# MODE OF ACTION OF DOTHISTROMIN

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## ABSTRACT

Dothistromin is a bright orange-red pigment produced by the pine-needle pathogen Dothistroma pini Hulbary, the causal agent of a necrotic disease known as dothistromal blight. This compound, implicated as a fungal toxin, has been isolated both from laboratory cultures of D. pini and from infected Pinus radiata foliage.

Detailed chemical investigation by Gallagher (1971) showed that dothistromin is a tri -α- hydroxyanthraquinone fused to a substituted tetrahydrobifuran ring system. The bifuran ring moiety is incorporated in other fungal metabolites including the extremely toxic and carcinogenic aflatoxin compounds. There is an increasing body of evidence to suggest that these fungal metabolites share a common biosynthetic origin.

Bioassay has demonstrated the toxicity of dothistromin to the unicellular green alga, Chlorella pyrenoidosa, and to tissue cultures of P. attenuata. The very low level of solubility of the compound in aqueous solutions has precluded bioassay using pine seedlings. This thesis reports an investigation of the biochemical changes induced by dothistromin in microbiological systems.

In the course of this investigation dothistromin has been shown to be toxic to a range of microorganisms in addition to its known toxicity to Chlorella pyrenoidosa. These studies have suggested possible ways of increasing the sensitivity of bioassays for dothistromin.

It was found that the addition of dothistromin to liquid cultures of Chlorella as an ethyl acetate solution caused reproducible levels of inhibition, provided that the ethyl acetate concentration was less than 0.5%. Batch culture techniques were used to establish the levels of dothistromin required for inhibition of growth of Chlorella. The ratio of dothistromin concentration to cell number was found to be an important factor in the inhibition response.

Utilization of synchronous culture techniques permitted the study of biochemical changes induced by dothistromin throughout the cell cycle of Chlorella. Results showed a marked inhibition of the rate of increase of total protein and RNA over the cell cycle with no significant alteration of the rate of DNA increase.

A dose-response curve for dothistromin inhibition of growth of Chlorella was established and a more detailed investigation of the action of dothistromin in inhibiting growth was undertaken using radioactive isotopes. By this means it was shown that $^{3}$H-uridine and $^{14}$C-phenylalanine incorporation into cell material

is inhibited within 30 mins of exposure to the toxin. Difficulties encountered in attempts to obtain satisfactory incorporation of label into Chlorella DNA-fractions prevented further investigation of the effect of dothistromin on DNA synthesis in this organism. This led to the investigation of other microorganisms as more suitable experimental systems for this study.

Bacillus megaterium KM, proved to be very sensitive to dothistromin and showed rapid incorporation of radioactive isotopes into protein and nucleic acid fractions. Growth curves established that the inhibitory ratios of dothistromin concentration to cell numbers for this organism were in the order of 0.25 $\mu g$/cell X $10^8$ (as compared to 2.0 $\mu g$/cell X $10^8$ for Chlorella). At this concentration, over the 30 min time course studied, dothistromin had no effect on the incorporation of $^3$H-thymidine into the DNA fraction. Inhibition of $^3$H-uridine incorporation was evident at 6 min and very marked by 10 min while the inhibition of $^{14}$C-phenylalanine incorporation into protein was not evident until considerably later. The effects of dothistromin in this system were compared with those of antibiotics with known sites of action. Dothistromin inhibition of $^3$H-uridine incorporation has a similar time course to that shown by actinomycin D, although marked inhibition by Actinomycin D is evident at 3 min, whereas dothistromin inhibition is not noticeable until 6 min.

On the basis of these results it is suggested that dothistromin interferes with RNA synthesis and that the observed inhibition of protein synthesis is a secondary effect of this impairment.

Confirmation of dothistromin action 'in situ' by administration of the compound to pine seedlings is necessary before any definitive statement can be made concerning its role in dothistromal blight. However these results indicate the possible importance of dothistromin in pathology of dothistromal needle blight of pines. Impairment of the RNA synthetic capacity in pine needle tissue by the toxin could rapidly lead to cell death and to the necrosis of needle tissue observed in diseased foliage.

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