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FUNGAL HYDROXYLATION OF ETHYL BENZENE AND DERIVATIVES

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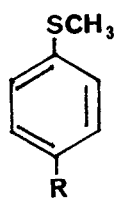
Abstract. The fungus Mortierella isabellina converts ethyl benzene and a number of para-substituted derivatives to the corresponding optically active 1-phenylethanols with enantiomeric excesses between 5 and 40%. Hydrogen removal from the substrate preceeds product formation and is stereochemically independent of it.

The fungus Mortierella isabellina NRRL 1757 is an efficient oxidiser of organic sulfides such as 1, giving the corresponding R sulfoxide in good chemical yield and in high enantiomeric excess.[1] We now report that the same fungus is capable of converting



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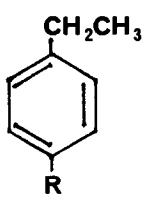

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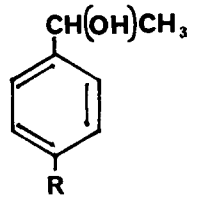

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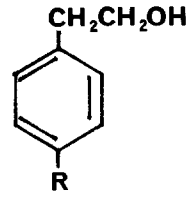

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ethyl benzene and its para- substituted derivatives 2 to the corresponding 1-phenylethanols 3. Ethyl benzene itself (2a) is one of the commonest of the non-halogenated aromatic solvents in industrial use, and its metabolism in mammalian systems (in which 3 is the first-formed of a series of oxidative metabolites) has recently been reported.[2] The fungal biotransformation reported herein serves both as a specific model for this, and as a general model for the oxidative metabolism of alkyl substituted PAHs.[3]

The isolated yields and enantiomeric excesses obtained are reported in Table 1. All incubations were performed as previously described[5] at substrate concentrations of 0.5-1.0 mg/mL, in stoppered 1L Erlenmeyer flasks for periods of 48-72 hours. Control experiments using acetophenone as substrate, and the results of incubations with 1,1-dideuteroethylbenzene (see below), confirm that both the enantiomeric alcohols are formed directly from ethyl benzene, and that reversible alcohol-carbonyl interconversion at C-1 does not occur during the incubation.

Table 1. Metabolism of ethyl benzene derivatives by M. isabellina

Compound	R	yield of 3 (%)	EE (%)*	configuration of** major isomer	other products (%)
2a	H	10	33	R	none
2b	CH_3	4	20	R	4b (0.1)
2c	C_2H_5	3	35	R	4c (1.0)
2d	F	5	26	R	4d (1.0)
2e	Cl	30	34	S	4e (10)
2f	Br	22	5	R	none
2g	OCH_3	10	29	S	4g (1.0)
2h	NO_2	40	9	R	none
2i	CN	60	39	R	none

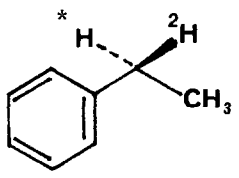
*Determined by ^1H nmr in the presence of $Eu(hfc)_3$

**Determined by optical rotation of 3 (reference 4)

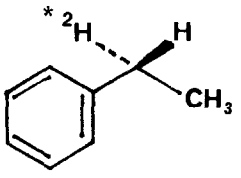
The enantiomeric excesses of the products 3 are generally lower than those observed with the corresponding sulfoxides[1], and in two cases (3e and 3g) the products are of opposite predominant chirality. The reasons for these differences are not clear, but may be related to the difference in steric size between -S- and $-CH_2-$, and to differences in the ways in which the reaction intermediates are influenced by the nature of the para-substitutent.

The addition of the protein synthesis inhibitor cycloheximide to incubations performed as described above resulted in the suppression of product formation in both ethylbenzene and phenyl methyl sulfide oxidations, indicating that the enzyme(s) responsible are inducible. By using standard cross induction techniques[6] we have demonstrated that the same enzyme of M. isabellina is responsible for the oxidation of both substrates; we have previously shown that this enzyme has the characteristics of a cytochrome P-450 dependent mono-oxygenase.[5]

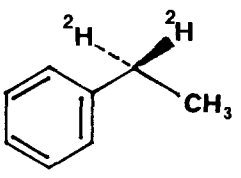
Since the hydroxylation of 2a is not stereospecific we considered the possibility that it might represent an example of a cyt. P-450 dependent hydroxylation in which partial inversion of substrate configuration occurs at the reacting centre, an unusual type of process which has recently been pivotal in delineating the mechanism of action of this class of enzyme.[7] We have investigated this possibility by using as substrates for the M. isabellina hydroxylase both the S and R enantiomers of 1-deuteroethylbenzene (5 and 6)



S-(+)-5



R-(-)-6



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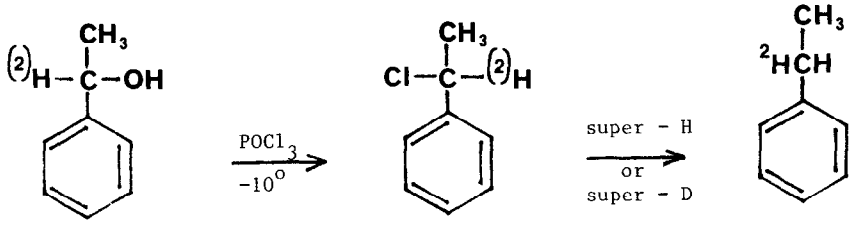
synthesized from S-1-phenylethanol or its 1-deutero analog by the route outlined below.[8]



The optical purities of the intermediate 1-phenylethyl chlorides were determined by both rotation[9] and chiral ^{1}H nmr shift reagent analysis. The optical purities of 5 and 6 were obtained by rotation measurements ($[\alpha]_D^{21}$ of 5 = +1.17°)[8]: the values so obtained were entirely consistent with their formation from 1-phenylethyl chloride by clean inversion of configuration.[10] Transformation of both 5 and 6 to 1-phenylethanol by M. isabellina proceeded with stereospecific loss of a single (pro-R, asterisked) hydrogen in each case (Table 2). Thus S-(+)-5 (EE 66%, S:R = 83:17) gave 3a containing 80% deuterium by ^{1}H nmr and ms analysis, and R-(-)-6 (EE 60%, R:S = 80:20) gave 3a containing 17% ^{2}H. Incubation of racemic 1-deuteroethylbenzene (5+6) demonstrated that removal of H or D is not complicated by a large primary intermolecular isotope effect, a situation common in biological hydroxylations.[11] The existence of a positive secondary isotope effect of $k_H/k_D \geq 56/44 = 1.27 \pm 0.05$ is indicated, a finding consistent with initial abstraction of

Table 2. Hydroxylation of labelled substrates

substrate	EE	% ^{2}H in 3a*	configuration of 3a
S(+)-5	66	80	R:S = 20:80
R(-)-6	60	17	R:S = 60:40
racemate 5+6	-	44	R:S = 40:60
7	-	100	R:S = 10:90
2a	-	-	R:S = 66:33

* ±2%

a hydrogen radical from the benzylic position analogous to that performed by the enzyme dopamine β-monooxygenase (sec. $k_H/k_D = 1.19 \pm 0.06$).[12]

We have also shown (Table 2) that the absolute stereochemistry of product 3, as determined by ^{1}H nmr chiral shift reagent analysis using authentic stereoisomers of 3 as standards, and by optical rotation measurements, is determined by the nature of substitution (H or D) on the intermediate (compare Table entries for 2 and 7). The product ratios presented in Table 2 are all consistent with this assumption. Thus the ratio resulting from 5, for example, is determined by the relative amounts of $Ph\dot{C}HCH_3$ (20%, giving R:S = 66:33) versus that of $Ph\dot{C}DCH_3$ (80%, giving R:S = 10:90). These data lead to a predicted R:S ratio of 21:79 (66x0.2 + 10x0.8:33x0.2 + 90x0.8).

One possible explanation for the dependence of product stereochemistry on radical substitution is the known effect of isotopic substitution on radical inversion rates, where a large isotope effect of a primary magnitude is operative.[13] Thus the rate of $Ph\dot{C}HCH_3$ inversion would be expected to exceed that of $Ph\dot{C}DCH_3$, perhaps to the extent that inversion of the latter radical may be slower than its rate of capture by the enzyme's oxidising species. In this way the stereochemistry of product formation from $Ph\dot{C}HCH_3$ and $Ph\dot{C}DCH_3$ could differ substantially.

We are currently exploring other substrates and enzyme systems to investigate the generality of this phenomenon.

Acknowledgements

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