Enantioselective Oxidation of 1,3-Dithioacetals Catalysed by Cyclohexanone Monooxygenase

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Cyclohexanone monooxygenase catalysed oxidation of dithioacetals in combination with kinetic resolution gives enantiomerically pure (*R*)-monosulfoxides.

1,3-Dithiane-1-oxide derivatives serve as chiral acyl anion equivalents for the enantioselective synthesis of a wide range of products. For example, 2-acyl-2-alkyl-1,3-dithiane-1-oxides give highly diastereoselective enolate alkylations and aminations, Mannich reactions, organometallic additions, heterocyclic cycloadditions and so on.[1] *trans*-1,3-Dithianedioxide can be transformed into thioesters, which act as starting materials for the synthesis of esters, amines, ketones and aldehydes.[2] Furthermore, thioacetals, thioacetal sulfoxides and thioacetal sulfones are present in natural products,[3] although little information is available on the stability and transformation of these functional groups during metabolism.[4]

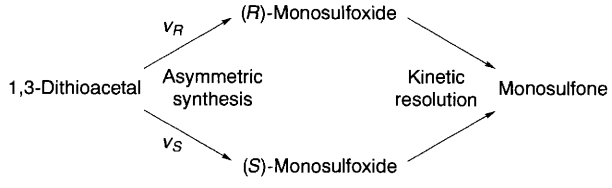
However, oxidation of unsubstituted 1,3-dithiane with the Kagan reagent,[5] or with isolated enzymes such as chloroperoxidase[6] occurs in low enantiomeric excess (20% e.e.). This prompted us to investigate the behaviour of cyclohexanone monooxygenase (CMO) from *Acinetobacter* NCIB 9871 as a potential enantioselective catalyst in the oxidation of 1,3-dithiane **1**, 1,3-dithiolane **2** and bis(methylthio)methane **3**. We were also interested in the structure of further oxidation products formed during this biotransformation.

Previously, we have shown that this enzyme can catalyse the asymmetric sulfoxidation of numerous alkyl aryl sulfides, dialkyl sulfides and dialkyl disulfides.[7] The structure of the sulfide dramatically influenced not only the enantioselectivity, but also the stereochemical course of the reaction, giving sulfoxides ranging from 99% e.e. with the (*R*)-configuration to 93% e.e. with the (*S*)-configuration. Similar results were obtained in the asymmetric oxidation of alkyl aryl sulfides with the alkyl chain functionalized with Cl, CN, vinyl or hydroxy groups.[8]

Yields and enantiomeric excesses of the CMO promoted sulfoxidation of the 1,3-dithioacetals **1–3** are reported in Table 1.

The oxidation of 1,3-dithiane by CMO, which gives the (*R*)-monosulfoxide in 81% chemical yield and ≥98% e.e., compares favourably with chemical and biochemical processes in terms of enantioselectivity. Indeed, Kagan obtained (*R*)-1,3-dithiane monosulfoxide in 20% e.e.[5] The oxidation of 1,3-dithiane to the corresponding monosulfoxide in the presence of growing cultures of *Aspergillus foetidus, Helminthosporium* species and *Mortierella isabellina* occurred in 17–24, 13–15 and 0% e.e., respectively.[4] Interestingly, the prevailing enantiomers with the first two fungi have different absolute configuration.[4]

With racemic 1,3-dithiane monosulfoxide the time course of the reaction with cyclohexanone monooxygenase was monitored by GC (conversion) and HPLC (e.e.). The (*S*)-enantiomer was oxidized to the corresponding monosulfone faster than the (*R*)-enantiomer, the *E* value being 20 (the enantiomeric ratio, *E*, was calculated according to Chen *et al.*[11]). As a consequence, the asymmetric synthesis [v_R/v_S = 12, *i.e.* the ratio of the rates of formation of (*R*) and (*S*)-sulfoxides, determined at low degrees of conversion by chiral HPLC] accompanied by kinetic resolution (see Scheme 1) led to enantiomerically pure (*R*)-1,3-dithiane monosulfoxide. The K_M and k_{cat} for **1** and the corresponding racemic sulfoxides are shown in Table 2.



Scheme 1 Pathway of the CMO catalysed oxidation of 1,3-dithioacetals to monosulfoxides and monosulfones

Table 1 Cyclohexanone monooxygenase catalysed oxidation of 1,3-dithioacetals to monosulfoxides and monosulfones[a]

1,3-Dithioacetal[b]	Monosulfoxide			Monosulfone yield (%)
	Yield (%)	E.e.[c] (%)		
1,3-Dithiane **1**	81	≥98		19
1,3-Dithiolane **2**	94	≥98		6
bis(Methylthio)methane **3**	92	≥98		8

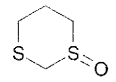
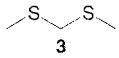
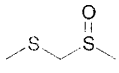
[a] The dithioacetal (17 mmol dm^{-3}) was reacted, at 25 °C, under stirring, in 5 ml of 50 mmol dm^{-3} Tris-HCl buffer, pH 8.6, containing NADPH (1 mmol dm^{-3}), glucose-6-phosphate (50 mmol dm^{-3}), 5 units of CMO (purified as described by Latham and Walsh[9]) and 50 units of glucose-6-phosphate dehydrogenase (glucose-6-phosphate and glucose-6-phosphate dehydrogenase served to regenerate NADPH[7]). After 16 h, the reaction mixture was freeze-dried and the residue extracted with Pr^iOH. The organic extract was dried and analysed by GC (conversion) and chiral HPLC or GC (e.e.). [b] The dithioacetals **1** and **3** were commercial products, and **2** was prepared as previously described.[10] [c] Determined by chiral HPLC on a Chiralcel OD column, using the proper mixture of *n*-hexane–Pr^iOH as the mobile phase, for **1** and **3**, and by chiral GC with a CP-cyclodextrin-β-2,3,6-M19 column, for **2**. All sulfoxide enantiomers were base-line separated. By comparison with specimens of known configuration, the absolute configuration of all the produced dithioacetal monosulfoxides was (*R*).

The lower value of K_M and the higher value of k_{cat} for 1,3-dithiane **1** are in line with the preference of the enzyme towards the dithiane **1** with respect to the corresponding monosulfoxide. This is confirmed by the fact that 1,3-dithiane monosulfone started to form only after the almost complete consumption of substrate **1**. It has to be pointed out that the further oxidation of 1,3-dithiane monosulfoxide by CMO led to the corresponding monosulfone whereas with the Kagan reagent the *trans*-1,3-dithiane disulfoxide was formed.[12] With acyclic 1,3-dithioacetals, disulfoxide formation was observed with *Aspergillus niger*,[13] whereas the bacterium *Corynebacterium equi* gave monosulfone, monosulfoxide monosulfone and disulfone.[14]

The CMO promoted oxidation of 1,3-dithiolane afforded the corresponding enantiomerically pure (*R*)-monosulfoxide with 94% chemical yield (Table 1). The high optical purity of the product is to be ascribed mainly to the asymmetric oxidation since the v_R/v_S value was 49. 2-Substituted-1,3-dithiolanes are oxidized with substantial enantioselectivity (70–80% e.e.) with Bu^{t}OOH, diethyl tartrate and Ti(OPr^i)$_4$.[15] Mono- and disulfoxide products were obtained after the addition of 1,3-dithiolane **2** to growing cultures of *Aspergillus foetidus*, *Mortierella isabellina* and a *Helminthosporium* species.[16] The highest e.e. observed in the formation of (*R*)-1,3-dithiolane monosulfoxide was 65%.[16] Very recently, high enantioselectivity was observed in bacterial dioxygenase catalysed oxidation of 2-methylbenzodithiole.[17]

The behaviour of bis(methylthio)methane **3** in the CMO promoted sulfoxidation was very similar to that already discussed for 1,3-dithiane. As shown in Table 1, the (*R*)-monosulfoxide was obtained in 92% chemical yield and ≥98% e.e. Also, in this case our method gave better results compared with Kagan's oxidation 40% e.e.[18] Under the usual reaction conditions the (*S*)-sulfoxide was oxidized faster than the (*R*)-enantiomer to the corresponding sulfone. Therefore, also in this case, the asymmetric synthesis (v_R/v_S = 39), together with the contribution of the kinetic resolution (the *E* value was 12), yielded enantiomerically pure (*R*)-methyl(methylthio) methylsulfoxide. As already seen for 1,3-dithiane **1**, the kinetic parameters were more favourable for the dithioacetal **3** than for the corresponding racemic sulfoxide (Table 2).

Table 2 Kinetic parameters for the cyclohexanone monooxygenase catalysed oxidation of dithioacetals and racemic dithioacetal monosulfoxides[a]

Substrate	K_M/μmol dm^{-3}	k_{cat}/min^{-1}
 **1**	33	450
	110	58
 **2**	41	309
 **3**	76	588
	1300	190

[a] The kinetic experiments were carried out in 0.05 mol dm^{-3} Tris-HCl buffer, pH 8.6 at 25 °C, in 1 ml cuvettes, 1 cm path length. The reaction mixture contained CMO (5–30 milliunits), 100 μmol dm^{-3} NADPH and 5–800 μmol dm^{-3} substrate. The consumption of NADPH was spectrophotometrically monitored at 340 nm. The K_M and k_{cat} values were obtained from the initial rate measurements using ENZFITTER.

In conclusion, we have shown that cyclohexanone monooxygenase from *Acinetobacter* is the catalyst of choice in the chemical and biochemical repertory for the enantioselective monosulfoxidation of the three types of 1,3-dithioacetal model compounds examined.

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