Communication to the editors

FUMIGACHLORIN, A NEW ANTIFUNGAL ANTIBIOTIC

Sir:

In the course of screening for antifungal antibiotics, a new antibiotic, named fumigachlorin, has been isolated from the fermentation broth of a fungus which was obtained from a soil sample collected in Izu Ohshima, Tokyo, and was identified as *Sartorya fumigata* var. *spinosa*[1] (*Aspergillus fischeri* var. *spinosus* RAPER *et* FENNELL[2]). The antibiotic, containing chlorine atoms, showed strongly inhibitory activities against filamentous fungi, especially, dermatophytes.

The organism was cultured in deep culture fermentors at 26°C and pH 6.0~6.5 in a medium containing 3.0% lactose, 2.0% Pharma media (Traiders Oil Mill Co.), 0.5% KH_2PO_4, 0.3% $MgSO_4$, and 0.1% NaCl. The maximum production (2~3 mcg/ml) was achieved in about 50 hours. The activity was determined by the agar cup-plate method using *Trichophyton asteroides* as test organism.

Fumigachlorin was extracted from the clarified broth with *n*-butyl acetate, and concentrated to an oily syrup. The purification was made by column chromatography of silicic acid (Mallinckrodt) using benzene-ethyl acetate (20:1) as a developer. Colorless needles were obtained from carbon tetrachloride-*n*-hexane. Fumigachlorin melts at 112~113°C and gives $[\alpha]_D^{26}$ −77.5 (*c* 1.0, $CHCl_3$). It is soluble in most organic solvents, but insoluble in water.

The ultraviolet absorption spectrum in methanol exhibits maxima at 238 mμ ($E_{1cm}^{1\%}$ 383), 273 mμ ($E_{1cm}^{1\%}$ 225), and 325 mμ ($E_{1cm}^{1\%}$ 217) as shown in Fig. 1. The infrared absorption spectrum is shown in Fig. 2. Elementary analysis proposed a molecular formula of $C_{16}H_{25}NO_4Cl_2$. (M. w. 366)

Found: C 52.31, H 6.66, N 3.80, Cl 19.38
Calcd: C 52.46, H 6.88, N 3.82, Cl 19.36

The presence of two chlorine atmos in a molecule was also confirmed by the observation in the mass spectrometry of three

Fig. 1. Ultraviolet absorption spectrum of fumigachlorin.

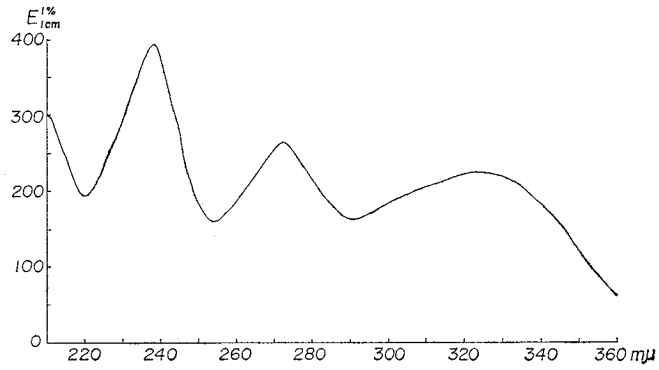
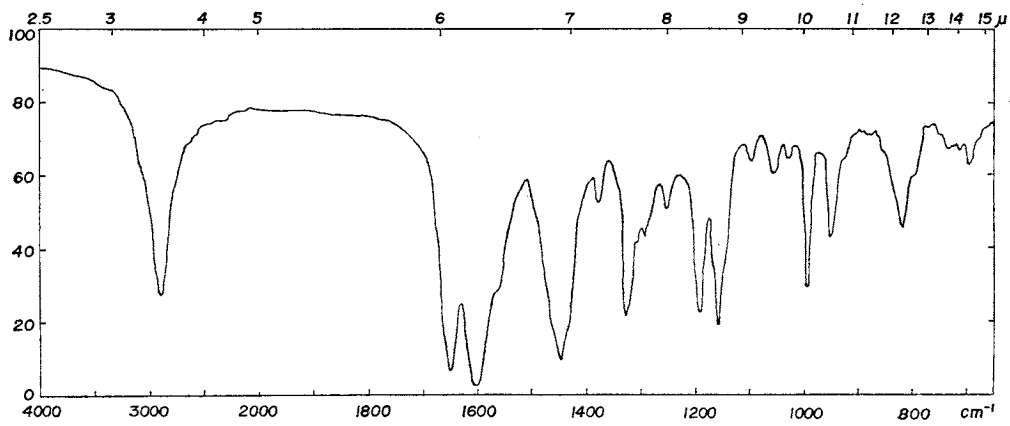


Fig. 2. Infrared absorption spectrum of fumigachlorin (KBr)



parent peaks at m/e 365 (M), 367 (M+2), and 369 (M+4).

It gives a positive ferric chloride reaction, but negative TOLLENS, EHRLICH, biuret, FEHLING, and 2,4-dinitrophenylhydrazine reactions.

On thin-layer chromatography using silicic acid (Kieselgel-G, Merck), Rf values are as follows: 0.65 with chloroform – ethyl acetate (3 : 1), and 0.42 with carbon tetrachloride – ethyl acetate – acetic acid (100 : 30 : 1).

The antibiotic is stable at acidic and neutral pH, slightly unstable at alkaline pH.

As shown in Table 1, fumigachlorin is principally active against filamentous fungi, but inactive against yeasts and bacteria.

Table 1. Antimicrobial spectrum of fumigachlorin

Test organisms	Medium	M.I.C. (mcg/ml)
Bacillus subtilis PCI 219	N	>100
Staphylococcus aureus FDA 209P	〃	>100
Micrococcus flavus	〃	>100
Sarcina lutea ATCC 1001	〃	>100
Mycobacterium ATCC 607	〃	>100
Pseudomonas aeruginosa	〃	>100
Escherichia coli NIHJ	〃	>100
Klebsiella pneumoniae PCI 602	〃	>100
Proteus vulgaris OX 19	〃	>100
Salmonella paratyphi A	〃	>100
Salmonella paratyphi B	〃	>100
Shigella sonnei E-33	〃	>100
Candida albicans ATCC 7491	S	>100
Saccharomyces cerevisiae	〃	>100
Mycotorula japonicus NI 6226	〃	>100
Torula utilis (towlepsisutilis)	〃	>100
Cryptococcus neoformans	〃	>100
*Cryptococcus neoformans**	〃	100
Penicillium chrysogenum Q-176	〃	100
Aspergillus niger ATCC 6275	〃	50
Aspergillus fumigatus IMA 2612	〃	3.2
Aspergillus PQMD 82	〃	>100
Trichophyton rubrum	〃	0.8
*Trichophyton rubrum**	〃	<0.05
*Trichophyton asteroides**	〃	<0.05
*Trichophyton interdigitale**	〃	<0.05
*Epidermophyton floccosum**	〃	<0.05
Microsporum gypseum	〃	1.6
*Microsporum gypseum**	〃	<0.05
Hormodendrum pedrosoi	〃	50
Hormodendrum pedrosoi	〃	100
Sporotricum SP*	〃	100
*Blastomyces braziliensis**	〃	100

* Clinical isolate
Media : N : nutrient agar at 37℃ for 16 hours
S : SABOURAUD's glucose agar at 30℃ for 42 hours

Table 2. Effect of serum on the antifungal activity of fumigachlorin against the clinical isolates.
(agar dilution method)

Test organisms	Serum conc. (%)	M.I.C. (mcg/ml) 2 days	4 days	7 days
Trichophyton asteroides	0	<0.1	<0.1	0.4
	10	0.2	1.6	3.2
Trichophyton rubrum	0	<0.1	<0.1	<0.1
	10	<0.1	1.6	3.2
Trichophyton interdigitale	0	<0.1	<0.1	0.2
	10	0.4	1.6	3.2
Epidermophyton floccosum	0	<0.1	<0.1	<0.1
	10	<0.1	<0.1	<0.1
Microsporum gypseum	0	<0.1	<0.1	0.2
	10	0.4	1.6	3.2
Microsporum canis	0	<0.1	0.2	6.25
	10	<0.1	12.5	>50

The antifungal activities were influenced by addition of serum (rat) as described in Table 2.

The subcutaneous, oral and intraperitoneal LD_{50} in mice were found to be 9.3 mg, 18.5 mg, and 4.6 mg per kg body weight, respectively.

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