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Steroidal monoglycosides from the Far Eastern starfish *Hippasteria kurilensis* and hypothetic pathways of polyhydroxysteroid biosynthesis in starfish

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ABSTRACT

Five new steroidal monoglycosides, kurilensosides E (**1**), F (**2**), G (**3**), H (**4**) and 15-*O*-sulfate of echinasteroside C (**5**) were isolated along with the previously known echinasteroside C (**6**) from the alcoholic extract of the Far Eastern starfish *Hippasteria kurilensis* collected near Kuril Islands. Compounds **1**–**3** were determined to contain unusual polyhydroxysteroidal aglycons lacking 6-hydroxy group. Aglycon moiety of kurilensoside H (**4**) was shown to be the first case of marine polar steroids containing 4,5-epoxy functionality. Hypothetic pathways of the biosynthesis of polyhydroxysteroids and related glycosides in starfish and the existence of the late C-6 oxidation pathway in *H. kurilensis* are discussed.

1. Introduction

Starfish are an especially rich source of structurally diverse and biologically active polar highly hydroxylated steroids and related glycosides. As a rule, these substances occur as complicated mixtures, very difficult for separation into individual components. In total, more then 500 polyhydroxylated steroid compounds were described from different starfish species studied up today [1–4]. Diversity of starfish polar steroids continues to open new their structural patterns and biological activities, while their biosynthesis remains to be almost completely unstudied. Only some data were obtained at experiments with homogenates of different starfish body components and radioactive sterol precursors showing that sterols and some hydroxysterols such as 6-hydroxycholesterols may be considered as biosynthetic precursors of highly hydroxylated steroids in starfish [5].

In this paper in continuation of our previous study [6] we report structures of five new monoglycosides from the Far Eastern spiny red starfish *Hippasteria kurilensis* (order Valvatida, family Goniasteridae) and discuss results of the computer analysis of all earlier described structures of this type to deduce the predominated sequence of oxidation stages during biosynthesis of similar steroids in starfish and peculiarities of this process in *H. kurilensis*.

2. Experimental

2.1. General methods

Optical rotations were determined on a PerkinElmer polarimeter Model 343. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer at 500 and 125.8 MHz, respectively, using signals of CD_3OD (3.30 ppm in ^{1}H NMR spectra and 49.0 ppm in ^{13}C NMR spectra) as the internal standard. MALDI-TOF mass spectra were recorded on a Bruker Biflex III laser-desorption mass spectrometer coupled with delayed extraction using a N_2 laser (337 nm). Samples of the tested compounds were dissolved in MeOH (1 mg/mL), and 1 μL aliquots were analyzed using α-cyano-4-hydroxy-cinnamic acid (CCA) matrix. LSI mass spectra were recorded on an AMD-604S mass spectrometer (AMD, Germany) with an accelerating voltage of 8 keV and energy of Cs^+ ions of 10–12 keV. For recording the mass spectra, a sample was dissolved in MeOH (10 mg/mL) and an aliquot (1 μL) was analyzed using glycerol (Sigma) as the matrix.

HPLC separations were carried out on a Agilent 1100 Series chromatograph equipped with a differential refractometer. Diasfer-110-C18 (10 μm, 250 mm × 15 mm) and Kromasil 100A-C18 (5 μm, 250 mm × 4.6 mm) columns were used. Low pressure column liquid chromatography was performed using Amberlite XAD-2 (20–80 mesh, Sigma, Chemical Co.), Si gel KSK (50–160 μm, Sorbpolimer, Krasnodar, Russia) and Florisil (200–300 mesh, Aldrich Chemical Co.). Sorbfil Si gel plates (4.5 cm × 6.0 cm, 5–17 μm, Sorbpolimer, Krasnodar, Russia) in the eluent system

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Table 1
^{1}H NMR data of **1**–**5** (500 MHz, CD_3OD, *J* in Hz)[a].

Position	**1**	**2**	**3**	**4**	**5**
1	1.73 m	1.49 m	1.88 m	1.55 m	1.77 m
	0.95 m	1.33 m	1.05 m	1.15 m	1.27 m
2	1.89 m	1.85 m	2.06 m	1.65 m	1.96 m
	1.62 m	1.63 m	1.62 m	1.56 m	1.76 m
3	3.61 m	4.21 ddd (3.8, 5.5, 12.2)	3.57 m	4.02 ddd (1.3, 4.4, 9.5)	4.18 ddd (2.0, 6.3, 9.7)
4	3.88 m	3.65 d (3.7)	4.30 d (3.3)	3.29 m[b]	5.62 br s
5	1.04 m				
6	2.10 m	1.99 m	5.63 dd (2.2, 5.2)	3.40 t (3.1)	4.29 t (3.1)
	1.21 m	1.79 m			
7	2.10 m	2.44 dt (4.3, 13.5)	2.42 dd (5.3, 19.2)	2.59 dd (3.1, 15.0)	2.65 dd (3.2, 15.0)
	1.46 m	1.10 m	2.22 dd (2.2, 19.2)	1.59 dd (3.2, 15.0)	1.46 dd (3.3, 15.0)
8					
9	0.82 m	1.59 m	1.12 m	1.22 m	1.02 m
10					
11	1.69 m	1.27 m	1.86 m	1.92 m	1.89 m
	1.40 m	0.92 m	1.43 m	1.43 m	1.47 m
12	1.93 m	1.93 m	1.99 m	1.94 m	1.99 m
	1.17 m	1.19 m	1.19 m	1.20 m	1.19 m
13					
14	1.07 d (10.9)	1.15 d (10.9)	1.07 d (10.9)	1.03 d (10.8)	1.21 d (10.8)
15	4.02 dd (2.5, 10.8)	4.03 dd (2.6, 10.9)	4.12 dd (2.6, 10.9)	4.16 dd (2.6, 10.7)	4.77 dd (2.3, 10.7)
16	3.96 dd (2.5, 7.6)	3.97 dd (2.6, 7.5)	3.98 dd (2.6, 7.7)	3.97 dd (2.5, 7.6)	4.32 dd (2.2, 7.8)
17	1.18 m	1.19 m	1.19 m	1.21 m	1.24 m
18	1.11 s	1.11 s	1.13 s	1.12 s	1.18 s
19	1.17 s	1.30 s	1.35 s	1.26 s	1.36 s
20	1.85 m	1.84 m	1.87 m	1.85 m	1.88 m
21	0.92 d (6.7)	0.92 d (6.7)	0.93 d (6.7)	0.92 d (6.7)	0.92 d (6.7)
22	1.56 m	1.57 m	1.57 m	1.57 m	1.62 m
	1.02 m	1.02 m	1.03 m	1.04 m	1.03 m
23	1.47 m	1.47 m	1.46 m	1.45 m	1.44 m
	1.21 m	1.22 m	1.21 m	1.22 m	1.21 m
24	1.42 m	1.42 m	1.40 m	1.42 m	1.39 m
	1.04 m	1.04 m	1.05 m	1.05 m	1.03 m
25	1.56 m	1.57 m	1.57 m	1.57 m	1.56 m
26	3.41 dd (6.0, 10.8)	3.41 dd (5.7, 10.6)	3.41 dd (5.8, 10.7)	3.41 dd (5.7, 10.6)	3.42 dd (5.7, 10.7)
	3.31 m[b]	3.31 m[b]	3.30 m[b]	3.31 m[b]	3.30 m[b]
27	0.90 d (6.7)	0.90 d (6.7)	0.90 d (6.7)	0.90 d (6.7)	0.89 d (6.7)
1′	4.42 d (7.6)	4.37 d (7.5)	4.36 d (7.5)	4.43 d (7.5)	4.41 d (7.5)
2′	2.88 dd (7.7, 9.0)	2.88 dd (7.5, 8.9)	3.19 t (9.0)	3.17 dd (7.6, 9.0)	2.81 dd (7.6, 9.1)
3′	3.34 t (8.0)	3.33 m[b]	3.31 m[b]	3.30 m[b]	3.30 m[b]
4′	3.46 m	3.47 m	3.47 m	3.47 m	3.46 m
5′	3.81 dd (5.5, 11.6)	3.82 dd (5.4, 11.6)	3.82 dd (5.4, 11.4)	3.82 dd (5.4, 11.4)	3.80 dd (5.4, 11.5)
	3.15 dd (9.7, 11.3)	3.14 dd (10.4, 11.3)	3.18 t (11.1)	3.20 dd (10.5, 11.4)	3.16 t (11.2)
2′-OMe	3.60 s	3.61 s			3.57 s

[a] Assignments from ^{1}H-^{1}H COSY, HSQC, HMBC and ROESY data.
[b] Overlapped with the solvent signal.

BuOH/EtOH/H_2O (4:1:2) were used for thin-layer chromatography.

2.2. Animal material

Specimens of *Hippasteria kurilensis* Fisher, 1911 (order Valvatida, family Goniasteridae) were collected by dredging in July 2003 at a depth of 100 m at near Matua Island (Kuril Islands) in the Sea of Okhotsk (research vessel *Akademik Oparin*, 29th scientific cruise). Species identification was carried out by Dr. A.V. Smirnov (Zoological Institute of the Russian Academy of Science, St. Petersburg, Russia). A voucher specimen [no. 029-26] is on deposit at the marine specimen collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

2.3. Extraction and isolation

The fresh animals (770 g) were chopped and extracted twice with EtOH at 20 °C. The water–ethanol layer was evaporated, and the residue was dissolved in H_2O (1 L). The H_2O-soluble fraction was passed through an Amberlite XAD-2 column (7 cm × 20 cm) and eluted with distilled H_2O until a negative chloride ion reaction was obtained, followed by elution with EtOH. The combined EtOH eluate was evaporated to give a brownish material (3.4 g). The resulting total fraction of steroidal compounds was chromatographed on a Si gel column (4 cm × 18 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:6) and than obtained fractions were purified on a Florisil column (2.5 cm × 15 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1 → 1:2). HPLC separation of collected subfractions, containing nonsulfated steroids, on a Kromasil 100A-C18