Berman, H. M., Sowri, A., Ginell, S., & Beveridge, D. (1988) *J. Biomol. Struct. Dyn. 5*, 1101–1110.
Chevrier, B., Dock, A. C., Hartmann, B., Leng, M., Moras, D., Thuong, M. T., & Westhof, E. J. (1986) *J. Mol. Biol. 188*, 707–719.
Domoradzki, J., Pegg, A. E., Dolan, M. E., Maher, V. M., & McCormick, J. J. (1984) *Carcinogenesis 5*, 1644–1647.
Fuji, F., Wang, A. H.-J., van der Marel, G., van Boom, J. H., & Rich, A. (1982) *Nucleic Acids Res. 10*, 7879–7892.
Gaffney, B. L., & Jones, R. A. (1989) *Biochemistry 28*, 5881–5889.
Gaffney, B. L., Marky, L. A., & Jones, R. A. (1984) *Biochemistry 23*, 5686–5691.
Gerson, S. L., Trey, J. E., Miller, K., & Benjamin, E. (1987) *Cancer Res. 47*, 89–95.
Gessner, R. V., Frederick, C. A., Quigley, G. J., Rich, A., & Wang, A. H.-J. (1989) *J. Biol. Chem. 264*, 7921–7935.
Ginell, S. L., Narendra, N., Jones, R., Berman, H. M., & Russu, I. M. (1990) *Biophys. J. 57*, 452a.
Hendrickson, W. A., & Konnert, J. H. (1979) in *Biomolecular Structure, Conformation, Function and Evolution* (Srinivasan, R., Ed.) pp 43–57, Pergamon, Oxford.
Ho, P. S., Quigley, G. J., Tilton, R. F., & Rich, A. (1988) *J. Chem. Phys. 92*, 939–945.
Hope, H. (1988) *Acta Crystallogr. B44*, 22–26.
Howard, A. J., Gilliland, G. L., Finzel, B. C., Poulos, T. L., Ohlendorf, D. H., & Salemme, F. R. (1987) *J. Appl. Crystallogr. 20*, 383–387.
Jones, T. A. (1978) *J. Appl. Crystallogr. 11*, 268–272.
Kuzmich, S., Marky, L. A., & Jones, R. A. (1983) *Nucleic Acids Res. 11*, 3393–3404.
Patel, D. J., Shapiro, L., Kozlowski, S., Gaffney, B. L., & Jones, R. A. (1986) *Biochemistry 25*, 1027–1036.
Pauley, G. T., Powers M., Pei, G. K., & Moschel, R. C. (1988) *Chem. Res. Toxicol. 1*, 391–397.
Singer, B., & Grunberger, D. (1983) *Molecular Biology of Mugatens and Carcinogens*, Plenum, New York.
Sussman, J. L., Holbrook, S. R., Church, G. M., & Kim, S.-H. (1977) *Acta Crsytallogr. A33*, 800–804.
Wang, A. H.-J., Quigley, G. J., Kolpak, F. J., Crawford, J. L., van Boom, J. H., van der Marel, G., & Rich, A. (1979) *Nature 282*, 680–686.
Wang, A. H.-J., Hakoshima, T., van der Marel, G., van Boom, J. H., & Rich, A. (1984) *Cell 37*, 321–331.
Westhof, E., Dumas, P., & Moras, D. (1985) *J. Mol. Biol. 184*, 119–145.
Zarbl, H., Sukumar, S., Arthur, A. V., Martin-Zanca, D., & Barbacid, M. (1985) *Nature 315*, 382–385.

Enantioselective Oxidations of Sulfides Catalyzed by Chloroperoxidase†

S. Colonna,* N. Gaggero, and A. Manfredi

Dipartimento di Chimica Organica e Industriale, Centro CNR, Università di Milano, Via Golgi 19, Milano, Italy

L. Casella and M. Gullotti

Dipartimento di Chimica Inorganica e Metallorganica, Centro CNR, Università di Milano, Via Venezian 21, Milano, Italy

G. Carrea and P. Pasta

Istituto di Chimica degli Ormoni, CNR, Via Mario Bianco 9, Milano, Italy

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ABSTRACT: The chloroperoxidase-catalyzed and horseradish peroxidase catalyzed oxidations of sulfides by *tert*-butyl and other peroxides have been investigated. The former metal enzyme afforded the corresponding sulfoxides having *R* absolute configuration in up to 92% enantiomeric excess (ee), whereas the latter gave racemic products. The various factors that control the enantioselectivity of the oxygenation have been examined.

Chloroperoxidase (CPO) is an enzyme produced by the marine fungus *Caldariomyces fumago* (Morris & Hager, 1966). It is a glycoprotein with M_r 42 000 containing ferriprotoporphyrin IX as the prosthetic group. A great deal of work, with a variety of spectroscopic techniques, has focused on the identification of the fifth axial ligand to the heme, and there is now strong proof that this ligand is a cysteine thiolate, which is bound to a high-spin five-coordinate ferric ion in the native protein (Dawson, 1988; Bangcharoenpaurong et al., 1986). Recently, amino acid and Edman sequence analysis revealed the axial ligand to be cysteine 29 (Blanke & Hager, 1988). The close similarity in the active site structure between chloroperoxidase and cytochrome P-450 corresponds to similar enzymatic activity in a number of instances, e.g., the N-demethylation of certain organic substrates (Kedderis et al., 1980; Padbury & Sligar, 1985) and the epoxidation of cyclohexene (McCarthy & White, 1983) and styrene (Ortiz de Montellano et al., 1987), but chloroperoxidase exhibits a broader spectrum of chemical reactivities, including the reactions typical of peroxidases (Thomas et al., 1970), the use of halide ions to halogenate a variety of organic acceptor molecules (Hager et al., 1966; Libby et al., 1982), and the catalase activity in the disproportionation of hydrogen peroxide (Frew & Jones, 1984). Therefore, chloroperoxidase shares similar properties with classical heme peroxidases, which catalyze the H_2O_2-dependent one-electron oxidation of organic and inorganic substrates (Dunford, 1982), and with P-450 monooxygenases, which transfer an oxygen atom from oxygen donors to their substrates (Ortiz de Montellano, 1986).

While there are a few reactions promoted by peroxidases

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that involve incorporation of oxygen into the product, the origin of the oxygen is generally not H_2O_2 (Ortiz de Montellano et al., 1987; Kobayashi et al., 1984; Kedderis et al., 1986). The only exception seems to be the sulfoxidation of thioanisoles, for which oxygen incorporation from peroxide has been demonstrated (Kobayashi et al., 1986, 1987). Since both chloroperoxidase (Kobayashi et al., 1986, 1987; Doerge, 1986) and cytochrome P-450 (Takata et al., 1983) perform such sulfoxidation, this may represent the only reaction promoted by the three hemoproteins that proceeds through a common transfer step.

We have been interested for some time in protein-promoted enantioselective oxidations of organic sulfides (Colonna et al., 1985, 1986), and in this paper we amplify upon our preliminary report on the asymmetric sulfoxidations catalyzed by chloroperoxidase (Colonna et al., 1988). In particular, we examined the various factors that control the enantioselectivity of the oxidation of sulfides to sulfoxides and the time course of the oxygenation:

$$\underset{\textbf{1–18}}{R\text{–}S\text{–}R'} \xrightarrow[CPO]{[O]} \underset{\textbf{19–36}}{R\text{–}SO\text{–}R'}$$

Materials and Methods

General Methods. The optical rotations were determined with a Perkin-Elmer R 241 polarimeter. The ^1H NMR spectra of the products were recorded in $CDCl_3$ on a Varian 390 instrument. Enantiomeric excesses were determined by ^1H NMR with the aid of $Eu(hfc)_3$ as a chiral shift reagent on a Varian XL 200 instrument or by HPLC analysis on a Daicel Chiralcel OB column (Ohta et al., 1989).

Materials. The sulfides used were methyl *p*-tolyl sulfide (**1**), methyl *o*-tolyl sulfide (**2**), *p*-methoxyphenyl methyl sulfide (**3**), *o*-methoxyphenyl methyl sulfide (**4**), methyl phenyl sulfide (**5**), *p*-chlorophenyl methyl sulfide (**6**), methyl *p*-nitrophenyl sulfide (**7**), ethyl *p*-tolyl sulfide (**8**), methyl 2-naphthyl sulfide (**9**), benzyl methyl sulfide (**10**), 1,3-dithiane (**11**), *n*-butyl methyl sulfide (**12**), methyl 2-pyridyl sulfide (**13**), *n*-octyl *p*-tolyl sulfide (**14**), *tert*-butyl 1-(*p*-tolylthio)acetate (**15**), isopropyl *p*-tolyl sulfide (**16**), benzyl *p*-tolyl sulfide (**17**), and *p*-acetamidophenyl methyl sulfide (**18**).

Sulfides **1**, **3**, **5**, **6**, **7**, and **18** were prepared according to the literature (Kobayashi et al., 1987). Sulfides **8**, **10**, **16**, and **17** were obtained as previously described (Sugimoto et al., 1981). Sulfides **4**, **9**, and **13** were obtained as already described (Pitchen et al., 1984). Sulfides **12** and **14** were prepared following Landini's et al. (1988) procedure. Methyl *o*-tolyl sulfide (**2**) and *tert*-butyl 1-(*p*-tolylthio)acetate (**15**) were obtained as previously reported (Vowinkel, 1974). 1,3-Dithiane (**11**) was a commercial product (Fluka).

Chloroperoxidase from *Caldariomyces fumago* (RZ 0.6) and horseradish peroxidase (type VI, mainly isoenzyme C) were obtained from Sigma.

Catalytic Oxidations: Typical Procedure. The sulfide (0.5 mmol) and CPO (8×10^{-6} mmol) were magnetically stirred in 50 mL of aqueous citrate buffer solution, pH 5 at 4 °C, for 5 min. Then the oxidant (1 mmol) in 5 mL of buffer solution, pH 5, was added in 1.5 h. The mixture was kept under stirring at 4 °C for the appropriate time, and the progress of the reaction was monitored by TLC. Extraction with four portions (60 mL each) of diethyl ether and then with two portions (60 mL each) of chloroform, evaporation of the organic layer, and drying gave the crude product. This was purified by flash chromatography (SiO_2) with mixtures of diethyl ether and methanol as eluents. In the recycling experiment the same procedure was followed; the aqueous solution of CPO, after extraction with organic solvents, was used instead of the fresh sample of the enzyme. When the oxidation of **1** with *t*-BuOOH was repeated with a concentration of the enzyme 5 times larger than that under the usual conditions for 4 days, the corresponding sulfoxide was obtained in 73% chemical yield and 80% ee. The yield refers to the conversion of the sulfides into the corresponding sulfoxides.

Table I: Oxidation of Methyl *p*-Tolyl Sulfide with Chloroperoxidase and Various Oxidants in Aqueous Buffer, pH 5 at 4 °C

oxidant	time (days)	yield (%)	abs config	ee (%)[a]
t-BuOOH	8	60	*R*-(+)	86
H_2O_2	0.5	48	*R*-(+)	35
cumyl-OOH	7	52	*R*-(+)	5
PhIO	11	49		0
Ph_3COOH	10	40		0
MCPBA	1	30		0

[a] Determined from the specific rotation of the product and/or by ^1H NMR spectroscopy with $Eu(hfc)_3$ as a chiral shift reagent.

Table II: Oxidation of Sulfides with *tert*-Butyl Hydroperoxide and Chloroperoxidase at 4 °C in Aqueous Buffer, pH 5

sulfide	time (days)	yield (%)	abs config	ee (%)[a]
(**1**) *p*-$CH_3C_6H_4SCH_3$	8	60	*R*-(+)	86
(**2**) *o*-$CH_3C_6H_4SCH_3$	10	27	*R*-(+)	19
(**3**) *p*-$CH_3OC_6H_4SCH_3$	8	71	*R*-(+)	92
(**4**) *o*-$CH_3OC_6H_4SCH_3$	9	33	*R*-(+)	25
(**5**) $C_6H_5SCH_3$	8	100	*R*-(+)	76
(**6**) *p*-$ClC_6H_4SCH_3$	8	44	*R*-(+)	85
(**7**) *p*-$NO_2C_6H_4SCH_3$	4	7	*R*-(+)	39
(**8**) *p*-$CH_3C_6H_4SCH_2CH_3$	10	40	*R*-(+)	30
(**9**) 2-naphthyl-SCH_3	9	0		0
(**10**) $C_6H_5CH_2SCH_3$	9	51	*R*-(−)	91
(**11**) 1,3-dithiane	10	63	*R*-(+)	20
(**12**) *n*-$C_4H_9SCH_3$	8	54	*R*-(−)	38
(**13**) 2-pyridyl-SCH_3	5	72	*R*-(+)	65
(**14**) *p*-$CH_3C_6H_4SC_8H_{17}{}^n$	15	13		0
(**15**) *p*-$CH_3C_6H_4SCH_2COOBu^t$	21	2		0
(**16**) *p*-$CH_3C_6H_4SC_3H_7{}^i$	22	24		0
(**17**) *p*-$CH_3C_6H_4SCH_2C_6H_5$	14	0		0
(**18**) *p*-$CH_3CONHC_6H_4SCH_3$	10	8		0

[a] Determined from the specific rotation of the product and/or by ^1H NMR spectroscopy with $Eu(hfc)_3$ as a chiral shift reagent.

When the oxidation of **1** with *t*-BuOOH was repeated with a concentration of the enzyme 5 times larger than that under the usual conditions for 4 days, the corresponding sulfoxide was obtained in 73% chemical yield and 80% ee. The yield refers to the conversion of the sulfides into the corresponding sulfoxides.

Oxidation in the Presence of BSA. BSA was dissolved in the aqueous buffer solution, pH 5 (50 mL), containing the sulfide at 4 °C under stirring. Then CPO and the oxidant were added to the reaction mixture following the usual procedure. The product was purified as indicated above.

Oxidation in the Presence of Acetone. The reactions were carried out under the conditions described for the typical procedure in the presence of 5 mL of acetone as a cosolvent.

Oxidation with HRP. The usual oxidation procedure was followed by replacing CPO with HRP. Concentration of the enzyme, reaction time, and chemical yield are reported in Table III.

Characteristics of the Sulfoxides. Sulfoxides **19–26** were all known to be in the optically active form, and the physical properties of our specimens were in agreement with those reported (Pitchen et al., 1984; Dunach & Kagan, 1985; Auret et al., 1983; Laur et al., 1965). Yields and ee are reported in Tables I and II.

Results and Discussion

The sulfoxidation of several organic sulfides by *tert*-butyl hydroperoxide, or other oxidants, in the presence of chloroperoxidase (1.6×10^{-5} molar equiv) was investigated in buffered aqueous solution at pH 5. The crude products were isolated by column chromatography and the enantiomeric

Table III: Oxidation of Sulfides with Horseradish Peroxidase[a] and Various Oxidants in Aqueous Buffer, pH 5 at 4 °C

sulfide	oxidant	time (days)	yield (%)[b]
1	*t*-BuOOH	8	30
1	H_2O_2	8	29
1	cumyl-OOH	1	50
3	*t*-BuOOH	2	24
3	H_2O_2	9	22
3[c]	*t*-BuOOH	1	27

[a] Molar ratio of HRP/substrate: 1.04×10^{-3}. [b] All the products were racemic. [c] With 1.5 molar equiv of oxidant and 1.56×10^{-3} molar equiv of enzyme.

excesses were determined by optical rotations, by ^1H NMR spectroscopy, and/or by HPLC analysis. Methyl *p*-tolyl sulfide was chosen as the standard substrate since the corresponding sulfoxide is optically stable to pyramidal inversion. Table I contains the results obtained in the oxidation of **1** with various oxidizing agents. They clearly show how critical is the choice of the oxidant both for the chemical yield and for the enantiomeric excess. *tert*-Butyl hydroperoxide gave the best results, while use of hydrogen peroxide led to a significant decrease of the optical yield. Sterically hindered oxidants, such as cumyl hydroperoxide and trityl hydroperoxide, or water-insoluble oxidants, such as iodosylbenzene and *m*-chloroperbenzoic acid (MCPBA), afforded racemic or almost racemic sulfoxide. The enzyme was not fully denaturated by the reaction medium when *t*-BuOOH was used since the recycle of its aqueous solution led to optically active **19**, although in lower chemical and optical yield (25% and 57% ee, respectively).

Substrate selectivity by chloroperoxidase was investigated on a series of aryl alkyl, dialkyl, and heterocyclic sulfides. Reaction time, chemical yield, absolute configuration, and ee of the resulting sulfoxides are reported in Table II. Electronic and particularly steric effects dramatically affected the outcome of the reaction. For instance, para substitution led to higher enantioselectivity and higher chemical yield with respect to ortho substitution, as shown by the reactivity of sulfides **1–4**. Activating or poorly deactivating substituents on the aromatic ring gave sulfoxides in high ee; on the contrary, strongly withdrawing groups such as the nitro group had deleterious effects on the chemical and optical yield, in line with the negative ρ value (−1.40) found by Kobayashi et al. (1986, 1987) for this CPO-dependent S-oxygenation. Steric effects of the alkyl groups bonded to sulfur are also very important: replacement of a methyl group in **1** by ethyl (**8**) or isopropyl (**16**) groups decreased the chemical yield and the degree of asymmetric induction. More sterically demanding substituents led to the recovery of unreacted sulfides even after a very long reaction time.

The enzyme gives rise to asymmetric induction also with dialkyl and heteroaryl sulfides. Particularly significant is the very high ee found in the oxidation of **10**, whose corresponding sulfoxide is known to undergo facile homolytic racemization. The cyclic sulfide **11** gave an optically active sulfoxide; in spite of the modest ee, chloroperoxidase is a better enantioselective catalyst for this reaction than the fungine monooxygenase *Mortierella isabellina* (20% vs 0% ee) (Holland, 1988). Chloroperoxidase favorably competes with *M. isabellina* also in the oxidation of sulfides **1**, **3**, **5**, and **7**.

In all cases examined the prevailing sulfoxide had *R* absolute configuration. This stereochemical course can be rationalized on the basis of the electrophilic attack of sulfur by the oxidant from the less hindered side.

It should be emphasized that sulfides can be aspecifically oxidized by peroxides to racemic sulfoxides, at a rate dependent on the concentration and nature of the substrate and oxidant, also in the absence of chloroperoxidase. Therefore, the outcome of the oxidation, in terms of yield and ee, is the result of two concomitant reactions, the first catalyzed by chloroperoxidase (reaction 1, where X is a compound I-like or compound II-like entity) and the second being the nonenzymatic oxidation (reaction 2).

$$\text{chloroperoxidase} + \text{oxidant} \rightleftharpoons \text{X} \xrightarrow{\text{R-S-R}'} \text{R-SO-R}' \text{ (varying ee)} \quad (1)$$

$$\text{oxidant} \xrightarrow{\text{R-S-R}'} \text{R-SO-R}' \text{ (racemic)} \quad (2)$$

The relative contribution of the catalyzed and uncatalyzed oxidation of **1** was quantitatively examined by HPLC. A chiral column was employed. The time course of the oxidation was monitored for 1 week in the presence and in the absence of the enzyme. In the reaction carried out in the presence of chloroperoxidase, the chemical yield of sulfoxide reached an approximate 60% value after 2–3 days, and then it remained almost constant, probably because of the decay of enzymatic activity. The initial ee was around 90% with a trend to slightly decrease with time. Instead, in the reaction carried out in the absence of the enzyme, the formation of the racemic sulfoxide increased after 3 days almost linearly up to 10% yield. Therefore, if the spontaneous reaction could be prevented, the ee of the catalyzed reaction would be close to 95%, suggesting an almost absolute stereoselectivity of chloroperoxidase with substrate **1**.

The influence of the pH (5 and 7) of the aqueous buffer, the reaction temperature, and the addition of acetone as a water-miscible cosolvent on the stereoselectivity of the oxidation of **1**, **3**, and **8** was also examined. The asymmetric synthesis was higher at pH 5 (about 20%), where the enzyme has the highest catalytic activity (Thomas et al., 1970), than at pH 7. The addition of acetone (10% v/v) decreased the optical yields of the sulfoxides (62% ee) without appreciable effects on the chemical yields. A similar negative effect on the enantioselectivity was observed by increasing the temperature: oxidation of **1** with *t*-BuOOH at 25 °C for 24 h gave **19** in 35% yield and 55% ee. It is interesting to note that the only known example of temperature-dependent enantiospecificity in an enzymatic reaction is very recent and deals with a secondary alcohol dehydrogenase (Pham et al., 1989).

We have also studied the influence of bovine serum albumin (BSA, 40 mg/ml), a globular protein that increases the solubility of hydrophobic substrates and stabilizes enzymes against denaturation. BSA almost doubled the optical and chemical yield for the less reactive sulfides **7** and **8**, without affecting the stereochemical course of the reaction, since **25** and **26** had *R*-(+) absolute configuration. In the case of the more reactive sulfide **1**, the higher chemical yield was accompanied by a lower enantioselectivity (74% ee for sulfoxide **19** instead of 86%), presumably due to the higher contribution of the aspecific reaction.

The oxidation of sulfides **1** and **3** by *t*-BuOOH, H_2O_2, or cumyl hydroperoxide in the presence of different amounts of horseradish peroxidase was also examined (Table III). Yields of sulfoxides **19** and **21** are generally modest, and in all cases no detectable asymmetric induction was observed.

In conclusion, although the degree of chloroperoxidase catalysis in the oxidation of sulfides is unknown in each case, we presume that the ee observed in the products reflects the minimum contribution to the enzymatic pathway.

Registry No. **1**, 623-13-2; **2**, 14092-00-3; **3**, 1879-16-9; **4**, 2388-73-0; **5**, 100-68-5; **6**, 123-09-1; **7**, 701-57-5; **8**, 622-63-9; **9**, 7433-79-6; **10**, 766-92-7; **11**, 505-23-7; **12**, 628-29-5; **13**, 18438-38-5; **14**, 3699-03-4; **15**, 36304-27-5; **16**, 14905-81-8; **17**, 5023-60-9; **18**, 10352-44-0; **19**,

1519-39-7; **20**, 84413-66-1; **21**, 93381-75-0; **22**, 84413-74-1; **23**, 4850-71-9; **24**, 28227-63-6; **25**, 93222-06-1; **26**, 1519-40-0; **28**, 2843-91-6; **29**, 63865-79-2; **30**, 51795-48-3; **31**, 93183-62-1; CPO, 9055-20-3; *t*-BuOOH, 75-91-2; H_2O_2, 7722-84-1; cumyl-OOH, 80-15-9; peroxidase, 9003-99-0.

REFERENCES

Auret, B. J., Boyd, D. R., Cassidy, E. S., Turley, F., Drake, A. F., & Mason, S. F. (1983) *J. Chem. Soc., Chem. Commun.*, 282–283.

Bangcharoenpaurong, O., Champion, P. M., Hall, K. S., & Hager, L. P. (1986) *Biochemistry 25*, 2374–2378.

Blanke, S. R., & Hager, L. P. (1988) *J. Biol. Chem. 263*, 18739–18743.

Colonna, S., Banfi, S., Fontana, F., & Sommaruga, M. (1985) *J. Org. Chem. 50*, 769–771.

Colonna, S., Banfi, S., Annunziata, R., & Casella, L. (1986) *J. Org. Chem. 51*, 891–895.

Colonna, S., Gaggero, N., Manfredi, A., Casella, L., & Gullotti, M. (1988) *J. Chem. Soc., Chem. Commun.*, 1451–1452.

Dawson, J. H. (1988) *Science 240*, 433–439.

Doerge, D. R. (1986) *Arch. Biochem. Biophys. 244*, 678–685.

Dunach, E., & Kagan, H. B. (1985) *Nouv. J. Chim.*, 1–3.

Dunford, H. B. (1982) *Adv. Inorg. Biochem. 4*, 41–68.

Frew, J. E., & Jones, P. (1984) *Adv. Inorg. Bioinorg. Mech. 3*, 176–212.

Hager, L. P., Morris, D. R., Brown, F. S., & Eberwein, H. (1966) *J. Biol. Chem. 241*, 1769–1777.

Holland, H. L. (1988) *Chem. Rev. 88*, 473–485.

Kedderis, G. L., Koop, D. R., & Hollenberg, P. F. (1980) *J. Biol. Chem. 255*, 10174–10182.

Kedderis, G. L., Rickert, D. E., Pandey, R. N., & Hollenberg, P. F. *(1986) J. Biol. Chem. 261*, 15910–15914.

Kobayashi, S., Sugioka, K., Nakano, H., Nakano, M., & Tero-Kubota, S. (1984) *Biochemistry 23*, 4589–4597.

Kobayashi, S., Nakano, M., Goto, T., Kimura, T., & Schaap, A. P. (1986) *Biochem. Biophys. Res. Commun. 135*, 166–171.

Kobayashi, S., Nakano, M., Kimura, T., & Schaap, A. P. (1987) *Biochemistry 26*, 5019–5022.

Landini, D., & Rolla, F. (1988) *Organic Syntheses* (Noland, W. E., Ed.) Collect. Vol. IV, pp 833–835, Wiley, New York.

Laur, P., Melillo, J. T., Simmons, T., & Ternay, A. L. (1965) *J. Am. Chem. Soc. 87*, 1958–1976.

Libby, R. D., Thomas, J. A., Kaiser, L. W., & Hager, L. P. (1982) *J. Biol. Chem. 257*, 5030–5037.

McCarthy, M.-B., & White, R. E. (1983) *J. Biol. Chem. 258*, 9153–9158.

Morris, D. R., & Hager, L. P. (1966) *J. Biol. Chem. 241*, 1763–1768.

Ohta, H., Matsumoto, S., Okamoto, Y., & Sugai, T. (1989) *Chem. Lett.*, 625–628.

Ortiz de Montellano, P. R., Ed. (1986) *Cytochrome P-450: Structure, Mechanism and Biochemistry*, Plenum Press, New York.

Ortiz de Montellano, P. R., Choe, Y. S., De Pillis, G., & Catalano, C. E. (1987) *J. Biol. Chem. 262*, 11641–11646.

Padbury, G., & Sligar, S. G. (1985) *J. Biol. Chem. 260*, 7820–7823.

Pham, V. T., Phillips, R. S., & Lijungdahl, L. G. (1989) *J. Am. Chem. Soc. 111*, 1935–1936.

Pitchen, P., Dunach, E., Deshmukh, M. N., & Kagan, H. B. (1984) *J. Am. Chem. Soc. 106*, 8189–8193.

Sugimoto, T., Kokubo, T., Miyazaki, J., Tanimoto, S., & Okano, M. (1981) *Biorg. Chem. 10*, 311–323.

Takata, T., Yamazaki, M., Fujimori, K., Kim, Y. H., Iyanagi, T., & Oae, S. (1983) *Bull. Chem. Soc. Jpn. 56*, 2300–2310.

Thomas, J. A., Morris, D. R., & Hager, L. P. (1970) *J. Biol. Chem. 245*, 3129–3134.

Vowinkel, E. (1974) *Synthesis*, 430–432.