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A POLYHYDROXYLATED STEROL AND A SAPONIN ISOLATED FROM THE STARFISH *CULCITA NOVAEGUINEAE*

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Abstract

Using various chromatographic methods, a polyhydroxylated sterol 5*α*-cholestane-3*β*,6*β*,7*α*,8*β*,15*α*,16*β*,26-heptol (**1**) and an asterosaponin sodium salt of 6α-[(*O*-*β*-D-fucopyranosyl-(l→2)-*O*-*β*-D-galactopyranosyl-(l→4)-*O*-[*β*-D-quinovopyranosyl-(l→2)]-*O*-*β*-D-xylopyranosyl-(l→3)-*O*-*β*-D-quinovopyranosyl)oxy]-5*α*-pregn-9(11)-ene-20-one (**2**), were isolated from the methanol extract of the starfish *Culcita novaeguineae*. Their structures were elucidated by 1D and 2D-NMR experiments and comparison of their NMR data with reported values. Compounds **1** was isolated from *C. novaeguineae* for the first time.

Keywords. *Culcita novaeguineae*, Oreasteridae, starfish, polyhydroxylated sterol, asterosaponin.

1. INTRODUCTION

Starfish are invertebrates belonging to the class Asteroidea, phylum Echinodermata. The secondary metabolites from starfish are characterized by a diversity of polar steroids, including polyhydroxylated steroids and steroid glycosides. These compounds have exhibited a variety of biological activities, such as cytotoxic, hemolytic, and anti-microbial effects [1-5].

As a part of our ongoing investigations on Vietnamese starfish, we address herein the isolation and structure identification of a polyhydroxylated sterol and an asterosaponin from the starfish *Culcita novaeguineae*.

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 (Waldbronn, Germany). 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) 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 sample of the starfish *C. novaeguineae* Muller & Troschel, 1842 was collected at Quangninh, Vietnam, in October 2013, and identified by Prof. Do Cong Thung. A voucher specimen (DAB-DG-CN-01/2013) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST, Vietnam.

2.3. Isolation

The fresh body walls of *C. novaeguineae* (10 kg) were cut into small pieces and extracted in hot methanol (three times for 6h each) to afford a MeOH residue (125 g, A) after removal of the solvent under reduced pressure. This extract was partitioned between H_2O and CH_2Cl_2 (3×1.0 L) to give CH_2Cl_2

extract (C, 15.2 g) and water layer. The CH_2Cl_2 extract (C, 15.2 g) was separated by silica gel MPLC using gradient elution of CH_2Cl_2–MeOH (100:1–1:1, v/v) to obtain nine fractions, C1–C9. Fraction C-8 (2 g) was separated into six subfractions, C-8.1–C8.6, by YMC RP-18 MPLC using gradient elution of MeOH–H_2O (1:1–5/1, v/v). Further separation of subfraction C-8.4 (0.17 g) by silica gel CC eluting with EtOAc–MeOH–H_2O (10:1:0.1, v/v), followed by YMC CC with MeOH–H_2O (1.5:1, v/v) to obtain compound **1** (5.4 mg). The latter was passed through Diaion HP-20 CC eluting with increasing concentration of MeOH in water (0, 25, 50, 75, and 100%) to obtain four fractions, W1–W4, after removal of the fraction eluted with water. Fraction W4 (6.5 g) was separated into five subfractions, W4A–W4D, by silica gel MPLC using gradient elution of CH_2Cl_2–MeOH (20:1–1:1, v/v). Subfraction W4D (1.5 g) was further separated on YMC RP-18 CC eluting with MeOH–H_2O (1:1, v/v), followed by silica gel CC using CH_2Cl_2–MeOH–H_2O (3:1:0.15, v/v) as eluent furnished compound **2** (4.5 mg).

5*α*-cholestane-3*β*,6*β*,7*α*,8*β*,15*α*,16*β*,26-heptol (**1**): White powder; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see table 1; ESI-MS: *m/z* 507 $[M+Na]^+$ and 519 $[M+Cl]^-$ ($C_{27}H_{48}O_7$, M = 484).

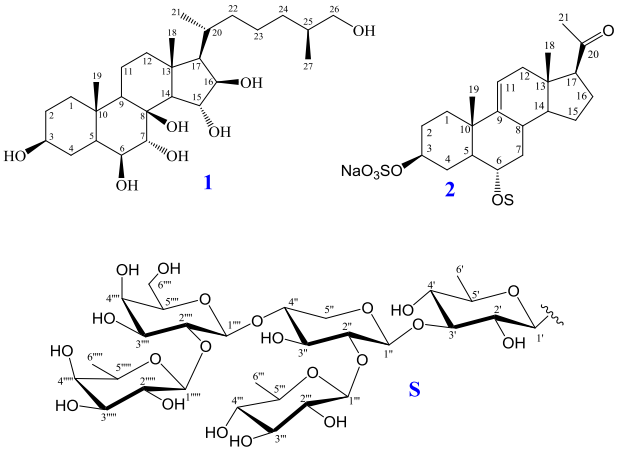

Figure 1: Chemical structures of **1** and **2**

Table 1: NMR data of **1** and reported compounds

C	$^a\delta_C$	$^b\delta_C$	δ_C ^{c,d}	δ_H ^{c,e} mult. (*J* in Hz)	**COSY** (H → H)	**HMBC** (H → C)
1	39.6	41.4	39.70	0.85 m/1.55 m	2	
2	31.5	31.7	30.80	1.32 m/1.60 m	1, 3	
3	72.3	72.5	69.99	3.40 m	2, 4	
4	32.3	35.8	35.09	1.35 m/1.65 m	3, 5	
5	44.5	42.8	40.64	1.40 m	4, 6	
6	68.9	**74.1**	**75.53**	3.53 br dd (3.0, 4.5)	5, 7	
7	76.5	**78.4**	**72.02**	3.67 t (3.0)	6	5, 9
8	77.7	77.8	76.53	-		
9	51.2	51.4	49.39	1.09 m	11	
10	37.8	36.4	34.78	-		
11	19.3	18.3	17.98	1.42 m/1.72 m	9, 12	
12	43.2	43.2	41.53	1.10 m/1.82 m	11	
13	45.5	45.6	43.66	-		
14	59.6	59.5	57.86	1.27 m	15	
15	79.3	80.1	78.25	3.98 m	14, 16	
16	82.7	82.8	80.18	3.82 dt (1.5, 7.0)	15, 17	13
17	61.4	61.6	59.61	1.08 m	16, 20	
18	16.6	16.8	16.19	1.03 s		12, 13, 14, 17
19	13.9	16.4	15.33	1.00 s		1, 5, 9, 10
20	30.6	30.6	28.74	1.77 m	17, 21, 22	
21	18.4	18.3	17.65	0.83 d (6.5)	20	17, 20, 22
22	37.1	37.0	35.32	0.96m/1.35 m	20, 23	
23	24.9	24.8	23.24	1.12 m/1.37 m	22, 24	
24	35.0	35.0	33.48	0.94 m/1.35 m	23, 25	
25	37.1	37.1	35.49	1.45 m	24, 26, 27	
26	68.5	69.6	66.25	3.15 m/3.24 m	25	14, 25, 27
27	17.4	17.2	17.00	0.81 d (6.5)	25	24, 25, 26
8-OH	-	-	-	4.07 s		7, 8, 14

$^a\delta_C$ of 5*α*-cholestane-3*β*,6*α*,7*α*,8*β*,15*α*,16*β*,26-heptol in CD_3OD [6], $^b\delta_C$ of 5*α*-cholestane-3*β*,6*β*,7*α*,8*β*,15*α*,16*β*,26-heptol in CD_3OD [7], ^{c}recorded in DMSO-d_6, ^{d}125 MHz, ^{e}500 MHz.

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

C	$^{a}\delta_C$	δ_C ^{b}	δ_H ^{b} mult. (*J* = Hz)	**C**	$^{a}\delta_C$	δ_C ^{b}	δ_H ^{b} mult. (*J* = Hz)
1	35.9	35.33	1.28 m/1.63 m	*Xyl*			
2	29.4	28.43	1.39 m/2.12 m	1″	104.4	102.65	4.53 d (7.0)
3	77.6	75.40	3.86 m	2″	82.2	82.69	3.35 ^{c}
4	30.7	29.57	1.08 m/2.37 m	3″	75.5	74.11	3.57 ^{c}
5	49.2	48.49	1.10 m	4″	79.2	77.17	3.60 ^{c}
6	80.2	78.06	3.47 m	5″	64.5	63.16	3.30/3.95 ^{c}
7	41.6	40.62	0.86 m/2.26 m	*Qui II*			
8	35.5	34.97	2.03 m	1‴	105.1	104.75	4.43 d (7.5)
9	146.0	145.78	-	2‴	75.4	74.99	3.07 dd (7.5, 9.0)
10	38.3	37.92	-	3‴	76.9	75.60	3.14 t (9.0)
11	116.0	115.57	5.31 d (4.5)	4‴	76.2	74.75	2.86 t (9.0)
12	40.6	40.09	2.20 m/2.28 m	5‴	73.9	72.17	3.22 ^{c}
13	42.5	41.99	-	6‴	18.5	17.40	1.18 d (6.0)
14	53.7	52.97	1.37 m	*Gal*			
15	23.1	22.38	1.58 m/2.03 m	1⁗	102.4	100.58	4.41 d (7.5)
16	25.6	25.01	1.20 m/1.73 m	2⁗	83.4	81.86	3.48 ^{c}
17	63.3	62.44	2.65 t (9.5)	3⁗	75.0	72.61	3.33 ^{c}
18	13.1	12.90	0.43 s	4⁗	69.0	67.48	3.67 br s
19	19.2	19.12	0.88 s	5⁗	76.8	75.34	3.42 ^{c}
20	208.3	208.75	-	6⁗	62.0	60.37	3.50 ^{c}
21	31.0	30.94	2.05 s	*Fuc*			
Qui I				1⁗′	107.2	105.73	4.21 d (7.0)
1′	105.1	102.85	4.31 d (8.0)	2⁗′	71.9	71.09	3.40 ^{c}
2′	74.1	73.17	3.13 ^{c}	3⁗′	75.0	73.21	3.51 ^{c}
3′	90.2	87.87	3.28 ^{c}	4⁗′	72.6	70.88	3.27 ^{c}
4′	74.5	73.08	2.91 t (9.0)	5⁗′	71.9	70.59	3.53 ^{c}
5′	71.9	70.59	3.27 ^{c}	6⁗′	17.2	16.76	1.12 d (6.0)
6′	17.9	17.96	1.14 d (6.0)				

$^{a}\delta_C$ of sodium salt of 6α-[(*O*-*β*-D-fucopyranosyl-(l→2)-*O*-*β*-D-galactopyranosyl-(l→4)-*O*-[*β*-D-quinovopyranosyl-(l→2)]-*O*-*β*-D-xylopyranosyl-(l→3)-*O*-*β*-D-quinovopyranosyl)oxy]-5*α*-pregn-9(11)-ene-20-one in pyridine-d_5 [8], ^{b}recorded in DMSO-d_6, ^{c}overlapped signals.

Sodium salt of 6α-[(*O*-*β*-D-fucopyranosyl-(l→2)-*O*-*β*-D-galactopyranosyl-(l→4)-*O*-[*β*-D-quinovopyranosyl-(l→2)]-*O*-*β*-D-xylopyranosyl-(l→3)-*O*-*β*-D-quinovopyranosyl)oxy]-5-*α*-pregn-9(11)-ene-20-one (**2**): White powder; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) see table 2; ESI-MS: *m/z* 1143 $[M–Na]^-$ ($C_{50}H_{79}NaO_{27}S$, M = 1166).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR features are typical for a polyhydroxylated sterol, one main constituent from starfish [1]. The ^{1}H-NMR spectrum revealed signals of two tertiary methyl [δ_H 1.03 (H-18) and 1.00 (H-19), each 3H, s] and two secondary methyl groups [δ_H 0.83 (H-21) and 0.81 (H-27), each 3H, d, *J* = 6.5 Hz]. In addition, five oxymethine groups [δ_C 69.99 (C-3), 75.65 (C-6), 72.02 (C-7), 78.25 (C-15), and 80.18 (C-16)/δ_H 3.40 (1H, m, H-3), 3.53 (1H, br dd, *J* = 3.0, 4.5 Hz, H-6), 3.67 (1H, t, *J* = 3.0 Hz, H-7), 3.98 (1H, m, H-15), and 3.82 (1H, dt, *J* = 1.5, 7.0 Hz, H-16)], one oxygenated quaternary carbon [δ_C 76.53 (C-8)], and one oxymethylene group [δ_C 66.25 (C-26)/δ_H 3.15 (1H, m, H_a-26) and 3.24 (1H, m, H_b-26)] were determined in the ^{1}H- and ^{13}C-NMR spectra of **1**. All ^{1}H-NMR data were assigned with

the relevant ^{13}C-NMR data by HSQC experiment and the results were shown in the table 1.

Analysis of ^{1}H-^{1}H COSY correlations led to identify the connectivities of H-1/H-2/H-3/H-4/H-5/H-6/H-7, H-9/H-11/H-12, H-15/H-16/H-17/H-20/H-22, H-23/H-24/H-25/H-26, H-20/H-21, and H-25/H-27. These data and the HMBC cross-peaks of H-18 (δ_H 1.03) with C-12 (δ_C 41.53), C-13 (δ_C 43.66), C-14 (δ_C 57.86), and C-17 (δ_C 59.61); H-19 (δ_H 1.00) with C-1 (δ_C 39.70), C-5 (δ_C 40.64), C-9 (δ_C 49.39), and C-10 (δ_C 34.78); and those of OH-8 (δ_H 4.07) with C-7 (δ_C 72.02), C-8 (δ_C 76.53)/C-14 (δ_C 57.86), clearly confirmed the planar structure of **1** (figure 2), which was also supported by comparison of the NMR data with the reported values [6, 7]. In the ROESY spectrum, the spatial proximities were observed between H-5 (δ_H 1.40) and H-3 (δ_H 3.40)/H-6 (δ_H 3.53), H-6 (δ_H 3.53) and OH-7 (δ_H 5.45), and H-16 (δ_H 3.82) and H-17 (δ_H 1.08) confirmed the *α*-orientation for H-3, H-6, OH-7, and H-16. Moreover, the correlations of H-15 (3.98) with H-7 (δ_H 3.67) and H-18 (δ_H 1.03), and those of OH-8 (δ_H 4.07) with H-7 (δ_H 3.67) and H-19 (δ_H 1.00) indicated *β*-orientation of H-7, OH-8, and H-15. Thus, compound **1** was elucidated as 5*α*-cholestane-3*β*,6*β*,7*α*,8*β*,15*α*,16*β*,26-heptol. This is the first report of **1** from the starfish *C. novaeguineae*. However, based on 2D-NMR experiments, the reported ^{13}C-NMR data at C-6 and C-7 [7] must be reversed as shown in the table 1.

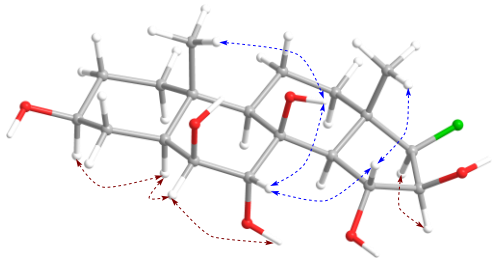

Figure 2: Key ROESY correlations of **1**

The NMR spectral data of **2** indicated an asterosaponin [2, 3] containing five sugar units. All carbon signals were assigned with relevant protons by mean of an HSQC experiment (Table 2). Comparison of the ^{13}C-NMR of **2** with published values and analysis of HMBC cross-peaks led to assignment of **2** as sodium salt of 6α-[(*O*-*β*-D-fucopyranosyl-(l→2)-*O*-*β*-D-galactopyranosyl-(l→4)-*O*-[*β*-D-quinovopyranosyl-(l→2)]-*O*-*β*-D-xylopyranosyl-(l→3)-*O*-*β*-D-quinovopyranosyl) oxy]-5*α*-pregn-9(11)-ene-20-one in pyridine-d_5 [8, 9]. The HMBC correlation of H-1′′′′′ (δ_H 4.21) with C-2′′′′ (δ_C 81.86), H-1′′′′ (δ_H 4.41) with C-4′′ (δ_C 77.17), H-1′′′ (δ_H 4.43) with C-2′′ (δ_C 82.69), and that of H-1′′ (δ_H 4.53) with C-3′ (δ_C 87.87) confirmed positions of all inner linkages. Moreover, anomeric proton H-1′ (δ_H 4.31) had an HMBC cross-peak with C-6 (δ_C 78.06) confirming the attachment of the pentaglycoside chain at C-3 (figure 3).

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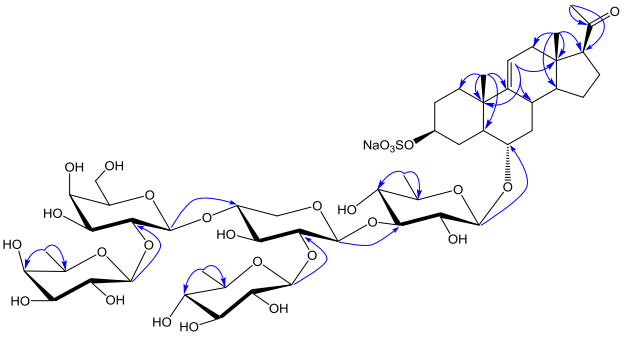

Figure 3: Key HMBC correlations of **2**

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