THE INVERSION OF CANE SUGAR BY INVERTASE. IV. THE INFLUENCE OF ACIDS AND ALKALIES ON THE ACTIVITY OF INVERTASE.

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Introduction.—The marked influence of the acidity of the medium on the activity of the enzyme invertase has been noticed by Kjeldahl,[1] O'Sullivan and Tompson,[2] Hudson,[3] and recently by Sorensen;[4] the results of these researches may be briefly summarized by the statement that, although alkalinity of any strength entirely prevents the action of invertase, slight acidity favors it, but strong acidity prevents it, and there is therefore a concentration of acid for which the enzymotic activity is a maximum. In preliminary experiments it was noticed that the action of acids and alkalies on invertase is twofold, for in some cases the enzyme is destroyed but in others it is only temporarily prevented from acting. For example, if a strongly active solution of invertase is made faintly alkaline to litmus and some cane sugar is dissolved in it no inversion of the sugar occurs even after several hours; if the alkaline solution is then made acid with acetic acid the invertase immediately begins to invert the sugar, showing that the enzyme has not been destroyed by the slight alkalinity but only held back or greatly retarded in its normal action. The word *inactivation* will be used to describe this phenomenon of retardation. On the other hand, if an invertase solution is made strongly alkaline the enzyme is permanently destroyed, as shown by the fact that the addition of acid does not cause any return of enzymotic activity. Invertase is also permanently destroyed by strong acidity. The present investigation was made to determine more exactly the conditions of acidity and alkalinity that cause an inactivation of the invertase and those that cause its total destruction.

The Preparation and Dialysis of the Invertase Solutions.—The invertase solutions were prepared from yeast by the rapid process previously described,[5] modified in certain particulars so that it now reads as follows: Crumble pure compressed yeast by hand and knead it with an equal weight of water at ordinary temperature, saturate the liquid with chloroform and keep it at 20 to 30° for forty-eight hours. Add neutral lead acetate to slight excess, filter the solution, remove the excess of lead from the filtrate with potassium oxalate and repeat the filtration. Then saturate this filtrate with toluene and preserve in an ice box.

In order to free the solution of invertase from salts and other impurities

[1] *Meddelelser fra Carlsberg Laboratoriet*, 1, 337 (1881).

[2] *J. Chem. Soc.*, 57, 854 (1890).

[3] THIS JOURNAL, 30, 1570 (1908).

[4] *Comptes rendus des travaux du Laboratoire de Carlsberg*, 8, 1–168 (1909).

[5] *J. Ind. Eng. Chem.*, 2, 143 (1910).

a thorough dialysis was performed. The solution was held in a cone of parchment paper which rested in a large funnel on porcelain rings affording a free passage for running tap water between the paper and the funnel. The marked changes which took place in the composition of the solution during dialysis are shown in Table I; they consist in a removal of large quantities of the total solids, including nearly all the nitrogenous and ash-bearing substances. The strong yellow color of the solution also disappears almost completely during dialysis.

TABLE I.—CHANGES IN PREPARED YEAST JUICE DURING DIALYSIS.

Date, 1909.	Activity. Natural.	Activity. Maximum.	Nitrogen. Per cent.	Total solids. Per cent.	Ash. Per cent.
November 8.....	260	1,100	3.300	7.50	.....
" 9.....	60	1,260	0.130	....	.....
" 10.....	80	1,200	0.073	1.14	0.200
" 11.....	210	1,150	0.042	0.56	0.064
" 12.....	200	....	0.022	0.44	0.036
" 13.....	250	1,030	0.013	0.32	0.024
" 22.....	160	930	0.008	0.22	0.004

A record of the enzymotic activity of the solutions is shown in the second and third columns. The natural activity is that of the solution without the addition of any acid to activate the invertase; this activity does not show the amount of invertase that is present in the solution because the activity depends greatly on the slight traces of acid or alkali that are present. The maximum activity is that of the solution after a few drops of acetic acid are added to bring the acidity to a point at which it is practically independent of slight changes in acidity; this maximum activity is a correct indication of the quantity of invertase that is present in the solution. The great changes in composition which occur during the dialysis of the solution involve little, if any, loss of invertase for the maximum activity remains nearly constant. The results show that invertase does not pass through dialyzing parchment paper. The natural activity, however, shows a peculiar change during dialysis, decreasing at first and later rising to about its original value. As the invertase does not disappear this change in natural activity must be ascribed to the changing acidity or alkalinity of the solution, and the explanation of its peculiar course doubtless lies in a rapid loss of acids through the parchment paper in the first stages of the dialysis, leaving alkaline substances behind which inactivate the invertase; as the dialysis proceeds these substances also pass through the filter, and the lessened alkalinity causes an activation of the invertase.

The values of the activity were measured as follows: 5 cc. of the solution, the activity of which was to be measured, were added to 100 cc. of a 0.2 formular solution of cane sugar, 5 cc. of water, or of acid in the experi-

ments on the effect of acids, were added and the mixture allowed to proceed in reaction in a thermostat at 30°. At the end of one or two hours 50 cc. of the mixture were removed, made alkaline with sodium carbonate to stop the action of the enzyme and complete the mutarotation of the invert sugar, and the rotation was read on the polariscope. The velocity coefficient of the reaction $k_1 = 1/t \log r_0 - r_\infty / r - r_\infty$ was calculated and its value, after multiplication by 10,000 to avoid decimals, was recorded as the activity of the given invertase solution. In the calculations the time (t) was expressed in hours and decimal logarithms were used. The determination of the rotation of the solutions after complete inversion (r_∞) was not made in each case, but instead the ratio r_∞ to r_0, usually known as the Clerget actor, was measured accurately in a special investigation,[1] which gave the value 0.317 for this factor at 20°, this being the same value as that found when hydrochloric acid is used as the inverting agent and the acid is neutralized before the polariscopic reading is made. The identity of the values of the factor for the two inverting agents shows that invertase accomplishes as complete a hydrolysis of cane sugar as does hydrochloric acid. Visser[2] has concluded from his similar experiments that invertase does not accomplish as complete an inversion of cane sugar as does hydrochloric acid, because his factor for invertase inversions was always smaller than that for the acid; but he overlooked the essential fact that the acid must be neutralized before the reading

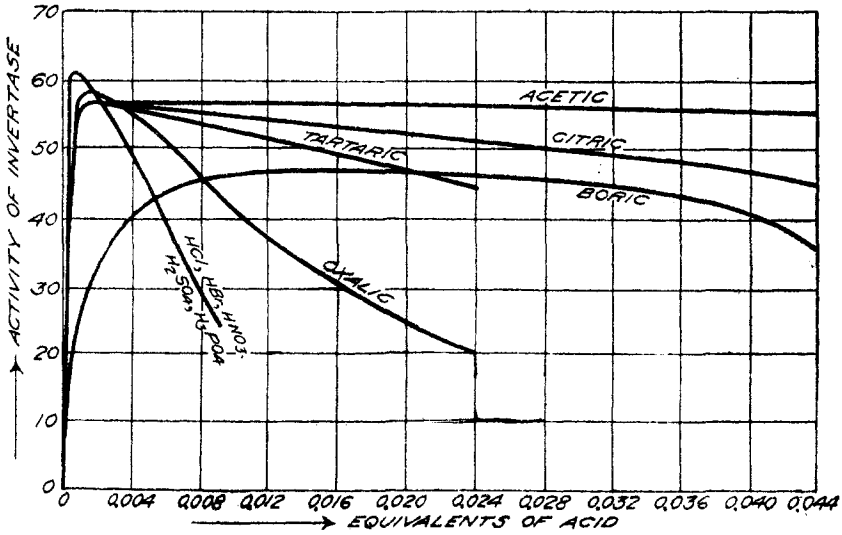

Fig. 1.--Influence of acids on the activity of invertase.

[1] *J. Ind. Eng. Chem.*, 2, 143 (1910).

[2] *Z. physik. Chem.*, 52, 275 (1905).

is made, if the results are to be compared with the reading of the neutral invertase solutions.

The Activity of Invertase in Various Acid Solutions.—The measurements of these activities were made in the manner described in the preceding section, and the values are recorded in Table II.

TABLE II.—ACTIVITY OF PURIFIED INVERTASE IN SOLUTIONS OF VARIOUS ACIDS.

	Acidity of invertase in the acids named.									
Concentration of acid. (Gram equivalents per liter)	Hydrochloric.	Nitric.	Sulphuric.	Phosphoric.	Hydrobromic.	Boric.	Oxalic.	Tartaric.	Citric.	Acetic.
Distilled water.....	4.8	4.5	4.5	5.8	4.3	5.0	9.0	9.0	13	15
0.00009.....	30	29	34	....	34	....	49	43	49	..
0.00012.....	....	....	....	27	....	....	....	....	..	..
0.0005.....	62	61	62	....	59	....	57	58	58	..
0.0007.....	....	....	....	59	....	....	....	....	..	..
0.0015.....	61	61	59	....	58	31	57	56	59	..
0.0021.....	....	....	....	62	....	....	....	....	..	..
0.003.....	58	55	51	....	53	....	....	56	..	..
0.004.....	....	....	....	54	....	....	55	....	..	..
0.008.....	37	28	19	....	31	45	46	54	56	..
0.010.....	(1)	(1)	(1)	....	(1)	....	....	....	..	57
0.011.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	..
0.014.....	(1)	(1)	(1)	(1)	(1)	47	34	50	54	..
0.020.....	(1)	(1)	(1)	(1)	(1)	....	25	48	52	..
0.023.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	56
0.025.....	(1)	(1)	(1)	(1)	(1)	....	21	45	50	..
0.028.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	..
0.030.....	(1)	(1)	(1)	(1)	(1)	45	....	....	50	..
0.035.....	(1)	(1)	(1)	(1)	(1)	43	....	....	48	..
0.040.....	(1)	(1)	(1)	(1)	(1)	41	....	....	46	..
0.045.....	(1)	(1)	(1)	(1)	(1)	35	....	....	45	..
0.072.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	54
0.12.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	54
0.24.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	50
0.35.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	44
0.54.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	37
0.96.....	(1)	(1)	(1)	(1)	(1)	....	....	....	..	21

In Fig. 1 the curves for the five strong acids—hydrochloric, hydrobromic, nitric, sulphuric, and phosphoric—fall so closely together that one unbroken line is shown for them all. The enzyme begins to be destroyed by these mineral acids at a concentration of about 0.01 normal and the measurements of the activity were not made above this limit. The falling of the curve (Fig. 1) with increasing acidity shows a characteristic progression in the order, strong mineral acids, oxalic, tartaric, citric, acetic, and boric, which is also the order of the strengths of these

[1] Destruction of enzyme occurs.

acids. If the ionization of the acids is taken into consideration, and the activity plotted against the actual hydrogen-ion concentration the various weaker acids all give curves agreeing closely with that for the strong mineral acids. The enzymotic activity is thus primarily a function of the hydrogen-ion concentration. The theoretical interpretation of this characteristic relation between the activity and the true acidity must be postponed until further investigations have been made.

The Destruction of Invertase by Acids and Alkalis at 30° C.—The destruction of invertase by acids and alkalis at 30° was measured by mixing a portion of well dialyzed invertase solution with the appropriate acid or alkaline solution, removing portions of this mixture after definite intervals of time and measuring their activities after bringing them to a uniform condition of slight acidity with acetic acid in order to find the maximum activity which is a measure of the quantity of invertase in the solution. If the activities of the successively removed samples showed a decrease it was concluded that the invertase was being destroyed by the acid or alkaline solution. The following typical experiment shows that the rate of this destruction follows the formula for unimolecular reactions, namely, $1/t \log A/A - x = k_2$, where A is the activity of the invertase at the beginning of the destruction, x is the activity after the destruction has proceeded for t minutes, and k_2 is a constant, the velocity coefficient. The concentration of acid in this experiment was 0.02 normal hydrochloric.

TABLE III.—THE UNIMOLECULAR ORDER OF THE DESTRUCTION OF INVERTASE.

Time. Minutes.	Activity (× 10000).	Rate of destruction (k_2).	Time Minutes.	Activity (× 10000).	Rate of destruction (k_2).
0	50.5	.....	70	24.9	0.0044
25	39.0	0.0045	85	22.0	0.0042
40	33.1	0.0046	115	17.7	0.0040
55	28.4	0.0045	...	....	.....

In Table IV the rate of destruction, that is, the velocity coefficient k_2 multiplied by 1000, is recorded for various strengths of hydrochloric acid and sodium hydroxide solutions at 30°; the results are also shown in Fig. 2.

TABLE IV.—RATE OF DESTRUCTION OF INVERTASE AT 30° BY ACID AND ALKALI.

Concentration (Gram molecules per liter.)	Rate of destruction of invertase (k_2 × 1000).	Concentration. (Gram molecules per liter.)	Rate of destruction of invertase (k_2 × 1000).
Normal hydrochloric acid:		Distilled water.....	0
0.05.....	365	Normal sodium hydroxide:	
0.04.....	96	0.01.....	3
0.03.....	42	0.02.....	11
0.02.....	4	0.03.....	50
0.015.....	1	0.04.....	245
0.01.....	0		

Summary.

Acids and alkalis are found to affect the purified enzyme invertase

in two ways: in small concentrations they influence its activity but do not permanently destroy it, in larger concentrations they accomplish its destruction. The destruction by acid at 30° reaches a barely noticeable rate at 0.01 normal acidity and increases rapidly with the acidity until it becomes almost instantaneous at 0.05 normal. The rate of destruction follows the formula for unimolecular reactions. The alkaline destruction begins a little below 0.01 normal and is almost instantaneous at 0.045 normal. The rates of destruction are shown in Fig. 2.

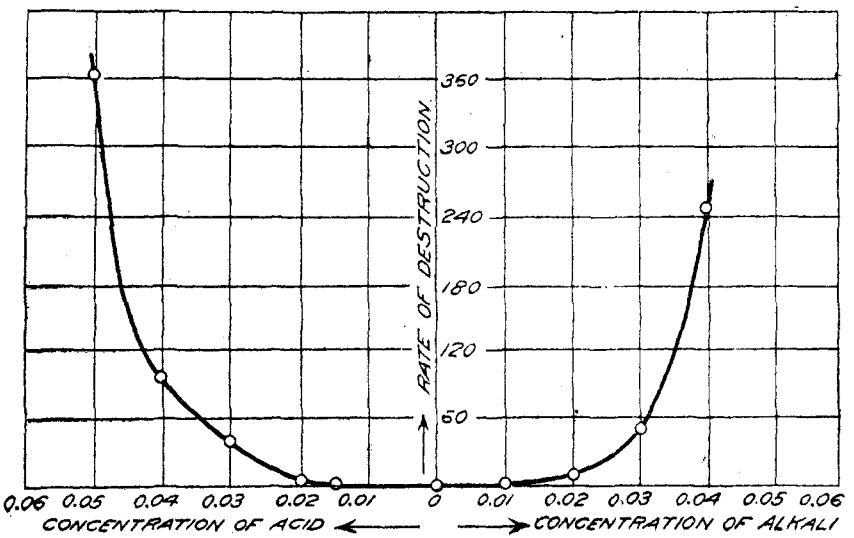

Fig. 2.—The rate of destruction of invertase at 30° C. by acid and alkali.

The activity of invertase in acid solutions which are not strong enough to destroy the enzyme was measured for hydrochloric, hydrobromic, nitric, phosphoric, sulphuric, boric, oxalic, tartaric, citric, and acetic acids; the activity depends almost entirely on the concentration of hydrogen ions in the acid solution and the various acids thus show typical differences which correspond with their recognized degrees of dissociation. The activity of invertase is zero in alkaline solutions, rises to a maximum in very weakly acid ones, and decreases with stronger acidity.

FEHLING'S SOLUTION.

A CONTRIBUTION TO THE HISTORY OF CHEMICAL REAGENTS.

By B. Herstein.

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A history of chemical reagents would form no small and not the least interesting part of the history of chemistry generally, and for obvious