Two New Cytotoxic Nonsulfated Pentasaccharide Holostane (=20-Hydroxylanostan-18-oic Acid γ-Lactone) Glycosides from the Sea Cucumber *Holothuria grisea*

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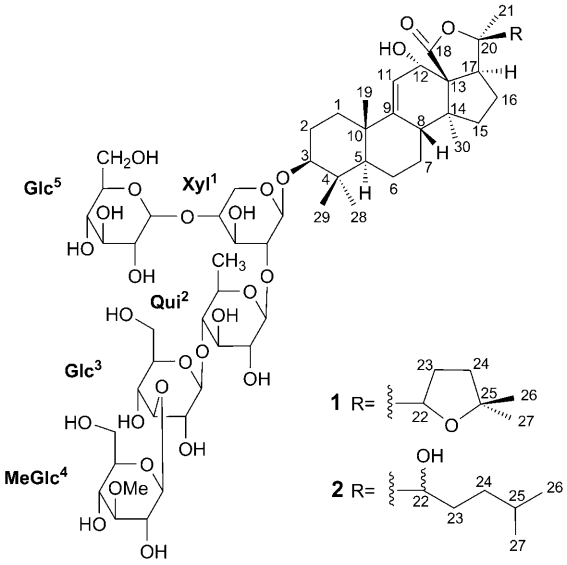
Two new lanostane-type nonsulfated pentasaccharide triterpene glycosides, 17-dehydroxyholothurinoside A (**1**) and griseaside A (**2**), were isolated from the sea cucumber *Holothuria grisea*. Their structures were elucidated by spectroscopic methods, including 2D-NMR and MS experiments, as well as chemical evidence. Compounds **1** and **2** possess the same pentasaccharide moieties but differ slightly in their side chains of the holostane-type triterpene aglycone. The structures of the two new glycosides were established as (3β,12α)-22,25-epoxy-3-{(*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[*O*-3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-β-D-glucopyranosyl-(1 → 2)]-β-D-xylopyranosyl)-oxy}-12,20-dihydroxylanost-9(11)-en-18-oic acid γ-lactone (**1**) and (3β,12α)-3-{(*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[*O*-3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-β-D-glucopyranosyl-(1 → 2)]-β-D-xylopyranosyl)oxy}-12,20,22-trihydroxylanost-9(11)-en-18-oic acid γ-lactone (**2**). The 17-dehydroxyholothurinoside A (**1**) and griseaside A (**2**) exhibited significant cytotoxicity against HL-60, BEL-7402, Molt-4, and A-549 cancer cell lines.

Introduction. – More than hundred triterpene glycosides found in holothurians have been described to date, and the majority of them are lanostane-type triterpenes with an 18,20-lactone moiety and a sugar chain linked to C(3) of the aglycone [1]. The carbohydrate chain consists mostly of disaccharides, tetrasaccharides, and hexasaccharides. These glycosides are the predominant secondary metabolites of holothurians and responsible for their general toxicity and have been reported to have a wide spectrum of biological activities, including cytotoxic, antifungal, hemolytic, and immunomodulatory effects [2][3]. *Holothuria grisea* SELENKA (family Holothuridae) is an abundant sea cucumber distributed from Florida south through the Bahamas to Brazil. Although previous studies of this sea cucumber have yielded a series of triterpene glycosides [4], knowledge on the constituents of *Holothuria grisea* remains fragmentary. Herein, we report the isolation and structure elucidation of two new constituents, 17-dehydroxyholothurinoside A (**1**) and griseaside A (**2**), as well as their potent cytotoxicities against various human tumor cell lines.

Results and Discussion. – The EtOH extract of *H. grisea* was partitioned between H_2O and BuOH. The BuOH extract was subjected to repeated chromatographic purification. Finally, reversed-phase HPLC on *Zobax SBC-18* afforded compounds **1** and **2**. DEPT and ^{13}C-NMR Spectral data of compounds **1** and **2** (*Table 1*) exhibited



that the carbohydrate moieties of these new glycosides were identical to each other and coincident with that of the earlier known holothurinoside A [5]. The relative configurations at all stereogenic C-atoms of the oligosaccharide chain were established by NOESY experiments, as shown in the *Figure*. Glycosides **1** and **2** were also treated with 2M CF_3COOH and gave D-quinovose (=6-deoxy-D-glucose; Qui), D-xylose (Xyl), 3-*O*-methyl-D-glucose (MeGlc), and D-glucose (Glc) in the ratio 1:1:1:2, together with a mixture of triterpene aglycones. The monosaccharides were identified by GC/MS in the form of the peracetates of the corresponding alditols [6].

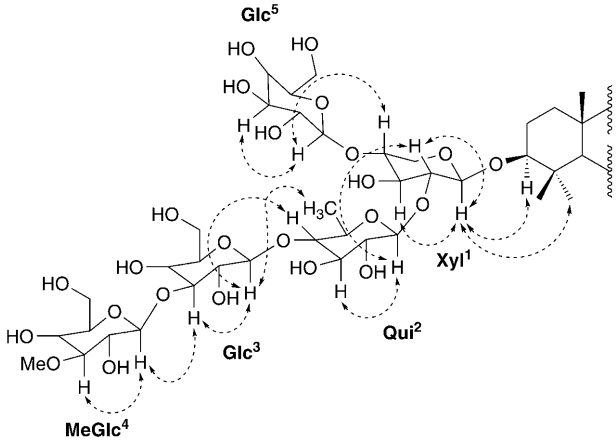


Figure. *^{1}H,^{1}H-NOESY of the carbohydrate moieties of* **1** *and* **2**

Table 1. *^{1}H- and ^{13}C-NMR Data and Selected HMBC Correlations of the Carbohydrate Moieties of* **1** *and* **2**. δ in ppm, J in Hz.

	δ(H)	δ(C)	^{1}H,^{13}C-HMBC
Xyl^1(1 → C(3))			
H–C(1)	4.78 (*d*, *J* = 7.2)	105.5	C(3)
H–C(2)	4.08–4.11 (*m*)	83.7	C(1)(Qui^2), C(1)(Xyl^1)
H–C(3)	4.29–4.33 (*m*)	76.0	C(2)(Xyl^1)
H–C(4)	4.07–4.10 (*m*)	78.1	C(1)(Glc^5)
CH$_2$(5)	3.68–3.72 (*m*), 4.43–4.46 (*m*)	64.4	C(1,3)(Xyl^1)
Qui^2(1 → C(2)(Xyl^1))			
H–C(1)	5.20 (*d*, *J* = 7.2)	105.7	C(2)(Xyl^1)
H–C(2)	4.18–4.20 (*m*)	76.1	C(4)(Qui^2)
H–C(3)	4.10–4.12 (*m*)	76.6	C(1)(Qui^2)
H–C(4)	3.70–3.73 (*m*)	87.6	C(1,6)(Glc^3)
H–C(5)	3.83–3.86 (*m*)	71.9	C(1,6)(Qui^2)
Me(6)	1.82 (*d*, *J* = 7.3)	18.4	C(4,5)(Qui^2)
Glc^3(1 → C(4)(Qui^2))			
H–C(1)	5.03 (*d*, *J* = 7.6)	103.5	C(4)(Qui^2)
H–C(2)	3.98–3.99 (*m*)	73.9	C(1,3)(Glc^3)
H–C(3)	4.28–4.32 (*m*)	88.3	C(1)(MeGlc^4), C(1)(Glc^3)
H–C(4)	4.07–4.11 (*m*)	70.1	C(3)(Glc^3)
H–C(5)	4.33–4.36 (*m*)	78.4	C(1)(Glc^3)
CH$_2$(6)	4.24–4.28 (*m*), 4.50–4.54 (*m*)	62.4	
MeGlc^4(1 → C(3)(Glc^3))			
H–C(1)	5.35 (*d*, *J* = 7.8)	105.8	C(3)(Glc^3)
H–C(2)	4.05–4.07 (*m*)	75.3	C(1)(MeGlc^4)
H–C(3)	3.76–3.79 (*m*)	88.2	C(4)(MeGlc^4), *Me*Glc^4
H–C(4)	4.20–4.24 (*m*)	70.8	C(5)(MeGlc^4)
H–C(5)	4.02–4.05 (*m*)	78.5	C(4)(MeGlc^4)
CH$_2$(6)	4.31–4.33 (*m*), 4.52–4.55 (*m*)	62.4	C(4)(MeGlc^4)
*Me*O–C(3)	3.92 (*s*)	61.0	C(3)(MeGlc^4)
Glc^5(1 → C(4)(Xyl^1))			
H–C(1)	5.09 (*d*, *J* = 7.4)	105.2	C(4)(Xyl^1)
H–C(2)	4.10–4.12 (*m*)	74.5	C(1)(Glc^5)
H–C(3)	3.90–3.93 (*m*)	79.0	C(2)(Glc^5)
H–C(4)	4.26–4.29 (*m*)	71.8	C(5)(Glc^5)
H–C(5)	4.28–4.31 (*m*)	77.5	C(4,6)(Glc^5)
CH$_2$(6)	4.37–4.39 (*m*), 4.60–4.63 (*m*)	62.7	C(4)(Glc^5)

The presence of five monosaccharide units in **1** and **2** was confirmed by the ^{13}C-NMR signals of the anomeric centers at δ(C) 105.5, 105.7, 103.5, 105.8, and 105.2, correlated by HMQC with the corresponding anomeric H-atoms at δ(H) 4.78 (*d*, *J* = 7.2 Hz), 5.20 (*d*, *J* = 7.2 Hz), 5.03 (*d*, *J* = 7.6 Hz), 5.35 (*d*, *J* = 7.8 Hz), and 5.09 ppm (*d*, *J* = 7.4 Hz). The large vicinal coupling constants of each anomeric H-atom indicated *trans*-diaxial orientations with respect to their coupling partners (β-configurations) [7]. A DQCOSY experiment allowed the sequential assignment of most of the resonances for each sugar moiety, starting from the easily distinguished signals due to the anomeric H-atoms. Complete assignments were achieved by a combination of HMQC, HMBC, DQCOSY, and TOCSY results. The HMQC experiment allowed us to correlate all sugar ^{1}H- and ^{13}C-NMR resonances. The data from these experiments (*Table 1*) indicated that the sugar residues were all in their pyranose form, and the D-configuration for the carbohydrate units was assumed also according to those most often encountered

among the sea-cucumber glycosides [8][9]. The interglycosidic linkages were deduced by analysis of the chemical shifts δ(C) for C(2) of Xyl^1 (83.7), for C(4) of Xyl^1 (78.1), for C(4) of Qui^2 (87.6), and for C(3) of Glc^3 (88.3), which were shifted downfield relative to the resonances expected for the corresponding methyl glycopyranosides [10]. The structure of the carbohydrate chain was corroborated by an HMBC experiment: a cross-peak between C(3) of the aglycone and H–C(1) of Xyl^1 indicated that this sugar residue was connected to C(3) of the aglycone. Similarly, the location of the interglycoside linkage in the oligosaccharide chain was confirmed on the basis of the cross-peaks H–C(1)(Qui^2)/C(2)(Xyl^1), H–C(1)(Glc^3)/C(4)(Qui^2), H–C(1)(MeGlc^4)/C(3)(Glc^3), and H–C(1)(Glc^5)/C(4)(Xyl^1). Therefore, the sugar sequence was elucidated as Glc-(1 → 4)-[MeGlc-(1 → 3)-Glc-(1 → 4)-Qui-(1 → 2)]-Xyl.

The 17-dehydroxyholothurinoside A (**1**), a colorless amorphous powder, tested positive in both the *Liebermann–Burchard* and *Molish* tests. The molecular formula was established as $C_{60}H_{96}O_{28}$ by the pseudomolecular ion $[M+Na]^+$ at *m/z* 1287.5991 in the HR-ESI-MS (positive-ion mode). The IR spectrum of **1** showed the presence of a γ-lactone moiety (1762), olefinic C=C bonds (1635), and strong, broad absorptions due to sugar OH functions (3376 cm^{-1}). The NMR data of the aglycon part of **1** (*Table 2*) were very close to those of the known holothurinoside A [5]. But the signals of C(16) and C(17) were shifted upfield (δ 23.9 and 47.9), correspondingly, due to the absence of an OH group at C(17). The absence of OH–C(17) was also confirmed by the upfield shift of the signal of C(20) (δ 83.8) [10]. On the basis of the ^{13}C-NMR spectrum of the aglycon part of **1**, it was concluded that this aglycon was identical to that of holothurin B_3 from the holothurian *H. polii* [11]. This structure was confirmed by extensive NMR spectroscopy (^{1}H- and ^{13}C-NMR, HMQC, HMBC, DQCOSY, TOCSY, and NOESY). In further confirmation of the carbohydrate-chain structure, the ESI-MS of **1** exhibited a pseudomolecular ion peak at *m/z* 1287 ($[M+Na]^+$) in the positive-ion mode, and a pseudomolecular ion peak at *m/z* 1263 ($[M-H]^-$) as well as peaks resulting from the loss of sugar moieties from the $[M-H]^-$ ion at *m/z* 1087 ($[M-H-MeGlc]^-$), 925 ($[1087-Glc]^-$), 763 ($[925-Glc]^-$), and 617 ($[763-Qui]^-$) in the negative-ion mode. All these data including results of the 2D-NMR experiments indicate that 17-dehydroxyholothurinoside A (**1**) is (3β,12α)-22,25-epoxy-3-{(*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[*O*-3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-β-D-glucopyranosyl-(1 → 2)]-β-D-xylopyranosyl)oxy}-12,20-dihydroxylanost-9(11)-en-18-oic acid γ-lactone.

Griseaside A (**2**), a colorless amorphous powder, tested positive in both the *Liebermann–Burchard* and *Molish* tests. The molecular formula was established as $C_{60}H_{98}O_{28}$ by the pseudomolecular ion $[M+Na]^+$ at *m/z* 1289.6139 in the HR-ESI-MS (pos.). ^{13}C-NMR and DEPT spectra of **2** (*Table 3*) showed that the signals of C(1) to C(18) are coincident with those of the aglycon part of 17-dehydroxyholothurinoside A (**1**). The ^{13}C-NMR spectrum of the side chain of the aglycon moiety of **2** was identical to that of the tetraoside holothurin A_1 [4]. The configuration at C(22) was not established.

The presence of an OH group at C(12) of **2** was indicated by the characteristic signal at δ 68.5. The Me(21) (δ(H) 1.90) exhibited a NOESY correlation with H_β–C(12) (δ(H) 4.72 (J = 6.1)), which indicated the α-configuration of the OH group at C(12) [12] (*Table 3*). On the other hand, the signals of the side chain differed from those of **1** by the absence of the signals at δ(C) 80.4 and 81.4, characteristic for a 22,25-epoxy group. Moreover, the signal of C(22) was observed at δ(C) 75.2, which indicated the presence of an OH group at this position of the side chain of griseaside A (**2**).

Table 2. *^{1}H- and ^{13}C-NMR Data and Selected HMBC and NOESY Correlations of the Aglycon Moieties of* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	^{1}H,^{1}H-NOESY	^{1}H,^{13}C-HMBC
CH_2(1)	1.53–1.57, 1.92–1.98 (2*m*)	36.7		
CH_2(2)	2.19–2.23, 1.98–2.03 (2*m*)	27.3	Me(19)	
H–C(3)	3.24 (*dd*, *J* = 4.0, 11.6)	88.8	H–C(1)(Xyl^1), H–C(5), Me(28)	C(1)(Xyl^1), C(4)
C(4)		40.2		
H–C(5)	1.09 (*d*, *J* = 6.9)	53.0	H–C(3), Me(28)	C(6), C(19), C(28)
CH_2(6)	1.62–1.66, 1.81–1.85 (2*m*)	21.4		
CH_2(7)	1.52–1.54, 1.85–1.89 (2*m*)	29.0		
H–C(8)	3.50–3.53 (*m*)	40.3	CH_2(15), Me(19)	C(9), C(11), C(14)
C(9)		153.4		
C(10)		39.8		
H–C(11)	5.82 (*d*, *J* = 5.1)	116.3	H–C(12), Me(19)	C(8), C(10), C(12), C(13)
H–C(12)	4.65 (*d*, *J* = 4.0)	68.1	H–C(11), H–C(12)	C(9), C(11), C(14), C(18)
C(13)		64.0		
C(14)		46.4		
CH_2(15)	1.51–1.55, 1.74–1.77 (2*m*)	37.5		
CH_2(16)	2.14–2.19, 2.43–2.48 (2*m*)	23.9	Me(30)	
H–C(17)	3.46–3.50 (*m*)	47.9	Me(21), H–C(24)	C(12), C(13), C(18), C(21)
C(18)		177.7		
Me(19)	1.50 (*s*)	22.7	CH_2(2), H–C(8), H–C(11), Me(29)	C(1), C(5), C(9), C(10)
C(20)		83.8		
Me(21)	1.69 (*s*)	21.0	H–C(12), H–C(17)	C(17), C(20), C(22)
H–C(22)	4.30–4.33 (*m*)	80.4		C(17), C(20), C(21)
CH_2(23)	1.97–2.03 (*m*)	27.3		C(20), C(24), C(25)
CH_2(24)	2.09–2.23, 1.70–1.75 (2*m*)	38.8	H–C(17)	C(22), C(23), C(26), C(27)
C(25)		81.4		
Me(26)	1.34 (*s*)	28.8		C(24), C(25), C(27)
Me(27)	1.30 (*s*)	27.5		C(24), C(25), C(26)
Me(29)	1.19 (*s*)	16.9	Me(19)	C(3), C(4), C(5), C(28)
Me(28)	1.35 (*s*)	28.3	H–C(3), H–C(5)	C(3), C(4), C(5), C(29)
Me(30)	1.38 (*s*)	22.3	H–C(16)	C(8), C(13), C(14), C(15)

The ESI-MS of **2** exhibited a pseudomolecular ion peak at *m*/*z* 1289 ($[M + Na]^+$) in the positive-ion mode, and pseudomolecular ion peaks at *m*/*z* 1265 ($[M - H]^-$) as well as peaks resulting from the loss of sugar moieties from the $[M - H]^-$ ion at *m*/*z* 1089 ($[M - H - MeGlc]^-$), 927 ($[1089 - Glc]^-$), 765 ($[927 - Glc]^-$), and 619 ($[765 - Qui]^-$) in the negative-ion mode. All these data including results of the 2D-NMR experiments indicate that griseaside A (**2**) is (3β,12α)-3-{(*O*-β-D-glucopyranosyl-(1 → 4)-*O*-[*O*-3-*O*-methyl-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 4)-6-deoxy-β-D-glucopyranosyl-(1 → 2)]-β-D-xylopyranosyl)oxy}-12,20,22-trihydroxylanost-9(11)-en-18-dioic acid γ-lactone.

Glycosides **1** and **2** were tested for *in vitro* cytotoxicity against four human tumor cell lines (A549, HL-60, BEL-7402, and Molt-4) by using the sulforhodamine B (SRB) method [13]; 10-hydroxycamptothecine (HCP) was used as reference compound (IC_{50}

Table 3. *^{1}H- and ^{13}C-NMR Data and Selected HMBC and NOESY Correlations of the Aglycon Moieties of* **2**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	^{1}H,^{1}H-NOESY	^{1}H,^{13}C-HMBC
CH_2(1)	1.54–1.58, 1.95–1.97 (2*m*)	36.6		C(3)
CH_2(2)	2.23–2.27 (*m*)	27.3	Me(19)	
H–C(3)	3.20–3.23 (*m*)	88.9	H–C(1)(Xyl^1), H–C(5), Me(28)	C(1)(Xyl^1), C(4)
C(4)		40.2		
H–C(5)	1.09 (*d*, *J* = 5.8)	53.0	H–C(3), Me(28)	C(6), C(19), C(28)
CH_2(6)	1.89–1.93 (*m*)	21.4		
CH_2(7)	1.78–1.82 (*m*)	29.1		
H–C(8)	3.53–3.57 (*m*)	40.4	CH_2(15), Me(19)	C(9), C(11), C(14)
C(9)		153.3		
C(10)		39.8		
H–C(11)	5.81 (*d*, *J* = 4.6)	116.4	H–C(12), Me(19)	C(8), C(12), C(13)
H–C(12)	4.72 (*d*, *J* = 6.1)	68.5	H–C(11), H–C(12)	C(9), C(11), C(14), C(18)
C(13)		64.2		
C(14)		46.5		
CH_2(15)	1.58–1.62, 1.77–1.79 (2*m*)	37.5		
CH_2(16)	2.41–2.45, 2.97–3.03 (2*m*)	23.9	Me(30)	
H–C(17)	3.66–3.71 (*m*)	47.9	Me(21), H–C(24)	C(13), C(14), C(18)
C(18)		177.9		
Me(19)	1.52 (*s*)	22.7	CH_2(2), H–C(8), H–C(11), Me(29)	C(1), C(5), C(9), C(10)
C(20)		85.8		
Me(21)	1.90 (*s*)	21.0	H–C(12), H–C(17)	C(17), C(20), C(22)
H–C(22)	4.05–4.08 (*m*)	75.2	H–C(23)	C(17), C(20), C(21)
CH_2(23)	1.84–1.88, 1.96–1.99 (2*m*)	31.0	H–C(22)	C(20), C(24), C(25)
CH_2(24)	1.40–1.43, 1.87–1.89 (2*m*)	36.5	H–C(17)	C(22), C(23), C(26), C(27)
H–C(25)	1.61–1.63 (*m*)	28.7		
Me(26)	0.98 (*s*)	23.1		C(24), C(25), C(27)
Me(27)	1.33 (*s*)	22.8		C(24), C(25), C(26)
Me(29)	1.19 (*s*)	16.9	Me(19)	C(3), C(4), C(5), C(28)
Me(28)	1.47 (*s*)	28.3	H–C(3), H–C(5)	C(3), C(4), C(5), C(29)
Me(30)	1.43 (*s*)	22.3	H–C(16)	C(8), C(13), C(14), C(15)

values in *Table 4*). The results showed that **1** and **2** exhibited significant cytotoxicity against the four cell lines with IC_{50} values in the range of 0.245–1.114 μM. Comparison of the activities of **1**, **2**, and the positive control showed that **1** and **2** are more active than HCP on these four cell lines. Based on these initially promising results, 17-dehydroxyholothurinoside A (**1**) and griseaside A (**2**) deserve further study as potent antitumor agents.

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Table 4. IC_{50} *Values of Compounds* **1** *and* **2** *against Human Tumor Cell Lines* in vitro

	IC_{50} [μM][a])			
	A-549	BEL-7402	HL-60	Molt-4
1	0.886 ± 0.042	0.973 ± 0.038	0.245 ± 0.006	0.340 ± 0.009
2	1.074 ± 0.053	1.114 ± 0.062	0.427 ± 0.009	0.521 ± 0.014
HCP[b])	2.335 ± 0.088	2.610 ± 0.133	1.978 ± 0.083	2.252 ± 0.083

[a]) IC_{50} Values are means from independent experiments (mean ± s.d.). [b]) Positive control.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; *Yantai*, P. R. China) and *Lobar Lichroprep RP-18* (40–63 μm; *Merck*). TLC: precoated silica gel GF_{254} plates (10–40 μm; *Yantai*), detection by spraying with 10% aq. H_2SO_4 soln. followed by heating. HPLC: *Agilent 1100* system equipped with a refractive-index detector; *Zorbax-300-SB-C18* column (250 × 9.4 mm i.d., 5 μm). M.p.: *XT5-XMT* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Bruker Vector-22* IR spectrometer; KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Varian Inova-600* spectrometer, at 600 and 150 MHz; δ in ppm, J in Hz; assignments supported by $^1H,^1H$-COSY, HMQC, HMBC, and NOESY experiments. ESI- and HR-ESI-MS: *Waters Q-TOF-Micromass* spectrometer. GC/MS: *Finnigan Voyager* apparatus with a *DB-5* column (0.25 mm × 30 m i.d.); in *m/z*. Cell lines: A-549 and Molt-4: ATCC; BL-60 and BEL-7402: Institute of Biochemistry and Cell Biology, SIBS, CAS.

Animal Material. Specimens of *H. grisea* were collected from Guangzhou (Guangdong Province) in the South China Sea in November 2005. The organisms were identified by Professor *Yu-Lin Liao* (Institute of Oceanology, Chinese Academy of Science, P. R. China). A voucher specimen was deposited with the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai, China, under the registration code number HG-2005-11.

Extraction and Isolation. The sea cucumbers (2.5 kg, dry weight) were crumbled and extracted with 65% aq. EtOH soln. (5 × 5 l) for 2 h each. The combined extract was concentrated, and the residue was dissolved in H_2O (4 l) and then partitioned with BuOH (6 × 4 l). The BuOH fraction (46 g) was subjected to CC (silica gel, $CHCl_3$/MeOH/H_2O 9:1:1 → 6.5:3.5:1 (lower phase)): *Fractions A–I. Fr. I* (7.9 g) mainly contained triterpene glycosides and was subjected to LPLC (*Lobar* column (*Lichroprep RP-18*, 40–63 μm; *Merck*), MeOH/H_2O 45:55 → 80:20): *Fr. I_1* and *I_2*. *Fr. I_1* (960 mg) was further purified by repeated semi-prep. HPLC (*Zorbax-300-SB-C18*; 67% aq. MeOH soln., 2 ml/min): pure **1** (860 mg, t_R 28.3 min). *Fr. I_2* was further purified by HPLC (*Zorbax-300-SB-C_{18}*; 59% aq. MeOH soln., 2 ml/min): pure **2** (15 mg, t_R 27.2 min).

17-Dehydroxyholothurinoside A (**1**): Colorless amorphous powder. M.p. 225–227°. $[\alpha]_D^{20} = -11.2$ (c = 0.785, MeOH). IR (KBr): 3376 (OH), 1762 (C=O), 1635 (C=C). ^1H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 1287 ($[M+Na]^+$). ESI-MS (neg.): 1263 ($[M-H]^-$), 1087 ($[M-H-MeGlc]^-$), 925 ($[1087-Glc]^-$), 763 ($[925-Glc]^-$), 617 ($[763-Qui]^-$). HR-ESI-MS (pos.): 1287.5991 ($[M+Na]^+$, $C_{60}H_{96}O_{28}Na^+$; calc. 1287.5986).

Griseaside A (**2**): Colorless amorphous powder. M.p. 230–232°. $[\alpha]_D^{20} = -10$ (c = 0.10, MeOH). IR (KBr): 3445 (OH), 1764 (C=O), 1632 (C=C). ^1H- and ^{13}C-NMR: *Tables 1* and *3*. ESI-MS (pos.): 1289 ($[M+Na]^+$). ESI-MS (neg.): 1265 ($[M-H]^-$), 1089 ($[M-H-MeGlc]^-$), 927 ($[1089-Glc]^-$), 765 ($[927-Glc]^-$), 619 ($[765-Qui]^-$). HR-ESI-MS (pos.): 1289.6139 ($[M+Na]^+$, $C_{60}H_{98}O_{28}Na^+$; calc. 1289.6142).

Acid Hydrolysis. The glycoside **1** or **2** (1 mg) was heated in 2M aq. CF_3COOH (1 ml) at 120° for 2 h. The mixture was concentrated and the residue partitioned between CH_2Cl_2 and H_2O. The aq. phase was evaporated to furnish a monosaccharide mixture. Pyridine (1 ml) and $NH_2OH \cdot HCl$ (2 mg) were added to the dried residue, and the mixture was heated at 90° for 30 min. Then, Ac_2O (0.8 ml) was added, and heating at 90° was continued for 1 h. The resulting soln. was concentrated, and the formed aldononitrile

peracetates were analyzed by GC/MS by using authentic reference samples for identification. The derivatives of D-xylose (Xyl), D-quinovose (Qui), D-glucose (Glc), and 3-*O*-methyl-D-glucose (MeGlc) were detected with the retention times (t_R) 5.53, 5.44, 6.76, and 6.57 min, resp. Glycoside **1** and **2** gave peaks of the derivatives of D-Xyl, D-Qui, D-Glc, and MeGlc in a ratio of 1:1:2:1 [9].

REFERENCES

[1] V. A. Stonik, G. B. Elyakov, in 'Bioorganic Marine Chemisty', Ed. P. J. Scheuer, Springer-Verlag, Berlin, 1988, p. 43–88.
[2] H. Chludil, C. C. Muniain, A. M. Seldes, M. S. Maier, *J. Nat. Prod*. **2002**, *65*, 860.
[3] M. S. Maier, A. J. Roccatagliata, A. Kuriss, H. Cludil, A. M. Seldes, C. A. Pujol, E. B. Damonte, *J. Nat. Prod*. **2001**, *64*, 732.
[4] G. K. Oleinikova, T. A. Kuznetsova, N. S. Ivanova, A. I. Kalinovskii, N. V. Rovnykh, G. B. Elyakov, *Chem. Nat. Compd.* 1**983**, *18,* 501.
[5] J. Rodriguez, R. Castro, R. Riguera, *Tetrahedron* **1991**, *47*, 4753.
[6] H. F. Tang, Y. H. Yi, L. Li, P. Sun, S. Q. Zhang, Y. P. Zhao, *Planta Med*. **2005**, *71*, 458.
[7] F. S. Shashkov, O. S. Chizhov, *Bioorg. Khim*. **1976**, *2*, 437.
[8] V. A. Stonik, V. I. Kalinin, S. A. Avilov, *J. Nat. Toxins* **1999**, *8*, 235.
[9] S. A. Avilov, V. I. Kalinin, A. V. Smirnov, *Biochem. Syst. Ecol*. **2004**, *32*, 715.
[10] E. Breitmaier, W. Voelter, 'Carbon-13 NMR Spectroscopy', VCH, Weinheim, 1987.
[11] A. S. Silchenko, V. A. Stonik, S. A. Avilov, V. I. Kalinin, A. I. Kalinovsky, A. M. Zaharenko, A. V. Smirnov, E. Mollo, G. Cimino, *J. Nat. Prod*. **2005**, *68*, 564.
[12] I. Kitagawa, T. Nishino, T. Matsuno, H. Akutsu, Y. Kyogoku, *Tetrahedron Lett*. **1978**, *11*, 985.
[13] P. Skehan, R. Storeng, D. Scudiero, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst*. **1990**, *82*, 1107.

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