Chemoenzymatic Synthesis of the 2,3- and 3,4-*cis*-Dihydrodiol Enantiomers of Monosubstituted Benzenes

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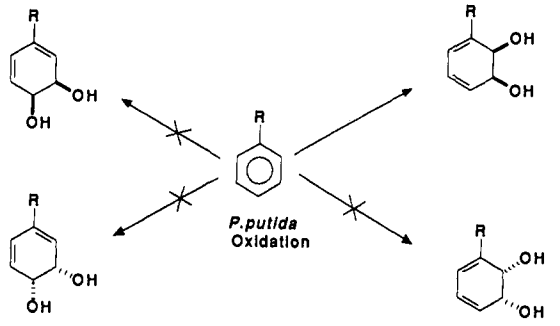
Received November 3, 1993

Mutant strains of *Pseudomonas putida* (e.g., UV4[1] and 39/D[2]) have earlier been used to produce 2,3-*cis*-dihydrodiols from monosubstituted arene substrates. Single enantiomers of the configurations shown for 2,3-*cis*-dihydrodiols in Scheme 1, with the exception of the *cis*-dihydrodiol from fluorobenzene,[3] are routinely obtained in good yields without the isomeric 3,4-*cis*-dihydrodiol enantiomers.

This report highlights how a series of disubstituted iodobenzene *cis*-diol metabolites, obtained using intact cells of *P. putida* UV4[1] and *Escherichia coli* JM109 (pDTG601, containing the *tod* C1C2BA genes encoding toluene dioxygenase from *P. putida* F1[4]), can be conveniently converted into novel and useful[5] *cis*-diols by facile removal of the iodine atom. The *cis*-dihydrodiol metabolites **1B–8B** obtained (Table 1) were analyzed for enantiomeric excess (% ee), by chiral stationary phase HPLC using a Chiralcel OJ column[6] and ^{1}H-NMR spectroscopy of the di-MTPA esters of the 4-phenyl-1,2,4-triazoline-3,5-dione adducts.[3] Absolute configurations were determined by X-ray crystallography (**4B**, **6B**), ^{1}H-NMR spectral analysis of the cycloadduct di-MTPA ester derivatives (**1B–7B**), and direct comparison of $[\alpha]_D$ values (**1C–3C**, **5C**, **7C**, and **8C**) with those of the corresponding enantiomers.[3]

Substituted iodobenzene substrates were selected for metabolism by *P. putida* UV4 and *E. coli* JM109 (pDTG601) since both mutant strains of bacteria are known to accumulate the corresponding *cis*-dihydrodiols due to the lack of any *cis*-diol dehydrogenase enzyme activity. Furthermore, from recent studies[6] it has been demonstrated that (i) a larger atom or substituent, e.g., an iodine atom, can be a controlling factor in facial selectivity during enzyme-catalyzed *cis*-dihydrodiol formation from mono- and 1,4-disubstituted arene oxidations, and (ii) an iodine substituent on a *cis*-dihydrodiol can be readily substituted by other groups.[7] Metabolism of the 1,4-disubstituted

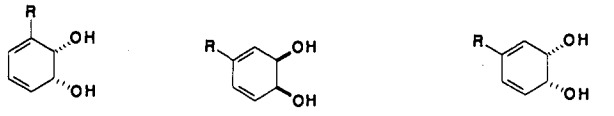
Scheme 1



benzene substrates **1A** and **2A** gave a strong preference for the *cis*-diol enantiomers (+)-**1B** (88%) and (+)-**2B** (80 and >98%) (Table 1).

Selective catalytic hydrogenolysis (H_2, 3% Pd/C, room temperature, 1 atm, MeOH containing NaOAc and traces of quinoline) of *cis*-dihydrodiols (+)-**1B** (88% ee) and (+)-**2B** (80 and >98% ee) gave the 2,3-*cis*-dihydrodiols of fluorobenzene ((+)-**1C**) and toluene ((−)-**2C**). Recrystallization of the chromatographically purified reaction mixture gave enantiopure samples of (+)-**1C** and (−)-**2C** of opposite absolute configuration to those reported as normal metabolites of fluorobenzene and toluene.[3]

Metabolism of the 1,2-disubstituted arenes **3A–6A** produced a preponderance of the *cis*-diol regioisomers **3B–6B** (*ca.* 95%, by ^{1}H-NMR and GC/MS analysis). This selectivity for the C=C bond proximate to the larger iodine substituent is consistent with the dominant regiodirecting effect of substituent size during enzyme-catalyzed oxidation, i.e., *cis*-dihydrodiol formation. Since the *cis*-dihydrodiol products **3B–6B** were found to be enantiopure, the marked influence of substituent size on facial stereoselectivity[6] is once again emphasized.



Replacement of the iodine substituent by a hydrogen atom on the *cis*-dihydrodiol regioisomers **3B–6B**, using the hydrogenolysis procedure, yielded the hitherto unavailable 3,4-*cis*-dihydrodiols **3C–6C**. Biotransformation of the 1,3-disubstituted arenes **7A** and **8A**, as expected, yielded *cis*-dihydrodiols mainly (*ca.* 95%) through oxidation of the C=C bond closest to the larger iodine substituent. The preferential regioselectivity and exclusive facial stereoselectivity shown during *cis*-dihydrodiol formation from 1,2-disubstituted (**3A–6A**) and 1,3-disubstituted (**7A**, **8A**) arenes, allied to the facial stereoselectivity observed during *cis*-dihydrodiol formation from monosubstituted[3] or 1,4-disubstituted arenes,[6] e.g., **1A** and **2A**, support the view that the larger iodine substituent exerts a dominant influence during dioxygenase enzyme-catalyzed *cis*-dihydrodiol formation in both *P. putida* UV4 and *E. coli* JM109 (pDTG601). Hydrogenolysis of the *cis*-diols **7B** and **8B** gave the opposite enantiomers (+)-**7C** and (−)-**8C** of the 3,4-*cis*-dihydrodiols (−)-**3C** and (+)-**5C**.

The results contained in this report demonstrate that it is now possible to obtain 2,3- and 3,4-*cis*-dihydrodiols of either enantiomeric form in a predictable manner by careful selection of the substrates and hydrogenolysis of the resulting metabolites. It is noteworthy that the major influence of larger substituents on the regio- and stereoselectivity of *cis*-dihydrodiol formation observed in *P. putida* UV4[6] also appears to be applicable to the substrates

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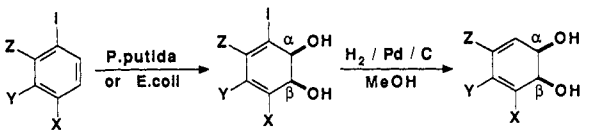
Table 1



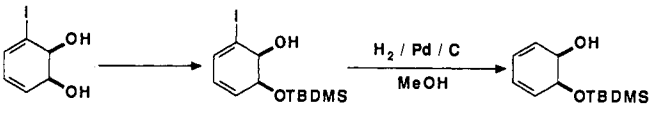
(A) (B) (C)

	A			B			C		
compd	X	Y	Z	$[\alpha]_D$ (MeOH)	e.e. (%)	confign (α,β)	$[\alpha]_D$ (MeOH)	yield (%)	confign (α,β)
1	F	H	H	+62[a]	88[a]	*S*,*R*[a]	+54[b,c]	70	*R*,*R*
2	Me	H	H	+3[a]	80[a]	*S*,*S*[a]	−56[b]	72	*R*,*S*
				+4[d]	>98[d]	*S*,*S*[d]	−70[b,c]	70	*R*,*S*
3	H	H	F	+24[a]	>98[a]	*S*,*S*[a]	−101[c,e]	75	*R*,*S*[e]
4	H	H	Cl	+81[a]	>98[a,f]	*S*,*S*[a,f]	+3.5[c]	67	*R*,*S*
				+81[d,f]	>98[d,f]	*S*,*S*[a,f]	+3.5[c]	65	*R*,*S*
5	H	H	Br	+75[a,f]	>98[a,f]	*S*,*S*[a,f]	+28[c,g]	65	*R*,*S*[g]
6	H	H	Me	+48[a,f]	>98[a,f]	*S*,*S*[a,c]	−16.7[c]	79	*R*,*S*
7	H	F	H	+74[a]	>98[a,f]	*S*,*S*[a,f]	+100[c,e]	75	*R*,*S*[e]
8	H	Br	H	+28[d]	>98[d,f]	*S*,*S*[c,d]	−30[c,g]	80	*R*,*S*[g]
9	H	H	H	+89[a,b,d,h]	>98[a,d,h]	*S*,*S*[a,d,h]	+48[b,c,i]	80[i]	*R*,*S*[i]

[a] Obtained from *P. putida* UV4. [b] In $CHCl_3$ solution. [c] >98% e.e. [d] Obtained from *E. coli* JM109 (pDTG601). [e] Enantiomeric pair (**3C** and **7C**). [f] Obtained on the major (>95%) regioisomer shown. [g] Enantiomeric pair (**5C** and **8C**). [h] Mono-TBDMS derivative, **9D**. [i] Mono-TBDMS derivative, **9E**.

metabolized to *cis*-dihydrodiols by *E. coli* JM109 (pDTG601). While the results obtained using intact cells of either strain appear to be similar, minor differences, e.g., the higher % ee value obtained for *cis*-diol **2B** using *E. coli* JM109 (pDTG601), were noted.

A previous successful attempt to desymmetrize the *cis*-diol metabolite of benzene, *cis*-cyclohexa-3,5-diene-1,2-diol, involved enzyme-catalyzed conversion to the β-monogalactosides using a β-galactosidase from *E. coli*, followed by HPLC separation of the diastereoisomers.[8] As an alternative approach, the *cis*-dihydrodiol metabolite of iodobenzene (**9A**) was converted exclusively to the mono *tert*-butyldimethylsilyl(TBDMS) ether derivative (>95% yield) at the β-position (**9D**). The latter compound was then hydrogenolyzed to yield the mono-TBDMS



(9B) (9D) (9E)

derivative of *cis*-cyclohexa-3,5-diene-1,2-diol (**9E**). The chiral TBDMS derivative of benzene 1,2-*cis*-dihydrodiol, **9E**, and the new range of 2,3- and 3,4-*cis*-dihydrodiols available from the present study (**1C**–**8C**) should prove useful chiral synthons for the synthesis of target molecules (e.g., sugars, cyclitols, alkaloids) of the type exemplified in recent reviews.[5]

Acknowledgment. We thank DED/Technology Board NI (N.D.S.) and the SERC Biotechnology Directorate (S.A.B. and J.C.) for financial support.

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