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Influence of Cosolutes upon the Conformation of Carbohydrates in Aqueous Solutions. II. Demonstration of the Anomeric Effect in Cellobiose and Maltose, and Proposal of a Mechanism for the Influence of Inorganic Ions upon its Magnitude

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In sulphuric acid at 70° and 40°, the ratio (K_β/K_α) of the rates of hydrolysis of cellobiose and maltose changed very little as the concentration of acid was increased up to 10 N, but then it increased by a factor of 2 as the acid-concentration was further increased to 16 N.

In hydrobromic acid at 40°, 50°, and 60°, increasing concentration of acid caused K_β/K_α first to decrease to about half its value in dilute acid, and then slightly to increase again. For a given concentration of hydrobromic acid, K_β/K_α first decreased, and then increased again, as the temperature was raised from 40° to 70°.

Zucker-Hammett and Arrhenius plots of the data, together with the results of experiments on the salting-in and salting-out of methyl cellulose, indicated that the changes in K_β/K_α were associated with changes in the degree of hydration of the disaccharides, dehydration causing it to increase, and enhanced hydration causing it to decrease.

The behaviour throughout was qualitatively similar to that of the methyl D-glucopyranosides. From this and earlier evidence, it is concluded that the changes in K_β/K_α represent changes in the magnitude of the anomeric effect, resulting from changes in the extent to which the dipole interaction between the ring- and glycosidic-oxygen atoms is quenched by hydrogen-bonding to water molecules. A lack of strict parallelism with the behaviour of the methyl glucopyranosides suggests, however, that at least in cellobiose, it is partly quenched also by intramolecular hydrogen bonding.

An attempt is made to interpret the phenomena in terms of the zonal model of Frank and Wen for the structure of water around an ion. It is suggested that anions, generally, orientate water molecules so that they are unable to form hydrogen bonds with the ring- and glycosidic-oxygen atoms, while cations, including the hydrogen ion, orientate them in such a way as to enhance such hydrogen-bonding. It would then follow that the behaviour in sulphuric acid is dominated by the anion throughout, while in hydrobromic acid, the net effect of the two ions is a sensitive function in concentration and temperature.

In Part I of this series,[1] it was shown that the ratio (K_β/K_α) of the rates of hydrolysis of β- and α-methyl D-glucopyranoside in sulphuric, phosphoric, hydrochloric, and hydrobromic acids at 70° depended upon both the concentration of acid and the identity of the anion. In all four acids at this temperature, it increased with increasing concentration of acid, but, 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.

Chromatography of the glucosides on silica gel, with the acids as mobile phases, showed that there was no significant salting-in or salting-out of one anomer relatively to the other, so that the phenomenon could not be attributed to primary salt effects. Similarly, studies of the dependence of the rates of hydrolysis upon the Hammett acidity function (H_0) showed that the anions were not participating directly in the reaction as nucleophiles. From this, and studies of the dependence of the activation parameters upon the concentration of acid, it was concluded that the phenomenon was caused by the anomeric effect, which would be expected to increase as the activity of water in the system decreased, leading to desolvation of the glycosides.[1]

The activity of water is a macroscopic quantity, and, for a given value of H_0, it is the same for all acids.[2] The magnitude of K_β/K_α, and, hence, the anomeric effect cannot therefore be a simple function in the activity of water alone. The present extension of the earlier work was undertaken in an attempt to understand, at the molecular level, why different anions differ in their capacity to desolvate the glycosides. Any such attempt must necessarily make use of existing knowledge about the influence of ions upon the structure of water, which is very incomplete. The conclusions will therefore have the status of a working hypothesis, which may require modification in the light of new evidence.

In the earlier paper,[1] brief mention was made of some unusual effects which had been noted in working with hydrobromic acid. This therefore seemed to provide a good starting-point for further work, especially since more is known about the effect of halide ions upon water than about other anions.

A second objective in the present work was to make the systems a little more closely analogous to the biological situation. To this end, temperatures lower than 70° have been investigated, and two new substrates, cellobiose and maltose, have been introduced, since these are better models for polysaccharides than the methyl glucosides.

THEORY

This section gives a documented summary of the principal theoretical concepts that will be used in discussing the results.

The anomeric effect as a measure of solvation. The chair form of an *O*-pyranoside in which O(1) is axial is more stable, and the one in which it is equatorial is less stable, than in the corresponding cyclohexane analogues.[3–5] This phenomenon is described as *the anomeric effect*. It arises because of a repulsive, dipole interaction between the ring- and glycosidic oxygen atoms

in the pyranoid compounds, which is stronger in the equatorial anomer than in the axial one.[3–5]

The *magnitude* of the anomeric effect has been defined [6–9] as the measured difference in free energy, $\Delta G^{\circ}_{\alpha,\beta}$, between an anomeric pair of glycosides, minus that, ΔG°_{s}, expected for the corresponding derivatives of cyclohexane, due attention being paid to the sign of the free-energy change in each case.

It has previously been determined mainly by measurement of the position of the equilibrium in anomerisation reactions.[3,4,6,9] For certain sugar derivatives and simpler derivatives of tetrahydropyran, whose free energies in the *C*-1 and 1-*C* conformations are not widely different, it has also been determined by using NMR spectroscopy to observe directly the relative amounts of the two conformers present at equilibrium.[7,8,10–14] In Part I of this series, it was measured by a kinetic method.[1]

It has long been known that the magnitude of the anomeric effect is dependent upon the solvent, and that any polar solvent has a capacity to diminish it.[3,9] Recently, however, Lemieux and his co-workers [12–14] have shown that the capacity of a solvent to quench the anomeric effect is not a simple function in its dielectric constant. Protic solvents, which are able to solvate the ring- and glycosidic-oxygen atoms, specifically by forming hydrogen bonds with them, have a much greater capacity to quench the anomeric effect than aprotic solvents with a similar dielectric constant. Water is an extreme example of such a solvent.[12–14]

It is obvious that water can solvate the hydroxyl groups of a glycoside in the same way, that is to say, by contributing a proton to form a hydrogen bond with the oxygen atom. These hydroxyl groups can, however, also form hydrogen bonds with water by themselves contributing their proton to the bond. The anomeric effect, as it has been defined,[6–9] does not measure this kind of solvation. This provides a possible basis for understanding why the magnitude of the anomeric effect need not necessarily be a simple function in the activity of water alone.

The ratio (K_{β}/K_{α}) *as a measure of the magnitude of the anomeric effect.* The proposition that the ratio of the rates of acid-hydrolysis of an anomeric pair of glycopyranosides under the same conditions is a simple function in the magnitude of the anomeric effect rests upon the validity of Edward's hypothesis.[15] It assumes that the transition state is common to both anomers, and that the difference in their rates of hydrolysis is therefore due solely to the difference in their free energies, $\Delta G^{\circ}_{\alpha,\beta}$, in the ground state. This idea has been discussed in detail, and justified in Part I.[1]

This difference in free energy will be a function in the magnitude of the anomeric effect, together with a steric contribution, ΔG°_{s}. If it can be assumed that ΔG°_{s} is independent of the medium, then K_{β}/K_{α} will be a *unique* function in the magnitude of the anomeric effect, insofar as this will be the only solvent-dependent variable.

This assumption must, however, be examined very critically. So far as carbon atoms and methine hydrogen atoms are concerned, tabulated values [16] for the conformational energies of alkyl groups do provide reasonable assurance that their van der Waals radii are virtually independent of the solvent. For hydroxyl groups, however, values ranging from 0.29 to 1.25 kcal mol^{-1} are

reported.[16] These seem to be more dependent upon the method of measurement than the solvent, but nevertheless, there is no overt reason for believing that the conformational size of an oxygen atom should be independent of whether or not it is hydrogen-bonded to the solvent.

In considering the possible solvent-dependence of ΔG°_{s}, it is convenient to divide it into two parts, (A) and (B). Part (A) represents the contribution from non-bonded interactions between O(1) and the rest of the glycose ring, which are present in the axial anomer but absent in the equatorial one, while part (B) results from interactions between the glycose ring and the aglycone.

For the glucopyranose ring, the significant interactions of type (A) are, importantly, all between O(1) and C(3), C(5), H(3), and H(5), since the *gauche* interaction with O(2) is common to both anomers. This means that any dependence of this part of the steric factor upon the medium will still be a unique function in the solvation of the glycosidic oxygen atom, and will be operationally indistinguishable from the anomeric effect itself.

With regard to interactions of type (B), consideration of models of α- and β-methyl D-glucopyranoside shows that the only interactions between the methyl group and the glycose ring that are not common to both anomers occur in the *A*-3 rotameric form [5] of the α-anomer, and again, these are with C(3), C(5), H(3), and H(5). No oxygen atom is involved which is not common to both anomers.

These arguments indicate that, whereas the measurement of K_{β}/K_{α} may possibly not give correct values for the absolute magnitude of the anomeric effect, it does, at least for the methyl glucopyranosides, provide a valid method for studying its dependence upon the concentration and identity of different anions.

The anomeric effect in disaccharides. The value of K_{β}/K_{α} for the glucopyranosides of all primary alcohols is, for hydrolysis in dilute acid, consistently between about 1.6 and 2.4, indicating that only the alcoholic methylene group interacts significantly with the glycose ring.[17,18] For glucopyranosides of secondary aliphatic alcohols, it is slightly lower,[17] while for α- and β-phenyl D-glucopyranoside, the familiar ratio is more than reversed,[15,19] the α-anomer being hydrolysed, in 2 N hydrochloric acid at 60°, about 4 times faster than the β-anomer.[19] Edward attributed this to a particularly strong steric interaction between the phenyl group and positions 3 and 5 in the α-anomer, pointing out that the $-O-Ph$ system would tend to be planar because of the partial, double-bond character of the $O(1)-Ph$ bond.[15] Overend *et al.*[19] subsequently found that the entropy of activation was higher for the α-anomer than for the β-anomer, so that highly restricted rotation about the $C(1)-O(1)$ bond is more likely to account for its higher free energy in the ground state.

For disaccharides, the only evidence obtained so far for the existence of the anomeric effect has been with the 1,6′-linked anomeric pair, gentiobiose and isomaltose, which exhibit the usual ratio characteristic for the glucopyranosides of primary alcohols.[20] For the other anomeric pairs of glucobioses, namely, cellobiose and maltose, laminaribiose and nigerose, and sophorose and kojibiose, K_{β}/K_{α} in dilute acid is in the range $0.3-0.7$, and the reason is unknown.[20] BeMiller [18] suggests that, as in the case of the phenyl

glucopyranosides, the steric factor for the α-anomer is simply larger than it is for the glucopyranosides of primary aliphatic alcohols.

There is, however, a serious reason for questioning whether the anomeric effect exists in these disaccharides at all. This is because of the possibility of intramolecular hydrogen-bonding between the ring- and glycosidic-oxygen atoms in the glycose moiety, and hydroxyl groups in the aglycone. Thus, the disaccharides may contain their own "solvent", which could partly replace the hydration which can be brought about by water.*

For example, the hydroxyl group at C(6) of the reducing unit could form a hydrogen bond with the glycosidic oxygen atom. This can be seen, not only from models, but also from the known fact that *pseudo*-cellobiouronic acid and polyuronides are hydrolysed, between pH 2 and pH 4, partly by intramolecular, general-acid catalysis.[21,22] In this reaction, the unionised carboxyl group at C(6) of the aglycone donates a proton directly to the glycosidic oxygen atom attached to it at C(4). It is obvious that an intramolecular hydrogen bond must be formed as an intermediate in this step.

The ring-oxygen atoms in the non-reducing glucose residues could also form intramolecular hydrogen bonds in these disaccharides. In cellobiose, the hydroxyl group at C(3) of the reducing unit would be ideally situated to act as the hydrogen-donor, and indeed, such a hydrogen bond is already postulated to exist in the Hermans "bent-chain" model for crystalline cellulose.[23] In the case of maltose, a model shows that the primary hydroxyl group in the reducing unit could form a hydrogen bond with the ring-oxygen atom of the non-reducing unit without any serious steric clashes.

The possible occurrence of such intramolecular hydrogen bonding in polysaccharides in aqueous solution has been much debated in recent years, and is clearly of key importance. Since it is now known that the magnitude of the anomeric effect can be varied at will, by carrying out hydrolysis in different acids of different concentrations,[1] it is possible to introduce a new kind of evidence into this area. Thus, if the higher rate of hydrolysis of maltose is due simply to a larger steric factor, then, even though the absolute values of K_β/K_α are lower for cellobiose and maltose than they are for β- and α-methyl D-glucopyranoside, a close parallelism in the response of these values to changes in the medium should still be observed for the two pairs of glucosides. If, however, there is any significant, intramolecular hydrogen-bonding of the type just discussed, it might be expected that the anomeric effect would be suppressed in the disaccharides, and that there would be no significant parallelism between the two sets of values of K_β/K_α.

The effect of ions upon the structure of water. There is much controversy about the detailed structure of liquid water, but it is undisputed that the molecules are associated by hydrogen-bonding, and that it is therefore a partial polymer.[24–27] The average degree of polymerisation decreases with increasing temperature. It is also undisputed that inorganic ions are, in general, hydrated in solution, and that, for closed-shell ions like the halides, the alkali-metal ions, and tetra-alkylammonium ions, the mechanism is simply dipole-attraction.[24,27,28]

* Since each oxygen atom can form 0,1 or 2 hydrogen bonds, nine different possible states of hydration can be formally recognised.

This mechanism is universally accepted to imply that, around an anion, the water molecules are orientated with their hydrogen atoms pointing, at least on an average, towards the anion, while around a cation, it is the oxygen atoms that are pointing inwards. The extent of this orientation, or "electrostriction", depends upon the electrostatic field at the surface of the ion, and is therefore inversely related to the ionic radius (distance from the nucleus). Thus, ions like F^- and Li^+ are powerful electrostrictors, while I^- and Cs^+, and especially tetra-alkylammonium ions, are weak ones.[24,27,28] Consistently with this, the proton is a strong electrostrictor,[24] and the hydronium ion, H_3O^+, is thought to be associated with at least four other water molecules in dilute solution.[2] The hydroxyl ion is also considered to be a strong electrostrictor.[24,27]

Attempts to develop this simple, electrostatic model through *a priori* calculations are complicated by the need to make simplifying assumptions about the distribution of charge in the water molecule. With one such model, Bernal and Fowler [24] concluded that a monovalent ion would be an electrostrictor only if its ionic radius were less than 1.6 Å. This implies that, among the halides, only F^- would be a significant electrostrictor, whereas, among the alkali-metal ions, only Cs^+ would not be. Later models,[28] by assuming a different charge-distribution, predict that anions, generally, should be stronger electrostrictors than cations of the same charge and radius. This comes about essentially because its smaller radius allows a hydrogen atom to get closer to an anion than an oxygen atom can get to a cation.[28]

Little has been stated about the sulphate ion, but from its double charge, its tendency to form hydrated salts, and its high position in the Hofmeister lyotropic series, it is reasonable to infer that it is a powerful electrostrictor. For metal ions with vacant orbitals, there is, of course, the possibility of co-ordination, but here again, the generalisation that the oxygen atoms of water point towards cations and away from anions holds true. For anions like HSO_4^- and $H_2PO_4^-$, the situation is clearly more doubtful.

Frank and Wen [29] have described the electrostricted layer of water molecules as "zone A". Nothing is known about its thickness, and there is no agreement as to how it should be defined, because different methods for measuring "hydration numbers" give widely different results.[27] It is only certain that, for a series like the halides at a given concentration and temperature, its thickness decreases with increasing ionic radius. It has been claimed that, for the iodide ion, it does not exist at all.[30]

Except for very dilute solutions, it follows as a direct consequence of the electrostatic model that the thickness of zone A will depend upon both the identity of the counterion, and the overall concentration of salt. This is because, as the concentration of salt increases, and the anions and cations approach one another more closely, each will start to neutralise the electrostatic field of the other, leading to a breaking-down of the respective A-zones.[31]

For certain ions, notably the larger halide and alkali-metal ions, there is evidence that, outside zone A, there exists a second zone, B, in which the molecules have a higher entropy, and a lower average degree of polymerisation, than they have in pure water at the same temperature.[29] Since the thickness of zone B seems to increase with increasing ionic radius, and hence to be

inversely related to the thickness of zone A, relevant ions like K^+, Rb^+, Cs^+, Br^-, and I^- are described [29] as "net structure breakers".* For dilute solutions, it is formally necessary to recognise that, outside zone B, there exists a third zone, C, which is the same as pure water.[29]

It seems to be possible to make the following inferences:

(*a*) For a given salt or acid at a given temperature, one ion will, in general, be a stronger electrostrictor than the other. As the salt is added to water, zone C will gradually disappear, and electrostricted water will be generated in the two A-zones, in proportion to their relative thicknesses. As the concentration increases still further, and the anions and cations approach one another, the A-zones will start to break down, that of the weaker electrostrictor disappearing first. Since this may be the origin of the B-zones, it cannot be inferred that the B-zones will disappear before the A-zones start to break down.

(*b*) For a given concentration of salt or acid, an increase in temperature will lead to an increase in the volume of the B-zones at the expense of the A-zones, and also at the expense of zone C, when it is present. Otherwise expressed, the A-zones, and the C-zone if any, will "melt". The A-zone of the weaker electrostrictor will melt first. The higher the concentration of salt, the lower should these "melting points" be, because, with the diminished net electrostatic field, the amount of energy required to break down the zones will be smaller.

A working hypothesis. Against this theoretical background, and on the basis of the results described in Part I,[1] the following two postulates are made:

(1) Anions generally, in water, increase the magnitude of the anomeric effect, and their capacity to do so is directly correlated with their electrostrictive power.[1] The magnitude of the anomeric effect is a direct measure of the extent to which the ring- and glycosidic oxygen atoms are solvated by water molecules, through hydrogen-bond formation with their hydrogen atoms.[12–14] At the periphery of a hydrated anion, the hydrogen atoms of the water molecules are pointing inwards, so that, in order to solvate these oxygen atoms, they would have to be re-orientated against the electrostatic field of the anion. This is why such solvation is diminished by anions. The glycosides are not excluded from the domain of the anions, that is to say, they are not significantly salted-out. Chromatographic evidence shows this.[1] This is because their hydroxyl groups are still able to form hydrogen bonds with the electrostricted water molecules, by themselves contributing the proton. Such hydrogen-bond formation should, in fact be promoted, because the density of electrons on the oxygen atoms of the electrostricted water molecules should be enhanced by the electrostatic field of the anion.

(2) Cations, generally, decrease the magnitude of the anomeric effect by orientating the water molecules so as to favour hydrogen-bond formation with the ring- and glycosidic oxygen atoms. They should also enhance the capacity of these molecules to act as hydrogen donors, by virtue of their electrostatic fields. These electrostricted water molecules are also able to form hydrogen bonds with the hydroxyl groups of the glycosides, the oxygen atoms of these groups acting as the proton acceptors.

* It is not apparent to this author that such a generalisation can be made without specifying the identity of the counterion, the total concentration of salt, and the temperature.

It is evident that it is not possible for postulate (1) to be correct without postulate (2) also being correct. A way to substantiate the truth of postulate (1) is therefore to seek evidence for the truth of postulate (2). This introduces the main purpose of the present work. To obtain the required evidence, it was clear that an acid should be chosen whose anion is a sufficiently weak electrostrictor to "allow" the hydronium cation, H_3O^+, to be the dominant electrostrictor. Ideally, hydrogen iodide should have been chosen, but it could not be used because the liberation of elementary iodine by dissociation and oxidation made the solutions too dark to be measured in the polarimeter.

The Zucker-Hammett plot[32] *as a measure of solvation.* This has been discussed in detail in Part I.[1] If the mechanism of hydrolysis is A-1, and if the activity coefficient of the substrate does not change relatively to that of the Hammett base as the concentration of acid increases, then a plot of the logarithms of the *pseudo*-first order rate-coefficients against the Hammett acidity function ($-H_0$) will have unit slope when the free energy of activation ($\Delta G^{\ddagger}$) is independent of acid-concentration. When $\Delta G^{\ddagger}$ increases with increasing acidity, the plot is sigmoid, and the slope at the point of inflexion is less than unity, and when $\Delta G^{\ddagger}$ decreases with increasing acidity, the plot is also sigmoid, but the slope at the point of inflexion is greater than unity.

Such a medium-dependence of $\Delta G^{\ddagger}$ has been identified with changes in the degree of hydration of the substrate in the ground state, *relative* to that in the transition state, by simultaneous studies of the medium-dependence of the activation parameters.[1] Thus, if the entropy of activation ($\Delta S^{\ddagger}$) decreases with increasing acidity, then the *difference* between the degree of hydration in the ground and transition states is decreasing, but if it increases, the opposite is true.

In the earlier study of the hydrolysis of the methyl glucopyranosides in sulphuric acid,[1] it was found that $\Delta S^{\ddagger}$ decreased sharply with increasing concentration of acid. Since this was accompanied by an increase in the magnitude of the anomeric effect, it was concluded that dehydration was taking place in the ground state. In order to understand why a parallel dehydration of the transition state was not taking place, it was necessary to assume that, in acid of any strength, the transition state is always less hydrated than the ground state. Specifically, it was suggested that the hydrate always decomposes when the molecule passes through the transition state, and that this is probably connected with the conformational change, from chair to half-chair, that is entailed in the formation of the transition state.

Insofar as this last assumption can be held to be generally true, it is seen that a decrease in $\Delta S^{\ddagger}$ will always mean that the ground state is being dehydrated, and that an increase in $\Delta S^{\ddagger}$ will always mean that it is being hydrated.

Theoretical aspects of the temperature of precipitation of methyl cellulose. By measuring the *ratio* (K_β/K_α) of the rates of hydrolysis of an anomeric pair of glucopyranosides, all medium effects on the rest of the molecule should be compensated for, and a specific measure obtained of hydrogen-bonding to the ring- and glycosidic-oxygen atoms. Because of the unusual results obtained in the present work with hydrobromic acid, it was desirable to seek con-

firmatory evidence of a more conventional kind, even though it would lack this compensatory feature.

Any suitable experimentation would necessarily entail some kind of measurement of solubility, and it was important that this should be carried out in the same temperature-range, namely, 40 – 70°. The idea of using the temperature at which methyl cellulose precipitates from aqueous solution [33] met this requirement, and possessed the additional feature that the methoxyl groups would resemble the ring- and glycosidic-oxygen atoms insofar as they could only be hydrated in the capacity of proton acceptors. This kind of approach to the study of solvation phenomena is well precedented, for example, in the experiments of Klotz [34] on the effect of salts on the temperature of precipitation ("cloud point") of polyvinylmethyloxazolidinone, and in the similar experiments of Ciferri and Orofino [35] on poly-L-proline. There are, however, theoretical difficulties in interpretation.

When a polymer dissolves in a solvent, there may be a net decrease in entropy because of an ordering effect upon the solvent molecules. In such a case, an increase in temperature will lead to a decrease in solubility. The phenomenon is a general one, and has been discussed in detail by Patterson.[36] The difficulty is that the solvent molecules may be ordered either because of a direct association with polar groups in the polymer, or because non-polar groups in the polymer give rise to "ice-like" structures in the solvent of the type discussed by Frank and Evans.[37] The ability of a cosolute to affect the temperature of precipitation is, therefore, not necessarily a measure of its ability to modify the extent to which the polar groups are solvated. The mechanism could consist simply in a modification of the free-energy change associated with the exposure of the non-polar groups to the solvent. This could come about, for example, through a change in the surface tension of the solvent.

Another difficulty in the present case is that fully methylated cellulose is insoluble in water, and the well-known temperature effect is observable only for partially methylated cellulose, with a degree of substitution in the range of 1.6 – 2.0 (Ref. 33). The possible effect of solvent upon the remaining hydroxyl groups must, therefore, also be considered.

In an attempt to distinguish between these three possibilities, auxiliary experimentation was carried out as described in the section on results. An interesting review of similar problems in the field of protein chemistry is given by Von Hippel and Schleich.[38]

EXPERIMENTAL

Kinetics of acid-hydrolysis. Apart from the following, additional details, the procedure and materials were exactly as described in Part I.[1]

β-Cellobiose was obtained from Theodor Schuchardt, München, and β-maltose monohydrate from Merck, A/G, Darmstadt. Both products were chromatographically homogeneous, had the correct specific rotations, and were used without further purification. This was also the case for methyl 2,3,4,6-tetra-*O*-methyl α-D-glucopyranoside, which was obtained from Koch-Light Laboratories, Ltd., Colnbrook, Buckinghamshire, England.

The initial concentration of the disaccharides in the reaction mixtures was 2 % w/v, calculated as anhydrous glucose. In each separate concentration of acid, and at each temperature, the "infinity" readings were obtained with freshly-prepared solutions of glucose, also at a concentration of 2 % w/v.

With the reducing disaccharides, the initial optical rotations were more sensitive to changes in acid-concentration than in the case of the methyl glucopyranosides, doubtless because of the anomeric effect. The earlier procedure of extrapolating the readings to zero time was therefore discarded in favour of taking the "initial" reading as the first reading to be taken after the establishment of thermal equilibrium. This is valid, because the rate-coefficient is independent of the concentration of substrate (*cf.* Overend *et al.*[19]).

Measurements of the temperature of precipitation of methyl cellulose. Methylated cellulose ("Methocel MC", U.S.P., 4000 cps.) was obtained from Fluka A/G, Buchs, Switzerland. An aqueous solution (1 % w/v) was centrifuged for 30 min at 40 000 *g*, and at 30°, before use. The other reagents were of Merck analytical grade.

For experiments with acids up to a concentration of 1 M, and with other reagents, the stock, 1 % w/v solution of methyl cellulose was mixed with an equal volume of reagent solution at room temperature, and placed in a test-tube in a large, insulated, well-stirred water-bath, which was heated at a rate of about 0.5° per min. With practice the temperature at which turbidity first appeared could be measured reproducibly to within 0.5°. The "cloud-point" was independent of thermal history, provided that the methyl cellulose was completely dissolved beforehand. Because of the difficulty in providing uniform conditions of agitation, no attempt was made to determine the temperature at which the precipitate redissolved upon cooling.

In experiments with acids at a concentration higher than 1 M, the foregoing procedure gave spuriously high results for the cloud-point, because of hydrolysis of the methyl cellulose during the period of heating. In these cases, more-approximate readings were obtained by varying the procedure as follows. When there was salting-in, the solutions of methyl cellulose and acid were heated separately to just below the cloud-point in water (about 52°), mixed, and then heated rapidly, at a rate of about 5° per min, until precipitation occurred. When there was salting-out, the temperature of precipitation was determined roughly by heating the solutions separately to a series of pre-determined temperatures, and observing the presence or absence of a precipitate immediately after mixing.

RESULTS

Cellobiose and maltose in sulphuric acid. In the first series of experiments, K_β/K_α for the disaccharides was studied as a function in the concentration of sulphuric acid at 70°, in order to compare the results directly with those obtained earlier[1] for the methyl glucopyranosides at the same temperature. It was, however, only possible to do this up to a concentration of 10 N acid, because the absolute rates of hydrolysis then became too high to be measured accurately. The results are shown in Fig. 1 (Curve *A*). Another series of experiments was then carried out at 40°, and in this case it was possible to work with concentrations of acid up to 16 N, before acid-catalysed dehydration-reactions started to cause serious departure from first order kinetics (*cf.* Part I).[1] The results are shown in Fig. 1 (Curve *B*).

In Fig. 2, the rate-coefficients obtained for cellobiose and maltose at 70° are shown separately, in the form of Zucker-Hammett plots. Unit slope is indicated by a broken line. Similar plots of the results obtained at 40° are shown in Fig. 3. All the values of $-H_0$ in these plots are taken from published tables,[2] and corrected for temperature with published values[2] of the temperature coefficient of $-H_0$.

Thin-layer chromatography of the disaccharides and of a Hammet base (*p*-nitroaniline) in various concentrations of sulphuric acid was carried out as described earlier,[1] and the results (Table 1) indicated that there was no tendency for the disaccharides to be salted-in or salted-out relatively to one another or to the Hammett base.

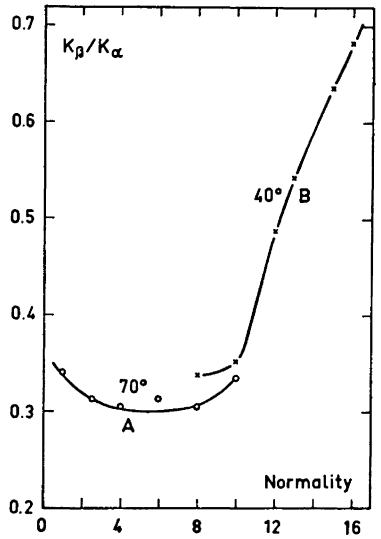

Fig. 1. Dependence upon acid-concentration of the ratio (K_β/K_α) of the rates of hydrolysis of cellobiose and maltose in sulphuric acid at 68.7° (○) and 39.6° (×).

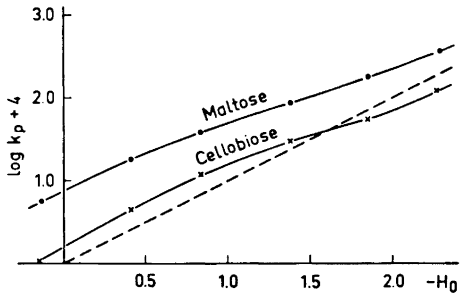

Fig. 2. Zucker-Hammett plots for hydrolysis of maltose (●) and cellobiose (×) in sulphuric acid at 68.7°. The rate-coefficients (k_p) were calculated by using logarithms to the base 10. The broken line indicates unit slope.

Table 1. Chromatographic mobilities (R_F values) of maltose, cellobiose and *p*-nitroaniline (PNA) on thin (0.75 mm) layers of silica gel, with various acids as the mobile phase.

Acid	R_F values of Maltose	Cellobiose	PNA
4 N H_2SO_4	0.94	0.95	0.83
5 N H_2SO_4	0.95	0.96	0.79
8 N H_2SO_4	0.95	0.95	0.76
10 N H_2SO_4	0.94	0.95	0.80
12 N H_2SO_4	0.95	0.95	0.80
16 N H_2SO_4	0.96	0.96	0.78
1 N HBr	0.99	0.99	0.76
3 N HBr	0.99	0.99	0.84
5 N HBr	0.99	0.99	0.81

Cellobiose and maltose in hydrobromic acid. There were no problems due to dehydration in this acid, but the range of acid-concentrations studied at each temperature was limited by the requirement that the absolute rates of hydrolysis should not be too high to be measured accurately, nor too low to be measured conveniently. The results obtained for K_β/K_α at 40°, 50°, 60°, and 70° are shown in Fig. 4.

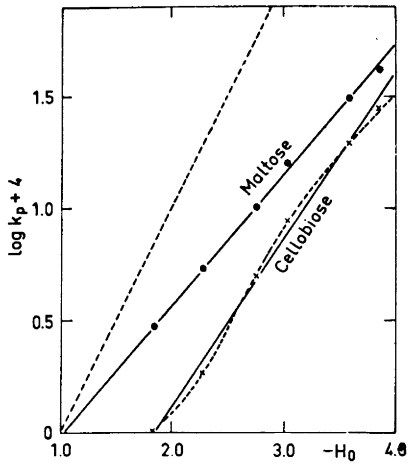

Fig. 3. Zucker-Hammett plots for hydrolysis of maltose (●) and cellobiose (×) in sulphuric acid at 39.6°.

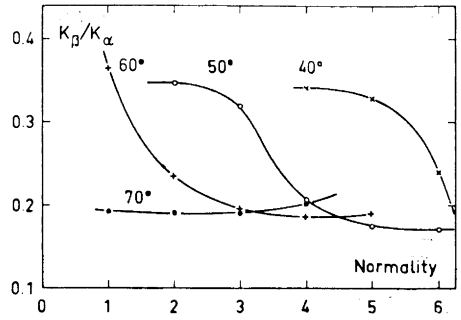

Fig. 4. Dependence upon acid-concentration of the ratio (K_β/K_α) of the rates of hydrolysis of cellobiose and maltose in hydrobromic acid at 39.5° (×), 49.4° (○), 58.5° (+) and 68.5° (●).

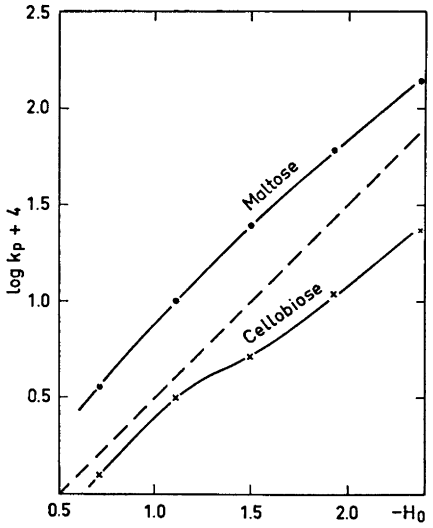

Fig. 5. Zucker-Hammett plots for hydrolysis of maltose (●) and cellobiose (×) in hydrobromic acid at 49.4°.

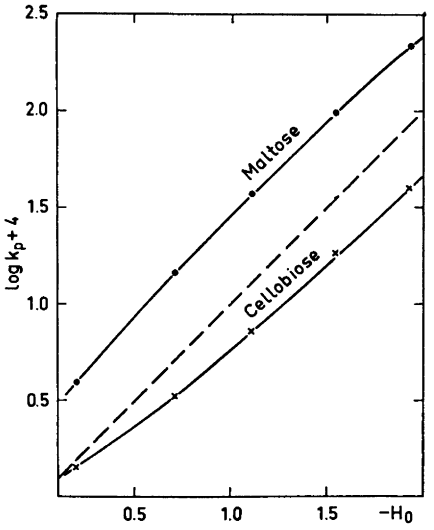

Fig. 6. Zucker-Hammett plots for hydrolysis of maltose (●) and cellobiose (×) in hydrobromic acid at 58.5°.

Since it was possible to study five different concentrations of acid at both 50° and 60°, Zucker-Hammett plots for these temperatures are shown in Figs. 5 and 6, respectively. The values of $-H_0$, taken from tables,[2] are valid at 25°, and are not corrected for temperature, because the temperature-coefficient of $-H_0$ is not reported for this acid. A preliminary study in this laboratory indicated that the temperature coefficient is negative (with respect to $-H_0$), and becomes more so with increasing acid-concentration, so that the slopes of the curves are a little too low. The matter was not pursued further, however, because the main purpose of Figs. 5 and 6 is to compare the behaviour of cellobiose with that of maltose.

Thin-layer chromatography of the disaccharides and of *p*-nitroaniline was also carried out in various concentrations of hydrobromic acid, and the results are included in Table 1. The R_F values of the disaccharides are so high in this acid, that it might reasonably be questioned whether the evidence is satisfactory. There was no flattening of the spots, however, which is usually indicative of complete exclusion from the stationary phase, and a proportionate salting-in was also observed for the Hammett base. Because of this, and of the similar results obtained for the methyl glucopyranosides, whose R_F values are lower,[1] it is very unlikely that the different behaviour of the two disaccharides is due to primary salt effects.

The kinetic data also allowed the temperature-dependence of K_β/K_α in 2 N, 3 N, 4 N, and 5 N hydrobromic acid to be illustrated, as shown in Fig. 7. There is an evident paucity of experimental points in the individual curves,

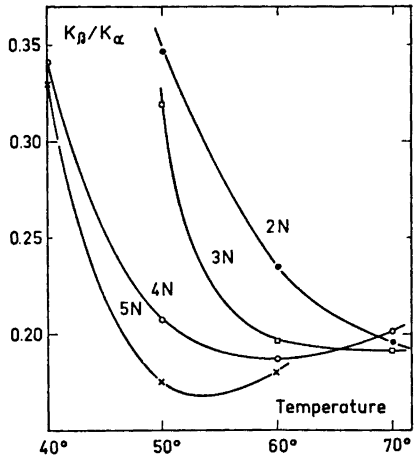

Fig. 7. Dependence upon temperature of the ratio (K_β/K_α) of the rates of hydrolysis of cellobiose and maltose in hydrobromic acid at concentrations of 5 N (×), 4 N (○), 3 N (□), and 2 N (●).

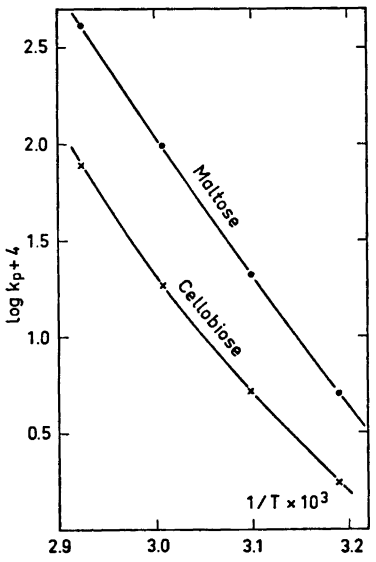

Fig. 8. Arrhenius plots for the hydrolysis of maltose (●) and cellobiose (×) in 4 N hydrobromic acid.

Table 2. Energies (E_A) and entropies ($\Delta S^{\ddagger}$) of activation for hydrolysis of maltose and cellobiose in 4 N hydrobromic acid at 40° and 70°.

	(E_A) 70°	(E_A) 40°	($\Delta S^{\ddagger}$) 70°	($\Delta S^{\ddagger}$) 40°
Maltose	34.4	30.0	+20.1	+6.8
Cellobiose	35.8	23.3	+20.9	−16.7

but qualitatively, the existence of a series of troughs is clear, even though their exact positions are not precisely known. With such a temperature-dependence, it is not surprising that the kinetics were non-Arrhenian. This is shown in Fig. 8, where the separate data for cellobiose and maltose in 4 N hydrobromic acid are plotted. Both plots are curved, and show that the energy of activation increases with increasing temperature for both disaccharides. The numerical values for the activation parameters were not very accurate, because they had to be measured from the slopes of tangents, but very approximate values are given in Table 2.

Methyl α- and β-D-glucopyranoside in hydrobromic acid. An almost identical series of experiments was carried out on the methyl glucopyranosides, and plots of K_β/K_α against normality of hydrobromic acid at the four different temperatures are shown in Fig. 9. They may be compared directly with the results for the disaccharides in Fig. 4. All the other plots of the data were sufficiently similar, in the qualitative sense, to those already shown for the disaccharides in this acid, that it is not necessary to show them. The relevant differences can be most clearly seen from Figs. 4 and 9.

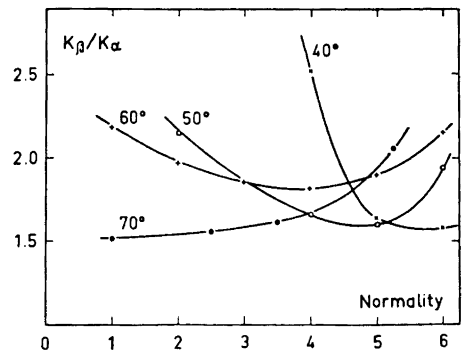

Fig. 9. Dependence upon acid-concentration of the ratio (K_β/K_α) of the rates of hydrolysis of the anomeric methyl D-glucopyranosides in hydrobromic acid at 39.5° (×), 49.5° (○), 58.5° (+) and 68.5° (●).

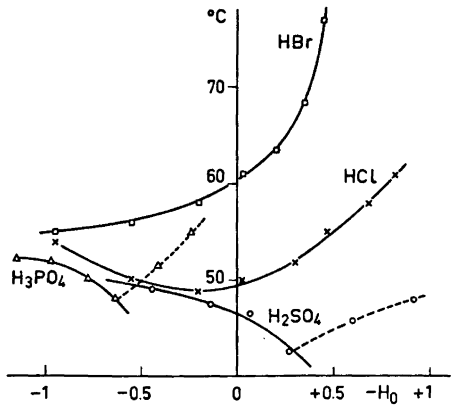

Fig. 10. Dependence upon acid-concentration of the cloud-point of methyl cellulose in hydrobromic acid (□), hydrochloric acid (×), phosphoric acid (△), and sulphuric acid (○). The points adjoined by broken curves are assumed to be spurious because of acid-degradation.

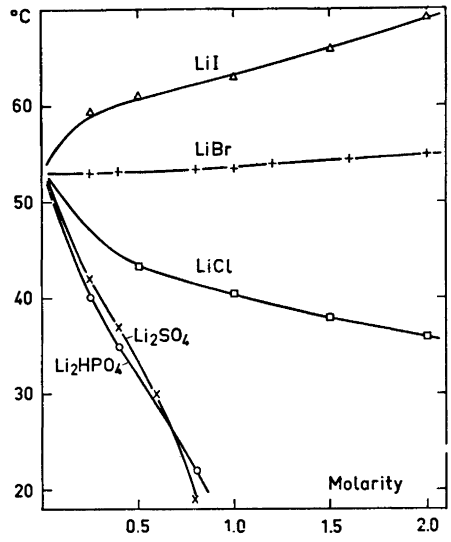

Fig. 11. Dependence upon salt-concentration of the cloud-point of methyl cellulose in lithium iodide (△), bromide (+), chloride (□), sulphate (×), and monohydrogen phosphate (○).

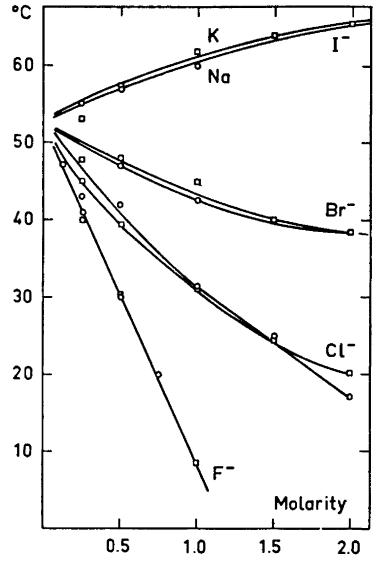

Fig. 12. Dependence upon salt-concentration of the cloud-point of methyl cellulose in the iodides, bromides, chlorides, and fluorides of sodium (○) and potassium(□).

Effect of the acids and of model cosolutes upon the temperature of precipitation of methyl cellulose. The results obtained for sulphuric, phosphoric, hydrochloric, and hydrobromic acids are shown in Fig. 10. The Hammett acidity function ($-H_0$) is chosen as the measure of acid-concentration, because this assures that, at a given value of $-H_0$, the samples of methyl cellulose are protonated to the same extent in the four different acids, so that their different solubilities cannot be due to differences in charge. In agreement with theory,[36] even mild acid-hydrolysis brought about a marked increase in the cloud-point, so that all the results are probably too high.

Because of this difficulty, it was of interest to replace the hydrogen ion by Li^+, which might be expected to behave similarly, because it is also a strong electrostrictor. Fig. 11 shows the results obtained with Li_2SO_4, LiH_2PO_4, LiCl, LiBr, and LiI, up to a concentration of 2 M. When a weaker electrostrictor than Li^+ was chosen as the cation, the bromide ion was no longer associated with salting-in, although the iodide ion still was. This and several other points of interest are shown in Fig. 12.

Experiments were then carried out to determine to what extent these effects could be due to changes in the hydration of the unmethylated hydroxyl groups. Since alkalis are good solvents for unmethylated polysaccharides, the behaviour in LiOH, NaOH, and KOH was determined. The results (Fig. 13) are shown with the H^- acidity function [2] as the measure of alkali-concentra-

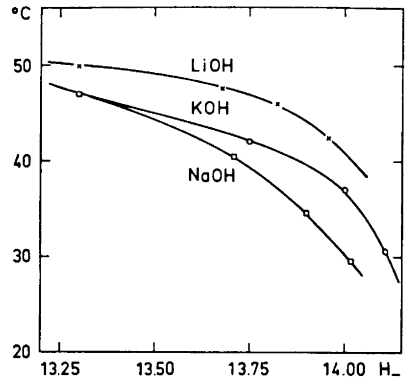

Fig. 13. Dependence upon alkali-concentration of the cloud-point of methyl cellulose in the hydroxides of lithium (×), sodium (□), and potassium (○).

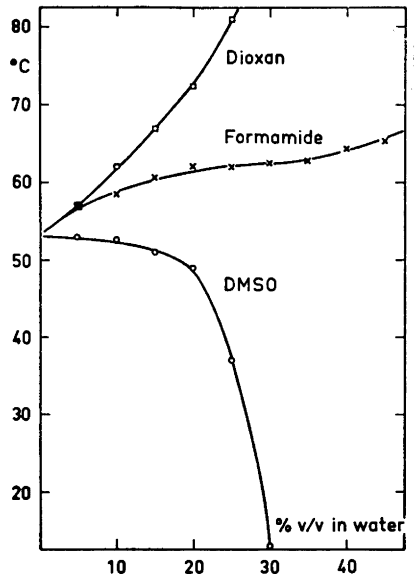

Fig. 14. Dependence of the cloud-point of methyl cellulose upon the concentration in water of dioxan (□), formamide (×) and dimethyl sulphoxide (○).

tion, since this assures that the hydroxyl groups are, for a given value of H^-, ionised to the same extent in the three alkalis.

Three organic solvents were next investigated: dioxan, because it is a good solvent for non-polar compounds; dimethyl sulphoxide, because it is a powerful proton-acceptor, and hence a good solvent for unmethylated polysaccharides; and formamide, because it is a good proton-donor. The results are shown in Fig. 14.

Table 3. Pseudo-first order rate-coefficients (k_p) and energies (E_A) and entropies ($\Delta S^{\ddagger}$) of activation for hydrolysis of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside in 1 N and 12 N sulphuric acid. The rate-coefficients (k_p) were calculated by using logarithms to the base 10.

Temp. (°C)	Normality of acid	$10^4 k_p$ (min^{-1})	E_A (kcal mol^{-1})	$\Delta S^{\ddagger}$ (e.u.)
87.4	1.0	5.04		+58.4
82.7	1.0	1.96	51.0	+58.4
78.1	1.0	0.75		+58.4
68.5	12.0	68.8		−0.8
57.9	12.0	14.4	30.7	−0.3
49.4	12.0	4.79		+0.1
39.6	12.0	1.04		+0.1

Finally, direct support was sought for the assumption that the methoxyl groups in methyl cellulose are hydrated in aqueous solution, with their oxygen atoms acting as proton-acceptors. This was done by taking methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside as a model, and measuring the activation parameters for hydrolysis in both 1.0 N and 12 N sulphuric acid. The results (Table 3) show that the decrease in both the energy and entropy of activation that occurs upon increasing the concentration of acid is even larger than in the corresponding unmethylated compound.[1]

DISCUSSION

The anomeric effect in cellobiose and maltose. The overall impression given by the results in sulphuric acid (Fig. 1) is that, whereas the molecules as a whole are, like the methyl glucopyranosides,[1] smoothly dehydrated by increasing concentrations of acid (Figs. 2 and 10), this is not associated with very much change in the magnitude of the anomeric effect until a transition, occurring only in cellobiose (Fig. 3), suddenly allows it to increase in that sugar. Whether a similar transition in maltose, or a second transition in cellobiose, would occur at a still-higher acid-concentration, cannot be determined with sulphuric acid because of decomposition of the sugars, but the use of a neutral salt as the dehydrating agent might possibly provide an answer to this question.

The effect of hydrobromic acid (Fig. 4) is more uniformly manifested in both disaccharides. This acid clearly enhances hydration (Table 2 and Fig. 10), and this is seen in Figs. 5 and 6 to lead to simultaneous stabilisation of cellobiose and destabilisation of maltose. Even here, however, the changes that occur are sharper and are displaced towards higher acid-concentrations compared to the corresponding changes in the methyl glucopyranosides (Fig. 9).

Decomposition of the disaccharides prevented the measurement of K_β/K_α in concentrations of sulphuric acid higher than 16 N, so that its upper limit is still not known. However, the total, measured range of variation in K_β/K_α, from the minimum of 0.17 in hydrobromic acid (Fig. 4) to the maximum of 0.68 in sulphuric acid (Fig. 1), corresponds to a variation in the magnitude of the anomeric effect, at 40°, of 860 cal mol^{-1}. This was calculated from eqn. (4) in Part I,[1] with the ratios of the activity coefficients set equal to unity, in accordance with the chromatographic evidence (Table 1).

This result may be compared with the corresponding figure of about 700 cal mol^{-1}, obtained earlier [1] for the methyl glucopyranosides at 70°. It is therefore reasonable to infer that the anomeric effect is just as strong in di- and poly-saccharides generally, as it is in simple glycosides.

Intramolecular hydrogen-bonding in cellobiose and maltose. The transition that occurs in cellobiose as the concentration of sulphuric acid increases from 10 N to 16 N at 40° (Fig. 3) is unlikely to be due to a sudden change in the steric factor, because the molecules have already been extensively dehydrated before it occurs (Fig. 2), and no similar change occurs in maltose, even though the steric factor should be larger for this disaccharide.

It is evident that further work on other disaccharides, and on derivatives in which specific hydroxyl groups are removed by substitution or reduction,

is needed to settle this question. However, it may be significant that a concentration of 16 N is not very much lower than that at which sulphuric acid becomes a good solvent for cellulose.[39]

The mechanism whereby inorganic ions influence the magnitude of the anomeric effect. One may begin by considering whether it could be the A-zone of the bromide ion that diminishes the magnitude of the anomeric effect. To do this, of course, it would have to be fundamentally different from the A-zones of the other anions that have been investigated,[1] and there is no theoretical or experimental basis for introducing such an assumption. It is therefore necessary first to enquire whether the facts can be accounted for within the framework of existing theories.

The second possibility is that it is the B-zone of the bromide ion that decreases K_β/K_α. The difficulty with this is that it does not explain the temperature-dependence (Figs. 7 and 8, and Table 2). Evidence was obtained in Part I that the water molecules that solvate the ring- and glycosidic-oxygen atoms, and the other oxygen atoms in the same manner, have a lower entropy than they have in the bulk of the solution, at least in dilute sulphuric acid.[1] Extrapolation to infinite dilution implies that this is also true for solutions in pure water. If, therefore, the water molecules in a B-zone have a higher entropy than they have in pure water at the same temperature, then an increase in temperature can only result in the dehydration of the ring- and glycosidic oxygen atoms of any glycoside molecule that is present in the zone.

The third possibility is that it is the A-zone of the hydrogen ions that diminishes the magnitude of the anomeric effect. If this is accepted, the other facts fall into place in a reasonably straightforward manner. Thus, if the A-zone of the hydrogen ions is larger than that of the bromide ions, as the electrostatic theory would predict, increasing concentration of hydrobromic acid should lead to a net increase in the hydration of the two glycosides, stabilising the β-anomer, and de-stabilising the α-anomer (Figs. 5 and 6). As the concentration of acid increases still further, the A-zones should start to break up. That of the bromide ion should break up first, which would diminish K_β/K_α still further, but that of the hydrogen ion should finally start to break up also, leading to an increase in K_β/K_α (Figs. 4 and 9).

The temperature-dependence of K_β/K_α (Fig. 7) can be interpreted by assuming that the troughs represent "melting points" of the A-zone of the bromide ion. Consistently with this, the melting point is lowest when the concentration of acid is highest. The melting points are not sharp, but this would not be expected if there are two or more layers of electrostricted water molecules surrounding the bromide ions. The final increase in K_β/K_α could be explained by assuming either that the A-zone of the hydrogen ion has started to melt, or that the B-zone has now become so large that the entropically favourable dehydration of glycoside molecules in this zone has become the dominant process.

Systems exhibiting non-Arrhenian kinetics are, of course, well known. The examples most commonly cited are reactions for which the energy of activation is very small,[40] or in which quantum-mechanical tunneling is possible.[41,42] Reactions which are heterogeneous, in the macroscopic sense, are also cited,[40] and of course there must exist a range of temperatures for which every enzymic

reaction is non-Arrhenian. It might be a useful generalisation to state that non-Arrhenian kinetics can always be expected, whenever one of the components of the system is undergoing a change of state. One can imagine, for example, the likely temperature-dependence of a reaction carried out in a solution containing ice. Another relevant example is the hydrolysis of sucrose in dilute acid, for which the activation energy decreases sharply as the temperature increases from 0° to 40° (Ref. 43). In this case, it may be supposed that the solvent sheath around the sucrose molecules is melting.

Independent evidence for the idea that the A-zone of the bromide ion is able to melt under the conditions of these experiments can be seen in the negative temperature-dependence of $-H_0$ for hydrobromic acid. The standard entropy-change that is associated with the proton-transfer reaction is positive,[44] so that, if the bromide ion were playing no part, the temperature coefficient of $-H_0$ would be expected to be positive. The fact that it is negative can be readily explained by assuming that the A-zone of the bromide ion melts, leading to an increase in the activity of water, and, hence, to a decrease in the activity of the hydrogen ions.[2]

In a series of experiments to be reported in detail elsewhere, the effect of hydrobromic and sulphuric acids upon the optical rotation of 2,3,4,6-tetra-*O*-methyl-D-glucose has been studied. This derivative was chosen because it cannot form furanoid or septanoid rings. Its optical rotation might therefore be expected to give an approximate measure of the relative amounts of α- and β-pyranoid forms present at equilibrium, and, hence, of the magnitude of the anomeric effect. Possible objections to this assumption will be discussed, but it is an empirical fact that a close parallelism was observed between the results of this static method, and the kinetic one described here.

A final possibility that must be considered is that anions are able to change the magnitude of the anomeric effect by associating directly with the glycoside molecules. This idea implies an equilibrium-controlled association between glycoside molecules and the anions, taking place competitively with water molecules.

In considering the likelihood of this, it must be remembered that, even in 16 N sulphuric acid, the ratio of water molecules to disaccharide molecules (at the used concentration of 2 % w/v) is still about 500:1, and the ratio of water molecules to anions is still more than 5:1. In 6 N hydrobromic acid, the molar ratio of water to disaccharide is 790:1, and the ratio of water molecules to anions is 7.5:1.

It must also be remembered that, for the methyl glucopyranosides, K_β/K_α reaches a maximum in 20 N sulphuric acid,[1] so that, for such a mechanism to be operative, it would be necessary to assume that all the glucoside molecules are fully complexed with anions in acid of that concentration. The chromatographic evidence[1] provides no support for this idea, and it is very unlikely that the association constant could be so large, when the mechanism of the association would necessarily be very similar to that whereby water molecules are themselves attracted to the anions.

Finally, it is, of course, impossible to explain both the initial decrease, and also the final increase, in K_β/K_α that takes place with increasing concentration of hydrobromic acid, on a basis of direct anion-substrate associa-

tion alone. Considered together, these facts leave little doubt that the observed effects are, at least primarily, hydration phenomena.

Relationship to solubility phenomena. As early as 1926, Kruyt and Robinson [45] suggested that, when a polar non-electrolyte orientates water molecules in the same sense as a particular ion, it will be salted-out by that ion, but salted-in by an ion of opposite charge.

In 1931, Meyer and Dunkel [46] classified the alkali-metal halides into "aquo-bases" or "aquo-acids", according to whether the smaller ion was an anion or a cation, respectively. They then sought to explain the solubilities of organic compounds in solutions of these salts by proposing that proton-acceptors, such as pyridine, dioxan and ethyl acetate, should be selectively bound to the solvation shells of cations, while compounds like phenol and carboxylic acids, which were expected to act primarily as proton-donors, should be bound to the solvent shells of anions. Conversely, it was expected that bases would be exluded from the solvent shells of anions, and acids from those of cations. The net effect of any salt was expected to be determined by the smaller ion.

The validity of these early ideas appears to have been challenged, only in the sense that, as an explanation for the solubility of organic compounds in aqueous salt solutions, they are incomplete. Mainly through the work of McDevit and Long,[47] it is now known that a well-defined salting-out order exists even for non-polar compounds like benzene, that it is connected with the changes in volume that occur when a salt is added to water, and that the mechanism consists in a physical "squeezing-out" of the organic solute, as the water molecules become selectively compressed in the domains of the anions and cations. Non-polar compounds can also be salted-in, most notably by large, organic ions, the mechanism being, almost certainly, direct ion-solute interaction.[48]

For polar non-electrolytes, very different salting-out orders have been noted, and salting-in is commonly brought about by simple, inorganic salts.[48] In a comprehensive review,[48] Long and McDevit have discussed these phenomena, with the fairly clear conclusion that, at least for weak organic acids and bases of low dielectric constant, the reversals in the salting-out order for non-polar compounds that are observed can best be explained by the water-orientation hypothesis of Kruyt and Robinson [45] and Meyer and Dunkel.[46]

The present study of the anomeric effect is not concerned with solubility as such, and it will be recognised that the working hypothesis advanced here consists essentially in the re-application of the ideas of Meyer and Dunkel to a system which is uncomplicated by other considerations. In seeking to confirm that hydrobromic acid enhances direct hydration of a glycoside, by having recourse to measurements of solubility, these complications are re-introduced.

The interpretation of the data obtained for the cloud-point of methyl cellulose (Figs. 10 – 14) is fortunately simplified by the evidence [47,48] that H^+, Li^+, Na^+, K^+, Br^-, and I^- do not normally salt-in non-polar compounds. The salting-in of methyl cellulose that is brought about by HBr (Fig. 10), by LiBr and LiI (Fig. 11), and by NaI and KI (Fig. 12) is therefore difficult to explain, unless it is assumed that direct hydration is being enhanced. Moreover, the data in Figs. 13 and 14, and in Table 3, indicate that it is mainly the methoxyl groups that are being hydrated. This is consistent with the idea

that the hydration is being brought about by the cations, and that the bromide ion is sufficiently large to "allow" both H^+ and Li^+ to be the dominant electrostrictor, while the iodide ion is large enough to allow even Na^+ and K^+ to be the dominant species.

It is tempting to interpret the instances of salting-out in Figs. 10–13, also in terms of the water-orientation hypothesis, but a useful warning is provided by the data in Fig. 13. Here it is seen that NaOH salts-out more effectively than KOH. This is contrary to the water-orientation hypothesis, but consistent with the solvent-compression hypothesis of McDevit and Long.[47] This makes it clear that, even for a highly oxygenated molecule like methyl cellulose, the effects described by McDevit and Long are still operative, and that all the data in Figs. 10–13 must be regarded as a manifestation of the two effects operating simultaneously.

All the experimental work in this paper was carried out by Kjersti Andresen, to whom I again express my warmest thanks. The continued interest and encouragement of Prof. N. A. Sørensen and Prof. A Haug are also deeply appreciated.

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