Steroid polyols from the Far-Eastern starfish *Henricia sanguinolenta*

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A new polyhydroxylated steroid (20*R*,24*S*)-5α-cholestane-3β,6β,15β,24-tetraol and a known glycoside, laeviuscoloside G, was isolated from the Far-Eastern starfish *Henricia sanguinolenta*, collected in the sea of Okhotsk, and characterized.

Key words: starfish *Henricia sanguinolenta*, polyhydroxysteroids, glycosides, aglycons, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, mass spectrometry.

While continuing the research into physiologically active steroids from starfishes, we studied steroids from the Far-Eastern starfish *Henricia sanguinolenta* (Spinulosida genus, Echinasteridae family). Here we report the isolation of two compounds; one of them (**1**) proved to be a new steroidal polyol, while the other (**2**) was a known glycosylated polyoxysteroid isolated previously from starfishes *Henricia laeviuscola*.[1]

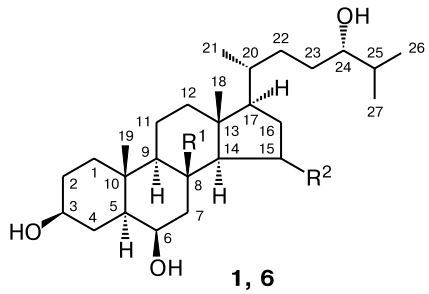
Results and Discussion

Steroid compounds **1** and **2** were isolated from an ethanolic extract of starfishes by repeated column chromatography on Polychrom, Sephadex LH-60, Florisil, and silica gel followed by HPLC on columns with re-

Table 1. ^{13}C NMR polyol **1** (Bruker DPX-500, C_5D_5N)

Atom	Fragment*	δ_C	Atom	Fragment*	δ_C
C(1)	CH_2	39.1	C(15)	CH	68.9
C(2)	CH_2	32.6	C(16)	CH_2	42.3
C(3)	CH	71.2	C(17)	CH	56.8
C(4)	CH_2	37.1	C(18)	CH_3	15.0
C(5)	CH	48.5	C(19)	CH_3	16.0
C(6)	CH	71.1	C(20)	CH	36.2
C(7)	CH_2	39.8	C(21)	CH_3	19.1
C(8)	CH	27.2	C(22)	CH_2	32.8
C(9)	CH	55.2	C(23)	CH_2	31.5
C(10)	C	36.0	C(24)	CH	76.2
C(11)	CH_2	21.4	C(25)	CH	33.9
C(12)	CH_2	41.7	C(26)	CH_3	17.3
C(13)	C	42.7	C(27)	CH_3	19.6
C(14)	CH	61.4			

* According to DEPT experiment.



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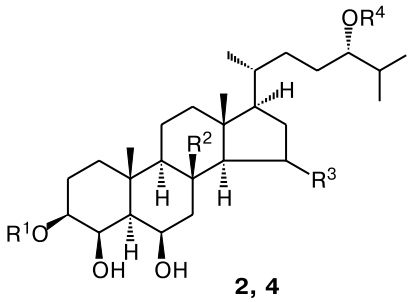

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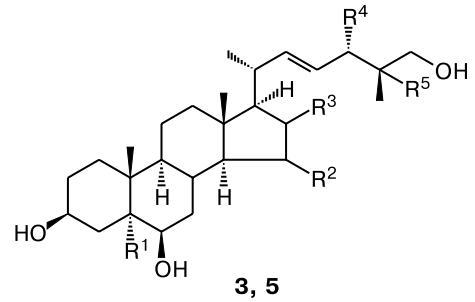

3, 5

1: R^1 = H; R^2 = β-OH
2: R^1 = 2,4-di-*O*-methyl-β-xylopyranosyl; R^2 = OH; R^3 = α-OH; R^4 = α-arabinofuranosyl
3: R^1 = R^4 = R^5 = H; R^2 = α-OH; R^3 = β-OH; 22,23-dihydro
4: R^1 = 2-*O*-methyl-β-xylopyranosyl; R^2 = H; R^3 = β-OH; R^4 = H
5: R^1 = R^5 = OH; R^2 = β-OH; R^3 = H; R^4 = Me
6: R^1 = OH; R^2 = α-OH

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Table 2. Data of ^{1}H NMR spectroscopy for polyoxysteroid **1** (Bruker DPX-500, Bruker AC-250) in various solvents

Proton or group	δ_H (*J*/Hz)	
	C_5D_5N	CD_3OD
$H(1)_{eq}$	1.77 (m)	—
$H(1)_{ax}$	1.12 (m)	—
$H(2)_{eq}$	2.14 (m)	—
$H(2)_{ax}$	1.87 (m)	—
H(3)	4.02 (m, Δw = 22.5)	3.55 (m)
$H(4)_{eq}$	2.08 (m)	—
$H(4)_{ax}$	2.45 (q, *J* = 12.5)	—
H(5)	1.35 (m)	—
H(6)	4.12 (q, *J* = 2.6)	3.78 (q, *J* = 2.6)
$H(7)_{eq}$	2.72 (m)	—
$H(7)_{ax}$	1.42 (m)	—
H(8)	2.74 (m)	—
H(9)	0.87 (m)	—
$C(11)H_2$	1.63 (m); 1.57 (m)	—
$C(12)H_2$	2.07 (m, H_{eq}); 1.23 (m, H_{ax})	—
H(14)	1.00 (dd, *J* = 5.8, 11.1)	—
H(15)	4.46 (ddd, *J* = 1.8, 5.3, 7.6)	4.17 (ddd, *J* = 1.8; 5.3; 7.6)
H(16)	2.58 (m)	—
H(16´)	1.76 (m)	—
H(17)	1.23 (m)	—
$C(18)H_3$	1.29 (s)	0.98 (s)
$C(19)H_3$	1.45 (s)	1.07 (s)
H(20)	1.74 (m)	—
$C(21)H_3$	1.10 (d, *J* = 7.0)	0.96 (d, *J* = 7.2)
$C(22)H_2$	1.31 (m); 2.05 (m)	—
$C(23)H_2$	1.60 (m); 1.82 (m)	—
H(24)	3.61 (m)	3.24 (m)*
H(25)	1.87 (m)	—
$C(26)H_3$	1.12 (d, *J* = 6.7)	0.89 (d, *J* = 6.8)
$C(27)H_3$	1.15 (d, *J* = 6.7)	0.91 (d, *J* = 6.8)

* The signals are partly overlapped by the solvent signals.

versed-phase ODS-A and Diasphere-110-C_{18} sorbents. Structural identification of the isolated compounds was elucidated by NMR spectroscopy and mass spectrometry (FABMS, MALDI-TOF) (Tables 1 and 2).

The quasi-molecular ion with *m/z* 459 $[M + Na]^+$ and fragment ions with *m/z* 401 $[M - 2\ H_2O + H]$ and 383 $[M - 3\ H_2O + H]$ in the positive ion FAB mass spectrum of steroid **1**, together with the data of ^{1}H and ^{13}C NMR spectroscopy established the molecular formula $C_{27}H_{48}O_4$. The ^{13}C NMR and DEPT spectra (see Table 1) indicate the presence of 27 carbon atoms including 5 methyl, 9 methylene, 11 methine, and 2 quaternary C atoms. In view of these data, we proposed that compound **1** is a saturated tetrahydroxycholestane steroid nucleus. The structure of polyol **1** was established by 2D NMR techniques including ^{1}H—^{1}H COSY and HSQC experiments (see Table 1).

The chemical shifts and the corresponding spin-spin coupling constants of C(3)H (δ 3.55) and C(6)H (δ 3.78, q, *J* = 2.6 Hz) (CD_3OD) protons in the NMR spectra of steroid **1**, together with the chemical shifts of the C(1)—C(7), C(9)—C(12), and C(19) atoms (C_5D_5N) coincide with the signals of similar atoms in the spectra of 5α-cholestane-3β,6β,15α,16β,26-pentaol (**3**) isolated from the starfish *Hacelia attenuata*;[2] this points to the presence of a 3β,6β-diol group in polyol **1**. The 15β-position of the third hydroxy group in steroid **1** follows from comparison of its NMR spectra with the spectra of known compounds: moniloside C (**4**) from the starfish *Fromia monilis*[3] and (22*E*)-24-methyl-5α-cholest-22-ene-3β,5,6β,15β,25,26-hexaol (**5**) from *Styracaster caroli*.[4] The chemical shift (see Table 2) and the multiplicity of the corresponding C(15)H signal in the ^{1}H NMR spectrum of steroid **1** (δ_H 4.17, ddd, *J* = 1.8, 5.3, 7.6 Hz) were in good agreement with these values in the spectra of **4** and **5**. In addition, the chemical shifts of the C(8)—C(27) atoms in the ^{13}C NMR spectrum of polyol **1** are close to the corresponding values for moniloside C, which implies that this molecular fragment is the same in both compounds.

The final confirmation of the proposed structure was obtained from analysis of the COSY and HSQC spectra, which showed the sequence of protons in fragment A (Fig. 1) of molecule **1**. The C(15)H and C(14)H coupling constant in the spectrum of **1** is also corresponding to the β-configuration of the hydroxy group ($J_{14,15}$ = 5.3—5.8 Hz, see Table 2). For 15a-hydroxysteroids, this coupling constant occurs[5,6] in the 9.5—11.0 Hz range. The signals of the protons of the side chain form intense cross-peaks in the ^{1}H—^{1}H COSY spectrum: C(25)H (δ_H 1.87)/C(24)H (δ_H 3.61) as well as $C(26)H_3$ (δ_H 1.12) and $C(27)H_3$ (δ_H 1.15)/C(25)H (δ_H 1.87). This confirms the presence of 24-hydroxycholestane side chain in compound **1**.

The configuration of C(24) is usually established by comparing the chemical shifts of the side-chain C atoms of the steroid with the corresponding values in the spectra of (24*S*)- and (24*R*)-24-hydroxycholesterol; the difference between the isomers equals[7] 0.02—0.04 ppm. Since

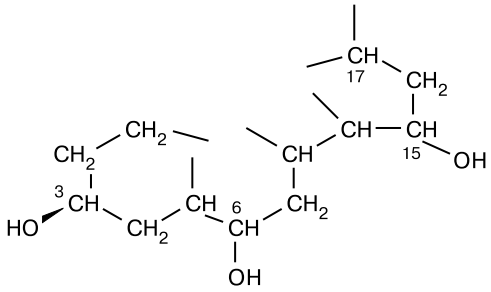

Fig. 1. Fragment A of molecule **1**.

the positions of signals of the C(22)—C(27) atoms in the spectrum of compound **1** (C_5D_5N) were found to coincide with the corresponding values for (24*S*)-5α-cholestane-3β,6β,8,15α,24-hexaol (**6**) from the starfish *Henricia.sp*,[8] we proposed the (*S*)-configuration for the C(24) atom in the compounds we isolated. The $C(21)H_3$ chemical shift is known[7] to depend on the configuration of C(20); for (20*R*)-isomers, these values vary in the range of 0.90—0.97 ppm (CD_3OD). In the ^1H NMR spectra (20*S*)-isomers, the $C(21)H_3$ signals shift upfield by 0.1 ppm and have[9] δ_H ~0.8. Since in the NMR spectrum of steroid **1** (see Table 2), the signal of $C(21)H_3$ occurs at δ_H 0.96, this compound was identified as (20*R*,24*S*)-5α-cholestane-3β,6β,15β,24-tetraol.

3β,6β-Hydroxylation is encountered rather frequently in polar steroids and in cyclic asterosaponins[10] from starfishes; however, the 3β,6β,15β-arrangement of hydroxy groups (as in the new polyhydroxysteroide **1**) has previously been unknown for these compounds.

Generally, steroid tetraols are uncommon for starfishes; penta-, hexa, and- heptaols are found more often. Steroid **1** is a new natural tetraol corresponding to the cholestane series.

The known glycoside **2** was identified as (24*S*)-3-*O*-β-D-(2,4-di-*O*-methyl)xylopyranosyl-24-*O*-α-L-arabinofuranosyl-5α-cholestane-3β,4β,6β,8,15α,24-hexaol (laeviuscoloside G) on the basis of its physical data and the MALDI-TOF and NMR spectra (see Experimental) coinciding with those for the glycoside isolated previously from the starfish *Henricia laeviuscola*.[1]

Experimental

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-250 (^1H, 250 MHz; ^{13}C, 62.9 MHz) and Bruker DPX-500 (^1H, 500 MHz; ^{13}C, 125.7 MHz) spectrometers. The optical rotation was measured on a Perkin—Elmer 141 polarimeter. MALDI-TOF mass spectra were run on a Biflex III mass spectrometer (Bruker, Germany) with laser ionization/desorption (N_2 laser, 337 nm). The sample was dissolved in MeOH (10 mg mL^{-1}) and a 1 μL aliquot was analyzed using α-cyano-hydroxycinnamic acid as the matrix. FAB and EI (70 eV) mass spectra were recorded on an AMD 6041S mass spectrometer (Germany). HPLC was carried out on a Du Pont Model 8800 chromatograph (with a refractometer as the detector) using columns with Diasphere-110-C_{18} (5 μm, 250×4 mm) and YMC-Pack ODS-A (5 μm, 12 nm, 250×10 mm). Melting points were measured on a Boetius stage. Thin layer chromatography was performed on Sorbfil plates with a silica gel CTX-1A (5—17 μm, Krasnodar, Russia) layer fixed on a foil. Preparative column chromatography was carried out on silica gel L (80—100 and 200—250 mesh, Chemapol, Czechia), Sephadex LH-60, Polychrom, and Florisil (200—250 mesh).

Animals. The starfishes *Henricia sanguinolenta* were collected by a trawl net in August 2001 (sea of Okhotsk, Onekotan island) in the 27th trip of the research ship Akademik Oparin from the depth 100—200 m and identified by Prof. V. S. Levin (Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences).

Extraction and isolation and overall fractions. Crushed starfishes (animal weight 0.390 kg) were exhaustively extracted with 95% EtOH at ~20 °C. The combined ethanolic extract was concentrated *in vacuo* to give a moist gum-like residue (50 g), which was cromatographed on a column (6×25 cm) with silica gel (80—100 mesh) in the $CHCl_3$—EtOH system (100 : 0 → 45 : 55). With an increase in the eluent polarity, two fractions of polyhydroxy steroids were isolated, a less polar fraction **I** (8.3 g), which contained polyoxy steroid **1** (TLC, BuOH—EtOAc—H_2O, 5 : 1 : 1, R_f 0.85), and a more polar fraction **II** (12.6 g), which contained glycoside **2** (TLC, R_f 0.45—0.40).

Isolation of compounds 1 and 2. Fraction **I** was dissolved in water and passed through a column with Polychrom (3×12 cm), the column being washed with water and 50% aqueous EtOH. The aqueous-ethanol eluate was concentrated *in vacuo* to give a brown gum-like residue (0.11 g), which was successively chromatographed on columns with Sephadex LH-60 (40×1.5 cm) in the $CHCl_3$—EtOH system (4 : 1) and Florisil (1.5×20 cm, 60—100 mesh) in a $CHCl_3$—EtOH system (6 : 1 → 5 : 1). The fraction (15 mg) containing polyol **1** with minor impurities was purified by HPLC on a column with ODS-A (10 mm×25 cm, 1 mL min^{-1}) in a 75 : 25 EtOH—water system and re-chromatographed on a Diasphere-110-C_{18} column (4.5 mm×25 cm, 0.3 mL min^{-1}) in a 65 : 35 MeOH—water system to give 4.3 mg (0.001% of the animal weight) of steroid **1**. More polar fraction **II** was purified on a column with Polychrom as described above. The water—ethanol eluate was concentrated and successively chromatographed on columns with silica gel (2×23 cm, 200—250 mesh) in the $CHCl_3$—EtOH system (6 : 1 → 6 : 3) and Florisil (60—100 mesh) in the same system. The fraction containing laeviuscoloside G (0.05 g) was subjected to HPLC on a column with ODS-A (1 mL min^{-1}) in the EtOH—water system (70 : 30) and to re-chromatography on Diasphere-110-C_{18} in the same system to give 8 mg (0.002%) of compound **2**.

(20*R*,24*S*)-5α-Cholestane-3β,6β,15β,24-tetraol (1), $C_{27}H_{48}O_4$, colorless crystals, m.p. 235.5—237 °C (from MeOH), $[\alpha]_D^{20}$ –4.5 (*c* 0.36, EtOH) was isolated in a yield of 0.001% of the animal weight. Since the intensity of the peak with *m/z* 459 $[M + Na]^+$ observed in the MALDI-TOF(+) mass spectrum is very low (1—2%), we give the FAB(+) and EI (70 eV) mass spectra. The FAB(+) spectrum contains a quasi-molecular ion with *m/z* 459 $[M + Na]^+$ (90%), and the electron impact mass spectrum exhibits fragment peaks corresponding to the loss of H_2O molecules with *m/z* 418 $[M - H_2O]$ and 400 $[M - 2\,H_2O]$. The ^1H NMR (MeOH) spectrum is presented in Table 2 and in the text, and the ^1H and ^{13}C NMR spectra (C_5D_5N) are in Tables 1 and 2.

Laeviuscoloside G (2), $C_{39}H_{68}O_{14}$, amorphous, $[\alpha]_D^{20}$ –0.8 (*c* 0.16, EtOH), yield 0.002%. MALDI-TOF(+) MS, *m/z*: 783 $[M + Na]^+$ (100). The ^1H and ^{13}C NMR spectra (MeOH) coincided with those described previously for the glycoside from *Henricia laeviuscola*.[1]

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