ISOLATION AND CHARACTERIZATION OF PAECILOMYCEROL, A NEW STEROIDAL ANTIVIRAL ANTIBIOTIC

STUDIES ON ANTIVIRAL AND ANTITUMOR ANTIBIOTICS. XXI

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A new antiviral substance, paecilomycerol (M. W. 428, $C_{27}H_{40}O_4$) has been isolated from the acetone extract of the mycelium of *Paecilomyces elegans*. It is steroidal and has no antibacterial or antifungal activity, but does have antiviral activity as measured by plaque inhibition assay.

Paecilomyces elegans IFO-6619 was selected in our program to screen for antiviral antibiotics from fungi. This fungus is classified as Fungi Imperfecti. According to H. L. BARNETT[1], *Paecilomyces* is very much like *Penicillium* except that phialides of the former are more divergent and tapering than that of the latter.

In primary screening study for antiviral antibiotics using the agar diffusion method[2], this fungus showed a strong antiviral activity. In this paper the production, isolation and some properties of an antiviral substance from the fungus are described.

Production and Isolation

One hundred ml of a medium consisting of 5% glucose, 0.5% peptone, 0.2% yeast extract, 0.06% KH_2PO_4, 0.1% NH_4Cl, 0.04% $MgSO_4 \cdot 7H_2O$ and 1% $CaCO_3$ was inoculated with *Paecilomyces elegans* IFO-6619 and shake-cultured at 26.5°C for 4 days. Three hundred ml of the culture thus obtained was transferred into 20 liters of the above medium in a 30-liter jar fermentor. The fermentor was stirred at 250 rpm and aerated at a rate of 15 liters per minute for 3 days at 27°C. The isolation of the antibiotic from the culture was performed according to the procedure outlined in Fig. 1. The culture was filtered to collect the mycelium. Acetone was added to the mycelial mass to extract intracellular products. Acetone was removed *in vacuo* and the extract was adjusted to pH 9.0 and was shaken several times with ethylacetate. The solvent phase from each extraction was collected, dried over anhydrous sodium sulfate and concentrated *in vacuo* to remove the ethylacetate. The crude extract thus obtained was chromatographed on a silicic acid (Mallincrodt) column. Two hundred and fifty grams of silicic acid with 50 g of Celite 545 were

suspended in benzene and put into a column of 5 cm in diameter. The crude extract in benzene was applied on the column. After washing with benzene, the column developed with a solvent mixture of benzene and methanol in the ratio of 90 : 10 (v/v). Eluate was collected. The solvent was removed by concentrating *in vacuo* and the residue dissolved in a small volume of methanol and allowed to stand at room temperature. The resulting white crystals were collected on a glass filter and dried *in vacuo*. The mother liquor was again chromatographed on a silicic acid column to obtain additional antibiotic. The concentrated mother liquor was applied on a column of silicic acid in benzene and washed with the same solvent. The elution of the colored materials with the solvent mixture of benzene and acetone (90 : 10, v/v) led us to obtain white crystalline powder from the top of the column, which was collected by turning the column upside down. The pure white crystals could be obtained by recrystallization from ethanol.

The name Paecilomycerol is suggested for this compound, after *Paecilomyces*, the fungi from which it was isolated.

Fig. 1. Isolation of paecilomycerol

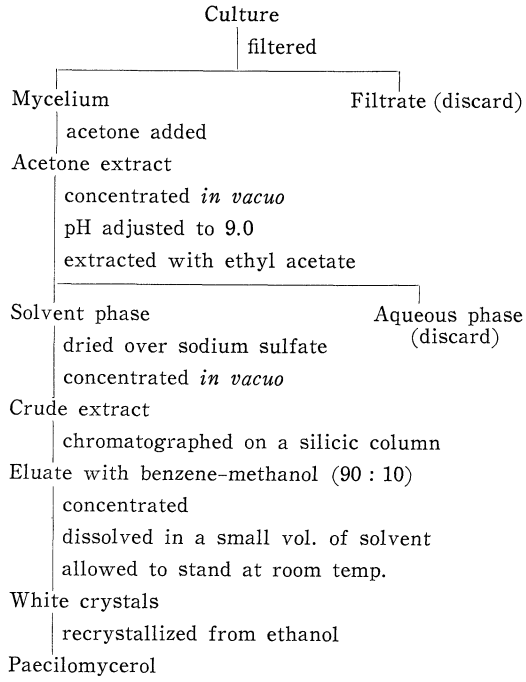
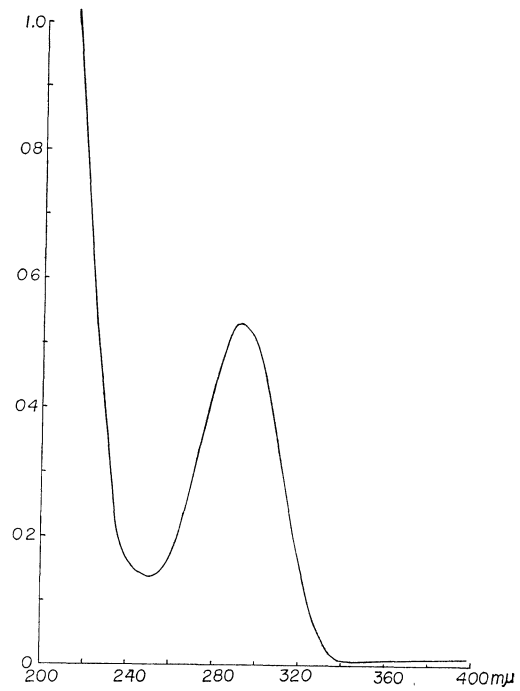


Fig. 2. Ultraviolet absorption spectrum in methanol.



Physical and Chemical Properties

Paecilomycerol was obtained as white needles, m. p. 193~194°C. It was soluble in pyridine and hot alcohol, sparingly soluble in cold alcohol and chloroform. The ultraviolet absorption spectrum given in Fig. 2 shows an absorption maximum at 293 mμ in methanol. The molar absorptivity (molar extinction coefficient) is 11,300. Fig. 3 shows the infrared absorption spectrum of paecilomycerol in nujol.

Fig. 3. Infrared absorption spectrum of paecilomycerol (nujol).

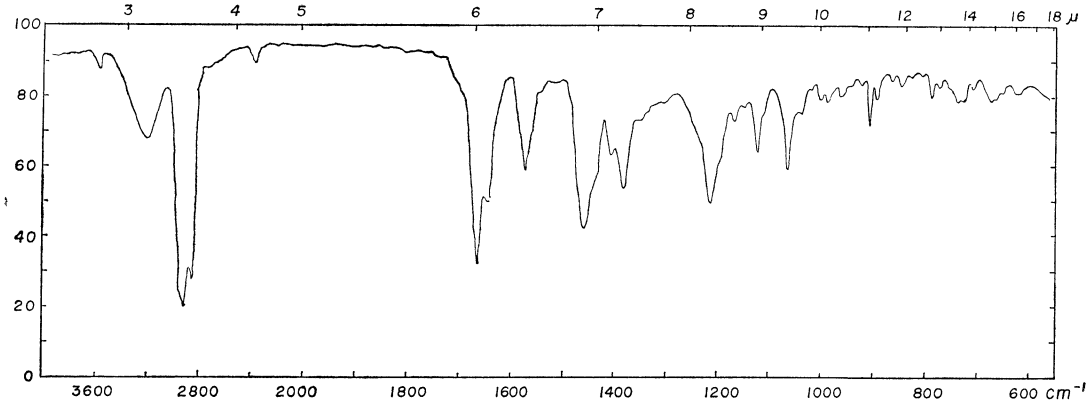


Fig. 4. Thin-layer chromatograms of paecilomycerol.

Solvents a. Benzene-acetone (2 : 1)
b. $CHCl_3$-methanol (7 : 1)
c. Ethylacetate

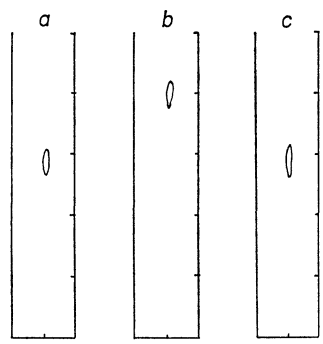


Table 1. Antiviral activity *in vitro* of paecilomycerol

μg/ml	CTZ	PFZ	
		NDV	HSV
125	—	>30	>30
62.5	—	>30	>30
32.0	—	>30	>30
16.0	—	>30	>30
8.0	—	28	25
4.0	—	25	23
2.0	—	22	23
1.0	—	17	16
0.5	—	15	16
0.25	—	15	16
0.125	—	+	+

CTZ: cytotoxic zone PFZ: plaque free zone

Anal. Found: C 75.20, 75.66; H 9.38, 9.24.
Calc'd for $C_{27}H_{40}O_4$: C 75.70, H 9.34.

The molecular weight obtained as a parent peak in the mass spectrum is 428 which agrees with that calculated from the elemental analysis. LIEBERMANN-BURCHARD reaction was positive (brown to green).

Paecilomycerol was studied for its behavior on the silica gel thin-layer chromatography. The chromatograms developed with three different solvent systems are presented in Fig. 4.

Biological Activity

Antimicrobial activity of paecilomycerol was studied using bacteria and fungi. No inhibitory effect on the all microorganisms tested was observed.

Antiviral activity of paecilomycerol *in vitro* was determined according to a method previously described[3]. The monolayers of chick embryo fibroblast in tissue culture were infected with either Newcastle disease virus (NDV) or herpes simplex virus (HSV) and overlaid with agar-medium. Paper disks 8 mm in diameter which had been dipped in the sample solution in methanol were put on the solidified medium and

incubated at 37°C for 48 hours. Antiviral activity is expressed as the diameter of the zone where plaque formation was inhibited. The cytotoxicity can also be measured at the same time, as a diameter of the zone where the color of the neutral red in the medium is reduced because cells had died. The results shown in Table 1 indicates that paecilomycerol has a strong antiviral activity *in vitro*. Careful observation of the plaque free zone (PFZ) indicates that the color of the cells within the PFZ is different from that of the typical viable cells. When microscopically observed, a part of the cells within such a zone were dead. Thus we concluded that paecilomycerol has only a weak cytotoxicity as only a part of the cell population was sensitive to it.

Discussion

Very few metabolites of *Paecilomyces* have been reported among which variotin is a biologically active metabolite of this family. Variotin was first isolated by H. YONEHARA *et al.*[4] from *Paecilomyces varioti* BAINIER var. *antibioticus*. Penicillin N was another antibiotic reported to be produced by *Paecilomyces persicinus* NICOT. Other known metabolites of this fungus are ustic acid and 4,6-dehydroxy-3-methoxyphthalic acid.

Paecilomycerol is a new antibiotic produced by *Paecilomyces elegans*. Though the result of LIEBERMANN-BURCHARD test of this antibiotic was weak to conclude that it is steroidal, mass spectral and NMR data support that hypothesis. In mass spectrum, a large fragment peak at m/e 257 appeared which is assumed to originate by simple cleavage of the C-17, C-20 bond with the loss of -OH in the ring. Other fragment ions are all consistent with steroidal structure. The details of the studies on its structure will be reported separately.

Paecilomycerol shows a large plaque free zone on the chick embryo monolayer infected with NDV. Though it does not show a usual cytotoxic zone in the tissue culture, paecilomycerol may have such an effect on the cells. A further study on its biological properties is under way.

Besides paecilomycerol, *Paecilomyces elegans* produces an antiviral substance which was isolated as an oil. The study on this substance will be reported elsewhere.

Acknowledgements

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