A NOTE ON THE ANTIFUNGAL ACTIVITY OF PENTACHLOROPHENYL DODECANOATE

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The antifungal activity of pentachlorophenyl dodecanoate against certain dermatophytes has been studied. The substance, in Sabouraud's maltose agar in 2 per cent concentration, inhibited the growth of *Microsporum canis*, *Trichophyton interdigitale*, *Trichophyton rubrum*, and *Microsporum audouini*. No activity was observed against *Candida albicans*, and only a slight inhibition in the growth of *Epidermophyton floccosum*. A 3 per cent concentration of the ester in an ointment base effected complete inhibition of *Trichophyton interdigitale* and *Microsporum canis* alone, and in mixed cultures.

The fungistatic activity of fatty acids, has been shown by Kiesel (1913) to increase with the number of carbon atoms, up to 11 or 12, maximum activity being obtained with 10-undecanoic acid. As a result of more critical tests, Golden and Oster (1947) demonstrated that this activity was relatively weak. Attempts to increase this by the formation of esters such as propylene glycol dipropionate, and propylene glycol dipelargonate, were not successful, the latter giving 58 per cent of treatment failures in cases of tinea capitis (Sullivan and Bereston, 1952). Since trichlorophenol is a clinically effective fungicide in superficial mycoses, but causes skin irritation (Hopkins, Fisher, Hillegas, Ledin and Camp, 1946), it was considered that esters of the type, $Me{\cdot}[CH_2]_n{\cdot}COOR$ or, $CH_2:CH{\cdot}[CH_2]_n{\cdot}COOR$, where n is less than 10 and R a halogenated phenol, might show antifungal activity. Further, esterification in this manner might lessen the toxicity of the phenol, since the *ortho*-alkyl derivatives of *p*-chlorophenol show a decrease in toxicity from the methyl to the n-heptyl, and at the same time an increase in antibacterial activity as the molecular weight increases (Klarman, Shternov and Gates, 1934).

During preliminary work on compounds of the type postulated above, it was found that the pentachlorophenyl dodecanoate was manufactured in large amounts as a fungicide for the textile industry. The present paper is a preliminary study of the activity of this substance against certain dermatophytes.

Materials and Methods

According to the manufacturers, the pentachlorophenyl dodecanoate is normally prepared and used in a liquid form at room temperature. For the present investigation, a purer form was made to Ministry of Supply Specification C.S. 2616. This is a solid with a melting point of 46·5°, and a setting point of 33°. The white, wax-like solid is insoluble in water and the lower alcohols, solubility beginning to be appreciable from isopropanol upwards. It is soluble in non-polar solvents such as acetone, benzene, and methyl ethyl ketone.

Organisms Used

Cultures of *Trichophyton rubrum* D 361, *Microsporum canis* 352, *Trichophyton interdigitale* 296, and *Trichophyton mentagrophytes* 336, were obtained from the London School of Hygiene and Tropical Medicine. Other organisms were: *Microsporum audouini*, *Candida albicans*, and *Epidermophyton floccosum*, which had been isolated during routine work in a Hospital Bacteriology Department. These were maintained on Sabouraud's maltose agar, pH 5·2 and kept at 25°. Subcultures were usually made at 6 to 8 weeks. Following suggestions by Golden and Oster (1947) and McCrea (1940) 10 to 15 day cultures were used in the assays so that all growth elements such as hyphae, micro- and macronidia and chlamydospores were present.

Experimental and Results

The ester was incorporated in Sabouraud's maltose agar by melting 200 ml. of the medium in a Koch steam steriliser, and adding 2 g. of pentachlorophenyl dodecanoate in thin shavings. During the addition, the mixture was stirred mechanically under sterile conditions, then 10 ml. aliquots were dispensed into sterile Universal containers. Since the specific gravity of the ester is 1·25, slopes were rapidly cooled to prevent deposition, and stored at 6°.

In preliminary assays with *T. rubrum* D361, pieces of mycelia from 15 day cultures of the organism were inoculated into 6 tubes of Sabouraud's maltose agar. At the same time, 6 tubes of medium containing 1 per cent of the ester were inoculated in a similar manner. At this stage, no attempts were made to standardise the size of the inoculum, although in each case mycelial strands about 5 mm. long were used. The periphery of each was then roughly outlined on the outer wall of the container with Indian ink, and the tubes incubated at 25°.

The results showed that there was slight growth in the medium containing 1 per cent pentachlorophenyl dodecanoate, after 6 days at 25°, as opposed to 2 days with the controls. After 14 days, no further growth could be detected, and the cultures had a shrunken and granular appearance. When the concentration of the ester was increased to 2 per cent, the growth of *T. rubrum* was inhibited for 14 days, one tube out of six, showed slight growth, and then no further growth could be detected.

A 2 per cent concentration of pentachlorophenyl dodecanoate, under similar test conditions, was found to inhibit the growth of *M. canis*, *T. interdigitale* and *M. audouini*. No action was found in plates using *C. albicans*, and only a slight inhibitory effect with a strain of *E. floccosum*.

Antifungal Activity in an Ointment Base

An ointment base consisting of liquid paraffin, 12; white soft paraffin, 30; and cetomacrogol emulsifying wax, 18 g. was prepared and sterilised at 150° for 1 hr. and allowed to cool to about 70°. Sterile Sabouraud's broth (140 ml.) was then added aseptically with mechanical stirring. Just before solidification, the emulsion was poured into sterile Petri

plates, which served as controls. Test plates were prepared by taking a similar mixture, heating it to 70°, and adding 6 g. of pentachlorophenyl dodecanoate in thin shavings, and then adding 134 ml. of sterile Sabouraud's broth.

Inoculum. Cultures of *T. interdigitale* and *M. canis* on Sabouraud's maltose agar were inoculated into Sabouraud's broth and incubated at 25° for 10 days. The mycelial pads in each were broken up, and the tubes shaken mechanically. Test plates were inoculated by flooding with 2 ml. of the cellular suspension, and incubated at 25°. Control plates without the fungicide were similarly treated. Table I shows the growth of both organisms without the test substance.

TABLE I

GROWTH PATTERNS IN AN EMULSION MADE WITH SABOURAUD'S BROTH WITHOUT PENTACHLOROPHENYL DODECANOATE

Culture	Days after Inoculation	
	4	12
T. interdigitale plus *M. canis*	Growth. Surface hyphae	Profuse growth over plate. Pale yellow pigment present
M. canis	Rapid growth. Pale yellow pigment	Growth over whole plate. Yellow pigment
T. interdigitale	Patches of white mycelium	Growth over whole plate

With a concentration of 3 per cent pentachlorophenyl dodecanoate, there was complete inhibition of *T. interdigitale* and *M. canis* alone and in mixed culture.

DISCUSSION

The difficulty of assaying water-insoluble compounds for antifungal activity has been noted by Golden and Oster (1947a) and by Reddish (1947). Golden and Oster (1947a) introduced an agar diffusion method, in which the substance under test is dissolved in ethanol and 1 ml. aliquots placed in cups cut in the culture plate. Although these authors found the method successful in assaying simple compounds such as phenol, benzoic acid and related products, in our hands the method was unsatisfactory. Pentachlorophenyl dodecanoate is not very soluble in ethanol, and the long incubation times necessary for growth of the cultures caused the solutions to evaporate.

The insolubility of the ester in water also caused difficulties when attempts were made to determine whether the action was fungistatic or fungicidal. After being in contact with the emulsion described previously, the pieces of mycelium, or mycelial pads cannot be washed free of the active material with Sabouraud's broth, before placing in fresh medium. For some types of compounds, Golden and Oster (1947b) used a 30 per cent v/v solution of acetone in water to effect removal, before subculturing. With pentachlorophenyl dodecanoate the procedure is not applicable.

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