Intervention of Oxygen in the Horseradish Peroxidase-catalysed Oxidation of Aromatic Tertiary Amines with Hydrogen Peroxide

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Summary The horseradish peroxidase-catalysed oxidation of aromatic tertiary amines with hydrogen peroxide leads to a secondary amine and an aldehyde, and with alkylmethylanilines demethylation is preferred to dealkylation; the reaction depends on the concentration of oxygen in the solution, probably as a consequence of the generation of peroxides by the reaction of oxygen with the radicals formed.

The horseradish peroxidase (HRP)-catalysed reaction of primary aromatic amines with hydrogen peroxide has been found by Saunders *et al.*[1] to yield polycondensation products in most cases.

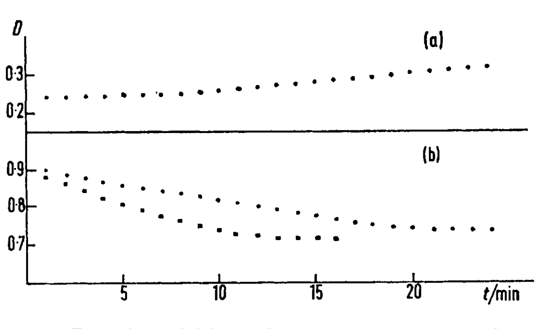

Figure 1. Reaction of (a) 4-$FC_6H_4NMe_2$ and (b) 4-$ClC_6H_4NMe_2$ with HRP and H_2O_2 in normal (●) and partially deoxygenated (■) solution, followed by monitoring the absorption at 290 nm and 275 nm respectively (D). The samples reach the asymptotic D_∞ value, corresponding to the demethylated amine.

We report the oxidation at room temperature of 15 aromatic tertiary amines (3×10^{-4} M in phosphate buffer, pH 6) with hydrogen peroxide ($4{\cdot}8 \times 10^{-5}$ M) using 50 μl/l of a solution of HRP (Böhringer Grade II, 1% in water) as a catalyst.

The reaction products were a secondary amine and an aldehyde, in analogy with the reaction of these substrates with the acetates of Pb^{IV}, Mn^{III}, Co^{III}, and Tl^{III}.[2] For alkylmethylanilines demethylation is preferred to dealkylation, as noted with the high-valence metal acetates[3] (Table). Control experiments showed that both HRP and hydrogen peroxide are necessary for the reaction. When a stoicheiometric amount of hydrogen peroxide was used according to equation (1), the reaction was shifted toward polycondensation products derived from further oxidation of the primary

$$X\text{–}C_6H_4\text{–}NMe_2 + H_2O_2 \xrightarrow{\text{HRP}} X\text{–}C_6H_4\text{–}NHMe + CH_2O + H_2O \qquad (1)$$

product. This, together with the inhibitory effect of added 4-t-butylcatechol and the high yields obtained with most substrates, suggested a radical reaction initiated by the activation of HRP by hydrogen peroxide[4] and propagated by the intervention of another oxidant. This point could be clarified by inspection of the ratio of tertiary to secondary amine in the reaction of 4-chlorodimethylaniline by measuring the change in absorption at 275 nm under normal reaction conditions and in a solution partially deoxygenated with a nitrogen stream. A lower oxygen concentration led to a slower reaction. The ratio of tertiary to secondary amine for the reaction of 4-fluorodimethylaniline was similarly obtained by observing the change in absorption at 290 nm (Figure 1). These values were used in a polarographic study of the dependence of the reaction rate for these two substrates upon the concentration of oxygen (Figure 2). These data indicated the intervention of oxygen in the reaction.

Aromatic tertiary amines undergo oxidative dealkylation with the intermediate formation of an aminium radical, a carbon-centred radical, and a carbonium ion [equations (2)—(4)]. In this case the oxidation occurs *via* catalysis by HRP. The enzyme reacts with hydrogen peroxide to give the oxidised species E_1, which is reduced by the amine substrate to the oxidising intermediate E_2 which restores the enzyme by intervention of another molecule of the substrate. The net result is an HRP-catalysed transfer of two electrons from the amine to hydrogen peroxide [equations (5)—(7)].

$$\text{R–C}_6\text{H}_4\text{–NMe}_2 \xrightarrow{\text{ox}} \text{R–C}_6\text{H}_4\text{–}\overset{+\cdot}{\text{N}}\text{Me}_2 \qquad (2)$$

$$\text{R–C}_6\text{H}_4\text{–}\overset{+\cdot}{\text{N}}\text{Me}_2 \longrightarrow \text{R–C}_6\text{H}_4\text{–NMeCH}_2\cdot + \text{H}^+ \qquad (3)$$

$$\text{R–C}_6\text{H}_4\text{–NMeCH}_2\cdot \xrightarrow{\text{ox}} \text{R–C}_6\text{H}_4\text{–NMeCH}_2^+ \longrightarrow \text{products} \qquad (4)$$

$$\text{HRP} + \text{H}_2\text{O}_2 + 2\,\text{H}^+ \longrightarrow \text{E}_1 + 2\,\text{H}_2\text{O} \qquad (5)$$

$$\text{E}_1 + \text{R}_3\text{N} \longrightarrow \text{E}_2 + \text{R}_3\text{N}^{+\cdot} \qquad (6)$$

$$\text{E}_2 + \text{R}_3\text{N} \longrightarrow \text{HRP} + \text{R}_3\text{N}^{+\cdot} \qquad (7)$$

TABLE. Products of the oxidation of tertiary amines with H_2O_2 with HRP catalyst.

Compound	Demethylation (%)	Dealkylation (%)	Starting material (%)	Reaction time/h
$4\text{-MeOC}_6\text{H}_4\text{NMe}$	76	—	12	0·25
$4\text{-MeC}_6\text{H}_4\text{NMe}_2$	40	—	17	0·50
PhNMe_2	20	—	14	1
$4\text{-FC}_6\text{H}_4\text{NMe}_2$	46	—	19	2
$3\text{-MeSC}_6\text{H}_4\text{NMe}_2$	42	—	24	8
$4\text{-ClC}_6\text{H}_4\text{NMe}_2$	49	—	16	17
$4\text{-IC}_6\text{H}_4\text{NMe}_2$	70	—	18	24
$3\text{-IC}_6\text{H}_4\text{NMe}_2$	18	—	8	15 days
$4\text{-NCC}_6\text{H}_4\text{NMe}_2$		No reaction		
$3\text{-O}_2\text{NC}_6\text{H}_4\text{NMe}_2$		,,		
$4\text{-O}_2\text{NC}_6\text{H}_4\text{NMe}_2$		,,		
$4\text{-MeOC}_6\text{H}_4\text{NMeBu}^n$	58	16	8	0·25
PhNMeBu^n	21	—	21	1
$4\text{-ClC}_6\text{H}_4\text{NMeEt}$	24	9	5	17
$4\text{-ClC}_6\text{H}_4\text{NMeBu}^n$	75	—	9	17

Oxygen could take part in the reaction through attack on the radicals formed with formation of peroxides. These are specific substrates for HRP and could yield the oxidised species E_1 and the reaction products with propagation of the radical reaction [equation (8)]. The different dependence of the reaction rate upon the concentration of oxygen

$$\text{R}\cdot + \text{O}_2 \rightarrow \text{R–O–O}\cdot \xrightarrow{\text{HRP}} \text{E}_1 + \text{products} \qquad (8)$$

for the two substrates tested indicates the influence of electronic factors in the reaction of the radicals with oxygen.

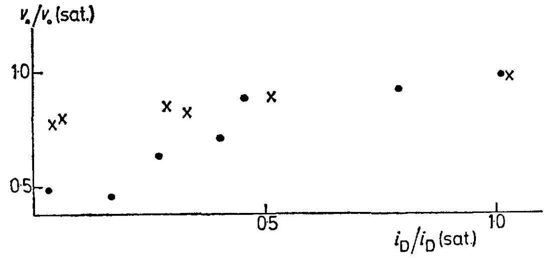

FIGURE 2. Plots of (v_0 for different oxygen concentrations)/v_0 in a saturated solution) *vs.* i_D/i_D (standard). in the reaction of $4\text{-FC}_6\text{H}_4\text{NMe}_2$ (X) and $4\text{-ClC}_6\text{H}_4\text{NMe}_2$ (●) with HRP and H_2O_2. Kinetic runs allow a correspondence between i_D/i_D (standard) and $c(O_2)/c(O_2)$(standard).

This work was supported by a grant from the Consiglio Nazionale delle Ricerche. We thank Miss A. Seccia Pesce for experimental assistance.

(*Received*, 27*th May* 1976; *Com.* 608.)

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