salts in the presence of sodium chloride are recorded in Table I. The ephedrine hydrochloride formed in *Method B* was then determined by nonaqueous titration (*Method C*) as a confirmatory procedure.

The proposed methods were applied to liquid dosage forms including solutions, an elixir, syrups, and an injection. The data are reported in Table II. Since the solutions contain sodium chloride, *Method A* could not be used. Favorable results were obtained with *Methods B*, *C*, and *D*.

The elixir reported in Table II was a commercial sample and when analyzed by *Method A* gave very high results. The manufacturer upon request was kind enough to supply the formula of the elixir which did contain appreciable amounts of sodium saccharin and sodium chloride. Quantitative results were obtained by *Methods B* and *C*. The elixir responded to the official assay for ephedrine sulfate solution.

The syrups yielded good results by the proposed methods as well as by the official assay procedure for the solution. Since the injection contains sodium chloride, *Methods B* and *C* were used.

Coloring agents found in the elixir and syrup do not interfere. They are readily washed from the column with water. Nonionic agents and acidic components in general, are not adsorbed by the column. Where synthetic sweeteners such as sodium saccharin are used in the formulation, *Method B* must be employed. Other organic bases and their salts will interefere with the proposed methods. However, if the base strength differs significantly from that of ephedrine, a nonaqueous differential titration may be possible.

Since four assays may be conveniently conducted at the same time, the proposed methods are less time consuming and less tedious than the official assay. The procedures are simple, accurate, and applicable to all commonly available liquid dosage forms of ephedrine salts. They should also be applicable to solid dosage forms and to combinations of ephedrine with other therapeutic agents such as barbiturates.

REFERENCES

(1) The "National Formulary," 11th ed., J. B. Lippincott Co., Philadelphia, Pa., 1960
(2) Hilty, W. W., THIS JOURNAL, **33**, 28(1944).
(3) Hilty, W. W., and Wilson, D. T., *ibid.*, **37**, 227(1948).
(4) The "National Formulary," 8th ed., J. B. Lippincott Co., Philadelphia, Pa., 1946.
(5) Higuchi, T., and Bodin, J. I., "Pharmaceutical Analysis," Interscience Publishers, New York, N. Y., 1961, p. 313.
(6) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," 3rd ed., Vol. IV, D. Van Nostrand Co., New York, N. Y., 1954, p. 35.
(7) Auerbach, M. E. *Drug. Std.*, **19**, 127(1951).
(8) Pifer, C. W., and Wollish, E. G., *Anal. Chem.*, **24**, 300(1952).
(9) Rink, M., and Lux, R., *Arch. Pharm.*, **294/66**, 117(1961).
(10) Ekeblad, P., *J. Pharm. Pharmacol.*, **4**, 636(1952).
(11) The "British Pharmacopoeia," General Medical Council, London, 1958, p. 248.
(12) Chatten, L. G., and Pernarowski, M., *Drug Std.*, **22**, 1(1954).
(13) Jindra, A., *J. Pharm. Pharmacol.*, **1**, 87(1949).
(14) Jindra, A., and Pohorsky, J., *ibid.*, **2**, 361(1950).
(15) Samuelson, O., "Ion Exchangers in Analytical Chemistry," John Wiley and Sons, New York, N. Y., 1953, p. 208.
(16) Saunders, L., Elworthy, P. H., and Fleming, R. *J. Pharm. Pharmacol.*, **6**, 32(1954).
(17) Vincent, M. C., Krupski, E., and Fischer, L., THIS JOURNAL, **46**, 85(1957).
(18) Huyck, C. L., *Am. J. Pharm.*, **122**, 228(1950).
(19) Blaug, S. M., and Zopf, L. C., THIS JOURNAL, **45** 9(1956).

Rate of Anaerobic Degradation of Ascorbic Acid in Aqueous Solution

By PER FINHOLT, ROLF B. PAULSSEN, and TAKERU HIGUCHI

The pH rate profile of the rate of disappearance of ascorbic acid from aqueous solution under anaerobic conditions has been determined at 96°. The dependency on pH is surprisingly low over pH range of 1–11. The profile shows a small but apparently real maximum at pH = pK_{a_1}, an effect which can be rationalized by assuming formation of a salt-acid complex in solutions. The anaerobic rate shows buffer dependency but relatively small ionic strength effect.

ALTHOUGH the oxidative route of degradation of ascorbic acid has been extremely well studied, relatively few papers have appeared dealing with degradative loss of ascorbic acid under anaerobic conditions. Since in practice reduction in the concentration of this vitamin in liquid pharmaceutical preparation appears to follow largely the latter route, serious study of the factors influencing its rate was felt needed. Even in instances where losses in the ascorbic acid concentration are of no major concern, the gas produced in the process often poses a problem.

Previous studies have been largely of qualitative nature. Reichstein and Grüssner (1), for example, showed that when ascorbic acid was heated with 0.2 *N* hydrochloric acid a decrease in the iodine consumption and furfural was formed. Their observation was part of a work on the synthesis of ascorbic acid from 2-keto-L-gulonic acid.

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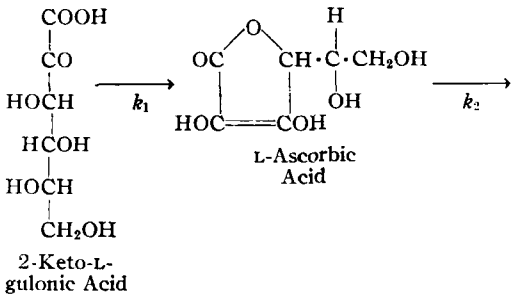
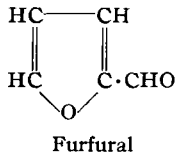
The enolization and lactonization of methyl 2-keto-L-gulonate into ascorbic acid could be brought about by sodium methylate and also by hydrochloric acid. Heating of 2-keto-L-gulonic acid with hydrochloric acid thus is presumed to lead to the consecutive reactions





Regna and Caldwell (2) have studied, again only in the strongly acid solution, the kinetics of this reaction and the kinetics of the transformation of some other 2-ketopolyhydroxy acids to their ascorbic acid analogs and the decomposition of these compounds. For the transformation of 2-keto-L-gulonic acid into L-ascorbic acid in 5 *M* hydrochloric acid at 59.9°, a first-order rate constant $k_1 = 2.53 \times 10^{-3}$ minutes^{-1} was found. For the decomposition of L-ascorbic acid into furfural under the same conditions, the first-order rate constant was 4.91×10^{-4} minutes^{-1}.

Taylor, Fowler, McGee, and Kenyon (3) noted that carbon dioxide is evolved when ascorbic acid was heated, again in 12% hydrochloric acid. They submitted kinetic data on the evolution of carbon dioxide from L-ascorbic acid and from D-glucuronic acid, D-galacturonic acid, pectic acid, alginic acid, celluronic acids, and oxidized starch. The first-order rate constant for the evalution of carbon dioxide from ascorbic acid was more than twice as great as that of its nearest neighbor in the series, D-galacturonic acid. This indicated the profound effect of an enediol structure on the stability of the adjacent lactonized carboxyl group. A rather rapid evolution of carbon dioxide occurs from ascorbic acid until approximately 28% by weight is lost, followed by slow evolution for many hours more, finally attaining a total value in excess of 30%. If only the carboxyl group supplied the carbon dioxide, the evolution should have been 25.0%. The results indicated that the carboxyl group decomposed rapidly and that there is a further slow decomposition evolving more carbon dioxide.

Von Euler and Hasselquist (4) found that by heating ascorbic acid with 1 *N* sodium hydroxide at 100° in nitrogen atmosphere the lactone ring was obviously opened and the salt of the corresponding hexuronic acid was formed.

For the anaerobic degradation of ascorbic acid acid at pH 1.10 Fischer-Jensen (5) found a first-order rate constant $k = 0.323$ hours^{-1} at 90° and $k = 0.801$ hours^{-1} at 100°, thus giving $Q_{10} = 2.5$. The decomposition was faster at pH 2.5 than at pH 6 and was independent of the ascorbic acid concentration.

By heating ascorbic acid solutions of different pH values below 5.5 under anaerobic conditions Cier, Nofre, and Drevon (6) showed that carbon dioxide, furfural, and xylose were formed. The rate of formation of only furfural was followed and was found to be very slow at pH 5 and higher; the rate, however, increased sharply by decreasing pH. The authors proposed the following scheme for the nonoxidative degradation of ascorbic acid in acid medium

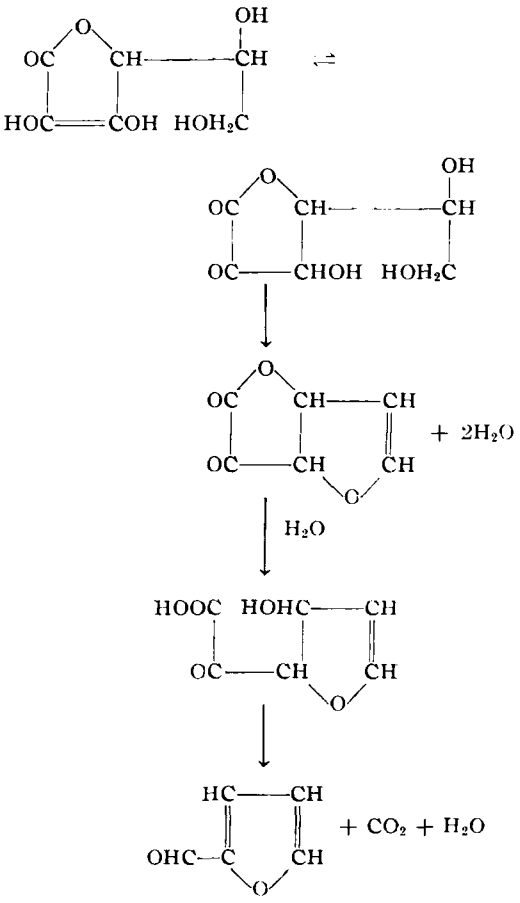


TABLE I.—COMPARISON OF RESULTS OBTAINED BY THE IODOMETRIC METHOD (A) AND BY THE DICHLOROPHENOL-INDOPHENOL METHOD (B) IN ANALYSIS OF ASCORBIC ACID SOLUTIONS OF DIFFERENT pH DEGRADED BY HEATING AT 96°C.

pH 1.08			pH 4.10			pH 5.20			pH 7.04			pH 10.40		
	Ascorbic Acid Concn. Found, $M/L. \times 10^3$			Ascorbic Acid Concn. Found, $M/L. \times 10^3$			Ascorbic Acid Concn. Found, $M/L. \times 10^3$			Ascorbic Acid Concn. Found, $M/L. \times 10^3$			Ascorbic Acid Concn. Found, $M/L. \times 10^3$	
Hrs. Heated	A	B	Hrs. Heated	A	B	Hrs. Heated	A	B	Hrs. Heated	A	B	Hrs. Heated	A	B
0	9.66	9.50	0	9.79	9.88	0	9.92	9.97	0	9.97	9.95	0	9.66	9.70
1	9.06	9.10	3	9.24	9.03	6	9.48	9.41	6	9.63	9.50	7	8.53	8.88
2	8.62	8.68	7.6	7.89	7.83	16.7	8.58	8.26	15	9.30	9.24	21	7.99	8.00
3.3	8.00	8.12	21.2	5.37	5.44	23.5	7.88	7.95	20	9.07	8.99	31.2	7.47	7.12
4.3	7.55	7.55	...	...	...	40.7	6.75	6.83	42.6	8.06	7.99	45	6.80	6.77
5.3	7.10	7.16	...	...	...	47.5	6.40	6.25	53	7.71	7.70	...	...	...
6	7.05	7.05	...	...	...	...	...	...	62	7.28	7.19	...	...	...
$t_{1/2}$	12.7 hr.	13 hr.		25 hr.	25 hr.		75 hr.	75 hr.		145 hr.	145 hr.		82 hr.	79 hr.

This scheme does not explain how the xylose is formed. The authors exclude the possibility of xylose being the precursor of furfural since xylose by heating in slightly acid medium only gives traces of furfural.

The present studies have been concerned with the exact rate of anaerobic degradation of ascorbic acid in acid and in alkaline medium and determination of the extent of catalysis by hydrogen and hydroxyl ions and by general acids and bases.

EXPERIMENTAL

Materials.—Ascorbic acid U.S.P. from Hoffmann-La Roche Co. was used. Iodometric titration of this product according to the method in U.S.P. XVI showed that it contained 99.85% $C_6H_8O_6$.

All reagents used 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 with 0.01 *N* iodine using a few drops of starch T.S. as indicator.

In some cases the ascorbic acid concentration was also determined by the dichlorophenol-indophenol method given by U.S.P. XVI for ascorbic acid injection.

Degradation Studies.—A 176-mg. quantity (0.001 mole) of ascorbic acid was dissolved in 100 ml. of the appropriate buffer solution containing a sufficient amount of sodium chloride to give the ascorbic acid solution an ionic strength $\mu = 0.50$. The solution was 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.

Determination of pKa_1 and pKa_2 of Ascorbic Acid at 96° and Ionic Strength $\mu = 0.5$.—pKa_1 was determined by measuring the pH at 96° of solutions containing equal amounts of ascorbic acid (A) and monohydrogen ascorbate (A^-) and a sufficient amount of sodium chloride to give $\mu = 0.50$. $pKa_1 = 3.94$ was found.

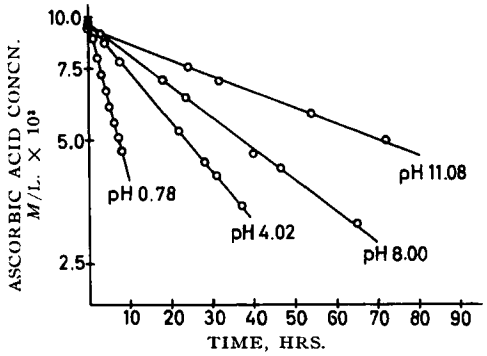


Fig. 1.—Plots showing the overall first-order character of the anaerobic degradation of ascorbic acid at different pH values and 96°C. For the actual runs 0.2 *N* perchloric acid (pH 0.78), 0.5 *M* acetate (pH 4.02), 0.15 *M* borate (pH 8.00), and 0.25 *N* NaOH (pH 11.08) were used.

TABLE II.—HALF-LIFE OF ASCORBIC ACID DEGRADATION AT 96°C. AND DIFFERENT pH VALUES AND DIFFERENT IONIC STRENGTH

Ionic Strength	Half-Life, hrs. pH 1.1	pH 4.0	pH 6.5	pH 10.2
0.1	13.2	31	140.3	35
0.2	13.1	30.5	139.8	35
0.3	...	30	139	36
0.4	12.9	30	...	36
0.5	...	30	137	36
0.6	13.1	...	...	...

pKa_2 was determined by measuring (under nitrogen) the pH at 96° of solutions prepared by mixing equal amounts of monohydrogen ascorbate (A^-) and ascorbate ($A^=$) and adding a sufficient amount of sodium chloride to give $\mu = 0.50$. The following equations were used for calculation of pKa_2

$$pKa_2 = pH - \log \frac{[A^-] - [OH^-]}{[A^-] + [OH^-]} \quad \text{(Eq. 1)}$$

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

The relationship between pH and $[OH^-]$ given by Eq. 2 was obtained as the result of pH measurements in solutions of sodium hydroxide of known hydroxyl ion concentration at 96° and $\mu = 0.50$.

It became difficult to determine pKa_2 with great accuracy. The average value of several determinations was $pKa_2 = 10.5$.

RESULTS AND DISCUSSION

Comparison of the Results Obtained by the Iodometric Method and by the Dichlorophenol-Indophenol Method.—Solutions of different pH were heated at 96° and the residual ascorbic acid concentration determined at appropriate intervals according to the iodometric method and the dichlorphenol-indophenol method. The half life ($t_{1/2}$) was found graphically. Table I shows that the two methods gave essentially the same results.

Order of Reaction with Respect to Ascorbic Acid.—The rate of disappearance of ascorbic acid from the solutions was found to be first order with respect to ascorbic acid at pH values from 0.4 up to 11.4. In all cases there was a linear relationship between time and logarithm of residual ascorbic acid concentration as shown in Fig. 1. The absence of lag time strongly suggests that the effective mechanism contains only one rate-determining step involving loss of the reducing capacity of the solutions. If ascorbic acid is initially converted to some other form still capable of reacting with iodine or the indophenol, this step would have to be exceedingly rapid. Since other data suggest that the vitamin is relatively stable under these conditions, it is unlikely that any irreversible formation of a readily oxidizable species takes place to a significant extent.

Primary Salt Effect.—A series of runs were made keeping pH, ascorbic acid concentration, and buffer concentration constant in each series but varying the ionic strength by addition of different amounts of sodium chloride. Table II shows that there was no primary salt effect at pH 1.1, 4.0, 6.5, or 10.2.

Catalytic Effect of General Acids and Bases on the Anaerobic Degradation of Ascorbic Acid.—The catalytic effect of different buffers was determined by making runs with each buffer varying the total buffer concentration by keeping the pH and the ionic strength constant ($\mu = 0.50$). Straight lines were obtained by plotting the observed k values against buffer concentration, the slopes of which were a measure of the catalytic activity of the buffers.

Figure 2 shows that phosphate buffers with pH 2.14, 2.60, and 3.05 have a catalytic effect on the anaerobic degradation of ascorbic acid. At pH $\lesssim$ 2.60, more than 95% of the total ascorbic acid will exist in the undissociated form. It is therefore reasonable to assume that the curves representing the runs at pH 2.14 and pH 2.60 give a picture of the catalytic effect of H_3PO_4 and $H_2PO_4^-$ on the undissociated ascorbic acid. If we assume this, it is possible to calculate the catalytic constants $k_{H_3PO_4}$ and $k_{H_2PO_4^-}$ of H_3PO_4 and H_2PO_4, using the equations

$$k_{obs} = k_o + k_{H_3PO_4} \cdot [H_3PO_4] + k_{H_2PO_4} - [H_2PO_4^-] \quad \text{(Eq. 3)}$$

$$\frac{[H_2PO_4^-]}{C - [H_2PO_4]} = \frac{ka_1}{[H^+]} \quad \text{(Eq. 4)}$$

where k_{obs} = the observed rate constant, k_o = the rate constant at zero buffer concentration = the intercept of the lines with the y-axis, C = total buffer concentration, and $ka_1 = 8.9 \times 10^{-3}$ at 96°, and $\mu = 0.50$.

On this basis, $k_{H_3PO_4} = 3.64 \times 10^{-2}$ hours^{-1} mole^{-1} L.; $k_{H_2PO_4^-} = 4.13 \times 10^{-2}$ hours^{-1} mole^{-1} L.

Figure 3 shows the catalytic effect of phosphate buffers pH 6.00 to 7.50. The zero slopes of the lines representing the runs at pH 7.04 and pH 7.50, where more than 99% of the total ascorbic acid exists as the monohydrogen ascorbate ion, indicates that both

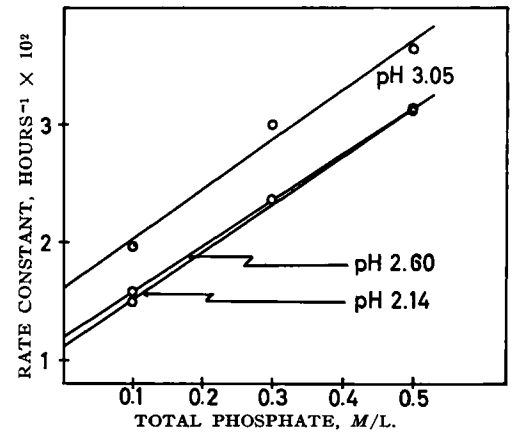
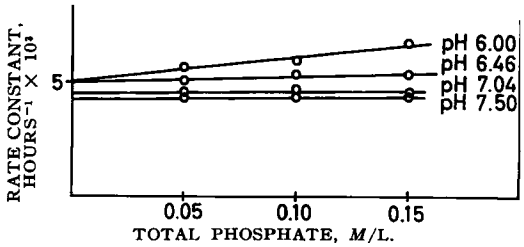


Fig. 2.—Plots showing the effect of phosphate concentration on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at fixed pH values. All the runs were made at 96°C. and an ionic strength of 0.50.



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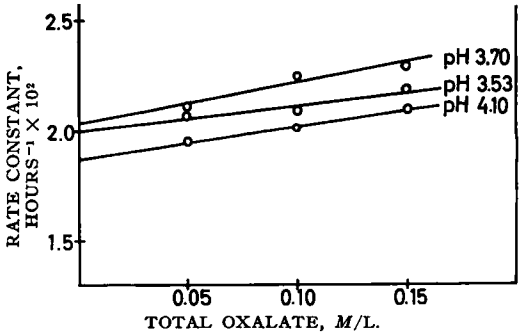

Fig. 4.—Plots showing the effect of oxalate concentration on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at fixed pH values. All the runs were made at 96°C. and an ionic strength of 0.50.

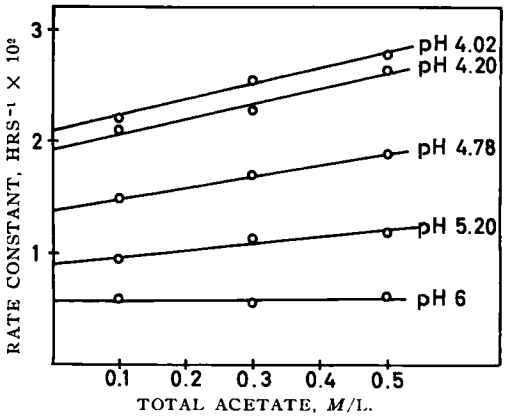

Fig. 5.—Plots showing the effect of acetate concentration on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at fixed pH values. All the runs were made at 96°C. and an ionic strength of 0.50.

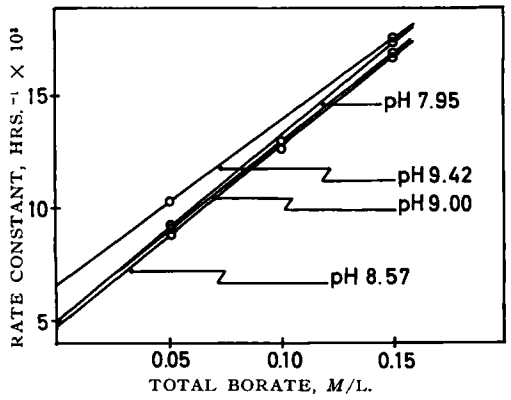

Fig. 6.—Plots showing the effect of borate concentration on the pseudo first-order rate constant of the anaerobic degradation of ascorbic acid at fixed pH values. All the runs were made at 96°C. and an ionic strength of 0.50.

$H_2PO_4^-$ and $HPO_4^=$ are noncatalytic with respect to this ion. The increasing slopes of the lines with decreasing pH may be attributed to the increase in the concentration of undissociated ascorbic acid with decreasing pH.

Oxalate buffers pH 3.7 to 4.1 and acetate buffers pH 4.02 to 6 have some catalytic effect (Figs. 4 and 5). Between pH 3.7 and 6 ascorbic acid will exist as undissociated acid and monohydrogen ascorbate ion. No attempt has been made to calculate the catalytic constants of the different species of these buffers with respect to different forms of ascorbic acid. The zero slope of the line on Fig. 5 representing the run at pH 6 seems to indicate, however, that CH_3COO^- is noncatalytic with respect to monohydrogen ascorbate ion. At pH 6, 99% of the total ascorbic acid will exist as the monohydrogen ascorbate ion, 95% of the total acetic acid will exist as the acetate ion.

The evaluation of the catalytic effect of borate buffers presents some problems because these buffers do not contain only undissociated H_3BO_3 and $H_2BO_3^-$ ions but also the ions BO_2^- and $B_4O_7^=$. The actual concentration of each of these ions cannot be readily determined. It is therefore quite difficult to make an accurate calculation of the ionic strength of the buffer solutions and to calculate the catalytic constant of each specie. The first of the difficulties mentioned may be handled by using the buffer in a relatively low concentration and adding a relatively great amount of a neutral salt. The ionic strength will then be determined mainly by the neutral salt. In our experiments the total buffer concentration never exceeded 0.15 *M* and sodium chloride was added to $\mu = 0.50$.

It is obvious from Fig. 6 that borate buffers have a pronounced apparent catalytic effect on the anaerobic degradation of ascorbic acid. At pH $\gtreqless$ 8.57, more than 98% of the total ascorbic acid will exist as the monohydrogen ascorbate ion. If we make the assumption that boric acid at pH $\gtreqless$ 8.57 will exist mainly as H_3BO_3 and $H_2BO_3^-$, it is possible to calculate the catalytic constants of these species with respect to the monohydrogen ascorbate ion.

We found $k_{H_3BO_3} = 8.03 \times 10^{-2}$ hours^{-1} mole^{-1} L.; $k_{H_2BO_3^-} = 7.81 \times 10^{-2}$ hours^{-1} mole^{-1} L.; pKa of boric acid was determined to be 8.59 at 96°; and $\mu = 0.50$.

It should be kept in mind that no attempt is made here to postulate the exact mode of catalysis. It is, of course, possible that an ester species may be involved with the borate system.

pH-Rate Profile of the Anaerobic Degradation of Ascorbic Acid.—The rate constants at zero buffer concentration can easily be picked from Figs. 2–6. The rate constants are shown in Fig. 7 as a function of pH. The rate constants given in Fig. 7 for pH<2 and pH>10 were found by making runs using perchloric acid solutions or sodium hydroxide solutions of known concentration. pH of the strongly acid solutions at 96° and $\mu = 0.5$ were calculated from

$$\text{pH} = -\log [H^+] + 0.08 \qquad \text{(Eq. 5)}$$

Equation 2 was used for the calculation of pH of the strongly basic solutions.

The pH rate profile (Fig. 7) suggests that the overall degradative rate represents a summation of a relatively large number of separate reactions. It is not possible from the experimental data to establish the correct reactions with certainty, but the following reactions would give a pH rate profile that appears to fit the experimental points well.

$$A + H^+ \xrightarrow{k_1} \text{products} \qquad \text{(Reaction 1)}$$

$$A \xrightarrow{k_2} \text{products} \qquad \text{(Reaction 2)}$$

$$A \cdot A^- \xrightarrow{k_3} \text{products} \qquad \text{(Reaction 3)}$$

$$A^- + A \xrightarrow{k_4} \text{products} \qquad \text{(Reaction 4)}$$

$$A^- \xrightarrow{k_5} \text{products} \qquad \text{(Reaction 5)}$$

$$A^= \xrightarrow{k_6} \text{products} \qquad \text{(Reaction 6)}$$

In these reactions, A = undissociated ascorbic acid, A^- = monohydrogen ascorbate ion, $A^=$ = ascorbate ion; and $A \cdot A^-$ = a complex of undissociated ascorbic acid and monohydrogen ascorbate ion.

The overall velocity is equal to the sum of the rates of all these reactions

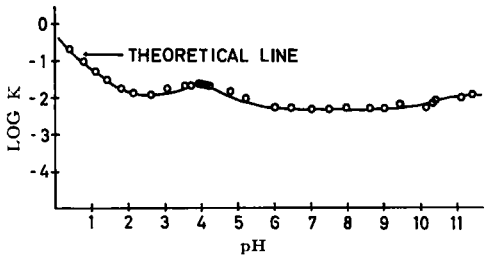

Fig. 7.—pH rate profile of the anaerobic degradation of ascorbic acid at 96°C. The circles represent the experimental results; the line corresponds to that expected theoretically from the six proposed reactions.

$$-\frac{d[A_T]}{dt} = k_1[A][H^+] + k_2[A] + k_3[A\cdot A^-] + k_4[A^-][A] + k_5[A^-] + k_6[A^=] \quad \text{(Eq. 6)}$$

$$[A_T] = [A] + [A^-] + [A^=] \quad \text{(Eq. 7)}$$

If we make the assumption that A^- will exist mainly bound as $A\cdot A^-$ at pH pKa_1, then $[A\cdot A^-]$ will be approximately equal to $[A^-]$.

At pH<pKa_1 $[A^=]$ will be negligible. This means that the following equation will be valid at pH<pKa

$$-\frac{d[A_T]}{dt} = k_1[A][H^+] + k_2[A] + k_3[A^-] + k_4[A^-][A] + k_5[A^-] \quad \text{(Eq. 8)}$$

Because of the overall first-order character of the reaction we have

$$-\frac{d[A_T]}{dt} = k\cdot[A_T] \quad \text{(Eq. 9)}$$

Combining Eqs. 7, 8, 9, and the equations

$$k_{a_1} = \frac{[H^+][A^-]}{[A]} \quad \text{(Eq. 10)}$$

$$k_{a_2} = \frac{[H^+][A^=]}{[A^-]} \quad \text{(Eq. 11)}$$

gives

$$k = \frac{k_1[H^+]^2 + k_2[H^+] + (k_3 + k_5)ka_1}{[H^+] + ka_1} + \frac{k_4ka_1[H^+]A_T}{([H^+] + ka_1)^2} \quad \text{(Eq. 12)}$$

Assuming A to exist mainly as $A\cdot A^-$ at pH>pKa_1, $[A\cdot A^-]$ will be approximately equal to $[A]$. At pH>pKa_1 $k_1[A][H^+]$ will be negligible compared to the other terms in Eq. 6 and we may write

$$-\frac{d[A_T]}{dt} = k_2[A] + k_3[A] + k_4[A^-][A] + k_5[A^-] + k_6[A^=] \quad \text{(Eq. 13)}$$

Combining Eqs. 7, 9, 10, 11, and 13 gives

$$k = \frac{(k_2 + k_3)[H^+]^2 + k_5ka_1[H^+] + k_6ka_1ka_2}{[H^+]^2 + ka_1[H^+] + ka_1ka_2} + \frac{k_4ka_1[H^+]^3A_T}{([H^+]^2 + ka_1[H^+] + ka_1ka_2)^2} \quad \text{(Eq. 14)}$$

From the experimental results the following k values have been calculated: $k_1 = 3.80 \times 10^{-1}$ hours^{-1}; $k_2 = 0.89 \times 10^{-2}$ hours^{-1}; $k_3 = 3.05 \times 10^{-2}$ hours^{-1}; $k_4 = 3.44 \times 10^{-1}$ hours^{-1} mole^{-1} L.; $k_5 = 5 \times 10^{-3}$ hours^{-1}; and $k_6 = 1.21 \times 10^{-2}$ hours^{-1}.

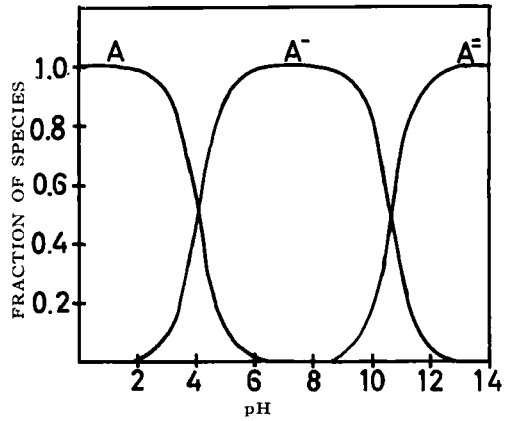

Fig. 8.—Fraction of ascorbic acid species in solution as functions of pH at 96°C. as calculated from the experimentally determined pKa_1 and pKa_2. A = Undissociated ascorbic acid, A^- = monohydrogen ascorbate ion, $A^=$ = ascorbate ion.

On the basis of the species profile shown in Fig. 8, the overall rate profile was calculated using Eq. 12 at pH<pKa_1 and Eq. 14 at pH>pKa_1; the result is shown as a solid line in Fig. 7.

Reaction 4 is suggested since it was found that the half life of the ascorbic acid degradation at pH 4 (= pKa_1 of ascorbic acid) was to some extent dependent on the total ascorbic acid concentration (Table III). No buffer was added in these experiments.

However, there is only a slight decrease in $t_{1/2}$ with increasing ascorbic acid concentration. This means that the apparent dominating reaction or reactions at pH 4 must be first order. Straight lines were obtained in each experiment by plotting the logarithm of residual ascorbic acid concentration against time.

Reaction 4 has nearly no influence on the shape of the calculated profile. At pH 4 where the influence is greatest, the calculated log k value is -1.65 with $k_4 = 3.44 \times 10^{-1}$, and -1.66 with $k_4 = 0$.

The relatively good agreement of the experimental data and the theoretical profile does not of course prove that the proposed Reactions 1–6 are the correct ones. Other reactions could lead to the same observed experimental dependencies.

At pH 6–9 the following three reactions would lead to the same log k/pH dependency

$$A^- \rightarrow \text{products}$$
$$A^= + H^+ \rightarrow \text{products}$$
$$A + OH^- \rightarrow \text{products}$$

The lack of primary salt effect at pH 6.5 indicates that the dominating reaction cannot be $A^= + H^+ \rightarrow$ products, but one of the other two. We have proposed the reaction $A^- \rightarrow$ products, but the reaction $A + OH^-$ cannot be eliminated.

At pH 10 to 11.5 the following two reactions would lead to the same log k/pH relationship

$$A^- + OH^- \rightarrow \text{products}$$
$$A^= \rightarrow \text{products}$$

TABLE III.—HALF-LIFE OF ANAEROBIC ASCORBIC ACID DEGRADATION AT 96°C. AND pH 4 AND DIFFERENT ASCORBIC ACID CONCENTRATIONS

Total Ascorbic Acid Concn., M/L.	$t_{1/2}$, hrs.
0.01	30.3
0.02	29.3
0.03	28.3
0.04	26.8
0.05	25.8

The lack of primary salt effect at pH 10.2 indicates that the dominating reaction cannot be $A^- + OH^- \rightarrow$ products but $A^- \rightarrow$ products.

Attempts to Prove the Existence of an Ascorbic Acid–Monohydrogen Ascorbate Complex.—Reaction 3 presupposes the existence of a complex between ascorbic acid and the monohydrogen ascorbate ion.

It is well known that organic acids are able to form complexes with their anions. Complex formation has been reported between mandelic acid and metal mandelate (7), salicylic acid and sodium salicylate (8), hippuric acid and potassium hippurate (8), succinic acid and potassium succinate (9), benzoic acid and sodium (10), and ammonium benzoate (11), *p*-hydroxybenzoic acid, dihydroxy benzoic acids, phenylacetic acid, adipic acid, saccharin, barbituric acid, barbital, phenobarbital, and their sodium salts (12).

The possible complex formation between ascorbic acid and monohydrogen ascorbate has been investigated by the authors by determination of freezing point depression, boiling point elevation, and solubility studies.

By calculation of the freezing point depression Δt, the following equation was used

$$\Delta t = p - q - 0.0125(p-q)(q-a)$$

where p = the freezing point of water, q = the freezing point of the solution, and a = the temperature to which the solution had been supercooled. Δt for a 0.5 M ascorbic acid solution was 0.943°. Δt for a 0.5 M ascorbic acid solution to which NaOH had been added to pH 4 was 1.301°.

For a 0.05 M ascorbic acid solution the corresponding values were 0.093 and 0.128°.

The boiling point elevation was determined according to Landsberger. There is by this method a condensation of vapor in the solution which is being studied. In our experiments we always used the same amount of a 0.5 M ascorbic acid solution and tried to get nearly the same amount of vapor condensed in each run. The final ascorbic acid concentration was therefore nearly the same in all experiments. Expressed as molality, it was 0.45. The average boiling point elevation Δt of the ascorbic acid solution with the final molality 0.45 was 0.19°. A solution of the same final molality but with NaOH added to pH 4 gave an average boiling point elevation $\Delta t = 0.27°$.

If the addition of sodium hydroxide to the ascorbic acid solutions (to pH 4) had caused no complex formation and if the influence of the ionic interactions in the solutions with pH 4 is neglected, the change in freezing point depression and boiling point elevation would have been 50%. The changes found were 38% for freezing point depression and 42% for the boiling point elevation. Taking into account the influence of the electrostatic attractions between the oppositely charged ions in the solutions with pH 4, our results show either that there is none or there is only a negligible complex formation between ascorbic acid and monohydrogen ascorbate, or the intermolecular forces in the complex are so weak that the complex formation cannot be detected by determination of freezing point depression or boiling point elevation.

Solubility studies were conducted according to the method of Higuchi and Lach (13). Five grams of ascorbic acid was added to 5 ml. of sodium monohydrogen ascorbate solutions of varying concentrations (0–3 M). The mixtures were placed in stoppered glass vials and rotated in a water bath at 30° ±0.1° overnight. An aliquot part of the clear solution was pipeted off and the ascorbic acid concentration determined by titration with 1 N sodium hydroxide.

Accurate results were impossible to obtain because of the high viscosity of the solutions. There was, however, a slight increase in the solubility of ascorbic acid with increasing salt concentration, thus indicating some complex formation. In our opinion it is difficult to draw definite conclusions from solubility studies in these very concentrated solutions and more so since an inseparable salting out effect cannot be excluded.

Our experiments do not prove the existence of a complex between ascorbic acid and monohydrogen ascorbate. On the other hand, they do not exclude this possibility. From a kinetical point of view a reaction that presupposes the existence of an ascorbic acid/ascorbate complex can explain the observed first-order character and the maximum in the pH rate profile at pH = pK_{a_1} of ascorbic acid.

REFERENCES

(1) Reichstein, T., and Grüssner, A., *Helv. Chim. Acta* **17**, 311(1934).
(2) Regna, P. P., and Caldwell, B. P., *J. Am. Chem. Soc.*, **66**, 246(1944).
(3) Taylor, E. W., Fowler, W. F., McGee, P. A., and Kenyon, W. O., *ibid.*, **69**, 342(1947).
(4) von Euler, H., and Hasselquist, H., *Arkiv Kemi*, **4**, 169(1952).
(5) Fischer-Jensen, E., *Dansk Tidsskr. Farm.*, **29**, 125 (1955).
(6) Cier, A., Nofre, C., and Drevon, B., *Bull. Soc. Chim. France*, **1959**, 74.
(7) Ross, J. D., and Morrison, T. J., *J. Chem. Soc.*, **1933** 1016.
(8) Hoitsema, C., *Z. Physik. Chem.*, **27**, 312(1898).
(9) Marshall, H., and Cameron, A. T., *J. Chem. Soc.*, **91**, 1519(1907).
(10) Kolthoff, I. M., and Bosch, W., *J. Phys. Chem.*, **36**, 1685(1932).
(11) Higuchi, T., Gupta, M., and Busse, L. W., THIS JOURNAL, **41**, 122(1952).
(12) Valinoti, M. R., and Bolton, S., *ibid.*, **51**, 201(1962).
(13) Higuchi, T., and Lach, J. L., *ibid.*, **43**, 349(1954).