Natural Product Sciences
13(2) : 132-134 (2007)

Antibacterial Sulfated Alkene from a Tunicate, *Styela clava*

So-Mi Yun, Jun-Ho Jang[1], Ji-Eun Ryu, Byeong-Dae Choi, and Jong-Soo, Lee*

Division of Marine Life Science and Technology, Institute of Marine Industry, Gyeongsang National University, Tongyeong, Gyeongnam 650-160, Korea
[1]Graduate School of Agricultural Science, Tohoku Univ., Sendai 981-8555, Japan

Abstract – An analog of antibacterial sulfated alkene against *Bacillus subtilis* was isolated from a species of tunicate (Mideoduck), *Styela clava*, cultured at Jindong Bay, Korea. The structure was determined as 4,8-dimethyl-3-nonenyl sulfate by MS and spectral analysis such as UV, IR and NMR.
Keywords – *Styela clava*, tunicate, 4,8-dimethyl-3-nonenyl sulfate, antibacterial activity

Introduction

Tunicates, classified as Urochordata animal, lives in coastal waters, have known as precious marine sources as well as sponges, to produce various biologically active compounds (Faulkner, 1996; Blunt *et al.*, 2005), including eudistomines (Rinehart *et al.*, 1987) and didemnins (Sakai *et al.*, 1995). Among them, sulfated C_9-alkane (2,6-dimethylheptyl sulfate, **2**), and sulfated C_{10}-alkenes (4,7-decadienyl sulfate and 3,6,9-decatrienyl sufate) were isolated as the antimicrobial active compounds from the hepatopancrea of tunicate (ascidian), *H. roretzi* and *H. aurantium* collected in Japan (Tsukamoto *et al.*, 1994), and also, 2,6-dimethylheptyl sulfate was found in ascidian, *Policitor adriaticus* collected at Croatia (Crispino *et al.*, 1995), respectively.

Sulfate esters have significance role in the elimination of secondary metabolites of animals and sometimes, high level of sulfate sterols are associated with diseases such as X-linked ichthyosis (Crispino *et al.*, 1995). Marine echinoderms including sea cucumbers and starfishes are rich sources of sulfated saponins, terpenes, sterols (Riccio *et al.*, 1987).

Three kinds of tunicates such as *Halocynthia roretzi*, *Styela clava* and *S. plicata* have been culturing at the southern coast of Korea and consuming as the foodstuffs due to those specific flavors. But, it is necessary to develop more effective and highly valuable products by using those over produced cultured tunicates.

In the course of our studies for the bioactive materials in the cultured tunicates, *Styela clava* showed antibacterial activity against *Bacillus subtilis*. We isolated a polar antibacterial component against *B. subtilis* from some active fractions guided by the formation of growth inhibition zone around the paper disc on the plate count agar and determined chemical structure as C_{11}-sulfated alkene(**1**). This paper, describes the isolation and structure elucidation of this component.

Experimental

Animal material – The cultured tunicates (Mideoduck in Korean name), *S. clava* were collected at Jindong Bay, Masan, Kyungnam Province, Korea in February, 2004.

Instruments and reagents – Measurement of NMR spectra were conducted in CD_3OD on a Varian Unity plus-500 (500 MHz, Varian, USA), and chemical shifts were referenced with residual solvent signal. UV spectrum was measured in MeOH on the UV-160A spectrophotometer (Shimadzu, Japan) and FT-IR was taken in KBr tablet on the IFS-66 IR spectrophotometer (Bruker, Germany). FAB-MS data(positive mode) were obtained by ESI method on the Finiganmat TSQ-700 MS spectrometer (Thermo Electron, USA).

Rhodizonate test for the coloring reaction were taken by heating about 120 °C after sprayed with 1% sodium rhodizonate and 12% Ammonia water (Feigl and Suter, 1942).

Extraction and isolation – After removed the tunic, only the edible part of *S. clava* (2.5 kg) were minced and extracted with acetone and filtrated. The residue was extracted further 2 times and those extracts was

*Author for correspondence
Fax: +82-55-640-3111; E-mail: leejs@gaechuk. gsnu.ac.kr

concentrated *in vacuo* under 40 °C. The concentrated extract (161.0 g) was dissolved in 80% MeOH (350 mL) and then partitioned with *n*-hexane (2 × 350 mL) to give hexane layer (oily residue 29.1 g). Aqueous MeOH was diluted to 40% MeOH and partitioned with $CHCl_3$ (3 × 500 mL) to afford $CHCl_3$ soluble fraction (2.7 g). After then, aqueous MeOH concentrated and partitioned between BuOH (4.0 g) and water layer (73.9 g), respectively.

$CHCl_3$ soluble fraction was subjected to basic alumina (70 - 230 μm, Merck) column and eluted with 1% NH_4OH-MeOH (1 : 1) after washed with $CHCl_3$-MeOH (1 : 1). 1% NH_4OH-MeOH (1 : 1) fraction was concentrated and charged on the silica gel (200 - 400 μm, Merck) column and eluted with $CHCl_3$, $CHCl_3$-MeOH (95 : 5), $CHCl_3$-MeOH (9 : 1), $CHCl_3$-MeOH (7 : 3), $CHCl_3$-MeOH (1 : 1) and MeOH, successively. $CHCl_3$-MeOH (9 : 1) and $CHCl_3$-MeOH (7 : 3) fraction was combined and concentrated (1.8 g). The concentrate was loaded on the ODS column (ODS-Q3, Fuji Gel, Japan) and stepwised with 50, 70, 85 and 100% MeOH, continuously. 50% MeOH (1.8 g) was concentrated and eluted on the same column with 30, 50, 100% MeOH. 50% MeOH fraction (0.52 g) was chromatographed on the HPLC attached ODS column (Develosil ODS-7, 1 × 25 cm, Nomura Chemical Co., Ltd., Japan) with 65% MeOH, monitoring at 215 nm and re-chromatographed to give active component **1** (218.9 mg, 4.9×10^{-3}% wet base).

4,8-dimethyl-3-nonenyl sulfate(1) – Amorphous white powder, UV λmax nm (Logε, MeOH): 215 (2.24); IR (KBr) ν cm^{-1} : 3400, 2950, 2930, 1227, 1100, 1020, 820; positive FAB-MS *m/z* $[M+Na]^+$ 295.10, $[M+K]^+$ 311.10, HR-FAB-MS *m/z* obsd. 295.0960(clcd. for $C_{11}H_{21}O_4SNa_2$, Δ-0.13 mmu); ^{1}H-NMR (500MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1.

Results and Discussions

Acetone extracts of cultured tunicate, *Styela clava*, showed growth inhibitory activity to the *B. subtilis*. It was re-extracted with $CHCl_3$ after removed non polar materials with hexane and was taken column chromatography on the basic alumina, silica gel and ODS column, successively. From the further purification by HPLC attached packed ODS column, most polar antibacterial component **1** was obtained (yield 4.9×10^{-3}% wet weight) and showed 11 mm clear zone of inhibition against *B. subtilis* at 0.2 mg/disk (0.8 cm diameter).

It was amorphous colorless powder and solved well in aqueous MeOH. UV spectrum exhibited maximum absorption band at 210 nm (in MeOH). Presence of sulfate group was suggested at the IR spectrum showing strong absorption wave number at 1227 and 810 cm^{-1} (weak) and gave positive pale violet color on the sodium rhodizonate coloring test.

Table 1. Assignment of ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR data (CD_3OD, 125 MHz) of 4,8-dimethyl-3-nonenyl sulfate **(1)**

Position	Chemical shift (δ : ppm)	
	^{1}H	^{13}C DEPT
1	3.94 (2H, tt, *J* = 6.9 Hz)	69.0 (t)
2	2.38 (2H, q, *J* = 8.4)Hz	29.3 (t)
3	5.19 (H, t, *J* = 9.8 Hz)	120.4 (d)
4	–	139.2 (s)
5	1.98 (2H, tt, *J* = 7.0 Hz)	41.0 (t)
6	1.41 (2H, qq, *J* = 10.5 Hz)	26.9 (t)
7	1.15 (2H, qq, *J* = 12.2 Hz)	39.7 (t)
8	1.54 (H, m, *J* = 7.0 Hz)	29.0 (d)
9	0.89 (3H, d, *J* = 6.3 Hz)	23.0 (s)
10	1.63 (3H, s)	16.1 (s)
11	0.89 (3H, d, *J* = 6.3 Hz)	23.0 (s)

The positive FAB-MS of **1** gave on $[M+Na]^+$ and $[M+K]^+$ ion peaks at *m/z* 295.10 and 311.10, respectively. On the HR-FAB-MS, $[M+Na]^+$ ion peak was observed at *m/z* 295.0960 corresponding to a formula $C_{11}H_{21}O_4SNa_2$ (clcd. △ -0.13 mmu).

^{1}H-NMR spectrum exhibited total 10 groups of protons (Table 1). Two doublet methyls overlapped at δ 0.89 (6H, *J* = 6.3 Hz), one singlet methyl at δ 0.91 (3H), five methylene signals at δ 2.38 (2H, qq, *J* = 8.4 Hz), 1.98 (2H, tt, *J* = 7.0 Hz), 1.41 (2H, qq, *J* = 10.50 Hz), 1.15 (2H, qq, *J* = 12.2 Hz), bearing a sulfate group at δ 3.94 (2H, tt, *J* = 6.9 Hz), two methine protons at δ 5.19 (H, t, *J* = 9.8 Hz) and 1.54 (H, m, *J* = 7.0 Hz), respectively.

^{13}C-NMR chemical shifts showed total 11 carbons included a quaternary carbon at δ 139.2 (s), 3 methyl carbons [δ 16.1 (s) and overlapped 2 carbons at δ 23.0 (s)], 5 methylene carbons [δ 26.9 (t), δ 29.3 (t), δ 39.7 (t), δ 41.0 (t) and 69.0 (t)] and 2 methine carbon [δ 29.0 (d) and δ 120.4 (d)] from the DEPT spectrum (Table 1).

2 methyls (C_9 and C_{11}) overlapped doublet signal at δ 0.89 were connected with C_8 methine and a methyl (C_{10}) singlet at δ 1.63 was connected to C_4 quaternary carbon which had observed no other contours neighbored protons from the HSQC (^{1}H-^{13}C COSY) spectrum.

3 partial structures such as from C_1 methylene to C_3 methine, C_{10} methyl and from C_5 methylene to C_8 methine coupled with 2 chemically equivalent methyls

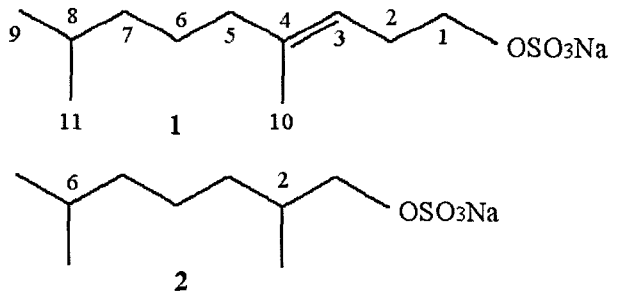

Fig. 1. Structure of 4,8-dimethyl-3-nonenyl sulfate **(1)** from *Styela clava* and 2,6-dimethylheptyl sulfate **(2)** from *Halocynthia roretzi.*

Fig. 2. Connection of protons and carbons by ^1H detected multiple bond connectivity (HMBC) of 4,8-dimethyl-3-nonenyl sulfate **(1)**.

were presumed on the ^1H-^1H COSY spectrum.

Interpretation of coupling between neighbored proton groups by those COSY spectra of **1** led to a gross structure as 4,8-dimethyl-3-nonenyl sulfate **(1)**.

All of those connectivities including the position of quaternary carbon at C_4 were confirmed well from the HMBC spectrum as Fig. 2.

Alkyl sulfate **(2)** or other alkenyl sulfates were known to contained only in hepatopancrea of some tunicate species of *Halocynthia spp.* and presumed may play certain physiological roles in the digestive glands (Tsukamoto *et al.*, 1994). While, Yasumoto *et al.* (2005) reported some aliphatic sulfates, kairomones, released from the crustacean, *Daphnia* induce morphological changes of phytoplankton at low concentration (10^{-9} M).

In case of *Styela clava*, alkenyl sulfate **(1)** was found only in edible part, which suggesting similar actions. It is necessary to investigate for the hygienic safety in the food, biochemical roles in the tunicate and biologically useful activities including antibacterial activity.

Those geometric stereochemistry around double bond and other activities containing minimum inhibition concentrations to various microbes are now under studying and will be reported elsewhere.

Acknowledgments

This work was supported by a Korea Institute of Marine Science & Technology Promotion (KIMST) Grant funded by the Ministry of Maritime Affairs and Fisheries (MOMAF), Republic of Korea (MNF22004012-3-2-SB010). The authors would like to thank to Professor Y. Oshima and Dr. M. Satake, Tohoku University, Japan, for the measurement of MS and NMR.

References

Blunt, J.W., Copp, B.R., Munro, H.G, Northeote, P.T., and Prinsep, M.R., Marine natural products, *Nat. Prod. Rep.* **22**, 15-61 (2005).

Crispino, A., De Giulio, A., De Rosa, S., De Strpano, S., and Milone, A., and N. Zavodnik., A sulfated normonoterpenoid from the ascidian *Polycltor adriaticus*, *J. Nat. Prod.* **57**, 1575-1577 (1994).

Faulkner, D.J., Marine natural products. *Nat. Prod. Rep.* **13**, 75-125 (1996).

Frits Feigl, F. and Suter, H.A., Analytical use of sodium rhodizonate. *Industrial and Engineering Chemistry* **14**, 840-842 (1942).

Sakai, R., Stroh, J.G, Sulins, D.W., and Rinehart, K.L., Seven new didemnins from the marine tunicate *Tridemnum solinum*, *J.Am. Chem. Soc.* **117**, 3734-3748 (1995).

Tsukamoto, S., Kato, H., Hirota, H., and Fusetani, N., Antibacterial and antifungal sulfated alkane and alkenes from the hepatopancreas of the ascidian *Halocynthia Roretzi. J. Nat. Prod.* **57**, 1606-1609 (1994).

Riccio, R., Iorizzi, M., and Minale, L., In Biologically active natural products (Ed. by Hostettmann, K. and Lea, P.J., Clarendon Press, Oxford, UK, 1987, pp. 153-165.

Rinehart, K.L. Jr., Kobayashi, J., Harbour, G.C., Mascal, M., Holt, T.G, Shield, L.S., and Lafargue, F., Eudistomins A-Q., β-Carbolines from the Antiviral Caribbean Tunicate *Eudistoma olivaceum, J. Am. Chem. Soc.* **109**, 3378-3387 (1987).

Yasumoto, K., Nishigami, A., Yasumoto, M., Kasai F., and Okada Y., Aliphatic sulfates released from *Daphnia* induce morphological defense of phytoplankton: isolation and synthesis of kairomones. *Tetrahedron Lett.* **46**, 4765-4767 (2005).

(Accepted May 16, 2007)