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STEROLS ISOLATED FROM THE SEA URCHIN *DIADEMA SAVIGNYI*

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Abstract

Using various chromatographic methods, six sterols namely cholest-6-ene-5*α*,8*α*-epidioxy-3*β*-ol (**1**), cholest-5-ene-3*β*,7*α*-diol (**2**), cholest-5-ene-3*β*,7*β*-diol (**3**), cholest-5-ene-7*β*-methoxy-3*β*-ol (**4**), cholest-5-ene-3*β*-ol (**5**), and cholest-5-ene-3*β*-sulfate sodium (**6**), were isolated from the methanol extract of the sea urchin *Diadema savignyi*. Their structures were elucidated by 1D- and 2D-NMR experiments and comparison of their NMR data with reported values. These compounds were isolated from *D. savignyi* for the first time.

Keywords. *Diadema savignyi*, Diadematidae, sea urchin, sterol.

1. INTRODUCTION

Natural products have many pharmacological applications, especially with regard to their potential for use in cancer chemoprevention. Natural marine products have recently become the focus of increased research interest due to their potential pharmacological activities and lower toxicity [1, 2]. Oxysterols, or oxygenated derivatives of cholesterol, are produced through autooxidation or *in vivo* enzymatic processes and have been identified in blood, mammalian tissues and cells, and processed foods. Oxysterols have emerged as intriguing substances with diverse biological activities [3, 4].

As a part of our ongoing investigations on Vietnamese echinoderms, we address herein the isolation and structure identification of six sterols including cholest-6-ene-5*α*,8*α*-epidioxy-3*β*-ol (**1**), cholest-5-ene-3*β*,7*α*-diol (**2**), cholest-5-ene-3*β*,7*β*-diol (**3**), cholest-5-ene-7*β*-methoxy-3*β*-ol (**4**), cholest-5-ene-3*β*-ol (**5**), and cholest-5-ene-3*β*-sulfate sodium (**6**) from the sea urchin *Diadema savignyi*.

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 samples of *D. savignyi* Michelin were collected in Nha Trang, Khanhhoa, Vietnam, in December 2011 and identified by MSc. Nguyen Thi My Ngan (Institute of Oceanography). Voucher specimens (No. DS-11-2011_01) were deposited at the Institute of Marine Biochemistry and Institute of Oceanography, VAST, Vietnam.

2.3. Isolation

Freshly collected specimens were immediately frozen and stored at −25 °C until used. The frozen starfish (4.5 kg) was chopped into pieces and

extracted with methanol at room temperature to afford a MeOH residue (8.1 g, A). This was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 fraction (3.2 g, B) was subjected to silica gel CC, using stepwise elution of CH_2Cl_2–MeOH (50:1, 20:1, v/v) to obtain four fractions (B-1 to B-4). Fraction B-1 (0.89 g) was subsequently subjected to Sephadex LH-20 CC (3.0 × 75 cm) eluting with CH_2Cl_2–MeOH (1:3, 1:2, v/v) to afford three subfractions (B-1.1 to B-1.3). Then, subfractions B-1.1 and B-1.2 were combined and subjected reversed-phase (RP) flash CC (YMC Gel ODS-A, 60 Å, 400/500 mesh), eluting with a gradient of MeOH–H_2O (from 6:4 to 9:1, v/v) to afford four smaller fractions (B-1.2a to B-1.2d). Purification of fraction B-1.2b (75.0 mg) on silica gel CC eluted with *n*-hexane–EtOAc (15:1, 12:1, v/v/v) to afford **5** (3.8 mg) and **6** (5.5 mg). Subfraction B-1.2c (42.0 mg) was further separated on an YMC RP-18 CC using a solvent system of acetone–H_2O (5:1, 3:1, v/v) as eluent to give **1** (6.5 mg). Subfraction B-3 (67.0 mg) was further separated by YMC RP-18 CC

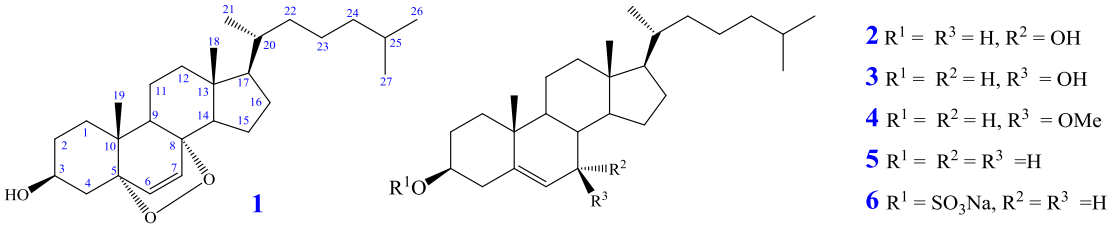

Figure 1: Chemical structures of compounds **1**–**5**

Table 1: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **1**–**3** and reported compounds in $CDCl_3$

C	[a]δ_C	**1**		[b]δ_C	**2**		[c]δ_C	**3**	
		δ_C	δ_H (J = Hz)		δ_C	δ_H (J = Hz)		δ_C	δ_H (J = Hz)
1	34.7	34.70	1.61/1.86 m	37.0	37.07	1.08/1.81 m	36.9	36.99	1.02/1.82 m
2	30.1	29.76	1.45/1.75 m	31.3	32.21	1.46/1.79 m	31.5	31.55	1.49/1.82 m
3	66.5	65.96	3.82 m	71.2	71.09	3.50 m	71.4	71.40	3.51m
4	37.0	36.73	1.82/2.01 m	42.0	41.97	2.24/2.28m	41.7	41.72	2.26/2.31m
5	82.1	82.32	-	146.2	146.30	-	143.4	143.57	-
6	135.4	135.49	6.42 d (8.0)	123.8	123.75	5.54 dd (5.0, 1.5)	125.4	125.46	5.26 t (2.2)
7	130.8	130.67	6.16 d (8.0)	65.3	65.32	3.79 ddd (5.0, 3.5, 1.5)	73.3	73.36	3.81 dt (8.0, 2.2)
8	79.4	79.57	-	37.5	37.54	1.43 m	40.8	40.90	1.37 m
9	51.1	51.04	1.41 m	42.2	42.27	1.18 m	48.2	48.32	1.00 m
10	37.0	36.92	-	37.4	37.43	-	36.4	36.50	-
11	23.4	23.38	1.14/1.43 m	20.7	20.75	1.40/1.48 m	21.0	21.14	1.52/1.43 m
12	39.4	39.41	1.86 m	39.1	39.22	1.04 m	39.5	39.57	1.05 m
13	44.5	44.73	-	42.1	42.18	-	42.9	42.99	-
14	51.7	51.55	1.48 m	49.4	49.43	1.38 m	55.4	55.51	1.05 m
15	20.6	20.58	1.34/1.54 m	24.3	24.31	1.09/1.66 m	26.4	26.43	1.10/1.78 m
16	28.2	28.22	1.31/1.92 m	28.2	28.34	123/1.85	28.5	28.62	1.77 m
17	56.4	56.39	1.10 m	55.8	55.92	1.11 m	55.9	56.02	1.10 m
18	12.6	12.59	0.73 s	11.6	11.68	0.63 s	11.8	11.89	0.66 s
19	18.2	18.14	0.81 s	18.2	18.29	0.94 s	19.1	19.21	1.02 s
20	35.2	35.20	1.34 m	35.7	35.83	1.35 m	35.7	35.81	2.19 m
21	18.6	18.54	0.83 d (7.0)	18.7	18.78	0.88 d (7.0)	18.7	18.84	0.89 d (7.0)
22	36.0	35.93	0.93/1.27 m	36.1	36.23	0.96/1.28 m	36.2	36.27	0.99/1.30 m
23	23.8	23.78	1.26/1.13 m	23.7	23.80	1.08 1.29/m	23.8	23.90	1.32/1.10 m
24	39.4	39.41	1.11 m	39.5	39.57	1.12/1.96 m	39.5	39.62	1.12/1.99 m
25	28.0	27.96	1.44 m	28.0	28.06	1.47 m	28.0	28.09	1.49 m
26	22.5	22.51	0.80 d (7.0)	22.6	22.62	0.83d (7.0)	22.5	22.63	0.84d (7.0)
27	22.8	22.78	0.79 d (7.0)	22.8	22.86	0.81 d (7.0)	22.8	22.89	0.82 d (7.0)

[a]δ_C of 5α,8α-epidioxy-cholest-6-en-3β-ol [5], [b]δ_C of cholest-5-ene-3β,7α-diol [6], [c]δ_C of cholest-5-ene-3β,7β-diol [6].

Table 2: ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **4**–**6** and reported compounds in $CDCl_3$

C	[a]δ_C	4		[b]δ_C	5		6	
		δ_C	δ_H (J = Hz)		δ_C	δ_H (J = Hz)	δ_C	δ_H (J = Hz)
1	36.9	36.79	1.15/1.80 m	37.5	37.33	1.05/1.82 m	37.16	1.02/1.79 m
2	31.6	31.50	1.50/1.82 m	31.6	31.70	1.51/1.81 m	28.85	1.57/1.99 m
3	71.6	71.48	3.60 m	71.3	71.85	3.49 m	79.09	4.14 m
4	42.5	42.15	2.27/2.33 m	42.4	42.38	2.22/2.27 m	39.22	2.28/2.45 m
5	146.2	146.19	-	141.2	140.82	-	140.02	-
6	120.9	120.80	6.06 dd (5.0, 1.5)	121.3	121.77	5.32 dd (1.5, 3.0)	122.53	5.30 br s
7	74.1	74.01	3.26 t (3.5)	32.0	31.97	1.41/1.99 m	31.96	1.46/1.90 m
8	37.3	37.25	1.47 m	32.0	31.97	1.47 m	31.07	1.36 m
9	42.9	42.80	1.28 m	50.5	50.20	0.90 m	50.07	0.85 m
10	37.6	37.50	-	36.5	36.57	-	36.22	-
11	20.9	20.88	1.39/1.44 m	21.2	21.16	1.42/1.48 m	21.07	1.43 m
12	39.2	39.12	1.20/1.94 m	40.0	39.86	1.98 m	39.77	1.47/1.90 m
13	42.2	42.35	-	42.4	42.38	-	42.35	-
14	49.2	49.12	1.48 m	56.9	56.23	1.07 m	56.19	1.03 m
15	24.4	24.34	0.96/1.60 m	24.3	24.36	1.05/1.54 m	24.32	1.08/1.27 m
16	28.8	28.32	1.85 m	28.3	28.31	1.23/1.81 m	28.26	1.19/1.78 m
17	55.9	55.85	1.14 m	56.5	56.83	0.98 m	56.74	0.92 m
18	11.6	11.54	0.63 s	12.0	11.94	0.65 s	11.88	0.61 s
19	18.4	18.33	0.96 s	19.4	19.47	0.98 s	19.31	0.93 s
20	36.0	35.87	1.37 m	35.8	35.86	1.37 m	35.83	1.33 m
21	18.9	18.81	0.89 d (7.0)	18.8	18.79	0.89 d (6.5)	18.73	0.85 d (6.5)
22	36.3	36.26	0.98/1.30 m	36.4	36.27	0.97/1.30 m	36.51	1.26/0.99 m
23	23.9	23.82	1.11/1.31 m	24.1	23.91	1.09/1.31 m	23.88	1.19/0.97 m
24	39.7	39.62	1.07/1.15 m	39.6	39.59	0.98/1.15 m	39.54	1.07/1.38 m
25	28.2	28.11	1.50 m	28.0	28.09	1.19 m	28.03	1.36 m
26	22.7	22.65	0.85 d (7.0)	22.6	22.64	0.84 d (6.5)	22.58	0.79 d (6.5)
27	23.0	22.90	0.83 d (7.0)	22.9	22.90	0.83 d (6.5)	22.84	0.80 d (6.5)
OMe	56.9	56.87	3.33 s					

[a]δ_C of cholest-5-ene-7β-methoxy-3β-ol [7], [b]δ_C of cholest-5-ene-3β-ol [8].

eluting with MeOH–H_2O (3.5:1, v/v), followed by silica gel CC with CH_2Cl_2–acetone (15:1, 13:1, v/v)to furnish compounds **3** (3.3 mg) and **4** (4.4 mg). And finally, fraction B-4 (0.69 g) was fractionated into three subfractions (B-4.1 to B-4.3) by YMC RP-18 CC using stepwise elution with acetone–H_2O (3:1 to 1:1). Subfraction B-4.2 (0.18 g) afforded compound **2** (4.5 mg) after subjecting it to silica gel CC eluting with CH_2Cl_2–acetone (9:1) and further separated by YMC RP-18 CC with acetone–H_2O (2.5:1).

Cholest-6-ene-5α,8α-epidioxy-3β-ol (**1**): White powder; $[\alpha]_D$: −34.4^o (*c*, 0.15, MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS: *m/z* 439 $[M+Na]^+$ ($C_{27}H_{44}O_3$, M = 416).

Cholest-5-ene-3β,7α-diol (**2**): White powder; $[\alpha]_D$: −91.5^o (*c*, 0.15, $CHCl_3$): ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS: *m/z* 403 $[M+H]^+$ ($C_{27}H_{46}O_2$, M = 402).

Cholest-5-ene-3β,7β-diol (**3**): White needles; mp: 178–179 ^{o}C; $[\alpha]_D$: +114.8^o (*c*, 0.15, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 1; ESI-MS: *m/z* 403 $[M+H]^+$ ($C_{27}H_{46}O_2$, M = 402).

Cholest-5-ene-7β-methoxy-3β-ol (**4**): White powder; $[\alpha]_D$: −72.4^o (*c*, 0.10, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 2; ESI-MS: *m/z* 417 $[M+H]^+$ ($C_{28}H_{42}O_2$, M = 416).

Cholest-5-ene-3β-ol (**5**): White powder; $[\alpha]_D$: +38.1^o (*c*, 1.0, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS: *m/z* 409 $[M+Na]^+$ ($C_{27}H_{46}O$, M = 386).

Cholest-5-ene-3β-sulfate sodium (**6**): White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) see table 2; ESI-MS: *m/z* 489 $[M+H]^+$ ($C_{27}H_{45}NaO_4S$, M = 488).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features indicated a sterol, one main constituents from echinoderm. The ^{1}H-NMR

spectrum revealed signals of two tertiary methyl [δ_H 0.73 (H-18) and 0.81 (H-19), each 3H, s] and three secondary methyl groups [δ_H 0.83 (H-21), 0.80 (H-26), and 0.79 (H-27), each 3H, d, J = 7.0 Hz] indicating a cholesterol-type sterol. In addition, signals of a methine group [δ_C 65.96 (C-3)/3.82 (1H, m, H-3)], two oxygenated quaternary carbons [δ_C 82.32 (C-5) and 79.59 (C-9)], and a 1,2-disubstituted double bond [δ_C 135.49 (d, C-6) and 130.67 (d, C-7)/6.42 (H-6) and 6.16 (H-7), each 1H, d, J = 8.0 Hz] were also determined in the ^{1}H and ^{13}C-NMR spectra of **1**. The signals of two oxygenated quaternary carbon were strongly shifted downfield suggesting for a peroxide bridge. The ^{13}C-NMR data of **1** essentially identical to those of 5α,8α-epidioxy-cholest-6-ene-3β-ol [5]. Detailed analysis of COSY correlation led to assignment of connectivities of H-1/H-2/H-3/H-4, H-6/H-7, H-9/H-11/H-12, H-14/H-15/H-16/H-17/H-20/H-22/H-23/H-24/H-25/H-26, H-21/H-22, and H-25/H-27 (figure 2). These data and HMBC cross-peaks of H-4 with C-5; H-6 with C-5 and C-10; H-7 with C-8 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 clearly confirmed the structure of **1** as 5α,8α-epidioxy-cholest-6-ene-3β-ol.

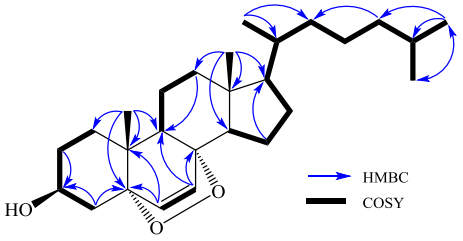

Figure 2: Key COSY and HMBC correlations of **1**

The NMR features of **2** and **3** also indicated cholesterol-type sterols. The easily visible changes are the presence of a trisubstituted double bond, an oxymethine and a methine groups in the spectra of **2** and **3** instead of a disubstituted double bond and two oxygenated quaternary carbon in **1** (table 1). The methyl proton H-19 had HMBC correlation with the quaternary olefinic carbon in **2** and **3** confirming the common position of the disubstituted double bond at C-5/C-6 of both compounds. In addition, the HMBC cross-peak of the oxymethine proton H-7 with the olefinic methine carbon C-6 was observed in both compounds indicating the position of the oxymethine group at C-7. However, the ^{13}C-NMR chemical shift for this oxymethine group at δ_C 65.32 (C-7) of **2** is quite different from that of **3** at δ_C 73.36 (C-7) confirming the two compounds have opposite configurations at C-7. Thus, compounds **2** and **3** were identified as cholest-5-ene-3β,7α-diol and cholest-5-ene-3β,7β-diol [6], respectively.

Compounds **4**–**6** were elucidated as cholest-5-ene-7*β*-methoxy-3*β*-ol [7], cholest-5-ene-3*β*-ol [8], and cholest-5-ene-3*β*-sulfate sodium [9] by comparison of their ^{13}C-NMR data with the reported values. This is the first report of these compounds from *D. savignyi*.

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