COMPARISON OF MICROBIAL AND HUMAN TRANSFORMATION OF BENZODIAZEPINES

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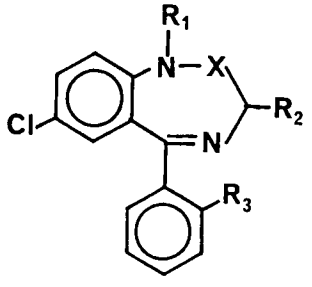
Microbial transformation may be exploited commercially in the manufacture of pharmaceuticals where chemical synthesis is either difficult or uses toxic or hazardous reagents. It has also been suggested that microbial transformation could mimic biotransformations observed in mammals and be useful models in drug metabolism and toxicology studies. Sewell (1982) and Gibson (1984) conducted preliminary studies on the microbial N-dealkylation of some benzodiazepines by *Cunninghamella sp.* and we report here further studies aimed at comparing the microbial transformation of diazepam(DZP), medazepam(MZP) and flurazepam(FZP) by *C. bainieri* with that observed in humans. The basic two stage fermentation protocol of Sewell(1982) was used, with modifications to the inoculum preparation to increase the N-dealkylation activity. Glucose depletion profiles indicated vigorous growth of the organisms. The complexity of the transformation necessitated the development of a new HPLC analytical method specifically to detect the more polar transformation products for each drug. The maximum concentration of the major transformation product was obtained in two days for medazepam and in five days for flurazepam but was not obtained after seven days for diazepam. Increase in the pH of the growth medium improved the yield of the major product of medazepam and flurazepam but not of diazepam. The hydrophobicity value($logP_k$) of each transformation product was calculated from the HPLC retention factor(logk') on the basis of a relationship established between logk' of the known compounds and logP values calculated using fragmental constants (Rekker,1977). The logP values for human metabolites(Bridge and Chasseaud,1984) were also calculated from fragmental constants for the structures given below.

logP for human metabolites and $logP_k$ for microbial transformation products.

R_1	R_2	R_3	X	logP	DZP	MZP	FZP	$logP_k$
H	OH	H	C=O	1.83	+	+		2.19
CH_3	OH	H	C=O	2.07	+	+		2.47
H	H	H	C=O	2.75	+++	+		2.99
CH_3	H	H	C=O	2.99		+		3.12
H	H	H	CH_2	3.32		+++		3.57
CH_2CHO	H	F	C=O	2.64			+	2.84
H	OH	F	C=O	2.60			+	2.64
CH_2CH_2OH	H	F	C=O	3.20			+	3.16
$CH_2CH_2NH_2$	H	F	C=O	3.27			+	3.37
H	H	F	C=O	3.53			+	3.71
$CH_2NHCH_2CH_3$	H	F	C=O	4.11			+++	4.13
UNKNOWN	-	-	-	-				3.66
UNKNOWN	-	-	-	-				3.79



	R_1	R_2	X	R_3
DZP	CH_3	H	C=O	H
MZP	CH_3	H	CH_2	H
FZP	$(CH_2)_2NEt_2$	H	C=O	F

A comparison of logP values for human metabolites and $logP_k$ values for transformation products shows that a good correlation exists (n=11,r=0.984,s=0.108). This suggests that the human biotransformation of the test benzodiazepines is mimicked to a large extent by that of *C. bainieri*. It is also evident that the major transformation product(+++) is a secondary amine formed probably by a mono-oxygenase system via oxidative N-dealkylation.

Sewell, G.J. (1982) Ph.D. Thesis, University of Bath, Bath ,U.K.
Gibson, M. (1984) Ph.D. Thesis, University of Bath, Bath ,U.K.
Rekker, R.F.(1977) "The Hydrophobic Fragmental Constant",Elsevier Sci.Pub.,N.Y.
Bridge, J.W. and Chasseaud, L.F. Ed(1984) "Progress in Drug Metabolism" Vol.8, Taylor & Francis,London.