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**.

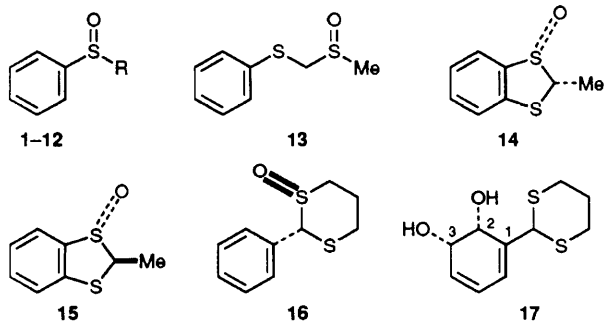
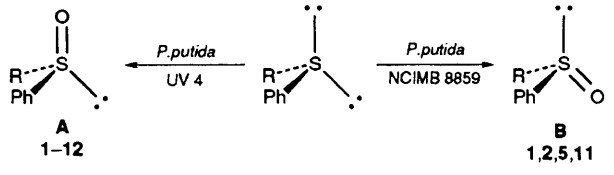


Table 1 Enantiomeric excess values (%ee), absolute configuration (Ab.con.), and isolated yields of sulfoxide metabolites from *P. putida* UV4

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	*o*-MeC$_6$H$_4$	86	S[b,c]	1
9	*p*-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.

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 enzymes[11] 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**



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 studies[18] 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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References

1 B. J. Auret, D. R. Boyd and H. B. Henbest, *J. Chem. Soc., Chem. Commun.*, 1966, 66.
2 B. J. Auret, D. R. Boyd, H. B. Henbest and S. Ross, *J. Chem. Soc. C*, 1968, 2371.
3 E. Abushanab, D. Reed, F. Suzuki and G. J. Sih, *Tetrahedron Lett.*, 1978, **19**, 3415.
4 H. L. Holland, H. Popperl, R. W. Ninniss and P. C. Chenchaiah, *Can. J. Chem.*, 1985, **63**, 1118.
5 H. Ohta, Y. Okamoto and G. Tsuchihashi, *Agric. Biol. Chem.*, 1985, **49**, 671.
6 H. Ohta, Y. Okamoto and G. Tsuchihashi, *Chem. Lett.*, 1984, 205.
7 H. Ohta, Y. Okamoto and G. Tsuchihashi, *Agric. Biol. Chem.*, 1985, **49**, 2229.
8 M. Mahmoadian and A. Michael, *J. Biotechnol.*, 1993, **27**, 173.
9 G. Carrea, B. Redigolo, S. Riva, S. Colonna, N. Gaggero, E. Battistel and D. Bianchi, *Tetrahedron Asymmetry*, 1992, **3**, 1063.
10 F. Secundo, G. Carrea, S. Dallavalle and G. Franzosi, *Tetrahedron Asymmetry*, 1993, **4**, 1981.
11 J. R. Cashman, L. D. Olsen, D. R. Boyd, R. A. S. McMordie, R. Dunlop and H. Dalton, *J. Am. Chem. Soc.*, 1992, **114**, 8772.
12 H. L. Holland, *Chem. Rev.*, 1988, **88**, 473.
13 D. R. Boyd, C. T. Walsh and Y. C. Chen, in *Sulfur Containing Drugs and Related Compounds*, Ellis-Horwood Ltd., Chichester, 1989, ch. 2, A, 67.
14 Y. Okamoto, H. Ohta and G. Tsuchihashi, *Chem. Lett.*, 1986, 2049.
15 H. L. Holland, F. M. Brown and B. G. Larsen, *Tetrahedron Asymmetry*, 1994, **5**, 1241.
16 D. R. Boyd, N. D. Sharma, P. J. Stevenson, J. Chima, D. J. Gray and H. Dalton, *Tetrahedron Lett.*, 1991, **32**, 3887.
17 L. P. Wackett, L. D. Kwart and D. T. Gibson, *Biochemistry*, 1988, **27**, 1360.
18 C. R. Allen, D. R. Boyd, H. Dalton, N. D. Sharma, I. Brannigan, N. Kerley, G. N. Sheldrake and S. C. Taylor, *J. Chem. Soc., Chem. Commun.*, 1995, 117.