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Steroid Compounds from Two Pacific Starfish of the Genus *Evasterias*

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Abstract—Three new steroid glycosides (evasteriosides C, D, and E) along with six known compounds were isolated from two Pacific starfish of the genus *Evasterias.* Evasterioside C from *E. retifera* collected from the Sea of Japan was identified as (20*R*,22*E*)-3-*O*-(β-*D*-xylopyranosyl)-24-nor-5α-cholest-22-ene-3β,6β,8,15α,26-pentaol 26-sulfate sodium salt. The structures of evasteriosides D and E from *E. echinosoma* (collected from the Gulf of Shelichov, the Sea of Okhotsk) were established as (20*R*,24*S*)-24-*O*-(β-*D*-glucopyranosyl)-5α-cholestane-3β,6α,8,15β,24-pentaol and (20*R*,24*S*)-3,24-di-*O*-(β-*D*-xylopyranosyl)-cholest-4-ene-3β,6β,8,15α,24-pentaol, respectively. In addition, the known compounds pycnopodiosides A and C, luridoside A, 5α-cholestane-3β,6α,8,15β,16β,26-hexaol. 5α-Cholestane-3β,6α,8,15β,24-pentaol 24-sulfate sodium salt and marthasterone sulfate sodium salt were identified in *E. echinosoma.* The structures of the isolated compounds were established on the basis of spectroscopic analyses, using 1D and 2D NMR techniques, mass spectrometry, and some chemical transformations.

Key words: Evasterias retifera, E. echinosoma, polyhydroxysteroids, starfish, steroid glycosides, FAB and MALDI TOF mass spectra, NMR spectra

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INTRODUCTION

We have recently described [1] isolation of two new steroid glycosides, evasteriosides A and B, from starfish *Evasterias retifera Verrill* Djakonov 1938 (order Forcipulata, family Asteriidae).[2] We continued the studies of starfish metabolites [2, 3] from extracts of two species of starfish from the genus *Evasterias* and isolated three new glycosides: evasterioside C (**I**) from *E. retifera* and evasteriosides D (**II**) and E (**III**) from *E. echinosoma* along with six earlier described polar steroids (**IV**)–(**IX**).

All nine isolated compounds are typical secondary metabolites of starfish, polyhydroxysteroids and their glycosylated and sulfated derivatives.

RESULTS AND DISCUSSION

New steroid compounds (**I**)–(**III**) together with the earlier known (**IV**)–(**VIII**) (scheme) and martasterone sulfate, (20*R*)-6α-hydroxy-23-oxocholesta-9(11),24-dien-3β-yl sodium sulfate (**IX**), were isolated from ethanolic extracts of starfish *E. retifera* and *E. echinosoma* by a column chromatography on Polychrome, Florisil, and silica gel with the subsequent purification by HPLC on columns with reversed-phase sorbents Diasphere-110-C_{18} and Zorbax Bonus-RP by the techniques described earlier [3]. Structural identification of the isolated compounds and their derivatives was carried out by spectral methods (NMR, FAB and MALDI TOF MS) and comparison of their physical constants with those described in literature.

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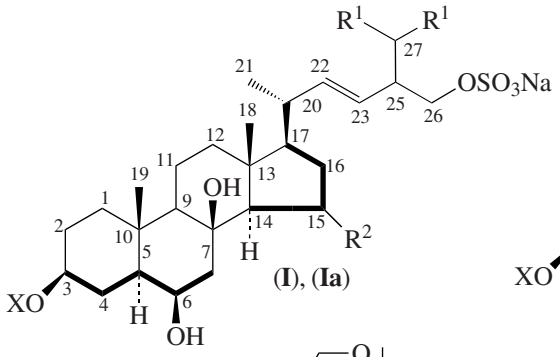
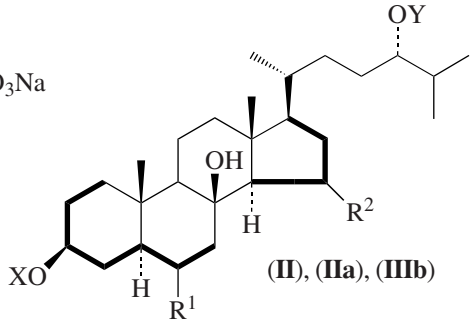
[2] Abbreviations: MALDI TOF MS, matrix-assisted laser desorption/ionization mass spectrometry; HMBC, heteronuclear CH correlation through multiple bonds; HSQC, heteronuclear single quantum coherence of the CH interaction through one bond; DEPT, Distortionless Enhancement by Polarization Transfer, an experiment that allows one to differentiate CH, CH_2, and CH_3 fragments according to the positive or negative polarity of signal; COSY, correlation spectroscopy, 2D correlations of shift through spin–spin coupling; TOCSY, total correlation spectroscopy that reveals all the proton couplings of the structure fragments in addition to those detected by COSY.

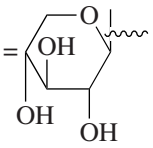


(**I**), (**Ia**)

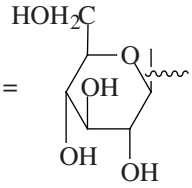


(**II**), (**IIa**), (**IIIb**)

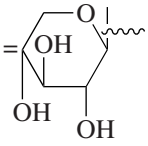
(**I**) R^1 = H, R^2 = αOH, X =



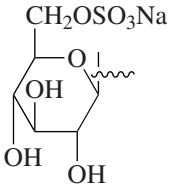
(**II**) R^1 = αOH, R^2 = βOH, X = H, Y =



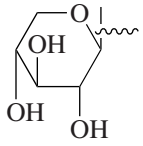
(**Ia**) R^1 = CH_3, R^2 = αOH, X =

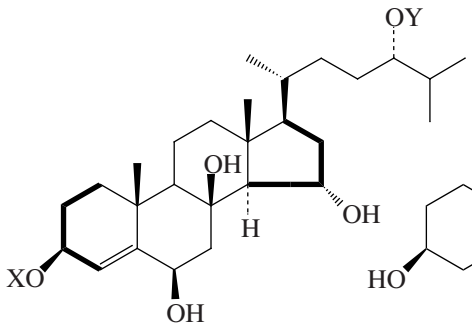


(**IIa**) R^1 = αOH, R^2 = βOH, X = H, Y =

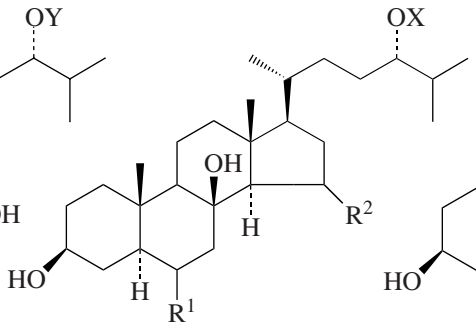


(**IIIb**) R^1 = βOH, R^2 = αOH, X = SO_3Na, Y =

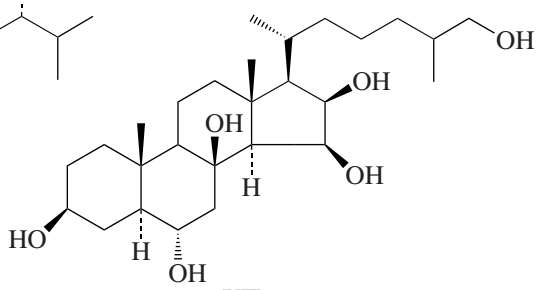




(**III**), (**IIIa**)

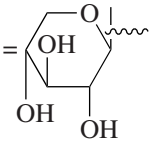


(**IV**)-(**VI**), (**VIII**)

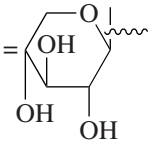


(**VII**)

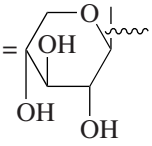
(**III**) X = Y =



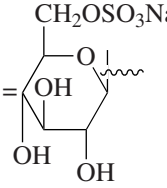
(**IV**) R^1 = αOH, R^2 = βOH, X =



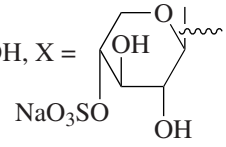
(**IIIa**) Y = SO_3Na, X =



(**V**) R^1 = αOH, R^2 = βOH, X =



(**VI**) R^1 = αOH, R^2 = βOH, X =



(**VIII**) R^1 = αOH, R^2 = βOH, X = SO_3Na

Scheme. Steroid compounds from starfish of genus *Evasterias*.

Mass spectra MALDI TOF and LSI (anion registration) of evasterioside C (**I**) from *E. retifera* displayed peak of $[M - Na]^-$ ion at *m/z* 647. At spectra also there were the fragmentation peaks at *m/z* 515 $[M - Na - Xyl]^-$ and 497 $[M - Na - Xyl–H_2O]^-$. In (+)-HR LSI mass spectrum of (**I**), the peak of a pseudo-molecular ion was observed at *m/z* 693.2863 $[M + Na]^+$. These data together with the data of NMR spectra corresponded to the molecular formula $C_{31}H_{51}O_9SO_3Na$ and proved the presence of sulfate group and pentose as a carbohydrate component of compound (**I**). In fact, an analysis of a ^{13}C NMR spectrum and DEPT specified (Table 1) the presence in (**I**) of 31 carbon atom, including 4 methyl, 9 methylene, and 15 methine groups, including 2 olefin carbon atoms, and also three quaternary carbon atoms, including one connected with oxygen.

In (–)-LSI mass spectrum, a more intensive peak at *m/z* 233 than in other spectra was observed. It is characteristic of steroid polyols containing no hydroxy groups at C4 and C7 [4]. The presence in NMR spectra of (**I**) of the signal of anomer carbon atom at (δ 103.2 ppm) and a doublet of anomer proton (δ_H 4.35 ppm, *J* 7.5 Hz) proves the presence of one monosaccharide residue in which H1 and H2 occupy *trans* position. Values of chemical shifts of C1–C21, C1'–C5', and also H3, H6, H15, 3 × H18, 3 × H19, 3 × H21, and H2' –$H_2$5' in ^{13}C-spectra and a ^1H NMR of glycoside (**I**) (Table 1) just as spin coupling constants of the corresponding protons practically coincided with the corresponding values in the spectra of asteriidoside L (**Ia**) from a starfish of family Asteriidae [5]. On this basis, a presumption was made that glycoside (**I**) contains a similar 3β,6β,8,15α-tetrahydroxycholestane nucleus with β-*D*-xylopyranose residue at the C3 atom. In fact, an acidic hydrolysis of glycoside (**I**) led to *D*-xylose identified by TLC and GLC methods (aldononitrile peracetates) and a comparison of value of $[\alpha]_D^{20}$ with standard compound. The bond of the monosaccharide residue to atom C3 was confirmed by correlation of anomeric proton H1'/C3 in HMBC spectrum (Table 1). The arrangement of hydroxy groups was also confirmed by HMBC- and COSY-correlations.

The following signals of the side chain are present in the NMR spectra of (**I**): two methyl doublets at δ 1.02 (*J* 6.8) and 0.97 (*J* 6.8) ppm (δ_C 20.9 and 17.1 ppm) and signals of olefin protons at δ_H 5.31 and 5.27 ppm (δ_C 130.5 and 138.2 ppm). The value of chemical shift of C20 atom (δ_C 40.6 ppm) and spin coupling constant $J_{22,23}$ of 15.3 Hz corresponded to a *E*-configuration of double bond (for *Z*-isomer, the corresponding signal of C20 is displayed at δ_C ~ 34.0 ppm [6]). These data and the signals of hydroxymethylene protons at δ_H 3.88 (*J* 6.0 and 9.4 Hz) and 3.74 (*J* 7.4 and 9.4) ppm indicated the Δ^{22E}-24-nor-26-hydroxycholestane side chain. The *R*-configuration has been attributed to the chiral center C20 on the basis of chemical shift of three H21 protons (δ 0.97 ppm) characteristic of steroids with such a configuration.

An analysis of spectral data corresponding to the steroid side chain and a comparison with the corresponding data for the known (20*R*,22*E*)-24-nor-5α-cholest-22-ene-3β,4β,6α,8,15β,26-hexaol (**Ib**) from *Acodontaster conspicuus* [7] showed that the signals of side chains of (**I**) and (**Ib**) are close, except for the signals of hydroxymethylene groups. Chemical shifts C26-H_2 in NMR spectra of (**Ib**) are δ_H 3.43 and 3.30, and δ_C 68.3 ppm [7] (whereas in spectra of (**I**), they are downfield displaced to δ_H 3.88 and 3.74, and δ_C 73.7 ppm), which corresponds to the shift of sulfation [8] and allows to assume the presence of sulfate group at C26. The structure of the side chain of evasterioside C was finally confirmed by cross peaks of H26' (δ_H 3.74 ppm) and H26 (δ_H 3.88 ppm)/H25 (δ_H 2.43 ppm), and also H25 (δ_H 2.43 ppm)/27-H_3 (δ_H 1.02 ppm) and H25/H23 (δ_H 5.31 ppm) in COSY spectra.

At all, the spectra COSY, HMBC, and HSQC allowed the assignment of signals of all atoms C and H in NMR spectra. In the formula (**I**), the lines indicating some fragments of molecule in which the sequences of protons were established by COSY experiments. In particular, in the spectrum ^1H–^1H-COSY, the presence of spin coupling between protons HC3–H_2C4–HC5–HC6–H_2C7 and HC14–HC15–H_2C16–HC17 was shown. On the basis of all the data, the structure of sodium salt 26-sulfate of (20*R*,22*E*)-3-*O*-(β-*D*-xylopyranosyl)-24-nor-5α-cholest-22-ene-3β,6β,8,15α,26-pentaol (**I**) was assigned to evasterioside C.

Marine 24-norsterols were found in phytoplankton [9], therefore, the identification of polar steroids similar to compound (**I**) with truncated oxidized side chains proves the opportunity of oxidation and glycosidation of these sterols at their getting up through a food chain in organisms of starfish.

The (+)HR-LSI mass spectrum of isolated from the Sea of Okhotsk starfish *E. echinosoma* evasterioside D (**II**) exhibits a peak of pseudo-molecular ion at *m/z* 637.3887 $[M + Na]^+$, which, in combination with the data of ^{13}C and ^1H NMR points out to the molecular formula $C_{33}H_{58}O_{10}$. For example, spectra of ^{13}C NMR and DEPT (Table 1) indicate in the molecule (**II**) the presence of signals of 33 carbon atoms, including 5 methyl, 10 methylene, 15 methine groups, 2 quaternary carbon atoms, and one such an atom connected to oxygen. The chemical shift of anomeric proton δ_H 4.29 ppm (*J* 7.7 Hz), anomeric carbon atom at δ_C 104.3 ppm, the atoms of carbon connected to oxygen at δ_C 63.1, 67.7, 71.1, 72.0, 72.2, 75.6, 77.5, 77.7, 78.2, and 86.2 ppm proved the presence in the molecule (**II**) of hexoside fragment with *trans* arrangement of H1 and H2 and a pentahydroxy substituted steroid aglycone with cholestane skeleton.

An analysis of ^{13}C and ^1H NMR spectra of glycoside (**II**) and their comparison with the spectra of pycnopodioside C (**IIa**) from starfish *Pycnopodia helianthoides*

Table 1. The data of NMR spectra of evasteriosides C (**I**) and D (**II**) (CD_3OD, DRX-500)[1]

Atom number	(**I**)			(**II**)		
	δ_C, mult.[2]	δ_H (*J*, Hz)	HMBC	@δ_C, мульт.[2]	δ_H (*J*, Hz)	HMBC
1	41.4, CH_2	1.72, m; 0.97, m		39.4, CH	0.97, m; 1.71, m	
2	30.0, CH_2	1.61, m; 1.82, m		31.5, CH_2	1.47, m; 1.73, m	
3	80.0, CH	3.69, m		72.2, CH	3.47, m	
4	32.9, CH_2	1.74, m; 1.82, m		32.3, CH_2	1.20, m; 2.18, m	
5	48.8, CH	1.20, m		53.8, CH	1.03, m	
6	74.2, CH	3.86, m	C8, C10	67.7, CH	3.69, dt (10.8; 4.1)	C8, C10
7	45.4, CH_2	1.57, dd (3.0; 14.5)	C5, C6, C8, C9	49.4, CH_2	1.27, m; 2.37, dd (4.2; 15.6)	C5, C6, C8, C9
		2.36, dd (3.0; 14.7)				
8	77.2, C			77.5, C		
9	57.1, CH	0.96, m		57.4, CH	0.83, m	
10	36.7, C			38.0, C		
11	19.8, CH_2	1.53, m; 1.83, m		19.8, CH_2	1.49, m; 1.81, m	
12	42.7, CH_2	1.25, m; 1.94, m		43.4, CH_2	1.16, m; 1.96, m	
13	45.4, C			44.3, C		
14	66.7, CH	1.20, d (9.5)	C13, C15, C18	62.6, CH	1.00, m	C13, C18
15	69.9, CH	4.24, dt (3.0; 9.3)		71.1, CH	4.40, уш.т@ (5.7)	
16	42.0, CH_2	1.56, m; 1.87, m		42.4, CH_2	1.39, m; 2.38, m	
17	55.6, CH	1.36, m		57.9, CH	0.99, m	
18	15.5, CH_3	0.97, s	C12, C13, C14, C17	16.5, CH_3	1.26, s	C12, C13, C17, C14
19	15.8, CH_3	1.16, s	C1, C5, C9, C10	14.1, CH_3	0.98, s	C1, C5, C9, C10
20	40.6, CH	1.98, m		36.3, CH	1.53, m	
21	20.9, CH_3	0.97, d (6.8)	C17, C20, C22	19.0, CH_3	0.92, d (0.7)	C17, C20, C22
22	138.2, CH	5.27, dd (15.4; 6.7)	C20, C24	32.6, CH_2	1.02, m; 1.61, m	
23	130.5, CH	5.31, dd (15.3; 8.0)	C20, C24, C27	28.6, CH_2	1.42, m; 1.60, m	
24				86.2, CH	3.43, m	
25	37.7, CH	2.43, m	C22, C23, C26	31.8, CH	1.87, m	
26	73.7, CH_2	3.74, dd (7.4; 9.4)	C23, C24, C27	18.4, CH_3	0.94, d (7.0)	C24, C25, C27
		3.88, dd (6.0; 9.4)				
27	17.5, CH_3	1.02, d (6.8)	C24, C26, C23	18.2, CH_3	0.92, d (7.0)	C24, C25, C26
1'	103.2, CH	4.35, d (7.5)	C3	104.3, CH	4.29, d (7.7)	C24
2'	75.0, CH	3.14, dd (7.6; 9.0)	C1'	75.6, CH	3.16, t (7.9)	
3'	77.9, CH	3.30[3], m		78.2, CH	3.33[3], m	
4'	71.3, CH	3.47, m		72.0, CH	3.26, t (8.7)	
5'	66.9, CH	3.18, dd (10.5; 11.3)	C4', C3', C1'	77.7, CH	3.23, m	
		3.82, dd (5.3; 11.6)				
6'				63.1, CH_2	3.84, dd (2.3; 11.7)	
					3.67, t (5.2; 11.7)	

Notes: 1. Signal assignment is made with application of 2D NMR spectroscopy ^1H-^1H-COSY, HMBC, and HSQC.
2. Multiplicity of signals was determined by DEPT technique.
3. Signal was overlaid with a signal of solvent.

[10] showed that they practically coincide, except for some signals of the monosaccharide residue. At the acidic hydrolysis of product (**II**), *D*-glucose was identified by TLC and GLC methods (aldononitrile peracetates) and by a comparison of the value of $[\alpha]_D^{20}$ with standard sample. For example, protons 6'–H_2 in the NMR spectrum of (**IIa**) containing 6-*O*-sulfated β-*D*-glucopyranose residue resonated under the influence of sulfate group in a low field at δ_H 4.18 ppm (dd, *J* 5 and 12 Hz) and 4.34 (dd, *J* 2 and 12 Hz) ppm. The signals of C6 atom have a chemical shift at δ_C 68.5 ppm [10]. For evasterioside D, these signals are shifted upfield to δ_H 3.67 and 3.84 ppm, and δ_C to 63.1 ppm. Taking into account the differences in chemical shifts of these atoms and the data [10] published earlier, we assumed that evasterioside D (**II**) differs from pycnopodioside C only by the absence of sulfate group at C6' in monosaccharide residue.

Such a structure of glycoside (**II**) was confirmed by the methods of bidimentional spectroscopy with the help of HMBC, ^{1}H–^{1}H-COSY and HSQC spectra, which allowed us to assign all the carbon and hydrogen atoms in NMR spectra (Table 1). A spin-coupling between the protons of carbohydrate chain H1'–H2'–H3'–H4'–H5'–2 × H6' is observed in the spectrum COSY-90 of (**II**) and the HMBC-correlation H1'/C24 confirms that β-*D*-glucopyranose residue is attached to C24 of aglycone (see Table 1). The *R*-configuration of chiral center C20 was attributed on the basis of size of chemical shift of protons 21-H_3 (CD_3OD, δ 0.92 ppm), and, for chiral center C24, the *S*-configuration by analogy to compound (**IIa**) is accepted. Thus, the new steroid glycoside (**II**) has the structure (20*R*,24*S*)–24-*O*-(β-*D*-glucopyranosyl)-5α-cholestane-3β,6α,8,15β,4-pentaol. In the formula (**II**), the bonds specifying the key fragments of molecule in which sequences of protons are established by COSY experiments are marked.

The peak of a pseudo-molecular ion at *m*/*z* 737 $[M + Na]^+$ and also the fragmentation peaks corresponding to the loss of pentoside fragment and a water molecule were seen in MALDI TOF and HR-LSI mass spectra (registration of cations) of steroid (**III**) (see the Experimental section). These data correspond to molecular formula $C_{37}H_{62}O_{13}$. The presence in the NMR spectra of signals of two anomeric carbon atoms (δ 104.9 and 104.3 ppm) and two doublets of anomeric protons with spin-coupling constants *J* of 7.6 and 7.5 Hz indicate the existence of two monosaccharide residues with *trans* arrangement of H1 and H2. At acid hydrolysis of evasterioside E, only one monosaccharide, *D*-xylose, was obtained; it was identified by TLC and GLC methods (aldononitrile peracetates) and by a comparison of value $[\alpha]_D^{20}$ with standard compound. An analysis of ^{13}C and ^{1}H NMR of glycoside (**III**) and a comparison with the corresponding spectral data for known pisasteroside D (**IIIa**) from starfish *Pisaster giganteus* [11] showed that the spectra practically coincide, except for some signals of a side chain of glycoside (**III**).

To confirm the structure of the side chain of (**III**), which we named as evasterioside E, we compared the spectral data with earlier known aphelasterioside A (**IIIb**) from *Aphelasterias japonica* [12]. Full concurrence of chemical shifts of all carbon atoms and protons, together with the corresponding spin-coupling constants confirmed the identity of their side chains and localization in position C24 of the second xylose residue.

The (20*R*,24*S*)-configurations for chiral centers of the side chain were accepted by analogy to other (24*S*)-hydroxyxylosides from starfish [12].

The HMBC-correlations H1'/C3 and H1''/C24 (Table 2) confirmed that evasterioside E (**III**) contains two β-*D*-xylopyranose residues located at C3 and C24. The methods of bidimentional spectroscopy with the help of HMBC, ^{1}H–^{1}H-COSY, 1D-TOCSY, and HSQC spectra allowed us to establish finally for evasterioside E the structure of (20*R*,24*S*)-3,24-di-*O*-(β-*D*-xylopyranosyl)-cholest-4-ene-3β,6β,8,15α,24-pentaol (**III**).

The identification of the before known polyoxysteroid (**IV**) was executed on the basis of coincidence of its spectral characteristics with the corresponding data for the earlier known pycnopodioside A [10]. Glycoside (**V**) was identified similarly as pycnopodioside C [10], and glycoside (**VI**) differed from (**IV**) by the presence of sulfate group at 4' in xylose residue and was also identified as earlier known luridoside A from starfish *Cosmasterias lurida* [13]. Compounds (**VII**)–(**IX**) were identified as (25*S*)-5α-cholestane-3β,6α,8,15β,16β,26-hexaol (**VII**), 5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate (**VIII**), and marthasterone sulfate (20*R*)-6α-hydroxy-23-oxocholesta-9(11),24-dien-3β-yl sodium sulfate (**IX**) by comparison of their ^{1}H and ^{13}C NMR spectra with the corresponding data for these compounds we earlier isolated [14, 15], and also by a direct comparison of their chromatographic behavior with available samples (TLC, HPLC).

Thus, we isolated from two species of starfish of the same genus earlier unknown glycosides named evasteriosides C, D, and E, which differ from each other by both monosaccharide residues, and their localization. They have an identical arrangement, but different stereochemistry of hydroxyl functions in the steroid nucleus. Evasteriosides C and D are monoglycosylated polyhydroxysteroids, and evasterioside E belongs to the group of 3,24-diglycosylated steroid polyols.

EXPERIMENTAL

Spectra ^{1}H and ^{13}C NMR were registered on spectrometers Bruker DPX-300 and DRX-500 with working frequencies of 300 and 500 MHz for protons, an internal standard was CD_3OD/δ_H 3.30 ppm, δ_C 49.0 ppm. Specific optical rotation was measured on a Perkin-Elmer 343 polarimeter; MALDI TOF MS, on a Bruker

Table 2. The data of NMR spectra of evasterioside E (**III**) (CD_3OD, DRX-500)[1]

Atom number	δ_C, mult.[1]	δ_H (*J*, Hz)	HMBC	Atom number	δ_C, mult.[1]	δ_H (*J*, Hz)	HMBC
1	39.7, CH_2	1.79, m		21	19.0, CH_3	0.89, d (6.6)	C17, C20, C22
2	27.7, CH_2	1.76, m; 1.96, m		22	32.8, CH_2	1.58, m; 0.97, m	
3	77.1, CH	4.19, m		23	28.6, CH_2	1.58, m; 1.35, m	
4	127.0, CH	5.67, brs	C2, C6, C10	24	86.2, CH	3.34, m	
5	148.3, C			25	31.9, CH	1.87, m	
6	76.4, CH	4.30, brt (3.0)	C8, C10	26	18.2[2], CH_3	0.93, d (6.5)	C24, C25, C27
7	44.5, CH_2	2.52, dd (3.0; 15.0); 1.51, m	C6, C9	27	18.3[2], CH_3	0.91, d (6.5)	C24, C25, C26
8	76.5, C			1'	104.3, CH	4.36, d (7.5)	C3
9	57.8, CH	1.03, dd (2.5; 13.0)		2'	75.0, CH	3.13, dd (7.8; 9.0)	
10	37.7, C			3'	78.0, CH	3.30[3]	
11	19.7, CH_2	1.84, m; 1.50, m		4'	71.2[2], CH	3.46, m	
12	42.8, CH_2	1.97, m; 1.22, m		5'	66.9, CH_2	3.82, dd (5.0; 11.3) 3.19, t (11.0)	
13	45.4, C			1"	104.9, CH	4.22, d (7.6)	C24
14	66.5, CH	1.15, m		2"	75.4, CH	3.14, dd (7.4; 9.1)	
15	70.1, CH	4.28, td (9.6; 3.2)		3"	78.0, CH	3.27, t (8.8)	
16	41.6, CH_2	2.00, m; 1.70, m		4"	71.4[2], CH	3.46, m	
17	55.9, CH	1.31, m		5"	66.8, CH_2	3.81, dd (5.3; 11.3) 3.14, t (11.0)	
18	15.3, CH_3	0.96, s	C12, C13, C14, C17				
19	22.6, CH_3	1.35, s	C1, C5, C9, C10				
20	36.2, CH	1.34, m					

Notes: 1. Signal assignment is made with application of 2D NMR spectroscopy ^1H-^1H-COSY, HMBC, and HSQC.
2. Equivocal assignment of signals.
3. Signal was overlaid with a signal of solvent.

Biflex III spectrometer (Germany) with laser ionization/desorption (the N_2-laser at 337 nm). A sample was dissolved in methanol (10 mg/ml) and aliquot of 1 μl was analyzed, using cyanohydroxycinnamic acid as a matrix. LSI (–) and (+)-mass spectra of the high resolution were obtained on an Intectra AMD-604S mass spectrometer (Germany) at the energy of cesium ions of 8 keV with glycerol as a matrix. The content of sodium was determined) on an atomic adsorption Nippon Jarrel Ash AA-780 spectrophotometer. HPLC was carried out on a Du Pont Model 8800 chromatograph using a refractometric detector and columns packed with Diasphere-110-C_{18} (5 μm, 4 × 250 mm) and Zorbax Bonus-RP (4.6 × 250 mm, 5 μm) in systems of 45 and 55% methanol–water (V = 1 ml/min), respectively. Melting points were determined on a Leica VMTG microtable. TLC was carried out on Sorbfil plates with a silica gel CTX-1A layer fixed on a foil (5–17 μm, Russia, Krasnodar) in 5 : 1 : 1 BuOH–EtOAc–H_2O system. Preparative column chromatography was carried out on a silica gel L (80–100 and 200–250 mesh, Chemapol, Czechia), Polychrome (Russia), and Florisil (200–250 mesh, Merck, Germany).

Animals. Samples of starfish *E. retifera* were collected in August, 2003 by divers at Gamov cape, Sea of Japan, from the depth of 60–150 m and were identified by Professor V.S. Levin (Pacific Institute of Bioorganic Chemistry, Pacific Division, Russian Academy of Sciences). Starfish *E. echinosoma* were collected by expedition of TINRO CENTER in August, 2004 during sailing of NIS Professor Kaganovsky (Shelikhov bay, Sea of Okhotsk) by a trawl from the depth of 100–200 m. The collector of the material is N.V. Bekova, samples were identified by Professor A.V. Smirnov (Zoological Institute, Russian Academy of Sciences).

Extraction and isolation of total fractions. Crushed starfish *E. retifera* were exhaustively extracted with 95% ethanol at room temperature. The combined

alcohol extract was evaporated in a vacuum up to a gum residue (0.826 kg), dissolved in water, and three times extracted with butanol. The combined butanol extracts were evaporated to a syrup and chromatographed on a silica gel (50–100 μm) column (6 × 25 cm) in chloroform–ethanol system (100 : 0 ⟶ 45 : 55). The fraction of steroid glycosides was obtained (2.3 g); it was eluted with 20 vol % ethanol in chloroform. The fraction was dissolved in water and was passed through a column with Polychrome (3 × 12 cm). The column was washed with water and 50% water ethanol. The water ethanol eluate was concentrated in a vacuum and chromatographed on a column with Florisil (200 mesh, 1.5 × 19 cm) in system chloroform–methanol (100 : 0 ⟶ 85 : 15). We obtained an enriched fraction (25 mg) containing glycoside (**I**). After the extraction of starfish *E. echinosoma*, the dry ethanolic extract (49 g) was separated on a column with Polychrome at eluted with 40, 50 and 100% ethanol–water; fractions 1 (0.5 g), 2 (0.615 g), and 3 (1.3 g) were obtained. From apolar fraction 1, chromatography on a silica gel column (50–100 μm) in a gradient chloroform–ethanol (100 : 0 to 80 : 20) gave 15 mg of the fraction containing compound (**IX**). A successive chromatography on silica gel columns eluted with a gradient chloroform–methanol (95 : 5 to 40 : 60), and then on a Florisil column (200–300 mesh, 2 × 50 cm) eluted with chloroform–methanol (95 : 5 to 65 : 35) gave from a more polar fraction 2 two fractions of steroid compounds: **A** (**II**)–(**V**) and **B** (**VI**)–(**VIII**).

Isolation of steroid compounds (I)–(IX). Fraction **A** consisting according to TLC of polar compounds, R_f = 0.8–0.7 in system butanol–ethyl acetate–water (5 : 1 : 1) was purified by HPLC on a Diasphere 110-C_{18} column under elution with 45% methanol–water and rechromatography on a Zorbax Bonus-RP column in system of 55% methanol–water (V = 1 ml/min). Under similar conditions, HPLC was carried out of the fraction mainly consisting of glycoside (**I**); there was obtained pure evasterioside C (3.5 mg). A less polar fraction **B** and the fraction containing compound (**IX**), was rechromatographed under similar conditions, using 55% methanol, and, for rechromatography, 65% methanol. There were obtained 4 mg of (**II**), 3.5 mg of (**III**), 3.1 mg of (**IV**), 3 mg of (**V**), 2.3 mg of (**VI**), and 3 mg of each (**VII**) and (**VIII**).

Hydrolysis of glycosides (I), (II), and (III) and identification of monosaccharides. Solutions of (**I**), (**II**), and (**III**) (2 mg of each compound) in 2 ml 2 M HCl were heated for 2 h on a boiling water bath. Xylose was identified in hydrolyzates of (**I**) and (**III**) by GLC (as aldononitrile peracetates) and paper chromatography (butanol–pyridine–water system, 10 : 3 : 3, Whatman no. 1). There were determined $[\alpha]_D^{20}$ +20 (from (**I**) (*c* 0.10, H_2O) and +26 (*c* 0.11, H_2O) (from (**III**)). For *D*-xylose, lit. $[\alpha]_D^{20}$ + 92 to19.0 (H_2O); for *L*-xylose $[\alpha]_D^{20}$ – 18.6 (H_2O) [16]. *D*-Glucose was similarly identified in hydrolyzate of (**II**): $[\alpha]_D^{20}$ + 45 (*c* 0.10, H_2O). Lit. [16]: *D*-glucose $[\alpha]_D^{20}$ +111.2 to +52.5 (H_2O).

Evasterioside C (I), amorphous, $[\alpha]_D^{20}$ – 1 (*c* 0.1, MeOH). In (+)-HR LSI-MS of (**I**), peak of pseudomolecular ion at *m/z* 693.2863 $[M + Na]^+$ (calc. for $C_{31}H_{51}O_9SO_3Na$, 693.2897); MS (+)-LSI: peak at *m/z* 233 [calc. for $C_{15}H_{21}O_2$, splitting of C8–C14, C13–C17] [4]. ^1H NMR spectra and ^{13}C NMR spectra (MeOH) are given in Table 1 and in the text.

Evasterioside D (II), amorphous, $[\alpha]_D^{20}$ + 18.6 (*c* 0.16, MeOH); (+) HR-LSI: *m/z* 637.3887 $[M + Na]^+$ (calc. for $C_{33}H_{58}O_{10}$ 637.3927), *m/z* 653 $[M + K]^+$. (–)-LSI MS: *m/z* 613 $[M – H]^–$; ^1H and ^{13}C NMR spectra (MeOH) are given in Table 1 and in the text.

Evasterioside E (III), amorphous, $[\alpha]_D^{20}$ – 2.7 (*c* 0.26, MeOH); (+) HR-LSI-MS, *m/z*: 737.4035 $[M + Na]^+$ (calc. for $C_{37}H_{62}O_{13}$ 737.4088), 753 $[M + K]^+$, 605 $[(M + Na)–Xyl]^+$ and 587 $[(M + Na) – Xyl – H_2O]^+$; (–)-LSI MS: *m/z* 713 $[M – H]^–$, 581 $[(M – H) – Xyl]^–$, 563 $[(M – H) – Xyl – H_2O]^–$. ^1H and ^{13}C NMR (MeOH) are given in Table 2 and discussed in the text.

Pycnopodioside A (IV), amorphous, $C_{32}H_{56}O_9$, $[\alpha]_D^{20}$ + 3.8 (*c* 0.5, MeOH); (+) MALDI TOF, *m/z*: 607 $[M + Na]^+$, FAB (–): *m/z* 583 $[M – H]^–$. It was identified by comparison of spectral characteristics with those given in the literature [10, 14].

Pycnopodioside C (V), amorphous, $C_{33}H_{57}O_{13}SNa$, $[\alpha]_D^{20}$ + 4 (*c* 0.5, MeOH); ^1H and ^{13}C NMR data and MALDI TOF (+) spectrum were identical with those for standard compound from *L. anthosticta* [14].

Luridoside A (VI), amorphous, $C_{32}H_{55}O_{12}SNa$, $[\alpha]_D^{20}$ + 3.1 (*c* 0.2, MeOH); MALDI TOF (+): *m/z* 709 $[M_{Na} + Na]^+$ (100). Spectral characteristics are identical to those in literature [13].

(25*S*)-5α-cholestane-3β,6α,8,15β,16β,26-hexaol (VII); colorless crystals (from MeOH), mp 260–262°C, $C_{27}H_{48}O_6$, $[\alpha]_D^{20}$ + 38.1 (*c* 0.1, MeOH); FAB (–): *m/z* 467 $[M – H]^–$. It is identified by a comparison of spectral characteristics with known steroid we earlier isolated from starfish *L. anthosticta* [14].

(24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol sulfate sodium salt (VIII), amorphous, $C_{27}H_{47}SO_3Na$, $[\alpha]_D^{20}$ + 8.1 (*c* 0.1, MeOH); FAB (–) *m/z* 531 $[M – H]^–$. It was identified by comparison of constants and spectral characteristics with standard compound [15].

Marthasteron sulfate, (20*R*)-6α-hydroxy-23-oxocholesta-9(11),24-dien-3β-yl sodium sulfate (IX), was identified by analogy to known compound [14].

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