Chemistry of Natural Compounds and Bioorganic Chemistry

Polyhydroxysteroids from the Far-Eastern starfish *Ctenodiscus crispatus*

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Four new polyhydroxysteroids, 5α-cholesta-3β,5,6β,15α,16β,25,26-heptaol, 24-ethyl-5α-cholesta-3β,5,6β,15α,28,29-heptaol-29-sulfate, (22*E*)-24-methyl-5α-cholest-22-ene-3β,5,6β,15α,25,26-hexaol-26-sulfate, 24-propyl-5α-cholesta-3β,5,6β,8,15α,28,29-heptaol, and the known 5α-cholesta-3β,5,6β,15α,16β,26-hexaol, have been isolated from the starfish *Ctenodiscus crispatus.*

Key words: polyhydroxysteroids, structure; starfish *Ctenodiscus crispatus.*

Steroid oligonucleosides and polyhydroxysteroids are the best known secondary metabolites of starfishes.[1–3] These structurally unique compounds may comprise up to 9 hydroxy groups in their molecules. Of all the natural compounds only some ecdysteroids (insect molting hormones[4]) can compare with the starfish polyhydroxysteroids in the content of hydroxy groups. These compounds are present in starfish tissues in the relatively small amounts of 0.001—0.0001 % of the animal weight.

In continuation of our study of the steroid fractions of the Far-Eastern starfish species[5,6] we have isolated five polyhydroxysteroids from the starfish *Ctenodiscus crispatus* (*Retzizis*). Four of these appeared to be new.

Results and Discussion

The structures of polyhydroxysteroids **1**—**5** (Scheme 1) have been determined mainly by ^{1}H and ^{13}C NMR spectroscopy (Tables 1—3).

In the ^{1}H NMR spectra of steroid **1** the chemical shifts of the signals and the proton spin coupling constants (SCC) coincide with those in the spectra of the steroid hexaol from the starfish *Luidia maculata.*[7] Therefore, compound **1** has been identified as 5α-cholesta-3β,5,6β,15α,16β,26-hexaol.

The spectral data for steroids **1** and **2** are in close agreement. The differences observed in their spectra for the carbon atoms C(23)—C(27) and protons HC(26), H'C(26), and C(27)H_3 give evidence for the existence of an additional hydroxy group at C(25) in steroid **2**. In fact, in the ^{1}H NMR spectra of compound **2**, as compared to that of compound **1**, the singlet at δ 1.47 appears instead of the doublet at δ 1.07 for the protons of the C(27)H_3 group, and the signals for the protons HC(26) and H'C(26) are moved downfield by 0.2 and 0.1 ppm, correspondingly. In the ^{13}C NMR spectra of compound **2** in the region, where the signals of the carbon atoms adjacent to the oxygen atoms appear, an additional signal at δ 70.5 emerges that has been as-

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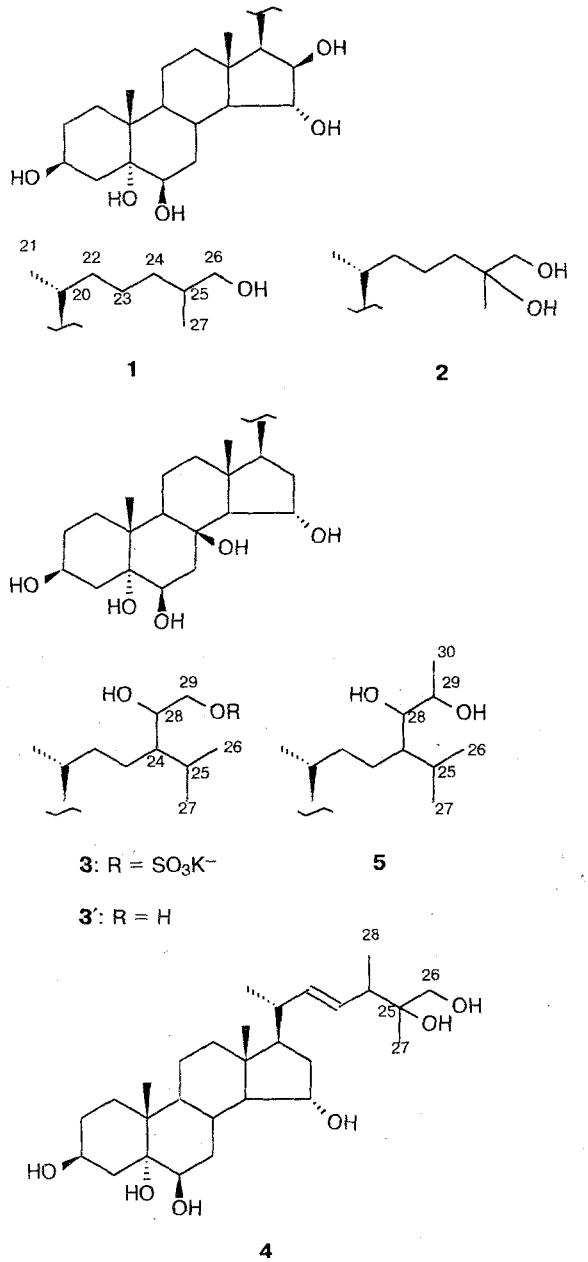
Scheme 1



4: R = SO_3^- (a mixture of K^+ and Na^+ salts

4′: R = H

signed to C(25) after the spectrum has been compared to that obtained by operating under the conditions of *J*-modulation. The structure of the side chain has also been confirmed using the nuclear Overhauser effect (NOE). Thus, on irradiation of the protons of the $C(27)H_3$ group the amplification of the signals of the protons HC(26) and H′C(26) is observed and, *vice versa*, on irradiation of these protons the signals for the protons of the $C(27)H_3$ group are enhanced. The structure

Table 1. ^{13}C NMR spectra of compounds **1**, **2**, **3**, **4′**, and **5** (C_5D_5N, δ,)*

Atom	1	2	3	4′	5
C(1)	31.1	31.1	31.8	31.1	34.5
C(2)	33.4	33.4	34.4	33.4	31.9
C(3)	67.4	67.3	67.2	67.3	67.4
C(4)	41.5	41.6	42.4**	42.1**	
42.5**					
C(5)	76.3	76.2	75.8	75.7	76.0
C(6)	75.8	75.7	77.8	76.2	78.0
C(7)	35.9	36.0	41.8	36.0	42.1
C(8)	33.3	33.2	76.5	32.4	76.8
C(9)	46.2	46.3	48.7	46.2	48.9
C(10)	39.5	39.3	39.1	39.2	39.3
C(11)	21.6	21.6	19.5	21.7	19.7
C(12)	42.7	42.7	42.5**	42.7	
42.6**					
C(13)	44.2	44.2	44.9	44.1	45.1
C(14)	61.1	61.1	66.3	63.6	66.6
C(15)	84.8	84.7	69.3	73.2	69.4
C(16)	82.4	82.3	40.4	40.9**	41.1
C(17)	59.6	59.7	55.0	54.3	55.4
C(18)	15.3	15.3	15.6	15.7	15.8
C(19)	17.3	17.3	18.1	17.3	18.3
C(20)	32.5	32.5	36.0	40.1	36.3
C(21)	18.5	18.5	19.0	21.2	19.2
C(22)	36.9	37.2	36.0	137.1	36.3
C(23)	24.4	21.2	23.4	130.6	23.5
C(24)	34.5	40.0 (39.8)	47.6	44.4	46.0
C(25)	36.7	72.6	29.4	74.3	29.9
C(26)	67.6	70.5 (70.2)	20.8	68.8	21.0
C(27)	17.5	24.7 (24.4)	20.5	22.5	20.5
C(28)			71.3	14.0	76.8
C(29)			72.5		69.4
C(30)					20.5

* Tetramethylsilane as the internal standard. ** Signal assignment is ambiguous.

of steroid **2** has been consequently assigned to be that of 5α-cholesta-3β,5,6β,15α,16β,25,26-heptaol. Compound **2** has been isolated as a mixture of two C(25)-epimers, since in its NMR spectra there are double signals for C(24), C(26), and C(27) carbon atoms and for the corresponding protons. A comparison of the intensities of the signals of the protons HC(26), H′C(26), and $C(27)H_3$ points to the 1:1 ratio of the epimers in the mixture.

The structure of steroid **3** has been solved by the comparison of its NMR spectra with those of the model compounds. The chemical shifts for the carbon atoms C(1)—C(19) in the polycyclic part of the molecule of this steroid coincide with the corresponding signals in the spectrum of the nodososide from the starfish *Protoreaster nodosus* that contains a steroid skeleton with five hydroxy groups on the 3β-, 5-, 6β-, 8-, and 15α-positions (see Ref. 8). The structure of the side chain of compound **3** has been determined using the spectral data for 29-*O*-(α-L-arabinofuranosyl)-5α-stigmasta-3β,6β,8,15α,16α-pentaol from the starfish *Patiria pectinifera*.[9] The spectrum of a side chain of

Table 2. ^{1}H NMR spectra of compounds **1**, **2**, **4**, (C_5D_5N, δ, *J*/Hz)

Proton (Group)	**1**	**2**	**4**
HC(3)	4.90 (м)	4.90 (м)	4.92 (м)
$H_AC(4)$	3.00 (dd, *J* = 11.0, 12.5)	3.01 (dd, *J* = 11.0, 12.5)	3.00 (dd, *J* = 11.0, 12.5)
$H_eC(4)$	2.35 (dd, *J* = 5.0, 12.5)	2.40 (dd, *J* = 5.0, 12.5)	2.32 (dd, *J* = 5.0, 12.3)
HC(6)	4.25 (t, *J* = 2.7)	4.25 (t, *J* = 2.7)	4.25 (t, *J* = 3.0)
HC(7)	2.83 (м)	2.83 (м)	2.80 (м)
H′C(7)	2.83 (м)	2.83 (м)	2.80 (м)
HC(14)	1.61 (t, *J* = 10.0)		
HC(15)	4.46 (dd, *J* = 2.3, 9.8)	4.46 (dd, *J* = 2.3, 9.8)	4.18 (td, *J* = 4.0, 9.0)
HC(16)	4.69 (dd, *J* = 2.3, 7.0)	4.70 (dd, *J* = 2.3, 7.0)	
HC(17)	1.53 (dd, *J* = 7.0, 10.5)		
Me(18)	1.30 (s)	1.30 (s)	0.81 (s)
Me(19)	1.73 (s)	1.74 (s)	1.71 (s)
Me(21)	1.10 (d, *J* = 6.0)	1.11 (d, *J* = 6.0)	1.08 (d, *J* = 6.0)
HC(26)	3.65 (dd, *J* = 6.5, 10.0)	3.85 (3.88) (s)	3.86 (d, *J* = 10.5)
H′C(26)	3.74 (dd, *J* = 5.2, 10.0)	3.85 (3.88) (s)	3.98 (d, *J* = 10.5)
Me(27)	1.07 (d, *J* = 6.5)	1.47 (1.48) (s)	1.46 (s)
Me(28)			1.28 (d, *J* = 7.0)
HC(22)			5.85 (dd, *J* = 8.5, 15.0)
HC(23)			5.40 (dd, *J* = 8.5, 15.0)

Note. Tetramethylsilane as the internal standard.

stigmasterol type (with the hydroxy group at C(29)) has been calculated taking into account the effects of glycosylation. Then the calculated data were compared with the spectrum of compound **3**. This made it possible to suggest the sulfation of the hydroxy group at C(29) and the presence of an additional hydroxy group at C(28). This suggestion has been proved as follows. First, on the successive irradiation of HC(28), HC(29), and H′C(29) protons the responses of the signals for the adjacent protons in the differential ^{1}H NMR spectra were observed. Second, in the ^{1}H NMR spectrum of the desulfated derivative **3a** the signals of the protons HC(29) and H′C(29) are moved upfield as compared to the analogous signals in the spectrum of steroid **3**, *i.e.*, from δ 4.74 to δ 4.08 and from δ 4.57 to δ 4.01, respectively. Finally, the nature of a cation bonded to the sulfate group followed from the mass-spectral data. In the (+)-FAB-MS of compound **3** there are peaks at *m/z* 653 $[M_K+Na]^+$ and 631 $[M_K+H]^+$, which confirm its empirical formula. In the (−)-FAB-MS of steroid **3** there is an intense peak of the $[M-cation]^-$ fragment at *m/z* 559. Based on these data, the structure of the potassium salt of 24-ethyl-5α-cholesta-3β,5,6β,8,15α,28,29-heptaol-24-sulfate has been assigned to steroid **3**.

The structure of the side chain of steroid **3** is rather unusual for the polyhydroxysteroids, since it has the hydroxy group at C(28) and the sulfated hydroxy group at C(29). A sole compound with a side chain of the stigmastane type but with the hydroxy group at C(29) and the sulfated hydroxy group at C(28) has been recently found in the deep-sea starfish *Styracaster caroli*.[1]

In the (−)FAB-MS of steroid **4** there is a peak of the $[M-cation]^-$ fragment at *m/z* 559. The presence of the peaks of $[M_K+H]^+$ (*m/z* 599) and $[M_{Na}+H]^+$ (*m/z* 583) fragments in the (+)-FAB-MS of compound **4** gives evidence that it is a mixture of potassium and sodium salts. Since compound **4** has been isolated in a small amount, its structure has been deduced mainly from the ^{1}H and ^{13}C NMR spectra of its desulfated analog **4′**

Table 3. ^{1}H NMR spectra of compounds **3** and **5**, (C_5D_5N, δ, *J*/Hz)

Proton (Group)	**3**	**5**
HC(3)	4.90 (m)	4.95 (m)
$H_aC(4)$	2.94 (dd, *J* = 11.0, 12.5)	2.98 (dd, *J* = 11.0, 12.5)
$H_eC(4)$	2.30 (dd, *J* = 5.5, 12.5)	2.30 (dd, *J* = 5.3, 12.5)
HC(6)	4.31 (t, *J* = 3.0)	4.35 (t, *J* = 3.0,)
HC(7)	3.13 (dd, *J* = 3.0, 14.6)	3.17 (dd, *J* = 3.0, 14.5)
H′C(7)	3.03 (dd, *J* = 2.5, 14.6)	3.03 (dd, *J* = 2.4, 14.5)
HC(14)	1.73 (d, *J* = 9.5)	1.76 (d, *J* = 9.5)
HC(15)	4.90 (m)	4.95 (m)
Me(18)	1.30 (s)	1.35 (s)
Me(19)	1.84 (s)	1.78 (s)
Me(21)	0.98 (d, *J* = 6.0)	1.09 (d, *J* = 6.0)
HC(25)	2.03 (m)	2.00 (m)
Me(26)	0.93 (d, *J* = 6.7)	1.11 (d, *J* = 6.6)
Me(27)	1.04 (d, *J* = 6.7)	1.17 (d, *J* = 6.6)
HC(28)	4.45 (m)	4.02 (dd, *J* = 2.7, 7.0)
HC(29)	4.74 (dd, *J* = 8.0, 10.2)	4.26 (кв, *J* = 6.2)
H′C(29)	4.57 (dd, *J* = 3.5, 10.2)	
Me(30)		1.66 (d, *J* = 6.0)

Note. Tetramethylsilane as the internal standard.

which has been isolated from the products of solvolysis of the total polyhydroxysteroid fraction. In the ^{13}C NMR spectrum of steroid **4'** the chemical shifts for the carbon atoms C(1)—C(13) are identical to those for steroid **1**, and the signals of C(14)—C(17) carbon atoms are close to the similar signals in the spectrum of the solasteroside S_1 from the starfish *Solaster dowsoni*.[6] The signals for the side chain carbon atoms of compound **4'** coincide with the analogous signals for the steroid pentaol from the starfish *Archaster typicus*.[10] The spin coupling constant (J = 15 Hz) for the protons HC(22) and HC(23) is characteristic of the *trans*-configuration of the double bond C(22)=C(23).[10] In the ^1H NMR spectrum of compound **4** the signals of HC(26) and H'C(26) protons (δ 3.86 and 3.98, respectively) are moved downfield as compared to the similar signals for compound **4'** (δ 3.86 and 3.98). This indicates that the sulfate group in steroid **5** is connected with the hydroxy group at C(26). Thus, compound **4** is (22*E*)-24-methyl-5α-cholest-22-ene-3β,5,6β,15α,25,26-hexaol-26-sulfate.

We were not able to isolate the other steroids from the polyhydroxysteroid fraction. Therefore, we have carried out the solvolysis of the fraction and, besides the above-mentioned compound **4'**, found a new steroid **5** among the products of mild desulfation. A comparison of the ^1H and ^{13}C NMR spectra of steroids **5** and **3** has demonstrated that they have similar polycyclic systems with the hydroxy groups at the positions 3β, 5, 6β, 8, and 15α but differ in their side chain structures. From the ^1H and ^{13}C NMR spectra of steroid **5** it follows that its side chain contains a propylic fragment with the hydroxy groups at C(28) and C(29), since there are signals of an additional methyl group and only one signal of C(29)-proton instead of two is observed, as in the case of compound **3**. These conclusions have been confirmed by using differential double resonance ^1H NMR: on the successive irradiation of HC(28), HC(29), and Me(30) protons the signals of the neighboring protons were observed in the differential spectra. Therefore, steroid **5** is 24-propyl-5α-cholesta-3β,5,6β,8,15α,28,29-heptaol.

24-Propyl steroids are very rare in nature. None of them has yet been found among the surface phytosteroids. The 24-propylcholesterol from microalgae and 24-isopropylcholesterol and its analogs from the sterol fractions of sponges are known.[2] Steroid **5** is the first 24-propylpolyhydroxysteroid found in starfish.

Experimental

^1H and ^{13}C NMR spectra were registered on a Bruker WM-250 spectrometer. Optical rotation was measured using a Perkin-Elmer 141 polarimeter. FAB-MS were obtained on a Finigan MAT 8340 mass-spectrometer (the glycerol matrix, Xe atoms, 8 kV). Melting points were determined using a Boetius melting block.

The samples were dredged aboard the scientific research ship «Akademik Oparin» in May 1991 in the Sea of Japan (Pociett Bay) at a depth of 4—20 m and identified by A. V. Smirnov (Zoologic Institute, Russian Academy of Sciences, St.-Petersburg).

Isolation of compounds 1—4. A methanolic extract obtained from the starfish (4.1 kg) was concentrated *in vacuo*. The residue was dissolved in water (1.5 L) and passed through an Amberlite XAD-2 column. The column was eluted successively with water and methanol. The methanolic eluate was evaporated and the resultant total steroid fraction was successively chromatographed through a Sefadex LH-20 column (1:1 chloroform—methanol mixture as the eluent) and through a silica gel column (gradient elution from 2:1 to 1:1 chloroform—methanol mixture). Further purification was carried out by HPLC on a Du Pont chromatograph with a refractometer as the detector. Fractions containing compounds **1** and **2** were chromatographed through a Zorbax ODS column (5 μ, 250×4.6 mm) using a 7:3 methanol—water mixture as the eluent to afford 35 mg ($8.5 \cdot 10^{-4}$ %) of compound **1** and 6 mg ($1.5 \cdot 10^{-4}$ %) of compound **2**. The fraction containing compounds **3** and **4** were chromatographed through an Altex Ultrasfere-Si column (5μ, 250×10.0 mm) using a 50:3:42 methanol—acetone—water mixture as the eluent to afford 27 mg ($6.5 \cdot 10^{-4}$ %) of compound **3** and 2 mg ($0.5 \cdot 10^{-4}$ %) of compound **4**.

Steroid 1: $C_{27}H_{48}O_6$, amorphous, $[\alpha]_{Hg}$ +20.2° (c 3.5, methanol). (+)-FAB-MS: m/z 450 $[M-H_2O]^+$. (−)-FAB-MS: m/z 467 $[M-H]^-$.

Steroid 2: $C_{27}H_{48}O_7$, m.p. 241—242 °C, $[\alpha]_{Hg}$ +19.0° (c 0.5, methanol). (+)-FAB-MS: m/z 507 $[M+Na]^+$. (−)-FAB-MS: m/z 483 $[M-H]^-$.

Steroid 3: $C_{29}H_{51}O_{10}SK$, amorphous, $[\alpha]_{Hg}$ +25.9° (c 0.8, methanol). (+)-FAB-MS: m/z 653 $[M_K+Na]^+$, 631 $[M_K+H]^+$. (−)-FAB-MS: m/z 591 $[M-cation]^-$.

Steroid 4: $C_{28}H_{47}O_6SKNa$, amorphous, $[\alpha]_{Hg}$ +12.9° (c 0.3, methanol). (+)-FAB-MS: m/z 599 $[M_K+H]^+$, 583 $[M_{Na}+H]^+$. (−)-FAB-MS: m/z 559 $[M-cation]^-$.

Isolation of compounds 4' and 5. The total steroid fraction was obtained as described above for compounds **3** and **4**. A sample (180 mg) of the total fraction (homogenous according to TLC) was dissolved in a 1:1 pyridine—dioxane mixture and heated to 85—95 °C for 2.5 h in an anhydrous atmosphere. The solvents were evaporated *in vacuo*, and the dry residue was chromatographed through a silica gel column using a 15:15:8 chloroform—ethyl acetate—methanol mixture as the eluent. Purification by HPLC on a Zorbax ODS column (5μ, 250×4.6 mm) using a 8:2 methanol—water mixture as the eluent yielded 8 mg ($2.0 \cdot 10^{-4}$ %) of compound **4'** and 8 mg ($2.0 \cdot 10^{-4}$ %) of compound **5**.

Desulfated steroid 4': $C_{28}H_{48}O_6$, amorphous, $[\alpha]_{Hg}$ +22.7° (c 0.9, methanol).

Desulfated steroid 5: $C_{30}H_{54}O_7$, amorphous, $[\alpha]_{Hg}$ +29.3° (c 0.5, methanol). (+)-FAB-MS: m/z 549 $[M+Na]^+$. (−)-FAB-MS: m/z 525 $[M-H]^-$.

Desulfated steroid 3a. Compound **3** (10 mg) in a 1:1 pyridine—dioxane mixture was heated to 85—95 °C for 2 h. Purification on a silica gel column using a 2:1 chloroform—methanol mixture as the eluent afforded 5 mg of compound **3a**, amorphous, $[\alpha]_{Hg}$ +17.0° (c 0.4, methanol).

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