concentration of PLA_2-FITC in the DPPC liposomes ("vesicles") and $[probe]_w$ is the concentration in the aqueous bulk phase ("water"). Such a mode of association of PLA_2-FITC may accord with the presence of an interfacial recognition site in PLA_2.[21] Figure 3, panel B, reveals that the L-32-Phy liposomes retained PLA_2-FITC more firmly than did the DPPC and DCPC liposomes at 50 °C and above. Although proteinaceous PLA_2-FITC is different from CF in size and affinity for the membranes, temperature similarly affected the leakage of both kinds of fluorophores (Figure 3).

The results presented here could be taken as substantiating the heat-resistant property of the L-32-Phy membranes. Since the L-32-Phy, DPPC, and DCPC membranes possess similar phosphorylcholine interfaces, the slow leakage in the L-32-Phy liposomes may be regarded phenomenologically as originating in the isoprenoid residues, the glyceryl ether linkage, and perhaps the prime monolayer membrane. It would be considered likely that the branched isoprenoid chains offer greater intramembrane resistance to the diffusing probes than the *n*-alkyl chains of DPPC and DCPC. This, in a sense, accords with the finding that the branched molecules diffuse via a lipid pathway more slowly than do small and simple compounds.[22] Biocompatible use of the newly synthesized membrane is under investigation.

Acknowledgment. We are grateful to Professors Kenkichi Sonogashira and Eiji Fuwa (this university) and Dr. Hiroshi Inoue (Osaka Municipal Technical Research Institute) for the fluorescence and DSC studies.

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(23) The gel chromatography efficiently removed the PLA_2-FITC probes that were located outside the liposomes. Namely, the fluorescence intensity ratio (eluents/Triton X-100 treated eluents) was 4/10 (emission at 520 nm). The fluorescence from the probes stored inside the vesicles is completely quenched because of the high concentration (>0.17 mM). The ratio, then, equals approximately a number ratio of the probes (outer aqueous phase/total probes in the eluents). Considering that the aqueous phase is about 5000 times the combined volume of the liposomes, the concentrations of the probes in the outer aqueous phase and interface were calculated to be negligibly low in comparison with the probe concentrations in the inner aqueous phase and interface, respectively. A concentration ratio (interface/aqueous phase) is about 18 at 25 °C as described in the Experimental Section.

(24) L-32-Phy was amorphous. The X-ray diffraction pattern from a film of L-32-Phy, which was prepared on the surface of a thin glass tube according to a reported procedure,[17] gave a layer repeat distance of 48.5 ± 0.5 Å. Though the molecular structure in the layer was not clear, it would be most likely that the dipolar lipid furnished a membrane that is a monolayer, by analogy with other dipolar amphiphiles.[5-7,10,18,19]

Oxygenation of Dialkyl Sulfides by a Modified Sharpless Reagent: A Model System for the Flavin-Containing Monooxygenase

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Abstract: The chemical and enzymatic S-oxygenation of 2-(*p*-methoxyphenyl)-1,3-dithiolane and 2-(*p*-cyanophenyl)-1,3-oxathiolane has been investigated. In the presence of chemical oxidizing agents (i.e., $NaIO_4$ or H_2O_2), modest diastereoselective formation of the trans *S*-oxide is formed with 0% enantioselectivity. In the presence of bovine serum albumin, the diastereoselectivity and enantioselectivity of $NaIO_4$-catalyzed S-oxygenation are increased. The maximum level of diastereoselective and enantioselective S-oxygenation was obtained in the presence of the Kagan modification of the Sharpless oxidation reagent, and the stereoselectivity mimicked that of the microsomal flavin-containing monooxygenase from hog liver. Thus, a marked preference for *pro-R* S-oxygenation of 2-(*p*-methoxyphenyl)-1,3-dithiolane was observed. Rat and mouse liver cytochrome $P\text{-}450_{PB\text{-}B}$ catalyzed the S-oxygenation of 2-(*p*-methoxyphenyl)-1,3-dithiolane preferentially at the *pro-S* sulfur atom. For 2-(*p*-cyanophenyl)-1,3-oxathiolane, a marked preference for *pro-S* S-oxygenation was observed for catalysis by the modified Sharpless reagent, hog liver microsomes, and highly purified hog liver flavin-containing monooxygenase, whereas for rat and mouse liver cytochrome $P\text{-}450_{PB\text{-}B}$, S-oxygenation of 2-(*p*-cyanophenyl)-1,3-oxathiolane occurs by attack on the *pro-R* sulfur atom. The modified Sharpless reagent is an efficient S-oxygenating catalyst that mimics the hog liver flavin-containing monooxygenase in diastereoselectivity and enantioselectivity of dialkyl sulfide S-oxygenation.

The dialkyl sulfide functionality is present in many important drugs, chemicals, and pesticides.[1] In principle, dialkyl sulfide containing drugs can be metabolized by S-dealkylation[2] or by S-oxygenation, but it is the latter biotransformation that is the main metabolic pathway for the dialkyl sulfide group. *S*-Oxide metabolites of sulfides are known to have interesting and useful biological and pharmacodynamic properties.[3] Among the various mammalian *S*-oxygenase enzymes responsible for sulfide oxidation,[4] there are three major monooxygenase enzyme systems. The flavin-containing monooxygenase (FMO),[1a] the cytochromes P-450,[5] and prostaglandin cyclooxygenase peroxidase[6] have been shown to be capable of S-oxygenating dialkyl sulfides. The hepatic flavin-containing monooxygenase from hog liver is the most carefully studied form of FMO, and the enzyme mechanism has

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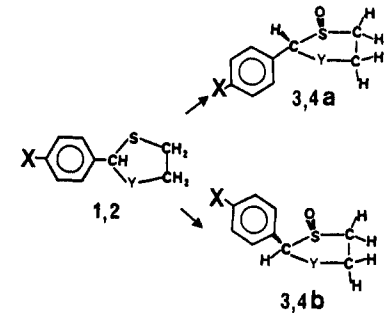
Table I. Stereoselective S-Oxygenation of Dialkyl Sulfides[a]

substrate	oxidation method	% diastereoselectivity[b] trans S-oxide[d]	% diastereoselectivity[b] cis S-oxide[d]	% enantioselectivity[c] trans S-oxide 1R,2R	trans S-oxide 1S,2S	cis S-oxide 1R,2S	cis S-oxide 1S,2R	ref
1	$NaIO_4$	12		0	0	0	0	17a
	$NaIO_4$/BSA	100		1				18a
	$Ti(OiPr)_4$/DET/tBuOOH/H_2O	100		76				18e
	hog liver FMO	100		100				17a
	hog liver microsomes	100		79.4 ± 1.1				17a
	rat liver cyt P-450$_{PB-B}$	100			96.5 ± 0.5			this study
	mouse liver cyt P-450$_{PB-B}$	100			100			this study
2	$NaIO_4$	26		0	0	0	0	17a
	$NaIO_4$/BSA	28			1 ± 1.0		6 ± 1.5	18a
	$Ti(OiPr)_4$/DET/tBuOOH/H_2O	64			54 ± 2.0		92 ± 2.2	18e
	hog liver FMO	58.1 ± 2.2			94.9 ± 2.4		100	30
	hog liver microsomes	100			81.4 ± 1.1			30
	rat liver cyt P-450$_{PB-B}$	54.2 ± 2.2		100		100		this study
	mouse liver cyt P-450$_{PB-B}$	70.7 ± 2.0		100		100		this study

[a] Reactions were performed as described in the references indicated. [b] The term percent diastereoselectivity is defined as the percent excess production of one diastereomeric S-oxide isomer (trans S-oxide) over another (cis S-oxide) = {([trans S-oxide] − [cis S-oxide])/([trans S-oxide] + [cis S-oxide])} × 100 = % trans S-oxide − % cis S-oxide. [c] Assuming a linear relationship between rotation and composition, percent "optical purity" is equated with the percent enantioselectivity or percent excess of one S-oxide enantiomer over the other, which we shall designate as enantiomeric excess (ee); that is, {([S] − [R])/([S] + [R])} × 100 = % S − % R, where S and R refer to the absolute stereochemistry of the S-oxide sulfur atom. [d] Each substrate diastereomeric S-oxide was completely characterized chemically, and the first designation refers to the absolute configuration of the sulfur atom.

been described in some detail.[7] A few chemical model systems have been described for FMO-catalyzed S-oxygenation reactions,[8] but stereochemical characterization of the products of these reactions is lacking. A few studies have reported that the cytochrome P-450 isozyme isolated from phenobarbital-pretreated animals (P-450$_{PB-B}$) is the cytochrome P-450 largely responsible for sulfide oxidation[9] and that the S-oxides produced are optically active, while S-oxides produced from other cytochrome P-450 isozymes do not always produce optically active S-oxides.[10] Extensive examples of model systems for cytochrome P-450 catalyzed epoxidations,[11] hydroxylations,[12] N-dealkylations,[13] and other oxidations[14] have been reported, but few biomimetic models for cytochrome P-450 related S-oxidation have been documented.[15] The oxidation of a few sulfides by prostaglandin cyclooxygenase peroxidase has been described,[6] but no enzyme model system for this process has been described, although use of other peroxidases may become useful alternatives to the chemical synthesis of dialkyl S-oxides.[16]

Scheme I. Oxidative (Bio)chemical Transformation of 2-(*p*-Methoxyphenyl)-1,3-dithiolane (**1**) and 2-(*p*-Cyanophenyl)-1,3-oxathiolane (**2**) to Their Corresponding Trans (**a**) and Cis (**b**) S-Oxides **3** and **4**[a]



[a] **1**, X = OCH_3, Y = S; **2**, X = CN, Y = O.

Aryl-1,3-oxathiolanes and aryl-1,3-dithiolanes are useful molecules to investigate the monooxygenase-catalyzed S-oxygenation of diastereotopic and enantiotopic sulfur atoms.[17] While a number of methods have been used to generate chiral S-oxides of thioacetals or other dialkyl sulfides,[18] no studies correlating the synthesis of chiral S-oxides with the production of S-oxides by purified hepatic monooxygenase systems have been reported. The purpose of this investigation was to study simple chiral S-oxygenation reagents in hopes of providing an efficient method useful for the production of chiral dialkyl S-oxides of relevance to the metabolites of chemicals, drugs, and pesticides. Results presented herein show that the Kagan modification of the Sharpless oxidation reagent is useful for the production of chiral dialkyl S-oxides, which are formed by the highly purified FMO from hog liver microsomes. The significance of the results is that, in principle, the major S-oxide metabolite arising from action of hepatic FMO on a large numbers of chemicals, drugs, and pes-

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Table II. CD Data and Absolute Configuration of Optically Active *S*-Oxides **3a**, **4a**, and **4b**[a]

S-oxide	% ee[b]	$[\theta]^c$ (λ_{max})	$\Delta\epsilon^d$	$[\theta]$ (λ)	configuration[e]
3a	76	+16 278 (245)	+4.9	+10 174 (250) infl, +7800 (230) tr, +11 869 (224) pk, +2713 (215) tr, +6782 (210) pk	1*R*,2*R*
4a	54	−19 907 (217)	−6.0	+9559 (247) pk, 0 (232) zero point, −7407 (228) infl, 0 (208) zero point, +25 463 (208) pk	1*S*,2*S*
4b	92	−19 565 (205)	−5.9	−13 587 (252) pk, 0 (235) zero point, +3261 (229) pk, 0 (225) zero point, −13 043 (215) sh, 0 (195)	1*S*,2*R*

[a] Circular dichroism spectra were taken with a JASCO J-500A spectropolarimeter of purified *S*-oxides (0.1–0.2 mM, CH_3CN) obtained from reactions with the Kagan modification of the Sharpless reagent. Insufficient amounts of pure **3b** were obtained for analysis. [b] Percent enantiomeric excess (defined in Table I) was determined by NMR analysis in the presence of chiral shift reagents, as described in the Experimental Section. [c] Specific molar ellipticity $[\theta]$ (deg M^{-1} cm^{-1}) values were calculated according to ref 35. [d] The change in extinction coefficient (ϵ) is calculated from $[\theta]/3300$ (ref 35). The abbreviations are inflection (infl), trough (tr), peak (pk), and shoulder (sh). [e] The assigned centers are as described in Table I.

ticides can be conveniently synthesized in enantiomerically enriched form.

Results

Reaction of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane with Chemical S-Oxidizing Reagents. At pH 7.0, the reaction of 2-(*p*-methoxyphenyl)-1,3-dithiolane (**1**) and 2-(*p*-cyanophenyl)-1,3-oxathiolane (**2**) with a slight excess of hydrogen peroxide or sodium metaperiodate produced the corresponding *S*-oxides **3** and **4**, respectively, as the only detectable products[17] (Scheme I). At extremely long reaction times, **3** and **4** were oxidized further and decomposed to the corresponding benzaldehydes. Of the diastereomers that could form from **1** and **2**, the major trans diastereomer **3a** or **4a**, respectively, was formed in greater than 90% yield. Oxidation of **1** and **2** with hydrogen peroxide or sodium metaperiodate led to *S*-oxide products with zero enantiomeric excess (ee) as defined in Table I. The optical activity and absolute configuration assignments of *S*-oxides[19] **3** and **4** were determined by NMR, CD, and HPLC methods, as described in the Experimental Section. The reaction was repeated in the presence of bovine serum albumin (BSA) and sodium metaperiodate in an attempt to induce stereoselective S-oxygenation by employing a chiral protein matrix. The presence of BSA markedly increased the diastereoselectivity of S-oxygenation for **1** but not for **2** and only very modestly increased the ee. Consequently, the asymmetric S-oxygenation of **1** and **2** was attempted by employing the chiral sulfoxidizing system consisting of titanium tetraisopropoxide, diethyl tartrate, *tert*-butyl hydroperoxide, and water[18c] (Table II). This system is analogous to the Kagan modification of the Sharpless reagent used by others to S-oxidize various sulfides. As shown in Table I, oxidation of **1** and **2** with the modified Sharpless reagent gave *S*-oxides with good diastereoselectivity and enantioselectivity. Thus, *S*-oxide **3a** was obtained in 79% ee, while **4a** and **4b** were obtained in 54% and 92% ee, respectively.

Reaction of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane with Microsomes and Purified Flavin-Containing Monooxygenase from Hog Liver. Preliminary studies showed that untreated hog liver microsomes supplemented with NADPH catalyze the S-oxygenation of **1** and **2** to the corresponding *S*-oxides **3** and **4** [20.0 and 7.5 nmol min^{-1} (mg of protein)$^{-1}$, respectively]. The formation of the *S*-oxide was a linear function of protein concentration (0–4 mg/mL) and of time for at least 5 min. Heat inactivation of hog liver microsomes under conditions that preserve 85% of the cytochrome P-450 activity but essentially completely destroy FMO activity almost completely (90%) abolished S-oxygenation of **1** and **2**. That the *S*-oxide product formed in hog liver microsomes is largely produced by an FMO-catalyzed reaction (i.e., 90% FMO catalyzed and 10% cytochrome P-450 catalyzed) is seen from inhibitor studies and analysis of the stereochemistry of the *S*-oxides formed. Reaction of hog liver microsomes with **1** and **2** in the presence of *n*-octylamine, a stimulator of hog liver FMO[1] and a potent inhibitor of cytochrome P-450,[20] does not decrease the S-oxygenation of **1** and **2**. As shown in Table I, the stereochemical preference for addition of an oxygen atom by hog liver microsomes to **1** is to the *pro-R* sulfur atom (i.e., 89.7% 1*R*,2*R* and 10.3% 1*S*,2*S*), and no cis *S*-oxide formation could be detected. For **2**, addition of an oxygen atom is to the trans *pro-S* sulfur atom by hog liver microsomes (i.e., 90.7% 1*S*,2*S* and 9.3% 1*R*,2*R*), and no cis *S*-oxide formation could be detected. The stereopreference for S-oxygenation of **1** and **2** is consistent with the suggestion that in hog liver microsomes FMO, and not the cytochromes P-450, is mainly responsible for S-oxygenation. In order to examine this point more carefully, the S-oxygenations of **1** and **2** were examined with highly purified hepatic microsomal monooxygenases.

Reactions of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane with Highly Purified Hog Liver FMO. The NADPH-dependent S-oxygenation of **1** and **2** catalyzed by highly purified hog liver FMO [372 and 125 nmol min^{-1} (mg of protein)$^{-1}$, respectively] is a linear function of protein concentration (0–200 μg of protein) and of time for at least 3 min. Formation of *S*-oxides **3** and **4** by the highly purified hog liver FMO was abolished by heat inactivation of FMO and significantly decreased by alternate competitive substrates for the FMO. As shown in Table I, the stereochemical preference for addition of an oxygen to **1** by the highly purified hog liver FMO is to the *pro-R* sulfur atom, and this occurs with 100% diastereoselectivity and enantioselectivity; no cis *S*-oxide formation could be detected. For **2**, addition of an oxygen atom by hog liver FMO to the *pro-S* sulfur atom was found to be 98.7% (i.e., 77.9% 1*S*,2*S* and 20.8% 1*S*,2*R*), with 1.3% addition to the *pro-R* sulfur atom (i.e., 1.3% 1*R*,2*R*). The fact that the stereopreference for S-oxygenation of **1** and **2** by the highly purified FMO from hog liver is similar to that observed for hog liver microsomes suggests that, at least in hog liver microsomes, S-oxygenation of **1** and **2** is largely catalyzed by FMO and not by hog liver microsomal cytochromes P-450. In order to confirm this suggestion, the S-oxygenation of **1** and **2** was investigated with highly purified cytochrome P-450 from rat and mouse liver.

Reaction of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane with Highly Purified Cytochrome P-450$_{PB-B}$ from Rat and Mouse Liver. Highly purified rat liver cytochrome P-450$_{PB-B}$ catalyzed S-oxygenation of **1** and **2** to **3** and **4** [11.1 and 10.8 nmol min^{-1} (nmol of protein)$^{-1}$, respectively] is linearly dependent on protein concentration (0–0.2 nmol of cytochrome P-450) and on time for at least 10 min. S-Oxygenation of **1** and **2** is dependent on cytochrome P-450 reductase and NADPH but only to a small extent on cytochrome b_5. As shown in Table I, the S-oxygenation of **1** and **2** by rat liver cytochrome P-450$_{PB-B}$ produces mainly trans *S*-oxide products. In contrast to what has been observed for microsomes and highly purified FMO from hog liver (discussed above), the stereopreference for addition of an oxygen atom to **1** and **2** by rat liver cytochrome P-450$_{PB-B}$ yields *S*-oxides with opposite enantioselectivity. For **1**, addition of oxygen by rat liver cytochrome P-450$_{PB-B}$ is to the *pro-S* sulfur atom (i.e., 98.2% 1*S*,2*S* and 1.8% 1*R*,2*R*). Compound **2** is also S-oxygenated by rat liver cytochrome P-450$_{PB-B}$, and although modest diastereoselectivity is observed,

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very high enantioselectivity is observed. Thus, addition of oxygen to **2** is only to the *pro-R* sulfur atom (i.e., 100% 1*R*,2*R* and 100% 1*R*,2*S*).

Like rat liver cytochrome P-450$_{PB-B}$, NADPH-dependent S-oxygenation of **1** and **2** to **3** and **4** with mouse liver cytochrome P-450$_{PB-B}$ gives largely trans *S*-oxide products with enantioselectivity almost identical with that observed for rat liver cytochrome P-450$_{PB-B}$ (Table I). Preliminary studies with mouse liver cytochrome P-450$_{PB-B}$ catalyzed S-oxygenation of **1** and **2** to **3** and **4** [9.2 and 8.5 nmol min^{-1} (nmol of protein)$^{-1}$, respectively] demonstrated that the reaction is linearly dependent on protein concentration (0–0.2 nmol of cytochrome P-450$_{PB-B}$) and on time for at least 10 min. S-Oxygenation of **1** and **2** is dependent on cytochrome P-450 reductase and NADPH but only slightly dependent on cytochrome b_5. In good agreement with rat liver cytochrome P-450$_{PB-B}$, mouse liver cytochrome P-450$_{PB-B}$ gives *S*-oxides with enantioselectivity opposite from that observed for hog liver FMO. Thus, for **1**, mouse liver cytochrome P-450 produces only the trans *S*-oxide diastereomer with 100% enantioselectivity (i.e., 100% 1*S*,2*S*). For **2**, mouse liver cytochrome P-450$_{PB-B}$ catalyzed addition of oxygen to the *pro-R* sulfur atom was found to be 100% (i.e., 100% 1*R*,2*R* and 100% 1*R*,2*S*). For cytochrome P-450$_{PB-B}$ catalyzed S-oxygenation of **1** and **2**, no sulfone or *S*,*S*′-dioxide formation could be detected during the short incubation time periods employed.

That concurrent nonenzymatic S-oxygenation of **1** and **2** does not contribute to the formation of *S*-oxides with either microsomal or purified monooxygenase preparations stems from the following observations: (1) the ee remains constant over the time course of the reaction, (2) the chemical oxidation (i.e., H_2O_2 or $NaIO_4$) is slow and gives zero ee, (3) incubations performed in the presence of catalase or glutathione do not change the de or ee observed, and (4) incubations performed in the absence of NADPH or active protein do not yield *S*-oxides. The conclusion that stereoselective reduction of *S*-oxides is not occurring during the monooxygenase-catalyzed reactions comes from the fact that *S*-oxides are quantitatively extracted from inactive enzyme preparations with their stereochemistry unchanged.

Discussion

Chemical S-Oxidation of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane. The reaction of **1** and **2** with ROOH or sodium metaperiodate in the presence of various chiral matrices was investigated in order to identify the diastereoselectivity and enantioselectivity of the products formed (Scheme I). In the presence of bovine serum albumin and sodium metaperiodate, marked diastereoselective S-oxygenation was observed in comparison to reactions performed in the absence of bovine serum albumin, although the degree of enantioselective S-oxygenation was very low.[18a,b] Sulfides **1** and **2** presumably bind to one or more of the binding sites of bovine serum albumin in a diastereoselective fashion. Attack by sodium metaperiodate must occur with almost equal facility at either enantiotopic lone pair of the sulfur atom trans to the aromatic ring. Another possibility is that the protein binds **1** and **2** at more than one distinct site, although attack of oxygen at the sulfur atom is similar. In an attempt to S-oxygenate **1** and **2** in an enantioselective fashion with an agent having one well-defined binding site, we investigated stereoselective S-oxygenation with the Kagan modification of the Sharpless reagent.

The structure of the Sharpless reagent has been established by X-ray crystallography;[21] each tartramide acts as a bidentate diolate to a titanium, where there is double bridging between the titaniums through the tartramide alkoxy groups. Presumably, **1** and **2** bind and orient with respect to the Sharpless oxidizing reagent in order to decrease steric repulsion interactions between the aromatic group and the titanium–tartramide backbone. Concomitant attack on the sulfur atom by the chiral titanium hydroperoxide produces mainly trans *S*-oxides with good overall enantioselectivity. That initial complexation of the sulfide to the tartramide prior to oxidation is not occurring is consistent with the observation that the enantioselectivity of the reaction is no different regardless of whether (+)- or (−)-diethyl tartrate is used in the reaction. Hydrogen bonding of the proximal substrate heteroatom to the modified Sharpless oxidizing reagent does not appear to be important, since much better enantioselectivity is observed for the poorly hydrogen-bonding dithiolane **1** compared to the oxathiolane **2**, which has the much more efficient hydrogen-bonding dialkyl ether group present. In good agreement with what has been proposed previously, we envisage the modified Sharpless reagent to be capable of chiral S-oxygenation, with enantioselectivity mainly determined by steric interactions.[18c]

Monooxygenase-Catalyzed S-Oxygenation of 2-(*p*-Methoxyphenyl)-1,3-dithiolane and 2-(*p*-Cyanophenyl)-1,3-oxathiolane. The S-oxygenation of **1** and **2** was investigated in vitro with microsomes and highly purified FMO from hog liver as well as with cytochrome P-450$_{PB-B}$ from mouse and rat liver. For all monooxygenase preparations examined, **1** and **2** were efficiently S-oxygenated. In general, a marked preference for trans *S*-oxide formation was observed[17,22] (Figure 1). In the presence of hog liver microsomes under conditions where both FMO and cytochrome P-450 activities are present, a stereochemical analysis of the *S*-oxides formed for both **1** and **2** supports the idea that FMO is mainly responsible for S-oxygenation. Thus, attack of oxygen on the sulfur atom of **1** or **2** gives stereochemistry similar to that observed for microsomes and highly purified FMO from hog liver but *S*-oxide stereochemistry opposite from that observed for cytochrome P-450$_{PB-B}$ from rat and mouse liver. From the data presented, it is clear that S-oxygenation of **1** and **2** with the modified Sharpless reagent gives *S*-oxide products similar to those produced by hog liver FMO. Although the modified Sharpless reagent employs a chiral titanium hydroperoxide[21] and hog liver FMO-mediated S-oxygenations require an isoalloxazine 4a-hydroperoxide,[7] it is remarkable that very similar stereochemistry is observed. This observation may have mechanistic as well as practical consequences. Nucleophilic attack by sulfides on electrophilic peroxides may occur either by an anionic nucleophilic mechanism or by a single-electron-transfer (SET) reaction mechanism. The data presented previously are consistent with a role of anionic nucleophilic mechanisms for FMO,[23] although a SET reaction cannot be completely ruled out.[17a,24] For cytochromes P-450, a SET mechanism has been implicated to explain the regio- and stereochemistry of the oxidation reactions.[25] The high degree of enantioselectivity observed for modified Sharpless reagent and FMO-catalyzed S-oxygenations is consistent with anionic nucleophilic mechanisms. For cytochrome P-450$_{PB-B}$ catalyzed S-oxygenations, the reactions are highly enantioselective. If SET reaction mechanisms are on the reaction path for cytochrome P-450, then collapse of sulfide radical cations for S-oxygenation of **1** and **2** must be extremely rapid to give the enantioselectivity observed. From a practical point of view, the work presented suggests that the Kagan modification of the Sharpless reagent may be a useful catalyst to synthesize chiral *S*-oxide metabolites of drugs or xenobiotics. This may be important, since it is widely recognized that FMO comprises a major metabolic route to dialkyl sulfide S-oxygenation in mammalian systems.[23] Another intriguing observation is that FMO and cytochrome P-450$_{PB-B}$ S-oxygenate **1** and **2** to produce opposite enantiotopic sulfoxide sulfur atoms.[10] While this is probably not the case for all dialkyl sulfide S-oxygenations, nevertheless, S-oxygenation of **1** or **2** could be a useful stereochemical probe of monooxygenase-catalyzed S-oxygenation in various mammalian tissues. Such a stereochemical probe should be highly useful to distinguish the activity of cytochromes P-450 and FMO in various tissue preparations under different experimental conditions. Recently, studies have been accomplished showing that rabbit lung FMO

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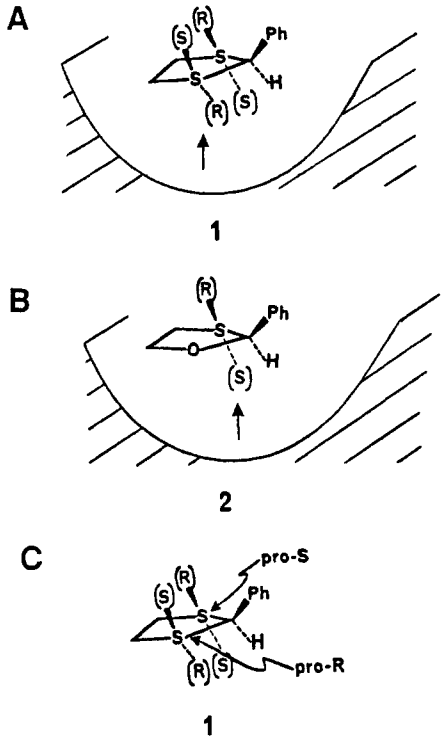

Figure 1. Stereoselective S-oxygenation of **1** or **2** by the flavin-containing monooxygenase or the modified Sharpless reagent (plates A and B). Plate C demonstrates the prochiral nature of compound **1**.

produces *S*-oxide enantiomers opposite from those produced by rabbit lung cytochrome P-450$_{IIB-4}$. In addition, S-oxygenation of **1** in rat microsomal preparations has been shown to be useful to investigate the changes in monooxygenase activities as a function of animal pretreatment.

Experimental Section

Chemicals. All chemicals used were reagent grade or better, and compounds **1–4** were fully characterized by IR, UV–vis, NMR, and mass spectrometry. 2-(*p*-Methoxyphenyl)-1,3-dithiolane (**1**) and 2-(*p*-cyanophenyl)-1,3-oxathiolane (**2**) as well as their corresponding *S*-oxides, were synthesized as previously described.[17] The relative configurations of the trans and cis *S*-oxides were assigned by high-field NMR studies and are in agreement with previous studies.[26] High-resolution mass spectrometry of synthetic *S*-oxides (i.e., ±5 ppm of the calculated mass) is in excellent agreement with the predicted structure (Table III).[17a] Chiral sulfoxides were synthesized by the method of Pitchen et al.[18c] With the combination of titanium tetraisopropoxide, (+)-diethyl tartrate, *tert*-butyl hydroperoxide, and water, *S*-oxides **3a** and **4a** were obtained in 76% and 54% ee, respectively. The optical purity of the purified cis and trans *S*-oxide products was determined by NMR analysis using chiral shift reagents: tris[3-(heptafluoropropyl)hydroxymethylene)-(−)-camphorato]europium-(III) for **1** and (+)-(*S*)-9-anthryl-2,2,2-trifluoroethanol for **2**. The absorption spectra of pure cis and trans *S*-oxides were determined in order to relate the optical purity of the products to the absolute configuration of dialkyl *S*-oxides independently correlated by other means.[27] Previous chemical and biochemical studies of thioketal *S*-oxides have established the relationship between the circular dichroism (CD) sign and the absolute stereochemistry of dialkl *S*-oxides.[28] A positive CD associated with the absorption between 285 and 195 nm can be correlated with an *R S*-oxide configuration.[27] Separation of each enantiomer of each diastereomer was accomplished by HPLC with a Chiralcel OD column (Daicel Chemical Industries, Ltd., New York) using an eluent of 2-propanol/hexane (18:82 v/v) and UV detection (240 nm).

Materials. All solvents and buffers were the purest commercially available products. Sodium metaperiodate, titanium tetraisopropoxide, (+)- and (−)-diethyl tartrate, *tert*-butyl hydroperoxide, *n*-octylamine, and hydrogen peroxide were obtained from Aldrich Chemical Co., Milwaukee, WI. NADP$^+$, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, dilauroylphosphatidylcholine, sodium dodecyl sulfate, and NADPH were obtained from Sigma Chemical Co.

Table III. Some Properties of 2-(*p*-Methoxyphenyl)-1,3-dithiolane, 2-(*p*-Cyanophenyl)-1,3-oxathiolane, and Their *S*-Oxides

compd	ret vol, mL[a]	λ_{max} (ϵ)[b]	high-resolution mass spectra[c] calcd	obsd	±ppm
1	4.2	234 (4697)			
3a, trans		236 (9200)	212.0504	212.0510	+1.6
3a, 1*S*,2*S*	14.6				
3a, 1*R*,2*R*	15.7				
3b, cis		236 (9100)	212.0504	212.0506	−0.6
3b, 1*S*,2*R*	16.7				
3b, 1*R*,2*S*	22.9				
2	5.7	231 (17500)			
4a, trans		236 (16750)	207.0358	207.0359	+2.6
4a, 1*S*,2*S*	26.4				
4a, 1*R*,2*R*	23.1				
4b, cis		234 (17750)	207.0358	207.0354	+0.1
4b, 1*S*,2*R*	32.8				
4b, 1*R*,2*S*	27.5				

[a] Retention volumes were measured by HPLC with a Chiralcel OD column as described in the Experimental Section. [b] UV spectra were recorded in methanol; λ (ϵ) values are in units of nm (M^{-1} cm^{-1}). [c] IR and NMR data are consistent with the predicted structures.[17a]

Metabolic and Enzymatic Incubations. Hog liver microsomes were a generous gift of Professor D. M. Ziegler (University of Texas at Austin). Hog liver FMO was purified according to a published procedure,[29] and both microsomes and highly purified FMO from hog liver used in this study were shown to N-oxygenate dimethylaniline[30] and S-oxygenate 2-(*p*-nitrophenyl)-1,3-oxathiolane.[22] The incubation mixture used has been described previously.[17a,31] Rat and mouse liver microsomes were isolated from phenobarbital-pretreated animals (60 mg ip for 4 days) by the method described previously.[32] The major phenobarbital-inducible cytochrome P-450 (P-450$_{PB-B}$) was purified from rat liver by the method of Waxman and Walsh[32] and from mouse liver by the method of Bornheim and Correia.[33] Both isozymes exhibited characteristically high pentoxyresorufin *O*-dealkylase and 16β-testosterone hydroxylase activities [6 and 3 nmol of product min^{-1} (nmol of cytochrome P-450)$^{-1}$, respectively] and were judged to be homogeneous by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Cytochrome P-450$_{PB-B}$ (0.1 nmol) was reconstituted in the presence of saturating amounts of NADPH–cytochrome P-450 reductase (600 units) and dilauroylphosphatidylcholine (50 μg) and allowed to stand at 4 °C for 10 min. Sodium phosphate buffer (50 mM, pH 7.4), a NADPH-generating system or NADPH (0.5 mM) and substrate (200 μM final concentration), and rat hepatic cytochrome b_5 (0.1 nmol) were added, for a total volume of 0.5 mL. Incubations were carried out for 10 min at 33 °C with constant shaking in air, and the reaction was terminated and prepared for HPLC analysis as previously described.[34] A portion of the reaction mixture extract was evaporated to dryness, taken up in hexane/2-propanol (85:15 v/v), and subjected to HPLC on a Chiralcel OD column, with UV detection at 240 nm. *S*-Oxide metabolites were compared with authentic synthetic *S*-oxides, and the integrated areas of the resolved peaks specifying each *S*-oxide enantiomer for each diastereomer were quantitated.

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