Callysponginol Sulfate A, an MT1-MMP Inhibitor Isolated from the Marine Sponge *Callyspongia truncata*[1]

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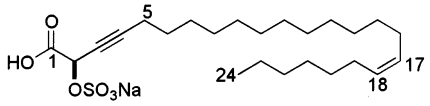
Callysponginol sulfate A (**1**) was isolated from the marine sponge *Callyspongia truncata* as a membrane type 1 matrix metalloproteinase (MT1-MMP) inhibitor. Its structure was elucidated by a combination of spectroscopic and chemical methods and found to be a new sulfated C_{24} acetylenic fatty acid. Compound **1** inhibited MT1-MMP with an IC_{50} of 15.0 μg/mL.

Membrane type 1 matrix metalloproteinase (MT1-MMP) is one of the key enzymes involved in tumor growth, migration, angiogenesis, invasion, and metastasis.[2] This enzyme plays important roles in degradation of basal membranes and extracellular matrices (ECM) by digesting type IV collagen, a major component of ECM. MT1-MMP also activates and recruits gelatinase A (MMP2) around the cell surface of the migration front by formation of a complex.[3] Therefore, MT1-MMP inhibitors are potential antimetastatic and antiangiogenic agents. Although a number of natural products and synthetic MMP inhibitors have been reported,[4] no specific inhibitors against MT1-MMP are known. In our search for MT1-MMP inhibitors from Japanese marine invertebrates, we found potent activity in both lipophilic and hydrophilic extracts of the marine sponge *Callyspongia truncata*, collected in western Japan.[5] Bioassay-guided fractionation afforded a sulfated C_{24} acetylenic fatty acid, callysponginol sulfate A (**1**), together with the known halistanol sulfates.[6] Here we report the isolation, structure elucidation, and biological activity of the new compound.



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The frozen specimen was extracted with H_2O, MeOH, $CHCl_3$, and EtOAc. The combined extracts were fractionated by solvent partitioning, ODS flash chromatography, gel filtration, and reversed-phase HPLC to afford callysponginol sulfate A (**1**).

Callysponginol sulfate A (**1**) had a molecular formula of $C_{24}H_{41}O_6SNa$ as established by HR-FABMS. The presence of a sulfate group was suggested by a fragment ion peak at *m*/*z* 97 in the negative FABMS. The ^{1}H NMR spectrum of **1** consisted of a terminal methyl (δ 0.87), three low-field methylenes [δ 2.00 (4H) and δ 2.18 (2H)], an oxymethine (δ 5.16), two olefinic protons (δ 5.32), and a methylene envelope (δ 1.25−1.32). A carbonyl carbon (δ_C 173.3) and two sp-hybridized carbons (δ_C 76.4 and 87.4) were observed in the ^{13}C NMR spectrum. Considering the molecular formula, **1** was determined to be a C_{24}-acetylenic fatty acid comprising a sulfate and olefin functionality.

The sulfate group could be placed on a methine carbon adjacent to the carboxylic acid on the basis of an HMBC correlation, H-2/C-1. This substructure was further connected to the acetylene, which was in turn linked to the deshielded methylene, as analyzed by COSY ($^5J_{2,5}$ = 2.3 Hz) and HMBC (H-2/C-3, 4 and H-5/C-3, 4) correlations (Table 1). The Δ^{17} double bond was evident from the prominent ion peaks at *m*/*z* 331 and 385 in the FABMS/MS analysis, which might have arisen by cleavage of the bonds between C-15 and C-16 and between C-19 and C-20. This was also substantiated by FABMS/MS data of the OsO_4 oxidation product **2**, where a daughter ion peak was observed at *m*/*z* 375 derived from cleavage between C-17 and C-18 (Figure 1). The *Z*-geometry of the double bond was inferred from a carbon chemical shift (δ_C 28.1) for both C-16 and C-19, which was typical of an allylic carbon of *Z*-olefins.[7] The absolute stereochemistry at C-2 was determined by the modified Mosher's method.[8] The sulfate group of **1** was hydrolyzed with acid, and the resulting secondary alcohol was converted to the MTPA esters [(*R*)-**3** and (*S*)-**3**]. The $\Delta\delta$ values were consistent with 2*R*-stereochemistry.

Acetylenic fatty acids isolated from marine sponges have exhibited a wide range of biological activities such as cytotoxicity,[9] antimicrobial[10] and antifouling[11] activities, and enzyme inhibition.[12] Callysponginol sulfate A (**1**) is the first example of an acetylenic acid containing a sulfate group from marine organisms. Compound **1** inhibited MT1-MMP with an IC_{50} of 15.0 μg/mL, while halistanol sulfate showed an IC_{50} of 19.0 μg/mL. The desulfated **1** did not show any inhibitory activity against MT1-MMP. Considering this result as well as the similar activity of structurally unrelated sulfated compounds, the MT1-MMP inhibition activity is probably a consequence of the sulfate.

Experimental Section

General Experimental Procedures. Optical rotation was measured on a Jasco DIP-1000 digital polarimeter. The UV spectrum was recorded on a Shimadzu BioSpec-1600 UV spectrophotometer. NMR spectra were recorded on a JEOL α600 NMR spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C. Chemical shifts of ^{1}H and ^{13}C NMR spectra were refer-

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Table 1. 1D and 2D NMR Data for Callysponginol Sulfate A (**1**)[a]

#	δ_C	δ_H mult. (*J* Hz)	COSY	HMBC
1	173.30			
2	70.01	5.16 t (2.3)	H-5[d]	C-1, 3, 4
3	76.42			
4	87.37			
5	19.47	2.18 dt (2.3, 7.3)	H-2,[d] 6	C-3, 4, 6
6	29.99	1.48 quint. (7.3)	H-5, 7	C-4, 5, 7
7	30.78	1.31[c]		
8−14	30.78	1.25[b]		
15	30.78	1.30[c]	H-16	
16	28.11	2.00 q (6.4)	H-15, 17	C-15, 17, 18
17	130.78	5.32 dd (4.6, 5.8)	H-16	C-16, 19
18	130.78	5.32 dd (4.6, 5.8)	H-19	C-16, 19
19	28.11	2.00 q (6.4)	H-18, 20	C-17, 18, 20
20	30.78	1.30[c]	H-19	
21−22	30.78	1.25[b]		
23	23.69	1.29[c]	H-22, 24	C-24
24	14.50	0.87 t (7.1)	H-23	C-22, 23

[a] Measured in CD_3OD. [b] Methylene envelope. [c] Overlapping on methylene envelope. [d] 5-Bond long-range correlation.

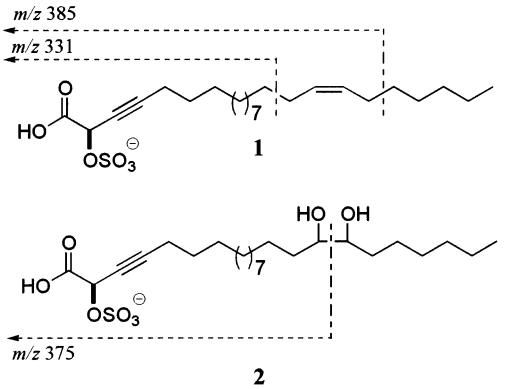

Figure 1. Negative ion mode FABMS/MS analysis of callysponginol sulfate A (**1**) and its OsO_4 oxidation product (**2**).

enced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD and δ_H 7.24 for $CDCl_3$. FAB mass spectra were measured with a JEOL JMX-SX102/SX102 tandem mass spectrometer using triethanolamine for the negative ion mode and glycerol for the positive ion mode as matrices. Negative ion mode HR-FABMS was obtained at a resolution of 5000 using PEG sulfate 600 as a marker.

Animal Material. The sponge was collected by hand using scuba at depths of 10−15 m at Hako Island off the Kii Peninsula (33°43′ N; 136°04′ E). The specimen was immediately frozen and preserved at −20 °C until extraction. The sponge was identified as *Callyspongia truncata* (Demospongiae, Haplosclerida, Callyspongiidae); a voucher specimen was deposited at the Zoological Museum, University of Amsterdam (ZMA POR 16716).

Extraction and Isolation. The frozen specimen (78 g) was homogenized and extracted with 50% aqueous MeOH, MeOH, $CHCl_3$/MeOH (1:1), and EtOAc (200 mL × 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 layers were combined and subjected to a modified Kupchan procedure;[13] first, between *n*-hexane and 90% aqueous MeOH and then between 60% MeOH and $CHCl_3$. The active $CHCl_3$ and 60% aqueous MeOH layers were combined and fractionated by ODS flash chromatography using stepwise elution with aqueous MeOH. Activity was found in fractions eluted with 70−100% aqueous MeOH, and these were combined and gel filtered on a Sephadex LH-20 column with 80% aqueous MeOH to obtain 10 fractions. Fractions 5−9 were combined and purified by reversed-phase HPLC on a Phenomenex Luna 5μ Phenyl-Hexyl column with 83% aqueous MeOH containing 250 mM $NaClO_4$ to yield callysponginol sulfate A (**1**; 19.8 mg, 2.5 × 10^{-2}% yield based on wet weight). ODS flash chromatography of the aqueous layer obtained from the crude extracts afforded halistanol sulfates (43.4 mg, 5.5 × 10^{-2}%).

Callysponginol sulfate A (1): white powder; $[\alpha]^{24}_D$ −0.16° (*c* 0.1, MeOH); UV (MeOH), end absorption; IR (film) ν_{max} 3599, 3019, 2926, 2854, 1635, 1216, 1112, 770, 669 cm^{-1}; NMR data, see Table 1; HR-FABMS (triethanolamine) *m/z* 457.2621 (calcd for $C_{24}H_{41}O_6S$ 457.2624).

MT1-MMP Inhibition Assay. The recombinant MT1-MMP,[14] which is the truncated transmembrane domain, 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 MT1-MMP was carried out by a modified procedure of Knight et al.[15] Test samples (2 μL) were added to wells of 96-well microtiter plates (eight concentrations from 3.13 to 400 μg/mL in triplicate), each well containing 100 μL of TNC buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM $CaCl_2$, 0.02% NaN_3, and 0.05% Brij-35). Aliquots of 50 μL of enzyme solution (40 ng/mL) were added to wells, and the mixtures were preincubated at 37 °C for 10 min. Finally, 50 μL aliquots of substrate solution (8 μM) were added to the mixtures 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.

OsO_4 Oxidation of 1. Compound **1** (1.0 mg) was dissolved in 1.5 mL of MeOH/MeCN (1:2), 100 μL of 0.1 M OsO_4 was added, and the mixture was stirred at room temperature overnight. The reaction mixture was dried in vacuo, redissolved in a small amount of MeOH, and directly subjected to the FABMS/MS analysis.

Preparation of MTPA Esters. Compound **1** (5.0 mg) was dissolved in 0.5 mL of 10% HCl/MeOH, and the solution was allowed to stand at 70 °C overnight. The solvent was removed in a stream of N_2 gas, and half of the residue was dissolved in a mixture of CH_2Cl_2/pyridine (5:1, 0.6 mL). A catalytic amount of DMAP and 15 μL of (*R*)-MTPACl were added to this solution. The mixture was kept at room temperature for 4 h, and the reaction was quenched by addition of MeOH (4 mL) and dried in vacuo. The residue was chromatographed on a silica gel column with hexane/ethyl acetate (10:1) to yield (*S*)-MTPA ester (*S*)-**3**. The (*R*)-MTPA ester (*R*)-**3** was obtained in the same manner.

(*R*)-3: ^{1}H NMR ($CDCl_3$) δ 7.60−7.40 (5H, m, MTPA), 5.84 (1H, t, J = 2.5 Hz, H-2), 5.33 (2H, t, J = 4.8 Hz, H-17, H-18), 3.83 (3H, s, 1-OMe), 3.64 (3H, s, MTPA), 2.18 (2H, dt, J = 2.3, 7.1, 7.1 Hz, H-5), 1.99 (4H, q, J = 6.5 Hz, H-16, H-19), 1.46 (2H, quint., J = 7.1, H-6), 1.23 (methylene envelope), 0.86 (3H, t, J = 6.9 Hz, H-24).

(*S*)-3: ^{1}H NMR ($CDCl_3$) δ 7.58−7.40 (5H, m, MTPA), 5.87 (1H, t, J = 2.3 Hz, H-2), 5.33 (2H, t, J = 5.0 Hz, H-17, H-18), 3.79 (3H, s, 1-OMe), 3.59 (3H, s, MTPA), 2.22 (2H, dt, J = 2.3, 7.3, 7.3 Hz, H-5), 2.00 (4H, q, J = 6.5 Hz, H-16, H-19), 1.49 (2H, quint., J = 7.3, H-6), 1.23 (methylene envelope), 0.86 (3H, t, J = 6.9 Hz, H-24).

$\Delta\delta$ values for the MTPA ester 3: −0.038 (OMe-1), +0.036 (H-5), +0.032 (H-6).

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