CHAETIACANDIN, A NOVEL PAPULACANDIN

I. FERMENTATION, ISOLATION AND CHARACTERIZATION

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Chaetiacandin, a new anti-yeast antibiotic structurally related to papulacandin, was isolated from a culture of *Monochaetia dimorphospora*. The fermentation, isolation, and physicochemical and biological properties are reported. The molecular formula of this compound was determined as $C_{43}H_{60}O_{16}$.

In the course of a screening program for inhibitors of fungal cell wall biosynthesis, we discovered an antibiotic, designated chaetiacandin, in the mycelial cake of *Monochaetia dimorphospora*.

Chaetiacandin belongs to the family of papulacandins[1,2,3] affecting glucan biosynthesis in yeast spheroplasts[4]. The strain was isolated from soil collected in Kyoto City, Japan, and was classified as *Monochaetia dimorphospora*[5] by taxonomic studies and direct comparison with the type strain. This paper describes the fermentation, isolation and biological and physico-chemical properties of chaetiacandin.

Production of Chaetiacandin

Production of the antibiotic is as follows: 30-liter fermentors with 20 liters of fermentation medium (Table 1) were inoculated using 1% of mature seed broth. Seed flasks (500 ml) containing 100 ml of the seed medium were inoculated with spores from slant cultures and incubated at 27°C on a rotary shaker with 7.6-cm throw at 180 rev/minute for 72 hours.

Fermentations were run for 72 hours under the following conditions: temperature 30°C; agitation 250 rpm; aeration 20 liters/minute; tank back pressure 1.0 kg/cm^2.

Table 1. Media used for production of chaetiacandin.

Seed medium		Fermentation medium	
Soluble starch	2.0%	Corn starch	3.0%
Glucose	1.0	Peanut powder	2.0
Corn steep liquor	0.5	Wheat germ	1.0
Dried yeast	0.5	Dried yeast	0.5
Cotton seed flour	0.5	NaI	0.5 μg/ml
$CaCO_3$	0.3	$CoCl_2 \cdot 6H_2O$	4.0 μg/ml
pH	6.0		

Fig. 1. UV spectra of chaetiacandin.

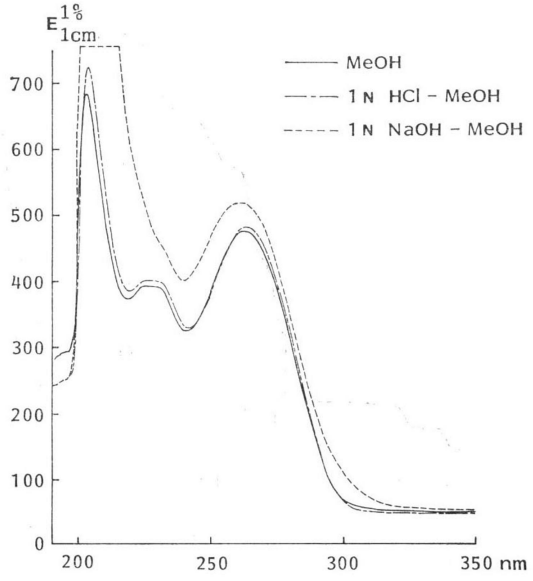

Fig. 2. IR spectrum of chaetiacandin in KBr disk.

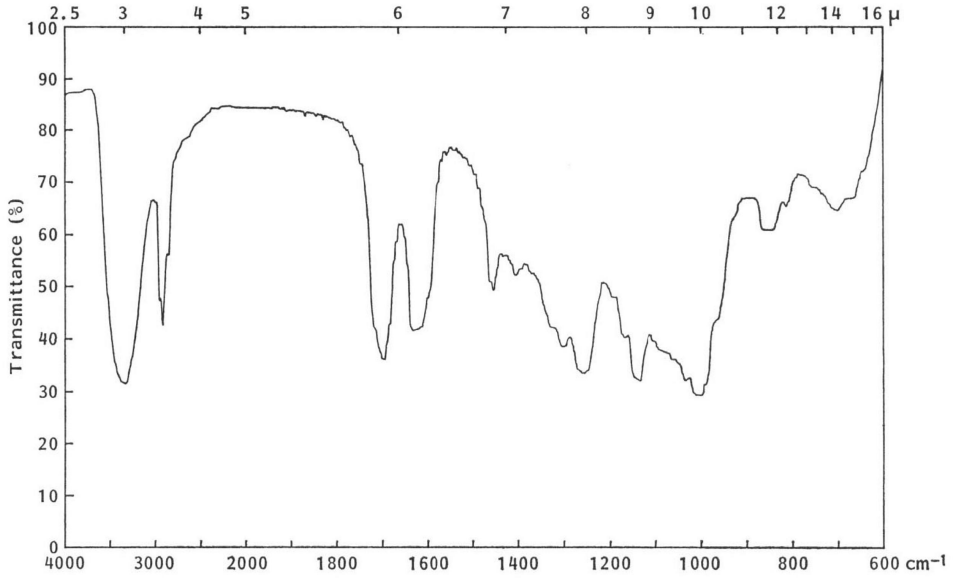

Fig. 3. ^{1}H NMR spectrum of chaetiacandin.

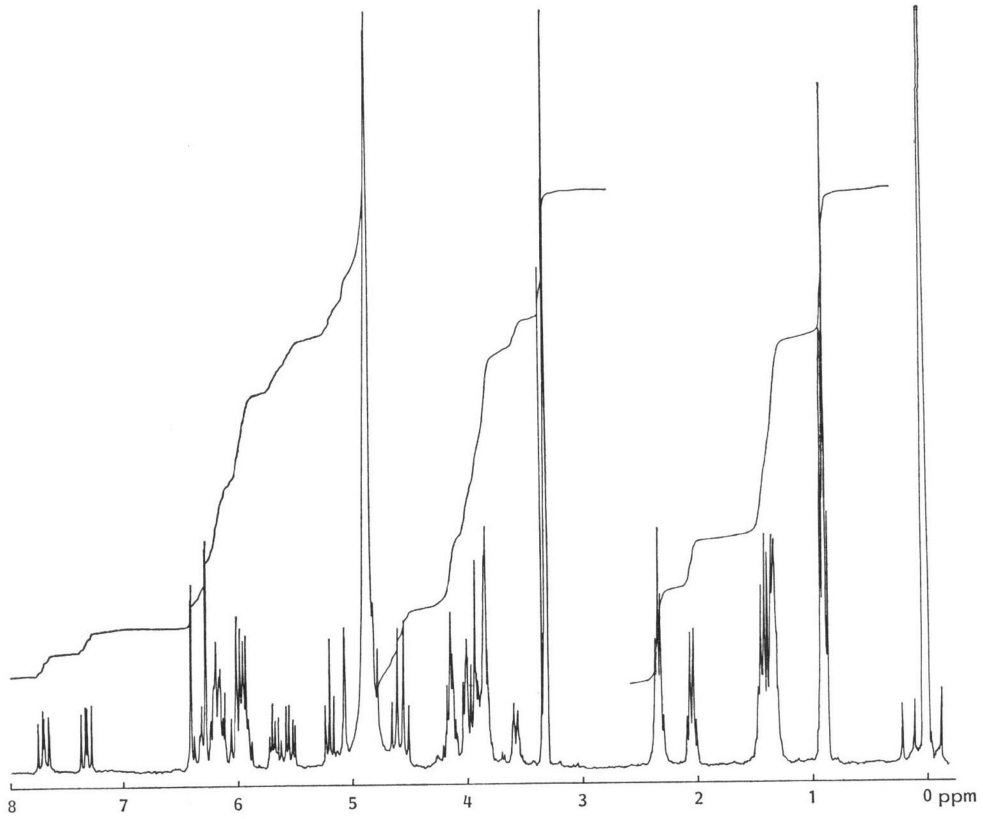

Fig. 4. ^{13}C NMR spectrum of chaetiacandin.

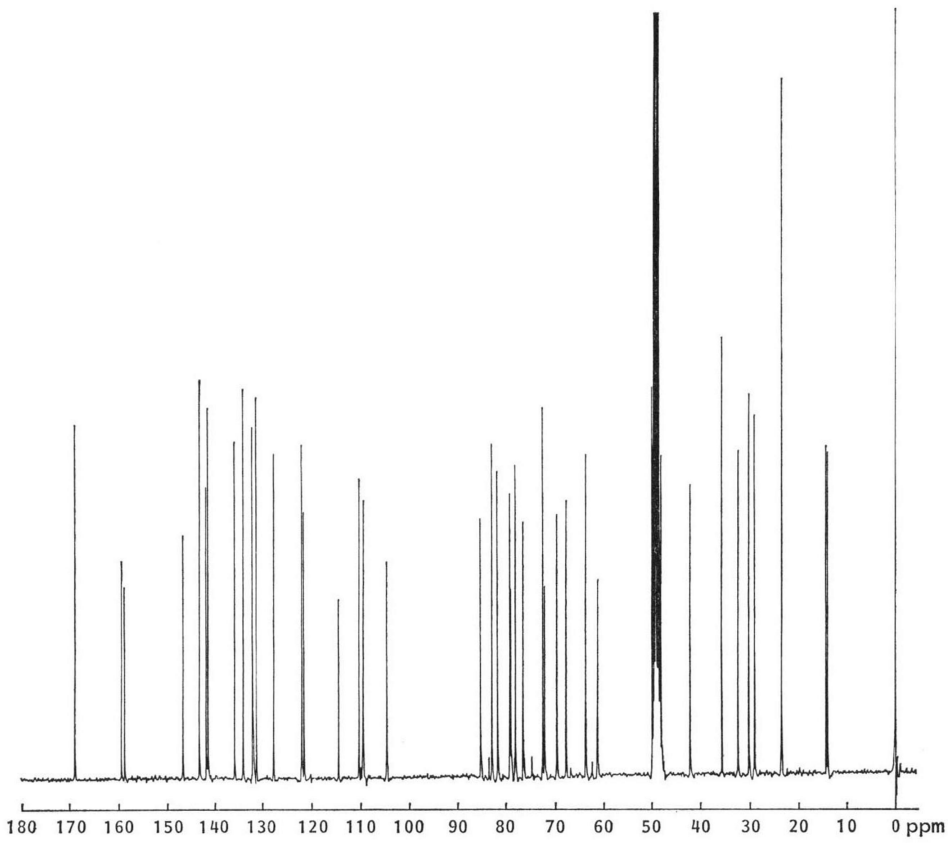

Table 2. Comparative activity of chaetiacandin and papulacandin B.

Test organisms		MIC (μg/ml)[b]	
		Chaetiacandin	Papulacandin B
Candida albicans	12-1[a]	0.025	0.05
〃	FP 614	0.2	0.1
〃	FP 615	0.1	0.1
〃	FP 616	0.1	0.1
〃	FP 622	0.1	0.1
Candida tropicalis	FP 583	0.2	0.2
Candida krusei	FP 585	0.2	0.2
Candida parakrusei	FP 586	0.2	0.2
Trichophyton asteroides		100	100
Aspergillus niger		100	100
Phoma sp.		100	100

[a] Supersensitive mutant to neopolyoxins.
[b] MIC determined by the agar dilution method.
The fungi were cultured in glucose 0.5%, Polypeptone 0.175%, yeast extract 0.175% and agar 1%.

Progress of the fermentation was monitored by diffusion plate assays performed on the whole broth. *Candida albicans* 12-1 (supersensitive mutant to neopolyoxins[6,7] or nikkomycins[8]) was the test organism for the bioassay.

Isolation Procedure

Most of the antibiotic activity was found in the mycelium extracts. The methanol extracts (3.5 liters) from the mycelium were concentrated to an aqueous oil (2.5 liters), which was extracted twice with ethyl acetate (2.5 liters). The ethyl acetate extracts were combined, dried over sodium sulfate and concentrated *in vacuo* to an oily residue (20 ml).

The active syrup was purified by chromatography on a column of silica gel (Merck) (300 ml) with chloroform containing an increasing amount of methanol as eluant. The active substance was eluted with chloroform - methanol (8: 1). The eluates were concentrated *in vacuo* to yield crude powder. Further purification was achieved by low pressure liquid chromatography on silica gel (Lobar Größe B (310-25) LiChroprep Si 60, Merck) using chloroform - methanol (8: 1). The active fractions were concentrated *in vacuo*, and then finally purified by preparative thin-layer chromatography on silica gel 60 F-254 (Merck) using chloroform - methanol (5: 1) as the total yielded 6 mg as a colorless solid.

Physico-chemical Properties of Chaetiacandin

Chaetiacandin is a weakly acidic colorless powder, soluble in lower alcohols, DMF and acetone, slightly soluble in ethyl acetate and chloroform, and insoluble in benzene, *n*-hexane and water. It decomposes at 128~132°C. The optical rotation is $[\alpha]_D^{22}$ $-1.5\pm1°$ (*c* 1, methanol). The antibiotic shows ultraviolet absorption maxima at 225 nm ($E_{1cm}^{1\%}$ 392), 230 (sh, 390) and 263 (475) in methanol; at 225 (401), 230 (sh, 400) and 263 (480) in methanol - 1 N HCl; at 230 (sh, 460) and 261 (520) in methanol - 1 N NaOH (Fig. 1).

Elemental analysis gave the following data;

Anal Calcd for $C_{43}H_{60}O_{16}$: C 62.00, H 7.26.
Found: C 61.88, H 7.38.

No molecular ion was visible in the FD mass spectrum. The IR spectrum in a KBr disk (Fig. 2) shows absorption bands at 3350 (OH) and 1695 and 1635 (unsaturated C=O). The ^1H NMR and ^{13}C NMR of the antibiotic in CD_3OD are shown in Fig. 3 and Fig. 4, respectively.

The Rf value on silica gel (Kieselgel 60 F-254, Merck) thin-layer chromatography with chloroform - methanol (5: 1) was 0.40, compared to papulacandin B Rf 0.32.

Biological Characteristics of Chaetiacandin

The antimicrobial spectrum of chaetiacandin was determined by the two-fold serial agar dilution method. The results are given in Table 2. Chaetiacandin has a high specific activity against yeasts, especially against *C. albicans* 12-1 (supersensitive mutant to neopolyoxins), but it is ineffective against bacteria.

Against divers *Candida* chaetiacandin exhibits a lower MIC than well known antifungal reagents (amphotericin B, 5-FC, *etc.*). Its activity is as high as papulacandin B, which has the highest activity against *Candida*.

However, addition of serum or blood to the medium greatly reduced the activity of chaetiacandin against test organisms.

The acute toxicity of chaetiacandin is very low. Intraperitoneal administration of 1 g/kg of chaetiacandin into mice did not result in any toxic symptoms. Considering its low toxicity and inductive capability of spheroplast formation in yeast, chaetiacandin seems to be an inhibitor of cell wall synthesis of yeasts.

Discussion

Chaetiacandin is similar to the papulacandins in chemical and biological properties from the above data, but differs from the papulacandins in the following points:

	Chaetiacandin	Papulacandin[1]				
		A	B	C	D	E
$[\alpha]_D^{22}$ (MeOH)	$-1.5\pm1°$	$+30\pm1°$	$+50\pm1°$	$+33\pm1°$	$+7\pm1°$	ND
UV λ_{max}^{MeOH} (nm)	225, 230 (sh), 263	232 (sh), 242, 265	232, 240, 268, 300 (sh)	232, 240, 268, 297 (sh)	230, 235, 261	230 (sh), 237 (sh), 267, 292 (sh)
Molecular formula	$C_{43}H_{80}O_{16}$	$C_{47}H_{86}O_{16}$	$C_{47}H_{84}O_{17}$	$C_{47}H_{84}O_{17}$	$C_{31}H_{42}O_{10}$	ND
MW	832	886	900	900	574	ND

Chaetiacandin is, therefore, concluded to be a new antibiotic.

Chaetiacandin showed strong antibiotic activities against yeasts and weak inhibitory effects against filamentous fungi, but had no effect on bacteria.

Studies on the structural determination of chaetiacandin are reported following paper[9].

Acknowledgment

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