CHEMISTRY LETTERS, pp. 1441-1444, 1980.

STEREOCHEMISTRY OF SULFOXIDES BY ENZYMATIC OXYGENATION OF SULFIDES WITH RABBIT LIVER MICROSOMAL CYTOCHROME P-450

Toshikazu TAKATA, Mayumi YAMAZAKI, Ken FUJIMORI,
Yong Hae KIM, Shigeru OAE*, and Takashi IYANAGI #

*Department of Chemistry, The University of Tsukuba,
#Division of Biochemistry, School of Medicine, The University of Tsukuba,
Sakura, Niihari, Ibaraki 305

Asymmetric induction and diastereomeric ratio in the enzymatic oxygenation of various sulfides to the corresponding sulfoxides with hepatic microsomal cytochrome P-450 obtained from phenobarbital pretreated rabbit were investigated in comparison with those of nonenzymatic oxidations with m-chloroperbenzoic acid and sodium metaperiodate. While substantial asymmetric inductions were observed in the sulfoxides formed by the enzymatic oxygenations, diastereomeric ratios of the sulfoxides formed were also quite different from those obtained by oxidation with m-chloroperbenzoic acid and sodium metaperiodate.

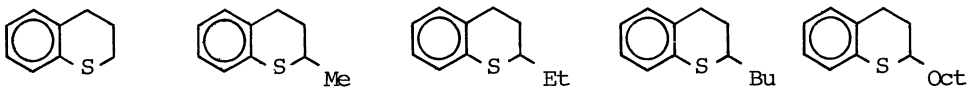
Cytochrome P-450, a monoxygenase having protohemin, is present in multiple forms in mammalian tissues.[1] Some cytochrome P-450's in adrenal gland, cytochrome $P\text{-}450_{11\beta}$ and cytochrome $P\text{-}450_{17\alpha}$, participate in the regiospecific and stereospecific hydroxylations of the steroids, while cytochrome P-450's in plants carry out specific biosyntheses of biologically important substances.[1] Meanwhile, one major role of cytochrome P-450 is known to be the oxygenation of various kinds of lipophilic xenobiotics, such as drugs, insecticides, food additives etc., to the more hydrophilic metabolites for excretion of these foreign substances.[1] In such metabolic oxygenations in liver microsomes stereospecificity may not be necessary because the main function of the oxygenation is to convert the foreign substances to other products which can be excreted readily out of living bodies. Since the cytochrome P-450 is knwon to play such a role, the enzyme should be less substrate selective and less stereospecific with regard of the binding site of the enzyme. No detailed stereochemical study, however, has appeared in the oxygenation by hepatic cytochrome P-450. Thus, we have investigated the stereochemistry of the oxygenation of various sulfides to the corresponding sulfoxides by rabbit liver microsomal cytochrome P-450 in both microsomal level and reconstituted system with purified cytochrome P-450.[2]

In order to induce the formation of the enzyme which oxidizes xenobiotics including sulfides, rabbits were pretreated with phenobarbital for 5 days.[3]

The sulfide(ca. 0.5 mmole) was incubated with rabbit liver microsomes(protein: 38 mg/ml, P-450: 2.67 nmole/mg protein)[2] together with NADPH generating system(G-6-P: 250 μmole, $NADP^+$: 25 μmole and G-6-P dehydrogenase: 20 units) in phosphate buffer(0.2 M, pH 7.4, total volume 17 ml) at 37°C for 1.5 h. After the incubation, acetone(ca. 20 ml) was added to the reaction mixture and then 3 M trichloroacetic acid was added to make the solution nearly pH 2.0. The resulted heterogeneous mixture was subjected to centrifugation at 3,000 rpm for 20 min to remove protein. The supernatant was collected and extracted with chloroform. The chloroform layer was washed with 2 M KOH and then water. The residue after evaporation of chloroform contained the sulfoxide(maximum 0.2 mmole) and the unreacted sulfide along with a small amount of impurity from protein. TLC fractionation of the residue(alumina, ethyl acetate - benzene) afforded the pure sulfoxide but no appreciable amount of sulfone and hydroxylated product were detected.

Ratios of the cis / trans isomers were determined by NMR spectra using a shift reagent, $Eu(DPM)_3$(Table I, No. 1 - 5 and 9) but some of sulfoxides(No. 6 - 8) were isolable in sufficient quantities to determine the ratios of axial and equatorial isomers. Enantiomeric excesses(Table II) for all the sulfoxides were determined from the values of specific rotation and the known value of maximum rotation and/or by NMR measurement using a shift reagent, $Eu(hfc)_3$. Selected results on stereochemical feature are listed in Table I and II.

Oxygen absorption during the reaction of some cyclic sulfides were also measured. The consumptions of oxygen showed typical sigmoid curves and reached 90 % completion of the reaction after 45 - 60 min in each case. The amount of oxygen consumption was found to be parallel to the yield of the sulfoxide. The yields of the sulfoxides in the microsomal oxygenations of a series of 2-substituted-5,6-thiochromanes with various alkyl groups are shown below.

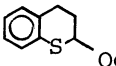
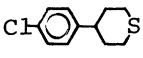


$\frac{\text{Product [mole]}}{\text{P-450 [mole]}}$	145	128	102	35	7

Obviously relative reactivities of these sulfides toward cytochrome P-450 decrease with the increase of the bulkiness of the alkyl substituent despite the increasing hydrophobicity.

These oxygenation reactions with liver microsomes are obviously caused by the catalytic action of cytochrome P-450, since carbon monoxide did inhibit the oxygenation in some extent, while neither DABCO, a singlet oxygen quencher, nor catalase, a catalyst of decomposition of hydrogen peroxide, affected the oxygenation at all, as we have shown earlier in the oxygenation of disulfide with the reconstituted system with purified cytochrome P-450.[2] Further, the identical stereochemical results were observed in the oxygenations of a few cyclic sulfides both by the liver microsomes and by the reconstituted system with purified cytochrome P-450 isolated from the same microsomes.[4] Namely, not only the ratio

Table I Cis/Trans Ratio of Sulfoxides by Enzymatic and Nonenzymatic Oxygenation

Entry No.,	Substrate,	cis / trans Microsomal Oxygenation,	MCPBA or	$NaIO_4$ Oxidation
1		18 / 82	56 / 44	55 / 45
2		18 / 82	43 / 57	-
3		16 / 84	44 / 56	-
4		-	51 / 49	49 / 51
5		19 / 81	47 / 53	48 / 52
6		37 / 63[a]	30 / 70[a]	-
7		39 / 71[a]	30 / 70[a]	-
8		33 / 67[a,b]	33 / 67[a,b]	76 / 24[a,c]
9	$PhCH_2SBu^{sec}$	34 / 66[d]	54 / 46[d]	58 / 42[e]

a) Ratio of ax/eq which was determined by isolated yields by column chromatography. b) ref. 5. c) ref. 6. d) Either ratio of threo/erythro or erythro/threo. e) Ratio by the oxidation with the sytem of $H_2O_2/MeOH/H_2SO_4$.

Table II Asymmetric Induction in Enzymatic Oxygenation of Sulfide

Entry No.,	Substrate,	$[\alpha]_D$(c, solvent)[a]	e.e.[b]	Abs. Confign.,	Direction of Oxygenation
1		-21.8°(1.06,acetone)	10.8%	-(R)[c]	(A)
2		-7.3°(1.10,acetone)	2.6	R	A
3	$PhCH_2SBu^t$	+129°(0.262, $CHCl_3$)	53.8	R	A
4	$PhCH_2SBu^n$	+13.5°(0.505, $CHCl_3$)	-	-(R)[d]	A
5	$PhCH_2STol\text{-}p$	-21.8°(0.248, $CHCl_3$)	22.0	S	A
6	$PhCH_2CH_2SBu^t$	+24.5°(0.188, $CHCl_3$)	-	-(R)[d]	A
7	$p\text{-}TolCH_2SBu^t$	+49.6°(0.488, $CHCl_3$)	19.8	R	A
8	$p\text{-}TolSBu^t$	-80.0°(0.135,acetone)	46.8	S	A
9	p-TolSMe	+25.6°(0.648 , $CHCl_3$)	14.1	R	A
10	$C_8H_{17}SBu^t$	+33.3°(0.102, $CHCl_3$)	-	-(R)[d]	A
11	$PhSC_6H_4OMe\text{-}o$	-21.2°(0.133, EtOH)	9.5	S	B

a) $[\alpha]_D$ value was obatined using 5 cm(length) quartz cell. b) Some of e.e. values were checked by NMR using shift reagent. c) R configuration is expected by comparing sign of specific rotation and CD spectra with those of No.2. d) R configuration is considered since most of (+)-sulfoxides like these have R configuration.

of cis/trans isomers obtained in the microsomal oxygenation of 2-methyl-2,3-dihydrobenzothiophene, but also the absolute configuration and the enantiomeric excess of the sulfoxide by the microsomal oxygenation of thiochromane were found to be identical to those with the reconstituted system with purified cytochrome P-450.

Inspection of data in Table I undoubtedly reveals that while nearly the same amounts of both cis and trans isomers were obtained in nonenzymatic oxidations of these sulfides, the formation of trans sulfoxide predominated over that of cis isomer in the enzymatic oxygenation of substituted cyclic sulfoxides(No. 1 - 5). These results can be rationalized in terms of that the electrophilic attack of very bulky porphyrin-oxenoide[1] on the divalent sulfur takes place predominantly along the less hindered side of the sulfide.

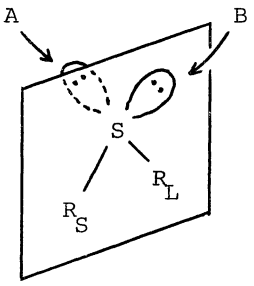


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Figure Direction of Oxygenation

Data in Table II show rather clearly that the oxygenation of the sulfide with phenobarbital pretreated rabbit liver microsomal cytochrome P-450, tends to proceed via a definite steric course.[7] Namely when R_L and R_S denote bulky group and less bulky one, respectively, in the prochiral sulfide(I), the enzymatic oxygenation along the direction of A appears predominant over that along the direction of B in all cases shown in Table II, where bulkiness of substituent is considered to decrease in the following order, t-Bu > p-TolCH_2 > $PhCH_2$ > p-Tol > Ph > Me.

Acknowledgement: This research was supported by Toray Science and Technology Prize and Grants for 1980 and Grant-in-Aid for Scientific Research B of The Ministry of Education, Science and Culture for 1980 which are gratefully acknowledged.

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(Received September 17, 1980)