Structure of ulososide A, a new triterpenoid glycoside from the *Ulosa* sp. sponge

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A new triterpenoid glycoside, (20*S*,22*S*,23*R*,24*S*)-3β,22,23-trihydroxy-3-*O*-[*O*-(β-D-glucuronopyranosyl)-(1→6)-(β-D-glucopyranosyl)]-14-nor-24-methyllanost-8(9)-en-31-oic acid (ulososide A), has been isolated from the sponge *Ulosa* sp. and characterized.

Key words: sponges; glycoside; triterpenoid; hydrolysis; periodate oxidation; reduction; genins; NMR spectra.

Quite recently, only Echinodermata have been known[1,2] to produce triterpenoid and steroid oligosides. Several years ago, we discovered similar compounds in sponges,[3] however, their structures could not be established then. Later, a series of triterpene glycosides of the 14α-norlanostane type named sarasinosides (**1**) were obtained from extracts of the sponge *Asteropus sarasinosus*.[4] In the present paper, the isolation and structure determination of a new triterpene glycoside, ulososide A (**2**), from the sponge *Ulosa* sp. are described.

Results and Discussion

Glycoside **2** was isolated from an extract of the Madagascarian sponge *Ulosa* sp. (*Demospongiae* class, *Hymeniacidonidae* family) by reversed-phase column chromatography on Polychrom-1, adsorption chromatography on silica gel, and HPLC on a Separon SGX-NH_2 column. The structure of this compound (Scheme 1) was established by NMR spectroscopy (Tables 1, 2) and routine chemical methods (acid hydrolysis, periodate oxidation, *etc.*).

The ^1H NMR spectrum of the glycoside demonstrated the presence of two monosaccaride residues in the carbohydrate chain. After acid hydrolysis of glycoside **2**, D-glucose and D-glucuronic acid were identified. The treatment of glycoside **2** with diazomethane afforded dimethyl ester **3**, whose ^1H NMR spectrum contained two singlets for the protons of the methoxy groups at δ 3.5 and 3.68. In the ^{13}C NMR spectrum, the chemical shifts of the carbon atoms of the carbohydrate chain of **3** were close to the corresponding signals of methyl 6-*O*-[methyl-(β-D-glucopyranosyl)uronate]-β-D-glucopyranoside,[5] which allowed one to propose the structure of this fragment of the molecule. In addition, the position of the signal of C(6′) at 70.3 ppm in the ^{13}C NMR spectrum of ester **3** attests to the presence of 6-*O*-substituted glucose in its carbohydrate chain. It is known that in the NMR spectra of glycosides a gradient of relaxation times along the carbohydrate chain takes place, and the monosaccharide residues located nearer to the terminus of the chain relax more slowly. Thus, if partially relaxed spectra are recorded with a 180°—τ—90° pulse sequence, one can determine the type of terminal monosaccharide.[6] The registration of the partially relaxed spectrum under conditions of deficient relaxation of the terminal monosaccharide residue demonstrated the absence of signals of glucuronic acid in the spectrum, thus attesting to its terminal position.

Reduction of compound **3** with $LiAlH_4$ gave derivative **4**, after its acid hydrolysis the only identified monosaccharide was glucose, and genins **5** and **6** were found and isolated.

Analysis of the NMR spectra of compounds **2**—**6** and their comparison with the literature data[4,7] attest to the 14α-norlanostane skeleton of the native aglycone of ulososide A with one additional methyl group, three hydroxyl groups, and one carboxylic group. The $\Delta^{8(14)}$- and $\Delta^{14(15)}$-isomers (**5** and **6**) are formed due to migration of the 8(9)-double bond to the 8(14)-position and then to the 14(15)-position during acid treatment of **4**, as is described for other $\Delta^{8(9)}$-14α-norlanostane derivatives.[4,7]

The 4β-position of the carboxylic group in the aglycone was determined principally from the nuclear Overhauser effect experiments (NOE). The results of these experiments are presented in Scheme 1 (formula **5**), where the interacting nuclei are connected with arrows. The β-position of the carboxylic group is also confirmed by the comparison of the chemical shift of C(30) (δ 24.0) in the spectrum of derivative **3** with the corresponding data for the model C(4)-epimeric compounds.[8]

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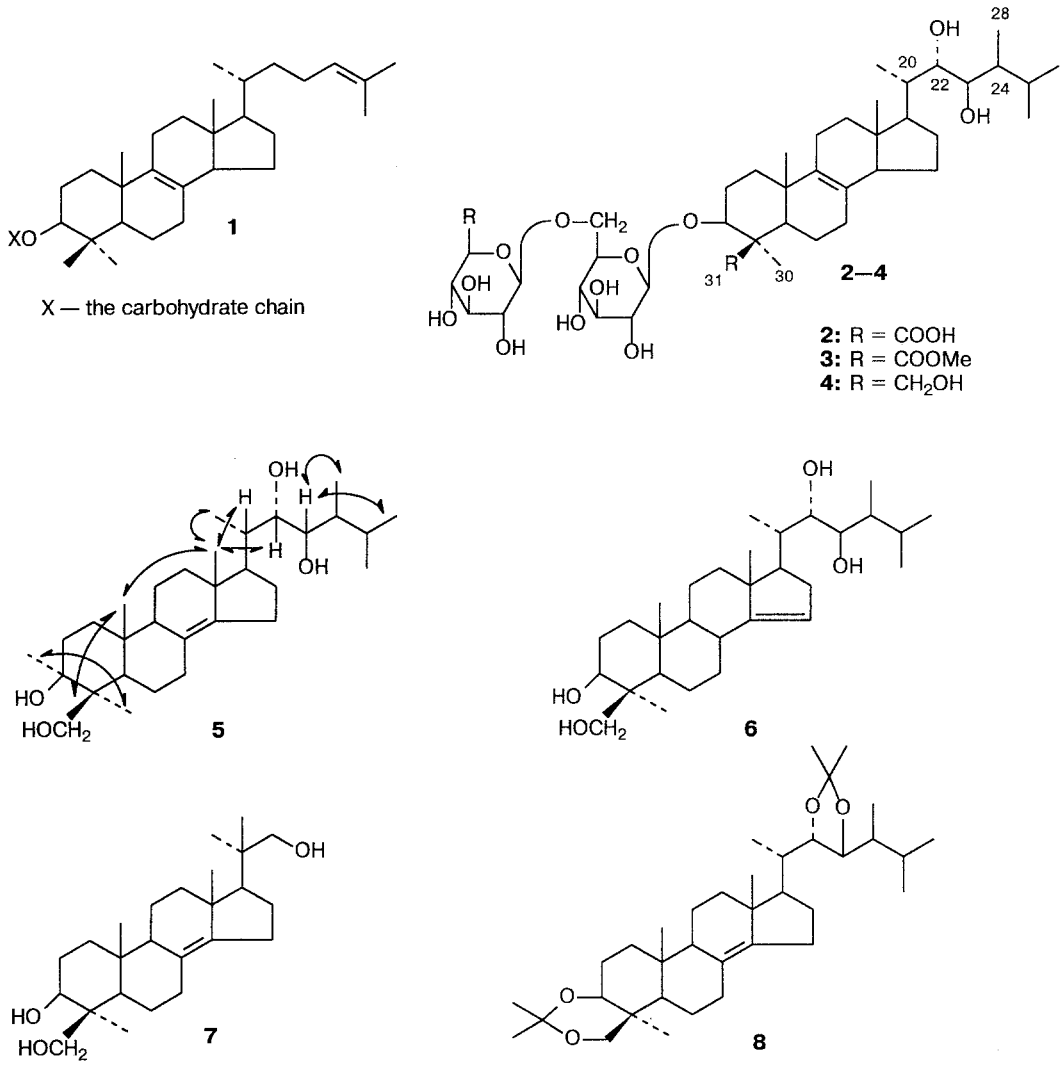
Scheme 1



The position of the hydroxyl groups at C(22) and C(23) of the lanostane nucleus of aglycone and the position of the additional methyl group at C(24) were established on a basis of the character of the signals of the corresponding protons and differential double resonance for genin **5**. In fact, irradiation of the H(20) signal at δ 2.50 results in the transformation of the doublet signal at δ 1.42 of Me(21) into a singlet and narrowing of the signal at δ 4.20 of C(22)H—OH is observed. Irradiation of C(24)H at 2.20 ppm transforms the doublet of Me(28) at δ 1.31 into a singlet, and the signal of C(23)H—OH at δ 4.32 converts into a doublet. The position of C(25)H (δ 2.04) was established following the irradiation of the doublets of Me(26,27) at 1.16 and 1.13 ppm. The relative configurations of the chiral centers C(20), C(22), C(23), C(24) were only tentatively assigned on the basis of NOE data following irradiation of the corresponding protons and analysis all the possible variants on molecular models.

In addition, positions 22 and 23 of the hydroxyl groups in the side chain of the aglycone were confirmed by periodate oxidation of derivative **3**, which afforded genin **7** with a shortened side chain (mass spectrum, *m/z*: 376 $[M]^+$) after treatment of the reaction products with sodium borohydride and acid hydrolysis. Preparation of diacetonide **8** from genin **5** in the reaction of the latter with 2,2-dimethoxypropane also attests to the vicinity of the hydroxyl groups in the side chain of the glycoside.

Ulososide A possesses the following unique structural features in comparison with the other glycosides of marine invertebrates. One of the two methyl groups at C(4) is absent, and a carboxyl group is there instead. The methyl group is located at C(24) in the side chain, which is not characteristic for derivatives of the lanostane series, and the α-diol fragment is also present. The glucuronic acid residue is a constituent of the carbohydrate chain.

Table 1. ^{13}C NMR spectra of ulososide A and its derivatives

Atom	Compound			
	2	**3**	**4**	**5**
C(1)	36.9	36.5	35.9	37.5
C(2)	28.8[a]	28.8[a]	28.7[a]	27.4
C(3)	88.8	88.8	88.9	80.3
C(4)	50.2	50.3	44.3	43.0[a]
C(5)	52.3[b]	50.5	51.4	54.8[a]
C(6)	20.8	20.4	20.3	22.6
C(7)	27.0	27.9	27.6	30.8
C(8)	128.1	127.8	127.6	126.2
C(9)	136.5	136.3	135.9	51.9[b]
C(10)	37.9	37.3	36.6	37.9
C(11)	22.7	22.5	22.4	20.3
C(12)	37.9	37.6	37.5	38.2
C(13)	42.8	42.5	42.4	43.0[a]
C(14)	52.6[b]	52.1	52.2	142.4
C(15)	24.7	24.0	24.0	26.1
C(16)	29.2[a]	28.9[a]	29.0[a]	28.9
C(17)	52.7[b]	51.8	51.9	55.7
C(18)	11.9	11.8	11.7	18.8
C(19)	18.9	18.3	19.0	15.6
C(20)	37.7	37.6	37.5	36.0
C(21)	12.7	12.6	12.8	12.8
C(22)	73.3[c]	72.9[b]	72.8[b]	72.8[d]
C(23)	72.4[c]	72.0[b]	71.7[b]	72.0[d]
C(24)	40.7	40.6	40.5	40.6
C(25)	31.7	31.7	31.6	31.7
C(26)	21.6[d]	21.6[c]	21.6[c]	21.6[c]
C(27)	20.8[d]	20.9[c]	21.0[c]	20.9[c]
C(28)	10.2	10.2	10.3	10.3
C(30)	24.1	24.0	23.1	23.7
C(31)	173.5	174.1	63.1	64.2
C(1′)	105.7[e]	107.2[e]	105.8	
C(2′)	75.3	75.2	75.2	
C(3′)	78.3	78.4	78.4	
C(4′)	71.0	71.4	71.7	
C(5′)	76.5	76.9	77.4	
C(6′)	69.9	70.3	70.0	
C(1″)	104.9[e]	105.5[e]	105.1[e]	C(1) 105.8*
C(2″)	74.5	74.7	75.0	C(2) 74.4*
C(3″)	78.0	77.5	78.2	C(3) 77.7*
C(4″)	73.3	72.9	71.7	C(4) 72.4*
C(5″)	76.8	77.2	78.2	C(5) 77.0*
C(6″)	177.8	170.1	62.7	C(6) 170.1*
COOMe		52.4		51.9*
OMe				56.9*

[a,b,c,d,e] Tentative assignment of signals.
* Signals of methyl (methyl-β-D-glucopyranoside)uronate.

It is interesting that from the point of view of its aglycone ulososide A is a biogenetic link between sarasinosides and the glycosides recently found[9] in the sponge *Erilus lendenfeldi*, whose aglycone belongs to the 4-methylcholestane series. In fact, in the aglycone of glycoside **2** one of the two methyl groups at C(4) is oxidized to carboxyl, whereas aglycones of *E. lendenfeldi* glycosides are apparently the products of further decarboxylation of the active metabolites that are similar to ulososide in the structure of the aglycone.

Table 2. ^1H NMR spectra of derivatives **5**, **6**, and **7** (C_5D_5N, δ, *J*/Hz)

Assign-	Compound		
ment	**5**	**6**	**7**
C(3)H	3.73 (m)	3.66 (m)	3.70 (m)
C(15)H	5.26 (m)		
C(20)H	2.50 (m)		
C(22)H	4.20 (d.d, ^1J = 9.2, ^2J = 1.0, A)	4.18 (d.d)	3.86 (d.d)
C(23)H	4.32 (d.d, ^1J = 9.2, ^2J = 1.5, B)	4.33 (d.d)	3.55 (d.d)
C(24)H	2.20 (m)		
C(31)H	4.60 (d, *J* = 10.8)	4.56 (d, *J* = 10.8)	4.55 (d, *J* = 10.8)
C(31′)H	3.73 (d, *J* = 10.8)	3.66 (d, *J* = 10.8)	3.65 (d, *J* = 10.8)
Me(18)	1.00 (s)	1.03 (s)	1.01 (s)
Me(19)	0.79 (s)	0.88 (s)	0.67 (s)
Me(21)	1.42 (d, *J* = 6.5)	1.41 (d, *J* = 6.6)	1.28 (d, *J* = 6.5)
Me(26)	1.16 (d, *J* = 6.6)	1.16 (d, *J* = 6.5)	
Me(27)	1.13 (d, *J* = 6.7)	1.13 (d, *J* = 6.7)	
Me(28)	1.31 (d, *J* = 6.7)	1.32 (d, *J* = 6.6)	
Me(30)	1.56 (s)	1.55 (s)	1.50 (s)

Experimental

The ^1H and ^{13}C NMR spectra were recorded with a Bruker WM-250 spectrometer (250 MHz). Chemical shifts are given in relation to tetramethylsilane as the internal standard. Optical rotations were determined with a Perkin-Elmer 141 polarimeter and a lamp with λ = 578 nm. GLC analysis of monosaccharide derivatives was carried out with a Tsvet-101 chromatograph (glass columns 1.5 m×3 mm with 3 % of QF-1 on Chromaton N-HMDS, argon as the carrier gas, 60 mL min^{-1}). Melting points were determined using a Boetius heating stage. The mass spectra were obtained on an LKB 9000S mass spectrometer (70 eV). For GLC—MS a glass column (3 m×5 mm with 3 % QF-1) at 140—230 °C, 5 °C min^{-1}, and a flame-ionization detector were used. Thin layer chromatography was carried out on glass plates (5×5 cm) with a fixed silica gel layer L (5—40 μm, Chemapol, Czechoslovakia). Polychrom 1 (Olaine, Latvia) was used for reversed-phase low-pressure chromatography. HPLC was performed with a DuPont Model 8800 chromatograph, equipped with 5 μm Separon-SGX-NH_2 (30×0.7 cm), Separon-SGX, and Separon-SGX-CN columns; the two latter columns were 15×0.7 cm (Tessek, Czechoslovakia). The animals were collected by diving to 20 m depth near the Northwestern coast of Madagascar Island in November, 1986, and classified by D. Sci. (Biol.) V. M. Koltun (Institute of Zoology, RAS, St. Petersburg).

Isolation of ulososide A (2). Lyophilized sponge tissues (360 g, dry weight) were extracted with boiling methanol (3×200 mL) and water (200 mL). The combined extracts were concentrated *in vacuo* at 70 °C, and the residue was chromatographed on a Polychrom-1 column (20×7.5 cm) with elution by water (1 L) and then 50 % aqueous ethanol (1 L). The glycoside fraction obtained from the aqueous ethanolic eluate after concentration *in vacuo* was purified by

column chromatography on silica gel (40/100 μm) using a chloroform—methanol—water system (65 : 25 : 4) and then separated by HPLC on a Separon-SGX-NH_2 column in 20 % 0.05 *M* $NaH_2PO_4 \cdot 2\ H_2O$ in methanol. Salts were removed from the eluate by repeated chromatography on Polychrom-1 (a 4 × 2 cm column), and elution with water (100 mL) and then with 50 % aqueous ethanol (100 mL) was carried out. Glycoside **2** (600 mg) was obtained from the aqueous ethanolic eluate as a colorless amorphous powder, m.p. 191—193 °C (precipitation with water from an ethanolic solution), $[\alpha]_{578}$ −4.5° (*c* 2.51, methanol). Found (%): C, 55.44; H, 7.77. $C_{42}H_{66}O_{16}Na_2 \cdot 2\ H_2O$. Calculated (%): C, 55.51; H, 7.71. IR (KBr, ν/cm^{-1}): 3450, 2930, 2870, 1700, 1640, 1240, 1100, 1050. The ^{13}C NMR spectrum is presented in Table 1.

Preparation of dimethyl ester 3. Glycoside **2** (300 mg) was dissolved in methanol and treated with excess diazomethane at *ca.* 20 °C for 10 min. After removal of the solvent, the residue was purified by HPLC on a Separon-SGX column in a chloroform—methanol—water (65 : 15 : 1.5) system to give ester **3** (307 mg), m.p. 196—198 °C (from aqueous methanol), $[\alpha]_{578}$ +17.9° (*c* 2.92, methanol). Found (%): C, 60.45; H, 8.25; $C_{44}H_{72}O_{16} \cdot H_2O$. Calculated (%): C, 60.41; H, 8.46. The ^{13}C NMR spectrum is presented in Table 1.

Reduction of dimethyl ester 3 with $LiAlH_4$. Compound **3** (300 mg) was treated with a mixture of pyridine and acetic anhydride (5 mL) for 12 h at *ca.* 20 °C. The solvent was removed *in vacuo*, the product was purified by HPLC on a Separon-SGX column in an ethyl acetate—hexane (1 : 1) system, and the peracetate (310 mg) was obtained. Found (%): C, 60.74; H, 7.38; $C_{60}H_{88}O_{24}$. Calculated (%): C, 60.40; H, 7.38. The peracetate was dissolved in 10 mL of dioxane and treated with excess $LiAlH_4$ at 100 °C. After the usual treatment of the reaction mixture and HPLC on a Separon-SGX-CN column in a methanol—water (3 : 2) system, compound **4** (145 mg) was obtained, m.p. 246—248 °C, $[\alpha]_{578}$ −13.1° (*c* 3.58, methanol). The ^{13}C NMR spectrum is presented in Table 1.

Acid hydrolysis of compound 4 and preparation of genins 5 and 6. Compound **4** (140 mg) was treated with a 2*N* H_2SO_4—benzene (2 : 1) mixture (6 mL) at 80 °C for 3 h. Each 20 min, the organic layer was separated and replaced with a new portion. The combined benzene extract was washed with water and concentrated *in vacuo*. A mixture of genins (35 mg) was obtained. The mixture was purified and separated by column chromatography on silica gel L (40/100 μm) (genins were eluted with ethyl acetate), and then by HPLC on a Separon-SGX column in a chloroform—methanol (175 : 4) system. As a result, genin **5** (7 mg) and genin **6** (14 mg) were obtained. Compound **5**, m.p. 254—256 °C (from aqueous methanol), $[\alpha]_{578}$ −12.3° (*c* 4.0, methanol); mass spectrum, *m/z* (I_{rel} (%)): 476 (15), 301 (8), 297 (10), 213 (14), 211 (20), 199 (100), 198 (50). Compound **6**, m.p. 254—256 °C (from aqueous methanol), $[\alpha]_{578}$ +30.1° (*c* 0.83, methanol): mass spectrum, *m/z* (I_{rel} (%)): 476 (20), 458 (16), 440 (6), 428 (14), 197 (100). The ^{13}C and ^1H NMR spectra are presented in Tables 1, 2.

Hydrolysis of compound 2 and identification of glucose and glucuronic acid. Compound **2** (5 mg) was treated with 10 % hydrochloric acid (1 mL) for 2 h at 90 °C. The reaction mixture was extracted with chloroform (3 × 0.7 mL), and the aqueous layer was separated and concentrated to dryness *in vacuo*. Glucuronic acid was identified by paper electrophoresis (Filtrak FN-15, 0.025 *M* pyridine—acetate buffer, pH 5.3). The determination of the D-configuration of both sugars was performed on basis of the overall optical rotation of its mixture: found $[\alpha]_D$ +17° (equilibrium), calculated for a mixture of D-glucose and D-glucuronic acid (1 : 1) +32°. The mixture of monosaccharides was redissolved in water (10 mL), the solution was neutralized with a Dowex (HCO_3^-) ion exchange resin, the monosaccharides were eluted from the resin with water, and after the usual workup[10] glucose was identified as gluconitrile acetate by GLC—MS.

Periodate oxidation of 4 followed by hydrolysis and isolation of 7. Compound **4** (32 mg) was treated with $NaIO_4$ (60 mg) in 3 mL of a water—*n*-butanol mixture (2 : 1) at *ca.* 20 °C for 24 h. The organic layer was separated, the solvent was removed *in vacuo*, the residue was treated with $NaBH_4$ (50 mg) in 3 mL of a methanol—water mixture (1 : 1) for 12 h at *ca.* 20 °C. After neutralization of the mixture with AcOH and evaporation to dryness *in vacuo* with periodic addition of methanol, a residue (30 mg) was obtained, which was hydrolyzed with a 1*N* H_2SO_4—*n*-butanol (2 : 1) mixture (3 mL) at 90 °C for 1 h. The organic phase was separated, washed with 1 mL of 5 % $NaHCO_3$, and after removal of the solvent *in vacuo* the residue was chromatographed on a column with silica gel L (40/100 μm) using a chloroform—methanol (35 : 3) mixture as the eluent. Then the crude genin was rechromatographed on a Separon-SGX column in a chloroform—methanol (35 : 0.4) system. As a result genin **7** (3.3 mg) was isolated, m.p. 249—252 °C (from aqueous methanol), $[\alpha]_{578}$ +36.4° (*c* 0.3, methanol); mass spectrum, *m/z* (I_{rel} (%)): 376 (100), 358 (16), 338 (6), 328 (10), 206 (16). The ^1H NMR spectrum is presented in Table 2.

Acetonation of 5. Compound **5** (5 mg) was treated with 2,2-dimethoxypropane (0.2 mL) and *p*-toluenesulfonic acid (2.5 mg) in acetone (1.5 mL) at *ca.* 20 °C for 30 min. Then the reaction mixture was neutralized with $NaHCO_3$, filtered, and concentrated *in vacuo*. The residue was extracted with benzene (3 × 3 mL), and the benzene extract was concentrated *in vacuo* and chromatographed on a column with silica gel L in a benzene—ethyl acetate (10 : 1) system. Derivative **8** was obtained, m.p. 76—79 °C, $[\alpha]_{578}$ +8.0° (*c* 0.5, methanol); mass spectrum, *m/z* (I_{rel} (%)): 556 (0.1), 541 (5), 526 (4), 515 (22), 500 (4), 498 (3), 171 (100). The ^1H NMR ($CDCl_3$, δ, *J*/Hz): 4.21 (br.d, J = 6.2, 1 H,); 4.15 (d, J = 11.5, 1 H,); 4.00 (d, ^1J = 6.2, ^2J = 10.5, 1 H,); 3.47 (d.d, ^1J = 4.5, ^2J = 11.5, 1 H,); 3.25 (d, J = 11.5, 1 H,); 1.46 (s, 3 H); 1.44 (s, 3 H); 1.39 (s, 3 H); 1.34 (s, 3 H); 1.28 (s, 3 H); 0.99 (d, J = 6.5, 3 H); 0.94 (d, J = 6.5, 3 H); 0.92 (d, J = 6.6, 3 H); 0.84 (s, 3 H); 0.82 (d, J = 6.6, 3 H).

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