

If acceptance can be accorded to the arguments put forward in this communication, some progress will have been made towards unravelling the very complex phenomena presented by fermentative changes.

[D 1820, 7050, 8010.]

“Studies on Enzyme Action. IV.—The Sucroclastic Action of Acids as contrasted with that of Enzymes.” By EDWARD FRANKLAND ARMSTRONG, Ph.D., Salters’ Company’s Research Fellow, and ROBERT JOHN CALDWELL, B.Sc., Clothworkers’ Scholar, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. ARMSTRONG, F.R.S. Received April 5,—Read April 28, 1904.

D 1820 Milk sugar, hydrolysis by acids.

D 7050 Hydrolysis of sugars by acids and by enzymes contrasted. Nature of active system and explanation of influence of concentration and of temperature.

D 8010 Enzyme action contrasted with that of acids.

Not only are the various bioses hydrolysed at very different rates by enzymes but they are also known to differ in their behaviour towards acids: cane sugar being hydrolysed with the greatest facility, whilst maltose is acted upon but slowly. The experiments described in this communication were instituted primarily with the object of ascertaining the behaviour of milk sugar, of which nothing was known.

The hydrolysis of cane sugar under the influence of acid was carefully investigated by Wilhelmy as far back as 1850, with the aid of the polariscope, then a new instrument. It was shown by him that nversion follows the logarithmic law

$$K = \frac{1}{t} \log \frac{a}{a-x},$$

which at a later date became regarded as the general law of mass action. Subsequent workers have studied the action more in detail, but the object of several of the latter inquiries has been rather to determine the relative activities of different acids and to study the application of the ionic hypothesis in such a case of hydrolysis.

Maltose has been shown by Sigmond* to exhibit the same general behaviour as cane sugar towards acids, whilst affording very different constants.

The only other glucose derivative which has been studied is salicin.†

* ‘Zeit. Phys. Chem.’ vol. 27, p. 385.

† Noyes and Hall, ‘Zeit. Phys. Chem.’ vol. 18, p. 240.

Since the above was written, a paper has appeared by Noyes and others* on the hydrolysis of maltose and dextrin by dilute acids, which in the main confirms Sigmond's results. The hydrolysis of starch by dilute acids under pressure at temperatures about 100°, was studied in detail by Rolfe and Defren in 1896.†

Although the behaviour of cane sugar in any particular solution is fairly in accordance with the law of mass action, marked departures from the law are observed in contrasting the effects produced by varying the concentration whether of sugar or of acid. Not only does the value of K increase as the concentration of the hydrolyte is increased; it also increases beyond the proportionate value when the concentration of the acid is increased.

The outcome of the experiments described in the following pages is, briefly stated, as follows: In the first place, it is shown that, within certain limits, milk sugar is hydrolysed in accordance with the logarithmic law; but that in somewhat concentrated solutions there is a marked tendency for "reversion" to take place, so that the course of change in the later stages of hydrolysis departs from this law. The rate at which milk sugar undergoes hydrolysis compared with that at which cane sugar is affected is shown to be remarkably slow: yet the effect of increasing the concentration is found to resemble that produced by changes of concentration in the case of cane sugar and maltose; moreover, the products of change are found to exercise an influence on the rate of change comparable with that exercised by milk sugar itself. Finally, it is shown that an increase in temperature has even more influence on the rate of change of milk sugar than on that of cane sugar.

Experimental Method.—The method adopted in determining the rate of change was practically that used by previous workers; but, as milk sugar is hydrolysed very slowly at ordinary temperatures, the experiments were carried out at 60° C. On account of the small change in the specific rotatory power which milk-sugar solutions exhibit when hydrolysed, it was necessary to work with strong solutions—the solution most frequently used contained 18 per cent. of sugar, *i.e.*, 0.5 gramme molecular proportion per litre; this was hydrolysed by an equivalent solution of chlorhydric acid. The solutions of acid and sugar, heated to 60° and then mixed, and the mixture maintained at 60°. Portions were taken out at stated intervals of time: each sample was quickly cooled and its optical rotatory power determined in a 2 dm. tube. As the heating was often extended over several days, it was necessary to cover the solution in the flask with a layer of molten paraffin. In order to make sure that no evaporation had taken place, the solution was titrated with alkali at the end of

* 'J. Am. Chem. Soc.,' 1904, vol. 26 p. 266.

† 'J. Am. Chem. Soc., vol. 18, p. 869.

each experiment. In order to determine the end value, a portion of the solution was heated on the water bath during 2 hours, a period which sufficed to effect complete hydrolysis without giving rise to any marked secondary change such as always occurs on prolonged heating at the temperatures at which the rate of change was studied. The ratio between the rotatory power at complete change and the initial value was found, as the mean of three accordant observations, to be 1.285.

The results of a complete series of observations are recorded in the following table. It is to be noted that in calculating the constant from the logarithmic equation, t is taken in minutes. To make the results comparable with those of other workers, the velocity constant K is expressed in terms of a gramme molecular proportion of acid per litre; this merely involves the multiplication of the constant deduced from the results approximately by two.

Table I.—18 per cent. (0.5 gramme molecule) Milk Sugar, 0.5007 gramme molecule HCl. Temperature $60^{\circ}1$.

Time in hours.	α_D .	Per cent. hydrolysed.	$\frac{10^4}{t} \log_{10} \frac{a}{a-x}$.
0	19°55	0.0	—
5	20.22	12.04	1.85
9	20.62	19.2	1.71
15	21.27	30.8	1.78
20	21.83	40.9	1.90
25	22.17	47.0	1.84
30	22.47	52.4	1.79
40	23.23	66.0	1.95
62	24.23	84.0	[2.14]
87	24.93	96.5	[2.81]
134	25.15		—
184	25.28		Mean 1.83
216	25.56		
Complete change	25.12		

$$K = 3.65.$$

In a second series of experiments, the value deduced from the first eight successive observations—during which about two-thirds of the sugar had undergone change—was $K = 3.42$.

In the interests of space, as the quotation of long series of values serves no useful purpose, in discussing our remaining observations it will suffice, as a rule, to give the mean value of K obtained in the manner described. It is, of course, to be understood that K is derived from not less than six accordant readings taken at regular intervals during a period of 30—40 hours.

It will be seen that after about 40 hours, when hydrolysis is half

completed, the values in the last column are no longer constant but increase; after 134 hours, the value of α_D actually rises above that corresponding to complete conversion into glucose and galactose. This increase was presumably to be ascribed to the formation of compounds of high specific rotatory power from the *products of change*: to test the correctness of this conclusion, experiments were made in which solutions of glucose and galactose were heated at 60° during several hours with chlorhydric acid of the strength used in the previous experiments. As Tables II and III show, in each case, a rise in α_D was observed but the rotatory power did not become constant until after about 250 hours, equilibrium being only slowly established between the monosaccharide and its reversion products. The departure during the later part of hydrolysis from the logarithmic law may therefore with some degree of probability be ascribed to some form of reversible change. It may be pointed out that Wohl* has observed a similar change in highly concentrated solutions of glucose and fructose when these are heated with small quantities of chlorhydric acid on the water bath; and that Fischer† prepared the biose isomaltose by the action of cold strong chlorhydric acid on glucose.

Table II.

27 per cent. Glucose.

Time in hours.	α_D .	Successive increase per hour.
0	28°·67	—
8	28·85	0·022
16	29·03	0·022
22	29·13	0·016
44	29·40	0·012
96	29·93	0·010
166	30·42	0·007
200	30·58	0·005

Table III.

27 per cent. Galactose.

Time in hours.	α_D .	Successive increase per hour.
0	42°·75	—
8	42·70	—
16	43·03	0·041
22	43·23	0·033
44	43·50	0·012
72	43·90	0·014
96	44·07	0·007
166	44·28	0·003

Hydrolysis of Maltose.—Owing to the great variation in the value of K produced by alterations in concentration and temperature, it was impossible to compare our results for milk sugar directly with those of Sigmond‡ for maltose. Accordingly, the value of K was determined for maltose under the conditions observed in our experiments with milk sugar.

* 'Ber.,' 1890, vol. 23, p. 2084.

† 'Ber.,' 1890, vol. 23, p. 3687.

‡ *Loc. cit.*

Table IV.—18 per cent. (0·5 gramme molecule) maltose, 0·5007 gramme molecule HCl. Temperature 60°·1.

Time in hours.	α_D .	Per cent. hydrolysed.	$\frac{10^4}{t} \log_{10} \frac{a}{a-x}$.
0	23°·28	0·0	—
8	20·02	23·5	2·43
16	17·92	38·7	2·22
24	16·12	51·7	2·20
50	12·58	77·3	2·15
72	11·22	87·1	[2·06]
96	10·45	92·7	[1·97]
144	9·85		
168	9·95		
216	9·94		
Complete change	9·44		—
			Mean 2·25

$$K = 4·49.$$

It will be noted that the values in the last column begin to fall after 50 hours, when about three-quarters has undergone change. As the hydrolysis of maltose is carried to an end, the value of α_D falls to a minimum, then rises slightly, finally remaining steady; the theoretical minimum rotation, however, is never attained. This is easily explicable as the consequence of the secondary changes brought about by the action of the acid on the glucose.

The following table shows at a glance the vast difference in the values of K for milk sugar and maltose as compared with that for cane sugar. This last value has been calculated with the aid of Arrhenius's temperature equation from the 47·6 value at 25° given by Ostwald.

Table V.

Sugar.	K at 60°·1.	Ratio.
Milk sugar	3·53	1·0
Maltose.....	4·49	1·27
Cane sugar	4378·0	1240·0

Although it is not yet possible to explain this very striking difference, it must be remembered that cane sugar has a peculiar structure: maltose and milk sugar are closely related in structure, one-half of the molecule still showing the properties of glucose; but cane sugar has none of the properties either of glucose or of fructose.

Influence of Concentration of Hydrolyte.—Whereas, in the case of enzymes, an increase in the concentration of the sugar never increases the rate of hydrolysis, in the case of acids an increase in the concentration of the hydrolyte actually hastens the rate of change. This

is known to be true both of cane sugar and of maltose. Our experiments show that it is equally true of milk sugar. Using a half normal ($\frac{1}{2}$ gramme molecular) solution of HCl, we have found that K has the following values :—

Table VI.

Concentration of sugar.		K.
9 per cent.	2·88
18 ,,	3·53
27 ,,	4·10

From these figures the following empirical law may be deduced connecting K with p , the concentration in grammes per 100 c.c. :

$$K = 2\cdot27 (1 + 0\cdot03 p).$$

Arrhenius has calculated a similar expression from Spohr's results for cane sugar, viz., $K = 19\cdot26 (1 + 0\cdot0131 p)$.

It would, therefore, appear that the rate at which hydrolysis takes place is more than twice as much influenced by changes in concentration in the case of milk sugar than in that of cane sugar.

Influence of the Products of Change.—The influence of the products of change on the rate of hydrolysis is of particular interest in view of the results obtained with enzymes. In the case of acids the products accelerate instead of retarding the change but they exercise no selective influence; moreover, about the same effect is produced by the addition of equal weights of glucose, of galactose or of milk sugar or even of the equivalent quantity of a neutral salt.

Table VII.

Amount added to 18 per cent. solution of milk sugar ($K = 3\cdot53$).		K.
9·0 grammes milk sugar	4·10
9·0 ,, glucose, 1st expt.	3·89
9·0 ,, ,, 2nd ,,	4·06
9·0 ,, galactose	4·14
3·7 ,, potassium chloride	3·79

Hydrolysis by Sulphuric Acid.—Several experiments were made, with an acid equivalent in strength to the chlorhydric acid used in the experiments previously referred to.

Table VIII.—18 per cent. (0·5 gramme molecule) Milk Sugar,
0·25 gramme molecule H_2SO_4 . Temperature 60°C .

Time in hours.	α_D .	Per cent. hydrolysed.	$\frac{10^4}{t} \log_{10} \frac{\alpha}{a-x}$.
0	$19^\circ 37$	0·0	—
6	$19^\circ 73$	6·5	0·81
17	$29^\circ 22$	15·4	0·71
42	$21^\circ 50$	38·5	0·84
66	$22^\circ 38$	54·5	0·86
90	$23^\circ 00$	65·7	0·86
140	$24^\circ 02$	84·2	0·96
Complete change	$24^\circ 89$		—
		Mean	0·84

$$K = 1\cdot68.$$

In a second experiment a value $K = 1\cdot70$ was obtained.

The value for K thus arrived at is much smaller than that deduced from the experiments with chlorhydric acid; but if the comparison be made for acids of the same molecular strength the results are nearly identical, since $2K = 2 \times 1\cdot69 = 3\cdot38$.

It will be noticed that in this instance there is little or no evidence of reversion.

Influence of Temperature.—We have determined the rate at which milk sugar undergoes hydrolysis at different temperatures in order to be able to contrast its behaviour in this respect with that of other sugars. The following table gives the values of K at three temperatures obtained on hydrolysing a solution containing 0·5 gramme-molecular proportion of milk sugar by means of a 0·5 gramme-molecular proportion chlorhydric acid.

Temperature.	K .
60°C	3·53
74°C	25·05
99°C (approx.)	334·2

It is apparent from these numbers that an increase of about 14 per cent. in the value of K takes place per degree and that the rate of hydrolysis of milk sugar is even more influenced by temperature than is that of maltose. The difference is better seen on comparing the constants calculated for each sugar from Arrhenius's temperature equation.

Sugar.	Acid.	$q/2$.
Cane sugar	HCl	12,820*
Maltose	HCl	17,127†
Milk sugar ..	HCl	19,105
„	H_2SO_4	18,424

* Arrhenius, *loc. cit.*

† Sigmond, *loc. cit.*

It will be seen that the value of the temperature constant for milk sugar is nearly half as great again as that for cane sugar.

That temperature changes have a similar effect on the hydrolysis of milk sugar by sulphuric acid is shown by the following figures, obtained by hydrolysing a 0.5 gramme-molecular proportion solution of the sugar by means of 0.25 gramme-molecular proportion acid.

Temperature.	K.
60°·1	3·36
74°·1	22·5

Lastly, the effect of adding a 0.5 gramme-molecular proportion of potassium chloride at the two temperatures was determined; it will be seen that this salt has a considerably greater accelerating influence at the higher temperature.

Temperature.	K with KCl.	K without KCl.
60°·1	3·79	3·53
74° 1	29·0	25·05

It is to be noted that maltose ($K = 4.49$) and milk sugar ($K = 3.53$) are hydrolysed at similar rates at 60°·1; as the rate of hydrolysis is affected to different extents by a rise of temperature in the two cases, there will be a temperature at which the two should be hydrolysed at the same rate; and above this temperature milk sugar should be hydrolysed more rapidly than maltose. The temperature at which the two sugars should behave alike, deduced from Arrhenius's equation, is about 77°; we intend comparing the hydrolysis of the two sugars at this temperature.

Theory of Hydrolysis by Acids.—When the action of enzymes on the sugars is contrasted with that of acids, the enormous difference in the rates at which enzyme and acid effect hydrolysis is very striking; thus, whereas an extract of lactase prepared as described on pp. 503 to 504, to which sufficient milk sugar has been added to give a 5-per-cent. solution, will hydrolyse about one-fourth of the sugar at 35° in about an hour, it takes twice normal hydrogen chloride at the same temperature about 5 weeks to effect the same amount of hydrolysis.*

Nevertheless, a very simple explanation of the action of acids may be given if the problem be considered from a point of view similar to that applied to enzyme action. In the first place, it may be assumed that an *active system* is formed by the combination of a part of the sugar with a part of the acid; and as the water molecules in the solu-

* Although no information is at present forthcoming admitting of a definite conclusion, it cannot be doubted that the amount of enzyme made use of in hydrolysing sugars, regarded as a molecular proportion, must be extraordinarily small. Any such conclusion as this makes their action appear all the more remarkable.

tion are attracting both sugar and acid molecules, that there is so to speak competition between the water and the sugar for the acid: there will, therefore, be at any given temperature an equilibrium between water, sugar and acid, depending on the relative proportions of these three constituents; a change in any one of them will necessarily also change the position of the equilibrium and therefore also the proportion of the combination of acid and sugar present.

At any moment during hydrolysis, the sugar is not susceptible to change as a whole: only the active system, formed by the combination of acid and sugar, including some limited number of water molecules, is concerned.

In the experiments hitherto made the proportion of acid molecules present has always been large, so that the magnitude of the active system present cannot have been negligible compared with the total amount of sugar: in such cases it was not to be expected that the change would prove to be a linear function of the time. If, however, a proportion of acid be used in some degree corresponding to the proportion of enzyme which is commonly used, it is to be expected that a linear period will be apparent.

To test this point experiments were made in which N/100 hydrogen chloride was used to hydrolyse a gramme-molecular proportion of cane sugar at 20°: in every case distinct indication was obtained that, at first, equal amounts of sugar were changed in successive equal intervals of time, change proceeding according to the logarithmic law only during the later stages.

It does not appear desirable to put forward our results relating to this question at present, as we are not satisfied with the degree of refinement we have reached in our experiments: to determine the law for very small proportions of acid, it will be necessary to make exact observations and especially to regulate the temperature with excessive care, as slight differences in temperature produce relatively great changes.

Effect of Altering the Amount of Acid.—It is well known in the case of cane sugar that an increase in the proportion of acid used to effect hydrolysis is followed by a more than proportionate increase in the rate of change. The effect of an increase in the amount of acid must obviously be to disturb the equilibrium in the direction of increasing the magnitude of the active system; it may be supposed that, on the whole, when the amount of acid is increased, the sugar is the greater gainer and that the increase in the active system is, therefore, more than proportionate to the increase in the amount of acid.

Effect of Altering the Amount of Hydrolyte.—Any considerable increase in the amount of sugar present must have the effect of diminishing the attraction exercised by the water upon the acid and consequently must increase the stability of the combination of sugar

with acid—in other words, it must materially increase the magnitude of the active system: this would lead to an increase in the rate of change.

Any substance having an attraction for water should exercise a similar influence: it is easy to understand that, in the case of milk sugar, as we have shown, the rate of change is as much accelerated by either glucose or galactose as it is by milk sugar and that the neutral salt, potassium chloride, exercises about the same influence as one of these sugars when used in equivalent amount. It is well known that the hydrolysis of cane sugar is hastened by neutral salts,* but it has not been noticed previously that the products of change exercise an accelerating influence.

Influence of Temperature.—The effect of temperature on the rate at which chemical changes generally take place has been discussed at length by Arrhenius.† Frankly recognising that the rate at which change increases as temperature rises is too great to be ascribed to an increase of the “ionisation” or to a diminution of the viscosity of the solution, Arrhenius has introduced the conception of an active part or mass but without in any way defining the nature of this mass. In applying this conception specially to the hydrolysis of cane sugar, he assumes that the formation of the active mass involves an absorption of heat, and, further, that although it is present in very small proportion relatively to the total sugar, the proportion increases rapidly as the temperature rises. Adopting the ordinary convention he supposes that the active mass undergoes hydrolysis at the expense of the hydrogen ions of the acid, at a rate, however, that is almost independent of the temperature. Availing himself of the equation which van't Hoff had put forward to express the alteration of equilibrium with temperature in the case of reversible interactions, he deduced the expression

$$K_{t_1} = K_{t_0} \cdot e^{\frac{q}{2} \cdot \frac{T_1 - T_0}{T_1 T_0}},$$

where K_{t_1} and K_{t_0} are the velocities at two temperatures and q is the heat absorbed in the formation of the active mass—this being an expression from which it is apparent that a rise in temperature should favour the formation of the active mass and *vice versa*. Arrhenius applied this expression to a large number of simple chemical changes, especially to cases of hydrolysis, for which the rate had been measured, and showed that it was in agreement with experimental facts.

The idea of an active mass was extended to explain the influence of salts: it was arbitrarily assumed by Arrhenius that these increased the proportion of the active mass, notwithstanding that they decreased the number of hydrogen ions.

* Spohr, 'Zeit. Phys. Chem.,' 1888, vol. 2, p. 194.

† 'Zeit. Phys. Chem.,' 1889, vol. 4, p. 226.

In a lengthy paper published 10 years later,* in which the results obtained in the meantime by other workers were discussed, he made an important addition to his theory by showing that the increase in K with concentration is in exact correspondence with the increase of osmotic pressure of the solution. He vaguely referred to the influence of neutral salts as being of the same order as that exercised by an increase in the concentration of the hydrolyte.

Exception was taken by Lippmann,† in 1900, to the explanation put forward by Arrhenius, on the ground that it was impossible, in the case of sugar, to understand how any change could take place which would render one part more active than another. Lippmann also sharply criticised the attempt made by Euler‡ to interpret the hydrolysis of sugar from a purely ionic standpoint. Euler's§ answer to these criticisms in no way served to remove the difficulties raised by Lippmann,|| but rather the contrary.

There can be no doubt that in discussing hydrolytic changes generally the conception of an active mass cannot be dispensed with; it remains only to give precision to the conception by defining the precise character of such a mass. On the assumption made in this and the two preceding papers, the active mass postulated by Arrhenius is neither more nor less than the system composed of the hydrolyte, the enzyme or acid and a certain limited number of water molecules. Although the formation of such a system is probably attended with a slight evolution of heat, its immediate breakdown should involve the absorption of heat and this change should be reversible. Etherification phenomena generally and the change of isodynamic systems into one another all satisfy these conditions. There can be little doubt that the breakdown of complex systems, such as are here contemplated, would be most materially influenced by changes in temperature, and that the rate of change would advance rapidly as temperature rose. The tendency to form such systems would obviously diminish somewhat as temperature rose.

In conclusion, it is desirable to lay emphasis on the differences noticeable in the behaviour of enzymes and acids respectively as hydrolytic agents: it appears not improbable that this difference is due mainly, if not wholly, (1) to the superior affinity of the enzymes for the carbohydrates, (2) to the very different behaviour of the two classes of hydrolysts towards water—which is a consequence of the colloid nature of the one and the crystalloid nature of the other. It appears possible to explain such differences as are apparent in their

* 'Zeit. Phys. Chem.,' 1899, vol. 28, p. 317.

† 'Ber.,' vol. 33, p. 3560.

‡ 'Ber.,' vol. 33, p. 3202, and 'Zeit. Phys. Chem.,' vol. 36, p. 641.

§ 'Ber.,' vol. 34, p. 1568.

|| 'Ber.,' vol. 34, p. 3747.

behaviour to these circumstances alone. If osmotic pressure be regarded as the outcome of some reciprocal interaction between solvent and dissolved substance rather than as a mechanical effect, the fact that the variation in osmotic pressure and in the value of K with concentration follow the same law may be taken as evidence that the increase in K with concentration when a sugar is hydrolysed with the aid of an acid is the consequence of a diminution in the influence exercised by the water.

*[D 7050, 8020 ; Q 1230, M 3010.]

“Enzyme Action as bearing on the Validity of the Ionic-Dissociation Hypothesis and on the Phenomena of Vital Change.” By HENRY E. ARMSTRONG, Ph.D., F.R.S. Received April 5,—Read April 28, 1904.

*D 8030 Formation of carbohydrates in protoplasm.

D 1810 } Glucosamine, significance of, in enzymes.
Q 1440 }

On several occasions of late years, I have protested against the dogmatic attitude assumed by the advocates of the ionic-dissociation hypothesis of chemical change and have remarked on the danger of allowing a purely mathematical treatment to supersede a careful, unbiassed consideration of the facts as these present themselves to the chemist. I have insisted on the limited application of the hypothesis—especially in explanation of the behaviour of the large majority of organic compounds; and have contended that an association hypothesis is preferable and of far wider application: yet, in so doing, I have always recognised that the dissociation hypothesis is often susceptible of numerical treatment in a way which places it at a great advantage.

Twenty years ago, I contended that the solvent played as important a part as the dissolved substance in electrolytic changes; then and for some time afterwards the dissociationists regarded the solvent as a mere screen. Gradually they have been led to recognise that the solvent plays an active part and “ionising” solvents are now freely spoken of: the admission has been made, however, tacitly and without recognition of the fact that the difference of opinion is now reduced to the one question—whether ions enjoy separate existence in solution: whether, for example, in a solution of hydrogen chloride, free hydrogen

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