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STRUCTURE ELUCIDATION OF FOUR STEROIDS FROM THE SOFT CORAL *SINULARIA NANOLOBATA*

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ABSTRACT

Four steroids namely 3*β*-hydroxyergosta-5,24(28)-diene-7-one (**1**), dissesterol (**2**), 16*α*-hydroxysarcosterol (**3**), and sarcophytosterol (**4**) were isolated from the soft coral *Sinularia nanolobata* using various chromatographic methods. Their structures were elucidated by detailed analysis of the 1D and 2D NMR data and comparison with the reported values.

Keywords: *Sinularia nanolobata*, soft coral, steroids.

1. INTRODUCTION

Soft corals are marine invertebrates of the order Alcyonacea, subclass Octocorallia, class Anthozoa, and phylum Cnidaria. Among these marine invertebrates, the genera *Cespitularia*, *Clavularia*, *Gersemia*, *Lobophytum*, *Nephthea*, *Sarcophyton*, and *Sinularia* are the most prolific [1]. The *Sinularia* soft corals are a rich source of hydroxylated steroids having cytotoxic effects [2 – 8]. Within the frame of our recent investigations on chemical constituents and biological activities of Vietnamese *Sinularia* soft corals [9, 10], we have recently reported six steroids from the soft coral *Sinularia nanolobata* and evaluation of their cytotoxic activity [11]. The current paper deals with detailed structure elucidation of four steroids from this species.

2. EXPERIMENTAL

2.1. General methods

The NMR spectra were recorded on a Bruker AVANCE III HD 500 spectrometer with TMS as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system. TLC was performed on Kieselgel 60 F_{254} (1.05715; Merck) or

RP-18 F_{254s} plates. Spots were visualized by spraying with 10% aqueous H_2SO_4 solution, followed by heating for 3 - 5 minutes. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) and YMC*GEL resins (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.).

2.2. Biological material

The samples of the soft coral *S. nanolobata* Verseveldt, 1977 were collected in Lang Co, Hue, Vietnam, in April 2015 and identified by Prof. Do Cong Thung (Institute of Marine Environment and Resources, VAST). Voucher specimens (No SN201504) were deposited at the Institute of Marine Biochemistry, VAST, Vietnam.

2.3. Extraction and isolation

Dried bodies of the soft coral *S. nanolobata* (1.5 kg) were extracted three times with methanol under ultrasonic condition. The resulting solutions were filtered, combined, and concentrated under reduced pressure to obtain the methanol residue (SNM, 140 g), which was suspended in water and extracted in turn with *n*-hexane and dichloromethane resulting in extracts of *n*-hexane (SNH, 47 g), dichloromethane (SND, 5 g), and an aqueous layer. Extract SNH (47 g) was crudely separated on silica gel MPLC using the mobile phase of *n*-hexane–acetone (gradient 50:1→1:1, v/v) to obtain six fractions, SNH1–SNH6. Fraction SNH4 (7.3 g) was further separated on silica gel MPLC using the mobile phase of *n*-hexane–ethyl acetate (gradient 5:1→1:1, v/v) to give seven subfractions, SNH4A-SNH4G. Subfraction SNH4B (2.1 g) was further separated into nine smaller fractions, SNH4B1-SNH4B9, by YMC CC eluting with methanol–water (2:1, v/v). Compounds **3** (5.0 mg) and **4** (3.5 mg) were obtained from subfraction SNH4B7 (300 mg) after purification by YMC CC eluted with acetone–water (3:1, v/v), followed by silica gel CC with *n*-hexane–acetone (3.5:1, v/v). Fraction SNH5 (5.0 g) was further separated on RP-18 MPLC with methanol–water (2:1, v/v) to give six subfractions, SNH5A-SNH5G. Finally, compounds **1** (6.0 mg) and **2** (2.6 mg) were purified from subfraction SNH5F (130 mg) by silica gel CC eluting with *n*-hexane–ethyl acetate (2:1, v/v).

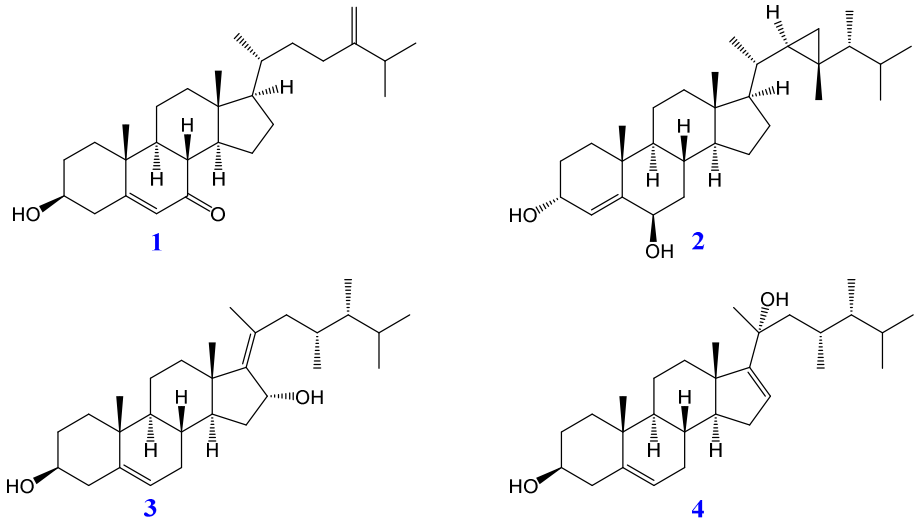

Figure 1. Chemical structures of compounds **1**–**4**.

*3β-Hydroxyergosta-5,24(28)-diene-7-one (**1**)*: White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 1.

*Dissesterol (**2**)*: White powder; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1.

Table 1. The NMR spectroscopic data of compounds **1** and **2**.

C	$^{a}\delta_C$	**1**		$^{e}\delta_C$	**2**	
		δ_C ^{b,c}	δ_H ^{b,d} mult. (*J* = Hz)		δ_C ^{c,f}	δ_H ^{d,f} mult. (*J* = Hz)
1	36.34	36.42	1.18 m /1.92 m	36.5	36.47	1.20 m/1.60 m
2	31.19	31.23	1.92 m/1.60 m	28.5	28.52	1.43 m/1.78 m
3	70.54	70.55	3.65 m	67.3	65.94	3.96 m
4	41.81	41.89	2.38 m/2.49 m	128.1	128.40	5.37 br s
5	165.07	165.34	-	147.1	145.17	-
6	126.13	126.14	5.66 s	73.6	72.10	4.02 br s
7	202.30	202.45	-	38.9	39.33	1.15 m/1.98 m
8	45.41	45.48	2.22 m	30.1	29.72	1.82 m
9	49.95	49.98	1.32 m	54.1	53.96	0.68 m
10	38.28	38.36	-	36.7	36.04	-
11	21.22	21.28	1.54 m/1.55 m	20.8	20.29	1.35 m/1.45 m
12	38.69	38.76	1.12 m/2.01 m	39.7	39.93	1.01 m/1.79 m
13	43.14	43.21	-	42.8	42.28	-
14	49.90	50.02	1.50 m	55.8	55.35	0.96 m
15	26.31	26.38	2.39 m/1.26 m	24.2	23.66	1.08 m/1.58 m
16	28.54	28.60	1.28 m/1.89 m	28.0	27.42	1.35 m/1.96 m
17	54.63	54.70	1.06 m	57.8	57.33	1.25 m
18	12.00	12.05	0.66 s	11.8	11.56	0.69 s
19	17.33	17.38	1.17 s	21.1	20.50	1.17 s
20	35.66	35.72	1.15 m	35.1	33.95	1.08 m
21	18.87	18.94	0.93 d (7.0)	21.1	20.53	0.98 d (7.0)
22	34.68	34.75	1.53 m	31.8	31.35	0.22 m
23	30.97	31.05	1.84 m/2.08 m	25.6	25.00	-
24	156.79	156.83	-	50.6	49.62	0.32 m
25	33.76	33.82	2.20 m	32.0	31.08	1.59 m
26	21.87	22.08	0.99 d (7.0)	21.2	20.45	0.85 d (7.0)
27	22.01	21.93	1.00 d (7.0)	21.8	21.60	0.94 d (7.0)
28	106.03	106.10	4.69 br s/4.62 br s	15.2	14.31	0.92 d (7.0)
29				14.0	13.82	0.91 s
30				20.9	20.12	–0.10 dd (4.5, 5.5) 0.45 dd (4.5, 9.0)

$^{a}\delta_C$ of 3*β*-hydroxyergosta-5,24(28)-diene-7-one [12], ^{b}recorded in $CDCl_3$, ^{c}125 MHz, ^{d}500 MHz, $^{e}\delta_C$ of dissesterol [13], ^{f}recorded in DMSO-d_6.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features indicated an ergosterol derivative, one main constituent of *Sinularia* soft corals [2]. The ^{1}H- and ^{13}C-NMR spectra of **1** showed typical signals of one oxymethine [δ_C 70.55 (C-3)/δ_H 3.65 (1H, m, H-3)], one trisubstituted endocyclic double bond [δ_C 165.34 (C, C-5) and 126.14 (CH, C-6)/δ_H 5.66 (1H, s,

H-6)], one ketone [δ_C 202.45 (C, C-7)], one 1,1-disubstituted double bond [δ_C 156.83 (C, C-24) and 106.10 (CH_2, C-28)/δ_H 4.62 and 4.69 (H-28), each 1H, br s], two *tert*-methyls [δ_C 12.05 (C-18) and 17.38 (C-19)/δ_H 0.66 (H-18) and 1.17 (H-19), each 3H, s], and three *sec*-methyls [δ_C 18.94 (C-21), 22.08 (C-26), and 21.93 (C-27)/δ_H 0.93 (H-21), 0.99 (H-26), and 1.00 (H-27), each 3H, d, J = 7.0 Hz]. The HMBC correlations (Fig. 2) of H-6 with C-4, C-8, and C-10; H-8 with C-7, and H-19 with C-1, C-5, C-9, and C-10, clearly confirmed positions of the double bond C-5/C-6 and ketone C-7. The detailed analysis of other HMBC correlations and an agreement of the ^{13}C-NMR chemical shifts of **1** with the reported data led to assignment of **1** as 3*β*-hydroxyergosta-5,24(28)-diene-7-one [12].

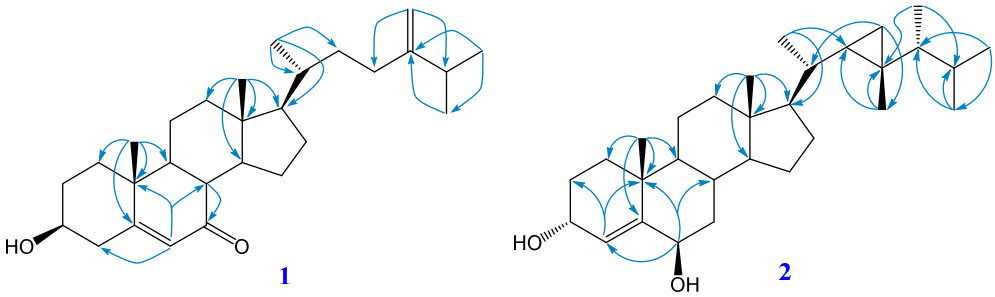

Figure 2. Key HMBC correlations of compounds **1** and **2**.

Compound **2** was also obtained as a white powder. The ^{13}C-NMR and HSQC spectra confirmed 30 carbon signals including seven methyls, eight methylenes, eleven methines, and four quaternary carbons. The signals at δ_C 11.56 (C-18), 20.50 (C-19), 20.53 (C-21), 20.45 (C-26), 21.60 (C-27), 14.31 (C-28), and 13.82 (C-29) indicated the presence of seven methyl groups. Moreover, two oxymethine groups [δ_C 65.94 (C-3) and 72.10 (C-6)] and a trisubstituted double bond [δ_C 128.40 (CH, C-4) and 145.17 (C, C-5)] were also identified. In the ^{1}H-NMR spectrum, the presence of four high-field protons at δ_H 0.22 (1H, m, H-22), 0.32 (1H, m, H-24), –0.10 (1H, dd, J = 4.5, 5.5 Hz, H_β-30), and 0.45 (1H, dd, J = 4.5, 9.0 Hz, H_α-30) is characteristic of a gorgosterol-type side chain possessing a cyclopropane ring [14]. The HMBC cross peaks of H-19 (δ_H 1.17) with C-1 (δ_C 36.47)/C-5 (δ_C 145.17)/C-9 (δ_C 53.96)/C-10 (δ_C 36.04) and H-6 (δ_H 4.02) with C-4 (δ_C 128.40)/C-8 (δ_C 29.72)/C-10 (δ_C 36.04) confirmed positions of the double bond at C-4/C-5 and the hydroxy group at C-6. The detailed analyses of other HMBC correlations (Fig. 2) and the comparison of the ^{13}C-NMR chemical shifts of **2** with those reported (Table 1) clearly identified compound **2** as dissesterol [13].

The other compounds were elucidated as 16*α*-hydroxysarcosterol (**3**) [15] and sarcophytosterol (**4**) [16] by detailed analysis of their 1D and 2D NMR data and in comparison with reported data.

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