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Steroids from the blood cockle *Anadara granosa*

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Abstract

Using various chromatographic experiments, four sterols as 5α,8α-epidioxy-24(*S*)-ethylcholest-6-en-3*β*-ol (**1**), (22*E*,24*S*)-5*α*,8*α*-epidioxy-24-ethyl-cholesta-6,22-dien-3*β*-ol (**2**), 7-oxocholesterol (**3**), and (22*E*)-3*β*-hydroxycholesta-5,22-dien-7-one (**4**) were isolated from the methanol extract of the blood cockle *Anadara granosa*. The structures of isolated compounds were elucidated by 1D and 2D-NMR experiments in comparison with reported data. This is the first report of these compounds from *A. granosa*.

Keywords. *Anadara granosa*, Arcidae, blood cockle, steroid.

1. INTRODUCTION

With more than 100,000 species, the phylum Mollusca is the second largest after the Arthropoda and, like the latter, the Mollusca includes as many marine species as terrestrial species. Of these, mollusk shells have been of considerable economic importance since antiquity [1].

The blood cockle *Anadara granosa* is one important sea product in Vietnam and some Asian countries. As a part of our investigations on chemical constituents of Vietnamese mollusks, we report herein the isolation and structure identification of four steroids from *A. granosa*.

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 with TMS used as an internal standard. 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, Darmstadt, Germany), YMC*GEL (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan), Sephadex LH-20 (Sigma-Aldrich, USA), and Diaion HP-20 (Supelco, USA) resins. For thin layer chromatography (TLC) there 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 blood cockle *A. granosa* were collected in Cat Ba, Hai Phong, Vietnam, in August 2014 and identified by Professor Do Cong Thung. Voucher specimens (No. VAST-TM09) were deposited at the IMBC, VAST, Vietnam.

2.3. Isolation

The muscle of blood cockle *Anadara granosa* was cut into small pieces and dried at 50^{o}C. The dried powder (1.5 kg) was extracted with MeOH in ultrasonic condition (3×1h). The obtained solutions were filtered, combined, and concentrated under reduced pressure to afford a MeOH residue (M, 150 g). This was suspended in water and partitioned in turn with *n*-hexane, CH_2Cl_2, and EtOAc to give the *n*-hexane (H, 50g), CH_2Cl_2 (D, 20g), EtOAc (E, 5g) extracts, and water layer (W).

Extract H (50 g) was crudely separated by silica gel MPLC with gradient concentrations of acetone in *n*-hexane (from 2 to 100 %) to yield five fractions, H1-H5. Fraction H2 (6 g) was separated by silica gel

CC eluting with *n*-hexane/acetone (7/1) to give three subfractions, H2a-H2c. Subfraction H2c (350 mg) was divided into two smaller fractions, H2c1 and H2c2, by YMC CC eluting with acetone/H_2O (5/1). Fraction H2c1 (45 mg) was purified by Sephadex LH-20 CC eluting with MeOH/H_2O (3/1) to give compounds **3** (5.0 mg) and **4** (4.0 mg). Compounds **1** (4.5 mg) and **2** (25 mg) were purified from fraction H2c2 (150 mg) using silica gel CC with *n*-hexane/EtOAc (4/1) as eluent.

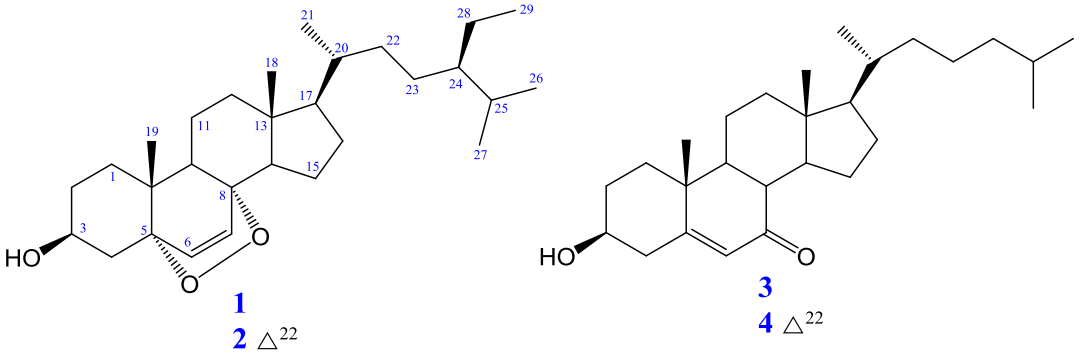

Figure 1: Chemical structures of compounds **1**–**4**

Table 1: ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) data of **1** and **2**

C	[a]δ_C	1		[b]δ_C	2	
		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	34.75	34.72	1.68 m/1.94 m	34.76	34.70	1.68 m/1.94 m
2	30.16	30.15	1.52 m/1.83 m	30.19	30.13	1.53 m/1.83 m
3	66.52	66.49	3.96 m	66.54	66.47	3.97 m
4	37.00	36.97	1.90 m/2.10 m	37.01	36.94	1.89 m/2.10 m
5	82.24	82.16	-	82.22	82.15	-
6	135.46	135.41	6.24 d (8.5)	135.46	135.41	6.24 d (8.5)
7	130.83	130.79	6.51 d (8.5)	130.83	130.76	6.50 d (8.5)
8	79.54	79.47	-	79.49	79.42	-
9	51.10	51.11	1.49 m	51.14	51.11	1.49 m
10	37.00	36.97	-	37.01	36.98	-
11	23.48	23.43	1.23 m/1.50 m	23.48	23.41	1.22 m/1.50 m
12	39.49	39.45	1.23 m/1.97 m	39.42	39.36	1.23 m/1.96 m
13	44.80	44.75	-	44.79	44.55	-
14	51.63	51.61	1.55 m	51.76	51.70	1.57 m
15	20.68	20.65	1.42 m/1.62 m	20.77	20.70	1.40 m/1.60 m
16	28.32	28.26	1.38 m/1.92 m	29.05	28.88	1.36 m/1.78 m
17	56.36	56.34	1.20 m	56.21	56.15	1.23 m
18	12.69	12.63	0.80 s	12.51	12.86	0.82 s
19	18.24	18.17	0.88 s	18.23	18.17	0.88 s
20	36.01	35.73	1.36 m	40.09	39.95	2.04 m
21	18.71	18.71	0.91 d (6.5)	21.16	21.12	1.01 d (6.5)
22	34.75	33.73	0.99 m/1.36 m	137.72	137.60	5.15 dd (8.5, 15.0)
23	26.11	26.41	1.02 m/1.30 m	129.97	129.97	5.04 dd (8.5, 15.0)
24	45.86	46.05	0.92 m	51.29	51.18	1.52 m
25	29.20	28.98	1.67 m	31.92	31.79	1.52 m
26	19.89	19.59	0.83 d (6.5)	21.22	20.90	0.83 d (6.5)
27	18.76	18.98	0.80 d (6.5)	19.00	18.94	0.78 d (6.5)
28	23.11	23.03	1.13 m/1.32 m	25.42	25.35	1.18 m/1.42 m
29	12.38	12.31	0.85 t (6.5)	12.92	12.44	0.82 t (6.5)

[a]δ_C of 5α,8α-epidioxy-24(*S*)-ethylcholest-6-en-3*β*-ol [2], [b]δ of (22*E*,24*S*)-5*α*,8*α*-epidioxy-24-ethyl-cholesta-6,22-dien-3*β*-ol [2].

Table 2: ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) data of **3** and **4**

C	$^{a}\delta_C$	3		$^{b}\delta_C$	4	
		δ_C	δ_H mult. (*J* = Hz)		δ_C	δ_H mult. (*J* = Hz)
1	36.46	36.36	1.36 m/1.97 m	36.3	36.37	1.20 m/1.95 m
2	31.29	31.22	1.63 m/1.97 m	31.2	31.20	1.62 m/1.94 m
3	70.61	70.55	3.67 m	70.5	70.52	3.68 m
4	41.90	41.83	2.42 m/2.52 m	41.8	41.83	1.82 m
5	165.23	165.04	-	165.0	165.10	-
6	126.27	126.15	5.69 s	126.1	126.44	568 d (1.5)
7	202.40	202.28	-	202.2	202.20	-
8	45.43	45.43	2.24 t (10.5)	45.4	45.40	2.23 m
9	50.07	49.99	1.35 m	50.0	49.97	1.51 m
10	38.38	38.29	-	38.3	38.29	-
11	21.34	21.24	1.01 m	21.2	21.22	1.59 m
12	38.82	38.73	1.17/2.04	38.6	38.60	1.17 m/2.00 m
13	41.90	43.12	-	43.0	43.02	-
14	50.07	49.96	1.53 m	51.2	50.06	1.34 m
15	26.41	26.33	1.25 m/2.41 m	51.2	26.35	1.24 m/2.39 m
16	28.60	28.55	1.09 m/1.95 m	28.8	28.75	1.28 m/1.78 m
17	54.91	54.83	1.13 m	54.6	54.68	1.14 m
18	12.07	11.98	0.68 s	12.2	12.20	0.69 (s)
19	17.40	17.32	1.20 s	17.3	17.32	1.20 (s)
20	35.80	35.72	1.40 m	39.9	39.89	2.05 m
21	18.96	18.88	0.92 d (6.5)	21.0	21.06	1.01 d (6.5)
22	36.28	36.20	1.01 m/1.25 m	137.9	137.88	5.25 m
23	23.92	23.84	1.18 m/1.36 m	126.4	126.11	5.28 m
24	39.58	39.49	1.17 m	41.9	41.95	2.39 m/2.50 m
25	28.09	28.01	1.13 m	28.5	28.56	1.59 m
26	22.64	22.56	0.86 d (6.5)	22.3	22.30	0.86 d (6.5)
27	22.88	22.81	0.86 d (6.5)	22.3	22.30	0.86 d (6.5)

$^{a}\delta_C$ of 7-oxocholesterol [3], $^{b}\delta_C$ of (22*E*)-3*β*-hydroxycholesta-5,22-dien-7-one [4].

5α,8α-Epidioxy-24(*S*)-ethylcholest-6-en-3*β*-ol (1): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 1.

(22*E*,24*S*)-5*α*,8*α*-Epidioxy-24-ethyl-cholesta-6,22-dien-3*β*-ol (2): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 1.

7-Oxocholesterol (3): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see Table 2.

(22*E*)-3*β*-Hydroxycholesta-5,22-dien-7-one (4): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$), see table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features indicated a steroid, one of main constituents of many marine organisms. The ^{1}H-NMR spectrum revealed typical signals of two *tert*-methyl [δ_H 0.80 (H-18) and 0.88 (H-19), each 3H, s], three *sec*-methyl [δ_H 0.91 (H-21), 0.83 (H-26), and 0.80 (H-27), each 3H, d, *J* = 6.5 Hz], and one *pri*-methyl [δ_H 0.85 (3H, t, *J* = 6.5 Hz, H-29)] groups suggesting a ethylcholesterol-type steroid. The proton signals of an oxymethine group [δ_H 3.96 (1H, m, H-3)] and a disubstituted endocyclic double bond [δ_H 6.24 (H-6) and 6.51 (H-7), each 1H, d, *J* = 8.5 Hz] were also observed. In addition, the ^{13}C-NMR spectrum of **1** revealed 29 signals containing one oxymethine group [δ_C 66.49 (C-3)], two oxygenated quaternary carbons [δ_C 82.16 (C-5) and 79.47 (C-9)], and a 1,2-disubstituted double bond [δ_C 135.41 (d, C-6) and 130.79 (d, C-7)]. The signals of two oxygenated quaternary carbons were strongly shifted downfield suggesting the presence of a peroxide bridge. The ^{13}C-NMR data of **1** were totally identical (table 1) to those of 5α,8α-epidioxy-24(*S*)-ethylcholest-6-en-3*β*-ol [2]. Moreover, the structure of **1** was further confirmed by HMBC experiment. The HMBC cross-peaks of H-4 with C-2, C-3, C-5 and C-10; H-6 with C-8 and C-10; H-7 with C-5 and

C-9; H-18 with C-12, C-13, C-14, and C-17; and those of H-19 and C-1, C-5, C-9, and C-10 (figure 2) clearly confirmed the structure of **1** as 5α,8α-epidioxy-24(*S*)-ethylcholest-6-en-3*β*-ol.

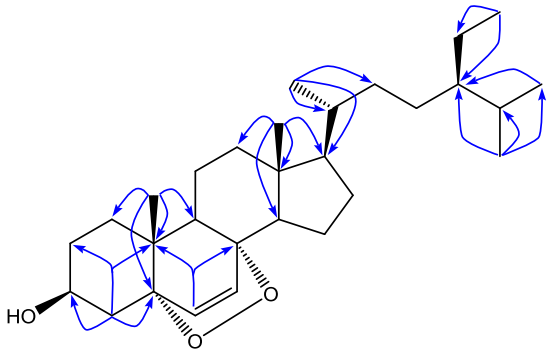

Figure 2: Key HMBC (H → C) correlations of **1**

The ^{1}H- and ^{13}C-NMR spectroscopic data of **2** were similar to those of **1**, except for an additional presence of a *trans* disubstitued double bond [δ_C 137.60 (C-22) and 129.97 (C-23)/δ_H 5.15 (H-22) and 5.04 (H-23), each 1H, d, *J* = 8.5, 15.0 Hz]. Analysis of the structure of **1** indicated a unique position of the additional double bond at C-22/C-23 in **2**, which was further confirmed by a good agreement of the ^{13}C-NMR data of **2** (table 1) with those of (22*E*,24*S*)-5*α*,8*α*-epidioxy-24-ethyl-cholesta-6,22-dien-3*β*-ol [2] and by 2D-NMR evidence.

Compounds **3** and **4** were also isolated as white powders. Their NMR features indicated 3*β*-hydroxycholesta-5-en-7-one steroid nucleus with signals of one oxymethine, one trisubstituted endocyclic double bond, and one α,β-unsaturated ketone. Similar as in case of **1** and **2**, compound **4** is different from **3** only with presence of a *trans* disubstitued double bond at C-22/C-23. Comparison of their ^{13}C-NMR values with those reported (table 2) and detailed analysis of HSQC and HMBC data confirmed these two compounds as 7-oxo-cholesterol (**3**) [3] and (22*E*)-3*β*-hydroxycholesta-5,22-dien-7-one (**4**) [4]. To the best of our knowledge, this is the first report of compounds **1**-**4** from blood cockle *A. granosa*.

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