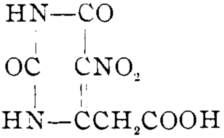
that has been found to have water of crystallization. It melted to an oil at 187–189°. It was dried over sulphuric acid for analysis.

Calculated for $C_8H_{10}O_4N_2.H_2O$: H_2O = 8.33; found, 8.25; N = 12.96; found, 12.97.

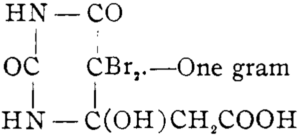
5-Nitrouracil-4-acetic acid,



.—Uracil-4-acetic acid was nitrated by dissolving 1 gram of the acid in 4 cc. of a mixture of two parts concentrated nitric acid and one of sulphuric acid. The solution was then heated on the steam bath. The product was insoluble in alcohol and difficultly soluble in hot water. It formed minute cubes which melted with effervescence at 153°.

Calculated for $C_6H_5O_6N_3$: N, 19.53; found, 19.39.

Dibromoxyhydrouracil-4-acetic acid,



.—One gram of uracil-4-acetic acid was dissolved in water and a large excess of bromine was added. On spontaneous evaporation colorless, elongated plates separated. They became dark colored above 180° and melted with effervescence at 240°. The substance appears to crystallize with a half molecule of water of crystallization.

Calculated for $C_6H_6O_5N_2Br_2.{}^1/_2H_2O$: H_2O = 2.54; found, 2.89. N = 7.87, found, 7.55.

A nitrogen determination in the case of the substance dried at 106° gave 8.43 per cent. nitrogen while the calculated is 8.10 per cent.

NEW HAVEN, CONN., May, 1908.

[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE, LABORATORY OF ANIMAL PHYSIOLOGICAL CHEMISTRY.]

THE INVERSION OF CANE SUGAR BY INVERTASE.[1]

BY C. S. HUDSON.

Received May 8, 1908.

It is the purpose of this preliminary communication to draw attention to a source of large error in the polariscopic measurement of the rate of inversion of cane sugar by invertase. This rate of inversion has been studied very often and the conclusion of all the observers except O'Sullivan and Tompson[2] is that the inversion does not follow the unimolecular reaction formula. This conclusion has received much notice and in most text books the inversion by invertase is said to follow a totally different

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[1] Published by permission of the Secretary of Agriculture.

[2] *J. Chem. Soc.*, **57**, 834–931 (1890).

law from the inversion by acids. In the author's opinion this conclusion is incorrect, for the reason that there is a systematic error in all the polariscopic measurements of this reaction except for those experiments that were made in 1890 by O'Sullivan and Tompson. That such an error should have been made by many independent observers is remarkable on account of the fact that O'Sullivan and Tompson described it clearly and showed how it may be avoided. Their measurements are accordingly free from the error, and it is interesting to note that they conclude that the inversion by invertase is a true unimolecular reaction like the inversion of cane sugar by acids. The error in question can best be expressed in the words of its discoverers, O'Sullivan and Tompson.[1]

"The dextrose formed by the action of invertase on cane sugar is initially in the birotary state, and, therefore, the optical activity of a solution undergoing inversion is no guide to the amount of inversion that has taken place. If a caustic alkali be added to a solution undergoing inversion, and the optical activity be allowed sufficient time to become constant, it is a true indicator of the amount of inversion that had taken place at the moment of adding the alkali."

The method of measurement of the progress of the inversion that was used by these authors is to add a drop of alkali to a sample from the inverting solution at regular intervals and then observe its rotation in the polariscope. The alkali stops the action of the invertase and also brings the mutarotation of the invert sugar to completion so that its normal rotatory value is shown. In this connection consider the following table, the first three columns of which are given by O'Sullivan and Tompson to show the different courses that the inversion by invertase apparently takes according as the mutarotation of the invert sugar is allowed to proceed at its usual rate in the neutral or only slightly acid invertase solution or is enormously accelerated by the addition of alkali before the readings. Columns two and three refer to samples from the same solution, one set being made alkaline before the reading, the other not.

TABLE I.

Time. (t)	Rotation.		$k = \frac{1}{t} \log. \frac{r_0 - r_\infty}{r - r_\infty}$	
	Without alkali.	With alkali.	Without alkali.	With alkali.
0	74.5°	69.4°	....	....
37 min	57.9	37.6	0.0021	0.0046
152 "	0.6	—20.4	0.0037	0.0074
268 "	—19.1	—24.8	0.0041	0.0058
∞ "	—27.8	—27.8	....	....

The starting time for the measurement of the reaction ($t = 0$) is taken several minutes after the addition of the invertase to the cane sugar solution, which accounts for the fact that the first rotations in columns

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[1] *Loc. cit.*, p. 927.

two and three are different. The velocity coefficients (k) of the unimolecular reaction formula have been calculated by the author and are given in columns four and five, r_0 and r_∞ being respectively the rotations at the time "o" and after complete inversion, and r the rotation at the time "t." Decimal logarithms are employed.

It is plain from these results that unless alkali is added before the reading, wholly incorrect values for the inversion are found, the error being as great as 20 per cent. in some cases. When no alkali is added, the coefficient "k" of the unimolecular reaction formula is not constant during the inversion, but increases regularly, a fact that has been observed by all experimenters in this work and has been wrongly interpreted as excluding this reaction from the unimolecular class. On the contrary it can be seen that the five measurements above, few as they are, nevertheless show that there is no regular variation in the coefficient when alkali is added. But this fact is better shown by the following three invertase inversion experiments at 15.5°, Table II, in which alkali was added before the reading, which are also due to O'Sullivan and Tompson.[1]

TABLE II.

A.			B.			C.		
Time. (t).	Per cent. inversion.[2] (x).	k.	Time. (t).	Per cent. inversion. (x).	k.	Time. (t).	Per cent. inversion. (x).	k.
5 min.....	3.1	0.0027	4 min....	4.9	0.0055	5 min....	3.1	0.0027
15 "	9.8	0.0030	19 "	19.2	0.0049	17 "	8.8	0.0024
30 "	19.2	0.0031	39 "	35.3	0.0040	35 "	18.3	0.0025
57 "	33.6	0.0031	59 "	49.5	0.0050	55 "	27.7	0.0026
90 "	45.8	0.0030	79 "	61.8	0.0053	78 "	37.3	0.0026
120 "	58.5	0.0032	99 "	71.0	0.0054	108 "	49.0	0.0027
150 "	67.4	0.0032	132 "	81.9	0.0056	139 "	59.2	0.0028
182 "	74.5	0.0033	164 "	88.7	0.0058	170 "	66.7	0.0028
210 "	79.8	0.0033				204 "	74.3	0.0029
240 "	84.4	0.0034				235 "	79.6	0.0029
270 "	87.3	0.0033				359 "	90.8	0.0029
381 "	93.5	0.0031						

The coefficient of the unimolecular order, $k = \frac{1}{t} \log \frac{100}{100-x}$, is constant in these inversions within the limits of error of the experiments[3] which proves that the reaction is a unimolecular one like the inversion

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[1] *Loc. cit.*, pp. 844, 845.

[2] This has been calculated from the polarimetric readings.

[3] After describing how the action of the invertase was stopped by the addition of caustic alkali before the polariscope readings were made, the authors state that a quarter of an hour should be allowed for the rotation to become perfectly constant; they continue: "We may add that we have only lately been acquainted with the necessity for this delay, and no doubt many of the minor irregularities in our results may be accounted for by not having regard to it."

of cane sugar by acids, although its normal course cannot in general be observed with the polariscope unless alkali is added. The coefficient is greater in Experiment B than in A or C because a greater concentration of invertase was used. It is generally agreed that the rate of the reaction is proportional to the concentration of the invertase.

The work of O'Sullivan and Tompson was published in 1890, and is one of the first investigations on the rate of the invertase inversion of cane sugar. Since its publication numerous investigations on the same subject have been made by others, and it is the unanimous conclusion of these later investigators that the rate of inversion does not follow the unimolecular formula. This disagreement has been strongly emphasized by Victor Henri in his book, "Lois générales de l'action des diastases," but the experiments of Henri contain the very error which O'Sullivan and Tompson had previously called attention to. Consider the following typical invertase inversion measurement[1] in which no alkali was added before the polariscopic readings. The rotations of the inverting solutions are not given in this experiment, but instead the per cent. inversion has been calculated from them by Henri[2] on the assumption that the polarimetric reading of the solution to which no alkali had been added was a correct measure of the extent of inversion. The temperature was 25°, and natural logarithms were used in calculating the velocity coefficient.

TABLE III.

Time (t).	Per cent. inversion (x).	$k = \frac{1}{t} \log. \frac{100}{100-x}$
0	0.0	....
28 min	6.1	0.00105
115 "	31.6	0.00143
182 "	48.7	0.00159
311 "	71.3	0.00174
373 "	78.9	0.00174
511 "	89.2	0.00181
630 "	93.2	0.00185
∞ "	100.00	.....

It is apparent from the regular increase of k in this inversion experiment, similar to the increase shown in columns four of Tables I and IV, that the mutarotation of the invert sugar has caused the polarimetric readings to give totally incorrect values of the extent of inversion, and has masked the true course of this unimolecular reaction.

In order to show conclusively that the inversion of cane sugar by invertase follows the unimolecular law, an experiment by the present author is given below, in which the inversion was measured by the polar-

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[1] Henri, "Lois générales de l'action des diastases," p. 55. Published by Hermann, Paris, 1903.

[2] *Loc. cit.*, p. 53.

iscope, and in two ways, first without the addition of any alkali to accelerate the mutarotation of the invert sugar, and second with the addition of a trace of strong caustic soda solution. A 10 per cent. solution of cane sugar was warmed to 30° and mixed at the time marked zero in Table IV, with approximately one-fourth of its volume of a liquid which had been pressed from fresh beer yeast and therefore contained invertase. With a portion of the resulting solution, a polariscope observation tube was filled, which was kept accurately at 30° by a rapid stream of water flowing through its mantle. The change of rotation of the solution in this tube during the inversion is given in column two of Table IV; it shows the course that the inversion apparently takes when no addition of alkali or other precaution is used to avoid the mutarotation of the invert sugar. The remainder of the original solution was kept in a constant temperature bath at 30°, and at intervals, 50 cc. were removed from it, mixed with one or two drops of 10 per cent. caustic soda solution and the rotation observed in a second polariscope tube. The values are given in column three of Table IV; they show the true extent of the inversion at the time the alkali was added, because the invertase is destroyed and the mutarotation of the invert sugar is brought to completion almost instantly by the alkali.

TABLE IV.—APPARENT AND REAL RATES OF INVERSION OF CANE SUGAR BY INVERTASE.

Time. (t).	Rotation.		$k = \frac{1}{t} \log. \frac{r_0 - r_\infty}{r - r_\infty}$.	
	Without alkali (apparent rate).	With alkali (real rate).	Without alkali.	With alkali.
0	24.50	24.50	....	....
30 min.	16.85	14.27	0.00396	0.00558
60 "	10.95	7.90	0.00399	0.00530
90 "	4.75	3.00	0.00464	0.00539
110 "	1.95	0.80	0.00482	0.00534
130 "	—0.55	—1.49	0.00511	0.00559
150 "	—2.20	—2.40	0.00522	0.00533
∞ "	—7.47	—7.47	....	....

In the last two columns are given the unimolecular velocity coefficients calculated from the data of columns two and three respectively. It is clear that the apparent rate of inversion gives a velocity coefficient which is not constant, but steadily increases, as has been found by Henri (see Table III). On the other hand, the real rate of inversion gives a constant coefficient, as was found by O'Sullivan and Tompson (see Table II). Also the variable coefficient of the apparent rate approaches the constant coefficient of the real rate as a limit. This experiment is conclusive proof that the inversion of cane sugar by invertase follows the law of a unimolecular reaction.

It may be asked why the rate of inversion of cane sugar by acids can be correctly measured in the polariscope without paying any attention to the mutarotation of the invert sugar. The answer is that the acids are strong catalyzers of the mutarotation of invert sugar and their presence in the solution causes the mutarotation to be completed so quickly that the invert sugar has at all times during the inversion practically its normal rotatory power. In this connection it should be mentioned that Meyer[1] has lately sought to show that even during the inversion by acids the polariscope gives incorrect values of the course of the reaction during its early stages, due to the mutarotation of the invert sugar. It can be shown, however, that these supposed irregularities in the polariscopic readings are much less than the errors of measurement and that Meyer's results give no indication of irregularity when they are properly interpreted.

The following table (V) is the one which Meyer has selected[2] as best showing the supposed irregular course of the inversion by hydrochloric acid in its early stages. Columns four and five of this table have been added by the present author.

TABLE V.

Time.	Rotation.	*k* (Meyer).	$\frac{\Delta r}{\Delta t}$.	*k* (Hudson).
0	40.45	....	......	....
6 min.	40.41	0.000123	0.0066	....
26 "	40.31	0.000100	0.0050	Start.
65 "	40.24	0.000086	0.0018	Omitted.
68 "	40.17	0.000076	0.0233	0.0000603
100 "	40.06	0.000072	0.0034	0.0000621
128 "	39.96	0.000071	0.0036	0.0000633
325 "	39.30	0.000066	0.0034	0.0000630
417 "	39.03	0.000065	0.0029	0.0000616
470 "	38.83	0.000065	0.0038	0.0000630
1419 "	35.77	0.000064	0.0032	0.0000630
1784 "	34.62	0.000064	0.0032	0.0000635
∞ "	—13.65	....	......	....

In column four is given the observed change of rotation per minute as calculated from columns one and two, and it is quite apparent that large errors have been made by the observer in the first five readings, though the later readings show a concordant rate of change. The first erroneous readings should evidently not be included in the valuation of *k*, and for this reason the beginning of the reaction is taken by the author at the time 26 instead of at 0 in calculating the values of *k* in column five. The start is taken at this instant, because although $\Delta r / \Delta t$ shows irregular values for the next two readings, these values are obviously due to the erroneous reading at the time 65, as $\Delta r / \Delta t$ for the interval between 26 and 68 has the normal value 0.0033. The reading at 65 is also accordingly omitted. When

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[1] *Z. physik. Chem.*, **62,** 59–88 (1908).

[2] *Loc. cit.*, pp. 78–81.

these measurements, which the internal evidence shows are untrustworthy, are thrown out, the inversion gives a constant coefficient (see column five), showing that the inversion of cane sugar by acids is of the unimolecular order even in its first stages, as it has always been considered to be. The other similar tables of Meyer contain the same error, and when the erroneous readings are excluded from them by the above unbiased method, they give satisfactory constancy for the inversion coefficient.

A complete discussion of Meyer's results is deferred to a later article which will present a detailed study that is now in progress of the inversion of cane sugar by invertase and acids. The present article is only a preliminary communication but it is thought that its immediate publication may be of service to investigators in this field, because the data that are here given are quite sufficient to show, first, that the inversion of cane sugar by acids follows, even in its first stages, the unimolecular order, and second, that the polarimetric measurement of the inversion of cane sugar by invertase involves a large source of error which can be avoided by adding alkali to the inverting solution before each reading of the polariscope. When this source of error is eliminated, as was done by O'Sullivan and Tompson in their inversion measurements, the rate follows the unimolecular order. The inversion of cane sugar by invertase is the most thoroughly studied of the numerous enzyme actions; in view of the foregoing proof of its unimolecular order may it not be that the other enzyme actions are, after all, quite similar to the usual types of chemical catalysis and that they do not constitute a group that has unique laws of catalytic action, as is now generally believed?

[CONTRIBUTION FROM THE NEW YORK AGRICULTURAL EXPERIMENT STATION.]

CONDITIONS AFFECTING THE PROPORTIONS OF FAT AND PROTEINS IN COW'S MILK.

BY LUCIUS L. VAN SLYKE.

Received April 18, 1908.

The interesting article of Mr. E. B. Hart on "Variations in the Amount of Casein in Cow's Milk" in THIS JOURNAL, 30, 281 has suggested that the subject is capable of much more extended treatment than was attempted and that its practical applications concern wider and more varied interests than those indicated. Thus, among these practical applications, we may mention the following: (1) The relation of fat and casein in milk to yield of cheese. (2) The relation of fat and casein in milk to composition and quality of cheese. (3) The relation of fat to proteins in milk as a basis for detecting skimmed milk. (4) The relation of fat to proteins in cheese as a basis for detecting cheese made from skimmed milk.

The accumulation of extensive data in the records of this station, bear-