VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 1-4 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-001

STEROID CONSTITUENTS FROM *LOBOPHYTUM CRASSUM*

Nguyen Phuong Thao[1], Nguyen Van Thanh[1], Nguyen Hoai Nam[1], Nguyen Xuan Cuong[1], Young Ho Kim[2], Phan Van Kiem[1], Chau Van Minh[1*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology*

[2]*College of Pharmacy, Chungnam National University, Korea*

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

Using various chromatographic methods, three sterols, (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol (**1**), ergosterol peroxide (**2**), and 3β-hydroxyandrost-5-ene-17-one (**3**), were isolated from the methanol extract of the soft coral *Lobophytum crassum*. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. Compounds **1**-**3** were isolated from *L. crassum* for the first time.

Keywords. *Lobophytum crassum*, Alcyoniidae, soft coral, sterol.

1. INTRODUCTION

Among marine organisms, soft corals are known to elaborate both 3β-monohydroxysterols and polyhydroxysterols, derived mainly from a 24-methylcholestane skeleton. Polyhydroxysterols of soft corals and other marine invertebrates occur mainly in either the free state or as the sulfate form, and examples of steroidal glycosides are rather rare, except for those found in starfishes [1]. As part of our continuing investigations to find bioactive compounds from Vietnamese marine organisms, this paper deals with the isolation from the soft coral *Lobophytum crassum*, and structure identification of three sterols: (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-en-3β-ol (**1**), ergosterol peroxide (**2**), and 3β-hydroxyandrost-5-ene-17-one (**3**).

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 specimens of *Lobophytum crassum* were collected in Conco, Quang Tri, Vietnam, during May 2013 and deep frozen until used. The sample was identified by Professor Do Cong Thung (Institute of Marine Environment and Resources). A voucher of specimen (No. LC0513) was deposited at the Institute of Marine Biochemistry and the Institute of Marine Resources and Environment, VAST, Vietnam.

2.3. Isolation

Freeze-dried bodies of the soft coral *L. crassum* (1.0 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 (75.0 g, A). This residue was suspended in water (1 L) and partitioned in turn with *n*-hexane and CH_2Cl_2 (3×1 L). The combined CH_2Cl_2 soluble portions were evaporated under reduced pressure to afford CH_2Cl_2 extract (6.02 g, B). The CH_2Cl_2 extract (C, 6.02 g) was separated

into five fractions, C1-C5, by a silica gel column chromatography (CC) using gradient elution of *n*-hexane–acetone (100:1–1:1, v/v). Fraction C3 (2.1 g) was further separated by YMC CC using methanol–acetone–water (2.5:1:1.1, v/v/v) to obtain six smaller fractions, C3A-C3F. Compounds **1** (9.5 mg) and **2** (8.2 mg) were purified from fraction C3D (0.55 g) by YMC RP-18 CC using methanol–acetone–water (3:1:1, v/v/v) as an eluent. Fraction C3C (0.39 g) was further separated by silica gel CC, eluted with chloroform–ethyl acetate (30:1, v/v) to give compound **3** (11.3 mg).

(22*R*,23*R*,24*R*)-5α,8α-Epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol (**1**): White powder; $[\alpha]_D$ + 30 (*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* 443 $[M+H]^+$ ($C_{29}H_{46}O_3$, M = 442).

Ergosterol peroxide (**2**): White powder; $[\alpha]_D$ −30 (*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* 429 $[M+H]^+$ ($C_{28}H_{44}O_3$, M = 428).

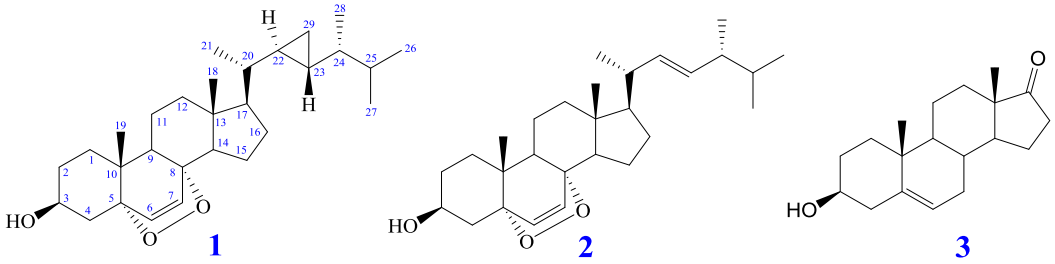

Figure 1: Chemical structures of compounds **1**–**3**

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

C	[a]δ_C	**1**[b]		[c]δ_C	**2**[b]	
		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	**39.3**	**34.70**	1.68 m/1.94 m	**30.20**	**34.74**	1.68 m/1.96 m
2	30.1	30.09	1.53 m/1.82 m	**34.77**	**30.17**	1.55 m/1.85 m
3	66.5	66.38	3.95 m	66.47	66.50	3.97 m
4	**51.1**	**36.94**	1.90 m/2.10 m	**39.44**	**37.01**	1.92 m/2.12 m
5	**79.5**	**82.14**	-	82.15	82.16	-
6	**130.8**	**135.39**	6.22 d (8.5)	135.22	135.44	6.24 d (8.5)
7	**135.4**	**130.75**	6.49 d (8.5)	130.77	130.78	6.51 d (8.5)
8	**82.2**	**79.48**	-	79.42	79.44	-
9	**34.7**	**51.10**	1.49 m	51.25	51.17	1.49 m
10	36.9	36.92	-	37.03	36.99	-
11	20.8	20.85	1.42 m/1.63 m	20.66	20.66	1.40 m/1.60 m
12	39.6	39.34	1.22 m/1.96 m	**37.03**	**39.40**	1.23 m/1.96 m
13	44.8	44.85	-	44.61	44.60	-
14	51.3	51.34	1.54 m	51.76	51.73	1.57 m
15	**28.4**	**23.40**	1.20 m/1.50 m	23.44	23.43	1.22 m/1.52 m
16	**23.4**	**28.47**	1.50 m/2.13 m	28.58	28.64	1.36 m/1.75 m
17	57.8	57.77	1.32 m	56.33	56.27	1.23 m
18	12.4	12.46	0.75 s	12.90	12.90	0.82 s
19	18.5	18.50	0.87 s	18.18	18.19	0.88 s
20	39.5	39.56	0.82 m	39.64	39.71	2.03 m
21	19.1	19.12	0.89 br s	20.90	20.90	1.00 d (6.5)
22	24.2	24.16	0.53 m	135.45	135.23	5.14 dd (8.5, 15.0)
23	25.1	25.12	0.30 m	132.39	132.36	5.22 dd (7.5, 15.0)
24	45.0	45.00	0.52 m	42.82	42.81	1.85 m
25	32.8	32.77	1.65 m	33.11	33.10	1.47 m
26	18.1	18.14	0.85 d (7.0)	19.64	19.65	0.82 d (6.5)
27	20.6	20.65	0.86 d (7.0)	19.93	19.95	0.83 d (6.5)
28	15.7	15.74	0.90 d (7.0)	17.56	17.57	0.90 d (6.5)
29	10.5	10.49	0.12 m			

[a]δ_C of (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol [2], [b]recorded in $CDCl_3$, [c]δ_C of ergosterol peroxide [3].

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

C	$^{a}\delta_C$	$\delta_C{}^{b,c}$	$\delta_C{}^{b,d}$ mult. (J = Hz)	HMBC (H → C)
1	37.8	37.20	1.10 m/1.86 m	
2	32.1	31.59	1.52 m/1.84 m	
3	71.3	71.59	3.53 m	
4	42.9	42.22	2.25 m/2.33 m	2, 3, 5, 6, 10
5	142.4	141.05	-	
6	120.7	120.91	5.38 m	
7	32.1	30.79	1.64 m/2.12 m	
8	32.1	31.52	1.68 m	
9	51.1	50.27	1.01 m	
10	37.2	36.65	-	
11	20.9	20.37	1.50 m/1.68 m	
12	31.2	31.46	1.28 m/1.84 m	
13	47.5	47.54	-	
14	52.2	51.79	1.28 m	
15	22.2	21.88	1.55/1.95	13
16	35.7	35.83	2.07 m/2.46 m	14, 15, 17
17	219.0	221.08	-	
18	13.5	13.54	0.89 s	12, 13, 14, 17
19	19.5	19.42	1.04 s	1, 5, 9, 10

$^{a}\delta_C$ of 3β-hydroxyandrost-5-ene-17-one [4], ^{b}recorded in $CDCl_3$, ^{c}125 MHz, ^{d}500 MHz.

3β-Hydroxyandrost-5-ene-17-one (**3**): White needles; mp. 140 °C; $[\alpha]_D$ –5 (*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* 289 $[M+H]^+$ ($C_{19}H_{28}O_2$, M = 288).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Its NMR features indicated a steroid, one main constituent of soft corals. The ^{1}H-NMR spectrum showed singlets at δ_H 0.75 (H-18) and 0.87 (H-19) assignable to two tertiary methyl groups, and doublets (J = 7.0 Hz) at δ_H 0.85 (H-26), 0.86 (H-27), and 0.90 (H-28) due to three secondary methyl groups. A sixth methyl signal appeared as a broad singlet at δ_H 0.89 and five high-field protons at δ_H 0.53 (1H, m, H-22), 0.30 (1H, m, H-23), 0.52 (1H, m, H-24), and 0.12 (2H, m, H-29), suggesting for an ergosterol-type side chain possessing a cyclopropane ring [5]. In addition, **1** was recognized as a 5α,8α-epidioxy sterol by the presence of the characteristic doublet (J = 8.5 Hz) proton signals for H-6 at δ_H 6.22 and H-7 at δ_H 6.49 [6]. The ^{13}C-NMR data of **1** (Table 1) were nearly identical to those of (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol [2]. However, based on HSQC and HMBC experiments (figure 2), the reported ^{13}C-NMR data at C-1, C-4, C-5, C-6, C-7, C-8, and C-9 [2] must be reassigned as shown in the table 1. Moreover, comparison of the ^{13}C-NMR chemical shifts at C-15 and C-16 of **1** with those reported for similar compounds [3, 6, 7] indicated that the published ^{13}C-NMR data at C-15 and C-16 of (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol [2] must be reversed as shown in the table 1.

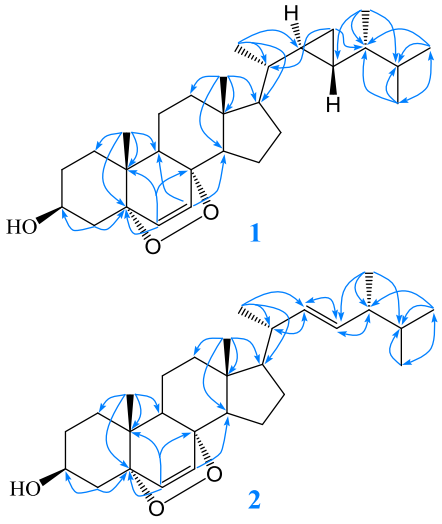

Figure 2: Key HMBC correlations of **1** and **2**

The NMR spectroscopic data of **2** were similar to those of **1** and also indicative of a 5α,8α-epidioxy sterol. The difference of NMR data between these

two compounds was only observed in the signals of the side chain with the presence of a disubstituted double bond at δ_H 135.23 (d, C-22) and 132.36 (d, C-23)/δ_H 5.14 (1H, dd, *J* = 8.5, 15.0 Hz, H-22) and 5.22 (1H, dd, *J* = 7.5, 15.0 Hz, H-23) in **2** instead of a cyclopropane ring in **1**. The large coupling constant (*J* = 15.0 Hz) of the olefinic protons indicated a *trans* geometry of the double bond. From the above evidence, the ^{13}C-NMR data of **2** were compared to those of ergosterol peroxide [3] and found to match (table 1). In addition, detailed analysis of HSQC and HMBC experiments led to determine compound **2** as ergosterol peroxide. As in case of compound **1**, based on HSQC and HMBC experiments (Figure 2), the reported ^{13}C-NMR data at C1, C-2, C-4, and C-12 of **2** [3] must be reassigned as shown in the table 1.

Compound **3** was elucidated as 3β-hydroxyandrost-5-ene-17-one [4] by an agreement of its ^{13}C-NMR data with the reported values and combination with 2D-NMR data (table 2).

4. CONCLUSION

Using combined chromatographic methods, three sterols including (22*R*,23*R*,24*R*)-5α,8α-epidioxy-22,23-methylene-24-methylcholest-6-ene-3β-ol (**1**), ergosterol peroxide (**2**), and 3β-hydroxyandrost-5-ene-17-one (**3**) were isolated from the methanol extract of the soft coral *Lobophytum crassum*. Their structures were elucidated by spectroscopic methods. This is the first report of compounds **1**-**3** from *L. crassum*.

Acknowledgement. *This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2012.37.*

REFERENCES

1. M. Kobayashi, F. Kanda, S. R. Damarla, D. V. Rao, C. B. Rao. *Marine sterols. XVII, Polyhdroxysterols of the soft corals of the Andaman and nicobar coasts. (2). Isolation and structures of three 16β-hydroxy steroidal glycosides from an Alcyonium sp. soft coral,* Chem. Pharm. Bull., **38(9)**, 2400-2403 (1990).
2. J. H. Sheu, K. C. Chang, C. Y. Duh. *A cytotoxic 5a,8a-epidioxysterol from a soft coral Sinularia species.* J. Nat. Prod., **63(1)**, 149-151 (2000).
3. J.-M. Yue, S.-N. Chen, Z.-W. Lin, H.-D. Sun. *Sterols from the fungus Lactarium volemus,* Phytochemistry, **56(8)**, 801-806 (2001).
4. J. W. Blunt, J. B. Stothers. *^{13}C-NMR spectra of steroids - a survey and commentary.* Org. Mag. Reson., **9(8)**, 439-464 (1977).
5. H. T. D'Armas, B. S. Mootoo, W. F. Reynolds. *Steroidal compounds from the Caribbean octocoral Eunicea laciniata,* J. Nat. Prod., **63(12)**, 1669-1671 (2000).
6. E. Ioannou, A. F. Abdel-Razik, M. Zervou, D. Christofidis, X. Alexi, C. Vagias, M. N. Alexis, V. Roussis. *5α,8α-Epidioxysterols from the gorgonian Eunicella cavolini and the ascidian Trididemnum inarmatum: Isolation and evaluation of their antiproliferative activity,* Steroids, **74(1)**, 73-80 (2009).
7. X. Luo, F. Li, P. B. Shinde, J. Hong, C. O. Lee, K. S. Im, J. H. Jung. *26,27-Cyclosterols and other polyoxygenated sterols from a marine sponge Topsentia* sp., J. Nat. Prod., **69(12)**, 1760-1768 (2006).

Corresponding author: **Chau Van Minh**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: cvminh@vast.vn.