Enantioselective dioxygenase-catalysed formation and thermal racemisation of chiral thiophene sulfoxides

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Substituted chiral thiophene 1-oxides and their cycloadducts of variable enantiopurity have been isolated as products of dioxygenase-catalysed sulfoxidation of the corresponding thiophenes using intact cells of *Pseudomonas putida*; thermal racemization ($\Delta G^{\ddagger}$ = 25.1 kcal mol^{-1}) of the enantiopure metabolite (1*R*)-2-methylbenzo[*b*]thiophene 1-oxide has been observed.

Toluene dioxygenase (TDO) and naphthalene dioxygenase (NDO), from different strains of the soil bacterium *Pseudomonas putida*[1,2] and a recombinant *E. coli* strain,[1] may catalyse the oxidation of alkyl aryl and diaryl sulfides in an enantiocomplementary manner. A similar sulfoxidation of the thiophene ring system, *e.g.* **1A–6A** → **1B–6B** is discussed herein.

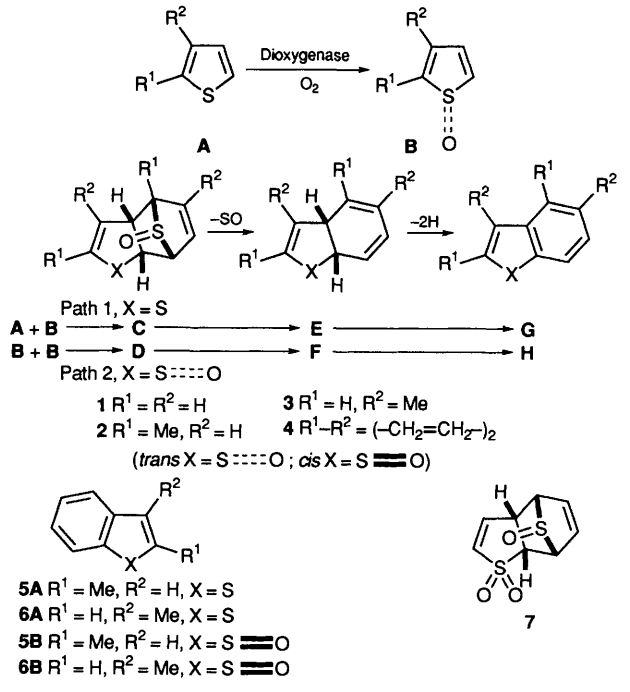
Peroxy acid oxidation of thiophene **1A** to yield the unstable thiophene 1-oxide **1B** and thiophene 1,1-dioxide is followed by a rapid cycloaddition reaction to yield thiophene sesquioxide **7**.[3] Benzo[*b*]thiophene **4A** was oxidised to yield the relatively unstable derivative, benzo[*b*]thiophene 1-oxide **4B**, using a peroxy acid or a monooxygenase biocatalyst.[4]

Thiophene sulfoxides **1B** and **4B** are unstable in comparison with some substituted thiophene 1-oxides *e.g.* the sulfoxides from 2,5-diphenyl-,[5] 2,5-di-*tert*-butyl-,[6] and 2,4-bis(1,1,3,3-tetramethylbutyl)-thiophene.[6] 2-Methylbenzothiophene **5B**[7] and 3-methylbenzothiophene **6B** are more stable and have previously been isolated in racemic form using non-enzymatic oxidation methods.

Biotransformation of thiophenes **1A–3A** and benzo[*b*]thiophenes **4A–6A** using *P. putida* UV4 (a source of TDO) yielded both heterocyclic dihydrodiol metabolites[8] and sulfoxidation products (Scheme 1). Indirect evidence for the dioxygenase-catalysed formation of thiophene 1-oxides **1B–3B** and benzo[*b*]thiophene 1-oxide **4B**, trapped as the cycloadducts **1C–3C** and **1D–3D**, and **4F–4H**, respectively, and direct evidence for the formation of optically active sulfoxide derivatives of benzo[*b*]-thiophenes, **5B** and **6B**, are shown below.

Thiophene substrates **1A–3A** with *P. putida* UV4 yielded both dihydrodiols (1–11% yield)[8] and unstable sulfoxides **1B–3B**, which were trapped *in situ* as Diels–Alder-type cycloadducts **1C** (11%), **2C** (12%) and **3C** (4%) by reaction with the parent thiophenes **1A–3A** and as dimers **1D** (45%), **2D** (4%) and **3D** (4%). The structures for the monosulfoxide (**1C–3C**) and disulfoxide cycloadducts (**1D–3D**) were deduced from ^{1}H NMR spectroscopy and X-ray crystallographic analysis (**1C**, **1D**, **3C**). It is noteworthy that despite the presence of five and six chiral centres in the monosulfoxides (**1C–3C**) and disulfoxides (**1D–3D**), respectively, only the diastereoisomer having the general structure and relative configuration shown was produced in each case. The monosulfoxides **1C–3C** were found to have an excess of one enantiomer (8–77% ee, Table 1) by chiral stationary phase HPLC analysis (Chiralpak AD), while the disulfoxides were found to be racemic by ^{1}H NMR analysis in the presence of (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TAE). The enantiomeric excess observed for cycloadducts **1C–3C** could arise *via* cycloaddition occurring in a chiral environment, *e.g.* the TDO system, or *via* an enzyme-catalysed kinetic resolution. The absolute configurations of the monosulfoxides **1C–3C** were not determined, but the similarity of their CD spectra indicated that the same configuration was preferred in all cases. Formation of the monosulfoxides **1C–3C** and the disulfoxides **1D–3D** is assumed to occur *via* separate routes, *i.e.* **A** + **B** → **C** (Path 1) and **B** + **B** → **D** (Path 2), respectively (Scheme 1).

Biotransformation of benzo[*b*]thiophene **4A** in *P. putida* UV4, previously reported to yield vicinal dihydrodiols at the 2,3- and 4,5-positions in *ca.* 50% isolated yield,[9] upon reinvestigation also gave a mixture of four minor separable



Scheme 1

Table 1 Data for chiral sulfoxides isolated from *P. putida* UV4

Sulfoxide	$[\alpha]_D$ ($CHCl_3$)	ee (%)	Abs. config.
1C	−31	77	*a*
2C	*a*	8	*a*
3C	−19	51	*a*
4H	−13	3	*a*
5B	−476	>98	*R*
	+267[b]	56[b]	S_b
6B	−181[b]	41[b]	R^b
4F$_{trans}$	+65[c]	6	*a*

[a] Not determined. [b] From NDO-catalysed oxidation of thiophenes in *P. putida* NCIMB 8859. [c] EtOH solvent.

metabolites $\mathbf{4F}_{trans}$ (2%), $\mathbf{4F}_{cis}$ (1%), **4G** (1%) and **4H** (3%). Their structures were determined by NMR ($\mathbf{4F}_{trans}$, $\mathbf{4F}_{cis}$, **4G**, **4H**) and X-ray crystallographic ($\mathbf{4F}_{trans}$) methods. The sulfoxides $\mathbf{4F}_{trans}$ ($[\alpha]_D$ +65, 6% ee) and **4H** ($[\alpha]_D$ −13, 3% ee) proved to be optically active, but of low enantiopurity by chiral stationary phase HPLC analysis (Chiralcel OJ). The configurational stability of thiophene sulfoxide **4H** was in accord with a recent *ab initio* computational study[10] which suggested that the barrier to pyramidal inversion of the sulfoxide centre in a dibenzothiophene 1-oxide would be relatively high ($\Delta G^{\ddagger}$ = 32.3 kcal mol^{-1}; 1 cal = 4.184 J). The dioxygenase-catalysed formation of the achiral dibenzothiophene 1-oxide has also been reported.[11]

Tetracyclic adducts $\mathbf{4F}_{trans}$, $\mathbf{4F}_{cis}$, **4G** and **4H** were again found when benzo[*b*]thiophene **4A** was oxidised using *m*-chloroperoxybenzoic acid. The tetracyclic products appeared to result from partial oxidation of benzothiophene **4A** to sulfoxide **4B** which combined to yield the cycloadduct sulfoxide **4C** (Path 1) or dimerised to yield disulfoxide **4D** (Path 2). Extrusion of sulfur monoxide to give the dihydrothiophenes **4E** and **4F**, followed by dehydrogenation during the biotransformation process can account for the formation of benzo[*b*]naphtho[1,2-*d*]thiophene **4G** and *S*-oxide **4H**, respectively.

Addition of 2-methylbenzo[*b*]thiophene **5A** as substrate to *P. putida* UV4 gave the corresponding 2,3- and 4,5-dihydrodiols as major metabolites (27% yield),[8] and 2-methylbenzo[*b*]thiophene 1-oxide **5B** as a minor metabolite (2% yield). This sample of thiophene *S*-oxide **5B**, ($[\alpha]_D$ −476, $CHCl_3$) was found to be enantiopure (>98% ee) from ^{1}H NMR analysis in the presence of TAE. X-Ray crystallographic analysis of sulfoxide **5B** confirmed the pyramidal nature, the enantiopurity and the (1*R*)-configuration of the sulfoxide chiral centre (Fig. 1).† Using *P. putida* NCIMB 8859 (a wild-type bacterium providing a source of NDO) in the metabolism of 2-methylbenzo[*b*]thiophene **5A**, the corresponding sulfoxide **5B**, of opposite configuration (1*S*, $[\alpha]_D$ + 270, $CHCl_3$) but of lower enantiopurity (56% ee), was isolated as the major metabolite (26% yield). The enantiocomplementary behaviour shown during dioxygenase-catalysed sulfoxidation of 2-methylbenzo(*b*]thiophene **5A**, is similar to that previously found during alkyl aryl sulfide sulfoxidations with TDO and NDO.[1]

No evidence of sulfoxidation was obtained when 3-methylbenzo[*b*]thiophene **6A** was metabolised by *P. putida* UV4, although traces of compound **6B** had previously been reported (GC–MS) among a mixture of metabolites from *P. aeruginosa*.[12] A further study using *P. putida* NCIMB 8859 did, however, yield an isolable quantity of 3-methylbenzo[*b*]-thiophene 1-oxide **6B**, (7% yield) in the presence of 3-hydroxymethylbenzo[*b*]thiophene (6% yield), and benzo[*b*]thiophene-3-carboxylic acid (5% yield). The sulfoxide metabolite **6B** ($[\alpha]_D$ − 181, $CHCl_3$), purified by PLC, was found to have an enantiomeric excess of 41% by ^{1}H NMR analysis in the presence of TAE. The CD spectra of sulfoxides (−)-**6B** and (−)-**5B** were very similar and were thus both assigned an *R* configuration (Table 1).

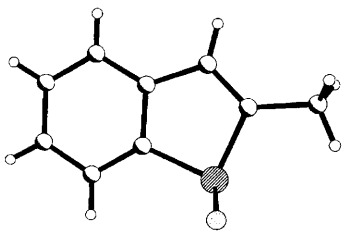


Fig. 1 X-Ray crystal structure of sulfoxide **5B**

The enzyme-catalysed synthesis of enantiomerically enriched samples of the chiral thiophene sulfoxides **5B**, **6B** and **4H** was of interest since it indicated that the barrier to racemisation was in each case >23 kcal mol^{-1}. The barrier to pyramidal inversion of the thiophene sulfoxide group obtained by ^{1}H NMR dynamic stereochemistry analysis of 2,5-di-1,1,3,3-tetramethylbutylthiophene 1-oxide ($\Delta G^{\ddagger}$ = 14.8 kcal mol^{-1}),[6] and by kinetic studies of the thermal racemisation of sulfoxides **5B** ($\Delta G^{\ddagger}$ = 25.1 kcal mol^{-1}) and **6B** ($\Delta G^{\ddagger}$ = 26.7 kcal mol^{-1}) at 50 °C in $CHCl_3$ solution, compare well with *ab initio* calculated values[10] for sulfoxides **1B** ($\Delta G^{\ddagger}$ = 11.2 kcal mol^{-1}) and **4B** ($\Delta G^{\ddagger}$ = 23.9 kcal mol^{-1}).

Two pathways involving thiophene sulfoxide bioproducts of type **B** are postulated: **A** → **B** → **C** → **E** → **G** (Scheme 1, path 1) and **A** → **B** → **D** → **F** → **H** (Scheme 1, path 2). Examples of each type of bioproduct except type **E** have been isolated and identified.

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Footnote

† *Crystal data* for **5B**: C_9H_8OS, M = 164.2, monoclinic, space group $P2_1$, a = 7.526(2), b = 11.199(3), c = 9.826(3) Å, β = 106.42(2)°, U = 794.4(4) Å^3, Z = 4, D_c = 1.373 Mg m^{-3}, μ(Cu-Kα) = 3.07 mm^{-1}, $F(000)$ = 344, light brown prisms, 0.5 × 0.5 × 0.6 mm; Siemens P3 diffractometer; 1637 independent data (and their Friedel opposites) were collected at 293 K in 2θ range 10–110°, $-8 \leq h \leq 8$, $0 \leq k \leq 11$, $0 \leq l \leq 10$; direct methods solution (SHELXS-86) and full-matrix least-squares refinement on F^2 (SHELXL-93), anisotropic temperature factors for non-hydrogens; hydrogens located but refined using riding model, $R1$ = 0.056, $wR2$ = 0.151 for 1612 data with $F_0 > 4\sigma(F_0)$, GOF = 1.01. The absolute configuration was established as (1*R*) from the Flack parameter 0.06(3). Refinement of the inverse structure gave Flack = 0.98(5). Atomic coordinates, bond lengths and bond angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See information for authors, Issue No. 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 182/224.

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