# Enzymatic Oxygenation of Sulfides with Cytochrome P-450 from Rabbit Liver. Stereochemistry of Sulfoxide Formation[1]

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Stereochemistry of enzymatic oxygenations of various diaryl, dialkyl and aryl alkyl sulfides to the corresponding sulfoxides with hepatic microsomes obtained from phenobarbital pretreated rabbit was investigated in comparison with those of nonenzymatic oxidations. Relative reactivity of the sulfides in the enzymatic oxygenation, based on the yields of the sulfoxides and the amount of oxygen absorption, depends upon the steric demand of the substituent in the oxygenation of 2-substituted thiochroman derivatives. However, such a trend was not observed in the enzymatic oxygenations of various acyclic sulfides. A substantial asymmetric induction on the sulfur atom was always observed (*e.g.*, 54% *e.e.* for benzyl *t*-butyl sulfide). Formation of the sulfoxide with *R*-configuration predominated over that with *S*-configuration. The enzymatic oxygenation of all the unsymmetrical sulfides examined proceeded *via* the same steric course. Diastereomeric ratios of the sulfoxides formed in the enzymatic oxygenations of various racemic sulfides bearing alkyl substituents at α-position are markedly favoured more for the trans isomers than for the cis ones, while the ratio was nearly fifty-fifty in the nonenzymatic oxidations with both MCPBA and $NaIO_4$. These results suggest that the enzymatic oxygenation takes place at the less hindered side of the sulfide. Both enzymatic and nonenzymatic oxidations of rigid six-membered cyclic sulfides, *trans*-thiadecalins and 4-(*p*-chlorophenyl)thiane, similarly occurred at the less hindered side to give mainly equatorial sulfoxides. The yields of the four stereoisomeric sulfoxides were nicely determined in the enzymatic oxygenation of racemic 2-methyl-2,3-dihydrobenzothiophene to elucidate the steric course of the oxygenation. Dependency of this microsomal oxygenation upon cytochrome P-450 enzyme was proved by several inhibition experiments and also by the identical stereochemical results of the oxygenation of a few selected sulfides to those with a reconstituted system with purified cytochrome P-450 and co-factors.

Cytochrome P-450, a monooxygenase, is known to be present in multiple forms in mammalian tissues.[2] Some cytochrome P-450s in adrenal grand, cytochrome $P\text{-}450_{11\beta}$ and cytochrome $P\text{-}450_{17\alpha}$, participate in the regiospecific and stereospecific hydroxylations of the steroids while cytochrome P-450s in plants carry out specific biosyntheses of biologically important substances.[2] One major role of cytochrome P-450 in mammalian liver is known to be the oxygenation of various kinds of lipophilic xenobiotics, such as drugs, insecticides, food additives, *etc.*, to the more hydrophilic metabolites in order for these xenobiotic substances to be excreted.[2] In such metabolic oxygenations in liver, stereospecificity may not be neccesary because the main function of the oxygenation is to convert the foreign substances to other more hydrophilic products which

$$[S] + NADPH + O_2 + H^+ \xrightarrow{\text{Cytochrome P-450}} [S\text{-}O] + NADP^+ + H_2O \quad (1)$$

can be excreted readily out of living bodies. Since the cytochrome P-450 is demonstrated to play such a role, the oxygenation with this enzyme would be less substrate selective and less stereospecific as compared to most other enzymes. However, practically no stereochemical study has appeared in the oxygenation of xenobiotic compounds by hepatic microsomal cytochrome P-450.

Meanwhile, organic sulfides are good substrates for studying the stereochemistry of the oxygenation with cytochrome P-450,[3] since chemical and biomimetic oxidations of sulfides to the sulfoxides and their stereochemistry have been extensively studied in our laboratory,[3-5] and hence stereochemical results obtained in the enzymatic oxygenation can be readily compared with our accumulated data.[3-5] Thus, we have investigated the stereochemistry of the oxygenation of various sulfides to the corresponding sulfoxides with rabbit liver microsomal cytochrome P-450 in both microsomal level and reconstituted system with purified liver microsomal cytochrome P-450[1] obtained from rabbit pretreated with phenobarbital for 5 d to induce the enzyme.

## Experimental

*General.* Melting points and infrared spectra were taken on a Yanaco instrument and a Hitachi 215 spectrometer, respectively, and uncorrected. NMR spectra were recorded on a Hitachi Perkin-Elmer R-20 spectrometer. FT-NMR spectra were obtained by a JEOL FX-90Q spectrometer at the chemical analysis center of this university. Mass spectra were taken with a Hitachi RMU-6MG mass spectrometer. A Shimadzu GC-6A instrument was used for gas chromatography using $N_2$ gas as a carrier gas. Specific rotations were calculated from the values of optical rotations which were measured by a JASCO DIP-140 polarimeter using a 5 cm (length) quartz cell. CD spectra were recorded on a JASCO J-20 spectrometer. Elemental analyses were carried out by the chemical analysis center of this university.

*Materials.* Liver microsomes were obtained from phenobarbital pretreated (5 d) male rabbits according to the reported method.[3b,6] Purified cytochrome P-450 was obtained from the same liver microsomes by Imai's method.[7] NADPH-cytochrome P-450 reductase was purified by the

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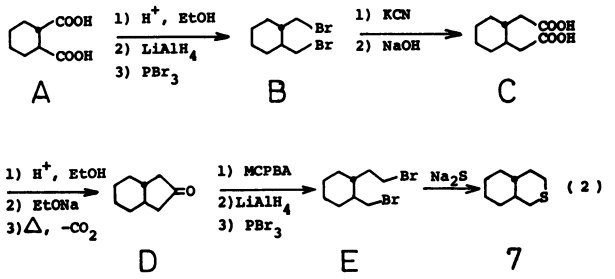
method of Iyanagi *et al.* with some modification.[8] NADP+, D-glucose 6-phosphate (G-6-P), and G-6-P dehydrogenase (G-6-PDH) were obtained from Oriental Yeast Co. NMR shift reagents, $Eu(dpm)_3$ and $Eu(hfc)_3$ were of Dojin Chemical Co. and Aldrich Chemical Co., respectively. SKF 525-A ((2-*N*,*N*-diethylamino)ethyl 2,2-diphenylpentanoate) was given from Tanabe Seiyaku Co.

*Sulfide and Sulfoxide.* Physical and spectral data, and elemental analyses data of various sulfides and sulfoxides used here are listed in Table 1.

Most of the sulfides were either commercially available or prepared by known method described below, and some were new compounds.

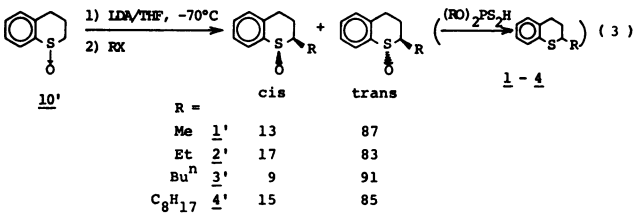
Common dialkyl and aryl alkyl sulfides (**9**, **12**—**19**) were prepared by the usual method, *i.e.* the reaction of the corresponding thiols and alkyl halides in the presence of a base,[9] while *t*-butyl *p*-tolyl sulfide (**17**) was obtained by treating *p*-toluenethiol with gaseous isobutylene.[10] The genuinely pure **17** was obtained by the oxidation of the crude sulfide with $H_2O_2$ in AcOH followed by reduction with *O*,*O*′-diethyl hydrogenphosphodithioate[11] after purification of the sulfoxide by column chromatography (eluent: hexane and then chloroform, on silica gel). Di-*t*-butyl sulfide (**21**) and thiane (**22**) were commercially available from Tokyo Kasei Kogyo Co. and Aldrich Chemical Co., respectively. *o*-Methoxyphenyl phenyl sulfide (**20**) was prepared by the reaction of *o*-methoxybenzenediazonium salt and thiophenol as reported previously.[12] *trans*-1-Thiadecalin (**6**) was also synthesized from 1,2-epithiocyclohexane *via* several reaction steps as reported.[13] 2,3-Dihydrobenzothiophene (**11**) was obtained by the reduction of benzothiophene with metallic sodium in ethanol[14] and purified by distillation using Kugelrohr.

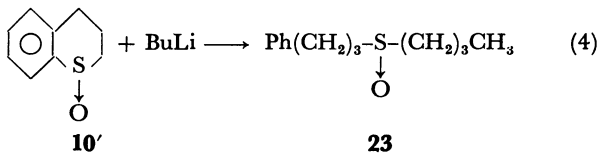
trans-*2-Thiadecalin* (**7**): The title sulfide, a new compound, was prepared from *trans*-1,2-cyclohexanedicarboxylic acid (**A**) through several steps as shown in Eq. 2.[15] Namely, esterification of the starting material **A**, reduction of the diester with $LiAlH_4$ and bromination of the diol with $PBr_3$ gave dibromide **B**. Substitution of **B** with $CN^-$ and alkaline hydrolysis of the dicyanide thus formed afforded dicarboxylic acid **C**, which was then subjected to the Dieckmann condensation after esterification to give the cyclic keto ester that was then decarboxylated to afford ketone **D**. The Baeyer-Villiger oxidation of **D**, reduction of the lactone with $LiAlH_4$ to the diol, and bromination of the diol, yielded dibromide **E** which was then converted to desired *trans*-2-thiadecalin (**7**) by treatment with $Na_2S$ (total yield 5%).[15]




*2-Methyl-2,3-dihydrobenzothiophene (5) and 1-Thiochroman (10)*: Cyclic sulfides **5** and **10** were obtained as an isomeric mixture in the thermal cyclization of allyl phenyl sulfide in quinoline, as reported by Anderson *et al.*[16] The mixture of **5** and **10** was separated by careful distillation, affording **10** and impure **5** containing **10**. The impure **5** was purified by oxidation with $H_2O_2$ in AcOH to the corresponding sulfone, followed by reduction with $LiAlH_4$, as reported.[10]

*2-Alkyl Thiochroman 1-Oxides (1′—4′) (Eq. 3)*: To a cooled solution (−70 °C) of **10′** (10 mmol) in dry THF (50—100 ml), diisopropylamine (12 mmol) and then BuLi (10% hexane solution, 10 mmol) were added under argon atmosphere. The clean colorless solution immediately turned to reddish black due to the formation of the anion of **10′**. After warming the solution up to −30 °C for several minutes the solution was cooled again to −70 °C. A dry alkyl halide (10 mmol, MeI, EtBr, *n*-BuBr, or $n$-$C_8H_{17}Br$) was added to the solution at −70 °C. The reaction mixture was gradually warmed up to 0 °C. The black color of the solution gradually faded out as the reaction proceeded, to give eventually a colorless solution. The reaction mixture was poured into cold dilute hydrochloric acid and extracted three times with chloroform. The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was subjected to column chromatography on silica gel (eluent: hexane–ethyl acetate–chloroform=4 : 1 : 1). The first fraction was a by-product. The second gave the desired alkylated product in 65—85% yield and the last fraction gave the starting material recovered. The by-product was then identified by NMR and MS spectra and elemental analysis to be butyl 3-phenylpropyl sulfoxide (**23**) which was a product by a direct reaction of **10′** with BuLi (Eq. 4).




Note the predominant formation of trans 2-substituted thiochromen 1-oxides over their cis isomers in the alkylations of **10′** (Eq. 3). The cis/trans ratio was determined from the NMR spectrum using $Eu(dpm)_3$, as mentioned later. This stereochemical result can be best explained in terms of the findings[28] that the alkylation of α-sulfinyl carbanion takes place from the opposite side of the chelated ring involving $Li^+$ and sulfinyl oxygen to give predominantly the trans isomer, as shown in Fig. 1.

*Butyl 3-Phenylpropyl Sulfoxide (23)*: Mp 44—47.5 °C from hexane; NMR (δ, $CDCl_3$), 0.91 (t, 3H, $CH_3$, $J$=6.8 Hz),

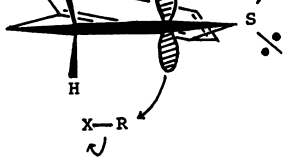



Fig. 1. Direction of alkylation of **10′** with LDA and RX (see Eq. 3). Stereospecific alkylation forms *trans*-sulfoxide predominantly.

TABLE 1. SULFIDES AND SULFOXIDES

| Compound | Physical and spectral data NMR ($\delta$, $CDCl_3$, $J$/Hz) | Calcd(%) C | Calcd(%) H | Found(%) C | Found(%) H |
|---|---|---|---|---|---|
| **1,** | bp 110 °C/3. 0 Torr (lit,[16a] 125—127 °C/9 Torr), NMR: 1.44 (d, $CH_3$, $J$=6.8), 1.66—3.10 (m, 5H), 7.14—7.90 (m, 9H). | 73.11 | 7.36 | 73.45 | 7.36 |
| **1′,** | (cis-trans mixture), oil, NMR: 1.44 66.62 (d, 3H, $J$=6.7), 1.69—3.38 (m, 5H), 6.96—7.87 (m,4H). | 66.62 | 6.71 | 66.75 | 6.76 |
| **2,** | oil, NMR: 1.04 (t, 3H, $J$=7.0), 1.40—3.45 (m, 7H), 6.83—7.08 (m, 4H). | 75.10 | 7.91 | 74.07 | 7.91 |
| **2′,** | (cis-trans mixture), oil, NMR: 1.09 (t, 3H, $J$=7.0), 1.73—3.10 (m, 7H), 7.00—7.60 (m, 4H). | 68.00 | 7.58 | 68.16 | 7.97 |
| **3,** | oil, NMR: 0.90 (t(br), 3H), 1.35—2.48 (m, 8H), 2.78 (m, 2H), 2.90 (m, 1H), 6.80—7.35 (m, 4H). | 75.65 | 8.79 | 75.75 | 8.89 |
| **3′,** | (cis-trans mixture), oil, NMR: 0.94 (t(br), 3H), 1.18—3.13 (m, 10H), 7.20—7.80 (m, 4H). | 70.22 | 8.16 | 69.98 | 8.25 |
| **4,** | oil, NMR: 1.03 (t(br), 3H, $-CH_3$), 1.43 (m, 12H, $-(C\underline{H}_2)_6-CH_2-$), 1.30—2.62 (m, 4H, $-C\underline{H}_2-(CH_2)-$ and $-S-C\underline{H}-CH_2-C\underline{H}_2-$), 2.80—3.10 (dd, 2H, $-S-C\underline{H}-C\underline{H}_2-$), 3.15—3.57 (bs, 1H, $-CH<$), 6.97—7.31 (m, 4H, arom). | 77.79 | 9.98 | 77.89 | 10.14 |
| **4′,** | (cis-trans mixture), oil, NMR: 0.88 (t(br), 3H), 1.10—3.10 (m, 22H), 7.24—8.10 (m, 4H). | 73.32 | 9.14 | 73.28 | 9.58 |
| **7,** | oil, MS: $m/e$ 156 ($M^+$). | 69.16 | 10.31 | 68.76 | 10.45 |
| **7a′,** | (axial), colorless crystals, mp 98—99 °C (hexane–ethyl acetate), IR (KBr, $cm^{-1}$): 1029 (S=O). | 62.74 | 9.23 | 62.61 | 9.36 |
| **7b′** | (equatorial), colorless crystals, mp 53—54 °C (hexane–ethyl acetate), IR (KBr, $cm^{-1}$):1036 (S=O). | 62.74 | 9.23 | 62.39 | 9.36 |
| **9,** | oil, bp 89 °C/3.0 Torr, NMR: 0.89 (t, 3H, $J$=2.9), 1.18 (d, 3H, $J$=6.8), 1.41 (m, 2H), 2.46 (m, 1H), 3.60 (s, 2H, $PhC\underline{H}_2-$), 7.15—7.55 (m, 5H). | 73.27 | 8.94 | 72.88 | 9.03 |
| **9′,** | (erythro-threo mixture), oil, NMR: 0.80—1.99 (m, 8H, $-CH_3$, $-CH_2-CH_3$), 2.13—2.73(m, 1H,$-CH<$), 3.80—3.96 (m, 2H, $PhC\underline{H}_2-$) 7.30 (s, 5H, arom). | 67.30 | 8.21 | 66.96 | 8.26 |
| **15,** | oil, bp 74—76 °C/2.0 Torr. | 74.17 | 9.34 | 74.01 | 9.45 |
| **15′,** | oil, NMR: 1.16 (s, 9H), 2.38—3.23 (m, 3H, $Ph-CH_2-CH_AH_B-(H_A$ or $H_B)$), 3.63 (t,1H, $H_B$ or $H_A$, $J$=6.3), 7.10—7.33 (br, 5H, arom.). | 68.53 | 8.63 | 68.44 | 8.60 |
| **16,** | oil, bp 106—110 °C/3.0 Torr. | 74.16 | 9.33 | 73.92 | 9.31 |
| **16′,** | oil, NMR: 1.34 (s, 9H), 2.36 (s, 3H), 3.58 (d, 1H, $J$=12.7), 3.90 (d, 1H, $J$=12.7), 7.10—7.33 (m, 4H). | 68.52 | 8.62 | 68.57 | 8.65 |
| **19′,** | oil, NMR: 1.18 (t(br), 3H), 1.90 (s, 9H, $Bu^t$), 1.87—2.63 (m, 12H, $-CH_2-(C\underline{H}_2)_6-CH_3$), 3.13 (t, 2H, $SO-CH_2-$, $J$=5.6). | 65.99 | 12.00 | 65.57 | 11.88 |
| **19,** | oil, bp 89 °C/1.5 Torr (lit,[24] 78—82 °C/0.6 Torr), NMR: 0.89 (t(br), 3H), 1.10—1.68 (m, 21H), 2.44 (t(br), 2H). | | | | |
| **5,** | bp 85—95 °C/3.5 Torr (lit,[16] 48—50 °C/0.2 Torr). | | | | |
| **5a′** | (cis), oil, NMR: 1.56 (d, 3H, $J$=6.6), 3.17—3.80 (bs, 3H), 7.91 (m, 4H) (lit,[16] 1.57 (d, 3H), 3.41 (bs, 3H), 7.55 (bs, 3H), 7.95 (m, 4H). | | | | |
| **5b′** | (trans), mp 40 °C (lit,[16] 42—43 °C), NMR: 1.43 (d, 3H, $J$=6.6), 2.94 (dd, 1H, $J$=6.6), 3.35—4.06 (m, 2H), 7.25—7.90 (m, 4H) (lit,[16] 1.43 (d, 3H), 2.95 (dd, 1H), 3.93 (dq, 1H), 3.60 (dq, 1H), 7.54 (bs, 3H), 7.90 (m, 1H). | | | | |
| **6,** | mp 17 °C/(lit,[17] 17—18 °C, lit,[13] 17.4 °C). | | | | |
| **6a′** | (axial), colorless crystals, mp 87—88 °C (hexane–ethyl acetate) (lit,[18] 82.5—86 °C), IR (KBr, $cm^{-1}$): 1026, 1018 (sh) (S=O). | | | | |
| **6b′** | (equatorial), colorless crystals, mp 71—72 °C (hexane–ethyl acetate) (lit,[18] 72—74 °C), IR (KBr, $cm^{-1}$): 1050 (sh), 1036, 1019 (sh) (S=O). | | | | |
| **10,** | oil, bp 90—100 °C/3.5 Torr (lit,[13] 254 °C, lit,[16] 82—87 °C/1 Torr). | | | | |
| **10′,** | oil, bp 155—165 °C/2.5 Torr (lit,[19] 117—120 °C/0.06 Torr), NMR: 1.09—3.50 (m, 6H), 7.20—7.90 (m, 4H). | | | | |
| **11,** | oil, bp 67—69 °C/2.0 Torr (lit,[13] 253.58 °C). | | | | |
| **11′,** | oil, 165 °C/2.0 Torr (lit,[20] 115 °C/0.09 Torr), NMR: 3.13—4.27 (m, 4H), 7.40—8.00 (m, 4H). | | | | |
| **12′,** | oil, bp 102—103 °C/3.0 Torr (lit,[21] 114 °C/12—13 Torr), NMR: 1.28 (s, 9H), 3.90 (s, 2H), 7.16 (bs, 5H). | | | | |
| **12′,** | mp 75—76 °C (hexane) (lit,[21] 75—76 °C), NMR: 1.46 (s, 9H), 3.56 (d, 1H, $J$=12.7), 3.82 (d, 1H, $J$=12.7), 7.30 (s, 5H). | | | | |
| **13,** | oil, bp 92 °C/2.5 Torr (lit,[22] 123 °C/14 Torr). | | | | |

(Continued)

| Compound | Physical and spectral data NMR ($\delta$, $CDCl_3$, $J$/Hz) | Calcd(%) C | Calcd(%) H | Found(%) C | Found(%) H |
|---|---|---|---|---|---|
| **13′,** | mp 62—63 °C (lit,[23] 63—64 °C), NMR: 0.90 (t(br), 3H), 1.10—2.13 (m, 4H), 2.59 (t(br). 2H), 3.96 (s, 2H), 7.23—7.50 (m, 5H). | | | | |
| **14,** | mp 43 °C (hexane) (lit,[21] 43—44 °C), NMR: 2.30 (s, 2H), 4.05 (s, 2H), 6.93—7.40 (m, 9H). | | | | |
| **14′,** | mp 137—138 °C (lit,[21] 136—139 °C), NMR: 2.38 (s, 3H), 3.96 (s, 2H), 7.10—7.63 (m, 9H). | | | | |
| **17,** | oil, bp 88 °C/4.0 Torr (lit,[21] 106 °C/10 Torr). | | | | |
| **17′,** | mp 88—89 °C (lit,[21] 88—89 °C). | | | | |
| **18,** | oil, bp 93 °C/2.5 Torr (lit,[21] 84 °C/0.8 Torr). | | | | |
| **18′,** | mp 41—43 °C (lit,[23] 40—42 °C). | | | | |
| **20,** | oil, bp 134—136 °C/4.0 Torr (lit,[25] 130—135 °C/2.0 Torr). | | | | |
| **20′,** | mp 99 °C (lit,[26] 98—99 °C). | | | | |

1.15—2.90 (m, 12H, $-CH_2-$), 7.00—7.43 (m, 5H, arom); MS (*m/e*), 224 ($M^+$); Calcd for $C_{13}H_{20}SO$: C, 69.59; H, 8.98. Found: C, 69.90; H, 9.18.

*2-Alkyl Thiochromans (1—4) from Sulfoxides (1′—4′)*: The sulfoxide (**1′**, **2′**, **3′**, or **4′**, 1 mmol) obtained above, and *O,O′*-diethyl hydrogenphosphodithioate (7—9 mmol)[11] were mixed at room temperature. After keeping the mixture standing till the sulfoxide disappeared (TLC), the mixture was subjected directly to column chromatography on silica gel using hexane as an eluent. Yields were nearly quantitative in all cases.

*Authentic Sulfoxides.* Authentic sulfoxides were prepared by treating the corresponding sulfides with one of the following oxidation systems, *i.e.* $H_2O_2$/AcOH, $H_2O_2/H_2SO_4$/MeOH, and *m*-chloroperbenzoic acid (MCPBA)/$CH_2Cl_2$. Physical and spectral data, and elemental analysis data of all the sulfoxides are shown in Table 1.

*Oxygenation of Sulfide with Microsomes.* A mixture of hepatic microsomes (190 mg protein), 250 μmol of G-6-P and 25 μmol of $NADP^+$ in phosphate buffer (0.2 M, pH 7.4) (final volume: 17 ml) was incubated with stirring with a magnetic stirrer at 37 °C for *ca.* 10 min. The substrate. sulfide (*ca.* 0.5 mmol) and then G-6-PDH (20 units) were added to the mixture and the resulting reaction mixture was incubated at 37 °C under air or pure oxygen atmosphere. After the incubation for 1.5 h, the reaction was stopped by adding 15—20 ml of acetone and 0.5 M trichloroacetic acid (to make the solution pH *ca.* 2.0) into the reaction mixture. The resulting mixture was left in a refrigerator (at *ca.* 4 °C) for 10 min to precipitate the protein, prior to centrifugation of the mixture (3000 $min^{-1}$ for 20 min). The supernatant collected was extracted three times with $CHCl_3$. The combined organic layer was washed with 2 M KOH and water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. GLPC analysis of the residue showed the presence of the recovered sulfide, the product sulfoxide and some impurities derived from the protein. The sulfoxide was purified by TLC fractionation (on alumina, eluent: benzene–ethyl acetate (2 : 1)), and identified by comparison with the authentic sample. Yields and other data are listed in Tables 3 and 4.

The purified sulfoxide was subjected to the measurements of NMR, CD and optical rotation. The cis/trans ratio of the sulfoxide was determined by measuring the NMR spectrum of the mixture of the isomeric sulfoxides and a shift reagent Eu $(dpm)_3$ (0.2—0.4 equiv). The results are shown in Table 3.

Enantiomeric excess was calculated from the integration ratio in NMR spectrum of the mixture of the enantiomeric sulfoxide and an optically active shift reagent Eu $(hfc)_3$ (0.4—0.6 equiv), and/or from the value of specific rotation and reported maximum rotation of the sulfoxide (Table 4).

CD spectra were measured for **5a′**, **5b′**, **10′**, and **11′** in ethanol, though the concentrations of the compounds were not measured (Fig. 5).

*Oxygen Consumption.* The oxygen consumption in the oxygenation with microsomes was measured with a gas bullet attached to the two-necked reactor in a constant temperature room (24 °C). The reaction conditions were same as that in the section of "*Oxygenation of Sulfides with Microsomes.*" The measurement of the oxygen consumption was started as soon as the reaction was initiated by the addition of the substrate and G-6-PDH through a serum cap into the reactor, which contained other co-factors and was thermally pre-equilibriated. The results are shown in Fig. 2.

*Control Experiments.* Relative reactivities were determined by following the yields of the sulfoxides formed in a given time. As for CO inhibition test, an atmospheric mixture of CO and $O_2$ gas (*ca.* 1 : 1) was used as a reaction atomsphere instead of pure oxygen or air. SKF 525-A (11.3 mg, 32 μmol), 1,4-diazabicyclo[2.2.2]octane (DABCO, 120 mg, 0.61 mmol) and catalase (134 nmol) were further added to the complete system which is described in "*Oxygenation of Sulfides with Microsomes,*" at the beginning of the reaction. Boiled microsomes prepared by heating for 10 min in boiled water, was used instead of microsomes. Results obtained according to the procedure described in "*Oxygenation of Sulfides with Microsomes,*" are shown in Table 2.

*Oxidation of Racemic Sulfides (1—5 and 9) to Mixture of Diastereomeric Sulfoxides with MCPBA and $NaIO_4$.* MCPBA (1.1 mmol) was slowly added to a solution of the sulfide (1 mmol) in 5—10 ml of $CH_2Cl_2$ and the mixture was stirred under cooling with an ice-water bath until the spot of the sulfide disappeared (TLC). The usual work-up afforded crude diastereomeric sulfoxides which then were purified by column chromatography on silica gel using an appropriate eluent.

Oxidation with $NaIO_4$ was carried out by adding slowly an aqueous solution (5—10 ml) of $NaIO_4$ (1.1 mmol) into an acetone or methanol solution (20—25 ml) of sulfide (1 mmol) under cooling with an ice-water bath. The mixture was warmed up to room temperature and kept stirring till the sulfide was completely consumed. The usual work-up and the subsequent column chromatography (on silica gel) of the crude products gave a mixture of two pure diastereomeric sulfoxides.

In both cases, the yields of the pure sulfoxides were ranging from 70 to 85%. The mixture of diastereomeric sulfoxides thus obtained were mixed with 0.1—0.4 equiv of $Eu(dpm)_3$ in order to determine the diastereomeric ratio by NMR spectrum in which protons at peri-position (7- or 8- position of **1′**—**5′**) appeared separately in a region between 8 and 12 ppm.

Although two separable protons due to diastereomers in NMR spectrum of **9′** using $Eu(dpm)_3$ (0.4 equiv) were confirmed it was not certain which one is threo or erythro.

Both **6′** and **7′** were also prepared by the method mentioned above, and two isomeric sulfoxides (axial and equatrial) could be separated two repeated careful column chromatography in which axial one was eluted faster than the equatorial one.

*Oxygenation with Reconstituted System Containing Purified Cytochrome P-450.* A phosphate buffer (7 ml; 0.2 M; pH 7.7) containing 60 nmol of cytochrome P-450 and 80 nmol of cytochrome P-450 reductase was incubated for 5 min at room temperature and combined with a solution of NADPH generating system (G-6-P: 250 μmol; $NADP^+$: 25 μmol and G-6-P-DH: 36 units) containing substrate (**5** or **10**) which was finely suspended into a phosphate buffer (30 ml: 0.2 M; pH 7.7) by ultrasonic vibrator. The reaction mixture was incubated understirring with a magnetic stirrer at 37 °C for 1.5 h and subjected to the same work-up procedure described in "*Oxygenation of Sulfides with Microsomes.*" Yields of **5′** and **10′** were 5.5 and 9.0 mg, respectively. The diastereomeric ratio of **5a′** and **5b′** was similarly determined to be *ca.* 25 : 75 by measuring the FT-NMR spectrum of the mixture of **5′** and $Eu(dpm)_3$ (*ca.* 0.3 equiv) in $CDCl_3$, while *e.e.* value of **10′** was *ca.* 7.5% from the integration ratio of the FT-NMR spectrum of the mixture of **10′** and $Eu(hfc)_3$ (0.5 equiv) in $CDCl_3$.

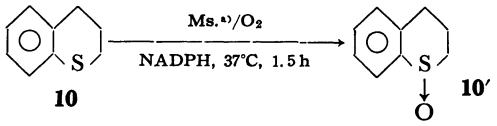
## Results and Discussion

The oxygenation of sulfides with microsomal cytochrome P-450 was carried out according to the method mentioned in Experimental section. In order to clarify co-factor requirements typical for the enzymatic oxygenation of sulfides to sulfoxides, at first control experiments were carried out.

*Control Experiments.* In order to examine whether or not the oxygenation of sulfides to sulfoxides with liver microsomes depends only on cytochrome P-450 enzyme, the following control experiments were carried out with thiochroman **10**. Carbon monooxide ($CO : O_2 = 1 : 1$ (v/v)) inhibited partially the oxygenation to the same extent as was found in our early study,[3b] while the reaction was not inhibited by SKF-525-A which is known to be a good inhibitor for a certain kind of dealkylation but does not inhibit the oxygenation of sulfur compounds.[27] DABCO (0.61 mmol), a singlet oxygen quencher, did not affect the oxygenation of **10**, which was either not affected by the addition of catalase (134 nmol) that catalyzes decomposition of hydrogen peroxide. Oxygenation either with boiled microsomes instead of microsomes or without NADPH generating system did not proceed, as was expected. Dependency of the oxygenation of sulfides with microsomes upon cytochrome P-450 enzyme, observed in these reactions, was also proved by the oxygenation with the reconstituted system with purified cytochrome P-450, as described later in this paper. Thus, the oxygenations of the various sulfides with liver microsomes obtained from phenobarbital pretreated rabbits are clearly due to the cytochrome P-450 enzyme.

*Oxygenation of Sulfide and Oxygen Consumption.* Various dialkyl, aryl alkyl, and diaryl sulfides were found to be oxygenated with the microsomal cytochrome P-450, as shown in Tables 3 and 4. However, benzothiophene was not oxygenated to the sulfoxide under the same conditions, but was recovered. Chloropromazine which is a well-known drug containing sulfur and is the first compound subjected to the oxygenation to the corresponding sulfoxide with microsomes,[3a] was also oxygenated but the yield of the sulfoxide was determined by high pressure liquid chromatography to be very small.

Table 2. Control Experiments. Relative reactivity was determined from the yield of the sulfoxide of **10** under various conditions




**10** **10′**

| System | Relative reactivity |
|---|---|
| Complete | 1.00 |
| $CO–O_2$ (1 : 1) | 0.60 |
| +SKF 525-A | 0.99 |
| +DABCO | 1.09 |
| +Catalase | 0.91 |
| −NADPH (generating sysem) | 0.15 |
| −Ms. +Boiled Ms. (100 °C, 10 min) | 0.00 |

* The oxygenation was carried out at 37 °C for 1.5 h.
a) Ms. : Microsomes.

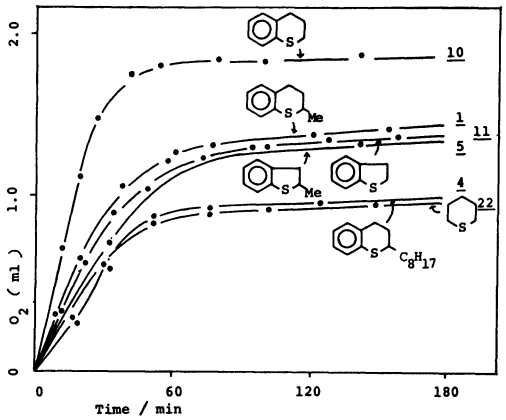
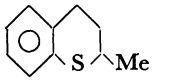
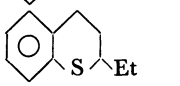
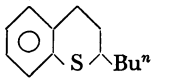
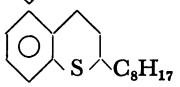
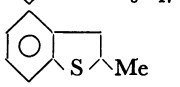
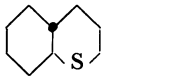
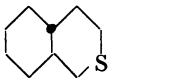
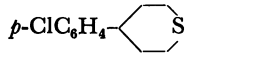



Fig. 2. Oxygen consumption in Enzymatic oxygenation of sulfides with microsomal cytochrome P-450 at 37 °C for 1.5 h was measured at a constant temperature room (24 °C).

Sulfoxides were the only products in the oxygenation. Activity of the enzyme toward various sulfides is expressed in terms of moles of product per 1.5 h per moles of cytochrome P-450, while as shown in Fig. 2, most enzymes died out after the incubation for 1 h. Since most sulfides are insoluble in water, the extent of the dispersion of the substrate into the reaction system may affect the yield of the sulfoxide. However, the yield of the sulfoxide did not change at all upon sufficient mixing of the sulfide with the buffer solution by ultrasonic vibration before the start of the oxygenation. This fact may indicate that the oxygenation takes place in a hydrophobic enzyme region.

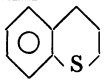
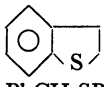
Oxygen consumption during the reaction was measured for the compounds, **1**, **4**, **5**, **10**, **11**, and **22** at a constant temperature room (Fig. 1), and was found to follow a typical mode till 90% completion in *ca.*

TABLE 3. DIASTEREOMERIC RATIO OF SULFOXIDES FORMED IN THE ENZYMATIC OXYGENATION OF RACEMIC SULFIDES WITH MICROSOMAL CYTOCHROME P-450 AT 37 °C FOR 1.5 h

| Entry No. | Substrate | | [Product/P-450][f] | $[\alpha]_D$/° | cis/trans ratio: Microsomal oxygenation, Microsomes | cis/trans ratio: Nonenzymatic oxidation, MCPBA | cis/trans ratio: Nonenzymatic oxidation, $NaIO_4$ |
|---|---|---|---|---|---|---|---|
| 1 |  | **1** | 128 | +4.0 | 18/82 | 56/44 | 55/45 |
| 2 |  | **2** | 102 | +1.8 | 18/82 | 43/57 | — |
| 3 |  | **3** | 35 | +14.3 | 16/84 | 44/56 | — |
| 4 |  | **4** | 7.6 | — | — | 51/49 | 49/51 |
| 5 |  | **5** | 71.7 | +13.1 | 19/81 | 47/53 | 48/52 |
| 6 |  | **6** | 172 | — | 37/63[a] | 30/70[a] | — |
| 7 |  | **7** | 138 | — | 29/71[a] | 30/70[a] | — |
| 8 |  | **8** | — | — | 33/67[a,b] | 33/67[a,b] | 76/24[a,c] |
| 9 | $PhCH_2SBu^s$ | **9** | 108 | +2.2 | 34/66[d] | 54/46[d] | 58/42[d,e] |

a) Ratio of axial/equatorial isomers which was calculated from isolated yields of the isomers separated by column chromatography. b) Ref. 29. c) Ref. 30. d) Ratio of threo and erythro sulfoxides (see text). e) Ratio for the oxidation with the system of $H_2O_2/MeOH/H_2SO_4$ f) Moles of product per 1.5 h per moles of cytochrome P-450 (see text).

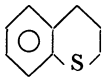
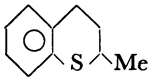
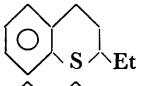
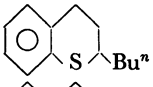
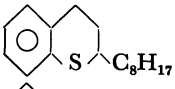
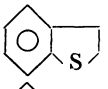
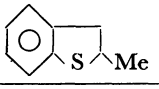
TABLE 4. ASYMMETRIC INDUCTION IN ENZYMATIC OXYGENATION OF SULFIDES WITH MICROSOMAL CYTOCHROME P-450 AT 37 °C FOR 1.5 h

| Entry No. | Substrate | | [Product/P-450][a] | $[\alpha]_D$/° | (*c*, solvent) | *e.e.*/% | Abs. confign. of sulfur | Max. rotation/° |
|---|---|---|---|---|---|---|---|---|
| 1 |  | **10** | 145 | −21.8 | (1.06, acetone) | 10.7[b] | (*R*)[c] | −202 (*R*)[e] |
| 2 |  | **11** | 120 | −7.3 | (1.10, acetone) | 2.6[b] | *R* | −285 (*R*)[20] |
| 3 | $PhCH_2SBu^t$ | **12** | 141 | +129 | (0.262, $CHCl_3$) | 53.8[b] | *R* | +240 (*R*)[21] |
| 4 | $PhCH_2SBu^n$ | **13** | 112 | +13.5 | (0.505, $CHCl_3$) | — | (*R*)[d] | — |
| 5 | $PhCH_2STol$-*p* | **14** | 44.4 | −21.8 | (0.248, $CHCl_3$) | 22.0 | *S* | +99 (*R*)[21] |
| 6 | $Ph(CH_2)_2SBu^t$ | **15** | 39.0 | +24.5 | (0.188, $CHCl_3$) | — | (*R*)[d] | — |
| 7 | *p*-$MeC_6H_4CH_2SBu^t$ | **16** | 93.0 | +49.6 | (0.488, $CHCl_3$) | 19.8[b] | *R* | −250 (*S*)[21] |
| 8 | *p*-$TolSBu^t$ | **17** | 10.5 | −80.0 | (0.135, acetone) | 46.8 | *S* | +171 (*R*)[21] |
| 9 | *p*-TolSMe | **18** | 188 | +25.6 | (0.648, $CHCl_3$) | 14.1 | *R* | +181 (*R*)[21] |
| 10 | $C_6H_{17}SBu^t$ | **19** | 26.5 | +33.3 | (0.102, $CHCl_3$) | — | (*R*)[d] | — |
| 11 | $C_6H_5SC_6H_4OMe$-*o* | **20** | 27.8 | −21.2 | (0.133, EtOH) | 9.6 | *S* | −221 (*S*)[31] |
| 12 | $Bu^tSBu^t$ | **21** | 138 | — | — | — | — | — |

a) Molar ratio. b) Some of the *e.e.* values were re-confirmed by NMR spectra using $Eu(hfc)_3$. c) *R* configuration is expected by comparing signs of its specific rotation and CD spectrum with those of **11′** (No. 2). d) *R* configuration is assumed, since most of (+)-sulfoxides like these have *R* configuration.[21] e) Calculated value from $[\alpha]_D$ and *e.e.* value.

45—60 min in all cases. This observation indicates that the lifetime of the enzyme is independent on the structure of the sulfide used. However, the amount and the rate of the oxygen consumption differed for each substrate but were parallel to the yields of the sulfoxides as shown in Tables 3 and 4. Since the rate of oxygen

Table 5. Relative reactivity of sulfides. Total turnover (product mol/cytochrome P-450 mol) and efficiency of oxygen used during the oxygenation (product mol/$O_2$ absorbed mol), in the oxygenation with microsomal cytochrome P-450 at 37 °C for 1.5 h

| Substrate | | Product (mol)[a] / P-450 (mol) | Product (mol)[a] / $O_2$(mol) |
|---|---|---|---|
|  | **10** | 145 | 35 |
|  | **1** | 128 | —[b] |
|  | **2** | 102 | —[b] |
|  | **3** | 35 | —[b] |
|  | **4** | 7 | 0 |
|  | **11** | 120 | 48 |
|  | **5** | 72 | 36 |

a) Product is sulfoxide. b) Not determined.

absorption into the reaction solution is sufficiently fast, the rate of oxygen consumption (initial slope) should be comparable to that of the oxygenation of each sulfide.

Meanwhile dependency of the yield of the sulfoxide on the substrate structure was confirmed with both thiochroman and 2,3-dihydrobenzothiophene derivatives. Relative reactivities of these sulfides toward cytochrome P-450 decrease with the increase of the bulkiness of the alkyl substituent (Table 5). Thus, in the oxygenations of these cyclic sulfides with microsomal cytochrome P-450, the reactivities of the sulfides seem not to depend on the hydrophobicity of the substituent but to depend largely on the steric hindrance of the substituent.

On the other hand, in the oxygenation of acyclic sulfides such clear structure-reactivity relationship was observed. Even bulky *t*-butyl group on the sulfur atom did not hinder the formation of the sulfoxide, *i.e.* *t*-butyl *p*-tolyl sulfide (**17**), di-*t*-butyl sulfide (**21**), and *t*-butyl octyl sulfide (**19**) were equally well oxygenated to the corresponding sulfoxides, although the oxygenation of 2-octylthiochroman (**4**) hardly proceeded. No good correlation between the yields of the sulfoxides and the structures of the sulfides, has also been reported for the *in vivo* oxygenations of the similar sulfides with *Aspergillus niger*.[23] This is comparable with the results obtained with *in vitro* oxygenation with microsomal cytochrome P-450 in this study. Therefore, based only on the yields of the sulfoxides in the oxygenations of both cyclic and acyclic sulfides, the steric hindrance by bulky substituents on sulfur atom may not always be crucial in the enzymatic oxygenation.

*Diastereotopic Differentiating Reactions.* There is always a possibility that two diastereomers are obtained in the oxidation of racemic sulfide which has an asymmetric center: for example, 2-substituted thiochromans and 2-substituted 2,3-dihydrobenzothiophene would give both cis and trans sulfoxides, while thiadecalins may give both axial and equatorial sulfoxides. In order to examine how much the diastereotopic differentiation would take place, these sulfides were oxidized enzymatically and nonenzymatically to the sulfoxides. In many cases, two diastereomeric sulfoxides were separated in pure forms, while in the others such a separation of two diastereomers was unsuccessful, and hence the ratio o the diastereomers was determined by NMR spectroscopy using an NMR shift reagent. NMR signals of the aromatic ring protons at 7-position of both cis and trans 2-methyl-2,3-dihydrobenzothiophene 1-oxides (**5a'** and **5b'**), appear in the same region. However, upon addition of an NMR shift reagent ($Eu(dpm)_3$) to the sulfoxide mixture, these signals of the cis and trans isomers shifted to down fields in different extents. Using pure authentic samples of *cis*-**5'** and *trans*-**5'** whose structures were determined by Anderson *et al.*[16] the 7-H signal of the trans isomer was found to appear at an extremely down field as compared to that of the cis isomer, so that the two signals of 7-H's of *cis*-**5'** and *trans*-**5'** can be seen separately on the NMR spectrum. Similarly, the cis/trans ratios of **1'**—**4'** obtained by both enzymatic and nonenzymatic oxidations of the corresponding sulfides were successfully determined by the relative intensities of the two NMR signals due to the protons at 8-position of the products in the presence of $Eu(dpm)_3$. The larger down field shift of the proton of the trans isomer of **1'**—**5'** than that of the cis one is presumably due to the distance between the hydrogen at 8-(or 7-) position and the europium complex bound to the oxygen of the sulfoxide. Actually, an examination with a CPK model reveals that the distance between the hydrogen of the trans sulfoxide and the shift reagent is shorter than that of the cis one. In Table 3, are summarized the results which reveal that the formation of the trans sulfoxide exceeds that of the cis isomer in the enzymatic oxygenation of the cyclic sulfides each bearing an alkyl group at $\alpha$-position. However, the nonenzymatic oxidation with MCPBA and $NaIO_4$ gave nearly equal amounts of both isomers. Both *trans*-thiadecalins (**6** and **7**) gave the equatorial sulfoxides predominantly in the enzymatic and the nonenzymatic oxidations. Probably the enzyme, which has a very bulky porphyrinoxenoid at the active site,[2] would interact with the sulfide in a certain geometry, prior to the oxygenation in the enzym pocket. NMR spectroscopic analysis using the shift reagent ($Eu(dpm)_3$) of both the enzymatic and the nonenzymatic oxidations of racemic benzyl *s*-butyl sulfide (**9**), revealed that two diastereomeric sulfoxides, *threo*- and *erythro*-**9'** were formed in the both oxidations. The diastereomeric ratio of **9'** obtained by the enzymatic oxygenation was found to be considerably different from that of the MCPBA oxidation product (Table 3). Unfortunately, it could not be assigned which diastereomer was threo or erythro, since no authentic sample of the pure diastereomer was available.

*Enantiotopic Differentiating Reactions.* The extent of asymmetric induction in the enzymatic oxygenation of prochiral sulfides was determined, and the absolute configurations of the sulfoxides formed were also examined. The values of enantiomeric excess (*e.e.*) of the sulfoxides obtained by the enzymatic oxygenations were determined by measuring their specific rotations in comparison with the reported maximum optical rotations and/or the NMR spectroscopies using an optically active shift reagent, $Eu(hfc)_3$. Although the *e.e.* values of some of the linear acyclic sulfoxides were difficult to be determined even with the use of the shift reagent, cyclic sulfoxides such as **10′** and **11′** were easily determined by the NMR spectroscopy. For example, the doublet signals due to the aromatic proton at 8-position of **10′** of *R*-configuration appeared in the down field than that of **10′** of *S*-configuration separately in the presence of the shift reagent. The results are listed in Table 4. Inspection of the data in Table 4 reveals clearly that the asymmetric induction took place in the enzymatic oxygenation of all the sulfides. The maximum *e.e.* value of the sulfoxide obtained was 53.8% (Entry No. 3) which is unexpectedly large despite the role of the enzyme to oxidize indiscriminatingly any xenobiotic substances only to be excreted. The small *e.e.* values in the oxygenations of cyclic sulfides (**10** and **11**) may be due to the difficulty in recognition of the prochiral sulfides by the enzyme, because of the structural similarity of these prochiral sulfides, *i.e.* the two substituents attached to sulfur atom. The *e.e.* values in Table 4 should indicate that the extent of the enantiotopic differentiation in the enzymatic oxygenation of sulfides increases with the increase of the bulkiness of the substituents bound to the sulfur atom. A similar trend also has been observed in the *in vivo* oxygenations of sulfides with *Aspergillus niger*.[21]

*R*-Configuration was assigned to the major enantiomer of **10′** (Table 4, No. 1) obtained by the enzymatic oxygenation based on the same signs of both its specific rotation and CD spectrum (Fig. 3) as those of *R*-2,3-dihydrobenzothiophene 1-oxide (*R*-**11′**) of which the absolute configuration was already known,[20] since there is no serious structural difference between *R*-**10′** and *R*-**11′**. As for sulfoxides whose absolute configurations are not yet known (Entry Nos. 4, 6, and 10), all their configurations are considered to be of *R*-form since the configurations of most of dextro-rotatory acyclic sulfoxides of similar structures are known to be of *R*-form.[23] Thus, in the oxygenation of sulfides, **1**—**11** with cytochrome P-450 enzyme the formation of sulfoxides of *R*-configuration is prefered. When the stereospecific oxygenations of sulfides (**12**, **14**, **16**, **17**, and **18**) are compared with those of *in vivo* oxygenations with *Aspergillus niger* (for 3, 5, 7, 8, and 9 of entry in Table 4),[23] signs of the absolute configurations of these sulfoxides except for **18** are opposite (for 3, 5, 7, and 8 of entry).

Figure 4 illustrates the two directions of the oxygenations on the sulfur atom of the prochiral sulfide ($R_S$–*S*–$R_L$), when a more bulky group ($R_L$) is placed in a rear side, regardless of the sequence rule. Actually, the order of the bulkinesses of the substituents is not always

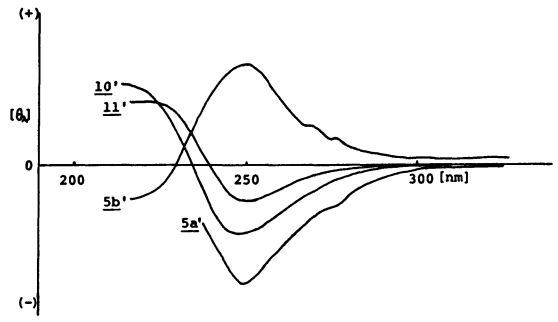



Fig. 3. CD spectra of sulfoxides obtained in the enzymatic oxygenation with microsomal cytochrome P-450 at 37 °C for 1.5 h. The spectra were recorded using EtOH as a solvent, though the concentration of the sulfoxide was not determined.

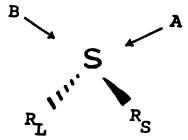



Fig. 4. Direction of the oxygenation of prochiral sulfide. More bulky substituent of the sulfide ($R_L$) was placed in rear side.

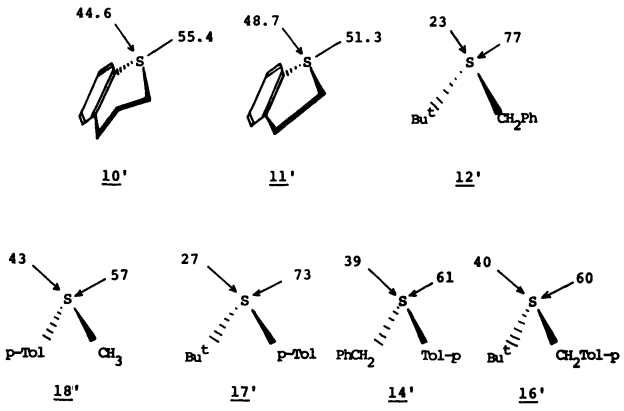



Fig. 5. Direction of the oxygenation of various prochiral sulfides with liver microsomes at 37 °C for 1.5 h. Numerical values indicate the ratios of the oxygenation at sulfur atom to produce *R* or *S*-sulfoxides.

consistent with that of the sequence rule in the *R*,*S*-nomenclature. Figure. 5 shows the actual directions of the oxygenations of various sulfides used in this study and indicates that "the direction A predominates over the direction B" in all cases (with only one exception, *i.e.* diaryl sulfide **21**), where the bulkiness is assumed to decrease in the following order: *t*-Bu>*p*-Tol$CH_2$> $PhCH_2$>*p*-Tol>Ph>Me.

Although the enzymatic action of the hepatic cytochrome P-450 induced by phenobarbital should be sterically insensitive because of its indiscriminative nature of the metabolism of various xenobiotics, the enzyme-catalyzed reaction prefers to take the same steric course "Direction A" and this enantiotopic differentiation becomes greater as the difference of the bulkiness between the two substituents ($R_S$ and $R_L$)

Table 6. Optical activity of separated diastereomers in the oxygenation with microsomal cytochrome P-450 at 37 °C for 1.5 h

| Sulfoxide | $[\alpha]_D^{25}$/° (*c*, solvent) | *e.e.*/% (Abs. confign.) |
|---|---|---|
| *cis*-**5′** | −37.5 ± 7.5 (0.08, acetone) | 40[a] ($R_sR_c$) |
| *trans*-**5′** | +15.8 ± 2.5 (0.24, acetone) | 12[a] ($S_sR_c$) |
| *axial*-**6′** | −22.0 ± 2.5 (0.236, $CHCl_3$) | — |
| *equatorial*-**6′** | −0.5 ± 1.5 (0.398, $CHCl_3$) | — |
| *axial*-**7′** | +2.5 ± 3.8 (0.160, $CHCl_3$) | — |
| *equatorial*-**7′** | +1.5 ± 1.5 (0.400, $CHCl_3$) | — |

a) Determined by NMR.

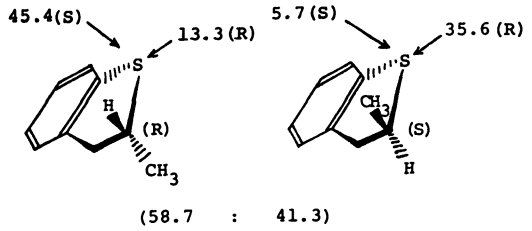



Fig. 6. Calculated ratio of the oxygenation products in the oxygenation of enantiomers of racemic sulfide **5** with microsomal cytochrome P-450 at 37 °C for 1.5 h.

bound to sulfur atom becomes larger.

*Concurrent Diastereotopic and Enantiomer Differentiating Reaction.* The earlier section dealt only with the ratios of the diastereomers in the enzymatic oxygenations of the racemic sulfides (**1**—**7** and **9**), despite that the mixtures of the cis and trans sulfoxides formed in this oxygenation were shown to be chiral and have some specific rotation values (Table 3). Table 6 shows the optical activities of the two separable diastereomers in the oxygenation with microsomal cytochrome P-450.

Determination of the absolute yields of the four stereoisomers was attempted in the enzymatic oxygenation of a racemic sulfide **5**. Two diastereomers of 2-methyl-2,3-dihydrobenzothiophene 1-oxides (*cis*-**5′** and *trans*-**5′**) were easily separated by TLC on alumina but diastereomeric mixtures of the other sulfoxides such as 2-substituted thiochroman 1-oxide derivatives were not separated. The values of *e.e.* of the two diastereomers were obtained from the FT-NMR spectra using $Eu(hfc)_3$. The optical activity of the *cis*-**5′** (40% *e.e.*) was relatively higher than that of the *trans*-**5′** (12% *e.e.*) (Table 6). From the CD spectra (Fig. 3) of the two diastereomers, the absolute configurations of the sulfur atoms of the enantiomerically excess sulfoxides of *cis*-**5′** and *trans*-**5′** are presumed to be of *R* for the cis form and of *S* for the trans form, respectively, on the basis of the comparison of the signs of the CD spectra with those of the other sulfoxides as shown in Fig. 3. Signs of the specific rotations also support the assignments. Thus, the extent of relative contributions of four possible steric courses in the oxygenation of racemic **5** can be shown in Fig. 6. The results shown in Fig. 6 reveal the following two features:

1) Enantiomer differentiation of racemic **5** is quite small; *i.e.* the enzymatic oxygenation of $R_c$-**5** is only 1.4 times greater than that of $S_c$-**5** (rate ratio, $S_c$-**5** : $R_c$-**5**=41 : 59 in Fig. 6).

2) In the enzymatic oxygenation of $S_c$-**5**, the formation of the *trans*-$R_sS_c$-**5**, predominates over that of the cis isomer by 6.2 folds (Course A : Course B=6.2 : 1, in Fig. 4). The formation of the *trans*-$S_sR_c$-**5′** predominates over that of cis isomer, however, only 3.4 folds (Course A: Course B=1 : 3.4 in Fig. 4) in the oxygenation of $R_c$-**5**. Apparently, the diastereotopic differentiation is more important than the enantiomer differentiation to determine the steric course in the enzymatic oxygenation of racemic **5**. Thus, the stereospecific enzymatic oxygenation of racemic **5** may be summarized at the following rules.

1) The oxidant approaches preferentially from the less hindered side, anti to α-methyl group.

2) When the sulfide **5** is placed in a manner as shown in Fig. 6, the oxygenation *via* the Course A is more favored than that *via* the Course B.

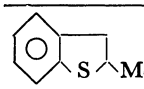
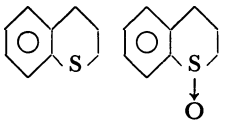
3) The rule 1 is more important than the rule 2.

As for *trans*-1- and 2-thiadecalin 1-oxides **6′** and **7′**, two diastereomers, *i.e.* axial and equatorial forms, were readily separated. All these diastereomers isolated were found to have some specific rotations (Table 6). However, unfortunately, neither the absolute configurations nor *e.e.* values of these optically active sulfoxides could be determined, because there were no authentic samples of the optically active sulfoxides.

*Oxygenation with Reconstituted System with Purified Cytochrome P-450 and Co-factors.*[32] In order to prove that the microsomal oxygenations depend upon the catalysis of cytochrome P-450 enzyme but not any other concomitant in the microsomes, the oxygenations of **5** and **10** with the reconstituted enzyme system containing co-factors, were carried out. The standard oxygenating system is made up with purified cytochrome P-450 (30 nmol), cytochrome reductase (80 nmol), G-6-P (250 μmol), $NADP^+$ (20 μmol), G-6-PDH (36 units), and 0.2 M phosphate buffer (pH 7.7, 30 ml) in a total volume of 37.5 ml. The oxygenation of racemic **5** with this system afforded a mixture of two diastereomeric sulfoxides (**5′**) with the ratio of cis/trans of 25/75 which is practically identical to that in the oxygenation with the microsomes (Table 7). The enantiomeric excess of the sulfoxide in the oxygenation of **10** with the reconstituted system with purified cytochrome P-450 was also identical to that with the microsomal oxygenation, and the sulfoxide of the *R*-form was produced preferentially over the *S*-form, as in the oxygenation with the microsomes (Tables 4 and 7).

Ratio of the yield of **5′** to that of **10′** (or total turnover ratio) in the oxygenation with the reconstituted system was nearly consistent with that in the microsomal oxygenation. Thus, it is concluded that cytochrome P-450 enzyme is responsible for the oxygenation of sulfide in the microsomal oxygenation in this work. Surprizing is the difference of the total turn-over

Table 7. Oxygenation of racemic **5** and **10** with reconstituted system with purified cytochrome P-450, at 37 °C for 1.5 h (see Experimental section)

| Substrate | Product (sulfoxide) | Turnover[a] ($min^{-1}$) | Total[b] turnover (—) | Total[c] turn-over with Ms. (—) |
|---|---|---|---|---|
|  **5** | *cis*-**5′** (25%)[d] / *trans*-**5′** (75%)[d] | 12 | 1104 | 72 |
|  **10** | **10′** | (*R*)[e] *e.e.* 7.5% | 20 | 1807 | 145 |

a) Product (mol)/cytochrome P-450 (mol)/min. b) Product (mol)/cytochrome P-450 (mol) with reconstituted system with purified cytochrome P-450. c) Product (mol)/cytochrome P-450 (mol) in the oxygenation with microsomes (see Tables 3 and 4). d) Ratio of *cis*-**5′** and *trans*-**5′**. e) Estimated absolute configuration.

number between the oxygenations with microsomes and with the purified cytochrome P-450. In the oxygenations with the reconstituted systems the total turn-over numbers are more than 10 times of those in the microsomal oxygenations (Tables 3, 4, and 7).

## Conclusion

Various dialkyl, aryl alkyl, and diaryl sulfides were oxygenated readily with the cytochrome P-450 enzyme to the corresponding sulfoxides while the sulfone or hydroxylated product was not obtained (or too small to be detected).[33] The amount of oxygen consumed in a set period was parallel to the reactivity of the sulfide or the yield of the sulfoxide. The yield of the sulfoxide depends on the structure of the sulfide in 2-substituted thiochroman derivatives, and the bulky substituent on the thiochroman markedly decreased the reactivity. Hydrophobicity appears not to be an important factor for the reactivity of sulfides, since it has no relation with the yields of the acyclic sulfoxides, as shown in Table 4. Based on the various inhibition experiments and the stereochemical results obtained by the liver microsomal oxygenation of sulfide consistent with those obtained by the reconstituted system with the purified liver microsomal cytochrome P-450, the oxygenation of sulfides with rabbit liver microsomes is concluded to be catalyzed undoubtedly by the cytochrome P-450 enzyme. Relatively large asymmetric inductions were obtained in the oxygenations of various sulfides. Assuming the following order of the bulkiness, *t*-Bu>*p*-Tol$CH_2$>Ph$CH_2$>*p*-Tol>Ph>Me, the oxygenation *via* the Course A (Fig. 4) predominates significantly over that *via* the Course B. The extent of this enantiotopic differentiation in the enzymatic oxygenation of the prochiral sulfide increases with the increase of the difference of the bulkiness between the two substituents on the sulfur atom. In the enzymatic oxygenation of racemic α-substituted cyclic sulfides, the diastereotopic differentiation is more important than the enantiomer differentiation in the stereochemical course of the oxygenation. Thus, the following stereoselectivity rule may be derived.

1) The enzymatic oxygenation of the cyclic sulfide takes place predominantly at the opposite side of the alkyl substituent at α-position, to form mainly the trans sulfoxide rather than the cis one.

2) When the sulfide is represented as shown in Fig. 4, the Course A is more preferable than the Course B.

3) The rule 1 is more important than the rule 2. Thus, the stereospecificity in the oxygenation of sulfides with the aid of cytochrome P-450 clearly reveals that although the enzymatic oxygenation of xenobiotic compounds in living boides may not require any stereospecificity because the metabolic oxygenation is supposed to increase only the hydrophobicity of the oxygenated compounds, the enzyme is assymetric and hence the enzymatic oxygenation of sulfides results in the partial asymmetric induction.

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33) A study on the oxygenation of a sulfoxide to a sulfone with cytochrome P-450 has been reported, see Ref. 4c as an example.