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New Sulfoalkylresorcinol from Marine-derived Fungus, *Zygosporium* sp. KNC52

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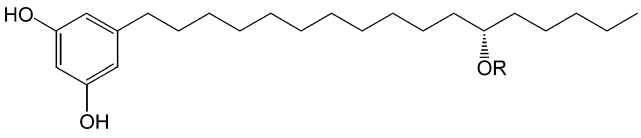
Abstract A new sulfoalkylresorcinol (**1**) was isolated from the marine-derived fungus, *Zygosporium* sp. KNC52. The structure of **1** was elucidated by spectroscopic methods including MS and NMR, and the absolute stereochemistry was determined by the modified Mosher's method. Compound **1** inhibited FtsZ polymerization *in vitro* and exhibited weak antimicrobial activity against multi-drug-resistant bacteria.

Keywords marine-derived fungus, sulfoalkylresorcinol, FtsZ

Marine microorganisms have recently gained attention as important sources of biologically active secondary metabolites for the development of new pharmaceutical agents [1]. In particular, marine-derived fungi have shown great potential as suggested by the diversity of secondary metabolites [2]. We have been searching for novel bioactive substances, including antitumor and antibacterial agents focusing on marine-derived fungi as the screening source [3,4]. In the course of screening for antimicrobial substances targeting FtsZ, which is a structural homolog of eukaryotic tubulin and has an important function in bacterial cell division [5,6], we found that an extract of marine-derived fungus *Zygosporium* sp. KNC52 inhibited FtsZ polymerization *in vitro*. A new sulfoalkylresorcinol (**1**) was isolated from a culture extract of this fungus. Although alkylresorcinols can be found in many different living organisms such as lower and higher plants, algae, fungi, bacteria, and animals, and are important in many aspects of cellular biochemistry and physiology [7], compound **1** is the sole alkylresorcinol derivative that has a sulfoalkyl side chain. This paper describes the structural determination of **1**, including its absolute stereochemistry, and its inhibitory activity against FtsZ polymerization and antimicrobial activity.

The **1**-producing fungus, *Zygosporium* sp. KNC52, was originally isolated from a hard coral in Republic of Palau. The fungus was cultured at 25°C for 14 days on an agar medium containing glucose 0.5%, glycerol 2.0%, yeast extract 0.2%, Pharmamedia® (Traders Protein) 2.0%, NaCl 0.25%, and agar 1.5%, adjusted to pH 6.5 before autoclaving. The cultured agar medium was extracted with



Sulfoalkylresorcinol(**1**): R=SO_3H
1,3-Dihydroxy-5-(12-hydroxyheptadecyl)benzene(**2**): R=H

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Me_2CO, and the resulting extract was filtered and concentrated *in vacuo*. The concentrated aqueous residue of the Me_2CO extract was partitioned with EtOAc. Inhibitory activity against FtsZ polymerization was found in the organic fraction; this fraction was concentrated to dryness and chromatographed on silica gel column, monitoring the inhibitory activity. The active fractions were combined and further purified by HPLC. Compound **1** (20 mg) was obtained as a colorless oil from 4.0 liters of the agar culture.

The molecular formula of **1** was determined to be $C_{23}H_{40}O_6S$ by HRFAB-MS ($[M+H]^+$ *m/z* 445.2591, calcd 445.2624), which was consistent with ^{1}H- and ^{13}C-NMR data. The intense IR absorption bands at 1400 and 1208 cm^{-1} suggested the presence of a sulfate moiety, and the negative ion ESI-MS/MS experiment, showing the unsulfated-fragment ion peak at *m/z* 363 ($[M-HSO_3]^-$) derived from the mother ion peak at *m/z* 443($[M-H]^-$). The fragment ion signal of *m/z* 97 (HSO_4^-) in the negative ESI-MS also indicated the presence of a sulfate group.

The ^{1}H-NMR spectrum indicated the presence of three meta-coupled aromatic protons at δ 6.01 (d, J=2.3 Hz, 2H) and δ 5.99 (t, J=2.3 Hz, 1H), and it also showed the signals due to a long alkyl side chain at around δ 1.2~1.5, a terminal methyl at δ 0.85 (t, J=6.8 Hz, 3H), an arylic methylene at δ 2.35 (t, J=7.5 Hz, 2H), and a sulfate-bearing methine proton at δ 4.00 (quintet, J=6.0 Hz, 1H). Interpretation of the ^{1}H-^{1}H COSY, HSQC and HMBC spectral data for **1** led to a gross structure of 1,3-dihydroxy-5-(12-sulfoheptadecyl)benzene. The detailed NMR data are listed in Table 1.

The unsulfated-derivative of **1**, 1,3-dihydroxy-5-(12-hydroxyheptadecyl)benzene (**2**) was incidentally produced during the NMR measurement of **1**. In the long-term NMR measurement of **1** (10 mM in DMSO-d_6), the signal of a sulfate methine proton at δ 4.00 decreased, and a new methine signal appeared at δ 3.34 (m). After 4 days, the former signal was completely replaced by the latter. This hydrolysis must have been caused by residual TFA contained in the HPLC solvent since neutralization (1 M

Table 1 NMR data of sulfoalkylresorcinol (**1**) and 1,3-dihydroxy-5-(12-hydroxyheptadecyl)banzene (**2**) (DMSO-d_6, 750 MHz for ^{1}H and 125 MHz for ^{13}C)

1					**2**				
Position	δ^{13}C		δ^1H		Position	δ^{13}C		δ^1H	
1	158.20	C	—		1	158.10	C	—	
2	100.01	CH	5.99	t, 2.25	2	99.91	CH	6.00	t, 2.25
3	158.20	C	—		3	158.10	C	—	
4	106.31	CH	6.01	d, 2.25	4	106.25	CH	6.01	d, 2.25
5	144.18	C	—		5	144.15	C	—	
6	106.31	CH	6.01	d, 2.25	6	106.25	CH	6.01	d, 2.25
1′	35.28	CH_2	2.35	t, 7.5	1′	35.22	CH_2	2.35	t, 7.5
2′	30.67	CH_2	1.47	m	2′	30.62	CH_2	1.47	m
3′	29.22*	CH_2	1.18~1.28$^{\$}$		3′	29.15*	CH_2	1.20–1.34$^{\$}$	
4′	29.04*	CH_2	1.18~1.28$^{\$}$		4′	29.02*	CH_2	1.20–1.34$^{\$}$	
5′	29.04*	CH_2	1.18~1.28$^{\$}$		5′	28.98*	CH_2	1.20–1.34$^{\$}$	
6′	29.02*	CH_2	1.18~1.28$^{\$}$		6′	28.95*	CH_2	1.20–1.34$^{\$}$	
7′	29.02*	CH_2	1.18~1.28$^{\$}$		7′	28.95*	CH_2	1.20–1.34$^{\$}$	
8′	28.89*	CH_2	1.18~1.28$^{\$}$		8′	28.84*	CH_2	1.20–1.34$^{\$}$	
9′	28.66*	CH_2	1.18~1.28$^{\$}$		9′	28.62*	CH_2	1.20–1.34$^{\$}$	
10′	24.52	CH_2	1.25$^{\#}$	m	10′	25.17	CH_2	1.24$^{\#}$, 1.35$^{\#}$	m
11′	33.83**	CH_2	1.42, 1.46	m	11′	37.12**	CH_2	1.26$^{\$}$, 1.30$^{\$}$	m
12′	76.16	CH	4.00	quint, 6.00	12′	69.49	CH	3.34	m
13′	33.78**	CH_2	1.42, 1.46	m	13′	37.10**	CH_2	1.26$^{\$}$, 1.30$^{\$}$	m
14′	24.18	CH_2	1.25$^{\#}$	m	14′	24.86	CH_2	1.24$^{\#}$, 1.35$^{\#}$	m
15′	31.47	CH_2	1.20	m	15′	31.43	CH_2	1.22	m
16′	22.09	CH_2	1.26	m	16′	22.09	CH_2	1.27	m
17′	13.93	CH_3	0.85	t, 6.75	17′	13.88	CH_3	0.85	t, 7.5

*: interchangeable, $^{\#}$, $^{\$}$: overlapping, **: interchangeable.

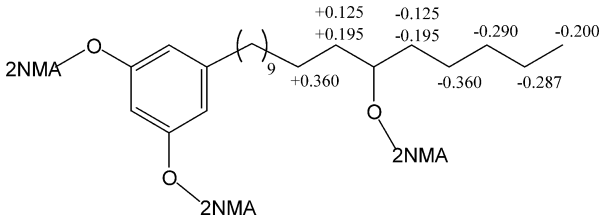

Fig. 1 ($\delta_R - \delta_S$) values obtained for the 2NMA esters of **2**.

NaOH) of the NMR solvent did not bring such a change. Compound **2**, $C_{23}H_{41}O_3$ (HRFAB-MS: $[M+H]^+$ *m/z* 365.3056, calcd 365.3073), was recovered from the NMR solution by HPLC.

The absolute stereochemistry of **1** was established by applying the modified Mosher's method [8,9] to **2**. Both the *R*- and *S*-2NMA esters of **2** were prepared, and the proton signals around the chiral center of both derivatives were assigned by ^{1}H-NMR, HSQC and HMBC. The $\Delta\delta$ (δ_R–δ_S) values obtained are shown in Fig. 1, indicating that the absolute configuration at C-12 to be *S*.

FtsZ is a structural homolog of eukaryotic tubulin and, similar to tubulin, is a GTPase that polymerizes in a GTP-regulated manner [10]. The inhibitory effects of **1** against FtsZ-GTPase and the polymerization of FtsZ were respectively measured by the methods of Margalit *et al*. [6] and Mukherjee *et al*. [11]. Compound **1** inhibited the GTPase activity of FtsZ by 50% at a concentration of 25 μg/ml, and almost completely inhibited FtsZ polymerization at this concentration. Compound **1** also exhibited mild antimicrobial activity against *Mycobcterium tuberculosis* (MDR-TB), *M. bovis* BCG, *M. avium*, *Pseudomonas aeruginosa* (MDRP), and *Staphylococcus aureus* (MRSA) with respective MIC values of 166, 166, 166, 50, and 12.5 μg/ml, but **1** did not exhibit cytotoxicity toward A549 up to 200 μg/ml.

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