UTILIZATION OF THE PROTEINS OF COTTON SEED BY MAN.

	Proteins, Grams.									
	Milk.	Corn meal.	Meat.	Cotton seed meal.	Total eaten.	Excreted.	Utilized.	Utilized from milk and corn.	Utilized from food tested.	Utilization value, Per cent.
Cotton seed meal, man 1	132.4	10.6	...	25.3	168.3	10.5	157.8	137.5	20.3	80.2
Cotton seed meal, man 2	142.6	38.6	...	50.3	221.5	22.5	199.0	161.4	37.6	74.7
Cotton seed meal, man 3	123.6	37.0	...	48.3	208.9	23.5	185.4	151.3	34.1	77.9
Cotton seed meal, average.....										77.6
Cotton seed flour, man 1	108.4	35.5	...	47.2	191.1	19.5	171.6	134.0	37.6	79.7
Cotton seed flour, man 2	122.0	41.4	...	59.6	223.4	22.3	201.1	153.9	47.2	79.2
Cotton seed flour, average.....										79.5
Meat, man 1.....	96.3	23.5	101.5	..	221.3	7.1	214.2	113.4	100.8	99.3
Meat, man 2.....	85.0	35.8	95.0	..	215.8	13.7	202.1	112.9	89.2	93.9
Meat, average.....										96.6

These results are higher than those obtained by Mendel and Fine with dogs, relatively (in comparison with meat) about 5%, and actually, about 7%.

It is generally considered that man's utilization of concentrated foods is equal to ruminants, but it is interesting to note that ruminants utilize 88% of the proteins of cottonseed, whereas man utilizes only 78%.

Summary.

The utilization values of the proteins of cotton seed meal and flour are the same and average 78.6%. Experiments with meat, on the same men, showed that 96.6% of the proteins was utilized.

Cotton seed proteins are utilized by man equally as well as those of legumes, nine-tenths as well as those of cereals, and eight-tenths as well as those of meat.

COLLEGE STATION, TEXAS.

[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK CITY.]

ON THE ACTION OF ACIDS UPON FRUCTOSE AND GLUCOSE.

BY M. M. HARRISON.

Received January 15, 1914.

1. Introductory.

It has long been known that the two hexoses of invert sugar are more or less rapidly acted upon by acids. The effect is of no little importance in studies of sugar hydrolysis, for the velocity coefficient of the reaction

can only be calculated on the basis of the rotation observed when inversion is complete. It seemed interesting, therefore, to investigate carefully, to just what extent and under what physico-chemical conditions the composition, and with it the rotatory power, of an invert sugar solution is appreciably modified by the action of such acids as are likely to be employed as catalyzers of sugar hydrolysis. Incidentally, it appeared desirable to reinvestigate the nature of the decomposition itself by physico-chemical methods, with a view to uncovering something of the mechanism of that decomposition. The effect of two acids was studied, *viz.*, hydrochloric and formic, the former as a representative of the strong mineral acids, the latter as a representative of the weaker organic acids, and also because it is the catalyzer employed in a reinvestigation of the mechanism of sugar hydrolysis at present carried on at Clark University. The effect of these acids was studied first on fructose alone and then on glucose alone. The physico-chemical changes attending the decomposition could be more conveniently followed in presence of the stronger than of the weaker acid, and consequently most of the experiments recorded below were carried out with hydrochloric acid only.

2. Decomposition of Fructose by Acids at 60°.

The decomposition of fructose by acids has been studied by a number of authors under a variety of conditions, the strength of the acid, for instance, varying from 0.01% to perhaps 40% in the case of hydrochloric acid. Other acids, too, including sulfuric, nitric, phosphoric and oxalic, have been employed in various degrees of concentration. The solutions studied have also been considerably varied in sugar concentration and the temperatures at which the action was allowed to take place. The present study was carried out, for the most part, at 60°, a temperature which permitted studying the kinetics of the change with more or less precision. A normality of about 0.7 of the hydrochloric acid was found, after some preliminary experimenting, to serve my purposes best.

The solutions were prepared by dissolving weighed amounts of Kahlbaum's fructose (not re-dried) in water, keeping the solution for a time at 60°, then adding the required amount of acid, making at once the necessary initial observation, then gradually observing the progress of the reaction in time. The thermostat in which the solutions were kept was maintained at 60 ± 0.05°. The polarimetric measurements were made with a Schmidt and Haensch saccharimeter, an incandescent Welsbach gas lamp, with a bichromate solution ray filter, being used as a source of light. The one decimeter jacketed Landolt observation tube was kept constantly at 35°, the work being carried on in midsummer. For an observation, a sample was pipetted out at a noted time, thoroughly cooled, and usually treated cold with boneblack, which diminished coloration and permitted of more precise polarimetric readings. A special experi-

ment showed that the boneblack treatment did not affect the rotation of the solutions.

The increasing acidity of the solution, which is due, as will be seen, to the appearance of formic and levulinic acids, was also measured by pipetting out sufficient samples at noted intervals of time and titrating with 0.05-normal barium hydroxide and phenolphthalein. The titrations were carried out in a closed vessel, and the solution was covered with a layer of purified toluene, to avoid any disturbing effect from atmospheric carbon dioxide—a practice which has been in use in the Clark University laboratories for some years past. A weighing buret was used throughout.

Finally, the increasing coloration of the solution, which is due to the formation of humin substances, was measured with the aid of an excellent Duboscq colorimeter.

The results of these measurements are recorded numerically in Tables I–IV and graphically in Figs. 1–3.

Conrad and Guthzeit[1] found, as far back as 1886, that fructose is entirely decomposed into levulinic acid, formic acid, and humin by heating with 5 or 10% hydrochloric acid. A few years later, Wohl,[2] working with a 92.3% solution of fructose containing about 0.01% hydrochloric acid, kept at 100° for one hour, was able to separate an extremely hygroscopic "dextrin-like substance," which he named *levulosin* and which he supposed to be a mixture of dehydration products of fructose. Its rotatory power he found to be about one-half that of fructose. He further states that in dilute solution levulosin is changed back to fructose by 0.6-normal hydrochloric acid. Still later, Kiermayer[3] found hydroxymethylfurfural, $C_4H_2O(CH_2OH)CHO$, as a constant intermediate product in the decomposition of fructose. This author believes that the mechanism of the reaction consists in the direct transformation of fructose into humin and hydroxymethylfurfural, the latter then gradually changing into formic and levulinic acids.

One of the first questions that suggested themselves in the present study was, whether Wohl's levulosin is formed, not only under the particular conditions of his experiments, but also under other conditions, including those chosen by myself. A further question that arose was, whether humin and hydroxymethylfurfural were formed directly from fructose, or, by a secondary reaction, from one of the intermediate products. Finally, it seemed desirable to attempt to learn something of the order of the reaction, to see whether each single molecule of fructose is attacked by itself or whether two or more molecules take part in the reaction together.

[1] *Ber.*, **19**, 2569 (1886).

[2] *Ibid.*, **23**, 2084 (1890).

[3] *Chem.-Ztg.*, **19**, 1004 (1895).

To establish the formation of levulosin under the conditions chosen, an attempt was made to actually separate this substance from one of the solutions. A solution of Schering's fructose in 0.7-normal hydrochloric acid, having a rotation of —28.2° in a 5 cm. tube, was heated at 60° for 84 hours; the precipitated humin was filtered off, and the filtrate neutralized with silver hydroxide. After removing the silver chloride formed and decomposing the soluble silver salts of the organic acids with hydrogen sulfide, the solution was decolorized with boneblack and the water distilled off at 30° *in vacuo*. When dry, the cream-colored residue was dissolved in asbolute alcohol and partly precipitated by adding 25%, by volume, of absolute ether. The precipitate, a waxy snow-white solid, was next washed with absolute ether and dried over phosphorus pentoxide in a vacuum desiccator. The product was extremely hygroscopic and formed a golden yellow solution in water. An analysis of the substance gave the following results:

	Found.		Theory for $C_6H_{10}O_5$.	Theory for $C_6H_{12}O_6$.
	I. Per cent.	II. Per cent.	Per cent.	Per cent.
C	41.9	41.7	44.4	39.9
H	6.66	6.67	6.2	6.7
O	51.4	51.6	49.4	53.4

These results point to the product being a mixture of fructose, $C_6H_{12}O_6$, and levulosin, which apparently has the formula $C_6H_{10}O_5$, the mixture under consideration containing about 57% of fructose and some 43% of levulosin. A molecular weight determination by the cryoscopic method was accordingly expected to give a figure slightly above 170. The result actually obtained was somewhat low, about 162; yet it indicates clearly enough the molecular magnitude of levulosin. The nature of the product was further indicated by a determination of its rotatory power. If it is a mixture of fructose and levulosin, containing somewhat more of the former than of the latter, its rotation should be intermediate between that of the two pure substances and somewhat nearer to that of fructose. (The rotatory power of levulosin is, according to Wohl, only about one-half that of fructose.) Measurement fully bore out this conclusion. A 2.32% soln. of the product gave, in a 20 cm. tube at 35°, a rotation of —8.2°, and a 4.24% solution, under similar conditions, gave a rotation of —14.8°. These figures would indicate the product to contain roughly about 60% of fructose and 40% of levulosin. When warmed with acid of the same strength (0.7-normal) as used in its formation, the product underwent a readily measurable change. The solution being made more dilute with respect to the product, that is, the concentration of the water being made greater than in the solution in which it had been formed, the result was an increase in the proportion of fructose present, as clearly indicated by the increase of the negative rotation.

It seems plain, therefore, that levulosin is a dehydration product of fructose and that it is formed from the latter and changed back into it by a reversible reaction.

As has been mentioned above, the decomposition products of fructose include, beside levulosin: hydroxymethylfurfural, formic and levulinic acids, and humin. The question now presented itself, are these substances formed, together with levulosin, directly from fructose itself, or are they decomposition products of levulosin? If the latter is the case, as seems more probable *a priori*, then one would expect, in the first place, the decrease of the negative rotation to run a peculiar course. At first, namely, the fall in rotation should be rapid, the amount of levulosin being formed which is necessary to hold equilibrium to the fructose in the reversible reaction. Then the fall in rotation should become strikingly slower, corresponding to the undoubtedly slow decomposition of levulosin into humin and the other substances mentioned. This is exactly what was observed, as shown by Curve A in Fig. 1, which represents graphically the data of Table I.

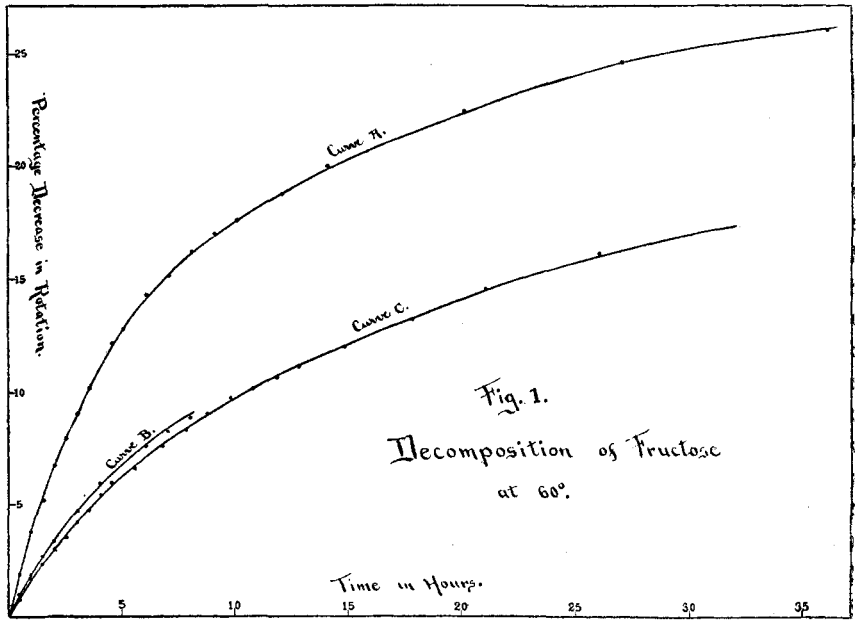


Fig. 1.

Decomposition of Fructose at 60°.

Some additional experiments indicated that, while under these conditions the solution loses about a fifth of its original rotation in a very few hours, loss of the remaining rotation would take at least two months.

That the formic and levulinic acids found among the final decomposition products of fructose originate, not from the sugar itself, but from an intermediate product, was more directly shown by the peculiar course of

TABLE I.—300 CC. OF THE SOLUTION CONTAINED 100 g. FRUCTOSE AND 231 g. WATER AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

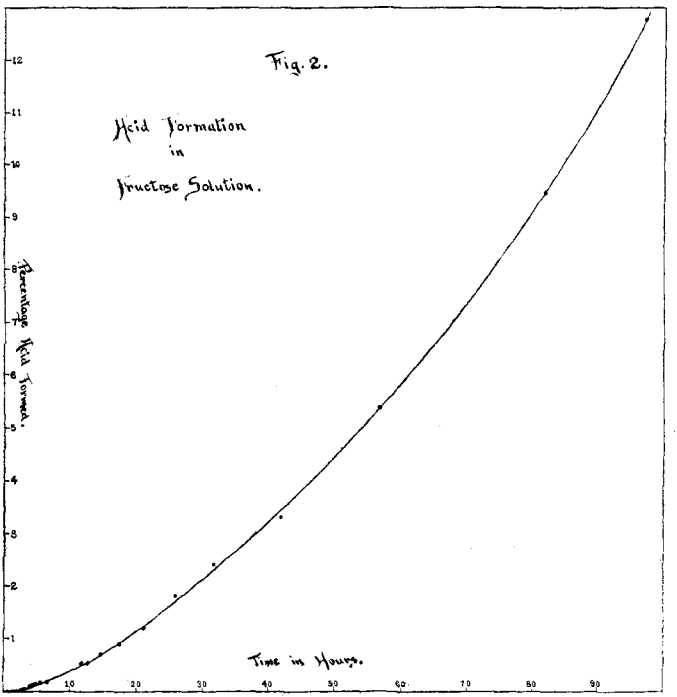
Time in hours.	Rotation.	Per cent. change.	Time in hours.	Rotation.	Per cent. change.
0.0	—84.5°	0	6.0	—72.45°	14.3
0.5	—82.9	1.9	7.0	—71.55	15.3
1.0	—81.3	3.8	8.0	—70.8	16.2
1.5	—79.9	5.4	9.0	—70.15	17.0
2.0	—78.75	6.8	10.0	—69.6	17.6
2.5	—77.7	8.0	12.0	—68.55	18.8
3.0	—76.75	9.1	14.0	—67.6	20.0
3.5	—75.85	10.2	20.0	—65.55	22.4
4.5	—74.3	12.1	27.0	—63.7	24.6
5.0	—73.65	12.8	36.0	—62.5	26.0

the gradual increase of acidity. If, namely, the acids were formed from fructose directly, their rate of formation would decrease with the sugar. Plotted graphically, the gradual increase of acidity would appear in the form of a curve concave downward toward the time-axis. If, on the contrary, the acids were slowly produced from a more rapidly forming intermediate product, then the curve of increasing acidity should exhibit two phenomena: it should be concave upward during a first considerable stretch, and at its origin, *i. e.*, at the point $t = 0$, its direction should be coincident with that of the time axis itself. The upward concavity of the curve would indicate an increasing rate of acid formation due to accumulation of the intermediate product from which the acids are formed. The tangency between the curve and the time axis at the very beginning would indicate that before any intermediate product has appeared and there is nothing but pure fructose present, the rate of acid formation is zero. *Fig. 2, representing the actual measurements, exhibits both of these phenomena.* The measurements are reproduced numerically in Table II.

TABLE II.—600 CC. OF THE SOLUTION CONTAINED 100 g. FRUCTOSE AND 526 g. WATER, AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in hours.	Percentage increase of acidity.	Time in hours.	Percentage increase of acidity.
0	0.00	14.75	0.72
0.5	0.00	17.75	0.87
1.0	0.00	21.00	1.2
1.5	0.01	26.00	1.8
2.0	0.04	32.00	2.4
2.5	0.10	42.00	3.3
3.5	0.15	57.00	5.4
4.0	0.15	82.00	9.5
4.5	0.16	97.00	12.8
5.5	0.17	120.00	15.5
6.75	0.19	144.00	17.9
11.75	0.58	168.00	20.4
12.75	0.60	192.00	24.4
		216.00	26.4

To express the increase in acidity in percentage form, I determined the total acid formed when decomposition was complete, which was attained after heating the solution at 75° for eighteen days. It appeared that



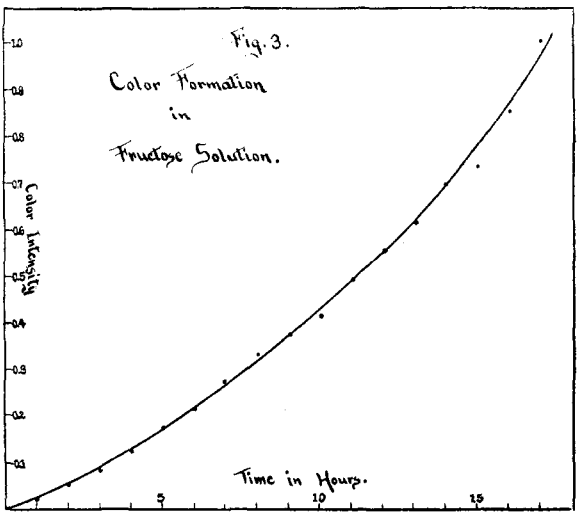
69.7% of the original fructose was finally turned into acids, the remaining 30% being presumably changed into humin.[1] The final normality of the organic acids formed was 1.289.

The rate of humin formation was studied next. The only method from which reasonable accuracy might be expected was the colorimetric method. The color intensity of the investigated solution, just before humin began settling out of it, was arbitrarily taken as unity, and the coloration gradually increasing to that point when expressed in fractions of that unit. The results are shown graphically by Fig. 3.

As in the case of acid formation, the curve of increase of color intensity is concave upward. This shows that, like formic and levulinic acids, humin is produced mainly from an intermediate product. If none were produced from fructose directly, the curve would also be tangent with the time axis at the origin, as in the case of the curve of acid formation.

[1] One of the sets of data given by Conrad and Guthzeit (*loc. cit.*) indicates that under their working conditions about the same percentages of the fructose are changed into acids and humin, respectively.

This tangency is not indicated by the figure. However, in view of the inaccuracy of colorimetric measurement in the early stages of the decom-



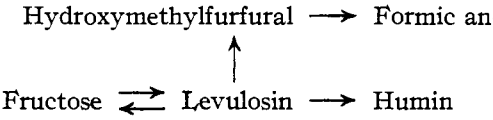
position, the question as to whether *absolutely* no humin is formed directly from fructose, remains undecided.

It remained to take into account the constant occurrence of hydroxymethylfurfural among the decomposition products of fructose, as observed by Kiermayer.[1] That, however, this substance, again, is not directly produced from fructose, appears from the following consideration: Hydroxymethylfurfural is present at any time during the decomposition only in small quantities. Kiermayer showed that, in the presence of acids, pure hydroxymethylfurfural is rather rapidly decomposed into formic and levulinic acids, which indicates that the acids of fructose decomposition are likewise the immediate offspring of hydroxymethylfurfural. If, now, hydroxymethylfurfural itself came directly from fructose, its rate of formation would diminish with the disappearance of the sugar, and the result would be a correspondingly diminishing rate of formation of the acids. As a matter of fact, the rate of acid formation, as shown above, is not decreasing, but increasing. Hence hydroxymethylfurfural appears to come, not from fructose itself, but from some substance into which the sugar is first transformed and whose amount in solution increases during the earlier stages of the decomposition.

Remembering, finally, that no humin-like substance is known to be produced from hydroxymethylfurfural, and barring the formation of still other intermediate products as yet undetected, the mechanism of the de-

[1] *Loc. cit.*

composition of fructose by acids may therefore be pictured by the following scheme:



Other substances than those mentioned in this scheme have, as just stated, not yet been isolated. That, however, the scheme does not fully represent the mechanism of fructose decomposition, and that at least one further intermediate compound—more or less ephemeral—must form, is clearly indicated by a further step to which the employment of the kinetic method has led. Early in the study I undertook to ascertain how many fructose molecules take part in the decomposition reaction. Two solutions were prepared. One (already mentioned above) contained, in a total volume of 300 cc., 231 g. of water and 100 g. of fructose. The second contained, in the same volume, again 231 g. of water and only 50 g. of fructose, the volume occupied in the first solution by the additional 50 g. of fructose, being in the second solution filled with 47.8 g. of mannitol. Both solutions were 0.7453-normal with respect to hydrochloric acid. This procedure for making the pair of solutions exactly equal in both acid and water concentration and differing in sugar concentration only, is similar to that recently employed by Rosanoff, Clark and Sibley[1] in a study of the order of the sugar hydrolysis reaction. The progress of the reaction in the first of the two solutions is already recorded in Table I, above, and graphically represented by Curve A, of Fig. 1. The progress of the reaction in the second solution is shown by Curve B of the same Fig. 1 and is numerically recorded in Table III:

TABLE III.—300 CC. OF THE SOLUTION CONTAINED 50 G. FRUCTOSE, 231 G. WATER AND 47.8 G. MANNITOL. AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in hours.	Rotation.	Per cent. change.	Time in hours.	Rotation.	Per cent. change.
0	—42.25°	0	3.0	—40.2°	4.8
0.5	—41.8	1.0	4.0	—39.7	6.0
1.0	—41.45	1.9	6.0	—39.0	7.7
1.5	—41.1	2.7	7.0	—38.75	8.3
2.0	—40.8	3.4	8.0	—38.5	8.8

In general, if n molecules of a substance take part in a reaction, and the initial concentrations of that substance in two given cases are respectively, a and b, then the rates of disappearance of the substance in the two cases are respectively described by the following equations:

$$\frac{dx}{dt} = k\,(a-x)^n \quad \text{and} \quad \frac{dx}{dt} = k\,(b-x)^n,$$

[1] THIS JOURNAL, 33, 1911 (1911).

where x is the amount that has disappeared at the time t, and k is the velocity coefficient of the reaction at the given temperature. If the rate of disappearance is expressed in *percentages* of the initial quantities, as is generally done, then the two equations must be written in the form:

$$\frac{d \log (a - x)}{dt} = k (a - x)^{n-1} \quad \text{and} \quad \frac{d \log (b - x)}{dt} = k (b - x)^{n-1}.$$

Denoting these two relative rates of change by the symbols V_a and V_b we get from the two equations, by sample algebraic transformation:

$$n = 1 + \left[\log \frac{V_a}{V_b} : \log \frac{a - x}{b - x}\right]$$

If the decomposition of fructose were not complicated by the consecutive reactions discussed above, this equation might be employed with reference to any moment in course of the decomposition. But in view of the complicating reactions that set in as soon as levulosin has made its appearance, the equation might be legitimately applied only with reference to the first instant of the fructose change, when nothing but pure fructose is present and that is just entering the first stage of the decomposition reaction. At that instant $x = o$ and the equation just given becomes:

$$n = 1 + \left[\log \frac{V_a}{V_b} : \log \frac{a}{b}\right]$$

In the solutions under consideration the initial concentrations, a and b, of fructose were respectively, 100 and 50 g. per 300 cc.; their ratio, therefore, was exactly 2. The ratio $V_a : V_b$ at the point $t = o$ was obtained with the aid of curves A and B of Fig. 1 by reading off the two x's (observed percents change) corresponding to the time $t = 2$ hours and dividing the greater x by the smaller, then reading off the x's for the time $t = 4$ hours, etc. These x *ratios* were next plotted against the corresponding times, and the resulting curve (nearly a straight line) was extrapolated to the axis $t = o$, the point of intersection of this axis giving the required ratio $V_a : V_b$ at the time $t = o$. This ratio was found to be about 2.03. Hence, by the equation just given, $n = 2.02$. In other words, *the first step in the change of fructose by acids is a bimolecular reaction.* Since, as is shown above, the molecular weight of levulosin corresponds to only $C_6H_{10}O_5$, the conclusion may be drawn with considerable certainty that the change of fructose into levulosin proceeds through the intermediate formation of a *disaccharide*, which changes reversibly into fructose as well as into levulosin. That this disaccharide should escape detectiou is not strange if one bears in mind that disaccharides usually undergo very nearly complete decomposition in the presence of acids.

In the kinetic study just outlined, two solutions were employed in which the concentrations of both acid and water were the same. Considered as reaction media, the two solutions were similar, on account of the physico-

chemical similarity of fructose and mannitol. The solutions were so made up for the purpose of avoiding any such catalytic effect of water as has been observed in the case of sugar hydrolysis, and in this manner I was able to determine exactly the order of the decomposition reaction with respect to fructose. In order, however, to find out whether water would really have any specific effect, a still further experiment was instituted, employing a solution containing the same relative amounts of acid and of fructose as the one into which mannitol had been introduced, but this time replacing the mannitol by water. The results are numerically recorded in Table IV and are graphically represented by Curve C of Fig. 1.

TABLE IV.—600 cc. OF SOLUTION CONTAINED 100 g. FRUCTOSE AND 526 g. WATER AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in hours.	Rotation.	Per cent. change.	Time in hours.	Rotation.	Per cent. change.
0.0	—41.5°	0.0	7.75	—38.0°	8.4
0.5	—41.15	0.8	8.75	—37.7	9.1
1.0	—40.8	1.7	9.75	—37.45	9.8
1.5	—40.5	2.4	10.75	—37.25	10.2
2.0	—40.2	3.1	11.75	—37.05	10.7
2.5	—40.0	3.6	12.75	—36.85	11.2
3.0	—39.7	4.3	14.75	—36.45	12.1
3.5	—39.5	4.8	17.75	—35.95	13.3
4.0	—39.2	5.5	21.00	—35.45	14.6
4.5	—39.0	6.0	26.00	—34.75	16.2
5.5	—38.7	6.7	32.00	—34.55	16.7
6.75	—38.3	7.7			

Curves B and C in Fig. 1 show that water has a distinctly depressing effect on the velocity of decomposition. This might be expected in view of the reversibility of the fructose-levulosin transformation, the fructose molecule being levulosin *plus* the elements of water. That, however, besides this expected mass action, water exerts also a special catalytic effect, is plain from the fact that Curves B and C start out at appreciably different angles from the very point $t = 0$. Since in the beginning no levulosin is present, the water can exert no mass action, and therefore its depressing effect upon the initial velocity of the reaction can only be considered as negatively catalytic. We have, then, here another reaction in which water acts as a negative catalyzer, as it does in the case of sugar hydrolysis.[1]

3. Decomposition of Fructose by Acids at Ordinary Temperatures.

The physico-chemical conditions employed were now changed to resemble more closely those likely to be maintained in sugar hydrolysis experiments. A new set of solutions was exposed to the temperature of the laboratory. The kinetic experiments described above had shown the decomposition of fructose to be a bimolecular reaction and hence to

[1] Rosanoff and Potter, THIS JOURNAL, 35, 248 (1913).

proceed at a relative speed proportional to the sugar cencentration. In view of this result, it was necessary to investigate a series of solutions varying in fructose content up to the highest limit that may be expected to occur in sugar hydrolysis work, namely, about 30% of fructose, corresponding to about 60% of uninverted cane sugar. Two acids were employed, for reasons already stated in the introductory section above. The strength of formic acid was varied up to four times normal, that of hydrochloric up to about one-half normal.

In the case of hydrochloric acid it will suffice to mention only the most significant results: (*a*) A solution containing about 19% of fructose and about 0.089 mols HCl per liter showed an initial rotation of —49.8°. Four days later the rotation was exactly the same. When the solution had stood for fifteen days, the observed rotation was —49.7°. Thus, when cane sugar is inverted with 0.1-normal hydrochloric acid at ordinary temperatures, the final rotation is attained long before an appreciable decomposition of the fructose present has set in, even if the original cane sugar solution be not less than 40% strong. No correction of the observed final rotation is required. (*b*) A solution containing about 20% of fructose (*i. e.*, corresponding to a 40% cane sugar solution), and 0.447 mols HCl per liter showed an initial rotation of —51.9°. It was found to change at the rate of about 0.09° Ventzke per 24 hours. With such a solution, a day is more than sufficient for obtaining the required final reading, and as a change of 0.09° V. in that final rotation has no appreciable influence upon the magnitude of the hydrolysis constant, it is plain that at least the fructose decomposition calls for no correction. In still stronger hydrochloric acid solutions a rapid appearance of color and decrease of rotation was observed. But solutions stronger than half-normal with respect to hydrochloric acid, and containing more than 40% of cane sugar, are not likely to be employed in studies of the velocity of hydrolysis.

In the case of formic acid it was found: (*a*) Strong fructose solutions 0.4-normal with respect to formic acid suffer no change in rotation in 77 days, probably in a much longer time yet; (*b*) Strong fructose solutions 0.8-normal with respect to formic acid likewise undergo no detectable change in 77 days. (*c*) *Double-normal* formic acid produced in 77 days no change in solutions containing respectively, 14%, 16% and 25% of fructose. Even in a 28% solution, corresponding to about 56% of cane sugar, the change produced by double-normal formic acid in 77 days was scarcely sufficient to affect the calculated velocity coefficient of sugar inversion. Moreover, as experience in the Clark University laboratories has shown, forty days are far more than sufficient for the attainment of the "infinity" reading in formic acids of usable strength.

4. The Decomposition of Glucose by Acids at 75°.

The experimental method exployed in the case of glucose was for the most part similar to that described above in connection with fructose. In the case of glucose it was found to be more convenient to use monochromatic light in measuring the changes of rotation. As a source of extremely brilliant sodium light a polarimeter lamp was used devised by M. A. Rosanoff and kindly placed by him at my disposal. The temperature of the thermostat, on account of the slower decomposition of glucose, was set at 75°, as compared with the 60° employed in the case of fructose. No boneblack was employed.

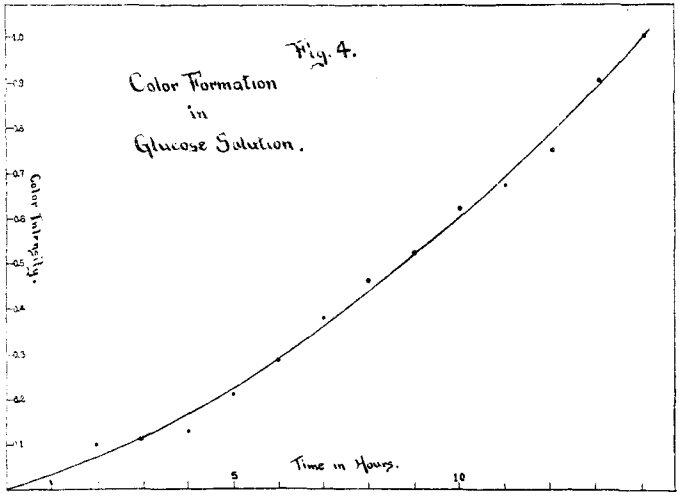
At lower temperatures and under the influence of concentrated hydrochloric acid, glucose condenses to the disaccharide isomaltose, as shown by Emil Fisher.[1]

The question now arose whether the same condensation would take place under the very different conditions of my own experiments. A solution containing per liter 500 g. of glucose and 0.7 mols HCl was kept for 48 hours at 75°. The insoluble humin formed was filtered off, and the dark brown filtrate was rendered neutral with an excess of silver hydroxide. The organic silver salts now in the solution were decomposed with hydrogen sulfide and the solution freed from the latter by boiling. After diluting and nearly neutralizing with sodium hydroxide, brewer's yeast was added and the solution allowed to stand at 32–35°. The rotation of the solution, originally 40°, was found at the end of 60 hours to have fallen to 18.9°. Subsequently no further change could be detected. A solution containing nothing but pure glucose, when similarly treated with yeast, lost its rotation completely. Obviously, the solution under investigation had contained another active substance beside glucose. To isolate that substance, the solution was separated from the yeast by filtration, evaporated *in vacuo*, to a thick sirup, and precipitated with 95% alcohol. The precipitate was redissolved in a small amount of water and about three-quarters of it again precipitated out with alcohol. The new precipitate was treated in the same way. The precipitate obtained in this last operation was again dissolved in water, alcohol was added to incipient precipitation, the first amount of precipitate formed was removed, then the precipitation was completed by the addition of an equal volume of ether. Finally, the precipitated sticky substance was transferred to a smaller vessel by once more dissolving in a small amount of water and then evaporating and drying the residue *in vacuo* over phosphorus pentoxide. The residue was identified as isomaltose, which is now generally accepted to be identical with the "gallisin" of Schmitt and Rosenhek.[2] Two cryoscopic determinations gave the molecular weights, respectively,

[1] *Ber.*, **23**, 3687 (1890).
[2] *Ibid.*, **17**, 2456 (1884).

326 and 332, in sufficient agreement with the theoretical molecular weight of isomaltose, 342. The specific rotation of the substance in 4.62% solution was found to be $[\alpha]_D = 84.1°$. According to a formula given Schmitt and Rosenhek, the rotation of a solution of that strength should be $[\alpha]_D = 84.4°$.

The question as to whether the humins are formed directly from glucose or from isomaltose was studied colorimetrically very much as in the case of fructose. The results are graphically represented by Fig. 4.



Since only glucose was present at first, the upward concavity of the curve demonstrates that humin is formed, not from glucose directly, but from some intermediate transformation product, possibly isomaltose itself, but perhaps more probably from an undetected transformation product of isomaltose, corresponding to the levulosin yielded by fructose. In this case again, if no humin whatever were formed from glucose directly, the curve would be tangent to the time axis at the origin. Such tangency is not indicated by the figure. The point, however, as in the case of fructose, must be considered as undecided in view of the comparative inaccuracy of the colorimetric observations in the early stages of the decomposition.

The formation of considerable amounts of isomaltose from glucose indicates that, unlike most disaccharides, which are hydrolyzed by aqueous acids all but completely, isomaltose undergoes hydrolysis to a distinctly limited extent, like esters. Under these circumstances it appeared possible to show that it is the isomaltose and not the glucose that changes into the later decomposition products, by studying the behavior of the two substances in separate solutions. Two solutions were prepared containing about 0.7 mol. of hydrochloric acid per liter and equivalent weights,

respectively, of isomaltose and glucose. The two were placed in a thermostat kept at 100° and their changing rotations were observed at short intervals of time. The results are reproduced in Tables V and VI, and are graphically shown by Fig. 5. Time was reckoned in minutes.

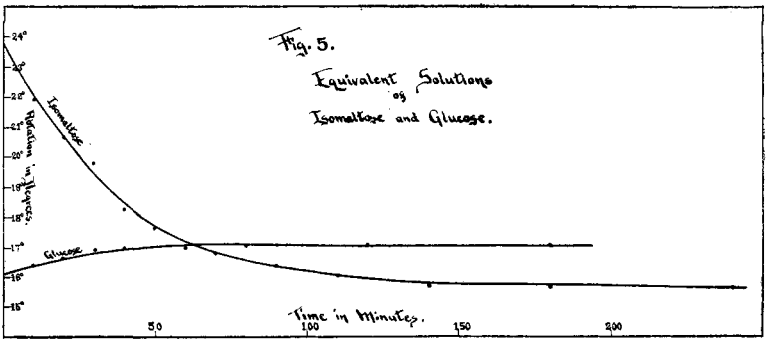
TABLE V.—100.36 g. SOLUTION CONTAINED 9.5919 g. ISOMALTOSE (2 × 0.0561 MOL) AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in minutes.	Rotation.	Time in minutes.	Rotation.
0	23.75°	90	16.4°
10	21.95	110	16.1
20	20.65	140	15.7
30	19.8	180	15.7
40	18.3	240	15.65
50	17.7	300	15.65
70	16.9		

TABLE VI.—100.36 g. SOLUTION CONTAINED 10.0965 g. GLUCOSE (0.0561 MOL) AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in minutes.	Rotation.	Time in minutes.	Rotation.
0	16.1°	60	17.0°
10	16.4	80	17.05
20	16.7	120	17.05
30	16.9	180	17.0
40	16.95		

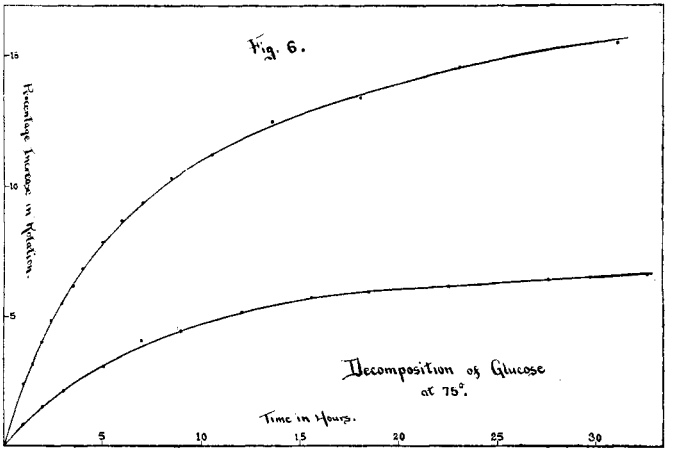
If either of the substances merely changed into the other and underwent no further decomposition, the same rotation would finally be attained in both solutions, corresponding to the equilibrium point of the reversible



reaction involved. In the figure, the two curves would run into coincidence with one and the same horizontal line. If it were the glucose that suffered decomposition, its curve would never rise high enough to attain that horizontal line, for the decomposition is attended with destruction of rotatory power. In that case, also, the curve corresponding to the isomaltose solution would be depressed. But while the depression for the glucose solution would, from the very beginning, be considerable, owing to the large amount of glucose present, and the consequently rapid rate

of its destruction, the depression of the curve of the isomaltose solution would in the early stages be very slight indeed, corresponding to the insignificant amount of glucose present and the consequently slight rate of its destruction. In other words, the upper curve in the figure would follow a practically unchanged course, while during the same time the lower curve would be considerably depressed. Instead, therefore, of soon merging into the horizontal equilibrium line, the two curves would be separated. But the figure indicates an entirely different phenomenon; the two curves *intersect* at an early stage of the process. This indicates that the actual change has depressed the upper curve far more than the lower one, *i. e.*, that the destruction of the rotatory power proceeded much faster in the isomaltose than in the glucose solution. The conclusion follows that it is the isomaltose that undergoes further decomposition and that any direct decomposition of glucose is very slight indeed, if it takes place at all.

In conclusion, the order of the glucose decomposition was investigated kinetically, to ascertain whether in this case also, as in that of fructose, the reaction is bimolecular in its early stages, indicating that the first step in the change is the formation of a disaccharide. Two solutions were prepared, containing, respectively, 150 and 75 g. of glucose per 300 cc. and both 0.7453-normal with respect to hydrochloric acid. The temperature was again 75°. Properly speaking, the two solutions should have also been made equal in water concentration, but this was not feasible on account of the limited solubility of mannitol, which I desired to employ as an equalizer of the volumes (as in the case of fructose above). The results are reproduced by Tables VII and VIII and by Fig. 6.



Owing to the larger amount of water in the more dilute solution, the lower curve is appreciably depressed (negatively catalytic effect of water),

and the initial order of the reaction appears to be, not 2, but about 2.4. However, in view of the results in the case of fructose, there can be no serious doubt but that the glucose decomposition is strictly bimolecular in its earliest stages. Both monosaccharides, then, begin by changing into disaccharides, and next, probably, both disaccharides, by further loss of water, split up into molecules with six carbon atoms. In the case of fructose it is the disaccharide that decomposes with great rapidity, while its decomposition product levulosin, $C_6H_{10}O_5$, is comparatively stable. In the case of glucose, on the contrary, it is the disaccharide isomaltose, $C_{12}H_{22}O_{11}$, that is comparatively stable.

TABLE VII.—300 CC. OF SOLUTION CONTAINED 150 G. GLUCOSE AND 195 G. WATER AND WAS 0.7453-NORMAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in hours.	Rotation.	Per cent change.	Time in hours.	Rotation.	Per cent. change.
0	79.15°	0	6.0	86.05°	8.7
1.0	81.1	2.5	7.0	86.60	9.4
1.5	81.7	3.2	8.5	87.35	10.4
2.0	82.35	4.1	10.5	88.10	11.3
2.5	83.0	4.9	13.5	89.05	12.5
3.0	83.55	5.6	18.0	89.8	13.5
3.5	84.1	6.3	23.0	90.8	14.7
4.0	84.6	6.9	31.0	91.5	15.6
5.0	85.4	7.9			

TABLE VIII.—ABOUT 300 CC. OF SOLUTION CONTAINED 75 G. GLUCOSE AND 244 G. WATER AND WAS 0.7453-NORNAL WITH RESPECT TO HYDROCHLORIC ACID.

Time in hours.	Rotation.	Per cent. change.	Time in hours.	Rotation.	Per cent. change.
0	39.0°	0	12.0	41.05°	5.3
1.0	39.35	0.9	15.5	41.25	5.8
2.0	39.6	1.5	18.5	41.35	6.0
3.0	39.85	2.2	22.5	41.45	6.3
5.0	40.20	3.1	27.5	41.6	6.6
7.0	40.60	4.1	32.5	41.65	6.8
9.0	40.75	4.5			

It may be added that in the stronger of the two glucose solutions just mentioned, the limit toward which the ratio *isomaltose: glucose* tends is about 2/3. In the more dilute solution that ratio appears to be roughly 1/5.

The final decomposition products of glucose, formic and levulinic acids, and a mixture of soluble and insoluble humins,[1] appear to be identical with those of fructose. On the other hand, as already stated above, the two acids are immediate decomposition products of hydroxymethylfurfural. The processes of decomposition in the case of glucose and of fructose would thus seem to be strictly analogous. The assumption that glu-

[1] Conrad and Guthzeit, *Loc. cit.* Kiermayer, *Loc. cit.* Van Ekenstein and Blanksma, *Ber.*, **43**, 2355 (1910).

cose is partly changed into fructose, recently made by H. Koenigsfeld,[1] is apparently without foundation.

5. Decomposition of Glucose by Acids at Ordinary Temperatures.

The results of the preceding section show that glucose decomposes far less rapidly than fructose, and since it was found that even the rate of fructose decomposition is not rapid enough to vitiate the final polarimetric reading in ordinary sugar hydrolysis, it was to be expected that danger from the decomposition of glucose was to be feared even less. Nevertheless, a few experiments were instituted to test the point directly.

A solution containing 0.179 mol HCl per liter and no less than 52% of glucose showed no change in rotation in 12 days; in 25 days the rotation was found to have changed only slightly over 0.3%. Even a solution nearly 0.9-normal with respect to hydrochloric acid and containing 27% of glucose was found to have changed its rotation only 0.25% in 25 days. A solution similarly strong in hydrochloric acid and containing no less than 43% of glucose showed no change of rotation in 7 days, and only 0.2% change in 12 days. Similarly, a solution containing 28% of glucose and two mols of formic acid per liter showed a change in rotation of only little over 0.2% in 76 days. Finally, a 24% solution of invert sugar containing two mols of formic acid per liter did not show the slightest change of rotation in 76 days. It is thus plain that under all ordinary circumstances the observed "final" rotation of an inverting sugar solution is entirely reliable and needs no correction.

In conclusion, I wish to thank Professor M. A. Rosanoff, of Clark University, for invaluable help in preparing this paper. It is further a pleasure to express my appreciation to Dr. W. G. Lyle and Mr. P. A. Kober of this laboratory for their kindly interest in the work.

WORCESTER, MASS.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF COLORADO.]

THE ACTION OF ACETIC ANHYDRIDE ON SOME BENZYLIDENE ANTHRANILIC ACIDS. III.

BY JOHN B. EKELEY AND L. CECIL SLATER.

Received January 10, 1914.

Ekeley and Dean[2] and Ekeley and Clinton[3] have shown that acetic anhydride reacts with benzylidene anthranilic acids with the formation of acetylketodihydrobenzmetoxazines, according to the following reactions:

[1] *Biochem. Z.*, **38**, 310 (1912).

[2] THIS JOURNAL, **34**, 161.

[3] *Ibid.*, **35**, 282.