Mechanisms of Reactions in the Sugar Series. Part I. The Acid-catalysed Hydrolysis of α- and β-Methyl and α- and β-Phenyl D-Glucopyranosides.

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By isotopic tracer methods and the criterion of molecularity based on Hammett's acidity function, the acid-catalysed hydrolysis of four glucopyranosides has been shown to proceed by unimolecular decomposition of their conjugate acids and to involve fission of hexose–oxygen bonds only. It is considered that all except two mechanisms are excluded by the observed facts.

ARMSTRONG and GLOVER (*Proc. Roy. Soc.*, 1908, *B*, **80**, 312), in one of the first investigations of the acid-catalysed hydrolysis of glycosides, measured the rates of hydrolysis of methyl α- and β-D-glucopyranosides and, on the basis of the five-membered ring formulæ then accepted, discussed the mechanism, suggesting that ring opening was involved. In a more extensive investigation the rates and activation energies of hydrolysis of a number of glucopyranosides were measured, at a fixed acid concentration, by Moelwyn-Hughes (*Trans. Faraday Soc.*, 1928, **24**, 309; 1929, **25**, 81, 503). He suggested that the activation energy rather than the rate coefficient was the true measure of reactivity, and he calculated the " number of degrees of freedom involved in the reactions " from activation energy data and the equation developed by Hinshelwood for unimolecular gas reactions. Heidt and Purves (*J. Amer. Chem. Soc.*, 1944, **66**, 1385) found that, irrespective of changes of structure within a group, the ease of hydrolysis at a particular acid concentration and at a particular temperature increased in the order glucopyranoside, fructopyranoside, fructofuranoside. More recently, Nath and Rydon (*Biochem. J.*, 1954, **57**, 1) measured the rates of hydrolysis, at a fixed acid concentration, of a number of substituted phenyl β-D-glucopyranosides and, certain exceptions apart, found that electron-withdrawing or electron-releasing substituents in the benzene ring produced, respectively, small increases and decreases in the rates of hydrolysis. These investigations concern the effect of structural changes on the rate or activation energy of hydrolysis, and it appears impossible to draw from them useful conclusions about the mechanisms of the reactions. This is not surprising since in acid-catalysed hydrolytic reactions the initial proton transfer is rarely rate-determining, so that a structural charge in the substrate may affect the overall rate and activation energy of the reaction in two ways : first, by changing the equilibrium concentration of the conjugate acid of the substrate; and, secondly, by changing its rate of breakdown. These two effects may operate in opposite directions, and predictions about them are especially difficult in the present case, partly because of the complexity of the substances involved and partly because of the multiplicity of possible reaction paths.

In this investigation we set out to determine (*a*) the positions of bond fission and (*b*) the molecularities of the rate-determining steps in four D-glucopyranosides which, on general grounds, might be expected to undergo hydrolysis by a common mechanism. As is shown later, the evidence obtained excludes all but two of the possible mechanisms for the hydrolysis of the four compounds studied.

RESULTS

The positions of bond fission were found by carrying out the reactions in water enriched in the isotope ^{18}O and measuring the excess abundance of ^{18}O in the methyl alcohol or phenol produced. The glucose produced in each reaction was isolated as the osazone and this was also isotopically analysed. The results, some of which have already been reported in a preliminary publication (Bunton, Lewis, Llewellyn, Tristram, and Vernon, *Nature*, 1954, **174**, 560), are given in Table 1. It is seen from these results that the hydrolysis of the compounds studied involves fission of the hexose–oxygen bonds, *e.g.*, R—O—Me + $H_2{}^{18}O$ ⟶ R—^{18}O—H + H—O—Me. It may also be concluded, from the results obtained with the glucosazones, that hydrolysis does not involve rupture of carbon–oxygen bonds at the 3-, 4-, 5-, or 6-position in the pyranoside ring.

TABLE 1.

D-Glucopyranoside	Acid	Temp.	% Excess abundance of ^{18}O: In solution	In MeOH or PhOH	In glucosazone *
Me α-	1·10N-HCl	80°	$0·54_0$	$0·00_5$	—
	1·81N-$HClO_4$	72·9	$0·83_2$	$0·01_2$	$0·00_6$
Me β-	1·10N-HCl	80	$0·54_0$	$0·00_8$	—
Ph α-	2·19N-$HClO_4$	72·9	$0·80_4$	$0·00_3$	$0·00_8$
Ph β-	2·19N-$HClO_4$	72·9	$0·79_3$	$0·00_3$	$0·00_6$

* Referred to a normal sample of glucosazone.

The molecularities of the reactions were determined by use of the criterion based on Hammett's acidity function, H_0 (Hammett, " Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, 1940, p. 273; Long and co-workers, see particularly McIntyre and Long, *J. Amer. Chem. Soc.*, 1954, **76**, 3240). This criterion distinguishes between the possibilities (*a*) and (*b*) in the scheme below, in which G and G^+H represent the glycoside and its conjugate acid respectively and G_1^+ represents some other entity formed from G^+H :

$$G + H_3O^+ \rightleftharpoons GH^+ + H_2O \quad \text{fast}$$

(*a*)
$$GH^+ \longrightarrow G_1^+ \quad \text{slow}$$
$$G_1^+ + H_2O \longrightarrow \text{Products} \quad \text{fast}$$

(*b*)
$$GH^+ + H_2O \longrightarrow \text{Products} \quad \text{slow}$$

In (*a*) the rate-determining step is unimolecular and does not involve a water molecule and it can be shown that an approximate proportionality between the logarithm of the first-order rate coefficient (log *k*) and H_0 should be observed. In (*b*) the rate-determining step is bimolecular and it may similarly be shown that an approximate proportionality between log *k* and pH (defined as $-\log c_{H_3O^+}$) should result. In Table 2 are shown the rates of hydrolysis of the four

TABLE 2. *Hydrolysis of glucopyranosides in aqueous perchloric acid.*

D-Glucopyranoside	Temp.	$[HClO_4]$ (N)	H_0	10^3k_1 (min.$^{-1}$)	D-Glucopyranoside	Temp.	$[HClO_4]$ (N)	H_0	10^3k_1 (min.$^{-1}$)
Me α-	72·9°	0·465	+0·32	0·227	Ph β-	72·9	0·573	+0·21	4·78
		0·936	−0·07	0·552			0·848	+0·02	7·88
		1·552	−0·46	1·25			1·156	−0·20	12·0
		2·209	−0·79	2·42			1·715	−0·55	24·7
		2·722	−1·01	3·95			2·175	−0·78	38·0
		3·720	−1·38	9·09			2·880	−1·08	72·9
		2·272 *	−0·23	0·725					
		3·262 *	−0·52	1·28	Ph α-	72·9	0·549	+0·22	13·9
						57·4	0·551	+0·22	1·76
Me β-	72·9	0·485	+0·31	0·493			1·046	−0·13	4·32
		0·934	−0·04	1·12			2·127	−0·75	15·8
		1·566	−0·46	2·56			2·680	−0·99	21·7
		2·102	−0·74	4·22					
		2·688	−0·99	7·71					

D-Glucopyranoside	Me α-	Me β-	Ph β-	Ph α-
Slope of log *k* against H_0	−0·95	−0·91	−0·91	−0·89

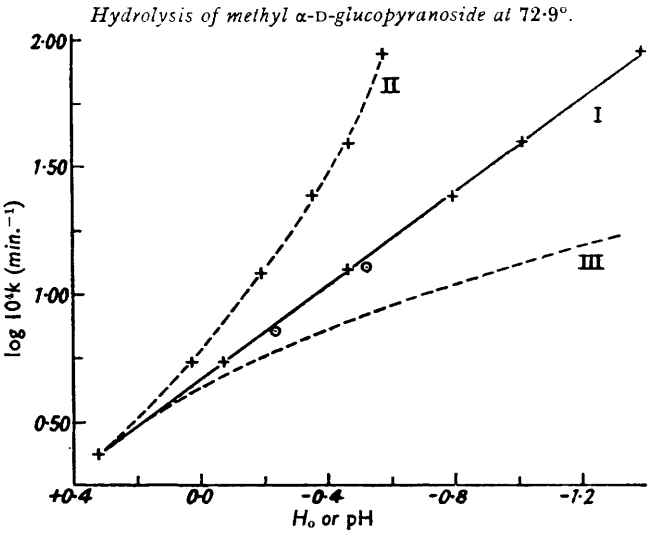
* Sulphuric acid was used in these experiments.

glucopyranosides in a range of acidities in which the numerical values of H_0 and pH differ sufficiently for diagnostic purposes. The Figure shows the rates for methyl α-D-glucopyranoside plotted against H_0 and against pH. It is clear that in all four cases log *k* and H_0 are nearly linearly related. The departures of the slopes from unity are small and have not been explicitly considered, particularly since the values of H_0 used are those measured at 25°, whereas the kinetic results have been obtained at higher temperatures. An analysis of factors leading to small departures from strict proportionality has been given by Long and McIntyre (*J. Amer. Chem. Soc.*, **1954**, **76**, 3243).

DISCUSSION

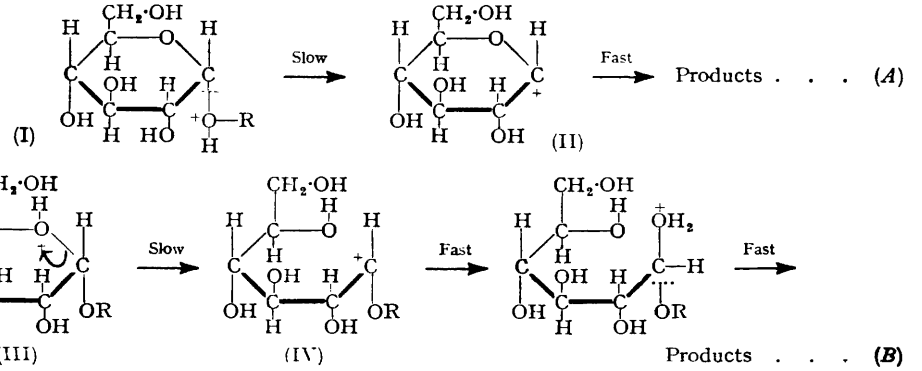
The results show that, in the hydrolysis of the four compounds studied, the initial proton transfers are fast and reversible, and that the conjugate acids so formed then undergo slow unimolecular reactions. This is the only hypothesis consistent with the observed dependence of rate on acidity. McIntyre and Long (*loc. cit.*) have drawn similar

conclusions about the acid-catalysed hydrolysis of methylal; the similarity of the reaction mechanisms of this compound to that of the pyranosides, which have a similar general structure, is not surprising especially since, in the pyranosides, bimolecular attack on $C_{(1)}$ is unlikely for steric reasons. The rate-determining, unimolecular steps may be formulated in two ways: first, as in scheme (*A*) in which the conjugate acid (I) undergoes heterolysis to form the carbonium ion (II), subsequent reaction with water being rapid; or, secondly, as in scheme (*B*) in which the conjugate acid (III) undergoes ring-opening between the oxygen atom and $C_{(1)}$ to form the ion (IV), subsequent rapid stages involving attack by a molecule and loss of methanol or phenol. It is also formally possible that the slow steps involve isomerisation to the corresponding furanosides. As it seems reasonable, however, to suppose that isomerisation would take place by way of ring-opening, as in scheme (*B*), no alternative reaction mechanism is provided by such considerations.



I, log *k* *plotted against* H_0. II, log *k* *plotted against* pH. III, *Hypothetical curve of* log *k* *plotted against* $[H_0]$ *for linear relation of* log *k* *and* pH.
\+ *Perchloric acid.* ○ *Sulphuric acid.*

Both reaction schemes are consistent with the evidence at present available, and it does not seem easy to devise a distinguishing experimental test. In principle a stereo-



chemical test might be employed since scheme (*B*) probably involves the production, under kinetic control, of the equilibrium mixture of α- and β-glucose, whereas this is probably not the case for scheme (*A*). In acidic aqueous solutions, where the mutarotation

of D-glucose is very rapid, such considerations have no diagnostic value; however, for other solvents, such as methanol, a possible distinction based on stereochemical considerations is being investigated. Foster and Overend (*Chem. and Ind.*, 1955, 566) discussed structural and conformational effects in the hydrolysis of glycosides only in terms of scheme (*A*); we think that no decision between the two possibilities can yet be made.

It is not our view that all glucopyranosides undergo hydrolysis by mechanisms exactly specified by one or other of the above alternatives. It is possible, for example, that a group which is very much more electron-releasing than methyl might cause alkyl–oxygen rather than hexose–oxygen fission; the reaction would, however, still be unimolecular. It is interesting in this connection that the hydrolysis of sucrose, one of the first reactions to be investigated by the criterion based on Hammett's acidity function, was shown to proceed by a unimolecular mechanism (Hammett and Paul, *J. Amer. Chem. Soc.*, 1934, **56**, 830). The position of bond fission in this reaction is, however, unknown.

Experimental

Materials.—Methyl α-D-*glucopyranoside.* A commercial sample, recrystallised several times from ethanol, had m. p. 164—165°, $[\alpha]_D^{25}$ +157·8° (*c* 3·0 in H_2O).

Methyl β-D-*glucopyranoside.* This was made by conventional methods from acetobromoglucose and recrystallised from ethyl acetate, to m. p. 107—108°, $[\alpha]_D^{25}$ −32·6° (*c* 2·7 in H_2O).

Phenyl α-D-*glucopyranoside.* The tetra-acetate (Montgomery, Richtmyer, and Hudson, *J. Amer. Chem. Soc.*, 1942, **64**, 690), $[\alpha]_D^{25}$ +168·8° (*c* 0·8 in $CHCl_3$), was catalytically deacetylated by sodium methoxide in methanol. The resulting glucopyranoside, recrystallised from water and dried *in vacuo*, had m. p. 169—170°, $[\alpha]_D^{25}$ +181·1° (*c* 0·65 in H_2O).

Phenyl β-D-*glucopyranoside.* The tetra-acetate (Montgomery *et al.*, *loc. cit.*), m. p. 127°, $[\alpha]_D^{25}$ −30·7° (*c* 2·0 in C_6H_6), gave on deacetylation the glucopyranoside which, dried *in vacuo*, had m. p. 173·5—174·5°, $[\alpha]_D^{25}$ −70·7° (*c* 2·0 in H_2O).

Kinetic Experiments.—The glucopyranoside (1—3 g.) was dissolved in water, a suitable amount of perchloric acid added, and the solution made up to 100 c.c. with water. The whole was suitably immersed in a thermostat and vigorously shaken until thermal equilibrium was reached. Volumes of about 10 c.c. were removed at appropriate intervals. In early experiments the reaction was stopped by pipetting known volumes of the mixture into known volumes of excess of alkali. This proved unnecessary for the runs at 73° and, because of decomposition of glucose in the presence of alkali, generally undesirable. The procedure finally adopted was to pipette a sample of the reaction mixture directly into a jacketed polarimeter tube maintained at 25°. The errors introduced into the time readings by this procedure were small since cooling was rapid and the reactions have high activation energies (Moelwyn-Hughes, *loc. cit.*). When thermal equilibrium was established, the optical rotatory power of the solution was determined. In a number of runs the calculated value of the " infinity " reading was compared with the observed " infinity " reading. The differences were in general less than 2%.

The values of the acidity function were calculated from data given by Hammett and Deyrup (*J. Amer. Chem. Soc.*, 1932, **54**, 2721) as corrected by Hammett and Paul (*ibid.*, 1934, **56**, 827).

In all the kinetic runs the first-order rate coefficients were found to be constant throughout the reaction. The following data for the hydrolysis of methyl β-D-glucopyranoside (3·0107 g./100 c.c.) in aqueous 1·566N-perchloric acid at 72·95° are typical.

Time (min.)	0	22·5	50·5	85	137·2	193	276	1300	∞
α_D^{25}	−0·925°	−0·791°	−0·623°	−0·455°	−0·227°	−0·010°	+0·267°	+1·364°	+1·470°
10^3k (min.$^{-1}$)	—	2·57	2·67	2·57	2·51	2·56	2·50	2·40	—

Isotope Experiments.—The following are typical of the procedures used.

(*a*) Methyl α-D-glucopyranoside (20 g.) was dissolved in 1·81N-perchloric acid (250 c.c.), the water in which contained 0·83$_2$% excess abundance of ^{18}O. The solution was heated in a sealed tube at 73° for 24 hr. Methanol was then distilled off through an efficient column of low hold-up. Experiments showed that isotopically normal methanol heated under these conditions and then isolated by distillation was not enriched in ^{18}O. After removal of methanol the residue was neutralised. Sodium acetate and phenylhydrazine hydrochloride were then added, and the whole was heated until the formation of glucosazone was complete. The glucosazone was recrystallised from aqueous ethanol.

(*b*) Phenyl β-D-glucopyranoside (6 g.) was dissolved in 2·19N-perchloric acid (100 c.c.),

the water in which contained $0{\cdot}79_3\%$ excess abundance of ^{18}O. The solution was heated for 24 hr. at 73°, and then extracted with ether. The ether extract was dried and the ether then distilled off. The phenol so obtained was purified by distillation.

The abundance of ^{18}O in the solvent water was determined by equilibration with carbon dioxide, and that in the organic products by pyrolysis to carbon monoxide *in vacuo* in a graphite tube heated in an R.F. induction furnace. Mass-spectrometric analyses were made on carbon monoxide for both methanol and phenol. The nitrogen evolved in the pyrolysis of glucosazone interferes with mass-spectrometric measurements on carbon monoxide. Therefore, the carbon monoxide from the glucosazones was converted into carbon dioxide on heated nickel catalyst, and the mass-spectrometric analyses were made on this gas.

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