SUPPLEMENTARY MATERIAL

Triterpene glycosides from the Vietnamese sea cucumber *Holothuria edulis*

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Abstract. From the MeOH extract of the Vietnamese sea cucumber *Holothuria edulis*, eight triterpene glycosides (1–8), including one new compound namely holothurin A5 (1), were isolated by using various chromatographic separations. Their structures were established by spectroscopic experiments including 1D, 2D NMR and HR-ESI-MS. Holothurin A5 (1) has a hydroperoxy group at C-25. To the best of our knowledge, this is the first report of this group in triterpene saponins obtained from sea cucumbers to date. In addition, the *in vitro* cytotoxicity against five human cancer cell lines (HepG2, KB, LNCaP, MCF7 and SK-Mel2) of all isolated compounds was also evaluated using SRB assays.

Keywords: *Holothuria edulis*, Holothuriidae, triterpene glycoside, cytotoxicity.
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## General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Tokyo, Japan). The HR-ESI-MS and ESI-MS/MS spectra were recorded on a Bruker Impact IIQ-TOF spectrometer (Germany). The $^1$HNMR (500 MHz) and $^{13}$CNMR (125 MHz) spectra were recorded on a Bruker AVANCE III HD 500 (MA, USA) FT-NMR spectrometer with tetramethylsilane (TMS) 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®GEL (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan) resins. TLC used pre-coated silica gel 60 F$_{254}$ (Merck) and RP-18 F$_{254S}$ plates (Merck), and compounds were visualized by spraying with aqueous 10% H$_2$SO$_4$ and heating for 3–5 min.
Figure S1. $^1$H NMR spectrum (pyridine-$d_5$, 500 MHz) of compound 1
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Figure S11. Plausible (−) ESI MS/MS fragmentation of compound 1
Table S1. $^1$H (pyridine-$d_5$, 500 MHz) and $^{13}$C NMR (pyridine-$d_5$, 125 MHz) spectroscopic data of 1.

| C   | $\delta^a$ | $\delta^b,c$ | $\delta^{b,d}$ mult. (J = Hz) | HMBC          |
|-----|------------|---------------|-------------------------------|---------------|
|     |            |               |                               | (H $\rightarrow$ C) |
| **Aglycon** |           |               |                               |               |
| 1   | 36.6       | 36.1          | 1.30 m/1.71 m                 |               |
| 2   | 27.1       | 26.7          | 1.80 m/1.99 m                 |               |
| 3   | 88.64      | 88.4          | 3.04 dd (4.0, 11.5)           | 1'            |
| 4   | 40.03      | 39.7          | -                             |               |
| 5   | 52.70      | 52.4          | 0.90 br d (11.0)              |               |
| 6   | 21.2       | 20.9          | 1.45 m/1.66 m                 |               |
| 7   | 28.0       | 28.1          | 1.43 m/1.70 m                 |               |
| 8   | 40.08      | 40.6          | 3.27 dd (5.5, 12.0)           |               |
| 9   | 153.95     | 153.8         | -                             |               |
| 10  | 39.71      | 39.4          | -                             |               |
| 11  | 115.54     | 115.2         | 5.57 br d (5.0)               | 8, 10, 12, 13 |
| 12  | 71.49      | 71.1          | 4.91 d (5.0)                  | 18            |
| 13  | 58.61      | 58.4          | -                             |               |
| 14  | 46.40      | 46.1          | -                             |               |
| 15  | 38.93      | 36.4          | 1.33 m/1.78 m                 | 17            |
| 16  | 36.0       | 35.6          | 2.28 m/2.64 m                 |               |
| 17  | 89.36      | 89.0          | -                             |               |
| 18  | 174.87     | 174.6         | -                             |               |
| 19  | 22.5       | 22.3          | 1.28 s                        | 1, 5, 9, 10   |
| 20  | 87.21      | 86.5          | -                             |               |
| 21  | 23.00      | 23.0          | 1.69 s                        | 17, 20, 22    |
| 22  | 36.6       | 41.3          | 2.64 m                        |               |
| 23  | 22.29      | 124.4         | 5.95 ddd (6.5, 9.0, 15.5)     | 22, 24, 25    |
| 24  | 39.71      | 139.1         | 6.01 d (15.5)                 | 22, 23, 25, 26, 27 |
| 25  | 28.00      | 81.1          | -                             |               |
| 26  | 22.61      | 24.8          | 1.49 s                        | 24, 25, 27    |
| 27  | 22.67      | 24.8          | 1.50 s                        | 24, 25, 26    |
| 30  | 16.7       | 16.5          | 0.98 s                        | 3, 4, 5, 31   |
| 31  | 28.00      | 27.8          | 1.19 s                        | 3, 4, 5, 30   |
| 32  | 20.1       | 19.8          | 1.57 s                        | 8, 13, 14, 15 |
| **Sulfo-Xyl** |         |               |                               |               |
| $1'$ | 105.1      | 105.0         | 4.62 d (7.5)                  | 3             |
| $2'$ | 83.0       | 82.7          | 4.00 dd (7.5, 9.0)            |               |
| $3'$ | 76.2       | 75.4          | 4.28 t (9.0)                  |               |
| $4'$ | 75.4       | 75.9          | 5.10 m                        |               |
| $5'$ | 63.9       | 64.2          | 3.71 dd (11.0, 11.5)          | 4.77 dd (5.0, 11.5) |
| **Qui** |           |               |                               |               |
| $1''$ | 105.1      | 105.0         | 5.01 d (8.0)                  | 2'            |
| $2''$ | 76.2       | 76.0          | 3.94 dd (8.0, 9.0)            |               |
| $3''$ | 75.6       | 75.3          | 4.02 t (9.0)                  |               |
| $4''$ | 87.0       | 86.8          | 3.59 t (9.0)                  |               |
| $5''$ | 71.7       | 71.5          | 3.67 dd (9.0, 6.0)            |               |
| $6''$ | 18.1       | 18.0          | 1.64 d (6.0)                  | $4'\,, 5''$   |
| C      | δ_c | δ_c^{b,c} | δ_{t}^{b,d} | HMBC    |
|--------|-----|----------|-------------|---------|
| Glc    |     |          | mult. (J = Hz) |         |
| 1’’    | 104.6 | 104.4   | 4.88 d (8.0) | 4’’     |
| 2’’    | 73.9   | 73.6     | 3.97 dd (8.0, 9.0) |         |
| 3’’    | 88.2   | 87.4     | 4.21 t (9.0) |         |
| 4’’    | 69.9   | 69.5     | 3.93*        |         |
| 5’’    | 77.7   | 77.6     | 3.94*        |         |
| 6’’    | 62.4   | 61.8     | 4.08 dd (5.5, 12.0) |         |
|        |        |          | 4.41 br d (12.0) |         |
| OMe-Glc|     |          |              |         |
| 1’’’   | 105.4 | 105.1    | 5.25 d (8.0) | 3’’     |
| 2’’’   | 74.9   | 74.8     | 3.92 dd (8.0, 9.0) |         |
| 3’’’   | 87.7   | 87.5     | 3.67 t (9.0) |         |
| 4’’’   | 70.8   | 70.4     | 3.98 t (9.0) |         |
| 5’’’   | 78.0   | 78.0     | 3.94 m       |         |
| 6’’’   | 62.4   | 61.9     | 4.14 dd (5.5, 12.0) |         |
|        |        |          | 4.43 dd (2.0, 12.0) |         |
| OMe    | 60.4   | 60.7     | 3.81 s       | 3’’’    |

\^δ_c of holothurin A2 in pyridine-d5 (Oleinikova et al. 1982), recorded in pyridine-d5, 125 MHz, 500 MHz, overlapped signals; All assignments were done by HSQC, HMBC, COSY, ROESY, and 1D and 2D TOCSY experiments.
Table S2. Cytotoxic activity of compounds 1–8

| Compounds | IC50 values (μM) |
|-----------|------------------|
|           | LNCaP            | HepG2           | KB              | MCF7            | SK-Mel2         |
| 1         | 66.22±6.32       | 57.53±6.27      | 46.65±2.28      | 49.08±6.44      | 63.53±3.49      |
| 2         | 0.96±0.09        | 0.76±0.06       | 0.75±0.09       | 0.81±0.07       | 0.84±0.05       |
| 3         | 82.75±3.91       | 75.76±7.60      | 67.31±6.93      | 76.45±6.29      | 68.55±3.18      |
| 4         | 57.61±5.54       | 59.59±3.38      | 64.72±4.94      | 55.99±6.43      | 61.65±5.67      |
| 5         | >100             | 93.56±4.95      | 91.27±5.41      | 91.47±3.30      | >100            |
| 6         | 1.30±0.18        | 2.03±0.49       | 1.79±0.33       | 2.29±0.47       | 2.49±0.21       |
| 7         | 2.74±0.29        | 2.63±0.28       | 2.75±0.31       | 3.35±0.47       | 3.66±0.41       |
| 8         | >100             | >100            | >100            | >100            | >100            |
| Ellipticine* | 1.71±0.24       | 1.67±0.28       | 1.42±0.12       | 1.54±0.20       | 1.50±0.16       |

*Ellipticine was used as positive control. Results are the means±SD of triplicate experiments.

Reference

Oleinikova GK, Kuznetsova TA, Rovnykh NV, Kalinovskii AI, Elyakov GB. 1982. Glycosides of marine invertebrates. XVIII. Holothurin A2 from the Caribbean holothurian *Holothuria floridana*. Chem Nat Comp. 18:501-502.