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Research Article

Isopropyl(ene)-type Cembrane Diterpene an Important Chemotaxonomical Marker in Bornean Soft Coral Genus *Sarcophyton*

Chin-Soon Phan, Charles Santhanaraju Vairappan*

Laboratory of Natural Products Chemistry, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
*Corresponding author: csv@ums.edu.my

Abstract
Two cembrane diterpenoids; (+)-11,12-epoxysarcophytol A (**1**) and sarcophytol W (**2**) were isolated from *Sarcophyton* sp. collected from Mantanani Island, Sabah. Secondary metabolites structures were elucidated based on spectroscopic data. This is the first record of cembranoid diterpenes isolated from the Bornean soft coral genus *Sarcophyton*. The isopropyl(ene)-type cembrane derivatives could be suggested as chemotaxonomical markers for soft coral genus *Sarcophyton*.

Keywords*:* Soft coral, *Sarcophyton*, cembrane, diterpene, chemotaxonomy, Mantanani Island

Introduction
The soft coral genus *Sarcophyton* consist about 35 species which are difficult to distinguish (Verseveldt, 1982). In addition, the soft coral *Sarcophyton* is known to be a rich source of variety structures of cembrane diterpenes (Jia et al., 2006). Soft corals are also known to synthesize and accumulate sesquiterpenes, steroids, fatty acids and amino acids (Rao et al., 1997; Rezenka & Dembisky, 2001; Feller et al., 2004; Grote et al., 2006; Lan et al., 2006). Due to their soft bodies and sedentary life, soft corals produce terpenoids as chemical defense compounds with ichthyotoxicity activity to avoid predatory fishes (Iwagawa et al., 1999). Therefore, many secondary metabolites isolated from soft corals have been reported to exhibit a diverse spectrum of biological activities such as anti-microbial, anti-fungal, anti-tumour, anti-viral, anti-fouling and anti-inflammatory (Wei et al., 2013). Associated with these biological activities, these secondary metabolites are believed to be of ecological importance to soft corals (Ishii et al., 2010a).

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Despite the advances in soft coral chemistry, there is shortage of information related to the chemistry of Bornean soft corals in Malaysia (Vairappan et al., 2012). To date, the only available chemical investigations of Bornean soft coral are of genus *Nephthea* and *Sinularia* collected from Sabah, which has led to isolation of three novel compounds from soft coral *Nephthea* sp. and several known compounds were isolated from *Sinularia flexibilis* (Ishii et al., 2009a, b; 2010b; Vairappan et al., 2012; Palaniveloo et al., 2014). Therefore, this paper reports the first discovery of two cembranoid diterpenes from the population of the soft coral genus *Sarcophyton* from Mantanani Island, Sabah.

Materials and Methods

Collection

The *Sarcophyton* sp. population was collected from a coral reef at 12 metre depth in waters of Mantanani Kecil (Mantanani Island, Sabah) (06°42.775"N, 116°19.758"E). The samples were photographed underwater; collected by Scuba divers and a representative specimen (BORMI0007) was deposited at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The samples were weighed and cut into pieces. The samples were then brought back to the laboratory under cool conditions (4 °C) (Ishii et al., 2010b).

Extraction and isolation

The specimen was chopped and soaked in methanol (MeOH) at room temperature for approximately four days. The resulting MeOH extract was concentrated *in vacuo* and the concentrate was partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc layer was further concentrated and partitioned between hexane (Hex) and 90 % MeOH yielding its resulting Hex and 90 % MeOH extracts. The Hex and 90 % MeOH extract was independently fractionated using silica gel column chromatography with a step gradient of Hex and EtOAc (9:1, 8:2, 7:3, 6:4 and 5:5), and $CHCl_3$:MeOH:H_2O (65:25:4). The resulting fractions were then further subjected to preparative thin layer chromatography (PTLC) to isolate the two isopropyl(ene)-type cembrene diterpenes **1** and **2**. Compounds **1** and **2** were subjected to ^{1}H-NMR, ^{13}C-NMR and 2D NMR measurements such as ^{1}H-^{1}H COSY, HSQC and HMBC. Other physical characteristics were obtained as described by Ishii et al. (2009a).

Results and Discussion

Approximately 1.3 kg of *Sarcophyton* sp. was collected from a population of soft corals at Mantanani Kecil Island (Figure 1). Upon chemical extraction and

partition as described above, 1.2 g of hexane extract and 31.9 g of 90 % MeOH extract were obtained.

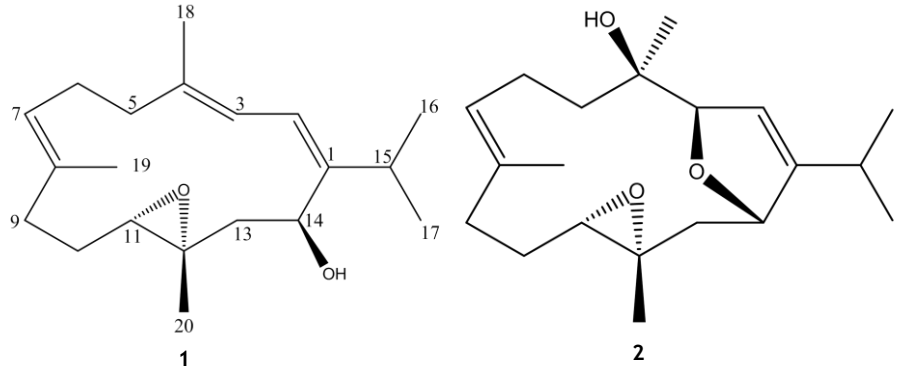
Figure 1. Underwater image of *Sarcophyton* sp.

A portion of fraction 2 (70.0 mg) was subjected to repetitive preparative TLC with $CHCl_3$-EtOAc (9:1) and toluene-EtOAc (8:2) to yield compound **1** (6.6 mg). Compound **2** (14.4 mg) was isolated from fraction 2 (175.0mg) under repetitive preparative TLC with toluene-EtOAc (8:2) and $CHCl_3$-EtOAc (9:1). The structures of **1** and **2** were elucidated based on extensive analysis of spectroscopic data and in comparison of those in previous literature data. Thus, (+)-11,12-epoxysarcophytol A (**1**) and sarcophytol W (**2**) were isolated and previously reported by Bowden et al. (1983) and Cao et al. (2013), respectively. The chemical structures of compounds **1** and **2** are shown in Figure 2.

Both compounds revealed a similar 14-membered ring with a terminal isopropyl moiety as shown in Figure 2. HSQC and ^{13}C-DEPT experiments as well as ^{13}C- and ^{1}H-NMR signals of compound **1** showed the presence of three pairs of double bond at δ_C 149.2 (C), 137.5 (C), 134.3 (C), 127.6 (CH), 120.2 (CH) and 119.0 (CH); δ_H 5.99 (1H, d), 5.76 (1H, d) and 5.11 (1H, t), five methyls at δ_C 24.9 (CH_3), 24.5 (CH_3), 20.1 (CH_3), 17.9 (CH_3) and 15.7 (CH_3); δ_H 1.74, 1.59, 1.30, 1.09 and 1.07 (each 3H), a trisubstituted epoxide moiety at δ_C 60.7 (C) and 59.3 (CH); δ_H 3.19 (1H, t), a secondary hydroxyl signal at δ_C 66.4 (CH); δ_H 4.73 (1H, dd), five methylenes and one methine.

On the other hand, HSQC and ^{13}C-DEPT experiments as well as ^{13}C- and ^{1}H-NMR signals of compound **2** revealed the presence of four olefinic carbons indicating two pairs of double bonds at δ_C 152.7 (C), 133.4 (C), 126.7 (CH) and 119.0 (CH); δ_H 5.44 (1H, q) and 5.35 (1H, d), five methyls at δ_C 23.6 (CH_3), 22.6 (CH_3), 21.9 (CH_3), 17.4 (CH_3) and 17.3 (CH_3); δ_H 1.59, 1.34, 1.12, 1.03 and 1.01 (each 3H), a trisubstituted epoxide moiety at δ_C 67.2 (CH) and 61.3 (C); δ_H 2.58(1H, dd), an isopropyl-bearing dihydrofuran moiety at δ_C 152.7 (C), 119.0 (CH), 88.2 (CH) and 83.5 (CH); δ_H 5.44 (1H, q), 4.80 (1H, dddd) and 4.54 (1H, dt), a hydroxyl-bearing quaternary carbon at δ_C 88.2 (C), five methylenes and one methine. The presence of isopropyl moiety of **1** and **2** at position 1 was confirmed by HMBC correlations. In the earlier publication of these compounds, coupling constant assignments and proton orientations were lacking, optical rotations were not reported and higher magnetic strength NMR spectroscopy was used to record the NMR spectroscopic data. The detailed spectroscopy data are given below:

Compound 1: colorless oil; $C_{20}H_{32}O_2$: $[\alpha]_D^{25}$ +66.5 ($CHCl_3$; 6.2); ^{13}C-NMR ($CDCl_3$, 150 MHz) δC: 149.2 (C-1), 119.0 (C-2), 120.2 (C-3), 137.5 (C-4), 39.1 (C-5), 25.6 (C-6), 127.6 (C-7), 134.3 (C-8), 37.1 (C-9), 24.8 (C-10), 59.3 (C-11), 60.7 (C-12), 42.8 (C-13), 66.4 (C-14), 28.3 (C-15), 24.9 (C-16), 24.5 (C-17), 17.9 (C-18), 15.7 (C-19), 20.1 (C-20); ^{1}H-NMR ($CDCl_3$, 600 MHz) δH: 5.99 (1H, d, *J* = 11.0 Hz, H-2), 5.76 (1H, d, *J* = 11.0 Hz, H-3), 2.17 (2H, m, H-5), 2.23 (2H, m, H-6), 5.11 (1H, t, *J* = 6.2 Hz, H-7), 2.25 (1H, m, H-9α), 2.08 (1H, m, H-9β), 1.86 (1H, m, H-10α), 1.49 (1H, dtd, *J* = 13.8, 7.6, 4.1, H-10β), 3.19 (1H, t, *J* = 6.9 Hz, H-11β), 1.98 (1H, dd, *J* = 15.1, 4.9 Hz, H-13α), 2.11 (1H, dd, *J* = 15.1, 7.6 Hz, H-13β), 4.73 (1H, dd, *J* = 7.6, 5.5 Hz, H-14β), 2.67 (1H, septet, *J* = 6.9 Hz, H-15), 1.09 (3H, d, *J* = 6.9 Hz, H-16), 1.07 (3H, d, *J* = 6.9 Hz, H-17), 1.74 (3H, s, H-18), 1.59 (3H, s, H-19), 1.30 (3H, s, H-20β).



1 **2**

Figure 2. Chemical structures of **1** and **2** from *Sarcophyton* sp.

Compound 2: colorless oil; $C_{20}H_{32}O_3$: $[\alpha]_D^{25}$ +16.1 ($CHCl_3$; 1.1); ^{13}C-NMR ($CDCl_3$, 150 MHz) δC: 152.7 (C-1), 119.0 (C-2), 88.2 (C-3), 75.5 (C-4), 41.4 (C-5), 22.5 (C-6), 126.7 (C-7), 133.4 (C-8), 37.3 (C-9), 25.8 (C-10), 67.2 (C-11), 61.3 (C-12), 46.3 (C-13), 83.5 (C-14), 26.9 (C-15), 21.9 (C-16), 22.6 (C-17), 23.6 (C-18), 17.3 (C-19), 17.4 (C-20); ^{1}H-NMR ($CDCl_3$, 600 MHz) δH: 5.44 (1H, q, *J* = 1.6 Hz, H-2), 4.54 (1H, dt, *J* = 5.5, 2.1 Hz, H-3α), 1.94 (1H, ddd, *J* = 14.1, 11.1, 1.9 Hz, H-5α), 1.61 (1H, dtd, *J* = 14.4, 6.2, 1.4 Hz, H-5β), 2.17 (1H, m, H-6α), 2.12 (1H, m, H-6β), 5.35 (1H, d, *J* = 4.8 Hz, H-7), 2.19 (2H, m, H-9), 1.79 (2H, m, H-10), 2.58 (1H, dd, *J* = 6.2, 2.8 Hz, H-11β), 2.19 (1H, m, H-13α), 1.01 (1H, m, H-13β), 4.80 (1H, dddd, *J* = 11.7, 5.3, 3.6, 1.7 Hz, H-14β), 2.19 (1H, m, H-15), 1.12 (3H, d, *J* = 6.9 Hz, H-16), 1.03 (3H, d, *J* = 6.9 Hz, H-17α), 1.01 (3H, s, H-18), 1.59 (3H, s, H-19), 1.34 (3H, s, H-20β).

Similar structure of compound **1** was reported by Bowden and coworkers (1983) which was isolated from *Lobophytum* sp. In addition, compound **1** was also isolated from soft coral genus *Sinularia* (Ahmed et al., 2008). Therefore, compound **1** could be the common metabolite that is present in several different genus of soft corals. However, compound **2** was first discovered by Cao and coworkers (2013) from *Sarcophyton* sp. To date, compound **2** was only reported in *Sarcophyton* sp. Thus, compound **2** might be suggested as a chemotaxonomical marker for soft coral genus *Sarcophyton*. The isopropyl(ene)-type cembrane derivatives are a class of diterpene commonly found in soft coral genus *Sarcophyton* (Dong et al., 2000; Yan et al., 2008; Jia et al., 2010; Aratake et al., 2012; Cao et al., 2013; Liang et al., 2013). Therefore, taking these two compound's biosynthetic pathway and structure relation, compounds 1 and 2 is suggested as chemotaxonomic markers of *Sarcophyton* sp.

In conclusion, chemical investigation of *Sarcophyton* sp. from Mantanani Island, Sabah, has led to isolation of (+)-11,12-epoxysarcophytol A (**1**) and sarcophytol W (**2**). These two isopropyl cembrenes have enriched our knowledge of the chemical constituents of Bornean soft coral *Sarcophyton* sp. To our knowledge, this is the first record of diterpenoids isolated from the Bornean soft coral *Sarcophyton* sp. Isopropyl(ene)-type cembrane derivatives might serve as chemotaxonomic markers for soft coral genus *Sarcophyton*.

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