Two New Sterol Sulfates from Marine Spider Conch *Lambis Lambis* Linnaeus, 1758

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Abstract

Two new (1 and 2) and six known (3-8) compounds were isolated from the methanolic extract of the Vietnamese marine spider conch *Lambis lambis* Linnaeus, 1758. Based on comprehensive spectroscopic analyses, particularly HRESIMS, and 1D- and 2D-NMR spectra, their chemical structures were determined to be 25,26,27-tri-nor-cholesta-2β,3α,24-triol-3,24-disulfate (1), 25,26,27-tri-nor-cholesta-2β,3α,24-triol-2,24-disulfate (2), 25,26,27-tri-nor-cholesta-2β,3α,24-triol-2,3,24-trisulfate (solomonsterol A, 3), 1-O-arachidonoyl-3-O-stearylglycerol (4), eicosapentaenoic acid (5), adenosine (6), adenine (7), and uracil (8). This is the first report of compounds 3-8 from *L. lambis*.

Keywords

Strombidae, *Lambis*, sterol sulfate, 25, 26, 27-tri-nor-cholesta-5-ene-2β, 3α, 24-triol-2, 24-disulfate, 25, 26, 27-tri-nor-cholesta-2β, 3α, 24-triol-3, 24-disulfate

Introduction

*Lambis* Linnaeus, 1758, commonly known as the spider conch, is a species of large sea snail, a marine gastropod mollusk in the family Strombidae.¹ The meat of *L. lambis* provides many nutrients, such as minerals (macro minerals, Na, K, Ca, P; micro minerals, Fe, ZN, Cu), protein, and vitamins.² Fucoidanase provides many nutrients (macro minerals, Na, K, Ca, P; micro minerals, Fe, ZN, Cu), protein, and vitamins.³ Fucoidanase was isolated from the digestive glands of *Lambis* sp. producing sulfated fucooligosaccharides.³ The mucus extract of *L. lambis* exhibited cytotoxic activity.⁴ Continuing our program of study on Vietnamese marine organisms,³⁸ we report in this paper the isolation and structural elucidation of two new sterol sulfates (1 and 2) and six known compounds (3-8) from the methanol extract of the sea spider conch *L. lambis*.

Results and Discussion

Compound 1 was obtained as a white amorphous solid with a molecular formula of *C*₂₄*H*₹₀*O*₂*S*₂*Na*₂ according to its HRESIMS ion at *m/z* 581.1844 [M+H]⁺ (calcd. for 
\[C₂₄H₹₀O₂S₂Na₂\]^⁺: 581.1825, \(\Delta = +3.3\) ppm) and at *m/z* 603.1637 [M+Na]⁺ (calcd. for \[C₂₄H₹₀O₂S₂Na₃\]^⁺: 603.1645, \(\Delta = −1.3\) ppm). The \(^1^H\) NMR spectrum of 1 showed the presence of two methyl singlets at δ_H 0.75 and 1.19 (each 3H), one methyl doublet at δ_H 0.99 (3H, d, J = 7.0 Hz), and one olefinic proton at δ_H 5.37 (1H, dd, J = 2.5, 2.5 Hz), along with two methine carbons at δ_C 4.04 and 4.46 (each 1H, br d, J = 2.5 Hz) and one oxygenated methylene group at δ_H 3.98 (2H, td, J = 7.0, 2.5 Hz). The \(^1^H\) NMR and HSQC spectra of 1 showed the presence of 24 carbons, including three quaternary carbons (δ_C 37.8, 43.5, 139.6), nine methylenes (δ_C 21.9, 25.3, 27.2, 29.2, 32.9, 33.2, 35.8, 39.0, 41.2), eight methines (δ_C 32.8, 36.9, 51.8, 57.5, 58.1, 69.6, 78.3, 123.5), and three methyls (δ_C 12.1, 19.1, 22.2) (Table 1). The above information suggested that 1 was a sulfated sterol compound having one

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## Table 1. NMR Spectroscopic Data for 1–3 (in CD₃OD).

| Pos. | δₖ (mult, J in Hz) | δₖ (mult, J in Hz) | δₖ (mult, J in Hz) |
|------|-------------------|-------------------|-------------------|
| 1    | 39.0 (1.60 (dd, 14.0, 2.5)) | 41.1 (1.37 (14.0, 2.5)) | 39.3 (1.44 (m), 2.09 (m)) |
| 2    | 78.3 (4.46 (br d, 2.5)) | 70.0 (4.09 (br d, 2.5)) | 76.6 (4.72 (br s)) |
| 3    | 69.6 (4.04 (br d, 2.5)) | 78.5 (4.42 (br d, 2.5)) | 76.2 (4.75 (br s)) |
| 4    | 35.8 (2.82 (dt, 14.3, 2.5)) | 30.3 (1.60 (m), 1.84 (m)) | 30.8 (1.61 (m), 1.28 (m)) |
| 5    | 139.6 (1.06 (m)) | 40.6 (1.06 (m)) | 40.2 (1.59 (m)) |
| 6    | 123.5 (5.37 (dd, 2.5, 2.5)) | 29.2 (1.25 (m), 1.30 (m)) | 29.5 (1.32 (m), 1.91 (m)) |
| 7    | 32.9 (1.62 (m), 1.97 (m)) | 33.2 (0.95 (m), 1.70 (m)) | 33.2 (1.12 (m), 1.72 (m)) |
| 8    | 32.8 (1.50 (m)) | 36.4 (1.41 (m)) | 36.3 (1.44 (m)) |
| 9    | 51.8 (1.06 (m)) | 56.6 (0.72 (m)) | 56.5 (0.73 (m)) |
| 10   | 37.8 (1.06 (m)) | 36.3 (1.06 (m)) | 36.3 (1.06 (m)) |
| 11   | 21.9 (1.21 (m), 1.54 (m)) | 22.0 (1.33 (m), 1.55 (m)) | 22.0 (1.47 (m), 1.45 (m)) |
| 12   | 41.2 (1.20 (m), 2.06 (m)) | 41.4 (1.16 (m), 2.02 (m)) | 41.4 (1.18 (m), 2.02 (m)) |
| 13   | 43.5 (1.06 (m)) | 43.8 (1.06 (m)) | 43.8 (1.06 (m)) |
| 14   | 58.1 (1.06 (m)) | 57.9 (1.07 (m)) | 57.8 (1.08 (m)) |
| 15   | 25.3 (1.10 (m), 1.62 (m)) | 25.2 (1.29 (m), 1.60 (m)) | 25.2 (1.09 (m), 1.51 (m)) |
| 16   | 29.2 (1.32 (m), 1.90 (m)) | 29.3 (1.33 (m), 1.88 (m)) | 29.2 (1.32 (m), 1.91 (m)) |
| 17   | 57.5 (1.13 (m)) | 57.6 (1.12 (m)) | 57.6 (1.14 (m)) |
| 18   | 12.1 (0.75 (s)) | 12.5 (0.71 (s)) | 12.5 (0.71 (s)) |
| 19   | 22.2 (1.19 (s)) | 14.6 (1.02 (s)) | 14.1 (0.99 (s)) |
| 20   | 36.9 (1.47 (m)) | 36.8 (1.46 (m)) | 36.8 (1.44 (m)) |
| 21   | 19.1 (0.99 (s)) | 19.1 (0.97 (s)) | 19.6 (0.97 (d, 6.5)) |
| 22   | 33.2 (1.13 (m), 1.56 (m)) | 33.1 (1.12 (m), 1.55 (m)) | 33.1 (0.99 (m), 1.54 (m)) |
| 23   | 27.2 (1.55 (m), 1.76 (m)) | 27.2 (1.57 (m), 1.77 (m)) | 27.2 (1.09 (m), 1.51 (m)) |
| 24   | 69.7 (3.98 (t, 7.0)) | 69.7 (3.97 (td, 7.0, 2.5)) | 69.7 (3.97 (m)) |

![Figure 1. Chemical structures of compounds 1-8.](image-url)
double bond, three oxygenated carbons, and which was structurally similar to solomonsterol A (3) (Figure 1).9,10 In the HSQC spectrum of 1, methyl protons at $\delta$H 0.75, 0.99, and 1.19 had cross peaks to carbons at $\delta$C 12.1, 19.1, and 22.2, respectively, while protons at $\delta$H 5.98, 4.40, 4.46, and 5.37 correlated with carbons at $\delta$C 69.7, 69.6, 78.3, and 123.5, respectively. In the $^1$H-$^1$H COSY spectrum of 1, correlations of H-1/H-2/H-3/H-4, H-7/H-8/H-9/H-11/H-12, H-8/H-14/H-15/H-16/H-20/H-21, and of H-20/H-22/H-23/H-24 (Figure 2) were observed. This evidence suggested that the A, B, C, and D rings of 1 were similar to that of (20R)-cholesta-5,24-diene-2β,3α,21-triol-2,21-disulfate,11 and the side chain was similar to that of solomonsterol.9,10 These suggestions were confirmed by the HMBC spectrum. HMBC correlations were observed from H3-19 ($\delta$H 1.19) to C-1 ($\delta$C 39.0)/C-5 ($\delta$C 139.6)/C-9 ($\delta$C 51.8)/C-10 ($\delta$C 43.5)/C-14 ($\delta$C 58.1)/C-17 ($\delta$C 57.5), from H3-18 ($\delta$H 0.75) to C-12 ($\delta$C 41.2)/C-13 ($\delta$C 43.5)/C-14 ($\delta$C 58.1)/C-17 ($\delta$C 57.5), from H3-21 ($\delta$H 0.99) to C-17/C-20 ($\delta$C 36.9)/C-22 ($\delta$C 33.2), and from H2-24 ($\delta$H 3.98) to C-22 ($\delta$C 33.2). In addition, the lower field signals of C-2 ($\delta$C 78.3) and C-24 ($\delta$C 69.7), together with the complete match of the NMR data of the A and B rings of 1 with those of (20R)-cholesta-5,24-diene-2β,3α,21-triol 2,21-disulfate,11 and of the side chain of 1 with that of solomonsterol,9,10 suggested two sulfate groups were at C-2 and C-24, and the hydroxy group was at C-3. These were further confirmed by the interpretation of $^1$H NMR, $^{13}$C NMR, $^1$H-$^1$H COSY, HSQC, HMBC, and NOESY spectra. The small proton coupling constants of H-3 and H$_2$-4 ($J$ = 2.0~2.5 Hz), H-2 and H$_2$-1 ($J$ = 2.0~2.5 Hz), and of H-2 and H-3 ($J$ = 2.5 Hz) confirmed that H-3 and H-2 were β/equatorial and α/equatorial, respectively. This was further indicated from the observation of the NOE correlation of H-2 ($\delta$H 4.46) and H-3 ($\delta$H 4.04). The other key NOE correlations of 1 are shown in Figure 3 indicating that its relative configuration was similar to that of (20R)-cholesta-5,24-diene-2β,3α,21-triol 2,21-disulfate.11 The CH$_3$-20 group was suggested as having an α-orientation due to the biosynthetic characteristics of all sterols.12 From the above evidence, compound 1 was determined to be a new compound, 25,26,27-tri-nor-cholest-5-ene-2β,3α,24-triol-2,24-disulfate.

The NMR spectra of 2 were similar to those of 1 and 3 suggesting that 2 was a sterol. The double bond signals of 1 were absent from the NMR spectra of 2. In addition, the molecular formula of 2 was determined as C$_{24}$H$_{40}$O$_9$S$_2$Na$_2$ from the exhibition of ions at $m/z$ 583.1977 [M+H]$^+$ (calcd C$_{24}$H$_{41}$O$_9$S$_2$Na$_2$ = 583.1982 $\Delta$ = −0.9 ppm) and $m/z$ 605.1808 [M+Na]$^+$ (calcd C$_{24}$H$_{40}$O$_9$S$_2$Na$_3$ = 605.1801 $\Delta$ = +1.2 ppm). The above data suggested that 2 was a disulfate sterol, similar to compounds 1 and 3.9,10 The NMR data of 2 were elucidated based on analyzing its $^1$H, $^{13}$C NMR, HSQC, $^1$H-$^1$H COSY, HMBC, and NOESY spectra in comparison with the corresponding data of 1 and solomonsterol A (3), measured in the same solvent (Table 1).9,10 In the HSQC spectrum, protons H-1 ($\delta$H 1.16/2.02), H-2 ($\delta$H 4.09), H-3 ($\delta$H 4.42), H-4 ($\delta$H 1.60/1.84) and H-5 ($\delta$H 1.60) correlated to carbons at $\delta$C 41.4, 70.0, 78.5, 30.3, and 40.6, respectively. In addition, COSY correlations of
H-1/H-2/H-3/H-5/H-5 were observed. This evidence indicated two oxygenated carbons were at C-2 and C-3. The complete agreement of the NMR data of the A, B, and C rings of 2 with those of (20R)-5α-choleno-24-ene-2β,3α,24-triol-3,24-disulfate (in the same solvent, CD3OD) suggested that one sulfate moiety was at C-3 and one hydroxy group was at C-2. The NMR data of the side chain of both H-2 and H-3. Thus, a compound NMR data of the side chain moiety was at C-3 and one hydroxy group was at C-2. The complete agreement of the NMR data of the A, B, and C rings of 2 are compared to those of 1 and solonomonsterol A (3) and found to match perfectly, suggesting that the other sulfate group was at C-24. The key HMBC correlations of 2 are shown in Figure 2. HMBC correlations from H-3 (δH 4.42) to C-5 (δC 40.6), from H-2 (δH 4.09) to C-10 (δC 36.3), from H3-21 (δH 0.97) to C-22 (δC 33.1), and from H-24 (δH 3.97) to C-22 (δC 33.1)/C-23 (δC 27.2) further confirmed the positions of the hydroxy group at C-2 and two sulfate groups at C-3 and C-24. Similar to 1, the small proton coupling constants between H-2 and H-2, and H-2 and H-3, as well as the observed NOE correlation between H-2 and H-3 clearly indicated equatorial positions of both H-2 and H-3. Thus, compound 2 was elucidated as a new compound, 25,26,27-tri-nor-cholesta-2β,3α,24-triol-3,24-disulfate...

**Materials and Methods**

**General Experimental Procedures**

Optical rotation was measured on a Jasco P-2000 polarimeter. HRESIMS were measured on an Agilent 6530 Accurate Mass Q-TOF system, and NMR spectra on a Bruker Avance III 500 MHz spectrometer. For preparative HPLC, an Agilent 1260 Infinity II system was used equipped with a YMC J’sphere ODS-H80 (20×250 mm, 4 μm) HPLC column. Flash column chromatography was performed using either silica gel or reversed phase (RP-18) resins as adsorbent. Thin layer chromatography was carried out on pre-coated silica gel 60 F254 and/or RP-18 F254S plates.

**Extraction and Isolation**

*L. lambis* (2.5 kg) was defrosted, then extracted with MeOH in an ultrasonic bath (3 times × 10.0 L MeOH, 40 °C, 3 h). The MeOH extract (121.1 g) was suspended in distilled water (2.5 L) and extracted with CH2Cl2 (2.5 L) to obtained the CH2Cl2 extract (LL1, 10.3 g). LL1 (10.0 g) was chromatographed on a silica gel column, eluting with a gradient solvent system of CH2Cl2/MeOH (100:0, 40:1, 20:1, 10:1) to obtain four fractions, LL1A-LL1D. LL1A (1.8 g) was chromatographed on a RP-18 column, eluting with acetone/water (4/1, v/v) to give compound 6 (23.1 mg). LL1C (4.3 g) was chromatographed on a RP-18 column, eluting with acetone/water (3.5/1, v/v) to obtain 5 fractions, LL1C1-LL1C5. LL1C2 (736 mg) was purified by HPLC (‘sphere ODS H-80, 250 mm×20 mm column), eluting with 35% acetonitrile (ACN) in H2O to yield 3 (6.1 mg, tR 37.8). LL1C3 (679 mg) was chromatographed on an HPLC (‘sphere ODS H-80, 250 mm×20 mm column), eluting with 45% acetonitrile (ACN) in H2O to yield 1 (5.2 mg, tR 42.5) and 2 (4.7 mg, tR 44.6). LL1D (2.7 g) was chromatographed on a RP-18 column, eluting with acetone/water (2/1, v/v) to give compound 7 (6.7 mg), 8 (4.6 mg), and 9 (7.2 mg).

**Conclusions**

From the methanol extract of the Vietnamese marine spider conch *Lambis Linnaeus*, 1758, two new (1 and 2) and six...
known compounds (3–8) were isolated. Their chemical structures were elucidated as 25,26,27-tri-nor-cholesta-5-ene-3β,3α,24-triol-2,24-disulfate (1), 25,26,27-tri-nor-cholesta-2β,3α,24-triol-3,24-disulfate (2), and 25,26,27-tri-nor-cholesta-2β,3α,24-triol-2,3,24-trisulfate (3), 1-O-arachidonoyl-3-O-stearylglycerol (4), eicosapentaenoic acid (5), adenosine (6), adenine (7), and uracil (8) based on extensive spectroscopic methods, including HRESIMS, 1D- and 2D-NMR spectra. Compounds 3–8 are reported herein from L. lambis for the first time.

**List of Abbreviations**

NMR, Nuclear Magnetic Resonance

COSY, Correlation Spectroscopy

HRESIMS, High-resolution electrospray ionization mass spectrometry

HMBC, Heteronuclear Multiple Bond Correlation

HSQC, Heteronuclear Single Quantum Coherence

**Author Contribution**

Research idea NT Dan, NX Nhiem, PV Kiem; Isolation, DTT Hang, BH Tai, CN Dinh, ND Hoi, VT Loan; Collection of samples: NT Dan, CN Dinh; Structure elucidation and writing, BH Tai, NT Dan, NX Nhiem, PV Kiem.

**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 Project KCB-TS-07, (grant number Project KCB-TS-07).

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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.

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