## Introduction

Triterpene gl cosides are the t pical predominant secondar metabolites in sea cucumbers. More than 100 of these gl cosides have been reported. The majorit of these saponins have a sugar chain of up to six monosaccharide units linked to the C-3 of the agl cone, which is usuall a triterpene of the lanosterol t pe with an 18(20)-lactone [1,2]. The saponins have a wide spectrum of biological functions, including antifungal, c totoxic, hemol tic, c tostatic and immunomodulator activities [3]. Sea cucumber, *Holothuria scabra* (Holothuriidae), is distributed abundantl in the South China Sea, and is used as a tonic in China [4]. As part of our research on biological secondar metabolites from echinoderms [5,6], we have focused our attention on the polar extracts of *H. scabra*. In this paper, we report the isolation and structure elucidation of two new sulfated triterpene gl cosides, scabraside A (**1**) and B (**2**) (**Fig. 1**), as well as their c totoxicit against four human tumor cell lines.

## Materials and Methods

### General experimental procedures

Melting points were determined on an XT5-XMT apparatus. Optical rotations were measured on a Perkin-Elmer-341 polarimeter. IR spectra were recorded on a Bruker Vector-22 apparatus. NMR spectra were recorded in $C_5D_5N$ on a Bruker Avance-II-600 spectrometer with TMS as internal standard. ESI- and HR-ESI-MS were acquired using a Q-TOF Micro LC-MS-MS mass spectrometer. GC-MS were acquired using a Finnigan Vo ager GC/MS apparatus with an ULTRA-2 column (50 m 0.2 mm). HPLC was carried out on an Agilent 1100 liquid chromatograph equipped with a refractive index detector using a Zorbax 300 SB-$C_{18}$ column (250 9.4 mm). Column chromatographic separations were performed on silica gel H (200–300 mesh, 10–40 m; Qingdao Marine Chemical Inc.) and Lobar Lichroprep RP-$C_{18}$ (40–63 m; Merck). Fractions were monitored b TLC on precoated silica gel $HSGF_{254}$ plates ($CHCl_3$-EtOAc-MeOH-$H_2O$, 4:4:2.5:0.5) or RP-C18 (MeOH-H

## Extraction and isolation

The sea cucumbers (3 kg, dr  weight) were powdered and refluxed four times each for 1 h with 60% ethanol (6 L  4). The extract was concentrated, and the residue (420 g) was suspended in $H_2O$, passed through a DA101 resin column (2 kg, 105  15 cm, i. d., Nankai Universit ) and then eluted with $H_2O$ (5 L), 70% EtOH (10 L), 95% EtOH (5 L), respectivel . The gl coside fraction was eluted with 70% ethanol. The combined extracts were concentrated. The gl coside fraction (crude gl coside-containing mixture, 70 g) was chromatographed over silica gel CC (200–300 mesh, 2100 g, 85  15 cm, i. d.), stepwise eluted with a $CHCl_3$-MeOH-$H_2O$ (8 : 2 : 1 to 6.5 : 3.5 : 1, lower phase) gradient to give fraction A (2.43 g), B (3 g), C (1.13 g), D (3.8 g) and E (2.23 g).

Fractions D and E were further purified b  MPLC with a column of reversed-phase silica (Lichroprep RP-$C_{18}$, 40–

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 36.3 | 1.39 m, 1.81 m | 36.7 | 1.37 m, 1.79 m |
| 2 | 27.0 | 1.86 m, 2.05 m | 27.1 | 1.91 m, 2.01 m |
| 3 | 88.7 | 3.13 dd (4.8, 12.0) | 88.6 | 3.14 dd (4.8, 12.0) |
| 4 | 40.0 | | 40.0 | |
| 5 | 52.7 | 0.97 d (10.2) | 52.6 | 0.82 d (10.2) |
| 6 | 28.3 | 1.69 m, 1.52 m | 28.1 | 1.68 m, 1.50 m |
| 7 | 21.2 | 1.45 m, 1.74 m | 21.1 | 1.48 m, 1.72 m |
| 8 | 40.9 | 3.33 brd (10.4) | 40.9 | 3.29 brd (10.2) |
| 9 | 154.0 | | 154.2 | |
| 10 | 39.7 | | 39.7 | |
| 11 | 115.6 | 5.57 d (4.2) | 115.3 | 5.59 d (4.2) |
| 12 | 71.3 | 4.95 dd (5.6,12.0) | 70.9 | 4.92 dd (5.6, 11.4) |
| 13 | 58.6 | | 58.5 | |
| 14 | 46.4 | | 46.2 | |
| 15 | 36.7 | 1.79 m, 1.36 m | 37.0 | 1.89 m, 1.40 m |
| 16 | 36.1 | 2.28 m, 2.61 m | 36.3 | 2.75 m, 2.87 m |
| 17 | 89.3 | | 92.1 | |
| 18 | 174.7 | | 173.6 | |
| 19 | 22.6 | 1.36 s | 22.5 | 1.34 s |
| 20 | 87.1 | | 87.4 | |
| 21 | 23.0 | 1.66 s | 21.4 | 1.89 s |
| 22 | 38.2 | 1.79 m, 1.85 m | 208.5 | |
| 23 | 22.4 | 0.83 m, 0.89 m | 39.0 | 2.10 m, 2.39 m |
| 24 | 38.4 | 1.92 m, 1.99 m | 32.4 | 1.78 m, 1.82 m |
| 25 | 145.5 | | 27.7 | 1.50 s |
| 26 | 110.8 | 4.66 s | 22.4 | 0.83 s |
| 27 | 22.2 | 1.67 s | 22.5 | 0.84 s |
| 30 | 16.7 | 1.03 s | 16.7 | 1.01 s |
| 31 | 28.1 | 1.23 s | 28.1 | 1.22 s |
| 32 | 20.1 | 1.65 s | 19.8 | 1.61 s |

[a] Assignments aided by DQFCOSY, TOCSY, HMQC, HMBC and NOESY experiments

**Table 1** $^1$H- (600 MHz) and $^{13}$C-NMR(150 MHz) data (δ value, *J* in Hz) for the aglyconemoiety of glycosides **1** and **2** in pyridine-$d_5$.[a]

determined graphicall for each experiment b curve fitting using Prism 4.0 software (GraphPad Software, Inc.) and the equation derived b DeLean et al. [8]. The $IC_{50}$ values for each treatment (**1, 2** and the control) were expressed as mean S.D. (n = 3), and the difference in the $IC_{50}$ values between the tested compound (**1** or **2**) and control was examined using Student's t-test. $P < 0.05$ was accepted as a significant difference.

## Results and Discussion

The 60% EtOH extract of *H. scabra* was successivel chromatographed on DA-101 resin, silica gel, and reversed-phase silica (Lichroprep RP-18, 40–63 m, Merck). Finall , reversed-phase HPLC on Zobax SB C-18 afforded scabraside A (**1**), scabraside B (**2**) and a structurall known compound **3**.

Scabraside A (**1**) was positive in the Liebermann-Burchard and Molish tests. Its molecular formula was determined as $C_{54}H_{85}O_{26}SNa$ from the pseudomolecular ion peak at *m/z* = 1227.4849 $[M + Na]^+$ in the positive-ion mode HR-ESI-MS and at *m/z* = 1181 $[M - Na]^-$ in the negative-ion mode ESI-MS. A fragment ion peak at *m/z* = 1107 $[M - OSO_3Na + Na - H]^+$ indicated the presence of a sulfate group in **1**, which was confirmed b the IR spectrum with absorption bands at 1255 and 1071 $cm^{-1}$. An examination of the $^1$H- and $^{13}$C-NMR spectra of **1** indicated the presence of a triterpene agl cone with six meth ls, two olefinic bonds and one lactone carbon l group, which had a close similarit to the agl cone of hemoiedemoside A [3], but **1** differed from hemoiedemoside A at C-17 and C-16. The h drox group was located at C-17 on the basis of a cross-peak [$\delta_H = 1.66/\delta_C = 89.3$ (H-21/C-17), $\delta_H = 2.28/\delta_C = 89.3$ (H-16/C-17)] in the HMBC spectrum (**Fig. 2**). The location of the meth lene at C-16 was deduced from the chemical shift of the H-16 signal (δ = 2.28), which showed coupling to signals at δ = 1.79 (H-15α), 1.36 (H-15β) in the TOCSY spectrum, and correlation with the meth lene signal at δ = 36.1 in the HMBC spectrum. The positions of a double bond at $\Delta^{9(11)}$ and a disubstituted terminal double bond at $\Delta^{25(26)}$ were deduced from the NMR signals at $\delta_C = 154.0$ (C-9), 115.6 (C-11); $\delta_H = 5.57$ (*d, J* = 4.2, H-11) and $\delta_C = 145.5$ (C-25), 110.8 (C-26); $\delta_H = 4.66$ (*s*, H-26) together with the anal sis of the TOCSY and HMBC experiments (**Fig. 2**). The HMBC spectrum showed the cross-peaks H-7/C-9, H-19/C-9, H-8/C-11, H-12/C-11, H-23/C-25, H-24/C-25, H-27/C-25 and H-27/C-26, and in the TOCSY spectrum, two protons [δ = 5.57 (H-11) and 4.95 (H-12)] and four protons [δ = 1.36 (H-15), 1.79 (H-15), 2.28 (H-16) and 2.61 (H-16)] comprising a two-spin and a four-spin s stem, respectivel . The $^1$H-NMR spectrum also showed an olefinic meth l signal [δ = 1.67 (*s*, H-27)] and five meth l groups [δ = 1.23 (*s*, H-31), 1.03 (*s*, H-30), 1.65 (*s*, H-32), 1.36 (*s*, H-19) and 1.66 (*s*, H-21)]. A signal characteristic for an ox genated methine [$\delta_C = 71.3$ (C-12); $\delta_H = 4.95$ (br. s, H-12)] in the holostane nucleus indicated α-configuration of the all lic OH group at C-12 [9]. Therefore, a 12-h drox lated $\Delta^{9(11)}$ terpenoid agl cone was identified.

The presence of four β-sugar units in **1** was deduced from the $^{13}$C- and $^1$H-NMR spectra, which showed four anomeric carbon and four anomeric protons (doublets) resonances with *J* values of

**Table 2** $^{1}$H- (600 MHz) and $^{13}$C-NMR(150 MHz) data (δ value, *J* in Hz) for the sugar moiety of glycosides **1** and **2** in pyridine-$d_5$.[a]

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| Xyl | | | | |
| 1 | 105.2 | 4.65 d (7.2) | 105.1 | 4.63 d (7.2) |
| 2 | 83.4 | 4.05 m | 83.2 | 4.02 m |
| 3 | 75.3 | 4.26 m | 75.0 | 4.24 m |
| 4 | 75.5 | 5.03 m | 75.4 | 5.01 m |
| 5 | 64.3 | 3.69 m | 64.2 | 3.67 m |
| | | 4.72 m | | 4.68 m |
| Qui | | | | |
| 1 | 105.4 | 5.01 brs | 105.3 | 4.48 d (7.8)[b] |
| 2 | 76.3 | 3.95 m | 76.3 | 3.94 m |
| 3 | 75.8 | 4.03 m | 75.8 | 4.05 m |
| 4 | 86.8 | 3.59 m | 87.0 | 3.58 m |
| 5 | 71.9 | 3.67 m | 71.9 | 3.66 m |
| 6 | 18.1 | 1.69 d (5.4) | 18.0 | 1.69 d (5.4) |
| Glc | | | | |
| 1 | 104.9 | 4.94 (overlapped) | 104.8 | 4.34 d (7.8)[b] |
| 2 | 73.9 | 4.01 m | 74.0 | 4.06 m |
| 3 | 87.9 | 4.21 m | 87.8 | 4.18 m |
| 4 | 69.5 | 3.99 m | 69.5 | 4.06 m |
| 5 | 77.8 | 3.91 m | 77.5 | 3.92 m |
| 6 | 61.8 | 4.46 m | 62.0 | 4.43 m |
| | | 4.27 m | | 4.22 m |
| MeGlc | | | | |
| 1 | 105.8 | 5.30 d (7.8) | 105.6 | 5.30 d (7.8) |
| 2 | 75.0 | 4.02 m | 75.0 | 3.97 m |
| 3 | 88.0 | 3.68 m | 88.0 | 3.69 m |
| 4 | 70.6 | 4.02 m | 70.5 | 4.00 m |
| 5 | 78.3 | 3.94 m | 78.2 | 3.96 m |
| 6 | 62.1 | 4.19 m | 61.7 | 4.19 m |
| | | 4.26 m | | 4.40 m |
| OMe | 60.8 | 3.84 s | 60.8 | 3.83 s |

[a] Assignments aided by DQFCOSY, TOCSY, HMQC, HMBC and NOESY experiments; [b] Measured at 600 MHz in DMSO-$d_6$

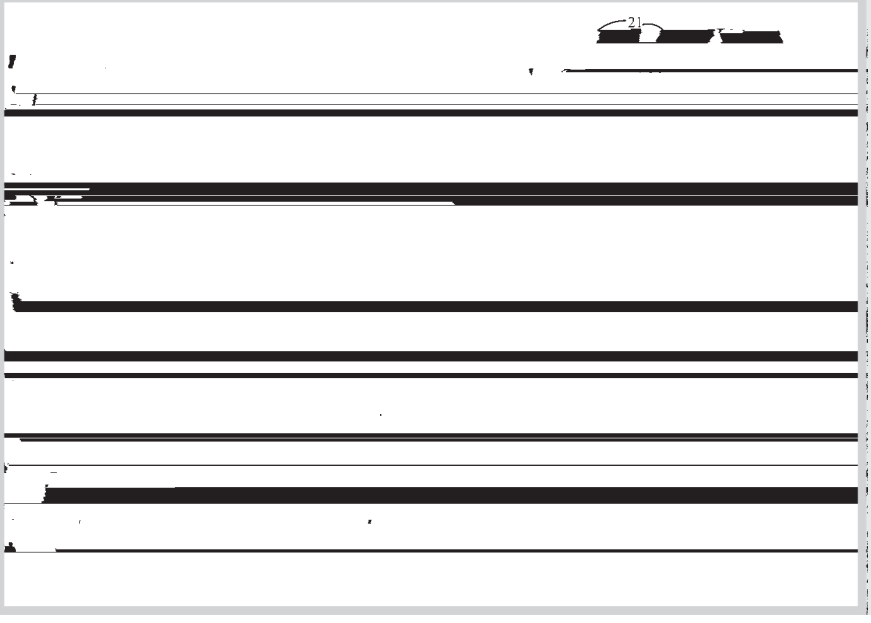

**Fig. 2** Key HMBC correlations of compound **1**.

7.2–7.8 H . The sugar moiet was confirmed to be D-x lose (X l), D-quinovose (Qui), D-glucose (Glc) and 3-*O*-meth lglucose (MeGlc) in a ratio of 1 : 1 : 1 : 1 b acidic h drol sis (aqueous 2 M $CF_3COOH$) followed b GC/MS anal sis of the corresponding aldononitrile peracetates and b comparing the GC retention time of the corresponding aldononitrile peracetates with those of the authentic samples prepared in the same manner [9]. The common D-configurations for the four carboh drate units were assumed according to those most often encountered among triterpene gl cosides from sea cucumbers [1].

The $^{1}$H- and $^{13}$C-NMR signals attributable to the sugar unit were assigned b the 2D NMR experiments and the data indicated that sugar residues were all in p ranose form. The sequence of the sugar residues in **1** was determined b anal sis of HMBC correlations: X l H-1/C-3 of the agl cone, Qui H-1/X l C-2, Glc H-1/Qui C-4 and MeGlc H-1/Glc C-3. The position of the sulfate group was determined b comparing $^{13}$C-NMR data of **1** with those of known gl cosides [10]. A downfield esterification shift was observed for the signal of X l C-4 (from δ 68.2 to 75.5). On the basis of the above data, the structure of **1** was deduced as 3-*O*-[3-*O*-meth l-*β*-D-glucop ranos l-(1 → 3)-*β*-