Triterpene Glycosides from the Deep-Water North-Pacific Sea Cucumber *Synallactes nozawai* Mitsukuri

Alexandra S. Silchenko,† Sergey A. Avilov,*,† Alexandr A. Antonov,† Vladimir I. Kalinin,† Anatoly I. Kalinovsky,† Alexey V. Smirnov,‡ Ricardo Riguera,§ and Carlos Jiménez⊥

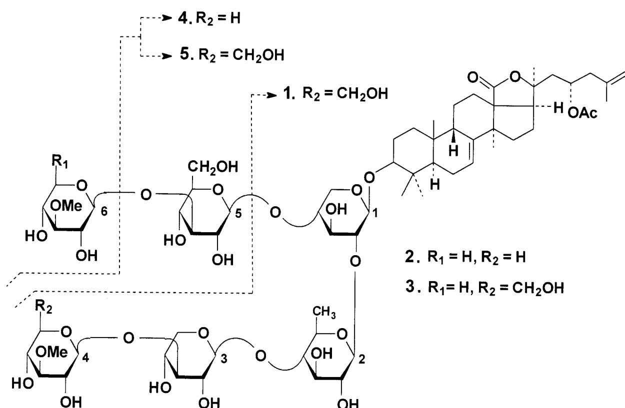
Pacific Institute of Bioorganic Chemistry, the Far East Division of the Russian Academy of Sciences, 690022, Vladivostok, Russia, Zoological Institute of the Russian Academy of Sciences, 199164, Saint Petersburg, Russia, Departamento de Química Orgánica and Instituto de Acuicultura, Universidad de Santiago, 15706 Santiago de Compostela, Spain, and Departamento de Química Fundamental, Facultad de Ciencias, Universidad de La Coruña, 15071 La Coruña, Spain

Received June 26, 2002

Five non-sulfated triterpene glycosides, synallactosides A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), and C (**5**), have been isolated from the sea cucumber *Synallactes nozawai*. Their structures have been deduced by extensive analysis of NMR and mass spectra. The glycosides **2**−**5** are new glycosides. Glycosides **2**−**4** have carbohydrate chains without precedent in the glycosides from sea cucumbers. This is the first time glycosides are found in members of the family Synallactidae.

Introduction

As a continuation of our studies on the triterpene glycosides from sea cucumbers,[1−3] we have investigated the triterpene glycosides from the North-Pacific deep-water sea cucumber *Synallactes nozawai* Mitsukuri (Synallactidae, Aspidochirotida). The organisms were collected in the southern part of the Sea of Japan at 540 m depth using an industrial fishing bottom trawl. In this paper we report the isolation of five non-sulfated glycosides: synallactosides A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**) and C (**5**).



Results and Discussion

The ethanolic extract of *S. nozawai* (302 g dry wt) was sequentially submitted to column chromatography on Polychrom-1 (powedered Teflon) and Si gel. Final separation of the polar glycosides and isolation of individual compounds were achieved by reversed-phase HPLC on Silasorb C_{18} to give synallactosides A_1 (**1**), A_2 (**2**), B_1 (**3**), B_2 (**4**), and C (**5**), numbered according to their increasing polarity on TLC. Structures of the glycosides have been elucidated by extensive analysis of ^{13}C, DEPT, and ^{1}H NMR spectra, 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY), and FABMS experiments.

^{13}C NMR spectral data of the aglycon parts of the glycosides **1**−**5** (Table 1) were found to be identical to each other and coincident with those of the aglycon of astichoposide C (Japanese name stichloroside C_2) isolated from the Caribbean sea cucumber *Astichopus multifidus,*[4−6] which had been previously identified as 23(*S*)-acetoxyholosta-7,25-dien-3β-ol. This structure for the glycosides **1**−**5** was confirmed by the ^{1}H NMR spectra and ^{1}H−^{1}H COSY, HMBC, and NOESY experiments (Table 1).

The presence of four monosaccharide units in the sugar chain of the glycoside **1** was easily deduced from its ^{13}C NMR and DEPT spectra, which showed four anomeric carbons at 105.06−105.59 ppm, correlated by HMQC to their corresponding anomeric protons at 4.83 d (J = 7.4 Hz), 4.85 d (J = 7.4 Hz), 5.18 (J = 7.0 Hz), and 5.30 d (J = 8.0 Hz) ppm (Table 2). The coupling constants of the anomeric protons were indicative in all cases of a β-configuration for the glycosidic bonds.[7] The monosaccharide units in **1** were identified as 3-*O*-methylglucose, xylose, and quinovose in a 1:2:1 ratio by acid hydrolysis with 2 N HCl followed by GC−MS analysis of the corresponding aldonitrile peracetates. The NMR spectral data of the carbohydrate part of glycoside **1** were coincident with those of thelenotoside A from *Thelenota ananas*[8] and cladoloside A from *Cladolabes* sp.,[9] indicating that these three glycosides contain the same carbohydrate chain.

The interglycosidic linkages in the tetrasaccharide chain of **1** and its bonding to the aglycon were confirmed by NOESY experiments (Table 2) that showed cross-peaks between H-1 of the first xylose residue and H-3 of the aglycon, between H-1 of quinovose and H-2 of the first xylose residue, between H-1 of the second xylose residue and H-4 of the quinovose residue, and between H-1 of 3-*O*-methylglucose and H-3 of the second xylose residue. The molecular formula of **1** was determined as $C_{55}H_{86}O_{22}$ by the pseudomolecular ion $[M + Na]^+$ at m/z 1121.5518 in the positive-ion mode HRFABMS. All these data indicate that synallactoside A_1 (**1**) is 3β-*O*-[3-*O*-methyl-β-D-gluco-

* To whom correspondence should be addressed. Fax: internat +7-(4232)-314050. E-mail: avilov@piboc.dvo.ru.
† Pacific Institute of Bioorganic Chemistry.
‡ Zoological Institute.
§ Universidad de Santiago de Compostela.
⊥ Universidad de La Coruña.

10.1021/np0202881 CCC: $22.00 © 2002 American Chemical Society and American Society of Pharmacognosy
Published on Web 10/22/2002

Table 1. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of Compounds **1**−**5**

position	δ_C mult.[a]	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	36.22 t	1.45 m, 1.50 m		
2	27.14 t	2.17 m, 2.09 m		
3	89.16d	3.28 dd (3.7, 11.6)	C: 1 Xyl1	H-5, H-Xyl1
4	39.46 s			
5	47.91 d	1.03 t (7.7)	C: 4, 10, 19, 30, 31	H-3, H-6
6	23.21 t	2.05 m		H-5, H-7, H-19
7	119.89 d	5.68 br s		H-6, H-32
8	146.45 s			
9	47.27 d	3.45 br d (13.7)		H-19, H-11
10	35.45 s			
11	22.78 t	1.77 m		H-9
12	30.12 t	1.91 m		
		1.87 m	C: 9, 13, 18	
13	58.33 s			
14	51.11 s			
15	33.73 t	1.64 m		
16	24.59 t	2.07 m		
		1.90 m		H-17
17	53.93 d	2.30 dd (4.1, 10.2)		H-16, H-21, H-32
18	179.68 s			
19	23.94 q	1.24 s	C: 1, 9, 10	H-9
20	83.02 s			
21	26.84 q	1.50 s	C: 17, 20, 22	H-17, H-23, H-32
22	43.09 t	2.20 m	C: 20, 21	
		1.97 m	C: 20, 21	
23	67.75 d	5.49 m		H-21, H-27
24	44.48 t	2.38 dd (7.8, 13.5),	C: 22, 23, 25, 26, 27	
		2.25 dd (5.2, 13.5)	C: 22, 23, 25, 26, 27	
25	141.54 s			
26	114.16 t	4.88 brs, 4.86 brs		
27	22.17 q	1.81 s	C: 24, 25, 26	H-23
30	17.21 q	1.15 s	C: 3, 4, 5, 31	
31	28.59 q	1.31 s	C: 3, 4, 5, 30	
32	30.79 q	1.07 s	C: 8, 13, 14, 15	H-7, H-17, H-21
O*C*OCH$_3$	170.38 s	2.12 s	OCO*C*H$_3$	
OCO*C*H$_3$	21.04 q			

[a] Recorded at 125.77 MHz in C$_5$D$_5$N. Multiplicity by DEPT. [b] Recorded at 500 MHz in C$_5$D$_5$N.

pyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-β-D-xylopyranosyl]-23(*S*)-acetoxyholosta-7,25-diene. Hence, glycoside **1** is the 25,26-dehydro derivative of thelenotoside A, isolated from *Thelenota ananas*[8] as a mixture with thelenotoside A. Synallactoside C (**5**) has molecular formula $C_{61}H_{96}O_{27}$, as indicated by the HRFABMS, which showed the pseudomolecular ion [M + Na]$^+$ at m/z 1283.6054. The presence in **5** of 3-*O*-methylglucose, glucose, xylose, and quinovose in a 1:1:2:1 ratio was established by acid hydrolysis with 2 N HCl followed by GC−MS analysis of the corresponding aldononitrile peracetates. The presence of five monosaccharide units in **5** was confirmed by its NMR spectra showing five anomeric carbons between 103.23 and 105.46 ppm and five anomeric protons at 4.75 d (J = 7.4 Hz), 4.85 d (J = 7.7 Hz), 5.03 d (J = 8.0 Hz), 5.15 d (J = 7.7 Hz), and 5.29 d (J = 7.7 Hz) ppm, with couplings indicative of β-glycosidic bonds.

The NMR spectral data of the sugar chain of **5** (Table 3) were very close to those of **1** but have signals corresponding to an additional terminal glucose residue. The location of this glucose moiety was deduced by taking into consideration the glycosidation shifts and NOESY correlations. Indeed, the downfield shift (by 6.49 ppm) of the C-4 signal and the upfield shift (by 2.36 and 2.54 ppm, respectively) of the C-3 and C-5 signals of the first xylose residue in the ^{13}C NMR spectrum of **5**, in comparison with those of **1**, indicated the attachment of the additional glucose to C-4 of the first xylose unit.[7] This was confirmed by the NOESY correlation between the anomeric proton of the glucose residue and H-4 of the first xylose residue. Further NOESY correlations (Table 3) were used to complete the sequence of sugars in **5**. Finally, the identical NMR spectral data of the sugar chain of **5** to those of cladoloside B from *Cladolabes* sp.[9] confirmed the structure of **5**. Hence, synallactoside C (**5**) is 3β-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl]-23(*S*)-acetoxyholosta-7,25-diene. The molecular formula of synallactoside B$_2$ (**4**) was established as $C_{60}H_{94}O_{26}$ by the pseudomolecular ion [M + Na]$^+$ at m/z 1253.5932 in the HRFABMS. Comparison of the NMR (^{1}H and ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, HMQC) data of the carbohydrate chain of **4** with those of **5** showed a very similar monosaccharide content and a sugar sequence that differed only in the presence of a terminal 3-*O*-methylxylose residue linked to C-3 of the second xylose unit in **4** in comparison with a terminal 3-*O*-methylglucose unit at that position in **5**. This was easily deduced by the absence of the signal at 62.02 ppm, corresponding to C-6 of the terminal 3-*O*-methylglucose unit, and by the upfield shift (11.18 ppm) of C-5 of the terminal sugar residue in the ^{13}C NMR and DEPT spectra of **4** in relation with those of **5** (Table 4). Moreover, the signals at 3.95 ppm (m, H5-MeGlu), 4.45 ppm (dd, H6′-MeGlu), and 4.25 ppm (m, H6″-MeGlu), characteristics of a terminal 3-*O*-methylglucose unit in **5**, are absent in the ^{1}H NMR of **4**, and instead new ones at 4.20 ppm (m, H5-MeXyl) and 3.63 ppm (H5′-MeXyl), characteristic for 3-*O*-methylxylose, are present.

The presence of a terminal 3-*O*-methylxylose unit in **4** was further confirmed by comparison of the ^{13}C NMR data of **4** with those of cucumarioside G$_1$ isolated from *Eupentacta fraudatrix*[10] and *E. pseudoquinquesemita*,[11] and cucumarioside G$_2$ isolated from *E. fraudatrix* and

Table 2. ^{13}C and ^{1}H NMR Chemical Shifts and Selected NOESY Correlations for the Sugar Units of Synallactoside A_1 (**1**)

position	δ_C mult.[a,b]	δ_H mult. (J in Hz)[c]	NOESY
Xyl1 (1→C-3)			
1	105.59 d	4.83 d (7.4)	H-3, H3,5-Xyl1
2	**84.13** d	4.07 m	H1-Quin
3	78.11 d	4.20 m	H1-Xyl1
4	**70.63** d	4.19 m	
5	66.62 t	4.33 m	H1-Xyl1
		3.71 m	H1-Xyl1
Qui (1→2Xyl1)			
1	105.46 d	5.18 d (7.0)	H3,5-Qui, H2-Xyl1
2	76.41 d	4.07 m	
3	75.40 d	4.05 m	H1-Qui
4	**85.85** d	3.65 t (8.9)	H1-Xyl2
5	71.64 d	3.77 m	H1-Qui
6	17.85 q	1.74 d (5.8)	
Xyl2 (1→4Qui)			
1	105.06 d	4.85 d (7.4)	H4-Qui, H3,5-Xyl2
2	73.34 d	3.97 m	
3	**87.26** d	4.16 m	H1-MeGlu, H1-Xyl2
4	68.93 d	4.02 m	
5	66.36 t	4.20 m	H1-Xyl2
		3.62 m	H1-Xyl2
MeGlu (1→3Xyl2)			
1	105.27 d	5.30 d (8.0)	H3-Xyl2, H3,5-MeGlu
2	74.93 d	3.98 m	
3	87.86 d	3.71 t (8.6)	H1-MeGlu
4	70.46 d	4.13 t (8.8)	
5	78.16 d	3.95 m	H1-MeGlu
6	62.02 t	4.46 br d (10.4)	
		4.26 m	
OMe	60.62 q	3.86 s	

[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.

Pentamera calcigera.[1] Finally, the full sequence of monosaccharides in the carbohydrate chain of **4** was confirmed by NOESY and HMBC correlations as shown in Table 4. Therefore, the structure of synallactoside B_2 (**4**) was determined as 3β-*O*-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl]-23(*S*)-acetoxyholosta-7,25-dien-3β-ol. The molecular formula of synallactoside A_2 (**2**) was determined as $C_{66}H_{104}O_{30}$ by HRFABMS, which showed the pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1399.6568. The presence of six monosaccharide units in the sugar chain of the glycoside **2** was deduced by the ^{13}C NMR and DEPT spectra of synallactoside A_2 (**2**), which showed six anomeric carbons at 102.70−106.02 ppm, correlated by HMQC to their corresponding signals for anomeric protons at 4.75 d ($J = 7.4$ Hz), 4.87 d ($J = 8.0$ Hz), 5.01 d ($J = 8.7$ Hz), 5.15 d ($J = 7.4$ Hz), 5.22 d ($J = 7.7$ Hz), and 5.26 d ($J = 7.7$ Hz) ppm, with coupling constants indicative of a β-configuration for the glycosidic bonds (Table 5).[7]

The comparison of the NMR spectral data (see Tables 4 and 5) of the carbohydrate chain of **2** with those of synallactoside B_2 (**4**) indicated the presence in **2** of an additional monosaccharide residue linked to the same sugar chain present in **4**. 1D and 2D NMR (^{1}H−^{1}H COSY, HMQC, and HMBC) experiments (see Table 5) allowed identification of this additional monosaccharide as a 3-*O*-methylxylose unit. Again, the glycosidation shifts along with the HMBC and NOESY correlations were used for the location of the additional sugar moiety in **2**. Indeed, the ^{13}C NMR of **2** shows that the C-3 signal of the glucose residue is shifted downfield (by 8.33 ppm) while C-2 and C-4 are shifted upfield (by 0.92 and 2.20 ppm, respectively)

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Sugar Units of Synallactoside C (**5**)

position	δ_C mult.[a,b]	δ_H mult. (J in Hz)[c]	NOESY
Xyl1 (1→C-3)			
1	105.13 d	4.75 d (7.4)	H-3,H3,5-Xyl1
2	**83.40** d	4.04 m	
3	75.75 d	4.25 m	H1-Xyl1
4	**77.12** d	4.30 m	H1-Glc
5	64.08 t	4.42 dd (4.9, 11.3)	H1-Xyl1
		3.66 t (11.3)	H1-Xyl1
Qui (1→2Xyl1)			
1	105.46 d	5.15 d (7.7)	H2-Xyl1, H3,5-Qui
2	76.38 d	4.05 m	
3	75.34 d	4.08 m	H1-Qui
4	**85.76** d	3.66 t (8.9)	H1-Xyl2
5	71.63 d	3.77 m	H1-Qui
6	17.86 q	1.73 d (6.1)	
Xyl2 (1→4Qui)			
1	105.03 d	4.85 d (7.7)	H4-Qui, H3,5-Xyl2
2	73.29 d	3.98 m	
3	**87.32** d	4.15 m	H1-MeGlu, H1-Xyl2
4	68.93 d	4.02 m	
5	66.37 t	4.19 m	
		3.65 t (10.9)	H1-Xyl2
MeGlu (1→3Xyl2)			
1	105.31 d	5.29 d (7.7)	H3-Xyl2, H3,5-MeGlu
2	74.93 d	3.99 m	
3	87.88 d	3.71 t (8.6)	H1-MeGlu
4	70.43 d	4.15 t (8.8)	
5	78.15 d	3.95 m	H1-MeGlu
6	62.02 t	4.45 dd (4.9, 11.7), 4.25 m	
OMe	60.62 q	3.86 s	
Glu (1→4Xyl1)			
1	103.23 d	5.03 d (8.0)	H4-Xyl1
2	74.14 d	4.03 m	
3	78.63 d	3.97 m	
4	71.42 d	4.20 m	
5	78.01 d	4.20 m	
6	62.35 t	4.54 brd (10.6), 4.33 dd (10.6, 5.5)	

[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.

with respect to the same signals in **4**, proving the attachment of the sixth monosaccharide unit to C-3 of the glucose residue.[7] This location was further confirmed by the HMBC cross-peaks between H1-MeXyl2 and C3-Glu and by NOESY correlations between H1-MeXyl2 and H3-Glu (Table 5).

Extensive HMBC and NOESY experiments allowed establishment of the full sequence of sugars, and hence, synallactoside A_2 (**2**) is a hexasaccharide with two terminal 3-*O*-methylxylose residues. On the basis of all above-mentioned data, the structure of synallactoside A_2 (**2**) was elucidated as 3β-*O*-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl]-23(*S*)-acetoxyholosta-7,25-diene. The HRFABMS of synallactoside B_1 (**3**) showed a pseudomolecular ion $[M + Na]^+$ at *m*/*z* 1429.6592, and this established its molecular formula as $C_{67}H_{106}O_{31}$. The presence of a hexaoxide sugar chain of synallactoside B_1 (**3**) was deduced from its ^{13}C NMR and DEPT spectra, showing six anomeric carbons between 102.7 and 106.03 ppm and the

Table 4. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Sugar Units of Synallactoside B_2 (**4**)

position	δ_C mult.[a,b]	δ_H mult. (J in Hz)[c]	HMBC (C)	NOESY
Xyl1 (1→C-3)				
1	105.13 d	4.75 d (7.1)	C: C-3	H-3, H3,5-Xyl1
2	**83.39** d	4.04 m	C: 1Xyl1, 1 Qui	
3	75.76 d	4.23 m	C: 4Xyl1	H1-Xyl1
4	**77.12** d	4.30 m		
5	64.08 t	4.42dd (4.9, 11.1)	C: 3Xyl1	H1-Xyl1
		3.66 m		H1-Xyl1
Qui (1→2Xyl1)				
1	105.46 d	5.15 d (7.7)	C: 2 Xyl1	H2-Xyl1, H3,5-Qui
2	76.37 d	4.03 m	C: 1 Qui, 3 Qui	
3	75.35 d	4.12 (t, 8.7)	C: 2 Qui	H1-Qui
4	**85.79** d	3.66 t (8.7)	C: 1 Xyl2	H1-Xyl2
5	71.64 d	3.77 m	C: 4 Qui	H1-Qui
6	17.86 q	1.75 d (5.8)	C: 4 Qui, 5 Qui	
Xyl2 (1→4Qui)				
1	105.08 d	4.87 d (7.7)	C: 4 Qui	H4-Qui, H3,5-Xyl2
2	73.60 d	3.98 m		
3	**86.53** d	4.12 t (8.3)	C: 1 MeXyl, 2 Xyl2	H1-MeXyl, H1-Xyl2
4	68.65 d	4.02 m		
5	66.54 t	4.20 m, 3.65 m		H1-Xyl2
MeXyl (1→3Xyl2)				
1	105.90 d	5.25 d (7.7)	C: 3 Xyl2	H3-Xyl2, H3,5-MeXyl
2	74.68 d	3.95 m	C: 1 MeXyl, 3 MeXyl	
3	87.73 d	3.60 t (8.8)	C: 2, 4, MeXyl, OMe	H1-MeXyl
4	69.97 d	4.08 m		
5	66.97 t	4.20 m, 3.63 m	C: 1 MeXyl, 4 MeXyl	H1-MeXyl
OMe	60.59 q	3.86 s	C: 3 MeXyl	
Glu (1→4Xyl1)				
1	103.24 d	5.04 d (7.7)	C: 4 Xyl1	H4-Xyl1, H3,5-Glu
2	74.15 d	4.02 m		
3	78.64 d	3.97 m		H1-Glu
4	71.43 d	4.21 m		
5	78.01 d	4.20 m	C: 4 Glc	H1-Glu
6	62.35 t	4.54 brd (11.7)		
		4.33 dd (11.7, 5.5)		

[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.

corresponding anomeric protons at 4.76 d (J = 7.4 Hz), 4.85 d (J = 8.0 Hz), 5.02 d (J = 8.2 Hz), 5.14 d (J = 7.4 Hz), 5.22 d (J = 7.7 Hz), and 5.30 d (J = 8.0 Hz) ppm observed in its ^{1}H NMR spectrum. As before, the couplings indicated a β-configuration of the glycosidic bonds (Table 6).[7]

The NMR spectral data of synallactoside B_1 (**3**) were similar to those of synallactoside A_2 (**2**) and indicated that the terminal 3-*O*-methylxylose of **2** had been substituted for a terminal 3-*O*-methylglucose in **3**. This was easily deduced by the presence in the NMR spectra of **3** of additional carbon and proton resonances at 62.02 ppm (C-6) and at 4.46 (dd, H′-6) and 4.27 (m, H″-6) assigned to position 6 of a terminal 3-*O*-methylglucose. The HMBC cross-peak between H1-MeGlu and C3-Xyl2 and the NOESY correlation between H1-MeGlu and H3-Xyl2 confirmed the attachment of the terminal 3-*O*-methylglucose to H-3 of the second xylose residue. This structural hypothesis was also supported by the comparison of ^{13}C NMR and DEPT spectra of synallactoside B_1 (**3**) to those of synallactoside C (**5**) (Table 3), which showed the presence of additional signals of a terminal 3-*O*-methylxylose in **3** in relation to **5** and allowed establishment of its attachment to C-3 of the glucose residue by glycosidation shifts. Thus, the signal of C-3 of the glucose residue in **3** is shifted downfield (by 8.33 ppm) in relation to that of **5**, while C-2 and C-4 are shifted upfield (by 0.84 and 2.19 ppm). These data, together with the HMBC cross-peak between H1-MeXyl and C3-Glu and the NOESY correlation between H1-MeXyl and H3-Glu, confirmed that the 3-*O*-methylxylose is linked to C-3 of the glucose residue.[7]

Finally, the sequence of the remaining monosaccharide units in the carbohydrate chain of **3** was confirmed by extensive HMBC and NOESY experiments, as shown in Table 6. Therefore, the structure of synallactoside B_1 (**3**) was established as 3β-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl]-23(*S*)-acetoxyholosta-7,25-diene.

The results described in this report constitute the first research on the triterpene glycosides of a sea cucumber belonging to the family Synallactidae (order Aspidochirotida). This novelty is reinforced by the fact that synallactosides A_2 (**2**), B_1 (**3**), B_2 (**4**), and C (**5**) are new natural products. Furthermore, several interesting features are found in their carbohydrate chains. The terminal 3-*O*-methylxylose residue in the sugar chain of glycosides **2**, **3**, and **4** is also found in glycosides of *Eupentacta fraudatrix*[10] and *E. pseudoquinquesemita*[11] (family Sclerodactylidae, order Derndrochirotida) and also in *Pentamera calcigera* (family Phyllophoridae, order Dendrochirotida).[1,2] However, the two terminal 3-*O*-methylxylose residues of the sugar chain of synallactoside A_2 (**2**) had never been found before in glycosides isolated from sea cucumbers. On the other hand, the carbohydrate chains of glycosides **3** and **4** have unprecedented structures among sea cucumber glycosides.[3] More specifically, glycoside **3** is characterized by an unprecedented branched hexaoside chain having two different terminal monosaccharide units (3-*O*-methylxylose and 3-*O*-methylglucose). Additionally, the carbohydrate chain of **4** bears a 3-*O*-methylxylose residue attached to C-3 of the second xylose residue instead of glucose as in the glycosides of *Eupentacta* spp. and *Pentamera calcigera*; an analogous situation is also observed in glycosides **2** and **3**.

Table 5. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Sugar Units of Synallactoside A_2 (**2**)

position	δ_C mult.[a]	δ_H mult. (J in Hz)[c]	HMBC	NOESY
Xyl1 (1→C-3)				
1	105.12 d	4.75 d (7.4)	C: C-3	H-3, H3,5-Xyl1
2	**83.44** d	4.04 m	C: 3 Xyl1, 1Xyl1	H1-Qui1
3	75.64 d	4.22 m	C: 2 Xyl1, 4Xyl1	H1-Xyl1
4	**77.25** d	4.25 m		H1-Glu
5	63.93 t	4.41dd (4.7, 11.6)	C: 3 Xyl1	H1-Xyl1
		3.65 m	C::3 Xyl1	H1-Xyl1
Qui (1→2Xyl1)				
1	105.48 d	5.15 d (7.4)	C: 2 Xyl1	H2-Xyl1, H3,5-Qui
2	76.35 d	4.03 m	C: 1 Qui2	
3	75.35 d	4.09 m	C: 4 Qui, 2 Qui	H1-Qui
4	**85.79** d	3.67 t (8.5)	C: 1 Xyl2, 5Qui, 3 Qui	H1-Xyl2
5	71.64 d	3.78 m		H1-Qui
6	17.85 q	1.75 d (6.0)	C: 4 Qui, 5 Qui	
Xyl2 (1→4Qui)				
1	105.07 d	4.87 d (8.0)	C: 4 Qui	H4-Qui, H3,5-Xyl2
2	73.60 d	3.97 m		
3	**86.54** d	4.12 m	C: 4 Xyl2, 1 MeXyl1	H1-MeXyl1, H1-Xyl2
4	68.65 d	4.02 m	C: 1 MeXyl1, 3 Xyl2	
5	66.53 t	4.20 m	C: 3 Xyl2	
		3.63 m	C: 3 Xyl2	H1-Xyl2
MeXyl1 (1→3Xyl2)				
1	105.89 d	5.26 d (7.7)	C: 3 Xyl2	H3-Xyl2, H3,5-MeXyl1
2	74.71 d	3.94 m	C: 1 MeXyl1, 3 MeXyl1	
3	87.73 d	3.60 t (8.8)	C: 1, 4, MeXyl1, OMe	H1-MeXyl1
4	69.97 d	4.08 m	C: 3 MeXyl1, 5 MeXyl1	
5	66.96 t	4.20 m, 3.63 m	C: 4 MeXyl1, 3 MeXyl1	H1-MeXyl1
OMe	60.58[d]q	3.86[e]s	C: 3 MeXyl1	
Glu (1→4Xyl1)				
1	102.70 d	5.01 d (8.7)	C: 4 Xyl1	H4-Xyl1, H3,5-Glu
2	73.23 d	3.99 m	C: 1 Glu, 3 Glu	
3	**86.97** d	4.18 m	C: 2 Glc, 4 Glu, 1 MeXyl2	H1-Glu, H1-MeXyl2
4	69.23 d	4.09 m	C: 3 Glu	
5	78.24 d	3.93 m		H1-Glu
6	61.92 t	4.46 brd (10.4)		
		4.23 m		
3MeXyl2 (1→3Glc)				
1	106.02 d	5.22 d (7.7)	C: 3 Glu	H3-Glu, H3,5-MeXyl2
2	74.66 d	3.93 m	C: 1 MeXyl2, 3 MeXyl2	
3	87.64 d	3.58 t (8.8)	C: 1, 4, MeXyl2, OMe	H1-MeXyl2
4	69.88 d	4.09 m	C: 3 MeXyl2, 5 MeXyl2	
5	66.94 t	4.20 m, 3.60 m	C: 4 MeXyl2, 3 MeXyl2	H1-MeXyl2
OMe	60.53[d]q	3.85[e]s	C: 3 MeXyl2	

[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,e] Interchangeable positions.

The glycosides of *Synallactes nozawai* have significant similarities to those isolated from sea cucumbers belonging to the family Stichopodidae.[3] Indeed, the aglycon of synallactosides is identical to that of glycosides isolated from members of the genus *Astichopus*, *Stichopus*, and *Thelenota*. Moreover, several structural features of the carbohydrate chain of the glycosides isolated from *S. nozawai* are also found in the glycosides from the family Stichopodidae: the presence of a hexasaccharide branched carbohydrate chain (as in **2** and **3**), the presence of a xylose residue as the third sugar unit, and the absence of sulfate groups. As we have noted above, glycoside **1** had been previously found in *Thelenota ananas*. Taking into consideration that there is a relationship between structure of glycosides and systematic position of the corresponding sea cucumbers,[3] these similarities undoubtedly indicate phylogenetic closeness between the families Synallactidae and Stichopodidae. However, the synallactosides can be distinguished from the stichopodid glycosides by the presence of 3-*O*-methylxylose terminal residues. Furthermore, the odd number of monosaccharide units present in glycosides **4** and **5** have not been found previously in glycosides from Stichopodidae. In our opinion, the structural peculiarities of glycosides of Stichopodidae and Synallactidae possibly arose as the result of parallel and independent evolution of these closely related taxonomical groups of sea cucumbers.

Traditionally, the three families Synallactidae, Stichopodidae, and Holothuriidae are included in the order Aspidochirotida.[12] Some morphological characteristics of the family Synallactidae, such as (1) absence of tentacle ampullae, (2) respiratory trees usually nonconnected with the alimentary canal through a rete mirabile, (3) stone canal attached to (embedded in) mediodorsal mesentery and usually in connection with the body wall, sometimes opening outward through it, (4) gonads on both sides of the mediodorsal mesentery, and (5) absence of Cuvierian organs, indicate that this family may be regarded as the most primitive one in the order. The last three characteristics suggest the existence of a closer phylogenetic relationship between Synallactidae and Stichopodidae than between Synallactidae and Holothuriidae. The structural similarity of the glycosides from these two families confirms that close relationship. Finally, the presence of 3-*O*-methylxylose residues in the glycosides of sinallactids, which were reported only from some species of the more ancient order Dendrochirotida, confirms that Synallactidae is the most primitive family of the Aspidochirotida.

Table 6. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugar Units of Synallactoside B_1 (**3**)

position	δ_C mult.[a,b]	δ_H mult. (J in Hz)[c]	HMBC (C)	NOESY
Xyl1 (1→C-3)				
1	105.12 d	4.76 d (7.4)		H-3, H3,5-Xyl1
2	**83.45** d	4.03 m	C: 1 Xyl1	H1-Qui1
3	75.65 d	4.22 m		H1-Xyl1
4	**77.25** d	4.25 m		H1-Glu
5	63.93 t	4.41dd (5.5, 11.5)	C: 1 Xyl1	H1-Xyl1
		3.63 m		H1-Xyl1
Qui (1→2Xyl1)				
1	105.49 d	5.14 d (7.4)	C: 2 Xyl1	H2-Xyl1, H3,5-Qui
2	76.35 d	4.05 m	C: 3 Qui	
3	75.34 d	4.09 m	C: 2 Qui, 4 Qui	H1-Qui
4	**85.76** d	3.66	C: 3 Qui	H1-Xyl2
5	71.64 d	3.77 m		H1-Qui
6	17.87 q	1.74 d (5.8)	C: 4 Qui, 5 Qui	
Xyl2 (1→4Qui)				
1	105.04 d	4.85 d (8.0)	C: 4 Qui	H4-Qui, H3,5-Xyl2
2	73.24 d	3.98 m		
3	**87.31** d	4.14 m		H1-MeGlc, H1-Xyl2
4	68.93 d	4.03 m		
5	66.37 t	4.20 m		
		3.62 m		H1-Xyl2
MeGlu (1→3Xyl2)				
1	105.31 d	5.30 d (8.0)	C: 3 Xyl2	H3-Xyl2, H3,5-MeGlu
2	74.93 d	3.98 m	C: 3 MeGlu	
3	87.88 d	3.71 t (8.7)	C: 2 MeGlu, OMe, 4 MeGlc	H1-MeGlu
4	70.43 d	4.13 t (8.7)	C: 3 MeGlu	
5	78.16 d	3.95		H1-MeGlu
6	62.02 t	4.46 dd (4.9, 10.8)		
		4.27 m		
Ome	60.62 q	3.86 s	C: 3 MeGlu	
Glu (1→4Xyl1)				
1	102.70 d	5.02 d (8.2)	C: 4 Xyl1	H4-Xyl1, H3,5-Glu
2	73.30 d	3.97 m	C: 1 Glu,3 Glu	
3	**86.96** d	4.18 m	C: 4 Glu	H1-Glu, H1-MeXyl
4	69.23 d	4.05 m	C: 3 Glu	
5	78.25 d	3.94 m		H1-Glu
6	61.92 t	4.45 brd (10.4)		
		4.27 m		
3MeXyl (1→3Glc)				
1	106.03 d	5.22 d (7.7)	C: 3 Glu	H3-Glu, H3,5-MeXyl1
2	74.71 d	3.93 m	C: 1 MeXyl	
3	87.65 d	3.60 t (8.8)	C: 2 MeXyl, Ome	H1-MeXyl1
4	69.89 d	4.09 m	C: 3 MeXyl, 5 MeXyl	
5	66.94 t	4.23 m, 3.63 m	C: 4 MeXyl, 3 MeXyl	H1-MeXyl1
OMe	60.54q	3.85 s	C: 3 MeXyl	

[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.

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. ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AMX 500 at 500.12 MHz for proton and 125.67 MHz for carbon in C_5D_5N with TMS as an internal reference ($\delta = 0$). The HRFABMS (positive-ion mode) were recorded using a Micromass model Autospec-M apparatus, on a glycerol/thyoglycerol matrix. HPLC was performed using a Dupont-8800 chromatograph equipped with a RIDK-102 differential refractometer (Czechoslovakia) on a Silasorb C_{18} column (3×150 mm), EtOH$-H_2O$ (55:45), 0.3 mL/min. GC analysis was carried out on a Tsvet-110 apparatus, using a 0.3×150 cm glass column with 1.5% QF-1 as stationary phase and the following experimental conditions: Ar as carrier gas, 60 mL/min, column temperarute 110 → 225° (5°/min). For GC−MS we used a LKB 9000S apparatus and a column (0.3×300 cm) with 1.5% QF-1 using a He as carrier gas (50 mL/min). The conditions selected for analysis were as follows: injection port 275°, molecular separator 265°, ion source 255°, column 110 → 210°, 4°/min, ionizing voltage 70 eV.

Animal Material. Specimens of *Synallactes nozawai* were collected by Drs. Y. M. Jakovlev and V. V. Gulbin at a depth of 540 m by an industrial fishing bottom trawl in the Sea of Japan (42−30′6″ N 133°39′57′ E) in May 1996 during a scientific expedition of the Institute of Marine Biology of the Far East Division of the Russian Academy of Sciences on r/v “Professor Kaganovsky” and kept in ethanol at room temperature. The sea cucumber was identified by Dr. A. V. Smirnov, and a voucher specimen, under reference 1996-1, is on deposit at the Zoological Institute, the Russian Academy of Sciences, St. Petersburg, Russia.

Extraction and Isolation. The sea cucumbers (302 g dried residue) were cut into pieces and extracted twice with refluxing ethanol. The combined extracts were concentrated, and the residue was dissolved in water. Desalting was carried out by passing this fraction through a Polychrom-1 column (powdered Teflon; Biolar, Latvia), eluting first the inorganic salts and polar impurities with H_2O and then the crude glycoside fraction with 50% acetone. The glycoside fraction was further chromatographed on Si gel eluting first with $CHCl_3$−EtOH (6:1) to give a fraction containing synallactoside A_1 (29 mg), then with $CHCl_3$−EtOH (5:1) to give a fraction containing A_2 (46 mg), with $CHCl_3$−EtOH (4:1) to give a fraction containing synallactosides B_1, B_2 (40 mg), and finally with $CHCl_3$−EtOH

(3:1) to give a fraction containing synallactoside C (52 mg). The fractions containing the synallactosides A_1 and A_2 were purified by chromatography on a DEAE-Sephadex column eluting with 55% ethanol, affording pure synallactosides A_1 (26 mg) and A_2 (42 mg). The other fractions were submitted to HPLC to give 5 mg of pure synallactoside A_1 (**1**), 17 mg of pure synallactoside A_2 (**2**), 11 mg of pure synallactoside B_1 (**3**), 10 mg of pure synallactoside B_2 (**4**), and 22 mg of pure synallactoside C (**5**).

Synallactoside A_1 (1): mp 220−222 °C; $[\alpha]^{20}_D$ −54° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 2; HRFABMS (positive-ion mode) *m*/*z* 1121.5518 $[M + Na]^+$ (calcd for $C_{55}H_{86}O_{22}Na$ 1121.5508).

Synallactoside A_2 (2): mp 184−186 °C; $[\alpha]^{20}_D$ −52° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 5; HRFABMS (positive-ion mode) *m*/*z* 1399.6568 $[M + Na]^+$ (calcd for $C_{66}H_{104}O_{30}Na$ 1399.6510).

Synallactoside B_1 (3): mp 177−179 °C; $[\alpha]^{20}_D$ −39° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 6; HRFABMS (positive-ion mode) *m*/*z* 1429.6592 $[M + Na]^+$ (calcd for $C_{66}H_{104}O_{30}Na$ 1429.6516).

Synallactoside B_2 (4): mp 196−197 °C; $[\alpha]^{20}_D$ −40°(*c* 0.1, pyridine); ^{13}C and ^{1}H MNR, see Tables 1 and 4; HRFABMS (positive-ion mode) *m*/*z* 1253.5932 $[M + Na]^+$ (calcd for $C_{60}H_{94}O_{26}Na$ 1253.5931).

Synallactoside C (5): mp 190−192 °C; $[\alpha]^{20}_D$ −35° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 3; HRFABMS (positive-ion mode) *m*/*z* 1283.6054 $[M + Na]^+$ (calcd for $C_{61}H_{96}O_{27}Na$ 1283.6037).

Sugar Analysis of Synallactoside A_1 (1). Compound **1** (5 mg) in 1 mL of 2 N HCl was heated at reflux for 2 h. Subsequently, 5 mL of H_2O was added to the mixture and the aglycon was 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 dry residue, and the mixture was heated at 100° for 1 h. Then, 1 mL of Ac_2O was added and the mixture was heated at 100° for 1 h. The solution was concentrated, the resulting aldononitrile peracetates were analyzed by GLC-MS, and xylose, quinovose, and 3-*O*-methylglucose (2:1:1 ratio) were identified. All the sugars in this and all other glycosides were related to the D-series by analogy with other sea cucumber glycosides.

Sugar Analysis of Synallactoside C (5). Compound **1** (5 mg) was treated as described above. Xylose, glucose, quinovose, and 3-*O*-methylglucose (2:1:1:1 ratio) were identified.

Acknowledgment. The authors wish to acknowledge Y. M. Jakovlev and V. V. Gulbin for the animal material. V.I.K. thanks the Science Support Foundation for the Grant for Talented Young Researchers. This work was partially supported by the Science Support Foundation and the Russian Foundation for Basic Researches (RFBR 00-15-97806, RFBR 01-04-96907, and 01-03-96903). C.J. acknowledges financial support from Xunta de Galicia (PGIDT00MAR10301PN) and from CICYT (MAR99-0287).

References and Notes

(1) Avilov, S. A.; Antonov, A. S.; Drozdova, O. A.; Kalinin, V. I.; Kalinovsky, A. I.; Riguera, R.; Lenis, L. A.; Jiménez, C. *J. Nat. Prod.* **2000**, *63*, 1349−1355.
(2) Avilov, S. A.; Antonov, A. S.; Drozdova, O. A.; Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A.; Riguera, R.; Lenis, L.; Jiménez C. *J. Nat. Prod.* **2000**, *63*, 65−71.
(3) Stonik, V. A.; Kalinin, V. I.; Avilov, S. A. *J. Nat. Toxins* **1999**, *8*, 235−248.
(4) Stonik, V. A.; Maltsev, I. I.; Kalinovsky, A. I.; Konde, K.; Elyakov, G. B. *Khim. Prirod. Soedin.* **1982**, 194−199.
(5) Kalinivsky, A. I.; Maltsev, I. I.; Antonov, A. S.; Stonik, V. A. *Bioorg. Khim.* **1984**, *10*, 1655−1663.
(6) Kitagawa, I.; Kobayashi, M.; Inamoto, T.; Yosuzawa, T.; Kyogoku, Y. *Chem. Pharm. Bull.* **1981**, *29*, 2387−2391.
(7) Shashkov, A. S.; Chizhov, O. S. *Bioorg. Khim.* **1976**, *2*, 437−497.
(8) Stonik, V. A.; Maltsev, I. I.; Elyakov, G. B. *Khim. Prirod. Soedin.* **1982**, 624−627.
(9) Avilov, S. A.; Stonik, V. A. *Khim. Prirod. Soedin.* **1988**, 764−765.
(10) Afiyatullov, S. S.; Tishchenko, L. Y.; Stonik, V. A.; Kalinovsky, A. I.; Elyakov, G. B. *Khim. Prirod. Soedin.* **1985**, 244−248.
(11) Kalinin, V. I.; Afiyatullov, S. S.; Kalinovsky, A. I. *Khim. Prirod. Soedin.* **1988**, 221−225.
(12) Smirnov, A. V. *Zool. Zhurn.* **1984**, *63*, 547−553.

NP0202881