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STEROLS ISOLATED FROM THE SOFT CORAL *SINULARIA DISSECTA*

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Abstract

Using various chromatographic methods, five sterols, gorgost-4-ene-3-one (**1**), ergost-4-ene-3-one (**2**), 24-methyleneergost-4-ene-3-one (**3**), ergost-4-ene-3,6-dione (**4**), and 24-methylenecholest-4-ene-3,6-dione (**5**), were isolated from the methanol extract of the soft coral *Sinularia dissecta*. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. These compounds were isolated from *S. dissecta* for the first time.

Keywords. *Sinularia dissecta*, Alcyoniidae, soft coral, sterol.

1. INTRODUCTION

Soft corals have been found to be storehouses of sterols, particularly in terms of unique side-chain structures and unusual functionalization [1, 2]. Marine sterols of *Sinularia* soft corals are often found in oxygenated forms, and such sterols sometimes shows a variety of biological and pharmacological activities [3].

Many novel sterols have been reported from the soft coral *S. dissecta* [4-7]. Previously, we reported a new gorgosterol-type sterol from this soft coral and its anti-inflammatory activity [8]. In this paper, we address the isolation and structure identification of five sterols including gorgost-4-ene-3-one (**1**), ergost-4-ene-3-one (**2**), 24-methyleneergost-4-ene-3-one (**3**), ergost-4-ene-3,6-dione (**4**), and 24-methylenecholest-4-ene-3,6-dione (**5**) from the same 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. dissecta* was collected during April 2010 at Hai Van - Son Cha, Hue, Vietnam and identified by Prof. Do Cong Thung (Institute of Marine Environment and Resources, VAST). A voucher specimen (SD042010_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. dissecta* (1.5 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 (9.15 g, A). This residue was suspended in water (0.5 L) and partitioned in turn with *n*-hexane (2×0.5 L) and CH_2Cl_2 (3×0.5 L). The combined dichloromethane soluble portions were evaporated under reduced pressure to afford CH_2Cl_2 extract (1.83 g, B). Extract B was crudely separated

by silica gel CC using gradient concentrations of ethyl acetate in *n*-hexane from 0 to 100 % to yield four fractions, B-1 to B-4. Fraction B1 (647 mg) was further separated on silica gel CC using *n*-hexane–EtOAc (25:1) as eluents, to give three subfractions, B1.1 to B1.3. Subfraction B1.1 (253 mg) was then chromatographed over silica gel CC using eluent of *n*-hexane–acetone (14:1), and further purified by YMC RP-18 CC eluting with LH-20 CC (MeOH-acetone 1:1). Subfraction B1.3 MeOH–acetone–H_2O (4:2:0.2) to afford **3** (110 mg). Compound **2** (20 mg) was purified from subfraction B1.2 (158 mg) by silica gel CC eluting with *n*-hexane–EtOAc (15:1) and followed by Saphadex (230 mg) afforded **1** (52 mg), after subjecting it to silica gel CC eluting with dichloromethane–acetone (21.5:1), followed by YMC RP-18 CC with MeOH–acetone (6.5:1).

Fraction B2 (80 mg) was separated by YMC RP-18 CC, using eluent of MeOH–acetone–H_2O (95:3:2) to yield three subfractions, B-2.1 to B-2.3. Subfraction B2.3 (28 mg) afforded compound **5** (17 mg), after subjecting it to silica gel CC eluting with *n*-hexane-EtOAc (8.5:1). Fraction B4 (740 mg) was passed through Sephadex LH-20 with MeOH–acetone (1:1) to yield five subfractions, B4.1 to B4.5. Subfraction B4.2 (46 mg) was further separated by silica gel CC eluting with CH_2Cl_2–MeOH (25:1), followed by Sephadex LH-20 with MeOH-acetone (70:30) to yield compound **4** (10 mg).

Gorgost-4-ene-3-one (**1**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS *m/z* 425 $[M+H]^+$ ($C_{30}H_{48}O$, M = 424).

Ergost-4-ene-3-one (**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* 421 $[M+Na]^+$ ($C_{28}H_{46}O$, M = 398).

24-methyleneergost-4-ene-3-one (**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* 397 $[M+H]^+$ ($C_{28}H_{44}O$, M = 396).

Ergost-4-ene-3,6-dione (**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* 435 $[M+Na]^+$ ($C_{28}H_{44}O_2$, M = 412).

24-methylenecholest-4-ene-3,6-dione (**5**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 2; ESI-MS *m/z* 433 $[M+Na]^+$ ($C_{28}H_{42}O_2$, M = 410).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The ^{1}H-NMR spectrum revealed signals of three tertiary methyl [δ_H 0.65 (H-18), 1.14 (H-19), and 0.87 (H-29), each 3H, s] and three secondary methyl groups [δ_H 0.82 (H-26), 0.92 (H-27), and 0.90 (H-28), each 3H, d, J = 7.0 Hz]. A seventh methyl signal appeared as a broad singlet at δ_H 0.96, which was overlapped with a methine multiplet of H-20, and four high-field protons at δ_H 0.13 (1H, m, H-22), 0.20 (1H, m, H-24), –0.16 (1H, dd, J = 4.0, 6.0 Hz, H_β-30), and 0.46 (1H, dd, J = 4.0, 9.0 Hz, H_α-30), is characteristic of a gorgosterol-type side chain possessing a cyclopropane ring [14, 15]. In addition, one olefinic proton was identified at δ_H 5.76 (1H, br s, H-4). The ^{13}C-NMR spectrum of **1** showed 30 carbon signals, of which even methyl groups were at δ_C 12.40 (C-18), 17.79 (C-19), 21.53 (C-21), 21.96 (C-26), 22.59 (C-27), 15.89 (C-28), and 14.70 (C-29). A good agreement of the ^{13}C-NMR data for the side chain of **1** (table 1) with those of gorgost-5-ene-3β,9α,11α-triol [9] and combination with the HMBC correlations (figure 2) confirmed the gorgosterol-type side chain. Moreover, one ketone group [δ_C 200.00 (C-3)] and a tri-substituted double bond [δ_C 124.16 (d, C-4)/172.06 (s, C-5)] were observed. The carbon signals of the ketone group was strongly shifted upfield suggesting its conjugated location with the double bond. The ^{13}C-NMR data for the steroidal skeleton of **1** were similar to those of 24-ethylcholest-4-ene-3-one [16]. Detailed analysis of other HMBC cross-peaks (figure 2) led to assignment of the structure of **1** as gorgost-4-ene-3-one [17]. This is the first report of the ^{13}C-NMR data of **1**.

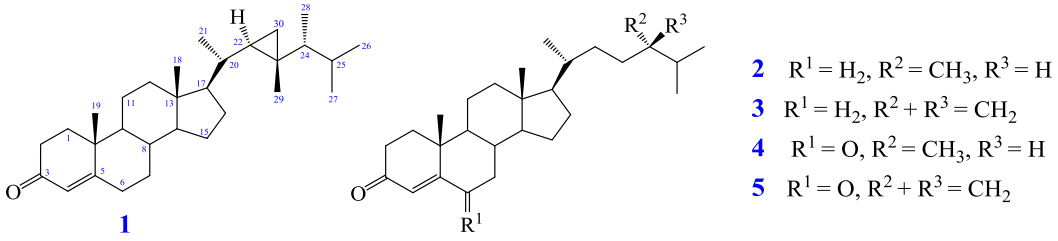

Figure 1: Chemical structures of compounds **1**–**5**

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

C	[a]δ_C	**1**[b]		[c]δ_C	**2**[b]		[d]δ_C	**3**[b]	
		δ_C	δ_H (*J* = Hz)		δ_C	δ_H (*J* = Hz)		δ_C	δ_H (*J*= Hz)
1		36.10	0.98 m 1.67 dd (5.0, 14.0)	35.7	36.22	1.66/1.97 m		36.23	1.66/1.97 m
2		34.40	2.38/2.30 m	34.0	33.48	2.22/2.34 m		33.45	2.22/2.34 m
3		200.00	-	199.6	200.08	-		199.92	-
4		124.16	5.76 brs	123.7	124.28	5.68 brs		124.28	5.68 brs
5		172.06	-	171.9	172.15	-		171.97	-
6		32.45	1.81/0.98 m	32.9	32.58	1.79/0.97 m		32.56	1.79/2.31 m
7		33.26	2.35/2.22 m	31.1	31.09	0.89/1.33 m		31.47	0.89/1.33
8		36.07	1.47 m	35.6	36.14	1.47 m		36.12	1.47 m
9		54.25	0.89 m	53.8	54.35	0.87 m		54.39	0.85 m
10		38.99	-	38.6	39.11	-		39.10	-
11		21.48	1.49/1.37 m	21.0	21.55	1.48/1.38 m		21.55	1.45/1.36 m
12		40.11	1.15/2.02 m	39.6	40.14	1.52/2.02 m		40.15	1.97/1.09 m
13		43.24	-	42.4	42.90	-		42.94	-
14		58.28	1.21 m	56.0	56.39	0.95 m		56.39	0.95 m
15		24.84	1.06/1.58 m	24.2	24.71	1.55/1.04 m		24.70	1.54/1.69m
16		28.60	1.99/1.29 m	28.1	28.68	1.80/1.23 m		28.69	1.81/1.21 m
17		56.15	0.99 m	55.9	56.46	1.07 m	55.7	56.47	1.06 m
18		12.40	0.65 s	11.9	12.49	0.66 s	11.7	12.49	0.64 s
19		17.79	1.14 s	17.4	17.91	1.13 s	18.3	17.90	1.11 s
20	35.2	35.67	0.97 m	36.1	36.68	1.31 m	35.8	35.12	1.09 m
21	21.1	21.53	0.96 brs	18.8	19.36	0.87 d 7.0)	18.8	19.17	0.88 d 7.0)
22	31.9	32.46	0.13 m	33.7	34.18	1.34/0.89 m	34.7	34.49	2.38/2.24 m
23	25.8	26.23	-	30.6	31.09	0.89/1.33 m	30.9	31.47	2.03/1.78 m
24	50.7	51.88	0.20 m	39.1	40.67	1.98 m	157.0	157.14	-
25	31.9	32.47	1.53 m	31.5	31.98	1.51 m	33.9	34.30	2.27 m
26	21.6	21.96	0.82 d (7.0)	17.6	18.14	0.73 d (7.0)	22.1	22.53	0.95 d (7.0)
27	22.2	22.59	0.92 d (7.0)	20.5	21.07	0.80 d (7.0)	22.1	22.40	0.95 d (7.0)
28	15.5	15.89	0.90 d (7.0)	15.4	15.99	0.86 d (7.0)	106.0	106.57	4.64 d (7.0) 4.58 d (7.0)
29	14.3	14.70	0.87 s						
30	21.3	21.73	-0.16 dd (4.0, 6.0) 0.46 dd (4.0, 9.0)						

[a]δ_C of gorgost-5-ene-3β,9α,11α-triol [9], [b]recorded in $CDCl_3$, [c]δ_C of ergost-4-ene-3-one [10],
[d]δ_C for the side chain of 3β,7α-dihydroxyergosta-5,24(28)-diene [11].

The [1]H- and [13]C-NMR data of **2** and **3** were similar to those of **1**, except for diffence in the data of the side chain. The most easily visible changes are the absence of four high-field proton signals and the presence of 28 carbon signals in **2** and **3** relative to **1**. Four secondary methyl proton signals (each 3H, d, *J* = 7.0 Hz) in the side chain of **2** were observed at δ_H 0.87 (H-21), 0.73 (H-26), 0.80 (H-27), and 0.86 (H-28) suggesting for the presence of an ergosterol-type side chain, which was further confirmed by an agreement of the [13]C-NMR data of **2** (table 1) with those of ergost-4-ene-3-one [10] and combination with HMBC correlations (figure 2). The presence of a 1,1-disubstituted double bond at δ_C 157.14 (s, C-24) and 106.57 (t, C-28)/δ_H 4.64 and 4.64 (each 1H, d, *J* = 7.0 Hz, H-28), and three secondary methyl groups at δ_H 0.88 (3H, d, *J* = 7.0 Hz, H-21) and 0.95 (6H, d, *J* = 7.0 Hz, H-26 and H-27) indicating a 24-methylene ergosterol-type side chain of **3** [11, 13].

Compounds **4** and **5** were elucidated as ergost-4-ene-3,6-dione [10, 12] and 24-methylenecholest-4-ene-3,6-dione [13] by comparison of their [13]C-NMR data with the reported values and combination with 2D-NMR data. This is the first report of compounds **1**–**5** from *S. dissecta*.

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

C	[a]δ_C	[b]δ_C	**4**[c]		[d]δ_C	**5**[c]	
			δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1		36.63	36.15	2.12/1.88 m	35.5	36.01	1.39/1.86 m
2		34.46	34.60	2.51/2.43 m	33.8	34.46	2.12/2.52 m
3		198.16	200.21	-	199.5	200.01	-
4		125.82	126.08	6.14 brs	125.5	125.94	6.12 brs
5		160.77	161.75	-	161.0	161.56	-
6		200.98	203.03	-	202.4	202.82	-
7		46.36	47.45	2.01/2.65 m	47.8	47.28	2.63/1.99 m
8		34.44	34.84	1.87 m	34.0	34.29	2.18 m
9		51.09	51.60	1.34 m	50.9	51.43	1.33 m
10		39.78	40.70	-	39.1	40.30	-
11		23.65	21.50	1.61/1.47 m	20.8	21.37	1.46/1.60 m
12		40.07	39.74	1.21/2.07 m	39.8	39.62	1.22/2.06 m
13		43.07	43.15	-	42.5	43.07	-
14		56.46	57.16	1.18 m	55.8	57.02	1.15 m
15		24.50	24.61	1.59/1.15 m	23.9	24.46	1.57/1.14 m
16		28.69	28.60	1.30/1.87 m	28.0	28.50	1.28/1.87 m
17	55.9	56.98	56.40	1.14 m	56.5	56.29	1.14 m
18	11.9	12.54	12.52	0.69 s	11.9	12.39	0.68 s
19	17.4	18.03	18.22	1.13 s	17.5	18.01	1.12 s
20	36.1	36.63	36.71	1.35 m	35.6	36.11	2.11 m
21	18.8	19.39	19.45	0.90 d (7.0)	18.6	19.14	0.92 d (7.0)
22	33.7	35.97	34.60	2.51/2.43 m	34.5	35.04	1.50/1.12 m
23	30.6	26.69	31.15	1.35/0.93 m	30.9	31.42	1.84/2.06 m
24	39.1	34.40	39.66	2.07 m	156.6	157.11	-
25	31.5	29.71	32.08	1.54 m	34.2	34.29	2.17 m
26	17.6	19.77	18.14	0.76 d (3.0)	21.8	22.50	0.99 d (3.5)
27	20.5	20.53	21.15	0.82 d (7.0)	22.0	22.37	0.97 d (3.5)
28	15.4	21.41	16.08	0.75 d (3.0)	106.1	106.61	4.68/4.61 brs

[a]δ_C for the side chain of ergost-4-ene-3-one [10], [b]δ_C of 24*S*-ergost-4-ene-3,6-dione [12], [c]recorded in $CDCl_3$, [d]δ_C of 24-methylenecholest-4-ene-3,6-dione [13].

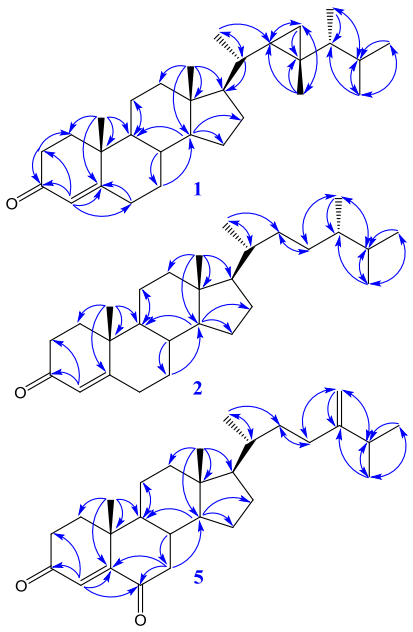

Figure 2: Key HMBC correlations of **1**, **2**, and **5**

4. CONCLUSION

Five sterols, gorgost-4-ene-3-one (**1**), ergost-4-ene-3-one (**2**), 24-methyleneergost-4-ene-3-one (**3**), ergost-4-ene-3,6-dione (**4**), and 24-methylenecholest-4-ene-3,6-dione (**5**), were isolated from the methanol extract of the soft coral *Sinularia dissecta*. Their structures were elucidated by 1D and 2D-NMR spectroscopic methods and comparison of their data with the published values. This is the first report of these compounds from *S. dissecta*.

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