

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 6 α -[(*O*- β -D-fucopyranosyl-(1 \rightarrow 2)-*O*- β -D-galactopyranosyl-(1 \rightarrow 4)-*O*-[β -D-quinovopyranosyl-(1 \rightarrow 2)]-*O*- β -D-xylopyranosyl-(1 \rightarrow 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. Compound **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 Asterozoa, 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 ¹H-NMR (500 MHz) and ¹³C-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 μ m, Fuji Silysia Chemical Ltd.). Thin layer chromatography (TLC) used pre-coated silica gel 60 F₂₅₄ (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck). Compounds were visualized by spraying with aqueous 10 % H₂SO₄ 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 H₂O and CH₂Cl₂ (3 \times 1.0 L) to give CH₂Cl₂

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-H₂O (1:1-5/1, v/v). Further separation of subfraction C-8.4 (0.17 g) by silica gel CC eluting with EtOAc-MeOH-H₂O (10:1:0.1, v/v), followed by YMC CC with MeOH-H₂O (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-H₂O (1:1, v/v), followed by silica gel CC using CH_2Cl_2 -MeOH-H₂O (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; ¹H-NMR (500 MHz, DMSO-*d*₆) and ¹³C-NMR (125 MHz, DMSO-*d*₆) see table 1; ESI-MS: *m/z* 507 [M+Na]⁺ and 519 [M+Cl]⁻ (C₂₇H₄₈O₇, M = 484).

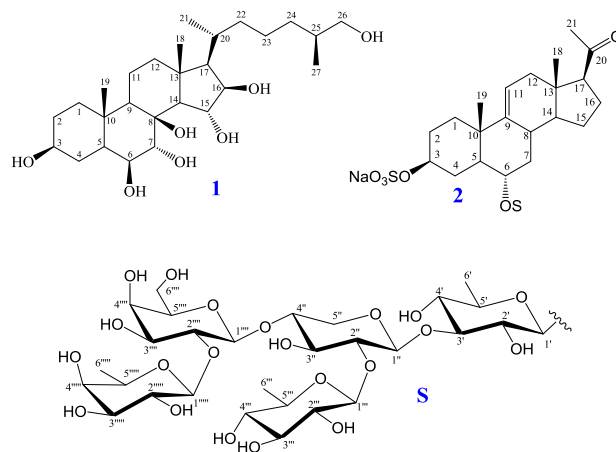


Figure 1: Chemical structures of **1** and **2**

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

C	^a δ _C	^b δ _C	^d δ _C ^{c,d}	^e 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

^aδ_C of 5 α -cholestane-3 β ,6 α ,7 α ,8 β ,15 α ,16 β ,26-heptol in CD₃OD [6], ^bδ_C of 5 α -cholestane-3 β ,6 β ,7 α ,8 β ,15 α ,16 β ,26-heptol in CD₃OD [7], ^crecorded in DMSO-*d*₆, ^d125 MHz, ^e500 MHz.

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

C	^a δ _C	δ _C ^b	δ _H ^b mult. (<i>J</i> = Hz)	C	^a δ _C	δ _C ^b	δ _H ^b mult. (<i>J</i> = Hz)
1	35.9	35.33	1.28 m/1.63 m	<i>Xyl</i>			
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	<i>Qui II</i>			
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	<i>Gal</i>			
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	<i>Fuc</i>			
<i>Qui I</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)				

^aδ_C of sodium salt of 6α-[(*O*-β-D-fucopyranosyl-(1→2))-*O*-β-D-galactopyranosyl-(1→4))-*O*-[β-D-quinovopyranosyl-(1→2)]-*O*-β-D-xylopyranosyl-(1→3))-*O*-β-D-quinovopyranosyl)oxy]-5α-pregn-9(11)-ene-20-one in pyridine-*d*₅ [8], ^brecorded in DMSO-*d*₆, ^coverlapped signals.

Sodium salt of 6α-[(*O*-β-D-fucopyranosyl-(1→2))-*O*-β-D-galactopyranosyl-(1→4))-*O*-[β-D-quinovopyranosyl-(1→2)]-*O*-β-D-xylopyranosyl-(1→3))-*O*-β-D-quinovopyranosyl)oxy]-5α-pregn-9(11)-ene-20-one (**2**): White powder; ¹H-NMR (500 MHz, DMSO-*d*₆) and ¹³C-NMR (125 MHz, DMSO-*d*₆) see table 2; ESI-MS: *m/z* 1143 [M-Na]⁻ (C₅₀H₇₉NaO₂₇S, 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 ¹H-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 ¹H- and ¹³C-NMR spectra of **1**. All ¹H-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 ^1H - ^1H 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 5α -cholestane- $3\beta,6\beta,7\alpha,8\beta,15\alpha,16\beta,26$ -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.

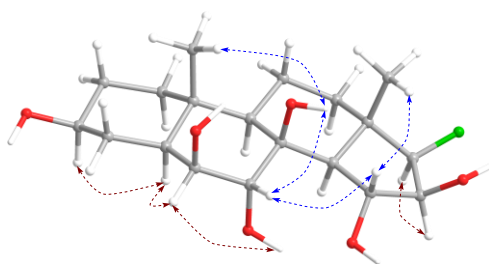


Figure 2: Key ROESY correlations of **1**

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-(1 \rightarrow 2)- O - β -D-galactopyranosyl-(1 \rightarrow 4)- O -[β -D-quinovopyranosyl-(1 \rightarrow 2)]- O - β -D-xylopyranosyl-(1 \rightarrow 3)- O - β -D-quinovopyranosyl)oxy]- 5α -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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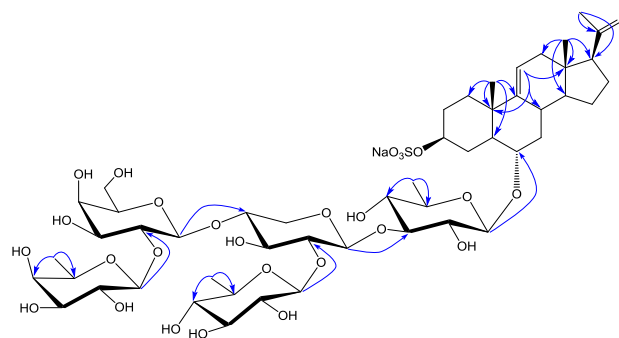


Figure 3: Key HMBC correlations of **2**

REFERENCES

1. G. Dong, T. Xu, B. Yang, X. Lin, X. Zhou, X. Yang, Y. Liu. *Chemical constituents and bioactivities of starfish*, Chem. Biodivers., **8**(5), 740-791 (2011).
2. M. Iorizzi, S. D. Marino, F. Zollo. *Steroidal oligoglycosides from the Asteroidea*, Curr. Org. Chem., **5**(9), 951-973 (2001).
3. N. V. Ivanchina, A. A. Kicha, V. A. Stonik. *Steroid glycosides from marine organisms*, Steroids, **76**(5), 425-454 (2011).
4. L. Minale, R. Riccio, F. Zollo. *Steroidal oligoglycosides and polyhydroxysteroids from echinoderms*, Fortschr. Chem. Org. Naturst., **62**, 75-308 (1993).
5. V. A. Stonik, N. V. Ivanchina, A. A. Kicha. *New polar steroids from starfish*, Nat. Prod. Commun., **3**, 1587-1610 (2008).
6. R. Riccio. *A novel group of highly hydroxylated steroids from the starfish Protoreaster nodosus*, Tetrahedron, **38**(24), 3615-3622 (1982).
7. I. Bruno, L. Minale, R. Riccio, S. La Barre, D. Laurent. *Isolation and structure of new polyhydroxylated sterols from a deep-water starfish of the genus Rosaster*, Gazz. Chim. Ital., **120**(7), 449-451 (1990).

8. Y. Itakura, T. Komori. *Biologically active glycosides from Asteroidea, IX. steroid oligoglycosides from the starfish Asterias amurensis [cf.] versicolor Sladen, 2. Structure elucidation of two new oligoglycoside sulfates, versicoside B and versicoside C*, Liebigs Annalen der Chemie, **1986(2)**, 359-373 (1986).
9. H. F. Tang, Y. H. Yi, L. Li, P. Sun, D. Z. Zhou, B. S. Liu. *A new asterosaponin from the starfish Culcita novaeguineae*, Chin. Chem. Lett., **16(5)**, 619-622 (2005).

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