VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 18-22 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-005

POLYHYDROXYLATED STEROLS FROM THE SOFT CORAL *SARCOPHYTON PAUCIPLICATUM*

Pham The Tung[1], Ninh Thi Ngoc[1], Nguyen Phuong Thao[1], Tran Thu Huong[2], Do Cong Thung[3], Nguyen Van Thanh[1], Nguyen Hoai Nam[1*], Nguyen Xuan Cuong[1], Young Ho Kim[4], Phan Van Kiem[1], and Chau Van Minh[1]

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

[2]*School of Chemical Engineering, Hanoi University of Science and Technology*

[3]*Institute of Marine Environment and Resources, VAST*

[4]*College of Pharmacy, Chungnam National University*

Received 23 January 2015; Accepted for Publication 18 March 2015

Abstract

The methanol extract of the soft coral *Sarcophyton pauciplicatum* afforded four sterols as (24*S*)-ergostane-3*β*,5*α*,6*β*,25-tetraol 25-monoacetate (**1**), (24*S*)-ergostane-3*β*,5*α*,6*β*,*25*-tetraol (**2**), (24*S*)-ergostane-1*β*,3*β*,5*α*,6*β*,25-pentaol 25-monoacetate (**3**), and (24*S*)-ergost-25-ene-1*β*,3*β*,5*α*,6*β*-tetraol (**4**) after subjecting it to various chromatographic experiments. The structures of isolated compounds 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. pauciplicatum*.

Keywords. *Sarcophyton pauciplicatum*, Alcyoniidae, soft coral, polyhydroxylated 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 a part of our investigations on chemical constitutents of Vietnamese soft corals, we report herein the isolation and structure identification of four polyhydroxylated sterols as (24*S*)-ergostane-3*β*,5*α*,6*β*,25-tetraol 25-monoacetate (**1**), (24*S*)-ergostane-3*β*,5*α*,6*β*,*25*-tetraol (**2**), (24*S*)-ergostane-1*β*,3*β*,5*α*,6*β*,25-pentaol 25-monoacetate (**3**), and (24*S*)-ergost-25-ene-1*β*,3*β*,5*α*,6*β*-tetraol (**4**) from the soft coral *Sarcophyton pauciplicatum*.

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. pauciplicatum* were collected in Hai Phong, Vietnam, in November 2013 and identified by Professor Do Cong Thung. Voucher specimens (No. SP-11-2013) were deposited at the IMBC, VAST, Vietnam.

2.3. Isolation

Freeze-dried bodies of the soft coral *S.*

pauciplicatum (1.0 kg) were well grinded and extracted three times with MeOH at room temperature for 7 days each to afford an extract (70.0 g, A), which was suspended in H_2O (1.5 L) and then partitioned in turn with hexane (3×0.8 L) and CH_2Cl_2 (3×0.8 L) to furnish extracts soluble in dried hexane (20.1 g, B) and CH_2Cl_2 (35.0 g, C). The CH_2Cl_2 fraction was crudely separated by silica gel MPLC using gradient concentrations of ethyl acetate in *n*-hexane (from 0 to 100 %). Fractions were pooled after TLC analysis to give seven combined fractions (C-1→C-7). Fraction C-6 (7.5 g) was separated by YMC RP-18 MPLC using mobile phase of methanol–H_2O (4:1) to obtain 9 subfractions, C6A–C6G. Subfraction C6C (1.2 g) was furhter separated by silica gel CC eluding with *n*-hexane–acetone (2:1) to give 7 smaller fractions C6C1–C6C7. Fraction C6C6 (130 mg) was purified by YMC RP-18 CC with methanol–H_2O (5:1) to furnish compound **2** (3.0 mg). Purification of subfraction C6G (300 mg) by Silica gel CC eluted with *n*-hexane–acetone (1.5:1) to obtain compound **1** (10 mg).

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**[d]	
		δ_C	δ_H (*J* = Hz)		δ_C	δ_H (*J* = Hz)
1	31.0	31.94	1.10/1.47 m	32.4	33.52	1.36 m/1.62 m
2	32.0	31.03	1.32 m	33.3	31.72	1.53 m
3	65.6	65.68	3.80 m	67.4	68.36	4.03 m
4	40.8	40.86	1.37/1.85 m	42.8	41.48	1.57 m/2.04 m
5	74.2	74.26	-	75.9	76.84	-
6	73.9	74.08	3.30 br s	76.3	76.57	3.48 br s
7	34.4	34.42	1.47/1.58 m	35.5	35.33	1.54 m/1.72 m
8	30.0	29.94	1.58 m	31.2	31.65	1.77 m
9	44.6	44.51	1.34 m	45.9	46.62	1.40 m
10	37.7	37.72	-	39.1	39.35	-
11	20.7	20.68	1.21/1.26 m	21.8	22.34	1.40 m
12	39.8	39.82	1.10/1.90 m	40.7	41.52	1.20 m/2.04 m
13	42.2	42.19	-	43.1	43.94	-
14	55.4	55.39	1.10 m	56.5	57.48	1.12 m
15	23.9	23.82	0.98/1.47 m	24.6	25.26	1.63 m
16	27.1	27.05	0.75/1.53 m	28.5	29.13	1.32 m/1.90 m
17	55.8	55.73	0.98 m	56.5	57.48	1.20 m
18	12.0	11.89	0.62 s	12.4	12.66	0.74 s
19	16.3	16.21	1.02 s	17.2	17.33	1.18 s
20	35.7	35.62	1.37 m	35.7	37.75	1.42 m
21	18.9	18.79	0.89 d (7.0)	19.3	19.61	0.98 d (6.5)
22	34.3	34.25	0.86/1.29 m	36.8	36.28	0.98 m/1.59 m
23	27.7	27.65	1.21/1.75 m	28.5	29.13	0.79 m/1.78 m
24	41.3	41.27	1.90 m	46.0	46.36	1.33 m
25	85.0	84.93	-	72.3	74.16	-
26	22.7	22.60	1.32 s	26.6	26.10	1.14 s
27	23.2	23.09	1.32 s	28.0	27.14	1.15 s
28	14.3	14.25	0.82 d (7.0)	15.4	15.24	0.91 d (6.5)
1′	169.6	169.48	-			
2′′	22.2	22.09	1.90			
3-OH		-	4.13 d (5.5)			
5-OH		-	3.77 s			
6-OH		-	4.35 d (6.0)			

[a]δ_C of (24*S*)-ergostane-3*β*,5*α*,6*β*,25-tetraol 25-monoacetate [2], [b]recorded in DMSO-d_6,
[c]δ of (24*S*)-ergostane-3*β*,5*α*,6*β*,*25*-tetraol [3], [d]recorded in CD_3OD.

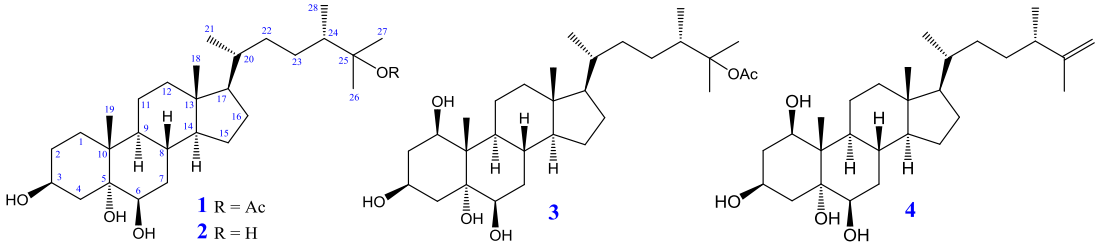

Figure 1: Chemical structures of compounds **1**–**4**

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

C	[a]δ_C	**3**[b]		**4**[b]	
		δ_C	δ_H mult. (*J* = Hz)	δ_C	δ_H mult. (*J* = Hz)
1	74.6	74.31	3.97 dd (4.5, 11.5)	74.31	0.96 dd (5.0, 11.5)
2	42.8	43.31	1.55/2.02 m	42.47	1.55/2.00 m
3	66.3	65.97	4.03 m	65.97	4.03 m
4	41.8	41.96	1.55/2.00 m	41.54	1.53/2.07 m
5	77.8	77.49	-	77.49	-
6	77.4	77.07	3.45 t (3.0)	77.08	3.45 t (3.0)
7	35.5	35.19	1.55/1.70 m	35.19	1.53/1.72 m
8	32.4	32.12	1.75 m	32.11	1.75 m
9	47.7	47.36	1.65 m	47.36	1.65 m
10	45.2	44.87	-	44.87	-
11	25.3	25.01	1.65 m/2.14 dd (3.5, 14.0)	25.01	1.62 m/2.13 m
12	42.4	42.47	1.55/2.02 m	41.98	1.18/1.98
13	43.6	43.34	-	43.35	-
14	57.8	57.53	1.13 m	57.53	1.11 m
15	29.4	29.06	1.30/1.84 m	29.10	1.25/1.82 m
16	25.8	25.55	1.20 m	25.54	1.11/1.62 m
17	57.8	57.53	1.13 m	57.81	1.11 m
18	12.9	12.62	0.74 s	12.61	0.73 s
19	10.5	10.17	1.11 s	10.17	1.15 s
20	37.8	37.54	1.42 m	36.98	1.41 m
21	19.8	19.48	0.91 d (7.0)	19.19	0.94 d (7.0)
22	36.3	36.00	0.96/1.55 m	34.87	0.96/1.35 m
23	29.0	28.75	0.85/1.65 m	32.30	1.20/1.45 m
24	43.6	43.31	1.99 m	42.86	2.12 m
25	87.7	87.39	-	151.05	-
26	23.5	23.18	1.40 s	110.08	4.68 br s/4.69 d (2.0)
27	24.1	23.81	1.42 s	18.79	1.65 s
28	15.2	14.89	0.91 d (7.0)	20.65	1.02 d (7.0)
1′	172.7	172.42	-		
2′′	22.7	22.44	1.97 s		

[a]δ_C of (24*S*)-24-methylcholestan-1*β*,3*β*,5*α*,6*β*,25-pentaol 25-monoacetate [4], [b]recorded in CD_3OD.

Moreover, fraction C-7 (1.0 g) was divided into six subfractions (C-7.1→C-7.6), by YMC RP-18 CC using stepwise elution with acetone–H_2O (1:2 to 1.5:1). Subfraction C-7.5 (0.25 g) afforded compounds **3** (5.7 mg) and **4** (7.5 mg) after subjecting it to silica gel CC eluting with CH_2Cl_2–MeOH (8.5:1).

(24*S*)-Ergostane-3*β*,5*α*,6*β*,25-tetraol 25-monoacetate (**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* 527 $[M+Cl]^-$ ($C_{30}H_{52}O_5$, M = 492).

(24*S*)-Ergostane-3*β*,5*α*,6*β*,*25*-tetraol (**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* 415 $[M-2H_2O+H]^+$ ($C_{30}H_{52}O_5$, M = 450).

(24*S*)-Ergostane-1*β*,3*β*,5*α*,6*β*,25-pentaol 25-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* 543 $[M+Cl]^-$ ($C_{30}H_{52}O_6$, M = 508).

(24*S*)-Ergostane-25-ene-1*β*,3*β*,5*α*,6*β*-tetraol (**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* 431 $[M–H_2O+H]^+$ ($C_{28}H_{48}O_4$, M = 448).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features indicated a polyhydroxylated sterol, one main constituent of soft corals [5]. Four tertiary methyl groups [δ_H 0.62 (3H, s, H-18), 1.02 (3H, s, H-19), and 1.32 (6H, s, H-26 and H-27)] and two secondary methyl groups [δ_H 0.89 (H-21) and 0.82 (H-28), each 3H, d, *J* = 7.0 Hz] were found in the ^{1}H-NMR spectrum suggesting for the presence of a 25-substituted ergosterol-type sterol. Typical signals of two oxymethine groups [δ_C 65.68 (C-3)/δ_H 3.80 (1H, m, H-3) and δ_C 74.08 (C-6)/δ_H 3.30 br s, H-6)] and two oxygenated quaternary carbons [δ_C 74.26 (C-5) and 84.93 (C-25)] were also identified. In addition, one acetyl group was confirmed by signals at δ_C 169.48 (s, C-1′) and δ_C 22.09 (q, C-2′)/1.90 (δ_H 3H, s, H-2′).

The HMBC cross-peaks of methyl protons H-26/H-27 (δ_H 1.32), and H-28 (δ_H 0.82) with C-25 (δ_C 84.93) confirmed location of one oxygenated quaternary carbon at C-25 (figure 2). The carbon signals of C-25 was strongly shifted downfield indicating the acetylation at this carbon. Detailed analysis of the other HMBC correlations and comparison of the ^{13}C-NMR data of **1** (table 1) with published values led to identification of **1** as (24*S*)-ergostane-3*β*,5*α*,6*β*,25-tetraol 25-monoacetate [2].

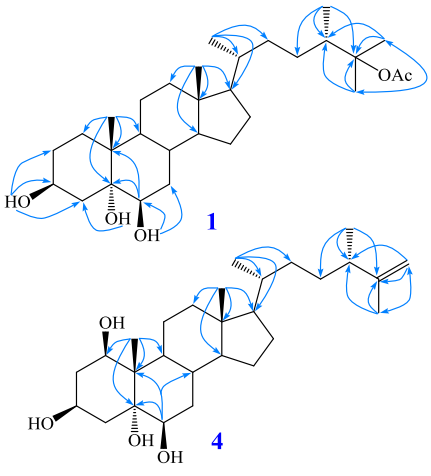

Figure 2: Key HMBC correlations of **1** and **4**

The ^{1}H and ^{13}C-NMR spectra of **2** were similar to those of **1**, except for the absence of the signals for the acetyl group. The good agreement of the ^{13}C-NMR data of **2** with the reported values confirmed its structure as (24*S*)-ergostane-3*β*,5*α*,6*β*,*25*-tetraol [3].

Compound **3** was also obtained as a white powder. The ^{1}H and ^{13}C-NMR data of **3** were similar to those of **1**, except for the additional presence of an oxymethine group [δ_C 74.31 (C-1) and δ_H 3.97 (1H, dd, *J* = 4.5 and 11.5 Hz, H-1)]. The HMBC cross-peak of H-19 (δ_H 1.11) with C-1 (δ_C 74.31) confirmed location of the additional oxymethine group at C-1, which was further confirmed by a good agreement of the ^{13}C-NMR data of **3** (Table 2) with those of (24*S*)-24-methylcholestan-1*β*,3*β*,5*α*,6*β*,25-pentaol 25-monoacetate [4].

The ^{1}H and ^{13}C-NMR data of **4** were similar to those of **3**, except for difference in the data for the side chain with the absence of the acetyl group and the presence of a 1,1-disustituted double bond at δ_C 151.05 (s, C-25)/δ_C 110.08 (t, C-26) in **4** instead of an oxygenated quaternary carbon and a tertiary methyl group in **3**. The HMBC cross-peaks of H-28 (δ_H 1.02) with C-25 (δ_C 151.05); H-26 (δ_H 4.68 and 4.69) with C-24 (δ_C 42.86), C-25 (δ_C 151.05) and C-27 (δ_C 18.79); and those of H-27 (δ_H 1.65) with C-24 (δ_C 42.86), C-25 (δ_C 151.05) and C-26 (δ_C 110.08) confirmed the position of the double bond at C-25/C-26. Thus compound **4** was identified as (24*S*)-ergost-25-ene-1*β*,3*β*,5*α*,6*β*-tetraol. This compound was previously isolated as a triacetate derivative from the acetylated fraction of the soft coral *Sarcophyton subviride* [2]. However, this is the first report of direct isolation of **4** from *S. pauciplicatum* and the NMR data were reported for the first time.

Acknowledgement. *This work was financially supported by Vietnam Academy of Science and Technology, code: VAST.TĐ.ĐAB.02/13–15.*

REFERENCES

1. M. Kobayashi, F. Kanda, S. R. Damarla, D. V. Rao, C. B. Rao. *Marine sterols. XVII. Polyhydroxy sterols of the soft coral of the Andaman and Nicobar coasts (2). Isolation and structures of three 16β-hydroxy steroidal glycosides from an Alcyonium sp. soft coral*, Chemical & Pharmaceutical Bulletin, **38(9)**, 2400-2403 (1990).
2. B. L. Raju, G. V. Subbaraju, M. C. Reddy, D. V. Rao, C. B. Rao, V. S. Raju. *Polyhydroxysterols from the soft coral Sarcophyton subviride of Andaman and Nicobar coasts*, Journal of Natural Products, **55(7)**, 904-911 (1992).
3. M. Vanisree, G. V. Subbaraju, R. C. Bheemasankara. *Alcyonacean metabolites VII - Chemical constituents of Lobophytum denticulatum and Lobophytum strictum of the Indian Ocean*, Journal of Asian Natural

Products Research, **2(2)**, 87-95 (2000).

4. JIA Rui, SHI Yan-Hong, HE Pei-Min, G. Yue-Wei. *Chemical Constituents of Sarcophyton tortuosum*, Chinese Journal of Natural Medicines, **8(5)**, 422-424 (2010).
5. N. S. Sarma, M. S. Krishna, S. G. Pasha, T. S. Rao, Y. Venkateswarlu, P. S. Parameswaran. *Marine metabolites: the sterols of soft coral*, Chemical Reviews, **109(6)**, 2803-2828 (2009).

Corresponding author: **Nguyen Hoai Nam**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: namnguyenhoai@imbc.vast.vn.