THE HYDROXYLATION OF TROPANE ALKALOIDS

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The role of cytochrome P450 as an enzyme responsible for hydroxylation reactions in mammalian systems is well documented (Wickramsinghe,1975). Bacteria and fungi have also been shown to contain this cytochrome and the majority of cases where the metabolic function of the enzyme is known are hydroxylation reactions (Wiseman,1977). Some instances of cytochrome P450 mediated hydroxylations in higher plants are also known, the most investigated system being the conversion of trans-cinnamic acid to its hydroxylated derivative, p-coumaric acid (Rich and Lamb,1977).

Datura species are known to produce a variety of tropane alkaloids, many of which are tigloyl or tropoyl esters of mono-, di- and trihydroxytropane. If cytochrome P450 is the enzyme responsible for the successive hydroxylation of the tropane nucleus then it should be possible to construct an in vitro system which would simulate the biosynthesis of the hydroxytropanes.

It was thus decided to investigate the possible synthesis of meteloidine from 3α-tigloyloxytropane, using the cytochrome P450 of rat liver microsomes. The experimental technique was based on that of Phillipson and others (1976).

Tropine labelled with ^{14}C in the N-methyl position was prepared by the method reported previously,(Basey and Woolley,1975), and esterified with tigloyl chloride. The labelled 3α-tigloyloxytropane (3αTT) was incubated with rat liver microsomes and an NADPH generating system at 37 C for 75min. Three controls were used: a) without cofactors b) in an atmosphere enriched with carbon monoxide c) with microsomes previously heated in boiling water for 3min. Following termination of the reaction a known amount of carrier meteloidine was added to each reaction mixture, and the alkaloids were extracted into chloroform after basification. The meteloidine was freed of 3αTT on an alumina column and converted to the picrate derivative. The activity of duplicate samples of each picrate was ascertained by liquid scintillation counting.

Preliminary results show that rat liver microsomes would catalyse the conversion of 3αTT to meteloidine. The reaction was dependent on NADPH and was inhibited by carbon monoxide, thus suggesting that cytochrome P450 is the enzyme responsible.

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