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# A New Sphingosine from a Marine Sponge *Haliclona* (*Reniera*) sp.

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**Abstract –** A new sphingosine **(1)** was isolated from the MeOH extract of a marine sponge *Haliclona* (*Reniera*) sp. by bioactivity-guided fractionation. The 1D and 2D NMR, and MS spectroscopic analyses were used to establish the planar structure of **1**. The stereochemistry of the compound was defined on the basis of modified Mosher's method, comparison of optical rotation and NMR data with those of the reported. Compound **1** was mildly cytotoxic to a panel of five human solid tumor cell lines.
**Keywords –** marine sponge, *Haliclona* (*Reniera*) sp., sphingosine, sterols, lysoPC

## Introduction

Sphingolipids are known to play important roles in signal transduction and cell regulation pathways (Enders *et al*., 2004). Sphingolipid biosynthesis starts by the codensation of acyl-CoA and serine to yield 3-ketosphinganine, which is reduced to yield sphinganine (2-amino-1,3-dihhyroxyalkane) (Gulavita *et al*., 1989). This basic structure can be further modified in chain length, degree of unsaturation, methyl branching, insertion of additional hydroxyl groups, and stereochemistry.

In the continuation of our search for cytotoxic metabolites from a marine sponge *Haliclona* (*Reniera*) sp., guided by brine shrimp lethality ($LD_{50}$ 27 mg/mL) (Mansoor *et al*., 2007), we have isolated a new sphingosine (**1**), six known sterols (**2 - 7**), and two known lysophosphatidylcholine derivatives (**8** and **9**), from the organic extracts of the sponge.

## Experimental

**General** – Optical rotation was measured using a JASCO P-1020 polarimeter. $^1$H- and $^{13}$C-NMR spectra were recorded on Varian INOVA 500 spectrometer. Chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peaks ($\delta_H$ 3.30 and $\delta_C$ 49.0 for $CD_3OD$; $\delta_H$ 7.27 and $\delta_C$ 77.0 for $CDCl_3$). FABMS data were obtained on a JEOL JMS SX-102A. HRFABMS data were obtained on JEOL JMS SX-101A. HPLC was performed with an YMC packed ODS column (250 × 10 mm, 5 μm, 120 Å) and a $C_{18}$-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) using a Gilson 133-RI detector.

**Animal Material** – The sponge, *Haliclona* (*Reniera*), was collected by hand using SCUBA (20 m depth) in Oct. 2001, off Ulleung Island, Korea. The collected sample was frozen immediately. The specimen (sample No. J01U-6) was identified as *Haliclona* (*Reniera*) sp. by Prof. Sim Chung Ja and has been described elsewhere (Mansoor *et al*., 2007).

**Extraction and Isolation** – The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract showed toxicity against brine shrimp larvae ($LD_{50}$ 126 μg/mL). The MeOH extract was partitioned between $CH_2Cl_2$ and water. The $CH_2Cl_2$ layer was further partitioned between aqueous MeOH ($LD_{50}$ 27 μg/mL) and *n*-hexane ($LD_{50}$ 45 μg/mL). The aqueous MeOH fraction was subjected to stepped gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 230 mesh), with a solvent system of 50 to 100% MeOH, to afford 10 fractions. Fraction 4 (506.8 mg), ($LD_{50}$ 85 μg/mL) was subjected to reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 85% MeOH, followed by another reversed-phase HPLC purification (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 55% MeOH, to afford compounds **1** (1.8 mg).

**(2*R*,3*R*,6*R*,7*Z*)-2-aminooctadec-7-ene-1,3,6-triol (1)** – light yellow oil; $[\alpha]^{22}_D$ +10° (*c* 0.1, MeOH); $^1$H and $^{13}$C

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NMR data, see Table 1; FABMS *m/z* 316 $[M+H]^+$, 338 $[M+Na]^+$, 298 $[(M+H)-H_2O]^+$, HRFABMS *m/z* 338.2645 (calcd. for $C_{18}H_{37}O_3NNa$, 338.2671).

**Preparation of *S*- and *R*-MTPA derivatives of 1** – (*S*)-α-Methoxy-α-(trifluoromethyl) phenylacetyl chloride (10 μl, 53 μM) was added to solutions of **1** (0.4 mg, 1.25 μM) in pyridine (50 μl, 0.6 μM). The mixture was then stirred at room temperature for 24 h, followed by purification on a reversed phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 80% MeOH, to obtain *R*-MTPA derivative. The progress of reaction was monitored by TLC. The same procedure was employed for *S*-MTPA derivative of **1**. $^1$H NMR data of *S*-MTPA derivative of **1** ($CDCl_3$, 500 MHz): δ 5.76 (H-7), 5.61 (H-8), 5.34 (H-6), 5.17 (H-3), 4.48 (H-2), 2.20 (H-9), 1.47 (H-4), 1.32 (H-5), 1.25-1.30 (H-10-H-17), 0.88 ($H_3$-18); $^1$H NMR data of *R*-MTPA derivative of **1** ($CDCl_3$, 500 MHz): δ 5.71 (H-7), 5.60 (H-8), 5.22 (H-6), 5.09 (H-3), 4.49 (H-2), 4.19 (H-1a), 4.08 (H-1b), 2.19 (H-9), 1.54 (H-4), 1.36 (H-5), 1.24-1.28 (H-10-H-17), 0.86 ($H_3$-18).

**Evaluation of Cytotoxicity** – Cytotoxicity assay was performed at Korea Research Institute of Chemical Technology. The sulforhodamine B (SRB) assay, developed for measuring the cellular protein content of the cultures, is applied for the measurement of the cytotoxicity of the compounds against tumor cells. The rapidly growing cells were harvested, counted, and inoculated at the appropriate concentrations (1 - 2 × $10^4$ cells/well) into 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate, followed by incubating for 48 h at 37 ºC under a 5% $CO_2$ atmosphere. The cultures fixed with cold TCA were stained by 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered tris base by gyratory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). Fifty percent inhibitory concentration ($ED_{50}$) was defined as the concentration that reduced absorbance by 50% of compared to the control level in the untreated wells.

## Results and Discussion

Compound **1** was isolated as a light yellow oil. Its molecular formula was established as $C_{18}H_{37}O_3N$ on the basis of the HRFABMS and $^{13}$C-NMR spectroscopic data. The LRFABMS showed the $[M+Na]^+$ ion peak at *m/z* 338. The HRFABMS provided the exact value of *m/z* 338.2645 for $[M+Na]^+$, which corresponded well with the molecular formula $C_{18}H_{37}O_3N$. In addition, LRFABMS also showed the ion peaks of $[M+H]^+$ and $[M-H_2O+H]^+$ at *m/z* 316 and 298, respectively. The $^1$H-NMR spectrum of **1** displayed signals at δ 4.35, 3.74, 3.64, and 3.62 for four protons attached to the oxygenated carbons. A doublet of triplets for one proton at δ 2.99, which was correlated to a carbon signal at δ 58.7, was assigned to a methine proton bonded to an amino-substituted carbon (Table 1). This pattern of the $^1$H-NMR spectrum suggested a sphingosine type structure with three oxygen functions. The signals for the methine proton at δ 2.99 showed COSY correlations with methylene proton signals at δ 3.74 and 3.62, which were correlated to a carbon signal at $\delta_C$ 60.9 (C-1), in the HSQC spectrum. This proton was also coupled with H-3 at δ 3.64, which in turn was coupled to protons at δ 1.52 (H-4a) and 1.42 (H-4b). The $^1$H-NMR spectrum also featured signals for a monoun-saturated long aliphatic chain. The COSY spectrum was helpful to determine the position of a double bond since the two allylic proton signals were clearly resolved. The protons at δ 1.55 (H-5a) and 1.35 (H-5b) were correlated with protons at δ 1.52 (H-4a) and 1.42 (H-4b), and allylic proton at δ 4.35 (H-7). The geometry of the double bond was ascertained to be *cis* on the basis of the characteristic coupling constant ($J$ = 11.0 Hz) of olefinic protons H-7 and 8, and the characteristic chemical shift of an allylic carbon (C-9) at δ 28.7. The NOE correlation between H-6 and H-9 also corroborated the *cis* configuration.

The stereochemistry at C-2, C-3, and C-6 was deduced by the NMR analysis of MTPA derivatives of **1**. Despite

**Table 1.** $^1$H- and $^{13}$C- NMR data of compound **1** ($CD_3OD$)

| position | $^1$H[a,b] | $^{13}$C[c] |
|---|---|---|
| 1 | 3.74 (dd, 11.5, 4.5 Hz) | 60.9 |
| | 3.62 (dd, 11.5, 7.0 Hz) | |
| 2 | 2.99 (dt, 7.0, 4.5 Hz) | 59.0 |
| 3 | 3.64 (m) | 69.3 |
| 4 | 1.52 (m) | 35.0 |
| | 1.42 (m) | |
| 5 | 1.55 (m) | 38.7 |
| | 1.35 (m) | |
| 6 | 4.35 (m) | 68.2 |
| 7 | 5.31 (dd, 11.0, 9.0 Hz) | 134.0 |
| 8 | 5.44 (dt, 11.0, 7.5 Hz) | 132.2 |
| 9 | 2.08 (m) | 28.7 |
| 10 - 16 | 1.28 - 1.37 (m) | 26.3-32.9 |
| 17 | 1.28 - 1.37 (m) | 23.7 |
| 18 | 0.90 (t, $J$ = 7.0 Hz) | 14.1 |

[a] Measured at 500 MHz. [b] Multiplicities and coupling constants are in parentheses. [c] Measured at 125 MHz.

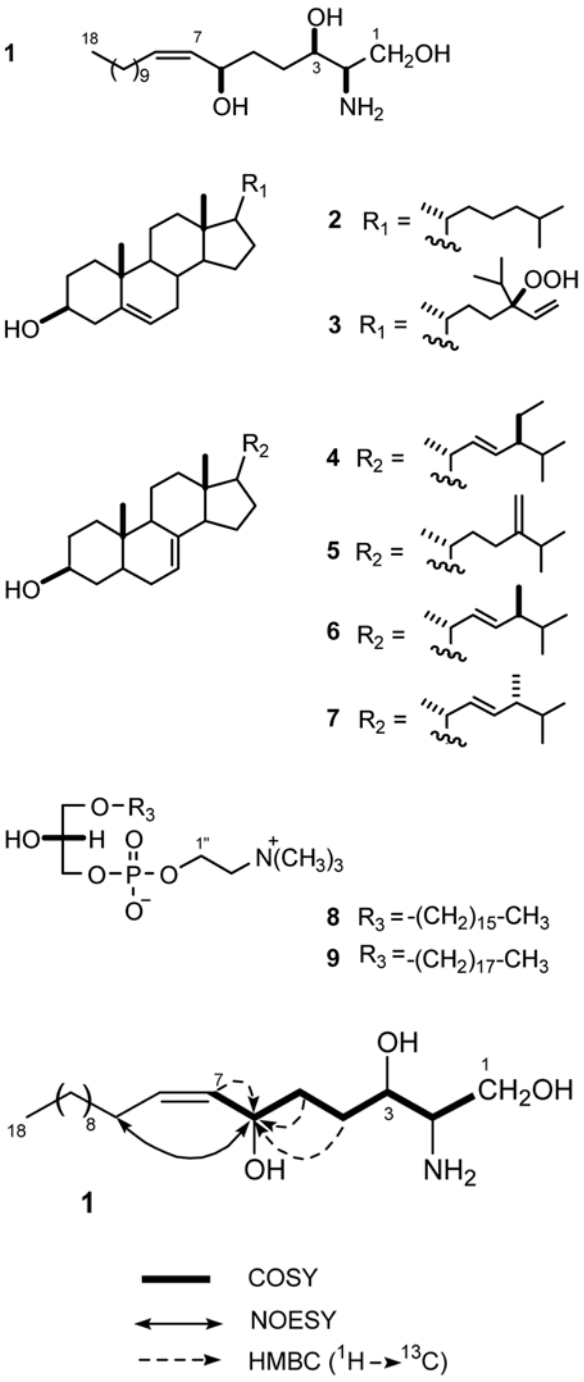


**Fig. 1.** Key COSY, NOESY, and HMBC correlations of compound **1**.

the potential complications of multiple MTPA groups, the reported $^1$H-NMR data of the four possible isomers were significantly different (Li *et al*., 1999). The $^1$H-NMR chemical shifts of H-2, H-3, and H-4 of *S*-MTPA derivatives of (2*S*,3*R*), (2*R*,3S), (2*S*, 3*S*), and (2*R*,3*R*), were reported as (δ 4.485/5.161/1.48), (δ 4.468/5.196/1.69), (δ 4.498/5.096/1.47), and (δ 4.489/5.197/1.67),

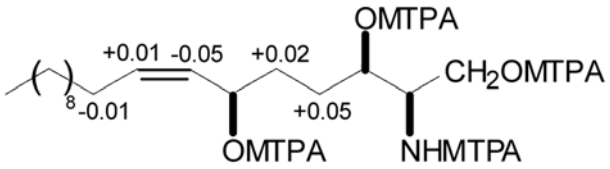


**Fig. 2.** Δδ ($\delta_S$ - $\delta_R$) values for the MTPA esters of compound **1**.

**Table 2.** Cytotoxicity Data of Compound **1**[a]

| compound | A549 | SK-OV-3 | SK-MEL-2 | XF498 | HCT15 |
|---|---|---|---|---|---|
| **1** | 4.33 | 4.82 | 5.20 | 5.75 | 5.40 |
| doxorubicin | 0.05 | 0.14 | 0.05 | 0.03 | 0.12 |

[a]Data expressed in $ED_{50}$ values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer.

respectively. The H-2, H-3, and H-4 of *S*-MTPA derivative of compound **1** appeared at δ 4.48, 5.17, and 1.64, respectively. On the basis of this comparison, the stereochemistries at C-2 and C-3 were established as (2*R*,3*R*). The analysis of $^1$H-NMR data of *S*- and *R*-MTPA derivatives of **1** further corroborated the stereochemistry at C-6. The significant differences in the chemical shift of H-7 (Δδ-0.05) alongwith similar differences in the neighboring protons (Fig. 2) aided in the assignment of absolute configuration at C-6 as *R*. In addition, the $^{13}$C-NMR data of C-1 to C-4, and the optical rotation data of **1** were almost identical to the previously reported 2*R*,3*R*,7*Z*-2-aminotetradec-7-ene-1,3-diol (C-1 at δ 60.8, C-2 at δ 58.7, C-3 at δ 69.9, and C-4 at δ 34.0; $[\alpha]^{20}_D$ + 19.7) (Devijver *et al*., 2000), supporting the stereochemical assignments. On the basis of these data, the structure of **1** was defined as (2*R*,3*R*,6*R*,7*Z*)-2-aminooctadec-7-ene-1,3,6-triol. The formation of 2-amino-octadec-7-ene-1,3-diol requires a $C_{16}$ fatty acid and L-serine, with the reduction of intermediary ketones along diastereomeric pathways. This is the first report on the isolation of OH-6 sphingosine derivative, while its counterpart has been previously reported from the unknown species of the same genus *Haliclona* (Devijver *et al*., 2000). The new features of **1** are the presence of hydroxyl group at C-6 and a longer chain length.

Six known sterols (**2** - **7**) (Sheikh *et al*., 1974; Seldes *et al*., 1985; Rovirosa *et al*., 1990; Luo *et al*., 2006; Santalova *et al*., 2004; Zielinski *et al*., 1982; Elenkov *et al*., 1999) and two known lysophosphatidylcholine derivatives (**8** and **9**) (Mansoor *et al*., 2005) have also been isolated from the organic extracts of the sponge and their structures have been characterized by comparison of

NMR data with those of reported. Of the isolated known compounds, sterol **3** and lysophosphatidylcholine derivatives have been reported for the first time from a sponge of the genus *Haliclona* (*Reniera*).

The sphingosine (**1**) was evaluated for cytotoxicity against a panel of five human solid tumor cell lines (Table 2), and showed moderate cytotoxicity.

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## References

Devijver, C., Salmoun, M., Daloze, D., Braekman, J.C., Weerdt, W.H.D., Kluijver, M.J.D., and Gomez, R., (2*R*,3*R*,7*Z*)-20-Aminotetradec-7-ene-1,3-diol, a new amino alcohol from the Caribbean sponge *Haliclona vansoesti*. *J. Nat. Prod.* **63**, 978-980 (1985).

Elenkov, I., Popov, S., and Andreev, S., Sterols from two Black Sea sponges (*Haliclona* Sp.). *Comp. Biochem. Phys. Part B: Biochem. Molec. Biology.* **123**, 357-360 (1999)

Enders, D. and Müller-Hüwen, A., Asymmetric synthesis of 2-amino-1,3-diols and D-erythro-sphinganine. *Eur. J. Org. Chem.* **2004**, 1732-1739 (2004).

Gulavita, N.K. and Scheuer, P.J., Two epimeric aliphatic amino alcohols from a sponge *Xestospongia* sp. *J. Org. Chem. J. Org. Chem.* **54**, 366-369 (1989).

Mansoor, T.A., Bae, B.H., Hong, J., Lee C., Im, K.S., and Jung, J.H., New fatty acid derivatives from *Homaxinella* sp., a marine sponge. *Lipids* **40**, 981-985 (2005).

Mansoor, T.A., Hong, J., Lee, C., Sim, C.J., Son, B.W., and Jung, J.H., Renierasides, new cerebrosides from a marine sponge *Haliclona (Reniera)* sp. *J. Nat. Prod.* Submitted (2007).

Li, S., Wilson, W.K., and Schroepfer, Jr. G.J., Chemical synthesis of D-*ribo*-phytosphingosine-1-phosphate, a potential modulator of cellular processes. *J. Lipid Res.* **40**, 117-125 (1999).

Luo, X., Li, F., Shinde, P.B., Hong, J., Lee, C., Im, K.S., and Jung, J.H., 26,27-Cyclosterols and other polyoxygenated sterols from a marine sponge *Topsentia* sp. *J. Nat. Prod.* **69**, 1760-1768 (2006).

Santalova, E.A., Makrieva, T.N., Gorshkova, I.A., Dmitrenok, A.S., Krasokhin, V.B., and Stonik, V.A., Sterols from six marine sponges. *Biochem. Biochem. Sys. Ecol.* **32**, 153-167 (2004).

Seldes, A.M., Rovirosa, J., San, M.A., and Gros, E.G., Sterols from the sponge *Haliclona chilensis*. *Comp. Biochem. Phys. Part B*: *Biochem. Molec. Biology* **81**, 119-122 (1985).

Sheikh, Y.M. and Djerassi, C., Steroids from sponges. *Tetrahedron*. **30**, 4095-4103 (1974).

Rovirosa, J., Vasques, M.D.L.L., and San-Martin A., Chemotaxonomic considerations in relation to sponges of the genera *Reniera* and *Haliclona*. *Biochem. Sys. Ecol.* **18**, 53-55 (1990).

Zielinski, J., Milkova, T., Popov, S., Marekov, N., and Djerassi, C., Minor and trace sterols in marine invertebrates 34. Isolation and structure elucidation of 24-methyl-5α-cholesta-7,9(11),24(28)-trien-3β-ol, the first naturally occurring $\Delta^{7,9(11)}$ unsaturated marine sterol. *Steroids* **39**, 675-680 (1982).

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