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SHORT COMMUNICATION

Montiporic acid D, a new polyacetylene carboxylic acid from scleractinian coral *Montipora digitata*

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A new polyacetylene carboxylic acid named montiporic acid D (**1**) was isolated along with a known polyacetylene alcohol, (*Z*)-13,15-hexadecadien-2,4-diyn-1-ol (**2**) from scleractinian coral *Montipora digitata*. The structures of compounds were determined by analyses of NMR and MS spectra.

Keywords: scleractinian coral; *Montipora digitata*; montiporic acid; NMR spectrum

1. Introduction

The presence of unique polyacetylene carboxylic acids including montiporic acids A–C were reported in scleractinian corals belonging to *Montipora* genus (Higa et al. 1990; Coll et al. 1994; Fusetani et al. 1996; Bae et al. 2000; Alam et al. 2001; 2002). Montiporic acids A and B were isolated from eggs of *Montipora digitata* as antibacterial and cytotoxic substances (Fusetani et al. 1996). Other montiporic acid-related compounds including montiporic acid C were isolated from the tissues of *M. digitata* collected in Korea (Bae et al. 2000). As a result of recent chemical investigation, we isolated a new polyacetylene carboxylic acid, (*Z*)-2-(hexadeca-13,15-dien-2,4-diynyloxy)acetic acid, named montiporic acid D (**1**, Figure 1a) along with a known polyacetylene alcohol, (*Z*)-13,15-hexadecadien-2,4-diyn-1-ol (**2**, Figure 1a) from the tissue of *M. digitata* (Higa et al. 1990). Here we describe isolation and structural determination of compound **1** and identification of compound **2**.

2. Results and discussion

Tissue samples of *M. digitata* (160 g), collected on the coral reef of Sesoko Island (Okinawa, Japan), were extracted with MeOH. The concentrated extract was subjected to open column chromatography using synthetic hydrophobic resin with stepwise elution of 20%, 60% and 100% MeOH. The MeOH fraction was subjected to HPLC separation using an ODS column to yield compound **1** (12.6 mg) and compound **2** (17.6 mg).

Montiporic acid D (**1**) had a molecular formula of $C_{18}H_{24}O_3$ as established by HR-ESI-MS (*m/z* 311.1596 for $C_{18}H_{24}O_3Na$, calculated value 311.1623, Δ −2.63 mmu). The ^{1}H-NMR spectrum in CD_3OD revealed seven methylenes, two oxygenated methylenes, a disubstituted olefin and a terminal monosubstituted olefin. The structure of **1** was determined by further analysis of NMR data including HMBC, HMQC and COSY spectra. The COSY spectrum revealed the sequence of three methylenes (H-6–H-8) and the connection from three methylenes

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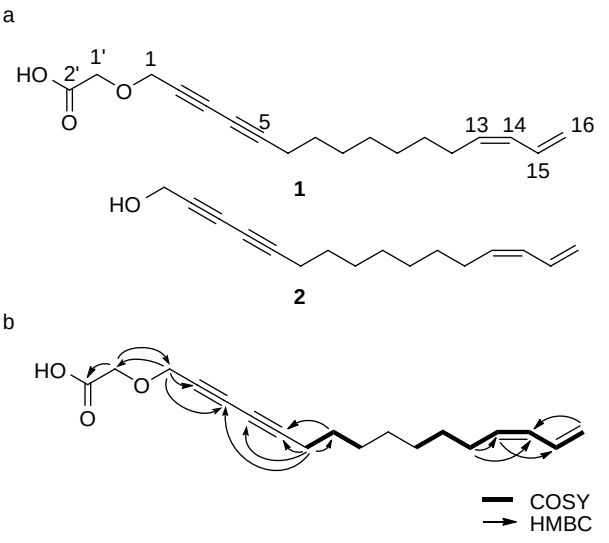

Figure 1. (a) Chemical structures of montiporic acid D (**1**) and (*Z*)-13,15-hexadecadien-2,4-diyn-1-ol (**2**) and (b) selective key NMR correlations of **1**.

(H-10–H-12) to the terminal conjugated diene (H-13–H-16). The ^{1}H and ^{13}C-NMR peaks at positions 8, 9 and 10 were not distinguishable because of overlapping. However, the area of peaks on ^{1}H-NMR indicated the presence of three methylenes. The ^{13}C-NMR spectrum indicated the presence of a diacetylenic unit (δ 82.5, 73.0, 70.5 and 64.8). HMBC cross peaks H-1/C-2, H-1/C-3, H-1/C-4, H-6/C-3, H-6/C-4 and H-6/C-5 (Figure 1b) revealed the presence of the conjugated diyne which connected to C-1 and C-6 methylene carbons. HMBC cross peaks H-12/C-13, H-12/C-14, H-13/C-15 and H-16/C-14 (Figure 1b) revealed that the conjugated diene was connected to C12 methylene carbon. The coupling constant (10.3 Hz) between H-13 and H-14 indicated that the olefin at position 13 and 14 had *Z*-configuration. The structure of 2-hydroxyacetate was indicated by the HMBC correlation H1′ to carboxyl carbon (C-2′). The connection between polyacetylene moiety (C-1–C-16) and 2-hydroxyacetate moiety (C-1′and C-2′) was determined by the HMBC correlations H1′/C1 and H1/C1′. Above all, the structure of montiporic acid D was determined as (*Z*)-2-(hexadeca-13,15-dien-2,4-diynyloxy)acetic acid (**1**).

Identification of compound **2** was accomplished by the analyses of ESI-MS and NMR spectra. The NMR chemical shift values of compound **2** in $CDCl_3$were identical with the literature values (Higa et al. 1990).

The antibacterial activities of compounds **1** and **2** were tested using the paper disk method (McCaffrey & Endean 1985). Compounds **1** and **2** showed week anti-bacterial activities against *Bacillus subtilis* and *Staphylococcus aureus* at the inoculation of 250 μg/disk. Both compounds did not show any activity against the testing microorganisms including *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Micrococcus luteus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Aspergillus niger* and *Mucor hiemalis* at the inoculation of 250 μg/disk.

The antioxidant activities of compounds **1** and **2** were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Sharma & Bhat 2009). Compounds **1** and **2** showed weak inhibitory activities with 35% and 25% inhibition at the concentration of 1 mg/mL, respectively.

3. Experimental

3.1 General methods

The chromatography system consisted of 2 HPLC pumps (Jasco, PU-980), a UV/VIS detector (Jasco, Model UV-970), and digital integration software (MacIntegrator II). ESI-TOF MS spectra were recorded using a JEOL JMS-T100LP mass spectrometer. ^{1}H-NMR spectra were obtained with a JEOL ECA-600 in CD_3OD at 27.0°C. The resonances of residual CHD_2OD at δ_H 3.31 ppm and at δ_C 49.2 ppm were used as internal references for NMR measurement in CD_3OD. The resonance of residual $CHCl_3$ at δ_H 7.26 ppm was used as internal reference for NMR measurement in $CDCl_3$. FT-IR spectrum was recorded using Avatar 360 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, USA).

3.2 Microorganism strains

Microorganisms including *E. coli* (NBRC 1002203), *P. aeruginosa* (NBRC 12689), *S. marcescens* (NBRC 1002204), *B. subtilis* (NBRC 13719), *S. aureus* (NBRC 1009110), *M. luteus* (NBRC 3333), *S. cerevisiae* (NBRC 2376), *S. pombe* (NBRC 0340), *A. niger* (NBRC 33023) and *M. hiemalis* (NBRC 9405) were obtained from the NBRC culture collection (NITE Biological Resource Centre, Japan). Cultivation of all bacteria was performed using ISP2 agar medium (Shirling & Gottlieb 1966) with incubation at 30°C.

3.3 Animal material

Coral specimens were collected in September 2011 from shallow water reefs around the Tropical Biosphere Research Center Sesoko Station of Ryukyu University in Sesoko Island (Okinawa, Japan) with permission No. 23-36 from the Okinawa prefectural government. The corals were immediately frozen after collection and kept at − 20°C until processed. The coral identification was done by Dr Irikawa of Shizuoka University according to Veron (2000). A voucher specimen (OKHC-MD1) is deposited at Shizuoka University, Graduate School of Science and Technology, Shizuoka, Japan.

3.4 Isolation of montiporic acid D from **M. digitata**

Tissue from *M. digitata* (160 g, wet weight) was crushed using pliers and extracted in 700 mL of MeOH. After filtration through a filter paper (Whatman No. 4 filter paper), the MeOH extract was evaporated to produce an aqueous residue. The concentrated extract was subjected to separation in an open column chromatography using a hydrophobic resin (CHP20P, Mitsubishi Chemical) with stepwise elution of 20%, 60% and 100% MeOH. The 100% MeOH fraction was subjected to HPLC purification using an ODS column (Nacalai Tesque, Cosmosil MSII 4.6 × 250 mm) with isocratic elution of aqueous 60% MeCN containing 0.05% TFA with the UV detection at 215 nm to yield compound **1** (12.6 mg) and compound **2** (17.3 mg).

3.5 Montiporic acid D (1)

^{1}H-NMR chemical shift values in CD_3OD: 1.34–1.40* (6H, m, H-8, H-9, H-10), 1.50 (2H, m, H-11), 1.53 (2H, m, H-7), 2.20 (2H, dt, J = 7.6, 6.8 Hz, H-12), 2.30 (2H, t, J = 6.9 Hz, H-6), 4.13 (2H, s, H-1′), 4.33 (2H, s, H-1), 5.05 (1H, br d, J = 10.3 Hz, H-16), 5.15 (1H, br d, J = 16.5 Hz, H-16), 5.45 (1H, dt, J = 10.3, 7.6 Hz, H-13), 5.99 (1H, dd, J = 11.0, 10.3 Hz, H-14), 6.66 (1H, ddd, J = 16.5, 11.0, 10.3 Hz, H-15), *indistinguishable; ^{13}C-NMR chemical shift values in CD_3OD: 19.8 (C-6), 28.7 (C-12), 29.4(C-7), 30.0 (C-11), 30.1*, 30.2*, 30.8* (C-8, C-9, C-10), 59.0 (C-1), 64.8 (C-4), 66.5 (C-1′), 70.5 (C-2), 73.0 (C-3), 82.4 (C-5), 130.7

(C-14), 133.7 (C-13), 133.8 (C-15), 117.2 (C-16), 171.8 (C-2′), *indistinguishable; UV absorption in MeOH at λ_{max} 228 nm (ε 3,460,000); IR (Microscopic transmission method) ν_{max} 3391, 3231, 2927, 2853, 2372, 2253, 1678, 1431, 1205, 1135, 958.

3.6 Antibacterial assay

Antibacterial activity was tested for using a standard agar plate assay–paper disk method (McCaffrey & Endean 1985). Each extract (50 μL) was added to the paper disk (i.d. 8 mm, thick type), air-dried and placed on agar plates seeded with microbes. After incubation at 30°C for 2 days, antibacterial activity was determined by the diameter of the inhibitory zone around the paper disk.

3.7 Antioxidant assay

Stock solutions of DPPH were prepared in MeOH. DPPH radical solution (0.1 mM) was mixed with various concentrations of extracts in methanol. The solution was then mixed vigorously and left for 30 min at 30°C in the dark. The absorbance was measured at 517 nm. Ascorbic acid was used as positive control (IC_{50} 10.3 μg/mL).

Supplementary material

Supplementary Figures S1–S8 relating to this article are available online.

Acknowledgements

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