VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 107-111 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-025

SESQUITERPENES AND STEROLS FROM THE SOFT CORAL *SINULARIA CRUCIATA*

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Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Two sesquiterpenes as sinularianin D (**1**) and 1*S**,4*S**,5*S**,10*R**-4,10-guaianediol (**2**), and three sterols as 24-methylenecholestane-3β,5α,6β-triol-6-monoacetate (**3**), 3β,7α-dihydroxyergosta-5,24(28)-diene (**4**), and 3β-hydroxyandrost-5-ene-17-one (**5**), were isolated from a methanol extract of the soft coral *Sinularia cruciata*. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. This is the first report of these compounds from *S. cruciata*.

Keywords. *Sinularia cruciata*, Alcyoniidae, soft coral, sesquiterpene, sterol.

1. INTRODUCTION

Soft corals are marine invertebrates of the order Alcyonacea, class Anthozoa, and phylum Cnidaria. These invertebrates are a rich source of secondary metabolites, such as diterpenoids and hydroxylated sterols [1]. Soft corals of the genera *Lobophytum*, *Sarcophyton*, and *Sinularia* are the most prolific [2]. *Sinularia cruciata* presents a little investigated species with only four sterols (sarcoaldesterol B, ergosta-1β,3β,5α,6β-tetraol, cholesterol peroxide, and cholesterol) and two alcohols (batilol and chimyl alcohol) reported to date [3, 4].

As a part of our ongoing investigations on Vietnamese soft corals, we report herein the isolation and structure elucidation of two sesquiterpenes as sinularianin D (**1**) and 1*S**,4*S**,5*S**,10*R**-4,10-guaianediol (**2**), and three sterols as 24-methylenecholestane-3β,5α,6β-triol-6-monoacetate (**3**), 3β,7α-dihydroxyergosta-5,24(28)-diene (**4**), and 3β-hydroxyandrost-5-ene-17-one (**5**) from the soft coral *S. cruciata*.

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. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system (SE-751 03 Uppsala, Sweden). 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 samples of soft coral *S. cruciata* were collected in Ha Long, Quang Ninh, Vietnam, in October 2013 and identified by Prof. Do Cong Thung (Institute of Marine Environment and Resources, VAST). Voucher specimens (No. SHM-21) were deposited at the Institute of Marine Environment and Resources and the Institute of Marine Biochemistry, VAST, Vietnam.

2.3. Isolation

Freeze-dried bodies of the soft coral *S. cruciata* (2.0 kg) were well grinded and extracted three times with MeOH in ultrasonic condition (3×1h) to obtain an extract (120.0 g, M), which was suspended in H_2O (2.0 L) and then partitioned with CH_2Cl_2 (3 × 2.0 L) to furnish CH_2Cl_2 residue (65.0 g, C) and water layer. Residue C (65 g) was crudely separated by silica gel MPLC using gradient concentrations of acetone in *n*-hexane (from 0 to 100 %) to give eight fractions (C-1→C-8). Fraction C-4 (5.0 g) was separated into five subfractions, C-4A to C-4E by YMC CC eluting with MeOH–H_2O (5:1). Compound **5** (2 mg) was purified from subfraction C-4B (0.38 mg) by silica gel CC using *n*-hexane–ethyl acetate (4:1) as eluent. Fraction C-5 (6.0 g) was separated by YMC RP-18 MPLC using mobile phase of methanol–H_2O (5:1) to obtain five subfractions, C5A–C5E. Purification of subfraction C-5B (0.78 g) by silica gel CC eluting with n-hexane-acetone (6:1) to obtain compounds **1** (5 mg) and **2** (7 mg). Fraction C-6 (12 g) was crudely separated into six subfractions C-6A-C-6E by YMC RP-18 MPLC using mobile phase of methanol–H_2O (5:1). Subfraction C6D (3.2 g) was further separated by silica gel CC eluding with *n*-hexane–ethyl acetate (3:1) to give four smaller fractions C6D1–C6D4. Fraction C6D3 (400 mg) furnished compounds **3** (6.0 mg) and **4** (3.6 mg) after subjecting it to silica gel CC eluted with dicholoromethane–acetone (6:1), followed by Sephadex LH-20 CC with MeOH–H_2O (3:1).

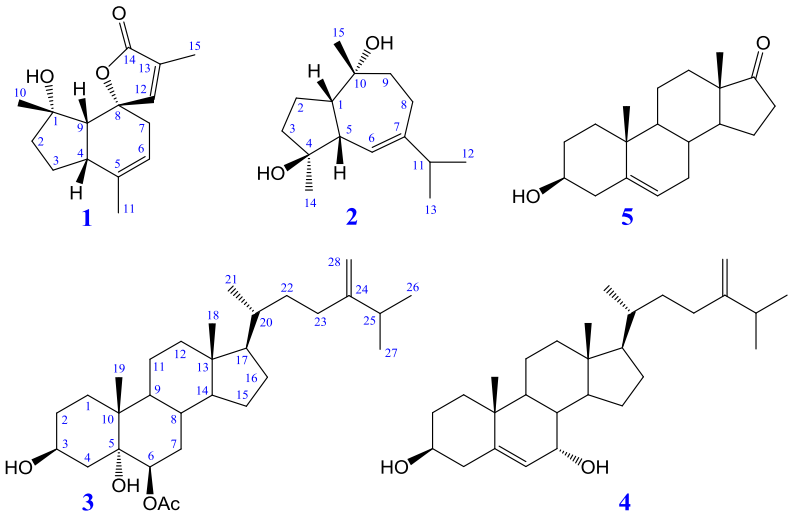

Figure 1: Chemical structures of compounds **1**-**5**

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

	[a]δ_C	[b]δ_C	**1**[c]		[d]δ_C	**2**[c]	
			δ_C	δ_H (*J* = Hz)		δ_C	δ_H (*J* = Hz)
1	78.5	78.4	78.36	-	50.7	50.74	1.88 m
2	41.8	43.1	43.10	1.80 m/1.94 m	21.5	21.54	1.65 m/1.77 m
3	25.3	25.9	25.92	1.23 m/2.03 m	40.4	40.51	1.63 m/1.70 m
4	40.9	41.8	41.85	2.91 m	80.2	80.25	-
5	137.3	137.5	137.49	-	50.3	50.37	2.17 m
6	117.2	117.2	117.20	5.25 dd (2.0, 4.0)	121.3	121.31	5.50 br d (2.0)
7	39.7	39.4	39.42	1.93 m/2.52 m	149.6	149.70	-
8	85.5	85.6	85.59	-	25.1	25.11	1.94 m/2.19 m
9	**56.6**	**55.5**	**55.50**	1.55 d (13.0)	42.6	42.65	1.48 m/1.81 m
10	**26.0**	**28.5**	**28.46**	1.27 s	75.2	75.27	-
11	20.2	20.5	20.46	1.74 s	37.3	37.32	2.24 m
12	152.2	150.9	150.93	7.03 d (1.5)	21.4	21.41	0.98 d (7.0)
13	129.1	129.7	129.67	-	21.3	21.20	0.98 d (7.0)
14	173.8	174.0	173.53	-	22.5	22.59	1.21 s
15	10.6	10.6	10.63	1.94 d (1.5)	21.2	21.44	1.29 s

[a]δ_C of sinularianin B [5], [b]δ_C of sinularianin D [6], [c]recorded in $CDCl_3$, [d]δ_C of 1*S**,4*S**,5*S**,10*R**-4,10-guaianediol [7].

Table 2: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **3**, **4**, and reported compounds

C	[a]δ_C	[b]δ_C	**3**[c]		[d]δ_C	**4**[c]	
			δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	31.9		31.99	1.56/1.43 m	37.1	37.04	1.86/1.14 m
2	30.6		30.75	1.83/1.50 m	31.4	31.40	1.86/1.52 m
3	67.2		67.24	4.07 m	71.4	71.37	3.58 m
4	40.5		40.45	1.59/1.82 m	42.1	42.04	2.19/2.24m
5	75.2		75.20	-	146.3	146.26	-
6	76.0		76.13	4.72 br s	123.9	123.89	5.60 dd (1.5, 5.0)
7	31.4		31.43	1.55/1.66 m	65.4	65.36	3.85 br s
8	31.0		30.55	1.61 m	37.6	37.55	1.48 m
9	44.9		45.25	1.33 m	42.3	42.30	1.23 m
10	38.4		38.43	-	37.4	37.42	-
11	20.9	21.0	21.11	1.33/1.40 m	20.8	20.73	1.50/1.54 m
12		39.5	39.92	1.16/2.00 m	39.2	39.21	1.18/2.01 m
13		42.9	42.77	-	42.2	42.21	-
14		55.2	55.83	1.08 m	49.5	49.45	1.45 m
15		26.3	24.09	1.02/1.54 m	24.3	24.30	1.15/1.72 m
16		28.5	28.19	1.26/1.84 m	28.3	28.26	1.31/1.92 m
17		55.9	56.03	1.12 m	55.7	55.71	1.21 m
18		11.8	12.18	0.68 s	11.7	11.65	0.69 s
19	16.4		16.43	1.14 s	18.3	18.26	1.00 s
20		35.7	35.77	1.40 m	35.8	35.73	1.43 m
21		18.7	18.64	0.93 d (7.0)	18.8	18.75	0.96 d (7.0)
22		34.6	34.69	1.53/1.14 m	34.7	34.70	1.57/1.18 m
23		30.9	31.03	2.10/1.86 m	30.9	30.86	2.18/1.89 m
24		156.8	156.82	-	157.0	156.88	-
25		33.7	33.79	2.23 m	33.9	33.83	2.23 m
26		22.0	21.99	1.02 d (7.0)	22.1	22.02	1.03 d (7.0)
27		21.8	21.86	1.01 d (7.0)	22.1	21.88	1.02 d (7.0)
28		105.8	105.97	4.65 d (0.5)/4.71 brs	106.0	105.99	4.66/4.71 brs
1′	170.6		170.44	-			
2′′	21.4		21.45	2.06 s			

[a]δ_C of punicin [8], [b]δ_C of 3*β*,7*β*-dihydroxyergosta-5,24(28)-dien [9], [c]recorded in $CDCl_3$, [d]δ_C of 3*β*,7*α*-dihydroxyergosta-5,24(28)-dien [9].

Sinularianin D (**1**): Colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 1; ESI-MS: *m/z* 247 $[M–H]^-$ ($C_{15}H_{20}O_3$, M = 248).

1*S**,4*S**,5*S**,10*R**-4,10-Guaianediol (**2**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 1; ESI-MS: *m/z* 237 $[M–H]^-$ ($C_{15}H_{26}O_2$, M = 238).

24-Methylenecholestane-3β,5α,6β-triol-6-monoacetate (**3**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 2; ESI-MS: *m/z* 475 $[M+H]^+$ ($C_{30}H_{50}O_4$, M = 474).

3β,7α-Dihydroxyergost-5,24(28)-diene (**4**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 2; ESI-MS: *m/z* 397 $[M–H_2O+H]^+$ ($C_{28}H_{46}O_2$, M = 414).

3β-Hydroxyandrost-5-ene-17-one (**5**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 1.10 (1H, m, H_a-1), 1.86 (1H, m, H_b-1), 1.52 (1H, m, H_a-2), 1.84 (1H, m, H_b-2), 3.53 (1H, m, H-3), 2.25 (1H, m, H_a-4), 2.33 (1H, m, H_b-4), 5.38 (1H, m, H-6), 1.64 (1H, m, H_a-7), 2.12 (1H, m, H_b-7), 1.68 (1H, m, H-8), 1.01 (1H, m, H-9), 1.50 (1H, m, H_a-11), 1.68 (1H, m, H_b-11), 1.28 (1H, m, H_a-12), 1.84 (1H, m, H_b-12), 1.28 (1H, m, H-14), 1.55 (1H, m, H_a-15), 1.95 (1H, m, H_b-15), 2.07 (1H, m, H_a-16), 2.46 (1H, m, H_b-16), 0.89 (3H, s, H-18), and 1.04 (3H, s, H-19); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 37.20 (C-1), 31.59 (C-2), 71.59 (C-3), 42.22 (C-4), 141.05 (C-5), 120.91 (C-6), 30.79 (C-7), 31.52 (C-8), 50.27 (C-9), 36.65 (C-10), 20.37 (C-11), 31.46 (C-12), 47.54 (C-13), 51.79 (C-14), 21.88 (C-15), 35.83 (C-16), 221.08 (C-17), 13.54 (C-18), and 19.42 (C-19); ESI-MS: *m/z* 289 $[M+H]^+$ ($C_{19}H_{28}O_2$, M = 288).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil. The ^{13}C-NMR spectrum exhibited 15 carbon signals indicating for presence of a sesquiterpene. The ^{1}H and ^{13}C-NMR spectra confirmed the presence of two quaternary oxygenated carbons [δ_C 78.36 (C-1) and 85.59 (C-8)], two trisubstituted double bonds [δ_C 137.49 (s, C-5) and 117.20 (d, C-6)/δ_H 5.25 (1H, dd, J = 2.0, 4.0 Hz, H-6) and δ_C 150.93 (d, C-12)/δ_H 7.03 (1H, d, J = 1.5 Hz, H-13)], three tertiary methyl groups [δ_C 28.46 (C-10), 20.46 (C-11), and 10.63 (C-15)/δ_H 1.27 (3H, s, H-10), 1.74 (3H, s, H-11), and 1.94 (3H, d, J = 1.5 Hz, H-15)], and a lactone carbonyl [δ_C 173.53 (C-14)]. Consideration of the ^{1}H-NMR chemical shifts of three methyl protons at δ_H 1.27, 1.74, and 1.94 suggesting their locations on a quaternary oxygenated carbon, on a double bond, and adjacent to the lactone carbonyl, respectively. From the above observation, the ^{13}C-NMR data of **1** were compared with those of sinularianin B [5] and found to be similar. However, a close inspection of their ^{13}C-NMR data revealed some significant difference: C-9 and C-10 were shifted from δ_C 56.6 to 55.50 and from δ_C 26.0 to 28.4, respectively (see table 1). This suggested that **1** is a configuration isomer of sinularianin B. The ^{13}C-NMR data of **1** were further compared with those of sinularianin D [6] and found to match. Moreover, detailed analysis of the HMBC correlations (see figure 2) confirmed the elucidation of **1** as sinularianin D.

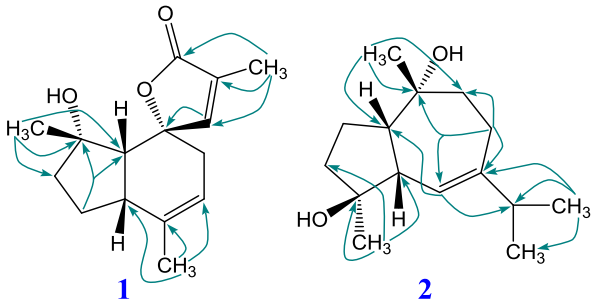

Figure 2: Key HMBC correlations of **1** and **2**

The NMR features of **2** were also indicative of a sesquiterpene. Two quaternary oxygenated carbons [δ_C 80.25 (C-4) and 75.27 (C-10)], a trisubstituted double bond [δ_C 121.31 (d, C-6)/δ_H 5.50 (1H, br d, J = 2.0 Hz, H-6)], and two tertiary methyl groups [δ_C 22.59 (C-14), and 21.44 (C-15)/δ_H 1.21 (H-14) and 1.29 (H-15), each 3H, s] were observed. In addition, two secondary methyl groups were identified by a proton signal at 0.98 (6H, d, J = 7.0 Hz, H-12 and H-13). The good agreement of the ^{13}C-NMR data of **2** with the reported values (see table 1) and combination with 2D-NMR data confirmed its structure as 1*S**,4*S**,5*S**,10*R**-4,10-guaianediol [7].

Compound **3** was obtained as a white powder. The ^{1}H and ^{13}C-NMR data were typical for a 24-methylenecholestane-type sterol [9] with typical signals of a terminal disubstituted double bond [δ_C 156.82 (s, C-24) and 105.97 (t, C-28)/δ_H 4.65 (1H, d, J = 0.5 Hz, H_a-28) and 4.71 (1H, br s, H_b-28)], two tertiary methyl [δ_C 12.18 (C-18) and 16.43 (C-19)/δ_H 0.68 (H-18) and 1.14 (H-19), each 3H, s], and three secondary methyl groups [δ_C 18.64 (C-21), 21.99 (C-26), and 21.86 (C-27)/δ_H 0.93 (H-21), 1.02 (H-26), and 1.01 (H-27), each 3H, d, J = 7.0 Hz].

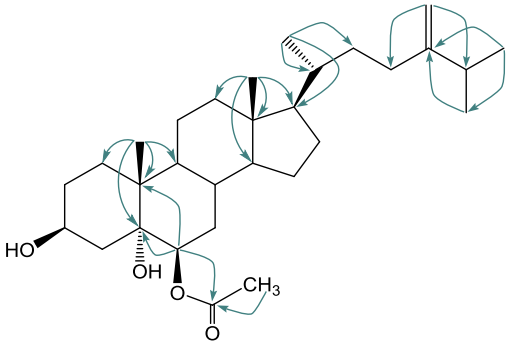

Figure 3: Key HMBC correlations of **3**

In addition, two oxymethine groups [δ_C 67.24 (C-3)/4.07 (1H, m, H-3) and 76.13 (C-6)/δ_H 4.72 (1H, br s, H-6)], a quaternary oxygenated carbon [δ_C 75.20 (C-5)], and an acetate group [δ_C 170.44 (s, C-1′) and 21.45 (q, C-2′)/δ_H 2.06 (3H, s, H-2′)] were identified in the ^{1}H and ^{13}C-NMR spectra of **3**. The oxygenated positions at C-3 and C-5 and acetylated position at C-6 were assigned by a good agreement of the ^{13}C-NMR data for the A and B rings of **3** with those of punicin [8], which was further confirmed by HMBC experiment (see figure 3). Thus, compound **3** was elucidated as 24-methylenecholestane-3β,5α,6β-triol-6-monoacetate. This compound was first isolated from the soft coral *Sinularia dissecta* in 1976 [10] and recently found from *Sinularia* sp. in 2012 [11]. However, this is the first report of **3** from *S. cruciata* and its full ^{1}H and ^{13}C-NMR were reported here for the first time.

Compounds **4** and **5** were elucidated as 3β,7α-dihydroxyergosta-5,24(28)-dien [9] and 3β-hydroxyandrost-5-en-17-one [12] by comparison of its ^{13}C-NMR data with the reported values and combination with 2D-NMR data. This is the first report of compounds **1-5** from the soft coral *S. cruciata*.

Acknowledgements. *This work was financially supported by Vietnam Academy of Science and Technology, codes: VAST.TĐ.ĐAB.02/13–15 and Project between IMBC and KIOST*

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