

Oxygen Activation by Iron(III)-Porphyrin/ NaBH_4 / $\text{Me}_4\text{N}\cdot\text{OH}$ System as Cytochrome P-450 Model. Oxygenation of Olefin, *N*-Dealkylation of Tertiary Amine, Oxidation of Sulfide, and Oxidative Cleavage of Ether Bond

Takashi MORI, Tomofumi SANTA, Tsunehiko HIGUCHI, Tadahiko MASHINO, and Masaaki HIROBE*

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

Received August 3, 1992

Oxygenation of olefin, *N*-dealkylation of tertiary amine, oxidation of sulfide, and oxidative cleavage of ether bond were conducted with tetraphenylporphyrinatoiron(III) ($\text{Fe}^{3+}\text{TPPCL}$), NaBH_4 , $\text{Me}_4\text{N}\cdot\text{OH}$, and molecular dioxygen in benzene–methanol solution. $\text{Fe}^{3+}\text{TPPCL}$, NaBH_4 , and molecular dioxygen were essential for these reactions and the yields were decreased when $\text{Me}_4\text{N}\cdot\text{OH}$ was absent. Olefins were converted to alcohols, which were not produced from the corresponding epoxides under the same conditions. In styrene oxygenation, an electron-donating substituent on the substrate decreased the reactivity, whereas in *N,N*-dimethylaniline demethylation, it enhanced the reactivity. Despite the use of the same reagents, the key intermediates of these two reactions are different. $\text{Fe}^{2+}\text{TPP}-\sigma$ -alkyl complexes produced from $\text{Fe}^{3+}\text{TPPCL}$, olefin, and NaBH_4 were identified as intermediates under anaerobic conditions. $\text{Fe}^{2+}\text{TPP}-\sigma$ -alkyl complex reacted with molecular dioxygen to give oxygenated products.

Examination of the relative reactivities of *p*-substituted *N,N*-dimethylanilines in the NaBH_4 reaction system revealed first, that the demethylation proceeded *via* one-electron abstraction, and second, that the reactive species of the demethylation reactions seems to be an iron-oxenoid.

Keywords tetraphenylporphyrinatoiron(III); cytochrome P-450 model; olefin oxygenation; *N*-demethylation; *S*-oxidation; sodium borohydride

Cytochrome P-450 (Cyt. P-450) plays an important role in metabolizing a wide variety of xenobiotics and biomolecules. This reaction is especially interesting since it produces a strong oxidant, iron-oxenoid, by the reductive activation of O_2 on its ironprotoporphyrin IX. Therefore, many Cyt. P-450 model reactions have been studied extensively.¹⁾ In most cases, however, previously activated oxygen sources such as iodosylbenzene, ClO^- , H_2O_2 , alkylhydroperoxide, *etc.*, have been used instead of O_2 and a reducing agent. Only a few reactions have involved the reductive activation of O_2 .^{1,2)} We and others have reported Cyt. P-450 model reactions involving the reductive activation of O_2 in the presence of NaBH_4 as a reductant.^{3–6)} Among the reductants used in the Cyt. P-450 model reactions, NaBH_4 is effective in olefin oxygenation, but other Cyt. P-450 type reactions such as tertiary amine dealkylation, *etc.*, have not been reported.

In most Cyt. P-450 model reactions, a metal-oxenoid is thought to be an active oxidant, as with Cyt. P-450 itself, and olefin is oxidized mainly to epoxide.¹⁾ The main products of Mn-,³⁾ Co-,⁴⁾ and Fe-porphyrin⁵⁾-catalyzed reactions were alcohols which were thought to be produced from the corresponding ketones, not *via* the epoxides, except in the case of Mn-porphyrin. In the Co-porphyrin reaction, it was proposed that the reaction proceeded *via* a carbon–cobalt intermediate, σ -alkyl complex, followed by the reaction with O_2 ,⁴⁾ whereas metal-oxenoid was thought to be an active oxidant in the Mn-porphyrin reaction.³⁾ The reaction mechanism in the tetraphenylporphyrinatoiron(III) ($\text{Fe}^{3+}\text{TPPCL}$) reaction is, therefore, of interest.

Lieber and Guengerich have reported that hydrogen atom migration and formation of carbonyl compounds could take place in the Cyt. P-450 catalysis of olefin oxidation.⁷⁾ Based on this, we proposed, in a previous paper, that the iron-oxenoid was the active oxidizing species of

olefin oxygenation in the $\text{Fe}^{3+}\text{TPPCL}/\text{NaBH}_4$ reaction.⁵⁾

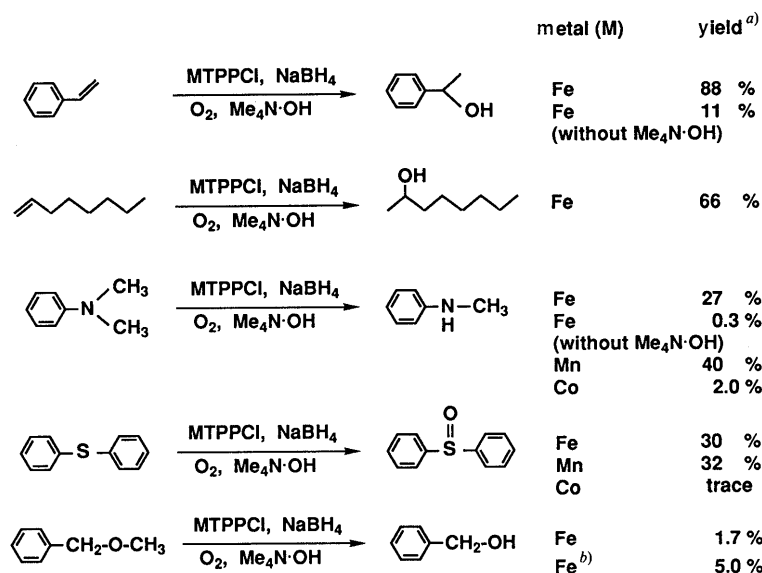
Recently, Setsune *et al.* have obtained the $\text{Fe}^{3+}-\sigma$ -alkyl complex from the reaction mixture of $\text{Fe}^{3+}\text{TPPCL}$, NaBH_4 , and olefin and identified it by ¹H-NMR.⁸⁾ However, we have detected $\text{Fe}^{2+}-\sigma$ -alkyl complex, not the $\text{Fe}^{3+}-\sigma$ -alkyl complex, under anaerobic conditions by UV–visible absorption spectroscopy,⁹⁾ and we would like to propose here an alternative mechanism for olefin oxygenation by the $\text{Fe}^{3+}\text{TPPCL}/\text{NaBH}_4$ reaction.

We also report Cyt. P-450 type *N*, *S*, and *O* oxidation reactions, tertiary amine dealkylation, sulfide oxygenation, and ether bond cleavage, catalyzed by the $\text{Fe}^{3+}\text{TPPCL}$, NaBH_4 , $\text{Me}_4\text{N}\cdot\text{OH}$, and O_2 system. Different from the olefin oxygenation, the reactive oxidant of these reactions seems to be an iron-oxenoid.

Results and Discussion

Olefin Oxygenation Styrene and 1-octene were converted to α -phenethyl alcohol (yield was 88% and catalyst turnover number was 528) and 2-octanol (66%), respectively, by $\text{Fe}^{3+}\text{TPPCL}$, NaBH_4 , $\text{Me}_4\text{N}\cdot\text{OH}$, and O_2 , as previously reported in a communication⁵⁾ (Chart 1). For this reaction, $\text{Fe}^{3+}\text{TPPCL}$, NaBH_4 , and O_2 were essential, and the yield was decreased in the absence of $\text{Me}_4\text{N}\cdot\text{OH}$. Other bases, KOH and NaOCH_3 , had the same effect. As we have already reported, $\text{Me}_4\text{N}\cdot\text{OH}$ prevented the formation of μ -oxo-dimer, which was less active.⁵⁾ When the amount of NaBH_4 was smaller or the reaction temperature was lower, acetophenone was also obtained from styrene. Epoxide yields were less than 0.5% and the epoxides were hardly reduced to the corresponding alcohols under our reaction conditions. These results indicated that the alcohols were formed for ketones, not through epoxides.

The following two considerations suggested that an

Chart 1. Cyt. P-450 Type *N*, *S*, and *O* Oxidations

MTPP: metal-tetraphenylporphyrin. *a*) Yields were based on substrate concentration. *b*) Tetrakis-(*o*-difluorophenyl)porphyrinatoiron was used as a catalyst.

TABLE I. Relative Reactivities of *p*-Substituted Styrenes

<i>p</i> -Substituent	Relative reactivity			
	-OCH ₃	-CH ₃	-H	-Cl
Fe ³⁺ TPPCL/NaBH ₄	0.68	0.94	1.0	1.4

Reaction conditions are given in Experimental.

electrophilic iron-oxenoid was not an active species in this olefin oxygenation. First, epoxides were not main products and yields were low in the NaBH₄ reaction, whereas epoxides were the main products with Fe³⁺TPPCL/iodosylbenzene, whose active oxidant was proposed to be an iron-oxenoid,^{1,10} and with which the alcohols or ketones were not detected. Second, the reactive species had a nucleophilic character in the NaBH₄ system (Table I) and an electrophilic character in the iodosylbenzene reaction,¹⁰ *i.e.*, an electron-withdrawing group at the *para* position of styrene enhanced the reactivity in the NaBH₄ system and decreased it in the iodosylbenzene reaction.

To detect the reactive intermediate, the spectrum of Fe³⁺TPPCL was taken with NaBH₄, Me₄N·OH and olefins under anaerobic conditions (Fig. 1). Fe³⁺TPPCL, NaBH₄, and Me₄N·OH mixture under an Ar atmosphere exhibited the absorption spectrum of Fe²⁺TPP (spectrum A in Fig. 1).⁵⁾ Addition of styrene and 1-octene to the Fe²⁺TPP and NaBH₄ solution changed the spectrum to B and C in Fig. 1, respectively. These were identified as being due to Fe²⁺TPP- σ -alkyl complexes (Fe²⁺-R), *i.e.*, Fe²⁺TPP-CH(CH₃)Ph (B) and Fe²⁺TPP-CH(CH₃)C₆H₁₃ (C).¹¹ Both olefin and NaBH₄ were essential for the formation of Fe²⁺-R from Fe²⁺TPP. Saveant *et al.* have reported that the spectrum of Fe²⁺-R produced from Fe²⁺TPP and alkyl halides was practically the same whatever the nature of R, but, for Fe²⁺-benzyl, the only change was a shift of the absorption band from 710 to 757 nm.¹¹ The spectra B and C in Fig. 1 exhibited absorption maxima at 757 and 710 nm, respectively, which strongly indicate that these spectra are due to Fe²⁺TPP-CH(CH₃)Ph and

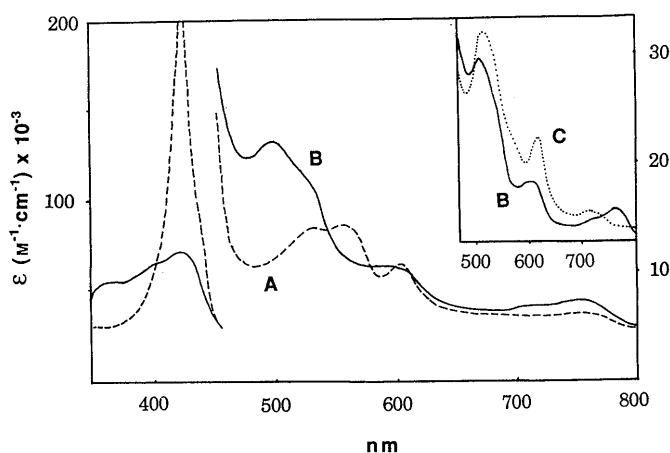


Fig. 1. UV-Visible Absorption Spectra of the Reaction Mixture

A mixture of Fe³⁺ TPPCL (15 mM), Me₄N·OH (37 mM), benzene (1.5 ml), and methanol (1.5 ml) was prepared under an Ar atmosphere. NaBH₄ (530 mM final concentration) was introduced anaerobically, then spectrum A was taken. Spectra B and C were taken at 5 min after the anaerobic addition of styrene and 1-octene, respectively, to the above mixture.

Fe²⁺TPP-CH(CH₃)C₆H₁₃, respectively. Setsune *et al.* have reported that the Fe³⁺- σ -alkyl(Fe³⁺-R) complex was obtained from the Fe³⁺TPPCL/NaBH₄/olefin reaction and identified by ¹H-NMR.⁸⁾ We would like to propose that the Fe²⁺-R is also formed in this reaction under anaerobic conditions, and then Fe²⁺-R reacts with O₂ to give Fe³⁺-R.

The reaction of Fe²⁺-R produced from alkyl halide/Fe²⁺-porphyrin or Grignard reagent/Fe³⁺-porphyrin with O₂ has been discussed by Saveant *et al.*¹¹ and Balch *et al.*¹² Fe²⁺-R is immediately oxidized by O₂ to Fe³⁺-R, which further reacts with O₂ and produces Fe³⁺-O-O-R (Chart 2). Fe³⁺-O-O-R decomposes mainly to Fe³⁺-OH⁻ and carbonyl compound (Chart 2).^{11,12}

In the case of olefin, for example, styrene (R = C₆H₅), the reaction proceeded in almost the same way after the formation of Fe²⁺-CH(CH₃)Ph. Acetophenone was a primary product, which was reduced by NaBH₄ to give α -phenethyl alcohol (Chart 2).

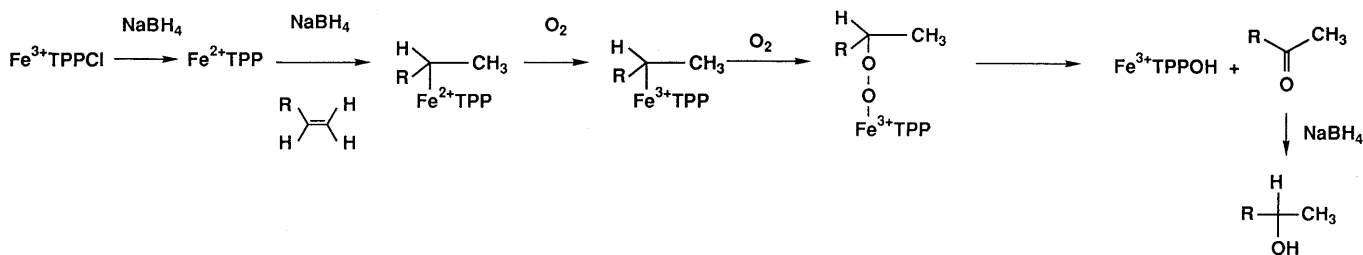


Chart 2

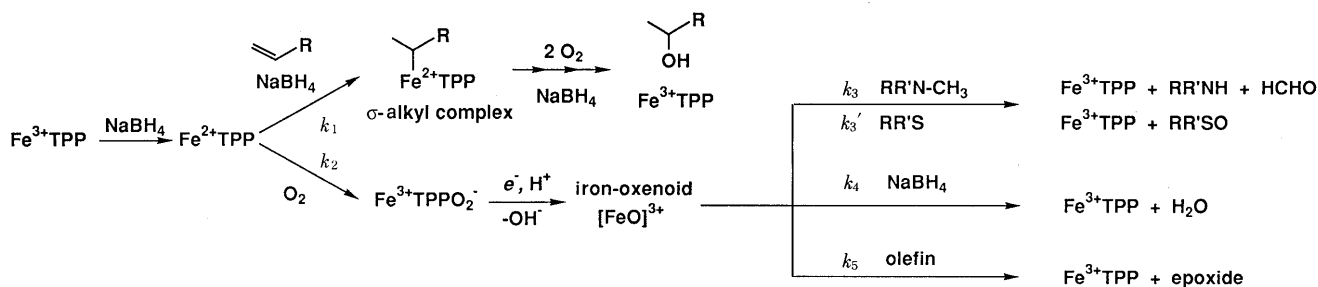


Chart 3

In the Cyt. P-450 catalysis of olefin oxidation, the iron- σ -alkyl complex has not been identified. But, recently $\text{Fe}^{3+}\text{-R}$ type complexes have been proposed as intermediates of the following Cyt. P-450 reactions: an anaerobic benzyl chloride reduction,¹³⁾ a porphyrin ring alkylation by mono-substituted hydrazine,¹⁴⁾ and reductive metabolism of the anesthetic halothane.¹⁵⁾ In addition, a non-heme iron- σ -alkyl intermediate was proposed in a lipoxigenase reaction.¹⁶⁾

Demethylation of *N,N*-Dimethylaniline, Oxidation of Diphenylsulfide, and Ether Bond Cleavage of Benzyl Methyl Ether Demethylation of *N,N*-dimethylaniline, oxidation of diphenylsulfide, and ether bond cleavage of benzyl methyl ether were also catalyzed by Fe^{3+}TPP , NaBH_4 , and O_2 . As with the olefin oxygenation, Fe^{3+}TPP , NaBH_4 , and O_2 were essential for these reactions, and the yield was decreased when $\text{Me}_4\text{N}\cdot\text{OH}$ was absent (Chart 1). When Fe^{3+}TPP was replaced by Mn^{3+}TPP , similar results were obtained in these reactions, but Co^{2+}TPP did not act as a good catalyst for the *N*-demethylation or the *S*-oxidation (Chart 1).

Relative reactivities of *p*-substituted *N,N*-dimethylanilines were estimated by a competition method in the Fe^{3+}TPP , NaBH_4 , $\text{Me}_4\text{N}\cdot\text{OH}$, and O_2 reaction (Fig. 2). An electron-donating substituent, the CH_3 group, enhanced the demethylation, whereas it decreased the reactivity in styrene oxygenation. This result clearly showed that the key intermediates of the dealkylation and the olefin oxygenation were different, despite the use of the same reagents. Relative reactivities of *p*-substituted *N,N*-dimethylanilines were also studied with the Fe^{3+}TPP /iodosylbenzene system, whose ultimate oxidant was reported to be an iron-oxenoid,^{1,10)} in the presence or absence of $\text{Me}_4\text{N}\cdot\text{OH}$. In both the NaBH_4 and the iodobenzene reactions, the reactivities correlated well with the Hammett σ_p^+ values, and in all reactions the ρ values were almost the same. These results indicated that first, the demethylation proceeded *via* one-electron abstraction in all the reactions, and second, the reactive species of all the reactions are

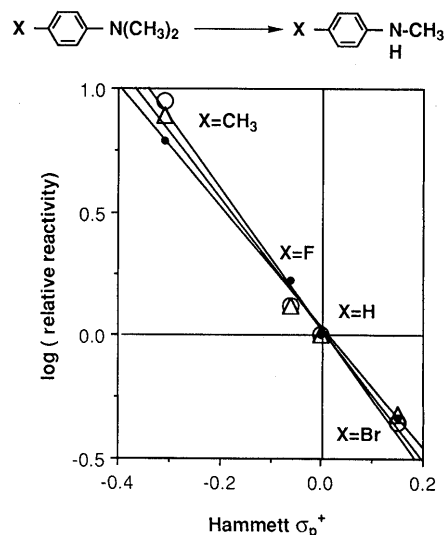


Fig. 2. Relative Reactivities of *p*-Substituted *N,N*-Dimethylanilines
 ○, Fe^{3+}TPP , NaBH_4 , O_2 , and $\text{Me}_4\text{N}\cdot\text{OH}$ system; △, Fe^{3+}TPP , iodobenzene, and $\text{Me}_4\text{N}\cdot\text{OH}$ system; ●, Fe^{3+}TPP and iodobenzene system.

similar, *i.e.*, the iron-oxenoid seems to be the ultimate oxidant of the demethylation by Fe^{3+}TPP / NaBH_4 reaction. Furthermore, the Fe-complex and Mn-complex gave similar results in the *N* and *S* oxidations (Chart 1), which also supported the production of iron-oxenoid in the iron-porphyrin and NaBH_4 reaction, because the oxidant of the Mn-complex/ NaBH_4 reaction has been elucidated as the oxenoid species. However, insufficient evidence is available as regards the iron-oxenoid formation, and further study may be required to clarify this point.

These conclusions seem to be inconsistent with the olefin oxygenation, since it proceeded *via* the σ -alkyl complex, and the iron-oxenoid did not act as an oxidant. The apparent inconsistency can be explained in terms of either or both of possibilities A and B. A: The Fe^{3+}TPP / NaBH_4 reaction produces the iron-oxenoid only in the absence of olefin ($k_1 > k_2$ in Chart 3). B: the iron-oxenoid

is produced even in the presence of olefin ($k_1 \approx k_2$, $k_1 < k_2$ in Chart 3), but NaBH_4 reduces the iron-oxenoid before olefin reacts with it ($k_4 > k_5$). This pathway is a non-productive consumption of iron-oxenoid. In this case, *N,N*-dimethylaniline and diphenylsulfide are thought to react with the iron-oxenoid before NaBH_4 quenches it ($k_3(k'_3) > k_4$ or $k_3(k'_3) \approx k_4$). The validity of explanations A and B is under investigation.

In conclusion, the $\text{Fe}^{3+}\text{TPP}\text{Cl}$, NaBH_4 , $\text{Me}_4\text{N}\cdot\text{OH}$, and O_2 system produced two completely different types of reactive species depending on the substrate. In the olefin oxygenation, the reaction proceeded *via* the σ -alkyl complex, whereas the iron-oxenoid may be the ultimate oxidant in Cyt. P-450 type *N*, *S*, and *O* oxidations.

Experimental

Preparation of $\text{Fe}^{3+}\text{TPP}\text{Cl}$ *meso*-TPP was prepared from propionic acid and benzaldehyde,¹⁷⁾ then TPP was reacted with $\text{FeCl}_2 \cdot n\text{H}_2\text{O}$ to give $\text{Fe}^{3+}\text{TPP}\text{Cl}$.¹⁸⁾

General Procedure of $\text{Fe}^{3+}\text{TPP}\text{Cl}$, NaBH_4 , O_2 , and $\text{Me}_4\text{N}\cdot\text{OH}$ Reaction⁵⁾ NaBH_4 (5.3 mmol) was added to a mixture of $\text{Fe}^{3+}\text{TPP}\text{Cl}$ (5.0 μmol), $\text{Me}_4\text{N}\cdot\text{OH}$ (0.87 mmol), substrate (3.0 mmol), benzene (4.0 ml), and methanol (4.0 ml), and the solution was stirred vigorously under air for 5 h at 20 °C. All products listed in Chart 1 were isolated and identified by examination of the NMR, IR, and mass spectra. The yields were determined by GLC (column: 10% polyethyleneglycol 6000, for 1-phenyl-1-ethanol, 2-octanol, and benzyl alcohol) or HPLC (column: Lichrosorb RP-18, for *N*-methylaniline and diphenylsulfide).

General Procedure of $\text{Fe}^{3+}\text{TPP}\text{Cl}$ /Iodosylbenzene Reaction Iodosylbenzene (1 mmol) was added to a mixture of $\text{Fe}^{3+}\text{TPP}\text{Cl}$ (5.0 μmol), $\text{Me}_4\text{N}\cdot\text{OH}$ (0.87 or 0 mmol), substrate (1.0 mmol), benzene (2.0 ml), and methanol (2.7 ml), and the solution was stirred vigorously for 5 h at 20 °C.

Determination of the Relative Reactivities of *p*-Substituted Styrenes and *N,N*-Dimethylanilines Reaction conditions were the same as described above, but two kinds of *p*-substituted styrenes or *N,N*-dimethylanilines in equal amount were added. The relative reactivities were estimated from the ratio of the corresponding α -phenethyl alcohols or *N*-methylanilines.

Measurement of Visible Spectra A mixture of $\text{Fe}^{3+}\text{TPP}\text{Cl}$ (15 μM), $\text{Me}_4\text{N}\cdot\text{OH}$ (37 mM), benzene (1.5 ml), and methanol (1.5 ml) was prepared under an Ar atmosphere. NaBH_4 (530 mM final concentration) was introduced anaerobically, then spectrum A in Fig. 1 was taken. Spectra B and C in Fig. 1 were taken at 5 min after the anaerobic addition of olefins to the above mixture.

Acknowledgements This work was supported in part by a Grant-in-Aid for Special Project Research from Ministry of Education, Science, and Culture, Japan.

References and Notes

- 1) P. R. Ortiz de Montellano (ed.), "Cytochrome P-450. Structure, Mechanism, and Biochemistry," Plenum Press, New York, 1986.
- 2) T. Santa, N. Miyata, and M. Hirobe, *Chem. Pharm. Bull.*, **32**, 1252 (1984); I. Tabushi, M. Koderu, and M. Yokoyama, *J. Am. Chem. Soc.*, **107**, 4466 (1985); I. Tabushi and M. Koderu, *ibid.*, **108**, 1101 (1986).
- 3) I. Tabushi and N. Koga, *J. Am. Chem. Soc.*, **101**, 6456 (1979); I. Tabushi and A. Yamazaki, *ibid.*, **103**, 7371 (1981).
- 4) T. Okamoto and S. Oka, *J. Org. Chem.*, **49**, 1589 (1984).
- 5) T. Santa, T. Mori, and M. Hirobe, *Chem. Pharm. Bull.*, **33**, 2175 (1985).
- 6) Y. Aoyama, T. Watanabe, H. Onda, and H. Ogoshi, *Tetrahedron Lett.*, **24**, 1183 (1983).
- 7) D. C. Lieber and F. P. Guengerich, *Biochemistry*, **22**, 5482 (1983).
- 8) J. Setsuno, Y. Ishimura, and A. Sera, *Chem. Lett.*, **1992**, 377; Kanou *et al.* also speculated that an $\text{Fe}^{3+}\text{-R}$ complex is formed in the reduction of styrene by $\text{Fe}^{3+}\text{TPP}\text{Cl}/\text{NaBH}_4$ under anaerobic conditions. K. Kanou, M. Takeuchi, S. Hashimoto, and Z.-I. Yoshida, *J. Chem. Soc., Chem. Commun.*, **1992**, 1728.
- 9) T. Mori, T. Santa, T. Higuchi, and M. Hirobe, Abstracts of Papers, The 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, April 1988, p. 83.
- 10) T. G. Traylar and F. Xu, *J. Am. Chem. Soc.*, **110**, 1953 (1988).
- 11) D. Lexa, J. Misspeiter, and J.-M. Saveant, *J. Am. Chem. Soc.*, **103**, 6808 (1981).
- 12) R. D. Arasasingham, A. L. Balch, C. R. Cornman, and L. Latos-Graazynski, *J. Am. Chem. Soc.*, **111**, 4357 (1989).
- 13) D. Munsuy and M. Fontecave, *Biochem. Pharmacol.*, **32**, 1871 (1983).
- 14) P. R. Ortiz de Montellano, O. August, F. Viola, and K. L. Kunze, *J. Biol. Chem.*, **258**, 8623 (1983).
- 15) H. H. Rub, H. Ahr, W. Nastainczyk, V. Ullrich, D. Mansuy, J.-P. Battioni, R. Montiel-Montoya, and A. Trautwein, *Biochemistry*, **23**, 5300 (1984).
- 16) E. J. Corey and R. Nagata, *J. Am. Chem. Soc.*, **109**, 8108 (1987).
- 17) A. D. Adler, F. R. Lougo, J. O. Finarell, J. Goldmacher, J. Assour, and L. Korsakoff, *J. Org. Chem.*, **32**, 476 (1967).
- 18) E. B. Fleisher, J. M. Palmer, T. S. Srivastava, and A. Chatterjee, *J. Am. Chem. Soc.*, **93**, 3162 (1971).