Steroid compounds from the Pacific starfish *Lysastrosoma anthosticta*

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Two new steroid compounds, 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one 3-sulfate and 3β,6α-dihydroxy-5α-cholest-9(11)-en-23-one 3-sulfate, were isolated from the Pacific starfish *Lysastrosoma anthosticta* as the corresponding sodium salts and identified. Two previously known glycosides of 24(*S*)-5α-cholestane-3β,6α,8,15β,24-pentol were also isolated and identified as sodium 24-*O*-β-D-glucopyranoside 6′-sulfate (pycnopodioside C) and sodium 24-*O*-β-D-xylopyranoside 4′-sulfate (luridoside A).

Key words: starfish, polyhydroxysteroids, glycosides, aglycons, *Lysastrosoma anthosticta*, NMR spectra.

Steroid oligoglycosides from starfishes (asterosaponins) known to possess hemolytic properties contain, as a rule, a steroid aglycon with the 3β,6α-diol group, the 9(11)-double bond, and the 23-carbonyl group. The oligosaccharide chain is attached to the O(6) atom and the hydroxy group at the C(3) atom is sulfated. The native aglycons of these glycosides, which are the monosulfates of the corresponding steroid diols, are usually prepared by mild acid or enzymatic hydrolysis of asterosaponins. However, one of these native aglycons, tornasterol A sulfate, has been found in ethanolic extracts from two species of Far-Eastern starfishes.[1] Recently, similar compounds, for example, a new steroid monosulfate called aphelaketotriol, was isolated by our research group from extracts of the starfish *Aphelasterias japonica*.[2]

As a continuation of the studies on physiologically active steroids from starfishes, we reinvestigated polar steroids from *Lysastrosoma anthosticta*. Previously,[3] we showed that acid hydrolysis of oligoglycoside from this invertebrate yields asterone (3β,6α-dihydroxypregn-9(11)-en-20-one), marthasterone (3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one), and dihydromarthasterone. In this study, we report the isolation of four steroid derivatives (**1a—4**); compounds **1a** and **2** proved to be new variants of the steroid diol sulfates and native aglycons of some known asterosaponins, while **3** and **4** are steroid monoglycosides not related to the asterosaponin series.

The structures of sulfates **1a** and **2** (Tables 1, 2) and glycosides **3** and **4** isolated were established by ^{1}H and ^{13}C NMR spectroscopy; the sodium ions were detected by atomic-absorption analysis.

Compound **1a** is an unsaturated steroid diol with a trisubstituted double bond in the nucleus (^{1}H NMR (CD_3OD), δ: 5.22 (br.d, 1 H, H(11), J = 4.8 Hz); ^{13}C NMR (CD_3OD), δ: 116.13 C(11), 146.1 C(9). The side chain contains a 24(25)-double bond and a keto group at position 23, which are manifested in the NMR spectra as a signal for the HC=C group at δ_H 6.16 (s, 1 H, H(24)) and a singlet for the carbonyl carbon (^{13}C NMR, δ: 200.6, C(23)). The protons of the methyl groups at C(26) and C(27) resonated at δ 1.90 (d, 3 H, H_3(26)) and δ 2.11 (d, 3 H, H_3(27)). In the ^{1}H NMR

Table 1. Data from the ^{1}H NMR spectra (C_5D_5N) of compounds **1a**, **1c**, and **2**

Proton, group	δ (J/Hz)			
	1a	**1c**	**2**	**2** (MeOH)
H_3C(18)	0.61 (s)	0.58 (s)	0.62 (s)	0.67 (s)
H_3C(19)	0.94 (s)	0.93 (s)	0.96 (s)	0.98 (s)
H_3C(21)	1.02 (d, J = 4.8)	1.02 (d, J = 4.8)	1.00 (d, J = 6.0)	0.90 (d, J = 6.5)
H_3C(26)	1.77 (s)	1.77 (s)	0.91 (d, J = 6.4)	0.91 (d, J = 6.5)
H_3C(27)	2.22 (s)	2.22 (s)	0.92 (d, J = 6.4)	0.91 (d, J = 6.5)
HC(24)	6.17 (s)	6.18 (s)		
HC(3)	4.95 (m)	4.94 (m)*	4.95 (m)	4.20 (m)
HC(6)	3.84 (m)	4.90 (m)*	3.84 (m)	3.52 (m)
H(11)	5.22 (m)*	*	5.22 (m)*	5.33 (br.d, J = 5)
CH_3COO		1.95 (s)		

* The signals superimposed on the solvent signal.

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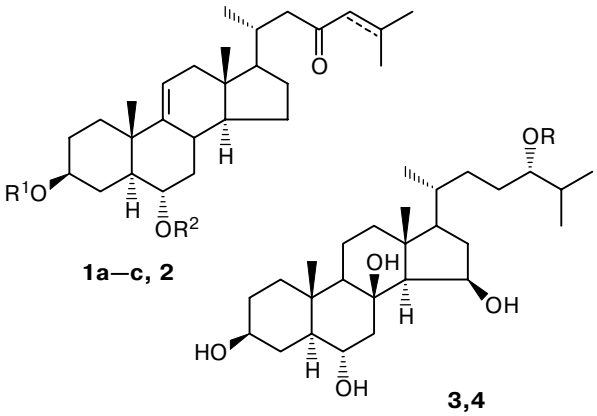
Table 2. Data from the ^{13}C NMR spectra (C_5D_5N) of compounds **1a** and **2**

Atom	δ		Atom	δ	
	1a	**2**		**1a**	**2**
C(1)	36.0	36.0	C(15)	25.3	25.3
C(2)	29.3	29.3	C(16)	28.9	28.5
C(3)	77.5	77.5	C(17)	56.4	56.1
C(4)	31.1	31.1	C(18)	11.5	11.5
C(5)	50.5	50.5	C(19)	19.1	19.1
C(6)	68.2	68.2	C(20)	32.9	32.3
C(7)	43.3	43.3	C(21)	19.4	19.4
C(8)	35.8	35.8	C(22)	51.4	51.4
C(9)	146.1	146.1	C(23)	200.6	209.5
C(10)	38.1	38.1	C(24)	124.7	52.2
C(11)	116.1	116.1	C(25)	153.8	24.3
C(12)	41.7	41.7	C(26)	27.1	22.4
C(13)	41.1	41.0	C(27)	20.3	22.3
C(14)	53.6	53.6			

spectrum of compound **1a**, the signals for the two CH—O protons are observed as multiplets at δ 4.20 (1 H, $W_{1/2}$ = 20 Hz), which is typical of the 3α-proton in steroid 3β-*O*-sulfates,[4] and δ 3.54 (m, 1 H), which is typical of 6β-H in steroid 6α-alcohols.[5] These results and the chemical shifts of the singlets for two methyl groups, CH_3(18) (δ 0.67) and CH_3(19) (δ 0.98), are in good agreement with the corresponding published data for the aglycon sulfate of marthasteroside B, found for the first time among the hydrolysis products of a mixture of glycosides from the starfish *Marthasterias glacialis*.[6] Marthasteroside B was isolated from several species of starfishes, namely, *Marthasterias glacialis*,[7] *Coscinasterias tenuispina*,[8] and *Luidia maculata*.[9]

Comparison of the ^{13}C NMR spectrum of steroid **1a** with that of tornasterone sulfate (3β,6α,20ξ-trihydroxy-5α-cholest-9(11)-en-23-one 3-sulfate), obtained upon enzymatic hydrolysis of glycosides from *Luidia maculata*,[5] revealed coincidence of the signals for C(1)—C(14) in these spectra, thus confirming that the polycyclic systems in these compounds are identical. In the spectra of marthasterone **1** and **1a**, the signals for C(15)—C(27) coincide. Hence, the compound isolated is marthasterone sulfate. Comparison of the ^1H NMR (CD_3OD) and ^{13}C NMR (C_5D_5N) spectra of steroid **1a** with the spectra of marthasteroside B,[7] which differs from **1a** in the presence of a carbohydrate chain at C(6) demonstrated that the spin-spin coupling constants and the chemical shifts of the protons of the aglycon moiety of marthasteroside B were completely identical to the corresponding values in the spectrum of **1a** (see Table 1). All the carbon chemical shifts in both compounds, except for those related to the site of attachment of the carbohydrate chain, also nearly coincided. Glycosylation of the hydroxy group at C(6) in steroids and triterpenes is known to induce a substantial downfield shift of the signal for C(6) (α-shift) and less pronounced upfield shifts of the signals for C(5) and C(7) (β-shift).[10] Indeed, in the spectrum of the glycoside, the signal for C(6) was shifted downfield (+12.07 ppm), while the signals for C(5) and C(7) were shifted upfield (−1.51 and −2.22 ppm, respectively) relative to those in the spectrum of **1a**. Relying on these data, compound **1a** was identified as the Na salt of 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one 3-sulfate or marthasterone sulfate.

Solvolytic desulfation of steroid **1a** yielded diol **1b**, which was identified as marthasterone based on the ^1H NMR spectrum. Acetylation of **1a** gave rise to monoacetate **1c**, whose ^1H NMR spectrum ($CDCl_3$) exhibited a signal for the acetate group with δ 2.03 (s, 3 H, C(6)OAc), while the signal for H(6) shifted downfield and was observed at δ 4.84 (m, 1 H). The other signals identified in this spectrum were at δ 0.64 (s, 3 H, C(18)H_3); 1.00 (s, 3 H, C(19)H_3); 1.20 (d, 3 H, C(21)H_3); 1.88, 2.13 (both s, each 3 H, C(26)H_3, C(27)H_3); 5.32 (br.d, 1 H, H(11), J = 5 Hz); and 6.04 (s, 1 H, H(24)).



1a: $R^1 = SO_3Na$, R^2 = H, $\Delta^{24(25)}$
1b: $R^1 = R^2$ = H, $\Delta^{24(25)}$
1c: $R^1 = SO_3Na$, R^2 = Ac, $\Delta^{24(25)}$
2: $R^1 = SO_3Na$, R^2 = H, 24,25-dihydro
3: R = β-glucopyranosyl-6-sulfate
4: R = β-xylopyranosyl-4-sulfate

Comparison of the spectral characteristics of steroid **2** and sulfate **1a** showed that the chemical shifts and spin-spin coupling constants of protons with the carbon atoms of the polycyclic fragment of the molecule are closely similar, the difference between the spectra being only in the absence of a signal for the trisubstituted double bond in the case of **2**. The signals for the protons and the C atoms in the side chain of **2** coincided with the corresponding signals for the aglycon of marthasteroside C from the starfish *Marthasterias glacialis*.[7] Thus, steroid **2** was identified as the Na salt of 3β,6α-dihydroxy-5α-cholest-9(11)-en-23-one 3-sulfate (or dihydromarthasterone 3-sulfate).

The structure of the known glycoside **3** was determined owing to the fact that its spectra coincided with the spectra of pycnopodioside C ((24*S*)-24-*O*-β-D-glucopyranosyl-5α-cholestane-3β,6α,8,15β,24-pentol 6′-sulfate Na salt), which we have isolated previously from the starfish *Distolasterias elegans*.[1] Compound **4**

was identified as luridoside A ((24*S*)-24-*O*-β-D-xylopyranosyl-5α-cholestane-3β,6α,8,15β,24-pentol 4′-sulfate Na salt), which was isolated previously from the starfish *Cosmasterias lurida*,[11] by comparing the spectral characteristics of **4** with relevant published data.

Since the new sulfated steroid diols isolated in this study had been encountered earlier only in the glycosylated form, their detection, together with the isolation of tornasterol sulfate,[1] can be regarded as evidence supporting the assumption that in the asterosaponin biosynthesis the step of aglycon sulfation precedes the glycosylation step.

Experimental

Melting points were determined on a Boetius hot stage. ^{1}H and ^{13}C 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 DuPont Model 8800 chromatograph (with refractometer as the detector) using columns with Zorbax ODS (5μ, 250×4.6 mm) and Silasorb C_{18} (13μ, 250×9.4 mm). Sodium ions in compounds **1a—4** were determined on an AA-780 atomic-absorption flame emission spectrophotometer.

TLC was performed on glass plates with a fixed layer of silica gel L (300 mesh, Chemapol, Czech Republic). Preparative column chromatography was also carried out on silica gel L (80—100 and 200—250 mesh).

The starfish specimens were gathered by a trawl net in August 1994 (Sea of Okhotsk, Kurils) during the 18th expedition of the research ship "Academician Oparin" at a depth of 100—200 m and identified by Prof. O. G. Kusakin (Institute of Marine Biology of the Far-Eastern Branch of the RAS, Vladivostok).

Isolation of compounds 1a and 2. Ground starfish (weight 15 kg) were subjected to exhaustive extraction with 95% ethanol at ~20 °C. The combined ethanolic extract was concentrated *in vacuo* to dryness and chromatographed on a column (6×20 cm) with silica gel (80—100 mesh) in the chloroform—methanol system (6 : 1 → 1 : 1). Two fractions of dihydroxysteroids were obtained as the polarity of the eluent increased, *viz.*, a less polar fraction **I** (9.85 g), which contained **1a** and **2** (TLC, BuOH—EtOAc—H_2O, 5 : 1 : 1, R_f 0.65—0.70), and a more polar fraction **II** (15.69 g), which contained glycosides **3** and **4** (TLC, R_f 0.25—0.30). Fraction **I** was dried, dissolved in water, and passed through a column with the Amberlite XAD-2 resin (3×17cm), the column was eluted with water (3 L) and with 50% aqueous methanol. The aqueous-methanolic eluate was concentrated to dryness and the residue containing a mixture of steroids (5.3 g) was chromatographed successively on columns with Sephadex LH-60 (0.9×50 cm) in the chloroform—methanol system (4 : 1), Florisil (1.5×20 cm, 60—100 mesh), and silica gel (2×26 cm, 200—250 mesh) in the chloroform—methanol system (20 : 1 → 15 : 1). A fraction containing a mixture of diols **1a** and **2** (225 mg) and a fraction containing **2** with slight impurities (100 mg) were purified by HLPC on a column with Zorbax ODS in the methanol—water system (55 : 45) and then subjected to re-chromatography on a column with Silasorb C_{18} in the ethanol—water system (42 : 58 and 47 : 53, respectively). This gave 25 mg (0.00016%) of compound **1a** and 18 mg (0.00012%) of compound **2**.

Isolation of glycosides 3 and 4. The polar fraction **II** was chromatographed on a column with Amberlite XAD-2 and then, successively, on columns with Sephadex LH-60, Florisil, and silica gel in the systems indicated above. The final purification was carried out by HPLC on a column with Silasorb C_{18} in the ethanol—water system (37 : 63) to give 40 mg (0.00026%) of **3** and 37 mg (0.00024%) of **4**.

3β,6α-Dihydroxy-5α-cholesta-9(11),24-dien-23-one 3-sulfate Na salt (1a) $C_{27}H_{41}NaO_6S$, colorless crystals, m.p. 126—128 °C (from MeOH—EtOAc), $[\alpha]_D^{20}$ +2.6 (*c* 1.2, EtOH). The ^{1}H NMR spectrum (MeOH) was used for the comparison with published data[5,7] and is discussed in the text. The ^{1}H and ^{13}C NMR spectra (C_5D_5N) are given in Tables 1 and 2.

Desulfation of 1a. A solution of sulfate **1a** (10 mg) in a dioxane—pyridine mixture (2 mL, 1 : 1) was heated for 2 h at 120 °C. The solution was concentrated *in vacuo* to dryness and the residue was chromatographed on a column with silica gel in the chloroform—methanol system (9 : 1 → 6 : 1) to give 5.3 mg of amorphous diol **1b**, $[\alpha]_D^{20}$ +2.84 (*c* 1.2, MeOH), which was identified by comparison of its spectral characteristics with those published for marthasterone.[6]

3β,6α-Dihydroxy-5α-cholesta-9(11),24-dien-23-one 3-sulfate 6-acetate Na salt (1c). Compound **1a** (8 mg) was treated with a $Ac_2O—C_5D_5N$ mixture (2 mL, 1 : 1, 16 h, ~20 °C). Chromatography on silica gel in the $CHCl_3$—EtOAc system (3 : 1) gave 4.6 mg of acetate **1c,** $C_{29}H_{43}NaO_7S$, amorphous, $[\alpha]_D^{20}$ +9.9 (*c* 1.2, $CDCl_3$). The ^{1}H NMR spectrum (C_5D_5N) is presented in Table 1; a more informative spectrum recorded in $CDCl_3$ is discussed in the text.

3β,6α-Dihydroxy-5α-cholest-9(11)-en-23-one 3-sulfate Na salt (2). $C_{27}H_{43}NaO_6S$, amorphous, $[\alpha]_D^{20}$ +9.5 (*c* 1.2, MeOH). The ^{1}H and ^{13}C NMR spectra are given in Tables 1 and 2.

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References

1. P. V. Andriyashchenko, E. V. Levina, and A. I. Kalinovskii, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 473 [*Russ. Chem. Bull.*, 1996, **45**, 455 (Engl. Transl.)]; A. A. Kicha, A. I. Kalinovskii, and V. A. Stonik, *Khim. Prirod. Soedinenii*, 1991, **4**, 520 [*Chem. Nat. Compds*, 1991, **4** (Engl. Transl.)].
2. N. V. Ivanchina, A. A. Kicha, A. I. Kalinovsky, P. S. Dmitrenok, V. A. Stonik, R. Rugiera, and C. Jimenez, *J. Nat. Prod.*, 2000, **63**, 1178.
3. G. B. Elyakov, E. V. Levina, V. S. Levin, V. A. Stonik, and I. I. Kapustina, *Comp. Biochem. Physiol.*, 1976, **55B**, 57.
4. F. De Simone, A. Dini, E. Finamore, L. Minale, C. Pizza, and R. Riccio, *Comp. Biochem. Physiol.*, 1979, **64B**, 25.
5. T. Komori, H. Chr. Krebs, Y. Itakura, R. Higuchi, K. Sakamoto, S. Taguchi, and T. Kawasaki, *Liebigs Ann. Chem.*, 1983, 2092.
6. D. S. H. Smith, A. B. Turner, and A. M. Mackie, *J. Chem. Soc., Perkin Trans. 1*, 1973, 1745.
7. A. Dini, F. A. Mellon, L. Minale, C. Pizza, R. Riccio, R. Self, and F. Zollo, *Comp. Biochem. Physiol.*, 1983, **76B**, 839.
8. R. Riccio, M. Iorizzi, and L. Minale, *Bull. Soc. Chim. Belg.*, 1986, **95**, 869.
9. L. Minale, R. Riccio, O. Squillace Greco, J. Pusset, and J. L. Menou, *Comp. Biochem. Physiol.*, 1985, **80B**, 113.
10. R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, 1977, **175**; K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, 1977, **179**.
11. M. S. Maier, A. Roccatagliata, and A. M. Seldes, *J. Nat. Prod.*, 1993, **56**, 6, 939.

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