employed (Table I). Preliminary evidence suggests that factors other than the nature of the metal halide may also influence the character of the electrophile.

It would be advisable at this stage to seriously consider an alternative interpretation entailing attack of the oxy radical upon the aromatic nucleus followed by oxidation of the resulting complex through the influence of cupric halide. Analogy may be found in the enhancement by cupric salt of intramolecular

$$i\text{-PrOCO}_2\cdot + \text{ArH} \rightleftharpoons i\text{-PrOCO}_2\text{ArH}\cdot \xrightarrow{\text{Cu}^{II}} i\text{-PrOCO}_2\text{Ar} + \text{H}^+ + \text{Cu}^I \quad (5)$$

oxygenation in the decomposition of di-*o*-phenylbenzoyl peroxide,[9] the role of ferric ion in hydroxylation with Fenton's reagent,[10] and the increased yield of biaryl effected by oxygen in phenylation of benzene with benzoyl peroxide.[11] Since the present investigations constitute the initial probings into unexplored areas, we deem it important to label the interpretations as tentative, and to defer a more detailed and rigorous treatment until our factual knowledge has advanced to a greater extent.

The procedural simplicity and extremely high yields of monooxygenated materials obtained by this novel method of aromatic substitution suggest possible synthetic usefulness. Further studies concerning the scope and mechanism of this intriguing reaction are underway.[12,13]

(9) J. K. Kochi and R. D. Gilliom, *J. Am. Chem. Soc.*, **86**, 5251 (1964).

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(11) M. Eberhardt and E. L. Eliel, *J. Org. Chem.*, **27**, 2289 (1962).

(12) The products were characterized by comparison with authentic substances.

(13) Relevance of this report to the analogous ferric chloride system [G. A. Razuvaev, N. A. Kartashova, and L. S. Boguslavskaya, *J. Gen. Chem. USSR*, 34, 2108 (1964)] is treated in a separate communication.

(14) We are grateful to the National Science Foundation for support of this work.

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## Oxidation by Molecular Oxygen. IV. A Possible Model Reaction for Some Amine Oxidases[1-3]

*Sir:*

Recent work has clearly shown that some amine oxidases[4] which catalyze the over-all reaction: $R_2CHNH_2 + O_2 + H_2O \rightarrow R_2CO + NH_3 + H_2O_2$, require pyridoxal phosphate and a metal ion (apparently copper) as cofactors.[5] During a search for a model reaction whose mechanism might be readily investigated we found that a number of amines, especially amino acids, are oxidized readily by $O_2$ at room temperature in slightly basic solution if catalytic amounts of both pyridoxal and manganese ions are present.[6] Some typical reaction conditions and results are shown in Figure 1. In other experiments it was found that the rate increases with pH and with increasing concentrations of alanine and pyridoxal (if the $Mn^{2+}$ concentration is greater than the pyridoxal concentration), but is essentially unchanged when the oxygen pressure is changed from 0.2 to 1.0 atm. A complexed form of $Mn^{3+}$ is probably the catalyst. On mixing solutions of $Mn^{2+}$ and pyridoxal (yellow) in the absence of $O_2$, very little color change occurs, but in the presence of $O_2$ the solution rapidly turns brown (but not turbid), and takes up 1 mole of $O_2$/4 moles of $Mn^{2+}$.

The stoichiometry of the oxidation (Table I), which is similar to the enzymic reactions, indicates that $H_2O_2$ should be another product. However, $H_2O_2$ rapidly reacts when added to the reaction mixture; some $O_2$

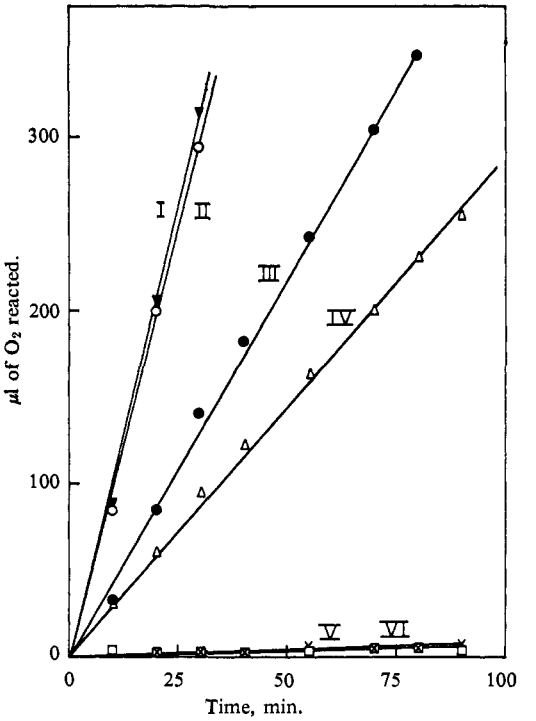



Figure 1. The oxidation of alanine by oxygen in the presence of pyridoxal and manganese ions. The oxygen uptake was measured by standard Warburg techniques. In each experiment the reaction solution was homogeneous, the temperature was 25.0°, the total volume was 3.0 ml, the oxygen pressure was 0.2 atm, the alanine concentration was 0.08 *M*, and the pH was 9.1. In expt II, III, IV, and V (×) the pyridoxal concentration was $5 \times 10^{-3}$ *M*; in expt I it was $2 \times 10^{-3}$ *M*; and in expt VI (□) there was no pyridoxal present. The concentration of $Mn(NO_3)_2$ in I, II, and VI was $1.7 \times 10^{-4}$ *M*; in III, $6.7 \times 10^{-5}$ *M*; in IV, $3.3 \times 10^{-5}$ *M*, and in V no manganese was present. In each experiment except VI the reaction was initiated at zero time by adding an aliquot of a pyridoxal solution; in expt VI the reaction was initiated by adding a $Mn^{2+}$ solution. Experiments similar to I-V but initiated by adding an aliquot of a $Mn^{2+}$ solution gave essentially the same rates of uptake of oxygen.

(1) Presented in part at the 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, Abstracts of Papers, Division of Biological Chemistry, p 38C.

(2) This research was supported by Public Health Service Research Grant GM-09585 from the Division of General Medical Sciences, Public Health Service.

(3) Part III: G. A. Hamilton and J. R. Giacin, *J. Am. Chem. Soc.*, **88**, 1584 (1966).

(4) For reviews of diamine and monoamine oxidases see E. A. Zeller, *Enzymes*, **8**, 313 (1963); H. Blaschko, *ibid.*, 337 (1963); also H. Blaschko, *Advan. Comp. Physiol. Biochem.*, 1, 67 (1962).

(5) (a) H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **238**, 2669 (1963); (b) H. Blaschko and F. Buffoni, *Proc. Roy. Soc.* (London), **B163**, 45 (1965); (c) B. V. Goryachenkova and E. A. Ershova, *Biokhimiya*, 30, 165 (1965); (d) B. Mondovi, A. Scioscia-Santoro, G. Rotilio, and M. T. Costa, *Enzymologia*, **28**, 228 (1965); (e) J. M. Hill and P. J. G. Mann, *Biochem. J.*, **91**, 171 (1964); (f) R. Kapeller-Adler, *Federation Proc.*, **24**, 757 (1965); (g) C. M. McEwen, Jr., *J. Biol. Chem.*, **240**, 2003 (1965); and references contained in these publications.

(6) M. Ikawa and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 4900 (1954), had observed a much less efficient oxidation with other metal ions.

**Table I.** The Stoichiometry of the Alanine Oxidation by Air at 25°

| Conditions | $O_2$ reacted,[a] μmoles/ml | Pyruvate formed,[b] μmoles/ml | $NH_3$ formed,[c] μmoles/ml |
|---|---|---|---|
| Tris buffer, pH 8.1[d] | 3.3[e] | 3.4[e] | ... |
| Alanine buffer, pH 9.1[f] | 3.0[e] | 1.3[e] | ... |
| Borate buffer, pH 9.5[g] | 4.4[h] | 3.3[h] | 4.2 |

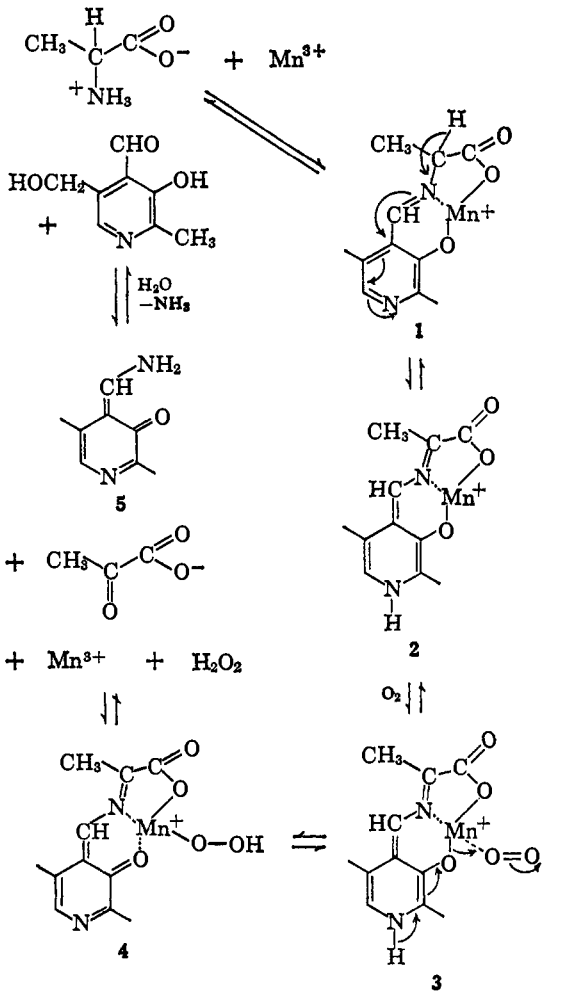
[a] Obtained after subtracting the amount which reacts in the absence of alanine. [b] Determined colorimetrically.[7] [c] Determined by Nesslerization after microdiffusion from the reaction which had gone to completion. [d] Initial concentrations: tris(hydroxymethyl)aminomethane, 0.1 *M*; alanine, 0.04 *M*; $Mn^{2+}$, $5 \times 10^{-3}$ *M*; pyridoxal, $5 \times 10^{-3}$ *M*. [e] Amount reacted or formed during the first hour; with alanine in excess, the $O_2$ uptake is essentially zero order during this time. [f] Initial concentrations: alanine, 0.08 *M*; $Mn^{2+}$, $3.3 \times 10^{-5}$ *M*; pyridoxal, $2 \times 10^{-3}$ *M*. [g] Initial concentrations: borate, 0.1 *M*; alanine, $5 \times 10^{-3}$ *M*; $Mn^{2+}$, $1 \times 10^{-3}$ *M*; pyridoxal, $5 \times 10^{-3}$ *M*. A precipitate formed on mixing the reagents. [h] Reacted or formed after 90 min; at this time the uptake of oxygen had stopped; presumably the alanine had all reacted.

is formed[8] but under our conditions appreciable amounts react with other components in the system. At the low concentrations expected in the amino acid oxidation, probably very little $O_2$ would be formed since its formation requires two molecules of $H_2O_2$. The low yields of pyruvate can be explained because pyruvate rapidly reacts with $H_2O_2$. Also, the $H_2O_2$ may react with the buffer or pyridoxal; we have found that pyridoxal is partially decomposed during the reaction.

Other results which have been obtained with this system include the following: salicylaldehyde and pyridoxine cannot replace pyridoxal but pyridoxal phosphate can; pyridoxamine is apparently not an intermediate because it is oxidized more slowly than alanine, and pyridoxal is a catalyst for pyridoxamine oxidation;[6] α-methylalanine, N-methylalanine, and lactic acid are not oxidized under conditions where alanine reacts readily; other amino acids and amino acid esters and amides can replace alanine but simple amines react slowly if at all with $O_2$; the rate of $O_2$ uptake is inhibited by ethylenediaminetetraacetic acid but is unaffected by light or by free radical inhibitors, such as phenols (thus, the oxidation presumably does not occur by a free-radical chain mechanism); glycine is oxidized five to six times more rapidly than α,α-dideuterioglycine.

These results and others are consistent with the mechanism outlined in Chart I. The formation of intermediates **1** and **2** is similar to what has been proposed to explain other pyridoxal-catalyzed reactions of amino acids.[9] It is suggested that **2** or some intermediate like **2** can complex with $O_2$ to give **3**. The transfer of a proton through the solvent and electrons through the complex, as shown, would lead to **4**, in which the oxygen has been reduced to hydrogen peroxide and the rest of the complex has been oxidized by two electrons. Compound **4** would be expected to be in equilibrium

**Chart I**



with pyruvic acid, $Mn^{3+}$, $H_2O_2$, and **5**. Compound **5** is a tautomer of the Schiff base of ammonia with pyridoxal and would be expected to give pyridoxal and ammonia readily. The $H_2O_2$ is thought to react in some unspecified way with various components in the system.

It is suggested that the mechanism of the enzymic oxidations of amines is similar to that proposed for the model oxidation. The reasons why manganese ion and amino acids give a conveniently studied model reaction, while copper ion and simple amines are involved in the enzyme reactions, follow from the proposed mechanism and from what is known about other pyridoxal-catalyzed reactions.[9] These will be discussed in subsequent publications.

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## Photoisomerization of Hexafluorobenzene

*Sir:*

Irradiation of substituted benzenes (which do not have the full $D_{6h}$ symmetry of the parent compound) has been shown[1-6] to give derivatives of Dewar ben-

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