Steroid glycosides from the starfish *Pentaceraster gracilis*

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ABSTRACT

Using combined chromatographic separations, two new steroid glycosides namely pentacerosides A (1) and B (2), and four known compounds were isolated from the methanol extract of the starfish *Pentaceraster gracilis*. Their structures were determined on the basis of spectroscopic data (\(^1\)H and \(^13\)C NMR, HSQC, HMBC, \(^1\)H-\(^1\)H COSY, ROESY, and FT-ICR-MS) and by comparing obtained results to the literature values. Among the isolated compounds, only maculatoside (5) showed significant cytotoxic effect against Hep-G2 (IC\(_{50}\) = 16.75 ± 0.69 μM) and SK-Mel2 (IC\(_{50}\) = 19.44 ± 1.45 μM) cell lines and moderate effect on KB (IC\(_{50}\) = 36.53 ± 0.78 μM), LNCaP (IC\(_{50}\) = 39.75 ± 3.34 μM), and MCF7 (IC\(_{50}\) = 47.34 ± 7.01 μM) cell lines.

1. Introduction

Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. In living echinoderms, starfish is a richest biological source of steroid glycosides, as any studied species contains a wide diversity of steroid glycosides [1]. *Pentaceraster* starfish (phylum Echinodermata, class Asteroidea, order Valvatida, family Oreasteridae) are little investigated species with few steroid glycosides [2,3], steroids [4], glycolipids [4], and glycosphingolipids [5] isolated and identified to date.

In continuation of our ongoing investigations on steroid glycosides of Vietnamese starfish [6,7], the present paper deals with the isolation, structure elucidation, and cytotoxic evaluation of six steroid glycosides (Figure 1), including two new compounds namely pentacerosides A (1) and B (2), from the starfish *Pentaceraster gracilis*.

2. Results and discussion

A methanol extract of the starfish *P. gracilis* revealed six steroid glycosides, including two new compounds. The known compounds were identified as nodososide (3) [8], (5α,25S)-cholestane-3β,6α,8α,15β,16β,26-hexol 3-O-[(2-O-methyl)-β-D-xylopyranoside] (4) [9],...
maculatoside (5) [3], and protoreasteroside (6) [3] by detailed analysis of their spectroscopic data (1D, 2D NMR, and MS) and comparison with previously reported values. These compounds were first isolated from *P. gracilis*.

Pentaceroside A (1) was isolated as a white powder. Its molecular formula, C_{37}H_{64}O_{14}, was determined by a quasi-molecular ion peak at m/z 755.4193 [M + Na]^+ on Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The 1D and 2D NMR features of 1 were indicative for a polyhydroxysteroid glycoside, one main constituent of starfish [10]. These data were similar to those of nodososide (3) [8], except for differences in the data of sugar moieties. The 1H and 13C NMR spectra for the aglycon of 1 confirmed the presence of four oxymethine groups [δC 68.2 (C-3), 77.9 (C-6), 70.1 (C-15), and 84.4 (C-24)/δH 4.07–4.14 (1H, m, H-3), 3.61 (1H, dd, J = 3.0, 3.5 Hz, H-6), 4.29 (1H, dt, J = 3.0, 10.0 Hz, H-15), and 3.31–3.35 (1H, m, H-24)] and two oxygenated quaternary carbons [δC 76.4 (C-5) and 77.4 (C-8)]. In addition, the signals of two tert-methyl [δC 15.4 (C-18) and 18.1 (C-19)/δH 0.97 (H-18) and 1.33 (H-19), each 3H, s] and three sec-methyl [δC 19.0 (C-21), 18.4 (C-26), and 18.1 (C-27)/δH 0.94 (H-21), 0.93 (H-26), and 0.92 (H-27), each 3H, d, J = 6.5 Hz] groups were also observed. The 1H-1H COSY spectrum confirmed connectivities of H2-1/H2-2/H-3/H2-4 and H-6/H2-7. This evidence and the heteronuclear multiple bond correlation (HMBC) cross-peaks of H_{J} 19 (δH 1.33) with C-1 (δC 34.4), C-5 (δC 76.4), C-9 (δC 49.2), and C-10 (δC 39.2); H-6 (δH 3.61) with C-8 (δC 77.4) and C-10 (δC 39.2); and that of H-7 (δH 2.11) with C-5 (δC 76.4) confirmed positions of the four hydroxy groups at C-3, C-5, C-6, and C-8. Detailed analysis of other COSY and HMBC correlations (Figure 2) clearly confirmed the planar structure of 1. The relative configurations for the aglycon of 1 were assigned to be identical to those of nodososide (3) [8] on the basis of a good agreement of their 1H and 13C NMR data as well as the coexistence in *P. gracilis*, which were also supported by ROESY experiment (Figure 3).

The presence of two sugar moieties was indicated by two anomeric carbon signals at δC 107.6 (C-1′) and 105.3 (C-1″), which have HSQC correlations with relevant anomeric proton
signals at $\delta_H$ 5.10 (1H, br s, H-1’) and 4.37 (1H, d, $J = 7.5$ Hz, H-1’). Detailed analysis of the HSQC, HMBC, and COSY experiments led to assignment of the $^1$H and $^{13}$C NMR data for both sugar moieties (Table 1). The HMBC cross-peaks of the anomic proton H-1” ($\delta_H$ 4.37) with C-2’ ($\delta_C$ 93.1) and H-1’ ($\delta_H$ 5.10) with C-24 ($\delta_C$ 84.4) confirmed the position of interglycosidic linkage and attachment of the disaccharide chain at C-24 of the aglycon. Careful comparison of the $^1$H and $^{13}$C NMR data for both sugar moieties of 1 with those of nodososide (3) [8] indicated that the difference between these two compounds is only in signals of the second sugar moiety with an absence of the methoxy group in 1 relative to 3. The $^{13}$C-NMR data for second sugar of 1 at $\delta_C$ 105.3 (C-1”), 75.0 (C-2”), 77.9 (C-3”), 71.1 (C-4”), and 67.1 (C-5”) are essentially identical to those for a $\beta$-D-xylopyranose of crossasteroside D at $\delta_C$ 105.2 (C-1”), 75.8 (C-2”), 77.4 (C-3”), 71.3 (C-4”), and 66.9 (C-5”) [11] and quite different from those for an $\alpha$-L-arabinopyranose of ilekudinchoside C at $\delta_C$ 105.8 (C-1”), 76.0 (C-2”), 73.4 (C-3”), 68.4 (C-4”), and 65.7 (C-5”) [12] clearly confirmed this sugar to be $\beta$-D-xylopyranose. Consequently, the structure of 1 was elucidated as 3$\beta$,5$\alpha$,6$\beta$,815$\alpha$,24(S)-hexahydroxycholestan-24-O-[$\beta$-D-xylopyranosyl-(1→2)-$\alpha$-L-arabinofuranoside].

Figure 2. Key COSY (---) and HMBC (▲) correlations of compound 1.

Figure 3. Key ROESY correlations of compound 1.
Table 1. $^1$H (500 MHz, CD$_3$OD) and $^{13}$C NMR (125 MHz, CD$_3$OD) spectroscopic data of compounds 1 and 2.

| Position | $\delta_C$ | $\delta_H$ mult. ($J$ in Hz) | $\delta_C$ | $\delta_H$ mult. ($J$ in Hz) |
|----------|------------|-------------------------------|------------|-------------------------------|
| Aglycon  |            |                               |            |                               |
| 1        | 34.4       | 1.38–1.42 m                   | 34.4       | 1.38–1.42 m                   |
|          |            | 1.67–1.73 m                   |            | 1.66–1.72 m                   |
| 2        | 30.9       | 1.56–1.61 m                   | 30.9       | 1.57–1.61 m                   |
|          |            | 1.78–1.81 m                   |            | 1.78–1.82 m                   |
| 3        | 68.2       | 4.07–4.14 m                   | 68.2       | 4.07–4.14 m                   |
| 4        | 41.1       | 1.58–1.62 m                   | 41.1       | 1.58–1.62 m                   |
|          |            | 2.15 dd (11.5, 13.0)          |            | 2.15 dd (11.5, 13.0)          |
| 5        | 76.4       | –                             | 76.5       | –                             |
| 6        | 77.9       | 3.61 dd (3.0, 3.5)            | 77.9       | 3.61 t (3.0)                  |
| 7        | 40.4       | 2.11 dd (3.0, 15.0)           | 40.4       | 2.10 dd (3.0, 15.0)           |
|          |            | 2.22 dd (3.5, 15.0)           |            | 2.20 dd (3.0, 15.0)           |
| 8        | 77.4       | –                             | 77.3       | –                             |
| 9        | 49.2       | 1.55–1.59 m                   | 49.0       | 1.56–1.60 m                   |
| 10       | 39.2       | –                             | 39.2       | –                             |
| 11       | 19.7       | 1.40–1.44 m                   | 19.7       | 1.40–1.44 m                   |
|          |            | 1.77–1.81 m                   |            | 1.78–1.81 m                   |
| 12       | 42.9       | 1.26–1.30 m                   | 42.9       | 1.25–1.31 m                   |
|          |            | 1.96–2.00 m                   |            | 1.97–2.00 m                   |
| 13       | 45.5       | –                             | 45.5       | –                             |
| 14       | 66.5       | 1.27 d (10.0)                 | 66.5       | 1.27 d (9.5)                  |
| 15       | 70.1       | 4.29 dt (3.0, 10.0)           | 70.1       | 4.29 dt (3.5, 9.5)            |
| 16       | 41.7       | 1.75 m                        | 41.7       | 1.73–1.77 m                   |
|          |            | 1.94 m                        |            | 1.91–1.95 m                   |
| 17       | 55.9       | 1.37 m                        | 55.9       | 1.35–1.39 m                   |
| 18       | 75.0       | 3.21 dd (7.5, 9.0)            | 75.0       | 3.21 dd (7.5, 9.0)            |
| 19       | 48.5       | 0.97 s                        | 48.5       | 0.97 s                        |
| 20       | 31.4       | 1.33 s                        | 31.4       | 1.33 s                        |
| 21       | 18.4       | 0.93 d (6.5)                  | 18.4       | 0.93 d (6.5)                  |
| 22       | 55.7       | 1.31–1.35 m                   | 55.7       | 1.33–1.37 m                   |
|          |            | 1.59–1.62 m                   |            | 1.60–1.63 m                   |
| 23       | 84.4       | 3.31–3.35 m                   | 84.4       | 3.32–3.36 m                   |
| 24       | 31.4       | 1.86–1.90 m                   | 31.4       | 1.84–1.89 m                   |
| 25       | 18.4       | 0.93 d (6.5)                  | 18.4       | 0.93 d (6.5)                  |
| 26       | 18.1       | 0.92 d (6.5)                  | 18.1       | 0.92 d (6.5)                  |
| Araf(f)  |            |                               |            |                               |
| 1'       | 107.6      | 5.10 br s                     | 109.4      | 4.94 d (1.5)                  |
| 2'       | 93.1       | 4.05 dd (1.0, 3.5)            | 83.9       | 3.98 dd (1.5, 4.0)            |
| 3'       | 77.3       | 4.03 dd (3.5, 7.5)            | 78.7       | 3.86 dd (4.0, 6.5)            |
| 4'       | 83.4       | 3.95–3.98 m                   | 85.0       | 4.99–4.02 m                   |
| 5'       | 62.4       | 3.67 dd (5.0, 12.0)           | 62.9       | 3.65 dd (5.0, 12.0)           |
|          |            | 3.81 dd (2.5, 12.0)           |            | 3.77 dd (3.0, 12.0)           |
| Xyl      |            |                               |            |                               |
| 1''      | 105.3      | 4.37 d (7.5)                  |            |                               |
| 2''      | 75.0       | 3.21 dd (7.5, 9.0)            |            |                               |
| 3''      | 77.9       | 3.31–3.35 m                   |            |                               |
| 4''      | 71.1       | 3.48–3.52 m                   |            |                               |
| 5''      | 67.1       | 3.19 dd (10.5, 12.0)          |            |                               |
|          |            | 3.85 dd (5.5, 12.0)           |            |                               |

Note: All assignments were done by HSQC, COSY, HMBC, and ROESY experiments.
The molecular formula of pentaceroside B (2) was determined as C$_{32}$H$_{56}$O$_{10}$ by FT-ICR-MS with a quasi-molecular ion peak at $m/z$ 623.3771 [M + Na]$^+$. The $^1$H and $^{13}$C NMR data of 2 were similar to those of 1, except for absence of the xylose moiety (Table 1). A good agreement of $^{13}$C NMR data for the sugar moiety of 2 at $\delta_C$ 109.4 (C-1'), 83.9 (C-2'), 78.7 (C-3'), 85.0 (C-4'), and 62.9 (C-5') with those of oreasteroside I [13] at $\delta_C$ 109.5 (C-1'), 83.8 (C-2'), 78.9 (C-3'), 85.4 (C-4'), and 63.0 (C-5'), and combination with the spin-coupling pattern of the sugar proton signals ($J_{1'-2'} = 1.5$ Hz, $J_{2'-3'} = 4.0$ Hz, and $J_{3'-4'} = 6.5$ Hz) are indicative for an $\alpha$-L-arabinofuranosyl moiety. Attachment of the arabinose moiety at C-24 was assigned by HMBC correlation of the anomeric proton H-1' ($\delta_H$ 4.94) with C-24 ($\delta_C$ 84.8). Thus, the structure (24S)-24-O-$\alpha$-L-arabinofuranosyl-cholestane-3$\beta$,5$\alpha$,6$\beta$,815$\alpha$,24-hexaol was elucidated for 2.

All isolated compounds were evaluated for their cytotoxic activity against five human cancer cell lines including HepG2 (hepatoma cancer), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma) using the sulforhodamine B method [14] and following the previously described protocols [15,16]. Among isolated compounds, maculatoside (5) showed significant cytotoxic effect against Hep-G2 (IC$_{50}$ = 16.75 ± 0.69 μM) and SK-Mel2 (IC$_{50}$ = 19.44 ± 1.45 μM) cell lines and moderate effect on KB (IC$_{50}$ = 36.53 ± 0.78 μM), LNCaP (IC$_{50}$ = 39.75 ± 3.34 μM), and MCF7 (IC$_{50}$ = 47.34 ± 7.01 μM) cell lines, relative to the positive control (ellipticine: IC$_{50}$ = 1.71 ± 0.16, 2.07 ± 0.12, 1.99 ± 0.16, 1.95 ± 0.12, and 2.15 ± 0.24 μM on Hep-G2, KB, LNCaP, MCF7, and SK-Mel2, respectively). The other compounds showed no cytotoxicity (IC$_{50}$ > 100 μM) against any of the tested cancer cell lines.

3. Experimental

3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). The high resolution mass spectra were gained using a Varian 910 FT-ICR mass spectrometer (Varian, CA, U.S.A). The $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded on a Bruker AM500 (Billerica, MA, U.S.A) with TMS used as an internal standard. Medium pressure liquid chromatography (MPLC) was carried out on a Biotage–Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Sigma–Aldrich, U.S.A), and YMC × GEL resins (ODS-A, 12 nm S-150 μm, YMC Co., Ltd.). Thin layer chromatography used pre-coated silica gel 60 F$_{254}$ (1.0555.4001, Merck, Darmstadt, Germany) and RP-18 F$_{254S}$ plates (1.15685.0001, Merck, Darmstadt, Germany), and compounds were visualized by spraying with aqueous 10% H$_2$SO$_4$ and heating for 3–5 min.

3.2. Biological material

The sample of the starfish *P. gracilis* (Lütken, 1871) was collected at Bac Van, Co To, Quangninh, Vietnam, in March 2014, and identified by Prof. Do Cong Thung. A voucher specimen (BV-SB1) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST, Vietnam.
3.3. Extraction and isolation

Dried body walls of the starfish *P. glacilis* (3.5 kg) were extracted three times with MeOH under ultrasonic condition to obtain 400 g residue after removal of MeOH in vacuum. This was suspended in water (2 L) and portioned three times with CH₂Cl₂ (2 L each time) to obtain CH₂Cl₂ residue (70 g) and water layer. The latter was passed through a Diaion HP-20 CC eluting with increasing concentration of MeOH in water (0, 25, 50, 75, and 100%) to obtain four fractions, W₁–W₄, after removal of the fraction eluted with water.

Fraction W₃ was crudely separated on RP-18 MPLC eluting with MeOH–H₂O (1:1, v/v) to obtain five subfractions, W₃A–W₃E. Subfraction W₃C (720 mg) was further separated by silica gel CC eluting with CH₂Cl₂–MeOH–H₂O (27:1:0.15, v/v), followed by Sephadex LH-20 CC with MeOH–H₂O (1:1, v/v) to furnish compound 6 (12 mg). Subfraction W₃D (1.3 g) was separated into two smaller fractions, W₃D₁ (210 mg) and W₃D₂ (700 mg), by silica gel CC eluting with CH₂Cl₂–MeOH–H₂O (2.5:1:0.15, v/v). Fraction W₃D₂ (700 mg) was purified by Sephadex LH-20 CC with MeOH–H₂O (1:1, v/v), followed by YMC CC with MeOH–H₂O (1.5:1, v/v) to give compound 5 (9 mg). Subfraction W₃E (900 mg) was further separated by silica gel CC eluted with CH₂Cl₂–MeOH–H₂O (4:1:0.15, v/v) to obtain five smaller fractions W₃E₁–W₃E₅. Fraction W₃E₃ (60 mg) was purified by silica gel CC eluted with EtOAc–MeOH–H₂O (5:1:0.1, v/v), followed by Sephadex LH-20 CC with MeOH–H₂O (1:1, v/v) to give compounds 2 (5 mg) and 4 (8 mg). Fraction W₃E₄ (100 mg) was purified by CH₂Cl₂–acetone–H₂O (1:3:0.1, v/v) to give compound 3 (15 mg). Finally, compound 1 (6 mg) was purified from fraction W₃E₅ (65 mg) after subjecting it on silica gel CC eluted with EtOAc–MeOH–H₂O (3.5:3:0.1, v/v).

3.3.1. Pentaceroside A (1)

Amorphous white powder; [α]ₚD°–25 (c 0.05, MeOH); IR (KBr) νmax: 3369, 2930, 1648, and 1042 cm⁻¹; for ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) spectroscopic data, see Table 1; FT-ICR-MS: m/z 755.4193 [M + Na]⁺ (calcd for C₃₇H₆₄O₁₄Na, 755.4188).

3.3.2. Pentaceroside B (2)

Amorphous white powder; [α]ₚD°+11 (c 0.05, MeOH); IR (KBr) νmax: 3366, 2946, 1650, and 1055 cm⁻¹; for ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) spectroscopic data, see Table 1; FT-ICR-MS: m/z 623.3771 [M + Na]⁺ (calcd for C₃₂H₅₆O₁₀Na, 623.3766).

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Disclosure statement

No potential conflict of interest was reported by the authors.
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