Mer-WF3010, A NEW MEMBER OF THE PAPULACANDIN FAMILY

I. FERMENTATION, ISOLATION AND CHARACTERIZATION

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Mer-WF3010†, a new member of the papulacandin family, was isolated from the mycelia of *Phialophora cyclaminis* Mer-WF3010 (FERM P-11475). The molecular formula of Mer-WF3010 was determined as $C_{45}H_{60}O_{16}$.

In the course of screening for antifungal antibiotics, we discovered Mer-WF3010. The producing microorganism was isolated from a soil sample collected in Fujisawa City, Japan, and was classified as *Phialophora cyclaminis*[1,2]. The structure of Mer-WF3010 was determined to be **1** (Fig. 1), a new member of the papulacandin family[3~6], whose mode of action is considered to be the inhibition of β-1,3-D-glucan synthase[7]. Mer-WF3010 is fungicidal to growing cells of *Candida albicans*, but is inactive on resting cells. This paper presents the fermentation, isolation, physico-chemical properties and biological activity of Mer-WF3010. Studies on the structure of Mer-WF3010 are reported in the following paper[8].

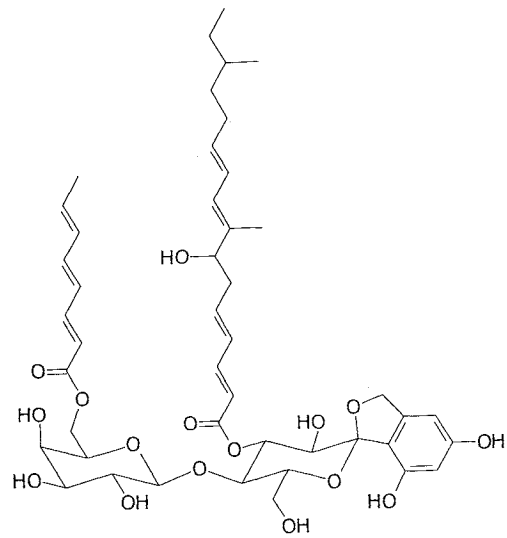
Fermentation

A loopful of a slant culture of *Phialophora cyclaminis* Mer-WF3010 was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of seed medium consisting of potato starch 2.0%, glucose 1.0%, soy bean meal (Ajinomoto Co., Inc.) 2.0%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% (pH not adjusted) and incubated on a rotary shaker at 28°C for 3 days. The seed culture broth was then transferred to a 10-liter jar fermentor containing 5 liters of production medium with the same composition as the seed medium and incubated at 30°C with agitation at 300 rpm and aeration at 5 liters per minute.

The typical time course of Mer-WF3010 production by *Phialophora cyclaminis* Mer-WF3010 grown in 10-liter fermentor is shown in Fig. 2.

Antifungal activity was detected by the paper disk-agar assay method using *Candida albicans* ATCC 10231 as the test organism.

Fig. 1. Structure of Mer-WF3010 (**1**).



† Mer-WF3010 has been described in Jpn. Kokai JK92-29995, Jan. 31, '92.

Fig. 2. Time course of fermentation of *Phialophora cyclaminis* Mer-WF3010.

○ Production of Mer-WF3010, △ pH, □ packed cell volume.

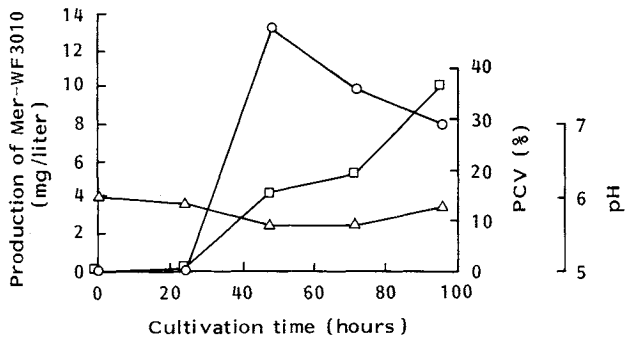


Table 1. Physico-chemical properties of Mer-WF3010.

Appearance	Colorless	Rf value[a]	
MP	163~166°C (dec.)	$CHCl_3$-MeOH (10:3)	0.6
Optical rotation		$CHCl_3$-MeOH (10:2)	0.4
$[\alpha]_D^{24}$ (*c* 1.0, MeOH)	+36.21°	Color reaction	
Molecular formula	$C_{45}H_{60}O_{16}$	Positive	Iodine, 10% H_2SO_4, anisaldehyde-H_2SO_4, phosphomolybdic acid
MW	856		
FAB-MS	857 $(M+H)^+$		
HRFAB-MS		Negative	Ninhydrin reagent, 2,4-dinitrophenylhydrazin, Rydon-Smith
Obsd.	857.3944		
Calcd. for $C_{45}H_{61}O_{16}$	857.3942		
UV λ (nm)	232, 239, 266, 297 (sh)	Solubility	
IR ν KBr cm^{-1}	3400, 1700, 1640, 1615, 1370, 1240, 1130	Soluble	MeOH
		Slightly soluble	$CHCl_3$
		Insoluble	H_2O

[a] Silica gel TLC (Merck $60F_{254}$).

The amount of Mer-WF3010 in the fermentation broth was determined by HPLC using YMC-pack A-312 ODS, 6 mm i.d. × 15 cm column with a mobile phase of 85% methanol.

Isolation

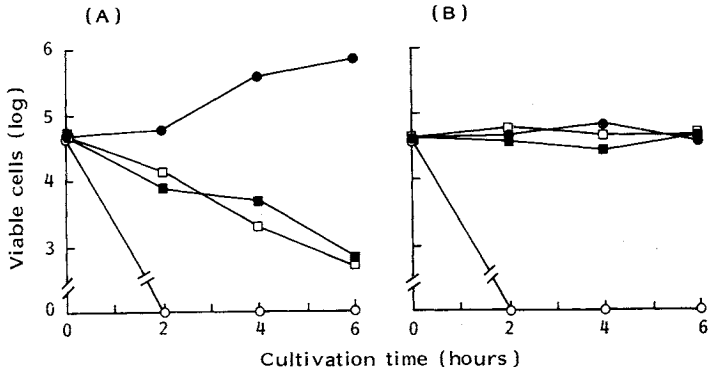
Mer-WF3010 was isolated mainly from the mycelia. The mycelial cake, obtained by filtration of the culture broth of the 48 hours, was soaked in 5 liters of methanol at 4°C for 60 minutes.

To detect Mer-WF3010, a disk diffusion-agar assay was used, along with TLC chromatography on E. Merck Silica gel $60F_{254}$ with chloroform-methanol (5:1).

The methanolic extract from the mycelia was concentrated to an aqueous oil (250 ml), which was extracted twice with an equal volume of ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The resulting oily residue was dissolved in 70 ml of chloroform and purified by chromatography on a silica gel column (Wako C-200, 550 ml). The active substance was eluated with 1 liter of chloroform-methanol (10:1). The active fractions were concentrated *in vacuo*, and dissolved in 4 ml of acetone. The acetone soluble fraction was again concentrated *in vacuo* and the residue, dissolved in chloroform, was chromatographed on a column of silica gel (300 ml) with the same conditions and solvent as before. The active fractions were collected and concentrated *in vacuo*, and the resulting residue was dissolved in methanol. Final purification was achieved by chromatography on a Sephadex

Fig. 3. Effect of Mer-WF3010 on viability of *Candida albicans*.

The cells were incubated in a medium (A), or in a buffer (B).
● Control (no antibiotics), ■ Mer-WF3010 (50 μg/ml), □ Mer-WF3010 (10 μg/ml), ○ nystatin (10 μg/ml).



LH-20 column (Pharmacia, 60 ml) using methanol. The total yield of 30 mg was obtained as a colorless powder.

Physico-chemical Properties

The physico-chemical properties are summarized in Table 1. The MW and molecular formula of Mer-WF3010 were determined by FAB-MS and HRFAB-MS. Mer-WF3010 is similar to the papulacandins in some aspects, *e.g.*, UV absorption, but obviously differs from them in its molecular formula.

Biological Activity

The antimicrobial activity of Mer-WF3010 was determined by a two-fold micro-dilution method with Sabouraud Dextrose broth after incubation at 28°C for 48 hours. As shown in Table 2, Mer-WF3010 has potent antifungal activity against *Candida* sp., being about as active as papulacandin B.

Table 2. Antimicrobial activity of Mer-WF3010 and papulacandin B.

	MIC (μg/ml)	
	Mer-WF3010	Papulacandin B
Candida albicans ATCC 10231	0.31	0.16
C. albicans IAM4905	0.63	0.31
C. albicans TIMM1623	0.63	0.63
C. albicans IFM40009	1.25	1.25
C. kefyr IAM4829	0.31	0.16
Cryptococcus neoformans TIMM0354	>40	>40
Aspergillus fumigatus TIMM0063	>40	>40
A. fumigatus IFM4942	>40	>40

To test the effect of Mer-WF3010 on the viability of *C. albicans*, 1-ml of YEPG (yeast extract 1.0%, peptone 2.0%, glucose 2.0%, pH 6.4) and/or 0.1 M phosphate buffer (pH 7.0), containing 0~50 μg of Mer-WF3010 per ml, were prepared and inoculated with *C. albicans* to give a final concentration of approximately 5×10^4 colony forming units (CFU). The tubes were incubated at 28°C, and samples taken at two hours interval to count the CFU.

Mer-WF3010 was fungicidal to growing cells at all levels tested, while nystatin was fungicidal to both growing and resting cells. The results are shown in Fig. 3. At a 24-hour time point viability cells were not detected when incubated in medium, even though cells were detected in buffer. There was no significant differences between Mer-WF3010 and papulacandin B (These data were not shown in Fig. 3.).

Mer-WF3010 did not produce any toxic symptoms in mice at a dose of 1,000 mg/kg when administered intraperitoneally.

Discussion

Chemical and biological data show that Mer-WF3010 is a new member of the papulacandin family. Some differences with other family members are listed below.

	Mer-WF3010	Papulacandin A	Papulacandin B	Chaetiacandin	L-687,781
UV: λ (nm)	232	232 sh	232	225	232 sh
MeOH-max	239	242	240	230 sh	240
	266	265	268	263	262
	297 sh		300 sh		
Molecular formula	$C_{45}H_{60}O_{16}$	$C_{47}H_{66}O_{16}$	$C_{47}H_{64}O_{17}$	$C_{43}H_{60}O_{16}$	$C_{47}H_{66}O_{17}$
MW	856	886	900	832	902

As shown in Fig. 1, Mer-WF3010 is therefore concluded to be a new antibiotic. Studies on structure determination are reported in the following paper[8].

RÖMMELE *et al.* discussed the effects of the side chains of papulacandin and showed that short fatty acid chain can affect penetration into the cell but is not required for inhibition of enzymatic activity[9]. The shortside chain fatty acid of Mer-WF3010 is shorter than that of any other known papulacandin, but the activity against *Candida* is as high as that of papulacandin B. This means that even though the length of the short fatty acid is C8, it is enough to show activity against the yeast.

Acknowledgments

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