Chiral Sulfoxidation by Biotransformation of Organic Sulfides

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Contents

Bert Holland is a native of Bolton, England. He studied chemistry and biochemistry at the University of Cambridge, where he received the B.A. degree in 1968. After working with Professor B. T. Golding at the University of Warwick, where he obtained the degree of M.Sc. in molecular enzymology, he moved to the Queen's University of Belfast, Northern Ireland, in 1969, where he first encountered the techniques of biotransformation. In the Department of Organic Chemistry at Queen's, headed by Professor H. B. Henbest, he obtained a Ph.D. degree in 1972, working under the direction of Dr. B. J. Auret. He then moved to Canada, joining the research group of Professor D. B. MacLean at McMaster University, Hamilton, as a postdoctoral fellow and worked on problems of alkaloid biosynthesis, synthesis, and structure elucidation until moving to Brock University as an Assistant Professor in 1976. He is now a Professor in the Chemistry Department at Brock. Since 1976 his research efforts have focused on synthetic and mechanistic aspects of the biotransformation of organic substrates, mainly steroids and sulfides. He is married with two children and outside the laboratory is known as a writer of articles on steam locomotive history and an expert builder of model steam engines.

I. Introduction

For many years, chiral sulfoxides have been prominent among the reagents studied by synthetic organic chemists in the search for higher stereoselectivity in reactions. The sulfoxide functional group activates adjacent carbon–hydrogen bonds toward attack by base, and the resulting anions can be alkylated[1,2] or acylated[2,3] with high diastereoselectivity. Reductions of α-keto sulfoxides,[4] and reactions at positions more remote from the sulfur chiral center,[5] can also proceed with high diastereoselectivity.

The above reactions, coupled with the facile thermal elimination of sulfoxides (which can additionally proceed with transfer of chirality from sulfur to carbon[6]) means that asymmetric sulfoxides have potential as chiral relay reagents. Their development in this role has been limited to date by the absence of a general method for the synthesis of chiral sulfoxides with high enantiomeric purities.[2]

The classical Andersen method, the addition of a Grignard reagent to a resolved sulfinate ester,[7] only gives high enantiomeric purities and acceptable chemical yields in *p*-tolyl-substituted cases (eq 1, R = *p*-

$$R^2MgX + RS(O)OR^1 \longrightarrow RS(O)R^2 \qquad (1)$$

tolyl).[7,8] Sulfoxidation in chiral environments (electrodes,[9] clays,[10] helices,[11] cyclodextrins,[12] solvents[13,14]) is either limited to specific substrates or gives only moderate to low enantiomeric excesses of product, and the use of chiral oxidants can be similarly unrewarding,[15,16] although the recent development of a modified Sharpless reagent by Kagan and co-workers gives synthetically useful chiral sulfoxides in some cases.[17,18] The chiral environment provided by the readily available protein bovine serum albumin (BSA) has been exploited in a preparation of chiral sulfoxides in low to moderate optical yields, which uses normal chemical reagents to perform sulfoxidation in the presence of BSA as a cosolute[19–23] or as a phase-transfer catalyst.[24]

An alternative approach to the preparation of chiral sulfoxides is to exploit the high regio- and stereoselectivity inherent in the reactivity of most enzymes by the use of oxidative enzymes themselves as reagents for sulfoxidation. The use of enzymes as reagents in organic synthesis provides exciting opportunities for the exercise of regio- and stereoselective control.[25] Isolated enzymes may be used as simply as chiral catalysts in many redox or hydrolytic applications,[25] but in the case of oxidative reactions catalyzed by oxygenase enzymes and occurring directly at unactivated carbon or sulfur atoms, whole microbial cells in an actively growing or

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TABLE I. Biotransformation of Aryl and Benzyl Sulfides by *A. niger*

substrate	sulfoxide yield, %	sulfoxide optical purity, %	sulfoxide confign
p-$CH_3C_6H_4S$-t-Bu	20–25	91–100	*S*
p-$CH_3C_6H_4SCH_2C_6H_4$-p-CH_3	4–12	88–100	*R*
$PhCH_2S$-t-Bu	24–65	77–91	*S*
p-$CH_3C_6H_4CH_2S$-t-Bu	44–49	78–88	*S*
p-$CH_3C_6H_4SCH_2Ph$	3–26	56–82	*R*
p-$CH_3C_6H_4S$-i-Pr	7–12	69–71	*R*
p-$CH_3C_6H_4SCH_3$	7–48	32–87	*R*
p-$CH_3C_6H_4S$-n-Bu	5–11	30–34	*R*
$PhCH_2SCH_3$	18	25–46	*R*
$PhCH_2SPh$	9–35	4–27	*S*
p-t-$BuC_6H_4SCH_2Ph$	8	13	*R*

resting state are commonly employed. This technique of "biotransformation" circumvents the problems associated with the isolation of the complex oxygenase enzyme systems and has been extensively used in the study of enzymic sulfoxidation.

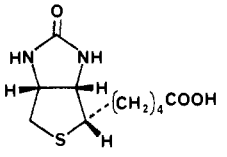
This review will discuss developments in this and other areas of enzymic sulfoxidation since the first intentional use of enzymic methodology to prepare chiral sulfoxides in the early 1960s and provides complete literature coverage to the end of 1986. The biotransformation of thioethers has been previously reviewed briefly in the overall context of either biotransformation,[25] or sulfoxide formation.[2,27-29] This review will include, in addition to synthetic aspects of enzymic sulfoxidation, a discussion of the nature and mechanism of action of the oxygenase enzymes that perform this reaction.

II. Enantioselective Enzymic Oxidation of Sulfides

A. By Fungi

1. Oxidation of Thioethers

The first indication that fungal cultures were capable of oxidizing thioethers to sulfoxides was provided by the observation that the addition of biotin (1) to the



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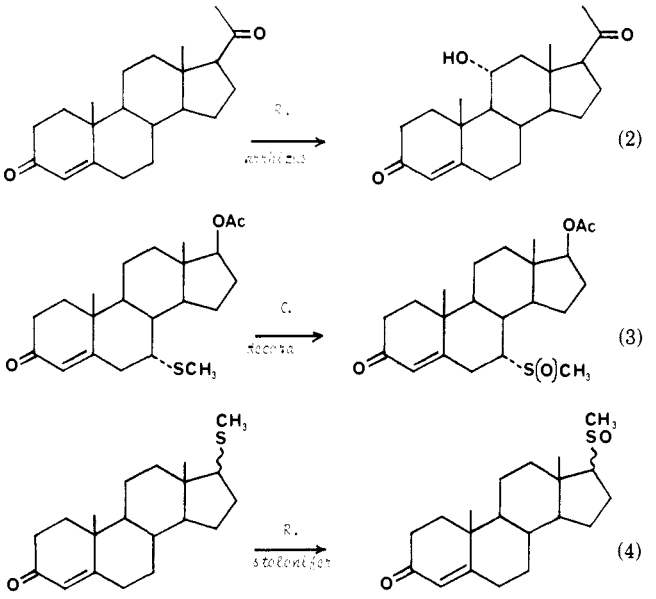
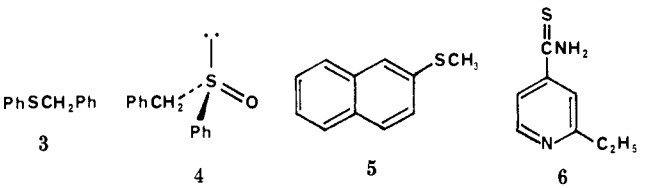
2 [(−)-1 *S*-oxide]

growth media of *Aspergillus niger* resulted in the formation of a new metabolite, identified as biotin *S*-oxide (2).[30] Subsequent investigation of other fungi of the *Rhodotorula*, *Penicillium*, and *Endomycopsis* species demonstrated that these microorganisms were also capable of the sulfoxidation of biotin, accompanied in some cases by partial oxidative degradation of the carboxyl side chain of the substrate.[31-33]

As part of the extensive investigation into the microbial hydroxylation of steroids carried out in the decade following the discovery that *Rhizopus arrhizus* could efficiently introduce the 11α-hydroxy group into pregnanes (e.g. eq 2),[34] the discovery was made that C-7- and C-17-thiomethyl-substituted steroids could be converted stereospecifically into the corresponding sulfoxides by *Calonectria decora*[35] and *Rhizopus stolonifer*,[36,37] respectively, although the absolute stereochemistry at the sulfur atom of the products was not determined in either case (eq 3 and 4).



In 1962, Dodson and co-workers reported the first enantioselective oxidation of an unsymmetrical sulfide, that of benzyl phenyl sulfide (3), to the (*S*)-sulfoxide 4, with an optical purity of 18%, by *A. niger*.[37] The same microorganism was also reported to perform stereoselective oxidation of methyl 2-naphthyl sulfide (5) and to give small quantities of the corresponding sulfone from each substrate. These observations were followed by a brief abstract reporting the "sulfoxidation" of the antitubercular agent 6 by *Septomyxa affinis*[38] and later by the first systematic study of the fungal oxidation of a series of unsymmetrical substituted thioethers, carried out in the laboratories of Professor H. B. Henbest.[39,40]



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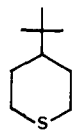
Working with the fungus *A. niger*, Henbest's group examined a series of aryl and benzyl sulfides as candidates for biotransformation. Diaryl sulfides were poor substrates, being recovered unchanged from incubation with *A. niger*, but biotransformation of aryl alkyl substrates was successful and gave the results summarized in Table I. The highest enantiomeric purities were obtained with *p*-tolyl *tert*-butyl sulfide, but although enantiomeric excess was generally dependent upon the steric size of substituents, a clearly predictive relationship did not emerge. In most cases, sulfones were also obtained. The use of an acetone powder preparation of fungus eliminated the formation of sulfone in several cases and in others led to an increased optical purity and material yield of sulfoxide (the upper end of the ranges shown in Table I). In the same study, diastereoselection (80% in favor of the trans isomer) was observed during the sulfoxidation of 4-*tert*-butylthiacyclohexane (7).



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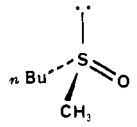
The Belfast group, in collaboration with research workers from Yugoslavia, later extended their study of several of the substrates listed in Table I to include different strains of *A. niger* and *Rhizopus* species and concluded that not only the degree of stereoselection but also the absolute configuration of sulfoxide formation was highly dependent upon the strain and species of fungus used.[41] Thus, benzyl phenyl sulfide (**3**) gave the (*S*)-sulfoxide 4 (ee 5%) with *A. niger* NRRL 337 but the (*R*)-sulfoxide (ee 86%) with *A. niger* NRRL 382, and different *Rhizopus* species could similarly be used to produce predominantly either *S* or *R* enantiomer (from *Rhizopus arrhizus* or *R. stolonifer*, respectively). Again, the highest enantiomeric excesses were obtained from *tert*-butyl-substituted alkyl aryl sulfides using *A. niger* (cf. Table I). This organism was also reported to produce the (*R*)-sulfoxide **8** in moderate enantiomeric



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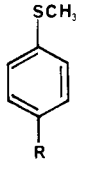
excess but very low yield (ca. 1%) from *n*-butyl methyl sulfide,[41] the first (and so far only) case of enantioselective fungal biotransformation of a dialkyl sulfide to be reported, although recent work has shown that *R. arrhizus* is capable of sulfoxidation of a number of thia fatty acid derivatives.[42] *A. niger* has also been reported to perform asymmetric sulfoxidation of poly(phenyl vinyl sulfides), but in very low chemical and optical yields. *Penicillium notatum* was equally inefficient in oxidizing these polymers.[43]

After a systematic search for microorganisms capable of high enantiospecificity in the oxidation of methyl *p*-tolyl sulfide (**9**, R = CH_3), Sih and co-workers reported in 1978 that *Helminthosporium* species converted this substrate to the *S* enantiomer **10** in 100%



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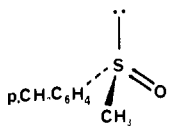

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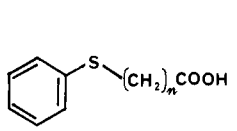
optical purity and 50% chemical yield.[44] Ethyl *p*-tolyl sulfide was transformed in a similarly efficient manner, and *Mortierella isabellina* was reported to produce the corresponding (*R*)-sulfoxides, also stereospecifically and in high yield.[44] The optical purities of the sulfoxides in this study were based on specific rotations; in a later study, Holland and co-workers, using both rotation and chiral ^1H NMR shift reagent data, reached quantitatively different conclusions about the optical purities of the sulfoxides produced by *M. isabellina*.[45] The latter group examined a variety of para- and S-substituted alkyl aryl sulfides,[46,47] and their data, together with that of Sih et al., are summarized in Table II. In general, optical and chemical yields were good, and in

TABLE II. Biotransformation of Alkyl Aryl Sulfides by *M. isabellina*[44-47]

substrate	sulfoxide		
	yield, %	optical purity, %	confign
$PhSCH_3$	65	56.5	*R*
p-$CH_3C_6H_4SCH_3$	52	46[45]	*R*
p-$CH_3C_6H_4SCH_3$	60	100[44]	*R*
p-$C_2H_5C_6H_4SCH_3$	20	90[45]	*R*
p-$CH_3C_6H_4SC_2H_5$	high	high[44]	*R*
p-*i*-$PrC_6H_4SCH_3$	30	82	*R*
p-*t*-$BuC_6H_4SCH_3$	27	76	*R*
p-$OCH_3C_6H_4SCH_3$	50	72	*R*
p-$NO_2C_6H_4SCH_3$	6	20	*R*
p-$FC_6H_4SCH_3$	45	70	*R*
p-$ClC_6H_4SCH_3$	69	90	*R*
p-$BrC_6H_4SCH_3$	66	100	*R*
p-$CNC_6H_4SCH_3$	35	80	*R*
$PhSC_2H_5$	70	84.5	*R*
PhS-*i*-Pr	55	83	*R*
PhS-*t*-Bu	15	60	*R*
PhS-*n*-Pr	52	100	*R*
$PhSCH_2CH{=}CHCH_3$	52		
$PhSCH_2$-c-C_6H_{11}	62		
$PhCH_2SCH_3$	58		
p-$CH_3C_6H_4CH_2SCH_3$	62		
p-$NO_2C_6H_4CH_2SCH_3$	14		

only two cases (p-$BrC_6H_4SCH_3$, p-$NO_2C_6H_4SCH_3$) was sulfone formation observed.

The range of substrates known to be susceptible to asymmetric sulfoxidation has been extended recently by a study of the use of thiophenoxy acids (e.g. **11**), esters, and ketones (e.g. **12**) as substrates for biotransformation by a range of fungi, including *A. niger* and *M. isabellina*.[48] This paper contains mainly qualitative data but claims moderate optical purities (15–45%) for sulfoxide formation.



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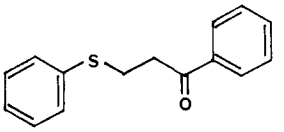

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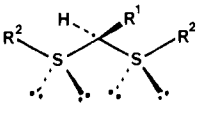
2. Oxidation of Thioacetals

The transformation of thioacetals to mono- or bis(sulfoxides) presents intriguing stereochemical possibilities. In a symmetric thioacetal of an aldehyde other than formaldehyde, the sulfur atoms are enantiotopic, and each contains two diastereotopic nonbonded electron pairs (see **13**). The acetaldehyde thioacetals **14** and **15** have been used as substrates for biotransformation



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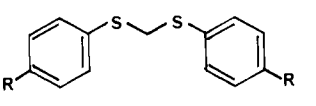
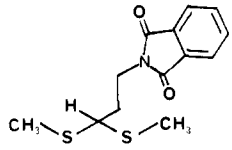


14, R = H
15, R = CH_3

and are converted to sulfoxides by several fungi, but no details of the product stereochemistry were given.[48] In a more detailed study, Balenovic et al., recognizing the implicit stereochemical possibilities, examined the biotransformation of the thioacetal **16** by *A. niger*[49] and identified the products as the pseudoasymmetric (*S*)-*meso*-bis(sulfoxide) **17** and the diastereomeric mono(sulfoxides) **18**/**19** and **20**/**21** formed in a diastereomeric excess (**18**/**19**:**20**/**21**) of 20% and an enantiomeric excess (**18**:**19** and **20**:**21**) of 46%.



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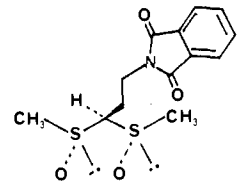

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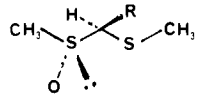

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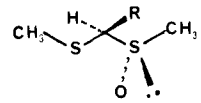

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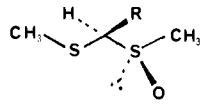

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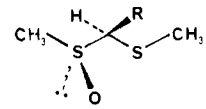

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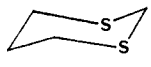
In a systematic study of thioacetal biotransformation, the Belfast group of Auret and Boyd examined firstly 1,3-dithiane (**22**) and the thioacetal **15** as substrates for



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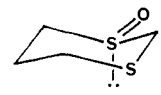

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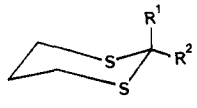
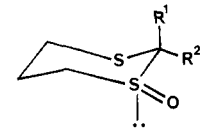
biotransformation by *Aspergillus foetidus* (*niger*), *M. isabellina*, and *Helminthosporium* species.[50] The latter substrate gave a low yield (3%) of low optical purity (*S*)-sulfoxide (20%), only with the latter fungus, and a racemic sulfoxide in very low yield from *A. foetidus*. Poor recoveries of products and starting materials were attributed to further reaction of the hydrolytically labile thioacetal oxides, producing water-soluble species. In contrast, dithiane **22** gave moderate yields of the (*R*)-sulfoxide **23** with *A. niger* but again in low optical purities (17–22%) and the corresponding (*S*)-sulfoxide in low optical and chemical yields from *Helminthosporium*. *M. isabellina*, although an efficient oxidizer of **22**, produced only racemic sulfoxide.

The same fungi were also used in a study of the sulfoxidation of substituted thioacetals **24–27**.[51,52] *Helminthosporium* gave predominantly the *trans*-monosulfoxide products **28** from **24** and **25** and exclusively



24, R^1 = H, R^2 = CH_3
25, R^1 = H, R^2 = *t*-Bu
26, R^1 = CH_3, R^2 = *t*-Bu
27, R^1 = R^2 = CH_3



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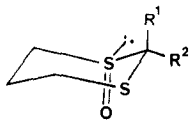
the trans product from **26**. The latter sulfoxide was racemic, but sulfoxides from **24** and **25** had predominantly the 1*S*,2*S* absolute configuration shown (ee 27 and 35%, respectively). The minor *cis*-sulfoxides **29**



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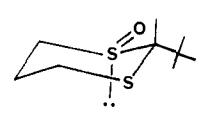

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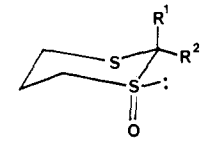

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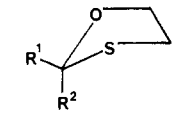
were predominantly the 1*S*,2*R* compounds shown. The symmetrical substrate **27** was oxidized to the (-)-sulfoxide in low optical purity (36%). The other fungi studied (*A. foetidus*, *M. isabellina*) gave similar results with **27** and similar cis to trans product ratios with substrates **24** and **25**, although the overall product recoveries were generally lower from *M. isabellina*. The *trans*-sulfoxide **30** was obtained in low ee (8%) from oxidation of **26** by *A. niger*, but no sulfoxidation of **26** by *M. isabellina* was apparent. The optical purities of the *trans*-sulfoxides produced by *A. foetidus* and *M. isabellina* were generally low (0–19%), and the absolute configuration of product formation was not related to substrate structure in any consistent manner. The *cis*-sulfoxides (**29**, **31**) were generally produced in low chemical and optical yields, although **25** gave the sulfoxide **29** in an enantiomeric purity of 72%.

3. Oxidation of Oxathiolanes

The fungal oxidation of a series of 1,3-oxathiolanes **32** has recently been studied.[54] The sulfoxides were generally unstable, but phenyl hydrogen, phenyl methyl, and phenyl *tert*-butyl substituted examples were isolated in low chemical (20–40%) and optical (0–10%) yields from incubation of the corresponding oxathiolanes with *M. isabellina*.



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4. Deoxygenation of Sulfoxides

In addition to the possibility that the optical purity of sulfoxides produced by fungal biotransformation can be dependent upon the degree of further oxidation to sulfone (see III), some fungi also have the capability of performing the reduction of some sulfoxides to sulfides, with selection for one stereoisomer of substrate, concomitant with the oxidation reaction.[50–53] Selective removal of one enantiomer of sulfoxide by *M. isabellina* has been demonstrated by the recovery of both thioacetal **22** and optically enriched starting materials following the use of racemic sulfoxide (±)-**23** as substrate for biotransformation.[50]

A similar result was obtained following incubations of racemic sulfoxides (±)-**28** (R^1 = H, R^2 = CH_3), (±)-**29** (R^1 = H, R^2 = CH_3), and (±)-**28** (R^1 = H, R^2 = *t*-Bu) with *A. foetidus* (but not *M. isabellina* or *Helminthosporium*),[52] but whether the optical enrichment of recovered sulfoxide was caused by stereoselective deoxygenation or by selective further oxidation (see III) was not determined. In any event, it is apparent that enzymic deoxygenation of sulfoxides is not a general process, but since it can significantly influence the optical purity of isolated sulfoxide in some cases, its possible contribution to the overall process cannot be ignored.

B. By Bacteria

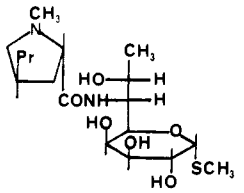
1. Oxidation of Thioethers

Sulfoxidation of the antibiotic lincomycin (**33**)[55] and the closely related clindamycin[56] by *Streptomyces*

TABLE III. Biotransformation of Sulfides by *C. equi*[57-59]

substrate	sulfoxide yield, %	sulfoxide optical purity, %	sulfoxide confign	sulfone, %
PhS-*n*-$C_{10}H_{21}$	25	99	*R*	31
PhS-*n*-C_4H_9	29	100	*R*	0
$PhSCH_3$	100	75	*R*	0
$PhSCH_2CH{=}CH_2$	38	100	*R*	27
p-$CH_3C_6H_4$S-*n*-$C_{10}H_{21}$	55	92	*R*	10
p-$CH_3C_6H_4$S-*n*-C_4H_9	79	87	*R*	3
p-$CH_3C_6H_4SCH_3$	33	82	*R*	0
p-$CH_3C_6H_4SCH_2CH{=}CH_2$	67	92	*R*	23
$PhSCH_2CH{=}CH_3$	22			35
$PhSCH_2CH{=}CH$-*n*-Pr	8			35
$PhSCH_2CH{=}CHPh$	7			37
p-$OCH_3C_6H_4SCH_2CH{=}CHPh$	41			1
o-$OCH_3C_6H_4SCH_2CH{=}CHPh$	0			0
$PhCH_2S$-*n*-$C_{10}H_{21}$	73	100	*R*	0

species provided the first indications that bacteria possessed enzyme systems capable of oxidation at

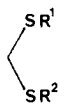


33

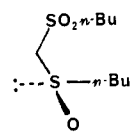
sulfur, but this process was not systematically investigated until relatively recently when Ohta et al. turned their attention to biotransformation of alkyl aryl sulfides by *Corynebacterium equi*.[57-59] Their data are summarized in Table III. In cases where optical purity is reported, a strong preference for production of the *R* enantiomer is evident. The production of sulfone in most incubations suggests that this selectivity may be due, at least in part, to stereospecific sulfoxide oxidation.[57] The possibility of optical enrichment by stereospecific sulfoxide reduction was not examined, but in other circumstances (reduction of biotin sulfoxide by *Pseudomonas*) specific reduction is known to occur.[60,61]

2. Oxidation of Thioacetals

The formaldehyde thioacetals **34–39** have been examined as substrates for biotransformation by *C. equi*.[62] In most cases, only sulfone products were formed, but **36** gave the chiral sulfone (*R*)-sulfoxide **40** in 70% chemical and >95% optical yields and **39** gave the corresponding (*R*)-mono(sulfoxide) in similarly good yields.



34, $R^1 = R_2 = Ph$
35, $R^1 = R^2 = n\text{-}C_8H_{17}$
36, $R^1 = R^2 = n\text{-}C_4H_9$
37, $R^1 = Ph$, $R^2 = n\text{-}C_8H_{17}$
38, $R^1 = Ph$, $R^2 = n\text{-}C_4H_9$
39, $R^1 = Ph$, $R^2 = CH_3$



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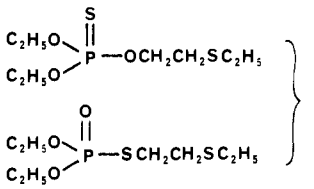
C. By Isolated Enzymes

Although the use of isolated oxidase enzymes cannot yet rival the technique of microbial biotransformation in preparative-scale applications, there has nevertheless been consistent study of the use of such enzymes of mammalian origin for chiral sulfoxidation. Interest in this field followed the early observations that oxidative enzymes are clearly implicated in the metabolic formation of sulfoxide derivatives of sulfur-containing pharmaceuticals or pesticides. Examples include the oxidation of chlorpromazine by peroxidase and catalase[63] and by guinea pig liver microsomes,[64] the metabolism of phenothiazine,[65] promazine,[66] and pergolide,[67] and the oxidation of the insecticide systox (**41**).[68]



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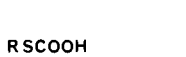
Enzymic sulfoxidation of sulfur containing amino acids such as cysteine derivatives[69] led to the partial purification of enzymes from rat liver homogenates capable of efficient sulfoxidation of a series of α-thiocarboxylic acids **42**;[70] mammalian livers had previously been identified as a rich source of oxidase enzymes,[71] capable of the sulfoxidation of thioethers.[41] Liver microsomal enzyme preparations cannot be assumed to be homogeneous unless so stated and may contain oxidizing enzymes of differing stereoselectivities and substrate specificities. The problems encountered in the use of crude enzyme preparations for sulfoxidations were recognized in an early paper on the oxidation of *p*-thioanisidine (**43**) by mammalian enzymes,[53] which identified in rat liver homogenate not only sulfoxidizing enzymes but also enzymes capable of oxidizing the (*R*)- and (*S*)-sulfoxides of **43** to sulfone (at different rates), in addition to an enzyme that selectively reduced the (*S*)-sulfoxide.



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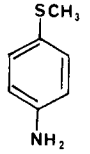
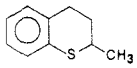
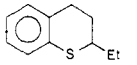
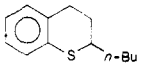
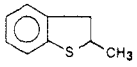
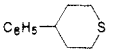
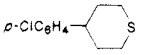


43

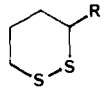
Working with purified preparations from rabbit liver, Iyanagi and Oae have studied both mechanistic and preparative aspects of sulfoxidation by an enzyme identified as a cytochrome P-450 dependent monooxygenase (see V.A).[72] The rabbit liver enzyme is capable of sulfoxidation of 1,2-dithianes **44** and thianes **45** and **46** to monosulfoxides and sulfones (in the case

TABLE IV. Biotransformation of Sulfides by Rabbit Liver Microsomal Cytochrome P-450

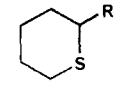
substrate	sulfoxide cis:trans	sulfoxide optical purity, %	sulfoxide confign
		11	R
	18:82		
	18:82		
	16:84		
		3	R
	19:81	40 (cis) 12 (trans)	R_SR_C S_SR_C
	37:63		
	29:71		
	34:66		
	33:67		
$PhCH_2S$-*sec*-Bu	34:66[a]		
$PhCH_2S$-*t*-Bu		54	*R*
$PhCH_2S$-*n*-Bu			*R*
$PhCH_2SC_6H_4$-*p*-CH_3		22	*S*
$PhCH_2CH_2S$-*t*-Bu			*R*
p-$CH_3C_6H_4CH_2S$-*t*-Bu		20	*R*
p-$CH_3C_6H_4S$-*t*-Bu		47	*S*
p-$CH_3C_6H_4SCH_3$		14	*R*
$C_8H_{17}S$-*t*-Bu			*R*
$PhSC_6H_4$-*o*-OMe		10	*S*

[a] Ratio of threo to erthyro sulfoxides.

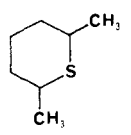
of **44**) and to sulfoxides (from **45** and **46**),[72,73] in yields between 10 and 30%.[73] The products from **45** were formed as cis/trans mixtures in almost equal amounts, but their absolute stereochemistry was not investigated. The same enzyme system was then used in a study of the sulfoxidation of *p*-thioanisoles **47**,[74,75] but again no details of absolute stereochemistry were given.



44



45, R = H, CH_3, C_2H_5, C_8H_{17}, OCH_3



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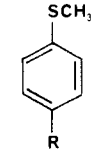
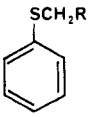


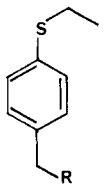
47, R = H, CH_3, OCH_3, Cl, NO_2

In a later study designed to investigate the stereochemical preferences of their enzyme, Iyanagi and Oae studied the range of substrates listed in Table IV.[76-78] The diastereomeric excesses observed in enzymic sulfoxidation of the 2-substituted thiochromans and benzodihydrothiophene were superior to those given by peracid reagents, and although a preference for *R* sulfoxidation was observed in most cases where absolute stereochemistry was examined, the optical purities were only moderate. The yield of sulfoxide in these studies was expressed only in terms of enzyme turnover parameters, and the same group identified a possible source of material loss when sulfides **48** were used as substrate. In the presence of an electron-withdrawing group (R = CN, *p*-$NO_2C_6H_4$, COPh), oxidation at the methylene carbon occurred along with sulfoxidation, leading to S-dealkylation via hemithioacetal hydrolysis.[79]

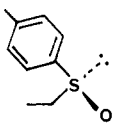


48, R = Ph, CN, *p*-$NO_2C_6H_4$, COPh

Cytochrome P-450 dependent enzymes purified from rat liver have been studied by Walsh and co-workers using ethyl *p*-tolyl sulfide (**49**) as substrate.[80] Two different isozymes both gave the (*S*)-sulfoxide **50** (ee 58 and 68%) as the predominant product, together with minor amounts of the benzyl alcohol **51**. In view of the fact that the racemic sulfoxide ((±)-**50**) was not oxidized to sulfone at a significant rate by the rat liver enzymes, the lack of stereospecificity in oxidation of **49** was attributed to an inherent lack of stereospecificity of the enzyme itself.[80]



49, R = H
51, R = OH



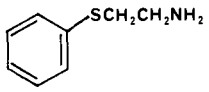
50

In contrast to the predominant formation of the (*S*)-sulfoxide **50** by oxidation of **49** with rat liver cytochrome P-450, a flavin-dependent monooxygenase from hog liver produced the corresponding (*R*)-sulfoxide in high optical purity (90%).[81] Walsh et al. have therefore suggested that the optical purity of sulfoxide formation by crude liver microsomes may reflect the relative activities of the flavin and cytochrome P-450 containing monooxygenases, present in the enzyme preparation,[81] and a method of distinguishing between these two activities based on the degree of S- vs. C-oxidation of phenacyl phenyl sulfide (**48**, R = COPh) has been developed.[82]

Other isolated enzymes known to oxidize sulfides are cyclohexanone monooxygenase from *Acinetobacter*, which produces **50** (ee 64%) from **49**,[81] and dopamine β-hydroxylase, which is thought to be stereospecific in converting the neuroamine analogue **52** to the (*S*)-sulfoxide.[83] Sulindac sulfide (**53**) is converted to the (+)-sulfoxide, provisionally assigned the *R* absolute stereochemistry, in high optical purity by hog liver flavin containing monooxygenase and less efficiently by rat liver microsomal enzymes.[81]

III. Enantioselective Enzymic Oxidation of Sulfoxides

As an alternative to chiral oxidation of sulfides, the enantioselective biotransformation of racemic sulfoxides



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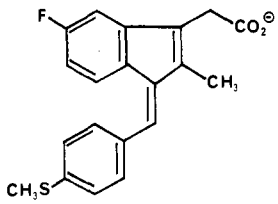


53

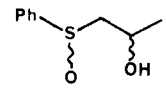
to sulfones has been studied as a method for the preparation of chiral sulfoxides. This process may accompany the sulfide–sulfoxide conversion, as discussed above, and in such cases the degree of sulfone formation can influence the optical purity of the isolated sulfoxide. However, as examples of sulfoxide–sulfone biotransformation have been reported showing no enantioselectivity for substrate conversion,[51] in addition to those that do (see below), no general conclusions regarding the steric interrelationship of the two oxidation steps are possible.

Working with the fungus *A. niger*, Henbest's group observed that the substrate stereoselectivity in the oxidation of several of the racemic sulfoxides of Table I was generally low (0–45%)[84] and dependent upon the strain of *A. niger* employed.[41] With one exception, the optical purity of recovered sulfoxide was inferior to that obtained by use of the corresponding sulfide as substrate.[40] The exception, benzyl methyl sulfoxide, gave a 12% recovery of high optical purity (ee 95%) (*S*)-sulfoxide from incubation with *A. niger* NRRL 382.[41] Although the other sulfoxides recovered from biotransformation of the racemates all had the *R* absolute configuration (unless racemic), this did not always correlate with the predominant enantiomer produced by sulfide oxidation (see Table I), suggesting that, in these cases, the enantioselection present in sulfoxide oxidation may be responsible for a lowering of the observed optical purity of sulfoxide isolated from sulfide biotransformation.

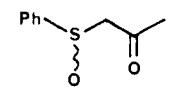
Low stereoenrichments were also observed in substrates recovered from metabolism of the sulfoxide (±)-**23** and the corresponding *cis*-bis(sulfoxide) by the fungi *A. foetidus*, *Helminthosporium* species, and *M. isabellina*[50] and attributed to preferential oxidation of one enantiomer to sulfone. The details of this process were obscured in the latter case by the concurrent stereoselective reduction of sulfoxide to sulfide by *M. isabellina* (see II.A.4), but with the other fungi studied the predominant absolute configurations of sulfoxide remaining after sulfone production were identical with those produced by sulfide oxidation, suggesting that the latter process may not be directly responsible for the isolation of optically enriched sulfoxide following biotransformation of **22**.[50] In contrast, racemic **28** (R^1 = R^2 = CH_3) recovered from incubations with all three fungi mentioned above showed no optical enrichment,[52] although it is likely that the optical enrichments observed in sulfoxides **28** (R^1 = H, R^2 = CH_3; R^1 = H, R^2 = *t*-Bu) and **29** (R^1 = H, R^2 = CH_3) recovered from incubation with *A. niger* are attributable to the preferential destruction of one enantiomer of the substrate by conversion to the readily hydrolyzed bis(sulfoxide).[52]

Of a range of microorganisms screened for ability to oxidize sulindac (**53** sulfoxide) to sulfone, two (*Aspergillus alliaceus*, *Nocardia corallina*) were able to carry out this transformation, but no stereochemical details were reported.[85] Several of the other microorganisms studied reduced the sulfoxide to give **53** (see II.A.4).

The use of other enzyme systems for the enantioselective oxidation of sulfoxides to sulfones remains largely undeveloped. A report of the conversion of sulfoxide **54** to sulfone by *C. equi*[86] records stereose-



54

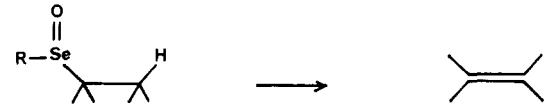


55

lection for the *R* enantiomer at the alcohol carbon of substrate but provides no details of the steric requirements at sulfur, although the corresponding enantiomeric keto sulfoxides **55** can be distinguished on the basis of their enzymic reduction at the carbonyl group by alcohol dehydrogenase enzymes of the same bacterium.[86] The rabbit liver monooxygenase enzyme system of Oae and Iyangi is capable of oxidizing dithiane sulfoxides 44[72] and the aryl sulfoxides corresponding to **48** to sulfones,[87] but no details of stereochemical preferences have been reported.

IV. Biotransformation of Selenides

Although most selenoxides undergo rapid and reversible acid-catalyzed hydration, with consequent racemization at selenium,[88] their decomposition to olefins (eq 5 and 6) may be even faster and may occur with transfer of chirality from selenium to carbon according to eq 6.[89] An efficient method of chiral selenoxide synthesis therefore has potential as a general route to chiral alcohols or olefins of defined stereochemistry.



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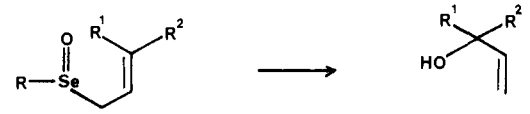


(6)

The biotransformation of selenides as a route to chiral selenoxides was first examined by Watson and Boyd,[90] who used phenyl benzyl selenide (**56**) as a substrate for *A. niger*, a fungus known to oxidize the corresponding sulfide to sulfoxide (Table I). The only isolable product, in low yield, was benzeneseleninic acid, formed by debenzylation, which probably proceeded via hydroxylation at the methylene carbon. In a more recent systematic investigation of selenide biotransformation by fungi, Holland and Carter first examined phenyl methyl selenide (**57**), using four different fungi of established sulfoxidizing capability.[47] Although control experiments established that phenyl methyl selenoxide was stable under the conditions used, no evidence was obtained for its formation from **57**, the predominant route for the latter's metabolism being by demethylation. A further series of experiments designed to investigate the formation of decomposition products following selenoxidation of **58** and **59** also failed to provide any evidence for microbial selenoxide formation.

The first example of enzymic oxidation of selenide to selenoxide was provided recently by Walsh et al., who

Ph—Se—R

56, R = CH_2Ph
57, R = CH_3
58, R = $CH_2CH{=}CHCH_3$
59, R = CH_2-c-C_6H_{11}
60, R = $CH_2C{\equiv}CH$
61, R = *cis*-$CH_2CH{=}CHCH_2CH_2CH_3$
62, R = *trans*-$CH_2CH{=}CHCH_2CH_2CH_3$

reported that cyclohexanone oxygenase of bacterial origin is capable of oxidizing the selenium atom of the selenides **60–62**.[91] The resultant selenoxides underwent spontaneous sigmatropic 2,3-rearrangements according to eq 6, but the alcohols resulting from **61** and **62** were achiral. The possibility that racemization at selenium may be faster than selenoxide decomposition, however, means that this result does not preclude stereoselective selenoxide formation by the enzyme.

V. Mechanisms of Enzymic Sulfur Oxidation

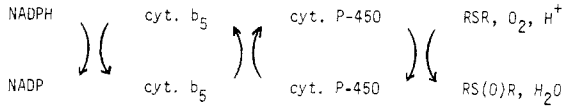
A. Nature of the Enzymes

In general, organic sulfides, along with other xenobiotic organic compounds, are oxidized both in vitro and in vivo by one or both of two types of monooxygenase enzymes, those dependent upon cytochrome P-450 for oxygen activation and transfer and those using a flavin molecule for this purpose. Thse enzymes are considered to be performing a detoxification role by enhancing the water solubility of the substrate, so the enzymes of mammalian origin are located primarily in the liver. Fungal and bacterial monooxygenases are thought to be carrying out a similar function. The oxygenation of organic xenobiotics by other types of enzymes (e.g. the copper-containing dopamine β-hydroxylase) is much rarer and probably nonphysiological and will not be considered here. The mammalian enzyme preparations referred to above (section II.C) have been well characterized, whereas enzymes of fungal origin (II.A) have not been isolated, and no information is available on the nature of the bacterial sulfoxidizing enzymes (section II.B).

The rabbit liver microsomal enzyme of Iyangi and Oae was characterized as a typical cytochrome P-450 dependent monooxygenase, functioning according to the stoichiometry of eq 7.[72] The activities of the cyto-

$$H^+ + NADPH + RSR + O_2 \longrightarrow NADP^+ + RS(O)R + H_2O \quad (7)$$

chrome P-450 oxygenase and the NADPH-cytochrome P-450 reductase enzymes of this liver microsomal complex have been separated, and the reconstituted system is an active sulfoxidizer,[72] which functions with the same relative and absolute stereochemical preferences as the cruder microsomal preparation.[76,78] The rat liver enzymes described by Walsh et al. are also cytochrome P-450 monooxygenases and function in a reconstituted system together with the necessary electron-transport proteins (eq 8).[80] In this case, the cytochrome P-450 proteins are chromatographically purified isozymes.[80]



(8)

The hog liver enzyme described by Walsh[81] and Oae[82] and the bacterial enzyme cyclohexanone monooxygenase[81] are flavin-dependent enzymes, which use

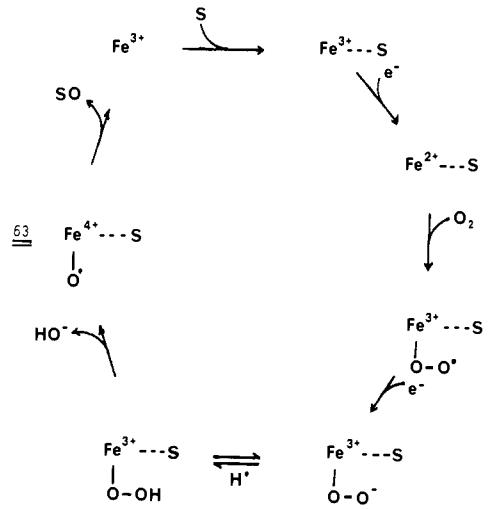


Figure 1. Catalytic cycle of cytochrome P-450 dependent monooxygenases (S = substrate).

flavin adenine dinucleotide (FAD) as the oxygen activating and transfer agent and also require NADPH for activity. These enzymes function according to eq 9, and both are highly purified, homogeneous proteins.

$$NADPH + H^+ + O_2 + RSR \xrightarrow[FAD]{} RS(O)R + H_2O + NADP \quad (9)$$

Although fungi are a rich source of cytochrome P-450 reactivity[92] and are capable of performing many of the same oxidative reactions as mammalian liver microsomal enzymes,[67,93] the technical difficulties associated with the isolation of oxygenases from fungi have frustrated their complete characterization. Some progress has recently been made in the purification of steroid hydroxylases from fungi,[94] but isolated sulfoxidizing enzymes of fungal origin have yet to be described. It has generally been assumed that the fungal enzymes responsible for oxidations of sulfides and sulfoxides are cytochrome P-450 containing monooxygenases, and the sulfoxidizing enzyme of *M. isabellina* has been shown to incorporate molecular oxygen directly into the product,[46] but in the absence of further data on the enzymes concerned, their exact nature remains speculative. Bacteria can contain both cytochrome P-450[95] and flavin-dependent[96] monooxygenases, but no data are available on the oxygenase enzymes of *C. equi* or the other bacteria discussed in section II.B.

B. Catalytic Cycles

1. Cytochrome P-450 Oxygenases

Cytochrome P-450 dependent monooxygenases have been intensively studied in recent years. Their catalytic cycle, which was originally deduced largely from a study of the camphor hydroxylase of *Pseudomonas putida* (Cyt-P-450$_{CAM}$), is presented in Figure 1.[97] The available evidence suggests that all cytochrome P-450 dependent monooxygenases function by a similar mechanism.[98] The first step, substrate binding, is necessary before reduction of the heme iron by one electron, provided ultimately by NADPH, can occur. Subsequently, oxygen binding is followed by transfer of the second electron from NADPH, also via the electron-transport system (flavin nucleotide, ferredoxin (iron–sulfur proteins), and/or cytochrome b_5), to generate, following internal electron transfers, a highly

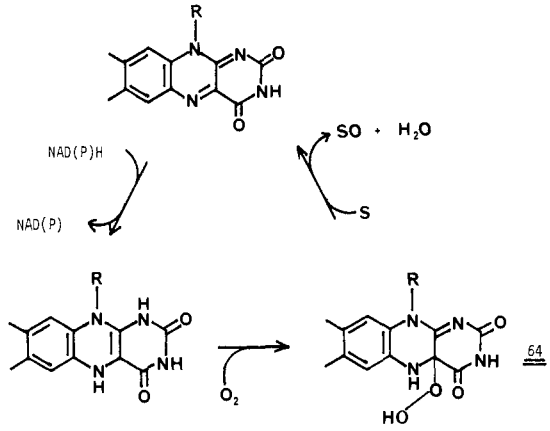


Figure 2. Catalytic cycle of flavin-dependent monooxygenases.

reactive oxidizing species here formulated as **63**. The nature of the possible reactions between **63** and a sulfide substrate is discussed in section V.D.

2. *Flavin-Dependent Oxygenases*

The flavin-dependent monooxygenases involve the intermediacy of a flavin–oxygen adduct, produced according to Figure 2, which then reacts directly with substrate. The nature of this adduct has been the subject of much speculation.[99] In the case of the sulfide-oxidizing enzymes, it has been proposed that the 4a-hydroperoxide **64**[82,100] is involved, but the possibility exists that the species actually reacting with the sulfide is not **64** itself, but another intermediate[99] derivable therefrom in a kinetically hidden step.[101]

C. Substrate Binding

The existence of a multitude of cytochrome P-450 enzymes that function with different substrate specificity and regio- and stereospecificities, and yet are all dependent on the same cofactors, is now well established in mammalian systems.[102] The close similarity between oxygenations performed by mammalian and fungal systems[103] suggests that a parallel state of affairs may exist in the microbial world.

The role of cofactors and polypeptide (apoenzyme) may be distinguished as follows: the cofactors are responsible for the binding of oxygen, its activation, and delivery to the substrate of the oxidizing species; and the apoenzyme is responsible for binding the substrate. The apoenzyme therefore controls the substrate specificity and regio- and stereospecificities of the oxygenation reaction, and presumably a variation in this portion of the enzyme is responsible for the range of substrate specificities and observed products. Substrate does not normally bind directly to the heme unit but is bound by the apoprotein in close proximity to the cofactor, although thiane (**45**, R = H) is reported to induce spectral changes in the rabbit liver cytochrome P-450 enzyme characteristic of a direct heme iron–sulfur interaction.[72]

The nature of the interaction between the apoenzyme and the substrate is the least well understood aspect of oxygenase reactions. A moderate-resolution X-ray structure is available for the P-450$_{CAM}$ enzyme–substrate complex, which shows substrate camphor fitting tightly into a hydrophobic cavity of the enzyme and oriented toward the cofactor by H-bonding between the enzyme and substrate carbonyl such that hydroxylation

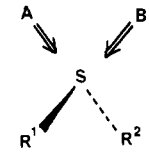


Figure 3. Stereoselectivity in enzymic sulfide oxidation.

becomes regio- and stereoselective.[104] In the case of steroidal substrates, binding by hydroxylase enzymes is thought to involve both hydrophobic interactions and interactions between the enzyme and the oxygen substituents of the substrate.[105]

The correlations that exist between the hydrophobic character of the substrate and both the rate of its oxidation and the spectral changes it induces on binding to the enzyme suggest that hydrophobic interactions play an important role in the binding of cytochrome P-450 substrates.[106] These relationships have been established for the thiane substrates **45** and the rabbit liver microsomal enzyme,[72] where the maximum oxidation rate was observed for **45** (R = C_8H_{17}), and a positive correlation was established between the hydrophobicity of the substrate, measured by the Hansch lipophilicity parameter π[107] and the V_{max} for sulfoxide formation.[73] The same reasoning has been used to explain the low rates of sulfoxide–sulfone oxidation observed with mammalian liver enzymes.[72,80] The observation from Tables I–III that fungal and bacterial oxidation of sulfides to sulfoxides is most efficient (in terms of overall yield) when the substrate carries large hydrophobic substituents (e.g., neopentyl, cyclohexyl, and *n*-decyl), and lowest when polar groups (e.g., NO_2) are present, is generally supportive of the conclusions derived from the mammalian enzymes.

If the assumption is made that hydrophobic binding interactions between enzyme and substrate are specific for either substituent, R^1 or R^2, and that the direction of oxygenation is fixed by the three-dimensional relationships between the bound substrate and the heme cofactor to occur either at the *pro-R* or *pro-S* position of sulfur (directions A or B, Figure 3), then the lack of absolute stereoselectivity observed for most enzymic sulfoxidations is potentially problematical. There are at least three alternative explanations for this phenomenon: First, in whole organisms or crude enzyme preparations there may be more than one sulfoxidizing enzyme. Simultaneous sulfide oxidation by two enzymes, operating with different stereospecificities and rates, could account for the production of both enantiomers of sulfoxide. Alternatively, there may be only a single enzyme, operating with either nonspecific substrate binding followed by specific oxidation or a nonspecific direction of oxidation following specific binding of the substrate.

The question of the involvement of several different isozymes, perhaps of different stereospecificities, has been considered for the mammalian liver enzyme preparations (section II.C). The sulfoxidizing cytochrome P-450 enzyme purified from rabbit liver showed relative and absolute stereochemical preferences during the oxidation of several sulfides, which were identical with those observed when a crude microsomal preparation was employed,[76,78] suggesting that if the crude preparation did contain several sulfoxidizing enzymes, then they possessed similar stereoselectivities. Both the

rat[80] and hog[81] liver enzymes described by Walsh were homogeneous proteins, but neither was entirely stereospecific in sulfur oxidation nor was the highly purified cyclohexanone monooxygenase.[81] These results suggest an intrinsic lack of absolute stereospecificity in the sulfoxidation reaction, but whether this comes about as a result of nonstereospecific substrate binding or nonstereospecific oxidation has yet to be determined, although recent data on the benzylic hydroxylation of ethyl benzene by a rat liver cytochrome P-450 enzyme suggest that the former explanation is probably correct.[108]

The stereochemical preferences for sulfoxide formation by the rabbit liver[78] and rat liver[80] cytochrome P-450 enzymes can be summarized by Figure 3 where R^2 is larger than R^1 and oxidation occurs preferentially but not exclusively from direction A. The FAD-containing hog liver enzyme, on the other hand, attacks preferentially from direction B, and it has been suggested that this stereochemical difference can be used as a means of characterizing the cofactor dependance of sulfoxidizing enzymes.[82]

In the absence of any data on isolated sulfoxidizing enzymes from fungi or bacteria, the question of oxidation by multiple enzymes cannot be definitively answered. The lack of any uniform correlation between the size of substituents and the predominant direction of oxidation by *A. niger* is apparent from Table I.[40] For example, benzyl phenyl sulfide gives predominantly the (*S*)-sulfoxide, but when both aromatic rings carry a para methyl substituent, the predominant configuration of sulfoxide formation is *R*. These results are difficult to explain on the basis of catalysis by a single enzyme but do not exclude the possibility: most of the oxidations listed in Table I are in fact compatible with Figure 3, where $R_2 > R^1$ and oxidation occurs predominantly from direction B.

The same parameters apply to sulfoxidation by *C. equi* (Table III)[56-58] and to most of the sulfoxidations carried out by *M. isabellina* (Table II).[44-47] In the case of the latter fungus, however, the exclusive formation of (*R*)-sulfoxides from a range of alkyl aryl sulfides argues strongly for a specific binding pocket for each substituent, with oxidation predominantly but not exclusively from one direction. This can be represented by the proposal of Figure 3 where R^1 = alkyl and R^2 = aryl. *M. isabellina* shows no variation in the enantioselectivity of oxidation of thioanisole over a temperature range from 18 to 30 °C and over a wide range of substrate concentrations, conditions over which the V_{max} and K_m of separate enzymes would be expected to vary, implying that a single enzyme of this fungus may indeed be responsible for the production of both enantiomers of sulfoxide.[109] The variation in optical purity of the sulfoxides produced by this fungus has been explained by a nonstereospecific oxidation of an intermediate sulfur radical cation.[45] This and other mechanistic aspects of the enzymic sulfoxidation reaction are discussed below.

D. Reaction of the Oxidizing Species with Sulfur

1. Cytochrome P-450 Monooxygenases

The mechanism by which cytochrome P-450 dependent monooxygenases perform in their most common catalytic role, hydroxylation at saturated carbon, has been the subject of intensive study, and the consensus of opinion favors the nonconcerted radical-rebound mechanism outlined in Figure 4.[97] This process is more consistent with some experimental data concerning loss of stereochemical integrity at carbon and with kinetic isotope effects for hydrogen loss than is the alternative earlier proposal of a concerted oxygen insertion into the C–H bond.[97]

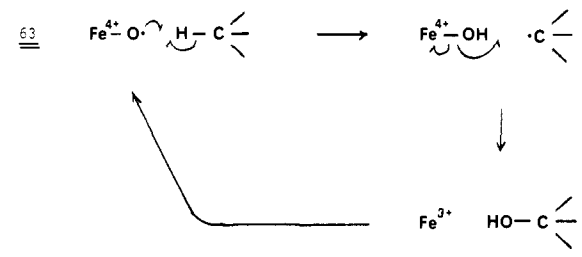


Figure 4. Hydroxylation reaction of cytochrome P-450 monooxygenases.

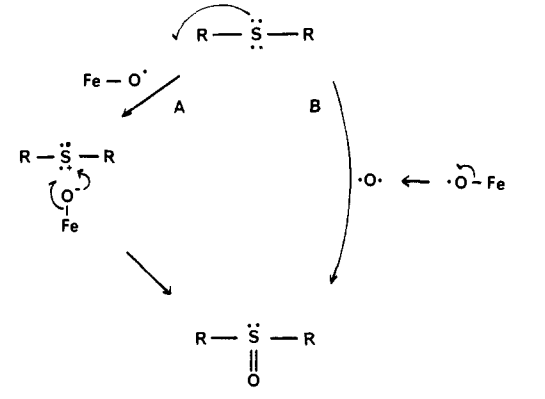


Figure 5. One- and two-electron routes for S-oxidation by cytochrome P-450 monooxygenases.

Unfortunately, neither of the above mechanistic probes is available in the case of the sulfoxide reaction. However, a close relationship between sulfur and carbon oxidation is evident from the fact that both can be catalyzed by the same enzyme.[75,79,80,109] If the reactive oxidizing species in sulfoxidation is assumed to be **63** (Figures 1 and 4), then two possible routes for its interaction with sulfur can be proposed. These involve initially either a one-electron abstraction (Figure 5, route A), analogous to the radical mechanism for hydroxylation, or a direct two-electron oxidation (Figure 5, route B), analogous to a concerted oxygen insertion into a C–H bond. Routes A and B therefore differ in the initial electron demand from sulfur (one electron via A, two electrons via B), and they have been experimentally distinguished on this basis, using the Hammett ρ value for the oxidation of a series of para-substituted thioanisoles as a parameter.

Experiments designed to determine the rates of oxidation of thioanisoles **47** by the sulfoxidizing enzyme from rabbit liver have been carried out, and the low ρ value obtained ($\rho^+ = -0.16$) attributed to a rate-determining one-electron removal from sulfur.[74,75] Correlation with ρ^+ rather than ρ is interpreted as supportive evidence for a polar intermediate such as the radical cation of route A, Figure 5.

With the exception of *p*-chlorothioanisole, there was also a linear correlation between V_{max} for the enzymic sulfoxidation and the one-electron oxidation potential

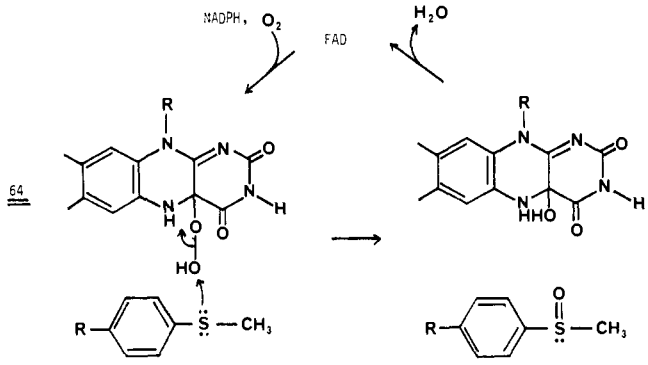


Figure 6. Proposed route for S-oxidation by flavin-dependent monooxygenases.

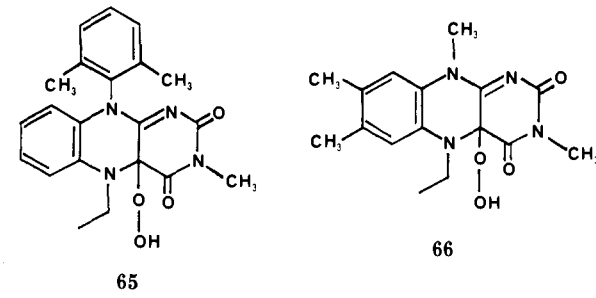
of the substrate. Similar data ($\rho^+ = -0.2$) were obtained for the oxidation of para-substituted sulfoxides to sulfones by the same enzyme.[87] The magnitudes of the ρ values obtained in the above studies were considered diagnostic of a one-electron transfer, since a ρ value of −1.13 has been reported for oxidation of thioanisoles by hydrogen peroxide, a concerted nonradical process.[110] The enzymic reaction rates in the above studies were determined only by monitoring NADPH consumption, however, and thus may not reflect the intrinsic rate effects caused by variation of substrate structure.

The rates of sulfoxidation of substrates **47** by the fungus *M. isabellina* have been determined by monitoring product formation and reported to proceed with a ρ value of −0.67.[46] This value is lower than that reported for oxidation with potassium persulfate (ρ = −0.87), a process thought to involve formation of a sulfonium ion intermediate,[111] and is not incompatible with the formation of a radical cation such as that of route A, Figure 5. The difference between this value and that reported for the isolated enzyme may reflect an additional degree of stabilization of the intermediate in the latter case[45] or the choice of inappropriate kinetic parameters in the assay of the isolated enzyme system.

The variation in the enantiomeric purities of sulfoxides produced from many of the substrates of Table II by *M. isabellina* has been interpreted on a mechanistic basis.[45] A comparison of the enantiomeric excesses of sulfoxides obtained from isosteric sulfides suggested that the ee may be dependent on the electron-withdrawing or -donating properties of the latter, i.e. on the electron density at sulfur during the reaction; thus, for the pairs *p*-H vs. *p*-F, *p*-OCH_3 vs. *p*-C_2H_5, and *p*-CH_3 vs. *p*-CN, a significantly larger ee is observed for the more electron-withdrawing member of the pair, an effect attributed to destabilization and consequent reduction in the lifetime of a stereochemically labile ionized intermediate.[45]

2. Flavin Monooxygenases

Investigation of the rates of sulfoxidation of para-substituted thioanisoles, with the synthetic oxidants **65** and **66** as models for the proposed enzymic intermediate **64** (Figures 2 and 6), have determined that these reactions proceed with ρ values of −1.67[112] and −1.32,[113] respectively. These values clearly reflect a rate-determining nucleophilic attack by sulfur at the terminal hydroperoxy oxygen of the model oxidants (cf. Figure 6).[112,113] Unfortunately, similar experiments using the FAD-containing sulfoxidase from pig liver found no



variation in the rate of the reaction (monitored only by NADPH consumption), with a change of para substituent of the substrate, a phenomenon attributed to the fact that substrate oxidation was not the rate-limiting step in the enzymic cycle.[82] In the absence of any definitive data on the enzyme itself, therefore, and with the uncertainties discussed above in section V.B.2 about the actual structure of the oxygen–flavin adduct, the model oxidations referred to above provide only indications of a possible enzymic oxidation mechanism.

VI. Potential of Enzymic Sulfoxidation as a Synthetic Tool

It will be apparent from the above discussion that enzymic methods do not provide a simple, high-yield, general route for the preparation of all chiral sulfoxides. However, the active site models discussed in section V.C do enable predictions to be made about the absolute stereochemistry of sulfoxidation in some cases, and complete enantiospecificity can be achieved with the appropriate substrates. In these cases, biotransformation is the most efficient route for the production of chiral sulfoxides. Provided that due care is taken over the choice and maintenance of microorganism,[41] reproducibility is assured.

The use of isolated enzymes suffers from the problems of scale discussed in the Introduction and also problems of reproducibility unless care is taken in the housing and maintenance of the experimental animals. Cytochrome P-450 monooxygenases are generally inducible, and the stereochemistry of sulfoxidation by these enzymes can be dependent upon the nature of the inducer used.[80] It should be remembered that the apparent intrinsic lack of stereospecificity of sulfoxidation displayed by the isolated enzymes refers to a single substrate only[80,81] and may not be a general property of these enzymes; nevertheless, the difficulties associated with the isolation of oxygenases will severely limit their synthetic utility for the foreseeable future.

Registry No. Oxygenase, 9037-29-0.

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