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Enzyme-catalysed oxygenation and deoxygenation routes to chiral thiosulfinates

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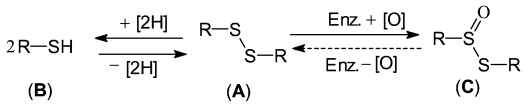
Enantioenriched thiosulfinates have been obtained by dioxygenase- and chloroperoxidase-catalysed oxidation of 1,2-disulfides and dimethyl sulfoxide reductase- catalysed deoxygenation.

The reduction of a 1,2-disulfide bond to the corresponding thiol group (**A** → **B**), and the reverse oxidation process (**B** → **A**) are common reactions in protein chemistry. By comparison, enzyme-catalysed monooxygenation of 1,2-disulfides (**A**) to yield isolable thiosulfinates (**C**) is uncommon while enzyme-catalysed deoxygenation of thiosulfinates (**C** → **A**) is unprecedented (Scheme 1).



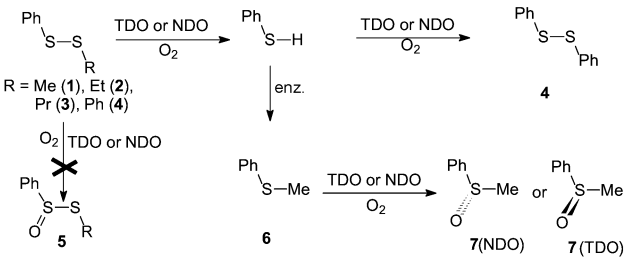
Scheme 1 Oxidation and reduction reactions of 1,2-disulfides.

The eucaryotic oxidation of naturally occurring 1,2-disulfides **A** (*e.g.* R = $CH_2C{=}CH_2$, and n-Pr)[1] to yield the corresponding thiosulfinates **C** using cytochrome P-450 and flavin-containing monooxygenases from human liver microsomes has recently been reported.[2] Prior to this study, only one report of a procaryotic enzyme-catalysed oxidation of 1,2-disulfides **A** to the corresponding thiosulfinates **C** has appeared.[3] Thus, stereoselective sulfoxidation of symmetrical 1,2-disulfides **A** (R = n-Bu, i-Pr, t-Bu, 22–97% enantiomeric excess, ee), utilising cyclohexanone monooxygenase from the bacterium *Acinetobacter calcoaceticus* was observed.[3]

As toluene dioxygenase (TDO), naphthalene dioxygenase (NDO) and chloroperoxidase (CPO) enzymes are known to catalyse the stereoselective sulfoxidation of aryl alkyl sulfides,[4–9] the focus of this study was to examine the potential of these enzymes to catalyse the asymmetric sulfoxidation of 1,2-disulfides (**A**) to yield the corresponding thiosulfinates (**C**). When methyl phenyl disulfide **1** was added to whole cell cultures of *Pseudomonas putida* UV4 (TDO), diphenyl disulfide **4** (28% yield) and (*R*)-methyl phenyl sulfoxide **7** (6–9% yield, 96% ee), rather than the anticipated thiosulfinate **5** (R = Me), were formed (Scheme 2). With whole cells of *P. putida* NCIMB 8859 (NDO) and substrate **1**, disulfide **4** (5% yield) and (*S*) methyl phenyl sulfoxide **7** (11% yield, 94% ee) were isolated. Furthermore, when 1,2-sulfides **2**, **3** and **4** were each added to *P. putida* UV4 or *P. putida* NCIMB 8859, diphenyl disulfide **4** (2–34% yield) and sulfoxide **7** (1–4% yield) of (*R*) and (*S*) configurations, respectively, were obtained. These results are consistent with an intracellular reductive cleavage of the S–S bond to yield the corresponding thiols. The less volatile benzene thiol was then converted to disulfide **4** or gave methyl phenyl sulfide **6** (after enzyme-catalysed methylation) and sulfoxide **7** (after dioxygenase-catalysed sulfoxidation of **6**). Addition of benzene thiol as a substrate to *P. putida* UV4 also yielded disulfide **4** and (*R*)-sulfoxide **7**. The dioxygenase-catalysed oxidation of methyl phenyl sulfide **1** had been found to give either the (*R*)- (> 98% ee, TDO) or (*S*)- isomer (91% ee, NDO) enantiomer of sulfoxide **7**.[5,7] Chemically synthesised thiosulfinate **5** (R = Me) was unstable and thus unlikely to survive the biotransformation conditions.

1,4-Dihydrobenzo-2,3-dithian **8**, and the corresponding thiosulfinate **9** were adopted as model compounds due to their greater stabilities. As NDO had earlier been found to catalyse sulfoxidation of dialkyl sulfides,[9] it was selected for the biotransformation of the cyclic 1,2-disulfide **8**; thiosulfinate **9** was the only metabolite isolated (11–23% yield, 10% excess of the *S* enantiomer by chiral stationary phase [CSP] HPLC analysis Whelk-O1 column, α 2.8, t-butyl methyl ether). Since thiosulfinate **9** had not been resolved or stereochemically assigned earlier, its enantiomers were separated by semi-preparative CSP HPLC and (+)-enantiomer **9** ($[\alpha]_D$ +250 $CHCl_3$) was found to have an (*S*) configuration by an X-ray crystal structure analysis[10] (Fig. 1).†

1,2-Disulfide **8**, as substrate to *P. putida* UV4, produced two unexpected metabolites; the less polar was identified as 2-thiophthalide **10** (17% yield) and the more polar as *cis*-dihydrodiol **11** ($[\alpha]_D$ +169, $CHCl_3$, 3% yield). The structure and (4*R*,5*S*) absolute configuration of diol **11** was determined by NMR spectral methods and by X-ray crystallographic analysis of the hydrogenated derivative **12** ($[\alpha]_D$ +20, $CHCl_3$; CCDC 185045).† Possible metabolic pathways involving TDO-catalysed benzylic hydroxylation to yield hemithioacetal **13**, followed by reductive cleavage of the S–S bond and loss of H_2S to give aldehyde **14** (in equilibrium with the cyclic hemiacetal **15**) are shown in Scheme 3. Dehydrogenation of the hemiacetal



Scheme 2 TDO- or NDO-catalysed oxidation products from 1,2-disulfides.

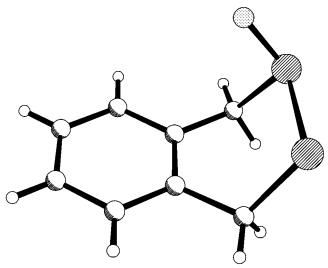


Fig. 1 X-Ray structure of the (*S*)-enantiomer of thiosulfinate **9**.

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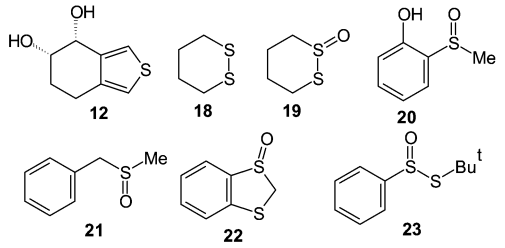
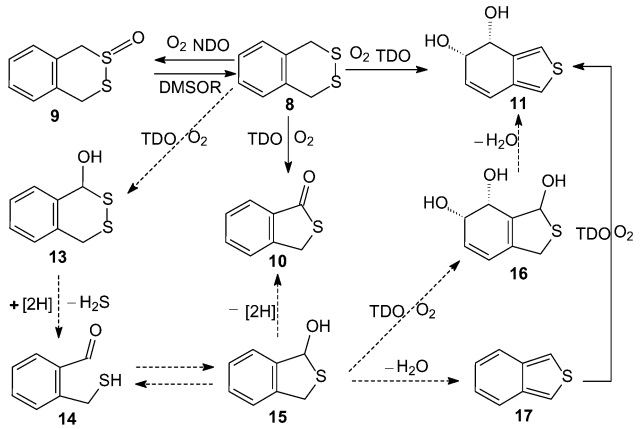


15 could account for the formation of 2-thiophthalide **10**. *cis*-Dihydrodiol **11** could be formed by a *cis*-dihydroxylation/dehydration sequence *via* the triol intermediate **16** or a dehydration/*cis*-dihydroxylation sequence *via* benzo[*c*]thiophene **17**; addition of benzo[*c*]thiophene **17** as a substrate to *P. putida* UV4 gave *cis*-dihydrodiol **11** of identical (4*R*,5*S*) configuration. Since dioxygenase-catalysed benzylic monol → triol → diol metabolic pathways are precedented using *P. putida* UV4,[11] the biosynthetic sequence **15** → **16** → **11** is preferred.

CPO enzyme from *Caldariomyces fumago*, a biocatalyst for the synthesis of enantiopure sulfoxides from sulfide precursors,[12–14] was applied to the asymmetric oxidation of 1,2-disulfides. The cyclic 1,2-disulfides **8** and **18** were selected as substrates for reaction with a suspension of CPO (Sigma) under previously reported conditions;[13,14] 1,2-disulfide **8** was biotransformed into thiosulfinate **9** (*ca*: 60% yield) with an excess of the (*S*) configuration (32–47% ee). The more water-soluble monocyclic 1,2-disulfide **18** was a better substrate for CPO; it gave the corresponding (*S*) thiosulfinate **19** in essentially quantitative yield and in almost enantiopure form (96% ee by CSP HPLC). The enantiomers of thiosulfinate **19** were separated by semi-preparative CSP HPLC (Whelk-01 column, α 1.4, t-butylmethyl ether : hexane; 1 : 1). The absolute configuration of (−) enantiomer **19** ($[\alpha]_D$ −338, $CHCl_3$) was assigned as (*R*) by circular dichroism spectral correlation.

A new range of wild-type anaerobic bacterial strains was found to catalyse the stereoselective deoxygenation of sulfoxides. Using one of these strains (DMSO-11) of the bacterium *Citrobacter braakii* containing dimethyl sulfoxide reductase (DMSOR), enantiomeric enrichment *via* kinetic resolution of the racemic sulfoxide substrates was achieved. Thus, enriched samples of alkylaryl sulfoxides (*e.g.* sulfoxide **20** [> 98% ee]), dialkyl sulfoxides (*e.g.* sulfoxide **21** [44% ee]) and cyclic sulfoxides (*e.g.* sulfoxide **22** [97% ee]) were obtained. When racemic thiosulfinate **9** was used as a substrate for the intact



Scheme 3 NDO- and TDO-catalysed oxidation products from 1,2-disulfide **8**

cells of *C. braakii* DMSO-11, the residual samples of substrate **9**, isolated after biotransformations, were consistently found to have an excess of the (*S*) enantiomer (17–77% ee). A time-course experiment with partially purified DMSOR enzyme isolated from *C. braakii* DMSO-11, and racemic thiosulfinate **9** allowed both the 1,2-disulfide bioproduct **8**, and the residual thiosulfinate **9** (95% ee, (*S*)), to be isolated (Scheme 3). Addition of racemic thiosulfinate **23** to whole cell cultures of *C. braakii* DMSO-11 showed some evidence of kinetic resolution (4% excess of the *R* configuration). Enantiomers of thiosulfinate **23** were separated by CSP HPLC using identical conditions to those used for thiosulfinate **19**. The (+) enantiomer of thiosulfinate **23** ($[\alpha]_D$ +150, $CHCl_3$) was found to have an (*R*) configuration by comparison with the (−)-(*S*) enantiomer whose configuration was assigned by X-ray crystal structure analysis (CCDC 185046).†

In conclusion, the preliminary results contained herein indicate that stereoselective oxygenation of 1,2-disulfides (using NDO and CPO) and deoxygenation of racemic thiosulfinates (using DMSOR) can yield enantiomerically enriched thiosulfinates. In addition novel metabolic pathways for 1,2-disulfides and thiosulfinates have been found.

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Notes and references

† See http://www.rsc.org/suppdata/cc/b2/b203139f/ for crystallographic files in .cif or other electronic format.

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10 *Crystal data* for (+)-(*S*)-thiosulfinate **9**: $C_8H_8OS_2$, M = 184.3, monoclinic, space group $P2_1$, a = 7.712(1), b = 4.705(1), c = 11.636(2) Å, β = 102.59(1)°, V = 412.1(1) Å^3, Z = 2, D_c = 1.485 g cm^{-3}, T = 300(2) K, $F(000)$ = 192, P4 diffractometer, 1067 observed reflections (> 2$\sigma(I)$), 101 parameters, $R1$ = 0.048, $wR2$ = 0.123 (all data), GoF = 1.08, $Flack\ x$ = −0.06(5). CCDC 183077.
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