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Stereoselective Enzyme-catalysed Oxidation–Reduction Reactions of Thioacetals–Thioacetal Sulphoxides by Fungi

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Enzymes present in the fungus *Mortierella isabellina* catalyse the transfer of an oxygen atom *to* the cyclic thioaceta 1,3-dithian and *from* 1,3-dithian 1-oxide, 1,3,5-trithian 1-oxide, and *cis*-1,3-dithian 1,3-dioxide. The oxidation of 1,3-dithian and the acyclic thioacetal bis-(*p*-tolylthio)methane to form the corresponding monosulphoxides occurred in the presence of growing cultures of *Aspergillus foetidus* and a *Helminthosporium* species. The degree and preferred direction of stereoselectivity occurring during the asymmetric oxidation and reduction steps was deduced from the enantiomeric excess (e.e.) and absolute stereochemistry of the isolated 1,3-dithian 1-oxide and *p*-tolylthio-(*p*-tolylsulphinyl)methane.

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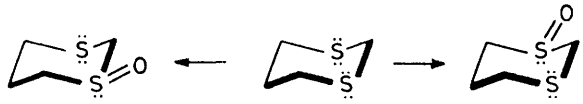
THIOACETAL derivatives of carbonyl compounds serve as protecting groups and also as a method of rendering the carbonyl group susceptible to both electrophilic and nucleophilic attack (umpolung [1]). Current interest in the potential of optically active thioacetal monosulphoxide groups as chiral acyl anion equivalents,[2,3] allied to a paucity of information concerning the stability or fate of thioacetal groups during metabolism has prompted the present report. This study also forms part of a programme from these laboratories concerned with the mechanism and stereochemistry of enzyme-catalysed oxygen atom-transfer processes in xenobiotic metabolism (in animal,[4] bacterial,[5] and fungal [6] systems).

Thioether substrates have previously been transformed into sulphoxide [6-8] and sulphone products [6,7,9] by fungi. While this microbial oxidation process is very common, the reverse (deoxygenation) step has rarely been observed.[10]

Three fungi which had previously been used to produce optically pure sulphoxides from the corresponding thioethers were again utilized in the present study [*Mortierella isabellina* NRRL 1757,[8] *Aspergillus foetidus* NRRL 337 (also described as *Aspergillus niger* NRRL 337 [6,7,9]), and a *Helminthosporium* species NRRL 4671 [8]].

Microbial metabolism of 1,3-dithian (1) using a shake culture method with each of the three fungi gave the thioacetal monosulphoxide (2) in moderate yield (12—52%) (Table 1). The optical yields and absolute configurations of the isolated sulphoxide (2) were determined by comparison with the results recently obtained [2] from the chemical resolution of (2). While the preferred conformation of (2) (chair form with the oxygen atom



equatorial) is indicated in the formula it is established that this conformer is in a rapid state of equilibration with the alternative form having an axial oxygen atom (84% equatorial : 16% axial at equilibrium).[11] The optical purities of the isolated metabolite (2) were relatively low (<25%) in comparison with alkyl–aryl thioether oxidations by these fungi.[6-9] Thus a preference for the (+)-(*R*)-enantiomer [17—22% enantiomeric excess (e.e.) from *A. foetidus*], for the (−)-(*S*)-enantiomer (13—15% e.e. from *Helminthosporium*) and a totally racemic sample of (2) (0% e.e. from *M. isabellina*) were obtained. The maximum observed optical yield (22%) was similar to that previously found from microbial oxidation of an acyclic dialkyl thioether (26% optically pure n-butyl methyl sulphoxide from n-butyl methyl thioether and *A. foetidus* [6]). This lower degree of stereoselectivity for dialkyl thioethers and thioacetals

TABLE 1

Peracid [a] and enzyme-catalysed asymmetric oxidation of (1) and (3)

Substrate	Oxidant	Yield of thioacetal recovered (%)	Yield of monosulphoxide (%)	$[\alpha]_{589}$ (°) (EtOH)	Optical yield (%)	Absolute configuration
(1)	O_2-*M. isabellina*	29, 34	16, 38	0, 0	0, 0	*R*/*S*
(1)	O_2-*Helminthosporium*	8, 5	12,27	−35, −29	15, 13	*S*
(1)	O_2-*A. foetidus*	32, 19, 42	49, 23, 52	+45, +51, +39	20, 22, 17	*R*
(1)	(+)-PCA	*b*	>90	+0.9	0.4	*R*
(3)	O_2-*M. isabellina*	*b*	*b*			
(3)	O_2-*Helminthosporium*	*b*	3	−15 [c]	20	*S*
(3)	O_2-*A. foetidus*	*b*	2	0 [c]	0	*R*/*S*
(3)	(+)-PCA	*b*	>90	+2 [c]	3	*R*

[a] (+)-Peroxycamphoric acid (PCA) in $CHCl_3$ solution at −5 °C. [b] No product could be detected. [c] In acetone solvent.

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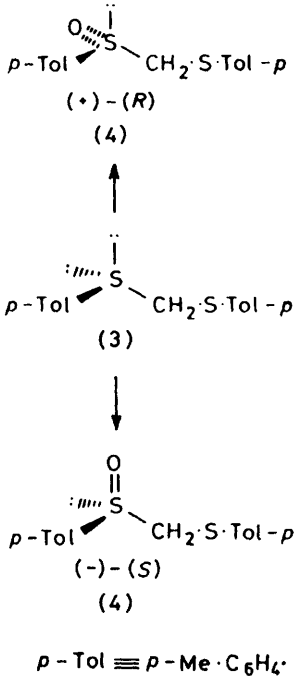
may be partially accounted for by the close similarity in size and nature of substituents attached to each sulphur atom and by attack from either above or below the molecular plane of (1) (on alternate sulphur atoms) to yield the same optical isomer. These factors are probably also reflected in the very low optical yield (0.4%) obtained by the chiral oxidizing agent (+)-peroxycamphoric acid (PCA).

When the acyclic thioacetal, bis-(*p*-tolylthio)methane (3), was added to growing cultures of *M. isabellina*, the normal extraction process (CH_2Cl_2) yielded neither starting material nor derived products. Similarly with *Helminthosporium* and *A. foetidus* no thioacetal could be recovered from the medium, but the monosulphoxide (4) was isolated in very low yield (2—3%). No stereoselectivity appears to have occurred in the formation of *p*-tolylthio-(*p*-tolylsulphinyl)methane (4) by oxidation of (3) in *A. foetidus*, while an excess of the (−)-(*S*)-enantiomer (20% e.e.) was obtained using *Helminthosporium*. This selectivity for the production of the (*S*)-enantiomer of (4) parallels the exclusive formation of the analogous (*S*)-enantiomer of methyl *p*-tolyl sulphoxide by this micro-organism, as observed by Sih and his co-workers.[8] As anticipated from the differing

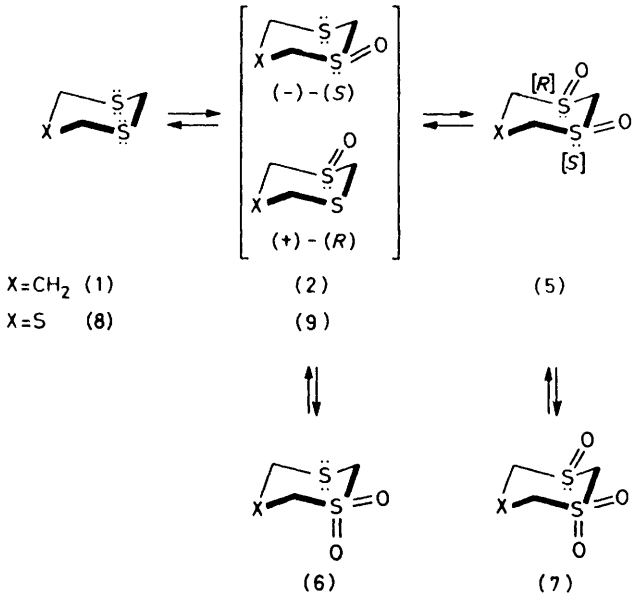


p-Tol ≡ p-Me·C6H4·

size and nature of substituents on each side of the sulphur atoms in (3), the optical yield of (4) obtained by (+)-PCA oxidation was proportionately much higher than for (2) under the same oxidation conditions.

The inability to recover any of the substrate (3), or only a very low yield (<5%) of the sulphoxide product (4) under the conditions required for microbial growth, suggested that both (3) and (4) were being hydrolysed and transformed into water-soluble products. This contrasts with the results obtained using substrate (1) for the three fungi, where the combined yields of (1) and (2) were in the range (20—94%). It was indeed surprising to find that in some cases relatively high combined recoveries of (1) and (2) could be obtained by dichloromethane extraction since the 1,3-dithian protecting group is reported to be readily hydrolysed (particularly after oxidation to a sulphoxide derivative).[1]

In order to determine the fate of the racemic sulphoxide (2) and the possible contribution of enzyme-catalysed enantioselectivity in the formation of optically active (2), relevant experiments (Table 2) were carried out.



When the racemic sulphoxide (2) was used as substrate, asymmetric destruction of one enantiomer was evident. Thus with *A. foetidus* and *Helminthosporium* cultures the recovered 1,3-dithian 1-oxide (2) showed an excess of the (+)-(*R*)- (10—13% e.e.) and (−)-(*S*)- (1—10% e.e.) enantiomers respectively. The same dominant enantiomer is obtained either from direct oxidation of (1) or from enantioselective removal from (2). At this stage it is not possible to identify unequivocally the metabolic pathway responsible for enantioselectivity since only the sulphoxide (2) could be recovered from the incubation medium. It is probable however, that the bis-sulphoxide (5) is formed but that it is rapidly hydrolysed and metabolised to water-soluble products. Thus when substrate (5) was added to cultures of *A. foetidus* or *Helminosporium* no derived products (nor starting material) could be extracted by dichloromethane.

An investigation of the degree of enantioselectivity found during metabolism of (2) by *M. isabellina* unexpectedly showed that the enzyme-catalysed removal of an oxygen atom from the sulphoxide group was a particularly favoured process. Thus when the mono- (2) or bis- (5) sulphoxides were substrates, enzymes present in *M. isabellina* produced (1) in yields of up to

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37% and 52% respectively. The enzyme-catalysed (sulphoxide reductase) abstraction of an oxygen atom from (5) was sufficiently fast to compete successfully with breakdown of the substrate. The bis-sulphoxide (5) appeared to be totally hydrolysed in the absence of microbial growth. Thus when added to cultures of *M. isabellina* which had been autoclaved no substrate could be recovered after shaking and dichloromethane extraction. Furthermore, evaporation of the extracted aqueous medium to dryness yielded no trace of (5). Enantioselective metabolism of (2) in the fungus *M. isabellina* resulted in an enrichment (1—16% e.e.) of the (+)-(*R*)-enantiomer, *i.e.* preferential removal of the (−)-(*S*)-form. The variation of optical yield in the results given in Table 2 is typical for enantioselective reactions where the microbial transformations may have been terminated at varying stages.

TABLE 2

Fungal metabolism of sulphoxides (2), (5), and (9)

Sulphoxide substrate	Oxidant	Yield of recovered sulphoxide (%)	Yield of recovered thioacetal (%)	$[\alpha]_{589}$ (°) (EtOH) of (2)	% Optical yield of (2)	Absolute configuration
(2)	O_2-*M. isabellina*	20, 8, 35 [a]	15, 27, 37 [b]	+37, +2, +36	16, 1, 16	*R*
(2)	O_2-*Helminthosporium*	24, 17, 12 [a]	*c*	−12, −1, −23	5, 0.5, 10	*S*
(2)	O_2-*A. foetidus*	53, 57 [a]	*c*	+30, +24	13, 10	*R*
(5)	O_2-*M. isabellina*	2, 7, 14 [a,d]	7, 40, 52 [b]	−21, −28, −5	9, 12, 2	*S*
(9)	O_2-*M. isabellina*	14 [e]	40 [f]			

[a] Monosulphoxide (2). [b] Thioacetal (1). [c] Thioacetal (1) undetected. [d] Bis-sulphoxide substrate (3) not recovered. [e] Monosulphoxide (9). [f] Thioacetal (8).

The recovery of the optically active monosulphoxide (2) from metabolic transformation of (5) in *M. isabellina* is noteworthy since a preference is found for the biosynthesis of the (−)-(*S*)-enantiomer of (2) (2—12% e.e.). This observation can most easily be rationalized in terms of a novel enantioselective reduction of the (*R*)-chiral centre in (5). The degree of stereoselectivity for either the (*R*)- or (*S*)-centre in (5) cannot be deduced directly from the observed $[\alpha]_D$ values for isolated samples of (2) in view of the further enantioselective reduction of the (−)-(*S*) form of (2) to (1). It appears probable however, that the selectivity for the (*R*)-centre during enzyme-catalysed reduction of (5) will be $\geqslant 12\%$.

The formation of the bis-sulphoxide (5) (or the corresponding *trans*-isomer) from metabolism of (1) or (2) in *M. isabellina* cannot be excluded. It is highly improbable however, that 1,3-dithian 1,1-dioxide (6) or 1,3-dithian 1,1,3-trioxide (7) are either formed or deoxygenated in *M. isabellina* since both appeared to be stable and were recoverable from the culture medium in moderate yield (35—51 and 36% respectively).

The facile deoxygenation reaction which occurred in *M. isabellina* on substrates (2) and (5) was also found to occur with 1,3,5-trithian 1-oxide (9) in good yield (40%). Since monosulphoxide (9) is achiral, no information about the stereochemistry of this reduction can be deduced. Surprisingly when substrate (8) was added to *M. isabellina* only the starting material could be recovered (70% yield). This latter result might be interpreted in terms of a very slow rate of oxidation of (8) followed by an extremely fast rate of reduction of (9).

Enzyme-catalysed deoxygenation reactions appear to be much less common than the reverse oxygen-atom transfer process according to the number of reports in the literature. Thus only a few isolated examples of amine oxide ⟶ amine,[12,13] epoxide ⟶ olefin,[14] arene oxide ⟶ arene,[15] and sulphoxide ⟶ sulphide [10,16] enzyme-catalysed deoxygenations in animal systems have been reported. The enzyme-catalysed removal of two oxygen atoms from a substrate molecule such as reported herein [(5) ⟶ (1)] appears to be without precedent. The reversibility of oxygen-atom transfer in metabolism may ultimately turn out to be a relatively common (although in many cases hidden) transformation. Studies of further aspects of the present investigations of reversibility in the microbial oxidation of organo-sulphur compounds are currently in progress in these laboratories.

EXPERIMENTAL

M.p.s were measured on a Reichert Kofler block. Optical rotations were determined at ambient temperature using a Perkin-Elmer automatic polarimeter (model 141). Sample concentrations of 10 mg ml^{-1} in ethanol or acetone were used for $[\alpha]$ measurements at 589 nm. Analytical t.l.c. was carried out on Merck Kieselgel $60F_{254}$ plates containing a fluorescent indicator. Plates were developed using ethanol–ether (1 : 1, v/v) as eluant and spots were located using u.v. light (Hanovia Chromatolite lamp) or iodine methods. R_F Values for compounds (1)—(9) are recorded in Table 3. I.r. spectra were recorded on a Perkin-Elmer double-beam grating spectrophotometer (model 157G). The sulphoxides (2), (4), (5), and (7) showed strong SO absorptions in the region 1 033—1 056 cm^{-1} while the SO_2 group of the sulphones (6) and (7) absorbed at 1 275—1 320 and 1 110 cm^{-1}. Mass spectra were recorded on an AEI MS902 instrument operating at 70 eV and showed molecular ions and fragmentation patterns consistent with structures (1)—(9). N.m.r. spectra were recorded at 60 and 90 MHz using JEOL JNM-PMX 60 and Bruker WH-90 spectrometers, respectively, for solutions in $CDCl_3$ with tetramethylsilane as internal reference. The n.m.r. spectra were consistent with the structures assigned [(1)—(9)] and with those previously reported.

Compounds (1) and (8) were obtained from Aldrich Chemical Co. Ltd. Compounds (2)—(7) and (9) were synthesized by reported methods and were purified by column chromatography and recrystallization. The

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physical properties and R_F values are given in Table 3. The strains of fungi used in the present work were *Aspergillus foetidus* NRRL 337, *Mortierella isabellina* NRRL 1757, and *Helminthosporium* sp. NRRL 4671. These were obtained from the Northern Regional Research Centre, Peoria, Illinois, U.S.A. A slope of *Helminthosporium sp.* NRRL 4671 was also kindly provided by Professor Sih. All fungi were maintained on malt agar slopes.

The microbial transformations were carried out using a shake culture technique. Mycelium from four slopes was transferred to four Erlenmeyer flasks (500 ml) containing

TABLE 3

Physical properties of thioacetals and derived racemic oxidation products

Compound	M.p. (°C) Found	M.p. (°C) Lit.	R_F
(1)	53—55	53—55 [a]	0.7 [b]
(2)	86—88	88—89.5 [c]	0.4 [b]
(3)	30—32	32 [d]	0.7 [e]
(4)	53—55	63—64 [d,f]	0.2 [e]
(5)	212—216 (decomp.)	215—216 (decomp.) [g]	0.1 [b]
(6)	137—140	139—140 [g]	0.5 [b]
(7)	198—199	*h*	0.2 [b]
(8)	216—218	216—218 [i]	0.6 [b]
(9)	184—186	186—187 [g]	0.4 [b]

[a] W. Autenreith and K. Wolff, *Ber.*, 1899, **32**, 1376. [b] Eluted with ethanol–ether (1 : 1, v/v). [c] F. A. Carey, O. D. Dailey, and O. Hernandez, *J. Org. Chem.*, 1976, **41**, 3979. Optically pure (2) has m.p. 108—109 °C, $[\alpha]_{589}$ +224° (in EtOH) (ref. 2). [d] M. Cinquini and S. Colonna, *Int. J. Sulfur Chem.*, 1976, **8**, 603. [e] Eluted with light petroleum (b.p. 40—60 °C)–ether (1 : 1, v/v). [f] Optically pure sample, $[\alpha]_{589}$ +77° (in acetone). [g] S. A. Khan, J. B. Lambert, O. Hernandez, and F. A. Carey, *J. Am. Chem. Soc.*, 1975, **97**, 1468. [h] Found: C, 28.6; H, 4.7. $C_4H_8O_3S_2$ requires C, 28.5; H, 4.8%. [i] E. Baumann, *Ber.*, 1890, **23**, 67.

Czapek Dox liquid medium (100 ml) and corn steep liquor (2 ml; Dista Products Ltd.). The mycelium in these intermediate flasks was grown for 2 days at 30 °C. Fifty Erlenmeyer flasks (1000 ml) each containing Czapek Dox liquid medium (100 ml) were inoculated with the mycelium grown in the intermediate flasks and were incubated at 30 °C for 48 h on a platform shaker (180 r.p.m.). The substrate (30 mg in 1 ml of ethanol) was injected into each flask and incubation was continued for 48 h at 30 °C. The aqueous culture medium was saturated with salt solution before being continuously extracted with dichloromethane (6 days). The concentrated and dried ($MgSO_4$) extracts were purified by column chromatography using silica gel (Crasfield sorbsil M60) as adsorbent and mixtures of light petroleum (b.p. 40—60°), ether, and methanol as eluants. The order of elution from the column was in accordance with the R_F values shown in Table 3.

Sulphoxide products were further purified by the formation of crystalline mercuric chloride adducts from aqueous ethanol and regenerated by reaction with NaOH in aqueous ethanol.

(+)-Peroxycamphoric acid used in asymmetric oxidation was prepared as reported.[17] Oxidation of the thioacetals (1) and (5) was carried out using equimolar quantities at −5 °C in $CHCl_3$ solution. Sulphoxide products from (+)-PCA oxidation were purified in a similar manner to the oxidation products of microbial metabolism.

The organosulphur compounds isolated from the microbial experiments had identical spectral characteristics to the authentic samples.

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