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The Enantioselective Oxidation of Sulfides to Sulfoxides with *Acinetobacter* sp. NCIMB 9871, *Pseudomonas* sp. NCIMB 9872, *Xanthobacter autotrophicus* DSM 431 (NCIMB 10811) and the Black Yeast NV-2

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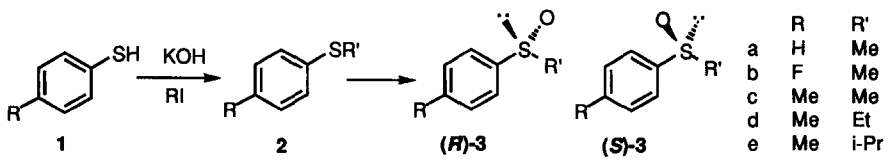
Abstract: Whole cell oxidation of aryl alkyl sulfides to sulfoxides with *Acinetobacter* sp. NCIMB 9871 is only slightly less enantioselective than isolated enzyme transformation with the cyclohexanone monooxygenase (CHMO) from the same species. *Pseudomonas* sp. NCIMB 9872 oxidises the same substrates with high and mostly opposite enantioselectivity (73-100%ee). CHMO activity was detected in the black yeast NV-2 and *Xanthobacter autotrophicus* DSM 431 (NCIMB 10811), but contrary to an earlier report this activity could not be detected in cell free extracts of the latter. Both species oxidised methyl phenyl sulfide exclusively to the corresponding (*R*)-sulfoxide (100% ee).

Flavin monoxygenases act essentially as a source of "enantioselective hydrogen peroxide". They mediate Baeyer-Villiger reactions, the oxidation of sulfides, amines and phosphines to the corresponding oxides and sundry other reactions typical of hydroperoxides[1]. The flavin monooxygenase which has been most widely investigated for synthetic purposes is cyclohexanone monooxygenase (CHMO) from *Acinetobacter* sp. NCIMB 9871. It has a single active site[2] and is an excellent catalyst for both Baeyer-Villiger reactions[3] and the oxidation of sulfides to sulfoxides, however the enantioselectivity of the latter reaction is highly substrate dependant.



	R	R'
a	H	Me
b	F	Me
c	Me	Me
d	Me	Et
e	Me	i-Pr

Most flavin monooxygenases investigated thus far utilise NADPH as a reductant[4]. This is less easily regenerated than NADH, which is a limitation on the maximum scale attainable. There is a need for new systems for the enantioselective oxidation[5] of sulfides which can be run on a large scale (preferably using whole cells), which utilise NADH as a reductant and which are enantiocomplementary to CHMO[6]. This *letter* describes our work towards these goals.

There have been several studies on the oxidation of sulfides by purified CHMO[7], but there is only one brief study with whole cells[8]. The isolated enzyme (Table 1, Column 2) is only marginally more enantioselective than whole cells (Table 1, column 4), except for the *p*-fluoro derivative **2b** and in all cases the yields were >95%. Cyclopentanone monooxygenase from *Pseudomonas* sp. NCIMB 9872[9] is a trimer (200KDa) of identical subunits comparable in size with cyclohexanone monooxygenase (59KDa). Like CHMO it is an FAD linked flavoenzyme with NADPH as a reductant[10]. Whole cell biotransformations with NCIMB 9872 were substantially more enantioselective (Table 1, Column 5) than CHMO. Moreover the oxidation of methyl phenyl sulfide **2a** by NCIMB 9872 was enantiocomplementary to that by CHMO, whereas ethyl *p*-tolyl sulfide **2d** was oxidised to the same sulphoxide (*S*)-**3d**.

Table 1 Enantiomeric excesses of sulfoxides **3** produced by oxidation of alkyl aryl sulfides **2** by *Acinetobacter* sp. NCIMB 9871 and *Pseudomonas* sp. NCIMB 9872

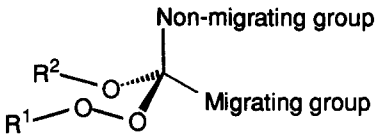
Substrate	*Acinetobacter* sp. NCIMB 9871 (IE)	*Acinetobacter* sp. NCIMB 9871 (WC)		*Pseudomonas* sp. NCIMB 9872 (WC)
Methyl phenyl sulfide **2a**	99 (*R*)[a]	85 (*R*)[b]	100 (*R*)	100 (*S*)
Methyl *p*-fluorophenyl sulfide **2b**	92 (*R*)	-	40 (*R*)	83 (*S*)
Methyl *p*-tolyl sulfide **2c**	37 (*S*)	26(*S*)[b]	34 (*S*)	84 (*S*)
Ethyl *p*-tolyl sulfide **2d**	89 (*S*)	-	80 (*S*)	100 (*S*)
i-Propyl *p*-tolyl sulfide **2e**	86 (*S*)	47(*S*)[b]	58 (*S*)	73 (*R*)

IE - isolated enzyme biotransformation results taken from reference 14.
WC - washed whole cell catalysed biotransformations.
a - We have repeated this transformation and obtained 100% ee (GC-FID, Lipodex D)
b - Results taken from reference 8

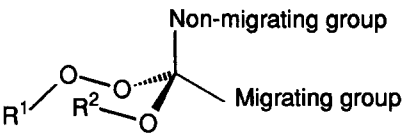
The biotransformation of methyl phenyl sulfide **2a** with two novel organisms was also investigated. *Xanthobacter autotrophicus* DSM 431[11] is reported to contain an NADH dependant CHMO[12]. Whole cells are able to utilise cyclohexanol as the sole carbon source and express Baeyer-Villiger activity with cyclohexanone and bicyclo[3.2.0]hept-2-en-6-one[11]. We were unable to demonstrate this activity in cell free systems using various cell disruption techniques, phosphate and non-phosphate buffers from pH 4-10, metals ions, trace elements or artificial electron donor systems; using ascorbate linked to a range of standard mediators (FMN, FAD, methyl viologen etc.). Addition of cyclohexanone and NADH to cell free systems resulted in consumption of NADH and the formation of cyclohexanol, but no ε-caprolactone was formed. The rate of consumption of NADH was comparable to that reported previously, consequently this reduction rather than CHMO activity may be the explanation for the data in the previous report[12]. Whole cells of *X. autotrophicus* oxidised methyl phenyl sulfide **2a** to the (*R*)-sulfoxide **3a** with 100% ee in 75% conversion. The corresponding sulfone could not be detected and the remainder of the material was accounted for by starting material **2a**.

NV-2 is an uncharacterised black dimorphic yeast isolated by screening on media containing cyclohexanol. It forms mycelia on agar plates, but grows as a yeast in liquid culture. It grows well on M9 medium supplemented with cyclohexanol which it is able to utilise as sole carbon source. However it is not able to utilise cyclohexanone or ε-caprolactone as sole carbon sources. Cell free extracts of NV-2, consume O_2, cyclohexanone and NADPH in the ratio 1:1:1 to give ε-caprolactone, which is consistent with the presence of an NADPH linked monooxygenase. Whole cells oxidised methyl phenyl sulfide to the corresponding (*R*)-sulfoxide **3a** with 100% ee (>98% yield by GC).

We have recently rationalised the stereoselectivity of the flavin monooxygenase catalysed Baeyer-Villiger ring expansion of ketones using an active site model in which the three oxygens of the Criegee intermediate (hydroxyperoxides or analogous structures) are in fixed positions relative to each other. Application of this analysis to the enzymes described above, classifies **all of them** as capable of stabilising Criegee intermediates with an "*S*-migration configuration", constrained to result in a *si*-face migration **(a)**. It might be anticipated that this stereoselectivity would correlate with that of sulfide oxidation. All of the oxidations of methyl phenyl sulfide **2a** give exclusively the (*R*)-sulfoxide **3a**, except for *Pseudomonas* sp. NCIMB 9872 which gives exclusively the (*S*)-sulfoxide **3a**. Similarly the oxidation of *i*-propyl *p*-tolyl sulfide **2e** gives enantiocomplementary results with NCIMB 9871 and 9872. Therefore it is apparent that the active site features that control the stereoselectivity of the Baeyer-Villiger oxidation of ketones are not applicable to the oxidation of sulfides[7].



(a) *Acinetobacter* sp. NCIMB 9871, *P. putida* NCIMB 9872, NV-2 *X. autotrophicus* DSM 431,



(b) *P. putida* NCIMB 10007 (MO1)

EXPERIMENTAL

Synthesis: Sulfides were prepared by alkylation of the requisite potassium thiophenolate with an alkyl iodide according to the method of Ipatieff *et al*[13]. Oxidation of the sulfides with hydrogen peroxide in acetic acid gave racemic sulfoxide standards. **2a** was purchased from Aldrich, yields for the other materials were as follows **3a** 84%, **2b** 75%, **3b** 86%, **2c** purchased from Aldrich, **3c** 84%, **2d** 81%, **3d** 86%, **2e** 78%, **3e** 92%. All materials were characterised by GC-MS and ^{1}H NMR spectroscopy.

Analysis: Methyl phenyl sulfoxide was resolved by GC-FID on a Machery-Nagel Lipodex D column (50m, 0.25mm id, injector and detector 250°C, oven 140°C isothermal, He carrier gas at 1.35 bar, split ratio 50:1). Retention times (mins): **2** 4.8, **(*R*)-3a** 35.18, **(*S*)-3a** 36.02. All other sulfoxides were resolved by HPLC using a 25cm Chiralcel OB column, eluted isocratically with hexane:propan-2-ol solvent mixtures and a 254nm UV detector. Enantiomerically pure samples of (*R*) and (*S*)-methyl *p*-tolyl sulfoxide **3c** were purchased from Aldrich. All assignments were made using the data in reference[14].

Table 2 HPLC retention times of analytes in minutes

Analytes	2	(*S*)-3	(*R*)-3	Ratio hexane : propan-2-ol	Flow rate (ml/min)
b	1.55	5.21	7.73	95:5	3
c	1.68	6.34	15.21	95:5	2
d	1.55	4.59	11.44	95:5	3
e	1.50	4.18	7.30	90:10	3

Microorganisms: *Acinetobacter* sp. NCIMB 9871, *X. autotrophicus* DSM431 and NV-2 were cultivated as described in reference 11. *Pseudomonas* sp. NCIMB 9872 was cultivated in the same way except that the carbon source was cyclopentanol at 2g/L.

Biotransformations: The following conditions were used for all the micro-organisms. Each isolate was grown until it had reached the late exponential phase of growth (6-8hrs). The cells were harvested, washed twice with phosphate buffer (50mM, KH_2PO_4/Na_2HPO_4, pH 7.1), separated by centrifugation at 20,000g for 10 minutes and suspended in phosphate buffer (25ml). The sulfide was added to a 250ml Erlenmeyer flask containing the cell suspension such that the final concentration was 10mM. The flask was sealed with a serum cap and incubated on a gyratory shaker at 200rpm, 30°C for 16-18hrs. Aliquots were analysed by GC or HPLC. The reaction mixture was extracted with ethyl acetate and the yield determined by GC or HPLC against standards. Biotransformations with *Acinetobacter* sp. NCIMB 9871, uniformly gave yields of circa 95% with no sulfide **2** or sulfone detected. The yields for *Pseudomonas* sp. NCIMB 9872 were **3a** >98%; **2b** 4%, **3b** 90%; **2c** 5%, **3c** 95%; **2d** 5%, **3d** 94%; **2e** 3%, **3e** 91%.

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