Cladielloides A and B: New Eunicellin-Type Diterpenoids from an Indonesian Octocoral Cladiella sp.

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Abstract: Two new eunicellin-type diterpenoids, cladielloides A (1) and B (2), which were found to possess a 2-hydroxybutyroxy group in their structures, were isolated from an Indonesian octocoral identified as Cladiella sp. The structures of eunicellins 1 and 2 were elucidated by spectroscopic methods. Cladielloide B (2) exhibited moderate cytotoxicity toward CCRF-CEM tumor cells and this compound displayed significant inhibitory effects on superoxide anion generation and elastase release by human neutrophils.
Keywords: cladielloide; eunicellin; octocoral; cytotoxicity; superoxide anion; elastase

1. Introduction

Previous chemical investigations on the octocorals belonging to the genus Cladiella have resulted in a series of interesting eunicellin-based (2,11-cyclized cembranoid) diterpenoids [1–6], and the compounds of this type have been found to possess complex structures and various bioactivities [1–3,5–11]. In continuation of our search for bioactive substances from the marine invertebrates distributed in the tropical West Pacific Ocean, an Indonesian octocoral identified as Cladiella sp. was studied, and its organic extract exhibited cytotoxicity toward DLD-1 (human colorectal adenocarcinoma), HL-60 (human promyelocytic leukemia cells), and P388D1 (macrophage-like murine tumor cells), with IC₅₀ = 2.7, 8.9, 7.2 μg/mL, respectively. Two new eunicellins, cladielloides A (1) and B (2), were isolated from this marine organism. In this paper, we report the isolation, structure determination, and bioactivity of the above new diterpenoids 1 and 2 (Scheme 1).

Scheme 1. The structures of cladielloides A (1) and B (2).

2. Results and Discussion

Cladielloide A (1) was isolated as a colorless oil and the molecular formula for this compound was determined to be C₂₆H₄₀O₇ (seven degrees of unsaturation) by HRESIMS (C₂₆H₄₀O₇ + Na, m/z 487.2674, calculated 487.2672). The IR spectrum of 1 showed bands at 3460 and 1734 cm⁻¹, consistent with the presence of hydroxy and ester groups. From the ¹H and ¹³C NMR spectra (Table 1), 1 was found to possess a trisubstituted olefin (δ₁ 5.43, 1H, m, H-12; δć 132.1, s, C-11; 122.2, d, C-12), an exocyclic carbon–carbon double bond (δ₁ 5.21, 1H, s, H-16a; 5.58, 1H, s, H-16b; δć 147.6, s, C-7; 115.2, t, C-16), an acetoxy group (δ₁ 2.14, 3H, s; δć 20.6, q; 171.1, s), and a 2-hydroxybutyrate (δ₁ 1.03, 3H, t, J = 7.2 Hz; 1.91, 2H, m; 4.86, 1H, dd, J = 6.8, 6.0 Hz; δć 9.3, q; 24.3, t; 74.1, d; 171.4, s) moiety. Thus, from the above data, four degrees of unsaturation were accounted for and compound 1 must be a tricyclic compound.

In the ¹H NMR spectrum of 1, two doublets at δ₁ 0.92 and 0.83 (each 3H, d, J = 6.4 Hz, H₃-19 and H₃-20) were deduced from two methyls of an isopropyl group. A singlet of the tertiary methyl bonded
to an oxygenated carbon was due to the resonance of signal at $\delta_{H}$ 1.37 (3H, s, H$_{3}$-15). In addition, a suite of resonances of proton signals at $\delta_{H}$ 2.74 (1H, m, H-1), 2.63 (1H, br s, H-10), 3.86 (1H, d, J = 8.0 Hz, H-2), 4.16 (1H, dt, J = 3.6, 3.2 Hz, H-9), and carbon signals at $\delta_{C}$ 39.7 (d, C-1), 44.6 (d, C-10), 87.1 (d, C-2), and 81.3 (d, C-9), indicated the presence of a tetrahydrofuran structural unit. Based on the above data, the proposed skeleton of 1 was suggested to be a eunicellin-based metabolite.

Table 1. $^1$H and $^{13}$C NMR data, $^1$H–$^1$H COSY, and HMBC correlations for diterpenoid 1.

| C/H | $^1$H a | $^{13}$C b | $^1$H–$^1$H COSY | HMBC (H→C) |
|-----|--------|------------|-----------------|-------------|
| 1   | 2.74 m | 39.7 (d) d | H-2, H-10, H-14 | C-2, -3, -10, -11, -14 |
| 2   | 3.86 d (8.0) c | 87.1 (d) | H-1 | C-3, -4, -9, -14, -15 |
| 3   | 74.6 (s) |          |     |             |
| 4   | 5.14 dd (4.4, 3.6) | 74.4 (d) | H-2-5 | C-3, -6, -15, -23 |
| 5α  | 2.97 ddd (16.0, 4.4, 2.8) | 37.2 (t) | H-4, H-5β, H-6 | C-3, -4 |
| β   | 1.75 ddd (16.0, 5.6, 3.6) | 72.6 (d) | H-4, H-5α, H-6 | C-4, -6, -7 |
| 6   | 4.21 br s | 72.6 (d) | H-2-5, OH-6 | n.o. e |
| 7   |        | 147.6 (s) |     |             |
| 8   | 2.35 br d (2.4) | 40.0 (t) | H-9 | C-6, -7, -9, -10, -16 |
| 9   | 4.16 dt (3.6, 3.2) | 81.3 (d) | H-2-8, H-10 | n.o. |
| 10  | 2.63 br s | 44.6 (d) | H-1, H-9 | C-11 |
| 11  |        | 132.1 (s) |     |             |
| 12  | 5.43 m | 122.2 (d) | H-2-13, H-3-17 | n.o. |
| 13α | 2.10 m | 22.8 (t) | H-12, H-13β, H-14 | n.o. |
| β  | 1.97 m | 39.0 (d) | H-1, H-2-13, H-18 | n.o. |
| 14  | 1.58 m | 22.4 (q) | H-16a | C-2, -3, -4 |
| 15  | 1.37 s | 115.2 (t) | H-16b | C-6, -8 |
| 16a | 5.21 s | 22.0 (q) | H-12 | C-10, -11, -12 |
| b   | 5.58 s | 28.8 (d) | H-14, H-3-19, H-3-20 | C-1, -14 |
| 17  | 1.68 d (0.8) | 21.3 (q) | H-18 | C-14, -18, -20 |
| 18  | 1.62 m | 20.5 (q) | H-18 | C-14, -18, -19 |
| 19  | 0.92 d (6.4) | 20.5 (q) | H-18 | C-14, -18, -19 |
| 20  | 0.83 d (6.4) | 20.5 (q) | H-18 | C-14, -18, -19 |
| OH-6 | 2.84 d (7.2) | 171.1 (s) | H-6 | n.o. |
| 3-OC(O)CH$_3$ | 21 22 | 171.4 (s) |     |             |
| 21  | 2.14 s | 20.6 (q) |     | C-21 |
| 3-OCC(=O)CH$_2$CH$_3$ | 24 25 26 23 | 171.0 (s) |     |             |
| 24  | 4.86 dd (6.8, 6.0) | 74.1 (d) | H-2-25 | C-23, -25, -26 |
| 25  | 1.91 m | 24.3 (t) | H-24, H-3-26 | C-23, -24, -26 |
| 26  | 1.03 t (7.2) | 9.3 (q) | H-2-25 | C-24, -25 |

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.

From the $^1$H–$^1$H COSY spectrum of 1, it was possible to identify the separate spin systems among H-1/H-2; H-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 HMBC correlations between H-1/C-2, -3, -10; H-2/C-3, -4, -9; H-4/C-3, -6; H$_{2}$-5/C-3, -4, -6, -7; and H$_{2}$-8/C-6, -7, -9, -10, established the connectivity from C-1 to C-10 within the ten-membered ring
An exocyclic carbon-carbon double bond at C-7 was confirmed by the HMBC correlations between H2-16/C-6, -7, -8 and H2-8/C-16. The hydroxy proton signal at δH 2.84 was revealed by its 1H–1H COSY correlations to H-6 (δH 4.21), indicating its attachment to C-6. The location of 2-hydroxybutyrate group in 1 was confirmed by an HMBC correlation between H-4 (δH 5.14) and the 2-hydroxybutyrate carbonyl (δC 171.4, s). Thus, the remaining acetate ester was at C-3, an oxygenated quaternary carbon which bonded to the C-15 tertiary methyl and is confirmed by the HMBC correlations between H-2/C-15; H-4/C-15; and H3-15/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 ten-membered ring at C-1 and C-10, was elucidated by the 1H–1H COSY correlations between H-12/H2-13/H-14/H-1; H-14/H-18; and H-18/H3-19(H3-20) and further supported by the HMBC correlations between H-1/C-11, -14; H-2/C-14; H-10/C-11; H3-17/C-10; and H-18/C-1. The vinyl methyl at C-11 was confirmed by the HMBC correlations between H3-17/C-10, -11, -12 and further supported by the allylic coupling between the olefin proton H-12 and H3-17 (J = 0.8 Hz). Therefore, the planar structure of 1 was established.

The relative configuration of 1 was elucidated from the interactions observed in a NOESY experiment. In the NOESY experiment of 1 (Table 2), the correlations between H-1 with H-4 and H-10, indicated that these protons are situated on the same face and assigned as β protons. H-2 exhibited interactions with H-14 and H3-15 and no correlation was found between H-1 and H-2, indicating that H-2, H-14, and Me-15 should be α-oriented. H-6 correlated with one proton of C-5 methylene (δH 2.97), but not with H-4, reflecting the α-orientation of H-6. Furthermore, H-9 correlated with H2-8 and H3-17. From consideration of molecular models, H-9 was found to be reasonably close to H2-8 and H3-17, when H-9 was α-oriented in 1. The stereochemistry of 1 was established using a NOESY experiment. The NOESY experiment of 1 (Table 2), the correlations between H-1 with H-4 and H-10, indicated that these protons are situated on the same face and assigned as β protons. H-2 exhibited interactions with H-14 and H3-15 and no correlation was found between H-1 and H-2, indicating that H-2, H-14, and Me-15 should be α-oriented. H-6 correlated with one proton of C-5 methylene (δH 2.97), but not with H-4, reflecting the α-orientation of H-6. Furthermore, H-9 correlated with H2-8 and H3-17. From consideration of molecular models, H-9 was found to be reasonably close to H2-8 and H3-17, when H-9 was α-oriented in 1. The relative configuration of 1 was elucidated from the interactions observed in a NOESY experiment. In the NOESY experiment of 1 (Table 2), the correlations between H-1 with H-4 and H-10, indicated that these protons are situated on the same face and assigned as β protons. H-2 exhibited interactions with H-14 and H3-15 and no correlation was found between H-1 and H-2, indicating that H-2, H-14, and Me-15 should be α-oriented. H-6 correlated with one proton of C-5 methylene (δH 2.97), but not with H-4, reflecting the α-orientation of H-6. Furthermore, H-9 correlated with H2-8 and H3-17. From consideration of molecular models, H-9 was found to be reasonably close to H2-8 and H3-17, when H-9 was α-oriented in 1.

Table 2. The stereoview of 1 (generated from computer modeling) and the calculated distances (Å) between selected protons having key NOESY correlations.

| Cladiellioide A (1) | H/H         | Å  |
|---------------------|-------------|----|
| H-1/H-4             | 2.59        |
| H-1/H-10            | 2.33        |
| H-2/H-14            | 2.45        |
| H-2/H3-15           | 2.32        |
| H-6/H-5α            | 2.44        |
| H-8α/H-9            | 2.44        |
| H-8β/H-9            | 2.50        |
| H-9/H3-17           | 2.61        |

In order to determine the absolute configuration, the eunicellin 1 was treated with (−) or (+)-MTPA chloride to yield the (S)- and (R)-MTPA esters 1a and 1b, respectively [12–14]. Comparison of the 1H NMR chemical shifts for 1a and 1b (Δ values shown in Figure 1) led to the assignment of the S-configuration at C-6. The C-24 hydroxy group in the 2-hydroxybutyrate moiety was also assigned as R-configuration. Therefore, the absolute configurations of all chiral centers of 1 were assigned as 1R, 2R, 3R, 4S, 6S, 9R, 10R, 14R, 24R.
Figure 1. The key $^1$H NMR chemical shift differences $\Delta \delta (\delta_S - \delta_H)$ in ppm for the MTPA esters of 1.

Cladielloide B (2) had the same molecular formula as that of 1, C$_{26}$H$_{40}$O$_7$, as determined by HRESIMS (C$_{26}$H$_{40}$O$_7$ + Na, m/z 487.2675, calculated 487.2672). The spectral data (1D, 2D NMR (Table 3), IR, and MS) were similar to those of 1. However, the polarity of 2, which was checked by TLC, was substantially different from that of 1, indicating that these two compounds are isomers. In the $^1$H NMR spectrum of 2, an acetate methyl was observed at $\delta$H 2.14 (3H, s). The additional acyl group was found to be a 2-hydroxybutyrate group, which showed six contiguous protons ($\delta$H 1.02, 3H, t, J = 7.2 Hz; 1.91, 2H, m; 4.87, 1H, dd, J = 6.8, 6.0 Hz). The $^{13}$C NMR signal at $\delta$C 170.2 (s) correlated with the signal of an oxymethine proton at $\delta$H 4.87 in the HMBC spectrum and was consequently assigned as the carbon atom of the 2-hydroxybutyrate carbonyl. A correlation observed in the HMBC experiment of 2 further revealed the connectivity between H-4 ($\delta$H 5.21) and the carbonyl carbon ($\delta$C 170.2) of 2-hydroxybutyrate unit and demonstrated the location of the 2-hydroxybutyrate to be at C-4. The position of acetoxy group at C-6 was also confirmed by the connectivity between the oxymethine proton at $\delta$H 4.66 (H-6) and the ester carbonyl at $\delta$C 171.6 (s) in the HMBC spectrum of 2. Thus, the remaining hydroxy group should be positioned at C-3. In addition, by comparison of the NOESY correlations of 2 with those of 1, the chiral centers of 2 were confirmed to be the same as those of 1.

Table 3. $^1$H and $^{13}$C NMR data, $^1$H–$^1$H COSY, and HMBC correlations for diterpenoid 2.

| Position | $^1$H a | $^{13}$C b | $^1$H–$^1$H COSY | HMBC (H→C) |
|----------|---------|-----------|----------------|-------------|
| 1        | 2.51 m  | 40.6 (d) d| H-2, H-10, H-14| C-10        |
| 2        | 3.90 d (3.6) c| 88.1 (d) | H-1 | C-1, -3, -4 |
| 3        | 74.8 (s) |           |                |             |
| 4        | 5.21 dd (8.0, 4.0) | 73.8 (d) | H-2-5 | C-6, -21 |
| 5α       | 2.48 m  | 34.2 (t)  | H-4, H-5β, H-6 | C-6, -7    |
| β        | 1.97 m  |           | H-4, H-5α, H-6 | n.o. e      |
| 6        | 4.66 dd (8.8, 3.2) | 83.8 (d) | H-2-5 | C-4, -7, -16, -25 |
| 7        | 144.2 (s) |         |                |             |
| 8α       | 2.65 dd (14.0, 4.8) | 41.4 (t) | H-8β, H-9 | C-6, -7, -9, -10, -16 |
| β        | 2.46 dd (14.0, 2.0) | 41.4 (t) | H-8α, H-9 | C-6, -7, -9, -10, -16 |
Table 3. Cont.

|    |  
|----|---|
| 9  | 4.06 br s | 82.4 (d) H₂-8, H₁₀ | n.o. |
| 10 | 2.58 br s | 44.7 (d) H₁-1, H₉ | C-8, -9, -11 |
| 11 | 131.1 (s) | | |
| 12 | 5.49 m    | 123.1 (d) H₂-13, H₃-17 | n.o. |
| 13α| 2.01 m    | 22.9 (t) H₁₂, H₁-13β, H₁-14 | n.o. |
| β | 1.80 m    | | |
| 14 | 1.39 m    | 39.8 (d) H₁-1, H₂-13, H₁₈ | C-2 |
| 15 | 1.33 s    | 22.8 (q) | C-2, -3, -4 |
| 16a| 5.26 s    | 117.7 (t) H₁-16b | C-6, -8 |
| b | 5.47 s    | | |
| 17 | 1.69 d (1.2) | 22.8 (q) H₁₂ | C-10, -11, -12 |
| 18 | 1.80 m    | 27.8 (d) H₁₄, H₃-19, H₃-20 | C-14, -19, -20 |
| 19 | 0.94 d (6.8) | 21.7 (q) H₁₈ | C-14, -18, -20 |
| 20 | 0.77 d (6.8) | 17.5 (q) H₁₈ | C-14, -19, -20 |

O₂22 23 24
4-OCCH₂CH₂CH₃
21 OH
6-OC(O)CH₃
25 26

2.14 s 20.6 (q) C-25

a Spectra measured at 400 MHz in CDCl₃ at 25 °C; b Spectra measured at 100 MHz in CDCl₃ 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.

The cytotoxicity of metabolites 1 and 2 toward various tumor cell lines, including DLD-1, HL-60, CCRF-CEM (human T-cell acute lymphoblastic leukemia), and P388D1 cells was evaluated. The results, in Table 4, show that eunicellin 2 exhibited moderate cytotoxicity toward CCRF-CEM cells.

Table 4. Cytotoxic data of diterpenoids 1 and 2.

| Compound | Cell lines IC₅₀ (µg/mL) | DLD-1 | HL-60 | CCRF-CEM | P388D1 |
|----------|-------------------------|-------|-------|----------|--------|
| 1        | >40                     | >40   | >40   | >40      | >40    |
| 2        | 10.2                    | >40   | 4.7   | >40      | 0.11   |
| Doxorubicin a | 0.09                | 0.03  | 0.18  | 0.11     |        |

a Doxorubicin was used as a reference compound.

The in vitro anti-inflammatory effects of metabolites 1 and 2 were tested. Metabolite 2 displayed significant inhibitory effects on superoxide anion generation and elastase release by human neutrophils at 10 µg/mL (Table 5).
Table 5. Inhibitory effects of diterpenoids 1 and 2 on superoxide anion generation and elastase release by human neutrophils in response to FMLP/CB.

| Compound | Superoxide anion IC₅₀ (μg/mL) a or (Inh. %) b | Elastase release IC₅₀ (μg/mL) or (Inh. %) |
|----------|-----------------------------------------------|------------------------------------------|
| 1        | (20.5 ± 5.0)                                  | (27.1 ± 4.8)                             |
| 2        | 5.9 ± 0.7                                     | 6.5 ± 1.9                                |
| DPI c    | 0.8 ± 0.2                                     | 30.8 ± 5.7                               |
| Elastatinal c |                                          |                                          |

a Concentration necessary for 50% inhibition (IC₅₀); b Percentage of inhibition (Inh %) at 10 μg/mL; c DPI (diphenylene indonium) and elastatinal were used as reference compounds.

3. Experimental

3.1. General Experimental Procedures

Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrometer. The NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ¹H and 100 MHz for ¹³C, in CDCl₃ at 25 °C. Proton chemical shifts were referenced to the residual CHCl₃ signal (δ₁H 7.26 ppm). ¹³C NMR spectra were referenced to the center peak of CDCl₃ at δ 77.1 ppm. ESIMS and HRESIMS 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 F₂₅₄ (0.25 mm, Merck) and spots were visualized by spraying with 10% H₂SO₄ solution followed by heating. HPLC was performed using a system comprised of a HITACHI L-7100 pump, a HITACHI photodiode array detector L-7455, and a RHEODYNE 7725 injection port. A normal phase column (Hibar 250 × 10 mm, Merck, silica gel 60, 5 μm,) was used for HPLC.

3.2. Animal Material

The octocoral 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 (NM MBA). This organism was identified by comparison with previous descriptions [15,16].

3.3. Extraction and Isolation

Slices of Cladiella sp. (wet weight 924 g) were extracted with a mixture of MeOH and CH₂Cl₂ (1:1) and the residue was partitioned between EtOAc and H₂O. 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 19 fractions A–S. Fractions K and N were repurified by normal phase HPLC, using the mixture of n-hexane/ethyl acetate to afford 2 (2.4 mg, 5.5:1) and 1 (7.9 mg, 3:1), respectively.

Cladielloide A (1). Colorless oil; [α]₁₀0° –24° (c 0.4, CHCl₃); IR (neat) νₘₐₓ 3460, 1734 cm⁻¹; ¹H (CDCl₃, 400 MHz) and ¹³C (CDCl₃, 100 MHz) NMR data, see Table 1; ESIMS m/z 487 (M + Na)⁺; HRESIMS m/z 487.2674 (calculated for C₂₆H₄₀O₇ + Na, 487.2672).
Cladielloide B (2). Colorless oil; [α]_D^20 = 10° (c 0.1, CHCl₃); IR (neat) v_max 3446, 1738 cm⁻¹; ^1H (CDCl₃, 400 MHz) and ^13C (CDCl₃, 100 MHz) NMR data, see Table 3; ESIMS m/z 487 (M + Na)^+; HRESIMS m/z 487.2675 (calculated for C₂₉H₄₀O₇ + Na, 487.2672).

3.4. Preparation of (S)- and (R)-MTPA Esters of Cladielloide A (1)

To a solution of 1 (1 mg) in pyridine (0.4 mL), R-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl (MPTA) chloride (25 μL) was added, and the mixture was allowed to stand for 24 h at room temperature. The reaction was quenched by addition of 1.0 mL of water, and the mixture was subsequently extracted with EtOAc (3 × 1.0 mL). The EtOAc-soluble layers were combined, dried over anhydrous MgSO₄ and evaporated. The residue was subjected to column chromatography over silica gel using n-hexane–EtOAc (13:2) to yield the (S)-MTPA ester, 1a (0.7 mg, 44%). The same procedure was used to prepare the (R)-MTPA ester, 1b (1.4 mg, 89%), from the reaction of (S)-MTPA chloride with 1 in pyridine. The key ^1H NMR chemical shift differences Δδ (δ_s − δ_h) in ppm for the MTPA esters of 1 are shown in Figure 1.

3.5. Cytotoxicity Testing

The cytotoxicity of compounds 1 and 2 was assayed with a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays was carried out according to the procedures described previously [17,18].

3.6. Human Neutrophil Superoxide Anion 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 [19,20]. 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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