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.

C.G.O. thanks the Ministry of Agriculture, Fisheries and Food for financial support.

Slade, P. & Smith, A. E. (1967). *Nature, Lond.*, **213**, 919.

The Bacterial Oxidation of *N*-Methylisonicotinate

By C. G. ORPIN,* M. KNIGHT and W. C. EVANS.
(*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

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

* Present address: Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge, U.K.

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.

C.G.O. thanks the Ministry of Agriculture, Fisheries and Food for financial support. The *N*-[¹⁴C]-methylisonicotinate was kindly provided by Dr A. Calderbank.

Slade, P. (1966). *Weed Res.* **6**, 158.

Wright, K. A. & Cain, R. B. (1970). *Biochem. J.* **118**, 52p.

Biosynthesis of Ergotamine

By R. A. BASSETT, E. B. CHAIN and K. CORBETT.
(*Department of Biochemistry and Medical Research Council Metabolic Reactions Research Unit, Imperial College of Science and Technology, London S.W.7, U.K.*)

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 -

amino acid carbon atoms of the ergotamine side chain.

The development of a relatively high-yielding strain of *Claviceps purpurea* (Fr.) Tul. (Tonolo, 1966) producing ergotamine as a principal alkaloidal component, when grown in submerged culture on a defined medium, has provided a suitable system for study.

Radioactive precursors were added to these cultures at 10 days, provided that the minimal alkaloid content was 300–500 µg/ml. Ergotamine was isolated by preparative t.l.c. after a further 24 h or 48 h fermentation in the presence of L-[U-¹⁴C]-phenylalanine, L-[U-¹⁴C]-proline and L-[U-¹⁴C]-alanine. Further, short-time incubation studies of 6.5 h duration were carried out with L-[U-¹⁴C]-alanine and DL-[1-¹⁴C]alanine. After acid or mild alkaline hydrolysis of the alkaloid, the degradation products were radioassayed as phenylalanine, proline, lysergic acid and the *p*-bromophenylhydrazine derivative of pyruvate, derived under these hydrolytic conditions from α -hydroxy- α -alanine.

L-Phenylalanine and L-proline were efficiently incorporated into the crude peptide alkaloid fraction (2–5%). Phenylalanine was found to specifically label the phenylalanyl moiety (93–96%) and proline the prolyl moiety (73–76%) of the side chain of ergotamine. An examination of the mycelial amino acid pool of *Claviceps purpurea* at 10 days showed that the radioactivity from proline appeared more rapidly in the other amino acids, whereas radioactivity from phenylalanine mainly appeared in tyrosine and phenylalanine.

Both L-[U-¹⁴C]alanine and DL-[1-¹⁴C]alanine were incorporated into the peptide alkaloid fraction with relatively low efficiency (0.07–0.34%). Radioactivity from L-alanine was found to be distributed mainly in the α -hydroxy- α -alanyl (52–54%), prolyl (21–25%) and lysergyl (20–23%) moieties of ergotamine. In contradistinction, DL-[1-¹⁴C]alanine labelled the α -hydroxy- α -alanyl carbon atoms specifically (84–89%). These results show that all three carbon atoms of alanine are incorporated into α -hydroxy- α -alanine of the side chain, whereas C-2 and C-3 are incorporated into proline and lysergic acid, following conventional pathways.

Agurell, S. (1966). *Acta pharm. suecica*, **3**, 71.

Mayer, J., Keybal, J. & Komersora, I. (1967). *Folia microbiol.*, Prague, **12**, 489.

Minghetti, A. & Arcamone, F. (1969). *Experientia*, **25**, 926.

Paul, A. G. (1957). Ph.D. Thesis: University of Connecticut.

Ramstad, E. (1968). *Lloydia*, **31**, 327.

Tonolo, A. (1966). *Nature, Lond.*, **209**, 1134.

Vining, L. C. & Taber, W. A. (1963). *Can. J. Microbiol.* **9**, 291.

The Metabolism *in vitro* of Phenothiazine Drugs

By D. S. HEWICK and A. H. BECKETT. (*Department of Pharmacology and Therapeutics, University of Dundee, Dundee DD1 4HN, U.K., and Chelsea School of Pharmacy, London S.W.3, U.K.*)

Beckett & Hewick (1967) found that *N*-oxidation was the major metabolic route for the biotransformation of [³⁵S]chlorpromazine *in vitro* by liver microsomal fraction from male rats. We have performed further studies on a series of medicinally used and experimental phenothiazine derivatives, to compare the results with chlorpromazine.

The compounds metabolized were: (1) 10-(2-dimethylaminoethyl)phenothiazine (fenethazine); (2) 10-(3-dimethylaminopropyl)phenothiazine (promazine); (3) 10-(4-dimethylaminobutyl)phenothiazine; (4) 2-chloro-10-(3-dimethylaminopropyl)phenothiazine (chlorpromazine); (5) 10-(3-dimethylaminopropyl)-2-trifluoromethylphenothiazine (trifluorpromazine); (6) 10-(2-dimethylaminopropyl)phenothiazine (promethazine); (7) 10-(2-dimethylamino-1-methylethyl)phenothiazine (isopromethazine); (8) 10-[3-(dimethylamino)-2-methylpropyl]phenothiazine (trimeprazine); (9) 10-(2-diethylaminoethyl)phenothiazine (diethazine); (10) 10-(2-diethylaminopropyl)phenothiazine (ethopropazine); (11) 2-chloro-10-(2-diethylaminopropyl)phenothiazine (chlorproethazine); (12) 10-[2-(1-pyrrolidinyl)ethyl]phenothiazine (pyrathazine); (13) 10-[3-(1-pyrrolidinyl)propyl]phenothiazine; (14) 2-chloro-10-[3-(1-pyrrolidinyl)propyl]phenothiazine; (15) 10-[3-(1-piperidinyl)propyl]phenothiazine; (16) 2-chloro-10-[3-(4-methylpiperazin-1-yl)propyl]phenothiazine (prochlorperazine); (17) 10-[3-(4-methylpiperazin-1-yl)propyl]trifluoromethylphenothiazine (trifluoperazine).

The basic procedures for metabolizing each compound (0.8 mM) and identifying metabolites were as described by Beckett & Hewick (1967). Where possible *N*-demethylation was measured by the method of Cochin & Axelrod (1959).

All the tested compounds underwent sulfoxidation and aromatic hydroxylation. Compounds containing *N*-methyl or *N*-ethyl groups (namely 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 16 and 17) were *N*-dealkylated. The percentage *N*-demethylation undergone by chlorpromazine was 10%. On normalization, taking the amount of *N*-demethylation occurring with chlorpromazine as unity, the relative amounts of *N*-demethylation undergone by compounds (1), (2), (3), (4), (5), (6), (7), (8), (16) and (17) were 1.3, 1.5, 1.9, 1.0, 1.0, 1.4, 2.3, 2.6, 0.9 and 1.0 respectively.

Demethylation tended to increase with increase in length of side chain (compare compounds 1, 2 and