Triterpene Tetraglycosides From *Stichopus Herrmanni* Semper, 1868

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Abstract

Using various column chromatographic methods, 5 triterpene tetraglycosides (1–5), including one new compound, namely holothurin A₆ (I), were obtained from the water soluble part of the methanol extract of the sea cucumber *Stichopus herrmanni*. Their structures were confirmed by careful analysis of the 1D and 2D NMR, and HR ESI QTOF mass spectra. Noteworthily, 24-dehydroechinoside A (3) showed potent cytotoxicity to 5 human cancer cell lines {HepG2 (hepatoma cancer), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma)} with IC₅₀ values ranging from 0.19 ± 0.03 to 1.17 ± 0.18 µM.

Keywords

*Stichopus herrmanni*, triterpene tetraglycosides, holothurin A₆, cytotoxic activity

Introduction

Within marine organisms, echinoderms are a source of a broad range of secondary metabolites having various biological activities. Of these metabolites, saponins represent the most abundant and diverse products in the phylum Echinodermata. Among these marine invertebrates, saponins are the most known and structurally diverse within sea cucumbers. The majority of them are holostane derivatives possessing an 18(20)-lanostane lactone as aglycon and a carbohydrate chain consisting of from 1 to 6 monosaccharide units. These compounds demonstrate interesting biological effects such as cytotoxic, ichthyotoxic, antifungal, and hemolytic activities, as well as a series of additional effects at subtoxic doses, including immunomodulatory and cancer preventive.¹,²

As a part of our ongoing investigations of saponins from Vietnamese sea cucumbers,³–⁶ this paper deals with the isolation and structural elucidation of 5 triterpene tetraglycosides (1–5), including one new compound, holothurin A₆ (I), from the sea cucumber *S. herrmanni* Semper, 1868. In addition, their cytotoxic activity was also evaluated against the human cancer cell lines HepG2 (hepatoma cancer), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma).

Results and Discussion

Using various column chromatographic separations, 5 triterpene tetraglycosides (1–5) were obtained from the water soluble part of the sea cucumber *Stichopus herrmanni* (Figure 1). By careful analysis of the 1D and 2D NMR and ESI-MS data, as well as by comparison with literature data, the known saponins were elucidated as 24-β-hydroxy-25-dehydroechinoside A (2), ³ 24-dehydroechinoside A (3),⁴ holothurin A₅ (4),⁵⁻⁶ and holothurin A₆ (5).⁶

Holothurin A₅ (3) was obtained as a white powder. Its HR ESI QTOF MS exhibited a quasi-molecular ion peak at *m/z* 1243.4767 [M + Na]⁺ (see Figure S9, Supplemental material), consistent with the molecular formula of C₅₄H₆₅NaO₂₇S. Similarly as in the case of 2–5, the ¹H and ¹³C NMR spectra of 1 were indicative for a triterpene tetraglycoside with characteristic signals of 4 anomeric protons/carbons at δ₁H 4.63 (1H, d, *J* = 7.2 Hz, H-1′)/δ₁C 105.1 (C-1′), δ₁H 5.03 (1H, d, *J* = 7.8 Hz, H-1″)/δ₁C 105.1 (C-1″), δ₁H 4.89 (1H, d, *J* = 7.8 Hz, H-1′′′)/δ₁C 104.6 (C-1′′′), and δ₁H 5.25

1 In vitro analysis of the 1D and 2D NMR, and HR ESI QTOF mass spectra, the known saponins were elucidated as 24-β-hydroxy-25-dehydroechinoside A (2), ³ 24-dehydroechinoside A (3),⁴ holothurin A₅ (4),⁵⁻⁶ and holothurin A₆ (5).⁶

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Extensive analysis of the HSQC, $^1$H–$^1$H COSY, HMBC, 1D and 2D TOCSY spectra allowed the assignment of the complete $^1$H and $^{13}$C NMR spectroscopic data for all 4 sugar units (see Table 1). These data were extremely similar to those of holothurin A5 (5), suggesting that 1 and 5 possess the same tetrasaccharide chain: 3-O-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-O-sodium sulfate-β-D-xylopyranoside. The D-configuration of all 4 sugar units was assigned due to biosynthetic reasons, and analogy with 2–5 coexistence in S. bermanni. The sequence of sugar units in the tetrasaccharide chain was assigned by HMBC interactions of H-1’ (δH 5.25) with C-3’ (δC 87.6), H-1’ (δH 4.89) with C-4’ (δC 86.9) and H-1” (δH 5.03) with C-2’ (δC 82.8) (Figure 1). This was also supported by ROESY correlations of H-1’ (δH 5.25) with H-3’ (δH 4.21), H-1’ (δH 4.89) with H-4’ (δH 3.60), and H-1” (δH 5.03) with H-2’ (δH 4.00). Moreover, the $^1$H and $^{13}$C NMR spectra for the triterpene part of 1 exhibited typical signals of 2 oxymethines [δC 88.4 (C-3) and 71.1 (C-12)/δH 3.07 (1H, dd, J = 3.6, 12.0 Hz, H-3) and 4.92 (1H, brd, J = 5.4 Hz, H-12)], 3 oxygenated quaternary carbons [δC 89.1 (C-17), 86.7 (C-20), and 69.6 (C-25)], one trisubstituted double bond [δC 153.9 (C, C-9) and 115.2 (CH, C-11)/δH 5.57 (1H, d, J = 4.2 Hz, H-11)], one trans disubstituted double bond [δC 120.6 (CH, C-23) and 143.5 (CH, C-24)/δH 6.60 (1H, m, H-23) and 5.96 (1H, d, J = 15.0 Hz, H-24)], one lactone carbonyl [δC 174.6 (C-18)], and 7 singlet methyls [δC 22.4 (C-19), 23.1 (C-21), 30.2 (C-26 and C-27), 16.6 (C-30), 27.9 (C-31), and 19.9 (C-32)/δH 1.30 (H-19), 1.70 (H-21), 1.50 (H-26), 1.49 (H-27), 1.01 (H-30), 1.19 (H-31), and 1.59 (H-32), each 3H, s]. The $^1$H and $^{13}$C NMR data for the triterpene part of 1 were also similar to those of holothurin A5 (5), except for the remarkable difference for the side chains. The $^{13}$C NMR signal for C-25 of 1 was shifted upfield to δC 69.6 versus that of holothurin A5 (5) at δC 81.1, indicating the presence of a hydroxy substituent at C-25.11,12 The relative configuration of the aglycon of 1 was assigned to be the same as those of 2–5 from a biosynthetic viewpoint with their coexistence in S. bermanni. This was also suggested by the similarity of their $^1$H and $^{13}$C NMR spectroscopic data and by the ROESY spectrum, with key spatial proximities of H-3 (δH 3.07) with H-5 (δH 0.92) and H-31 (δH 1.19), H-19 (δH 1.30) with H-8 (δH 3.29) and H-30 (δH 1.01), and H-12 (δH 4.92) with H-21 (δH 1.70) (see Figure 2). Finally, HMBC interaction of the anomeric proton H-1’ (δH 4.63) with C-3 (δC 88.4) and the ROE-correlation of H-1’ (δH 4.63) with H-3 (δH 3.07) confirmed the location of the tetrasaccharide chain at C-3 of the aglycon. Thus, the structure (23E)-3β-O-[3-O-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)-β-D-quinovopyranosyl-(1→2)-4-O-sodium sulfate-β-D-xylopyranosyl]-holost-9(11),23E-diene-12α,17α,25-triol was elucidated for 1 (Supplemental Figures S1-S9).

The cytotoxic activity of compounds 1–3 against the human cancer cell lines HepG2 (hepatoma cancer), KB (epidermoid carcinoma), LNCaP (prostate cancer), MCF7 (breast cancer), and SK-Mel2 (melanoma) was evaluated by SRB assay.13
following previously described steps. Potent cytotoxicity was observed for 24-dehydroechinoside A (3) (IC\textsubscript{50} values from 0.19 ± 0.03 to 1.17 ± 0.18 µM) against all the 5 cell lines, comparable to that of the positive control (ellipticine: IC\textsubscript{50} values from 1.50 ± 0.16 to 1.30 ± 0.16 µM) (Table 2). Similar cytotoxic effect was also reported for holothurin A2 (4) (IC\textsubscript{50} values from 0.75 ± 0.09 to 0.96 ± 0.09 µM). Strong cytotoxic activity was observed for 24ξ-hydroxy-25-dehydroechinoside A (2) on all 5 cell lines (IC\textsubscript{50} values from 3.99 ± 0.58 to 11.67 ± 1.19 µM) and for holothurin A6 (1) on MCF7 (IC\textsubscript{50} = 14.31 ± 1.17 µM) and SK-Mel2 (IC\textsubscript{50} = 13.87 ± 1.39 µM) cell lines. Compound 1 exhibited moderate activity against LNCaP (IC\textsubscript{50} = 48.57 ± 2.49 µM) and HepG2 (IC\textsubscript{50} = 52.62 ± 3.45 µM) cell lines, and weak effect on the KB (IC\textsubscript{50} = 97.86 ± 1.50 µM) cell line. Previously, holothurin A5 (5) was found to exhibit either moderate or weak activity against the tested cell lines (IC\textsubscript{50} values

Table 1. \textsuperscript{1}H and \textsuperscript{13}C NMR Spectroscopic Data of 1.

| C          | \(\delta_c\) | \(\delta_H\) mult. (\(J\) in Hz) | C          | \(\delta_c\) | \(\delta_H\) mult. (\(J\) in Hz) |
|------------|--------------|---------------------------------|------------|--------------|---------------------------------|
| Aglycon    |              |                                 | Glc        |              |                                 |
| 1          | 36.2         | 1.28 m/1.73 m                   | 1′         | 105.1        | 4.63 d (7.2)                   |
| 2          | 26.8         | 1.83 m/2.02 m                   | 2′         | 82.8         | 4.00 dd (7.2, 9.0)             |
| 3          | 88.4         | 3.07 dd (3.6, 12.0)             | 3′         | 75.6         | 4.30 t (9.0)                   |
| 4          | 39.8         |                                 | 4′         | 75.9         | 5.14 m                         |
| 5          | 52.5         | 0.92 br d (10.2)                | 5′         | 64.3         | 3.71 dd (9.0, 11.4)            |
| 6          | 21.0         | 1.49 m/1.70 m                   | Qui        | 105.1        | 5.03 d (7.8)                   |
| 7          | 28.2         | 1.45 m/1.71 m                   | 1′′        | 104.6        | 4.89 d (7.8)                   |
| 8          | 40.7         | 3.29 m                          | 2′′        | 73.7         | 3.98 dd (7.8, 9.0)             |
| 9          | 153.9        |                                 | 3′′        | 87.6         | 4.21 t (9.0)                   |
| 10         | 39.5         |                                 | 4′′        | 86.9         | 3.60 d (9.0)                   |
| 11         | 115.2        | 5.57 br d (4.2)                 | 5′′        | 71.6         | 3.68 dd (9.0, 5.4)             |
| 12         | 71.1         | 4.92 br d (5.4)                 | 6′′        | 18.0         | 1.66 d (6.0)                   |
| 13         | 58.5         |                                 |           |              |                                 |
| 14         | 46.2         |                                 |           |              |                                 |
| 15         | 36.5         | 1.35 m/1.78 m                   | 1′′′       | 103.5        | 5.25 d (7.8)                   |
| 16         | 35.6         | 2.30 m/2.70 m                   | 2′′′       | 74.9         | 3.93 dd (7.8, 9.0)             |
| 17         | 89.1         |                                 | 3′′′       | 87.7         | 3.68 d (9.0)                   |
| 18         | 174.6        | -                               | 4′′′       | 69.6         | 3.94*                         |
| 19         | 22.4         | 1.30 s                          | 5′′′       | 77.7         | 3.95*                         |
| 20         | 86.7         |                                 | 6′′′       | 62.0         | 4.10 dd (4.2, 11.4)            |
| 21         | 23.1         | 1.70 s                          | 1′′′′      | 105.3        | 4.42 br d (11.4)               |
| 22         | 41.3         | 2.68 m                          | 2′′′′      | 74.9         | 4.42 br d (11.4)               |
| 23         | 120.6        | 6.60 m                          | 3′′′′      | 87.7         | 4.42 br d (11.4)               |
| 24         | 143.5        | 5.96 d (13.0)                   | 4′′′′      | 70.5         | 4.01 t (9.0)                   |
| 25         | 69.6         | -                               | 5′′′′      | 78.2         | 3.95 m                         |
| 26         | 30.2         | 1.50 s                          | 6′′′′      | 62.0         | 4.18 dd (4.8, 11.4)            |
| 27         | 30.2         | 1.49 s                          | OMe       | 60.7         | 3.82 s                         |
| 30         | 16.6         | 1.01 s                          |           |              |                                 |
| 31         | 27.9         | 1.19 s                          |           |              |                                 |
| 32         | 19.9         | 1.59 s                          |           |              |                                 |

\(\textsuperscript{*}\)Overlapped signals. All data were confirmed by HSQC, COSY, HMBC, ROESY, and 1D and 2D TOCSY spectra.

Table 2. The Cytotoxicity of 1–3.

| Compounds | IC\textsubscript{50} (µM) |
|-----------|--------------------------|
|           | LNCaP                    | HepG2        | KB          | MCF7        | SK-Mel2     | HEK-293A   |
| 1         | 48.57 ± 2.49             | 52.62 ± 3.45 | 97.86 ± 1.50 | 14.31 ± 1.17 | 13.87 ± 1.39 | >100       |
| 2         | 3.99 ± 0.58              | 7.42 ± 0.38  | 11.67 ± 1.19 | 6.30 ± 0.69  | 5.59 ± 0.52  | 7.70 ± 0.24 |
| 3         | 0.19 ± 0.04              | 0.30 ± 0.05  | 1.17 ± 0.18  | 0.19 ± 0.03  | 0.32 ± 0.03  | 0.27 ± 0.03 |
| Ellipticine\(\textsuperscript{*}\) | 1.50 ± 0.16              | 1.26 ± 0.16  | 1.83 ± 0.20  | 1.30 ± 0.16  | 1.58 ± 0.20  | 1.30 ± 0.16 |

\(\textsuperscript{*}\)Positive control. Data are the means ± SD of triplicate experiments.
from 46.65 ± 2.28 to 66.22 ± 6.32 µM). In addition, 1 showed no cytotoxic effect (IC₅₀ > 100 µM) on the normal human embryonic kidney cell line (HEK-293A), whereas 2 and 3 revealed cytotoxicity with IC₅₀ values of 7.70 ± 0.24 and 0.27 ± 0.03 µM, respectively.

Experimental

General

Refer to Supplemental Material.

Marine Organism Material

*S. berrmanni* Semper, 1868 samples were collected at Bai Giau island (coordinates: 12°40′44″−109°20′33″) and Khai Luong island (coordinates: 12°35′06″−109°24′44″) in Van Phong, Khanhhoa, Vietnam, during July 2020. Its scientific name was identified by one of our authors, Prof Do Cong Thung. Voucher specimens (No. DLB-2020-DG06) were deposited at the IMBC and the IMER, VAST.

Extraction and Isolation

Refer to Supplemental Material.

Holothurin A₆ (1). White amorphous powder. 

\[ \alpha_d^{20} = -21 (c 0.1, \text{MeOH}) \]; 

\(^1\text{H}\) (pyridine-d₅, 600 MHz) and \(^{13}\text{C}\) NMR (pyridine-d₅, 150 MHz) data given in Table 1; 

HR ESI QTOF MS \( m/z \) 1243.4767 [M + Na]⁺ (calcd. for C₅₄H₄₅Na₂O₂₇S⁺, 1243.4783).

Cytotoxic Assays

Refer to Supplemental Material.

Conclusions

Five triterpene tetracyglcosides, with one new compound holothurin A₆ (1), were isolated and structurally elucidated from the Vietnamese sea cucumber *S. berrmanni*. Among the isolated compounds, 24-dehydroechinoside A (3) and holothurin A₂ (4) showed potent cytotoxicity on the human cancer cell lines HepG2, KB, LNCaP, MCF7, and SK-Mel2. Consideration of the cytotoxicity and structures of 1−5 suggested that the side chain might play an important role in the cytotoxicity of these saponins against the tested cancer cell lines and the presence of an oxygenated substituent in the side chains might reduce the cytotoxicity of these compounds.

Acknowledgments

This study was supported by Vietnam Academy of Science and Technology (grant number TDDB0.02/20-22). The authors are thankful to the Institute of Chemistry, VAST for the NMR spectral measurements and Dr Bui Huu Tai, Institute of Marine Biochemistry, VAST for the HR-QTOF mass spectrum measurement.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Our institution does not require ethical approval for reporting individual cases or case series.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Vietnam Academy of Science and Technology, (grant number TDDB0.02/20-22).

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Statement of Human and Animal Rights
This article does not contain any studies with human or animal subjects.

Statement of Informed Consent
There are no human subjects in this article and informed consent is not applicable.

Supplemental Material
Supplemental material for this article is available online.

References
1. Kamayab E, Kellermann MY, Kunzmann A, Schupp PJ. Chemical Biodiversity and Bioactivities of Saponins in Echinodermata With an Emphasis on Sea Cucumbers (Holothuroidea). In YOUMARES 9 — The Oceans: Our Research, Our Future: Proceedings of the 2018 Conference for YOung MArine REsearcher in Oldenburg. Springer International Publishing; 2020:121-157.
2. Kalinin VI, Silchenko AS, Avilov SA, Stonik VA. Progress in the studies of triterpene glycosides from sea cucumbers (holothuroidea, echinodermata) between 2017 and 2021. Nat Prod Commun. 2021;16(10):1-24. DOI:10.1177/1934578X211053934
3. Cuong NX, Vien LT, Hanh TT, et al. Cyotoxic triterpene saponins from Coriophora anops. Bioorg Med Chem Lett. 2015;25(16):3151-3156. DOI:10.1016/j.bmcl.2015.06.005
4. Cuong NX, Vien LT, Hoang L, et al. Cyotoxic triterpene diglycosides from the sea cucumber Stichopus horrens. Bioorg Med Chem Lett. 2017;27(13):2939-2942. DOI:10.1016/j.bmcl.2017.05.003
5. Vien LT, Hoang L, Hanh TTH, et al. Triterpene tetracylicgoisides from the sea cucumber Stichopus horrens. Nat Prod Res. 2018;32(9):1039-1043. DOI:10.1080/14786419.2017.1378206
6. Hoang L, Vien LT, Hanh TTH, et al. Triterpene glycosides from the Vietnamese sea cucumber Holothuria edulis. Nat Prod Res. 2020;34(8):1061-1067. DOI:10.1080/14786419.2018.1548451
7. Bhatnagar S, Dudouet B, Ahond A, et al. Marine invertebrates of the new cedalian lagoon. IV. Saponins and sapogenins from a sea cucumber, Actinopyga flamma. Bull Soc Chim Fr. 1985;1985(1):124-129.
8. Kitagawa I, Kobayashi M, Kyogoku Y. Marine natural products. IX. Structural elucidation of triterpenoidal oligoglycosides from the Bahamean sea cucumber Actinopyga agassizi selenka. Chem Pharm Bull. 1982;30(6):2045-2050. DOI:10.1248/cpb.30.2045.
9. Thanh NV, Dang NH, Kiem PV, Cuong NX, Huong HT, Minh CV. A new triterpene glycoside from the sea cucumber Holothuria scabra collected in Vietnam. ASEAN J Sci Tech Develop. 2006;23(4):253-259. DOI:10.2903/ajstd.113.
10. Oleinikova GK, Kuznetsova TA, Rovnykh NV, Kalinovskii AI, Elyakov GB. Glycosides of marine invertebrates. XVIII. Holothurin A2 from the Caribbean holothurian Holothuria floridana. Chem Natl Compd. 1982;18(4):501-502. DOI:10.1007/BF00579666
11. Silchenko AS, Stonik VA, Avilov SA, et al. Holothurins B2, B3, and B4, new triterpene glycosides from Mediterranean sea cucumbers of the genus Holothuria. J Nat Prod. 2005;68(4):564-567. DOI:10.1021/np049631n
12. Silchenko AS, Avilov SA, Kalinovskiy AI, et al. Psolusosides C3 and D2-D5, five novel triterpene hexaosides from the sea cucumber Psolus fabricii (psolidae, dendrochirotida): chemical structures and bioactivities. Nat Prod Commun. 2019;14(7):1-12. DOI:10.1177/1934578X19861253
13. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst. 1991;83(11):757-766. DOI:10.1093/jnci/83.11.757