



(1R,7R,8R,11R,12R)-7,8:11,12-Bisepoxyembrene A: A novel cembranoid from octocoral *Sinularia* sp.

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ABSTRACT

A novel cembranoid, (1R,7R,8R,11R,12R)-7,8:11,12-bisepoxyembrene A (**1**), was isolated from an octocoral *Sinularia* sp. The structure of **1** was elucidated using spectroscopic methods, and its absolute configuration was confirmed by a single-crystal X-ray diffraction analysis.

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Introduction

Octacorals of the genus *Sinularia* have been recognized as a rich source of novel oxygenated terpenoids. Among these metabolites, cembrane analogues are the major components of *Sinularia* spp., and compounds of this type have been found to possess extensive bioactivities [1,2]. During the course of our research on natural substances from the marine invertebrates distributed in Taiwanese waters, where the Kuroshio current and South China Sea surface current converge to provide high biodiversity. We analyzed the organic extract from an octocoral identified as *Sinularia* sp., [3] in

the hope of identifying extracts that exhibit interesting and meaningful signals in NMR studies, which led to the isolation of a novel cembranoid, (1R,7R,8R,11R,12R)-7,8:11,12-bisepoxyembrene A (**1**) (Fig. 1). We described herein the isolation and structure determination of **1**.

Results and discussion

(1R,7R,8R,11R,12R)-7,8:11,12-Bisepoxyembrene A (**1**) was isolated as crystal prisms that gave an $[M + Na]^+$ ion peak at m/z 327.22935 in HRESIMS, indicating the molecular formula $C_{20}H_{32}O_2$ (calcd. for $C_{20}H_{32}O_2 + Na$, 327.22945), with 5° of unsaturation. From the 1H and ^{13}C NMR data, in combination with the DEPT spectrum (Table 1), the presence of an exocyclic and a trisubstituted carbon-carbon double bonds were deduced from the signals of four olefin carbons at δ_C 148.1 (C-15), 134.2 (C-4), 126.1 (CH-3) and 110.5 (CH₂-16), and further supported by three olefin

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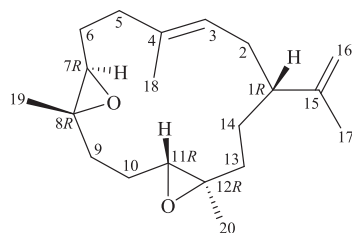


Fig. 1. Structure of (1R,7R,8R,11R,12R)-7,8:11,12-bisepoxycembrene A (**1**).

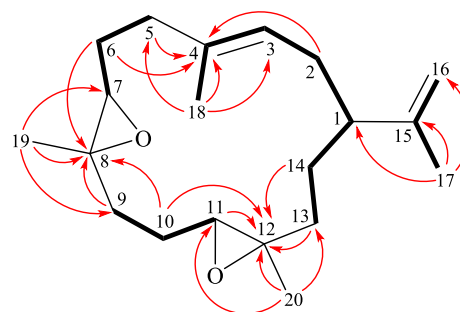


Fig. 2. Key COSY (—) and HMBC (---) correlations of **1**.

Table 1

¹H (400 MHz, CDCl₃) and ¹³C (100 MHz, CDCl₃) NMR data for **1**.

Position	δ_{H} (J in Hz)	δ_{C} , Mult. ^a
1	2.08 dddd (12.0, 12.0, 3.2, 3.2)	48.4, CH
2	2.05 m	33.0, CH ₂
3	5.27 ddq (10.4, 5.2, 0.8)	126.1, CH
4		134.2, C
5	2.24 m	36.8, CH ₂
6	1.90 m; 1.48 m	25.1, CH ₂
7	2.71 dd (7.6, 3.2)	63.6, CH
8		60.3, C
9	1.78 m; 1.54 m	35.0, CH ₂
10	2.01 m; 1.40 dddd (14.8, 8.0, 8.0, 2.8)	24.9, CH ₂
11	3.05 dd (8.0, 6.0)	61.7, CH
12		61.7, C
13	1.82 m; 0.92 ddd (13.2, 9.6, 4.0)	36.6, CH ₂
14	1.56 m; 1.17 m	26.5, CH ₂
15		148.1, C
16	4.73 br s; 4.71 br s	110.5, CH ₂
17	1.66 dd (1.2, 0.8)	19.2, CH ₃
18	1.65 br s	15.5, CH ₃
19	1.30 s	17.3, CH ₃
20	1.17 s	17.0, CH ₃

^a Multiplicity deduced from DEPT and HSQC experiments.

proton signals at δ_{H} 4.73 (1H, br s, H-16), 4.71 (1H, br s, H-16) and 5.27 (1H, ddq, J = 10.4, 5.2, 0.8 Hz, H-3). The presence of two trisubstituted epoxides, both containing a methyl substituent, was established from the signals of four oxygenated carbons at δ_{C} 63.6 (CH-7), 60.3 (C-8), 61.7 (CH-11) and 61.7 (C-12), and further supported by the proton signals of two methyl singlets at δ_{H} 1.30 (3H, s, H₃-19) and 1.17 (3H, s, H₃-20) and two oxymethine protons at δ_{H} 2.71 (1H, dd, J = 7.6, 3.2 Hz, H-7) and 3.05 (1H, dd, J = 8.0, 6.0 Hz, H-11). From the ¹³C NMR data and degrees of unsaturation, **1** was established as a tricyclic diterpenoid. Moreover, two vinyl methyls (δ_{H} 1.66, 3H, dd, J = 1.2, 0.8 Hz, H₃-17; 1.65, 3H, br s, H₃-18), seven pairs of aliphatic methylene protons (δ_{H} 2.05, 2H, m, H₂-2; 2.24, 2H, m, H₂-5; 1.90, 1H, m/1.48, 1H, m, H₂-6; 1.78, 1H, m/1.54, 1H, m, H₂-9; 2.01, 1H, m/1.40, 1H, dddd, J = 14.8, 8.0, 8.0, 2.8 Hz, H₂-10; 1.82, 1H, m/0.92, 1H, ddd, J = 13.2, 9.6, 4.0 Hz, H₂-13; 1.56, 1H, m/1.17, 1H, m, H₂-14), one aliphatic methine proton (δ_{H} 2.08, 1H, dddd, J = 12.0, 12.0, 3.2, 3.2 Hz, H-1) were observed in the ¹H NMR spectrum (Table 1).

The ¹H NMR coupling information gained from the COSY spectrum enabled identification of H-1/H₂-2/H-3, H₂-5/H₂-6/H-7, H₂-9/H₂-10/H-11, H₂-13/H₂-14/H-1, H-3/H₃-18 (by long-range allylic coupling, J = 0.8 Hz) and H₂-16/H₃-17 (by long-range allylic coupling, J = 1.2, 0.8 Hz) couplings (Fig. 2). These data, together with key HMBC correlations between protons and non-protonated carbons, such as H₂-2, H₂-5, H₂-6/C-4; H₂-6, H₂-9, H₂-10/C-8; and H₂-10, H-11, H₂-13, H₂-14/C-12 (Fig. 2), enabled the establishment of the 14-membered macrocyclic molecular skeleton of **1**. The presence of a propenyl group at C-1 was confirmed by analysis of HMBC correlations from H₃-17 to C-1, C-15 and C-16. The presence of methyl groups (Me-18, Me-19 and Me-20) on C-4, C-8 and C-12

was substantiated by HMBC correlations from H₃-18/C-3, C-4, C-5; H₃-19/C-7, C-8, C-9; and H₃-20/C-11, C-12, C-13, respectively.

Due to the conformational mobility of the 14-membered macrocycle, the stereochemistry of the stereogenic centers of **1** could not be fully determined from a NOESY experiment. Fortunately, single crystals of **1** could be obtained by slow evaporation of a methanol solution. Single-crystal X-ray diffraction analysis was then carried out and the ORTEP drawing of the structure of **1** is shown in Fig. 3, which unambiguously confirmed the structure of **1**; the stereogenic carbons of **1** were elucidated as (1R,7R,8R,11R,12R). The geometry of the C-3/4 double bond in **1** was found to be of an *E*-configuration.

Cembrene A was proven to be a common cembrene derivative in plant [4], soft coral [5], termite soldier [6] and alligator [7], and its diepoxy-containing derivatives were also found in soft coral [8–11]. To the best of our knowledge, this is the first time to obtain a cembrene-type diterpenoid featuring with epoxide groups at C-7/8 and C-11/12, respectively.

The cytotoxicity of **1** toward HT-29 (human colorectal adenocarcinoma) and HL-60 (human promyelocytic leukemia) tumor cells was assayed using MTT method, and it was found that cembranoid **1** was not to be cytotoxic (IC₅₀ > 20 μ M) toward these two tumor cell lines. Other possible biological activities of **1** will be assayed in the future.

Experiments

General experimental procedures

Optical rotation values were measured using a JASCO P-1010 digital polarimeter. IR spectra were obtained with a Thermo Scien-

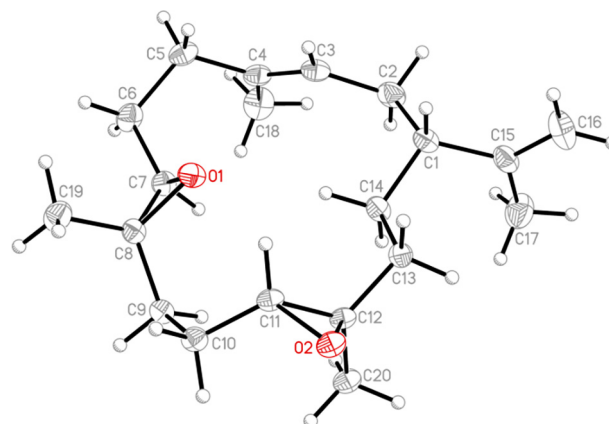


Fig. 3. ORTEP revealing the structure of (1R,7R,8R,11R,12R)-7,8:11,12-bisepoxycembrene A (**1**).

tific Nicolet iS5 FT-IR spectrophotometer. NMR spectra were recorded on a 400 MHz Jeol ECZ NMR spectrometer using the residual CHCl_3 (δ_{H} 7.26 ppm) and CDCl_3 signals (δ_{C} 77.0 ppm) as internal standards for ^1H and ^{13}C NMR, respectively; coupling constants (J) are presented in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solarix FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck). TLC was performed on plates precoated with DC-Fertigfolien Alugram® Xtra SIL G/UV₂₅₄ (0.20-mm-thick) and RP-18 W/UV₂₅₄ (0.15-mm-thick, Macherey-Nagel), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots. Reverse-phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-2130 pump, a Hitachi L-2455 photodiode array detector and a Rheodyne 7725i injection port with a reverse-phase column (Luna, 5 μm , C18 (2) 100 Å, 250 × 21.2 mm).

Animal material

Specimens of *Sinularia* sp. were collected on Turtle Island, Yilan County, Taiwan. The samples were then stored in a freezer at –20 °C until extraction. Identification of this organism was performed by comparison with previous descriptions [3].

Extraction and isolation

Freeze-dried and sliced bodies (wet/dry weight = 510/172 g) of the coral specimens were extracted with a 1:1 mixture of MeOH and CH_2Cl_2 to give 17.8 g of crude extract, which was partitioned between EtOAc and H_2O . The EtOAc extract (6.8 g) was applied in a silica gel column chromatography (Si C.C.) and eluted with gradients of *n*-hexane/EtOAc (100% *n*-hexane – 100% EtOAc, stepwise) to furnish 14 sub-fractions A ~ N. Fraction F was chromatographed by Si C.C. and eluted with gradients of CH_2Cl_2 /EtOAc (100% CH_2Cl_2 – 100% EtOAc, stepwise) to obtain 16 sub-fractions F1 ~ F16. Fraction F12 was re-purified by RP-HPLC using a mixture of MeOH/ H_2O (70:30; flow rate = 5 mL/min) to yield 15 sub-fractions F12A ~ F12O. Fraction F12N was re-purified by RP-HPLC using a mixture of MeOH/ H_2O (90:10; flow rate = 5.0 mL/min) to afford **1** (0.7 mg).

(1R,7R,8R,11R,12R)-7,8:11,12-Bisepoxycembrene A (**1**)

Colorless prisms; $[\alpha]_{\text{D}} + 151$ (c 0.23, CHCl_3); ^1H (400 MHz, CDCl_3) and ^{13}C (100 MHz, CDCl_3) NMR data (see Table 1); ESIMS: m/z 327 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 327.22935 (calcd. for $\text{C}_{20}\text{H}_{32}\text{O}_2 + \text{Na}$, 327.22945).

Single-crystal X-ray crystallography of (1R,7R,8R,11R,12R)-7,8:11,12-Bisepoxycembrene A (**1**)

Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.407 × 0.065 × 0.046 mm³) belongs to the orthorhombic system, space group $P2_12_12_1$ (# 19), with $a = 6.0442(2)$ Å, $b = 11.3546(3)$ Å, $c = 27.0874(8)$ Å, $V = 1858.99$ (10) Å³, $Z = 4$, $D_{\text{calcd}} = 1.088$ Mg/m³, and λ (Mo K α) = 0.71073 Å. Intensity data were measured on a Bruker D8 Venture diffractometer up to θ_{max} of 24.996°. All 10,600 reflections were col-

lected. The structure was solved by direct methods and refined using a full-matrix least-squares procedure. The refined structural model converged to a final $R1 = 0.0439$; $wR2 = 0.0979$ for 2,919 observed reflections [$I > 2\sigma(I)$] and 203 variable parameters. The absolute configuration was determined from the Flack parameter, $x = -0.1(9)$ [12,13]. Crystallographic data for the structure of **1** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 2105023. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tetlet.2021.153628>.

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