Asterosaponins and glycosylated polyhydroxysteroids from the starfish Culcita novaeguineae and their cytotoxic activities

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Using combined chromatographic methods, two asterosaponins (compounds 1 and 2), including a new compound novaeguinoside E (compound 1), and six glycosylated polyhydroxysteroids (compounds 3–8) were isolated from a methanol extract of the starfish Culcita novaeguineae. 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 HRESI-MS) and by comparison with the literature values. The new compound 1 represents the third example of asterosaponins containing the 5α-cholesta-9(1l)-en-3β,6α,20,22-tetraol aglycone. Among isolated compounds, 4–7 exhibited moderate to weak cytotoxic activities against five human cancer cell lines such as Hep-G2 (hepatoma), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma).

Keywords: Culcita novaeguineae; Oreasteridae; starfish; novaeguinoside E; cytotoxic activity

1. Introduction

Starfish are invertebrates belonging to the class Asteroidea, phylum Echinodermata. The secondary metabolites from starfish are characterized by a diversity of polar steroids, including polyhydroxylated steroids and steroid glycosides. There are two main structural groups of steroid glycosides from starfish, namely asterosaponins and glycosylated polyhydroxysteroids. These compounds have exhibited a variety of biological activities, such as cytotoxic, hemolytic, and anti-microbial effects [1–5].

As a part of our ongoing investigations to catalog the chemical constituents and biological effects of Vietnamese starfish [6–11], we studied to this concern the species C. novaeguineae. The present study addresses the isolation and structure elucidation of two asterosaponins, including a new compound novaeguinoside E (1), and six glycosylated polyhydroxysteroids (Figure 1) from this starfish. Their in vitro cytotoxic activities against five human cancer cell lines were also evaluated using the sulforhodamine B (SRB) method.

2. Results and discussion

A methanol extract (125 g) of C. novaeguineae was suspended in water and partitioned with dichloromethane. Two asterosaponins (1 and 2) and six
glycosylated polyhydroxystereoids (3–8) were isolated from the water layer and dichloromethane residue using combined chromatographic methods. Detailed analysis of the spectroscopic data (1D, 2D NMR, and MS) and comparison with previously reported values led to the elucidation of the known compounds to be sodium salt of 6α-[(O-β-D-fucopyranosyl-(1 → 2)-O-β-D-galactopyranosyl-(1 → 4)-O-β-D-quinoxyranosyl-(1 → 2)]-O-β-D-xyllopyranosyl-(1 → 3)-O-β-D-quinoxyranosyl-ox]-5-α-preg-9-(11)-en-20-one (2) [12,13], linckoside B (3) [14], halilurosides A (4) [15–19], halilurosides B (5) [15–19], culcitoside C5 (6) [17], halilurosides D (7) [15,17], and halilurosides E (8) [15–19]. Among the isolated compounds, 3 was isolated from *Culcita* species for the first time.

Novaeguinoside E (1) was isolated as a white amorphous powder. Its molecular formula was determined as C_{56}H_{91}NaO_{25}S by high-resolution electrospray ionization mass spectrometry (HRESI-MS) at m/z 1273.5257 [M + Na]^+. The NMR features indicated an asterosaponin, one of the main constituents of starfish. The $^1$H and $^{13}$C NMR data (in DMSO-$d_6$) of compound 1 were similar to those of protoreasteroside [20], except for differences in the data of sugar moieties. Analysis of 1D and 2D NMR spectra confirmed that the aglycone of compound 1 contained three oxymethine groups [δC 75.2 (C-3), 78.1 (C-6), and 76.2 (C-22)/δH 3.83–3.87 (H-3), 3.45–3.47 (H-6), and 3.04–3.06 (H-22), each 1H, m], one oxygenated quaternary carbon atom [δC 75.1 (C-20)], two trisubstituted double bonds [δC 145.2 (s, C-9) and δC 115.7 (d, C-11)/δH 5.24 (1H, d, J = 5.0 Hz, H-11); δC 124.0 (d, C-24)/δH 5.20 (1H, t, J = 7.0 Hz, H-24) and δC 130.4 (s, C-25)], and five tertiary methyl groups [δC 13.0 (C-18), 19.0 (C-19), 19.9 (C-21), 17.8 (C-26), and 25.6 (C-27)/δH 0.72 (H-18), 0.88 (H-19), 1.06 (H-21), 1.55 (H-26), and 1.65 (H-27), each 3H, s]. Locations of the oxygenated quaternary carbon atom at C-20, one oxymethine group at C-22, and one double bond at C-24/C-25 were identified by $^1$H–$^1$H correlation spectroscopy (COSY) peaks of H-22/H-23/H-24 and combination with heteronuclear multiple-bond correlation (HMBC) cross-peaks of H-21 (δH 1.06) with C-17 (δC 53.8)/C-20 (δC 75.1)/C-22 (δC 76.2) and those of H-26

![Figure 1. The structures of compounds 1–8.](image-url)
(\(\delta_C 1.55\))/H-27 (\(\delta_H 1.65\)) with C-24 (\(\delta_C 124.0\))/C-25 (\(\delta_C 130.4\)). Detailed analysis of the other HMBC and COSY peaks (Figure 2) unambiguously identified the planar structure of the aglycone. In the rotating frame Overhause effect spectroscopy (ROESY), the correlation of H-3 (\(\delta_H 3.83–3.87\)) with H-5 (\(\delta_H 1.05–1.07\)) suggested an \(\alpha\)-orientation of H-3. Spatial proximities were observed between H-6 (\(\delta_H 3.45–3.47\)) and H-8 (\(\delta_H 1.97–1.99\))/H-19 (\(\delta_H 0.88\)) as well as H-8 (\(\delta_H 1.97–1.99\)) and H-18 (\(\delta_H 0.72\)), indicating the \(\beta\)-orientation of H-6 (Figure 2). The \(^{13}\)C NMR chemical shift of C-21 at \(\delta_C 19.9\) (in DMSO-\(\text{d}_6\)) indicated the relative \(R^*\) configuration at C-20 [20]. For determination of the stereochemistry at C-22, the \(^1\)H NMR spectrum of compound 1 was recorded again in pyridine-\(\text{d}_5\). The \(^1\)H NMR chemical shift of H-21 at \(\delta_H 1.64\) (pyridine-\(\text{d}_5\)) clearly indicated the relative \(S^*\) configuration at C-22 [20].

In addition, the \(^{13}\)C NMR spectrum of compound 1 contained five anomeric carbon signals at \(\delta_C 102.7\) (C-1''), 102.5 (C-1'''), 104.5 (C-1''''), 100.1 (C-1'''''), and 105.6 (C-1'''''''); which correlated with the corresponding anomeric protons (each 1H, d, \(J = 7.5\) Hz) at \(\delta_H 4.31\) (H-1''), 4.53 (H-1'''), 4.44 (H-1'''''), and 4.21 (H-1''''''') in the heteronuclear single quantum coherence (HSQC) spectrum, confirming the presence of five sugar moieties. A detailed comparison of the \(^1\)H and \(^{13}\)C NMR data for the oligosaccharide chain of compound 1 with those of protoreasteroside [20] and a combination of the 1D total correlation spectroscopy (TOCSY), COSY, HMBC, HSQC, and ROESY data.
(Figure 2) indicated that the difference between these two compounds was only observed in the fourth sugar moiety. The $^{13}$C NMR chemical shifts of this sugar in DMSO-$d_6$ at $\delta_C$ 100.1 (C-1), 81.5 (C-2), 72.4 (C-3), 70.2 (C-4), 70.1 (C-5), and 16.5 (C-6) were similar to those of novaeguinoside A in pyridine-$d_5$ at $\delta_C$ 102.0 (C-1), 82.8 (C-2), 74.9 (C-3), 71.7 (C-4), 71.6 (C-5), and 16.9 (C-6) [13] and quite different from those of protoreastosteroside in pyridine-$d_5$ at $\delta_C$ 101.2 (C-1), 84.0 (C-2), 75.9 (C-3), 77.3 (C-4), 73.4 (C-5), and 18.2 (C-6) [20], respectively, indicating the fourth sugar is fucose (Table 1). This was also supported by hypothetic biosynthesis with the coexistence of compound 1 and novaeguinoside A [13], containing the same pentaglycoside chain, in the starfish *C. novaeguineae*. The HMBC correlations of the anomeric proton H-1$^{\beta\prime\prime}$ ($\delta_H$ 4.21) with C-2$^{\alpha\prime\prime}$ ($\delta_C$ 81.5), H-1$^{\beta\prime\prime}$ ($\delta_H$ 4.42) with C-4$^{\alpha\prime\prime}$ ($\delta_C$ 76.5), H-1$^{\alpha\prime}$ ($\delta_H$ 4.44) with C-2$^{\beta\prime}$ ($\delta_C$ 82.7), and H-1$^{\alpha\prime}$ ($\delta_H$ 4.53) with C-3$^{\beta\prime}$ ($\delta_C$ 87.9) confirmed attachment position of the fucose II at C-2$^{\alpha\prime\prime}$, fucose I at C-4$^{\alpha\prime\prime}$, quinovose II at C-2$^{\beta\prime}$, and xylose at C-3$^{\beta\prime}$, respectively. Moreover, the anomeric proton H-1$^{\alpha\prime}$ ($\delta_H$ 4.31) of quinovose I had an HMBC cross-peak with C-6 ($\delta_C$ 78.1), indicating the common attachment position of the pentaglycoside chain at C-6 of the steroidal aglycone (Figure 2). The large coupling constant ($J = 7.5$ Hz) of the anomeric protons indicated all $\beta$-glycosidic linkage. Configuration of all five sugars was assigned as D by analogy with novaeguinoside A [13] and all the reported asterosaponins. Consequently, the structure of novaeguinoside E (1) was elucidated as sodium (20R*,22S*)-6α-O-[(β-d-fucopyranosyl(1 → 2)-β-d-fucopyranosyl(1 → 4)-[β-d-quinovopyranosyl(1 → 2)]-β-d-xylopyranosyl(1 → 3)-β-d-quinovopyranosyl]-3β,6α,20,22-tetrahydroxy-5α-cholesta-9(11),24-dien-3β-yl sulfate. The presence of 5α-cholesta-9(11)-en-3β,6α,20,22-tetraol aglycone in asterosaponins is quite rare. To the best of our knowledge, novaeguinoside E (1) represents the third example of asterosaponins containing this aglycone [20,21] (Table 1).

All isolated compounds were evaluated for their cytotoxic activity against five human cancer cell lines, such as Hep-G2 (hepatoma), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma), using the SRB method [22]. Among isolates, halitlylosides B and D (5 and 7) showed moderate cytotoxicity against KB, LNCaP, MCF7, and SK-Mel2 cell lines (Table 2) with the IC$_{50}$ values of 31.80 ± 1.59–50.09 ± 4.06 μM and weak effect on Hep-G2 cell line with the IC$_{50}$ values of 80.22 ± 3.67 and 75.01 ± 4.11 μM, respectively. Moderate cytotoxicity against LNCaP (IC$_{50}$ = 48.59 ± 2.30 μM) and MCF7 (IC$_{50}$ = 51.61 ± 2.70 μM) cells and weak effects on KB (IC$_{50}$ = 70.70 ± 3.56 μM) and SK-MEL2 (IC$_{50}$ = 73.99 ± 3.10 μM) cells were observed for halitlyloside A (4). Culcitoside C$_{5}$ (6) showed weak cytotoxicity against KB, LNCaP, MCF7, and SK-Mel2 cell lines with the IC$_{50}$ values of 57.08 ± 1.81–92.04 ± 2.84 μM; the positive control (ellipticine) had IC$_{50}$ values of 2.07 ± 0.12, 1.99 ± 0.16, 1.95 ± 0.12, and 2.15 ± 0.24 μM, respectively (Table 2). Compounds 1–3 and 8 did not exhibit any significant cytotoxic effect on all five tested cell lines (IC$_{50}$ > 100 μM).

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). High-resolution mass spectra were recorded on a MicroQ-TOF III mass spectrometer (Bruker Daltonics, Bremen, Germany). The $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded on Bruker AM500 (Billerica, MA, USA). TMS was 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) and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F<sub>254</sub> (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F<sub>254S</sub> plates.

Table 1. <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of compound 1 in DMSO-<em>d</em><sub>6</sub>.

| Position | δ<sub>C</sub><sup>a</sup> | δ<sub>H</sub><sup>b</sup> mult. (J in Hz) | Position | δ<sub>C</sub><sup>a</sup> | δ<sub>H</sub><sup>b</sup> mult. (J in Hz) |
|----------|-----------------|-------------------------------|----------|-----------------|-------------------------------|
| Aglycon  | Qui I           |                               |          |                 |                               |
| 1        | 35.1            | 1.25–1.27 m                   | 1<sup>1</sup> | 102.7          | 4.31 d (7.5)                 |
|          | 161–1.63 m      |                               |          |                 |                               |
| 2        | 28.3            | 1.34–1.38 m                   | 2<sup>1</sup> | 73.0           | 3.18                           |
|          | 2.11–2.13 m     |                               |          |                 |                               |
| 3        | 75.2            | 3.83–3.87 m                   | 3<sup>1</sup> | 87.9           | 3.28                           |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 4        | 29.5            | 1.03–1.05 m                   | 4<sup>1</sup> | 72.9           | 2.91 t (9.0)                  |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 5        | 48.4            | 1.05–1.07 m                   | 5<sup>1</sup> | 70.7           | 3.27                           |
|          | 2.26–2.28 m     |                               |          |                 |                               |
| 6        | 78.1            | 3.45–3.47 m                   | 6<sup>1</sup> | 17.8           | 1.16 d (6.5)                  |
|          | 2.26–2.28 m     |                               |          |                 |                               |
| 7        | 40.6            | 0.82–0.84 m                   | Xyl      |                 |                               |
|          | 2.26–2.28 m     |                               |          |                 |                               |
| 8        | 34.7            | 1.97–1.99 m                   | 1<sup>1</sup><sup>1</sup> | 102.5 | 4.53 d (7.5) |
|          | 2.16–2.22 m     |                               |          |                 |                               |
| 9        | 145.2           | 1.14–1.16 m                   | 2<sup>1</sup><sup>1</sup> | 82.7 | 3.35 dd (7.5, 9.5) |
|          | –               |                               |          |                 |                               |
| 10       | 37.7            | 1.09–1.09 m                   | 3<sup>1</sup><sup>1</sup> | 74.0 | 3.58 t (9.5) |
|          | 2.12–2.14 m     |                               |          |                 |                               |
| 11       | 115.7           | 5.24 d (5.0)                  | 4<sup>1</sup><sup>1</sup> | 76.5 | 3.58-3.61 m |
|          | 2.16–2.20 m     |                               |          |                 |                               |
| 12       | 42.0            | 1.94–1.97 m                   | 5<sup>1</sup><sup>1</sup> | 63.1 | 3.31 c |
|          | 2.16–2.20 m     |                               |          |                 |                               |
| 13       | 40.5            |                               | Qui II   |                 |                               |
| 14       | 53.4            | 1.14–1.16 m                   | 1<sup>1</sup><sup>1</sup><sup>1</sup> | 104.5 | 4.44 d (7.5) |
|          | 2.16–2.22 m     |                               |          |                 |                               |
| 15       | 24.5            | 1.09–1.11 m                   | 2<sup>1</sup><sup>1</sup><sup>1</sup> | 74.8 | 3.06 dd (7.5, 9.0) |
|          | 2.12–2.14 m     |                               |          |                 |                               |
| 16       | 21.1            | 1.61–1.63 m                   | 3<sup>1</sup><sup>1</sup><sup>1</sup> | 75.6 | 3.12 t (9.0) |
|          | 1.74–1.76 m     |                               |          |                 |                               |
| 17       | 53.8            | 1.82–1.85 m                   | 4<sup>1</sup><sup>1</sup><sup>1</sup> | 74.6 | 2.86 t (9.0) |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 18       | 13.0            | 0.72 s                        | 5<sup>1</sup><sup>1</sup><sup>1</sup> | 72.4 | 3.22 dd (6.0, 9.0) |
|          | –               |                               |          |                 |                               |
| 19       | 19.0            | 0.88 s                        | 6<sup>1</sup><sup>1</sup><sup>1</sup> | 17.3 | 1.19 d (6.0) |
|          | –               |                               |          |                 |                               |
| 20       | 75.1            |                               | Fuc I    |                 |                               |
| 21       | 19.9            | 1.06 s                        | 1<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 100.1 | 4.42 d (7.5) |
|          | –               |                               |          |                 |                               |
| 22       | 76.2            | 3.04–3.06 m                   | 2<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 81.5 | 3.44 c |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 23       | 29.0            | 1.74–1.76 m                   | 3<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 72.4 | 3.50 c |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 24       | 124.0           | 5.20 t (7.0)                  | 4<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 70.2 | 3.45 c |
|          | 2.34–2.36 m     |                               |          |                 |                               |
| 25       | 130.4           |                               | 5<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 70.1 | 3.58 c |
|          | –               |                               |          |                 |                               |
| 26       | 17.8            | 1.55 s                        | 6<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 16.5 | 1.14 d (6.0) |
|          | –               |                               |          |                 |                               |
| 27       | 25.6            | 1.65 s                        | Fuc II   |                 |                               |
|          | 12<sup>1</sup><sup>1</sup><sup>1</sup><sup>1</sup> | 105.6 | 4.21 d (7.5) |
|          | 2.34–2.36 m     |                               |          |                 |                               |

Note: All assignments were done by 1D TOCSY, HSQC, COSY, HMBC, and ROESY experiments.

<sup>a</sup>125 MHz.
<sup>b</sup>500 MHz.
<sup>c</sup>Overlapped signals.
(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 Culcita novaeguineae Muller & Troschel, 1842 was collected at Quang Ninh, Vietnam, in October 2013, and identified by Prof. Do Cong Thung. A voucher specimen (DAB-DG-CN-01/2013) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST, Vietnam.

3.3 Extraction and isolation

The fresh body walls of C. novaeguineae (10 kg) were cut into small pieces and extracted in hot methanol (three times for 6 h each) to yield a MeOH residue (125 g, A) after removal of the solvent under reduced pressure. This extract was partitioned between H$_2$O and CH$_2$Cl$_2$ (3 × 1.0 L) to give CH$_2$Cl$_2$ extract (C, 15.2 g) and water layer. The latter was passed through Diaion HP-20 CC eluting with an 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 W4 (6.5 g) was separated into five subfractions, W4A–W4E, by silica gel MPLC using gradient elution of CH$_2$Cl$_2$—MeOH (20:1–1:1, v/v). Subfraction W4D (1.5 g) was further separated on YMC RP-18 CC eluting with MeOH–H$_2$O (1:1, v/v), followed by silica gel CC using CH$_2$Cl$_2$—MeOH–H$_2$O (3:1:0.15, v/v) as eluent to furnish compounds 1 (2.7 mg) and 2 (4.5 mg). The CH$_2$Cl$_2$ extract (C, 15.2 g) was separated by silica gel MPLC using gradient elution of CH$_2$Cl$_2$—MeOH (100:1–1:1, v/v) to obtain nine fractions, C1–C9. Fraction C-8 (2 g) was separated into six subfractions, C-8.1–C8.6, by YMC RP-18 CC using gradient elution of MeOH–H$_2$O (1:1–5:1, v/v). Further separation of subfraction C-8.5 (0.56 g) by silica gel CC eluting with EtOAc—MeOH—H$_2$O (10:1:0.1, v/v) gave seven smaller fractions, C-8.5A–C8.5G. Purification of fraction C-8.5E (76 mg) on silica gel CC eluted with CH$_2$Cl$_2$—MeOH—H$_2$O (5:1:0.1, v/v) furnished compounds 3 (4.0 mg), 7 (7.5 mg), and 8 (9.5 mg). Fraction C-8.5F (150 mg) yielded compounds 4 (5.0 mg), 5 (3.5 mg), and 6 (2.5 mg), after subjecting it to Sephadex LH-20 CC eluted with acetone–H$_2$O (1:1, v/v).

3.3.1 Novaeguinoside E (1)

Amorphous white powder; [α]$_D^{20}$ + 4.5 (c 0.05, MeOH); IR (KBr) $\nu_{max}$: 3366, 2946, 1747, 1650, 1376, and 1055 cm$^{-1}$; for $^1$H NMR (DMSO-$d_6$, 500 MHz) and $^{13}$C NMR (DMSO-$d_6$, 125 MHz) spectroscopic

| Compounds | IC$_{50}$ values (µM) |
|-----------|-----------------------|
| 4         | 48.59 ± 2.30          |
| 5         | 39.68 ± 2.65          |
| 6         | 57.08 ± 1.81          |
| 7         | 31.80 ± 1.59          |
| Ellipticine$^a$ | 1.99 ± 0.16 |

Note: Results are expressed as mean ± SD of independent experiments performed in triplicate.

$^a$Positive control.
data, see Table 1; HR-ESI-MS: \( m/z \) 1273.5257 \([M + \text{Na}]^+\) (calcd for \( \text{C}_{56}\text{H}_{91}\text{Na}_2\text{O}_{27}\text{S}_2 \% \), 1273.5258).

### 3.4 Cytotoxic assays

Cytotoxic evaluations were performed by following the protocols described previously [23,24].

### Supplementary data

1D, 2D-NMR, and HR-ESI mass spectra for the new compound 1.

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