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CXXIV.—*Studies on Enzyme Action. 1. The Correlation of the Stereoisomeric α - and β -Glucosides with the corresponding Glucoses.*

By E. FRANKLAND ARMSTRONG (Salters' Company's Research Fellow).

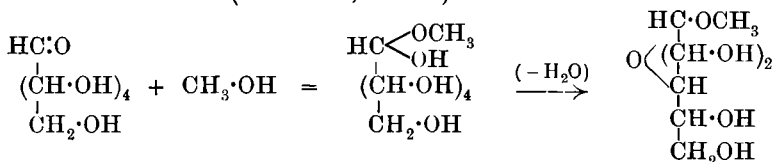
OF the explanations put forward of the change in rotatory power which glucose undergoes in solution—a phenomenon discovered by Dubrunfaut in 1846—the most probable is that suggested by Lowry (Trans., 1899, 75, 213), which connects the change of rotatory power of glucose with a reversible isomeric change. When this explanation was first given, it was not possible to decide definitely what formulæ were to be assigned to the dynamic isomerides. In view, however, of observations made during the investigation of various glucoside derivatives (Fischer and Armstrong, *Ber.*, 1901, 34, 2885; 1902, 35, 833, 3144 and 3153), in the course of which the existence of two undoubtedly stereoisomeric series of glucose derivatives was discovered

and their relationship to the α - and β -methylglucosides established, it now seems probable that a lactonic structure may be assigned to the isomeric glucoses: these isomerides are, in fact, the two possible stereoisomeric lactones. From this point of view, the labile forms of glucose of high and low rotatory powers isolated by Tanret (*Compt. rend.*, 1895, 120, 1060), and called by him α - and γ -glucose, are the parent substances of the α - and β -glucosides whereas, as already pointed out by Lowry, his stable β -form of glucose is a mixture of the α - and γ -isomerides in the proportion in which they are in equilibrium. Assuming this to be the case, the changes taking place in glucose—which occur slowly in simple aqueous solution but instantaneously in the presence of a small quantity of alkali—involve the passage from one or other form of the lactone into a mixture of both.

Lactonic structure of glucosides.—The following arguments may be adduced in support of the view that the α - and β -glucosides are γ -lactones and that the isomerism is stereochemical.

(1) They do not combine with phenylhydrazine and therefore cannot contain an aldehyde group (E. Fischer, *Ber.*, 1893, 26, 2400).

(2) Even assuming that in the initial stage of the alkylation of glucose the latter is present in the aldehydic form, the separation of water at a subsequent stage necessarily involves the participation of a hydroxyl group attached to a carbon atom other than the terminal atom; in other words, it leads to the formation of a compound of lactonic structure (E. Fischer, *loc. cit.*).



(3) To judge from the non-formation of glucosidic derivatives from compounds such as benzoylcarbinol and benzoin—which contain the group $\text{CO}\cdot\text{CH}\cdot\text{OH}$ —an α -lactonic or ethylene oxide structure is practically excluded (E. Fischer, *loc. cit.*).

(4) On the other hand, the evidence accumulated by Fittig and others brings into prominence the tendency of hydroxy-acids to form γ -lactones wherever possible. Gluconic and similar acids have therefore been generally regarded as γ -lactones and the argument may fairly be extended to glucosides. The view put forward by Tollens (*Ber.*, 1883, 16, 921) that sugars in solution have a lactonic structure was based on this argument.

(5) W. H. Perkin, sen. (*Trans.*, 1902, 81, 177), in the course of his investigations on the magnetic rotatory power of the sugars, has

shown that glucose, galactose and the disaccharides maltose and lactose exhibit a rotatory power in agreement with that calculated on the assumption that they possess a lactonic structure.

(6) Inasmuch as the passage from glucose to the glucoside involves the production of a fifth asymmetric carbon atom, the existence of stereochemical isomerides may be anticipated.

(7) α -Acetylchloroglucose and similar compounds are very easily converted into the β -modifications and *vice versa*: judging from the readiness with which this reversible change takes place, it is highly probable that the relationship is stereochemical (Fischer and Armstrong, *Ber.*, 1901, 34, 2885).

Conversion of the Glucosides into the corresponding Glucoses.

Assuming that Tanret's α - and γ -glucoses represent the two possible stereoisomerides, it is only necessary, in order to determine their relationship to the α - and β -glucosides, to convert the latter into the former by some method which does not involve the occurrence of isomeric change. Enzymes appeared to be the most likely agents to effect the desired changes without giving rise to secondary interactions; it is from this point of view that I have studied the action of emulsin, maltase, and invertase on a series of glucosides.

The α - and β -alkylglucosides differ considerably in optical rotatory power, thus:

α -methylglucoside =	$[\alpha]_D = +157.5^\circ$	β -methylglucoside =	-32°
α -ethylglucoside =	$+150.6^\circ$	β -ethylglucoside =	-30°

It is to be supposed that the same order of difference will be met with in the case of the glucoses. Obviously, Tanret's observations are in accord with this assumption and favour the view that α - and γ -glucoses are to be correlated with the α - and β -glucosides. Inasmuch as both forms are unstable in solution, the rotatory power of the glucose formed from a glucoside should change, falling considerably in the case of the α -glucose and rising in that of the β -modification; as alkali brings about such a change at once, a sudden fall in the rotation on the addition of a trace of alkali would denote the presence of the α -compound, whereas a sudden rise would indicate the β -compound.

The experiments made have in every case confirmed these views. Thus, in the case of the hydrolysis of α -methylglucoside by means of maltase, the marked fall in the rotatory power, which was observed on adding ammonia, not only proves the formation of α -glucose but also affords proof that the ordinary crystalline glucose of high rota-

tory power is α -glucose, and should accordingly be correlated with the α -glucosides.

In the case of experiments with β -methylglucoside and emulsin, a fall in rotatory power was observed on the addition of alkali; this observation shows that β -glucose is the immediate product of hydrolysis and makes it possible to correlate Tanret's solid γ -glucose with the β -glucosides.

The nature of the sugar first formed on hydrolysing some di- and tri-saccharides has been investigated in a similar manner.

When maltose—which has long been recognised as an α -glucoside—is hydrolysed with maltase, a glucose is formed the rotatory power of which diminishes on the addition of ammonia; this again is a proof that the initial product of hydrolysis of α -glucosides is α -glucose.

When, in like manner, cane sugar—also an α -glucoside—is hydrolysed with invertase, a mixture of glucose and α -glucose is formed. The addition of ammonia to such solutions causes a marked diminution in the rotatory power, mainly due, no doubt, to the conversion of the α -glucose initially formed into the mixture of constant rotatory power. Moreover, the change in the α -glucose initially formed undoubtedly affects the rotatory power, but judging from the very small change in rotatory power which takes place in freshly prepared α -glucose solutions, the magnitude of the change is relatively small. This observation confirms that previously made by O'Sullivan and Thompson (*Trans.*, 1890, 57, 920).

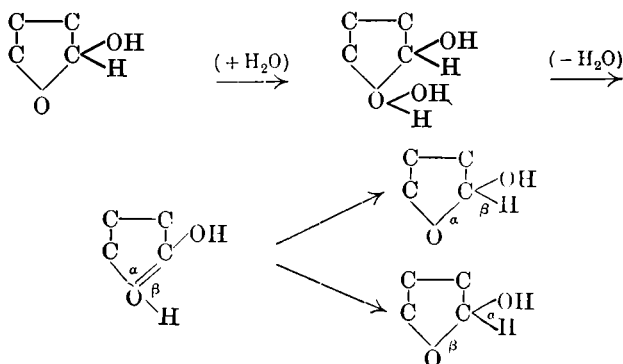
Lastly, when the trisaccharide, raffinose (derived from galactose, glucose and α -glucose), is hydrolysed by invertase, a mixture of α -glucose with the disaccharide melibiose is formed, which diminishes in rotatory power on the addition of ammonia. This result is evidence that melibiose is an α -sugar; it may be mentioned that this is the first occasion on which the mutarotation of melibiose has been recorded.

In studying the action of diastase on cold starch paste, Brown and Morris (*Trans.*, 1895, 67, 309) noticed that a rise in rotatory power took place on adding alkali, showing that the maltose produced was in the β -form. This conclusion involves the assumption that in the case of maltose, indeed of the sugars generally, the modification of low rotatory power is the β -form. The rotatory power of the only known maltoside, β -methylmaltoside ($[\alpha]_D = +70^\circ$ approximately), is such as to support this supposition (Fischer and Armstrong, *Ber.*, 1901, 34, 2896; Königs and Knorr, *ibid.*, 3443). The ordinary solid form of maltose ($[\alpha]_D +118^\circ$ initially, changing to $[\alpha]_D +138^\circ$) must, from the same point of view, be regarded as the β -form; under normal conditions maltose separates from solution in this modification.

Other glucosides and polysaccharides are being tested in the same direction; the results obtained are already sufficient to show that glucosides and polysaccharides on hydrolysis form sugars of corresponding structure and that the sugars which exhibit the phenomenon of mutarotation have a lactonic structure.

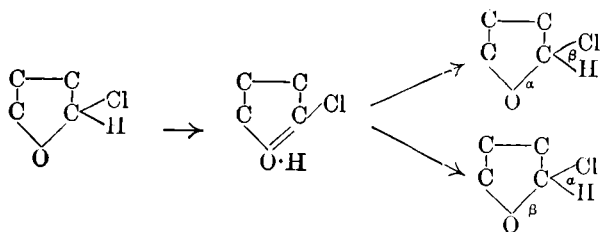
Nature of the Isomeric Changes, α - \rightleftharpoons - β -Glucoses.

On the assumption that the glucoses are lactones, the isomerism may obviously arise in two ways: either it may be conditioned by the different relative position of the hydrogen atom and the hydroxyl group attached to the carbon atom which is in association with the lactonic oxygen, or it may arise from a difference in the position of the hydrogen atom relatively to the lactonic oxygen atom. The manner in which the change may be assumed to take place is represented by the following formulae:

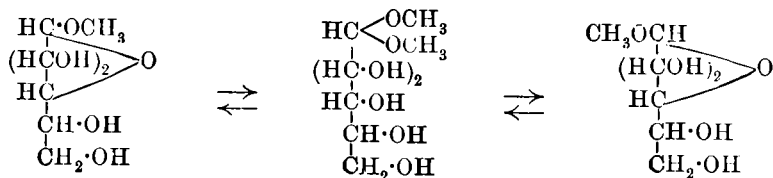


Apparently, the possibility of the latter form of isomerism has not hitherto been considered, although the behaviour of acetylchloroglucose is such as almost to place the existence of this relationship beyond doubt.

When α -acetylchloroglucose, $[\alpha]_D + 193^\circ$, dissolved in dry chloroform, is placed in contact with sodium hydroxide and a trace of water, it is to a large extent converted, in the course of a few weeks, into what must undoubtedly be regarded as the stereoisomeric β -compound, $[\alpha]_D + 165^\circ$ (Fischer and Armstrong, *Ber.*, 1901 34, 2885). In this case, a change from the lactonic to the enolic type would involve the elimination and subsequent addition of hydrogen chloride, a migration which is inconceivable under such conditions. Isomerism must, therefore, be regarded as being conditioned by the interchange in position of the lactonic oxygen and hydrogen groups, somewhat in the following manner:



A similar explanation may be given of the partial conversion of β -pentacetylglucose into the α -derivatives under the influence of zinc chloride (Erwig and Königs, *Ber.*, 1889, **22**, 1464, 2207) and of α - into β -methylglucosides and *vice versa* (*Ber.*, 1895, **28**, 1146). In the latter case Fischer has assumed that an intermediate compound of the acetal type is produced and that the lactone ring is opened :



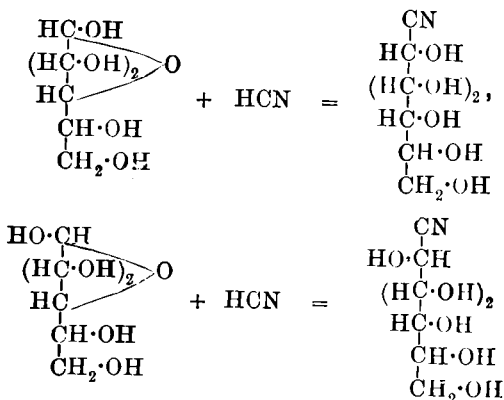
Jungius (*Proc. K. Akad. Wetensch. Amsterdam*, 1903, **6**, 99), who has recently determined the velocity with which the change takes place, however, considers it very improbable that an acetal is formed as an intermediate product; he concludes that the two glucosides are directly converted into each other. This conclusion is in harmony with that deduced above in so far as it concerns the disruption of the lactone ring.

It should be pointed out in the case of glucose that, besides the above-mentioned changes occurring in the relative positions of the groups attached to the terminal carbon atom, another series of changes may take place involving enolisation and an interchange in the relative positions of the groups attached to the terminal and penultimate carbon atoms. The conversion of glucose into mannose and fructose probably involves changes of this order.

The view here established, namely, that glucose in solution is a mixture of two stereoisomeric lactones, affords a very simple explanation of several experimental facts observed in the sugar group.

Thus it is probable that the formation of two isomeric nitriles in unequal quantities when glucose, dissolved in water, combines with hydrogen cyanide merely involves the direct addition of hydrogen cyanide to the lactone ring of both modifications as shown below; it is not necessary to assume that the hydrogen cyanide combines

eslectively, as must be the case if it be supposed to combine with an aldehyde group :



As the action of enzymes on α - and β -methylglucosides is selective, it is to be supposed that differences will be met with in the physiological behaviour of the α - and β -modifications of sugars : thus it is conceivable that the one form of glucose would be fermented at least more easily than the other ; it is proposed to test this view experimentally. Brown (British Association Report, Dover, 1899, p. 703) has already suggested that maltose in the form in which it is initially present when produced from starch may be fermented at a different rate than the optically stable form.

EXPERIMENTAL.

The solution of the enzyme having been prepared by extracting the dried enzyme with water and filtering several times, was mixed with a solution of the glucosides, and kept at a constant temperature. At stated intervals, about 7 c.c. were withdrawn by means of a pipette, rapidly filtered and examined in the polarimeter. In the case of invertase and similar rapidly acting enzymes, it was necessary to stop the hydrolysis before putting the solution in the polarimeter tube : this was effected by adding a drop of salicylaldehyde. After noting the angle of rotation, a drop of dilute ammonia was introduced into the solution and a new reading taken. Control experiments were made to ascertain the effect of minute quantities of salicylaldehyde or ammonia on the specific rotatory power of sugar solutions. Salicylaldehyde has no effect ; ammonia, however, causes a slight diminution in the rotatory power of the reducing sugars, although it has no action on the glucosides.

Thus the rotatory power of a 10 per cent. glucose solution in a

1-dm. tube fell from $+4.38^{\circ}$ to 4.32° on adding a drop of ammonia. In these experiments, however, as the quantity of reducing sugar present is always very small, the ammonia causes at the most a diminution of $2'$ or $3'$ in the rotatory power.

β -Methylglucoside and Emulsin.

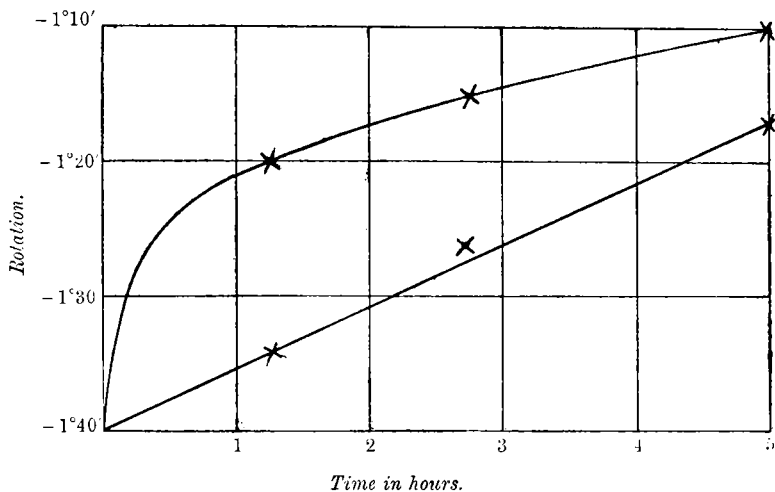
Concentration of glucoside = 5.4 per cent. Temperature = 37° .

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0	$-1^{\circ}40'$	$-1^{\circ}40'$	rise 0
1.25 hours	$-1^{\circ}34'$	$-1^{\circ}20'$	„ 14'
2.75 „	$-1^{\circ}26'$	$-1^{\circ}16'$	„ 10'
5 „	$-1^{\circ}17'$	$-1^{\circ}10'$	„ 7'
23 „	$-0^{\circ}44'$	$-0^{\circ}44'$	„ 0'

Concentration of glucoside = 10 per cent. Temperature = 37° .

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0 hour	$-2^{\circ}30'$	$-2^{\circ}30'$	rise 14'
0.25 „	$-2^{\circ}23'$	$-2^{\circ}9'$	„ 12'
0.5 „	$-2^{\circ}18'$	$-2^{\circ}6'$	„ 10'
1.0 „	$-2^{\circ}10'$	$-2^{\circ}0'$	„ 10'

The change in rotatory power on the addition of alkali is very clearly expressed in the following curve.



α -Methylglucoside and Maltase.

Concentration of glucoside = 10 per cent. Temperature = 27°.

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0 hour	+ 15°6'	+ 15°6'	
0.5 "	+ 15°	+ 14°41'	fall 19'
1 "	+ 14°50'	+ 14°16'	" 34'
2 hours	+ 14°36'	+ 13°40'	" 56'

Maltose and Maltase.

Concentration of disaccharide = 10 per cent. Temperature = 27°.

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0 hour	+ 11°38'	+ 11°33'	fall 5'
0.5 "	+ 11°14'	+ 10°52'	" 22'
1 "	+ 11°0'	+ 10°24'	" 36'
2 hours	+ 10°33'	+ 10°1'	" 32'

Cane Sugar and Invertase.

Concentration of disaccharide = 10 per cent. Temperature = 17°.

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0 hour	+ 5°12'	+ 5°12'	
0.5 "	+ 4°32'	+ 3°59'	fall 33'
1 "	+ 3°48'	+ 3°1'	" 47'
2 hours	+ 2°1'	+ 1°20'	" 41'
3 "	+ 0°30'	- 0°4'	" 34'
4.75 "	- 1°9'	- 1°27'	" 18'

Raffinose and Invertase.

Concentration of trisaccharide = 5 per cent. Temperature = 17°.

Time.	Rotatory power.		
	Initial.	Alkali added.	Difference.
0.5 hour	+ 5°23'	+ 4°47'	fall 36'
1.0 "	+ 4°50'	+ 4°18'	" 32'
2.5 hours	+ 3°42'	+ 3°20'	" 22'

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