Steroidal polyols from Far-Eastern starfishes *Henricia sanguinolenta* and *H. leviuscula leviuscula*

E. V. Levina, A. I. Kalinovskii, V. A. Stonik, and P. S. Dmitrenok*

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100-letiya Vladivostoka, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050. E-mail: piboc@stl.ru

Two new compounds were isolated from two Far-Eastern starfish species, *Henricia sanguinolenta* and *H. leviuscula leviuscula,* collected in the Sea of Okhotsk, *viz.*, the glycoside sanguinoside C, (20*R*,22*E*,24*R*,25*S*)-3-*O*-(2,3,4-tri-*O*-methyl-β-xylopyranosyl)-24-methyl-5α-cholest-22-ene-3β,4β,6β,8β,15α,26-hexol, and a steroidal ketone, (20*R*,24*S*)-3β,6β,24-trihydroxy-5α-cholestan-15-one. They exhibit moderate cytostatic activity with respect to the eggs of sea urchin *Strongylocentrotus intermedius*.

Key words: starfish, *Henricia sanguinolenta, H. l. leviuscula,* polyhydroxysteroids, glycosides, aglycons, ^{1}H and ^{13}C NMR spectra.

At present, a series of polyhydroxy steroids and their sulfated and glycosylated derivatives from starfishes of the Echinasteridae family are known.[1—3] Most of glycosides of this group contain a Δ^4-3β,6β,8,15α,16β-pentahydroxysteroid aglycon; they differ in the structures of side chains and in the nature of the monosaccharide residue. While continuing the study of physiologically active steroid derivatives from Far-Eastern starfishes, we determined the structures of two new compounds present in the extracts from *Henricia sanguinolenta* and *H. leviuscula leviuscula* (the Spinulosida order, Echinasteridae family), namely, monoglycosylated polyhydroxysteroid **1** and polyhydroxylated steroidal ketone **2**.

Results and Discussion

New steroid compounds **1** and **2** were isolated from ethanolic extracts of starfishes *Henricia sanguinolenta* and *H. leviuscula leviuscula* by chromatography on Amberlite XAD-2, Sephadex LH-60, Florisil, or silica gel followed by reversed-phase HPLC on columns with ODS-A and Silasorb C_{18}, as described previously.[4] Structural identification of the isolated compounds was performed by NMR spectroscopy (Table 1) and mass spectrometry (MALDI-TOF, EIMS).

A peak of an $[M + Na]^+$ pseudomolecular ion with *m/z* 677 recorded in the MALDI-TOF positive-ion mass spectrum and the ^{13}C and ^{1}H NMR data for compound **1** point to the molecular formula $C_{36}H_{62}O_{10}$. Indeed, the ^{13}C NMR and DEPT spectra (see Table 1) indicate the presence of 36 carbon atoms (8 CH_3, 8 CH_2, and 17 CH groups and 3 C atoms carrying no protons). The chemical shift of the anomeric C atom (δ 102.6) and the spin—spin coupling constant of the anomeric proton (J = 7.4 Hz) observed in the ^{1}H NMR spectrum (CD_3OD) of compound **1** (see Table 1) attest to the β-configuration of the glycosidic bond. Acid hydrolysis of **1** yielded 2,3,4-tri-*O*-methylxylose, which was identified by TLC and paper chromatography (PC) in comparison with an authentic sample. A comparison of the ^{1}H and ^{13}C NMR data for methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside and glycoside **1** demonstrated full coincidence of the signals corresponding to the carbohydrate component in compound **1** with those of the methyl glycoside.

The positions of signals for the C(1)—C(19) and for C(3)H, C(4)H, C(6)H, C(15)H, C(18)H_3, and C(19)H_3 atoms and the spin—spin coupling constants of the corresponding protons in the ^{13}C and ^{1}H NMR spectra (CD_3OD) of compound **1** were the same as those for the previously described forbeside I (**3**) from the starfish *Asterias forbesi*.[5] This implies that the polycyclic moieties in these compounds are identical and that the carbohydrate residue is located at C(3). In particular, the signals at δ 70.0, 74.6, 76.1, 77.1, and 80.5 in the ^{13}C NMR spectrum of glycoside **1** were assigned to the methine C atoms linked to the OH groups of the pentahydroxycholestane nucleus. The signals for the anomeric carbon atom (δ 102.6) and the signals at δ 58.8, 60.8, 60.9, 64.1, 80.9, 84.5, and 86.6 were ascribed to the carbon atoms of the monosaccharide fragment.

The signals for the side-chain protons and C atoms coincided with those found in the spectra of two starfish steroids having identical side chains, namely, (20*R*,22*E*,24*R*,25*S*)-24-methyl-5α-cholesta-8(14),22-diene-3β,6α,15β,26-tetrol (**4**) from *Acodontaster conspicuus*[6] and desulfated echinasteroside A (**5**) from *Echinaster*

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 7, pp. 1539—1543, July, 2003.

1066-5285/03/5207-1623 $25.00

Table 1. Data of the NMR spectra of glycoside **1** (CD_3OD) and steroid **2** (C_5D_5N)

Atom	**1**		**2**		
	δ_C (mult)*	δ_H (*J*/Hz)	δ_C (mult)*	δ_H (*J*/Hz)	HMBC
1	40.9 t		38.9 t	1.05 m (*a*) 1.70 m (*e*)	
2	25.2 t	1.69 m (*a*)	38.4 t	1.84 m (*a*) 2.13 br.d (*e*) (*J* = 10.6)	
3	80.5 d	3.63 m	71.1 d	4.00 m	
4	74.6 d	4.26 m	36.9 t	2.45 q (*a*) (*J* = 12.6) 2.06 m (*e*)	C_3, C_5
5	50.5 d	1.28 m	48.2 d	1.28 m	
6	76.1 d	4.27 m	70.6 d	4.07 q (*J* = 2.5)	
7	45.2 t	1.57 m 2.40 dd (*J* = 3.0, *J* = 14.8)	39.2 t	1.30 m (*a*); 3.39 dt (*e*) (*J* = 13.7, *J* = 3.2)	
8	77.1 s		27.5 d	2.47 m	
9	57.7 d	0.98 m	54.1 d	1.75 m	
10	36.9 s		35.9 s		
11	19.3 t	1.47 m; 1.90 m	20.8 t	1.36 m (*a*); 1.56 m (*e*)	
12	42.6 t	1.25 m; 1.96 m	39.8 t	1.36 m, 2.07 m	
13	45.4 s		42.3 s		
14	66.7 d	1.18 m	65.4 d	1.80 d (*J* = 10.7)	C_8, C_{15}, C_{16}, C_{18}
15	70.0 d	4.25 m	214.9 s		
16	42.1 t	1.87m	41.9 t	1.89 dd (*J* = 9.7, *J* = 18.4); 2.52 dd (*J* = 8.4, *J* = 18.2)	C_{15} C_{13}, C_{15}
17	55.8 d	1.36 m	51.4 d	1.62 q (*J* = 9.6)	
18	15.5 q	0.98 s	12.8 q	0.71 s	C_{12}, C_{13}, C_{14}, C_{17}
19	18.6 q	1.44 s	15.8 q	1.39 s	C_1, C_5, C_9, C_{10}
20	40.7 d	1.98 m	35.7 d	1.53 m	
21	21.1 q	0.98 d (*J* = 6.6)	19.2 q	1.05 d (*J* = 6.5)	C_{17}, C_{22}
22	133.7 d	5.21 m	32.7 t	1.90 m; 1.21 m	
23	136.9 d	5.22 m	31.4 t	1.55 m; 1.75 m	
24	39.6 d	2.07 m	76.1 d	3.57 m	
25	42.0 d	1.51 m	34.0 d	1.85 m	
26	66.7 t	3.26 m; 3.57 m	17.3 q	1.14 d (*J* = 6.5)	C_{24}, C_{25}, C_{27}
27	14.0 q	0.87 d (*J* = 6.8)	19.5 q	1.12 d (*J* = 6.5)	C_{24}, C_{25}, C_{26}
28	17.1 q	0.92 d (*J* = 6.9)			
1′	102.6 d	4.45 d (*J* = 7.4)			
2′	84.5 d	2.95 dd (*J* = 7.4, *J* = 8.6)			
3′	86.6 d	3.12 t (*J* = 8.5)			
4′	80.9 d	3.25 m			
5′	64.1 t	3.12 m 3.98 dd (*J* = 4.0, *J* = 10.7)			
OMe	60.8 q	3.58 s			
	60.9 q	3.59 s			
	58.8 q	3.44 s			

* The multiplicity was measured in the DEPT experiment.

sepositus,[7] where the stereochemistry of the side-chain double bond and the asymmetric centers had been determined previously. In the ^1H NMR spectrum of glycoside **1**, the multiplets for the protons at the double bond (δ 5.21 and 5.22, both m, CD_3OD, see Table 1) are well resolved only in C_5D_5N; in this case, they are located at δ 5.40 (dd, *J* = 15.0 and 6.8 Hz) and δ 5.36 (dd, *J* = 15.0 and 6.6 Hz). The spin—spin coupling constant of the olefinic protons and the carbon chemical shifts (see Table 1) correspond to the *trans*-configuration of the

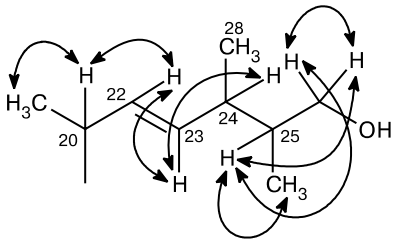


Fig. 1. COSY-45 for the side chain in sanguinoside C (**1**).

C(22)H=C(23)H bond in the side chain of glycoside **1**. The chemical shift of the C(20) atom (δ_C 40.7.) also corresponds to the *E* configuration of the double bond; for *Z*-isomer, this signal would appear at δ ~35.0.[8]

The structure of the side chain of glycoside **1** was confirmed by the COSY-45 spectrum (Fig. 1), while the structure of the whole molecule can be derived from the COSY-45 and HSQC data, which allowed the assignment of all carbon and hydrogen signals in the NMR spectra. The *R* configuration was assigned to the asymmetric center C(20) based on the chemical shift of C(21)H_3 (δ 0.98). These chemical shifts are known to depend on the configuration of the C(20) atom, being equal to δ 0.90—0.97 (CD_3OD) for the (20*R*)-isomers.[9] In the ^{1}H NMR spectra of the (20*S*)-isomers, these signals are shifted upfield by 0.1 ppm, the chemical shifts being equal to δ ~0.8.[10]

Based on the whole set of obtained data, glycoside **1**, which we called sanguinoside C, was identified as (20*R*,22*E*,24*R*,25*S*)-3-*O*-(2,3,4-tri-*O*-methyl-β-xylopyranosyl)-24-methyl-5α-cholest-22-ene-3β,4β,6β,8β,15α,26-hexol.

Analysis of the ^{1}H and ^{13}C NMR spectra of compound **2** showed that its molecule contains 27 carbon atoms, in particular, 5 methyl groups, 9 methylene groups, 10 methine groups, 1 carbonyl group, and 2 quaternary C atoms. The signals at δ_C 70.6, 71.1, and 76.1 were assigned to the C atoms bearing hydroxy groups, and the signal at δ_C 214.9 was attributed to the carbonyl function (see Table 1). The electron impact mass spectrum (EIMS) (70 eV) exhibits a peak of a molecular ion with *m/z* 434 $[M]^+$ and several peaks due to fragmentation (see Experimental). Based on these results, we proposed a saturated trihydroxycholestane structure with a carbonyl group and with the molecular formula $C_{27}H_{46}O_4$ for compound **2**. The positions of functional groups in the cyclic part of the molecule were established by comparing the ^{1}H and ^{13}C NMR spectra (CD_3OD) of steroid **2** with those of a known polyhydroxysteroid, 5α-cholestane-3β,6β,15α,16β,26-pentol (**6**) from the starfish *Hacelia attenuata*.[11] Good agreement of the chemical shifts of the C(1)—C(12) and C(19) atoms and those of the corresponding protons, C(3)H (δ 4.00, m), C(6)H (δ 4.07, q, J = 2.5 Hz), and C(19)H_3 (δ 1.39, s), confirms the presence of a 3β,6β-diol group in steroid **2**. The presence of the oxo group at C(15) is indicated by the doublet at δ 1.80 (J = 10.7 Hz) for the C(14)H methine proton and by the signals at δ 2.52 (dd, J = 8.4 and 18.2 Hz) and δ 1.89 (dd, J = 9.7 and 18.4 Hz) for the C(16)H_2 methylene protons.

Further structure determination was carried out using COSY-45, COSY RCT, HSQC, and HMBC experiments, which demonstrated the presence of the corresponding hydrocarbon sequences in those fragments of molecule **2** that are marked by thick lines in its formula shown in Scheme 1. These experiments allowed also the assignment of signals for all the carbon and hydrogen atoms in the NMR spectra of compound **2**.

The doublet for the proton adjacent to the carbonyl group was assigned to H(14) rather than to H(9), judging from the H(14)/H(18) cross-peak observed in the COSY-45 spectrum of compound **2**; a H(17)/H(18) cross-peak can also be found. In the HSQC spectrum, the C(14) is responsible for a low-field signal at δ 65.4; this was also confirmed by HMBC correlation.[12] The spin—spin coupling constant ($J_{H(14),H(18)}$ = 10.7 Hz) is indicative of *trans*-fusion of rings C and D. Thus, the data presented here confirm that the oxo group occupies position 15.

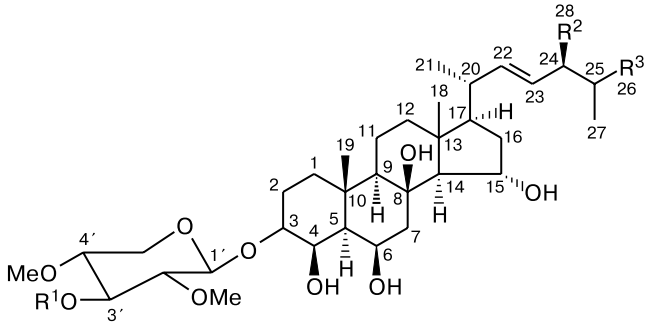
The chemical shifts of the signals for the C(20)—C(27) atoms in ketone **2** (C_5D_5N) were the same as those found for polyhydroxysteroid (**7**) from the starfish *Henricia sp.*,[13] which suggests the identity of the side chains in the two compounds and the (24*S*)-configuration for ketone **2**. The (20*R*)-configuration was ascribed to the C(20) asymmetric center on the same grounds as in the case of glycoside **1**.

On the basis of the results obtained, compound **2** was identified as (20*R*,24*S*)-3β,6β,24-trihydroxy-5α-cholestan-15-one.

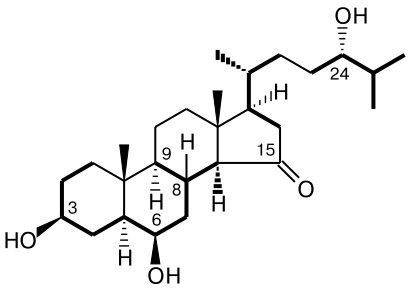
For the isolated compounds, we determined the minimum concentration (C_{min}) that inhibits division of the fertilized eggs of the sea urchin *Strongylocentrotus intermedius* in the first cell-division step. Glycoside **1** and steroid **2** showed equal moderate cytostatic activities: $C_{min} = 4.4 \cdot 10^{-5}$ and $4.5 \cdot 10^{-5}$ mol L^{-1}, respectively. These results are consistent with the data we obtained previously[4] for steroid derivatives from starfishes and confirm the conclusion that the cytostatic action on the sea-urchin eggs depends appreciably on whether or not sulfate residues are present and on the arrangement of hydroxy groups.

Only several starfish steroids containing a carbonyl function in the core are currently known; they have been found mainly in Antarctic starfish species. These include asterasterols B and C [14] from Asteriidae starfishes and polyol **8** [15] from Echinasteridae starfishes. A new group of polyhydroxysteroids **9** with the carbonyl group at C(6) was found in *Styracaster caroli*.[16] The steroid **2** we isolated is of interest due to its stereochemistry. It has an oxo group in position 15 and *trans*-fused rings C and D. The

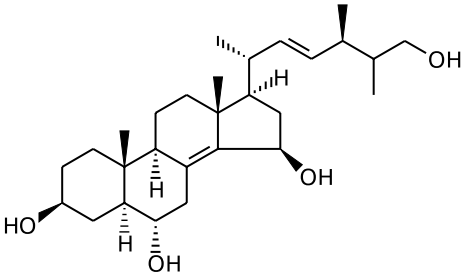
Scheme 1



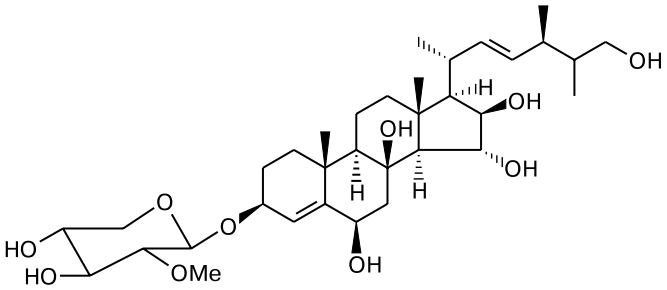
1, **3**



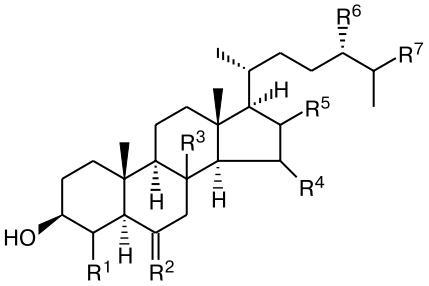
2



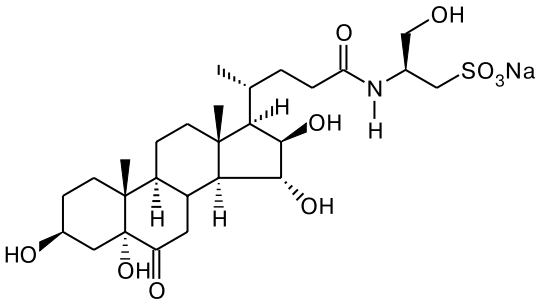
4



5



6—8



9

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	Me	Me	CH_2OH				
3	H	α-OH	Me 22,23-dihydro				
6	H	H, β-OH	β-H	α-OH	β-OH	H	CH_2OH
7	β-OH	H, β-OH	β-OH	β-OH	H	α-OH	Me
8	H	O	β-H	α-OH	β-OH	H	CH_2OH

cis-fusion of rings C and D is often more stable in 15-oxosteroids than the *trans*-fusion.[17] Six marine steroids with *cis*-fused rings C and D are known, in particular, contignasterol from the sponge *Petrosia contignata*,[18] xestobergsterols A—C from the sponges *Xestospongia berquistia*[19] and an *Ircinia* sp.[20], haliclonostanol sulfate from the sponge *Haliclona* sp.,[21] and 14β-tamosterone sulfate from sponges of the new genus *Oceanapia*;[22] all these contain an oxo group in position 15. However, tamosterone sulfate, which contains a 15-oxo group and *trans*-fused rings C and D, is a quite stable compound, like steroid **2**.

The discovery in starfishes of polyols with the oxo group confirms the presence of the corresponding oxido reductases responsible for the oxidation of hydroxy groups in the steroid substrate to oxo groups. Previously,[23,24] a study of the biosynthesis of steroids in various starfish species showed the presence of not only steroid dehydro-

genase but also hydroxylase and reductase. Therefore, steroids containing a carbonyl functional group can be either intermediate or final products of the steroid metabolism in these invertebrates.

The permethylated residue of the β-xylose, which we discovered in compound **1**, has not been found previously in starfish glycosides.

Experimental

^{1}H and ^{13}C NMR spectrum was recorded on Bruker DRX-500, Bruker DPX-300, and Bruker AC-250 spectrometers operating at 500, 300, and 250 MHz, respectively using Me_4Si as the internal standard. Optical rotation was measured on a Perkin—Elmer 141 polarimeter. MALDI-TOF mass spectrum was run on a Biflex III mass spectrometer (Bruker, Germany) with laser ionization/desorption (N_2 laser, 337 nm). The sample was dissolved in methanol (10 mg mL^{-1}) and a 1 μL aliquot portion was analyzed using *p*-hydroxy-α-cyanocinnamic acid as a matrix. EI mass spectra were run on an AMD-604S mass spectrometer (AMD, Germany), the energy of ionizing electrons was 70 eV. HPLC was carried on a Du Pont Model 8800 chromatograph using a refractometric detector and Zorbax ODS (5 μm, 4.6×250 mm) and Silasorb C_{18} (13 μm, 9.4×250 mm) as stationary phases. Melting points were determined on a Leica VMTG hot stage. TLC was carried out on Sorbfil plates with a CTX-1A layer fixed on the foil (5—17 μm, Russia, Krasnodar). Preparative column chromatography was carried out on silica gel L (80—100 and 200—250 mesh, Chemapol, Czechia) and Florisil (60—100 and 200—250 mesh, Merck, Germany).

Animals. Specimens of the starfishes *H. sanguinolenta* and *H. leviuscula leviuscula* were collected in August 1999 (sea of Okhotsk, Kuril Islands) during the 22nd trip of the research ship "Akademik Oparin" from a 100—200 m depth using a drag-net and identified by V. S. Levin (Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences).

Bioassays. The cytostatic activity of compounds **1** and **2** with respect to the fertilized eggs of the sea urchin *Strongylocentrotus intermedius* was determined by a previously described method.[4].

Extraction and isolation of total fractions. Crushed starfishes *H. sanguinolenta* (the animal weight was 0.32 kg) were exhaustively extracted with 95% EtOH at ~20 °C. The combined ethanolic extracts were concentrated *in vacuo* to give a crude resinous residue (30 g), which was chromatographed on a column (6×25 cm) with silica gel (80—100 mesh) in a $CHCl_3$—EtOH system (100 : 0 → 45 : 55) to give two fractions of polyhydroxysteroids, less polar fraction **I** (0.83 g), consisting of steroids **1** and **2**, and polar fraction **II** (4.5 g) containing known leviusculoside G and some minor components.

A similar procedure carried out for 52 g of a dry residue from an ethanolic extract of the starfish *H. leviuscula leviuscula* (the animal weight was 0.23 kg) gave 0.72 g of fraction **I** and 3 g of fraction **II**.

Isolation of compounds 1 and 2. Fraction **I** (*H. sanguinolenta*) was dissolved in water and passed through a column with Polychrom (3×12 cm), the column being eluted with water and 50% aqueous EtOH. The water—ethanol eluate was concentrated *in vacuo* to form a brown resinous residue (0.1 g), which was successively chromatographed on columns with Sephadex LH-60 (40×1.5 cm) in a 4 : 1 $CHCl_3$—EtOH solvent mixture and with Florisil (1.5×20 cm, 60—100 mesh) in a 6 : 1 → 5 : 1 $CHCl_3$—EtOH mixture to give a fraction containing glycoside **1** (6.5 mg) and a fraction containing steroid **2** (5 mg). This fraction was finally purified by HPLC on a column with Diaspher-110-C_{18} (5 μm, 4×250 mm) followed by re-chromatography on a YMC-Pack ODS-A column (5 μm, 12 nm, 10×250 mm) to give 2 mg of glycoside **1** (0.0006% of the animal weight) and 2.4 mg of steroid **2** (0.0007%). The separation of fraction **I** obtained from the starfish *H. leviuscula leviuscula* by a procedure described above gave 1 mg of glycoside **1** (0.0004% of the animal weight) and 2.5 mg of steroid **2** (0.001%).

(20*R*,22*E*,24*R*,25*S*)-3-*O*-(2,3,4-Tri-*O*-methyl-β-xylopyranosyl)-24-methyl-5α-cholest-22-ene-3β,4β,6β,8β,15α,26-hexol (sanguinoside C) (1), colorless crystals, m.p. 178—179.5 °C (from MeOH), $[\alpha]^{20}_D$ −13 (*c* 0.1, EtOH). The ^{1}H and ^{13}C NMR spectra (CD_3OD) are presented in Table 1; some data from the ^{13}C NMR spectrum (C_5D_5N) were used for comparison with published data and are given in the text. The interpretation of the COSY-45 spectrum for the side chain of **1** is shown in Fig. 1. MS MALDI-TOF (+) (I_{rel} (%)): *m/z* 677 [M + Na]$^+$ (100).

Hydrolysis of glycoside 1. Glycoside (2 mg) was dissolved in 2 mL of 2 *M* HCl and heated for 2 h at 100 °C. The hydrolyzate was analyzed by TLC on silica gel impregnated with 0.02 *M* NaH_2PO_4 using a 4 : 1 : 5 butanol—acetone—water solvent system and by paper chromatography in a 6 : 4 : 40 butanol—pyridine—water system. 2,3,4-Tri-*O*-methylxylose was identified. The ^{1}H and ^{13}C NMR (MeOH) spectra of glycoside **1** were compared with the spectra of methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside.

(20*R*,24*S*)-3β,6β,24-Trihydroxy-5α-cholestan-15-one (2), colorless crystals, m.p. 93—94.5 °C (from MeOH), $[\alpha]^{20}_D$ + 9.1 (*c* 0.6, EtOH). The ^{1}H and ^{13}C NMR spectra (C_5D_5N) are shown in Table 1. The EI mass spectrum (I_{rel} (%)) contains a peak of a molecular ion with *m/z* 434 [M]$^+$ (35) and other peaks corresponding to elimination of H_2O molecules: *m/z* 416 [M − H_2O] (30) and 398 [M − $2H_2O$] (25), methyl groups: 383 [M − $2H_2O$ − CH_3] (10), and the side chain: 305 [M − $C_8H_{17}O$] (12), 287 [M − $C_8H_{17}O$ − H_2O] (15) and 269 [M − $C_8H_{17}O$ − $2H_2O$] (10).

The authors are grateful to E. V. Evtushenko (Pacific Institute of Bioorganic Chemistry) for providing a sample of authentic methyl 2,3,4-tri-*O*-methyl-β-D-xylopyranoside used in the study and to N. G. Prokof´eva (Pacific Institute of Bioorganic Chemistry) for determining the cytotoxic action on the eggs of the sea urchin *Strongylocentrotus intermedius*.

This work was supported by the Russian Foundation for Basic Research (Projects No. 02-04-49491).

References

1. M. Iorizzi, F. DeRiccardis, L. Minale, and R. Riccio, *J. Nat. Prod.*, 1993, **56**, 2149.
2. L. Minale, M. Iorizzi, E. Palagiano, and R. Riccio, *Adv. Exp. Med. Biol.,* 1996, **404**, 335.

3. V. A. Stonik, *Usp. Khim.*, 2001, **70**, 763 [*Russ. Chem. Rev.*, 2001, **70** (Engl. Transl.)].
4. E. V. Levina, P. V. Andriyashchenko, A. I. Kalinovskii, and V. A. Stonik, *Izv. Akad. Nauk, Ser. Khim.*, 2001, 300 [*Russ. Chem. Bull., Int. Ed.*, 2001, **50**, 313].
5. J. A. Findlay and He Zheng-Quan, *J. Nat. Prod.*, 1991, **54**, 428.
6. S. De Marino, M. Iorizzi, F. Zollo, L. Minale, C. D. Amsler, B. J. Baker, and J. B. McClintock, *J. Nat. Prod.*, 1997, **60**, 959.
7. F. Zollo, E. Finamore, and L. Minale, *Gazz. Chim. Ital.*, 1985, **115**, 303.
8. J. L. C. Wright, A.G. Shimizu, S. Smith, J. A. Walter, D. Idler, and W. Khalil, *Can. J. Chem.*, 1978, **56**, 1898.
9. C. Pizza, L. Minale, D. Laurent, and J. L. Menou, *Gazz. Chim. Ital.*, 1985, **115**, 585.
10. D. J. Vanderah and C. Djerassi, *J. Org. Chem.*, 1978, **43**, 1442.
11. L. Minale, C. Pizza, F. Zollo, and R. Riccio, *Tetrahedron Lett.*, 1982, **23**, 1841.
12. H. Eggert and C. Djerassi, *J. Org. Chem.*, 1973, **38**, 3788.
13. A. A. Kicha, A. I. Kalinovskii, N. V. Gorbach, and V. A. Stonik, *Khim. Prirod. Soedin.*, 1993, **2**, 241 [*Chem. Nat. Compd.*, 1993 (Engl. Transl.)].
14. S. De Marino, E. Palagiano, F. Zollo, L. Minale, and M. Iorizzi, *Tetrahedron*, 1997, **53**, 8625.
15. M. Iorizzi, S. De Marino, L. Minale, F. Zollo, V. Le Bert, and C. Roussakis, *Tetrahedron,* 1996, **52**, 10997.
16. M. Iorizzi, F. De Riccards, L. Minale, E. Palagiano, R. Riccio, C. Debitus, and D. Duhet, *J. Nat. Prod.*, 1994, **57**, 1361.
17. N. L. Allinger, R. B. Hermann, and C. Djerassi, *J. Org. Chem.*, 1960, **25**, 922.
18. D. L. Burgoyone, R. J. Andersen, and T. M. Allen, *J. Org. Chem.*, 1992, **57**, 525.
19. N. Shoji, A. Umeyama, K. Shin, K. Takeda, S. Arihara, J. Kobayashi, and M. Takei, *J. Org. Chem.,* 1992, **57**, 2996.
20. J. Kobayashi, H. Shinonaga, H. Shigemori, A. Umeyama, N. Shoji, and S. Arihara, *J. Nat. Prod.*, 1995, **58**, 312.
21. S. Sperry and P. Crews, *J. Nat. Prod.,* 1997, **60**, 29.
22. X. Fu, M. L. G. Ferreira, F. J. Schmitz, and M. Kelly, *J. Org. Chem.*, 1999, **64**, 6706.
23. E. V. Levina and I. I. Kapustina, *Vses. simp. po bioorganicheskoi khimii (Vladivostok, 8—15 Sentyabrya, 1975) All-Union Symp. on Bioorganic Chem. (Vladivostok, September 8—15, 1975)*, *Abstrs.*, Vladivostok, 1975, 23 (in Russian).
24. H. J. N. Schoenmakers, *Comp. Biochem. Physiol. B*, 1979, **63**, 179.

Received February 27, 2003