Rate Studies on the Anaerobic Degradation of Ascorbic Acid II

Rate of Formation of Carbon Dioxide

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It is shown that the rate of carbon dioxide formation in aqueous solutions of ascorbic acid under oxygen-free conditions closely parallels the loss of the acid from the system at a pH lower than 7. In strongly alkaline solutions, less than one molecule of carbon dioxide seems to be produced from each molecule of the acid. In addition to the rate data, temperature dependencies under varying pH conditions are reported.

THIS PAPER is concerned with the rate of evolution of carbon dioxide during breakdown of ascorbic acid in oxygen-free aqueous solutions. In a previous communication (1), the over-all loss of the acid from these systems was treated. The production of the gas itself aside from the loss of the vitamin is of serious concern in pharmaceutical formulation since it represents a hazard in tightly sealed containers.

Several authors have shown that carbon dioxide is one of the products formed by the decomposition of ascorbic acid. Taylor *et al.* (2) followed the evolution of the gas while heating ascorbic acid in 12% hydrochloric acid in a stream of nitrogen. Carbon dioxide evolved was absorbed in absorption tubes, which were weighed at 0.5 hour intervals. The evolution of carbon dioxide from ascorbic acid rapidly reached 28% by weight, after which it was slow for many hours more, finally attaining a total value in excess of 30%. If only the lactonized carboxyl group supplied carbon dioxide, the evolution should have been 25.0%. The results indicate that the carboxyl group decomposes rapidly and that there is a further slow decomposition evolving more carbon dioxide.

Flores and Brunner (3) tested the stability of partially neutralized 10% ascorbic acid solutions by heating in an autoclave at 120° for 1 hour. Carbon dioxide formed was absorbed in 20% sodium hydroxide solution in a Lunge nitrometer. The authors found the maximum amount of carbon dioxide formed and the maximum decrease in the ascorbic acid concentration at pH 4. As no buffer substances were added to the solutions, there was in all cases a change in pH during the sterilization process.

Huelin (4) studied the anaerobic degradation of ascorbic acid in aqueous solution at pH 2.2–6.0. A maximum rate was found at pH 3–4. Carbon dioxide formed during storage of the ascorbic acid solution in an evacuated Florence flask was determined in the following way. The Florence flask was connected to a second flask containing a measured amount of 0.05 *N* barium hydroxide. Carbon dioxide absorbed in this solution was determined by titration with standard acid. Ascorbic acid solutions (0.25%) kept at 100° for 10 days showed 93% decrease in the ascorbic acid concentration and a change of pH from 3.0 to 7.2. For each mole of ascorbic acid destroyed, 1.05 moles of carbon dioxide were produced. Experiments made at 30° with 0.01 *M* ascorbic acid solutions containing buffers gave the following results. After 104 weeks, the decrease in the ascorbic acid concentration was 55% at pH 2.2, 58% at pH 4.0, and 14% at pH 6.0. The amount of carbon dioxide formed at pH 2.2 was 1.04 ± 0.04 moles per mole of ascorbic acid destroyed; at pH 4.0, it was 0.66 ± 0.03 mole; and at pH 6.0, it was 0.70 ± 0.15 mole. The author concludes that the anaerobic degradation of ascorbic acid to carbon dioxide predominates at high temperatures or acidities, while other reactions become relatively more important with lowering of temperature or acidity.

The scope of the present study was to determine the rate of formation of carbon dioxide by the anaerobic degradation of ascorbic acid at different pH values. By comparing the rate of formation of carbon dioxide with the over-all rate of loss of ascorbic acid, some information about the importance of the carbon dioxide-producing reaction by the anaerobic degradation of ascorbic acid should be obtained.

An additional purpose of this study was to determine the temperature dependency of rate of

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inactivation of ascorbic acid under anaerobic conditions at different pH values.

EXPERIMENTAL

The degradation studies and the determination of the residual ascorbic acid concentration of the heated solutions were performed in the same way as described in a previous paper (1). The amount of carbon dioxide formed when the ascorbic acid solutions were heated at 96° in sealed ampuls was determined according to a method worked out by Finholt and Paulssen (5). This method was based on determination of the carbon dioxide concentration of the water phase in the ampul, using the microdiffusion technique of Conway (6), and calculation of the total amount of the gas in the ampul. When the ascorbic acid solution was so acid that the amount of HCO_3^- in the heated solution was negligible, Eq. 1 was used for the calculation.

$$x = \frac{y}{a} \cdot \frac{T_1 f_1 a + 273b}{T_1 f_1} \qquad \text{(Eq. 1)}$$

where

x = the total amount of CO_2 in the ampul (milligrams),
y = the amount of CO_2 in the water phase (milligrams),
T_1 = the absolute temperature of the ampul,
a = the volume of the water phase (milliliters),
b = the volume of the gas in the ampul (milliliters),
f_1 = the absorption coefficient of CO_2 in the water phase at T_1 °K,
$\frac{y}{a}$ (milligrams of CO_2 per milliliter of the water phase) was determined by the analysis of the water phase, and x was calculated.

When the pH of the ascorbic acid solution was such that the heated solutions contained both CO_2 and HCO_3^-, Eq. 2 was used.

$$x = \frac{T_1 f_1 a(y + z) + 273by}{T_1 f_1 a} \qquad \text{(Eq. 2)}$$

where

x = the total amount of CO_2 and HCO_3^- in the ampul, expressed as milligrams of CO_2,
y = the amount of free CO_2 in the water phase (milligrams),
z = the amount of HCO_3^- in the water phase, expressed as milligrams of CO_2.

$y + z$ (the total amount of CO_2 and HCO_3^- in the water phase) was determined by the analysis of the water phase. Knowing $y + z$, y can be calculated from Eq. 3.

$$\frac{a_{H^+} \cdot z}{y} = ka_1 \qquad \text{(Eq. 3)}$$

where

a_{H^+} = the hydrogen ion activity in the water phase, and
ka_1 = the primary effective dissociation constant of $H_2CO_3 \approx 10^{-6.2}$ at 25° and ion strength 0.5.

The procedure for determination of carbon dioxide was the following. Two-tenths of a milliliter of 1 N sulfuric acid was introduced into the outer chamber of the Conway unit, and 1.00 ml. of 0.1 N barium hydroxide solution was run into the central chamber. The lid, smeared with a mixture of Carbowax 1500 and 1 N sulfuric acid, immediately was placed in position. The lid then was displaced horizontally to provide a small opening into the outer chamber sufficient to allow the introduction of the tip of a pipet containing 5.00 ml. of the sample solution. The fluid was run in quickly and the lid immediately replaced. The contents of the chamber were mixed by rotation and the unit set aside for 120 minutes at room temperature. The fluid in the central chamber then was titrated quickly under stirring with a magnetic stirrer. A blank was run.

TABLE I.—SOLUTIONS USED AS SOLVENT FOR ASCORBIC ACID

	Soln.	pH at 20° C.	pH at 96° C.
1	Perchloric acid, 0.50 N	...	0.38
2	Phosphate buffer, 0.10 M	1.78	2.10
3	Acetate buffer, 0.10 M	3.92	4.10
4	Phosphate buffer, 0.15 M	5.40	5.40
5	Phosphate buffer, 0.15 M	7.20	7.20
6	Sodium hydroxide, 0.03 N	...	10.18
7	Sodium hydroxide, 0.25 N	...	11.10
8	Sodium hydroxide, 0.50 N	...	11.40

The absorption coefficient of carbon dioxide in the water phase of the ampuls depended on the electrolyte concentration of the solution used as the solvent for ascorbic acid. Table I shows the composition of the solutions used. Sodium chloride was added to solutions *2–5* to ionic strength 0.5.

The absorption coefficient of carbon dioxide in solution *1* (0.50 N perchloric acid) is, according to Markham and Kobe (7), equal to the absorption coefficient of carbon dioxide in water ($f = 0.878$ at 20°). Since in solutions *2* and *3* sodium chloride amounted to more than 90% of the total electrolyte contents, the absorption coefficient of carbon dioxide in 0.5 M sodium chloride at 20° was used ($f = 0.750$). In solution *4*, sodium chloride did not amount to more than about 60% of the total electrolyte contents. The absorption coefficient of carbon dioxide in this solution therefore was determined experimentally. Ampuls were filled with solution *4*, and a known amount (about 2 mg.) of sodium bicarbonate was added to each ampul. The ampuls were sealed at once, heated, and stored overnight to obtain equilibrium between carbon dioxide in the gas phase and carbon dioxide in the aqueous phase of the ampul. The total amount of carbon dioxide and bicarbonate in the water phase ($y + z$ in Eq. 2) was determined. Knowing the total amount of carbon dioxide and bicarbonate in the ampul (x in Eq. 2) and the pH of the buffer solution, the absorption coefficient of carbon dioxide (f_1) was calculated from Eqs. 2 and 3. As an average of several determinations a value of $f_1 = 0.65$ was found. At the pH of solution *5* (pH = 7.2), the amount of bicarbonate in the water phase (z) is about 10 times the amount of carbon dioxide (y) in the water phase. By normal filling of a 5-ml. ampul, the volume (a) of the solution in the ampul will be about five times the volume (b) of the gas in the ampul. The ratio of the amount of carbon dioxide + bicarbonate in the water phase ($y + z$) to the total amount of carbon dioxide + bicarbonate in the ampul (x) may be calculated from Eqs. 2 and 3. The value of the

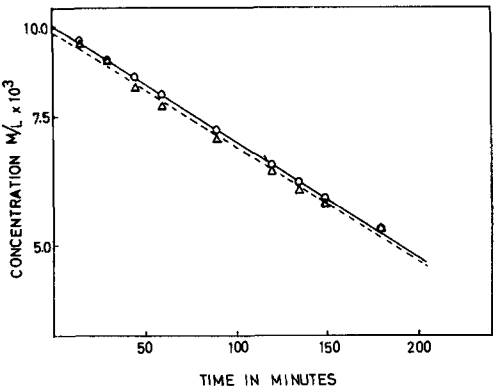


Fig. 1.—First-order disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 0.38 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, initial ascorbic acid concentration − concentration of CO_2 formed.

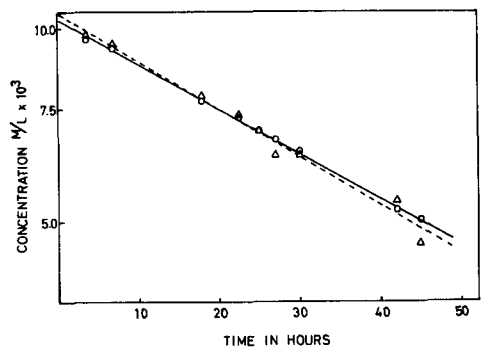


Fig. 2.—First-order disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 2.10 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, initial ascorbic acid concentration − concentration of CO_2 formed.

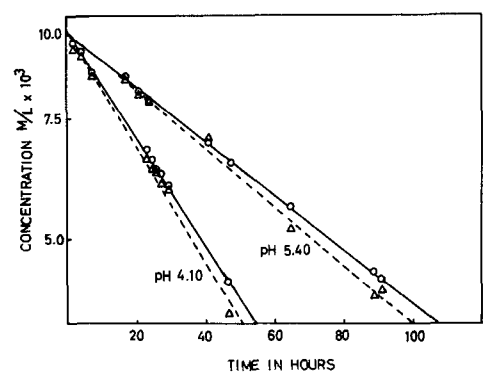


Fig. 3.—First-order disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 4.10 and pH 5.40 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, initial ascorbic acid concentration − concentration of CO_2 formed.

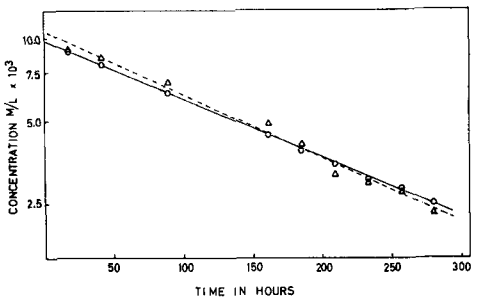


Fig. 4.—First-order disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 7.20 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, initial ascorbic acid concentration − concentration of CO_2 formed.

TABLE II.—EXPERIMENTAL RATE CONSTANTS OF THE ANAEROBIC DEGRADATION OF ASCORBIC ACID AT 96° C. AND DIFFERENT PH VALUES

pH	$k\alpha$, Hr.$^{-1}$ × 10^{2} [a]	$k_{CO_2}\beta$, Hr.$^{-1}$ × 10^{2} [b]	$\frac{k_{CO_2}}{k}$
0.38	22.0	21.8	0.99
2.10	1.61	1.73	1.08
4.10	1.79	1.92	1.07
5.40	0.912	0.976	1.07
7.20	0.495	0.533	1.08
10.15	0.510	0.398	0.78
11.10	0.889	0.316	0.36
11.40	1.22	0.376	0.31

[a] α, Rate constant of the over-all reaction. [b] β, Rate constant of the CO_2 producing reaction.

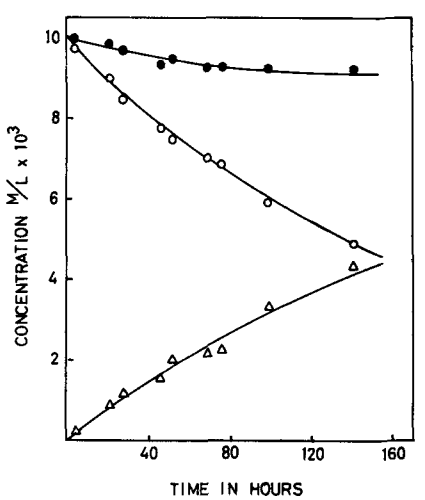


Fig. 5.—Disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 10.15 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, concentration of CO_2 formed; —●—●—, residual ascorbic acid concentration + concentration of CO_2 formed.

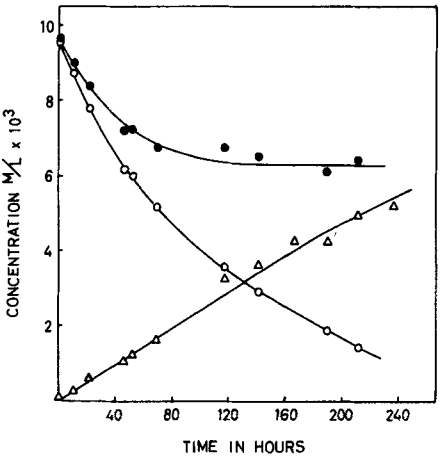


Fig. 6.—Disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 11.10 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, concentration of CO_2 formed; —●—●—, residual ascorbic acid concentration + concentration of CO_2 formed.

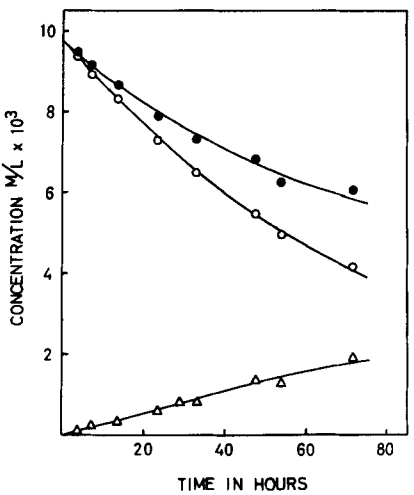


Fig. 7.—Disappearance of ascorbic acid and appearance of CO_2 by the anaerobic degradation of ascorbic acid at pH 11.40 and 96°C. Key: —O—O—, residual ascorbic acid concentration; —Δ—Δ—, concentration of CO_2 formed; —●—●—, residual ascorbic acid concentration + concentration of CO_2 formed.

absorption coefficient (f_1) has little influence on this ratio:

$$f_1 = 0.7 \text{ gives } \frac{y+z}{x} = 0.974$$

$$f_1 = 0.6 \text{ gives } \frac{y+z}{x} = 0.970$$

$$f_1 = 0.5 \text{ gives } \frac{y+z}{x} = 0.964$$

$$f_1 = 0.4 \text{ gives } \frac{y+z}{x} = 0.955$$

Assuming the absorption coefficient of carbon dioxide

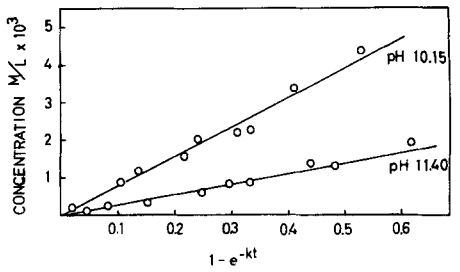


Fig. 8.—Plots showing the relation between concentration of CO_2 formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 10.15 and pH 11.40 and 96°C.

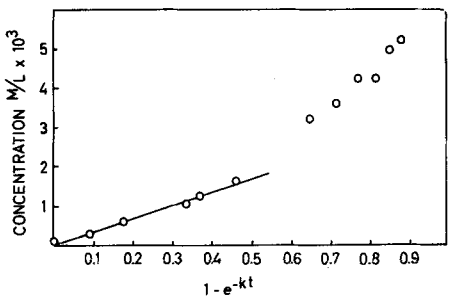


Fig. 9.—Plot showing the relation between concentration of CO_2 formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 11.10 and 96°C.

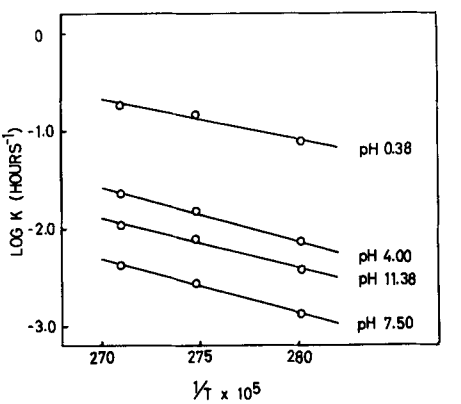


Fig. 10.—Arrhenius-type plots for the anaerobic degradation of ascorbic acid at pH 0.38, 4.00, 7.50, and 11.38.

in solution *5* to be about 0.6, the concentration of carbon dioxide formed when ascorbic acid was heated in this solution was calculated by dividing the concentration of carbon dioxide found in the heated solution by 0.97.

When ascorbic acid is heated in solutions *6*, *7*, or *8*, the total amount of carbon dioxide formed will be found in the water phase of the ampul.

RESULTS AND DISCUSSION

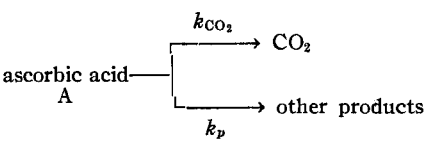
Runs were made at different pH values. The results obtained at pH 0.38, 2.10, 4.10, 5.40, and 7.20 are given in Figs. 1–4. In these figures two plots are made: logarithm of residual ascorbic acid concentra-

tion [A] *versus* time and logarithm of the difference between the initial concentration of ascorbic acid $[A]_0$ and the concentration of carbon dioxide formed $[CO_2]$ *versus* time. $[CO_2]$ is the total amount (x) of carbon dioxide found in the ampul divided by the volume (a) of the water phase.

Figures 1–4 seem to indicate that the main route of the anaerobic degradation of ascorbic acid in acid or neutral medium is a reaction (or reactions) leading to the formation of one mole of carbon dioxide per mole of ascorbic acid decomposed.

From the plots on Figs. 1–4 the apparent first-order rate constant of the over-all reaction (k) and of the carbon dioxide producing reaction (k_{CO_2}) were calculated (Table II). An average value of $k_{CO_2}/k = 1.06$ was found. This suggests that the lactonized carboxyl group in the ascorbic acid molecule decomposes relatively fast yielding 1 mole of carbon dioxide per mole of ascorbic acid decomposed and that there is a further slow decomposition evolving more carbon dioxide.

The results obtained at pH 10.15, 11.10, and 11.40 are given in Figs. 5–7. In these figures [A], $[CO_2]$, and ([A] + $[CO_2]$) have been plotted *versus* time. It can be seen that ([A] + $[CO_2]$) decreases with time. This means that at pH >10 the rate of formation of carbon dioxide is slower than the rate of disappearance of ascorbic acid. A possible explanation of this fact could be that ascorbic acid in alkaline medium decomposes *via* two parallel reactions, one leading to the formation of carbon dioxide and one leading to the formation of other products.



(Eq. 4)

Assuming both reactions to be first order with respect to ascorbic acid, the following equation is valid.

$$k[A] = k_{CO_2}[A] + k_p[A] \qquad \text{(Eq. 5)}$$

or

$$k = k_{CO_2} + k_p \qquad \text{(Eq. 6)}$$

$$kt = \ln \frac{[A]_0}{[A]} \qquad \text{(Eq. 7)}$$

or

$$[A] = [A]_0 e^{-kt} \qquad \text{(Eq. 8)}$$

where $[A]_0$ is the initial concentration of ascorbic acid and [A] is the concentration at any time t. The rate of formation of carbon dioxide is

$$\frac{d[CO_2]}{dt} = k_{CO_2} \cdot [A] = k_{CO_2} \cdot [A]_0 e^{-kt} \qquad \text{(Eq. 9)}$$

This equation gives by integration

$$[CO_2] = -\frac{k_{CO_2}[A]_0 e^{-kt}}{k} + \text{constant} \qquad \text{(Eq. 10)}$$

The constant may be calculated easily since $[CO_2] = 0$ when $t = 0$.

TABLE III.—HEATS OF ACTIVATION BY THE ANAEROBIC DEGRADATION OF ASCORBIC ACID AT DIFFERENT pH VALUES

pH	Apparent Heat of Activation, Kcal./Mole
0.38	19
4.00	25
7.50	24
11.38	23

$$\text{constant} = \frac{k_{CO_2}[A]_0}{k} \qquad \text{(Eq. 11)}$$

and

$$[CO_2] = \frac{k_{CO_2}[A]_0}{k}(1 - e^{-kt}) \qquad \text{(Eq. 12)}$$

If our assumptions were correct, then a plot of $[CO_2]$ found experimentally *versus* $(1 - e^{-kt})$ should yield a straight line through the origin. Figures 8 and 9 show that the experimental points fall reasonably well on straight lines passing through the origin up to a value of $(1 - e^{-kt})$ around 0.5. For higher values of $(1 - e^{-kt})$, the experimental points do not fit (Fig. 9).

It is possible that the two parallel reactions in Eq. 4 are the dominating ones during the initial part of the degradation process but that other reactions producing more carbon dioxide later become important.

k_{CO_2} was calculated from the slopes of the straight lines on Figs. 8 and 9 using

$$\text{slope} = \frac{k_{CO_2}[A]_0}{k} \qquad \text{(Eq. 13)}$$

The values found are shown in Table II for pH 10.15, 11.10, and 11.40.

Determination of Heats of Activation by the Anaerobic Degradation of Ascorbic Acid.—The temperature dependency of rate of inactivation of ascorbic acid under anaerobic conditions was determined at pH 0.38, 4.00, 7.50, and 11.38. At pH 0.38, 4.00, and 11.38, no buffer was added to the ascorbic acid solutions. At pH 7.50, a phosphate buffer was used.

The results are shown in Fig. 10, where the logarithms of the rate constants are plotted against the reciprocals of the corresponding absolute temperatures. From the slopes of the lines, the apparent heats of activations at the different pH values have been determined (Table III).

The heats of activation found correspond to temperature coefficients between 2 and 2.5 in the temperature range 100–120°.

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