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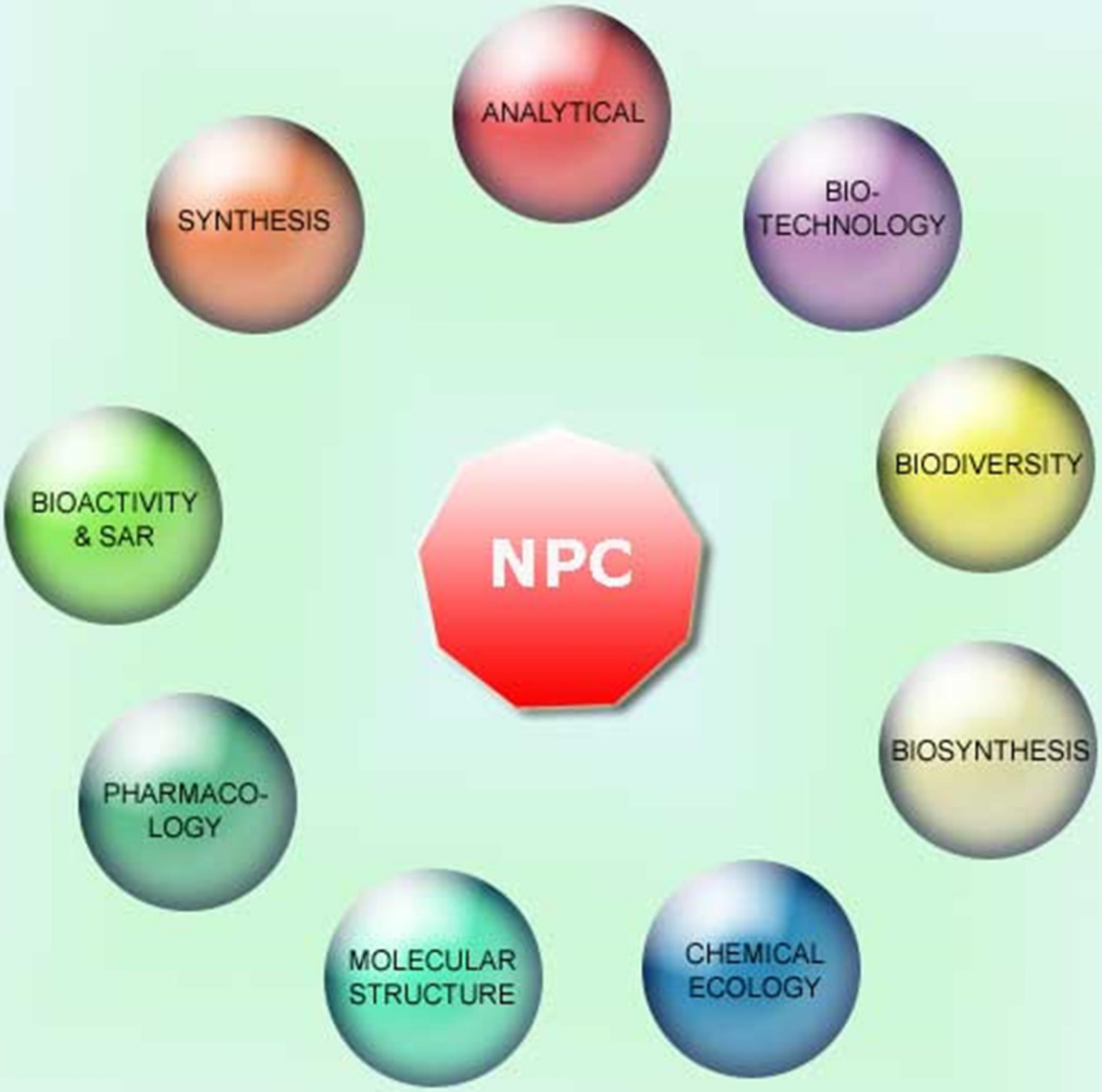

Volume 9. Issue 9. Pages 1223-1398. 2014
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
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NPC Natural Product Communications

2014
Vol. 9
No. 9
1269 - 1274

Three New Steroid Biglycosides, Plancisides A, B, and C, from the Starfish *Acanthaster planci*

Alla A. Kicha[a*], Thi H. Dinh[b], Natalia V. Ivanchina[a], Timofey V. Malyarenko[a], Anatoly I. Kalinovsky[a], Roman S. Popov[a], Svetlana P. Ermakova[a], Thi T. T. Tran[b] and Lan P. Doan[b]

[a]*G.B. Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Pr. 100 let Vladivostoku 159, Vladivostok, 690022, Russian Federation*

[b]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Nighiado, Caugiay, Hanoi, Vietnam*

kicha@piboc.dvo.ru

Received: June 20th, 2014; Accepted: July 2nd, 2014

Three new steroid biglycosides, plancisides A-C (**1-3**), were isolated from the ethanolic extract of the starfish *Acanthaster planci*. The structures of **1-3** were determined by extensive NMR and ESI-MS techniques, as (24*S*)-28-*O*-[β-D-galactofuranosyl-(1→5)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol (**1**), (24*S*)-28-*O*-[α-L-fucopyranosyl-(1→2)-3-*O*-methyl-β-D-xylopyranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol (**2**) and (24*S*)-28-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol 6-*O*-sulfate (**3**), respectively. Compound **2** is the first steroid glycoside containing an α-fucopyranose unit found from starfish. Compound **1** slightly inhibits cell proliferation of HCT-116, T-47D, and RPMI-7951 cancer cell lines, but has no effect on colony formation of these cells in a soft agar clonogenic assay.

Keywords: Steroids, Glycosides, Starfish, *Acanthaster planci*, Proliferation, Clonogenic assay.

Starfish are a rich source for the discovery of marine polar steroid glycosides with new structures. Besides rare glycosides with cyclic carbohydrate chains, starfish contain two main structural groups of steroid glycosides, namely asterosaponins and glycosylated polyhydroxysteroids [1–6]. Asterosaponins are steroid oligoglycosides containing 3-*O*-sulfated $\Delta^{9(11)}$-3β,6α-dihydroxysteroid aglycons and carbohydrate chains with usually five or six sugars attached to C-6. Starfish glycosylated polyhydroxysteroids have highly oxygenated steroid aglycons with the number of hydroxyl groups from three to nine, and one, two and rarely three monosaccharide residues attached to C-3 (or C-15) in a steroid nucleus and/or to C-24, or C-26, C-28, C-29 in the side chain. Starfish polar steroid glycosides have been reported to show a wide spectrum of biological activities, including hemolytic, cytotoxic, antiviral, antibacterial, antibiofouling, neuritogenic, and antifungal effects [1–6].

The crown-of-thorns starfish, *Acanthaster planci* Linnaeus, 1758 (order Valvatida, family Acanthasteridae) is found throughout the Indo-Pacific region. This species is one of the largest sea stars in the world. The adult *A. planci* is a carnivorous predator that usually preys on reef coral polyps. The toxic properties of the crown-of-thorns spines proved to be partly due to the presence of polar steroid glycosides, mainly asterosaponins, in the starfish tissues [1]. The polar steroid compounds from *A. planci* have been studied previously by Japanese and Italian research groups. Thornasteroside A, a major steroid glycoside from *A. planci*, is the first asterosaponin whose structure was fully elucidated by Kitagawa and Kobayashi [7]. Later Itakura and Komori *et al.* carried out the isolation and structural study of six asterosaponins: acanthaglycosides A–F and the known marthasteroside A_1 [8,9], whereas Pizza *et al.* encountered in this species three polyhydroxysteroid biglycosides: the known nodososide, isonodososide, and 5-deoxyisonodososide [10].

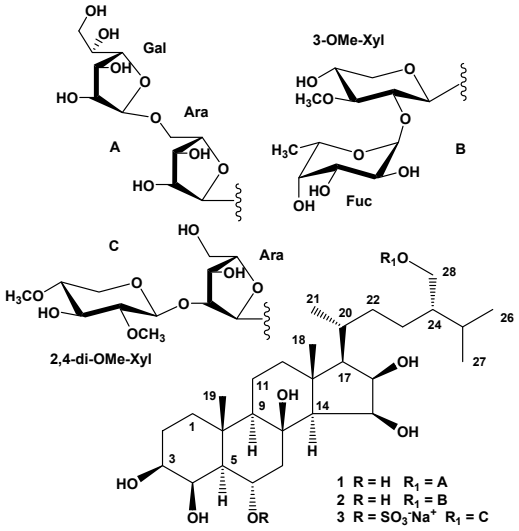

Figure 1: The structures of **1-3**.

Herein, we report the results of studies on the polyhydroxysteroid glycoside fraction from the ethanolic extract of the starfish *A. planci*, collected from Van Phong Bay near Nha Trang (Khanh Hoa province, Vietnam), and describe the structures of three new steroid biglycosides, named as plancisides A–C (**1**–**3**).

The concentrated ethanol extract of *A. planci* was partitioned with *n*-hexane, chloroform, and methanol. The methanol extract was subjected to sequential separation by chromatography on columns of Polychrom-1, Si gel, and Florisil, followed by HPLC on semi-preparative Diasfer-110-C18 and Discovery C18 columns to give three new polyhydroxysteroid glycosides, named as plancisides A–C (**1**–**3**) (Figure 1).

Table 1: ^{1}H and ^{13}C NMR data, and HMBC and NOESY correlations of compound **1**[a] in CD_3OD (δ in ppm, *J* in Hz).

position	δ_C	δ_H	HMBC	NOESY
1	39.7	1.70 m	C3, C5	H19
		0.97 m		
2	26.2	1.82 m		H19
		1.54 m		
3	73.7	3.42 m		H5
4	69.1	4.26 br s	C2, C10	
5	57.2	0.94 m	C6, C7, C10, C19	H3, H7
6	64.8	4.18 dt (4.5, 11.0)		H19
7	50.1	2.46 dd (4.4, 12.2)	C5, C6, C8, C9	
		1.35 t (11.8)		H5, H9, H14
8	77.1			
9	58.4	0.82 dd (3.2, 12.4)		H7, H14
10	38.1			
11	18.9	1.76 m		H18
		1.40 m		
12	43.4	1.94 m	C9, C14	H18
		1.11 m		H21
13	44.5			
14	61.2	1.01 d (5.6)	C8, C9, C12, C13, C15, C17	H7, H9
15	71.2	4.37 dd (5.6, 7.0)	C13, C16, C17	
16	72.8	4.20 t (7.0)	C14, C15	
17	62.8	0.96 m		
18	17.9	1.23 s	C12, C13, C14, C17	H11, H12, H21
19	17.0	1.15 s	C1, C5, C9, C10	H1, H2, H6
20	31.4	1.90 m		
21	18.6	0.95 d (6.6)	C17, C20, C22	H18, H12
22	34.8	1.75 m		
		1.11 m		
23	26.0	1.47 m		H27
		1.20 m		
24	45.9	1.40 m	C26, C27	H27
25	29.5	1.83 m	C24, C26, C27	
26	20.0	0.91 d (6.6)	C24, C25, C27	
27	19.9	0.89 d (6.6)	C24, C25, C26	H23, H24, H28
28	70.2	3.71 m	C23, C24, C25	H1′, H27
		3.32 m	C23, C24, C25	H1′, H27
Ara				
1′	109.7	4.82 d (1.7)	C28, C3′, C4′	H28
2′	83.8	3.95 dd (1.7, 3.9)	C3′	
3′	79.1	3.89 dd (3.8, 6.5)	C4′, C5′	
4′	83.4	4.01 m		
5′	68.0	3.83 dd (5.1, 11.2)	C3′, C4′	H1″
		3.66 dd (3.7, 11.2)	C3′, C4′	H1″
Gal				
1″	109.6	4.94 d (1.5)	C5′, C2″, C3″, C4″	H5′
2″	83.0	3.98 m		
3″	78.9	3.99 m		
4″	84.8	3.98 m		
5″	72.5	3.71 m	C6″	
6″	64.4	3.63 dd (5.8, 11.3)	C4″, C5″	
		3.62 dd (6.8, 11.1)		

[a] – Assignments from ^{1}H-700.13 MHz, ^{13}C-176.04 MHz, ^{1}H-^{1}H COSY, 1D TOCSY, HMBC, NOESY (270 msec), and H2BC data.

The molecular formula of planciside A (**1**) was determined to be $C_{39}H_{68}O_{16}$ from the $[M + Na]^+$ sodiated-molecular ion peak at *m/z* 815.4410 in the (+)-HR ESI-MS and the $[M - H]^-$ molecular anion peak at *m/z* 791.4444 in the (–)-HR ESI-MS. The ^{1}H, ^{13}C and DEPT NMR spectra of **1** showed the presence of 39 carbon atoms, including 5 methyl groups, 10 methylenes, 21 methines, 2 quaternary carbons, and 1 oxygenated tertiary carbon. The ^{1}H NMR spectrum of **1** indicated two resonances in the downfield region due to anomeric protons at δ_H 4.82 (*J* = 1.7 Hz) and 4.94 (*J* = 1.5 Hz) that correlated in the HSQC experiment with carbon signals at δ_C 109.7 and 109.6, respectively. The (+)-ESI-MS/MS of the ion $[M + Na]^+$ at *m/z* 815 contained the fragment ion peaks corresponding to the loss of hexose at *m/z* 653 $[(M + Na) - C_6H_{10}O_5]^+$, the simultaneous loss of hexose and pentose at *m/z* 521 $[(M + Na) - C_6H_{10}O_5 - C_5H_8O_4]^+$, and a disaccharide chain at *m/z* 317 $[C_6H_{10}O_5 + C_5H_8O_4 + Na]^+$. Accordingly, the (–)-ESI-MS/MS of the ion $[M - H]^-$ at *m/z* 791 exhibited the fragment ion peaks obtained due to the loss of hexose at *m/z* 629 $[(M - H) - C_6H_{10}O_5]^-$ and the simultaneous loss of hexose and pentose at *m/z* 497 $[(M - H) - C_6H_{10}O_5 - C_5H_8O_4]^-$. Along with NMR spectra, these data revealed the existence of two monosaccharide residues, one hexose and one pentose, and a heptahydroxysubstituted 24-methylcholestane moiety in **1**. The ^{1}H–^{1}H COSY and HSQC cross-peaks confirmed the corresponding sequences of protons at C-1 to C-7, C-9 to C-12 through C-11, C-14 to C-17, C-17 to C-20, C-20 to C-21, C-20 to C-22 and to the end of the side chain, and C-24 to C-28. The HMBC correlations supported the total structure of the steroid moiety of **1** (Table 1). The key NOESY cross peaks, such as H-3/H-5; H_{ax}-7/H-5, H-9, H-14; H_3-18/H_{ax}-11; and H_3-19/H_{ax}-2, H-6, confirmed the 5α-cholestane stereochemistry of the steroid nucleus of **1**. Analysis of the found carbon and proton chemical shifts and the corresponding coupling constants of the aglycon part of **1** in the ^{1}H and ^{13}C NMR spectra allowed us to suggest the 3β,4β,6α,8,15β,16β-hexahydroxysubstituted steroid nucleus and 28-*O*-hydroxylated 24-methylcholestane side chain [11,12].

Irradiation of anomeric protons in the 1D TOCSY spectra gave the chemical shifts and coupling constants of H-1–H-6 of a galactose residue and of H-1–H-5 of an arabinose residue. ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY experiments led to the assignment of all the proton and carbon signals of the carbohydrate chain of **1**, except for two carbon chemical shifts at δ_C 83.0 and 84.8 of the galactose residue, which correlated in the HSQC spectrum with one multiplet at δ_H 3.98 of the protons H-2″ and H-4″. A H2BC procedure was used to assign chemical shifts of these carbons. Based on the presence of the H2BC correlation of anomeric proton H-1″ at δ_H 4.94 with the neighboring carbon C-2″, the value of its chemical shift was found as δ_C 83.0 and the value of the chemical shift of C-4″ was determined as δ_C 84.8. The obtained NMR spectral data of the oligosaccharide moiety of **1** (Table 1) strictly coincided with those of terminal β-D-galactofuranosyl and internal 5-*O*-substituted α-L-arabinofuranosyl residues in the earlier reported NMR spectra of methyl β-D-galactofuranoside and previously known starfish glycosides [13–15]. The attachment of the carbohydrate chain to the steroid aglycon and the position of the interglycosidic linkage were deduced from long-range correlations in the NOESY and HMBC spectra. There were the cross-peaks between H-1′ of Ara_f and H_2-28 (C-28) of the aglycon, and H-1″ of Gal_f and H-5′ (C-5′) of Ara_f. The (20*R*)-configuration was assumed on the basis of the NOESY correlations of H_3-18/H_3-21 and H_3-21/H_{eq}-12 [16], as well as the chemical shift of H_3-21 at δ_H 0.95 (more than δ_H 0.90 for 20*R*-steroids with saturated side chain [17]). Acid hydrolysis of **1** with aqueous 2 M CF_3COOH gave a mixture of steroid derivatives, from which the individual aglycon **1a** was isolated by HPLC on a Ascentis RP-Amide column. The stereochemistry of C-24 was determined as *S* because, in the ^{1}H NMR spectrum of **1a**, the protons H_2-28 appeared as two well separated signals at δ_H 3.52 (dd, *J* = 6.3, 11.0 Hz) and 3.54 (*J* = 6.1, 11.0 Hz), and the protons H_3-27 at δ_H 0.870 (*J* = 6.7 Hz) and H_3-26 at δ_H 0.874 (*J* = 6.7 Hz) had close chemical shifts, whereas, in the case of the 24*R* epimer, the protons H_2-28 resonated as a broad doublet at δ_H 3.52 (br d, *J* = 5.0 Hz) and the H_3-26 and H_3-27 signals appeared more separated [1].

We presumed the L-series for the α-arabinose unit and D-series for the β-galactose unit in the carbohydrate moiety of **1** by analogy with steroid glycosides isolated previously from *A. planci* and other species of starfish [1–9,14]. Hence, the structure of planciside A was elucidated as (24*S*)-28-*O*-[β-D-galactofuranosyl-(1→5)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol (**1**). The terminal β-D-galactofuranose residue and the glycosidic bond (1→5) are very rare in starfish polyhydroxysteroid glycosides. Moreover, the disaccharide chain β-D-Gal_f-(1→5)-α-L-Ara_f is found in this group of steroid metabolites for the first time.

Table 2: ^{1}H and ^{13}C NMR data, NOESY (or ROESY) correlations[a] of compounds **2** and **3** in CD_3OD (δ in ppm, *J* in Hz).

Position	**2**			**3**		
	δ_C	δ_H	NOESY	δ_C	δ_H	ROESY
1	39.6	1.70 m		39.4	1.72 m	
		0.97 m			1.00 m	H9
2	26.2	1.82 m		26.5	1.82 m	
		1.55 m			1.57 m	
3	73.7	3.42 m	H5	72.9	3.45 m	H5
4	69.1	4.26 br s		68.9	4.30 br s	
5	57.2	0.94 m	H3, H7	56.0	1.13 m	H3, H7, H9
6	64.8	4.18 dt (4.5, 11.0)	H19	74.5	4.92 dt (4.4, 11.1)	H19
7	50.0	2.46 dd (4.6, 12.0)		47.9	2.72 dd (4.4, 12.2)	H15
		1.35 t (12.2)	H5, H9, H14		1.54 t (12.0)	H5, H9, H14
8	77.1			77.2		
9	58.4	0.82 dd (3.2, 12.4)	H7, H14	58.1	0.84 dd (3.0, 12.5)	H1, H5, H7, H12, H14
10	38.2			38.7		
11	18.9	1.76 m		18.8	1.76 m	H18, H19
		1.41 m			1.40 m	
12	43.4	1.94 m	H18, H21	43.4	1.94 m	H21
		1.11 m	H17		1.11 m	H9, H21
13	44.5			44.6		
14	61.2	1.01 d (5.7)	H7, H9	61.0	1.03 d (5.6)	H7, H9
15	71.2	4.38 dd (5.6, 7.1)		71.1	4.38 dd (5.6, 6.8)	H7
16	72.8	4.20 t (7.1)	H22	72.9	4.20 t (6.8)	H22
17	63.0	0.94 m	H12	62.9	0.94 m	
18	17.9	1.23 s	H12, H20	17.9	1.23 s	H11, H20
19	17.0	1.15 s	H6	16.9	1.23 s	H6, H11
20	31.5	1.90 m	H18	31.4	1.91 m	H18
21	18.6	0.94 d (6.5)	H12	18.6	0.95 d (6.8)	H12, H23
22	35.0	1.73 m	H16	34.8	1.75 m	H16
		1.08 m			1.12 m	H24
23	26.6	1.46 m	H27	26.1	1.46 m	H21
		1.10 m			1.24 m	
24	46.3	1.37 m	H27, H28	45.9	1.40 m	H22, H28
25	28.9	1.86 m		29.8	1.83 m	
26	20.1	0.89 d (6.8)	H28	20.1	0.89 d (7.0)	H28
27	19.2	0.87 d (6.8)	H23, H24, H28	19.8	0.91 d (7.0)	
28	71.6	3.78 dd (6.5, 9.5)	H1′, H24, H26, H27	70.2	3.72 dd (5.8, 9.6)	H1′, H24, H26
		3.42 m	H24, H26, H27		3.28 m	H1′
		3-OMe-Xyl			**Ara**	
1′	103.8	4.31 d (7.2)	H28, H3′, H5′	108.3	4.96 d (1.5)	H28
2′	76.3	3.42 dd (7.1, 8.6)		91.9	4.05 dd (1.5, 4.0)	H1″
3′	88.2	3.27 t (8.5)	H1′	77.6	3.95 dd (4.0, 7.6)	
4′	71.7	3.57 m		84.0	3.88 m	
5′	66.7	3.82 dd (5.7, 11.7)		62.7	3.76 dd (3.1, 12.1)	
		3.20 dd (9.8, 11.5)	H1′		3.62 dd (5.7, 12.1)	
OMe	60.8	3.61 s	H3′, H1″			
		Fuc			**2,4-di-OMe-Xyl**	
1″	100.1	5.21 d (3.0)	H2′, OMe	104.8	4.41 d (7.6)	H2′, H3″, H5″
2″	70.2	3.70 dd (3.3, 10.1)		84.8	2.86 dd (7.6, 9.0)	
3″	71.7	3.72 dd (2.9, 10.0)	H5″	76.5	3.38 t (9.0)	H1″
4″	73.8	3.65 br s	H5″, H6″	80.9	3.17 ddd (5.0, 8.7, 10.0)	
5″	67.6	4.33 br q (6.7)	H3″, H4″	64.5	4.01 dd (5.1, 11.3)	
					3.12 dd (10.0, 11.3)	H1″
6″	16.8	1.17 d (6.6)	H4″			
2″-OMe				61.2	3.55 s	
4″-OMe				59.0	3.45 s	

[a] – Assignments from ^{1}H-700.13 MHz, ^{13}C-176.04 MHz, ^{1}H-^{1}H COSY, 1D TOCSY, HMBC, and NOESY (or ROESY) data.

The molecular formula of planciside B (**2**) was determined to be $C_{40}H_{70}O_{15}$ from the $[M + Na]^+$ sodiated-molecular ion peak at *m/z* 813.4580 in the (+)-HR ESI-MS and the $[M – H]^–$ molecular anion peak at *m/z* 789.4651 in the (–)-HR ESI-MS. The fragment ion peaks at *m/z* 667 $[(M + Na) – C_6H_{10}O_4]^+$ and 521 $[(M + Na) – C_6H_{10}O_4 – C_6H_{10}O_4]^+$ in the (+)-ESI-MS/MS of the ion at *m/z* 813 $[M + Na]^+$ and the fragment ion peaks at *m/z* 643 $[(M – H) – C_6H_{10}O_4]^–$ and 497 $[(M – H) – C_6H_{10}O_4 – C_6H_{10}O_4]^–$ in the (–)-ESI-MS/MS of the ion at *m/z* 789 $[M – H]^–$ corresponded to the successive loss of deoxyhexose and *O*-methyl-pentose units. The detailed comparison of the ^{1}H and ^{13}C NMR data of **2** with those of co-occurring **1** clearly indicated that glycoside **2** contained the same 24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol as aglycon and differed from compound **1** only in the structure of the disaccharide chain (Tables 1 and 2).

The ^{1}H NMR spectrum of **2** exhibited two resonances in the downfield region due to anomeric protons at δ_H 4.31 (*J* = 7.2 Hz) and 5.21 (*J* = 3.0 Hz) that correlated in the HSQC experiment with carbon signals at δ_C 103.8 and 100.1, respectively. The presence of one 6-deoxyhexose unit was supported by the methyl doublet at δ_H 1.17. The irradiation of anomeric protons in the 1D TOCSY experiments gave the chemical shifts and coupling constants of H-1′–H-5′ of an *O*-methyl-pentose residue and of H-1″–H-4″ of a 6-deoxyhexose residue, whereas irradiation of the resonance of the corresponding methyl group H_3-6″ gave the signal of H-5″ of a 6-deoxyhexose residue. The application of 2D NMR experiments allowed the assignment of all chemical shifts that were attributable to the disaccharide part of **2** (Table 2). A HMBC cross-peak between H-3′/OMe of the *O*-methyl-pentose showed that the *O*-methyl group was at the sugar C-3′ position. The carbon and proton signals and the corresponding coupling constants of the *O*-methyl-pentose unit were similar to those of a nonsubstituted 3-*O*-methyl-β-xylopyranosyl residue, except for the signals C-2′ and H-2′ that were downfield shifted from δ_C 74.5 to 76.3 and from δ_H 3.23 to 3.42 due to the effect of glycosylation at sugar C-2′ position [18]. The small *J* value between H-1″/H-2″ (3.0 Hz) of the 6-deoxyhexose residue indicated an equatorial orientation for H-1″; the large *J* value between H-2″/H-3″ (10.0 Hz) showed an axial orientation for both protons, and the small *J* value between H-3″/H-

4″ (2.9 Hz) exhibited an equatorial orientation for H-4″. These data, together with the NOESY cross-peak H-3″/H-5″, allowed us to suggest that the second monosaccharide is α-fucopyranose. Indeed, ^{13}C NMR data of this monosaccharide residue coincided well with those already reported for a terminal α-fucopyranose unit [20]. The position of the interglycosidic linkage and the attachment of the carbohydrate chain to the aglycon were determined from the NOESY and HMBC spectra, where cross-peaks between H-1′ of 3-OMe-Xyl$_p$ and H$_2$-28 (C-28) of the aglycon, and H-1″ of Fuc$_p$ and H-2′ (C-2′) of 3-*O*-Me-Xyl$_p$ were detected. Thus, the structure of planciside B was defined as **2**. This is the first record of the α-fucose residue in starfish steroid glycosides. The β-D-fucose unit is generally present in this class of metabolites. Earlier, the α-L-fucopyranose residue was found in steroid glycosides from some other marine sources, such as soft corals and marine sponges [19–21]. Therefore, by analogy with these steroid glycosides, we assumed the L-series for the α-fucopyranose unit in **2**. The D-series of the 3-*O*-methyl-β-xylopyranose residue in **2** was preferred as the β-D-xylopyranose, and *O*-methyl- or di-*O*-methyl-β-D-xylopyranose residues are the most often encountered ones among starfish steroid glycosides [1–6].

The molecular formula of planciside C (**3**) was determined to be $C_{40}H_{69}O_{18}SNa$ from the $[M + Na]^+$ sodiated-molecular ion peak at *m/z* 915.4013 in the (+)-HR ESI-MS and the $[M - Na]^-$ molecular anion peak at *m/z* 869.4226 in the (–)-HR ESI-MS. The fragment ion peaks at *m/z* 795 $[(M + Na) - NaHSO_4]^+$ in the (+)-ESI-MS/MS of the ion at *m/z* 915 $[M + Na]^+$ and at *m/z* 97 $[HSO_4]^-$ in the (–)-ESI-MS/MS of the ion at *m/z* 869 showed the presence of a sulfate group in **3**. The fragment ion peaks at *m/z* 635 $[(M + Na) - NaHSO_4 - C_7H_{12}O_4]^+$, 503 $[(M + Na) - NaHSO_4 - C_7H_{12}O_4 - C_5H_8O_4]^+$, and 315 $[C_7H_{12}O_4 + C_5H_8O_4 + Na]^+$ in the (+)-ESI-MS/MS of the ion at *m/z* 915 $[M + Na]^+$ and the fragment ion peaks at *m/z* 709 $[(M - Na) - C_7H_{12}O_4]^-$ and 577 $[(M - Na) - C_7H_{12}O_4 - C_5H_8O_4]^-$ in the (–)-ESI-MS/MS of the ion at *m/z* 869 corresponded to the successive loss of di-*O*-methyl-pentose and pentose units. A detailed comparison of the ^{1}H and ^{13}C NMR data of **3** with those observed in co-occurring **1** clearly indicated that glycoside **3** contained the same 28-*O*-glycosylated 3β,4β,6α,8,15β,16β,28-heptahydroxy-substituted 24-methyl-5α-cholestane aglycon, which differed from the aglycon of **1** only in the presence an additional sulfate group at C-6 (Tables 1 and 2). So, downfield shifts of the signals H-6 from δ_H 4.26 to δ_H 4.92 and C-6 from δ_C 69.1 to δ_C 74.5 in the NMR spectra of compounds **1** and **3**, respectively, indicated the C-6 position of the sulfate group in **3**. All proton and carbon resonances of **3** were derived from ^{1}H–^{1}H COSY, HSQC, HMBC, and ROESY experiments and established the structure of the aglycon and carbohydrate moieties. The found proton and carbon signals of the terminal monosaccharide residue of **3** coincided well with those of the 2,4-di-*O*-methyl-β-D-xylopyranosyl unit, and the spectral data of the internal monosaccharide residue corresponded well with those of the 2-substituted α-L-arabinofuranosyl unit reported for other starfish steroid glycosides [22,23]. The presence of the cross-peaks in the ROESY and HMBC spectra between H-1′ of Ara$_f$ and H$_2$-28 (C-28) of the aglycon and H-1″ of 2,4-di-*O*-Me-Xyl$_p$ and H-2′ (C-2′) of Ara$_f$ confirmed a (1→2) interglycosidic linkage and attachment of the carbohydrate chain at C-28. Thus, the structure of planciside C was determined as **3**.

The *in vitro* cytotoxicity of glycoside **1** against human colon cancer HCT-116, breast cancer T-47D, and melanoma RPMI-7951 cell lines was evaluated by the MTS method. As a positive control, we used cisplatin. Both glycoside **1** and cisplatin were non-toxic up to 120 μM against T-47D cells. Glycoside **1** exhibited a moderate cytotoxicity against HCT-116 and RPMI-7951 cells with IC_{50} = 36 and 58 μM, respectively, while cisplatin demonstrated similar cytotoxic effects against HCT-116 and RPMI-7951 cells with IC_{50} = 75 and 43 μM, respectively.

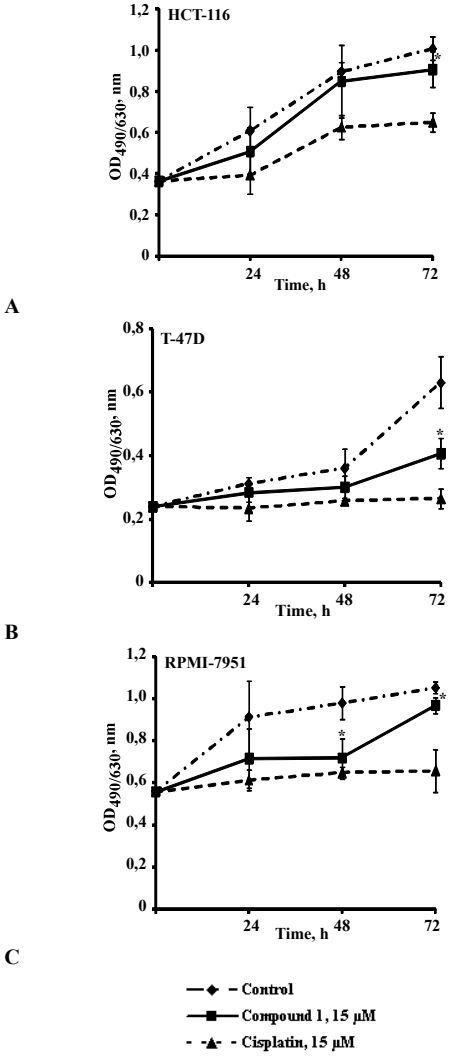

Figure 2: The time-dependent cell proliferation of HCT-116 (A), T-47D (B), and RPMI-7951 (C) cells. The CellTiter 96 AQueous One Solution cell proliferation assay kit was used to assess cell proliferation at 24, 48, and 72 h of culture. The cells were treated with either compound **1** (15 μM) or cisplatin (15 μM) for the indicated time. The absorbance is directly proportional to the number of living cells. Each bar indicates the mean ± S.D. of values obtained from triplicate experiments. The significant differences were evaluated using Student's t test. *, $p < 0.05$.

Next, we examined the effect of compound **1** and cisplatin at a non-cytotoxic concentration of 15 μM on the proliferation of HCT-116, T-47D, and RPMI-7951 cell lines (Figure 2). Figure 2A illustrates that glycoside **1** slightly inhibited the growth of HCT-116 cells compared with cisplatin. Compound **1** inhibited the proliferation of T-47D cells after 72 h by 35 %, and the proliferation of RPMI-7951 cells after 48 h by 27 % (Figure 2B and C). Cisplatin almost completely inhibited the growth of T-47D and RPMI-7951 cells (Figure 2B and C).

To evaluate the effect of **1** on colony formation we carried out soft agar clonogenic assays using T-47D, HCT-116, and RPMI-7951 cells. The cells were treated with compound **1** at 15 μM in soft agar matrix and incubated at 37°C in a 5% CO_2 incubator for 4 weeks. Our results indicated that **1** had no effect on colony formation of

T-47D, HCT-116, and RPMI-7951 cells, while cisplatin at 4 μM completely inhibited colony formation of these cells. Based on all observations, we showed that the planciside A (**1**) inhibited cell proliferation of HCT-116, T-47D, and RPMI-7951 cancer cell lines, but had no effect on colony formation of these cells.

Experimental

General: Optical rotations were determined on a Perkin-Elmer 141 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer at 500.13 and 125.76 MHz, respectively, and a Bruker AVANCE III 700 spectrometer at 700.13 and 176.04 MHz, respectively, with tetramethylsilane used as the internal standard. The NMR spectra of compounds **1a** and **3** were obtained in a Shigemi ampoule in CD_3OD. The HR ESI mass spectra were recorded on an Agilent 6510 Q-TOF LC/MS mass spectrometer; the samples were dissolved in MeOH (c 0.001 mg/mL). HPLC separations were carried out on an Agilent 1100 Series chromatograph that was equipped with a differential refractometer; Diasfer-110-C18 (10 μm, 250 × 15 mm), Discovery C18 (5 μm, 250 × 10 mm), and Ascentis RP-Amide (5 μm, 250 × 4.6 mm) columns were used. Low pressure column liquid chromatography was performed with Polychrom 1 (powdered Teflon, Biolar, Latvia), Si gel KSK (50÷160 μm, Sorbpolimer, Krasnodar, Russia), and Florisil (200÷300 mesh, Aldrich Chemical Co.). Sorbfil Si gel plates (4.5 × 6.0 cm, 5÷17 μm, Sorbpolimer, Krasnodar, Russia) were used for thin-layer chromatography.

Animal material: Specimens of *A. planci* were collected in May 2012 from Van Phong Bay near Nha Trang (Khanh Hoa province, Vietnam), at a depth of 5-10 m, and were identified by Dr. Do Cong Thung, the Institute of Marine Resources and Environment. A voucher specimen [No. **SBG 05-2012**] was deposited at the Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and isolation: The fresh animals (10 kg) were cut into small pieces and extracted thrice with EtOH. The extract (100 g) concentrated *in vacuo* was treated sequentially with *n*-hexane (3 × 500 mL), chloroform (3 × 500 mL), and methanol (3 × 500 mL), and the *n*-hexane and chloroform layers were separated. The methanol layer was evaporated, and the residue (50 g) was dissolved in H_2O (1 L). The H_2O-soluble fraction was passed in two portions through a Polychrom 1 column (7.5 × 26 cm) and eluted with distilled H_2O until a negative chloride ion reaction was obtained, followed by elution with MeOH. The combined MeOH eluate was evaporated to give a brownish material (35 g). The resulting total fraction was chromatographed on a Si gel column (6 × 16 cm) using CH_2Cl_2 – MeOH (stepwise gradient, 9:1 to 5:1), CH_2Cl_2 – MeOH – H_2O (stepwise gradient, 5:1:0.1 to 1:1:0.2), MeOH – H_2O (stepwise gradient, 15:1 to 5:1) to yield 9 main fractions (SBG 1–SBG 9). Fraction SBG 4 (4.5 g) was chromatographed on a Si gel column (4 × 8.5 cm) using $CHCl_3$ – EtOH (stepwise gradient, 2:1 to 1:2) to yield sub-fractions 4.1–4.13. Sub-fractions 4.4 and 4.5 were purified on a Florisil column (4 × 5 cm) using $CHCl_3$ – EtOH (stepwise gradient, 2:1 to 1:2) and submitted to HPLC on a Diasfer-110-C18 column (10 μm, 250 × 15 mm, 2.5 mL/min) and then on a Discovery C18 column (5 μm, 250 × 10 mm, 1.2 mL/min) with EtOH – H_2O – 1 M NH_4OAc (60:24:1) as an eluent system to give pure **1** (7.0 mg, t_R 24.5 min), **2** (1.9 mg, t_R 25.2 min), and **3** (0.3 mg, t_R 20.3 min).

Planciside A [(24*S*)-28-*O*-[β-D-galactofuranosyl-(1→5)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol] (**1**)
Amorphous powder.
Rf: 0.67 (BuOH – EtOH – H_2O, 4:1:2).
$[\alpha]_D$: –30.7 (*c* 0.7, MeOH).
^{1}H NMR (700.13 MHz, C_5D_5N): Table 1.
^{13}C NMR (125.76 MHz, C_5D_5N): Table 1.
(+)-HR ESI-MS: *m/z* $[M + Na]^+$ calcd for $C_{39}H_{68}O_{16}Na$: 815.4400; found: 815.4410.
(–)-HR ESI-MS: *m/z* $[M – H]^–$ calcd for $C_{39}H_{67}O_{16}$: 791.4435; found: 791.4444.
(+)-ESI-MS/MS of the ion at *m/z* 815: *m/z* 797 $[(M + Na) – H_2O]^+$, 653 $[(M + Na) – C_6H_{10}O_5]^+$, 635 $[(M + Na) – C_6H_{10}O_5 – H_2O]^+$, 521 $[(M + Na) – C_6H_{10}O_5 – C_5H_8O_4]^+$, 503 $[(M + Na) – C_6H_{10}O_5 – C_5H_8O_4 – H_2O]^+$, 317 $[C_6H_{10}O_5 + C_5H_8O_4 + Na]^+$.
(–)-ESI-MS/MS of the ion at *m/z* 791: *m/z* 629 $[(M – H) – C_6H_{10}O_5]^–$, 611 $[(M – H) – C_6H_{10}O_5 – H_2O]^–$, 497 $[(M – H) – C_6H_{10}O_5 – C_5H_8O_4]^–$, 479 $[(M – H) – C_6H_{10}O_5 – C_5H_8O_4 – H_2O]^–$.
Yeild of **1**: 7.0 mg.

Planciside B [(24*S*)-28-*O*-[α-L-fucopyranosyl-(1→2)-3-*O*-methyl-β-D-xylopyranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol] (**2**)
Amorphous powder.
Rf: 0.65 (BuOH – EtOH – H_2O, 4:1:2).
$[\alpha]_D$: –37.9 (*c* 0.2, MeOH).
^{1}H NMR (700.13 MHz, CD_3OD): Table 2.
^{13}C NMR (125.76 MHz, CD_3OD): Table 2.
(+)-HR ESI-MS: *m/z* $[M + Na]^+$ calcd for $C_{40}H_{70}O_{15}Na$: 813.4607; found: 813.4580.
(–)-HR ESI-MS: *m/z* $[M – H]^–$ calcd for $C_{40}H_{69}O_{15}$: 789.4642; found: 789.4651.
(+)-ESI-MS/MS of the ion at *m/z* 813: *m/z* 795 $[(M + Na) – H_2O]^+$, 667 $[(M + Na) – C_6H_{10}O_4]^+$, 649 $[(M + Na) – C_6H_{10}O_4 – H_2O]^+$, 521 $[(M + Na) – C_6H_{10}O_4 – C_6H_{10}O_4]^+$.
(–)-ESI-MS/MS of the ion at *m/z* 789: *m/z* 771 $[(M – H) – H_2O]^–$, 643 $[(M – H) – C_6H_{10}O_4]^–$, 625 $[(M – H) – C_6H_{10}O_4 – H_2O]^–$, 497 $[(M – H) – C_6H_{10}O_4 – C_6H_{10}O_4]^–$, 479 $[(M – H) – C_6H_{10}O_4 – C_6H_{10}O_4 – H_2O]^–$.
Yield of **2**: 1.9 mg.

Planciside C [(24*S*)-28-*O*-[2,4-di-*O*-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-24-methyl-5α-cholestane-3β,4β,6α,8,15β,16β,28-heptol**]** 6-*O*-sulfate (**3**)
Amorphous powder.
Rf: 0.63 (BuOH – EtOH – H_2O, 4:1:2).
$[\alpha]_D$: +3.3 (*c* 0.03, MeOH).
^{1}H NMR (700.13 MHz, CD_3OD): Table 2.
^{13}C NMR (125.76 MHz, CD_3OD): Table 2.
(+)-HR ESI-MS: *m/z* $[M + Na]^+$ calcd for $C_{40}H_{69}O_{18}SNa_2$: 915.3995; found: 915.4013.
(–)-HR ESI-MS: *m/z* $[M – Na]^–$ calcd for $C_{40}H_{69}O_{18}S$: 869.4210; found: 869.4226.
(+)-ESI-MS/MS of the ion at *m/z* 915: *m/z* 795 $[(M + Na) – NaHSO_4]^+$, 777 $[(M + Na) – NaHSO_4 – H_2O]^+$, 635 $[(M + Na) – NaHSO_4 – C_7H_{12}O_4]^+$, 503 $[(M + Na) – NaHSO_4 – C_7H_{12}O_4 – C_5H_8O_4]^+$, 315 $[C_7H_{12}O_4 + C_5H_8O_4 + Na]^+$.
(–)-ESI-MS/MS of the ion at *m/z* 869: *m/z* 851 $[(M – Na) – H_2O]^–$, 709 $[(M – Na) – C_7H_{12}O_4]^–$, 691 $[(M – Na) – C_7H_{12}O_4 – H_2O]^–$,577 $[(M – Na) – C_7H_{12}O_4 – C_5H_8O_4]^–$, 97 $[HSO_4]^–$.
Yield of **3**: 0.3 mg.

Acid hydrolysis of 1: A solution of glycoside **1** (2 mg) in aq. 2 M CF_3COOH (1 mL) was heated at 80-100°C for 1 h in a sealed vial. The reaction mixture was evaporated *in vacuo,* and the residue was dissolved in H_2O and extracted twice with $CHCl_3$. The $CHCl_3$ layer was evaporated *in vacuo* and the resulting fraction of steroid

aglycons was chromatographed on an Ascentis RP-Amide column (5 μm, 250 × 4.6 mm, 1 mL/min) with MeOH – H_2O (9:1) as the eluent to yield aglycon **1a** (0.2 mg, t_R 41.8 min): selected ^{1}H NMR (CD_3OD, 700.13 MHz) δ 0.870 (3H, d, *J* = 6.7 Hz, H_3-27), 0.874 (3H, d, *J* = 6.7 Hz, H_3-26), 3.52 (1H, dd, *J* = 6.3, 11.0 Hz, H′-28), 3.54 (1H, dd, *J* = 6.1, 11.0 Hz, H-28).

Bioassays: Cell cytotoxicity, cell proliferation, and soft agar clonogenic assays were performed as previously reported [24].

Acknowledgments – Financial support was provided by the FEB RAS Program "Far East", Grant 14-04-93008 Viet_a from the RFBR, FEB RAS Grant 12-III-A-05-058, Grant 148.2014.4 supporting the leading Russian scientific schools, and the Vietnam - India Project 40/2012/HD-NĐT.

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Natural Product Communications
2014
Volume 9, Number 9

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