Natural Product Sciences
9(4) : 232-234 (2003)

A New Glycerol Ether from a Marine Sponge *Stelletta* Species

Seung-Yeon Lee,[1] Qingchun Zhao,[2] Kyutaek Choi,[1] Jongki Hong,[3] Dong Seok Lee,[4] Chong-O. Lee,[5] and Jee H. Jung[1,*]

[1]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*
[2]*Shenyang Northern Hospital, Shenyang, P.R. China*
[3]*Korea Basic Science Institute, Seoul, Korea*
[4]*Department of Biomedical Science and Engineering, Inje University, Gimhae, Korea*
[5]*Korea Research Institute of Chemical Technology, Daejon, Korea*

Abstract – A new glycerol ether has been isolated from a marine sponge *Stelletta* sp. by bioactivity-guided fractionation. The structure was established on the basis of NMR and MS analyses. The compound was evaluated for cytotoxicity against a small panel of five human tumor cell lines and exhibited significant cytotoxicity.
Keywords – marine sponge, *Stelletta* sp., glycerol ether, cytotoxicity.

Introduction

Some sterols (Guerriero *et al.*, 1991; Li *et al.*, 1994; Yan *et al.*, 2001), terpenoids (McCormick *et al.*, 1996; Oku *et al.*, 2000; Ryu *et al.*, 1996), and alkaloids (Matsunaga *et al.*, 1999; Nazawa *et al.*, 2001; Tsukamoto *et al.*, 1999) were reported from the marine sponges of the genus *Stelletta.* In the course of screening for cytotoxic constituents of a marine sponge *Stelletta* sp. collected from Korean waters, we have noticed significant brine shrimp lethality in the crude MeOH extract. In subsequent bioactivity-guided fractionation, new acetylenic acids (Zhao *et al.*, 2003a), lysophosphatidylcholines (Zhao *et al.*, 2003b), and cyclitol derivatives (Zhao *et al.*, 2003c) have been isolated. In the continuing study of cytotoxic constituents of the same sponge, a new glycerol ether was isolated. The isolation and structure elucidation of the glycerol ether are described herein.

Experimental

General experimental procedures – Optical rotation was obtained using a JASCO DIP-370 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC200 (Analytik gmbh, Silberstreifen, Germany) and a Varian UNITY Inova 500 (Palo Alto, California, U.S.A.). Chemical shifts were reported in reference to the respective solvent peaks (δ_H 3.3 and δ_C 49.0 for CD_3OD; δ_H 7.27 and δ_C 77.0 for $CDCl_3$). COSY, HMQC, and HMBC spectra were recorded on an UNITY Inova 500. FAB-CID tandem MS data were obtained using a JEOL JMS SX-102A. Gel filtration chromatography was performed with Sephadex LH-20 (Pharmacia Biotech AB). HPLC was performed on a Gilson (Villiers-le-Bel, France) 370 pump with a YMC ODS-H80 (preparative, 250×20 mm, 4 µm, 80Å) column, a YMC ODS-H80 (250×10 mm, 4 µm, 80Å) column and a YMC-Pack CN (250×10 mm, 5 µm, 120Å) column using a Shodex RI-71 detector (Minato-ku, Tokyo, Japan) at a flow rate of 1.5 mL/min and 1.0 mL/min.

Animal material – The sponge was collected by hand using SCUBA (20 m in depth) in October 2001, off Ullung Island, Korea. The specimen was identified as *Stelletta* sp. by Prof. C. J. Sim, Hannam University. It has cup-shaped crater of 9 cm height, 14×11 cm width. The surface was rough owing to the projecting brushes of orthotriaenes. The exterior was a shade of dark gray and the interior was beige. The texture was tough like a stone. The skeleton was composed of megascleres, oxea (2,200-3,000 µm×50 µm), orthotriaene (1,000-1,500 µm×50 µm, sometimes abnormal dichotriaene), microscleres, large oxyaster (70-85 µm in diameter), thin oxyaster (30-40 µm in diameter), small oxyaster (15-25 µm in diameter), weakly spined strongylaster (10-14 µm in diameter), and thin strongylaster (7-10 µm in diameter). A voucher specimen (registry No. Spo. 37) was deposited at the Natural History Museum, Hannam University, Korea.

Extraction and isolation – The frozen sponge (15 kg,

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

wet weight) was extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay (Meyer, B. N. *et al.*, 1982), the MeOH extract was partitioned between water and dichloromethane (CH_2Cl_2). The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to afford 5.2 g of the aqueous MeOH. The aqueous MeOH fraction was subjected successively to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with a step gradient solvent system of 50 to 0% H_2O/MeOH to obtain 22 fractions (Fr.1-Fr.22). These fractions were evaluated for activity in the brine shrimp assay, and fractions Fr.8-Fr.16 were found active. Fraction Fr.14 was further separated by a Sephadex LH-20 column chromatography eluting with MeOH, to afford 17 fractions (Fr.14-1-Fr.14-17). Guided by the brine shrimp lethality assay, fractions Fr.14-2-Fr.14-10 were combined (Fr.14a) and purified by a reversed-phase HPLC (YMC ODS-H80, 250×20 mm, 4 μm, 80 Å) eluting with 5% H_2O/MeOH to yield a glycerol ether (5 mg).

1-*O*-(10-methylhexadecyl)-*sn*-glycerol: light yellow oil; $[\alpha]^{21}_{D}$ +1°, (*c* 0.30, MeOH); ^{1}H and ^{13}C NMR, see Table 1. FAB-CID MS/MS *m/z* 353 [M + Na]$^+$ (100), 323 (0.2), 295 (0.2), 281 (0.3), 267 (0.2), 239 (0.3), 225 (0.1), 182 (0.2), 169 (0.1), 147 (0.1), 133 (0.4), 119 (0.2), 113 (0.2), 89 (1.1), 88 (0.4), 63 (0.2), 23 (3.1); HRFABMS *m/z* 353.3038 (calcd for $C_{20}H_{42}O_3Na$, 353.3032).

Table 1. ^{1}H NMR and ^{13}C NMR Data (CD_3OD)[a]

position	δ_H[b]	δ_C[c]
1	3.56 (dd, 10.5, 5.5)	73.2
	3.50 (dd, 10.5, 4.5)	
2	3.73 (quint, 5.5)	72.6
3	3.49 (dd, 10.5, 4.5)	64.6
	3.40 (dd, 10.0, 6.0)	
1'	3.45 (td, 6.5, 1.5)	72.2
2'	1.56 (quint, 6.5)	30.6-31.0
3'	1.251.35 (m)	27.2
4'-7'	1.251.35 (m)	30.6-31.0
8'	1.251.35 (m)	28.1
9'	1.251.35 (m)	38.2
	1.09 (m)	
10'	1.251.35 (m)	33.9
11'	1.251.35 (m)	38.2
	1.09 (m)	
12'	1.251.35 (m)	28.1
13'	1.251.35 (m)	30.6-31.0
14'	1.251.35 (m)	33.0
15'	1.251.35 (m)	23.7
16'	0.89 (t, 6.5)	14.4
17'	0.85 (d, 6.5)	20.1

[a]Multiplicities and coupling constants (in Hz) are in parentheses.
[b]Measured at 500 MHz.
[c]Measured at 50 MHz.

Results and Discussion

The MeOH extract of the sponge showed toxicity to brine shrimp larvae (LD_{50}, 296 μg/mL). Guided by the brine shrimp lethality assay, the MeOH extract was further partitioned between water and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 layer between aqueous MeOH and *n*-hexane. The aqueous MeOH layer was subjected successively to reversed-phase flash column chromatography, Sephadex LH-20 gel permeation chromatography, CN HPLC, and ODS HPLC to afford a new glycerol ether.

The compound was isolated as a colorless oil. The molecular formula was established as $C_{20}H_{42}O_3$ on the basis of HRFABMS. The [M+Na]$^+$ ion peak was observed at *m/z* 353.3038 ($C_{20}H_{42}O_3Na$, Δ+0.6 mmu). The ^{1}H NMR spectrum exhibited signals for a methyl branched (δ 0.85, d, *J* = 6.5 Hz, δ 20.1) aliphatic chain and signals associated with a glycerol monoether moiety. Three pairs of oxymethylene protons and one oxymethine proton signals were observed: a two proton multiplet (an apparent triplet of doublets, *J* = 6.5, 1.5 Hz) at δ 3.45, two doublets of doublets centered at δ 3.56 (*J* = 10.5, 5.5 Hz) and δ 3.50 (*J* = 10.5, 4.5 Hz), two doublets of doublets centered at δ 3.49 (*J* = 10.5, 4.5 Hz) and δ 3.40 (*J* = 10.0, 6.0 Hz), and a pseudo quintet at δ 3.73 (*J* = 5.5 Hz) for an oxymethine proton. The ^{13}C NMR spectrum was in agreement with glycerol ether structure and showed three oxymethylene carbon signals at δ 73.2 (C-1), 72.2 (C-1'), and 64.6 (C-3), and an oxymethine carbon signal at δ 72.6 (C-2) (Quijano *et al.*, 1994). The configuration at C-2 was established to be *S* from the positive optical rotation, which is a general feature of the long chain 1-*O*-alkyl-*sn*-glycerols (Costantino *et al.*, 1993; Barbara *et al.*, 1983). The methyl branching position in the alkyl chain was clearly recognized from FAB-CID tandem mass spectrum. The fragmentations involved parallel pathways of sequential losses of CH_2 groups differing by one carbon except for the fragmentations occurring at the branching point, where the significant losses of CH_2 groups differ by two carbons causing an obvious interruption in the main series of peaks. Thus, the location of the methyl branching was clear from the 28-mass gap between the fragment ion

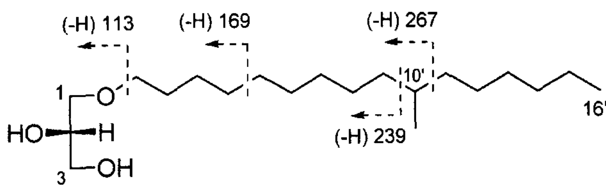

Fig. 1. Key FAB-CID tandem mass fragmentations of the [M + Na]$^+$ ion.

Table 2. Cytotoxicity (ED_{50}, μg/mL) of the Compound against Human Solid Tumor Cells[a]

	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
compound	4.5	6.2	4.4	8.7	6.4
doxorubicin	0.03	0.13	0.06	0.19	0.29

[a]A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT 15: human colon cancer.

peaks at *m/z* 239 and 267 (Fig. 1). The stereochemistry of the methyl branch was undetermined. Although the same methyl-branched alkyl chain was previously reported as an alkyl substituent of a cyclitol derivative from a marine sponge *Sarcotragus* sp. (Liu *et al.*, 2002), this is the first report of the designated glycerol ether from natural source. The compound was evaluated for cytotoxicity against a small panel of five human solid tumor cell lines and exhibited significant cytotoxicity (Table 2).

Acknowledgments

Our thanks are due to Prof. Chung Ja Sim of Hannam University for the identification of the sponge. This study was supported by a grant from the Korea Science and Engineering Foundation through the Biohealth Products Research Center, Inje University.

References

Barbara, L. and Phillp, C. Chiral ether glycerides from a marine sponge. *J. Org. Chem.*, **48**, 3585-3587 (1983).

Bergquist, P., Lawson, M. P., Lavis, A. and Cambie, R. C. Fatty acid composition and the classification of the Porifera. *Biochem. Syst. Ecol.*, **12**, 63-84 (1984).

Costantino, V., Fattorusso, E., Mangoni, A., Aknin, M., Fall, A., Samb, A. and Miralls, J. An unusual ether glycolipid from the Senegalese sponge *Trikentrion loeve* carter. *Tetrahedron*, **49**, 2711-2716 (1993).

Guerriero, A., Debitus, C. and Pietra, F. On the first marine stigmastane sterols and sterones having a 24,25-double bond. Isolation from the sponge *Stelletta* sp. of deep coral sea. *Helv. Chim. Acta*, **74**, 487-494 (1991).

Li, H., Matsunaga, S. and Fusetani, N. Bioactive marine metabolites. 62. A new 9,11-secosterol, stellettasterol from a marine sponge *Stelletta* sp. *Experientia*, **50**, 771-773 (1994).

Liu, Y., Lee, C. O., Hong, J. and Jung, J. H. Cyclitol derivatives from the sponge *Sarcotragus* species. *Bull. Korean Chem. Soc.*, **23**, 1467-1469 (2002).

Matsunaga, S., Yamashita, T., Tsukamoto, S. and Fusetani, N. Three new antibacterial alkaloids from a marine sponge *Stelletta* sp. *J. Nat. Prod.*, **62**, 1202-1204 (1999).

McCormick, J. L., McKee, T. C., Cardellina, J. H., Leid, M. and Boyd, M. R. Cytotoxic triterpenes from a marine sponge *Stelletta* sp. *J. Nat. Prod.*, **59**, 1047-1050 (1996).

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.*, **45**, 31-34 (1982).

Nazawa, D., Takikawa, H. and Mori, K. Synthesis and absolute configuration of stellettadine A: A marine alkaloid that induces larval metamorphosis in Ascidians. *Bioorg. Med. Chem. Lett.*, **11**, 1481-1483 (2001).

Oku, N., Matsunaga, S., Wada, S. I., Watabe, S. and Fusetani, N. New isomalabaricane triterpenes from the marine sponge *Stelletta globostellata* that induce morphological changes in rat fibroblasts. *J. Nat. Prod.*, **63**, 205-209 (2000).

Quijano, L., Cruz, F., Navarrete, I., Gomez, P. and Rios, T. Alkyl glycerol monoethers in the marine sponge *Desmapsamma anchorata*. *Lipids*, **29**, 731-734 (1994).

Ryu, G., Matsunaga, S., Fusetani, N. Globostellatic acids A-D, new cytotoxic isomalabaricane triterpenes from the marine sponge *Stelletta globostellata*. *J. Nat. Prod.*, **59**, 512-514 (1996).

Tsukamoto, S., Yamashita, T., Matsunaga, S. and Fusetani, N. Bistellettadines A and B: Two bioactive dimeric stellettadines from a marine sponge *Stelletta* sp. *J. Org. Chem.*, **64**, 3794-3795 (1999).

Yan, S. J., Su, J. Y., Zhang, G. W., Wang, Y. H. and Li, H. Ketosterols from *Stelletta tenuis*. *Zhongshan Daxue Xuebao*, **40**, 54-57 (2001).

Zhao, Q., Lee, S. Y., Hong, J., Lee, C. O., Im, K. S., Sim, C. J., Lee, D. S. and Jung, J. H. New acetylenic acids from a marine sponge *Stelletta* species. *J. Nat. Prod.*, **66**, 408-411 (2003a).

Zhao, Q., Mansoor, T. A., Hong, J., Lee, C. O., Im, K. S., Sim, C. J., Lee, D. S. and Jung, J. H. New cytotoxic lysophosphatidylcholines and monoglycerides from a marine sponge *Stelletta* species. *J. Nat. Prod.*, **66**, 725-728 (2003b).

Zhao, Q., Liu, Y., Hong, J., Lee, C. O., Park, J. H., Lee, D. S. and Jung, J. H. A new cyclitol derivative from a sponge *Stelletta* species. *Nat. Prod. Sci.*, **9**, 18-21 (2003c).

(Accepted October 14, 2003)