backwards attack, so that the reaction has the characteristics of a nucleophilic substitution.

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Effect of the Interaction between Chelating Agents on their Fungitoxicity

THE role of chelation in the mechanism of action of 8-hydroxyquinoline ('oxine') against fungi has been reviewed by Horsfall[1] and Hollingshead[2]. The increased bactericidal and fungicidal activity of this compound in the presence of Cu^{2+} has been ascribed to the rapid penetration of the cell wall by the highly lipophilic 1 : 2 copper : oxine complex[3,4], which may dissociate within the cell to a more toxic substance or substances.

More recently, Zentmyer and Rich[5] demonstrated that the toxicity of oxine and copper oxinate to spores and mycelium of *Aspergillus niger* van Tiegh. was reversed on Czapek's agar by L-histidine (0·5 per cent) or L-cysteine (0·5 per cent), both of which were shown to compete for the copper and liberate free oxine. A similar biological effect was reported by Heath and Clark[6], who showed that the inhibition of root growth of wheat seedlings by 10^{-5} *M* oxine was partially reversed by ethylenediamine tetraacetic acid (EDTA) at a concentration as low as 10^{-11} *M*.

We have examined the fungistatic properties of a series of 5-*n*-alkyl oxines[7] towards *A. niger*. The fungus was grown on a glucose–mineral salts–agar medium (*p*H 5·0) without added trace metals. The compounds were incorporated in the medium at a final concentration of 10^{-4} *M*, and the growth of the mycelium (at 25°) was assessed over the 2–4 day period after inoculation[8]. The fungistatic activity of the compounds rose to a maximum at a chain-length of 5 or 6 carbon atoms. In another experiment, the addition of Cu^{2+} (10^{-4} *M*) markedly reduced the activity of 5-*n*-amyl- and 5-phenyl-oxine (10^{-4} *M*). This effect could be reversed by the addition of the disodium salt of ethylenediamine tetraacetic acid (10^{-3} *M*), which presumably liberated the free oxines. Oxine itself, by contrast, was active in the presence of Cu^{2+}, and inactive in the presence of excess ethylenediamine tetraacetic acid. All three compounds showed intermediate activity in the basal medium alone, in which the only trace metals present arose from impurities in the constituents. Neither Cu^{2+} nor ethylenediamine tetraacetic acid reduced growth significantly in the absence of oxines (Table 1).

Table 1. MEAN RADIAL MYCELIAL GROWTH (MM./DAY) OF *A. niger* IN THE PRESENCE OF THREE 8-HYDROXYQUINOLINES (10^{-4} *M*)

Compound	Cu^{2+} and EDTA added	Cu^{2+} added	EDTA added	No addition
Oxine	12·6	0·0	12·9	4·4
5-Phenyl oxine	0·0	12·2	0·0	5·4
5-Amyl oxine	0·0	9·8	0·0	2·0
Control	12·8	13·3	13·6	12·9

Least significant difference between means ($P = 0·05$) : 2·70
($P = 0·01$) : 3·70
($P = 0·001$) : 5·04
The interactions Cu^{2+}/oxines and oxines/EDTA are both significant ($P < 0·001$)

The fungistatic activity of the 5-*n*-alkyl oxines follows the pattern normally encountered in such a homologous series[9]. Owing to their greater lipoid solubility, unchelated molecules of 5-amyl and 5-phenyl oxines are able to penetrate the fungal cell wall more rapidly than those of oxine itself, which is more active in the form of its 1 : 2 copper complex[4]. It is suggested that the decrease in the activity of 5-amyl and 5-phenyl oxines in the presence of Cu^{2+} may arise from too low an aqueous solubility of the corresponding 1 : 2 copper : oxine complexes.

Experiments on the effect of ethylenediamine tetraacetic acid on the toxicity of a wider range of chelating agents to the mycelium of *A. niger* will be described elsewhere. Preliminary results indicate that the addition of an excess of ethylenediamine tetraacetic acid to the basal medium results in a significant increase in the fungistatic activity of a number of compounds, including 5 : 7-dichloro-oxine (10^{-5} *M*) and 2-mercaptobenzthiazole (10^{-4} *M*). It is of interest that both these compounds have already been reported to be more active in their unchelated form than as their copper or zinc chelates respectively[10,11].

In order to test an unchelated molecule *per se* it is first necessary to remove metal ions from the culture medium. This may virtually be achieved, and fungal growth still supported, by the incorporation of an excess of a second chelating agent, itself non-toxic. Such a procedure may prove valuable, not only in studies on the mode of biological action of chelating agents, but also in their application in the control of bacteria and fungi.

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