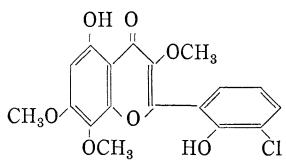
Communications to the editors

CHLORFLAVONIN, A NEW ANTIFUNGAL ANTIBIOTIC

Sir:

We have isolated and characterised a novel antibiotic substance, which we have called chlorflavonin, from cultures of some strains of *Aspergillus candidus* LINK. Low concentrations of the substance in liquid or solid culture media inhibit growth of *Aspergillus fumigatus* and a few other species of mould fungi.

Chlorflavonin is a yellow microcrystalline solid of m. p. 212°C. It has low solubility in water (8 mcg/ml) but is more soluble in organic solvents, *e. g.* 100 mcg/ml in ethanol, 25 mg/ml in chloroform, and 35 mg/ml in dimethylformamide. The molecular formula, $C_{18}H_{15}O_7Cl$, was obtained by high resolution mass spectroscopy. Colour tests and the infrared and nuclear magnetic resonance spectra of chlorflavonin and various derivatives indicate that it is a flavone, and structure I (3′-chloro-5,2′-dihydroxy-3,7,8-trimethoxy flavone) was established by identification of the products from alkaline hydrolysis of chloroflavonin and its dimethyl ether.



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Chlorflavonin is apparently the first fully characterised flavone to be isolated as a fungal metabolite[1] and the first naturally occurring chlorinated flavone from any source. Also we believe there are no previous reports of a chlorinated flavone exhibiting antifungal properties.

The antibiotic was first discovered in laboratory fermentations carried out with *Aspergillus candidus* BRL 274, (ATCC 20022, IMI 127259) in the following procedure. Spores from an agar slant culture of the fungus were inoculated into 500 ml Erlenmeyer flasks containing 100 ml of corn-steep liquor dextrose broth (Corn-steep liquor 50 ml, anhydrous dextrose B. P. 50 g, chalk 2 g, and water to 1 litre; pH adjusted to pH 6.6 before sterilization). The flasks were incubated at 26°C for five days on a rotary shaker. Filtered samples of culture liquor taken at this time on dilution with MYGP culture broth[2] prevented growth of inoculum of *Aspergillus fumigatus* while having no observable effect on inocula of *Trichophyton mentagrophytes*, *Candida albicans* and *Cryptococcus neoformans*. This specificity of action indicated the presence in the fermentations of a novel substance. The activity could be extracted from culture liquor with chloroform, toluene and other organic solvents. The active substance occurred in roughly equal quantities in the cell-free culture liquor and in the mycelial fraction.

Subsequently larger scale brewing was carried out in small fermenters and the following method established for isolating the chlorflavonin. Harvested whole broth (mycelium and culture liquor) was acidified to pH 4.5. At this pH about 90 % of the

Table 1. Antibiotic activity of chloroflavonin against various fungi *in vitro*

Species tested	Minimum inhibitory concentration (mcg/ml)*
Alternaria tenuis	>20
Aspergillus amstelodami	0.08
Aspergillus fumigatus	0.08
Aspergillus nidulans	>20
Aspergillus niger	>20
Aspergillus ochraceous	0.08
Aspergillus repens	>20
Aspergillus terreus	>20
Aspergillus versicolor	>20
Botrytis cinerea	5
Candida albicans	>20
Cryptococcus neoformans	>20
Fusarium oxysporum	>20
Mucor pusillus	>20
Nocardia asteroides	>20
Paecilomyces variotii	2.5
Saccharomyces cerevisiae	>20
Scopulariopsis brevicaulis	>20
Trichophyton mentagrophytes	>20
Verticillium albo-atrum	>20

* In SABOURAUD's glucose agar or MYGP broth.

antibiotic was precipitated and could be filtered off with the mycelium. The moist mycelial cake was extracted with mixtures of aromatic and aliphatic hydrocarbon solvents. Evaporation of the extract precipitated chlorflavonin about 50% pure. This crude product was recrystallized from benzene and petroleum ether to give pure chlorflavonin.

Chlorflavonin exhibits a remarkable degree of specificity in its antibiotic activity (Table 1). A few species of fungi are highly sensitive to the compound, their growth being completely inhibited by less than one microgram per millilitre of culture medium, but most of the fungi tested are not suppressed by chlorflavonin.

More detailed accounts of the properties and production of chlorflavonin are to be published.

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