J. Antibiot. 59(9): 591–596, 2006

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Paecilaminol, a New NADH-Fumarate Reductase Inhibitor, Produced by *Paecilomyces* sp. FKI-0550

Hideaki Ui, Kazuro Shiomi, Hideaki Suzuki, Hiroko Hatano, Hiromi Morimoto, Yuichi Yamaguchi, Rokuro Masuma, Kimitoshi Sakamoto, Kiyoshi Kita, Hideto Miyoshi, Hiroshi Tomoda, Haruo Tanaka, Satoshi Ōmura

Received: July 12, 2006 / Accepted: August 24, 2006

Abstract A new NADH-fumarate reductase inhibitor, paecilaminol, was isolated from the cultured broth of a fungus *Paecilomyces* sp. FKI-0550. It is an amino alcohol compound, the structure being established as 2-amino-14,16-dimethyl-3-octadecanol. Paecilaminol exhibited an IC_{50} value of 5.1 μM against *Ascaris suum* NADH-fumarate reductase.

Keywords paecilaminol, electron transport enzyme inhibitor, NADH-fumarate reductase

Introduction

Microorganisms produce many useful antiparasitic antibiotics [1]. In the course of screening for anthelmintic antibiotics, we have been interested in the differences in energy metabolisms between the host and helminths [2]. The NADH-fumarate reductase (NFRD) system, which is found in many anaerobic organisms, is part of a special respiratory system in parasitic helminths [3]. The system is composed of complex I (NADH-rhodoquinone oxidoreductase) and complex II (rhodoquinol-fumarate oxidoreductase). Electrons from NADH are accepted by rhodoquinone through complex I, and then transferred to fumarate through complex II. This anaerobic electron transport system can provide ATP in the absence of oxygen. During our screening for inhibitors of NFRD using *Ascaris suum* (roundworm) mitochondria, we obtained both nafuredin and atpenins. Nafuredin is a selective inhibitor of helminth complex I which showed anthelmintic activity *in vivo* [4, 5]. Atpenins are complex II inhibitors, and the inhibition is non-selective between helminths and mammals [6]. They are the most potent complex II inhibitors and are expected to be useful tools for biochemical studies.

Further screening for NFRD inhibitors led to the isolation of a new compound, paecilaminol (**1**, Fig. 1), which was produced by a cultured broth of *Paecilomyces* sp. FKI-0550 [7]. In this report, we describe the taxonomy of the producing strain and the fermentation, isolation, structure elucidation, and biological properties of **1**.

K. Shiomi (Corresponding author)**, S. Ōmura** (Corresponding author)**, H. Ui, R. Masuma:** Kitasato Institute for Life Sciences and Graduate School of Infection Control Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, E-mail: shiomi@lisci.kitasato-u.ac.jp and omura-s@kitasato.or.jp
H. Morimoto, H. Tomoda: School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan
K. Shiomi, H. Suzuki, H. Hatano, Y. Yamaguchi, H. Tanaka, S. Ōmura: The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan
K. Sakamoto, K. Kita: Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
H. Miyoshi: Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

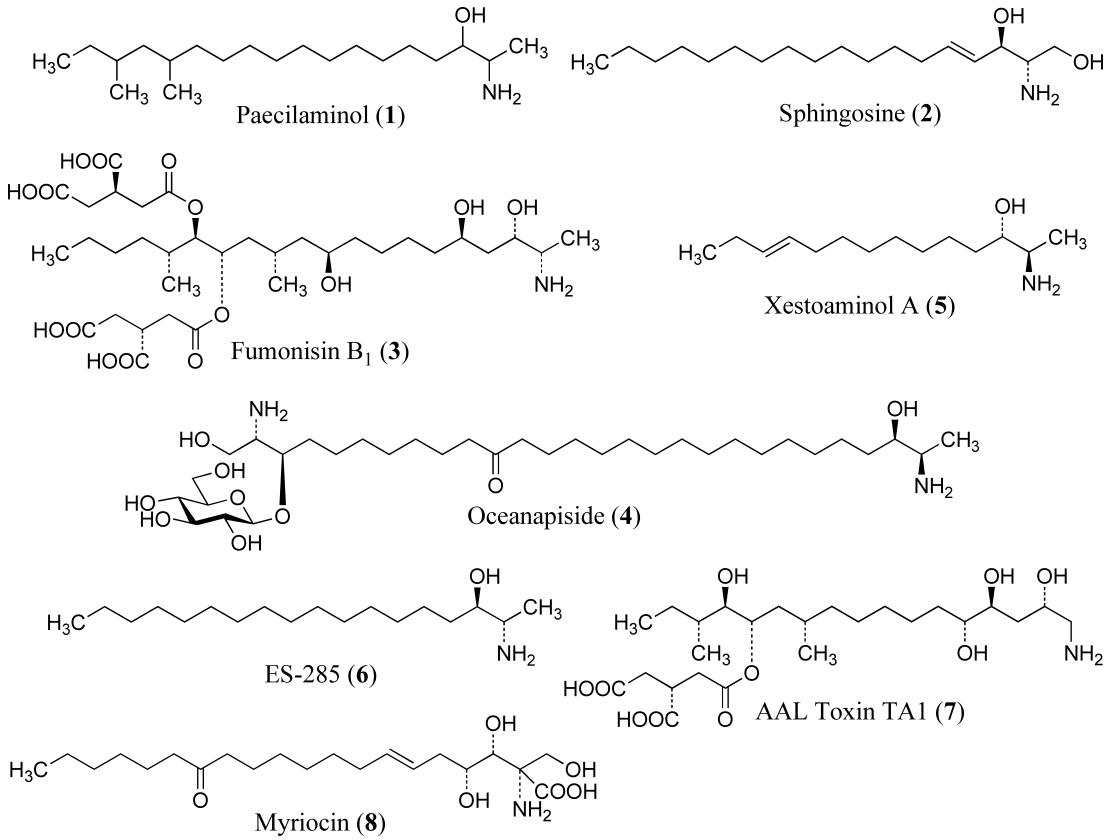

Fig. 1 Structures of paecilaminol (**1**) and related amino alcohols (**2**~**8**).

Results and Discussion

Taxonomy of Producing Strain FKI-0550

Strain FKI-0550 was originally isolated from a soil sample collected on Miyakojima Island, Okinawa Prefecture, Japan. The strain was taxonomically determined as genus *Paecilomyces* sp. The strain has been deposited at the International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan, as FERM BP-7785.

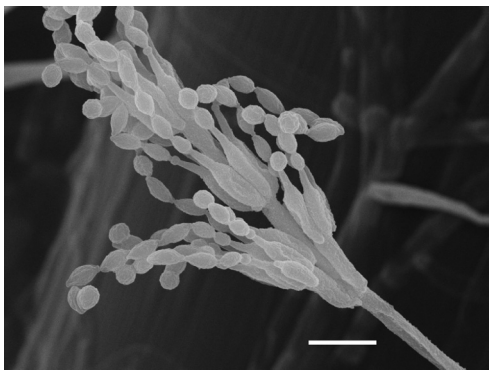

Fig. 2 Scanning electron micrograph of strain FKI-0550. Bar represents 5 μm.

Fermentation and Isolation of Paecilaminol

A stock culture of the strain FKI-0550 was inoculated into two 500-ml Erlenmeyer flasks containing 100 ml of a seed medium and incubated on a rotary shaker at 27°C for 3 days. One milliliter of the seed culture was transferred into each of one hundred 500-ml Erlenmeyer flasks containing 100 ml of a production medium. The fermentation was carried out on a rotary shaker at 27°C for 9 days.

Mycelia were collected from the cultured broth (10 liters) by centrifugation. They were treated with methanol, the extract removed and the methanol was evaporated. The aqueous extract was partitioned with ethyl acetate, and the organic layer was concentrated to dryness *in vacuo* to afford a crude material (461 mg). This was applied on a silica gel column (Merck Art. 7734) and washed with $CHCl_3$ - methanol (10 : 1). Active fractions eluted with $CHCl_3$ - methanol (1 : 1) were concentrated to yield a crude material (120 mg), which was then chromatographed over another silica gel column. The column was washed with

ethyl acetate and eluted with ethyl acetate - methanol - concd NH_4OH (80 : 9 : 1). The elution afforded a pale yellow oil of **1** (70.1 mg).

Structure Elucidation of Paecilaminol

Physico-chemical properties of **1** are summarized in Table 1. The molecular formula of **1** was established as $C_{20}H_{43}NO$ by HR-FAB-MS. The IR absorbances observed at 2958, 2924, 2852, 1464, and 1377 cm^{-1} suggested the presence of saturated methyl and methylene groups. The absorbance at 1574 cm^{-1} suggested an amino group, and the absorbances at 3300 and 1080 cm^{-1} suggested a hydroxyl and/or amino group. As the index of hydrogen deficiency of **1** was 0, **1** was suggested to be an alkyl amino alcohol. This was further supported by the analysis of IR absorbances.

Chemical shifts of **1** in the ^1H and ^{13}C NMR are shown in Table 2. Analysis of the ^1H NMR, ^{13}C NMR, DEPT, and HMQC spectra revealed the presence of four methine, twelve methylene, and four methyl carbons. ^1H-^1H-COSY of **1** indicated the presence of 3-amino-2-hydroxybutyl (C-1 to C-4) and 2,4-dimethylhexyl (C-13 to C-18) moieties (Fig. 3). Long-range couplings from 3-H (δ 3.16) and 4-H_2 (δ 1.33 and 1.46) to C-5 (δ 25.8) suggested the bond between C-4 and C-5, and the couplings from 13-H_2 (δ 1.01 and 1.26) and 14-H (δ 1.45) to C-12 (δ 26.9) suggested the bond between C-12 and C-13 in HMBC. Though the remaining six methylenes could not be assigned as their protons showed almost the same chemical shifts, they should be linked to the two moieties. Thus, the structure of **1** was elucidated as 2-amino-14,16-dimethyl-3-octadecanol (Fig. 1). After our patent publication [7], **1** was reported to be isolated from *Fusarium avenaceum* as a cytotoxic compound [8].

Table 1 Physico-chemical properties of **1**

Appearance		pale yellow oil
$[\alpha]_D^{25}$		+26.7° (*c* 0.24, MeOH)
Molecular formula		$C_{20}H_{43}NO$
Molecular weight		313.57
HR-FAB-MS (*m/z*)	found	314.3424 $(M+H)^+$
	calcd	314.3423 for $C_{20}H_{44}NO$
UV λ_{max}^{MeOH} nm (ε)		203 (8,250), 220 (sh, 4,400), 246 (2,040), 265 (sh, 1,570)
IR ν_{max} (KBr) cm^{-1}		3300, 2958, 2924, 2852, 1574, 1464, 1377, 1080
Solubility	soluble	$CHCl_3$, EtOAc, MeOH, CH_3CN
	insoluble	*n*-hexane
Color reaction	positive	ninhydrin, H_2SO_4

Table 2 ^1H and ^{13}C data (in $CDCl_3$) of **1**[a]

Position	δ_C (mult)	δ_H (int, *J* (Hz))
1	20.9 q	1.09 d (3H, 6.3)
2	51.1 d	2.72 dq (1H, 6.4, 6.3)
3	75.6 d	3.16 m (1H)
4	34.2 t	1.33 m (1H), 1.46 m (1H)
5	25.8 t	1.33 m (1H), 1.46 m (1H)
6	30.03[b] t	1.25 m (12H)
7∼10	29.62 t	
	29.63 t	
	29.65 t	
	29.69 t	
11	29.8[b] t	
12	26.9 t	1.26 m (2H)
13	36.9 t	1.01 m (1H), 1.26 m (1H)
14	30.01 d	1.45 m (1H)
14-CH_3	20.3 q	0.82 d (3H, 6.6)
15	44.7 t	0.88 ddd (1H, 14.0, 7.0, 7.0), 1.20 m (1H)
16	31.6 d	1.40 m (1H)
16-CH_3	19.7 q	0.82 d (3H, 6.6)
17	29.2 t	1.05 m (1H), 1.33 m (1H)
18	11.2 q	0.84 dd (3H, 7.4, 7.4)

[a] NMR spectra were recorded on a Varian Inova 600 spectrometer. Chemical shifts are shown in δ values (ppm) relative to $CDCl_3$ at 7.26 ppm for ^1H NMR and at 77.0 ppm for ^{13}C NMR.
[b] The chemical shifts are interchangeable.

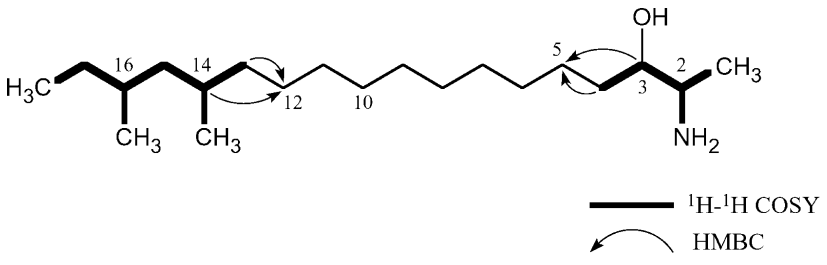

Fig. 3 Selected ^1H-^1H COSY and HMBC correlations of **1**.

Table 3 Inhibition of electron transport enzymes by **1**

Enzyme	Complex	IC_{50} (μM)
NADH-fumarate reductase (*A. suum*)	I+II	5.1
NADH oxidase (bovine heart)	I+III+IV	19.8
NADH-rhodoquinone oxidoreductase (*A. suum* SMP)	I	23
Rhodoquinol-fumarate oxidoreductase (*A. suum* SMP)	II	35
NADH-ubiquinone oxidoreductase (bovine heart SMP)	I	16
Succinate-ubiquinone oxidoreductase (bovine heart SMP)	II	>100
Ubiquinol-cytochrome *c* oxidoreductase (bovine heart SMP)	III	20

Biological Activities of Paecilaminol

Though the *A. suum* NFRD inhibitory activity of **1** was moderate (IC_{50}=5.1 μM, Table 3), the inhibition was about 4 times more potent than that of bovine heart NADH oxidase (complexes I+III+IV). In the screening of NFRD inhibitors, we added 30 mg/ml of bovine serum albumin to the NFRD assay system to eliminate the effect of non-specific inhibition by fatty acids and acylglycerols. It is interesting that bovine serum albumin did not affect the NFRD inhibition of **1** (IC_{50}=3.2 μM). Generally, it reduced NFRD inhibition significantly. In the case of nafuredin, inhibition was reduced about 100-fold by bovine serum albumin.

We evaluated inhibitory activities of **1** against each complex using submitochondrial particles (SMP) of *A. suum* and bovine heart (Table 3). Compound **1** inhibited NADH-rhodoquinone oxidoreductase (complex I) and rhodoquinol-fumarate oxidoreductase (complex II) of *A. suum* SMP in similar concentration. It also inhibited NADH-ubiquinone oxidoreductase (complex I) and ubiquinol-cytochrome *c* oxidoreductase (complex III) of bovine heart SMP in similar concentration. However, the inhibition against bovine succinate-ubiquinone oxidoreductase (complex II) was weak. Therefore, **1** showed similar inhibitory activities against complexes I, II, and III of *A. suum* and bovine heart, except bovine complex II. It is not common for electron transport inhibitors to show such low selectivity. The only group that shows such wide inhibitions are the 2-alkyl-4,6-dinitrophenols, but their inhibitory activity against complex II is also weaker than that for complexes I and III [9]. The low selectivity of **1** may be due to its linear structure, because both amino and hydroxyl groups can freely rotate and be attached to enzymes.

Compound **1** is an amino alcohol, and its structure is similar to sphingosine (**2**), a long-chain base of sphingolipids. However, NFRD inhibition of **2** was very weak (Table 4). Fumonisin B_1 (**3**) [10], a fungal amino alcohol, did not inhibit NFRD at 100 μM. A simple alcohol 2-decanol also showed no inhibition against NFRD at 100 μM.

Table 4 NFRD inhibition by compounds related to **1**

Enzyme	IC_{50} (μM)
Sphingosine (**2**)	28
Fumonisin B_1 (**3**)	>100
2-Decanol	>100

Table 5 Antimicrobial activity of **1**

Microorganisms	MIC (μg/ml)
Staphylococcus aureus ATCC6538P	12.5
Bacillus subtilis ATCC6633	6.25
Micrococcus luteus ATCC9341	6.25
Mycobacterium smegmatis ATCC607	25
Escherichia coli NIHJ	>100
Escherichia coli IFO12734	>100
Pseudomonas aeruginosa IFO3080	>100
Xanthomonas campestris pv. *oryzae* KB88	>100
Candida albicans KF1	>100
Saccharomyces cerevisiae KF26	>100
Aspergillus niger ATCC6275	100
Mucor racemosus IFO4581	100

Nematocidal and insecticidal activities of **1** were studied by a microplate assay using free-living nematode *Caenorhabditis elegans* and brine shrimp *Artemia salina*. Minimum growth inhibitory concentrations of **1** against *C. elegans* and *A. salina* were 20 μg/ml and 5 μg/ml, respectively. As shown in Table 5, it exhibited moderate antimicrobial activity against Gram-positive bacteria.

Though **2** is a 2-aminoalkene with 1,3-diol residues, **1**

lacks 1-hydroxyl residue. Such 2-amino-3-alkanols or 2-amino-3-alkenols have been isolated from natural origins. Most of them were obtained from marine sponges, such as xestoaminols A~C isolated from *Xestospongia* sp. [11] and oceanapiside (**4**) isolated from *Oceanapia phillipensis* [12]. Xestoaminol A (**5**) showed nematocidal, antibacterial, and antifungal activity and inhibited reverse transcriptase [11]. Compound **4** showed antifungal activity [12]. It also exhibits inhibitory activity against a mycobacterial detoxification enzyme, mycothiol-*S*-conjugate amidase [13]. ES-285 (spisulosine 285, **6**) was isolated from the clam *Mactromeris polynyma*, and it disrupts the cytoskeleton of cancer cells [14]. Compound **6** may decrease the activity of the GTP-binding protein Rho. It is currently being evaluated in phase I clinical trials for advanced solid tumors. Fumonisins are fungal 2-amino-3-alkanols produced by *Fusarium verticillioides*. They are toxic and carcinogenic for animals and humans as a contaminant of grains [10, 15]. The analogous compounds, AAL toxins (AAL Toxin TA1=**7**), are 1-amino-2-alkanols and produced by the phytopathogenic fungus *Alternaria alternata* f. sp. *lycopersici* [16]. Both mycotoxins inhibit ceramide synthase (sphingosine *N*-acyltransferase) [15, 17]. Myriocin (ISP-I, **8**) is a 2-aminoalkan-1,3-diol compound produced by fungi, *Myriococcum albomyces* and *Isaria sinclairii* [18, 19]. It is a potent immunosuppressant inhibiting serine palmitoyltransferase, the enzyme that catalyzes the first step of sphingolipid biosynthesis [19, 20]. The derivative of **8**, FTY720, is in phase II clinical trials as an immunosuppressant [21].

As described above, amino alcohols have various biological activities. However, only a few have been reported to have an effect on electron transport enzymes. Galactosylsphingosine (psychosine), glucosylsphingosine, and **2** showed more than 50% inhibition against complex IV (cytochrome *c* oxidase) at 5 μM [22]. Though 3% of bovine serum albumin did not affect the NFRD inhibition of **1**, 1% of human serum albumin completely abolished complex IV inhibition of **2** and its glycosides [22]. As for complex I, *N*-acetylsphingosine and *N*-palmitoyl-sphingosine were reported to inhibit the enzyme [23]. However, the inhibition was weak (IC_{50}=20~40 μM), which is comparable with the NFRD inhibition of **2**. Therefore, **1** is the first amino alcohol that has NFRD inhibitory activity.

Experimental

General

NMR spectra were recorded on a Varian Inova 600 spectrometer ($^{2\text{-}3}J_{CH}$=8 Hz in HMBC). Chemical shifts are shown in δ values (ppm) relative to $CDCl_3$ at 7.26 ppm for ^{1}H NMR and at 77.0 ppm for ^{13}C NMR. Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. The UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. Optical rotations were recorded on a JASCO model DIP-181 polarimeter.

Taxonomic Studies of the Producing Organism

Morphological observations of the paecilaminol producing strain were carried out using an Olympus Vanox-S AH-2 microscope and a JEOL JSM-5600 scanning electron microscope.

Media

The seed medium consisted of glucose 2.0%, Polypepton (Nihon Pharmaceutical Co.) 0.5%, yeast extract (Oriental Yeast Co.) 0.2%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and agar 0.1%, pH 5.7. The production medium consisted of glycerol 2.0%, sucrose 1.0%, ammonium acetate 0.5%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05%, Cultivater #100 (fish extract, Yaizu Suisankagaku Industry Co.) 0.2%, and agar 0.1%, pH 6.0.

Biological Studies

NFRD activity was measured as described previously [24]. The other enzyme assays were performed as described previously [4]. The assay method for nematocidal and insecticidal activities was reported previously [25]. The antimicrobial activity was measured by agar dilution method.

Acknowledgements We are grateful to Dr. Achim Harder of Bayer HealthCare AG, Animal Health-Research & Development-Parasiticides for valuable discussions. We also thank Ms. Akiko Nakagawa and Ms. Chikako Sakabe of School of Pharmaceutical Sciences, Kitasato University for measurements of mass spectra. This work was supported by a Grant-in-Aid for Scientific Research (14593006 to K.S. and 13854011 to K.K.) and a Grant-in-Aid for Encouragement of Young Scientists (12771373 to H.U.). This work was also supported in part by the Grant of the 21st Century COE Program, Ministry of Education, Culture, Sports, Science, and Technology.

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