New polyhydroxysteroids and steroid glycosides from the Far East starfish *Ceramaster patagonicus*

A. A. Kicha, A. I. Kalinovsky, and V. A. Stonik*

Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100-letiya Vladivostoka, 690022 Vladivostok, Russian Federation. Fax: 007 (423 2) 31 4050

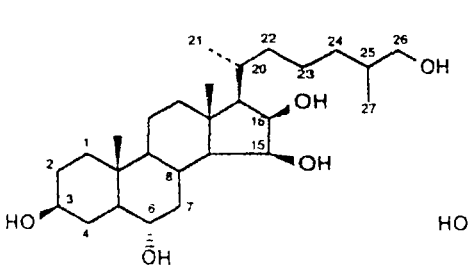
Two new polyhydroxysteroids and five new glycosides were isolated from the starfish *Ceramaster patagonicus* and their structures were elucidated: 5α-cholestane-3β,6α,15β,16β,26-pentol, (22*E*)-5α-cholest-22-ene-3β,6α,8,15α,24-pentol, (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,4β,6α,8,15β,16β,28-heptol (ceramasteroside C_1), (22*E*)-28-*O*-[*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,6α,8,15β,16β,28-hexol (ceramasteroside C_2), (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,6α,8,15β,16β,28-hexol (ceramasteroside C_3), (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,26-hexol (ceramasteroside C_4), and (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-xylopyranosyl]-5α-cholest-22-ene-3β,6α,8,15β,24-pentol (ceramasteroside C_5)). Three known polyhydroxysteroids (24-methylene-5α-cholestane-3β,6α,8,15β,16β,26-hexol, 5α-cholestane-3β,6α,8,15β,16β,26-hexol, and 5α-cholestane-3β,6β,15α,16β,26-pentol) were also isolated.

Key words: starfish, *Ceramaster patagonicus*; polyhydroxysteroids, glycosides; ^1H and ^{13}C NMR spectra.

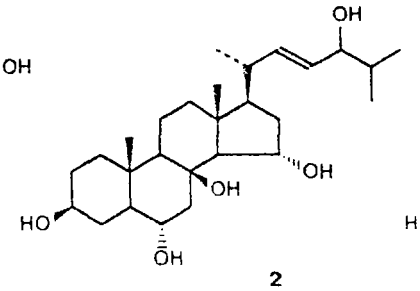
In continuation of our study of steroids of Far-East starfishes,[1–3] we isolated five polyhydroxysteroids (**1—5**) (two of them, **1** and **2**, were new) and five new steroid glycosides (**6—10**) from the starfish *Ceramaster patagonicus*.

The structure of polyhydroxysteroids **1—5** and glycosides **6—10** was established by ^1H and ^{13}C NMR spectroscopy (Tables 1—6).

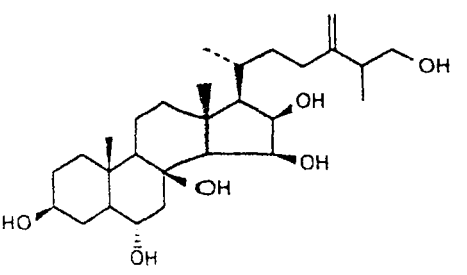
To determine the position and the configuration of hydroxy substituents in compound **1**, we used the



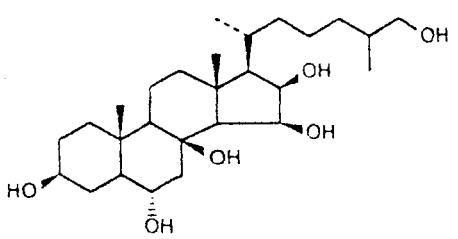
1



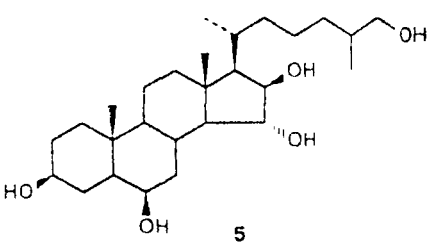
2



3



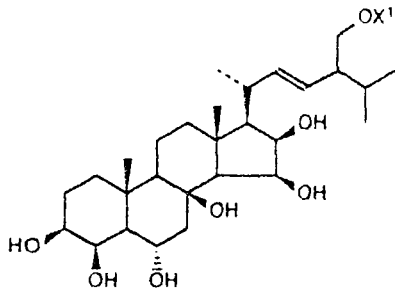
4



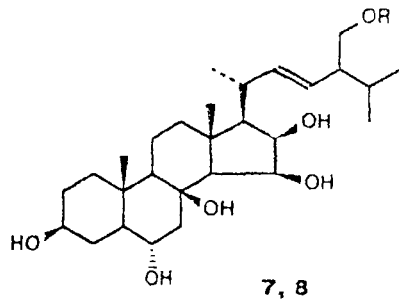
5

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 1, pp. 190—195, January, 1997.

1066-5285/97/4601-0186 $18.00

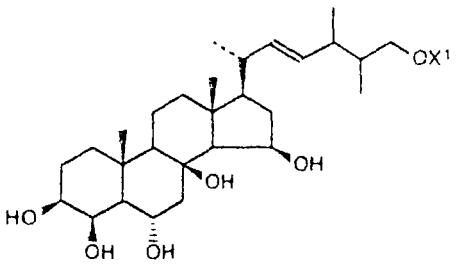


6

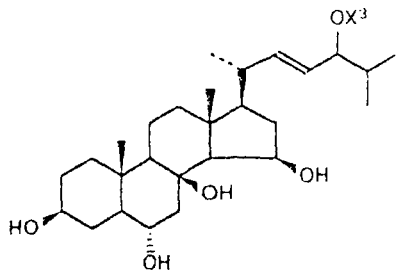


7, 8

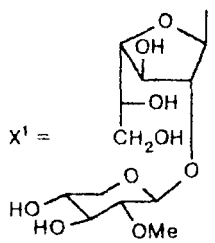
R = X^2 (7), X^1 (8)

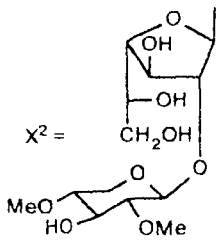


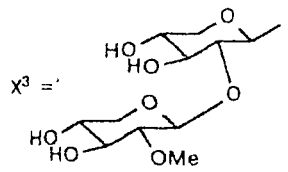
9



10







method of differential decoupling.* The chemical shifts (δ) of C atoms were assigned by comparing the ^{13}C NMR spectrum with spectra of the known compounds with the hydroxy groups at positions 3β, 6α, 15β, and 16β (see Refs. 4—6). The signals in the NMR spectra of the C and H atoms of the side chain of compound **1** coincided with the corresponding signals in the spectra of polyhydroxysteroids from the starfish *Patiria pectinifera*.[7] The structure of the side chain was confirmed by selective decoupling. For example, preirradiation of both $H_2C(26)$ and $H_3C(27)$ protons resulted in changes in the same multiplet of HC(25) (δ 1.87). Preirradiation of this proton, in its turn, transforms the signals of HC(26) into an AB quartet, and the signal of $H_3C(27)$ into a singlet. The structure of 5α-cholestane-3β,6α,15β,16β,26-pentol was assigned to compound **1** on the basis of the above experiments.

* The result of subtraction of the spectrum obtained by incomplete suppression of spin coupling with one or several protons upon small power irradiation from the routine spectrum.

The spectral data for the steroid backbone of compound **2** coincided with those for the polycyclic fragment of asterosaponin P_1 from the starfish *Patiria pectinifera*,[5] which proves the identity of the polycyclic systems in both compounds. The structure of the side chain was established by the spin decoupling experiments: sequential preirradiation of the HC(22), HC(23), HC(20), and $H_3C(26)$ protons results in the appearance of signals of the adjacent protons in the differential spectra. The value of spin coupling constant for the HC(22) and HC(23) protons (J = 15 Hz) correspond to the *trans*-configuration of the C(22)=C(23) bond,[8] which made it possible to assign the structure of (22*E*)-5α-cholest-22-ene-3β,6α,8,15α,24-pentol to steroid **2**.

The structure of the previously known polyhydroxysteroids **3**, **4**, and **5** was determined by comparing their ^1H NMR spectra with published ones. We identified compounds **3**, **4**, and **5** as 24-methylene-5α-cholestane-3β,6α,8,15β,16,26-hexol, 5α-cholestane-3β,6α,8,15β,16β,26-hexol, and 5α-cholestane-3β,6β,15α,16β,26-pentol, which have been isolated pre-

Table 1. ^{1}H NMR spectra of compounds **1** and **2** (in C_5D_5N)

Proton (group)	δ (*J*/Hz)	
	1	**2**
HC(3)	3.95 (m)	4.03 (m)
H_{ax}C(4)	1.69 (q, *J* = 11.0)	1.86 (q, *J* = 11.0)
H_{eq}C(4)	3.04 (m)	3.15 (m)
HC(5)	1.46 (m)	1.56 (m)
HC(6)	3.82 (td, *J* = 4.0, 10.3)	4.38 (td, *J* = 4.0, 10.3)
H_{ax}C(7)	1.46 (q, *J* = 10.5)	2.21 (dd, *J* = 11.6, 13.0)
H_{eq}C(7)	3.04 (m)	3.42 (dd, *J* = 4.0, 13.0)
HC(8)	2.26 (qd, *J* = 4.0, 10.7)	
HC(14)	0.98 (dd, *J* = 5.5, 11.0)	1.75 (d, *J* = 10.0)
HC(15)	4.43 (dd, *J* = 5.5, 7.0)	4.80 (td, *J* = 3.3, 10.0)
HC(16)	4.51 (t, *J* = 7.0)	
HC(17)	1.12 (dd, *J* = 7.3, 11.0)	
HC(20)		2.23 (m)
HC(22)		5.77 (dd, *J* = 7.6, 15.6)
HC(23)		5.71 (dd, *J* = 5.5, 15.6)
HC(24)		4.12 (t, *J* = 5.5)
HC(25)	1.87 (m)	1.93 (m)
HC(26)	3.68 (dd, *J* = 6.4, 10.1)	
H′C(26)	3.80 (dd, *J* = 5.8, 10.3)	
H_3C(18)	1.27 (s)	1.35 (s)
H_3C(19)	0.93 (s)	1.46 (s)
H_3C(21)	1.12 (d, *J* = 6.7)	1.12 (d, *J* = 6.7)
H_3C(26)		1.09 (d, *J* = 7.0)
H_3C(27)	1.13 (d, *J* = 6.8)	1.15 (d, *J* = 7.0)

Table 2. ^{13}C NMR spectra of compounds **1** and **2** and the aglycon part of compounds **6**—**10** (in C_5D_5N)

Atom	δ					
	1	**2**	**6**	**7, 8**	**9**	**10**
C(1)	38.1	39.4	39.5	39.1	39.5	39.2
C(2)	32.3	32.0	26.8	32.1	26.8	32.1
C(3)	71.0	71.3	72.9	71.3	73.0	71.2
C(4)	33.6	33.2	68.8	33.2	68.9	33.2
C(5)	53.0	53.8	57.3	54.0	57.4	54.0
C(6)	68.8	66.5	63.8	66.5	63.8	66.5
C(7)	42.1*	55.1	50.8	50.2	50.4	50.0
C(8)	31.0	75.5	76.3	76.4	76.6	76.7
C(9)	54.9	57.0	57.9	56.9	57.9	56.9
C(10)	36.8	37.2	37.8	37.5	37.7	37.5
C(11)	21.3	19.3	18.5	18.9	18.8	19.3
C(12)	42.0*	42.3	42.8	42.8	42.6	42.6
C(13)	42.8	44.8	43.9	43.8	43.7	43.7
C(14)	59.9	67.0	60.6	60.5	62.2	62.0
C(15)	69.4	68.0	72.3	70.8	70.3	70.3
C(16)	72.1	42.3	72.3	72.3	43.2	43.0
C(17)	62.3	55.1	62.7	62.8	56.9	56.9
C(18)	16.2	15.8	18.1	18.0	16.7	16.7
C(19)	13.7	14.5	17.2	14.3	17.2	14.3
C(20)	30.7	39.4	34.1	34.2	40.0	39.8
C(21)	18.7	20.8	20.1	20.2	21.0	20.7
C(22)	36.8	137.2	139.5	139.6	136.3	139.6
C(23)	24.5	130.7	127.5	127.5	132.6	127.5
C(24)	34.5	77.2	49.4	49.5	38.9	86.8
C(25)	36.7	35.0	29.0	29.0	38.9	33.2
C(26)	67.5	18.7	21.2	21.2	71.7	19.0
C(27)	17.5	19.0	18.8	19.0	14.3	18.3
C(28)			70.1	70.2	19.0	

* The assignment of signals may be interchanged.

viously from starfishes *Dermasterias imbricata*[9], *Crossaster papposus*,[10] and *Hacelia attenuata*,[11] respectively.

D-Galactose and 2-*O*-methyl-D-xylose were identified after acid hydrolysis of glycosides **6** and **8**. Proton and carbon chemical shifts, as well as $J_{H,H}$ of the carbohydrate moiety of these glycosides, coincided with the corresponding values for crossasterosides P_1 and P_2 from the starfish *C. papposus*,[12] which establishes the complete identity of their carbohydrate chains. The glycosylation sites in the molecules of **6** and **8** were confirmed by nuclear Overhauser effect (NOE) experiments. Preirradiation of the HC(2′) proton of the galactofuranose residue resulted in the enhancement of the HC(1″) anomeric proton signal of the 2-*O*-methylxylopyranose unit. Preirradiation of the HC(1′) anomeric proton in the galactose residue, in turn, enhances the HC(28) and H′C(28) proton signals.

The position and the configuration of hydroxy groups in the aglycon part of compound **6** were established using differential decoupling. The chemical shifts of carbon and hydrogen atoms and $J_{H,H}$ for the aglycon in the spectra of glycoside **6** coincide with the corresponding values in the spectra of culcitoside C_2 from the starfish *Culcita novaeguineae*,[6] except for the signals for the C(22)=C(23) double bond, because it is absent in culcitoside C_2. The structure of (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,4β,6α,8,15β,16β,28-heptol was assigned to glycoside **6** on the basis of these data.

Table 3. ^{13}C NMR spectra of the carbohydrate part of compounds **6**—**10** (in C_5D_5N)

Atom	δ			Atom	δ		
	6, 8, 9	**7**	**10**		**6, 8, 9**	**7**	**10**
C(1′)	107.5	107.5	102.5*	C(1″)	104.2	104.2	104.0*
C(2′)	91.3	91.3	81.1	C(2″)	84.8	84.8	85.2
C(3′)	77.5*	77.7	78.2	C(3″)	77.6*	76.2	77.3
C(4′)	84.2	84.1	71.4	C(4″)	71.0	80.6	70.9
C(5′)	72.6	72.6	66.7	C(5″)	67.0	64.2	66.6
C(6′)	64.8	64.8		OCH_3	60.6	60.6, 58.7	60.4

* The assignment of signals may be interchanged.

Acid hydrolysis of glycoside **7** gave D-galactose and 2,4-di-*O*-methyl-D-xylose. The assignment of signals for protons of the aglycon part and the carbohydrate chain in its ^{1}H NMR spectrum was carried out using differential decoupling. The results obtained showed

Table 4. ^{1}H NMR spectra of the aglycon part of compounds **6—8** (in C_5D_5N)

Proton (group)	δ (*J*/Hz)	
	6	**7, 8**
HC(3)	3.95 (m)	4.00 (m)
$H_{ax}C(4)$		1.84 (q, *J* = 11.0)
$H_{eq}C(4)$	5.23 (t, *J* = 2.5)	3.12 (m)
HC(5)	1.46 (dd, *J* = 2.1, 11.0)	1.53 (m)
HC(6)	5.06 (td, *J* = 4.0, 11.0)	4.39 (td, *J* = 4.3, 10.7)
$H_{ax}C(7)$	1.88 (dd, *J* = 11.6, 12.5)	1.83 (dd, *J* = 10.8, 12.0)
$H_{eq}C(7)$	3.14 (dd, *J* = 4.2, 10.5)	3.04 (dd, *J* = 4.0, 12.0)
HC(14)	1.09 (d, *J* = 5.7)	1.08 (d, *J* = 5.5)
HC(15)	4.68 (dd, *J* = 5.4, 6.7)	4.67 (dd, *J* = 5.0, 7.0)
HC(16)	4.45 (t, *J* = 6.7)	4.46 (t, *J* = 7.0)
HC(17)	1.10 (dd, *J* = 7.4, 11.0)	1.10 (dd, *J* = 6.7, 11.6)
HC(20)	2.95 (m)	2.95 (m)
HC(22)	5.93 (dd, *J* = 7.4, 15.3)	5.95 (dd, *J* = 7.2, 15.0)
HC(23)	5.56 (dd, *J* = 8.7, 15.1)	5.58 (dd, *J* = 8.0, 15.0)
HC(24)	2.34 (m)	
HC(28)	5.58 (dd, *J* = 7.4, 10.0)	3.59 (dd, *J* = 6.7, 10.0)
H′C(28)	3.99 (dd, *J* = 7.9, 10.0)	4.01 (dd, *J* = 7.0, 9.8)
HC(25)	1.93 (m)	
$H_3C(18)$	1.63 (s)	1.63 (s)
$H_3C(19)$	1.83 (s)	1.37 (s)
$H_3C(21)$	1.21 (d, *J* = 6.7)	1.22 (d, *J* = 6.5)
$H_3C(26)$	0.91 (d, *J* = 7.0)	0.93 (d, *J* = 7.0)
$H_3C(27)$	0.92 (d, *J* = 7.0)	0.94 (d, *J* = 7.0)

Table 5. ^{1}H NMR spectra of the aglycon part of compounds **9** and **10** (in C_5D_5N)

Proton (group)	δ (*J*/Hz)	
	9	**10**
HC(3)	3.95 (m)	4.02 (m)
$H_{ax}C(4)$		1.84 (q, *J* = 11.0)
$H_{eq}C(4)$	5.22 (t, *J* = 2.5)	3.12 (m)
HC(5)	1.45 (dd, *J* = 2.1, 11.0)	1.54 (m)
HC(6)	5.03 (td, *J* = 4.3, 10.7)	4.38 (td, *J* = 4.5, 10.8)
$H_{ax}C(7)$	1.86 (dd, *J* = 11.0, 12.0)	1.79 (dd, *J* = 11.0, 12.5)
$H_{eq}C(7)$	3.15 (dd, *J* = 4.0, 12.0)	3.05 (dd, *J* = 4.0, 12.0)
HC(14)	1.16 (d, *J* = 6.0)	1.12 (d, *J* = 5.7)
HC(15)	4.76 (m)	4.68 (m)
HC(20)	2.25 (m)	2.32 (m)
HC(22)	5.28 (m)	5.62 (m)
HC(23)	5.28 (m)	5.62 (m)
HC(24)	2.25 (m)	4.06 (t, *J* = 6.1)
HC(25)	1.85 (m)	2.05 (m)
HC(27)	3.35 (dd, *J* = 7.7, 9.1)	
H′C(27)	3.99 (dd, *J* = 6.4, 9.5)	
$H_3C(18)$	1.65 (s)	1.63 (s)
$H_3C(19)$	1.83 (s)	1.39 (s)
$H_3C(21)$	1.10 (d, *J* = 6.5)	1.11 (d, *J* = 6.5)
$H_3C(26)$	0.96 (d, *J* = 6.8)	1.08 (d, *J* = 7.0)
$H_3C(27)$		1.13 (d, *J* = 7.0)
$H_3C(28)$	0.98 (d, *J* = 6.8)	

that the backbone of the aglycon has a structure similar to that of culcitoside C_3 from the starfish *C. novaeguineae*.[6] The coincidence of chemical shifts for carbon atoms of the side chains in the ^{13}C NMR spectra of glycosides **6** and **7** indicates the attachment of the carbohydrate chain in compound **7** to OC(28) of aglycon. NOE experiments showed that preirradiation of the HC(2′) proton of the galactosyl residue resulted in the appearance of a signal for the anomeric proton of

Table 6. ^{1}H NMR spectra of carbohydrate part of compounds **6—10** (in C_5D_5N)

Proton	δ (*J*/Hz)		
	6, 8, 9	**7**	**10**[a]
HC(1′)	5.60 (d, *J* = 1.6)	5.58 (d, *J* = 1.5)	4.87 (dd, ΔW = 7.0 Hz)
HC(2′)	4.91 (dd, *J* = 1.8, 4.0)	4.88 (dd, *J* = 1.5, 4.0)	
HC(3′)	(H_2O)[b]	(H_2O)[b]	
HC(4′)	4.75 (dd, *J* = 3.7, 7.7)	4.77 (dd, *J* = 3.7, 7.5)	
HC(5′)	4.57 (td, *J* = 3.5, 5.7)	4.59 (td, *J* = 3.7, 6.1)	
HC(6′)	4.37 (dd, *J* = 5.5, 11.0)	4.39 (dd, *J* = 6.0, 11.0)	
H′C(6′)	4.40 (dd, *J* = 5.5, 11.0)	4.41 (dd, *J* = 6.2, 11.0)	
HC(1″)	5.07 (d, *J* = 7.9)	5.01 (d, *J* = 7.6)	5.60 (d, *J* = 7.5)
HC(2″)	3.45 (dd, *J* = 7.5, 8.5)	3.39 (dd, *J* = 7.5, 8.7)	3.57 (dd, *J* = 7.5, 8.5)
HC(3″)	3.99 (t, *J* = 8.5)	3.93 (t, *J* = 8.7)	4.12 (t, *J* = 8.5)
HC(4″)	4.18 (m)	3.58 (m)	4.20 (m)
HC(5″)	3.57 (t, *J* = 10.5)	3.33 (t, *J* = 10.5)	3.65 (t, *J* = 10.5)
H′C(5″)	4.28 (dd, *J* = 5.0, 10.5)	4.22 (dd, *J* = 5.0, 11.0)	4.37 (dd, *J* = 5.0, 11.0)
OCH_3	3.77 (s)	3.53 (s); 3.73 (s)	3.90 (s)

[a] Signals of the HC(2′)—HC(5′) protons were not identified due to overlapping with signals of other protons.

[b] Signals of the HC(3′) protons are overlapped with the residual signal of water.

the 2,4-di-*O*-methylxylosyl residue, HC(1''), which establishes the presence of the (1→2) bond between the monosaccharide units in compound **7**. Examination of signals of carbon atoms and of $J_{H,H}$ corresponding to the carbohydrate chain of glycoside **7** in the NMR spectra and their comparison with the related data for glycoside **6** showed that 2,4-di-*O*-methyl-β-D-xylopyranosyl residue is attached to the O(2') atom of the β-galactofuranosyl residue. Hence, glycoside **7** is (22*E*)-28-*O*-[*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,6α,8,15β,16β,28-hexol.

The structure of glycoside **8** was determined using procedures similar to those described above for glycosides **6** and **7**. The NMR spectra of the aglycon part of compound **8** coincided completely with the related data for glycoside **7**, and the spectral parameters for the carbohydrate moiety of compound **8** coincided with those for the carbohydrate part of glycoside **6**. Thus, glycoside **8** is (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-hydroxymethyl-5α-cholest-22-ene-3β,6α,8,15β,16β,28-hexol.

The structure of glycoside **9** was determined by differential proton decoupling and confirmed by comparing its spectral data with the parameters of model compounds. The signals of carbon atoms of the side chain of compound **9** coincided with the corresponding signals in the ^{13}C NMR spectrum of crossasteroside P_3 from the starfish *C. popposus*.[10] The chemical shifts of carbon and hydrogen atoms and $J_{H,H}$ in the spectra of glycoside **9** were the same as those of the polycyclic part of culcitoside C_1,[13] which indicated the identity of the substitution in rings *A*, *B*, *C*, and *D* of these compounds. The spectral parameters of the carbohydrate moiety of glycoside **9** coincided completely with the corresponding data for the carbohydrate moieties of glycosides **6** and **8**. Hence, the structure of (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-galactofuranosyl]-24-methyl-5α-cholest-22-ene-3β,4β,6α,8,15β,26-hexol was assigned to compound **9**.

Acid hydrolysis of glycoside **10** resulted in formation of D-xylose and 2-*O*-methyl-D-xylose. The signals of carbon atoms of the carbohydrate chain in the ^{13}C NMR spectrum of glycoside **10** corresponded to the signals of halityloside A from the starfish *Halityle regularis*[14]; it thus follows that the 2-*O*-methyl-β-D-xylopyranosyl residue is attached to the residue of β-D-xylopyranose by the (1→2)-glycosidic linkage. The signals of carbon atoms of the aglycon part of glycoside **10** coincided with the corresponding signals in the spectrum of asterosaponin D_2 from the starfish *Distolasterias nippon*,[15] except for the signals of C(2), C(3), and C(4), because an additional monosaccharide residue is attached to OC(3) in asterosaponin D_2. Thus, it was established that a *trans*-C(22)=C(23) double bond is present in the aglycon, and the hydroxy groups are at the 3β, 6α, 8, and 15β-positions. The structure of the aglycon was confirmed also by differential decoupling. The glycosylation site OC(24) in glycoside **10** was established by recording the NOE signal of the HC(24) proton upon preirradiation of the HC(1') anomeric proton of the xylosyl residue. Thus, compound **10** is (22*E*)-28-*O*-[*O*-(2-*O*-methyl-β-D-xylopyranosyl)-(1→2)-β-D-xylopyranosyl]-5α-cholest-22-ene-3β,6α,8,15β,24-pentol.

We determined the stereochemistry of the C(20) atom in the compounds isolated as *R* by comparing the chemical shifts of the $H_3C(21)$ atoms in the NMR spectra with the data for the known polyhydroxysteroids.[16] In the present work, the stereochemistry of the C(24) atoms in the molecules of **2** and **6**—**10** and C(25) in the molecules of **1**—**5** and **9** was not determined.

The structure of the carbohydrate chains of glycosides **6**—**9** is rarely encountered in starfish steroids. Previously, the β-D-galactofuranosyl residue was found only in four glycosides,[1,12,17] and three of these compounds were isolated from Far-East species of starfishes *Solaster dowsoni* and *C. papposus*. Thus, *C. patagonicus* is yet another starfish species, which contains steroid glycosides with β-D-galactofuranose in their carbohydrate chains.

Experimental

The ^1H and ^{13}C NMR spectra were recorded with a Bruker WM-250 spectrometer using $SiMe_4$ as the internal standard. Optical rotations were measured with a Perkin—Elmer 141 polarimeter. HPLC was carried out with a DuPont Model 8800 chromatograph equipped with a refractometric detector and using Silasorb C_{18} (13 μ, 250×9.4 mm) and Altex Ultrasphere-Si (5 μ, 250×10.0 mm) columns.

Thin layer chromatography (TLC) was carried out on glass plates (4.5×6.0 cm) with a fixed layer of silica gel L (Chemapol, Czech Republic).

The starfishes were collected during run No. 7 of the "Academician Oparin" scientific research boat in June, 1988, near Shiashkotan Island (Kurile Islands, the Sea of Okhotsk) from 100—200 m depth and identified by E. N. Gruzov (Institute of Zoology of the Russian Academy of Sciences, St.Petersburg).

Isolation of compounds 1—10. Freshly collected starfishes (animal weight 4.0 kg) were ground and extracted exhaustively with 95% ethanol at *ca.* 20 °C. The combined extracts were concentrated *in vacuo*, the residue was dissolved in water (1.0 L), and the solution was passed through a column with an Amberlite XAD-2 ion-exchange resin (6×30 cm). The column was washed with water (1.5 L) and then with methanol (3.0 L), and the methanolic eluate was concentrated. The resulting total fraction of steroid compounds was successively chromatographed on a Sephadex LH-20 column (3×50 cm) in a 2 : 1 methanol—water system and on silica gel columns (4×18 cm) (twice) in a 4 : 1 → 2 : 1 chloroform—methanol system. As the polarity of the eluent increased, the following fractions were eluted, which contained polyhydroxysteroids **1** (TLC, toluene—ethanol, 9 : 5, R_f 0.58), **2** (R_f 0.54), **3** (R_f 0.64), **4** (R_f 0.58), and **5** (R_f 0.49), and

glycosides **6** (R_f 0.23), **7** (R_f 0.38), **8** (R_f 0.28), **9** (R_f 0.23), **10** (R_f 0.36). Fractions containing polyhydroxysteroids **1**, **3**, and **4** were additionally purified on a Florisil column (2×15 cm) in a chloroform—methanol gradient (20 : 1→15 : 1) and by HPLC on a Silasorb C_{18} column eluted with a 4 : 1 methanol—water mixture as the eluent. This gave compound **1** (19 mg, 0.0003%), $C_{27}H_{48}O_5$, $[\alpha]_D$ +13.8° (*c* 1.4, methanol); compound **3** (20 mg, 0.0005%), $[\alpha]_D$ +16.8° (*c* 0.9, methanol); and compound **4** (9 mg, 0.0002%), $[\alpha]_D$ +30.8° (*c* 0.6, methanol). Fractions containing polyhydroxysteroids **2** and **5** and glycosides **6—10** were purified by HPLC on Altex Ultrasphere-Si (chloroform—methanol, 3.5 : 1 and 2 : 1, respectively) and Silasorb C_{18} columns (methanol—water, 80 : 20 and 73 : 27, respectively) to give compound **2** (5 mg, 0.0001%), $C_{27}H_{46}O_5$, $[\alpha]_D$ 0° (*c* 0.2, methanol); compound **5** (8 mg, 0.00021%), $C_{27}H_{48}O_5$, $[\alpha]_D$ 0°; compound **6** (6 mg, 0.0002%), $C_{40}H_{68}O_{16}$, $[\alpha]_D$ −26.7° (*c* 0.7, methanol); compound **7** (5 mg, 0.0001%), $C_{41}H_{70}O_{15}$, $[\alpha]_D$ −22.0° (*c* 0.5, methanol); compound **8** (7 mg, 0.0002%), $C_{40}H_{68}O_{15}$, $[\alpha]_D$ −25.1° (*c* 0.7, methanol); compound **9** (7 mg, 0.0002%), $C_{40}H_{68}O_{15}$, $[\alpha]_D$ −24.8° (*c*, 0.4, methanol); and compound **10** (9 mg, 0.0002%), $C_{37}H_{64}O_{13}$, $[\alpha]_D$ −4.9°; (*c* 0.8, methanol).

Hydrolysis of glycosides 6—10. Acid hydrolysis was carried out in 2 *M* HCl at 100 °C for 2 h. 2-*O*-Methylxylose and galactose were identified in hydrolysates of compounds **6**, **8**, and **9**; 2,4-di-*O*-methylxylose and galactose were identified in hydrolysate of compound **7**, and 2-*O*-methylxylose and xylose were found in hydrolysate of compound **10** by TLC (butanol—acetone—water, 4 : 5 : 1) and GLC (as aldononitrile acetates). Monosaccharides in glycosides **6**, **8**, and **9** were referred to the D-series based on the specific rotations of mixtures of monosaccharides obtained upon acid hydrolysis of the glycosides, $[\alpha]_D$ +22°, +23°, and +25°, respectively (in all cases, *c* 0.1, H_2O). These data almost coincided with the specific rotations of mixtures of monosaccharides from crossasterosides P_1 ($[\alpha]_D$ +27°) and P_2 ($[\alpha]_D$ +24°) isolated by us previously,[12] from which D-galactose and 2-*O*-methyl-D-xylose were isolated in the individual state by preparative paper chromatography. The monosaccharides in glycosides **7** and **10** were referred to the D-series only by analogy with other starfish glycosides, because exclusively D-enantiomers of galactose, xylose, 2-*O*-methylxylose, and 2,4-di-*O*-methylxylose have been found so far in these glycosides.[16]

This work was financially supported by the International Science Foundation and the Government of the Russian Federation (Grant J78 100).

References

1. A. A. Kicha, A. I. Kalinovsky, N. V. Ivanchina, and V. A. Stonik, *Izv. Akad. Nauk, Ser. Khim.*, 1993, 980 [*Russ. Chem. Bull.*, 1993, **42**, 943 (Engl. Transl.)].
2. A. A. Kicha, A. I. Kalinovsky, N. V. Ivanchina, Yu. N. El'kin, and V. A. Stonik, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 1821 [*Russ. Chem. Bull.*, 1994, **43**, 1726 (Engl. Transl.)].
3. A. A. Kicha, A. I. Kalinovsky, and V. A. Stonik, *Izv. Akad. Nauk, Ser. Khim.*, 1995, 1164 [*Russ. Chem. Bull.*, 1995, **44**, 1125 (Engl. Transl.)].
4. H. Eggert, C. L. Van-Antwerp, N. S. Bhacca, and C. Djerassi, *J. Org. Chem.*, 1976, **41**, 71.
5. A. A. Kicha, A. I. Kalinovsky, E. V. Levina, V. A. Stonik, and G. B. Elyakov, *Tetrahedron Lett.*, 1983, **24**, 3893.
6. A. A. Kicha, A. I. Kalinovsky, P. V. Andriyashchenko, and E. V. Levina, *Khim. Prir. Soedin.*, 1986, 592 [*Chem. Nat. Compd.*, 1986 (Engl. Transl.)].
7. A. A. Kicha, A. I. Kalinovsky, E. V. Levina, V. A. Stonik, and G. B. Elyakov, *Bioorg. Khim.*, 1983, **9**, 975 [*Sov. J. Bioorg. Chem.*, 1983, **9** (Engl. Transl.)].
8. R. Riccio, M. Santaniello, O. Squillace Greco, and L. Minale, *J. Chem. Soc., Perkin Trans. I*, 1989, 823.
9. I. Bruno, L. Minale, and R. Riccio, *J. Nat. Prod.*, 1990, **53**, 366.
10. A. A. Kicha, A. I. Kalinovsky, and V. A. Stonik, *Khim. Prir. Soedin.*, 1990, 218 [*Chem. Nat. Compd.*, 1990 (Engl. Transl.)].
11. L. Minale, C. Pizza, F. Zollo, and R. Riccio, *Tetrahedron Lett.*, 1982, **23**, 1841.
12. A. A. Kicha, A. I. Kalinovsky, and V. A. Stonik, *Khim. Prir. Soedin.*, 1989, 669 [*Chem. Nat. Compd.*, 1989 (Engl. Transl.)].
13. A. A. Kicha, A. I. Kalinovsky, E. V. Levina, and P. V. Andriyashchenko, *Khim. Prir. Soedin.*, 1985, 801 [*Chem. Nat. Compd.*, 1985 (Engl. Transl.)].
14. M. Iorizzi, L. Minale, R. Riccio, M. Debray, and J. L. Menou, *J. Nat. Prod.*, 1986, **49**, 67.
15. I. I. Kapustina, A. I. Kalinovsky, S. G. Polonik, and V. A. Stonik, *Khim. Prir. Soedin.*, 1987, 250 [*Chem. Nat. Compd.*, 1987 (Engl. Transl.)].
16. L. Minale, R. Riccio, and F. Zollo, in *Progress in the Chemistry of Organic Natural Products*, Eds. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, and Ch. Tamm, Springer-Verlag, Wien—New York, 1993, **62**, 75.
17. R. Riccio, L. Minale, S. Bano, and V. U. Ahmad, *Tetrahedron Lett.*, 1987, **28**, 2291.

Received January 24, 1996;
in revised form October 7, 1996