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"Studies on Enzyme Action. V.—Hydrolysis of Isomeric Glucosides and Galactosides by Acids and Enzymes." By EDWARD FRANKLAND ARMSTRONG, Ph.D., Salters' Company's Research Fellow, Chemical Department, City and Guilds of London Institute, Central Technical College. Communicated by Professor H. E. ARMSTRONG, F.R.S. Received August 26, 1904.

In view of the use constantly made, in contrasting the action of sucroclastic enzymes, of the stereoisomeric α- and β-methyl glucosides and the corresponding galactosides as test materials, it was desirable to gain some idea of the relative stability of these four compounds in presence of acids and wherever possible towards enzymes, a knowledge of their behaviour being of importance, both as throwing light on their intrinsic properties and for the purpose of correlating the activities of the various compounds amenable to hydrolysis.

As already pointed out,* in studying the hydrolysis of sugars under the influence of enzymes, it is difficult to institute just comparisons, as not only, as a rule, is a different enzyme required for each sugar, but we have no means at present of determining the amount of enzyme used; and sooner or later, it will be necessary to accumulate data correlating one or more analytical factors (nitrogen percentage, etc.) with hydrolytic activity. The difficulty spoken of is enhanced by the fact that, usually, several enzymes occur together—so that no ordinary analytical process can suffice for the determination of the amount of a particular enzyme present in a solution.

On the other hand, it will be of importance to determine whether any one enzyme is capable of hydrolysing several different compounds or whether each particular hydrolysis is ascribable to some one particular enzyme. There is only one case at present known which can be discussed with any degree of certainty. It has been urged by some French workers, especially by Bourquelot and Herissey,† that the action of emulsin on milk sugar, is due to the presence of small quantities of lactase, together with the emulsin proper. The following facts, brought forward in Nos. 2 and 3 of this series of papers, may, however, be urged against this view.

(1) The curve expressing the rate at which milk sugar changes is not of the form to be expected if only a very small quantity of enzyme (lactase) were present: in that case a linear expression should apply during the early stages; actually the curve is only of this form when small quantities of emulsin are used.

* 'Roy. Soc. Proc.,' 1904, vol. 73, p. 515.

† 'Compt. Rend.,' 1903, vol. 137, pp. 56, 59.

(2) The action of emulsin on milk sugar is most retarded by glucose, and only to a slight extent by galactose, whereas galactose alone affects the action of lactase. This, again, would appear to afford proof that the emulsin is directly active.

(3) The curves for emulsin fall off very much more rapidly than those for lactase, showing that the action of the products in removing the enzyme is greater in the former case.

It therefore appears that the differences apparent in the behaviour of emulsin and lactase towards milk sugar are such as to render it improbable that the action of the emulsin is due to the presence of a small proportion of lactase; it would seem, rather, that emulsin is capable of acting on β-galactosides as well as on β-glucosides.*

A different enzyme being required, as a rule, for each sugar, the relati ve activities of enzymes cannot well be reported in terms of any particular sugar taken as standard; the only method open to us at present is to determine the activity of a particular acid towards the various sugars and to estimate, by direct comparison, the activity of the enzyme in terms of this standard acid. But, unfortunately, acids compare very unfavourably with enzymes as hydrolysts: so that, in order to effect hydrolysis at any reasonable rate, it is necessary, except in the case of cane sugar, to operate at elevated temperatures, at which a direct comparison between acid and enzyme is impossible. The experiments here described are to be regarded merely as a first attempt in the direction indicated.

Acid Activity.—The method adopted is substantially that previously described. Solutions of the glucoside containing 3 grammes per 100 c.c. were hydrolysed by means of a half gramme molecular proportion of hydrogen chloride at 74°. The following tables give the results obtained with the various hexosides. The values of the velocity constant K, expressed in the last column, are calculated on the assumption that the change is mono-molecular.

* Pottevin's ('Ann. Inst. Pasteur,' 1903, vol. 17, p. 31) investigations seem to show, however, that *Aspergillus niger* contains an enzyme which is capable of hydrolysing β-glucosides but not β-galactosides or milk sugar. It remains an open question whether this "emulsin" is identical with that obtained from almonds.

Table I. Table II.

α-Methyl glucoside.

Temperature, 74°·1.			Temperature, 74°·8.		
Time in hours.	Change in rotation = x.	$K = \frac{1}{t} \log \frac{a}{a-x}$.	Time in hours.	x.	K.
	mins.			mins.	
2	15	0·00955	2	18	0·0115
4	30	0·00975	4	34	0·0111
7	52	0·01000	6	48	0·0107
17·5	117	0·01010	24	174	0·0124
21	137	0·01027			
24	146	0·00977			
29	176	0·01047			
Total change	$a = 350$	Mean K = 0·01		$a = 350$	K = 0·0114

Table III. Table IV.

β-Methyl glucoside.

Temperature, 74°·1.			Temperature, 74°·8.		
Time.	x.	K.	Time.	x.	K.
	mins.			mins.	
1	11	0·0177	2	28	0·0233
2	22	0·0181	4	50	0·0218
4	41	0·0178	6	70	0·0213
6	60	0·0178	24	193	0·0219
7	69	0·0179	48	253	0·0228
	$a = 275$	K = 0·0179		$a = 275$	K = 0·0220

Table V.

α-Methyl galactoside.

Temperature, 74°·1.		
Time.	x.	K.
	mins.	
2	72	0·0537
4	125	0·0518
5	150	0·0528
7	196	0·0562
10	240	0·0567
	$a = 329$	K = 0·0542

Table VI.

β-Methyl galactoside.

Temperature, 74°·8.		
Time.	x.	K.
	mins.	
2	42	0·1066
4	85	0·1059
5½	105	0·1041
7	121	0·1077
	$a = 154$	K = 0·1061

Table VII.

Salicin. Temperature, 74°·1.

t.	x.	K.
	mins.	
1	48	0·0612
2	85	0·0576
3	124	0·0601
4	161	0·0632
6	205	0·0597
8	244	0·0600
	$a = 365$	mean 0·0603

Table VIII.

t.	x.	K.
	mins.	
0·5	26	0·0642
1·5	65	0·0535
2·5	105	0·0589
3·5	141	0·0606
5·5	199	0·0622
7·5	235	0·0598
	$a = 365$	mean 0·0599

The mean values of K are collected in the following table, which also contains Sigmond's value* for maltose :—

Table IX.

Hexoside.	$K = \frac{1}{t} \log \frac{a}{a-x}$.	
	Temperature, 74°·1.	Temperature, 74°·8.
α-Methyl glucoside	0·0100	0·0114
β-Methyl glucoside	0·0179	0·0220
α-Methyl galactoside	0·0542	0·0650*
β-Methyl galactoside	0·0884*	0·1061
Salicin	0·0601	
Maltose	0·0740	

* 'Zeit. Phys. Chem.,' 1898, vol. 27, p. 385.

It will be noticed that the various hexosides vary widely in stability: the β-glucosides undergoing hydrolysis more rapidly than the stereoisomeric α-compounds—a fact already noted by Alberda von Ekenstein—whilst the galactosides are more rapidly attacked than the corresponding glucosides. These conclusions are in harmony with the well-known fact that the α-compound preponderates in the mixtures obtained in preparing the methyl glucosides and galactosides* and also serve to explain the circumstance that, in separating methyl galactoside (by E. Fischer's method), it is necessary to avoid the presence of acid far more carefully than in separating methyl glucoside.

In the case of the α- and β-glucosides and galactosides, the stereoisomerism in each pair of compounds is confined to the terminal carbon atom; it is, perhaps, noteworthy that there should be so considerable a difference between compounds so related.

But it is even more surprising that a change in the general configuration at the fourth carbon atom, affecting only the nature of the attachment of the oxygen atoms within the ring, such as occurs when glucose passes into galactose, should have so marked an influence on the activity of the group associated with the terminal carbon atom. Such a result enhances the probability of the conclusion that the active system within which the change takes place† is formed by the association of acid-water molecules with the oxygen atom in the pentaphane ring: in other words, that this oxygen atom is the attractive centre. The argument here made use of renders it desirable that the behaviour of the isomeric mannosides towards acids should also be studied, in order that it may be possible eventually to define more or less accurately the functions of the different oxygen atoms in the molecule.

Enzyme Activity.—At present, the experiments have been confined to two substances, maltose and α-methyl glucoside, which both undergo hydrolysis under the influence of the enzymes contained in ordinary yeast maltase.

Fifty cubic centimetres of a solution containing 5 grammes of α-methyl glucoside was mixed with 50 c.c. of a maltase extract prepared from dried yeast;‡ the mixture was kept at 22°. Samples were withdrawn every hour and polarimetrically examined. Considerable difficulty was at first experienced in obtaining sufficiently clear solutions for this purpose, owing to the impossibility of removing the suspended proteid matter by mere filtration; it was eventually discovered that the liquids could be clarified by means of sodium acetate. The method at present

* E. Fischer, 'Ber.,' vol. 26, p. 2400; Jungius, 'Proc. K. Akad. Wetensch.,' Amsterdam, 1903, vol. 6, p. 99.

† Compare Armstrong and Caldwell, 'Roy. Soc. Proc.,' vol. 73, p. 526.

‡ 'Roy. Soc. Proc.,' vol. 73, p. 504.

adopted consists in mixing 5 c.c. of water at 100°, containing 0·5 gramme of sodium acetate, with the 5 c.c. withdrawn, then shaking with charcoal and filtering through a double filter.

Table X.

Time in hours.	Percentage changed = x.	$K = \frac{1}{t} \log \frac{100}{100-x}$.
	mins.	
1	8·7	0·0395
2	16·6	0·0394
3	24·0	0·0397
4	31·3	0·0407
5	37·0	0·0401

To effect a direct comparison of the activity of maltase towards maltose and α-methyl glucoside, the two substances were hydrolysed by the same yeast extract under precisely similar conditions. The extract used in this case was prepared by digesting 5 grammes of dried yeast with 100 c.c. of water at 22° during 1 hour.

Table XI.

Time in hours.	α-Methyl glucoside.		Maltose.	
	x.	K.	x.	K.
	mins.		mins.	
1	5·55	0·0248	21·2	0·098
2	10·5	0·0241	36·0	0·0969
3	14·8	0·0232	43·0	0·0814
4	18·5	0·0222	49·4	0·0722
5	22·2	0·0218	53·6	0·0667
6	25·9	0·0217	58·2	0·0631

It will be seen that the maltose was hydrolysed very much more quickly than the α-methyl glucoside. In both cases the velocity coefficient K diminishes as action proceeds; but to a far greater extent in the case of maltose. This difference is obviously due to the different influence exercised by the products of change in the two cases. It is to be remembered that two molecules of glucose are produced by the hydrolysis of maltose but only one from α-methyl glucoside; any retardation, therefore, which glucose can effect should be less obvious in the case of the glucoside.

On comparing the results recorded in the above tables with those given on pp. 190—191, representing the action of acid, it is obvious that the enzyme was much more active than the acid. About 40 per cent. of the glucoside was changed in 5 hours at 22° by the enzyme; whereas, when acid was used, even in so large a proportion as three molecules of hydrogen chloride to one of glucoside, the same amount of change was effected in only about 20 hours at 75°. As the enzymes are undoubtedly of high molecular weight and the proportion of maltase in the yeast extract is certainly small, it would seem to follow that the relative molecular activity of the enzyme is very great compared with that of the acid. But, as pointed out in an earlier paper,* inasmuch as only a small proportion of the acid is actually active, it is probable that the enzyme owes its apparent activity to its greater affinity for the sugar and that, in reality, the acid has the greater hydrolytic activity.

On account of the rapid alteration in the values of K, it is difficult to make any exact numerical comparison between maltose and methyl glucoside. The initial value of K, in the case of the glucoside, may be estimated at about 0·025; in view of the results previously obtained,† which throw considerable light on the behaviour of maltose during the early stages of hydrolysis, the corresponding value for this sugar may be set at 0·12 or even higher. Comparing these initial rates, it would appear that maltose is hydrolysed from five to six times as rapidly as α-methyl glucoside, a result of the same order as that deduced in comparing the action of chlorhydric acid on the two hexosides.

Taking into account both the superior stability of the methyl glucoside and the greater influence exercised by the products of change in the case of maltose, the difference in the behaviour of the two compounds on hydrolysis seems to be satisfactorily accounted for.

* Part 4, *loc. cit.*

† 'Roy. Soc. Proc.,' vol. 73, p. 508.