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DITERPENOID CONSTITUENTS FROM *SINULARIA MAXIMA*

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Abstract

Three diterpenoids including isomandapamate (**1**), sethukarailin (**2**), and (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol (**3**) were isolated and structurally elucidated from the methanol extract of the soft coral *Sinularia maxima* by using combined chromatographic and spectroscopic experiments. Of the isolated compounds, **3** was isolated from *S. maxima* for the first time.

Keywords. *Sinularia maxima*, Alcyoniidae, soft coral, diterpene.

1. INTRODUCTION

Soft corals are a group of colonial invertebrates which form a significant set of marine organisms occurring widely in the coral reefs throughout the world [1, 2]. Among the Alcyonacean soft corals, genus *Sinularia* is one of the most widely distributed soft coral genera, constituting a dominant portion of the biomass in the tropical reef environment. *Sinularia* species are rich sources of structurally unique and biologically active diterpenoids [1]. As part of our ongoing investigations to find bioactive compounds from Vietnamese marine invertebrates, we have reported nine new diterpenoids from the soft coral *Sinularia maxima* [3]. The current paper addresses the isolation and structural elucidation of three diterpenoids including isomandapamate (**1**), sethukarailin (**2**), and (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol (**3**) 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 sample of soft coral *S. maxima* was collected at Nha trang Bay, in 11/2010 and identified by Prof. Do Cong Thung (Institute of Marine Environment and Resources, VAST). A voucher specimen (SM112010_01) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST.

2.3. Isolation

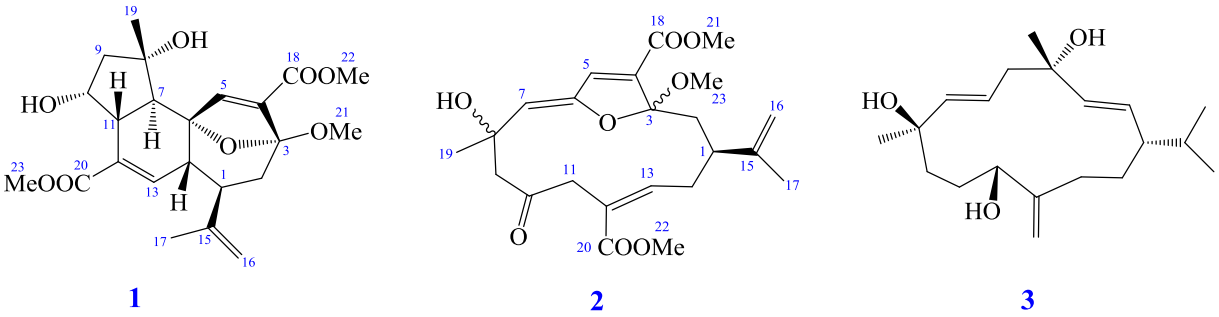
Fresh frozen samples of the soft coral *S. maxima* (2.5 kg) were well grinded and extracted three times with hot MeOH (at 50 °C for 5 h each time). The obtained solutions were filtered, combined, and concentrated under reduced pressure to yield a dark brown viscous residue (38.75 g, A). This residue was suspended in water (2 L) and partitioned in turn with CH_2Cl_2 (3 × 2 L) and EtOAc (3 × 2 L). The combined dichloromethane soluble portions were evaporated under reduced pressure to afford CH_2Cl_2 extract (12.24 g, B). Extract B was crudely separated by silica gel column chromatography (CC) using gradient concentrations of ethyl acetate in *n*-hexane from 0 to

100% to yield four fractions, B-1 to B-4. Fraction B4 (2.05 g) was fractionated into five subfractions, B4.1 to B4.5, by YMC RP-18 CC using stepwise elution with acetone/H_2O (1/3 to 1/1). Subfraction B4.1 (0.38 g) afforded compound **3** (12 mg) after subjecting it to silica gel CC eluting with CH_2Cl_2/acetone (6/1). Compound **1** (15 mg) was obtained from subfraction B4.3 (0.57 g) by silica gel CC using CH_2Cl_2/EtOAc (3.5/1) as eluent. Subfraction B4.5 (0.39 g) was chromatographed on silica gel CC eluting with $CHCl_3$/MeOH/acetone (25/1/0.6) and further separated by YMC RP-18 CC with acetone/H_2O (1/2.5) to obtain compound **2** (7 mg).

Isomandapamate (**1**): Colorless oil; $[\alpha]_D$ +75 (*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* 457 $[M+Na]^+$ ($C_{23}H_{30}O_8$, M = 434).

Sethukarailin (**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* 457 $[M+Na]^+$ ($C_{23}H_{30}O_8$, M = 434).

(1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol (**3**): Colorless solid; $[\alpha]_D$ +70 (*c* 0.1, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 2; ESI-MS *m/z* 345 $[M+Na]^+$ ($C_{20}H_{34}O_3$, M = 322).



1 **2** **3**

Figure 1: Chemical structures of compounds **1**–**3**

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

C	$^a\delta_C$	$^b\delta_C$	**1**[c]		$^d\delta_C$	**2**[c]	
			δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	43.2	43.4	43.86	2.19 m	41.4	42.01	1.74 m
2	33.4	33.5	33.90	1.61 m/1.90 m	**33.2**	**40.27**	2.54 dd (6.0, 14.5)
							2.03 dd (5.0, 14.5)
3	111.1	111.2	111.66	-	115.9	116.43	-
4	134.8	135.0	135.33	-	131.2	131.73	-
5	**154.4**	**150.5**	**151.15**	6.65 s	138.9	139.57	6.94 s
6	84.7	84.8	85.32	-	150.0	150.63	-
7	52.6	52.8	53.21	2.68 d (13.0)	116.9	117.54	5.14 d (1.5)
8	77.5	77.5	77.93	-	70.7	71.30	-
9	51.3	51.5	51.99	2.20 m	51.4	51.99	3.86 d (18.5)
				1.97 br d (15.0)			2.67 dd (1.5, 18.5)
10	67.6	67.5	68.17	4.58 m	210.6	211.39	-
11	46.9	47.2	47.59	2.45 br d (13.0)	40.9	41.48	3.32 d (18.5)/3.52 d (18.5)
12	133.5	133.6	134.12	-	128.1	128.63	-
13	143.2	143.2	144.02	6.63 t (3.0)	143.3	143.95	6.98 t (7.0)
14	43.2	43.4	43.78	2.14 m	**39.8**	**33.71**	2.14 m/2.21 m
15	144.2	144.5	144.75	-	145.8	146.37	-
16	113.4	113.6	114.20	4.82 s/4.72 s	112.8	113.44	4.72 s/4.56 s
17	19.6	19.8	20.33	1.62 s	19.5	20.11	1.60 s
18	162.5	162.0	163.22	-	162.0	162.61	-
19	26.8	27.0	27.55	1.51 s	27.3	27.88	1.34 s
20	166.6	166.8	167.34	-	167.3	167.91	-
21	51.8	51.1	52.11	3.33 s	51.8	52.49	3.73 s
22	52.3	51.8	52.45	3.74 s	52.2	52.76	3.76 s
23	52.0	51.9	52.70	3.70 s	49.9	50.59	3.07 s

$^a\delta_C$ of mandapamate [4], $^b\delta_C$ of isomandapamate [5], ^{c}recorded in $CDCl_3$, $^d\delta_C$ of sethukarailin [6].

Table 2: NMR data of **3** and reported compound

C	[a]δ_C	δ_C[b,c]	δ_C[b,d] mult. (*J* = Hz)	HMBC (H → C)
1	49.2	49.66	1.65 m	
2	130.2	129.90	5.32 m	1, 3
3	138.2	138.01	5.35 d (16.0)	2, 4
4	73.0	73.05	-	
5	45.7	45.46	2.22 m/2.34 dd (14.0, 4.0)	4, 18
6	123.6	124.92	5.51[e]	5, 7
7	139.3	139.24	5.50[e]	6, 8
8	73.2	73.16	-	
9	37.0	37.27	1.49 m/1.30 m	8, 10
10	29.4	28.70	1.58 m/1.28 m	11
11	76.6	75.21	4.11 t (6.2)	12, 13, 20
12	151.4	151.14	-	
13	29.1	29.64	1.57 m/1.16 m	11, 12, 14
14	31.2	28.97	1.87 m/1.96 m	1
15	32.2	32.31	1.54 m	
16	19.7	19.71	0.82 d (7.0)	1, 15, 17
17	20.6	20.67	0.85 d (7.0)	1, 15, 16
18	30.7	30.77	1.29 s	3, 4, 5
19	30.6	28.93	1.26 s	7, 8, 9
20	111.3	110.43	4.81 br s/4.92 br s	11, 12, 13

[a]δ_C of (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol [7], [b]recorded in $CDCl_3$, [c]125 MHz, [d]500 MHz, [e]overlapped signals.

3. RESULTS AND DISCUSSION

The NMR features of **1** indicated a diterpenoid, one main constituent of soft corals. The ^{1}H-NMR spectrum of **1** exhibited typical signals of two tertiary methyl [δ_H 1.62 (H-17) and 1.51 (H-19), each 3H, s], one oxymethine [δ_H 4.58 (1H, m, H-10)], three methoxyl groups [δ_H 3.33 (H-21), 3.74 (H-22), 3.70 (H-23), each 3H, s], and two olefinic protons [δ_H 6.65 (1H, s, H-5) and 6.63 (1H, t, *J* = 3.0 Hz, H-13)]. In addition, a terminal olefinic methylene was identified by singlet proton signals at δ_H 4.72 (H_a-16)/4.82 (H_b-16), which was correlated with the relevant carbon at δ_C 114.20 (C-16). The presence of two methyl [δ_C 20.33 (C-17) and 27.55 (C-19)], one oxymethine [δ_C 68.17], three methoxyl carbons [δ_C 52.11 (C-21), 52.45 (C-22), and 52.70 (C-23)], and two trisubstituted double bonds [δ_C 135.33 (s, C-4)/151.15 (d, C-5) and 134.12 (s, C-12)/144.02 (d, C-13)] was also confirmed by ^{13}C-NMR spectrum of **1**. Moreover, carbon signals of one dioxygenated quaternary [δ_C 111.66 (C-3)], two oxygenated quaternary [δ_C 85.35 (C-6) and 77.93 (C-8)], and two carbonyl [δ_C 163.22 (C-18) and 167.34 (C-20)] carbons were also observed. From above evidence, the ^{13}C-NMR data of **1** (Table 1) were found to be similar to those of isomandapamate, a diterpene previously isolated from *S. maxima* [5]. Detailed analysis of the HMBC cross-peaks also confirmed the structure of **1**. Finally, the ^{13}C-NMR chemical shift for C-5 of **1** at δ_C 151.15 was similar to that of isomandapamate at δ_C 150.5 [5] and quite different from that of mandapamate at δ_C 154.4 [4] confirmed that compound **1** is isomandapamate.

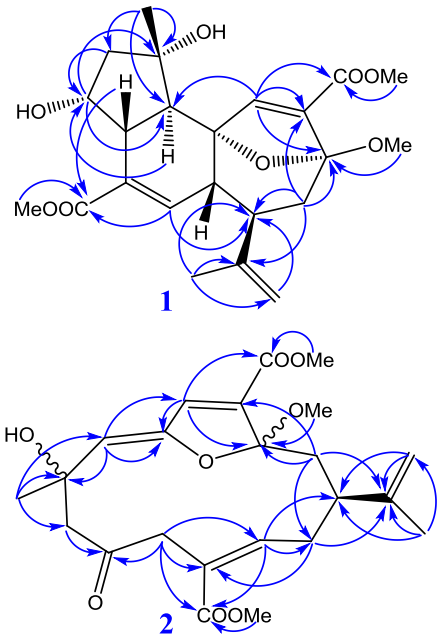


Figure 2: Key HMBC correlations of **1** and **2**

The ^{1}H and ^{13}C-NMR data of **2** were similar to those of **1** with the presence of two tertiary methyl [δ_H 1.60 (H-17) and 1.34 (H-19), each 3H, s], three methoxyl [δ_H 3.73 (H-21), 3.76 (H-22), 3.07 (H-23), each 3H, s], a terminal olefinic methylene groups [δ_H 4.56 and 4.72, each 1H, s, H-16/113.44 (C-16)], and two carbonyl carbons [δ_C 162.61 (C-18) and 167.91 (C-20)]. The easily visible difference between these two compounds is the absence of an oxygenated quaternary and an oxymethine carbons and the additional presence of a trisubstituted double bond and a ketone group in the spectra of **2** relative to those of **1**. The good agreement of the ^{13}C-NMR data of **2** (table 1) with the reported values [6] and combination with the HMBC data (figure 2) confirmed compound **2** as sethukarailin. However, based on HSQC and HMBC experiments (figure 2), the published ^{13}C-NMR data at C-2 and C-14 of sethukarailin [6] must be reversed as shown in the table 1.

An agreement of the ^{13}C-NMR data with the reported values and combination with HMBC data (Table 2) led to identification of compound **3** as (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol [7]. This compound was first isolated from *S. maxima*.

4. CONCLUSION

Using combined chromatographic and spectroscopic methods, three diterpenoids including isomandapamate (**1**), sethukarailin (**2**), and (1*S*,2*E*,4*S*,6*E*,8*S*,11*R*)-2,6,12(20)-cembratriene-4,8,11-triol (**3**) were isolated and structurally elucidated from the methanol extract of the soft coral *Sinularia maxima*. This is the first report of compound **3** from this soft coral, while compounds **1** and **2** were previously isolated from *S. maxima* confirming our taxonomic identification.

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