New Taurine Derivatives from a Starfish and a Sponge

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Abstract – A new (2) and a known (1) acylated taurine derivatives were isolated from the MeOH extract of the starfish Certonaardoa semiregularis. Another new acylated taurine derivative (3) was isolated from the MeOH extract of the sponge Erylus nobilis. The structures were determined on the basis of spectral analysis.

Keywords – starfish, Certonaardoa semiregularis, sponge, Erylus nobilis, acylated taurine.

Introduction

Starfish have been a rich source of marine sterols and saponins with new structures. In our study on the cytotoxic constituents of the starfish Certonaardoa semiregularis (Family: Linckidiæ) collected from Korean waters, 16 saponins and 15 polyhydroxysterols have been isolated (Wang et al., 2002; Wang et al., 2003a; Wang et al., 2003b). In our continuing study on the metabolites from the same starfish, we have isolated one new (2) and one known (1) acylated taurine derivatives. Another new acylated taurine derivative (3) was isolated from the MeOH extract of the sponge Erylus nobilis. Taurine derivatives exist in higher animals usually as a moiety of bile salts or as taurine conjugates. However, only a few taurine derivatives are reported from starfishes and they are all present as taurine amides of steroids (Finanore et al., 1991; De Riccardis et al., 1993). The closest structural relative in marine organisms appears to be taurosporin A, a fatty acid taurine amides from the sponge Hippospongia species (Ishiyama et al., 1996). Tauroacidins A and B are the other example of natural products with taurine amide residue from sponge (Kobayashi et al., 1997). In addition, taurine amide residue was also found in compounds isolated from shellfish such as brevetoxin B1 (Ishida et al., 1995), and in microbial metabolites such as saccharomycins A and B (Kong et al., 1998). Among the above mentioned compounds, taurosporin A showed inhibitory activity against c-erbB-2 kinase and exhibited no cytotoxicity. Taurine derivatives exist in higher animals usually as a moiety of bile salts or as taurine conjugates. Compounds 1 and 3 are first described from natural source though compound 1 and its analogues have been synthesized and were employed in pharmaceutics and cosmetics to improve the solubility of drugs or as a pharmacologically acceptable carrier, emulsifier, surfactant, or occasionally as the effective component. (Zappia et al., 1988; Zappia et al., 1989; Tsubone et al., 1996; Suzuki et al., 2001).

Experimental

Animal material – The starfish Certonaardoa semiregularis were collected in July 2000, at depths of 5-10 m off the coast of Gomun Island, Korea, and has been described elsewhere (Wang et al., 2002). The sponge Erylus nobilis was collected in August 1998, at the depth of 18 m off the coast of Jeju Island, Korea. The starfish specimen was identified by Prof. Sook Shin, Sahmyook University, Seoul, Korea. The sponge specimen was identified by Prof. Chung Ja Sim, Hannam University, Daejeon, Korea. The voucher specimens (starfish: J00K-4; sponge: J98J-4) were deposited in the Marine Natural Product Laboratory, Pusan National University, Busan, Korea.

General procedures – IR spectra were recorded on a JASCO FT/IR-410 Spectrometer. FABMS data were obtained on a JEOL JMS-SX-102A double-focusing spectrometer. ¹H and ¹³C NMR spectra were measured on a Bruker AC200 or a Varian Inova 500. Chemical shifts were reported with reference to the respective solvent peaks (δH 3.30 and δC 49.0 for CD3OD). HPLC was performed with a C18–5E Shodex packed column (250×10 mm, 5 µm, 100 Å), and

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a YMC-Pack ODS column (250×10 mm, 5 μm, 80 Å) using a Shodex RI-71 detector.

**Extraction and isolation** – The frozen starfish (9 kg) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay, the MeOH extract was partitioned between H₂O and CH₂Cl₂. The CH₂Cl₂ layer was further partitioned between aqueous MeOH and n-hexane to afford aqueous MeOH (14 g) and n-hexane soluble (39 g) fractions. The aqueous MeOH fraction was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with MeOH-H₂O (gradient, 1:2→1:0) to obtain fractions (1-13). Fraction 4 (1.19 g) was very active in the brine shrimp assay (LD₅₀, 15 ppm) and was subjected to chromatotron (Silica gel 60 PF₂₅₄) eluting with CHCl₃-MeOH (gradient, 5:1→0:1) to afford sixty fractions (1-60). The sub-fractions 12-34 were repeatedly chromatographed on HPLC [column: C_{18}-5E Shodex packed; mobile phase: MeOH-H₂O (80:20); flow rate: 1 mL/min] to give compounds 1 (1.7 mg, t₈ 39 min) and 2 (2.3 mg, t₈ 33 min).

The frozen sponge (8 kg) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay, the MeOH extract was partitioned between H₂O and BuOH. The BuOH layer was then dissolved in Et₂O to yield precipitate (27 g). The precipitate was active in the brine shrimp assay (LD₅₀, 161 μg/mL) and was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 500/400 mesh, 60 Å) eluting with MeOH-H₂O (gradient, 1:5→1:0) to afford 25 fractions. Fraction 14 was subjected to YMC-Pack ODS column eluted with 83% MeOH, followed by further purification on the same column eluting with 50% MeCN (flow rate: 1 mL/min), to give compound 3 (0.5 mg, t₈ 22 min).

**Acid hydrolysis of compounds 1 and 2** – Each solution of 1 (0.5 mg) and 2 (0.5 mg) in aqueous HCl (10%, 0.5 mL) was heated at 110°C in a stoppered reaction vial. After 2 h, TLC analysis [Silica gel with CHCl₃-MeOH (4:1)] showed that the original spot had disappeared. The hydrolysate was cooled and examined by co-TLC [n-BuOH-HOAc-H₂O (60:15:25)] with the authentic taurine. The spray reagent for the taurine was 1% ninhydrin in acetone.

**Compound 1** white amorphous powder; IR ν_{max} (film) cm⁻¹: 3300, 2926, 1643, 1555, 1455, 1207, 1060, 712; FABMS (+ve) m/z: 456 [M+Na]^+; 434 [M+H]^+; FABMS (ve) m/z: 410 [M-Na]^-; HRFABMS (+ve) m/z: 456.2155 (calc for C₂₉H₂₃NNa₂O₅S, 456.2160); H-NMR (500 MHz, CD₂OD) δ: 2.96 (2H, t, J = 7.0 Hz, H-1), 3.59 (2H, t, J = 7.0 Hz, H-2), 2.21 (2H, t, J = 7.5 Hz, H-2'), 1.67 (2H, quint, J = 7.5 Hz, H-3'), 2.10 (2H, m, H-4'), 5.31-5.39 (8H, m, H-5', 6', 8', 9', 11', 12', 14', and 15'), 2.80-2.85 (6H, m, H-7', 10', and 13'), 2.05 (2H, m, H-16'), 1.27-1.38 (6H, m, H-17'19'), 0.90 (3H, t, J = 6.8 Hz, H-20'). ¹³C-NMR (50 MHz, CD₂OD) δ: 51.5 (C-1), 36.5 (C-2), 176.1 (C-1'), 36.7 (C-2'), 26.8 (C-3'), 27.7 (C-4'), 128.8, 128.9, 129.2, 129.5, 129.8, 130.1, 131.2 (C-5, 6', 8', 9', 11', 12', 14', and 15'), 26.5, 26.6, and 26.6 (C-7', 10', and 13'), 28.2 (C-16'), 30.5 (C-17'), 32.7 (C-18'), 23.6 (C-19'), 14.4 (C-20').

**Compound 2** – white amorphous powder; IR ν_{max} (film) cm⁻¹: 3239, 2931, 1643, 1559, 1455, 1207, 1056, 742; FABMS (+ve) m/z: 454 [M+Na]^+; FABMS (ve) m/z: 408 [M-Na]^-. H-NMR (500 MHz, CD₂OD) δ: 2.95 (2H, t, J = 7.0 Hz, H-1), 3.58 (2H, t, J = 7.0 Hz, H-2), 2.19 (2H, t, J = 7.5 Hz, H-3'), 1.66 (2H, quint, J = 7.5 Hz, H-3'), 2.10 (2H, m, H-4'), 5.28-5.40 (10H, m, H-5', 6', 8', 9', 11', 12', 14', 15', 17', and 18'), 2.80-2.86 (8H, m, H-7', 10', 13', and 16'), 2.06 (2H, m, H-19'), 0.96 (3H, t, J = 7.5 Hz, H-20'). ¹³C-NMR (50 MHz, CD₂OD) δ: 51.5 (C-1), 36.7 (C-2), 175.7 (C-1'), 36.6 (C-2'), 26.8 (C-3'), 27.7 (C-4'), 128.2, 128.9, 129.1, 129.1, 129.2, 129.3, 129.5, 129.7, 130.2, 132.8 (C-5', 6', 8', 9', 11', 12', 14', 15', 17', and 18'), 26.4, 26.5, 26.6, and 26.6 (C-7', 10', 13', and 16'), 21.5 (C-19'), 14.6 (C-20').

**Compound 3** – white amorphous powder; FABMS (+ve) m/z: 420 [M+Na]^+ (45), 376 (2.0), 334 (4.5), 280 (5.5), 266 (5.0), 211 (15.0); H-NMR (500 MHz, CD₂OD) δ: 2.95 (2H, t, J = 7.0 Hz, H-1), 3.58 (2H, t, J = 7.0 Hz, H-2), 2.17 (2H, t, J = 7.5 Hz, H-2'), 1.60 (2H, quint, J = 7.5 Hz, H-3'), 1.25-1.37 (14H, m, H-4'-7', H-12'-14'), 2.03 (4H, m, H-8', 11'), 5.34 (2H, m, H-9', 10'), 1.51 (1H, m, J = 6.5 Hz, H-15'), 0.87 (6H, d, J = 6.5 Hz, H-16', 17'); ¹³C-NMR (50 MHz, CD₂OD) δ: 51.5 (C-1), 36.7 (C-2), 175.8 (C-1'), 36.8 (C-2'), 26.8 (C-3'), 30.0 30.9 (C-4'-7' and C-12', 13'), 27.8, 27.9 (C-8', 11'), 130.6, 130.6 (C-9', 10'), 40.3 (C-14'), 29.1 (C-15'), 23.0 (C-16', 17').

![Fig. 1. Structures of compounds 1-3.](image-url)
Results and Discussion

Compound 1 was isolated as white amorphous powder. The molecular formula of compound 1 was deduced to be C$_{29}$H$_{38}$NNaO$_{12}$S on the basis of the pseudomolecular ion peak at m/z 456.2155 [M + Na]$^+$ (caled for C$_{29}$H$_{38}$NNaO$_{12}$S, 456.2160, Δ-0.5 mmu) in the HRFABMS spectrum. The negative FABMS gave a pseudomolecular ion peak at m/z 410 [M-Na]$^-$. The gross structure was determined by the aid of COSY, HMOC, and HMBC experiments. In the $^1$H NMR spectrum, the two methylene triplets at δ 2.96 and 3.59, coupled to each other by 7.0 Hz, were observed. The intense absorptions in the FT IR spectrum at 1643 and 1555 cm$^{-1}$, and absorptions at 1207 and 1060 cm$^{-1}$ were suggestive for the presence of an amide function and a sulfonic acid salt, respectively (Finamore et al., 1991; Pavia et al., 2001). All these data indicated the presence of the taurine residue. Acid hydrolysis (10% HCl in H$_2$O) followed by TLC comparison of the hydrolysate with the authentic taurine gave further support to the proposed structure. The acyl moiety was defined as a polyunsaturated fatty acid on the basis of NMR data. The double bonds were flanked by a methylene group as indicated by diallylic methylene protons (δ 2.80-2.85) which integrated to six protons. The location of the double bonds were determined by the COSY correlations among the well resolved H-2', 3', 4', and 5' signals. The geometry of the double bond was deduced to be cis based on the chemical shifts of the allylic carbons (δ 27.7 and 28.2) and the diallylic carbons (δ 26.5 and 26.6), which were typical for a methylene shielded by one or two cis double bonds (Amico et al., 1982; Doi et al., 1994).

Compound 2 was isolated as white amorphous powder. The FABMS gave a pseudomolecular ion peak at m/z 454 [M + Na]$^+$. Comparison of the spectral data with those of compound 1 showed that it is 17,18-didehydro derivative of compound 1, that is a sodium salt of (all-Z)-2-(icos-5,8,11,14,17-pentaenoylamino)ethane sulfonic acid. Compound 2 was previously undescribed, though the free acid (all-Z)-icos-5,8,11,14,17-pentaenoic acid (EPA), corresponding to the fatty acid moiety of compound 2, is widespread in cold deep water fishes. EPA is known to possess various physiological and biological functions for mammals and may play important ecological roles in the marine environment (Suzuki et al., 1996).

Compound 3 was isolated in trace amount as white amorphous powder. The structure of compound 3 was derived from $^1$H NMR spectroscopy, FABMS data, COSY and HSQC experiments, and comparison with compounds 1 and 2. The molecular formula of compound 3 was deduced to be C$_{29}$H$_{38}$NNaO$_{12}$S on the basis of the pseudomolecular ion peak at m/z 420 [M + Na]$^+$ in the FABMS spectrum. In the $^1$H NMR spectrum, the characteristic signals for acylated taurine derivative (2.95, 2H, t, J = 7.0 Hz, CH$_2$SO$_3$; 3.58, 2H, t, J = 7.0 Hz, NH-CH$_2$) were observed as in those of 1 and 2. In the COSY spectrum, a 6H doublet at δ0.87 showed correlation with a methine signal at δ1.15, which indicated the presence of the terminal isopropyl group. The position of the double bond was ascertained at C-9' on the basis of the prominent allylic cleavages at m/z 334 and 280 in the FAB-CID tandem mass spectrum (Fig. 2). The geometry of the double bond was deduced to be cis based on the allylic carbon signals at about 828 (Doi et al., 1994). The free acid, 15-methyl-9Z-hexadecenoic acid, corresponding to the fatty acid moiety of compound 3, has been found in the sponge Dysidea fragilis (Christie et al., 1992), and often in the phospholipids from sponges (Carballeira et al., 1993; Carballeira et al., 1998; Carballeira et al., 2001).

The isolated compounds have been tested for cytotoxicity against a small panel of human solid tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF498, and HCT15) to exhibit insignificant activity (LD$_{50}$ > 30 μg/mL).

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References


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