Triterpene Glycosides from the Far-Eastern Sea Cucumber *Pentamera calcigera*. 1. Monosulfated Glycosides and Cytotoxicity of Their Unsulfated Derivatives

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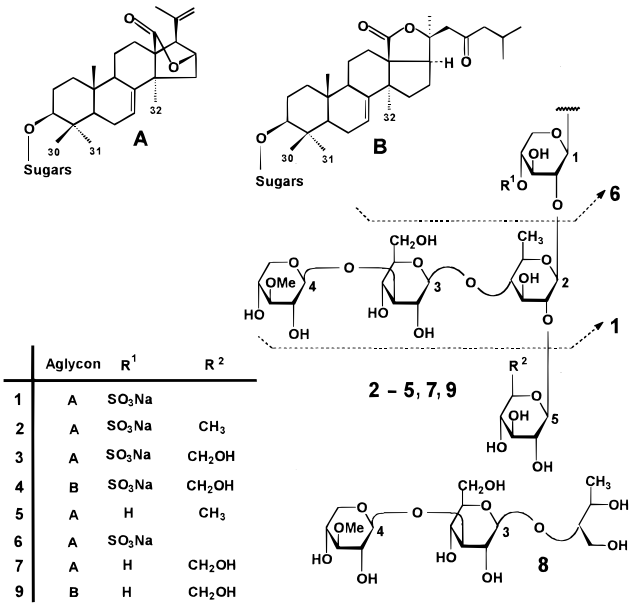
Three new monosulfated triterpene glycosides, calcigerosides B (**2**), C_1 (**3**), and C_2 (**4**), along with the known cucumarioside G_2 (**1**), have been isolated from the sea cucumber *Pentamera calcigera*. Their structures have been deduced from extensive spectral analysis (NMR and MS) and chemical evidence. Compounds **2**−**4** present a novel pentasaccharide chain never reported before in sea cucumber triterpene glycosides. The desulfated derivatives of calcigerosides B, C_1, and C_2 (**5**, **7**, and **9**, respectively) showed moderate cytotoxicity (IC_{50} = 5 μg/mL) against a selection of four human and mouse tumor cell lines.

As a continuation of our studies on the triterpene glycosides from the holothurians belonging to the order Dendrochirotida,[1−4] we have turned our attention to the glycoside content of the Far Eastern sea cucumber *Pentamera calcigera* (Stimpson) (Thyoninae, Phyllophoridae), collected by trawling on the 18th scientific cruise of the r/v *Akademik Oparin* in the southwestern area of the Peter the Great Gulf. In this paper we report the isolation of four monosulfated glycosides: calcigerosides B (**2**), C_1 (**3**), and C_2 (**4**), along with the known cucumarioside G_2 (**1**).



	Aglycon	R^1	R^2
1	A	SO_3Na	
2	A	SO_3Na	CH_3
3	A	SO_3Na	CH_2OH
4	B	SO_3Na	CH_2OH
5	A	H	CH_3
6	A	SO_3Na	
7	A	H	CH_2OH
9	B	H	CH_2OH

Results and Discussion

The ethanolic extract of *Pentamera calcigera* (425 g dry wt) was sequentially submitted to column chromatography on Polychrom-1 (powdered Teflon) and Si gel. Final separation and isolation was achieved by reversed-phase HPLC on Silasorb C_{18} to give cucumarioside G_2 (**1**), calcigeroside B (**2**), calcigeroside C_1 (**3**), and calcigeroside C_2 (**4**).

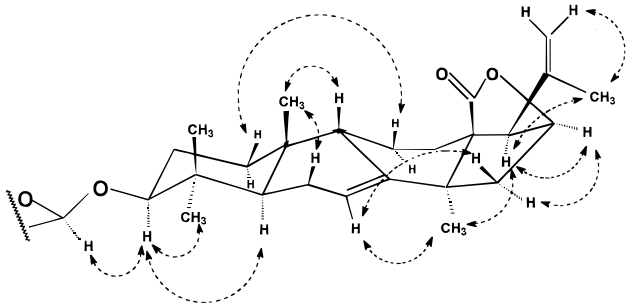


Figure 1. NOESY correlations of the aglycon moieties of the cucumarioside G_2 (**1**) and calcigerosides B (**2**) and C_1 (**3**).

The presence of 3-*O*-methylxylose, xylose, quinovose, and glucose in a 1:1:1:1 ratio in glycoside **1** was deduced by acid hydrolysis with aqueous 1N trifluoroacetic acid followed by GC−MS analysis of the corresponding aldononitrile peracetates. The NMR spectral data and all of the physical constants of this compound were found to be identical in all respects to the known cucumarioside G_2 (**1**), a compound isolated earlier as a minor glycoside from the sea cucumber *Eupentacta fraudatrix*.[5] Thus, compound **1** was identified as the known 18(16)-lactone-23,24,25,26,27-pentanor-3-*O*-[3-*O*-methyl-β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl]-lanosta-7,20(22)-dien-3β-ol (cucumarioside G_2).

The aglycon part of calcigeroside B (**2**) was found to be the same as that of cucumarioside G_2 (**1**) as deduced by extensive NMR spectroscopy (^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC) (Table 1). Thus, the structure of the aglycon moiety was identified as 23,24,25,26,27-pentanor-lanosta-7,20(22)-dien-3β-ol (posietogenin)[5] and its relative stereochemistry established by NOESY experiments as shown in Figure 1.

Acid hydrolysis of calcigeroside B (**2**) gave D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-xylose (1:2:1:1), identified by GC−MS as their aldononitrile peracetates. The presence of five monosaccharide units in **2** was deduced

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Table 1. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Aglycon Moiety of Calcigeroside B (**2**)

position	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	HMBC (C)	NOESY (H)
1	35.63 t	1.31 m		11
2	26.59 t	1.87 m	4	
3	88.73 d	3.16 dd (11.5, 3.4)	30, 31, 1Xyl	5, 31, H1 Xyl
4	39.21 s			
5	47.22 d	0.82 br d (12.2)	4, 19	3
6	23.06 t	1.93 m		19
7	122.43 d	5.56 d (6.5)	9, 14	15, 32
8	147.26 s			
9	46.26 d	2.80 br d (13.4)		19
10	35.25 s			
11	21.59 t	1.97 m, 1.39 m	10	1
12	19.87 t	2.25 m, 1.84 m	9, 13, 14	
13	56.72 s			
14	46.00 s			
15	43.55 t	2.10 d (13.6), 1.99 d (13.7)	8, 14, 16, 17, 32	7, 16
16	80.96 d	4.78 s	13	15, 17
17	59.05 d	2.95 s	13, 14, 18, 20, 21, 22	16, 21, 32
18	181.87 s			
19	23.70 q	0.85 s	1, 5, 9, 10	6, 9
20	139.90 s			
21	23.01 q	1.72 s	17, 20, 22	17, 22
22	113.76 t	4.94 s, 4.88 s	17, 21	
30	17.14 q	0.96 s	3, 4, 5, 31	
31	28.52 q	1.15 s	3, 4, 5, 30	3
32	33.93 q	1.33 s	8, 13, 14, 15	7, 17

[a] Recorded at 125 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).

by the presence of five anomeric carbons, between 102.3 and 105.2 ppm in the ^{13}C NMR spectrum, and five doublets, due to the anomeric protons, at δ_H 4.71 ($J = 6.9$ Hz), 4.85 ($J = 7.8$ Hz), 5.03 ($J = 7.7$ Hz), 5.11 ($J = 7.8$ Hz), and 5.13 ppm ($J = 8.7$ Hz) in the ^{1}H NMR spectrum (Table 2). The *J* values for the anomeric protons indicate a *β*-configuration of all the glycoside bonds.[6]

The pseudomolecular ions at *m/z* 1195 [$M_{Na} - Na$] in APIES−MS (negative ion mode) and at *m/z* 1241 [$M_{Na} + Na$] in FABMS (positive ion mode), corresponding to the molecular formula $C_{54}H_{83}O_{27}SNa$ for calcigeroside B (**2**), and the fragment ion at *m/z* 1139 [$M_{Na} - SO_3Na + H + Na$] in the FABMS (positive ion mode) indicate that this compound is a monosulfated pentaoside. The presence of a sulfate group in the glycoside was confirmed by solvolytic desulfation of calcigeroside B (**2**) to give the desulfated derivative **5**, and by the positive test of the acid hydrolysis products from glycoside **2** with $BaCl_2$.

The location of the sulfate group on the xylose residue was deduced from the comparison of the monossacharide analysis of the products obtained by periodate oxidation of calcigeroside B (**2**) and its desulfated derivative (**5**). Thus, while periodate oxidation of **2** led to degradation of one quinovose residue, the same treatment on its desulfated derivative (**5**) led to degradation of both xylose and quinovose. This was confirmed by FABMS studies of **2**. Its FABMS (positive ion mode) showed peaks at *m/z* 623 [(Agl−O−Xyl−SO_3Na) − H + Na] and 639 [(aglycon−O−Xyl−SO_3Na−O) − H + Na], and its APIES−MS (negative ion mode) (Figure 2) displayed ions at *m/z* 596 [(Agl−O−Xyl−SO_3Na−O) + 2H − Na]. The position of the sulfate group at C-4 of the first xylose residue was deduced by the comparative study of the ^{13}C NMR spectra of the carbohydrate parts of calcigeroside B (**2**) (Table 2) and its desulfated derivative (**5**). Indeed, the signal due to C-4 of xylose in glycoside **2** was shifted downfield by 6.4 ppm and those due to C-3 and C-5 were shifted to higher field by 2.02 and 1.78 ppm, respectively, due to the α- and *β*-effects of sulfate groups[6] in relation to those of desulfated derivative **5**.

The sequence of monosaccharide units in calcigeroside B (**2**) was determined by careful analysis of the data

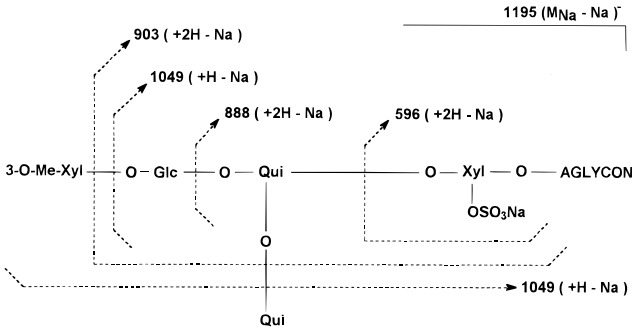

Figure 2. Fragmentation of calcigeroside B (**2**) in the APIES−MS (negative ion mode).

obtained by NMR (^{13}C, HMBC, $^{1}H-^{1}H$ COSY) (Table 2) and by FABMS and APIES−MS (Figure 2). The double degradation of calcigeroside B (**2**) by the method of Smith proved to be very useful to confirm the proposed sequence. The first degradation of the carbohydrate chain of glycoside **2** led to a compound that contained a sulfated xylose, quinovose, glucose, and 3-*O*-methylxylose in a 1:1:1:1 ratio, indicating the oxidation of one quinovose unit. Its NMR data were identical to those of cucumarioside G_2 (**1**). Degradation of cucumarioside G_2 (**1**) led to sulfated xyloside **6** (confirmed by solvolytic desulfation and monosaccharide analysis) and bioside **8**.

On the basis of the data discussed above, the structure of calcigeroside B (**2**) was determined as 18(16)-lactone-23,24,25,26,27-pentanor-3-*O*-{3-*O*-methyl-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-quinovopyranosyl-(1→2)]-*β*-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-*β*-D-xylopyranosyl}-lanosta-7,20(22)-dien-3*β*-ol.

The molecular formula of **3** was deduced to be $C_{54}H_{83}O_{28}SNa$ on the basis of pseudomolecular ions at *m/z* 1211 [$M_{Na} - Na$] in the FABMS (negative ion mode) and *m/z* 1257 [$M_{Na} + Na$] in the FABMS (positive ion mode). The structure of the aglycon of **3** was determined to be 18(16)-lactone-23,24,25,26,27-pentanor-lanosta-7,20(22)-dien-3*β*-ol[5] by analysis of its spectroscopic data and comparison with those of **2** and cucumarioside G_2 (**1**).

Table 2. ^{13}C NMR Chemical Shifts for the Sugar Moieties of Calcigerosides B (**2**) and Its Desulfated Derivative (**5**) and ^{1}H NMR Chemical Shifts, HMBC, and NOESY Correlations for the Sugar Moieties of Calcigeroside B (**2**)

	2	**5**		**2**	
position	δ_C mult[a,b]	δ_C mult[a,b]	δ_H mult[c] (J in Hz)	HMBC	NOESY
Xyl(1→C-3)					
1	104.52 d	104.64 d	4.71 d (6.9)	3	H-3
2	*82.06* d	*83.04* d	3.91 m	1 and 3 Xyl, 1 Qui_1	
3	74.91 d	76.93 d	4.26 t (9.0)	2 and 4 Xyl	
4	**75.87** d	69.47 d	4.97 m		
5	64.09 t	65.87 t	4.80 m, 3.83 m	1 Xyl	
Qui_1(1→2Xyl)					
1	102.37 d	102.97 d	5.13 d (8.7)	2 Xyl	H2 Qui_1
2	*83.43* d	*83.41* d	3.88 m	1 Qui_1	H1 Qui_1
3	75.07 d	75.07 d	3.99 m		
4	*85.65* d	*85.54* d	3.55 m		H1 Glu
5	70.90 d	70.99 d	3.57 m	1, 4 and 6 Qui_1	H6 Qui_1
6	17.83 q[d]	17.87 q	1.58 d (5.4)	4 and 5 Qui_1	H5 Qui_1
Glu(1→4 Qui_1)					
1	103.89 d	103.88 d	4.85 d (7.8)	4 Qui_1	H2 Glu
					H4 Qui_1
2	73.53 d	73.54 d	3.91 m	1 and 3 Glu	H1 Glu
3	*86.46* d	*86.48* d	4.11 m	2 and 4 Glu	
	69.03 d	69.04 d	3.82 m	3 and 5 Glu	
	77.07 d	77.07 d	3.88 m	6 Glu	H6 Glu
	61.46 t	61.49 t	4.31 br d (10.9), 3.99 m		
MeXyl(1→3Glu)					
1	105.14 d	105.13 d	5.11 d (7.8)	3 Glu	H2 MeXyl
					H3 Glu
2	74.12 d	74.11 d	3.79 m		H1 MeXyl
3	86.56 d	86.55 d	3.57 m	2 MeXyl, OMe	
4	69.59 d	69.59 d	3.95 m	3 and 5 MeXyl	
5	66.26 t	66.26 t	4.13 m, 3.63 m	1 MeXyl	
OMe	60.56 q	60.58 q	3.79 s	2, 3 and 4 MeXyl	
Qui_2(1→2Qui_1)					
1	105.21 d	105.23 d	5.03 d (7.7)	2 Qui_1	H2 Qui_1
2	75.75 d	75.83 d	3.88 m	1 Qui_2	H1 Qui_2
3	76.40 d	76.24 d	3.98 m		
4	75.55 d	75.58 d	3.55 m		
5	73.30 d	73.36 d	3.65 m	6 Qui_2	H6 Qui_2
6	17.92 q[d]	17.87 t	1.55 d (5.9)	4 and 5 Qui_2	H5 Qui_2

[a] Recorded at 125 MHz in $C_5D_5N-D_2O$ (4:1); multiplicity by DEPT. [b] Italics = interglycosidic positions, bold = sulfate positions. [c] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1). [d] Interchangeable.

NMR analysis of the sugar part of **3** showed the presence of five monosaccharide units. Five anomeric carbon signals were observed in the ^{13}C NMR spectrum at δ_C 102.52, 104.31, 104.56, 105.61, and 105.79 ppm. In addition, five doublet signals due to anomeric protons with a β-configuration of the glycosidic bonds[6] were found in the ^{1}H NMR spectrum at δ_H 4.79 (J = 7.0 Hz), 4.88 (J = 7.8 Hz), 5.16 (J = 7.4 Hz), 5.16 (J = 7.4 Hz), and 5.17 (J = 6.8 Hz) ppm (Table 3). Monosaccharide analysis of **3** allowed us to identify those units as D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-xylose in a 1:1:2:1 ratio.

The presence of a sulfate group in **3** was first suggested by the ion at m/z 1155 [$M_{Na} - SO_3Na + H + Na$] observed in the FABMS (positive ion mode) and later demonstrated by its solvolytic desulfation to **7** and the positive test with $BaCl_2$ of the hydrolyzate. The location of the sulfate group in the first xylose residue of **3** was deduced, as in **2**, from periodate oxidation of **3** and **7** and by FABMS (positive ion mode) studies. Treatment of **3** with $NaIO_4$, subsequent hydrolysis, and monossacharide analysis showed that the glucose residue had been destroyed. Similar treatment of the desulfated derivative **7** led to the degradation of the glucose and xylose residues. Furthermore, the FABMS (positive ion mode) of glycoside **3** showed fragments at m/z 639 [(Agl−O−Xyl−SO_3Na−O) − H + Na], and the FABMS (negative ion mode) (Figure 3) showed fragments, at m/z 577 [(Agl−O−Xyl−SO_3Na) − H − Na] and 595 [(Agl−O−Xyl−SO_3Na−O) + H − Na].

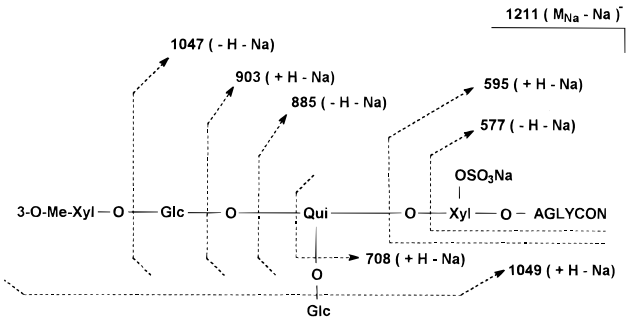

Figure 3. Fragmentation of calcigeroside C_1 (**3**) in the negative FABMS (negative ion mode).

The attachment of a sulfate group at C-4 of the first xylose residue in glycoside **3** was deduced by comparison of ^{13}C NMR and DEPT spectra of carbohydrate chains of calcigeroside C_1 (**3**) with those of its desulfated derivative (**7**) (Table 3). Indeed, the signal due to C-4 of the xylose unit of glycoside **3** was shifted downfield by 5.84 ppm, and those of C-3 and C-5 were shifted higher field by 1.42 and 1.65 ppm, respectively, in relation to those of **7** due to the α- and β-effects of the sulfate groups.[6]

The sequence of monosaccharide units in glycoside **3** was determined by extensive spectroscopic analysis using ^{1}H and ^{13}C NMR, DEPT, HMQC, HMBC, $^{1}H-^{1}H$ COSY (Table 2), and FABMS (Figure 3). The double degradation of **3** by the Smith method confirmed the suggested sequence. Thus, the first degradation of **3** led to a compound whose NMR

Table 3. ^{13}C NMR Chemical Shifts for the Sugars Moieties of Cucumarioside G_2 (**1**), Calcigerosides C_1 (**3**) and C_2 (**4**), and Their Desulfated Derivatives **7** and **9**[a,b]

carbon	**1**	**3**	**4**	**7**	**9**
Xyl(1→C-3)					
1	105.3 d	104.56 d	104.62 d	104.66 d	104.51 d
2	*82.9* d	*82.17* d	*82.80* d	*82.83* d	*82.74* d
3	75.8 d	75.02 d	74.88 d	76.44 d	76.21 d
4	**75.8** d	**75.58** d	**75.36** d	69.74 d	69.44 d
5	64.5 t	64.21 t	64.28 t	65.86 t	65.71 t
Qui(1→2Xyl)					
1	105.3 d	102.52 d	102.86 d	102.85 d	102.65 d
2	76.6 d	83.68 d	83.98 d	83.52 d	83.36 d
3	76.4 d	74.35 d	74.41 d	74.30 d	74.84 d
4	*86.9* d	*86.02* d	*86.05* d	*85.69* d	*85.62* d
5	71.9 d	70.87 d	70.92 d	71.00 d	70.60 d
6	19.1 q	17.85 q	17.87 q	17.84 q	17.50 q
Glu_1(1→4Qui)					
1	104.9 d	104.31 d	104.46 d	104.14 d	104.03 d
2	74.2 d	73.61 d	73.63 d	73.60 d	73.27 d
3	*87.6* d	*86.83* d	*86.89* d	*86.79* d	*86.51* d
4	70.2 d	69.78 d	69.82 d	70.50 d	70.26 d
5	77.9 d	77.54 d	77.65 d	78.61 d	78.52 d
6	62.4 t	61.70 t	61.76 t	61.93 t	61.73 t
MeXyl(1→3Glu_1)					
1	105.9 d	105.79 d	106.02 d	105.47 d	105.36 d
2	74.7 d	75.07 d	75.05 d	75.16 d	74.05 d
3	87.4 d	87.20 d	87.42 d	87.01 d	86.99 d
4	69.8 d	69.18 d	69.23 d	69.68 d	69.40 d
5	67.1 t	66.69 t	66.82 t	66.56 t	66.41 t
OMe	60.8 q	60.54 q	60.54 q	60.56 q	60.18 q
Glu_2(1→2Qui)					
1		105.61 d	105.77 d	105.63 d	105.54 d
2		75.98 d	76.10 d	75.91 d	75.83 d
3		76.96 d	76.96 d	76.85 d	76.61 d
4		70.42 d	70.21 d	69.15 d	68.84 d
5		78.34 d	78.40 d	77.45 d	77.29 d
6		61.57 t	61.31 t	61.66 t	61.40 t

[a] Recorded at 125 MHz in $C_5D_5N-D_2O$ (4:1). [b] Italics = interglycosidic positions, bold = sulfate positions.

data were identical to those of cucumarioside G_2 (**1**). Subsequent degradation of **1** by the same method yielded the sulfated xyloside **6** and bioside **8**.

On the basis of the data discussed above, the structure of calcigeroside C_1 (**3**) was determined as 18(16)-lactone-23,24,25,26,27-pentanor-3-*O*-{3-*O*-methyl-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-*β*-D-xylopyranosyl}-lanosta-7,20(22)-dien-3*β*-ol.

The molecular formula of compound **4** was established by FABMS (positive ion mode). The structure of the aglycon moiety of **4** was deduced by its spectroscopic data and by comparison of these data with those of known terpenes. The ^{13}C NMR spectrum indicated the presence of a 7(8)-double bond (signals at δ_C 119.78 and 146.54 ppm), an 18-(20)-lactone (C-18 and C-20 signals at δ_C 179.82 and 82.50 ppm, respectively), a saturated side chain bearing a keto group (six signals due to saturated carbons and the signal due to C-23 at δ_C 207.46 ppm), and the signal due to C-3 connected with the carbohydrate chain (signal at δ_C 88.86 ppm). On the other hand, the ^1H NMR spectrum showed an isolated AB system at C-22 due to the presence of doublet signals for H-22 and H-22′, at δ_H 3.03 (J = 18.2 Hz) and 3.10 (J = 18.2 Hz) ppm, respectively, (Table 5), and a nine-spin proton system at C-24−C-27, correlated by $^1H-^1H$ COSY. Comparison of these carbon and proton resonances with the literature data for other saponins showed that the aglycon of **4** is holosta-7-one-23-en-3*β*-ol (synaptogenin), a compound identified earlier in glycosides from *Synapta maculata*[7] and *Cucumaria echinata*.[8] HMBC and HMQC experiments on **4** confirmed the proposed

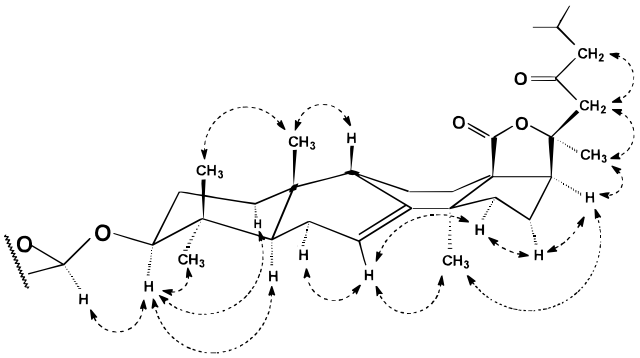

Figure 4. Proton correlations in NOESY for aglycon parts of the calcigeroside C_2 (**4**).

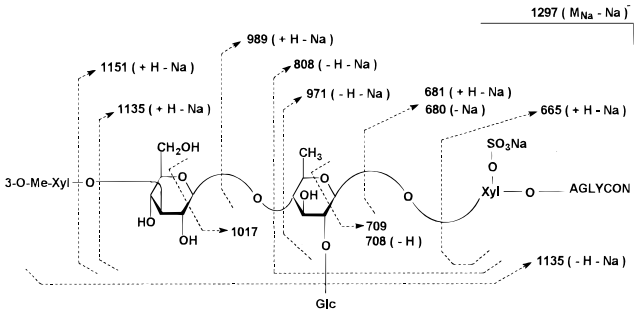

Figure 5. Fragmentation of calcigerosiede C_2 (**4**) in the negative FABMS (negative ion mode).

structure. The NOESY spectrum of calcigeroside C_2 (**4**) (Figure 4) was very useful for the elucidation of the stereochemical peculiarities of the aglycon in the glycoside.

Acid hydrolysis of the calcigeroside C_2 (**4**) gave D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-xylose in a 1:1:2:1 ratio. Solvolytic desulfation of calcigeroside C_2 (**4**) gave the desulfated derivative **9**. Comparison of the ^{13}C NMR and DEPT spectra of the carbohydrate part of **4** and its desulfated derivative (**7**) with those of **3** and **9**, respectively, suggests that they have an identical carbohydrate chain (Table 4). The sequence of monosaccharide units was confirmed by FABMS (Figure 5).

Thus, the structure of calcigeroside C_2 (**4**) was determined as 3-*O*-{3-*O*-methyl-*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-quinovosyl-(1→2)-4-*O*-sodium sulfate-*β*-D-xylopyranosyl}-3*β*-hydroxy-holosta-7-en-23-one. The cation associated with the sulfate groups of all glycosides described was identified as Na^+ by atomic absorption spectroscopy.

This work represents the first study of the glycosidic content of a sea cucumber belonging to the genera *Pentamera* and subfamily Thyoninae (family Phyllophoridae, order Dendrochirotida). Calcigerosides B (**2**), C_1 (**3**), and C_2 (**4**) are new triterpene glycosides. The majority of known sea cucumber glycosides (more than 70 examples are known) are holostane derivatives characterized by an 18-(20)-lactone. Recently, seven “nonholostane” glycosides have been isolated that do not contain a lactone or, alternatively, contain an 18(16)-lactone.[4] Calcigerosides B (**2**) and C_1 (**3**) are “nonholostane” glycosides and present a posietogenin aglycon characterized by an 18(16)-lactone and a shortened side chain. Many sea cucumber glycosides having a pentasaccharide carbohydrate chain branched at the second monosaccharide unit (quinovose) are known. In all cases xylose is the terminal unit attached to C-2 of quinovose. Calcigerosides **2**−**4** constitute the first examples of a new sugar chain where xylose is not the terminal unit: calcigeroside B (**2**) has quinovose in that position,

Table 4. ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugars Moieties of Calcigerosides C_1 (**3**) and C_2 (**4**)[a]

	3			**4**		
proton	δ mult (J in Hz)	HMBC (C)	NOESY	δ mult (J in Hz)	HMBC (C)	NOESY
Xyl(1→C-3)						
1	4.79 d (7.2)	3, 5 Xyl	H3 H2 Xyl	4.83 d (6.7)	3	H3
2	3.95 m	1 Xyl and 1 Qui	H1 Qui H1 Xyl	3.91 m		H1 Qui
3	4.48 m	2 and 4 Xyl		4.47 m		
4	5.07 m			5.09 m		
5	4.76 m, 3.87 m	1 Xyl		4.77 dd	1 Xyl	
Qui(1→2Xyl)						
1	5.17 m (6.8)	2 Xyl, 2 and 5 Qui	H2 Xyl H2 Qui	5.11 m (7.6)	2 Xyl	H2 Xyl
2	4.02 m	1 Glu_2, 1 and 4 Qui	H1 Glu_2 H1 Qui	4.00 m	1 Qui	H1 Qui
3	3.87 m	2 Qui		3.91 m		
4	3.54 m		H1 Glu_1	3.54 m		H1 Glu_1
5	3.56 m	3 Qui	H6 Qui	3.62 m		
6	1.60 d (4.7)	4 and 5 Qui	H5 Qui	1.65 d (5.6)	4 and 5 Qui	
Glu_1(1→4Qui)						
1	4.88 d (7.8)	4 Qui	H2 Glu_1 H4 Qui	4.92 d (7.8)	4 Qui	H4 Qui
2	3.95 m	1 and 3 Glu_1	H1 Glu_1	3.97 m		
3	4.14 m	1 and 4 Glu_1	H1 MeXyl	4.19 m		H1 MeXyl
4	4.02 m			4.08 m		
5	3.95 m		H6 Glu_1	3.98 m		
6	4.46 m, 4.12 m		H5 Glu_1	4.47 m, 4.16 m		
MeXyl(1→3Glu_1)						
1	5.16 d (7.4)	3 Glu_1	H3 Glu_1 H2 MeXyl	5.19 m	3 Glu_1	H3 Glu_1
2	3.98 m	1 MeXyl	H1 MeXyl	3.90 m		
3	3.56 d	OMe, 2 and 4 MeXyl		3.56 m		
4	3.95 m			3.97 m		
5	4.16 m, 3.60 m	3 and 4 MeXyl		4.20 m	3 and 4 MeXyl	
OMe	3.81 s	2 and 3 MeXyl		3.83 s	3 MeXyl	
Glu_2(1→2Qui)						
1	5.16 d (7.4)	2 Qui	H2 Xyl H2 Glu_2	5.19 m	2 Qui	H2 Qui
2	3.98 m	1 Glu_2	H1 Glu_2	3.92 m		
3	4.09 m			4.12 m		
4	4.09 m			4.10 m		
5	3.81 m		H6 Glu_2	3.80 m		
6	4.46 m, 4.26 m		H5 Glu_2	4.47 m, 4.31 m		

[a] Recorded at 500 MHz in $C_5D_5N-D_2O$ (4:1).

while calcigerosides C_1 (**3**) and C_2 (**4**) have glucose. Furthermore, calcigeroside B (**2**) is the first sea cucumber glycoside that posseses two quinovose units in the carbohydrate chain.

When the glycosides isolated from *Pentamera calcigera* and the derivatives described in this paper were individually tested in vitro against standard mouse and human tumor cell lines (P-388, A-549, HT-29, and Mel-28), only the desulfated derivatives of calcigerosides B, C_1 and C_2 (compounds **5**, **7**, and **9**) showed cytotoxic activity (IC_{50} = 5 μg/mL).

Experimental Section

General Experimental Procedures. All melting points were determined using a Kofler Thermogenerate apparatus. Optical rotations were measured using 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/D_2O (4:1) with TMS as an internal reference (δ = 0). The FABMS (positive and negative ion modes) were recorded using a Micromass apparatus, model Autospec-M, on a glycerol/thioglycerol matrix. APIES−MS spectra were obtained on a Hewlett−Packard 1100 MSD apparatus by direct injection. HPLC was performed using an Yanako L-2000L chromatograph equipped with a differential refractometer on a Silasorb C_{18} column (10 × 150 mm). GC analysis was carried out using a Tsvet-110 apparatus, with a glass column (0.3 × 150 cm) and 1.5% QF-1 as the stationary liquid phase and Chromatrone N-HMDS as the stationary solid phase. The following experimental conditions were used: carrier gas, Ar at 60 mL/min; column temperature, from 150 to 225 °C at 5 °C/min. GLC−MS was performed using an LKB 9000s apparatus with a glass column (0.3 × 300 cm) with 1.5% OF-1 as the stationary liquid phase and Chromatrone N−HMDS as the stationary solid phase and He at 50 mL/min as the carrier gas. The following conditions were selected: injection port 275 °C, molecular separator 265 °C, ion source 255 °C, column 110−210 °C at 4 °C/min, ionizing voltage 70 ev. The atomic absorption spectroscopy data were obtained using a Jarrell Ash AA-780 spectrometer. The cations in all the glycosides were determined to be Na^+.

Animal Material. Specimens of *Pentamera calcigera* were collected at a depth of 70−80 m by Sigsby trawl in the southwestern area of the Peter the Great Gulf (Sea of Japan) between Furugelm Island and Gamov Cape in September 1995, during the 18th scientific cruise of r/v *Akademik Oparin* and stored in ethanol at room temperature. The sea cucumber was identified by Dr. V. S. Levin (Kamchatka Institute of Fishery and Oceanography, Petropavlovsk-Kamchtsky, Russian Federation). A voucher specimen [no. 018-68(13)] is on a deposit at the marine specimen collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russian Federation.

Table 5. ^{1}H and ^{13}C NMR Chemical Shifts and HMBC and NOESY Correlations of the Aglycon Moiety of Calcigeroside C_2 (**4**)

position	δ_C mult[a]	δ_C mult[b] (*J* in Hz)	HMBC (C)	NOESY (H)
1	36.01 t	1.48 m, 1.30 m		3
2	26.82 t	2.00 m, 1.82 m		
3	88.86 d	3.23 dd (11.5, 3.0)	30, 31, 1 Xyl	1, 5, 31, 1 Xyl
4	39.39 s			
5	47.84 d	0.99 br t		3
6	23.18 t	2.05 m, 1.72 m		7
7	119.78 d	5.66 br s		6, 15, 32
8	146.54 s			
9	47.21 d	3.42 d (13.6)		19
10	35.39 s			
11	22.66 t			
12	29.95 t	2.00 m, 1.70 m		
13	57.47 s	2.01 m, 1.91 m		
14	51.24 s			
15	34.04 t	1.68 m		7, 16
16	25.49 t	1.96 m, 1.71 m		15, 17
17	53.35 d	2.72 dd (10.1, 3.8)	13, 20, 21	16, 21, 32
18	179.82 s			
19	23.87 q	1.18 s	1, 9, 10	9, 30
20	82.50 s			
21	27.05 s			
22	51.84 t	3.10 d (18.2), 3.03 d (18.2)	17, 18, 21, 23	21, 24
23	207.46 s			
24	52.02 t	2.30 d (6.8), 2.15 m	23, 25, 26, 27	22
25	24.32 d	0.86 m		
26	22.26 q	0.87 d (2.0)	24, 25, 27	
27	22.26 q	0.86 d (2.1)	24, 25, 26	
30	17.33 q	1.09 s	4, 5, 31	19
31	28.61 q	1.25 s	4, 5, 30	3
32	30.62 q	1.05 s	8, 13, 14, 16	7, 17

[a] Recorded at 125 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).

Extraction and Isolation. The sea cucumbers (the weight of dried residue was 425 g) were cut into pieces and extracted twice with refluxing ethanol. The combined extracts were concentrated, and the extract 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 glycoside fraction (3.5 g) with 65% aqueous methanol. The glycoside fraction was further chromatographed on Si gel, eluting with $CHCl_3-MeOH-H_2O$ (65:25:4) and $CHCl_3-MeOH-H_2O$ (650:300:54) to give fractions A (450 mg), B (450 mg), and C (600 mg). Final purification of these fractions was achieved by HPLC: fraction A afforded 270 mg of pure cucumarioside G_2 (**1**) using $MeOH-H_2O$ (1:1) as the mobile phase and a flow of 2.5 mL/min. Fraction B gave 75 mg of pure calcigeroside B (**2**) using $MeOH-H_2O$ (7:8). Fraction C yielded 180 mg of pure glycoside **3** and 75 mg of pure glycoside **4** using $MeOH-H_2O$ (7:8).

Cucumarioside G_2 (1): mp 235−236 °C, $[\alpha]^{20}_D$ − 46.5° (*c* 0.1, pyridine); ^{13}C NMR identical to that of the known compound cucumarioside G_2 (**1**).[5]

Calcigeroside B (2): mp 234−236 °C, $[\alpha]^{20}_D$ − 49.0° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 2; FABMS (positive ion mode) *m/z* (rel int) 1241 ($[M_{Na} + Na]^+$, 2.6), 1139 ($[M_{Na} + H - SO_3Na + Na]^+$, 4.3), 1095 ($[M_{Na} - Qui + H + Na]^+$ or $[M_{Na} - 3\text{-O-Me-Xyl} + H + Na]^+$, 1.1), 639 ([{Agl-O-Xyl-SO_3Na-O} − H + Na]$^+$, 6.26), 623 ([{Agl-O-Xyl-SO_3Na} − H + Na]$^+$, 5.64), 132 (100.0); APIES−MS (negative ion mode) (*i*-PrOH−H_2O + NH_4OH) *m/z* (rel int) 1195 ($[M_{Na} - Na]^-$, 100.0), 1149 ($[M_{Na} - Qui + H - Na]^-$ or $[M_{Na} - 3\text{-O-Me-Xyl} + H - Na]^-$, 18.1), 903 ($[M_{Na} - Qui - 3\text{-O-Me-Xyl} + 2H - Na]^-$, 14.8), 888 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc} + 2H - Na]^-$, 10.0), 596 ([{Agl-O-Xyl-SO_3Na-O} + 2H − Na]$^-$, 11.2).

Calcigeroside C_1 (3): mp 204−206 °C, $[\alpha]^{20}_D$ − 60.0° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 3 and 4; FABMS (positive ion mode) *m/z* (rel int) 1257 ($[M_{Na} + Na]^+$, 27.3), 1155 ($[M_{Na} - SO_3Na + H + Na]^+$, 15.4), 1009 ($[M_{Na} - SO_3Na - 3\text{-O-Me-Xyl} + 2H + Na]^+$, 7.0), 771 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc-O} + 2H + Na]^+$, 12.5), 639 ([{Agl-O-Xyl-O-SO_3Na-O} − H + Na]$^+$, 22.9) 384 ([Agl-O + H]$^+$, 100.0); FABMS (negative ion mode) *m/z* (rel int) 1211 ($[M_{Na} - Na]^-$, 100.0), 1049 ($[M_{Na} - Glc + H - Na]^-$, 8.5), 1047 ($[M_{Na} - 3\text{-O-Me-Xyl-O} - H - Na]^-$, 12.0), 903 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc} + H - Na]^-$, 12.5), 885 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc-O} + H - Na]^-$, 6.9), 708 ([{Agl-O-Xyl-O-SO_3Na-O-Qui} + H − Na]$^-$, 6.9), 595 ([{Agl-O-Xyl-O-SO_3Na-O} + H − Na]$^-$, 26.4), 577 ([{Agl-O-Xyl-O-SO_3-Na} − H − Na]$^-$, 14.3).

Calcigeroside C_2 (4): mp 226−228 °C, $[\alpha]^{20}_D$ −39.0° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 3, 4, and 5; FABMS (positive ion mode) *m/z* (rel int) 1343 ($[M_{Na} + Na]^+$, 5.1), 1241 ($[M_{Na} - SO_3Na + H + Na]^+$, 4.3), 625 ([{Agl-O-Xyl-O_2} + 2H + Na]$^+$, 20.0), 494 ([Agl-O + 2H + Na]$^+$, 56.9), 392 (100.0). FABMS (negative ion mode); *m/z* (rel int) 1297 ($[M_{Na} - Na]^-$, 27.3), 1151 ($[M_{Na} - 3\text{-O-Me-Xyl} + H - Na]^-$, 5.5), 1135 ($[M_{Na} - Glc - H - Na]^-$, 5.5), 989 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc} + H - Na]^-$, 7.1), 971 ($[M_{Na} - 3\text{-O-Me-Xyl-O-Glc-O} + H - Na]^-$, 6.6), 681 ([{Agl-O-Xyl-OSO_3Na-O} + H − Na]$^-$, 38.7), 665 ([{Agl-O-Xyl-OSO_3Na + H − Na]$^-$, 17.8), 219 (100.0).

Hydrolysis of Cucumarioside G_2 (1) and Calcigerosides B (2), C_1 (3), and C_2 (4). The glycoside (5 mg) was dissolved in 5 mL of 1N trifluoroacetic acid and heated in an ampule at 100 °C for 1 h. The solution was decanted from an aglycon residue and concentrated. Then, 1 mL of pyridine and 2 mg of $NH_2OH\cdot HCl$ were added to the dry residue, and the mixture was heated at 100 °C for 1 h. After that time, 1 mL of Ac_2O was added and heating at 100 °C was continued for a further 1 h. After removing the solvents, the resulting aldononitrile peracetates were analyzed by GC−MS using standard aldononitrile peracetates as reference samples. Xylose, quinovose, glucose, and 3-*O*-Me-xylose were identified for the four glycosides in a ratio of (1:1:1:1) for cucumarioside G_2 (**1**), (1:2:1:1) for calcigeroside B (**2**), and (1:1:2:1) for calcigerosides C_1 (**3**) and C_2 (**4**).

Determination of the D-Configuration of the Monosaccharide Units. The glycosides were hydrolyzed as described above and the sugars separated by preparative paper chromatography on Filtrak FN-15 with BuOH−pyridine−H_2O (6:4:3) as eluent. Their optical rotations were found to correspond to the D series.

Desulfation of Calcigeroside B (2). The glycoside **2** (30 mg) was dissolved in a mixture of pyridine−dioxane (1:1), heated under reflux for 1 h, and the mixture was then

concentrated under reduced pressure. The residue was chromatographed on a Si gel column with $CHCl_3$−MeOH−H_2O (700:125:7) to give desulfated derivative **5** (16 mg), mp 201−203 °C, $[\alpha]^{20}_D$ −19.0 (*c* 0.07, pyridine).

Periodate Oxidation of Calcigeroside B (2). The glycoside **2** (5 mg) was dissolved in 15 mL of water, and 30 mg of $NaIO_4$ was added. The mixture was kept for 24 h at room temperature and then loaded onto a Polychrom-1 column, washed with water, and eluted with ethanol. The ethanol eluate was concentrated under reduced pressure, and the residue was hydrolyzed as described above. The monosaccharides were identified by the method described above as the aldononitrile peracetates of xylose, quinovose, glucose, and 3-*O*-Me-xylose (1:1:1:1 ratio).

Periodate Oxidation of Desulfated Derivative 5. The derivative **5** (5 mg) was dissolved in a mixture of H_2O−BuOH (2:1) (15 mL), and 30 mg of $NaIO_4$ was added. The mixture was stirred for 24 h at room temperature. The BuOH layer was decanted off, and the water layer was washed twice with 3 mL of BuOH. The combined BuOH layers were concentrated under reduced pressure and analyzed as described above showing the presence of quinovose, glucose, and 3-*O*-Me-xylose (1:1:1 ratio).

Double Degradation of Calcigeroside B (2) by the Smith Method. The glycoside **2** (20 mg) was treated with 30 mg of $NaIO_4$ in 10 mL of H_2O for 24 h. The solution was loaded onto a Polychrom-1 column, washed with H_2O, and the product was recovered by elution with EtOH. $NaBH_4$ (25 mg) was then added to the aqueous EtOH eluate, and the mixture was stirred at room temperature for 2 h, adjusted to pH 5.0 by the addition of HOAc, and concentrated under reduced pressure. The residue was dissolved in 10 mL of 0.5 N HCl, allowed to stand at room temperature for 2 h and subjected to chromatography on a Polychrom-1 column affording cucumarioside G_2 (**1**) after elution with ethanol. Monosaccharide analysis of **1**, using the method described above, showed the presence of xylose, quinovose, glucose, and 3-*O*-Me-xylose (1:1:1:1 ratio). Smith degradation of **1** (10 mg) followed by chromatography on a Polychrom-1 column, afforded bioside **8** in the aqueous eluates and glycoside **6** in the alcoholic fractions. Monosaccharide analysis showed the presence of xylose in progenin **6** and glucose and 3-*O*-Me-xylose (1:1 ratio) in **8**.

Desulfation of Calcigeroside C_1 (3): The glycoside (**3**) (50 mg) was desulfated as described above and chromatographed on a Si gel column using $CHCl_3$−MeOH−H_2O (700:125:7) to give the desulfated derivative **7** (22 mg), mp 194−196 °C, $[\alpha]^{20}_D$ − 50.0 (*c* 0.1, pyridine).

Periodate Oxidation of Calcigerioide C_1 (3). The glycoside **3** (5 mg) was oxidized as described above, and xylose, quinovose, glucose, and 3-*O*-Me-xylose (1:1:1:1 ratio) were identified as oxidation products.

Periodate Oxidation of the Desulfated Derivative 7. Compound **7** (5 mg) was oxidized as described above and quinovose, glucose, and 3-*O*-Me-xylose (1:1:1 ratio) were identified as reaction products.

Double Degradation of Calcigeroside C_1 (3) by the Smith Method. The glycoside **3** (20 mg) was degraded by the Smith method as described above to give cucumarioside G_2 (**1**). This was degraded to give a xylose-containing progenin **6**, and the bioside derivative **8**, which produced 3-*O*-Me-xylose and glucose (1:1) upon monosaccharide analysis.

Desulfation of Calcigeroside C_2 (4). The glycoside **4** (40 mg) was desulfated as described above. The product of solvolysis was purified on a Si gel column using $CHCl_3$−MeOH−H_2O (700:125:7) to give the desulfated derivative **9** (17 mg), mp 188−190 °C, $[\alpha]^{20}_D$ − 27.0 (*c* 0.1, pyridine).

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