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Rate Studies On the Anaerobic Degradation of Ascorbic Acid III

Rate of Formation of Furfural

By PER FINHOLT, INGER ALSOS, and TAKERU HIGUCHI*

Under acidic and anaerobic conditions, a molecule of furfural appears to be produced from each molecule of ascorbic acid. For nonhydrogen ion-catalyzed reaction, the aldehyde production seems to be substantially less than the mole-for-mole relationship. A pH profile for furfural production has been determined. The rate of disappearance of the aldehyde itself from oxygen-free aqueous solutions also has been studied.

IN PREVIOUS papers, the rate of the anaerobic loss of ascorbic acid (1) and the rate of formation of carbon dioxide by the same degradation (2) were reported. Since furfural is produced at the same time, it was of interest to study the rate of formation of this compound in the same system.

Reichstein and Grüssner (3) were the first to show that furfural was formed by the anaerobic decomposition of ascorbic acid. They heated ascorbic acid with 0.2 *N* hydrochloric acid in the absence of air and identified furfural as one of the decomposition products.

The formation of furfural during anaerobic loss of ascorbic acid was studied more thoroughly by Huelin (4). In his preliminary experiments, solutions of 0.25% ascorbic acid in distilled water were sealed in Florence flasks *in vacuo* and held at 100° for 10 days. During this period, 93% of the ascorbic acid decomposed, and the pH increased from 3.0 to 7.2. For each mole of ascorbic acid destroyed, 0.43 mole of furfural was produced. The furfural was identified by color reaction with aniline acetic acid, melting points of phenylhydrazone (99°) and dinitrophenylhydrazone (200°), and spectral absorption of dinitrophenylhydrazone in alkaline solution, and was determined as phloroglucide. More detailed measurements were made at 30° with 0.01 *M* ascorbic acid solutions containing buffers. The solutions were kept in sealed glass tubes under nitrogen for 2 years. Furfural was determined colorimetrically with aniline in acetic acid according to the method of Duncan (5). The amount of furfural formed at pH 2.2 was 0.48 mole per mole of ascorbic acid destroyed; at pH 3.0, it was 0.17 mole; and at pH 4.0, it was 0.03 mole. At pH 5.0 and 6.0, no furfural could be detected. The author concludes that a plausible interpretation of the experimental results is that the formation of furfural does not occur in the primary reaction but is determined by the relative rate of various secondary reactions.

Cier *et al.* (6) heated ascorbic acid solutions at pH 2.5–5.5 under anaerobic conditions for 24 hours at 100° and determined the furfural concentration of the heated solutions colorimetrically with benzidine in acetic acid. At pH 5.5, no furfural could be detected. With a decrease in pH, the furfural concentration of the heated solutions increased sharply.

Quite recently, Coggiola (7) isolated the acids formed when a 5% solution of ascorbic acid in water was incubated at 100° under an atmos-

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phere of carbon dioxide for 10 days until decomposition was practically complete. The predominant acid isolated was considered to be 2,5-dihydro-2-furoic acid.

The present study was undertaken to determine the rate of formation of furfural by the anaerobic degradation of ascorbic acid in acid, neutral, and alkaline media. Since the furfural formed may undergo some subsequent decomposition, it was felt necessary to determine the rate of degradation of furfural under the same experimental conditions used by the earlier ascorbic acid studies (1). Little work has been done hitherto on the kinetics of the anaerobic decomposition of furfural. Williams and Dunlop (8) heated furfural in 0.1 *N* sulfuric acid and in 0.05 *N* and 0.10 *N* hydrochloric acid in sealed Pyrex tubes at 160° and found the reaction to be first order with respect to furfural. The rate constant was proportional to the hydrogen ion concentration. Formic acid was identified as one of the decomposition products.

EXPERIMENTAL

Materials.—All reagents used were of analytical grade. The furfural used was distilled *in vacuo* at 1.5 mm. Hg and stored in ampuls under nitrogen protected from light. The aniline used was distilled *in vacuo* at 10 mm. Hg and stored under nitrogen in light-resistant containers. The water used was distilled water redistilled from a neutral glass still, boiled, and cooled under oxygen-free nitrogen.

Determination of Furfural.—Furfural was determined colorimetrically according to the method of Duncan (6), slightly modified by the authors. An amount of the furfural-containing solution corresponding to 0.005 to 0.03 mg. of furfural was pipeted into a 50-ml. volumetric flask. Ten milliliters of a solution containing 0.012 Gm. of oxalic acid and 0.025 Gm. of disodium phosphate (Na_2HPO_4) was added, and the solution was diluted to approximately 25 ml. with distilled water. Aniline reagent (25.0 ml.) was added; the mixture was diluted to the mark with distilled water. The flask was placed in a water bath at 20° and covered so that almost all the light was excluded. Fifty to sixty minutes after the addition of the aniline reagent, the absorbance at 518 mμ was measured in a Beckman model B spectrophotometer. The amount of furfural in the reaction mixture was read from the standard curve (Fig. 3).

Aniline Reagent.—Freshly distilled aniline (10.0 ml.) and approximately 90 ml. of glacial acetic acid were mixed in a 100-ml. volumetric flask and cooled to 20°. Glacial acetic acid was added to the mark.

The red compound formed when furfural reacts with aniline in 50% acetic acid solution has maximum absorbance at 518 mμ. A_{518} mμ depends on time and the amount of aniline added (Fig. 1). The color develops faster and becomes less stable with increasing temperature. The time necessary for obtaining the maximum value of A_{518} increases with

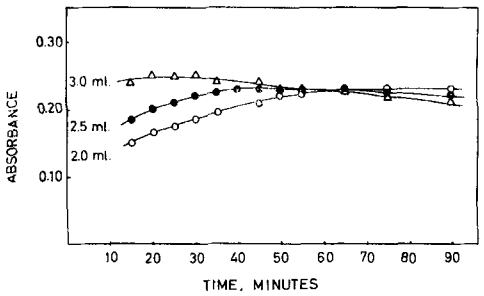


Fig. 1.—Influence of time and milliliters of aniline added on the absorbance at 518 mμ by the colorimetric determination of furfural.

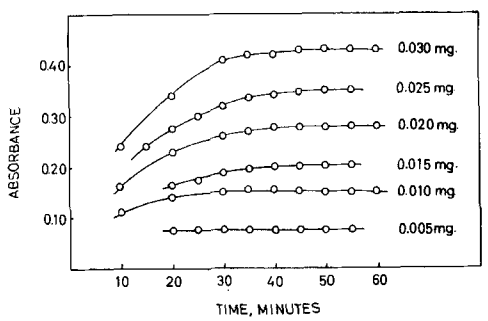


Fig. 2.—Influence of time and milligrams of furfural in the reaction mixture (50 ml.) on the absorbance at 518 mμ by the colorimetric determination of furfural.

an increase in furfural concentration (Fig. 2). For amounts of furfural between 0.005 and 0.030 mg. in the reaction mixture, maximum values of A_{518} mμ will be found when the readings are done 50–60 minutes after the addition of the aniline reagent (Fig. 2).

Rate Studies on the Anaerobic Degradation of Furfural in Aqueous Solution.—The rate of the anaerobic degradation of furfural in aqueous solution was determined under the same experimental conditions used by the studies of the anaerobic degradation of ascorbic acid (1). A furfural concentration of 0.001 mole per liter was used.

A 9.6-mg. quantity (0.0001 mole) of furfural was dissolved in 100 ml. of the appropriate solvent. The solution was heated under nitrogen in 5-ml. ampuls at 96° in a constant-temperature bath. At appropriate intervals, ampuls were taken out of the bath, cooled on ice, and the residual furfural concentration determined. As solvents for furfural, 0.002–0.50 *N* perchloric acid, phosphate buffers (pH 5.95 and 7.50), borate buffers (pH 8.56 and 9.00), and 0.01–0.10 *N* sodium hydroxide were used. All solvents were adjusted to an ionic strength of 0.5 with sodium chloride.

Rate Studies on the Formation of Furfural by the Anaerobic Degradation of Ascorbic Acid.—The degradation studies and the determination of the residual ascorbic acid concentration of the heated solutions were done in the same way as described in a previous paper (1). The furfural concentration of the heated solutions was determined according to the colorimetric method. The following solvents were

used: 0.05–0.50 *N* perchloric acid, 0.1 *M* phosphate buffers (pH 2.24 and 3.05), 0.1 *M* acetate buffers (pH 3.94 and 5.30), 0.05 *M* phosphate buffers (pH 5.97 and 7.00), 0.05 *M* borate buffer (pH 8.56), and 0.03 *N* sodium hydroxide.

RESULTS AND DISCUSSION

Precision of the Assay for Furfural.—One-milliliter aliquots of solutions containing 0.010 mg., 0.020 mg., and 0.030 mg. of furfural per milliliter were analyzed according to the colorimetric method. The mean values of absorbance and the coefficients of variation of the results are given in Table I. In Fig. 3, the mean values of absorbance are plotted *versus* milligrams of furfural in the reaction mixture (50 ml.). The curve was used as the standard curve. Ascorbic acid had no influence on the results, even if the weight ratio of ascorbic acid to furfural was as high as 4000:1.

By the method reported here 10 mcg. of furfural in 5 ml. of 0.01 *M* ascorbic acid solutions may be determined easily. This means that a 0.2% decomposition of ascorbic acid can be determined, provided that 1 mole of furfural is formed per mole of ascorbic acid destroyed.

Results from the Rate Studies on the Anaerobic Degradation of Furfural in Aqueous Solution.—Anaerobic degradation of furfural in aqueous solutions was found to be first order with respect to furfural at pH values from 0.4 up to 10.18. In all cases there was a linear relationship between time

TABLE I.—DETERMINATION OF FURFURAL IN AQUEOUS SOLUTION

Furfural, mg.	Determinations, No.	Mean Value of Absorbance	Coefficient of Variation, %
0.010	20	0.137	2.89
0.020	20	0.279	1.60
0.030	19	0.423	1.62

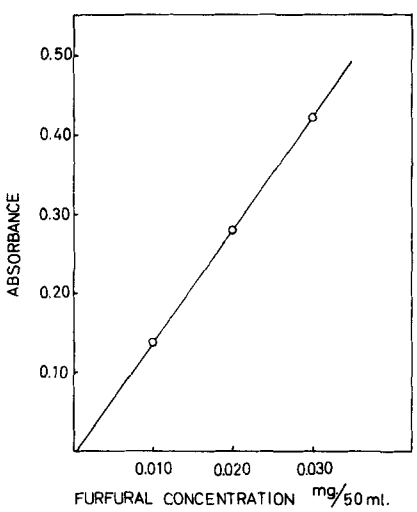


Fig. 3.—Relation between furfural concentration of the reaction mixture and absorbance at 518 mμ by the colorimetric determination of furfural.

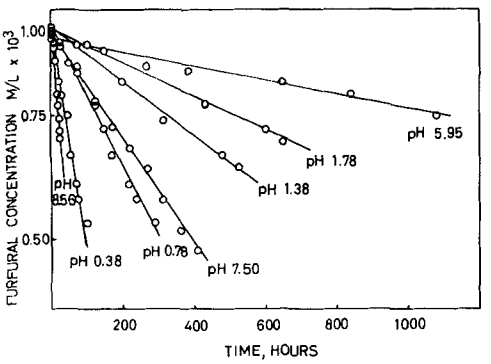


Fig. 4.—Plots showing the over-all first-order character of the anaerobic degradation of furfural in aqueous solution at different pH values and 96° C. For the actual runs 0.5 *N* perchloric acid (pH 0.38), 0.2 *N* perchloric acid (pH 0.78), 0.05 *N* perchloric acid (pH 1.38), 0.02 *N* perchloric acid (pH 1.78), 0.05 *M* phosphate buffer (pH 5.95), 0.05 *M* phosphate buffer (pH 7.50), and 0.05 *M* borate buffer (pH 8.56) were used.

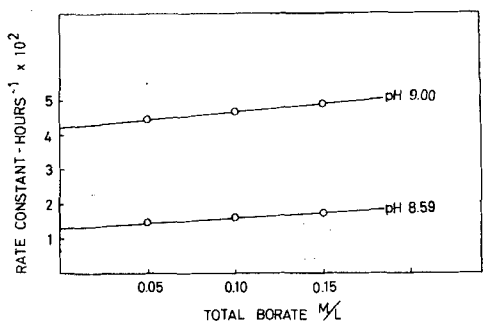


Fig. 5.—Effect of borate concentration on the pseudo first-order rate constant of the anaerobic degradation of furfural at fixed pH values. All runs were made at 96° C. and an ionic strength of 0.50.

and logarithm of residual furfural concentration, as shown in Fig. 4.

The catalytic effect of the buffers used was determined by experiments at constant pH, constant ionic strength ($\mu = 0.50$), and constant furfural concentration, varying only the total buffer concentration at a given pH. Borate buffers (pH 8.59 and 9.00) had some catalytic effect (Fig. 5), whereas phosphate buffer (pH 7.50) was noncatalytic.

In Fig. 6, the logarithms of the rate constants at zero buffer concentration and the logarithms of the rate constant found by the experiments where perchloric acid or sodium hydroxide solutions were used as solvents are plotted *versus* pH. pH of the strongly acid and strongly basic solutions at 96° and $\mu = 0.50$ were calculated from Eqs. 1 and 2, which were derived in a previous paper (1).

$$pH = -\log[H^+] + 0.08 \qquad \text{(Eq. 1)}$$

$$pH = \log [OH^-] + 11.70 \qquad \text{(Eq. 2)}$$

The shape of the pH rate profile may be explained by assuming that the following reactions take place at zero buffer concentration:

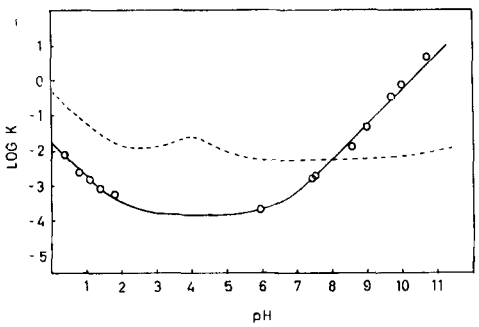


Fig. 6.—pH-rate profile of the anaerobic degradation of furfural at 96° C. Key: O, experimental results; ——, results expected theoretically from the three proposed reactions; ----, pH-rate profile of the anaerobic degradation of ascorbic acid at 96° C.

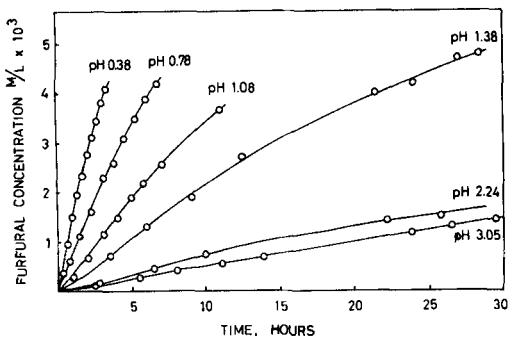


Fig. 7.—Formation of furfural by the anaerobic degradation of ascorbic acid at different pH values and 96° C.

$$F + H^+ \xrightarrow{k_{H^+}} \text{products} \quad \text{Reaction } 1$$

$$F + OH^- \xrightarrow{k_{OH^-}} \text{products} \quad \text{Reaction } 2$$

$$F + H_2O \xrightarrow{k_{H_2O}} \text{products} \quad \text{Reaction } 3$$

where F = furfural.

The over-all velocity is equal to the sum of the rates of these reactions:

$$-\frac{d[F]}{dt} = k_{H^+}[F][H^+] + k_{OH^-}[F][OH^-] + k_{H_2O}[F][H_2O] \quad \text{(Eq. 3)}$$

Because of the over-all first-order character of the reaction, the following reaction is valid

$$-\frac{d[F]}{dt} = k[F] \quad \text{(Eq. 4)}$$

Combining Eqs. 3 and 4 gives

$$k = k_{H^+}[H^+] + k_{OH^-}[OH^-] + k_{H_2O}[H_2O] \quad \text{(Eq. 5)}$$

From the experimental results, the following k values have been calculated:

$$k_{H^+} = 1.43 \times 10^{-2} \text{ hr.}^{-1} \text{ mol.}^{-1} \text{ L.}$$

$$k_{OH^-} = 29 \text{ hr.}^{-1} \text{ mol.}^{-1} \text{ L.}$$

$$k_{H_2O} = 2.62 \times 10^{-6} \text{ hr.}^{-1} \text{ mol.}^{-1} \text{ L.}$$

The theoretical line in Fig. 6 has been calculated by substituting the above k values into Eq. 5.

The pH rate profile of the anaerobic degradation of ascorbic acid (1) also is given in Fig. 6, from which it can be seen that furfural is more stable than ascorbic acid at pH < 8 but less stable at pH > 8.

Results from the Rate Studies on the Formation of Furfural by the Anaerobic Degradation of Ascorbic Acid.—In Figs. 7 and 8, the concentration of furfural found in the heated ascorbic acid solutions are plotted *versus* time of heating. At pH $\leqq$ 7, no furfural could be detected in the heated ascorbic acid solutions. Figures 7 and 8 seem to indicate that there is a short lag time in the formation of furfural, especially at the higher pH values.

If the only reactions taking place were

$$\text{ascorbic acid} \xrightarrow{k_1} \text{furfural} \xrightarrow{k_2} \text{products}$$

the furfural concentration might be calculated from

$$[F] = \frac{[A]_0 k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \quad \text{(Eq. 6)}$$

where

[F] = concentration of furfural
$[A]_0$ = initial concentration of ascorbic acid
t = time

When 50% of the ascorbic acid is degraded, *i.e.*,

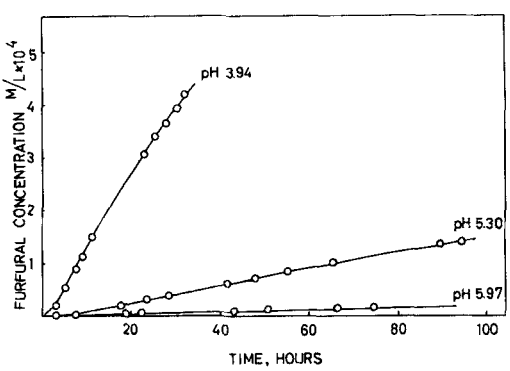


Fig. 8.—Formation of furfural by the anaerobic degradation of ascorbic acid at different pH values and 96° C.

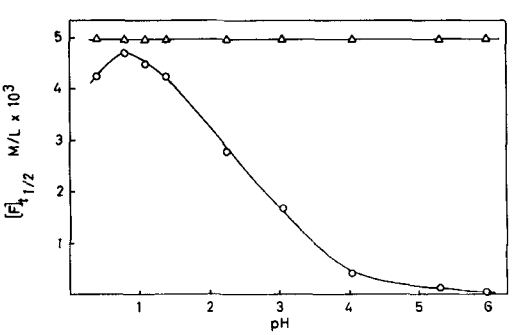


Fig. 9.—Furfural concentration, $[F]_{t_{1/2}}$, of 50% degraded 0.01 *M* ascorbic acid solutions of different pH. Key: O, experimental results; △, values calculated from Eq. 7.

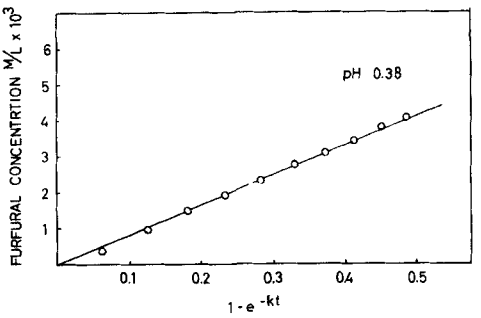


Fig. 10.—Relation between concentration of furfural formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 0.38 and 96° C.

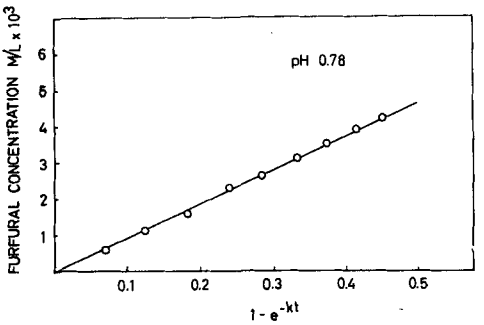


Fig. 11.—Relation between concentration of furfural formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 0.78 and 96° C.

when $t = \frac{0.693}{k_1}$, the furfural concentration, $[F]_{t_{1/2}}$, would be

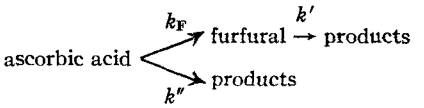
$$[F]_{t_{1/2}} = \frac{[A]_0 k_1}{k_2 - k_1}\left(e^{-0.693} - e^{-0.693\frac{k_2}{k_1}}\right) \quad \text{(Eq. 7)}$$

Assuming k_1 to be the specific rate of the over-all degradation of ascorbic acid and k_2 to be the specific rate of the degradation of furfural, k_1 and k_2 for different pH values may be picked easily from Fig. 6 and the corresponding values of $[F]_{t_{1/2}}$ calculated. In Fig. 9, $[F]_{t_{1/2}}$ calculated is plotted (as triangles) *versus* pH. It will be seen that $[F]_{t_{1/2}}$ calculated at all pH values is very close to $5 \cdot 10^{-3}$ molar, *i.e.*, equal to $[A]_0/2$. At pH < 6, k_2 is so small, compared to k_1, that Eq. 7 may be written

$$[F]_{t_{1/2}} = [A]_0 (e^0 - e^{-0.693}) = \frac{[A]_0}{2} \quad \text{[Eq. 8)}$$

$[F]_{t_{1/2}}$ found experimentally also is plotted in Fig. 9 (the circles). The experimental values of $[F]_{t_{1/2}}$ are lower than the calculated ones, and the deviations increase with increasing pH. This means that the furfural-producing reaction is not the only reaction by which ascorbic acid is decomposed. Other reactions also take place and these reactions become more important with increasing pH.

It may be assumed that ascorbic acid under anaerobic conditions decomposes *via* two parallel first-order reactions, one leading to the formation of furfural and one leading to the formation of other products.



[F] may be calculated from the following equation [Rodiguin and Rodiguina (9)]:

$$[F] = [A]_0 \frac{k_F}{k - k'}(e^{-k't} - e^{-kt}) \quad \text{(Eq. 9)}$$

where $k = k_F + k''$.

At pH < 6, k' is so small, compared to k, that we may write

$$[F] = \frac{A_0 k_F}{k}(1 - e^{-kt}) \quad \text{(Eq. 10)}$$

If the assumption is correct a plot of [F] found experimentally *versus* $(1 - e^{-kt})$ should yield a straight line through the origin. Figures 10–14 show that the experimental points fit quite well to a straight line through the origin at pH 0.38–pH 5.97.

The specific rate of the furfural producing reaction (k_F) may be calculated from the slopes of the lines in Figs. 10–14 using

$$\text{slope} = \frac{k_F [A]_0}{k} \quad \text{(Eq. 11)}$$

In Fig. 15, the logarithms of the rate constants (k_F) of the furfural producing reaction are plotted *versus* pH. The shape of this pH rate profile may be explained by assuming furfural to be formed by the two following reactions:

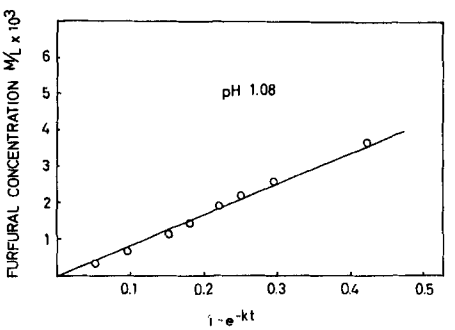


Fig. 12.—Relation between concentration of furfural formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 1.08 and 96° C.

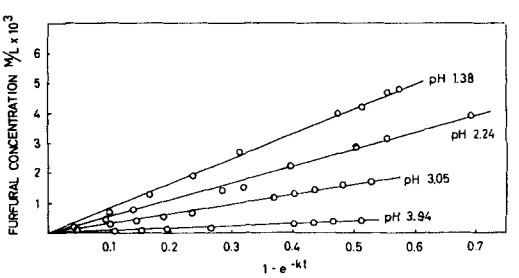


Fig. 13.—Relation between concentration of furfural formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at different pH values and 96° C.

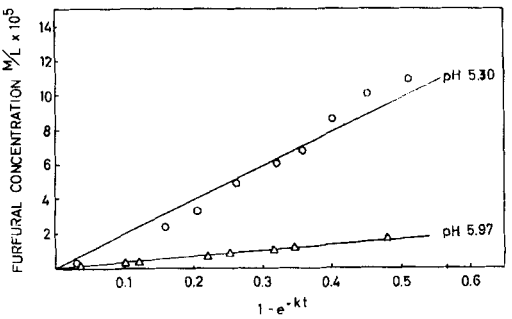


Fig. 14.—Relation between concentration of furfural formed and $(1 - e^{-kt})$ by the anaerobic degradation of ascorbic acid at pH 5.30 and pH 5.97 and 96° C.

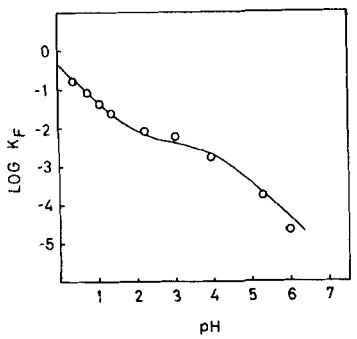


Fig. 15.—pH-rate profile of the furfural producing reactions by the anaerobic degradation of ascorbic acid at 96° C. Key: O, experimental results; ——, results expected theoretically from the two proposed reactions.

$$A + [H^+] \xrightarrow{k_1} F$$

$$A \xrightarrow{k_2} F$$

where A = undissociated ascorbic acid and F = furfural.

$$-\frac{d[A_T]}{dt} = k_1[A][H^+] + k_2[A] \quad \text{(Eq. 12)}$$

$$[A_T] = [A] + [A^-]^{1} \quad \text{(Eq. 13)}$$

where A^- = monohydrogen ascorbate ion.

$$-\frac{d[A_T]}{dt} = k_F[A_T] \quad \text{(Eq. 14)}$$

[1] At pH < 6, the ncentration of A^-, ascorbate ion, will be negligible.

Combining Eqs. 12–14 and the equation

$$ka_1 = \frac{[H^+][A^-]}{[A]} \quad \text{(Eq. 15)}$$

gives

$$k_F = \frac{k_1[H^+]^2 + k_2[H^+]}{[H^+] + ka_1} \quad \text{(Eq. 16)}$$

The theoretical line in Fig. 15 has been calculated by substituting the following values for k_1, k_2, and ka_1 into Eq. 16:

$$k_1 = 3.8 \times 10^{-1} \text{ hr.}^{-1} \text{ mol.}^{-1} \text{ L.}$$

$$k_2 = 4 \times 10^{-3} \text{ hr.}^{-1}$$

$$ka_1 = 1.15 \times 10^{-4}$$

The value of k_1 is the same value found by a previous study (1) for the reaction

$$A + H^+ \rightarrow \text{products}$$

The value of k_2, however, is smaller than the one found earlier (1) for the reaction

$$A \rightarrow \text{products}$$

The rate constant of this reaction was determined to be 8.9×10^{-3} hr.$^{-1}$.

CONCLUSION

From the experimental results, the following conclusion may be drawn. The rate of formation of furfural by the hydrogen ion-catalyzed anaerobic degradation of undissociated ascorbic acid is equal to the rate of disappearance of ascorbic acid. One mole of furfural is formed per mole of ascorbic acid destroyed. By the noncatalyzed cleavage of undissociated ascorbic acid, the rate of formation of furfural is slower than the rate of disappearance of ascorbic acid. It is possible that the reason for this is that ascorbic acid here decomposes *via* two parallel reactions, one leading to the formation of furfural and one leading to the formation of other products. Monohydrogen ascorbate ion (A^-) and ascorbate ion ($A^{=}$) do not seem to give furfural by anaerobic degradation.

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