Synaptosides A and A_1, Triterpene Glycosides from the Sea Cucumber *Synapta maculata* Containing 3-*O*-Methylglucuronic Acid and Their Cytotoxic Activity against Tumor Cells

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Two novel triterpene holostane glycosides, synaptosides A (**1**) and A_1 (**2**), have been isolated from the Vietnamese sea cucumber *Synapta maculata* (Synaptida, Apodida). Their structures were elucidated by spectroscopic methods (NMR and MS) and chemical transformations. Glycosides **1** and **2** have rare branched pentasaccharide carbohydrate chains featuring a 3-*O*-methylglucuronic acid residue not previously reported in glycosides from sea cucumbers and a 6-*O*-sulfated glucose. Glycoside **2** has an oxo group at C-7 and a 8(9)-double bond. All these structural features are unknown in glycosides from sea cucumbers. Glycoside **1** has moderate cytotoxic activity (IC_{50} 8.6 μg/mL) and glycoside **2** is inactive against HeLa tumor cells.

Triterpene glycosides from sea cucumbers (Holothurioidea, Echinodermata) have been the subjects of long-term systematic investigations.[1–3] Most of these glycosides have been isolated from sea cucumbers belonging to the orders Aspidochirotida and Dendrochirotida. Only one species belonging to the order Apodida, *Synapta maculata,* has been studied. The aglycon structure of the major glycoside was established to be holost-7-en-3β-ol-23-one, but the full structure of the glycoside was unknown.[4] Here we report the full structures of two new triterpene glycosides, synaptosides A (**1**) and A_1 (**2**), isolated from the glycosidic fraction of *S. maculata* collected in the South China Sea near the Vietnamese shore.

Results and Discussion

An ethanolic extract of *S. maculata* was evaporated *in vacuo*, and the residue was sequentially submitted to column chromatography on Polychrom-1 (powdered Teflon, Latvia) and silica gel to give a glycoside fraction. This fraction was submitted to HPLC on a Diasphere C-8 column to give pure synaptoside A (**1**) as a major component and synaptoside A_1 (**2**) as a minor component. Structures of the isolated glycosides were elucidated on the basis of spectroscopic data (^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOESY, TOCSY, and MALDI TOF MS) and chemical transformations.

The HR MALDI TOF MS (positive ion mode) of synaptoside A (**1**) exhibited a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 1409.5147, calculated for $C_{60}H_{92}O_{31}SNa_3$ as 1409.5036 *m/z*, that allowed determination of the molecular formula of synaptoside A (**1**) as $C_{60}H_{92}O_{31}SNa_2$. In the MALDI TOF MS (positive ion mode) of synaptoside A (**1**) the ion peaks $[M + Na - SO_3Na + H]^+$ at *m/z* 1307.4 and [M + Na − 3-*O*-methylglucuronic acid sodium salt + H]$^+$ at *m/z* 1197.4, indicating the loss of a sulfate group and the terminal monosaccharide residue, respectively, were observed along with a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 1409.4. The MALDI TOF MS (negative ion mode) showed a pseudomolecular ion peak $[M - Na]^-$ at *m/z* 1363.3 along with fragmentary ion peaks [M − Na − 3-*O*-methylglucuronic acid sodium salt − glucose + H]$^-$ at *m/z* 989.3 and [M − Na − 3-*O*-methylglucuronic acid sodium salt − glucose − quinovose + H]$^-$ at *m/z* 843.3, indicating the sequential cleavage of a carbohydrate chain of synaptoside A (**1**).

^{13}C NMR and DEPT spectra of the aglycon part of synaptoside A (**1**) (Table 1) are coincident with those of cucumechinosides C and F isolated from the sea cucumber *Cucumaria echinata*[5] and calcigerosides C_2 and D_2 from the sea cucumber *Pentamera calcigera*.[6,7] ^{13}C NMR and DEPT spectra of synaptoside A (**1**) show signals at 180.8 (C-18), 120.0 (C-7), and 146.5 (C-8) ppm, characteristic of holostane aglycones having a 7(8)-double bond. The signal of a quaternary carbon at 209.18 ppm (C-23) in the ^{13}C NMR spectrum along with the correlation H-24/C-23 in the HMBC spectrum confirmed the presence of a keto group in the aglycon side chain of synaptoside A (**1**). The structure of the aglycon was also confirmed by the analysis of NOESY and COSY spectra (Table 1).

A comparison of NMR data for the carbohydrate moiety in synaptoside A (**1**) with those of known glycosides revealed that the sugar chain of **1** was new and that some signals in the spectra were not characteristic of typical monosaccharide units in triterpene glycosides from sea cucumbers.

The presence of five monosaccharide units in the sugar chain of the glycoside **1** was easily deduced from its ^{13}C NMR and DEPT spectra. These spectra showed signals of five anomeric carbons at 102.9–104.8 ppm correlated with the corresponding anomeric protons at δ 4.79 (d, J = 7.2 Hz), 5.12 (d, J = 7.8 Hz), 5.00 (d, J = 7.9 Hz), 5.31 (d, J = 7.9 Hz), and 4.97 (d, J = 7.9) ppm in the HSQC spectrum (Table 2). The coupling constants of the anomeric protons indicated a β-configuration for all of the glycosidic bonds.[8]

Correlations in the NOESY spectrum of **1** between the anomeric proton of a xylose residue (monosaccharide I) and H-3 and H-31 of the aglycon moiety showed that the xylose residue was attached to C-3 of the aglycon (Table 2). Localization of the interglycosidic linkages of the xylose residue were established using the glycosylation shifts of C-2 and C-4 in the ^{13}C NMR spectrum of synaptoside A (**1**) to 82.3 and 77.9 ppm, corresponding with the signals of C-2 (75.1 ppm) and C-4 (71.1 ppm) in the spectrum of the model 3-*O*-[β-D-xylopyranosyl]-16-oxo-holost-9(11)-en-3β-ol obtained by acid hydrolysis of psolusoside A from *Psolus fabricii*.[9] Moreover, the cross-peak between H-2 of the xylose unit at 4.07 ppm and C-1 of the second monosaccharide unit at 104.7 ppm in the HMBC spectrum along with the analogous cross-peak between H-2 of the xylose residue and an anomeric proton of the second sugar unit at 5.12 ppm in the NOESY spectrum confirmed the attachment of

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Table 1. ^{1}H and ^{13}C NMR Data and Selected HMBC and NOESY Correlations of the Aglycon Moieties of Synaptoside A (**1**), Its Desulfated Derivative **3**, and Desulfated Methylated Derivative **4**

position	δ_C[a]	δ_H mult. (*J* in Hz)[b]	HMBC	NOESY
1	36.1	1.46 m		
2	26.9	1.94 m (a), 2.12 m (e)		
3	89.2	3.31 dd (3.9, 12.0)		H-5, H-1 Xyl1
4	34.9			
5	47.9	1.03 dd (6.0, 10.0)		H-3
6	23.2	2.04 m		H-19, H-30, H-31
7	120.0	5.76 m		
8	146.5			
9	47.3	3.41 m		H-19
10	35.4			
11	22.7	1.54 m, 1.80 m		H-1, H-12
12	30.0	2.04 m		H-17
13	57.7			
14	51.3			
15	34.1	1.85 m, 1.63 m		
16	25.5	2.07 m, 1.72 m		
17	53.5	2.79 dd (3.5, 10.5)	C: 20	H-12, H-21, H-32
18	180.8			
19	23.9	1.22 s	C: 1, 5, 9, 10	H-6, H-9
20	83.2			
21	27.1	1.66 s	C: 17, 20, 22	H-17, H-22
22	51.8	3.20 d (18.0) 3.13 d (18.0)		H-21, H-24 H-21, H-22
23	209.2			
24	52.2	2.43 d (6.5)	C: 23, 25, 26, 27	H-22
25	24.5	2.18 m		
26	22.4	0.96 d (7.0)	C: 24, 25, 27	
27	22.3	0.96 d (7.0)	C: 24, 25, 26	
30	17.2	1.11 s	C: 3, 4, 5, 31	H-6
31	28.6	1.27 s	C: 3, 4, 5, 30	H-6, H-1 Xyl
32	30.7	1.14 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125.77 MHz in C_5D_5N/D_2O (4:1). [b] Recorded at 500 MHz in C_5D_5N/D_2O (4:1).

this sugar to C-2 of the xylose residue. The analysis of 1D TOCSY, COSY, HSQC, HMBC, and NOESY spectra showed that the second monosaccharide unit (monosaccharide II) in the carbohydrate moiety of synaptoside A (**1**) was quinovose (Table 2).

The presence of the cross-peak between C-4 of the xylose at 77.9 ppm and H-1 of the sulfated glucose in the HMBC spectrum and the analogous correlation between H-4 of the xylose residue at 4.26 ppm and H-1 of the sulfated glucose residue at 4.97 ppm in the NOESY spectrum of **1** indicated that the fourth carbon of the xylose residue was linked by a β-glycosidic bond with an anomeric carbon of a sulfated glucose residue (monosaccharide V).

The presence of a sulfate group at C-6 of this glucose residue was confirmed by comparison of the ^{13}C NMR spectrum of the synaptoside A (**1**) carbohydrate moiety with that of the known synallactoside C from the sea cucumber *Synallactes nozawae*.[10] Both have similar glucose residues linked to C-4 of the first xylose unit. The C-6 and C-5 signals of the glucose residue in the ^{13}C NMR spectrum of **1** were shifted downfield by 5.1 ppm and shifted upfield by 2.2 ppm, respectively, when compared with shifts of synallactoside C, due to α- and β-shifting effects of sulfation.[8]

One more monosaccharide unit in the carbohydrate moiety of synaptoside A (**1**) (monosaccharide III) was identified as a glucose attached by a β-(1→4) linkage to the quinovose residue. It was confirmed by cross-peaks H-4 Qui/C-1 Glc and H-1 Glc/C-4 Qui in the HMBC and H-4 Qui/H-1 Glc in the NOESY spectra. The signals H-1−H-6 and C-1−C-6 of this residue were identified by the analysis of TOCSY, NOESY, and HSQC spectra (Table 2). The third carbon of this glucose unit was linked by a glycosidic bond with a terminal monosaccharide unit. This was confirmed by the presence of cross-peaks between C-3 of glucose at 86.8 ppm and H-1 of the terminal sugar unit at 5.31 ppm observed in the HMBC spectrum of synaptoside A (**1**).

Analysis of the 1D TOCSY spectrum allowed assignment of the signals of H-1−H-5 in the terminal monosaccharide unit (monosaccharide IV) of the carbohydrate chain of **1**. The signals of H-4 (at 4.07 ppm) and H-5 (at 4.13 ppm) of the terminal monosaccharide unit were correlated with the signal of a quaternary carbon at 175.4 ppm in the HMBC spectrum of **1**. Such a chemical shift (δ_C) is characteristic of the carboxyl group in uronic acids. The signal at 60.4 ppm in the ^{13}C NMR and the corresponding proton signal at 3.89 ppm in the ^{1}H NMR spectrum indicated the presence of an O-methyl group attached to C-3 [the cross-peak H_3 (OMe)/C-3 MeGlcUa in the HMBC spectrum] of the terminal monosaccharide unit. According to these data, 3-*O*-methylglucuronic acid residue was identified in the carbohydrate moiety of synaptoside A (**1**) as the terminus. Therefore, the carbohydrate moiety of **1** was a pentasaccharide, branched at C-4 of the first xylose residue and containing a 6-*O*-sulfated glucose and a 3-*O*-methylglucuronic acid as terminal units.

In order to confirm the position of the sulfate group in the carbohydrate moiety of synaptoside A (**1**), we obtained desulfated derivative **3** by solvolysis of **1** in a dioxane/pyridine mixture. The signals of the carbohydrate chain in the ^{13}C NMR spectrum of **3** were compared with those of synaptoside A (**1**) (Table 3). Signals of C-5 and C-6 of the terminal glucose residue in the ^{13}C NMR spectrum of **3** were shifted downfield by 1.6 ppm and upfield by 5.0 ppm, correspondingly, due to the absence of α- and β-effects of a sulfate group, as compared with the same signals in the spectrum of **1**.[8] These data confirm the presence of a sulfate group attached to C-6 of the glucose residue in the carbohydrate moiety of glycoside **1**.

To confirm the presence of a carboxyl group in the 3-*O*-methylglucuronic acid in the carbohydrate chain of **1**, methylated derivative **4** was obtained by methylation of derivative **3** with diazomethane. Comparison of ^{13}C NMR spectra of **3** and **4** showed the difference in the values of chemical shifts of the fourth terminal monosaccharide residue only (Table 3). The characteristic signal of the carboxyl group of 3-*O*-methylglucuronic acid at 175.7 ppm was absent in the ^{13}C NMR spectrum of **4**. Instead of this signal, there was a signal of a quaternary carbon at 170.4 ppm (C-6) and an additional signal of an *O*-methyl group at 52.4 ppm. These data

Table 2. ^{1}H and ^{13}C NMR Data and Selected HMBC and NOESY Correlations of the Carbohydrate Moieties of Synaptosides A (**1**) and A_1 (**2**)

position	δ_C[a,b,c]	δ_H mult. (*J* in Hz)[d]	HMBC	NOESY
Xyl (1→C-3)				
1	104.8	4.79 d (7.2)	C: 3	H-3,5-Xyl, H-3, H-31
2	**82.3**	4.07 t	C: 1 Qui	H-1 Qui
3	75.1	4.28 t (9.0)	C: 4 Xyl	H-1 Xyl
4	**77.9**	4.26 m		H-1 Glc2
5	63.6	4.51 m	C: 3 Xyl	H-1 Glc 2
		3.76 m		H-1 Xyl
Qui (1→2Xyl)				
1	104.7	5.12 d (7.8)	C: 2 Xyl	H-5 Qui, H-2 Xyl
2	75.7	3.99 t (7.5)	C: 3 Qui	
3	75.1	4.10 t (8.7)	C: 4 Qui	
4	**86.4**	3.66 t (8,7)	C: 1 Glc 1, 3 Qui	H-6 Qui, H-1 Glc 1
5	71.5	3.79 m		H-1 Qui
6	17.9	1.74 d (6.0)	C: 4,5-Qui	H-4 Qui
Glc 1 (1→4Qui)				
1	104.1	5.00 d (7.9)	C: 4-Qui	H-4 Qui, H-3,5 Glc 1
2	73.8	4.05 m	C: 1, 3 Glc 1	
3	**86.9**	4.37 t (8.8)	C: 1-MeGlcUa, 2,4 Glc 1	H-1
MeGlcUa, H-1 Glc 1				
4	69.5	4.02 t (9.5)	C: 5 Glc 1	
5	77.3	4.08 m		H-1 Glc 1
6	61.7	4.46 brd (10.3)		
		4.14 m		
MeGlcUA (1→3Glc 1)				
1	104.4	5.31 d (7.9)	C: 3-Glc 1	H-3 Glc 1, H-3,5 MeGlcUa
2	74.2	3.97 t (8.5)	C: 1, 3 MeGlcUa	
3	86.6	3.74 t (8.7)	C: 2,4 MeGlcUa, OMe	H-1 MeGlcUa
4	72.6	4.07 t (9.5)	C: 6 MeGlcUa	
5	74.4	4.13 d (10.0)	C: 1,4,6 MeGlcUa	H-1 MeGlcUa
6	175.4			
OMe	60.4	3.89 s	C:3 -MeGlcUa	
Glc 2 (1→4 Xyl)				
1	102.9	4.97 d (7.9)	C: 4-Xyl	H-5 Glc 2, H-4, 5 Xyl
2	73.8	3.95 t (8.2)	C: 1,3 Glc 2	
3	76.8	4.24 t (9.0)	C: 2,4 Glc 2	
4	70.7	4.07 m		
5	75.8	4.15 m		H-1 Glc 2
6	*67.4*	5.12 brd (10.7) 4.86 dd (11.2; 7.9)	C:5 Glc 2	

[a] Recorded at 125 MHz in C_5D_5N/D_2O (4:1). [b] Bold = interglycosidic positions. [c] Italic = sulfate position. [d] Recorded at 500 MHz in C_5D_5N/D_2O (4/:1).

Table 3. ^{13}C NMR Data of the Carbohydrate Moieties for the Desulfated Derivative of Synaptoside A (**3**), Desulfated Methylated Derivative of Synaptoside A (**4**), and Synthetic Methyl (Methyl-3-*O*-methyl-β-D-glucopyranoside)uronate (**5**)

	δ_C[a,b]					
position	**3**	**4**	position	**3**	**4**	**5**
Xyl (1→C-3)			MeGlcUA			
1	104.9	104.8	1	104.3	104.6	105.1
2	**82.8**	**82.7**	2	74.1	74.1	73.7
3	75.2	75.3	3	86.4	86.2	86.3
4	**77.8**	**77.8**	4	72.5	71.9	72.1
5	63.7	63.6	5	74.4	76.0	76.2
			6	175.7	170.4	170.6
Qui (1→2Xyl)			OMe→3MeGlcUA	60.5	60.9	60.8
1	104.9	104.8	OMe→6MeGlcUA		52.4	52.5
2	75.6	75.6	Glc 2 (1→4 Xyl)			
3	75.3	75.2	1	102.8	102.8	
4	**86.5**	**86.5**	2	74.1	74.**2**	
5	71.5	71.5	3	77.2	77.3	
6	17.9	17.9	4	71.0	71.0	
Glc 1 (1→4Qui)			5	77.4	77.4	
1	104.0	104.1	6	62.4	61.9	
2	73.7	73.7				
3	**87.2**	**86.8**				
4	69.5	69.2				
5	77.3	77.3				
6	61.8	61.7				

[a] Recorded at 125 MHz in C_5D_5N/D_2O (4:1). [b] Bold = interglycosidic positions.

indicated selective methylation of a carboxyl group in the 3-*O*-methylglucuronic acid residue.

The presense of 3-*O*-methylglucuronic acid as a terminal monosaccharide residue was also confirmed by the coincidence of the corresponding signals in the ^{13}C NMR spectra of methylated derivative **4** and synthetic methyl (methyl-3-*O*-methyl-β-D-glucopyranoside)uronate (**5**) (Table 3). Reference substance **5** was synthesized from commercial methyl-β-D-glucopyranoside by oxidation at atmospheric air on Pt/carbon catalyst followed by etherification of the carboxyl group by methanol and methylation of the obtained derivative by diazomethane in the presence of $SbCl_3$.

Acid hydrolysis of synaptoside A (**1**) with TFA was carried out to ascertain its monosaccharide composition. The mixture of sugars obtained was submitted to HPLC to give individual monosaccharides. Subsequent alcoholysis of each monosaccharide 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 synaptoside A (**1**) (xylose, quinovose, glucose, and 3-*O*-methylglucuronic acid). The reference sample of 3-*O*-methylglucuronic acid was synthesized in our laboratory.[11] Hence, synaptoside A (**1**) is 3-*O*-{6-*O*-sodium-3-*O*-methyl-β-D-glucopyranosyluronate-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[4-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl}-23-oxo-holost-7-en-3β-ol.

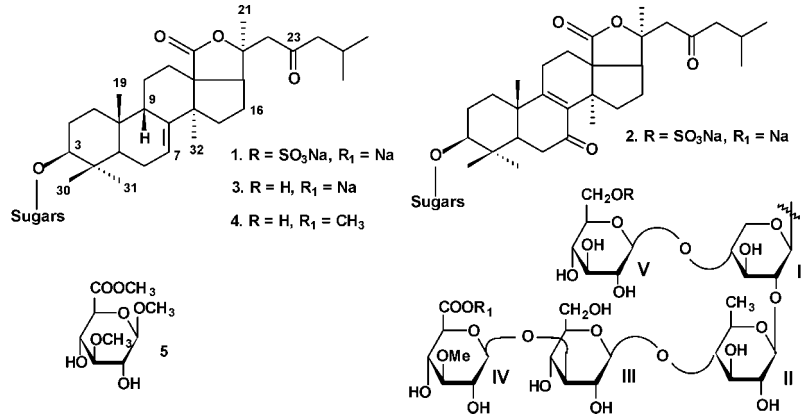
The HR MALDI TOF MS (positive ion mode) of synaptoside A_1 (**2**) exhibited a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 1423.4694, calculated for $C_{60}H_{90}O_{32}SNa_3$ as 1423.4829 *m/z*, that

Table 4. ^{1}H and ^{13}C NMR Data and Selected HMBC and NOESY Correlations of the Aglycon Moiety of Synaptoside A_1 (**2**)

position	δ_C[a]	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	34.8	1.81 m 1.29 m		H-11
2	26.5	1.93 m, 2.14 m		
3	87.7	3.23 dd (3.9, 11.2)		H-5, H-31
4	39.3			
5	50.2	1.68 m		H-3, H-31
6	36.5	2.60 m		H-19, H-30, H-31
7	200.0			
8	135.2			
9	167.5			
10	39.7			
11	24.0	2.62 m 2.48 m		H-1
12	26.9	2.27 m		H-17, H-21
13	58.4			
14	46.9			
15	34.3	2.68 m, 1.83 m		
16	26.4	2.04 m, 1.72 m		
17	49.3	2.74 dd (2.8, 10.3)	C-18	H-12, H-21, H-32
18	178.7			
19	17.9	1.39 s	C: 1, 5, 9, 10	H-6
20	83.4			
21	27.6	1.70 s	C: 17, 20, 22	H-12, H-17, H-22
22	52.1	3.20 d (18.0), 3.13 d (18.0)	C: 23	H-21, H-24
23	209.1			
24	52.1	2.42 dd (3.0, 7.2)	C: 23, 26, 27	H-22
25	24.5	2.18 m	C: 23	
26	22.3	0.96 d (6.5)	C: 24, 25, 27	
27	22.3	0.96 d (6.5)	C: 24, 25, 26	
30	16.0	1.03 s	C: 3, 4, 5, 31	H-6
31	26.9	1.14 s	C: 3, 4, 5, 30	H-3, H-5, H-6
32	25.7	1.16 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125.77 MHz in C_5D_5N/D_2O (4:1). [b] Recorded at 500 MHz in C_5D_5N/D_2O (4:1).

Chart 1



allowed us to determine a molecular formula of synaptoside A_1 (**2**) as $C_{60}H_{90}O_{32}SNa_2$. The MALDI TOF MS (positive ion mode) peaks of synaptoside A_1 (**2**) were analogous to the peaks of synaptoside A (**1**) $[M + Na - SO_3Na + H]^+$ at *m/z* 1321.4 and $[M + Na -$ 3-*O*-methylglucuronic acid sodium salt $+ H]^+$ at *m/z* 1211.3. The MALDI TOF MS (negative ion mode) of synaptoside A_1 (**2**) showed a pseudomolecular ion peak $[M - Na]^-$ at *m/z* 1377.4 and fragmentary ion peaks $[M - Na -$ 3-*O*-methylglucuronic acid sodium salt $+ H]^-$ at *m/z* 1165.3, $[M - Na -$ 3-*O*-methylglucuronic acid sodium salt $-$ glucose $+ H]^-$ at *m/z* 1003.3, and $[M - Na -$ 3-*O*-methylglucuronic acid sodium salt $-$ glucose $-$ quinovose $+ H]^-$ at *m/z* 857.3, demonstrating the sequential loss of monosaccharide residues in the carbohydrate chain of synaptoside A_1 (**2**).

The analysis of ^{13}C NMR spectral data of the aglycon moiety of synaptoside A_1 (**2**) showed the presence of the signal of C-18 at 178.7 ppm characteristic for the 18(20)-lactone. The signals of carbons C-22−C-27 were coincident with the corresponding signals in the ^{13}C NMR spectrum of synaptoside A (**1**). This indicated their side chains have a ketone group at C-23 (the signal of the quaternary carbon at 209.1 ppm in the ^{13}C NMR spectrum of **2**) (Table 4). However, in the downfield region of the ^{13}C NMR spectrum of **2**, signals at 135.2 and 167.5 ppm are present. Such signals were not characteristic for a 7(8)- or 9(11)-double bond. The DEPT and HSQC spectra of synaptoside A_1 (**2**) indicated that these signals are signals of quaternary carbons belonging to a 8(9)-double bond. In the downfield region of the ^{13}C NMR spectrum of **2**, an additional signal of a quaternary carbon at 200.0 ppm was found due to a ketone group in the polycyclic system of the aglycon.

The typical position of a ketone group in the sea cucumber saponin aglycons is C-16.[2] However, the presence of a keto group at C-16 is impossible in **2** because of the signal of a secondary carbon, CH_2-16, at 26.4 ppm in the ^{13}C NMR and DEPT spectra. Moreover, in the COSY spectrum protons of two methylene groups [H_2-15 at 2.68 (m) and 1.83 (m) ppm; H_2-16 at 2.04 (m) and 1.72 (m) ppm)] and a methine group [H-17 at 2.74 (dd, J = 2.8, 10.3 Hz)] of the ring D formed an isolated spin system $CH_2(15)-CH_2(16)-CH(17)$.

Comparison of the ^{13}C NMR spectrum of synaptoside A_1 (**2**) with that of the 7-keto derivative of lanosterol[12] reveals considerable

coincidence of the carbon signals of rings A, B, and C of polycyclic systems of these compounds. This allows us to suggest an additional keto group occurs at C-7 of the aglycon of synaptoside A_1 (**2**). The aglycon structure proposed for synaptoside A_1 (**2**) containing an 8(9)-double bond and a 7-ketone group is confirmed by COSY, NOESY, and HMBC spectral data (Table 4).

Comparison of the NMR spectra of carbohydrate moieties of synaptosides A (**1**) and A_1 (**2**) (Table 2) shows coincidence of all monosaccharide residue signals. This indicates the identity of the sugar parts of the glycosides.

Therefore, synaptoside A_1 (**2**) is 3-*O*-{6-*O*-sodium-3-*O*-methyl-β-D-glucopyranosyluronate-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-[4-*O*-sodium sulfate-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranosyl}-7,23-dioxo-holost-8-en-3β-ol.

Synaptosides A (**1**) and A_1 (**2**) were studied as potential cytotoxic agents using the HeLa human tumor cell line. Glycoside **1** showed moderate cytoxicity, with an IC_{50} of 8.6 μg/mL. Glycoside **2** was not active against these tumor cells in concentrations of 14.1 μg/mL. The absence of activity of glycoside **2** may be explained by the different position of the double bond in the aglycon nucleus, 8(9), but not 7(8). Hence the cytotoxic activity depends on the configuration of the aglycon nucleus.

Sugar moieties of sea cucumber glycosides are comprised of two to six monosaccharide residues. The first is always xylose and the rest of the monosaccharides are usually glucose, quinovose, 3-*O*-methylglucose, and rarely 3-*O*-methylxylose.[1–3] Aglycons of the majority part of the glycosides are referred to a holostane type, having an 18(20)-lactone along with 7(8)- or 9(11)-double bonds.

Synaptosides A (**1**) and A_1 (**2**) are characterized by uncommon chemical features in the carbohydrate moieties. These glycosides are the first representatives of triterpene glycosides from sea cucumbers containing methylated glucuronic acid as one of monosaccharide residues in carbohydrate chains. 3-*O*-Methylglucuronic acid was found in capsule polysaccarides of bacteria,[13,14] but has never been identified in sea cucumber triterpene glycosides or any other triterpene glycosides. Moreover, synaptoside A_1 (**2**) contains an unusual polycyclic nucleus having a 7-oxo-8(9)-en system that is unique in sea cucumber triterpene glycosides.

Taxonomists consider the order Apodida as evolutionarily distant from other orders in the class based on morphological[15–18,20] and paleontological[21,22] data and partially on analysis of 18S rRNA.[19,23] The presence of unique 3-*O*-methylglucuronic acid residues as terminal monosaccharides in both **1** and **2** correlates well with this point of view.

Lanosterol is a triterpenoid characteristic of the animal kingdom that is a precursor of sterols. It has also been suggested to be biosynthetic[24,25] and most probably a phylogenetic precursor of aglycons from sea cucumber triterpene glycosides. The presence of the 8(9)-double bond in the aglycon moiety of synaptoside A_1 (**2**), like in lanosterol, suggests the Apodida (including the family Synaptidae) is the most primitive order in the class Holothurioidea. Such a point of view was supported earlier by morphological,[15–18,20] paleontological,[21,22] and 18S rRNA gene sequence[19,23] data. This point of view correlates well with the data on the structures of triterpene glycosides of *Synapta maculata*. The absence of cytotoxic activity of synaptoside A_1 (**2**) having an 8(9)-double bond in the aglycon moiety also reveals the primitive position of the order Apodida because membranolytic activity (including cytotoxicity) of triterpene glycosides ought to be increased during sea cucumber evolution as an adaptive response against evolving predatory fish.[27]

Glucuronic acid in water solution may easily form glucurone, 6(3)-lactone.[26] The presence of a 3-*O*-methyl group in the glucoronic acid residue completely prevents the formation of this lactone and correspondingly decreases the hydrophilicity of glycoside. It may be adaptive because maintaining the optimal hydrophilicity may contribute to the ability of glycosides to increase microviscosity of sea cucumber oocyte membranes, to block (reversibly) calcium transportation, and to inhibit oocyte maturation before the spawning season in order to synchronize reproductive processes in a population.[27] When oxidation of the terminal glucose residue into glucuronic acid was lost during further evolution of sea cucumbers, the 3-*O*-methyl groups of terminal monosaccharide residues were conserved because they increased membranolytic activity of glycosides that was useful for protection against predatory fish.[27] Glycosides having no 3-*O*-methyl at the terminal monosaccharide unit are less active as membranolytic toxins compared to similar substances having a 3-*O*-methyl group.[28]

Experimental Section

General Experimental Procedures. All melting points were determined with a Kofler-Thermogenerate apparatus. Specific rotation was measured on a Perkin-Elmer 343 polarimeter. NMR spectra were recorded on a AMX Bruker 500 spectrometer at 500.12/125.67 MHz ($^1H/^{13}C$) in C_5D_5N with TMS as an internal reference ($\delta = 0$). GLC analysis was carried out on an Aligent 6850 Series apparatus, carrier gas He (1.7 mL/min) at 100 °C (0.5 min) → 250 °C (5 °C/min, 10 min), capillary column HP-5 MS (30 m × 0.25 mm); temperatures of injector and detector were 150 and 280 °C, respectively. The MALDI TOF MS (positive and negative ion modes) were recorded using a Bruker mass spectrometer, model BIFLEX III, with impulse extraction of ions, on an α-cyano-4-hydroxycinnamic acid matrix. HPLC was performed using an Agilent 1100 chromatograph equipped with a differential refractometer on a Diasphere C_8 (4.6 × 250) column.

Animal Material. Specimens of the sea cucumber *Synapta maculata* (family Synaptidae; order Apodida) were collected in January 2005 during the 30th scientific cruise of the research vessel *Akademik Oparin* in Van Phong Bay (Vietnam), South China Sea, using scuba at a depth of 2–12 m. The sea cucumber was identified by Dr. V. S. Levin (Pacific Institue of Bioorganic Chemistry), and a voucher specimens is on deposit in the collection of the Zoological Institute, the Russian Academy of Sciences, Saint Petersburg.

Extraction and Isolation. The sea cucumbers were minced and extracted twice with refluxing 70% EtOH. The dry wt of the residue after extraction was 34.5 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 $CHCl_3$/EtOH/H_2O (100:150:50 and 100:125:25) solvent systems to give 563 mg of glycoside fraction A, as an individual spot on TLC. The glycosides were separated by HPLC on a Diasphere C_8 (4 × 250) column, with 50% MeOH as mobile phase, to give 150 mg of synaptoside A (**1**) as a major compound. A minor fraction was rechrogmatographed in the same conditions with 55% MeOH as mobile phase to give 7 mg of synaptoside A_1 (**2**).

Synaptoside A (1): mp 285–287 °C; $[\alpha]_D^{20}$ –16 (*c* 0.1, pyridine); for ^1H and ^{13}C NMR data, see Tables 1 and 2; MALDI TOF MS (positive ion mode) *m/z* (rel int) 1409.4 ($C_{60}H_{92}O_{31}SNa_3$ [M + Na]$^+$, 1), 1307.4 ([M + Na − SO_3Na + H]$^+$, 0.3), 1197.4 ([M + Na − 3-*O*-methylglucuronic acid sodium salt + H]$^+$, 0.2); MALDI TOF MS (negative ion mode) *m/z* (rel int) 1363.3 ($C_{60}H_{92}O_{31}SNa$ [M − Na]$^-$, 1), 989.3 ([M − Na − 3-*O*-methylglucuronic acid − glucose + H]$^-$, 0.3), 843.3 ([M − Na - 3-*O*-methylglucuronic acid sodium salt − glucose − quinovose + H]$^-$, 0.2); HR MALDI TOF MS (positive ion mode) [M + Na]$^+$ at *m/z* 1409.5147, calculated for $C_{60}H_{92}O_{31}SNa_3$ as 1409.5036 *m/z*.

Synaptoside A_1 (2): mp 268–270 °C; $[\alpha]_D^{20}$ –3 (*c* 0.1, pyridine); for ^1H and ^{13}C NMR data, see Tables 1 and 4; MALDI TOF MS (positive ion mode) *m/z* (rel int) 1423.4 ($C_{60}H_{90}O_{32}SNa_3$ [M + Na]$^+$, 1), 1321.4 ([M + Na − SO_3Na + H]$^+$, 0.3), 1211.3 ([M + Na − 3-*O*-methylglucuronic acid sodium salt + H]$^+$, 0.2), 1109.3 ([M + Na − SO_3Na − 3-*O*-methylglucuronic acid + H]$^+$, 0.2); MALDI TOF MS (negative ion mode) *m/z* 1377.4 ($C_{60}H_{90}O_{32}SNa$ [M − Na]$^-$, 1), 1165.3 ([M − Na − 3-*O*-methylglucuronic acid sodium salt + H]$^-$, 0.3), 1003.3 ([M − Na − 3-*O*-methylglucuronic acid sodium salt − glucose + H]$^-$, 0.2), 857.3 ([M − Na − 3-*O*-methylglucuronic acid − glucose sodium salt − quinovose + H], 0.2); HR MALDI TOF MS (positive ion mode) [M + Na]$^+$ at *m/z* 1423.4694, calculated for $C_{60}H_{90}O_{32}SNa_3$ as 1423.4829 *m/z*.

Desulfation of Synaptoside A (1). A sample of synaptoside A (**1**, 5 mg) was dissolved in a mixture of pyridine/dioxane (1:1) and refluxed for 1 h. The obtained mixture was concentrated *in vacuo*. The residue was chromatographed on a Si gel column with $CHCl_3$/EtOH/H_2O (100:50:4) to give 4 mg of the desulfated derivative **3**; see Tables 1 and 3 for NMR data.

Methylation of Desulfated Derivative 3 with Diazomethane. A sample of derivative **3** (4 mg) was dissolved in 2 mL of MeOH and placed on a magnetic stirrer. A solution of CH_2N_2 (2 mL) in diethyl ether was added to the solution, and the mixture was concentrated *in vacuo*. The residue was chromatographed on a Si gel column with $CHCl_3$/EtOH/H_2O (100:75:10) to give 3.5 mg of the desulfated methylated derivative **4**; see Tables 1 and 3 for NMR data.

Synthesis of Methyl (Methyl-3-*O*-methyl-β-D-glucopyranoside)-uronate (5). To a solution of 0.49 g of commercial methyl-β-D-glucopyranoside in 10 mL of H_2O was added 15 mg Pt/carbon catalyst, and the reaction mixture was stirred during 5 h at 60 °C as atmospheric air bubbled through the reaction mixture. To the reaction mixture was added 2.5 mL of 1 N H_2O solution of $NaHCO_3$ in several portions, maintaining a pH of 7.0–8.5. The catalyst was removed by filtration, the solution was deionized by cation exchange resin KU-2 (H^+), and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in absolute MeOH, refluxed 1 h, and evaporated to dryness. The residue was chromatographed on a Si gel column (MeOH/$CHCl_3$, 1:9) to give 0.16 g of methyl (methyl-β-D-glucopyranoside)uronate, mp 149–150 °C, $[\alpha]_D^{20}$ –25.2 (*c* 0.8, MeOH). To the solution of 0.12 g of methyl (methyl-β-D-glucopyranoside)uronate in 1.5 mL of MeOH were added 0.01 mM $SbCl_3$ and 5 mL of a 0.5 N solution of CH_2N_2 in CH_2Cl_2. The solution was kept for 2 h at room temperature and evaporated *in vacuo*. The residue was chromatographed on a Si gel column (MeOH/$CHCl_3$, 0.5:9.5) to give 0.07 g of methyl (methyl-3-*O*-methyl-β-D-glucopyranoside)uronate (**5**), syrup, $[\alpha]_D20 = -22.4$ (*c* 0.5, MeOH); for ^{13}C NMR see Table 3.

Acid Hydrolysis and Isolation of Individual Monosaccharides from Synaptoside A (1). The acid hydrolysis of synaptoside A (**1**) (7 mg) was conducted 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 extracted with $CHCl_3$ (3 × 0.5 mL) and concentrated *in vacuo*. The mixture of resulting sugars was submitted to HPLC on a NH_2 9.4 column (4 × 250) with CH_3CN/H_2O/TFA (65:35:0.1) as the mobile phase to give only individual 3-*O*-methylglucuronic acid and a mixture of the remaining sugars. This mixture was rechromatographed on the same column with CH_3CN/H_2O (90:10) as the mobile phase to isolate xylose, quinovose, and glucose, identified as described below.

Synthesis of 3-*O*-Methyl-D-glucuronic Acid. 3-*O*-Methyl-1,2-isopropiliden-α-D-glucuronic acid (0.2 g) was obtained from commercial 1(2),5(6)-diisopropilidene-α-D-glucofuranoside by Haworth metalation[29] with $(CH_3)_2SO_4$ in NaOH solution followed by hydrolysis with CH_3COOH, and oxidation by atmospheric air expulsion through the reaction mixture in the presence of Pt/carbon catalyst as was described by Marsh[11] It was dissolved in 20% HCO_2H (6 mL), hydrolyzed for 1.5 h at 60 °C, and evaporated *in vacuo* to give 3-*O*-methyl-D-glucuronic acid: 0.16 g, syrup; $[\alpha]_D^{20}$ +39.1 (*c* 1.2, H_2O); ^{13}C NMR (D_2O) δ 173.8, 172.9, 96.7, 93.0, 85.3, 82.8, 75.1, 73.7, 71.5, 71.3, 71.2, 71.2, 60.8, 60.5.

Determination of the Absolute Configuration of Monosaccharides. One drop of concentrated TFA and 0.2 mL of (−)-2-octanol (Aldrich) were added to the dry residue of each sugar, and the ampules were sealed and then heated on a glycerol bath at 130 °C for 6 h. The derivatives obtained from each monosaccharide were evaporated *in vacuo* and treated with a mixture of pyridine/acetic anhydride (1:1, *V* = 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-glucuronic acid treated by the same procedure. The following peaks were detected: D-xylose (retention times 24.51, 24.70, and 25.02 min), D-quinovose (retention times 24.03, 24.24, 24.66, and 24.86 min), D-glucose (retention times 28.32, 29.01, 29.24, and 29.44 min), and 3-*O*-methyl-D-glucuronic acid (retention times 35.24, 35.52, and 36.14 min). Retention times of authentic samples were as follows: D-xylose (retention times 24.51, 24.71, and 25.00 min), D-quinovose (retention times 24.02, 24.24, 24.64, and 24.86 min), D-glucose (retention times 28.32, 29.03, 29.24, and 29.46 min), and 3-*O*-methyl-D-glucuronic acid (retention times 35.24, 35.52, and 36.14 min).

Tumor Cells Viability Assay. The effect of compounds **1** and **2** on cell viability was evaluated using MTS reduction into its formazan product.[30] The HeLa cells were cultured for 12 h in 96-well plates (6000 cells/well) in RPMI media (100 μL/well) containing 10% FBS. The media was replaced with 5% FBS-RPMI containing known concentrations of the compounds, and the cells were incubated for 22 h. A 20 μL amount of MTS reagent was added into each well, and MTS reduction was measured 2 h later spectrophotometrically at 492 and 690 nm as background using a μQuant microplate reader (Bio-Tek Instruments, Inc.). The means ± SD from six samples of two independent experiments were calculated. The statistical computer program Statistica 6.0 for Windows (StatSoft, Inc., 2001) was used to compute SD and IC_{50} in corresponding experiments. The IC_{50} value for **1** is 8.6 μg/mL. Glycoside **2** was not active in concentrations of 14.1 μg/mL.

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