Formation of Hydroxyl Radicals during the Facile Aerial Oxidation of a Phenolic Porphyrin

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ESR spectroscopy with a spin-trap shows that porphyrin **1** undergoes aerial oxidation in basic solution to produce the two-electron oxidised compound **3** and hydrogen peroxide: the porphyrin-phenoxyl-radical **2** is formed by further reaction of **3** with base and **1**.

Porphyrin **1** undergoes facile two-electron aerial oxidation in basic[1] and acidic[2] solutions, to give the porphodimethene-like compound **3**. This reaction has been used to model the redox activity of haem in peroxidases and cytochrome P450,[1d] and makes **1** a promising oxygen-reduction catalyst.[1e,f] In basic solutions, the phenoxyl-radical **2** has been observed by ESR spectroscopy and assumed to be an intermediate on the way to **3**.[1e–g] If so, then oxygen should simultaneously be reduced to superoxide.[1g] Indeed, superoxide has previously been detected by cyclic voltammetry of **1** and its zinc complex in dimethylformamide.[1c]

We now report the use of ESR spectroscopy with the spin-trap, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO[3]) to study the aerial oxidation of **1** in basic solutions. The presence of spin-adducts, due to radical species other than superoxide, reveals a richer chemistry than was originally supposed.[1e–g] Hydrogen peroxide, not superoxide, is probably the initial reduced oxygen species, suggesting that the two-electron aerial oxidation of **1** could be a concerted process. We also show that **2** originates from further reactions of **3**, not by direct oxidation of **1**.

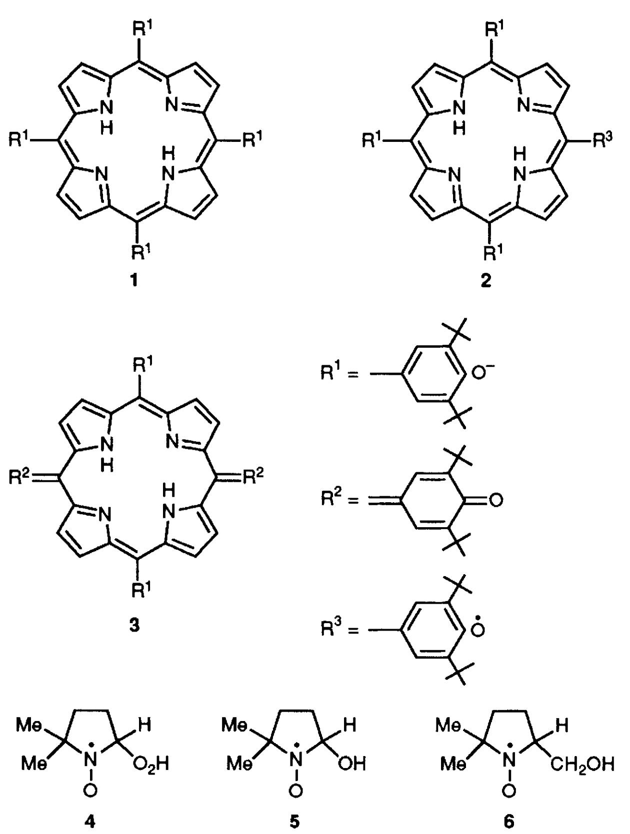
Porphyrin **1** was synthesised and purified as before.[1] Oxygen was bubbled through solutions of **1** (10^{-2} mol dm^{-3}) in ethanol and dichloromethane for 30 min. To 1 ml of these solutions was added aqueous DMPO solution (1 mol dm^{-3}; 0.05 ml) followed rapidly by the base tetra-n-butylammonium hydroxide in methanol (1 mol dm; 0.1 ml). The shaken mixture rapidly changed colour (from red, through brown–green, to deep blue; signifying formation of the radical **2**[1]), during which time (30 s) a small volume was transferred to an ESR flat cell, which was then positioned within the cavity (at 20 °C) of a Bruker ER 200D ESR spectrometer.

The ESR spectra in ethanol and CH_2Cl_2 were virtually identical, consisting of a triplet [**2**[1e–g]: $a_H = 0.133$ mT, $\Delta H_{pp} = 0.053$ mT, $g = 2.009(6)$] straddled by bands due to a mixture of DMPO spin-adducts.[3] These consisted of ten main bands, with some distorted by the strong, phenoxyl triplet [Fig. 1(*a*)]. Repeated scanning showed the decay of three of these bands [Fig. 1(*b*)] to leave six main bands that decayed more slowly. These spectra could not be assigned to the DMPO spin-adduct of superoxide **4**, whose twelve-line ESR spectrum has previously been characterised.[3a] No ESR spectra were observed without addition of base.

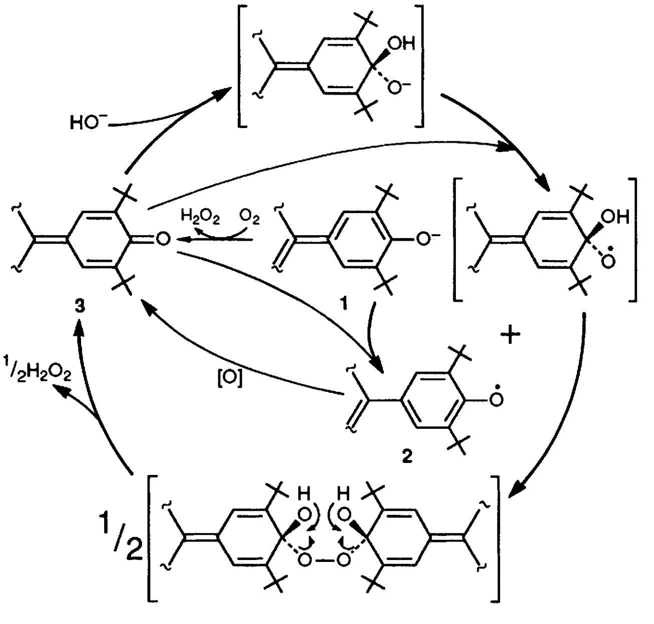
The spectra could be assigned to a mixture of DMPO spin-adducts of ·OH [**5**, $a_N = 1.40$ mT, $a_H = 1.48$ mT, $g = 2.009(5)$], and a carbon-centred species [**6**, in ethanol; $a_H = 2.23$ mT, $a_N = 1.55$ mT, $g = 2.009(8)$: in CH_2Cl_2; $a_H = 2.13$ mT, $a_N = 1.51$ mT, $g = 2.010(2)$] originating from attack by ·OH on solvent, *e.g.* $CH_3\dot{C}HOH$ (from ethanol), ·$CHCl_2$ (from CH_2Cl_2) and ·CH_2OH (from methanol used as solvent for the base $Bu^n{}_4NOH$).[3e]

The strong phenoxyl triplet masks and distorts some of these bands, and those assigned to **5** are not in the expected 1:2:2:1 ratio;[3] the bands decrease in intensity with higher field. This shows the instability of **5** under the largely non-aqueous, basic conditions of the aerial oxidation. Also, the *g*-values obtained are different from those obtained previously in aqueous solutions (*e.g.* for **5** $g = 2.005$–2.006:[3f] we suspect this is due to the generation of **5** under essentially non-aqueous conditions). Nevertheless, generation of ·OH would account for: (*a*) the presence of **6** (by attack of ·OH on solvent); and (*b*) the subsequent loss of the signal due to **5** (by attack of ·OH on **5**).[3d]

The most likely source of ·OH is hydrogen peroxide, which



Scheme 1



Scheme 2

is known to decompose rapidly in basic non-aqueous media giving substantial amounts of superoxide and ·OH.[4a] Using ESR spectroscopy and DMPO, only ·OH (and the carbon-centred species generated from methanol) would be detected as these react several orders of magnitude faster than superoxide with the spin-trap.[5] This would explain why no superoxide was detected by spin-trapping, but was observed earlier by cyclic voltammetry.[1c] Also, superoxide is known to be stable in aprotic media.[4b] We have repeated these experiments in acetonitrile and obtained similar results, *i.e.* spin-adducts whose ESR spectra are assignable to **5** and carbon-centred species **6**. Thus the presence of **5** is unlikely to be due to the prior formation of **4** which subsequently decomposes to **5**. The production of **4** would also preclude generation of **6** which is formed by attack of ·OH on solvent.

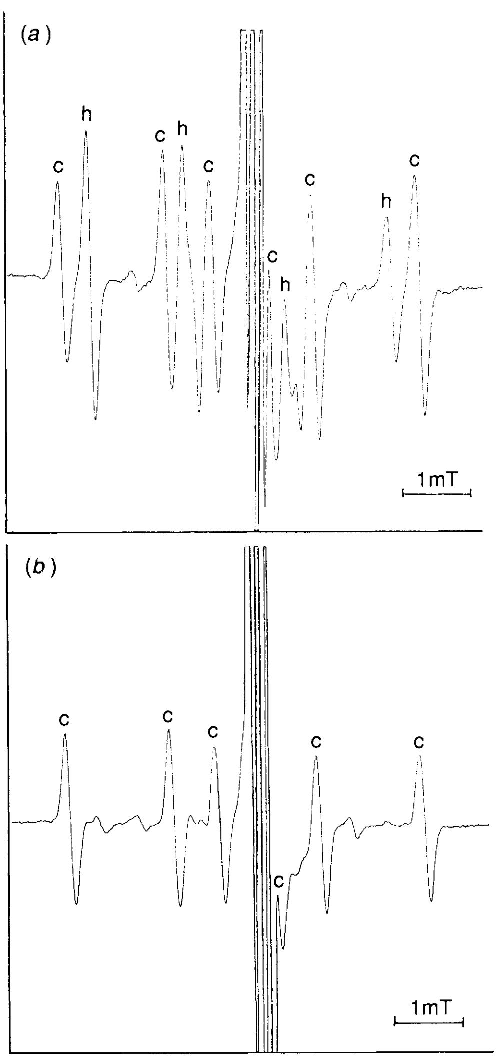


Fig. 1 (*a*) ESR spectrum of **1** in CH_2Cl_2 and ethanol in the presence of base and DMPO. Triplet [**2**; $a_H = 0.133$ mT, $\Delta H_{pp} = 0.053$ mT, $g = 2.009(6)$]: [**5** marked 'h'; in ethanol, $a_N = 1.40$ mT, $a_H = 1.48$ mT, $g = 2.009(5)$]: [**6** marked 'c'; in ethanol, $a_H = 2.23$ mT, $a_N = 1.55$ mT, $g = 2.009(5)$; in CH_2Cl_2, $a_H = 2.13$ mT, $a_N = 1.51$ mT, $g = 2.010(2)$]. Spectrometer conditions: microwave power, 10 dB; microwave frequency, 9.77 GHz; modulation amplitude, 0.2 mT; scan width, 10 mT; and gain, 4 × 10^5. (*b*) Ten minutes later (same spectrometer conditions). Notice loss of peaks due to DMPO-OH spin-adduct.

In order to generate peroxide, **1** cannot be oxidised *via* long-lived **2** as previously supposed,[1] but must undergo concerted two-electron oxidation to **3**. This raises the question of how **2** is generated. Before addressing this point, we present evidence which suggests peroxide is the initial reduced-oxygen species from the aerial oxidation of **1**.

Fig. 2(*a*) shows the ESR spectrum obtained on addition of hydrogen peroxide (10^{-2} mol dm^{-3}; 0.1 ml) to methanolic base (1 mol dm^{-3} $Bu^n{}_4NOH$, 2 ml) containing DMPO (1 mol dm^{-3}; 0.05 ml). Apart from the absence of the strong triplet due to **2** (and its concomitant effect on the splitting constants and the *g*-values[3f]), this spectrum bears a striking resemblance to Fig. 1(*a*) and consists of a combination of spin-adducts **5**; [$a_N = 1.47$ mT, $a_H = 1.52$ mT, $g = 2.009(7)$, the peaks are not in the usual 1 : 2 : 2 : 1 ratio], and **6** [$a_H = 2.11$ mT, $a_N = 1.57$ mT, $g = 2.007(2)$].

Returning to **2**, two routes to its generation are feasible. First, we have previously shown that **3** comproportionates with **1** in degassed acidified and basified solutions, to generate

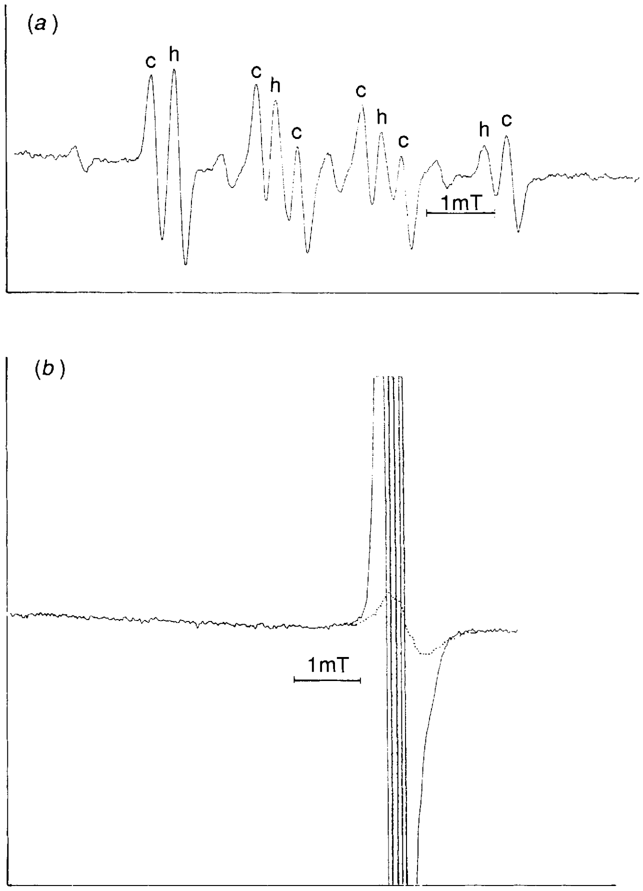


Fig. 2 (*a*) ESR spectrum obtained on addition of hydrogen peroxide to a basic solution of ethanol containing DMPO: [**5**, marked 'h'; a_N = 1.47 mT, a_H = 1.52 mT, g = 2.009(7)]; [**6**, marked 'c'; a_H = 2.11 mT, a_N = 1.57 mT, g = 2.007(2); same spectrometer conditions as Fig. 1]. (*b*) ESR spectrum of **3** in degassed CH_2Cl_2 on addition of base (dotted line is the spectrum of **3** in degassed neutral CH_2Cl_2). Same spectrometer conditions.

respectively, π-cation and phenoxyl-radical species in high yield.[2b] An alternative (or concurrent) route to **2** is by analogy with the known chemistry of anthraquinones in basic non-aqueous media. Semiquinone radical anions are formed, along with hydrogen peroxide, *via* another radical species.[6] The extended quinonoid structure of **3** should, on addition of base, make a similar reaction sequence possible (Scheme 2) for the formation of **2**. Thus, **1** undergoes rapid, two-electron oxidation to **3** (with production of hydrogen peroxide), which then undergoes nucleophilic attack by hydroxide anion. The product reacts further with **3** to produce two radicals; long-lived **2** and another species that rapidly dimerises.[6] The dimer collapses to regenerate **3** and more hydrogen peroxide. Meanwhile, comproportionation of **1** with **3** produces **2**, which slowly oxidises to **3**.

Supporting evidence for Scheme 2 is that neutral degassed CH_2Cl_2 and ethanol solutions of **3** are virtually ESR-silent. However, on addition of base, a strong triplet spectrum appeared [Fig. 2(*b*)], indicating formation of the phenoxyl radical **2**. Addition of the spin-trap also led to the appearance of bands due to DMPO spin-adducts.

In conclusion, ESR spin-trapping experiments suggest that aerial oxidation of **1** in basic solutions is probably a two-electron process initially generating hydrogen peroxide and **3**. The former rapidly disproportionates to ·OH and superoxide, while **3** goes on to form **2** by comproportionation with **1** and/or further reaction with base. We continue to investigate the mechanism of this reaction and are currently examining possible applications for this facile generation of reduced oxygen species.

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