Triterpene Glycosides from the Far Eastern Sea Cucumber *Cucumaria conicospermium*

Sergey A. Avilov,*,† Alexandr S. Antonov,† Alexandra S. Silchenko,† Vladimir I. Kalinin,† Anatoly I. Kalinovsky,† Pavel S. Dmitrenok,† Valentin A. Stonik,† Ricardo Riguera,‡ and Carlos Jimenez§

Pacific Institute of Bioorganic Chemistry, the Far East Division of the Russian Academy of Sciences, 690022, Vladivostok, Russian Federation, Departamento de Química Orgánica and Instituto de Acuicultura, Universidad de Santiago, 15706 Santiago de Compostela, Spain, and Departamento de Química Fundamental, Facultad de Ciencias, Universidad de La Coruňa, La Coruňa 15071, Spain

Four new triterpene glycosides, cucumariosides A_2-5 (**1**), A_3-2 (**2**), A_3-3 (**3**), and isokoreoside A (**4**), along with the previously isolated koreoside A (**5**), have been found in the sea cucumber *Cucumaria conicospermium*. Glycoside **1** was isolated as a native substance, while glycosides **2**−**5** were identified through their desulfated derivatives. Their structures have been deduced by extensive spectral analysis (NMR and MS) and chemical evidence. All the glycosides contain the same branched pentasaccharide carbohydrate chain but differ in the number and positions of the sulfate groups. Glycoside **1** has one, glycosides **2** and **3** have two, and glycosides **4** and **5** have three sulfate groups. Glycosides **2**−**5** are non-holostane derivatives; their aglycons lack the 18(20)-lactone and are characterized by shortened side chains, which is a very rare feature among the sea cucumber glycosides.

Results and Discussion

In continuation of our studies of sea cucumbers belonging to the genus *Cucumaria*,[1–4] we became interested in the study of the triterpene glycosides from the recently described sea cucumber *Cucumaria conicospermium* Levin et Stepanov,[5] collected in September 1997 in the Sea of Japan near Sosunov Cape during the 20th scientific cruise on the research vessel *Akademik Oparin*. In this paper we describe the structural elucidation of four new mono-, di-, and trisulfated glycosides named cucumariosides A_2-5 (**1**), A_3-2 (**2**), A_3-3 (**3**), and isokoreoside A (**4**), along with the known compound koreoside A (**5**)[1] previously found in *Cucumaria koraiensis*.

The ethanolic extract of *C. conicospermium* (197 g of dry wt) was sequentially submitted to column chromatography on Polychrom-1 (powdered Teflon) and Si gel to give three fractions of glycoside mixtures that contained the same carbohydrate chain but a different number of sulfate groups. In this way, fractions containing monosulfated glycosides (cucumariosides belonging to the A_2 group), disulfated glycosides (cucumariosides belonging to the A_3 group), and trisulfated glycosides (glycosides belonging to the A_7 group) in approximately equal amounts were obtained.

The first glycoside fraction containing the monosulfated cucumariosides belonging to the A_2 group was submitted to reversed-phase HPLC on Silasorb C_{18} to afford pure cucumarioside A_2-5 (**1**) as the main component. The MALDI-TOF MS of **1** exhibited a pseudomolecular ion at *m/z* 1401 $[M_{Na} + Na]^+$ in the positive ion mode and a pseudomolecular ion at *m/z* 1355 $[M_{Na}- Na]^-$ in the negative ion mode (Figure 1), which also showed the fragment ions at *m/z* 845 (corresponding to the loss of the aglycon) and at *m/z* 1179 and 1223 (due to the loss of the terminal monosaccharide residues). The HRFABMS (positive ion mode) of the

pseudomolecular ion $[M_{Na} + H]^+$ at *m/z* 1379.5574 ($C_{61}H_{96}$-$O_{31}NaS$, Δ 2.0 mmu), allowed us to determine the molecular formula of cucumarioside A_2-5 (**1**) as $C_{61}H_{95}O_{31}NaS$.

The aglycon moiety of glycoside **1** was found by extensive NMR spectroscopy (¹H and ¹³C NMR, ¹H−¹H-COSY, HMBC, and NOESY) (Table 1), to be the same as that of cucumarioside A_0-1, isolated earlier from *Cucumaria japonica*.[6] Thus, the structure of the aglycon part of **1** was identified as 16β-acetoxy-3β-hydroxyholost-7-en-23-one.

The presence of 3-*O*-methylglucose, glucose, quinovose, and xylose in a 1:1:1:2 ratio in the glycoside **1** was established by acid hydrolysis with 2 N HCl followed by GC−MS analysis of the corresponding aldononitrile per-acetates.[7] The existence of five monosaccharide residues in glycoside **1** was confirmed by its ¹³C NMR and DEPT spectra (Table 2), which exhibited five signals for anomeric carbons between 102.36 and 105.54 ppm and by its ¹H NMR spectrum that showed the signals corresponding to five anomeric protons at 4.77 d (*J* = 6.4), 4.91 d (*J* = 7.7), 5.27 d (*J* = 7.4), 5.28 d (*J* = 7.4), and 5.31 d (*J* = 6.8) ppm (Table 2), displaying in all cases coupling constants indicative of a β-configuration for the glycosidic bonds.[8] The NMR data of the carbohydrate part of **1** were coincident with those of the pentaoside cucumarioside A_2-2 having a sulfate at C-4 of the first xylose residue, previously isolated from *Cucumaria japonica* and having holosta-7,25-diene-3β-ol-16-one as an aglycon.[9] The structure of the carbohydrate chain of **1** was corroborated by HMBC and NOESY correlations. Thus, the interglycosidic bonds were confirmed by NOESY cross-peaks between H-1 of the first xylose residue and H-3 of the aglycon; between H-2 of the first xylose residue and H-1 of quinovose; between H-2 of quinovose and H-1 of the terminal xylose residue; between H-4 of quinovose and H-1 of glucose; and between H-3 of glucose and H-1 of the terminal 3-*O*-methylglucose (Table 2). The sequence of monosaccharides in the carbohydrate chain was confirmed by the above-mentioned data of MALDI-TOF-MS (Figure 1).

Hence, cucumarioside A_2-5 (**1**) is 3-*O*-{3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-[β-D-xy-lopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→2)-4-*O*-so-

* To whom correspondence should be addressed. Fax: internat+7-(4232)-314050. E-mail: avilov@piboc.dvo.ru.
† Pacific Institute of Bioorganic Chemistry.
‡ Universidad de Santiago.
§ Universidad de La Coruňa.
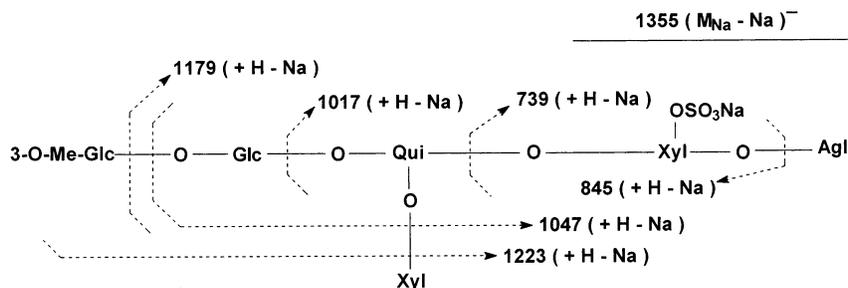
Figure 1. Fragmentation of cucumarioside A_2-5 (**1**) in MALDI-TOF MS (negative ion mode).

Chart 1

	Aglycon	R^1	R^2	R^3
1	A	H	H	SO_3Na
2	B	H	SO_3Na	SO_3Na
3	C	H	SO_3Na	SO_3Na
4	C	SO_3Na	SO_3Na	SO_3Na
5	B	SO_3Na	SO_3Na	SO_3Na
6	B	H	H	H
7	C	H	H	H
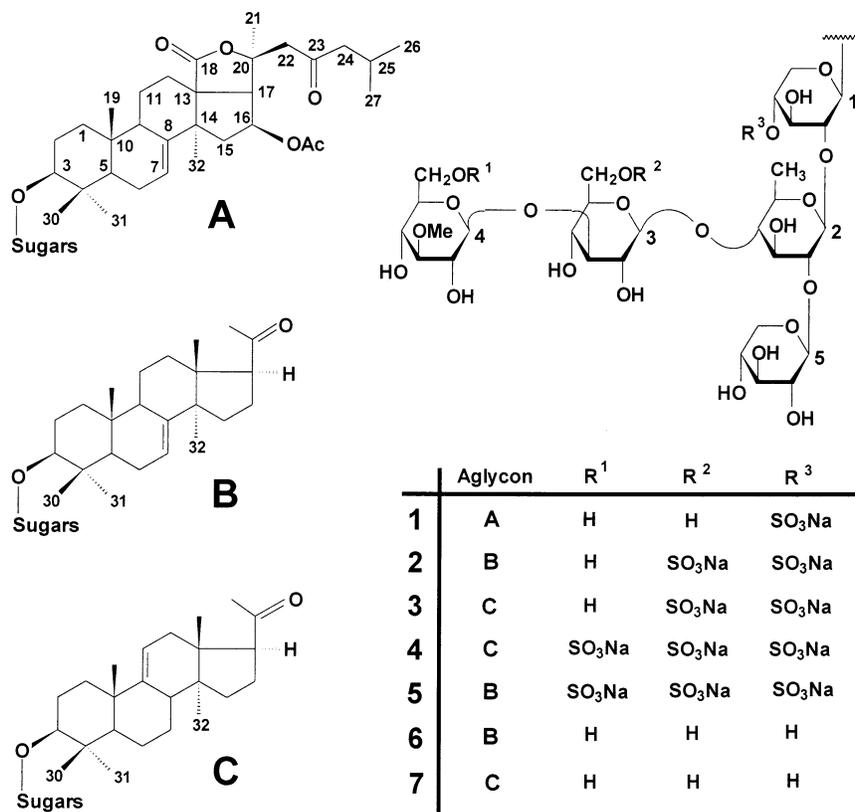
dium sulfate-β-D-xylopyranosyl}-16β-acetoxy-3β-hydroxy-holost-7-en-23-one.

The second glycoside fraction contained the disulfated cucumariosides belonging to the A_3 group (ΣA_3) and was difficulte to separate into its individual components by chromatographic methods. The ^{13}C NMR spectral data corresponding to the carbohydrate chain (Table 3) of this fraction (ΣA_3) were similar to those of cucumarioside A_3, previously isolated from *Cucumaria japonica*.[2] Indeed, acid hydrolysis of ΣA_3 led to xylose, glucose, quinovose, and 3-*O*-methylglucose in a 2:1:1:1 ratio identified by GC–MS as aldononitrile peracetates.

Solvolytic desulfation of the mixture (ΣA_3) with 1:1 pyridine–dioxan under reflux led to a mixture of desulfated derivatives (**DS**-ΣA_3) that afforded the desulfated derivatives **6** and **7** after HPLC chromatography on Silasorb C_{18}. Furthermore, ^{13}C NMR data of **DS**-ΣA_3 were identical to those of the desulfated derivative of cucumarioside A_2-2 from *Cucumaria japonica*.[9]

The comparison of the ^{13}C NMR data of ΣA_3 and **DS**-ΣA_3 confirmed the number and position of the sulfates as characteristic for the glycosides of A_3 group, namely, at C-4 of the first xylose residue and C-6 of the glucose residue.

Indeed, the signals of C-5 and C-6 of the glucose residue in the glycoside fraction were shifted upfield (2.66 ppm) and downfield (5.77 ppm), respectively, in relation to those of the mixture of desulfated derivatives due to β- and α-shift effects of a sulfate at C-6. In a similar way, the signals of C-4, C-3, and C-5 of the first xylose residue were shifted downfield (6.05 ppm) and upfield (2.78 and 2.45 ppm) respectively, due to the presence of a sulfate group at C-4 of that sugar (α- and β-shift effects of a neighboring sulfate group).[8]

These data indicated that all the components of this fraction have the same carbohydrate chains, identical with that of cucumarioside A_3.[2]

The MALDI-TOF MS (positive ion mode) of **6**, the desulfated derivative of the native cucumarioside A_3-2 (**2**), exhibited pseudomolecular ion peaks at m/z 1145 $[M + K]^+$ and at m/z 1129 $[M + Na]^+$. These data together with the HRFABMS (positive ion mode) of the second pseudo-molecular ion at m/z 1129.5421 ($C_{53}H_{86}O_{24}Na$, Δ 1.4 mmu) determined the molecular formula of **6** as $C_{53}H_{86}O_{24}$. The structure of **6** was elucidated by extensive analysis of ^1H, ^{13}C NMR spectra and HMBC and NOESY data (Tables 4 and 5).

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of Cucumarioside A$_2$-5 (**1**)

position	δ_C mult.[a,]	δ_H mult. (*J* in Hz)[b]	HMBC	NOESY
1	35.40 t	1.38 m		
2	26.79 t	2.06 m		
3	88.93 d	3.23 m		H-5, H-Xyl1
4	39.39 s			
5	47.71 d	0.98 t (7.8)	C: 1, 6, 30	H-3
6	23.17 d	2.04 m		
7	120.32 s	5.62 br s		H-32, H-15
8	145.40 s			
9	46.92 d	3.40 br d (12.9)		H-19
10	35.87 s			
11	22.36 t	1.47 m		
12	30.97 t			
13	57.62 s			
14	47.41 s			
15	43.52 t	2.69 dd (7.2, 12.1), 1.59 m	C: 32	H-7
16	76.07 d	5.76 m		H-17, H-21, H-32
17	55.03 d	3.23 d (8.9)		H-16, H-21, H-32
18	179.23 s			
19	23.81 q	1.18 s	C: 1, 5, 9, 10	H-9
20	82.02 s			
21	29.44 q	1.56 s	C: 17, 20, 22	H-16, H-17, H-32
22	52.62 t	3.89 d (18.8), 3.14 d (18.8)	C: 20, 23	
23	207.68 s			
24	51.40 t	2.38 dd (6.6, 15.9), 2.22 dd (7.4, 5.9)	C: 23, 25, 26, 27	
25	24.22 d	2.14 m	C: 26, 27	
26	22.36 q[c]	0.85 d (6.5)[d]	C: 25	
27	22.17 q[c]	0.87 d (6.7)[d]	C: 25	
30	17.27 q	1.10 s	C: 3, 4, 5, 31	
31	28.55 q	1.24 s	C: 3, 4, 5, 30	
32	31.98 q	1.05 s	C: 8, 13, 14, 15	H-7, H-16, H-17, H-21
O*C*OCH$_3$	169.03 s			
OCO*C*H$_3$	21.10 q	1.99 s	*C*OCH$_3$	

[a] Recorded at 125 MHz in C$_5$D$_5$N. Multiplicity by DEPT. [b] Recorded at 500 MHz in C$_5$D$_5$N. [c,d] Interchangeable signals.

Table 2. ^{13}C and ^{1}H NMR Chemical Shifts and NOESY Correlations for the Sugar Moiety of Cucumarioside A$_2$-5 (**1**)

position	δ_C mult.[a,b,c]	δ_H mult. (*J* in Hz)[d]	NOESY
Xyl1 (1→C3)			
1	104.69 d	4.77 d (6.4)	H-3, H3,5b-Xyl1
2	**81.66** d	3.97 m	H1-Qui, H4,5a-Xyl1
3	75.40 d	4.37 t (8.3)	H1-Xyl1
4	*75.91* d	5.10 m	H2,5a-Xyl1
5	64.04 t	4.75 m	H2,4-Xyl1
		3.81 t (6.6)	H1-Xyl1
Qui (1→2Xyl1)			
1	102.36 d	5.28 d (7.4)	H2-Xyl1, H3,5-Qui
2	**82.57** d	4.09 m	H1-Xyl2
3	74.97 d	4.10 m	H1-Qui
4	**86.57** d	3.58 m	H1-Glu, H6-Qui
5	70.82 d	3.64 m	H1-Qui
6	17.86 q	1.63 d (5.8)	H4-Qui
Glu (1→4Qui)			
1	104.59 d	4.91 d (7.7)	H4-Qui, H3,5-Glu
2	73.49 d	3.98 m	
3	**87.73** d	4.22 t (6.7)	H1-MeGlu, H1-Glu
4	70.21 d	3.98 m	
5	77.65 d	3.99 m	H1-Glu
6	61.83 t	4.43 br d (12.3), 4.15 m	
MeGlu (1→3Glu)			
1	105.41 d	5.27 d (7.4)	H3-Glu, H3,5-MeGlu
2	74.85 d	3.98 m	
3	87.77 d	3.68 t (8.9)	H1-MeGlu
4	70.41 d	4.09 m	
5	78.13 d	3.97 m	H1-MeGlu
6	62.00 t	4.45 br d (9.8), 4.22 m	
OMe	60.61 q	3.84 s	
Xyl2 (1→2Qui)			
1	105.54 d	5.31 d (6.8)	H2-Qui, H3-Xyl2
2	69.53 d	3.99 m	
3	76.47 d	4.06 m	H1-Xyl2
4	70.41 d	4.04 m	
5	66.49 t	4.28 m, 3.60 m	

[a] Recorded at 125 MHz in C$_5$D$_5$N. Multiplicity by DEPT. [b] Bold = interglycosidic positions. [c] Italic = sulfate position. [d] Recorded at 500 MHz in C$_5$D$_5$N.

The ^{13}C NMR spectrum of the aglycon part of **6** (Table 4) showed the presence of 24 carbons, consistent with the aglycon being a 22,23,24,25,26,27-hexanortriterpene derivative. Characteristic signals at 122.68 ppm (C-7) and at 147.36 ppm (C-8) were indicative of a Δ^7 double bond, while the signal at 208.99 ppm was assigned to a keto group at C-20.

The ^{1}H NMR spectrum of the aglycon moiety of **6** (Table 4) showed signals corresponding to an olefinic proton at 5.70 ppm (m, H-7), the signal of the α-proton to the keto group at C-20 at 2.79 ppm (t, *J* = 7.6 Hz, H-17α), and also signals for six methyl groups at 0.92 (s, CH$_3$-18), 1.06 (s, CH$_3$-19), 2.12 (s, CH$_3$-21), 1.18 (s, CH$_3$-30), 1.33 (s, CH$_3$-31), and 1.11 (s, CH$_3$-32) ppm. These data were identical with those of koreoside A, isolated from *Cucumaria koraiensis*,[2] and the structure was confirmed by HMBC and NOESY experiments (Table 4). Hence, the aglycon of cucumarioside A$_3$-2 (**2**) is 3β-hydroxy-22,23,24,25,26,27-hexanorlanosta-7-en-20-one.

The ^{13}C NMR spectrum of the carbohydrate part of **6** showed the signals of five anomeric carbons between 102.99 and 105.82 ppm, which correlate by HMQC with the corresponding anomeric protons at 4.87 d (*J* = 7.2 Hz), 4.94 d (*J* = 7.9 Hz), 5.23 d (*J* = 7.4 Hz), 5.31 d (*J* = 7.9 Hz),

and 5.40 d (*J* = 7.0 Hz) ppm (Table 6). The coupling constants were indicative of a β-configuration of the glycosidic bonds in all cases. The sequence of monosaccharide units in the carbohydrate chain of **6** was established by HMBC and NOESY correlations (Table 5). Thus, NOESY cross-peaks between H-1 of the first xylose residue and H-3 of the aglycon; H-2 of the first xylose residue and H-1 of quinovose; H-4 of quinovose and H-1 of glucose; and H-3 of glucose and H-1 of terminal 3-*O*-methylglucose allowed us to determine the interglycosidic bonds. The NOESY cross-peak due to the correlation between H-2 of quinovose and H-1 of the terminal xylose overlapped with the cross-peak corresponding to the correlation between H-1 of the terminal xylose and H-3 of the same monosaccharide unit. However, the HMBC cross-peaks between H-2 of quinovose and C-1 of the terminal xylose residue and also between H-1 of this xylose and C-2 of quinovose established the attachment of the terminal xylose residue to C-2 of quinovose. Hence, the structure of cucumarioside A$_3$-2 (**2**) is 3β-hydroxy-22,23,24,25,26,27-hexanor-3-*O*-{3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-[-β-D-xylopyranosyl-(1→2)]-β-D-quinovopyranosyl-

Table 3. ^{13}C NMR Chemical Shifts for Carbohydrate Chains of Initial (ΣA_3) and Desulfated (**DS**-ΣA_3) Fractions of the A_3 Group Glycosides

position	δ_C mult.^{a,c,d} (ΣA_3)	δ_C mult.^{b,c} (**DS**-ΣA_3)
Xyl1 (1→C3)		
1	104.45 d	105.12 d
2	**82.04** d	**82.56** d
3	74.98 d	77.76 d
4	*76.25* d	70.20 d
5	64.05 t	66.50 t
Qui (1→2Xyl1)		
1	102.45 d	102.99 d
2	**82.60** d	**83.25** d
3	74.98 d	75.60 d
4	**86.50** d	**86.65** d
5	71.03 d	70.96 d
6	19.2 q	17.9 q
Glu (1→4Qui)		
1	104.06 d	104.72 d
2	73.65 d	73.49 d
3	**86.93** d	**87.86** d
4	69.17 d	69.56 d
5	75.28 d	77.94 d
6	*67.47* t	61.70 t
MeGlu (1→3Glu)		
1	104.45 d	105.51 td
2	74.58 d	74.88 d
3	85.94 d	87.86 d
4	70.02 d	70.39 d
5	77.51 d	78.19 d
6	61.65 t	61.99 t
OMe	60.86 q	60.60 q
Xyl2 (1→2Qui)		
1	105.23 d	105.81 d
2	74.98 d	75.60 d
3	76.43 d	77.05 d
4	70.40 d	70.39 d
5	66.55 t	66.93 t

^{a} Recorded at 75 MHz in $C_5D_5N-D_2O$ (4:1). Multiplicity by DEPT. ^{b} Recorded at 75 MHz in C_5D_5N. Multiplicity by DEPT. ^{c} Bold = interglycosidic posititions.

(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl}-lanost-7-en-20-one.

The molecular formula of **7** was established as $C_{53}H_{86}O_{24}$ by HRFABMS (positive ion mode), which showed the pseudomolecular ion [M + Na]$^+$ at *m/z* 1129.5417 ($C_{53}H_{86}O_{24}$-Na, Δ 5.5 mmu) and indicated that compound **7** must be an isomer of compound **6**. This was confirmed by its MALDI-TOF MS (positive ion mode), which showed the pseudomolecular ions at *m/z* 1129 [M + Na]$^+$ and 1145 [M + K]$^+$ being identical to that of **6**.

Comparison of the ^{1}H and ^{13}C NMR data of compound **7** with those of **6** (see Tables 4−7) showed that both compounds share the same carbohydrate chain (confirmed by HMBC and NOESY correlations as shown in Table 7) and a similar aglycon structure that differed in the location of the double bond. Indeed, the signals in the ^{13}C NMR spectrum of **7** at 149.13 s (C-9) and 114.04 t (C-11) ppm (Table 6) clearly indicated the 9(11)-position for the double bond. The structure of the aglycon of **7** was also confirmed by HMBC and NOESY experiments (Table 6). In particular, the location of the double bond between carbons C-9 and C-11 was proven by the HMBC correlation between the olefinic quaternary carbon C-9 at 149.13 ppm and the Me-19 protons at 1.05 ppm.

On the basis of the above-mentioned data, the aglycon of **7** was established as 3β-hydroxy-22,23,24,25,26,27-hexanorlanost-9(11)-en-20-one. Taking into account the spectral data for the glycoside fraction of cucumariosides belonging to the A_3 group and the structure elucidated for

Table 4. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of the Desulfated Derivative **6**

position	δ_C mult.^{a}	δ_H mult. (J in Hz)^b	HMBC	NOESY
1	35.70 t	1.46 m		
2	27.13 t	1.94 m, 2.22 m		
3	88.80 d	3.34 dd (3.5, 11.5)		H-5, H-31, H1-Xyl1
4	39.48 s			
5	48.75 d	1.03 dd (3.6, 11.4)		H-3, H-31
6	23.21 t	1.99 m, 2.03 m		H-7
7	122.68 d	5.70 m		H-6, H-15, H-16, H-32
8	147.36 s			
9	48.18 d	2.27 m		H-18, H-19
10	35.70 s			
11	22.43 t	1.53 m, 1.68 m		
12	33.46 t	2.03 m, 1.74 m		H-17, H-18
13	44.70 s			
14	53.17 s			
15	33.38 t	1.65 m		H-7
16	22.34 t	2.49 m, 1.76 m		H-7
17	61.85 d	2.97 t (7.6)		H-12, H-32
18	24.75 q	0.92 s	C: 12, 13, 14, 17	H-9, H-12, H-21
19	22.45 q	1.06 s	C: 1, 5, 9, 10	H-2, H-9
20	208.70 s			
21	30.39 q	2.12 s	C: 20	H-18
30	17.38 q	1.18 s	C: 3, 4, 5, 31	H-2
31	28.65 q	1.33 s	C: 3, 4, 5, 30	H-3, H-5
32	30.39 q	1.11 s	C: 8, 13, 14, 15	H-7, H-17

^{a} Recorded at 125 MHz in C_5D_5N. Multiplicity by DEPT. ^{b} Recorded at 500 MHz in C_5D_5N.

the desulfated derivative **7**, the structure of cucumarioside A$_3$-3 (**3**) was established as 3β-hydroxy-22,23,24,25,26,27-hexanor-3-*O*-{3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl}-lanost-9(11)-en-20-one.

Acid hydrolysis of the fraction containing the trisulfated glycosides belonging to the A_7 group (ΣA_7) led to xylose, glucose, quinovose, and 3-*O*-methylglucose in a 2:1:1:1 ratio identified by GC−MS as aldononitrile peracetates. All our attempts to separate the components of this fraction by reversed-phase HPLC failed and led to an inseparable mixture of glycosides **4** and **5**. The ^{13}C NMR spectral data of this fraction showed signals of a carbohydrate chain (Table 8) identical to those of cucumariosides A$_7$-1, A$_7$-2, and A$_7$-3 from *Cucumaria japonica*[10] and koreoside A from *Cucumaria koraiensis.*[1] Thus, the sugar chain of glycosides belonging to the A_7 group must be a branched pentasaccharide with sulfate groups at C-4 of the first xylose residue, C-6 of the glucose residue, and C-6 of the 3-*O*-methylglucose terminal residue. Fortunately, solvolytic desulfation of the mixture **4** and **5** with pyridine−dioxan (1:1) followed by RP-HPLC separation led to two desulfated glycoside derivatives whose ^{13}C and ^{1}H NMR spectral data were identical to those of the desulfated derivatives **7** and **6** (Tables 4−7).

The comparison of the ^{13}C NMR data of the mixture of **4** and **5** (Table 8) and those of the desulfated derivatives **6** and **7** (Tables 5 and 7) confirmed the number and position of the sulfates as characteristic for the glycosides of the A_7 group, namely, at C-4 of the first xylose residue, C-6 of glucose residue, and C-6 of 3-*O*-methylglucose. Indeed, the signals of C-5 and C-6 of the 3-*O*-methylglucose residue in the mixture of **4** and **5** were shifted upfield by 1.67 and downfield by 5.51 ppm, respectively, and the signals of C-5

Table 5. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugar Moiety of Desulfated Derivative **6**

position	δ_C mult.[a,b]	δ_H mult. (J in Hz)[c]	HMBC (C)	NOESY
Xyl1 (1→C3)				
1	105.11 d	4.87 d (7.4)	C: 3, 3,5 Xyl1	H-3,H3,5-Xyl1
2	**83.26** d	3.95 t (8.5)	C: 1 Qui, 1 Xyl1	H1-Qui1
3	77.76 d	4.23 m		H1,5-Xyl1
4	70.40[d] d	4.15 m		
5	66.51 t	4.36 m	C: 1 Xyl1	H1,3-Xyl1
		3.73 m		
Qui (1→2Xyl1)				
1	102.99 d	5.23 d (7.4)	C: 2 Xyl1, 3,5 Qui	H2-Xyl1, H3,5-Qui
2	**82.60** d	4.16 m	C: 1 Xyl2, 1 Qui	
3	75.65[g] d	4.12 m		H1-Qui
4	**86.58** d	3.62 t (8.5)	C: 1 Glu	H1-Glu
5	70.98 d	3.68 m		H1-Qui
6	17.88 q	1.72 d (6.0)	C: 4,5 Qui	
Glu (1→4Qui)				
1	104.70 d	4.94 d (7.9)	C: 4 Qui	H4-Qui, H3,5-Glu
2	73.51 d	4.01 m		
3	**87.85** d	4.25 t (9.0)	C: 1 MeGlu	H1-MeGlu, H1-Glu
4	69.55 d	4.07 m		
5	77.96[e] d	4.01 m	C: 1 Glu	H1-Glu
6	61.85[f] t	4.46 br d (11.5),		
		4.20 m		
MeGlu (1→3Glu)				
1	105.51 d	5.31 d (7.9)	C: 3,5 MeGlu, 3 Glu	H3-Glu, H3,5-MeGlu
2	74.88 d	4.00 m	C: 1 MeGlu	
3	87.85 d	3.72 t (8.5)		H1-MeGlu
4	70.20[d] d	4.16 t (8.7)		
5	78.19[e] d	3.97 m		H1-MeGlu
6	61.98[f] t	4.48 br d (11,5),		
		4.28 dd (11.5, 5.5)		
OMe	60.60 q	3.87 s	C: 3 MeGlu	
Xyl2 (1→2Qui)				
1	105.82 d	5.40 d (7.0)	C: 5 Xyl2, 2 Qui	H2-Qui, H3,5-Xyl2
2	75.59[g] d	4.07 m		
3	77.06 d	4.13 m		H1-Xyl2
4	70.40[d] d	4.16 m		
5	66.92 t	3.66 m		H1-Xyl2
		4.3 m,	C: 1 Xyl2	

[a] Recorded at 125 MHz in C_5D_5N. Multiplicity by DEPT. [b] Bold = interglycosidic positions. [c] Recorded at 500 MHz in C_5D_5N. [d-g] Interchangeable positions.

and C-6 of the glucose residue were shifted upfield by 2.69 and downfield by 5.91 ppm, respectively, in relation to those of the desulfated derivative **6** (β- and α-shift effects of a sulfate group).[8] In a similar way, the signals of C-4, C-3, and C-5 of the first xylose residue were shifted downfield (4.87 ppm) and upfield (2.90 and 2.53 ppm), respectively, due to α- and β-shift effects of a sulfate at C-4 of that sugar. A similar result was obtained through a comparison with the corresponding shifts of the desulfated derivative **7**. All these data indicated that the glycosides **4** and **5** bear the same carbohydrate chain.

On the other hand, a comparison of the ^{1}H and ^{13}C NMR spectral data corresponding to the aglycon part of the glycoside mixture of **4** and **5** to similar reported glycosides allowed us to identify compound **5** as koreoside A, isolated previously from *Cucumaria koraiensis*,[1] while glycoside **4** was found to be its Δ^9 isomer, a new natural product that was named isokoreoside A (**4**). The structure of isokoreoside A (**4**) is 3β-hydroxy-22,23,24,25,26,27-hexanor-3-*O*-{6-*O*-sodium sulfate-3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-[β-D-xylopyranosyl-(1→2)]-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl}-lanost-9(11)-en-20-one.

In conclusion, herein we report the isolation of five glycosides from *Cucumaria conicospermium* with four of them (**1**–**4**) being new compounds. From a structural point of view, glycosides **2**–**5** belong to the "non-holostane" series and are characterized by an aglycon with a shortened side chain lacking the 18(20)-lactone, which is a very rare feature among the sea cucumber glycosides.[4] Furthermore,

C. conicospermium exemplifies the first case of the presence of several isomeric glycosides in the same sea cucumber, distinguished only by the position of the double bond in the aglycon nucleus (pairs of compounds **2/3** and **4/5**).

On the other hand, some generalizations may be deduced from this study. It is interesting to note that the glycoside composition of the sea cucumbers belonging to the genus *Cucumaria* studied thus far is specific for each species, while in sea cucumbers belonging to the order Aspidochirotida such glycoside specificity reaches only the super genus level.[4] Moreover, all the glycosides found in sea cucumbers belonging to the genus *Cucumaria* have similar carbohydrate chains, while the glycosides bearing a trisulfated pentasaccharide chain branched at quinovose residue have been found in all species.

Experimental Section

General Experimental Procedures. All melting points were determined using a Kofler Thermogenerate apparatus. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on a AMX-Bruker 500 spectrometer at 500.12/125.67 MHz (^{1}H/^{13}C) and a Bruker DPX 300 spectrometer at 300/75 MHz (^{1}H/^{13}C) in C_5D_5N/H_2O (4:1) or C_5D_5N with TMS as an internal reference ($\delta = 0$). The MALDI TOF MS (positive and negative ion modes) were recorded using a Bruker apparatus, model BIFLEX III, with impulse extraction of ions, on an α-cyano-4-hydroxycinnamic acid matrix. HRFABMS (positive ion mode) were recorded using a Micromass apparatus, model Autospec-M, on a glycerol/thioglycerol matrix. HPLC was performed using a Dupont-

Table 6. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of the Desulfated Derivative **7**

position	δ_C mult.[a]	δ_H mult. (*J* in Hz)[b]	HMBC	NOESY
1	36.33 t	1.75m, 1.46 m		
2	26.98 t	2.25 m, 1.93		
3	88.56 d	3.26 dd (4.1, 11.8)	C: 1 Xyl1	H-5, H-31, H1-Xyl1
4	39.27 s			
5	52.82 d	0.93 br d (11.4)		H-3, H-31
6	21.19 t	1.72 m, 1.41 m		
7	28.38 t	1.62 m, 1.30 m		
8	41.44 d	2.15 m		H-18, H-19
9	149.13 s			
10	39.76 s			
11	114.04 t	5.29 br s		
12	35.95 t	2.30 br d (18.1), 1.96 m	C: 13, 14	
13	45.90 s			
14	47.55 s			
15	33.91 t	1.44 m, 0.88 t (7.1)		
16	21.86 t	2.52 m, 1.66 m		
17	59.71 d	2.97 t (8.6)		H-32
18	16.42 q	0.62 s	C: 12, 13, 14, 17	H-8, H-19, H-21
19	22.20 q	1.05 s	C: 1, 4, 9, 10	H-8, H-18
20	208.86 s			
21	30.78 q	2.11 s	C: 17, 20	H-18
30	16.66 q	1.11 s	C: 3, 4, 5	
31	28.00 q	1.28 s	C: 3, 4, 5	H-3, H-5
32	18.61 q	0.80 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125 MHz in C_5D_5N. Multiplicity by DEPT. [b] Recorded at 500 MHz in C_5D_5N.

8800 chromatograph equipped with a differential refractometer RIDK-102 (Czechoslovakia) on a Silasorb C_{18} column. GC

analysis was carried out on a Tsvet-110, using a glass column (0.3 × 150 cm) with 1.5% QF-1 as stationary liquid phase. The following experimental conditions were used: Ar as carrier gas at 60 mL/min; column temperature, from 110 to 225 °C at 5 °C/min. GC−MS was performed using a LKB 9000S apparatus using a glass column (0.3 × 300 cm) with 1.5% QF-1 as stationary liquid phase and He as the carrier gas at 50 mL/min. The following conditions were selected for analysis: injection port 275 °C, molecular separator 265 °C, ion source 255 °C, column 110→210 °C at 4 °C/min, ionizing voltage 70 eV.

Animal Material. Specimens of *Cucumaria conicospermium* Levin et Stepanov were collected by Dr. S. N. Fedorov and V. R. Stepanov at a depth of 85 m by Sigsbi trawl in the Sea of Japan near Sosunov Cape (46°24′08″ N 138°19′08″ E) in September 1997, during scientific cruise No. 20 of *r/v Akademik Oparin* and stored in ethanol at room temperature. The sea cucumber was identified by Dr. V. S. Levin of the Kamchatka Scientific Research Institute of Fishery and Oceanography, Petropavlovk-Kamchatsky, Russia Federation, where a voucher specimen is on deposit under reference KNIRO-B-005.

Extraction and Isolation. The sea cucumbers (197 g, dried weight) were cut into pieces and extracted twice with refluxing ethanol. The combined extracts were concentrated, and the extracted residue was dissolved in water. Desalting was carried out by passing this fraction through a Polychrom-1 column (powdered Teflon; Biolar, Latvia), first eluting the inorganic salts and polar impurities with H_2O and, then, the crude glycoside fraction (1.5 g) with 50% aqueous acetone. This fraction was further chromatographed on a Si gel column eluted first with $CHCl_3$−MeOH−H_2O (4:8:1) to give two fractions containing cucumariosides belonging to the A_2 (120 mg) and A_3 (190 mg) groups and finally eluted with $CHCl_3$−^iPrOH−H_2O (10:30:4) to give a third fraction containing glycosides belonging to the A_7 group (145 mg). The first fraction containing the cucumariosides belonging to the A_2 group was

Table 7. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugar Moiety of Desulfated Derivative **7**

position	δ_C mult.[a,b]	δ_H mult. (*J* in Hz)[c]	HMBC (C)	NOESY
Xyl1 (1→C3)				
1	105.12 d	4.84 d (7.4)	C: 3	H-3, H3,5- Xyl1
2	**83.21** d	3.93 m	C: 1Qui	H1-Qui1
3	77.91 d	4.18 m		H1-Xyl1
4	70.25 d	4.12 m		
5	66.92 t	4.33dd (5.5, 11.5)		
		3.71 m		H1-Xyl1
Qui (1→2Xyl1)				
1	102.98 d	5.21 d (7.4)	C: 2 Xyl1	H2-Xyl1, H3,5-Qui
2	**82.59** d	4.12 m	C: 1Xyl2, 1Qui	
3	75.57 d	4.06 m		H1-Qui
4	**86.60** d	3.60 m	C: 1 Glu	H1-Glu
5	70.98 d	3.64 m		H1-Qui
6	17.91 q	1.70 d (5.8)		
Glu (1→4Qui)				
1	104.68 d	4.92 d (7.7)	C: 4 Qui	H4-Qui, H3,5-Glu
2	73.52 d	3.97 m		
3	**87.83** d	4.23 m	C: 1 MeGlu	H1-MeGlu, H1-Glu
4	70.19 d	3.98 m		
5	77.75 d	3.99 m		H1-Glu
6	61.86 t	4.46 m, 4.18 m		
MeGlu (1→3Glu)				
1	105.49 d	5.29 d (8.0)	C: 3 Glu	H3-Glu, H3,5-MeGlu
2	74.88 d	3.98 m	C: 1 MeGlu	
3	87.84 d	3.68 t (8.7)?		H1-MeGlu
4	70.39 d	4.15 t (8.7)?		
5	78.18 d	3.97 m		H1-MeGlu
6	62.00 t	4.46 m, 4.25 m		
OMe	60.61 q	3.85 s	C: 3 MeGlu	
Xyl2 (1→2Qui)				
1	105.81 d	5.39 d (7.1)	C: 2 Qui	H2-Qui, H3-Xyl2
2	75.55 d	4.06 m		
3	77.04 d	4.12 m		H1-Xyl2
4	70.39 d	4.15 m		
5	66.48 t	4.3 m, 3.64 m		

[a] Recorded at 125 MHz in C_5D_5N. Multiplicity by DEPT. [b] Bold = interglycosidic positions. [c] Recorded at 500 MHz in C_5D_5N.

Table 8. ^{13}C NMR Chemical Shifts for the Initial Glycoside Fraction Belonging to the A_7 Group (ΣA_7) and the Mixture of **4** (Isokoreoside A) and **5** (Koreoside A)

position	δ_C mult.^{a,b,c} (ΣA_7)	δ_C mult.^{a,b,c} (**4+5**)
Xyl1 (1→C3)		
1	104.64 d	104.61
2	**82.23** d	**82.24** d
3	74.88 d	74.86 d
4	*75.30* d	*75.27* d
5	64.00 t	63.98 t
Qui (1→2Xyl1)		
1	102.59 d	102.59 d
2	**82.59** d	**82.59** d
3	74.66 d	74.65 d
4	**86.35** d	**86.33** d
5	71.17 d	71.21 d
6	18.85 q	18.85 q
Glu (1→4Qui)		
1	103.99 d	103.99 d
2	73.60 d	73.64 d
3	**86.53** d	**86.33** d
4	69.25 d	69.24 d
5	75.30 d	75.27 d
6	*67.74* t	*67.76* t
MeGlu (1→3Glu)		
1	104.64 d	104.61 d
2	74.34 d	74.31 d
3	86.53 d	86.33 d
4	70.08 d	70.08 d
5	76.51 d	76.52 d
6	*67.46* t	*67.49* t
OMe	60.84 q	60.86 q
Xyl2(1→2Qui)		
1	105.21 d	105.20 d
2	75.05 d	75.05 d
3	76.51 d	76.52 d
4	69.87 d	69.87 d
5	66.58 t	66.57 t

^{a} Recorded at 75 MHz in C_5D_5N:D_2O (4:1). Multiplicity by DEPT. ^{b} Bold = interglycosidic positions. ^{c} Italic = sulfate positions.

submitted to HPLC on a Silasorb C_{18} column (3 × 150 mm) using CH_3COCH_3–H_2O (43:57) as the mobile phase to give 43 mg of pure cucumarioside A_2-5 (**1**).

Cucumarioside A_2-5 (1): mp 207–209 °C; $[\alpha]^{20}_D$ −44° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 2; MALDI TOF MS (negative ion mode) *m/z* 1355 ([M_{Na} − Na]$^-$, 100), 1223 ([M_{Na} − Xyl + H − Na]$^-$, 12), 1179 ([M_{Na} − 3-O-MeGlc + H − Na]$^-$, 59), 1047 ([M_{Na} − 3-O-MeGlc − Xyl + H − Na]$^-$, 9), 1017 ([M_{Na} − 3-O-MeGlc-O-Glc + H − Na]$^-$, 5), 845 ([M_{Na} − 3-O-MeGlc-O-Qui-O-OSO$_3$Na-Xyl − Xyl + H − Na]$^-$, 4), 739 ([M_{Na} − 3-O-MeGlc-O-Glc-O-Qui − Xyl + H − Na]$^-$, 8); MALDI TOF MS (positive ion mode) *m/z* 1417 ([M_{Na} + Na]$^+$, 52), 1401 ([M_{Na} +K]$^+$, 39), 1315 ([M_{Na} − SO$_3$Na + H + K]$^+$, 49), 1299 ([M_{Na} − SO$_3$Na + H + K]$^+$, 100), 1167 ([M_{Na} − Xyl − SO$_3$Na + 2H + Na]$^+$, 28), 1123 ([M_{Na} − 3-O-Me-Glc − SO$_3$Na + 2H + Na]$^+$, 44); HRFABMS (positive ion mode) *m/z* 1379.5574 [M + H]$^+$ (calcd for $C_{61}H_{96}NaO_{31}S$, 1379.5554).

Sugar Analysis of Cucumarioside A_2-5 (1).[7] The glycoside **1** (5 mg) was dissolved in 5 mL of 2 N HCl and heated under reflux for 2 h. Then, 5 mL of H_2O was added to the mixture and the aglycon removed by extraction with CHCl$_3$. The aqueous layer was neutralized with Dowex (HCO$_3^-$), the resin filtered off, and the H_2O layer concentrated. Pyridine (1 mL) and $NH_2OH\cdot HCl$ (2 mg) were added to the dried residue and the mixture was heated at 100 °C for 1 h. After that time, 1 mL of Ac$_2$O was added and the heating at 100 °C was continued for a further 1 h. The solution was concentrated, and the resulting aldononitrile peracetates were analyzed by GC–MS using standard aldononitrile peracetates obtained in the Laboratory of Carbohydrate Chemistry of the Pacific

Institute of Bioorganic Chemistry as reference samples. Xylose, quinovose, glucose, and 3-*O*-methylglucose were identified in a ratio of 2:1:1:1.

Desulfation of the Cucumariosides Belonging to the A_3 Group. The cucumariosides mixture (190 mg) belonging to the A_3 group was dissolved in 15 mL of water, and 200 mg of cation resin Dowex (H$^+$) was added to the solution. The solution was kept at room temperature for 20 min, and the resin was filtered off and then washed with H_2O (2 × 5 mL) and ethanol (2 × 5 mL). Then, 0.2 mL of pyridine was added to the combined solution and concentrated to dryness. The residue was dissolved in 10 mL of a mixture of pyridine and dioxane (1:1) and refluxed for 1 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified on a Si gel column eluting with CHCl$_3$–EtOH (5:2). The resulting mixture of desulfated derivatives **6** and **7** was separated by reversed-phase HPLC on an Octadecyl C18 column (4.6 × 250 mm), EtOH–H_2O (55:45), 3.5 mL/min, to give the derivatives **6** (8 mg) and **7** (33 mg).

Sugar Analysis of the Cucumariosides Belonging to the A_3 Group. Sugar analysis was carried out using the method described above, and xylose, quinovose, glucose, and 3-*O*-methylglucose (2:1:1:1 ratio) were identified.

Desulfated derivative 6: mp 214–216 °C; $[\alpha]^{20}_D$ −35° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 5 and 6; MALDI TOF MS (positive ion mode) *m/z* 1129 ([M + Na]$^+$, 100) and 1145 ([M + K]$^+$, 11); HRFABMS (positive ion mode) *m/z* 1129.5421 [M + Na] (calcd for $C_{53}H_{86}O_{24}Na$, 1129.5407).

Desulfated Derivative 7: mp 208–210 °C; $[\alpha]^{20}_D$ −10° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 7 and 8; MALDI TOF MS (positive ion mode) *m/z* 1129 ([M + Na]$^+$, 100) and 1145 ([M + K]$^+$, 12); HRFABMS (positive ion mode) *m/z* 1129.5417 [M + Na] (calcd for $C_{53}H_{86}O_{24}Na$ 1129.5407).

Sugar Analysis of the Glycosides Belonging to the A_7 Group. Sugar analysis was carried out using the method described above, and xylose, quinovose, glucose, and 3-*O*-methylglucose (2:1:1:1 ratio) were identified.

Desulfation of the Glycosides Belonging to the A_7 Group. A 145 mg portion of the fraction was desulfated and separated as described above to give the derivatives **6** (7 mg) and **7** (24 mg).

References and Notes

(1) Avilov, S. A.; Kalinovsky, A. I.; Kalinin, V. I.; Stonik, V. A.; Riguera, R.; Jiménez, C. *J. Nat. Prod.* **1997**, *60*, 808–810.
(2) Drozdova, O. A.; Avilov, S. A.; Kalinin V. I.; Kalinovsky, A. I.; Stonik, V. A.; Riguera, R.; Jiménez, C. *Liebigs Ann.* **1997**, 2351–2356.
(3) Avilov, S. A.; Drozdova, O. A.; Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A.; Gudimova, E. N.; Riguera, R.; Jiménez, C. *Can. J. Chem.* **1998**, *76*, 137–141.
(4) Stonik, V. A.; Kalinin, V. I.; Avilov, S. A. *J. Nat. Toxins* **1999**, *8*, 235–248.
(5) Levin, V. S.; Stepanov, V. G. *Biol. Morya.* **2002**, *28*, 66–69.
(6) Drozdova, O. A.; Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. W. *Khim. Prirod. Soedin.* **1993**, 242–248.
(7) Easterwood, V. M.; Huff, B. J. L. *Svensk Paperstidniong.* **1969**, *72*, 768–772.
(8) Shashkov, A. S.; Chizhov, O. S. *Bioorgan. Khim.* **1976**, *2*, 437–497.
(9) Avilov, S. A.; Stonik, V. A.; Kalinovsky, A. I. *Khim. Prirod. Soedin.* **1990**, 787–792.
(10) Drozdova, O. A.; Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. W. *Khim. Prirod. Soedin.* **1993**, 369–374.