

Sulfoxides of High Enantiopurity from Bacterial Dioxygenase-catalysed Oxidation

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Selected strains of the bacterium *Pseudomonas putida* (previously shown to effect dioxygenase-catalysed asymmetric *cis*-dihydroxylation of alkenes) have been found to yield chiral sulfoxides from the corresponding sulfides with a strong preference for the (*R*)- or (*S*)-configurations but without evidence of sulfone formation; similar results obtained using an *Escherichia coli* clone (pKST11, containing the Tod C1 C2 B and A genes encoding toluene dioxygenase from *P. putida* NCIMB 11767) are again consistent with a stereoselective dioxygenase-catalysed sulfoxidation.

Enzyme-catalysed sulfoxidations have previously yielded a relatively small number of enantiopure sulfoxides using either intact fungal^{1–4} or bacterial^{5–8} cells or purified enzymes.^{9–11} While microbial oxidation can provide a simple route to enantiopure alkyl aryl sulfoxides of (*R*)-configuration, which are of value for synthetic studies, this method has been less successful in the stereoselective sulfoxidation of other sulfides including thioacetals^{12,13} or diaryl sulfides^{12,13} and in the production of (*S*)-sulfoxides. The results obtained in this study, using selected strains of *Pseudomonas putida*, and a recombinant strain of *Escherichia coli* expressing toluene dioxygenase, indicate that whole-cell sulfoxidations catalysed by dioxygenase enzymes, can offer distinct advantages over previously reported microbial sulfoxidations which are generally catalysed by other enzyme systems *e.g.* monooxygenases.

Using shake flask cultures of a mutant strain (UV4) of *P. putida*, both alkyl aryl (1–6,10) and diaryl (7–9) sulfoxides were

produced (Table 1). The alkyl aryl sulfoxide metabolites, 1–5 and 10, were found to have a marked preference (>94% ee) for the (*R*)-enantiomer (A, Scheme 1). Both yield and optical purity were found to decrease with increasing size of substituent *e.g.* sulfoxide 6 having a bulky Bu^t group gave a lower yield (2%) and lower enantiomeric excess (62% ee). The novel sulfoxidation of the diaryl sulfides 7–9 also occurred in lower yield (≤10%) but showed a similar strong preference (86 to >98% ee) for configuration A.

The pyridyl (10), vinyl (11) and thioacetal (12) members of the alkyl aryl sulfoxide series were all obtained with high ee values (≥94%) and contained useful functional groups for further synthetic manipulations. When a change in sequence rule priorities is taken into account for sulfoxides 8, 9 and 12 [all having an (*S*)-configuration], it becomes apparent that all members (1–12) of the series shown in Table 1 have the same absolute configuration A. Earlier attempts, to obtain enantiopure thioacetal sulfoxides by enzyme-catalysed oxidation, have generally been unsuccessful.^{12,13} (Methylthio)methyl phenyl sulfide and 2-methylbenzo-1,3-dithiole being exceptions since the derived acyclic-[13, >95% ee, (*R*)] and cyclic-[14, >98% ee, (1*S*,2*R*)] sulfoxide enantiomers were reported as metabolites from intact cells of *Corynebacterium equi*¹⁴ and from purified mammalian monooxygenase enzymes¹¹ respectively. In contrast with cultures of *C. equi*¹⁴ and chemical oxidation of (methylthio)methyl phenyl sulfide, where the dialkyl sulfoxide 13 was formed preferentially, sulfoxidation in *P. putida* UV4 strongly favoured the alkylaryl over the dialkyl sulfur atom to give thioacetal sulfoxide 12. The stereoselectivity associated with thioacetal sulfoxidation reactions in *P. putida* UV4 is further illustrated by oxidation of 2-methylbenzodithiole to yield both *cis*-[14, >98% ee, (1*S*,2*R*), 40% yield] and *trans*-[15, >98% ee, (1*S*,2*S*), 5% yield] sulfoxide isomers. Oxidation of a single dialkyl sulfur atom in 2-phenyl-1,3-dithiane using cultures of *P. putida* UV4 also gave one dithioacetal sulfoxide enantiomer 16 [*trans*-2-phenyl-1,3-dithiane-1-oxide, >98% ee, (1*S*,2*S*)]. Evidence of the reluctance of the bacterial enzyme to oxidize a dialkyl sulfur atom was thus provided by (i) the lower yield (7%) of thioacetal sulfoxide 16 and (ii) the formation of *cis*-dihydrodiol 17 [>98% ee, (2*R*,3*S*)] as a major metabolite (18% yield). *cis*-Dihydrodiol metabolites were found in trace quantities (≪ 1%) during the formation of several alkyl aryl sulfoxides *e.g.* 1,5,6. The absolute configurations of the new metabolites 12, 16 and 17 were determined by methods which will be discussed elsewhere. The formation of only one enantiomer of the thioacetal sulfoxides 14 [(1*S*,2*R*)], 15

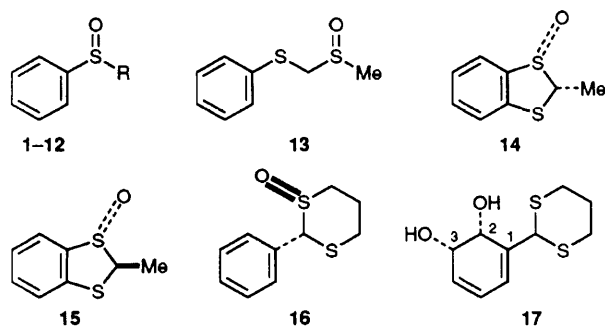
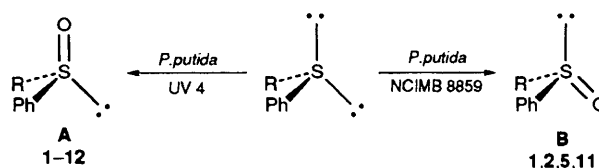


Table 1 Enantiomeric excess values (%ee), absolute configuration (Ab.con.), and isolated yields of sulfoxide metabolites from *P. putida* UV4

Compound	R	%ee ^a	Ab.con.	% Yield
1	Me	>98	R ^b	95
2	Et	>98	R ^b	64
3	Pr	>98	R ^b	5
4	Bu	97	R ^b	7
5	Pr ⁱ	97	R ^b	27
6	Bu ^t	62	R ^b	2
7	Ph	—	—	10
8	<i>o</i> -MeC ₆ H ₄	86	S ^{b,c}	1
9	<i>p</i> -MeC ₆ H ₄	>98	S ^{b,c}	<1
10	Me ^d	94	R	20
11	CH ₂ =CH	>98	R ^b	38
12	MeSCH ₂	97	S ^c	20

^a Determined by chiral stationary phase HPLC (Chiralcel OD). ^b Based upon previously reported configurations. ^c Absolute configurations appear to be reversed due to a change in sequence rule priorities. ^d The phenyl group has been replaced by a 2-pyridyl group in this example.



Scheme 1

[(1*S*,2*S*), and **16** [(1*S*,2*S*)] is consistent with exclusive enzyme-catalysed oxidation of the *pro*-S lone pair on a prochiral sulfur atom (**14**, **15**, **16**) and the *pro*-S sulfur atom on a prochiral carbon atom (**16**).

In contrast to the majority of reports on bacterial and fungal sulfoxidations,^{12,13,15} where sulfone formation was also observed, sulfoxides **1–11** and thioacetal sulfoxides **12**, **14–16** were obtained from *P. putida* UV4 without any evidence of sulfones. The formation of sulfoxides, in growing cultures of *P. putida* UV4, could imply that the oxidations were catalysed by a monooxygenase as reported in past microbial studies.^{12,13} However, results obtained from these and other laboratories have indicated that monooxygenation reactions can be catalysed by the toluene dioxygenase from this organism.^{16,17} To establish the possibility that sulfoxidation reactions in *P. putida* UV4 might be catalysed by a dioxygenase we used an *Escherichia coli* clone (pKS T11 constructed by PCR amplification from published sequence information) expressing the toluene dioxygenase enzyme from *P. putida* NCIMB 11767 (a wild-type from which the UV4 mutant was derived). Biotransformation with *E. coli* (pKS T11) yielded the acyclic sulfoxides **1**, **2** and **11** [95–100% ee, (*R*)] and cyclic thioacetal sulfoxides **14** [97% ee, (1*S*,2*R*)] and **15** [40% ee, (1*S*,2*S*)] of identical stereochemistry to the bioproducts obtained using *P. putida* UV4. Since only trace amounts of the sulfoxides, in essentially racemic form, were produced using the non-recombinant *E. coli* parent strain (JM 109), it is assumed that the same dioxygenase enzyme was responsible for the stereoselective sulfoxidations in both *P. putida* UV4 and *E. coli* pKS T11. Based upon the limited results obtained in this study, the absence of any sulfone metabolites appears to be a characteristic of the dioxygenase-catalysed oxidation of sulfides.

The preferred (*R*)-configurations found for the alkyl aryl sulfoxides **1–6**, **10**, using *P. putida* UV4, are also the most common enantiomers previously reported in microbial biotransformations of alkyl aryl sulfides.^{12,13} When, however, a naphthalene-utilizing wild-type strain (NCIMB 8859) of *P. putida* was employed for sulfoxidation, the phenyl sulfoxides **1** (91% ee), **2** (84% ee), **5** (76% ee) and **11** (91% ee) were isolated with a preference for the (*S*)-configuration (**B**, Scheme 1). Similarly the cyclic sulfoxides **14** [82% ee, (1*R*,2*S*)] and **15** [38% ee, (1*R*,2*R*)] isolated from the bacterial oxidation of the corresponding thioacetals, using *P. putida* NCIMB 8859, were of opposite absolute configuration to those found using *P. putida* UV4. Recent studies¹⁸ have shown that the latter two strains of *P. putida* can also yield enantiopure *cis*-diols of opposite configuration by dioxygenase-catalysed *cis*-dihydroxylation of a series of bicyclic alkenes. Although *P. putida* NCIMB 8859 frequently gave metabolites of opposite configuration to *P. putida* UV4, sulfoxides **3** and **12** were found to have identical configurations and similar enantiopurities when produced by either strain.

In conclusion, this communication indicates: that (*i*) a range of enantiopure alkyl aryl, diaryl and thioacetal sulfoxides can be obtained by microbial oxidation using *P. putida* UV4, (*ii*) this bacterial sulfoxidation process occurs preferentially on alkyl aryl sulfides, without evidence of sulfone formation, as a result of dioxygenase-catalysed sulfoxidation, (*iii*) the dioxygenase

enzyme can exhibit exclusive stereoselectivity during sulfoxidation of both prochiral lone pairs on a sulfur atom and prochiral sulfur atoms on a carbon atom (*iv*) different strains of *P. putida* can often yield sulfoxides of high enantiopurity and of opposite absolute configuration from a range of sulfide substrates. A comparable type of enantiocomplementarity has recently been observed during dioxygenase-catalysed asymmetric *cis*-dihydroxylation of cyclic alkenes using the same strains.¹⁸ Although the absolute configuration was found to be identical at the sulfoxide and benzylic chiral centres of *cis*-diols produced for a specific strain, *e.g.* UV4, speculation on the similarity in binding of sulfide and alkene substrates at the active site can only be confirmed when the pure dioxygenases are available.

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