THE STRUCTURE OF DOTHISTROMIN

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by

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ABSTRACT

The fungus Dothistroma pini Hulbary is a needle pathogen of Pinus radiata and other pines, producing a necrotic disease commonly known as Dothistroma needle blight. The fungus is widely distributed in major pine forests throughout the world; it was reported in New Zealand on P. radiata in 1964.

The species P. radiata, P. ponderosa, and P. nigra (laricio) are all grown extensively in New Zealand and unfortunately are all highly susceptible to the disease. Because forestry plays an important role in the New Zealand economy, the disease is of economic significance to New Zealand. The disease can be controlled by spraying with insoluble compounds of copper.

Following a suggestion that D. pini might produce a toxin responsible for host cell death, a red pigment was isolated from D. pini cultures and shown to be toxic to Chlorella pyrenoidosa, a unicellular green alga. This thesis is concerned with a detailed investigation into the nature and characterisation of the red pigment.

D. pini was cultured in the laboratory on an aqueous malt medium, and red pigment extracted from the cultures and purified by thin layer chromatography was shown by mass spectroscopy to be a mixture of two closely related compounds of molecular formula $C_{18}H_{12}O_9$ and $C_{18}H_{12}O_8$. The former compound which was present in greater amount, was named dothistromin, and the latter was named deoxydothistromin. Both dothistromin and deoxydothistromin were shown to be present in extracts of D. pini infected P. radiata needles.

A detailed chemical investigation using chemical reactions (including the classical degradative technique of zinc dust distillation), derivative formation, infrared spectroscopy, electronic absorption spectroscopy, nuclear magnetic resonance spectroscopy,

and mass spectroscopy, allowed elucidation of the structure of dothistromin. Dothistromin was shown to be a tri-α-hydroxyanthraquinone onto which was fused a substituted tetrahydrodifuro ring system.

A major feature of the structure of dothistromin is the substituted tetrahydrofuro[2,3-b]benzofuran moiety. Fungal metabolites known to incorporate this structural feature include the toxic and potently carcinogenic aflatoxins, and the carcinogenic sterigmatocystin. A discussion on the possible carcinogenicity of dothistromin, its co-metabolites, and artefacts is included.

The strong green-yellow fluorescence of solutions of the red pigment and dothistromin, when irradiated with ultraviolet light is attributable to the 1,4-dihydroxyanthraquinone chromophoric nucleus of dothistromin.

Another important structural feature of dothistromin is the reactive hemiacetal group, allowing dothistromin to undergoe facile acid catalysed mono-alkylation and mono-acetylation.

The probability that in solution dothistromin exists as a complex equilibrium mixture, was discussed.

The mass spectrum of dothistromin shows a characteristic loss of the formyl radical CHO$^{\bullet}$ (m/e 29), and the neutral fragment C_2H_4O (m/e 44). The same loss of a formyl radical, and a homologous neutral fragment was also shown by a number of dothistromin derivatives. Two fragmentation schemes were proposed to rationalise the mass-spectral fragmentation of dothistromin.

During the course of the investigation, a number of crystalline, optically active derivatives of dothistromin were prepared; these included dothistromin penta-acetate and dothistromin ethyl ether tetra-acetate. The structure and absolute configuration of a crystalline heavy atom derivative of dothistromin was determined

by an x-ray crystallographic diffraction study. This confirmed the structures proposed in this thesis, and also allowed the absolute configuration of the cis-fused furo rings of dothistromin to be deduced.

Deoxydothistromin was assigned one of two structures, and the nature of other co-metabolites was briefly considered. The synthesis and biosynthesis of dothistromin was also discussed.

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