BIOSYNTHESIS OF HYDROXYTROPANES

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We reported previously (Major, Davies & Woolley 1978) that the conversion of 3α-tigloyloxytropane to meteloidine was catalysed by the cytochrome P-450 of rat liver microsomes. The presence of cytochrome P-450 in roots of Datura stramonium has now been demonstrated (Major 1981). Since this enzyme is known to catalyse hydroxylation reactions in higher plants (West 1980) it was thought possible that it might be responsible for some or all of the hydroxylation stages in the metabolism of tropine and its tigloyl ester into the di- and trihydroxytropanes.
3α-Tigloyloxytropane-[N-14C] was incubated with Datura stramonium root microsomes and an NADPH generating system. After 1hr at 37°C the reaction was terminated and carrier meteloidine was added. The alkaloids were extracted and the meteloidine purified by column chromatography.The base was converted to its picrate and then recrystallised to constant specific activity. Simultaneous incubations were performed using conditions selected to inhibit cytochrome P-450. In a second experiment the labelled substrate, (3αTt),was incubated with the post-mitochondrial enzymes from a Datura stramonium root homogenate, together with an NADPH generating system. 6β-Hydroxy-3α-tigloyloxytropane, not meteloidine, was added as carrier in this experiment. Again, simultaneous incubations were performed under conditions selected to inhibit cytochrome P-450.

It was found that the hydroxylation of 3αTt to its 6β-hydroxy derivative could be catalysed by the enzyme fraction used, and that the reaction was inhibited by SKF 525A (a known inhibitor of P-450). However whilst it was also found that the root microsomes could effect the conversion of 3αTt to meteloidine, this reaction was not inhibited by SKF 525A neither by carbon monoxide nor by absence of NADPH. This suggests that the monohydroxylation of 3αTt is catalysed by a cytochrome P-450 system. Furthermore it would appear that this reaction is not in the pathway from 3αTt to meteloidine and that this path is independent of cytochrome P-450.

Earlier work (Beresford & Woolley 1975) implied that 3αTt was at a branch point in the pathways of hydroxytropane biosynthesis. The work reported above thus supports that view but is at variance with the opinion that the biosynthesis of meteloidine from 3αTt requires a 6β-hydroxy intermediate (Leete & Lucast 1976).

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