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Tandem enzyme-catalysed oxidations of alkyl phenyl sulfides and alkyl benzenes: enantiocomplementary routes to chiral phenols

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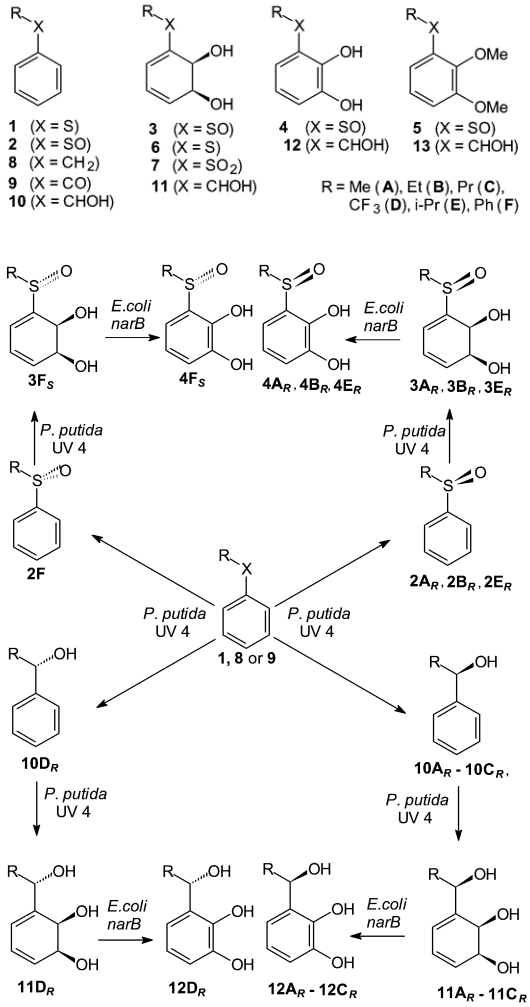
Dioxygenase-catalysed trioxygenation of alkyl phenyl sulfides and alkyl benzenes yields enantiopure *cis*-dihydrodiol sulfoxides and triols respectively; naphthalene *cis*-dihydrodiol dehydrogenase-catalysed aromatisation of these diastereoisomers gives enantiopure catechols of either configuration.

Toluene dioxygenase (TDO), from *Pseudomonas putida* UV4, has been found to catalyse stereoselective monooxygenation (benzylic hydroxylation or sulfoxidation),[1–3] dioxygenation (*cis*-dihydroxylation or *bis*-benzylic hydroxylation),[4–6] and trioxygenation (benzylic hydroxylation/*cis*-dihydroxylation).[7,8] Two new approaches to trioxygenated arenes using *P. putida* UV4 *i.e.* monosulfoxidation/*cis*-dihydroxylation *e.g.* **1A** → **2A** → **3A** and ketone reduction/*cis*-dihydroxylation *e.g.* **9D** → **10D** → **11D**, are presented in this report. The trioxygenated products **3** and **11** were all found to be substrates for naphthalene *cis*-diol dehydrogenase (NDD) present in *Escherichia coli* DH5α (pUC129::*narB*), a recombinant strain (*E. coli narB*) constructed using the NDD gene expressed by *Rhodococcus sp.* NCIMB 12038.[9]

Using whole cells of *P. putida* UV4, a source of TDO, stereoselective sulfoxidation is the preferred biotransformation pathway of alkyl, aryl and diaryl sulfides.[1] Conversely, *cis*-dihydroxylation of a phenyl group was strongly favoured over sulfoxidation of a benzyl alkyl sulfide by this strain.[3] It has now been found that using the standard method for triol formation,[8] allied to an extended period of biotransformation (> 18 h) and the reported improved isolation procedure (involving complete removal of the water under mild conditions),[3] with alkyl aryl sulfides as substrates, *e.g.* **1A**, **1B**, **1E**, TDO-catalysed tandem trioxygenation (**1**→ **2** → **3**) produces the *cis*-dihydrodiol sulfoxide diastereoisomers $\mathbf{3A}_{\boldsymbol{R}}$, $\mathbf{3B}_{\boldsymbol{R}}$, and $\mathbf{3E}_{\boldsymbol{R}}$ in *ca.*: 80% (30 mmol), 80% (32 mmol) and 20% (5 mmol) isolated yields respectively (Scheme 1).[10] Formation of the enantiopure metabolite $\mathbf{3F}_{\boldsymbol{S}}$ from the diaryl sulfide **1F** provides a novel example of enzyme-catalysed stereodifferentiation between prochiral phenyl groups *i.e.* preferential *cis*-dihydroxylation of the *pro-S* group. TDO-catalysed oxygenation reactions in *P. putida* UV4 show a marked preference for one of the two prochiral lone pairs,[1] sulfur atoms,[11] hydrogen atoms,[8] methylene groups[5] and now phenyl groups. The formation of a remarkably stable *cis*-dihydrodiol sulfone bioproduct **7B** of sulfide **1B** (15% isolated yield) using *P. putida* UV4, brings to light a new type of TDO-catalysed polyoxygenation, *i.e.* tetraoxygenation (sulfoxidation/*cis*-dihydroxylation/sulfonidation).

Mixtures of the *cis*-dihydrodiol sulfoxide diastereoisomers $\mathbf{3A}_{\boldsymbol{R}}/\mathbf{3A}_{\boldsymbol{S}}$, $\mathbf{3B}_{\boldsymbol{R}}/\mathbf{3B}_{\boldsymbol{S}}$, $\mathbf{3E}_{\boldsymbol{R}}/\mathbf{3E}_{\boldsymbol{S}}$ or $\mathbf{3F}_{\boldsymbol{R}}/\mathbf{3F}_{\boldsymbol{S}}$[10] were prepared from: (i) biotransformation of the corresponding racemic, **2A**, **2B** and **2E**, or meso, **2F**, sulfoxide substrates and (ii) dimethyl dioxirane oxidation of the corresponding *cis*-dihydrodiol sulfides **6A**, **6B**, **6E** and **6F** obtained by a chemoenzymatic method.[3,12] Separation of the *cis*-dihydrodiol sulfoxide diastereoisomeric pairs $\mathbf{3E}_{\boldsymbol{R}}/\mathbf{3E}_{\boldsymbol{S}}$ or $\mathbf{3F}_{\boldsymbol{R}}/\mathbf{3F}_{\boldsymbol{S}}$ was achieved by PLC (6% MeOH in $CHCl_3$). The diastereoisomers $\mathbf{3A}_{\boldsymbol{R}}/\mathbf{3A}_{\boldsymbol{S}}$ or $\mathbf{3B}_{\boldsymbol{R}}/\mathbf{3B}_{\boldsymbol{S}}$ were separable by HPLC. The absolute configurations of the enantiopure *cis*-diol sulfoxide metabolites $\mathbf{3A}_{\boldsymbol{R}}$, $\mathbf{3B}_{\boldsymbol{R}}$, $\mathbf{3E}_{\boldsymbol{R}}$, $\mathbf{3E}_{\boldsymbol{S}}$, $\mathbf{3F}_{\boldsymbol{R}}$ and $\mathbf{3F}_{\boldsymbol{S}}$ were determined by circular dichroism (CD) spectral comparison (and stereochemical correlations) with the corresponding catechols **4** and their dimethoxy derivatives **5** with alkyl aryl sulfoxides of established configuration (Table 1).[1] Absolute configurations and enantiopurity values were also



Scheme 1 Major bioproducts formed from arenes **1**, **8** and **9**.

Table 1 Optical rotations, ($[\alpha]_D$, $CHCl_3$), and absolute configurations (*R*/*S*) of compounds (Cpd) **5A**, **5B**, **5E**, **5F** and **13A-D**

Cpd	5A	5B	5E	5F	13A	13B	13C	13D
(+)-$[\alpha]_D$	220 (*R*)	256 (*R*)	230 (*R*)	159 (*S*)	23 (*R*)	9 (*R*)	15 (*R*)	6 (*R*)
(−)-$[\alpha]_D$			231 (*S*)	161 (*R*)	25 (*S*)	9 (*S*)	16 (*S*)	5 (*S*)

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determined by ^{1}H-NMR analysis of the corresponding boronate derivatives formed using (*R*)- and (*S*)-2-(1-methoxyethyl) benzeneboronic acids as reported for other trioxygenated biopoducts.[3,8]

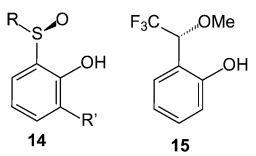
Aromatisation (dehydration) of the individual *cis*-diol sulfoxide diastereoisomers **3A$_R$**, **3B$_R$**, **3E$_R$**, **3E$_S$**, **3F$_R$** and **3F$_S$**, either thermally or in the presence of acid, yielded a mixture of *ortho*-and *meta*-phenols with evidence, in some cases, of partial racemisation of the sulfoxide stereogenic centre. A milder enzyme-catalysed approach to aromatisation was thus adopted.

Recently we have shown[9] that the NDD enzyme present in the recombinant strain, *E. coli narB*, accepts naphthalene *cis*-dihydrodiol as substrate. This new strain has not however been tested with other types of *cis*-dihydrodiol substrates. *E. coli narB* was thus used with the *cis*-diol sulfoxide substrates **3A$_R$**, **3B$_R$**, **3E$_R$** and **3F$_S$** (from the corresponding sulfide precursors **1A**, **1B**, **1E** and **1F**), **3E$_S$** and **3F$_R$** (from the *cis*-dihydrodiol sulfides **6E** and **6F**). The corresponding catechol enantiomers **4A$_R$**, **4B$_R$**, **4E$_R$**, **4E$_S$**, **4F$_R$** and **4F$_S$** were obtained as metabolites (50–70% yield) using a general procedure.† Due to their variable stability in solution, the catechols were characterised as the stable dimethoxy or the diacetoxy derivatives. The absolute configurations of all the catechol metabolites were determined by stereochemical correlation. These assignments were confirmed for catechols **4A$_R$**, **4B$_R$**, **4E$_R$**, **4E$_S$** and **4F$_S$** by comparison of the CD spectra of the dimethoxy derivatives **5A$_R$**, **5B$_R$**, **5E$_R$** and **5E$_S$**, with those of the corresponding alkylphenyl sulfoxides of known configurations (Table 1).

The tandem conversion (TDO-catalysed oxidation using *P. putida* UV4) of the alkylbenzene substrates **8A**–**8C** *via* the corresponding monol intermediates **10A$_R$**–**10C$_R$** to the triol metabolites **11A$_R$**–**11C$_R$** was carried out using the reported method[8] and the improved isolation procedure.[3] Addition of the commercially available benzylic alcohol enantiomers **10A$_S$**–**10C$_S$** as substrates yielded the corresponding triol diastereoisomers **11A$_S$** (4 mmol, 79%), **11B$_S$** (5 mmol, 89%) and **11C$_S$** (1 mmol, 65%). A novel biotransformation pathway was observed when ketone **9D** was used as substrate with whole cells of *P. putida* UV4; the only bioproducts observed were the separable triol diastereoisomers **11D$_R$**/**11D$_S$** (95 : 5, 230 mmol, 50% yield).[10] It was evident that a stereoselective dehydrogenase-catalysed reduction of ketone **9D** had occurred and that the transient benzylic alcohol products **10D$_R$**/**10D$_S$** were rapidly oxidised to yield triols **11D$_R$**/**11D$_S$**.

Addition of the triols **11A$_R$**–**11D$_R$** and **11A$_S$**–**11D$_S$** to *E. coli nar B* cultures,† gave the corresponding catechols **12A$_R$**–**12D$_R$** and **12A$_S$**–**12D$_S$** (50–70% yield). These enantiopure catechols were also characterised as their more stable dimethylethers **13A$_R$**–**13D$_R$** and **13A$_S$**–**13D$_S$** and as triacetates (Table 1).

Enantiopure catechols **4** and derivatives **14** are currently



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being evaluated as chiral ligands for asymmetric alkylation and other reactions. The acetonide derivative of triol **11D$_R$** was methylated, and aromatised/deprotected under acid conditions to give enantiopure phenol **15**.[10] This phenol proved to be a promising new reagent for the determination of enantiopurity of chiral carboxylic acids *e.g.* the pharmaceutical intermediate ketoprofen, by ^{1}H-NMR (OMe signal) and ^{19}F-NMR (CF_3 signal) spectral analysis of the derived esters. It was also found to be a good resolving agent (TLC or HPLC separation) when tested on racemic samples of chiral acid *e.g.* 2-arylpropanoic acids.

In conclusion, enantiopure *cis*-dihydrodiol sulfoxides **3** and triols **11**, produced in good yields by enzyme-catalysed and chemoenzymatic reactions of sulfides **1**, alkyl benzenes **8** and a ketone **9D** have been used to develop enantiocomplementary routes to a series of new enantiopure phenols. These have already shown potential as new chiral ligands, reagents for diastereoisomeric resolution and determination of enantiopurity.

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Notes and references

† *E. coli narB* was grown at 37 °C in Luria broth with ampicillin (0.1 mg/cm^3) and isopropyl-β-D-thiogalactopyranoside (IPTG, 0.05 mg/cm^3); cells were harvested, in late exponential growth phase, by centrifugation, washed and resuspended (shake flasks; OD_{600} = 5–10) in potassium phosphate buffer (0.05 M, pH 7.2) for performing biotransformations at 30 °C. Substrates were added at concentrations between 0.2–0.5 mg/cm^3 and the reactions terminated after 18 h. The catechols were isolated, from the aqueous medium after saturating it with sodium chloride, by repeated extractions with EtOAc.

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