Metabolism in Mouse Liver and Photooxidation of the Insecticidal *cis*- and *trans*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithianes†

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cis- and *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithianes and 11 of their S-oxidation products undergo NADPH-dependent oxidation by cytochrome P-450 in mouse liver microsomes. The cis isomers (2-aryl group axial) are metabolized more rapidly than the trans isomers to form up to seven distinct S-oxidation products. The metabolic pathways for the *cis*- and *trans*-dithianes converge on one major dioxidized product, *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane *cis*-1,*trans*-3-dioxide. The observed oxidation stereospecificity is rationalized on the basis of the orientation of the sulfur lone pairs relative to the perferryl oxidizing species in the P-450 active site. Some of the same S-oxidation products are observed in the livers of dithiane-treated mice and in thin films of the compounds exposed to sunlight. The metabolic oxidations provide both activation and detoxification mechanisms for the dithianes as insecticides and inhibitors of the GABA-gated chloride channel.

INTRODUCTION

cis- and *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithianes ($Ar_{ax}SS$ and $Ar_{eq}SS$) (Figure 1) are potent insecticides (Elliott et al., 1992) and noncompetitive γ-aminobutyric acid (GABA) antagonists (Wacher et al., 1992). Twelve of their S-oxidation products (Table I) are known from reactions with peracid and permanganate, and they vary over a wide range in potency as insecticides and inhibitors of the GABA-gated chloride channel in mouse brain and housefly head membranes (Wacher et al., 1992). One or more of these products are expected as metabolites in the microsomal cytochrome P-450 or flavin-containing monooxygenase (FMO) system (Damani, 1989a; Holland, 1988; Kinsler et al., 1988, 1990; Waxman et al., 1982).

Metabolic S-oxidation of 1,3-dithianes has previously been studied in fungi (Auret et al., 1981, 1985) but not in mammalian systems. Thianes, 1,2-dithianes, thiochromanes, and thiadecalins undergo S-oxidation by microsomal cytochrome P-450, forming only monooxidized products that are poor substrates for further oxidation (Fukushima et al., 1978; Takahashi et al., 1978; Takata et al., 1980, 1983). Similar results are observed for FMO-mediated metabolism of the related 2-aryl-1,3-dithiolanes (Cashman and Olsen, 1990; Cashman and Williams, 1990; Cashman et al., 1990a).

The relative contributions of P-450 and FMO to the metabolism are determined using selective inhibitors: piperonyl butoxide (PB) to inhibit P-450 (Casida, 1970); thiourea (TU) to competitively inhibit FMO (Cashman et al., 1990a; Venkatesh et al., 1991); and *n*-octylamine (NOA) to weakly induce FMO activity in mice (Sabourin and Hodgson, 1984) and inhibit P-450 (Jefcoate et al., 1969).

This investigation considers the metabolic oxidation of $Ar_{ax}SS$ and $Ar_{eq}SS$ and their S-oxidation products by mouse liver microsomes and the in vivo relevance of these oxidations. The metabolites are analyzed by normal-phase high-pressure liquid chromatography (HPLC) with identification by cochromatography with authentic standards. Gas chromatography/mass spectrometry (GC/MS) was not used for analysis as the dithiane S-oxidation products are

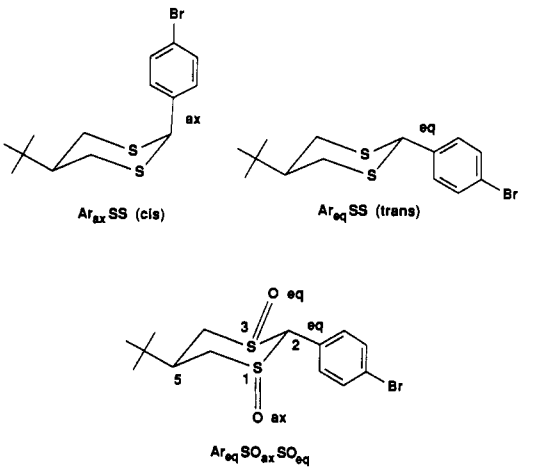

Figure 1. Stereochemistries of *cis*- and *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane and their S-oxidation products (one to four oxygens; two oxygens illustrated). Ax or axial and eq or equatorial define the orientation of the aryl group and the stereochemistry of *S*-oxidation. For example, $Ar_{eq}SO_{ax}SO_{eq}$ designates the disulfoxide in which the aryl group is equatorial and one S=O bond is axial and the other S=O bond is equatorial.

Table I. HPLC Retention Times for *cis*- and *trans*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithiane and Their S-Oxidation Products

compd		HPLC, Rt,[a] min	
cis	trans	cis	trans
$Ar_{ax}SS$	$Ar_{eq}SS$	1.4	1.8
$Ar_{ax}SSO_{eq}$	$Ar_{eq}SSO_{eq}$	8.8	32.4
$Ar_{ax}SSO_{ax}$	$Ar_{eq}SSO_{ax}$	33.3	17.3
$Ar_{ax}SSO_2$	$Ar_{eq}SSO_2$	4.3	11.1
$Ar_{ax}SO_{eq}SO_{eq}$[b]	$Ar_{eq}SO_{eq}SO_{eq}$	36.2	42.0
$Ar_{ax}SO_{ax}SO_{eq}$	$Ar_{eq}SO_{ax}SO_{eq}$	35.0	43.2
	$Ar_{eq}SO_{eq}SO_2$[c]		35.2
	$Ar_{eq}SO_2SO_2$		29.6
	$Ar_{eq}SO_2SO_2$–OH[b]		39.6

[a] Conditions used for microsomal metabolites. [b] Not available as authentic standards but proposed as microsomal metabolites. [c] $Ar_{eq}SO_{ax}SO_2$ is available as an authentic standard but is not detected as a metabolite.

† Supported by National Institute of Environmental Health Sciences Grant P01 ES00049.

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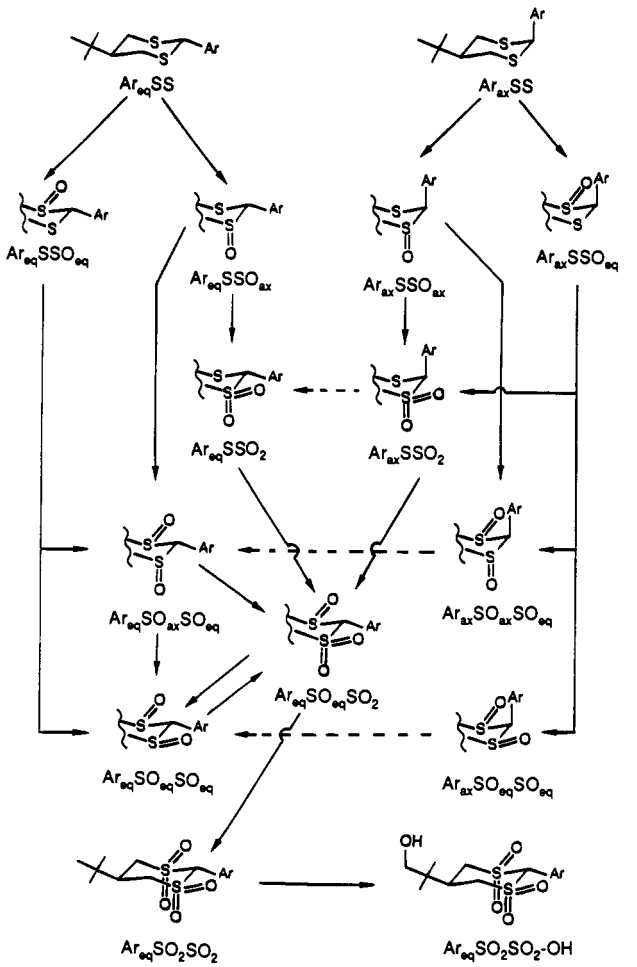

Figure 2. Convergent metabolic pathways for *cis*- and *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithianes and their S-oxidation products. Nonenzymatic epimerization is shown by a broken line (Ar = 4-bromophenyl).

not amenable to GC. The findings establish the metabolic pathways and stereochemistries for the dithianes and their S-oxidation products as shown in Figure 2.

MATERIALS AND METHODS

Chemicals. $Ar_{ax}SS$, $Ar_{eq}SS$, and several of their S-oxidation products were available from previous preparations (Wacher et al., 1992) (Table I). Tetrahydrofuran (THF) (HPLC grade; from EM Science, Gibbstown, NJ) was purified by refluxing over lithium aluminum hydride followed by distillation under nitrogen.

Microsomal Metabolites. *Preparation of Microsomes.* Microsomes were prepared from the livers of male albino Swiss-Webster mice (Simonsen Laboratories, Gilroy, CA) using phosphate buffer (100 mM, pH 7.4) and standard differential centrifugation procedures (Cole et al., 1991). The washed microsomal pellet was suspended in buffer, the protein concentration determined (Bradford, 1976), and the suspension stored at −80 °C until used.

Microsomal Incubation. Standard reaction mixtures contained 30 μM substrate, 0.3 mg/mL microsomal protein, 0.5 mM NADPH, and 1 mM diethylenetriaminepentaacetic acid (DETPAC; to ensure metal-free conditions) in 1 mL of phosphate buffer (100 mM, pH 7.4) with incubation for 20 min at 37 °C. All experiments compared samples with NADPH to a control with no NADPH. Sonication was used to dissolve the candidate inhibitors NOA and PB in buffer prior to their addition to the incubation mixture. Centrifuge tubes (10 mL) containing the incubation mixtures in ice-cold buffer were transferred to a water bath at 37 °C 2 min prior to initiation of the metabolism by addition of the substrate as an acetone solution (20 μL).

Extraction and Metabolite Recovery. Microsome reactions were stopped by addition of 1:1 dichloromethane–hexane (2 mL) containing methyl 4-hydroxybenzoate (3 μM) as the internal standard; the addition of hexane to the dichloromethane shifted the organic phase above the protein plug formed at the solvent interface for convenient recovery. Extraction involved vortexing and then centrifugation (100*g*, 10 min). The organic phase was transferred to a sample tube and then evaporated to dryness under argon for sealed storage under argon at −4 °C until analyzed; there was no change in product ratios during sample preparation and storage up to 4 days. One extraction step was adequate since two or three extractions did not improve recoveries. Extraction efficiencies for the dithianes and their S-oxidation products, as well as 4-bromobenzaldehyde and 4-bromobenzoic acid, were 89 ± 11% for the parent compounds from incubation mixtures without NADPH and 77 ● 11% for the substrate plus metabolites when NADPH was included.

Metabolites in the Liver. Mice as above were treated by intraperitoneal (ip) injection with the test compound (400 μg) in dimethyl sulfoxide (100 μL) to give a dithiane dose of 20 mg/kg. The liver was removed after 20 min and homogenized in acetone (2 mL). This homogenate was washed into a centrifuge tube (10 mL) with a further 2 mL of acetone and then centrifuged (100*g*, 5 min). The supernatant was transferred to a second centrifuge tube, dichloromethane (2 mL) added, and the centrifugation repeated. The aqueous phase was removed, and then the organic phase was dried ($MgSO_4$) and filtered and the solvent evaporated under vacuum. Samples were stored under argon until analyzed by HPLC.

HPLC Analysis. HPLC utilized a Waters 600E multisolvent delivery system and a Waters 994 programmable photodiode array detector set at 230 (λ_{max} for the dithianes and their S-oxidation products) and 258 nm (to detect products from ring opening including bromobenzaldehyde and bromobenzoic acid). Reversed phase (RP) HPLC was not suitable due to the limited solubility of the compounds in typical RP-HPLC solvents such as methanol–water and acetonitrile–water mixtures. Normal-phase HPLC used a Merck LiChroCART Si 60 analytical cartridge (125 mm × 4 mm i.d., 5-μm particle size) fitted with a precolumn (4 × 4 mm i.d., 5 μm) to trap dust and macromolecules. Gradient elution involved THF–hexane at a flow rate of 0.85 mL/min. Freshly distilled solvent minimized the baseline absorbance of THF at 230 nm so it did not affect quantitation of metabolites. The initial concentration of THF (20% for microsomes, 10% for liver extracts) was maintained for 20 min and then raised (2%/min for microsomes, 2.5%/min for liver extracts) to a final concentration of 60%, which was maintained for 10 min.

Metabolites were identified by HPLC retention times (Rt's) (Table I) and cochromatography with authentic standards. They were quantitated by dissolving the samples in 2:1 THF–hexane (100 μL) and examining a 20-μL aliquot by HPLC comparing peak areas to standard curves prepared with each authentic dithiane and S-oxidation product. The percentage recovery of each compound was calculated on a molar equivalent basis without correction for extraction efficiency. All experiments, including standard curve determinations, were carried out in triplicate with sample variation reported as standard deviation (SD). The internal standard was used to compare peak areas among triplicates of the same experiment as well as extraction differences between experiments, establishing an insignificant variation in these respects.

Photooxidation. $Ar_{ax}SS$ and $Ar_{eq}SS$ were individually exposed for 4 h as thin films (2.6 μg/cm²) in Pyrex Petri dishes to ambient July sunlight in Berkeley, CA. Similar films were examined with 4-bromobenzaldehyde (0.13 μg/cm²), as a candidate sensitizer. The photoproducts were dissolved in dichloromethane (1 mL) and analyzed by HPLC as above. Alternatively, $Ar_{ax}SS$ and $Ar_{eq}SS$ were individually irradiated in acetonitrile solution with Rose bengal sensitizer (2% w/w relative to the dithiane), continuous oxygen flow, and exposure to an incandescent lamp as well as in hexane solutions at 254 nm with analysis by TLC, NMR, and gas chromatography/mass spectrometry (GC/MS).

RESULTS

Microsomal Metabolism of $Ar_{ax}SS$, $Ar_{eq}SS$, and Their S-Oxidation Products. Optimal conditions established with $Ar_{ax}SS$ (60 μM) as the substrate were 0.3

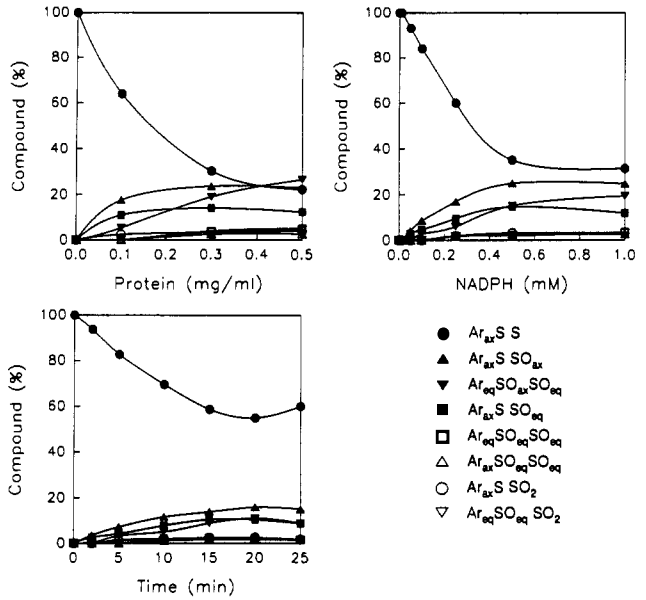

Figure 3. Effect of microsomal protein level, NADPH concentration, and incubation time on the metabolites of *cis*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane. Incubations involved 60 μM Ar$_{ax}$SS, 0.3 mg/mL microsomal protein, 0.5 mM NADPH, and 20 min at 37 °C unless indicated otherwise.

Table II. Microsomal Metabolism of *cis*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithiane and the Effect of Selected Inhibitors

	recovery with indicated inhibitor, % (SD)			
compd	alone	4 mM NOA	1 mM TU	1 mM PB
Ar$_{ax}$SS	34.4 (6.4)	93.7 (0.5)	57.0 (1.7)	88.7 (0.3)
Ar$_{ax}$SSO$_{eq}$	13.3 (3.4)		4.4 (1.1)	3.5 (0.1)
Ar$_{ax}$SSO$_{ax}$	21.7 (1.2)	6.3 (0.5)	13.0 (1.1)	7.8 (0.1)
Ar$_{ax}$SSO$_2$	3.4 (0.5)		2.6 (0.1)	
Ar$_{eq}$SO$_{eq}$SO$_{eq}$	3.6 (0.6)		3.1 (0.4)	
Ar$_{eq}$SO$_{ax}$SO$_{eq}$	17.0 (1.6)		13.8 (0.4)	
Ar$_{eq}$SO$_{eq}$SO$_2$	3.5 (0.4)		3.4 (0.7)	
Ar$_{ax}$SO$_{eq}$SO$_{eq}$[a]	3.1 (0.2)		2.7 (0.4)	

[a] Proposed product.

mg/mL microsomal protein, 0.5 mM NADPH, and 20 min of incubation time; at each of these points both the number of metabolites (seven) and the percentages of monooxidized compounds reached a maximum (Figure 3). The substrate was not metabolized in the absence of protein or at ≤0.01 mM NADPH. The percentage metabolism decreased with higher Ar$_{ax}$SS levels, i.e., 64, 59, and 45% at 15, 30, and 60 μM, respectively. In each case the first products observed were monooxidized compounds (2 min) and then dioxidized (5 min), with all seven metabolites evident after 10 min. Metabolism of Ar$_{ax}$SS was strongly inhibited by NOA and PB, whereas TU had minimal effect (Table II).

Comparisons were made of both dithiane isomers and all of the S-oxidation products observed from their metabolism by incubating them individually at 30 μM using the standard conditions. Each substrate was metabolized with microsomes and NADPH but not in the absence of either microsomes or NADPH. NOA very effectively inhibited the metabolism of Ar$_{ax}$SSO$_{eq}$ and Ar$_{ax}$SSO$_{ax}$ (Table III) and completely blocked the metabolism of Ar$_{eq}$SS (Table IV). The UV spectra of all product peaks were identical to those obtained for the authentic standards.

Microsomal Metabolites of Ar$_{ax}$SS (Table II). Ar$_{ax}$SS yielded seven S-oxidation products under the standard conditions (Figure 4). Ar$_{ax}$SSO$_{ax}$ and Ar$_{ax}$SSO$_{eq}$ constituted 35% of the extracted material in a 62:38 isomer ratio;

Table III. Microsomal Metabolites of S-Oxidation Products of *cis*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithiane

	recovery from indicated substrate, % (SD)			
compd	Ar$_{ax}$SSO$_{eq}$[a,b]	Ar$_{ax}$SSO$_{ax}$[c,d]	Ar$_{ax}$SSO$_2$[e]	Ar$_{ax}$SO$_{ax}$SO$_{eq}$[f]
Ar$_{ax}$SSO$_{eq}$	8.5 (0.8)			
Ar$_{ax}$SSO$_{ax}$		47.7 (0.6)		
Ar$_{eq}$SSO$_2$	3.8 (0.2)		16.0 (1.9)	
Ar$_{ax}$SSO$_2$	10.1 (3.6)	3.5 (0.2)	43.2 (4.0)	
Ar$_{eq}$SO$_{eq}$SO$_{eq}$	12.4 (0.7)	2.8 (0.5)		
Ar$_{eq}$SO$_{ax}$SO$_{eq}$	47.0 (3.6)	40.7 (0.7)		97.2 (0.6)
Ar$_{ax}$SO$_{eq}$SO$_{eq}$[g]	10.5 (0.2)			
Ar$_{eq}$SO$_{eq}$SO$_2$	7.7 (0.4)	5.3 (0.5)	40.1 (2.4)	2.8 (0.6)
Ar$_{eq}$SO$_2$SO$_2$			0.7 (0.06)	

[a] After 2 min: Ar$_{ax}$SSO$_{eq}$ 76.3 (0.7); Ar$_{ax}$SSO$_2$ 6.4 (0.4); Ar$_{eq}$SO$_{eq}$SO$_{eq}$ 3.8 (0.07); Ar$_{eq}$SO$_{ax}$SO$_{eq}$ 7.1 (0.2); Ar$_{ax}$SO$_{eq}$SO$_{eq}$ 2.7 (0.07); Ar$_{eq}$SO$_{eq}$SO$_2$ 3.7 (0.1). [b] With 4 mM NOA: Ar$_{ax}$SSO$_{eq}$ 84.9 (1.8); Ar$_{eq}$SO$_{eq}$SO$_{eq}$ 4.7 (1.3); Ar$_{eq}$SO$_{ax}$SO$_{eq}$ 10.4 (0.7). [c] After 2 min: Ar$_{ax}$SSO$_{ax}$ 84.2 (0.04); Ar$_{eq}$SO$_{eq}$SO$_{eq}$ 4.6 (0.01); Ar$_{eq}$SO$_{ax}$SO$_{eq}$ 7.4 (0.07); Ar$_{eq}$SO$_{eq}$SO$_2$ 3.8 (0.07). [d] With 4 mM NOA: Ar$_{ax}$SSO$_{ax}$ 90.1 (0.4); Ar$_{eq}$SO$_{ax}$SO$_{eq}$ 9.9 (0.4). [e] After 2 min: Ar$_{ax}$SSO$_2$ 86.2 (0.2); Ar$_{eq}$SSO$_2$ 4.5 (0.4); Ar$_{eq}$SO$_{eq}$SO$_2$ 8.7 (0.6); Ar$_{eq}$SO$_2$SO$_2$ 0.6 (0.07). [f] In the absence of NADPH: Ar$_{eq}$SO$_{ax}$SO$_{eq}$ 97%; Ar$_{ax}$SO$_{ax}$SO$_{eq}$ 3%. [g] Proposed product.

Table IV. Microsomal Metabolites of *trans*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithiane and Its S-Oxidation Products

	recovery from indicated substrate, % (SD)			
compd	Ar$_{eq}$SS[a]	Ar$_{eq}$SSO$_{eq}$	Ar$_{eq}$SSO$_{ax}$	Ar$_{eq}$SSO$_2$
Ar$_{eq}$SS	64.9 (2.0)			
Ar$_{eq}$SSO$_{eq}$	11.3 (1.8)	76.6 (1.5)		
Ar$_{eq}$SSO$_{ax}$	17.8 (0.8)		80.9 (1.0)	
Ar$_{eq}$SSO$_2$			6.8 (1.0)	84.3 (1.0)
Ar$_{eq}$SO$_{eq}$SO$_{eq}$	1.8 (0.3)	8.4 (0.3)		
Ar$_{eq}$SO$_{ax}$SO$_{eq}$	4.2 (0.1)	14.6 (1.2)	10.0 (0.5)	
Ar$_{eq}$SO$_{eq}$SO$_2$		0.4 (0.06)	2.3 (0.3)	15.2 (1.0)
Ar$_{eq}$SO$_2$SO$_2$				0.5 (0.03)

	recovery from indicated substrate, % (SD)			
compd	Ar$_{eq}$SO$_{eq}$SO$_{eq}$	Ar$_{eq}$SO$_{ax}$SO$_{eq}$	Ar$_{eq}$SO$_{eq}$SO$_2$	Ar$_{eq}$SO$_2$SO$_2$
Ar$_{eq}$SO$_{eq}$SO$_{eq}$	96.4 (0.3)	2.0 (0.06)	2.0 (0.16)	
Ar$_{eq}$SO$_{ax}$SO$_{eq}$		96.0 (0.18)		
Ar$_{eq}$SO$_{eq}$SO$_2$	3.6 (0.3)	2.0 (0.12)	97.2 (0.18)	
Ar$_{eq}$SO$_2$SO$_2$			0.8 (0.03)	95.7 (0.2)
Ar$_{eq}$SO$_2$SO$_2$-OH[b]				4.3 (0.2)

[a] Metabolism of Ar$_{eq}$SS is completely inhibited by 4 mM NOA. [b] Proposed product.

although not illustrated, this ratio did not vary significantly with time, protein level, or NADPH concentration. Ar$_{eq}$SO$_{ax}$SO$_{eq}$ was the major dioxidized product, with smaller amounts of Ar$_{eq}$SO$_{eq}$SO$_{eq}$, Ar$_{ax}$SSO$_2$, and the trioxidized product Ar$_{eq}$SO$_{eq}$SO$_2$. An additional metabolite, proposed to be Ar$_{ax}$SO$_{eq}$SO$_{eq}$, is discussed below since it was formed exclusively via Ar$_{ax}$SSO$_{eq}$ as an intermediate.

Microsomal Metabolites of S-Oxidation Products of Ar$_{ax}$SS (Table III). Ar$_{ax}$SSO$_{eq}$ underwent the most extensive metabolism of all compounds tested, with only 9% unmetabolized substrate, Ar$_{eq}$SO$_{ax}$SO$_{eq}$ as the major product, and Ar$_{ax}$SSO$_2$, Ar$_{eq}$SO$_{eq}$SO$_{eq}$, Ar$_{ax}$SO$_{eq}$SO$_{eq}$, and Ar$_{eq}$SO$_{eq}$SO$_2$ in smaller but similar amounts. Formation of Ar$_{ax}$SSO$_2$ was sufficiently high that its epimerization product Ar$_{eq}$SSO$_2$ was also observed. The product tentatively designated Ar$_{ax}$SO$_{eq}$SO$_{eq}$ (10%) formed concurrently with the other dioxidized products (from a comparison of metabolic profiles at 2 and 20 min; Table III) and had an Rt consistent with a disulfoxide but different from authentic Ar$_{ax}$SO$_{ax}$SO$_{eq}$ (Table I); in addition, the UV spectrum was almost identical to that for Ar$_{ax}$SO$_{ax}$SO$_{eq}$, leading to the tentative structural assignment. Ar$_{ax}$SSO$_{ax}$ yielded Ar$_{eq}$SO$_{ax}$SO$_{eq}$ as the major product with

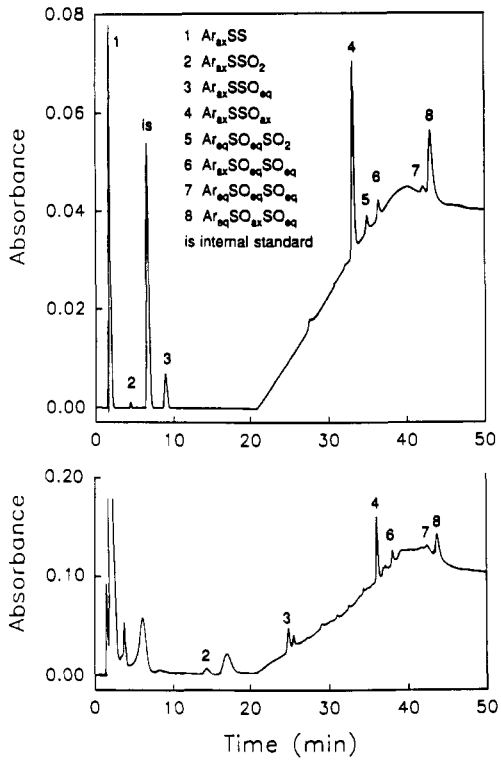

Figure 4. HPLC chromatograms for metabolites of *cis*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane. (Top) Microsomal oxidase metabolites; (bottom) metabolites in the liver 20 min after ip treatment of mice at 20 mg/kg. Unlabeled peaks are endogenous materials. These extractives from liver obscured the first 6 min of the HPLC trace.

$Ar_{ax}SSO_2$, $Ar_{eq}SO_{eq}SO_{eq}$, and $Ar_{eq}SO_{eq}SO_2$ in substantially smaller quantities; $Ar_{ax}SO_{eq}SO_{eq}$ was not observed. The major metabolite from $Ar_{ax}SSO_2$ was $Ar_{eq}SO_{eq}SO_2$, with only a trace amount of $Ar_{eq}SO_2SO_2$, consistent with the poor oxidation of the trioxidized compound described below; epimerization to $Ar_{eq}SSO_2$, which was not enzyme dependent, accounted for 16% of the recovered products. $Ar_{ax}SO_{ax}SO_{eq}$ epimerized almost immediately in buffer (>95% in 2 min) in a nonenzymatic process, yielding predominantly $Ar_{eq}SO_{ax}SO_{eq}$ with a small amount of $Ar_{eq}SO_{eq}SO_2$ from further enzymatic oxidation.

Microsomal Metabolites of $Ar_{eq}SS$ and Its S-Oxidation Products (Table IV). $Ar_{eq}SS$ was less sensitive to microsomal oxidation than $Ar_{ax}SS$ and gave two major monosulfoxides ($Ar_{eq}SSO_{ax}$ and $Ar_{eq}SSO_{eq}$ in a 61:39 ratio), two minor disulfoxides ($Ar_{eq}SO_{ax}SO_{eq}$ and $Ar_{eq}SO_{eq}SO_{eq}$), and no $Ar_{eq}SSO_2$.

$Ar_{eq}SSO_{eq}$ and $Ar_{eq}SSO_{ax}$ were significantly less susceptible to microsomal metabolism than the corresponding 2-axial isomers with recoveries of unmetabolized substrate of 77% from $Ar_{eq}SSO_{eq}$ and 81% from $Ar_{eq}SSO_{ax}$. The major metabolites from $Ar_{eq}SSO_{eq}$ were $Ar_{eq}SO_{ax}SO_{eq}$ and $Ar_{eq}SO_{eq}SO_{eq}$ (63:37), whereas those from $Ar_{eq}SSO_{ax}$ were $Ar_{eq}SO_{ax}SO_{eq}$ and $Ar_{eq}SSO_2$ (60:40); a small amount of $Ar_{eq}SO_{eq}SO_2$ was the only other metabolite observed in each case.

The dioxidized compounds were poorly metabolized with recoveries of unmetabolized substrate of 84% for $Ar_{eq}SSO_2$ and 96% for $Ar_{eq}SO_{eq}SO_{eq}$ and $Ar_{eq}SO_{ax}SO_{eq}$; they were each converted to $Ar_{eq}SO_{eq}SO_2$ with a small amount of $Ar_{eq}SO_2SO_2$ also formed from $Ar_{eq}SSO_2$. In addition to S-oxidation, $Ar_{eq}SO_{ax}SO_{eq}$ underwent NADPH- and enzyme-dependent epimerization. Trioxidized compound $Ar_{eq}SO_{eq}SO_2$ showed only trace oxidation to the disulfone ($Ar_{eq}SO_2SO_2$), whereas, unexpectedly, reduction to $Ar_{eq}SO_{eq}SO_{eq}$ was preferred. $Ar_{eq}SO_2SO_2$ was metabolized to a single product which was substantially more polar and had the dithiane ring intact; this compound is proposed to be a hydroxylation product, possibly at the *tert*-butyl group ($Ar_{eq}SO_2SO_2$–OH) (Cole et al., 1991).

Table V. Comparison of Relative Metabolite Levels in the Liver with Those in the Liver Microsomal Oxidase System for *cis*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithiane and Its Monosulfoxides

	metabolite ratio from indicated starting material					
	$Ar_{ax}SS$[a]		$Ar_{ax}SSO_{eq}$		$Ar_{ax}SSO_{ax}$	
metabolite	liver	microsome[b]	liver	microsome[c]	liver	microsome[c]
$Ar_{ax}SSO_{eq}$	0.8	0.6	*a*			
$Ar_{ax}SSO_{ax}$	1.0	1.0			*a*	
$Ar_{eq}SSO_2$				0.1		
$Ar_{ax}SSO_2$	0.5	0.2	1.0	0.2	0.6	0.1
$Ar_{eq}SO_{eq}SO_{eq}$	0.3	0.2	0.2	0.3		0.1
$Ar_{eq}SO_{ax}SO_{eq}$	0.8	0.8	0.7	1.0	1.0	1.0
$Ar_{ax}SO_{eq}SO_{eq}$[d]	0.3	0.1	0.4	0.2		
$Ar_{eq}SO_{eq}SO_2$		0.2		0.2		0.13

[a] The substrate $Ar_{ax}SS$ is obscured by endogenous extractives. Substrate is the largest HPLC peak with $Ar_{ax}SSO_{eq}$ and $Ar_{ax}SSO_{ax}$. [b] Derived from data in Table II. [c] Derived from data in Table III. [d] Proposed product.

Table VI. Photoproducts from *cis*- and *trans*-2-(4-Bromophenyl)-5-*tert*-butyl-1,3-dithianes Exposed as Thin Films to Sunlight

	photoproduct recoveries,[a] %	
compd	$Ar_{ax}SS$	$Ar_{eq}SS$
$Ar_{ax}SS$	43 (95)[b]	
$Ar_{ax}SSO_{eq}$	7 (1)	
$Ar_{ax}SSO_{ax}$	31 (3)	
$Ar_{ax}SSO_2$	4	
$Ar_{eq}SS$		96 (99)[b]
$Ar_{eq}SSO_{eq}$	5 (0.5)	2 (0.8)
$Ar_{eq}SSO_{ax}$	4 (0.5)	
$Ar_{eq}SSO_2$	3	1 (0.2)
$Ar_{eq}SO_{eq}SO_{eq}$	2	0.5
$Ar_{eq}SO_{ax}SO_{eq}$	1	0.5

[a] Product recoveries refer to dithianes only. The photolysis of $Ar_{ax}SS$ but not $Ar_{eq}SS$ yields a large but unknown amount of an unidentified ring-opened compound. [b] Values in parentheses are dark controls.

Metabolites in the Liver from $Ar_{ax}SS$ and Its Monosulfoxides (Table V). Metabolites in the liver from $Ar_{ax}SS$ (Figure 4), $Ar_{ax}SSO_{eq}$, and $Ar_{ax}SSO_{ax}$ were the same as those from the microsomal system. The number of metabolites decreased in the order $Ar_{ax}SS > Ar_{ax}SSO_{eq} > Ar_{ax}SSO_{ax}$ both in vivo and in vitro. The proportion of monosulfone was much greater in the liver than in the enzyme system under the standard conditions. Although not tabulated, $Ar_{eq}SS$ was barely metabolized in vivo, giving only a small amount of $Ar_{eq}SSO_{ax}$.

Photooxidation (Table VI). $Ar_{eq}SS$ underwent only a small amount of sulfoxidation on exposure as a thin film to sunlight for 4 h. In contrast, $Ar_{ax}SS$ was extensively oxidized with no enhancement by 4-bromobenzaldehyde as a possible photosensitizer; the products obtained were those noted above for the metabolic studies plus epimerized derivatives (i.e., $Ar_{eq}SSO_{eq}$, $Ar_{eq}SSO_{ax}$, and $Ar_{eq}SSO_2$). Photooxidation of $Ar_{ax}SS$ (λ_{max} = 230 nm) yielded another, as yet unidentified, derivative with λ_{max} = 258 nm which was not 4-bromobenzaldehyde or 4-bromobenzoic acid. Some oxidation of $Ar_{ax}SS$ was observed in the absence of sunlight.

The Rose bengal sensitized photooxidation of $Ar_{ax}SS$ and $Ar_{eq}SS$ yielded $Ar_{eq}SSO_{eq}$ plus non-dithiane decomposition products. On photoloysis as hexane solutions exposed to 254-nm light both $Ar_{ax}SS$ and $Ar_{eq}SS$ yielded

4-*tert*-butyl-1,2-dithiolane and 4-bromobenzaldehyde with further decomposition to 4-bromobenzoic acid, benzaldehyde, and benzoic acid (each identified by GC/MS comparison to authentic standards). The dithiolane and 4-bromobenzaldehyde were also formed on acid hydrolysis of the mono- and disulfoxides of $Ar_{ax}SS$ and $Ar_{eq}SS$ in acetonitrile.

DISCUSSION

Figure 2 presents the most extensive study to date on the stereoselectivity of microsomal S-oxidation reactions of a sulfur-containing insecticide. The cis isomers are metabolized more readily than the trans to form up to seven distinct S-oxidation products. Two of the compounds also undergo reactions other than S-oxidation. $Ar_{eq}SO_{eq}SO_2$ is converted to a disulfoxide, which is unusual as metabolic reduction of sulfones is rare (Damani, 1989b). $Ar_{eq}SO_2SO_2$ undergoes hydroxylation tentatively proposed to be at the *tert*-butyl substituent by analogy with the related insecticidal 4-*tert*-butyl-1-(4-chlorophenyl or 4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane (Cole et al., 1991); the alternative hydroxylation at C-2 would yield ring-opened compounds (see below), which is not the case, while hydroxylation of the aromatic ring would be inconsistent with findings on related aryl-containing S-heterocycles (Cashman and Olsen, 1990; Cashman and Williams, 1990; Cashman et al., 1990a; Takata et al., 1980, 1983).

P-450-mediated S-oxidation proceeds by abstraction of an electron from sulfur, yielding a sulfenium cation radical that is subsequently S-oxidized by the electrophilic perferryl species. A sulfenium cation radical adjacent to a carbon with an acidic proton can rearrange with loss of the proton to generate a carbon radical which is subsequently hydroxylated, resulting in S-dealkylation (Guengerich and Macdonald, 1984; Oae et al., 1985; Watanabe et al., 1980, 1981, 1982). Electrochemical studies establish that this reaction sequence converts a dithiane to the parent aldehyde and the 1,2-dithiolane (Platen and Steckhan, 1980). Thus, the absence of ring-opened products indicates that dithiane metabolism does not involve hydroxylation at C-2. P-450-generated hydrogen peroxide (Ortiz de Montellano, 1986) is not believed to contribute to the observed metabolic oxidations as we find that $Ar_{eq}SS$ is not oxidized by hydrogen peroxide in aqueous acetone.

Cytochrome P-450 rather than FMO mediates the metabolism of both the dithianes and their S-oxidation products since their degradation is strongly inhibited by NOA and PB and minimally by TU. While metabolism of $Ar_{eq}SS$ was completely blocked by NOA, a small amount of S-oxidation was observed for $Ar_{ax}SS$ and its monooxidized products in the presence of NOA and PB. The residual S-oxidation probably results from incomplete inhibition of P-450 rather than a contribution by FMO since NOA serves as only a weak inducer of FMO in mice (Sabourin and Hodgson, 1984). In contrast, FMO-rich microsomal preparations and purified FMO mediate the monosulfoxidation of the related 2-aryl-1,3-dithiolanes but not further S-oxidation (Cashman and Olsen, 1990; Cashman and Williams, 1990; Cashman et al., 1990a). These differences may in part reflect the thermal instability of FMOs (Ziegler, 1988), leading to their inactivation under the pH and temperature conditions of the present experiments.

The stereoselectivity of the P-450-mediated dithiane oxidations in vitro and in vivo differs from that for *m*-chloroperoxybenzoic acid (MCPBA) oxidation on the basis of the isomer ratios observed (Table VII) (Wacher et al., 1992). S-Oxidation in the P-450 active site is assumed to occur from one fixed direction such that the *S*-oxide stereochemistry is determined by the orientation of the substrate relative to the perferryl oxidizing species (Cashman and Williams, 1990; Cashman et al., 1990b; Holland and Munoz, 1988; Holland et al., 1985) (Figure 5). Positioning of the substrate in the same plane as the oxidizing agent (mode A) would lead to axial oxygenation, while an "edge on" orientation (mode B) would result in an equatorial S=O bond. Mode A oxidation should be favored for dithianes and compounds with only equatorial S=O bonds, where axial approach of the oxidizing species is unhindered. Alternatively, mode B should be preferred for compounds with an axial S=O bond. For compounds in which one sulfur is oxidized, either the sulfide (mode B-1) or the sulfoxide moiety (mode B-2) can be placed adjacent to the oxidizing agent. Sulfoxides are less readily oxidized than sulfides (Watanabe et al., 1982); hence, mode B-1 should be favored. For the 2-axial compounds, an alternative to mode B orientation is the possibility that the active site distorts the ring into a twist-boat (mode C). This explanation is disfavored since ^1H NMR studies show that the dithianes and their S-oxidation products in organic solvents are conformationally stable at 37 °C and above (Elliott et al., 1992; Wacher et al., 1992). In addition, the active site of the enzyme would be required to exert significant restraint over the substrate, which is inconsistent with previous results (Holland, 1988; Holland et al., 1985; Yang and Lu, 1987). Table VIII summarizes the

Table VII. Comparative Stereochemistries of the Dithiane S-Oxidation Products Formed by Microsomes, Mouse Liver, and Peracid

	stereochemistry of monooxygenation, %					
	microsome		liver		MCPBA[a]	
substrate	ax	eq	ax	eq	ax	eq
$Ar_{eq}SS$	61	39	100	0	0	100
$Ar_{ax}SS$	62	38	56	44	67	33
$Ar_{eq}SSO_{eq}$	63	37			22	78

[a] Wacher et al. (1992).

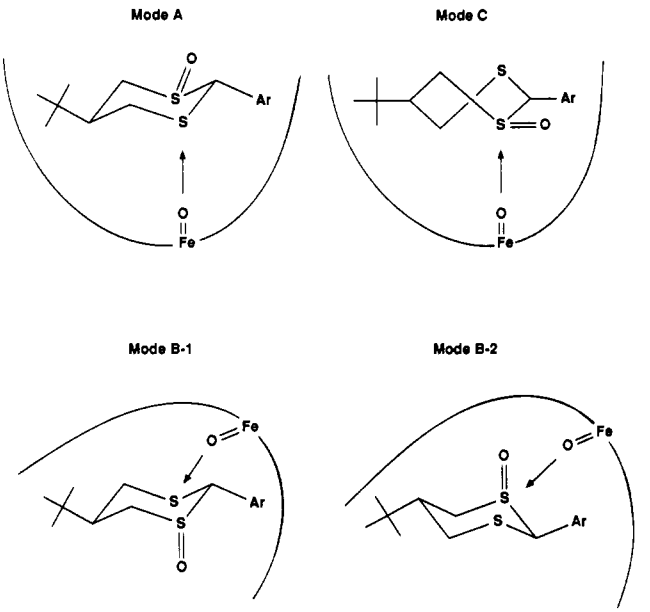

Figure 5. Models for cytochrome P-450-mediated S-oxidation of *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane 1-oxide by the perferryl species in the active site. Modes B-1 and B-2 may also represent oxidation of different enantiomers that are positioned with the ring in the same orientation rather than rotated as shown.

Table VIII. Preferred Modes of Dithiane S-Oxidation by Cytochrome P-450 Resulting in the Observed Stereoselectivities

	mode of S-oxidation to indicated product						
substrate[a]	SSO_{eq}	SSO_{ax}	SSO_2	$SO_{eq}SO_{eq}$	$SO_{ax}SO_{eq}$	$SO_{eq}SO_2$	SO_2SO_2
SS[b]	B-1	A					
SSO_{eq}[b]			A	B-1[c]	A[d]		
SSO_{ax}			B-2		B-1[d]		
SSO_2						B-1[e]	
$SO_{eq}SO_{eq}$						A	
$SO_{ax}SO_{eq}$						B-2	
$SO_{eq}SO_2$							A

[a] Partial structural abbreviations since mode of oxidation is independent of aryl group orientation. [b] $Ar_{eq}SS$, $Ar_{ax}SS$, and $Ar_{eq}SSO_{eq}$ show the same ratio of axial vs equatorial (mode A vs mode B) monosulfoxidation in microsomes (Table VII). [c] $Ar_{ax}SSO_{eq}$ is first oxidized to $Ar_{ax}SO_{eq}SO_{eq}$ and subsequently epimerizes to $Ar_{eq}SO_{eq}SO_{eq}$. [d] $Ar_{ax}SSO_{eq}$ and $Ar_{ax}SSO_{ax}$ are almost certainly converted initially to $Ar_{ax}SO_{ax}SO_{eq}$ with subsequent epimerization to $Ar_{eq}SO_{ax}SO_{eq}$. [e] $Ar_{ax}SSO_2$ oxidation likely proceeds via formation of $Ar_{ax}SO_{eq}SO_2$ followed by nonenzymatic epimerization to $Ar_{eq}SO_{eq}SO_2$.

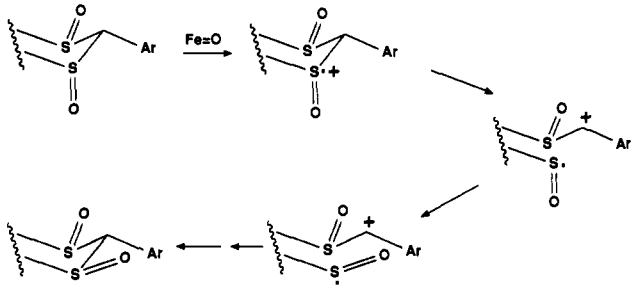

Figure 6. Proposed mechanism of cytochrome P-450-catalyzed isomerization of *trans*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane *cis*-1, *trans*-3-dioxide. A similar mechanism is applicable to *cis*-2-(4-bromophenyl)-5-*tert*-butyl-1,3-dithiane *cis*-1- or *trans*-1-oxide.

suggested preferred modes of oxidation leading to the observed stereoselectivities.

Enzyme-catalyzed epimerization is an unexpected reaction of $Ar_{eq}SO_{ax}SO_{eq}$ to form $Ar_{eq}SO_{eq}SO_{eq}$, possibly involving ring opening of the sulfenium cation radical intermediate in the active site (Platen and Steckhan, 1980) to give a cation at C-2 and a sulfur radical as shown in Figure 6. The rearrangement and ring closure must be very rapid since S-dealkylation products were not observed. A similar ring opening may also contribute to the metabolism of $Ar_{ax}SSO_{eq}$ and $Ar_{ax}SSO_{ax}$ to the 2-equatorial disulfoxides and may explain the presence of $Ar_{eq}SO_{eq}SO_{eq}$ without formation and epimerization of $Ar_{ax}SO_{eq}SO_{eq}$.

The relevance of the enzyme studies to the organismal level is established by the similar metabolite profiles in the microsomal system and the liver of treated mice. These metabolic S-oxidation reactions serve as both activation and detoxification mechanisms. A large number of metabolites have insecticidal activity, mammalian toxicity, and high potency at the GABA-gated chloride channel, so toxicological evaluation of any individual compound in the metabolic pathway is influenced by its further metabolites. Qualitative listings of mouse and housefly toxicities are as follows (Wacher et al., 1992): synergized housefly toxicity $Ar_{eq}SO_{eq}SO_{eq} < Ar_{eq}SO_{ax}SO_2 < Ar_{eq}SO_{eq}SO_2 < Ar_{eq}SS = Ar_{ax}SS < Ar_{eq}SSO_{eq} = Ar_{eq}SO_{ax}SO_{eq} = Ar_{ax}SSO_{ax} < Ar_{ax}SO_{ax}SO_{eq} < Ar_{ax}SSO_{eq} = Ar_{eq}SSO_{ax} = Ar_{ax}SSO_2 < Ar_{eq}SSO_2 = Ar_{eq}SO_2SO_2$; mouse toxicity (ip) $Ar_{eq}SS = Ar_{eq}SSO_{ax} < Ar_{ax}SS < Ar_{ax}SSO_{eq} = Ar_{eq}SSO_2 < Ar_{eq}SSO_{eq} = Ar_{ax}SSO_{ax} < Ar_{ax}SSO_2$.

Synergistic ratios comparing toxicity to houseflies in the presence and absence of PB provide a measure of the degree of oxidative detoxification (Wacher et al., 1992). Comparison of the metabolite profiles with mouse microsomes to the synergistic ratios suggests clear differences between the mouse and the housefly, with the most striking example provided by $Ar_{eq}SO_2SO_2$. In mouse liver microsomes the disulfone is largely unmetabolized; however, the synergistic ratio for its toxicity to houseflies is >2500, indicating extensive oxidative detoxification in the insect. A second major difference is that $Ar_{eq}SS$ and its S-oxidation products undergo less metabolism than $Ar_{ax}SS$ and its S-oxidation products in mouse liver microsomes, yet the 2-equatorial compounds have higher synergistic ratios in the fly than the 2-axial isomers (indicative of greater oxidative metabolism). In comparisons of the metabolite profiles in vitro and in vivo in mouse liver, there was more sulfone found in vivo than with the microsomal system. Differing pathways and degrees of metabolism provide a potential means to achieve selectively toxic dithianes.

Dithianes undergo photochemical as well as metabolic S-oxidation to yield essentially the same products but in a different ratio. Toxicological and environmental studies on the dithianes must therefore take their bioactive *S*-oxides into consideration.

ABBREVIATIONS USED

Ax, axial; DETPAC, diethylenetriaminepentaacetic acid; eq, equatorial; FMO, flavin-containing monooxygenase; GABA, γ-aminobutyric acid; GC, gas chromatography; HPLC, high-pressure liquid chromatography; ip, intraperitoneal; MCPBA, *m*-chloroperoxybenzoic acid; MS, mass spectrometry; NOA, *n*-octylamine; PB, piperonyl butoxide; P-450, cytochrome P-450; RP, reversed-phase; Rt, retention time; SD, standard deviation; THF, tetrahydrofuran; TU, thiourea.

ACKNOWLEDGMENT

V.W. was supported in part by the Gowrie Scholarship Trust Fund (Australia) and a University of California Regents Fellowship.

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Received for review August 6, 1992. Accepted November 20, 1992.