THE BIOSYNTHESIS OF DOTHISTROMIN

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G. JOHN SHAW

Massey University

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ABSTRACT

This thesis is concerned with the biosynthesis of dothistromin (2,3,3a,12a tetrahydro-2,3a,4,6,9 pentahydroxy-anthra[2,3-b]furo[3,2-d]furan-5,10 dione) by the fungus Dothistroma pini. The biosynthesis of related secondary metabolites is reviewed and as a working hypothesis it is proposed that dothistromin is solely acetate-derived.

In the preliminary phases of the investigation strains of the organism giving high yields of the metabolite were sought and isolated from natural sources. Some growth media were tested for their ability to support growth, promote sporogenesis and sustain high yields of dothistromin. A medium containing malt and dried whole yeast was chosen. The growth characteristics of the organism in this medium were studied and the temporal relationship between growth and pigment production for a variety of cultural conditions was found. The findings of these experiments suggested times when it would be favourable to add possible precursors.

Incorporation studies with [1-^{14}C]-sodium acetate revealed that dothistromin incorporated isotope from this precursor and disclosed that the lipids heavily incorporated the label. Subsequent experiments were concerned with examining the effects that precursor concentration, time of precursor addition and time of metabolite harvesting had on the isotope enrichment and yield of dothistromin.

It was found that the optimisation of these two parameters were mutually exclusive processes and compromise conditions which were compatible with obtaining both reasonably good enrichments and yields of dothistromin had to be selected.

Initially attempts were made to determine the distribution of isotope in dothistromin, which had incorporated isotopically labelled acetate, by chemical degradation. Potassium tertiary-butoxide/water cleavage of the anthraquinone ring of the pentamethyl derivative of dothistromin labelled by [1-^{14}C]-acetate yielded 1,4-dimethoxybenzene which had a molar specific radioactivity that was 0.33 times that of the starting material. This finding was consistent with the formation of dothistromin from nine molecules of acetate.

Subsequently ^{13}C-NMR techniques were used to determine the distribution of isotope in dothistromin derived from [1-^{13}C] and [2-^{13}C]-acetates. Pulsed Fourier transform ^{13}C-NMR spectra of the monoethyl acetal derivative of dothistromin were obtained using broad-band proton decoupling and off-resonance proton decoupling. By comparison with the ^{13}C-NMR spectra of a number of model compounds the resonances in the spectrum of the dothistromin derivative were assigned in most cases to specific carbon atoms and in a few instances to two or three alternatives.

The ^{13}C-NMR spectra of the dothistromin derivatives which had been enriched by isotope from the carbon-13 labelled acetates showed nine resonances with intensities enhanced by enrichment

from the carboxyl carbon of acetate, eight from the methyl carbon and one resonance of uncertain origin. The distribution of isotope in the anthraquinone moiety of the molecule was consistent with its formation from a polyketide precursor but this was not proven because of the equivocal assignment of some of the NMR signals. The distribution of isotope in the furofuran ring moiety and its relation to the distribution in the anthraquinone part was the same as that reported by others for corresponding structures in aflatoxin B_1 and sterigmatocystin.

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