Stereochemisty of Oxygenation of Organic Sulphides with Pig Liver Microsomal FAD-containing Mono-oxygenase: Comparison with Cytochrome P-450$_{PB}$ Oxidations

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The enantiotopic differentiating ability of pig liver microsomal FAD-containing mono-oxygenase (EC 1.14.13.8) in the oxygenation of nine unsymmetrical sulphides has been investigated. By this enzymatic oxygenation, the sulphides are converted into the corresponding optically active sulphoxides with varying degrees of enantiomeric excess (96–12%).

Three cyclic sulphides have been studied in order to establish the diastereotopic differentiating ability of the enzyme. In the oxygenation of racemic 2-methyl-2,3-dihydrobenzo[*b*]thiophene both enantiomeric and diastereotopic differentiation appears to take place concurrently with high selectivity. The enantiotopic, diastereotopic, and enantiomeric differentiating abilities of the FAD-containing mono-oxygenase are all higher than those of the phenobarbital-induced rabbit liver microsomal cytochrome P-450.

Two liver microsomal mono-oxygenases, *i.e.* FAD-containing mono-oxygenase (EC 1.14.13.8)[1] and phenobarbital-induced cytochrome P-450 (cytochrome P-450$_{PB}$),[2] are known to promote oxygenation of lipophilic foreign substances prior to excretion. The former possesses a flavin adenine dinucleotide (FAD) as a prosthetic group and the latter a protoheme IX. Both mono-oxygenases have similar substrate specificities. However, cytochrome P-450$_{PB}$ covers a much wider range of substances than the FAD-containing mono-oxygenase. The FAD-containing mono-oxygenase adds oxygen to compounds which possess nucleophilic heteroatoms, such as trivalent nitrogen and divalent sulphur. The FAD-containing mono-oxygenase has been purified and characterized for the first time by Ziegler and his co-workers.[1] Organic sulphides are good substrates for the two enzymes and are converted into sulphoxides at the expense of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen [equation (1)];[1d,3–6] however, the mechanisms of these two enzymatic oxygenations are quite different.[6]

$$R\text{–}S\text{–}R' + NADPH + O_2 + H^+ \longrightarrow R\text{–}\overset{\overset{O}{\uparrow}}{S}\text{–}R' + NADP^+ + H_2O \quad (1)$$

The oxygenation of a sulphide with the FAD-containing mono-oxygenase involves direct nucleophilic substitution on the peroxidic oxygen of the flavin hydroperoxide contained in the active site of the enzyme, while that with the cytochrome P-450 is initiated by a single-electron oxidation of the sulphide to form a sulphenium cation radical which then combines with the oxygen atom attached to the heme iron at the active site of the enzyme.[6]

Previously we reported detailed stereochemical studies of the oxygenation of sulphides to the corresponding sulphoxides with rabbit liver microsomal cytochrome P-450$_{PB}$.[5] However, stereochemical studies with the FAD-containing mono-oxygenase have been reported for only one tertiary amine, *i.e.* *N*-ethyl-*N*-methylaniline[7] and three sulphides.[8,9] Of these the oxygenation of ethyl *p*-tolyl sulphide is the only example in which both the absolute configuration and the optical yield of the resulting products were reported.[8]

Thus, we have carried out stereochemical studies on the oxygenation of organic sulphides with pig liver microsomal FAD-containing mono-oxygenase focussing our attention on the following two points. One is a comparison of the stereoselectivities and stereospecificities of the oxygen addition to various sulphides by the FAD-containing mono-oxygenase with those by the phenobarbital-induced cytochrome P-450. The other is the influence of octylamine and pH on the stereochemistry of the oxygenation with the FAD-containing mono-oxygenase. Although tertiary and secondary amines are excellent substrates for this enzyme, primary amines are not oxygenated with the FAD-containing mono-oxygenase; instead, certain primary amines bearing a long alkyl chain, *e.g.* n-octylamine, are known to accelerate this enzymatic oxygenation.[1a] Both the rate of the reaction and the stimulating effect of n-octylamine depend on the pH of the medium. The effect of n-octylamine reaches a maximum at pH 9.0, while at pH 7.4 the oxygenation proceeds at the same rate regardless of either the presence of the absence of octylamine.[10] Thus, the stereochemistry of the enzymatic oxygenation has been investigated both at low and high pH and with and without octylamine.

Experimental

Materials.—Nicotinamide adenine dinucleotide phosphate ($NADP^+$), glucose 6-phosphate (G6P), and G6P-dehydrogenase were purchased from Oriental Yeast. Catalase was purchased from Boehringer Mannheim. Unsymmetrical sulphides and sulphoxides were prepared by the method reported in our previous paper.[5b] The pig liver microsomal FAD-containing mono-oxygenase was purified by the method of Ziegler and Poulsen,[1b] except that laurate AH-Sepharose 4B column[11] was used for the final step. Tris(dipivaloylmethanato)europium(III), Eu(dpm)$_3$, and tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III), Eu(hfc)$_3$, were from Dojin Chemical and Aldrich, respectively. Alumina for thin layer chromatography (aluminium oxide G, type 60/E) was obtained

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Table 1. Stereochemical results of oxygenation of unsymmetrical sulphides with pig liver microsomal FAD-containing mono-oxygenase.[a]

Substrate[h]	Reaction conditions: pH	Reaction conditions: Octylamine (3 mM)	Sulphoxide formed: Optical rotation $[\alpha]_D^{25°}$ (*c*, solvent)	Sulphoxide formed: Enantiomer[b] excess (%)	Sulphoxide formed: Absolute configuration
(1)	7.4	−	+155° (0.60, $CHCl_3$)	96	*R*[d]
	7.4	+	+109° (0.45, $CHCl_3$)	96	*R*
	9.0	−	+141° (0.29, $CHCl_3$)	96	*R*
	9.0	+	+107° (0.28, $CHCl_3$)	96	*R*
(2)	7.4	−		65	*R*[d]
	9.0	−	+160° (2.89, $CHCl_3$)	61	*R*
	9.0	+	+118° (0.49, $CHCl_3$)	59	*R*
(3)	7.4	−	−33° (0.75, $CHCl_3$)	33	*S*[d]
(4)	7.4	−	+31° (1.19, $CHCl_3$)	12.4	*R*[d]
	7.4	+	+32° (0.63, $CHCl_3$)	14.8	*R*
(5)	7.4	−	−87° (0.72, Me_2CO)	51[c]	*S*[d]
(6)	7.4	−	+141° (0.40, EtOH)	64[c]	*R*[e]
(7)	7.4	−	−210° (1.04, Me_2CO)	77	*R*[f]
(8)	7.4	−	−122° (1.44, Me_2CO)	60	*R*[g]
	7.4	+	−101° (0.40, Me_2CO)	58	*R*
	9.0	−		56	*R*
	9.0	+		56	*R*

[a] At 37 °C. [b] Determined by FT-NMR method. [c] Determined by optical rotation. [d] B. J. Auret, D. R. Boyd, H. B. Henbest, and S. Ross, *J. Chem. Soc. C*, 1968, 2371. [e] D. R. Rayner, A. J. Gordon, and K. Mislow, *J. Amer. Chem. Soc.*, 1968, **90**, 4854. [f] F. G. Yamagishi, D. R. Rayner, E. T. Zwicker, and D. J. Cram, *J. Am. Chem. Soc.*, 1973, 95, 1916. [g] Ref. 5. [h] Structures of sulphides are shown in Table 2.

from Merck. Triton X-100 was purchased from Nakarai Chemical.

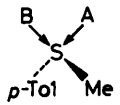
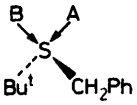
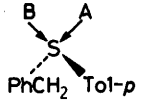
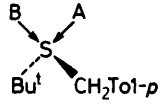
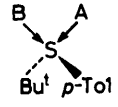
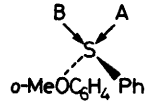
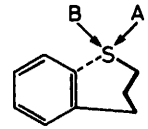
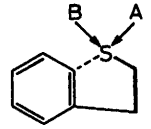
Stereochemistry of the Oxygenation of Sulphide.—To a 100-ml round-bottomed flask fitted with a magnetic stirring bar were added 0.1M potassium phosphate buffer (pH 7.4; 25 ml) or tris(hydroxymethyl)aminomethane (Tris)-HCl buffer (pH 9.0), the NADPH generating system [consisting of G6P (117 mg, 343 μmol) and 50 mM $NADP^+$ (1.2 ml, 60 μmol)], and 10 μM catalase (0.1 ml). The pH of the solution was adjusted to 7.4 or 9.0 by adding small amounts of 0.5M K_2HPO_4 or 0.5M Tris, respectively. The flask was placed in a water-bath thermostatted at 37 °C and to it were added 1.3 μM FAD-containing mono-oxygenase, 50 μl of G6P-dehydrogenase (5 units), and 0.5 ml of methanol solution containing 240 μM (*ca.* 30 mg) of an unsymmetrical sulphide. The mixture was stirred for 2 h while O_2 was bubbled through it. The metabolites were extracted as follows. The reaction mixture was saturated with NaCl and extracted 6 times each with 23-ml portions of chloroform. The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness to give a mixture of the starting material and the sulphoxide. The sulphoxide was then isolated from the crude mixture by alumina TLC (eluant: chloroform). The purified sulphoxide was subjected to optical rotation measurement with a JASCO digital polarimeter model DIP-140 to determine which isomer was preferentially afforded. The sulphoxide was recovered to determine the enantiomeric excess (e.e.) by Fourier transform nuclear magnetic resonance spectroscopy (FT-NMR) using a chiral shift reagent, $Eu(hfc)_3$. The protons of the sulphoxides utilized for this purpose are as follows: methyl *p*-tolyl sulphoxide, *S*-protons; benzyl t-butyl sulphoxide, t-butyl protons; *p*-methylbenzyl t-butyl sulphoxide, t-butyl protons; 2,3-dihydrobenzo[*b*]thiophene 1-oxide, the proton at the 7-position; benzo[*b*]thiophene 1-oxide, the proton at the 8-position; 2-methyl-2,3-dihydrobenzo[*b*]thiophene 1-oxide, the proton at the 7-position and the 2-methyl protons. In the oxygenation of racemic 2-methyl-2,3-dihydrobenzo[*b*]thiophene, (*R,S*)-(**9**), the diastereoisomeric ratio (*cis*:*trans*) of the sulphoxide formed was determined by FT-NMR spectrometry using $Eu(dpm)_3$ and utilizing the signal for 7-H. The two diastereoisomers were separated by TLC on alumina and subjected to FT-NMR analysis using $Eu(hfc)_3$ to determine the e.e. values. NMR spectra were recorded on Hitachi R-6000 and JEOL FX-100 FT-NMR instruments. The results are listed in Table 1. In all cases, the sulphoxides obtained were identified with authentic samples.

Results and Discussion

The following three modes of stereochemical differentiation have been looked at in the enzymatic oxygenation of sulphides with pig liver microsomal FAD-containing mono-oxygenase. (*a*) Since an unsymmetrical sulphide has an enantiotopic face, the enzyme differentiates the enantiotopic face to result in preferential formation of one enantiomeric sulphoxide over the other. (*b*) When a sulphide has a diastereotopic face, the mono-oxygenase recognizes the face to give a predominance of one diastereoisomeric sulphoxide. (*c*) When a racemic sulphide which possesses an asymmetric carbon is added to the mono-oxygenase, one of the enantioisomers should be oxygenated preferentially over the other to give, eventually, kinetic resolution of the racemic sulphide.

Enantiotopic Differentiating Oxygenation.—The e.e. value of the sulphoxide formed by the enzymatic oxygenation of a prochiral sulphide was determined by means of FT-NMR spectrometry using a chiral shift reagent. Only when suitable protons were not present in the sulphoxide molecule to be analysed by this NMR technique, was the e.e. value estimated on the basis of the optical rotation. Direction of the oxygen addition to unsymmetrical sulphides by this enzyme are compared with those with the cytochrome $P\text{-}450_{PB}$ [5] as shown in Table 2. There are two possible directions to add oxygen to the prochiral sulphur atom of an unsymmetrical sulphide, A (from right) and B (from left) as illustrated in Figure 1. In Table 2, the unsymmetrical sulphides are illustrated so that the preferred direction of the oxygen addition is in direction A. Inspection of data in Table 2 reveals that the α-carbon of the substituent (R_L) in the rear side is more highly branched than that of the front side substituent (R_S). The same trend has been observed earlier in the oxygenation of unsymmetrical sulphides with cytochrome $P\text{-}450_{PB}$. However, the oxygenation with the

Table 2. Enantiotopic differentiation in the oxygenation of unsymmetrical sulphides in 0.1M potassium phosphate buffer (pH 7.4) at 37 °C.

								
Substrate	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
FAD-containing mono-oxygenase	2:98(*R*)	17:83(*R*)	34:66(*S*)	44:56(*R*)	25:75(*S*)	18:82(*R*)	12:88(*R*)	15:85(*R*)
PB-induced rabbit[a] liver microsomes	43:57(*R*)	23:77(*R*)	39:61(*S*)	40:60(*R*)	27:73(*S*)	55:45(*S*)	49:51(*R*) (47:53(*R*)[b]	45:55(*R*)

[a] Ref. 5. [b] Oxygenation was carried out by the reconstituted system with the purified cytochrome P-450$_{PB}$.

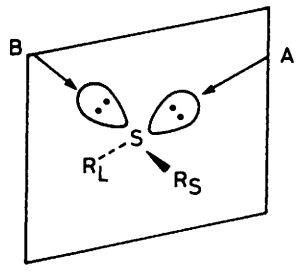


Figure 1. Steric courses of oxygen addition.

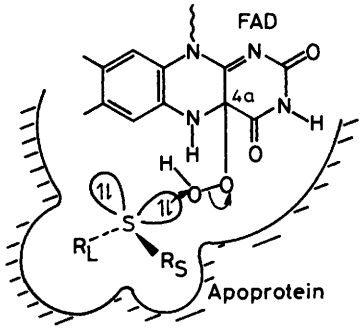


Figure 2. Model of active site for FAD-containing mono-oxygenase.

FAD-containing mono-oxygenase is sterically much more highly controlled than that with cytochrome P-450$_{PB}$. For example, the FAD-containing mono-oxygenase can recognize the enantiotopic face very well in some cases with nearly 100% selectivity [methyl *p*-tolyl sulphide, (**1**)], while the highest e.e. value observed in the oxygenation with the cytochrome P-450 is only 54% [benzyl t-butyl sulphide (**2**)]. The other remarkable difference in the enantiotopic differentiating abilities of these two liver microsomal mono-oxygenases is that higher e.e. values for sulphoxides are observed in the oxygenation of smaller sulphide molecules than big ones in the oxygenation with the FAD-containing mono-oxygenase, while cytochrome P-450$_{PB}$ can better differentiate the enantiotopic face of the relatively large molecules than that of sulphides bearing small substituents. This trend is further exemplified in the oxygenation of the sulphide (**9**) as mentioned later. These stereochemical observations suggest that the size of the binding pocket for the FAD-containing mono-oxygenase is smaller than that for cytochrome P-450$_{PB}$. Hence a substrate whilst it is tightly fixed in the enzyme cavity of the FAD-containing mono-oxygenase, is only loosely so in that of the cytochrome P-450$_{PB}$; a substrate therefore has more freedom to rotate in cytochrome P-450$_{PB}$ than in the FAD-containing mono-oxygenase.

One attractive explanation may be as follows. The actual reactive site in the FAD-containing mono-oxygenase reaction has been suggested by Poulsen and Ziegler[1f] and Beaty and Ballou[11] to be the hydroperoxy group of the enzyme 4a-hydroperoxyflavin intermediate, E(4a-FlOOH). The oxygen addition to a divalent sulphur atom occurs by direct nucleophilic attack of the sulphur atom on the terminal peroxidic oxygen of the enzyme hydroperoxide.[6] Since the reactive hydroperoxy group juts from the chiral 4a-carbon of the flavin plane, whose absolute configuration is not yet known, the apoprotein can tightly surround this active terminal peroxidic oxygen to provide a relatively tight chiral substrate binding pocket forming a rigid E(4a-FlOOH)·sulphide complex (Figure 2). This assumption is in accord with quite small K_m values for FAD-containing mono-oxygenase catalysed reactions.[1d,10,12] On the other hand, the protoheme IX oxenoid is believed to be the actual oxidant in the cytochrome P-450 promoted oxygenation.[13–16] The oxygen addition step to a sulphide has been suggested to be the coupling of the sulphenium cation radical and iron(IV) oxenoid formed by the single electron transfer from the sulphide to the iron(V) oxenoid which is equivalent to the horseradish peroxidase compound I.[4] The active oxygen in the iron(IV) oxenoid which corresponds to the horseradish peroxidase compound II is bound directly to the centre of the large heme plane. The substrate binding pocket of the cytochrome P-450$_{PB}$ constructed with flat heme and the apoprotein is rather large, forming a relatively loose cytochrome P-450-sulphide complex; it has therefore only a moderate enantiotopic plane of the prochiral sulphenium cation radical (Figure 3). Indeed, the apparent K_m values of the oxygenation of sulphides with cytochrome P-450$_{PB}$ are 1–2 orders of magnitude greater than those with the FAD-containing mono-oxygenase.[3b,10] Further, the dissociation constants of the cytochrome P-450$_{PB}$ substituted thiane complexes are known to decrease with an increase in the size of the alkyl substituent on the thiane ring despite the increase of the bulkiness.[3b]

When the pH of the medium is changed from 7.4 to 9.0 (optimum pH), v_{max} of the oxygenation of (**1**) increases 3 times at 24 °C.[10] Octylamine, which is known to accelerate this enzymatic reaction, did not affect the rate at pH 7.4, but accelerated it at higher pH, the effect becoming optimum at pH 9.0. Thus, in order to examine the effect of pH and octylamine on the stereochemistry of the oxygenation of sulphides, four sulphides were selected from the sulphide listed in Table 1.

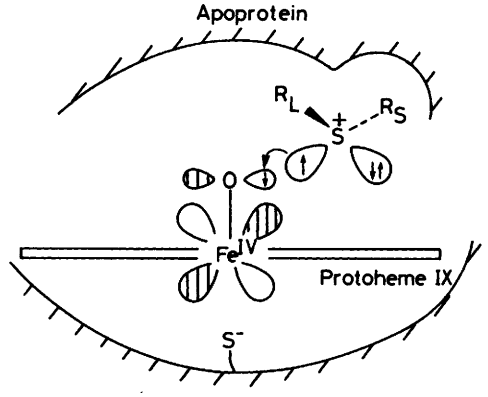


Figure 3. Model of active site for PB-induced cytochrome P-450.

Those are (**1**) whose oxygenation is highly stereoselective, (**2**) and 2,3-dihydrobenzo[*b*]thiophene (**8**) whose oxygenation is moderately stereoselective, and t-butyl *p*-methylbenzyl sulphide (**4**) the oxygenation of which is poorly stereoselective at pH 7.4 without octylamine. Neither the change of pH nor the presence of octylamine was found to change the e.e. values of the sulphoxides formed in the oxygenation of these sulphides (Table 1); this suggests that these changes of the oxygenation conditions do not alter the size and the shape of the substrate binding pocket of the FAD-containing mono-oxygenase.

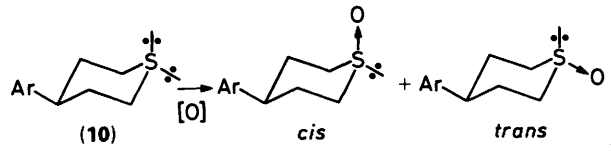
Diastereotopic Differentiating Oxygenation.—Two cyclic sulphides, (**9**) and 4-*p*-chlorophenylthiane (**10**) were examined in order to observe the diastereotopic differentiating ability of the FAD-containing mono-oxygenase (Schemes 1, 2). The results are summarized in Table 3 together with those of cytochrome P-450$_{PB}$ and the enzyme model systems.

Diastereotopic differentiation is one good way to examine the stereochemical features of the oxygenation of sulphides by achiral or racemic enzyme model systems.[17,18] Both FAD-containing mono-oxygenase and racemic 3-methyl-5-ethyl-4a-

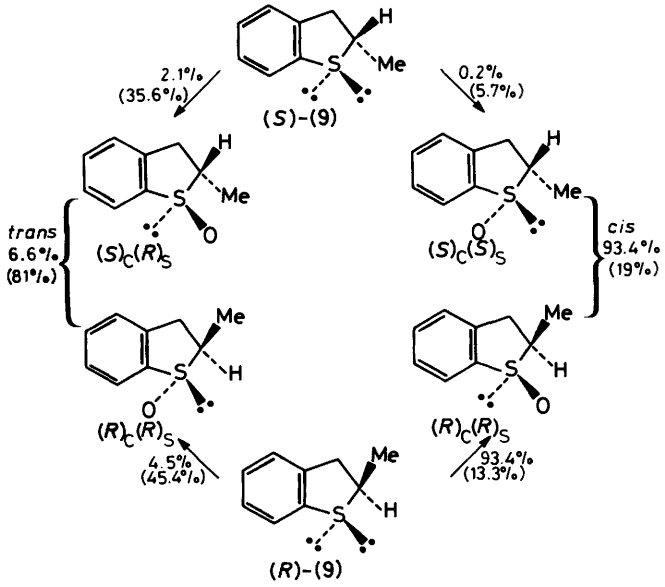
Table 3. Diastereotopic differentiating oxygenation of cyclic sulphides, (**10**), (*R*)-(**9**) and (*S*)-(**9**), with FAD-containing mono-oxygenase and PB-induced P-450 and enzyme model systems.

Starting sulphide	*cis*:*trans* Ratio of sulphoxide formed					
	FAD-containing[a] mono-oxygenase	4a-FlEtOOH[b]	mCPBA	H_2O_2[c]	PB-induced[d] P-450	$TPPFe^{III}Cl/Im/H_2O_2$[e]
(**10**)	6:94	33:67	33:67[c]	30:70	34:66	
(*R*)-(**9**)	95:5	18:82	47:53[d]	23:77	23:77	24:76
(*S*)-(**9**)	9:91	18:82	47:53[d]		14:86	24:76

[a] This work. [b] Ref. 18. [c] C. R. Johnson and D. McCant, Jr., *J. Am. Chem. Soc.*, 1965, **87**, 1109. [d] Ref. 5. [e] Ref. 17.



Scheme 1. Ar = C_6H_4Cl-*p*.



Scheme 2. Concurrent enantiomer and diastereotopic differentiating oxygenation of racemic sulphide, (*R*,*S*)-(**9**), with pig liver microsomal FAD-containing mono-oxygenase[a] and rabbit liver PB-induced P-450[b] in 0.1M potassium phosphate buffer, pH 7.4 at 37 °C.

a Conversion of starting (*R*,*S*)-(**9**) is 9%. *b* Stereochemical results for PB-induced P-450 are shown in parenthesis (Takata *et al.*, 1983).

hydroperoxyflavin (4a-FlEtOOH), a model of the active species of the FAD-containing mono-oxygenase, which was prepared at first by Kemal and Bruice,[19] can add oxygen to the sulphide (**10**) from the sterically less hindered equatorial direction to afford the *trans* sulphoxide preferentially. Other typical electrophilic oxidants, *m*-chloroperbenzoic acid (*m*CPBA) and hydrogen peroxide, gave the same stereochemical results as the FAD-containing mono-oxygenase, although the stereoselectivity is less than that of the enzymatic oxygenation with the FAD-containing mono-oxygenase. Cytochrome $P\text{-}450_{PB}$ also gave preferentially the *trans* sulphoxide with lower stereoselectivity than with the FAD-containing mono-oxygenase.

All simple electrophilic oxidants (*m*CPBA, H_2O_2, 4a-FlEtOOH), cytochrome $P\text{-}450_{PB}$, and chloromesotetraphenylporphinatoiron(III)/imidazole/hydrogen peroxide ($TPPFe^{III}Cl/Im/H_2O_2$), an enzyme model for cytochrome P-450, add oxygen in a manner such as to avoid steric hindrance by the α-methyl group and afford the *trans* sulphoxide. However, the FAD-containing mono-oxygenase differentiates the diastereotopic face of (*R*)-(**9**) and (*S*)-(**9**) in opposite manners; *i.e.* (*R*)-(**9**) gives the *cis*-sulphoxide but (*S*)-(**9**) affords the *trans* sulphoxide in high diastereoisomeric excess, 90 and 82%, respectively. This suggests that the α-methyl group does not control primarily the stereochemistry of the oxygenation of (**9**) with the FAD-containing mono-oxygenase. This will be discussed further below.

Concurrent Enantiomeric and Diastereotopic Differentiating Oxygenation of Racemic 2-Methyl-2,3-dihydrobenzo[b]thiophene, (*R*,*S*)-(**9**).—In order to determine the inherent reactivities of (*R*)-(**9**) and (*S*)-(**9**) in the oxygenation with the FAD-containing mono-oxygenase, the oxygenation of the racemic sulphide (*R*,*S*)-(**9**) was stopped at a low conversion in order to avoid a change in the ratio of concentrations of (*R*)-(**9**) and (*S*)-(**9**) with the progress of the reaction by kinetic resolution. When the incubation of (*R*,*S*)-(**9**) with the FAD-containing mono-oxygenase was stopped after 9% of the starting sulphide had been consumed, a mixture of 93% of the *cis* sulphoxide and 7% of the *trans* sulphoxide was obtained. The *cis* and *trans* isomers were separated by TLC. The *cis* sulphoxide was found to be almost completely levorotatory by FT-NMR spectrometry using a shift reagent. In the oxygenation of the *trans* isomer, the levorotatory sulphoxide was again found to be the major component. According to the assignment of the absolute configuration in our previous work,[5] these levorotatory sulphoxides are $(R)_C(R)_S$ and $(R)_C(S)_S$ isomers, respectively. The steric courses of the oxygenation of (*R*,*S*)-(**9**) with the FAD-containing mono-oxygenase are illustrated in Scheme 2 together with cytochrome $P\text{-}450_{PB}$. The stereochemistry of the oxygenation of (*R*,*S*)-(**9**) with the FAD-containing mono-oxygenase is completely different from that with the cytochrome $P\text{-}450_{PB}$. The following two factors may determine the stereochemistry of the oxygenation of (*R*,*S*)-(**9**) with the FAD-containing mono-oxygenase. (*a*) Regardless of the configuration of the α-carbon bearing methyl group, the oxygen addition take place highly preferentially on the *pro-R* lone pair of the prochiral sulphur atom, suggesting that the 2,3-dihydrobenzo[*b*]thiophene skeleton controls the direction of the oxygenation of (**9**). This skeleton is quite similar to that of the sulphide (**1**) in which the oxygenation takes place on the *pro*-(*R*) lone pair of the divalent sulphur atom with high selectivity. (*b*) The methyl group at the α-carbon in (*S*)-(**9**) greatly decreases the reactivity of (*S*)-(**9**). Consequently, (*R*)-(**9**) is oxidized 43 times as fast as (*S*)-(**9**) by the FAD-containing mono-oxygenase. This enantiomeric differentiation (kinetic resolution) should leave the starting sulphide (**9**) enriched with the less reactive (*S*)-(**9**). In fact, the sulphide which was recovered after 30% conversion of (**9**) exhibited optical activity, $[\alpha]_D^{24}$ −14° (*c* 9.7, $CHCl_3$).

On the other hand, the stereochemistry of the oxygenation of (*R*,*S*)-(**9**) with cytochrome P-450 is primarily controlled by the steric effect of the methyl group at the α-carbon; *i.e.* the oxygen addition takes place preferentially from the opposite, less

hindered, side to the methyl group to afford the *trans* sulphoxides in the oxygenation of both (*R*)-(**9**) and (*S*)-(**9**). These results also support our argument on the stereoselectivity dependence on the substrate binding pockets of the enzymes. Namely, the oxygenation of (**9**) is tightly controlled in a relatively small cavity of the active site of the FAD-containing mono-oxygenase, while in the relatively large cavity on the plane of the protoheme IX in the cytochrome P-450, the sulphide (**9**) can orient freely so that the addition to both (*R*)-(**9**) and (*S*)-(**9**) occurs eventually from the less hindered side to afford the *trans* sulphoxide regardless of the configuration of the α-carbon.

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