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CEMBRANOID CONSTITUENTS FROM *LOBOPHYTUM CRASSUM*

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Abstract

Five cembranoids, 2,16:7*S*,8*S*-diepoxy-1,3,11,15-cembratetraene (**1**), 7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester (**2**), (1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-triene-4-ol (**3**), (2*S*,7*S*,8*S*)-sarcophine (**4**), and (2*S*,7*S*,8*S*)-sarcophytoxide (**5**), were isolated from the methanol extract of the soft coral *Lobophytum crassum* by using various chromatographic methods. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. Compounds **1** and **3** were isolated from *L. crassum* for the first time.

Keywords. *Lobophytum crassum*, Alcyoniidae, soft coral, cembranoid.

1. INTRODUCTION

Soft corals are marine invertebrates of the order Alcyonacea, subclass Octocorallia, class Anthozoa, and phylum Cnidaria. These marine invertebrates are a rich source of secondary metabolites, especially diterpenoids and hydroxylated sterols [1]. As part of our ongoing investigations to find bioactive compounds from Vietnamese marine invertebrates, we have reported eight new cembranoids from the soft coral *Lobophytum crassum* [2, 3]. The current paper deals with the isolation and structural identification of five cembranoids including 2,16:7*S*,8*S*-diepoxy-1,3,11,15-cembratetraene (**1**), 7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester (**2**), (1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-trien-4-ol (**3**), (2*S*,7*S*,8*S*)-sarcophine (**4**), and (2*S*,7*S*,8*S*)-sarcophytoxide (**5**) from this soft coral.

2. EXPERIMENTAL

2.1. General experimental procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer, TMS was used as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 minutes.

2.2. Marine materials

The specimens of *Lobophytum crassum* were collected in Conco, Quang Tri, Vietnam, during May 2013 and deep frozen until used. The sample was identified by Professor Do Cong Thung (Institute of Marine Environment and Resources). A voucher of specimen (No. LC0513) was deposited at the Institute of Marine Biochemistry and the Institute of Marine Resources and Environment, VAST, Vietnam.

2.3. Isolation

Freeze-dried bodies of the soft coral *L. crassum* (1.0 kg) were well chopped and extracted three times with hot MeOH (at 50 °C for 5 h each time). The obtained solutions were filtered, combined, and concentrated under reduced pressure to yield a dark brown viscous residue (75.0 g, M). This residue was suspended in water (1 L) and partitioned with *n*-hexane and CH_2Cl_2 (3 × 3 L). The combined *n*-hexane soluble portions were evaporated under reduced pressure to afford a *n*-hexane fraction (36.2 g, H). Fraction H was crudely separated on silica gel column chromatography (CC) with gradient

concentrations of ethyl acetate in *n*-hexane from 0 to 100% as eluent to yield six fractions, H-1 to H-6. Fraction H2 (1.5 g) was further separated on silica gel CC using *n*-hexane/EtOAc (25/1) as eluent, to give three sub-fractions, H-2.1 to H-2.3. Sub-fraction H-2.1 (0.3 g) was then separated on silica gel CC with eluent of *n*-hexane/acetone (30/1) and further purified by YMC RP-18 CC eluting with MeOH/acetone/H_2O (9/1/1) to afford **1** (6.8 mg). Compound **5** (8.2 mg) was purified from sub-fraction H-2.3 (0.8 g) using silica gel CC with *n*-hexane/EtOAc (20/1) as eluent. Fraction H-4 (2.5 g) was separated on YMC RP-18 CC with eluent of MeOH/acetone/H_2O (95/2/3) to yield four subfractions, H-4.1 to H-4.4. Subfraction H-4.1 (0.8 g) was further separated on YMC RP-18 CC, eluting with MeOH/H_2O (8/1) to yield **4** (9.8 mg). Sub-fraction H-4.2 (0.5 g) was separated into four smaller fractions, H-4.2a to H-4.2d, using YMC RP-18 CC with acetone/water (85/15) as eluent. Subfraction H-4.2a (0.17 g) afforded compound **3** (9.8 mg) and **2** (10.4 mg), after subjecting it to silica gel CC with eluent of dichloromethane/acetone (35/1).

2,16:7*S*,8*S*-diepoxy-1,3,11,15-cembratetraene (**1**): Colorless oil; $[\alpha]_D$ + 35 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS *m/z* 323 $[M+Na]^+$ ($C_{20}H_{28}O_2$, M = 300).

7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester (**2**): Colorless oil; $[\alpha]_D$ +15 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1; ESI-MS *m/z* 355 $[M+Na]^+$ ($C_{21}H_{32}O_3$, M = 332).

(1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-triene-4-ol (**3**): Colorless oil; $[\alpha]_D$ –70 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS *m/z* 313 $[M+Na]^+$ ($C_{20}H_{34}O$, M = 290).

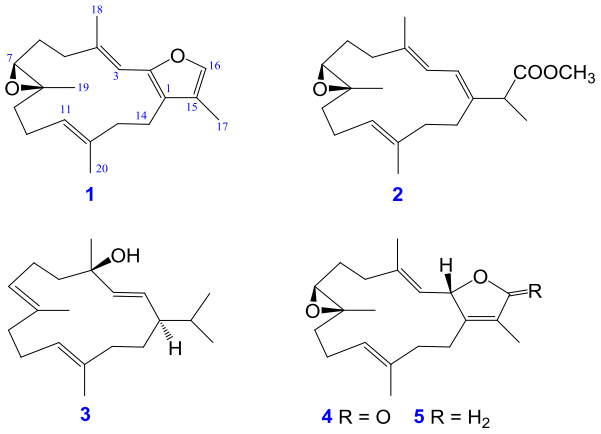

Figure 1: Chemical structures of compounds **1**–**5**

Table 1: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **1**–**3** and reported compounds

C	**1**[a]		[b]δ_C	**2**[a]		[c]δ_C	**3**[a]	
	δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	122.04	-	139.7	139.62	-	46.3	46.14	1.63 m
2	149.50	-	122.9	122.82	6.19 d (11.0)	127.3	126.99	5.27 dd (9.0, 15.5)
3	113.46	5.91 d (1.0)	121.0	120.93	5.96 dd (11.0, 1.0)	138.8	138.78	5.55 d (15.5)
4	133.93	-	136.6	136.54	-	73.7	73.80	-
5	36.99	2.31 m	35.9	35.82	2.27 m	44.1	44.04	1.60 m/1.88 m
6	25.58	1.75 m/1.81 m	25.7	25.61	1.71 m/1.80 m	23.6	23.48	2.08 m/2.33 m
7	60.47	2.88 dd (5.5, 6.5)	61.3	61.21	2.83 t (5.5)	127.7	127.79	5.20 t (7.0)
8	60.39	-	60.0	60.00	-	132.8	132.83	-
9	36.20	1.75 m/1.81 m	36.9	37.20	1.59 m/1.89 m	39.1	39.01	2.07 m/2.13 m
10	21.76	1.92 m	22.3	22.28	2.00 m	23.9	23.68	2.08 m/2.33 m
11	126.47	5.03 dt (6.5, 1.0)	126.1	126.04	5.07 dt (6.5, 1.0)	124.9	124.83	5.03 dt (6.5, 1.0)
12	134.60	-	135.3	135.23	-	132.5	132.57	-
13	38.34	2.13 m/2.20 m	38.2	38.46	2.00 m/2.23 m	37.0	36.86	2.02 m
14	22.78	2.47 m/2.56 m	29.0	28.94	2.33 m	28.2	27.95	1.28 m/1.61 m
15	120.53	-	46.3	46.24	3.20 d (7.5)		33.00	1.50 m
16	137.40	7.12 br s	175.3	175.24	-	19.4	19.34	0.81 d (7.0)
17	8.21	1.97 d (1.5)	16.8	16.73	1.31 d (7.5)	20.6	20.65	0.84 d (7.0)
18	18.12	2.03 br s	17.2	17.21	1.77 br s	29.4	29.30	1.26 s
19	18.72	1.27 s	18.1	18.03	1.26 s	15.2	15.11	1.58 s
20	17.49	1.42 br s	17.1	17.01	1.58 br s	14.9	14.79	1.52 s
OMe			51.8	51.77	3.67 s			

[a]recorded in $CDCl_3$, [b]δ_C of 7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester [4],
[c]δ_C of (1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-trien-4-ol [5].

Table 2: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **4**, **5**, and reported compounds

C	[a]δ_C	**4**[b]		[c]δ_C	**5**[b]		
		δ_C	δ_C mult. (*J* = Hz)		δ_C	δ_C mult. (*J* = Hz)	HMBC (H → C)
1	162.2	162.14	-	133.2	133.21	-	
2	78.8	78.66	5.57 dd (1.5, 10.0)	83.6	83.64	5.54 d (10.0)	
3	120.6	120.50	5.03 dd (1.5, 10.0)	126.2	126.26	5.23 d (10.0)	1, 5, 18
4	144.0	143.87	-	138.9	139.21	-	
5	37.4	37.24	2.38 m	37.5	37.61	2.35 m	
6	25.2	25.12	1.90 m/1.68 m	25.2	25.27	1.65 m/1.92 m	
7	61.5	61.25	2.68 t (4.5)	61.7	61.78	2.71 t (4.0)	
8	59.9	59.76	-	59.6	59.81	-	
9	39.0	38.85	1.10 dt (3.0, 13.0) 2.11 m	39.6	39.76	1.01 dt (13.0, 3.0) 3.13 m	8, 10, 11, 19
10	23.3	23.17	2.27 m/1.93 m	23.4	23.49	1.90 m/2.27 m	
11	124.9	124.82	5.14 dd (5.5, 9.0)	123.6	123.60	5.10 dd (5.5, 10.0)	10, 13, 20
12	135.5	135.37	-	136.5	136.77	-	
13	36.4	36.20	2.20 m/2.03 m	36.5	36.63	1.83 m	
14	27.5	27.40	2.75 m/2.10 m	25.9	26.06	1.68 m/2.56 m	1, 2, 13, 15
15	122.9	122.75	-	127.7	127.82	-	
16	175.0	174.51	-	78.2	78.34	4.50 m	1, 15
17	8.9	8.86	1.85 t (1.5)	10.0	10.13	1.65 s	1, 15, 16
18	16.1	16.00	1.90 s (1.0)	15.4	15.54	1.82 s	3, 4, 5
19	17.7	17.05	1.28 s	16.8	16.88	1.27 s	7, 8, 9
20	15.5	15.32	1.62 s	15.0	15.05	1.60 s	11, 12, 13

[a]δ_C of (2*S*,7*S*,8*S*)-sarcophine [6], [b]recorded in $CDCl_3$, [c]δ_C of (2*S*,7*S*,8*S*)-sarcophytoxide [7].

(2*S*,7*S*,8*S*)-sarcophine (**4**): Colorless oil; $[\alpha]_D$ +90 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 2; ESI-MS *m/z* 339 $[M+Na]^+$ ($C_{20}H_{28}O_3$, M = 316).

(2*S*,7*S*,8*S*)-sarcophytoxide (**5**): Colorless solid; $[\alpha]_D$ +130 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m/z* 325 $[M+Na]^+$ ($C_{20}H_{30}O_2$, M = 302).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil. Its NMR features indicated a cembranoid, one main constituent of soft corals. The ^{1}H-NMR spectrum exhibited typical signals of three olefinic [δ_H 5.91 (1H, d, *J* = 1.0 Hz, H-3), 5.03 (1H, dt, *J* = 6.5, 1.0 Hz, H-11), and 7.12 (1H, br s, H-16)] and four tertiary methyl groups [δ_H 1.97 (3H, d, *J* = 1.5 Hz, H-17), 2.03 (3H, br s, H-18), 1.27 (3H, s, H-19), and 1.42 (3H, br s, H-20)]. Moreover, the presence of one epoxy group was determine by signals at δ_C 60.47 (d, C-7)/δ_H 2.88 (1H, dd, *J* = 5.5, 6.5 Hz, H-7) and δ_C 60.39 (s, C-8). The ^{1}H-^{1}H correlation spectroscopy (COSY) led to assignment of the following connectivities: H-5/H-6/H-7, H-9/H-10/H-11, and H-13/H-14. The COSY evidence and the HMBC cross-peaks of H-3 (δ_H 5.91) with C-1 (δ_C 122.04)/C-4 (δ_C 133.93)/C-5 (δ_C 36.99), H-16 (δ_H 7.12) with C-1 (δ_C 122.04)/C-2 (δ_C 149.50)/C-15 (δ_C 120.53), H-17 (δ_H 1.97) with C-1 (δ_C 122.04)/C-15 (δ_C 120.53)/C-16 (δ_C 137.40), H-18 (δ_H 2.03) with C-3 (δ_C 113.46)/C-4 (δ_C 133.93)/C-5 (δ_C 36.99), H-19 (δ_H 1.27) with C-7 (δ_C 60.47)/C-8 (δ_C 60.39)/C-9 (δ_C 36.20), and those of H-20 (δ_H 1.42) with C-11 (δ_C 126.47)/C-12 (δ_C 134.36)/C-13 (δ_C 38.34), confirmed the structure of compound **1** as 2.16: 7*S*,8*S*-diepoxy-1,3,11,15-cembratetraene. This compound was previously isolated from a *Sarcophyton* soft coral [7]. However, this is the first report of this compound from *L. crassum* and the ^{13}C-NMR data of **1** were reported here for the first time.

The NMR data of **2** were similar to those of **1** indicating that this compound is also a cembranoid. The easily visible difference of the NMR data between these two compounds is the additional presence of a methoxy group [δ_C 51.77/δ_H 3.67 (3H, s)] and replacement of a carbonyl [δ_C 175.24 (C-16)], a methine [δ_C 46.24 (C-15)/δ_H 3.20 (1H, d, *J* = 7.5 Hz, H-15)] and a secondary methyl groups [δ_C 16.73 (C-17)/δ_H 1.31 (3H, d, *J* = 7.5 Hz, H-17)] in **2** for a fully substituted double bond and a tertiary

methyl group in **1**. From the above evidence, the ^{13}C-NMR data of **2** were compared to those of 7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester [4] and found to match (table 1). The structure of **2** was further confirmed by HMBC experiment (figure 2). This compound was previously isolated from *L. crassum* [4] demonstrating our taxonomic identification.

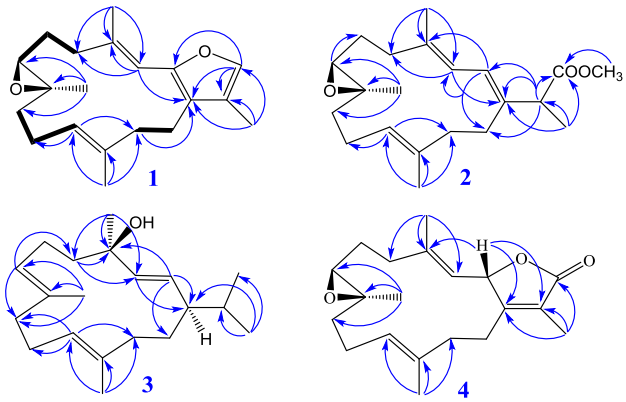

Figure 2: Key COSY (▬) and HMBC (→) correlations of **1**–**4**

The other compounds were elucidated as (1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-triene-4-ol (**3**) [8], (2*S*,7*S*,8*S*)-sarcophine (**4**) [6], and (2*S*,7*S*,8*S*)-sarcophytoxide (**5**) [7] by agreement of their ^{13}C-NMR data with the reported values and combination with 2D-NMR data (tables 1 and 2).

4. CONCLUSION

Using combined chromatographic methods, five cembranoids including 2,16:7*S*,8*S*-diepoxy-1,3,11,15-cembratetraene (**1**), 7*S*,8*S*-epoxy-1,3,11-cembratriene-16-oic acid methyl ester (**2**), (1*R*,4*R*,2*E*,7*E*,11*E*)-cembra-2,7,11-triene-4-ol (**3**), (2*S*,7*S*,8*S*)-sarcophine (**4**), and (2*S*,7*S*,8*S*)-sarcophytoxide (**5**) were isolated from the methanol extract of the soft coral *Lobophytum crassum*. Their structures were elucidated by 1D and 2D-NMR experiment. This is the first report of the ^{13}C-NMR data of **1** and compounds **1** and **3** were firstly isolated from *L. crassum*.

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