the most part the agreement between different determinations was as good as could be expected from the accuracy of the temperature measurements. The necessity of the complete saturations of the solutions with air has already been discussed. There is no reason to believe that any appreciable error arose in this connection.

Summary

Apparatus, including a new form of potentiometer, which is specially adapted to the measurement of the freezing points of very dilute solutions, is described.

For seven electrolytes of different valence types, freezing-point data between 0.01 *M* and 0.001 *M* show rather remarkable agreement with values derived from the formula of Debye and Hückel.

URBANA, ILLINOIS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, NO. 476]

MUTAROTATION AS A FACTOR IN THE KINETICS OF INVERTASE ACTION

BY J. M. NELSON AND OSCAR BODANSKY

RECEIVED MAY 20, 1924 PUBLISHED JUNE 5, 1925

It is known that in the hydrolysis of sucrose by invertase from yeast, the invert sugar formed exerts a retarding influence on the velocity of the reaction.

In the present paper an attempt has been made to study this effect by considering the relation between the rate of hydrolysis and the initial concentration of sucrose as the hypothetical hydrolysis of sucrose in the absence of invert sugar. The retarding effect of the invert sugar can thus be noted by comparing this hypothetical hydrolysis with the actual hydrolysis of sucrose in the presence of the reaction products. The paper then proceeds to deal with the influence of mutarotation by comparing the actual hydrolysis with another hypothetical hydrolysis in which the reaction products are considered to be completely mutarotated immediately upon their formation. The results obtained are summarized at the end of the article.

Magnitude of Retardation by Invert Sugar at Any Point in the Course of an Hydrolysis

When the amount of sucrose hydrolyzed, in the case, say, of a 10% sucrose solution, is plotted against the time of hydrolysis, then a curve such as *A* in Fig. 1 is obtained. The different points, P_1, P_2, P_3, etc., in this hydrolysis curve correspond to solutions containing definite amounts of sucrose and invert sugar; the tangents to the curve at these points

represent the rates of hydrolysis at these particular compositions, or the velocities with which solutions, corresponding to these compositions, should begin to hydrolyze. Thus, for example, the composition of the solution corresponding to P_1 (where the solution has undergone 80% hydrolysis) is 2 g. of sucrose per 100 cc. and 8.421 g. (equivalent to 8 g. of sucrose as demanded by the stoichiometric relation; this relation is observed throughout the paper) of invert sugar per 100 cc., and the rate with which such a solution should begin to hydrolyze corresponds to the rate of hydrolysis at P_1. Similar statements may be made of the other points—P_2, P_3, etc Curve B, Fig. 2, shows the rates of hydrolysis at the different points in the course of an hydrolysis.

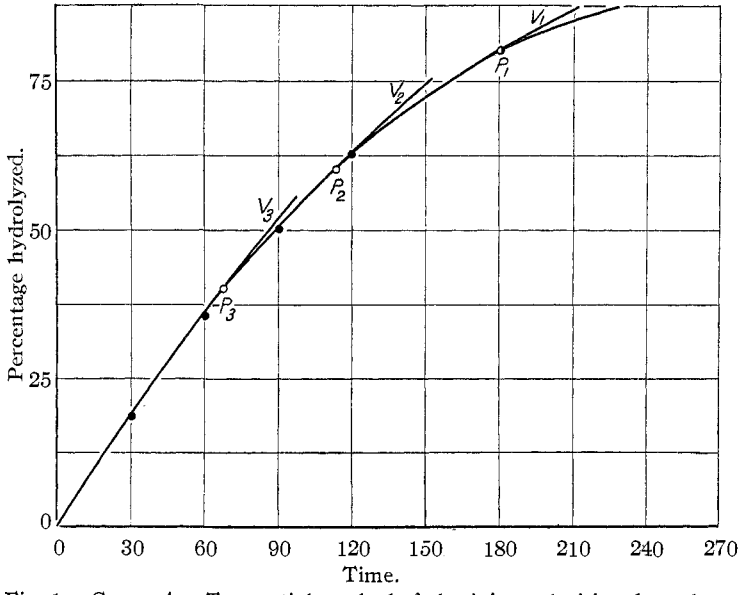

Fig. 1.—Curve A. Tangential method of obtaining velocities throughout the course of hydrolysis.

If solutions be prepared, differing from the solutions corresponding to P_1, P_2, P_3 in only one respect—namely, that the invert sugar be absent, and if, as in the case of the corresponding solutions, the tangents at the beginning of the hydrolysis of each solution be determined, a curve C, Fig. 2, is obtained. This Curve C differs from Curve B in that the retarding influence of the invert sugar has been eliminated. The data necessary for the determination of such a curve as C already exist in the literature. Nelson and Bloomfield[1] have determined the rates of hydrolysis at different concentrations of sucrose.

[1] Nelson and Bloomfield, THIS JOURNAL, **46**, 1025 (1924).

The Ideal Hydrolysis.—This hypothetical hydrolysis in the absence of invert sugar, represented by its curve *C* (Fig. 2), has been designated as an ideal hydrolysis—ideal in the sense that the curve would then represent the course of an hydrolysis unaffected by the presence of the reaction products. Similar curves may be drawn for the ideal hydrolyses of solutions of initial concentrations other than 10 g. of sucrose per 100 cc.

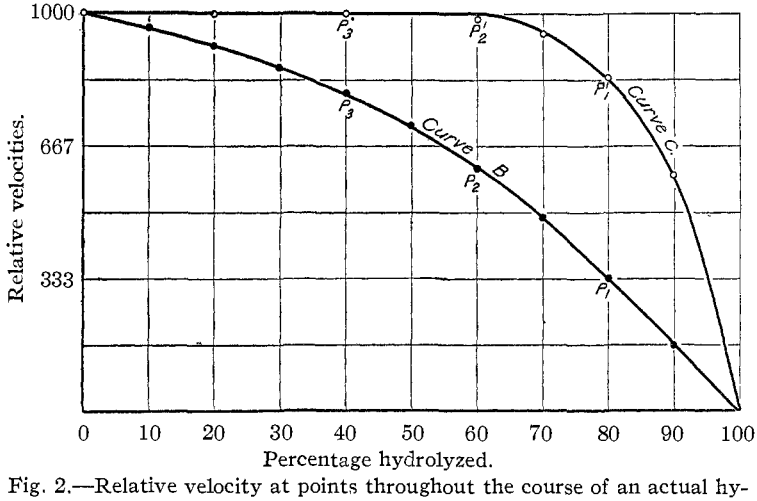

Fig. 2.—Relative velocity at points throughout the course of an actual hydrolysis (Curve *B*) and an ideal hydrolysis (Curve *C*).

Table I contains experimental data for the ideal hydrolyses of solutions of 8, 10 and 12 g. of sucrose per 100 cc. of solution. These data are represented graphically by the curves *I* in Figs. 3, 4 and 5. The data are those of Nelson and Bloomfield.[1]

TABLE I

RELATIVE VELOCITIES AT POINTS THROUGHOUT THE COURSE OF AN IDEAL HYDROLYSIS

The values of the "Actual" velocities in Part A were obtained by reading the ordinates, corresponding to the various sucrose concentrations, on the curve marked *B* = 4.7 in Fig. 2 of Nelson and Bloomfield's paper. These were changed to relative velocities on the basis of 1000. Since in an "Ideal" hydrolysis the invert sugar is considered to be removed immediately upon its formation, the values of the relative velocities at various points in the course of such an hydrolysis (Part B) will be the relative velocities at various sucrose concentrations and, hence, the same as those given in Part A, from which they were taken.

PART A

Sucrose per 100 cc., g.	12	10	9	7	5	4	3	2	1	0
Actual velocity	335	335	335	335	335	330	316	280	200	000
Relative velocity	1000	1000	1000	1000	1000	985	943	836	597	000

PART B IDEAL HYDROLYSES
For 12% sucrose soln.

Sucrose hydrol., g.	0	1	3	6	7	8	9	10	11	12
%	0.0	8.3	25.0	50.0	58.3	66.7	75.0	83.3	91.7	100.0
Sucrose left, g.	12	11	9	6	5	4	3	2	1	0
Rel. vel.	1000	1000	1000	1000	1000	985	943	836	597	000

TABLE I (*Concluded*)

For 10% sucrose soln.

Sucrose hydrol., g.	0	1	2	3	5	6	7	8	9	10
%	0	10	20	30	50	60	70	80	90	100
Sucrose left, g.	10	9	8	7	5	4	3	2	1	0
Rel. vel.	1000	1000	1000	1000	1000	985	943	836	597	000

For 8% sucrose soln.

Sucrose hydrol., g.	0	1	2	3	4	5	6	7	8
%	0.0	12.5	25.0	37.5	50.0	62.5	75.0	87.5	100.0
Sucrose left, g.	8	7	6	5	4	3	2	1	0
Rel. vel.	1000	1000	1000	1000	985	943	836	597	000

The Actual Hydrolysis.—No general law has yet been formulated which describes the velocity of hydrolysis of sucrose in the presence of invertase. Nelson and Hitchcock,[2] however, developed an empirical equation for the hydrolysis of a 10% sucrose solution, at the usual hydrogen-ion concentrations and temperatures.

$$(\mathrm{R})\ t = 1/n\left[\log\frac{100}{100-p} + 0.002642p - 0.000008860p^2 - 0.0000001034p^3\right] \quad (1)$$

Nelson and Hollander[3] and Nelson and Kerr[4] showed that the equation held for all stable preparations of invertase made from bottom yeast. Moreover, it was found that Nelson and Hitchcock's equation is applicable to the hydrolysis of 8 and 12% sucrose solutions. Upon differentiation of this equation, the following expression is obtained.

$$\frac{\mathrm{d}p}{\mathrm{d}t} = \frac{n}{\frac{0.434}{100-p} + 0.002642 - 0.00001772\,p - 0.0000003102\,p^2} \quad (2)$$

When values of p and n are substituted in this expression, the corresponding values for $\mathrm{d}p/\mathrm{d}t$ can be calculated.

The velocities at several points in the course of an actual hydrolysis were obtained in the way described above, employing the data from experiments in which 8, 10 and 12% sucrose solutions were hydrolyzed (Table I). For example, in the sucrose hydrolysis ($n = 446 \times 10^{-5}$) of the initial concentration 10 g. per 100 cc. at the point where 8 g. of sucrose has been hydrolyzed and 2 g. is left in solution, the value 80 was substituted for p in Equation 2. This gave $\mathrm{d}p/\mathrm{d}t = 21.3$ mg. per minute as the rate of hydrolysis. Similar results were obtained at other points. The highest velocity was 63.9 mg. per minute at p equals 0. This was made equal to 1000 and all other velocities in that specific hydrolysis were made relative to it. Since relative velocities had been used to describe the ideal hydrolysis a method was thus afforded of comparing the curve representing the velocities at any point in the course of an actual hydrolysis. This method of calculating relative rates of hydrolysis has been followed for all

[2] Nelson and Hitchcock, THIS JOURNAL, **43**, 2632 (1921).
[3] Nelson and Hollander, *J. Biol. Chem.*, **58**, 291 (1923).
[4] Nelson and Kerr, *ibid.*, **59**, 495 (1924).

the values used in this paper. Curves A in Figs. 3, 4 and 5 represent the hydrolysis of sucrose solutions of 8, 10 and 12% concentration.

Reference to Fig. 2 will show that at P_1 (in the course of an actual hydrolysis) where the composition of the solution is 2% of sucrose and 8.421% of invert sugar, the relative rate of hydrolysis is $V_1 = 333$. At P'_1 (in the course of the ideal hydrolysis) where the composition is 2% of sucrose and no invert sugar, the rate of hydrolysis is $V'_1 = 836$. The difference between these two rates of hydrolysis, $V'_1 - V_1 = 503$, has been considered to be due to the presence of the invert sugar. Similar considerations hold for all other points in that or any other hydrolysis.

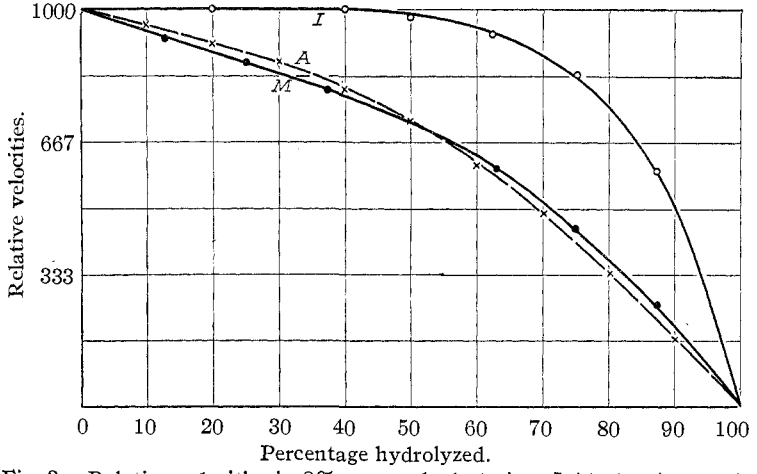

Fig. 3.—Relative velocities in 8% sucrose hydrolysis. I, ideal; A, actual; M, mutarotated.

Nelson and Hitchcock's equation is limited to describing the hydrolyses of sucrose solutions ranging in concentration from 8 g. to 12 g. of sucrose per 100 cc. Outside of that range, the rates of hydrolysis can be obtained only by the graphical method of drawing tangents to the curve "amount hydrolyzed versus time" (as in Fig. 1, Curve A); this method is, of course, less exact than substitution in Equation 2. In any case, however, a method has been described of showing the magnitude of the retarding effect, due to the presence of the invert sugar, at any point in the course of an hydrolysis.

Different Retarding Effects of Mutarotated and Nascent Invert Sugar

From Fig. 4, it can be seen that at P'_1 (in the course of the ideal hydrolysis) where the composition of the solution is 2% of sucrose and no invert sugar, the rate of hydrolysis is $V'_1 = 836$. At P_1 (in the course of the actual hydrolysis) where the composition of the solution is 2% of sucrose and 8.421% of invert sugar, the rate of hydrolysis is $V_1 = 333$. This

invert sugar has come, of course, from the hydrolysis of what was initially a 10% sucrose solution. Invert sugar, formed in the sucrose solution undergoing hydrolysis, which has not had sufficient time for complete mutarotation, has been designated as "nascent" invert sugar. This term distinguishes this form of invert sugar from invert sugar which, under the conditions of the experiment, has had ample time for complete mutarotation. When a solution was prepared consisting of 2% of sucrose and 8.421% of *mutarotated* invert sugar, it was found that its initial rate of hydrolysis, $v = 387$, determined by drawing a tangent to the curve "amount hydrolyzed versus time" at the beginning, was different from the rate of hydrolysis, $V_1 = 333$, of the 2% sucrose and 8.421% *nascent* invert sugar solution.

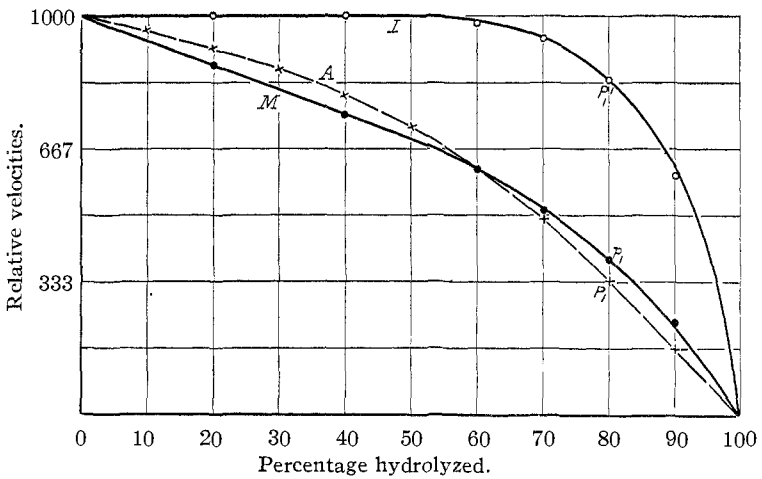

Fig. 4.—Relative velocities of 10% sucrose hydrolysis. *I*, ideal; *A*, actual; *M*, mutarotated.

Similar solutions (Series G, Table II) of sucrose and invert sugar were prepared so that when the invertase was added and the hydrolysis begun, their initial compositions were 6% of sucrose and 4.21% of invert sugar, 4% of sucrose and 6.316% of invert sugar, etc.—always complementary to 10% of sucrose. In each hydrolysis, several samples were taken at five-minute intervals after the beginning of the inversion, read in the polariscope and the courses of the reaction plotted. The slope of the tangent at the origin of this curve represented the initial velocity of the reaction. The highest of these velocities in this set of experiments was taken as 1000 and the other velocities referred to it (Table II). Similar sets of experiments were run in which the amounts of the invert sugar and the sucrose were always complementary to 8% (Series K) and to 12% sucrose (Series M). On plotting the values in Table II, it will be noted that the

curve representing the velocities throughout an actual hydrolysis (Curves *A*, Figs. 3, 4 and 5) is different from the curve (Curves *M* in Figs. 3, 4 and

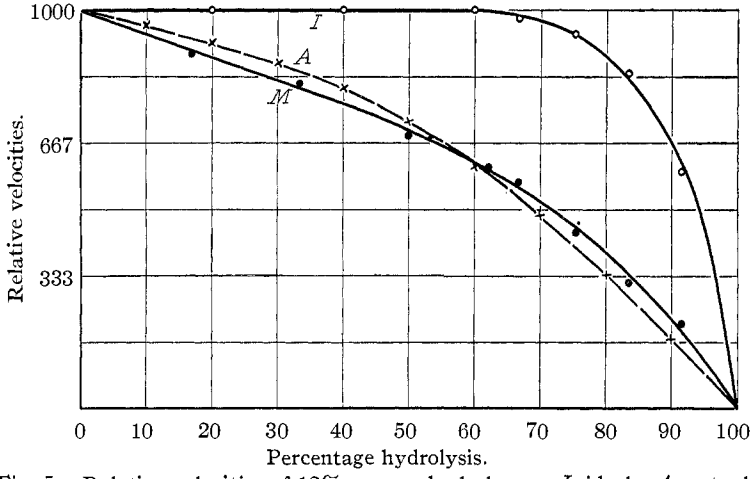

Fig. 5.—Relative velocities of 12% sucrose hydrolyses. *I*, ideal; *A*, actual; *M*, mutarotated.

5) representing the velocities in the corresponding hypothetical hydrolysis in which the invert sugar was mutarotated immediately upon its formation.

Three types of hydrolyses have now been developed: (1) the hypo-

TABLE II

RELATIVE VELOCITIES AT POINTS THROUGHOUT THE COURSE OF MUTAROTATED HYDROLYSES. TEMP. 25°. P_H = 4.7 TO 4.8

The weight of the invert sugar actually used was 360/342 of the values given. Hence, the values for invert sugar given in Column 2 are in terms of sucrose.

SERIES K—8% SUCROSE HYDROLYSIS

Expt.	Sucrose-invert sugar	% Hydr'd.	Rel. vel.
K8	8–0	0.0	1000
K7	7–1	12.5	930
K6	6–2	25.0	868
K5	5–3	37.5	793
K4	4–4	50.0	723
K3	3–5	62.5	603
K2	2–6	75.0	445
K1	1–7	87.5	260
	0–8	100.0	000

SERIES G—10% SUCROSE HYDROLYSIS

Expt.	Sucrose-invert sugar	% Hydr'd.	Rel. vel.
G10	10–0	0.0	1000
G8	8–2	20.0	873
G6	6–4	40.0	749
G4	4–6	60.0	618
G3	3–7	70.0	509
G2	2–8	80.0	387
G1	1–9	90.0	230
	0–10	100.0	000

SERIES M—12% SUCROSE HYDROLYSIS

M12A-B	12–0	0.0	1000
M10	10–2	16.7	887
M8	8–4	33.3	810
M6	6–6	50.0	688
M4.5	4.5–7.5	62.5	609
M4	4–8	66.7	573
M3	3–9	75.0	439
M2	2–10	83.33	310
M1	1–11	91.7	211
	0–12	100.0	000

thetical "ideal hydrolysis" which represents the hydrolysis of sucrose when the invert sugar is considered as being eliminated immediately upon its formation; (2) the "actual hydrolysis" which represents the ordinary observed course of hydrolysis of sucrose, the invert sugar being "nascent;" (3) the hypothetical "mutarotated hydrolysis" which represents the course of the hydrolysis of sucrose when the invert sugar is considered mutarotated immediately upon its formation.

Discussion of Errors.—A consideration of the precision of these results shows that any of the curves *A* in Figs. 3, 4 and 5 is distinct from the corresponding *M* curve. The use of the Hitchcock and Nelson equation or of its derivative involves a maximum error of 0.7%; that of the method of determining initial velocities, by means of tangents, an error of about 1%. Thus, in the specific case of the 10% mutarotated hydrolysis, the velocity at the 20% hydrolyzed point is 873. This value may be in error by 9 and may range, therefore, anywhere from 882 to 864; the corresponding velocity in the actual hydrolysis, 922, may be in error by 7 and may range, therefore, anywhere from 929 to 915. This region, however, and the one from 882 to 864 are mutually exclusive. In general, when regions of error are drawn about the actual and mutarotated curves at each of the three percentages of initial sucrose concentration considered, these regions are found to be mutually exclusive for the greater part of the length of these curves.

Moreover, to take a pair of curves at any particular percentage, if the set of values which represents the mutarotated hydrolysis was not distinct from that set which represents the actual hydrolysis, then the members of these sets would scatter about one another indiscriminately throughout the entire set. As is the case, however, a certain number of the consecutive members of one set are all *below* the corresponding consecutive members of the other set; the remaining members of that first set are then all *above* the remaining members of that second set. This order in the arrangement of the members argues for the distinctness of the sets.

Discussion of the Difference between the Mutarotated and Actual Hydrolyses

Different Mutameric Forms of Glucose and Fructose.—*d*-Glucose exists in two forms, α-*d*-glucose, $[\alpha]_D = +111.2°$, and β-*d*-glucose, $[\alpha]_D = +17.5°$.[5] Each of these forms undergoes mutarotation when dissolved in water, yielding an equilibrium mixture $[\alpha]_D = +52.5°$, which corresponds to about 37% of the alpha and 63% of the beta forms. β-*d*-Fructose, $[\alpha]_D = -130.8°$, when dissolved in water also mutarotates, yielding an equilibrium mixture, $[\alpha]_D = -88.0°$ at 25°. Since it mutarotates, there must be another form of *d*-fructose besides the beta form. So far,

[5] Nelson and Beegle, THIS JOURNAL, **41**, 559 (1919).

no one has been successful in isolating, in pure crystalline form, this other variety of fructose.

Hudson[6] considers that both the glucose and the fructose occur in sucrose in their alpha forms and, hence, when liberated as invert sugar, they undergo mutarotation. Irvine[7] holds that the fructose in cane sugar is a specially reactive form, a γ-fructose. Likewise, Haworth and Law[8] explain a more complex change in the fructose liberated in the hydrolysis of sucrose as follows.

$$\begin{array}{ccccccc} & & \text{A} & & \text{B} & & \text{C} \\ \text{Sucrose} & (\leftrightarrows) & \alpha\text{-glucose} & + & \alpha\text{-fructose} & (\rightleftarrows) & \alpha\text{-fructose} \\ & & \Updownarrow & & \Updownarrow & & \Updownarrow \\ & & \beta\text{-glucose} & & \beta\text{-fructose} & (\leftrightarrows) & \beta\text{-fructose} \\ & & \text{(Butylene} & & \text{(Ethylene} & & \text{(Butylene} \\ & & \text{oxide form)} & & \text{oxide form)} & & \text{oxide form)} \end{array}$$

The final products consist of the equilibrium mixture of A and C together with, perhaps, a small proportion of B.

Effect of Different Mutameric Forms.—Michaelis and Menten[9] studied the retarding effect of invert sugar but made no mention as to whether the hexoses employed in determining this retarding effect on the hydrolysis of sucrose were alpha, beta or mutarotated. In other words, their experiments did not take into account the possibility of the retarding powers of the mutameric forms of glucose (or of fructose) being different.

From the relative values of the initial rates of hydrolysis given in Table II and shown graphically by means of the curves M in Figs. 3, 4 and 5, which describe the course of mutarotated hydrolyses (that is, the hypothetical hydrolysis of sucrose in the presence of completely mutarotated invert sugar) it can be seen that completely mutarotated invert sugar has a retarding effect which is different from that of the invert sugar released in the course of hydrolysis and, therefore, only partially mutarotated. A specific illustration may be taken from Fig. 4. The rate of hydrolysis in an ideal hydrolysis at P' (2 g. of sucrose per 100 cc., 8.421 g. of invert sugar considered removed) is, in terms of the relative velocities already described, 836. The rate of hydrolysis in the actual hydrolysis at P_1 (2 g. of sucrose per 100 cc., and 8.421 g. of *nascent* invert sugar) is 333. The rate of hydrolysis in the mutarotated hydrolysis at P_1 (2 g. of sucrose per 100 cc. and 8.421 g. of *completely mutarotated* invert sugar) is 387.

The same conclusion becomes evident upon consideration of another series of experiments (Table III). A definite amount of α-glucose added directly to a solution of sucrose at the beginning of an hydrolysis retarded differently from the same amount of β-glucose added in the same way to

[6] Hudson, THIS JOURNAL, **30**, 1564 (1908); **31**, 655 (1909).
[7] Irvine and Robertson, *J. Chem. Soc.*, **109**, 1305 (1916).
[8] Haworth and Law, *ibid.*, **109**, 1314 (1916).
[9] Michaelis and Menten, *Biochem. Z.*, **49**, 333 (1913).

a similar sucrose solution. The different mutameric forms of fructose also retarded differently.

TABLE III

RETARDATION BY DIFFERENT FORMS OF GLUCOSE AND FRUCTOSE

Expts. 61, 62, 63: 10 g. of sucrose; 5.263 g. of glucose; 11.11 cc. of No. 1 invertase per 100 cc. of solution. Temp., 25°. P_H = 4.75.

Time	α-Glucose		Mutarot. glucose		β-Glucose	
Mins.	Rotation	Diff.	Rotation	Diff.	Rotation	Diff.
0.0	18.49	..	18.49	..	18.46	0.0
2.5	18.19	0.30	18.20	0.29	18.22	.24
7.0	17.61	.88	17.65	.84	17.67	.79
17	16.35	2.14	16.40	2.09	16.43	2.03
39	13.79	4.70	13.83	4.66	13.93	4.53
69	10.80	7.69	10.85	7.64	10.91	7.55

Expts. 64, 65: 5 g. of sucrose; 5.263 g. of glucose; 11.11 cc. of No. 1 invertase per 100 cc. Temp., 25°. P_H = 4.73, 4.94, respectively.

Time	α-Glucose		Mutarot. glucose	
Mins.	Rotation	Diff.	Rotation	Diff.
0	12.02	..	12.02	..
5	11.41	0.61	11.45	0.57
15	10.24	1.78	10.32	1.70
30	8.72	3.30	8.87	3.15
51	7.05	4.97	7.24	4.78
75	5.77	6.25	5.89	6.13

Expts. 66, 67: 2.5 g. of sucrose; 5.263 g. of glucose; 11.11 cc. of No. 1 invertase per 100 cc. Temp., 25°. P_H = 4.55.

Time	α-Glucose		Mutarot. glucose	
mins.	Rotation	Diff.	Rotation	Diff.
0	8.76	..	8.76	..
5	8.24	0.52	8.30	0.46
15	7.36	1.40	7.49	1.27
25	6.63	2.13	6.84	1.92
40	5.90	2.86	6.04	2.72
60	5.29	3.47	5.39	3.37

Expts. 71, 72: 13 g. of sucrose; 20 g. of *d*-fructose; 50 cc. of No. Hb invertase and 200 cc. of water. Temp., 0°. P_H = 4.83. The fructose used in Expt. 71 was in the beta form, while that used in Expt. 72 was in the mutarotated form.

Time	Expt. 71		Expt. 72	
Mins.	Rotation	Diff.	Rotation	Diff.
0.0	353.15 (extr.)	..	353.15 (extr.)	..
3.5	352.58	0.57	352.79	0.36
13.5	351.20	1.95	351.54	1.61
23.5	350.12	3.03	350.55	2.60
28.5	349.62	3.53	350.08	3.07
38.5	348.86	4.29	349.32	3.83
53.5	347.94	5.21	348.32	4.83
93.5	346.47	6.68	346.69	6.46

In Expt. 71 the invertase was added and the hydrolysis begun 1 minute and 10 seconds after the water had been added. The α- or β-glucose was added to the solution of sucrose; and in all cases, eight minutes after the glucose was dissolved the invertase was added and the hydrolysis begun.

Further confirmation of the fact that the mutameric forms of the hexoses retard differently is offered by similar experiments (unpublished) carried out by Beegle in this Laboratory several years ago. When the amount hydrolyzed in a solution containing 2% of sucrose and 2% of α-glucose was plotted against times of hydrolysis, it was found that such a curve did not superimpose upon a curve that represented the hydrolysis of a solution containing 2% of sucrose and 2% of β-glucose.

Simons[10] reports an experiment which also seems to indicate that the nascent and added invert sugar retard differently. He prepared a solution containing cane sugar and invert sugar in such proportions as to correspond to a 3% sugar solution that had undergone hydrolysis to the extent of 42%. The invert sugar thus introduced naturally mutarotated although the experiment did not state to what extent. This solution was allowed to hydrolyze and the course of its hydrolysis from the beginning was compared with the course of hydrolysis of a 3% sucrose solution from its 42% point to completion. When the amount of sucrose hydrolyzed was plotted against the time, it was found that the two curves did not superimpose. In the first case, the 42% invert sugar was added to the solution before the hydrolysis was begun. In the second case, the 42% invert sugar was formed in the course of the hydrolysis. The extent of mutarotation of the invert sugar in the two cases was undoubtedly different. Hence, the difference in the courses of the two actions was probably due to a difference in the extent of the mutarotation of the invert sugar in the two cases.

After the present work had been in progress for some time, Kuhn[11] reported experiments similar to those of Beegle, and stated that α-glucose had no retarding effect. Euler and Josephson[12] have also recently reported experiments in which the retarding effects of α- and β-glucose are of about the same order, that of β-glucose being slightly larger. These authors attribute the discrepancy between their experiments and those of Kuhn to a difference in the kinds of invertases employed. They point out, moreover, that in those cases where a particular enzyme preparation is used, of such character that the different mutameric forms of glucose or of fructose retard differently, the velocity of mutarotation exerts a particular influence on the kinetics of hydrolysis. For example, these authors state that the curve representing the hydrolysis at a low concentration of invertase, or a slow hydrolysis, has a different form from, or does not superimpose upon, a curve representing a fast hydrolysis induced by a high concentration of the same invertase. Thus, they say on

[10] Simons, "A Study of the Initial Velocity in the Hydrolysis of Sucrose by Invertase," *Dissertation*, Columbia University, **1921**.

[11] Kuhn, *Z. physiol. Chem.*, **129**, 57 (1923).

[12] Euler and Josephson, *ibid.*, **132**, 301 (1924).

p. 325: "Bei hoher Enzymkonzentration muss unter Verwendung einer Saccharase vom Typus der Kuhnschen die prozentische Steigerung der Inversion-konstanten grösser sein als bei geringer Enzymkonzentration."

The same idea had naturally presented itself upon a preliminary consideration of the experimental data obtained in the course of the present investigation. Nelson and Hitchcock[2] found that their equation was applicable to hydrolyses where the invertase from brewery yeast ranged in concentration so that in one case 90% hydrolysis took place in 70 minutes to another case where 90% took place in 12 hours. Invertase Hb used in the present work is very probably similar to the kind used by Kuhn, since the different mutameric forms of fructose retard its action differently (Table III). This invertase preparation gave constant values for n when 90% hydrolysis took place in two hours. In the following experiments (Table IV) very dilute concentrations of this invertase preparation were used. In one case, 86% inversion required three weeks; in another, 90% inversion took place in four weeks. Yet it was found that at these extremely low concentrations of the invertase Nelson and Hitchcock's equation held

TABLE IV

APPLICATION OF NELSON AND HITCHCOCK'S EQUATION TO SLOW HYDROLYSES

Expt. 81. Invertase was diluted approximately 400 times. 250 mg. of gelatin was added to 1000 cc. of this invertase solution to prevent possible inactivation.[a] 10 g. of sucrose and 5.55 cc. of invertase per 100 cc. solution. Temp., 25°. $P_H = 4.86$.

Time Mins.	Rotation	% Hydrolyzed	$n \times 10^7$
0.0	13.01	...	...
2520	11.10	11.34	318.2
5395	9.06	23.44	315.8
8215	7.16	34.72	318.9
11406	5.22	46.23	317.7
16585	2.50	62.37	319.4
22759	0.18	76.13	319.1
29900	−1.57	86.54	323.2
∞	−3.84		
			Mean 318.9
			Av. dev. 0.40%

[a] Nelson and Kerr, *J. Biol. Chem.*, **59**, 495 (1924).

Expt. 82. Invertase Hb was diluted approximately 400 times. No gelatin was used and all other conditions were the same as in Expt. 81.

0.0	13.01	...	...
2520	11.24	10.50	292.6
5320	9.37	21.60	296.4
8607	7.32	33.77	295.2
13779	4.43	50.92	294.7
21320	1.24	69.85	293.8
27365	−0.56	80.54	297.3
37353	−2.17	90.08	292.9
∞	−3.84		
			Mean 294.7
			Av. dev. 0.41%

just as well (Table IV) as it did at the higher. Therefore, under these conditions, at least, Euler and Josephson's prediction does not seem to hold.

Discussion of the Interdependence of the Processes of Mutarotation and Hydrolysis.—That the form of the function in a fast hydrolysis appears to be the same as the form in a slow hydrolysis does not necessarily mean that mutarotation does not play a role in the hydrolysis of sucrose by invertase. The fact that the mutarotated hydrolysis (the hydrolysis of sucrose in the presence of completely mutarotated invert sugar) is different from the course of the actual hydrolysis (Figs. 3, 4 and 5) and the fact that in direct experiments (Table III) the mutameric forms of glucose (or of fructose) retard differently are sufficient evidence that mutarotation does enter into the kinetics of the reaction. But it must be noted that the best method available for the determination of functional similarity—Nelson and Hitchcock's equation—is after all an empirical one. In such an equation there is no assumption as to the nature of the causes operating. Conformance of different sets of observations to this equation to within the required degree of precision does not necessarily signify identity, either in number or nature, of the causes giving rise to these different sets of observations.

The holding of the Nelson and Hitchcock equation despite variation in factors which affect mutarotation—such as hydrogen-ion concentration, temperature, and the length of time of the hydrolysis—has the following significance. The effect of any one factor or the resultant effect of any number of these factors may be so small as to be obscured by the 0.7% error allowed to the criterion (which is empirical in nature). On the other hand, the holding of the Nelson and Hitchcock equation despite variation in factors which affect mutarotation may mirror the existence of a real functional similarity. For the rate of mutarotation or change from alpha sugars to the beta form is dependent naturally on the amount of the alpha sugars. But these amounts are, in turn, dependent on the rate of hydrolysis. Moreover, conditions such as hydrogen-ion concentration and temperature not only affect the rate of hydrolysis directly, but also the rate of liberation of the invert sugar and therefore indirectly the rate of mutarotation.

This complexity of effects may be exactly compensatory, for the form of the function will remain the same even when factors affecting the rate of mutarotation are varied. An attempt to consider these possibilities in detail necessitates a mathematical formulation of the dependence of the mutarotation of invert sugar upon the rate of hydrolysis.

Intersection of the Curves for Actual and Mutarotated Hydrolyses.—As stated above, Curves *A* in Figs. 3, 4 and 5 represent the change in velocity as the hydrolyses of 8, 10 and 12% sucrose solutions progress. The

curves marked *M* in these figures represent the change in velocity of the hypothetical mutarotated hydrolyses of these same solutions. These hydrolyses are termed mutarotated because the invert sugar is considered to be mutarotated instantly upon its formation. The *I* curves also represent the change in velocity during the progress of hypothetical hydrolyses in which the invert sugar is considered to be removed immediately upon its formation. It will be noticed that the *M* and *A* curves cross at a point corresponding to a concentration of sucrose of about 4 to 5 g. per 100 cc. of the solution, and that this is the concentration of sucrose in the ideal hydrolyses where the velocity begins to decrease.

By further inspection of the *M* curves it will be seen also that the portions corresponding to sucrose concentrations greater than 4 to 5% are apparently straight lines. If this is so then the retardation of the mutarotated hydrolyses is directly proportional to the complementary amount of added mutarotated invert sugar, and this relationship between the extent of retardation and amount of mutarotated invert sugar persists until the mutarotated hydrolyses have progressed so far that the concentration of sucrose is below 4 to 5%.

This relationship is also evident from the data given in Table II. Thus in the case of the mutarotated hydrolysis of the 10% sucrose solution: when the composition is 10 g. of sucrose and 0 g. of invert sugar per 100 cc., the relative rate is 1000; at 8 g. of sucrose and 2.11 g. of invert sugar the rate is 873; at 6 g. of sucrose and 4.21 g. of invert sugar the rate is 749; and at 4 g. of sucrose and 6.32 g. of invert sugar the rate is 618. Hence, for each additional increase of 2.11 g. of invert sugar per 100 cc. and the complementary decrease in sucrose the decreases in velocity of the reaction are 127, 124 and 131, respectively. On the other hand, when the sucrose concentration is below 4 to 5% the drop in velocity for each additional increase of 2.11 g. of invert sugar is 231 and 387, respectively. The latter decrease in velocity is much greater and not constant. This same relation between the extent of retardation and the relative concentrations of the components of the complementary mixtures of sucrose and mutarotated invert sugar was found to exist in the case of the 8 and 12% sucrose solutions. Sucrose solutions of concentrations higher than 12% were not studied because it is known that the velocity of hydrolyses for concentrated sucrose solutions decreases with increase of sucrose. This decrease in velocity has been indicated by Curve I, Fig. 2, in a paper by Nelson and Vosburgh.[13]

Experimental Details

Preparation of Materials.—The preparation of Invertase Hb, used in this investigation, is described by Nelson and Hollander.[3] The other preparation, No. 1, is described in the article by Nelson and Hitchcock.[2]

[13] Nelson and Vosburgh, THIS JOURNAL, **39**, 803 (1917).

The best commercial sugar was used. Its solution in distilled water was stirred with charcoal, filtered and recrystallized by shaking according to the procedure of Bates and Jackson.[14] Its rotation agreed within 0.13% with that calculated from the formula of Landolt and Schönrock.[15] The glucose was a very pure commercial grade, obtained from the Corn Products Company of New York and was recrystallized by means of acetic acid according to the method of Hudson and Dale.[16] In order to remove the last traces of acetic acid, the drying was repeated over stick sodium hydroxide. The glucose was then washed with alcohol and dried at 50° over sulfuric acid at 0.04 mm. pressure. The fructose was recrystallized from acetic acid and twice from 95% alcohol according to Vosburgh.[17] The fructose was dried in the same manner as the glucose, except that the temperature was maintained at 30°. Both the fructose and the glucose exhibited the correct rotations.

Apparatus and Procedure.—The apparatus and procedure have become well standardized in the course of their use for several years in this Laboratory. A full description of thermostat, polariscope, hydrogen-ion control and measurement, and hydrolyzing procedure are to be found, therefore, in any article such as that of Nelson and Bloomfield.[1]

Summary

1. A method has been described for showing the magnitude of the effect, due to the presence of the invert sugar, on the rate of hydrolysis at any point in the course of the reaction.

2. It has been shown that the invert sugar has a different retarding effect upon the rate of hydrolysis according to whether it is in a freshly liberated or in a final, mutarotated form. In other words, if the invert sugar were mutarotated immediately upon its liberation, the course of hydrolysis followed would be different from the ordinary, observed course of the reaction.

3. It has been shown that, if the hydrolysis of a 10% sucrose solution is considered to consist of two simultaneous and continuous reactions, namely, the hydrolysis of the sucrose present and the mutarotation of the invert sugar which is being formed, a change in the relative rates of these two reactions has no apparent effect upon the form of the course of the hydrolysis.

4. It has been found, by comparing the actual and mutarotated hydrolyses of 8, 10 and 12% sucrose solutions, that in the portion of the hydrolysis where the sucrose concentration is greater than 4 to 5 g., the retardation of the "nascent" invert sugar is less than that of the mutarotated, while where the sucrose concentration is less than 4 to 5 g. the retardation of the nascent invert sugar is greater than that of the mutarotated.

NEW YORK, N. Y.

[14] Bates and Jackson, *Bur. Standards Sci. Papers*, **268, 75** (1916).

[15] Browne's "Handbook of Sugar Analysis," John Wiley and Sons Co., New York, **1912**, pp. 177–178.

[16] Hudson and Dale, THIS JOURNAL, **39**, 320 (1917).

[17] Vosburgh, *ibid.*, **42**, 1696 (1920).