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A New Asterosaponin from the Starfish *Culcita novaeguineae*

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Abstract: A new asterosaponin named novaeguinoside A, along with a known saponin, asteronyl pentaglycoside sulfate, was isolated from the starfish *Culcita novaeguineae*. The new compound was identified to be sodium 6α-O-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-5α-pregn-9(11)-en-20-one-3β-yl-sulfate by extensive spectral analysis and chemical evidence.

Keywords: Starfish, *Culcita novaeguineae*, steroidal glycoside, asterosaponin, novaeguinoside A.

Steroidal glycosides are the predominant metabolites of starfish and responsible for their general toxicity[1]. According to the chemical structures they have been subdivided into three main groups: asterosaponins, cyclic steroidal glycosides and glycosides of polyhydroxylated steroids. The first group are usually $\Delta^{9(11)}$-3β, 6α-dioxyl and a sulfate group at C_3 and the penta- or hexaglycosides at C_6[2]. Cushion star (*Culcita novaeguineae*) are abundant starfish distributed in South China Sea. Iorizzi and Kicha have reported the isolation of thirteen polyhydroxysteroid glycosides and five polyhydroxysteroids from the species[3-5]. However, no asterosaponin has been obtained up to date. In the course of search for new bioactive compounds from echinoderms under the guidance of *Pyricularia oryzae* bioassay[6], we found that an *n*-BuOH extract from *C. novaeguineae* collected in 2002 at Sanya Bay showed significant activitiy against *P. oryzae* mycelia. In this report, we described the identification of a new asterosaponin named novaeguinoside A (**1**) isolated from the *n*-BuOH extract of *C. novaeguineae*, along with a known saponin, asteronyl pentaglycoside sulfate (**2**), *i.e.* 6α-O-{β-D-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-5α-pregn-9(11)-en-20-one-3β-yl-sulfate[7].

Compound **1**, a white crystalline powder, mp 213-215°C, $[\alpha]_D^{20}$+6 (*c* 0.1, methanol), was positive to Liebermann-Burchard and Molish tests. The negative HRESI-MS and positive ESI-MS showed a quasi molecular ion peak at *m/z* 1127.4547 ($[M-Na]^-$, calcd. 1127.4580) and a quasi molecular ion peak at *m/z* 1173$[M+Na]^+$, respectively, indicating the molecular formula of $C_{50}H_{79}O_{26}SNa$. This was supported by the NMR data. The data of the ^1H, ^{13}C-NMR and DEPT spectra of **1** suggested the presence of a steroidal aglycon with a 9(11)-double bond [δ_C146.6 (s, C-9) and 115.9 (d, C-11); δ_H 5.09 (brs, H-11)], one sulfated oxomethine

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[δ_C77.6 (d, C-3); δ_H4.73 (m, H-3)], one oxomethine [δ_C80.3 (d, C-6); δ_H3.65 (m, H-6)], and one ketone carbonyl group (δ_C208.3, s, C-20). The assignments of the NMR signals associated with the aglycon moiety (**Table 1**) were derived from ^{1}H-^{1}H COSY, TOCSY, HMQC and HMBC experiments. Comparison of the NMR spectra of **1** with those of **2** indicated that these two glycosides possess the same aglycons, *i.e.* 6α-hydroxypregn-9(11)-en-20-one-3β-yl-sulfate (asterone sulfate). Glycosidation at C-6 was supported by the downfield shift of the C-6 signal in the ^{13}C-NMR spectrum with respect to asterosaponin aglycons of the 3β, 6α-dihydroxy oxidation pattern[8]. Because the chemical shift of C-19 is usually affected by the configuration of 6-OH, and the methyl signal at δ_C19.3 (C-19) in **1** was in good agreement with the natural and synthetic aglycons with 6α-OH[9], C-6 was confirmed as α configuration.

The presence of D-quinovose, D-xylose and D-fucose in a 2:1:2 ratio in **1** was established by acid hydrolysis with 2 mol/L CF_3COOH followed by GC-MS analysis of the corresponding aldononitrile peracetates[10]. The five anomeric carbons and protons in **1** was confirmed by its ^{13}C- and ^{1}H-NMR spectra at δ_C105.1, 104.5, 104.8, 102.0, 106.9 and δ_H4.76 (d, J=6.4Hz), 5.02 (d, J=7.2Hz), 5.04 (d, J=7.2Hz), 4.79 (2H, d, J=7.2Hz) (**Table 1**). Assignment of the sugar moieties was performed by the ^{1}H-^{1}H COSY, TOCSY, HMQC spectra combined with HMBC spectrum. The location of the inter-glycosidic linkages was deduced from the chemical shifts of QuiI C-3 (δ90.5), Xyl C-2 (δ81.9), Xyl C-4 (δ78.8) and FucI C-2 (δ82.8), which were downfield relative to shifts expected for the corresponding methyl glycopyranosides. Correlations from QuiI H-1 to C-6, Xyl H-1 to QuiI C-3, QuiII H-1 to Xyl C-2, FucI H-1 to Xyl C-4 and FucII H-1 to FucI C-2 in the HMBC spectrum, and cross-peaks between H-6 and QuiI H-1, Xyl H-1 and QuiI H-3, QuiII H-1 and Xyl H-2, FucI H-1 and Xyl H-4, FucII H-1 and FucI H-2 in the NOESY spectrum indicated that a pentasaccharide β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl moiety was located at C-6 of the aglycon.

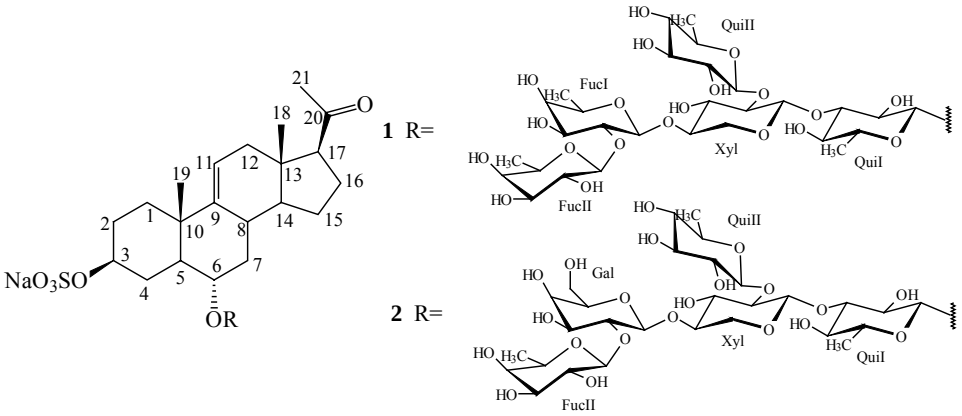
Hence, the structure of novaeguinoside A (**1**) was detemined as sodium 6α-O-{β-D-fucopyranosyl-(1→2)-β-D-fucopyranosyl-(1→4)-[β-D-quinovopyranosyl-(1→2)]-β-D-xylopyranosyl-(1→3)-β-D-quinovopyranosyl}-5α-pregn-9(11)-en-20-one-3β-yl-sulfate. Some authors have questioned whether the asterone glycosides are natural saponins[11], nevertheless, the others regarded these asterone glycosides as naturally occurring asterosaponins[1].

Table 1 ^{1}H(400MHz) and ^{13}C(100MHz) NMR data of novaeguinoside A (**1**) and asteronyl pentaglycoside sulfate (**2**) in C_5D_5N (δ in ppm, J in Hz)

Position	δ_C		Position	δ_H	δ_C
	1	**2**		**1**	
1	36.0 t	36.2 t	QuiI		
2	29.4 t	29.5 t	1	4.70[a] / 4.76 d (6.4)[c]	105.1
3	77.6 d	78.1 d	2	3.84 m	74.1
4	30.7 t	30.9 t	3	3.66 m	90.5
5	49.3 d	49.3 d	4	3.42 t (8.8)	74.5
6	80.3 d	80.1 d	5	3.54 m	72.0
7	41.4 t	41.6 t	6	1.46[b] / 1.52 d (6.0)[c]	18.4
8	35.6 d	35.8 d	Xyl		
9	146.6 s	146.4 s	1	4.87 d (7.2) / 5.02 d (7.2)[c]	104.5
10	38.3 s	38.6 s	2	3.94 m	81.9
11	115.9 d	116.3 d	3	4.02 m	75.6
12	40.6 t	40.8 t	4	3.96 m	78.8
13	42.5 s	42.7 s	5	4.35 brd (8.4), 3.65 m	64.5
14	53.7 d	54.0 d	QuiII		
15	23.3 t	23.5 t	1	5.16 d (6.4) / 5.04 d (7.2)[c]	104.8
16	25.5 t	25.8 t	2	3.91 m	76.2
17	63.4 d	63.7 d	3	3.93 m	76.8
18	13.1 q	13.4 q	4	3.89 m	75.5
19	19.3 q	19.5 q	5	3.56 m	73.6
20	208.3 s	209.6 s	6	1.64 d (6.0) / 1.74 d (6.0)[c]	17.8
21	30.9 q	31.4 q	FucI		
			1	4.75[a] / 4.79 d (7.2)[c]	102.0
			2	4.21 m	82.8
			3	3.98 m	74.9
			4	3.87 m	71.7
			5	3.67 m	71.6
			6	1.34 d (6.4) / 1.39 d (6.4)[c]	16.9
			FucII		
			1	4.74[a] / 4.79 d (7.2)[c]	106.9
			2	4.25 m	73.8
			3	3.86 m	75.0
			4	3.82 m	72.5
			5	3.51 m	71.9
			6	1.34 d (6.4) / 1.33 d (6.4)[c]	17.1

[a,b]overlapped with the signals of H_2O and H_3-21, respectively. [c]recorded in C_5D_5N/D_2O (4:1).

Figure 1 The structure of compound **1**



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