From the PROCEEDINGS OF THE ROYAL SOCIETY, VOL. 73.

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[D 1810, 8010; Q 1230.]

"Studies on Enzyme Action. III.—The Influence of the Products of Change on the Rate of Change conditioned by Sucroclastic 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 April 5,—Read April 28, 1904.

D 1810 Hexoses—power of sucroclastic enzymes to combine with.
D 8010 Enzymes—activity of correlated with configuration of hydrolyte.
D 8010, Q 1240 Emulsin, lactase, maltase—varying influence of glucoses and glucosides on their activity.

In the previous paper, it has been shown that, in order to explain the action of sucroclastic enzymes, it is necessary to assume not only that the enzyme combines with the hydrolyte but that it is also more or less affected by—and presumably combines with—the product of change.

At present there is but little information available bearing on this latter contention. The experiments to be described have been made with the object of ascertaining by direct observation whether and to what extent the action of a given enzyme is affected by one or more of the products formed under its influence. They establish very clearly the existence of a close relationship between the configuration of the hexose and the enzyme in those cases in which a retarding influence is apparent: it is difficult to explain such a result except on the assumption that the enzyme and hexose combine together in some intimate manner.

Historical.—The observation made by Würtz in 1879,* when studying the action of Papaïn on fibrin, that the enzyme was in some way retained, probably first gave rise to the conception that the primary action of enzymes was additive in character. The first definite evidence of combination, however, appears to be that advanced by O'Sullivan and Thomson in 1890.† It was shown by these observers that in the presence of cane sugar invertase will withstand without injury a temperature fully 25° higher than it will in its absence. They pointed to this as a very striking fact which was difficult to explain except on the assumption that the invertase enters into combination with the sugar; they further supposed that it was capable of combining with the *invert* sugar.

Systematic experiments were made by Tamman in 1892,‡ who showed that the hydrolysis of amygdalin and salicin by emulsin is materially retarded in either case on adding any one of the products of their change in advance.

The effect of glucose on the hydrolysis of maltose by maltase was studied by Croft Hill in 1898.§ The retardation observed was attributed by him, perhaps not quite logically, merely to a reverse action by which the glucose underwent conversion into maltose: it may be added that the investigation was undertaken by him from this point of view.

A more definite step forward was taken by Henri in 1901, who showed that the retarding effect of invert sugar was mainly due to the fructose, glucose having little or no effect on the action of invertase on cane sugar. Evidence that the effect was due to a specific action of the invert sugar was adduced in the same year by Adrian Brown,‖ who showed that whilst the rate of hydrolysis of cane sugar was materially reduced by invert sugar, the corresponding amount of milk sugar had little or no effect, thus precluding the conclusion that the retardation was due to increased viscosity of the liquid.

In the course of his classic researches on the action of enzymes on the stereochemically related glucosides commenced by Emil Fischer¶ in 1894, it was clearly established that the closest relationship exists between the configuration of the hydrolyte and that of the particular enzyme which can affect it.

In a course of lectures delivered at the Pharmaceutical Society in 1892,** my father discussed fermentation phenomena generally from the point of view that hydrolysis was conditioned by the association of

* 'C. R.,' vol. 81, p. 425 and vol. 91, p. 787.
† 'Chem. Soc. Trans.,' vol. 57, p. 919.
‡ 'Zeit. physiol. Chem.,' vol. 16, p. 291.
§ 'Chem. Soc. Trans.,' 1898, vol. 23, p. 634.
‖ 'Chem Soc. Trans.,' 1902, vol. 81, p. 373
¶ Summary in 'Zeit. physiol. Chem.,' vol. 26, p. 60.
** 'Pharmaceutical Journal,' vol., 22, pp. 495, 596, 659, and 757.

the enzyme with both the sugar and water; this theme was further developed in his Presidential Address to the Chemical Society in 1895.*

Hydrolysis of Milk Sugar by Lactase.

Table 1.—Solutions were compared containing equal amounts of milk sugar to one of which an equal weight of a mixture of equal parts of glucose and galactose was added: it will be seen that this addition reduced the rate to nearly half its value. In this and all subsequent tables the figures indicate the percentage of biose hydrolysed:—

Time in hours.	10 grammes milk sugar per 100 c.c.	+ 5 grammes glucose and 5 grammes galactose.
1	1·3	0·88
2	2·2	1·34
4	3·37	1·82
6	3·8	2·22
24	5·55	3·19
48	6·45	3·64

Table 2.—The effect of glucose, of galactose and of an equal weight of a mixture of both on a 10-per-cent. solution of milk sugar are contrasted in the following table:—

Time in hours.	+ 10 grammes galactose.	+ 5 grammes galactose and 5 grammes glucose.	+ 10 grammes glucose.
19	18·2	22·8	22·9
24	21·0	25·0	25·6
44	25·6	29·5	29·5
67	30·9	34·8	34·8
190	38·7	44·8	44·8

Table 3.—That the retardation is produced almost entirely by the galactose and that glucose or fructose are almost without influence is shown by the following comparisons:—

Time in hours.	5 grammes milk sugar per 100 c.c.	+ 5 grammes glucose.	+ 5 grammes fructose.	+ 5 grammes galactose.
4	18·0	17·6	18·0	16·0
22	59·2	59·6	59·6	47·4
28	65·6	65·4	65·4	52·0
69	81·4	78·4	80·2	61·6

* 'Trans.,' vol. 67, p. 1136.

Table 4.—The effect of increasing the amount of galactose is illustrated by the following figures :—

Time in hours.	5 grammes milk sugar per 100 c.c.	+ 5 grammes fructose.	+5 grammes galactose.	+10 grammes galactose.	+15 grammes galactose.
5	18·8	18·8	16·0	15·2	9·2
23	59·6	56·0	38·0	28·4	18·0
48	67·2	68·0	47·4	37·0	19·2

Hydrolysis of Milk Sugar by Emulsin.

Table 5.—A comparison of the relative influence of glucose and of galactose, as well as of an equal weight of a mixture of these in equal proportions, on the rate of hydrolysis shows that the glucose has the greater effect on this enzyme. Each solution contained 10 grammes of milk sugar in 100 c.c. :—

Time in hours.	+ 10 grammes glucose.	+5 grammes glucose and 5 grammes galactose.	+ 10 grammes galactose.
1	1·0	1·0	1·3
2	2·8	3·2	3·5
3	3·8	4·0	4·3
23	7·4	8·7	8·7
46	9·3	11·1	12·0
70	17·7	20·7	21·3
140	34·4	39·0	42·7
380	45·8	50·1	54·0

Table 6.—The following results, representing the influence of glucose, galactose and fructose, show that whilst glucose most retards hydrolysis, galactose also exercises a retarding influence, fructose having little if any effect, the differences noticed being within the limits of error :—

Time in hours.	5 grammes milk sugar per 100 c.c.	+ 5 grammes fructose.	+ 5 grammes galactose.	+ 5 grammes glucose.
22	17·0	17·0	16·0	13·6
46	32·0	30·4	23·2	20·0
70	46·0	43·8	31·2	27·0
94	58·0	54·6	42·0	34·8

Hydrolysis of Maltose by Maltase.

Table 7.—In this case, glucose retards the hydrolysis considerably; galactose also has a slight retarding influence, but the effect of fructose is not appreciable :—

Time in hours.	5 grammes maltose per 100 c.c.	+ 5 grammes fructose.	+ 5 grammes galactose.	+ 5 grammes glucose.
3	25·6	25·8	25·2	14·0
5	34·0	34·8	28·8	18·0
24	73·8	75·2	64·0	23·0

Correlation of Differential Action of Enzymes with Configuration of Hydrolyte.

Combining my results with Emil Fischer's earlier observations the following table is arrived at:—

Enzyme.	Corresponding hydrolyte.	Effect of hextose on rate of change.		
		Glucose.	Galactose.	Fructose.
Lactase ..	β-Galactosides (*i.e.*, milk sugar, β-alkyl galactosides).	No influence.	Retards.	No influence.
Emulsin .	β-Glucosides (*i.e.*, most natural glucosides, β-alkyl glucosides).	Retards considerably.	Retards slightly.	No influence.
	β-Galactosides (as above).			
Maltase ..	α-Glucosides (*i.e.*, maltose, α-alkyl glucosides).	Retards considerably.	Retards slightly.	No influence.
	α-Galactosides (*i.e.*, α-alkyl galactosides).			
Invertase	Fructosides* (*i.e.*, cane-sugar, raffinose, gentianose, manneotetrose).	No influence.	—	Retards.

The compounds entered in the second column of the table are those which, according to Emil Fischer, are alone hydrolysed by the particular enzymes indicated in the first column. My own observations on the specific retarding influence of the various hexoses are entered in the remaining columns. It is clear that the only hexoses which retard hydrolysis by any given enzyme are those derived from

* It is probable that these compounds are not derivatives of fructose of the —OR type corresponding to the simple glucosides; probably the linkage is of a peculiar character, two centres being concerned. In this connection, it may be mentioned that invertase has no action on methyl fructoside, a substance in every way analogous to methylglucoside. This point will be more fully dealt with in a later communication.

the hexosides* which undergo hydrolysis under the influence of that enzyme.

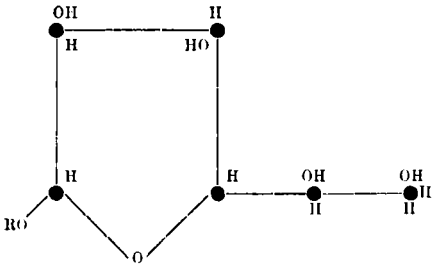
A more absolute proof of the close correlation in configuration between enzyme and hydrolyte cannot well be imagined: it is difficult to interpret such behaviour in any other way than as evidence that the enzyme combines with the hexose in some special, peculiarly intimate manner and is thereby withdrawn from the sphere of action. The retardation cannot well be due to reversion, as in the case of milk sugar the retardation is effected chiefly by glucose when emulsin is the active agent but by galactose alone when lactase is used to effect hydrolysis.

Emil Fischer's researches have brought to light the remarkable fact that the naturally occurring hexoses or derivatives of these, *i.e.*, glucose, mannose, galactose and fructose, are the only compounds affected either by the organisms which condition alcoholic fermentation or by any of the sucroclastic enzymes. The stereoisomeric hexoses produced by artificial means cannot be fermented; this is true also of the "lower" and "higher" sugars, whether derived from natural products (arabinose, xylose, etc.) or prepared artificially from the natural products by reducing or adding to the number of carbon atoms in the chain. The fact that resistant materials such as straw, the gums, etc., are pentose derivatives is of interest in this latter connection. Three of the four hexose sugars referred to are fermented readily and apparently with equal ease; the fourth, galactose, is only slowly fermented: such being the case, it is noteworthy that the formulæ ordinarily assigned to glucose, mannose and fructose are reducible to one common enolic form. It is conceivable that this enolic form is the substance actually fermented to carbon dioxide and alcohol.

CHO	CHO	$CH_2.OH$	CH.OH
HĊ.OH	HO.Ċ.H	ĊO	Ċ.OH
HO.ĊH	HO.Ċ.H	HO.ĊH	HO.ĊH
HĊ.OH	HĊ.OH	HĊ.OH	HĊ.OH
HĊ.OH	HĊ.OH	HĊ.OH	HĊ.OH
$ĊH_2.OH$	$ĊH_2.OH$	$ĊH_2.OH$	$ĊH_2.OH$
Glucose.	Mannose.	Fructose.	Enolic form.

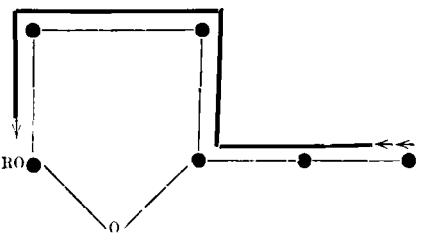
* The term "hexoside" is used as a general expression to include all compounds of a glucosidic character derived from a hexose.

But it is necessary to attribute a γ-oxide formula to glucose and its derivatives,* thus:—



When R = H, the formula is that of glucose itself; when R is an alkyl radicle, it represents one of the alkyl glucosides; when R is a hexose residue ($C_6H_{12}O_6 - H$), it represents a biose such as maltose. The carbon atom to which the group RO is attached in this formula is that which is figured as the superior carbon atom in the formula previously given.

Attachment of enzyme to hydrolyte.—The effect of hydrolysis, whether by acids or enzymes, is to remove the radicle R and displace it by hydrogen; apparently, this change need in no way affect the oxygen linkage in the ring. In the case of acids, although the attack may be regarded as located on the OR radicle, it probably proceeds from the neighbouring oxygen atom of the ring;† but there is every reason to suppose that, in the case of enzymes, the enzyme becomes in some way attached *along the line of carbon atoms*, thus:



* It is to be borne in mind that ordinary glucose in solution consists almost wholly of two stereoisomeric compounds in equilibrium, together with a very small proportion, at the most, of the enolic form, the presence of which must be assumed in order, among other reasons, to explain the results arrived at by Lobry de Bruyn, which establish a reciprocal relationship between glucose, fructose and mannose in solution. The argument which makes it necessary to attribute the γ-oxide formula to glucose and allied compounds is fully stated in the first of this series of papers ('Chem. Soc. Trans.,' 1903, vol. 85, p. 1305) and in a paper by Dr. Lowry (*loc. cit.*, p. 314). The conclusions at which Dr. Lowry and I arrived have received independent confirmation from the recently published work of Behrend and Roth ('Annalen,' 1904, vol. 331, p. 361).

† See Part I, *loc. cit.*

In proof of this contention the following facts may be adduced :—

It is to be remembered that each hexose can give rise to two stereoisomeric hexosides (*e.g.*, α- and β-methyl glucoside), which differ only in the fact that the RO group and an atom of hydrogen are attached to the carbon atom in different relative positions : nevertheless, these require different enzymes to effect their hydrolysis. It is obvious, therefore, that but a slight shifting of the one radicle in space is sufficient to throw the enzyme out of action.

Yet it would seem that the enzyme is only out of harmony with the glucoside at the terminal point, inasmuch as the action of emulsin on milk sugar is hindered not only by glucose but also, although to a less extent, by α-methyl glucoside, which is not in the least attacked by emulsin, whereas the corresponding β-glucoside is readily hydrolysed.

Time in hours.	10 grammes milk sugar per 100 c.c.	+ 10 grammes α methyl glucoside.
3	11·2	8·3
6	15·2	12·1
25	32·7	24·5

[*Note added May* 30, 1904.—Since this paper was presented a series of observations have been made with glucosides. A second experiment with the α-glucoside confirms the results arrived at in the first.

Time in hours.	10 grammes milk sugar per 100 c.c.	+ 10 grammes α-methyl glucoside.
5	7·9	5·7
23	23·3	16·9
28	27·1	19·0

In a similar manner, α-methyl galactoside, which is itself unaffected by the enzyme, hinders the hydrolysis of milk sugar by lactase practically to the same extent as galactose itself does.

Time in hours.	10 grammes milk sugar per 100 c.c.	+ 10 grammes α-methyl galactoside.	+ 10 grammes galactose.
2	7·9	5·3	4·8
5	14·9	9·7	9·6
26	27·8	20·2	20·0

On the other hand α-methyl glucoside like glucose has no retarding influence on the hydrolysis of milk sugar by lactase.

Lastly, β-methyl glucoside was found to retard the action on maltose of maltase, which easily hydrolyses the α-glucoside though it is entirely without action on the β-glucoside.

Time in hours.	10 grammes maltose per 100 c.c.	+ 10 grammes β-methyl glucoside.
2	10·2	7·7
4	14·4	10·7
6	21·0	17·0]

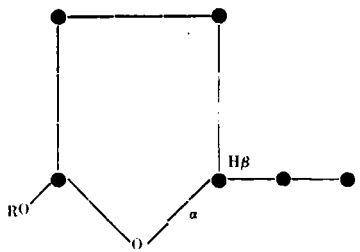
One of the most remarkable conclusions to be derived from Fischer's researches is that glucose and galactose are the only hexoses which afford glucosidic derivatives attackable by sucroclastic enzymes: any alteration in configuration other than that involved in the passage from the one of these hexoses into the other or any shortening or lengthening of the chain is sufficient to confer on the derivative complete immunity from attack. Thus the methyl mannosides, the methyl arabinosides and xylosides and the methyl glucoheptosides cannot be hydrolysed by enzymes. Inasmuch as hydrolysis affects only the OR radicle, this inhibition of hydrolysis by the very slightest change in configuration must mean that the enzyme not only becomes attached but that the attachment takes place along the entire chain of the hexosides: in other words, *enzyme and hydrolyte must be in complete correlation.* Probably this union is effected through the agency of the hydroxyl groups and it may be of basic groups in the enzyme—perhaps their union takes place not immediately but through the intervention of water molecules.

The only case in which there is evidence of partial or incomplete attachment is that afforded by the inhibiting influence of α-methyl glucoside on the hydrolysis of milk sugar by emulsin: in this case it would seem that there is no interruption along the chain, the correlation of hydrolyte and enzyme being incomplete only at the terminal point. This argument is applicable not only to hydrolyte but also to enzyme. Inasmuch as glucose inhibits the action of emulsin and of maltase, it is to be supposed that the general configuration of these enzymes is comparable with that of glucose: the departure must be of such a character that the terminal element of the enzyme is deflected in the one case in the direction which brings it into harmony with the α-OR radicle whilst in the other it coincides in its space orientation with the β-OR radicle. It may be anticipated that β-methyl glucoside will be found to inhibit the action of maltase on maltose.

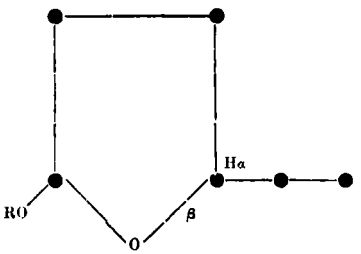
[*Note added May* 30, 1904.—This has since been found to be the case, and as before mentioned whilst α-methyl galactoside hinders the action of lactase, α-methyl glucoside is without influence on this enzyme.]

Extent to which Configuration may be modified.—Galactose differs from glucose merely in having the radicles attached to the fourth carbon atom in the reversed order: as galactose is fermented less readily than glucose and galactosides are less easily hydrolysed by enzymes than are the corresponding glucosides, the change in configuration, although sufficient to hinder action, does not prevent it.

Writing the skeleton formulæ of the two compounds side by side, it would seem that they differ but slightly in configuration, the alteration being one which only concerns the ring structure.



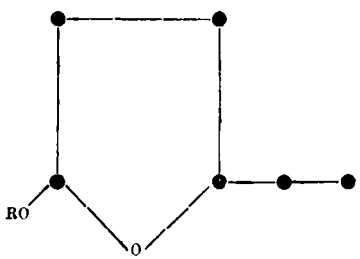
Glucoside.



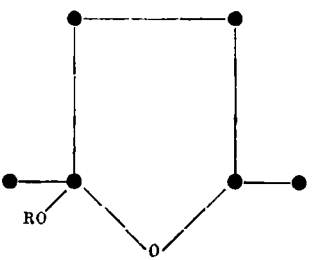
Galactoside.

Assuming that the attachment of the enzyme is in some way dependent on the hydroxyl groups, assuming also that the enzyme is provided with points of attachment which bring it into relation with the hydroxyl groups rather than with the oxygen atom in the ring, it may be supposed that the alteration in the configuration of the ring which is involved in the passage from glucose to galactose—following the establishment of a β- instead of an α-linkage in the ring—would be of less consequence than any shifting of the hydroxyl groups relatively to the ring plane. It is proposed to discuss the question of the relation of glucose to galactose more fully in a separate communication dealing with the relative stability of derivatives of the two compounds in presence of acids.

It remains only to consider why fructose, which is so closely related to glucose, should have no inhibiting influence except on invertase. Inasmuch as this enzyme is the only one at present known to us which can effect the separation of fructose from higher carbohydrates and inasmuch as it is influenced by fructose alone, it must be supposed that the structure of invertase is in close correlation with that of fructose. Assuming that fructose is also a γ-oxide, its structure is essentially different from that of glucose, as will be seen on comparing their formulæ.



Glucose.



Fructose.

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.

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