Inorganica Chimica Acta, 23 (1977) 221–225

Spectroscopic and Kinetic Studies of the Reactions of Iron(III) Porphyrins with Alkyl Thiols

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Received October 21, 1976

The reaction of toluene solutions of FeTPPCl with RSH (R = CH_3 or $(CH_3)_3C$) in the presence of pyridine has been studied by epr and electronic spectra. The reaction results in the reduction of FeTPPCl to $FeTPP(Py)_2$ and the oxidation of RSH to R_2S_2. Kinetic data obtained for the reaction shows the observed rate law to be:

$$-d[FeTPPCl]/dt = k[FeTPPCl][RSH][Py]^3$$

Epr studies show the involvement of two paramagnetic, S = 1/2, intermediates. The first intermediate is assigned as FeTPP(Py)(SR), while the second intermediate is postulated as $FeTPP(Py)_2(RS)$. Possible structures for this second intermediate are discussed. A mechanism is proposed which is consistant with the observed data. The autocatalytic nature of this system is also described.

Introduction

The reaction of thiols with metal complexes, specifically iron porphyrins, has been known for over 50 years [1]. Interest in the reaction of thiols with metals focuses on the biological importance of the sulfhydral groups and the possible relationship of model systems to cyctochrome P-450. Several kinetic studies of metal salts with mercaptans have been done to determine the degree of oxidation of the thiol and the conditions governing the reaction [2–8]. The relationship of cyctochrome P-450 to an iron porphyrin interacting with a sulfur ligand was first suggested by Mason *et al.* [9]. Low spin iron(III) epr spectra, similar to those of cyctochrome P-450, have been observed by reacting myoglobin, hemoglobin [10], or iron porphyrins [11] with various mercaptans.

Model systems for cytochrome P-450 and isolable iron porphyrins with sulfur bonded ligands have only recently been characterized [12–18]. In this paper we report the kinetics for the reaction of alkyl thiols with tetraphenylporphinato iron(III) chloride in the presence of pyridine and on spectroscopic observation of several intermediates.

Experimental

Materials

Tetraphenylporphyrin was purchased from Strem Chemicals and the iron complexes were prepared by literature methods [19]. Purification was effected by chromatography on Alumina Oxide, Woelm neutral, activity grade 1. This procedure resulted in the formation of $(FeTPP)_2O$, which was converted to the chloride by shaking with HCl [20]. Pyridine was purchased from Fisher Scientific and was purified by drying over $CaSO_4$ and then distilled from BaO. Methanethiol was purchased from Eastman and 2-methyl-2-propanethiol was purchased from K & K Laboratories. Both were used without further purification. Myoglobin was purchased from Gallard–Schelesinger Chemical Mfg.

Procedure

For the kinetic experiments done with 2-methyl-2-propanethiol, the following procedure was used. Toluene, pyridine, and 2- methyl-2-propanethiol were deoxygenated by bubbling argon through each bottle which was equipped with a serum cap. A volumetric flask equipped with serum cap was flushed with Argon and then weighed. An aliquot of 2-methyl-2-propanethiol was then transferred to the volumetric flask by use of a syringe, weighed and then filled with solvent. A similar procedure was repeated for preparing solutions of pyridine. A stock solution of FeTPPCl was prepared by weighing a sample of FeTPPCl into a volumetric flask, flushing with argon and then filling with deoxygenated toluene. The concentration of FeTPPCl as directly determined was checked by use of extinction coefficients at 5080 Å for FeTPPCl. Solutions were stored by replacing the serum cap with a screw cap. This procedure was done in a glove bag filled with argon to ensure the exclusion of oxygen. The samples were then placed in a dessicator under an atmosphere of Argon. For a kinetic run, a cuvette was equipped with a serum cap and flushed with argon. One milliliter of the pyridine solution was transferred to the cuvette by the use of a syringe, and similarly one milliliter of the FeTPPCl solution was transferred. The spectrum was then

recorded to check the concentration of FeTPPCl and to check for the presence of $(FeTPP)_2O$. Finally one milliliter of the 2-methyl-2-propanethiol solution was transferred. The reaction was then observed for at least three half-lives.

Kinetic studies using methanethiol required a different procedure. Stock solutions of FeTPPCl and pyridine were prepared and two milliliters of each were transferred to the reaction vessel. The solution was then degassed by the usual freeze–pump–thaw cycle. The spectrum of the solution was then recorded to determine the concentration of FeTPPCl. Methanethiol gas was then trapped in a second compartment of the reaction vessel. Just prior to the beginning of the experiment the reaction vessel was placed in an ice–water bath and the methanethiol was allowed to condense for ten seconds. The reaction vessel was then transferred to the spectrophotometer and the reaction was observed for three half-lives.

Both sets of kinetic experiments were carried out at 298.5 °K and followed by the disappearance of the 5080 Å band of FeTPPCl. All kinetic experiments were done so that the FeTPPCl disappeared in a pseudo-first-order fashion. The rate constants k were determined by plotting ln[FeTPPCl] *vs.* time, and then refined by using an unweighted linear least squares calculation. The rate orders for pyridine, 2-methyl-2-propanethiol, and methanethiol were determined by plotting lnk *vs.* ln[concentration] for each reactant. This data was then refined with an unweighted linear least squares calculation.

NMR samples were prepared by placing 2 mg of FeTPPCl into an nmr tube. The sample was placed on a vacuum line and 0.4 milliliters of d_8 toluene was distilled in, followed by a stoichiometric amount of methanethiol and the n.m.r. spectrum recorded. Pyridine, d_5, was then distilled into the tube and the spectra was recorded again. For experiments using olefins for radical traps, the olefin was added and the nmr spectra recorded before and after the addition of pyridine.

EPR samples were prepared on the vacuum line in quartz epr tubes with high vacuum stopcocks. FeTPPCl was placed in the tube as a solid, evacuated, and then toluene and pyridine were distilled into the tube and mixed thoroughly. The sample was then frozen and the thiol was distilled into the epr tube. Before warming the epr spectra was recorded at 130 °K. The sample was then briefly warmed and refrozen at 77 °K and the epr spectra was recorded again. This procedure was repeated until no epr signal was observed.

EPR samples of myoglobin were prepared by placing an aqueous solution of myoglobin into an epr tube equipped with a high vacuum stopcock. The sample was then degassed by the usual freeze–pump–thaw cycle and the epr spectra was then recorded at 130 °K. Methanethiol was then distilled into the epr tube and the spectra of this solution was then recorded at 130 °K. The sample was then warmed and allowed to react for 72 hours, after which the epr spectra was recorded at 130 °K. Pyridine was then added to this solution and the resulting epr spectra was recorded at 130 °K.

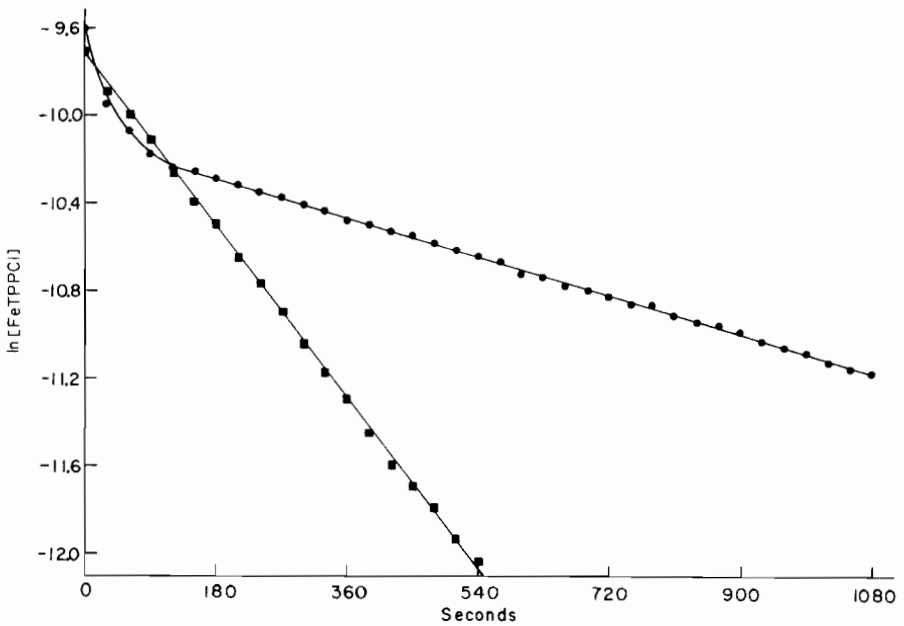

Fig. 1. Representative plots of ln[FeTPPCl] *vs.* time for a toluene solution of FeTPPCl reacting with RSH in the presence of pyridine (T = 298.5 °K). (■–■– R = CH_3, ●–●– R = $(CH_3)_3C$)

Instrumentation

Electronic spectra were recorded on a Cary Model 14 recording spectrometer, equipped with a thermostated cell. The temperature was kept constant at 298.5 °K ± 0.05 by use of a Wilkes–Anderson heater–cooler water bath regulated with a Standard Scientific Thermoregulator.

NMR spectra were recorded on a JEOL PS100, operating at 100 MHz for proton spectra operating in the FT mode.

EPR spectra were taken on a Varian Model V-4502 X-band spectrophotometer at 130 °K, equipped with a Field Dial Mark I, Hewlett-Packard Model 7001 AM X–Y recorder, and a Hewlett–Packer frequency meter, model X 532 B. Cooling was provided by using cold nitrogen gas and the temperature was controlled to ± 2°, using a Varian V-4557 variable temperature accessory. The field and frequency calibrations were made using DPPH powder (g = 2.0036), field dial, and X 532 B frequency meter.

Results and Discussion

Tetraphenylporphinato iron(III) chloride reacts with alkyl thiols in the presence of pyridine to form bis pyridine complexes of tetraphenylporphinato iron(II) and the dialkyl disulfide by reaction 1.

$$FeTPPCl + 3Py + RSH \rightarrow FeTPP(Py)_2 + HClPy + 1/2R_2S_2 \quad (1)$$

$FeTPP(Py)_2$ is characterized by electronic spectral bands at 5600, 5300, and 4235 Å and by nmr chemical shifts of −8.82, −8.12, and −7.45 ppm downfield from TMS. The dimethyl disulfide was characterized by nmr and had a chemical shift of −2.01 ppm downfield from TMS. Integration of the proton nmr intensities demonstrates that the conversion of thiol to disulfide is quantitative. Representative kinetic data and rate constants k, for reaction 1, where R = methyl or t-butyl, are presented in Figure 1 and Tables I and II. The experimental rate law observed for reaction 1 is

$$-d[FeTPPCl]/dt = d[FeTPP(Py)_2]/dt = k[FeTPPCl][RSH][Py]^3$$

A plot of ln[FeTPPCl] *vs.* time for the CH_3SH reaction is linear for the entire reaction time; however, a similar plot for the $(CH_3)_3CSH$ reaction shows curvature during the initial period of the reaction, but then becomes linear for about 85% of the reaction. The curvature is attributed to a mechanism containing a pre-equilibrium step that is only slightly faster than the rate-determining step. Once the pre-equilibrium is established the observed experimental rate law for reaction 1 is the same for CH_3SH and $(CH_3)_3CSH$.

TABLE I. Rate Constants[a] for the Reaction of Toluene Solutions of FeTPPCl[b] with CH_3SH in the Presence of Pyridine.

Py (*M*)	Methanethiol	k rate (min^{-1})[c]	$k/[Py]^3[RSH]$ (M^{-4} min^{-1})[d]
2.96	0.30	0.254	0.0326
2.96	0.45	0.311	0.0266
2.96	0.60	0.543	0.0349
2.47	0.30	0.136	0.0301
2.47	0.45	0.197	0.0291
1.47	0.45	0.040	0.0280
			0.0302 ± 0.0024

[a]Reactions were studied using pseudo-first-order conditions for FeTPPCl, T = 298.5 °K. [b]Initial [FeTPPCl] = 7×10^{-5} *M*. [c]Pseudo-first-order rate constant. [d]Rate constant obtained by dividing pseudo-first-order rate constant by the observed rate law.

TABLE II. Rate Constants[a] for the Reaction of Toluene Solutions of FeTPPCl[b] with $(CH_3)_3CSH$ in the Presence of Pyridine.

Py (*M*)	t-Butylthiol (*M*)	k rate (min^{-1})[c]	$k[Py]^3[RSH]$ (M^{-4} min^{-1})[d]
4.138	0.1208	0.058	0.0067
4.138	0.1637	0.068	0.0059
4.138	0.1800	0.076	0.0060
4.138	0.6524	0.281	0.0061
3.202	0.6524	0.136	0.0063
2.361	0.6524	0.059	0.0069
			0.0063 ± 0.0003

[a]Reactions were studied using pseudo-first-order conditions for FeTPPCl, T = 298.5 °K. [b]Initial [FeTPPCl] = 8×10^{-5} *M*. [c]Pseudo-first-order rate constant. [d]Rate constant obtained by dividing the pseudo-first-order rate constant by the observed rate law.

Epr spectra studies have been used in detecting paramagnetic intermediates in reaction 1. Figure 2a shows the epr spectrum of the high spin Fe(III) complex FeTPPCl in the presence of pyridine prior to addition of $(CH_3)_3CSH$. Distilling $(CH_3)_3CSH$ into the sample and immediately freezing to 130 °K results in observation of a new paramagnetic species in addition to residual FeTPPCl. Epr g values of 2.316, 2.233 and 1.946 for Intermediate I are very similar to those reported for low spin Fe(III) complexes of the form Fe(por)(:B)(SR) [10, 11, 18]. This species can be confidently assigned as FeTPP(Py)$(SC(CH_3)_3)$. Warming the sample and refreezing to 130 °K several times resulted in the appearance and sequential increase in relative intensity for an epr signal from a second S = 1/2 species, (intermediate II). This new signal is at least 120 gauss wide and overlaps

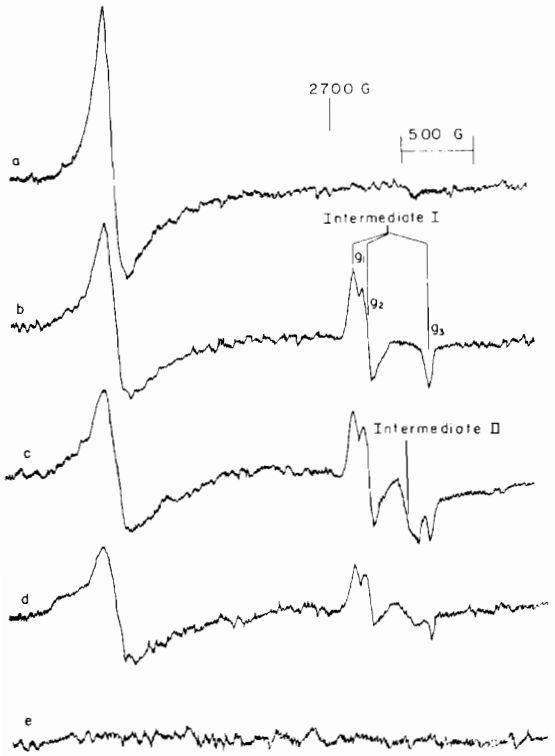

Fig. 2. EPR spectra, as a function of time, for the reaction of toluene solutions of FeTPPCl with $(CH_3)_3CSH$ in the presence of pyridine. (a) FeTPPCl + Py (130 °K). (b) FeTPPCl + Py + $(CH_3)_3CSH$ immediately frozen to 130 °K. (c) Solution b warmed to 298 °K for several minutes and refrozen to 130 °K. (d) Solution b warmed several times to 298 °K and refrozen to 130 °K. (e) EPR spectra after reaction of solution b is complete (130 °K).

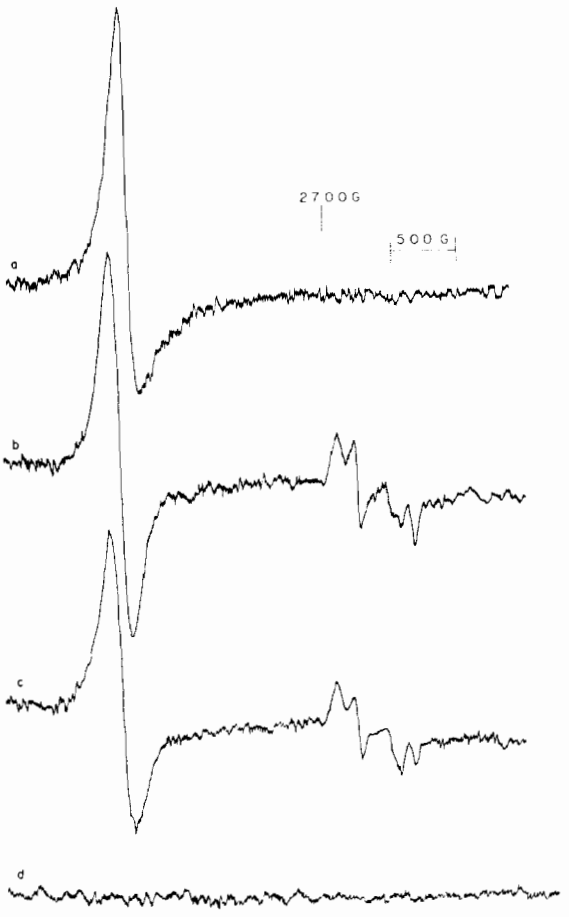

Fig. 3. EPR spectra, as a function of time, for the reaction of toluene solution of FeTPPCl with CH_3SH in the presence of pyridine. (a) FeTPPCl + Py (130 °K). (b) FeTPPCl + Py + CH_3SH frozen immediately to 130 °K. (c) Solution b warmed briefly to 298 °K and refrozen to 130 °K. (d) EPR spectra after reaction of solution b is complete (130 °K).

the epr spectra of FeTPP(Py)($(CH_3)_3CS$). The stepwise appearance of epr signals for the two intermediates and curvature of the ln[FeTPPCl] *vs.* time plot indicates the presence of a pre-equilibrium step only slightly faster than the rate-determining step. The epr spectral intensities for FeTPPCl, and the two intermediates, achieve a constant ratio and mutually decline in intensity as the reaction proceeds to completion. This suggests that FeTPPCl and the two epr detectable intermediates are in equilibrium.

Mixing CH_3SH with a toluene solution of FeTPPCl in the presence of pyridine and immediately freezing to 130 °K results in the epr spectra in Figure 3b. These spectra are consistent with the rapid formation of two new paramagnetic species. The transition at $g_1 = 2.37$, $g_2 = 2.23$ and $g_3 = 1.94$ are closely related to intermediate I in the $(CH_3)_3CSH$ case and are clearly associated with the low spin Fe(III) complex FeTPP(Py)(SCH_3). The additional broad transition centered at $g \sim 2.06$ appears closely related to intermediate(II) in the $(CH_3)_3CSH$ reaction. The rapid simultaneous appearance of the epr for two paramagnetic intermediates and their mutual decline in epr intensity indicates that FeTPPCl and the two intermediates are involved in a fast pre-equilibrium.

One possible mechanism by which reaction 1 may occur is given by reaction steps 2–6.

$$\text{FeTPPCl} + \text{Py} \rightleftharpoons \text{FeTPP(Cl)(Py)} \quad (2)$$

$$\text{RSH} + \text{Py} \rightleftharpoons \text{(RS)(HPy)} \quad (3)$$

$$\text{(RS)(HPy)} + \text{FeTPP(Cl)(Py)} \rightleftharpoons \text{FeTPP(RS)(Py)} + \text{HClPy} \quad (4)$$

$$\text{FeTPP(Py)(RS)} + \text{Py} \rightleftharpoons \text{FeTPP(Py)}_2\text{(RS)} \quad (5)$$

$$\text{FeTPP(Py)}_2\text{(RS)} \xrightarrow{\text{slow}} \text{FeTPP(Py)}_2 + 1/2\text{R}_2\text{S}_2 \quad (6)$$

The derived law for this mechanism is

$$-\text{d[FeTPPCl]}/\text{dt} = k[\text{FeTPPCl}][\text{RSH}][\text{Py}]^3/[\text{HClPy}]$$

and is consistent with the observed rate laws $-\text{dFeTPPCl/dt} = k[\text{FeTPPCl}][\text{RSH}][\text{Py}]^3$. The

inverse dependence of PyHCl could not be experimentally verified due to low solubility of PyHCl in toluene. The relatively slow pre-equilibrium step in the 2-methyl-2-propanethiol reaction is proposed to occur in reaction step 5. The species denoted $FeTPP(Py)_2(RS)$ is the S = 1/2 species observed in epr spectra 2b and 3c. The exact formulation of this species is not known at the present time. It could be a cationic species such as $[FeTPP(Py)_2]^+[RS]^-$. This type of species is expected to be very similar to that of the previously reported $FeTPP(Im)_2^+Cl^-$ [21]; however, attempts to observe epr spectra for cationic species of the form $[FeTPP(Py)_2]^+X^-$ in frozen toluene media have been unsuccessful. Another possibility for the species $FeTPP(Py)_2(RS)$ is an associated radical species $Fe^{II}TPP(Py)_2(RS\cdot)$. An unassociated free radical [22] is unlikely because the g values would be smaller and the lines narrower than observed (Figure 2b, 3c). Attempts to observe isotropic epr spectra for an RS radical or to trap an RS radical by using various olefins were unsuccessful.

The reaction of Fe^{III} myoglobin with CH_3SH is consistent with this mechanism. Distilling CH_3SH into an aqueous solution of Fe^{III} myoglobin results in formation of a low spin Fe^{III} myoglobin species. Frozen solution (130 °K) epr spectra indicate g values of 2.39, 2.22 and 1.95, indicative of the low spin species Fe^{III} myoglobin(SR). This species in aqueous solution at 298 °K does not change over a period of 72 hours. The direct interaction of RS^- with the metal center results in a stable complex, and does not directly reduce the metal center. Addition of pyridine or imidazole to the aqueous solution of Fe^{III} myoglobin(RS) results in the immediate disappearance of low spin Fe(III) epr signal and production of R_2S_2 and a low spin Fe(II) complex. Myoglobin contains only one imidazole that can strongly bond the Fe(III) center and thus cannot achieve an intermediate like that in reaction step 4 of the proposed mechanism for reduction of FeTPPCl by RSH. Addition of a nitrogen donor molecule can produce an intermediate of this type and leads to the reduction of Fe(III) to Fe(II).

Substituting imidazole for pyridine in reaction 1 results in the rapid reduction of Fe(III) without detection of an intermediate of the form FeTPP(Im)(RS). FeTPPCl interacts with imidazole to directly form the low spin Fe(III) complex $[Fe(TPP)(Im)_2]^+Cl^-$. This species can then react with the alkyl thiol directly to form $Fe(TPP)(Im)_2 + R_2S_2$ without going through an intermediate with an $Fe^{III}SR$ linkage.

The oxidation of thiols to disulfides by Fe(III) porphyrins becomes catalytic when oxygen is present. The series of reactions has been studied by observing the changes in electronic spectra by sequential addition of reagents. The sequence of reactions in the autocatalysis process is given by equations

$$FeTPPCl + 3Py + RSH \rightarrow FeTPP(Py)_2 + 1/2R_2S_2 + HClPy \quad (7)$$

$$2FeTPP(Py)_2 + 1/2\,O_2 \rightarrow (FeTPP)_2O + 4Py \quad (8)$$

$$(FeTPP)_2O + 2RSH + 4Py \rightarrow 2FeTPP(Py)_2 + R_2S_2 + H_2O \quad (9)$$

The overall reaction is $2RSH + 1/2O_2 \rightarrow R_2S_2 + H_2O$. The catalytic process is found to cycle through the μ-oxo-bis(tetraphenylporphinatoiron(III), $[(FeTPP)_2O]$. This type of reaction may be related to redox cycles in biological systems.

Acknowledgment

This research was supported by PHS Grant AM 17533 and OMR 76-0068.

References

1 D. C. Harrison, *Biochem. J., 18,* 1009 (1924).
2 C. G. Overberger, K. H. Burg and W. H. Daley, *J. Am. Chem. Soc., 85,* 4125 (1965).
3 J. Hill and A. McAuley, *J. Chem. Soc. A,* 2405 (1968).
4 J. Hill and A. McAuley, *J. Chem. Soc. A,* 156 (1968).
5 C. J. Weschler, J. C. Sullivan and E. Deutsch, *Inorg. Chem., 13,* 2360 (1974).
6 D. K. Lanallee, J. C. Sullivan and E. Deutsch, *Inorg. Chem., 12,* 1440 (1973).
7 K. C. Elliot, *Biochem. J., 24,* 310 (1930).
8 H. Lamfrom and S. O. Nielson, *J. Am. Chem. Soc., 79,* 1966 (1957).
9 H. S. Mason, J. C North and M. Vanneste, *Federation Proc., 24,* 1164 (1965).
10 E. Bayer, H. A. O. Hill, A. Roder and R. J. P. Williams, *Chem. Comm.,* 109 (1969).
11 A. Roder and E. Bayer, *Eur. J. Biochem., 11,* 80 (1969).
12 J. O. Stern and J. Peisash, *J. Biol. Chem., 249,* 7495 (1974).
13 J. P. Collman, B. M. Hoffman and T. N. Sorrel, *J. Am. Chem. Soc., 97,* 913 (1975)
14 J. P. Collman and T. N. Sorrell, *J. Am. Chem. Soc., 97,* 4133 (1975).
15 S. Koch, S. C. Tang, R. H Holm and R. B. Frankel, *J. Am. Chem. Soc., 97,* 914 (1975).
16 S. Kock, S. C. Tang, R. H. Holm, R. B. Frankel and J. A. Ibers, *J. Am. Chem. Soc., 97,* 916 (1975).
17 H. Ogoshi, H. Sugimato and Z. Yoshida, *Tetra. Lett., 27,* 2289 (1975).
18 J. C. Tang, S. Koch, G. C. Papaefthymion, S. Foner, R. B. Frankel, J. A. Ibers and R. H. Holm, *J. Am. Chem. Soc., 98,* 2414 (1976).
19 P. Rothermund and A. R. Menetti, *J. Am. Chem. Soc., 70,* 1808 (1948).
20 E. B. Fleisher and T. S. Srivastava, *J. Am. Chem. Soc., 91,* 2403 (1969).
21 D. M. Collins, R. Countrymen and J. L. Hoard, *J. Am. Chem. Soc., 94,* 2066 (1972).
22 "ESR in Chemistry", P. B. Ayscough, Methuen, London, 1967.