

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 ¹H-NMR (500 MHz) and ¹³C-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 F₂₅₄ (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H₂SO₄ 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 CH₂Cl₂ (3×0.5 L). The combined dichloromethane soluble portions were evaporated under reduced pressure to afford CH₂Cl₂ 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–H₂O (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 Sphadex (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–H₂O (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 CH₂Cl₂–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; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see table 1; ESI-MS *m/z* 425 [M+H]⁺ (C₃₀H₄₈O, M = 424).

Ergost-4-ene-3-one (**2**): White powder; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see table 1; ESI-MS *m/z* 421 [M+Na]⁺ (C₂₈H₄₆O, M = 398).

24-methyleneergost-4-ene-3-one (**3**): White powder; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see table 2; ESI-MS *m/z* 397 [M+H]⁺ (C₂₈H₄₄O, M = 396).

Ergost-4-ene-3,6-dione (**4**): White powder; ¹H-

NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see table 2; ESI-MS *m/z* 435 [M+Na]⁺ (C₂₈H₄₄O₂, M = 412).

24-methylenecholest-4-ene-3,6-dione (**5**): White powder; ¹H-NMR (500 MHz, CDCl₃) and ¹³C-NMR (125 MHz, CDCl₃) see table 2; ESI-MS *m/z* 433 [M+Na]⁺ (C₂₈H₄₂O₂, M = 410).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The ¹H-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 ¹³C-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 ¹³C-NMR data for the side chain of **1** (table 1) with those of gorgost-5-ene-3 β ,9 α ,11 α -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 ¹³C-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 ¹³C-NMR data of **1**.

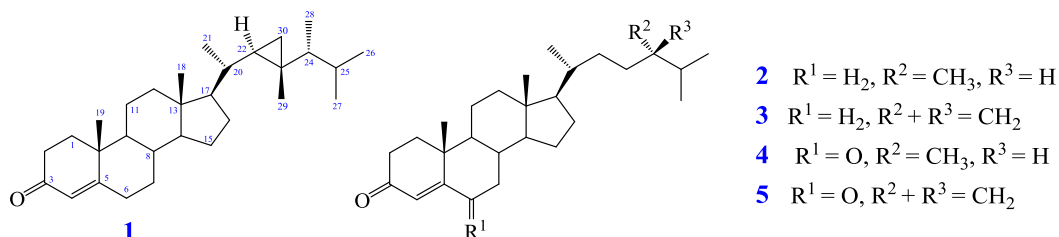


Figure 1: Chemical structures of compounds 1–5

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

C	$^a\delta_{\text{C}}$	1^b		$^c\delta_{\text{C}}$	2^b		$^d\delta_{\text{C}}$	3^b	
		δ_{C}	δ_{H} ($J = \text{Hz}$)		δ_{C}	δ_{H} ($J = \text{Hz}$)		δ_{C}	δ_{H} ($J = \text{Hz}$)
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	1.54/1.69 m
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)						

$^a\delta_{\text{C}}$ of gorgost-5-ene-3 β ,9 α ,11 α -triol [9], b recorded in CDCl_3 , $^c\delta_{\text{C}}$ of ergost-4-ene-3-one [10],

$^d\delta_{\text{C}}$ for the side chain of 3 β ,7 α -dihydroxyergosta-5,24(28)-diene [11].

The ^1H - and ^{13}C -NMR data of **2** and **3** were similar to those of **1**, except for difference 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).

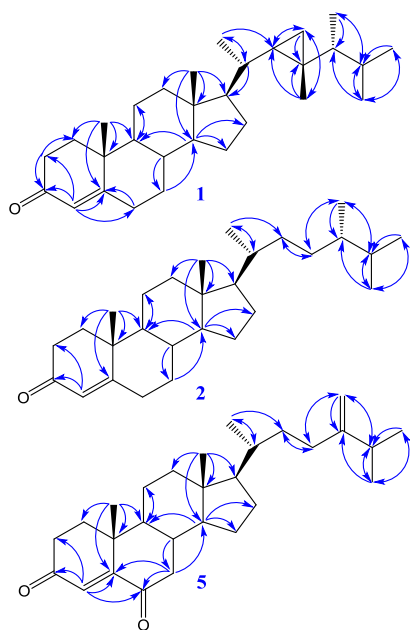
The presence of a 1,1-disubstituted double bond at δ_{C} 157.14 (s, C-24) and 106.57 (t, C-28)/ δ_{H} 4.64 and 4.64 (each 1H, d, $J = 7.0$ Hz, H-28), and three secondary methyl groups at δ_{H} 0.88 (3H, d, $J = 7.0$ Hz, H-21) and 0.95 (6H, d, $J = 7.0$ Hz, H-26 and H-27) indicating a 24-methylene ergosterol-type 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: $^1\text{H-NMR}$ (500 MHz) and $^{13}\text{C-NMR}$ (125 MHz) data of **4**, **5**, and reported compounds

C	$^a\delta_{\text{C}}$	$^b\delta_{\text{C}}$	4^{c}		$^d\delta_{\text{C}}$	5^{c}	
			δ_{C}	δ_{H} mult. ($J = \text{Hz}$)		δ_{C}	δ_{H} mult. ($J = \text{Hz}$)
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

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

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

4. CONCLUSION

Five sterols, ergost-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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REFERENCES

1. M. V. D'Auria, L. Minale, R. Riccio.

- Polyoxygenated steroids of marine origin*, Chem. Rev., **93(5)**, 1839-1895 (1993).
2. N. S. Sarma, M. S. Krishna, S. G. Pasha, T. S. P. Rao, Y. Venkateswarlu, P. S. Parameswaran. *Marine metabolites: The sterols of soft coral*, Chem. Rev., **109(6)**, 2803-2828 (2009).
 3. V. Lakshmi, R. Kumar. *Metabolites from Sinularia species*, Nat. Prod. Res., **23(9)**, 801-850 (2009).
 4. B. M. Jagodzinska, J. S. Trimmer, W. Fenical, C. Djerassi. *Sterols in marine invertebrates. 49. Isolation and structure elucidation of eight new polyhydroxylated sterols from the soft coral Sinularia dissecta*, J. Org. Chem., **50(9)**, 1435-1439 (1985).
 5. B. M. Jagodzinska, J. S. Trimmer, W. Fenical, C. Djerassi. *Sterols in marine invertebrates. 51. Isolation and structure elucidation of C-18 functionalized sterols from the soft coral Sinularia dissecta*, J. Org. Chem., **50(16)**, 2988-2992 (1985).
 6. P. Jin, Z. Deng, Y. Pei, H. Fu, J. Li, L. Van Ofwegen, P. Proksch, W. Lin. *Polyhydroxylated steroids from the soft coral Sinularia dissecta*, Steroids, **70(8)**, 487-493 (2005).
 7. P. Ramesh, Y. Venkateswarlu. *Novel steroid constituents of the soft coral Sinularia dissecta*, Steroids, **64(11)**, 785-789 (1999).
 8. N. P. Thao, N. H. Nam, N. X. Cuong, B. H. Tai, T. H. Quang, N. T. T. Ngan, B. T. T. Luyen, S. Y. Yang, C. H. Choi, S. Kim, D. Chae, Y.-S. Koh, P. V. Kiem, C. V. Minh, Y. H. Kim. *Steroid constituents from the soft coral Sinularia dissecta and their inhibitory effects on lipopolysaccharide-stimulated production of pro-inflammatory cytokines in bone marrow-derived dendritic cells*, Bull. Korean Chem. Soc., **34(3)**, 949 - 952 (2013).
 9. H. T. D'Armas, B. S. Mootoo, W. F. Reynolds. *Steroid compounds from the Caribbean octocoral Eunicea laciniata*, J. Nat. Prod., **63(12)**, 1669-1671 (2000).
 10. W.-R. Abraham, G. Schmeda-Hirschmann. *(24S)-3 β -hydroxy-ergost-5-en-6-one from Cyttaria johowii*, Phytochemistry, **36(2)**, 459-461 (1994).
 11. F. De Riccardis, L. Minale, M. Iorizzi, C. Debitus, C. Lévi. *Marine sterols. Side-chain-oxygenated sterols, possibly of abiotic origin, from the new Caledonian sponge Stelodoryx chlorophylla*, Journal of Natural Products, **56(2)**, 282-287 (1993).
 12. K. A. Eshbakova, B. Tashkhodzhaev, Z. I. Tursunov, K. K. Turgunov, K. M. Bobakulov, N. D. Abdullaev. *Structure of a new sterol 24S-4-en-3,6-dione from Aconitum septentrionale*, Chem. Nat. Comp., **47(1)**, 73-75 (2011).
 13. A. Migliuolo, V. Piccialli, D. Sica. *Steroid ketones from the sponge Geodia cydonium*, J. Nat. Prod., **53(5)**, 1262-1266 (1990).
 14. H. T. D'Armas, B. S. Mootoo, W. F. Reynolds. *Steroid compounds from the Caribbean octocoral Eunicea laciniata*. J. Nat. Prod., **63(12)**, 1669-1671 (2000).
 15. A. Rueda, E. Zubía, M. a. J. Ortega, J. Salvá. *Structure and cytotoxicity of new polyhydroxylated sterols from the Caribbean gorgonian Plexaurella grisea*, Steroids, **66(12)**, 897-904 (2001).
 16. P. Georges, M. Sylvestre, H. Ruegger, P. Bourgeois. *Ketosteroids and hydroxyketosteroids, minor metabolites of sugarcane wax*, Steroids, **71(8)**, 647-652 (2006).
 17. S. Popov, R. M. K. Carlson, A. Wegmann, C. Djerassi. *Minor and trace sterols in marine invertebrates I. General methods of analysis*, Steroids, **28(5)**, 699-732 (1976).

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