Two New Holostan-Type Triterpene Glycosides from the Sea Cucumber *Bohadschia marmorata* JAEGER

Weihua YUAN,[a] Yanghua YI,*[,a] Haifeng TANG,[b] Mei XUE,[a] Zenglei WANG,[a] Guoquan SUN,[a] Wen ZHANG,[a] Baoshu LIU,[a] Ling LI,[a] and Peng SUN[a]

[a] *Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University; 325 Guohe Road, Shanghai 200433, P.R. China: and* [b] *Department of Pharmacy, Xijing Hospital, Fourth Military Medical University; Xi'an 710032, P. R. China.* Received April 18, 2008; accepted May 23, 2008; published online May 27, 2008

Two new holostan-type triterpene glycosides, 17-hydroxy fuscocineroside B (1) and 25-hydroxy fuscocineroside B (2), together with a known triterpene glycoside, fuscocineroside B (3) were isolated from the sea cucumber *Bohadschia marmorata* JAEGER. The structures of the new triterpene glycosides were elucidated on the basis of spectroscopic analyses and chemical reactions. Compounds 1 and 3 showed considerable antifungal activities against six strains.

Key words sea cucumber; *Bohadschia marmorata*; triterpene glycoside, antifungal active

In order to find new biologically active substances from marine organisms, we have been studying kinds of sea cucumbers.[1—6] Triterpene glycosides are the predominant secondary metabolites of the sea cucumber, and exhibit wide spectra of biological activity, such as antifungal, cytotoxic effects,[4,7] and they are usually of the lanosterol-type with a 18(20)-lactone and the sugar chain of up to six monosaccharide units is generally linked to the C-3 of the aglycone, which are composed by D-xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-glucose.[8] In this paper, we report the isolation and the antifungal activity of the three glycosides from the sea cucumber *Bohadschia marmorata* JAEGER (family *Bohadschia*) [distribute abundantly in the South China Sea], and structural elucidation of the new triterpene glycosides.

Results and Discussion

The 60% EtOH extracts of *B. marmorata* JAEGER (3.5 kg, dried) were suspended in H_2O, partitioned with *n*-BuOH. The *n*-BuOH extract was sequentially subjected to column chromatography on Si gel and ODS, and followed by HPLC on a Silasorb C-18 column to afford the pure 17-hydroxy fuscocineroside B (**1**), 25-hydroxy fuscocineroside B (**2**) and fuscocineroside B (**3**).

The known compound **3** was identified as fuscocineroside B, originally isolated from the sea cucumber *Holothuria fuscocinerea*, by comparison of physical and spectroscopic data (MS and 2D-NMR) with literature values.[5]

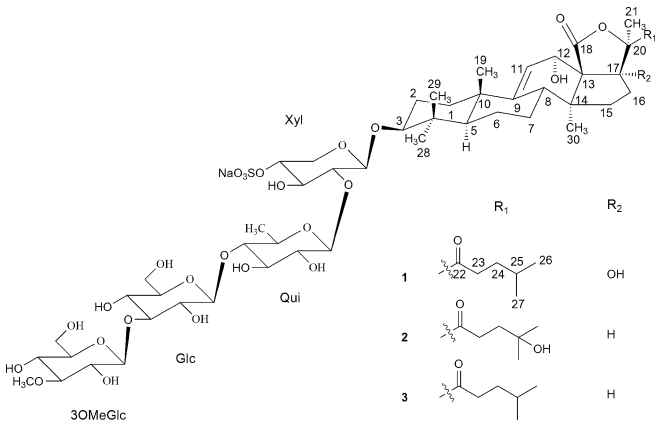

Fig. 1. The Structures of Compounds **1**—**3**

17-Hydroxy fuscocineroside B (**1**), colorless amorphous powder, was positive to Liebermann–Burchard and Molish tests. The molecular formula was established as $C_{54}H_{85}O_{27}NaS$ by the pseudomolecular ion $[M+Na]^+$ at *m/z* 1243.4805 in the HR-ESI-MS (positive-ion mode). A fragment ion peak at *m/z* 1123 $[M+Na-NaHSO_4]^+$ in the positive ion mode ESI-MS indicated the presence of a sulfate group in **1**, which was confirmed by the IR spectrum with absorption bands at 1265 and 1205 cm^{-1}. The IR spectrum also showed the presence of hydroxyl (3421 cm^{-1}), carbonyl (1775, 1717 cm^{-1}), olefinic (1651 cm^{-1}).

The ^1H-, ^{13}C-NMR, and DEPT spectra displayed resonances due to seven tertiary methyl groups, one olefinic bond (δ_H 5.69; δ_C 154.3, 115.5), one lactone carbonyl group (δ_C 173.8), and one doublet (δ_H 5.06; δ_C 71.1) that was ascribed to a methine proton linked to a carbon bearing a hydroxy group and suggested that the aglycone of **1** had a holostane triterpenoid skeleton with a 9(11)-en-12-ol moiety.[9]

The location of the ketone carbonyl group at C-22 was deduced from the chemical shift of the C-22 signal, which showed long-range correlation with the protons at δ_H 2.00 (s, CH_3-21) in the HMBC spectrum. The aglycone of **1** is similar to that of fuscocineroside B in spite of a hydroxyl instead of a α-H at C-17.[5] The signal of C-17 was shifted downfield from 46.9 to 87.6 ppm. The carbon C-17 (δ_C 87.6) showed long-rang correlation with the protons at δ_H 2.00 (s, CH_3-21) and δ_H 5.06 (12-CH) in the HMBC spectrum.

The relative stereochemistry of all chiral centers of the aglycone was established with the aid of a NOESY experiment. Thus, as is depicted in Fig. 2, H-3 showed correlations with H-1 (Xyl), H-1α (1.47 ppm), H-5α and H-28 confirming the β configuration at C-3. The 12α configuration of the hydroxyl group was confirmed by a cross-peak at δ_H 5.06 (H-12)/2.00 (H-21) in the NOESY spectrum and from the coupling constant for H-12 with H-11 (4.0 Hz).[7] A correlation between H-12 and H-21 in the NOESY spectrum, evidenced the α configuration of the hydroxyl group at C-17,[10] see Fig. 2. Complete assignments were achieved by studying the results of HMQC, HMBC, COSY, TOCSY and NOESY experiment (see Table 1, Fig. 2). Thus, the structure of the aglycone part of **1** was identified as a 22-oxo-9(11)-

* To whom correspondence should be addressed. e-mail: yiyanghua@126.com

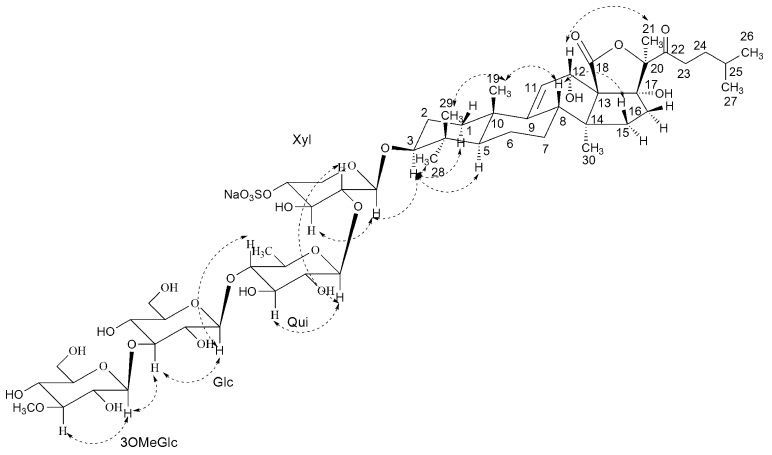

Fig. 2. The Key NOESY Correlations and Relative Configuration of **1**

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts and Selected HMBC Correlations for the Aglycone Moiety of **1** and **2** (in Pyridine-d_5, 400/100 MHz)

	1			**2**		
C	δ_H mult. (J in Hz)	δ_C	HMBC	δ_H mult. (J in Hz)	δ_C	HMBC
1	1.47, 1.90 m	36.5		1.51, 1.93 m	36.6	
2	2.02, 2.19 m	27.2		2.20 m	27.2	
3	3.22 (overlap)	88.9	4,28, 29, 1′	3.22 (overlap)	88.9	4,28, 29, 1′
4	—	40.1		—	40.2	
5	1.07 m	52.8	3, 4, 6, 10, 19, 28	1.07 m	52.9	3, 4, 6, 10, 19, 28
6	1.56, 1.80 m	21.3		1.78 m	21.4	
7	1.58, 1.81 m	28.5		1.78 m	29.0	
8	3.37 m	41.1	7, 9, 11, 14, 30	3.39 m	40.3	7, 9, 11, 14, 30
9	—	154.3		—	153.3	
10	—	39.9		—	39.8	
11	5.69 d (4.0)	115.5	8, 10, 12, 13	5.79 d (4.1)	116.1	8, 10, 12, 13
12	5.06 d (4.0)	71.1	9, 11, 14, 18	4.63 d (4.1)	67.9	9, 11, 14, 18
13	—	58.7		—	64.0	
14	—	46.4		—	46.7	
15	1.47, 1.89 m	36.9		1.47, 1.64 m	37.2	
16	2.24, 2.51 m	39.2		2.25 m	27.4	
17	—	87.6		3.73 m	47.0	
18	—	173.8		—	176.9	
19	1.45 s	22.7	1, 5, 9, 10	1.47 s	22.7	1, 5, 9, 10
20	—	92.3		—	90.6	
21	2.00 s	21.6	17, 20, 22	1.87 s	25.1	17, 20, 22
22	—	208.7		—	211.6	
23	2.87, 2.98 m	37.2		3.24 m	35.3	
24	1.70 m	32.6		2.20 m	37.4	
25	1.62 m	27.9		—	69.0	
26	0.95 d (6.5)	22.6	24, 25, 27	1.48 s	30.2	24, 25, 27
27	0.95 d (6.5)	22.6	24, 25, 26	1.48 s	29.9	24, 25, 26
28	1.32 s	28.2	3, 4, 5, 29	1.32 s	28.2	3, 4, 5, 29
29	1.12 s	16.9	3, 4, 5, 28	1.13 s	16.9	3, 4, 5, 28
30	1.72 s	20.0	8, 13, 14, 15	1.30 s	22.0	8, 13, 14, 15

holostene-3β,12α,17α-triol.

The sugar moieties of **1** were determined to be D-xylose, D-glucose, D-quinovose and 3-*O*-methyl-D-glucose in a 1 : 1 : 1 : 1 ratio by acidic hydrolysis with aqueous 2 M trifluoroacetic acid and preparation of the corresponding aldononitrile peracetates, which were analyzed by GC-MS using standard aldononitrile peracetates as reference samples. The common D-configuration for the four carbohydrate units was assumed also according to those most often encountered among the sea cucumber glycosides.[11,12]

The ^{1}H-, ^{13}C-NMR and DEPT spectra displayed signals for four anomeric protons and their corresponding carbons, δ_H 4.76 (δ_C 105.4), δ_H 5.12 (δ_C 105.6), δ_H 5.06 (δ_C 105.0) and δ_H 5.42 (δ_C 106.0). All anomeric protons were doublet with $^{3}J_{H\text{-}1/H\text{-}2}$=6.4—7.6 Hz, the large vicinal coupling constants of each anomeric proton indicated a *trans*-diaxial orientation with respect to their coupling partners (β-configuration).

Complete assignment was achieved by studying the results of HMQC, HMBC, COSY and TOCSY experiment (Table 2). The HMQC experiment correlated all proton resonances with those of their corresponding carbons. The COSY exper-

Table 2. ^{1}H- and ^{13}C-NMR Chemical Shifts and Selected HMBC Correlations for the Sugar Moiety of **1** and **2** (in Pyridine-d_5, 400/100 MHz)

	1			**2**		
C	δ_H mult. (*J* in Hz)	δ_C	HMBC	δ_H mult. (*J* in Hz)	δ_C	HMBC
Xyl						
1	4.76 d (6.4)	105.4	C-3 (Aglycone)	4.76 d (6.7)	105.4	C-3 (Aglycone)
2	4.12 m	83.5		4.12 m	83.5	
3	4.36 m	75.4		4.38 m	75.4	
4	5.20 m	76.0		5.20 m	76.0	
5	3.84, 4.79 m	64.4		3.81, 4.83 m	64.4	
4-O-SO_3Na						
Qui						
1	5.12 d (6.8)	105.6	C-2 (Xyl)	5.12 d (7.1)	105.5	C-2 (Xyl)
2	4.22 m	75.5		4.19 m	75.5	
3	4.07 m	76.5		4.06 m	76.4	
4	3.76 m	86.8		3.73 m	86.8	
5	3.82 m	72.1		3.82 m	72.1	
6	1.79 d (4.9)	18.2	C-4, 5 (Qui)	1.79 d (5.2)	18.2	C-4, 5 (Qui)
Glc						
1	5.06 d (7.0)	105.0	C-4 (Qui)	5.06 d (7.3)	105.0	C-4 (Qui)
2	4.20 m	74.2		4.16 m	74.2	
3	4.37 m	88.1		4.36 m	88.1	
4	4.23 m	69.6		4.16 m	69.7	
5	4.12 m	77.8		4.12 m	77.8	
6	4.33, 4.53 m	61.9		4.31, 4.55 m	62.0	
3OMeGlc						
1	5.42 d (7.6)	106.0	C-3 (Glc)	5.41 d (7.7)	106.0	C-3 (Glc)
2	4.13 m	75.1		4.12 m	75.2	
3	3.78 m	88.1		3.78 m	88.1	
4	4.16 m	70.7		4.16 m	70.8	
5	4.08 m	78.4		4.07 m	78.4	
6	4.34, 4.55 m	62.2		4.33, 4.56 m	62.3	
OMe	3.94 s	61.0		3.94 s	61.0	

iment allowed the sequential assignment of most of the resonances for each sugar ring, staring from the easily distinguished signals due to anomeric protons. By the HMBC experiment, the location of the interglycosidic linkages was deduced, the signals of them were downfield relative to shifts expected for the corresponding methyl glycopyranosides: δ_C 83.5 (Xyl C-2), δ_C 86.8 (Qui C-4), δ_C 88.1 (Glc C-3),[13] and a cross-peak between the H-1 of Xyl and C-3 of the aglycone indicated that the Xyl was connected to C-3 of the aglycone, then the interglycosidic linkages could be deduced by the same way. The site of linkage of the sulfate group in the sugar units was determined by studying the NMR chemical shifts of the sugar moiety, C-4 (Xyl) shifted downfield to 76.0 ppm, indicating that the sulfate group was located at C-4 of the xylose unit.[5] The structure of the carbohydrate chain of **1** was corroborated by HMBC, COSY and NOESY correlations, see Fig. 2.

Combined with NMR data, it was showed that **1** and **3** have the same sugar sequence.[5] Thus, the structure of **1** was established to be 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-β-D-xylopyranosyl]-22-oxo-9(11)-holostene-3β,12α,17α-triol.

25-Hydroxy fuscocineroside B (**2**), colorless amorphous powder, was positive to Liebermann–Burchard and Molish tests. The molecular formula was established as $C_{54}H_{85}O_{27}NaS$ by the pseudomolecular ion $[M+Na]^+$ at *m*/*z* 1243.4790 in the HR-ESI-MS (positive-ion mode). A fragment ion peak at *m*/*z* 1123 $[M+Na-NaHSO_4]^+$ in the positive ion mode ESI-MS indicated the presence of a sulfate group in **2**, which was confirmed by the IR spectrum with absorption bands at 1262 and 1208 cm^{-1}. The IR spectrum also showed the presence of hydroxyl (3421 cm^{-1}), carbonyl (1773, 1716 cm^{-1}), olefinic (1651 cm^{-1}).

The aglycone of **2** differs from **3** by the presence of a hydroxyl at C-25,[5] the signals of C-24 (CH_2), C-25 (C), C-26 (CH_3) and C-27 (CH_3) were shifted downfield to 37.4, 69.0, 30.2 and 29.9 ppm. The HMBC spectrum showed cross-peaks H_3-26/C-25, H_3-26/C-24, H_3-27/C-25, H_3-27/C-24, H_3-26/C-27 and H_3-27/C-26, together with the chemical shift of C-25 supported the presence of the two methyl groups attached to C-25 bearing the hydroxyl group. Complete assignments were achieved by studying the results of HMQC, HMBC, COSY, and TOCSY experiment (Table 1). The α configuration of the H at C-17 was confirmed by cross-peaks at δ_H 3.19 (H-17)/1.57 (H-21) and δ_H 3.19 (H-17)/1.28 (H-30) in the NOESY spectrum[10] (see Fig. 3). Thus, the structure of the aglycone part of **2** was identified as a 22-oxo-9(11)-holostene-3β,12α, 25-triol.

Combined with acid hydrolysis and NMR (see Table 2), it was found that the compounds **1**, **2** and **3** all have the same sugar sequence.[5] Thus, the structure of **2** was established to be 3-*O*-[3-*O*-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-*O*-sodiumsulfato-β-D-xylopyranosyl]-22-oxo-9(11)-holostene-3β,12α, 25-triol.

The Antifungal Activities Some triterpene glycosides hitherto isolated from sea cucumber exhibited significant antifungal activity.[7] In this paper, we report the antifungal activities of three triterpene glycosides isolated from *B. mar-*

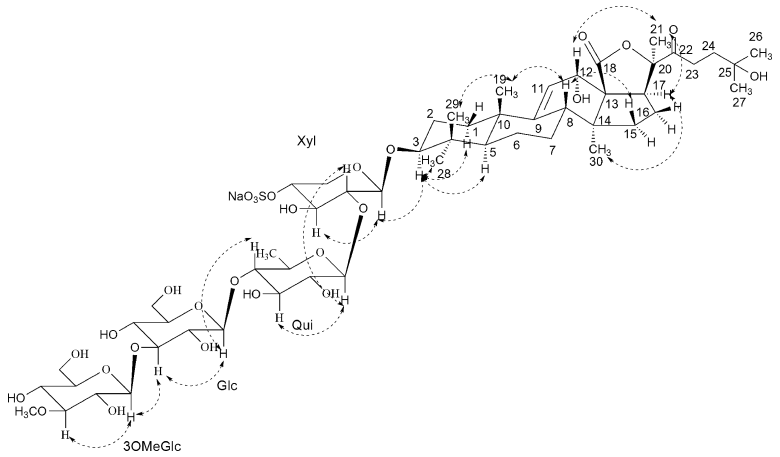

Fig. 3. The Key NOESY Correlations and Relative Configuration of **2**

Table 3. Antifungal Activity of the Compounds **1**—**3** (MIC_{80},[a] μg/ml)

	Candida albicans	*Cryptococcus neoformans*	*Aspergillus fumigatus*	*Trichophyton rubrum*	*Candida tropicalis*	*Candida krusei*
1	64	16	16	16	64	64
2	>64	>64	>64	>64	>64	>64
3	64	16	16	16	64	64
TBNF	4	1	0.0625	0.0625	0.0625	>64
KCZ	0.0625	0.0625	1	0.0625	0.0156	0.25
AMB	0.25	0.25	4	4	0.25	2
FCZ	0.25	0.25	>64	4	0.25	4

a) MIC_{80}: the minimum concentration to inhibit ≤80% growth for **1**—**3** against six fungi strains.

morata against six strains: *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Trichophyton rubrum*, *Candida tropicalis* and *Candida krusei*. Terbinafine (TBNF), Ketoconazole (KCZ), Amphotericin B (AMB), Fluconazole (FCZ) were used as positive controls, and the results are shown in Table 3.

Earlier studies on the triterpene glycosides isolated from *B. bivittata* revealed that the presence of the 12α-OH function seemed to be essential for exhibiting antifungal activities.[7] As we see in Table 3, **1** differs from **3** by the presence of a hydroxyl instead of a α-H at C-17, the antifungal activities were at the same level, but when the hydroxyl attaches to C-25 in the side chain, the antifungal activities decreased remarkably. It was envisioned that the better for the hydrophilic property of the side chain, the lower for the antifungal activities.

Experimental

General Melting points were determined on an XT5-XMT apparatus. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Bruker Vector 22 infrared spectrometer. NMR spectra were recorded in C_5D_5N on a Varian Inova-400 spectrometer, and the 2D NMR spectra were obtained using standard pulse sequences. ESI-MS and HR-ESI-MS were recorded on a Micromass Quattro mass spectrometer. GC/MS were performed on a Finnigan Voyager apparatus using a DB-5 column (30 m×0.25 mm i.d., 0.25 μm) with an initial temperature of 150 °C for 2 min and then temperature programming to 300 °C at a rate of 15 °C /min. Semipreparative HPLC was carried out on an Agilent 1100 liquid chromatograph equipped with a refractive index detector using a Zorbax 300 SB-C18 column (25 cm×9.4 mm i.d.).

Column chromatographies were performed on silica gel (200—300 mesh, 10—40 mm; Yantai, P. R. China) and ODS (40—63 mm; Merck) and Sephadex LH-20 (Pharmacia). Fractions were monitored by TLC (precoated silica-gel GF254 plates (10—40 mm; Yantai), and spots were visualized by heating Si gel plates sprayed with 10% H_2SO_4 in EtOH.

Six strains: *Candida albicans* (ATCC76615), *Cryptococcus neoformans* (ATCC32609), *Aspergillus fumigates* (Clinic Strains), *Trichophyton rubrum* (Clinic Strains), *Candida tropicalis* (Clinic Strains) and *Candida krusei* (Clinic Strains). Positive controls: Terbinafine (TBNF), Ketoconazole (KCZ), Amphotericin B (AMB), Fluconazole (FCZ).

Animal Material Specimens of *B. marmorata* were collected from offshore waters of Hainan island in the South China Sea in March 2006, and authenticated by Mr. Yu-Lin Liao (Institute of Oceanology, Chinese Academy of Science, P. R. China). A voucher specimen (No. BM-2006-3) was deposited at the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai.

Extraction and Isolation The dried sea cucumbers (3.5 kg) were chopped and refluxed with 60% EtOH (3 l×6, 3 h/time), and evaporated under reduced pressure, and then suspended in 6 l water, partitioned with *n*-BuOH for three times. The *n*-BuOH extract (90 g) was subjected to column chromatography on Si gel eluting with a $CHCl_3$: MeOH : H_2O (10 : 1 : 0.1; 9 : 1 : 0.1; 8 : 2 : 0.2; 7 : 3 : 0.5; 6 : 4 : 0.8) gradient to give 12 fractions. Fraction 10 (11 g) was further purified by reversed-phase silica MPLC eluting with an aq. CH_3OH (40—80%) gradient to give 8 fractions. And then the fraction 7 (0.5 g) was purified by HPLC (Zorbax 300 SB-C18; 70% aq. MeOH, 1.5 ml/min) to afford the compounds **1** (16 mg), **2** (15 mg) and **3** (35 mg).

17-Hydroxy Fuscocineroside B (**1**): Colorless amorphous powder; mp 224—226 °C; $[\alpha]_D^{20}$ −0.2 (c=0.675, pyridine); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS (positive-ion mode) *m/z* 1243 $[M+Na]^+$, 1123 $[M+Na-NaHSO_4]^+$; ESI-MS (negative-ion mode) *m/z* 1197 $[M-Na]^-$. IR (KBr) ν_{max}: 3421, 1775, 1717, 1651, 1265, 1205, 1076 cm^{-1}. HR-ESI-MS (positive ion mode) *m/z* 1243.4805 $[M+Na]^+$, (Calcd for $C_{54}H_{85}O_{27}Na_2S$, 1243.4794).

25-Hydroxy Fuscocineroside B (**2**): Colorless amorphous powder; mp 219—221 °C; $[\alpha]_D^{20}$ +8.3 (c=0.41, pyridine); ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; ESI-MS (positive-ion mode) *m/z* 1243 $[M+Na]^+$, 1123 $[M+Na-NaHSO_4]^+$; ESI-MS (negative-ion mode) *m/z* 1197 $[M-Na]^-$. IR (KBr) ν_{max}: 3421, 1773, 1716, 1651, 1262, 1208, 1074 cm^{-1}. HR-ESI-MS (positive ion mode) *m/z* 1243.4790 $[M+Na]^+$, (Calcd for $C_{54}H_{85}O_{27}Na_2S$, 1243.4794).

Acid Hydrolysis of 1—3 Each glycoside (5 mg) was heated in an ampule with 5 ml of aqueous 2 M CF_3COOH at 120 °C for 2 h. The aglycone was extracted with dichloromethane, and the aqueous residue was evaporated under reduced pressure. Then 1 ml of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the dry residue, and the mixtures were heated at 90 °C for 1 h. After the reaction mixtures were cooled, 1.5 ml of Ac_2O was added and the mixtures were heated at 90 °C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples. D-Xylose, D-quinovose, D-glucose, and 3-*O*-methyl-D-glucose were identified for each of **1** and **2** in a 1 : 1 : 1 : 1 ratio (D-quinovose: t_R=5.44 min; D-xylose: t_R=5.53 min; 3-*O*-methyl-D-glucose: t_R=6.57 min; D-glucose: t_R=6.75 min).

Bioassays The antifungal activities of the compounds **1**—**3** were tested against six strains: *Candida albicans* (ATCC76615), *Cryptococcus neoformans* (ATCC32609), *Aspergillus fumigates* (Clinic Strains), *Trichophyton rubrum* (Clinic Strains), *Candida tropicalis* (Clinic Strains) and *Candida krusei* (Clinic Strains). The antifungal activity data were evaluated by mensurating optical delnsity (OD) at 630 nm using Automatic Microplate Reader.[14] The drug MIC_{80} was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well. The data represented the means of three independent experiments in which each compound concentration was tested in three replicate wells. Terbinafine (TBNF), Ketoconazole (KCZ), Amphotericin B (AMB), Fluconazole (FCZ) were used as positive controls.

Acknowledgment This work was financially supported by National High-Tech Research and Development Project (863 Project, 2006AA09Z417 and 2006AA09Z423) and Chinese National Natural Science Foundation (Grant number: 20502035).

References

1) Sun P., Liu B. S., Yi Y. H., Li L., Gui M., Tang H. F., Zhang D. Z., Zhang S. L., *Chemistry & Biodiversity*, **4**, 450—457 (2007).
2) Zhang S. Y., Tang H. F., Yi Y. H., *Fitoterapia*, **78**, 283—287 (2007).
3) Han H., Yi Y. H., Li L., Wang X. H., Liu B. S., Sun P., Pan M. X., *Chinese Chem. Lett.*, **18**, 161—164 (2007).
4) Zou Z. R., Yi Y. H., Wu H. M., Yao X. S., Du L. J., Wu J. H., Liaw C. C., Lee K. H., *J. Nat. Prod.*, **68**, 540—546 (2005).
5) Zhang S. Y., Yi Y. H., Tang H. F., *J. Nat. Prod.*, **69**, 1492—1495 (2006).
6) Zou Z. R., Yi Y. H., Wu H. M., Wu J. H., Liaw C. C., Lee K. H., *J. Nat. Prod.*, **66**, 1055—1060 (2003).
7) Kitagawa I., Kobayashi M., Hori M., Kyogoku Y., *Chem. Pharm. Bull.*, **37**, 61—67 (1989).
8) Stonik V. A., Elyakov G. B., "Bioorganic Marine Chemisty," Vol. 43, ed. by Scheuer P. J., Springer, Berlin, 1988.
9) Kitagawa I., Kobayashi M., Inamoto T., Fuchida M., Kyogoku Y., *Chem. Pharm. Bull.*, **33**, 5214—5224 (1985).
10) Murray A. P., Muniain C., Seldes A. M., Maier M. S., *Tetrahedron*, **57**, 9563 (2001).
11) Stonik V. A., Kalinin V. I., Avilov S. A., *J. Nat. Toxins*, **8**, 235 (1999).
12) Avilov S. A., Kalinin V. I., Smirnov A. V., *Biochem. Syst. Ecol.*, **32**, 715—733 (2004).
13) Breitmaier E., Voelter W., Carbon-13 NMR Spectroscopy [M], VCH, Weinheim, 1987.
14) Zhang J. D., Xu Z., Cao Y. B., S. Chen H., Yan L., An M. M., Gao P. H., Wang Y., Jia X. M., Jiang Y. Y., *J. Ethnopharmacol.*, **103**, 76—84 (2006).