YM-170320, a Novel Lipopeptide Antibiotic Inducing Morphological Change of Colonies in a Mutant of *Candida tropicalis* pK233

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There is substantial interest in discovering inhibitors of ergosterol biosynthesis in fungi, since ergosterol is an essential constituent of the fungal cytoplasmic membrane. Such inhibitors as the azole antifungals have been clinically useful[1]. Using a mutant of *Candida tropicalis* pK233 strain[2], which produced abnormally shaped colonies when exposed to an inhibitor of ergosterol biosynthesis, we have screened microbial fermentations for novel inhibitors to find that an unidentified fungal strain YL-03706F induced the morphological change of colonies in this mutant, but not in the wild type of *C. tropicalis*. In this report, we describe the fermentation, isolation, structure elucidation, and biological properties.

The producing strain YL-03706F was isolated from a deadwood sample collected in Aomori, Japan. Colonies grew slowly on malt extract agar reaching 20 mm in diameter after two weeks at 24°C. The surface of the colonies was white to gray, and the reverse was colored pale yellowish brown. The strain produced no reproductive structures even on sterilized natural substrates. The absence of morphological characteristics implied that this fungus could be treated as a deuteromycete in the Agonomycetes[3].

YM-170320 (**1**) was produced by solid state fermentation. Vegetative mycelia of strain YL-03706F growing on potato-dextrose agar were used to prepare the fermentation inoculum in a 500-ml Erlenmeyer flask containing 100 ml of a seed medium consisting of glucose 1%, potato starch 2%, yeast extract 0.5%, Polypepton (Nihon Pharmaceutical Co., Ltd.) 0.5% and $CaCO_3$ 0.4%. This seed culture was incubated at 24°C for three days on a rotary shaker at 220 rpm. The production medium consisting of the same components as described above was prepared in distilled water. The pH of the medium was adjusted to 7.0 before sterilization. For inoculation, two ml of the seed culture was mixed with 90 ml of the production medium in a 500-ml Erlenmeyer flask. The inoculated medium was supplemented with 1.4 g of agar in the flask, and then incubated under static condition at 24°C for ten days.

The solid fermentations from 40 flasks were lyophilized and extracted with acetone/H_2O (1 : 1). After removal of the organic solvent, the extract was further extracted with EtOAc. The EtOAc extract (840 mg) was successively separated by silica gel flash chromatography ($CHCl_3$-MeOH) and by ODS flash chromatography (MeOH-H_2O). The active fractions were combined, and then subjected to centrifugal partition chromatography with *n*-hexane/EtOAc/MeOH/H_2O (1 : 1 : 1 : 1) using the upper layer as the mobile phase. Final purification was done by ODS HPLC on an *L*-column (Chemicals Inspection and Testing Inst., Japan) with CH_3CN/H_2O/THF (5 : 4 : 1) to give 3.9 mg of YM-170320 (**1**).

The physico-chemical properties of **1** are listed in

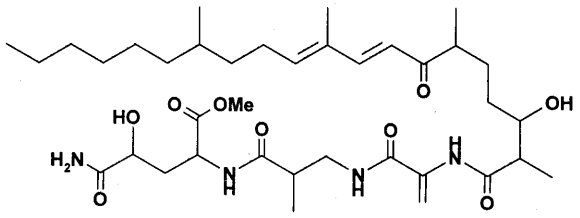

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Table 1. Physico-chemical properties of YM-170320 (**1**).

Appearance	White powder
Molecular weight	706
Molecular formula	$C_{37}H_{62}N_4O_9$
HRFAB-MS (*m/z*)	
Found:	729.4411 ($M+Na^+$)
Calcd:	729.4415
$[\alpha]_D^{25}$	−38.0° (*c* 0.10, MeOH)
UV (MeOH) λ_{max} nm (ε)	214 (9200), 262 (13200)
IR ν_{max} (film) cm^{-1}	3310, 2930, 1740, 1650, 1510, 1460

Table 2. ^{1}H and ^{13}C NMR data of YM-170320 (**1**) in CD_3OD.

	No.	^{13}C	^{1}H
Fatty acid	1	177.2 (s)	
	2	48.8 (d)	2.43 (dq, 7.0, 7.0)
	3	74.3 (d)	3.62 (ddd, 7.0, 7.0, 0.9)
	4	33.3 (t)	1.54 (m)
			1.40 (m)
	5	30.3 (t)	1.73 (m)
			1.59 (m)
	6	45.1 (t)	2.89 (m)
	7	207.0 (s)	
	8	123.8 (d)	6.24 (d, 15.9)
	9	149.8 (d)	7.29 (d, 15.9)
	10	134.5 (s)	
	11	145.5 (d)	6.04 (br t, 7.6)
	12	27.7 (t)	2.23 (m)
	13	37.3 (t)	1.45 (m)
			1.26 (m)
	14	33.8 (d)	1.45 (m)
	15	38.0 (t)	1.34 (m)
			1.15 (m)
	16	30.8 (t)	1.29 (m)
	17	28.1 (t)	1.34 (m)
			1.29 (m)
	18	33.1 (t)	1.31 (m)
	19	23.8 (t)	1.31 (m)
	20	14.5 (q)	0.90 (t, 7.0)
	21	14.9 (q)	1.13 (d, 7.0)
	22	17.2 (q)	1.10 (d, 7.3)
	23	12.3 (q)	1.82 (br s)
	24	20.0 (q)	0.90 (d, 6.7)
*Δ*Ala	CO	166.9 (s)	
	α	137.4 (s)	
	β	107.9 (t)	5.88 (br s)
			5.53 (br s)
*β*Aib	CO	177.2 (s)	
	α	41.4 (d)	2.69 (qt, 6.7, 6.1)
	β	44.1 (t)	3.35 (d, 6.1)
	α-Me	15.6 (q)	1.12 (d, 6.7)
OHGln	CO	173.9 (s)	
	α	50.9 (d)	4.63 (dd, 6.4, 6.4)
	β	37.2 (t)	2.29 (ddd, 14.3, 6.4, 4.6)
			2.01 (ddd, 14.3, 7.6, 6.4)
	γ	69.8 (d)	4.12 (dd, 7.6, 4.6)
	γ-CO	179.6 (s)	
	OMe	49.8 (q)	3.34 (s)

Table 1. **1** had a molecular formula of $C_{37}H_{62}N_4O_9$, which was established by high-resolution FABMS and NMR data. Hydrolysis of **1** with 6N HCl (110°C, 17 hours) followed by standard amino acid analysis[4] revealed the presence of three unusual amino acid residues. Since **1** gave well-resolved ^{1}H NMR signals in CD_3OD (Table 2) rather than in $CDCl_3$ (Table 3), an initial attempt to assign the structure was made using NMR spectra recorded in CD_3OD. Analysis of 2D NMR spectra including COSY, HOHAHA, HSQC, and HMBC led to the assignment of three amino acids as **a**, **b** and **c**, and a C_{20} unsaturated fatty acid as **d** (Fig. 1). Taking into account the molecular formula, the presence of a methoxyl group and a primary amide was inferred, and the structure should be acyclic.

Amino acids **a** and **b** were unambiguously identified to be dehydroalanine (*Δ*Ala) and *β*-aminoisobutyric acid (*β*Aib), respectively. The NMR data for amino acid **c** were similar to those of glutamic acid[5,6] except for the chemical shift of the C-*γ* methine (δ_C 69.8 and δ_H 4.12), suggesting that a hydroxyl group was placed at this position. The (8*E*,10*E*)-geometry of the diene system in **d** was suggested by the size of the proton-proton coupling constant ($^{3}J_{8,9}$ = 15.9 Hz) and the carbon chemical shift of the vinylic methyl on C-10 (δ 12.3).

Although the HMBC spectrum in CD_3OD gave little information connecting these residues, a ROESY (rotating-frame Overhauser enhancement spectroscopy) spectrum in $CDCl_3$ provided correlations with NH protons through all amide bonds (Fig. 2). Unfortunately, any of the HMBC and ROESY spectra measured in CD_3OD or $CDCl_3$ gave no definite information to specify the location of the primary amide and the methoxyl group. Therefore, difference NOE experiments were conducted in $CDCl_3$, in which irradiation of one of the primary amide protons (δ 5.88) gave enhancement of H-*γ* (δ 4.20) in **c** (Fig. 2), strongly suggesting that the primary amide was placed adjacent to C-*γ* in **c** to form *β*-hydroxyglutamine (OHGln). By default, the remaining methoxyl group should be attached to the *α*-carbonyl of OHGln to establish the overall structure of **1**. The stereochemistry for **1** remains to be determined.

Table 3. ^{1}H NMR data of YM-170320 (**1**) in $CDCl_3$.

	No.	^{1}H		No.	^{1}H
Fatty acid	2	2.40 (dq, 7.0, 7.0)	⊿Ala	βa	6.03 (br s)
	3	3.58 (m)		βb	5.58 (br s)
	4	1.56 (m)		α-NH	8.38 (br s)
		1.43 (m)	βAib	α	2.80 (m)
	5	1.81 (m)		β	3.58 (m)
		1.53 (m)			3.22 (m)
	6	2.80 (m)		α-Me	1.16 (d, 6.7)
	8	6.14 (d, 15.3)		β-NH	7.46 (br t, 6.1)
	9	7.24 (d, 15.3)	OHGln	α	4.68 (m)
	11	5.98 (br t, 6.7)		β	2.44 (m)
	12	2.22 (m)			2.16 (m)
	13	1.43 (m)		γ	4.20 (m)
		1.22 (m)		OMe	3.49 (s)
	14	1.43 (m)		α-NH	6.86 (br d, 7.3)
	15	[a]		NH_2	6.98 (br s)
	16	[a]			5.88 (br s)
	17	[a]			
	18	[a]			
	19	1.27 (m)			
	20	0.88 (t, 6.1)			
	21	1.19 (d, 6.7)			
	22	1.14 (d, 6.7)			
	23	1.79 (br s)			
	24	0.89 (d, 6.1)			

[a] Signals were overlapped between δ 1.22 and 1.33.

Fig. 1. Partial structures **a**~**d**.

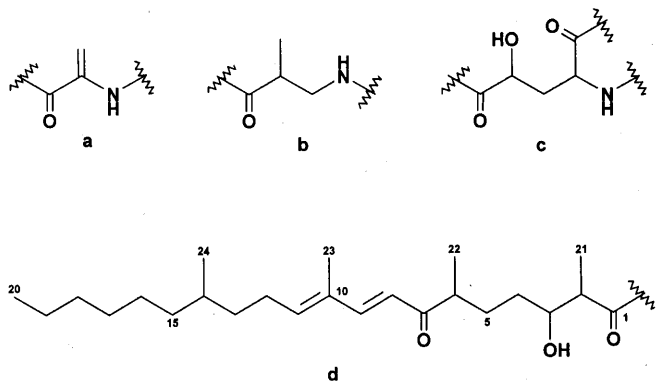
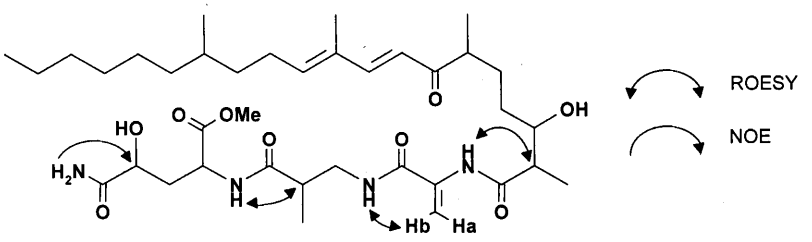


Fig. 2. ROESY correlations and NOE enhancement observed in the difference NOE experiment.



Evaluation of biological activity was performed using a stereomicroscope by obesrvation of the effect of tested substances on colony morphology of *C. tropicalis* pK233. The mutant, which normally produced oval or ellipsoid colonies with smooth surfaces, gave abnormal ones with echinulate surfaces when exposed to miconazole at 0.1 μg/ml. YM-170320 (**1**) induced this morphological change at concentrations up to 100 μg/ml, whereas the growth of typical yeasts and filamentous fungi was not inhibited by **1** at these concentrations.

YM-170320 (**1**) belongs to a novel class of ergosterol-biosynthesis inhibitors, and the mode of action is now under investigation.

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