Chemical Mechanism of Dioxygen Activation by *trans*-$[FeCl_2(cyclam)]^+$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) in the Presence of Reducing Agents such as Aliphatic Aldehydes or Linolenic Acid†

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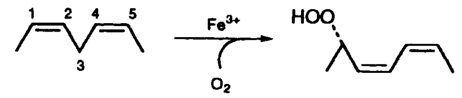
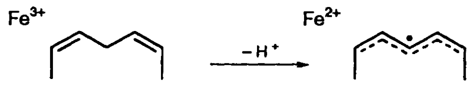
The complex *trans*-$[FeCl_2(cyclam)]^+$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) was found to activate dioxygen in the presence of reducing agents such as an aliphatic aldehyde or linolenic acid (octadeca-9,12,15-trienoic acid), leading to degradation of tetraphenylcyclopentadienone. The activity of the corresponding *cis* isomer is almost negligible. The origin of the high activity of the *trans* compound is discussed in terms of the results of extended-Hückel molecular-orbital calculations performed for the assumed intermediate. It is concluded that the formation of hydrogen bonding between dioxygen and the ligand is very important for activation of dioxygen in this system.

Lipoxygenase (linoleate:oxygen oxidoreductase, E.C. 1.13.11.12) is a non-haem iron dioxygenase which catalyses the dioxygenation of polyunsaturated fatty acids containing the (1*Z*,4*Z*)-penta-1,4-diene system (Scheme 1).[1] Interest in the mechanism of action of lipoxygenase has been stimulated in the last few years by the implication of lipoxygenase in the biosynthesis of leukotrienes which have an important role in immediate hypersensitivity and inflammation.[2] Native lipoxygenase-1 from soy bean is colourless (Fe^{2+}) and virtually ESR-silent, and the active form of the enzyme contains iron in the oxidation state III. Two hypotheses for the mechanism of lipoxygenase catalysis are currently proposed. In the first, abstraction of a hydrogen atom at C^{11} of linolenic acid (octadeca-9,12,15-trienoic acid) is proposed to be a rate-determining step, and is supported by the occurrence of a primary kinetic deuterium isotope effect.[3] Hydrogen-atom abstraction with reduction of the iron would result in formation of a pentadienyl radical as an intermediate (Scheme 2); this was assumed to account for the fact that the active enzyme when treated with linolenic acid in the absence of oxygen lost its characteristic ESR signal at $g = 6$.[4] However, it seems unlikely that the observed ESR data completely support the hypothesis described above. Thus, an alternative mechanism has recently been proposed[5] in which electron transfer to the iron does not take place; this mechanism involves an organoiron intermediate (Scheme 3) in place of the pentadienyl radical and iron(II). As mentioned above, the chemical mechanism for dioxygen activation by lipoxygenase remains unknown at present.

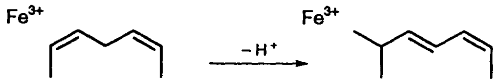
In a previous communication[6] we reported that a mixture of *trans*-$[FeCl_2(cyclam)]ClO_4$ (cyclam = 1,4,8,11-tetraazacyclotetradecane) and linolenic acid is highly efficient in degrading tetraphenylcyclopentadienone (tpcdo), a singlet-oxygen ($^1\Delta_g$) scavenger,[7] and that peroxidation of linolenic acid proceeds in the solution containing *trans*-$[FeCl_2(cyclam)]^+$; however, the corresponding *cis* isomer exhibits no activity for both reactions. Later, we found that the *trans* isomer can degradate the ketone in the presence of an aliphatic aldehyde, such as cyclohexanecarbaldehyde. As it is known that the aldehyde behaves as a reducing agent,[8] it seems reasonable to assume that linolenic acid may act as a reducing agent in the lipoxygenase, and thus a study of the dioxygen activation by *trans*-$[FeCl_2(cyclam)]^+$ in the presence of reducing agents such as linolenic acid or aliphatic aldehydes is important for the elucidation of the chemical mechanism of dioxygen activation by native lipoxygenase. In this article we report the results of such a study.



Scheme 1



Scheme 2



Scheme 3

Experimental

The iron(III) compounds used, *trans*-$[FeCl_2(cyclam)]ClO_4$,[9] *cis*-$[FeCl_2(cyclam)]Cl$,[9] $[Fe(tpma)Cl_2]ClO_4$[10] [tpma = tris(2-pyridylmethyl)amine] and $[Fe(bpmg)Cl_2]$[10] [Hbpmg = *N*,*N*-bis(2-pyridylmethyl)glycine] were obtained according to the literature methods.

The degradation of tpcpo was evaluated by measuring the time course of the absorbance at 505 nm of a mixture of solutions of tpcpo (25 cm^3 of 1/1500 mol dm^{-3} in 1,2-dichloroethane), linolenic acid (1.5 cm^3) and iron(III) compound [2 cm^3 of 1/250 mol dm^{-3} in dimethyl sulfoxide (dmso)]. Its degradation in the presence of the iron(III) compound and an aldehyde was evaluated similarly.

The extent of peroxidation of linolenic acid in the presence of iron(III) compounds was evaluated by means of the thiobarbituric acid (tba) (1*H*,5*H*-2-thioxopyrimidine-4,6-dione) method.[11] In a typical run a solution containing an iron(III) compound (1 cm^3 of 1/250 mol dm^{-3} in dmso), 1,2-dichloroethane (25 cm^3), and linolenic acid (0.5 cm^3) was allowed to

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† *Supplementary data available* (No. SUP 57026, 7 pp.): experimental electronic absorbance and ESR data. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1994, Issue 1, pp. xxiii–xxviii.

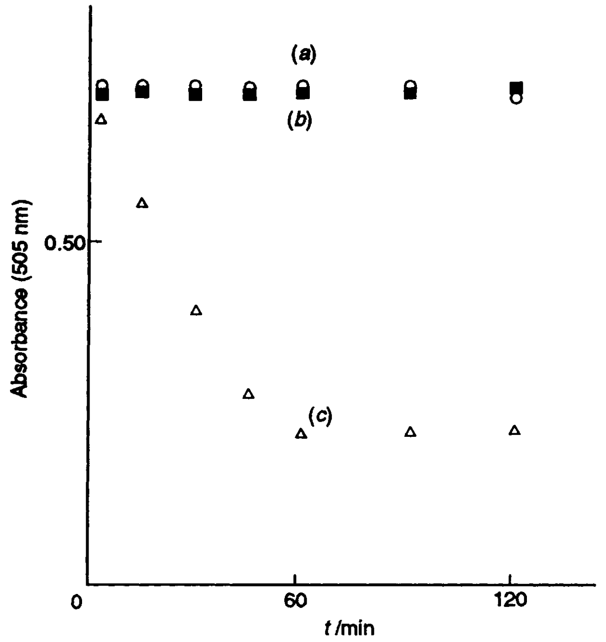


Fig. 1 Time course of the absorbance at 505 nm for reaction mixtures of tpcpo, linolenic acid, and (a) *cis*-[FeCl$_2$(cyclam)]$^+$, (b) no iron(III) complex and (c) *trans*-[FeCl$_2$(cyclam)]$^+$

stand for several hours at room temperature. At appropriate times, 5 cm^3 of the solution were extracted into water (10 cm^3). To 4 cm^3 of this solution was added 2 mol dm^{-3} HCl solution (1 cm^3) containing tba (40 mg) and the mixture heated at 95 °C for 20 min. After cooling, the absorbance at 532 nm was recorded.

X-Band ESR spectra were obtained at liquid-nitrogen temperature (77 K) by the use of a JEOL model RE-2X spectrometer. Cyclic voltammetry (CV) of the iron(III) compounds was performed in the usual way in dmso solution, with 0.1 mol dm^{-3} tetra(*n*-butyl)ammonium tetrafluoroborate as supporting electrolyte and 0.001 mol dm^{-3} metal compounds at 25 °C. The potentials were referenced to a saturated sodium chloride calomel electrode (SSCE). Measurements in the presence of O$_2$ were performed as follows; dioxygen gas was bubbled into the reaction mixture for 20 min at 25 °C, and then CV measurements were made as described above. The concentration of dioxygen in the reaction mixture was evaluated to be 2.1 mmol dm^{-3} based on the work of Sawyer and co-workers.[12]

Results

Fig. 1 shows the time course of the absorbance at 505 nm of reaction mixtures of tpcpo, an iron(III) compound and linolenic acid. It is well known that tpcpo shows a strong absorption band at 507 nm, and reacts with singlet oxygen ($^1\Delta_g$) to yield the corresponding end peroxide with decolouration.[7] In the case of *cis*-[FeCl$_2$(cyclam)]$^+$, no decrease in absorbance at 505 nm was detected within several hours [Fig. 1(*a*)]. On the other hand a rapid decrease in the absorbance was observed with *trans*-[FeCl$_2$(cyclam)]$^+$ [Fig. 1(*c*)]. A similar behaviour was found for the system containing iron(III)–cyclam compounds and cyclohexanecarbaldehyde, the decrease in absorbance at 505 nm being detected only for the mixture containing the *trans* isomer (not shown). Among the iron(III) complexes with tripodal ligands, the activity of the tpma compound was the highest for degradation of tpcpo, but its activity was much lower than that of *trans*-[FeCl$_2$(cyclam)]$^+$. These facts all imply that there is a singlet ($^1\Delta_g$)-like active oxygen species in the solution containing *trans*-[FeCl$_2$(cyclam)]$^+$ and linolenic acid or aliphatic aldehydes; this is very similar to the system of bis(acetylacetonato)nickel(II) and an aliphatic aldehyde.[13]

Table 1 Electrochemical data for iron(III) compounds

Compound	Fe^{III}–Fe^{II} Reduction potential, *E*/V *vs.* SSCE
trans-[FeCl$_2$(cyclam)]$^+$	+0.01
cis-[FeCl$_2$(cyclam)]$^+$	+0.04
[Fe(tpma)Cl$_2$]$^+$	+0.42
[Fe(bpmg)Cl$_2$]	+0.12

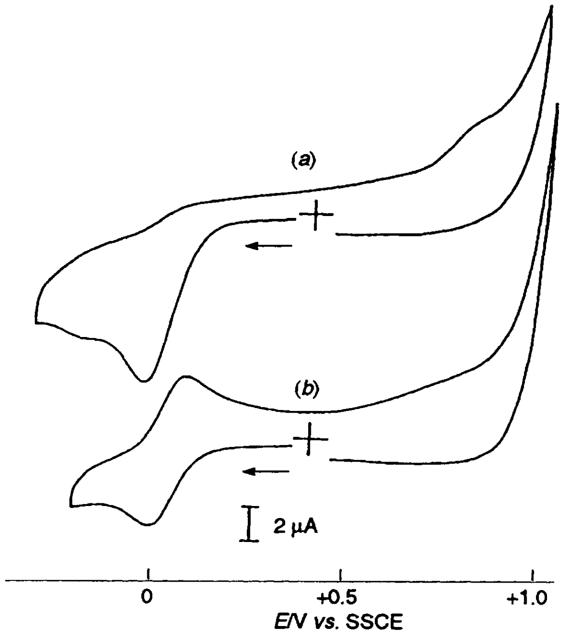


Fig. 2 Cyclic voltammograms of iron(III) compounds under an oxygen atmosphere (25 °C, in dmso, scan speed 100 mV s^{-1}, *cf.* Experimental section): (*a*) *trans*-[FeCl$_2$(cyclam)]$^+$, (*b*) *cis*-[FeCl$_2$(cyclam)]$^+$

We have examined the reaction mixture containing an iron(III) complex and unsaturated fatty acid by the tba method;[11] this method determines the quantity of malonaldehyde in solution, which should be derived from the peroxides of the unsaturated fatty acid. The results in Fig. 2 in ref. 6 are clearly consistent with those described above, *i.e.* only *trans*-[FeCl$_2$(cyclam)]$^+$ can efficiently catalyse peroxidation of unsaturated fatty acids such as linolenic or linoleic acid (octadeca-9,12-dienoic acid).

Table 1 summarizes the electrochemical (CV) data for the iron(III) compounds; under an argon atmosphere, all the processes are quasi-reversible. The reduction potential, $\mathrm{Fe^{III}} \longrightarrow \mathrm{Fe^{II}}$, of the *trans* isomer is more negative than that of the *cis*. This means that *cis*-[FeCl$_2$(cyclam)]$^+$ is reduced more easily to an iron(II) state than is the *trans* isomer, suggesting that this reduction process is not the main source of the observed high activity of the *trans* isomer towards dioxygen in the presence of reducing agents. In order to determine the reason for the different activities observed for *trans*- and *cis*-[FeCl$_2$(cyclam)]$^+$ we measured their CVs under an oxygen atmosphere. As shown in Fig. 2, the presence of dioxygen did not result in any change in the cyclic voltammogram of the *cis* isomer, on the other hand a drastic change was observed for the *trans* isomer, the reoxidation step from Fe^{II} to Fe^{III} becoming irreversible [Fig. 2(*a*)]. This indicates that an iron(II) species derived from the *trans* isomer reacts with dioxygen, but that from the *cis* isomer does not. Recently, Valentine and co-workers[14] investigated the reactivity of the *trans*-[Fe^{II}(cyclam)]$^{2+}$ compound in the presence of several oxidizing reagents, and observed that only H$_2$O$_2$ and not RO$_2$H gave epoxidation of cyclohexene under the same experimental conditions. They concluded that epoxidation occurs by a direct

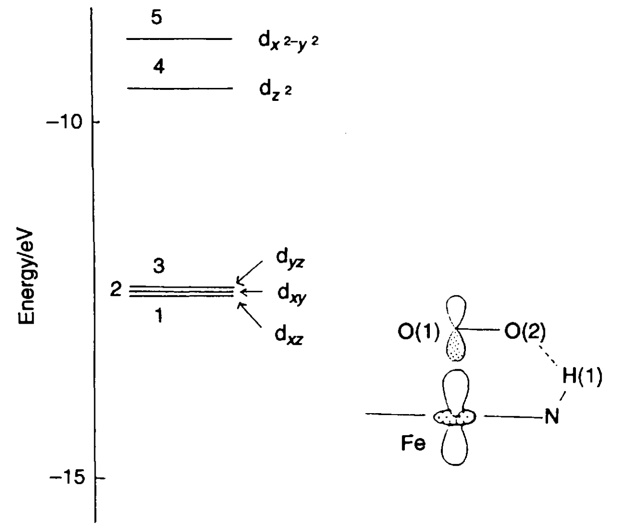
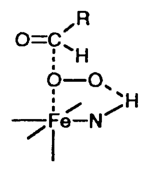


Fig. 3 Molecular-orbital scheme for $[Fe(NH_3)(O_2)]^{3+}$ calculated by the EHMO method. Bond distances are Fe–O(1) 2.10, O(1)–O(2) 1.456, O(2)–H(1) 1.852 and Fe–N (NH_3) 2.20 Å. Orbital 4 can be expressed approximately as $-0.871d_{z^2} - 0.349p_z[O(1)] - 0.006p_z[O(2)] + 0.446p_z(N)$

reaction of the olefin with co-ordinated O_2H^-;[14] such a possibility had previously been discussed by the present authors.[15] The hypothesis that an HO_2^- complex with iron–cyclam may be an intermediate is also supported by molecular modelling, which suggests strongly that the preferred conformation of the cyclam ligand presents the HO_2^- with an axial N–H bond that is well suited for hydrogen bonding.[14] The present CV results under an oxygen atmosphere can be explained by the same assumption of Valentine and co-workers; that is, an iron(II)–cyclam species with *trans* conformation is favourable for hydrogen bonding with dioxygen, leading to facile or rapid reaction between them. In the case of the *cis* isomer the corresponding iron(II) species seems to be inactive towards dioxygen, for reasons which are not clear at present.

Discussion

We have shown that *trans*-$[FeCl_2(cyclam)]^+$ shows a quite different reactivity towards dioxygen in the presence of reducing agents from that of the corresponding *cis* isomer, and that the reduction of Fe^{III} to Fe^{II} is not the most important step in the reactivity. Based on these facts, it seems reasonable that the activation of dioxygen by the *trans* isomer in the presence of reducing agents should occur *via* intermediate **I**: this is formed



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only when the three reagents, aldehyde, dioxygen and iron(III) complex, are present simultaneously. It is noteworthy that the activation of dioxygen is assumed to proceed without change in oxidation state of the iron(III) ion. In the intermediate the dioxygen is co-ordinated to an iron(III) atom and also hydrogen bonded with the chelate ring, as assumed by Valentine and co-workers,[14] and the formation of this intermediate may be rationalized by the fact that the chloride ion is substitution labile in the present iron complex, as will be shown later. In order to obtain more information on the intermediate, we carried out an extended-Hückel molecular orbital (EHMO) calculation for $[Fe(NH_3)_5(O_2)]^{3+}$ as a model for the species assumed by Valentine and co-workers; for the parameters and method used see ref. 16. The results shown in Fig. 3 suggest that the dioxygen molecule behaves as an oxo oxygen atom, because the contribution of atom O(2), which interacts with the hydrogen atom of the ligand system, is nearly zero in the molecular orbital 4, consisting of a metal d_{z^2} orbital and a π^* orbital of the dioxygen. We may anticipate that the highest occupied molecular orbital (HOMO) of an aldehyde molecule[17a] (calculated by the MNDO/PM3 method[17b]) (see Fig. 5) and dioxygen interact through the formation of this intermediate, leading to an organic peracid (in the case of unsaturated acids and dioxygen, to an alkyl hydroperoxide).

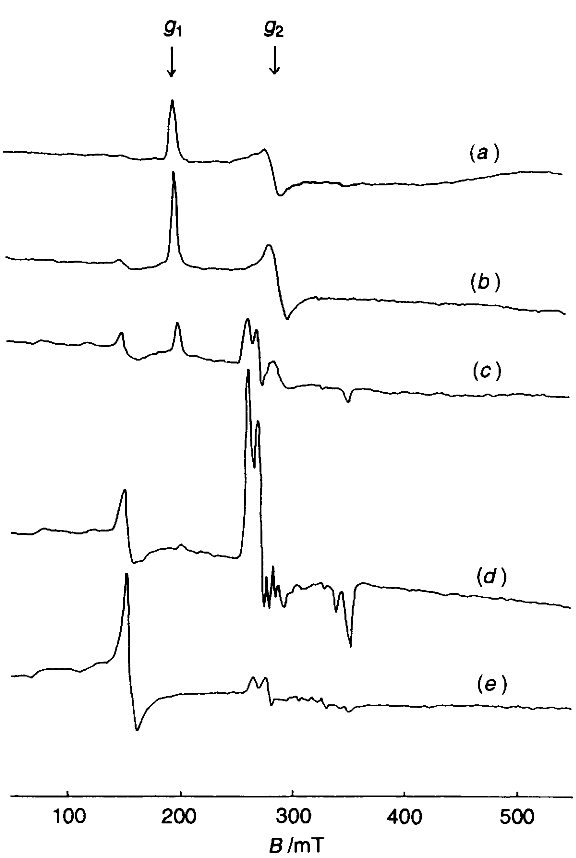


Fig. 4 Time course of the ESR spectrum of a mixture of *trans*-$[FeCl_2(cyclam)]^+$ (1 cm^3 of 1/250 mol dm^{-3} in dmso), 1,2-dichloroethane (12.5 cm^3) and linoleic acid (1 cm^3): (*a*) 1, (*b*) 11, (*c*) 21, (*d*) 31 and (*e*) 132 min

Table 2 The ESR parameters of low-spin species in the reaction mixture of *trans*-$[FeCl_2(cyclam)]^+$ and linoleic acid

Species	g_1	g_2	g_3
1	2.44	2.32	1.87
2	2.29	2.23	1.94

In order to obtain experimental support for the above discussion, we measured the ESR spectrum of the reaction mixture. Fig. 4 shows the time course of the spectra of the system containing *trans*-$[FeCl_2(cyclam)]^+$ and linoleic acid. First a signal due to the original low-spin species is observed [$g_1 = 3.30, g_2 = 2.28$, Fig. 4(*a*)]. After 11 min signals due to a high-spin species ($g = 4.3$) appeared, and the intensity of the original low-spin species gradually decreased [Fig. 4(*c*)]; this may be due to dissociation of chloride ions from the co-ordination sphere. After 21 min a signal due to a new low-spin species appeared, and there are at least two species in the solution (ESR parameters of the low-spin species are summarized in Table 2). After 60 min the signal intensities of

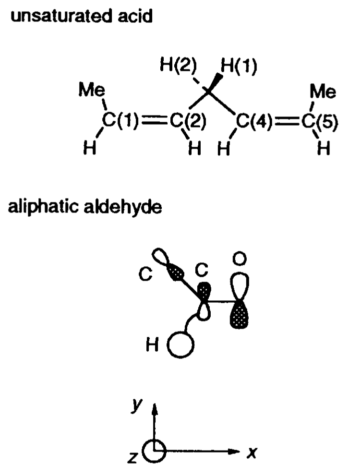
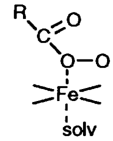


Fig. 5 Diagram showing the unsaturated fatty acid containing (1*Z*,4*Z*)-penta-1,4-diene; the HOMO calculated by the EHMO method can be expressed as $0.445p_z[C(1)] + 0.398p_z[C(2)] - 0.169p_z[C(3)] + 0.398p_z[C(4)] + 0.445p_z[C(5)] + 0.218s[H(1)] - 0.218s[H(1)]$. The HOMO of an aliphatic aldehyde is shown below[17]



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these low-spin species decreased [Fig. 4(*e*)]. Similar but more rapid ESR spectral changes were observed for the system containing *trans*-$[FeCl_2(cyclam)]^+$ and linolenic acid. Interestingly, similar changes were also observed for the system containing *trans*-$[FeCl_2(cyclam)]^+$ and cyclohexanecarbaldehyde (not shown); this suggests that the intermediate complex in the system with linolenic acid should be very similar to that in the system with an aliphatic aldehyde. The HOMO of an unsaturated fatty acid containing a (1*Z*,4*Z*)-penta-1,4-diene moiety is very similar to that of an aldehyde, as illustrated in Fig. 5. As the ESR parameters ($g_1 = 2.44, g_2 = 2.29, g_3 = 1.88$) of the low-spin species formed in the reaction mixture of *trans*-$[FeCl_2(cyclam)]^+$ and *tert*-butyl hydroperoxide are similar to those observed for the solution containing linolenic acid or cyclohexanecarbaldehyde, it seems likely that the low-spin species in the latter solutions may be an iron(III)–alkyl hydroperoxide or organic peracid, **II**. A species containing Cl^- ion instead of a solvent molecule (solv) is also possible; this may explain the presence of two low-spin species in the reaction mixture as described above. The fact that a mixture containing *trans*-$[FeCl_2(cyclam)]^+$ and Bu^tO_2H can degrade tpcpo also supports the above discussion.

Since it is known that an organic peracid or alkyl hydroperoxide can catalyse olefin epoxidation in the presence of metal complexes,[18] the high activity of the present *trans* isomer for dioxygen in the presence of reducing agents such as aliphatic aldehydes or linolenic acid should be due to the formation of an intermediate as shown before. In the case of the *cis* isomer, the addition of linolenic acid or aliphatic aldehydes does not cause any change in the original ESR spectrum of high-spin character ($g = 4.3$). This is also consistent with the observed facts that this isomer is inactive for dioxygen activation in the presence of linolenic acid or aliphatic aldehydes. Our recent observations that a mixture of *trans*-$[FeCl_2(cyclam)]^+$ and cyclohexanecarbaldehyde can catalyse epoxidation of olefins such as styrene or *trans*-stilbene[19] also support the present discussion.

References

1 G. A. Veldink and J. F. G. Vliegenhart, *Adv. Inorg. Biochem.*, 1984, **6**, 139.
2 B. Samuelsson, *Science*, 1983, **220**, 568.
3 M. R. Egmond, G. A. Veldink, J. F. G. Vliegenhart and J. Boldingh, *Biochem. Biophys. Res. Commun.*, 1973, **54**, 1178.
4 J. J. M. C. de Groot, G. J. Garssen, J. F. G. Vliegenhart, J. Boldingh and B. F. van Gelder, *Biochim. Biophys. Acta*, 1975, **337**, 71; **326**, 279.
5 E. J. Corey and R. Nagata, *J. Am. Chem. Soc.*, 1987, **109**, 8107.
6 Y. Nishida, N. Tanaka and S. Takahashi, *Chem. Lett.*, 1993, 411.
7 H. W.-S. Chan, *J. Am. Chem. Soc.*, 1971, **93**, 2357.
8 T. Yamada, T. Takai, O. Rhode and T. Mukaiyama, *Chem. Lett.*, 1991, 1.
9 P. Chan and C. K. Coon, *J. Chem. Soc., Dalton Trans.*, 1976, 858.
10 D. D. Cox and L. Que, jun., *J. Am. Chem. Soc.*, 1988, **110**, 8085; T. Kojima, R. A. Leising, S. Yan and L. Que, jun., *J. Am. Chem. Soc.*, 1993, **115**, 11328.
11 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, London, 1985, ch. 4.
12 D. T. Sawyer, T. S. Calderwood, Y. Yamaguchi and C. T. Angelis, *Inorg. Chem.*, 1983, **22**, 2577; D. T. Sawyer and J. L. Roberts, *J. Electroanal. Chem.*, 1966, **12**, 90.
13 Y. Nishida, T. Fujimoto and N. Tanaka, *Chem. Lett.*, 1992, 1291.
14 W. Nam, R. Ho and J. S. Valentine, *J. Am. Chem. Soc.*, 1991, **113**, 7052.
15 Y. Nishida and M. Takeuchi, *Z. Naturforsch., Teil B*, 1987, **42**, 52; Y. Nishida and K. Takahashi, *J. Chem. Soc., Dalton Trans.*, 1988, 2003; Y. Nishida and T. Yokomizo, *Inorg. Chim. Acta*, 1989, **163**, 9.
16 R. Hoffmann, M. M.-L. Chen and D. L. Thorn, *Inorg. Chem.*, 1977, **16**, 503.
17 (*a*) Y. Nishida, H. Itoh and A. Yamazaki, *Polyhedron*, in the press; (*b*) J. J. J. Stewart, *J. Comput. Chem.*, 1989, **10**, 209.
18 H. Mimoun, *The Chemistry of Peroxides*, ed. by S. Patai, Wiley, New York, 1983, ch. 15.
19 Y. Nishida and N. Tanaka, unpublished work.

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