[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

A Kinetic Study of the Formation of the Cobalt–Glycylglycine–Oxygen Complex[1,2]

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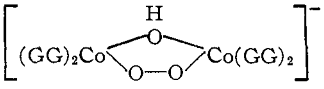
RECEIVED APRIL 1, 1954

The rate of formation of the stable red complex between cobalt, glycylglycine (GG) and molecular oxygen has been investigated over the range of *p*H 7 to 12.5. It appears that the formation of this complex requires as an intermediate a complex containing two hydroxyl ions, $[(OH)(GG)_2Co\text{–}O_2\text{–}Co(GG)_2(OH)]^-$. Below *p*H 8 this intermediate is highly unstable and its presence can be deduced only from the pronounced *p*H-dependence of the reaction rate. At higher *p*H values the intermediate becomes increasingly stable and can be observed by its intense brown color. The important step in the formation of the intermediate is the addition of oxygen to $Co(GG)_2(OH)_2^-$, with a rate constant about 6×10^4 (moles/liter)$^{-1}$ min.$^{-1}$, at ionic strength 1.0 and 25°. The transformation of this intermediate probably involves the formation of a second Co–Co bridge, with the elimination of at least one OH^- ion. It follows a first-order law, $-dc/dt = (1.65 \times 10^{-2} + 1.5 \times 10^9[H^+])c$ (moles/liter)$^{-1}$ min.$^{-1}$.

The formation of a red complex in alkaline solutions containing cobalt and glycylglycine (GG)[3] was first observed by Smith,[4] who believed that it might be an intermediate in the cobalt-catalyzed enzymatic hydrolysis of GG. The complex was isolated and crystallized by Gilbert, Otey and Price,[5] who showed that it could not play the role envisaged for it by Smith. These authors also made the important discovery that oxygen was taken up in the formation of the complex, and that it could not be formed in the absence of oxygen. They obtained analytical data to show that the complex contained Co, GG and molecular O_2, in the ratio 2:4:1, and these figures were confirmed during the present study. This complex is thus similar to the cobalt–histidine–oxygen complexes described by Hearon, Burk and co-workers[6,7] and to the peroxo cobalt ammines described by Werner and Mylius.[8] Possible structures for the complex in solution, compatible with the analytical data of Gilbert, Otey and Price,[5] are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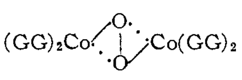
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The latter formulation is similar to one suggested for the cobalt–histidine–oxygen complex.[7] Since the present study is primarily a kinetic one, and the precise structure of the product does not enter into formulation of the rate equations, alternative structures for the red complex are not excluded.[9]

The main purpose of this paper is to describe the kinetics of formation of this red complex over the range of *p*H 7 to 12.5. The study is conveniently divided into two parts: at low *p*H, where the red complex is formed directly, and at high *p*H where, as already observed by Gilbert, Otey and Price,[5] a brown-colored intermediate occurs. Throughout the entire *p*H range, *i.e.*, whether or not the brown intermediate is observed, the final product, in solution, is identical, spectroscopically, with the products of Smith[4] and of Gilbert, Otey and Price.[5] Its principal absorption maximum is at 520 mμ, and its molar extinction coefficient, $\epsilon_1 = \log I_0/I$ for a 1 *M* solution and a 1-cm. light path, is equal to 768.

Experimental

C.P. reagents were used throughout. GG was obtained from the Pfanstiehl Chemical Co., Waukegan, Ill., and from the H. M. Chemical Co., Santa Monica, Calif. The material from both suppliers contained no detectable moisture, and gave the predicted nitrogen analyses and titration curves.

All *p*H measurements were made with a Beckman Model G *p*H meter, using external electrodes, at 25.0°. Light absorption was measured with a Beckman Model DU spectrophotometer, equipped with thermospacers, which maintained the cell compartments at 25.0°. Measurements not requiring temperature control were sometimes made with a Cary recording spectrophotometer. Oxygen absorption was measured with a Warburg apparatus, at 30.1°.

Rate Studies between *p*H 7 and 8.—In this *p*H region the brown intermediate does not appear in detectable amounts. The conversion to the red product and the absorption of oxygen both occur relatively slowly. That they occur simultaneously was demonstrated by placing 16 identical solutions (0.252 *M* GG, 0.121 *M* KOH, 0.012 *M* $Co(NO_3)_2$, *p*H 8.02) in a Warburg apparatus, allowing O_2 absorption to take place. At appropriate intervals these solutions were removed, their accumulated oxygen uptake recorded and the optical density at the absorption maximum of the red complex measured. The results are shown in Fig. 1. In another experiment a solution in which reaction had proceeded to completion was progressively diluted, and the absorption at 520 mμ was measured. Strict adherence to Beer's law was observed. These experiments show that the absorption at 520 mμ is an accurate measure of the concentration of the red product and that, in this *p*H range, it can be used to measure the rate of progress of its formation.

Quantitative measurements were made on solutions saturated either with oxygen or with air. Separate solutions were prepared, one containing GG, KOH and KNO_3, the other containing $CO(NO_3)_2$, the concentrations being such that the desired initial composition would be attained on mixing. Both solutions were presaturated by passage of a rapid stream of oxygen or air through them. The two solutions were then mixed without interruption of the gas stream, and, at appropriate intervals, samples were withdrawn for determination of light absorption at 520 mμ. The gas stream was continued throughout a run. The reaction vessel was a 50-ml. cylinder immersed in a 25° bath.

The oxygen or air used to saturate the reaction mixture

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[1] Abstracted from the Ph.D. thesis of David C. Kirk, Jr., State University of Iowa, February 1953, and from the M.S. thesis of Miran K. Chantooni, Jr., State University of Iowa, February 1954.

[2] This investigation was supported by research grants RG-2350 and H-1619 from the National Institutes of Health, Public Health Service, and by a grant from the National Science Foundation.

[3] Throughout this paper, GG will stand for the word "glycylglycine." In chemical formulas, $GGH^{\pm}$ will represent the zwitterion form of this molecule, and GG^- the anionic form.

[4] E. L. Smith, *J. Biol. Chem.*, **173**, 571 (1948).

[5] J. B. Gilbert, N. C. Otey and V. E. Price, *ibid.*, **190**, 377 (1951).

[6] J. Z. Hearon, *J. Natl. Cancer Inst.*, **9**, 1 (1948).

[7] J. Z. Hearon, D. Burk and L. A. Schade, *ibid.*, **9**, 337 (1949).

[8] A. Werner and A. Mylius, *Z. anorg. chem.*, **16**, 245 (1898).

[9] Titration studies showed no uptake of acid or base between *p*H 3 and 12 indicating that both the carboxyl and amino groups of GG are bonded to cobalt. A small amount of acid was taken up reversibly below *p*H 3, with an accompanying reversible shift in the absorption peak. This acid uptake may represent the addition of a hydrogen ion to the OH bridge of IA.

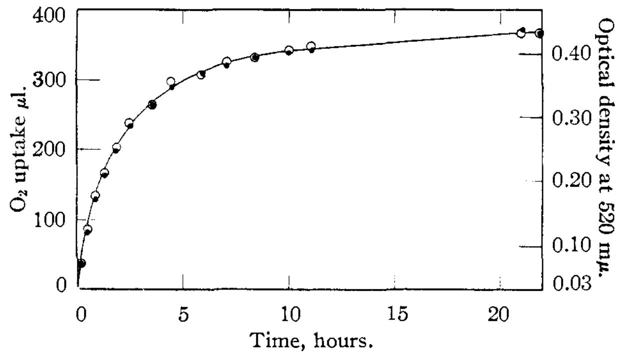

Fig. 1.—Oxygen uptake (●) and light absorption at 520 mμ (O), measured simultaneously; (0.012 *M* Co^{++}, *p*H 8.02, $\mu = 0.16$, $T = 30°$).

was passed through a solution of KNO_3 of the same ionic strength as the reaction mixture, so that its water content was approximately in equilibrium with it. When this gas stream was passed for several hours through a copper sulfate solution of about the same ionic strength no appreciable change in light absorption could be detected.

Most of the studies reported here were confined to *initial rates*. The optical density, $E = \log I_0/I$, was measured as a function of time, and the initial rate was determined from the relation, $dc_1/dt = (dE/dt)/(\epsilon_1 - 2\epsilon_0)$ where c_1 is the number of moles of the red product per liter, ϵ_1 is its molar extinction coefficient at 520 *m* (given above), and ϵ_0 is the extinction coefficient per mole of cobalt of the initial unoxidized mixture, at the same wave length. The latter was found, by extrapolation of curves of *E vs. t* to $t = 0$, to be small ($\epsilon_0 = 10$ to 20) and to increase slightly with increasing *p*H, presumably because of more complete complexing of the unoxidized cobalt at higher *p*H.

Measurements of *p*H were made at intervals during a run. In the range of *p*H here under consideration no *p*H changes were ever observed, because of the excellent buffering action of the excess GG.

Rate Studies at High *p*H.—Above *p*H 8, as previously mentioned, a brown-colored species begins to be formed as a transient intermediate early in the reaction. As the *p*H is increased the brown color appears more rapidly and becomes more intense, until, at sufficiently high *p*H, its appearance is instantaneous, and its initial intensity becomes independent of *p*H. At the same *p*H the absorption of oxygen also becomes instantaneous, the amount absorbed being 0.5 mole per mole of cobalt, *i.e.*, the same amount as is present in the final red product. It can therefore be concluded that at high *p*H all of the cobalt is instantaneously converted into the brown species, and that this species already contains the Co–O–O–Co structure present in the final red product. The precise *p*H at which instantaneous conversion to the brown species is complete depends on the initial concentrations and the ionic strength. At ionic strength 1.0 and total cobalt concentration 2.43×10^{-3} *M*, conversion is essentially complete at *p*H 9.7, as shown by the fact that the *initial* optical densities at 350 mμ, of solutions containing the same amount of cobalt, had exactly the same values at all *p*H values between *p*H 9.7 and 12.5, independent of whether the solutions were saturated with air or oxygen. From these initial optical densities, as well as the values given in Fig. 7 for solutions with varying cobalt concentration, Beer's law was found to be obeyed, and a molar extinction coefficient of about 8000 at 350 mμ was calculated for the brown species (assuming two cobalt atoms per molecule). Because of the relatively rapid transformation of the brown species to the red product, a good complete spectrum was not obtained. It appears, however, that the spectrum is similar to the charge-transfer spectra exhibited by many loose molecular complexes,[10] with an intense absorption peak well in ultraviolet.

The rate of transformation of the brown species to the final red product was measured by following the optical density at 350 mμ, at which wave length the molar extinction coefficient of the red product is less than 80. The rate of reaction could not be conveniently followed at 520 mμ, because the molar extinction coefficient of the brown species at that wave length is almost as great as that of the red and the over-all change in optical density at that wave length is therefore small. Within experimental error, however, identical results were obtained in runs in which measurements were made simultaneously at 350 and 520 mμ. No evidence could be found in these experiments to indicate that absorbing species other than the brown and red forms might exist.

In the high *p*H region fluctuations in *p*H up to 0.2 *p*H units occurred during some runs. The values reported represent average values.

Reaction in the Absence of Oxygen.—In the absence of oxygen the only reaction is the formation of cobaltous-GG complexes. Equilibrium in this reaction is attained essentially instantaneously. For the present study the equilibrium constants at ionic strength 1.0 are required, and these were obtained potentiometrically by the Bjerrum method.[11] The experiments were performed at the same time as those dealing with the combination of cobalt with α-amino acids, described in a previous paper.[12] The utmost precautions were taken to exclude oxygen from the reaction mixtures.

Equilibria in the Absence of Oxygen

The formation curve for cobalt–GG complex formation at 25° and ionic strength 1.0 is shown in Fig. 2. It can be seen that each cobalt ion combines with a maximum of two GG^- ions. Since the coördination number of Co^{++} is six, as shown by its combination with six molecules of NH_3[11] or with three molecules of α-amino acids,[12] this result indicates an unusually small formation constant for $Co(GG)_3^-$, either for steric reasons or because GG^- acts as a tridentate ligand, using, presumably, the amino and carboxyl groups and the amide nitrogen.

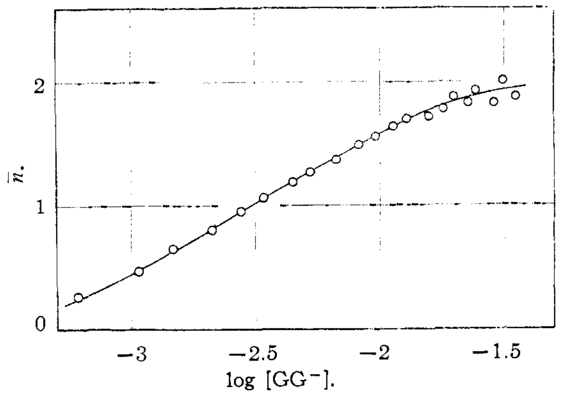

Fig. 2.—Formation curve for Co^{++}–GG complexing in the absence of oxygen; (∼0.01 *M* Co^{++}, 0.25 *M* GG, *p*H < 7.5, $\mu = 1.0$, $T = 25°$).

By means of Bjerrum's method of successive approximation,[11] the two association constants were determined. They are listed in Table I, together with the acid dissociation constant of $GGH^{\pm}$, which is required in making the calculations.

Table I

Constants for the Association Between Cobaltous Ion and GG in the Absence of Oxygen ($\mu = 1.0$, $T = 25.0°$)

$GGH^{\pm} \rightleftarrows GG^- + H^+$; pK_H	8.24
$Co^{++} + GG^- \rightleftarrows CoGG^+$; $\log K_1$	2.73
$CoGG^+ + GG^- \rightleftarrows Co(GG)_2$; $\log K_2$	2.29

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(10) R. S. Mulliken, This Journal, **74**, 811 (1952); *J. Phys. Chem.*, **56**, 801 (1952).

(11) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(12) C. Tanford and W. S. Shore, This Journal, **75**, 816 (1953).

To assure the validity of these calculations, it is necessary to consider the possibility of hydrolysis, *i.e.*, the formation of complexes containing OH^- as ligand. Such complexes undoubtedly exist in the solutions studied, as will be indicated below, but in amounts too small to affect the cobalt–GG complexing. From studies on the solubility of $Co(OH)_2$, Gayer and Garrett[13] obtained the equilibrium constant, 1.6×10^9, for the reaction $Co^{++} + 2OH^- \rightleftarrows Co(OH)_2(aq.)$. If one assumes that the equilibrium constant for the reaction $Co^{++} + OH^- \rightleftarrows CoOH^+$ is approximately the square root of Gayer and Garrett's constant, then the amount of $CoOH^+$ present in all solutions with $\bar{n} < 1.5$ is quite negligible, for the *p*H in these solutions was always less than 6.8. Higher *p*H values (up to *p*H 7.5) were required in the extension of the formation curve to $\bar{n} = 2$. In this *p*H range Gayer and Garrett's constant would lead to a value for $[CoOH^+]/[Co^{++}]$ up to 0.012. However, the high ionic strength used in the present study would decrease the association. In addition, the cobalt is present largely as $Co(GG)_2$. Hydroxyl ions can be introduced into this species only by first rupturing one of the Co–GG bonds, *i.e.*, there would be a further decrease in the association. The possible amount of hydroxy forms thus becomes less than 1%, which is too small to affect, within the experimental error, the data on Co–GG complex formation.

In connection with the rate studies in the low *p*H region, it was found desirable to be able to compute the concentration of the predominant form, $Co(GG)_2$, in any solution. Accordingly, Fig. 3 shows curves of the ratio, $f = [Co(GG)_2]/[Co]_T$, where $[Co]_T$ represents the total concentration of divalent cobalt in the solution. This ratio was calculated from the constants of Table I. Since Fig. 3 extends to *p*H 8, a correction was made for the presence of hydroxy forms, intelligent guesses being made for the values of the appropriate equilibrium constants. As would be expected from the preceding discussion the magnitude of this correction was actually quite negligible. Even at *p*H 8 it amounts to only 1% change in the value of *f*. Since the error in experimental rate determinations, and in the values for $[OH^-]$, is well in excess of 1%, this correction plays no part in the conclusions reached in the kinetic study.

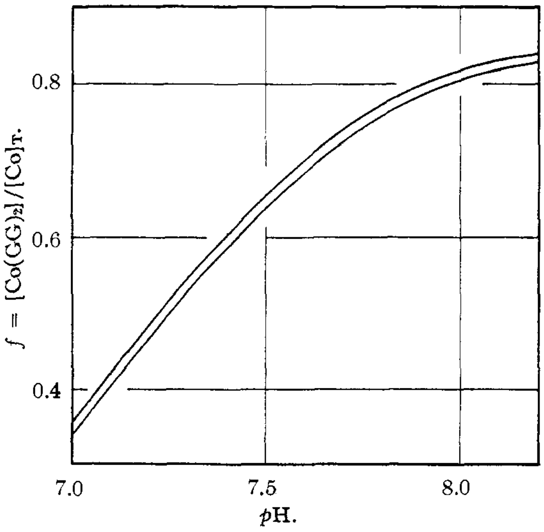

Fig. 3.—The fraction of cobaltous ion present as $Co(GG)_2$ under conditions prevailing in the kinetic studies at low *p*H: total GG = 0.0757 *M*, total Co^{++} = 0.00081 *M* (upper curve) or 0.00405 *M* (lower curve), $\mu = 1.0$, $T = 25°$.

As was indicated above, there are present in the solutions here under discussion small amounts of hydroxy forms. The forms $Co(GG)_2OH^-$ and $Co(GG)_2(OH)_2^=$ will be seen to be of particular importance. These species can be formed by substitution of OH^- at one of the coördination sites occupied by GG^-, most likely that involving the amide nitrogen. This leaves the GG^- as a bidentate ligand, as in the red product I. If we designate the equilibrium constants, $[Co(GG)_2OH^-]/[Co(GG)_2][OH^-]$ and $[Co(GG)_2(OH)_2^=]/[Co(GG)_2OH^-][OH^-]$, respectively, by K_3 and K_4, then, in terms of the factor *f*, and the total concentration, $[Co]_T$, of divalent cobalt

$$[Co(GG)_2OH^-] = K_3f[Co]_T[OH^-] \quad (1)$$

and

$$[Co(GG)_2(OH)_2^=] = K_3K_4f[Co]_T[OH^-]^2 \quad (2)$$

The values of K_3 and K_4, as previously indicated, are unknown. It is unlikely that they will be larger than about 3×10^4.

Rate Studies; *p*H 7.0 to 8.2

Results.—The results of measurements of the *initial rate* of formation of the red product as a function of *p*H, cobalt concentration and oxygen pressure, all in the presence of excess glycylglycine and KNO_3, with a total ionic strength of 1.0, are shown in Table II. They are plotted in Fig. 4 and show at once that at constant *p*H the reaction rate is proportional to the first power of the oxygen pressure and, roughly, to the 3/2 power of the total cobalt concentration. From the analytical result

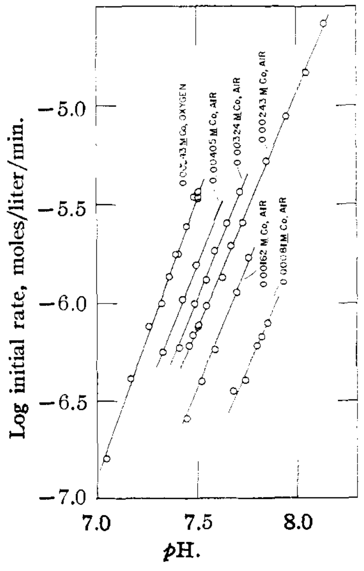

Fig. 4.—Summary of all initial rate data in the low *p*H region.

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(13) K. H. Gayer and A. B. Garrett, THIS JOURNAL, **72**, 3921 (1950).

that the final product contains 1 molecule of O_2 and 2 cobalt atoms, one would expect a reaction of second-order in cobalt if the reaction occurs in a single step. The observed 3/2 order thus indicates the reversible formation of an intermediate preceding conversion to the final product.

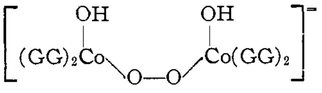
TABLE II

OBSERVED AND CALCULATED INITIAL RATES AT LOW *p*H ($\mu = 1.0$, $T = 25°$)

$[Co]_T$, mole/l. × 10^3	*p*H	Oxidizing gas	Rate × 10^6 moles/l./min. Obsd.	Calcd. by eq. 6	Dev., %
0.81	7.68	Air	0.355	0.310	14.6
.81	7.74	Air	.405	.436	7.2
.81	7.80	Air	.61	.615	0.8
.81	7.825	Air	.675	.709	4.8
.81	7.855	Air	.795	.840	5.3
1.62	7.45	Air	0.256	0.268	4.4
1.62	7.52	Air	.40	.402	0.4
1.62	7.59	Air	.585	.616	5.0
1.62	7.70	Air	1.13	1.154	2.0
1.62	7.76	Air	1.70	1.599	6.3
2.43	7.465	Air	0.61	0.575	6.4
2.43	7.48	Air	.69	.624	10.5
2.43	7.51	Air	.775	.749	3.4
2.43	7.51	Air	.755	.749	0.8
2.43	7.51	Air	.755	.749	0.8
2.43	7.55	Air	.97	.947	2.6
2.43	7.63	Air	1.45	1.500	3.2
2.43	7.67	Air	1.96	1.881	4.4
2.43	7.73	Air	2.54	2.61	2.7
2.43	7.85	Air	5.25	5.05	4.1
2.43	7.95	Air	8.85	8.62	2.7
2.43	8.05[a]	Air	14.7	14.57	0.5
2.43	8.14[a]	Air	25.8	23.7	9.1
2.43	7.045	O_2	0.160	0.162	0.9
2.43	7.17	O_2	.413	.398	3.9
2.43	7.26	O_2	.76	.737	3.1
2.43	7.325	O_2	1.00	1.132	11.5
2.43	7.365	O_2	1.37	1.475	7.1
2.43	7.395	O_2	1.77	1.768	0.3
2.43	7.41	O_2	1.78	1.945	8.3
2.43	7.45	O_2	2.45	2.51	2.2
2.43	7.49	O_2	3.45	3.16	9.1
2.43	7.51	O_2	3.40	3.56	4.5
2.43	7.51	O_2	3.67	3.56	3.1
2.43	7.51	O_2	3.45	3.56	3.2
2.43	7.51	O_2	3.63	3.56	2.0
2.43	7.51	O_2	3.67	3.56	3.1
3.24	7.415	Air	0.595	0.660	9.8
3.24	7.49	Air	.995	1.035	3.8
3.24	7.55	Air	1.315	1.477	11.0
3.24	7.59	Air	1.84	1.860	0.9
3.24	7.65	Air	2.54	2.62	2.8
3.24	7.72	Air	3.68	3.81	3.4
4.05	7.33	Air	0.56	0.546	2.7
4.05	7.43	Air	1.045	1.008	3.7
4.05	7.50	Air	1.56	1.524	2.5
				Mean dev.	4.4

[a] A trace of brown coloration was briefly observed in the run at *p*H 8.05, and more definitely in the run at *p*H 8.14. Because of the very large extinction coefficient of the brown species, the concentrations corresponding to this absorption are quite small (*cf*. Table III). The run at *p*H 8.14 was, however, omitted in the statistical analysis of the data of this table.

What was most unexpected about the data of Table II and Fig. 4 is the strong dependence on *p*H, the apparent order with respect to $[OH^-]$ being about 2.5. Only a small part of this *p*H dependence can be accounted for by the effect of *p*H on the initial equilibrium extent of formation of $Co(GG)_2$, the factor f varying roughly as $[OH^-]^{1/2}$. Hence it must be concluded that hydroxyl ions are specifically involved in the reaction. Presumably, therefore, $Co(GG)_2$ is incapable of reaction with molecular O_2 and the forms $Co(GG)_2OH^-$ or $Co(GG)_2(OH)_2^=$ must be the reactive forms. The only logical structure for the reaction intermediate indicated by the fractional reaction order is then that given by the structure II.



II

Numerous other structures were considered, but had to be rejected as incompatible with the results here reported.

Mechanism and Rate Law.—The possible initial steps of the reaction are therefore

$$Co(GG)_2OH^- + O_2 \overset{k_1}{\underset{k_2}{\rightleftharpoons}} Co(GG)_2OH{\cdot}O_2^- \quad (a)$$

$$Co(GG)_2(OH)_2^= + O_2 \overset{k_3}{\underset{k_4}{\rightleftharpoons}} Co(GG)_2OH{\cdot}O_2^- + OH^- \quad (b)$$

The product $Co(GG)_2OH{\cdot}O_2^-$ contains an odd electron and would be very unstable. It would react further to form the binucleate complex II previously designated as a likely structure for the principal intermediate in the reaction. This complex may again be formed in two ways

$$Co(GG)_2OH{\cdot}O_2^- + Co(GG)_2OH^- \overset{k_5}{\underset{k_6}{\rightleftharpoons}} II \quad (c)$$

$$Co(GG)_2OH{\cdot}O_2^- + Co(GG)_2(OH)_2^= \overset{k_7}{\underset{k_8}{\rightleftharpoons}} II + OH^- \quad (d)$$

It was shown by Fig. 1 that in the low *p*H region the rate of formation of the final red product is equal to the rate of oxygen uptake. The form II is therefore presumably also unstable and decays at once to the final product. Since this product is so unusually stable, the final reaction must be essentially irreversible. The reaction presumably involves the formation of a second Co–Co bridge, and is therefore likely to be slow. It may also very well involve a hydrogen ion, since elimination of at least one OH^- ion is required. One may therefore write two reactions.

$$II \xrightarrow{k_9} \text{product} + OH^- \text{ (or } 2\ OH^-) \quad (e)$$

$$II + H^+ \xrightarrow{k_{10}} \text{product (possibly} + OH^-) \quad (f)$$

The results at high *p*H will show that only reaction *f* will be appreciably rapid in the *p*H region here under consideration.

By applying a steady state treatment to the two unstable forms, $Co(GG)_2OH{\cdot}O_2^-$ and II, and substituting equations 1 and 2 for the concentrations of the initial reactive species, the following rate equation is easily obtained[14]

$$\frac{\mathrm{d(product)}}{\mathrm{d}t} = \frac{K_3{}^2K_{\mathrm{O}_2}k_1k_5}{k_2}\,\frac{f^2[\mathrm{Co}]_\mathrm{T}{}^2[\mathrm{OH}^-]^2P_{\mathrm{O}_2}}{\left\{\dfrac{1 + \gamma[\mathrm{OH}^-] + \beta\gamma[\mathrm{OH}^-]^2}{1 + \beta[\mathrm{OH}^-]}\right\} + \dfrac{K_3k_5}{k_2}\,\dfrac{f[\mathrm{Co}]_\mathrm{T}[\mathrm{OH}^-]}{1 + \alpha[\mathrm{OH}^-]}} \quad (3)$$

where K_{O_2} is the solubility constant for oxygen, giving the molar concentration in equilibrium with a partial pressure, P_{O_2}, above the solution. The parameters α, β and γ are equal to[15]

$$\begin{aligned} \alpha &= K_4k_3/k_1 = k_4/k_2 \\ \beta &= K_4k_7/k_5 = k_8/k_6 \\ \gamma &= k_6/K_\mathrm{w}k_{10} \end{aligned} \quad (4)$$

where K_w represents the concentration product, $[\mathrm{OH}^-][\mathrm{H}^+]$. Mathematically, equation 3 has five arbitrary parameters, three of which, α, β and γ, are involved solely in describing the effect of $[\mathrm{OH}^-]$ on the reaction rate. The experimental data are not sufficiently accurate to permit the evaluation of so many parameters. Accordingly, equation 3 has been reduced to one of three parameters only by reducing the number solely involving $[\mathrm{OH}^-]$ to one. Two such equations may be written as

$$\frac{\mathrm{d(product)}}{\mathrm{d}t} = \frac{Lf^2[\mathrm{Co}]_\mathrm{T}{}^2[\mathrm{OH}^-]^2P_{\mathrm{O}_2}}{1 + M[\mathrm{OH}^-] + Nf[\mathrm{Co}]_\mathrm{T}[\mathrm{OH}^-]} \quad (5)$$

and

$$\frac{\mathrm{d(product)}}{\mathrm{d}t} = \frac{Lf^2[\mathrm{Co}]_\mathrm{T}{}^2[\mathrm{OH}^-]^2P_{\mathrm{O}_2}}{1 + M[\mathrm{OH}^-] + Qf[\mathrm{Co}]_\mathrm{T}} \quad (6)$$

In these equations the term in brackets in equation 3 has been replaced by $M[\mathrm{OH}^-]$. In equation 5 it has been assumed that $\alpha[\mathrm{OH}^-] \ll 1$; in equation 6 that $\alpha[\mathrm{OH}^-] \gg 1$. It can be seen that

$$\begin{aligned} L &= \frac{K_3{}^2K_{\mathrm{O}_2}k_1k_5}{k_2} = \frac{K_3{}^2K_4K_{\mathrm{O}_2}k_3k_5}{k_4} \\ N &= \frac{K_3k_5}{k_2} \\ Q &= \frac{K_3k_5}{k_4} \end{aligned} \quad (7)$$

Equations 5 and 6, and several additional equations derivable from equation 3 with different assumptions were applied to the initial rate data shown in Table II. Best values for the arbitrary parameters were found by a least squares method (minimizing the *relative* difference in rate). The average deviation between computed and observed initial rates was then calculated. It was found that equation 6 gave the best fit of the data, the average deviation between computed and experimental rates, using this equation, was only ±4.4% (*cf.* Table II). A better agreement could hardly have been expected. The reproducibility of most of the rate measurements was about 2 or 3%: for the fastest runs, the limiting initial slopes could be measured with a precision of only about 5%. The value of $[\mathrm{OH}^-]$ was estimated from *p*H measurements,[14] which, being correct to only 0.01 *p*H unit, introduce an error of 5% into the value of $[\mathrm{OH}^-]^2$ appearing in the numerator of equation 6.

The average deviation in the case of equation 5, with the best values of L, M and N, was about 8%. Several other equations were tried, none of which were able to describe the experimental data with the accuracy of equation 6.

The following were the best values of L, M and Q found for equation 6: $L = 2.045 \times 10^{13}$, $M = -2.39 \times 10^5$, $Q = 3.60 \times 10^2$, these values applying to concentration units of moles/liter with P_{O_2} in atmospheres.[16]

From these constants it is possible to calculate an approximate value for the rate constant k_3. Since, by equations 7, $L/Q = K_3K_4K_{\mathrm{O}_2}k_3 = 5.7 \times 10^{10}$, and since $K_3K_4 \sim 10^9$, and $K_{\mathrm{O}_2} = 9.2 \times 10^{-4}$ moles/liter/atm.[17] one obtains for k_3 the value of $\sim 6 \times 10^4$ (moles/liter)$^{-1}$ min.$^{-1}$.

Placing $K_3 \sim 3 \times 10^4$, one obtains from the value of Q(or L): $k_5/k_4 = 0.012$. Individual values for k_5, k_4 and other rate constants cannot be obtained without further information.

Final Stages of the Reaction.—To prove the validity of equation 6 it is necessary to demonstrate that it describes not only the observed variation of initial rate with initial concentrations, but also the progress of the reaction to its completion. This is especially necessary since the observed progress of the reaction, especially at the higher *p*H limit of the range here under discussion, undergoes a sharp break after about 80–90% completion. Accordingly, equation 6 was integrated to give the extent of reaction as a function of time

$$Qf \ln\frac{a}{a - x} + (1 + M[\mathrm{OH}^-])\left(\frac{1}{a - x} - \frac{1}{a}\right) = 4Lf^2[\mathrm{OH}^-]^2p_{\mathrm{O}_2}t \quad (8)$$

where $2a$ is the initial total concentration of cobalt, and x is the concentration of red product at time t. The factors p_{O_2} and $[\mathrm{OH}^-]$ are constants throughout the reaction; f is very nearly constant (Fig. 3), and has been assumed constant in the integration.

Figure 5 shows that this equation, with the values of L, M and Q obtained from initial rate measurements, does indeed fit the experimental data through the entire course of the reaction.

The Effect of Ionic Strength.—Figure 6 shows the effect of ionic strength on the reaction rate under otherwise identical conditions, *p*H 7.50, $[\mathrm{Co}]_\mathrm{T} = 2.43 \times 10^{-3}\,M$, in equilibrium with air. A theoretical computation of the value of the ionic strength effect at the limit of $\mu = 0$ can be made in the usual

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[14] All rate equations are given in terms of concentrations. However, *p*H meter readings have been used directly as a measure of $[\mathrm{H}^+]$ and $[\mathrm{OH}^-]$; *i.e.*, we have used the relations $[\mathrm{H}^+] = 10^{-p\mathrm{H}}$ and $[\mathrm{OH}^-] = 10^{p\mathrm{H}-14.00}$. In actual fact these quantities differ from the ion concentrations by factors involving the activity coefficients of the ions and the liquid junction potentials in the *p*H measuring system. At constant ionic strength, however, these factors are likely to be constants, and may be incorporated in the appropriate rate constants: *i.e.*, k_4, k_8 and k_{10} each contain a factor $1/\gamma'$, which, as a rough approximation, may be set equal to the reciprocal of the activity coefficient of a single univalent ion. Similarly, the concentration equilibrium constants K_3 and K_4 each contain a factor $1/\gamma'$ and the constant $K\mathrm{w}$ contains a factor $(\gamma')^2$.

[15] The equations $K_4k_3/k_1 = k_4/k_2$ and $K_4k_7/k_5 = k_8/k_6$ arise from the fact that identical equilibrium conditions must apply to the formation of $\mathrm{Co(GG)_2OH{\cdot}O_2}^-$ and of II, independent of the path of reaction.

[16] The *negative* value of M implies that the predominant term in the bracketed expression in the denominator of equation 3 is $1 + \beta[\mathrm{OH}^-]$, *i.e.*, it implies that $\beta > \gamma$.

[17] Calculated from appropriate solubility data given by A. Seidell, "Solubilities of Inorganic and Metal Organic Compounds," Vol. 1, 3rd ed., D. Van Nostrand Book Co., New York, N. Y., 1940.

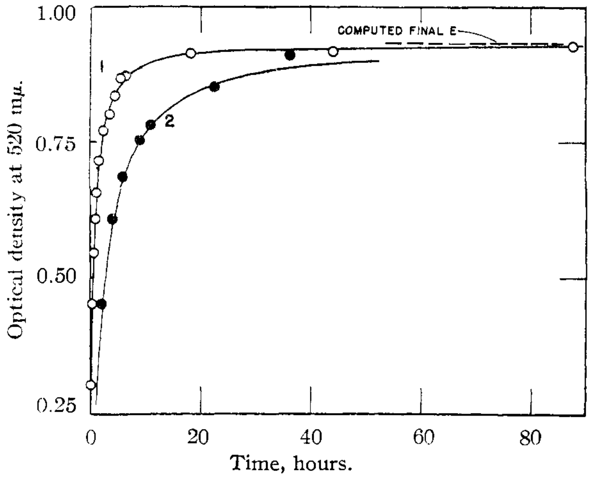

Fig. 5.—Final stages of the reaction at low *p*H. The circles represent experimental points; the curves are computed from equation 8, with the constants obtained from *initial* rate studies. Curve 1 and open circles: 0.00243 *M* Co^{++}, 0.0757 *M* GG, *p*H 8.14, μ = 1.0, 25°. Curve 2 and solid circles: same conditions, but at *p*H 7.89.

way by means of the limiting Debye–Hückel expression, which for the activity coefficient, γ, of any ion, is of the form, $\log \gamma = -0.5\, Z\sqrt{\mu}$, at 25° in water, where Z is the charge of the ion. Using this expression, one finds that the concentration equilibrium constants K_H, K_1, K_2, K_3 and K_w, and the rate constants k_4, k_5, k_7, k_8 and k_{10} all depend on ionic strength, in addition to which there is an ionic strength effect on the factors discussed in footnote 18. Using equations 7, one can then estimate the dependence of f, L and Q on ionic strength. The result of such an estimate is that the denominator of equation 6 should be essentially independent of ionic strength. The reaction rate is therefore influenced principally by the effect of ionic strength on the numerator. The predicted effect is that, at the limit of $\mu = 0$, $d \log \text{rate}/d\mu^{1/2} \simeq 1.5$. Figure 6 is in agreement with this prediction to the extent that the rate increases with ionic strength. The observed value of $d \log \text{rate}/d\mu^{1/2}$ is, however, about 0.5, rather than 1.5. This is at least partly due to the fact that the ionic strength region covered is well outside the limits of applicability of the Debye–Hückel law.

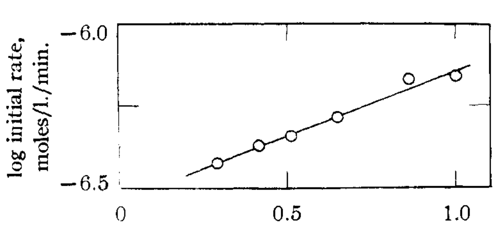

Fig. 6.—The effect of ionic strength on the initial rate at low *p*H (0.00243 *M* Co^{++}, 0.0757 *M* GG, *p*H 7.49, in air, ionic strength adjusted with KNO_3).

Rate Studies at High *p*H

The experimental results in the low *p*H region required that the existence of an unstable intermediate be postulated. This intermediate was assigned the formula II. The reaction mechanism deduced from the low *p*H data indicated that the formation of this intermediate would be greatly accelerated by increasing *p*H. Its rate of disappearance, however, if it goes by reaction *f*, would decrease with increasing *p*H. The intermediate thus becomes increasingly stable with increasing *p*H.

As mentioned in the experimental section, a brown-colored compound, containing the structure Co–O–O–Co, begins to make an appearance in the reaction mixture at *p*H 8. At sufficiently high *p*H (above *p*H 9.7 in the series of experiments at ionic strength 1.0) all of the cobalt is instantaneously converted into this brown compound. This suggests at once the hypothesis that this brown-colored compound is identical with the unstable intermediate of the low *p*H region, *i.e.*, that it, too, is represented by structure II. This conclusion will be confirmed by the data of Table III below.

As was shown in the Experimental section the rate of transformation of the brown species to the red product can be measured by following the light absorption at 350 mμ. Figure 7 shows typical plots of $\log E_{350}$ *vs.* time at *p*H 12 and ionic strength 0.32. The plots are seen to be linear, and the slopes independent of cobalt concentration: the decay of the brown species is therefore a first order reaction. The first-order rate constant is 7.2×10^{-3} min.$^{-1}$.

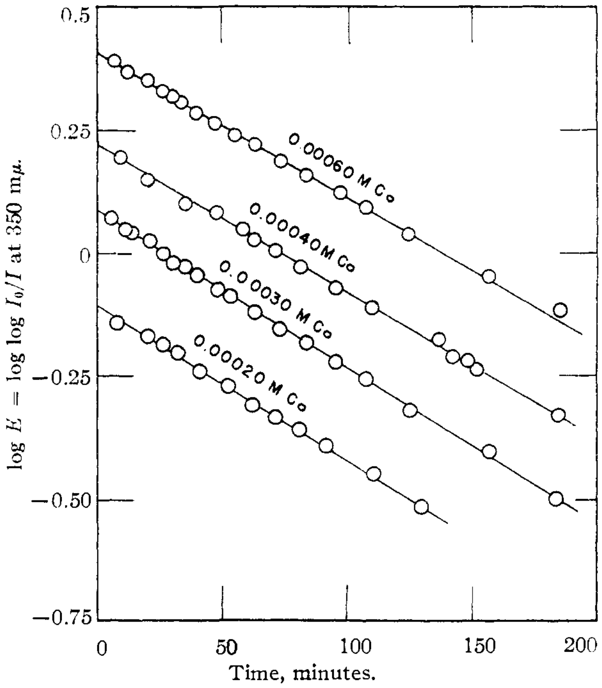

Fig. 7.—Decay of the brown species: typical runs showing the first-order disappearance of the brown color at various cobalt concentrations (0.3 *M* GG, *p*H 11.9–12.0, μ = 0.32, in air).

Figure 8 shows the rate constants obtained from similar plots at ionic strength 1.0, at constant cobalt concentration (2.43×10^{-4} *M*) and varying *p*H and oxygen pressure. The rate constant is seen to be independent of the oxygen pressure, and, above *p*H 11, independent of *p*H. Below *p*H 11, however, the rate constant increases with decreasing *p*H, aproaching a first-order dependence on the hydrogen ion concentration. The rate of transformation can be described adequately by the relation $-\mathrm{d}c/\mathrm{d}t = (1.65 \times 10^{-2} + 1.5 \times 10^{9}\,[\mathrm{H}^+])c$ in moles/liter/min. The data are relatively poor in precision, in part because the solutions in this *p*H range possess only poor buffering capacity.

The rate constant at *p*H 12 at ionic strength 1.0 obtained from Fig. 8 is 1.65×10^{-2} min.$^{-1}$, compared with the value of 7.2×10^{-3} min.$^{-1}$ obtained at ionic strength 0.32. The uncatalyzed decomposition of the brown species is therefore strongly affected by ionic strength, such that d log rate/d$\mu^{1/2} \simeq 0.8$. In view of the identification of the brown species with formula II, this is a reasonable relation, since the uncatalyzed reaction must involve the close approach of the two hydroxyl ions to one another. (The effect of ionic strength on the acid-catalyzed reaction should be in the opposite direction. No data on this question were obtained, because of the difficulty of maintaining constant *p*H in this *p*H region.)

Confirmation of the Identity of the Brown Species with the Low *p*H Intermediate.—Nowhere in this study have we as yet made use of the *p*H-dependence of the initial concentration of the brown species. If our assumption is correct, that the brown species is identical with the low *p*H intermediate, II, then the rate of formation of the red product at any *p*H, including the low *p*H region, must obey the same rate law

$$\frac{\mathrm{d(product)}}{\mathrm{d}t} = (k_9 + k_{10}[\mathrm{H}^+])[\mathrm{II}] = (1.65 \times 10^{-2} + 1.5 \times 10^{9}[\mathrm{H}^+])[\mathrm{II}] \quad (9)$$

This equation shows at once that k_9 is negligible with respect to $k_{10}[\mathrm{H}^+]$ below *p*H 9, as has already been assumed in the derivation of the low *p*H rate equation.

Equation 9 can now also be used to calculate the *p*H-dependence of the initial steady-state concentration of II, *i.e.*, if our assumption is correct, of the brown species, for by equation 9, in the low *p*H region

$$[\mathrm{II}] = \frac{1}{1.5 \times 10^{9}[\mathrm{H}^+]}\,\frac{\mathrm{d(product)}}{\mathrm{d}t} \quad (10)$$

Table III shows the appropriate calculations, using experimental initial rates from Table II. The anticipated optical density at 350 mμ, indicative of the intensity of brown color to be observed, begins to exceed the intensity of the initial pink coloration (E_{520} mμ ~0.04) at *p*H 8.0. At and above this *p*H a brown color should be observed.

TABLE III

IDENTITY OF BROWN SPECIES WITH LOW *p*H INTERMEDIATE

Initial $[\mathrm{Co}]_\mathrm{T} = 2.43 \times 10^{-3}$, air oxidation, $\mu = 1.0$

*p*H	Obsd. rate, moles/l./min. × 10^6	[II] = [Brown] by eq. 10 × 10^6	Anticipated opt. dens. at 350 mμ; E = 8000 [II]
7.55	0.97	0.23	0.002
7.73	2.54	.91	.007
7.95	8.85	5.2	.042
8.05	14.7	10.0	.080
8.14	25.8	23.9	.191

This agrees exactly with our experimental observations (*cf.* Table II).

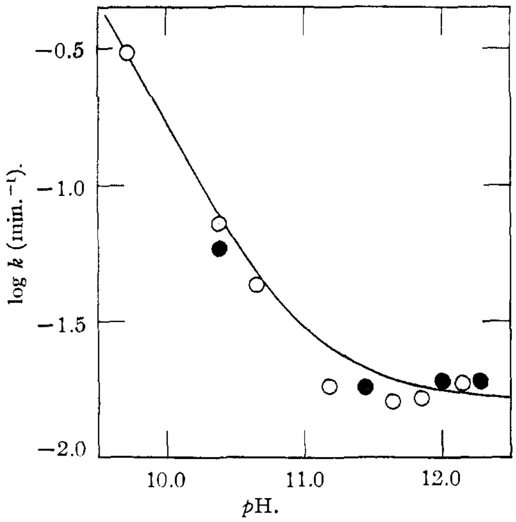

Fig. 8.—Effect of *p*H on the first-order decomposition of the brown species: runs in air (○) and oxygen (●); 0.000243 *M* Co^{++}, $\mu = 1.0$. The curve represents the equation $k = 1.65 \times 10^{-2} + 1.5 \times 10^{9}\,[\mathrm{H}^+]$.

Reversibility.—Whereas the formation of the red product is irreversible, that of the brown complex is readily reversed by the addition of acid. It was of particular interest to observe what would happen when a freshly prepared solution of the brown complex was brought to within the range of *p*H 7 to 8.[18] For in this *p*H range the red product is the stable final form, and it might be formed in two ways: directly by reaction *f*, or indirectly, by a reversal of reactions *a* to *d*, followed by the slow conversion of the unoxidized cobalt. Our experiments showed that about 20% of the reaction goes by the direct path, about 80% of the cobalt being returned to the divalent state, and subsequently oxidized at a rate appropriate to the final *p*H and cobalt concentration, as given by equation 6. These percentage figures are independent of the final *p*H, indicating that the choice of paths occurs during mixing, before the final *p*H is attained, presumably near *p*H 8.

Thus, near *p*H 8, $k_6 + k_8\,[\mathrm{OH}^-] \simeq 4\,k_{10}[\mathrm{H}^+]$, a result from which a minimum value may be computed for the constant β. Introduction of the expressions for β and γ from equation 4 gives $\gamma(1 + \beta\,[\mathrm{OH}^-]) \simeq 4[\mathrm{H}^+]/K_\mathrm{w}$. It was shown earlier[16] that $\beta > \gamma$ so that $\beta(1 + \beta[\mathrm{OH}^-]) > 4[\mathrm{H}^+]/K_\mathrm{w}$. Substituting $[\mathrm{OH}^-] = [\mathrm{H}^+] = 10^{-7}$ and $K_\mathrm{w} = 10^{-14}$, this shows that $\beta > 1.5 \times 10^{6}$. Since $K_4 \sim 3 \times 10^{4}$ or somewhat smaller, therefore from equation 4, $k_7/k_5 > 50$, *i.e.*, $\mathrm{Co(GG)_2(OH)_2^{=}}$ is apparently much more reactive than $\mathrm{Co(GG)_2OH^-}$.

This same conclusion is reached from the fact that the best fit of the experimental data at low *p*H was obtained by equation 6 rather than equation 5, *i.e.*, with the assumption that $\alpha[\mathrm{OH}^-] \gg 1$ between *p*H 7 and 8, for this implies that $k_3/k_1 \gg 100$.

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(18) This was accomplished by preparing the brown complex at *p*H 11 in approximately 0.01 *M* concentration, and then diluting at once by adding an aliquot to a GG solution in the range of *p*H 7 to 8. Because of the superior buffering capacity of GG in this range, a final *p*H between 7 and 8 was obtained.

It was pointed out earlier that the spectrum of the brown complex is qualitatively of the charge-transfer type. If charge-transfer forces contribute to the formation of the peroxo bridge, therefore, the present results suggest that these forces are much stronger with two OH^- ions in the coördination sphere of Co^{++} than if only one OH^- ion is present.

These conclusions do not imply that reactions *a* and *c* are unimportant in the formation of II, but, rather, that reactions *b* and *d* are at least equally important as reactions *a* and *c*, despite the fact that the equilibrium concentration of $Co(GG)_2(OH)_2^{=}$ must be much smaller than that of $Co(GG)_2OH^-$.

Changes in *p*H.—Some of the experiments in the unbuffered range of *p*H 10 to 11 were conducted with a view to measuring *p*H changes, rather than light absorption. The solutions were prepared in the complete absence of oxygen, and their *p*H values measured. Oxygen was then bubbled through the solutions, and the usual brown color rapidly was formed. During this process a sharp drop in *p*H was observed. As the brown color was transformed to the red product, however, an accompanying *p*H increase was found. Qualitatively, this result is in accord with the mechanism proposed in this paper, for the formation of II requires the absorption of OH^- ions from the solution, and these are released again in the transformation to I.

Acknowledgment.—The authors wish to express their appreciation to Dr. George Kalnitsky for providing the apparatus for measurement of oxygen absorption, and to Dr. Y. Kobayashi for assistance in its use.

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The Photolysis of Biacetyl

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RECEIVED JANUARY 25, 1954

The vapor phase photochemical decomposition of biacetyl has been studied over a wide range of temperature, wave length, concentration and light intensity. Quantum yields are reported for the products, carbon monoxide, ethane, methane and acetone, all of which were determined mass spectrometrically. Ketene and possibly 2,3-pentanedione were found as products. Some photolyses were conducted in the presence of iodine vapor. In these cases the products were carbon monoxide, methyl iodide and a small quantity of acetone. The results may be explained by assuming two primary processes and secondary reactions involving free radicals. The importance of the various secondary reactions changes considerably with temperature and under the influence of iodine as a free radical trap. Revised values of 7.7 and 6.6 kcal./mole are reported for the activation energies of the acetone and methane forming reactions, respectively, both of which involve a methyl radical attack on the biacetyl molecule. At room temperature the primary quantum yield is related to the sum of the ethane and acetone quantum yields. At temperatures of 100° and above, the primary quantum yield for the uninhibited photolyses is shown to be equal to the ethane quantum yield, and that for the iodine inhibited photolyses is shown to be equal to one-half the quantum yield of carbon monoxide. All values of the primary quantum yield are much less than unity.

The photochemical decomposition of biacetyl has been studied in the second absorption region.[1] The preliminary results, obtained at 2654 Å., and a mechanism based on those results were presented in a previous report.[2] Additional data, including further evidence in support of the proposed mechanism, have been obtained by conducting the photolysis over a range of temperature, wave length, concentration and intensity and also by conducting the photolysis in the presence of iodine vapor. Iodine is an effective radical trap and inhibits normal secondary reactions. Experiments in which iodine was added for this purpose are designated herein as iodine inhibited photolyses.

Experimental

Apparatus and Procedure.—The apparatus and experimental procedure used in these experiments were essentially as described previously.[2] The reaction system consisted of a cylindrical quartz reaction cell, 30 mm. inside diameter and 20 cm. in length, and two precision stopcocks, all of which were mounted in an air thermostat of such construction that it could be heated to 200°. The reaction system was joined to a biacetyl supply and to a gas train which was utilized to fractionate the products at the end of a run.

Monochromatic light was supplied by a constant pressure mercury arc and a crystal quartz monochromator. Intensities were measured at all temperatures by means of a photocell–thermopile combination. The desired vapor pressure was obtained by observing the photocell response. In no case was more than 2% of the vapor photolyzed. At the end of an experiment, the excess biacetyl and products were fractionated in the gas train, and the residual biacetyl was condensed in a trap at Dry Ice temperature. The trap was removed from the system and the contents weighed; the true concentration of biacetyl was calculated from this weight and the volume of the reaction system. The weight of the products condensed at −80° and the weight of the biacetyl lost in the fractionation were negligible.

For the iodine-inhibited photolyses, a trap for introducing the iodine and a magnetic pump for circulating the biacetyl and iodine vapors were added to the reaction system inside the air thermostat. The procedure for the iodine work was similar to that described by others.[3] Extra precautions were take to ensure that the reaction system was anhydrous and free of mercury vapor. Iodine vapor concentrations were determined from the weight of iodine introduced into the system. During the fractionation at the end of a run, the excess iodine and biacetyl both condensed at −80°. The quantity of biacetyl was obtained by subtracting the weight of iodine from the weight of the condensable fraction.

Appreciable absorption by the iodine vapor, with a maximum at 2804 Å., was observed. Others also have observed an absorption spectrum for iodine in this region.[4-6] This absorption by the iodine was assumed not to affect the quantum yield calculations because the cell-empty intensities were measured behind the reaction cell after vaporiza-

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(1) The first absorption region of biacetyl extends from about 4600 to 3500 Å., and the second from 3200 to below 2200 Å.

(2) F. E. Blacet and W. E. Bell, *Faraday Soc. Disc.*, **14**, 70 (1953).

(3) F. E. Blacet and J. Heldman, THIS JOURNAL, **64**, 889 (1942).

(4) A. Coehn and K. Stuckardt, *Z. physik. Chem.*, **91**, 722 (1916).

(5) W. C. Price, *Phys. Rev.*, **48**, 477 (1935).

(6) D. T. Warren, *ibid.*, **47**, 1 (1935).