Rate Studies on the Anaerobic Degradation of Ascorbic Acid IV

Catalytic Effect of Metal Ions

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The effect of different metal ions on the rate of the anaerobic degradation of ascorbic acid in aqueous solution has been studied. Among the bivalent metal ions tested, Pb^{2+} was the most powerful catalyst, followed by Zn^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+}, and Mg^{2+}. Also the trivalent metal ions Al^{3+} and Cr^{3+} catalyzed the process, Al^{3+} being more active than Cr^{3+}. The experimental results seem to indicate that the metal ions form complexes with ascorbic acid.

ALTHOUGH the catalytic effect of metals on the oxidative degradation of ascorbic acid has been well studied (1–6), no papers have appeared dealing with the influence of metals on the anaerobic degradation. Since the degradation of ascorbic acid in many liquid pharmaceutical preparations appears to follow largely the non-oxidative route, and since these preparations may contain or may be contaminated by metals, a study of the effect of metal ions on the rate of the anaerobic degradation of ascorbic acid in aqueous solution was felt necessary.

Several authors (7–11) have shown that ascorbic acid is decarboxylated when heated in aqueous solution under anaerobic conditions. It is known from other papers that metal ions may catalyze the decarboxylation of keto-acids (12, 13). By the decarboxylation of dimethyloxaloacetic acid in the presence of heavy metals a complex between the metal ion and the diion of the acid is formed and this complex undergoes a rapid decarboxylation (12).

EXPERIMENTAL

Materials.—The following metal salts were used in this study: $Ca(NO_3)_2$, $MgSO_4$, $NiSO_4$, $MnSO_4$, $Mn(NO_3)_2$, $FeSO_4$, $CoCl_2$, $ZnSO_4$, $Zn(NO_3)_2$, $Pb(CH_3COO)_2$, $Pb(NO_3)_2$, $CuSO_4$, $AlCl_3$, and $CrCl_3$. Solutions of the metal salts were standardized when necessary with sodium ethylenediaminetetraacetate.

The ascorbic acid used was of P. Nord. (Pharmacopoea Nordica) quality. The metal salts and all reagents were of analytical grade. The water used was distilled water redistilled from a neutral glass still, boiled, and cooled under oxygen-free nitrogen.

Assay.—The residual ascorbic acid concentration of the heated solutions was determined iodometrically. An aliquot part of the sample, usually 5.00 ml., was acidified with diluted sulfuric acid and titrated under nitrogen with 0.01 *N* iodine using a few drops of starch T.S. as indicator. The metal salts added did not interfere.

Kinetic Studies.—Quantities of 0.01 *M* ascorbic acid solutions, buffered or unbuffered, containing the actual metal salt, and a sufficient amount of sodium chloride or potassium nitrate[1] to give the solutions an ionic strength of 0.5, were prepared. The solutions were filled into 5-ml. ampuls, and the air in the ampuls was replaced by nitrogen. The ampuls were sealed and heated at 96° in a constant-temperature bath. At appropriate intervals ampuls were taken out from the bath, cooled on ice, and the solution analyzed.

RESULTS AND DISCUSSION

Order of Reaction with Respect to Ascorbic Acid.—The anaerobic degradation of ascorbic acid in the presence of each of the metal salts tested was found to be strictly first order with respect to ascorbic acid.

Catalytic Effect of Bivalent Metal Ions on the Anaerobic Degradation of Ascorbic Acid.—Figure 1 shows the effect of Mn^{2+}, Fe^{2+}, Co^{2+}, Zn^{2+}, and Pb^{2+} on the rate of the anaerobic degradation of ascorbic acid at pH 2–6. (Amount of metal salt added, 0.05 moles/L.; buffer, 0.3 *M* acetate.) The effect of Ni^{2+}, Ca^{2+}, and Mg^{2+} was also tested, but the results for these cations are omitted in Fig. 1. Ni^{2+} showed very close to the same effect as Mn^{2+}. Ca^{2+} and Mg^{2+} had virtually no effect.

No rate studies were carried out at pH >6, because most metals caused precipitations in the ascorbic acid solutions at pH >6.

Experiments with Cu^{2+} as catalyst showed that ascorbic acid at pH >2 was oxidized by this metal even in the absence of air. At pH 0.4 addition of 0.01 moles/L. of $CuSO_4$ had a slight catalytic effect on the anaerobic degradation of ascorbic acid.

Figure 1 shows that all active metals have the highest effect at pH 4–6. Because of this all further studies of the catalytic effect of the different metals were made at pH values within this region.

The effect of addition of different amounts of

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[1] Potassium nitrate was used in experiments involving lead because of the low solubility of lead chloride. In experiments involving the other metals tested sodium chloride was used, if not otherwise stated.

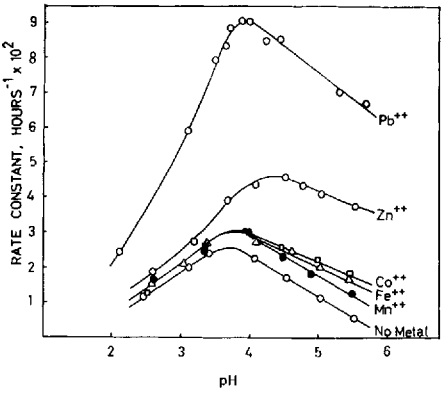

Fig. 1.—Effect of bivalent metal ions on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at different pH values and 96°. Metal salts used: $MnSO_4$, $FeSO_4$, $CoCl_2$, $ZnSO_4$, and $Pb(CH_3COO)_2$. Amount of metal salt added, 0.05 moles/L.; buffer, 0.3 *M* acetate; ionic strength, 0.50.

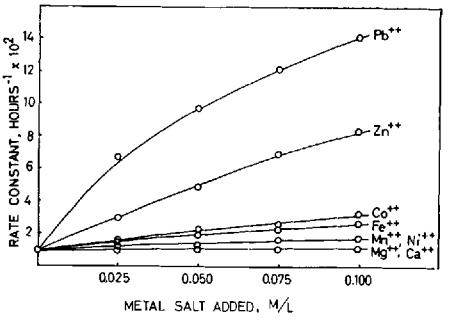

Fig. 2.—Effect of bivalent metal ions on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at pH 5 and 96°. Metal salts used: $Ca(NO_3)_2$, $MgSO_4$, $NiSO_4$, $MnSO_4$, $FeSO_4$, $CoCl_2$, $ZnSO_4$, and $Pb(CH_3COO)_2$. Buffer, 0.1 *M* acetate; ionic strength, 0.50.

bivalent metal salts on the rate of the anaerobic degradation of ascorbic acid at pH 5 (0.1 *M* acetate as buffer) is shown in Fig. 2. The results given for Zn^{2+} in Fig. 2 are from two parallel series of runs, one series made with NaCl, the other with KNO_3 for correction of ionic strength. There was no difference in the results from the two series of runs.

Since acetate may form complexes with metal ions, the two most active metals, lead and zinc, and one of the less active metals, manganese, were also tested in buffer-free solutions. The nitrates of the metals were used because nitrate ions have a very low tendency to form complexes with metal ions. For comparison lead was also tested as acetate and zinc as sulfate. The studies were made at pH 4. This pH value was chosen because ascorbic acid has such a buffer capacity at pH 4 that no addition of buffer was needed to keep the pH of the ascorbic acid solutions constant during the experiments.

The solutions were made up to an ionic strength of 0.5 with KNO_3. The results are found in Fig. 3. It will be seen that the nitrate of lead is a little more active than the acetate. The reason for this must

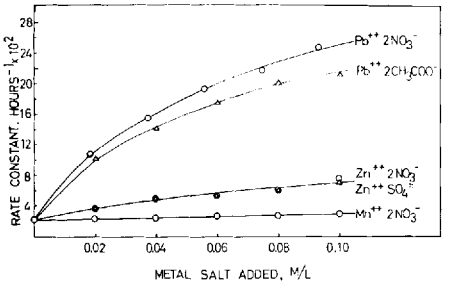

Fig. 3.—Effect of different salts of bivalent metals on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at pH 4 and 96°. Ionic strength, 0.50; no buffer added.

be that the lead ions in the acetate are partly bound as complex. The nitrate and the sulfate of zinc have the same effect. Manganese nitrate is nearly noncatalytic.

Most of the lines in Figs. 2 and 3 are slightly curved. This may be explained by assuming a weak complex formation between ascorbic acid (HA) and metal ions (Me^{2+}) and a subsequent degradation of ascorbic acid in these complexes.

The following reactions may be assumed to take place at pH 2–6:

$$HA + Me^{2+} \rightleftharpoons AMe^+ + H^+ \quad \text{Reaction } 1$$

$$HA \xrightarrow{k_1} \text{products}$$

$$A^- \xrightarrow{k_2} \text{products}$$

$$AMe^+ \xrightarrow{k_3} \text{products}$$

The following equations are valid:

$$-\frac{d[A_T]}{dt} = k_1[HA] + k_2[A^-] + k_3[AMe^+] \qquad \text{(Eq. 1)}$$

$$A_T = [HA] + [A^-] + [AMe^+] \qquad \text{(Eq. 2)}$$

$$-\frac{d[A_T]}{dt} = k[A_T] \qquad \text{(Eq. 3)}$$

$$\frac{[AMe^+][H^+]}{[HA][Me^{2+}]} = k' \qquad \text{(Eq. 4)}$$

$$\frac{[A^-][H^+]}{[HA]} = k_a \qquad \text{(Eq. 5)}$$

$$[Me^{+2}] + [AMe^+] = b \qquad \text{(Eq. 6)}$$

In the experiments the amount (b) of metal salt added (in moles per liter) is 2 to 10 times the total ascorbic acid concentration. Since the lines in Figs. 2 and 3 are but *slightly* curved, only a small part of the ascorbic acid has formed complex with the metal ions even at the highest metal salt concentrations. For these reasons it is evident that $[Me^{2+}] \gg [AMe^+]$ in all the experiments. We may, therefore, without introducing any great error, put $[Me^{2+}] = b$ in Eq. 4. Doing so and combining Eq. 4 with Eqs. 1, 2, 3, and 5 give

$$k = \frac{k_1[H^+] + k_2k_a + k_3k'b}{[H^+] + k_a + k'b} \qquad \text{(Eq. 7)}$$

TABLE I.—STABILITY CONSTANTS (k') OF METAL ION ASCORBIC ACID COMPLEXES AND RATE CONSTANTS (k_3) FOR THE DEGRADATION OF ASCORBIC ACID IN THESE COMPLEXES

Metal Ion	pH	$k' \times 10^3$	k_3 hr.$^{-1}$ × 10
Pb^{2+}	4	3.5	4
Zn^{2+}	4	3.8	1.1
Zn^{2+}	6	0.9	1.9

The experiments, the results of which are given in Figs. 2 and 3, are made at constant pH and ionic strength. $[H^+]$ is therefore a constant and Eq. 7 becomes the equation of a hyperbola with k and b as the two variables. Consequently, plots of rate constants found *versus* amount of metal salt added have to yield curved lines.

From the experiments with $Pb(NO_3)_2$ at pH 4 (Fig. 3) k_3 and k' in Eq. 7 could be calculated. The earlier found values for k_1 and k_2 were used. ($k_1 = 8.9 \times 10^{-3}$ hr.$^{-1}$, $k_2 = 5 \times 10^{-3}$ hr.$^{-1}$) (14). The calculation gave $k_3 = 4 \times 10^{-1}$ hr.$^{-1}$ and $k' = 3.5 \times 10^{-3}$. According to this calculation, the rate of degradation of ascorbic acid in the lead complex is about 50 times faster than the rate of degradation of the free ascorbic acid. (Table I.)

Similar calculations based on the experiments with $Zn(NO_3)_2$ at pH 4 (Fig. 3) gave $k_3 = 1.1 \times 10^{-1}$ hr.$^{-1}$ and $k' = 3.8 \times 10^{-3}$.

The stability constant (k') is nearly the same for the zinc–ascorbic acid complex as for the lead–ascorbic acid complex. The rate of degradation of ascorbic acid, however, is considerably faster in the lead complex than in the zinc complex.

In Fig. 4 the relation between amount of $ZnSO_4$ added and rate constant found at pH 6 is given. Addition of buffer proved not to be necessary to keep pH constant during the runs. At pH 6 the term $k_1[H^+]$ in the numerator of Eq. 7 becomes negligible compared to the other terms in the numerator and may be omitted, thus giving

$$k = \frac{k_2 k_a + k_3 k' b}{[H^+] + k_a + k' b} \qquad \text{(Eq. 8)}$$

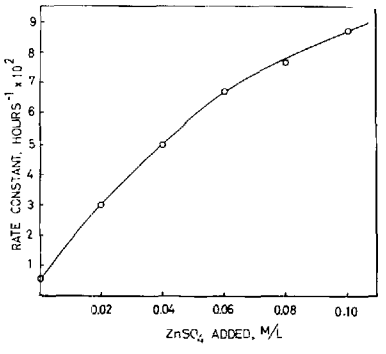

Fig. 4.—Effect of $ZnSO_4$ on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at pH 6 and 96°. Ionic strength, 0.50; no buffer added.

At pH 6 $[H^+] \ll ka_1$, and $[H^+]$ may be omitted:

$$k = \frac{k_2 k_a + k_3 k' b}{k_a + k' b} \qquad \text{(Eq. 9)}$$

Equation 9 may also be written

$$\frac{k - k_2}{k_3 - k} = \frac{k'}{k_a} \cdot b \qquad \text{(Eq. 10)}$$

Inserting the results from the experiments with 0.04 moles/L. and 0.10 moles/L. of $ZnSO_4$ added, gave $k_3 = 1.9 \times 10^{-1}$ hr.$^{-1}$ and $k' = 9 \times 10^{-4}$. These values for k_3 and k' deviate somewhat from the values found at pH 4 ($k_3 = 1.1 \times 10^{-1}$ hr.$^{-1}$ and $k' = 3.8 \times 10^{-3}$). There may be several reasons for these deviations: other reactions than those postulated may occur. Zinc may form basic compounds at pH 6; k_3 may be pH dependent.

In Fig. 5 $k - k_2/k_3 - k$ is plotted *versus* b. In accordance with Eq. 10, a straight line is obtained. The slope of this line is $k'/k_a = 8$.

Experiments with lead similar to those made with

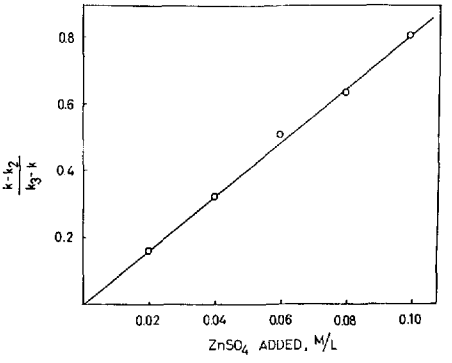

Fig. 5.—Relationship between amount of $ZnSO_4$ added and $k - k_2/k_3 - k$ (*cf.* Eq. 10) by the anaerobic degradation of ascorbic acid at pH 6 and 96° Ionic strength, 0.50; no buffer added.

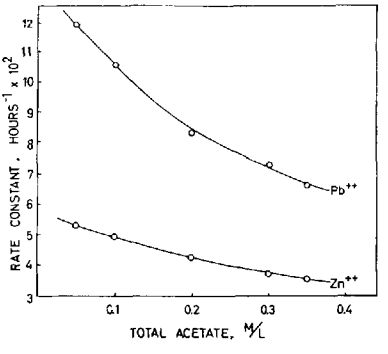

Fig. 6.—Effect of acetate buffer concentration on the pseudo first-order rate constant of the lead and zinc catalyzed anaerobic degradation of ascorbic acid at pH 5 and 96°. A quantity of 0.05 moles of $Pb(CH_3COO)_2$ or $ZnSO_4$ added per liter. Ionic strength, 0.50.

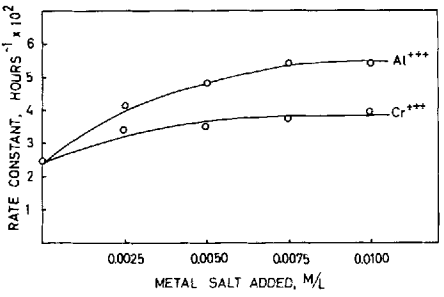

Fig. 7.—Effect of trivalent metal ions on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at pH 4 and 96°. Metal salts used, $AlCl_3$ and $CrCl_3$; buffer, 0.3 *M* acetate; ionic strength, 0.50.

zinc (that is at pH 6 without buffer), were impossible because of the formation of precipitate.

Effect of Acetate on the Lead and Zinc Catalyzed Degradation of Ascorbic Acid.—A series of runs were made keeping pH, ionic strength, and lead concentration constant, but varying the acetate buffer concentration. The results of the runs are found in Fig. 6. There is a decrease in rate with increasing buffer concentration, probably due to an increasing complexation of lead by the buffer. Similar runs made with zinc instead of lead gave similar results (Fig. 6).

Since acetate has such a strong effect on the rates of the lead and zinc-catalyzed degradation of ascorbic acid, Eq. 7, which is applicable only to the process in buffer free solution, cannot be used for calculation of the rate constant at different pH values in solutions containing acetate. It is thus not possible to use Eq. 7 for calculation of a theoretical line fitting the experimental points in Fig. 1.

Attempts to Prove the Formation of Ascorbic Acid Metal Complexes.—According to Reaction *1*, the complex formation between ascorbic acid and metal ions in aqueous solution should cause a decrease in pH. Some experiments were made to test if this was the case.

It became necessary to use more concentrated ascorbic acid solutions than 0.01 *M* to get any measurable change in pH. A 25.00 ml. quantity of 0.1 *M* ascorbic acid solution made up to an ionic strength of 1.5 with KNO_3 and having an initial pH of 2.65, was titrated with 0.5 *M* $Pb(NO_3)_2$ solution to which nitric acid had been added to pH 2.65. The pH of the mixture decreased during the titration and reached a value of 2.29 after addition of 25.00 ml. of 0.5 *M* $Pb(NO_3)_2$ solution. A similar experiment carried out with zinc nitrate instead of lead nitrate gave a decrease in pH from 2.54 to 2.44.

The pH decreases observed may be an indication of complex formation between ascorbic acid and lead and zinc. It is of course not possible from these experiments, which are made at 25° and at an ionic strength of 1.5, to make any calculation of the stability constants of the metal–ascorbic acid complex at 96° and ionic strength of 0.5.

Catalytic Effect of Trivalent Metal Ions on the Anaerobic Degradation of Ascorbic Acid.—The evaluation of the catalytic effect of the trivalent ions Al^{3+} and Cr^{3+} presented some problems. If the aluminum concentration was 0.01 moles/L. and pH >4.1 a precipitate was formed in the ascorbic acid solutions during the runs. At pH 4 (0.3 *M* acetate as buffer) precipitations occurred at aluminum concentrations >0.015 moles/L. At the same time pH dropped 0.2–0.3 units. At aluminum concentrations $\leq$0.01 moles/L. there was no precipitation and the pH drop was only about 0.1 unit. The results of experiments at these low metal salt concentrations are given in Fig. 7. It will be seen that aluminum is a powerful catalyst at pH 4. An addition of 0.005 moles of $AlCl_3$ per liter doubles the rate of the anaerobic degradation of ascorbic acid. The shape of the curve may indicate that the major part of the ascorbic acid exists as a complex at an aluminum concentration of 0.01 moles/L.

At pH 3 (0.3 *M* acetate as buffer) aluminum showed nearly no catalytic effect.

With chromium as catalyst it turned out to be impossible to keep the pH of the ascorbic acid solutions constant during the runs when the chromium concentration was more than 0.02 moles/L. At pH 4 (0.3 acetate as buffer) and chromium concentrations $\leq$0.01 moles/L. there was only a slight decrease in pH (about 0.05 units). The results of runs at this pH are given in Fig. 7. It will be seen that the catalytic effect of Cr^{3+} is less than that of Al^{3+}.

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