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Novel Ceramide 1-Sulfates, Potent DNA Topoisomerase I Inhibitors Isolated from the Bryozoa *Watersipora cucullata*

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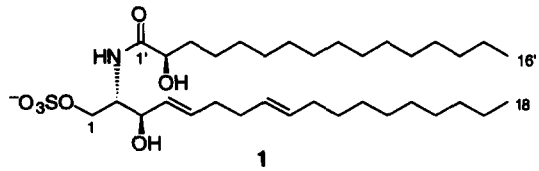
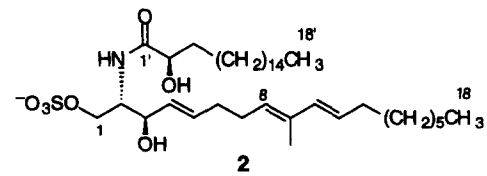
Abstract: Two ceramide 1-sulfates **1** and **2** have been isolated from the Japanese Bryozoa *Watersipora cucullata* as new potent inhibitors of a human DNA topoisomerase I. The gross structures of **1** and **2** were determined by spectroscopic analysis, and their absolute stereochemistry was elucidated by the optical data of degradation products. © 1997 Elsevier Science Ltd.

DNA topoisomerase I (topo I) is a nuclear enzyme that controls the topological state of DNA and is implicated in various genetic processes including replication, transcription, and recombination.[1] Since this enzyme has been recognized as a principal target of the anticancer drugs camptothecins,[2] topo I-targeted chemotherapy of human cancers has become of interest in recent years.[3] At present, the camptothecins are the major specific inhibitors of topo I and reports on other types of inhibitors from natural sources are limited.[4] In the course of our screening studies on new topo I inhibitors from marine organisms,[5] a group of ceramide 1-sulfates has been isolated as potent topo I inhibitors from the Japanese Bryozoa *Watersipora cucullata*. Ceramide 1-sulfates, though known as synthetic compounds,[6] have not yet been isolated as natural products. In this paper we describe the isolation and structural elucidation of two novel ceramide 1-sulfates.



1



2

The animal *W. cucullata* (5.9 kg, wet wt), collected in Aichi Prefecture, Japan, was extracted with MeOH. The MeOH extract was subjected to solvent partition to afford EtOAc, BuOH, and aqueous fractions. The BuOH fraction, which exhibited topo I inhibitory activity with an IC_{50} of 53 μg/mL, was chromatographed on Sephadex LH20 ($CHCl_3$/MeOH step gradient) and then silica gel (i. $CHCl_3$/acetone/MeOH step gradient; ii. toluene/EtOAc/MeOH step gradient) to give a mixture of ceramide 1-sulfates (IC_{50} = 2 μg/mL). The mixture was further separated by reversed-phase HPLC (90:6:4 MeOH/MeCN/H_2O, 20 mM NH_4OAc, pH 7) to afford two ceramide 1-sulfates **1** and **2**, which contained a slight amount of impurities. Each compound was rechromatographed by reversed-phase HPLC under similar conditions with pH 4.5 to give pure **1** (1.6×10^{-3}% yield based on wet weight) and **2** (7.8×10^{-4}% yield) as white powders. These compounds **1** and **2** showed the inhibitory activity against a human topo I with IC_{50}s of 0.4 and 0.2 μM, respectively.[7]

Ceramide 1-sulfate **1**, $[\alpha]^{25}_D$ +17 (*c* 0.064, MeOH), has the molecular formula of $C_{34}H_{65}NO_7S$ (free acid), which was determined by high-resolution negative FABMS [*m/z* 630.4415 $(M-H)^-$, Δ +1.1 mmu]. We

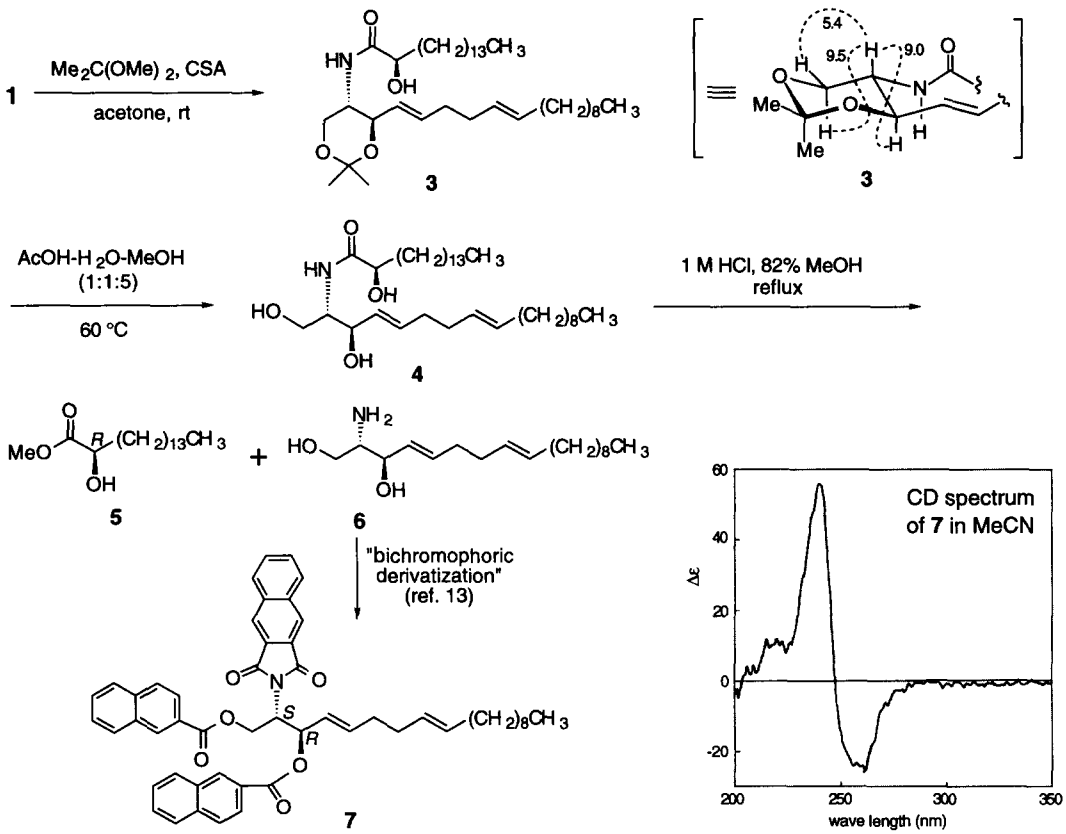
Table 1. NMR Data for Ceramide 1-Sulfates **1** and **2** in Methanol-d_4.

Position	1		2	
	$^1H^a$	$^{13}C^b$	^1H	^{13}C
1	4.11 dd (10.3, 2.9) 4.28 dd (10.3, 4.9)	67.6 t	4.12 br d (10.0) 4.28 dd (10.0, 3.8)	67.6 t
2	4.00 m	54.0 d	4.02 m	54.1 d
3	4.12 m	72.6 d	4.14 m	72.6 d
4	5.47 dd (15.3, 7.4)	131.0 d	5.50 dd (15.0, 7.0)	131.3 d
5	5.74 br d (15.3)	134.5 d	5.75 dt (15.0, 6.4)	134.4 d
6	2.06 br s	33.7 t[c]	2.10 m	33.5 t
7	2.06 br s	33.3 t[c]	2.21 m	28.7 t
8	5.42 br s	130.6 d[d]	5.35 t (7.0)	130.3 d
9	5.42 br s	131.9 d[d]	-	135.2 s
10	1.98 m	33.6 t	6.03 d (15.5)	136.1 d
11	1.2-1.4 m	30.3-33.0 t	5.55 dt (15.5, 7.0)	128.5 d
12	1.2-1.4 m	30.3-33.0 t	2.07 m	33.9 t
13	1.2-1.4 m	30.3-33.0 t	1.40 m	30.3-33.1 t
14-16	1.2-1.4 m	30.3-33.0 t	1.2-1.4 m	30.3-33.1 t
17	1.32 m	23.7 t	1.32 m	23.7 t
18	0.90 t (6.6)	14.4 q	0.90 t (6.5)	14.4 q
1'	-	177.1 s	-	177.1 s
2'	3.98 dd (7.8, 3.7)	73.0 d	4.00 m	73.0 d
3'	1.53, 1.69 m	35.7 t	1.55, 1.70 m	35.8 t
4'	1.40 m	26.2 t	1.40 m	26.2 t
5'-14'	1.2-1.4 m	30.3-33.0 t	1.2-1.4 m	30.3-33.1 t
15'	1.32 m	23.7 t	1.2-1.4 m	30.3-33.1 t
16'	0.90 t (6.6)	14.4 q	1.2-1.4 m	30.3-33.1 t
17'	-	-	1.32 m	23.7 t
18'	-	-	0.90 t (6.6)	14.4 q
9-Me	-	-	1.71 s	12.8 q

[a] Recorded at 400 MHz. Coupling constants in Hz are in parenthesis. [b] Recorded at 100 MHz. [c,d] Signals are interchangeable within the same superscripts.

first assumed the alternative molecular formula of $C_{34}H_{66}NO_7P$ for **1** because of the close values of the exact molecular weights between two possible molecular formulae. However, the presence of a sulfur atom was evidently demonstrated not only by FABMS data (high-resolution MS measured at the resolution of 10000 and the relative intensities of isotope peaks) but also by elementary analysis.[9] The intense IR bands at 1230 and 1070 cm^{-1} suggested the existence of a sulfate group. The presence of a secondary amide group was suggested by IR bands at 1640 and 1540 cm^{-1} and a ^{13}C NMR signal at δ 177.1. The strong signal at δ 1.2-1.4 in the ^1H NMR spectrum indicates that **1** is a lipid. Analysis of COSY and ^{13}C-^1H COSY spectra of **1** allowed us to assign ^1H and ^{13}C chemical shifts (Table 1) and to construct a ceramide-like structure that comprised an 8,9-dehydrosphingosine base and a saturated α-hydroxy fatty acid. The location of the sulfate group was determined to be at the C1 position from low-field shifts of the methylene signals of H1 (δ 4.28 and 4.11) and C-1 (δ 67.6).[10] The carbon chain length of two lipid components in **1** was clarified by the observation of a strong fragment peak at *m/z* 376 in the linked scan negative FABMS spectrum, which corresponded to the sphingosine 1-sulfate part resulting from amide bond cleavage. This was also confirmed by degradation experiments (vide infra).

The absolute stereochemistry of **1** was elucidated by chemical derivatization (Scheme 1). The sulfate group of **1** was easily removed by treatment with 2,2-dimethoxypropane under acidic conditions to furnish acetonide **3**. The relative stereochemistry of C2-C3 in **3** was determined to be *anti* on the basis of vicinal coupling constants (in Hz) of **3** as shown in Scheme 1. Acid hydrolysis of **3** afforded ceramide **4**, in which the



Scheme 1. Chemical derivatization of ceramide 1-sulfate **1**

signals of H1 were observed at higher fields (δ 3.97 and 3.73) than those of **1**, supporting that the sulfate group in **1** was connected to the C1 position. Acid hydrolysis of **4** under the conditions reported for cleaving ceramides[11] yielded methyl 2-hydroxyhexadecanoate (**5**) and 8,9-dehydrosphingosine (**6**). The absolute configuration of **5** was determined to be *R* from its specific rotation.[12] A chemical and circular dichroic method was developed by Nakanishi and coworkers for the microscale determination of the absolute configuration of sphingoid bases.[13] We applied this method to determine the absolute stereochemistry of **6**. Thus, naphthimide formation at the amino group of **6** followed by naphthoylation of two hydroxyl groups afforded the bichromophoric derivative **7**. The CD spectrum of **7** (Scheme 1) indicated a negative cotton effect similar to that reported for a D-*erythro* sphingosine derivative,[14] establishing the D-*erythro* (2*S*,3*R*) configuration of the sphingosine part of **1**. From these findings the stereostructure of **1** was determined as shown in the formula.

The compound **2**, $[\alpha]^{25}_{D}$ +16 (*c* 0.05, MeOH), was considered to be a congener of **1** by the comparison of the NMR (Table 1) and IR spectra between **1** and **2**. The presence of a sulfur atom and the molecular formula of $C_{37}H_{69}NO_7S$ was determined by high-resolution FABMS data [*m/z* 670.4724 $(M\text{-}H)^-$, Δ +0.8 mmu] and elementary analysis. The COSY experiment of **2** revealed the presence of two substructures C1–9-Me and C10–C18. The observation of an 8% NOE between H8 and H10 suggests the connectivity of the above substructures and the *E* geometry of the trisubstituted olefin at C8-C9. The trisubstituted butadiene structure was supported by

a UV band at 235 nm. The carbon chain length of two lipid components was determined by the observation of an intense fragmentation at the amide bond giving the *m/z* 388 peak (sphingoshine 1-sulfate part) in a linked-scan negative FABMS experiment. Both **1** and **2** show quite similar spectral data (specific rotations and ^{1}H NMR signals of H1, H2, and H3), suggesting that the absolute stereochemistry of **2** is identical with that of **1**.

It is reported that certain lipids bind to topo I and inhibit its DNA binding activity,[15] whereas an intermediate (enzyme-DNA cleavable complex) is known to accumulate upon the topo I inhibition by camptothecin.[2] The mode of action of ceramide 1-sulfates **1** and **2**, though unclear yet, seems to be the same as that of the lipid-type inhibitors judging from the structural similarity. Since, among the lipid-type inhibitors reported so far, **1** and **2** are most potent inhibitors, the inhibition mechanism, specificity, and other biological activities are of interest in connection with cancer chemotherapy.

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