

STUDIES ON THE MICROBIAL N-DEALKYLATION OF DIAZEPAM

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The potential benefits of microbial N-dealkylation in the preparation of drug intermediates has been described in a previous communication (Sewell et al., 1979). It is believed that microbial transformations occur on substrate dissolved in the aqueous microbial incubation medium (Wodzinski et al 1974). In attempts to increase the microbial transformation yield of substrates with low aqueous solubility, Chien and Rosazza (1980) used pharmaceutical solubilisation adjuvants, particularly polyvinylpyrrolidone (PVP). Our studies have investigated the effect of solubilisation on N-demethylation of the poorly soluble drug diazepam by the fungus *C. echinulata*.

Solubilisation systems investigated included o/w emulsions, two-phase systems with organic solvents, co-solvent and solubilisation systems with propylene glycol, PVP, or Triton X-100 and hydrotropic salt formation with sodium salicylate. Since these systems would not support microbial growth they were incorporated in a basal-salts medium in which diazepam (1 mM) was incubated with resting cells of *C. echinulata* at 27°C for 10 days.

These systems were compared with conventional transformation methods in which diazepam was incubated with growing *C. echinulata* cultures in a) chemically defined medium and b) a complex soya/glucose based medium. N-demethylation of diazepam in each transformation system was determined by GLC analysis. The results are shown in Table 1.

Table 1. Diazepam solubility and transformation by *C. echinulata* in various solubilised/incubation medium systems.

Transformation system	diazepam solubility (%)	transformation(%)
Resting cells: PVP (10% w/v)	42	1.4
Other systems	35-100	0
Growing cells: Defined medium	9	7.5
Soya/glucose medium ^a	19	12.0
Soya/glucose medium ^b	19	38.5
^a pelleted fungal growth form		^b filamentous fungal growth form

Only one resting cell/solubilised system, that containing 10% PVP (M.W. 44,000), supported diazepam transformation and then only in low yields. In growing cultures the highest yields of N-desmethyldiazepam were obtained in the complex soya/glucose medium with a filamentous fungal growth form. This finding was attributed to increased diazepam solubility, possibly arising from the higher protein content of the complex medium, and the increased surface area of the fungal mycelium.

Sewell, G.J., Soper, C.J. and Parfitt, R.T. (1979) *J. Pharm. Pharmacol.* 31: 90P.
 Wodzinski, R.S. and Coyle, J.E. (1974) *Appl. Microbiol.* 21: 1081.
 Chien, M.M. and Rosazza, J.P. (1980) *Appl. Environ. Microbiol.* 40: 741.