Triterpene Glycosides from Antarctic Sea Cucumbers. 2. Structure of Achlioniceosides A_1, A_2, and A_3 from the Sea Cucumber *Achlionice violaecuspidata* (=*Rhipidothuria racowitzai*)

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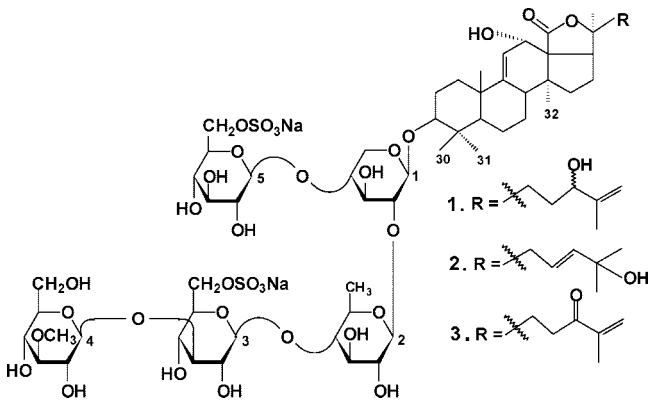
Three new triterpene glycosides, achlioniceosides A_1 (**1**), A_2 (**2**), and A_3 (**3**), have been isolated from the Antarctic sea cucumber *Achlionice violaecuspidata*. The glycoside structures were elucidated using extensive NMR spectroscopic analysis including one-dimensional ^{1}H and ^{13}C spectra, ^{1}H−^{1}H-COSY, HMBC, HMQC, and NOESY and mass spectrometry. Gycosides **1**−**3** are disulfated pentaosides that are branched at the first xylose residue. The sulfates are attached to C-6 of the glucose residues. Glycosides **1**−**3** are the first triterpene glycosides isolated from a sea cucumber belonging to the order Elasipodida.

Holostane oligoglycosides [a 18(20) lactone lanostane skeleton of aglycone] and nonholostane triterpene oligoglycosides have been isolated from many sea cucumber species (Holothurioidea, Echinodermata) collected from the tropical Pacific, Indian, and Atlantic Oceans, the Mediterranean Sea, the North Atlantic, and North Pacific.[1−3] However, the only sea cucumber species collected in the Antarctic waters, *Staurocucumis liouvillei*, was shown to contain holostane glycosides.[4,5] All the previously studied sea cucumbers in which triterpene glycosides were found belong to the orders Aspidochirotida, Dendrochirotida,[1,2] Molpadiida,[6] and Apodida.[7] Triterpene glycosides from the sea cucumber belonging to the order Elasipodida that occurs at mainly great depth and in Antarctic waters have not be studied earlier.



As a continuation of our search for new triterpene glycosides in Antarctic sea cucumbers,[4] we have investigated the polar extract of the sea cucumber *Achlionice violaecuspidata* (=*Rhipidothuria racowitzai*) (order Elasipodida). Specimens were collected in the Weddell Sea by Agassiz trawl and epibenthic sledge on board the R/V *Polarstern* from the Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany) during the Antarctic expedition ANT XXI/2 in December 2003. Here we report the isolation and structural investigation of three new sulfated triterpene glycosides, achlioniceosides A_1 (**1**), A_2 (**2**), and A_3 (**3**).

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Results and Discussion

The concentrated ethanol extract was chromatographed on a column with Teflon powder Polychrom-1 for desalting and elimination of polar substances and then on a Si gel column. The resulting glycoside fraction was separated by HPLC on a Supelco C-18 column [EtOH−H_2O−NH_4OAc (1 N water solution), 45:55:1; 40:60:1.5] to yield achlioniceosides A_1 (**1**), A_2 (**2**), and A_3 (**3**).

The ^{13}C NMR spectra of the carbohydrate portions of the glycosides **1**, **2**, and **3** were identical. The carbohydrate chain of **1**−**3** consists of five monosaccharide residues, as deduced from the ^{13}C NMR spectra, which indicated the signals of five anomeric carbons at 102.8−104.7 ppm. This was correlated by the HSQC spectrum with the corresponding signals of anomeric protons at 4.73 (d, J = 7.4 Hz), 4.85 (d, J = 8.0 Hz), 4.96 (d, J = 7.9 Hz), 5.09 (d, J = 7.6 Hz), and 5.34 (d, J = 7.9 Hz) ppm (Table 1). The coupling constants of the anomeric protons indicated the glycosidic bonds had a β-configuration in all cases.[8]

The ^{13}C NMR and DEPT spectra of the carbohydrate portion of **1**−**3** are similar to those of holothurinoside A from *Holothuria forskalii*,[9] having a pentasaccharide carbohydrate chain branched at the first monosaccharide (D-xylose) and containing D-quinovose, D-glucose, and 3-*O*-methyl-D-glucose (terminal) as the second, third, and fourth sugars, respectively. The fifth (terminal) monosaccharide residue of holothurinoside A is D-glucose. The difference in the spectra of **1**−**3** was in the fifth monosaccharide residue (terminal glucose), in which the signal of C-6 was downfield shifted to 5.3 ppm and the signal of C-5 was upfield shifted to 2.4 ppm, corresponding to α- and β-shifted effects[8] of sulfate groups (Table 1). Another discrepancy was in the signals of the third monosaccharide residue (glucose), where the signal of C-6 was downfield shifted to 4.8 ppm and the signal of C-5 was upfield shifted to 3.4 ppm, corresponding to α- and β-shifted effects of sulfate groups.[8]

This suggests the carbohydrate chains of **1**−**3** contain additional sulfates at C-6 of both glucose residues. The positions of these sulfate groups in the glucose residues were confirmed by TOCSY experiments (tm = 100 ms). The cross-peaks between all carbohydrate protons (CHO) were observed including H-1 at 4.96 ppm and H_2-6 at 5.12 and 4.82 ppm of the fifth monosaccharide residue and between all carbohydrate protons (CHO) including H-1 at 4.85 ppm and H_2-6 at 5.11 and 4.72 ppm of the third monosaccharide residue.

The positions of interglycosidic linkages in **1**−**3** were deduced from NOESY and HMBC spectra (Table 1). Cross-peaks were observed between H-1 of the xylose residue and H-3 (C-3 in the

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Table 1. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and NOESY Correlations of the Carbohydrate Moieties in Glycosides **1**−**3**

atom	δ_C mult.[a,b,c]	δ_H mult. (*J* in Hz)[d]	HMBC	NOESY
Xyl (1→C-3)				
1	104.7 CH	4.73 d (7.4)	C: 3	H-3, H-3,5 Xyl, H-31
2	**82.2** CH	4.03 t (8.0)		H-1 Qui
3	75.1 CH	4.26 t (8.6)	C: 4 Xyl	H-1,5 Xyl
4	**77.8** CH	4.24 m		H-1 Glc 5
5	63.6 CH_2	4.48 m		H-1 Glc 5
		3.75 m		H-1,3 Xyl
Qui (1→2Xyl)				
1	104.5 CH	5.09 d (7.6)	C: 2 Xyl	H-2 Xyl, H-3,5 Qui
2	75.4 CH	3.98 t (8.7)	C: 3,4 Qui	
3	75.0 CH	4.05 t (8.7)		H-1,5 Qui
4	**87.0** CH	3.51 t (8.9)		H-1 Glc3
5	71.3 CH	3.76 m		H-1,3 Qui
6	17.8 CH_3	1.71 d (6.0)		H-4 Qui, H-1 Glc3
Glc (1→4Qui)				
1	104.2 CH	4.85 d (8.0)	C: 4 Qui	H-4,6 Qui, H-3,5 Glc3
2	73.7* CH	3.96 t (8.6)		
3	**85.9** CH	4.31 t (9.1)	C: 2,4 Glc3, 1 MeGlc	H-1,5 Glc, H-1 MeGlc
4	69.3 CH	3.93 t (8.8)		
5	74.9 CH	4.21 m		H-1,3 Glc3
6	*67.3* CH_3	5.11 brd (9.3)		
		4.72 dd (6.5, 11.5)		
MeGlc (1→3Glc)				
1	104.5 CH	5.34 d (7.9)	C: 3 Glc3	H-3 Glc3, H-3,5 MeGlc
2	74.5 CH	3.98 dd (8.5)		
3	86.9 CH	3.80 t (9.0)	C: 4 MeGlc	H-1 MeGlc
4	70.3 CH	4.02 m	C: 5 MeGlc	
5	77.5 CH	4.02 m		H-1 MeGlc
6	61.7 CH_2	4.47 brd (11.1)		
		4.17 m	C: 5 MeGlc	
OMe	60.7 CH_3	3.92 s		
Glc (1→4Xyl)				
1	102.8 CH	4.96 d (7.9)	C: 4 Xyl	H-4,5 Xyl, H-3,5 Glc5
2	73.5* CH	3.95 t (8.5)		
3	76.7 CH	4.22 t (9.0)	C: 2,4 Glc5	H-1 Glc5
4	70.7 CH	4.09 t (9.1)		
5	75.7 CH	4.15 m		H-1 Glc5
6	*67.5* CH2	4.82 dd (6.2, 11.5) 5.12 brd (9.3)		

* Signals may be interchanged. [a] Recorded at 125 MHz in $C_5D_5N-D_2O$ (4:1). Multiplicity by DEPT, [b] Bold = interglycosidic positions. [c] Italic = sulfate position. [d] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1).

HMBC) of the aglycone. Similarly, correlations were seen between H-1 of the quinovose and H-2 (C-2) of the xylose residue and between H-1 of the glucose (third monosaccharide residue) and H-4 (C-4) of the quinovose unit. Finally, H-1 of the 3-*O*-methylglucose and H-3 (C-3) of the glucose unit as well as H-1 of the glucose (the fifth monosaccharide residue) and H-4 (C-4) of xylose showed correlations.

The ^{13}C NMR data of the aglycone portions of glycosides **1**−**3** (Tables 2−4) were very similar, especially in the signals of C-1−C-20 and C-30−C-32, and are very close to the corresponding signals in the spectra of the known bivittoside D from the sea cucumber *Bohadschia bivittata*, having a holostane aglycone with a 12α-hydroxy-9(11)-en fragment.[10] Indeed, the signals of C-9 at 153.2 ppm, C-11 at 115.6 ppm, and C-12 at 68.1 ppm are characteristic for a 12α-hydroxy-9(11)-ene fragment, and the signals of C-18 at 178.0 ppm and C-20 at 85.5 ppm are characteristic for a 18(20)-lactone; these were observed in the spectra of **1**, **2**, and **3**.

The signals of the aglycone side chain of glycoside **1** in the ^{13}C NMR spectrum were similar to the signals of 24(*S*)-hydroxy-25-dehydroechinoside A known from the sea cucumber *Actinopyga flammea*[11] and indicated the presence of a 24-hydroxy group and terminal double bond in the side chain. Indeed, the HMBC correlations between olefinic H_2-26 and the hydroxylated C-24 and between H_3-27 and C-24 clearly confirmed the position of the hydroxy group at C-24 and the terminal position of the double bond. Unfortunately it is impossible to determine the configuration of the C-24 hydroxyl by ^{13}C NMR because the chemical shift differences for the corresponding signals between similar substances with 24(*R*)- and 24(*S*)-hydroxyls are quite small.

The ESIMS (negative ion mode) of achlioniceoside A_1 (**1**) exhibited pseudomolecular ion peaks at *m/z* 711.2550 (calc 711.2539) $[M_{2Na} - 2Na]^{2-}$, 1423.5167 (calc 1423.5152) $[M_{H,Na} - Na]^-$, and 1445.4986 (calc 1445.4971) $[M_{2Na} - Na]^-$. That along with the ^{13}C NMR spectroscopic data allowed the determination of the molecular formula of **1** as $C_{60}H_{94}O_{34}S_2Na_2$.

The sequence of monosaccharide units in the carbohydrate chain was confirmed by (−) ESIMS/MS data. Indeed, the MS/MS spectrum of the ion $[M_{2Na} - 2Na]^{2-}$ at *m/z* 711.27 of **1** indicated the peaks for fragment ions at *m/z* 1325.55 $[M_{2Na} - 2Na - HSO_4]^-$, 1181.51 $[M_{2Na} - 2Na - Glc - SO_3 + H]^-$, 1149.48 $[M_{2Na} - 2Na - MeGlc - SO_4 - 2H]^-$, 1005.44 $[M_{2Na} - 2Na - MeGlc - Glc - SO_3 + H]^-$, 859.38 $[M_{2Na} - 2Na - MeGlc - Glc - SO_3 - Qui + H]^-$, 623.22 $[M_{2Na} - 2Na - MeGlc + H]^{2-}$, 563.13 $[M_{2Na} - 2Na - Glc - SO_3 - Xyl - OAgl - H]^-$, 519.11 $[M_{2Na} - 2Na - MeGlu - Glc - SO_3-OAgl]^-$, 417.08 $[M_{2Na} - 2Na - Glc - SO_3 - Xyl - Qui - OAgl - H]^-$, and 241.06 $[M_{2Na} - 2Na - MeGlc - Glc - SO_3 - Qui - Xyl - OAgl]^-$ (Figure 1).

Acid hydrolysis of **1** with TFA was carried out to ascertain the monosaccharide composition. Subsequent alcoholysis of the resulting monosaccharide mixture by (*R*)-(−)-2-octanol followed by acetylation, GLC analysis, and comparison with standard monosaccharides allowed us to determine the absolute D-configuration of all monosaccharide residues comprising the carbohydrate moiety of **1** (xylose, quinovose, glucose, and 3-*O*-methylglucose). All these data indicated that achlioniceoside A_1 (**1**) is 3β-*O*-{[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)]-[6-*O*-sodium sulfate-β-D-

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and NOESY Correlations of the Aglycone Moiety of the Glycoside **1**

position	δ_C mult.[a]	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	36.2 CH_2	1.83 m		H-11, H-19
		1.47 m		H-3, H-11
2	26.7 CH_2	2.10 m		
		1.92 m		H-19, H-30
3	88.6 CH	3.15 dd (4.4, 11.9)		H1-Xyl. H-1, H-5,
4	39.7 C			
5	52.5 CH	0.97 brd (13.0)		H-3
6	21.0 CH_2	1.70 m		H-31
		1.52 m		
7	28.6 CH	1.79 m		
		1.42 m		
8	40.0 CH	3.32 m		H-19
9	153.2 C			
10	39.4 C			
11	115.6 CH	5.76 dd (1.8, 5.7)	C: 10	H-1
12	68.1 CH	4.59 brd (5.6)		H-21
13	64.2 C			
14	46.4 C			
15	37.0 CH_2	1.68 m, 1.35 m		
16	24.0 CH_2	2.16 m, 2.05 m		
17	46.8 CH	3.22 dd (3.0, 10.3)		H-21, H-32
18	178.0 C			
19	22.2 CH_3	1.36 s	C: 1, 5, 9, 10	H-1, H-2, H-8
20	85.5 C			
21	26.4 CH_3	1.71 s	C: 17, 20, 22	H-12, H-17
22	35.4 CH_2	2.13 m, 1.98 m		
23	29.8 CH_2	1.84 m,		
24	75.1 CH	4.39 t (6.3)	C: 26	
25	148.1 C			
26	111.2 CH_2	5.26 brd (1.6)	C: 24, 27	
		5.00 brs	C: 24, 27	
27	17.8 CH_3	1.90 s	C: 24, 25, 26	
30	16.5 CH_3	1.07 s	C: 3, 4, 5, 31	H-2
31	27.9 CH_3	1.23 s	C: 3, 4, 5, 30	H1-Xyl, H-3, H-6
32	21.7 CH_3	1.30 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125.77 MHz in $C_5D_5N-D_2O$ (4:1). Multiplicity by DEPT. [b] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1).

glucopyranosyl-(1→4)]-β-D-xylopyranosyl}holosta-9(11),25(26)-diene-12α,24(ζ)-diol.

The ^{13}C NMR signals of the side chain in the aglycone moiety of glycoside **2** are similar to the signals of holothurin B_4 known from the sea cucumber *Holothuria polii*[12] and indicated the presence of a 23(24)-double bond and a hydroxy group at C-25 in the side chain (Table 3). Indeed the HMBC correlations between H_3-26 and C-24 and H_3-27 and C-24 as well as NOESY correlations between the H_3-21 and H_2-22 allyl protons clearly confirmed the 23(24)-position of the double bond. The HMBC correlations between H_3-26 (H_3-27) and the hydroxylated C-25 carbon confirmed the position of a hydroxy group at C-25.

The ESIMS (negative ion mode) of achlioniceoside A_2 (**2**) exhibited pseudomolecular ion peaks at *m/z* 711.2561 (calc 711.2539) $[M_{2Na} - 2Na]^{2-}$, 1423.5137 (calc 1423.5152) $[M_{H,Na} - Na]^-$, and 1445.4967 (calc 1445.4971) $[M_{2Na} - Na]^-$. That along with the ^{13}C NMR spectroscopic data allowed the determination of the molecular formula of **2** as $C_{60}H_{94}O_{34}S_2Na_2$.

The sequence of monosaccharide units in the carbohydrate chain was confirmed by (−) ESIMS/MS data. Indeed, the MS/MS spectrum of the ion of $[M_{2Na} - 2Na]^{2-}$ at *m/z* 711.26 of **2** indicated the peaks for fragment ions were similar to the corresponding ions in the spectrum of **1** (Figure 1) because glycosides **1** and **2** are isomers. All these data indicated that achlioniceoside A_2 (**2**) is 3β-*O*-{[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)]-[6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl}holosta-9(11),23(24)-diene-12α,25-diol.

Table 3. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and NOESY Correlations of the Aglycone Moiety of the Glycoside **2**

position	δ_C mult.[a]	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	36.2 CH_2	1.84 m		H-11
		1.48 m		H-3, H-11,
2	26.7 CH_2	2.10 m		
		1.93 m		H-19, H-30
3	88.6 CH	3.15 dd (4.1, 11.8)		H1-Xyl, H-1, H-5, H-31
4	39.7 C			
5	52.5 CH	0.97 brd (11.5)		H-3, H-7, H-31
6	21.0 CH_2	1.72 m		H-31
		1.52 m		
7	28.7 CH	1.81 m		H-5, H-32
		1.43 m		
8	40.0 CH	3.33 m		H-15, H-19
9	153.2 C			
10	39.4 C			
11	115.5 CH	5.76 dd (1.6, 5.5)		H-1
12	68.0 CH	4.58 brd (5.6)	C: 9, 11, 14, 18	H-21
13	64.2 C			
14	46.4 C			
15	36.9 CH_2	1.70 m		H-8
		1.41 m		
16	24.2 CH_2	2.12 m		H-22
17	47.0 CH	3.26 dd (3.2, 9.8)		H-32
18	177.9 C			
19	22.2 CH_3	1.36 s	C: 1, 5, 9, 10	H-2, H-8, H-30
20	84.9 C			
21	26.5 CH_3	1.70 s	C: 17, 20, 22	H-12, H-22
22	42.1 CH_3	2.62 brd (7.5)	C: 20, 23, 24	H-16, H-21
23	120.3 CH	5.91 dt (15.5, 7.0)	C: 24, 25	
24	143.7 CH	6.03 d (15.5)	C: 22, 23, 25	
25	70.0 C			
26	29.8 CH_3	1.56 s	C: 24, 25, 27	
27	29.8 CH_3	1.56 s	C: 24, 25, 26	
30	16.5 CH_3	1.06 s	C: 3, 4, 5, 31	H-2, H-19
31	27.9 CH_3	1.23 s	C: 3, 4, 5, 30	H1-Xyl, H-3, H-5, H-6
32	21.7 CH_3	1.26 s	C: 8, 13, 14, 15	H-7, H-17

[a] Recorded at 125.77 MHz in $C_5D_5N-D_2O$ (4:1). Multiplicity by DEPT. [b] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1).

The ^{13}C NMR signals of the aglycone side chain of glycoside **3** are similar to the signals of frondoside A_2-2 known from the sea cucumber *Cucumaria frondosa*[13] and indicated the presence of a 25(26)-double bond and a keto group at C-24 in the side chain (Table 3). Indeed, the HMBC correlations between the H-26 vinyl protons and H-27 methyl protons and C-24 clearly confirmed the 25(26)-position of the double bond and the presence of a 24-keto group. The presence of an α,β-unsaturated ketone fragment in the side chain was independently confirmed by the UV spectrum of glycoside **3** (λ_{max} = 255.4 nm).

The ESIMS (negative ion mode) of achlioniceoside A_3 (**3**) exhibited pseudomolecular ion peaks at *m/z* 710.2490 (calc 710.2461) $[M_{2Na} - 2Na]^{2-}$, 1421.4985 (calc 1421.4995) $[M_{H,Na} - Na]^-$, and 1443.4878 (calc 1443.4815) $[M_{2Na} - Na]^-$. That along with the ^{13}C NMR data allowed the determination of the molecular formula of **3** as $C_{60}H_{92}O_{34}S_2Na_2$. It is worth mentioning that the pseudomolecular ion peaks listed above were accompanied by peaks at *m/z* 710.7504 $[M_{2Na} - 2Na + H]^{2-}$, 711.2540 $[M_{2Na} - 2Na + 2H]^{2-}$, 1422.5079 $[M_{HNa} - Na + H]^-$, 1423.5152 $[M_{HNa} - Na + 2H]^-$, 1444.4898 $[M_{2Na} - Na + H]^-$, and 1445.4971 $[M_{2Na} - Na + 2H]^-$ in the MS of **3**. MS/MS analysis of **3** showed that the satellite signals were caused by aglycone transformation in the ESI ion source.

The sequence of monosaccharide units in the carbohydrate chain was confirmed by (−) ESIMS/MS data. Indeed, the MS/MS spectrum of the ion $[M_{2Na} - 2Na]^{2-}$ at *m/z* 710.25 of **3** gave peaks for fragment ions at *m/z* 1323.54 $[M_{2Na} - 2Na - HSO_4]^-$, 1179.54

Table 4. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and NOESY Correlations of Aglycone Moiety of the Glycoside **3**

position	δ_C mult.[a]	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	36.2 CH_2	1.85 m		H-11
		1.48 m		H-3, H-11,
2	26.7 CH_2	2.12 m		
		1.93 m		H-19, H-30
3	88.6 CH	3.16 dd (4.2, 12.0)		H1-Xyl. H-1, H-5, H-31
4	39.7 C			
5	52.5 CH	0.98 brd (12.0)	C: 6, 19, 29	H-3, H-31
6	21.0 CH_2	1.72 m, 1.52 m		
7	28.5 CH	1.81 m		
		1.42 m		
8	40.0 CH	3.32 m		H-19
9	153.2 C			
10	39.4 C			
11	115.5 CH	5.76 dd (1.8, 5.7)	C: 10	H-1
12	68.1 CH	4.59 brd (5.7)	C: 9, 11, 14, 18	H-21
13	64.2 C			
14	46.4 C			
15	36.9 CH_2	1.70 m, 1.37 m		
16	23.8 CH_2	2.03 m		
17	46.9 CH	3.28 dd (4.5, 9.6)	C: 18	H-21, H-32
18	177.7 C			
19	22.2 CH_3	1.37 s	C: 1, 5, 9, 10	H-2, H-8, H-30
20	84.7 C			
21	26.2 CH_3	1.73 s	C: 17, 20, 22	H-12, H-17
22	33.5 CH_2t	2.26 m, 2.19 m		
23	32.5 CH_2	2.98 m		
24	201.8 C			
25	143.9 C			
26	126.2 CH_2	6.21 brs	C: 24, 27	
		5.97 brs	C: 24, 27	
27	17.5 CH_3	1.97 s	C: 24, 25, 26	
30	16.5 CH_3	1.07 s	C: 3, 4, 5, 31	H-2, H-19
31	27.9 CH_3	1.24 s	C: 3, 4, 5, 30	H1-Xyl, H-3, H-5
32	21.7 CH_3	1.30 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125.77 MHz in $C_5D_5N-D_2O$ (4:1). Multiplicity by DEPT. [b] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1).

$[M_{2Na} - 2Na - Glc - SO_3 + H]^-$, 1147.46 $[M_{2Na} - 2Na - MeGlc - SO_4 - 2H]^-$, 1003.42 $[M_{2Na} - 2Na - MeGlc - Clc - SO_3 + H]^-$, 857.37 $[M_{2Na} - 2Na - MeGlc - Glc - SO_3 - Qui + H]^-$, 622.22 $[M_{2Na} - 2Na - MeGlc + H]^{2-}$, 563.13 $[M_{2Na} - 2Na - Glc - SO_3 - Xyl - OAgl - H]^-$, 519.11 $[M_{2Na} - 2Na - MeGlc - Glc - SO_3 - OAgl]^-$, 417.08 $[M_{2Na} - 2Na - Glc - SO_3 - Xyl - Qui - OAgl - H]^-$, and 241.06 $[M_{2Na} - 2Na - MeGlu - Glc - SO_3 - Qui - Xyl - OAgl]^-$ (Figure 2). All these data indicated that achlioniceoside A_3 (**3**) is 3β-*O*-{[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)]-[6-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl}holosta-9(11),25(26)-diene-12α-ol-24-one.

In spite of the fact that the aglycones of the isolated glycosides **1**−**3** are similar to the aglycone of bivittoside D and related aglycones of other glycosides from sea cucumbers belonging to the family Holothuriidae,[2,3,12] the aglycones described herein are new and differ from each other in details of the structures of the side chains. Nevertheless, the side chain of the aglycone of achlioniceoside A_3 (**3**) contains a 25(26)-en-24-one α,β-unsaturated ketone fragment. This is only the second such finding in sea cucumber glycosides after frondoside A_2-2 from *Cucumaria frondosa*.[13]

The carbohydrate moiety, identical in all these glycosides, branched at the first xylose residue and having two sulfates, has never been found in sea cucumber triterpene glycosides. Achlioniceosides A_1 (**1**), A_2 (**2**), and A_3 (**3**) are structurally related to the glycosides from sea cucumbers belonging to the family Holothuriidae from Aspidochirotida, especially to holothurinosides from *Holothuria forskalii*.[9] Nevertheless, they seem to be the result of parallel and independent evolution of glycosides in different higher taxa of sea cucumbers because plural sulfation of carbohydrate chains at C-6 glucose residues is not characteristic for saponins from holothurians belonging to the family Holothuriidae and other families of the order Aspidochirotida.

Isolation of the glycosides **1**−**3** from the sea cucumber *Achlionice violaecuspidata* (=*Rhipidothuria racowitzai*) in the family Elpidiidae, order Elasipodidae, is the first finding of triterpene glycosides in holothurians belonging to this order. Taking into account that triterpene oligoglycosides have been found in sea cucumbers belonging to the orders Aspidochirotida, Dendrochirotida,[1,2] Molpadida,[6] and Apodida (=Synaptida),[7] the present results show that holothurians from all the extant orders of the class Holothurioidea contain these compounds. Therefore, the presence of triterpene oligoglycosides may be considered as a taxonomic character for this class.

Experimental Section

General Experimental Procedures. The UV spectrum was recorded on a CECIL, CE 7250, 7000 Series spectrophotometer in an H_2O solution. NMR spectra were recorded on a DRX-500 Bruker spectrometer at 500.13/125.75 MHz ($^1H/^{13}C$) in $C_5D_5N-D_2O$ (4:1) with TMS as an internal reference ($\delta = 0$). The ESIMS and CID MS/MS (negative ion mode) were recorded using a Agilent 6510 Q-TOF LC/MS apparatus; sample concentration was 0.01 mg/mL CH_3CN-H_2O (1:1) as the solvent. HPLC was performed using an Agilent 1100 chromatograph equipped with a differential refractometer on a Supelco C-18 (10 × 250 mm, 5 μm) column. GLC analysis was carried out with an Agilent 6850 Series apparatus, carrier gas He (1.7 mL/min) at 100 °C (0.5 min) → 250 °C (5 °C/min, 10 min), a capillary column HP-5 MS (30 m × 0.25 mm), and temperatures of injector and detector of 150 and 280 °C, respectively.

Animals. A total of 125 specimens (100 from station #232 and 25 from station #400) of the sea cucumber *Achlionice violaecuspidata* Gutt, 1990 (=*Rhipidothuria racowitzai* Herouard, 1901)[14,15] (family Elpidiidae; order Elasipodida) were collected at two sampling stations in the Weddell Sea by Agassiz trawl [11°24,13′ W, 70°47,88′S (station #232)] and epibentic sledge [10°48,04′ W, 70°56,98′ S (station #400)] at 1525 and 407 m depth, respectively. Sampling was performed on board the R/V *Polarstern* [Alfred Wegener Institute for Polar and Marine Research (Bremenhaven, Germany)] during the Antarctic expedition ANT XXI/2. Sampling was carried out on December 10, 2003, and on December 13, 2003, by Dr. C. Avila and Dr. M. Ballesteros. Sea cucumbers were identified by A. Bosch and Dr. M. Ballesteros (University of Barcelona, Spain). Voucher specimens are preserved in the collection of the Department of Animal Biology (Invertebrates), Faculty of Biology, University of Barcelona.

Extraction and Isolation. The sea cucumbers were minced and extracted twice by refluxing in 70% EtOH. The dry weight of the residue was 20 g. The combined extracts were concentrated to dryness *in vacuo*, dissolved in H_2O, and chromatographed on a Polychrom-1 column (powdered Teflon, Biolar, Latvia), eluting first inorganic salts and polar impurities with H_2O and then the glycosides with 60% acetone. The latter fraction was submitted to sequential chromatography on Si gel columns eluting with a $CHCl_3-EtOH-H_2O$ (100:100:17) solvent system to give 28 mg for the glycoside fraction as an individual spot on TLC. The fraction was separated by HPLC on a Supelco C-18 column with $EtOH-H_2O-NH_4OAc$ (1 N water solution) (45:55:1 and 40:60:1.5) as mobile phase (1.5 mL/min) to give 1.2 mg of achlioniceoside A_1 (**1**), 1.6 mg of achlioniceoside A_2 (**2**), and 1.3 mg of achlioniceoside A_3 (**3**).

Achlioniceoside A_1 (1): mp 228−229 °C; see Tables 1 and 2 for NMR data; ESIMS (−), *m*/*z* 711.2671 (calc 711.25395) $[M_{2Na} - 2Na]^{2-}$; ESIMS/MS (−) of the ion $[M_{2Na} - 2Na]^{2-}$ at *m*/*z* 711.27, *m*/*z* is presented in the Results and Discussion and in Figure 1.

Achlioniceoside A_2 (2): mp 229−230 °C; see Tables 1 and 3 for NMR data; ESIMS (−), *m*/*z* 711.2594 (calc 711.25395) $[M_{2Na} - 2Na]^2$; ESIMS/MS (−) of the ion $[M_{2Na} - 2Na]^{2-}$ at *m*/*z* 711.26 is similar to the corresponding spectrum of **1** (Figure 1).

Achlioniceoside A_3 (3): mp 225−227 °C; UV (in H_2O) $\lambda_{max} = 255.4$ nm; see Tables 1 and 4 for NMR data; ESIMS (−), *m*/*z* 710.2461 (calc 710.2483) $[M_{2Na} - 2Na]^{2-}$, 1421.5006 (calc 1421.5001) $[M_{H,Na} - Na]^-$ and 1443.4825 (calc 1443.4919) $[M_{2Na} - Na]$; ESIMS/MS (−) of the

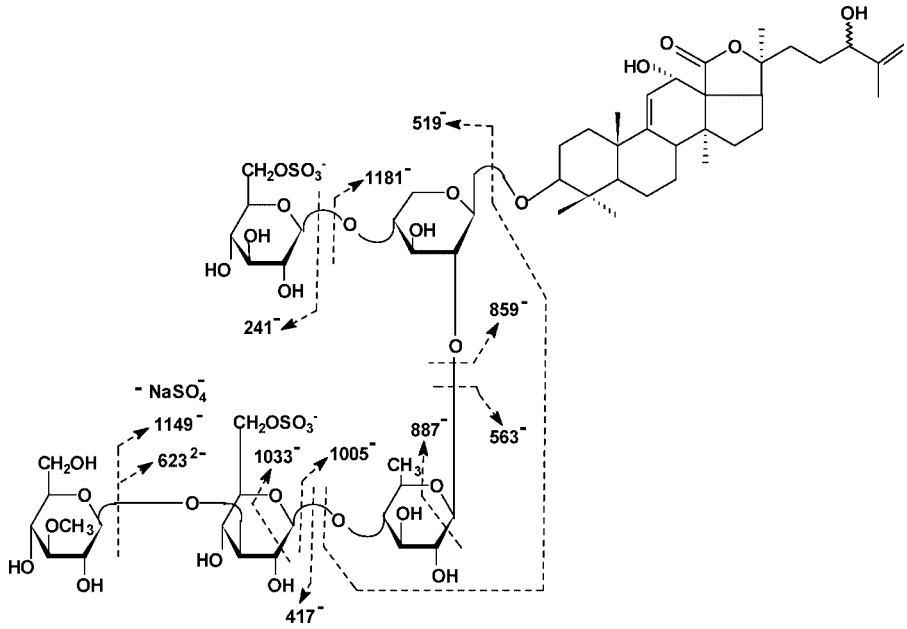

Figure 1. Main CID-type fragmentations in (−) ESIMS/MS of the ion $[M_{2Na} - 2Na]^{2-}$ at *m/z* 711.27 of achlioniceoside A_1 (**1**).

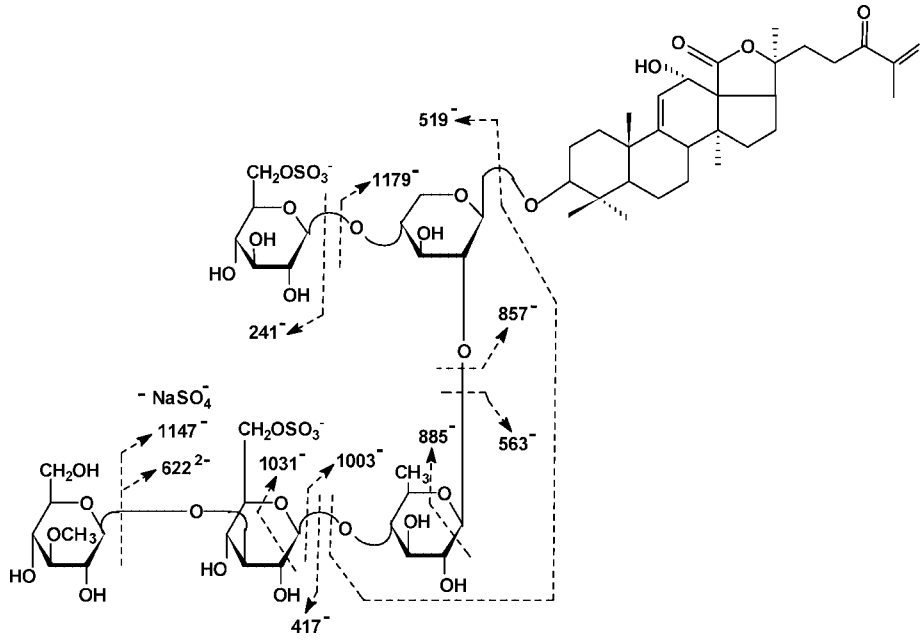

Figure 2. Main CID-type fragmentations in (−) ESIMS/MS of the ion $[M_{2Na} - 2Na]^{2-}$ at *m/z* 710.25 of achlioniceoside A_3 (**3**).

ion $[M_{2Na} - 2Na]^{2-}$ at *m/z* 710.25 is presented in the Results and Discussion and in Figure 2.

Acid Hydrolysis and Determination of the Absolute Configuration of Monosaccharides in Achlyoniceoside A_1 (1). Acid hydrolysis of achlioniceoside A_1 (**1**) (1 mg) was carried out in a solution of 0.2 M trifluoroacetic acid (TFA) (0.3 mL) in a stoppered vial on a H_2O bath at 100 °C for 30 min. The H_2O layer was washed with $CHCl_3$ (3 × 0.5 mL) and concentrated *in vacuo*. One drop of concentrated TFA and 0.2 mL of (−)-2-octanol (Aldrich) were added to the sugar mixture. The ampule was sealed and then heated on a glycerol bath at 130 °C for 6 h. The mixture was evaporated *in vacuo* and treated with a mixture of pyridine−acetic anhydride (1:1, 0.6 mL) for 24 h at room temperature. The acetylated (−)-2-octylglycosides were analyzed by GLC using the corresponding authentic samples D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-glucose treated by the same procedure. The following peaks were detected: D-xylose (retention times 24.46, 24.67, and 24.95 min), D-quinovose (retention times 24.04, 24.22, and 24.63 min), D-glucose (retention times 28.27, 28.94, and 29.16 min), and 3-*O*-methyl-D-glucose (retention times 28.23 and 28.55 min). Injections of the sample with authentic samples of D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-glucose did not split the corresponding peaks in the mixture.

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