by column chromatography. Elution with EtOAc/hexane/Et_3N (20/78/2 v/v/v; to elute dimethoxytritanol), followed by EtOAc/hexane/Et_3N (50/48/2 v/v/v), afforded the DMT derivative of compound **9**: 0.9 g (65%); R_f (TLC hexane/EtOAc/Et_3N 80/19/1 v/v/v) 0.24, positive DMT+ test; ^1H NMR ($CDCl_3$) ppm 0.02 (s, Me_2Si), 0.87 (s, *t*-BuSi), 1.30-1.62 (m, $2(CH_2)_4$), 3.04 (t, CH_2OC), 3.40 (m, $2CH_2N$), 3.56 (t, CH_2OSi), 3.74 (s, 2MeO), 6.12 (m, 2NH), 6.8 (d, 4 H [J_{HH} 8.8 Hz], H3 and H5 of anisyl), 7.2-7.5 (m, rest of DMT), 7.77 (s, terephthalyl).

***N*-(6-((Dimethoxytrityl)oxy)hexyl]-*N'*-(6-hydroxyhexyl)terephthalamide (10).** A portion of compound **9** (0.61 g, 0.78 mmol) was stirred with Bu_4NF (1 mmol) in dry THF (25 mL) for 3 h, and then the mixture was concentrated under reduced pressure and chromatographed to give 0.52 g (98%) of compound **10**: mp 89-90 °C; R_f (TLC, EtOAc/Et_3N 98/2 v/v) 0.25; FAB MS, M + 1 667, M calcd 666; ^1H NMR ($CDCl_3$) ppm 1.30-1.62 (m, $2(CH_2)_4$), 3.04 (t, CH_2OC), 3.46 (m, $2CH_2N$), 3.63 (m, CH_2-hydroxyl) 3.78 (s, $2CH_3O$), 6.19 (t, HN), 6.30 (t, HN), 6.8 (d, 4 H [J_{HH} 8.8 Hz], H3 and H5 of 2 anisyls), 7.2-7.5 (m, rest of DMT), 7.77 (s, terephthalyl). Anal. Calcd for $C_{41}H_{50}O_6N_2 \cdot 0.5H_2O$: C, 72.89; H, 7.56; N, 4.15. Found: C, 72.65; H, 7.78; N, 4.11.

Hydrogen Phosphonate Derivative of Compound 10 (11). To imidazole (0.73 g, 11 mmol) in MeCN (20 mL) at ice temperature was successively added with stirring PCl_3 (0.28 mL, 3.2 mmol), Et_3N (1.6 mL, 11 mmol), and compound **10** (0.50 g, 0.75 mmol in 5 mL of MeCN). After removing the ice bath, stirring for 4 h, addition of water (5 mL), and stirring for 30 min, the mixture was evaporated under reduced pressure, coevaporated with pyridine/Et_3N, and partitioned between $CHCl_3$ and water. Purification of the recovered solid by chromatography ($CHCl_3$/MeOH/Et_3N, 87/10/3, v/v/v) gave 0.535 g (84%) of the desired triethylammonium hydrogen phosphonate salt (compound **11**): R_f (TLC, $CHCl_3$/MeOH/Et_3N 87/10/3 v/v/v). The ^{31}P NMR spectrum showed two peaks (+4.95 and -0.12 ppm) at 121 MHz for (P resonance split by H).

Cyanoethyl *N,N*-Diisopropylphosphoramidite Derivative of Compound 10 (12). Chloro-β-cyanoethoxy-*N,N*-diisopropylaminophosphine (0.23 mL, 1 mmol) was slowly added to a solution of compound **10** (500 mg, 0.75 mmol), diisopropylamine (0.28 mL, 2 mmol), and 4-(dimethylamino)pyridine (10 mg) in tetrahydrofuran (10 mL). After 4 h (as indicated by TLC, the reaction was virtually complete) the mixture was poured into ethyl acetate (100 mL, prewashed with brine) and washed successively with dilute sodium bicarbonate and brine. The ethyl acetate layer was dried (sodium sulfate) and concentrated, and the product was isolated by chromatography on a silica gel column with a solvent of ethyl acetate/hexane/triethylamine 47/50/3 v/v/v: yield 390 mg (60%); R_f (Merck silica plates) 0.5 in the solvent used for the preparative separation; ^1H NMR ($CDCl_3$) ppm 1.12-1.29 (m, 6Me of 2*i*Pr), 1.35-1.64 (m, $8CH_2$), 2.64 (m, 2 CH of 2*i*Pr), 3.40 (m, $CH_2OPOCH_2CH_2CN$), 3.45 (m, $2CH_2N$), 3.40 (t, CH_2O-DMT), 3.79 (s, $2CH_3O$), 6.15-6.27 (m, 2 NH), 6.80-7.44 (m, 13 aromatic H of DMT), 7.80 (s, terephthalyl); ^{31}P NMR 145.4 ppm (85% H_3PO_4 as external reference).

Synthetic Oligonucleotides. Oligonucleotides were synthesized on long chain alkylamine controlled pore glass supports (80-100 mesh, 500 A, from Glen Research) using nucleoside cyanoethyl phosphoramidite reagents (Milligen/Biosearch) for addition of nucleotide units and hydrogen phosphonate reagent **11** (preparation of compounds **2-4** and **6**) or phosphoramidite reagent **12** (preparation of compounds **1** and **5**) for addition of the linker. Conventional protocols for syntheses with phosphoramidite reagents[10] and hydrogen phosphonate reagents[11] were followed, starting with 1 μmol of loaded nucleoside. A Cyclone DNA synthesizer was employed for the amidite couplings. For the hydrogen phosphonate coupling with compound **11**, the support was removed from the DNA synthesizer and coupling carried out manually by the syringe technique. The yields in the coupling steps, as indicated by the DMT+ test, averaged >97% for the cycles with phosphoramidite reagents and 90% for condensations with the hydrogen phosphonate linker reagent. The oligonucleotides, worked up in a conventional manner and isolated by reversed phase HPLC, were better than 95% pure by analysis by HPLC and polyacrylamide gel electrophoresis. That the terephthalamide linkage is stable to ammonium hydroxide under the synthesis conditions was demonstrated by treating compounds **1** and **10** with concentrated ammonium hydroxide at 55 °C for 15 h. Analysis by TLC and HPLC showed that the compounds were unchanged (<5% degradation).

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Stereoselectivity of Enzymatic and Chemical Oxygenation of Sulfur Atoms in 2-Methyl-1,3-benzodithiole

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Abstract: S-Monooxygenation of 2-methyl-1,3-benzodithiole, to yield *cis*- and *trans*-2-methyl-1,3-benzodithiole 1-oxide, has been studied as a new stereochemical probe using achiral and chiral chemical oxidants and enzymes from microbial and animal sources. Chemical oxidants showed a preference for trans *S*-oxide formation, and this preference was enhanced in the presence of a chiral matrix. The ability of pure enzymes to stereodifferentiate between geminal lone pairs on a prochiral sulfur atom, or geminal sulfur atoms on a prochiral carbon atom, has been observed. Thus, intact fungal and bacterial oxidations showed a marked selectivity (87-96%) for formation of the cis *S*-oxide isomers. In addition, highly purified hog liver and rabbit lung flavin-containing monooxygenases showed a marked preference for formation of the cis *S*-oxide and the *pro-R* sulfur atom. Other monooxygenase-catalyzed sulfoxidations of 2-methyl-1,3-benzodithiole, including cytochrome P-450 2B1 ($P450_{PB-B}$), showed a markedly lower stereoselectivity.

Introduction

Sulfoxidation is a very common biotransformation process which has been widely observed in xenobiotic metabolism.[1] The ability of monooxygenase enzymes to stereodifferentiate between prochiral lone pairs on a sulfur atom has previously been examined using fungal, bacterial, and animal enzymes.[1,2] The two sulfur atoms present in the thioacetal group provide a more rigorous challenge to the monooxygenase enzymes in stereoselecting between each geminal lone pair on a prochiral sulfur atom and between geminal sulfur atoms attached to a prochiral carbon atom.

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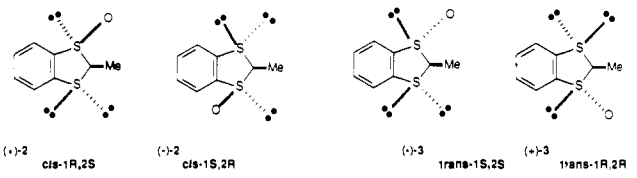
Previous reports from these laboratories have examined the ability of intact fungal cells to sulfoxidize a series of 2-substituted 1,3-dithianes[3] and 2-substituted 1,3-dithiolanes.[4] Thus, the fungi *Aspergillus foetidus*, *Mortierella isabellina*, and a *Helminthosporium* species were found to yield the corresponding monosulfoxides.[3,4] The enantiomeric excess values of the latter sulfoxides were relatively modest in both the 1,3-dithiane (33–72%) and the 1,3-dithiolane (32–54%) series. Unfortunately the latter types of sulfoxides are not of the alkyl–aryl category whose enantiomers are more readily separable by chiral stationary phase (CSP) HPLC. Furthermore, the sulfoxide metabolites of the 1,3-dithiane and 1,3-dithiolane substrates[3,4] are difficult to observe using a UV detector in CSPHPLC analysis due to the absence of a strong chromophore. Because pure enzyme-catalyzed oxidations from these laboratories are generally conducted on a small scale, the quantities of sulfoxide metabolites available are expected to be very limited. An alternative type of thioacetal substrate which would be more suitable for UV detection, i.e., 2-methyl-1,3-benzodithiole, has now been examined using a range of chemical, prokaryotic, and enzyme-catalyzed oxidations. A primary objective of this study was to show the value of this new stereochemical probe for chiral sulfoxidation over previously available substrates.

The enzymatic oxidation of sulfur-containing compounds by bacteria, fungi, and mammalian tissues is largely dependent upon two types of monooxygenases: the cytochrome P-450 and the flavin-containing monooxygenases. Flavin-containing- and cytochrome P-450-monooxygenase-catalyzed S-oxygenations can be distinguished by the use of various inhibitors and pH conditions and by the greater susceptibility of hepatic flavin-containing monooxygenases to thermal inactivation.[5] These two monooxygenase types may also be distinguished by the stereoselective nature of their enzyme action because it has been reported that cytochrome P-450 and flavin-containing monooxygenases form *S*-oxides with opposite absolute stereochemistry.[6–8] The S-oxygenase activities of cytochrome P-450 2B1 ($P\text{-}450_{PB\text{-}B}$) and flavin-containing monooxygenases were examined to investigate the similarity and/or dissimilarity in stereoselectivity of S-oxygenation of 2-methyl-1,3-benzodithiole.

As a prelude to this study, the relative and absolute configurations of *cis*- and *trans*-2-alkyl-1,3-benzodithiole 1-oxides (**2** and **3**) were determined. Thus, using a combination of chromatographic (CSPHPLC), spectral (^{1}H-NMR), chiroptical (circular dichroism), X-ray crystallographic, and stereochemical correlation methods, the absolute configurations shown have recently been established.[9]



The sulfoxides $\mathbf{2}_{cis\text{-}1R,2S}$, $\mathbf{2}_{cis\text{-}1S,2R}$, $\mathbf{3}_{trans\text{-}1S,2S}$, and $\mathbf{3}_{trans\text{-}1R,2R}$ were separated using CSPHPLC and thus allowed a direct stereochemical analysis.

Experimental Procedures

^{1}H-NMR spectra were recorded using a Bruker WH 250 instrument. Ultraviolet spectra were recorded on a Perkin-Elmer 559A spectrometer. Optical rotations were determined using a Perkin-Elmer 241 automatic polarimeter. HPLC analyses were carried out using a Perkin-Elmer 3B liquid chromatograph coupled to a Hewlett Packard 3380S integrator.

Enantiomeric excess values were determined using (i) a Pirkle Type analytical column (250 × 4.6 mm) containing a *N*-(3,5-dinitrobenzoyl) derivative of D-phenylglycine covalently bonded to a Spherisorb SSNH column (Analchem) and (ii) a Chiralcel OD column (10 μm, 250 × 4.6 mm) containing cellulose carbamate coated on silica gel (Daicel Chemical Industries Ltd.). Preliminary studies on sulfoxides **2** and **3** were carried out using a Chiralcel OB column, but this column was found to be unstable and deteriorated rapidly, despite using only recommended solvents.

1,2-Benzenedithiol, acetaldehyde, sodium periodate, *n*-octylamine, (*R*,*R*)-diethyl tartrate, *tert*-butyl hydroperoxide, titanium tetraisopropoxide, and the buffers were purchased from the Aldrich Chemical Company. $NADP^+$, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, phosphatidylcholine, and bovine serum albumin were purchased from the Sigma Chemical Company. Aminobenzotriazole was a generous gift from Professor Paul Ortiz de Montellano (University of California, San Francisco). 2-Methyl-1,3-benzodithiole was synthesized in 97% yield from benzenedithiol and acetaldehyde.[10]

Liver Preparations. Microsome fractions were isolated by the method previously described.[11] Hog liver microsomes were a generous gift of Professor Dan Ziegler, University of Texas, Austin. Cytochrome P-450 2B1 ($P\text{-}450_{PB\text{-}B}$) and the enzymes and cofactors required were generously provided by Dr. L. Bornheim, (University of California, San Francisco). Rabbit lung microsomes and highly purified rabbit lung FMO from pregnant female rabbits were kindly provided by Professor David Williams (Oregon State University). Purification of the flavin-containing monooxygenase was accomplished by the method of Sabourin et al.[12] The concentration of protein was determined by the method of Lowry et al.[13] or as described below. The microsomal protein and purified flavin-containing monooxygenase used in this study have previously been shown to N-oxygenate dimethylaniline[14] and S-oxygenate other sulfides.[15]

Stereoselective Synthesis of Thioacetal *S*-Oxides. (A) Chemical Oxidation Using Sodium Periodate. Sodium metaperiodate (2.8 g, 13 mmol) in water (60 mL) was added dropwise with stirring to a cooled (0 °C) solution of 2-methyl-1,3-benzodithiole (1.71 g, 10 mmol) in methanol (150 mL), and the mixture was stirred overnight at room temperature. The sodium iodate was filtered off and washed with chloroform. The combined filtrate was concentrated and extracted with chloroform. After drying ($MgSO_4$), the chloroform solvent was removed in vacuo to give a crude mixture of *cis*-2-methyl-1,3-benzodithiole 1-oxide (**2**, 33%) and *trans*-2-methyl-1,3-benzodithiole 1-oxide (**3**, 67%), whose composition was determined by ^{1}H-NMR analysis (Table I, entry A). The cis and trans *S*-oxide diastereomers were separated by semipreparative HPLC (Zorbax SIL, 9.4 × 250 mm) using an eluant of 0.2% propan-2-ol in dichloromethane at a flow rate of 7 mL min^{-1} (α 1.21) in a total isolated yield of 75%.

(a) *cis*-2-Methyl-1,3-benzodithiole 1-Oxide (2). Sulfoxide **2** was obtained as the early eluting isomer: mp 89–91 °C (aqueous MeOH) (lit.[9] mp 88–90 °C); ^{1}H-NMR δ_H (250 MHz) 1.84 (3 H, d, *J* 7.0 Hz, Me), 4.45 (1 H, q, *J* 7.0 Hz, 2-H), 7.28–7.34 (1 H, m, ArH), 7.42–7.49 (2 H, m, ArH), 7.85 (1 H, d, *J* 7.7 Hz, ArH).

(b) *trans*-2-Methyl-1,3-benzodithiole 1-Oxide (3). Sulfoxide **3** was obtained as the late eluting isomer: mp 130–133 °C (aqueous MeOH) (lit.[9] mp 132–134 °C) ^{1}H NMR δ_H (250 MHz) 1.80 (3 H, d, *J* 7.3 Hz, Me), 4.59 (1 H, q, *J* 7.3 Hz, 2-H), 7.28–7.34 (1 H, m, ArH), 7.42–7.49 (2 H, m, ArH).

(B) Chemical Oxidation with Sodium Metaperiodate in the Presence of Bovine Serum Albumin. Using conditions previously reported for the chiral oxidations of other sulfides,[16] bovine serum albumin (3.3 g) and aqueous borate buffer solution (pH 9.2, 12.5 mL) were added to a solution of 2-methyl-1,3-benzodithiole (**1**, 0.17 g, 1 mmol) in acetone (0.5 mL). Stirring was continued for 2 h prior to the addition of sodium metaperiodate (0.43 g, 2 mmol) and then for 3 days at ambient temperature. The reaction mixture was extracted (diethyl ether); the extract was dried ($MgSO_4$), concentrated in vacuo, and purified by preparative TLC (silica gel, eluted with diethyl ether) to give a mixture of *cis*- and

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Table I. S-Oxidation of 2-Methyl-1,3-benzodithiole by Chemical and Enzyme-Catalyzed Methods

	relative percent yield of total sulfoxides formed						
	cis sulfoxide **2**			trans sulfoxide **3**			
oxidant	(−)-1*S*,2*R*	(+)-1*R*,2*S*	% ee	(+)-1*R*,2*R*	(−)-1*S*,2*S*	% ee	% *pro-R*
A. $NaIO_4$	16	16	0	34	34	0	50
B. $NaIO_4$/BSA	11	17	21	61	11	69	72
C. tBuOOH/DET/$Ti(Pro)_4$	10	4	43	9	77	76	19
D. *A. foetidus*	84	3	93	6	7	8	90
E. *P. putida*	96	0	100	0	4	100	92
F. hog liver microsomes[a]	73 (3.0)	12 (0.5)	72	5 (0.3)	9 (0.4)	29	78
G. hog liver − NOA[b]	28 (1.2)	8 (0.3)	56	35 (2.0)	28 (1.3)	11	63
H. rat liver microsomes	43 (0.9)	10 (0.2)	62	28 (0.6)	19 (0.4)	19	71
I. rat liver microsomes − NOA[b]	27 (0.4)	7 (0.1)	59	33 (0.5)	33 (0.5)	0	60
J. hog liver FMO	80 (170.3)	6 (13.0)	86	4 (8.3)	10 (22.5)	43	84
K. hog liver FMO − NOA[b]	81 (164.0)	5 (9.9)	88	4 (8.1)	10 (24.1)	43	85
L. rat liver FMO	32 (73.6)	4 (9.2)	78	39 (89.7)	25 (57.5)	22	71
M. rat liver FMO − NOA[b]	30 (60.0)	2 (4.0)	87	39 (78.0)	29 (58.0)	15	69
N. rabbit lung FMO	100 (33.2)	0[c]	100	0[c]	0[c]	0	100
O. rat liver $P\text{-}450_{PB\text{-}B}$[d,e]	26 (0.3)	14 (0.2)	30	20 (0.2)	40 (0.5)	33	46
P. rat liver $P\text{-}450_{PB\text{-}B}$ − cytochrome b_5[d,e]	24 (0.3)	15 (0.2)	23	20 (0.2)	41 (0.5)	34	44

[a] Enzymatic incubations were carried out in the presence of a 0.5 mM NADPH-generating system, 400 μM substrate, phosphate buffer (pH 8.4), and enzyme (1.6 mg of hog liver microsomes, 2.0 mg of rat liver microsomes, 0.46 mg of rabbit lung microsomes, 0.05 mg of hog liver flavin-containing monooxygenase, 68 μg of rabbit lung flavin-containing monooxygenase, and 0.15 mg of rat liver flavin-containing monooxygenase. Each value is the average of two to four determinations incubated for 5 min, 33 °C with constant shaking. Product (when measured, in parenthesis) expressed as nmol/min/mg of protein. *n*-Octylamine (NOA) was present at a concentration of 4.5 mM. [b] −NOA, incubation carried out in the absence of *n*-octylamine. [c] Not detected, the limit of detection was 10 pmol/min/mg of protein. [d] Incubation carried out in the presence of 0.1 nmol of cytochrome P-450, 600 IU of cytochrome P-450 reductase, 50 μg of phosphatidylcholine, 0.1 nmol of cytochrome b_5, 100 μM substrate, and 1.0 M NADPH in 0.1 M phosphate buffer, pH 7.4. [e] Product expressed as nmol *S*-oxide/min/nmol cytochrome P-450.

trans-2-methyl-1,3-benzodithiole *S*-oxides (**2** and **3**) in a total isolated yield of 37%.

The ratio of sulfoxides **2** and **3** (28:72) was determined by ^{1}H-NMR analysis. Addition of (+)-(*S*)-9-anthryl-2,2,2-trifluoroethanol as a chiral auxiliary to the NMR sample in a $CDCl_3$ solution allowed the enantiomeric excess (ee) to be determined. Both the cis–trans *S*-oxide separation and ee determinations were confirmed by CSPHPLC analysis using (i) a covalent Pirkle column (250 × 4.8 mm, 2 mL min^{-1} flow rate, 10% propan-2-ol in hexane as eluant), which gave an elution sequence (min) of sulfoxides, $\mathbf{2}_{cis\text{-}1S,2R}$ (23.88), $\mathbf{2}_{cis\text{-}1R,2S}$ (24.29), $\mathbf{3}_{trans\text{-}1R,2R}$ (28.16), $\mathbf{3}_{trans\text{-}1S,2S}$ (29.38), and (ii) a Chiralcel OD column (250 × 4.8 mm, 2 mL min^{-1} flow rate, 10% propan-2-ol in hexane as eluant), which gave an elution sequence (min) of sulfoxides, $\mathbf{2}_{cis\text{-}1R,2S}$ (11.77), $\mathbf{2}_{cis\text{-}1S,2R}$ (15.01), $\mathbf{3}_{trans\text{-}1R,2R}$ (19.49), $\mathbf{3}_{trans\text{-}1S,2S}$ (21.99).

(C) Chemical Oxidation with *t*-BuOOH in the Presence of Titanium Isopropoxide and (*R*,*R*)-Diethyl Tartrate. The Kagan method,[17] previously used in the chiral oxidation of other sulfides, was applied to the sulfoxidation of 1-methyl-1,3-benzodithiole (**1**). Thus, titanium tetraisopropoxide (0.3 mL, 1 mmol) and (*R*,*R*)-diethyl tartrate (0.34 g, 2 mmol) were dissolved in dry dichloromethane (50 mL) at ambient temperature under nitrogen. Water (0.02 mL, 1 mmol) was added, and stirring was continued until the solution became homogeneous (ca. 30 min). 2-Methyl-1,3-benzodithiole (**1**, 0.17 g, 1 mmol) was added, the solution was cooled to −20 °C, and *t*-BuOOH (1.2 mmol) was added. The reaction mixture was maintained at −20 °C for 2 days. Water (0.2 mL) was added dropwise, and the reaction mixture was stirred for 1 h at −20 °C and then for a further 1 h at room temperature. The mixture was filtered, and the filtrate was stirred with sodium hydroxide (5% aqueous solution); the dichloromethane solution was then separated, dried ($MgSO_4$), and concentrated to give a mixture of cis and trans *S*-oxides of 2-methyl-1,3-benzodithiole (**2** and **3**). The sulfoxides **2** and **3** were purified by preparative TLC to give a total yield of 81%. The absolute configurations and ee values for sulfoxides **2** and **3** were determined by methods reported in part B and are listed in Table I.

(D) Enzyme-Catalyzed Oxidation Using *Aspergillus foetidus* NRRL337. Enzyme-catalyzed oxidation of 2-methyl-1,3-benzodithiole (**1**) was carried out using intact cultures of *A. foetidus* NRRL337 and methods similar to those previously reported for S-monooxygenations of 1,3-dithianes[3] and 1,3-dithiolanes.[4] In a typical experiment, 50 Erlenmeyer flasks (1000 mL) containing Czapek Dox liquid medium (100 mL) were innoculated with a suspension of *A. foetidus* and incubated for 48 h at 30 °C using a platform shaker (180 rev/min). Each flask was then injected with a 1-mL solution of 2-methyl-1,3-benzodithiole (**1**, 0.03 g in 1 mL of EtOH). After shaking at 30 °C for a further 48 h, the mycelium was separated by filtration and washed with dichloromethane. The aqueous culture medium was saturated with NaCl and continuously extracted with dichloromethane for 7 days. The dichloromethane extract and washings were combined, dried ($MgSO_4$), and concentrated in vacuo to give a crude product mixture. Purification by preparative TLC on silica gel gave a mixture of sulfoxides **2** and **3** in a total yield of 37%. The relative yield, absolute configuration, and ee were determined by the ^{1}H-NMR and HPLC methods reported in part B (Table I).

(E) Enzyme-Catalyzed Oxidation Using *Pseudomonas putida* UV4. Enzyme-catalyzed sulfoxidation of 2-methyl-1,3-benzodithiole (**1**) was carried out in a manner similar to that reported[18] for the oxidation of alkane (benzylic hydroxylation) and alkene (*cis*-diol formation) substrates. Thus, 2-methyl-1,3-benzodithiole (**1**, 200 mg) was added to a culture of *P. putida* UV4 previously grown on a mineral salts medium containing gluconate (12% w/v), and the mixture was resuspended in the same medium using pyruvate rather than gluconate. The biotransformation was carried out in Erlenmeyer flasks (1000 mL) containing culture medium (60 mL) using an orbital shaker (400 rev/min) at a temperature of 30 °C over a period of 25 h. The culture medium was then centrifuged, saturated with sodium chloride, and extracted with diethyl ether. The extract was dried ($MgSO_4$) and concentrated to yield the crude product, which was in turn purified (24% yield) by preparative TLC. The relative yield of sulfoxide isomers, absolute configurations, and ee values were determined by the ^{1}H-NMR and HPLC methods described in part B (entries A–C, Table I).

(F–P) Enzyme-Catalyzed Oxidations Using Mammalian Enzyme Preparations. The incubation contained potassium phosphate buffer (50 mM, pH 8.4), 0.5 mM $NADP^+$, 2.0 mM glucose 6-phosphate, 1 IU of glucose-6-phosphate dehydrogenase, and 0.5–4.0 mg/mL of microsomes or 45–200 μg of purified flavin-containing monooxygenase. Heat-inactivated microsomes were prepared following the method previously described.[14,19] Incubations were performed by the addition of enzyme to a cold preequilibrated solution containing the NADPH-generating system. Reactions were initiated by addition of substrate. At the time intervals indicated, the reactions were stopped by the addition of 4 volumes of cold dichloromethane and the resulting extract was separated by a brief centrifugation and filtration through a nylon filter. The extracts were evaporated to dryness, taken up in acetonitrile, and analyzed by HPLC (IBM Model 9000) with a UV detector set at 253 nm, fitted with a precolumn and a 5-μm Beckman Ultrasphere ODS column (2.0 × 250 mm). The mobile phase consisted of solvents 90% A and 10% B. Solvent A contained water/acetonitrile (2:1, v/v), while solvent B was acetonitrile. This HPLC system efficiently separated thioacetal **1**, cis thioacetal *S*-oxide **2**, and trans thioacetal *S*-oxide **3**, which gave retention volumes of 9.7, 1.3, and 1.1 mL, respectively. Metabolites were quan-

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tified by comparison of sulfoxide and substrate peak areas after adjusting for differences in extinction coefficients. The recovery of metabolites, as judged by HPLC, was 92% for thioacetal **1** and 97% for the thioacetal *S*-oxides **2** and **3**.

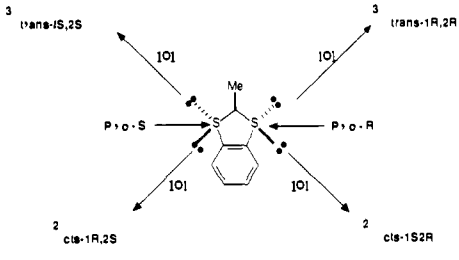
Activity of rat liver cytochrome P-450 2B1 ($P\text{-}450_{PB\text{-}B}$) was measured by determining *S*-oxide product formation as described above. Cytochrome P-450 2B1 was reconstituted as follows: 50 μg of dilauroylphosphatidylcholine was combined with 600 units of rat cytochrome P-450 reductase and 100 pmol of P-450 2B1. After a 10-min equilibration at 4 °C, the mixture was diluted with potassium phosphate buffer (50 μM, pH 7.4); substrate was added (100 μM final concentration in 10 μL of methanol), and then 100 pmol of rat liver cytochrome b_5 was added. The reaction was immediately initiated by the addition of a 0.5 mM NADPH-generating system in a final volume of 1 mL. After a 10-min incubation at 37 °C with constant shaking in air, the reaction was stopped by the addition of 3 volumes of cold CH_2Cl_2 and the reaction mixture was prepared for HPLC as described above. Rat liver cytochrome P-450 2B1 exhibited characteristically high pentoxyresorufin O-dealkylase and 16 β-testosterone hydroxylase activities [6 and 3 nmol of product/(min·nmol of P-450), respectively] shown to be characteristic of the phenobarbital-induced isozymes and was judged to be homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

The concentration of protein was determined by the method of Bradford[20] or by the BCA method (Pierce, Rockford, IL).

Results and Discussion

While numerous reports have appeared on the ability of chemicals and enzymatic oxidants to stereodifferentiate between geminal lone pairs on a prochiral sulfur atom, few studies have been carried out using a substrate which allows *both* geminal lone pairs on a sulfur atom and geminal sulfur atoms on a prochiral carbon atom. 2-Methyl-1,3-benzodithiole (**1**) provides a new stereochemical probe for such studies. Because a baseline separation of monosulfoxide products can be obtained by chiral stationary phase HPLC analysis utilizing UV detection methods, comparative studies of the stereoselectivity of a variety of chemical, microbial, and enzyme systems using this new stereochemical probe were undertaken.

Chemical Oxidations. The oxidation of 2-methyl-1,3-benzodithiol (**1**) using sodium metaperiodate in aqueous solution gave both cis ($\mathbf{2}_{cis\text{-}1R,2S}$ and $\mathbf{2}_{cis\text{-}1S,2R}$, 32%) and trans ($\mathbf{3}_{trans\text{-}1S,2S}$ and $\mathbf{3}_{trans\text{-}1R,2R}$, 68%) isomers in racemic form (Table I, entry A). When this oxidation was repeated in the presence of bovine serum albumin, a preference for the trans (72%) over the cis (28%) isomer was again observed. A further preference for the $\mathbf{3}_{trans\text{-}1R,2R}$ enantiomer (69% ee) and the $\mathbf{2}_{cis\text{-}1R,2S}$ enantiomer (21% ee) was observed, probably due to hydrophobic binding between the thioacetal substrate and the chiral environment of the protein. Preferential oxidation of the *pro-R* sulfur atom in thioacetal **1**



was reflected in the combined proportion of the sulfoxides $\mathbf{2}_{cis\text{-}1S,2R}$ and $\mathbf{3}_{trans\text{-}1R,2R}$ (72%). Conversely, the *pro-S* sulfur atom was oxidized to a lesser degree, yielding sulfoxides $\mathbf{2}_{cis\text{-}1R,2S}$ and $\mathbf{3}_{trans\text{-}1S,2S}$ (28%) (Table I, entry B).

The Kagan modification of the Sharpless method for chiral oxidation,[17] when applied to thioacetal **1** showed a stronger preference for the trans isomers (86%) and a greater degree of enantioselectivity toward $\mathbf{3}_{trans\text{-}1S,2S}$ (76% ee) and $\mathbf{2}_{cis\text{-}1S,2R}$ (43% ee). The combined results indicate that this chiral oxidant was very selective toward the *pro-S* sulfur atom (81%) (Table I, entry C).

Microbial Oxidations. Previous reports from these laboratories on the ability of enzymes in fungi to differentiate between *pro R* and *pro S* sulfur atoms in 2-alkyl-substituted 1,3-dithianes[3] and 1,3-dithiolanes[4] showed that the stereopreference was relatively low (57% *pro S* → 66% *pro R*). Using intact cultures of the fungus *A. foetidus* with thioacetal **1** as substrate gave mainly cis sulfoxides (87%) with a dominance (93% ee) of the (1*S*,2*R*) enantiomer ($\mathbf{2}_{cis\text{-}1S,2R}$). The minor (13%) trans sulfoxides were found to show a slight excess (8% ee) of the (1*S*,2*S*) enantiomer ($\mathbf{3}_{trans\text{-}1S,2S}$). On the basis of the relative proportions of the four stereoisomeric metabolites, it could be concluded that the enzyme(s) responsible for sulfoxidation with intact cultures of *A. foetidus* (probably monooxygenase(s)) show(s) a strong preference (ca. 90%) toward the *pro-R* sulfur atom (Table I, entry D).

When a racemic mixture of the cis ($\mathbf{2}_{cis\text{-}1R,2S}$, $\mathbf{2}_{cis\text{-}1S,2R}$) and trans ($\mathbf{3}_{trans\text{-}1S,2S}$ and $\mathbf{3}_{trans\text{-}1R,2R}$) *S*-oxides was added as substrate to *A. foetidus* cultures (i.e., a mixture of 40% cis and 60% trans sulfoxide isomers was added and metabolized for 2 days under the same conditions described in part D), no evidence of preferential removal of one enantiomer (kinetic resolution) of the trans sulfoxide was apparent from the recovered trans isomer. However, evidence of a minor degree of kinetic resolution was obtained from the cis sulfoxide under similar biotransformation conditions. Thus, the recovered cis isomer was enriched (22% ee) in $\mathbf{2}_{cis\text{-}1S,2R}$. In view of this modest degree of kinetic resolution observed for the major cis isomer of the racemic sulfoxide mixture, the determination of overall preference for the *pro-R* sulfur atom shown by this fungal method of oxidation (90% *pro-R*) will be less accurate. However, no evidence of enzyme-catalyzed deoxygenation of sulfoxides was obtained with the microbial systems examined (*A. foetidus*, *P. putida*).

As part of a comprehensive program using a mutant strain (UV4) of the bacterium *P. putida* as a chiral oxidizing system,[21-24] a series of organosulfur substrates have been examined.[25] The results obtained using 2-methyl-1,3-benzodithiole (**1**) and intact cultures of *P. putida* UV4 are shown in Table I (entry E). It is noteworthy that while the chemical oxidants yielded an excess (68–86%) of the trans sulfoxide **3** (Table I, entries A–C), the microbial enzymes responsible for sulfoxidation (a monooxygenase (D) or possibly a dioxygenase (E)) produced a marked preference (87–96%) for the cis sulfoxide **2** (Table I, entries D and E). Enzymes in *P. putida* were found to produce homochiral (100% ee) samples of both the cis ($\mathbf{2}_{cis\text{-}1S,2R}$) and trans ($\mathbf{3}_{trans\text{-}1S,2S}$) sulfoxide metabolites. The latter enzymatic oxidations (entries D and E) also appeared to show a marked preference (90–92%) for the *pro-R* sulfur atom. Because some evidence of kinetic resolution of sulfoxides has also been observed with *P. putida*,[25] the preference for oxidation (i.e., % ee) shown for the *pro-R* sulfur atom should again be treated as only an approximate value. Despite the uncertain mechanistic origin of the enantiomeric excess in the sulfoxides **2** and **3** produced by microbial metabolism (entries D and E) and the unconfirmed nature of the enzymes involved, this enzymatic approach combined with chemical entries (B and C) provides useful synthetic routes to both trans 86% relative yield, 76% ee, entry C) and cis (96% relative yield, 100% ee, entry E) sulfoxides **3** and **2**, respectively. The production of enantiomerically pure sulfoxides (e.g., $\mathbf{2}_{cis\text{-}1S,2R}$ and $\mathbf{3}_{trans\text{-}1S,2R}$ and other[25] enantiopure thioacetal sulfoxides) by *P. putida* appears to have considerable advantages over other microbial methods. Sulfoxides of high optical purity (>90% ee) have been isolated from cultures of *A. foetidus* (3 examples),[1] *Mortierella isabellina* (4 examples),[2] and *Corynebacterium equi* (6 examples).[1,2,26] The latter microbial

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Table II. Biotransformation of 2-Methyl-1,3-benzodithiole (1) Using Hog and Rat Livier Microsomes

conditions	formation of sulfoxides 2 and 3 (nmol/min/mg of protein)			
	hog liver microsomes		rat liver microsomes	
	2[a]	3[b]	2[a]	3[b]
complete	12.5	2.1	1.0	0.9
-NADPH	d	d	d	d
+heat inactivation	d	d	d	d
-n-octylamine	2.3	4.1	0.5	0.9
+thiourea (0.5 mM)	0.8	1.6	0.3	1.1
+aminobenzotriazole[e] (0.5 mM)	6.2	1.4	0.6	0.5

[a] cis Isomers $2_{cis\text{-}1S,2R}$ + $2_{cis\text{-}1R,2S}$. [b] trans Isomers $3_{trans\text{-}1S,2S}$ + $3_{trans\text{-}1R,2R}$. [c] The complete incubation system contained 0.5 mM NADPH-generating system, 300 μM substrate, 1.6 mg of hog liver or 2.0 mg of rat liver microsomes, 4.5 mM n-octylamine, and phosphate buffer, pH 8.4. The values are the average of three to four experiments as determined by HPLC. [d] Not detectable. [e] Aminobenzotriazole was preincubated with the microsomes for 10 min before initiation of the reaction.

sulfoxidation systems appear to be among the most stereoselective enzymatic systems thus far available. The present observation of homochiral sulfoxide formation from 2-methyl-1,3-benzodithiole using *P. putida* UV4 extends the number of highly stereoselective (>90% ee) oxidations catalyzed by *P. putida* to >10 examples.[25] The excellent yields in some cases allied to the generally high % ee values obtained and total absence of sulfone metabolites formed (sulfones are observed in the metabolism of sulfides by other microbial oxidation systems) suggest that *P. putida* UV4 may be the most stereoselective and efficient microbial sulfoxidizing system currently available. The synthetic potential of the enantiomerically pure thioacetal sulfoxides produced by *P. putida* is currently being examined because base-catalyzed substitution at the C-2 carbon and asymmetric synthesis (using the chiral sulfoxide group) are possible.

Mammalian Monooxygenase Oxidations. The presence of strong chromophores in both the thioacetal substrate **1** and the sulfoxides **2** and **3** allowed very small quantities of the latter metabolites from mammalian enzyme sources to be stereochemically assigned using the CSPHPLC method in conjunction with UV detection and CD spectroscopy. Thus, a series of in vitro experiments using rat and hog liver microsomes (entries F–I) and pure monooxygenase enzymes isolated from rat and hog liver (entries J–M, O, P) and rabbit lung (entry N) was carried out.

Preliminary studies showed that hog and rat liver microsomes supplemented with NADPH catalyzed the oxidation of thioacetal **1** to the corresponding monosulfoxides **2** (Table I, entries F–I). Although the microsomes used were competent to yield bis(sulfoxide) products, during the short incubation times used in this study, they were not detected. The formation of the monosulfoxide products **2** and **3** was a linear function of microsomal protein concentration (1–6 mg/mL). 2-Methyl-1,3-benzodithiole 1-oxide (**2** and **3**) formation was linear for at least 8 min and was dependent on active microsomal protein and NADPH (Table II). Heat inactivation of the protein under conditions which completely destroyed flavin-containing monooxygenase activity[19,27] but left the cytochromes P-450 essentially intact[19] prevented any detectable level of thioacetal S-oxidation. *n*-Octylamine, a positive stimulator of the flavin-containing monooxygenase[19] and an inhibitor of cytochromes P-450[28] caused an increase in the amount of *S*-oxide formed, and this was quite marked for hog and less apparent for rat liver microsomes. The relative sensitivity of hepatic monooxygenases to *n*-octylamine has been reported previously,[29] and it is generally observed that rat liver flavin-containing monooxygenase is less sensitive to the stimulatory effects of *n*-octylamine than is hog liver flavin-containing monooxygenase. However, it is also possible that the variable effect of *n*-octylamine on flavin-containing monooxygenase activity is due to the presence of multiple forms of the enzyme, each of which respond to *n*-octylamine differently.[27]

In the presence of rat and hog liver microsomes, *n*-octylamine has a marked effect on stereoselective formation of 2-methyl-1,3-benzodithiole *S*-oxides (**2** and **3**). Thus, in the presence of *n*-octylamine, more of the cis *S*-oxide (**2**) is formed. In the absence of *n*-octylamine, cytochromes P-450 apparently make a greater contribution to formation of the trans *S*-oxide (**3**). *n*-Octylamine does not markedly change the stereoselectivity of S-oxygenation by the highly purified flavin-containing monooxygenase, although it tends to increase the rate of 2-methyl-1,3-benzodithiole S-oxygenation. Taken together, the results suggest that the flavin-containing monooxygenase is primarly responsible for 2-methyl-1,3-benzodithiole S-oxidation in hog liver microsomes. In contrast to the case of hog liver microsomes, in the presence of rat liver microsomes, cytochromes P-450 make a more important contribution to S-oxidation of 2-methyl-1,3-benzodithiole. For microsomal or purified mammalian monooxygenase systems, addition of thioacetal *S*-oxides **2** and **3** to the buffer or to the quenched reaction medium resulted in the quantitative recovery of the *S*-oxides. This suggests that reduction or other transformations of **2** and **3** do not occur during the short incubation periods employed.

Data in Table II show the effects of several metabolic inhibitors on the formation of thioacetal *S*-oxides **2** and **3**. Thiourea,[19] a well documented alternate substrate competitive inhibitor of the flavin-containing monooxygenase markedly decreased the amount of sulfoxide formed. There is significant stereoselectivity in the inhibition of *S*-oxide formation by thiourea. Thus, in hog and rat liver microsomes, predominant inhibition of the cis *S*-oxide (**2**) was observed. The potent mechanism-based cytochrome P-450 inhibitor, aminobenzotriazole[30] also inhibits thioacetal *S*-oxide formation but appears to inhibit trans *S*-oxide (**3**) formation in both rat and hog liver microsomes to a greater extent than the cis sulfoxide (**2**) formation.

The degree of stereoselectivity during S-oxidation using the mammalian liver microsomal fractions (entries F–I, Table I) was greater for the cis (**2**, 56–72% ee) than for the trans (**3**, 0–29% ee) sulfoxides. The preference shown for the *pro-R* sulfur atom was in the range 60–78%.

Use of the highly purified flavin-containing monooxygenase (entries J–N, Table I) again showed low stereoselectivity in the formation of the trans sulfoxides (**2**, 15–43% ee) but a much higher degree of stereoselectivity for the cis sulfoxides (**2**, 78–100% ee). An exclusive preference for the *pro-R* sulfur atom was observed using rabbit lung flavin-containing monooxygenase (entry N, Table I). This is the first example of total stereoselectivity observed during an enzyme-catalyzed sulfoxidation of a geminal pair of sulfur atoms on a prochiral carbon atom.

The results of the stereoselective S-oxygenation studies with highly purified monooxygenases are in agreement with those of previous studies which suggest that rabbit pulmonary flavin-containing monooxygenase places greater constraints on substrate S-oxygenation than does the rat or hog liver enzyme. As observed previously,[31] cytochromes P-450 appear to be less stereoselective than either of the flavin-containing monooxygenases examined. Furthermore, the fact that only cis S-oxygenation was observed for the rabbit pulmonary enzyme and that both cis and trans S-oxygenation was observed for rat and hog liver flavin-containing monooxygenase suggests that the pulmonary enzyme possesses a much smaller binding channel than the hepatic form of the enzyme.[6-8,32,33] The ability to distinguish S-oxygenation ste-

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reoselectivity with **1** as a substrate may be particularly useful in predicting the composition of flavin-containing monooxygenases in crude preparations where multiple forms of the enzyme may exist.[27,34]

Highly purified cytochrome P-450 2B1 (in the presence or absence of cytochrome b_5) from rat liver showed much lower stereopreferences for the cis sulfoxides **2** (23–30% ee) and slightly higher values for the trans sulfoxides **3** (33–34% ee) in comparison with the results obtained using crude liver microsomal fractions. Cytochrome b_5 may facilitate cytochrome P-450 oxidation of substrates. Apparently, however, cytochrome b_5 does not play a significant role in cytochrome P-450 2B1-mediated S-oxidation, as we have previously observed for other sulfides.[8,31,33]

The stereoselectivity studies show that the contribution of the flavin-containing monooxygenase enzymes to the sulfoxidation of thioacetal **1** is dominant during hog liver microsomal metabolism in vitro. This is also suggested in the case of the rat liver enzyme studies where preference for the $\mathbf{2}_{cis\text{-}1S,2R}$ isomer is greater (78–87% ee) using flavin-containing monooxygenase (entries L and M) compared with P-450 2B1 (30% ee, entry O) and P-450 2B1 without cytochrome b_5 (23% ee, entry P). This conclusion is further emphasized during formation of the trans isomer **3**, where flavin-containing-monooxygenase-catalyzed oxidations gave a preference for $\mathbf{3}_{trans\text{-}1R,2R}$ (15–22% ee, entries L and M), while the other enantiomer $\mathbf{3}_{trans\text{-}1S,2S}$ was produced in excess (33–34% ee) using P-450 2B1 (entries O and P). These observations illustrate the value of 2-methyl-1,3 benzodithiole (**1**) as a substrate in probing the stereoselectivity of sulfoxidizing enzymes and the relative contributions of particular enzymes in microsomal preparations. The results also suggest that the active site of cytochrome P-450 2B1 is more accommodating than the rather rigid substrate-binding channel of the flavin-containing monooxygenase.[6-8,31,33] On the other hand, if cytochrome P-450-catalyzed (or other hemoprotein) S-oxidation of **1** is observed to be highly stereoselective, a possible prediction is that the active site is more constrained than that of cytochrome P-450 2B1.

Previous sulfoxidation studies of 1,3-thioacetal substrates[3,4] were limited by the quantities of sulfoxide metabolites produced and because CSP-HPLC methods were unsuitable for 1,3-dithiane 1-oxide and 1,3-dithiolane 1-oxide enantiomer separation. Product analysis was thus restricted to ^{1}H-NMR methods using a chiral auxiliary and a minimum quantity of ca. 5 mg of sulfoxide metabolite. The CSP-HPLC method described in the present work provides a marked improvement over the NMR method in terms of sensitivity. Precise determination of S-oxidation stereoselectivity using 2-methyl-1,3-benzodithiole as substrate with fungal (90% *pro R*, entry D), bacterial (92% *pro-R*, entry E), and pure monooxygenase enzyme (100% *pro R*, entry N) shows the utility of these biological catalysts as biosynthetic tools. The 2-methyl-1,3-benzodithiole system has thus shown advantages in terms of accuracy, sensitivity of measurement, and stereoselectivity (→ 100% *pro R*) and may be widely applicable to examine the stereoselectivity of other biological oxidation systems.

Conclusions

A new type of thioacetal system, 2-methyl-1,3-benzodithiole (**1**), has been used to examine the relative value of chemical oxidants in the production of cis or trans thioacetal 1-oxides with a preference for one enantiomer. The trans isomers ($\mathbf{3}_{trans\text{-}1R,2R}$ and $\mathbf{3}_{trans\text{-}1S,2S}$) were in all cases preferentially formed (64–86%) using chemical oxidants. By contrast, the intact fungal and bacterial oxidations showed a marked selectivity (87–96%) for the cis isomers ($\mathbf{2}_{cis\text{-}1S,2R}$ and $\mathbf{2}_{cis\text{-}1R,2S}$). The production of enantiomerically pure sulfoxides $\mathbf{2}_{cis\text{-}1S,2R}$ and $\mathbf{3}_{trans\text{-}1S,2S}$ provides a useful chiral synthon for further synthetic studies.

With the exception of rabbit lung and hog liver flavin-containing-monooxygenase-catalyzed sulfoxidations, where a preference for the $\mathbf{2}_{cis\text{-}1S,2R}$ enantiomer and the *pro-R* sulfur atom was observed, all other sulfoxidations examined using animal liver enzymes showed a significant propensity to form the trans isomer ($\mathbf{3}_{trans\text{-}1R,2R}$ and $\mathbf{3}_{trans\text{-}1S,2S}$). The flavin-containing monooxygenase enzymes have been observed to play a dominant role during liver microsomal S-oxygenation biotransformations, in vivo. The flavin-containing monooxygenase may have biosynthetic potential as well. Because it is possible to construct a fluidized-bed reactor covalently linking the hog liver flavin-containing monooxygenase to glass beads,[35] it is likely that such as catalyst could be useful in the preparation of significant quantities of sulfoxide metabolites possessing high % ee that may be applicable to asymmetric chemical syntheses. The pulmonary flavin-containing monooxygenase may be particularly useful in this regard because it can be isolated as a complex with the calcium binding protein, calreticulin, which apparently renders this monooxygenase quite thermally stable.[36]

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