[D 7050, 8010.]

"Studies on Enzyme Action. VI.—The Sucroclastic Action of Acids as contrasted with that of Enzymes. Part II." By EDWARD FRANKLAND ARMSTRONG, Ph.D., Salters' Company's Research Fellow and ROBERT JOHN CALDWELL, Clothworkers' Scholar, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. ARMSTRONG, F.R.S. Received August 26, 1904.

D 7050. *Hydrolysis of Cane Sugar by very Dilute Acids.*

In accordance with the theory put forward in our former paper,* it was to be expected that on hydrolysing cane sugar with sufficiently dilute acids the course of the change would not follow the simple logarithmic law but that it would approximate, during the earlier period, to a linear function of the time. This supposition has been confirmed by experiments made very carefully to test this point.

Experimental Method:—In order that hydrolysis should be about half completed in 10 hours by N/500 chlorhydric acid, it was necessary to work at about 40°. At this temperature, when so weak an acid is used, the solution does not show any trace of colour even after 4 days, which may be regarded as evidence that no decomposition of the levulose into acid substances has occurred. In order to maintain the temperature at 40°, a stream of water was passed through the jacket of the polarimeter tube in which the hydrolysis was carried out. The water was taken from the mains at nearly 18° and passed through a metal vessel, about 2 litres in capacity, in which it was heated by a Bunsen burner to 30°. It was then passed through a metal vessel holding about 5 litres containing an Ostwald thermoregulator; in this the temperature of the water was raised to about 39°. The final adjustment was performed by a very sensitive Ostwald thermoregulator with fluted sides to make it respond quickly to changes in temperature; this was placed in a cylindrical Dewar vacuum vessel, which it almost filled. After passing through a thin copper drum holding about 150 c.c., the water circulated through the regulator, cooling being prevented by the vacuum jacket; the temperature of the water in the drum was maintained at 40° by means of a flame controlled by the regulator. This arrangement proved to be eminently satisfactory in observing slow rates of change, although the extreme variation of the temperature was nearly $\pm \frac{1}{10}$th of a degree, the period of the variations was only 1 minute, so that in experiments extending over several hours the temperature variation was of no account.

* 'Roy. Soc. Proc.,' vol. 73, p. 526.

The strength of the acid selected was N/500 hydrogen chloride; the two sugar solutions used contained 171 and 342 grammes sucrose per litre. The polarimeter tube had been in use for a considerable time with acid solutions. The solutions of sugar and acid were accurately measured out from burettes into a small flask into which steam had been passed, according to Ostwald's directions, to free it from alkali. The mixture was then rapidly filtered into the polarimeter tube. The first reading was taken 10 or 15 minutes after mixing, when the temperature had attained to that of the thermostat.

As under these conditions the rotatory power of the solution fell quickly, the polarimeter readings had to be taken very rapidly, so that the possible error in the tabulated values (although amounting to only 0·1 per cent.) is about 0·03°, making a possible error of 3 units in the third column of differences.

By taking a very large number of points, such minor errors are eliminated and when the velocity constant K is calculated according to the usual logarithmic law a very steady series of values is obtained.

From Tables I—IV it will at once be obvious that the value of the velocity coefficient K steadily increases during about the first 4 or 5 hours of the change and then remains constant, the figures obtained being in striking contrast with those of Ostwald and also with those given later in this paper, Tables VI, VII, for experiments in which stronger solutions of acid were used: in all these experiments the successive values of K vary up and down on either side of a mean value.

The fact that the value of K rises is an indication that change proceeds faster than the mass-action law requires. On the other hand, the figures do not offer definite evidence that the change proceeds at a strictly linear rate, although in all cases, especially that recorded in Table II, the approximation to a straight line law during the first $2\frac{1}{2}$ hours is very close: indeed, if the possible error in the difference be taken into account, the first 10 or 15 per cent. of the change is practically linear. When the values are plotted on rectangular co-ordinates, the curve obtained falls between the straight line and the mass-action curve.

Furthermore, Tables III and IV indicate that the approximately linear portion of the change persists the longer the larger the proportion of sugar to acid.

It is obvious from these results that the analogy between acid and enzyme action is complete. In both cases, when the proportion of hydrolyst is relatively small the change is at first approximately a linear function of the time and subsequently a logarithmic function; whilst when a larger proportion of hydrolyst is present, the change is from the first a logarithmic function which may become modified by secondary causes. The association theory of hydrolysis put forward in

Table I. Table II.

Time in minutes.	α_D.	Average difference per 30 minutes.	K.	α_D.	Average difference per 30 minutes.	K.
0	22·22	..	..	22·12	..	..
15	21·83	..	412	21·73	..	414
30	21·40	82	437	21·35	77	411
45	21·00	..	437	20·97	..	412
60	20·63	81	430	20·58	76	417
75	20·18	..	445	20·22	..	415
90	19·77	80	449	19·82	77	422
105	19·45	..	438	19·45	..	423
120	19·00	80	449	19·03	76	431
135	18·63	..	448	18·72	..	425
150	18·27	72	447	18·37	69	425
165	17·92	..	446	18·00	..	427
180	17·52	75	451	17·60	77	433
195	17·18	..	449	17·23	..	436
210	16·83	70	450	16·90	67	435
225	16·47	..	451	16·60	..	433
240	16·17	68	448	16·27	65	433
255	15·78	..	453	15·93	..	434
270	15·43	73	454	15·60	66	435
285	15·05	..	459	15·27	..	437
300	..	..	..	14·93	65	439
315	..	..	..	14·63	..	438
330	..	..	..	14·30	65	441
345	13·87	54	455	13·95	..	444
360	13·57	..	454	13·68	61	442
375	13·25	62	455	13·35	..	445
390	12·95	..	456	13·10	55	443
405	12·65	57	457	12·83	..	442
420	12·42	..	454	12·53	58	444
435	12·13	55	454	12·23	..	445
450	11·85	..	453	11·97	53	445
465	11·58	54	455	11·73	..	443
480	11·32	..	455	11·48	50	443
495	11·07	52	454	11·23	..	443
510	10·80	..	455	11·03	45	440
525	10·58	49	453	..	..	..
Complete change	−5·37	..	..	−5·37	..	..

Table III.

Time in minutes.	α_D.	Average difference per 30 minutes.	K.
0	46·03	..	..
10	45·47	..	428
20	44·83	..	461
30	44·20	..	471
40	43·62	185	468
50	42·97	..	478
60	42·37	..	479
70	41·80	182	477
80	41·17	..	482
90	40·57	..	485
100	40·07	175	488
110	39·45	..	483
120	38·85	..	486
130	38·28	176	487
140	37·67	..	491
150	37·13	..	490
160	36·55	173	492
170	36·03	..	491
180	35·43	..	495
190	34·93	167	494
200	34·45	..	492
210	33·87	..	495
220	33·38	156	494
230	32·87	..	494
240	32·38	..	494
250	..	152	..
260	31·28	..	499
270	30·80	..	499
280	30·38	154	496
290	29·87	..	498
300	29·48	..	495
310	28·83	144	502
320	28·42	..	500
330	27·93	..	501
340	27·43	146	503
350	26·97	..	503
360	26·55	..	503
370	26·12	134	503
380	25·65	..	504
390	25·25	..	504
400	24·87	126	502
410	24·42	..	503
420	24·02	..	503
430	23·62	124	503
440	23·17	..	504
450	22·80	..	503
460	22·38	123	504
470	21·93	..	506
480	21·53	..	507
490	21·13	123	507
500	20·77	..	507
510	20·40	..	507
520	20·00	112	508
530	19·68	..	506
540	19·27	..	508
Complete change..	−11·12	..	..

Table IV.

α_D.	Average difference per 30 minutes.	K.
44·63	..	..
44·02	..	481
43·37	..	489
42·75	..	493
42·20	183	487
41·58	..	492
40·93	..	500
40·30	186	505
..	..	..
39·10	..	507
38·47	183	512
37·87	..	513
37·28	..	515
36·70	178	516
36·13	..	516
35·53	..	519
34·95	177	521
34·33	..	525
33·78	..	526
33·25	168	525
32·73	..	525
32·25	..	523
31·65	158	527
31·13	..	527
30·65	..	526
30·13	154	527
29·62	..	528
29·08	..	530
28·60	152	530
28·15	..	529
27·63	..	530
27·22	142	528
26·72	..	530
26·28	..	529
25·83	138	529
25·32	..	532
24·88	..	532
24·47	135	531
24·02	..	532
23·60	..	531
23·15	130	532
22·73	..	532
22·35	..	531
−10·79		

these papers gives a very satisfactory explanation of the observed phenomena; as before stated, the differences between acid and enzyme action can all be attributed to the crystalloid nature of the former and the colloid nature of the latter.

Influence of the Products of Change.—In view of the theoretical importance of the influence of the products of change,* it appeared desirable to extend our experiments in this direction to cane sugar. Accordingly the effect of adding 9 grammes of glucose or fructose to 100 c.c. of 17·1 per cent. sucrose containing half a gramme molecule of hydrogen chloride has been determined in the manner previously described by one of us for β-camphor sulphonic acid at 20°.

The results are incorporated in the following tables:—

Table V.

Time.	17·1 grammes sucrose per 100 c.c.	
	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.
0	+21·55	..
15	20·40	[11·83]
30	19·30	[11·81]
45	18·27	11·70
60	17·33	11·50
75	16·40	11·44
90	15·43	11·56
105	14·60	11·45
120	13·75	11·46
135	12·95	11·44
150	12·10	11·55
165	11·37	11·51
180	10·65	11·51
195	9·93	11·54
210	9·30	11·49
225	8·62	11·54
240	8·02	11·52
Complete change	−7·18	..
	Mean ..	11·52

Table VI.

	17·1 grammes sucrose per 100 c.c.	
	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.
	+21·62	..
	20·42	[12·32]
	19·47	11·23
	18·48	11·14
	17·48	11·23
	16·55	11·21
	15·53	11·46
	14·70	11·37
	13·90	11·29
	13·12	11·25
	12·35	11·25
	11·53	11·35
	10·87	11·27
	10·13	11·34
	9·42	11·39
	8·90	11·25
	8·25	11·29
	−7·18	..
	Mean ..	11·29

Table VII.

	17·1 grammes sucrose + 9·0 grammes glucose per 100 c.c.	
	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.
	+30·72	..
	29·53	[10·93]
	28·37	12·37
	27·22	12·55
	26·17	12·50
	25·25	12·25
	24·25	12·34
	23·33	12·32
	22·38	12·43
	21·45	12·55
	20·67	12·49
	19·87	12·51
	19·10	12·53
	18·30	12·63
	17·65	12·57
	17·03	12·52
	16·42	12·49
	+2·03	..
	Mean ..	12·47

* *Loc. cit.*, p. 534.

Table VIII. Table IX. Table X.

Time.	17·1 grammes sucrose + 9·0 grammes glucose per 100 c.c.		17·1 grammes sucrose + 9·0 grammes fructose per 100 c.c.		17·1 grammes sucrose + 9·0 grammes fructose per 100 c.c.	
	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.	α_D.	$\frac{10^4}{t}\log\frac{a}{a-x}$.
0	+ 30·63	..	+ 4·35	..	+ 4·24	..
15	29·45	12·20	3·18	12·07	3·13	12·59
30	28·25	12·58	2·03	12·22	1·97	12·55
45	27·13	12·60	+ 0·95	12·19	+ 0·92	12·31
60	26·15	12·33	− 0·10	12·22	− 0·10	12·22
75	25·12	12·39	1·08	12·17	1·10	12·22
90	24·12	12·46	2·05	12·20	2·13	12·37
105	23·15	12·54	2·97	12·20	3·05	12·26
120	22·23	12·58	3·85	12·20	3·90	12·29
135	21·33	12·65	4·70	12·21	4·82	12·41
150	20·45	12·74	5·55	12·27	5·65	12·43
165	19·67	12·72	6·30	12·23	6·43	12·43
180	19·00	12·59	7·07	12·27	7·20	12·45
195	18·18	12·73	7·82	12·32	7·93	12·47
210	17·48	12·73	8·48	12·28	8·63	12·48
225	16·80	12·75	9·07	12·20	9·25	12·43
240	16·13	12·80	9·77	12·29	9·93	12·49
Complete change	+ 2·03	..	−24·30	..	−24·30	..
	Mean ..	12·59	Mean ..	12·22	Mean ..	12·38

Calculating from the equation

$$K = K_1 [1 + 0{\cdot}0131\ \rho]$$

given by Arrhenius as that expressing the influence of concentration ρ on the constant of hydrolysis, it follows that in the case of a solution containing 17·1 + 8·55 grammes of cane sugar per 100 c.c.,

$$K = 12{\cdot}45$$

The results obtained may thus be summarised—

Table XI.

		Mean value of K.
(i)	9·0 grammes glucose.....	12·53
(ii)	9·0 „ fructose	12·30
(iii)	9·0 „ invert sugar (mean of (i) and (ii))...	12·42
(iv)	8·55 „ cane sugar (calc.)	12·45

It will be seen that about the same increase in the value of K is produced by equimolecular proportions of glucose and fructose, whilst the molecular effect of the biose cane-sugar is about twice the molecular effect of the monose.

The acceleration brought about by the addition of sugars may be attributed to a withdrawal of water by the sugar and the consequent increase in the amount of the "active system," as pointed out in our previous paper.

"Studies on Enzyme Action: The Effect of 'Poisons' on the Rate of Decomposition of Hydrogen Peroxide by Hæmase." By GEORGE SENTER, Ph.D., B.Sc. (Lond.). Communicated by Professor E. H. STARLING, F.R.S. Received June 2, 1904.

Schönbein* was the first to observe that most animal and vegetable juices have the property of decomposing hydrogen peroxide into water and oxygen, as well as the power of developing a blue colour in tincture of guaiacum containing a little hydrogen peroxide. Since these properties belonged to all the enzymes then known, *e.g.*, emulsin and diastase, Schönbein regarded them as characteristic of enzymes in general, and used them in his numerous investigations as tests for the presence of these bodies.

A good many years afterwards Jacobson,† working with impure emulsin and pancreatin, showed that the power of these enzymes to catalyse hydrogen peroxide could be destroyed without affecting seriously the specific ferment action. A year or two ago, Loew‡ suggested that the power of plant and animal juices to decompose hydrogen peroxide is due to an enzyme of very wide distribution, which he has named catalase. According to this view, Jacobson's impure emulsin contained some catalase, which is less resistant against heat than the emulsin, and can be rendered inactive without affecting seriously the other ferment.

Since Schönbein's time, the properties which blood possesses of decomposing hydrogen peroxide and of giving the guaiacum reaction have formed the subject of numerous investigations by Schmidt,§ Bergengrün,‖ Spitzer,¶ Schär,** Cotton,†† Ville and Moitessier,‡‡ and others, and this is not surprising when we consider the importance of the guaiacum test for the detection of small amounts of blood.

Spitzer§§ concluded that the guaiacum reaction and the catalysis

* 'Journ. f. prakt. Chemie,' vol. 89, p. 334 (1863).
† 'Zeit. f. physiol. Chemie,' vol. 16, p. 340 (1892).
‡ Loew, "Catalase," 'U.S. Dept. of Agriculture Report,' No. 68, 1901.
§ Pflüger's 'Archiv,' vol. 6, p. 413 (1872).
‖ 'Inaug. Dissertation,' Dorpat, 1888.
¶ Pflüger's 'Archiv,' vol. 67, p. 615 (1897).
** 'Zeit. für Biologie,' vol. 6, p. 467 (1870).
†† Cotton, 'Bull. Soc. Chim.,' vol. 25, p. 255 (1901).
‡‡ 'Bull. Soc. Chim.' [3], vol. 27, p. 1003 (1902).
§§ *Loc. cit.*