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ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 6294–6296

Cephalimysin A, a potent cytotoxic metabolite from an Aspergillus species separated from a marine fish

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Received 29 May 2007; revised 2 July 2007; accepted 5 July 2007
Available online 30 July 2007

Abstract—Cephalimysin A (**1**) was isolated from a strain of *Aspergillus fumigatus* originally separated from the marine fish *Mugil cephalus*, and its absolute stereostructure was elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and some chemical transformations including the modified Mosher's method. This compound exhibited significant cytotoxicity against cultured P388 cells and HL-60 cells.

Based on the fact that some of the bioactive materials isolated from marine animals have been produced by bacteria, we have focused our attention on new antitumor materials from microorganisms separated from marine organisms.[1–3] As part of this study, we have made a search for antitumor compounds from a strain of *Aspergillus fumigatus* OUPS-T106B-5 originally obtained from the marine fish *Mugil cephalus* and found a new cytotoxic metabolite designated as cephalimysin A (**1**)[4] from the culture broth of this fungal strain. This metabolite exhibited significant cytotoxic activity against the murine P388 leukemia cell line and the human HL-60 leukemia cell line. We describe herein the absolute stereostructure and biological activities of **1**.

The microorganism from *M. cephalus* fish was cultured at 27 °C for 6 weeks in a medium (50 l) containing 1% soluble starch and 0.1% casein in 50% artificial seawater adjusted to pH 7.4. After incubation, the AcOEt extract of the culture filtrate was purified by bioassay-directed fractionation (cytotoxicities against P388 cell line) employing stepwise combination of Sephadex LH-20, silica gel column chromatography, and reverse phased HPLC to afford cephalimysin A (**1**) as pale yellow oil.

Cephalimysin A (**1**) had the molecular formula $C_{22}H_{25}NO_6$ established by the $[M+Na]^+$ peak in HRFABMS. The UV spectrum of cephalimysin A (**1**) exhibited absorption bands characteristic of a conjugated carbonyl group and a phenyl group. In addition, IR spectrum exhibited absorption bands at 3330, 2195, 1733, and 1717 cm^{-1}, characteristic of a hydroxy group and an amide. A close inspection of the ^1H and ^{13}C NMR spectra of **1** (Table 1) by DEPT and ^1H-^{13}C correlation spectroscopy (HSQC) experiments revealed the presence of one primary methyl (C-15), one olefinic methyl (C-16), one methoxyl group (8-OCH_3), three sp^3-hybridized methylenes (C-10, C-11, and C-14), one oxygen-bearing sp^3-methine (C-9), two oxygen-bearing quaternary sp^3-carbon (C-5 and C-8), seven sp^2-methine (C-12, C-13, C-19, C-20, C-21, C-22, and C-23), three quaternary sp^2-carbon (C-2, C-3, and C-18) including one oxygen-bearing quaternary carbon (C-2), two conjugated carbonyl groups (C-4 and C-17), one amido (C-6 and N-7) and one hydroxy group. The ^1H–^1H COSY analysis of **1** led to three partial structural units (C-9–9-OH, C-10–C-15, and C-19–C-23) as shown by bold-faced lines in Figure 1. These results were supported by HMBC correlations. The geometrical configuration of the double bond moieties (C-12–C-13) was deduced as trans from the coupling constants of the olefinic protons ($J_{12,13} = 15.1$ Hz) and NOEs (H-14/H-12 and H-11/H-13). The connection of these units and the remaining functional groups was determined on the basis of the key HMBC correlations summarized in Figure 1, and the planar structure of **1** was elucidated.

Since the relative stereochemistry for **1** could not be deduced from NOESY experiments, compound **1** was derived to the acetonide of the product which was obtained by the reduction of **1** as is shown in Scheme 1. Compound **1** was first reduced with $NaBH_4$

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doi:10.1016/j.tetlet.2007.07.024

Table 1. NMR spectral data of cephalimysin A (**1**) in $CDCl_3$

Position	δ_H[a]	*J*/Hz	^1H–^1H COSY	δ_C	HMBC (C)[b]
2				190.54 (s)	
3				111.06 (s)	
4				197.11 (s)	
5				91.52 (s)	
6				165.75 (s)	
7	7.41 br s				5, 6, 8, 9
8				89.62 (s)	
9	4.59 d	12.5 (9-OH)	9-OH	74.06 (d)	4, 5, 8, 17
10	2.62 m		11	29.36 (t)	2, 3, 11, 12
11	2.35 m		10, 12	29.04 (t)	2, 10, 12, 13
12	5.40 dt	15.1 (13), 6.9 (11)	11, 13	126.04 (d)	10, 13, 14
13	5.54 dt	15.1 (12), 6.2 (14)	12, 14	134.36 (d)	11, 12, 14, 15
14	1.97 m		13, 15	25.44 (t)	12, 13, 15
15	0.93 t	7.1 (14)	14	13.61 (q)	13, 14
16	1.68 s			5.67 (q)	2, 3, 4
17				194.58 (s)	
18				132.41 (s)	
19	8.31 d	7.9 (20)	20	130.55 (d)	17, 20, 21
20	7.48 t	7.9 (19, 21)	19, 21	128.67 (d)	18,19, 21, 22
21	7.64 t	7.9 (20, 22)	20, 22	134.52 (d)	19, 23
22	7.48 t	7.9 (21, 23)	21, 23	128.67 (d)	18, 20, 21, 23
23	8.31 d	7.9 (22)	22	130.55 (d)	17, 21, 22
9-OH	4.06 d	12.5 (9)	9		8, 9
8-OCH_3	3.40 s			51.60 (q)	8

[a] ^1H chemical-shift values (δ ppm from $SiMe_4$) followed by multiplicity and then the coupling constants (*J*/Hz). Figures in parentheses indicate the proton coupling with that position.

[b] Long range ^1H–^{13}C correlations from H to C observed in the HMBC experiment.

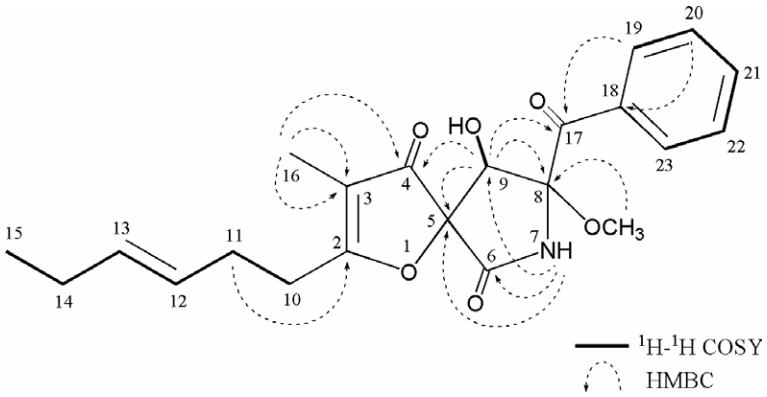
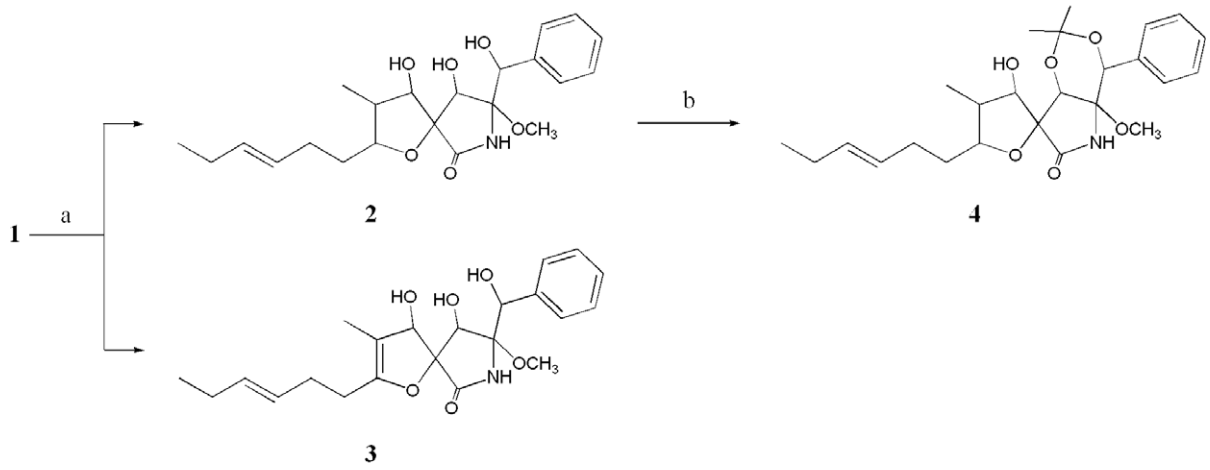


Figure 1. Selected ^1H–^1H COSY and HMBC correlations in cephalimysin C (**1**).

in the presence of $CeCl_3$ to give **2** and **3** (yields 21% and 58%, respectively). The reaction mixture was diluted with water and extracted with AcOEt, and the AcOEt extract was chromatographed on ODS HPLC, and no diastereoisomer of the reduction product except 2 and 3 was detected. The mechanism for the stereoselectivity of this reduction is not be revealed, but it is suggested that $CeCl_3$ plays a catalytic role as Lewis acid since the reduction of 1 without $CeCl_3$ gave some diastereoisomers of the reduction products. These products were further treated to obtain their acetonides with the aim of deducing the relative configuration for the benzyl moiety (C-17–C-23). Compound **2** was treated with 2,2-dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate to give **4**.[5] However, acetonide could not be derived from compound **3**. In NOESY experiments for **4**, NOEs for H-2/H-3, H-2/H-4, H-2/H-9, H-3/H-4, and H-3/H-9 (Fig. 2) suggested that H-2 is arranged cis to H-3, H-4, and C-5–C-9 bond in A ring. In addition, NOEs were observed from one of the isopropylidene methyl group (δ_H 1.40) to both H-4 and H-17 and from another of the isopropylidene methyl group (δ_H 1.52) to both H-9 and 8-OCH_3. From this evidence, C-4–C-5 bond is arranged trans to both H-9 and 8-OCH_3in B ring. Thus, the relative stereostructure of **4** was elucidated as shown in Figure 2. Therefore the relative stereostructure of cephalimysin A (**1**) was determined. The modified Mosher's method[6] was applied to compound **4** for determination of the absolute configuration of cephalimysin A (**1**). The ^1H chemical-shift differences between the (*R*)- and (*S*)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid (MTPA) esters **5a** and **5b** of compound **4** are shown in Figure 3. The result suggested 4*R* configuration, and hence allowed assignment



Scheme 1. Reagents and conditions: (a) $CeCl_3$, $NaBH_4$, MeOH, 20 °C, 2 h; (b) 2,2-dimethoxypropane, PPTS, 20 °C, 1 h.

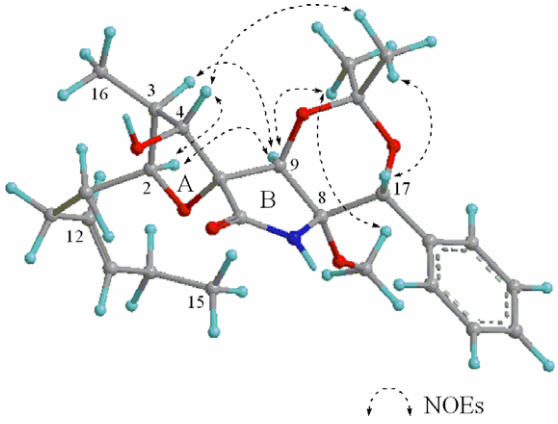


Figure 2. Key NOEs correlations in **4** (graphical representation using the program CHEM 3D).

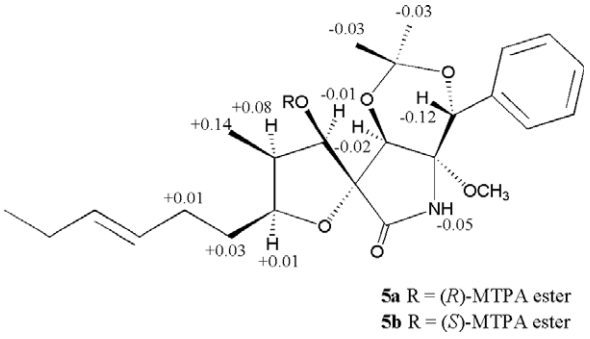


Figure 3. ^1H chemical-shift differences ($\Delta\delta = \delta_S - \delta_R$) between the (*R*)- and (*S*)-MTPA esters (**5a** and **5b**) of acetonide **4**.

of absolute stereostructure **4** with the 2*S*, 3*S*, 4*R*, 5*R*, 8*S*, 9*R*, and 17*R* configuration. The above evidence led to the absolute stereostructure for cephalimysin A (**1**).

The cancer cell growth inhibitory properties of cephalimysin A (**1**) were examined using the murine P388 leukemia cell line and the human HL-60 leukemia cell line. This compound **1** exhibited significant cytotoxic activity against P388 and HL-60 cell lines (IC_{50} 15.0 and 9.5 nM, respectively).

In future, cancer cell growth inhibitory properties of cephalimysin A (**1**) will be examined using a disease-oriented panel of 39 human cell lines and molecular target inhibitory activities of the substance will also be tested using protein kinase, histone deacetylase, farnesyl transferase, telomerase, and proteasome.

Acknowledgments

We thank Dr. T. Ito (National Institute of Technology and Evaluation, Biological Resource Center) for the identification of the fungal strain. We are grateful to Ms. M. Fujitake and Dr. K. Minoura of this university for the MS and NMR measurements, respectively.

References and notes

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3. Iwamoto, C.; Yamada, T.; Ito, Y. M.; Minoura, K.; Numata, A. *Tetrahedron* **2001**, *57*, 2997–3004.
4. Cephalimysin A (**1**): pale yellow oil, $[\alpha]_D$ +3.5 (*c* 0.11, EtOH); UV λ_{max} (EtOH)/nm: 208 (log ε 3.88), 252 (3.95), 277 (3.74); IR ν_{max} (liquid)/cm^{-1}: 3330, 2915, 1733, 1717, 1684; HRFABMS *m/z*: 422.1578 $[M+Na]^+$ (calcd for $C_{25}H_{35}NO_6Na$: 422.1579).
5. Acetonide derivative **4**: pale yellow oil, HRFABMS *m/z*: 468.2356 $[M+Na]^+$ (calcd for $C_{22}H_{25}NO_6Na$: 468.2358); ^1H NMR (500 MHz, $CDCl_3$, δ_H): 0.94 (t, $J = 7.2$ Hz, H-15), 1.08 (d, $J = 7.0$ Hz, H-16), 1.40 (s, acetonide–$CH_3\alpha$), 1.48 (dddd, $J = 13.8$, 9.8, 7.0, 3.8 Hz, H-10A), 1.52 (s, acetonide–$CH_3\beta$), 1.75 (dtd, $J = 13.8$, 10.5, 5.1 Hz, H-10B), 1.98 (m, H-14), 2.13 (m, H-11), 2.39 (quint d, $J = 7.0$, 5.8 Hz, 1H), 3.07 (s, 3H), 4.15 (s, H-9), 4.53 (d, $J = 5.0$ Hz, 8-OH), 4.68 (t, $J = 5.0$ Hz, H-4), 4.84 (s, H-17), 5.41 (dt, $J = 15.1$, 6.0 Hz, H-12), 5.45 (dt, $J = 15.1$, 6.0 Hz, H-13), 7.29–7.31 (m, 3H Ar), 7.47–7.49 (m, 2H Ar).
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