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Asterosaponins from the Far Eastern starfish *Leptasterias ochotensis* and their anticancer activity

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ABSTRACT

Six new asterosaponins, leptasteriosides A–F (**3**–**8**), one new and one previously known asterogenins (**1**, **2**) were isolated from the alcoholic extract of the Far Eastern starfish *Leptasterias ochotensis*. The structures of **1**–**8** were elucidated by extensive NMR and ESI-MS techniques. Compounds **2**–**8** showed slight or moderate cytotoxic activities against cancer cell lines RPMI-7951 and T-47D. The asterosaponins **3**–**5** demonstrated a significant inhibition of RPMI-7951 and T-47D cell colony formation in soft agar clonogenic assay in nontoxic doses.

1. Introduction

The low molecular weight metabolites from the starfish (phylum Echinodermata, class Asteroidea) are characterized by a remarkable diversity of polar steroids, including polyhydroxysteroids and related mono- and biosides as well as steroid oligoglycosides named as asterosaponins [1–6]. These substances usually represent very complicated mixtures, whose separations into individual components are rather difficult. The majority of asterosaponins contains 3β,6α-dihydroxysteroid aglycones with the 9(11)-double bond and an *O*-sulfate group at C-3. Their carbohydrate chains include, as a rule, five or six monosaccharide units attached to C-6 of the aglycon. Steroid metabolites from starfish, especially glycosides, have been reported to show a wide spectrum of biological activities, including cytotoxic, antiviral, antibacterial, antibiofouling, neuritogenic and antifungal effects [1–6]. Recently, we have shown that some of the new asterosaponins from the starfish *Hippasteria kurilensis*, *Asteropsis carinifera* and *Lethasterias fusca* exhibit a significant inhibition of the human tumor HT-29, HCT-116, RPMI-7951, and T-47D cells colony formation in soft agar clonogenic assay [7–9].

So far, polar steroids of the Far Eastern starfish *Leptasterias ochotensis* (order Forcipulatida, family Asteriidae) have not been studied. Herein, we report results of our study on the asterosaponin fractions from the alcoholic extract of animals collected off the Sea of Okhotsk near Shantar Islands. We describe the structures of new steroid compounds **1**, **3**–**8**, which were isolated along with known asterogenin **2**, as well as their cytotoxic properties against human cancer cell lines RPMI-7951 and T-47D, and the capabilities of compounds **3**–**5** to inhibit the colony formation of RPMI-7951 and T-47D cells in a soft agar clonogenic assay *in vitro*.

2. Experimental

2.1. General methods

Optical rotations were determined on a PerkinElmer 343 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 700 spectrometer at 700.13 and 176.04 MHz, respectively, with tetramethylsilane used as an internal standard. 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). The CD spectrum was determined on a Jasco 500A spectropolarimeter in MeOH. HPLC separations were carried out on an Agilent 1100 Series chromatograph that was equipped with a differential refractometer; the Diasfer-110-C18 (10 μm, 250 mm × 15 mm) and Discovery C_{18} (5 μm, 250 mm × 10 mm) columns were used. GC analysis was conducted on an Agilent 6580 Series apparatus, with the carrier gas He (1.7 mL/min) at 100 °C (0.5 min) to 250 °C (5 °C/min, 10 min) and the capillary

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column HP-5 MS (30 m × 0.25 mm), the length of the program was 45 min. The temperatures of the injector and the detector were 150 and 280 °C, respectively. Low-pressure column liquid chromatography was performed with the Polychrom 1 (powdered Teflon, Biolar, Latvia), Si gel KSK (50–160 μm, Sorbpolimer, Krasnodar, Russia). Sorbfil silica gel plates (4.5 × 6.0 cm, 5–17 μm, Sorbpolimer, Krasnodar, Russia) were used for thin-layer chromatography. The mediums Minimum Essential Medium Eagle (MEM), Roswell Park Memorial Institute (RPMI-1640), fetal bovine serum (FBS), L-glutamine, and gentamicin were purchased from Biolot (Russia). The CellTiter 96 nonradioactive cell proliferation assay kit was purchased from Promega (Madison, WI).

2.2. Animal material

Specimens of *L. ochotensis* Brandt, 1851 (order Forcipulatida, family Asteriidae) were collected at a depth of 20–40 m in the Sea of Okhotsk near the Island of Bolshoy Shantar during the research vessel *Akademik Oparin* 29th scientific cruise in August 2003. Species identification was carried out by Dr. A.V. Smirnov (Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia). A voucher specimen [no. 029-052] is on deposit at the marine specimen collection of the G.B. Elyakov Pacific Institute of Bioorganic Chemistry of the FEB RAS, Vladivostok, Russia.

2.3. Extraction and isolation

The fresh animals (0.35 kg) were chopped and extracted twice with EtOH at 20 °C. The H_2O/EtOH layer was evaporated, and the residue was dissolved in H_2O (0.5 L). The H_2O-soluble materials were passed through a Polychrom 1 column (6.5 cm × 21 cm), eluted with distilled H_2O until a negative chloride ion reaction was obtained, and eluted with EtOH. The combined EtOH eluate was evaporated to give a brownish residue (4.8 g). This material was chromatographed over a silica gel column (4 cm × 20 cm) using $CHCl_3$/EtOH (stepwise gradient, 8:1–1:2) to yield six fractions, 1–6, that were then analyzed by TLC on silica gel plates in the eluent system BuOH/EtOH/H_2O (4:1:2). Fractions 1, 2, 4, and 5 mainly contained the free polyhydroxysteroids and related glycosides, fraction 3 mainly contained the free sulfated asterogenins, and fraction 6 mainly contained the asterosaponins. HPLC separation of fraction 3 on a Diasfer-110-C18 column (10 μm, 250 mm × 15 mm, 2.5 mL/min) with EtOH/H_2O (65:35) as an eluent system followed by the further separation on a Discovery C_{18} column (5 μm, 250 mm × 10 mm, 2.5 mL/min) with MeOH/H_2O (70:30) as an eluent system yielded pure **1** (2.0 mg, t_R 16.5 min, R_f 0.77) and **2** (1.3 mg, t_R 22.4 min, R_f 0.79). HPLC separation of fraction 6 on a Diasfer-110-C18 column (10 μm, 250 mm × 15 mm, 2.5 mL/min) with EtOH/H_2O (60:40) as an eluent system and further separation on a Discovery C_{18} column (5 μm, 250 mm × 10 mm, 2.5 mL/min) with MeOH/H_2O (70:30) as an eluent system gave pure **3** (1.8 mg, t_R 12.3 min, R_f 0.48), **4** (1.7 mg, t_R 13.8 min, R_f 0.48), **5** (4.5 mg, t_R 11.7 min, R_f 0.47), **6** (1.5 mg, t_R 11.3 min, R_f 0.48), **7** (1.8 mg, t_R 13.1 min, R_f 0.46), and **8** (1.8 mg, t_R 14.2 min, R_f 0.46).

2.4. Spectral data of new compounds

2.4.1. (23S)-6α,23-Dihydroxy-5α-cholesta-9(11),20(21)-dien-3β-yl sulfate, sodium salt (*1*)

$C_{27}H_{43}O_6SNa$, amorphous powder; $[\alpha]_D^{25}$ − 8.5° (c 0.2, MeOH); the ^1H and ^{13}C NMR data are listed in Tables 1 and 2; (+)HR ESI-MS *m/z* 541.2587 $[M+Na]^+$ (calcd. for $C_{27}H_{43}O_6SNa_2$, 541.2583); (−)HR ESI-MS *m/z* 495.2771 $[M-Na]^-$ (calcd. for $C_{27}H_{43}O_6S$, 495.2780).

2.4.2. (22E)-6α-Hydroxy-5α-cholesta-9(11),20(22)-dien-23-one-3β-yl sulfate, sodium salt (*2*)

$C_{27}H_{41}O_6SNa$, amorphous powder; $[\alpha]_D^{25}$ − 12.0° (c 0.2, MeOH); ^1H and ^{13}C NMR data are listed in Tables 1 and 2; (+)HR ESI-MS *m/z* 539.2441 $[M+Na]^+$ (calcd. for $C_{27}H_{41}O_6SNa_2$, 539.2414); (−)HR ESI-MS *m/z* 493.2625 $[M-Na]^-$ (calcd. for $C_{27}H_{41}O_6S$, 493.2629).

2.4.3. Leptasterioside B (*3*)

$C_{57}H_{93}O_{26}SNa$, amorphous powder; $[\alpha]_D^{25}$ + 3.0° (c 0.18, MeOH); ^1H and ^{13}C NMR data are listed in Tables 1–3; (+)ESI-MS/MS of the ion $[M+Na]^+$ at *m/z* 1271: 1151 $[(M+Na)-NaHSO_4]^+$, 1005 $[(M+Na)-NaHSO_4-C_6H_{10}O_4]^+$, 859 $[(M+Na)-NaHSO_4-2\times C_6H_{10}O_4]^+$, 713 $[(M+Na)-NaHSO_4-3\times C_6H_{10}O_4]^+$; (+)HR ESI-MS *m/z* 1271.5481 $[M+Na]^+$ (calcd. for $C_{57}H_{93}O_{26}SNa_2$, 1271.5466); (−)ESI-MS/MS of the ion $[M-Na]^-$ at *m/z* 1225: 1079 $[(M-Na)-C_6H_{10}O_4]^-$, 933 $[(M-Na)-2\times C_6H_{10}O_4]^-$, 787 $[(M-Na)-3\times C_6H_{10}O_4]^-$, 655 $[(M-Na)-3\times C_6H_{10}O_4-C_5H_8O_4]^-$, 509 $[(M-Na)-4\times C_6H_{10}O_4-C_5H_8O_4]^-$, 491 $[(M-Na)-4\times C_6H_{10}O_4-C_5H_8O_4-H_2O]^-$, 97 $[HSO_4]^-$; (−)HR ESI-MS *m/z* 1225.5678 $[M-Na]^-$ (calcd. for $C_{57}H_{93}O_{26}S$, 1225.5681).

2.4.4. Leptasterioside A (*4*)

$C_{56}H_{91}O_{26}SNa$, amorphous powder; $[\alpha]_D^{25}$ + 7.5° (c 0.17, MeOH); ^1H and ^{13}C NMR data are listed in Tables 1–3; (+)ESI-MS/MS of the ion $[M+Na]^+$ at *m/z* 1257: 1137 $[(M+Na)-NaHSO_4]^+$, 1111 $[(M+Na)-C_6H_{10}O_4]^+$, 991 $[(M+Na)-NaHSO_4-C_6H_{10}O_4]^+$, 965 $[(M+Na)-2\times C_6H_{10}O_4]^+$, 845 $[(M+Na)-NaHSO_4-2\times C_6H_{10}O_4]^+$; (+)HR ESI-MS *m/z* 1257.5302 $[M+Na]^+$ (calcd. for $C_{56}H_{91}O_{26}SNa_2$, 1257.5309); (−)ESI-MS/MS of the ion $[M-Na]^-$ at *m/z* 1211: 1065 $[(M-Na)-C_6H_{10}O_4]^-$, 919 $[(M-Na)-2\times C_6H_{10}O_4]^-$, 773 $[(M-Na)-3\times C_6H_{10}O_4]^-$, 641 $[(M-Na)-3\times C_6H_{10}O_4-C_5H_8O_4]^-$, 495 $[(M-Na)-4\times C_6H_{10}O_4-C_5H_8O_4]^-$, 477 $[(M-Na)-4\times C_6H_{10}O_4-C_5H_8O_4-H_2O]^-$, 97 $[HSO_4]^-$; (−)HR ESI-MS *m/z* 1211.5519 $[M-Na]^-$ (calcd. for $C_{56}H_{91}O_{26}S$, 1211.5525).

2.4.5. Leptasterioside C (*5*)

$C_{58}H_{95}O_{27}SNa$, amorphous powder; $[\alpha]_D^{25}$ + 15.7° (c 0.45, MeOH); ^1H and ^{13}C NMR data are listed in Tables 1–3; (+)ESI-MS/MS of the ion $[M+Na]^+$ at *m/z* 1301: 1181 $[(M+Na)-NaHSO_4]^+$, 1155 $[(M+Na)-C_6H_{10}O_4]^+$, 1035 $[(M+Na)-NaHSO_4-C_6H_{10}O_4]^+$, 1009 $[(M+Na)-2\times C_6H_{10}O_4]^+$, 889 $[(M+Na)-NaHSO_4-2\times C_6H_{10}O_4]^+$, 743 $[(M+Na)-NaHSO_4-3\times C_6H_{10}O_4]^+$, 701 $[(M+Na)-3\times C_6H_{10}O_4-C_6H_{10}O_5]^+$; (+)HR ESI-MS *m/z* 1301.5548 $[M+Na]^+$ (calcd. for $C_{58}H_{95}O_{27}SNa_2$, 1301.5571); (−)ESI-MS/MS of the ion $[M-Na]^-$ at *m/z* 1255: 1109 $[(M-Na)-C_6H_{10}O_4]^-$, 963 $[(M-Na)-2\times C_6H_{10}O_4]^-$, 817 $[(M-Na)-3\times C_6H_{10}O_4]^-$, 655 $[(M-Na)-3\times C_6H_{10}O_4-C_6H_{10}O_5]^-$, 509 $[(M-Na)-4\times C_6H_{10}O_4-C_6H_{10}O_5]^-$, 491 $[(M-Na)-4\times C_6H_{10}O_4-C_6H_{10}O_5-H_2O]^-$, 97 $[HSO_4]^-$; (−)HR ESI-MS *m/z* 1255.5788 $[M-Na]^-$ (calcd. for $C_{58}H_{95}O_{27}S$, 1255.5787).

2.4.6. Leptasterioside D (*6*)

$C_{57}H_{91}O_{27}SNa$, amorphous powder; $[\alpha]_D^{25}$ + 9.2° (c 0.15, MeOH); ^1H and ^{13}C NMR data are listed in Tables 1–3; (+)ESI-MS/MS of the ion $[M+Na]^+$ at *m/z* 1285: 1165 $[(M+Na)-NaHSO_4]^+$, 1139 $[(M+Na)-C_6H_{10}O_4]^+$, 1019 $[(M+Na)-NaHSO_4-C_6H_{10}O_4]^+$, 993 $[(M+Na)-2\times C_6H_{10}O_4]^+$, 873 $[(M+Na)-NaHSO_4-2\times C_6H_{10}O_4]^+$, 847 $[(M+Na)-3\times C_6H_{10}O_4]^+$, 727 $[(M+Na)-NaHSO_4-3\times C_6H_{10}O_4]^+$, 685 $[(M+Na)-3\times C_6H_{10}O_4-C_6H_{10}O_5]^+$; (+)HR ESI-MS *m/z* 1285.5260 $[M+Na]^+$ (calcd. for $C_{57}H_{91}O_{27}SNa_2$, 1285.5258); (−)ESI-MS/MS of the ion $[M-Na]^-$ at *m/z* 1239: 1093 $[(M-Na)-C_6H_{10}O_4]^-$, 947 $[(M-Na)-2\times C_6H_{10}O_4]^-$, 801 $[(M-Na)-3\times C_6H_{10}O_4]^-$, 639 $[(M-Na)-3\times C_6H_{10}O_4-C_6H_{10}O_5]^-$, 493 $[(M-Na)-4\times C_6H_{10}O_4-C_6H_{10}O_5]^-$, 475 $[(M-Na)-4\times C_6H_{10}O_4-C_6H_{10}O_5-H_2O]^-$, 97 $[HSO_4]^-$; (−)HR ESI-MS *m/z* 1239.5470 $[M-Na]^-$ (calcd. for $C_{57}H_{91}O_{27}S$, 1239.5474).