PRELIMINARY INVESTIGATIONS INTO MICROBIAL N-DEALKYLATION

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Removal of alkyl groups from nitrogen in drug molecules is often an important intermediate step in drug synthesis, for example in the variation of N-substituents to afford agonist and antagonist morphine congeners. Chemical N-dealkylation is usually difficult, low yielding and requires toxic reagents. An alternative method of N-dealkylation is desirable and the objective of this project is to examine the feasibility of using microorganisms to N-dealkylate medicinal tertiary amines.

Initial screening was carried out with methylotrophic Pseudomonads. The enzyme systems of Ps.aminovorans in particular have been studied and a mono-oxygenase likely to be specific for N-methyl groups has been found (Large 1971). The compounds studied were dimethylamine, trimethylamine, N-methyl piperidine and codeine. Ps.aminovorans was grown in optimal, defined media, with the test amine at a concentration of 20m.Mol.L^{-1} as sole carbon source, at 30°C for 5 days. Growth was monitored spectrophotometrically at 470 nm. As expected, Ps.aminovorans grew at the expense of dimethylamine and trimethylamine, demethylating them to the primary and secondary amine respectively. Trace amounts of readily utilised substrates such as glucose prevented amine utilisation. When the tertiary nitrogen was contained in a ring structure, as in N-methyl piperidine and codeine, no growth occurred. Ps. aeruginosa, Ps. fluorescens, Ps. putida and Ps. testosteroni were unable to grow on any amine substrate tested.

In an attempt to N-dealkylate larger molecules, Streptomyces and Cunninghamella species were screened since these organisms have been shown capable of N and O demethylating antibiotics (Argoudelis & others 1969) and various alkaloids (Bellet & others 1970, Davis & others 1976). In this case the amine under test was not the sole carbon source, but was offered for attack by cells in logarithmic growth. The compounds screened were codeine, pentazocine, and other selected benzomorphans. The test organism was grown in glucose/soya media incubated at 27°C. The test amine was added to a 24 hour secondary culture to give a concentration of 1.25 m.Mole.L^{-1}, and incubation was continued for 5 days. The beer was then filtered, basified, extracted into 25% isobutanol in 1,2,dichloroethane and evaporated to dryness. The residue was dissolved in 5 ml. methanol or T.H.F. and analysed by GLC using on-column acetylation with suitable standards. The 2m. glass column was packed with 3% SE30 Ultraphase on Chromosorb W HP 100/120 mesh.

Analysis of extracts from cultures screened with pentazocine showed that the N substituent (dimethylallyl) had not been removed. In contrast, with codeine as the drug substrate N-demethylation was clearly indicated when GLC comparison was made with standard codeine derivatives.

Large, P.J. (1971) Xenobiotica 1, 457-467
Argoudelis, A.D., Coats, J.M. & others (1969) J. Antibiotics 22, 309-314
Bellet, P., Van Thuong, T. (1970) Ann. Pharm. Franc. 28, 245-249
Davis, P.J., Wiese, D. & Rosazza, J.P. (1977) J.Chem. Soc. P1, 1-6.