ANTIMYCOTIC EFFECTS OF THE NOVEL ANTITUMOR AGENTS FOSTRIECIN (CI-920), PD 113,270 AND PD 113,271

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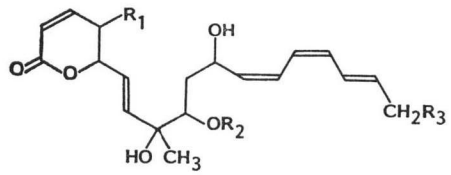
The novel fermentation products fostriecin and analogs PD 113,270 and PD 113,271 are structurally related polyene lactone phosphates that have antitumor activity *in vitro* and *in vivo*. They have no antibacterial effects, but they were inhibitory to yeasts (agar diffusion method) with MICs of 3~300 μg/ml. Fostriecin or its analogs were active vs. 29 of 46 yeast species (11 genera). Ten of 12 cultures of *Candida* sp. were not sensitive to any of the analogs, while 11 of 14 cultures of *Saccharomyces* sp. were inhibited by one or more of the agents. Sensitivity patterns were of three types: Twelve cultures were sensitive only to PD 113,270; fostriecin and PD 113,271 (but not PD 113,270) were active vs. 7 cultures; and 9 cultures were sensitive to all three compounds. Dephosphorylation of the compounds resulted in the loss of antimycotic effects. Activity vs. the yeasts was related to studies of uptake and activity against cancer cells.

Fostriecin (CI-920, PD 110,161), PD 113,270 and PD 113,271 are structurally related fermentation products that possess *in vitro* and *in vivo* antitumor activity[1~3]. These analogs have a polyene lactone phosphate structure which differ by substituting hydrogen and hydroxyl groups at two sites on the molecule (Fig. 1). Fostriecin or its analogs had no effect against nine bacteria and two fungi at a concentration of 500 μg/ml[3]. Although these agents were inactive against prokaryotes, further investigations revealed that all three analogs exhibit significant antimycotic effects vs. certain yeasts, with one analog showing inhibition at 3~10 μg/ml. The purpose of this paper is to report on the spectrum and nature of the antimycotic activities of these novel antitumor antibiotics.

Fig. 1. Structure of fostriecin and its analogs.



Fostriecin	R_1=H	R_2=PO_3	R_3=OH
PD 113,270	R_1=H	R_2=PO_3	R_3=H
PD 113,271	R_1=OH	R_2=PO_3	R_3=OH
PD 114,631	R_1=H	R_2=H	R_3=OH
PD 116,243	R_1=OH	R_2=H	R_3=OH

Materials and Methods

Test Cultures

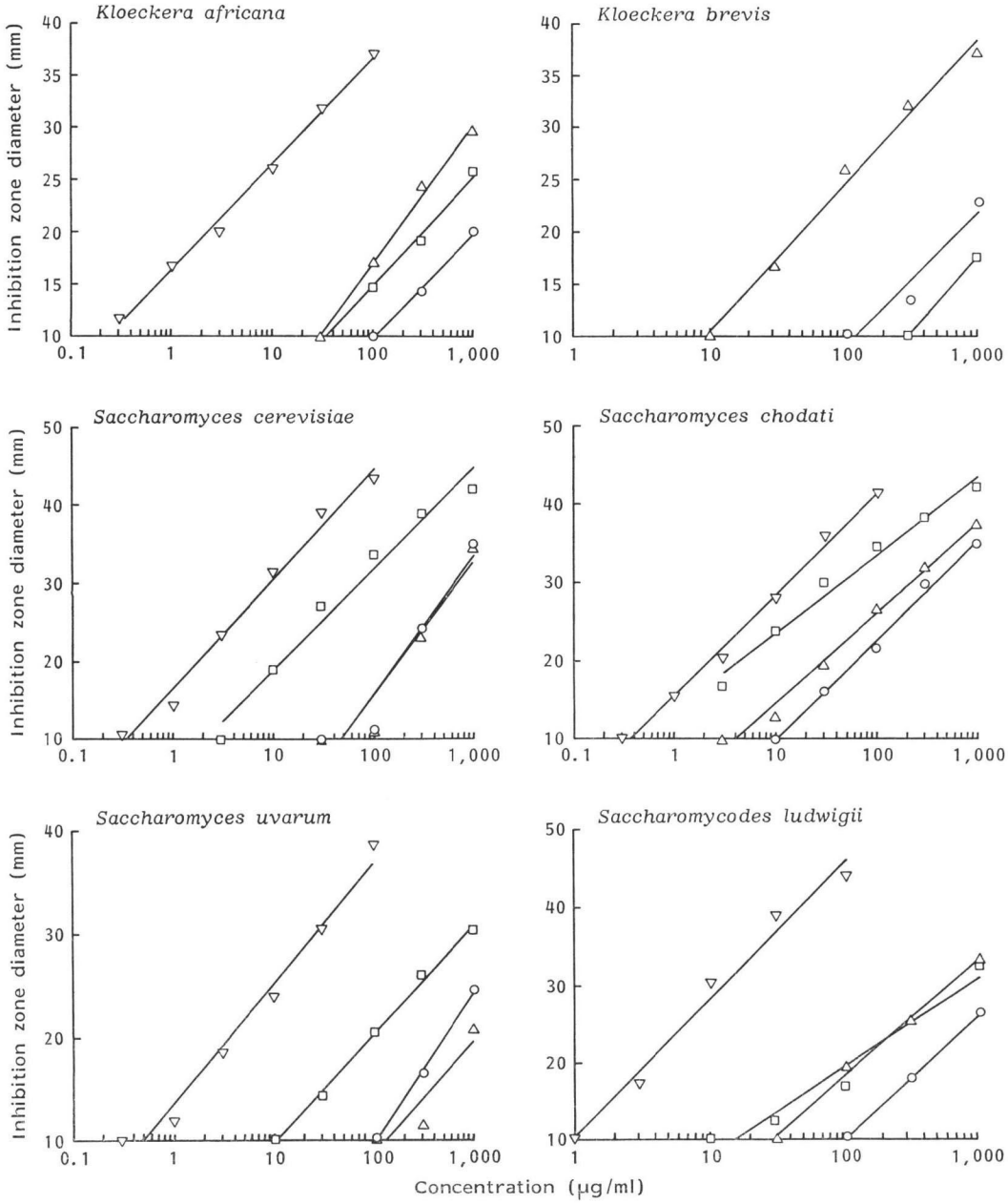
A total of 46 yeast cultures from the Warner-Lambert/Parke-Davis culture collection were used in these evaluations. All cultures were maintained on Sabouraud Dextrose Agar (SDA) (Difco Laboratories, Detroit, MI).

Antimicrobial Evaluation

Antimicrobial susceptibility tests employed disk-agar or well-agar procedures. Each culture

Fig. 2. Inhibition of the yeasts *Kloeckera africana*, *K. brevis*, *Saccharomyces cerevisiae*, *S. chodati*, *S. uvarum* and *Saccharomycodes ludwigii* by fostriecin (○), PD 113,270 (△), PD 113,271 (□) and cycloheximide (▽) in well-agar diffusion assays (10 mm wells).

Each point represents the mean of three replicate samples (semilogarithmic regression plots).



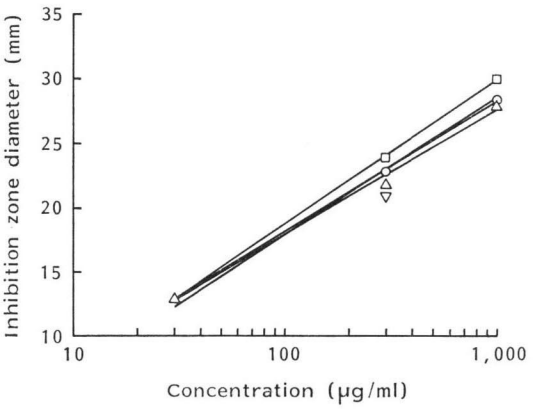
was washed from SDA slants and suspended in 0.85% saline, which was adjusted spectrophotometrically to an appropriate inoculum density (generally, 14% transmission on a Perkin-Elmer Model 35 spectrophotometer set at 550 nm wavelength). Molten agar (50°C) was inoculated with an aliquot (1~4 ml) of the saline suspension, and plates were poured. Plating agar was Parke-Davis No. 77 (a yeast maintenance/assay medium)[4] except for *Kloeckera brevis*, which was plated in Parke-Davis

agar No. 69, a seed layer medium for assay of azaserine[5], and *Torulopsis albida*, which was plated in 6-diazo-5-oxo-L-norleucine (DON) assay agar[6].

Solutions of each compound were freshly prepared at concentrations of 1,000, 300, 100, 30, 10 and 3 μg/ml using sterile distilled water. Powdered fostriecin and PD 113,271 were of better than 98% purity; PD 113,270 was approximately 85% pure but was free of contamination by the other two analogs.

Paper disks of 12.7 mm diameter (Schleicher and Schuell No. 740) were placed on the surface of the agar and were impregnated with 80 μl of a given solution. Alternatively, wells of 10 mm diameter were cut in the agar, and 80 μl of a given solution were added per well. Plates were incubated at 28°C for 36~48 hours, and inhibition zones were measured. In quantitative evaluations, the minimal inhibitory concentration (MIC) was the lowest test concentration that yielded a zone of inhibition.

Fig. 3. Inhibition of the yeasts *Mycoderma decolorans* (○), *Pichia membranaefaciens* (△), *Rhodotorula glutinis* (□) and *Torulopsis albida* (▽) by PD 113,270 in disk-agar diffusion assays. (semilogarithmic regression plots).



Effect of Reduced Folates on Antimycotic Susceptibility

To determine if folate metabolism was in any way involved in the antimycotic effects of fostriecin or its analogs, selected isolates were plated in both regular assay media and in agar supplemented with 10 mg/ml of calcium leucovorin (Sigma). Disks impregnated with 80 μl of 1 mg/ml solutions of each analog were spotted onto the agar plates.

Effect of Dephosphorylation on Antimycotic Susceptibility

To demonstrate the involvement of the phosphate group in antimycotic activity, dephosphorylated analogs of fostriecin and PD 113,271 (PD 114,631 and PD 116,243, respectively) were prepared by treatment with bovine alkaline phosphatase (Sigma). The resulting alcohols then were spot-tested vs. selected yeasts at 1 mg/ml concentrations.

Results

Of the 46 yeasts evaluated, 12 were members of *Candida* sp., and 14 were members of *Saccharomyces* sp. The remaining cultures belonged to one of 9 other genera.

The results of disk-agar tests with 1 mg/ml solutions of fostriecin, PD 113,270 and PD 113,271 are shown in Table 1. Only two of the 12 *Candida* cultures showed any sensitivity to any of the analogs. Of the remaining 36 cultures, 27 were sensitive to one or more of the analogs, including 11 of 14 *Saccharomyces* cultures.

Interestingly, the sensitive cultures showed three distinct patterns of sensitivity. PD 113,270 was the only active analog vs. 12 cultures; fostriecin and PD 113,271 (but not PD 113,270) were active vs. 7 cultures; and, all three analogs were active vs. 9 cultures. PD 113,271 produced markedly larger zones of inhibition than the other two analogs against 8 of the 9 cultures sensitive to all 3 agents (Table 1). It should be noted, however, that the zone diameters may have been influenced by such factors as diffusion rates of the compounds and their interactions with components of the agar (D. A. STEVENS, personal communication).

Table 1. Antimycotic effects of fostriecin and its analogs (1 mg/ml solutions) in disk-agar diffusion assays.

Organism	Inhibition zone diameter (mm)[a]		
	Fostriecin	PD 113,270	PD 113,271
Candida sp.			
C. albicans	0	0	0
C. chalmersi	0	0	0
C. flareri	0	0	0
C. guilliermondii	0	0	0
C. krusei	0	0	0
C. lipolytica M1367	0	0	0
C. lipolytica M1580	0	0	0
C. monosa	0	22	0
C. parapsilosis	0	0	0
C. pulcherrima	0	0	0
C. stellatoidea	0	0	0
C. zeylanoides	0	20	0
Debaryomyces matruchoti	0	17	0
Endomycopsis fibuligera	0	25	0
Hansenula anomala	0	0	0
H. saturnus	0	0	0
Kloeckera africana	22	26	28
K. brevis	20	30	24
Mycoderma cerevisiae	0	28	0
M. decolorans	0	31	0
M. vini	0	23	0
Pichia alcoholophila	0	0	0
P. chodati	0	25	0
P. membranaefaciens	0	30	0
Rhodotorula glutinis	0	30	0
R. pallida	0	24	0
Saccharomyces sp.			
S. acidifaciens	22	0	34
S. carlsbergensis	17	0	29
S. cerevisiae	26	21	35
S. chodati	30	29	36
S. ellipsoideus	19	16	30
S. fragilis	0	0	0
S. globosus M1387	0	0	0
S. globosus M1577	0	0	0
S. italicus	21	18	33
S. logos	16	0	29
S. marxianus	16	0	22
S. pastorianus	20	0	34
S. rosei	18	0	28
S. uvarum	24	20	29
Saccharomycodes ludwigii	28	26	35
Torulopsis albida	16	28	0
T. dattila	0	24	0
T. rotundata	0	0	0
Zygosaccharomyces japonicus	18	0	34
Z. lactis	16	19	22

[a] Using 12.7 mm paper disks.

Table 2. Effects of calcium leucovorin on the antimycotic activity of fostriecin, PD 113,270 and PD 113,271.

Organism	Fostriecin (1 mg/ml)		PD 113,270 (1 mg/ml)		PD 113,271 (1 mg/ml)	
	−LV[a]	+LV	−LV	+LV	−LV	+LV
Kloeckera africana	21[b]	21	29	29	28	28
K. brevis	21	17	34	33	21	18
Mycoderma decolorans	29	30	43	43	0	0
Pichia membranaefaciens	18	16	34	33	0	0
Rhodotorula glutinis	0	0	29	26	0	0
Saccharomyces cerevisiae	25	26	23	25	37	38
Torulopsis albida	0	0	25	26	0	0

[a] LV: Leucovorin, 10 mg/ml.
[b] Inhibition zones obtained using 12.7 mm paper disks.

Table 3. Effects of dephosphorylation on the antimycotic activity of fostriecin and PD 113,271.

Organism	Fostriecin (1 mg/ml)	PD 114,631[a] (1 mg/ml)	PD 113,271 (1 mg/ml)	PD 116,243[b] (1 mg/ml)
Kloeckera africana	22[c]	0	28	0
K. brevis	20	0	24	0
Rhodotorula glutinis	0	0	0	0
Saccharomyces cerevisiae	26	0	35	0
S. chodati	30	0	36	0
S. uvarum	24	0	29	0

[a] Dephosphorylated analog of fostriecin.
[b] Dephosphorylated analog of PD 113,271.
[c] Inhibition zones obtained using 12.7 mm paper disks.

Figs. 2 and 3 show the results of quantitative (multiple-concentration) well- or disk-agar tests vs. selected yeast cultures. By these methods, the MIC for fostriecin and PD 113,270 vs. any sensitive culture ranged from 30~300 μg/ml. PD 113,271 generally was more active than the other two analogs, with MICs of 3~30 μg/ml. The marked decrease in inhibition zone diameters observed with decreasing concentration contrasts with the potency of the antifungal agent cycloheximide.

Calcium leucovorin has been shown to block cytotoxicity of the analogs to L1210 leukemia cells *in vitro*[7]. The results of testing the three analogs both in the absence and presence of calcium leucovorin (Table 2) show essentially no effect of leucovorin on the activity of fostriecin or its analogs vs. the representative yeast cultures.

FRY *et al.*[7] and LEOPOLD *et al.*[8] demonstrated an involvement of the phosphate group of fostriecin in L1210 cell uptake and L1210 cell killing, respectively. A comparison of fostriecin and PD 113,271 and their corresponding dephosphorylated analogs (PD 114,631 and PD 116,243, respectively), demonstrated the need for the phosphate group in exerting antimycotic effects (Table 3). None of the cultures evaluated showed any sensitivity to the dephosphorylated analogs.

Discussion

FRY *et al.*[9] found that fostriecin markedly inhibits macromolecular synthesis in L1210 cells. FRY *et al.*[7] also reported that reduced folates, such as leucovorin, protect L1210 cells from the cytotoxic effects of fostriecin by competing with the antibiotic for the reduced folate carrier system. Fos-

triecin and PD 113,270 were more cytotoxic than PD 113,271 to L1210 leukemia cells and HCT-8 colon carcinoma cells *in vitro*[1,8]. The cytotoxicity vs. these mammalian cell systems appeared to correlate with the uptake of the compounds[1,7,8]. In the yeast systems, PD 113,271 appeared to be more active (lower MICs) than fostriecin and PD 113,270. Furthermore, in testing fostriecin and its analogs in both the absence and presence of leucovorin, no reductions in antimycotic effects were observed. These results indicate that neither the mechanisms of cell uptake or of protection by leucovorin in mammalian cells are applicable to yeast cells. The phosphate group of fostriecin was shown to be required for activity both in the yeast and mammalian systems.

Evidence has been presented that fostriecin and its analogs inhibit the activity of eukaryotic type II DNA topoisomerase[1]. Moreover, BORITZKI *et al.*[10] observed that PD 113,271 was the most potent topoisomerase inhibitor of the three analogs. This is consistent with the MIC results observed for the yeasts. It is possible that inhibition of type II DNA topoisomerase by fostriecin and its analogs is involved in the cytotoxic effects of these agents in both the yeast and mammalian cell systems. It is speculated further that the differential antimycotic effects of these compounds may be a manifestation of differential specificities for the type II DNA topoisomerases of the various yeast genera and species; whereas, the varying MIC values of the analogs may be related to cell penetration or uptake. Additional studies of the mechanisms of antimycotic activity of these analogs would be needed in order to support this hypothesis.

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