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Stereoselective *S*-Oxygenation of 2-Aryl-1,3-dithiolanes by the Flavin-containing and Cytochrome P-450 Monooxygenases

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SUMMARY

The reaction of $NaIO_4$, highly purified flavin-containing monooxygenase (EC 1.14.13.8), and microsomes from hog liver with 2-aryl-1,3-dithiolanes and 2-aryl-1,3-dithiolane *S*-oxides was investigated. The initial rates determined for the microsome- and purified flavin-containing monooxygenase-catalyzed rate of *S*-oxidation of *para*-substituted 2-aryl-1,3-dithiolanes were similar, demonstrating that *S*-oxidation of these substrates occurred with similar velocities at saturating concentrations of substrate and, at least for the first *S*-oxidation, the reaction was insensitive to the nature of the *para*-substituent. The diastereoselectivity of *S*-oxygenation of 2-aryl-1,3-dithiolanes was determined and, in general, a marked preference for addition of oxygen to the sulfide sulfur atom was observed to occur *trans* to the aryl groups. In all cases examined, enantioselective enzymatic *S*-oxidation was observed. For *S*-oxide formation in microsomes, the data provided evidence for a minor role of cytochrome P-450 in *S*-oxide formation, but the flavin-containing monooxygenase was mainly responsible for production of *S*-oxide. In contrast to previous reports, the enantioselectivity of *S*-oxidation catalyzed by highly purified cytochrome P-450IIB-1 and cytochrome P-450IIB-10 was not always opposite to that catalyzed by hog liver flavin-containing monooxygenase activity. 2-Aryl-1,3-dithiolane *S*-oxides were also oxidized a second time by $NaIO_4$, microsomes, or highly purified flavin-containing monooxygenase from hog liver but not cytochrome P-450IIB-1 or P-450IIB-10. The rate of the second oxidation was 10–15-fold slower than the corresponding first *S*-oxidation and *S*,*S*′-dioxide formation was markedly dependent on the electronic nature of the *para*-substituent (Hammett correlation ρ value of −1.3 and −1.1 for microsomes and highly purified flavin-containing monooxygenase from hog liver, respectively). The large dependence of the rate of *S*,*S*′-dioxide formation on the nature of the *para*-substituent demonstrates that velocity values at saturating concentrations of *S*-oxide were not the same for all 2-aryl-1,3-dithiolane *S*-oxides and suggests that the chemical nature of the 2-aryl-1,3-dithiolane *S*-oxide contributes to the rate-determining step of this enzymatic reaction.

The FMO (EC 1.14.13.8, dimethylaniline monooxygenase) is present in a variety of mammalian tissues (1) and catalyzes oxygenation of nucleophilic nitrogen- (1, 2), phosphorous-, and sulfur-containing chemicals, drugs, and pesticides (2–4). For substrates containing both nucleophilic nitrogen and sulfur atoms, FMO preferentially oxygenates the sulfur atom. FMO could be more accurately classified as a nucleophilic heteroatom-oxygenase (2).

FMO is concentrated in hepatic tissue and is present in especially high concentration in human (1, 5) and hog liver (1). Careful kinetic studies have been performed most extensively with the hog liver enzyme (6–8) and the kinetic data are consistent with an ordered Ter-Bi mechanism. The overall enzymatic mechanism is postulated to occur with an irreversible step between the second and third substrates, where NADPH is added first, followed by molecular oxygen and the oxygenatable substrate. After FAD reduction by NADPH, molecular oxygen adds to the reduced flavin, forming a relatively stable, long lived peroxyflavoenzyme. Next, the oxygenatable substrate attacks the electrophilic peroxyflavin to form the hydroxyflavin (or flavin pseudobase) and oxygenated product. Release of oxygenated product and release of $NADP^+$ are predicted to be fast. Elimination of pseudobase water to form oxidized flavin is postulated to be slow, and it is this step that is thought to be rate limiting. The mechanism predicts that all good substrates should have similar V_{max} values, because the postulated rate limiting step is thought to occur after oxygenated product is formed (6–8). It has been suggested that primary amines such as *n*-octylamine accelerate the overall reaction by facilitating the base-catalyzed elimination of flavin pseudobase water (1, 2). Thus, the V_{max} of hog liver FMO is generally increased severalfold in the presence of *n*-octylamine (2, 9). In contrast, stimulation of rat liver FMO is variable (10), suggesting that other enzymatic steps may also be partially rate limiting for FMO.

The cytochromes P-450 catalyze a wide variety of oxygena-

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ABBREVIATIONS: FMO, flavin-containing monooxygenase; HPLC, high pressure liquid chromatography; TLC, thin layer chromatography.

tions of drugs, chemicals, and pesticides (11, 12). Studies of the enzyme mechanism show that substrate binding to the hemoprotein initiates the transfer of one electron from NADPH to heme iron (13). After the substrate-hemoprotein complex is reduced, oxygen binds and this subsequent iron-oxo species is reduced by transfer of a second electron from cytochrome P-450 reductase. Sulfur-containing substrates are readily oxidized by the powerful Fe^{+3}-O_2^{2-} complex of the hemoprotein (14). The mechanism of sulfoxidation has not been as thoroughly studied as other cytochrome P-450-catalyzed oxidations, but it is thought to involve one-electron oxidation forming a sulfenium cation radical, which can accept oxygen from the iron-oxo species, yielding a sulfoxide, or lose an α proton followed by radical rearrangement, generating a carbon-centered radical. Addition of oxygen to the carbon-centered radical yields the α-hydroxyl sulfide, which dealkylates to the thiol and aldehyde (15). In general, for non-electron-deficient sulfides, *S*-oxide formation predominates over *S*-dealkylation in the presence of cytochromes P-450 (16).

Studies to date suggest that the isozyme induced by phenobarbital in rat (17) and rabbit (18) liver is the primary cytochrome P-450 responsible for the oxidation of sulfides to sulfoxides. Cytochrome P-450 catalyzed *S*-oxygenation is not completely stereoselective but, for the few cases examined, cytochrome P-450 preferentially forms the S(+) (*S*)-oxide enantiomer (18–20). In contrast, hog liver FMO demonstrates a greater degree of stereoselectivity and predominantly forms the *S*-oxide enantiomer (21).

In this paper we describe a systematic analysis of kinetic and product studies of FMO-R(−) and cytochrome P-450IIB-catalyzed sulfoxidation reactions. The *S*-oxygenase activities of FMO and cytochrome P-450 were examined to investigate the similarities and/or dissimilarities in stereoselectivities of *S*-oxygenation of common substrates by these monooxygenases. The objectives of this study were to synthesize a series of *para*-substituted 2-aryl-1,3-dithiolanes and *para*-substituted 2-aryl-1,3-dithiolane *S*-oxides and investigate the electronic and stereoselectivity requirements for *S*-oxygenation by hog liver microsomes and highly purified hog liver FMO and rat and mouse liver cytochrome P-450IIB. The results of our studies with 2-aryl-1,3-dithiolanes provide a clear view of the kinetic and stereochemical consequences of substrate structural alterations on FMO- and cytochrome P-450-mediated *S*-oxygenation reactivity and provide insight into a number of FMO and cytochrome P-450IIB enzymatic steps.

Experimental Procedures

Materials. *Para*-substituted benzaldehydes, *para*-toluenesulfonic acid, 1,2-ethanedithiobis(trimethylsilane), sodium borohydride, sodium *meta*-periodate, and *n*-octylamine were obtained from Aldrich Chemical Co. Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, $NADP^+$, and NADPH were purchased from Sigma Chemical Co. All other reagents and buffers were obtained in the highest quality available from commercial sources. Hog liver microsomes were a generous gift of Prof. D. M. Ziegler (University of Texas at Austin) and FMO was isolated and purified from hog liver microsomes by a procedure previously described (22).

Rat liver cytochrome $P\text{-}450_{PB\text{-}B}$ (P-450IIB-1) (23), cytochrome b_5, and NADPH-cytochrome P-450 reductase were purified to homogeneity by a method previously described (24). Mouse liver cytochrome $P\text{-}450_{PB\text{-}B}$ (P-450IIB-10) (23) was purified as previously described (25). Rat cytochrome P450IIB-1 and mouse cytochrome P-450IIB-10 had specific contents of 19.5 and 11.7 nmol of P-450/mg of protein, respectively. The specific content of cytochrome b_5 was 32.8 nmol of b_5/mg of protein and the reductase preparation had an activity of 66 μmol of cytochrome *c*/min/mg of protein in 0.3 M potassium phosphate buffer (pH 7.7) at 25°.

The major phenobarbital-inducible cytochrome P-450 from mouse and rat liver exhibited high pentoxyresorufin *O*-dealkylase and 16β-testosterone hydroxylase activities (6 and 3 nmol of product/min/nmol of cytochrome P-450, respectively) shown to be characteristic of the phenobarbital-induced isozymes and were judged to be homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (26).

Synthesis of 2-aryl-1,3-dithiolanes and *S*-oxides. The synthesis of *para*-substituted 2-aryl-1,3-dithiolanes followed the general procedure previously described (27–29). All synthetic materials were fully characterized by NMR, IR, mass spectrometry, UV-vis, and HPLC analysis. The chemical properties of 2-aryl-1,3-dithiolanes (**1–5**) are listed in Tables 1 and 2. The synthesis of 2-aryl-1,3-dithiolane *S*-oxides (**6–10**) was accomplished by combining 1.2 equivalents of sodium *meta*-periodate (in water) with 1 equivalent of 2-aryl-1,3-dithiolane (in methanol). The crude product was placed on silica gel preparative TLC plates (Merck silica gel, 60F-254 plates, 1.0-mm thickness) and the diastereomers were separated with three or four developments with CH_2Cl_2/CH_3OH (98:2, v/v). The chemical properties of 2-aryl-1,3-dithiolane *S*-oxides (**6–10**) are listed in Tables 1 and 2. 2-Aryl-1,3-dithiolane *S*,*S*′-dioxides (**11–15**) were synthesized by treating the corresponding *S*-monooxide with 1.2 equivalents of sodium *meta*-periodate. The *S*,*S*′-dioxide diastereomeric products **11** and **12** were isolated after chromatography on silica gel preparative TLC with three or four developments with CH_2Cl_2/CH_3OH (95:5, v/v), and **13–15** were isolated after chromatography on alumina preparative TLC [Sigma WN-3 alumina (grade II) plates, 1.0-mm thickness] with three or four developments with CH_2Cl_2/CH_3OH (90:10, v/v). The chemical properties of the 2-aryl-1,3-dithiolane *S*,*S*′-dioxides **11–15** are shown in Tables 1 and 2. In all cases, the compounds were low melting solids or waxes and the yields reported were isolated yields after purification.

Metabolism incubations and enzyme assays. Activity of the FMO or cytochrome P-450 was measured by monitoring *S*-oxide product formation by HPLC. Unless otherwise indicated, the incubation medium contained 50 mM potassium phosphate (pH 8.4 for FMO or pH 7.4 for cytochrome P-450) and 0.5 mM NADPH or an NADPH-generating system consisting of 0.5 mM $NADP^+$, 2.0 mM glucose-6-phosphate, and 1 IU of glucose-6-phosphate dehydrogenase. After a 5-min equilibration, the reaction mixture was cooled and FMO or microsomes from hog liver were added. The reaction was initiated by addition of substrate at the concentration specified. For reactions with cytochrome P-450, reactions were initiated by addition of NADPH. At various time intervals, the reaction was stopped by the addition of 4 volumes of cold CH_2Cl_2 and, after thorough mixing, the insoluble material was separated by a brief centrifugation. After filtration through a 4-μm nylon filter and evaporation, the extract was taken up in acetonitrile for separation and quantification by HPLC (27, 28) (IBM model 9000 with UV detector, fitted with a C-18 precolumn and 5-μm C-18 Altex Ultrasphere ODS reverse phase analytical column). The mobile phase efficiently separates 2-aryl-1,3-dithiolanes, 2-aryl-1,3-dithiolane *S*-oxides, and 2-aryl-1,3-dithiolane *S*,*S*′-dioxides (Table 3). The recovery of metabolites, as judged by HPLC, was greater than 90%, and 95% of this material was the dithiolane, dithiolane *S*-oxide, or dithiolane *S*,*S*′-dioxide. Quantification was accomplished by comparing the integrated HPLC peak areas of the dithiolane, dithiolane *S*-oxide, or dithiolane *S*,*S*′-dioxide, after taking into consideration the extinction coefficient values of each species at the specified wavelength (Table 3).

Determination of 2-aryl-1,3-dithiolane *S*-oxide stereochemistry. The absolute stereochemistry of 2-aryl-1,3-dithiolane *S*-oxide metabolites was determined after correlating the *S*-oxide metabolites obtained with authentic synthetic *S*-oxides. The synthesis of optically active dithiolane *S*-oxides followed the general procedure of Pitchen *et*

TABLE 1
Chemical properties of synthetic 2-aryl-1,3-dithiolanes,2-aryl-1,3-dithiolane S-oxides, and 2-aryl-1,3-dithiolane S,S′-dioxides

Compound		Diastereomer trans (a)	Diastereomer cis (b)	Overall yield		UV-visible λ_{max} (ε)	EIMS[a]
		%		%		nm (cm^{-1})	m/z (relative intensity)
2-Aryl-1,3-dithiolanes							
1	4-CH_3O—			64		234 (4,697)	
2	4-H—			55		260 (606)	
3	4-Cl			33		216 (6,687)	
4	4-CN—			34		260 (4,442)	
5	4-NO_2—			58		260 (4,129)	
2-Aryl-1,3-dithiolane S-oxides							
6a,b	4-CH_3O—	56	44	91		236 (9,200)	228 (68%), 165 (4%), 152 (100%)
7a,b	4-H—	92	8	66		260 (519)	198 (56%), 121 (100%), 105 (10%)
8a,b	4-Cl—	98	2	38		226 (9,394)	232 (1.4%), 156 (100%), 135 (6%)
9a,b	4-CN—	60	40	63		264 (5,600)	223 (71%), 147 (100%), 100 (19%)
10a,b	4-NO_2—	70	30	98		260 (7,003)	227 (7%), 167 (100%), 121 (31%)
2-Aryl-1,3-dithiolane S,S′-dioxides							
11a,b	4-CH_3O—	90	10	11	**11a**	238 (3,000)	244 (1%), 168 (18%), 135 (100%)
					11b	237 (3,030)	244 (2%), 168 (37%), 136 (100%)
12a,b	4-H—	92	8	38	**12a**	240 (1,024)	137 (20%), 122 (33%), 108 (100%)
					12b	240 (2,252)	137 (20%), 122 (97%), 108 (69%)
13a,b	4-Cl—	80	20	32	**13a**	228 (5,282)	249 (4%), 232 (63%), 108 (100%)
					13b	224 (9,448)	249 (1%), 232 (3%), 108 (100%)
14a,b	4-CN—	91	9	26	**14a**	260 (13,189)	162 (14%), 146 (68%), 108 (100%)
					14b	264 (3,481)	239 (2%), 146 (65%), 108 (100%)
15a,b	4-NO_2—	93	7	24	**15a**	260 (3,477)	259 (4.5%), 166 (56%), 150 (100%)
					15b	260 (8,963)	259 (1%), 167 (55%), 150 (100%)

[a] EIMS, electron impact mass spectrometry.

al. (29). The optical purity of the product *S*-oxides was determined by NMR analysis and the configuration was established by correlating the CD absorption spectra, as described before (28). A previous study of 1,3-dithiolane *S*-oxides established that a positive CD can be correlated with an (*R*)-sulfoxide configuration (30, 31). With synthetic chiral *S*-oxides to serve as standards, an efficient HPLC method to separate each *cis*- and *trans*-*S*-oxide enantiomer was developed. HPLC was performed with an IBM model 9000, fitted with a Daicel Chiralcel OD (25 cm × 0.46 cm i.d.) analytical column (Daicel Chemical Industries, New York). The mobile phase consisted of isopropanol/hexane (18:82, v/v). This system efficiently separates the various enantiomers, as shown in Table 4. Quantification was accomplished by comparing the integrated area of the *S*-oxide HPLC peak, after taking into consideration the extinction coefficient values of each species at the wavelength monitored (Table 2). Dichloromethane extracts of metabolic reactions (as described above) were evaporated to dryness, reconstituted in isopropanol/hexane (18:82, v/v), and analyzed by Chiralcel OD HPLC.

Other analytical methods. Heat-inactivated microsomes were prepared by the method previously described (1, 27, 28). The concentration of protein used in this study was determined by the method of Bradford (32) or by the Pierce protein assay method.

Results

Reaction of 2-aryl-1,3-dithiolanes and 2-aryl-1,3-dithiolane *S*-oxides with sodium *meta*-periodate. At neutral pH, the reaction of 2-aryl-1,3-dithiolanes with an equimolar concentration of sodium *meta*-periodate produced the corresponding 2-aryl-1,3-dithiolane *S*-oxides (see scheme in Fig. 1) in reasonable overall yield (Table 1). In each case the 2-aryl-1,3-dithiolane *S*-oxide was the only detectable product formed, although at extremely long reaction times or in the presence of acid the *S*-oxide decomposed to the corresponding benzaldehyde.

Of the two possible 2-aryl-1,3-dithiolane *S*-oxide diastereomers that could form (e.g., see Fig. 1), in all cases examined the *trans*-diastereomer was the major product formed (Table 1). Compounds **6–10** were quite stable between the pH values of 6 and 9 employed for these investigations. Based on studies of similar compounds (33), we estimate that the aqueous half-life (pH 7.4) for a 2-aryl-1,3-dithiolane *S*-oxide is approximately 100 hr. 2-Aryl-1,3-dithiolane *S*-oxides can be stored in benzene at −70° with minimal chemical decomposition.

The reaction of the major *trans*-2-aryl-1,3-dithiolane *S*-oxide with one equivalent of sodium *meta*-periodate produced the corresponding 2-aryl-1,3-dithiolane *S,S′*-dioxide diastereomers. The finding that sequential oxygenation occurs on the sulfide sulfur and not on the *S*-oxide sulfur was confirmed by high field proton NMR and mass spectrometric measurements (Tables 1, 3, and 5). Although the isolated yields reported for the chemical 2-aryl-1,3-dithiolane-*S,S′*-dioxides were low, this is mainly due to the extensive chromatographic purification of these materials. As in the case of dithiolane monooxygenation, of the two diastereomers that could form, a major *trans,trans*-*S,S′*-dioxide diastereomer was formed in greater than 80% yield (Table 1). Like the 2-aryl-1,3-dithiolane *S,S′*-dioxides, the extensive chromatography required to separate 2-aryl-1,3-dithiolane *S*-oxides led to decomposition of **9** and **10**. Thus, **6–8** were used in further chemical and biotransformation studies. In order to demonstrate that the instability of **9** and **10** was due to the chromatographic conditions and not to some inherent chemical property, the stability of **9a** toward configurational isomerization was examined. NMR studies demonstrated that no ionization and configurational isomerization of **9a** was apparent, even in the presence of a small amount of base for prolonged periods (data not shown).

Stereoselective hog liver microsome-mediated *S*-oxygenation of 2-aryl-1,3-dithiolanes. The biotransformation of **1–5** was studied *in vitro*. Studies showed that hog liver microsomes supplemented with NADPH catalyzed the rapid and temperature-dependent *S*-oxygenation of 2-aryl-1,3-dithiolanes (Table 6). The formation of 2-aryl-1,3-dithiolane *S*-oxides

TABLE 2

NMR spectral data for 2-aryl-1,3-dithiolanes, 2-aryl-1,3-dithiolane *S*-oxides, and 2-aryl-1,3-dithiolane *S,S′*-dioxides[a]

2-Aryl-1,3-dithiolanes

1 δ7.50 (bd, J = 8.2 Hz, 2 H, C2- and C5-H), 6.84 (bd, J = 8.25 Hz, 2 H, C3- and C4-aromatic H), 5.63 (s, 1 H, C6-H), 3.8 (s, 3 H, CH_3O), 3.50 (2 overlapping dd, J = 15.1 Hz, J = 11.7 Hz, 2 H, C7- and C9-H), 3.34 (2 overlapping dd, J = 15.1 Hz, J = 11.7 Hz, 2 H, C8- and C10-H)

2 δ7.52 (d, J = 7.3 Hz, 2 H, C2- and C5-aromatic H), 7.31 (t, J = 7.4 Hz, 2 H, C3- and C4-aromatic H), 7.26 (t, J = 7.4 Hz, 1 H, C1-aromatic H), 5.63 (s, 1 H, C6-H), 3.50 (2 overlapping dd, J = 17.4 Hz, J = 11.5 Hz, 2 H, C7- and C9-H), 3.35 (2 overlapping dd, J = 17.4 Hz, J = 11.5 Hz, 2 H, C8- and C10-H)

3 δ7.49 (bd, J = 8.4 Hz, 2 H, C2- and C5-aromatic H), 7.31 (bd, J = 8.4 Hz, 2 H, C3- and C4-aromatic H), 5.62 (s, 1 H, C6-H), 3.50 (2 overlapping dd, J = 20.0 Hz, J = 12.1 Hz, 2 H, C7- and C9-H), 3.36 (2 overlapping dd, J = 20.0 Hz, J = 12.1 Hz, 2 H, C8- and C10-H)

4 δ7.60 (bs, 4 H, C2-, 3-, 4-, and 5-aromatic H), 5.59 (s, 1 H, C6-H), 3.50 (2 overlapping dd, J = 17.0 Hz, J = 12.2 Hz, 2 H, C7- and C9-H), 3.38 (2 overlapping d, J = 17.0 Hz, J = 12.2 Hz, 2 H, C8- and C10-H)

5 δ8.16 (d, J = 7.5 Hz, 2 H, C2- and C5-aromatic H), 7.67 (d, J = 7.4 Hz, 2 H, C3- and C4-aromatic H), 5.56 (s, 1 H, C6-H), 3.51 (unresolved m, 2 H, C7- and C9-H), 3.41 (unresolved m, 2 H, C8- and C10-H)

2-Aryl-1,3-dithiolane *S*-oxides

6a δ7.39 (d, J = 8.5 Hz, 2 H aromatic C2- and C5-H), 6.87 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 5.36 (s, 1 H, C6-H), 3.80 (ddd, $J_{8,7}$ = 11.0 Hz, $J_{8,10}$ = 11.0 Hz, $J_{8,9}$ = 5.0 Hz, 1 H, H_8), 3.79 (s, 3 H, OCH_3), 3.58 (ddd, $J_{7,8}$ = 11.0 Hz, $J_{7,10}$ = 7.5 Hz, $J_{7,9}$ = 2.0 Hz, 1 H, H_7), 3.32 (ddd, $J_{9,10}$ = 14.5 Hz, $J_{9,7}$ = 2.0 Hz, $J_{9,8}$ = 5.0 Hz, 1 H, H_9), 2.89 (ddd, $J_{10,9}$ = 14.5 Hz, $J_{10,8}$ = 11.0 Hz, $J_{10,7}$ = 7.5 Hz, 1 H, H_{10})

6b δ7.49 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 6.91 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 5.28 (s, 1 H, C6-H), 3.93 (ddd, $J_{8,7}$ = 10.5 Hz, $J_{8,10}$ = 10.5 Hz, $J_{8,9}$ = 5.5 Hz, 1 H, H_8), 3.80 (s, 3 H, OCH_3), 3.51 (ddd, $J_{9,10}$ = 13.5 Hz, $J_{9,8}$ = 5.5 Hz, $J_{9,7}$ = 3.0 Hz, 1 H, H_9), 3.42 (ddd, $J_{7,8}$ = 10.5 Hz, $J_{7,10}$ = 7.0 Hz, $J_{7,9}$ = 3.0 Hz, 1 H, H_7), 3.12 (ddd, $J_{10,9}$ = 13.5 Hz, $J_{10,8}$ = 10.5 Hz, $J_{10,7}$ = 7.0 Hz)

7a δ7.50 (d, J = 7.1 Hz, 2 H, aromatic C3- and C4-H), 7.41 (t, J = 7.1 Hz, 2 H, aromatic C2- and C5-H), 7.37 (buried t, J = 7.1 Hz, 1 H, C1-H), 5.45 (s, 1 H, C6-H), 3.87 (ddd, $J_{8,7}$ = 11.3 Hz, $J_{8,10}$ = 11.3 Hz, $J_{8,9}$ = 5.6 Hz, 1 H, H_8), 3.65 (ddd, $J_{7,8}$ = 11.3 Hz, $J_{7,10}$ = 7.0 Hz, $J_{7,9}$ = 3.51, 1 H, H_7), 3.38 (ddd, $J_{9,10}$ = 12.6 Hz, $J_{9,8}$ = 5.6 Hz, $J_{9,7}$ = 3.5 Hz, 1 H, H_9), 2.92 (ddd, $J_{10,9}$ = 12.6 Hz, $J_{10,8}$ = 11.3 Hz, $J_{10,7}$ = 7.0 Hz, 1 H, H_{10})

7b δ7.61 (bd, J = 4.0 Hz, 2 H, aromatic C3- and C4-H), 7.43 (bs, 3 H, aromatic C1-, C2-, and C3-H), 5.35 (s, 1 H, C6-H), 4.02 (ddd, $J_{8,7}$ = 10.5 Hz, $J_{8,10}$ = 10.5 Hz, $J_{8,9}$ = 5.4 Hz, 1 H, H_8), 3.59 (ddd, $J_{9,10}$ = 12.6 Hz, $J_{9,8}$ = 5.4 Hz, $J_{9,7}$ = 2.0 Hz, 1 H, H_9), 3.51 (ddd, $J_{7,8}$ = 10.5 Hz, $J_{7,10}$ = 6.0 Hz, $J_{7,9}$ = 2.0 Hz, 1 H, H_7), 3.18 (ddd, $J_{10,9}$ = 12.6 Hz, $J_{10,8}$ = 10.5 Hz, $J_{10,7}$ = 6.0 Hz, 1 H, H_{10})

8a δ7.47 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.39 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 5.38 (s, 1 H, C6-H), 3.87 (ddd, $J_{8,7}$ = 11.3 Hz, $J_{8,10}$ = 11.3 Hz, $J_{8,9}$ = 5.4 Hz, 1 H, H_8), 3.64 (ddd, $J_{7,8}$ = 11.3 Hz, $J_{7,10}$ = 7.1 Hz, $J_{7,9}$ = 2.2 Hz, 1 H, H_7), 3.39 (ddd, $J_{9,10}$ = 13.5 Hz, $J_{9,8}$ = 5.4 Hz, $J_{9,7}$ = 2.2 Hz, 1 H, H_9), 2.91 (ddd, $J_{10,9}$ = 13.5 Hz, $J_{10,8}$ = 11.3 Hz, $J_{10,7}$ = 7.1 Hz, 1 H, H_{10})

9a δ7.68 (d, J = 8.4 Hz, 2 H, aromatic C2- and C5-H), 7.63 (d, J = 8.4 Hz, 2 H, aromatic C3- and C4-H), 5.37 (s, 1 H, C6-H), 3.80 (ddd, $J_{8,7}$ = 11.0 Hz, $J_{8,10}$ = 11.0 Hz, $J_{8,9}$ = 6.5 Hz, 1 H, H_8), 3.65 (ddd, $J_{7,8}$ = 11.0 Hz, $J_{7,10}$ = 7.0 Hz, $J_{7,9}$ = 2.0 Hz, 1 H, H_7), 3.41 (ddd, $J_{9,10}$ = 13.5 Hz, $J_{9,8}$ = 6.5 Hz, $J_{9,7}$ = 2.0 Hz, 1 H, H_9), 2.91 (ddd, $J_{10,9}$ = 13.5 Hz, $J_{10,8}$ = 11.0 Hz, $J_{10,7}$ = 7.0 Hz, 1 H, H_{10})

9b δ7.69 (s, 4 H, aromatic H), 5.33 (s, 1 H, C6-H), 4.03 (ddd, $J_{8,7}$ = 10.5 Hz, $J_{8,10}$ = 10.5 Hz, $J_{8,9}$ = 4.5 Hz, 1 H, H_8), 3.60 (ddd, $J_{9,10}$ = 13.0 Hz, $J_{9,8}$ = 4.5 Hz, $J_{9,7}$ = 2.5 Hz, 1 H, H_9), 3.51 (ddd, $J_{7,8}$ = 10.5 Hz, $J_{7,10}$ = 7.0 Hz, $J_{7,9}$ = 2.5 Hz, 1 H, H_7), 3.14 (ddd, $J_{10,9}$ = 13.0 Hz, $J_{10,8}$ = 10.5 Hz, $J_{10,7}$ = 7.0 Hz, 1 H, H_{10})

10a δ8.23 (d, J = 8.3 Hz, 2 H, aromatic C2- and C5-H), 7.70 (d, J = 8.3 Hz, 2 H, aromatic C3- and C4-H), 5.43 (s, 1 H, C6-H), 3.88 (ddd, $J_{8,7}$ = 11.9 Hz, $J_{8,10}$ = 11.9 Hz, $J_{8,9}$ = 6.2 Hz, 1 H, H_8), 3.67 (ddd, $J_{7,8}$ = 11.9 Hz, $J_{7,10}$ = 8.1 Hz, $J_{7,9}$ = 2.5 Hz, 1 H, H_7), 3.43 (ddd, $J_{9,10}$ = 13.3 Hz, $J_{9,8}$ = 6.2 Hz, $J_{7,9}$ = 2.5 Hz, 1 H, H_9), 2.91 (ddd, $J_{10,9}$ = 13.3 Hz, $J_{10,8}$ = 11.9 Hz, $J_{10,7}$ = 8.1 Hz, 1 H, H_{10})

10b δ8.24 (d, J = 8.0 Hz, 2 H, aromatic C2- and C5-H), 7.75 (d, J = 8.0 Hz, 2 H, aromatic C3- and C4-H), 5.41 (s, 1 H, C6-H), 4.05 (ddd, $J_{8,7}$ = 10.5 Hz, $J_{8,10}$ = 10.5 Hz, $J_{8,9}$ = 6.0 Hz, 1 H, H_8), 3.61 (ddd, $J_{9,10}$ = 14.5 Hz, $J_{9,8}$ = 5.0 Hz, $J_{9,7}$ = 3.0 Hz, 1 H, H_9), 3.55 (ddd, $J_{7,8}$ = 10.5 Hz, $J_{7,10}$ = 7.6 Hz, $J_{7,9}$ = 3.0 Hz, 1 H, H_7), 2.92 (ddd, $J_{10,9}$ = 14.5 Hz, $J_{10,8}$ = 10.5 Hz, $J_{10,7}$ = 7.6 Hz, 1 H, H_{10})

2-Aryl-1,3-dithiolane *S,S′*-dioxides

11a δ7.20 (d, J = 8.6 Hz, 2 H, aromatic C2- and C5-H), 7.00 (d, J = 8.6 Hz, 2 H, aromatic C3- and C4-H), 4.98 (s, 1 H, C6-H), 3.97 (bs, 2 H, CHCH), 3.95 (bd, J = 4.5, 1 H, CH), 3.88 (s, 3 H, OCH_3), 3.79 (bd, J = 7.09, 1 H, CH)

11b δ7.57 (d, J = 8.4 Hz, 2 H, aromatic C2- and C5-H), 7.06 (d, J = 8.4 Hz, 2 H, aromatic C3- and C5-H), 4.50 (s, 1 H, C6-H), 4.03–3.99 (m, 2 H, CHCH), 3.89 (s, 3 H, OCH_3), 3.68 (bd, J = 6.58 Hz, 2 H, CHCH)

12a δ7.45 (bt, J = 5.45 Hz, 2 H, aromatic C3- and C4-H), 7.25 (m, 3 H, aromatic C1-, C2-, and C5-H), 4.93 (s, 1 H, C6-H), 3.97–3.95 (m, 3 H, CH_2CH), 3.78 (bd, J = 7.0 Hz, 1 H, CH)

12b δ7.61 (bdd, J = 5.2 Hz, J = 1.0 Hz, 2 H, aromatic C3- and C4-H), 7.50 (bt, J = 5.2 Hz, 3 H, aromatic C1-, C2-, and C5-H), 5.24 (s, 1 H, C6-H), 3.98 (m, 2 H, CH), 3.67 (m, 2 H, CH)

13a δ7.42 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.17 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 4.89 (s, 1 H, C6-H), 3.97–3.94 (m, 3 H, CH_2CH), 3.79 (m, 1 H, CH)

13b δ7.59 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.53 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 4.49 (s, 1 H, C6-H), 4.06–4.00 (m, 2 H, CHCH), 3.77–3.69 (m, 2 H, CHCH)

14a δ7.73 (d, J = 7.5 Hz, 2 H, aromatic C2- and C5-H), 7.35 (d, J = 7.5 Hz, 2 H, aromatic C3- and C4-H), 4.92 (s, 1 H, C6-H), 4.09–3.92 (m, 3 H, CH_2CH), 3.84 (m, 1 H CH)

14b δ7.75 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.69 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 4.46 (s, 1 H, C6-H), 4.02–3.98 (m, 2 H, CHCH), 3.71–3.67 (m, 2 H, CHCH)

15a δ8.23 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.36 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 4.91 (s, 1 H, C6-H), 3.98–3.91 (m, 3 H, CH_2CH), 3.79 (bd, J = 8.5 Hz, 1 H, CH)

15b δ8.26 (d, J = 8.5 Hz, 2 H, aromatic C2- and C5-H), 7.70 (d, J = 8.5 Hz, 2 H, aromatic C3- and C4-H), 4.48 (s, 1 H, C6-H), 3.99–3.87 (m, 2 H, CHCH), 3.69–3.58 (m, 2 H, CHCH)

[a] HNMR data ($CDCl_3$) relative to tetramethylsilane.

was a linear function of protein concentration (0–1.2 mg/ml) and of incubation time for at least 4 min, except for compounds **4** and **5**, which showed linear S-oxide production for at least 7 min. For hog liver microsomes that had previously been heat inactivated in the absence of NADPH under conditions that destroy the FMO (1, 34) but that do not inactivate cytochrome P-450 (34), the formation of *S*-oxide was significantly reduced (Table 6).

n-Octylamine, a known positive effector for the FMO (1) and an inhibitor of cytochromes P-450 (35), generally stimulated the formation of the *S*-oxide 20–30%, except for compound **1**, where a 30% inhibition was observed (data not shown). During the short incubation times employed in these studies, 2-aryl-1,3-dithiolane *S*-oxide metabolites were the only materials detected in dichloromethane extracts analyzed by HPLC. After brief exposure of authentic 2-aryl-1,3-dithiolane *S*-oxides (**6a** to **10a**) to the complete active microsomal incubation reaction, the *S*-oxides were quantitatively recovered unchanged. *S*-Oxygenation of 2-aryl-1,3-dithiolanes may produce either *cis*- or *trans*-*S*-oxide diastereomers. As shown in Table 6, stereoselec-

TABLE 3

HPLC properties of 2-aryl-1,3-dithiolanes, 2-aryl-1,3-dithiolane S-oxides, and 2-aryl-1,3-dithiolane S,S′-dioxides

	Compound	Retention volume	Solvent system[a]	Wavelength monitored
		ml		*nm*
2-Aryl-1,3-dithiolanes				
1	4′-CH_3O—	11.8	A	240
2	4′-H—	12.6	A	260
3	4′-Cl—	21.2	A	240
4	4′-CN—	8.9	A	260
5	4′-NO_2—	11.2	A	260
2-Aryl-1,3-dithiolane S-oxides				
6a	4′-CH_3O—	6.7	B	240
6b	4′-CH_3O—	5.9	B	240
7a	4′-H—	6.1	B	260
7b	4′-H—	5.3	B	260
8a	4′-Cl—	10.7	B	240
8b	4′-Cl—	9.0	B	240
9a	4′-CN—	8.5	C	260
9b	4′-CN—	7.9	C	260
10a	4′-NO_2—	9.9	D	260
10b	4′-NO_2—	9.1	D	260
2-Aryl-1,3-dithiolane S,S′-oxides				
11a	4′-CH_3O—	6.6	E	240
11b	4′-CH_3O—	5.6	E	240
12a	4′-H—	5.7	F	260
12b	4′-H—	5.1	F	260
13a	4′-Cl—	4.8	F	240
13b	4′-Cl—	4.3	F	240
14a	4′-CN—	5.7	E	260
14b	4′-CN—	5.0	E	260
15a	4′-NO_2—	5.4	F	260
15b	4′-NO_2—	4.9	F	260

[a] Solvent systems: A, 56% CH_3CN/H_2O; B, 40% CH_3CN/H_2O; C, 29% CH_3CN/H_2O; D, 32% CH_3CN/H_2O; E, 20% CH_3CN/H_2O; F, 25% CH_3CN/H_2O.

TABLE 4

Chiralcel OD HPLC separation of 2-aryl-1,3-dithiolane S-oxide enantiomers

HPLC conditions for separation of enantiomers are given in Experimental Procedures.

2-Aryl-1,3-dithiolane S-oxide		Retention volume			
		trans-S-Oxide		*cis*-S-Oxide	
		(1*S*, 2*S*)	(1*R*, 2*R*)	(1*S*, 2*R*)	(1*R*, 2*S*)
		ml			
6	4-OCH_3—	14.6	15.7	16.7	22.9
7	4-H—	14.5	15.7	17.1	18.4
8	4-Cl—	17.3	19.0	21.4	23.5
9	4-CN—	29.9	31.6	24.0	29.0
10	4-NO_2—				

tive *S*-oxygenation for electron-rich 2-aryl-1,3-dithiolanes (i.e., compounds **1** and **2**) was considerably greater than the stereoselectivity observed for *S*-oxygenation of electron-deficient 2-aryl-1,3-dithiolanes (i.e., compounds **4** and **5**). In the presence of *n*-octylamine, an interesting reversal of the configurational stereoselectivity of *S*-oxygenation is observed for the electron-deficient 2-aryl-1,3-dithiolanes.

Highly purified FMO-mediated *S*-oxygenation of 2-aryl-1,3-dithiolanes. Without exception, 2-aryl-1,3-dithiolanes **1–5** were excellent substrates for the purified FMO from hog liver microsomes (Table 7). Preliminary studies showed that hog liver FMO supplemented with NADPH catalyzed extremely efficient *S*-oxygenation of 2-aryl-1,3-dithiolanes. The only initial product detected corresponded to the *S*-oxide. The formation of *S*-oxide was a linear function of protein concentration (0–0.3 mg/ml) and of reaction incubation time for at least 4 min.

For substrates **1**, **3**, and **5**, FMO catalyzed the formation of the 2-aryl-1,3-dithiolane *S*-oxide up to 1.2-fold when *n*-octylamine was added to the reaction (Table 7). In agreement with studies employing hog liver microsomes, *S*-oxygenation of *para*-methoxyaryl-1,3-dithiolane was decreased in the presence of *n*-octylamine. These results can be interpreted to suggest that the stimulatory effect of *n*-octylamine on *S*-oxygenation may be suppressed in some cases, and this will be discussed in greater detail below.

As shown in Tables 8 and 9, stereoselective *S*-oxygenation by microsomes and by the highly purified hog liver FMO for electron-rich 2-aryl-1,3-dithiolanes (e.g., compounds **1–3**) was greater than the stereoselectivity observed for *S*-oxygenation of electron-deficient 2-aryl-1,3-dithiolanes (e.g., compounds **4** and **5**). In agreement with what was observed for *S*-oxygenation in the presence of hog liver microsomes, the configurational stereoselectivity of FMO-mediated *S*-oxygenation of electron-deficient 2-aryl-1,3-dithiolane was reversed in the presence of *n*-octylamine.

S-Oxygenation of *para*-cyanophenyl-1,3-dithiolane was investigated in order to determine the influence of NADPH and *n*-octylamine on the kinetic pattern of *S*-oxygenation by the highly purified FMO from hog liver. Initial velocity measurements for *S*-oxygenation of *para*-cyanoaryl-1,3-dithiolane are shown in Figs. 2 and 3. Double-reciprocal plots of initial velocities of *S*-oxygenation with variable concentrations of NADPH and *para*-cyanoaryl-1,3-dithiolane gave sets of parallel lines. In the presence of 250 μM NADPH, K_m and V_{max} values of 26.3 μM and 495 nmol/min/mg of protein, respectively, were obtained for *para*-cyanoaryl-1,3-dithiolane *S*-oxygenation (Fig. 2). However, at saturating concentrations of *n*-octylamine and variable concentrations of NADPH and *para*-cyanoaryl-1,3-dithiolane, the double-reciprocal plots intersect (Fig. 3). The V_{max} value (495 nmol/min/mg of protein) was unchanged but a $K_{m_{app}}$ of 50 μM was calculated. In parallel with the kinetic measurements, stereochemical investigations were carried out. The relative configuration of the *S*-oxide products for initial velocity studies are shown in Figs. 4 and 5. As shown in Fig. 4, in the presence of subsaturating concentrations of NADPH the ratio of *trans*- to *cis*-*S*-oxide formed is markedly dependent upon the concentration of substrate present. In contrast, at higher concentrations of NADPH (e.g., 12.5, 25, and 250 μM NADPH) (Fig. 2) the only product detected from incubations with the dithiolane at all concentrations of substrate employed was the (*trans*)-*para*-cyanoaryl-1,3-dithiolane *S*-oxide (data not shown). In the presence of saturating concentrations of *n*-octylamine and various concentrations of NADPH and substrate, the ratio of *cis*-*S*-oxide to *trans*-*S*-oxide was considerably different. Thus, in the presence of 25 μM NADPH and various concentrations of substrate approximately 25–35% of the product was the *cis*-*S*-oxide, whereas in the presence of 250 μM NADPH and various concentrations of substrate approximately 85–95% of the product was the *cis*-*S*-oxide. To investigate this further, the *S*-oxygenation of *para*-cyanoaryl-1,3-dithiolane was studied in the presence of saturating concentrations of substrate and NADPH. As shown in Fig. 5, increasing the concentration of *n*-octylamine increased the percentage of *cis*-*S*-oxide formed. The approximate EC_{50} was calculated to be slightly less than 2 mM.

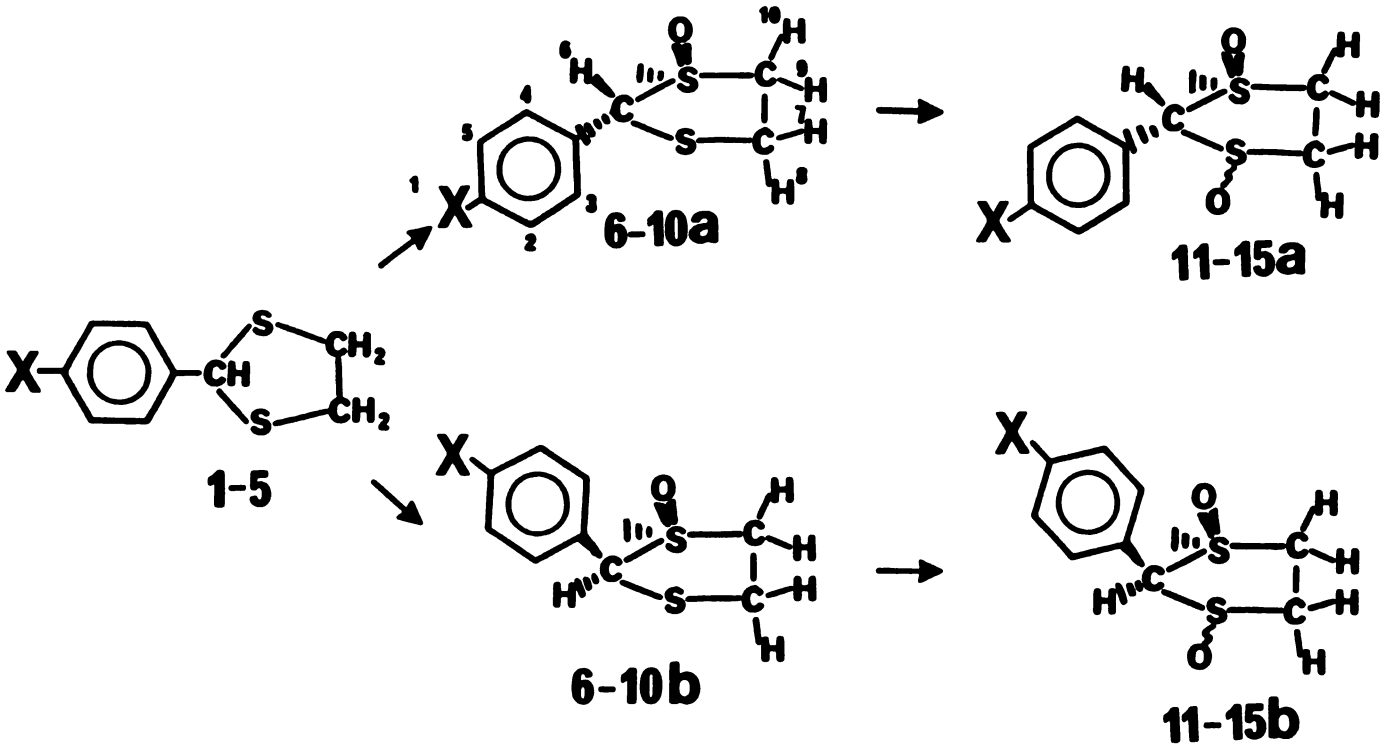

Fig. 1. Overall *S*-oxidative biotransformation of 2-aryl-1,3-dithiolanes (**1–5**) to 2-aryl-1,3-dithiolane *S*-oxides (**6a,b–10a,b**) and, ultimately, to 2-aryl-1,3-dithiolane *S,S′*-dioxides (**11a,b–15a,b**).

TABLE 5

High resolution mass spectral characteristics of 2-aryl-1,3-dithiolane *S*-oxides and *S,S′*-dioxides

High resolution mass spectra were obtained using a modified Kratos MS-25 in the electron impact mode. Relative intensity pertains to the percentage of total ions relative to the base peak.

Compound		Formula	Atomic mass		HREIMS[a]
			Calculated	Observed ± ppm	
			amv		*m/z (relative intensity)*
2-Aryl-1,3-dithiolane *S*-oxides					
6a	4-OCH_3—	$C_{10}H_{12}S_2O_2$	228.0283	228.0283 + 1.9	228 (65%), 152 (100%), 108 (6%)
6b	4-OCH_3—	$C_{10}H_{12}S_2O_2$	228.0283	228.0283 + 2.0	228 (50%), 152 (100%), 108 (6%)
7a	4-H—	$C_9H_{10}S_2O$	198.0174	198.0174 + 1.0	198 (52%), 149 (4%), 122 (100%)
7b	4-H—	$C_9H_{10}S_2O$	198.0174	198.0174 + 0.5	198 (60%), 149 (5%), 122 (100%)
8a	4-Cl—	$C_9H_9ClS_2O$	231.9788	231.9788 + 1.8	232 (71%), 162 (10%), 156 (100%)
9a	4-CN—	$C_{10}H_9S_2NO$	223.0139	223.0139 + 5.8	223 (49%), 207 (1%), 147 (100%)
9b	4-CN—	$C_{10}H_9S_2NO$	223.0139	223.0139 + 4.6	223 (66%), 174 (6%), 147 (100%)
10a	4-NO_2—	$C_9H_9S_2NO_3$	227.0089	227.0089 + 6.2	227 (13%), 194 (24%), 167 (100%)
2-Aryl-1,3-dithiolane *S,S′*-dioxides					
11a	4-OCH_3—	$C_{10}H_{12}S_2O_3$	244.0237	244.0237 + 3.7	244 (2%), 152 (55%), 136 (100%)
11b	4-OCH_3—	C_8H_8SO[b]	152.0309	152.0309 − 1.4	244 (0.6%), 152 (89%), 135 (100%)
12a	4-H—	$C_9H_{10}S_2O_2$	215.0198	215.0198 − 1.1	215 (1%), 137 (35%), 121 (100%)
12b	4-H—	$C_9H_{10}S_2O$[c]	199.0197	199.0197 − 4.9	215 (0.1%), 198 (28%), 121 (81%)
13a	4-Cl—	$C_9H_9ClS_2O$[d]	231.9797	231.9797 + 5.9	248 (1.3), 232 (13.2), 108 (100%)
14a	4-CN—	$C_{10}H_9S_2NO_2$	240.0146	240.0146 − 2.9	240 (1%), 162 (18%), 146 (57%)
14b	4-CN—	$C_{10}H_9S_2NO_2$	240.0146	240.0146 + 1.9	240 (1%), 162 (26%), 146 (89%)
15a	4-NO_3—	$C_9H_9S_2NO_3$[e]	227.0089	227.0089 + 6.2	243 (4.5), 166 (56%), 150 (100%)
15b	4-NO_2—	$C_9H_9S_2NO_4$	243.0038[f]		243 (15.2%), 166 (29%), 150 (45%)

[a] High resolution electron impact mass spectrometry.
[b] The atomic composition was determined with a major molecular ion (i.e., C_8H_8SO).
[c] The atomic composition was determined with a major molecular ion (i.e., $C_9H_{10}S_2O$).
[d] The atomic composition was determined with a major molecular ion (i.e., $C_9H_9ClS_2O$).
[e] The atomic composition was determined with a major molecular ion (i.e., $C_9H_9S_2NO_3$).
[f] High resolution electron impact mass spectrometry was not possible with this compound.

Enantioselective *S*-oxygenation of 2-aryl-1,3-dithiolanes by FMO and microsomes from hog liver. The *S*-oxygenation of **1–5** by highly purified FMO and microsomes from hog liver was repeated in the presence and absence of *n*-octylamine. For microsomes in the presence of *n*-octylamine, electron-deficient 2-aryl-1,3-dithiolanes such as compound **4** preferentially produced the *cis*-*S*-oxide (Table 8). In contrast to compounds **1–3**, however, in the absence of *n*-octylamine the enantioselectivity of *S*-oxygenation of **4** is reversed. The same stereoselectivity is observed for the *S*-oxygenation of **1–**

TABLE 6

S-Monooxygenation of 2-aryl-1,3-dithiolanes by hog liver microsomes

2-Aryl-1,3-dithiolane	S-Oxide formed (diastereomeric excess)	
	Complete[a]	Heat-inactivated[b]
	nmol/min/mg of protein	
1 4-OCH_3—	18.75 ± 1.7 (92%)[c]	4.7 ± 0.6 (9.3%)[d]
2 4-H—	8.9 ± 1.8 (44%)[c]	0.9 ± 0.3 (89.7%)[d]
3 4-Cl—	18.9 ± 2.4 (94%)[c]	3.5 ± 0.3 (100%)[c]
4 4-NO_2—	4.3 ± 0.5 (28%)[d]	1.3 ± 0.2 (20%)[c]

[a] Hog liver microsomal preparations (200 μg of protein/incubation) were incubated in the presence of an NADPH-regenerating system, *n*-octylamine (5 mM), and substrate (200 μM) at 33° for 2 min in pH 8.4 phosphate buffer (0.2 M).
[b] Microsomes were heated at 55° for 1 min in the absence of an NADPH-regenerating system. Control experiments demonstrated that 85% of the cytochrome P-450 activity was retained, yet essentially no FMO activity remained.
[c] Diastereomeric excess (*trans*-*S*-oxide). Percentage of diastereoselectivity was defined as the percentage of excess production of diastereomeric *S*-oxide isomer (e.g., *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.
[d] Diastereomeric excess (*cis*-*S*-oxide).

TABLE 7

S-Monooxygenation of 2-aryl-1,3-dithiolanes by the highly purified FMO from hog liver

2-Aryl-1,3-dithiolane	S-Oxide formed (Diastereomeric excess)	
	Complete[a]	−NOA[b]
	nmol/min/mg of protein	
1 4-OCH_3—	372 ± 11.9[c] (96%)[d]	548 ± 20.3 (94%)[d]
2 4-H—	324 ± 10.6 (78%)[d]	326 ± 2.4 (80%)[d]
3 4-Cl—	307 ± 23.8 (94%)[d]	262 ± 15.9 (94%)[d]
4 4-CN—	287 ± 17.0 (38%)[d]	268 ± 25.1 (92%)[d]
5 4-NO_2—	336 ± 5.4 (26%)[e]	293 ± 12.7 (56%)[d]

[a] The incubation mixture contained the same materials as described in Table 6, except that 50 μg of highly purified FMO were used.
[b] NOA, *n*-octylamine (5.0 mM).
[c] Each value is the average of three determinations ± standard deviation.
[d] *Trans* diastereomeric excess.
[e] *Cis* diastereomeric excess.

4 with the highly purified hog liver FMO (Table 9). In the presence of highly purified hog liver FMO or hog liver microsomes, a marked preference for addition of oxygen to the *pro*-*R*-sulfur atom occurs for compounds **1–3**, whereas in the presence or absence of *n*-octylamine compounds **3** and **4** demonstrated a preference for addition of oxygen to the *pro*-*S*-sulfur atom (Tables 8 and 9).

Stereoselective *S*-oxygenation of 2-aryl-1,3-dithiolanes by cytochrome P-450. The *S*-oxygenation of sulfides **1–5** was investigated with highly purified cytochrome P-450IIB in order to determine the rate of *S*-oxide formation as well as the diastereoselectivity and enantioselectivity of the reaction. Cytochrome P-450IIB was used because previous studies demonstrated that this cytochrome P-450 isozyme is principally responsible for chiral *S*-oxygenation (17–19).

For *S*-oxygenation with purified cytochrome P-450IIB-1 and P-450IIB-10, experiments demonstrated that *S*-oxide formation was linearly dependent upon cytochrome P-450 protein concentration (0–400 pmol/ml) and time (0–10 min). *S*-Oxygenation had an absolute requirement for cytochrome P-450IIB, NADPH, and cytochrome P-450 reductase activity but did not demonstrate a strong dependence on cytochrome b_5.

During the short incubation periods employed in this study, no formation of *S*,*S*′-dioxygenated products or other decomposition products was observed. The conclusion that the diastereoselectivity and enantioselectivity for *S*-oxygenation of 2-aryl-1,3-dithiolanes were due solely to enzymatic reactions and not to nonenzymatic oxidation reactions comes from the following observations: 1) to effect the same rate of oxidation of aryl dithiolanes, large concentrations of $NaIO_4$ (i.e., 100–200 mM) or H_2O_2 were required (33) and these chemical oxidizing agents gave 0% enantiomeric excess of *S*-oxide product (31) and 2) the diastereoisomeric excess and enantiomeric excess of *S*-oxide product formed were the same during the entire time course of the reaction.

In the presence of rat or mouse liver cytochrome P-450IIB, 2-aryl-1,3-dithiolanes were converted to a major *trans*-*S*-oxide diastereomer in all cases examined. As shown in Tables 10 and 11, the rates of *S*-oxide formation do not follow a strict Hammett-type dependence (36) on the nature of the aryl substituent, but there was a tendency for electron-deficient 2-aryl-1,3-dithiolanes **3–5** to be oxidized by cytochrome P-450IIB to a greater extent than electron-rich 2-aryl-1,3-dithiolanes **1** and **2**. In each case examined, the *trans*-*S*-oxide product had an excess of one enantiomer. The stereochemistry of *S*-oxygenation catalyzed by rat liver cytochrome P-450IIB-1 was deter-

TABLE 8

S-Oxygenation of 2-aryl-1,3-dithiolanes with hog liver microsomes

The complete system is as described in Table 6. The results are the average of three or four determinations ± standard deviation.

Substrate	Diastereomeric excess[a]		Enantiomeric excess[b]			
			trans-*S*-Oxide		*cis*-*S*-Oxide	
	trans-*S*-Oxide	*cis*-*S*-Oxide	(1*S*, 2*S*)	(1*R*, 2*R*)	(1*S*, 2*R*)	(1*R*, 2*S*)
	%		%			
+*n*-Octylamine						
1 4-OCH_3—	100			100		
2 4-H—	52.6 ± 1.2			90.5 ± 2.0	100	
3 4-Cl—	100		100			
4 4-CN—		36.8 ± 16.9	100			89.2 ± 2.4
−*n*-Octylamine						
1 4-OCH_3—	100			79.4 ± 1.1		
2 4-H—	61.4 ± 1.4			75.9 ± 0.6		100
3 4-Cl—	100		100			
4 4-CN—	100		100			

[a] Diastereomeric excess as defined in Table 6.
[b] Enantiomeric excess was determined as follows: (e.g., {[*S*] − [*R*]/[*S*] + [*R*]} × 100 = % *S* − % *R*, where *S* and *R* refer to the absolute stereochemistry of the *S*-oxide sulfur atom, assuming a linear relationship between rotation and composition percentage of "optical purity."

TABLE 9

S-Oxygenation of 2-aryl-1,3-dithiolanes with hog liver FMO

The complete system is as described in Table 6. The results are the average of three or four determinations ± standard deviation.

Substrate	Diastereomeric excess[a]		Enantiomeric excess[b]			
			trans-*S*-Oxide		*cis*-*S*-Oxide	
	trans-*S*-Oxide	*cis*-*S*-Oxide	(1*S*, 2*S*)	(1*R*, 2*R*)	(1*S*, 2*R*)	(1*R*, 2*S*)
	%		%			
+*n*-Octylamine						
1 4-OCH_3—	100			100		
2 4-H—	75.4 ± 0.7			100		100
3 4-Cl—	100		100			
4 4-CN—		100			100	
−*n*-Octylamine						
1 4-OCH_3—	100			100		
2 4-H—	69.9 ± 1.0			100		100
3 4-Cl—	100		100			
4 4-CN—	100		100			

[a] Diastereomeric excess as defined as Table 6.
[b] Enantiomeric excess as defined in Table 8.

mined and indicated a preference for formation of the 1*S*,2*S*-*trans*-*S*-oxide (Table 10). Preference for addition of molecular oxygen to the sulfur atom catalyzed by mouse liver cytochrome P-450IIB-10 was less enantiospecific (Table 11). Thus, **1** and **2** demonstrated a marked preference for attack of oxygen at the *pro*-*S* sulfur atom, whereas **3** and **4** demonstrated a preference for attack of oxygen at the *pro*-*R* sulfur atom.

***S*-Oxygenation of 2-aryl-1,3-dithiolane *S*-oxides by hog liver microsomes and highly purified FMO.** The microsomal metabolism of compounds **6a–10a** was carried out in order to investigate the enzyme system(s) involved, the effect of *para*-substituents, and the nature of the sulfur *S*-oxidized. In contrast to the microsomal oxygenation of compounds **1–5**, the microsomal *S*-oxygenation of **6a–10a** was slower and markedly more sensitive to the electronic nature of the *para*-substituents. Preliminary studies indicated that *S*-oxygenation of **6a–10a** was linearly dependent upon microsomal protein (from 0 to 2 mg/ml) and time up to 10 min. In the presence of *n*-octylamine, only a modest enhancement of *S*,*S*′-dioxygenation was observed (Table 12). As discussed above, 2-aryl-1,3-dithiolane *S*-oxides **6a–8a** were the only *S*-oxides employed in further studies, because of the extensive decomposition of the individual diastereomers of **9** and **10** during the extensive chromatography required for separation after chemical synthesis. In the presence of heat-inactivated microsomes under conditions that destroy FMO activity (34), absolutely no *S*-oxygenation of **6a–8a** was observed (Table 12). Compared with the *S*-oxygenation of **1–5**, *S*-oxygenation of **6a–8a** was considerably less efficient and highly dependent upon the electronic nature of the *para*-substituent (36). Thus, compared with **1–3**, 2–4.5-fold slower rates of *S*-oxygenation were observed for *S*-oxides **6a–8a**. In addition, a negative ρ value ($\rho = -1.3$) was calculated for the *S*-oxygenation of **6a–8a** from a plot of σ versus the logarithm of the rate of the microsomal *S*-oxygenation (plot not shown), which is in agreement with previous work (33). Results shown in Table 12 indicate that 2-aryl-1,3-dithiolane *S*-oxides were converted to their corresponding *S*,*S*′-dioxides in a process solely dependent on FMO. During the short incubation periods employed, *S*,*S*′-dioxides **11–13** were the only products observed.

The *S*-oxygenation of **6a–8a** by the highly purified FMO from hog liver demonstrated results similar to the microsome metabolism studies (see Table 12). As shown in Table 13, *S*-oxygenation of **6a–8a** with purified hog liver FMO is 15–20-fold slower than *S*-oxygenation of their corresponding sulfides. Preliminary results showed that *S*,*S*′-dioxide formation was linearly dependent upon purified protein concentration (0.03–0.2 mg/ml) and incubation time up to 7 min. As shown in Table 13, formation of **11–13** is, in general, markedly stimulated by the presence of *n*-octylamine. In the presence of active FMO activity, no detectable amount of sulfone metabolite (or more likely, the decomposition product benzaldehyde) could be detected under any reaction conditions. Results shown in Tables 12 and 13 demonstrate that FMO catalyzed the *S*,*S*′-dioxygenation of **6a–8a** and that no oxygenation of electrophilic *S*-oxide sulfur could be demonstrated. In close agreement with the results employing hog liver microsomes (Table 12), *S*-

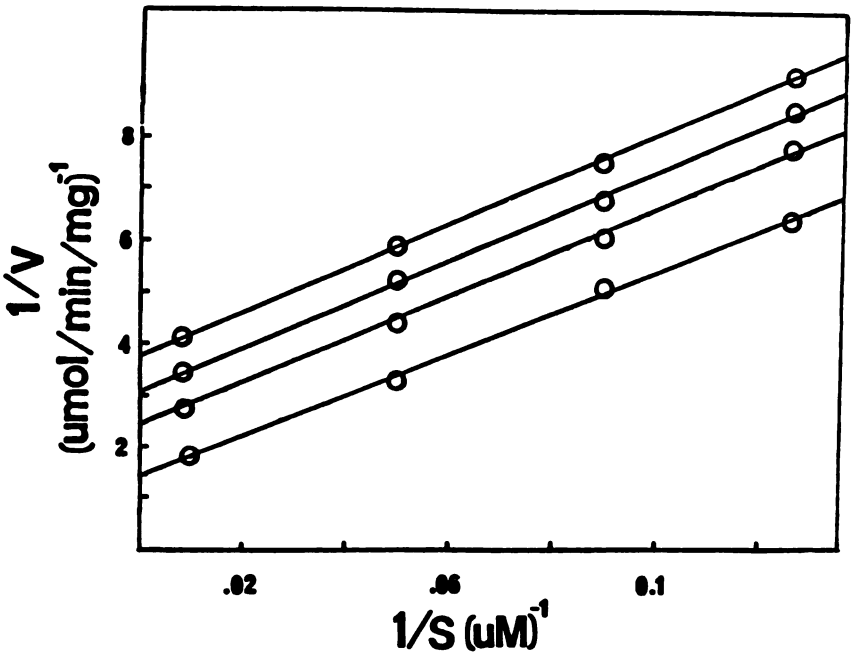

Fig. 2. Double-reciprocal plots of initial velocities with variable NADPH and 2-(4-cyanoaryl)-1,3-dithiolane. Concentrations of NADPH (*top* to *bottom*) are 6.25, 12.5, 25, and 250 μM. In addition to the dithiolane, the reaction contained 0.5 mM glucose-6-phosphate and 1 IU of glucose-6-phosphate dehydrogenase, in 0.1 M potassium phosphate buffer at pH 7.4 and 33°. Reactions were initiated by the addition of 0.04 mg of the purified FMO.

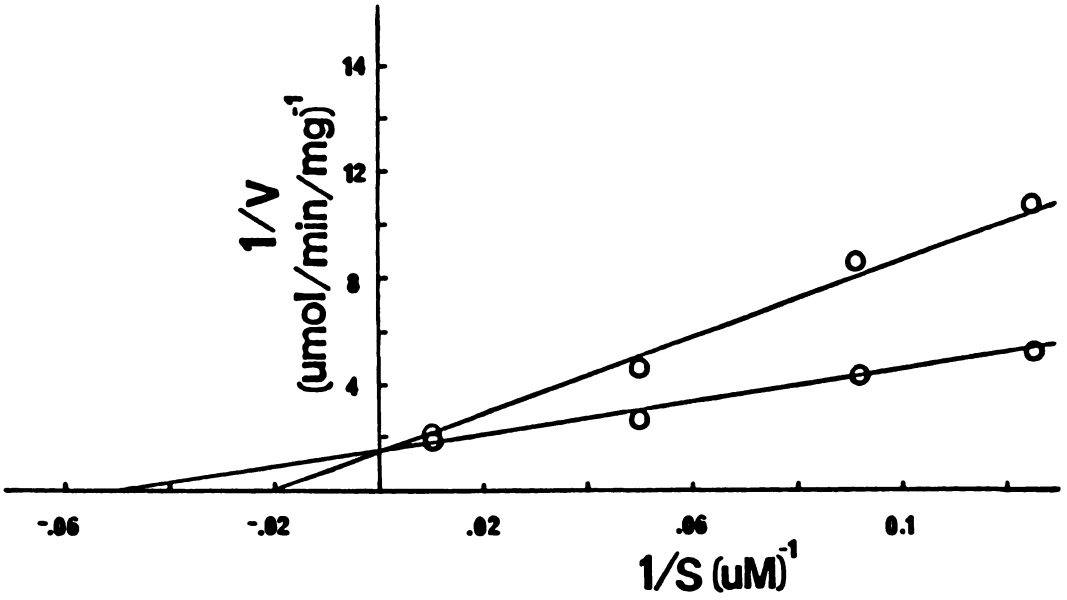

Fig. 3. Double-reciprocal plots of initial velocities with variable 2-(4-cyanoaryl)-1,3-dithiolane. Concentration of NADPH is 250 μM. The reaction contained 5 mM *n*-octylamine (*top*) or no *n*-octylamine (*bottom*; otherwise, conditions are as described in Fig. 2.

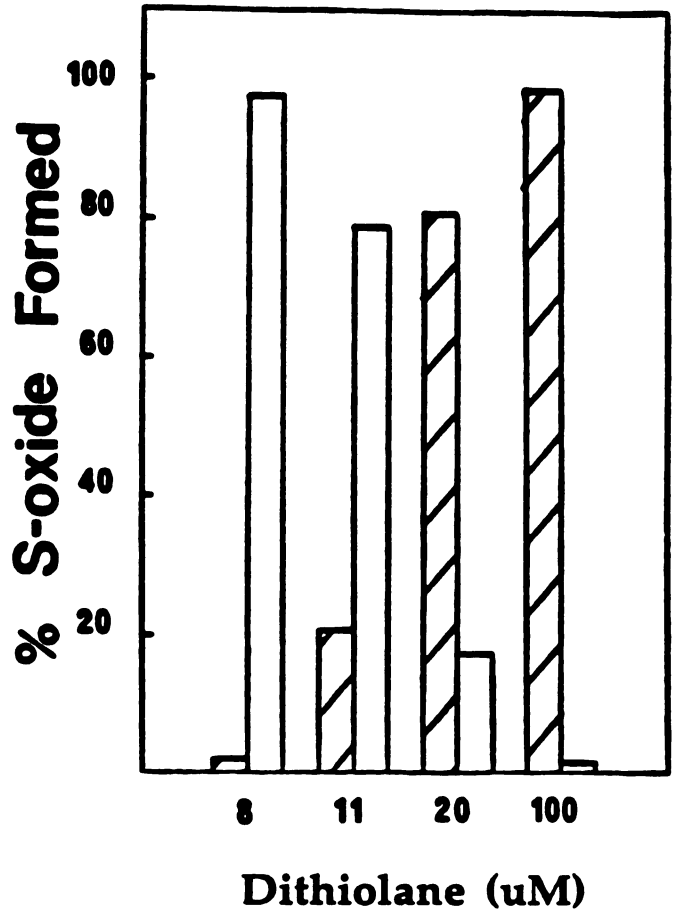

Fig. 4. Relative stereochemistry of 2-(4-cyanoaryl)-1,3-dithiolane *S*-oxide formation determined from Fig. 2 (*top line*). The concentration of NADPH was 6.25 μM and variable dithiolane concentrations were used. The reaction conditions are described in the legend to Fig. 2. ▨, *cis*-*S*-Oxide; □, *trans*-*S*-oxide.

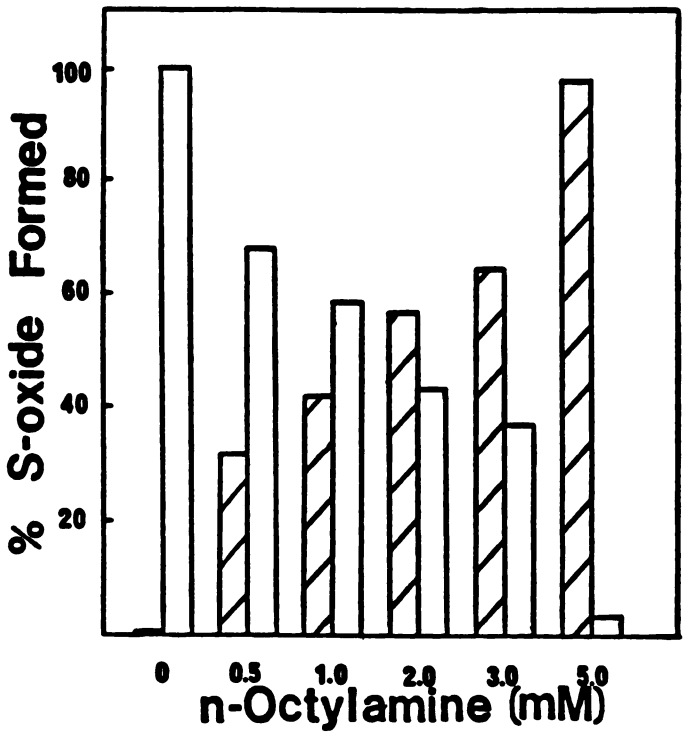

Fig. 5. Relative stereochemistry of 2-(4-cyanoaryl)-1,3-dithiolane *S*-oxide formation determined at variable concentrations of *n*-octylamine. Dithiolane was present at 0.4 mM and NADPH at 0.5 mM. Reaction conditions are described in the legend to Fig. 2. ▨, *cis*-*S*-Oxide; □, *trans*-*S*-oxide.

oxygenation of **6a–8a** with highly purified FMO activity indicated that those 2-aryl-1,3-dithiolane *S*-oxides with electron-donating substituents were oxidized faster than those with electron-withdrawing substituents (Table 13). The ρ value obtained from a plot of σ versus the logarithm of the rate of *S*,*S*′-dioxide formation in the presence or absence of *n*-octylamine was −1.1.

***S*-Oxygenation of 2-aryl-1,3-dithiolane *S*-oxides by cytochrome P-450.** We could not detect the *S*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides by cytochrome P-450IIB-1 from rat and cytochrome P-450BII-10 from mouse liver. During the short incubation periods used in our experiments, the 2-aryl-1,3-dithiolane *S*-oxides were recovered unchanged.

Discussion

***S*-Oxygenation of 2-aryl-1,3-dithiolanes.** The overall chemical and enzymatic *S*-oxygenation of 2-aryl-1,3-dithio-

TABLE 10

S-Oxygenation of 2-aryl-1,3-dithiolanes by rat liver cytochrome P-450IIB-1

The incubation system is as described in Experimental Procedures and the results are the average of three or four determinations ± standard deviation.

	Substrate	S-Oxide formed		Diastereomeric excess[a]	Enantiomeric excess[b]			
		cis-S-Oxide	trans-S-Oxide		trans-S-Oxide		cis-S-Oxide	
					(1S, 2S)	(1R, 2R)	(1S, 2R)	(1R, 2S)
		nmol/min/nmol of P450		%	%			
1	4-OCH_3—	0.2 ± 0.1	10.9 ± 1.4	97	96.5			
2	4-H—	0.1[c]	1.3 ± 0.2	97	96.5			
3	4-Cl—	0.3[c]	5.0 ± 0.7	85	85.0			
4	4-CN—	6.3 ± 1.1	25.0 ± 3.4	60	77.3			100
5	4-NO_2—[d]	5.0 ± 0.9	11.6 ± 1.1	40				

[a] Diastereomeric excess as described in Table 6.
[b] Enantiomeric excess as described in Table 8.
[c] The range of values was between 0 and 0.1 nmol/min/nmol of P-450IIB-1.
[d] 2-(4-Nitrophenyl)-1,3-dithiolane S-oxide enantiomers could not be separated.

TABLE 11

S-Oxygenation of 2-aryl-1,3-dithiolanes by mouse liver cytochrome P-450IIB-10

The incubation system is as described in Experimental Procedures. The results are the average of three or four determinations ± standard deviation.

	Substrate	S-Oxide formed		Diastereomeric excess[a]	Enantiomeric excess[b]			
		cis-S-Oxide	trans-S-Oxide		trans-S-Oxide		cis-S-Oxide	
					(1R, 2R)	(1S, 2S)	(1R, 2S)	(1S, 2R)
		nmol/min/nmol of P450						
1	4-OCH_3—	ND[c]	5.5 ± 0.4	100		100		
2	4-H—	2.9 ± 0.2	6.6 ± 0.8	39		81.3		10.5
3	4-Cl—	ND	2.9 ± 0.2	75.9	87.5			
4	4-CN—	0.5 ± 0.1	4.2 ± 0.9	100	100			20.0
5	4-NO_2—[d]	0.7 ± 0.1	11.7 ± 2.1	88.7				

[a] Diastereomeric excess as defined in Table 6.
[b] Enantiomeric excess as defined in Table 8.
[c] ND, not detectable.
[d] 2-(4-Nitrophenyl)-1,3-dithiolanes S-oxide enantiomers could not be separated.

TABLE 12

S-Oxygenation of 2-aryl-1,3-dithiolane S-oxides with hog liver microsomes

The incubation mixture contained the same materials as indicated in Table 6, except that 650 μg of protein/incubation was used.

	2-Aryl-1,3-dithiolane S-oxide	S,S′-Dioxide formed (Diastereomeric excess)[a]		
		Complete	—NOA[b]	Heat-inactivated
		nmol/min/mg of protein		
6a	4-OCH_3—	10.7 ± 0.9 (100%)[c]	11.6 ± 0.7 (100%)	ND[d]
7a	4-H—	6.86 ± 0.9 (67%)	6.29 ± 1.2 (67%)	ND
		1.38 ± 0.2[e]	1.25 ± 0.2[e]	ND
8a	4-Cl—	1.15 ± 0.2 (15%)	0.91 ± 0.1 (9%)	ND
		0.86 ± 0.1[e]	0.76 ± 0.1[e]	ND

[a] Diastereomer excess as defined in Table 6.
[b] NOA, n-octylamine.
[c] One diastereomer formed.
[d] ND, not detected.
[e] trans-cis-S,S′-Dioxide formed.

lanes by microsomes or by highly purified FMO from hog liver or highly purified cytochrome P-450IIB-1 from rat or cytochrome P-450IIB-10 from mouse liver can be described by the scheme in Fig. 1, in which each step has been investigated and represents a process that converts the sulfide initially to the corresponding 2-aryl-1,3-dithiolane *S*-oxide and ultimately to the 2-aryl-1,3-dithiolane *S,S′*-dioxide. Based on preliminary studies[2] and previous results (27, 33), the putative 2-aryl-1,3-dithiolane *S,S,S′*-trioxide is unstable and rapidly decomposes to the corresponding benzaldehyde. We have observed that air oxidation of 2-aryl-1,3-dithiolane *S,S′*-dioxides yields the corresponding benzaldehyde, as previously observed for other similar systems (27, 33). However, during the short time periods employed in the reactions, no *para*-substituted benzaldehyde was ever observed.

[2] Unpublished observations.

TABLE 13

S-Oxygenation of 2-aryl-1,3-dithiolane S-oxides by the highly purified hog liver FMO

The incubation contained the same materials as described in Table 6, except that 50 μg of highly purified FMO protein/incubation was used.

	2-Aryl-1,3-dithiolane S-oxide	S,S′-Dioxide formed (Diastereomeric excess)[a]	
		Complete	—NOA[b]
		nmol/min/mg of protein	
6a	4-OCH3	54.1 ± 2.6 (100%)	31.0 ± 2.9 (100%)
7a	4-H	30.7 ± 2.0 (70%)	20.9 ± 3.1 (68%)
		5.4 ± 0.3[c]	3.9 ± 0.6[c]
8a	4-Cl	19.5 ± 1.8 (36%)	12.0 ± 0.6 (24%)
		9.4 ± 1.3[c]	7.4 ± 0.5[c]

[a] Diastereomeric excess as defined in Table 6.
[b] NOA, n-octylamine.
[c] trans-cis-S,S′-dioxide formed.

For the *S*-oxygenation of 2-aryl-1,3-dithiolanes by hog liver microsomes, the major route of enzymatic *S*-oxygenation is due to the FMO. Cytochrome P-450 may contribute to the *S*-oxidation of **1**, **3**, and especially **5**, because *n*-octylamine does not markedly stimulate *S*-oxygenation (data not shown). Heat inactivation of hog liver microsomes is another effective way to distinguish the contribution of each hepatic monooxygenase enzyme to *S*-oxygenation activities (1). After hog liver microsomes were treated under conditions that heat inactivate the FMO but preserve approximately 85% of the cytochrome P-450 activity (34), small but significant amounts of *S*-oxygenase activity for some of the 2-aryl-1,3-dithiolanes are observed (Table 6). Use of *n*-octylamine and heat inactivation are standard methods for distinguishing between hepatic FMO- and cytochrome P-450-related *S*-oxygenation (1). 2-Aryl-1,3-dithiolanes are excellent substrates for the highly purified FMO from hog liver and cytochrome P-450IIB from rat and mouse liver, and the rates of *S*-monooxygenation compare favorably with the rate of *S*-oxygenation of other good sulfide-containing substrates for those enzymes (1, 3, 17–19).

For *S*-oxygenation, varying the electronic nature of the *para*-substituent of 2-aryl-1,3-dithiolanes did not produce a discern-

ible Hammett-type dependence (36) when microsomal *S*-oxygenase activity was determined (Table 6). Differences in *S*-oxygenation of **1** to **5** are apparent, but this may simply be a consequence of the enhanced lipophilicity of the substrate rather than of any electronic property of the substrate. In agreement with microsomal studies, no apparent Hammett-type correlation was observed for the *S*-oxygenation of 2-aryl-1,3-dithiolanes by the highly purified FMO from hog liver. The results of these studies are in accord with previous work (6–9) that suggests that, at least for highly nucleophilic sulfur-containing substrates, the rate-limiting step of the enzymatic reaction is loss of water from the FMO flavin pseudobase.

Although it is not possible to distinguish a single-electron transfer from a nucleophilic mechanism for 2-aryl-1,3-dithiolane *S*-oxygenation by means of Hammett-type substituent correlations alone (37), the data, nevertheless, suggest that, if single-electron transfer reactions are on either the FMO or cytochrome P-450IIB reaction pathway, collapse of active oxygen species with sulfide cation radical is more rapid than rearrangement or isomerization of a sulfur cation radical, at least for this class of substrate. No *para*-substituted benzaldehyde arising from sulfide cation radicals was ever detected in oxidations of dithiolanes with FMO or cytochrome P-450IIB.

Stereochemical analyses of monooxygenase-catalyzed *S*-oxide products. The stereochemical outcome of *S*-oxygenation of 2-aryl-1,3-dithiolanes was investigated. The aim of this study was to determine the diastereotopic and enantiotopic preference for cytochrome P-450IIB- and FMO-mediated *S*-oxygenation. Determination of the preference for *cis*- versus *trans*-*S*-oxygenation could reveal details about the chemical nature of the *S*-oxygenation step, as well as information about the active site of FMO and cytochrome P-450IIB. Previous studies have shown that FMO *S*-oxygenates sulfides with as much as 93–95% enantioselectivity, in a process where the minor *S*-oxide enantiomer arises from incomplete chiral processing by the enzyme (21). Recently, we demonstrated that as much as 99% diastereoselectivity can be observed for the *S*-oxygenation of racemic 2-aryl-1,3-oxathiolanes (33). These studies and others (33, 34) demonstrate that the FMO can differentiate between *cis*- and *trans*-heteroatom oxygenation. For example, others have demonstrated an overwhelming preference for formation of *trans*-nicotine *N*-oxide (38) by hepatic FMO. Although nicotine is a tertiary amine, it, nevertheless, bears certain structural relatedness to the 2-aryl-1,3-dithiolanes used in the present study. The finding that hepatic FMO forms any *cis*-aryl-1,3-dithiolane *S*-oxides and *cis*-nicotine *N*-oxides (38) suggests that the active site is substantially larger than in the pulmonary FMO, which apparently has a very narrow substrate binding product, producing only *trans*-2-aryl-1,3-dithiolane *S*-oxides (39) and mainly *trans*-nicotine *N*-oxides (40, 41). *S*-Oxygenation of compounds **1** to **5** by rat and mouse liver cytochrome P-450IIB is, in general, highly diastereoselective. In general, *trans*-aryl-1,3-dithiolane *S*-oxide is the major product formed, but a significant amount of *cis*-aryl-1,3-dithiolane *S*-oxide is formed in *S*-oxygenations catalyzed by rat liver cytochrome P-450IIB-1 and especially in the case of *S*-oxygenations catalyzed by mouse liver cytochrome P-450IIB-10.

As shown in Tables 8 and 9, 2-aryl-1,3-dithiolane *S*-oxygenation catalyzed by microsomes or highly purified FMO from hog liver is highly enantioselective, whether the reactions were performed in the presence or absence of *n*-octylamine. For compounds **1** and **2**, a marked preference for hog liver microsome- and highly purified FMO-catalyzed *S*-oxygenation at the *pro-R* sulfur atom was observed. In the absence of *n*-octylamine, compounds **3** and **4** had a marked preference for oxygenation at the *pro-S* sulfur atom. Thus, for **1**, addition of an oxygen atom by hog liver microsomes and purified FMO is 100% enantioselective (i.e., 100% *trans*-1*R*,2*R*-*S*-oxide is observed) in the presence of *n*-octylamine. In the absence of *n*-octylamine, preference for addition of oxygen to the *pro-R* sulfur atom is almost as great.

Rat liver cytochrome P-450IIB-1 *S*-oxygenates the dithiolane at the *pro-S* sulfur atom position. Mouse liver cytochrome P-450IIB-10 exhibits a preference for *S*-oxygenation of the *pro-S* sulfur atom for **1** and **2** but, in contrast, *S*-oxygenation of **3** and **4** exhibits a preference for *S*-oxygenation of the *pro-R* sulfur atom. Previously, it was observed that the FMO and cytochromes P-450IIB produced *S*-oxides of opposite enantioselectivity (18–21). From the data presented herein, that observation is supported (e.g., for **1** and **2**) but for other cases (e.g., most notably **3** and **4**) the similarity of FMO- and cytochrome P-450IIB-catalyzed enantioselectivity observed suggests that this is not an absolute rule. However, for **1**, *S*-oxygenation enantioselectivity is a useful property to distinguish between FMO- and cytochrome P-450-catalyzed *S*-oxygenations. This compound has found use as a probe to investigate for the presence of cytochromes P-450 and FMO-*S*-oxygenase activity in various tissue preparations (28).

In the presence of chemical oxidizing agents, hog liver microsomes, or purified FMO, stereoselective *trans*-*S*-oxide formation occurs preferentially, especially for electron-rich 2-aryl-1,3-dithiolanes (i.e., compounds **1**–**4**), in either the presence or the absence of *n*-octylamine. In contrast, 2-aryl-1,3-dithiolanes with electron-deficient 2-aryl-1,3-dithiolane sulfur atoms are oxidized with only modest diastereoselectivity. Surprisingly, in the presence of *n*-octylamine, both hog liver microsomes and highly purified FMO *S*-oxygenate *para*-nitro-2-aryl-1,3-dithiolane to give predominantly the *cis*-*S*-oxide. However, in the absence of *n*-octylamine, stereoselective formation of *trans*-*para*-cyano- and *trans*-*para*-nitro-2-aryl-1,3-dithiolane *S*-oxide was observed. We chose to investigate *para*-cyano-2-aryl-1,3-dithiolane *S*-oxygenation in some detail, because the *S*-oxide was considerably more stable than the *para*-nitro-substituted homolog. As shown in Fig. 2, in the absence of *n*-octylamine, the kinetic data are consistent with the ordered Ter-Bi mechanism for *S*-oxygenation described previously (6–9), where NADPH is added first, followed by molecular oxygen, and substrate (6–9). In the presence of *n*-octylamine, apparent inhibition of *S*-oxide formation by NADPH is observed, which increases the $K_{m_{app}}$ while maintaining the V_{max} constant (Fig. 3). We interpret these data to suggest that *n*-octylamine distorts the active site (possibly near the NADPH-binding domain) and prevents substrate binding and/or substrate turnover. To investigate this more thoroughly, we determined the *cis* to *trans* ratio for each data point of Fig. 2. For each of the bottom three lines (Fig. 2), the *cis* to *trans* ratio was constant. However, in the presence of 6.25 μM NADPH, the ratio of *cis*- to *trans*-*S*-oxide increased to almost 100% as the substrate was increased to 100 μM (Fig. 4). These data suggest that the preferred mode of *S*-oxygenation of **4** in the presence of concentrations of NADPH near the K_m is for *S*-oxygenation in the *trans* config-

uration, although this preference is lost at higher concentrations of NADPH and substrate. Although a major change in the configuration of the *S*-oxide is observed for compound **4**, the rate of the reaction in the presence or absence of *n*-octylamine remains approximately the same. This suggests that the K_m and V_{max} values for production of *cis*- or *trans*-*S*-oxide are the same. The configurational preference for FMO *S*-oxygenation of **4** in the presence or absence of *n*-octylamine cannot be rationalized based on kinetics alone. *n*-Octylamine does not act as a delivery vehicle or participate in a micellar effect, because such an effect would occur at concentrations significantly higher than 2 mM, the EC_{50} value of the effect (Fig. 4). Further, we do not view this as an active site-substrate hydrogen-bonding effect, because the *para*-methoxy substituent is certainly competent to hydrogen bond and no such *n*-octylamine effect is seen for this substrate. It is possible that *n*-octylamine stabilizes an enzyme active site anionic group that would otherwise make contact with the $NADP^+$-binding domain. This putative interaction may impinge on the substrate binding site to provide an impedance to preferred *trans*-*S*-oxygenation. In the absence of *n*-octylamine, electrostatic interactions between the *para*-nitro and *para*-cyano groups that are not present in compounds **1–3** may decrease the rate of *S*-oxygenation by destabilization of the 4a-hydroperoxyflavin via the postulated substrate-$NADP^+$ interaction. Others have shown that 4a-hydroperoxyflavin of FMO can be destabilized by structural alteration of $NADP^+$ (42). In summary, at low concentrations of NADPH stereoselectivity is a function of substrate concentration, whereas at high NADPH concentrations stereoselectivity is independent of substrate concentration but a function of *n*-octylamine concentration.

***S*-Oxygenation of 2-aryl-1,3-dithiolane *S*-oxides.** The *S*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides was investigated with rat liver cytochrome P-450IIB-1, mouse liver cytochrome P-450IIB-10, and microsomes and highly purified FMO from hog liver. Because compounds **14** and **15** were unstable to configurational isomerization, we investigated only the formation of **11–13**. In every case examined, the rate of *S*,*S*′-dioxygenation was markedly reduced, compared with the rate of *S*-oxygenation of the corresponding sulfide. A reduced rate for *S*,*S*-dioxygenation has been observed before (2, 3). Cytochrome P-450IIB-catalyzed *S*,*S*′-dioxygenation of **11–13** was not observed, and investigations of cytochrome P-450IIB-catalyzed *S*,*S*′-dioxygenation were not continued. The finding that FMO is solely responsible for *S*-oxide *S*′-oxygenation is apparent from the fact that heat inactivation completely abolishes and *n*-octylamine markedly stimulates the formation of 2-aryl-1,3-dithiolane *S*,*S*′-dioxide (Tables 12 and 13). In contrast to *S*-monooxygenation, *S*,*S*′-dioxygenation is sensitive to the nature of the *para*-substituent, demonstrating a negative Hammett correlation (i.e., ρ value of −1.3 and −1.1 for hog liver microsomes and purified hog liver FMO, respectively). Chemical *S*-oxidation of similar *S*-oxides likewise shows a dependence on the electronic nature of the *para*-substituent [e.g., the ρ value for the oxidation of oxathiolanes was −0.32 (33)]. In all cases examined, the major product obtained for the oxidation of 2-aryl-1,3-dithiolane *S*-oxides was the *S*,*S*′-dioxide with a *trans*,*trans*-configuration with respect to the *S*-oxide and the aromatic ring. In agreement with the *S*-monooxygenation studies reported above, the percentage of *trans*,*cis*-*S*-oxide product obtained was highly dependent upon the electronic character of the *para*-substituent. Thus, no *trans*,*cis*-*S*,*S*-′-dioxide could be detected for formation of **11**, whereas **13** was formed in a *trans*,*cis* to *trans*,*trans* ratio of 0.72. The observation that the *trans*,*cis* to *trans*,*trans* ratio does not change for *S*-oxygenation of **6a–8a** in the presence or absence of *n*-octylamine (Tables 12 and 13) suggests that, at least for this class of substrate, the site of *n*-octylamine action is at a step distinct from *S*,*S*′-dioxide product formation. The finding that *n*-octylamine stimulates *S*,*S*′-dioxygenation suggests that *n*-octylamine may function as a general base catalyst to eliminate FMO flavin pseudobase water (1, 2). For *S*,*S*′-dioxygenation, the role of *n*-octylamine at the substrate site may be offset by the zwitterionic nature of the *S*-oxide group of the substrate. The large dependence on the nature of the *para*-substituent demonstrates that, at least for **6a–8a**, the chemical nature of the *para*-substituent and sulfur atom contributes to the overall rate-determining step of the enzyme reaction. Thus, and in contrast to other substrates for FMO activity (1, 2), the maximal velocity at saturating concentrations of substrate for *S*-oxygenation of **6a** to **8a** is not invariant but, rather, is strongly dependent upon the chemical nature of the oxygenatable sulfur atom.

Conclusions

The mechanism of *S*-oxygenation of 2-aryl-1,3-dithiolanes is comparable to the *S*-oxygenation of other dialkyl sulfides catalyzed by hog liver FMO (1, 33). Stereochemical analysis of 2-aryl-1,3-dithiolane *S*-oxide formation suggests that 1) *S*-oxygenation is preferentially directed at *trans* attack by peroxyflavin oxygen but sufficient space is available in the active site in order to accommodate *cis*-*S*-oxygenation, and 2), for some 2-aryl-1,3-dithiolane substrates, the presence of *n*-octylamine may determine the relative configuration of *S*-oxide formation, demonstrating that *n*-octylamine plays a role in substrate binding or substrate orientation in the active site, in addition to its role as a postulated general base. In contrast, *S*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides must involve at least a partial contribution of attack by the substrate in the enzymatic rate-limiting step (e.g., the maximal velocity at saturating concentrations of substrate for all substrates is not the same). Because the "puckered" nature of the dithiolane ring should be decreased for dithiolane *S*-oxides, the influence of the *para*-substituent and the neighboring sulfoxide group should be more readily transmitted to the *S*-oxide sulfide sulfur atom and the inductive effect of the *para*-substituent on sulfide nucleophilicity should be manifested.

The strong dependence of rate of *S*-oxygenation catalyzed by FMO on sulfoxide nucleophilicity has been observed previously (43). The positive effector *n*-octylamine accelerates the rate of *S*,*S*′-dioxygenation, at least for purified FMO, but does not change the relative configuration of the *S*,*S*′-dioxide product. We interpret these results to suggest that *n*-octylamine acts as a general base catalyst to accelerate flavin pseudobase decomposition. We propose that the zwitterionic nature of the *S*-oxide sulfur of **6a–8a** prevents *n*-octylamine from approaching the substrate-binding domain of the active site.

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