Sodium 1-(12-Hydroxy)octadecanyl Sulfate, an MMP2 Inhibitor, Isolated from a Tunicate of the Family Polyclinidae

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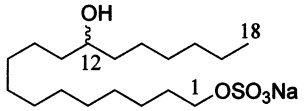
Sodium 1-(12-hydroxy)octadecanyl sulfate (**1**) was isolated from a marine tunicate as a matrix metalloproteinase 2 (MMP2) inhibitor.[1] The structure was elucidated by a combination of spectroscopic and chemical methods. Compound **1** inhibited MMP2 with an IC_{50} value of 9.0 μg/mL.

Solitary ascidians of the families Ascidiidae and Pyuridae often contain simple sulfated alkanes and alkenes, which are cytotoxic or antimicrobial.[2] Recently, more complex polysubstituted alkyl sulfates have been isolated from colonial ascidians.[3,4] In the course of our program on the discovery of potential cancer chemotherapeutics, we found that the extract from an ascidian collected in western Japan inhibited matrix metalloproteinase 2 (MMP2).[5] Bioassay-guided fractionation of the extract afforded an active compound whose structure was elucidated to be an alkyl sulfate on the basis of spectroscopic and chemical methods. The present paper deals with the isolation and structure elucidation of the new compound.



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The frozen specimen was extracted with MeOH, $CHCl_3$/MeOH, and EtOAc. The combined extracts were successively fractionated by solvent partitioning, reversed-phase flash chromatography, gel filtration, silica gel chromatography, and reversed-phase HPLC. The active fractions were finally purified by ODS HPLC using aqueous MeCN containing 250 mM $NaClO_4$ to afford an active compound, **1**, in a yield of 4.2×10^{-4}% based on wet weight.

The molecular formula of **1** was established as $C_{18}H_{37}O_5SNa$ on the basis of HR-FABMS. The presence of a sulfate group was suggested by a fragment ion peak at *m*/*z* 97 in the negative ion FABMS. The ^{1}H NMR spectrum of **1** consisted of a terminal methyl (δ 0.90), an oxymethine (δ 3.49), an oxymethylene (δ 3.99), and a methylene envelope (δ_H 1.28−1.34). NMR data indicated its linear alkanic nature containing both a primary and a secondary hydroxyl group, one of which was sulfated. A ^{13}C isotope shift experiment placed the sulfate group on C-1 ($\Delta\delta$ 0.030; $\Delta\delta$ 0.146 for oxymethine; Figure 1). The position of the secondary hydroxyl group could not be determined from NMR data. Fortunately, the negative charge of the sulfate group at the terminus of the molecule enabled us to carry out charge remote fragmentation analysis by tandem mass spectrometry; the negative ion mode FAB-MS/MS exhibited

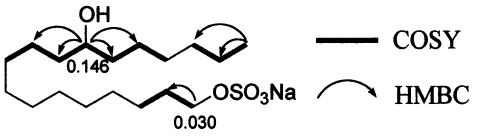

Figure 1. Key COSY and HMBC correlations and deuterium shift values [$\delta_C(CD_3OH) - \delta_C(CD_3OD)$].

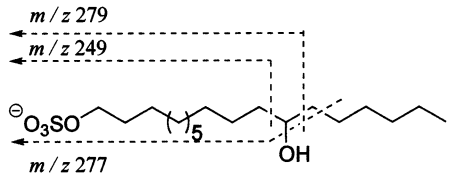

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Figure 2. Negative mode FAB-MS/MS analysis of **1**.

prominent fragment ion peaks at *m*/*z* 249 and 279, thereby placing a hydroxyl group on C-12 (Figure 2).

The absolute stereochemistry at C-12 was determined by the modified Mosher's method.[6] Compound **1** was derivatized to (*R*)- and (*S*)-MTPA esters [(*R*)-**2** and (*S*)-**2**, respectively], the ^{1}H NMR spectra of which exhibited two terminal methyl signals, thus indicating that **1** was a mixture of two isomers. To confirm this, we synthesized **1** from (*R*)-12-hydroxystearic acid (**3**) by the following scheme. The secondary hydroxyl group of **3** was protected with a THP group, followed by reduction of the carboxylic acid to the primary alcohol with $LiAlH_4$. The resulting alcohol was sulfated with SO_3/Py, and deprotection yielded optically pure **1**. The (*R*)- and (*S*)-MTPA esters [(*R*)-**2** and (*S*)-**2**] of the synthetic **1** exhibited only one terminal methyl signal and showed a clear distribution of $\Delta\delta$ values. On the other hand, the ^{1}H NMR spectrum of the mixture of the synthetic (*R*)-**2** and (*S*)-**2** showed two methyl signals which were almost superimposable to those of the natural **2**. These ^{1}H NMR data for the MTPA esters indicated that natural **1** was a 55:45 mixture of 12*R* and 12*S* isomers.

Compound **1** is structurally related to the forbesins[7] isolated from the starfish *Asterias forbesi* and several alkyl sulfates[2,4] isolated from the tunicates *Ascidia mentula* and *Sidnyum turbinatum*. Both natural and synthetic **1** inhibited MMP2 with an IC_{50} value of 9.0 μg/mL; thus the stereochemistry of the hydroxyl group did not influence the activity.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Jasco DIP-1000 digital polarimeter in

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MeOH. UV spectra were recorded on a Shimadzu BioSpec-1600 UV spectrophotometer in MeOH. Fluorescence measurements of enzyme inhibition assay were performed on a Molecular Devices SPECTRA MAX GEMINI spectrofluorometer. NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were referenced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD. FAB mass spectra were measured on a JEOL JMX-SX102/SX102 tandem mass spectrometer using triethanolamine as a matrix. Negative ion mode HR-FABMS were obtained at the resolution of 5000 using PEG sulfate 600 as a marker.

Animal Material. The tunicate was collected by hand at a depth of 3−5 m off the Kii Peninsula (34°19′ N; 136°41′ E). The specimen was immediately frozen and preserved at −20 °C until extraction. As the material for identification was poorly preserved, determining its specific or even generic affiliation was impossible; it may possibly belong to the family Polyclinidae. The living colony is almost black according to its color photograph. This coloration may be reminiscent of *Sidneioides snamoti* (Oka), *S. japonense* Redikorzev, or some species of the genus *Polyclinum*, which however should remain only a very tentative suggestion. Examination of a defrosted and ethanol-preserved small piece of colony reveals that. The tunic is semitransparent and dull white, with its surface coated sparsely with fine sand grains. The zooidal layer of the colony is ca. 10 mm thick. Common cloacal apertures are large with extensive cavities and surrounded by many pale brownish zooids. The zooid is composed of a ca. 5 mm long thorax and an elongated mass of tissue, consisting probably of abdomen and postabdomen. The thorax has a large atrial aperture, whose languet is obscure, and more than 10 rows of stingmata; further details of the branchial are unknown. The voucher (T96-002) was deposited at the Nagoya University Museum, Nagoya, Japan.

Extraction and Isolation. The frozen specimen (800 g) was homogenized and extracted with MeOH, $CHCl_3$/MeOH (1:1), and EtOAc (1.5 L × 2, each). The combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O, and the aqueous layer was further extracted with *n*-BuOH. The $CHCl_3$ and *n*-BuOH extracts were combined and partitioned between *n*-hexane and MeOH/H_2O (9:1). The water content of the aqueous MeOH layer was increased to 40% and extracted with $CHCl_3$.[8] The active $CHCl_3$ layer was fractionated by ODS flash chromatography using stepwise elution of aqueous MeOH. Fractions eluted with 70−100% aqueous MeOH were combined and gel filtered on a Sephadex LH-20 column with MeOH to obtain eight fractions. Fractions 4−6 were combined and fractionated by silica gel column chromatography by a stepwise elution with $CHCl_3$/MeOH/H_2O systems to afford five fractions. Three active fractions were further purified by HPLC on Inertsil ODS-3 with 60% aqueous MeCN containing 250 mM $NaClO_4$ followed by 50% aqueous MeCN containing 250 mM $NaClO_4$ to yield the sodium 1-(12hydroxy)octadecanyl sulfate (**1**: 3.4 mg, 4.2 × 10^{-4}% yield based on wet weight).

Sodium 1-(12-hydroxy)octadecanyl sulfate (1): white powder; $[\alpha]^{24}_D$ −0.9° (*c* 0.1, MeOH); UV (MeOH) no absorption maximum above 200 nm; NMR data see Table 1; HR-FABMS (triethanolamine) *m*/*z* 365.2361 (calcd for $C_{18}H_{37}O_5S$, 365.2362).

MMP2 Inhibition Assay. Purified recombinant MMP2 which was expressed by insect cells and fluorescent substrate MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2 purchased from Peptide Institute Inc., Osaka, were used. The inhibition assay for MMP2 was carried out by the modified procedure of Knight et al.[9] Test samples (2 μL) were added to wells of microtiter plates, each of which contained 100 μL of TNC buffer (50 mM Tris-HCl pH 7.5 + 150 mM NaCl + 10 mM $CaCl_2$ + 0.02% NaN_3 + 0.05% Brij-35). Aliquots of 50 μL of the enzyme solution (5 ng/mL) were added to this solution and preincubated at 37 °C for 10 min. Then, 50 μL of substrate solution (10 μM) was added to the mixture to begin the reaction. The fluorescence values were measured at an excitation of 328 nm and an emission of 393 nm after incubation at 37 °C for 1 h.

Preparation of MTPA Esters 2. Compound **1** (1.0 mg) was dissolved in 0.1 mL of pyridine, to which was added 10 μL of (*R*)- or (*S*)-MTPACl. The mixture was kept at room temperature for 30 min and then diluted with 4 mL of H_2O and extracted with EtOAc. The EtOAc fraction was separated by ODS HPLC (CAPCELL PAK UG-120; 60% *n*-PrOH + 0.05% TFA) to yield (*S*)- or (*R*)-MTPA esters ((*S*)-**2** and (*R*)-**2**), respectively.

Table 1. ^{1}H and ^{13}C NMR Data for **1**[a]

#	δ_H mult. (J (Hz))	δ_C
1	3.99 t (6.5)	69.13
2	1.54 dt (15.0, 6.7)	30.45
3	1.38 m	26.91
4[b]	1.28−1.34	30.72
5[b]	1.28−1.34	30.72
6[b]	1.28−1.34	30.72
7[b]	1.28−1.34	30.72
8[b]	1.28−1.34	30.72
9[b]	1.28−1.34	30.72
10	1.31[c]	26.80
11	1.42 m	38.43
12	3.49 quint. (3.75)	72.43
13	1.42 m	38.43
14	1.31	26.80
15[b]	1.28−1.34	30.72
16	1.29[c]	33.08
17	1.32[c]	23.72
18	0.90 t (7.1)	14.44

[a] Measured in CD_3OD. [b] Methylene envelope. [c] Overlapping on methylene envelope.

(*R*)-2: ^{1}H NMR data (CD_3OD) δ 7.42−7.53 (5H, m, Ph), 5.08 (1H, quint., *J* = 6.3 Hz, H-12), 3.98 (2H, t, *J* = 6.7 Hz, H-1), 3.53 (3H, s, OMe), 1.65 (2H, quint., *J* = 6.2 Hz, H-2), 1.64, 1.55 (4H, q, *J* = 7.6 Hz, H-11, H-13), 1.40 (2H, quint., *J* = 7.2 Hz, H-3), 1.30, 1.24 (overlapped, H-10, H-14), 1.31, 1.28 (overlapped, H-17), 1.28 (methylene envelope), 0.90, 0.87 (3H, t, *J* = 6.5 Hz, H-18). ^{1}H NMR data of the (*S*)-**2** was essentially identical with that of (*R*)-**2**, except for minor intensity differences.

Synthesis of Chiral Sodium 1-(12-Hydroxy)octadecanyl Sulfate (1). To a solution of (*R*)-12-hydroxystearic acid (**3**: 1.0 g) in THF (5 mL) were added dihydropyrane (3 mL) and PPTS (10 mg), and the mixture was stirred at room temperature for 2 h. An additional 10 mg of PPTS was added to the mixture, and the mixture stirred at 40 °C for 2 h. To the reaction mixture was added 300 μL of triethylamine, and the mixture was partitioned between H_2O and EtOAc. The organic phase was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue was dissolved in THF (5 mL), to which was added 3.5 mL of 1 M $LiAlH_4$ diethyl ether solution, and the mixture was stirred for 2 h at room temperature. The reaction was quenched by the addition of 50 mL of H_2O, and the mixture was extracted with EtOAc. The organic layer was dried, evaporated, and dissolved in 5 mL of pyridine. To the solution was added sulfur trioxide pyridine complex (500 mg), and the mixture was stirred at room temperature for 2 h. An additional 100 mg of sulfur trioxide pyridine complex was added and the mixture stirred for another 1 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with $CHCl_3$. The organic layer was dried over anhydrous $MgSO_4$, evaporated, and dissolved in a mixture of THF/MeOH/H_2O (4:2:1, 14 mL). To the mixture was added 20 mg of PPTS, and the mixture was stirred at 40 °C for 3 h. The reaction mixture was evaporated to remove the solvent, and the residue was subjected to the modified Kupchan solvent partitioning procedures.[8] The $CHCl_3$ fraction was concentrated and fractionated by silica gel column chromatography with $CHCl_3$/MeOH (5:1). The product was finally purified by HPLC on Inertsil ODS-3 with 60% MeOH containing 300 mM $NaClO_4$ to furnish synthetic **1** in a yield of 33%.

Synthetic 1: white powder; $[\alpha]^{24}_D$ −1.0° (*c* 0.1, MeOH); UV (MeOH) no absorption maximum above 200 nm; NMR data superimposable to natural **1**; HR-FABMS (triethanolamine) *m*/*z* 365.2376 (calcd for $C_{18}H_{37}O_5S$, 365.2362).

Preparation of Synthetic MTPA Esters 2. MTPA esters of synthetic **1** were prepared as described above.

Synthetic (*R*)-2: ^{1}H NMR data (CD_3OD) δ 7.37−7.53 (5H, m, Ph), 5.08 (1H, quint. J = 6.2 Hz, H-12), 3.98 (2H, t, J = 6.5 Hz, H-1), 3.53 (3H, s, OMe), 1.65 (2H, quint., J = 6.5 Hz, H-2), 1.63 (2H, q, J = 6.9 Hz, H-13), 1.55 (2H, q, J = 6.5 Hz, H-11), 1.39 (2H, quint., J = 7.4 Hz, H-3), 1.28 (methylene envelope), 0.87 (3H, t, J = 7.3 Hz, H-18).

Synthetic (*S*)-2: ^{1}H NMR data (CD_3OD) δ 7.32−7.53 (5H, m, Ph), 5.08 (1H, quint., J = 6.2 Hz, H-12), 4.00 (2H, t, J = 6.5 Hz, H-1), 3.53 (3H, s, OMe), 1.66 (2H, quint., J = 6.9 Hz, H-2), 1.63 (2H, q, J = 6.5 Hz, H-11), 1.54 (2H, q, J = 7.0 Hz, H-13), 1.38 (2H, quint., J = 7.6 Hz, H-3), 1.28 (methylene envelope), 0.89 (3H, t, J = 7.2 Hz, H-18).

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Supporting Information Available: Photograph of the ascidian. This material is available free of charge via the Internet at http://pubs.acs.org.

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