung@nmmba.gov.tw; sheu@mail.nsysu.edu.tw

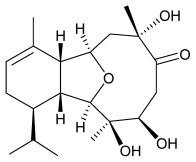
Table 1. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **1**



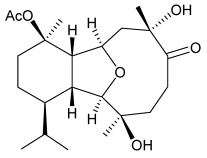
Cladieunicellin A (**1**): R = H
Cladieunicellin B (**2**): R = OH



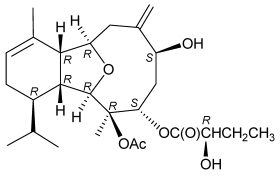
Cladieunicellin C (**3**)



Cladieunicellin D (**4**)



Cladieunicellin E (**5**)



Cladielloide A (**6**)

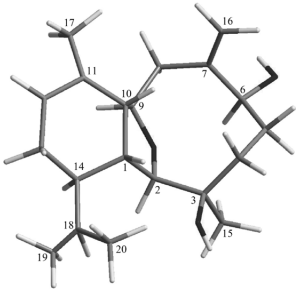
C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1	2.37 ddd (10.0, 8.0, 3.6) [c]	41.0 (d) [d]	H-2, H-10, H-14	n.o. [e]
2	3.78 d (3.6)	89.2 (d)	H-1	C-1, -4, -9, -14, -15
3		74.1 (s)		
4α	1.52 m	33.4 (t)	H-4β, H$_2$-5	C-2, -3, -5
β	1.86 m		H-4α, H$_2$-5	C-2, -3, -5, -6
5α	2.18 m	33.0 (t)	H$_2$-4, H-5β, H-6	C-6, -7
β	1.83 m		H$_2$-4, H-5α, H-6	C-3, -4, -6
6	4.35 dd (9.2, 3.6)	73.6 (d)	H$_2$-5	C-7, -16
7		150.6 (s)		
8α	2.73 dd (14.0, 4.8)	41.4 (t)	H-8β, H-9	C-6, -7, -9, -10, -16
β	2.44 dd (14.0, 2.0)		H-8α, H-9	C-6, -7, -16
9	4.06 ddd (8.0, 4.8, 2.0)	82.5 (d)	H$_2$-8, H-10	C-7, -11
10	2.65 br t (8.0)	44.9 (d)	H-1, H-9	C-1, -8, -9, -11, -12
11		131.4 (s)		
12	5.49 m	122.9 (d)	H$_2$-13, H$_3$-17	n.o.
13α	2.01 m	23.0 (t)	H-12, H-13β, H-14	C-1, -11, -12
β	1.69 m		H-12, H-13α, H-14	C-1, -11, -12
14	1.39 m	39.5 (d)	H-1, H$_2$-13, H-18	C-1, -2, -19, -20
15	1.30 s	27.1 (q)		C-2, -3, -4
16a	5.15 s	115.6 (t)	H-16b	C-6, -7, -8
b	5.46 s		H-16a	C-6, -7, -8
17	1.70 d (1.2)	23.1 (q)	H-12	C-10, -11, -12
18	1.78 m	27.8 (d)	H-14, H$_3$-19, H$_3$-20	C-1, -13, -14, -19, -20
19	0.93 d (6.8)	21.8 (q)	H-18	C-14, -18, -20
20	0.78 d (6.8)	16.8 (q)	H-18	C-14, -18, -19

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *c*) *J* values (in hertz) in parentheses. *d*) Attached protons were deduced by DEPT and HMQC experiments. *e*) n.o.=not observed.

C-11 was confirmed by the HMBC correlations between H$_3$-17/C-10, -11, -12 and further supported by the allylic coupling between the olefin proton H-12 and Me-17. Therefore the planar structure of **1** was established.

The relative configuration of **1** was elucidated from the interactions observed in a nuclear Overhauser effect spectroscopy (NOESY) experiment. In the NOESY experiment of **1** (Table 2), the correlations between H-1 with H-10 and a proton of a C-4 methylene (δ_H 1.86) indicated that these protons are situated on the same face and assigned as β protons. A small coupling constant was found between H-1 and H-2 (J=3.6 Hz), and no correlation was found between these two protons in the NOESY experiment, indicating that H-2 should be α-oriented. H-2 exhibited interactions with H-14 and H$_3$-15, and H$_3$-15 correlated with H-6, showing that the hydroxy groups at C-3 and C-6 and the isopropyl group at C-14 are β-oriented. Furthermore, H-9 correlated with H$_2$-8 and H-14. From consideration of molecular models, H-9 was found to be reasonably close to H$_2$-8 and H-14, when H-9 was α-oriented in **1**. Thus the structure of **1** was established.

Table 2. The Stereoview of **1** (Generated from Computer Modeling) and the Calculated Distances (Å) between Selected Protons Having Key NOESY Correlations

Cladieunicellin A (**1**)	H/H	(Å)
	H-1/H-4β	2.58
	H-1/H-10	2.30
	H-2/H-14	2.51
	H-2/H$_3$-15	2.37
	H-6/H$_3$-15	2.34
	H-8α/H-9	2.43
	H-8β/H-9	2.50
	H-9/H-14	2.81

The HR-ESI-MS of **2** (cladieunicellin B) exhibited a pseudomolecular ion at *m/z* 359.2196 $[M+Na]^+$, with the

Table 3. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **2**

C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1	2.62 m [c]	40.0 (d) [e]	H-2, H-10, H-14	C-2, -9, -10, -11, -13, -14, -18
2	3.91 d (6.0) [d]	87.9 (d)	H-1	C-1, -3, -4, -9, -14, -15
3		75.0 (s)		
4	3.85 br s	69.3 (d)	H_2-5	n.o. [f]
5α	2.69 ddd (15.2, 5.2, 2.8)	39.7 (t)	H-4, H-5β, H-6	C-3, -4
β	1.85 m [c]		H-4, H-5α, H-6	C-6
6	4.38 br d (5.2)	72.7 (d)	H_2-5	C-7
7		148.7 (s)		
8α	2.48 dd (14.4, 4.0)	40.1 (t)	H-8β, H-9	C-6, -7, -9, -10, -16
β	2.36 dd (14.4, 3.2)		H-8α, H-9	C-6, -7, -9, -16
9	4.12 td (4.0, 3.2)	82.0 (d)	H_2-8, H-10	C-2
10	2.60 m [c]	44.6 (d)	H-1, H-9	C-1, -2, -8, -9, -11, -14, -17
11		131.9 (s)		
12	5.46 m	122.5 (d)	H_2-13, H_3-17	n.o.
13α	2.08 m	22.9 (t)	H-12, H-13β, H-14	n.o.
β	1.91 m [c]		H-12, H-13α, H-14	n.o.
14	1.49 m	39.5 (d)	H-1, H_2-13, H-18	C-2, -10, -12, -18, -19, -20
15	1.31 s	22.2 (q)		C-2, -3, -4
16a	5.16 s	115.1 (t)	H-16b	C-6, -8
b	5.51 s		H-16a	C-6, -8
17	1.69 d (1.2)	22.4 (q)	H-12	C-10, -11, -12
18	1.66 m	28.5 (d)	H-14, H_3-19, H_3-20	C-1, -13, -14, -19, -20
19	0.92 d (6.8)	21.5 (q)	H-18	C-14, -18, -20
20	0.82 d (6.8)	19.2 (q)	H-18	C-14, -18, -19
3-OH	2.18 s			C-2, -3, -4

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *c*) The proton signals for H-1 and H-10; and H-5β and H-13β are overlapped, respectively. *d*) *J* values (in hertz) in parentheses. *e*) Attached protons were deduced by DEPT and HMQC experiments. *f*) n.o.=not observed.

Table 4. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **3**

C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1	2.01 m	40.0 (d) [d]	H-2, H-10, H-14	n.o. [e]
2	3.74 br s	88.5 (d)	H-1	C-3, -4, -9, -14, -15
3		84.7 (s)		
4α	1.57 m	27.8 (t)	H-4β, H_2-5	n.o.
β	2.22 m		H-4α, H_2-5	C-2, -3
5α	2.23 m	34.5 (t)	H_2-4, H-5β, H-6	n.o.
β	1.69 m		H_2-4, H-5α, H-6	n.o.
6	4.36 dd (11.2, 3.6) [c]	72.7 (d)	H_2-5	C-7, -16
7		150.5 (s)		
8α	2.92 dd (14.0, 4.8)	41.2 (t)	H-8β, H-9	C-7, -9, -10, -16
β	2.45 dd (14.0, 1.2)		H-8α, H-9	C-6, -7, -16
9	4.41 ddd (9.2, 4.8, 1.2)	80.5 (d)	H_2-8, H-10	n.o.
10	2.58 br t (9.2)	42.4 (d)	H-1, H-9	C-8, -9, -14, -17
11		55.6 (s)		
12	3.11 br s	61.4 (d)	H_2-13	C-13, -14
13α	2.01 m	22.9 (t)	H-12, H-13β, H-14	n.o.
β	1.49 m		H-12, H-13α, H-14	C-14
14	1.47 m	35.7 (d)	H-1, H_2-13, H-18	n.o.
15	1.63 s	21.7 (q)		C-2, -3, -4
16a	5.12 s	115.8 (t)	H-16b	C-6, -7, -8
b	5.41 s		H-16a	C-6, -7, -8
17	1.38 s	24.3 (q)		C-10, -11, -12
18	1.95 m	26.7 (d)	H-14, H_3-19, H_3-20	C-13, -14, -19, -20
19	0.93 d (6.8)	21.6 (q)	H-18	C-14, -18, -20
20	0.71 d (6.8)	16.3 (q)	H-18	C-14, -18, -19
3-OAc		169.8 (s)		
	1.91 s	22.6 (q)		Acetate carbonyl

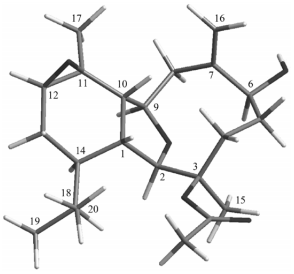
a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *c*) *J* values (in hertz) in parentheses. *d*) Attached protons were deduced by DEPT and HMQC experiments. *e*) n.o.=not observed.

molecular formula $C_{20}H_{32}O_4$, implying five degrees of unsaturation. The IR absorption of **2** showed the presence of a hydroxy group at 3387 cm^{-1}. By comparison of the 1D- and 2D-NMR data of **2** (Table 3) with those of **1**, it was found that one proton of the C-4 methylene in **1** was replaced with a hydroxy group in **2**. In addition, by comparison of the NOESY correlations of **2** with those of **1**, H-4 exhibited correlations with H-1 and H-10, indicating that the 4-hydroxy

group in **2** should be α-oriented and the remaining chiral centers of **2** were confirmed to be the same as those of **1**. Based on the above observations, compound **2** was found to be the 4α-hydroxycladieunicellin A.

Cladieunicellin C (**3**) was isolated as a colorless oil and had the molecular formula $C_{22}H_{34}O_5$ according to its HR-ESI-MS at *m/z* 401.2302 (Calcd, $C_{22}H_{34}O_5Na$, 401.2304). The IR spectrum of **3** showed bands at 3438 and 1732 cm^{-1}, consistent with the presence of hydroxy and ester carbonyl groups. From the ^{1}H- and ^{13}C-NMR spectra of **3** (Table 4), the presence of an acetoxy group (δ_H 1.91, 3H, s; δ_C 169.8, s; 22.6, q) was deduced. A trisubstituted epoxide containing a methyl substituent was deduced from the signals of an oxymethine (δ_H 3.11, 1H, br s, H-12; δ_C 61.4, d, C-12), an oxygen-bearing quaternary carbon (δ_C 55.6, s, C-11), and a methyl singlet at δ_H 1.38 (3H, s, H_3-17). From the ^{1}H–^{1}H COSY and HMBC correlations (Table 4), the epoxy group positioned at C-11/C-12 was established. It was found that the NMR data of **3** were similar to those of **1**. However, the 1D- and 2D-NMR spectra revealed that the signals corresponding to the 3-hydroxy and C-11/C-12 olefin in **1** were not present but had been replaced by those of an acetoxy group and an epoxy group in **3**, respectively. Based on the correlations observed in the NOESY experiment of **3** (Table 5), the relative stereochemistry of **3** was confirmed to be the same as that of **1**, and the signal of H_3-17 showed a correlation with H-9, indicating the 11,12-epoxy group should be β-oriented.

Table 5. The Stereoview of **3** (Generated from Computer Modeling) and the Calculated Distances (Å) between Selected Protons Having Key NOESY Correlations

Cladieunicellin C (**3**)	H/H	(Å)
	H-1/H-4β	2.39
	H-1/H-10	2.36
	H-2/H-14	2.55
	H-2/H_3-15	2.44
	H-6/H_3-15	2.59
	H-8α/H-9	2.45
	H-8β/H-9	2.57
	H-9/H-14	2.79
	H-9/H_3-17	2.60

Cladieunicellin D (**4**) was obtained as a colorless oil. The HR-ESI-MS of **4** exhibited a peak at *m/z* 375.2146, its molecular formula is $C_{20}H_{32}O_5$ based on the pseudomolecular ion of $[M+Na]^+$. Thus five degrees of unsaturation were deduced for **4**. The IR absorptions at 3447 and 1715 cm^{-1} revealed the presence of hydroxy and ketone groups in **4**. Resonances in the ^{13}C-NMR spectrum of **4** showed the presence of 20 carbon signals (Table 6), which were characterized by DEPT and heteronuclear multiple-quantum coherence (HMQC) spectra as five methyls, three sp^3 methylenes, seven sp^3 methines (including three oxymethines), two sp^3 oxygen-bearing quaternary carbons, an sp^2 methine, an sp^2 quaternary carbon, and an sp^2 carbonyl. A ketone carbonyl at δ_C 212.2 (s, C-6) was assigned from the ^{13}C-NMR spectrum. On the basis of the above data, eunicellin **4** was found to be tricyclic. The ^{1}H-NMR spectrum of **4** (Table 6) showed two tertiary methyls bonded to oxygenated carbons (δ_H 1.31, 1.28, each 3H×s, H_3-15, H_3-16) and two secondary methyls (δ_H 0.93, 0.77, each 3H, d, J=6.8 Hz, H_3-19, H_3-20) of an isopropyl moiety. Signals at δ_H at 2.20 (1H, m, H-1), 4.23 (1H, s, H-2), 4.02 (1H, ddd, J=12.0, 8.0, 4.0 Hz, H-9), and 2.72 (1H, br t, J=8.0 Hz, H-10) and δ_C 43.4 (d, C-1), 83.5 (d, C-2), 80.0 (d, C-9), and 50.2 (d, C-10), suggested the presence of a tetrahydrofuran structural unit.

The ^{1}H–^{1}H COSY and HMBC correlations were used to

Table 6. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **4**

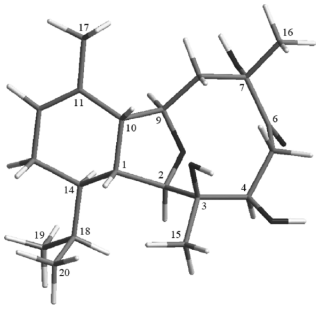
C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1	2.20 m	43.4 (d)[d]	H-2, H-10, H-14	C-3, -9, -10, -13, -14
2	4.23 s	83.5 (d)	H-1	C-1, -3, -9, -10, -14, -15
3		75.3 (s)		
4	3.87 dd (8.0, 7.2)[c]	76.3 (d)	H_2-5	C-6
5α	2.33 dd (13.6, 8.0)	33.7 (t)	H-4, H-5β	C-3, -4, -6
β	3.58 d (13.6)		H-4, H-5α	C-4, -6
6		212.2 (s)		
7		78.1 (s)		
8α	2.24 dd (14.0, 4.0)	48.0 (t)	H-8β, H-9	C-7
β	2.63 dd (14.0, 12.0)		H-8α, H-9	C-9, -10
9	4.02 ddd (12.0, 8.0, 4.0)	80.0 (d)	H_2-8, H-10	n.o.[e]
10	2.72 br t (8.0)	50.2 (d)	H-1, H-9	C-8, -9, -11, -12
11		131.5 (s)		
12	5.45 m	121.4 (d)	H_2-13, H_3-17	C-13, -17
13α	1.37 m	22.6 (t)	H-12, H-13β, H-14	n.o.
β	1.98 m		H-12, H-13α, H-14	n.o.
14	1.40 m	38.1 (d)	H-1, H_2-13, H-18	n.o.
15	1.31 s	26.6 (q)		C-2, -3, -4
16	1.28 s	25.5 (q)		C-6, -7, -8
17	1.62 d (1.2)	22.9 (q)	H-12	C-10, -11, -12
18	1.90 m	27.6 (d)	H-14, H_3-19, H_3-20	C-1, -14, -19, -20
19	0.93 d (6.8)	21.7 (q)	H-18	C-14, -18, -20
20	0.77 d (6.8)	15.1 (q)	H-18	C-14, -18, -19
4-OH	3.81 d (7.2)		H-4	n.o.
7-OH	4.08 s			C-8

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *c*) *J* values (in hertz) in parentheses. *d*) Attached protons were deduced by DEPT and HMQC experiments. *e*) n.o.=not observed.

establish the molecular skeleton of **4** (Table 6). The downfield chemical shifts for H_3-15 (δ_H 1.31) and H_3-16 (δ_H 1.28) determined the position of hydroxy groups at C-3 and C-7, respectively. The placement of a ketone group at C-6 was supported by the HMBC correlations between H-4, H_2-5, and H_3-16 and the carbon resonating at δ_C 212.2 (s, C-6). Therefore the planar structure of **4** was established unambiguously.

In the NOESY spectrum of **4** (Table 7), observation of the NOESY correlations between H-10 with a proton of C-8 methylene (δ_H 2.63) and H-1 and H_3-16 with H_2-8 (δ_H 2.24, 2.63) suggested the β-orientation of H-1, H-10, and H_3-16. Moreover, H-2 correlated with H_3-15, and H_3-15 exhibited a correlation with H-4, suggesting that H-2, H-4, and H_3-15 were α-oriented. H-14 exhibited a correlation with H-2, indicating that the isopropyl group at C-14 was β-oriented. Thus the structure of diterpenoid **4** was established.

Table 7. The Stereoview of **4** (Generated from Computer Modeling) and the Calculated Distances (Å) between Selected Protons Having Key NOESY Correlations

Cladieunicellin D (**4**)	H/H	(Å)
	H-1/H-10	2.33
	H-2/H-14	2.71
	H-2/H_3-15	2.37
	H-4/H_3-15	2.67
	H-8α/H-9	2.42
	H-8α/H_3-16	2.51
	H-8β/H-10	2.28
	H-8β/H_3-16	2.54

Cladieunicellin E (**5**) was isolated as a colorless oil that gave a pseudomolecular ion peak at *m/z* 419.2407 $[M+Na]^+$ in HR-ESI-MS, appropriate for the molecular formula $C_{22}H_{36}O_6$ requiring five degrees of unsaturation. The gross structure of **5** was established by 1D- and 2D-NMR experiments (Table 8) and was found to be similar to that of **4**. The ^{1}H- and ^{13}C-NMR spectra of **5** displayed a set of signals at δ_H 2.19 (1H, m, H-1), 3.56 (1H, s, H-2), 3.98 (1H, ddd, J=10.4, 6.8, 4.0 Hz, H-9), and 3.05 (1H, br t, J=6.8 Hz, H-10) and δ_C 42.8 (d, C-1), 90.0 (d, C-2), 75.0 (d, C-9), and 52.8 (d, C-10), which were determined to be the signals of a tetrahydrofuran functionality by comparison with the proton and carbon chemical shifts of **4**. In the ^{13}C-NMR spectrum of **5**, an additional ester carbonyl resonance appeared at δ_C 170.2 (s) and further confirmed the presence of an ester group in **5**. In the ^{1}H-NMR spectrum, an acetyl methyl was observed (δ_H 2.01, 3H, s). The location of the acetoxy group at C-11 could be confirmed by comparing the related ^{13}C-NMR data with those of the known eunicellin derivatives klysimplexins A—G,[8] which were found to possess 11β-acetoxy groups in their structure.

In our previous study, the absolute configuration of a known eunicellin analogue, cladielloide A (**6**), which was isolated from the same organism, was determined using the modified Mosher's method.[9] Thus the new eunicellins cladieunicellins A—E (**1**—**5**) are assumed to have the same absolute configuration as cladielloide A (**6**) because these compounds were isolated from the same organism.

The cytotoxicity of metabolites **1**—**5** toward a limited panel of tumor cell lines, including DLD-1, HL-60, CCRF-CEM (human T-cell acute lymphoblastic leukemia), and P388D1 cells was evaluated (Table 9). The results showed

Table 8. ^{1}H- and ^{13}C-NMR Data, ^{1}H–^{1}H COSY, and HMBC Correlations for Diterpenoid **5**

C/H	δ_H [a]	δ_C [b]	^{1}H–^{1}H COSY	HMBC (H→C)
1	2.19 m [c]	42.8 (d) [e]	H-2, H-10, H-14	C-9
2	3.56 s	90.0 (d)	H-1	C-1, -3, -9, -10, -14, -15
3		72.7 (s)		
4α	2.12 m	37.7 (t)	H-4β, H_2-5	C-15
β	1.78 dd (14.4, 8.4) [d]		H-4α, H_2-5	C-2, -3, -6, -15
5α	1.98 m	29.8 (t)	H_2-4, H-5β	C-3, -4, -6
β	3.22 br t (12.0)		H_2-4, H-5α	C-3, -4, -6
6		213.3 (s)		
7		78.0 (s)		
8α	2.20 m [c]	47.8 (t)	H-8β, H-9	C-6, -7, -9
β	2.60 dd (14.0, 10.4)		H-8α, H-9	C-9, -10
9	3.98 ddd (10.4, 6.8, 4.0)	75.0 (d)	H_2-8, H-10	n.o. [f]
10	3.05 br t (6.8)	52.8 (d)	H-1, H-9	C-8, -9, -11, -12
11		82.1 (s)		
12α	2.33 dd (10.4, 2.8)	31.0 (t)	H-12β, H_2-13	n.o.
β	1.32 m		H-12α, H_2-13	C-17
13	1.37 m (2H)	17.7 (t)	H_2-12, H-14	n.o.
14	1.19 m	41.5 (d)	H-1, H_2-13, H-18	n.o.
15	1.16 s	29.0 (q)		C-2, -3, -4
16	1.24 s	25.7 (q)		C-6, -7, -8
17	1.44 s	24.7 (q)		C-10, -11, -12
18	1.65 m	28.6 (d)	H-14, H_3-19, H_3-20	C-14, -19, -20
19	0.91 d (6.8)	21.5 (q)	H-18	C-14, -18, -20
20	0.78 d (6.8)	15.0 (q)	H-18	C-14, -18, -19
7-OH	4.20 s			C-6, -7, -8
11-OAc		170.2 (s)		
	2.01 s	22.6 (q)		Acetate carbonyl

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. *b*) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. *c*) The proton signals for H-1 and H-8α are overlapped. *d*) *J* values (in hertz) in parentheses. *e*) Attached protons were deduced by DEPT and HMQC experiments. *f*) n.o.=not observed.

Table 9. Cytotoxic Data of Diterpenoids **1**—**5**

Compounds	Cell lines IC_{50} (μg/ml)			
	DLD-1	HL-60	CCRF-CEM	P388D1
1	26.5	>40	>40	>40
2	2.0	>40	>40	>40
3	>40	18.4	>40	>40
4	>40	>40	>40	>40
5	>40	2.7	31.1	>40
Doxorubicin[a)]	0.09	0.03	0.18	0.11

a) Doxorubicin was used as a reference compound.

Table 10. Inhibitory Effects of Diterpenoids **1** and **3**—**5** on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to FMLP/CB

Compounds	Superoxide anion IC_{50} (μg/ml)[a)] or (Inh %)[b)]	Elastase release (Inh %)
1	(22.8±6.3)	(25.9±6.7)
3	8.1±0.3	(49.4±6.2)
4	(41.7±6.2)	(48.2±7.0)
5	(36.9±5.2)	(12.7±7.3)
DPI[c)]	0.8±0.2	
Elastatinal[c)]		30.8±5.7

a) Concentration necessary for 50% inhibition (IC_{50}). *b*) Percentage of inhibition (Inh %) at 10 μg/ml concentration. *c*) DPI (diphenylene indonium) and elastatinal were used as reference compounds.

that cladieunicellins B (**2**) and E (**5**) exhibited significant cytotoxicity against DLD-1 and HL-60 cells. The *in vitro* anti-inflammatory effects of metabolites **1** and **3**—**5** were tested (Table 10). In this assay, metabolite **3** displayed a significant inhibitory effect against superoxide anion generation by human neutrophils.

Experimental

General Optical rotation values were measured with a JASCO P-1010 digital polarimeter. IR spectra were obtained on a Varian Diglab FTS 1000 Fourier transform-IR (FT-IR) spectrophotometer. NMR spectra were recorded on a Varian Mercury Plus 400 FT-NMR at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR, respectively, in $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ_H 7.26 ppm). ^{13}C-NMR spectra were referenced to the center peak of $CDCl_3$ at δ_C 77.1 ppm. ESI-MS and HR-ESI-MS data were recorded on a Bruker Apex II mass spectrometer. Column chromatography was performed on silica gel (230—400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system composed of a Hitachi L-7100 pump, Hitachi photo diode array detector L-7455, and Rheodyne 7725 injection port. A normal-phase column (Hibar 250×25 mm, LiChrospher Si 60, 5 μm) was used for HPLC.

Animal Material *Cladiella* sp. were collected from Indonesia in 2004 and stored in a freezer until extraction. A voucher specimen was deposited in the National Museum of Marine Biology and Aquarium, Taiwan (NMMBA). This organism was identified by comparison with previous descriptions.[10,11)]

Extraction and Isolation The sliced bodies of *Cladiella* sp. (wet weight 924 g) were extracted with a mixture of MeOH and CH_2Cl_2 (1 : 1), and the residue was partitioned between EtOAc and H_2O. The EtOAc layer was subjected to silica gel column chromatography and eluted using a mixture of *n*-hexane and EtOAc (stepwise, 100 : 1 to pure EtOAc) to obtain the 19 fractions A—S. Fraction G was purified over silica gel using a mixture of *n*-hexane/EtOAc (stepwise, 100 : 1 to pure EtOAc) to obtain the 21 fractions G1—21. Fractions G12 and G14 were separated using normal-phase HPLC using a mixture of *n*-hexane/ethyl acetate to afford **2** (1.3 mg, 7 : 1). Fraction G19 was separated using normal-phase HPLC using CH_2Cl_2/acetone (13 : 1) to afford **3** (1.3 mg), **1** (10.2 mg), **5** (2.6 mg), and **4** (1.2 mg), respectively.

Cladieunicellin A (**1**): Colorless oil; $[\alpha]_D^{23}$ −10° (*c*=0.51, $CHCl_3$); IR (neat) ν_{max} 3427 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data: see Table 1. ESI-MS *m*/*z*: 343 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 343.2246 (Calcd for $C_{20}H_{32}O_3Na$, 343.2249).

Cladieunicellin B (**2**): Colorless oil; $[\alpha]_D^{23}$ −47° (*c*=0.07, $CHCl_3$); IR (neat) ν_{max} 3387 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data: see Table 3. ESI-MS *m*/*z*: 359 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 359.2196 (Calcd for $C_{20}H_{32}O_4Na$, 359.2198).

Cladieunicellin C (**3**): Colorless oil; $[\alpha]_D^{23}$ −29° (*c*=0.07, $CHCl_3$); IR (neat) ν_{max} 3438, 1732 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data: see Table 4. ESI-MS *m*/*z*: 401 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 401.2302 (Calcd for $C_{22}H_{34}O_5Na$, 401.2304).

Cladieunicellin D (**4**): Colorless oil; $[\alpha]_D^{23}$ −5° (*c*=0.06, $CHCl_3$); IR (neat) ν_{max} 3447, 1715 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data: see Table 6. ESI-MS *m*/*z*: 375 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 375.2146 (Calcd for $C_{20}H_{32}O_5Na$, 375.2147).

Cladieunicellin E (**5**): Colorless oil; $[\alpha]_D^{23}$ +10° (*c*=0.13, $CHCl_3$); IR (neat) ν_{max} 3436, 1730 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) data: see Table 8. ESI-MS *m*/*z*: 419 $(M+Na)^+$; HR-ESI-MS *m*/*z*: 419.2407 (Calcd for $C_{22}H_{36}O_6Na$, 419.2409).

Cytotoxicity Testing The cytotoxicity of compounds **1**—**5** was assayed with a modification of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method. Cytotoxicity assays were carried out according to the procedures described previouly.[12,13)]

Human Neutrophil Superoxide Generation and Elastase Release Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide generation and elastase release were carried out according to the procedures described previously.[14,15)] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Elastase release experiments were performed using MeO-Suc-Ala-Ala-Pro-Valp-nitroanilide as the elastase substrate.

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