483. *Oxidations of Organic Compounds by Cupric Salts. Part I. Kinetics of the Oxidations of Glucose and Acetoin.*

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The oxidations of both glucose and acetoin by alkaline solutions of complexed cupric salts are preceded by an induction period, and thereafter are substantially of zero order with respect to Cu^{2+} and of first order with respect to the organic substrate. Detailed analysis of the kinetics of the oxidation of acetoin indicates that the latter is oxidised *via* its ene-diol which apparently forms a chelated cuprous complex that is easily attacked by cupric ions and gives biacetyl and two equivalents of cuprous oxide.

In the induction period the above cuprous complex is promptly re-oxidised by oxygen, but if air-free solutions are used then the cuprous complex is very slowly formed from a direct reaction between cupric ions and the ene-diol of acetoin. Precipitation of cuprous oxide, when the limiting solubility of Cu^+ has been reached, is the ultimate rate-controlling feature of the whole oxidation process.

KINETIC studies [1] of the mechanism of oxidation of aliphatic aldehydes and ketones by one-electron-abstracting reagents [Ce^{4+}, Mn^{3+}, $V(OH)_3^{2+}$, $\cdot O \cdot N(SO_3)_2^{2-}$, $Fe(CN)_6^{3-}$] have consistently shown that the slow enolisation of the carbonyl group precedes the easy oxidation of the enol to the mesomeric radical, $-\overset{\overset{\displaystyle O\cdot}{|}}{C}=\overset{|}{C}- \longleftrightarrow -\overset{\overset{\displaystyle O}{\|}}{C}-\overset{|}{\underset{\cdot}{C}}-$ and it was expected therefore that oxidation of aldehydes and α-hydroxy-ketones by Fehling's solution and analogous reagents might proceed in a similar manner.

Though oxidations of reducing sugars by various alkaline solutions of complexed cupric salts can be developed into reproducible analytical procedures, the oxidations themselves are not stoicheiometric reactions and scarcely any kinetic studies of such oxidations have been made. Singh, Krishna, and Ghosh [2] have examined the rates of oxidation of some simple monosaccharides with alkaline cupric tartrate and citrate solutions but their observations do not seem to be very accurate, for they adopted a very cumbersome analytical technique. They decided that the oxidations were alkali-catalysed, of first order with respect to the sugar but of zero order with respect to the cupric salt, and not affected by the presence of an excess over one molar equivalent of either tartrate or citrate. The oxidation seemed to set in after an induction period and did not appear to be a surface reaction.

In a preliminary study we have oxidised alkaline glucose solutions by cupric ions complexed with picolinic acid and in substance confirm the conclusions of Singh, Krishna, and Ghosh. Iodometric titration of residual cupric salt, after acidification of samples with dilute sulphuric acid, is accurately reproducible and not affected by the presence of cuprous oxide or other reaction products. Fig. 1 shows that the oxidation of glucose, in stoppered flasks, does not set in for some minutes after the reagents are mixed, though later, when the reaction mixture has become turbid, the cupric salt is steadily reduced to cuprous oxide. With respect to the cupric salt, much of the reaction is a zero-order process which eventually accelerates, possibly on account of further oxidation of initial reaction products. Table 1 and Fig. 2 indicate the dependence of the reaction velocity on the glucose and alkali concentrations respectively.

Since the chemical nature of alkaline solutions of glucose is uncertain, and time-dependent, a more detailed study of this one-electron oxidation has been made by using acetoin, $Me \cdot CH(OH) \cdot COMe$, which, by the action of cupric ions complexed by either citrate

[1] See papers by Waters and his co-workers, *J.*, 1953—1959.
[2] Singh, Krishna, and Ghosh, *Z. phys. Chem.*, 1955, **204**, 1; 1956, **205**, 285, 294; 1958, **208**, 265, 273.

or malate in the presence of excess of sodium carbonate, is oxidised stoicheiometrically to biacetyl:

$$\text{Me·CH(OH)·COMe} + 2\text{Cu}^{2+} + \text{H}_2\text{O} \longrightarrow \text{Me·CO·COMe} + \text{Cu}_2\text{O} + 4\text{H}^+$$
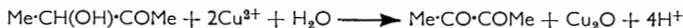
The characteristic features of this oxidation are shown in Fig. 3. Curve (*a*) of this figure exemplifies a typical kinetic experiment carried out in a stoppered flask, immersed in a

FIG. 1. *The oxidation of glucose by alkaline cupric picolinate at 25°.*
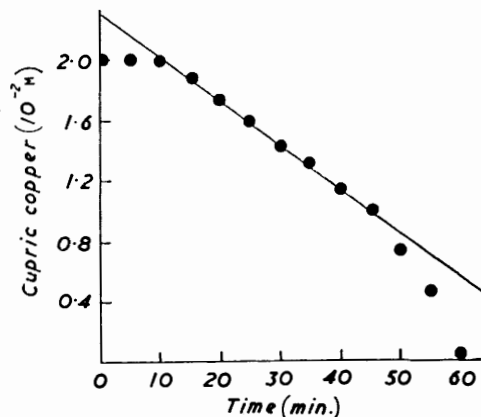
The reaction mixture contained 0·02M-copper sulphate, 0·06M-picolinic acid, 0·35M-sodium hydroxide, and 0·1M-glucose.

FIG. 2. *The effect of sodium hydroxide concentration on the rate of oxidation of glucose by cupric picolinate at 25°.*
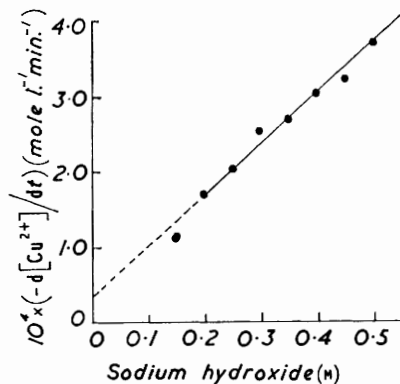
Each reaction mixture contained 0·02M-copper sulphate, 0·06M-picolinic acid, and 0·1M-glucose.

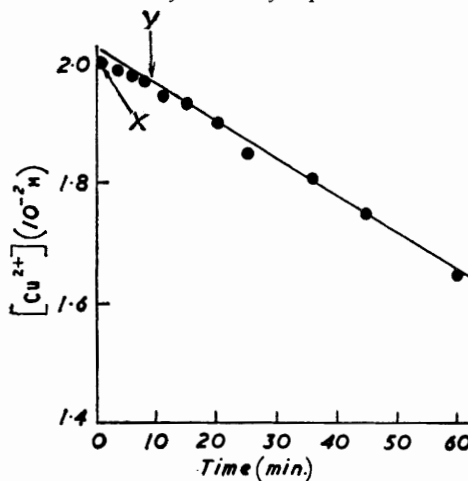FIG. 3. *The oxidation of acetoin by alkaline cupric citrate at 40°.*
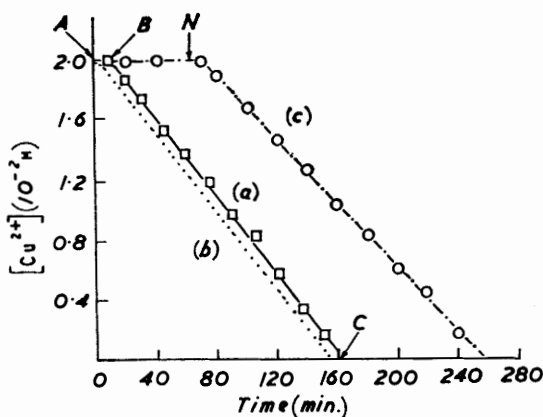
Each reaction mixture contained 0·02M-copper sulphate, 0·06M-sodium citrate, 0·1M-sodium carbonate, and 0·1M-acetoin.
(*a*) Reaction in a stoppered flask. (*b*) Reactants flushed with nitrogen before mixing and during the reaction. (*c*) Oxygen blown through the reaction solution until *N*, then nitrogen blown through the solution. The rate of nitrogen bubbling, and thus the degree of agitation of the solution, did not affect the reaction velocity.
Slopes × 10⁴ (in mole l.⁻¹ min.⁻¹): (*a*) 1·31; (*b*) 1·31; (*c*) 1·08.

FIG. 4. *The autocatalytic stage in the oxidation of acetoin by cupric citrate.*

Temp. 30°. The reaction mixture contained 0·02M-copper sulphate, 0·06M-sodium citrate, 0·1M-sodium carbonate, and 0·1M-acetoin. The reaction was carried out under nitrogen.

thermostat at 40°. Curve (*b*) shows the effect of flushing the separate reactants and then the reacting mixture with oxygen-free nitrogen, and curve (*c*) the effect of saturating the reacting mixture with oxygen and then sweeping it out with nitrogen at time *N*.

Evidently the initial induction period, (A—B) of curve (a) is due to atmospheric oxidation of a primary reaction product, but, even in the absence of oxygen, the reduction of the cupric complex has a brief autocatalytic stage before the reaction settles down to being a simple zero-order process. This is shown more clearly in Fig. 4. During this autocatalytic stage the clear blue solution becomes greenish and turbid. Solid cuprous oxide has always been deposited by the time the reactions reach their final steady state.

The induction period can be eliminated by adding copper powder to the reaction mixture, or by adding a portion of a partly reacted mixture from a previous experiment, but not by adding washed, precipitated cuprous oxide. It may be inferred, therefore, that

FIG. 5. *The effect of the concentration of added sodium carbonate on the rate of oxidation of acetoin by complexed cupric ions when the complexing agent is* (a) *trisodium citrate,* (b) *malic acid.*

$10^2 \times$ Slopes (in min.$^{-1}$): (a) 1·40; (b) 1·38.

Temp. 40°. Each reaction mixture contained 0·02M-copper-sulphate, 0·06M-complexing agent, and 0·1M-acetoin. The OH⁻ values for acetoin-free solutions were measured potentiometrically, a glass electrode being used.

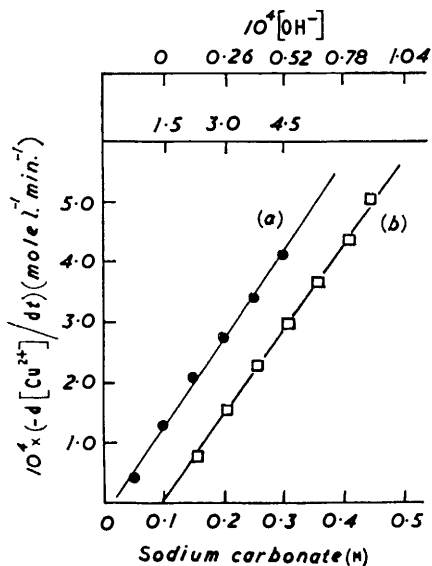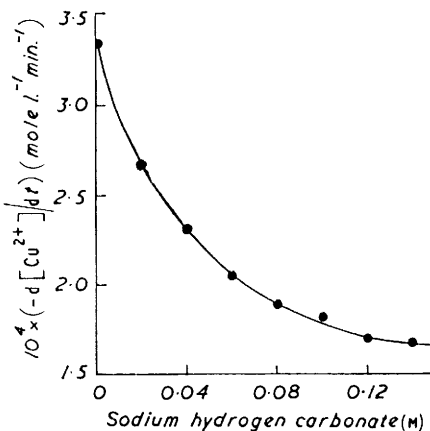FIG. 6. *The effect of bicarbonate concentration on the rate of oxidation of acetoin at constant carbonate concentration.*

Temp. 40°. The reaction mixtures contained 0·02M-copper sulphate, 0·06M-sodium citrate, 0·25M-sodium carbonate, and 0·1M-acetoin.

the main reaction (B—C) requires the presence of dissolved cuprous ions which accumulate to a limiting concentration, during the autocatalytic stage (X—Y of Fig. 4), and can be removed by oxygen. The rate of the steady reaction would, on this hypothesis, be limited by the solubility of the dissolved cuprous complex. The eventual rate of oxidation of the oxygenated solution is slightly slower than (a) or (b), showing that some slow oxidation of acetoin is effected by oxygen before the cuprous ions reach a sufficient concentration for precipitation to be detectable. Closer investigation of the induction period, measured by extrapolating portions B—C of reaction (a) (Fig. 3) back to zero reduction of Cu^{2+}, shows that its duration is inversely proportional to both the acetoin and the sodium carbonate concentration, as shown by Tables 2 and 3.

The main sections (*e.g.*, B—C) of each of the curves of Fig. 3 show that the dissolved cupric salt is being reduced by a homogeneous zero-order reaction, not affected by the progressive separation of cuprous oxide or by the degree of agitation of the solution. Table 4

shows that the oxidation is of first order with respect to acetoin, and Table 5 that excess of the complexing agent has no effect. Fig. 5 shows that the rate of oxidation is proportional to the concentration of carbonate anions and is identical in value for both the citrate and the malate complexes when allowance is made for the neutralisation of carbonate by both the organic acid and the sulphuric acid equivalent to the copper sulphate originally taken. Neverthless, corresponding solutions which are oxidised at the same rate differ considerably in pH. Though this could indicate that the reaction is carbonate-dependent but not hydroxyl-dependent, if the alkalinity of citrate solutions of constant carbonate concentration is varied by the addition of sodium hydrogen carbonate, then the oxidation rate decreases as shown in Fig. 6. Measurements of the pH of these solutions show that the oxidation is of first order with respect to hydroxyl, though the plot does not pass through
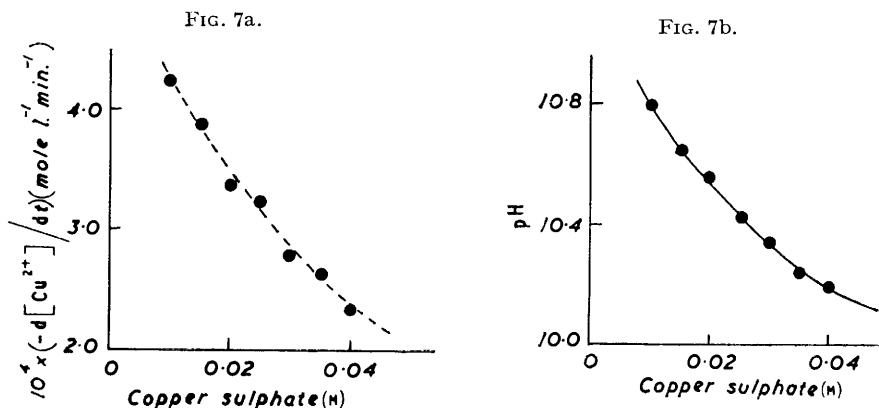

FIG. 7a.   FIG. 7b.

FIG. 7. *The effect of he initial cupric concentration on* (a) *the rate of oxidation of acetoin,* (b) *the pH of the reaction solutions.*
Temp. 40°. The reaction mixtures contained 0·06M-sodium citrate, 0·25M-sodium carbonate, and 0·1M-acetoin.

the origin (Table 6). In the concentration range of Fig. 5 there is a linear relation between carbonate concentration and alkalinity (Table 7). pH changes may also account for the fact that the actual oxidation rate is dependent on the initial Cu^{2+} concentration when both citrate and carbonate are kept constant (Fig. 7).

TABLE 1. *The effect of the initial glucose concentration on the rate of oxidation of glucose by cupric picolinate at 25°.*

Glucose (M)	0·025	0·050	0·075	0·100	0·125
$10^4 \times -d[Cu^{2+}]/dt$ (mole l.$^{-1}$ min.$^{-1}$)	0·78	1·37	2·00	2·68	3·50
$10^3 \times -d[Cu^{2+}]/dt/[Glucose]$	3·12	2·74	2·67	2·68	2·80

Each solution contained 0·02M-copper sulphate, 0·06M-picolinic acid, and 0·35M-sodium hydroxide.

TABLE 2. *The effect of the initial acetoin concentration on the induction period in the oxidation of acetoin by cupric citrate at 40°.*

$10 \times$ [Acetoin] (M)	0·25	0·50	0·75	1·00	1·25	1·50
Induction period (min.)	32·5	15·0	12·0	9·0	6·0	5·0
[Acetoin] × Induction period	0·71	0·75	0·90	0·90	0·75	0·82

Each solution contained 0·02M-copper sulphate, 0·06M-sodium citrate, and 0·1M-sodium carbonate.

TABLE 3. *The effect of the added sodium carbonate concentration on the induction period in the oxidation of acetoin by cupric citrate at 40°.*

Sodium carbonate (M)	0·05	0·10	0·15	0·20	0·25	0·30
Induction period (min.)	20·0	10·0	7·0	3·5	3·0	3·0
$[Na_2CO_3] \times$ Induction period	1·00	1·00	1·05	0·70	0·75	0·90

Each solution contained 0·02M-copper sulphate, 0·06M-sodium citrate, and 0·1M-acetoin.

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TABLE 4. *The effect of the initial acetoin concentration on the rate of oxidation of acetoin by cupric citrate at 40°.*

10 × [Acetoin] (M)	0·25	0·50	0·75	1·00	1·25	1·50
$10^4 \times$ —d[Cu²⁺]/dt (mole l.⁻¹ min.⁻¹)	0·30	0·66	1·00	1·31	1·68	1·94
$10^3 \times$ —d[Cu²⁺]/dt/[Acetoin]	1·20	1·32	1·33	1·31	1·34	1·29

Each solution contained 0·02M-copper sulphate, 0·06M-sodium citrate, and 0·1M-sodium carbonate.

TABLE 5. *The effect of the concentration of the complexing agent on the rate of oxidation of acetoin by cupric citrate at 40°.*

Sodium citrate (M)	0·06	0·09	0·12
$10^4 \times$ —d[Cu²⁺]/dt (mole l.⁻¹ min.⁻¹)	3·36	3·34	3·32

Each solution contained 0·02M-copper sulphate, 0·25M-sodium carbonate, and 0·1M-acetoin.

TABLE 6. *The effect of hydroxide-ion concentration on the rate of oxidation of acetoin at constant carbonate concentration.*

$10^4 \times$ —d[Cu²⁺]/dt (mole l.⁻¹ min.⁻¹)	1·12*	1·67	1·71	1·82	1·88	2·04	2·32
10^5[OH⁻]	0·00	5·12	5·62	6·24	7·16	9·02	12·16
(Rate — 1·12 × 10⁻⁴*)/[OH⁻]	—	1·07	1·05	1·12	1·08	1·02	0·98

* By extrapolation.

The mixtures contained 0·02M-copper sulphate, 0·06M-sodium citrate, 0·25M-sodium carbonate, and 0·1M-acetoin. The OH⁻ concentration was varied by the addition of sodium hydrogen carbonate.

TABLE 7. *The effect of the added sodium carbonate concentration on the alkalinity of a solution of cupric citrate at 40°.*

Sodium carbonate (M)	0·30	0·25	0·20	0·15	0·10	0·05	0·00
10^4[OH⁻]	3·89	3·63	2·95	2·57	1·62	0·72	1·59 × 10⁻⁹
10^3[OH⁻]/[Na₂CO₃]	1·30	1·45	1·47	1·71	1·62	1·44	—

Each solution contained 0·02M-copper sulphate and 0·06M-sodium citrate.
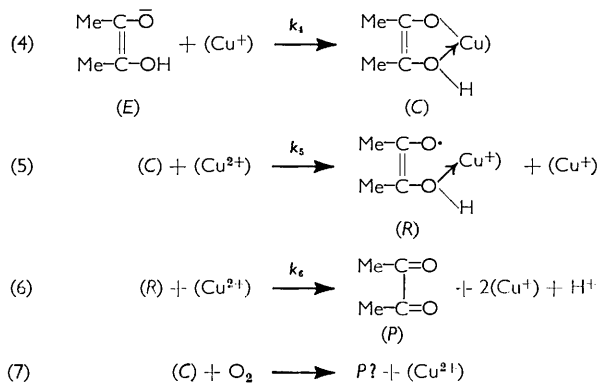
DISCUSSION

(1) *Enolisation prior to Oxidation.*—The rate of oxidation of acetoin is of first order with respect to both [Acetoin] and [Hydroxide] during both the induction period and the subsequent main reaction. These observations indicate that the acetoin is probably oxidised in the form of its enol or enol-anion, following base-catalysed reactions, of which only (1) need be considered as a rate-determining process.

(1) Me·CH(OH)·COMe + B $\rightleftharpoons$ Me·C̄(OH)·COMe + HB⁺

(2) Me·C̄(OH)·COMe $\longleftrightarrow$ Me·C(OH):C(Ō)·Me (Mesomerism)

(3) Me·C(OH):C(Ō)·Me + HB⁺ $\rightleftharpoons$ Me·C(OH):C(OH)·Me + B

(2) *Reduction of* Cu²⁺.—Zero-order reactions of aldehydes and ketones with respect to other reagents, *e.g.*, halogens or Mn³⁺, usually indicate that the respective enols are being destroyed immediately they have been formed, but such reactions promptly set in at their full velocity. The existence of induction and autocatalytic phases of slow oxidation, linked to the formation of a cuprous compound, as shown by the effect of adding metallic copper, and by the constancy of the reaction velocity when cuprous ions are being removed by precipitation of cuprous oxide, indicates that the formation and destruction of cuprous complexes are concerned in the regulation of the reaction velocity. Our system is kinetically similar to one observed by Calvin,[3] who oxidised hydrogen by cupric chloride dissolved in quinoline and noted that cuprous acetate was a catalyst for the oxidation. He concluded that the formation of a co-ordinated cuprous compound was essential for the oxidation of hydrogen. Our reaction can be explained along similar lines (4—7) if it is postulated that the ene-diol of acetoin is most prone to attack in the form of its cuprous chelate complex (C). Parentheses have been placed round Cu⁺ and Cu²⁺ to indicate that citrate, malate, or other complexed copper ions are involved. Reaction (4) can be viewed

[3] Calvin, *Trans. Faraday Soc.*, 1938, **34**, 1181; *J. Amer. Chem. Soc.*, 1939, **61**, 2230.

(4) $\text{Me-C-}\bar{\text{O}}$, Me-C-OH (E) $+ (\text{Cu}^+)$ $\xrightarrow{k_4}$ [Cu chelate] (C)

(5) $(C) + (\text{Cu}^{2+})$ $\xrightarrow{k_5}$ (R) $+ (\text{Cu}^+)$

(6) $(R) + (\text{Cu}^{2+})$ $\xrightarrow{k_6}$ Me-C=O, Me-C=O (P) $+ 2(\text{Cu}^+) + \text{H}^+$

(7) $(C) + \text{O}_2$ $\longrightarrow$ $P? + (\text{Cu}^{2+})$

as a slow displacement of one chelating group by another. This would accord with our findings that the citrate and malate complex ions effect oxidations at different rates at the same pH. From steady-state equations:

$$[C] = (k_4/k_5)([E] . [\text{Cu}^+]/[\text{Cu}^{2+}])$$
$$[R] = (k_5/k_6)[C]$$

so

$$-\text{d}[\text{Cu}^{2+}]/\text{d}t = [\text{Cu}^{2+}]\{k_5 C + k_6 R\} = 2k_4[E][\text{Cu}^+]$$

Thus, once $[\text{Cu}^+]$ has reached the solubility limit for the deposition of cuprous oxide from the solution, the oxidation velocity would become constant at a value proportional to the ene-diol concentration, provided that reaction (4) is slower than the rate-determining stage (1) of the base-catalysed enolisation of acetoin. This is most probable since $[\text{Cu}^+]$ is limited by the solubility of cuprous ions in alkaline solution and must be very small indeed.

The above equations, however, do not explain how the reaction is initiated in homogeneous solution under nitrogen. The structure (R), however, is that of a resonance hybrid, *i.e.*,

(i) $\longleftrightarrow$ (ii)
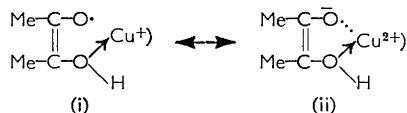
form (i) being a radical–cuprous complex and form (ii) that of an enol–cupric complex. If we suppose that cupric ions (Cu^{2+}) complex with the ene-diol anion (E) to a very minute extent only, giving the hybrid $R = $ (i) $\longleftrightarrow$ (ii) above, then reaction (6) could be regarded as the initial oxidation step that generates the trace of (Cu^+) which allows reaction (4) to set in. Were it unnecessary to propound some explanation of initiation of the reaction then equations (5) and (6) could be combined as:

$$C + 2(\text{Cu}^{2+}) \longrightarrow P + 3(\text{Cu}^+)$$

to give the kinetic sequence suggested by Calvin for his oxidation.

Radical–oxidant complex formation has previously been suggested by one of us for oxidation of aldehydes and ketones by alkaline ferricyanide,[4] a reagent which approximates in its redox potential to complexed cuprous–cupric systems: mesomerism, of the type (i) $\longleftrightarrow$ (ii), can explain the stability of such complexes and the failure to detect free organic radicals in these oxidations.

EXPERIMENTAL

Materials.—Acetoin (B.D.H.) was washed with alcohol until colourless, then with ether, and air-dried by suction. The dimeric solid had a sharp m. p. if heated rapidly in a sealed

[4] Speakman and Waters, *J.*, 1955, 40.

tube and gave a satisfactory ultimate analysis. Standard solutions (0·5M) were prepared in distilled water, through which nitrogen had been bubbled, at frequent intervals; these were stored in the dark. It is known that the solid dimer rapidly dissociates to the monomer in solution.[5] All other reagents, except picolinic acid, were of " AnalaR " quality. Copper sulphate ($CuSO_4,5H_2O$) was used as the source of Cu^{2+}; 0·0025 mole of sulphuric acid was added to 0·1 mole of copper sulphate to assist solution. Trisodium citrate was used as the dihydrate, and malic acid as the pure anhydrous DL-acid, m. p. 128—129°.

Quantitative Oxidation.—Acetoin (0·2216 g., 0·00252 mole) was oxidised at 40° for 24 hr. with an excess of alkaline cupric citrate (0·01 mole of copper sulphate, 0·03 mole of sodium citrate, and 0·15 mole of sodium carbonate), and the residual cupric salt in solution was then titrated as described below; 0·00529 mole of Cu^{2+} had been reduced.

From various experiments the deposit of cuprous oxide was collected, washed with distilled water, alcohol, and ether, dried *in vacuo*, and analysed (Found: Cu, 85·5. Calc. for Cu_2O: Cu, 88·8%).

Titration Procedure.—Samples (5 ml.) of reacting solutions were withdrawn and added to 2N-sulphuric acid (5 ml.) in a narrow-mouthed flask. Immediately thereafter, excess of potassium iodide solution was added and the free iodine solution was titrated with standard thiosulphate (*ca.* 0·005N), with starch indicator, 1 ml. of 10% potassium thiocyanate being added just before the end-point was reached to prevent " after-blueing." [6] In the studies of the glucose reaction, carbonate was added with potassium iodide so that the carbon dioxide liberated would effectively check any autoxidation during the estimation. The other reacting mixtures contained carbonate.

Whenever gases were passed through the solutions they were presaturated by bubbling through water and an identical reacting solution.

⁵ Lowry and Baldwin, *J.*, 1935, 704.
⁶ Belcher and Wilson, " New Methods in Analytical Chemistry," Chapman and Hall, London, 1955, p. 224.