Chemistry of Natural Compounds and Bioorganic Chemistry

Steroid compounds from the Pacific starfishes *Luidia quinaria* and *Distolasterias elegans*

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One new and four previously known steroid compounds were identified from the Pacific starfishes *Luidia quinaria* and *Distolasterias elegans*. The structure of the new steroid was established from spectral data and chemical correlations with other steroids such as 5α-cholestane-3β,5,6β,15α,16β,26-hexaol 3-sulfate (**1**). The previously known compounds were identified as 5α-cholestane-3β,5,6β,15α,26-pentaol 15-sulfate (**2**) from *Luidia quinaria* and sodium (24*S*)-*O*-[β-D-glucopyranosyl]-5α-cholestane-3β,6α,8,15β,24-pentaol 6′-sulfate (**3**), sodium (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate (**4**), and sodium tornasterol A sulfate (**5**).

Key words: polyhydroxysteroids, starfish, *Luidia quinaria*, *Distolasterias elegans*, structure, NMR spectra.

Starfishes are one of the richest sources of polyhydroxysteroids with new, unusual structures. All known steroid polyols from starfishes may be classified in several groups differing in the type of hydroxylation.[1] The majority of them contain the common fragment of 3β,6α(or β),8,15α(or β),16β-pentahydroxycholestane; additional hydroxylation of the latter may occur at the 4β, 5α, 7α (or β), and, sometimes, 14α positions. Sulfation of hydroxyls most often occurs at the 3β, 6α, 15α, and 26 positions. The most abundant side chains are C_8-side chains having a hydroxyl function at C(26) (25*S*-configuration) or else 24*S*-hydroxylated (a small group of compounds).

In continuation of studies of Pacific starfish steroid compounds, we isolated five compounds from *Luidia quinaria* and *Distolasterias elegans*; one of them was found to be new.

Results and Discussion

The structures of steroid compounds **1**—**5** (Scheme 1) were established mainly by ^1H and ^{13}C NMR spectroscopy.

New compound **1** is a saturated steroid hexaol: its ^1H NMR spectrum contains two doublets of doublets at δ

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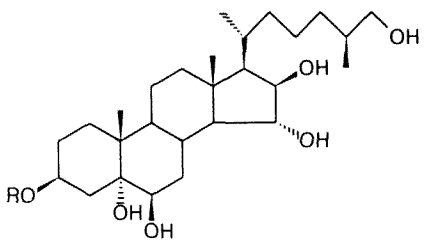
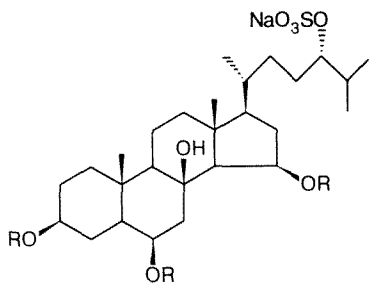
Scheme 1



1: R = SO_3Me
1a: R = H



4: R = H
4a: R = Ac

3.76 (J = 10.5; 3 Hz) and that of CH(16) at 4.00 (J = 7.5; 3 Hz) and part A of the ABX spin system ($CH-CH_2OH$) at δ 3.45 (J_{AB} = 11.0 Hz; J_{AX} = 5.5 Hz; part B is covered by the residual signal of the solvent). These signals are observed in the spectra of many polyols containing the 15α,16β,26-trihydroxysteroid fragment.[2] Two three-proton doublets of Me(21) and Me(27) at δ 0.94 and 0.98 also indicate 26-hydroxycholestane structure of the side chain. Solvolysis of **1** by heating in a dioxane—pyridine mixture afforded desulfated steroid **1a**. The spectral data on **1** and **1a** are similar, and the differences observed in the spectra of these compounds in the C(2)—C(4) carbons and the HC(3) protons indicate the presence of the sulfo group at C(3). In fact, the lowfield shift of the signal of the HC(3) proton from the corresponding signal of **1a** (+0.75 ppm) was observed in the ^1H NMR spectrum of compound **1**. In the ^{13}C NMR spectrum of compound **1**, the signals of the C(2) and C(4) atoms are shifted upfield relatively to those in **1a** by 2.5 and 2.2 ppm, respectively, and C(3) is shifted downfield by 8.7 ppm. These data are in a good agreement with β- and α-effects of sulfation reported in the literature for 3β-sulfated steroids.[3,4] In the same time, the chemical shifts (δ) of carbons and protons, as well as spin-coupling constants (J) of protons in the spectra of steroid **1a** coincide with the corresponding values in the spectra of steroid hexaol from the starfish *Luidia maculata*.[5] Hence, the structure of 5α-cholestane-3β,5,6β,15α,16β,26-hexaol 3-sulfate was assigned to compound **1**.

We corrected the assignment of signals of C(1) and C(2) in the carbon spectra of **1a**: the signal at δ 33.6, which remains unchanged after desulfation, corresponds to C(1), and the signal at δ 31.7 shifted downfield is assigned to C(2); the assignment in Ref. 5 was opposite.

The comparison of spectral data on sulfated polyol **2** with those for **1** showed that **2** had the similar structure of side chain and steroid nucleus bearing three hydroxyl groups at 3β, 5α, and 6β positions, while sulfated hydroxyl could be located only in the ring D. The carbon and proton chemical shifts and J_{HH} of steroid sulfate **2** coincide with the corresponding values reported in the literature for the sulfated pentaol from *Myxoderma platyacanthum*.[6] Thus, compound **2** is 5α-cholestane-3β,5,6β,15α,26-pentaol 15-sulfate. For sulfates **1** and **2**, the nature of the cation is not determined.

Up to now, *ca.* 10 polyoxysteroids from starfishes of the Luididae family have been isolated,[5,7] while most of them belong to the subgroup with 3β,6β,15α,16β-hydroxylated nucleus and 26-hydroxycholestane side chain; the additional hydroxylation at 5α or 7α positions and the sulfation at 15α, 16β, or 26 positions in this group were observed. Steroids **1** and **2** isolated from *Luidia quinaria* possess the same structural features, however, the sulfation at the C(3) position was found by us for such polyols for the first time. Until now, the only known starfish 3β-sulfates were 3β,6α-disulfated triols from a "living fossil", the starfish *Tremaster novaecaledonia*,[8] which are structurally similar to aglycones of starfish oligoglycosides.

Steroid compounds **3**—**5** were obtained by multiple chromatography of the methanolic extract of *Distolasterias elegans*, and their structures were also established by spectral methods. Steroid glycoside **3** contains a sulfate group and sodium ions (IR spectrum, atomic-absorption analysis), and in its ^{13}C NMR spectrum, the signal of anomeric carbon atom at δ 106.3 indicating the presence of monosaccharide residue was observed. The only monosaccharide, D-glucose (TLC, GLC, $[\alpha]_D^{20}$) was identified after acid hydrolysis. The structure of glycoside was determined by ^1H and ^{13}C NMR spectra. The proton and carbon signals in the spectra of glycoside coincide with those for pycnopodioside C from the starfish *Pycnopodia helianthoides*.[9] Thus, glycoside **3** was identified as sodium (24*S*)-24-*O*-β-D-glucopyranosyl-5α-cholestane-3β,6α,8,15β,24-pentaol 6′-sulfate.

For compound **4**, containing a sulfate group (IR: 1250 cm^{-1}) and Na ions (atomic-absorption analysis), complete coincidence of the signals of the C(1)—C(22) atoms in the ^{13}C NMR spectrum with the corresponding values of glycoside **3** was observed, and the difference in the signals of carbon atoms of side chains indicated the localization of the sulfate group in the side chain. In fact, comparison of the spectra of previously known 5α-cholestane-3β,6α,8,15β,24-pentaol from the starfish *Comophia watsoni*[10] and those of sulfate **4** isolated by us shows that they have similar structures and that the sulfate group is located at C(24). The upfield shifts of the

signals of the C(22), C(23), and C(25) atoms and downfield shifts of the signals of C(24) and HC(24) due to sulfation are in accord with the literature data on the 24-sulfated side chain.[11] Therefore, compound **4** is sodium (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate. Acetylation of **4** with acetic anhydride in pyridine affords triacetate **4a**, in the ^{1}H NMR spectrum of which the signals of three acetate groups (δ 2.0, 2.01, 2.06, 3 s, 9 H) and four methyne protons (δ 4.61 m, H(α)C(3); δ 5.03 m, H(β)C(6); δ 5.2 m, H(α)C(15); δ 4.12 m, HC(24)) are observed. The structure of **4a** can be represented as sodium 3,6,15-tri-*O*-acetyl-(24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate.

Recently, the sulfated polyhydroxysteroid from the starfish *Astropecten scoparius* has been isolated,[12] whose structure is in accord with that of compound **4**. The comparison of the spectral data demonstrated a complete identity of these compounds.

Polyhydroxysteroids obtained by us from *Distolasterias elegans* belong to the same group as the steroid derivatives from the starfish *Distolasterias nippon*[13] isolated previously: 3β,6α,8,15β,24-hydroxylation and different glycosylation patterns at C(3) and C(24) are characteristic of them. Steroid sulfate **4** has an unusual structure of side chain; such steroids are more frequent as aglycone in starfish glycosides with carbohydrate chain at C(24). At present, only three free 24-hydroxysteroid are known, and sulfate **4** (isolated by us[13] and Italian authors[12] from different starfish species) has no analogs yet.

The ^{13}C NMR spectrum of compound **5** is identical to that of 3-sulfate of tornasterine A, which was obtained for the first time by hydrolysis of versicoside A from the starfish *Asterias amurensis*.[14] Solvolytic desulfation of **5** gave compound **5a**, identical to tornasterine A by the ^{1}H NMR spectrum.[15] Since the δ(^{13}C) values of the side chain coincide with those of synthetic (20*S*)-tornasterine A, the structure of sodium (20*S*)-23-oxo-5α-cholest-9(11)-ene-3β,6α,20-triol 3-sulfate can be assigned to compound **5**. Recently, a glycoside, tornasteroside A aglycone **5**, was found in the starfish *Distolasterias nippon*.[13] The isolation of native compound **5** in related animal species may indicate that in the biosynthesis of asterosaponins, sulfation of aglycone precedes its glycosylation.

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker WM-250 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. GLC analysis of monosaccharide derivatives was carried out with a Tsvet-101 chromatograph (glass columns, 3 % of QF-1 on Chromaton N-HMDS). Sodium ions in compounds **3**—**5** were determined with an AA-780 atomic-absorption flame-emission spectrophotometer.

Starfish samples of *Distolasterias elegans* were collected by a crested dredge of the "Breeze" trawler in July, 1990, near Onekotan Island (Kuril Islands, the Sea of Okhotsk) from 100 m depth; samples of *Luidia quinaria* were collected by a trawl net during run No. 13 of the "Academician Oparin" scientific research boat in July, 1991, in Possuet Gulf (the Sea of Japan) from 20—100 m depth and identified by A. V. Smirnov (Institute of Zoology, RAS, St.Petersburg).

TLC was carried out on glass plates (4.5×6.0 cm) with a fixed layer of silica gel L (300 mesh, Chemapol, Czechoslovakia).

Isolation of compounds 1 and 2. Ground samples of *Luidia quinaria* (animal weight 10.0 kg) were extracted with ethanol three times (3×6 L) under reflux on a water bath (100 °C). The combined ethanolic extract (18 L) was evaporated *in vacuo* to dryness, the dry residue (80.5 g) was dissolved in water (700 mL), and the aqueous solution was extracted with *n*-butanol in a separatory funnel (4×300 mL). The combined butanolic extract was evaporated to dryness, and the residue (32 g) was dissolved in water (100 mL). The solution was passed through a column with Amberlite XAD-2 ion exchange resin (2.5×18 cm) by eluting the column with water (2 L) and then 50 % aqueous methanol (1.5 L). The aqueous methanolic eluate was evaporated to dryness, and the residue (1.53 g), containing a mixture of steroid compounds, amino acids, pigments, and other admixtures, was chromatographed successively on columns with Florisil (1.8×15 cm, 60—100 mesh) and silica gel (2×23 cm, 200—250 mesh) using a chloroform—methanol (9 : 1→1 : 1) system. The combined fraction of steroid compounds (692 mg) obtained was chromatographed on columns with Sephadex LH-20 (2×60 cm) using a chloroform—methanol system (4:1) and with silica gel using a chloroform—methanol (7:2→7:4) system. The volume of the fractions was 10—15 mL; the elution was controlled by TLC in a chloroform—methanol—water (2 : 1 : 0.7) system. Further separation of the mixture was performed by HPLC (a Du Pont chromatograph, refractometric detector) on a Zorbax ODS column (5μ, 250×4.6 mm) using methanol—water as the eluent (55 : 45). Steroid **1** (24 mg, yield 0.0002 %) and steroid **2** (179 mg, yield 0.0017 %) were obtained.

5α-Cholestane-3β,5,6β,15α,16β,26-hexaol 3-sulfate (1). $C_{27}H_{47}O_9SMe$, amorphous, $[\alpha]_D$ +38.5° (*c* 1.2, methanol), the ^{1}H and ^{13}C NMR spectra are presented in Tables 1 and 2.

Desulfation of 1. Sulfate **1** (12 mg) was heated in a dioxane—pyridine mixture (3 mL, 1 : 1) at 120 °C for 2 h. The solution was evaporated *in vacuo* to dryness and chromatographed on a column with silica gel in a chloroform—methanol (7 : 3) system. Hexaol **1a** (5 mg) was obtained,

Table 1. ^{13}C NMR spectra (CD_3OD) of compounds **1** and **1a**

Atom	^{13}C NMR, δ		Atom	^{13}C NMR, δ	
	1	**1a**		**1**	**1a**
C(1)	33.6	33.5	C(14)	61.0	61.0
C(2)	29.2	31.7	C(15)	85.1	85.1
C(3)	77.3	68.4	C(16)	83.0	83.0
C(4)	39.3	41.6	C(17)	60.0	60.0
C(5)	76.5	76.6	C(18)	15.2	15.2
C(6)	76.5	76.7	C(19)	17.3	17.3
C(7)	35.3	35.3	C(20)	31.0	31.0
C(8)	31.2	31.2	C(21)	18.8	18.8
C(9)	46.6	46.7	C(22)	37.5	37.5
C(10)	39.3	39.5	C(23)	24.9	24.9
C(11)	22.0	22.0	C(24)	35.0	35.0
C(12)	42.0	42.1	C(25)	37.0	37.0
C(13)	44.9	44.9	C(26)	68.5	68.6
			C(27)	17.2	17.2

Table 2. ^{1}H NMR spectra (CD_3OD) of compounds **1**, **1a**, **4**, and **4a**

Proton	^{1}H NMR, δ (*J*/Hz)			
	1	**1a**	**4** (C_5D_5N)	**4a**
HC(3)	4.79 (m)	4.04 (m)*	4.02 (m)	4.61 (m)
HC(6)	3.50 (br.s)	3.50 (br.s)	4.39 (td)	5.03 (m)
HC(15)	3.76 (dd, *J* = 10.5, 3)	3.76 (dd, *J* = 10.5, 3)	4.78 (td)*	5.2 (m)
HC(16)	4.00 (dd, *J* = 7.5, 3)	4.00 (dd, *J* = 7.5, 3)*		
HC(24)			4.78 (td)*	4.12 (m)
HC(26)	3.35 (dd)**	3.45 (dd, *J* = 11, 5.5)*		
H′C(26)	3.44 (dd, *J* = 10.8, 5.5)	3.45 (dd, *J* = 11, 5.5)*		
HC(27)	0.94 (d, *J* = 7)	0.94 (d, *J* = 7)		0.92 (d, *J* = 7.0)
Me(18)	0.94 (s)	0.94 (s)	1.60 (s)	1.09 (s)
Me(19)	1.18 (s)	1.21 (s)	1.39 (s)	1.03 (s)
Me(21)	0.98 (d, *J* = 7)	0.98 (d, *J* = 7)	1.04 (d)	0.95 (d)
HC(4a)			1.86 (td)	
HC(4e)			3.15 (dm)	
HC(7a)			2.24 (dd)	
HC(7e)			3.51 (dd)	

* The signal is covered by the residual signal of the solvent. ** Signals are partially overlapped.

amorphous, $[\alpha]_D$ +11.3° (*c* 1.5, methanol). It was identified by comparison of the spectral data with those published in the literature.

Isolation of compounds 3 — 5. A total fraction of steroid polyols was isolated from an ethanolic extract of the starfish *Distolasterias elegans* (animal weight 20 kg) as described above. Chromatography on a Sephadex LH-20 column in a chloroform—methanol (4 : 1) system gave the fraction containing polar compounds (40 mg) and the fraction of less polar steroids (22 mg), after HPLC (methanol—water, 65 : 35), the latter gave 15 mg (yield 0.005 %) tornasterine A sulfate (**5**). Pycnopodioside C (**3**) (8 mg, yield 0.00008 %) and sulfate **4** 18 mg (yield 0.0009 %) were obtained from the polar fraction by the HPLC method.

Sodium 3,6,15-tri-*O*-acetyl-(24*S*)-5α-cholestane-3β,6α,8,15β,24-pentaol 24-sulfate (4a). Compound **4** (10 mg) in a mixture of acetic anhydride and pyridine (2 mL, 1 : 1) was allowed to stay at ambient temperature overnight. Chromatography on silica gel in a chloroform—ethyl acetate (3 : 1) system gave acetate **4a** (9 mg), $C_{33}H_{50}O_{11}SNa$, amorphous, $[\alpha]_D$ +4.1 (*c* 7.3, methanol). The spectral data are presented in Tables 1 and 2.

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