Natural Product Sciences
9(4) : 241-244 (2003)

New Taurine Derivatives from a Starfish and a Sponge

Weihong Wang[1], Yoon Mi Lee[1], Jongki Hong[2], Chong-Ok Lee[3], Jong Hee Park[1], and Jee H. Jung[1,*]

[1]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*
[2]*Basic Science Institute, Seoul 136-701, Korea*
[3]*Korea Research Institute of Chemical Technology, Daejon, Korea*

Abstract – A new (**2**) and a known (**1**) acylated taurine derivatives were isolated from the MeOH extract of the starfish *Certonardoa 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, *Certonardoa 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 *Certonardoa semiregularis* (Family: Linckiidae) 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 starfish and they are all present as taurine amides of steroids (Finamore *et al.*, 1991; De Riccardis *et al.*, 1993). The closest structural relative in marine organisms appears to be taurospongin 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 saccharomicins A and B (Kong *et al.*, 1998). Among the above mentioned compounds, taurospongin 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).

*Author for correspondence
Fax: +82-51-513-6754, E-mail: jhjung@pusan.ac.kr

Experimental

Animal material – The starfish *Certonardoa 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, Daejon, 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 ^{13}C NMR spectra were measured on a Bruker AC200 or a Varian Inova 500. Chemical shifts were reported with reference to the respective solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). HPLC was performed with a C18-5E Shodex packed column (250×10 mm, 5 μm, 100 Å), and

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 H_2O and CH_2Cl_2. The CH_2Cl_2 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-H_2O (gradient, 1:2→1:0) to obtain 13 fractions (1-13). Fraction 4 (1.19 g) was very active in the brine shrimp assay (LD_{50}, 15 ppm) and was subjected to chromatotron (Silica gel 60 PF_{254}) eluting with $CHCl_3$-MeOH (gradient, 5:1→0:1) to afford sixty fractions (1-60). The sub-fractions 12-34 were repeatedly chromatographed on HPLC [column: C18-5E Shodex packed; mobile phase: MeOH-H_2O (80:20); flow rate: 1 mL/min] to give compounds **1** (1.7 mg, t_R 39 min) and **2** (2.3 mg, t_R 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 H_2O and BuOH. The BuOH layer was then dissolved in Et_2O to yield precipitate (27 g). The precipitate was active in the brine shrimp assay (LD_{50}, 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-H_2O (gradient, 1:5→1:0) to afford 25 fractions. Fraction 14 was submitted 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, t_R 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 $CHCl_3$-MeOH (4:1)] showed that the original spot had disappeared. The hydrolysate was cooled and examined by co-TLC [*n*-BuOH-HOAc-H_2O (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 (calcd for $C_{22}H_{36}NNa_2O_4S$, 456.2160); ^{1}H-NMR (500 MHz, CD_3OD) 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′). ^{13}C-NMR (50 MHz, CD_3OD) 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, 129.2, 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}: 3329, 2931, 1643, 1559, 1455, 1207, 1056, 742; FABMS (+ve) *m/z*: 454 $[M + Na]^+$, FABMS (-ve) *m/z*: 408 $[M-Na]^-$; ^{1}H-NMR (500 MHz, CD_3OD) 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-2′), 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′). ^{13}C-NMR (50 MHz, CD_3OD) 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); ^{1}H-NMR (500 MHz, CD_3OD) 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′). ^{13}C-NMR (50 MHz, CD_3OD) 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′).

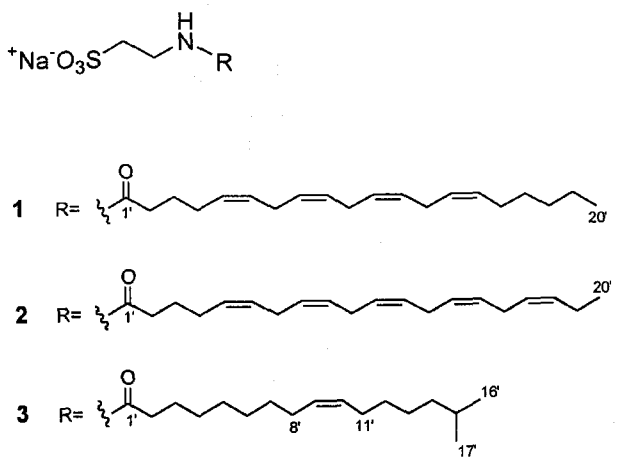

Fig. 1. Structures of compounds **1-3**.

Results and Discussion

Compound **1** was isolated as white amorphous powder. The molecular formula of compound **1** was deduced to be $C_{22}H_{36}NNaO_4S$ on the basis of the pseudomolecular ion peak at *m/z* 456.2155 $[M + Na]^+$ (calcd for $C_{22}H_{36}NNa_2O_4S$, 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, HMQC, and HMBC experiments. In the ^1H 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_2O) 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 (δ2.80-2.85) 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 (δ27.7 and 28.2) and the diallylic carbons (δ26.5 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-(icosa-5,8,11, 14,17-pentaenoylamino)ethane sulfonic acid. Compound **2** was previously undescribed, though the free acid (all-*Z*)-icosa-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 ^1H 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 $C_{19}H_{36}NNaO_4S$ on the basis of the pseudomolecular ion peak at *m/z* 420 $[M + Na]^+$ in the FABMS spectrum. In the ^1H NMR spectrum, the characteristic signals for acylated taurine derivative (2.95, 2H, t, J = 7.0 Hz, $C\underline{H}_2$-SO_3; 3.58, 2H, t, J = 7.0 Hz, NH-$C\underline{H}_2$) 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 δ1.51, 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 δ28 (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).

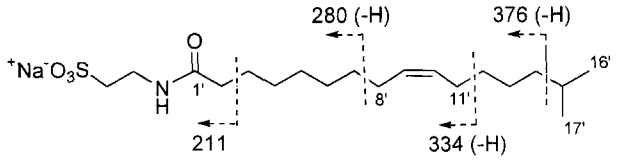

Fig. 2. Key fragmentations of the $[M+Na]^+$ ion of **3** in FAB-CID MS/MS.

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).

Acknowledgments

The authors wish to thank Prof. Sook Shin and Prof. Chung Ja Sim of Sahmyook University and Hannam University, respectively, for the identification of the animal specimens. A research grant by the Korea Research Foundation (2001-041-F00279) is gratefully acknowledged.

References

Amico, V., Nicolosi, G., Oriente, G., Piattelli, M., and Tringali, C., A novel acylphloroglucinol from the brown alga *Zonaria tournefortii*. *Phytochemistry*, **21**, 739-741 (1982).

Carballeira, N. M., Reyes, E. D., and Shalabi, F. Identification of novel iso/anteiso nonacosadienoic acids from the phospholipids of the sponges *Chondrosia remiformis* and *Myrmekioderma styx*. *J. Nat. Prod.*, **56**(10), 1850-1855 (1993).

Carballeira, N. M., Pagan, M., and Rodriguez, A. D. Identification and total synthesis of novel fatty acids from the Caribbean sponge *Calyx podatypa*. *J. Nat. Prod.*, **61**(8), 1049-1052 (1998).

Carballeira, N, M., and Pagan, M., New methoxylated fatty acids from the Caribbean sponge *Callyspongia fallax*. *J. Nat. Prod.*, **64**(5), 620-623 (2001).

Christie, W. W., Brechany, Elizabeth Y., Stefanov, K., and Popov, S. The fatty acids of the sponge *Dysidea fragilis* from the Black Sea. *Lipids*, **27**(8), 640-644 (1992).

De Riccardis, F., Minale, L., Riccio, R., Iorizzi, M., Debitus, C., Duhet, D., and Monniot, C., A novel group of polyhydroxycholanic acid derivatives from the deep water starfish *Styracaster caroli*, *Tetrahedron Lett.*, **34**(27), 4381-4384 (1993).

Doi, Y., Ishibashi, M., and Kobayashi, J., Isolation and structure of shimofuridins B-G from the Okinawan marine tunicate *Aplidium multiplicatum*. *Tetrahedron*, **50**, 8651-8656 (1994).

Finamore, E., Minale, L., Riccio, R., Rinaldo, G., and Zollo, F., Novel marine polyhydroxylated steroids from the starfish *Myxoderma platyacanthum*, *J. Org. Chem.*, **56**(3), 1146-1153 (1991).

Ishida, H., Nozawa, A., Totoribe, K., Murmatsu, N., Nukaya, H., Tsuji, K., Yamaguchi, K., Yasumoto, T., Kaspar, H., Berkett, N., and Kosuge, T., Brevetoxin B1, a new polyether marine toxin from the New Zealand shellfish *Austrovenus stutchburyi*, *Tetrahedron Lett.*, **36**(5), 725-728 (1995).

Ishiyama, H., Ishibashi, M., and Kobayashi, J., Taurospongin A, a novel acetylene derivative from Okinawan marine sponge *Hippospongia sp.*, *Tennen Yuki Kagobutsu Toronkai Keon Yoshishu*, **38**, 469-474 (1996).

Kobayashi, J., Inaba, K., and Tsuda, M., Tauroacidins A and B, new bromopyrrole alkaloids possessing a taurine residue from *Hymeniacidon* sponge, *Tetrahedron*, **53**(49), 16679-16682 (1997).

Kong, F., Zhao, N., Siegel, M. M., Janota, K., Ashcroft, J. S., Keohn, F. E., Borders, D. B., and Carter, G. T., Saccharomichins, novel heptadecaglycoside antibiotics effective against multidrug-resistant bacteria, *J. Am. Chem. Soc.*, **120**(51), 13301-13311 (1998).

Oshima, Y., Yamada, S., Matsunaga, K., Moriya, T., and Ohizumi, Y., A monogalactosyl diacylglycerol from a cultured marine dinoflagellate, *Scrippsiella trochoidea*, *J. Nat. Prod.*, **57**(4), 534-536 (1994).

Pavia, D. L., Lampman, G. M., and Kriz, G. S., Introduction to spectroscopy (3rd edition), Harcourt College Publishers, U. S. A., 2001, p. 68 and 80.

Suzuki, M., Wakana, I., Denboh, T., and Tatewaki, M., An allelopathic polyunsaturated fatty acid from red algae, *Phytochemistry*, **43**(1), 63-65 (1996).

Suzuki, N., Terasaki, H., Kaneko, Y., and Fujii, A., Pearly substances for cosmetics, *Jpn. Kokai Tokkyo Koho*, 2001, p. 5.

Tsubone, K., Cosmetics containing *N*-acyltaurine salts as emulsifiers, *Jpn. Kokai Tokkyo Koho*, 1996, p. 5.

Wang, W., Li, F., Alam, N., Liu, Y., Hong, J., Lee, C. O., Im, K. S., and Jung, J. H., New saponins from the starfish *Certonardoa semiregularis*, *J. Nat. Prod.*, **65**(11), 1649-1656 (2002).

Wang, W., Li, F., Hong, J., Lee, C. O., Kong, J. Y., Shin, S., Im, K. S., and Jung, J. H., Bioactive sterols from the starfish *Certonardoa semiregularis*. *J. Nat. Prod.*, **66**(3), 384-391 (2003a).

Wang, W., Li, F., Hong, J., Lee, C. O., Cho H. Y., Shin, S., Im, K. S., and Jung, J. H., Four new saponins from the starfish *Certonardoa semiregularis*. *Chem. Pharm. Bull.*, **51**(4), 435-439 (2003b).

Zappia, V. and De Rosa, M. Pharmaceutical compositions containing ubidecarenones and *N*-acylaminoethanesulfonic acid, *PCT Int. Appl.*, 1988, p. 11.

Zappia, V. and De Rosa, M. Lipophilic salts of *S*-adenosyl-L-methionine with acylated taurine derivatives, useful in pharmaceuticals and cosmetics, *PCT Int. Appl.*, 1989, p. 19.

(Accepted October 14, 2003)