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Stereoselective reductase-catalysed deoxygenation of sulfoxides in aerobic and anaerobic bacteria

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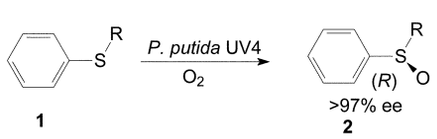
Direct and indirect evidence, of unexpected stereoselective reductase-catalysed deoxygenations of sulfoxides, was found. The deoxygenations proceeded simultaneously, with the expected dioxygenase-catalysed asymmetric sulfoxidation of sulfides, during some biotransformations with the aerobic bacterium *Pseudomonas putida* UV4. Stereoselective reductase-catalysed asymmetric deoxygenation of racemic alkylaryl, dialkyl and phenolic sulfoxides was observed, without evidence of the reverse sulfoxidation reaction, using anaerobic bacterial strains. A purified dimethyl sulfoxide reductase, obtained from the intact cells of the anaerobic bacterium *Citrobacter braakii* DMSO 11, yielded, from the corresponding racemates, enantiopure alkylaryl sulfoxide and thiosulfinate samples.

Introduction

Heteroatom oxidation of sulfides to yield sulfoxides is among the most common and facile metabolic steps in eukaryotic and prokaryotic organisms.[1–22] This reaction has been catalysed by a range of enzyme systems including peroxidases,[14,15] monooxygenases,[1–8,11,12] and dioxygenases.[8–10,17–22] Particular emphasis has recently been placed on the application of dioxygenases, *e.g.* toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO), as biocatalysts in chiral sulfoxidation.[8,9,17–22] The dioxygenases, present in different strains of the soil bacterium *Pseudomonas putida*, *e.g.* UV4 (a source of TDO), NCIMB 8859 (a source of NDO) and 9816/11 strains (a source of NDO), have been found to catalyse the stereoselective sulfoxidation of a wide range of alkylaryl and diaryl sulfides.[8–10,17–22] To date, > 40 sulfoxides have been isolated with high enantiomeric excess values (> 90% ee), using TDO and NDO enzymes. The dioxygenases are, thus, among the best enzymes currently available for the production of enantiopure sulfoxides. Typical examples of enantiopure sulfoxides, obtained using the *P. putida* UV4 mutant strain, are shown in Scheme 1.



1 **2**

R = Me, Et, Pr, Bu, Pr^{i}, CH=CH$_2$, CH$_2$OMe, CH$_2$SMe

Scheme 1

Asymmetric sulfoxidation of methylphenyl sulfide **1** (R = Me) substrate was of particular interest since it yielded either the (*R*)-sulfoxide enantiomer **2** (R = Me, > 98% ee; 90% yield),[17] using TDO or the (*S*) enantiomer (> 98% ee; 98% yield) using NDO.[9,10] Further noteworthy examples, of enantiocomplementary biocatalysis using TDO and NDO, have been found during the sulfoxidation of bicyclic *bis*-sulfides where the resulting enantiopure sulfoxides proved to be of value in synthesis.[22] Although the stereoselective TDO-catalysed sulfoxidation, of alkylaryl and diaryl sulfides in *P. putida* UV4, has been very successful, sulfoxidation of dialkyl sulfides has proved to be much more difficult, with this biocatalyst.[18]

Based on the sulfoxidation results, obtained using whole cell *P. putida* mutant strains containing dioxygenases,[9,10,17] it could be assumed that the remarkably high stereoselectivity and yield often observed was entirely due to a process of asymmetric oxidation (**A** ⟶ [+]-**B** or [−]-**B**, Scheme 2). Preliminary studies,[17] with whole cell cultures of *P. putida* UV4, however, indicate that kinetic resolution could also be responsible for the residual enantioenriched (*R*)-alkylaryl sulfoxides recovered from some racemic precursors without corresponding bioproducts being isolated. The kinetic resolutions could be explained by several mechanistic pathways including: (i) a non-stereoselective slow deoxygenation process (**B** ⟶ **A**) and a concomitant fast stereoselective sulfoxidation step to yield the (*R*) enantiomer (**A** ⟶ **B**, Scheme 2) or (ii) the stereoselective removal of the (*S*) enantiomer, *e.g.* by *cis*-dihydroxylation, resulting in the formation of very water-soluble metabolites that could not be isolated by solvent extraction (ethyl acetate).

Precedence for the production of an enantioenriched alkylaryl sulfoxide, where enzyme-catalysed oxygenation and deoxygenation mechanisms both occur, is found in an earlier report[23] of a fungal biotransformation of sulfide **A** (R = 4-H$_2$N.C$_6$H$_4$, R′ = Me) to yield the corresponding sulfoxide **B**; this was accompanied by a simultaneous, but slower, stereoselective enzyme-catalysed deoxygenation of sulfoxide **B** (Scheme 2) to give sulfide **A**.[23] Under these circumstances, where a fast sulfoxidation step (and possibly other enzyme-catalysed oxidations) happens simultaneously with a relatively slow deoxygenation process, it is very difficult to detect the sulfide and thus obtain direct evidence of the reduction reaction. The present study is concerned with: (i) the quest for both direct and indirect evidence of a stereoselective sulfoxide deoxygenation mechanism during aerobic and anaerobic bacterial biotransformations, and (ii) the potential application of the enzymatic deoxygenation process in the kinetic resolution of racemic sulfoxides which are difficult to obtain as single enantiomers by enzymatic asymmetric oxidation methods.

Several biotransformation pathways, involving enzyme-catalysed deoxygenation, which could account for the kinetic resolution of racemic sulfoxides, are shown in Scheme 2. These include: (i) sulfoxide deoxygenation to yield a sulfide (**B** ⟶ **A**), (ii) sulfoxide deoxygenation–monooxygenation to yield a sulfide alcohol (**B** ⟶ **A** ⟶ **E**), (iii) sulfoxide deoxygenation–

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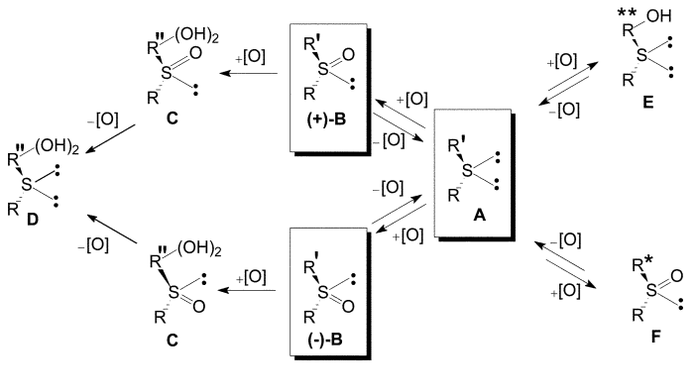


Scheme 2

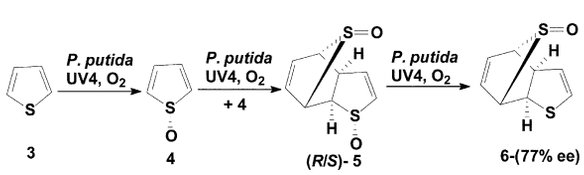
monooxygenation to yield a new sulfoxide diastereoisomer (**B** ⟶ **A** ⟶ **F**) and (iv) sulfoxide dioxygenation–deoxygenation to yield a sulfide *cis*-dihydrodiol (**B** ⟶ **C** ⟶ **D**) (Scheme 2). Employing *P. putida* UV4 whole cells, and a series of racemic alkylaryl sulfoxide substrates, evidence for each of these mechanisms is presented in the results and discussion section.

Bacterial dimethyl sulfoxide (DMSO) reductase enzymes play an important role in the environment, by regulating the level of DMSO and dimethyl sulfide (DMS) in the global sulfur cycle. Thus, the deoxygenation of DMSO, formed by photo-oxidation of volatile DMS in the atmosphere, is catalysed by DMSO reductases found mainly in ocean-dwelling bacteria. The second part of this study is focused, exclusively, on potential application of such facultative anaerobic bacteria which have been shown to contain sulfoxide reductase enzymes. To date, few reports are available on the use of sulfoxide reductase enzymes in the kinetic resolution of racemic sulfoxides, and none on the application of these enzymes in the production of single enantiomer thiosulfinates. These biocatalysts have now been found to deoxygenate, stereoselectively, a new range of sulfoxides, including a thiosulfinate, without evidence of the reverse sulfoxidation reaction occurring.

Results and discussion

(A) Enzyme-catalysed deoxygenation of racemic sulfoxides, using the UV4 mutant strain of the aerobic bacterium *P. putida*

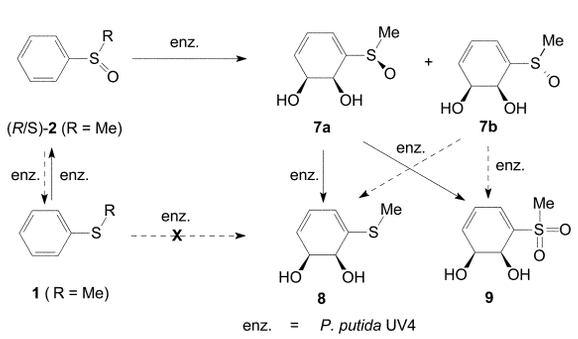
The biotransformation, of thiophene **3** in *P. putida* UV4, has recently been found to yield the corresponding achiral sulfoxide intermediate **4**.[20,21] This unstable thiophene oxide **4** was found to dimerise to yield the racemic *bis*-sulfoxide **5** (Scheme 3). Stereoselective and regioselective deoxygenation of one sulfoxide group of the dimer was observed as a further step (**5** ⟶ **6**) during the biotransformation of thiophene **3**, and also when racemic *bis*-sulfoxide **5** was added as a substrate, to yield an excess (77% ee) of one enantiomer of monosulfoxide **6** (Scheme 3).[21]



Scheme 3

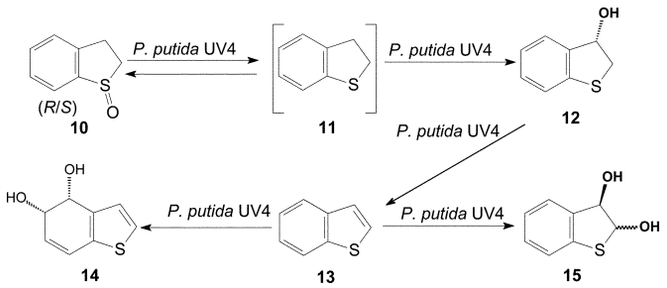
This direct evidence of a dioxygenase-catalysed sulfoxidation (**3** ⟶ **4**), and an enzyme-catalysed deoxygenation (**5** ⟶ **6**) operating in tandem, was assumed to be due to the very slow rate of the reverse TDO-catalysed sulfoxidation of a dialkyl sulfide (**6** ⟶ **5**). While stereoselective dioxygenase-catalysed (*P. putida* UV4) sulfoxidation reactions, of a wide range of alkylaryl sulfides, have been reported,[8,9,17,18,22] no other direct evidence, of the reverse deoxygenation reaction, had been obtained in earlier studies of the racemic alkylaryl sulfoxides.[17] In our current study, the biotransformations of alkylaryl sulfoxides have been re-examined in light of this one example of a deoxygenation (**5** ⟶ **6**)[21] and the recent improvements in down-stream processing of bioproducts from *P. putida* UV4.[18] The latter process involved removal of most of the water under vacuum, from the centrifuged culture medium containing the metabolites, prior to solvent extraction. With this method, previously undetected metabolites, including water-soluble sulfoxide *cis*-dihydrodiols, have been isolated.[18]

When racemic methylphenyl sulfoxide **2** (R = Me, Scheme 1) was earlier added to whole-cell cultures of *P. putida* UV4, the normal extraction procedure was adopted for the isolation of metabolites (saturating the aqueous culture medium with common salt followed by its repeated extractions with EtOAc).[17] Under these conditions, only a low yield (7%) of enantioenriched residual sulfoxide (2*R*, 85% ee) was recovered, without any trace of methylphenyl sulfide **1** or other metabolites.[17] The biotransformation of racemic sulfoxide **2** was repeated during the present study, in the hope of finding other metabolites, which would help in understanding the mechanism of this kinetic resolution. The new protocol involved: (i) an extended period of biotransformation and (ii) the improved work-up procedure.[18] Using this modified technique, no residual sulfoxide **2** was recovered; the resulting crude mixture of metabolites, on ^{1}H NMR spectral analysis, showed the presence of *cis*-diol sulfoxides (**7a**, 34%),**7b** (26%), *cis*-diol sulfide **8** (30%) and *cis*-diol sulfone **9** (10%) (Scheme 4). The mixture of *cis*-diol sulfoxide diastereoisomers **7a/7b** could not be separated by PLC, but pure samples of the sulfide diol **8** and sulfone diol **9** were obtained by this PLC. Compounds **7a**, **8** and **9** were reported earlier [17,19,24] only as minor *cis*-dihydrodiol metabolites or derivatives.

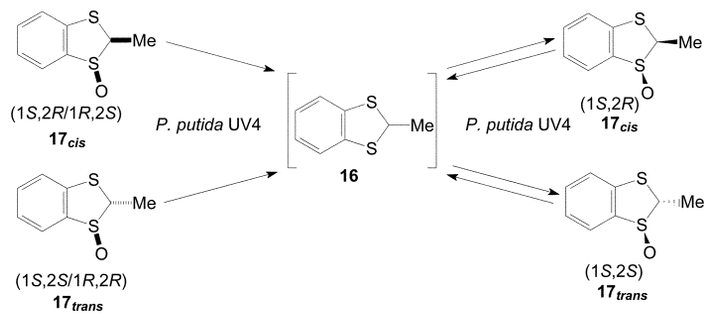


Scheme 4

Application of similar biotransformation and work-up conditions, and methylphenyl sulfide **1** as substrate, yielded *cis*-diol sulfide **8** as the sole isolable metabolite. These observations are consistent with either a single-step mechanism (sulfide **1** ⟶ *cis*-diol sulfide **8**) or a three-step mechanism (sulfide **1** ⟶ sulfoxide **2** ⟶ *cis*-diol sulfoxide **7a/7b** ⟶ *cis*-diol sulfide **8**, Scheme 4). In order to elucidate the mechanism, a further experiment with



Scheme 5



Scheme 6

sulfide **1** was conducted using a shorter time of biotransformation. On this occasion, the enantiopure *cis*-diol sulfoxide intermediate **7a** was intercepted as the only bioproduct. When sulfoxide diol **7a** was, in turn, added as substrate, both sulfide diol **8** and sulfone diol **9** were formed. These results clearly indicate that an unprecedented three-step sequence (**1** ⟶ **2** ⟶ **7a** ⟶ **8**, Scheme 4), including an enzyme-catalysed deoxygenation step, is preferred to the single-step (**1** ⟶ **8**) mechanism. The observation also supports the premise that dioxygenase-catalysed sulfoxidation of alkylaryl sulfides generally occurs much faster than dioxygenase-catalysed arene-*cis*-dihydroxylation. Conversely, *cis*-dihydroxylation of dialkyl sulfides, *e.g.* methylbenzyl sulfide,[18] proceeds at a much faster rate than sulfoxidation.

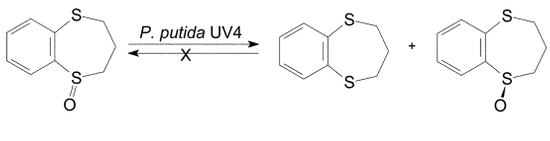
It is, thus, possible that the kinetic resolution earlier observed for sulfoxide **2** (R = Me)[17] was mainly due to *cis*-dihydroxylation to yield the water-soluble *cis*-diol sulfoxide metabolites **7a/7b**; the water-soluble metabolites were not isolated due to the less efficient extraction procedure. While dioxygenase–catalysed sulfoxidations[8–10,17–22] occur readily, sulfones *e.g.* metabolite **9**, have rarely been observed as bioproducts from these biotransformations. A *cis*-diol sulfone metabolite, of similar structure and configuration to compound **9**, was however recently obtained when ethylphenyl sulfide was used as substrate.[19] The enzyme, responsible for sulfone formation in *P. putida* UV 4, has not yet been identified.

It was noted that neither of the direct deoxygenation reactions, found using *P. putida* UV4 (**5** ⟶ **6** and **7a** ⟶ **8**), involved an alkylaryl sulfoxide substrate. While *cis*-dihydroxylation may play a major role in the kinetic resolution of the racemic alkylaryl sulfoxide **2**, the possibility of a competing pathway, *i.e.* slow deoxygenation of compound **2** followed by a fast sulfoxidation of the resulting sulfide **1**, could not at this stage be discounted.

The first evidence, of a slow deoxygenation process in alkylaryl sulfoxides, was obtained when the racemic bicyclic compounds **10**, $\mathbf{17}_{cis}$, $\mathbf{17}_{trans}$ and **18** were used as substrates (Schemes 5–7). Biotransformation of 2,3-dihydrobenzothiophene **11** had been found to yield the sulfoxide (1*R*)-**10** (26% ee, **11** ⟶ **10**, Scheme 5);[22] it was also accompanied by 3-hydroxy-2,3-dihydrobenzo[*b*]-thiophene **12**, as a result of TDO-catalysed benzylic hydroxylation (**11** ⟶ **12**). When racemic sulfoxide **10** was added to *P. putida* UV4, during the present study, the residual substrate (1*R*)-**10** (19% yield, 3% ee) was isolated along with the minor metabolites, 3-hydroxy-2,3-dihydrobenzo[*b*]-thiophene **12** (5% yield) and *cis*-dihydrodiols **14** (3% yield) and **15** (2% yield) (Scheme 5). The enantioenriched (83% ee) metabolite (+)-(3*S*)-**12** was assumed to have an identical absolute configuration (allowing for Sequence Rule priorities) to that found for alcohols obtained earlier by TDO-catalysed (*P. putida* UV4) benzylic hydroxylations of benzocycloalkanes, benzocycloheteroalkanes, and benzocycloalkenes.[25–34] The minor *cis*-dihydrodiol metabolites **14** and **15** had earlier been isolated as bioproducts from benzo[*b*]thiophene **13**; compound **13** was obtained by dehydration of 3-hydroxy-2,3-dihydrobenzo[*b*]-thiophene **12**.[35,36] The structures and stereochemical assignments of *cis*-dihydrodiols **14** and **15** (which spontaneously isomerise to the corresponding *trans*-isomers)[36] will be discussed elsewhere. Although the sulfide bioproduct **11**, derived from deoxygenation of sulfoxide substrate **10**, was not observed directly, the formation of mono- (**12**) and dihydroxylation products (**14** and **15**) provides indirect evidence of a slow reductase-catalysed deoxygenation of the alkylaryl sulfoxide **10** (**B** ⟶ **A** ⟶ **E**, Scheme 2).

Biotransformation of 1,3-disulfide **16** had been found to yield the corresponding (1*S*,2*R*)-($\mathbf{17}_{cis}$) and (1*S*,2*S*)-($\mathbf{17}_{trans}$) sulfoxide enantiomers (> 98% ee, Scheme 6).[8] During the present study, the racemic sulfoxides $\mathbf{17}_{cis}$ and $\mathbf{17}_{trans}$ were, separately, added as substrates to *P. putida* UV4 (Scheme 6); no direct evidence for reductase-catalysed deoxygenation of racemic sulfoxides $\mathbf{17}_{cis}$ and $\mathbf{17}_{trans}$ was obtained. However, isolation of residual (1*S*,2*R*)-$\mathbf{17}_{cis}$ (40% recovered yield, 47% ee), from racemic substrate, is indicative of a kinetic resolution process in operation. Furthermore, isolation of diastereoisomer (1*S*,2*S*)-$\mathbf{17}_{trans}$ (> 98% ee), as a metabolite of racemic substrate $\mathbf{17}_{cis}$, is consistent with its deoxygenation to form sulfide **16** followed by TDO-catalysed asymmetric sulfoxidation (**B** ⟶ **A** ⟶ **F**, Scheme 2).[8] Similar indirect evidence, of reductase-catalysed stereoselective deoxygenation, was observed with racemic substrate $\mathbf{17}_{trans}$: the residual (1*S*,2*S*)-sulfoxide $\mathbf{17}_{trans}$ was found to be enantioenriched (18% ee) and the diastereoisomeric metabolite (1*S*,2*R*)-$\mathbf{17}_{cis}$ was enantiopure (> 98% ee), as expected[8] from TDO-catalysed oxidation of sulfide **16**. The very low yields (1–2%) of diastereoisomeric sulfoxide metabolites $\mathbf{17}_{trans}$ and $\mathbf{17}_{cis}$, derived from racemic samples of $\mathbf{17}_{cis}$ and $\mathbf{17}_{trans}$, respectively, again suggests that the deoxygenation step is slow.

The bicyclic alkylaryl sulfide **19** was exceptional in not being oxidized to the corresponding sulfoxide **18** by whole cells of



Scheme 7

P. putida UV4.[22] However, biotransformation of racemic sulfoxide **18** followed by isolation of residual sulfoxide (+)-(1*R*)-**18** (72% ee), showed that kinetic resolution had occurred. In contrast with the other alkylaryl sulfoxides studied (**10**, $\mathbf{17}_{cis}$ and $\mathbf{17}_{trans}$), only the biotransformation of sulfoxide **18** yielded an isolable sample of the corresponding sulfide metabolite (**19**). This is the first direct evidence of a reductase-catalysed deoxygenation of an alkylaryl sulfoxide using *P. putida* UV4 (**B** ⟶ **A**, Scheme 2). The absolute configuration of (−)-sulfoxide **18** had been determined as (1*S*) by X-ray crystallography.[22]

The evidence, presented herein, confirms the recent preliminary conclusion that *P. putida* UV4 contains a reductase enzyme.[21] While this strain can deoxygenate both dialkyl and alkylaryl sulfoxides, it also contains a dioxygenase enzyme that can catalyse the reverse reaction on alkylaryl sulfides. The reductase enzyme also showed a similar stereopreference toward one enantiomer of the racemic bicyclic sulfoxides. Thus, the residual sulfoxides **10** (3% ee, 1*R*), $\mathbf{17}_{cis}$ (47% ee, 1*S*), $\mathbf{17}_{trans}$ (25% ee, 1*S*) and **18** (72% ee, 1*R*) had an enantiomeric excess favouring the same absolute configuration (allowing for Sequence Rule priorities). Although *P. putida* UV4 undoubtedly contains a reductase enzyme, the relatively slow rate of deoxygenation, compared with the faster reverse process, would limit its use in the kinetic resolution of racemic sulfoxides. In view of this limitation, alternative strains of bacteria were sought with minimal dioxygenase-catalysed sulfoxidation activity and maximal sulfoxide reductase activity.

(B) Enzyme-catalysed deoxygenation of racemic sulfoxides using the anaerobic bacteria *Rhodobacter capsulatus*, *Escherichia coli*, *Proteus vulgaris*, *Citrobacter braakii*, *Klebsiella sp.* and *Serratia sp.*

Despite the well-established role of DMSO reductases in the environment, relatively few studies have been carried out to assess their value in the kinetic resolution of racemic sulfoxides.[37–39] One study had shown that chiral sulfoxides, including methyl *p*-tolyl sulfoxide **20**, could be stereoselectively deoxygenated to yield sulfide **21** using *Rhodobacter sphaeroides* f.sp. *denitrificans* IL 106 (Scheme 8).[37,38] Similar stereochemical results from one of our laboratories were also reported with *R. capulatus* DSM 938 and sulfoxide **20** as substrate.[39] *E. coli* ATCC 33694, and *P. vulgaris* NCIMB 67 strains were again found to reduce sulfoxide **20** but with opposite enantioselectivity. Several enantiopure sulfoxides were thus obtained by this kinetic resolution approach involving DMSO reductase-catalysed deoxygenation.[39] In our study, the use of reported, and new, sulfoxide reductases in kinetic resolutions of sulfoxides has been evaluated with the help of a wider range of anaerobic bacteria and substrates.

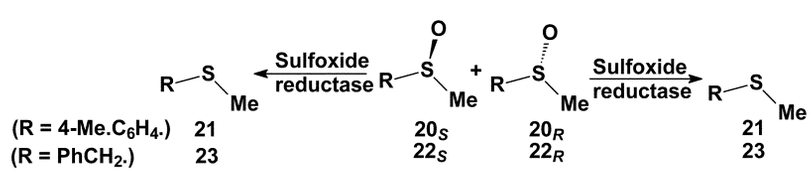
The commercially available racemic and enantiopure forms of methyl *p*-tolyl sulfoxide **20** were, initially, selected as model substrates for a range of facultative anaerobic bacteria. *R. capulatus* DSM 938 was studied first, due to its capability to reduce several sulfoxides through the action of a periplasmic DMSO reductase that had already been purified and fully characterized by X-ray crystallography.[39,40] The other bacterial strains that showed most promise, in terms of stereoselective deoxygenation of sulfoxide **20** (ee values 32 to > 98%), are shown in Table 1. Although attempts were made in each case to follow the course of biotransformations by HPLC analysis, and to terminate the biotransformation after ~50% conversion of substrate, it proved difficult, in some cases, due to the different growth characteristics, solubilities and biotransformation rates associated with the range of bacteria and substrates. Thus, the enantiopurity figures shown in Table 1 merely represent the values observed, upon termination of the biotransformation, which were not always optimal.

A comparison of results, obtained using whole cells, and pure DMSO reductases from *R. capsulatus* and the newly isolated bacterium *C. braakii*, on biotransformations of sulfoxide **20**, showed that the progress of reaction with pure enzymes could be more easily monitored, and generally gave higher ee values. The DMSO reductase enzymes, in *R. capsulatus* and *R. sphaeroides*, had each been purified and were found to selectively deoxygenate the (*S*) enantiomer of methyl *p*-tolyl sulfoxide **20** yielding, exclusively, the (*R*) enantiomer.[37–39] With whole cells of either *R. capsulatus* or *R. sphaeroides*, and ensuring that the biotransformation was stopped at ~50% completion, it was also possible to obtain the (*R*)-sulfoxide **20** in enantiopure form (>98% ee). Other strains, examined for comparison purposes, included *E. coli* HB 101 and *P. vulgaris* NCIMB 67, that had earlier been found to preferentially metabolize the (*R*) enantiomer of sulfoxide **20** yielding enantiopure samples of the (*S*) sulfoxide.[39] During the study, the *E. coli* strain gave (*S*)-**20** of lower enantiopurity (60% ee after a 42% substrate conversion), while *P. vulgaris* showed >98% ee (after 50% substrate conversion) for the residual (*S*) sulfoxide, by chiral stationary phase HPLC (CSPHPLC) analysis.

Three new bacterial strains, isolated from soil and marine environments,[41] were also found to deoxygenate racemic sulfoxide **20** in a stereoselective manner. These bacteria were provisionally identified as *C. braakii* DMSO 11 (from canal sediment, Coventry), *Klebsiella sp.* DMSO 7 (from the North Sea), and *Serratia* sp. DMSO 10 (from the North Sea). The *Klebsiella* DMSO 7 strain gave an excess of the residual (*R*) sulfoxide **20** (54% ee after a 39% substrate conversion) and thus a similar stereochemical preference to *R. capsulatus* and *R. sphaeroides*. By contrast, an excess of the residual (*S*) sulfoxide **20** was found after biotransformations with the *Serratia* sp. DMSO 10 (>98% ee) and *C. braakii* DMSO 11 (32% ee).

The DMSO reductase enzymes, present in *R. capsulatus*[38,39] and *R. sphaeroides*,[37] were also found to deoxygenate chiral dialkyl sulfoxides in a stereoselective manner. Thus, methylbenzyl sulfoxide **22** was recovered with a 50% excess of the (*R*) enantiomer (*R. sphaeroides*)[37] and methylthiomethylmethyl sulfoxide ($MeSCH_2SOMe$) was recovered as a single enantiomer of unknown configuration (*R. capsulatus*).[39] When racemic methylbenzyl sulfoxide **22** was used as substrate with five bacterial strains (Table 1), the residual dialkyl sulfoxide **22** was consistently found to have the (*S*) configuration (26–77% ee) – an opposite configuration to that found using *R. sphaeroides*.

It appears, from the results in Table 1, that the *P. vulgaris* strain is among the most stereoselective strains for the

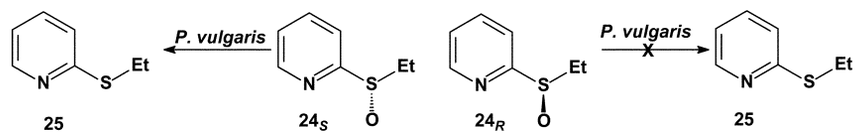


Scheme 8

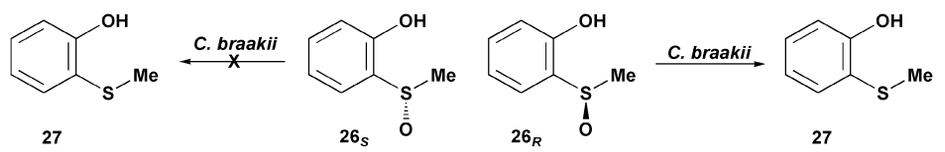
Table 1 Enantiopurity (% ee) and absolute configuration (Ab. con.) of residual sulfoxides **20** and **22** after stereoselective DMSO reductase-catalysed deoxygenation to yield the corresponding sulfides **21** and **23**

	Sulfoxide **20**		Sulfoxide **22**	
	% ee	Ab. con.	% ee	Ab. con.
Rhodobacter capsulatus 938	58 (>98)[a, b]	*R* (*R*)[ab, b]	—	—
Escherichia coli HB 101	60 (>98)[a]	*S* (*S*)[a]	68	*S*
Proteus vulgaris NCIMB 67	>98 (>98)[a]	*S* (*S*)[a]	77	*S*
Klebsiella sp. DMSO 7	54	*R*	44	*S*
Serratia sp. DMSO 10	>98	*S*	37	*S*
Citrobacter braakii DMSO 11	32 (>95)[b]	*S* (*S*)[b]	26	*S*

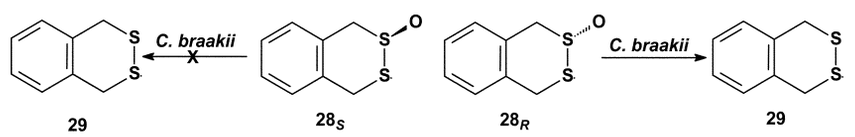
[a] From earlier whole cell time course studies.[39] [b] By using pure DMSO reductase.



Scheme 9



Scheme 10



Scheme 11

deoxygenation of both alkylaryl and dialkyl sulfoxides. This strain was also found to yield enantiopure (CSPHPLC analysis) ethyl-2-pyridyl sulfoxide **24** of undetermined absolute configuration.[39] Biotransformation (*P. vulgaris*) of racemic ethyl-2-pyridyl sulfoxide **24** to yield sulfide **25** was repeated on a larger scale (2.0 g); the residual sulfoxide **24** (45% recovery) was found to have the (−)-(*R*) configuration (>98% ee) by comparison of its optical rotation with the literature value (Scheme 9).[42]

The new *C. braakii* DMSO 11 strain, appeared to deoxygenate a wider range of racemic sulfoxides, and in a more stereoselective manner, than the other strains listed in Table 1. An enantiopure sample of 2-methylsulfinyl phenol **26** was required as a chiral ligand for a separate study of asymmetric alkylation reactions of aldehydes. Initial attempts to obtain enantiopure sulfoxide **26**, *via* TDO- or NDO-catalysed sulfoxidation of the parent sulfide, were unsuccessful. DMSO reductase enzymes, present in *P. vulgaris* NCIMB 67 and *R. capsulatus* DSM 938, were used to partially resolve sulfoxide **26** (32% and 48% ee respectively) *via* deoxygenation. However, whole cells of *C. braakii* DMSO 11 deoxygenated, selectively, the (*R*) enantiomer yielding enantiopure (−)-(*S*)-2-methylsulfinyl phenol **26** (Scheme 10). The configuration (*S*) was determined by methylation (CH_2N_2) and comparison of the CD spectrum of the resulting 2-methoxy analogue with that of (−)-(*S*)-methylphenyl sulfoxide **2**.[17]

Attempts were made to obtain enantiopure sulfoxide (thiosulfinate) **28**, as part of an earlier investigation of the enzyme-catalysed asymmetric oxidation of 1,2-disulfides, using monooxygenases, dioxygenases and peroxidases.[19] Unfortunately, these efforts were only partially successful in the production of enantioenriched thiosulfinate **28** (11% ee with NDO and 58% ee using chloroperoxidase).[19] As an alternative approach, the racemic thiosulfinate **28** was added as substrate to the anaerobic bacteria shown in Table 1; only *C. braakii* DMSO 11 showed promise in the stereoselective deoxygenation of 1,4-dihydrobenzo-2,3-dithian-2-oxide **28** (Scheme 11). The residual (+)-thiosulfinate **28** was found to have a modest excess of the (*S*) enantiomer (18% ee, 46% yield). The (+)-(*S*) absolute configuration for sulfoxide **28** had been determined by X-ray crystallography of an enantiopure sample separated by semi-preparative CSPHPLC.[19]

Fortunately the enzyme responsible for sulfoxide deoxygenation reactions in *C. braakii* DMSO 11 was isolated, identified and characterised as a periplasmic DMSO reductase.[41] When the purified DMSO reductase enzyme was used with racemic methyl *p*-tolyl sulfoxide **20** and 1,4-dihydrobenzo-2,3-dithian-2-oxide **28**, as substrates, it was found that essentially only single enantiomers of the residual sulfoxide (−)-(*S*)-**20** (> 98% ee) and thiosulfinate (+)-(*S*)-**28** (> 95% ee) remained and the corresponding sulfides **20** and **29** were formed. This appears to be the first example of the formation of an enantiopure thiosulfinate *via* an enzyme-catalysed kinetic resolution of a racemate.

Conclusion

Methods for the detection of a stereoselective reductase-catalysed deoxygenation of sulfoxides, while operating simultaneously with an oxygenase-catalysed sulfoxidation of sulfides, have been developed. Evidence has been gathered of enzyme-catalysed: (i) sulfoxide deoxygenation to yield an isolable sulfide, (ii) sulfoxide deoxygenation and benzylic monooxygenation to form a sulfide alcohol, (iii) sulfoxide deoxygenation and sulfoxidation to yield a diastereoisomeric sulfoxide, and (iv) sulfoxide dihydroxylation and deoxygenation to furnish a *cis*-dihydrodiol sulfide. A remarkable enzyme-catalysed sulfoxidation-*cis*-dihydroxylation–deoxygenation sequence has been observed during the biotransformation of an alkylaryl sulfide to yield the corresponding *cis*-dihydrodiol.

The stereoselective reductase-catalysed deoxygenation method, utilizing both established and new bacterial strains and purified enzymes, has been used in kinetic resolution studies to yield functionalised sulfoxide and thiosulfinate enantiomers that are not readily available by the enzyme-catalysed asymmetric sulfoxidation method.

Experimental

^{1}NMR spectra of compounds were recorded on Bruker Avance DPX-300 and DPX-500 instruments. Column 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 TLC. Optical rotation ($[\alpha]_D$) measurements were carried out with a Perkin-Elmer 214 polarimeter at ambient temperature (*ca.* 20 °C) and are given in units of 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded using a JASCO J-720 instrument and spectroscopic grade methanol as solvent. Unless mentioned otherwise the enantiopurity of sulfoxides was determined by CSPHPLC using specified Chiralcel columns and 10% 2-propanol in hexane as eluent.

Authentic samples of sulfides **1** (R = Me), **11**, **16**, **19**, **21**, **23**, **25**, **27** and **29** and racemic sulfoxides **2**, **10**, **17**$_{cis}$, **17**$_{trans}$, **18**, **20**, **22**, **24**, **26**, **28** were available. Some were obtained commercially (**1**, **2**, **20**, **21**, **23**, **27**), others from earlier synthetic studies in these laboratories (**10**,[22] **11**,[22] **16**,[22] **17**$_{cis}$,[22] **17**$_{trans}$,[22] **18**,[22] **19**,[22] **22**,[22] **24**,[39] **25**,[39]) and the rest were synthesized by literature methods (**26**–**29**). All sulfoxide substrates showed a characteristic strong S=O group absorption in the IR spectra (ν_{max}/cm^{-1} 1023–1094).

Synthesis of 2-(methylsulfinyl)phenol 26

A stirred solution of 2-(methylsulfanyl)phenol **27** (5 g, 35.7 mmol), in acetone (50 cm^3) maintained at 0 °C, was oxidised by the dropwise addition of an acetone solution of dimethyldioxirane (0.08 M) prepared in accordance with the literature procedure.[43] The progress of the oxidation reaction was followed by TLC analysis (60% EtOAc in hexane) and upon completion, acetone was evaporated off, the residue dried (vaccum pump) and crystallised, to furnish sulfoxide **26** (5.30 g, 95%) as a colourless crystalline solid; mp 123 °C (from MeOH) (lit.[44] 127–128 °C); R_f 0.23 (5% MeOH in $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 2.96 (3 H, s, Me), 6.93 (1 H, m, Ar), 6.97 (1 H, d, *J* 7.7, Ar), 7.07 (1 H, d, *J* 7.8, Ar), 7.37 (1 H, m, Ar); δ_C (125 MHz, $CDCl_3$) 41.9, 119.71, 119.86, 123.07, 124.83, 133.05; *m*/*z* (EI) 156 (M$^+$, 100%), 141 (95%).

Synthesis of 1,4-dihydrobenzo-2,3-dithian 29 and racemic 1,4-dihydrobenzo-2,3-dithian-2-oxide 28

α,α′-Dibromo-*o*-xylene (6.6 g, 25.0 mmol) and thiourea (4.75 g, 62.4 mmol) were dissolved in ethanol (62.5 cm^3) and the solution refluxed for 6 h. The ethanol was removed under reduced pressure and the remaining solid was dissolved in water (125 cm^3). A solution of NaOH (4 g) dissolved in water (37.5 cm^3) was added and the reaction mixture refluxed for a further 6 h. After cooling and addition of sulfuric acid (2 M) until neutral pH, the aqueous phase was extracted (CH_2Cl_2). The organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give crude *o*-xylene-*α,α′*-dithiol as a malodorous low melting solid (4.2 g, 100%). It was identified spectrally and used directly without further purification. R_f 0.10 (10% EtOAc in hexane); mp 33–35 °C (lit.,[45] 44 °C); (Found: M$^+$, 170.0228. $C_8H_{10}S_2$ requires 170.0224) *m*/*z* (EI) 170 (M$^+$, 40%), 137 (100), 123 (80), 104 (97), 90 (47), 76 (47), 63 (82), 51 (89), 45 (97), 39 (85); δ_H (500 MHz, $CDCl_3$), 1.85 (2 H, t, $J_{1,2}$ 7.1, 2 × SH), 3.86 (4 H, d, $J_{2,1}$ 7.1, 2 × CH_2SH), 7.20–7.23 (2 H, m, 2 × Ar–H), 7.27–7.29 (2 H, m, 2 × Ar–H); δ_C (125 MHz, $CDCl_3$) 25.94, 127.92, 129.75, 138.71.

Potassium permanganate/copper sulfate pentahydrate catalyst (10.5 g, 1 : 1) was added to a stirred solution of *o*-xylene-α,α′-dithiol (4.2 g, 24.7 mmol) in acetone (250 cm^3). After stirring for 2 h, the mixture was filtered, concentrated and purified by column chromatography (30% EtOAc in hexane) to afford 1,4-dihydrobenzo-2,3-dithian **29** as a white crystalline solid (3.7 g, 98%). R_f 0.68 (10% ethyl acetate in hexane); mp 70–71 °C ($CHCl_3$/hexane) (lit.,[46] 77–78 °C); (Found: C, 57.0; H, 4.7. $C_8H_8S_2$ requires C, 57.1; H, 4.8%); *m*/*z* (EI) 168 (M$^+$, 99%), 134 (34), 104 (100), 91 (14), 78 (51), 64 (24), 51 (23); δ_H (500 MHz, $CDCl_3$) 4.07 (4 H, s, $(SCH_2)_2$), 7.06–7.10 (2 H, m, 2 × Ar–H), 7.15–7.18 (2 H, m, 2 × Ar–H); δ_C (125 MHz, $CDCl_3$) 34.57, 126.80, 130.14, 132.86.

A solution of sodium periodate (0.12 g, 0.55 mmol) in water (10 cm^3) was added dropwise to a stirred solution of 1,4-dihydrobenzo-2,3-dithian **29** (0.084 g, 0.50 mmol) in methanol (30 cm^3) at 0 °C. The mixture was allowed to stir overnight and then filtered. The filtrate was concentrated and extracted ($CHCl_3$); the extracts were dried (Na_2SO_4) and concentrated to yield the crude product. Purification by column chromatography (30% ethyl acetate in hexane) gave 1,4-dihydrobenzo-2,3-dithian-2-oxide **28** as a solid (2.47 g, 75%); R_f 0.18 (30% ethyl acetate in hexane); mp 130–132 °C ($CHCl_3$/hexane) (lit.,[47] 128–129 °C); (Found: C, 51.7; H, 4.3. $C_8H_8OS_2$ requires C, 52.1; H, 4.4%);ν_{max}/cm^{-1} 1072 (S=O); *m*/*z* (EI) 184 (M$^+$, 9%), 168 (4), 136 (63), 135 (100), 104 (87), 91 (25), 78 (37); δ_H (500 MHz, $CDCl_3$) 3.96 (1 H, d, $J_{A,B}$ 13.5, CH_AH$_B$S(O)), 4.23 (1 H, d, $J_{A,B}$ 13.1, CH_AH$_B$S), 4.32 (1 H, d, $J_{B,A}$ 13.1, CH$_A$$H_B$S), 4.37 (1 H, d, $J_{B,A}$ 13.5, CH$_A$$H_B$S(O)), 7.37–7.43 (4 H, m, 4 × Ar–H); δ_C (125 MHz, $CDCl_3$) 33.04, 59.69, 127.73, 127.86, 128.47, 129.34, 131.76, 135.56.

Typical biotransformations of racemic sulfoxides using aerobic and anaerobic bacteria

(i) Aerobic bacteria: *P. putida* UV4. Biotransformations with *P. putida* UV4 were carried out using the shake-flask conditions described earlier for the sulfoxidation of both acyclic and cyclic sulfides.[8,17,18,22] The biotransformation times involved were in some cases modified as described in the text. The improved work-up procedure involved removal of most of the water, from the aqueous centrifuged culture medium, under reduced pressure and relatively low temperatures (≤ 40 °C) and repeated extraction of the residual viscous concentrate with EtOAc. The combined extracts were dried (Na_2SO_4) and the solvent evaporated to yield the crude mixture of bioproducts.

Biotransformation of racemic methylphenyl sulfoxide ***2*** *(R = Me) using P. putida* UV4. Biotransformation of sulfoxide **2** (R = Me, 0.2 g, 1.43 mmol) followed by concentration, extraction (EtOAc), and separation by PLC on silica gel (8% MeOH in $CHCl_3$; two elutions) gave *cis*-dihydrodiol sulfide **8**, *cis*-dihydrodiol sulfone **9** and an inseparable mixture of the corresponding *cis*-dihydrodiol sulfoxides **7a/7b**.

(1S,2S)-1,2-Dihydroxy-3-methylsulfanylcyclohexa-3,5-diene ***8***. Light yellow coloured solid (0.01 g, 4%); R_f 0.10 (5% MeOH in $CHCl_3$); mp 58–60 °C (lit.,[17] mp 57–61 °C); $[\alpha]_D$ +63 (*c* 1.29, MeOH); (Found: M$^+$, 158.0399. $C_7H_{10}O_2S$ requires 158.0402); *m*/*z* (EI) 158 (M$^+$, 20%), 140 (100), 125 (41), 97 (66), 53 (42), 39 (60), 29 (47); δ_H (500 MHz, $CDCl_3$) 1.94 (1 H, br s, OH), 2.33 (3 H, s, Me), 2.47 (1 H, br s, OH), 4.18–4.26 (1 H, dd, $J_{OH,2}$ 8.3, $J_{2,1}$ 5.9, 2-H), 4.29 (1 H, m, 1-H), 5.52 (1 H, d, $J_{4,5}$ 5.7, 4-H), 5.81–5.84 (1 H, dd, $J_{6,5}$ 9.5, $J_{6,1}$ 4.1, 6-H), 6.00–6.04 (1H, dd, $J_{5,6}$ 9.4, $J_{5,4}$ 5.7, 5-H); δ_C (125 MHz, $CDCl_3$) 14.08, 68.20, 71.50, 113.43, 124.59, 125.36, 142.30.

(1S,2S)-1,2-Dihydroxy-3-methylsulfonylcyclohexa-3,5-diene ***9***. Colourless crystals (0.020 g, 6.0%); R_f 0.3 (5% MeOH in $CHCl_3$); mp 126–128 °C (from $CHCl_3$/$(CH_3)_2CO$) (Found: C, 44.1; H, 5.4. $C_7H_{10}O_4S$ requires C, 44.20; H, 5.30%); $[\alpha]_D$ −5.3 (*c* 0.73, MeOH); (Found: M$^+$, 190.0291. $C_7H_{10}O_4S$ requires 190.0300); δ_H (500 MHz, $CDCl_3$) 2.75–2.95 (2 H, m, OH), 3.08 (3 H, s, Me), 4.58 (1 H, dd, $J_{1,2}$ 6.3, $J_{1,6}$ 2.4,1-H), 4.64 (1 H, d,

$J_{2,1}$ 6.3, 2-H), 6.17 (1 H, m, $J_{5,4}$ 5.5, $J_{5,6}$ 9.5 5-H), 6.25 (1 H, dd, $J_{6,5}$ 9.5, $J_{6,1}$ 2.4, 6-H), 7.05 (1H, d, $J_{4,5}$ 5.5, 4-H).

*(1S,2S)-1,2-Dihydroxy-3-(R and S)-methylsulfinyl-cyclohexa-3,5-diene (**7a** and **7b**).* Colourless viscous oil (0.1 g, 40%, 56 : 44); R_f 0.35 (15% MeOH in $CHCl_3$); δ_H (300 MHz, $CDCl_3$) 2.81 (3 H, s, Me_{7a}), 2.83 (3 H, s, Me_{7b}), 3.65 (1 H, br s, OH), 3.85 (1 H, br s, OH), 4.03 (2 H, br s, 2 × OH), 4.34 (1 H, dd, $J_{1,2}$ 6.0, $J_{1,6}$ 2.8, 1-H_{7a}), 4.43 (1 H, d, $J_{1,2}$ 5.9, 1-H_{7b}), 4.53 (1 H, d, $J_{2,1}$ 5.9, 2-H_{7b}), 4.60 (1 H, d, $J_{2,1}$ 6.0, 2-H_{7a}), 6.09–6.19 (4 H, m, 5-H_{7a}, 5-H_{7b}, 6-H_{7a}, 6-H_{7b}), 6.51 (1 H, m, 4-H_{7a}), 6.60 (1 H, m, 4-H_{7b}).Biotransformation, of methyl phenyl sulfide **1** (R = Me, 0.2 g, 1.6 mmol), was repeated but using a higher cell density and a longer biotransformation time (20 h) compared with the earlier experiment.[17] Using the improved work-up procedure, (1*S*,2*S*)-1,2-dihydroxy-3-methylsulfanyl-cyclohexa-3,5-diene **8** (0.06 g, 24%) was isolated as the sole metabolite. When the latter biotransformation was repeated again but using a shorter (8 h) biotransformation period, a pure sample of (1*S*,2*S*)-1,2-dihydroxy-3-(*R*)-methylsulfinyl-cyclohexa-3,5-diene **7a** was intercepted (80% yield), $[\alpha]_D$ +200 (*c* 1.36, MeOH); (Found: M^+,174.0355 $C_7H_{10}OS$ requires 174.0351); ν_{max}/cm^{-1} 1098 (S=O); *m/z*: 174 (M^+, 38), 157 (22), 111(90); CD data (MeOH) λ/nm 214 ($\Delta\varepsilon$ −2.50) and 278 ($\Delta\varepsilon$ 2.56). Addition of the (1*S*,2*S*)-*cis*-diol-3-(*R*)-sulfoxide **7a** (0.25 g, 1.44 mmol) as substrate to *P. putida* UV4 yielded a mixture of *cis*-diol sulfide **8** and *cis*-diol sulfone **9** which proved to be identical to the samples obtained when sulfoxide **2** was used as the substrate.

*Biotransformation of racemic 2,3-dihydrobenzo[b]thiophene-1-oxide **10** using P. putida UV4.* Racemic 2,3-dihydrobenzo[*b*]-thiophene-1-oxide **10** (0.20 g, 1.32 mmol) yielded unreacted (−)-(*R*)-2,3-dihydrobenzo[*b*]thiophene-1-oxide **10** (0.038 g, 19%), (+)-3-(*S*)-hydroxy-2,3-dihydrobenzo[*b*]thiophene **12** (0.009 g, 5%) which were separated, by column chromatography ($CHCl_3$), from a mixture (0.01 g) of dihydrodiols, 2,3-dihydro-2,3-dihydroxybenzo[*b*]thiophene $\mathbf{15}_{cis}$, 2,3-dihydro-2,3-dihydroxybenzo[*b*]thiophene $\mathbf{15}_{trans}$ and (4*R*,5*S*)-4,5-dihydro-4,5-dihydroxybenzo[*b*]thiophene **14**. Metabolites $\mathbf{15}_{cis}$, $\mathbf{15}_{trans}$, and **14**, although not separated, were identified by ^{1}H NMR spectral comparisons with samples isolated earlier from the biotransformation of benzo[*b*]thiophene.[36]

(−)-(R)-2,3-Dihydrobenzo[b]thiophene-1-oxide $\mathbf{10}_R$. Ee 3%, Chiralcel OB column (30% 2-propanol in hexane, α = 1.8, early *R*).

*(+)-3-(S)-Hydroxy-2,3-dihydrobenzo[b]thiophene **12**.* Colourless viscous oil; R_f 0.41 (2% MeOH in $CHCl_3$); $[\alpha]_D$ +18 (*c* 0.61, $CHCl_3$); (Found: M^+, 152.0293. C_8H_8OS requires 152.0296); *m/z* (EI) 152 (M^+, 100%), 135 (56), 122 (26), 121 (37), 91 (46), 77 (41), 57 (88), 45 (51); δ_H (500 MHz, $CDCl_3$) 2.08 (1H, d, $J_{OH,3}$ 7.4, OH), 3.31 (1 H, dd, $J_{2A,2B}$ 12.0, $J_{2A,3}$ 3.8, CH_2), 3.60 (1 H, dd, $J_{2B,2A}$ 12.0, $J_{2B,3}$ 6.2, CH_2), 5.36 (1 H, m, 3-H), 7.10–7.13 (1 H, ddd, *J* 7.8, *J* 4.8, *J* 3.3, 7-H), 7.25–7.26 (2 H, m, 5 and 6-H), 7.38–7.40 (1 H, d, *J* 7.6, 4-H); δ_C (125 MHz, $CDCl_3$) 41.53, 70.58, 122.81, 124.75, 125.31, 129.72, 140.85, 140.93; ee 83%, Chiralcel OD column (α = 1.1, early *S*).

Biotransformation of racemic cis-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$ by P. putida UV4. Racemic *cis*-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$ (0.113 g, 0.62 mmol) gave *trans*-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$ (0.003 g, 2%), and residual (−)-(1*S*,2*R*)-*cis*-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$ (0.045 g, 40%) after separation by PLC (diethyl ether). Sulfoxides $\mathbf{17}_{cis}$ and $\mathbf{17}_{trans}$ were spectrally indistinguishable from authentic samples obtained earlier.

(−)-(1S,2R)-2-Methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$. R_f 0.46 (diethyl ether); $[\alpha]_D$ −165 (*c* 3.0, $CHCl_3$) (lit.,[48] −69, in EtOH, 25% ee); ee 47%, Chiralcel OD column (α = 1.2, late [1*S*,2*R*])

(−)-(1S,2S)-2-Methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$. R_f 0.36 (diethyl ether); ee > 98%, Chiralcel OD column (α = 1.2, early[1*S*,2*S*])

Biotransformation of racemic trans-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$ by P. putida UV4. Racemic 2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$ (0.20 g, 1.09 mmol) yielded recovered (−)-(1*S*,2*S*)-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$ (0.069 g, 35%), (−)-(1*S*,2*R*)-*cis*-2-methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$ (0.002 g, 1%) after PLC separation (diethyl ether).

(−)-(1S,2S)-2-Methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{trans}$. R_f 0.36 (diethyl ether); $[\alpha]_D$ −32.2 (*c* 1.41, $CHCl_3$) (lit.,[48] −28 in EtOH, 25% ee); ee 18%, Chiralcel OD column (α = 1.1, late [1*S*,2*S*]).

(−)-(1S,2R)-2-Methyl-1,3-benzodithiole-1-oxide $\mathbf{17}_{cis}$. R_f 0.46 (diethyl ether); ee > 98%, Chiralcel OD column (α = 1.2, late [1*S*,2*R*]).

*Biotransformation of racemic 1,2,3,4-tetrahydro-2H-1,5-benzodithiepine-1-oxide **18** by P. putida UV4.* Racemic 1,2,3,4-tetrahydro-2*H*-1,5-benzodithiepine-1-oxide **18** (0.131 g, 0.66 mmol) yielded (+)-(*R*)-1,2,3,4-tetrahydro-2*H*-1,5-benzodithiepine-1-oxide $\mathbf{18}_R$ (0.004 g, 6%) and 3,4-dihydro-2*H*-1,5-benzodithiepine **19** (0.002 g, 2%), after separation by column chromatography ($CHCl_3$).

(+)-(R)-1,2,3,4-Tetrahydro-2H-1,5-benzodithiepine-1-oxide $\mathbf{18}_R$. $[\alpha]_D$ +52.0 (*c* 0.28, $CHCl_3$) (lit.,[22] −82, 93% ee); ee 72%, Chiralcel OB column (30% 2-propanol in hexane, α = 1.3, late *R*).

*3,4-Dihydro-2H-1,5-benzodithiepine **19**.* Metabolite **19** was spectrally identical to an authentic sample.

(ii) Biotransformations using the anaerobic bacteria: *R. capsulatus* 938, *E. coli* HB 101, *P. vulgaris* NCIMB 67, *Klebsiella sp.* DMSO 7, *Serratia* sp. DMSO 10 and *C. braakii* DMSO 11. *Typical procedure.* The bacterium was grown anaerobically in a supplemented basal salts medium. DMSO (40 mmol) was added as the electron donor and glucose (5 g L^{-1}) as the carbon source for growth. Bacteria were grown in Suba-sealed flasks (250 cm^3) containing growth medium (200 cm^3). The cultures were flushed with nitrogen and incubated at 30 °C on a rotary shaker (250 rev min^{-1}) for 16 h. Following growth, bacteria were harvested by centrifugation (5000 rpm for 15 min) and washed in potassium phosphate buffer (25 mmol, pH 7.4). The cell pellets were then re-suspended in fresh phosphate buffer to give a 10-fold concentration of the initial cell density. The cell suspension (20 cm^3 from a 200 cm^3 culture) was transferred to a Suba-sealed flask (50 cm^3 growth medium) containing racemic sulfoxide (1 mg cm^{-3}) and glucose (10 mmol) under nitrogen. The reaction flasks were incubated at 30 °C on a rotary shaker (250 rev min^{-1}) until about 50% of the sulfoxide substrate had been deoxygenated. After centrifugation (5000 rpm, 15 min), the metabolites from the remaining aqueous supernatant, were extracted in a similar manner to the *P. putida* UV4 biotransformations.

*Biotransformation of racemic 2-(methylsulfinyl) phenol **26** by C. braakii* DMSO 11. Sulfoxide **26** (0.5 g), was used as a substrate and progress of the biotransformation was monitored by RP-HPLC (30% H_2O in MeOH). The biotransformation was terminated after ~50% of the substrate had been metabolised to the corresponding sulfide **27**. The crude metabolite was purified by PLC (5% MeOH in $CHCl_3$) to give (−)-(*S*) sulfoxide **26** (0.135 g, 27%); $[\alpha]_D$ −189 (*c* 0.5, $CHCl_3$) (lit.[49] + 188, $CHCl_3$); ee > 98%, Chiralcel OB column (α = 1.60, early *S*).

*Biotransformation of racemic 1,4-dihydrobenzo-2,3-dithian-2-oxide **28** by C. braakii* DMSO 11. Biotransformation of 1,4-dihydrobenzo-2,3-dithian-2-oxide **28** (0.25 g, 1.36 mmol) followed by ethyl acetate extraction of the centrifuged medium yielded residual (+)-(*S*)-1,4-dihydro-2,3-benzodithian-2-oxide $\mathbf{28}_S$ (0.058 g, 46%) after purification by PLC (3% MeOH in $CHCl_3$); $[\alpha]_D$ +43.1 (*c* 0.56, $CHCl_3$), ee 18% by CSPHPLC, Whelk-01 column (*t*-BuOMe, α = 2.8, early *S*).

The 1,4-dihydrobenzo-2,3-dithian-2-oxide **28** (0.005 g, 0.027 mmol) was used as substrate with purified DMSO reductase from *C. braakii* DMSO 11.[41] Ethyl acetate extraction, of the centrifuged medium, yielded unreacted (+)-(*S*)-1,4-dihydro-2,3-benzodithian-2-oxide $\mathbf{28}_S$ and 1,4-dihydrobenzo-2,3-dithian **29**. Separation and purification by PLC (3% MeOH in $CHCl_3$) gave thiosulfinate $\mathbf{28}_S$ (0.002 g, 40%, > 95% ee) and 1,2-disulfide **29** (0.0005 g, 10%).

*Biotransformation of racemic ethyl-2-pyridyl sulfoxide **24** by P. vulgaris* NCIMB 67. The aqueous mixture of bioproducts, obtained when racemic ethyl-2-pyridyl sulfoxide **24** (2.0 g) was metabolised with *P. vulgaris* NCIMB 67, was extracted with EtOAc. PLC of the crude product yielded (−)-(*R*)-ethyl-2-pyridyl sulfoxide **24** (0.90 g, 45%); $[\alpha]_D$ −164 (*c* 1.6, MeOH) (lit.[42] −168, MeOH); ee > 98%, Chiralcel OB column ($\alpha = 1.95$).

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