THE KINETICS OF THE HYDROLYSIS OF CERTAIN GLUCOSIDES, PART II.:—TREHALOSE, α-METHYLGLUCOSIDE AND TETRAMETHYL-α-METHYLGLUCOSIDE.

By Emyr Alun Moelwyn-Hughes.

Communicated by Professor W. C. M. Lewis.

Received 23rd November, 1928.

It has been shown in a previous publication * by the writer [1] that the critical increment for the hydrolysis of aromatic glucosides by means of acids is a much more significant quantity than velocity itself, in that it lends itself more readily to comparative treatment. This is equally true in the case of the disaccharides, for it may be inferred from the work of Sigmund [2] on maltose, the data of von Bleyer and Schmidt [3] and of Stothart [4] for lactose, and the results of Jones and Lewis [5] in the case of sucrose that the critical increment, calculated by means of the equation of Arrhenius,[6] is independent of temperature, of the degree of hydration of the reactants and of the activity of the catalysing hydrogen ion. The experimental work described in the present paper is concerned with the determination of the critical increment for three additional Glucosides.† Trehalose has been chosen on account of its structural similarity to sucrose. α-methylglucoside and tetramethyl-α-methylglucoside have been selected as prototypes of the natural and the methylated Glucosides respectively.

The Hydrolysis of Trehalose.

Trehalose is a non-reducing diglucose, the potentially aldehydic group in each monose being involved in the biose link. Winterstein [7] observed that trehalose "is only hydrolysed by acids with considerable difficulty," and Ivanov [8] incorrectly states that "trehalose can be completely hydrolysed to glucose only by prolonged boiling with sulphuric acid." As far as it is known, no experiments have been conducted on the methylation of trehalose, presumably because there is no dispute concerning the linkage joining the two glucose units. On analogy with the normal glucosides, it is reasonable

* The publication referred to dealt with the hydrolysis of salicin, arbutin and phloridzin. For convenience it will be referred to as Part I.

† The term Glucoside (spelt with a capital G) is used to denote any hydrolysable carbohydrate or its substituted derivative; the term glucoside (small g) is reserved for any compound which yields glucose on hydrolysis.

[1] Moelwyn-Hughes, *Trans. Faraday Soc.*, **24**, 309 (1928).
[2] Sigmund, *Z. physikal. Chem.*, **27**, 385 (1898).
[3] Van Bleyer and Schmidt, *Biochem. Z.*, **135**, 546 (1923).
[4] Dr. S. N. H. Stothart, *Unpublished work carried out in this Laboratory* (1924-26).
[5] Jones and Lewis, *Trans. Chem. Soc.*, **117**, 1120 (1920).
[6] Arrhenius, *Z. physikal. Chem.*, **4**, 226 (1889).
[7] Armstrong, *Simple Carbohydrates and Glucosides*, *4th Ed.*, 124 (1924).
[8] Ivanov, *Biochem. Z.*, **162**, 445 (1925).

to assume that trehalose is a symmetrical biose, the two monose units possessing the amylene-oxide ring structure.[9]

The trehalose used in the present work was supplied by the Digestive Ferments Company. It was a colourless, crystalline dihydrate, readily soluble in water. By extrapolation from the values given in Table I., the

TABLE I.

Grams Trehalose Dihydrate per 100 c.c. of Solution.	Specific Rotation.	
	Trehalose Dihydrate.	Trehalose.
0·8678	+ 178·8°	+ 197·8°
8·1920	+ 179·4°	+ 198·3°
19·9932	+ 180·1°	+ 199·0°

specific rotation of a 5 per cent. solution of trehalose dihydrate is + 179·2 and of anhydrous trehalose is + 198·1°, which are in good agreement with the accepted values of + 178·3° and + 197·0°.[10]

From the observations of Winterstein and of Ivanov[8] it was anticipated that the hydrolysis of trehalose would offer serious experimental difficulties, and that concentrated acid at high temperatures would be needed. As a preliminary experiment, a 0·6507 per cent. solution was kept in *N* HCl at 80° C. for 18 hours, during which the optical rotation fell from + 2·31° to + 0·86°, indicating 87 per cent. hydrolysis. The rotation of the solution after 40 hours was observed to be + 0·66°, which compares very favourably with the value + 0·65° calculated from the concentration of glucose theoretically present. The hydrolysis of 5 per cent. solutions of trehalose by *N* HCl at 80° C. and 90° C. proceeds smoothly, as indicated by the results given on Table II., the reaction being unimolecular, as for Glucoside

TABLE II.—5 Per Cent. Trehalose Dihydrate. Normal HCl.

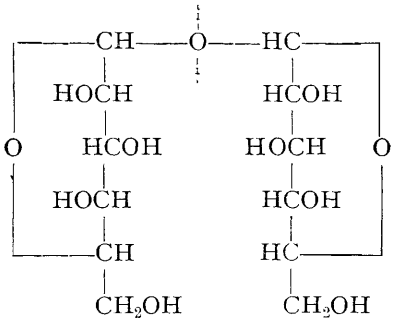
Time (Minutes).	Rotation (Degrees).	$k \times 10^5$ (Seconds^{-1}) at 80° C.	Time (Minutes).	Rotation (Degrees).	$k \times 10^4$ (Seconds^{-1}) at 90° C.
0	35·96	—	0	35·84	—
115	31·80	2·53	83	23·64	1·30
200	29·04	2·58	112	20·80	1·32
335	25·48	2·57	131	19·24	1·33
400	24·04	2·56	154	17·88	1·30
580	20·64	2·56	180	16·64	1·28
Inf.	10·00	—	Inf.	10·12	—
	Mean = 2·56			Mean = 1·31	
	Mean value of duplicate = 2·74			Mean value of duplicate = 1·27	
	Average value of k at 80° C. = $2·65 \times 10^{-5}$ seconds^{-1}.			Average value of k at 90° C. = $1·29 \times 10^{-4}$ seconds^{-1}.	

hydrolyses generally. As far as can be ascertained, no measurements of the rate of hydrolysis of this sugar have previously been made. Trehalose is a particularly stable diglucose, the rate of hydrolyses at 60° C. in the presence of hydrogen ion of unit activity being approximately 1/20th of the value for

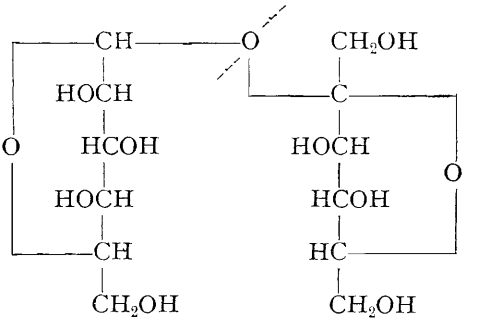
[9] Haworth, *Ann. Reports, Chem. Soc.*, **23**, 87 (1926).
[10] Hudson, *Scientific Papers of Bureau of Standards*, No. 533, 227 (1926).

maltose under the same conditions, and about 1/17,000th of the value for sucrose. Of all the hydrolysable carbohydrates hitherto examined, sucrose is the most readily hydrolysed, and trehalose is the most resistant to hydrolytic attack. Both are non-reducing disaccharides, and both contain one normal glucose unit.

These results are of great interest on account of the similarity in structure of sucrose and trehalose, and because of their bearing on a suggestion made by Armstrong [11] to aacount for the instability of sucrose in the presence of acids. It will be observed that both disaccharides contain the rare atomic arrangement – O . C . O . C . O –, a fact which holds



Trehalose.

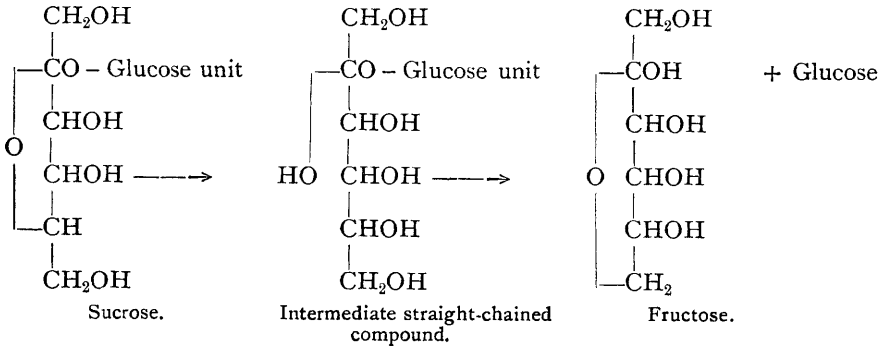


Sucrose.

good although occasion should arise for a revision of the oxide-ring structures involved, and which depends upon the well-established observations that (*a*) both disaccharides are non-reducing and (*b*) the oxygen atom of the closed ring (whether this be propylene-, butylene- or amylene-oxide) in any monose is concerned with the potentially aldehydic carbon atom. Armstrong attributes the ease of hydrolysis of sucrose to this atomic arrangement: the results given here for trehalose, which possesses the same conjugation as sucrose and is the most stable disaccharide examined, render Armstrong's suggestion inapplicable. It is thought that the factor which contributes to the lability of sucrose towards acids is the presence in the molecule of a γ-fructose residue, a view which is supported by previous evidence [1] in favour of the assumption that the primary attack during the hydrolysis of sucrose by acids involves the temporary opening of the fructoside link. This result is in harmony with, and indeed offers a mechanism for, the behaviour of sucrose during hydrolysis. It is now known with a fair degree of certainty that the fructose portion *in* sucrose is a 5-membered unit, and that the fructose which is present in the solution after hydrolysis is complete contains a 6-membered ring.[12] The mechanism of hydrolysis of fructosides which therefore seems the most probable is the opening of the 5-membered ring, followed by fission of the disaccharide molecule, the last stage being the closing of the temporarily straight chained fructose molecule to form a different, more stable, ring structure containing 6 members, as represented in the scheme on p. 84.

The critical increment for the hydrolysis of trehalose is found to be 40,180 calories, a value which, compared with that of 30,970 calories for maltose, gives an indication of the comparative stability of the non-reducing diglucose.

[11] As for 7; p. 123. [12] Irvine, *Chemical Reviews*, **4**, 303 (1927).



The Hydrolysis of α-Methylglucoside.

In view of the great theoretical importance attached to α- and β-methylglucosides, it is unfortunate that, beyond Armstrong's pioneer work,[13] little attention has been paid to the acidic hydrolysis of these glucosides. The critical increment for the hydrolysis of α-methylglucoside, calculated from Armstrong's data,[14] is 44,740 calories. No great reliance can be placed on this value, however, since the two temperatures at which hydrolysis were carried out differed by only 0·7° C.

The α-methylglucoside employed in this work has been prepared according to Fischer's method [15] by condensing glucose with methyl alcohol in the presence of dry hydrogen chloride in the cold. The product obtained was a colourless, soluble, crystalline solid possessing a well-defined melting-point and a high positive specific rotation, the purity of which may be gauged from the data of Table III.

TABLE III.

Authority.	Specific Rotation.	Melting-Point.
Fischer [15]	+ 157·5° (10 per cent. solution)	165°-166° C.
Purdie and Bridgett [16] . .	+ 159·5° (5 ,, ,,)	164° C.
Moelwyn-Hughes . .	+ 157·0° (4 ,, ,,)	164·5° C.

5 per cent. solutions of α-methylglucoside have been hydrolysed by N HCl at 60° C. and 80° C., the values of the velocity constants being given in Table IV. Previous work [17] has shown that in the case of solutions containing a low concentration of glucose, condensation into diglucose is negligibly small, so that calculated values for the infinity rotation may be legitimately used to calculate the velocity constants. A preliminary experiment with a 1·1797 per cent. solution of a α-methylglucoside fully confirmed this view. α-methylglucoside is fairly resistant to hydrolysis by acids, its rate of hydrolysis at 60° C. being roughly 1/12 of the value for maltose and about 1·7 times the value for trehalose. The critical increment of the reaction is found to be 38,190 calories, a value slightly

[13] Armstrong, *Trans. Chem. Soc.*, **83**, 1305 (1903).
[14] Armstrong, *Proc. Roy. Soc.*, **74B**, 188 (1904).
[15] Fischer, *Berichte*, **26**, 2405 (1893).
[16] Purdie and Bridgett, *Trans. Chem. Soc.*, **83**, 1039 (1903).
[17] Moelwyn-Hughes, *Trans. Faraday Soc.*, **24**, 321 (1928).

TABLE IV.—5 Per Cent. α-Methylglucoside. Normal HCl.

Time (Hours).	Rotation (Degrees).	$k \times 10^6$ (Seconds^{-1}) at 60° C.	Time (Minutes).	Rotation (Degrees).	$k \times 10^5$ (Seconds^{-1}) at 80° C.
0	31·32	—	0	31·44	—
38	27·70	1·37	75	28·28	3·50
65	25·62	1·32	250	22·28	3·58
85	24·10	1·33	300	21·24	3·53
133	20·74	1·34	410	18·48	3·53
200	18·18	1·31	500	17·08	3·60
Inf.	9·74	—	550	16·36	3·60
			Inf.	9·74	—
	Mean = 1·33			Mean = 3·56	
	Mean value for duplicate = 1·31			Mean value for duplicate = 3·46	
	Average value of k at 60° C. = $1·32 \times 10^{-6}$ seconds^{-1}.			Average value of k at 80° C. = $3·51 \times 10^{-5}$ seconds^{-1}.	

lower than that of 40,180 calories found for trehalose, and considerably greater than the mean value of 31,120 calories for the hydrolysis of the three glucosides maltose, salicin and arbutin.* It may be noted here that, in general, a Glucoside for which the velocity of hydrolysis is comparatively large requires a critical increment which is relatively small, and *vice versa*.

The Hydrolysis of Tetramethyl-α-Methylglucoside.

The tetramethyl-α-methylglucoside used in this work has been prepared from α-methylglucoside according to the method of Haworth.[18] The final product, a colourless, odourless, mobile syrup distilling at 119° C./2·3 mm., was identified by means of its specific rotation, refractive index and relative density, as indicated in Table V.

TABLE V.

Authority.	$[\alpha]_D^{25°}$ C.	$n_D^{25°}$ C.	$D_{4°}^{15°}$ C.
Purdie and Irvine [19] . .	+ 147·4° (water) + 153·9° (alcohol)	1·4464 —	— —
Haworth [18] . . .	—	1·4454	1·1082
Moelwyn-Hughes . .	+ 151·1° (water)	1·4467	1·1044

It was shown by Purdie and Irvine [19] that tetramethyl-α-methylglucoside is hydrolysed by dilute HCl into methyl alcohol and tetramethyl-α-glucose, which mutarotates rapidly into an equilibrium mixture of α- and β-tetramethylglucose having a value of + 83·3° for its specific rotation. A 2·82 per cent. solution of the tetramethyl-α-methylglucoside prepared was kept in *N*.HCl at 80° C., when the rotation was observed to fall from the initial value of + 4·26° to a constant value of + 2·22°, which corresponds to a specific rotation of + 83·39° for the product. A sample of tetramethyl-α-glucose has also been isolated from the products of hydrolysis and identified by means of its melting-point.

* The mean value given in Part I. has here been corrected by taking $R = 1·98$ calories per degree.

[18] Haworth, *Trans. Chem. Soc.*, **107**, 8 (1915).

[19] Purdie and Irvine, *ibid.*, **85**, 1049 (1904).

Table VI. gives the unimolecular constants governing the rate of hydrolysis of approximately 5 per cent. solutions * of tetramethyl-α-methyl-

TABLE VI.—5 Per Cent. Tetramethyl-α-Methylglucoside. Normal HCl.

Time (Minutes).	Rotation (Degrees).	$k \times 10^5$ (Seconds $^{-1}$) at 80° C.	Time (Minutes).	Rotation (Degrees).	$k \times 10^5$ (Seconds $^{-1}$) at 90° C.
0	33·20	—	0	32·72	—
180	30·44	2·13	97	29·40	4·42
220	30·04	2·04	295	24·72	4·47
260	29·60	2·00	355	23·64	4·55
350	28·60	2·00	395	23·32	4·34
450	27·40	2·10	498	22·08	4·34
Inf.	19·80	—	Inf.	18·08	—
		Mean = 2·05			Mean = 4·42
		Mean value for duplicate = 2·05			Mean value of duplicate = 4·48
		Average value of k at 80° C. = $2{\cdot}05 \times 10^{-5}$ seconds $^{-1}$.			Average value of k at 90° C. = $4{\cdot}45 \times 10^{-5}$ seconds $^{-1}$

glucoside by *N*.HCl at 80° C. and 90° C. It has been found that the substitution of four methyl groups into α-methylglucoside has caused the velocity of hydrolysis (at 60° C.) to increase threefold and the critical increment to decrease by about a half, the value for tetramethyl-α-methylglucoside being 19,840 calories as compared with 38,190 calories for the parent α-methylglucoside. It is clear that, as far as the critical increment may be taken as an indication of stability, tetramethyl-α-methylglucoside is more labile than any of the unsubstituted hexose derivatives which have been examined.

The Relative Rates of Hydrolysis of Glucosides.

It is apparent from the approximate constancy of the values in the last column of Table VII. that the rates of hydrolysis of sucrose [4] and maltose [20]

TABLE VII.

Composition of Solution (Grams per 100 c.c.).	$k_{25°} \times 10^5$.	a_{H+}.	$\left(\frac{k}{a_{H+}}\right) \times 10^4$.
5 sucrose	1·30	0·088	1·48
5 sucrose + 12·6 glycerol	1·45	0·098	1·48
5 ,, + 31·5 ,,	1·67	0·116	1·44
	$k_{60°} \times 10^5$.	a_{H+}.	$\left(\frac{k}{a_{H+}}\right) \times 10^5$.
10 maltose	1·63	0·954	1·71
10 maltose + 10 glucose	1·83	1·091	1·68
10 ,, + 20 ,,	2·16	1·254	1·72
10 ,, + 30 ,,	2·53	1·431	1·77
	$k_{60°} \times 10^6$.	a_{H+}	$\left(\frac{k}{a_{H+}}\right) \times 10^6$.
10 glucose	4·20	0·97	4·33
20 ,,	4·76	1·11	4·29
30 ,,	5·51	1·27	4·34
40 ,,	6·39	1·46	4·38
50 ,,	7·66	1·67	4·58

* The solution examined contained 5 c.c. of tetramethyl-α-methylglucoside per 100 c.c.

[20] Dr. A. J. Kieran, *Unpublished work carried out in this Laboratory* (1921-23).

and the rate of condensation of glucose[17] are governed in each case by the activity of the hydrogen ions.

Although it is by no means clear why the rate of hydrolysis is more closely related to the activity of the hydrogen ion than to its concentration, the linear relation between velocity coefficients and the activity of H^+ is useful in that it allows us to define the standard of acidic catalysis as that which is conditioned by hydrogen ion of unit activity. In Table VIII., the rates of hydrolysis of various glucosides at 25° C. and 60° C. are given, k for α-methylglucoside being given the arbitrary value of 100 at both temperatures.

TABLE VIII.

Glucoside Hydrolysed.	$\frac{k_{60°\,C.}}{a_{H^+}}$.	$\frac{k_{25°\,C.}}{a_{H^+}}$.	Relative k's taking $k_{\alpha\text{-methylglucoside}} = 100$ at both Temperatures.		
			At 60° C.	At 25° C.	Armstrong's Values.[21]
Trehalose . .	$8{\cdot}64 \times 10^{-7}$	$6{\cdot}74 \times 10^{-10}$	59	42	—
Amygdalin[22] (biose)	$1{\cdot}21 \times 10^{-6}$	$3{\cdot}16 \times 10^{-9}$	85	195	—
α-Me-Glucoside .	$1{\cdot}46 \times 10^{-6}$	$1{\cdot}62 \times 10^{-9}$	100	100	100
Tetra Me-α-Me-Glucoside . . .	$4{\cdot}09 \times 10^{-6}$	$1{\cdot}19 \times 10^{-7}$	280	5,851	—
Lactose . . .	$1{\cdot}66 \times 10^{-5}$	$1{\cdot}38 \times 10^{-7}$	1,138	8,504	583
Maltose . . .	$1{\cdot}68 \times 10^{-5}$	$6{\cdot}75 \times 10^{-8}$	1,150	4,167	740
Salicin . . .	$1{\cdot}80 \times 10^{-5}$	$6{\cdot}43 \times 10^{-8}$	1,233	3,970	601
Arbutin . . .	$4{\cdot}34 \times 10^{-5}$	$1{\cdot}81 \times 10^{-7}$	2,973	11,170	—
Phloridzin . .	$1{\cdot}16 \times 10^{-4}$	$1{\cdot}96 \times 10^{-6}$	7,945	120,800	—
Melezitose . .	$4{\cdot}94 \times 10^{-3}$	$5{\cdot}17 \times 10^{-5}$	338,300	3,193,000	—
Raffinose . . .	$1{\cdot}12 \times 10^{-2}$	$1{\cdot}23 \times 10^{-4}$	767,000	6,017,000	—
Sucrose . . .	$1{\cdot}46 \times 10^{-2}$	$1{\cdot}48 \times 10^{-4}$	1,000,000	9,118,000	722,500

It will readily be seen that comparisons of rates of hydrolysis such as those made by Armstrong[14] and by Caldwell,[23] are subject to a limitation enforced by the fact that the ratio of the rates of hydrolysis for any pair of Glucosides at a given temperature differs from the same ratio at any other temperature except in the particular case where both Glucosides possess the same critical increment, so that only in specialised cases of groups of Glucosides which possess similar values for the critical increment (*e.g.*, salicin, arbutin, and maltose; sucrose, raffinose and melezitose) is it legitimate to consider the effect of substitution and molecular rearrangement on the value of the velocity constant.

The object of Table VIII. is, in short, to emphasise the error which may be entailed in regarding velocity constants as anything other than a very *qualitative* measurement of structural stability. The critical increment, on the other hand, has already been shown (*cf.* Part I.) to be a much more reliable guide. It should, perhaps, be pointed out that in Table VIII. the value 100 has been ascribed to the velocity constant of α-methylglucoside at both temperatures, *i.e.*, the values in columns 4 and 5 of the Table do not give the effect of temperature on any single Glucoside.

Finally, it should be noted that the interpretation of the results given above for the hydrolysis of trehalose as evidence for the inapplicability of the presence of the conjugated system —O . C . O . C . O— to account for the

[21] As for 7; p. 140.
[22] Caldwell and Courtauld, *Trans. Chem. Soc.*, **91**, 666, (1907).
[23] Caldwell, *Brit. Assoc. Reports*, 267 (1906).

lability of sucrose is not invalidated by the limitation referred to, since the unique positions occupied by trehalose as the most stable disaccharide and by sucrose as the most labile disaccharide remain unaltered over a wider range of temperature than that hitherto covered by experiment.

The Possible Bearing of Glucoside Hydrolysis Results on the Mechanism of Mutarotation.

Two rival mechanisms have been advanced for the mutarotation of glucose. "Lowry[24] considers the formation of the aldehyde or of its hydrate, which involves the opening of the ring, to be an intermediate stage in the process; E. F. Armstrong has formulated the change as taking place without any disruption of the oxide ring."[25] Lowry's mechanism has been opposed on theoretical grounds by Baker, Ingold and Thorpe,[26] who regard mutarotation as a tautomeric change involving no intervention of water. Lowry has, however, replied to this criticism.[27] The present work contains two results which are interpreted as evidence in favour of Armstrong's mechanism.

(*a*) $T_{k=1}$ for a catalytic reaction in solution is defined as the temperature at which the reaction, under standard catalytic conditions, proceeds at unit rate. Consider the mutarotation of glucose as catalysed by hydrogen ion. Hudson[28] finds that the rate of mutarotation of 20 per cent. solutions of glucose by decinormal HCl at 24·7° C. is $1{\cdot}36 \times 10^{-3}$ seconds^{-1} This composite velocity coefficient ($k_1 + k_2$) is the sum of the rates of mutarotation of α-glucose and β-glucose. The equilibrium constant $K = \frac{[\alpha\text{-glucose}]}{[\beta\text{-glucose}]}$, calculated from the values + 113°, + 19°, and + 52·5° for the specific rotations of α-glucose, β-glucose and equilibrium solutions of α-β-glucose respectively, is 0·554. From the relation $K = \frac{k_{\beta\text{-glucose}}}{k_{\alpha\text{-glucose}}}$, the rate of mutarotation of α-glucose under these conditions is found to be $8{\cdot}75 \times 10^{-4}$ seconds^{-1}. The activity of the hydrogen ion in the solution considered is approximately 0·115 gram ion per litre,[29] so that $\frac{k_{24{\cdot}7^\circ\text{ C.}}}{a_{H+}}$ is $7{\cdot}63 \times 10^{-3}$ seconds^{-1}. The mean value of five critical increments for the mutarotation of glucose, calculated from the results of Hudson and Dale,[30] is 17,110 calories. It should be noted here that, since the equilibrium constant K does not vary perceptibly with temperature over the range considered, the critical increments for the mutarotation of α- and β-glucoses will be identical, being in each case equal to the observed critical increment. From these data, $T_{k=1}$ in the case of the mutarotation of α-glucose* is 358°A., a value which is low compared with those of $T_{k=1}$ found for the hydrolysis of glucosides, *viz.*, 433°A., and for the hydrolysis of γ-butyrolactone[31] (414°A.). The hydrolysis of the lactone is known to

* The numerical value of $T_{k=1}$, is obtained by means of the equation :—

$$ln\frac{k_1}{k_2} = \frac{E}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right).$$

[24] Lowry, *Trans. Chem. Soc.*, **85**, 1565 (1904).
[25] As for 7; p. 46.
[26] Baker, Ingold and Thorpe, *Trans. Chem. Soc.*, 268 (1924).
[27] Lowry, *ibid.*, 1371 (1925).
[28] Hudson, *J. Amer. Chem. Soc.*, **29**, 1571 (1907).
[29] Moran and Lewis, *ibid.*, **121**, 1613 (1922).
[30] Hudson and Dale, *ibid.*, **39**, 320 (1917).
[31] Dr. W. H. Garrett, *Unpublished work carried out in this Laboratory* (1921-23).

involve the opening of an oxide ring; and it is felt that the proximity in the values of $T_{k=1}$ for the hydrolyses of glucosides and the lactone, and the divergence in the values of $T_{k=1}$ for the mutarotation of glucose and for the hydrolysis of the lactone respectively indicate that glucoside hydrolysis involves the opening of the oxide ring whereas mutarotation of glucose does not.

(*b*) Maltose bears the same relation to α-glucose as lactose does to β-galactose. If the hydrolysis of the disaccharides and the mutarotation of the monosaccharides both involve rupture of the oxide ring, it is to be expected that the difference in the critical increments for the hydrolysis of maltose and lactose (30,970 − 26,900 = + 4070 calories) should equal the difference in the critical increments for the mutarotation of α-glucose and β-galactose (17,110 − 17,340* = − 230 calories). This, however, is not true, and it is concluded that the mechanism of mutarotation differs from that of glucoside hydrolysis in that mutarotation can proceed while the amylene oxide ring is intact.

The Possible Bearing of a Collision Theory of Unimolecular Reactions on Glucoside Hydrolysis Results.

An equation put forward by Hinshelwood[33] as an attempt to account for the rate of unimolecular reactions on the basis of a collision theory,[34] in which the critical increment of activation E is considered as being distributed among many degrees of freedom, takes the form

$$k = Z \cdot e^{-\frac{E}{RT}} \cdot \frac{\left(\frac{E}{RT}\right)^{\frac{1}{2}n-1}}{\lfloor \frac{1}{2}n - 1} \quad . \quad . \quad . \quad . \quad (1)$$

in which Z is a constant, and n is the total number of energy terms involved. Regarding the catalytic hydrolysis of glucosides as the unimolecular decomposition of a glucoside-hydrogen-ion complex, it is inferred that this is the type of reaction to which Hinshelwood's equation might conform, the complexity of the reactants leading us to anticipate a large value for n.† The justification for attempting to reconcile the data for glucoside hydrolysis with the above theoretical equation, which has been deduced for reactions in the gaseous phase, lies in the fact that the unimolecular reaction for which the most accurate data are available (decomposition of N_2O_5) proceeds according to the same mechanism in the gaseous state[36] as in solution.[37] Similarly, the racemisation of pinene[38] gives the same velocity constant in the gaseous and liquid states as in solution of three different organic solvents. The catalytic hydrolysis of Glucosides is strictly analogous to these two unimolecular reactions in the sense that, provided due correction is made for the change in activity of the hydrogen ion, the rate of hydrolysis is independent of the nature of the solvent medium. Thus, as shown in Table VII., in solutions containing hydrogen ion of unit activity, the rate of hydrolysis

* Calculated from the data of Worley and Adams.[32]

† n for the decomposition of propionic aldehyde[35] is 12-14, whence the number of degrees of freedom involved ($\frac{1}{2}n - 1$) is 5-6.

[32] Worley and Adams, *J. Physical Chem.*, **32**, 307 (1928).

[33] Hinshelwood, *Proc. Roy. Soc.*, **113A**, 230 (1927).

[34] Christiansen, *Proc. Camb. Phil. Soc.*, **23**, 438 (1928); Fowler and Rideal, *Proc. Roy. Soc.*, **113A**, 570 (1927).

[35] Hinshelwood and Thompson, *ibid.*, **113A**, 221 (1927).

[36] Daniels and Johnston, *J. Amer. Chem. Soc.*, **43**, 53 (1921).

[37] Lueck, *ibid.*, **44**, 757 (1922).

[38] Smith, *ibid.*, **49**, 43 (1927).

of a given Glucoside is the same in water as in glucose-water or glycerol-water mixtures.

(I.) For the closely related glucosides salicin and arbutin, we can assume that Z is not very different for each glucoside, so that Z_s/Z_a is almost unity, and that n is identical in each case, so that equation (1) may be written in the form :—

$$\frac{k_s}{k_a} = e^{\frac{E_a - E_s}{RT}} \cdot \left(\frac{E_s}{E_a}\right)^{\frac{1}{2}n - 1}, \quad . \quad . \quad . \quad . \quad (2)$$

which enables us to calculate n at any temperature. It is found that n at 60° C. is 34 ; and at 25° C. also, $n = 34$. This means that 16 (*i.e.* $\frac{1}{2}n - 1$) degrees of freedom come into play during the hydrolysis of these glucosides and that the same number functions over the range of temperature considered. On account of the error involved in the determination of E, stress is laid not so much on the actual numerical value of n as on the fact that, on the basis of the configuration of the glucosides, this number is not unreasonable.

(II.) It follows as a corollary from equation (1) that

$$E \quad = E_{\text{observed}} + (\tfrac{1}{2}n - 1)RT \quad . \quad . \quad . \quad (3)$$

The correction to be applied to the observed critical increment necessitated by this equation is large (*e.g.*, E_{true} for salicin becomes 40,200 calories) and will vary from case to case; but it is not likely to alter the relative values of the critical increments. The critical increment values used in equation (2) are, of course, observed values ; but since n for salicin and arbutin are *ex hypothesi* identical, $(E_a - E_s)$ will have the same value whether true or observed critical increments are considered.

It is well known that $E_{\text{obs.}}$ for the enzymic hydrolysis of a glucoside is generally lower than the corresponding value for its hydrolysis by acids, *e.g.*, $E_{\text{obs.}}$ for the hydrolysis of sucrose by invertase is 6,000 to 11,000 calories [39] as opposed to 25,600 calories for its hydrolysis by acids.[40, 1] Similarly, $E_{\text{obs.}}$ for the hydrolysis of salacin by emulsin is 15,000 calories [41] as contrasted with 31,630 calories for its hydrolysis by HCl.[1] It is also well known that $E_{\text{obs.}}$ for the enzymic hydrolysis of a glucoside does not give an accurate indication of the variation with temperature of the rate of hydrolytic change; the observed value of E is too low, its depression being due to (*a*) the fact that the glucoside-enzyme complex is dissociated more at the higher temperature than at the lower, and (*b*) the fact that irreversible decomposition of the enzyme is enhanced by rise in temperature. The magnitude of the difference between the observed critical increments for the enzymic and non-enzymic hydrolysis of a given glucoside is, however, so large that it is probable that, when the observed critical increment for enzymic hydrolysis is corrected for the two effects considered, the corrected $E_{\text{obs.}}$ for enzymic hydrolysis will still be considerably lower than the value of $E_{\text{obs.}}$ for acidic hydrolysis.

An interesting relation between the enzymic and non-enzymic hydrolyses of a given hydrolyst can be shown if it be assumed that E_{true} for the enzymic and non-enzymic hydrolyses of a given substrate is the same in both cases.

[39] O'Sullivan and Thompson, *Trans. Chem. Soc.*, 57 (1890); Euler and Ugglas, *Z. physiol. Chem.*, **65**, 124, (1910); Euler and Laurin, *ibid.*, **110**, 55 (1920); Euler, *Chemie der Enzyme*, **1**, 277 (1925).

[40] Spohr, *Z. physikal. Chem.*, **2**, 194 (1888); Euler, *ibid.*, **47**, 353 (1904).

[41] Tammann, *ibid.*, **18**, 426 (1895).

Such an assumption is not entirely without corroboration. Euler [42] has recently emphasised the analogy between the hydrolyses of sucrose by enzyme and by acid. "If we compare an enzymatic and a non-enzymatic solution, hydrolysing the same quantity of sugar with the same velocity, we find that the concentrations of the interacting molecules sugar-enzyme on the one hand and sugar-acid on the other are of the same order of magnitude, and that even the specific reactivities rq of both kinds of interacting molecules are not very different. . . ." This result "leads to the assumption that the reacting part of the substrate in both cases is changed in a similar manner as regards to structure." It must be admitted that Euler's conclusion is based on calculations which are liable to considerable error, and that "specific reactivity," like reaction rate itself, is but a qualitative indication of structural stability. On the other hand, experience of numerous catalytic reactions in solution, of a similar character and considered under comparable conditions, leads us to anticipate for two reactions proceeding at the same rate, values of critical increments which lie very close together. It is not probable that the critical increment necessary to activate, say, a sucrose-hydrogen-ion complex will be the same as that required to activate a sucrose-invertase complex, but if activation in the enzyme case be considered as localised to the "reacting part" of the substrate, then it is conceivable that the unit undergoing hydrolysis will, at the instant prior to hydrolytic cleavage, be activated to the same extent in the presence of an enzyme as in acid solution.

Assuming, however, without further speculation, that (*a*) E for enzymic hydrolysis, duly corrected, is less than $E_{\text{obs.}}$ for non-enzymic hydrolysis, and (*b*) E_{true} for both enzymic and non-enzymic hydrolyses is identical, then it follows from equation (3) that n for the enzymic hydrolysis of a glucoside must be greater than n for the hydrolysis of the same glucoside by acid. It would appear, therefore, that the function of an enzyme is to allow a greater number of degrees of freedom of internal energy of the reacting unit to contribute to the energy of activation than would be possible in its absence.

A possible mechanism whereby such a result is brought about is afforded by the so-called molecular distortion theory.[43] As Fischer first pointed out,[44] in the case of hydrolysis by enzymes the specificity of the catalyst towards the substrate suggests that a large part of the hydrolyst molecule fits into the enzyme surface. This is another way of stating that adsorption of the substrate by the enzyme takes place at more than one point, or that complex formation during enzymic hydrolysis is an example of multiple adsorption. Unless the distances between the various groups of the substrate molecules which are adsorbed are *exactly* equal to the distances between the points on the enzyme surface at which attachment takes place, adsorption involves distortion of the adsorbed molecule, and the act of distortion, in turn, implies that the substrate molecule has received a certain amount of energy, say W calories per gram mole. Being thus partially activated through contact with the enzyme, it would appear from the molecular distortion theory that the hydrolyst molecule would now require an energy increment of only $(E - W)$ calories before it can undergo hydrolytic cleavage. If, however, the suggestion that n for enzymic hydrolysis is greater than n for acidic hydrolysis be true, then

[42] Euler, *Trans. Faraday Soc.*, **24**, 661 (1928); Euler and Ölander, *Z. anorg. Chem.*, **156**, 143 (1926).
[43] Burk, *J. physical Chem.*, **30**, 1134 (1926); *ibid.*, **33**, 1613 (1928).
[44] Fischer; as for 7, p. 182.

the true critical increment E is still required to activate the hydrolyst unit, except that in the case of enzymic hydrolysis the term W now becomes available as a contribution to the total energy of activation E. In other words, the molecular distortion theory offers a mechanism for the suggestion considered if it be assumed that the enzyme, by its work in distorting the substrate molecule, induces certain internal degrees of freedom of the substrate molecule (*e.g.* the vibrations between adjacent carbon atoms, —C—C—), that are inoperative during acidic hydrolysis, to contribute to the total energy of activation.

Summary.

(1) The rates of hydrolysis of trehalose, α-methylglucoside and tetramethyl-α-methylglucoside by means of N. HCl have been determined polarimetrically at two temperatures.

(2) A comparison drawn between the rates of hydrolysis of the non-reducing disaccharides sucrose and trehalose shows that the lability of sucrose towards acids cannot be associated with the atomic arrangement —O . C . O . C . O—. It is much more probable that the lability of sucrose is due to the γ-fructose moiety, the act of hydrolysis for all Glucosides requiring fission of the oxide ring. Results based on relative rates of hydrolysis are limited in their applicability, because the ratio of velocity coefficients varies with the temperature except in specialised cases. It is shown that the critical increment is a more reliable guide.

(3) Incidentally, in connection with the mechanism of mutarotation, evidence is advanced that this process probably does not involve the rupture of the oxide ring.

(4) The idea that internal degrees of freedom of the hydrolyst molecule can contribute to the energy of activation is not irreconcilable with the data available for Glucoside hydrolysis, and can be utilised as a means of differentiating between enzymic and non-enzymic hydrolyses. It is tentatively suggested that the function of an enzyme is to allow a greater number of internal degrees of freedom to come into play in the process of activation than is possible in its absence.

The writer is indebted to the Department of Scientific and Industrial Research for a maintenance grant, and to Imperial Chemical Industries, Ltd., for a grant made to the Department of Physical Chemistry of this University.

Muspratt Laboratory,
University of Liverpool.