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A new hexahydroxysteroid from the Far Eastern starfish *Luidiaster dawsoni*

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A new polyhydroxysteroid was isolated from the starfish *Luidiaster dawsoni*; the structure of the product was established as (24*S*,25*R*)-24-methylcholestane-3β,5α,6β,15α,16β,26-hexaol. A mixture of methyl-α- and -β-D-glucopyranosides was also isolated from the extract of this starfish.

Key words: starfish, *Luidiaster dawsoni*, polyhydroxysteroid.

The starfish, unlike other animals, contains highly oxidized steroids with five to nine hydroxyl groups in a molecule.[1] As a continuation of studies dealing with highly hydroxylated steroid metabolites of the Far Eastern starfish,[2,3] we isolated a new steroid hexaol (**1**) in a yield of $9 \cdot 10^{-5}$% of the crude animal weight from a methanolic extract of the starfish *Luidiaster dawsoni*. Compound **1** was prepared and purified using successive chromatography on Amberlite XAD-2, Sephadex LH-20, silica gel, and Phlorisil followed by high-performance liquid chromatography (HPLC) on the inverted phase Silasorb C_{18}.

The structure of compound **1** was established by ^{1}H NMR spectroscopy. The arrangement of the hydroxyl groups in the steroid nucleus was determined in spin-decoupling experiments, whose results are presented in Fig. 1. The configurations of substituents were established based on the spin-spin coupling constants of protons (see Experimental). Based on the results obtained, we found that the hydroxyl groups in the steroid nucleus of compound **1** occupy the 3β,5α,6β,15α,16β positions. The structure of the steroid nucleus was confirmed by a comparison of the proton chemical shifts and spin-spin coupling constants in the ^{1}H NMR spectra (C_5D_5N) of hexaol **1** and the polyhydroxysteroids with a similar steroid nucleus that we isolated previously from the *Ctenodiscus crispatus* starfish.[3]

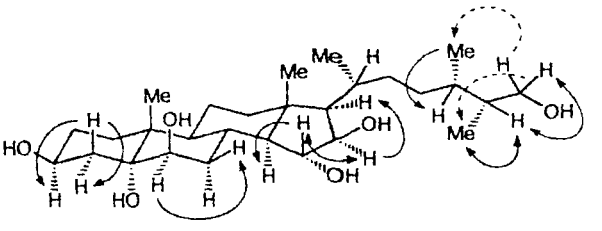

Fig. 1. Arrangement of hydroxyl groups in the steroid nucleus. The arrows show the difference spin decoupling and the dashed arrows correspond to the nuclear Overhauser effect.

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The structure of the side chain in compound **1** was determined using the difference spin decoupling method and the nuclear Overhauser effect (NOE) (see Fig. 1). Irradiation of the $H_2C(26)$, $H_3C(27)$, and $H_3C(28)$ protons caused response of the signals of the neighboring protons in the difference spectra. Irradiation of the $H_2C(26)$ protons resulted in the NOE signals for the $H_3C(27)$ and $H_3C(28)$ protons.

The chemical shifts of the side-chain protons HC(26) δ 3.49, H′C(26) δ 3.38, $H_3C(27)$ δ 0.82, and $H_3C(28)$ δ 0.82 in the ^1H NMR spectrum (CD_3OD) of compound **1** coincided with those of the corresponding signals in the spectrum of (24*S*,25*R*)-26-hydroxy-24-methylsteroid, one of the four possible stereoisomers of 26-hydroxy-24-methylsteroids synthesized in order to establish the configuration of the C(24) and C(25) asymmetric centers in the side chain and the chemical shifts of the protons at C(26), C(27), and C(28), which differ from one another.[1]

Thus, the isolated compound **1** was identified as (24*S*,25*R*)-24-methylcholestane-3β,5α,6β,15α,16β,26-hexaol.

In addition to compound **1**, a mixture of methyl-α- and -β-D-glucopyranosides present in a ratio of 2 : 1 ($4 \cdot 10^{-5}$%) was isolated from the methanolic extract of *L. dawsoni* using similar techniques; these products were identified by a comparison of their ^1H and ^{13}C NMR spectra and ^1H NMR spectra of their acetates with the spectra of reference compounds. In starfishes, glucose residues are incorporated into steroid glycosides,[3] cerebrosides,[4] and sialoglycolipids[5]. Glucose had not been isolated previously from starfish either free or as the corresponding methylglycosides. In our opinion, most likely, methylglycosides are native compounds in this case, although their artifact origin, *viz.*, formation from glucose during isolation, also cannot be ruled out.

Experimental

^1H NMR spectra were recorded on a Bruker WM-250 spectrometer using $SiMe_4$ as the internal standard. Optical rotation was measured on a Perkin—Elmer 141 polarimeter. HPLC was performed on a Du Pont Model 8800 chromatograph (with refractometer as the detector) using a column with Silasorb C_{18} (13 mm, 250×9.4 mm).

Thin layer chromatography (TLC) was carried out on glass plates (4.5×6.0 cm) with a fixed layer of Silica gel L (Chemapol, Czech Republic).

Starfish specimens were gathered in June 1993 onboard the ship "Akademik Oparin" (trip No. 17) near the Atlasov island (the Kuril Islands, the Sea of Okhotsk) at a depth of 250 m and identified by A. V. Smirnov (Zoological Institute of the RAS, St. Petersburg).

Isolation of steroid 1. Ground and lyophilically dried starfishes (the weight of the animals was 2.3 kg) were subjected to exhaustive extraction with methanol at ~20 °C. The combined extracts were concentrated *in vacuo*, and the residue was dissolved in 1.0 L of water and passed through a column filled with the Amberlite XAD-2 resin (6×30 cm). The column was washed with water (1.5 L) and methanol (3.0 L), and the methanolic eluate was concentrated. The resulting total fraction of steroid compounds was successively chromatographed on columns with Sephadex LH-20 (3×50 cm) in the methanol—water (2 : 1) system, silica gel (4×18 cm) in the chloroform—methanol system (5 : 1 → 1 : 2), and Phlorisil (2×15 cm) in the chloroform—methanol (5 : 1) system. This gave a fraction containing compound **1** (TLC, chloroform—methanol—water, 30 : 15 : 2, R_f 0.7). Then the fraction was purified by HPLC on a column with Silasorb C_{18}; the product was eluted with a methanol—water mixture (3 : 1). This gave 3 mg of compound **1**, $C_{28}H_{50}O_6$, $[\alpha]_D$ ±0° (*c* 0.2, methanol).

^1H NMR (C_5D_5N), δ: 0.84 (d, 3 H, Me(28), J = 6.5 Hz); 0.92 (d, 3 H, Me(27), J = 6.5 Hz); 1.11 (d, 3 H, Me(21), J = 6.3 Hz); 1.30 (s, 3 H, Me(18)); 1.74 (s, 3 H, Me(19)); 1.86 (m, 1 H, HC(24)); 2.83 (m, 2 H, $H_2C(7)$); 3.00 (dd, 1 H, $H_aC(4)$, J = 10.9, 12.5 Hz); 3.73 (m, 2 H, $H_2C(26)$); 4.25 (t, 1 H, HC(6), J = 2.9 Hz); 4.45 (dd, 1 H, HC(15), J = 1.8, 10.4 Hz); 4.68 (dd, 1 H, HC(16), J = 1.8, 7.6 Hz); 4.83 (m, 1 H, HC(3)).

MS (EI, 70 эВ), m/z (I_{rel} (%)): 464 $[M-H_2O]^+$ (25), 446 $[M-2\ H_2O]^+$ (38), 428 $[M-3\ H_2O]^+$ (50), 410 $[M-4\ H_2O]^+$ (25), 371 (38), 354 (50), 214 (100).

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