THE OPTICAL ROTATION OF MIXTURES OF SUCROSE, GLUCOSE AND FRUCTOSE.

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In studying the kinetics of sucrose hydrolysis it has been the usual procedure to follow the course of the reaction by measuring the optical rotation at different stages, the amount of sugar hydrolyzed being assumed to be a linear function of the change in rotation. Hudson[2] has pointed out that strict proportionality between change in rotation and the amount hydrolyzed would be possible only if the specific rotation of sucrose, glucose and fructose did not change with concentration, a condition which is only approximately fulfilled in the case of the pure sugars. In the first 2 the variation is negligible but in the case of fructose it is appreciable in magnitude and the variation is in such a direction as to cause an apparent increase in the velocity coefficients when the latter are calculated by the unimolecular law, an effect which has been noticed by several observers.

On the other hand, Nelson and Beegle[3] have found that the specific

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[1] National Research Fellow.

[2] Hudson, THIS JOURNAL, **32**, 885 (1910).

[3] Nelson and Beegle, *ibid.*, **41**, 572 (1919).

rotation of fructose is affected by the presence of sucrose. This effect is in such a direction as to compensate the variation in specific rotation with concentration in the case of the hydrolysis of sucrose. The question is thus raised as to whether or not these 2 errors exactly compensate each other.

Ostwald[1] carried out an experiment on the practicability of using the polariscope to determine the amount of sugar inverted. By mixing portions of a sucrose solution with an invert sugar solution he showed that the rotation was a linear function of the amount of invert sugar present for the case where the initial sucrose concentration is 25 g. per 100 cc.

Rosanoff[2] also has investigated this question and comes to the conclusion that for the conditions of his experiments, namely, a large sugar concentration in presence of formic acid, the rotation of the solution is a linear function of the sugar inverted. He claims that the specific rotation of fructose does not change with concentration when the water concentration is kept constant by substituting sucrose for part of the fructose.

Thus it appears that within the errors of the experiments mentioned above the amount of sugar inverted can be calculated as a linear function of the optical rotation. The present investigation was undertaken to determine with a greater degree of precision the errors in following a sugar hydrolysis by means of the polariscopic method and to investigate further the effect of the presence of other sugars on the specific rotation of fructose.

The information obtained by the present investigation can be summarized as follows.

1. The specific rotations of glucose and fructose when mixed in equal proportions in solution (invert sugar), are those which the sugars would have if *each were present alone* at a concentration *equal to the total invert sugar concentration.* In other words, the angular rotation of an invert sugar solution is equal to $1/2$ the algebraic sum of the rotations of solutions of glucose and fructose of the same concentration.

2. The specific rotations of glucose and sucrose in mixtures of the 2 are those which the sugars would have if each were present alone at a concentration equal to the total sugar concentration.

3. This relationship is only approximate for mixtures of fructose and sucrose in which case the angular rotation is a little smaller (or larger numerically if negative) than that calculated upon its assumption.

4. The polariscopic determination of the per cent. of sucrose replaced by invert sugar gives slightly high results.

5. Conclusion 4 applies whether the light source in the polariscopic measurements is a sodium flame or a mercury-vapor lamp with suitable

[1] Ostwald. *J. prakt. Chem.,* **29,** 390 (1884).
[2] Rosanoff, THIS JOURNAL, **33,** 1911 (1911).

purification of the light.   In the latter case, however, the error is not as great as in the former.

6. The presence of a constant amount of hydrochloric acid at a concentration of 0.1 molar, sodium chloride at a concentration of one molar, or sodium carbonate at a concentration of about 0.02 molar has no effect on the determination of the per cent. of sucrose replaced by invert sugar when the rotations of the pure sucrose and invert sugar are determined under the same conditions as in the case of the mixtures.

7. The error introduced by the use of the polariscopic method of analysis is small enough to be neglected in most sucrose hydrolysis investigations both in hydrolysis by acids and hydrolysis by invertase when properly carried out in the latter case.   It is such as to cause neither increasing nor decreasing velocity coefficients but would cause the coefficients to be too high by about 4 parts in a thousand when sodium light is used and quite appreciably less in error if mercury-vapor light is used in the polariscopic measurements.

Preparation of Materials.

Sucrose.—The starting material was rock candy which was known to have been made from the best commercial sucrose. It was dissolved and reprecipitated according to the method of Cohen and Commelin[1] only alcohol distilled from quicklime being used. The alcohol was removed from the crystals by heating at 50° in a stream of dry air and the sucrose then dried *in vacuo* over conc. sulfuric acid at 50°. It was preserved in glass-stoppered bottles sealed with paraffin. The specific rotation was found to agree with that calculated by means of Landolt's formula.[2]

Glucose.—The best crystalline glucose manufactured by the Corn Products Refining Co. was purified by reprecipitation. The original material was pure white and gave a colorless solution. Reprecipitation was carried out according to the method of Hudson and Dale[3] for the preparation of α-glucose. It was dried *in vacuo* over conc. sulfuric acid at 50° and preserved in glass-stoppered bottles kept in a desiccator over sulfuric acid. The specific rotation at 25° for a concentration of 10.006 g. per 100 cc. was found to be 52.89° as compared with 52.75° calculated from the formula of Browne.[4]

Fructose.—Most of the fructose used was that described in connection with an investigation of the specific rotation of fructose.[5] A large number of different preparations were needed because of the poor keeping qualities of the pure dry substance. Preparations 1 and 2 were used principally in the present investigation. Preparation 3 was used in Expts. 1b and 2b of Table III and in all the solutions corresponding to 30% inverted in Table I. Preparation 4 was used in 4b of Table IV. Preparation 5 was used in the experiments of Series 3 of Table V. The fructose used in Series 1 and 2 of Table V and all the experiments of Tables VI and VII was prepared by the same method, 2 recrystallizations being made. The formula proposed for the relation of specific rotation of fructose to concentration, namely

$$[\alpha]_D^{25} = -(88.50 + 0.150\ c + 0.00086\ c^2)$$

[1] Cohen and Commelin, *Z. physik. Chem.*, **64,** 29 (1908).

[2] Landolt, "Das Optische Drehingsvermögen," 1898, p. 420.   Browne. "A Handbook of Sugar Analysis," 1912, p. 178.

[3] Hudson and Dale, THIS JOURNAL, **39,** 323 (1917).

[4] Browne, *J. Ind. Eng. Chem.,* **2,** 521 (1910).

[5] Vosburgh, THIS JOURNAL, **42,** 1696 (1920).

can therefore, be used for the calculation of the specific rotation under the conditions required for this investigation with a minimum of error.

Measurement of the Angular Rotation.

A Schmidt and Haensch polarimeter sensitive to $0.01°$ was used for the measurement of the angular rotation. The light source was unpurified sodium light except in the experiments of Tables V, VI and VII, in which a mercury-vapor lamp was used as the light source, the light being purified by passing through a Wratten No. 74 filter.

The temperature was kept constant at $25°$ by the thermostat described by Nelson and Beegle.[1] The polariscope tubes were measured and the observed rotations were corrected to apply to a 200 mm. tube.

Polariscopic Analysis of Mixtures of Sucrose and Invert Sugar.

Solutions corresponding to a sucrose solution at different stages of hydrolysis were made up by weighing out the required amount of sucrose, glucose and fructose, dissolving in water and making up to 100 cc. at $25°$ in calibrated volumetric flasks. All solutions containing glucose and fructose were set aside overnight at room temperature to complete the mutarotation.[2]

The angular rotation was then measured by means of a polariscope as described above. Each mixture was made up in duplicate giving the 2 values in Table I for the angular rotation. The mean of the 2 was used in the calculations.

The per cent. of invert sugar (calculated as sucrose) or "per cent. inverted" was calculated for each mixture by the formula,

$$\text{per cent. invert sugar} = 100 \, \frac{a_s - a}{a_s - a_i}$$

where a_s is the rotation of the pure sucrose, a is the rotation of the mixture under consideration, and a_i is the rotation of the pure invert sugar. The results thus obtained allow a comparison of the known composition of the solution with the composition as determined by the polariscopic method. Also, in order to compare the observed angular rotation with that calculated by assuming the above equation the true per cent. of invert sugar was used and the rotation of the mixture treated as the unknown giving the figures in the column headed "calculated angular rotation."

[1] *Loc. cit.*

[2] A calculation from the results of Nelson and Beegle (*loc. cit.*) showed that the mutarotation of glucose, which is the slower, should be 99.9% complete after 13 hours at $15°$ or after 5 hours at $25°$. At least 17 hours elapsed from the time the solutions were made up until their rotation was determined, so no errors could have been caused by incomplete mutarotation. An experiment showed that no measurable hydrolysis of sucrose took place under the conditions of the experiments during this time.

TABLE I.

Determination of Invert Sugar in Known Mixtures.

A. Concentration Equivalent to 15 g. Sucrose per 100 cc.

Sucrose. G.	Glucose. G.	Fructose. G.	Angular rotation.[a]			Per cent. inverted.	
			1.	2.	Calc.	Obs.	Actual.
15.000	...	...	19.94	19.94	..	...	0
13.500	0.970	0.970	17.33	17.35	17.35	10.04	10
12.000	1.579	1.579	14.73	14.75	14.76	20.09	20
10.500	2.369	2.369	12.13	12.12	12.17	30.19	30
9.000	3.158	3.158	9.56	9.54	9.58	40.13	40
6.000	4.737	4.737	4.35	4.37	4.41	60.18	60
3.000	6.316	6.316	—0.81	—0.81	—0.77	80.13	80
..	7.895	7.895	—5.95	—5.95	..	...	100

B. Concentration Equivalent to 10 g. Sucrose per 100 cc.

10.000	...	...	13.29	13.29	..	...	0
9.000	0.526	0.526	11.57	11.58	11.57	9.97	10
8.000	1.053	1.053	9.84	9.84	9.85	20.06	20
7.000	1.579	1.579	8.11	8.10	8.13	30.15	30
6.000	2.105	2.105	6.41	6.40	6.41	40.04	40
4.000	3.158	3.158	2.98	2.96	2.97	60.02	60
2.000	4.210	4.210	—0.46	—0.47	—0.47	79.99	80
..	5.263	5.263	—3.91	—3.90	..	...	100

C. Concentration Equivalent to 5 g. Sucrose per 100 cc.

5.000	...	...	6.44	6.44	..	...	0
4.500	0.263	0.263	5.77	5.77	5.78	10.15	10
4.000	0.526	0.526	4.91	4.91	4.93	20.19	20
3.500	0.790	0.790	4.05	4.06	4.07	30.16	30
3.000	1.053	1.053	3.19	3.21	3.21	40.14	40
2.000	1.579	1.579	1.49	1.50	1.50	60.02	60
1.000	2.105	2.105	—0.23	—0.22	—0.22	80.10	80
..	2.631	2.631	—1.93	—1.93	..	...	100

Unpurified light from a sodium flame used.

In Table I the agreement of the calculated with the observed per cent. inverted is close, but in all but 4 cases the observed value is the higher. The calculated values for the rotation of the mixtures are correspondingly higher than the observed values. The differences between the observed and calculated rotations are most noticeable in the most concentrated mixtures, namely in A. In B and C, where the differences are nearer in magnitude to the experimental error, the deviation from the linear relationship is less apparent and might be questioned. However, in the latter cases, when any appreciable differences exist between the observed and calculated they are all in the same direction. The differences could have been caused by a high value for the rotation of the pure sucrose, but that is not probable, because the values for pure sucrose all agree well with the values calculated from the formula of Landolt. Apparently, the specific rotations of the sugars in the mixture vary slightly

with concentration in such a way that the amount of invert sugar appears too high.

The Specific Rotation of Sugars in Mixtures.

Nelson and Beegle[1] showed that when glucose and sucrose are present in a solution the rotation is the sum of the rotations due to the 2 sugars calculated as if each were present alone. However, in the case of fructose and sucrose, they found that the observed rotation was greater than the calculated by an amount which for constant fructose concentration was proportional to the amount of sucrose present. The experimental work of Nelson and Beegle was carried out at 37° and it was desirable to check their conclusions at 25° and to determine more definitely the relationship between the rotations of solutions of the separate sugars and solutions of their mixtures.

Glucose and Sucrose.—The rotations of glucose and sucrose separately in the concentrations indicated in Table II and also those of the mixture of the 2 were measured and the rotations of the mixtures are compared in Table II with values calculated in 2 different ways. The first calculated value was obtained by adding the observed values for the rotations of the 2 sugars. The second was calculated on the assumption that the specific rotation is dependent on the total concentration of sugar in the solution instead of on the concentration of the particular sugar in question. To this end the specific rotations for the concentrations 15 g. per 100 cc. for the first experiment and 20 g. per 100 cc. for the second experiment were calculated[2] and from these the rotations due to the separate sugars under the conditions of the experiments were calculated and added.

TABLE II.

Rotation of Mixtures of Glucose and Sucrose.

No.	Glucose. G/100 cc.[a]	Sucrose. G./100 cc.[a]	Observed rotation.[b]			Calculated.	
			Glucose.	Sucrose.	Mixture.	1.	2.
1a	5.003	10.006	5.28	13.29	18.56	18.56	18.59
1b	5.003	10.006	5.27	13.29	18.60	...	...
2a	10.006	10.006	10.59	...	23.93	23.87	23.94
2b	10.006	10.006	10.57	...	23.93	...	...

[a] Weights reduced to a vacuum.

[b] Unpurified light from a sodium flame used.

The rotations calculated by both methods agree with the first value

[1] *Loc. cit.*

[2] These were calculated by the formula of Landolt (*loc. cit.*) for sucrose and by the formula of Browne (*loc. cit.*) for glucose. The observed values for glucose at concentrations 5 g. and 10 g. per 100 cc. are slightly higher than those calculated by Browne's formula, the differences being 0.01° and 0.02° respectively in the cases under consideration. The values calculated for concentrations of 5 g. and 10 g. on the basis of the specific rotations at concentrations of 15 g. and 20 g. respectively, were corrected by adding these differences.

within the experimental error of the first experiment, the second calculated value being closer to the mean. The second method of calculation gives a value in better agreement with the observed in the second experiment. Evidently the specific rotations of glucose and sucrose do not change enough with concentration to make much difference as to which method of calculation is used.

Fructose and Sucrose.—Table III gives experiments on mixtures of fructose and sucrose similar to the experiments in Table II. The 2 calculated values for the mixtures were arrived at by the same 2 assumptions, respectively, as were used for the calculated values for mixtures of glucose and sucrose.

<div align="center">TABLE III.</div>

<div align="center">Rotation of Mixtures of Fructose and Sucrose.</div>

No.	Fructose. G./100 cc.[a]	Sucrose. G./100 cc.[a]	Observed rotation.[b]			Calculated.	
			Fructose.[c]	Sucrose.	Mixture.	1.	2.
1a	5.003	5.003	—8.93	6.64	—2.39	..	..
1b	5.003	5.003	—8.93	6.64	—2.38	—2.29	—2.35
2a	5.003	10.006	—8.93	13.29	4.20	..	..
2b	5.003	10.006	—8.93	13.29	4.19	4.36	4.24

[a] Weights reduced to a vacuum.

[b] Unpurified light from a sodium flame used.

[c] Calculated by means of formula given above.

On the assumption that abnormalities in the rotation of the mixture may be ascribed to the fructose[1] the experiments show that the specific rotation of fructose in the mixture is slightly larger than that corresponding to fructose at a concentration equal to the total sugar concentration in the solution. Replacement of part of the fructose in a solution by an equal weight of sucrose slightly increases the specific rotation of the remaining fructose.

Invert Sugar.—In the case of mixtures of glucose and fructose the second method of calculation gives results which agree well with the observed, as Table IV shows. The 2 calculated values for each mixture were obtained in the manner explained for mixtures of glucose and sucrose. It is probable, therefore, that the specific rotations of fructose in the presence of glucose and also glucose in the presence of fructose, are those which the pure sugars would have if present alone at concentrations equal to the total sugar concentration in the mixture.

The specific rotation of invert sugar can be calculated within 0.01° to 0.02° for any concentration up to 20 g. per 100 cc. at least, by taking $^1/_2$ the algebraic sum of the specific rotations of the glucose and fructose at the same concentration (twice the concentration at which they actually

[1] This assumption was made by Nelson and Beegle (*loc. cit.*). It is made here as an aid in visualizing the results and has little bearing on the conclusions.

TABLE IV.

Rotation of Mixtures of Glucose and Fructose.

No.	Glucose. G./100 cc.[a]	Fructose. G./100 cc.[a]	Observed rotation.[b]			Calculated.	
			Glucose.	Fructose.[c]	Mixture.	1.	2.
1a	2.632	2.632	2.77	—4.68	—1.93	—1.91	—1.93
1b	2.632	2.632	...	...	—1.93	..	..
2a	5.266	5.266	5.54	— 9.40]	—3.91	—3.86	—3.92
2b	5.266	5.266	...	...	—3.90	..	..
3a	7.900	7.900	8.34	—14.16	—5.95	—5.82	—5.94
3b	7.900	7.900	...	...	—5.95	...	..
4a	10.006	10.006	10.59	—17.99	—7.63	—7.41	—7.61
4b	10.006	10.006	10.57	...	—7.62	..	..

[a] Weights reduced to a vacuum.

[b] Unpurified sodium light used.

[c] Calculated by means of formula given above.

exist in the invert sugar) as Ost[1] has pointed out. It is apparent that as a rule the specific rotations of sugars in a mixture are equal to the specific rotations which the pure sugars would have at concentrations equal to the total sugar concentration in the mixture. This rule seems to hold well for mixtures of glucose and sucrose, and of glucose and fructose, but is approximate only for mixtures of fructose and sucrose.

Effect of Light Source in the Polariscopic Measurements on the Above Relationships.

After the above determinations were completed a mercury-vapor lamp was installed in this laboratory for use in polariscopic measurements. It is not to be expected that the light source would have much effect on the above relationships, but it was thought well to repeat some of the experiments of Table I, using light of wave length $\lambda = 546.1\mu\mu$ instead of sodium light. The solutions were made up and the angular rotation measured as described above, using mercury-vapor light which was purified by passing through a Wratten No. 74 filter. The results are shown in Table V. Some further experiments using portions of the solutions of Series 1 and 2 are described below.

The observed per cents. in the experiments of Table V are nearer the actual than the observed ones of Table I A, but here also they are all slightly high. It is to be concluded, therefore, that the polariscopic method of determining the composition of mixtures of sucrose and invert sugar can be used without serious error when purified mercury-vapor light is used for illumination.

[1] Ost, Ber. **24**, 1639 (1891). This proposition is more accurately demonstrated in Table IV than by Ost's work. The accuracy of Ost's conclusions on this subject is impaired (1) by his using glucose whose specific rotation did not agree with the formula of Tollens in making up his invert sugar, while for the calculated values the formula of Tollens was used, and (2) by his using his own formula for the specific rotation of fructose which as the author has shown (*loc. cit.*) does not agree as well as possible with his experimental results.

TABLE V.

Repetition of Some of the Experiments of Table I A Using Light of Wave Length $\lambda = 546.1 \ \mu\mu$.

Actual per cent. inverted.[a]	Angular rotation.			Calc.	Observed per cent. inverted.
	1.	2.	3.		
0	23.48	23.49	23.50[b]	...	...
20	17.36	17.33	17.36	17.38	20.11
30	14.29	14.31	(14.37)[c]	14.33	30.10
40	11.25	11.26	11.27	11.27	40.06
60	5.16	5.16	5.16	5.17	60.04
80	...	—0.95	...	—0.93	80.05
100	—7.04	—7.03	7.04[d]	..	...

[a] The composition of these solutions is given in Table I A.

[b] Calculated 23.49° for $c = 15.009$ (concentration reduced to vacuum) from Landolt's formula and the data of Bates and Jackson, Bur. Standards, *Bull.* **13,** 110 (1916).

[c] This determination is apparently in error and the result is not used in the calculations.

[d] Calculated —7.03° from the formula of Jackson, Bur. Standards, *Bull.* **13,** 652 (1916), for glucose and the formula of the author for fructose by the method demonstrated above.

Effect of the Presence of Acids and Salts.

The results of the above experiments are not directly applicable to the measurement of the velocity of sucrose hydrolysis without further assumptions. If the hydrolysis is catalyzed by acids the observations of the rotation must either be made in the presence of the acid or, if the acid in the samples is neutralized to avoid the difficulty of determining a rotation which is changing, the determination is made in the presence of a salt. If the hydrolysis is catalyzed by invertase it is necessary[1] to remove samples and add them to a small amount of a sodium carbonate solution before determining the rotation. In either case, therefore, a constant quantity of a foreign substance, either acid or salt, will be present in all of the samples of any one hydrolysis. Acids and salts are known to have an effect on the specific rotation of sugars, especially on fructose.[2] Therefore, for the above relationships to apply to the actual determination of sucrose hydrolyzed requires the assumption that the constant amount of acid or salt, although by its presence altering the specific rotations of the sugars somewhat, changes the specific rotation in each case by the same fraction of its value whatever the concentration of the sugar. In other words, if the presence of an acid or a salt does not interfere with the condition that the specific rotations of the 3 sugars be constant throughout all the samples the above relationships will apply to the actual determination of sucrose hydrolyzed in either hydrolysis by acid or invertase provided that errors due to incomplete mutarotation are

[1] Hudson, THIS JOURNAL, **30,** 1564 (1908).

[2] Browne, *loc. cit.,* p. 185.

avoided in the latter case. To ascertain whether or not this assumption is correct required additional experiments similar to those of Tables I and V, but in which the solutions contained a constant amount of either hydrochloric acid, sodium chloride or sodium carbonate.

Hydrochloric Acid.—When hydrochloric acid is added to a solution containing sucrose, glucose and fructose hydrolysis of the sucrose starts immediately and the rotation at the start must be determined by extrapolation after measuring the rotation from time to time during the early stages of the change. In order that the observed rotations of the changing solution be sufficiently accurate the acid concentration must not be too high. It can be as high, however, as the acid concentration of an actual hydrolysis which is followed polariscopically with equal accuracy without neutralization. Armstrong and Caldwell,[1] working at 40°, used 0.002 molar hydrochloric acid and remark that the accuracy of the polariscopic readings is somewhat impaired by the progress of the reaction during reading. At 25° a velocity about equal to that obtained by Armstrong and Caldwell under the above conditions is given by 0.1 molar hydrochloric acid, and this strength was, therefore, chosen for the following work.

Solutions were prepared of sucrose, glucose and fructose similar to those of Table I A except that they were at first made up to somewhat less than 75 cc. and allowed to stand overnight at 25° in order that most of the mutarotation might be completed. Then at time $t = 0$, 25 cc. of 0.4 molar hydrochloric acid was added, the solution quickly made up to 100 cc. and a sample placed in a polariscope tube. An experiment using this procedure with only glucose and fructose as shown in Table VI ("100% inverted") gave a small change in rotation during the first 10 minutes due probably to the mutarotation made necessary by the dilution. Consequently in the other experiments, excepting those starting with pure sucrose, no readings were taken until 10 minutes had elapsed. A large number of readings were then taken during the next 50 minutes and the time of each reading noted. The results were plotted and the time-rotation curve found to be a straight line[2] within the limits of error, making the extrapolation to $t = 0$ simple.

The experimental results and a summary of the initial rotations for the various mixtures as found by extrapolation and also the "per cent. inverted" or per cent. of invert sugar calculated as sucrose on the basis of the experimental results, are given in Table VI. Comparison of the observed "per cent. inverted" column of Table VI with that of Table V shows that the presence of 0.1 molar hydrochloric acid has no effect as regards the relation between change in rotation and fraction of sucrose replaced by an equivalent amount of invert sugar.

[1] Armstrong and Caldwell. *Proc. Roy. Soc. (London)*, **74**, 195 (1904).

[2] The fact that these curves are straight lines has no significance with respect to whether or not the reaction follows the unimolecular law. The portion of the curve covered in an hour is so small that the curve calculated by the unimolecular law for the same conditions is a straight line also within the limits of error of the experiments.

TABLE VI.
Determination of Invert Sugar in Known Mixtures Containing a Constant Amount of Hydrochloric Acid.

Sugar concentration equivalent to 15 g. of sucrose per 100 cc., composition of the solutions as to sugars at time $t = 0$ being that given in Table I A. All solutions are in addition 0.1 molar with respect to hydrochloric acid. Time given in minutes. Mercury vapor light used.

Initial rotation.				20% inverted.				40% inverted.			
1.		2.		1.		2.		1.		2.	
t.	Rot.	t.	Rot.	t.	Rot.	t.	Rot.	t.	Rot.	t.	Rot.
3.8	23.41	5.5	23.34	10.0	17.18	10.0	17.19	9.2	11.13	10.0	11.10
5.2	23.38	7.3	23.33	11.5	17.16	12.0	17.14	10.5	11.10	12.0	11.08
6.8	23.34	8.9	23.29	12.5	17.13	14.5	17.09	11.5	11.08	14.5	11.04
12.5	23.22	11.0	23.22	15.2	17.10	17.0	17.05	14.3	11.02	17.0	11.00
14.2	23.16	13.0	23.17	18.5	17.02	26.0	16.86	15.9	11.01	22.5	10.92
15.5	23.14	20.3	23.00	21.2	16.97	28.0	16.82	21.2	10.92	27.0	10.86
18.0	23.07	22.0	22.95	25.0	16.89	34.0	16.69	22.8	10.89	29.5	10.83
24.5	22.91	28.4	22.82	28.5	16.81	35.5	16.66	27.3	10.83	34.0	10.73
26.5	22.88	30.0	22.77	33.5	16.72	37.0	16.63	28.3	10.82	37.5	10.68
27.9	22.84	38.8	22.55	34.8	16.70	51.0	16.35	29.8	10.79	40.0	10.65
30.6	22.78	41.9	22.46	40.5	16.57	53.0	16.31	35.5	10.73	47.5	10.54
32.1	22.74	50.2	22.23	42.2	16.55	..	...	37.0	10.71	49.0	10.52
33.8	22.69	53.5	22.16	45.7	16.47	..	...	43.0	10.61	53.0	10.43
∞	—7.08	∞	—7.08	47.3	16.45	..	...	45.2	10.58	55.0	10.43
..	...	..	...	52.0	16.33	..	...	50.5	10.50	58.0	10.38
..	...	..	...	53.5	16.32	..	...	54.0	10.45	61.0	10.35
..	...	..	...	59.3	16.20	..	...	57.0	10.39	..	...
..	...	..	...	60.5	16.17	..	...	60.0	10.35	..	...

60% inverted.				80% inverted.				100% inverted.			
9.5	5.04	10.0	5.04	10.0	—0.99	10.0	—1.03	6.0	—7.05	∞	—7.07
10.8	5.03	12.5	5.02	11.5	—1.01	12.0	—1.05	6.5	—7.05	..	..
12.0	5.01	15.0	4.98	12.5	—1.02	14.0	—1.05	9.5	—7.06	..	..
14.0	5.00	17.0	4.96	13.5	—1.02	17.0	—1.07	11.0	—7.07	..	..
16.5	4.98	23.5	4.90	17.5	—1.04	23.3	—1.11	14.0	—7.07	..	..
22.5	4.91	25.0	4.88	18.5	—1.05	25.0	—1.12	15.5	—7.07	..	..
24.0	4.89	33.5	4.79	24.0	—1.09	27.0	—1.13	27.0	—7.06	..	..
28.5	4.84	36.0	4.77	25.5	—1.10	35.3	—1.15	33.0	—7.06	..	..
30.5	4.81	42.0	4.71	29.8	—1.11	38.0	—1.17	39.0	—7.06	..	..
32.0	4.80	45.0	4.68	31.5	—1.12	41.0	—1.19	42.0	—7.06	..	..
37.3	4.75	53.0	4.59	43.5	—1.17	45.0	—1.21	57.0	—7.06	..	..
39.0	4.74	55.0	4.57	45.3	—1.19	48.0	—1.23	58.0	—7.06	..	..
44.5	4.69	57.5	4.54	46.7	—1.19	52.0	—1.24	59.0	—7.07	..	..
46.0	4.67	60.0	4.52	53.0	—1.23	56.0	—1.26	61.0	—7.07	..	..
53.8	4.58	..	...	55.0	—1.23	60.5	—1.29	..	...	..	..
55.8	4.56	..	...	60.3	—1.28	..	...	..	...	..	..
58.0	4.53	..	...	62.0	—1.29	..	...	..	...	..	..
60.0	4.51	..	...	..	...	..	...	..	...	..	..

SUMMARY.

Actual % inverted.	0.	20.	40.	60.	80.	100.
Rotation by extrapolation......... { 1	23.50	17.40	11.25	5.14	—0.95	—7.07
2	23.50	17.39	11.26	5.14	—0.98	—7.07
Observed % inverted.................	...	19.97	40.06	60.06	80.03	..

Sodium Chloride and Sodium Carbonate.—To investigate the effect of the presence of sodium chloride and sodium carbonate on the determination of the amount of sucrose replaced by invert sugar, solutions similar in all respects to those described in Table I A were prepared. These correspond quite closely to samples of a solution undergoing hydrolysis by invertase and for determining the effect of sodium carbonate were treated in the manner which has been recommended by Hudson,[1] for the measurement of the amount of sucrose hydrolyzed in the case of hydrolysis by invertase.

A 50 cc. portion of each solution was added to 5 cc. of 0.2 molar-sodium carbonate and the rotation determined within 2 hours. The results are given in Table VII. For determining the effect of sodium chloride a 25-cc. portion of each original solution was added to 1.465 g. of sodium chloride making the solution approximately one molar[2] with respect to the salt. After standing overnight the rotation was determined giving the results in Table VII. The remaining 25 cc. of original solution was allowed to stand overnight and the rotation determined giving the results tabulated in Series 1 and 2 of Table V.

Comparison of Table VII with Table V shows that the observed per cent. of sucrose replaced by invert sugar bears the same relation to the actual within the limits of error whether determined in solutions containing nothing but the sugars in question or in solutions containing in addition one-molar sodium chloride or 0.02—molar sodium carbonate, provided that the salt concentration is kept constant throughout the series of solutions.

TABLE VII.

Determination of Invert Sugar in Known Mixtures Containing Constant Amounts of Sodium Chloride and Sodium Carbonate.

For experiments with sodium chloride 25 cc. portions of Solutions 1 and 2 of Table V were added to 1.465 g. of NaCl.

For experiments with sodium carbonate 50 cc. portions of Solutions 1 and 2 of Table V were added to 5 cc. portions of 0.2 M Na$_2$CO$_3$.

	Sodium chloride.			Sodium carbonate.		
Actual per cent. inverted.	Rotation.[a]		Per cent. inverted.	Rotation.[a]		Per cent. inverted.
	1.	2.		1.	2.	
0	22.69	22.71	...	21.30	21.29	...
20	16.64	16.65	20.02	15.75	15.77	20.02
30	13.61	13.62	30.04	12.96	12.99	30.09
40	10.60	10.60	40.01	10.20	10.22	40.09
60	4.53	4.55	60.05	4.70	4.69	60.04
80	—1.50	—1.50	80.03	—0.82	—0.84	80.02
100	—7.54	—7.54	...	—6.35	—6.36	...

[a] Mercury-vapor light used.

Discussion.

The small difference between the observed per cent. of invert sugar and the actual in Tables I and V is explainable in the light of the results

[1] *Loc. cit.*

[2] Strictly one mole of sodium chloride to a liter of the sugar solution.

for mixtures of sucrose and fructose. For the 2 to agree would require that the specific rotation of the 3 sugars remained constant throughout the various mixtures. Table II shows for mixtures of sucrose and glucose and Table IV for invert sugar that the specific rotations of the sugars in these mixtures can be considered as depending on the total sugar concentration. For the case under consideration, namely, a practically constant total sugar concentration, the specific rotations should be constant as far as these mixtures are concerned. However, in mixtures of sucrose and fructose the specific rotation of the fructose is larger than that which fructose would have at a concentration equal to the total sugar concentration, as shown in Table III. Therefore, while sucrose is present the fructose has a greater specific rotation than it has after the sucrose has all disappeared. The observed value of the rotation for all mixtures containing sucrose and fructose consequently has a smaller value if positive (or a greater value if negative) than that calculated on the basis of the smaller specific rotation of the fructose in the invert sugar.

Hudson[1] has suggested that the increase in the velocity coefficients for the hydrolysis of sucrose by hydrochloric acid in the work of Armstrong and Caldwell[1] may be due to an error in method rather than to a true exception to the law of mass action. Errors due to incomplete mutarotation at the time of observation account, according to Hudson, for only a negligible part of the deviation from the unimolecular law. The principal error referred to is the one illustrated in Tables I, V, VI and VII, namely, the difference between the actual per cent. inverted and that determined polariscopically.

In order to show the effect of this difference on the velocity coefficients calculated according to the unimolecular law, Table VIII was calculated from the results of Table I. The results given in Table I were used because the difference between the calculated and observed per cents. inverted is greater than in Tables V, VI and VII, due possibly to the use of sodium light. The fact that the solutions in the experiments of Table I contain no hydrochloric acid will not affect the conclusions as it has been shown that the presence of a constant amount of hydrochloric acid does not sensibly alter the difference between the observed and actual per cents. inverted. Table I was considered as the results of a sugar hydrolysis of which the velocity coefficient was taken as 0.000400, approximately the velocity coefficient for hydrolysis with 0.1 molar hydrochloric acid at 25°. Assuming a true unimolecular reaction, the times of sampling were calculated from the assumed constant and the actual per cent. inverted by the formula, $t = (1/k) \log (1/1 - x)$, where x is the *actual* fraction of sucrose which has been replaced by an equivalent amount of invert sugar. Then using the time so calculated, the velocity

[1] *Loc. cit.*

coefficients based on the *observed* "per cents. inverted" were calculated, giving the results in Table V. The differences between the constants so calculated and the assumed constant, 0.000400, are due only to the differences between the observed and actual per cent. inverted.

TABLE VIII.

Effect on the Velocity Coefficients of the Difference between Actual and Observed Per Cent. Inverted in Table I.

$$k = \frac{1}{t} \log \frac{1}{1-x} \text{ from obs. per cent.}$$

Actual %. inverted.	Times t. Calc.[a]	A. ($c=15$).	B. ($c=10$).	C. ($c=5$).
10	114.4	0.0004017	0.0003986	0.0004063
20	242.3	4020	4013	4042
30	387.3	4030	4023	4025
40	554.6	4017	4005	4018
60	994.8	4020	4002	4002
80	1747.5	4016	3999	4012

[a] Calculated from the actual per cent. inverted and the assumed velocity coefficient, $k = 0.000400$.

An inspection of the velocity coefficients calculated from the observed per cents. shows that an apparent deviation from the unimolecular law resulting in increasing velocity coefficients in the hydrolysis of sucrose by acids cannot be explained as due to errors in the method of determining the amount of sucrose hydrolyzed if the possibility of errors due to incomplete mutarotation is excluded. The difference between the observed and actual per cents. of invert sugar in Table I has the effect of making the velocity coefficients too high by about 4 parts per thousand, but causes no increase or decrease in the coefficients as the amount of invert sugar increases, the amount of the decrease in the case of 5 g. per 100 cc. being within the experimental error. The use of mercury-vapor light would give velocity coefficients with quite appreciably less error according to the results of Tables V, VI and VII, as compared with Table I A.

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