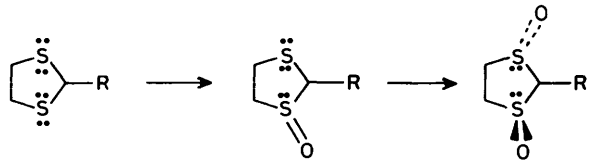
Stereoselectivity during Fungal Sulphoxidations of 1,3-Dithiolanes

Barbara J. Auret, Derek R. Boyd,* and Robert Dunlop
Department of Chemistry, The Queen's University of Belfast, Belfast BT9 5AG
Alex F. Drake*
Department of Chemistry, Birkbeck College, London WC1 H0AJ

Mono- and di-sulphoxide products were obtained after addition of 1,3-dithiolane (**1**), 2-methyl-1,3-dithiolane (**4**), and 2-t-butyl-1,3-dithiolane (**7**) as substrates to growing cultures of *Aspergillus foetidus*, *Mortierella isabellina*, and a *Helminthosporium* species. Enzyme-catalysed stereodifferentiation between prochiral lone pairs on a sulphur atom gave optically active 1,3-dithiolane 1-oxide (**2**) (10—65% e.e.). The derived optically pure *trans*-1,3-dithiolane-1,3-dioxide (**3**) was obtained by fractional recrystallization. A stereopreference for the *pro-R* sulphur atom (61 and 66%) was observed during microbial oxidation of 2-methyl-1,3-dithiolane (**4**) and 2-t-butyl-1,3-dithiolane (**7**) respectively. The absolute stereochemistry of the dextrorotatory sulphoxide metabolites (**2**), *t*-(**5**), *t*-(**8**), and (**3**) was assigned the *R* configuration at the chiral sulphur atoms from c.d. spectroscopy.

The ability of mono-oxygenase enzymes to discriminate between prochiral lone pairs or faces during sulphoxidation reactions is well documented.[1] Recent studies[2] have also indicated that fungal enzymes can show a combination of enzyme specificity[3] by their ability to differentiate between prochiral (diastereotopic) lone pairs on a sulphur atom, and prochiral (enantiotopic) sulphur atoms during sulphoxidation of 2-substituted 1,3-dithianes. Unfortunately, the stereopreference found for a prochiral sulphur atom was relatively low (57% *pro*-S) and the maximum optical yield obtained for a sulphoxide was ⩽72% e.e. Although evidence of further oxidation was obtained during biotransformations of acyclic 1,3-disulphides by a fungus (disulphoxide formation from *Aspergillus niger*[4]) and a bacterium (monosulphone, monosulphoxide monosulphone, and disulphone formation from *Corynebacterium equi*[5]), only monosulphoxide metabolites of 1,3-dithianes were found using three fungal species.[2,6] The present report thus is a continuation of the earlier[2,6] studies on cyclic 1,3-disulphides in the quest for a higher degree of stereoselective sulphoxidation and for evidence of multiple addition of oxygen atoms to the thioacetal substrates.



R	*trans*	*cis*	
H (**1**)	(**2**)	(**2**)	(**3**)
Me (**4**)	*t*-(**5**)	*c*-(**5**)	(**6**)
Bu^t (**7**)	*t*-(**8**)	*c*-(**8**)	(**9**)

The 1,3-dithiolanes (**1**), (**4**), and (**7**) and the derived mono-(**2**), (**5**), and (**8**) and disulphoxide (**3**) were all obtained in racemic form by the literature routes.

The parent 1,3-dithiolane (**1**) proved to be an acceptable substrate for sulphoxidation in growing shake cultures of *Aspergillus foetidus* (30—40% recovered yield) and a *Helminthosporium* species (25—30% recovered yield). Incubation of the thioacetal (**1**) with *Mortierella isabellina* (this fungus was previously found to be effective in sulphoxidation reactions of 1,3-dithianes[2]) gave only a trace of the sulphoxide product.

The optical purity of the product sulphoxide (**2**) (Table 1) was determined by n.m.r. analysis (250 MHz, $CDCl_3$) using a chiral solvating agent [(+)-(*S*)-9-anthryl-2,2,2-trifluoroethanol]. The monosulphoxide (**2**) was obtained with maximum optical yields of 65% (*A. foetidus*) and 10% (*Helminthosporium*). The maximum value previously observed for sulphoxides derived from enzymatic oxidation of thioacetal substrates in growing cultures of *A. foetidus* was 22% e.e.[2,6] When the racemic monosulphoxide (**2**) was used as substrate with either *A. foetidus* or *Helminthosporium* the recovered unchanged sulphoxide was again racemic. This result indicates that optical activity in the original metabolite (**2**) was the result of asymmetric oxidation of 1,3-dithiolane (**1**) rather than enantioselective destruction of the product (**2**).

Table 1. Fungal metabolism of the 1,3-dithiolane (**1**), 2-methyl-1,3-dithiolane (**4**), and 2-t-butyl-1,3-dithiolane (**7**)

Fungus	Dithiolane	Sulphoxide product ($[\alpha]_D^a$, % e.e.[b])
A	(**1**)	(**2**)[c] (+80°, 40; +100°, 54; +114°, 65)
H	(**1**)	(**2**)[c] (+20°, 10)
A	(**4**)	*t*-(**5**)[d] (+18°, 28); *c*-(**5**)[c] (0, 0)
H	(**4**)	*t*-(**5**)[d] (−3°, 4); *c*-(**5**)[c] (0, 0)
M	(**4**)	*t*-(**5**)[d] (+9°, 15); *c*-(**5**)[c] (0, 0)
A	(**7**)	*t*-(**8**)[e] (0, 0)
H	(**7**)	*t*-(**8**)[e] (0, 0)
M	(**7**)	*t*-(**8**)[e] (+6°, 14; +9°, 25; +13°, 32)

[a] In ethanol solvent. [b] N.m.r. analysis in presence of (+)-(*S*)-9-anthryl-2,2,2-trifluoroethanol. [c] Isolated yields 30—40%. [d] Isolated yields 20—25%, *ca.* 75% *trans*:25% *cis*. [e] Isolated yields 7—20%.

In addition to the major (95%) sulphoxide metabolite (**2**) formed from (**1**) in *A. foetidus*, in one experiment a minor (5%) low R_F metabolite was identified as the *trans*-disulphoxide (**3**). The optical purities of the monosulphoxide (**2**) and disulphoxide (**3**) metabolites from *A. foetidus* were identical (54% e.e.). This result eliminates the possibility of enantioselectivity occurring during the second oxygenation step. Fractional recrystallization of the *trans* disulphoxide (**3**) ($[\alpha]_D$ +205°) from ethanol yielded optically pure material ($[\alpha]_D$ +412°). The latter metabolite is the first example of an optically pure sulphoxide derivative of a thioacetal to have been obtained by enzyme-catalysed sulphoxidation.

An essential feature of the comparison of chiroptical properties in a series of compounds is the need to relate to transitions of the same origin. The absorption spectra of sulphoxides are generally characterized by a steadily rising absorption between 260 and 185 nm with associated, clearly

Table 2. C.d. data and absolute configuration of optically active 1,3-dithiolane 1-oxides (**2**), *t*-(**5**), and *t*-(**8**) and 1,3-dithiolane 1,3-dioxide (**3**)

			C.d. data[a]						
Sulphoxide	$[\alpha]_D$/°[b]	% E.e.[c]	λ/nm	Δε	λ/nm	Δε	λ/nm	Δε	Configuration
(**2**)	+80	54	255	−0.4	217	+2.13	188	+2.6	1*R*
t-(**5**)	+18	28	257	−0.07	216	+3.7	198	+2.8	1*R*,2*R*
t-(**8**)	+13	32	*d*	*d*	240	+1.3	217	+2.2	1*R*,2*R*
(**3**)	+412	100	*d*	*d*	240	+28.0	220	+14.0	1*R*,3*R*

[a] Acetonitrile solvent. [b] See footnote *a* in Table 1. [c] See footnote *b* in Table 1. [d] Not observed.

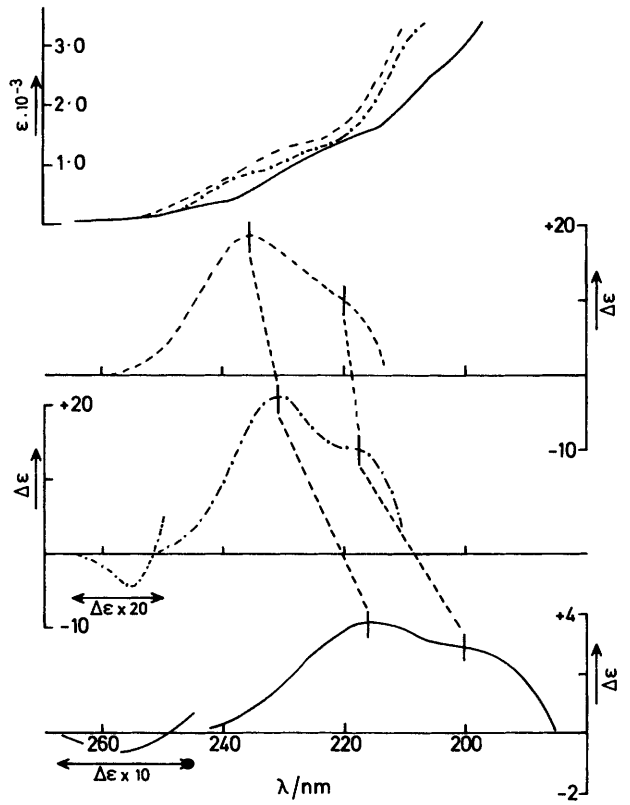


Figure. C.d. spectra taken in MeCN for: – – – – – (+)-(1*R*,2*R*)-*trans*-2-methyl-1,3-dithiane 1-oxide, – · – · – · – (+)-(1*R*,2*S*)-*cis*-2-methyl-1,3-dithiane 1-oxide, and ———— (+)-(1*R*,2*R*)-*trans*-2-methyl-1,3-dithiolane 1-oxide *t*-(**5**)

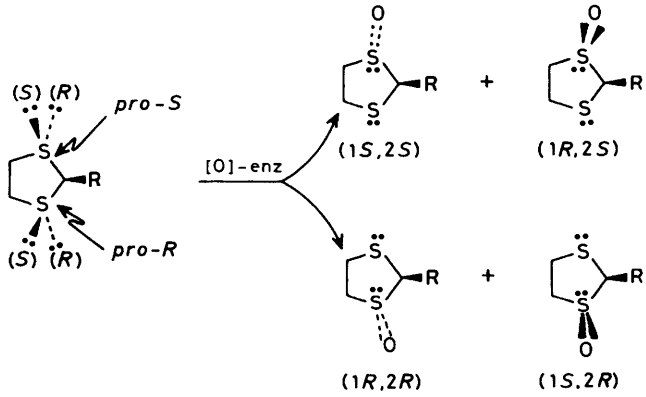
developed, shoulders. In all the sulphoxides we have examined (see ref. 2) one such shoulder is always identifiable between 240 and 215 nm with an extinction coefficient between 800 and 1 300. This corresponds to an absorption band covering two excitations of the same c.d. sign which carry the information required for configuration correlation. A lower energy (>245 nm) weak dichroism of opposite sign is sometimes observed. However, this is usually apparently overlaid by the major two components.

An earlier study[2] of the c.d. of a range of optically active 1,3-dithiane 1-oxides (the majority of whose absolute configurations had been unequivocally assigned by alternative means) established the relationship between c.d. sign and absolute stereochemistry. A positive circular dichroism associated with the two electronic excitations defined above can be correlated with an *R* sulphoxide configuration. A typical graphical relationship between the five- and six-membered cyclic 1,3-disulphide 1-oxides is illustrated in the Figure. Accordingly, the dextrorotatory enantiomers of (**2**), *t*-(**5**), *t*-(**8**), and (**3**) can all be assigned the *R* sulphoxide configuration. The 2-substituent configuration is defined by the geometric isomerism determined by n.m.r. spectroscopy.

Fungal oxidations of the 2-alkyl-1,3-dithiolanes (**4**) and (**7**) were found to occur in the presence of each of the three fungi to give the corresponding monosulphoxides with isolated yields within the range 7—25%. The relative configurations (*cis*–*trans*) of the monosulphoxide metabolites (**5**) and (**8**) had previously been assigned on the basis of ^{13}C n.m.r. analysis[7] and was confirmed by both ^1H decoupling and n.O.e. difference spectroscopy in the present study. The ratio of *cis* *c*-(**5**):*trans* *t*-(**5**) monosulphoxides obtained after $NaIO_4$ oxidation of 2-methyl-1,3-dithiolane (**4**) was estimated by ^1H n.m.r. analysis to be (40:60%). The isomeric mixture of sulphoxide metabolites obtained by fungal oxidation in each case contained a higher proportion of the *trans*-isomer *t*-(**5**) (75%). The *cis* and *trans* isomers *c*-(**5**) and *t*-(**5**) were separated by preparative t.l.c. on silica gel with propan-2-ol (15%) in carbon tetrachloride as eluant.

Using ^1H n.m.r. analysis in association with (+)-(*S*)-9-anthryl-2,2,2-trifluoroethanol as chiral solvent, it was found that the *cis*-sulphoxide metabolite *c*-(**5**) produced by each of the three fungi was racemic. The *trans* sulphoxide product *t*-(**5**) did, however, have an excess of one enantiomer in each case with a maximum value of 28% e.e. from *A. foetidus*. The c.d. spectrum of the sulphoxide *t*-(**5**) ($[\alpha]_D$ +18°, 28% e.e.), indicates an *R*-configuration at the chiral sulphur atom (Table 2). Addition of a mixture of racemic sulphoxides *c*-(**5**) (40%):*t*-(**5**) (60%) as substrates to either of the fungi showed no stereopreference for individual geometric or optical isomers in the recovered sulphoxide.

From this knowledge of the relative yield, optical purity, isomer distribution, and absolute configuration it was possible to estimate the degree of selectivity for an enantiotopic sulphur atom during enzyme-catalysed sulphoxidation of 2-methyl-1,3-dithiolane (**4**) in *A. foetidus* (Scheme). Thus, preference for addition of an oxygen atom to the *pro-R* sulphur atom was



Scheme.

found to be 60.5% (48% 1*R*,2*R*; 12.5% 1*S*,2*R*) with 39.5% attack at the *pro-S* sulphur atom (27% 1*S*,2*S*; 12.5% 1*R*,2*S*).

Microbial oxidation of 2-methyl-1,3-dithiolane (**4**) by enzymes in *A. foetidus* yielded mainly the monosulphoxide isomers [98%, *t*-(**5**):*c*-(**5**)] but was accompanied by a very minor proportion (*ca.* 2%) of the *trans*-disulphoxide metabolite (**6**). No reliable $[\alpha]_D$ value nor meaningful optical purity determination could be carried out on the disulphoxide metabolite (**6**) owing to the very small sample available.

Fungal oxidation of 2-t-butyl-1,3-dithiolane (**7**) by each of the three fungi gave only the *trans* sulphoxide *t*-(**8**) in similar isolated yields to those found during sulphoxidation of the thioacetal (**4**) (7—20%). From these fungi only the *M. isabellina* species produced optically active sulphoxide *t*-(**8**), *i.e.* optical yields in the range (14—32%) (Table 1). The c.d. curve for the (+)-enantiomer of the sulphoxide *t*-(**8**) indicates an *R* sulphoxide configuration using a similar rationale to that employed for the metabolites (**2**) and *t*-(**5**). When a racemic sample of the sulphoxide *t*-(**8**) was added as substrate to *M. isabellina* only racemic sulphoxide was recovered, indicating that the enantiomeric excess of sulphoxide *t*-(**8**) found after fungal oxidation was entirely due to asymmetric synthesis. The maximum % e.e. observed (32% e.e.) was thus due to a preference for the dextrorotatory 1*R*,2*R* enantiomer (66%) over the 1*S*,2*S* enantiomer (34%). This result also indicates an optimal stereopreference for the *pro-R* sulphur atom (66%) compared with the *pro-S* sulphur atom (34%) (Scheme).

The *trans*-disulphoxide (**9**) was not detected as a metabolite of the thioacetal (**7**) but the *trans*-2-t-butyl-1,3-dithiolane *cis*-1,3-dioxide isomer was identified as a racemic oxidation product derived from the racemic sulphoxide substrate *t*-(**8**) formed in the presence of the *Helminthosporium* species.

The variation in optical yields of sulphoxide metabolites recovered from 'repeated' experiments, *e.g.* (**2**) (40—65% e.e.) and (**7**) (14—32% e.e.), cannot readily be accounted for by the selective enzymatic removal of one sulphoxide enantiomer, but may result from minor changes in microbial growth conditions or the differing levels of enzyme activity at the time of substrate addition.

The results from this study which indicate a maximum stereopreference for the *pro-R* sulphur atom during oxidation of the thioacetals (**4**) (61%) and (**7**) (66%) in the fungi *A. foetidus* and *M. isabellina* respectively, may be compared with the earlier[2] findings where the *pro-S* sulphur atom of 2-t-butyl-1,3-dithiane was preferentially sulphoxidized (57%) by the *Helminthosporium* species.

Experimental

N.m.r. spectral data were obtained using a Bruker WH 250 MHz instrument with $CDCl_3$ as solvent, and tetramethylsilane as standard reference compound. Optical purity determinations were carried out using (*S*)-(+)-9-anthryl-2,2,2-trifluoroethanol as chiral co-solvent in $CDCl_3$ solution (±3% e.e.).

Optical rotations were obtained at 589 nm using a Perkin-Elmer Automatic Polarimeter, Model 241 and ethanol as solvent. C.d. spectra were obtained using a Jasco model J40CS and acetonitrile as solvent.

The strains of fungi and the microbial transformation conditions employed were identical with those reported earlier.[2,6] The crude products obtained from continuous dichloromethane extraction of the culture medium were purified by silica-gel column chromatography followed by preparative t.l.c. on silica gel using ether–methanol (9:1) as eluant. Optical rotation and optical purity measurements were determined directly after chromatographic purification and prior to recrystallization.

The thioacetal substrates 1,3-dithiolane (**1**), 2-methyl-1,3-dithiolane (**4**), and 2-t-butyl-1,3-dithiolane (**7**) were synthesized by literature methods.[8,9] Samples of 1,3-dithiolane-1-oxide (**2**),[10] *trans*-dithiolane 1,3-dioxide (**3**),[11] *cis*-2-methyl-1,3-dithiolane 1-oxide *c*-(**5**),[7] *trans*-2-methyl-1,3-dithiolane 1-oxide *t*-(**5**),[7] and *trans*-2-t-butyl-1,3-dithiolane 1-oxide *t*-(**8**)[7] were all obtained by oxidation of the corresponding 1,3-dithiolanes using literature procedures.

Sodium metaperiodate oxidation of an isomeric mixture of 2-methyl-1,3-dithiolane 1-oxide, *t*-(**5**) and *c*-(**5**), yielded mainly *trans*-2-methyl-1,3-dithiolane 1,3-dioxide (**6**) as a colourless solid, m.p. 148—152 °C (EtOH) (Found: C, 31.7; H, 5.3. $C_4H_8O_2S_2$ requires C, 31.6; H, 5.3%); δ(250 MHz) 1.64 (3 H, d, $J_{Me,H}$ 7.9 Hz, Me), 3.56—3.67 (2 H, m, 4-H), 3.75—3.83 (2 H, m, 5-H), and 4.04 (1 H, q, $J_{H,Me}$ 7.9 Hz, 2-H). Using a similar procedure, *trans*-2-t-butyl-1,3-dithiolane *cis*-1,3-dioxide was also obtained as a colourless solid, m.p. 170—172 °C (EtOH) (Found: *M*, 194.043 39. $C_7H_{14}O_2S_2$ requires *M*, 194.043 51); δ_H(250 MHz) 1.28 (9 H, s, Bu^t), 3.36 (2 H, m, 4-H), 3.59 (1 H, s, 2-H), and 3.76—3.88 (2 H, m, 5-H).

A sample of (+)-1,3-dithiolane 1-oxide (**2**) ($[\alpha]_D$ +100°, 54% e.e.) obtained as a microbial metabolite was oxidized using $NaIO_4$ to yield (+)-*trans*-1,3-dithiolane 1,3-dioxide (**3**) ($[\alpha]_D$ +205°, 54% e.e.). Fractional crystallization from ethanol yielded the optically pure disulphoxide (**3**), m.p. 167—169 °C, $[\alpha]_D$ +412°.

Acknowledgements

We thank D.E.N.I. for a Postgraduate Studentship to R. D. and Mrs. Fiona Turley for assistance with the microbial work.

References

1 D. R. Boyd, C. T. Walsh, and Y. C. J. Chen in 'Sulphur Drugs and Related Organic Chemicals. Chemistry, Biochemistry and Toxicology,' A. Damani, Ed., Ellis Horwood, London, vol. III, 1988, in the press.
2 B. J. Auret, D. R. Boyd, E. S. Cassidy, R. Hamilton, F. Turley, and A. F. Drake, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1547.
3 J. B. Jones, *Tetrahedron*, 1986, **42**, 3351.
4 M. Poje, O. Nota, and K. Balenović, *Tetrahedron*, 1980, **36**, 1895.
5 Y. Okamoto, H. Ohta, and G. I. Tsuchihashi, *Chem. Lett.*, 1986, 2049.
6 B. J. Auret, D. R. Boyd, F. Breen, R. M. E. Greene, and P. M. Robinson, *J. Chem. Soc., Perkin Trans. 1*, 1981, 930.
7 F. A. Carey, O. D. Dailey, Jr., and T. E. Fromuth, *Phosphorus Sulfur*, 1981, **10**, 163.
8 A. Hoppman, P. Weyerstahl, and W. Zummack, *Ann. Chem.*, 1977, 1547.
9 R. E. Stevenson, G. Bhat, C. H. Bushweller, and W. G. Anderson, *J. Am. Chem. Soc.*, 1974, **96**, 1067.
10 D. T. Gibson, *J. Chem. Soc.*, 1930, 12.
11 G. M. Burnett and E. S. Statham, *J. Org. Chem.*, 1931, 1684.

Received 5th January 1988; *Paper* 8/00061A