CXXV.—*Studies of Dynamic Isomerism. I. The Mutarotation of Glucose.*

By T. MARTIN LOWRY, D.Sc.

THE gradual change in the rotatory power of freshly prepared aqueous solutions of glucose, observed by Dubrunfant in 1846, was at first regarded as being due to purely physical causes. The chemical character of the phenomenon was not recognised until Emil Fischer, in 1890, discovered that similar changes of rotatory power were produced by the partial hydrolysis, in aqueous solution, of optically active lactones and suggested that the mutarotation of glucose might be due to the conversion of the sugar by combination with water into a heptahydric alcohol of lower rotatory power :

This theory explained all that was known of the mutarotatory sugars until Tanret, in 1896, showed that it was possible to isolate, not only ordinary α -glucose, $[\alpha]_D +105^\circ$, and the β -glucose, $[\alpha]_D +52.5^\circ$, into which it passes in solution, but also a γ -glucose of low rotatory power, $[\alpha]_D +22^\circ$, which was also convertible into β -glucose; γ -glucose proved to be isomeric with α -glucose, not the hydrate which Fischer's theory indicated.

Three years later, nitrocamphor was observed to undergo changes of rotatory power which were undoubtedly due to reversible isomeric change; it then became clear that mutarotation was a normal accompaniment of dynamic isomerism in optically active substances, although similar effects might be produced by reversible polymeric change or by association with the solvent. The application to the case of glucose was obvious: Tanret's α - and γ -glucose were dynamic isomerides; his β -glucose was not a third isomeride, as he had supposed, but a mixture in equilibrium of the α - and γ -forms.

At that time, it was not possible to assign definite formulæ to the isodynamic glucoses; in addition to the aldehydic formula, two oxide formulæ, II and III, had been proposed, each representing two stereo-isomerides, and an enolic modification, IV, was not impossible; any two of these possible compounds might exhibit dynamic isomerism and give rise to mutarotation :

For purely chemical reasons, the ethylene oxide formula for glucose has been generally abandoned; the enolic form, as will be shown later, can scarcely be present except in minute quantities; it is therefore probable that the main constituents have an aldehydic or a γ -oxide structure: indeed this view has been held by Lippmann, Tanret and others. Quite recently, Simon (*Compt. rend.*, 1901, 132, 487—490) has suggested that α - and γ -glucoses are the lower homologues of α - and β -methylglucosides respectively, and supports this view by referring to the specific rotatory power of the four substances:

α -Methylglucoside	+ 157°	α -Glucose.....	+ 105°
β -Methylglucoside.....	- 32	γ -Glucose.....	+ 22
	Mean + 62.5°		+ 63.5°

This theory accords with the fact that α - and γ -glucoses can both be crystallised from solution, a property specially characteristic of sugar derivatives containing a γ -oxide ring, and is, moreover, much more probable than the alternative view that the aldehyde is in equilibrium with only one of the stereoisomeric γ -oxides. A direct proof of the glucosidic structure of α - and γ -glucoses was, however, wanting; this is now afforded by the experiments which Dr. E. F. Armstrong describes in the preceding paper. The actual production from β -methylglucoside of a glucose of low initial rotatory power, whilst the α -glucosides yield a sugar of high rotatory power, affords very strong evidence in favour of the γ -oxide formulæ which had been provisionally assigned to α - and γ -glucoses.

Simon, like Lippmann (*Ber.*, 1896, 29, 203) and Tanret (*Bull. Soc. Chim.*, 1896, iii, 15, 195), regards β -glucose as a third isomeride, ascribing the mutarotation of α - and γ -glucoses to their (complete) conversion into the isomeric aldehyde. Such a view is directly opposed to the theory of dynamic isomerism, which postulates a reversible isomeric change in which both isomerides are converted into a mixture in equilibrium of the two forms; it is, moreover, not in agreement with the experimental data, for if the change into the aldehyde were complete it would be impossible by mere crystallisation to reconvert this into the α -glucose which normally separates from the solution.

But whilst the mutarotation of the glucoses must be regarded as being mainly due to the establishment of equilibrium between the stereoisomeric γ -oxides, it is not improbable that the aldehyde may also be present in the mixture, either in the free state or as a hydrate. This conclusion is based on the view that it is impossible to interchange directly the points of attachment of the H and OH radicals; the simplest mechanism by which this can be brought about is that which involves the breaking of the γ -oxide ring and the formation of the aldehyde or its hydrate as an intermediate product:

If this view be correct the conversion of α - into γ -glucose involves a two-fold change, and can only proceed rapidly if the aldehyde be formed with considerable velocity and be present in appreciable quantity in the solution.

A similar argument may be used to show that only traces of the enolic form are present. Glucose, fructose and mannose have a common enolic form:

and, as Lobry de Bruyn has shown (*Rec. trav. chim.*, 1895, 14, 201), are convertible into one another in alkaline solution. But whilst α - and γ -glucose reach a condition of equilibrium in a few hours when merely dissolved in water or instantaneously in the presence of a mere trace of alkali, mannose, glucose and fructose are changed only slowly and incompletely in the presence of large amounts of the catalyst. This excessively slow conversion indicates clearly that the enolic form can be produced only at a very slow rate and in the most minute proportions.

The mutarotation of nitrocamphor in neutral solvents having been found to depend on the presence of traces of some third substance (*Trans.*, 1899, 75, 219), it was of interest to find whether the

mutarotation of aqueous solutions of glucose were not also conditioned by associated impurities. In view of the great sensitiveness of glucose to alkaline catalysts and the impossibility of preparing and keeping "pure" water in glass vessels, the preparation of a "pure" solution of glucose which should not exhibit mutarotation was not undertaken. But as neutral salts have little or no influence on the change, it was thought that the mutarotation might be checked or altogether prevented by neutralising any acid or basic impurities present in the solution.

Before attempting such a neutralisation, it was necessary to ascertain (1) the extent to which the activity of acids and bases could be destroyed by neutralisation; (2) the amount of acid or base required to account for the rate of change normally observed. On the former point, the evidence was conclusive: the rotatory power of a 5 per cent. solution of glucose in normal potassium chloride decreased somewhat less rapidly than that of a simple aqueous solution, the change at 11° being half completed in 1.9 hours in the former and in 1.8 hours in the latter case; a decinormal solution showed a greater retardation but this was perhaps due to some slight contamination of the aqueous solution used for comparison.

Quantitative experiments on the influence of acids, the results of which are plotted in Fig. 1 (p. 1318), showed that whilst the change in a 5 per cent. solution of glucose in $N/10$ hydrochloric acid was half complete in 0.27 hour at 14° , the corresponding period for $N/100$ hydrochloric acid was 0.7 hour, and for a simple aqueous solution, 0.9 hour at 15° . Osaka (*Zeit. physikal. Chem.*, 1900, 35, 661—706) has shown that the effects produced by acids are proportional to the square root of the concentration, so that doubling the amount of acid increases the velocity by about 40 per cent.; as the actual increase produced by $N/100$ hydrochloric acid was only 30 per cent., if the mutarotation were dependent on acid impurities these must be present to a greater extent than $N/100$ in the 5 per cent. solution. This is obviously not the case, inasmuch as not only is the solution not appreciably acid but it responds readily to the addition of quantities of alkali which would be completely neutralised by much smaller proportions of acid impurity.

Quantitative experiments on the influence of alkali brought out in a very striking way the extraordinary sensitiveness of glucose to these agents, a fact to which numerous experimenters have called attention. Whilst $N/100$ hydrochloric acid produced only a slight acceleration, the equivalent quantity of caustic potash brought about so rapid a change that equilibrium had already been attained when the first reading of the polarimeter was taken only four minutes after preparing the solution. A solution in $N/1000$ caustic potash showed an equally remarkable acceleration: the isomeric change at 11° was half com-

pleted at the end of five minutes, and equilibrium was reached in little more than half an hour. A distinct acceleration was produced even by $N/10000$ caustic potash, the action being half completed in 0.8 hour, whilst a solution in $N/100000$ caustic potash and a comparison solution in water each required 1.5 hours. Osaka has shown that the effects produced by alkalis are directly proportional to the concentration, a result which is confirmed by the values given above for $N/1000$ and $N/10000$ caustic potash. The velocity of

FIG. 1.—Influence of acids on the mutarotation of glucose.

isomeric change is doubled by the addition of $N/10000$ caustic potash, so that if the mutarotation of the aqueous solution were due to an alkaline impurity this need only be present to the extent of $N/10000$ in the 5 per cent. solution. Even this small amount of alkali could not be derived from the water, which had a specific conductivity of only 0.000002 reciprocal ohms per cubic centimetre at 18° , whilst $N/10000$ caustic potash gave the value 0.000056. The glucose solution, how-

ever, in spite of an increase of some 10 per cent. in its viscosity as compared with water, had a conductivity 0.000052 at 18°. This is probably due in part to the feebly acid properties of the sugar but might have been caused by associated alkali. Attempts were therefore made to check the mutarotation by the addition of acids: in a comparative experiment, using 5 per cent. solutions of glucose in (1) water, (2) *N*/10000 hydrochloric acid and (3) *N*/1000 hydrochloric acid—

FIG. 2.—Influence of alkalis on the mutarotation of glucose.

the amounts of acid present were too small to produce any specific acceleration, but more than sufficient to neutralise all alkaline impurities—the numerical results were so closely in agreement that it was not possible to represent them by separate curves.*

* At the end of three hours, the solutions gave identical values (67.0, 67.0, and 66.8), but the readings at one hour and two hours indicated that the aqueous solution had changed a trifle more rapidly and was perhaps five minutes in advance of

It is therefore clear that the mutarotation of glucose cannot be checked by neutralisation and is not dependent on the presence of acid or basic impurities in the aqueous solution. This result was somewhat unexpected and indicates a behaviour quite different from that of nitrocamphor dissolved in chloroform. The difference may perhaps be ascribed to the fact that water is an ionising solvent. When solvents of this class were used, labile solutions of nitrocamphor were never obtained, and indeed such solutions could scarcely be expected to withstand the catalytic action of the strongly acid *pseudonitro*-compound. It may, however, be pointed out that, apart from its ionising properties, water is probably directly concerned in the isomeric change of glucose since this change appears to depend upon a process of reversible hydrolysis; chloroform, on the other hand, merely brings the nitrocamphor into the liquid state and cannot assist directly in the isomeric change.

Whilst the experiments now described show that the mutarotation of glucose is not due to alkaline or acid impurities, they give no indication of the probable behaviour of pure glucose dissolved in pure water: if the solution could by extreme purification be rendered non-conducting, it is possible that the mutarotation would not take place.

EXPERIMENTAL.

In order to obtain comparable results at atmospheric temperatures, the method adopted was to observe the rate of change of rotatory power of a 5 per cent. solution of glucose containing a known quantity of acid, base, or salt, and to compare it with that of a simple aqueous solution prepared at the same time. The change was usually complete in about 8 hours, and a series of half a dozen readings of each tube was taken at intervals of about an hour; the solutions which changed more rapidly were observed at shorter intervals, and the curve for *N*/1000 caustic potash was plotted from observations made at intervals of less than a minute. As the change of specific rotatory power amounts to over 50° , no difficulty was experienced in obtaining smooth curves.

The *glucose* used was a commercial "pure" sample from Kahlbaum; the solutions prepared from it gave normal values for the specific rotatory power, and no alteration was produced by recrystallising twice from dilute methyl alcohol. As recrystallisation produced no retardation in the velocity of change, the commercial sample was used in all the remaining experiments.

The *water* had been purified (for conductivity measurements) by a

the acid solutions; this may be merely accidental, but might also indicate that a trace of alkaline dirt, amounting to about *N*/200000, had been neutralised and the velocity of isomeric change thus slightly reduced, in the acid solutions.

process of continuous fractional distillation, and had a conductivity of 0.000002 reciprocal ohms per cubic centimetre.

The *potassium chloride* was purified by crystallising until it gave solutions of constant conductivity; the normal solution prepared from the fused salt had a conductivity which agreed exactly with that given by Kohlrausch, Holborn, and Dusseldorf.

The *hydrochloric acid* was prepared by absorbing a weighed quantity of the dry gas in purified water.

The *caustic potash* was the purest commercial alkali containing no alumina or silica, and was freed from carbonate by means of purified baryta.

The values observed in 5 per cent. solutions of glucose were as follows:

I.

(a) Commercial glucose.		(b) Recrystallised glucose.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
6 m.	98.5°	—	—
10 m.	96.0	10 m.	98.3°
55 m.	76.7	49 m.	80.3
1 h. 38 m.	67.2	1 h. 38 m.	68.7
2 h. 18 m.	61.7	2 h. 18 m.	62.7
4 h. 10 m.	54.7	4 h. 0 m.	55.2

Temperature 17.5°.

Half-period..... (a) 0.82 h. (b) 0.71 h.

II.

(a) Water.		(b) N/10 KCl.		N/10 HCl.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
11 m.	94.8°	9 m.	100.8°	4 m.	97.3°
15 m.	92.5	14 m.	96.0	6 m.	92.3
26 m.	87.8	31 m.	87.8	8 m.	87.3
42 m.	81.3	47 m.	82.8	13 m.	81.8
1 h. 3 m.	75.7	1 h. 9 m.	77.7	34 m.	67.5
1 h. 40 m.	67.7	1 h. 48 m.	70.7	48 m.	62.2
2 h. 22 m.	63.0	2 h. 29 m.	66.3	1 h. 12 m.	56.0
3 h. 48 m.	57.7	3 h. 53 m.	59.7	1 h. 40 m.	54.3
6 h. 24 m.	53.0	6 h. 32 m.	53.7	2 h. 20 m.	54.2
next day.	52.0	next day.	52.3	next day.	53.3

Temperature 14°.

Half-period intervals..... (a) 0.82 h. (b) 1.0 h. (c) 0.27 h.

III.

(a) Water.		(b) Normal KCl.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
8 m.	104.7°	8 m.	105.5°
17 m.	100.0	20 m.	101.0
51 m.	90.8	52 m.	91.7
2 h. 22 m.	74.3	2 h. 24 m.	75.5
3 h. 18 m.	68.5	3 h. 21 m.	69.2
4 h. 15 m.	63.8	4 h. 17 m.	64.7
4 h. 59 m.	61.0	5 h. 7 m.	61.5
6 h. 39 m.	57.2	6 h. 42 m.	57.2
next day.	53.3	next day.	52.7

Temperature 11°.

Half-period..... (a) 1.8 h. (b) 1.9 h.

IV.

(a) Water.		(b) N/100 HCl.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
6 m.	102.3°	8 m.	98.5°
13 m.	96.6	13 m.	94.7
1 h. 33 m.	68.6	1 h. 32 m.	64.0
2 h. 30 m.	60.0	2 h. 30 m.	55.7
3 h. 30 m.	55.0	3 h. 30 m.	53.3

Temperature 14°.

Half-period..... (a) 0.9 h. (b) 0.7 h.

There was some uncertainty as to the zero reading of the polarimeter during this series, but this would affect the values for both solutions to an equal extent.

V.

(a) Water.		(b) <i>N</i> /10000 HCl.		(c) <i>N</i> /1000 HCl.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
10 m.	101·5°	10 m.	102·3°	10 m.	101·8°
58 m.	84·8	58 m.	85·5	58 m.	86·5
2 h. 4 m.	73·3	2 h. 2 m.	75·0	2 h. 3 m.	74·8
3 h. 5 m.	67·0	3 h. 5 m.	66·8	3 h. 5 m.	67·0
4 h. 24 m.	61·5	4 h. 24 m.	61·2	4 h. 24 m.	60·8
next day.	52·8	next day.	53·3	next day.	52·8

VI.

(a) Water.		(b) <i>N</i> /100000 KOH.		(c) <i>N</i> /10000 KOH.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
9 m.	102·5°	6 m.	103·7°	6 m.	98·5°
33 m.	92·8	33 m.	94·5	16 m.	93·5
1 h. 4 m.	86·5	1 h. 3 m.	85·0	32 m.	86·3
1 h. 43 m.	77·2	1 h. 43 m.	77·5	1 h. 2 m.	75·2
2 h. 51 m.	68·2	2 h. 52 m.	69·0	1 h. 42 m.	67·0
next day.	52·7	next day.	54·2	2 h. 50 m.	60·2
				next day.	52·7

(d) <i>N</i> /1000 KOH.		<i>N</i> /1000 KOH.		(e) <i>N</i> /100 KOH.	
Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.	Time.	$[\alpha]_D$.
1·5 m.	97·0°	7 m.	74·0°	4 m.	53·8°
2 m.	92·0	8 m.	71·2		
2·5 m.	89·8	9 m.	69·8		
3 m.	86·7	10 m.	67·8		
3·5 m.	86·2	15 m.	61·7		
4 m.	83·8	20 m.	56·3		
5 m.	80·0	26 m.	54·7		
6 m.	77·0	next day.	52·7		

Temperature 11° C.

Half period (a) and (b) 1·50 h. (c) 0·8 h. (d) 0·08 h.
 (e) probably about 0·008 h = 30 secs.

CHEMICAL DEPARTMENT,
 CITY AND GUILDS INSTITUTE,
 CENTRAL TECHNICAL COLLEGE.