STEROIDAL DIGLYCOSIDES FROM THE STARFISH *ANTHENEA SIBOGAE*

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ABSTRACT

Two steroidal diglycosides namely anthenoside R (**1**) and anthenoside S (**2**) were isolated from the methanol extract of the starfish *Anthenea sibogae*. Their structures were elucidated by means of 1D, 2D-NMR, HR-MS techniques and comparison of their NMR data with reported values. These compounds (**1** and **2**) were isolated from starfish *Anthenea sibogae* for the first time.

Keywords: Anthenea sibogae, Oreasteridae, starfish, steroid diglycoside, polyhydroxysteroid.

1. INTRODUCTION

Anthenea sibogae is an echinoderm species belonging to the genus *Anthenea*, family Oreasteridae, order Valvatidae, and class Asteroidea. The genus of *Anthenea* has more than 20 species, distributed mainly in the Indian Ocean and Pacific Ocean [1]. Scientific studies on the chemical composition and biological activity of the genus *Anthenea* are very limited. Recently, six new polyhydroxysteroidal glycosides, Anthenosides S1-S6 were isolated and identified from the starfish *A. sibogae* by our research group [2].

Herein, we report the isolation and structure identification of two steroidal diglycosides, anthenoside R (**1**) and anthenoside S (**2**) from the methanol extract of starfish *A. sibogae* collected from Da Den Island in Bai Tu Long Bay, Vietnam.

2. MATERIALS AND METHODS

2.1. General

Optical rotations were determined on a PerkinElmer polarimeter Model 343 (Waltham, MA, USA) in MeOH. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 and 125 MHz, respectively, using tetramethylsilane as internal standard. The HR-ESI-MS spectra were recorded on an Agilent 6510 Q-TOF LC/MS. HPLC separation were carried out on Agilent 1100 (Santa Clara, CA, USA) with Diasorb-130-C16T (250 x 16 mm id, 11 µm) and Diasfer-110-C18 (250 x 4.6 mm id, 5 µm) columns were used columns. Thin-layer chromatography was performed using Sorbfil Si gel (4,5 x 6,0 cm; 5-17 µm, Sorpolimer).

2.2. Materials

Anthenea sibogae was collected at Da Den Island, Bai Tu Long Bay (Vietnam) in March 2015 and identified by Assoc. Prof. Do Cong Thung - Institute of Marine Resources and Environment, VAST. The starfish specimen (SB017) has been deposited at the Institute of Natural Products Chemistry, VAST.

2.3. Extraction and isolation

Fresh *A. sibogae* (2 kg) were chopped into small pieces and extracted four times with MeOH at 45 ^{o}C and then removed all solvent to get 100 g crude extract. The crude extract was extracted with CH_2Cl_2 (3 × 500 mL), and the CH_2Cl_2 layer was separated. The remaining residue (80 g) was dissolved in water (1.0 L). The water-soluble fraction was applied to the Polychrom-1 column chromatography, eluted with distilled H_2O until a negative chloride ion reaction was obtained. Then, this column was eluted with EtOH (3.0 L) and dried under reduced pressure to give a brownish residue (10.5 g). The brownish residue was separated on a silica gel column using CH_2Cl_2/MeOH (stepwise gradient, 9/1→1/5, v/v) and MeOH/H_2O (stepwise gradient, 15/1→5/1, v/v) as eluent to give 8 fractions (F1-F8). Fraction F6 (10.05 g) was chromatographed on silica gel column using CH_2Cl_2/MeOH (stepwise gradient, 15/1→9/1) as eluent to give 5 fractions (F6.1-F6.5). Fraction F6.3(1.58 g)was chromatographed on a silica gel column using $CHCl_3$/EtOH (3/1, v/v) as eluent to give 5 sub-fractions F6.3.1-F6.3.5 (87.1 mg). Further purification of F6.3.5 (87.1 mg)by semi preparative high-performance liquid chromatography (HPLC) [using an isocratic solvent system of MeOH/H_2O (85:15); flow rate 0.5 mL/min; Diasfer-110-C18 column] resulted in the isolation of 1 (2.3 mg) and 2 (2.9 mg).

Anthenoside R (1): white amorphous powder; $[\alpha]_D^{20}$ –52 (*c* 0.1, MeOH); HR-ESI-MS *m/z* 807.4496 $[M+Na]^+$ (calcd. for $C_{41}H_{68}O_{14}Na$: 807.4501); ^{1}H- (500 MHz, CD_3OD-d_4) and ^{13}C-NMR (125 MHz, CD_3OD-d_4) data, see Table 1.

Anthenoside S (2): white amorphous powder; $[\alpha]_D^{20}$ –31 (*c* 0.1, MeOH); HR-ESI-MS *m/z* 821.4652 $[M+Na]^+$ (calcd. for $C_{42}H_{70}O_{14}Na$: 821.4658);^{1}H- (500 MHz, CD_3OD-d_4) and ^{13}C-NMR (125 MHz, CD_3OD-d_4) data, see Table 1.

3. RESULTS AND DISCUSSION

Compound **1** was isolated as white amorphous powder. The molecular formula of compound **1** was determined to be $C_{41}H_{68}O_{14}$ by the HR-ESI-MS at *m/z* 807.4496 $[M+Na]^+$. The ^{1}H-, ^{13}C-NMR and HSQC spectra of **1** showed the presence of a tetracyclic aglycon with 2

methyl groups attached to quaternary carbon [δ_H 0.91 (H_3-18), δ_C 20.2 (C-18), δ_H 0.84 (H_3-19) and δ_C 15.4 (C-19)], a double bond at δ_C 126.6 (C-8) and 147.6 (C-14), four oxygenated methine groups [δ_H 4.07 (H-3), δ_C 67.5 (C-3), δ_H 3.62 (H-6), δ_C 75.2 (C-6), δ_H 4.23 (H-7), δ_C 78.6 (C-7), δ_H 4.62 (H-16) and δ_C 79.6 (C-16)] and two monosaccharide bonded to CH-7 and CH-16. The chemical shifts and coupling constants of the CH-3, -6, -7, -16 groups and the width of the multiplet of H-3 (about 10 Hz) were similar to the NMR signals of anthenoside Q isolated from *Antheneaaspera* starfish [3] with the tetracyclic *3α, 6β, 7β, 16α*-tetrahydroxysteroidal having sugar moieties at positions 7 and 16. The key correlation in ^{1}H-^{1}H COSY and HSQC spectra allowed to establish the sequences of protons from C1 to C-7, C-9 to C-12, C-15 to C-17. The HMBC correlations from H-6 to C-8 and C-10; H-7 to C-9 and C-14; H-9 to C-8; H-15 to C-8, C-13 and C-14; H-17 to C-13 and C-18; H-18 to C-12, C-13, C-14 and C-17; and from H-19 to C-1, C-5, C-9 and C-10 confirmed the overall structure of the $\Delta^{8(14)}$–steroid moiety of **1** (Table 1) [3]. The ^{1}H-NMR spectra of the side chain revealed the presence of the three secondary methyl groups [δ_H 1.11 (H_3-21), δ_C 24.1 (C-21), δ_H 0.88 (H_3-26), δ_C 22.9 (C-26), δ_H 0.89 (H_3-27) and δ_C 22.8 (C-27)] and a double bond [δ_H 5.79 (H-22), δ_C 138.1 (C-22), δ_H5.34 (H-23) and δ_C 128.8 (C-23)] (Table 1). The ROESY correlations of H_3-18/H-20 and H-21; H_3-21/H_β-12, H-22 and H-16 and the chemical shifts of H_3-21 (δ_H 1.11) allowed to determine the *20R*-configuration [4]. The *trans* configuration of the 22 (23)-double bond were confirmed by $J_{22,23}$ = 15.0 Hz. Therefore, the aglycon side chain of glycoside **1** was found to be (20*R*, 22*E*) cholest-22(23)-en.

The ^{1}H-, ^{13}C-NMR and HSQC spectra of **1** also exhibited two sugar moieties [δ_H 4.33 (H-1′), δ_C103.1 (C-1′)], δ_H 5.02 (H-1″) and δ_C108.5 (C-1″)] (Table 1). The first sugar moiety was determined as 3-*O*-methyl-*β*-glucopyranosyl by comparison of ^{1}H- and ^{13}C-NMR data with reported values [3]. The *β*-configuration of the glycosidic bond was suggested by the coupling constant (J = 8.0 Hz) of the anomeric proton. The correlations of H-1′ (δ_H 4.33) to C-16 (δ_C 79.6) in the HMBC and H-1′ to H-16 in the ROESY spectrum suggested that the 3-*O*-methyl-*β*-glucopyranosyl residue linked to the steroidal aglycon at C-16 (Figure 1). The second sugar moiety was determined as 6-*O*-methyl-*β*-galactofuranosyl by analysis of ^{1}H-, ^{13}C-NMR, ^{1}H-^{1}H COSY, HSQC and HMBC spectra. The *β*-configuration of the glycosidic bond was confirmed by comparison of literature value (δ_C109.2 for *β*-*O*-methylgalactofuranoseand δ_C 103.1 for *α*-configuration) [3]. The cross-peak between H-1″ (δ_H 5.02) of the sugar and C-7 (δ_C 78.6) of the aglycon in the HMBC spectrum proved that the second sugar moiety attached to aglycon at C-7 (Figure 1). Thus, compound **1** was determined as anthenoside R by combination of all NMR, HR-MS data and by comparison with reported values [3].

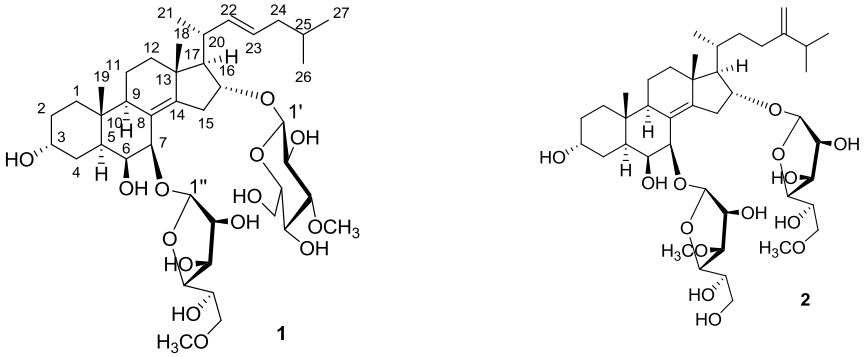

Figure 1. Chemical structure of compounds **1** and **2**.

Table 1. ^{1}H- and ^{13}C-NMR spectroscopic data for compounds **1**, **2** and reported values [3].

Position	**1**		**Anthenoside R** δ_C [a] [3]	**2**		**Anthenoside S** δ_C [a] [3]
	δ_H (ppm) (J in Hz)	δ_C (ppm)		δ_H(ppm) (J in Hz)	δ_C (ppm)	
1	1.53; m 1.31; m	34.6; CH_2	34.6; CH_2	1.53, m 1.29; m	34.5; CH_2	34.5; CH_2
2	1.61; m	29.7; CH_2	29.7; CH_2	1.61; m	29.6; CH_2	29.7; CH_2
3	4.07; m	67.5; CH	67.5; CH	4.07; m	67.5; CH	67.5; CH
4	1.96; td (14.0; 3.0) 1.37; dd (14.0; 3.0)	33.3; CH_2	33.3; CH_2	1.96, td (13.5; 2.5) 1.38; m	33.1; CH_2	33.1; CH_2
5	2.12; dt (14.0; 3.0)	38.0; CH	38.0; CH	2.12; dt (13.5; 2.5)	38.0; CH	38.0; CH
6	3.62; m	75.2; CH	75.2; CH	3.62; t (2.5)	75.2; CH	75.2; CH
7	4.23; d (2.5)	78.6; CH	78.6; CH	4.22; d (2.5)	78.3; CH	78.3; CH
8	2.26; m	126.6; C	126.7; C		127.0; C	127.0; C
9	1.64; m	45.9; CH	46.0; CH	2.26; m	45.8; CH	45.9; CH
10		38.9; C	38.9; C		38.8; C	38.8; C
11	1.65; m 1.53; m	19.4; CH_2	19.5; CH_2	1.65; m 1.53; m	19.5; CH_2	19.5; CH_2
12	1.81; dt (12.0; 3.2) 1.25; m	37.3; CH_2	37.2; CH_2	1.80; dt (12.5; 3.5) 1.25; m	37.1; CH_2	37.2; CH_2
13		45.3; C	45.4; C		44.9; C	44.9; C
14		147.6; C	147.6; C		147.2; C	147.2; C
15	2.86; ddd (17.5; 9.0; 3.0) 2.64; ddd (17.5; 5.0; 2.0)	34.4; CH_2	34.5; CH_2	2.87; ddd (17.0; 9.0, 3.0) 2.58; ddd (17.0; 6.0; 2.0)	33.3; CH_2	33.3; CH_2
16	4.62; td (9.0; 5.0)	79.6; CH	79.6; CH	4.45; td (9.0; 6.0)	76.9; CH	76.9; CH
17	1.53; (overlap)	62.8, CH	62.8, CH	1.45; dd (9.0; 4.0)	62.7, CH	62.8, CH
18	0.91; s	20.2; CH_3	20.2; CH_3	0.92; s	20.1; CH_3	20.1; CH_3
19	0.84; s	15.4; CH_3	15.4; CH_3	0.85; s	15.4; CH_3	15.4; CH_3
20	2.41; m	37.3; CH	37.3; CH	1.65; m	32.7; CH	32.8; CH
21	1.11; d (7.0)	24.1; CH_3	24.0; CH_3	1.04; d (7.0)	21.4; CH_3	21.4; CH_3
22	5.79; dd (15.0; 9.0)	138.1; CH	138.1; CH	1.79; m 1.42; m	33.5; CH_2	33.5; CH_2

23	5.34; dd (15.0; 7.0)	128.8; CH	128.8; CH	2.22; m 1.92; m	33.8; CH_2	33.8; CH_2
24	1.91; m	43.2; CH_2	43.2; CH_2		157.6; C	157.7; C
25	1.59; m	29.9; CH	29.9; CH	2.27; m	34.8; CH	34.8; CH
26	0.88; d (7.0)	22.9; CH_3	22.9; CH_3	1.04; d (7.0)	22.5; CH_3	22.5; CH_3
27	0.89; d (7.0)	22.8; CH_3	22.9; CH_3	1.04; d (7.0)	22.3; CH_3	22.3; CH_3
28				4.74; br s 4.71, br s	107.2; CH_2	107.2; CH_2
1′	4.33; d (8,0)	103.1; CH	103,1; CH	4.98 d (1.5)	108.5; CH	108.5; CH
2′	3.23; dd (9.0; 8.0)	75.2; CH	75.2; CH	3.92; dd (3.5; 1.5)	83.4; CH	83.4; CH
3′	3.09; t (9.0)	87.9; CH	87.9; CH	3.95; dd (6.0; 3.5)	78.8; CH	78.8; CH
4′	3.38; m	71.6; CH	71.6; CH	3.88; dd (6.0; 3.5)	84.9; CH	85.0; CH
5′	3.27; ddd (10.0; 5.5; 2.5)	77.7; CH_2	77.7; CH_2	3,85 dd (6.0; 3.5)	70.8; CH	70.8; CH
6′	3.88; dd (11.5; 2.5) 3.72; dd (11.5; 5.5)	63.2; CH	63.2; CH	3.53; d (5.5)	75.5; CH_2	75.6; CH_2
OMe	3.63; s	61.0; CH_3	61.0; CH_3	3.39; s	59.3; CH_3	59.3; CH_3
1″	5.02; d (2.0)	108.5; CH	108.5; CH	4.98; d (1.5)	108.1; CH	108.2; CH
2″	3.90; m	83.3; CH	83.3; CH	4.07; dd (3.0; 1.5)	81.2; CH	81.2; CH
3″	3.94; dd (6.0; 3.5)	78.8; CH	78.8; CH	3.74; dd (6.0; 3.0)	88.5; CH	88.6; CH
4″	3.90; m	85.1; CH	85.1; CH	3.97; dd (6.0; 3.0)	84.0; CH	84.0; CH
5″	3.83; ddd (7,0; 4.5; 2.0)	70.8; CH	70.8; CH	3.69; dd (6.0; 3.0)	73.1; CH	73.1; CH
6″	3.52; m	75.5; CH_2	75.5; CH_2	3.59; d (6.0)	65.2; CH_2	65.2; CH_2
OMe	3.38; s	59.4; CH_3	59.4; CH_3	3.40; s	58.1; CH_3	58.2; CH_3

[a]Assignment from 176.04 MHz.

Compound **2** was isolated as white amorphous powder. The molecular formula of **2** was found to be $C_{42}H_{70}O_{14}$ by the HR-ESI-MS at *m/z* 821.4652 $[M+Na]^+$. The ^1H- and ^{13}C-NMR spectra of **2** differed from those of **1** in signals of the steroidal side chain and the sugar moieties. The side chain of compound **2** contained a 24(28) double bond [δ_H4.74 and 4.71, δ_C 107.2 (C-28)

and 157.6 (C-24)] (Table 1). Furthermore, the ^{1}H- and ^{13}C-NMR spectra revealed the presence of two *O*-methylhexose moieties characterized by two anomeric protons at δ_H4.98 (H-1′ and H-1″) and the corresponding carbon signals at δ_C 108.5 (C-1′) and 108.1 (C-1″). Two methoxy groups [δ_H 3.39/δ_C 59.3 and δ_H 3.40/δ_C 58.1] were also observed in the ^{1}H- and ^{13}C-NMR spectra (Figure 1). The attachment positions of two *O*-methylhexose moieties were determined by the HMBC correlations of H-1′ (δ_H4.98) to C-16 (δ_C76.9) and H-1″(δ_H4.98) to C-7 (δ_C78.3). The protons and carbons chemical shifts of these sugars were agreed with those of 6-*O*-methyl-β-D-galactofuranosyl and 3-*O*-methyl-β-D-galactofuranosyl [4]. A combination of the NMR spectra 1 and 2D and comparison with reported valuesallowed confirming that compound **2** is Anthenoside S which was isolated previously from starfish *A. aspera* [3, 5].

4. CONCLUSIONS

From starfish *Anthenea sibogae*, collected in Bai Tu Long Bay, QuangNinh province, two diglycoside steroids, anthenoside R (**1**) and anthenoside S (**2**) were isolated from methanol extract by HPLC technique. Although these compounds had been known in other species but this is the first time they were isolated and identified from the starfish *Anthenea sibogae*.

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REFERENCES

1. Fresenius H. - Jahrbucher des NassauischanVereins fur Naturkunde, Wiesbaden, Verlag von J. F. Bergmann, 1915, pp. 21-38.
2. Kicha A. A., Ha D. T., Ivanchina N. V., Malyarenko T. V., Kalinovsky A. I., Dmitrenok P. S., Ermakova S. P., Malyarenko O. S., Hung N. A., Thuy T. T. T., Long P. Q. - Six new polyhydroxysteroidal glycosides, Anthenosides S1 - S6, from the starfish *Anthenea sibogae*. Chem. Biodivers. **15** (3): e1700553 (2018), doi: 10.1002/cbdv.201700553.
3. Malyarenko T. V., Kharchenko S. D., Kicha A. A., Ivanchina N. V., Dmitrenok P. S., Chingizova E. A., Pislyagin E. A., Evtushenko E. V., Antokhina T. I., Minh C. V, Stonik V. A. - Anthenoside L–U, steroidal glycoside with unusual structural features from the starfish *Anthenea aspera*, J. Nat. Prod. **79** (12) (2016) 3047-3056.
4. Vanderah D. J., Djerassi C. - Marine natural products - Synthesis of four naturally occurring 20. beta.-H cholanic acid derivatives, J. Org. Chem. **43** (7) (1978) 1442-1448.
5. Ma N., Tang H. F., Qiu F., Lin H. W., Tian X. R., Yao M. - Polyhydroxysteroidal glycosides from the starfish *Anthenea chinensis*, J. Nat. Prod. **73** (4) (2010) 590-597.