· Research Papers ·

Triterpene Glycosides from Sea Cucumber *Holothuria scabra* with Cytotoxic Activity

HAN Hua[1, 2], LI Ling[2], YI Yang-hua[2*], WANG Xiao-hua[3], PAN Min-xiang[4]

1. College of Animal Sciences, Zhejiang University, Hangzhou 310058, China
2. Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University, Shanghai 200433, China
3. Department of Pharmacy, 202th Hospital of PLA, Shenyang 110003, China
4. Department of Pharmacy, 306th Hospital of PLA, Beijing 100101, China

Abstract: **Objective** To study the new triterpene glycosides from sea cucumber *Holothuria scabra* with cytotoxic activity. **Methods** Triterpene glycosides from *H. scabra* were separated and purified by chromatography on DA-101, silica gel, and reversed-phase silica gel column, as well as RP-HPLC. Their structures were elucidated on the basis of spectral data and chemical evidence. **Results** Three triterpene glycosides were identified as scabraside D (**1**), fuscocineroside C (**2**), and 24-dehydroechinoside A (**3**). Their inhibition on P-388, A549, MKN-28, HCT116, and MCF-7 cells were significant. **Conclusion** Scabraside D (**1**) is a new triterpene glycoside, and compounds **2** and **3** are isolated from *H. scabra* for the first time. The glycosides **1**—**3** show the *in vitro* cytotoxicity against five human tumor cell lines in comparison to 10-hydroxycamptothecin.

Key words: cytotoxicity; 24-dehydroechinoside A; fuscocineroside C; *Holothuria scabra*; scabraside D; triterpene glycoside
DOI: 10.3969/j.issn.1674-6384.2012.03.002

Introduction

Sea cucumbers, *Holothuria scabra* Jaeger (Holothuriidae), are widely distributed in Atlantic and Pacific Oceans and have been used in traditional Chinese medicine as tonics and delicacies for a long time. Triterpene glycosides are the most important secondary metabolites in sea cucumbers because of their biological activities, including antifungal, cytotoxic, hemolytic, cytostatic, and immuno-modulatory effects (Habermehl and Volkvein, 1971; Kitagawa *et al*, 1989; Stonik, Kalinin, and Avilov, 1999; Chludil *et al*, 2002; Zhang, 2011a; 2011b). More than 100 compounds have been isolated from sea cucumbers up to date. Most of the known sea cucumber glycosides have lanostane aglycones with an 18(20)-lactone and a sugar chain composed of up to six monosaccharide units linked to the C-3 of the aglycone, which is composed of *D*-xylose, *D*-quinovose, *D*-glucose, and 3-*O*-methyl-*D*-glucose (Stonik and Elyakov, 1988; Maier *et al*, 2001). Sea cucumber is abundantly distributed in the South China Sea. Some triterpene glycosides have been isolated from *H. scabra* (Liao, 1997; Han *et al*, 2009a; 2009b). In a preceding paper, we also reported the antifungal and cytotoxic activities of this sea cucumber. As a part of our research on biological secondary metabolites from echinoderms (Zou *et al*, 2003; Tang *et al*, 2005; Zhang, Yi, and Tang, 2006; Yi *et al*, 2006; Han *et al*, 2007; Sun *et al*, 2007) and a continuation of studies on this sea cucumber, we present here the isolation and structure elucidation of a new sulfated triterpenoid glycoside, named scabraside D (**1**), as well as the cytotoxicities of the three glycosides against five human tumor cell lines.

* Corresponding author: Yi YH Address: School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China
Tel: + 86-21-6538 4988 Fax: +86-21-6548 3662 E-mail: hanhua@zju.edu.cn; yiyanghua@126.com
Received: December 19, 2011; Revised: May 2, 2012; Accepted: May 23, 2012
Fund: National High-Tech Research and Development Project (863 Project, 2006AA09Z417) and National Natural Science Foundation of China (20772155)
Online time: July 18, 2012 Online website: http://www.cnki.net/kcms/detail/12.11410.R.20120718.1719.001.html

Materials and methods

Equipments

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-600 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 was performed on a Finnigan Voyager Apparatus using a DB-5 column (30 m × 0.25 mm, 0.25 μm) with an initial temperature of 150 ℃ for 2 min and then temperature programming to 300 ℃ at a rate of 15 ℃/min. Semi-preparative HPLC was carried out on an Agilent 1100 Liquid Chromatograph equipped with a Refractive Index Detector using a Zorbax 300 SB-C_{18} column (25 cm × 9.4 mm). Column chromatographies were performed on silica gel (200－300 meshes, 10－40 μm; China) and ODS (40 － 63 μm; Merck, Germany) and Sephadex LH-20 (Pharmacia). Fractions were monitored by thin layer chromatography (TLC) [precoated silica gel GF_{254} plates (10－40 μm; China)], and spots were visualized by heating silica gel plates sprayed with 15% H_2SO_4 in EtOH.

Experimental materials

Specimens of *Holothuria scabra* Jaeger were collected from offshore water of Hainan Island in the South China Sea in May, 2006, and authenticated by Prof. LIAO Yu-lin (Institute of Oceanology, Chinese Academy of Sciences, China). A voucher specimen (HY200605) was deposited at the Research Center for Marine Drugs, School of Pharmacy, Second Military Medical University.

Extraction and isolation

The sea cucumbers (3 kg, dry weight) were powdered and refluxed for four times with 60% ethanol (6 L × 4, each time for 1 h). The extract was concentrated, and the residue (420 g) was suspended in H_2O, passed through a DA101 resin column (2 kg, 105 cm × 15 cm, Nankai University, China) and then eluted with H_2O (5 L), 70% EtOH (10 L), and 95% EtOH (5 L), respectively. The glycoside fraction was eluted with 70% ethanol. The combined extracts were concentrated. The glycoside fraction (crude glycoside-containing mixture, 70 g) was separated over silica gel column chromatography (CC, 200－300 meshes, 2.1 kg), stepwise eluted with $CHCl_3$- MeOH-H_2O (8:2:1 to 6.5:3.5:1, lower phase) gradient to give Frs. A (2.43 g), B (3 g), C (1.13 g), D (3.8 g), and E (2.23 g).

Fr. E was subjected to CC (ODS RP-C_{18}; MeOH-H_2O 54:46) and gave subfractions E_1 and E_2. Subfraction E_1 was purified by HPLC (Zorbax 300 SB-C_{18}; 59% MeOH, 1.5 mL/min) to afford compounds **1** (36.6 mg; t_R = 20.1 min) and **2** (11 mg; t_R = 26.7 min). Subfraction E_2 gave 110 mg of pure glycoside **3** (t_R = 29.3 min) using MeOH-H_2O (62:38) as the mobile phase and a flow rate of 1.5 mL/min.

Acid hydrolysis of compounds 1－3

Each of the glycoside (1 mg) was heated with 2 mol/L trifluoroacetic acid (1 mL) at 120 ℃ for 2 h. The reaction mixture was evaporated to dryness and the residue was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was concentrated under reduced pressure. Then pyridine (1 mL) and NH_2OH·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 was continued at 90 ℃ for 1 h. The solution was concentrated and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples. *D*-xylose (Xyl), *D*-quinovose (Qui), *D*-glucose (Glc), and *D*-3-*O*-methylglucose (MeGlc) were identified in a 1:1:1:1 ratio for all the glycosides (*D*-Xyl: t_R = 5.53 min; *D*-Qui: t_R = 5.44 min; *D*-Glu: t_R = 6.75 min; *D*-3-*O*-MeGlc: t_R = 6.57 min).

Bioassay

The cytotoxicities of compounds **1**－**3** (95% purity) against mouse leukemic cell (P-388), human lung cancer cell (A-549), gastric cancer cell (MKN-28), human colorectal cancer cell (HCT-116) and human breast cancer cell (MCF-7) (Shanghai Institute of Materia Medica, Chinese Academy of Sciences) were evaluated by sulforhodamine-B (SRB) assay (Skehan *et al*, 1990), with the anticancer agent 10-hydroxy-camptothecin (HCP, 98% purity; Knowshine Pharmaceuticals Inc.; China) as a positive control. IC_{50} was determined graphically for each experiment by curve fitting using Prism 4.0 software (GraphPad software, Inc.) and the equation derived by DeLean,

Munson, and Rodbard (1978). The results showed that the compounds exhibited significant cytotoxicity against the five tumor cell lines.

Results and discussion

Structure elucidation

Scabraside D (**1**) was positive in the Libermann-Burchard and Molish tests. Its molecular formula was determined as $C_{54}H_{87}O_{27}SNa$ from pseudomolecular ion peak at *m*/*z* 1245.5864 $[M + Na]^+$ (calcd. for $C_{54}H_{87}O_{27}S^+Na_2$: 1245.5860) in positive-ion mode HR-ESI-MS and at *m*/*z* 1199 $[M-Na]^-$ in negative-ion mode ESI-MS. A fragment ion peak at *m*/*z* 1125 $[M-OSO_3Na + Na-H]^+$ indicated the presence of a sulfate group in compound **1**, which was confirmed by the IR spectrum with absorption bands at 1266 and 1074 cm^{-1}. An examination of ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** indicated the presence of a triterpene aglycone with seven methyls, one olefinic bond and one lactone carbonyl group, which had a close similarity to the aglycone of echinoside A (Kitagawa *et al*, 1985) and holothurin A_3 (Dang *et al*, 2007), but compound **1** differed from echinoside A at C-25 and holothurin A_3 at C-22. The position of a hydroxyl group at C-25 was deduced from the NMR signals at δ 81.4 (C-25), 38.5 (C-24), and δ 1.62 (m, H-24) together with the analyses of TOCSY and HMBC experiments. The HMBC spectrum showed cross-peaks H-27/C-25, H-24/C-25, and H-26/C-25. The double bond at $\Delta^{9(11)}$ was deduced from the NMR signals at δ 153.8 (C-9), 115.6 (C-11); and δ 5.60 (br d, *J* = 10.4 Hz, H-11) together with the analyses of TOCSY and HMBC experiments. The HMBC spectrum showed cross-peaks H-9/C-11, H-19/C-9, H-8/C-11, and H-12/C-11, and in the TOCSY spectrum, two protons [δ 5.60 (H-11) and 4.95 (H-12)] comprised a two-spin system. A signal characteristic for an oxygenated methine [δ 71.6 (C-12) and 4.95 (m, H-12)] in the holostane nucleus indicated α-configuration of the allylic OH group at C-12 (Silchenko *et al*, 2005). Therefore, a 12- hydroxylated $\Delta^{9(11)}$ terpenoid aglycone was identified.

The presence of four β-sugar units in compound **1** was deduced from the ^{13}C-NMR and ^{1}H-NMR spectra, which showed four anomeric carbons and four anomeric protons resonances with coupling constant doublets (*J* = 7.2－8.0 Hz). The sugar moieties were confirmed to be *D*-Xyl, *D*-Qui, *D*-Glc, and 3-*O*-methylglucose (MeGlc) at a ratio of 1:1:1:1 by acidic hydrolysis (2 mol/L CF_3COOH) followed by GC-MS analysis of the corresponding aldononitrile peracetates and by comparing the GC retention time of the corresponding aldononitrile peracetates with those of the authentic samples prepared in the same manner (Silchenko *et al*, 2005). The ^{1}H-NMR and ^{13}C-NMR signals attributable to the sugar units were assigned by the 2D NMR experiments and the data indicated that sugar residues were all in pyranose form. The sequence of the sugar residues in compound **1** was determined by analysis of HMBC correlations: Xyl H-1/C-3 of the aglycone, Qui H-1/Xyl C-2, Glc H-1/Qui C-4, and MeGlc H-1/Glc C-3. The position of the sulfate group was determined by comparing ^{13}C-NMR data of compound **1** with those of known glycosides (Breitmaier and Voelter, 1987). A downfield esterification shift was observed for the signal of Xyl C-4 (from δ 68.2 to 75.7). On the basis of the above data, the structure of compound **1** was deduced as 3-*O*-[3-*O*-methyl-β-*D*-glucopyranosyl-(1→3)-β-*D*-glucopyranosyl-(1→4)-β-*D*-quinovo-pyranosyl-(1→2)-4-*O*-sulfate-β-*D*-xylopyransyl]-holosta-9(11)-ene-3β,12α,17α,25α-tetrol and named scabraside D (Figs. 1 and 2).

Cytotoxicity

Some triterpene glycosides hitherto isolated from sea cucumber exhibited cytotoxic activity. Glycosides **1**－**3** isolated from the sea cucumber *H. scabra* were tested for *in vitro* cytotoxicity against five tumor cell lines (P-388, A549, MKN-28, HCT116, and MCF-7). HCP was used as a positive control. The results in Table 1 indicated that three glycosides showed cytotoxic activity against five tumor cell lines with IC_{50} in the range of 0.93－2.60 μmol/L. On the basis of the data available, the cytotoxic activity of sea cucumber glycosides is very sensitive to their precise functionalization, and perhaps they show different sensitivities against different cell lines. Therefore, more extensive studies are needed before a clear structure-activity relationship could be reached. Based on these promising preliminary results, glycosides **1**－**3** need further study to be potential anticancer agents.

Compound **1**: colorless amorphous powder; mp 268 － 270 ℃, $[\alpha]_D^{20}$ −12.4° (*c* 0.4, MeOH); IR ν_{max}^{KBr} (cm^{-1}): 3417, 1773, 1632, 1266, 1074; ESI-MS

(+) mode: *m/z*: 1245 $[M+Na]^+$, (−) mode: *m/z*: 1199 $[M-Na]^-$; HR-ESI-MS (+) mode: *m/z*: 1245.5864 $[M + Na]^+$ (calcd. for $C_{54}H_{87}O_{27}S^+ Na_2$: 1245.5860). ^{1}H-NMR and ^{13}C-NMR are in Table 2.

Compound **2**: white crystal. It gave a positive reaction to Libermann-Burchard and Molish tests. mp 254 − 255 ℃, $[\alpha]_D^{20}$ −3.5° (*c* 0.82, pyridine); IR ν_{max}^{KBr} (cm^{-1}): 3433, 1756, 1673, 1264, 1217; ESI-MS (+) mode: *m/z* 1227 $[M + Na]^+$ ($C_{54}H_{85}O_{26}S^+Na_2$), ESI-MS (−) mode: *m/z* 1181 $[M-Na]^-$. It was deduced as fuscocineroside C by comparison of the NMR spectra with those of fuscocineroside C (Zhang, Yi, and

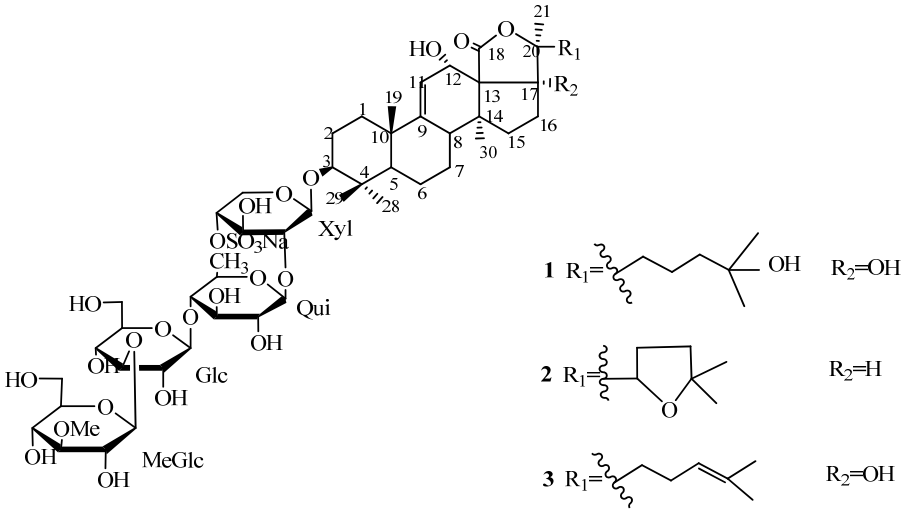

Fig. 1 Structures of compounds 1−3

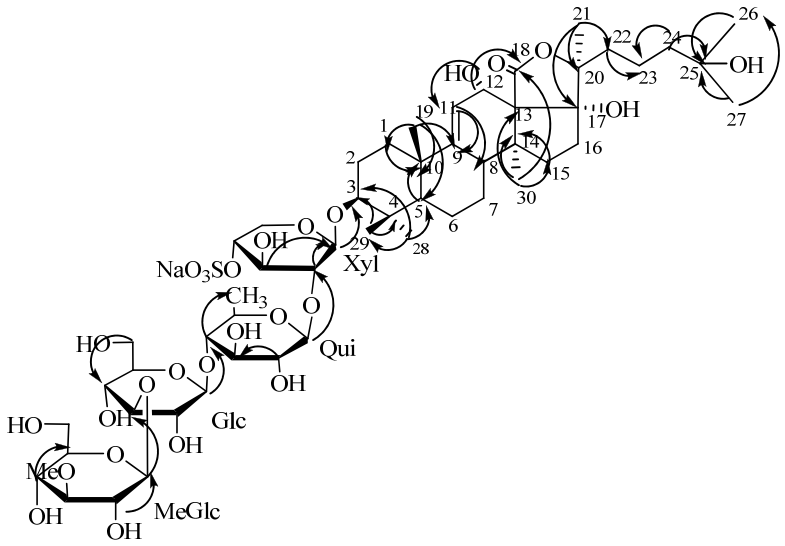

Fig. 2 Key HMBC correlations of compound 1

Table 1 Cytotoxicity of glycosides 1−3 against five tumor cell lines *in vitro* ($\bar{x} \pm s$, *n* = 3)

Cell lines	IC_{50} / (μmol·L^{-1})			
	1	**2**	**3**	HCP
P-388	0.96 ± 0.10	0.94 ± 0.11	1.23 ± 0.15	0.41 ± 0.13
A-549	1.72 ± 0.14	1.69 ± 0.12	1.41 ± 0.16*	0.84 ± 0.05
MKN-28	1.27 ± 0.13*	0.93 ± 0.10*	2.18 ± 0.32	0.77 ± 0.19
HCT-116	1.63 ± 0.17	1.70 ± 0.18	1.11 ± 0.13*	1.21 ± 0.14
MCF-7	1.80 ± 0.23*	2.60 ± 0.44	1.79 ± 0.21	1.17 ± 0.13

*$P < 0.05$ *vs* control

Table 2 ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data for glycoside 1 in C_5D_5N

Position	δ_H (*J*)	δ_C	Position	δ_H (*J*)	δ_C	Position	δ_H (*J*)	δ_C
1	1.37 m, 1.82 m	36.4	21	1.74 s	18.9	4	3.64 m	86.8
2	1.89 m, 2.08 m	27.0	22	1.68 m	38.0	5	3.74 m	71.9
3	3.13 dd (4.2, 12.0 Hz)	88.7	23	2.03 m	28.4	6	1.70 d (6.0 Hz)	18.0
4		39.9	24	1.62 m	38.5	Glc		
5	0.98 m	52.8	25		81.4	1	4.96 d (7.8 Hz)	105.2
6	1.50 m, 1.76 m	21.2	26	1.19 s	28.7	2	4.09 m	75.4
7	1.72 m, 1.54 m	28.1	27	1.17 s	27.4	3	4.24 m	88.0
8	3.34 brd (10.2 Hz)	40.9	28	1.05 s	16.7	4	4.10 m	69.5
9		153.8	29	1.24 s	28.1	5	4.00 m	77.7
10		39.7	30	1.66 s	20.3	6	4.45 m, 4.42 m	61.8
11	5.60 brd (10.4 Hz)	115.6	Xyl			MeGlc		
12	4.95 m	71.6	1	4.66 d (7.2 Hz)	104.8	1	5.31 d (8.0 Hz)	105.8
13		58.9	2	4.02 m	83.4	2	4.03 m	75.0
14		45.9	3	4.26 m	75.5	3	3.68 m	88.0
15	1.82 m, 1.39 m	36.8	4	5.10 m	75.7	4	4.07 m	70.6
16	2.39 m, 2.97 m	35.6	5	3.70 m, 4.69 m	64.3	5	3.97 m	78.3
17		89.8	Qui			6	4.20 m, 4.46 m	62.1
18		174.5	1	5.02 d (7.8 Hz)	105.4	OMe	3.84 s	60.8
19	1.36 s	22.5	2	3.96 m	76.3			
20		86.7	3	4.08 m	74.0			

Tang, 2006).

Compound **3**: white crystal. It gave a positive reaction to Libermann-Burchard and Molish tests. mp 235−236 ℃, $[\alpha]_D^{20}$−11.5° (*c* 0.4, MeOH); IR ν_{max}^{KBr} (cm^{-1}): 3421, 1761, 1653, 1256, 1073; ESI-MS (+) mode: *m*/*z* 1227 $[M + Na]^+$ ($C_{54}H_{85}Na_2O_{26}S^+$), 1125 $[M-SO_3Na + H + Na]^+$, ESI-MS (−) mode: *m*/*z* 1181 $[M-Na]^-$. It was deduced as 24-dehydroechinoside A by comparison of the NMR spectra with those of 24-dehydroechinoside (Kitagawa, Kobayashl, and Kyogoku, 1982; Kobayashi *et a1*, 1991).

Acknowledgments

Thanks are given to Prof. LIAO Yu-lin for the taxonomic identification of the sea cucumber.

References

Breitmaier E, Voelter W, 1987. *Carbon-13 NMR Spectroscopy*. 3rd Edition. VCH: Weinheim.

Chludil HD, Muniain CC, Seldes AM, Maier MS, 2002. Cytotoxic and antifungal triterpene glycosides from the Patagonian sea cucumber *Hemoiedema spectabilis*. *J Nat Prod* 65: 860-865.

Dang NH, Thanh NV, Kiem PH, Huong LM, Minh CV, Kim YH, 2007. Two new triterpene glycosides from the Vietnamese sea cucumber *Holothuria scabra*. *Arch Pharm Res* 30(11): 1387-1391.

DeLean AD, Munson PJ, Rodbard D, 1978. Simultaneous analysis of families of sigmoidal curves: Application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* 235: E97-E102.

Habermehl G, Volkvein G, 1971. Aglycones of the toxins from the cuverian organs of *Holothuria forskali* and a new nomenclature for the aglycones from Holothurioidea. *Toxicon* 9: 319-326.

Han H, Yi YH, Li L, Liu BH, La MP, Zhang HW, 2009a. Antifungal active triterpene glycosides from sea cucumber *Holothuria scabra*. *Acta Pharm Sin* 44(6): 620-624.

Han H, Yi YH, Li L, Wang XH, Liu BS, Sun P, Pan MX, 2007. A new triterpene glycoside from sea cucumber *Holothuria leucospilota*. *Chin Chem Lett* 18: 16l-164.

Han H, Yi YH, Xu QZ, La MP, Zhang HW, 2009b. Two new cytotoxic triterpene glycosides from sea cucumber *Holothuria scabra*. *Planta Med* 75: 1608-1612.

Kobayashi M, Hori M, Kan K, Yasuzawa T, Matsui M, Suzuki S, Kitagawa I, 1991. Marine natural products XXVII. Distribution of lanostane-type triterpene oligoglycosides in ten kinds of okinawan sea cucumbers. *Chem Pharm Bull* 39(9): 2282-2287.

Kitagawa I, Kobayashi M, Hori M, Kyogoku Y, 1989. Marine natural products. XVIII. Four lanostane-type triterpene oligoglycosides, bivittosides A, B, C, and D, from the okinawan sea cucumber *Bohadschia bivittata* Mitsukuri. *Chem Pharm Bull* 37(1): 61-67.

Kitagawa I, Kobayashi M, Kyogoku Y, 1982. Marine natural products IX. Structural elucidation of triterpenoidal oligoglycosides from the bahamean sea cucumber *Actinopyga agassizi* Selenka. *Chem Pharm Bull* 30(6): 2045-2050.

Kitagawa I, Kobayashi M, Inamoto T, Fuchida M, Kyogoku Y, 1985. Marine natural products. XIV. Structure of echinosides A and B, antifungal lanostane-oligosides from the sea cucumber *Actinopyga echiinites* (Jaeger). *Chem Pharm Bull* 33(12): 5214-5224.

Liao YL, 1997. *Chinese Fauna Echinodermata Holothuroidea*. Science Press: Beijing.

Maier MS, Roccatagliata AJ, Kuriss A, Cludil H, Seldes AM, Pujol CA, Damonte EB, 2001. Two new cytotoxic and virucidal trisulfated triterpene glycosides from the Antarctic sea cucumber *Staurocucumis liouvillei*. *J Nat Prod* 64: 732-736.

Silchenko AS, Stonik VA, Avilov SA, Kalinin VI, Kalinovsky AI, Zaharenko AM, Smirnov AV, Mollo E, Cimino G, 2005. Holothurins B(2), B(3), and B(4), new triterpene glycosides from Mediterranean sea cucumbers of the genus *Holothuria*. *J Nat Prod* 68: 564-567.

Skehan P, Storeng R, Scudiero D, Monks A, Mcmahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR, 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 82: 1107-1112.

Stonik VA, Elyakov GB, 1988. *Bioorganic Marine Chemistry*. In: Scheuer PJ. *Secondary Metabolites from Echinodens as Chemotaxonomic Markers*. Springer: Berlin.

Stonik VA, Kalinin VI, Avilov SA, 1999. Toxins from sea cucumbers (Holothuroids): Chemical structures, properties, taxonomic distribution, biosynthesis and evolution. *J Nat Toxins* 8: 235-248.

Sun P, Liu BS, Yi YH, Li L, Gui M, Tang HF, Zhang DZ, Zhang SL, 2007. A new cytotoxic lanostane-type triterpene glycoside from the sea cucumber *Holothuria impatiens*. *Chem Biodiv* 4(3): 450-457.

Tang HF, Yi YH, Li L, Sun P, Zhang SQ, Zhao YP, 2005. Three new asterosaponins from the starfish *Culcita novaeguineae* and their bioactivity. *Planta Med* 71: 458-463.

Yi YH, Xu QZ, Li L, Zhang SL, Wu HM, Ding J, Tong YG, Tan WF, Li MH, Tian F, Wu JH, Liaw CC, Lee KH, 2006. Philinopsides A and B, two new sulfated triterpene glycosides from sea cucumber *Pentacta quadrangularis*. *Helv Chim Acta* 89: 54-63.

Zhang JJ, 2011a. Extraction, isolation, and structure elucidation of two new triterpene glycosides from sea cucumber *Holothuria nobilis*. *Chin Tradit Herb Drugs* 42(8): 1467-1472.

Zhang JJ, 2011b. Two new triterpene glycosides from *Stichopus variegates*. *Chin Tradit Herb Drugs* 42(1): 10-14.

Zhang SY, Yi YH, Tang HF, 2006. Bioactive triterpene glycosides from the sea cucumber *Holothuria fuscocinerea*. *J Nat Prod* 69: 1492-1495.

Zou ZR, Yi YH, Wu HM, Wu JH, Liaw CC, Lee KH, 2003. Intercedensides A－C, three new cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens* Lampert. *J Nat Prod* 66: 1055-1060.