DILATOMETRIC STUDIES IN ENZYME ACTION. PART III.

Contraction constants of enzyme-substrate reactions.

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GLUCOSIDES.

The present communication deals with the hydrolysis of four well-known glucosides, amygdalin, arbutin, aesculin and salicin by emulsin, the reactions being conducted in the dilatometer described in an earlier paper (*J. Indian Inst. Sci.*, 1932, **15A**, 17), which gives the experimental procedure in detail. In the present study, the fall in the dilatometric column was measured at intervals; the degree of hydrolysis was also determined by an independent analysis of the reaction mixture, 5 c.c. aliquots of which, were drawn from a control flask maintained for the purpose. The amount of sugar thus determined gives the amount of glucoside hydrolysed. The contraction constant per gram molecule of the glucoside can therefore be calculated from the quantity hydrolysed and the corresponding dilatometric depression observed.

EXPERIMENTAL.

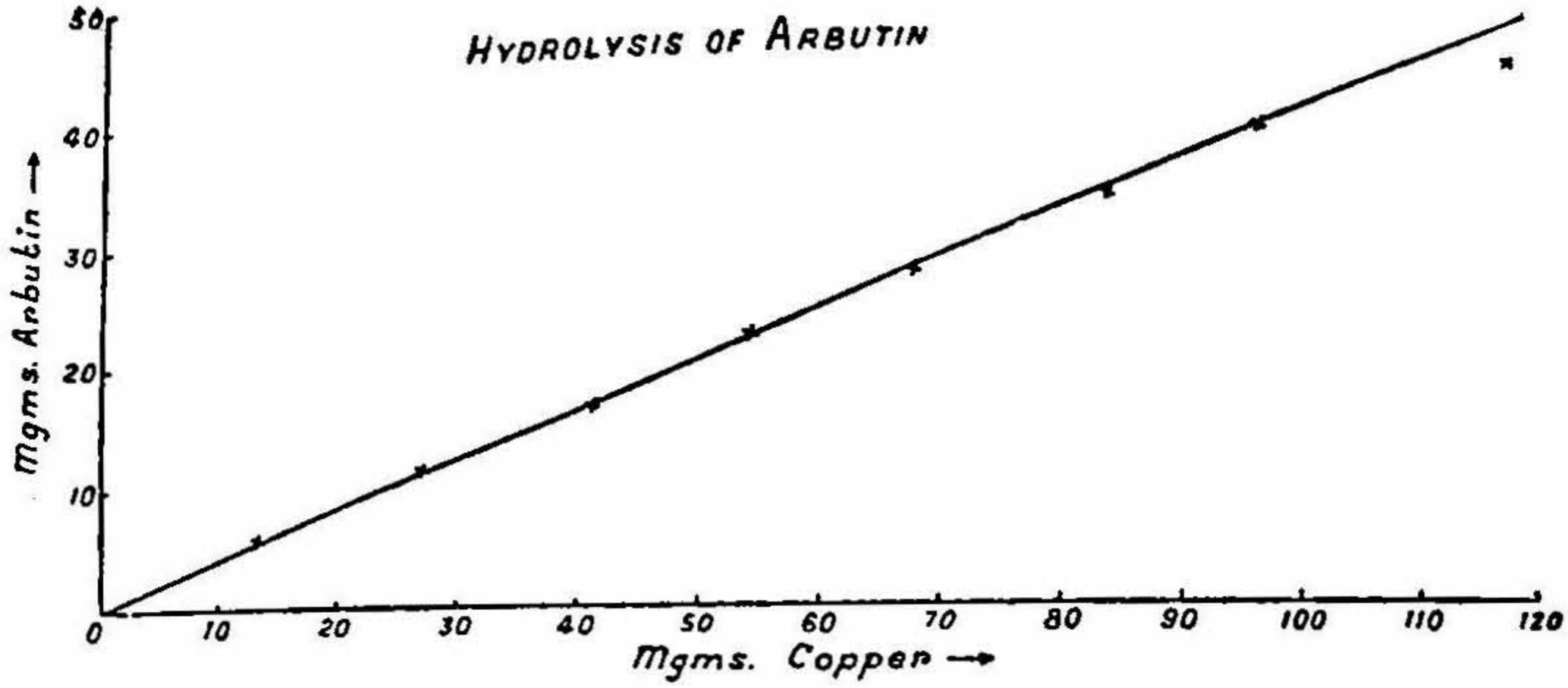
Mixtures containing 50 c.c. of about 1 per cent. solution of the glucoside dissolved in Sörensen's phosphate buffer (P 5.2) and 5 c.c. of 1 per cent. solution of almond emulsin (B.D.H.) were usually employed for the experiments. Aesculin and arbutin were preparations of Theodor Schuchardt, amygdalin, Kahlbaum's, and salicin, B.D.H.

Sugar formed in the hydrolysates was estimated by Bertrand's method. In the case of arbutin, however, the estimation had to be carried out immediately, since the hydroquinone accompanying the sugar in the hydrolysate became rapidly oxidised to quinone particularly in alkaline solutions. In calculating the amount of arbutin from the experimental copper value, the combined reducing action of sugar and hydroquinone have to be considered.

Copper values for mixtures of the two reducing constituents in the proportion in which they occur in arbutin were determined, and the values given in Table I are represented in the graph, from which, given the copper value of arbutin hydrolysate, the amount of glucoside hydrolysed can be directly read.

TABLE I

	1	2	3	4	5	6	7	8
Mixture, c.c. ...	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Copper reduction, mg. ...	13.8	27.1	41.4	54.7	68.5	84.8	97.1	118.3
Arbutin, mg. ...	5.6	11.2	16.8	22.4	27.5	33.5	39.1	44.7



Reduction values should be determined immediately after pipetting the arbutin hydrolysate or the artificially prepared sugar-hydroquinone mixture into the alkaline copper solution. Atmospheric oxygen quickly oxidises the hydroquinone in alkaline media as revealed by the following table, in which the results of back-titrating unused ferric iron according to the Bertrand method at different intervals, are given.

TABLE II

Time of estimation.	$KMnO_4$ ($N/10$) required in c.c.
Immediate.	5·95
After 1 hour.	4·70
After aeration for 1 hour.	2.85

TABLE III

Emulsin—amygdalin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Amygdalin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression
			Exptl.	Control	
23	28.6	165.8	2.8	16.7	0.0
46	47.0	330.3	2.8	16.7	0.0
78	51.6	363.8	2.8	16.7	0.0
135	59.8	424.5	2.8	16.8	0.0

TABLE IV

Emulsin—arbutin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Arbutin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression
			Exptl.	Control	
100	15.3	70.4	4.3	12.2	0.0
194	25.2	116.1	4.3	12.2	0.0
342	39.2	176.0	4.3	12.2	0.0
610	62.3	279.4	4.3	12.2	0.0
1230	83.8	375.1	4.4	12.3	0.0

TABLE V

Emulsin—Salicin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Salicin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression cms.	Contraction constants
			Exptl.	Control		
...	...	...	4.2	5.4	...	...
28	9.8	89.8	5.2	5.4	1.29	4.12
80	17.8	172.4	7.1	5.4	2.46	4.08
236	14.4	139.2	8.6	5.4	2.00	4.11

Since a preliminary study of the emulsin-aesculin system revealed no volume-change while the hydrolysis proceeded to the equilibrium point, no contraction constant can be obtained for this system.

DISCUSSION.

Of the four glucosides, the hydrolyses of which have been dilatometrically investigated, salicin is the only one which gives a depression and has a contraction constant. The absence of any volume-change during the enzymatic splitting of the other three glucosides is noteworthy.

To eliminate the possibility of counter volume-changes which might accompany the solution of the reaction products in petroleum, experiments were conducted with the old type of instrument which eliminates the use of the solvent. Neither this gave any depression, showing that the factor of solubility in petroleum does not affect the result. Attention should be drawn to the fact that the volume-change measured in the dilatometer is the net result of a series of physical and chemical changes accompanying the hydrolysis, such as the solubility of the hydrolytic products in the reaction media, the disappearance of water during hydrolysis, and the configurational readjustment of the new molecules resulting from hydrolysis. It is difficult at this stage to apportion the change to each of the above factors, but an attempt will be made on a future occasion when a greater variety and number of substances would have been investigated.

SUMMARY.

The hydrolysis of four glucosides, aesculin, amygdalin, arbutin and salicin by emulsin has been investigated in the dilatometer.

Emulsin-salicin gives a contraction constant of 4.1 while other systems do not reveal any volume-change during hydrolysis.

POLYSACCHARIDES.

A dilatometric study of the enzymatic hydrolysis of the colloidal polysaccharides starch and glycogen is described in the present communication. As previously mentioned (*J. Indian Inst. Sci.*, 1932, **15A**, 17) the contraction constant is expressed in cubic centimetres per 100 grams of the colloidal substrate. The preparation of solutions of these collodial substrates under standard and reproducible conditions is of great importance in these experiments. For example, the hydration of a given starch is regulated by hydrogen ion, the presence or absence of salts, and temperature. Since this hydration itself is accompanied by certain volume changes, the starch solutions were kept in the thermostat for 19 hours, so that the hydration equilibrium was attained before mixing the substrate and the enzyme.

EXPERIMENTAL.

Lintner's soluble starch (B.D.H.) was used throughout the experiments : glycogen was a Pfansteil's c.p. sample. They were subjected to the action of four different diastases, widely varying in origin. Pancreatin and malt diastase were Merck's preparations ; ptyalin was prepared in the laboratory, and Taka-diastase was a product of Parke Davis.

Except in the case of glycogen, all hydrolyses were conducted at four different concentrations of the substrate. Completion of the reaction was determined by the dilatometric column reaching a steady

value, usually in the course of 4 to 5 hours. The degree of hydrolysis was independently determined by estimating the reducing value of the hydrolysate by Bertrand's method. For both series of studies, the respective optimal reactions were maintained with Sörensen's phosphate buffer :—Pancreatin, 7.0 ; ptyalin, 6.6 ; malt and Taka-diastase, 5.3.

TABLE I

Starch

Substrate concentration per cent.		0.25	0.50	1.0	2.0	Average constant
Pancreatin	Observed depression in cm.	0.65	1.45	2.7	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	8.9	17.2	34.3	73.8	
	Contraction constant	0.64	0.71	0.66	0.71	0.68
Ptyalin	Observed depression in cm.	0.7	1.6	2.65	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	...	...	41.34	80.14	
	Contraction constant	0.69	0.65	0.65	0.71	0.67
Diastase	Observed depression in cm.	0.8	1.60	3.2	6.5	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	10.8	...	38.2	85.2	
	Contraction constant	0.79	0.79	0.79	0.79	0.79
Taka-diastase	Observed depression in cm.	1.6	3.15	6.25	13.0	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	16.5	40.55	67.4	136.1	
	Contraction constant	1.57	1.55	1.55	1.59	1.57

TABLE II

Glycogen

Substrate concentration per cent.		0.25	0.5	Average constant
Pancreatin	Observed depression in cm.	0.45	0.95	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.1	11.8	
	Contraction constant	0.44	0.47	0.45
Ptyalin	Observed depression in cm.	0.85	1.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.6	13.8	
	Contraction constant	0.83	0.88	0.85
Diastase	Observed depression in cm.	0.55	1.1	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	7.7	15.3	
	Contraction constant	0.54	0.54	0.54
Taka-diastase	Observed depression in cm.	1.5	2.9	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	23.0	42.4	
	Contraction constant	1.47	1.43	1.45

DISCUSSION.

Tables I and II reveal that highest depressions are obtained with Taka-diastase at all concentrations of the substrates tested. The highest degree of hydrolysis is brought about by Taka-diastase as indicated by the corresponding copper values also. A strict proportionality (within reasonable limits of experimental error) is maintained between the various concentrations not only with respect to dilatometric depressions but also with regard to the copper values of the hydrolysate. The contraction constants of each system obtained at various concentrations of the substrate show good agreement and therefore these constants can be used for the estimation of starch or glycogen. Taka-diastase, giving the highest constant, should prove most useful in the analytical dilatometry of starch or glycogen.

The results show that the degree of hydrolysis of polysaccharides can be followed in the dilatometer, although it may be difficult to follow the disruption of the molecule; there is no doubt that each of these diastases act differently on starch. We are dealing here with a complex substrate consisting of amylopectin and amylose, the former according to Pringsheim being closely related to glycogen. The enzyme preparations represent a mixture of several saccharifiers and one liquefying component. Therefore a detailed study of this system by effecting a separation of the components of the substrate and enzyme systems is being made.

SUMMARY.

1. Starch and glycogen in concentrations of 0.25, 0.5, 1.0 and 2 per cent. have been separately subjected to the action of four diastases differing widely in origin, pancreatin, ptyalin, malt and Taka-diastase, in the dilatometer.

2. Contraction constants for each of these eight systems have been determined and the possibility of their being employed for the dilatometric estimation of starch and glycogen with particular reference to Taka-diastase, has been indicated.

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