Enantioselective Bacterial Biotransformation Routes to *cis*-Diol Metabolites of Monosubstituted Benzenes, Naphthalene and Benzocycloalkenes of Either Absolute Configuration

Christopher C. R. Allen,[a] Derek R. Boyd,*[b] Howard Dalton,*[a] Narain D. Sharma,[b] Ian Brannigan,[b] Nuala A. Kerley,[b] Gary N. Sheldrake[c] and Stephen C. Taylor[d]

[a] *Department of Biological Sciences, University of Warwick, Coventry, UK CV4 7AL*
[b] *School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[c] *Zeneca Specialities, PO Box 42, Hexagon House, Blackley, Manchester, UK M9 3DA*
[d] *Zeneca Bio Products, PO Box 1, Billingham, Cleveland, UK TS23 1LB*

Enzyme-catalysed kinetic resolution and asymmetric dihydroxylation routes to enantiopure *cis*-diol metabolites of arenes and benzocycloalkenes of either absolute configuration have been developed using appropriate strains of the bacterium *Pseudomonas putida*.

Biotransformation of monosubstituted benzenes (*e.g.* **1**, X = H) and disubstituted benzenes (*e.g.* **1**, X = F, Cl, Br, Me) in growing cultures of a mutant strain (UV4) of *Pseudomonas putida* has been reported[1–3] to yield the corresponding *cis*-dihydrodiols **4** (X = I) and **2**, **3** (X = F, Cl, Br, Me). Enantiopure (>98% ee) *cis*-dihydrodiols **4** having a (3*S*)-configuration are the normal metabolites of monosubstituted arenes (fluorobenzene being an exception since the (3*R*) enantiomer is also present[2]) while both enantiomers (*e.g.* **2**, **3**, X = F, Cl, Br, Me) are produced from 1,4-disubstituted arenes[1,3] (Table 1). Recent studies have shown that *cis*-dihydrodiol derivatives of iodobenzenes can be chemically modified by substitution of the iodine atom.[1,4] Thus, catalytic hydrogenolysis (H_2, Pd/C, 1 atm., MeOH containing NaOAc)[4] of the disubstituted *cis*-dihydrodiols **2**, **3** (X = F, Cl, Br, Me) provided a route to both (3*S*)-**4** and (3*R*)-**5** enantiomers of the monosubstituted *cis*-dihydrodiols (X = F, Cl, Br, Me) as shown in Table 1.

Metabolism of the mixture of *cis*-diol enantiomers **4**, **5** (X = F, Cl, Br, Me), obtained by the above chemoenzymatic route, in the presence of growing cultures of a wild-type strain of *P. putida* (NCIMB 8859, initially grown with naphthalene as a carbon source), resulted in the selective removal of the (3*S*) enantiomer **4** leaving the (3*R*) enantiomer **5** intact in the culture medium (*ca.* 30% recovered yield, Scheme 1). Since *P. putida* NCIMB 8859 is a wild type strain (unrelated to the mutant strain UV4) which is presumed to contain a *cis*-dihydrodiol dehydrogenase enzyme, it is reasonable to assume that only the (3*S*) enantiomer **4** is further metabolized *via* the corresponding catechol. This kinetic resolution procedure thus leads to a new enzymatic route to the elusive (3*R*)-*cis*-dihydrodiol enantiomer **5** of fluorobenzene, chlorobenzene, bromobenzene and toluene,

Table 1 Ee values and absolute configurations of *cis*-dihydrodiols **2**–**5**

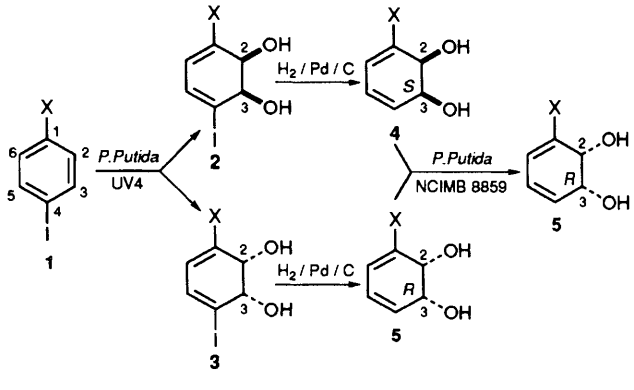
Substrate **1**	Product diols **2**, **3**		Substrate diols **4**, **5**		Product **5**
X	% ee[a]	Config.[a]	% ee[b]	Config.[b]	Config.[c,d]
F	88	2*R*:3*S*	88	2*R*:3*R*	2*R*:3*R*[e]
Cl	15	2*R*:3*S*	15	2*R*:3*R*	2*R*:3*R*
Br	22	2*R*:3*S*	22	2*R*:3*R*	2*R*:3*R*
Me	80	2*S*:3*S*	80	2*S*:3*R*	2*S*:3*R*

[a] Major *cis*-dihydrodiol enantiomer **3** from biotransformation of arene **1** in *P. putida* UV4. Ee values and absolute configurations determined by reported methods.[4] [b] Major *cis*-dihydrodiol enantiomer **5** from catalytic hydrogenolysis of dihydrodiols **2**, **3**.[4] [c] *Cis*-dihydrodiol enantiomer **5** isolated (>98% ee) after kinetic resolution using *P. putida* NCIMB 8859 and biotransformation conditions similar to those reported for *P. putida* UV4.[11] [d] Abbreviated to (3*R*) configuration in the text. [e] Fluorobenzene as substrate also yielded the corresponding mixture of *cis*-dihydrodiol enantiomers **4**, **5** (X = F)[2] and the (2*R*:3*R*) enantiomer **5** was again obtained exclusively by an enzyme-catalysed kinetic resolution in the presence of *P. putida* NCIMB 8859.

and complements the direct oxidation pathway which produces the normal (3*S*) enantiomer **4** through arene metabolism using *P. putida* UV4.[2] Isolation of the (3*R*)-*cis*-dihydrodiol enantiomer of toluene **5** (X = Me) and fluorobenzene **5** (X = F) exclusively by the kinetic resolution method provides an alternative to the fractional crystallization procedure previously reported for diols **5**, **4** of high enantiopurity [*e.g.* X = Me (80% ee) or F (88%ee), Table 1], which were obtained by hydrogenolysis of the corresponding diols **3**, **2** (X = Me and F)[2,4] and would be useful for obtaining a wider range of the abnormal (3*R*)-*cis*-dihydrodiol enantiomers.

The racemic *cis*-1,2-dihydrodiol derivative of naphthalene **9**, **10** was synthesised in four steps, from 1,4-naphthoquinone, by the literature method.[5] Kinetic resolution of *cis*-diols **9**, **10** was examined using two wild-type strains of *P. putida i.e.* NCIMB 11767 (the parent strain isolated using benzene as a carbon source and from which the mutant strain UV4 was derived) and the unrelated strain NCIMB 8859. In both cases the (1*R*:2*S*) enantiomer **10** was removed selectively to yield enantiopure *cis*-diol **9** of (1*S*:2*R*) configuration (*ca.* 40% recovered yield from the racemate). When naphthalene was metabolized by *P. putida* UV4 it yielded exclusively the (1*R*:2*S*) isomer **10**. Our preliminary study of the enzyme-catalysed kinetic resolution method suggests that it may also be applicable to other members of the polycyclic aromatic and heteroaromatic series where both enantiomers are available.[6–8] The (1*S*:2*R*) diol enantiomer **9** had earlier been obtained by kinetic resolution of the racemate using a diol dehydrogenase enzyme from *P. putida* 119.[5,9]

The value of *P. putida* NCIMB 8859 in the synthesis of enantiopure *cis*-diol metabolites **7** or **8** (*n* = 1–3) was further explored first by using the benzocycloalkenes **6** and then the racemic *cis*-diols **7**, **8** as substrates (Scheme 2). Enzyme-catalysed asymmetric dihydroxylation of the alkenes **6** (*n* = 1–3), using a substrate concentration of *ca.* 0.5–1.0 mg ml^{-1}, yielded exclusively (>98% ee, *n* = 2 or 3) or mainly (*ca.* 85–90% ee, *n* = 1) the (1*R*:2*S*) enantiomer **8** (*ca.* 10–30% yield). Our past studies have shown that the *opposite* (1*S*:2*R*)

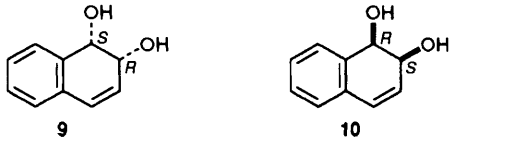


Scheme 1

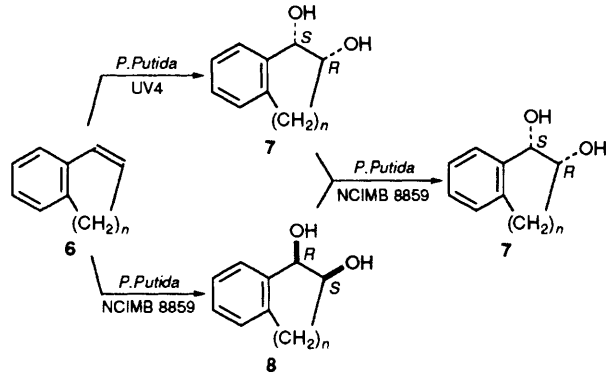
cis-diol enantiomer **7** was formed exclusively (>98% ee, n = 2 or 3) or preferentially (20% ee, n = 1) by dioxygenase-catalysed asymmetric dihydroxylation of alkenes **6** (n = 1–3) with *P. putida* UV4.[10,11]

Results obtained using both the UV4 and NCIMB 8859 strains of *P. putida* for dioxygenase-catalysed sulfoxidation have again demonstrated an enantiocomplementary relationship.[12] Thus, in many cases the oxidation products (sulfoxides) were of high enantiopurity but of *opposite* absolute configuration using either strain.

Addition of the racemic *cis*-diols **7**, **8** (obtained by chemical synthesis) as substrates for *P. putda* NCIMB 8859, but using a lower concentration (0.2–0.4 mg ml^{-1}) compared with earlier conditions, resulted in the asymmetric destruction of one enantiomer. The recovered *cis*-diols **7**, **8** (n = 1–3) were enriched (35–>98% ee) in the (1*S*:2*R*)-**7** configuration (*ca.* 35% yield). The relative rates of kinetic resolution decreased with increasing ring size of the benzocycloalkene **6**. The recorded % ee values were obtained after a fixed incubation period (24 h) and thus could in principle be >98% under optimal conditions (Table 2).



9 **10**



Scheme 2

Table 2 Ee values and absolute configurations of *cis*-diols **7** and **8**

Alkene **6**	Product diol **8**[a]		Substrate diols **7**, **8**	Product diol **7**[b]		
n	% ee[c]	Config.	n	% ee[c]	t/h[d]	Config.
1	85–90[e,f]	1*R*:2*S*	1	>98	24	1*S*:2*R*
2	>98	1*R*:2*S*	2	91	24	1*S*:2*R*
3	>98	1*R*:2*S*	3	35	24	1*S*:2*R*

[a] Major *cis*-diol enantiomer **8** from biotransformation of alkene **6** in *P. putida* NCIMB 8859 (24 h, substrate concentration, 0.5–1.0 mg ml^{-1}). [b] Major *cis*-diol enantiomer **7** isolated after kinetic resolution of diols **7**, **8** using *P. putida* NCIMB 8859 (24 h, substrate concentration, 0.2–0.4 mg ml^{-1}). [c] Ee values were obtained by ^{1}H-NMR analysis of diMTPA ester derivatives and chiral stationary phase HPLC. [d] Time interval between addition of substrate and product isolation. [e] Higher ee value (90%) after shorter biotransformation times. [f] Accompanied by a minor product resulting from benzylic hydroxylation (inden-1-ol).

On the basis of the observations and results contained in this communication we conclude that by using *P. putida* NCIMB 8859: (*i*) the *cis*-dihydrodiol derivatives of monosubstituted benzenes (and naphthalene) can be obtained exclusively as the abnormal (3*R*)-**5** [and (1*S*:2*R*), **9**] enantiomers by an enzymatic kinetic resolution procedure; (*ii*) *cis*-diol derivatives of benzocycloalkenes **6** may be obtained with an excess of the (1*R*:2*S*) enantiomer **8** by dioxygenase-catalysed asymmetric dihydroxylation or the (1*S*:2*R*) enantiomer **7** by kinetic resolution of the racemic *cis*-diols **7**, **8**. The enzyme-catalysed routes to enantiopure samples of the (*R*)- and (*S*)-*cis*-diols **4**, **5**, **7**–**10** complement the recent improvements in asymmetric dihydroxylation of alkenes (using OsO_4 and chiral auxiliaries)[13–15] although the latter chemical route appears to be less stereoselective for *cis*-alkenes and is not applicable to the asymmetric dihydroxylation of arenes.

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