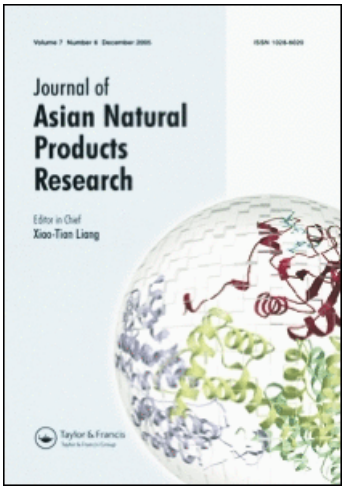

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A new cembranoid from the Hainan soft coral *Sinularia* sp.

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A new cembrane-type diterpene, diepoxycembrene A (**1**), has been isolated from the Hainan soft coral *Sinularia* sp. Its structure was elucidated on the basis of detailed analysis of its spectroscopic data, and by comparison of its NMR spectral data with those of the related model compounds.

Keywords: soft coral; *Sinularia* sp; cembranoid; diterpenoid

1. Introduction

Soft corals of the genus *Sinularia*, marine invertebrates in the family Alcyoniidae, are a rich source of structurally diverse diterpenes [1,2], notably cembranoid diterpenes, of which many were reported to exhibit ichthyotoxic [3], cytotoxic, anti-inflammatory, anti-arthritic [4,5], Ca-antagonistic, and antimicrobial properties [6], which attracted considerable attention of natural product chemists and pharmacologists. In the course of our research on biologically active substances from South China Sea invertebrates [7–9], a sample of the soft coral *Sinularia* sp. was recently collected off the Lingshui Bay, Hainan province, China, and was chemically investigated. Separation of the Et_2O-soluble fraction of the acetone extract of the animal led to the isolation of a new cembrane-type diterpenoid, diepoxycembrene A (**1**). This paper describes the isolation and structure elucidation of the new compound.

2. Results and discussion

Freshly collected animals (dry weight 244.3 g) were immediately stored at −20°C and kept frozen until the extraction. Frozen material was cut into small pieces and subsequently extracted with acetone. The acetone extract was then partitioned between Et_2O and H_2O. The Et_2O-soluble portion was subjected to repeated column chromatography (silica gel, Sephadex LH-20, and RP-C18 silica gel) to afford compound **1**.

Compound **1** was obtained as colorless oil. The molecular formula of **1** was established to be $C_{20}H_{32}O_2$ by positive HR-ESI-MS quasi-molecular ion peaks at *m/z* 327.2293 ($[M + Na]^+$; calculated for 327.2300) in combination with the ^{13}C NMR spectral data. The IR absorption bonds of **1** at 1645, 1252, and 885 cm^{-1} indicated the presence of olefin and epoxide functionalities. The ^1H NMR spectrum (Table 1) of **1** displayed the presence of four methyls at δ 1.23 (3H, s, H-20), 1.27 (3H, s, H-18), 1.63 (3H, s, H-19), and 1.67 (3H, s, H-17), one olefinic proton at δ 5.18 (1H, t, $J = 6.1$ Hz, H-7), two trisubstituted epoxide groups at δ 2.72 (1H, dd, $J = 3.1$, 9.7 Hz, H-11) and 2.82 (1H, dd, $J = 3.9$, 10.4 Hz, H-3). The corresponding carbons were assigned through HMQC correlations.

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Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1**[a] and ^{13}C NMR spectral data of compounds **2** and **3** (δ in ppm, J in Hz).

Position	**1**		**2** [10]	**3** [11]
	δ_H, J (Hz)	δ_C	δ_C	δ_C
1	2.14 (m)	41.8 (d)	40.2 (d)	150.9 (s)
2α	1.34 (m)	31.8 (t)	33.6 (d)	125.8 (d)
2β	1.98 (m)			
3	2.82 (dd, 3.9, 10.4)	61.2 (d)	63.3 (d)	59.3 (d)
4	–	60.3 (s)	60.8 (s)	61.7 (s)
5α	2.10 (m)	22.6 (t)	23.6 (t)	22.3 (t)
5β	2.11 (m)			
6α	2.01 (m)	37.3 (t)	38.2 (t)	40.2 (t)
6β	1.51 (m)			
7	5.18 (t, 6.1)	126.0 (d)	123.9 (d)	121.2 (d)
8	–	134.0 (s)	135.1 (s)	134.9 (s)
9α	2.28 (m)	35.9 (t)	39.5 (t)	36.8 (t)
9β	2.06 (m)			
10α	1.40 (m)	24.6 (t)	24.3 (t)	24.5 (t)
10β	2.05 (m)			
11	2.72 (dd, 3.1, 9.7)	61.6 (d)	124.2 (d)	62.1 (d)
12		60.7 (s)	137.3 (s)	61.2 (s)
13α	1.72 (m)	36.9 (t)	34.6 (t)	38.0 (t)
13β	1.30 (m)			
14α	1.58 (m)	26.6 (t)	29.7 (t)	26.0 (t)
14β	1.48 (m)			
15	–	147.0 (s)	148.5 (s)	73.4 (s)
16α	4.63 (d, 1.2)	110.8 (t)	110.6 (t)	29.7 (t)
16β	4.78 (t, 1.6)			
17	1.67 (s)	20.4 (q)	18.4 (q)	29.7 (q)
18	1.27 (s)	17.5 (q)	16.9 (q)	18.0 (q)
19	1.63 (s)	15.6 (q)	15.8 (q)	14.7 (q)
20	1.23 (s)	17.5 (q)	17.1 (q)	16.1 (q)

[a] ^{1}H NMR ($CDCl_3$, 500 MHz); ^{13}C NMR ($CDCl_3$, 125 MHz); chemical shifts (in ppm) are referenced to $CHCl_3$ ($\delta_H = 7.26$, $\delta_C = 77.2$).

The ^{13}C NMR and DEPT spectroscopic data (Table 1) were in good agreement with the above analysis. The ^{1}H and ^{13}C NMR spectral data in combination with the molecular composition highly showed compound **1** to be a cembrane-type diterpene.

Analysis of ^{1}H–^{1}H COSY (Figure 2) and HMQC spectra in combination with ^{13}C NMR spectral data readily allowed to identify three spin–spin systems [**a** (C-13 to C-1 to C-3), **b** (C-5 to C-7), and **c** (C-9 to C-11)]. Furthermore, a series of significant HMBC (Figure 2) correlations between H-3 (δ 2.82), H_2-5, and H_3-18 (δ 1.27)/C-4; H-7 (δ 5.18), H_2-9, and H_3-19 (δ 1.63)/C-8; H-11 (δ 2.72), H_2-13, and H_3-20 (δ 1.23)/C-12 suggested that the partial structures **a**–**c** were connected to each other to form a 14-membered ring through the quaternary carbons C-4, C-8, and C-12. The connectivity of C-1 (δ 41.8) to C-15 (δ 147.0) was revealed by the HMBC cross-peaks of H_2-16/C-1, C-15, and C-17, and H_3-17/C-1 and C-15. Thus, the gross structure of **1** was established as depicted in Figure 1.

The relative stereochemistry of **1** was deduced from careful comparison of its ^{13}C NMR spectral data with those of the model compound **2** [(1*R*,3*R*,4*S*,7*E*,11*E*)-3,4-epoxycembra-7,11,15-triene] [10] and sinugibberol

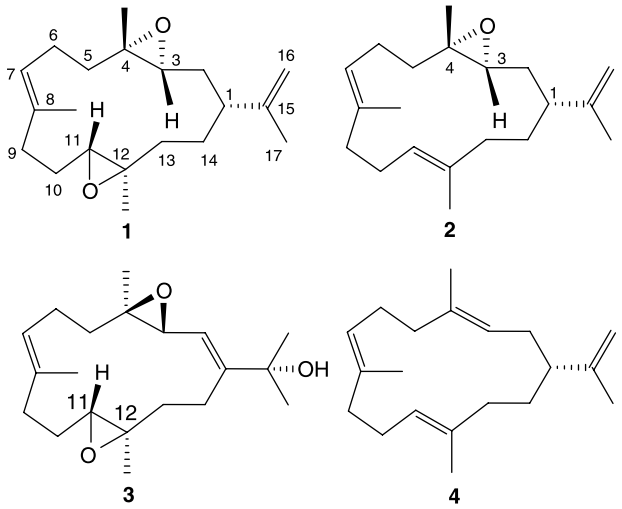

Figure 1. Structures of compounds **1**–**4**.

(**3**) [11], and by analysis of its ROESY spectrum. Thus, the characteristic upfield ^{13}C NMR chemical shifts of C-18, C-19, and C-20 ($\delta < 20$ ppm) [12,13] implied *E* stereochemistry for both $\Delta^{7(8)}$ olefin and two epoxide rings. Moreover, the ^{13}C NMR chemical shifts of **1** and those of the compound **2** were very similar from C-1 to C-8, C-18, and C-19, suggesting that the relative configurations at C-1, C-3, and C-4 of **1** are the same as those of **2**. Finally, the relative stereochemistry of the second epoxy ring at C-11 and C-12 of **1** was tentatively assigned the same as that of **3**, based mainly on the ^{13}C NMR chemical shifts from C-9 to C-14, and that of C-20 (Table 1) showing similar δ values in **1** and **3**, supported by the presence of ROESY correlations between H-1 and H-3, H-3 and H_3-18, and H-11 and H_3-18 (Figure 2). On the basis of above evidence, the structure of **1** was established as (1*R**,3*R**,4*S**,11*R**,12*R**)-3,4,11,12-diepoxycembra-7,15-diene.

Cembrane diterpenes containing epoxy ring are frequently encountered in soft corals. However, the cembranoids with two epoxide moieties in their molecules are relatively rare. Biogenetically, **1** may be formally derived from compound **4** (cembrene A), which is frequently isolated from the soft corals of the genus *Sinularia* [14], by the oxidation of both olefins at $\Delta^{3(4)}$ and $\Delta^{11(12)}$.

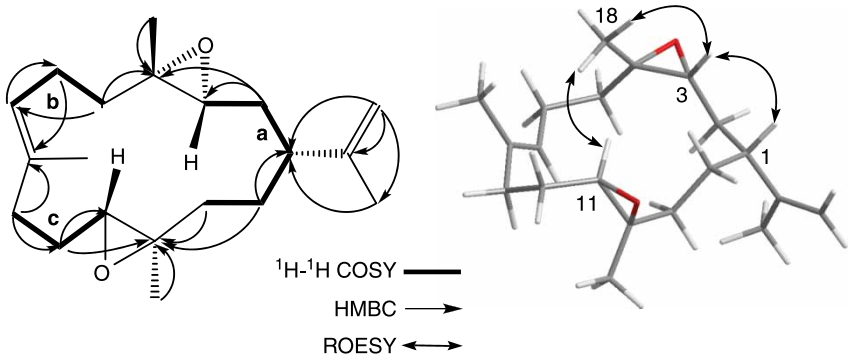

Figure 2. Selected ^{1}H–^{1}H COSY, HMBC and ROESY correlations for compound **1**.

The new compound **1** was tested for the inhibitory activities against hPTP1B (human protein tyrosine phosphatase 1B), a key target for the treatment of Type II diabetes and obesity [15]. But, it showed no inhibitory effect. **1** was also tested for cytotoxicity against A-549 and HL-60 tumor cell lines, but they were inactive at a concentration of 20 μg/ml. Other bioassays such as antibacterial and anti-inflammatory are currently ongoing.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectrum was recorded on a Nicolet Magna FT-IR 750 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. Chemical shifts (δ) are reported in ppm relative to an internal TMS standard, coupling constant (J) in Hz. ^{1}H and ^{13}C NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, HMBC, and ROESY experiments. The ESI-MS was recorded on a Q-TOF-Micro-LC-MS-MS spectrometer. Commercial silica gel plates (Qing Dao Hai Yang Chemical Group Co. Qingdao, China) were used for TLC. The chromatograms were detected by an UV lamp at 254 nm, and successively sprayed with 0.1% $Ce(SO_4)_2$ in 2 N H_2SO_4 and heating at 80°C for 5 min.

3.2 Collection of biological material

Specimens of the soft coral *Sinularia* sp. were collected off the Lingshui Bay, Hainan province, China, in July 2004, and identified by Associate Prof. Hui Huang of South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (LS-286) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

3.3 Extraction and isolation

The frozen animals (dry weight 244.3 g) were cut into small pieces and exhaustively extracted with acetone (3 × 3 l). The organic extract was evaporated to give a residue, which was partitioned between Et_2O and H_2O. The Et_2O solution was concentrated under reduced pressure to give a dark brown residue (12.7 g), which was fractionated by gradient silica gel column chromatography [0–100% acetone in light petroleum ether (PE)], yielding five fractions (A–G). The fraction B eluted by PE/Me_2CO (99:1) was further purified on a second silica gel column chromatography eluting with PE-Et_2O (95:5) to afford **1** (4.3 mg).

3.3.1 Diepoxycembrene A (*1*)

Colorless oil; IR ν_{max} (KBr) cm^{-1}: 3021, 1645, 1252, 885; ^{1}H and ^{13}C NMR spectral data: (Table 1) HR-ESI-MS: m/z 327.2293 $[M + Na]^+$ (calculated for $C_{20}H_{32}O_2Na$, 327.2300).

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