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Enantioselective S-Oxygenation of 2-Aryl-1,3-dithiolanes by Rabbit Lung Enzyme Preparations

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SUMMARY

Pulmonary microsomes, highly purified pulmonary flavin-containing monooxygenase, and highly purified pulmonary cytochrome P-450$_{IIB-4}$ from pregnant female rabbits catalyze the NADPH-dependent *S*-oxygenation of a series of 2-aryl-1,3-dithiolanes. The *S*-oxide is the only detectable product formed during the short time period of the enzymatic reactions. Studies on the biochemical mechanism for *S*-oxygenation of 2-aryl-1,3-dithiolanes suggest that this reaction is catalyzed preferentially by the flavin-containing monooxygenase, although cytochromes P-450 also contribute to *S*-oxygenation. This conclusion is based on the effects of a cytochrome P-450 inhibitor, aminobenzotriazole, as well as on studies of the stereoselectivity of the reaction. Although both purified rabbit pulmonary cytochrome P-450$_{IIB-4}$ and purified flavin-containing monooxygenase have identical diastereoselectivity, producing the (*trans*)-*S*-oxide, these monooxygenases possess opposite *S*-oxygenation enantioselectivity. Pulmonary cytochrome P-450$_{IIB-4}$ *S*-oxygenates 2-aryl-1,3-dithiolanes almost exclusively at the *pro*-*S*-sulfur atom, whereas pulmonary flavin-containing monooxygenase *S*-oxygenates 2-aryl-1,3-dithiolanes exclusively at the *pro*-*R*-sulfur atom. 2-Aryl-1,3-dithiolane *S*-oxides are *S*-oxygenated a second time on the *S'*-sulfide sulfur atom but only by rabbit lung microsomes and pulmonary flavin-containing monooxygenase and not by cytochrome P-450$_{IIB-4}$. That pulmonary flavin-containing monooxygenase only catalyzes formation of (*trans*)- and not (*cis*)-2-aryl-1,3-dithiolane *S*-oxide formation suggests that the active site of pulmonary flavin-containing monooxygenase exerts great steric limitations on 2-aryl-1,3-dithiolane *S*-oxygenation.

The dialkylsulfide (or thioether) group occurs widely in drugs, chemicals, and pesticides (1). Among the routes of metabolism available to dialkylsulfides are oxidation to the sulfoxide or *S*-dealkylation. *S*-Dealkylation is a minor route of biotransformation (2–4). Once formed, dialkylsulfide *S*-oxides may be further oxidized to dialkylsulfones or, alternatively, reduced to the parent sulfide. Several examples of important sulfide-containing drugs or chemicals metabolized by these routes both *in vitro* and *in vivo* have been described (5–8).

Dialkylsulfoxide metabolites are generally chemically stable to hydrolysis, rearrangement, or racemization, although elimination reactions are possible (9). Sulfone metabolites are, likewise, generally chemically stable, although the amount of sulfone formed *in vivo* from the sulfide is usually substantially less than the amount of sulfoxide. This may be a consequence of the relative hydrophilicity of the sulfoxide metabolite or the relative nucleophilicity of the sulfoxide sulfur, compared with sulfide sulfur, towards *S*-oxygenation. A decrease in the relative hydrophilicity and nucleophilicity of the sulfur atom, as seen in the case of sulfoxide sulfur, generally results in a decrease in monooxygenase-catalyzed *S*-oxygenation (8, 10). Reductive metabolic processes may also help determine the amount of sulfone formed, because sulfoxides are easily reduced whereas sulfones are not reduced at all (4). The three monooxygenase systems primarily involved in dialkylsulfide *S*-oxygenation are cytochrome P-450, flavin-containing monooxygenase, and prostaglandin synthetase. Generally, highly nucleophilic dialkylsulfide sulfur atoms are *S*-oxygenated by the flavin-containing monooxygenase (8, 11, 12), prostaglandin synthetase (13), and cytochrome P-450, whereas nonnucleophilic sulfur atoms are also *S*-oxygenated by cytochrome P-450 (14, 15). The relative contribution to the *S*-oxygenation of a dialkylsulfide by each monooxygenase has been investigated in only a few cases (11, 15–18).[1]

Recently, another form of the flavin-containing monooxygenase was isolated from rabbit lung and found to be immunochemically and catalytically distinct from the liver enzyme (19, 20), in agreement with earlier studies that suggested that the lung possessed some unusual metabolic properties (21). In

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[1] J. R. Cashman, L. D. Olsen, C. E. Lambert, and M. J. Presas, Enantioselective *S*-oxygenation of *para*-methoxy-phenyl-1,3-dithiolane by various tissue preparations: effect of estradiol. Submitted for publication.

ABBREVIATION: HPLC, high pressure liquid chromatography.

contrast to hepatic systems (12, 15–17),[1] to date, the enzymatic basis for the studies of the stereoselective *S*-oxygenation of sulfides in rabbit lung has not been reported. The purpose of this investigation, therefore, was to determine the stereoselective *S*-oxygenation of a model sulfide with rabbit lung microsomes and compare these results with the major purified *S*-monooxygenases present in lung microsomes from pregnant rabbits, namely pulmonary flavin-containing monooxygenase and pulmonary cytochrome P-450$_{IIB-4}$. The *para*-substituted aryl-1,3-dithiolanes were employed in this study because they possess several features useful for investigation of *S*-oxygenase activity; (a) *para*-substituted aryl-1,3-dithiolanes are good substrates for both purified cytochrome P-450$_{IIB-4}$ and purified flavin-containing monooxygenase, (b) enzymatic *S*-oxygenation of **1–5** may occur with diastereotopic selectivity and may also result in enantioenrichment of an *S*-oxide product by selection between two enantiotopic sulfur atom lone pairs that are stable to pyramidal inversion, and (c) during the short incubation times used in these studies, the aryl-1,3-dithiolane *S*-oxides formed did not undergo further *S*-oxygenation, reduction, or decomposition to other products.

In this report we determine the diastereoselectivity and enantioselectivity of *S*-oxygenation of 2-aryl-1,3-dithiolanes catalyzed by microsomes and highly purified flavin-containing monooxygenase and cytochrome P-450$_{IIB-4}$ from female rabbit lung. In addition, we examine the *S*-oxygenation of 2-aryl-1,3-dithiane *S*-oxides. The results of our study demonstrate the utility of investigating cytochrome P-450- and flavin-containing monooxygenase-catalyzed *S*-oxygenations of aryl-1,3-dithiolanes as mechanistic and stereochemical probes of enzyme function.

Experimental Procedures

Methods and materials. 2-Aryl-1,3-dithiolanes, 2-aryl-1,3-dithiolane *S*-oxides, and 2-aryl-1,3-dithiolane *S,S'*-dioxides were synthesized as previously described.[2] Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP$^+$, NADPH, and dilaurylphosphatidyl choline were purchased from Sigma Chemical Co. All other reagents and buffers were obtained in the highest quality from commercially available sources. Aminobenzotriazole was a gift of Professor Paul Ortiz de Montellano of this department.

Chiral aryl-1,3-dithiolane *S*-oxides were synthesized by the method of Pitchen *et al.* (22). The optical purity of the purified (*cis*)- and (*trans*)-*S*-oxide products were determined by NMR analysis using a chiral shift reagent.[1] The absorption spectra of pure (*cis*)- and (*trans*)-*S*-oxide were determined in order to relate the optical purity of the products to the absolute configuration correlation of dialkyl *S*-oxides independently correlated by other means (23). Previous studies of thioketal *S*-oxides have established the relationship between the CD sign and the absolute stereochemistry of the dialkyl *S*-oxides (24). A positive CD associated with the absorption between 285 and 195 nm can be correlated with an (*R*)-*S*-oxide configuration (23). Separation of each enantiomer of each diastereomer was accomplished by HPLC with a Chiralcel OD column, as described below. 2-Aryl-1,3-dithiolane *S*-oxides from enzymatic incubations were compared with synthetic chiral *S*-oxides by HPLC for determination of absolute stereochemistry.

Rabbit lungs were obtained from Pel-Freez Biological and were from pregnant (25–30 days of gestation) New Zealand white rabbits. Rabbit lung cytochrome P-450$_{IIB-4}$ was isolated and purified from pregnant female rabbits as previously described (25). Rabbit lung cytochrome P-450$_{IIB-4}$ had a specific content of 14.2 nmol of P-450/mg of protein. Rabbit cytochrome P-450$_{IIB-4}$ had characteristically high benzphetamine *N*-demethylase activity (26) (30.4 nmol/min/nmol of enzyme) and was a single band (M_r 49,500) estimated to be greater than 95% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (27). Rat cytochrome b_5 and NADPH-cytochrome P-450 reductase were purified to apparent homogeneity by a method previously described (28). 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 buffer (pH 7.7) at 25°.

Rabbit lung flavin-containing monooxygenase was isolated and purified from pregnant female rabbits by a method previously described (29). Rabbit lung flavin-containing monooxygenase had a specific content of 13.4 nmol of flavin/mg of protein. Rabbit lung flavin-containing monooxygenase had characteristically high *N,N*-dimethylaniline *N*-oxygenase activity (30) (33.6 nmol/min/nmol of protein) and was judged to be homogeneous (greater than 98% pure) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (27).

Metabolic incubations and enzyme assays. *S*-Oxygenation of 2-aryl-1,3-dithiolanes by microsomes, highly purified flavin-containing monooxygenase from rabbit lung, or highly purified cytochrome P-450$_{IIB-4}$ from rabbit lung was measured by monitoring *S*-oxide product formation by HPLC (31). For studies with rabbit lung microsomes, the incubation media contained 50 mM potassium phosphate (pH 8.0), an NADPH-generating system consisting of 0.5 mM NADP$^+$, 2.0 mM glucose-6-phosphate, and 1 IU of glucose-6-phosphate dehydrogenase, and 0.76 mg of rabbit lung microsomes. The reaction was initiated by the addition of substrate (400 μM) and after 5 min the reaction was stopped by the addition of cold CH_2Cl_2 and analyzed for products by HPLC, as described below. For studies with highly purified rabbit lung flavin-containing monooxygenase, the above protocol was used except a buffer consisting of 50 mM potassium phosphate (pH 9.0) was employed. The major phenobarbital-inducible rabbit lung cytochrome P-450 (P-450$_{IIB-4}$) was purified from rabbit lung by the method of Williams *et al.* (25). Cytochrome P-450$_{IIB-4}$ (0.1 nmol) was reconstituted in the presence of dilaurylphosphatidylcholine (25 μg) and saturating amounts of rat cytochrome P-450 reductase (600 units) and was allowed to stand at 4° for 10 min. Sodium phosphate buffer (50 mM, pH 7.4), the NADPH-generating system, substrate (400 μM final concentration), and rat liver cytochrome b_5 (0.1 nmol) were added for a total volume of 1.0 ml. Incubations were carried out for 10 min at 33° with constant shaking in air and the reactions were terminated and prepared for HPLC analysis as previously described (31).[1,2]

The reactions were stopped by the addition of 2 volumes of cold CH_2Cl_2. 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 quantitation by HPLC (Rainin system with UV detector set at 240 nm, with a C-18 precolumn and 5-μm C-18 Altex Ultrasphere ODS reversed phase analytical column). The mobile phase (40% CH_3CN/H_2O, v/v) efficiently separates 2-aryl-1,3-dithiolanes, 2-aryl-1,3-dithiolane *S*-oxides, and 2-aryl-1,3-dithiolane *S,S'*-dioxides.[2] The recovery of metabolites as judged by HPLC was greater than 88% and 96% of this material was the dithiolane, dithiolane *S*-oxide, or dithiolane *S,S'*-dioxide. The HPLC traces are remarkably clean and the chromatograms show essentially only starting material and *S*-oxide. This result suggests that other transformations of the dithiolanes are not taking place. Quantitation was accomplished by comparing the integrated area of the dithiolane, dithiolane *S*-oxide, or dithiolane *S,S'*-dioxide HPLC peak after taking into consideration the extinction coefficient values of each species.[2]

After analysis of the reaction products by HPLC as described above, the remaining reaction products were evaporated to dryness and taken up in isopropanol/hexane (18:82, v/v) for separation and quantitation by chiral HPLC [IBM model 9000 with UV detector set at 240 nm, fitted with a Chiracel OD analytical column (25 cm × 0.15 cm, i.d.)

[2] J. R. Cashman, and L. D. Olsen. Stereoselective *S*-oxygenation of 2-aryl-1,3-dithiolanes by the flavin-containing monooxygenase. Submitted for publication.

from DAICEL Chemical Ind.] This system efficiently separates the starting material and enantiomers of (*cis*)- and (*trans*)-*S*-oxide diastereomers.[3] Quantitation was accomplished by comparing the integrated area for each enantiomer, taking into consideration the extinction coefficient values of each species, as described previously.[3] The concentration of protein was determined by the method of Bradford (32).

Statistics. Data are expressed as the mean ± standard error. Statistical analysis was performed by the Student *t* test for evaluation of the difference between two means.

Results

***S*-Oxygenation of 2-aryl-1,3-dithiolanes.** The biotransformation of **1–5** was studied *in vitro* with pulmonary microsomes, highly purified flavin-containing monooxygenase, and highly purified cytochrome P-450$_{IIB-4}$ from female rabbits. Preliminary studies demonstrated that pulmonary microsomes supplemented with NADPH catalyze the *S*-oxygenation of 2-aryl-1,3-dithiolanes to their *S*-oxides (Table 1). The formation of dithiolane *S*-oxide was a linear function of microsome protein concentration (0–1.4 mg of protein) and of incubation time for at least 7 min. Rabbit lung microsomes that were treated with aminobenzotriazole, a mechanism-based inactivator of cytochromes P-450 (33), inhibited the *S*-oxygenation of **1** and **2** but actually increased the *S*-oxygenation of 3–5 (Table 1). Although the specificity of aminobenzotriazole towards inhibition of pulmonary cytochromes P-450 is not completely understood (34), aminobenzotriazole does not appear to inhibit hepatic or pulmonary flavin-containing monooxygenase-mediated *S*- or *N*-oxygenation (35–37)[1] and in some cases actually stimulates hetero atom oxygenation (34, 35). It is possible that, in addition to its role as an inactivator of cytochromes P-450, aminobenzotriazole may bind to an effector site and stabilize the flavin-containing monooxygenase. The decreased rate of *S*-oxygenation of **1** and **2** in the presence of aminobenzotriazole suggests that rabbit lung microsomal cytochrome P-450 may contribute to *S*-oxygenation of **1** and **2**. In order to investigate this aspect more carefully, the *S*-oxygenation of **1** to **5** was investigated with highly purified rabbit lung cytochrome P-450$_{IIB-4}$.

As shown in Table 2, cytochrome P-450$_{IIB-4}$ efficiently converts 2-aryl-1,3-dithiolanes to their corresponding *S*-oxides. From the data presented, there is not a strong Hammett-type dependence on the electronic character of the aromatic *para*-substituent (38) of the rate of *S*-oxygenation, although it appears that 2-aryl-1,3-dithiolanes with strongly electron-withdrawing *para*-substituents are in general better substrates than 2-aryl-1,3-dithiolanes with electron-donating *para*-substituents (Table 2). Preliminary studies showed that *S*-oxygenation of **1–5** catalyzed by reconstituted cytochrome P-450$_{IIB-4}$ supplemented with NADPH is a linear function of protein concentration (0–0.2 nmol of protein) and of incubation time for at least 10 min. Formation of 2-aryl-1,3-dithiolane *S*-oxide was strictly dependent on the presence of cytochrome P-450 reductase, dilaurylphosphatidylcholine, NADPH, and cytochrome P-450$_{IIB-4}$ but was only modestly dependent on the presence of cytochrome b_5 (data not shown).

The highly purified flavin-containing monooxygenase from rabbit lung catalyzes the NADPH-dependent *S*-oxygenation of 2-aryl-1,3-dithiolanes to their corresponding *S*-oxide (Table 2). Preliminary studies showed that *S*-oxygenation of **1–5** by the highly purified rabbit lung flavin-containing monooxygenase was a linear function of protein concentration (0–0.6 nmol of protein) and of incubation time for at least 5 min. As shown in Table 2, *S*-oxygenation of **1–5** was observed to occur at a uniformly high rate and no apparent Hammett-type dependence (38) on the electronegativity of the dithiolane aryl *para*-substituent was observed. These results are in agreement with other studies that demonstrate that nonaromatic five-membered ring systems that contain a hetero atom are among the best substrates known for the pulmonary rabbit flavin-containing monooxygenase (15, 19, 37).[1] The lack of a Hammett-type substituent dependence (38) of the rate of *S*-oxygenation is similar to that observed for *S*-oxygenation of 2-aryl-1,3-dithiolanes with other flavin-containing monooxygenase enzyme systems, as well as other studies with dialkylsulfides, which suggest that all good substrates for the flavin-containing monooxygenase (8, 39, 40) have similar V_{max} values.

That concurrent nonenzymatic *S*-oxygenation of aryl-1,3-dithiolanes does not contribute to the formation of the *S*-oxides stems from the observations that (a) the ratio of diastereomers remains constant over the time course of the reaction, (b) chemical oxidation (i.e., oxidation of **1–5** to *S*-oxides in the

TABLE 1

S-Oxygenation of 2-aryl-1,3-dithiolanes by rabbit lung microsomes

Substrate		Microsomes + ABT[a]			Microsomes − ABT[b]		
		S-Oxide formed		DE[c]	S-Oxide formed		DE
		(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide		(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide	
		nmol/min/mg of protein		%	*nmol/min/mg of protein*		%
1	4-OCH_3	0.2[d]	2.7 ± 0.2	86	1.0 ± 0.2[e]	3.3 ± 1.2	53
2	4-H	0.1	1.6 ± 0.2	88	2.0 ± 0.3[e]	3.9 ± 1.1[f]	34
3	4-Cl	ND[g]	6.5 ± 0.5	100	0.9 ± 0.1[e]	1.6 ± 0.1[e]	28
4	4-CN	ND	1.3 ± 0.2	100	ND	0.5 ± 0.1[e]	100
5	4-NO_2	0.1	6.1 ± 1.0	96	0.8 ± 0.2[e]	4.3 ± 0.9	69

[a] Rabbit lung microsomes (0.76 mg of protein/incubation) were incubated in the presence of an NADPH-generating system, aminobenzotriazole (ABT), and substrate (400 μM) in potassium phosphate buffer (pH 8.0) at 33° for 3 min. Each value is the average of four determinations ± standard error.
[b] Incubation performed as described above in the absence of aminobenzotriazole.
[c] Product diastereoselective excess [% (*trans*)-*S*-oxide- % (*cis*)-*S*-oxide].
[d] The range of values is 0–0.3 nmol/min/mg of protein.
[e] $p < 0.001$ versus microsomes plus aminobenzotriazole.
[f] $p < 0.05$ versus microsomes plus aminobenzotriazole.
[g] ND, Not detectable.

TABLE 2

S-Oxygenation of 2-aryl-1,3-dithiolanes by rabbit lung flavin-containing monooxygenase and cytochrome P-450$_{IIB-4}$

Substrate		Flavin-containing monooxygenase[a]			Cytochrome P-450$_{IIB-4}$[a]		
		S-Oxide formed		DE[b]	S-Oxide formed		DE
		(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide		(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide	
		nmol/min/nmol of protein		%	*nmol/min/nmol of protein*		%
1	4-OCH_3	ND[c]	22.3 ± 2.0	100	ND	6.9 ± 1.4	100
2	4-H	ND	24.4 ± 6.1	100	ND	1.0 ± 0.1	100
3	4-Cl	ND	17.1 ± 1.9	100	ND	2.7 ± 0.8	100
4	4-CN	ND	26.4 ± 1.1	100	ND	19.3 ± 0.9	100
5	4-NO_2	NA[d]	NA		ND	9.5 ± 1.8	100

[a] Incubations were performed as described in Experimental Procedures and the values are the average of 4 determinations ± SE.
[b] Diastereoselective excess as described in Table 1.
[c] ND, not detectable.
[d] NA, not available.

[3] J. R. Cashman, L. D. Olsen, and L. M. Bornheim. Enantioselective *S*-oxygenation by flavin-containing and cytochrome P-450 monooxygenases. Submitted for publication.

presence of H_2O_2 or $NaIO_4$) is extremely slow (40) and requires a large concentration of oxidizing agents, and (c) incubations performed in the presence of catalase gave the same stereoselectivity of *S*-oxidation as those incubations performed in the absence of catalase (data not shown). That **1–5** are not oxidized by lipid peroxides or endogenous H_2O_2 during the short incubation times employed can be seen from a comparison of the diastereoselectivity of H_2O_2- or $NaIO_4$ catalyzed *S*-oxygenation of **1–5** and the diastereoselectivity of the microsome-catalyzed *S*-oxygenation of **1–5**. The average percentage diastereoselectivity for $NaIO_4$ and H_2O_2-catalyzed *S*-oxidation of **1–5** is 39 and 31%, respectively (data not shown). For pulmonary microsomes, the average percentage diastereoselectivity for *S*-oxygenation of **1–5** in the presence and absence of aminobenzotriazole is 94 and 57%, respectively (Table 1). The data suggest that nonenzymatic processes do not contribute to *S*-oxygenation of **1–5** and that in the presence of aminobenzotriazole the diastereoselectivity is markedly increased.

***S*-Oxygenation of 2-aryl-1,3-dithiolane *S*-oxides.** The *S*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides with various rabbit lung monooxygenase preparations was performed in order to determine the products of the reactions as well as to determine the enzymes involved in the transformations. In the presence of rabbit lung microsomes supplemented with NADPH, 2-aryl-1,3-dithiolane *S*-oxides are *S*-oxygenated to the corresponding *S,S′*-dioxide (Table 3). In contrast to initial *S*-oxygenation of dithiolanes, the rate of rabbit lung microsome catalyzed *S,S′*-dioxygenation is significantly decreased and a tendency for a modest Hammett-type substituent dependence (38) of the rate of *S′*-oxygenation is observed. Thus, compounds **6a** and **7a** are *S*-oxygenated at an elevated rate, compared with compounds **9a** and **10a**. A modest Hammett-type substituent dependence of the *S′*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides is observed for the purified flavin-containing monooxygenase from rabbit lung (Table 3). As observed for rabbit lung microsomes, the rate of *S*-oxygenation catalyzed by purified rabbit flavin-containing monooxygenase of compounds **6a–10a** is significantly decreased, in comparison with the parent sulfide. Compounds **6a** -**8a** are *S*-oxygenated more efficiently than **9a** and **10a**. Incubations performed with **6a–10a** in the presence of rabbit lung cytochrome P-450_{IIB-4} gave variable and only very small amounts of *S,S′*-dioxide products. As a result, the amount of *S,S′*-dioxygenation catalyzed by rabbit cytochrome P-450_{IIB-4} was not quantitated.

In a control experiment, addition of aryl-1,3-dithiolane *S*-oxides **6a–10a** to inactive rabbit pulmonary microsomes or to active microsomes in the absence of NADPH did not result in any transformation of **6a–10a**. This result suggests that reduction or other transformation of **6a–10a** does not occur during the short time period of the incubation. That no aryl-1,3-dithiolane (from reduction) or *para*-substituted benzaldehyde (from oxidation) is observed in the metabolic reactions of **6a–10a** shows that *S*-oxygenation of **6a–10a** is the only detectable biotransformation taking place.

Stereoselectivity of 2-aryl-1,3-dithiolane *S*-oxygenation. As shown in Table 4, a slight stereochemical preference for addition of an oxygen atom by rabbit lung microsomes is to the *pro-S*-sulfur atom for **1** and **2** and to the *pro-R*-sulfur atom for **3** and **4**. Thus, for **2**, addition of an oxygen to the *pro-S*-sulfur atom was found to be 59% (i.e., 40% 1*S*,2*S*; 19% 1*S*,2*R*), with 41% addition of oxygen to the *pro-R*-sulfur atom (i.e., 26% 1*R*,2*R*; 15% 1*R*,2*S*). In the presence of aminobenzotriazole, the increased enantioselectivity for *S*-oxygenation of **2** shows a preference for addition of oxygen to the *pro-S*-sulfur atom (i.e., 64% 1*S*,2*S*), with 36% addition of oxygen to the *pro-R*-sulfur atom (i.e., 36% 1*R*,2*R*). For **3** and **4**, a marked preference for addition of oxygen to the *pro-R*-sulfur atom was observed (i.e., average value of 73% 1*R*,2*R*), with 27% addition of oxygen to the *pro-S*-sulfur atom (i.e., 12% 1*S*,2*S* and 15% 1*S*,2*R*). In the presence of aminobenzotriazole, the enantioselective *S*-oxygenation of **1**, **3**, and **4** was significantly increased (i.e., average value of 88% 1*R*,2*R* and 12% 1*S*,2*S*).

For rabbit lung cytochrome P-450_{IIB-4}, a marked preference for addition of oxygen to the *pro-S*-sulfur atom of **1–4** was observed (i.e., average percentage enantioselectivity of 91% 1*S*,2*S* and 9% 1*R*,2*S*). In strong contrast, almost exclusive preference for addition of oxygen to the *pro-R*-sulfur atom was observed for the highly purified rabbit pulmonary flavin-containing monooxygenase (Table 4). The results clearly demonstrate that rabbit lung cytochrome P-450_{IIB-4} and flavin-containing monooxygenase *S*-oxygenate **1–4** with opposite enantioselectivity.

That nonenzymatic *S*-oxygenation is not occurring can be seen from a comparison of enzymatic versus H_2O_2-catalyzed *S*-oxidation of **1–5**. As shown in Table 4, enantioselective *S*-oxidation of **1–5** is quite large. In contrast, H_2O_2-catalyzed *S*-oxygenation of **1–5** has zero enantioselectivity (data not shown).

TABLE 3

S-Oxygenation of 2-aryl-1,3-dithiolane S-oxides by rabbit lung microsomes and rabbit lung flavin-containing monooxygenase

Each value is the average of four determinations ± standard error.

Substrate		*S,S′*-Dioxide formed			
		Rabbit lung microsomes[a]		Rabbit lung flavin-containing monooxygenase[a]	
		(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide	(*cis*)-*S*-Oxide	(*trans*)-*S*-Oxide
		nmol/min/mg of protein		nmol/min/mg of protein	
6a	4-OCH_3	0.9 ± 0.2	2.8 ± 0.4	0.2[b]	2.0 ± 0.3
7a	4-H	ND[c]	4.1 ± 0.6	0.9 ± 0.2	3.6 ± 0.9
8a	4-Cl	0.7 ± 0.2	2.2 ± 0.3	0.9 ± 0.2	1.6 ± 0.3
9a	4-CN	ND	ND	ND	0.1[b]
10a	4-NO_2	0.1[b]	1.9 ± 0.3	0.6 ± 0.1	1.3 ± 0.2

[a] Incubations performed as described in Experimental Procedures.
[b] The range of values is 0–0.2 nmol/min/mg of protein or nmol/min/nmol of protein.
[c] ND, not detectable.

Discussion

The overall enzymatic *S*-oxygenation of 2-aryl-1,3-dithiolanes with microsomes or highly purified flavin-containing monooxygenase or cytochrome P-450_{IIB-4} from rabbit lung can be described by the scheme in Fig. 1. Each step has been investigated with five *para*-substituted 2-aryl-1,3-dithiolanes and represents a process that converts the dithiolane to the corresponding dithiane *S*-oxide and, in a second reaction, converts the *S*-oxide to the dithiolane *S,S′*-dioxide. At extremely long reaction times, the *S,S′*-dioxide is not indefinitely stable and is converted to the corresponding benzaldehyde (in a process that presumably involves formation of the dithiolane *S,S,S′*-trioxide) (40), but during the short incubation times employed in this study decomposition of *S*-oxide reaction prod-

TABLE 4

Stereoselective S-oxygenation of 2-aryl-1,3-dithiolanes by various rabbit lung enzyme preparations

Absolute stereochemistry was determined from reactions shown in Tables 1 and 2, as described in Experimental Procedures. Each value is the average of four determinations ± standard error.

	Substrate	S-Oxide formed[a]											
		Flavin-containing monooxygenase				Cytochrome P-450$_{IIB-4}$				Rabbit lung microsomes[a]			
		(*cis*)-*S*-Oxide		(*trans*)-*S*-Oxide		(*cis*)-*S*-Oxide		(*trans*)-*S*-Oxide		(*cis*)-*S*-Oxide		(*trans*)-*S*-Oxide	
		(1*R*,2*S*)[b]	(1*S*,2*R*)	(1*S*,2*S*)	(1*R*,2*R*)	(1*R*,2*S*)	(1*S*,2*R*)	(1*S*,2*S*)	(1*R*,2*R*)	(1*R*,2*S*)	(1*S*,2*R*)	(1*S*,2*S*)	(1*R*,2*R*)
		%											
1	4-OCH_3	ND[c]	ND	0	100	ND	ND	90 ± 3	10 ± 3	75.5 ± 11	24.5 ± 11	56.1 ± 1	43.9 ± 1
2	4-H	ND	ND	3.2 ± 2.0	96.7 ± 2.0	ND	ND	92 ± 2	8 ± 2	43.2 ± 3	56.8 ± 4	60.5 ± 2	39.5 ± 2
3	4-Cl	ND	ND	2.4 ± 1.7	97.6 ± 1.6	ND	ND	90 ± 4	10 ± 4	57.6 ± 8	42.4 ± 9	32.0 ± 2	68.0 ± 2
4	4-CN	ND	ND	1.5 ± 0.3	98.5 ± 0.4	ND	ND	91 ± 3	9 ± 3	ND	ND	26.8 ± 1	73.2 ± 1

[a] Experiment performed in the absence of aminobenzotriazole.
[b] The first designation is for the absolute stereochemistry of the sulfur atom.
[c] ND, Not detectable.

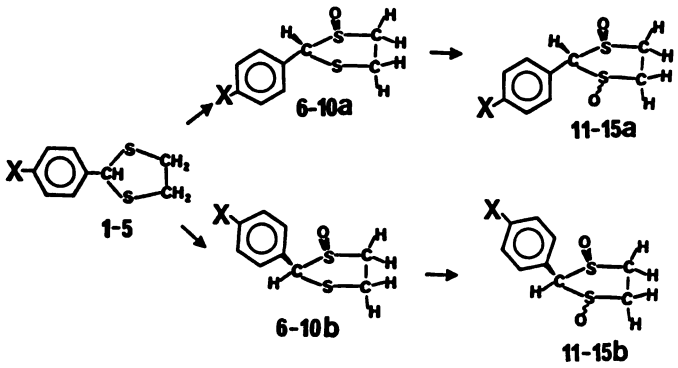

Fig. 1. Overall *S*-oxidative biotransformation of the 2-aryl-1,3-dithiolanes. The *trans*-diastereomers and the *cis*-diastereomers are designated as **a** and **b**, respectively.

ucts did not pose a problem and no *para*-substituted benzaldehyde was ever observed.

For the *S*-oxygenation of 2-aryl-1,3-dithiolanes by rabbit lung microsomes, a significant contribution to the *S*-oxygenation of **1** and **2** may be due to cytochromes P-450 whereas **3**, **4**, and **5** are largely *S*-oxygenated by the flavin-containing monooxygenase. This conclusion is based on the results of the effects of aminobenzotriazole (Table 1) and from the determination of the absolute stereochemistry of the *S*-oxide products obtained (Table 4) (see below). It is possible, however, that another unidentified aminobenzotriazole-sensitive reaction is also in part responsible for *S*-oxygenation of **1** and **2**. Although aminobenzotriazole is known to stimulate rabbit lung microsomal oxygenation of substrates of the flavin-containing monooxygenase (34), this does not preclude other processes from contributing to substrate oxidation.

Aminobenzotriazole, a mechanism-based inactivator of cytochromes P-450 (33), significantly inhibits the formation of **6** and **7** from **1** and **2** (Table 1). Stereochemical analysis (Table 1) of the products formed from rabbit lung microsomes demonstrates that, in the presence of aminobenzotriazole, the diastereoselectivity for (*trans*)-*S*-oxide formation increases. For **2**, in the absence of aminobenzotriazole, the increased enantioselectivity [i.e., mainly (*pro-S*)-*S*-oxide] is consistent with the involvement of cytochrome P-450 in the *S*-oxygenation (data not shown). That the *S*-oxide enantiomer that is increased in the presence of aminobenzotriazole is mainly the (*pro-R*)-*S*-oxide for **3** and **4** suggests that the flavin-containing monooxygenase is primarily responsible for the *S*-oxygenation of **3** and **4** in rabbit lung microsomes. Compound **1**, at least in part, appears to be *S*-oxygenated by cytochromes P-450 but in the presence of aminobenzotriazole **1** is almost exclusively *S*-oxygenated by the flavin-containing monooxygenase [i.e., 97% (*trans*)-(1*R*,2*R*)-*S*-oxide is formed; data not shown]. This may suggest that rabbit lung microsomes contain a cytochrome P-450 that is particularly sensitive to the inhibitory properties of aminobenzotriazole (34) and this isozyme is the major contributor to cytochrome P-450-dependent *S*-oxygenation of **1**. Whereas a specific aminobenzotriazole-sensitive cytochrome P-450 isozyme may participate in the *S*-oxygenation of **1**, the flavin-containing monooxygenase is largely responsible for *S*-oxygenation of **1** in rabbit lung microsomes.

For both highly purified rabbit lung flavin-containing monooxygenase and cytochrome P-450$_{IIB-4}$, 2-aryl-1,3-dithiolanes **1–5** are efficiently *S*-oxygenated (Table 2). *S*-Oxygenation of **1–5** by the purified monooxygenases is highly diastereoselective for formation of the (*trans*)-*S*-oxide diastereomer. Varying the electronic nature of the *para*-substituent of 2-aryl-1,3-dithiolanes does not influence the rate of flavin-containing monooxygenase-catalyzed *S*-oxygenation. These results are in agreement with other studies that suggest that at least for this class of substrate the rate-determining step occurs after product formation (15). In contrast, cytochrome P-450$_{IIB-4}$ tends to *S*-oxygenate electron-deficient *para*-substituted 2-aryl-1,3-dithiolanes more efficiently than electron-rich *para*-substituted 2-aryl-1,3-dithiolanes in disagreement with other studies employing hepatic enzyme systems (41) (Table 2). The results with cytochrome P-450 tend to support the notion that electron-deficient dialkylsulfides are *S*-oxygenated more efficiently than electron-rich dialkylsulfides (41).

The *S*-oxygenation of 2-aryl-1,3-dithiolane *S*-oxides was investigated with microsomes and highly purified flavin-containing monooxygenase and cytochrome P-450$_{IIB-4}$ from rabbit lung. As shown in Table 3, microsomes and purified flavin-containing monooxygenase are competent to *S*-oxygenate **6a–10a**. Attempts to demonstrate cytochrome P-450$_{IIB-4}$-catalyzed *S*-oxygenation of **6a–10a** were unsuccessful. From the limited data, it appears that *S*-oxide *S'*-oxygenation is largely dependent on flavin-containing monooxygenase activity, although compared with *S*-monooxygenation the rate of *S,S'*-dioxide formation is markedly decreased. In contrast to *S*-monooxygenation, *S,S'*-dioxygenation is somewhat sensitive to the nature of the *para*-

substituent, demonstrating a Hammett correlation (i.e., ρ value of 0.3 and 0.27 for microsomes and purified flavin-containing monooxygenase from rabbit lung, respectively) (Table 3). Based on the results presented, a distinction between a single-electron transfer mechanism and an anionic nucleophilic mechanism for *S*-oxygenation cannot be made solely on the basis of Hammett-type correlations (40, 43). Stereochemical analysis of the products of *S,S'*-dioxygenation, in principle, could provide insight into enzyme mechanism. However, the relative instability of the *S,S'*-dioxides precluded careful examination of the absolute stereochemistry of **11–15**. Absolute stereochemistry assignments of *S*-monooxide metabolites of **1–4** were investigated and this has led to an understanding of rabbit pulmonary monooxygenase mechanism.

A summary of the results of *S*-oxygenation catalyzed by microsomes and purified cytochrome P-450$_{IIB-4}$ and flavin-containing monooxygenase from rabbit lung is shown in Tables 1, 2, and 4. From the results shown in Tables 1 and 2 it is apparent that the *S*-oxygenation of **1–5** is highly stereoselective and the (*trans*)-*S*-oxide product is formed in large excess of the (*cis*)-*S*-oxide. For rabbit lung microsomes the diastereoselective excess was modest and varied between 100% and 28%, with an average value of 57% (Table 1). In the presence of aminobenzotriazole, a mechanism-based inactivator of cytochromes P-450 (33) and an agent that does not inhibit pulmonary flavin-containing monooxygenase activity (34, 37), a marked increase in diastereoselectivity is observed. The diastereoselective excess varied between 100% and 86%, with an average value of 94%. We interpret these results to suggest that the contribution to dithiolane *S*-oxygenation from rabbit lung cytochrome P-450 is significant and the low diastereoselective excess observed for lung microsomes in the absence of aminobenzotriazole reflects an involvement of cytochrome P-450 in (*cis*)-*S*-oxide formation. For the 2-aryl-1,3-dithiolanes studied, both the purified flavin-containing monooxygenase and the purified cytochrome P-450$_{IIB-4}$ from rabbit lung catalyze exclusive formation of the (*trans*)-*S*-oxide (Table 2). Because the flavin-containing monooxygenase catalyzes only (*trans*)-*S*-oxide formation of **1–5** this suggests that some other monooxygenase [presumably cytochrome P-450 IVA4 (pg-ω) or IVB1 (form 5), which are the other major isoenzymes present] catalyzes formation of the (*cis*)-*S*-oxide of **1–5**.

As shown in Table 4, the absolute stereochemistry of *S*-oxygenation of **1–4** catalyzed by microsomes, flavin-containing monooxygenase, and cytochrome P-450$_{IIB-4}$ from rabbit lung was determined. It is clear that rabbit lung flavin-containing monooxygenase has a marked preference for forming (*trans*)-(*1R,2R*)-*S*-oxides. In contrast, cytochrome P-450$_{IIB-4}$ *S*-oxygenates **1–4** to produce exclusively (*trans*)-(*1S,2S*)-*S*-oxides. From inspection of the stereochemistry of *S*-oxide products from experiments employing rabbit lung microsomes, it is apparent that cytochrome P-450 is preferentially responsible for *S*-oxygenation of **2**, whereas flavin-containing monooxygenase activity is mainly responsible for *S*-oxygenation of **1, 3, 4**, and **5**. Thus, attack by the peroxyflavin of rabbit lung flavin-containing monooxygenase is directed to the *pro-R*-sulfur atom, whereas attack by the cytochrome P-450 iron-oxo species is directed to the (*pro-S*)-sulfur atom. It is possible that the 2-aryl-1,3-dithiolanes are *S*-oxygenated by rabbit pulmonary cytochrome P-450 in an "edge-on" orientation with respect to the dithiolane group and the heme moiety, whereas rabbit pulmonary flavin-containing monooxygenase requires alignment of the dithiolane substrate in a deep narrow cleft where steric interactions between the peroxyflavin and the aryl group constrain the substrate into a *trans*-orientation (Fig. 2). Compared with hog liver flavin-containing monooxygenase, which can produce (*cis*)-2-aryl-1,3-dithiolane *S*-oxide products as well as (*trans*)-2-aryl-1,3-dithiolane *S*-oxide products (40),[1] it would appear that the rabbit lung flavin-containing monooxygenase is highly stereoselective and presumably possesses a much smaller binding pocket than the hepatic form of the enzyme. That a large degree of enantioselectivity is observed for both monooxygenase-catalyzed reactions suggests that, if single-electron transfer reactions are on the 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 (43). However, an alternative mechanism involving nucleophilic attack via anionic mechanisms is favored for the flavin-containing monooxygenase (40, 42).

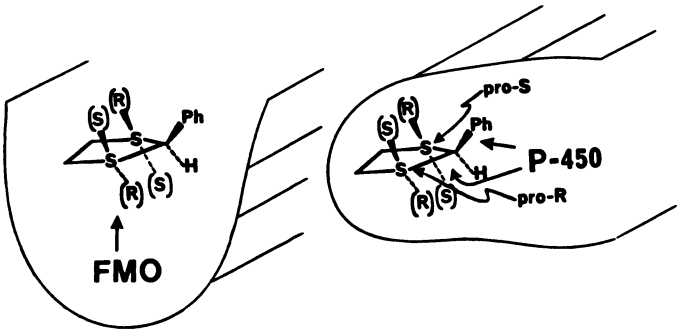

Fig. 2. *S*-oxygenation of 2-aryl-1,3-dithiolanes by cytochrome P-450-catalyzed "edge-on" *S*-oxygenation and flavin-containing monooxygenase (*FMO*)-catalyzed *S*-oxygenation.

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