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16-Hydroxycembra-1,3,7,11-tetraene, a new Cembrane Diterpene from Malaysian Soft Coral Genus *Sarcophyton*

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One new cembrane diterpene, 16-hydroxycembra-1,3,7,11-tetraene (**1**), along with three known compounds, 15-hydroxycembra-1,3,7,11-tetraene (**2**), sarcophine (**3**) and sarcophytoxide (**4**) were isolated from *Sarcophyton* sp. collected from Karah Island, Terengganu, West Malaysia. Their structures were elucidated based on spectroscopic data. Activities of these compounds against antibacterial resistant clinical bacteria are reported. Only **1** exhibited inhibition against *Staphylococcus aureus.*

Keywords: *Sarcophyton* sp., Alcyoniidae, Soft coral, Diterpene, Cembrane.

The soft coral genus *Sarcophyton* (Alcyoniidae) consists of about 35 species, which are difficult to distinguish [1]. They are known to be a rich source of cembrane diterpenes [2]. Despite the advance in the chemistry of soft corals, there is a shortage of information related to the chemistry of Malaysian soft corals. To date, the available chemical investigation of Malaysian *Sarcophyton* was reported from Sabah [3]. In our recent investigation, one population of Malaysian *Sarcophyton* was collected from Karah Island and has led to the isolation of one new cembranoid, 16-hydroxy cembra-1,3,7,11-tetraene (**1**), along with three known cembrane diterpenes, 15-hydroxycembra-1,3,7,11-tetraene (**2**), sarcophine (**3**) and sarcophytoxide (**4**) (Figure 1). This paper reports the isolation, structure elucidation and antibacterial activities of these compounds.

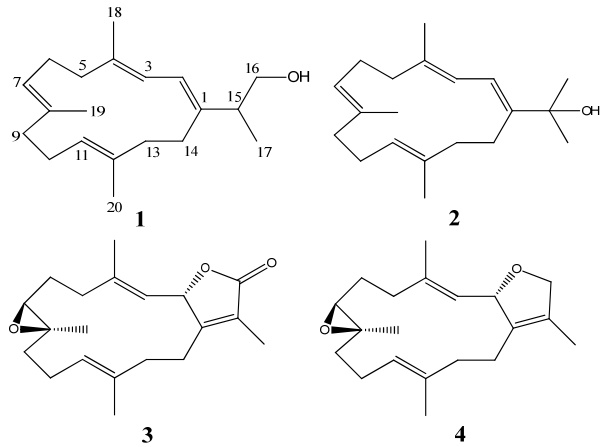

Figure 1: Structures of compounds **1**-**4**.

Compound **1** was isolated as a colorless oil, $[\alpha]_D^{25}$: -9.3 (*c* 0.18, $CHCl_3$). The molecular formula, $C_{20}H_{32}O$, was deduced based on HR-MS (*m/z* 289.2486 $[M + H]^+$, calcd. 289.2526), accounting for five degrees of unsaturation. The IR absorption band at 3418 cm^{-1} indicated the presence of a hydroxyl group. The ^1H- and ^{13}C-NMR spectral data (Table 1), as well as HSQC and ^{13}C-DEPT experiments, showed the presence of four pairs of double bonds at δ_C 141.7(C), 136.8 (C), 135.2 (C), 135.0 (C), 125.6 (CH), 124.9 (CH), 122.9 (CH) and122.3 (CH); δ_H 6.08 (1H, d), 5.96 (1H, d), 5.04 (1H, t) and 4.99 (1H, t), three vinyl methyls at δ_C 18.1 (CH_3), 17.6(CH_3) and 16.2 (CH_3); δ_H 1.74, 1.60 and 1.49 (each 3H, s), one secondary methyl at 17.5 (CH_3); δ_H 1.05 (3H, d), an oxygenated methylene signal at δ_C 67.2 (CH_2); δ_H 3.50 (2H, m), six methylenes and one methine. These signals explained four degrees of unsaturation, implying a monocyclic ring in the molecule of **1**. The ^1H–^1H COSY experiment revealed the sequences of the correlations depicted by the bold lines in Figure 2. The chemical shift values at C-16 (δ_c 67.2; δ_H 3.50) clearly indicated that a hydroxyl group was attached to it, as supported by HR-MS and IR data. The spectroscopic data of **1** closely matched that of **2**, but the main difference was the location of the hydroxyl group, which was transferred from C-15 in **2** to C-16 in **1**. The isopropyl alcohol moiety attached to C-1 was confirmed by HMBC correlations of H_3-17 to C-1, C-15 and C-16. The 14-membered cembrane of **1** was

Table 1: ^1H- and ^{13}C-NMR data (600 MHz and 150 MHz, $CDCl_3$) for **1** (δ in ppm, *J* in Hz).

Position	δ_C	δ_H (mult., *J* in Hz)
1	141.7 (s)	
2	122.9 (d)	6.08 d (11.0)
3	122.3 (d)	5.96 d (11.0)
4	136.8 (s)	
5	40.0 (t)	2.14 m
6	26.1 (t)	2.18 m
7	125.6 (d)	4.99 t (6.1)
8	135.2 (s)	
9	39.6 (t)	2.10 m
10	25.1 (t)	2.14 m
11	124.9 (d)	5.04 t (5.5)
12	135.0 (s)	
13	38.5 (t)	2.14 m
14	28.4 (t)	2.34 br t (6.9)
15	43.0 (d)	2.42 sextet (6.9)
16	67.2 (t)	3.50 t (6.2)
17	17.5 (q)	1.05 d (6.9)
18	17.6 (q)	1.74 s
19	16.2 (q)	1.49 s
20	18.1 (q)	1.60 s

connected based on the key HMBC correlations of H_3-18 to C-3, C-4 and C-5; H_3-19 to C-7, C-8 and C-9; H_3-20 to C-11, C-12 and C-13; and H_2-15 to C-1, C-2 and C-14. Therefore, the gross structure of **1** was determined as shown in Figure 2.

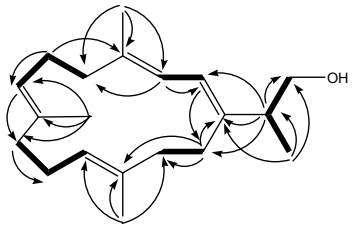

Figure 2: ^{1}H-^{1}H COSY and key HMBC correlations of **1**.

The relative stereochemistry of compound **1** was deduced from the NOESY experiment, as well as the ^{13}C NMR chemical shifts. The ^{13}C NMR chemical shifts of vinyl methyls at C-18, C-19 and C-20 at δ_C 17.6, 16.2 and 18.1, respectively, suggested that all the trisubstituted double bonds had *E* configurations [4,5]. NOE correlations observed between H-3/H_2-5, H-7/H_2-9 and H-11/H_2-13 further supported this deduction. Determination of the absolute stereochemistry at C-15 was not possible on the basis of the reported data. Therefore, compound **1** was reported as (3*E*,7*E*,11*E*)-16-hydroxycembra-1,3,7,11-tetraene. Compounds **2-4** were identified as 15-hydroxycembra-1,3,7,11-tetraene (**2**), sarcophine (**3**) and sarcophytoxide (**4**) by comparing their spectroscopic data with those reported in the literature [6-8].

Compounds **1**-**4** were tested for their antibacterial activities against antibiotic resistant clinical bacterial strains such as *Staphylococcus aureus* (ATCC 6538DR) and *Escherichia coli* (ATCC 25253). Only **1** exhibited inhibition against *Staphylococcus aureus*. Its MBC and MIC values were calculated to be 75 μg mL^{-1} and 25 μg mL^{-1}, respectively. The MBC/MIC ratio was calculated to be 3.0 and indicated that **1** exhibits bactericidal activity. Compounds **1** and **2** were very similar except for the position of the hydroxy functional group. The antibacterial activity of structure **1** could possibly be due to the presence of the allyl alcohol functional group.

In conclusion, chemical investigation of *Sarcophyton* sp. from Karah Island, Terengganu, has led to the isolation of a new cembrane diterpene, 16-hydroxycembra-1,3,7,11-tetraene (**1**), with bactericidal potential. This is the first record of diterpenoids isolated from a *Sarcophyton* species from the east coast of peninsula Malaysia .

Experimental

General: ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL ECA 600 NMR spectrometer using $CDCl_3$ with TMS as an internal standard. The high-resolution mass spectrum was acquired via LCMS-IT-TOF (Shimadzu). An AUTOPOL IV automatic polarimeter (Rudolph Research Analytic) and FTIR (Thermo Nicolet) were used to acquire their physical data. Preparative TLC was performed with silica gel glass plates (Merck, Kieselgel 60 F_{254}), and column chromatography (CC) with silica gel (Merck, Kieselgel 60, 70-230 mesh).

Biological material: A specimen of *Sarcophyton* sp. was collected from Karah Island, Terengganu, (5°35'52.6"N, 103°03'47.0"E, 5°35'57.9"N, 103°03'56.5"E), West Malaysia. The voucher specimen (BORM48086) was deposited in the BORNEENSIS Collection of the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Extraction and isolation: Fresh soft coral (2.40 kg wet wt) was extracted in MeOH at room temperature for 5 days. The resulting MeOH extract was concentrated *in vacuo* and partitioned between EtOAc/H_2O. The EtOAc fraction was further partitioned with *n*-hexane/90% MeOH. The *n*-hexane fraction was subjected to CC eluting with a gradient of *n*-hexane and EtOAc with increasing polarity. Fraction 3 obtained in *n*-hexane-EtOAc (7:3) gave compound **1** (1.8 mg; 0.5 %), **3** (14.4 mg; 4.0 %) and **4** (98.0 mg; 27.2 %) after purification by preparative TLC using $CHCl_3$. Fraction 4, obtained in *n*-hexane-EtOAc (1:1), was subjected to preparative TLC with *n*-hexane-EtOAc (2:1) to yield compound **2** (4.4 mg; 0.8 %). Percentages of compounds were the average of the respective compounds in the *n*-hexane fraction.

Antibacterial activity: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays were performed by the microdilution method against *Staphylococcus aureus* (ATCC 6538DR) and *E. coli* (ATCC 25253) according to Clinical and Laboratory Standards Institute (CLSI) [9]. Pure compounds were added to 100 μL Tryptic Soy Broth (TSB) with 100 μL of bacterial inoculum (1.0 x 10^4 CFU per well), and serial diluted to the desired concentration in a microtitre plate. The mixtures in the microplates were incubated for 24 h at 37°C.

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