Mollisosides A, B_1, and B_2: Minor Triterpene Glycosides from the New Zealand and South Australian Sea Cucumber *Australostichopus mollis*

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Three new monosulfated triterpene glycosides, mollisosides A (**2**), B_1 (**3**), and B_2 (**4**), have been isolated from the sea cucumber *Australostichopus mollis*. Their structures were determined by NMR and mass spectra. The presence of sulfated glycosides in sea cucumbers belonging to the family Stichopodidae is uncommon.

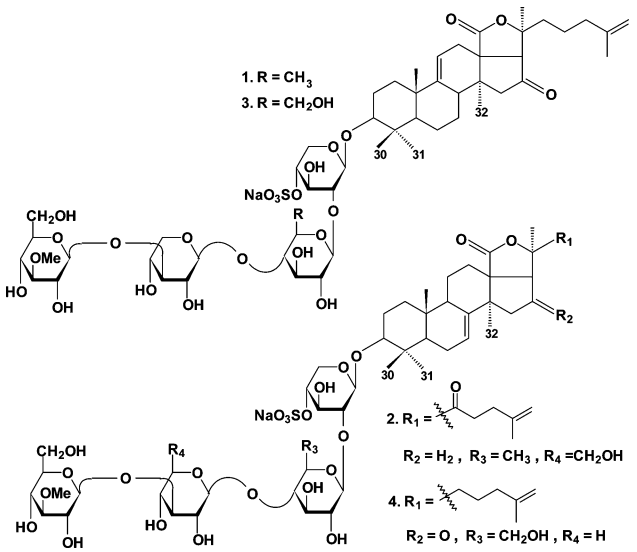
Sea cucumbers belonging to the family Stichopodidae (order Aspidochirotida, class Holothurioidea) are well known in Asia as trepang (edible holothurians) and are popular both as a culinary delicacy and for their medicinal properties. *Australostichopus* (=*Stichopus*) *mollis* (Hutton) is found in the shallow waters of New Zealand and southern Australia and has also been successfully farmed in New Zealand.

In an earlier publication we reported the isolation of the major triterpene glycoside (**1**) from this sea cucumber.[1] This compound was found to be identical to neothyonidioside reported earlier from the sea cucumber *Neothyonidium magnum* (Phyllophoridae, Dendrochirotida).[2] It was the first example of a sulfated glycoside isolated from sea cucumbers belonging to the family Stichopodidae.[1] On the basis of the chemical and morphological differences between *S. mollis* and the other studied species of the genus *Stichopus* and other representatives of the family Stichopodidae, we revised the systematic position of *S. mollis* and placed this species into a new genus, *Australostichopus* Levin.[1] In this paper we report the isolation of three new monosulfated triterpene glycosides, mollisosides A (**2**), B_1 (**3**), and B_2 (**4**), from the same animal.

Results and Discussion

The methanolic extract of *Australostichopus mollis* (717 g of frozen specimen) was sequentially chromatographed on HP20 (PSDVB), Amberchrom (PSDVB), and silica gel. The final separation and purification of individual compounds was achieved by reversed-phase HPLC on a Silasorb C_{18} column to give neothyonidioside (**1**)[1,2] and three novel triterpenes glycosides, mollisosides A (**2**), B_1 (**3**), and B_2 (**4**). Structures of these glycosides were elucidated by extensive analysis of 1D NMR (^{13}C, DEPT, ^{1}H) and 2D NMR (^{1}H−^{1}H COSY, NOESY, HSQC, HMBC) spectra and MALDI-TOF MS.

An inspection of the ^{13}C NMR spectrum revealed that the aglycon of mollisoside A (**2**) was similar to the aglycons of pseudostichoposides A and B (from *Pseudostichopus trachus*),[3] which have a 7(8)-double bond and a 22-keto group. The downfield resonance at 209.9 ppm for C-22 and



the HMBC correlations from H-21 to C-17, C-20, and C-22 confirm the position of the keto group in the side chain at C-22. The aglycon of **2** differs from the aglycon of pseudostichoposides A and B by the presence of a 25,26-double bond, as evidenced by the downfield signals at 144.4 and 110.5 ppm for C-25 and C-26, respectively. ^{1}H resonances for H_2-24 at 2.40 ppm t (J = 7.5 Hz), downfield resonances at 2.96 ppm dt (J = 18.5, 7.8 Hz) for H-23 and at 2.86 ppm dt (J = 18.5, 7.4 Hz) for H-23′, and the COSY correlations between these resonances show the presence of an isolated A_2XY spin system. The remaining part of the aglycon structure was confirmed by ^{1}H NMR spectra and ^{1}H−^{1}H COSY, HMBC, and NOESY experiments (Table 1). On the basis of the spectral data, the structure of the aglycon of mollisoside A (**2**) was assigned as 22-keto-holosta-7, 25-diene-3β-ol.

The ^{13}C NMR spectrum of the carbohydrate chain of mollisoside A (**2**) (Table 2) was identical to those of glycosides belonging to the holothurin A group, which have a linear tetrasaccharide carbohydrate chain containing D-xylose sulfated at C-4, D-quinovose, D-glucose, and D-3-*O*-methylglucose.[4−6] The presence of four monosaccharide units in the carbohydrate chain of glycoside **2** was deduced from the ^{13}C and ^{1}H NMR spectra, which showed four anomeric carbons at 104.7−105.6 ppm and corresponding

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

position	δ_C [a]	DEPT	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	36.1	CH_2	1.45 m		
2	27.0	CH_2	2.12 m		
			1.90 m		
3	89.0	CH	3.26 dd (3.7, 12.0)		H-5, H-31, H1-Xyl
4	39.4	qC			
5	47.7	CH	1.05 m		H-3, H-31
6	23.1	CH_2	2.05 m		
7	120.4	CH	5.68 m		
8	146.1	qC			
9	47.3	CH	3.42 brd (14.4)		H-19
10	35.4	qC			
11	22.5	CH_2	1.51 m		
			1.80 m		
12	29.3	CH_2	2.03 m		H-21
			1.96 m		
13	58.2	qC			
14	51.2	qC			
15	34.0	CH_2	1.75 m		
			1.65 m		
16	27.6	CH_2	2.16 m		
			1.43 m		
17	52.4	CH	2.65 dd (10.4, 5.0)		H-21, H-32
18	179.3	qC			
19	23.9	CH_3	1.22 s	C: 1,5, 9, 10	H-9
20	89.3	qC			
21	24.3	CH_3	1.57 s	C: 17, 20, 22	H-17, H-12
22	209.9	qC			
23	37.3	CH_2	2.96 dt (18.5, 7.8)		
		qC	2.86 dt (18.5, 7.4)		
24	30.6	CH_2	2.40 t (7.5)	C: 22, 23, 25, 26, 27	
25	144.4	qC			
26	110.5	CH_2	4.78 m		
27	22.4	CH_3	1.67 s	C: 24, 25, 26	
30	17.1	CH_3	1.11 s	C: 3, 4, 5, 31	H6-Qui
31	28.5	CH_3	1.27 s	C: 3, 4, 5, 30	H-3, H-5, H1-Xyl
32	30.6	CH_3	1.09 s	C: 8, 13, 14, 15	H-17

[a] Recorded at 125.77 MHz in C_5D_5N. [b] Recorded at 500 MHz in C_5D_5N.

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

position	δ_C [a,b]	DEPT	δ_H mult. (J in Hz)[c]	HMBC	NOESY
Xyl (1→C-3)					
1	104.9	CH	4.73 d (7.2)	C: 3	H-3, H-31, H3,5-Xyl
2	**83.4**	CH	4.01 m		H1-Qui
3	75.4	CH	4.32 t (8.7)	C: 2,4Xyl	H1,5-Xyl
4	*75.4*	CH	5.16 m		
5	64.2	CH_2	3.76 m		H1,3-Xyl
			4.78 m	C: 3Xyl	
Qui (1→2Xyl)					
1	105.2	CH	5.04 d (7.4)	C: 2Xyl	H2-Xyl, H3,5-Qui
2	76.0	CH	3.97 m		H4-Qui
3	75.4	CH	4.09 m	C: 2Qui	H1-Qui
4	**86.8**	CH	3.65 t (9.0)	C: 5Qui	H1-Glu, H2,6-Qui
5	71.6	CH	3.75 m		H1-Qui
6	17.9	CH_3	1.73 d (5.5)	C: 4,5Qui	H-30, H1-Glu, H4-Qui
Glu (1→4Qui)					
1	104.7	CH	4.99 d (7.8)	C: 4Qui	H4,6-Qui, H3,5-Glu
2	73.6	CH	4.10 m		
3	**87.7**	CH	4.27 t (9.0)	C: 1MeGlu	H1-Glu, H1-MeGlu
4	69.5	CH	4.11 t (9.0)		
5	77.7	CH	4.03 m		H1-Glu
6	61.8	CH_2	4.48 brd (11.6)		
			4.23 m		
MeGlu (1→3Glu)					
1	105.6	CH	5.33 d (7.9)	C: 3Glu	H3-Glu, H3,5-MeGlu
2	74.8	CH	4.04 m	C: 3MeGlu	
3	87.8	CH	3.72 t (8.5)	C: OMe, 4MeGlu	H1-MeGlu
4	70.3	CH	4.15 t (8.5)	C: 3MeGlu	
5	78.1	CH	3.98 m		H1-MeGlu
6	61.9	CH_2	4.48 brd (11.6)		
			4.30 m		
OMe	60.6	CH_3	3.87 s	C: 3MeGlu	

[a] Recorded at 125 MHz in C_5D_5N. [b] Bold = positions of interglycosidic linkages, italic = sulfate position. [c] Recorded at 500 MHz in C_5D_5N.

anomeric protons at 4.73 d (J = 7.2), 4.99 d (J = 7.8), 5.04 d (J = 7.4), and 5.33 d (J = 7.9) ppm (Table 2). The coupling constants of the anomeric protons were indicative of β-configurations of the glycosidic bonds.[7]

The interglycosidic linkages in the tetrasaccharide chain of **2** and its connectivity to the aglycon were confirmed by HMBC experiments (Table 2), which showed cross-peaks between H-1 of xylose and C-3 of the aglycon, H-1 of

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of Mollisoside B_1 (**3**)

position	δ_C [a]	DEPT	δ_H mult. (*J* in Hz)[b]	HMBC	NOESY
1	36.0	CH_2	1.39 m		
			1.82 m		
2	26.7	CH_2	1.91 m		
			2.16 m		
3	88.4	CH	3.19 dd (4.4, 11.1)		H-5, H-31, H1-Xyl1
4	39.5	qC			
5	52.6	CH	0.87 m		H-3
6	20.8	CH_2	1.44 m		
			1.59 m		
7	28.2	CH_2	1.54 m		
8	38.5	CH	3.23 m		H-15β, H-19
9	151.0	qC			
10	39.6	qC			
11	110.9	CH	5.30 m		
12	31.9	CH_2	2.48 m		H-17, H-21
13	55.5	qC			
14	41.8	qC			
15	51.8	CH_2	2.20 d (15.5)		H-8
			2.36 d (15.5)		H-32
16	212.9	qC			
17	61.1	CH	2.80 s		H-12, H-21, H-32
18	175.7	qC			
19	21.8	CH_3	1.35 s	C: 1,5, 9, 10	H-8, H-30
20	82.9	qC			
21	26.6	CH_3	1.39 s	C: 17, 20, 22	H-17, H-12
22	38.3	CH_2	1.65 m		
			1.81 m		
23	22.2	CH_2	1.52 m		
			1.81 m		
24	37.8	CH_2	1.98 m		
25	145.4	qC			
26	110.3	CH_2	4.78 brs		
27	22.1	CH_3	1.69 s	C: 24, 25, 26	
30	16.5	CH_3	1.03 s	C: 3, 4, 5, 31	H-19
31	27.9	CH_3	1.18 s	C: 3, 4, 5, 30	H-3, H1-Xyl1
32	20.4	CH_3	0.88 s	C: 8, 13, 14, 15	H-17, H-15α

[a] Recorded at 125.77 MHz in C_5D_5N. [b] Recorded at 500 MHz in C_5D_5N.

quinovose and C-2 of xylose, H-1 of glucose and C-4 of quinovose, and H-1 of 3-*O*-methylglucose and C-3 of glucose. These data were also confirmed by the NOESY spectrum. The pseudomolecular ion $[M_{Na} + Na]^+$ at *m/z* 1209.4825 in the positive-ion mode HR MALDI-TOF MS was consistent with the molecular formula $C_{54}H_{83}O_{25}SNa$ for **2**. The sequence of the monosaccharide residues in the sugar chain was also confirmed by ion peaks at *m/z* 987.5 $[M_{Na} - Na -$ 3-*O*-methylglucose $+ H]^-$, 825.5 $[M_{Na} - Na -$ 3-*O*-methylglucose $-$ glucose $+ H]^-$, and 679.3 $[M_{Na} - Na -$ 3-*O*-methylglucose $-$ glucose $-$ quinovose $+ H]^-$ in the negative-ion mode MALDI-TOF MS. The assignment of the sugars to the D-series was done by analogy with all known sea cucumber triterpene glycosides. All these data indicate that mollisoside A (**2**) is 3β-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodium sulfate-β-D-xylopyranosyl]-22-keto-holosta-7,25-diene.

The ^{13}C NMR spectral data of the aglycon part of mollisoside B_1 (**3**) (Table 3) were found to be identical to those of the aglycons which were first identified in holotoxins A_1 and B_1 (from *Apostichopus japonicus*) as holosta-9,25-dien-3β-ol-16-one.[8] This aglycon is widely distributed in sea cucumber glycosides[6] and was also found - in the glycoside **1** from A. mollis.[1] The structure of the aglycon of **3** was confirmed by ^{1}H NMR spectra and $^{1}H-^{1}H$ COSY, HMBC, and NOESY experiments (Table 3).

The ^{13}C NMR spectrum of the carbohydrate chain of mollisoside B_1 (**3**) was quite similar to that of eximisoside A (from *Psolus eximius*),[9] which has a linear tetrasaccharide chain containing D-xylose, D-glucose, D-xylose, and D-3-*O*-methylglucose (Table 4). A comparison of the ^{13}C NMR data of the two carbohydrate chains, however, revealed a downfield shift of 4.6 ppm for C-4 and an upfield shift of 2.8 and 2.5 ppm for C-3 and C-5, respectively, of the first xylose residue of **3**. These shifts account for the presence of a sulfate group at C-4 of the first xylose (α- and β-shift effects of a neighboring sulfate group).[7] The presence of four monosaccharide units in the sugar chain of glycoside **3** was deduced from the ^{13}C and ^{1}H NMR spectra, which showed four anomeric carbons at 104.7–105.3 ppm and corresponding anomeric protons at 4.75 d (J = 7.2), 5.11 d (J = 7.6), 5.21 d (J = 7.7), and 5.30 d (J = 7.9) ppm (Table 4). The coupling constants of the anomeric protons were indicative of β-configurations of the glycosidic bonds.[7]

The interglycosidic linkages in the tetrasaccharide chain of **3** and its bonding to the aglycon were established by HMBC experiments (Table 4), which showed cross-peaks between H-1 of the first xylose and C-3 of the aglycon, H-1 of glucose and C-2 of the first xylose, H-1 of the second xylose and C-4 of glucose, and H-1 of 3-*O*-methylglucose and C-3 of the second xylose residue. The positions of glycosidic bonds and the sequence of monosaccharide units were also confirmed by the NOESY spectrum. The molecular formula $C_{53}H_{81}O_{25}SNa$ was assigned for **3**, which was consistent with the pseudomolecular ion $[M_{Na} + Na]^+$ at *m/z* 1195.4516 in the positive-ion mode HR MALDI-TOF MS. The sequence of the monosaccharide residue in the sugar chain was further confirmed by ion peaks at *m/z* 973.5 $[M_{Na} - Na -$ 3-*O*-methylglucose $+ H]^-$, 841.4

Table 4. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugar Moiety of Mollisoside B_1 (**3**)

position	δ_C [a,b]	DEPT	δ_H mult. (*J* in Hz)[c]	HMBC	NOESY
Xyl1 (1→C-3)					
1	104.9	CH	4.75 d (7.2)	C: 3	H-3, H-31, H3,5-Xyl1
2	**82.6**	CH	4.12 t (8.2)	C: 4Xyl1	H1-Glu
3	75.1	CH	4.37 t (8.7)	C: 2,4Xyl1	H1,5-Xyl1
4	*75.5*	CH	5.15 m		
5	64.1	CH_2	3.78 m		H1,3-Xyl1
			4.78 m		
Glu (1→2Xyl1)					
1	105.1	CH	5.21 d (7.7)	C: 2Xyl1	H2-Xyl1, H3,5-Glu
2	76.2	CH	4.03 m		H4-Glu
3	75.3	CH	4.19 t (9.3)	C: 4,5Glu	H1-Glu
4	**80.1**	CH	4.38 t (9.3)		H2-Glu, H1-Xyl2
5	76.5	CH	3.80 m		H1,3-Glu
6	60.9	CH_2	4.41 dd (2.1, 11.0)		
			4.59 brd (11.0)		
Xyl2 (1→4Glu)					
1	104.7	CH	5.11 d (7.6)	C: 4Glu	H4-Glu, H3,5-Xyl2
2	73.5	CH	4.02 m		
3	**87.2**	CH	4.08 m	C: 4Xyl2	H1-Xyl2, H1-MeGlu
4	68.8	CH	4.08 m		
5	66.4	CH_2	3.59 t (10.5)		H1-Xyl2
			4.21 m		
MeGlu (1→3Xyl2)					
1	105.3	CH	5.30 d (7.9)	C: 3Xyl2	H3-Xyl2, H3,5-MeGlu
2	74.9	CH	4.01 m	C: 3MeGlu	
3	87.8	CH	3.73 t (9.0)	C: OMe, 2,4MeGlu	H1-MeGlu
4	70.4	CH	4.15 m		
5	78.1	CH	3.98 m		H1-MeGlu
6	61.8	CH_2	4.48 dd (2.2, 11.3)		
			4.28 dd (5.4, 11.3)		
OMe	60.6	CH_3	3.87 s	C: 3MeGlu	

[a] Recorded at 125 MHz in C_5D_5N. [b] Bold = positions of interglycosidic linkages, italic = sulfate position. [c] Recorded at 500 MHz in C_5D_5N.

$[M_{Na} - Na - 3\text{-}O\text{-methylglucose} - xylose + H]^-$, and 679.4 $[M_{Na} - Na - 3\text{-}O\text{-methylglucose} - xylose - glucose + H]^-$ in the negative-ion mode MALDI-TOF MS. All these data indicate that mollisoside B_1 (**3**) is 3*β*-*O*-[3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→2)-4-*O*-sodium sulfate-*β*-D-xylopyranosyl]-16-keto-holosta-9,25-diene.

The ^{13}C NMR spectral data of the aglycon part of mollisoside B_2 (**4**) (Table 5) were found to be identical to those of the aglycon of cucumarioside A_2-2 (from *Cucumaria japonica*), which has been previously identified as holosta-7,25-dien-3*β*-ol-16-one.[10] The structure of the aglycon of glycoside **4** was confirmed by the ^{1}H NMR spectra and $^{1}H-^{1}H$ COSY, HMBC, and NOESY experiments (Table 5).

The ^{13}C NMR spectrum of the carbohydrate chain of mollisoside B_2 (**4**) was identical to that of glycoside **3**, having the linear tetrasaccharide chain containing sulfated D-xylose, D-glucose, D-xylose, and D-3-*O*-methylglucose (Table 6). The presence of four monosaccharide units in the sugar chain of **4** was deduced from the ^{13}C and ^{1}H NMR spectra, which showed four anomeric carbons at 104.7–105.3 ppm and corresponding anomeric protons at 4.76 d (J = 7.1), 5.11 d (J = 7.6), 5.19 d (J = 7.8), and 5.28 d (J = 7.9) ppm (Table 6). The coupling constants of the anomeric protons were indicative of *β*-configurations of the glycosidic bonds.[7]

The interglycosidic linkages in the tetrasaccharide chain of **4** and its bonding to the aglycon were established by HMBC experiments (Table 6), which showed cross-peaks between H-1 of the first xylose and C-3 of the aglycon, H-1 of glucose and C-2 of the first xylose, H-1 of the second xylose and C-4 of glucose, and H-1 of 3-*O*-methylglucose and C-3 of the second xylose residue. The positions of glycosidic linkages and monosaccharide sequences were also confirmed by NOESY data. The molecular formula $C_{53}H_{81}O_{25}SNa$ was assigned to **4**, which is consistent with the pseudomolecular ion $[M_{Na} + Na]^+$ at *m/z* 1195.4502, observed in the positive-ion mode HR MALDI-TOF MS. The sequence of the monosaccharide residues in the sugar chain was further confirmed by the ion peaks at *m/z* 973.4 $[M_{Na} - Na - 3\text{-}O\text{-methylglucose} + H]^-$, 841.4 $[M_{Na} - Na - 3\text{-}O\text{-methylglucose} - xylose + H]^-$, and 679.3 $[M_{Na} - Na - 3\text{-}O\text{-methylglucose} - xylose - glucose + H]^-$ in the negative-ion mode HR MALDI-TOF MS. All these data confirm the structure of mollisoside B_2 (**4**) as 3*β*-*O*-[3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-*β*-D-xylopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→2)-4-*O*-sodium sulfate-*β*-D-xylopyranosyl]-16-keto-holosta-7,25-diene.

Mollisosides A (**2**), B_1 (**3**), and B_2 (**4**) are new natural products. The aglycon of mollisoside A (**2**) containing a 22-keto group and a 25(26)-terminal double bond in the side chain is novel for sea cucumber glycosides. Glycosides containing glucose as the second monosaccharide in the charbohydrate chain are very rare in sea cucumbers. Such carbohydrate moieties have previously been reported only in thelenotoside B (and its 25,26-dehydro derivative) from *Thelenota ananas*,[11] stichoposide D and its 25,26-dehydro analogue (also known as stichloroside B_1 and B_2)[12] isolated from sea cucumbers belonging to the genera *Astichopus*, *Stichopus*, and *Thelenota*,[6,13] eximisoside A from *Psolus eximius*,[9] and intercedenside C from *Mensamaria intercedens*.[14] Glycosides containing glucose as the second sugar unit and 4-O-sulfation at the first xylose residue in the carbohydrate chain have been previously reported only in *Mensamaria intercedens.* However, the formula of intercedenside C with an excessive CH_2 group between the monosaccharide cycle and sulfate group was erroneous.[14] Obviously, our finding of the glycosides having glucose as the second sugar unit and 4-O-sulfation at the first xylose residue in the carbohydrate chain may be considered as the first reliable one. The presence of a sulfate group in the carbohydrate chain, as in glycosides **2**, **3**, and **4**, is uncharacteristic for glycosides isolated from sea cucumbers belonging to the genus *Stichopus* and the family Sticho-

Table 5. ^{13}C and ^{1}H NMR Chemical Shifts and Selected HMBC and NOESY Correlations for the Aglycon Moiety of Mollisoside B_2 (**4**)

position	δ_C [a]	DEPT	δ_H mult. (J in Hz)[b]	HMBC	NOESY
1	35.7	CH_2	1.40 m		H-3
			1.45 m		
2	26.9	CH_2	1.87 m		
			2.09 m		
3	88.4	CH	3.25 dd (4.4, 11.6)		H-1, H-5, H-31, H1-Xyl1
4	39.3	qC			
5	48.7	CH	0.97 m		H-3
6	23.1	CH_2	1.94 m		
7	121.6	CH	5.63 m		H-15, H-32
8	143.9	qC			
9	46.9	CH	3.66 brd (13.0)		H-19
10	35.5	qC			
11	22.3	CH_2	1.52 m		H-32
			1.85 m		
12	29.4	CH_2	2.05 m		H-17, H-21
			2.24 m		
13	56.4	qC			
14	45.5	qC			
15	51.8	CH_2	2.45 brd (16.0)	C: 14, 29	
			2.36 d (16.0)	C: 13, 17, 32	H-7, H-32
16	212.7	qC			
17	63.4	CH	2.82 s	C: 12, 16, 18, 21	H-12, H-21, H-32
18	178.4	qC			
19	23.9	CH_3	1.20 s	C: 1,5, 9, 10	H-9
20	83.1	qC			
21	26.0	CH_3	1.43 s	C: 17, 20, 22	H-17, H-12
22	38.3	CH_2	1.68 m		
			1.81 m		
23	22.3	CH_2	1.52 m		
			1.85 m		
24	37.8	CH_2	2.00 m		
25	145.4	qC			
26	110.3	CH_2	4.79 brs	C: 24	
27	22.1	CH_3	1.70 s	C: 24, 25, 26	
30	17.2	CH_3	1.07 s	C: 3, 4, 5, 31	
31	28.5	CH_3	1.21 s	C: 3, 4, 5, 30	H-3, H1-Xyl1
32	31.7	CH_3	1.15 s	C: 8, 13, 14, 15	H-17, H-11, H-15, H-17

[a] Recorded at 125.77 MHz in C_5D_5N. [b] Recorded at 500 MHz in C_5D_5N.

Table 6. ^{13}C and ^{1}H NMR Chemical Shifts and HMBC and NOESY Correlations for the Sugar Moiety of Mollisoside B_2 (**4**)

position	δ_C [a,b]	DEPT	δ_H mult. (*J* in Hz)[c]	HMBC	NOESY
Xyl1 (1→C-3)					
1	104.9	CH	4.76 d (7.1)	C: 3	H-3, H-31, H3,5-Xyl1
2	**82.6**	CH	4.11 t (7.4)	C: 3Xyl1	H1-Glu
3	75.3	CH	4.37 t (8.0)	C: 2Xyl1	H1,5-Xyl1
4	*75.5*	CH	5.16 td (10.2, 5.5)		
5	64.1	CH_2	3.79 m	C: 3Xyl1	H1,3-Xyl1
			4.81 dd (5.5, 11.7)		
Glu (1→2Xyl1)					
1	105.3	CH	5.19 d (7.8)	C: 2Xyl1	H2-Xyl1, H3,5-Glu
2	76.2	CH	4.03 m		H4-Glu
3	75.2	CH	4.18 t (9.2)	C: 4Glu	H1,5-Glu
4	**80.1**	CH	4.38 t (9.5)	C: 1Xyl2, 3,5,6Glu	H2-Glu, H1-Xyl2
5	76.7	CH	3.80 m	C: 1Glu	H1-Glu
6	60.9	CH_2	4.41 brd (12.0)		
			4.59 brd (12.0)		
Xyl2 (1→4Glu)					
1	104.7	CH	5.11 d (7.6)	C: 4Glu	H4-Glu, H3,5-Xyl2
2	73.5	CH	4.02 m		
3	**87.2**	CH	4.07 m	C: 4Xyl2	H1-Xyl2, H1-MeGlu
4	68.8	CH	4.07 m		
5	66.4	CH_2	3.59 t (11.0)	C: 4Xyl2	H1-Xyl2
			4.20 dd (5.0, 11.0)		
MeGlu (1→3Xyl2)					
1	105.2	CH	5.28 d (7.9)	C: 3Xyl2	H3-Xyl2, H3,5-MeGlu
2	74.9	CH	4.01 m	C: 1MeGlu	
3	87.9	CH	3.73 t (9.0)	C: 2,4MeGlu	H1-MeGlu
4	70.4	CH	4.16 m		
5	78.1	CH	3.97 m		H1-MeGlu
6	61.9	CH_2	4.47 dd (2.2, 11.3)		
			4.27 dd (5.4, 11.3)		
OMe	60.6	CH_3	3.87 s	C: 3MeGlu	

[a] Recorded at 125 MHz in C_5D_5N. [b] Bold = positions of interglycosidic linkages, italic = sulfate position. [c] Recorded at 500 MHz in C_5D_5N.

podidae. The isolation of glycosides **2**, **3**, and **4** from *Australostichopus mollis* further justifies its assignment to a new genus as it was reported previously.[1]

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 the internal reference ($\delta = 0$). The HR MALDI-TOF MS (positive- and negative-ion modes) were recorded using a Bruker apparatus, model BIFLEX III, with impulse extraction of ions, on an α-cyano-4-hydroxycinnamic acid matrix. HPLC was performed using a Dupont-8800 chromatograph equipped with a RIDK-102 differential refractometer (Czechoslovakia) on a ODS C-18 Diasphere column (250 × 4 mm), MeOH/H_2O/$Na_2HPO_4 \cdot 12H_2O$ (540 mg in 100 mL of water) (60:39:1), 0.3 mL/min.

Animal Material. Specimens of *Australostichopus mollis* were collected in August 2002 by scuba divers R. Keyzers and D. Mazon in Wellington Harbor, New Zealand, near Mokopuna Island, 41°15′ S, 174°52′ E, depth 3.5−8.0 m, and kept in methanol at room temperature. The sea cucumber was identified by Dr. V. S. Levin (Pacific Institute of Bioorganic Chemistry, Vladivostok), and voucher specimens (PIBOC-NZ-01, -02, -03) are deposited at the Pacific Institute of Bioorganic Chemistry, Far East Division of the Russian Academy of Sciences, Vladivostok, Russian Federation.

Extraction and Isolation. A 717 g sample of frozen *A. mollis* was minced and extracted twice with methanol at room temperature. The methanolic extract was concentrated to dryness, and an aqueous solution was loaded onto a HP20 (PSDVB) column. The column was first washed with water to desalt the sample and then eluted with 60% acetone to yield 280 mg of a fraction, containing mainly glycosides. This fraction was repeatedly chromatographed on silica gel columns using chloroform/ethanol/water (100:75:10) to obtain 170 mg of a glycoside fraction. This fraction was further separated by HPLC under conditions described above and then desalted using a Teflon (or HP20) column to yield 50 mg of glycoside **1**, 1.2 mg of glycoside **2**, 1.2 mg of glycoside **3**, and 1.0 mg of glycoside **4**.

Mollisoside A (2): mp 233−235 °C; $[\alpha]^{20}_D$ −37° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 1 and 2; HR MALDI-TOF MS (positive-ion mode) *m/z* 1209.4825 $[M_{Na} + Na]^+$ (calcd for $C_{54}H_{83}O_{25}SNa_2$ 1209.4738).

Mollisoside B_1 (3): mp 245−247 °C; $[\alpha]^{20}_D$ −51° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 3 and 4; HR MALDI-TOF MS (positive-ion mode) *m/z* 1195.4516 $[M_{Na} + Na]^+$ (calcd for $C_{53}H_{81}O_{25}SNa_2$ 1195.4581).

Mollisoside B_2 (4): mp 240−242 °C; $[\alpha]^{20}_D$ −28° (*c* 0.1, pyridine); ^{13}C and ^{1}H NMR, see Tables 5 and 6; HR MALDI-TOF MS (positive-ion mode) *m/z* 1195.4502 $[M_{Na} + Na]^+$ (calcd for $C_{53}H_{81}O_{25}SNa_2$ 1195.4581).

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