COMMUNICATION

Toluene dioxygenase-mediated oxidation of aromatic substrates with remote chiral centers

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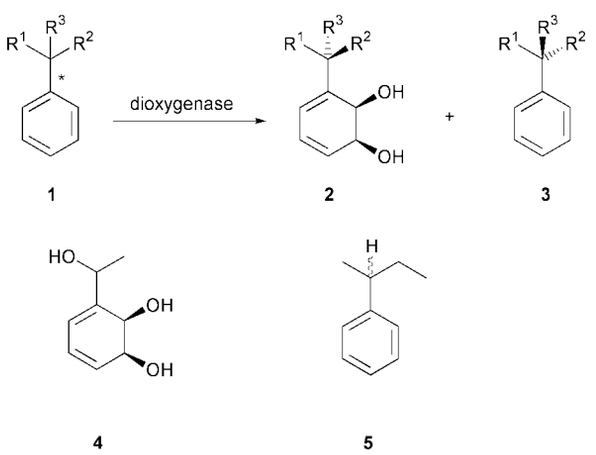
Several aromatic substrates containing remote chiral centers were subjected to toluene and naphthalene dioxygenase expressed in blocked mutants and recombinant organisms yielding *cis*-diol metabolites with little or no kinetic resolution.

The utility of *cis*-dihydroxydihydrobenzene metabolites of aromatic hydrocarbons in asymmetric synthesis is firmly established. Several recent reviews highlight the growing activity in this field[1] while investigations continue to explore new compounds as potential substrates for the enzyme(s).[2] To date, over three hundred homochiral diols have been identified from the oxidations of simple, fused, and biphenyl-type aromatics.[1]

Reports of metabolites containing additional chiral centers and arising from kinetic resolution of substrates of type **1** (Scheme 1) are rare. To our knowledge only five instances of



Scheme 1 Biooxidation of substituents containing benzylic chiral centers.

biooxidation of such compounds have been reported. Gibson *et al.* reported the production of diol **4** in conjunction with their studies of di- *vs.* monohydroxylation.[3] This has been further elaborated by Cripps and co-workers.[4] Several studies were recently conducted by Boyd *et al.* on 1-phenylethan-1-ol and other substrates.[5] Ribbons and Ahmed[6] studied the biooxidation of phenyl ethanols (*R*-, *S*-, and racemate) with blocked mutants and reported a slight preference for the *S*-enantiomer. Racemic *sec*-butylbenzene (**5**) and racemic 1-phenylpropan-1-ol were also oxidized but the stereochemical outcome was not reported. With these limited examples we nevertheless investigated several substrates with one or more remote centers by means of parallel experiments with both blocked mutants and recombinant organisms.[7] The results of these experiments are shown in Table 1.

Our interest in these issues is related to ongoing activities in the synthesis of morphine alkaloid.[8] One such approach already published[9] employed a dienediol generated from biphenyl. We reasoned that a suitably functionalized phenylcyclohexanone or phenylcyclohexanol of type **18** or **19** would, if successfully oxidized, furnish a "resolved" synthon in which the biochemically set chirality would later be destroyed to provide the dibenzofuran core of the alkaloid (Scheme 2). Clearly, the best models for such an undertaking are compounds **10** and **12** (Table 1). To provide a background against which any potential kinetic resolution could be measured we repeated the published experiments with 1-phenylethan-1-ol and also compared several organisms used in the biooxidations.

Repetition of Ribbons' and Ahmed's experiments with phenethyl alcohol gave confirmation of their earlier observation except in the case of fermentation with *Escherichia coli* JM109 (pDTG601A). While *Pseudomonas putida* F39/D organism recognizes the *R*-enantiomer of the alcohol to a lesser extent, the recombinant clone produced the diol metabolite without any kinetic resolution. Although surprising, since the same dioxygenase enzyme is expressed in both organisms, this result may be rationalized by slight differences in the enzyme topology resulting from post-translational folding. All of the fermentations were carried out with freshly grown cells[10] of either organism. For all runs with *P. putida* F39/D the enzyme expression was induced with toluene prior to substrate addition, while in the fermentations with *E. coli* such expression was induced with IPTG. Substrates were added as neat compounds at a concentration of 0.05–0.1%. The disruption of the aromatic ring was monitored by an increasing absorption between 260 and 270 nm. In general, work-up was performed by centrifuging the cells followed by extraction of the supernatant with ethyl acetate, drying and evaporation. Products were isolated by recrystallisation and/or flash chromatography and were fully characterized by spectral and physical methods.

With the *E. coli* JM109 (pDTG601) both enantiomers of racemic 1-phenylethan-1-ol (**6a**,**b**) were fully converted to two compounds **4a** and **4b** in a 1:1 ratio assumed to be the diastereomeric mixture as revealed by both ^{1}H and ^{13}C NMR spectroscopy. Analyses of aliquots from fermentations for the non-converted 1-phenylethan-1-ol on a chiral support GLC did not reveal any difference in the rate of conversion of the two enantiomers. Moreover, in separate runs both the *R*- and the *S*-enantiomers were oxidized at the same rate (UV-monitoring at $\lambda = 266$ nm). In contrast, when *P. putida* F39/D was used the *S*-enantiomer was oxidized at a faster rate than the *R*-antipode according to UV-monitoring. When the racemic mixture was used as substrate a 60:40 ratio of diastereomers resulted with a preference for the dihydroxylation of the *S*-enantiomer as evident by ^{1}H NMR spectroscopy as well as analysis of the non converted compound.

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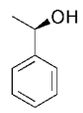
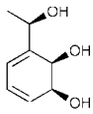
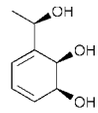
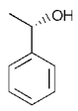
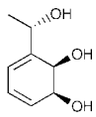
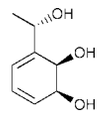
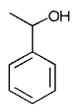
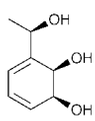
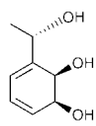
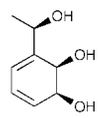
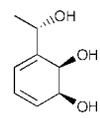
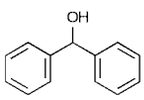
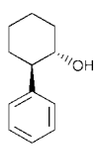
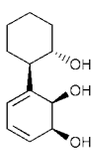
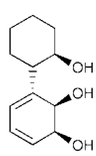
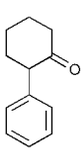
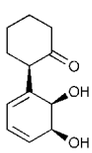
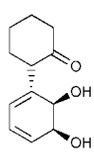
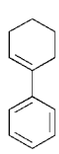
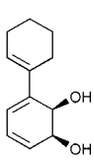
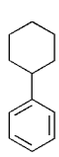
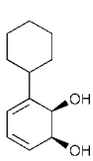
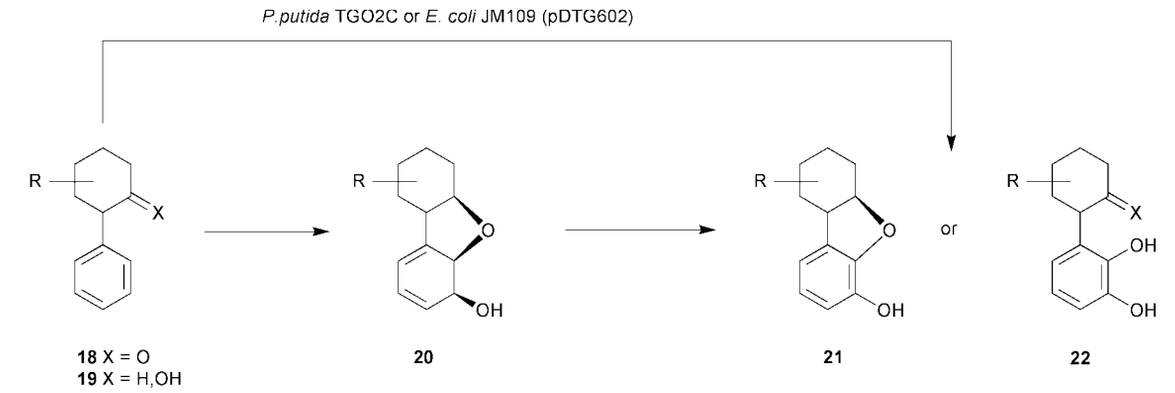
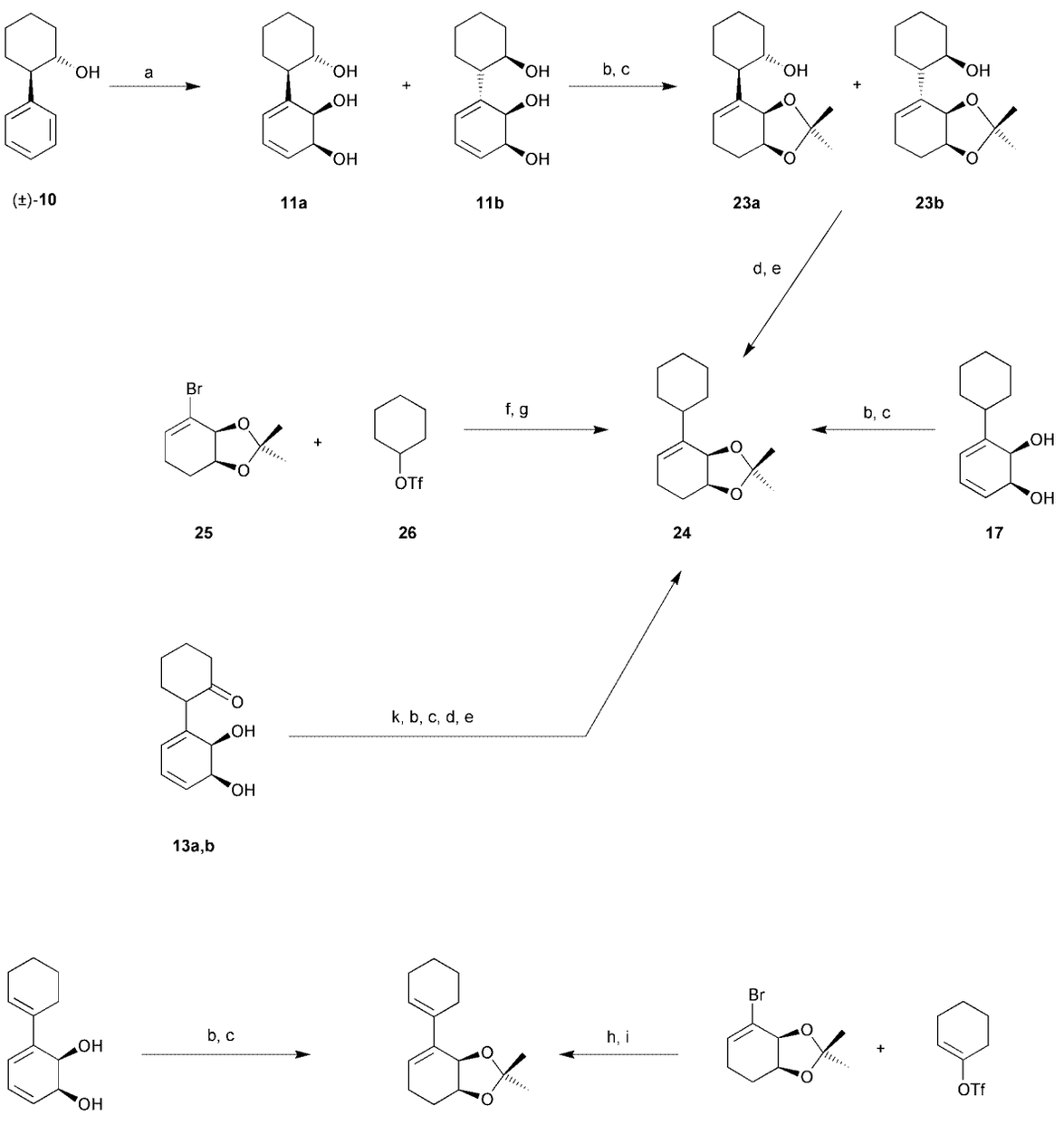
Table 1 Comparison of biooxidation of substrates with different organisms[a]

Entry	JM109 (pDTG601) TDO	JM109 (pDTG141) NDO	*Pp* F39/D
 **6a**	 **4a** [1.0]	—	 **4a** [1.0]
 **6b**	 **4b** [1.0]	—	 **4b** [1.0]
 **6a,b**	 **4a**  **4b** [5.0] (1 : 1)	—	 **4a**  **4b** [1.0] (4 : 6)
 **7**	No conversion	No conversion	—
 **8**	No conversion	No conversion	—
 **9**	No conversion	No conversion	—
 (±)-**10**	 **11a**  **11b** [1.0] (1 : 1)	—	No conversion
 **12**	 **13a**  **13b** [1.6] (1 : 1)	—	No conversion
 **14**	 **15** [3][b]	—	—
 **16**	 **17** [1.8][c]	—	—

[a] A dash implies that the experiment has not been performed. Numbers in parentheses are ratios, numbers in brackets are yields (g L^{-1}) and are not optimised. For mixtures the numbers refer to the yield for both isomers. [b] $[a]_D^{28} = +47.5$ (*c* 0.05, MeOH). [c] $[a]_D^{30} = -73.8$ (*c* 1.0, CH_2Cl_2).



Scheme 2 Chemoenzymatic approach to morphine synthesis—introduction of catechol to ring A.



Scheme 3 Correlation of absolute stereochemistry of metabolites. *Reagents and conditions*: a) *E. coli* JM109 (pDTG602); b) PAD, MeOH, HOAc, 0 °C; c) DMP, TsOH, rt; d) Ph_3P, CBr_4, CH_2Cl_2, 0–5 °C; e) Bu_3SnH, AlBN, THF, Δ; f) Mg (act.), ether; g) Li_2CuCl_4, −78 °C; h) *sec*-BuLi, THF, −78 °C; then Me_3SnCl, rt; i) $(CH_3CN)_2PdCl_2$, DMF, rt; j) PCC, CH_2Cl_2, 0–5 °C; k) $NaBH_4$, EtOH, rt.

To explore the potential of the kinetic resolution we tested other substrates with an oxygen substituent in the benzylic position. Because of the known overlap in recognition of certain aromatics by both toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO), we performed the fermentations of 1,2,3,4-tetrahydro-1-naphthol (**7**), benzhydryl alcohol (**8**), and 1,4-dihydro-1,4-epoxynaphthalene (**9**) with both enzymes. However, neither **7**, **8** or **9** produced detectable amounts of dihydroxylated metabolites with TDO or NDO (the latter expressed by *E. coli* JM109 (pDTG141)). It is known that indane, indan-1-ol, indan-2-ol, indan-1-one, and indan-2-one are not dihydroxylated,[11] but undergo benzylic hydroxylation

instead. It may well be that this is a general trend for the *ortho*-fused ring systems, at least with the use of organisms described in this manuscript, and this may explain why there is no dihydroxylation for 1,2,3,4-tetrahydro-1-naphthol and 1,4-dihydro-1,4-epoxynaphthalene.[12] It is however known that *P. putida* UV4 oxidizes a number of *ortho*-fused ring systems.[13]

The substrate of most interest to us is *trans*-2-phenylcyclohexanol (**10**) which has two neighboring stereogenic centers and which most closely resembles the intended morphine models **18** or **19**. However, racemic **10** was converted to the corresponding dienediol as a 1:1 mixture of diastereomers indicating no preference of the enzyme for either enantiomer. Three other substrates, 1-phenylcyclohexanone (**12**), 1-phenylcyclohexene (**14**) and phenylcyclohexane (**16**) were converted to the corresponding dienediols for the purpose of correlation of absolute stereochemistry in **11a/11b**. Even though the kinetic resolution in **10** did not occur, the utility of this type of substrate in approaches to morphine is still viable and compounds of this structural type with additional chiral centers, such as **18** and **19**, will be investigated, if for no other reason, because of the facile introduction of the catechol unit to the morphine nucleus.

The absolute stereochemistry of the *vic*-diols in **11a**, **11b**, and **13a**, **13b**, **15** and **17** were proven according to the reactions in Scheme 3. The mixture of triols **11a** and **11b** was reduced to the alkenes **23a**,**b** with potassium azodicarboxylate (PAD). Protection of the *vic*-diol as the ketals, bromination with CBr_4–Ph_3P and reduction of the bromides with Bu_3SnH yielded one compound which was identical to **24** as prepared from **17**. Since **17** was obtained from the biooxidation of phenylcyclohexane followed by PAD reduction and protection with 2,2-dimethoxypropane (DMP), the absolute stereochemistry of the diol in both **17** and **11** is identical. It is also of the same absolute configuration as the diol derived from bromobenzene. Coupling of bromide **25** of known[14] stereochemistry with cyclohexyl triflate **26** was achieved *via* the transformation of the bromide to the Grignard compound in the presence of Li_2CuCl_4.[15] The product was identical by all means, including optical rotation, with the material prepared from **11a** and **11b**, the products of the biotransformation of **10**. The absolute stereochemistry of **15** was proven by a similar sequence using the bromide **25**, but coupling with cyclohexenyl triflate **28** according to the work of Stille *et al.*[16] giving diene **27**, identical in all respects to the one derived from **15**, the biotransformation product of **14**. Finally, the absolute stereochemistry of **13** was proven by conversion to **24**.

It appears that the substrates chosen for this study were not processed by the enzymes as separate enantiomeric entities. While no significant kinetic resolution was observed the investigation of compounds of type **18** and **19** remains of interest in the production of synthons containing the ring A of morphine. Because compounds of type **19** will be enantiomerically pure it appears that the merit of their oxidation will be in the biocatalytic conversion of the phenyl ring directly to catechol when the clone containing catechol diol dehydrogenase is used. We will report on these studies as well as further details connected to the work presented here in the near future.

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12 A good model to test this assumption would be *o*-methylphenylethan-1-ol.

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