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Isolation and structural characterisation of five new and 14 known metabolites from the commercial starfish *Archaster typicus*

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ABSTRACT

From the commercially available starfish *Archaster typicus*, five new (**1**–**5**) and 14 known (**6**–**19**) metabolites were isolated and identified. Detailed 1D and 2D NMR spectroscopic data, including ^{1}H, ^{13}C, DEPT, HSQC, HMBC, and NOESY, established the structures of the new metabolites as sodium 5α-cholesta-9(11),24-dien-3β,6α,20β-triol-23-one 3-sulphate (**1**), sodium 5α-cholesta-9(11)-en-3β,6α,20β-triol-23-one 3-sulphate (**2**), sodium (25*R*)-5α-cholestane-3β,4β,6α,8,14α,15β,26-heptaol-15-sulphate (**3**), sodium (25*R*)-5α-cholestane-3β,6α,8,14α,15β,26-hexaol 15-sulphate (**4**), and sodium cholest-25(27)-ene-3β,4β,5α,6α,7β,8β,14α,15α,24,26-decanol 6-sulphate (**5**). Other spectroscopic techniques, including IR, ESI–MS, and HR-ESI–MS, were also adopted to further confirm the structures of the metabolites. These five steroids (**1**–**5**) are reported in nature for the first time. All of the steroids found in *A. typicus* (**1**–**12**) were tested for anticancer activities against MDA-MB-435 and Colo205 tumour cells. However, only sodium 5α-cholesta-9(11)-en-3*β*,6α,20*β*-triol-23-one 3-sulphate (**2**) and 27-nor-5α-cholestane-3*β*,4*β*,5,6α,7*β*,8,14,15α,24α-nonaol (**6**) exhibited weak activities.

1. Introduction

Starfish (sea stars) are invertebrates that belong to the class Asteroidea, phylum Echinodermata, of which over 1500 species are widely distributed in most of the oceans of the world (Han, Yuang, Cong, & Fan, 2006). Since they are rich in nutritional elements, including various proteins, amino acids, unsaturated fatty acids, trace elements, and vitamins, starfish have long been used in food preparation in China (Guo, Cheng, Liu, Ren, & Lin, 2004; Pan, Guo, Liu, & Dong, 2006; Xu et al., 1995; Zhou and Gu, 2000). In the seashore provinces of China, such as Guangdong, starfish are used by local inhabitants to make many kinds of soups because they believe that such soups can improve their health and prevent cancer and other diseases (Tang, Cheng, et al., 2009; Tang, Yi, et al., 2009; Xu, Song, Lu, Su, & Fu, 2004; Xu et al., 1997).

Archaster typicus Muller et Troschel, one of the most popular sea stars found in the South China Sea, is the only species from the family Archasteridae, order Valvatida, class Asteroidea (Huang, 1994). In the 1990s, nine highly hydroxylated steroids were isolated from this starfish collected off New Caledonia (Riccio, Santaniello, Squillace Greco, & Minale, 1989; Riccio, Squillace Greco, Minale, Laurent, & Duhet, 1986). Since then, no further investigations of *A. typicus* have been reported. In our current search for novel natural products from the crude extracts of marine foods, 19 metabolites have been isolated and identified from *A. typicus*, including five new (**1**–**5**) (Fig. 1) and 14 known (**6**–**19**) compounds. In the present work, we undertook the isolation, purification, and structure elucidation of the five new metabolites from the commercial starfish *A. typicus*, using a variety of spectroscopic techniques. Moreover, the antitumour activities of all the steroids (**1**–**12**) against MDA-MB-435 and Colo205 cells were also uncovered.

2. Materials and methods

2.1. General

Optical rotations were recorded using a Perkin–Elmer 341 polarimeter. IR spectra were recorded on a Bruker Vector 22 spectrometer with KBr pellets. NMR spectra were obtained on a Bruker Avance 500 NMR spectrometer, using TMS as the internal standard. ESI mass spectra were acquired on an Agilent LC/MSD Trap XCT

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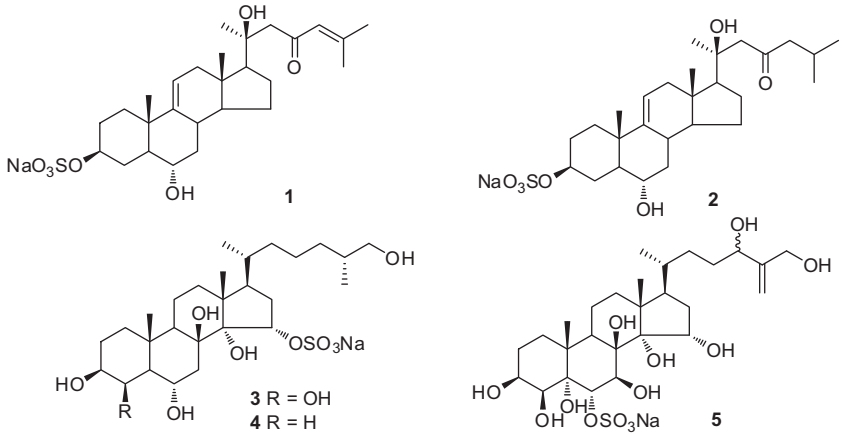

Fig. 1. Chemical structures of compounds **1**–**5**.

mass spectrometer, while HRESI mass spectra were measured using a Waters Q-TOF Micromass spectrometer. Materials for column chromatography were silica gel (100–200 mesh; Huiyou Silical Gel Development Co. Ltd., Yantai, China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-Gel ODS-A (50 μm; YMC, Milford, MA). Preparative TLC (0.4–0.5 mm) was conducted with glass plates precoated with silica gel GF_{254} (Yantai). Compounds were visualised by exposure to UV light at 254 nm.

2.2. Sample material

The starfish *A. typicus* (5 kg, dry weight) were bought from the Qingping Market in Guangzhou in March 2008 and authenticated by Prof. Qing-Chao Chen from the South China Sea Institute of Oceanology. A voucher specimen (0803005) was deposited at the Key Laboratory of Marine Bio-resources Sustainable Utilisation, South China Sea Institute of Oceanology, Chinese Academy of Sciences.

2.3. Extraction and isolation

Starfish material was extracted thrice with 70% ethanol for 24 h at room temperature. The extract was then concentrated to a small volume and partitioned with $CHCl_3$ (10 l). The residue was subjected to column chromatography (CC) over macroreticular resin D101, eluting with EtOH–H_2O to afford a crude extract (60.2 g). The latter was then chromatographed on silica gel eluting with gradient $CHCl_3$–MeOH to give nine fractions (Fr. 1–Fr. 9). Fraction Fr. 1 was purified by Sephadex LH-20, eluting with $CHCl_3$–MeOH (1:1) to give **13** (230 mg). Fraction Fr.5 was purified by LH-20, eluting with MeOH, followed by preparative TLC ($CHCl_3$–MeOH–H_2O, 5:1:0.1), to give **14** (10.2 mg) and **2** (8.4 mg). Fraction Fr.6 was first chromatographed over ODS with gradient MeOH–H_2O, purified by Sephadex LH-20, eluting with $CHCl_3$–MeOH (1:1), and then subjected to preparative TLC (EtOAc–MeOH–H_2O, 5:1:0.1) to afford **1** (3.3 mg) and **6** (279.6 mg). Using similar procedures, **7** (172.6 mg), **8** (98.0 mg), and **12** (21.0 mg) were isolated from fraction Fr.7; **3** (23.4 mg), **4** (50.4 mg), **5** (8.0 mg), **9** (33.1 mg), **10** (9.4 mg), and **11** (10.0 mg) were obtained from fraction Fr.8. The $CHCl_3$ extract (622 g) was subjected to CC over silica gel, eluting with a gradient petroleum ether (PE)–EtOAc (100:0 → 0:100) to give three fractions (Fr.C1–Fr.C3). Fraction Fr.C3 was chromatographed on ODS with gradient MeOH–H_2O, followed by repeated chromatography on Sephadex LH-20 with MeOH and/or $CHCl_3$–MeOH (1:1) and preparative TLC ($CHCl_3$–MeOH, 20:1) to yield **15** (47.2 mg), **16** (15.2 mg), **17** (27.7 mg), **18** (82.0 mg), and **19** (9.9 mg).

Sodium 5α-cholesta-9(11),24-dien-3β,6α,20β-triol-23-one 3-sulphate (**1**). Amorphous powder; $[\alpha]_D^{20}$ 0 (*c* 0.33, MeOH); IR (KBr) ν_{max} 3451, 2926, 2871, 1727, 1672, 1611, 1445, 1382, 1225, 1064, 996, 961, 835, 632 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive ion) *m*/*z* 555.7 $[M + Na]^+$; ESIMS (negative ion) *m*/*z* 509.6 $[M-Na]^-$; HRESIMS (positive ion) *m*/*z* 555.2363 $[M + Na]^+$ (calcd. for $C_{27}H_{41}O_7Na_2S$, 555.2363).

Sodium 5α-cholesta-9(11)-en-3β,6α,20β-triol-23-one 3-sulphate (**2**). Amorphous powder; $[\alpha]_D^{20}$ + 3.6 (*c* 3.00, MeOH); IR (KBr) ν_{max} 3304, 2956, 2927, 2871, 1678, 1585, 1467, 1242, 1064, 995, 960, 834, 629 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive ion) *m*/*z* 557.7 $[M + Na]^+$; ESIMS (negative ion) *m*/*z* 511.7 $[M-Na]^-$; HRESIMS (positive ion) *m*/*z* 557.2528 $[M + Na]^+$ (calcd. for $C_{27}H_{43}O_7Na_2S$, 557.2519).

Sodium (25*R*)-5α-cholestane-3β,4β,6α,8,14α,15β,26-heptaol-15-sulphate (**3**). Amorphous powder; $[\alpha]_D^{20}$ −6.6 (*c* 0.83, MeOH); IR (KBr) ν_{max} 3320, 2926, 2852, 1451, 1250, 1118, 1064, 1001, 917, 828, 774, 590 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (negative ion) *m*/*z* 563.4 $[M-Na]^-$; HRESIMS (positive ion) *m*/*z* 587.2814 $[M + H]^+$ (calcd. for $C_{27}H_{48}O_{10}NaS$, 587.2860).

Sodium (25*R*)-5α-cholestane-3β,6α,8,14α,15β,26-hexaol 15-sulphate (**4**). Amorphous powder; $[\alpha]_D^{20}$ + 45.2 (*c* 4.57, MeOH); IR (KBr) ν_{max} 3453, 2948, 2869, 1642, 1548, 1512, 1385, 1224, 1005, 954, 914, 824, 597 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive ion) *m*/*z* 593.8 $[M + Na]^+$; ESIMS (negative ion) *m*/*z* 547.7 $[M-Na]^-$; HRESIMS (positive ion) *m*/*z* 593.2757 $[M + Na]^+$ (calcd. for $C_{27}H_{47}O_9Na_2S$, 593.2731).

Sodium cholest-25(27)-ene-3β,4β,5α,6α,7β,8β,14α,15α,24,26-decanol 6-sulphate (**5**). Amorphous powder; $[\alpha]_D^{20}$ + 50.9 (*c* 0.93, MeOH); IR (KBr) ν_{max} 3440, 2952, 2872, 1744, 1640, 1607, 1451, 1384, 1252, 1061, 1015, 974, 914, 834, 755 cm^{-1}; ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; ESIMS (positive ion) *m*/*z* 655.7 $[M + Na]^+$; ESIMS (negative ion) *m*/*z* 609.7 $[M-Na]^-$; HRESIMS (positive ion) *m*/*z* 655.2393 $[M + Na]^+$ (calcd. for $C_{27}H_{45}O_{13}Na_2S$, 655.2371).

2.4. Preparation of MTPA esters

The *R*-(−) and *S*-(+)-MTPA esters of compound **3** were prepared as described previously (Kicha, Ivanchina, Kalinovsky, Dmitrenok, & Stonik, 2009). Briefly, two aliquots (1.0 mg) of **3** were treated respectively with *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) chloride (10 μl) in dry pyridine