u.v., visible and i.r. spectra, by its conversion in acidic ammonium acetate into 6-methyl-2-picolinate, and by its rapid hydrolysis by extracts of *Ps. aeruginosa* T1 to yield pyruvate and acetate (Ribbons, 1966b). 2,6-Dioxoheptenoate is also known to be the product of 3-methylcatechol oxidation catalysed by catechol 2,3-oxygenase of other pseudomonads (Dagley, Chapman, Gibson & Wood, 1964; Ribbons, 1966a, b; Catelani, Fiechi & Galli, 1968). 3-Methylcatechol is not oxidized by cells or extracts of 2,3-dihydroxybenzoate-grown *Ps. fluorescens* 23D-1, nor is it formed anaerobically from 2,3-dihydroxy-*p*-toluate. 2,3-Dihydroxy-*p*-toluate is not an inducer for the oxygenase, nor a growth substrate for *Ps. fluorescens*. The results are consistent only with a ring cleavage of 2,3-dihydroxy-*p*-toluate in the 3,4-position, and thereby indicates that the site of cleavage of 2,3-dihydroxybenzoate is at the 3,4-position.

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Inhibition by Streptomycin of the Biosynthesis of Cyclic Depsipeptides in *Serratia marcescens*

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An acetone-soluble lipoamino acid, serratamic acid, was isolated from *Serratia marcescens* during a study of the biosynthesis of the tripyrrolic pigment prodigiosin (Cartwright, 1955); it was shown to consist of a linear amide of L-serine and β -hydroxydecanoic acid (Cartwright, 1957). A dimer of serratamic acid, serratamolide, was later discovered (Wasserman, Keggi & McKeon, 1961) and proved to have a cyclic depsipeptide structure that gave rise to two molecules of serratamic acid on mild alkaline hydrolysis. It has been speculated that serratamolide is the naturally occurring compound, serratamic acid being an artifact of extraction procedures.

Recent experiments in this laboratory have confirmed this speculation and strongly suggest that two further cyclic depsipeptides (consisting of serine esterified to β -hydroxydecanoic acid and β -hydroxydodecanoic acid respectively) are also present (M. A. C. Bermingham, B. S. Deol & J. L. Still, unpublished work).

No connexion has been established thus far between the biosynthesis of these compounds and prodigiosin formation, but 'lipoamino acid' content of *S. marcescens* is greatest in highly pigmented strains, and growth conditions (e.g. temperature, carbon source and pH) that favour pigment production also favour lipoamino acid biosynthesis (Deol, Bermingham & Still, 1969).

The formation of prodigiosin in the mutant strain 933 is 75% inhibited by streptomycin (10 μ g/ml of medium) although growth is not affected (Williams & Gott, 1964). We have investigated the effect of streptomycin on the biosynthesis of prodigiosin and cyclic depsipeptide (red strain 330, bacto-peptone medium) and find that very low concentrations (2.5–5.0 μ g/ml of medium) do not affect growth or pigmentation under these conditions but cause a sevenfold decrease in cyclic depsipeptide content (measured as acetone-soluble bound serine). In the white mutant strain 214 (no pigmentation) biosynthesis of cyclic depsipeptide is completely inhibited by streptomycin.

Extraction and fractionation of lipid from control (C) and streptomycin-grown (S) cells indicate that, although total lipid weight [chloroform-methanol (2:1, v/v)-soluble] is unaffected (C, 138.8mg/g dry wt. of cells; S, 131.6mg/g dry wt. of cells), the neutral lipid (acetone-soluble, containing cyclic depsipeptides) is decreased (C, 62.2mg/g; S, 37.8mg/g) and consequently the polar lipid is increased. The phosphorus content of the total lipid is increased from 1.58% (C) to 3.76% (S).

T.l.c. of the acetone extracts [silica gel G; chloroform-methanol-7M-NH₃ (65:25:4, by vol.)] confirmed that the cyclic depsipeptide content was markedly decreased by streptomycin. When feeding experiments were carried out with [U-¹⁴C]serine (specific radioactivity 667 μ Ci/mg) at a concentration of 0.012 μ Ci/ml of medium, radioactivity was incorporated very rapidly into cyclic depsipeptides, and this incorporation was decreased almost to zero by streptomycin treatment. The results will be discussed.

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