

STEROLS FROM THE SPONGE *Haliclona varia*

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Received 21 September 2015; Accepted for publication 25 April 2016

Abstract

Using combined chromatographic methods, four sterols, 3 β -hydroxycholest-5-en-7-one (**1**), 22(*E*)-3 β -hydroxycholesta-5,22-dien-7-one (**2**), 3 β ,7 α -dihydroxycholest-5-ene (**3**), and 3 β ,7 β -dihydroxycholest-5-ene (**4**) were isolated from the methanol extract of the sponge *Haliclona varia*. Their structures were elucidated by 1D and 2D-NMR spectroscopic methods as well as in comparison with the previous literature. Compounds **2-4** were reported from the genus *Haliclona* for the first time.

Keywords. *Haliclona varia*, sponge, sterol.

1. INTRODUCTION

Marine sponges are incredible source of novel pharmacologically active compounds which have shown efficiency against various diseases such as anticancer and anti-inflammatory [1]. Up to date, there are a lot of studies on chemical constituents and biological activities of the genus *Haliclona*. Main chemical constituents of this genus have been found as terpenoids [2-4], alkaloids [5, 6] and the polyacetylene [7, 8]. However, the chemical investigation of *Haliclona varia* (Chalinidae) has not been reported yet. We report herein the isolation and structure elucidation of four sterols from *H. varia*.

2. MATERIAL AND METHODS

2.1. Sponge materials

Haliclona varia (Bowerbank, 1875) was collected in Coto Island, Quang Ninh, Vietnam during March, 2014. The scientific name was identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources, VAST. A voucher specimen (HM27) was deposited at Institute of Marine Biochemistry.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker

AM500 FT-NMR spectrometer (500 MHz for ¹H and 125 MHz for ¹³C-NMR), chemical shifts are reported in ppm using TMS as an internal standard. ESI-MS spectra were recorded on Agilent 1100. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter (Jasco, Tokyo, Japan). Column chromatography (CC) was performed on silica gel 230-400 mesh or RP-18 resins (150 μ m, Fuji Silysia Chemical Ltd.). Compounds were visualized by spraying with aqueous 10% H₂SO₄ and heating for 5 minutes.

2.3. Extraction and isolation

The fresh frozen samples of the sponge *H. varia* (1.5 kg) were well grinded and extracted with hot MeOH three times (5L, 3h each) and then concentrated under reduced pressure to give MeOH extract (HV, 53.0 g). This extract was suspended in water and then partitioned with dichloromethane to give the dichloromethane (HV1, 18.0 g) and water residue (HV2, 30.0 g). The HV1 layer was chromatographed on a silica gel column and eluted with a gradient elution of *n*-hexane – acetone (100/1→0/1, v/v) to yield three fractions, HV1A (3.5 g), HV1B (5.0 g), and HV1C (2.0 g). The HV1A fraction was chromatographed on a silica gel column eluted with *n*-hexane - acetone (2.5/1, v/v) to give two fractions, HV1A1 (900 mg) and HV1A2

(450.0 mg). Compounds **1** (14.0 mg), **3** (8.0 mg), and **4** (17.0 mg) were obtained from HV1A1 by RP-18 column chromatography, eluted with methanol - water (1.5/1, v/v). The HV1B fraction was chromatographed on RP-18 column using acetone - water (2/1, v/v) as a mobile phase to give two smaller fractions, HV1B1 (900.0 mg) and HV1B2 (500.0 mg). Compound **2** (14.0 mg) was obtained from HV1B2 fraction using silica gel column, eluted dichloromethane - acetone (6/1, v/v).

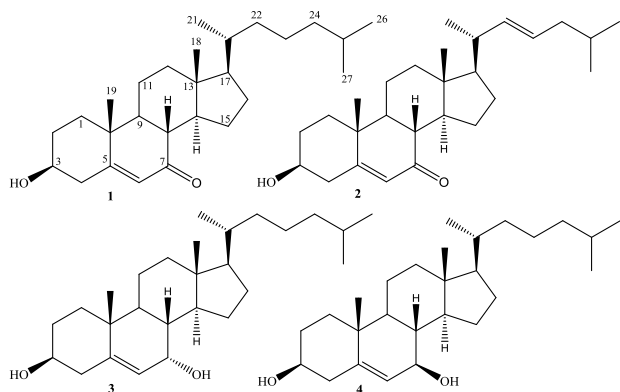


Figure 1: Chemical structures of compounds **1-4**

3β-Hydroxycholest-5-en-7-one (1): white amorphous; $[\alpha]_D^{25}$: -78.0 (c 0.3, CHCl_3); $\text{C}_{27}\text{H}_{44}\text{O}_2$; $M = 400.3$; ESI-MS: m/z 401 $[\text{M}+\text{H}]^+$; $^1\text{H-NMR}$ (CDCl_3), $^{13}\text{C-NMR}$ (CDCl_3), see table 1.

22(E)-3β-Hydroxycholesta-5,22-dien-7-one (2): white amorphous; $[\alpha]_D^{25}$: -93.0 (c 0.2, CHCl_3); $\text{C}_{27}\text{H}_{42}\text{O}_2$; $M = 398$; ESI-MS: m/z 399 $[\text{M}+\text{H}]^+$. $^1\text{H-NMR}$ (CDCl_3 , 500 MHz), $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz), see table 1.

3β,7α-Dihydroxycholest-5-ene (3): white amorphous; $[\alpha]_D^{25}$: -130.2 (c 0.2, CHCl_3) molecular formula: $\text{C}_{27}\text{H}_{46}\text{O}_2$; $M = 402$; APCI-MS: m/z 367 $[\text{M}+\text{H}-2\text{H}_2\text{O}]^+$; $^1\text{H-NMR}$ (CDCl_3) and $^{13}\text{C-NMR}$ (CDCl_3), see table 1.

3β,7β-Dihydroxycholest-5-ene (4): white amorphous; $[\alpha]_D^{25}$: $+30.0$ (c 0.1, CHCl_3); $\text{C}_{27}\text{H}_{46}\text{O}_2$; $M = 402$; APCI-MS: m/z 367 $[\text{M}+\text{H}-2\text{H}_2\text{O}]^+$; $^1\text{H-NMR}$ (CDCl_3) and $^{13}\text{C-NMR}$ (CDCl_3), see table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous and its molecular formula was determined as $\text{C}_{27}\text{H}_{44}\text{O}_2$ by ESI-MS ion peak at m/z 401 $[\text{M}+\text{H}]^+$ and $^{13}\text{C-NMR}$. The $^1\text{H-NMR}$ of **1** showed the signals of five methyl groups at δ_{H} 0.68 (3H, s, H-18), 1.21 (3H, s, H-19), 0.92 (3H, d, $J = 6.5$ Hz, H-21) and 0.87 (6H, d, $J = 6.5$ Hz, H-26 and H-27), one

oxymethine proton at δ_{H} 3.63 (1H, m, H-3), and one olefinic proton at δ_{H} 5.67 (1H, s, H-6). The $^{13}\text{C-NMR}$ and DEPT spectra of **1** exhibited the signals for 27 carbons, including one carbonyl, three quaternary, eight methine, ten methylene, and five methyl carbons. Analytical $^1\text{H-}$ and $^{13}\text{C-NMR}$ data of **1** indicated that NMR data were similar to those of 3β-hydroxycholest-5-en-7-one [9]. The HMBC correlations between H-26/H-27 (δ_{H} 0.87) and C-24 (δ_{C} 39.47)/C-25 (δ_{C} 27.98); H-21 (δ_{H} 0.92) and C-20 (δ_{C} 35.69); H-18 (δ_{H} 0.68) and C-12 (δ_{C} 38.71)/C-13 (δ_{C} 41.82)/C-14 (δ_{C} 49.95)/C-17 (δ_{C} 54.81); H-19 (δ_{H} 1.21) and C-1 (δ_{C} 36.36)/C-5 (δ_{C} 165.23)/C-9 (δ_{C} 49.97)/C-10 (δ_{C} 38.38) indicated the positions of methyl groups at C-10, C-13, C-20 and C-25/C-25. The HMBC correlations from H-3 (δ_{H} 3.63) to C-5 (δ_{C} 165.23); from H-8 (δ_{H} 2.22)/H-9 (δ_{H} 1.52) to C-7 (δ_{C} 202.34) suggested the positions of double bond and carbonyl group to at C-5/C-6 and C-7, respectively. The hydroxyl group at C-3 was determined by HMBC correlations between H-4 (δ_{H} 2.42/2.52) and C-3 (δ_{C} 70.46). Thus, compound **1** was elucidated to be 3β-hydroxycholest-5-en-7-one. This compound was reported from *Haliclona* sp. by Li and co-author [10].

The $^1\text{H-NMR}$ of **2** showed the signals for five methyl groups at δ_{H} 0.68 (3H, s, H-18), 1.20 (3H, s, H-19), 1.01 (3H, d, $J = 6.5$ Hz, H-21), and 0.88 (6H, d, $J = 6.5$ Hz, H-26 and H-27), one oxygenated methine proton at δ_{H} 3.68 (1H, m, H-3), three olefinic protons at δ_{H} 5.69 (1H, m, H-6), 5.25 (1H, dd, $J = 8.0, 16.0$ Hz, H-22) and 5.28 (1H, dt, $J = 6.5, 16.0$ Hz, H-23). The $^{13}\text{C-NMR}$ and DEPT spectra of **2** exhibited the signals for 27 carbons, including one carbonyl, three quaternary, ten methine, eight methylene, and five methyl carbons. The NMR data of **2** were similar to the corresponding of **1** except for additional signals of double bond at C-22/C-23. In addition, the position of C-22/C-23 double bond was confirmed by HMBC correlations between H-21 (δ_{H} 1.01) and C-22 (δ_{C} 137.88); H-23 (δ_{H} 5.28) and C-20 (δ_{C} 39.89)/C-24 (δ_{C} 41.95). The *E*-configuration of the double bond was based on the coupling constant between H-22 and H-23, $J = 16.0$ Hz. Based on the ESI-MS result and the above evidence, compound **2** was determined as 22(*E*)-3β-hydroxycholesta-5,22-dien-7-one, a compound known from the sponge *Cliona copiosa* [9]. However, this is the first report from the genus *Haliclona*.

The $^1\text{H-NMR}$ spectrum of **3** showed the presence of one olefinic proton at δ_{H} 5.62 (1H, s, H-6), two tertiary methyl groups at δ_{H} 0.68 (3H, s, H-18) and 1.05 (3H, s, H-19), three secondary methyl at δ_{H} 0.92 (3H, d, $J = 6.5$ Hz, H-21), 0.85 (3H, d, J

Table 1: ^1H - and ^{13}C -NMR data of compounds **1-4** and reference compounds

C	1			2		3		4		
	$\delta_{\text{C}}^{\#}$	δ_{C}	$\delta_{\text{H}}(J, \text{Hz})$	δ_{C}	$\delta_{\text{H}}(J, \text{Hz})$	δ_{C}	$\delta_{\text{H}}(J, \text{Hz})$	δ_{C}^{\S}	δ_{C}	$\delta_{\text{H}}(J, \text{Hz})$
1	36.5	36.36	1.21 (m) 1.95 (m)	36.37	1.20 (m) 1.95 (m)	36.18	1.02 (m) 1.33 (m)	36.6	36.97	
2	31.3	31.16	1.62 (m) 1.94 (m)	31.20	1.62 (m) 1.94 (m)	31.38	1.52 (m) 1.86 (m)	31.5	31.60	
3	70.6	70.46	3.63 (m)	70.52	3.68 (m)	71.34	3.61 (m)	71.4	71.46	3.55 (m)
4	41.9	43.10	2.42 (m) 2.52 (m)	41.83	2.39 (m) 2.51 (m)	42.03	2.29 (m) 2.34 (m)	41.7	41.76	
5	165.2	165.23	-	165.10	-	146.25	-	143.4	143.49	
6	126.3	126.05	5.67 (s)	126.44	5.69 (s)	123.87	5.60 (d, 5.5)	125.4	125.47	5.30 (d, 8.5)
7	202.4	202.34	-	202.20	-	65.36	3.85 (br s)	73.3	73.38	3.85 (br d, 8.0)
8	45.5	45.41	2.22 (m)	45.40	2.23 (m)	37.54	1.47 (m)	40.8	40.95	
9	50.1	49.97	1.34 (m)	49.97	1.34 (m)	42.29	1.24 (m)	48.2	48.30	
10	38.4	38.38	-	38.29	-	37.41	-	36.4	36.46	
11	21.3	21.21	1.58 (m)	21.22	1.59 (m)	20.72	1.49 (m) 1.51 (m)	21.0	21.10	
12	38.8	38.71	1.14 (m) 2.02 (m)	38.60	1.17 (m) 2.00 (m)	39.20	1.18 (m) 2.01 (m)	39.5	39.52	
13	41.9	41.82	-	43.02	-	42.16	-	42.9	42.95	
14	50.1	49.95	1.52 (m)	50.06	1.50 (m)	49.44	1.43 (m)	55.4	55.50	
15	26.4	26.31	1.25 (m) 2.41 (m)	26.35	1.24 (m) 2.39 (m)	28.27	1.32 (m) 1.92 (m)	26.4	26.40	
16	28.6	28.52	1.28 (m) 1.88 (m)	28.75	1.28 (m) 1.78 (m)	24.30	1.16 (m) 1.71 (m)	28.5	28.55	
17	54.9	54.81	1.08 (m)	54.68	1.14 (m)	55.89	1.18 (m)	55.9	55.99	
18	12.1	11.96	0.68 (s)	12.20	0.68 (s)	11.64	0.68 (s)	11.8	11.84	0.68 (s)
19	17.4	17.30	1.21 (s)	17.32	1.20 (s)	18.25	1.05 (s)	18.7	18.79	1.21 (s)
20	35.8	35.69	1.41 (m)	39.89	2.05 (m)	35.78	1.41 (m)	35.7	35.74	
21	19.0	18.86	0.92 (d, 6.5)	21.06	1.01 (d, 6.5)	18.75	0.92 (d, 6.5)	19.1	19.17	0.92 (d, 6.5)
22	36.3	36.18	1.01 (m)	137.88	5.25 (dd, 8.0, 16.0)	37.03	1.12 (m) 1.86 (m)	36.2	36.23	
23	23.9	23.82	1.12 (m) 1.33 (m)	126.11	5.28 (dt, 6.5, 16.0)	23.73	1.16 (m) 1.34 (m)	23.8	23.86	
24	39.6	39.47	1.12 (m)	41.95	1.82 (m)	39.53	1.12 (m) 1.18 (m)	39.5	39.59	
25	28.1	27.98	1.52 (m)	28.56	1.59 (m)	28.02	1.52 (m)	28.0	28.02	
26	22.6	22.54	0.87 (d, 6.5)	22.30	0.88 (d, 6.5)	22.57	0.85 (d, 6.5)	22.5	22.56	0.87 (d, 6.5)
27	22.9	22.79	0.87 (d, 6.5)	22.30	0.88 (d, 6.5)	22.80	0.86 (d, 6.5)	22.8	22.82	0.87 (d, 6.5)

$^{\#}\delta_{\text{C}}$ of 3β -hydroxycholest-5-en-7-one [9] in CDCl_3 , $^{\S}\delta_{\text{C}}$ of $3\beta,7\beta$ -dihydroxycholest-5-ene [11] in CDCl_3 .

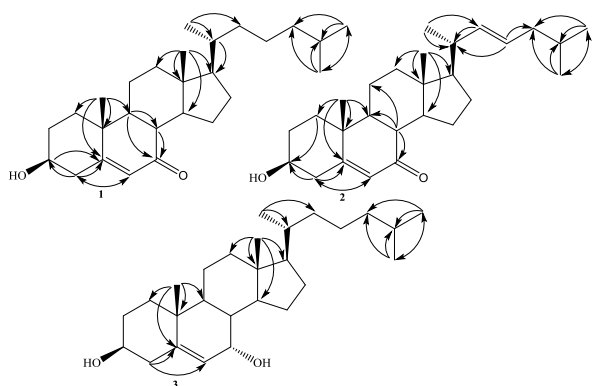


Figure 2: The important HMBC correlations of **1-3**

= 6.5 Hz, H-26) and 0.86 (3H, d, J = 6.5 Hz, H-27), two oxymethine groups at δ_{H} 3.61 (1H, m, H-3) and 3.85 (1H, br s, H-3). The ^{13}C -NMR and DEPT spectra of **3** exhibited the signals for 27 carbons, including three quaternary, eleven methine, eight methylene and five methyl carbons. The NMR data

of **3** were similar to those of $3\beta,7\alpha$ -dihydroxycholest-5-ene [11]. The broad singlet at 3.85 (H-7) confirmed α -configuration of hydroxyl group at C-7 [11]. The HMBC correlations between H-19 (δ_{H} 1.05) and C-1 (δ_{C} 36.18)/C-5 (δ_{C} 146.25)/C-9 (δ_{C} 42.29)/C-10 (δ_{C} 37.41); H-4 (δ_{H} 2.29 and 2.34) and C-5 (δ_{C} 146.25)/C-6 (δ_{C} 123.87) confirmed the double bond at C-4/C-5. Based on the evidence of ESI-MS and NMR data, **3** was determined to be $3\beta,7\alpha$ -dihydroxycholest-5-ene. The NMR spectra of compounds **4** and **3** were almost similar. The difference only is signal of oxymethine group [(δ_{C} 73.38, C-7)/(δ_{H} 3.85, br d, J = 8.0 Hz, H-7) in compound **4** and (δ_{C} 65.36, C-7)/(δ_{H} 3.85, br s, H-7) in compound **3**]. In addition, the ESI-MS and NMR data of **4** were found to match with those of $3\beta,7\beta$ -dihydroxycholest-5-ene [12]. Thus, **4** was elucidated as $3\beta,7\beta$ -dihydroxycholest-5-ene. Compounds **3** and **4** were known from the sponge *Cliona copiosa* [9].

Acknowledgment. *This research was supported by Vietnam Academy of Science and Technology under grant number VAST.TĐ.ĐAB.01/13-15.*

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