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Biotransformation of organic sulfides — VIII. A predictive model for sulfoxidation by *Helminthosporium* species NRRL 4671

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Abstract: The fungus *Helminthosporium* species NRRL 4671 converts a wide range of prochiral sulfides to the corresponding chiral sulfoxides, the majority of which have (*S*) configuration at sulfur. The formation of a series of chiral cyclopentyl alkyl, cyclohexyl alkyl, benzyl alkyl, and methyl alkyl sulfoxides by biotransformation of the corresponding sulfides using *Helminthosporium* species is described. The analysis of over 90 such biotransformations has resulted in the development of a model based on restrictive space descriptors that has been used to rationalize these reactions, and that is proposed as a predictor of the outcome of *Helminthosporium*-catalyzed sulfoxidations. © 1997 Elsevier Science Ltd. All rights reserved.

Introduction

The demand for chiral sulfoxides in asymmetric synthesis[1] has engendered a continuing interest in their preparation. Among the most direct and versatile methods for chiral sulfoxide synthesis are those involving the enzymic catalysis of sulfide oxidation. The range of isolated enzymes capable of this transformation continues to grow, and now includes chloroperoxidase,[2] cyclohexanone monoxygenase,[3,4] cytochrome P-450 dependent monoxygenases,[5–7] flavin dependent monoxygenases,[8] horseradish peroxidase,[9,10] lactoperoxidase,[11] microperoxidase,[12] and dioxygenases.[13] Chiral sulfoxides can also be conveniently prepared by biotransformation using whole cell biocatalysts,[14] one of the most versatile of which is the fungus *Helminthosporium* species NRRL 4671. This microorganism produces chiral sulfoxides from 1,3-dithiolanes,[15] 1,3-dithianes,[16] cycloalkyl methyl sulfides,[17] benzyl alkyl sulfides,[18–21] phenyl alkyl sulfides,[19,22] and substituted alkyl methyl sulfides.[23] In many cases sulfoxides are obtained in good yield and high enantiomeric purity, without the complication of sulfone formation,[19–23] suggesting an application for *Helminthosporium* in the preparative-scale production of such chiral sulfoxides.

The ability to predict the regio- and stereochemical outcome of a biotransformation is an important factor in assessing the synthetic utility of that reaction. Unfortunately, the predictability of biocatalytic sulfoxidations has traditionally been low, largely as a result of an imperfect understanding of the nature of the relevant enzymes. For those enzymes for which sequence and/or structure information is available, a more fundamental understanding of enzyme–substrate interactions is emerging which will lead to increased predictability.[7,9] For others, such as the *Helminthosporium* sulfoxidising system, an alternative approach can be taken based on determination of the active site topography of the relevant enzyme by investigation of the biotransformation of a range of different substrates.

The latter approach has been validated by its application to isolated enzymes where correlation with structural information is possible, such as *Aspergillus niger* glucoamylase,[24] cytochrome P-450_{CAM},[25,26] and phospholipase A_2,[27] and successfully extended to enzymes such as cyclohexanone monooxygenase for which no physical structure data are available.[4,28,29] It has also been successfully applied to whole-cell biocatalyses, such as the hydrolysis of esters by *Rhizopus nigricans*,[30] benzylic

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Table 1. Biotransformation of phenyl alkyl sulfides with *Helminthosporium*[19,22]

ENTRY	SUBSTRATE		SULFOXIDE		REFERENCE
		yield (%)	config.	e.e. (%)	
1	Ph-S-CH_3	30	*S*	48	19
2	Ph-S-C_2H_5	40	*S*	84	19
3	Ph-S-n.C_3H_7	57	*S*	33	19
4	Ph-S-i.C_3H_7	42	*R*	32	19
5	Ph-S-n.C_4H_9	39	*S*	16	19
6	Ph-S-t.C_4H_9	1	*R*	15	19
7	Ph-S-n.C_5H_{11}	19	*S*	10	19
8	Ph-S-CH=CH_2	22	*R*	45	19
9	Ph-S-n.C_6H_{13}	7	*R*	25	19
10	1-naphthyl-S-CH_3	7	*S*	26	19
11	2-naphthyl-S-CH_3	18	*S*	75	19
12	p.CH_3-Ph-S-CH_3	10	*S*	27	22
13	p.F-Ph-S-CH_3	56	*S*	60	22
14	p.Cl-Ph-S-CH_3	35	*S*	68	22
15	p.Br-Ph-S-CH_3	69	*S*	90	22
16	p.CN-Ph-S-CH_3	80	*S*	92	22
17	p.NO_2-Ph-S-CH_3	55	*S*	48	22
18	p.NH_2-Ph-S-CH_3	13	*S*	52	22
19	p.$COCH_3$-Ph-S-CH_3	38	*S*	32	22
20	p.OCH_3-Ph-S-CH_3	83	*S*	80	22
21	p.SCH_3-Ph-S-CH_3	64	*S*	80	22
22	p.S(O)CH_3-Ph-S-CH_3	21	*S*	95	22

hydroxylations[31] and epoxidations[32] by *Mortierella isabellina*, and hydroxylations by *Beauveria sulfurescens* and other fungi.[33]

This report presents a predictive model for chiral sulfoxidation by *Helminthosporium* species NRRL 4671 based on an analysis of the sulfoxidation of a large number of substrates (>90) by this microorganism.

Results and discussion

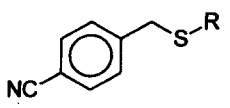
Previously published data used in this analysis are summarized in Tables 1–3.

Hitherto unreported data obtained from biotransformation of substrates **2–4**, **6–8**, **9–12**, **14–16**, **18–21**, **23** and **26** are listed in Table 4. As in previous studies,[19–23] the assignment of product structures relied heavily on NMR information, ^1H and ^{13}C spectral data being diagnostic of the oxidation state at sulfur. These, together with supporting mass spectral data, are presented at length in the experimental section. The predominant configuration and enantiomeric excesses of sulfoxide products were determined by analysis of their ^1H NMR spectra in the presence of the chiral shift reagent (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) and confirmed by correlation with optical rotation data where available. Use of the NMR shift reagent gives consistent, configurationally dependent chemical shift patterns that have been correlated with data obtained from both (*R*) and (*S*) sulfoxides of established configuration.[23,34] The shift reagent causes the S-methyl group signals of methyl sulfoxides to appear as a pair of singlets with $\Delta\delta$ values of the order of 0.02–0.03 ppm, cleanly resolved at 300–500 mHz. In all examples studied to date,[18–22,34,35] the signal from the methyl protons α to sulfur in the (*S*) enantiomer is at the higher field. Configurationally dependent shifts are also observed for other hydrogens α to sulfur,[19,34,35] and have been used for stereochemical analyses of the products listed in Table 4. The configurational assignments of the table are in full agreement with the complexation model proposed for MPAA interaction with sulfoxides.[34]

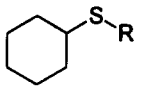
Table 2. Biotransformation of benzyl alkyl sulfides by *Helminthosporium*[18–21]

ENTRY	SUBSTRATE		SULFOXIDE		REFERENCE
		yield (%)	config.	e.e. (%)	
1	$PhCH_2$-S-CH_3	68	*S*	62	18
2	$PhCH_2$-S-C_2H_5	55	*S*	51	18
3	$PhCH_2$-S-n.C_3H_7	12	*S*	10	18
4	$PhCH_2$-S-i.C_3H_7	22		0	18
5	$PhCH_2$-S-n.C_4H_9	38	*S*	25	18
6	$PhCH_2$-S-t.C_4H_9	5	*S*	7	18
7	$PhCH_2$-S-n.C_5H_{11}	20	*S*	70	19
8	$PhCH_2$-S-n.C_6H_{13}	22	*S*	82	19
9	$PhCH_2$-S-n.C_7H_{15}	11	*S*	>95	19
10	$PhCH_2$-S-cyc.C_6H_{11}	12		0	19
11	$PhCH_2$-S-i.C_4H_9	61	*S*	23	19
12	$PhCH_2$-S-i.C_5H_{11}	38	*S*	45	19
13	$PhCH_2$-S-Ph	4		0	19
14	$PhCH_2$-S-Ph-p.CH_3	3		0	19
15	$PhCH_2$-S-$(CH_2)_2$Ph	14	*S*	6	19
16	$PhCH_2$-S-$(CH_2)_3$Ph	11	*S*	10	19
17	p.CH_3-$PhCH_2$-S-CH_3	70	*S*	52	20
18	p.C_2H_5-$PhCH_2$-S-CH_3	42	*S*	74	20
19	p.n.C_3H_7-$PhCH_2$-S-CH_3	45	*S*	85	20
20	p.n.C_4H_9-$PhCH_2$-S-CH_3	74	*S*	90	20
21	p.i.C_3H_7-$PhCH_2$-S-CH_3	77	*S*	80	20
22	p.t.C_4H_9-$PhCH_2$-S-CH_3	58	*S*	76	20
23	p.F-$PhCH_2$-S-CH_3	82	*S*	80	21
24	p.Cl-$PhCH_2$-S-CH_3	71	*S*	90	21
25	p.Br-$PhCH_2$-S-CH_3	75	*S*	88	21
26	p.CN-$PhCH_2$-S-CH_3	96	*S*	98	21
27	p.NO_2-$PhCH_2$-S-CH_3	95	*S*	92	21
28	p.NH_2-$PhCH_2$-S-CH_3	62	*S*	95	21
29	p.$COCH_3$-$PhCH_2$-S-CH_3	62	*S*	84	21
30	p.OCH_3-$PhCH_2$-S-CH_3	86	*S*	80	21
31	p.CF_3-$PhCH_2$-S-CH_3	68	*S*	68	21
32	p.CO_2H-$PhCH_2$-S-CH_3	21	*S*	48	21
33	p.$NHCOCH_3$-$PhCH_2$-S-CH_3	50	*S*	92	21
34	p.$OCOCH_3$-$PhCH_2$-S-CH_3	40	*S*	84	21
35	p.OH-$PhCH_2$-S-CH_3	35	*S*	80	21

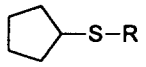
The capacity of *Helminthosporium* to enantioselectively oxidise prochiral sulfides to chiral sulfoxides is described by the model shown in Figure 1. *Helminthosporium* is capable of a range of oxidase-catalysed biotransformations, including efficient sulfoxidation and hydroxylation reactions.[20] Preliminary evidence indicates that a single protein in *Helminthosporium* may be responsible for the sulfoxidase activity observed in whole-cell biotransformations,[37] but whether the same enzyme is capable of carbon hydroxylation is not known. Analysis of the hydroxylation reactions carried out by *Helminthosporium* is complicated by the presence of oxidoreductase enzymes that can oxidise secondary alcohols to ketones in an enantioselective manner.[38]



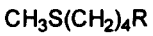
1; R = CH_3
2; R = C_2H_5
3; R = n.C_3H_7
4; R = n.C_4H_9



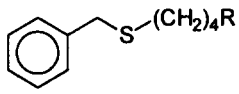
5; R = CH_3
6; R = C_2H_5
7; R = n.C_3H_7
8; R = n.C_4H_9



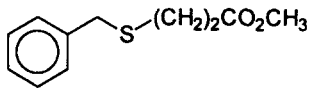
9; R = CH_3
10; R = C_2H_5
11; R = n.C_3H_7
12; R = n.C_4H_9



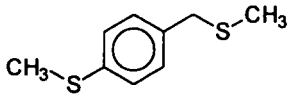
13; R = N=C=S
14; R = C(O)CH_3
15; R = CN
16; R = CH_3



17; R = CH_3
18; R = C(O)CH_3
19; R = CN
20; R = CO_2H
21; R = CO_2CH_3



22



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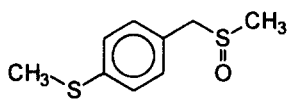

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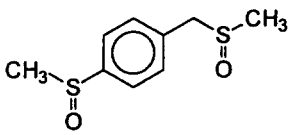
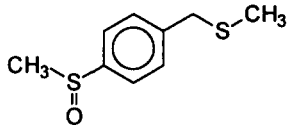
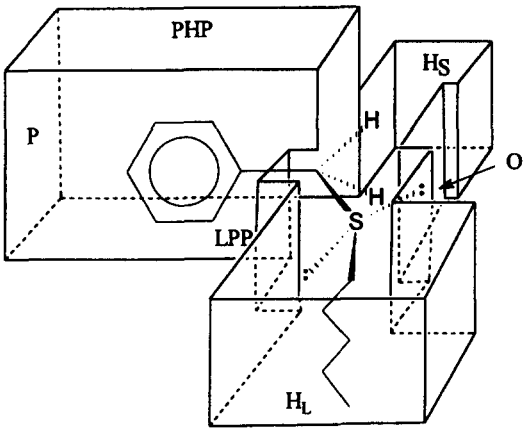


25



26

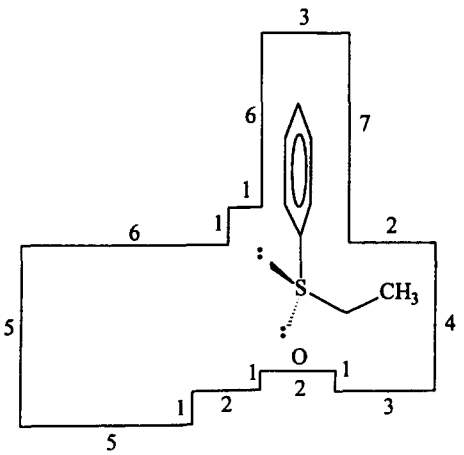
The model of Figure 1 was developed from energy-minimized (MM^+) structures of substrates produced by Hyperchem©. As a starting point, the substrates listed in Tables 1 and 2 were analyzed by fixing at arbitrary points both the sulfur atom and the aromatic region of the molecule. Compounds were then divided into groups of acceptable (>10% yield sulfoxidation, e.g. entries 1–5, 13–17, and 19–21 of Table 1) and non-acceptable (<10% sulfoxidation, e.g. entries 6, 9, and 10 of Table 1; 5, 13 and 14 of Table 2) substrates. This grouping, together with the stereochemical outcome of the sulfoxidation, was then used to define the limits of two non-polar binding pockets, H_L and H_S, taking into account molecular shape and size (including Van der Waal's radii of hydrogens). The model as developed thus far explains the stereochemical inversion seen for sulfoxidation of some phenyl alkyl sulfides (e.g. entries 4, 8 and 9 of Table 1), illustrated in Figs 2 and 3, in which the larger alkyl groups cannot be easily accommodated in H_S, and the stereochemical trend in the series of benzyl alkyl sulfoxides (entries 1–9, Table 2), illustrated in Figs 4–6. For the latter series, small alkyl groups bind preferentially in H_L with the benzylic methylene occupying H_S and the non-reacting lone pair in LPP, whereas larger n.alkyl groups (c.f. entries 8 and 9) can bind only in H_L. Those compounds with a large carbon substituent having a branch α to sulfur (entries 6, 10, 13, and 14, Table 2) are



H_S: small hydrophobic pocket
H_L: large hydrophobic pocket
PHP: polar hydrophobic pocket
P: polar binding site
LPP: lone pair pocket
O: oxidation centre

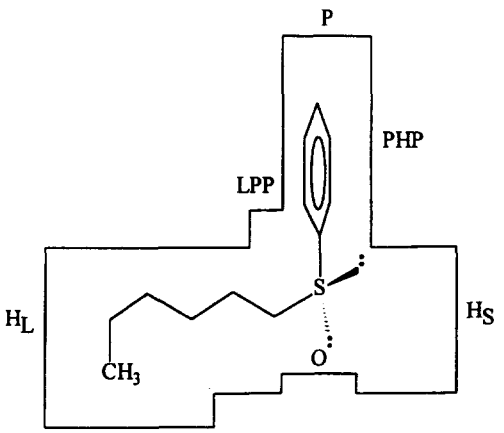
optimum P to O distance, 8 -10 Å

Figure 1. Model for sulfoxidation by *Helminthosporium*



binding leading to (*S*) sulfoxide
phenyl ethyl sulfoxide, (*S*), ee 84%
dimensions shown in Angstrom

Figure 2.

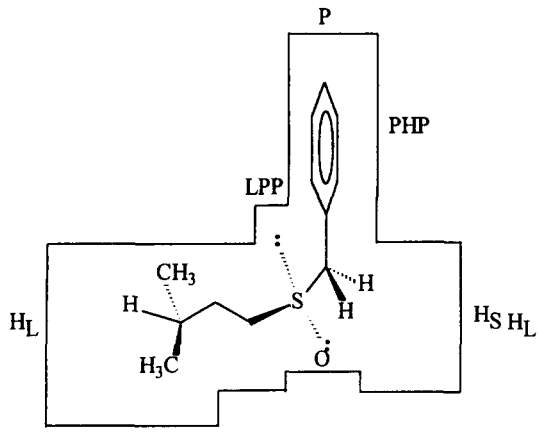


binding leading to (*R*) sulfoxide
phenyl hexyl sulfoxide, (*R*), ee 25%

Figure 3.

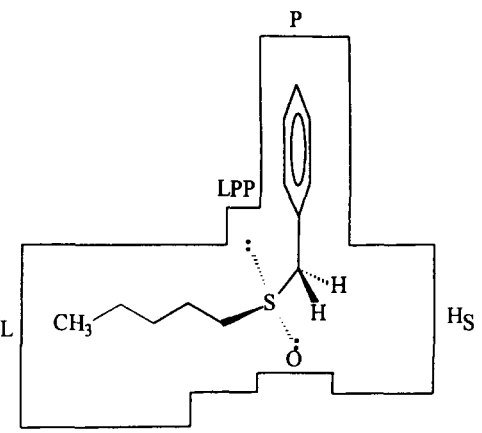
uniformly poor substrates, but when the branch is more remote from sulfur (entries 11 and 12) they can be accommodated by the enzyme. This suggests a restriction in H_L close to the oxidising centre, as shown in Figure 1. Benzyl i.propyl sulfide (entry 4, Table 2) is therefore equally restricted in binding into either H_L or H_S as shown in Figure 6, resulting in a low yield of racemic sulfoxide, whereas benzyl i.pentyl sulfide (entry 12, Table 2) is a more acceptable substrate, as shown in Figure 4. This analysis is illustrated from the top dimension of the model for representative substrates of Tables 1 and 2 in Figs 2–6. Figure 2 indicates the approximate dimensions of the model in Å.

The importance of a polar site, P, located within the hydrophobic pocket labeled PHP, in the binding of aryl substrates is evident from the results from substrates which contain a group capable of interacting with this site (Table 1, entries 13–16, and 20–22; Table 2, entries 23–31 and 33–35). In all these cases, moderate to good yields and high e.e.'s of sulfoxide are obtained. Sulfoxidation of p-alkyl-substituted benzyl methyl sulfides (entries 18–22 of Table 2) is accompanied by hydroxylation of the p.alkyl group,[20] placing these substrates also in the category of those capable of interaction with such a site if hydroxylation precedes sulfoxidation.



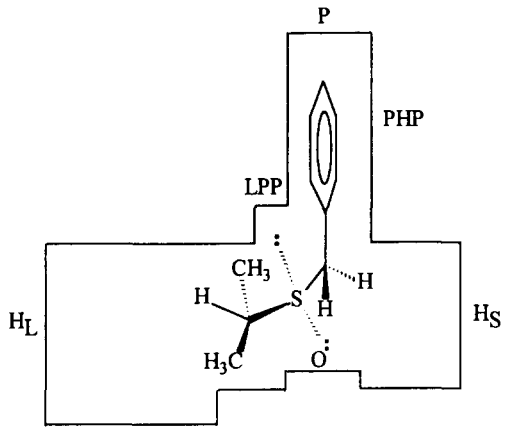
binding leading to (*S*) sulfoxide
benzyl 3-methylbutyl sulfoxide, ee 45%

Figure 4.

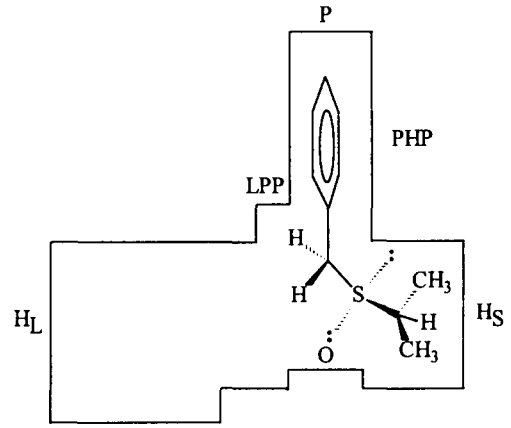


binding leading to (*S*) sulfoxide
benzyl n.pentyl sulfoxide, ee 70%

Figure 5.



binding leading to (*S*) sulfoxide
benzyl isopropyl sulfoxide: e.e. 0

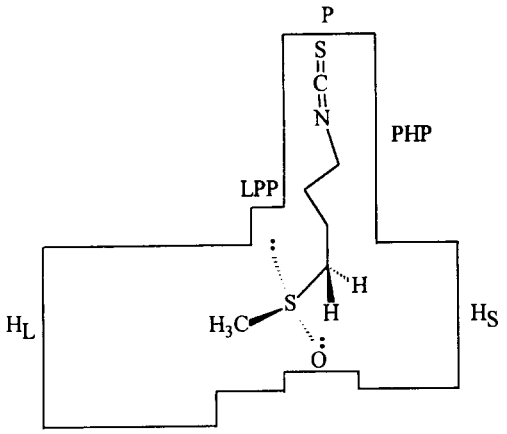


binding leading to (*R*) sulfoxide

Figure 6.

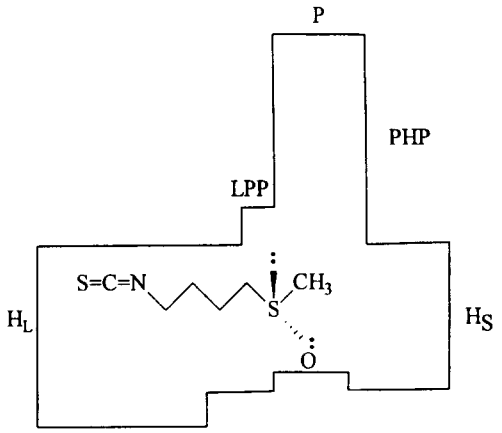
In order to gain insight into the nature of the site P, molecular modelling of the electron densities of the substrates listed in Tables 1 and 2 was performed using both Hyperchem© and Spartan© software. A clear correlation emerged between the presence of non-bonded electron density in the *para* position of benzyl sulfides and the ability of *Helminthosporium* to oxidize those sulfides in good yield and high e.e. (see Table 2, entries 23–31 and 33–35). A similar trend is also apparent, but less pronounced, in the phenyl series (see Table 1, entries 13–16 and 20–22). There is no apparent correlation between efficient sulfoxidation and either the hydrogen bonding or electron donating or withdrawing properties of the para substituent.

The importance of such an 'anchoring' group in the substrate is also suggested by the biotransformation of non-aromatic substrates (Table 3), where sulfides that contain such a group (entries 12–19) are oxidised with uniformly high e.e.'s. The binding of such a substrate is exemplified by Figure 7: the location of P towards the rear of PHP does not necessarily imply that it limits the depth of this



binding leading to (*S*) sulfoxide, ee 93%

Figure 7.



alternate binding leading to (*S*) sulfoxide

Figure 8.

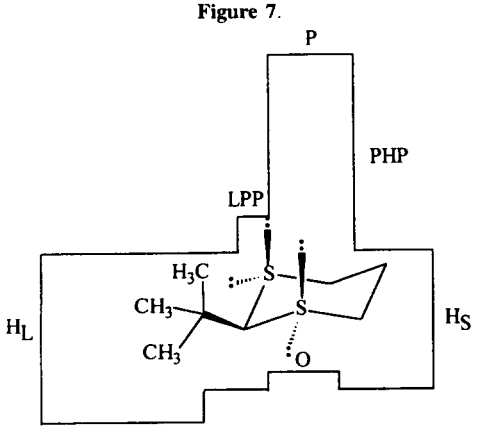


Figure 9.

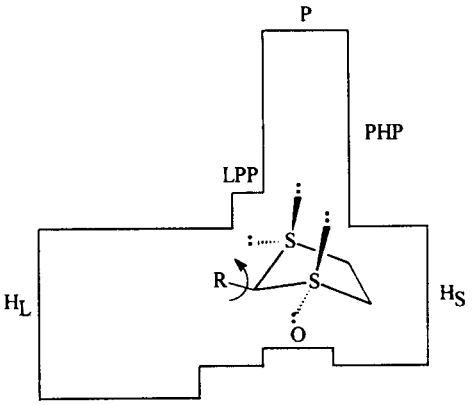
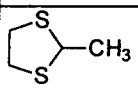
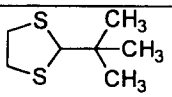
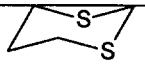
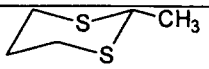
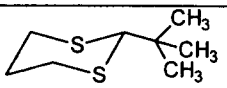
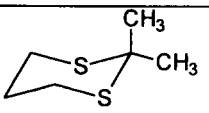
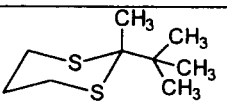
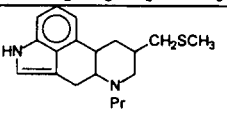
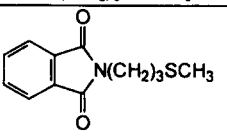
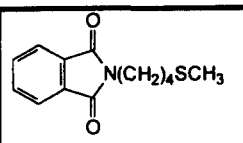
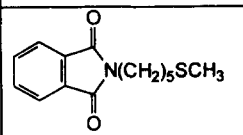
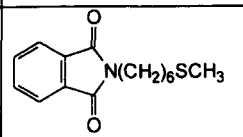


Figure 10.

Table 3. Biotransformation of miscellaneous sulfides by *Helminthosporium*[15–17,19,23,36]

ENTRY	SUBSTRATE		SULFOXIDE		REFERENCE
		yield (%)	config.	e.e. (%)	
1		30-40	1*R*	10	15
2		trans 15-20 cis 5-8	1*S*,2*S*	4 0	15 15
3		trans 7-20		0	15
4		12-27	*S*	14	36
5		trans 28 cis 2	1*S*,2*S* 1*R*,2*S*	31 33	16 16
6		trans 21 cis 4	1*S*,2*S* 1*S*,2*R*	25 68	16 16
7		27-30	1*S*	16-36	16
8		trans 24		0	16
9	$PhCH_2CH_2$-S-CH_3	72	*S*	30	19
10	$PhCH_2CH_2CH_2$-S-CH_3	73	*S*	34	19
11		61	unknown		17
12	SCN-$(CH_2)_3$-S-CH_3	24	*S*	68	23
13	SCN-$(CH_2)_4$-S-CH_3	45	*S*	93	23
14	SCN-$(CH_2)_5$-S-CH_3	34	*S*	88	23
15	SCN-$(CH_2)_6$-S-CH_3	33	*S*	90	23
16		47	*S*	>95	23

17		61	*S*	88	23
18		50	*S*	>95	23
19		62	*S*	>95	23

pocket, illustrated by the efficient biotransformations of entries 16–19, Table 3. An alternate binding mode, presented in Figure 8, implies the presence of a polar site in H_L and is discounted for reasons discussed below. Measurement of parameters from energy-minimized models suggests an optimum distance from site P to the oxidizing centre of 8–10 Å.

The predominant regio- and stereochemistry of oxidation of substituted dithianes and dithiolanes (entries 1–8, Table 3) can also be accounted for by the model as shown in Figure 9. Binding of the substrate with a large C-2 alkyl group in H_L and the ring residues in H_S leads to attack occurring, as observed, preferentially at the equatorial lone pair of the pro-*S* sulfur, shown in Figure 9 for entry 6. The formation of racemic and close to racemic products from entries 1–3 may be accounted for by a rotation of 180° of the near-planar and relatively small dithiolane groups within H_S as shown in Figure 10 along the alkyl–C-2 bond, whereas formation of racemic product from entry 8 may be attributable to the inability of this substrate to bind preferentially as shown in Figure 9 due to the extra bulk of the C-2 methyl group in the restricted region of H_L. The sulfoxidation of pergolide (entry 11) gives sulfoxide of unknown stereochemistry, but the distance from sulfur to the putative binding site (the indole nitrogen) of 9.5 Å is within the limits of the model.

The biotransformations listed in Table 4 were carried out in order to test several of the features of this model. Sulfoxidations of **2–4** were performed to examine the trend apparent in the biotransformation of the analogous unsubstituted benzyl alkyl sulfides (entries 1–3 and 5, Table 2), where particularly low enantiomeric excesses were observed for n.propyl- and n.butyl-substituted examples. The results presented in Table 2, entry 26 and Table 4, entries 2–4, confirm this trend, which may be attributable to a propitious fit of $n.C_3$ and $n.C_4$ alkyl substituents into H_S leading to the formation of substantial amounts of the *R* sulfoxides. The higher overall enantiomeric excesses observed for the *para*-cyano substituted series also serves to substantiate the importance of the polar binding site P. The latter phenomenon is also influential in sulfoxidations of **14** to **16**, where replacement of the terminal substituent by a methyl group results in a reduction in optical purity of the sulfoxide (c.f. Table 4, entry 13 vs. entries 11 and 12).

The possibility of a polar site in H_L referred to in Figure 8 was examined by the use of substrates **18–21**, which carry a terminal substituent capable of acting as a probe for such a site. The carboxylic acid **20** and its methyl ester **21** underwent substantial β-oxidation during biotransformation, resulting in the isolation of an inseparable mixture of the sulfoxides of **21** and **22**, but **18** and **19** were converted cleanly to the corresponding sulfoxides by *Helminthosporium*. The formation of both these products in very low enantiomeric purities (entries 14 and 15, Table 4), implies the absence of a specific binding site in H_L capable of interacting with the polar substituents present in **18** and **19** (cf. Figure 8); indeed, the low enantiomeric purities of these products (4% and 16% e.e.), compared with that obtained from

Table 4. Biotransformations of sulfides **2–4**, **6–8**, **9–12**, **14–16**, **18–21**, **23** and **26** by *Helminthosporium*

ENTRY	SUBSTRATE		SULFOXIDE		OTHER PRODUCTS (%)
		yield (%)	config.	e.e. (%)	
1	**2**	65	*S*	>95	sulfone (1)
2	**3**	64	*S*	50	sulfone (8)
3	**4**	52	*S*	30	sulfone (6)
4	**6**	25	*S*	72	
5	**7**	25	*S*	26	
6	**8**	10	*S*	33	
7	**9**	20	*S*	10	
8	**10**	45	*S*	84	
9	**11**	25	*S*	26	
10	**12**	44	*S*	16	
11	**14**	61	*S*	92	
12	**15**	70	*S*	80	
13	**16**	65	*S*	54	
14	**18**	46	*S*	4	
15	**19**	21	*S*	16	
16	**20**	10*	unknown		**22** (20)
17	**21**	15*	unknown		**22** (16)
18	**23**	**24** 62 **25** 15	*S* $(\pm)_{phenyl\text{-}S}$,$S_{benzyl\text{-}S}$	76 0, 76	
19	**26**	75	$(\pm)_{phenyl\text{-}S}$,$S_{benzyl\text{-}S}$	0, 66	

* mixture of **21** and **22** sulfoxides obtained after treatment with diazomethane (see Experimental)

benzyl n.pentyl sulfide (70% e.e.: entry 7, Table 2) suggests that substantial binding of the polar substituent group of **18** and **19** at site P in the PHP region, with binding of the benzyl group in H_L, may be occurring, resulting in the formation of *R* sulfoxide.

The cycloalkyl sulfides **5** to **12** were all converted by *Helminthosporium* to *S*-sulfoxides in a consistent manner, with highest optical purity being observed for the ethyl-substituted examples **6** and **10**. This parallels the results obtained from phenyl alkyl sulfides (cf. entries 1–3 and 5, Table 1) and suggests that the cycloalkyl sulfides bind in a manner analogous to that shown in Figure 2, with optimal binding of the alkyl substituent in H_S occurring for the ethyl-substituted examples. High enantiomeric excesses are thus observed for **6**, **10**, and ethyl phenyl sulfide (entry 2, Table 1).

The disulfide **23** was examined to assess the propensity of *Helminthosporium* for sulfoxidations at the benzylic and homobenzylic positions. The major product, **24**, clearly demonstrates a preference for the latter mode of reaction. The bis(sulfoxide) **25** was also obtained as a minor product, but no material arising from monosulfoxidation at the benzylic sulfur was detected. This regioselectivity is difficult to explain on the basis of a steric model, and may be due to electronic differences between the sulfur atoms.

The successful application of a model such as that shown in Figure 1 for the rationalization of whole cell biotransformations is predicated on an assumed relationship between the model and the physical structure of the enzyme responsible for catalysis of the reaction under investigation. Unfortunately, no data are currently available for the *Helminthosporium* sulfoxidase, but as preliminary investigations suggest that a single protein is responsible for these reactions,[37] the proposal of Figure 1 as an active site model is credible. Active site regions that contain a combination of two or more spatially defined hydrophobic binding regions with a polar binding site are common to oxidative enzymes of known (e.g. cyt. $P\text{-}450_{CAM}$)[7,26,39] and unknown (e.g. CMO)[4,28,40] structure, and have frequently been proposed as part of whole-cell derived models.[33] The present proposal for the *Helminthosporium* sulfoxidase fits this pattern, and we therefore suggest Figure 1 as an active site model for the sulfoxidase of

Helminthosporium. Further studies aimed at the characterization of this enzyme and the extension of its use for preparative sulfoxidations are in progress.

Experimental

Apparatus, materials and methods

Melting points were determined on a Kofler heating stage. Infrared spectra were recorded with an Analect 6260FX spectrometer. NMR spectra were recorded at 200 MHz (routine ^1H) or 50 MHz (^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ as internal standard. Enantiomeric ratios were determined at 500 MHz (Bruker AC500) by ^1H NMR analysis in the presence of 3 equivalents of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA).[34] Optical rotations were obtained in the stated solvent at ambient temperature with a Rudolph Autopol III polarimeter. Mass spectra (EI mode) were obtained with a Kratos 1S instrument. Thin layer chromatography was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh.

Maintenance of microorganisms

Helminthosporium species NRRL 4671, obtained from the US Department of Agriculture, Northern Regional Research Laboratories, Peoria, Ill., was maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C.

Preparation of substrates

Benzylalkyl sulfides (**1–4, 17, 19**) were prepared by reaction of the corresponding benzyl chloride with 1.1 equivalents of sodium thioalkylate in ethanol for 4 h at reflux followed by conventional work-up. *Cyclopentyl- and cyclohexyl alkyl sulfides* (**5–12**) were prepared by reaction of the appropriate thiol with an iodoalkane as previously described.[41] *6-Methylthio-2-hexanone* (**14**) was obtained from reaction of 4-cyanobutyl methyl sulfide (**15**) with trimethylaluminium as described.[21] *6-Benzylthio-2-hexanone* (**18**) was similarly obtained from the nitrile **19**. *5-(Benzylthio)pentanoic acid* (**20**) was prepared by conventional (KOH/ethanol/water) hydrolysis of **19**, and the ester **21** obtained therefrom by treatment with diazomethane. All the above compounds gave satisfactory spectral (^1H NMR, ^{13}C NMR, mass) and other analytical data. New compounds were additionally characterised by h.r.m.s. data.

p.Methylthiobenzyl methyl sulfide (**23**). p.Methylthiobenzyl chloride (2.5 g) (obtained by treatment of the corresponding alcohol with thionyl chloride) was added to a solution of sodium thiomethoxide (prepared from 0.34 g of sodium and 0.78 g of methanethiol) in ethanol (20 mL) and the resulting mixture refluxed for 4 h. The solvent was then removed by evaporation and the residue extracted with ether, washed (5% KOH, water), dried and evaporated. Purification by chromatography (2% acetone/hexane, silica gel) gave 2.3 g of pure product, ^1H NMR δ 1.95 (3H, s, CH_2–S–CH_3), 2.4 (3H, s, Ar–S–CH_3), 3.60 (2H, s, CH_2) and 7.1–7.3 (4H, ABq, Ar–H) ppm; ^{13}C NMR δ 17.1, 30.4 (2C), 127.0, 129.3, 129.7, and 145.5 ppm; MS m/z(%) 184(27), 137(100), 122(13).

p.Methylsulfinylbenzyl methyl sulfide (**26**). A solution of sodium thiomethoxide (prepared from 0.15 g of sodium and 0.32 g of methanethiol) in ethanol (10 mL) was added slowly to a solution of p.methylsulfinylbenzyl chloride (1.2 g) in ethanol (8 mL). The mixture was then refluxed for 5 h and worked up as described above to give 1.0 g of product, ^1H NMR δ 1.91 (3H, s, CH_2–S–CH_3), 2.64 (3H, s, Ar–S(O)–CH_3), 3.62 (2H, s, CH_2) and 7.38/7.52 (4H, ABq, Ar–H) ppm; ^{13}C NMR δ 16.1, 39.3, 46.1, 125.1, 131.2, 144.1 and 146.5 ppm; MS m/z(%) 200(100), 185(85), 169(22), 153(60), 138(90), 107(52).

Biotransformations with H. *species*

Two slopes of *Helminthosporium* species NRRL 4671 were used to inoculate 15 1 L Erlenmeyer flasks each containing 200 mL of an autoclaved medium composed of V-8 vegetable juice (200 mL) and calcium carbonate (3 g) per L of distilled water, adjusted to pH 7.2 by the addition of 1 M sodium hydroxide prior to sterilization. The flasks were allowed to stand overnight at 27°C, then placed on

a rotary shaker at 180 rpm, and growth continued for a further 72 h at 27°C. The fungus was then harvested by vacuum filtration (Büchner funnel), and resuspended in 15 1 L Erlenmeyer flasks each containing 200 mL of distilled water, resulting in ca. 90 g (wet weight) of mycelial growth per flask. Substrate (1 g in 30 mL of 95% ethanol) was then distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, 27°C for a further 48 h. The fungus and aqueous medium were then separated by filtration as before, the aqueous medium extracted with dichloromethane (continuous extraction, 72 h), and the fungus discarded. Concentration of the medium extract gave the crude product, which was treated as described below.

Isolation and characterization of products

The crude biotransformation extracts obtained as described above were examined by TLC, using ether or 10% methanol/ether as solvent, and then submitted to flash chromatography using a benzene–ether 10% stepwise gradient, followed by an ether–methanol 5% stepwise gradient. The yields and e.e. values quoted in the tables refer to purified, homogeneous material and, unless otherwise stated, arise from the combination of (only) homogeneous column fractions without further purification (e.g. crystallization) that could lead to changes in stereochemical enrichment values. Products were identified by a combination of NMR and mass spectral analysis. Spectral and optical rotation data for products obtained in this study are listed below under the appropriate substrate heading. Yields and enantiomeric excesses are reported in the tables.

p.Cyanobenzyl ethyl sulfide (2)

p.Cyanobenzyl ethyl sulfone; mp 93–95°C; ^{1}H NMR δ 1.40 (3H, t), 2.94 (2H, q), 4.28 (2H, s), and 7.55/7.70 (2H, Abq) ppm; MS m/z(%) 209(5), 116(100). p.Cyanobenzyl ethyl sulfoxide; mp 107–109°C; ^{1}H NMR δ 1.36 (3H, t), 2.55–2.7 (2H, m), 3.91/3.97 (2H, Abq), and 7.40/7.68 (4H, Abq) ppm; ^{13}C NMR δ 6.7, 44.9, 56.9, 112.4, 118.8, 130.6, 132.5, and 135.6 ppm; MS m/z(%) 193(10), 177(10), 116(100); $[\alpha]_D$ +137.8 (c=1.33, EtOH), +41.6 (c=0.9, chloroform), (*S*), e.e. >95%.

p.Cyanobenzyl n.propyl sulfide (3)

p.Cyanobenzyl n.propyl sulfone; mp 100–102°C; ^{1}H NMR δ 1.08 (3H, t), 1.90 (2H, sext.), 2.87 (2H, t), 4.26 (2H, s) and 7.50/7.70 (2H, Abq) ppm; MS m/z(%) 223(5), 116(100). p.Cyanobenzyl n.propyl sulfoxide; mp 110–114°C; ^{1}H NMR δ 1.09 (3H, t), 1.85 (2H, m), 2.58 (2H, m), 3.90/3.98 (2H, Abq), and 7.40/7.65 (4H, Abq) ppm; ^{13}C NMR δ 13.3, 16.3, 53.6, 57.6, 112.3, 118.5, 130.8, 132.5, and 135.9 ppm; MS m/z(%)207(5), 191(7), 116(100); $[\alpha]_D$ +11.7 (c=0.64, EtOH), +5.8 (c=0.6, chloroform), (*S*), e.e. 50%.

p.Cyanobenzyl n.butyl sulfide (4)

p.Cyanobenzyl n.butyl sulfone; mp 136–138°C; ^{1}H NMR δ 0.97 (3H, t), 1.45 (2H, m), 1.82 (2H, m), 2.40 (2H, t), 4.28 (2H, s), and 7.55/7.72 (4H, Abq) ppm; MS m/z(%) 253(1), 237(4), 116(100). p.Cyanobenzyl n.butyl sulfoxide; mp 73–75°C; ^{1}H NMR δ 0.94 (3H, t), 1.45 (2H, m), 1.72 (2H, m), 2.58 (2H, t of t), 3.90/3.98 (2H, Abq), and 7.38/7.65 (4H, Abq) ppm; ^{13}C NMR δ 13.6, 21.9, 24.5, 51.3, 57.3, 111.9, 118.4, 130,9, 132.4 and 135.9 ppm; MS m/z(%) 237 (0.5), 221(3), 116(100); $[\alpha]_D$ +43.0 (c=1.0, EtOH), +5.6 (c=0.57, chloroform), (*S*), e.e. 30%.

Cyclohexyl ethyl sulfide (6)

Cyclohexyl ethyl sulfoxide; oil, ^{1}H NMR δ 1.2–1.5 (8H, m, including 1.33, t, 3H), 1.65–1.71 (1H, m), 1.79–1.88 (2H, m), 1.88–1.93 (1H, m), 2.06–2.11 (1H, m), 2.48–2.55 (1H, t ot t, CH–S), and 2.57–2.71 (2H, m, CH_2–S) ppm; ^{13}C NMR δ 6.7, 24.6, 25.0, 25.3, 25.4, 26.3, 41.9 and 58.0 ppm; MS m/z(%) 160(8), 144(5), 83(100); $[\alpha]_D$ −27.2 (c=1.0, EtOH), (*S*), e.e. 72%.

Cyclohexyl n.propyl sulfide (7)

Cyclohexyl n.propyl sulfoxide; oil, ^{1}H NMR δ 0.98 (3H, t), 1.2–2.1 (13H, m, including 1.60, sext.), 2.50–2.56 (1H, t of t, CH–S) and 2.60–2.75 (2H, m, CH_2–S) ppm, MS m/z(%) 174 (0.2), 158(2), 83(100); $[\alpha]_D$ −12.6 (c=0.6, EtOH), (*S*), e.e. 45%.

Cyclohexyl n.butyl sulfide (8)

Cyclohexyl n.butyl sulfoxide; oil, ^{1}H NMR δ 1.06 (3H, t), 1.56–2.0 (12H, m), 2.1–2.2 (1H, m), and 2.55–2.62 (4H, m) ppm; MS m/z(%) 204(4), 188(10), 106(70), 83(100); $[\alpha]_D$ −9.6 (c=0.8, EtOH), (*S*), e.e 33%.

Cyclopentyl methyl sulfide (9)

Cyclopentyl methyl sulfoxide; oil; ^{1}H NMR δ 1.4–1.9 (8H, m), 2.10 (1H, m) and 2.45 (3H, s) ppm; MS m/z(%) 132(5), 116 (2), 69(100); $[\alpha]_D$ +6.5 (c=1.8, chloroform), (*S*), e.e. 10%.

Cyclopentyl ethyl sulfide (10)

Cyclopentyl ethyl sulfoxide; oil; ^{1}H NMR δ 1.33 (3H, t), 1.56–1.73 (6H, m), 1.85–1.95 (2H, m), 2.1–2.2 (1H, m); 2.5–2.7 (2H, m, CH_2–S) and 3.0 (1H, m, CH–S) ppm; ^{13}C NMR δ 6.6, 25.1, 25.4, 25.9, 27.1, 43.8 and 58.6 ppm; MS m/z(%) 146 (11), 130(1), 78(100), 69(50). $[\alpha]_D$ −26.6 (c=1.4, EtOH), (*S*), e.e. 84%.

Cyclopentyl n.propyl sulfide (11)

Oil, ^{1}H NMR δ 1.05 (3H, t), 1.5–2.2 (10H, m), 2.56 (2H, t ot t, CH_2–S), and 3.0 (1H, m, CH–S) ppm; ^{13}C NMR δ 13.3, 16.4, 25.4, 25.5, 26.0, 27.3, 52.9 and 59.7 ppm; MS m/z(%) 160(10), 144(3), 92(100); $[\alpha]_D$ −3.4 (c=2.05, EtOH), (*S*), e.e. 26%.

Cyclopentyl n.butyl sulfide (12)

Oil, ^{1}H NMR δ 0.97 (3H, t), 1.3–2.2 (12H, m), 2.60 (2H, t ot t, CH_2–S), and 3.0 (1H, m, CH–S) ppm; ^{13}C NMR δ 13.3, 21.8, 24.6, 25.2, 25.3, 25.9, 27.2, 50.4, and 59.4ppm; MS m/z(%) 174 (12), 158 (4), 69(100); $[\alpha]_D$ −6.9 (c=1.84, EtOH), (*S*), e.e. 16%.

6-Methylthio-2-hexanone (14)

6-Methylsulfinyl-2-hexanone; oil; ^{1}H NMR δ 1.4–1.7 (4H, m), 1.96 (3H, s, $COCH_3$), and 2.2–2.7 (total 10H, m, including 2.36, 3H, s) ppm; ^{13}C NMR δ 21.9, 22.5, 29.8, 38.3, 42.7, 54.0 and 208.0 ppm; MS m/z(%) 162(3), 145(7), 99(100); $[\alpha]_D$ +79.5 (c=1.84, chloroform), +89.6 (c=1.38, EtOH), (*S*), e.e. 92%.

4-Cyanobutyl methyl sulfide (15)

4-Cyanobutyl methyl sulfoxide; oil; ^{1}H NMR δ 1.7–2.1 (4H, m), 2.44 (2H, t), 2.62 (3H, s) and 2.75 (2H, d of t) ppm; ^{13}C NMR δ 16.8, 21.7, 24.3, 38.4, 53.0 and 119.2 ppm; MS m/z(%) 145(20), 82(38), 64(48), 55(100); $[\alpha]_D$ +106.1 (c=1.2, EtOH), (*S*), e.e. 80%.

Methyl n.pentyl sulfide (16)

Methyl n.pentyl sulfoxide; oil; ^{1}H NMR δ 0.92 (3H, t), 1.3–1.55 (4H, m), 1.76 (2H, m), 2.60 (3H, s) and 2.75 (2H, d of t) ppm; ^{13}C NMR δ 12.9, 21.1, 21.3, 30.0, 37.7 and 53.7 ppm; MS m/z(%) 134(4), 117(86), 64(100); $[\alpha]_D$ +66.1 (c=0.5, chloroform), +85.6 (c=0.6, EtOH), (*S*), e.e. 54%.

6-Benzylthio-2-hexanone (18)

6-Benzylsulfinyl-2-hexanone; mp 76–68°C; ^{1}H NMR δ 1.5–1.8 (4H, m), 2.08 (3H, s), 2.40 (2H, t), 2.54 (2H, d of t), 3.90/4.0 (2H, Abq) and 7.2–7.4 (5H, m) ppm; ^{13}C NMR δ 22.0, 22.6, 29.9, 42.8,

50.4, 58.1, 128.3, 128.9, 129.9 and 130.0 ppm; MS m/z(%) 238(3), 222(1), 131(4), 91(100); $[\alpha]_D$ −8.2 (c=0.92, chloroform); 0 (c=0.6 EtOH); (*S*), e.e. 4%.

*Benzyl 4-cyanobutyl sulfide (**19**)*

Benzyl 4-cyanobutyl sulfoxide; oil; ^{1}H NMR δ 1.6–2.1 (4H, m), 2.4 (2H, t), 2.6 (2H, t), 3.95/4.1 (2H, Abq), and 7.2–7.55 (5H, m) ppm; ^{13}C NMR δ 17.4, 22.3, 25.0, 50.0, 58.9, 119.4, 128.9, 129.5, 129.9 and 130.4 ppm; MS m/z(%) 221(2), 205(0.5), 91(100); $[\alpha]_D$ −16.2 (c=0.625, chloroform), +3.1 (c=0.8, EtOH), (*S*), e.e. 16%

*5-(Benzylthio)pentanoic acid (**20**) and methyl 5-(benzylthio)pentanoate (**21**)*

Treatment of the crude extract with excess diazomethane followed by chromatography gave methyl 3-(benzylthio)propanoate; oil; ^{1}H NMR δ 2.3–2.8 (4H, m), 3.5–3.8 (5H, m, including 3.65, s) and 7.1–7.5 (5H, m) ppm; MS m/z(%) 210(30), 176(10), 123(36), 91(100) followed by a mixture of methyl 3-(benzylsulfinyl)propanoate and methyl 5-(benzylsulfinyl)pentanoate; oil; ^{1}H NMR contained signals at δ 3.48, 3.68 (each s, OCH_3) and 3.95/4.06 and 4.0/4.11 (each Abq, $PhCH_2S(O)$); MS M^+ 226 and 254.

*p.Methylthiobenzyl methyl sulfide (**23**)*

p.Methylthiobenzyl methyl sulfoxide; mp 72–74°C; ^{1}H NMR δ 2.45 (3H, s), 2.48 (3H, s), 3.90/3.98 (2H, Abq) and 7.18/7.20 (4H, Abq) ppm; ^{13}C NMR δ 15.0, 36.5, 59.1, 126.0, 126.2, 130.1 and 139.0 ppm; MS m/z(%) 200(0.2), 170(1), 137(100); $[\alpha]_D$ +70.6 (c=0.79, EtOH), −46.3 (c=1.09, chloroform), (*S*), e.e. 76%, and p.methylsulfinylbenzyl methyl sulfoxide; mp 109–112°C; ^{1}H NMR δ 2.50 (3H, s), 2.78 (3H, s), 4.0/4.1 (2H, Abq) and 7.5/7.7 (4H, Abq) ppm; ^{13}C NMR δ 37.3, 43.6, 59.0, 123.7, 130.8, 132.8 and 145.8 ppm; MS m/z(%) 216(2), 199(1), 153(100), 138(22), 107(80); $[\alpha]_D$ +112.5 (c=0.85, EtOH), ($\pm_{phenyl-S}$, $S_{benzyl-S}$), e.e. 76%.

*p.Methylsulfinylbenzyl methyl sulfide (**26**)*

p.Methylsulfinylbenzyl methyl sulfoxide; mp 100–104°C; ^{1}H and ^{13}C NMR, MS as above; $[\alpha]_D$ +102.8 (c=1.0, EtOH), ($\pm_{phenyl-S}$, $S_{benzyl-S}$), e.e. 66%.

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