Role of Copper Dimers and the Participation of Copper(III) in the Copper-catalysed Autoxidation of Ascorbic Acid. Part II.[1] Kinetics and Mechanism in 0.100 mol dm^{-3} Potassium Nitrate

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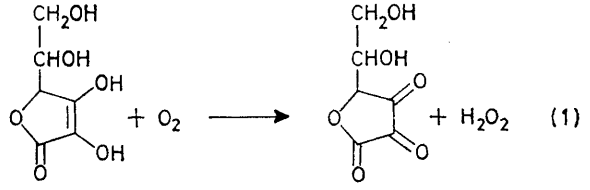
The kinetics and mechanism of the copper(II)-catalysed oxidation of ascorbic acid by dissolved oxygen in the presence of nitrate ions have been reinvestigated in the pH range 2.0—3.5. The rate law for the disappearance of oxygen is as in (i) where L^{2-} is the ascorbate anion, and on the basis of this and on thermodynamic evidence a chain

$$-d[O_2]/dt = k'[Cu^{2+}][HL^-][O_2]^{\frac{1}{2}} \quad (i)$$

mechanism is proposed in which copper(II) ascorbate dimers are the reactive species. This mechanism involves a two-electron transfer to oxygen (the latter binding across a Cu—Cu binuclear site), as the primary electron-transfer process, and thus could be said to involve formal copper(III) intermediates. The catalytic activity of these copper dimers parallels that of the oxygen-binding sites of a number of copper-containing oxidases (*e.g.* ceruloplasmin, laccase, ascorbic acid oxidase, *etc.*) for which ascorbate is a natural substrate. The extension of the mechanism proposed for the model system to that of the oxidases is discussed, and it is suggested that electron transfer to oxygen might also occur as the primary process in the latter [likewise requiring the participation of what is formally copper(III) in the enzyme mechanism].

THE copper(II)-catalysed oxidation of ascorbic acid by molecular oxygen has been the subject of a number of previous investigations [2-4] (and references quoted therein). Interest in the reaction stems largely from the importance of this ligand as a biological reducing agent and especially from its role as a substrate for certain copper-containing oxidase enzymes (*e.g.* ceruloplasmin, laccase, ascorbic acid oxidase, *etc.*) which reduce molecular oxygen to water. The most detailed mechanistic study, made by Taki Khan and Martell,[3] reported first-order dependences on the concentrations of oxygen, ligand, and catalyst, and the apparent simplicity of the kinetics prompted us to utilise the reaction to test methods and apparatus (notably the oxygen-sensitive electrode) for use in a general study of metal-catalysed autoxidations of ligands such as adrenalin and L-dopa [3-(3,4-dihydroxyphenyl)alanine]. Far from reproducing their results, we have obtained a significantly different oxygen dependence for copper-catalysed ascorbate autoxidation which requires their mechanism [in which an ascorbate–copper(II)–oxygen monomeric complex undergoes internal one-electron transfer in a rate-determining step with participation of the Cu^{II}–Cu^{I} redox couple] to be abandoned in favour of a two-electron-transfer mechanism involving dimeric copper ascorbate complexes as the reactive species and possibly proceeding *via* the Cu^{III}–Cu^{II} couple. The oxygen dependence found in many of the investigations prior to that of Taki Khan and Martell deviated from first order, although no attempt was made to fit it to any other rate law. Plots of $[O_2]$ against time obtained in this study using an oxygen-sensitive electrode have been shown to conform to a rate law which is exactly half order in $[O_2]$ and is one of the few known examples of half-order reactions in solution. A preliminary report of our kinetics and proposed mechanism has been published,[1,5] while the half-order oxygen dependence has recently been confirmed manometrically by Shtamm and Skurlatov,[6] although the latter presented different mechanistic interpretations [7,8] which we cannot reproduce by steady-state arguments.

The stoicheiometry of the reaction is generally accepted as conforming to (1). In the present work the final



Ascorbic acid (H_2L) Dehydroascorbic acid (L')

ratio of moles of ascorbic acid oxidised to moles of oxygen reduced has been found to vary between 1.0 and 1.3 : 1, but the deviation from 1 : 1 stoicheiometry only becomes significant after *ca.* 85% completion of reaction. The kinetics have been measured in 0.100 mol dm^{-3} potassium nitrate, 0.100 mol dm^{-3} potassium chloride, and 0.100 mol dm^{-3} sodium bromide as well as in 0.100 mol dm^{-3} ionic-strength mixtures of chloride and nitrate. Considerable differences in rate laws have been found in all systems studied, implying a dependence of the mechanism on the nature and concentration of the added electrolyte, and we report here the kinetics and mechanism with 0.100 mol dm^{-3} potassium nitrate as the supporting electrolyte.

RESULTS

The acid-association constant, K_2^H, for the reaction $H^+ + HL^- \rightleftharpoons H_2L$, where L^{2-} is the ascorbate anion, was measured by potentiometric titration and $\log_{10} K_2^H$ was found to be 4.045 ± 0.005 at 25 °C and an ionic strength of 0.100 mol dm^{-3} (adjusted with $K[NO_3]$).

Oxygen Dependence.—The rate law in the pH range 2—3.5

[1] Part I, R. F. Jameson and N. J. Blackburn, *J. Inorg. Nuclear Chem.*, 1975, **37**, 809.
[2] H. Nord, *Acta Chem. Scand.*, 1955, **9**, 442.
[3] M. M. Taki Khan and A. E. Martell, *J. Amer. Chem. Soc.*, 1967, **89**, 4176.
[4] L. Pekkarinen, *Suomen Kem.*, 1970, **B43**, 305.
[5] R. F. Jameson and N. J. Blackburn, *Proc. 16th Internat. Conf. Co-ordination Chem.*, Dublin, 1974, paper 1.19.
[6] E. B. Shtamm and I. Skurlatov, *Zhur. fiz. Khim.*, 1974, **48**, 1454.
[7] E. B. Shtamm, A. P. Purmal, and I. Skurlatov, *Zhur. fiz. Khim.*, 1974, **48**, 2229.
[8] E. B. Shtamm, A. P. Purmal, and I. Skurlatov, *Zhur. fiz. Khim.*, 1974, **48**, 2233.

was obtained from the dependence of the rate of disappearance of oxygen (*a*) on the total ascorbic acid concentration, $[L]_T$, at constant total copper concentration, $[Cu]_T$, and

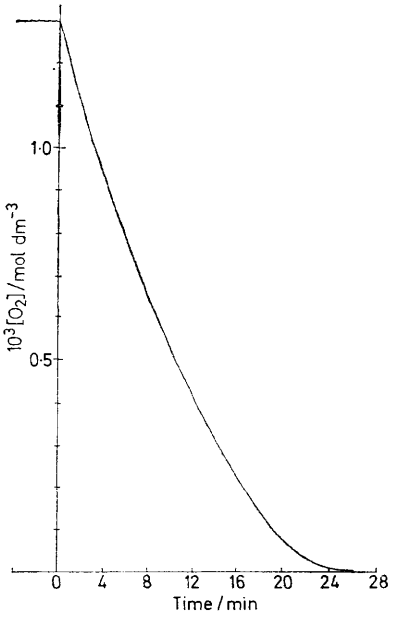


FIGURE 1 Typical concentration against time curve for the disappearance of oxygen at 25 °C. pH 2.55, $[L]_T = 0.0396$ mol dm^{-3}, $[Cu]_T = 2.59 \times 10^{-5}$ mol dm^{-3}, $I = 0.100$ mol dm^{-3} ($K[NO_3]$)

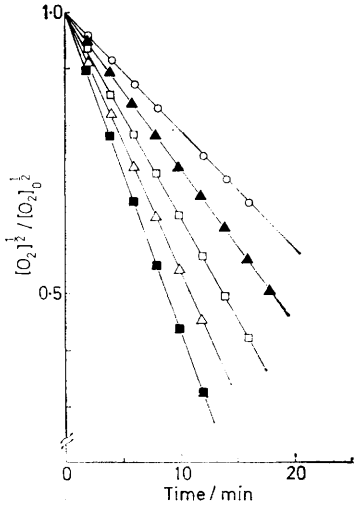


FIGURE 2 Oxygen dependence of the rate ($[O_2]^{\frac{1}{2}}/[O_2]_0^{\frac{1}{2}}$) against time at 25 °C. pH 2.55; $[Cu]_T = 1.30 \times 10^{-5}$ mol dm^{-3}; $[L]_T = 0.0203$ (○), 0.0283 (▲), 0.0396 (□), 0.0596 (△), and 0.0792 mol dm^{-3} (■); $[O_2]_0 = 0.0013$ mol dm^{-3} (assumed); $I = 0.100$ mol dm^{-3} $K[NO_3]$

(*b*) on $[Cu]_T$ at constant $[L]_T$. Figure 1 shows a representative plot of $[O_2]$ against time. In every case the rate of oxygen uptake was half order in $[O_2]$, *i.e.* as in equation

$$-d[O_2]/dt = k_{obs.}[O_2]^{\frac{1}{2}} \quad (2)$$

(2) at constant $[L]_T$, $[Cu]_T$, and pH, and Figure 2 illustrates the exellent linearity obtained when the square root of the oxygen concentration (normalised to unity) was plotted as a function of time according to the integrated form of (2), namely (3) where $[O_2]_0$ is the initial oxygen concentration.

$$\frac{[O_2]^{\frac{1}{2}}}{[O_2]_0^{\frac{1}{2}}} = 1 - \frac{k_{obs.}\,t}{2[O_2]_0^{\frac{1}{2}}} \quad (3)$$

$k_{obs.}$ Is therefore equal to twice the gradient of the straight lines in Figure 2 multiplied by the initial oxygen concentration and is tabulated as a function of total ascorbic acid concentration at pH 2.10, 2.30, 2.55, 2.80, and 3.00 (Table 1).

TABLE 1

'Pseudo'-half-order constants ($k_{obs.}$) * for the rate of disappearance of O_2 with time in 0.100 mol dm^{-3} $K[NO_3]$; $[Cu]_T = 2.59 \times 10^{-5}$ mol dm^{-3}, 25.00 °C

pH	$[L_T]$/mol dm^{-3}	$10^4 k_{obs.}$/mol$^{\frac{1}{2}}$ dm$^{-\frac{3}{2}}$ min^{-1}
2.10	0.0284	8.73
	0.0395	12.44
	0.0623	16.30
	0.0906	24.42
2.30	0.0204	9.88
	0.0288	11.30
	0.0398	16.38
	0.0571	21.08
	0.0805	27.18
2.55	0.0203	15.16
	0.0283	20.12
	0.0396	26.30
	0.0576	32.92
	0.0689	37.88
	0.0792	40.44
2.80	0.0167	21.72
	0.0230	27.02
	0.0342	36.04
	0.0575	49.20
3.10	0.0125	31.82
	0.0161	40.20
	0.0223	49.80
	0.0342	70.80

* $k_{obs.}$ Was calculated from the gradients of the functions $[O_2]^{\frac{1}{2}}/[O_2]_0^{\frac{1}{2}}$ against time according to the integrated form of the half-order rate law, $[O_2]^{\frac{1}{2}}/[O_2]_0^{\frac{1}{2}} = 1 - (k_{obs.}/2[O_2]_0^{\frac{1}{2}})t$; $[O_2]_0$, the initial oxygen concentration, was assumed to be 0.0013 mol dm^{-3}.

Ligand Dependence.—Although plots of $k_{obs.}$ against $[L]_T$ were smooth curves through the origin, plots of $1/k_{obs.}$ against $1/[L]_T$ gave good straight lines (Figure 3). The

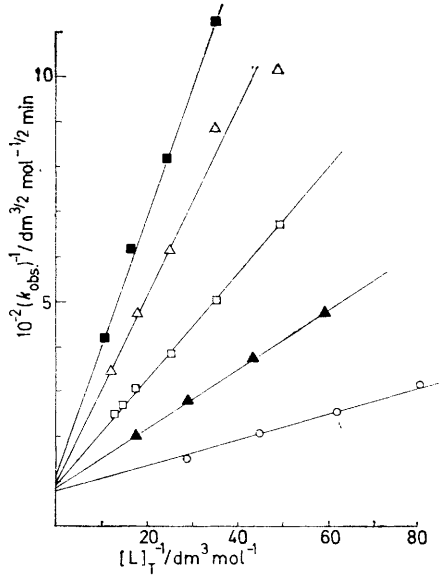


FIGURE 3 Ligand dependence of the rate; plots of $1/k_{obs.}$ against $1/[L]_T$ at various pH values. $[Cu]_T = 2.59 \times 10^{-5}$ mol dm^{-3}; pH 2.10 (■), 2.30 (△), 2.55 (□), 2.80 (▲), and 3.10 (○)

linearity of these Lineweaver–Burke plots indicates the presence of pre-equilibrium copper–ascorbate complex formation, and, in agreement with the previously reported observation that ascorbic acid (H_2L) forms [M(HL)] complexes with the majority of non-catalytically active metal ions so far investigated,[9] we find that $[Cu(HL)]^+$ is likely to be the predominant complex although at low pH values the form $[Cu(H_2L)]^{2+}$ is also important (see below). It will now be shown that analysis of the data in Figure 3 leads to the rate law (4)

$$-d[O_2]/dt = k'[Cu^{2+}][HL^-][O_2]^{\frac{1}{2}} \quad (4)$$

where $$k' = k_{obs.}/[Cu^{2+}][HL^-] \quad (5)$$

Rate law (4) is expressed in terms of the concentrations of free copper and free monodeprotonated ligand. The addition of the second proton to the ascorbate anion may be written as in (6). We may also write equation (8), for the range pH 2—4, and combining (7) and (8) we obtain (9).

$$H^+ + HL^- \rightleftharpoons H_2L \quad (6)$$

with $$K_2^H = [H_2L]/[H^+][HL^-] \quad (7)$$

$$[L]_T = [HL^-] + [H_2L] \quad (8)$$

$$[HL^-] = [L_T]/(1 + K_2^H[H^+]) \quad (9)$$

If it is now assumed that the predominant complexes are $[Cu(HL)]^+$ and $[Cu(H_2L)]^{2+}$, then we can write (10) and (12).

$$Cu^{2+} + HL^- \rightleftharpoons [Cu(HL)]^+ \quad (10)$$

with $$K^M = [Cu(HL)^+]/[Cu^{2+}][HL^-] \quad (11)$$

$$H^+ + Cu^{2+} + HL^- \rightleftharpoons [Cu(H_2L)]^{2+} \quad (12)$$

with $$K^{MH} = [Cu(H_2L)^{2+}]/[H^+][Cu^{2+}][HL^-] \quad (13)$$

Expressions (14)—(16) may be written, and substitution of

$$[Cu]_T = [Cu^{2+}] + [Cu(HL)^+] + [Cu(H_2L)^{2+}] \quad (14)$$

$$= [Cu^{2+}](1 + K_2^H[H^+] + K^M[L]_T + K^{MH}[H^+][L]_T)/(1 + K_2^H[H^+]) \quad (15)$$

$$[Cu^{2+}] = [Cu]_T(1 + K_2^H[H^+])/(1 + K_2^H[H^+] + K^M[L]_T + K^{MH}[H^+][L]_T) \quad (16)$$

(9) and (16) into (5) leads to (17) and (18).

$$k_{obs.} = k'[Cu]_T[L]_T/(1 + K_2^H[H^+] + K^M[L]_T + K^{MH}[H^+][L]_T) \quad (17)$$

$$\frac{1}{k_{obs.}} = \frac{1 + K_2^H[H^+]}{k'[Cu]_T[L]_T} + \frac{K^M + K^{MH}[H^+]}{k'[Cu]_T} \quad (18)$$

Thus the gradients of the plots in Figure 3 should be equal to $(1 + K_2^H[H^+])/k'[Cu]_T$, or, in logarithmic form as in (19), and this is verified up to a pH of at least 2.8 in Figure 4

$$\log_{10}(\text{Gradient}) = \log_{10}(1 + K_2^H[H^+]) - \log_{10} k'[Cu]_T \quad (19)$$

where a straight line is drawn with the theoretical gradient of unity [equation (19)]. Similarly, the intercepts in Figure 3 should vary linearly with $[H^+]$ [equation (20)]

$$\text{Intercept} = \frac{K^{MH}[H^+]}{k'[Cu]_T} + \frac{K^M}{k'[Cu]_T} \quad (20)$$

and this is verified in Figure 5.

[9] P. Ulmgren and O. Wahlberg, *Acta Chem. Scand.*, 1971, **25**, 1000.

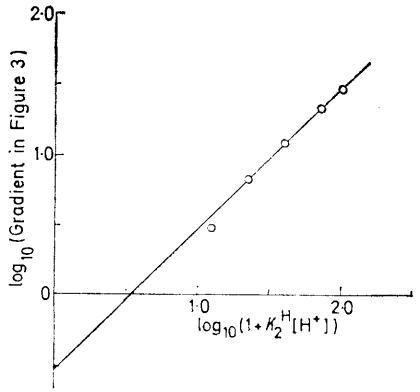


FIGURE 4 Plot of $\log_{10}$ (Gradient of Figure 3) against $\log_{10}$ $(1 + K_2^H[H^+])$. The straight line has unit gradient and intercept $= -0.52 = -k'[Cu]_T$ (data in Table 3)

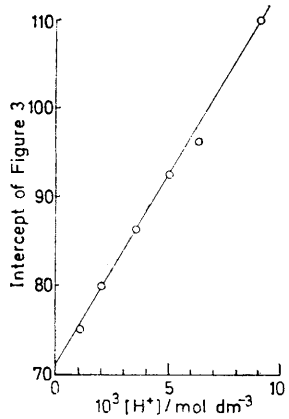


FIGURE 5 Plot of (Intercept of Figure 3) against $[H^+]$: gradient $= 4.3 \times 10^3$ $dm^{\frac{9}{2}}$ $mol^{-\frac{5}{2}}$ $min = K^{MH}/k'[Cu]_T$; Intercept $=$ 71.3 $dm^{\frac{3}{2}}$ $mol^{-\frac{1}{2}}$ $min = K^M/k'[Cu]_T$ (data in Table 2)

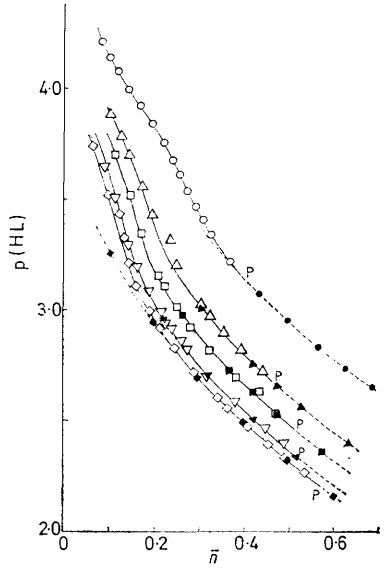


FIGURE 6 ' Formation ' curves [$\bar{n}$ against p(HL)] for the copper(II)–ascorbate system. $I = 0.100$ mol dm^{-3} $K[NO_3]$, 25.00 °C, $[Cu]_T = 2.113 \times 10^{-4}$ mol dm^{-3} (initial volume, 35.00 cm^3). $[L]_T : [Cu]_T = 5.1$ (○), 10 : 1 (△), 15 : 1 (□), 25 : 1 (▽), and 50 : 1 (◇) (Filled symbols represent points calculated using data in Table 3; P indicates where precipitation occurred)

TABLE 2

Gradients and intercepts from Figure 3

pH *	10^3[H$^+$]/mol dm^{-3}	Gradient/ mol$^{\frac{1}{2}}$ dm$^{-\frac{3}{2}}$ min	$\log_{10}$ (Gradient)	$\log_{10}(1 + K_2^H[H^+])$	Intercept/ dm$^{\frac{3}{2}}$ mol$^{-\frac{1}{2}}$ min
2.10	9.15	28.8	1.46	2.01	110
2.30	6.31	21.1	1.32	1.86	96
2.55	3.55	11.9	1.08	1.61	86
2.80	2.00	6.70	0.83	1.35	80
3.10	1.00	4.88	0.46	1.09	75

* [H$^+$] was calculated from the pH after activity correction using the experimentally determined empirical relation pH = $-\log_{10}$ [H$^+$] + 0.10, I = 0.100 mol dm^{-3} K[NO$_3$], 25.00 °C.

Since [Cu]$_T$ is known, k' can be obtained from the intercept in Figure 4, and thus K^{MH} and K^M may be determined from the gradient and intercept of Figure 5 respectively. Thus the ligand and metal dependence of the rate are consistent with the rate law [equation (4)] and the data from Figure 3 are presented in Table 2, and the calculated values of k', K^M, and K^{MH} are listed in Table 3. However, k', although independent of the concentrations of ligand, catalyst, O_2, and H$^+$, is not a true specific rate constant since it contains the equilibrium constant for the kinetically active complex, the nature of which cannot be uniquely determined from the above treatment of kinetic data.

TABLE 3

Values of rate and equilibrium constants estimated from kinetic data and determined potentiometrically (25.00 °C, I = 0.100 mol dm^{-3} K[NO$_3$])

	Kinetic data	E.m.f. data
log K^{MH}	4.2	3.94 ± 0.2
log K^M	2.4	2.32 ± 0.02
log K^{DH}		6.33
log K^D		0.05 (K^D 1.13 dm^3 mol^{-1})
k'	1.29 × 10^5 dm$^{\frac{9}{2}}$ mol$^{-\frac{3}{2}}$ min^{-1}	

Equilibrium Studies.—Varying ratios of Cu^{II} to ascorbic acid were titrated potentiometrically with base under an atmosphere of pure nitrogen, and the results used to calculate formation curves [$\bar{n}$ against p(HL)] on the basis that the uncomplexed ligand was the only source of protons. The solution turned apple-green above $\bar{n} \approx 0.25$ and copper metal was always precipitated at $\bar{n}$ *ca.* 0.5. The formation curves (Figure 6) demonstrate (*a*) the presence of polymers [which we conclude (below) to be dimers] because the curves do not normalise for differing ratios of [Cu]$_T$: [L]$_T$, and (*b*) the presence of fully protonated complexes because a definite inflexion occurs at $\bar{n} \approx 0.25$.

Although titrations of solutions containing a 1 : 1 mole ratio of Cu^{II} to ascorbic acid were not very reproducible, the application of the method used by Rajan and Martell [10] over a limited titration range gave some confidence that above $\bar{n} = 0.25$ the only complexes present were [Cu(HL)]$^+$, [{Cu(HL)}$_2$]$^{2+}$, and [(CuL)$_2$]. The following approximate values for K^M, K^{DH}, and K^D were obtained where K^M is defined by (10) and (11) and the other constants are as shown in expressions (21)—(24).

$$K^M \approx 220 \text{ dm}^3 \text{ mol}^{-1} \quad (20)$$

$$2\ Cu^{2+} + 2\ HL^- \rightleftharpoons [Cu_2(HL)_2]^{2+} \quad (21)$$

$$K^{DH} = [Cu_2(HL)_2^{2+}]/[Cu^{2+}]^2[HL^-]^2 \approx 10^6 \text{ dm}^9 \text{ mol}^{-3} \quad (22)$$

$$2\ Cu^{2+} + 2\ HL^- \rightleftharpoons [Cu_2L_2] + 2\ H^+ \quad (23)$$

$$K^D = [Cu_2L_2][H^+]^2/[Cu^{2+}]^2[HL^-]^2 = 1 \gtrsim 2 \text{ dm}^3 \text{ mol}^{-1} \quad (24)$$

Since the formation of [Cu$_2$L$_2$] involves the liberation of two protons [equation (23)] not accounted for in our formulation of $\bar{n}$ and p(HL), it was possible to calculate approximate correction terms involving K^D *e.g.* for $\bar{n}$ we can write (25) and (26). The value of K^M was obtained by extrapolation of the p(HL) value at $\bar{n} = 0.5$ as the ligand to metal ratio increased [even by 50 : 1 the correction term to p(HL)$_{0.5}$ due to liberation of protons *via* equation (23) was extremely low]. It was then a fairly rapid cyclic calculation to obtain values of [Cu^{2+}], [HL$^-$], K^{DH}, and K^D *via* expressions for [Cu]$_T$ and [L]$_T$ that reproduced the data at specific $\bar{n}$ values. Finally, computed values of $\bar{n}$ and p(HL) using the constants summarised in Table 3 are also indicated in Figure 6, and satisfactorily reproduce the data.

$$\bar{n}_{exact} = \bar{n}_{calc.} - 2[Cu_2L_2]\{(1 + K^H[H^+])/K_2^H[H^+]\}/[Cu]_T \quad (25)$$

$$= \bar{n}_{calc.} - 2K^D\frac{[Cu^{2+}]^2[HL^-]^2}{[Cu]_T}\left\{\frac{1 + K_2^H[H^+]}{K_2^H[H^+]^3}\right\} \quad (26)$$

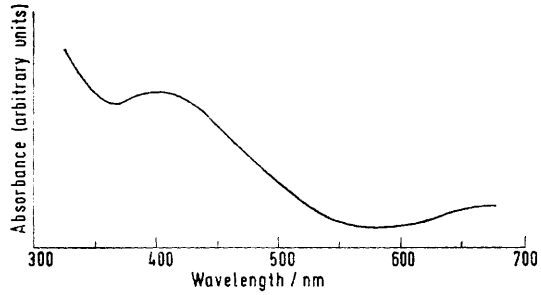


FIGURE 7 Absorption spectrum of the copper(II)–ascorbate complex system

The low-pH region was next investigated using an Orion copper(II)-ion-sensitive electrode. Reproducibility was not good except at very low pH, but the values of free copper concentrations thus obtained for a series of titrations covering a 20-fold change in concentration of ascorbic acid and a five-fold change in copper concentration enabled the evaluation of the formation constant K^{MH} for the fully protonated complex [equations (12) and (13)]. Unfortunately it had to be assumed that [Cu(H$_2$L)]$^{2+}$ was the only fully protonated species present in order to obtain the value quoted in Table 3.

Spectrum.—The spectrum of the green solution is shown in Figure 7 and has $\lambda_{max.}$ = 410 nm. It closely resembles the absorption spectrum of coppper(II) complexes of ligands containing a similar *o*-dihydroxy group, *e.g.* catechol [11] and L-dopa [12] where the band is attributed to low-energy charge transfer from ligand to metal.

[10] K. S. Rajan and A. E. Martell, *J. Inorg. Nuclear Chem.*, 1967, **29**, 463.
[11] R. F. Jameson and M. F. Wilson, *J.C.S. Dalton*, 1972, 2617.
[12] J. E. Gorton and R. F. Jameson, *J. Chem. Soc.* (*A*), 1968, 2615.

Copper Dependence.—Analysis of the dependence of $k_{obs.}$ on $[L]_T$ has shown that the kinetic data can be satisfactorily fitted to first-order kinetics in both the free copper and, at pH $\leqslant$ 2.80, the monodeprotonated ascorbate anion, HL^-, with complexes $[Cu(HL)]^+$ and $[Cu(H_2L)]^{2+}$ present in the system. While dimer formation has been demonstrated potentiometrically, the concentration is small under the 'kinetic' conditions of $>10^2$-fold excess of ligand over catalyst; in any case if significant quantities of dimeric species were present deviations from first-order kinetics in $[Cu^{2+}]$ would occur. (This, of course, does not imply that the dimers are not the kinetically active species.) Consequently the functions $k_{obs.}$ against $[Cu]_T$ at constant $[L]_T$ and pH should be straight lines passing through the origin; $k_{obs.}$ was measured for a series of differing total copper concentrations at pH 2.30, 2.50, 2.80, 2.90, and 3.30 and the results are given in Figure 8. It is possible to calculate

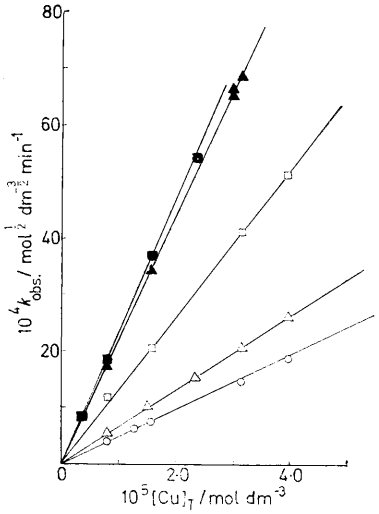


FIGURE 8 Copper dependence of the rate: pseudo-half-order rate constants, $k_{obs.}$ (for the disappearance of O_2), plotted against $[Cu]_T$. (○), pH 2.30, $[L]_T$ = 0.0303 mol dm^{-3}; (△), pH 2.50, $[L]_T$ = 0.0303 mol dm^{-3}; (□), pH 2.80, $[L]_T$ = 0.0303 mol dm^{-3}; (▲), pH 2.90, $[L]_T$ = 0.0521 mol dm^{-3}; (■), pH 3.30; $[L]_T$ = 0.0260 mol dm^{-3}. The full lines were calculated from equation (17)

the expected gradients of the graphs of $k_{obs.}$ against $[Cu]_T$ from (17) using the measured values of K_2^H, K^{MH}, K^M, and k'. These calculated gradients are drawn as full lines in Figure 8 and provide an excellent fit to the data. Thus the copper dependence of the rate is clearly as predicted.

DISCUSSION

Rate law (4) contains a considerable amount of mechanistic information. First, half-order kinetics in oxygen demands a chain mechanism in which the oxygen molecule (at some stage of reduction) is bound within a radical which undergoes unimolecular propagation and bimolecular termination.[13] Secondly the fact that the order with respect to copper is twice that with respect to oxygen requires this radical to be formed or propagated in a step which involves two coppers per oxygen atom. The latter requirement is reminiscent of the mechanism first proposed by George[14] for the oxidation of Fe^{II} by molecular oxygen in acid solution where the reaction is first order in $[O_2]$ but second order in $[Fe^{II}]$. George proposed that the 'reducing power' of one Fe^{II} was insufficient to overcome the unfavourable redox potential for a one-electron transfer to oxygen (−0.32 V),[15] but that participation of a second Fe^{II} (with perhaps a bridging O_2) facilitated the more favourable two-electron transfer.

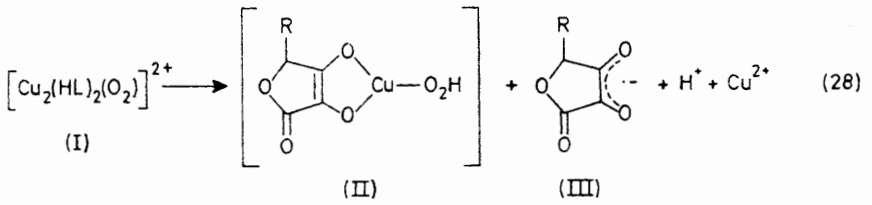
$$Fe^{II} + O_2 \longrightarrow [Fe^{II}\text{–}O_2] \qquad (27a)$$

$$[Fe^{II}\text{–}O_2] + Fe^{II} \longrightarrow 2\ Fe^{III} + [O_2]^{2-} \qquad (27b)$$

Such two-electron transfers are also known for the Pu^{III}–O_2 and V^{II}–O_2 systems,[16] and a similar type of mechanism may be invoked for the ascorbate system with one electron donated from each of two copper atoms, thereby completing a two-electron transfer to oxygen. It is pleasing to find that copper ascorbate dimers are actually present in the system (although at rather low concentration) and a George-type mechanism consistent with the observed kinetics may be proposed with these as the reactive species, but complying with the requirements of half-order kinetics outlined above. If $[Cu_2(HL)_2]^{2+}$ is assumed to be the most important reactive complex (see below), then we might suppose the oxygen molecule to bond across the Cu–Cu binuclear site and furthermore suggest that the mode of binding is perpendicular to the Cu–Cu axis. The π_x and π_y orbitals on O_2 may then interact with the d_{xz} and d_{yz} metal orbitals respectively, such that the degeneracy of the π^* orbital is split by the symmetry of the ligand field.[17] The spin restrictions for reaction of triplet-ground-state O_2 with the singlet organic substrate to form singlet products is thereby lifted, and the thermodynamically unfavourable one-electron transfer to oxygen is also avoided since two electrons may be transferred, as a pair, into the now vacant π^* orbital. Interaction of the π system of the copper–ascorbate complex with that of O_2 itself will further facilitate electron transfer to the oxygen molecule, resulting in the break up of the ascorbate–copper–oxygen complex to radical products which can act as chain propagators.

Direct involvement of the dimer, rather than a two-step mechanism of the type proposed by George[14] [equations (27)], is further supported by the change to a half-order dependence on ligand when the species present is not a simple dimer but contains only one ligand per two copper ions as is the case in chloride media.[1] In (28), (III) is the ascorbate semiquinone while (II) is formally an ascorbate–copper(II)–peroxide complex, and it is this peroxide-containing radical which is presumed to undergo unimolecular propagation and bimolecular termination. The detailed mechanism is given below, in which L^{2-} denotes the doubly deprotonated ligand, L^-

[13] K. J. Laidler, 'Chemical Kinetics,' 2nd edn., McGraw-Hill, 1965.
[14] P. George, *J. Chem. Soc.*, 1954, 4349.
[15] S. Fallab, *Angew. Chem. Internat. Edn.*, 1967, **6**, 496.
[16] H. Taube, *J. Gen. Physiol.*, 1965, **49**, 29.
[17] J. S. Griffith, *Proc. Roy. Soc.*, 1956, **A235**, 23.



the ascorbate semiquinone, (III), and L′ represents dehydroascorbic acid. Applying the steady-state hypothesis, we obtain equations (36) and (37). On adding (36) and (37), we obtain equations (38)—(40), where

$$Cu^{2+} + HL^{-} \overset{K^{M}}{\rightleftharpoons} [Cu(HL)]^{+} \quad (10)$$

$$2[Cu(HL)]^{+} \overset{K^{DH}}{\rightleftharpoons} [Cu_2(HL)_2]^{2+} \quad (29)$$

$$[Cu_2(HL)_2]^{2+} + O_2 \overset{K^{O_2}}{\rightleftharpoons} \underset{(I)}{[Cu_2(HL)_2(O_2)]^{2+}} \quad (30)$$

$$[Cu_2(HL)_2(O_2)]^{2+} \xrightarrow{k_1} \underset{(II)}{[CuL(O_2H)]^{\bullet}} + Cu^{2+} + L^{\bullet} + H^{+} \quad (31)$$

$$\underset{(III)}{L^{\bullet}} + \underset{(I)}{[Cu_2(HL)_2(O_2)]^{2+}} \xrightarrow{k_2} \underset{(II)}{[CuL(O_2H)]^{\bullet}} + [Cu(HL)]^{+} + L' \quad (32)$$

$$\underset{(II)}{[CuL(O_2H)]^{\bullet}} \xrightarrow{k_3} \underset{(III)}{L^{\bullet}} + [HO_2]^{-} + Cu^{2+} \quad (33)$$

$$2\underset{(II)}{[CuL(O_2H)]^{\bullet}} \xrightarrow{k_4} \text{Termination products} \quad (34)$$

$$H^{+} + [HO_2]^{-} \rightleftharpoons H_2O_2 \quad (35)$$

$$d[CuL(O_2H)^{\bullet}]/dt = 0 = k_1[Cu_2(HL)_2(O_2)^{2+}] + k_2[L^{\bullet}][Cu_2(HL)_2(O_2)^{2+}] - k_3[CuL(O_2H)^{\bullet}] - 2k_4[CuL(O_2H)^{\bullet}]^2 \quad (36)$$

$$d[L^{\bullet}]/dt = 0 = k_1[Cu_2(HL)_2(O_2)^{2+}] - k_2[L^{\bullet}][Cu_2(HL)_2(O_2)^{2+}] + k_3[CuL(O_2H)^{\bullet}] \quad (37)$$

thesis, we obtain equations (36) and (37). On adding (36) and (37), we obtain equations (38)—(40), where

$$k_1[Cu_2(HL)_2(O_2)^{2+}] = k_4[CuL(O_2H)^{\bullet}]^2 \quad (38)$$

$$[CuL(O_2H)^{\bullet}] = (k_1/k_4)^{\frac{1}{2}}[Cu_2(HL)_2(O_2)^{2+}]^{\frac{1}{2}} \quad (39)$$

$$= (k_1/k_4)^{\frac{1}{2}}(K^{M}K^{DH}K^{O_2})^{\frac{1}{2}}[Cu^{2+}][HL][O_2]^{\frac{1}{2}} \quad (40)$$

K^{M} and K^{DH} are defined in (11) and (22), and K^{O_2} may be expressed as in (41).

$$K^{O_2} = \frac{[Cu_2(HL)_2(O_2)^{2+}]}{[O_2][Cu_2(HL)_2^{2+}]} \quad (41)$$

If it is assumed that either the unimolecular decomposition of radical(II) (step k_3) is rate determining, or that it represents the only reaction where oxygen is converted into products and thereby removed from the system [the latter assumption is reasonable since the only other step in which H_2O_2 is produced is the termination reaction (k_4) which, if it were important in removing oxygen, would certainly end the chain reaction!], then we may write (42) and (43) which give the observed rate

$$-d[O_2]/dt = k_3[CuL(O_2)^{\bullet}] \quad (42)$$

$$= k_3(k_1/k_4)^{\frac{1}{2}}(K^{M}K^{DH}K^{O_2})^{\frac{1}{2}}[Cu^{2+}][HL^{-}][O_2]^{\frac{1}{2}} \quad (43)$$

law with (44). Hence the proposed mechanism is entirely

$$k' = k_3(k_1/k_4)^{\frac{1}{2}}(K^{M}K^{DH}K^{O_2})^{\frac{1}{2}} \quad (44)$$

consistent with the observed kinetics.

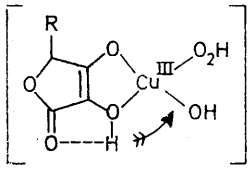
The changes in the oxidation state of copper occurring during the catalytic cycle are rather ill defined. Spectral evidence suggests that in the absence of oxygen there is some degree of charge transfer within the copper–ascorbate complexes, but that the complete one-electron redox reaction (45) does not proceed. Thus the spectrum

$$[\text{copper(II)—ascorbate}] \longrightarrow [\text{copper(I)} \cdots \text{semiquinone}] \quad (45)$$

(Figure 7) shows no band at 360 nm (ε 3 700 dm^3 mol^{-1} cm^{-1}), due to the ascorbate semiquinone [18] and resembles closely that of the straightforward copper(II) complexes of other ene–diol-containing ligands. It is therefore reasonable to assume that the oxygen molecule binds to what is essentially a copper(II)–ascorbate dimer and that two-electron reduction of oxygen occurs as the primary electron-transfer process. This would leave two formal copper(III)–ascorbate units, one of which dissociates, the other remaining bound to peroxide. Such a precise formulation of oxidation states is, of course, invalid with ligands as ' non-innocent ' as ascorbate and dioxygen, and one might equally well describe the product of this primary process as a copper(II)–semiquinone–peroxide species. [A copper(I)–dehydroascorbic acid description would seem to be unacceptable, however, since in this case one would expect that the reactive complex would dissociate to products, thus leaving no dioxygen-containing radicals to propagate the chain reaction as required by half-order O_2 kinetics.] The proposed mechanism thus differs from all previous ones in that no formal copper(I) species are invoked as intermediates. This also implies that no change of stereochemistry at the metal ion is necessary (Cu^{III} can also adopt square-planar geometry); it is to be noted that involvement of Cu^{I} would require a change to linear or tetrahedral stereochemistry. Furthermore, in the copper(III) formalism the stability of the $[CuL(O_2H)]^{\bullet}$ radical may be attributed at least in part to the kinetic inertness of square-planar d^8 complexes, while the preference of the ' harder ' peroxide to bind to metals in higher oxidation

[18] B. H. Bielski and A. O. Allen, *J. Amer. Chem. Soc.*, 1970, **92**, 3793.

states (*e.g.* Ti^{IV}, V^{V}, *etc.*)[19] may also serve to increase the relative stability of a copper(III)–peroxide radical. Finally, if the ascorbate ion were to remain protonated, then the radical could be formulated as $[Cu(HL)(OH)(O_2H)]^{\bullet}$, thus further stabilising the high oxidation state in an analogous manner to the way in which hydrolysis dominates the aqueous solution chemistry of Fe^{III}, V^{V}, Ti^{IV}, and Cr^{VI}. The resulting copper(III) ion may then react by intramolecular α-hydrogen-atom abstraction (46) as suggested by Anbar [20] in the catalytic oxidative deamination of 1,2-diaminoethane and related ligands in which Cu^{III}, and certainly not Cu^{I}, is thought to be an intermediate. (Similarly, the reactive dimer may itself



$$\xrightarrow{k_3} Cu^{2+} + L^{\cdot} + [HO_2]^{-} \qquad (46)$$

be hydroxo-bridged since metal complexes bridged by both $[OH]^-$ and O_2 are well known amongst the cobalt oxygen carriers.[21])

The deviation from linearity shown in the pH dependence of the rate above pH 2.80 can be explained on the basis of the equilibrium evidence since by pH *ca.* 3.3 there are equimolar amounts of $[\{Cu(HL)\}_2]^{2+}$ and $[(CuL)_2]$ present and it is certain that the deprotonated dimer is also reactive, oxidation of the latter proceeding *via* an identical mechanism.

Both Taki Khan and Martell [3] and Shtamm *et al.*[7] reported a measurable uncatalysed reaction in the pH range 3—6 in which the ascorbate semiquinone was produced as an intermediate. Using extremely pure materials, we were unable to reproduce this result, the oxygen concentration always remaining absolutely constant over the period prior to the addition of catalyst. Our kinetics, which have recently been corroborated,[6,8] demand a chain mechanism likewise involving the free ascorbate semiquinone as a propagating radical. Taki Khan and Martell also reported [3] that it is possible to subtract the uncatalysed from the catalysed reaction, but this is clearly invalid if both produce common chain carriers.

A number of copper-containing oxidases (*e.g.* ceruloplasmin, laccase, and ascorbic acid oxidase) contain spin paired Cu–Cu binuclear units which are thought to be the site of oxygen binding.[5,22] These enzymes catalyse the oxidation of catechols, hydroquinone, ascorbic acid, and other similar ligands with associated four-electron reduction of oxygen to water. The copper(II)-catalysed autoxidation of ascorbic acid also involves dimeric copper complexes as reactive species with two-electron transfer to oxygen occurring as the primary event, leaving a formal copper(III) intermediate. The enzyme mechanism is generally deemed to involve re-oxidation of Cu^{I} formed by substrate reduction, but the ' model ' system strongly suggests that initial oxidation of the oxygen binding site will occur even in low-molecular-weight complexes, provided sufficiently electron-rich ligands are also bound as ligands. The mechanistic implications for enzyme action suggested by the copper(II)-catalysed reaction have been fully discussed in a previous communication,[5] and we reiterate our original suggestion that formally higher oxidation-state intermediates ought to be seriously considered in the enzyme case, especially since the latter is able to provide a variety of nitrogen- and/or sulphur-donor ligands at the dioxygen binding site which would help reduce the formal positive charge on copper.

It is worth mentioning that a variety of stable square-planar complexes of Cu^{III} have been prepared with nitrogen and with sulphur donors which include the oxamide and biuret [23] (N donor) and dialkyldithiocarbamate [24] (S donor). A $[Cu^{III}F_6]^{3-}$ complex [25] can also be isolated as a purple crystalline solid where, however, the stability is a consequence of strong electrostatic rather than non-innocent interactions.*

EXPERIMENTAL

Ascorbic acid was Fisons AnalaR grade and was used without further purification. All copper solutions were prepared by dissolving known weights of AnalaR copper foil in pure nitric acid and diluting to the mark. The resulting solutions were checked by complexiometric titration for $[Cu^{2+}]$ with ethylenediaminetetra-acetate and standardised potentiometrically for $[H^+]$. Experiments were made in 0.100 mol dm^{-3} $K[NO_3]$ and the pH was adjusted with standard 0.100 mol dm^{-3} nitric acid. A thermostatted oil-bath was used to maintain the temperature (for both kinetic and thermodynamic work) at 25.00 °C.

A Radiometer E 5046 oxygen-sensitive electrode was used to measure the rate of oxygen consumption. The pH was measured with a Russell CMAT microcombination electrode and was maintained at a constant value in kinetic runs by a Radiometer TT1 pH-stat fitted with an SBR 2 recorder. 0.05 mol dm^{-3} Potassium hydrogenphthalate (pH 4.008) was used to calibrate the pH meter. Pure oxygen was bubbled through the cell containing ascorbic acid in 0.100 mol dm^{-3} $K[NO_3]$ until the $[O_2]$ had reached its saturated value. The cell was then closed to the atmosphere and after a stable $[O_2]$ reading had been achieved the catalyst solution was added through a microsyringe. The resulting $[O_2]$ against time curve was recorded on a Watanabe pen recorder. For potentiometric titrations, a Beckman AS7LB glass elec-

* *Note added in proof:* More recently Margerum has prepared stable copper(III) complexes of deprotonated amides and peptides with remarkably low redox potentials for the Cu^{III}–Cu^{II} couple. This low value of the redox potential (*e.g.* 0.631 V for the deprotonated tetraglycine complex) taken in conjunction with the fact that the complexes are only stable if deprotonated (*i.e.* without an α-hydrogen atom) would seem to lend added weight to our proposed reaction scheme [see also equation (46)] (D. W. Margerum, *Chem. Eng. News*, 1975, **53** (49), 26).

[19] J. A. Connor and E. A. Ebsworth, *Adv. Chem. Radiochem.*, 1964, **6**, 279.
[20] M. Anbar, *Adv. Chem. Ser.*, 1965, **49**, 126.
[21] F. Millar and R. G. Wilkins, *J. Amer. Chem. Soc.*, 1970, **92**, 2687.
[22] R. Malkin and B. G. Malmstrom, *Adv. Enzymology*, 1970, **33**, 177.
[23] J. J. Bows, P. J. M. Birker, and J. J. Steggerder, *Inorg. Chem.*, 1971, **10**, 1202.
[24] J. O. H. Van der Linden and P. J. H. Geurts, *Inorg. Nuclear Chem. Letters*, 1972, **8**, 903.
[25] G. C. Allen and K. D. Warren, *Inorg. Chem.*, 1969, **8**, 1895.

trode, a Radiometer K401 calomel electrode, and an Oriol copper-sensitive electrode were used in conjunction with a Beckman Research pH meter and all titrations were made under an atmosphere of pure nitrogen [oxygen content reduced by use of vanadium(II) sulphate solution].

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