30 and 50% saturation with ammonium sulphate. One mol of 2,5-dihydropyridine required 1 mol of oxygen for conversion into 1 mol of maleamate and 1 mol of formate.

The supernatant fraction of cell-free extracts after centrifugation at 38000g for 30 min oxidized maleamate with the liberation of ammonia and maleate. In the presence of 10 mM-*meso*-tartrate both compounds were converted stoichiometrically into fumarate. No cofactors were found to be required for either reaction.

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The Bacterial Oxidation of *N*-Methylisonicotinate

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N-Methylisonicotinate, a photodegradation product of Paraquat (1,1'-dimethyl-4,4'-bipyridylium ion) (Slade, 1966), has been shown to be degraded aerobically by *Achromobacter* D (Wright & Cain, 1970). The pathway involves the partial reduction of the ring and ring-fission leads to the production of methylamine, succinate and CO₂. We now report evidence supporting the existence of a second pathway, in a Gram-positive rod, isolated from soil, capable of utilizing *N*-methylisonicotinate as sole carbon source.

Whole cells grown on *N*-methylisonicotinate were capable of the immediate oxidation of 2-hydroxy-*N*-methylisonicotinate. Crude cell-free extracts oxidized neither *N*-methylisonicotinate nor its 2-hydroxy derivative, though 2-hydroxyisonicotinate was oxidized rapidly with the uptake of 0.5 mol of oxygen/mol. The activity was associated with a particulate fraction sedimenting at 100000g. On the cessation of oxygen uptake, a compound with $\lambda_{\max}$ 338 nm (at pH 7.0) was present. A scaled-up reaction mixture was used to isolate sufficient material to identify this compound, by u.v. and i.r. spectra, chromatography and melting point as 2,6-dihydroxyisonicotinate. The reaction could occur anaerobically in the presence of Methylene Blue.

The 38000g supernatant fraction of cell-free

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extracts deamidated maleamate, and converted maleate into fumarate. In the presence of 10 mM-*meso*-tartrate, 1 mol of maleamate was converted into 1 mol each of fumarate and ammonia. Fumarate and methylamine were not oxidized by either whole cells or cell-free extracts.

Whole cells incubated with *N*-[¹⁴C]-methylisonicotinate released more than 50% of the added radioactivity as [¹⁴C]formaldehyde, and no [¹⁴C]-methylamine could be detected chromatographically. When whole cells were incubated with *N*-methyl[carboxyl-¹⁴C]isonicotinate, 95% of the added radioactivity was released as ¹⁴CO₂.

These data suggest that the first step in the pathway is a hydroxylation to yield 2-hydroxy-*N*-methylisonicotinate, which is demethylated to 2-hydroxyisonicotinate with the release of the methyl group as formaldehyde. The 2-hydroxyisonicotinate is hydroxylated to 2,6-dihydroxyisonicotinate. No information has been obtained on either the ring-fission substrate or product, but it seems that the carboxyl group of *N*-methylisonicotinate is not incorporated into maleamate, or more ¹⁴C would be incorporated via fumarate into cellular constituents from *N*-methyl[carboxyl-¹⁴C]isonicotinate.

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Biosynthesis of Ergotamine

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Numerous investigations (for reviews see Agurell, 1966; Ramstad, 1968) of the biosynthesis of the ergoline nucleus suggests that the primary precursors are tryptophan, mevalonate and the methyl group of methionine. There have been few reports on the biosynthesis of the side chain of the tripeptide alkaloids, for example, ergotamine.

The ¹⁴C-labelled amino acids phenylalanine and alanine were found to be incorporated into ergotamine (Paul, 1957; Vining & Taber, 1963), but the exact distribution of radioactivity in the alkaloid was not determined. Further studies (Minghetti & Arcamone, 1969; Mayer, Keybal & Komersora, 1967), using L-[U-¹⁴C]alanine, have shown that relatively more label entered the phenylalanyl, prolyl and lysergyl moieties than the α -hydroxy- α -