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Influence of Cosolutes upon the Conformation of Carbohydrates in Aqueous Solutions. I. Dependence upon the Anion of the Relative Rates of Hydrolysis of the Anomeric Methyl Glucopyranosides in Aqueous Mineral Acids

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The ratio (K_β/K_α) of the rates of hydrolysis of β- and α-methyl D-glucopyranoside in aqueous sulphuric, phosphoric, hydrochloric, and hydrobromic acids increased markedly with increasing concentration of acid, and, for a given value of the Hammett acidity function (H_0), the magnitude of the effect decreased in the order in which the acids are named.

For values of $-H_0$ between 1.0 and 3.0, separate plots of log (K_β) and log (K_α) against $-H_0$ were virtually rectilinear in each acid, but they had different slopes. A more detailed study of hydrolysis in sulphuric acid showed that, outside this range, the plots were parallel. In the region in which the slopes deviated, the respective activation energies, E_β and E_α, both decreased with increasing acidity, but E_β decreased more than E_α. Simultaneously, the entropies of activation decreased, but to the same extent.

The increase in K_β/K_α was attributed to the anomeric effect, which, as the activity of water decreases, would be expected to stabilise the α-anomer and de-stabilise the β-anomer relative to the transition state.

The overall decrease in E_β and E_α was attributed to the stripping of water of solvation from the hydroxyl groups, leading to a decrease in their effective conformational size. The concomitant decrease in the entropy of activation supports this, and implies that water of solvation is released in passing through the transition state.

In an attempt to learn more about the biological role of carbohydrates in marine organisms, a study was undertaken of the effect of inorganic ions and other relevant cosolutes upon the conformation of polysaccharides and simple, model glycosides in aqueous solutions. In the first instance, attention was focussed upon the effect of inorganic ions upon reactions of simple glycosides, whose mechanisms are well understood, and which are known to entail conformational changes in the transition state. The work now described is

concerned with the influence of anions, and consists simply in a study of the relative rates of hydrolysis of α- and β-methyl D-glucopyranoside in different mineral acids.

EXPERIMENTAL

Materials. The two glycosides were commercial products, and were recrystallised to constant melting point from absolute ethanol. The acids were of Merck analytical grade. The normalities of the acids were determined by titration at 20° with standard sodium hydroxide, prepared from Merck ampoules. The indicator was methyl red.

Method. Hydrolysis was carried out in a 2 dm, centre-filling polarimeter tube, fitted with a heavy copper heating jacket, which was insulated with cotton wool. Water from a thermostatically controlled water-bath was pumped through the jacket at a rate of 1.5 l/min. The temperature was measured with a thermometer fitted into the polarimeter tube, and could be held constant to within ±0.02°. A conventional Zeiss polarimeter was used, which permitted measurement of the angle of rotation to within 0.01°.

To start an experiment, the polarimeter tube was first brought to the desired temperature. Identical volumes (measured at 20°) of a 2 % w/v aqueous solution of the glycoside and of the acid were then separately heated in the water-bath to the same temperature. The two solutions were then rapidly mixed, and transferred to the polarimeter tube. Complete thermal equilibrium was normally established inside the tube within 2 min, after which the first readings were taken. In experiments with very concentrated acids, there was some heat of dilution liberated upon mixing the two reactants. In these cases, prior to mixing, the reactants were warmed to a lower temperature than that desired, the exact temperature being determined by trial and error.

To avoid errors due to the acid-catalysed reversion and dehydration of the liberated glucose, only the first 50 – 70 % of the hydrolysis was followed, and normally, 30 to 50 readings were taken in the time (30 to 240 min) required for this. An "infinite time" reading was obtained with a freshly prepared solution of D-glucose in the acid under the same conditions. These experiments additionally confirmed that the mutarotation of D-glucose was virtually instantaneous in all the acids studied, and that the rate of destruction of the glucose was not of significant importance compared to its rate of liberation from the parent glycosides under the same conditions.

In the more concentrated acids, the "initial" optical rotation differed significantly from that in water at the same temperature, and was estimated by extrapolation of the hydrolysis-curve to zero time.

Thin-layer chromatography of the glycosides and of p-*nitroaniline in strong mineral acids.* This was carried out at room temperature on glass plates (20 × 5 cm) coated with a 0.75 mm thick layer of silica gel (Merck Kieselgel nach Stahl). Portions (200 μg) of material were applied to the origin as 1 % w/v solutions in methanol. After the acid had ascended 15 – 18 cm, the plates were placed horizontally on asbestos mats, and heated about 30 min in an oven at 110°. The glycosides were then revealed as dark spots, due to charring.

In low concentrations of acid, the *p*-nitroaniline migrated as a visible, yellow spot, and its position was marked on the developed plate before heating. In high concentrations of acid, it was colorless, but could be localised after heating by examination under UV light, when it appeared as a black spot. In low concentrations of acid, the glycosides did not char sufficiently upon heating to be clearly visible, but in these cases they were plainly visible, after heating, as fluorescent spots under UV light.

When chromatography was carried out in pure water as the solvent, the developed plates were first dried completely in the oven, then sprayed with 10 N sulphuric acid, and then heated again. When the acids themselves were chromatographed in water, they were localised by spraying the dried plates with aqueous sucrose (20 % w/v), followed by further heating.

THEORY

Mechanism of hydrolysis. The extensive literature on the mechanism of hydrolysis of glycosides has been comprehensively reviewed by BeMiller.[1]

It is generally accepted that, for pyranosides, hydrolysis takes place by the "cyclic", A-1 mechanism (Fig. 1), in which a rapid, equilibrium-controlled protonation of the glycosidic oxygen atom to give the conjugate acid (I) is followed by a unimolecular, rate-controlling heterolysis of the bond between this oxygen atom and C(1) of the pyranose ring. This liberates the aglycone, and generates a carbonium ion at C(1) of the glycose moiety, forcing it into the highly strained, half-chair form (II). This then reacts rapidly with water to give free glucose and a proton.

The Edward hypothesis. It is also widely accepted [1] that the well-known difference in the rates of hydrolysis of anomeric pairs of glycopyranosides is due to the effect first suggested by Edward,[2] and now generally known as *the anomeric effect*.[3,4]

The anomeric effect can be most simply regarded as a special case of the general proposition that a polar molecule in a non-polar solvent will tend to adopt a conformation in which the net dipole moment is as small as possible. This tendency will normally be opposed by other non-bonded interactions, and will weaken as the polarity of the solvent increases, and polar solvent-solute interactions become dominant.

Edward [2] pointed out that a glycopyranoside in which the glycosidic oxygen atom is axial should have a lower net dipole moment than one in which it is equatorial. This should stabilise the *C-1* conformation in methyl α-D-glucopyranoside relative to the *C-1* conformation in methyl β-D-glucopyranoside. Edward further assumed that the transition state in the heterolysis step would be the same for both anomers, and would correspond to the carbonium ion (II) in Fig. 1. The β-anomer would then be expected to hydrolyse faster than the α-anomer, as is the case.

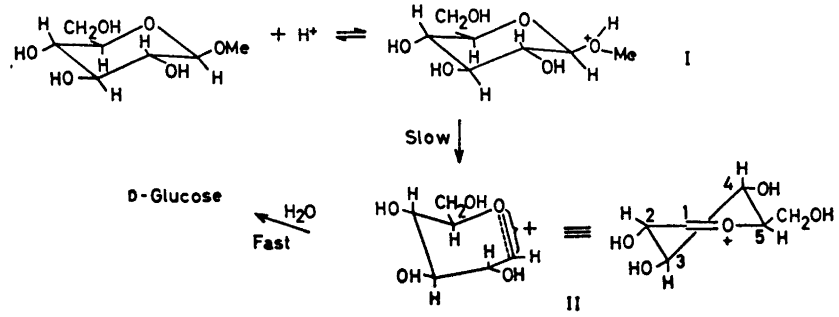

Fig. 1. Accepted mechanism for the acid hydrolysis of the methyl glucopyranosides.

In connection with a discussion of the preferred position of protonation in glycosidic hydrolysis, Lemieux and Morgan [5] subsequently pointed out that the stability of the conjugate acid would be governed by the reverse of the anomeric effect, essentially because, after protonation, the dipole along the C(1)–O(1) bond is in the opposite direction. This implies that the β-anomer, after protonation at the glycosidic oxygen atom, would be more stable than

the α-anomer, protonated in the same position. Expressed in other terms, the β-anomer is the stronger base, and the concentration of its conjugate acid at equilibrium is therefore higher.

There is no evidence that Lemieux and Morgan [5] regarded these statements as amounting to a negation of Edward's hypothesis in its original form, but their acceptance by others has led to the assumption that the β-anomer is hydrolysed faster, simply because it is the stronger base.[6] This is wrong, because the rate of the reaction is determined not only by the concentration of the conjugate acid at equilibrium, but also by the free energy of activation of the heterolysis step.

The theory of the transition state provides that the rate of reaction is proportional to the concentration of molecules in that state. This can be expressed in terms of the concentration of the conjugate acid at equilibrium, together with the free energy of activation of the heterolysis, but it can also be expressed in terms of the activities of the reactants, the activity coefficient of the conjugate acid, and the total change in free energy incurred in passing from the unprotonated glycoside to the transition state. This last quantity is the sum of the change in free energy upon protonation and the free energy of activation of the heterolysis.

Thus, we have: $G + H^+ \rightleftharpoons GH^+$; $GH^+ \rightarrow$ Products.

Whence:

$$K = \frac{\{GH^+\}}{\{G\}\{H^+\}} \times \frac{f_{GH^+}}{f_G f_{H^+}} \tag{1}$$

and

$$\text{Rate} = kC^{\ddagger} = k'\{GH^+\} \tag{2}$$

Hence:

$$\text{Rate} = k'K\{G\}\{H^+\}(f_G f_{H^+}/f_{GH^+}) \tag{3}$$

But $k' = (\varkappa kT/h)e^{-\Delta F^{\ddagger}/RT}$ and $K = K_0 e^{-\Delta F/RT}$, from which it is seen that the product, $k'K$ in eqn. (3) contains the relevant energetic term, $\exp[-(\Delta F^{\ddagger} + \Delta F)/RT]$.

The ratio of the rates of hydrolysis of the two anomers is then given by:

$$\frac{K_\beta}{K_\alpha} = \frac{(f_G)_\beta (f_{GH^+})_\alpha}{(f_G)_\alpha (f_{GH^+})_\beta} \times \exp[(\Delta F_\alpha^{\ddagger} - \Delta F_\beta^{\ddagger} + \Delta F_\alpha - \Delta F_\beta)/RT] \tag{4}$$

It is, therefore, a valid hypothesis to suggest that the β-anomer is hydrolysed faster because, in its unprotonated state, it is less stable than the unprotonated α-anomer. It rests, of course, upon the stated assumption that the transition state is identical for both anomers, and it also implies the assumption that the term containing the activity coefficients in eqn. (4) is unity. For hydrolysis in dilute mineral acids, this last assumption is plausible, but it is not known to be true, and it is certainly not known to be true in strong mineral acids. An attempt was therefore made in the present work to show whether or not it is true.

It is not a valid alternative to the hypothesis of Edward to suggest that the β-anomer is the stronger base without making any statement about the

free energy of activation. This applies, regardless of whether the reason for the greater basicity is that advanced by Lemieux and Morgan,[5] or whether, as others[1] have suggested, the glycosidic oxygen atom of the β-anomer is more easily protonated because it is "more accessible".

Alternatives to Edward's hypothesis. It must be explained at this point that it is not our purpose to question, or seek further evidence for, the existence of the anomeric effect.[3,4] We shall investigate the effect of various anions upon the relative rates of hydrolysis of the two glucosides, and in order to understand these effects, it will be necessary to know, in every instance, whether the observed difference in rates is due solely to the anomeric effect, or to what extent it is due also to other effects.

It must be recognised that Edward's hypothesis requires not merely that the β-anomer be less stable than expected on the basis of van der Waals forces alone, or the α-anomer more stable than otherwise expected, but that the β-anomer be less stable than the α-anomer in the absolute sense, even in dilute, aqueous mineral acid. Water is known to have a strong capacity to quench the anomeric effect, apparently because it is able to form hydrogen bonds with the ring and glycosidic oxygen atoms.[7,8]

We may begin by questioning Edward's first assumption, namely, that the transition state is identical for both anomers. Assuming that the mechanism is A-1 in both instances, it is difficult to see how it could not be. There can be no doubt that the half-chair form of the carbonium ion represents an energy maximum in passing from conjugate acid to products. It is significant, however, that the most definitive mechanistic studies favouring the A-1 mechanism have been carried out on α-anomers.[9,10,11] If the possibility that the β-anomer is hydrolysed, even partly, by a bimolecular mechanism is admitted, the situation changes. Moreover, there is a serious reason for believing that it may do so.

This is because of the Hassel-Ottar effect, a hypothesis originally advanced to explain why, in the hydrohalogenolysis of penta-*O*-acetyl-hexopyranosides, the α-glycopyranosyl halide is formed to the almost complete exclusion of the corresponding β-anomer.[12] This reaction is thought to proceed by an S_N2 mechanism, entailing Walden inversion at the anomeric centre. Both anomeric forms of the penta-*O*-acetate react, but since the only product is the α-halide, the α-acetate must first anomerise to the β-acetate before reaction can occur. Expressed in other terms, the β-anomer is again the more reactive, but in this case overwhelmingly so.

Hassel and Ottar pointed out that, for the α-acetate to react, the attacking halide ion would have to approach the ring on the side which would bring it into a *cis*-configuration with respect to C(6), and that there would be a particularly strong steric interaction with this atom in the transition state, because of the relative shortness of the bonds adjoining C(5), O(5) and C(1). In fact, they went further, and suggested that, in order to make room for the acetoxyl ion to leave, the ring would temporarily have to convert into the alternative chair-conformation, thus making the interaction a *syn*-diaxial one. On the other hand, there would be no such interaction in the conversion of the β-acetate into the α-halide.

The corollary of ring-conversion would appear to be a necessary one, because, in the case of glucose, for example, conversion of the β-acetate into the α-halide would, in the transition state, entail a strong interaction between the entering halide ion and O(2). This can be seen in Fig. 1.

The existence of the Hassel-Ottar effect, which is now generally regarded as referring to the especially strong syn-diaxial interaction between substituents at C(1) and C(5), is, of course, just as indisputable as the existence of the anomeric effect. It is interesting, however, that this particular reaction, which Hassel and Ottar sought to explain in terms of such an interaction, has a rival explanation. Thus, the proposed mechanism implies kinetic control, but if it is assumed that anomerisation of acetylglycopyranosyl halides can occur rapidly under the conditions of the reaction, the reaction will be equilibrium-controlled, and the high yield of the α-halide would then be a direct result of the anomeric effect.

Returning to the question of glycosidic hydrolysis, it is evident that the molecularity of the hydrolysis of the β-anomer must be examined very carefully. The attacking species could be either the anion of the mineral acid used for hydrolysis, or it could be a water molecule, in which case the mechanism would be an A-2 one.

Turning to the second assumption implied by Edward's hypothesis, namely, that the term containing the activity coefficients in eqn. 4 is unity, we come to a consideration of primary salt effects. Even if there were no difference between the free energies of the two glycosides in the unprotonated state, the β-anomer would still be hydrolysed faster than the α-anomer if its activity coefficient were higher than that of the α-anomer, or if the activity coefficient of its conjugate acid were lower than that of the conjugate acid of the α-anomer. It is already known [1] that the activation energy for hydrolysis of the β-anomer is lower than that of the α-anomer, so it is certain that the facts cannot be explained solely in terms of salt effects. It will still be necessary, however, to ascertain to what extent any new results are due to salt effects.

Finally, brief comment must be made regarding the possibility that the effects to be described are spurious, that is, an artefact of the polarimetric method of analysis. This could come about if anomerisation occurred as a reaction competitive with hydrolysis. Such anomerisation could occur either through ring-scission between O(5) and C(1), or as a result of a cave effect, that is, recombination of the carbonium ion (II in Fig. 1) with the molecule of methanol before it has escaped from the site of reaction. Against this may be cited the following facts:

(*a*) The position of the equilibrium between the α- and β-glycosides would certainly be different from that between the α- and β-anomers of free glucose.[3,4] Therefore, the kinetics indicated by the polarimetric method would not be of the first order.

(*b*) The kinetics of the acid-hydrolysis of simple glycosides and disaccharides, measured both polarimetrically and by chemical methods, have invariably been found to be of the first order, with good agreement between the two methods. This holds true, even for hydrolysis in concentrated mineral acids (see, for example, results given for cellobiose and maltose in 18 N sulphuric acid by Freudenberg *et al.*[13]). Clearly, if anomerisation occurred, neither method

would indicate first-order kinetics, and nor would they agree.

As a corollary to these well-known facts, it may be pointed out that they also exclude the "acyclic" mechanism of hydrolysis that has been discussed [1] as an alternative to the "cyclic" one. If the "acyclic" mechanism operated, anomerisation would be expected to take place as a reaction competitive with hydrolysis, and it could be expected to be at least as fast, if not more so.*

Criteria of molecularity. To determine whether or not the observed results can be attributed in any degree at all to the Hassel-Ottar effect, it is necessary to have a sensitive criterion of molecularity. If the assumptions of the Zucker-Hammett hypothesis [15] held true, a plot of the logarithm of the first-order rate-coefficient against $-H_0$ would give a straight line of unit slope for an A-1 mechanism. For an A-2 mechanism, a curve would be obtained whose slope decreased as the numerical value of $-H_0$ increased. This would happen, of course, because the activity of the water molecules decreases with increasing values of $-H_0$ (increasing concentration of acid). If, on the other hand, bimolecularity resulted because of nucleophilic attack by the anion of the acid, a curve would be expected whose slope increased with increasing values of $-H_0$.

In practice, it has been found that, for glycosides and other acetals in strong mineral acids, the plots are usually linear, but not of unit slope. [15] This has led some authors [16] to describe the criterion as "useless". There are, however, a number of additional points to be considered before reaching such a conclusion.

The first of these was raised by McIntyre and Long,[17] who pointed out that deviation from unit slope would be expected if either the activity coefficient of the unprotonated substrate relative to that of the unprotonated base changed with increasing acid-concentration, or if the activity coefficients of the protonated substrate and Hammett base changed relative to one another with increasing acid-concentration.

Thus, starting with the Brønsted equation for the dependence of the first-order rate-coefficient upon changes in the medium:

$$k_h = k_{h0}' C_{H^+}(f_{H^+} f_S / f_{S^+}) \tag{5}$$

in which f_S and f_{S^+} are the activity coefficients of the unprotonated and protonated substrate respectively, McIntyre and Long [17] deduced an expanded expression of the Zucker-Hammett relationship:

$$\log k_h = -H_0 + \log \frac{f_S f_{BH^+}}{f_{S^+} f_B} + \text{const.} \tag{6}$$

in which f_{BH^+} and f_B are the activity coefficients of the protonated and free Hammett base, respectively.

* Reference is made to Lindberg's work [14] on the acetolysis of the anomeric ethyl glucopyranosides. This reaction is definitely known to proceed partly by the "acyclic" mechanism, because a small amount of hepta-*O*-acetyl-glucose is formed. The catalytic species in this reaction is the acetonium ion instead of a proton, and steric hindrance may account for the different mechanism. The rate of anomerisation was extremely high compared to the rate of acetolysis.

These authors then studied the effect of neutral salts upon the activity coefficient of methylal, by measuring its distribution ratio between benzene and the aqueous phase, and also upon the activity coefficient of *p*-nitroaniline, by measurements of solubility.[18] They then studied the hydrolysis of methylal in dilute hydrochloric acid containing various proportions of salt, and found that the correlation between rate and H_0 could be satisfactorily expressed by eqn. (6), on the assumption that f_{BH^+}/f_{S^+} was consistently unity.[18] No attempt was made to measure these last quantities, but the work nevertheless shows that the Hammett criterion of molecularity can still be used if measurements of activity coefficients are also carried out.

Apart from primary salt effects, it is also necessary, in any complete analysis of molecularity, to consider the possibility that changes in the medium may bring about changes in the total free energy of activation ($\Delta F^{\neq} + \Delta F$). This could come about, without implying any change of mechanism, if the possibility is admitted that the substrate may exist in solution as one or more hydrated species as well as an unhydrated molecule. As the concentration of acid increases, and the activity of the water molecules decreases, the relative proportions of these species would be expected to change. Moreover, it is readily seen why both the enthalpy and entropy of activation might be different for these different species, because the conformational change (from chair to half-chair) that takes place on passing through the transition state might well entail decomposition of the hydrate.

For simplicity, we may consider a two-component system of unhydrated glycoside, G, and a hydrate, $G(H_2O)_n$, which will be referred to as GS for purposes of notation. Then, $G + nH_2O \rightleftharpoons G(H_2O)_n$ whence $K_{solv} = a_{GS}/a_G(a_{H_2O})^n = [(1-\alpha)/\alpha(a_{H_2O})^n]f_{GS}/f_G$ where α represents the degree of dissociation of the hydrate.

It follows that $\alpha = [1 + K_{solv}(a_{H_2O})^n f_G/f_{GS}]^{-1}$, and hence:

$$\text{Rate} = k_1\alpha C_{H^+}(f_G f_{H^+}/f_{GH^+}) + k_2(1-\alpha)C_{H^+}(f_{GS}f_{H^+}/f_{GSH^+}) \quad (7)$$

Applying the unmodified assumptions of the Zucker-Hammett hypothesis, we now get:

$$\log\ (\text{Rate}) = -H_0 + \log[k_1\alpha + k_2(1-\alpha)] = \\ = -H_0 + \log\left[\frac{k_1 + k_2 K_{solv}(a_{H_2O})^n f_G/f_{GS}}{1 + K_{solv}(a_{H_2O})^n f_G/f_{GS}}\right] \quad (8)$$

On the assumption that f_G/f_{GS} is independent of the medium, examination of eqn. (8) shows that there are two special cases in which a plot of log (Rate) against $-H_0$ would give a straight line of unit slope. Thus, when $k_1 = 0$ and K_{solv} is large, eqn. (8) simplifies to:

$$\log\ (\text{Rate}) = -H_0 + \log\ k_2 \quad (9)$$

and when $k_2 = 0$ and K_{solv} is small:

$$\log\ (\text{Rate}) = -H_0 + \log\ k_1 \quad (10)$$

Two other special cases of interest arise when $k_1 = 0$ and K_{solv} is small:

$$\log (\text{Rate}) = -H_0 + n \log (a_{H_2O}) + \log k_2 + \text{const.} \quad (11)$$

and when $k_2 = 0$ and K_{solv} is large:

$$\log (\text{Rate}) = -H_0 - n \log (a_{H_2O}) + \log k_1 + \text{const.} \quad (12)$$

Eqns. (11) and (12) are identical with those arrived at empirically by Bunnett,[19–22] n being, in these particular cases, identical with his parameter, w. Bunnett tried to interpret all examples of acid-hydrolysis in terms of these two special cases, and hence in terms of a single parameter, w.

In the general case, all curves described by eqn. (8) are sigmoid in shape, and have unit slopes at both low concentrations of acid ($-H_0$ less than zero) and very high concentrations of acid ($-H_0$ greater than about 3.5 – 5.0, depending on the value of n). In the intermediate range of acid-concentration, which is normally chosen in mechanistic studies, the slopes of the curves are greater than unity when $k_1 > k_2$, and less than unity when $k_1 < k_2$. Two examples are shown in Fig. 2. Bearing in mind that in this figure, rather extreme

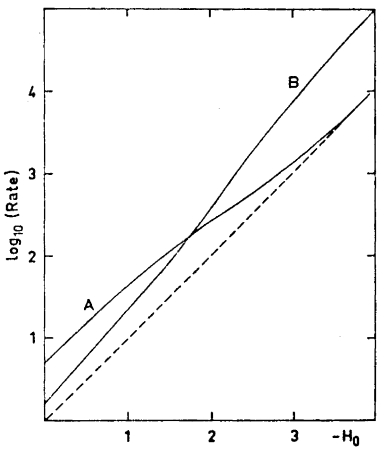

Fig. 2. Theoretical plots of the logarithm of *pseudo*-first-order rate-coefficients against $-H_0$, according to eqn. (8). In curve A, $k_1 = 1$, $k_2 = 10$, and $K_{solv} = 1$, while in curve B, $k_1 = 10$, $k_2 = 1$, and $K_{solv} = 10$. In both curves, $n = 4$, and values for the activity of water are taken from the table compiled by Bunnett.[29]

examples have been chosen for purposes of illustration, and the existence of experimental error, it is readily understood why straight-line plots are usually reported: *it is because they are drawn through points of inflexion in what are really sigmoid curves.*

Given that a study of the type described by McIntyre and Long [17,18] allows possible salt effects to be either discounted or corrected for, it is now necessary to decide how a plot of log (Rate) against $-H_0$ will be helpful in determining the reason for the higher rate of hydrolysis of the β-glucoside. With regard to the possibility of direct participation by the anion of the acid, the criterion is fortunately simple: without participation, the slope will eventually, with increasing values of $-H_0$, return to unity, while with participation, it will increase indefinitely.

As regards our ability to detect the operation of an A-2 mechanism, the situation is impossible. This is because eqns. (7) and (8) refer not only to the hydrolysis, by an A-1 mechanism, of mixtures of free and solvated molecules, but also to the hydrolysis of a single species that reacts partly by an A-1 mechanism, and partly by an A-2 mechanism (or a multimolecular mechanism when $n > 1$). The only difference is that, in the latter case, K_{solv} would refer to the transition state. This proves that it is impossible, from kinetic data alone, to distinquish between a reaction in which water acts as a nucleophile and one in which closely-associated water undergoes some other change of state as the molecule passes through the transition state. Bunnett clearly saw this, in his third paper.[21]

If it is objected that at least the high entropy of activation characteristic of the hydrolysis of glycopyranosides [1] is evidence for an A-1 mechanism, as has been suggested,[23] we must point out that this idea also ignores entropic changes in the water of hydration. Results to be described in this paper will show that the argument is invalid.* Bunnett [21] has previously noted an inverse correlation between $\Delta S^{\ddagger}$ and his parameter, w.

The only operational criterion of an A-2 mechanism is the ability to demonstrate a Walden inversion at the carbon atom attacked, and, since anomerisation is extremely fast in strong acids, there is no possibility of doing this. (The possibility of nucleophilic attack by water on the aglycone is, of course, ruled out by the ^{18}O tracer studies of Bunton *et al.*,[24] and the fact that isomerisation of sugar residues does not occur during the acid-hydrolysis of polysaccharides).

This situation fortunately does not prevent a reasonably definitive answer to our question. If the higher rate of hydrolysis of the β-glucoside is due to a Hassel-Ottar effect associated with an A-2 mechanism, then, even though K_{solv} may be very large, it is at least certain that the *ratio* of the rates of hydrolysis of the β- and α-anomers (K_β/K_α) will not increase as $-H_0$ increases. If it can be additionally shown that K_{solv} is not large, and that K_β/K_α does not *decrease* with increasing acid-concentration, this will confirm that the higher rate of hydrolysis of the β-anomer is not due to an A-2 mechanism, or to the presence of closely-associated water of any kind.

RESULTS

The principal result that prompted this paper is shown in Fig. 3, where K_β/K_α is plotted against molarity for hydrolysis in sulphuric, phosphoric, hydrochloric, and hydrobromic acids. In Fig. 4, the same data are plotted against $-H_0$. The remainder of the work consisted in an attempt to understand (*a*) why K_β/K_α increases with increasing acid-concentration, (*b*) why, for a given value of $-H_0$, the magnitude of the effect is different for the four different acids, and (*c*) why, for a particular glucoside at a given value of $-H_0$, the absolute rate of hydrolysis is different in the four different acids. (This last effect had previously been noted by Timell for the α-glucoside.[25])

* Even a negative entropy of activation, such as is found in the acid-hydrolysis of glycofuranosides,[1] cannot prove an A-2 mechanism, because it is possible for water of hydration to become more highly ordered in the transition state.

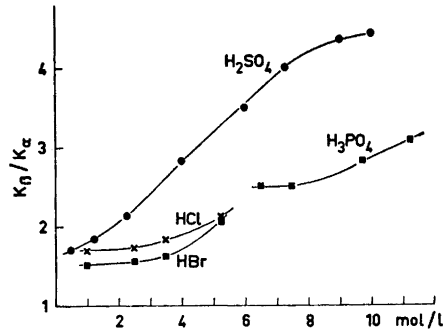

Fig. 3. Dependence upon acid-concentration of the ratio (K_β/K_α) of the rates of hydrolysis of the anomeric methyl glucopyranosides at 68.5°.

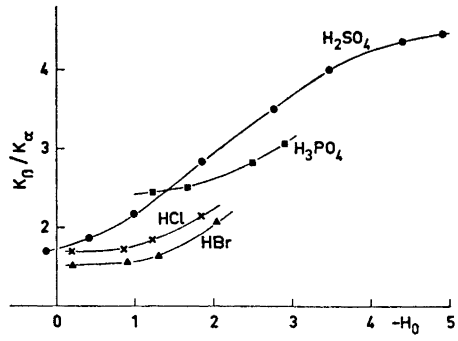

Fig. 4. The data of Fig. 3, re-plotted with $-H_0$ as the measure of acid-concentration.

The following, preliminary conclusions could be drawn: (*i*) Since the activity of water at a given value of $-H_0$ is known to be independent of the nature of the anion,[26,27] the phenomenon is not a simple function in the activity of water alone.

(*ii*) The magnitude of the effect increases in proportion to the affinity of the anion for water, as judged by its position in the Hofmeister lyotropic series, and the heat of dilution of the acid.

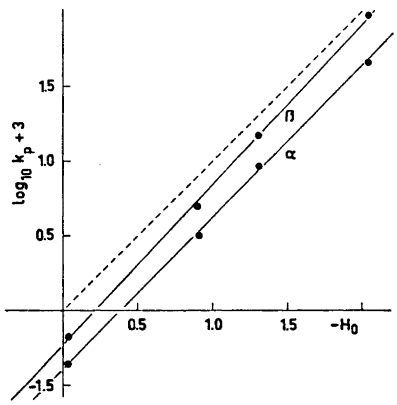

Fig. 5. Hydrolysis of the anomeric methyl glucopyranosides in hydrobromic acid at 68.5°. The *pseudo*-first-order rate-coefficients (k_p) were obtained by use of logarithms to the base 10, and were expressed in min^{-1}. The values of $-H_0$ were taken from published tables [28] and are valid at 25°. The broken line indicates unit slope.

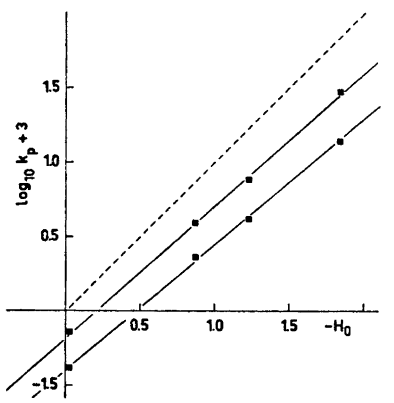

Fig. 6. Hydrolysis of the anomeric methyl glucopyranosides in hydrochloric acid at 68.4°. The values of $-H_0$ were taken from published tables [18] and were corrected, to be valid at 68.4°, from published values of the temperature coefficient of H_0 in this acid.[18]

(*iii*) The bromide ion, which is the strongest nucleophile, has the weakest effect.

Investigation of the possibility that the phenomenon is due to a Hassel-Ottar effect. In Figs. 5 – 8, the logarithms of the *pseudo*-first order rate-coefficients

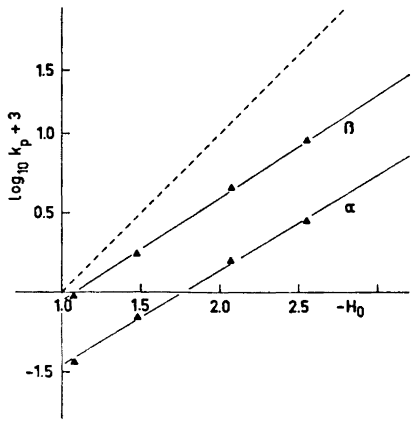

Fig. 7. Hydrolysis of the anomeric methyl glucopyranosides in phosphoric acid at 68.5°. The values of $-H_0$ were corrected [28] to be valid at that temperature.

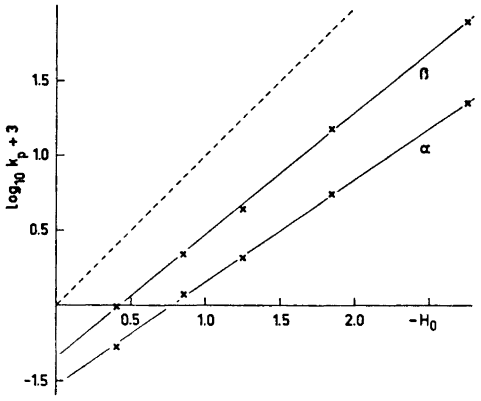

Fig. 8. Hydrolysis of the two anomers in moderately concentrated sulphuric acid at 68.5°. The values of $-H_0$ were corrected for temperature.[28]

are plotted against $-H_0$ for hydrolysis in the four acids at 70°. Straight lines are drawn through the points, because this is uncontroversial, and because an insufficient number of points was obtained to justify any other action. The reader will decide for himself whether or not to believe our claim that the curves are really sigmoid. The following comments must, however, be made:

(A) The sizes of the experimental points give a realistic estimate of the accuracy of the ordinates at about the 2σ level.

(B) The values of H_0 are corrected for temperature from published values [28] for the temperature coefficient of H_0, except in the case of hydrobromic acid, for which the temperature coefficient could not be found. In that case, the plotted values are valid at 25°.

(C) Although they were unbiassed by theoretical considerations, De Bruyne and Wouters-Leysen [16] concluded, from a very detailed study of the hydrolysis of α-methyl-D-glucopyranoside and β-phenyl-D-glucopyranoside in hydrochloric and sulphuric acids that the plots showed slight curvature, the slope decreasing with increasing acid-concentration. They did not investigate acidities higher than about $-H_0 = 2$.

(D) A careful examination of the data of Timell [25] will perhaps suggest that the experimental points could be more easily accommodated in a sigmoid curve than a straight one. In every case, and again in Figs. 6 – 8,* the slope

* But not Fig. 5, which seems to do the opposite. This may be due to uncertainties about the correct values of H_0 at 70°, but our experience suggests that hydrobromic acid is very different also in other ways, and should be studied separately.

initially decreases with increasing acidity, and then increases again. The magnitude of the effect is greatest for sulphuric and phosphoric acids.

Because of the importance of attaching the correct significance to the slopes of these curves, a special study was carried out of hydrolysis in sulphuric acid at very low and also at very high acid-concentrations. This could not be done at the same temperature of 70°, however, because the rate of hydrolysis was then too low to be measured conveniently in weak acid, and too high to be measured accurately in strong acid.

Hydrolysis in weak sulphuric acid was therefore carried out at 80°, 90°, and 100°, and, from the activation energy obtained, the expected rate at 70° was calculated. The results (Fig. 9) show that the slopes are almost parallel,

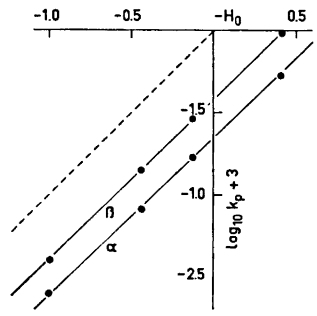

Fig. 9. Hydrolysis of the two anomers in dilute sulphuric acid at 68.5°. The values of k_p for $-H_0 = 0.41$ were measured directly at 68.5°. The others were calculated to be valid for 68.5°, from measurements made at 77.9°, 86.6°, and 95.4°.

and very close to unity. The value of K_β/K_α at infinite dilution is apparently 1.6 as judged by this experiment. (In hydrobromic acid, the ratio initially *decreases* to about 1.5, and then slowly increases again with increasing acid-concentration. A special study of this will be reported elsewhere. In any acid the actual value of K_β/K_α is, of course, temperature-dependent, because the energies of activation are different for the two anomers.[25] The results just reported refer to a temperature of 70°.)

Hydrolysis in very concentrated sulphuric acid (normality greater than 12) was carried out at 30°, 40°, and 50°, and in one case at 40°, 50°, and 60°. At concentrations higher than 15 N, plots of log $a/(a-x)$ against time deviated from strict linearity after degrees of hydrolysis (x/a) of about 50 – 70 %. This is illustrated in Fig. 10, which compares the results obtained at 50° in 15 N and 18 N sulphuric acid. Such effects were accompanied by darkening of the reaction mixture. There is little doubt that they were due to further modification of the liberated glucose, rather than of the glycoside, because the β-anomer was always less affected; this would be expected, since it hydrolyses faster. The initial slope was therefore taken as a correct measure of the rate-coefficient.

The expected rates at 70° were calculated from the activation energies, and the results are shown in Fig. 11. Again, straight lines have been drawn through the points, to avoid theoretical bias. Their slopes are about 0.85 in both cases. It is at least clear that the slope for the α-anomer is now higher,

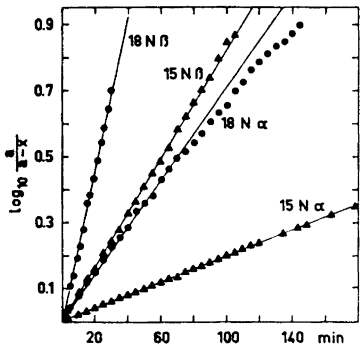

Fig. 10. Illustration of the onset of serious departure from first-order kinetics in very concentrated sulphuric acid. The temperature in all four experiments was 49.3°.

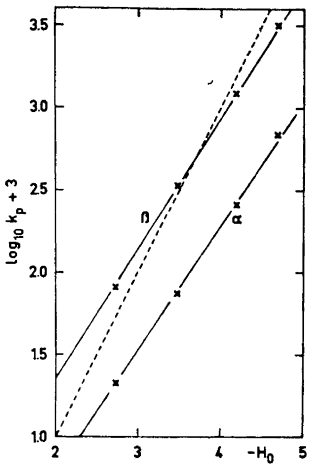

Fig. 11. Hydrolysis of the two anomers in very concentrated sulphuric acid at 68.5°. The values of $-H_0$ were corrected for temperature.[28] The values of k_p for $-H_0 = 2.67$ were measured at the stated temperature. The others were calculated to apply at that temperature from measurements made at 39.6°, 49.3°, and 58.6°.

and that the plots have become almost parallel again. The final value of K_β/K_α at 70° is about 4.6. It is clearly impossible to say whether or not the slopes would eventually increase to unity. With the onset of serious deviation from first-order kinetics, it was not worthwhile to investigate still-higher concentrations of acid.

The entire behaviour in sulphuric acid can be summarised as follows. Even in 0.1 N acid, the β-anomer is hydrolysed faster than the α-anomer. For values of $-H_0$ between -1 (pH = $+1$) and $+0.4$ (corresponding to about 2.5 N acid), plots of log (Rate) against $-H_0$ are linear and of unit slope. In this region, the activity of water suffers only a 10 % decrease.[29]

For values of $-H_0$ between 1 and 3, the plots are approximately linear. The average slope for the β-anomer is 0.82, and that for the α-anomer is 0.68. The activity of water in the same region decreases from about 0.85 to about 0.35. As $-H_0$ increases further up to 5, the slope of the plot for the α-anomer increases markedly, until the two plots become virtually parallel again. The activity of water at $-H_0 = 5$ is about 0.1. The "final" rate of hydrolysis of the β-anomer is about 30 %, and that of the α-anomer is about 10 %, of the rate at which they would have been hydrolysed, had unit slope been maintained throughout.

It will be noted that, throughout the entire range studied, the concentration of the anion has increased by a factor of 200, while the activity of water has decreased to 10 %.

Investigation of primary salt effects. This was undertaken, firstly to determine whether the increase in K_β/K_α with increasing acidity could be due to any change in the term containing the activity coefficients in eqn. (4), and secondly to determine to what extent the deviations from unit slope in Figs. 5 – 8 should be attributed to the effects described by McIntyre and Long.[17,18]

McIntyre and Long found that methylal was, relatively to the Hammett base studied, salted-out by strong acids [17] and neutral salts,[18] and therefore the plot of log (Rate) against $-H_0$ had a slope greater than unity. The methyl glucopyranosides are, compared to methylal, very polar compounds, and it is therefore possible that they may be salted-in relatively to the Hammett base, thus giving slopes less than unity.

The method of investigation was chromatographic. Preliminary experiments showed that thin-layer chromatography on silica gel could be carried out successfully with aqueous mineral acids or even water as the mobile phase. The quality of the separations was generally comparable with that obtainable in conventional solvent-systems. The selection of R_F values shown in Table 1 makes it clear that separation takes place on a basis of the polarity of the compounds, with the silica gel acting as the less-polar phase.

Table 1. Chromatographic mobilities of various carbohydrates in 10 N sulphuric acid. The stationary phase was silica gel, and the mobilities are calculated relative to the solvent front.

Compound	R_F value
Glycolaldehyde	0.81
D-Glucose	0.88
2-Deoxy-D-glucose	0.89
3-*O*-Methyl-D-glucose	0.89
2-Deoxy-D-ribose	0.82
6-*O*-Methyl-D-galactose	0.70
Methyl β-L-arabinopyranoside	0.69
2,3,4,6-Tetra-*O*-methyl-D-glucose	0.34
Methyl 2,3,4,6-Tetra-*O*-methyl-D-glucopyranoside	0.14
Maltose	0.95
Cellobiose	0.96

In agreement with this, it was next found that, when sulphuric, phosphoric, hydrochloric, or hydrobromic acid was chromatographed with water as the mobile phase, the acid was completely excluded from the silica gel, migrating at the solvent front. This implied that, when chromatography of organic compounds is carried out with these acids as the mobile phase, the silica gel would act like an immiscible organic solvent. Partition would then occur between this and a mobile phase whose composition could be varied at will. Any tendency for the acids to salt-in or salt-out one compound relatively to another should then be reflected in a change in the relative chromatographic mobilities of the two compounds.

The results obtained with the two methyl glucopyranosides and a Hammett base (*p*-nitroaniline) are shown in Table 2. Although the absolute mobilities vary a little, as could be expected, the relative mobilities are remarkably insensitive to both the nature of the anion and its concentration.

Table 2. Chromatographic mobilities of α- and β-methyl D-glucopyranoside (α/β-Me-G), *N*-acetyl-D-glucosamine (GNAc), and *p*-nitroaniline (p-NA) in various acids. The stationary phase was silica gel.

Acid	α-Me-G	β-Me-G	GNAc	p-NA
2 N H_2SO_4	0.87	0.88	0.86	0.87
4 N H_2SO_4	0.81	0.82	0.89	0.83
6 N H_2SO_4	0.78	0.80	0.89	0.79
8 N H_2SO_4	0.74	0.78	0.91	0.76
10 N H_2SO_4	0.75	0.79	0.91	0.80
12 N H_2SO_4	0.80	0.82	0.91	0.80
14 N H_2SO_4	0.75	0.77	0.91	0.79
16 N H_2SO_4	0.77	0.78	0.94	0.78
4 M H_3PO_4	0.78	0.81	0.93	0.74
6 M H_3PO_4	0.76	0.78		0.72
8 M H_3PO_4	0.75	0.76	0.90	0.71
11 M H_3PO_4	0.80	0.81		0.76
1 N HBr	0.82	0.82		0.76
3.6 N HBr	0.81	0.82		0.84
5 N HBr	0.83	0.83	0.90	0.81
2.5 N HCl	0.85	0.86		0.84
3.5 N HCl	0.82	0.85		0.84
5 N HCl	0.74	0.75	0.89	0.76
Water	0.83	0.83		0.75

The purpose of the experiment was to find out not only whether the unprotonated glycosides and Hammett base were salted-in or salted-out relatively to one another, but also whether this occurred with their conjugate acids. This was the purpose in varying the concentration of mineral acid in the mobile phase over a wide range. This was undoubtedly successful with regard to the Hammett base, whose yellow colour faded as the concentration of acid in the mobile phase increased.

The glycosides are, however, much weaker bases than *p*-nitroaniline, and it is doubtful whether they would have been sufficiently highly protonated even in the most concentrated acid for their mobilities to be significantly affected by any change in the activity coefficients of their conjugate acids. This is why data for 2-acetamido-2-deoxy-D-glucose are also included in Table 2. The reasoning was as follows.

The behaviour of *p*-nitroaniline was in good agreement with the general experience [30] that protonation of a relatively large organic, basic molecule has little effect upon its activity coefficient. To ascertain whether or not this was also likely to be true for a glycoside, it was necessary to find a molecule that was structurally very similar to a glycoside, but a stronger base. Amides are

well known to be highly protonated in moderately concentrated mineral acids.[31] The chromatographic behaviour of 2-acetamido-2-deoxy-D-glucose (Table 2) therefore indicates that any change in the activity coefficients of the protonated glycosides, relatively to one another and to *p*-nitroaniline, with increasing acid-concentration, is very unlikely.

Dependence of activation parameters upon acid-concentration. Table 3 gives the energies and entropies of activation for hydrolysis of both anomers in sulphuric acid of different concentrations. The results given for hydrolysis in 2.5 N and 12.0 N acid are based upon measurements of the rate of hydrolysis at five different temperatures, while the others are based upon measurements at three different temperatures. The limits of error were estimated by drawing the two "worst possible" straight lines through the plots of log (Rate) against $1/T$, and measuring the deviation of their slopes from that of the best straight line.

Table 3. Activation parameters for hydrolysis of α- and β-methyl D-glucopyranoside in sulphuric acid at 70°. The possible error in the activation energies (E_α and E_β) is consistently about ± 0.5 kcal mol^{-1}, while that in the entropies of activation ($\Delta S_\alpha^{\ddagger}$ and $\Delta S_\beta^{\ddagger}$) is about ± 1 e.u. The "theoretical" values of $E_\alpha - E_\beta$ are calculated from the ratio (K_β/K_α) of the rates of hydrolysis of the two glucosides on the assumption that the difference in the rates of hydrolysis is due solely to differences in the energy (and hence enthalpy) of activation.

Normality	K_β/K_α	E_α	E_β	$\Delta S_\alpha^{\ddagger}$	$\Delta S_\beta^{\ddagger}$	$E_\alpha - E_\beta$ Found	$E_\alpha - E_\beta$ Calc.
0.100	1.60	35.6	35.3	19.9	20.0	0.3	0.32
0.500	1.65	35.8	35.4	20.3	20.3	0.4	0.34
1.00	1.70	34.8	34.5	17.4	17.6	0.3	0.36
2.50	1.84	34.6	34.2	16.7	16.7	0.4	0.41
12.00	3.50	32.5	31.7	7.7	7.8	0.8	0.85
15.00	4.00	32.3	31.4	5.9	6.3	0.9	0.94
18.00	4.35	32.0	31.0	4.2	4.4	1.0	1.00
20.00	4.42	31.8	30.7	3.3	3.1	1.1	1.01

It is at least clear that, as the concentration of acid increases, the entropy of activation goes down for both anomers, the energy of activation goes down for both anomers, and the energy of activation of the β-anomer goes down significantly more than that of the α-anomer.

If the assumption is now permitted, that the estimated limits of error correspond to about 3σ, and that the probable error is about two-ninths of this, a further conclusion is strongly indicated. This is that, *at any acid-concentration, the higher rate of hydrolysis of the β-anomer is due entirely to its lower activation energy*. In other words, the phenomenon is entirely enthalpic.*

* It may be noted that, for small substituents attached to a fairly rigid pyranoid ring, the difference in free energy between two configurational isomers would be expected to be almost entirely enthalpic in nature.

Accepting this, and that salt effects are unimportant, eqn. (4) can be re-written:

$$K_\beta/K_\alpha = \exp[(E_\alpha - E_\beta)/RT] \quad (13)$$

From the experimental values for K_β/K_α at 70° (Figs. 3 and 4), expected values of $E_\alpha - E_\beta$ were calculated from this equation, and are included in Table 3 for comparison with the experimental values.

DISCUSSION

The possibility that the higher rate of hydrolysis of the β-anomer is due to direct nucleophilic attack by the anion of the acid on the anomeric centre, and to a consequent Hassel-Ottar effect, is discounted for the following reasons:

(*a*) Even in 0.1 N acid, the β-anomer is hydrolysed faster, and the value of K_β/K_α does not change significantly as the normality of the acid increases by a factor of 10 to 1 N (Fig. 9).

(*b*) The value of K_β/K_α tends to become constant again as the normality of the sulphuric acid increases from 15 to 20 (Figs. 3 and 11). In this region, the slope of the plot of log (Rate) against $-H_0$ increases more for the α-anomer than for the β-anomer (Figs. 8 and 11).

(*c*) The bromide ion, which is the strongest nucleophile, has the weakest effect. Both sulphuric and phosphoric acid are relatively weak nucleophiles.

(*d*) If the sulphate or bisulphate ion selectively attacked the β-anomer as a nucleophile, it is not very likely that the entropies of activation for the two anomers would be consistently the same at all concentrations of acid (Table 3).

The possibility that the higher rate of hydrolysis of the β-anomer is due to a primary salt effect is discounted for the following reasons:

(*a*) A close examination of the R_F values in Table 2 suggests that there is a slight, but probably significant, tendency for the α-anomer to be salted out, relatively to the β-anomer and the Hammett base, by sulphuric and phosphoric acid. On this basis, one would expect the α-anomer to be hydrolysed slightly faster than the β-anomer.

(*b*) On the basis of a fairly reasonable assumption about experimental accuracy, the data in Table 3 indicate that the higher rate of hydrolysis of the β-anomer is due largely, if not completely, to its lower energy of activation.

The increase in K_β/K_α with increasing acidity cannot be attributed to a Hassel-Ottar effect arising from an A-2 mechanism, because on this basis, K_β/K_α would have to be at a maximum when the activity of water is maximal, that is, in dilute acid. In the case of hydrobromic acid, the plot of log (Rate) against $-H_0$ is very close to unity, showing that K_{solv} in eqn. (8) is very large for this acid. In this particular acid, therefore, it would be possible for an A-2 mechanism to operate throughout the range of acidity studied. In the other acids, however, the plots are sigmoid, and for these it is certain that any contribution that an A-2 mechanism may make to the total rate of hydrolysis would have to diminish with increasing acidity. Any associated Hassel-Ottar effect would therefore also decrease with increasing acidity, and in sulphuric

acid, it would be expected to disappear entirely as $-H_0$ increases above 3 (Fig. 11).

The possibility that the higher rate of hydrolysis of the β-anomer in *dilute* acid may be due *partly* to a Hassel-Ottar effect, associated with an A-2 mechanism, cannot be completely excluded, but two facts oppose it:

(i) If, in dilute acid, the β-anomer were *selectively* hydrolysed by an A-2 mechanism, because of the Hassel-Ottar effect, it would be surprising to find that the entropies of activation were the same for the two anomers, as is the case. If there is any evidence against the A-2 mechanism, it is perhaps this lack of *selectivity* in the entropies of activation, rather than their absolute magnitudes.

(ii) As has been briefly mentioned, and will be reported in detail elsewhere, the value of K_β/K_α in hydrobromic acid initially goes *down* with increasing acid-concentration. In terms of the anomeric effect, this can be explained by assuming that low concentrations of bromide ion increase the activity of water, but it is impossible to explain on the assumption that a Hassel-Ottar effect is operating.

Finally, before attributing the entire phenomenon to the anomeric effect alone, it is necessary to consider whether accepted values for the magnitude of the anomeric effect are able to account for it. Angyal [4] has estimated that, on the basis of van der Waals interactions alone, the β-anomer should be *more* stable than the α-anomer by about 0.9 kcal mol^{-1}. In order to explain the present results, for hydrolysis in sulphuric acid at 70°, entirely in terms of the anomeric effect, it would therefore be necessary to assume that it has a magnitude of about 1.2 kcal mol^{-1} in dilute acid, and about 1.9 kcal mol^{-1} in 20 N acid.

It is, of course, known [32] that, for pyranosyl halides in inert solvents, the anomeric effect can have a magnitude of 3.2 kcal mol^{-1} or more, but this is not very helpful. A more realistic picture is given by the following facts:[3,4]

(a) The optical rotation, at equilibrium, of glucose in water indicates that the anomeric affect has a value of 0.55 kcal mol^{-1} while in anhydrous methanol it has a value of 0.9 kcal mol^{-1}.

(b) It is well known that the magnitude of the anomeric effect is higher for glycosides than for reducing sugars.

(c) An indication of how much higher it may be is given by the fact [3] that a solution of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranose in methanolic hydrogen chloride anomerises, and gives, at equilibrium, the α- and β-anomers in a ratio of about 3:1. Depending slightly on the temperature, this indicates a value for the anomeric affect of about 1.6 kcal mol^{-1}.

If, therefore, the anomeric effect in methanol is 0.7 kcal mol^{-1} higher for the glycoside than for the reducing sugar, it is a reasonable speculation that a similar increment will apply in water. Accepting this, the expected value for the glycoside in water would be $0.55+0.7=1.25$ kcal mol^{-1}, as was found.

It is now possible to suggest answers to the three questions asked at the beginning of the section on results:

(a) The value of K_β/K_α increases with increasing acidity because the magnitude of the anomeric effect increases as the activity of water in the system decreases, leading to desolvation of the glycosides.

(b) For a given value of H_0, that is to say, for a given activity of water, the magnitude of the effect is different for different anions because the anions differ in their capacity to desolvate the glycosides. This must be explained further. The quantity, K_{solv}, in eqn. (8) will be a constant, independent of the medium, only if the activity coefficients, f_G and f_{GS} of the free and solvated glycoside are measured, with a dilute solution in pure water as the reference state. It is, however, impossible to do this, and therefore it is operationally meaningless to keep f_G/f_{GS} as a term, separate from K_{solv}. It is therefore better to set f_G/f_{GS} equal to a constant, or to unity, and to regard K_{solv} as an operational parameter, dependent upon the medium. It is not yet known whether K_{solv} is dependent upon the concentration of the anion as well as its identity, but the ability of eqn. (8) to explain the experimental results suggests that it may be nearly independent of concentration.

(c) For a given glycoside, at a given value of H_0, the absolute rate of hydrolysis is different in the presence of different anions because the glycoside exists in solution as a mixture of solvated and desolvated species, which are hydrolysed at different rates. The relative amounts of the solvated and desolvated species are different in the presence of different anions, for the reason just discussed. In the case of the α-anomer, the solvated species is hydrolysed about 10 times faster than the desolvated species, while in the case of the β-anomer, it is hydrolysed 3 times faster. The solvated species is hydrolysed faster than the desolvated species because its higher enthalpy of activation is more than offset by the very favourable entropic change associated with the release of solvent in the transition state.

It is not known, of course, that desolvation of the glycosides is complete in 20 N sulphuric acid, when K_β/K_α has again become constant, but it would certainly appear that those water molecules that diminish the magnitude of the anomeric effect, by forming hydrogen bonds with the ring and glycosidic oxygen atoms, have been removed in acid of that strength. It is possible that other water molecules, more firmly bound than these, are still present, and that an expanded form of eqn. (8), including more than one solvation constant, would give a better fit with the experimental data. In particular, this would explain why the slopes of the plots of log (Rate) againts $-H_0$ in Fig. 11 have not returned to unity, even though they are almost parallel. To judge from the value of n that would make the term $(a_{H_2O})^n$ in eqn. (8) vanishingly small for values of $-H_0$ between 4 and 5, it would appear that four, or possibly three, molecules of water are removed from the glycosides relatively easily, while a smaller number, if present, may be removed with greater difficulty.

It must be recognised that, in dilute, aqueous solutions, all these water molecules of solvation are in a very different state from those of the ambient solvent. Their entropy is much lower, which points to the existence of a fairly well-defined, ordered structure. This structure stabilises the chair form of the pyranoid ring, relative to the half-chair form of the transition state, to the extent of about 3 kcal per mol of glycoside. Exactly how it does this, is not known, but effectively, water can be regarded as increasing the conformational size of the oxygen atoms attached to the pyranoid ring.

Concentrated solutions of mineral acids do not exist in Nature, but sulphated polysaccharides are ubiquitous in marine plants and animals, while

phosphorylated sugars are important in intermediary metabolism, teichoic acids and nucleic acids. In the immediate vicinity of a sulphate half-ester group, or a phosphate mono- or di-ester group, the activity of water must be very low. The analogy with moderately concentrated solutions of sulphuric and phosphoric acids is perhaps quite good, because the principal anions in these solutions are the bisulphate and the dihydrogen phosphate ions, respectively.

The analogy breaks down, of course, insofar as the only cations in the present systems were hydrogen ions. A logical extension of the work would therefore be to introduce metallic and organic cations. Previous work in this laboratory by Haug and Smidsrød [33,34] on the salting-out and salting-in of anionic and neutral polysaccharides has demonstrated a remarkable sensitivity to both the nature of the anionic group and the identity of the cation. These solubility phenomena may or may not, in some cases, be associated with changes in the conformation of the pyranoid ring, but it is certain, in every case, that they represent profound changes in the state of the solvent molecules in the vicinity of the chains, and the purpose of future papers in this series will be to explore all the consequences of this.

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