Novel polyhydroxysteroidal glycoside from the starfish *Leptasterias polaris acervata*

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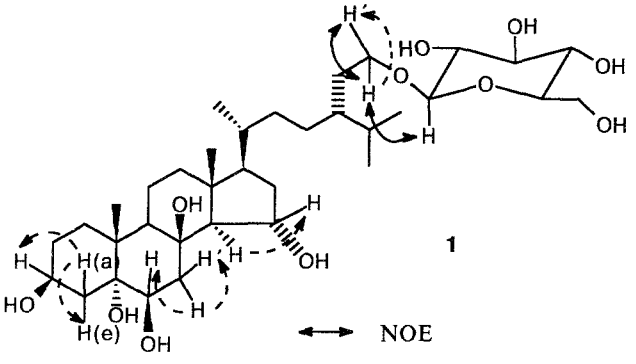
Leptasteroside L ((24*R*)-29-*O*-(β-D-glucopyranosyl)-24-ethyl-5α-cholestane-3β,5,6β,8,15α,29-hexaol), a novel polyhydroxysteroidal glycoside, was isolated from the starfish *Leptasterias polaris acervata* and characterized. Deoxyguanosine, thymidine, deoxyadenosine, tryptophan, and tyramine were isolated from the extracts.

Key words: starfish, *Leptasterias polaris*, glycoside, polyhydroxysteroid, nucleosides, tyramine, tryptophan.

In continuation of our studies on highly hydroxylated steroidal starfish metabolites,[1,2] we isolated a total fraction of polyhydroxylated steroids from the methanolic extract of the starfish *Leptasterias polaris acervata (Stimpson)* using column chromatography on Amberlite XAD-2, Sephadex LH-20, silica gel, and Florisil. However, subsequent high-performance liquid chromatography (HPLC) on a Zorbax ODS column failed to prepare pure compounds. Hence, the subfractions obtained by this method were subjected to solvolytic desulfation. Repeated separation of one of them on a Florisil column afforded a new steroidal glycoside leptasteroside L (**1**) (0.0003 % per crude weight of animal tissue).



1

The structure of glycoside **1** was established mainly by the ^1H and ^{13}C NMR spectroscopy methods. Acid hydrolysis of **1** afforded a monosaccharide, which was identified as D-glucose. In the ^{13}C NMR spectrum of glycoside **1**, the signals of the monosaccharide fragment are close to the corresponding signals of methyl β-D-glucopyranoside (see Experimental), thus attesting to β-configuration of the glycosidic bond and the presence of the pyranose ring in the monosaccharide residue of compound **1** (*cf.* Ref. 3). The positions of hydroxyl groups in aglycone and the relative configurations of the corresponding chiral centers were determined by comparison of the chemical shifts (δ) of carbon and hydrogen atoms and spin-coupling constants (*J*) in the spectra of **1** and model compounds, *viz.*, 24-ethyl-5α-cholestane-3β,5,6β,8,15α,28,29-heptaol-29-sulfate (**2**) from the starfish *Ctenodiscus crispatus*[2] and solasteroside S_2 (**3**) from the starfish *Solaster dawsoni*.[1] Thus, δ of C(1)—C(19) and of the characteristic signals of the Me(18), Me(19), HC(3), HC(6), and HC(15) protons and *J* in the spectra of compound **1** coincide with those of polycyclic part of the spectra of **2** suggesting the identity of substitution in A, B, C, and D rings of compounds **1** and **2**. The signals of carbon atoms in the side chain of glycoside **1** coincide with the corresponding signals in the ^{13}C NMR spectrum of compound **3**, thus indicating the presence (24*R*)-24-ethylcholestane side chain with the oxygen function at C(29) in **1**. The irradiation of the HC(29) proton under conditions of the nuclear Overhauser effect (NOE) causes increase in H'C(29) and HC(1') signal intensity (the latter signal belongs to the carbohydrate fragment). This fact indicates an attachment of the glucopyranosyl residue at C(29) in the side chain.

The 3β,5,6β,8,15α-positions of five hydroxyl groups in **1** were confirmed by difference decoupling. These experimental data are presented in Fig. 1. On the basis of the data obtained, the structure of (24*R*)-29-*O*-(β-D-glucopyranosyl)-24-ethyl-5α-cholestane-3β,5,6β,8,15α,29-hexaol was assigned to glycoside **1** (Fig. 1).

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 6, pp. 1164—1166, June, 1995.

1066-5285/95/4406-1125 $12.50

In addition to glycoside **1**, we isolated several nucleosides and some known metabolites from the methanolic extract of *L. polaris acervata*. The compounds were identified by comparison of their ^{1}H NMR and mass spectra with those of reference compounds. Thus, deoxyguanosine (0.0022 %), thymidine (0.00028 %), and deoxyadenosine (0.00012 %) were obtained by multiple column chromatography on silica gel. Application of HPLC on a Zorbax ODS column (after chromatography on silica gel) gave tyramine (0.0003 %), and introduction of an additional stage of HPLC on silica gel afforded tryptophan (0.00012 %).

Glycoside **1** has the structure of the side chain being rare for starfish steroidal glycosides. Previously, the side chain of 29-hydroxystigmastane type glycosylated with glucopyranose residue has been found only in pisasteroside F from *Pisaster giganteus*.[4] However, this glycoside differs from that of isolated by us in the polycyclic part of the aglycone. The nucleosides were isolated previously from the starfishes *Acantaster planci* [5] and *Dermasterias imbricata*,[6] free tryptophan and tryptamine from the starfish *Nardoa navaecaledonia*,[7] and tyramine in conjugate with sulfated steroid from the extract of *Asterina pectinifera*.[8]

Experimental

The ^{1}H and ^{13}C NMR spectra were registered with a Bruker WM-250 spectrometer. HPLC was carried out with a Du Pont model 8800 chromatograph (refractometric detector) using Zorbax ODS (5μ, 250×4.6 mm) and Altex Ultrasphere-Si (5μ, 250×10.0 mm) columns.

The samples of starfishes were collected by a dredge of the trawler "Dmitrov" in June, 1990, near Onecotan Island (Kuril Islands, Pacific Ocean) from 20—100 m depth and were classified by A. V. Smirnov (Institute of Zoology, RAS, St. Petersburg).

Thin layer chromatography (TLC) was carried out on glass plates (4.5×6.0 cm) with a fixed layer of silica gel L (Chemapol, Czecho-Slovakiya).

Isolation of leptasteroside L (1). The methanolic extract of starfishes (5.0 kg of biomass) was concentrated *in vacuo*; the residue was dissolved in 1.0 L of water and passed through a column with Amberlite XAD-2 resin. The column was washed with water and then with methanol. The methanolic eluate was evaporated. The obtained total fraction of steroidal compounds of nucleosides, amino acids, and other accompanying compounds was chromatographed sequentially on columns with Sephadex LH-20 in a chloroform—methanol (1 : 1) system and with silica gel in a chloroform—methanol (10 : 1—1 : 1) gradient system. Increase in polarity of the eluent gave fractions containing thymidine, deoxyadenosine, deoxyguanosine, tryptophan, tyramine, and sulfated leptasteroside L (TLC, butanol—ethanol—water, 4 : 1 : 2, R_f = 0.5). The subsequent purification of the fraction containing sulfated leptasteroside L was carried out on a Florisil column with a chloroform—methanol system (2 : 1) and by HPLC on a Zorbax ODS column with methanol—water (17 : 3). In accordance with the ^{1}H NMR data, the chromatographically isolated fraction contained steroidal glycoside bearing the sulfate group presumably at the monosaccharide residue and an admixture of the other compound. This fraction was desulfated by dissolution in a pyridine—dioxane mixture (1 : 1) and heating in dry atmosphere for 2.5 h at 85—95 °C. The completion of the reaction was determined by increase in chromatographic mobility of the major product *vs.* the parent compound (TLC). The solvents were removed *in vacuo*, the residue was chromatographed on a Florisil column in a chloroform—methanol system (7:3). Amorphous compound **1** (16 mg, 0.0003 %), $C_{35}H_{62}O_{11}$, $[\alpha]_D$ −3.3° (c 0.15, methanol) was obtained.

^{1}H NMR (C_5D_5N), δ: 0.79 (d, 3 H, Me(26), J = 6.7 Hz); 0.81 (d, 3 H, Me(27), J = 6.7 Hz); 0.98 (3 H, Me(21), J = 6.0 Hz); 1.33 (s, 3 H, Me(18)); 1.75 (d, 1 H, HC(14), J = 9.5 Hz); 1.85 (s, 3 H, Me(19)); 2.31 (d.d, 1 H, H(e)C(4), ^1J = 5.5 Hz, ^2J = 12.5 Hz); 2.95 (d.d, 1 H, H(a)C(4), ^1J = 11.0 Hz, ^2J = 12.5 Hz); 3.03 (d.d, 1 H, HC(7), ^1J = 3.0 Hz, ^2J = 14.6 Hz); 3.15 (d.d, 1 H, H'C(7), ^1J = 3.0 Hz, ^2J = 14.6 Hz); 3.75 (t.d, 1 H, HC(29), ^1J = 6.4 Hz, ^2J = 9.1 Hz, ^3J = 9.1 Hz); 4.27 (m, 1 H, H'C(29)); 4.33 (t, 1 H, HC(6), J = 3.0 Hz); 4.93 (m, 1 H, HC(3)); 4.93 (m, 1 H, HC(15)); 4.90 (d, 1 H, HC(1'), J = 8.2 Hz); 4.07 (d.d, 1 H, HC(2'), ^1J = 7.8 Hz, ^2J = 8.6 Hz); 4.27 (m, 1 H, HC(3')); 4.27 (m, 1 H, HC(4')); 4.0 (m, 1 H, HC(5')); 4.40 (d.d, 1 H, HC(6'), ^1J = 2.7 Hz, ^2J = 11.9 Hz); 4.58 (d.d, 1 H, H'C(6'), ^1J = 5.2 Hz, ^2J = 11.9 Hz).

^{13}C NMR (C_5D_5N), δ: 31.6 C(1); 34.2 C(2); 67.2 C(3); 42.4* C(4); 75.7 C(5); 78.1 C(6); 41.7 C(7); 76.4 C(8); 48.6 C(9); 39.0 C(10); 19.4 C(11); 42.2* C(12); 44.8 C(13); 66.3 C(14); 69.1 C(15); 40.8 C(16); 55.1 C(17) 15.5 C(18); 18.0 C(19); 35.6 C(20); 18.9 C(21); 34.0 C(22); 27.6 C(23); 41.4 C(24); 29.8 C(25), 19.4 C(26); 19.6 C(27); 31.5 C(28); 69.0 C(29); 104.6 C(1'); 75.1 C(2'); 77.7 C(3'); 71.9 C(4'); 78.5 C(5'); 63.0 C(6').

Hydrolysis of glycoside 1. Acid hydrolysis of compound **1** was carried out in 2 *N* HCl at 100 °C for 2 h. D-Glucose was identified in the hydrolyzate by TLC (silica gel, butanol—acetone—water, 4 : 5 : 1) and GLC (as the aldononitrile acetate).

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* The assignment of the signals may be interchanged.

Received December 1, 1994;
in revised form February 1, 1995