

Stereoselectivity of Prochiral Thioalkyl Groups in the Enzymatic Oxidation of Thioacetals by Fungi

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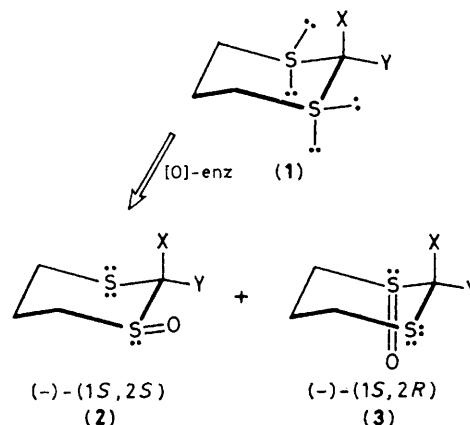
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Enzymatic oxidation of 2-substituted 1,3-dithians in cultures of a *Helminthosporium* species shows a stereo-preference for equatorial oxidation and for (1*S*)-monosulphoxide products.

Each sulphur atom of 1,3-dithian (**1a**) and its 2-alkyl- (**1b,c**) and 2,2'-dialkyl-derivatives (**1d,e**) bears both a *pro-R* and a *pro-S* lone pair of electrons, and the lone pair with a given prochirality has an equatorial orientation at one sulphur atom but an axial orientation at the other. Previous studies¹⁻³ have shown that the mono-oxygenase enzymes produced by fungi distinguish between a *pro-R* and a *pro-S* lone pair of electrons of a sulphur atom in the oxidation of (**1a**) and its analogues without a prochiral 2-carbon atom. A concomitant axial-equatorial enzymatic discrimination is reported here at *pro-R* and *pro-S* thioalkyl groups in an extension of the studies to 2-prochiral-1,3-dithians (**1b,c,d**).

The thioacetals (**1b-d**) were added individually as substrates to cultures of a *Helminthosporium* species (NRRL 4671) and the *trans*-monosulphoxide (**2**) and *cis*-monosulphoxide (**3**) products were separated and purified by column chromatography on silica gel, followed by preparative h.p.l.c.⁴ (yields 35–65% overall from the substrate). In each case, the *trans*-monosulphoxide (**2b,c,d**) is found to be the major product (82–100%) (Table 1), showing the steric preference of the fungal mono-oxygenase enzyme(s) for the equatorial lone pairs of the corresponding 1,3-dithian (**1b,c,d**). The achiral oxidant NaIO₄ similarly differentiates between the diastereotopic lone pairs of the sulphur atoms in (**1b,c,d**), affording the corresponding *trans*-monosulphoxide (**2b,c,d**) as the major product (90–100%).

In order to evaluate the stereopreference of the enzymatic oxidation for the *pro-R* or *pro-S* thioalkyl groups, the (+)-(1*R*,2*R*)-*trans*-monosulphoxide (**2b**) (enantiomer) ([α]_D + 62°) and the (+)-(1*R*,2*S*)-*cis*-isomer (**3b**) (enantiomer)



- a; X = H, Y = H
 b; X = H, Y = Me
 c; X = H, Y = Bu^t
 d; X = Me, Y = Bu^t
 e; X = Me, Y = Me

([α]_D + 201°) were synthesised by the 2-methylation of (+)-(1*R*)-1,3-dithian 1-oxide⁵ (**2a**) ≡ (**3a**) (enantiomer) ([α]_D + 227°, EtOH). A comparison of the [α]_D rotations and the c.d. spectra of the synthetic (+)-(1*R*)-monosulphoxides with the corresponding values for the products of the enzymatic oxidation of (**1b**) in the cultures of the *Helminthosporium* species shows that the major metabolites are the (-)-(1*S*,2*S*)-

Table 1. Relative yield and $[\alpha]_D$ (EtOH) of the monosulphoxide metabolites (**2b,c,d**) and (**3b,c**) and the enantiomeric excess (e.e.) and configuration of the preferred enantiomer assigned from comparisons of the c.d. spectra.

Sulphoxide	Yield, ^a %	$[\alpha]_D^{b/c}$	E.e., ^c %	Configuration	λ/nm	$\Delta\epsilon^d$
(2b)	86	-17	27	(1 <i>S</i> , 2 <i>S</i>)	235	-19.7
					203	+29.0
(3b)	14	-71.5	36	(1 <i>S</i> , 2 <i>R</i>)	231	-23.0
					202	+9.6
(2c)	82	-13	35	(1 <i>S</i> , 2 <i>S</i>)	237	-23.4
					203	+27.4
(3c)	18	+83	72	(1 <i>S</i> , 2 <i>R</i>)	245	-3.6
					215	-35.0
(2d)	100	0	0	racemic		

^a Estimated by duplicate n.m.r. analyses of the product mixture (250 MHz, CDCl₃). ^b Obtained from product samples after isolation by preparative h.p.l.c. ^c Obtained by n.m.r. analysis (ref. 6) and duplicated by $[\alpha]_D$ comparisons. ^d The c.d. values refer to optically pure isomers in cyclohexane solution; comparison values obtained from synthesised enantiomers are, for the (-)-(1*S*)-isomers, $\Delta\epsilon_{233} -19.5$, $\Delta\epsilon_{202} +13.5$ (**2a**) \equiv (**3a**), and $\Delta\epsilon_{232} -25.6$, $\Delta\epsilon_{215} +21.8$ (**2e**) \equiv (**3e**), together with the values recorded for (**2b**) and (**3b**).

enantiomer (**2b**) and the (-)-(1*S*,2*R*)-enantiomer (**3b**), produced in an enantiomeric excess of 27% and 36%, respectively (Table 1). The same values for the enantiomeric excess of (**2b**) and (**3b**) were estimated by the n.m.r. method of Pirkle and coworkers,⁶ using solutions in (-)-phenyl- or 1-(+)-9-anthryl-2,2,2-trifluoroethanol, diluted with CDCl₃, to distinguish the ¹H n.m.r. signals of the 2-alkyl groups in each enantiomer. The optical yields of the major metabolites (**2c**) and (**3c**) from (**1c**) were estimated by the n.m.r. method, and the respective (1*S*,2*S*) and (1*S*,2*R*) configurations were assigned by comparisons of the c.d. spectra (Table 1).

Only the *trans*-monosulphoxide (**2d**) in racemic form was isolated from the enzymatic oxidation of the thioacetal (**1d**), the most sterically crowded of the 1,3-dithians examined. In contrast, the less crowded thioacetal (**1e**), in which the bulk of the 2-*t*-butyl group is not buttressed by another alkyl group at the 2-carbon atom, as in (**1d**), gives the largest enantiomeric excess (72%) of the series investigated in the minor *cis*-monosulphoxide product (**3c**) from the enzymatic oxidation (Table 1). The lack of chiral discrimination in the enzymatic oxidation of (**1d**) does not arise from 2,2'-dialkyl substitution, since the thioacetal (**1e**) in cultures of the *Helminthosporium* species was oxidised to (**2e**) \equiv (**3e**), with a 35% enantiomeric excess of the (-)-(1*S*)-isomer, comparable to the optical yields of the (-)-(1*S*)-enantiomers of (**2b**) and (**3b**) from the corresponding oxidation of the thioacetal (**1b**) (Table 1).

The partial recovery of racemic monosulphoxides after their incubation with growing cultures of the *Helminthosporium* species eliminates the possibility of optical activity resulting from selective removal of one enantiomer during metabolism.

Overall, the product analyses suggest that the enzymatic oxidation of the thioacetals (**1b,c**) has a stereopreference of *ca.* 27–35% for equatorial oxidation of the *pro-S* thioalkyl group or *ca.* 36–72% for axial oxidation of the *pro-R* thioalkyl group. These results (and methods of stereochemical assignment) are of particular relevance in view of earlier work on other thioacetal sulphoxides which have also been produced under enzymatic control.^{3,7}

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