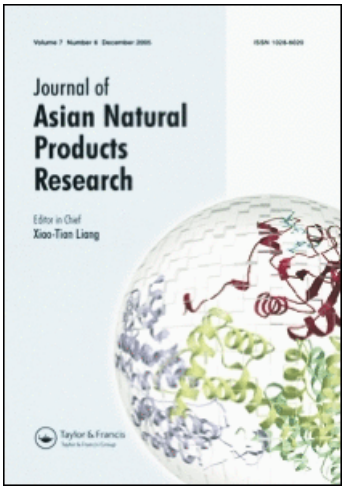

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A new diterpenoid from the south China sea soft coral *Lobophytum* sp.

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A new cembrane-type diterpene, 11,12-epoxy-sarcophytoxide (**1**), along with five related diterpenoids (**2**, and **4**–**7**), has been isolated from the soft coral *Lobophytum* sp. The structure of the new compound **1** was elucidated on the basis of detailed analysis of its spectroscopic data, and by comparing its NMR spectral data with those of the model compounds.

Keywords: soft coral; *Lobophytum* sp; cembranoid; 11,12-epoxy-sarcophytoxide

1. Introduction

Soft corals of the genus *Lobophytum*, a marine invertebrate in the family Alcyoniidae, are a rich source of structurally diverse diterpenes [1,2]. Numerous cembranoids have been reported to exhibit cytotoxic properties [3], and recently a cembrane diterpene from *Lobophytum cristagalli* has been reported to inhibit Ras farnesyl transferase [4]. In the course of our research on biologically active substances from Chinese marine invertebrates [5–7], a sample of the soft coral *Lobophytum* sp. has recently been collected off the Lingshui Bay, Hainan Province, China, and 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, 11,12-epoxy-sarcophytoxide (**1**), and five known analogues. This paper describes the isolation and structure elucidation of the new compound.

2. Results and discussion

Freshly collected animals (dry weight 410.5 g) from the South China Sea 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 extract was evaporated, and the residue was partitioned between Et_2O and H_2O. On evaporation, the Et_2O extract yielded a dark brown crude residue (9.65 g), which was subjected to repeated column chromatography (silica gel, Sephadex LH-20 and RP-C18 silica gel) to give compounds **1**, **2**, and **4–7**, respectively.

The known compounds were readily identified as (2*R*,7*R*,8*R*)-sarcophytoxide (**2**) [8], 2-[1*R*,4,8,12-trimethyl-3*E*,7*E*,11*E*-lyclotetradecatrien-1-yl]-prop-2-en-l-ol (**4**) [9], (−)-*trans*-cembranolide (**5**) [10], thunbergol (**6**) [11], and sarcophine (**7**) [12] by comparison of their spectral data with previously reported values.

Compound **1** was obtained as optically active colorless oil $[\alpha]_D^{24}$ −71 (*c* 0.11, $CHCl_3$). The molecular formula of **1** was established to be $C_{20}H_{30}O_3$ using ESIMS pseudo-molecular ion peaks at *m/z* 319.3 $[M + H]^+$ and 341.2 $[M + Na]^+$ in combination with the ^{13}C NMR spectral data, indicating six degrees of

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unsaturation. The IR absorptions of **1** at 1665 and 1254 cm^{-1} indicated the presence of olefin and epoxide functionalities. The ^1H NMR spectrum (Table 1) of **1** displayed the presence of four methyls [δ 1.26 (3H, s, H-20); 1.32 (3H, s, H-19); 1.67 (3H, s, H-17); 1.75 (3H, s, H-18)], one olefinic proton [δ 5.20 (1H, d, $J = 10.1$ Hz, H-3)], and two trisubstituted epoxide groups [δ 2.52 (1H, dd, $J = 2.8$, 10.6 Hz, H-11); 2.78 (1H, dd, $J = 4.2$, 4.2 Hz, H-7)]. The corresponding carbons were assigned through HMQC correlations. The ^{13}C NMR and DEPT spectroscopic data (Table 1) were in good agreement with the above analysis. The ^1H and ^{13}C NMR spectral data, in combination with the molecular composition, highly showed compound **1** to be a cembrane-type diterpene. The final structure of **1** was deduced to correspond to 11,12-epoxy-sarcophytoxide, as elucidated on the basis of extensive spectroscopic data analysis and by comparison with the NMR spectral data of co-occurring compound **2**, an epoxy cembranoid isolated from several marine organisms [13,14] (Figure 1).

Analysis of ^1H–^1H COSY (Figure 2) and HMQC spectra in combination with ^{13}C NMR spectral data readily identified four spin–spin systems [**a** (C-2 to C-3), **b** (C-5 to C-7), **c** (C-9 to C-11), and **d** (C-13 to C-14)]. Furthermore, a series of significant HMBC (Figure 2) correlations between H-2 (δ 5.44) and H_2-14/C-1, H_2-5 and H_3-18 (δ 1.75)/C-4, H-7, H_2-9, and H_3-19 (δ 1.32)/C-8, H-11, H_2-13,

Table 1. ^1H 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** [8]	**3** [8]
	δ_H, J (Hz)	δ_C	δ_C	δ_C
1		131.6 (s)	133.5 (s)	132.6 (s)
2	5.44 (m)	83.2 (d)	83.8 (d)	83.4 (d)
3	5.20 (d, 10.1)	126.9 (d)	126.4 (d)	126.6 (d)
4		138.9 (s)	139.2 (s)	139.9 (s)
5α	2.37 (m)	37.3 (t)	37.6 (t)	38.9 (t)
5β	2.82 (m)			
6α	1.69 (m)	24.9 (t)	25.4 (t)	24.3 (t)
6β	1.87 (m)			
7	2.78 (dd, 4.2, 4.2)	61.8 (d)	61.9 (d)	125.7 (d)
8		58.9 (s)	59.8 (s)	133.2 (s)
9α	1.18 (m)	36.2 (t)	39.7 (t)	36.7 (t)
9β	2.20 (m)			
10α	1.36 (m)	24.7 (t)	23.5 (t)	23.8 (t)
10β	2.02 (m)			
11	2.52 (dd, 2.8, 10.4)	62.4 (d)	123.7 (d)	62.3 (d)
12		61.6 (s)	136.7 (s)	61.4 (s)
13α	0.95 (m)	36.7 (t)	36.7 (t)	37.4 (t)
13β	2.24 (m)			
14α	2.46 (m)	23.5 (t)	26.0 (t)	22.5 (t)
14β	2.46 (m)			
15		129.0 (s)	127.5 (s)	128.36 (s)
16α	4.50 (br s)	78.4 (t)	78.4 (t)	78.3 (t)
16β	4.50 (br s)			
17	1.67 (s)	10.1 (q)	10.1 (q)	9.9 (q)
18	1.75 (s)	15.4 (q)	15.6 (q)	14.6 (q)
19	1.32 (s)	16.4 (q)	17.0 (q)	14.7 (q)
20	1.26 (s)	15.8 (q)	15.2 (q)	15.7 (q)

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

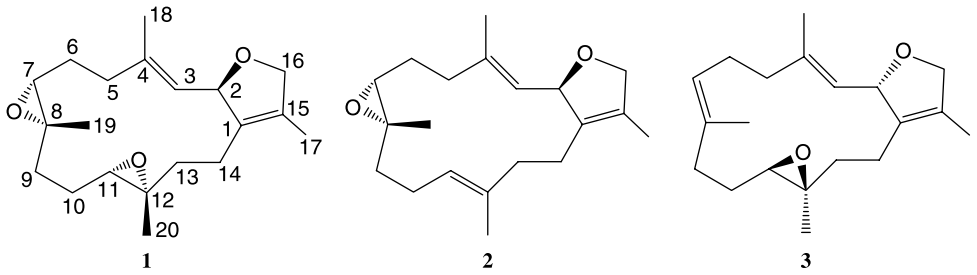

Figure 1. Structures of compounds **1**–**3**.

and H_3-20 (δ 1.26)/C-12 suggested that the four spin–spin systems (**a**–**d**) were connected to each other through the quaternary carbons C-1, C-4, C-8, and C-12. The connectivity of C-1 (δ 131.6) to C-15 (δ 129.0) was revealed by the HMBC cross-peaks of H_2-16/C-1, C-2, C-15, and C-17, and H_3-17/C-1, C-15, and C-16. Thus, the gross structure of **1** was established as shown 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 compounds **2**, **3** [8], and analysis of its ROESY spectrum. The characteristic upfield ^{13}C NMR chemical shifts of C-18, C-19, and C-20 ($\delta < 20$ ppm) [15,16] implied *E* stereochemistry for both $\Delta^{3,4}$ olefin and two epoxide rings. In fact, the ^{13}C NMR chemical shifts of **1** and those of the co-occurring compound **2** were very similar from C-1 to C-8, C-18, and C-19; thus, the relative stereochemistry at C-2, C-7, and C-8 was established to be the same as that of **2**. The relative stereochemistry of **1** at C-11 and C-12 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 almost identical δ values in **1** and **3**, and supported by the presence of ROESY correlation between H-7 and H-11.

As can be seen from the cembranoid molecular structure reported herein, the compound **1** contains a $\Delta^{1,15}$ double bond rather than the usually encountered $\Delta^{15,17}$ olefin identified in the previous constituents from *Lobophytum* sp. [14]. To the best of our knowledge, this is the first report that *Lobophytum* sp. produces a cembranoid diterpene possessing both a $\Delta^{1,15}$ double bond and two epoxide functionalities in the molecule.

All the new and known compounds were tested for the inhibitory activities against hPTP1B (human protein tyrosine phosphatase 1B), a key target for the treatment of type-II diabetes and obesity [17]. But, they all showed no inhibitory effects. Further study should be conducted to understand the real biological/ecological role of these metabolites in the life cycle of the invertebrate as well as to test their biological activities such as cytotoxic, anti-inflammatory and anti-fouling activities.

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

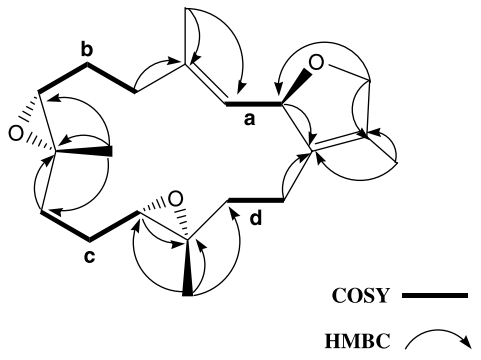

Figure 2. Selected ^{1}H–^{1}H COSY and HMBC correlations for compound **1**.

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 ESIMS 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

Specimen of the soft coral *Lobophytum* 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-255) is available for inspection at the Herbarium of Shanghai Institute of Materia Medica, CAS.

3.3 Extraction and isolation

The frozen animals (dry weight 410.5 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 (9.65 g), which was fractionated by gradient Si gel column chromatography [0–100% acetone in light petroleum ether (PE)], yielding five fractions (A–G). The fraction B eluted by PE/Me_2CO (98:2) was further purified on a second Si gel column chromatography eluting with PE-Et_2O (95:5) to afford **1** (2.9 mg) and **2** (42.2 mg). Fraction D, eluted by PE/Me_2CO (9:1), was further chromatographed on a Si gel column, eluting with PE/Me_2CO (95:5), and successively further purified by RP-HPLC [semi-preparative OSD-HG-5 (5 μm, 250 × 10 mm)] to yield **5** (11.2 mg) and **7** (16.7 mg). Fraction F, eluted by PE/Me_2CO (3:2), was treated in the same way as that for fraction C by further eluting with PE-Me_2CO from 85:15 to 5:5 to give compounds **4** (12.1 mg) and **6** (3.8 mg), respectively.

11,12-Epoxy-sarcophytoxide (1). Colorless oil; $[\alpha]_D^{24}$ −71 (*c* 0.11, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3021, 1665, 1372, 1254; ^{1}H and ^{13}C NMR spectral data: see Table 1; HRESIMS: *m/z* 341.2102 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2093); ESIMS: *m/z* 319.3 $[M + H]^+$ and 341.2 $[M + Na]^+$.

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