Degradation of *p*-Methoxybenzoate by Cell-Free Extracts of *Pseudomonas fluorescens*

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Bacteria grown on *p*-methoxybenzoate are induced to metabolize *p*-methoxybenzoate, *p*-hydroxybenzoate and protocatechuate (Cartwright & Buswell, 1969; Cartwright *et al.*, 1970).

A cell-free system from *Pseudomonas fluorescens* strain PM3, grown on *p*-methoxybenzoate as sole carbon and energy source, catalysed the oxidative demethylation, hydroxylation and subsequent ring cleavage of *p*-methoxybenzoate. Demethylation, to yield *p*-hydroxybenzoate, involved absorption of 1 mol of O₂/mol of *p*-methoxybenzoate and required reduced nicotinamide nucleotide (either NADH or NADPH) as cofactor. *p*-Hydroxybenzoate, the first detectable intermediate, was hydroxylated to protocatechuate by an NADPH-dependent *p*-hydroxybenzoate hydroxylase (Hosokawa & Stanier, 1966) with the absorption of 1 mol of O₂/mol of substrate. Protocatechuate was oxidized by *ortho* fission to 3-oxoadipate.

The methyl group of *p*-methoxybenzoate was removed as formaldehyde, and oxidized to formate and carbon dioxide by formaldehyde dehydrogenase, which required GSH and NAD⁺, and formate dehydrogenase, which required NAD⁺. Each step accounted for the absorption of 0.5 mol of O₂/mol of substrate. All enzymic activity was present in the supernatant fraction prepared by centrifugation of sonically disrupted cells at 270000g for 3 h.

The ether-cleaving enzyme in *Ps. fluorescens* strain PM3 is specific for a substituent *para* to a carboxyl group. Of the methoxylated compounds examined only *p*-methoxybenzoate, isovanillate (3-hydroxy-4-methoxybenzoate) and veratrate (3,4-dimethoxybenzoate), resulting in the accumulation of vanillate, were oxidized. It is noteworthy that the demethylase system catalysed the oxidation of piperonylate (3,4-methylenedioxybenzoate) with the absorption of 2 mol of O₂/mol of substrate. Activity was NADH-dependent and resulted in the accumulation of Rothera-positive material, the 2,4-dinitrophenylhydrazone of which was chromatographically identical with the laevulinate derivative.

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Studies on the Biosynthesis of Cyclic Depsipeptides in *Serratia marcescens*

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Almost all the important features of the biosynthesis of cyclic depsipeptides are unknown (Taylor, 1970); these include the activation of the component moieties, the mechanism of formation and the way in which the order of the residues is controlled. The biosynthesis of these compounds is of particular interest since studies with chloramphenicol (Katz *et al.*, 1965) have shown that cyclic depsipeptides are formed by a system that is quite different to that of protein synthesis and probably non-ribosomal. The cyclic depsipeptides of *Serratia marcescens* (Bermingham *et al.*, 1970a) are among the simplest representatives of this group of lipids, and the major component, serratamolide (which contains only L-serine and D-3-hydroxydecanoate), offers one of the best opportunities for biosynthetic studies. These have been attempted by (a) feeding of radioactive precursors to *S. marcescens* and (b) inhibition studies with streptomycin (which blocks serratamolide biosynthesis; Bermingham *et al.*, 1970b).

3-Hydroxy[¹⁴C]decanoate (isolated from the rhamnolipid of [¹⁴C]acetate-grown *Pseudomonas aeruginosa*; Hauser & Karnovsky, 1954) and [¹⁴C]acetate were fed to *S. marcescens* N.T.C.C. 1377 in the presence and in the absence of streptomycin (10 µg/ml of medium) and the resulting radioactive lipids were analysed by t.l.c. as previously described (Bermingham *et al.*, 1970b). Radioactivity was incorporated readily from acetate into serratamolide and this incorporation was blocked by streptomycin, but no incorporation into cyclic depsipeptide was found from 3-hydroxy[¹⁴C]decanoate even though this compound was taken up and metabolized into phosphatidylethanolamine and phosphatidylglycerol. Subsequent feeding of [1-¹⁴C]octanoate did give rise to [¹⁴C]serratamolide. It appears that some precursor of 3-hydroxydecanoate rather than the acid itself is utilized for serratamolide biosynthesis.

G.l.c. analysis of the fatty acids from a purified cyclic depsipeptide fraction of *S. marcescens* grown in the presence of streptomycin showed complete inhibition of the synthesis of 3-hydroxydecanoate with the simultaneous appearance of two non-hydroxy fatty acids (not present in the control cells), which were identified as dodecanoate (laurate) and tetradecanoate (myristate). It may be that, in the absence of a demand for 3-hydroxydecanoate for serratamolide production, this acid is not released from the fatty acid-synthesizing complex.

We have previously shown that L-[¹⁴C]serine