U.S. Department of Commerce, Bureau of Standards

RESEARCH PAPER RP611

*Part of Bureau of Standards Journal of Research, Vol. 11, November 1933*

# THE STABILITY OF LEVULOSE IN AQUEOUS SOLUTIONS OF VARYING pH

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ABSTRACT

Levulose was subjected at five temperatures to the action of buffer solutions whose pH varied from −2.6 to 14.2. In its earlier stages the decomposition reaction is unimolecular and approaches an equilibrium which cannot be realized but by a new method can be calculated. The equilibrium values are a function of pH and give evidence of the nature of the reaction. In acid solutions levulose is dehydrated to form levulosans of negative rotation, in alkaline solutions the familiar Lobry de Bruyn-van Ekenstein transformation occurs, and in intermediate reactions both transformations occur. Velocity constants of the early stages of the decomposition were measured in 50 experiments. Polariscopic, total reducing sugar, and selective levulose analyses gave approximately the same velocity constants. A plot of these velocity constants against pH showed that the stability of levulose passed through a maximum at pH 3.3. Neutral solutions are alkaline with respect to this maximum. The temperature coefficient of the reaction at constant pH is shown to be relatively high. The periods of time required for a 1 percent decomposition of levulose are tabulated as a function of pH and temperature.

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## I. INTRODUCTION

Levulose is one of the more reactive sugars and in aqueous solution is particularly unstable at the extreme ranges of pH. In the process of its preparation from the juices of the Jerusalem artichoke or other natural sources by the method described by Jackson, Silsbee, and Proffitt [1] the sugar is necessarily subjected to relatively high acidity during the hydrolysis of the polysaccharides, to high alkalinity during the calcium levulate precipitation, and to intermediate reactions after the pure substance has been isolated in aqueous solution. It therefore becomes important to determine the degree of stability of levulose under the conditions to which it is subjected in order to evaluate and as far as possible minimize the losses. It is expected, moreover, that the data secured in the present investigation will have a more general applicability since levulose is a constituent of practically all fruit juices and occurs in a great variety of commercial sirups and other products.

[1] B. S. Sci. Papers No. 519, vol. 20, p. 519, 1926.

Nef [2] has shown that the prolonged action of relatively strong alkali leads to a complete disintegration of the levulose molecule with the possible formation of about 116 products. Lobry de Bruyn and van Ekenstein [3] showed that action of mild alkali leads to formation of an equilibrium between levulose, dextrose, and mannose, established by rearrangement of the common dienol.

Strong acid solutions have been shown by Pictet and Chavan [4] to yield by dehydration heterolevulosan as a primary product. This substance in the same series of reactions condenses in part to the dimer, diheterolevulosan. More prolonged action of acid solutions results in the formation of levulinic and formic acids, oxymethyl furfural, and humus substances of high carbon content.[5] In none of the work cited has the reaction velocity been measured.

In the present investigation we have sought to measure the velocities of the respective reactions in their initial stages as functions of pH and temperature and, by computing the time required for decomposition of the first 1 percent of levulose, to state in quantitative terms the stability of the sugar under the specified conditions.

We emphasize the fact that the reactions here studied are not susceptible to exact measurement since they are in many cases accompanied by competitive, consecutive, and secondary reactions. We have based our conclusions upon statistics drawn from a large number of observations, none of which has been rejected. That the conclusions are fundamentally correct is attested by the regularity with which the experimental data, all acquired independently, fit into a system of curves.

## II. METHODS

Pure crystalline levulose or levulose sirups of about 99 percent purity were mixed with buffer solutions of systematically varying pH and subjected to the influence of carefully controlled temperatures for such periods of time as permitted measurements of the rates of decomposition. The concentration of levulose in the reaction mixture was varied between 2 and 10 grams per 100 ml.

The change in the reaction mixture was followed in every instance polariscopically, and in selected cases by change in reducing sugar, and in levulose determined selectively.

Polariscopic measurements were made with a Bates type quartz-wedge saccharimeter in water-jacketed polariscope tubes. Total reducing sugars were determined by Lane and Eynon's volumetric method [6] and levulose selectively by Jackson and Mathews' [7] modification of Nyns' method. The copper reduction methods were carried out in duplicate and were controlled by analyses of standard solutions of pure levulose.

The buffering substances were those recommended by Clark and by Sørensen. The concentrations of buffer in the buffer-sugar mixtures were, however, made double those given by Clark [8] in order that the decomposition products of levulose should alter the pH as slightly as possible. Borate buffers were found unsuitable because

[2] Ann., vol. 335, p. 191, 1904; vol. 357, p. 214, 1907; vol. 376, p. 1, 1910; vol. 403, p. 204, 1914.
[3] Rec. trav. Chim., vol. 14, p. 156 and 203, 1895; vol. 16, p. 256, 1897.
[4] Helv. Chim. Acta, vol. 9, p. 809, 1926.
[5] v. Lippmann, Die Chemie der Zuckerarten, vol. I, p. 837, 1904. Vieweg und Sohn, Braunschweig.
[6] J.Soc.Chem.Ind., vol. 42, p. 32, 1923.
[7] B. S. Jour. Research, vol. 8 (RP426), p. 422, 1932; J. Assoc. Off. Agri. Chemists, vol. 15, p. 198, 1932.
[8] The Determination of Hydrogen Ions. Williams and Wilkins, Baltimore, Md.

the reaction between the sugar and the borate ion caused a change in the pH of the buffer mixture. Phosphate, phthalate, and glycine buffers showed no change in pH upon addition of levulose.

The pH of the buffer-sugar mixture was determined at intervals during the course of the reaction either by the colorimetric method or with the quinhydrone electrode at the temperature of the room, and the pH found was corrected to the temperature of the reaction by temperature coefficients accompanying the buffer tables. In some experiments a slight but progressive change of pH occurred as a result of acid formation. In such cases a weighted average was accepted for tabulation.

Sodium hydroxide solutions were used to obtain reaction mixtures of hydroxyl ion concentration greater than that represented by pH 13.5, the upper limit of the glycine buffers. For such a reaction mixture the pH was not determined by direct measurement, but was calculated on the assumption that the activity of the hydroxyl ion in the levulose solution was the same as that in the pure aqueous sodium hydroxide solution. Such an assumption is not strictly correct, for levulose acts as a very weak acid [9] (dissociation constant is $9\times10^{-13}$) and unites with 1 molecule of the hydroxide. However, the change in the pH of the solution on the addition of sugar was assumed to be negligible, especially when the molar concentration of the hydroxide was greater than that of the sugar.

Hydrochloric and sulphuric acids of the proper normality were used to maintain hydrogen ion concentrations greater than that represented by pH 1.2. Here again the pH was calculated on the assumption that the sugar was without effect on the activity of the acid.

The method of calculating pH from the activities is as follows:

For acids

$$\mathrm{pH}=\log 1/\gamma N$$

where $\gamma$ is the activity coefficient of the hydrogen ions in an $N$ normal solution of the acid.

For alkalis

$$\mathrm{pH}=\log 1/K_w-\log 1/\gamma N$$

in which $K_w$ is the dissociation constant of water, and $\gamma$ the activity coefficient of hydroxyl ion in an $N$ normal solution of the alkali.

The activity coefficients are given by Lewis and Randall.[10] It should be noted that pH as used in the present paper is a measure of activity and not of concentration.

The types of buffers and the ranges for which they were used were as follows:

| Type of buffer | pH |
|---|---|
| Hydrochloric or sulphuric acids | −2. 6 to 1. 2 |
| Glycine-hydrochloric acid | 1. 4 to 2. 0 |
| Phthalate-hydrochloric acid | 3. 0 |
| Phthalate-sodium hydroxide | 4. 0 to 6. 0 |
| Phosphate-sodium hydroxide | 6. 0 to 8. 0 |
| Glycine-sodium hydroxide | 8. 0 to 13. 4 |
| Sodium hydroxide | 13. 7 to 14. 4 |

[9] Michaelis and Rona, Biochem. Zeit., vol. 49, p. 232, 1913.

[10] Thermodynamics and the Free Energy of Chemical Substances. McGraw-Hill Book Co., Inc., New York, 1923.

The reaction mixtures were kept at constant temperature during the course of the reaction, samples being withdrawn for analysis at proper intervals of time. In a few instances the reaction was followed by direct observation in a water-jacketed polariscope tube. When the rate of reaction was high the reaction was stopped by adding to a measured sample a definite quantity of acid or alkali which would give a pH near neutrality. The whole was then made to a definite volume with water and analyzed. All the polarizations for any one run were made under identical conditions, where possible, to eliminate

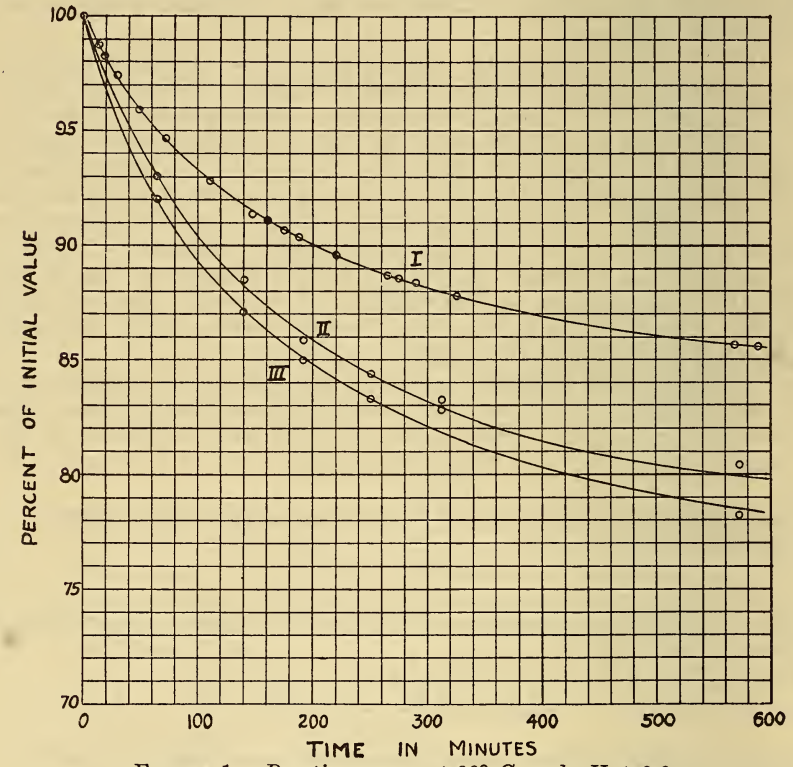



FIGURE 1.—*Reaction curves at 20° C. and pH −2.0.*

(5 g levulose per 100 ml; buffer, 8.31 *N* HCl. Curve I shows change in polarization; II, reducing sugar; III, levulose.)

errors that might have been introduced by correcting the readings for temperature and dilution. Rapid reactions were followed to the point where the development of color or turbidity made polarization difficult. This point was reached when the polarization had fallen from 10 to 30 percent, which change was sufficient for the calculation of the velocity constants. Slower reactions were followed until the polarization had changed enough to give velocity constants of sufficient accuracy.

## III. TYPICAL REACTION CURVES AND THE DETERMINATION OF THE EQUILIBRIUM

By the methods described above, 50 experiments were made to determine the rates of decomposition of levulose under widely varying conditions of pH and temperature.

The courses of two typical reactions are given in figures 1 and 2, the former showing the decomposition in strongly acid solution, the latter in alkaline solution. The experimental observations are given in tables 1 and 2.

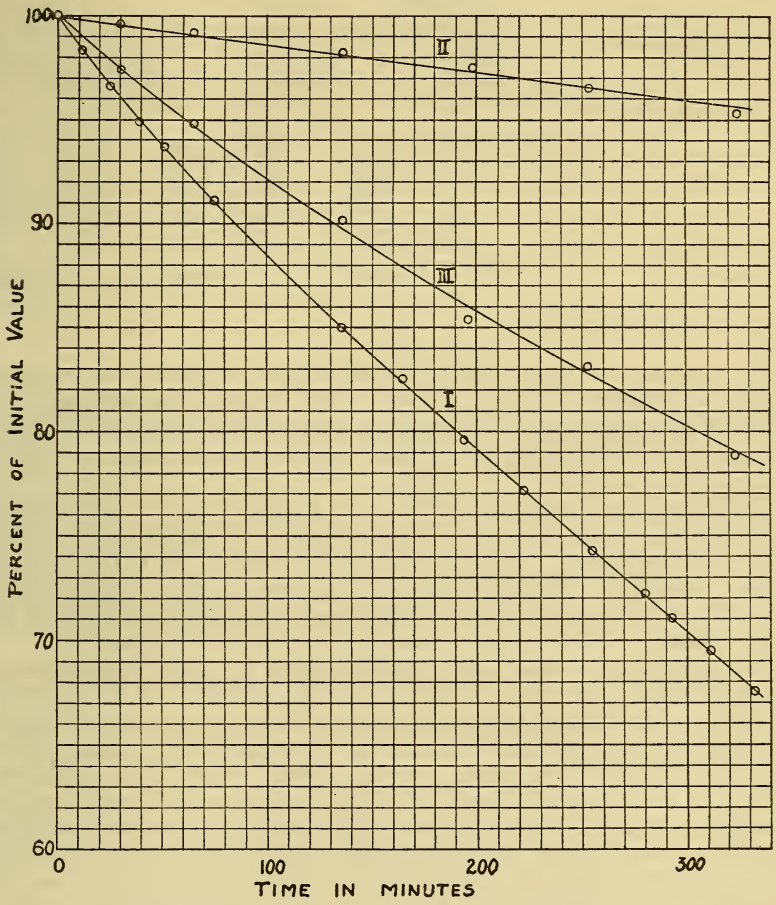



FIGURE 2.—*Reaction curves at 20° C. and pH 13.4.*
(5 g levulose per 100 ml; buffer, glycine-sodium hydroxide. Curve I shows change in polarization; II, reducing sugar; III, levulose.)

TABLE 1.—*Decomposition of levulose at 20° C. in 8.3 N HCl, pH—2.0*
(5 g levulose per 100 ml)

| Time (in minutes) | Polarization | | Reducing sugar | | Levulose | |
|---|---|---|---|---|---|---|
| | Percent of initial reading | *K* | Percent of initial concentration | *K* | Percent of initial concentration | *K* |
| 0 | 100.0 | | 100.0 | | 100.0 | |
| 64 | 94.9 | 0.0028 | 93.0 | 0.0022 | 92.0 | 0.0026 |
| 140 | 91.6 | .0026 | 88.5 | .0024 | 87.1 | .0022 |
| 192 | 90.3 | .0024 | 85.9 | .0025 | 84.8 | .0021 |
| 251 | 89.1 | .0023 | 84.3 | .0025 | 83.2 | .0019 |
| 313 | 88.0 | .0023 | 83.3 | .0024 | 82.9 | .0016 |
| 573 | 85.7 | .0022 | 80.5 | .0016 | 78.2 | .0019 |
| ∞ | [1] (85.1) | | [1] (75.5) | | [1] (74.8) | |
| Average | | 0.0024 | | 0.0023 | | 0.0021 |

[1] Values in parentheses are calculated.

TABLE 2.—*Decomposition of levulose at 20° C. in a glycine-sodium hydroxide buffer, pH 13.4*

(5 g levulose per 100 ml)

| Time (in minutes) | Polarization | | Reducing sugar | | Levulose | |
|---|---|---|---|---|---|---|
| | Percent of initial reading | $K$ | Percent of initial concentration | $K$ | Percent of initial concentration | $K$ |
| 0 | 100.0 | | 100.0 | | 100.0 | |
| 30 | 95.8 | 0.00081 | 99.6 | 0.00074 | 97.4 | 0.00069 |
| 65 | 92.0 | .00073 | 99.2 | .00071 | 94.8 | .00063 |
| 136 | 85.0 | .00069 | 98.2 | .00068 | 90.1 | .00059 |
| 198 | 79.2 | .00074 | 97.5 | .00097 | 86.4 | .00058 |
| 253 | 74.4 | .00071 | 96.5 | .00081 | 83.0 | .00059 |
| 323 | 68.3 | .00071 | | | 78.8 | .00061 |
| ∞ | [1] (23.0) | | (91.9) | | (41.6) | |
| Average | | 0.00073 | | 0.00078 | | 0.00062 |

[1] Values in parentheses are calculated.

The number of polarimetric observations exceeds the number indicated in these two tables, but all of the observed polarizations are plotted in figures 1 and 2. These two figures show diagrammatically the changes in polarization, reducing sugar, and levulose during the course of the reaction.

The form of the curves in figures 1 and 2 suggested that the reaction was of the unimolecular type and approached an equilibrium. That the reaction is indeed unimolecular is supported by that fact that the velocity constants obtained under this assumption proved to be essentially independent of the concentration of sugar, at least in the dilute solutions which alone were studied in this investigation.

It was, however, found to be impracticable to run the reactions to the equilibrium and thus obtain the final rotation of the solution because the coloring materials formed made an accurate polarization impossible. The clarification and decolorization of the dark solution by lead acetate or activated carbon would remove an unknown amount of the products and introduce errors which could not be evaluated. Indeed, it is doubtful if the final polarization at equilibrium would be of value in calculating the velocity constant for the initial reaction, since the equilibrium is undoubtedly influenced by secondary and consecutive reactions. Under such circumstances a calculated value for the equilibrium is more significant, inasmuch as it shows the equilibrium which the initial reaction would approach if it could be entirely separated from the secondary reactions. It is impossible to separate the various reactions in practice, but if only the initial reaction is considered, that is, only the disappearance of levulose during the initial period of reaction, the influence of the secondary reactions will be small, and the resulting velocity constant should approximate that which would be found if the initial reaction could be isolated.

Calculations of equilibrium values were made by application of the formula for unimolecular reactions. The method of calculation is illustrated by its application to polarization changes, but is equally applicable to the reducing sugar and levulose data.

Polarizations are observed or read from a curve at exactly equal intervals of time. If $a$, $b$, $c$, $d$, etc., are the polarizations at the times,

$t_1$, $t_2$, $t_3$, $t_4$, etc., the time intervals between observations being equal, we can set up a series of equations of the form,

$$K=\frac{1}{t_2-t_1}\log\frac{a-x}{b-x} \tag{1}$$

in which $K$ is the unknown velocity constant and $x$ the polarization at equilibrium.

Elimination of $K$ between two of these equations gives

$$\frac{1}{t_2-t_1}\log\frac{a-x}{b-x}=\frac{1}{t_4-t_3}\log\frac{c-x}{d-x}$$

But as $t_2-t_1=t_4-t_3$,

$$\frac{a-x}{b-x}=\frac{c-x}{d-x}$$

Solving for $x$,

$$x=\frac{ad-bc}{a+d-b-c} \tag{2}$$

Equation (2) was used in the calculation of equilibrium polarizations. It was found that the polarizations from the initial portions of the velocity curves gave constant values for the equilibrium polarizations, but extension of the calculations to the polarizations made at later periods of the reaction, when much color had developed, gave values for the equilibrium polarization that were progressively smaller, indicating that secondary reactions had begun to interfere. The average value from the initial portion of the reaction curve was used in the calculation of the velocity constant.

## IV. THE VARIATION OF EQUILIBRIUM VALUES WITH pH

Eight experiments in which the reaction products were determined by three methods of analysis were carried out at such a distribution of pH as permitted plotting continuous curves of the equilibrium values throughout the whole range. Since it proved true that the equilibria were dependent solely upon the pH and were independent of temperature, such curves enabled us to determine by interpolation the equilibrium values for the large number of experiments which were required to cover the whole range of pH and temperature.

The equilibrium values expressed in terms of percent of initial concentration or polarization, together with the velocity constants calculated from the three independent series of analyses, are shown in table 3. The three velocity constants are, with some exceptions, in satisfactory agreement, showing that the rates of change indicated by the three methods of analysis are essentially the same. The general agreement of the three sets of measurements is shown by the close agreement of the averaged values. These averages of course have no other significance.

Since the polariscopic method furnished a reliable measure of reaction rate, it alone was used for the remaining measurements.

TABLE 3.—*Decompositions for which the equilibrium values of polarization, reducing sugar, and levulose were determined*

(Equilibrium values expressed as percent of initial value, velocity constants in terms of minutes and common logs)

| Run no. | Temperature | pH | Type of buffer | Polarization | | Reducing sugar | | Levulose | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Equilibrium value | $K$ | Equilibrium value | $K$ | Equilibrium value | $K$ |
| | °C. | | | Percent | | Percent | | Percent | |
| 1 | 20 | −2.0 | 8.3 *N* HCl | 85.1 | 0.00254 | 75.5 | 0.00232 | 74.8 | 0.00213 |
| 2 | 45 | −1.2 | 5.45 *N* HCl | 80.5 | .0084 | 75.5 | .0120 | | |
| 3 | 100 | 1.4 | HCl-glycine | 55.3 | .00127 | 81.2 | .00183 | 73.2 | .00190 |
| 4 | 100 | 4.0 | NaOH-phthalate | 46.5 | .000059 | 86.9 | .000068 | 72.7 | .000057 |
| 5 | 100 | 7.3 | NaOH-$KH_2PO_4$ | 43.5 | .00564 | 93.1 | .00484 | 65.3 | .00517 |
| 6 | 45 | 10.2 | NaOH-glycine | 36.7 | .00110 | 93.4 | .00109 | 55.3 | .00108 |
| 7 | 45 | 10.4 | do | 37.1 | .00148 | 94.4 | .00120 | 55.0 | .00145 |
| 8 | 20 | 13.4 | do | 23.0 | .00078 | 91.9 | .00076 | 41.6 | .00062 |
| Average (excluding run no. 2) | | | | | .00184 | | .00173 | | .00184 |

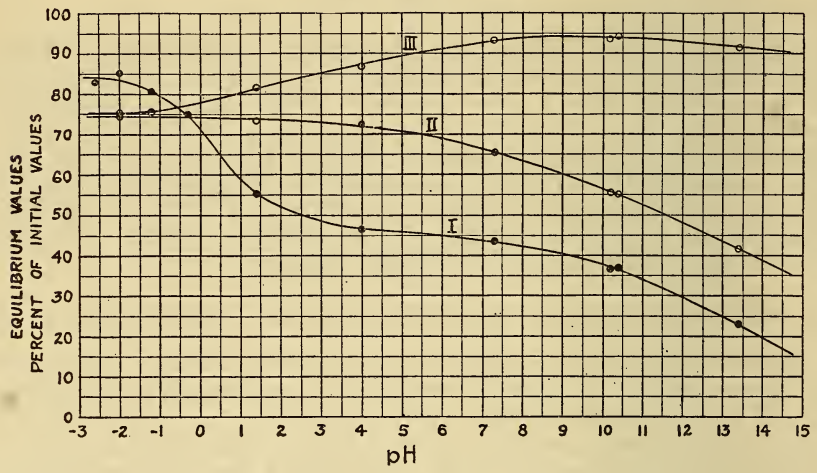



FIGURE 3.—*Showing the variation with pH of the calculated values of polarization, reducing sugar, and levulose at the equilibrium approached by the initial reaction.*

Curve I, polarization; II, levulose; III, reducing sugar.

Figure 3 shows the equilibrium values of polarization, reducing sugar, and levulose as functions of pH. In order to fix the polarization curve with greater accuracy in the range from pH −2.6 to pH 2.0, several additional points were calculated from the data of experiments in which the course of the reaction was followed by polarization only.

In general these curves indicate the type of the reaction which takes place at the various concentrations of hydrogen and hydroxyl ions. At high acidities the polarization falls by a comparatively small amount, while the loss in reducing sugar and levulose is greater. At the equilibrium the concentrations of levulose and of reducing sugar are the same, indicating that levulose is the sole reducing sugar occurring in the equilibrium mixture. The predominant reaction is, then, the formation of nonreducing substances, whose rotations are negative, but less negative than that of levulose. The average specific

rotation, $[\alpha]_D$, of the products in experiment 1 can be roughly estimated to be $-42$. Pictet and Chavan [11] found the rotation of heterolevulosan to be $-45$ and its dimer $-65$. Thus the predominant reaction is dehydration to heterolevulosan and its dimer both of which are nonreducing. Side reactions show their effects, for the rotation is slightly lower than would be expected if the two anhydro sugars were the sole products.

Thus it appears that these fructosans, which Pictet and Chavan designated heterolevulosan and diheterolevulosan, play an important role in the chemistry of levulose. Not only are they formed under the conditions of high acidity which Pictet and Chavan employed in their preparation, but they are formed in mild acidities whose pH is as high as 2. They are probably introduced in all operations in which levulose is subjected to treatment by strong or even mild acidities. Indeed it is even doubtful if cane sugar can be hydrolyzed by acid without introducing small quantities of these fructosans. Jackson and Gillis [12] determined by stop-watch experiments the minimum conditions of time and temperature for the inversion of sucrose at varying concentrations of hydrochloric acid and extrapolated their curve to zero concentration of acid. They found that twice the rotation of the half normal weight of sucrose after inversion was $-32.0°$ S. at 20° C., whereas Paine and Balch [13] found the value $-32.1°$ S. by use of invertase. The more drastic methods of acid inversion which are in common use yield still lower values. Zerban [14] found that commercial invert sugar which had been prepared by acid inversion contained reversion products which were hydrolyzable by acid and not by invertase. It is probable that these reversion products are these same fructosans.

We have repeated Pictet and Chavan's preparation, following their procedure in detail, and have isolated the crystalline fructosans having essentially the properties which they describe.

In highly alkaline solutions of levulose the predominant reaction is the Lobry de Bruyn and van Ekenstein transformation. A rough estimation of the products obtained in experiment 8 (pH 13.4) can be made. The equilibrium rotation is 23 percent of the initial rotation of levulose, hence $[\alpha]_D = -21$.

Assuming that glucose and mannose are in the ratio 5 to 1 [15] and combining the rotation of such a mixture with the rotation of the levulose remaining in the equilibrium, that is, $\frac{41.6}{91.9}$ or 45 percent of the reducing sugar, we should observe a specific rotation of $-17$ for the products of decomposition, in rough agreement with the $-21$ found. The results are sufficiently in agreement to indicate that we are dealing with the familiar Lobry de Bruyn-van Ekenstein transformation.

In the range between high hydrogen and high hydroxyl ion concentrations this transformation is favored above pH 3, while below this pH, dehydration is predominant. There is no clear-cut division point between the two types of reactions, since both can apparently take place together, the preference for the one or the other being dependent upon the pH of the solution.

[11] See footnote 4.
[12] B.S. Sci. Papers No. 375, vol. 16, p. 166, 1920.
[13] Ind. Eng. Chem., vol. 17, p. 240, 1925.
[14] J. Assoc. Off. Agri. Chemists, vol. 8, p. 396, 1925; vol. 9, p. 171, 1926; vol. 12, p. 162, 1929.
[15] Nef, Ann., vol. 357, p. 214, 1907.

In acid reactions an agreement among the three velocity constants determined by the three methods of analysis is to be expected since the changes in polarization, reducing sugar, and levulose are all

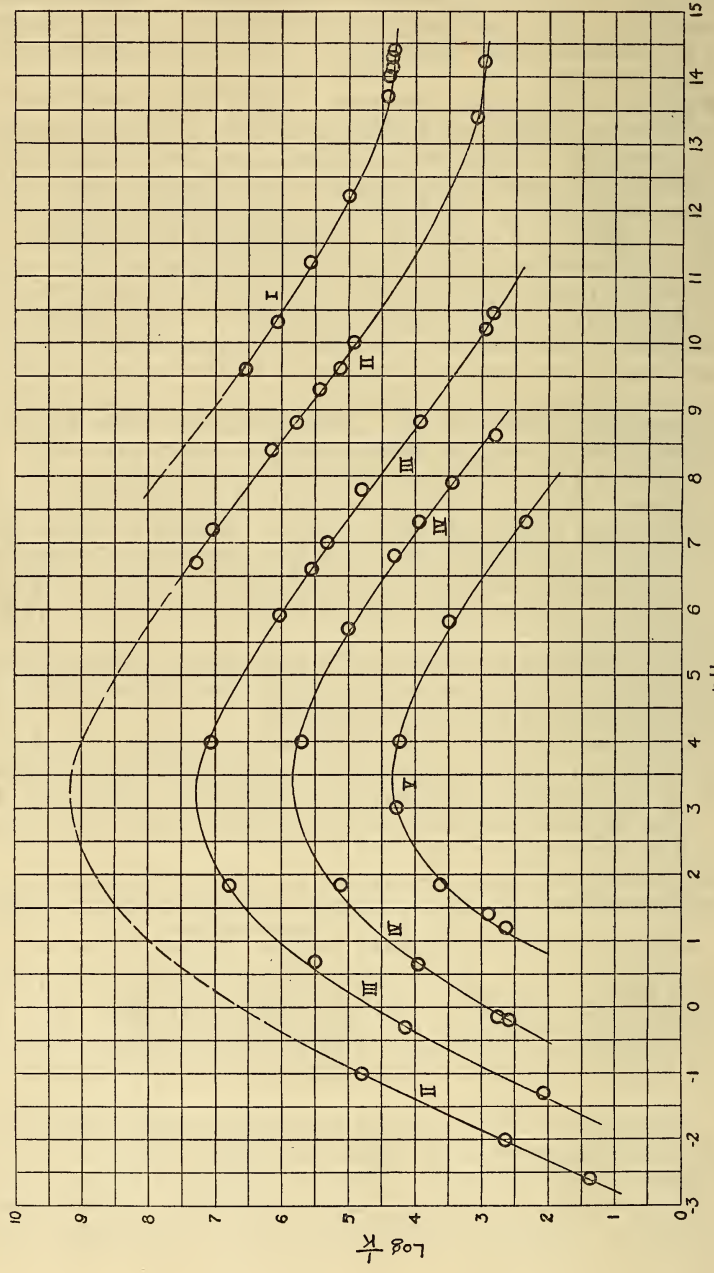



FIGURE 4.—*Variation of log 1/K with pH at I, 4° C.; II, 20° C.; III, 45° C.; IV, 70° C.; V, 100° C.*
(Levulose is most stable in solutions of pH 3.3; in more acid solutions it is transformed to levulosans; in alkaline solutions it undergoes the Lobry de Bruyn-van Ekenstein rearrangement to dextrose and mannose.)

largely measures of the same reaction, that is, the conversion of levulose into heterolevulosan. In alkaline reactions an agreement among the three constants cannot be predicted, for while the change in polar-

ization and the decrease in levulose are largely measures of the rate of conversion of levulose into glucose and mannose, the loss of reducing power is largely a measure of the rate of side reactions. The rates of change of polarization and levulose should then be approximately equal, but it does not follow a priori that loss in reducing power must proceed at the same rate. Actually, however, the rates of change of the three independent measurements are approximately equal.

## V. THE STABILITY OF LEVULOSE

In table 4 are given the results obtained in 50 experimental determinations of the rates for the decomposition of levulose in the early stages. The fifth column gives the velocity constants calculated from the observed changes in polarization. In making the calculations of these constants, the equilibrium values of the polarization were read directly from the interpolation curve in figure 3 plotted from data obtained in this series of experiments.

TABLE 4.—*Velocity constants*

| Temperature, °C. | pH | Type of buffer | Concentrations of levulose | $K$ (minutes and common logs) | Time for loss of first 1 percent of total levulose |
|---|---|---|---|---|---|
| | | | *g/100 ml* | | *Minutes* |
| 4 | 9.6 | NaOH-glycine | 2 | $2.90\times10^{-7}$ | 37,000 |
| 4 | 10.3 | do | 2 | $8.6\times10^{-7}$ | 11,500 |
| 4 | 11.2 | do | 2 | $2.71\times10^{-6}$ | 3,310 |
| 4 | 12.2 | do | 2 | $1.01\times10^{-5}$ | 850 |
| 4 | 13.7 | 0.1 *N* NaOH | 2 | $3.70\times10^{-5}$ | 190 |
| 4 | 14.0 | 0.2 *N* NaOH | 2 | $3.93\times10^{-5}$ | 174 |
| 4 | 14.1 | 0.3 *N* NaOH | 2 | $4.40\times10^{-5}$ | 158 |
| 4 | 14.3 | 0.4 *N* NaOH | 2 | $4.43\times10^{-5}$ | 151 |
| 4 | 14.4 | 0.5 *N* NaOH | 2 | $4.80\times10^{-5}$ | 138 |
| 20 | −2.6 | 12 *N* HCl | 10 | $4.15\times10^{-2}$ | 0.42 |
| 20 [1] | −2.0 | 8.3 *N* HCl | 5 | $2.54\times10^{-3}$ | 6.9 |
| 20 | −1.0 | 4.0 *N* HCl | 10 | $1.55\times10^{-5}$ | 1,000 |
| 20 | 6.7 | NaOH-$KH_2PO_4$ | 10 | $5.35\times10^{-8}$ | 248,000 |
| 20 | 7.2 | do | 10 | $9.0\times10^{-8}$ | 141,000 |
| 20 | 8.4 | NaOH-glycine | 10 | $7.3\times10^{-7}$ | 15,900 |
| 20 | 9.3 | do | 10 | $3.60\times10^{-6}$ | 3,050 |
| 20 [1] | 13.4 | do | 5 | $7.37\times10^{-4}$ | 10.0 |
| 20 | 14.2 | 2 *N* NaOH | 4 | $1.04\times10^{-3}$ | 6.6 |
| 25 | 8.8 | NaOH-glycine | 5 | $3.13\times10^{-6}$ | 3,600 |
| 25 | 9.6 | do | 5 | $1.24\times10^{-5}$ | 870 |
| 25 | 10.0 | do | 5 | $2.11\times10^{-5}$ | 468 |
| 45 [1] | −1.2 | 5.45 *N* HCl | 2 | $8.4\times10^{-3}$ | 2.05 |
| 45 | −0.3 | 2.0 *N* HCl | 8 | $7.0\times10^{-5}$ | 245 |
| 45 | 0.7 | 0.3 *N* HCl | 4 | $3.30\times10^{-6}$ | 5,200 |
| 45 | 1.85 | HCl-glycine | 10 | $1.64\times10^{-7}$ | 102,000 |
| 45 | 4.0 | NaOH-phthalate | 10 | $8.7\times10^{-8}$ | 186,000 |
| 45 | 5.9 | do | 10 | $9.3\times10^{-7}$ | 15,500 |
| 45 | 6.6 | NaOH-$KH_2PO_4$ | 10 | $2.91\times10^{-6}$ | 4,780 |
| 45 | 7.0 | do | 10 | $4.81\times10^{-6}$ | 2,750 |
| 45 | 7.8 | NaOH-glycine | 10 | $1.55\times10^{-5}$ | 790 |
| 45 | 8.8 | do | 10 | $1.16\times10^{-4}$ | 100 |
| 45 [1] | 10.2 | do | 7 | $1.10\times10^{-3}$ | 9.1 |
| 45 [1] | 10.4 | do | 3.5 | $1.48\times10^{-3}$ | 6.6 |
| 70 | −0.2 | 5.4 *N* $H_2SO_4$ | 2 | $2.57\times10^{-3}$ | 6.7 |
| 70 | −0.15 | 1.5 *N* HCl | 5 | $1.75\times10^{-3}$ | 10.0 |
| 70 | 0.65 | 0.4 *N* HCl | 5 | $1.09\times10^{-4}$ | 158 |
| 70 | 1.85 | HCl-glycine | 10 | $8.0\times10^{-6}$ | 2,130 |
| 70 | 4.0 | NaOH-phthalate | 10 | $2.05\times10^{-6}$ | 7,900 |
| 70 | 5.7 | NaOH-$KH_2PO_4$ | 10 | $1.01\times10^{-5}$ | 1,480 |
| 70 | 6.8 | do | 10 | $4.89\times10^{-5}$ | 275 |
| 70 | 7.3 | do | 10 | $1.16\times10^{-4}$ | 112 |
| 70 | 7.9 | NaOH-glycine | 10 | $3.60\times10^{-3}$ | 33 |
| 70 | 8.6 | do | 10 | $1.67\times10^{-3}$ | 6.9 |
| 100 | 1.2 | 0.2 *N* HCl | 4.5 | $2.41\times10^{-3}$ | 7.2 |
| 100 [1] | 1.4 | HCl-glycine | 5 | $1.27\times10^{-3}$ | 13.5 |
| 100 | 1.85 | do | 5 | $2.40\times10^{-4}$ | 71 |
| 100 | 3.0 | HCl-phthalate | 5 | $5.37\times10^{-5}$ | 309 |
| 100 [1] | 4.0 | NaOH-phthalate | 5 | $5.90\times10^{-5}$ | 275 |
| 100 | 5.8 | do | 5 | $3.20\times10^{-4}$ | 45.5 |
| 100 [1] | 7.3 | NaOH-$KH_2PO_4$ | 10 | $4.67\times10^{-3}$ | 2.75 |

[1] These reactions were followed by determinations of reducing sugar and levulose as well as by polarizations.

Figure 4 shows diagrammatically the variation of log $1/K$ with the pH at five temperatures. The 4° isotherm was determined only in the range of high alkalinities, and the 20° curve was not determined in the middle range, because the time required at these temperatures for a measurable decomposition is extremely long. Some of the slower reactions at both 4° and 20° gave such small decompositions, even after 10 weeks, that the calculated velocity constants may be in error by large percentages.

All the isotherms pass through a maximum at approximately pH 3.3 at which point the stability of levulose is at its maximum. It follows that pH 7 is highly alkaline with respect to the maximum stability point of levulose and considerable decomposition of the sugar may be expected in neutral solutions at the higher temperatures or over extended periods of time. In harmony with the results of the present investigation, Lobry de Bruyn found that levulose in pure aqueous solution suffered decomposition at elevated temperatures. It is of interest to note that the pH of honeys and fruits which contain large percentages of levulose lies between 3 and 4.

In the cane sugar refining industry the juices which always contain invert sugar are kept approximately neutral during the refining process. Blake [16] has shown that in these products there is a progressive destruction of levulose.

On the acid side of the maximum point the slope of the curve changes rapidly until in strong acids it becomes 2, indicating that the rate of the purely acid decomposition of the sugar is proportional to the square of the hydrogen ion activity. In the range between the neutral point and mild alkalinities the rate of decomposition is a little less than proportional to the concentration of the hydroxyl ions. The slope of the curve is here about 0.75, an indication that the rate is proportional to the 0.75 power of the hydroxyl ion activity. In the high alkalinities, the rate tends to become constant as is shown by the flattening of the curve. This is in agreement with the observations of Powell [17] who found that the rate of volume change accompanying decomposition of levulose in normal sodium hydroxide was only four times the rate in tenth normal.

In the last column of table 4 is given the time in minutes required for the loss of the first 1 percent of the total levulose in the solution at the given temperature and pH. The time required for the loss of the first 1 percent of total levulose is different from that required for the reaction to proceed 1 percent of the way toward equilibrium, since at equilibrium a large fraction of the levulose remains unchanged. The latter time is given by the equation

$$t=\frac{1}{K}\log\frac{100}{100-1} \tag{3}$$

while the time required for loss of 1 percent of the total levulose is

$$t=\frac{1}{K}\log\frac{100-L}{99-L} \tag{4}$$

in which $L$ is the percent of the initial amount of levulose remaining in the reaction mixture at equilibrium. $L$ can be read directly from

[16] Ind. Eng. Chem., vol. 12, p. 1104, 1920.
[17] Powell, J.Chem.Soc., vol. 107, p. 1335, 1915.

the interpolation curve of figure 3. The distinction between the equations is important since the two times may differ by several hundred percent. For practical purposes the time given by equation (4) is the more significant and is given in the sixth column of table 4.

## VI. THE TEMPERATURE COEFFICIENTS

Temperature coefficients of the decomposition reactions were determined directly from the curves in figure 4 at either the experimentally determined points or points near them. By substituting in the Arrhenius equation

$$A=\frac{2.303T_1T_2}{T_2-T_1}\left(\log\frac{1}{K_{T_1}}-\log\frac{1}{K_{T_2}}\right)$$

in which $K_{T_1}$ and $K_{T_2}$ are the velocity constants at a fixed pH and at the absolute temperatures, $T_1$ and $T_2$, a series of nineteen values of $A$ was obtained between the various experimental temperatures and at different pH's. $A$ was found essentially independent of the temperature and varied only with the pH. The experimentally calculated values of $A$ were plotted against the pH and a curve constructed from which adjusted values of $A$ were read. The numerical values of this constant are given in table 5.

TABLE 5.—*The variation with pH of the values of A in the Arrhenius equation*

| pH | $A$ | pH | $A$ | pH | $A$ |
|---|---|---|---|---|---|
| −2 | 19, 200 | 4 | 14, 400 | 10 | 15, 400 |
| −1 | 18, 200 | 5 | 14, 300 | 11 | 15, 600 |
| 0 | 17, 200 | 6 | 14, 500 | 12 | 15, 800 |
| 1 | 16, 200 | 7 | 14, 700 | 13 | 16, 000 |
| 2 | 15, 300 | 8 | 14, 900 | 14 | 16, 200 |
| 3 | 14, 700 | 9 | 15, 100 | 15 | 16, 400 |

By means of the adjusted values of $A$, all experimental values of log $1/K$ at other than 20° were corrected to 20° C. These corrected values, together with those actually determined at 20°, were plotted against the pH. All points were in satisfactory agreement with a smooth curve, the 20° C isotherm of figure 4.

From this composite curve were read the values of log $1/K_{20}$ from which the corresponding values of log $t_{20}$ were calculated ($t$ being the time for decomposition of the first 1 percent of the total levulose). Values of log $t$ at other temperatures were then calculated from the Arrhenius equation in the form,

$$\log t_T=\log t_{20}-\frac{A(T-293)}{2.303\times 293T}$$

in which $A$ must correspond to the pH for which the calculation is made.

The temperature coefficients of both the alkaline and acid decompositions are greater than those ordinarily encountered. In general, rates are doubled or trebled for a 10° increase in temperature, and $A$ has a value of 10,000 to 12,000. For decomposition of levulose, rates are increased from 5 to 10 times for a 10° rise in temperature, and the value of $A$ varies from 14,000 to 19,000.

The great variations in the stability of levulose under the different conditions which may be encountered are emphasized in table 6.

TABLE 6.—*Time required for the decomposition of the first 1 percent of the total levulose in aqueous solutions at integral units of pH and 10° intervals of temperature*

| pH | Temperature °C. | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0° | 10° | 20° | 30° | 40° | 50° | 60° | 70° | 80° | 90° | 100° |
| −2 | 17 h | 87 m | 8.7 m | 1 m | | | | | | | |
| −1 | 75 d | 165 h | 19 h | 145 m | 21 m | 3.5 m | 0.6 m | | | | |
| 0 | 8 y | 310 d | 40 d | 135 h | 22 h | 240 m | 49 m | 11 m | 2.6 m | | |
| 1 | | 13 y | 2 y | 110 d | 20 d | 98 h | 21 h | 310 m | 80 m | 22 m | 6.8 m |
| 2 | | | 11 y | 2.5 y | 140 d | 32 d | 127 h | 47 h | 13 h | 240 m | 78 m |
| 3 | | | 24 y | 4.7 y | 365 d | 85 d | 520 h | 141 h | 43 h | 810 m | 275 m |
| 4 | | | 20 y | 4.0 y | 320 d | 76 d | 480 h | 138 h | 42 h | 810 m | 280 m |
| 5 | | | 8 y | 1.6 y | 130 d | 31 d | 200 h | 56 h | 17 h | 245 m | 108 m |
| 6 | | 11 y | 1.9 y | 135 d | 30 d | 165 h | 44 h | 12 h | 225 m | 71 m | 25 m |
| 7 | 14 y | 2.1 y | 132 d | 25 d | 130 h | 30 h | 7.6 h | 126 m | 38 m | 12 m | 4.1 m |
| 8 | 2.5 y | 129 d | 21 d | 95 h | 20 h | 4.6 h | 69 m | 18.5 m | 5.5 m | 1.7 m | .6 m |
| 9 | 190 d | 21 d | 83 h | 15 h | 3.1 h | 42 m | 10 m | 2.7 m | .8 m | .2 m | |
| 10 | 29 d | 95 h | 15 h | 2.6 h | 30 m | 6.9 m | 1.7 m | .4 m | | | |
| 11 | 185 h | 20 h | 3.0 h | 32 m | 6.0 m | 1.3 m | .3m | | | | |
| 12 | 40 h | 5.2 h | 47 m | 8 m | 1.5 m | .4m | | | | | |
| 13 | 13 h | 102 m | 15 m | 2.5 m | .5 m | | | | | | |
| 14 | 6 h | 45 m | 6.3 m | 1.0 m | | | | | | | |
| 15 | 4 h | 29 m | 4.0 m | | | | | | | | |

m=minutes; h=hours; d=days; y=years.

The time required for the loss of the first 1 percent of the total levulose is given at unit intervals of pH and 10° intervals of temperature. This table is serviceable for the estimation of small losses of levulose under specified conditions. The result will be accurate when the decomposition is small, that is, not more than 1 or 2 percent of the total sugar, and although accuracy decreases when larger decompositions are involved, the results will be significant for decompositions as great as 5 percent. If very large decompositions are to be estimated, a special calculation must be made using the velocity constant and the equilibrium value of levulose at the pH of the reaction.

## VII. SUMMARY

1. The rates of the decomposition of levulose in the early stages of the reaction have been determined over a range of temperature from 4° to 100° C., and of pH from −2.6 to 14.2, that is, from 12 *N* hydrochloric acid to 2 *N* sodium hydroxide.

2. The decomposition rate is independent of the concentration of levulose within the limits used in the experiments, and in the early stages of the reaction the decomposition curves follow closely those for unimolecular reactions.

3. A method for calculating the equilibrium of a reaction, when the equilibrium cannot be reached in practice because of interfering secondary reactions, is described.

4. The calculated equilibrium values indicate that in acid solution the primary decomposition of levulose results in the formation of heterolevulosan and its dimer, and that in alkaline solution the Lobry de Bruyn and van Ekenstein transformation to glucose and mannose is the predominant reaction.

5. The stability of levulose is maximum in solutions of pH 3.3. The point of maximum stability is independent of the temperature.

6. The temperature coefficients of decomposition are higher than those ordinarily encountered and depend upon the pH, being greatest in strong acid solutions.

7. In order to facilitate practical application of the results, the times required for 1 percent decomposition are tabulated for integral pH's at 10-degree intervals of temperature.

Washington, August 5, 1933.