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STRUCTURE ELUCIDATION AND CYTOTOXIC ACTIVITY OF ATTENUATOSIDE B-1, PLANCISIDE A, AND CULCITOSIDE C_2 FROM THE STARFISH *ACANTHASTER PLANCI*

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Abstract. Detailed analysis of the 1D and 2D NMR data and comparison with the reported values, the structures of attenuatoside B-1 (**1**), planciside A (**2**), and culcitoside C_2 (**3**) from the starfish *Acanthaster planci* were clearly elucidated. Culcitoside C_2 (**3**) showed weak cytotoxicity against five human cancer cell lines including HepG2, KB, LNCaP, MCF7, and SK-MEL-2.

Keywords: *Acanthaster planci*, starfish, steroid glycoside, cytotoxic activity.

Classification numbers: 1.1.1; 1.2.1; 1.5.1.

1. INTRODUCTION

Steroid glycosides are a class of wide-spread natural products having either terrestrial or marine origins. The marine steroid glycosides were mainly isolated from invertebrates such as echinoderms, sponges, and soft corals. Among marine invertebrates, starfish is the richest source of steroid glycosides, at that any studied species contains a wide diversity of this kind of compounds [1]. The crown-of-thorns starfish *Acanthaster planci* is distributed in tropical waters of the Indian and Pacific Oceans, Red Sea, Australia, Seychelles, Madagascar, Philippines, and abundantly found in Vietnam sea. Prior investigations demonstrated that steroids, steroid glycosides, asterosaponins, carotenoids, ceramides, cerebrosides, and gangliosides are main constituents of this starfish [2]. Recently, investigations on this species (collected in Van Phong bay, Khanh Hoa province, Vietnam) resulted in the isolation of four new steroid glycosides [3, 4]. As parts of our ongoing investigations on chemical constituents and biological activities of Vietnamese starfish, we have recently reported steroid glycosides [5, 6], asterosaponins [7], and pyrrole oligoglycosides [8] from the starfish *A. planci*. The current paper deals with the detailed structural elucidation and cytotoxic activity of three steroid glycosides as attenuatoside B-1 (**1**), planciside A (**2**), and culcitoside C_2 (**3**) from this species.

2. EXPERIMENTAL

2.1. General methods

Optical rotations were determined on a JASCO P-2000 polarimeter. The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AVANCE III HD 500 spectrometer with TMS used as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage - Isolera One system. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck) and YMC*GEL resins (ODS-A, 12 nm S-150 μm, YMC Co., 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), and compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 min.

2.2. Biological materials

The samples of the starfish *Acanthaster planci* Linnaeus (order Valvatida, family Acanthasteridae) were collected near the Con Co Island, Quang Tri province, Viet Nam, in December 2015, and identified by Prof. Do Cong Thung (Institute of Marine Environment and Resources, VAST, Vietnam). A voucher specimen (DG-AP-12/2015) was deposited at the Institute of Marine Biochemistry, VAST, Vietnam.

2.3. Extraction and isolation

The fresh samples of *A. planci* (10 kg) were cut into small pieces and extracted in hot methanol (three times for 6 h each) to afford a MeOH residue (202 g, M) after removal of the solvent under reduced pressure. This residue was suspended in water (2.0 L) and partitioned with CH_2Cl_2 (3 × 2.0 L) to give CH_2Cl_2 extract (C, 55 g) and water layer. The latter was passed through Diaion HP-20 CC eluting with increasing concentration of MeOH in water (stepwise gradient: 0, 25, 50, 75, and 100 %) to obtain four fractions W1–W4. Fraction W3 (8.5 g) was separated into eight subfractions, W3A–W3H, by RP-18 MPLC using mobile phase of MeOH–H_2O (1:1, v/v). Further separation of fraction W3G (300 mg) by silica gel CC eluting with EtOAc–MeOH–H_2O (3.5:1:0.1, v/v) gave two subfractions, W3G1 and W3G2. Purification of subfraction W3G1 (40 mg) by YMC CC using MeOH–H_2O (2:1, v/v) as eluent furnished compounds **1** (5.0 mg) and **3** (4.5 mg). Compound **2** (4.2 mg) was purified from subfraction W3G2 (50 mg) after subjecting it on YMC CC eluted with MeOH–H_2O (2:1, v/v), following by silica gel CC with CH_2Cl_2–MeOH–H_2O (4/1/0.1, v/v).

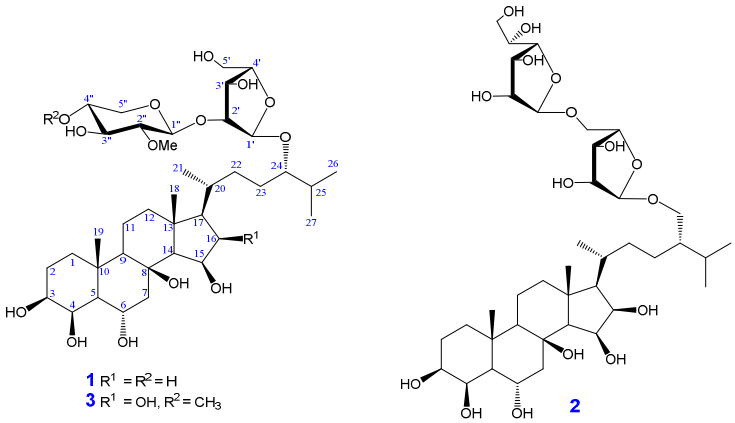

Figure 1. Chemical structures of compounds **1**–**3**.

Table 1. The NMR spectroscopic data of compounds **1** and **2**.

C	Attenuatoside B-1		**1**		Planciside A		**2**	
	[a]δ_C	[a]δ_H mult. (J = Hz)	δ_C [b,c]	δ_H [b,d] mult. (J = Hz)	[e]δ_C	[e]δ_H mult. (J = Hz)	δ_C [c,f]	δ_H [d,f] mult. (J = Hz)
1	39.5		39.41	0.97 m/1.72 m	39.7	0.97 m/1.70 m	39.63	1.00 m/1.73 m
2	26.8	2.40 qd	25.95	1.58 m/1.82 m	26.2	1.54 m/1.82 m	26.15	1.57 m/1.84 m
3	72.8	3.97 m	73.30	3.45 m	73.7	3.42 m	73.68	3.45 m
4	68.7	5.26 t	68.83	4.26 br s	69.1	4.26 br s	69.06	4.28 br s
5	57.1	1.48 dd (10.7, 2.2)	57.04	0.95 m	57.2	0.94 m	57.21	0.96 m
6	63.7	5.08 td (10.6, 4.2)	64.51	4.18 dt (4.0, 11.0)	64.8	4.18 dt (4.5, 11.0)	64.76	4.20 dt (4.0, 11.5)
7	50.4	3.14 dd (12.2, 4.3)	49.52	2.47 dd (4.0, 12.0)	50.1	1.35 t (11.8)	50.05	1.38 dd (11.5, 12.5)
				1.31 dd (11.0, 12.0)		2.46 dd (4.4, 12.2)		2.48 dd (4.5, 12.5)
8	76.4		77.13	-	77.1	-	77.14	-
9	57.8		58.24	0.82 dd (3.0, 13.0)	58.4	0.82 dd (3.2, 12.4)	58.38	0.85 dd (3.0, 12.5)
10	37.7		37.89	-	38.1	-	38.14	-
11	18.8		18.99	1.43 m/1.79 m	18.9	1.40 m/1.76 m	18.92	1.43 m/1.78 m
12	42.6		43.07	1.15 m/1.98 m	43.4	1.11 m/1.94 m	43.42	1.13 m/1.96 m
13	43.8		44.18	-	44.5	-	44.52	-
14	61.9	1.09 d (5.7)	62.54	0.99 m	61.2	1.01 d (5.6)	61.15	1.04 d (5.0)
15	70.1	4.75 m	70.94	4.43 m	71.2	4.37 dd (5.6, 7.0)	71.21	4.40 dd (5.0, 6.5)
16	42.2	2.63 dt (14.0, 7.5)	42.19	1.41 m/2.37 m	72.8	4.20 t (7.0)	72.77	4.25 t (6.5)
17	57.3		57.77	0.98 m	62.8	0.96 m	62.82	0.98 m
18	16.6	1.63 s	16.44	1.27 s	17.9	1.23 s	17.90	1.26 s
19	17.2	1.85 s	16.82	1.16 s	17.0	1.15 s	16.98	1.18 s
20	35.6		36.11	1.53 m	31.4	1.90 m	31.38	1.94 m
21	19.0	1.05 d (6.5)	18.99	0.93 d (6.5)	18.6	0.95 d (6.6)	18.55	0.98 d (6.5)
22	32.3		32.72	1.00 m/1.61 m	34.8	1.11 m/1.75 m	34.82	1.13 m/1.77 m
23	28.3		28.38	1.32 m/1.57 m	26.0	1.20 m/ 1.47 m	25.97	1.24 m/1.50 m
24	83.2	3.65 m	84.30	3.33 m	45.9	1.40 m	45.90	1.43 m
25	30.8	2.06 m	31.27	1.85 m	29.5	1.83 m	29.48	1.85 m
26	18.2	1.02 d (6.7)	18.28	0.91 d (6.5)	20.0	0.91 d (6.6)	19.98	0.94 d (6.5)
27	18.2	0.99 d (6.7)	18.15	0.89 d (6.5)	19.9	0.89 d (6.6)	19.92	0.92 d (6.5)
28					70.2	3.32 m/3.71 m	70.21	3.35 m/3.73 m
1′	107.2	5.73 d (1.0)	107.47	5.11 br s	109.7	4.82 d (1.7)	109.70	4.85 d (1.0)
2′	92.5	4.84 dd (1.0, 4.0)	92.37	4.08 dd (1.0, 3.5)	83.8	3.95 dd (1.7, 3.9)	83.72	3.98 dd (1.0, 4.0)
3′	77.6	4.90 m	77.53	4.02 dd (3.5, 7.0)	79.1	3.89 dd (3.8, 6.5)	79.12	3.92 dd (4.0, 6.5)
4′	84.2	4.76 m	83.60	3.97 m	83.4	4.01 m	83.00	4.01 m
5′	62.5	4.43 dd (3.0, 12.0)	62.42	3.67 dd (4.5, 11.5)	68.0	3.66 dd (3.7, 11.2)	68.00	3.69 dd (3.5, 11.5)
		4.29 dd		3.77 dd (3.0, 11.5)		3.83 dd (5.1, 11.2)		3.85 dd (5.0, 11.5)
1″	104.7	4.99 d (7.5)	104.97	4.43 d (7.5)	109.6	4.94 d (1.5)	109.57	4.96 br s
2″	84.7	3.46 dd (7.5, 9.0)	84.44	2.90 dd (7.5, 9.0)	83.0	3.98 m	83.47	4.05 m
3″	77.4	4.01 t (8.5)	77.20	3.35 t (9.0)	78.9	3.99 m	78.88	4.02 m
4″	70.9	4.33 m	70.89	3.51 m	84.8	3.98 m	84.75	4.02 m
5″	66.9	4.29 dd (5.0, 11.2)	66.64	3.84 dd (5.5, 11.5)	72.5	3.71 m	72.46	3.74 m
		3.56 t		3.16 dd (10.5, 11.5)				
6″					64.4	3.62 dd (6.8, 11.1)	64.43	3.65 m
						3.63 dd (5.8, 11.3)		
2″-OMe	60.5	3.78 s	61.03	3.59 s				

[a]δ_C and δ_H of attenuatoside B-1 in pyridine-d_5 [9], [b]recorded in CD_3OD+$CDCl_3$, [c]125 MHz, [d]500 MHz, [e]δ_C and δ_H of planciside A in CD_3OD [3], [f]recorded in CD_3OD.

*Attenuatoside B-1 (**1**)*: White powder; $[\alpha]_D$ −15 (*c* 0.10, MeOH); ^{1}H-NMR (500 MHz, CD_3OD+$CDCl_3$) and ^{13}C-NMR (125 MHz, CD_3OD+$CDCl_3$) see Table 1.

*Planciside A (**2**)*: White powder; $[\alpha]_D$ −30 (*c* 0.10, MeOH); ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Its NMR data are indicative for a glycosylated polyhydroxysteroid, one main constituent of starfish [10]. The ^{1}H- and ^{13}C-NMR data for the aglycone of **1** confirmed a C_{27}-steroid skeleton with presence of five oxymethines [δ_C 73.30 (C-3), 68.83 (C-4), 64.51 (C-6), 70.94 (C-15), and 84.30 (C-24)/δ_H 3.45 (1H, m, H-3), 4.26 (1H, br s, H-4), 4.18 (1H, dt, *J* = 4.0, 11.0 Hz, H-6), 4.43 (1H, m, H-15), and 3.33 (1H, m, H-24)] and one oxygenated quaternary carbon [δ_C 77.13 (C-8)]. In addition, the signals of two *tert*-methyl [δ_C 16.44 (C-18) and 16.82 (C-19)/δ_H 1.27 (H-18) and 1.16 (H-19), each 3H, s] and three *sec*-methyl [δ_C 18.99 (C-21), 18.28 (C-26), and 18.15 (C-27)/δ_H 0.93 (H-21), 0.91 (H-26), and 0.89 (H-27), each 3H, d, *J* = 6.5 Hz] groups were also observed. The ^{13}C-NMR spectrum of **1** contained two anomeric carbon signals at δ_C 107.47 (C-1′) and 104.97 (C-1″); which correlated with the corresponding anomeric protons at δ_H 5.11 (1H, br s, H-1′) and 4.43 (1H, d, *J* = 7.5 Hz, H-1″) in the HSQC spectrum, confirming the presence of two sugar moieties. The ^{1}H- and ^{13}C-NMR data for the aglycone of **1** also indicated six oxymethine, two oxymethylene, and one methoxy groups (see Table 1). Detailed analysis of the HMBC correlations (Figure 1) and a good agreement of the ^{13}C-NMR data with published values (Table 1) determined compound **1** to be attenuatoside B-1 [9].

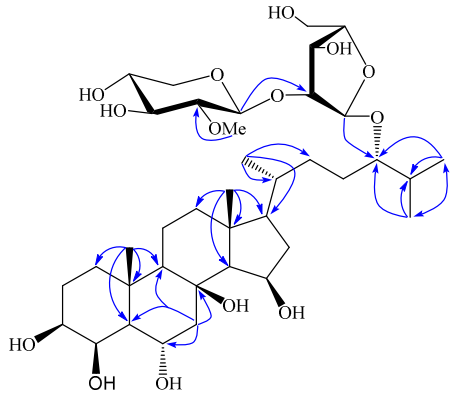

Figure 2. Key HMBC correlations of compounds **1**.

The NMR data of **2** are also indicative for a glycosylated polyhydroxysteroid. The ^{1}H- and ^{13}C-NMR data (Table 1) for the aglycone of **2** confirmed a C_{28}-steroid skeleton with presence of five oxymethines, one oxygenated quaternary carbon, one oxymethylene, two *tert*-methyls, and three *sec*-methyls. In addition, the ^{1}H- and ^{13}C-NMR data for the sugar part of **2** confirmed 11 carbon atoms including nine oxymethine and two oxymethylene groups. Detailed analysis of HMBC experiment and the comparison of the ^{1}H- and ^{13}C-NMR chemical shifts of **2** with those reported (Table 1) clearly identified compound **2** as planciside A [3]. Compound **3** was identified as culcitoside C_2 [5, 11] by detailed analysis of its 1D- and 2D-NMR data and comparison of them with those reported.

Compounds **1**–**3** were evaluated for their cytotoxicity against five human cancer cell lines, including HepG2 (hepatoma cancer), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-MEL-2 (melanoma) using the sulforhodamine B method [12] and following the previously described protocols [13, 14]. As the results, only culcitoside C_2 (**3**) showed weak cytotoxic activity on the above cancer cell lines with corresponding IC_{50} values of 80.08 ± 6.87, 63.87 ± 3.53, 59.96 ± 3.11, 51.05 ± 3.87, and 65.77 ± 5.15 µM, compared to the reference compound ellipticine (IC_{50} values of 1.38 ± 0.28, 1.79 ± 0.28, 1.95 ± 0.20, 1.34 ± 0.16, and 1.91 ± 0.20 µM, respectively). The other compounds did not show any significant cytotoxicity (IC_{50} > 100 µM) against the five tested cancer cell lines.

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