Stereodirecting Substituent Effects during Enzyme-catalysed Synthesis of *cis*-Dihydrodiol Metabolites of 1,4-Disubstituted Benzene Substrates

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The *cis*-dihydrodiol enantiomer preferentially formed, by dioxygenase-catalysed oxidation of 1,4-disubstituted benzene substrates in growing cultures of *Pseudomonas putida* UV4, is found to be largely controlled by the relative size of the substituents.

The biotransformation of aromatic substrates by mutant strains of the soil bacterium *Pseudomonas putida* can provide high yields of *cis*-dihydrodiol derivatives *via* a dioxygenase-catalysed oxidation.[1,2] The remarkable synthetic versatility of the *cis*-dihydrodiol of benzene and monosubstituted benzenes is increasingly being exploited[2,3] owing to their commercial availability. The development of general methods for the determination of stereochemistry [enantiomeric excess (e.e.) and absolute configuration[4,5]] and for the replacement of substituents on the parent[5,6] or protected *cis*-dihydrodiol[7] has greatly increased their value as chiral synthons.

In this communication we report that (*i*) 1,4-disubstituted benzene substrates, in cultures of *P. putida* UV4, can be converted to the corresponding *cis*-dihydrodiol metabolites whose % e.e. can be determined both indirectly, using the method developed for the *cis*-diol metabolites of monosubstituted arenes,[4,5] and directly by a new method involving chiral stationary phase (CSP) HPLC analysis; (*ii*) the absolute configurations of *cis*-dihydrodiol metabolites have been determined by stereochemical correlations involving halogen substitution reactions and circular dichroism (CD) spectral comparison; (*iii*) the absolute stereochemistry of the *cis*-dihydrodiols can be predicted from a consideration of the relative steric parameters of the two substituents.

When a series of monosubstituted benzene substrates **A** (S = H, L = Me, Cl, Br, I, CF_3) were added to growing cultures

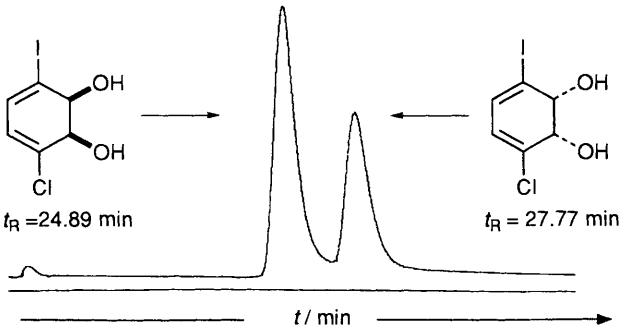
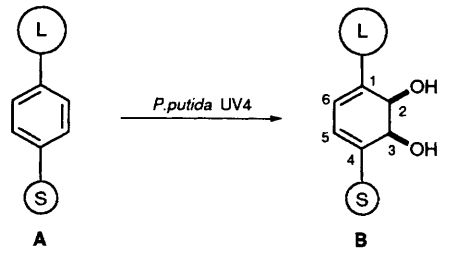


Fig. 1 HPLC trace (254 nm) of *cis*-dihydrodiol metabolite **9** [Chiralcel OJ column (25 × 0.46 cm); eluent, propan-2-ol(10) : hexane(90); flow rate 0.5 cm^3 min^{-1}]



of *P. putida* UV4, a single *cis*-diol enantiomer **B** was obtained in each case.[4] In contrast, while enantiomer **B** (S = H, L = F) was again the major product, a significant proportion (*ca.* 20%, see Table 1) of the opposite enantiomer was isolated when fluorobenzene was utilized as substrate.[4] The preference for a single *cis*-dihydrodiol enantiomer **B** (S = H, L = F, Me, Cl, Br, I, CF_3), was shown in each case by analysis of the corresponding di-MTPA esters of the 4-phenyl-1,2,4-triazoline-3,5-dione cycloadducts[4,5] [MTPA = α-methoxy-α-(trifluoromethyl)phenylacetic acid]. The absolute configurations were determined by a combination of methods, involving ^1H NMR spectroscopy, X-ray crystallographic analysis on the di-MTPA esters of the cycloadducts[4,5] and stereochemical correlation methods.[5]

Using a selected group of nine 1,4-disubstituted benzene substrates **A** (L/S = CF_3/Me, CF_3/I, I/F, Me/F, I/Me, Br/Me, I/Br, I/Cl, Cl/Me) in the current study, the corresponding *cis*-dihydrodiols **B** were isolated as the sole metabolites (Table 1). Although the isolated yields obtained (60–13%) were lower than those previously reported from monosubstituted benzene substrates,[4,5] these have not been optimized. The 4-phenyl-1,2,4-triazoline-3,5-dione cycloadducts were isolated in quantitative yields as single diastereoisomers from *cis*-diols **1**, **3–7**, **10** and subsequently converted to the corresponding di-MTPA esters using the reported method.[4] The rate of formation of di-MTPA esters of *para*-disubstituted cycloadducts was, in general, slower than that of the corresponding cycloadducts derived from the *cis*-dihydrodiols of monosubstituted arenes. Thus, owing to the slow rate of formation of cycloadducts of *cis*-dihydrodiols **2**, **8** and **9**, and lower stability of the di-MTPA esters, it was not possible to isolate pure samples of these esters for ^1H NMR spectral analysis. The e.e. of *cis*-dihydrodiols **1** and **3–7**, **10** was, however, readily determined from the diastereoisomeric excess which was evident from the diagnostic ^1H NMR (MeO) signals in the spectrum of the corresponding di-MTPA esters. The e.e. values for all of the *cis*-dihydrodiols shown in Table 1 could be confirmed by the first applicatrion of CSP HPLC analysis to this type of enantiomer. The separation factor (α) for enantiomers was in the range 1.1–1.2. Fig. 1 depicts a typical elution profile of the enantiomers of metabolite **9**. This direct method of analysis has also proved to be applicable to the *cis*-dihydrodiols of monosubstituted arenes.[8]

Table 1 Isolated yield, optical rotation, e.e., and absolute configuration of *cis*-dihydrodiol metabolites **B**

Compounds	L	S	Yield (%)[a]	$[\alpha]_D$[a,b]	% e.e.[c]	Config.[d]
1	CF_3	Me	28	−119	>98	2*R*:3*S*[e]
2	CF_3	I	50	−55	>98	2*R*:3*R*
3	I	F	60	+62	88	2*S*:3*R*
4	Me	F	21	+132	83	2*R*:3*R*[e]
5	I	Me	24	+3	80	2*S*:3*S*
6	F	H	[f]	−33	60	2*S*:3*S*
7	Br	Me	13	−9	37	2*S*:3*S*
8	I	Br	22	+5.5	22[g]	2*S*:3*R*[e]
9	I	Cl	25	+6	15	2*S*:3*R*
10	Cl	Me	20	−12	15[g]	2*S*:3*S*

[a] Based upon uncrystallized material, purified by preparative TLC on silica-gel. [b] MeOH solvent. [c] Average value obtained by CSP HPLC analysis and ^1H NMR spectral analysis of di-MTPA esters. Reproducibility of e.e. value between repeated biotransformations was found to be within ±3%. [d] Established unequivocally by replacement of a halogen atom and stereochemical correlation. The specified configuration refers to the major enantiomer formed (structure **B**) and any apparent change in configuration at C-2 and C-3 is due to a change of group priorities in the Sequence Rule. [e] Determined by CD spectral comparison. [f] Commercial sample. [g] Estimated by CSP HPLC analysis only.

The absolute configurations of all the *cis*-dihydrodiols derived from 1,4-disubstituted benzene substrates shown in Table 1 (with the exception of the *cis*-dihydrodiols **1**, **4** and **8**) were determined unequivocally by making use of the substitution reactions[5,8] of the halogen atoms (Cl, Br, I) present on the *cis*-dihydrodiol metabolites. *cis*-Dihydrodiols **1**, **4** and **8** were configurationally assigned by comparison of their CD spectra with similar *cis*-dihydrodiols of established configurations *e.g.* **2**, **3**, **5–7**, **9** and **10**. From the stereochemical correlation study it was concluded that in every case the configuration of the major enantiomer was identical to that shown in structure **B**, where L and S signify the large and small groups respectively, using either the Taft (E'_s) or Charton (ν) steric parameters.[9] The size of substituents (E'_s or ν) were assumed to increase in the sequence H < F < Me < Cl < Br < I < CF_3. In the monosubstituted benzene series **A** (S = H) all substituents were larger than a hydrogen atom and therefore the *cis*-dihydrodiol product in every case (with the exception of L = F) had an exclusive (>98%) preference for enantiomer **B**. The smaller size differential between fluorine and hydrogen atoms is reflected in a diminished preference for enantiomer **B** (60% e.e. in *cis*-dihydrodiol **6**).

The data in Table 1 show that within the limitations of accuracy [± 3%] in the determination of e.e., and of the validity of the steric parameters as an indicator of size, a qualitative correlation exists between the difference in size of *para*-substituents and the enantiomeric preference. Furthermore, with the marked exception of metabolite **3**, a rough correlation can be observed between the resultant polarity of the two substituents (dipole) and the e.e. value. While the role of other factors cannot be excluded, the steric effect appears to be a dominant factor in the control of facial selectivity of the dioxygenase enzymes in *P. putida* UV4 and as such could assist in the design of new *cis*-dihydrodiols bearing the required functionalities for use as chiral synthons. The unavailability of reliable methods for the determination of e.e. of *cis*-dihydrodiols from substituted benzene substrates (*e.g.* CSP HPLC analysis and ^1H NMR spectral analysis of di-MTPA esters[4]) may have limited the accuracy of earlier work which led to the conclusion that the dioxygenase enzyme system in growing cultures of *P. putida* was unable to differentiate between Me-, Cl- and Br-substituents during the formation of *cis*-dihydrodiol metabolites from 4-chloro- and 4-bromo-toluene.[10] It has recently been suggested[11] that there

may be a subtle but significant difference in the active sites of the toluene dioxygenases in *P. putida* UV4 and the strains used by Gibson and coworkers. This may also account for the dissimilar e.e. values.

The qualitative effect of substituent size upon facial selectivity during *cis*-dihydrodiol formation from mono- and 1,4-di-substituted benzene substrates using *P. putida* UV4 is apparent from the data in Table 1. Preliminary experiments from a comprehensive study to establish whether a similar type of steric control is operative during selective attack at different bonds of 1,2-disubstituted benzene substrates (regioselectivity) have been carried out.[8] Thus, using F, Cl, Br, I and Me substituents at the 1- and 2-position, the regioisomeric *cis*-dihydrodiol products obtained clearly indicate that substituent size is again a dominant factor in the selection of which bond is preferentially oxidized and therefore in the prediction of which diol is likely to be formed.

The accessibility of a new range of *cis*-dihydrodiol metabolites from 1,2-, and 1,4-disubstituted benzene substrates *via* dioxygenase enzyme-catalysed oxidation in *P. putida* UV4, (of predictable absolute configuration and regiochemistry) supplemented by chemical methods for the replacement of substituents[5–8] will greatly facilitate the synthesis of hitherto unobtainable members of the chiral *cis*-dihydrodiol series. It should in principle be possible to obtain *cis*-dihydrodiols of monosubstituted benzene derivatives but with a reversed chirality. Similarly, while oxidation does not occur at the 3,4-bond of monosubstituted benzene substrates in *P. putida* UV4, this new *cis*-dihydrodiol type should be available using the above chemo-enzymatic method.

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