

Oxidised metallophenolicporphyrins as models for Compound I of the peroxidases. Reduction of oxidised iron(III), nickel(II) and zinc(II) complexes of *meso*-tetrakis(3,5-di-*t*-butyl-4-hydroxyphenyl)porphyrin by the peroxidase substrate *p*-cresol

Niamh Nic Daeid and Kevin B. Nolan*

Department of Chemistry, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2 (Ireland)

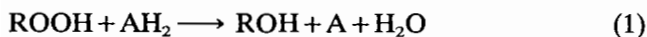
(Received March 1, 1993; revised June 4, 1993)

Abstract

Oxidised metal complexes of *meso*-tetrakis(3,5-di-*t*-butyl-4-hydroxyphenyl)porphyrin (3), M-TBHPP_{ox}, react with peroxidase substrates such as *p*-cresol regenerating the parent metalloporphyrins cleanly and quantitatively. The kinetics of the reduction of the oxidised iron(III), nickel(II) and zinc(II) porphyrin complexes in chloroform and methanol have been investigated. Generally rate expressions of the form, rate = { $k_1 + k_2[p\text{-cresol}]$ }[M-TBHPP_{ox}] were observed where the k_1 term represents an auto- or solvent-assisted reduction pathway while the k_2 term represents a reduction pathway involving *p*-cresol. For the reduction of Ni-TBHPP_{ox} in chloroform however a rate expression of the form rate = { $k_1 + k_3[p\text{-cresol}]^2$ }[Ni-TBHPP_{ox}] was observed, the second order dependence of rate on *p*-cresol concentration being attributed to pre-equilibrium formation of a *p*-cresol dimer which in the rate determining step is the active reductant. Rate constants for the above reactions are reported as well as activation parameters for the reduction of the oxidised nickel(II) complex in both solvents.

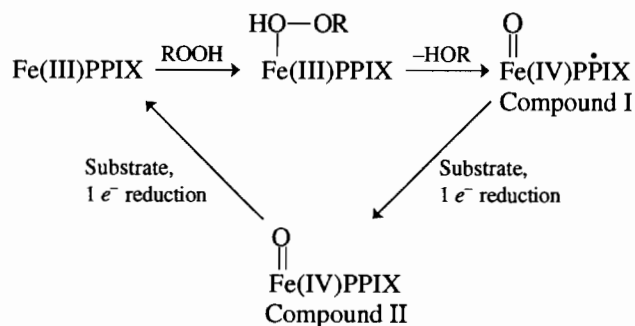
Introduction

The peroxidases are haemoproteins which catalyse the oxidation of a variety of organic and inorganic compounds by hydrogen peroxide and by alkyl and acyl hydroperoxides, eqn. (1) [1].



Much of our understanding of the mechanism of peroxidase catalysed reactions emanates from horseradish peroxidase (HPR), which has been extensively studied because of its availability from the root of the horseradish plant. This enzyme like most peroxidases contains a high spin iron(III)-protoporphyrin IX prosthetic group and has the imidazole side chain of a histidyl residue from the protein also complexed to the metal.

The mechanism of peroxidase catalysed oxidations proceeds via two intermediates, Compounds I and II as shown in Scheme 1 [2]. Complexation of a peroxide or a peracid to horseradish peroxidase is followed by heterolytic fission of the O–O bond producing Compound I, an oxyferryl porphyrin π radical species in which one oxidising equivalent of the oxidant has been transferred to the metal and the second to the porphyrin.



PPIX = protoporphyrin IX, PPIX \cdot = porphyrin cation radical

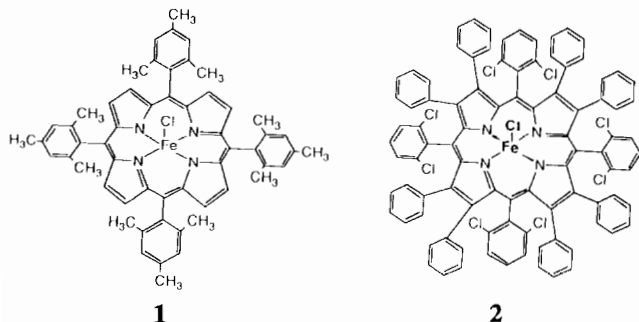
Scheme 1.

Compound I undergoes two successive one-electron reductions by an oxidisable substrate to reproduce the original enzyme. The product of the first reduction is Compound II, an oxyferryl porphyrin species.

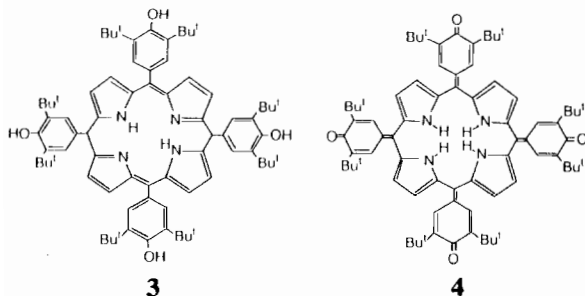
Previous attempts to mimic Compound I both structurally and chemically have met with varying degrees of success. The main difficulty in this work is due to the general instability of iron(III) porphyrins to oxidative degradation on treatment with peracids/peroxides and their propensity to form binuclear μ -oxo complexes [3]. However Groves *et al.* have successfully prepared an

*Author to whom correspondence should be addressed.

oxyferryl porphyrin cation radical intermediate by the reaction of *m*-chloroperbenzoic acid (*m*-CPBA) with **1** at $-46\text{ }^{\circ}\text{C}$ in CH_2Cl_2 solution and at $-78\text{ }^{\circ}\text{C}$ in $\text{CH}_2\text{Cl}_2/\text{MeOH}$ solution [4, 5]. This produced a green intermediate having broad bands in its visible spectrum at 406 and 645 nm and was characterised as an oxoiron(IV) porphyrin cation radical complex by a combination of NMR, Mössbauer, ESR and electronic spectra. A relatively stable oxoiron(IV) porphyrin cation radical species has also been prepared by the action of *m*-CPBA on the octachlorooctaphenylporphyrin complex (**2**) in CH_2Cl_2 at $8\text{ }^{\circ}\text{C}$ [6].



Yet another approach in the attempted formation of high valent iron porphyrin cation radical complexes has involved porphyrins with phenolic substituents such as 2H-TBHPP (**3**). The presence of phenolic groups in these porphyrins was expected to accommodate radical formation with the bulky *t*-butyl substituents offering steric protection to the radicals so formed from intermolecular association as is the case in the stabilisation of the 2,4,6-tri-*t*-butylphenoxy radical [7]. Aerial oxidation of **3** under basic conditions gives a porphyrin-phenoxy radical intermediate which decays firstly to a porphodimethene type compound and then to the tetraquinonemethideporphyrinogen (**4**) on neutralising the reaction solution [7, 8]. Oxidation of **3** in acid solution gives a long lived π cation radical species [9].



It was found that several metallo derivatives of **3** can undergo facile two-electron aerial oxidation producing oxidants which mimic the chemical properties of Compound I of horseradish peroxidase [7, 10]. However only some of the metallo derivatives of the porphyrin undergo such oxidation and this is related to whether

or not the macrocyclic core is planar or puckered [10]. The oxidised metalloporphyrin complexes are reduced by peroxidase substrates such as *p*-cresol in a manner similar to Compound I of the peroxidase enzyme and the original metalloporphyrin species is regenerated. The kinetics of oxidation of *p*-cresol by the oxidised iron(III), nickel(II) and zinc(II) porphyrin complexes is reported in this paper.

Experimental

Oxidation of metalloporphyrins, *M*-TBHPP; *M* = Fe(III), Ni(II), Zn(II)

The porphyrin 2H-TBHPP and its metal complexes, Ni(II)-TBHPP, Zn(II)-TBHPP and Fe(III)-TBHPP, were prepared according to previously reported methods [7, 11]. To a dilute solution of the metalloporphyrin ($\sim 10^{-4}\text{ M}$) in chloroform (25 cm^3) in a separating funnel, was added a few drops of a 40% aqueous solution of tetrabutyl ammonium hydroxide. This resulted in an immediate colour change from wine red to mauve. An equal volume of deionised water was added and the mixture shaken vigorously and then allowed to separate. The aqueous phase was removed and the organic layer, which was now green, was washed twice more with deionised water and finally dried over anhydrous sodium sulfate (Aldrich), and filtered. This was used as a stock solution for the kinetic studies. The electronic spectra of Zn(II)-TBHPP and its oxidation product are shown in Fig. 1.

In order to investigate the ability of the oxidised metalloporphyrins to mimic the behaviour of peroxidase Compound I, *p*-cresol, which is oxidised to Pummerer's ketone by the enzyme [1], was chosen as a substrate. Commercially available *p*-cresol (Aldrich) was recrystallised from petroleum ether (40–60). Solutions of the required concentrations for the kinetic studies were prepared in both methanol and chloroform. The solutions were freshly prepared prior to kinetic measurements and the reactions were studied without prior deoxygenation since rates were found to be the same in deoxygenated solutions.

The kinetics of the reduction of the oxidised metalloporphyrins by *p*-cresol were investigated spectrophotometrically on a Philips PU 8700 series UV-Vis spectrophotometer. Temperature was maintained constant ($\pm 0.2\text{ }^{\circ}\text{C}$) using a Haake D1 circulating water bath. Before a kinetic run the appropriate *p*-cresol solution was allowed to temperature equilibrate for at least 5 min in the spectrophotometer. After this time, a small quantity of the stock solution of the oxidised metalloporphyrin was added, the solutions thoroughly mixed and the reappearance of the Soret band of the parent metalloporphyrin recorded over time. The re-

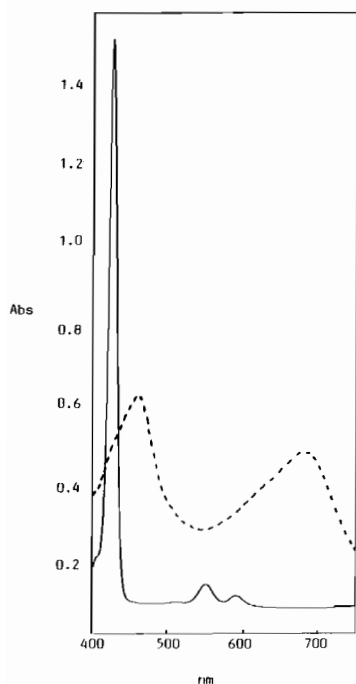


Fig. 1. Visible absorption spectra of Zn(II)-TBHPP (solid line, λ_{\max} 427, 552, 592 nm) and its air oxidation product (λ_{\max} 462, 680 nm) in chloroform.

actions were studied under pseudo first order conditions using a vast excess of *p*-cresol at various concentrations. The concentrations of metalloporphyrin in the reaction solutions were typically $\sim 5 \times 10^{-6}$ M. Each reaction was followed for at least two and a half to three half lives and each kinetic run was performed in duplicate or triplicate.

Results and discussion

The phenolic porphyrin (**3**) undergoes oxidation in basic chloroform solution to give a radical species which decays to the porphyrinogen (**4**) on neutralisation [8]. This porphyrinogen was also prepared by oxidation of **3** with *m*-CPBA in the presence of a catalyst and a spectrophotometric titration showed the reaction to be a $2e^-$ oxidation [7]. A crystal structure determination showed that **4** contains four coplanar quinonemethide groups with a highly puckered porphyrinogen core [12].

Some metal complexes of the porphyrin (**3**) also undergo facile aerial oxidation in basic solution and give the same product after washing the organic phase with water as that using *m*-CPBA as oxidant [7, 10]. The spectra of the various oxidised metalloporphyrins are virtually identical, suggesting that the site of oxidation is the same in each case and since the zinc(II) porphyrin complex contains a metal which cannot be oxidised, the $2e^-$ oxidation must therefore be occurring

on the porphyrin ring. The oxidised metalloporphyrins are therefore metal complexes of the porphyrinogen (**4**) or of its tautomers. Metal complexes of porphyrinogens have recently been reported [13].

Metal incorporation into the porphyrin affects the planarity of the core and hence the ease of oxidation of the complex. A planar metalloporphyrin is estimated to have a central hole radius of 2.01 Å and hence if the metal nitrogen bond distance is close to this value the porphyrin remains planar [10]. In this situation, *meso*-aryl substituents are positioned perpendicular to the porphyrin plane so that little or no interaction between the π clouds of the macrocycle and the substituents occurs and aerial oxidation is impeded. Such is the case for the Pd(II) complex of **4** in which the M-N bond lengths are 2.022 Å whereas complexes with Fe(III), Co(II), Ni(II) and Zn(II) are readily oxidised [10]. These metal ions have ionic radii smaller than that of Pd(II) and in order to complex the porphyrin is forced to undergo contraction giving rise to a puckering of the macrocyclic core. The non-planarity of the porphyrin allows the aryl substituents to move from the perpendicular positions towards the plane, interactions occur between the π -electron clouds and facile aerial oxidation results. In the case of the Ni(II) complex the M-N bond distance is 1.91 Å, longer than normal for Ni-N bonds with imine type nitrogens but shorter than the ideal M-N distance for porphyrin planarity, giving a highly puckered porphyrin which is oxidised readily by air under basic conditions.

Because of the difference between the electronic spectra of the oxidised and reduced forms of the metalloporphyrins, the kinetics of reduction of the oxidised metalloporphyrins by *p*-cresol were followed spectrophotometrically by monitoring the reappearance of the Soret band of the metalloporphyrin as a function of time. In the case of nickel(II) and zinc(II) the parent metalloporphyrin complexes Ni(II)-TBHPP and Zn(II)-TBHPP were regenerated quantitatively in the reaction and for iron(III) the complex Fe(III)-TBHPP(*p*-cresol) was obtained (this is also obtained when Fe(III)-TBHPP(Cl) is dissolved in *p*-cresol solutions of the same concentrations as those employed in the kinetic studies). The spectral changes observed during the reduction of the oxidised Ni(II)-TBHPP complex are shown in Fig. 2. Plots of $\ln(\text{Abs}_\infty - \text{Abs}_t)$ versus t were constructed using a computerised iterative program and good linear plots were obtained over 2-3 half lives of reaction. Values of k_{obs} the pseudo first order rate constants were obtained from the slopes of these plots and are presented in Table 1.

In all cases, with one exception, plots of k_{obs} versus *p*-cresol concentration were found to be linear as shown by the typical plot in Fig. 3 and expressed by eqn. (2).

TABLE 1. Values of k_{obs} for the reduction of the oxidised metalloporphyrin complexes by *p*-cresol in chloroform and methanol at 298 K. All values are $\pm 3\%$

| Complex | Chloroform | | Methanol | |
|-----------------------------|--|--|--|--|
| | [<i>p</i> -cresol] (mol dm ⁻³) | k_{obs} (s ⁻¹) | [<i>p</i> -cresol] (mol dm ⁻³) | k_{obs} (s ⁻¹) |
| Ni(II)-TBHPP _{ox} | 2.0 | 3.10×10^{-3} | 2.0 | 1.93×10^{-3} |
| | 1.5 | 1.68×10^{-3} | 1.5 | 1.53×10^{-3} |
| | 1.0 | 7.56×10^{-4} | 1.0 | 1.05×10^{-3} |
| | 0.5 | 2.59×10^{-4} | 0.5 | 5.56×10^{-4} |
| | 0.25 | 8.77×10^{-5} | 0.25 | 3.22×10^{-4} |
| Fe(III)-TBHPP _{ox} | 0.25 | 2.64×10^{-2} | 0.15 | 4.53×10^{-2} |
| | 0.15 | 1.54×10^{-2} | 0.10 | 2.63×10^{-2} |
| | 0.05 | 4.35×10^{-3} | 0.05 | 9.07×10^{-3} |
| | 0.025 | 1.99×10^{-3} | 0.03 | 5.21×10^{-3} |
| Zn(II)-TBHPP _{ox} | a | a | 0.15 | 2.06×10^{-2} |
| | | | 0.10 | 1.41×10^{-2} |
| | | | 0.051 | 9.88×10^{-3} |
| | | | 0.025 | 5.70×10^{-3} |

^aReliable data could not be obtained for the reduction of Zn(II)-TBHPP_{ox} in chloroform.

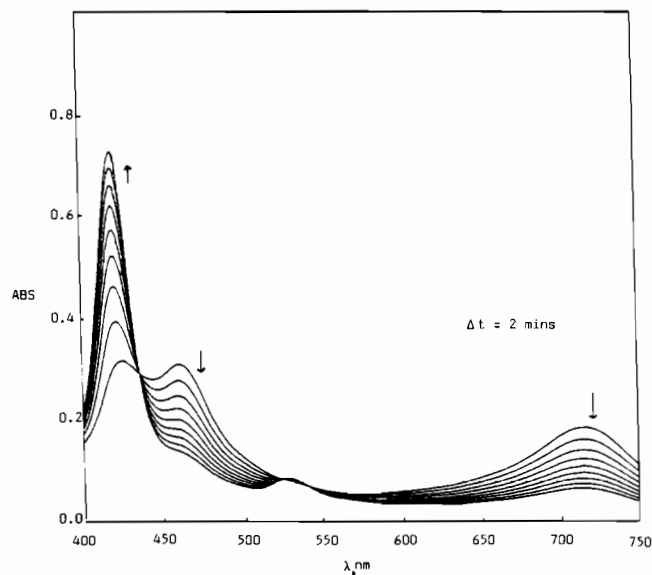


Fig. 2. Spectral changes accompanying the reduction of Ni(II)-TBHPP_{ox} by *p*-cresol in methanol (2 M) at 25 °C.

$$k_{\text{obs}} = k_1 + k_2[\textit{p}\text{-cresol}] \quad (2)$$

For the reduction of the oxidised iron(III) complex $k_1=0$. The two term rate law implies two parallel reaction pathways, one (k_1) involving auto- or solvent-assisted reduction of the oxidised metalloporphyrin, the other (k_2) involving the reductant. For the oxidised Ni(II)-TBHPP complex in chloroform it was found that k_{obs} varied linearly with the square of the *p*-cresol concentration, Fig. 4. The second order dependence of rate on *p*-cresol concentration suggests a reaction mechanism involving pre-equilibrium formation of an

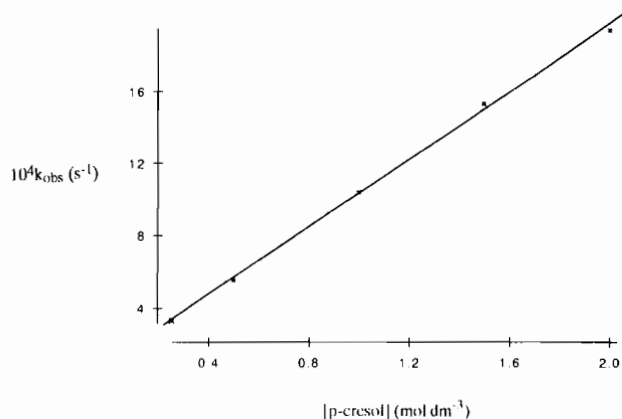


Fig. 3. Plot of k_{obs} vs. [*p*-cresol] for the reduction of Ni(II)-TBHPP_{ox} in methanol at 25 °C.

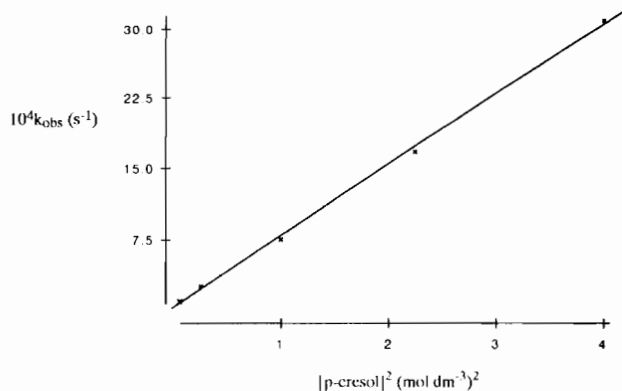
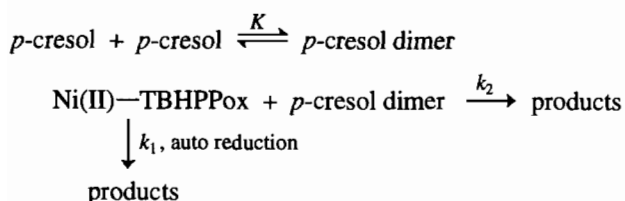


Fig. 4. Plot of k_{obs} vs. [*p*-cresol]² for the reduction of Ni(II)-TBHPP_{ox} in chloroform at 25 °C.

TABLE 2. Rate data for the reduction of M-TBHPP_{ox} by *p*-cresol in chloroform and methanol at 298 K. All values $\pm 3\%$

| Complex | Chloroform | | | Methanol | |
|---------------|-----------------------------|---|---|-----------------------------|---|
| | k_1 (s ⁻¹) | k_2 (M ⁻¹ s ⁻¹) | k_3 (M ⁻² s ⁻¹) | k_1 (s ⁻¹) | k_2 (M ⁻¹ s ⁻¹) |
| Ni(II)-TBHPP | 3.04×10^{-5} | | 7.58×10^{-4} | 9.95×10^{-5} | 9.32×10^{-4} |
| Fe(III)-TBHPP | | 1.09×10^{-1} | | | 3.40×10^{-1} |
| Zn(II)-TBHPP | | | | 3.306×10^{-3} | 1.14×10^{-1} |



Scheme 2.

H-bonded, *p*-cresol dimer [14] which serves as the active reductant, Scheme 2.

The rate expression for the reaction in Scheme 2 is given by eqn. (3) [15].

$$\text{rate} = [\text{Ni(II)-TBHPP}_{\text{ox}}] \{k_1 + k_2 K [p\text{-cresol}]^2\} \quad (3)$$

This under the pseudo first conditions employed becomes

$$\text{rate} = [\text{Ni(II)-TBHPP}_{\text{ox}}] k_{\text{obs}} \quad (4)$$

where $k_{\text{obs}} = k_1 + k_2 K [p\text{-cresol}]^2$ and $k_3 = k_2 K$.

Values obtained for k_1 , k_2 and k_3 for each of the systems studied are shown in Table 2.

A comparison of the effect of solvent on rates of reaction of oxidised metalloporphyrins can only be made for the iron(III) complex since reliable data for the reduction of the oxidised zinc(II) complex in chloroform could not be obtained and in the case of the nickel(II) complex different rate expressions were found to apply for the different solvents. The reduction of the iron(III) porphyrin complex by *p*-cresol in methanol is 2 to 3 times faster than that in chloroform, the difference in rate may be due to the more facile formation of an outer sphere complex between the reactants (involving H-bonded solvent molecules) in methanol. The rate of reaction was also found to be dependent on the nature of the metal ion in the porphyrin with the order of reactivity: Fe(III)-TBHPP_{ox} > Zn(II)-TBHPP_{ox} > Ni(II)-TBHPP_{ox} in methanol.

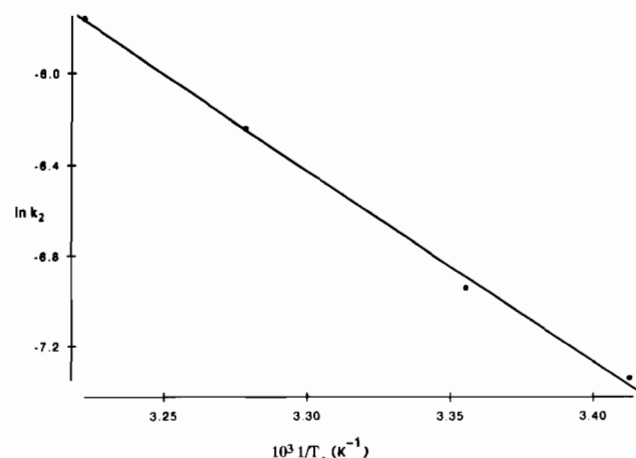
The reactivity of oxidised Fe(III)-TBHPP relative to Ni(II)-TBHPP in methanol is of the order of 10², and relative to Zn(II)-TBHPP in the same solvent is of the order of 2-3. The greater reactivity of the oxidised iron(III) complex may be due to the fact that electron transfer from *p*-cresol to the oxidised porphyrin in the

iron complex may occur via the metal ion for which the +II oxidation state is readily accessible.

In the case of the reduction of the oxidised Ni(II)-TBHPP activation parameters were obtained from the dependence of rate constants on temperature. Values of k_{obs} as a function of [*p*-cresol] and temperature are listed in Table 3 and the Arrhenius plot of $\ln k_2$ versus $1/T$ in methanol is shown in Fig. 5. For this reaction in chloroform $\Delta H^\ddagger = 67.3 \pm 2.5 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -77 \text{ J K}^{-1} \text{ mol}^{-1}$ and in methanol $\Delta H^\ddagger = 67.7 \pm 2.7 \text{ kJ mol}^{-1}$, $\Delta S^\ddagger = -78 \pm 7 \text{ J K}^{-1} \text{ mol}^{-1}$, all values at 298 K and standard errors obtained by a literature method [16].

TABLE 3. Rate constants for the reduction of oxidised Ni(II)-TBHPP by *p*-cresol at various temperatures

| Temperature (K) | Chloroform k_3 (M ⁻² s ⁻¹) | Methanol k_2 (M ⁻¹ s ⁻¹) |
|-----------------|---|---|
| 293 | 4.34×10^{-4} | 6.49×10^{-4} |
| 298 | 7.58×10^{-4} | 9.32×10^{-4} |
| 305 | 1.42×10^{-3} | 1.99×10^{-3} |
| 310 | 2.20×10^{-3} | 3.16×10^{-3} |

Fig. 5. Arrhenius plot for the reduction of oxidised Ni(II)-TBHPP_{ox} by *p*-cresol in methanol.

Acknowledgements

We thank the Research Committee of the Royal College of Surgeons in Ireland and the Irish Science and Technology Agency (EOLAS) for financial support, Professor D.A. Brown, Department of Chemistry, University College Dublin for useful discussions and the Microanalysis Laboratory at the same address for carrying out analytical work.

References

- 1 L.J. Marnett, P. Weller and J.R. Battista, in P.R. Ortiz de Montellano (ed.), *Cytochrome p-450: Structure, Mechanism and Biochemistry*, Plenum, New York, 1986, Ch. 7, pp. 29–77, and refs. therein.
- 2 T.J. McMurray and J.T. Groves, in P.R. Ortiz de Montellano (ed.), *Cytochrome p-450: Structure, Mechanism and Biochemistry*, Plenum, New York, 1986, Ch. 1, pp. 1–27.
- 3 A. Nanthakumar and H.M. Goff, *J. Am. Chem. Soc.*, *112* (1990) 4047.
- 4 J.T. Groves, R.C. Hanshalter, M. Nakamura, T.E. Nemo and B.J. Evans, *J. Am. Chem. Soc.*, *103* (1981) 2884.
- 5 J.T. Groves and Y. Watanabe, *J. Am. Chem. Soc.*, *110* (1988) 8443.
- 6 S. Tsuchiya, *J. Chem. Soc., Chem. Commun.*, (1991) 716.
- 7 T.G. Traylor, K.B. Nolan and R. Hildreth, *J. Am. Chem. Soc.*, *105* (1983) 6149.
- 8 L.R. Milgrom and W.D. Flitter, *J. Chem. Soc., Chem. Commun.*, (1991) 1492.
- 9 L.R. Milgrom, W.D. Flitter and E.L. Short, *J. Chem. Soc., Chem. Commun.*, (1991) 788.
- 10 L.R. Milgrom, *Tetrahedron*, *39* (1983) 2895.
- 11 A.J. Golder, L.R. Milgrom, K.B. Nolan and D.C. Povey, *J. Chem. Soc., Chem. Commun.*, (1987) 1788.
- 12 A.J. Golder, L.R. Milgrom, K.B. Nolan and D.C. Povey, *J. Chem. Soc., Chem. Commun.*, (1989) 1751.
- 13 D. Jacoby, C. Floriani, A. Chiesi-Villa and C. Rizzoli, *J. Chem. Soc., Chem. Commun.*, (1991) 790.
- 14 R.M. Silverstein and G.C. Bassler, *Spectrometric Identification of Organic Compounds*, Wiley, New York, 2nd edn., 1967, p. 84.
- 15 P.W. Atkins, *Physical Chemistry*, Oxford University Press, Oxford, 1978, Ch. 26.
- 16 J.O. Edwards, F. Monacelli and G. Ortaggi, *Inorg. Chim. Acta*, *11* (1974) 47.