Cytotoxic and Antifungal Triterpene Glycosides from the Patagonian Sea Cucumber *Hemoiedema spectabilis*

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Two new sulfated triterpene glycosides, hemoiedemosides A (**1**) and B (**2**), have been isolated from the Patagonian sea cucumber *Hemoiedema spectabilis*. Their structures have been established by a combination of spectroscopic analysis (NMR and FABMS) and chemical transformations. Both glycosides present the same aglycon and differ in the degree of sulfation of the tetrasaccharide chain. Hemoiedemoside B (**2**) is a new example of a small number of trisulfated triterpene glycosides from sea cucumbers belonging to the family Cucumariidae. Glycosides **1** and **2** exhibit considerable antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum*, while the semisynthetic desulfated derivative **1a** is less active.

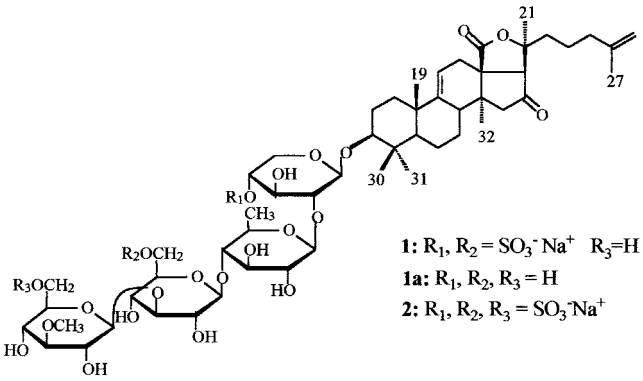
Sea cucumbers (class Holothuroidea) have been shown to contain a variety of triterpene glycosides of the lanosterol type with a 18(20) lactone and a sugar chain of up to six monosaccharide units linked to C-3 of the aglycon.[1] These substances have a wide spectrum of biological effects, including antifungal, cytotoxic, hemolytic, cytostatic, and immunomodulatory activities.[2] Recently, we have demonstrated the virucidal activity of two new trisulfated triterpene glycosides from the Antarctic sea cucumber *Staurocucumis liouvillei*.[3]

As a continuation of our search for new bioactive compounds from cold water echinoderms of the South Atlantic,[4−6] we have investigated the polar extracts of the sea cucumber *Hemoiedema spectabilis* (Ludwig 1882) (Dendrochirotida, Cucumariidae) collected off the Patagonian coast of Argentina. We report here the isolation and structure elucidation of two new sulfated triterpene glycosides, hemoiedemosides A (**1**) and B (**2**), as well as the results of the cytotoxic and antifungal activities of these compounds and their semisynthetic desulfated derivative.

Results and Discussion

The *n*-BuOH extract of *H. spectabilis* exhibited brine shrimp toxicity and antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum*. Antifungal bioautographic assay-guided fractionation of the extract led to the isolation of an active fraction containing sulfated triterpene glycosides. Purification of this fraction by Si gel C_{18} reversed-phase chromatography and finally by reversed-phase HPLC on Bondclone C_{18} yielded two new sulfated triterpene glycosides, hemoiedemosides A (**1**) and B (**2**). Both compounds present the same aglycon moiety and differ in the degree of sulfation of the oligosaccharide chain.

Hemoiedemoside A (**1**) was obtained as a white amorphous powder. An examination of the ^{1}H and ^{13}C NMR spectra of **1**, the major component of *H. spectabilis*, suggested the presence of a triterpenoid aglycon with two olefinic bonds, a keto group, and one lactone carbonyl group



bonded to an oligosaccharide chain composed of four sugar units. The assignment of the NMR signals associated with the aglycon moiety (Table 1) was derived from ^{1}H−^{1}H COSY, HETCOR, and NOESY experiments and showed a close similarity to those reported for 3β-hydroxyholosta-9(11),25-dien-16-one, the aglycon of several triterpene glycosides isolated from the sea cucumbers *Stichopus japonicus*,[7] *Psolus fabricii*,[8] *Pentacta australis*,[9] *Neothyonidium magnum*,[10] and *Cladolabes* sp.[11] It is to be noted that ^{1}H NMR signals of this aglycon were unambiguously assigned for the first time by application of the standard 2D NMR methods mentioned above.

The ^{1}H and ^{13}C NMR and DEPT spectra of **1** showed resonances for a keto group at δ_C 213.4 (s, C-16), a carbonyl lactone group at δ_C 176.1 (s, C-18), a trisubstituted 9(11)-double bond [δ_C 151.0 (s, C-9) and 111.0 (d, C-11); δ_H 5.32 (1H, bd, J = 5.3, H-11)], and a disubstituted terminal double bond [δ_C 145.4 (s, C-25) and 110.4 (t, C-26); δ_H 4.75 (2H, m, H-26)]. The ^{1}H NMR spectrum also showed a vinyl methyl signal at δ 1.68 (s, H-27) and five methyl groups characteristic of a holostane skeleton at δ 0.92 (s, H-32), 1.01 (s, H-30), 1.20 (s, H-31), 1.38 (s, H-19), and 1.44 (s, H-21). ^{1}H−^{1}H COSY and HETCOR spectra allowed the assignment of all proton and carbon resonances. The relative stereochemistry of all chiral centers of the aglycon was established with the aid of a NOESY experiment. Thus, as shown in Figure 1, H-3 showed correlations with H-1′, H-5, and H_3-31, confirming the β configuration at C-3. Correlations between H_3-19/H_3-30 and H_3-19/H-8β allowed

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Table 1. ^{1}H and ^{13}C NMR Data for the Aglycon Moiety of Hemoiedemosides A (**1**) and B (**2**)

carbon	δ_C mult[a]	δ_H mult[b] (J in Hz)	^{1}H−^{1}H COSY correlations	^{1}H−^{1}H NOESY correlations
1	36.1 t	Hα 1.50 m	H-1β	
		Hβ 1.82 m	H-1α, H-2β	
2	26.8 t	Hα 1.93 m	H-2β, H-1β	H_3-19
		Hβ 2.13 m	H-3α, H-2α, H-1α	
3	88.4 d	3.17 dd (4.1, 11.9)	2.13, H-2β	H-1′, H-5, H_3-31
4	39.5 s[c]			
5	52.7 d	0.90 m	H-6α, H-6β	
6	20.9 t	1.48 m, 1.68 m		
7	28.3 t	1.64 m		
8	38.6 d	3.22 bd (12.5)		H-15β, H_3-19
9	151.0 s			
10	39.7 s[c]			
11	111.0 d	5.32 bd (5.3)	H-12β	H_3-19, H-1β
12	31.9 t	Hα 2.58 bd (17.5)	H-12β	H-17, H_3-21, H-12β, H-11
		Hβ 2.51 dd (17.5, 5.5)	H-12α, H-11	H-12α, H_3-21, H-11
13	55.7 s			
14	41.9 s			
15	51.9 t	Hα 2.44 d (15.5)	H-17, H-15β	
		Hβ 2.21 d (15.5)	H-15α, H_3-32	
16	213.4 s			
17	61.2 d	2.87 s	H-15α, H-15β	H-12α, H_3-21, H_3-32
18	176.1 s			
19	21.9 c	1.38 s	H-1α	H_3-30, H-11, H-1β, H-2β
20	83.2 s			
21	26.7 c	1.44 s		H-12β, H-17
22	38.3 t			
23	22.2 t	1.55 m	H_2-24	
24	37.9 t	1.97 m	H_2-23	
25	145.4 s			
26	110.4 t	4.75 m	H_3-27, H_2-24	
27	22.1 c	1.68 s	H_2-26	
30	16.4 c	1.01 s	H_3-31	H_3-19
31	27.8 c	1.20 s	H_3-30	H-3α, H-5
32	20.5 c	0.92 s	H-15β	H-17, H-15α

[a] Recorded at 125 MHz in pyridine-d_5−D_2O (5:1); multiplicity by DEPT. [b] Recorded at 500 MHz in pyridine-d_5−D_2O (5:1). [c]Assignments may be reversed.

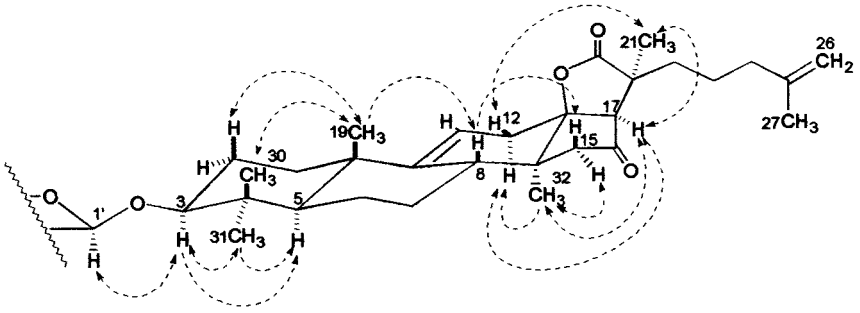

Figure 1. NOESY correlations of the aglycon moiety of hemoiedemoside A (**1**).

unambiguous assignment of the angular methyl group, while correlations between H-12β/H_3-21 and H-17/H_3-21 confirmed the *S* configuration at C-20. Correlation of H-8β with a signal at δ 2.44, corresponding to one of the H-15 protons, allowed assignment of this signal to H-15β. Further correlations between H-12α/H-17, H-17/H_3-32, and H-12α/H_3-32 indicated that they were on the same side of the molecule and allowed unambiguous assignment of H_3-32.

In addition to the aglycon signals, the ^{1}H NMR spectrum (Table 2) showed four anomeric proton signals at δ 5.29 (d, J = 7.8 Hz, 3-*O*-methylglucose), 4.92 (d, J = 7.7 Hz, quinovose), 4.76 (d, J = 7.7 Hz, glucose), and 4.69 (d, J = 7.1 Hz, xylose), a doublet at δ 1.63 (J = 6.1 Hz) due to the methyl group of the quinovose unit, and a singlet at δ 3.85 ppm corresponding to the methoxy group of 3-*O*-methylglucose. Signals at δ_C 17.8 (c, C-6″) and 60.6 (c, OCH_3) in the ^{13}C NMR spectrum support the presence of a 6-deoxyhexose and a methoxy group. The β stereochemistries at the anomeric carbons were deduced from the coupling constant values (J = 7.1−7.8 Hz). The presence of xylose, quinovose, glucose, and 3-*O*-methylglucose in a ratio 1:1:1:1 was confirmed by acid hydrolysis with aqueous 2 N trifluoroacetic acid followed by GC analysis of the corresponding alditol peracetates.

The molecular formula of hemoiedemoside A (**1**) was determined as $C_{54}H_{82}O_{28}S_2Na_2$ (*m*/*z* 1288) by pseudomolecular ions at *m*/*z* 1265 $[M - Na]^-$ in the FABMS (negative ion mode) and at *m*/*z* 1311 $[M + Na]^+$ in the FABMS (positive ion mode). Fragment ion peaks at *m*/*z* 1209 $[M - SO_3Na + H + Na]^+$ and 1083 $[M - 2SO_3Na + H]^+$ indicated the presence of two sulfate groups in the glycoside. This was also confirmed by solvolytic desulfation of hemoiedemoside A (**1**) to give the desulfated derivative **1a**. Comparison of ^{13}C NMR data of glycoside **1** with those of its desulfated derivative **1a** (Table 2) confirmed the presence of two sulfate groups. Esterification shifts were observed at the signals of C-4′ (xylose) (from 70.3 to 75.8) and C-6‴ (glucose) (from 61.8 to 67.5). The sequence of monosaccharides in the carbohydrate chain of hemoiedemo-

Table 2. ^{1}H and ^{13}C NMR Data for the Sugar Moieties of Hemoiedemosides A (**1**) and B (**2**) and ^{13}C NMR Data for the Sugar Moiety of the Desulfated Analogue **1a**

	1		**2**		**1a**
carbon	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]	δ_H mult[b] (*J* in Hz)	δ_C mult[a]
1′	104.9 d	4.69 d (7.1)	104.3 d	4.70 d	105.1 d
2′	82.4 d	3.96 m	81.8 d	3.98 m	83.1 d
3′	74.8 d	4.27 dd (9, 8.7)	74.1 d	4.25 dd (8.9, 8.7)	77.4 d
4′	75.8 d	5.11 m	75.8 d	4.94 m	70.3 d
5′	63.9 t	Hα: 3.72 m	63.3 t	Hα: 3.75 m	66.2 t
		H*β*: 4.75 m		H*β*: 4.75 m	
1″	104.6 d	4.92 d (7.7)	104.1 d	4.95 d (7.5)	105.0 d
2″	75.5 d	3.88 dd	74.8 d	3.88 dd	75.4 d
3″	75.6 d	3.97 m	74.5 d	3.93 m	75.8 d
4″	87.8 d	3.44 dd (8.7, 8.9)	86.6 d	3.46 dd (8.9, 8.7)	86.7 d
5″	71.3 d	3.66 m	71.0 d	3.64 m	71.4 d
6″	17.8 c	1.63 d (6.1)	17.4 c	1.62 d (6.15)	17.9 c
1‴	104.6 d	4.76 d (7.7)	103.7 d	4.78 d (7.9)	104.8 d
2‴	74.3 d	3.95	73.0 d	3.87 dd (7.9, 9.3)	73.6 d
3‴	86.5 d	4.25 m	85.9 d	4.13 m	87.3 d
4‴	69.9 d	3.79	68.9 d	3.82 dd (8.9, 9)	70.3 d
5‴	74.7 d	4.21	74.2 d	4.15 dd (8.9, 9)	77.4 d
6‴	67.5 t	Hα: 4.68 m	67.1 t	Hα: 4.59 dd (7, 10.9)	61.8 t
		H*β*: 5.14 dd (2, 10.7)		H*β*: 4.98 m	
1⁗	104.9 d	5.29 d (7.8)	104.2 d	5.19 d (7.9)	104.3 d
2⁗	74.5 d	3.96 m	73.8 d	3.81 m	74.6 d
3⁗	87.4 d	3.71 m	85.9 d	3.68 dd (8.9)	87.3 d
4⁗	70.3 d	4.02 dd (8.9, 9.3)	69.3 d	4.03 m	69.4 d
5⁗	77.9 d	3.95 m	74.9 d	4.05 m	77.9 d
6⁗	61.8 t	H_A: 4.43 dd (2, 11.9)	66.7 t	Hα: 4.75 m	61.7 t
		H_B: 4.19 m		H*β*: 4.94 m	
OMe	60.6 c	3.85 s	60.2 c	3.81 s	60.6 c

[a] Recorded at 125 MHz in pyridine-d_5−D_2O (5:1); multiplicity by DEPT. [b] Recorded at 500 MHz in pyridine-d_5−D_2O (5:1).

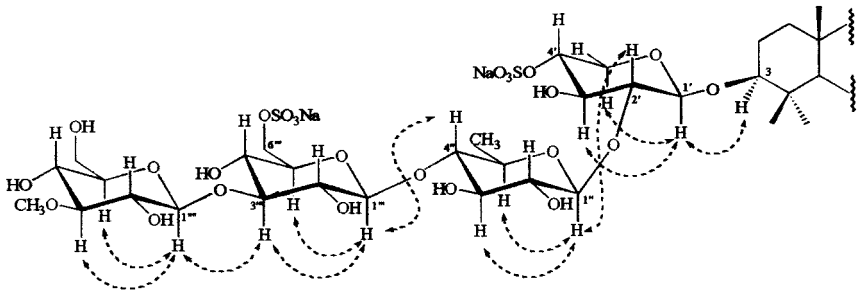

Figure 2. NOESY correlations of the oligosaccharide chain of hemoiedemoside A (**1**).

side A (**1**) was determined by fragments at *m*/*z* 1089, 870, 725, and 474 in the FABMS (negative ion mode), corresponding to the sequential losses of 3-*O*-methylglucosyl, sulfated glucosyl, quinovosyl, and sulfated xylosyl units, respectively.

All proton and carbon chemical shifts of the oligosaccharide chain of **1** (Table 2) could be assigned using ^{1}H−^{1}H COSY and HETCOR experiments. Location of the interglycosidic linkages in the oligosaccharide chain was deduced from the chemical shifts of C-2′ (*δ* 82.4), C-4″ (*δ* 87.8), and C-3‴ (*δ* 86.5) assigned on the basis of cross-peaks at *δ* 3.96/82.4 (H-2′/C-2′), 3.44/87.8 (H-4″/C-4″), and 4.25/86.5 (H-3‴/C-3‴) in the HETCOR spectrum. The carbons involved in the interglycosidic linkages gave values shifted downfield from those expected for unsubstituted pyranoses.[12] These results were confirmed by methylation of the desulfated derivative **1a** followed by hydrolysis and GC−MS analysis of the partially methylated alditol acetates[13] derived from 2-linked xylopyranose, 4-linked quinovopyranose, 3-linked glucopyranose, and terminal 3-*O*-methylglucopyranose.

The NOESY spectrum of **1** showed the correlations H-1″/H-2′, H-1‴/H-3″, H-1″/H-5″; H-1‴/H-4″, H-1⁗/H-3‴, H-1‴/H-5‴; and H-1⁗/H-3‴, H-1⁗/H-3⁗, H-1⁗/H-5⁗ (Figure 2), confirming the interglycosidic linkages in the oligosaccharide chain. We assume a D-configuration for the monosaccharide units of hemoiedemoside A (**1**) on the basis of GC analysis of the mixture of 1-[(*S*)-*N*-acetyl-(2-hydroxypropylamino)]-1-deoxyalditol acetate derivatives obtained by hydrolysis of liouvilloside A, a trisulfated triterpene glycoside containing the same sugar chain as hemoiedemoside A (**1**).[3]

On the basis of all the above data the structure of hemoiedemoside A (**1**) was established as 3-*O*-{3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-6‴-*O*-sodium sulfate-*β*-D-glucopyranosyl-(1→4)-*β*-D-quinovopyranosyl-(1→2)-4′-*O*-sodium sulfate-*β*-D-xylopyranosyl}holosta-9(11),25-dien-16-one. The oligosaccharide part of **1** is identical to the sugar chain of cucumechinosides A and C, isolated from the sea cucumber *Cucumaria echinata*.[14]

The ^{1}H and ^{13}C NMR spectra of hemoiedemoside B (**2**), the minor component of *H. spectabilis*, suggested the presence of the same aglycon as hemoiedemoside A (**1**) bonded to an oligosaccharide chain composed by four sugar units. The assignments of the NMR signals associated with the aglycon moiety (Table 1) were derived from ^{1}H−^{1}H COSY, HETCOR, and NOESY experiments. Acid hydrolysis of **2** with aqueous 2 N trifluoroacetic acid followed by GC analysis of the corresponding alditol peracetates de-

termined the presence of xylose, quinovose, glucose, and 3-*O*-methylglucose in a ratio 1:1:1:1.

The molecular formula of **2** was determined as $C_{54}H_{81}O_{31}S_3Na_3$ (*m*/*z* 1390) on the basis of pseudomolecular ions at *m*/*z* 1367 $[M - Na]^-$ in the FABMS (negative ion mode) and *m*/*z* 1413 $[M + Na]^+$ in the FABMS (positive ion mode). Fragment ion peaks at *m*/*z* 1311 $[M - SO_3Na + H + Na]^+$, 1209 $[M - 2SO_3Na + 2H + Na]^+$, and 1107 $[M - 3SO_3Na + 3H + Na]^+$ indicated the presence of three sulfate groups in the glycoside. This was confirmed by solvolytic desulfation of **2**, which rendered the same desulfated derivative (**1a**) as hemoiedemoside A (**1**), indicating that both glycosides differed only in the degree of sulfation of the oligosaccharide chain. Comparison of ^{13}C NMR data of **2** and those of its desulfated derivative **1a** (Table 2) allowed determination of the site of linkage of the sulfate groups. Esterification shifts were observed at the signals of C-4′ (xylose) (from 70.3 to 75.8), C-6‴ (glucose) (from 61.8 to 67.1), and C-6⁗ (3-*O*-methylglucose) (from 61.7 to 66.7). Thus, glycoside **2** contained an additional sulfate group at C-6⁗.

The sequence of monosaccharides in the carbohydrate chain of hemoiedemoside B (**2**) was confirmed by fragments at *m*/*z* 1071, 825, and 679 in the FABMS (negative ion mode) corresponding to the sequential losses of sulfated 3-*O*-methylglucosyl, sulfated glucosyl, and quinovosyl units. The position of the interglycosidic attachments was confirmed by methylation of the desulfated derivative followed by hydrolysis and GC−MS analysis of the partially methylated alditol acetates derived from 2-linked xylopyranose, 4-linked quinovopyranose, 3-linked glucopyranose, and terminal 3-*O*-methylglucopyranose.

Hence, the structure of hemoiedemoside B (**2**) was determined as 3-*O*-{6⁗-*O*-sodium sulfate-3-*O*-methyl-*β*-D-glucopyranosyl-(1→3)-6‴-*O*-sodium sulfate-*β*-D-glucopyranosyl-(1→4)-*β*-D-quinovopyranosyl-(1→2)-4′-*O*-sodium sulfate-*β*-D-xylopyranosyl}holosta-9(11),25-dien-16-one. The oligosaccharide part of **2** is identical to the sugar chain of liouvillosides A and B, isolated from the Antarctic sea cucumber *Staurocucumis liouvillei*.[3] Hemoiedemoside B (**2**) is one of the few and rare trisulfated triterpene glycosides isolated previously from five *Cucumaria* species, i.e., *C. echinata*,[14] *C. japonica*,[15] *C. koraiensis*,[16] *C. miniata*,[17] and *C. frondosa*,[18] and from *S. liouvillei*.[3]

Because several triterpene glycosides isolated from sea cucumbers have been reported to show antifungal properties,[2,6] sulfated tetraglycosides **1** and **2** and their semisynthetic desulfated analogue **1a** were evaluated for antifungal activity against the phytopathogenic fungus *Cladosporium cucumerinum* by a bioautographic technique.[19] Benomyl, a commercially available fungicide, was used as reference compound. As shown in Figure 3, the three glycosides were active in a concentration-dependent manner. The natural glycosides hemoiedemosides A (**1**) and B (**2**) were more active than their desulfated analogue **1a**, which was inactive at the lowest concentrations (1.5−5 *μ*g/spot) and weakly active at the higher ones (7.5−50 *μ*g/spot). The disulfated glycoside **1** was more active than benomyl at the higher concentrations (20−50 *μ*g/spot), while trisulfated glycoside **2** was slightly more active than the reference compound at 40 and 50 *μ*g/spot. Recently, we have reported the antifungal activity of patagonicoside A, a disulfated tetraglycoside isolated from the holothuroid *Psolus patagonicus*.[6] Patagonicoside A contains the same oligosaccharide chain as hemoiedemoside A (**1**) and a triterpenoid aglycon with a 18(20)-lactone, a Δ^7 double bond, and two hydroxyl functions at C-12 (α) and C-17 (α). On comparing the antifungal activities of hemoiedemoside A (**1**) and patagonicoside A against the phytopathogenic fungus *Cladosporium cucumerinum*, glycoside **1** showed inhibition zones of 8−33 mm at the tested concentrations (1.5−50 *μ*g/spot), while patagonicoside A was less active (8−19 mm) at the same concentrations. On the other hand, hemoiedemoside B (**2**), differing from **1** in the presence of a third sulfate group at C-6⁗, is less active than **1** (Figure 3). These results suggest that both the structure of the triterpenoidal aglycon and the presence and number of sulfate groups at the oligosaccharide chain may play an important role in the antifungal activity of these saponins.

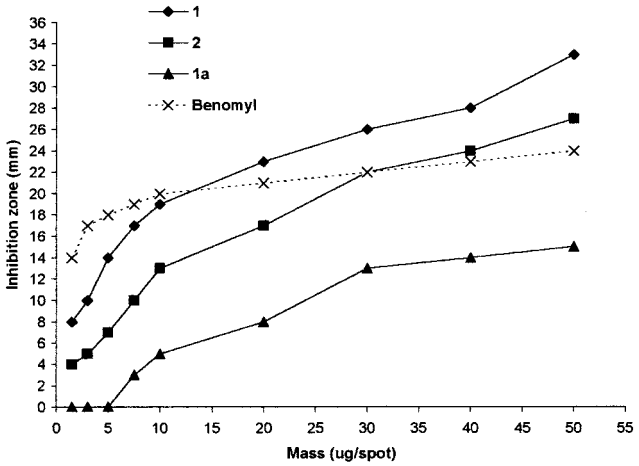

Figure 3. Dose−response curves for the antifungal activity of hemoiedemoside A (**1**), B (**2**), desulfated hemoiedemoside A (**1a**), and benomyl against *C. cucumerinum*.

The zootoxicities of glycosides **1** and **2** and their desulfated derivative **1a** were evaluated using the brine shrimp (*Artemia salina* L.) larvae mortality bioassay.[20] Hemoiedemoside A (**1**) showed a noteworthy toxicity in this assay (LC_{50} 18.7 ppm). Hemoiedemoside B (**2**) was 2 times less active (LC_{50} 47.5 ppm) than glycoside **1** and nearly 10 times more active than the desulfated derivative **1a** (LC_{50} 424.5 ppm). These results correlate with data on antifungal activity.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded in $C_5D_5N-D_2O$ (5:1) on a Bruker AM 500 spectrometer. The FABMS (positive and negative ion modes) were obtained on a VG-ZAB mass spectrometer, on a glycerol matrix. Optical rotations were measured on a Perkin-Elmer 343 polarimeter. Preparative HPLC was carried out on an SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector, and a refractive index detector using a C_{18} Bondclone 10 *μ*m column (30 cm × 7.8 mm i.d.). TLC was performed on precoated Si gel F254 (*n*-BuOH−HOAc−H_2O (12:3:5) and C_{18} reversed-phase plates (60% MeOH−H_2O). GC was performed on a Hewlett-Packard 5890A chromatograph equipped with a flame-ionization detector and an ULTRA-2 column (30 m × 0.2 mm i.d.). GC−MS was performed on a TRIO-2 VG mass spectrometer coupled to a Hewlett-Packard 5890A chromatograph and an SP-2330 column (25 m × 0.2 mm i.d.).

Animal Material. Seven specimens of *H. spectabilis* were collected in October 2000 off the Golfo San Jorge near Comodoro Rivadavia, on the Argentine Patagonian coast. The organisms were collected by scuba divers at 3 m in depth and identified by C.C.M. of the Museo de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina, where a voucher specimen is preserved (MACN No. 34813).

Extraction and Isolation. The sea cucumbers (0.3 kg wet weight) were defrosted, cut into small pieces, homogenized in EtOH, and centrifuged. The EtOH extract was evaporated, and

the aqueous residue was partitioned between MeOH−H_2O (9:1) and cyclohexane. The MeOH extract was evaporated, and the aqueous residue was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract was evaporated under reduced pressure to give a glassy material (0.9 g), toxic to the brine shrimp *Artemia salina* L. (LC_{50} 193 ppm). The *n*-BuOH extract was subjected to vacuum-dry column chromatography on Davisil C-18 reversed-phase (35−75 μm) using H_2O, H_2O−MeOH mixtures with increasing amounts of MeOH, and finally MeOH as eluents. The fraction eluted with 50% MeOH contained the sulfated triterpene glycosides. This fraction was finally submitted to repeated reversed-phase HPLC (ODS, MeOH−H_2O 50%) to give the pure glycosides **1** (22.6 mg, t_R = 20.8 min) and **2** (10.6 mg, t_R = 10.1 min).

Hemoiedemoside A (1): white amorphous powder, mp 225−227 °C, $[\alpha]^{20}_D$ −29.6° (*c* 0.4, pyridine); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive ion mode) *m*/*z* 1311 [M + Na]$^+$, 1209 [M − SO_3Na + H + Na]$^+$, 1136 [M − 3-*O*-Me-Glc + 2H + Na]$^+$, 1083 [M − 2SO_3Na + H]$^+$, 1033 [1136 − SO_3Na]$^+$, 870 [M − 3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na + Na]$^+$, 725 [M − 3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na-*O*-Qui + H + Na]$^+$, 474 [M − 3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na-*O*-Qui-*O*-Xyl-OSO$_3$Na-*O* + Na]$^+$; FABMS (negative ion mode) *m*/*z* 1265 [M − Na]$^-$, 1164 [M − SO_3Na + 2H − Na]$^-$, 1089 [M − 3-*O*-Me-Glc + H − Na]$^-$, 825 [M − 3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na + H − Na]$^-$, 807 [M − 3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na-*O* − H − Na]$^-$, 679 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O*-Glc-OSO$_3$Na-*O*-Qui + H − Na]$^-$, 663 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O*-Glc-OSO$_3$Na-*O*-Qui-*O* + H − Na]$^-$, 579 [3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na-*O*-Qui-*O* − H − Na]$^-$, 433 [3-*O*-Me-Glc-*O*-Glc-OSO$_3$Na-*O* − H − Na]$^-$.

Hemoiedemoside B (2): white amorphous powder, mp 230−232 °C, $[\alpha]^{20}_D$ −28.9° (*c* 0.5, pyridine); ^{1}H and ^{13}C NMR, see Tables 1 and 2; FABMS (positive ion mode) *m*/*z* 1413 [M + Na]$^+$, 1311 [M − SO_3Na + H + Na]$^+$, 1209 [M − 2SO_3Na + 2H + Na]$^+$, 1107 [M − 3SO_3Na + 3H + Na]$^+$; FABMS (negative ion mode) *m*/*z* 1367 [M − Na]$^-$, 1265 [M − SO_3Na + H − Na]$^-$, 1163 [M − 2SO_3Na + 2H − Na]$^-$, 1071 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O* − H − Na]$^-$, 825 [M − 3-*O*-Me-Glc-OSO$_3$-Na-*O*-Glc-OSO$_3$Na + H − Na]$^-$, 807 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O*-Glc-OSO$_3$Na-*O* − H − Na]$^-$, 679 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O*-Glc-OSO$_3$Na-*O*-Qui + H − Na]$^-$, 663 [M − 3-*O*-Me-Glc-OSO$_3$Na-*O*-Glc-OSO$_3$Na-*O*-Qui-*O* + H − Na]$^-$.

Acid Hydrolysis of Hemoiedemoside A (1) and Hemoiedemoside B (2). Each glycoside (1.5 mg) was heated in a screwcap vial with 2 N trifluoroacetic acid (0.6 mL) at 120 °C for 2 h. The aglycon was extracted with CH_2Cl_2, and the aqueous residue was evaporated under reduced pressure. Each sugar mixture was treated with 0.5 M NH_3 (0.4 mL) and $NaBH_4$ (3 mg) at room temperature for 20 h. After acidification with 1 M AcOH, each reaction mixture was treated with MeOH (0.5 mL) and evaporated under reduced pressure. Each alditol mixture was peracetylated with Ac_2O (0.2 mL) and pyridine (0.2 mL) at 100 °C for 45 min. The reaction mixtures were cooled and poured into $CHCl_3$−H_2O (1:1) (1 mL), and the aqueous phases were extracted with $CHCl_3$ (2 × 0.5 mL). The combined chloroform extracts were washed with H_2O (0.5 mL), saturated $NaHCO_3$ solution (0.5 mL), and H_2O (2 × 0.5 mL) and evaporated to dryness under nitrogen. Each mixture of peracetylated alditols was analyzed by GC using standard peracetylated alditols as reference samples.

Desulfation of Hemoiedemoside A (1). A solution of glycoside **1** (13 mg) in pyridine (0.3 mL) and dioxane (0.3 mL) was heated at 120 °C for 2.5 h. The reaction mixture was partitioned between H_2O and *n*-BuOH. The *n*-BuOH extract was evaporated to dryness at reduced pressure and purified by reversed-phase HPLC (ODS, MeOH−H_2O 80%), to give the pure desulfated glicoside **1a** (6 mg).

Desulfated Hemoiedemoside A (1a): white amorphous powder, mp 258−261 °C, $[\alpha]^{20}_D$ −65° (*c* 0.5, pyridine); ^{1}H NMR (C_5D_5N−D_2O (5:1), 500 MHz) aglycon δ 5.34 (1H, bd, *J* = 5.9 Hz, H-11), 4.76 (1H, bd, H-26 under anomeric proton signal), 3.25 (1H, m, H-3α), 2.88 (1H, s, H-17), 2.42 (1H, d, *J* = 15.5, H-15β), 2.20 (1H, d, *J* = 15.5, H-15α), 1.68 (3H, s, H_3-27), 1.45 (3H, s, H_3-21), 1.38 (3H, s, H_3-19) 1.27 (3H, s, H_3-31), 1.01 (3H, s, H_3-30), 0.92 (3H, s, H_3-32); sugars δ 5.28 (1H, d, *J* = 7.9 Hz), 5.12 (1H, d, *J* = 7.4 Hz), 4.94 (1H, d, *J* = 7.9 Hz), 4.78 (1H, d, *J* = 8 Hz, under olefinic proton signal), 3.85 (3H s, OCH_3), 1.72 (3H, d, *J* = 6.15 Hz, H_3-6 quinovose); ^{13}C NMR (C_5D_5N−D_2O (5:1), 125 MHz) aglycon δ 213.6 (s, C-16), 176.2 (s, C-18), 150.9 (s, C-9), 145.3 (s, C-25), 110.9 (d, C-11), 110.3 (t, C-26), 88.6 (d, C-3), 83.3 (s, C-20), 61.0 (d, C-17), 55.6 (s, C-13), 52.6 (d, C-5), 51.8 (t, C-15), 41.8 (s, C-14), 39.7 (s, C-10), 39.4 (s, C-4), 38.5 (d, C-8), 38.1 (t, C-22), 37.7 (t, C-24), 36.0 (t, C-1), 31.8 (t, C-12), 28.2 (t, C-7), 27.8 (c, C-31), 26.8 (t, C-2), 26.5 (c, C-21), 22.0 (c, C-27), 22.0 (t, C-23), 21.8 (c, C-19), 20.8 (t, C-6), 20.4 (c, C-32), 16.1 (c, C-30); sugars, see Table 2; FABMS (negative ion mode) *m*/*z* 1083 [M − Na]$^-$.

Methylation of Glycoside 1a Followed by Hydrolysis. A solution of **1a** (2 mg) in anhydrous DMSO (1 mL) was treated with NaOH (25 mg) and stirred at room temperature for 20 min. The reaction mixture was treated with CH_3I (0.15 mL) and stirred for a further 30 min. After addition of water (2 mL) the mixture was extracted with $CHCl_3$ (2 mL) and evaporated to dryness under nitrogen. The permethylated glycoside was heated in a screwcap vial with 2 N trifluoroacetic acid (0.3 mL) at 120 °C for 1.5 h. After extraction with $CHCl_3$, the aqueous residue was evaporated and further coevaporated with H_2O (2 × 0.5 mL) and MeOH (0.5 mL). The sugar mixture was treated with 0.5 M NH_3 (0.3 mL) and $NaBH_4$ (3 mg) at room temperature for 20 h. After acidification with 1 M AcOH, the reaction mixture was treated with MeOH (0.5 mL) and evaporated under reduced pressure. The alditol mixture was acetylated with Ac_2O (0.2 mL) and pyridine (0.2 mL) at 100 °C for 45 min. The reaction mixture was cooled and poured into $CHCl_3$−H_2O (1:1) and the aqueous phase extracted with $CHCl_3$. The combined chloroform extracts were washed with H_2O (0.5 mL), saturated $NaHCO_3$ solution (0.5 mL), and H_2O (2 × 0.5 mL) and evaporated to dryness under nitrogen. The mixture of methylated alditol acetates was analyzed by GC−MS. The following carbohydrates could be detected: 2-linked xylose (1,2,5-tri-*O*-acetyl-3,4-di-*O*-methylxylitol; t_R = 30.6 min; *m*/*z* (%) = 189 (27.9), 129 (84.4), 117 (100), 101 (33.8), 87 (37.5), 71 (6.9), 43 (79.7)); 4-linked quinovose (1,4,5-tri-*O*-acetyl-2,4-di-*O*-methylquinovitol; t_R = 29.5 min; *m*/*z* (%) = 203 (30.4), 143 (68.9), 117 (100), 101 (56.6), 87 (14.8), 45 (10.3), 43 (99.0)); 3-linked glucose (1,3,5-tri-*O*-acetyl-2,4-di-*O*-methylglucitol; t_R = 31.5 min; *m*/*z* (%) = 233 (20.7), 161 (48.1), 129 (65.9), 117 (100), 101 (30.8), 87 (11.5), 71 (9.7), 45 (39.5), 43 (89.7)); terminal 3-*O*-methylglucose (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol; t_R = 28.6 min; *m*/*z* (%) = 205 (26.2), 161 (76.6), 145 (46.8), 129 (43.3), 117 (46.2), 101 (100), 87 (26.9), 71 (19.4), 45 (60.2), 43 (94.7)).

***Artemia salina* Bioassay.** Geometric dilutions of the *n*-BuOH extract and the natural and semisynthetic compounds were freshly prepared from 1 to 10 mg/mL stock solutions in an appropriate solvent. Aliquots of these solutions (0.5 mL) were added to vials containing 10 shrimp/vial in marine water [3.8% (wt/vol) marine salts in distilled water], and the volume was adjusted to 5 mL/vial. The percentage of larvae mortality was determined after exposure to the *n*-BuOH extract and compounds **1**, **2**, and **1a** for 24 h at 25 °C. The LC_{50} for the *n*-BuOH extract and each compound tested were calculated with data from three independent experiments by using the standard procedure of probit analysis.

Sample Preparation for *C. cucumerinum* Bioautographic Assay. Geometric dilutions were obtained from freshly prepared stock solutions of isolated and semisynthetic compounds and of benomyl as reference compound at concentrations of 1.5−3.5 mg/mL in an appropriate solvent. Of these solutions 2, 5, and 10 μL were applied on the TLC plates using graduated capillaries.

Bioautographic Assay. After application of the samples on a Si gel 60 F_{254} Al sheet plate (Merck), the plate was sprayed with a suspension of *C. cucumerinum* in a nutritive medium and incubated 2−3 days at 25 °C in a glass box with a moist atmosphere. Clear inhibition zones appeared against a dark gray background.

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References and Notes

(1) Stonik, V. A.; Elyakov, G. B. Secondary Metabolites from Echinoderms as Chemotaxonomic Markers. In *Bioorganic Marine Chemistry*; Scheuer P. J., Ed.; Springer: Berlin, 1988; Vol. 2, pp 43−86.
(2) Kalinin, V. I.; Anisimov, M. M.; Prokofieva, N. G.; Avilov, S. A.; Afiyatullov, Sh. Sh.; Stonik, V. A. Biological activities and biological role of triterpene glycosides from holothuroids (Echinodermata). In *Echinoderm Studies*; Jangoux, M., Lawrence, J. M., Eds.; A. A. Balkema: Rotterdam, 1996; Vol. 5, pp 139−181.
(3) Maier, M. S.; Roccatagliata, A. J.; Kuriss, A.; Chludil, H.; Seldes, A. M.; Pujol, C. A.; Damonte, E. B. *J. Nat. Prod.* **2001**, *64*, 732−736.
(4) Maier, M. S.; Kuriss, A.; Seldes, A. M. *Lipids* **1998**, *33*, 825−827.
(5) Comin, M. J.; Maier, M. S.; Roccatagliata, A. J.; Pujol, C. A.; Damonte, E. B. *Steroids* **1999**, *64*, 335−340.
(6) Murray, A. P.; Muniain, C.; Seldes, A. M.; Maier, M. S. *Tetrahedron* **2001**, *57*, 9563−9568.
(7) Kitagawa, I.; Yamanaka, H.; Kobayashi, M.; Nishino, T.; Yosioka, I.; Sugawara, T. *Chem. Pharm. Bull.* **1978**, *26*, 3722−3731.
(8) Garneau, F.-X.; Simard, J.-L.; Harvey, O.; ApSimon, J. W.; Girard, M. *Can. J. Chem.* **1983**, *61*, 1465−1471.
(9) Miyamoto, T.; Togawa, K.; Higuchi, R.; Komori, T.; Sasaki, T. *J. Nat. Prod.* **1992**, *55*, 940−946.
(10) Zurita, M. B.; Ahond, A.; Poupat, C.; Potier, P. *J. Nat. Prod.* **1986**, *49*, 809−813.
(11) Stonik, V. A.; Kalinin, V. I.; Avilov, S. A. *J. Nat. Toxins* **1999**, *8*, 235−248.
(12) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, Germany, 1987.
(13) Björndal, H.; Lindberg, B.; Svensson, S. *Carbohydr. Res.* **1967**, *5*, 433−440.
(14) Miyamoto, T.; Togawa, K.; Higuchi, R.; Komori T.; Sasaki T. *Liebigs Ann. Chem.* **1990**, 453−460.
(15) Drozdova, O. A.; Avilov, S. A.; Kalinovsky, A. I.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. V. *Khim. Prirod. Soedin* **1993**, 369−374.
(16) Avilov, S. A.; Kalinovsky, A. I.; Kalinin, V. I.; Stonik, V. A.; Riguera, R.; Giménez, C. *J. Nat. Prod.* **1997**, *60*, 808−810.
(17) Drozdova, O. A.; Avilov, S. A.; Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A.; Riguera, R.; Jiménez, C. *Liebigs Ann./Recl.* **1997**, 2351−2356.
(18) Avilov, S. A.; Drozdova, O. A.; Kalinin, V. I.; Kalinovsky, A. I.; Stonik, V. A.; Gudimova, E. N.; Riguera, R.; Jiménez, C. *Can. J. Chem.* **1998**, *76*, 137−141.
(19) Homans, A. L.; Fuchs, A. *J. Chromatogr.* **1970**, *51*, 327−329.
(20) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.

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