EPR evidence for one-electron oxidation of phenols by a dimeric manganese(IV/IV) triazacyclononane complex in the presence and absence of hydrogen peroxide

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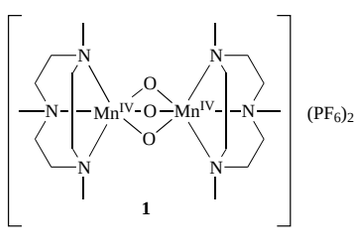
The reaction of $[L_2Mn_2^{IV}(\mu\text{-}O)_3](PF_6)_2$, L = 1,4,7-trimethyl-1,4,7-triazacyclononane (1) with a range of phenols (2–5) in aqueous solution at pH 10.5 has been investigated. At least for electron-rich substrates, the reaction proceeds *via* a rapid overall one-electron process from the phenolate ion to the Mn^{IV}/Mn^{IV} species (1) to give, initially, a Mn^{III}/Mn^{IV} species (detected *via* its characteristic 16-line EPR spectrum in frozen solution at 77 K) and the corresponding phenoxyl radical, detected directly for Trolox (3) by EPR spectroscopy in aqueous solution. The dimeric Mn^{III}/Mn^{IV} species is ultimately converted to monomeric Mn^{II}. In the presence of H_2O_2, reoxidation of the manganese species is accompanied by an increase in the rate of formation of the phenoxyl radicals. In similar reactions, 4-methoxyphenol (4) and 2,6-dimethoxyphenol (5) are converted into polyphenols (with the formation of phenoxyl radicals trapped in a polymer matrix). Kinetic EPR and UV–VIS studies provide additional evidence of the reaction mechanism in the presence and absence of H_2O_2. The mechanism of phenolate oxidation by 1 in the presence and absence of H_2O_2 is discussed.

Introduction

Manganese ions are involved in a number of fundamental biochemical processes,[1,2] for example, the photosynthetic conversion of CO_2 and H_2O into carbohydrates and dioxygen in green plants, algae and some bacteria,[3] for which it is believed that a tetranuclear manganese cluster, in photosystem II (PS II), catalyses the oxidation of water to dioxygen.[4] The exact structure of PS II remains unknown and attracts intensive research efforts. Manganese catalases catalyse the disproportionation of cytotoxic H_2O_2 to water and dioxygen; the most thoroughly characterised example is that of *Lactobacillus plantarum*[5] and a mechanism has been proposed[1,2] in which dimeric Mn^{II}/Mn^{II} and Mn^{III}/Mn^{III} complexes are involved. Studies of synthetic mono- and oligo-meric manganese model complexes[6–8] provide important insights into the structure and function of the more complex biological systems.[1,4]

Structurally related monomeric and dimeric Mn complexes with the 1,4,7-trimethyl-1,4,7-triazacyclononane ligand (TMTACN, see **1**) have recently been reported to act as potent catalysts for the selective oxidation of alkenes, alcohols and DNA by hydrogen peroxide.[8–14] They also serve as efficient low-temperature bleaching catalysts for tea stain oxidation by H_2O_2,[9] and the oxidation of catechol (a tea stain mimic).[9]

In order to obtain more information about the reactions of **1**, we have investigated its oxidation of a range of phenolic substrates including phenol (**2**), Trolox (**3**, a sterically hindered example and vitamin E analogue, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 4-methoxyphenol (**4**) and 2,6-dimethoxyphenol (**5**). Our intention was to explore the possible mechanisms of phenol oxidation by **1** in the presence and absence of H_2O_2, with initial emphasis on water-soluble compounds with relatively low oxidation potentials, some of which would give stabilised or long-lived phenoxyl radicals should this be an important pathway. We have employed EPR and UV–VIS spectroscopy to monitor the nature of paramagnetic intermediates formed and a stopped-flow approach to obtain preliminary kinetic information.



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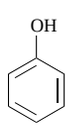

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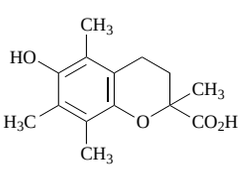

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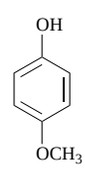

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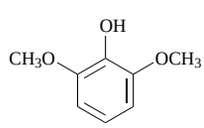

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Results and discussion

Spectroscopic studies of the reactions of 1 with 2–5 in aqueous solution (pH 10.5) at room temperature in the absence of H_2O_2

Results. Initial experiments involved mixing equal volumes of equimolar aqueous solutions of **1** and Trolox (**3**) both at pH 10.5 to give concentrations, after mixing, of 10^{-3} mol dm^{-3} (except where indicated otherwise, all concentrations are those after mixing). These produced characteristic EPR spectra from the fluid and frozen (77 K) solutions. For example, when mixed solutions of **1** and **3** were frozen shortly after mixing (*ca.* 1 min), the EPR spectrum recorded at 77 K displayed the characteristic and relatively isotropic 16-line signal shown in Fig. 1, which is typical of Mn^{III}/Mn^{IV} mixed valence complexes, with

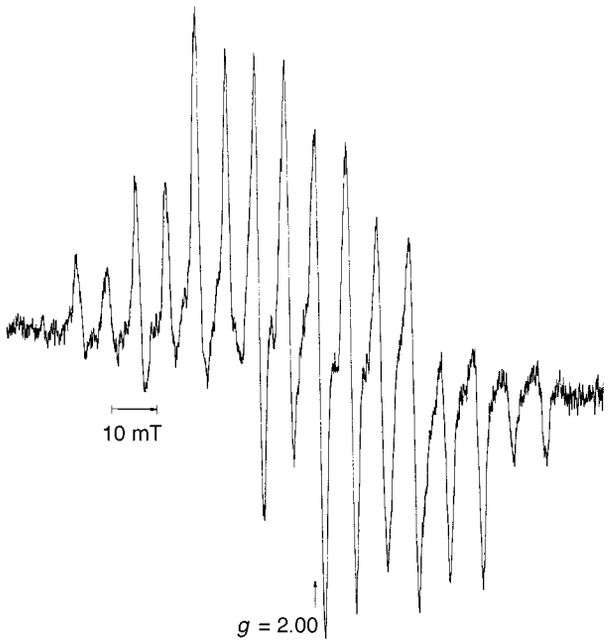
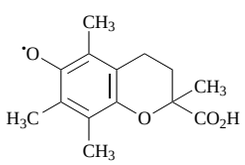


Fig. 1 Sixteen-line EPR spectrum from a Mn^{III}/Mn^{IV} species formed in the reaction of **1** (10^{-3} mol dm^{-3}) and Trolox (**3**) (10^{-3} mol dm^{-3}) at pH 10.5 and recorded at 77 K

splittings from both manganese nuclei.[1,15,16] The hyperfine splittings ($A_{Mn^{IV}} = 6.9$, $A_{Mn^{III}} = 13.8$ mT) are identical to those reported by Hage *et al.* for the Mn^{III}/Mn^{IV} species obtained by electrochemical reduction of **1** in acetonitrile.[16] When the time between mixing and freezing was extended to *ca.* 15 min in experiments with excess of the phenol, a typical six-line mononuclear Mn^{II} spectrum was detected, with $a_{Mn} = 9.6$ mT (Fig. 2). Liquid solution spectra also confirmed the formation of Mn^{II} on this time-scale and allowed quantification of the amount of Mn^{II} formed: for example, in the reaction of **1** (10^{-3} mol dm^{-3}) with **3** (10^{-1} mol dm^{-3}), all of the manganese in **1** had been converted to Mn^{II} after 60 min.

When the reaction of **1** (10^{-5} mol dm^{-3}) with **3** (10^{-3} mol dm^{-3}) was explored in fluid solution using stopped-flow in conjunction with EPR spectroscopy, characteristic isotropic signals from the Trolox phenoxyl radical (**6**) were observed (see below).



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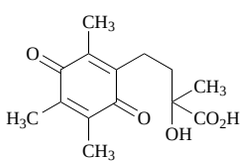

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The build-up and decay of the phenoxyl radical is shown in Fig. 3(*a*). Stronger signals were observed in the presence of H_2O_2 [see Fig. 3(*b*) and Fig. 4 and below]; reaction of **3** with H_2O_2 itself gave relatively weak signals [Fig. 3(*c*)] which nevertheless allowed extra hyperfine splittings to be resolved (see Fig. 4, inset). The spectrum shows quartet splittings of 0.386 and 0.517 mT from the *ortho*-methyl protons, with a doublet splitting of 0.086 mT and a small quartet (0.0265 mT) from the protons of the remaining ring methyl-group [the simulated spectrum is shown in Fig. 4(*b*)]. (NB the parameters differ significantly from those observed for the analogous α-tocopherol phenoxyl radical and those previously reported for a relatively poorly resolved spectrum of **6**.[17]) The observation of a relatively large doublet splitting (0.086 mT) from a single proton in the methylene group is consistent with that expected, on the basis of a $\beta\cos^2\theta$-type interaction, of a (pseudo)axial proton in a cyclohexene-type ring in a half-chair conformation.

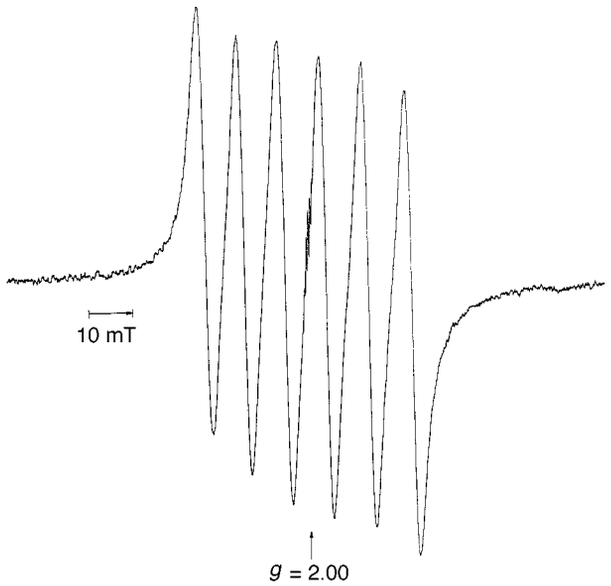

Fig. 2 Fluid solution EPR spectra of Mn^{II}, with the signal from the Trolox phenoxyl radical (**6**) superimposed, resulting from the reaction of **1** (10^{-3} mol dm^{-3}) with Trolox (**3**) (10^{-1} mol dm^{-3}), recorded immediately after mixing

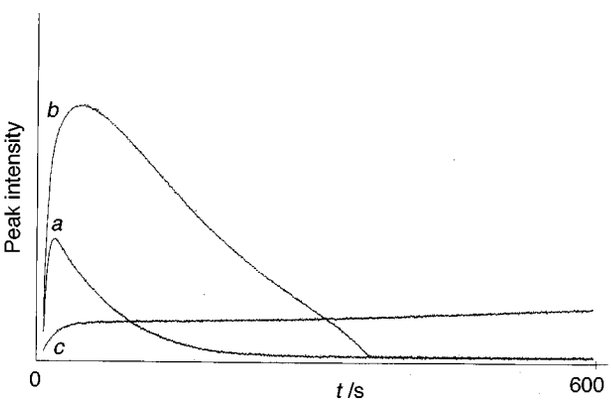

Fig. 3 Stopped-flow EPR time-traces of the intensity of the EPR signal from Trolox phenoxyl radical (**6**) in the reaction of Trolox with **1** in the presence and absence of H_2O_2 (all concentrations after mixing): (*a*) 10^{-5} mol dm^{-3} **1** with 10^{-3} mol dm^{-3} Trolox (**3**), (*b*) as for (*a*) but in the presence of 10^{-2} mol dm^{-3} H_2O_2, (*c*) as for (*b*) but in the absence of **1**

Because the Trolox phenoxyl radical is relatively long-lived, the EPR experiments allowed the detection of radical **6** and Mn^{II} simultaneously in the reaction mixture (see Fig. 2 which shows the EPR spectrum of the Trolox phenoxyl radical superimposed on the six-line Mn^{II} spectrum, generated from the reaction of **1** and **3** immediately after mixing).

Confirmation of the conclusions from the EPR studies was obtained with stopped flow UV–VIS experiments involving the mixing of **1** (10^{-5} mol dm^{-3}) and **3** (10^{-3} mol dm^{-3}), using the characteristic absorbance at 435 nm ($\varepsilon = 7100$ dm^3 mol^{-1} cm^{-1}) of the Trolox phenoxyl radical (**6**).[17] These gave a kinetic profile for the build-up and decay of **6** (Fig. 5), which closely matches the equivalent EPR results (Fig. 3). Furthermore, the decrease in the concentration of radical **6** was accompanied by the appearance of a new absorbance at 260 nm which originates from an oxidation product of Trolox [2-hydroxy-2-methyl-4-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dienyl)butanoic acid, **7**].[18,19]

When 4-methoxyphenol (**4**) (10^{-1} mol dm^{-3}) was mixed with **1** (10^{-3} mol dm^{-3}), followed by immediate quenching to 77 K, a strong 16-line spectrum was also obtained. In contrast, reaction of **5** led to a strong Mn^{II} signal under similar conditions. The

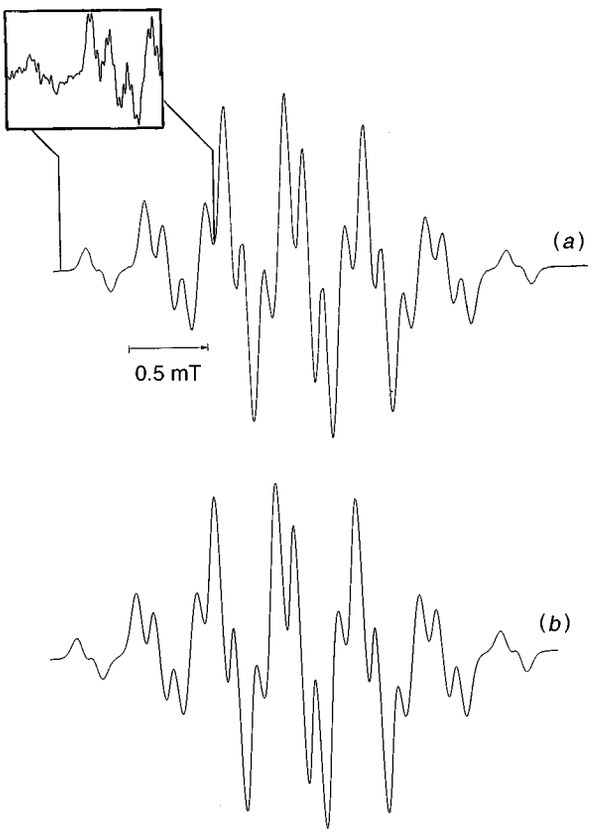

Fig. 4 (*a*) Isotropic EPR spectrum of the Trolox phenoxyl radical ($a_{o\text{-Me}}$ 0.386, $a_{o\text{-Me}}$ 0.517, a_{H} 0.0860 and $a_{m\text{-Me}}$ 0.0265 mT) obtained by mixing (to give final concentrations) **1** (10^{-5} mol dm^{-3}), Trolox (**3**) (2×10^{-3} mol dm^{-3}) and H_2O_2 (10^{-2} mol dm^{-3}), pH 10.5. [Inset: high resolution spectrum of **6** from reaction of **3** (2×10^{-3} mol dm^{-3}) with H_2O_2 (10^{-2} mol dm^{-3})]. (*b*) Simulation of the EPR spectrum from **6** using the parameters above.

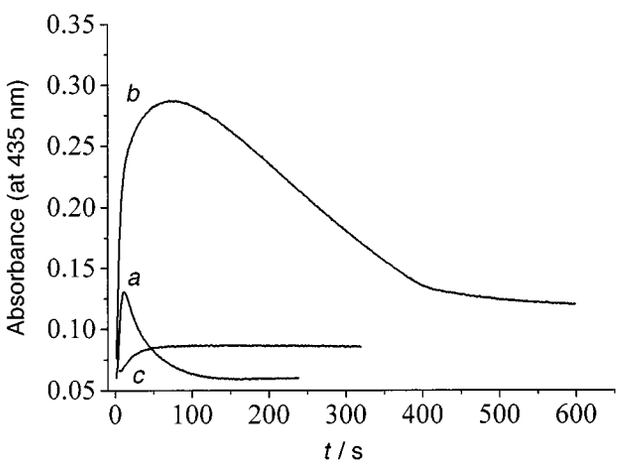

Fig. 5 Stopped-flow UV–VIS kinetic time-traces (monitored at 435 nm) of the reaction of: (*a*) 10^{-5} mol dm^{-3} **1** with 10^{-3} mol dm^{-3} Trolox (**3**), (*b*) as for (*a*) but in the presence of 10^{-2} mol dm^{-3} H_2O_2, (*c*) as for (*b*) but in the absence of **1**

reaction of 4-methoxyphenol (**4**) eventually led, after 60 min at room temperature, to *ca.* 30% conversion of **1** to Mn^{II} (as determined by EPR analysis; see Experimental section), with the remaining Mn probably in the form of MnO_2 (observed as a black precipitate).

Mix–freeze experiments involving the reaction of **1** (10^{-3} mol dm^{-3}) with **5** at much lower phenol concentrations (10^{-3} mol dm^{-3}), and frozen immediately after mixing, showed the characteristic Mn^{III}/Mn^{IV} spectrum described above; at slightly higher concentrations (3×10^{-3} mol dm^{-3}) both Mn^{II} and Mn^{III}/Mn^{IV} signals were observed. A similar result was obtained with Trolox.

Table 1 pK_a Values and oxidation potentials of the phenols and the reduction potential of the Mn^{IV}/Mn^{IV} complex **1** used in this study

Compound	pK_a	$E°$/V (SCE)
Phenol	10.0[a]	0.55[b]
4-Methoxyphenol	10.1[a]	0.30[b]
2,6-Dimethoxyphenol	10.2[c]	0.18[d]
Trolox	11.9[e]	−0.05[f]
$[L_2Mn_2^{IV}(\mu\text{-}O)_3](PF_6)_2$		−0.1[g]

[a] Ref. 21. [b] Ref. 22, pH 11–12. [c] Ref. 23. [d] Ref. 24, pH 13.5. [e] Ref. 25. [f] Ref. 26, pH 13.5. [g] Ref. 16, pH 7.0–10.0.

No radicals could be observed in fluid solution experiments with **4** and **5**, even when using the stopped-flow system, either by EPR or UV–VIS spectroscopy. When phenol (**2**) was used as the substrate no EPR signals were obtained either in fluid solution (even 60 min after mixing) or when the mixed solutions were rapidly quenched to a glass and studied in solid solution at 77 K.

Mechanistic interpretation. The rapid appearance of the Trolox phenoxyl radical (**6**), revealed by the stopped-flow EPR and UV–VIS studies on the reaction of Trolox (**3**) with the Mn^{IV}/Mn^{IV}–TMTACN complex, coupled with the simultaneous formation of the Mn^{III}/Mn^{IV} species, observed in parallel mix and freeze EPR experiments, strongly suggests that the reaction between the dinuclear Mn^{IV}/Mn^{IV} complex and phenols involves an initial one-electron process: this conclusion is reinforced by the detection of the same Mn^{III}/Mn^{IV} species in the reactions of **4** and **5**. Attempts to detect the phenoxyl radicals directly in the latter reactions were unsuccessful; this, perhaps, is not unexpected, even with the assumption that phenoxyl radicals are formed, given the much longer lifetime of **6** than those of phenoxyl radicals with unsubstituted *ortho*- or *para*-positions.[20]

Following the initial one-electron transfer step, the Mn^{III}/Mn^{IV} complex is evidently further reduced to Mn^{II} when the phenol is in excess. This effectively requires three electrons and may involve both mono- and di-nuclear manganese species (although none of these could be detected). The rate and extent of Mn^{II} formation from complex **1** is dependent on the structure and concentration of the phenol. Thus, a 100-fold excess of either of the two most reactive substrates, **3** and **5**, led to a rapid and complete reduction to Mn^{II}, whereas the less reactive 4-methoxyphenol (**4**) gave 30% Mn^{II} together with manganese dioxide over a period of one hour. It is notable that phenol itself is inert under these conditions. The relatively long lifetime of **6** makes it possible to detect the EPR spectrum of the organic radical simultaneously with that of the Mn^{II} species in liquid solution at room temperature. Reaction of **1** using a smaller excess of **5** or equimolar quantities in the case of **3**, resulted in a lower rate of reduction and the detection of the Mn^{III}/Mn^{IV} complex in the 77 K EPR experiments.

Table 1 records the pK_a values of the phenols and the redox potentials of their phenolate ions together with those of the Mn^{IV}/Mn^{IV} complex **1**. From these data, it is clear that both the phenols and their anions will be present in the reaction mixtures at pH 10.5 and that the ease of reduction of **1** by the phenols observed in this study [phenol(inert) < 4-methoxyphenol < 2,6-dimethoxyphenol < Trolox] parallels that predicted from the redox potentials of the phenolate ions. It is also clear from the reduction potential of **1** that, although the products from the initial step are evidently a Mn^{III}/Mn^{IV} species and a phenoxyl radical, the reactions are unlikely to occur by a simple reversible electron-transfer between **1** and the phenolate ions. We believe that although **1** is a relatively weak oxidant (−0.1 V *vs.* SCE), it may be able to oxidise phenolate anions with oxidation potentials up to 0.3 V *vs.* SCE due to a fast subsequent reaction of the initially formed Mn^{III}/Mn^{IV} species.[27] This conclusion is sup-

ported by the studies of Hage *et al.*[16] which show that the electrochemical reduction of **1** in aqueous solution is irreversible. Alternatively, initial complexation between the phenolate ion and **1** may generate a new Mn^{IV}/Mn^{IV} species, possibly with two μ-oxo bridges, with a higher reduction potential than **1** which brings about the observed one-electron process (typically Mn^{IV}/Mn^{IV} species with two oxygen bridges have reduction potentials >0.6 V *vs.* SCE[28]).

Spectroscopic studies of the reactions of 1 with 2–5 in aqueous solution (pH 10.5) at room temperature in the presence of H_2O_2

Results. The experiments described above were repeated in the presence of H_2O_2; whilst a range of concentration ratios was explored most of the experiments employed an excess of H_2O_2 (typically 1 mol dm^{-3}) with [phenol] typically in the range of 10^{-1}–10^{-3} mol dm^{-3} and [**1**] 10^{-3}–10^{-5} mol dm^{-3}.

Examination, by EPR spectroscopy, of the reaction of **4** or **5** (10^{-1} mol dm^{-3}) with **1** (10^{-3} mol dm^{-3}) and H_2O_2 (1 mol dm^{-3}) in fluid and frozen solution showed the appearance of a long-lived, broad singlet ($g = 2.0046$) superimposed on the signals originating from manganese(II). The formation of these radicals was accompanied by the precipitation of solids which were identified by EIMS as mixtures of polyphenols (see Experimental section). The formation of yellow and purple precipitates from **4** and **5**, respectively, indicated that diphenoquinones were also formed during the reaction of **4** and **5** with H_2O_2 and **1**: for example 3,3′,5,5′-tetramethoxydiphenoquinone was isolated as the major product from the reaction of **5** (comparison of ^1H NMR spectrum and mp with those of an authentic sample).

The reaction of **3** (10^{-3} mol dm^{-3}) with **1** (10^{-5} mol dm^{-3}) and H_2O_2 (10^{-1} mol dm^{-3}) resulted in the detection of the isotropic EPR signal from the Trolox phenoxyl radical (**6**) in fluid solution; the intensity was significantly higher than that in the absence of H_2O_2 (see Fig. 3, line *b*). It was also clear that **6** decays more slowly in the presence of H_2O_2. (In contrast to **4** and **5**, oxidation of Trolox did not result in the formation of a precipitate from the aqueous reaction solution.) The abrupt end of the reaction after *ca.* 4–5 min, as judged by the time-dependent concentration of **6**, is attributed to the complete oxidation of Trolox by the catalytically active species: further addition of Trolox at this point led to the regeneration of **6**. This observation shows that the catalyst is still active and by repeated reaction we estimate that it is capable of oxidising up to 300 equivalents of **3**. Similar investigations with **4** also showed that approximately 300 equivalents of phenol can be oxidised by **1**. Interestingly, the addition of 1,4,7-trimethyl-1,4,7-triazacyclononane, after the reaction had finally stopped (presumably on account of catalyst inactivity), resulted in the further oxidation of Trolox and the regeneration of **6** as judged by UV–VIS spectroscopy.

The kinetic EPR and UV–VIS spectroscopy time traces in Fig. 3(*c*) and Fig. 5(*c*) show that there is also a small background reaction between **3** and H_2O_2 in the absence of **1**. This was not affected by the addition of EDTA to the system to complex trace metals.

Mechanistic interpretation. When the reactions between **1** and the electron-rich phenols are carried out in the presence of hydrogen peroxide, our results show that they become catalytic in manganese. Thus, the stopped-flow kinetic time traces (EPR and UV–VIS) for the reaction of **3** show that the presence of H_2O_2 leads to an increase in the concentration of **6**, indicating that the peroxide is capable of rapid regeneration of the active species and subsequently more **6**. The fact that **1** with H_2O_2 brought about the oxidation of approximately 300 molar equivalents (300 turnovers) of **3** and **4** provides further evidence for the catalytic behaviour of the system. A limiting factor in the catalytic process is the destruction of the ligand, TMTACN, as is evident from the observation that the addition of fresh ligand to the oxidation mixtures, after 300 turnovers, restored the activity of the catalyst. The latter experiment also shows that the Mn^{II} ions are complexed by free ligand under the reaction conditions.

Another potential limitation to the application of these systems in catalytic oxidations is the competitive oxidation of the H_2O_2 with the active oxidant (*cf.* the catalase model for the disproportionation of the H_2O_2)[8,29] which results in the non-productive consumption of the peroxide.

The higher yields from the catalytic reactions made it possible to detect and identify the polyphenol and diphenoquinone oxidation products from **4** and **5**. These are typical coupling products from phenoxyl radicals and provide further support for the one-electron steps in these reactions. The EPR signals observed during the catalytic oxidations of these substrates, however, lack fine structure and are too broad and long-lived to be derived from a simple organic radical: we attribute them to polyphenoxyl radicals trapped in a polyphenol polymer matrix. In contrast, the catalysed and uncatalysed oxidations of Trolox did not lead to a polyphenol precipitate and gave the EPR spectrum of **6** rather than of a polyphenoxyl radical since, as has been noted before, coupling of sterically hindered phenoxyl radicals such as **6** is unfavourable.

The fate of the Trolox radical (**6**) in aqueous solution in the absence of an added oxidant has been shown to be disproportionation to **3** and the 1,4-benzoquinone (**7**) ($\lambda_{max} = 260$ nm).[18,19] We believe that the same product (detected by its UV absorption) is also formed in the oxidation of **3** by **1**, both in the presence and absence of H_2O_2, and that this occurs by two sequential single electron-transfers *via* **6**, brought about by a manganese complex or hydrogen peroxide.

From our studies it is clear that, in the absence of H_2O_2, the dinuclear complex **1** is reduced by phenols *via* a Mn^{III}/Mn^{IV} species to mononuclear Mn^{II} and that addition of H_2O_2 regenerates an active oxidant. The nature of the active oxidant(s) in the catalytic system cannot yet be identified: possible candidates are high-valent mono- or di-nuclear Mn–TMTACN complexes, including their oxo, hydroperoxo or μ-peroxo derivatives. A plausible interpretation is that, in the catalytic system, phenol oxidation is brought about by a mononuclear Mn–TMTACN complex derived from the reaction of the Mn^{II}–TMTACN and H_2O_2. However, Hage has shown (EPR spectroscopy)[30] that aqueous solution of the Mn^{II} sulfate and the TMTACN ligand can be oxidised by H_2O_2 to a Mn^{III}/Mn^{IV} species; likewise Bein and co-workers[10] have reported EPR spectroscopy evidence for the generation of a binuclear Mn^{III}/Mn^{IV} complex from the oxidation of Mn^{II} with TMTACN in a zeolite, consequently an alternative mechanism could involve a dinuclear manganese complex as the active oxidant. Further studies are currently underway aimed at resolving these mechanistic details.

Experimental

Materials

Commercially available materials were purchased from Aldrich Chemical Co. Ltd. and used without further purification. Hydrogen peroxide was determined to be 31.1% by weight and was regularly checked during the course of this research by iodometric titration. **1** was provided by R. Hage (Unilever, Holland). Deionised water was used throughout the study.

Methods

EPR experiments were carried out using a Bruker ESP 300 spectrometer equipped with an X-band klystron and at 100 kHz modulation. For the mixing experiments a stopped-flow apparatus with 10 cm^3 drive syringes was fitted to the EPR cavity. For those solutions involving frozen mixtures at 77 K the EPR sample tube was placed in the EPR cavity in a dewar flask containing liquid nitrogen.

Quantitative analysis of the Mn^{II} formed in the reaction of **1** with **3**–**5** was achieved by comparison of the EPR spectrum of

a standard $MnSO_4$ solution with that from the reaction; using the measured intensity of the fourth line in the spectrum calculated with the formula, $I = (\Delta H^2)h$, where ΔH is the width of the hyperfine line and h is its height.[31] UV–VIS spectra and kinetics data were recorded on Hewlett Packard 8452A and 8453 diode array spectrometers. For the study of the reaction of **1** with **3** and H_2O_2 an Applied Photophysics RX 1000 stopped-flow apparatus equipped with 2.5 cm^3 drive syringes and a dual path-length (10/2 mm) cuvette was attached to the spectrometer. The syringes and the reaction chamber were thermostatted at 30 °C. pH measurements were made with a Corning 220 pH meter equipped with a Reagecom combined pH/reference electrode.

EI-Mass spectra were recorded on a VG Analytical Autospec instrument. ^1H NMR spectra were recorded on a Bruker 300 spectrometer (300 MHz) using D_2O and $CDCl_3$ with DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) and TMS as references, respectively.

Reaction and kinetic procedures

Stopped-flow EPR spectroscopy of the reaction of 1 with 3 in aqueous solution (pH 10.5) in the presence and absence of H_2O_2. The stopped-flow apparatus attached to the EPR cavity consisted of three 10 cm^3 syringes which were filled with solutions of the reactants in aqueous borate buffer (pH 10.5, 10^{-2} mol dm^{-3}). The two (three) reactants were injected into the EPR cell and the 120 s scan started immediately. In order to obtain the kinetic EPR time trace, the increase and decrease in intensity of the centre signal of the radical **6** was monitored over 10 min.

Stopped-flow UV–VIS spectroscopy of the reaction of 1 with 3 in aqueous solution (pH 10.5) in the presence and absence of H_2O_2. The 2.5 cm^3 two drive syringes of the stopped-flow apparatus were filled with solutions of the reactants in pH 10.5 aqueous borate buffer (10^{-2} mol dm^{-3}). When **1** was reacted with **3** and H_2O_2, **1** was mixed with H_2O_2 in one drive syringe and **3** was placed in the other. The reactants were then injected into the stopped-flow UV–VIS cell and the scanning at 435 and 260 nm started immediately. The reaction was followed for 10 min. The kinetic information was processed with a PC using a Hewlett Packard UV–VIS ChemStation software package and transferred into a maths programme (Origin®) to draw the kinetic time plots.

Reaction of 1 with 2, 4 or 5 in the presence of H_2O_2 in aqueous solution (pH 10.5) at room temperature. Stock solutions of the reactants in pH 10.5 aqueous borate buffer (10^{-2} mol dm^{-3}) solution were prepared and mixed in a sample tube before being transferred to an EPR flat-cell. The spectra were recorded over 320 s.

Rapid freezing (77 K) reaction of 1 with 2, 4 or 5 in the presence of H_2O_2 in aqueous solution (pH 10.5). The reaction mixtures were prepared in a sample tube (as above) and then, after the required reaction time, methanol was added to the mixture (33% w/w, to obtain a water–methanol glass) and the mixture was immediately frozen in liquid nitrogen at 77 K.

Isolation and characterisation of 3,3′,5,5′-tetramethoxydiphenoquinone from the oxidation of 2,6-dimethoxyphenol. Addition of 2,6-dimethoxyphenol to hydrogen peroxide and the Mn^{IV}/Mn^{IV} complex (**1**) in aqueous borate buffer (10^{-2} mol dm^{-3}), to give final concentrations of 10^{-1}, 1 and 10^{-3} mol dm^{-3}, respectively, in a total volume of 3 cm^3, gave an immediate purple precipitate. Filtration, washing of the precipitate with deionised water (3 × 10 cm^3) and drying (100 °C under vacuum for 1 h) gave the diphenoquinone. Mp 290 °C (dec) [lit. value,[32] 287 °C (dec)]; δ_H(300 MHz, $CDCl_3$) 3.95 (s, 12H) 6.71 (s, 4H) (Found: C, 63.15; H, 5.29. $C_{16}H_{16}O_6$ requires: C, 63.51, H, 5.33%).

Isolation and characterisation of polyphenol product from the oxidation of 4-methoxyphenol. The reaction of 4-methoxyphenol was carried out as described above for 2,6-dimethoxyphenol and the yellow–brown precipitate was filtered, washed (3 × 10 cm^3 deionised water) and dried (100 °C under vacuum for 1 h) to give the polyphenol. This was characterised by EIMS, *m/z* 856 [1, $(ArO)_7$], 734 [12, $(ArO)_6$], 718 (1), 612 [36, $(ArO)_5$], 594 (5), 490 [56, $(ArO)_4$], 474 (17), 368 [67, $(ArO)_3$], 352 (34), 246 [100%, $(ArO)_2$] and 230 (68).

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References

1 *Manganese Redox Enzymes*, ed. V. L. Pecoraro, VCH, New York, 1992.
2 G. C. Dismukes, *Chem. Rev.*, 1996, **96**, 2909.
3 L. Stryer, *Biochemistry*, W. H. Freeman, New York, 1988.
4 V. K. Yachandra, K. Sauer and M. P. Klein, *Chem. Rev.*, 1996, **96**, 2927.
5 Y. Kono and I. Fridovich, *J. Biol. Chem.*, 1983, **258**, 6015.
6 K. Wieghardt, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 1153.
7 K. Wieghardt, U. Bossek, B. Nuber, J. Weiss, J. Bonvoisin, M. Corbella, S. E. Vitols and J. J. Girerd, *J. Am. Chem. Soc.*, 1988, **110**, 7398.
8 R. Hage, *Recl. Trav. Chim. Pays-Bas*, 1996, **115**, 385.
9 R. Hage, J. E. Ilburg, J. Kerschner, J. H. Koek, E. L. M. Lempers, R. J. Martens, U. S. Racherla, S. W. Russel, T. Swarthoff, M. R. P. van Vliet, J. B. Warnaar, L. van der Wolf and B. Krijnen, *Nature* (*London*), 1994, **369**, 637.
10 D. E. De Vos, J. L. Meinershagen and T. Bein, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 2211.
11 D. E. De Vos and T. Bein, *J. Organomet. Chem.*, 1996, **520**, 195.
12 C. Zondervan, R. Hage and B. L. Feringa, *Chem. Commun.*, 1997, 419.
13 D. E. De Vos and T. Bein, *Chem. Commun.*, 1996, 917.
14 T. Kobayashi, K. Tsuchiya and Y. Nishida, *J. Chem. Soc., Dalton Trans.*, 1996, 2391.
15 K. Wieghardt, U. Bossek, L. Zsolnai, G. Huttner, G. Blondin, J. J. Girerd and F. Babonneau, *J. Chem. Soc., Chem. Commun.*, 1987, 651.
16 R. Hage, B. Krijnen, J. B. Warnaar, F. Hartl, D. J. Stufkens and T. L. Snoeck, *Inorg. Chem.*, 1995, **34**, 4973.
17 M. J. Davies, L. G. Forni and R. L. Willson, *Biochem. J.*, 1988, **255**, 513.
18 M. J. Thomas and B. H. J. Bielski, *J. Am. Chem. Soc.*, 1989, **111**, 3315.
19 C. Suarna, D. C. Craig, K. J. Cross and P. T. Southwell-Keely, *J. Am. Chem. Soc.*, 1988, **53**, 1281.
20 W. G. B. Huysmans and W. A. Waters, *J. Chem. Soc. B*, 1966, 1047.
21 E. P. Serjeant and B. N. Dempsey, *Ionisation Constants of Organic Acids in Aqueous Solution*, Pergamon, Oxford, 1979.
22 J. Lind, X. Shen, T. E. Eriksen and G. Merenyi, *J. Am. Chem. Soc.*, 1990, **112**, 479.
23 E. Chapoteau, B. P. Czech, A. Kumar, A. Pose, R. A. Bartsch, R. A. Holwerda, N. K. Dalley, B. E. Wilson and J. Weining, *J. Org. Chem.*, 1989, **54**, 861.
24 S. V. Jovanovic, M. Tosic and M. G. Simic, *J. Phys. Chem.*, 1991, **95**, 10 824.
25 S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic and M. G. Simic, *J. Am. Chem. Soc.*, 1994, **116**, 4846.
26 S. Steenken and P. Neta, *J. Phys. Chem.*, 1982, **86**, 3661.
27 L. Eberson, *Electron transfer reactions in organic chemistry*, Springer-Verlag, Berlin, 1987.
28 H. H. Thorp and G. W. Brudwig, *New J. Chem.*, 1991, **15**, 479.
29 U. Bossek, M. Saher, T. Weyhermuller and K. Wieghardt, *J. Chem. Soc., Chem. Commun.*, 1992, 1780.
30 R. Hage, personal communication.
31 C. Rossi, F. Laschi, R. Pogni, E. Tiezzi, R. Basosi and L. Pogliani, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 3331.
32 D. A. Rockcliffe and A. E. Martell, *J. Mol. Catal. A: Chem.*, 1996, **106**, 211.

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