Toluene and naphthalene dioxygenase-catalysed sulfoxidation of alkyl aryl sulfides

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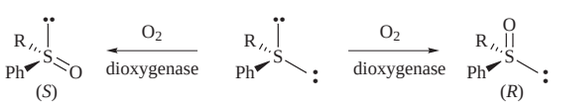
A series of alkyl aryl sulfides were metabolised, using selected strains of the soil bacterium *Pseudomonas putida* containing either toluene dioxygenase (TDO) or naphthalene dioxygenase (NDO), to give chiral sulfoxides. Alkyl aryl sulfoxides 2a–2k, 4a–4j and 4l, having enantiomeric excess (ee) values of >90%, were obtained by use of the appropriate strain of *P. putida* (UV4 or NCIMB 8859). Enantiocomplementarity was observed for the formation of sulfoxides 2a, 2b, 2d, 2j, 4a, 4b and 4d, with TDO-catalysed (UV4) oxidation favouring the (*R*) enantiomer and NDO-catalysed oxidation (NCIMB 8859) the (*S*) enantiomer. Evidence of involvement of the TDO enzyme was obtained using a recombinant strain of *Escherichia coli* (pKST 11). The marked degree of stereoselectivity appears to be mainly due to enzyme-catalysed asymmetric sulfoxidation, however the possibility of a minor contribution from kinetic resolution, in some cases, cannot be excluded.

Introduction

The sulfoxide group has been widely used in asymmetric synthesis due to its ability to act as a stereodirecting group capable of stabilizing negative charge on an adjacent carbon atom.[1–4] Chiral sulfoxides can be obtained by the stereoselective enzyme-catalysed oxidation of sulfides.[5] Early reports, on the production of enantiopure sulfoxides, by enzyme-catalysed oxidation of sulfides, involve the use of whole cell systems from both fungi *e.g. Aspergillus niger* (*foetidus*),[6] *Helminthosporium* sp.,[7] *Mortierella isabellina*[7–9] and bacteria *e.g. Corynebacterium equi*.[10–12] The majority of examples of stereoselective microbial sulfoxidation (>90% ee), prior to 1988, were carried out using fungal enzymes of unspecified type.[5] While intact cell fungal biotransformations of sulfides continue to be an important route to chiral sulfoxides,[13,14] bacterial enzymes *e.g.* monooxygenases and dioxygenases, have been more widely used to yield enantiopure sulfoxides over the last decade. Recent whole-cell bacterial sulfoxidations include the use of a wide range of bacteria.[13,15–17] Previous reports have shown that selected strains of the soil bacterium *Pseudomonas putida* containing dioxygenase enzymes[17,18] (and pure dioxygenase enzymes[19]) may yield a small number of enantiopure sulfoxides from the corresponding sulfide substrates. The range of sulfides which can be metabolized using (i) a mutant strain (UV4) containing TDO but no *cis*-dihydrodiol dehydrogenase, (ii) a recombinant strain (pKST 11) of *Escherichia coli* containing TDO but no *cis*-dihydrodiol dehydrogenase, and (iii) a wild type strain (NCIMB 8859) containing both NDO and a *cis*-dihydrodiol dehydrogenase, has now been extended to yield more than twenty sulfoxides of high ee (Tables 1 and 2).

Results and discussion

Although microbial sulfoxidations of alkyl aryl sulfides to yield enantiopure sulfoxides have been known for more than three decades,[6] this method has not yet been widely exploited in chemoenzymatic synthesis. A major limitation of microbial oxidation has been the inability of any particular strain to yield more than a few sulfoxides with high ee values (>90%); other disadvantages include failure to oxidize some types of sulfides *e.g.* diaryl sulfides, problems of scale-up, production of only one absolute configuration and the possibility of losses both in terms of yield and stereoselectivity due to further oxidation of the sulfoxide to sulfone. The preliminary studies using *P. putida* UV4,[17,18] and the additional results contained herein, form part of our continuing quest for enzyme systems which would be suitable for large-scale production of individual sulfoxide enantiomers having synthetic potential. The technology has already been developed to produce *cis*-dihydrodiols *via* biotransformation of arenes on the multi-kilogram and even multi-ton scale using whole cells of the UV4 constitutive mutant strain of *P. putida*.[20,21] To date however the latter strain has not been used for the large-scale production of chiral sulfoxides.



R

a CH_3
b CH_2CH_3
c $(CH_2)_2CH_3$
d $CH(CH_3)_2$

R

e $(CH_2)_3CH_3$
f $(CH_2)_4CH_3$
g $(CH_2)_5CH_3$
h $(CH_2)_6CH_3$

R

i $(CH_2)_7CH_3$
j $CH{=}CH_2$
k CH_2OCH_3

The simple alkyl aryl sulfides **1a**–**1i** were first examined as substrates for the dioxygenase enzymes present in intact cells of *P. putida* UV4 (a TDO source) and NCIMB 8859 (an NDO source). The results shown in Table 1 confirm that all were oxidised to the corresponding sulfoxides, **2a**–**2i**, in variable yields depending on the nature of substrate and enzyme. The yields obtained using TDO were generally higher with sulfides bearing smaller alkyl groups (**2a**–**2d**, 95–5%). The most efficient large scale biotransformation carried out during this study involved using *P. putida* UV4 and substrate **1a**, to give multigram quantities (*ca.* 8 g) of enantiopure sulfoxide **2a** in high yield (90%). The observation that yields of sulfoxides **2a**–**2i** generally decreased with increasing size of the alkyl group using

Table 1 Isolated yields, absolute configurations and enantiomeric excess values of sulfoxides **2a**–**k** from TDO- and NDO-catalysed oxidation of sulfides **1a**–**k**

	P. putida UV4			*P. putida* NCIMB 8859		
Sulfoxide	Yield (%)	Abs. con.[a]	ee (%)	Yield (%)	Abs. con.[a]	ee (%)
2a	90[b]	*R*	>98	33[c]	*S*	91
2b	64	*R*	>98	27[c]	*S*	84
2c	5	*R*	>98	33	*R*	86
2d	27	*R*	97	24	*S*	76
2e	7	*R*	98	79[c]	*R*	>98
2f	6.5[c]	*R*	57	21[c]	*R*	98
2g	<1[c]	*R*	78	76	*R*	>98
2h	<1[c]	*R*	12	43	*R*	>98
2i	<1[c]	*R*	22	25[c]	*R*	98
2j	38	*R*	>98	6	*S*	91
2k	36[c]	*S*[d]	>98	4[c]	*S*[d]	10

[a] Absolute configuration was determined by CD studies and literature data. [b] *cis*-Dihydrodiol was also obtained in trace amounts. [c] Corrected for recovered substrate. [d] Absolute configurations appear to be reversed due to a change in the substituent priority according to the Sequence Rules.

Table 2 Isolated yields, absolute configurations and enantiomeric excess values of sulfoxides **4a**–**l** from TDO- and NDO-catalysed oxidation of sulfides **3a**–**l**

	P. putida UV4			*P. putida* NCIMB 8859		
Sulfoxide	Yield (%)	Abs. con.[a]	ee (%)	Yield (%)	Abs. con.[a]	ee (%)
4a	41	*R*	>98	4[b]	*S*	69
4b	20	*R*	94	18[b]	*S*	35
4c	—[c]	—[c]	—[c]	5	*S*	95
4d	31	*R*	78	4	*S*	91
4e	2[d]	*S*	72	5[d]	*S*	>98
4f	2[d]	*S*	73	35	*S*	>98
4g	<1	*S*	70	3[b]	*S*	90
4h	3	*S*	18	17	*S*	>98
4i	23[b]	*S*	10	35[b]	*S*	94
4j	2	*S*	76	3[b]	*S*	>98
4k	—[c]	—[c]	—[c]	<1	*S*	73
4l	30	*S*	98	53	*S*	97

[a] Absolute configuration was determined by CD studies. [b] Corrected for recovered substrate. [c] Sulfoxide metabolite not detected. [d] *cis*-Dihydrodiol also obtained.

the TDO enzyme system might be accounted for by relative solubilities of substrates **1a**–**1i**. However, by contrast the NDO system often gave higher yields of sulfoxides with the larger alkyl groups (**2e**–**2i**, 21–77%). Thus, it is evident that additional factors including the growth conditions (*e.g.* cell density, growth phase), toxicity of both substrate and bioproducts, and the nature and size of the active site of the enzyme may also affect the isolated yields.

P. putida UV4, containing TDO, has generally been found to oxidize smaller arene substrates *e.g.* monocyclic arenes to the corresponding arene *cis*-dihydrodiols.[22] By contrast the *P. putida* NCIMB 8859 strain contains naphthalene dioxygenase which prefers to catalyse the oxidation of larger substrates *e.g.* *cis*-dihydroxylation of bicyclic or tricyclic arenes.[23] The effect of the nature and size of the dioxygenase active site is likely to be a factor in the isolated yield of both *cis*-dihydrodiol and sulfoxide metabolites. Thus the general decrease in yield with increasing size of alkyl groups along the series **2a** to **2i** using TDO, and the general increase in yield of sulfoxides having larger alkyl groups *e.g.* **2e**–**2i** using NDO, may be attributed in part to different affinities of substrates for the active sites of the dioxygenases.[23]

The enantiopurities of the sulfoxides **2a**–**2i**, and other sulfoxides mentioned in this study were determined using both chromatographic and spectroscopic methods. Thus, chiral stationary phase high pressure liquid chromatography (CSPHPLC), and ^{1}H NMR analysis in the presence of the chiral solvating agent (+)-(*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol, showed characteristic peaks for each enantiomer. Using CSPHPLC and NMR methods, all of the sulfoxides, **2a**–**2i**, obtained either by the TDO (**2a**–**2e**) or the NDO (**2a**, **2e**–**2j**) enzyme systems, were found to be formed with high ee values (>90%). The circular dichroism (CD) spectra of all the chiral sulfoxide metabolites **2a**–**2i** showed a peak in the region 239–253 nm due to the alkyl aryl sulfoxide chromophore having an (*R*) absolute configuration. The latter assignment is based upon the CD rules for chiral alkyl aryl sulfoxides devised by Mislow *et al.*[24] The absolute configurations and ee values were, in many cases, confirmed by comparison with the $[\alpha]_D$ values of chiral sulfoxides of known configuration and enantiopurity (Table 3).

Evidence that the TDO enzyme, present in the mutant strain of *P. putida* (UV4), prefers a sulfoxidation rather than a *cis*-dihydroxylation pathway was found when sulfoxide products **2b**–**2i** were obtained exclusively from sulfides **1b**–**1i**. The corresponding *cis*-dihydrodiol of sulfide **1a**, (+)-*cis*-1,2-dihydroxy-3-methylsulfanylcyclohexa-3,5-diene, was found in trace amounts (<1%) in the presence of the major sulfoxide **2a** during larger scale biotransformations. The *cis*-dihydrodiol derivatives of sulfides **1a**–**1i** were not detected when *P. putida* NCIMB 8859 was used for biotransformations. The latter observation was anticipated since the NDO enzyme system is generally unable to catalyse the *cis*-dihydroxylation of monocyclic arenes.[23]

It is noteworthy that the sulfoxide metabolites **2a**–**2j** (obtained using TDO) and **2c**, **2e**–**2i** (obtained using NDO) had the same (*R*) configuration. A stereopreference for the (*S*) configuration was found for the sulfoxides **2a**, **2b**, **2d** and **2j** formed by NDO. The degree of enantiocomplementarity was optimal for sulfoxide **2a**, (*R*) >98% ee with TDO and (*S*) 91% ee with NDO.

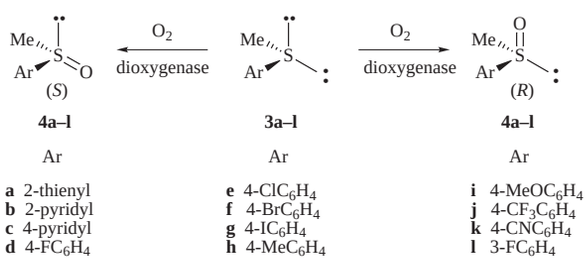
Table 3 Optical rotation, separation factor, enantiomeric excess, absolute configuration, dioxygenase type and literature reference for chiral sulfoxides **2a**–**2k** and **4a**–**4l**

Sulfoxide	$[\alpha]_D$	α[a]	ee (%)[a]	Abs. con.	Dioxygenase	Ref.[b]
2a	+162	1.36	>98	*R*	TDO	47
2b	+207	1.36	>98	*R*	TDO	47
2c	+201	1.34	>98	*R*	TDO	47
2d	+209	1.31	>98	*R*	TDO	47
2e	+200	1.37	>98	*R*	TDO	47
2f	+195	1.41	98	*R*	NDO	14
2g	+98	1.38	>98	*R*	NDO	14
2h	+127	1.44	>98	*R*	NDO	—[c]
2i	−39	1.35	>98	*R*	NDO	—[c]
2j	+393	1.41	>98	*R*	TDO	14
2k	+241	1.39	>98	*S*	TDO	50
4a	+72	1.06	>98	*R*	TDO	47
4b	+55	1.11	94	*R*	TDO	51
4c	−109	—[d]	95	*S*	NDO	48
4d	−120	1.12	91	*S*	NDO	14
4e	−154	1.06	98	*S*	NDO	47
4f	−158	1.06	98	*S*	NDO	47
4g	−123	—[d]	90	*S*	NDO	46
4h	−154	1.13	98	*S*	NDO	47
4i	+134	1.13	94	*S*	NDO	47
4j	−131	1.10	98	*S*	NDO	49
4k	−187	—[d]	70	*S*	NDO	14
4l	−138	1.25	97	*S*	NDO	52

[a] Chiralcel OD column and hexane–propan-2-ol (9:1) as eluent. [b] Literature reference to enantioenriched sulfoxides. [c] No literature reference found to enantioenriched sulfoxides. [d] Determined by ^{1}H NMR analysis in the presence of (+)-(*S*)-1-(9-anthryl)-2,2,2-trifluoroethanol as enantiomers did not separate by CSPHPLC.

The origin of the stereoselectivity in sulfoxides shown in Table 1 is assumed to be mainly due to asymmetric sulfoxidation. Addition of selected racemic sulfoxides **2a**–**2d**, to either *P. putida* UV4 or *P. putida* NCIMB 8859 did, however, show some evidence of kinetic resolution. Thus, the recovered sulfoxides showed varying degrees of enantioselectivity *i.e.* **2a** (*R*, 85% ee), **2b** (*S*, 4% ee), **2d** (*S*, 7% ee) with the TDO system, and **2a** (*S*, 16% ee), **2c** (*S*, 10% ee) with NDO. Although a large excess (>85%) of the (*R*) enantiomer of sulfoxide **2a** was obtained when either sulfide **1a** or racemic sulfoxide **2a** was used as substrate with *P. putida* UV4, the isolated yield of sulfoxide **2a** (90%) from sulfide **1a** obtained using TDO is more consistent with asymmetric synthesis. On the basis of the latter results it is considered likely that the ee, found in the chiral sulfoxide metabolites of sulfides **1a**–**1k**, is exclusively or mainly due to asymmetric synthesis. When the racemic sulfoxides **2a**–**2d** were used as substrates with either TDO or NDO systems no further metabolites, *e.g.* sulfoxide *cis*-dihydrodiols or sulfones, were observed. The possibility of both slow sulfoxide deoxygenation occurring simultaneously with fast sulfoxidation, which has previously been observed in fungal systems,[53] cannot be excluded.

Confirmation that TDO was the biocatalyst, responsible for the sulfoxidation of sulfides **1a**, **1b** and **1j**, was sought with the assistance of an *E. coli* clone (pKST 11). This recombinant strain contained the same TDO as was found in the wild type strain, *P. putida* 11 767, from which the mutant strain, *P. putida* UV4, was derived. Sulfoxides **2a** (*R*, >98% ee,), **2b** (*R*, 95% ee) and **2j** (*R*, 98% ee) formed using the *E. coli* strain were of identical configuration and similar enantiopurity to those found using *P. putida* UV4. The slightly lower ee values of the sulfoxides are explained by the concomitant formation of trace quantities of racemic sulfoxides by an enzyme system found to be present in the non-recombinant *E. coli* parent strain (JM 109). Further experiments[23] using labelled oxygen, $^{18}O_2$, and the purified TDO from *P. putida* F1 and NDO from a *Pseudomonas* sp. NCIMB 9816/4, have provided confirmation that sulfoxides **2a** (*R*, >98% ee, from TDO; *S*, >98% ee, from NDO) and **2b** (*R*, >98% ee, from TDO; *S*, 93% ee, from NDO) are formed *via* a dioxygenase-catalysed sulfoxidation process when intact bacterial cells of *P. putida* UV4 and NCIMB 8859 are used.



The range of substrates was extended from the simple alkyl phenyl thioethers (**1a**–**1i**) to sulfides containing functional groups *e.g.* alkene (**1j**), oxathioacetal (**1k**), heterocyclic rings *e.g.* thiophene (**3a**), pyridine (**3b, 3c**) and substituted phenyl rings *e.g.* (**3d**–**3l**). The sulfoxides **2j**, **2k**, **4a**, **4b** were isolated as metabolites with high ee values (94 to >98%) having the same configuration (*R* for compounds **2j**, **4a** and **4b**, but *S* for sulfoxide **2k** due a change in the Sequence Rule priorities) when the TDO-containing bacterial system was used. The NDO enzyme present in *P. putida* NCIMB 8859 gave almost exclusively (>90% ee) the (*S*) enantiomer of sulfoxides **4c**–**4j** (Table 2). The TDO system generally proved to be a less efficient biocatalyst for the production of single enantiomers of the 4-substituted phenyl methyl sulfoxides (**4d**–**4k**). Judicious selection of the appropriate dioxygenase enzyme (TDO or NDO) gives sulfoxides **4a**–**4j** of high enantiopurity (>90% ee).

A primary objective of the present study was to evaluate TDO and NDO as biocatalysts for sulfoxidation in terms of stereoselectivity and substrate type. Sulfoxidation appears to be the preferred oxidative pathway for the substrates **1a**–**1k** and **3a**–**3l** despite the variable yields of sulfoxides obtained (Tables 1–3). No evidence of other types[21] of dioxygenase-catalysed oxygenation of *e.g.* the alkene group in sulfide **1j** (alkene *cis*-dihydroxylation), the activated methylene group in sulfide **1k** (alkane monohydroxylation), the ring heteroatom in sulfide **3a** (sulfoxidation), or the arene group in any of the sulfides (arene monohydroxylation or *cis*-dihydroxylation) was detected. The only additional products detected were the *cis*-dihydrodiol derivatives of sulfides **1e** and **1f** in trace amounts. From Table 3 it is clear that a significant proportion of the sulfoxides **2a**–**2k**,

4a–**4j** produced were obtained as essentially single enantiomers using the dioxygenase systems. All the sulfoxides in Table 3 have previously been reported in enantioenriched form with the possible exception of compounds **2h** and **2i**. Further sulfoxidation studies using TDO and NDO as biocatalysts on diaryl sulfides,[17] thioacetals[17,18] and thiophene rings[25,26] have again shown a marked degree of stereoselectivity although dialkyl sulfides devoid of a benzene ring proved to be poor substrates. The remarkable versatility of the dioxygenase enzyme systems present in *P. putida* UV4 and *P. putida* NCIMB 8859 is also evident from their ability to catalyse benzylic hydroxylation, alkene *cis*-dihydroxylation and arene *cis*-dihydroxylation yielding enantiopure products in the majority of cases.[17,21,27]

Pure enzyme systems, *e.g*. cyclohexanone monooxygenase (CMO)[28–30] and chloroperoxidase (CPO)[19,31,32] have also been used in the oxidation of sulfides to yield sulfoxides of high ee (>90%) *e.g*. sulfoxides **2a**,[19,28,30,31] **2b**,[19] **2j**,[29] **4b**,[31] **4d**,[28,31] **4e**,[31] **4h**[31] and **4i**.[31] The current whole cell studies of sulfoxidations using dioxygenase enzymes suggest that they can be readily scaled up without the requirement for recycling of expensive co-factors, *e.g*. NADPH or NADH, which are required when purified monooxygenase and dioxygenase enzymes are used. The range of stereoselective dioxygenase-catalysed sulfoxidations recorded in Tables 1–3 compares favourably with other intact cell or pure enzyme biotransformations of acyclic alkyl aryl sulfides. Applications of the dioxygenase enzymes to other types of organosulfur substrates including dialkyl and diaryl sulfides, thioacetals, oxathioacetals, disulfides and thiophenes, and synthetic uses for the enantiopure sulfoxides isolated from such TDO- and NDO-catalysed oxidations, will be reported elsewhere.

Experimental

^{1}H NMR spectra were recorded using General Electric QE 300, GNΩ-500 instruments using $CDCl_3$ as solvent. Flash chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$ respectively. Merck Kieselgel $60F_{254}$ analytical plates were used for normal TLC. Optical rotation ($[\alpha]_D$) measurements are given in units of 10^{-1} deg cm^2 g^{-1} and were carried out with a Perkin-Elmer 214 polarimeter at ambient temperature (*ca*. 20 °C) at a concentration of 0.005 g cm^{-3}. Circular dichroism spectra were obtained using a JASCO J-720 instrument and spectroscopic grade acetonitrile. Enantiopurity of sulfoxides was determined by CSPHPLC (Chiralcel OD column, hexane–propan-2-ol, 9 : 1).

Biotransformation procedures

Substrates were metabolized on a small scale using shake flask cultures of the constitutive mutant strain *P. putida* (UV4) according to the reported method.[33] *P. putida* NCIMB 8859, a wild type strain, acquired from the National Cultures of Industrial and Marine Bacteria, Aberdeen, was grown on naphthalene (10 g l^{-1}) as the sole carbon source and inducer. Small scale biotransformations (0.05–0.5 g) with *P. putida* NCIMB 8859 were carried out using cells in the late exponential phase of growth. The excess naphthalene was filtered off using glass wool, the filtrate was centrifuged, and the collected cells were resuspended in potassium phosphate buffer (50 mM). Sodium pyruvate (0.5%) was added as co-substrate with the sulfide substrates (0.2–1.0 g l^{-1}).

Typical small-scale biotransformation: sulfoxidation of *n*-butyl phenyl sulfide 1e using *P. putida* NCIMB 8859. *n*-Butyl phenyl sulfide **1e** (0.2 g, 1.2 mM) was added in a minimum volume of ethanol (0.001 g substrate ml^{-1} of culture medium) to the 500 ml Erlenmeyer shake flask cultures which were in turn incubated at 30 °C, on an orbital shaker (200 rpm) for 24 h. The contents of the flasks were centrifuged (9000 rpm, 10 min) and the aqueous supernatant was decanted off. The bioproducts were harvested by solvent extraction (EtOAc) of the sodium chloride-saturated aqueous solution containing the biotransformed material, and concentration of the combined extracts under reduced pressure. ^{1}H NMR spectra of the crude mixture showed that sulfoxide **2e** was a major component. Purification by PLC on silica gel (methanol–chloroform, 5 : 95) afforded (+)-(*R*)-*n*-butyl phenyl sulfoxide **2e** (0.164 g, 79%); $[\alpha]_D$ +200 (*c* 3.0 in $CHCl_3$) and recovered substrate **1e** (0.01 g, 5%). The ^{1}H NMR spectrum was identical to an authentic sample; the enantiomeric excess was found to be >98% (CSPHPLC : Chiralcel OD, propan-2-ol : hexane; 1 : 9, separation factor $\alpha = 1.37$).

General procedure for large-scale biotransformations using a fermenter

Large scale biotransformations (2–10 g) using *P. putida* UV4 or NCIMB 8859 were carried out in a 10 l fermenter (pH 7.0, 30 °C, 350 rpm) using the same media as for the small scale experiments described above. Cell cultures were not centrifuged for the large scale biotransformations and D-glucose was used as a co-substrate (0.5 g l^{-1}). The rate of addition of both substrate (0.2–1.0 g l^{-1}) and co-substrate was controlled in order to maintain the oxygen tension in the fermenter at >50%.

Typical large-scale biotransformation using *P. putida* UV4: sulfoxidation of methyl phenyl sulfide 1a. Methyl phenyl sulfide **1a** (7.8 g, 63 mM) was added, neat, in aliquots (*ca*. 0.4 g), over a 5 h period to the filtered cell cultures (*ca*. 8 l) while monitoring the oxygen tension in the fermenter. The biotransformation was continued for a further 1 h period; the contents were centrifuged (9000 rpm, 10 min) and the aqueous supernatant was decanted off. The concentrated supernatant solution (under reduced pressure) was extracted (EtOAc) to yield crude methyl phenyl sulfoxide **2a** which on purification by flash chromatography (silica gel, chloroform as eluent) afforded (+)-(*R*)-methyl phenyl sulfoxide **2a** (7.84 g, 90%), $[\alpha]_D$ +154 (*c* 1.0 in $CHCl_3$). ^{1}H NMR analysis showed the product to be indistinguishable from an authentic sample and to have an ee value of >98% using chiral shift reagent. CSPHPLC analysis (Chiralcel OD, propan-2-ol–hexane, 1 : 9, $\alpha = 1.36$) confirmed the ee value. A more polar minor metabolite (0.05 g, 0.5%) was identified as (+)-(1*S*,2*S*)-*cis*-1,2-dihydroxy-3-methylsulfanylcyclohexa-3,5-diene having identical spectral and chiroptical characteristics to those reported.[27,33]

E. coli JM109(pKST 11) was constructed using PCR amplification of the todC1C2BA gene cluster from *P. putida* NCIMB 11767. A product of 4 kb was obtained which was the correct size for the expected tod gene cluster. The polymerase chain reaction product was treated with T4 kinase and inserted by blunt end ligation with T4 ligase into the pKK223-3 ampicillin-resistant expression vector. The plasmid containing the tod gene insert (pKST 11) was transformed into *E. coli* JM109 by electroporation and the resultant cells were shown to produce indigo dye when grown on nutrient agar containing indole.

Biotransformations using *E. coli* JM109(pKST 11) were carried out using cells grown on minimal medium supplemented with glucose (20 mM), thiamine (1 mM) and ampicillin (100 μg ml^{-1}) in a 10 l fermenter at 37 °C. Toluene dioxygenase activity was induced by addition of 1 mM isopropyl-β-thiogalactopyranoside (IPTG). After 2 h the cells were suspended by centrifugation, washed and resuspended in the same volume of phosphate buffer (50 mM, pH 7.5) supplemented with 20 mM glucose. Aliquots (500 ml) were transferred to 2 l conical flasks and biotransformation substrates added before shaking at 37 °C for between 10 and 24 h. Metabolites were extracted, purified and characterised in the manner described earlier for *P. putida* biotransformations of sulfides.

Sulfides **3a**, **3d**, **3e**, **3g**, **3h** and **3k** were obtained from commercial sources. The remaining sulfides were prepared by standard literature methods.[34–39] The corresponding racemic sulfoxides were obtained by oxidation using sodium periodate in aqueous methanol and were found to have similar physical and spectral properties to those reported in the literature.[40–46]

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