The Mutarotation of Some Aldoses

A thesis presented to the University of Glasgow

for the degree of

DOCTOR OF PHILOSOPHY

by

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To my parents.

Acknowledgements

It is my pleasure to express my gratitude to Dr. B. Capon for his advice and guidance in the course of these studies, and for his timely encouragement in times of darkness. I should also like to express my sincere thanks to Mrs. G. Wilson for the expert manner in which she typed this thesis. My thanks are also due to the department and its staff for the use of the excellent services and equipment and to the Science Research Council for an Industrial Studentship which has supported me during the course of this thesis. Finally I should like to express my most sincere gratitude to my fiance whose patience "in the cause of science" has been remarkable!

Abstract

The kinetics of the mutarotation of a series of nine 5-substituted aldoses of the D-glucose configuration and five 2-substituted glucoses, catalysed by a wide range of catalysts has been studied. It was found that substituents in the 5-position of the pyranose ring had a much greater effect on the kinetics of mutarotation than substituents in the 2-position of the ring. Electron withdrawing substituents (in the 5-position) were found to enhance the rate of the base catalysed mutarotation but to decrease the rate of acid and water catalysed mutarotation. Reasons for this are discussed with reference to the possible mechanisms of mutarotation. Steric hindrance of general base catalysis of mutarotation has been observed with the bases 2,6-lutidine, diethanolamine and tris-hydroxymethyl methylamine as catalysts. Attempts have been made to establish conclusively the reasons for this steric hindrance and to use it as a tool in distinguishing between kinetically indistinguishable mechanisms of mutarotation.

The kinetic data of the acid and base catalysed mutarotation of the 5-substituted aldoses have been fitted to linear free-energy relationships (Taft and Hammett Equations) and found to give, at best, only a moderately good correlation. Reasons for this have been suggested.

The spontaneous mutarotation of the anions of 6-deoxy-α-D-glucohepturonic acid and 6-O-(o-hydroxyphenyl)-β-D-glucose have

been found to be faster than anticipated on the basis of the inductive effects of the substituent on the 5-position of the ring. It is proposed that these rapid mutarotations result from intramolecular catalysis by the carboxylate and phenolate groups respectively. The possible mechanisms are discussed and a preference expressed for that mechanism which involves intramolecular general acid catalysis of the sugar anion in the rate determining step, rather than the kinetically equivalent true intramolecular general base catalysis.

A brief comment is made on the possibility of testing the hypothesis of "tautomeric catalysis".

Finally, the appendix describes the attempted preparation of suitable substrates for a kinetic investigation of the enzyme ribonuclease A. Although a substrate for ribonuclease was prepared in situ, the difficulty in preparation and isolation of such substrates proved too great.

Contents

Introduction

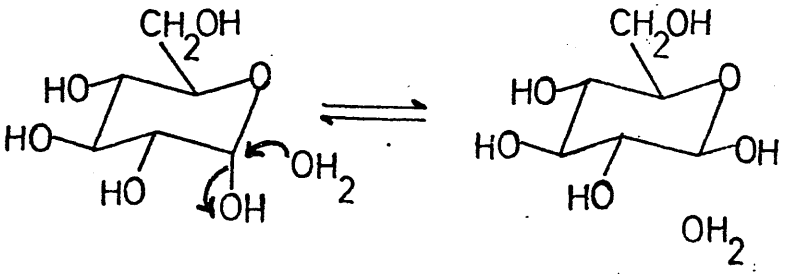
1.1 Historical

The phenomenon of mutarotation in sugars has a long history. The discovery of this phenomenon has been credited to Dubrunfaut[1], who in 1846 first observed the change in optical rotation of a freshly prepared aqueous solution of α-D-Glucose. Subsequent studies by early workers[2-4] established a similar change in solutions of other sugars such as galactose and lactose. The term "mutarotation" was introduced by Lowry[5] in 1899, and is a general term denoting any change in the optical rotation of a solution. As Pigman and Isbell[6] point out in their review, mutarotations have now been observed for a wide range of compounds such as gelatin, α-amino nitriles, menthyl benzoylformate as well as for many aldoses and ketoses. The historical background to many facets of the development of mutarotation in sugar chemistry has been included in the review of Isbell and Pigman which is in two parts[6,7], and so further information on the historical aspects of mutarotation should be sought there. Suffice to say that despite the enormous amount of work carried out on the subject, there is still much debate on certain aspects of the mechanism of mutarotation (see later).

1.2 The Basis of Mutarotation

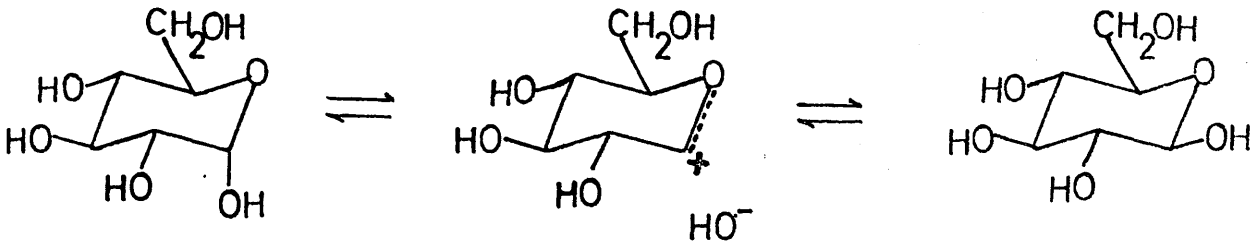
It is now clearly established that mutarotation in aldoses and ketoses results from the interconversion of cyclic and acyclic forms of the sugar. The rotational change shown by the α - and β- pyranose

forms of glucose[5,8], xylose[9] and several other sugars[10] obey the rate law for a first order reversible reaction, hence the mutarotation reaction is presumably the interconversion of these two pyranose forms. If the reaction is to proceed without ring opening then the mechanism of mutarotation would require to be direct exchange of the hydroxy-group at C(1) with water (eq 1)



(1)

or the formation and subsequent capture by water of a cyclic carbonium ion (eq 2) analogous to the mechanism for anomerisation of glycosides[11-14] and sugar acetates[15].

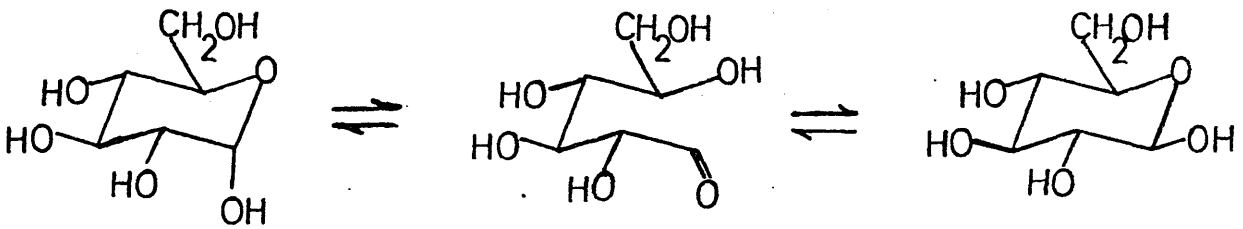


(2)

Both these mechanisms are excluded by the observation[16] that the rate of oxygen exchange of glucose -1-^{18}O with water is about 30 times slower than the rate of mutarotation. Either of the above mechanisms imply that the rate of oxygen exchange should equal the rate of

mutarotation.

Thus it has been widely accepted that the basis of mutarotation of glucoses is the interconversion of the α- and β- pyranose forms via an acyclic form which is present in very small (barely detectable[52]) concentrations (eq 3).



(3)

As Capon[16] points out, this mechanism is easily rationalised with the observed rate of oxygen exchange, by virtue of the aldehydo-group of the acyclic intermediate being captured by the hydroxy-group at C(5) to reform the hemiacetal sugar linkage. Approximately one time in 15 however, the aldehydo-group is captured by water to yield the hydrated acyclic aldehyde which undergoes oxygen exchange as statistically expected one time in two. Isbell[17] invokes a new and more complicated concept of the same thing by postulating that the acyclic aldehydo-forms are "pseudo-acyclic" intermediates with conformations similar to the parent cyclic sugars. No evidence has been obtained yet to support the validity of this concept, but it presents an attractive possible hypothetical model for mutarotation reactions.

As recently as 1966, there has been the suggestion[75] that ring opening to the acyclic aldehyde does not form the basis of

mutarotation of pyranoses, but that reaction occurs via a carbanion intermediate derived by removal of the C(1) anomeric proton by base leaving the hemiacetal ring intact. This clearly requires that hydrogen-deuterium exchange at C(1) be at least as fast as mutarotation and that the isotope effect resulting from the substitution of deuterium for hydrogen at C(1) be of the order commonly observed for the rate determining transfer of a carbon bound hydrogen atom (typically 6-7). Dean[76] has pointed out the improbability of the high exchange rate in the light of the absence of exchange of the more acidic proton of triethylorthoformate in stong base[77]. Quite clearly the carbanion postulate is impossible in the knowledge that the rate of mutarotation of glucose-1-H is almost the same (in fact very slightly slower) as the rate of mutarotation of glucose-1-D in water and deuterium oxide as solvents[73]. The authors of this last piece of work[73] also showed that no exchange of the C(1)-hydrogen atom occurred in deuterium oxide thus corroborating Dean's objection to the carbanion mechanism.

The mutarotations which form the studies of this thesis are "simple" mutarotations, i.e. of the type discussed above. Many aldoses and ketoses undergo "complex" mutarotations which differ from those described above, in that other forms of the sugar in question, such as the furanose, aldehydo and aldehydrol forms, are present to a significant extent, and interconversions between these forms, and between them and the pyranose forms of the sugar, render the kinetics of mutarotation rather complex. The basis of mutarotation in these

sugars again involves formation of an acyclic intermediate as in eq. 3, but capture of the aldehydo-group by the hydroxy-group at C(4) now competes effectively with capture by the hydroxy-group at C(5) to form stable furanose forms of the sugar.

Many methods have been used to study mutarotation reactions. The change in composition of the sugar solution as mutarotation proceeds has been followed by the accompanying change in density[18], refractive index[19], infra red[20] and NMR[21] spectra, pH[22], solubility,[23] gas-liquid chromotography[71,72] and of course optical rotation. The last named is by far the most useful and has been used in all the studies in this thesis.

1.3 Catalysis of mutarotation

The mutarotation reaction in a way is a landmark in the concepts of general acid and general base catalysis. As early as the 1880's, it was appreciated that acids[24] and bases[25] accelerated mutarotation. Later workers such as Brönsted and Guggenheim[26] showed that catalysis was not restricted to protons and hydroxide ions but was a property of many acids and bases such as undissociated carboxylic acids and their dissociated anions. The catalysis by these molecules of mutarotation was one of the first observations of the now widely known concepts of general acid and general base catalysis. It was shown that in a buffer solution the rate constant could be expressed as

$$k_{obsd} = k_o + k_{H_3O^+}(H_3O^+) + k_{OH^-}(OH^-) + k_A(A) + k_B(B) \qquad (4)$$

where A and B are the acidic and basic forms of the buffer. It was

also realised that catalysis was much more effective when both an acid and a base catalyst were present and that therefore amphoteric solvents such as water were much better catalysts than aprotic solvents. Thus the rate of mutarotation of 2,3,4,6-tetra-O-methyl-α-D-glucose is much greater in mixtures of pyridine and cresol than in either or these solvents alone[27]. In fact mutarotation in dry pyridine or dry cresol is very slow while mutarotation in pure water proceeds at an appreciable rate. This is due to the amphoteric nature of water.

Clearly by varying the buffer and pH conditions suitably, certain terms in equation (4) become negligible and allow calculation of the catalytic coefficients. All previous studies of this nature[26,28,29,31] have shown that mutarotation is considerably more susceptible to basic catalysis than to acidic catalysis. For example, Brönsted and Guggenheim[26] determined k_o and $k_{H_3O^+}$ to be 8.8×10^{-5} sec^{-1} and 2.4×10^{-3} l. mol^{-1} sec^{-1} at 18° while Los and Simpson[28] estimated k_{HO^-} to be 25 l.mol^{-1} sec^{-1} at 15° (for α-D-glucose).

In accordance with this observation, Isbell[7] points out that the basic catalytic function of water predominates over the acidic catalytic function. The most difficult of the catalytic coefficients to measure accurately is k_{HO^-} since in alkaline solution the sugar itself is appreciably ionised and the resulting glucosate ion is catalytically active[30]. Early measurements of k_{HO^-} were wildly incorrect due to lack of recognition of this fact, but more recent determinations of k_{HO^-} are more accurate.

An interesting example of base catalysed mutarotation was reported[32] recently. It represents the first reported case of an intramolecularly catalysed mutarotation. The authors report that the mutarotation of glucose 6-phosphate at pH 7.0 proceeds at a rate 240 times that of α-D-glucose. This they ascribe to intramolecular general base catalysis principally by the monohydrogen orthophosphate ion HPO_4^{2-}. They present evidence for this postulate by calculating the "effective concentration" of phosphate arcund the C(1) of glucose 6-phosphate and then measuring the rate of mutarotation of α-D-glucose in this concentration of inorganic phosphate buffer. Good agreement between this rate and the rate of mutarotation of the glucose 6-phosphate was obtained. Further evidence was the similarity in the activation energies for the mutarotation of glucose (22.2 kcal) and glucose 6-phosphate (21.8 kcal) which eliminates the possibility of the phosphate group increasing the ring strain of the sugar, thereby lowering the activation energy for the reaction. The existence of intramolecular catalysis in mutarotation is one to which further attention will be given in this thesis.

One of the proposals often made to explain the remarkable efficiency of enzyme catalysis is that of concerted general acid-base catalysis in two-proton transfer reactions[33]. Although such concerted catalysis has frequently been postulated, particularly for reactions in non polar solvents (see later this section),

evidence for such a mechanism in aqueous solution has been scant. In recent years the catalysis by bifunctional catalysts, such as bicarbonate and phosphate, of the hydrolysis of 4-hydroxybutyranilide has been ascribed[34] to a cyclic concerted general acid-base catalysis of the breakdown of the tetrahedral intermediate (Fig.1)

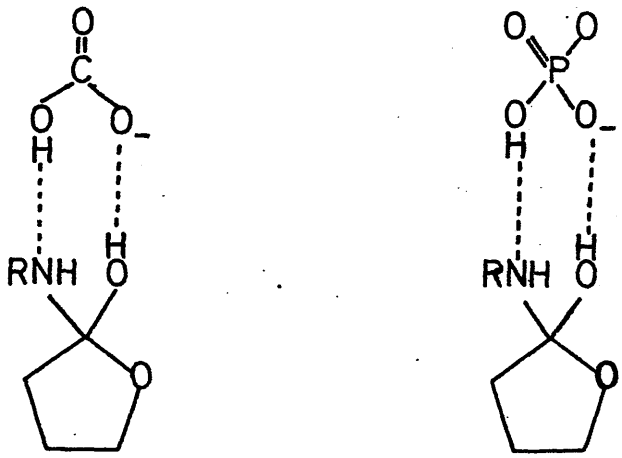


Fig. 1

Similar bifunctional concerted acid-base catalysis has been postulated in the hydration of acetaldehyde [44] and carbon dioxide[45] and in several other reactions[46]. The existence of third order terms in the rate equations required to explain the kinetics of enolization of ketones[35] and the ketonisation of oxalacetic acid[36] are taken by many authors to mean that a pathway involving concerted general acid-base catalysis contributes at least to some extent to the mechanism of these reactions.

Not surprisingly, since mutarotation is a two-proton transfer reaction, workers have attempted to find evidence in support of such concerted catalysis in mutarotation. Despite a detailed

examination, Swain et.al.[37] could find no evidence for a third order term in the mutarotation of α-D-glucose in pyridine/pyridinium aqueous acetone buffers. Lienhard and Anderson[38] investigated the catalysis of the mutarotation of α-D-glucose by the monoanions of dicarboxylic acids, and found no unusually high catalytic effect which could be interpreted as evidence of concerted cyclic general acid-base catalysis. The catalysis was of the magnitude expected by summing the catalytic effect of an ionised and an unionised carboxy-group. Westheimer[39] had earlier obtained similar results in the amino-acid catalysed mutarotation of α-D-glucose.

However it appears that concerted general acid-base catalysis is more likely to be of significant mechanistic importance in non aqueous solvents. The observation of Swain and Brown[40], that the rate of mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in dry benzene was only slightly enhanced by the addition of catalysts such as pyridine or phenol, but was greatly increased if both these catalysts were added (cf. earlier observation by Lowry and Faulkner[47]), was interpreted by these authors to mean that concerted acid-base catalysis was operating. They predicted that such catalysis would be much more efficient if the phenolic and amine group were constructed, with the necessary geometry for action, in the same molecule. The now classical studies[41] of the mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in benzene catalysed by 2-pyridone confirmed this prediction with striking

results. In 0.05M 2-pyridone the "observed rate" is more than 50 times the "total rate" with a mixture of 0.05M pyridine and 0.05M phenol, while with 0.001M 2-pyridone the rate was 7000 times that calculated for 0.001M mixtures of pyridine and phenol. The authors further point out that this large effect is obtained despite the fact that 2-pyridone is one ten thousandth as strong a base as pyridine and one hundredth as stong an acid as phenol. These results have made this reaction the classic example of concerted general acid-base catalysis and a model for a possible source of the efficiency of enzyme catalysis.

However several authors have disagreed with the concept of concerted general acid-base catalysis postulated in the pyridine-phenol catalysed reaction. The studies of Pocker[42] and more recently Rony[43], have led these authors to postulate that catalysis of mutarotation by pyridine-phenol mixtures is not due to concerted acid-base catalysis but to general base catalysis by the phenoxide ion within a pyridinium-phenoxide ion pair. It is well known that the nucleophilic strength of ionic species is greatly increased in non polar solvents and that the mere presence of ion pairs often greatly accelerates rates in non polar solvents. Therefore it might not require a very high concentration of the pyridinium-phenoxide ion pair to account for the increased rate of mutarotation. Indeed evidence for a general base catalysed pathway in benzene solution seems reasonable in that Rony also observes catalysis by various aliphatic amine bases, and in addition notes that the

efficiency of the catalysis falls with increasing steric hindrance of the amine. For example, 1,4-diazabicyclo (2,2,2) octane though a weaker base (pK_a = 9.6) is sterically less hindered than triethylamine (pK_a = 10.7) and is approximately ten times more effective a catalyst. Such steric hindrance to general base catalysis is well known[48,49].

Clearly the results observed by Swain and Brown for the 2-pyridone catalysed mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in benzene cannot be explained by this ion pair hypothesis. But again Rony[50] disagrees with the authors' postulate of concerted acid-base catalysis and suggests that 2-pyridone derives its catalytic powers from the fact that it is a tautomeric molecule and that such catalysis be preferably named "tautomeric catalysis". Under this classification would come the examples of bifunctional catalysis by phosphate and bicarbonate already cited in this section, as well as many other examples of bifunctional catalysis[50]. One of the strongest pieces of evidence for the postulate of tautomeric catalysis is that 2-aminophenol has been reported as being a very much poorer catalyst than 2-pyridone both in the mutarotation reaction[51] and in the aminolysis of p-nitrophenyl acetate[50]. Since 2-aminophenol is a much stronger acid and base than 2-pyridone, Rony suggests that it is not the acid or base strengths of these molecules which determine their catalytic activity but their ability to exist as two tautomeric species.

However, Rony does agree with Swain and Brown in as much as he suggests that the proton transfers involved in his postulated tautomeric catalysis are concerted[50]. A possible test of the postulate of tautomeric catalysis will be discussed later (see page 297).

Bifunctional catalysis by 2-pyridone is by no means restricted to mutarotation. It is well known as a catalyst in the chemical synthesis of amides[53] and in peptide synthesis[54,55].

Catalysis of mutarotation in aqueous solution has also been observed when Lewis acids are present in solution[56,57]. The catalytic effect is proportional to the strength of the Lewis acid (aluminium chloride > zinc chloride > magnesium chloride) and the catalysis is entirely associated with a more favourable entropy term. Heterogeneous catalysis of mutarotation by finely dispersed copper has also been observed[58]. Finally the mutarotation of α-D-glucose in pyridine has been found to be autocatalytic[59].

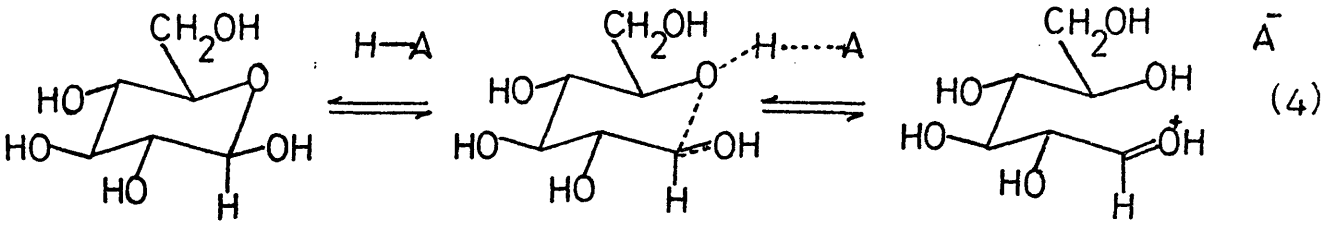
1.4 Isotope effects in mutarotation reactions

Isotope effects, arising from two sources - kinetic and solvent isotope effects - yield information about the mechanisms of many reactions. The kinetic isotope effect arises from differences in the energy required to stretch the normal and isotopic band in the corresponding transition states. The solvent isotope effect arises when the isotopic compound is used

both as a reactant and a solvent. It is well known that when reactions are studied in water and in deuterium oxide, a large kinetic isotope effect k_H/k_D will result if the bond joining the isotopic atom to the substrate is formed or broken in the rate determining step. Thus rate determining proton transfer is associated with a large kinetic isotope effect (typically of the order of 2-6). Similarly since the solvated deuteron is a stronger acid than the solvated proton[66], reactions which involve a proton transfer from catalyst to substrate prior to the rate determining step, are often characterised by an isotope effect which is less than unity. Thus the size of the isotope effect can give information about the timing of proton transfer steps in a mechanism.

Many studies have been made of the mutarotation of α-D-glucose in deuterium oxide and in water. It has been found that the value of k_H/k_D is dependent on the strength of the catalyst. For example, typical values for k_H/k_D are 1.37 for catalysis by H_3O^+, 2.60 for catalysis by acetic acid and 3.87 for catalysis by water[7].

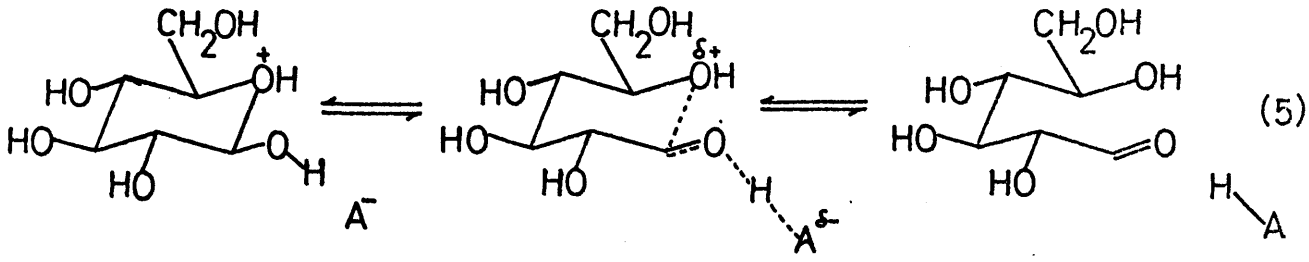
Explanations for these observed isotope effects vary. Bonhoeffer[60] and Bell[61] proposed that the k_H/k_D for H_3O^+ catalysis could be explained by a slow proton transfer from the acid to the ether oxygen of the sugar concerted with ring opening (Equation 4).



(4)

Since the DA bond will be stronger than the HA bond the ratio k_H/k_D will be greater than unity as observed.

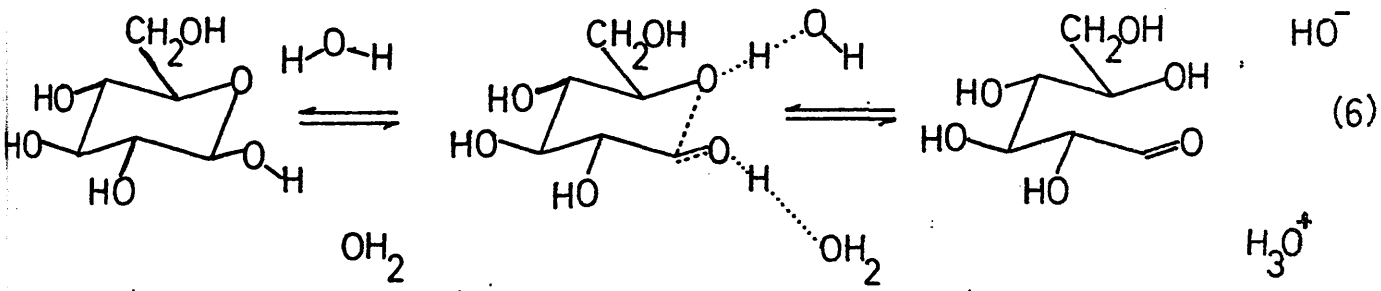
However other workers[62,63] prefer a mechanism which involves pre-equilibrium proton transfer to the ether oxygen with as a consequence a relatively larger concentration of the sugar conjugate acid in deuterium oxide. Were this the only factor bearing on the isotope effect, it would be expected to be less than unity. However the rate determining step which follows this pre-equilibrium protonation, involves the breaking of the anomeric O-H bond in water (O-D bond in deuterium oxide) with simultaneous ring opening (eq. 5), and this will obviously counterbalance the previous effect with a resultant isotope effect greater than one.



(5)

The theory behind these isotope effects has been laid out in detail by Long and Bigeleisen[63] and they show that the observed increase in the isotope effect with decreasing strength of the catalysing acid is wholly predicted by theory.

The very much higher isotope effect observed for water catalysis may arise from a mechanism in which water molecules act as both acid and base in a concerted mechanism (eq.6)



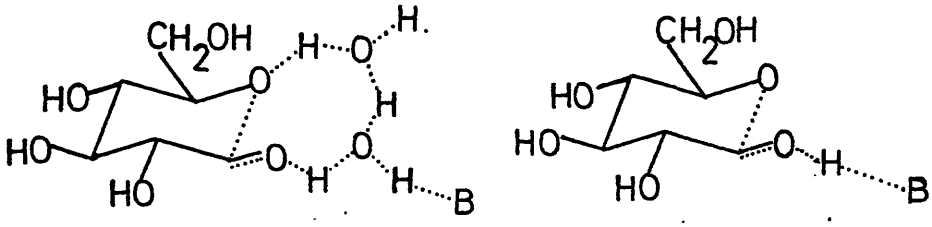
(6)

Clearly two proton (deuteron) transfers occur in the transition state and the isotope effect will therefore be high.

On the other hand it may arise merely through the operation of the mechanism depicted by eq. 5, the large isotope effect being anticipated because water is such a weak acid and the effect discussed by Long and Bigeleisen would be expected to be at a maximum.

The more recent developments in isotope effects in mutarotation have in the main been in the study of solvent isotope effects in mixtures of water and deuterium oxide. The detailed theoretical basis of such studies is complex and well documented[64,65]. Recent

studies have been made by Long[67], Huang[68] and co-workers on the mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in mixed H_2O-D_2O solvents with a variety of catalysts. The main purpose of such studies is to endeavour to distinguish between mechanisms which involve solvent molecules usually in a cyclic synchronous mechanism (see Fig. 2a) and those which do not invoke the presence of solvent molecules (Fig. 2b). Fig. 2 illustrates these mechanisms for the transition state of a base catalysed mutarotation.



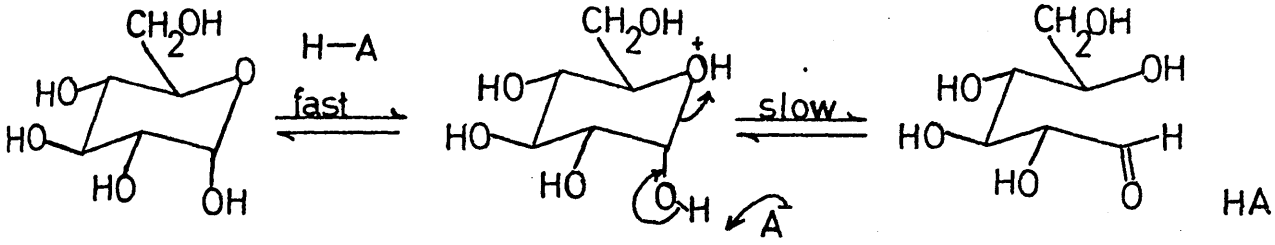
(a) Fig. 2 (b)

Such cyclic synchronous mechanisms have been invoked by several authors for mutarotation and for the closely analogous hydration (dehydration) of aldehydes (hydrated). More will be said of this in the subsequent section on the mechanism of mutarotation, but suffice to say that studies in mixed H_2O-D_2O solvents endeavour to find evidence for the number of water molecules (if any) present in the transition state of any particular mechanism by observing the change in k_n/k_H with $\underline{n}$ (where $\underline{n}$ = atom fraction of deuterium in the solvent and k_n/k_H = rate constant observed in water containing atom fraction $\underline{n}$ of deuterium divided by the rate constant observed in water). The studies of Huang found no particular cyclic transition state

(either with one, two or three water molecules) led to a better fit of the observed results than did the conventional mechanism of mutarotation. However the cyclic synchronous mechanisms do lead to better values for the "fractionation factors" (to some extent a reflection of the acidity of an exchangeable hydrogen atom in the transition state) than the conventional mechanism. The doubt about the true physical significance of the fractionation factor for a hydrogen atom being transferred in the transition state, and the presence of several adjustable parameters in the calculations makes meaningful conclusions from these studies unlikely. The difficulty in distinguishing between kinetically indistinguishable mechanisms involving solvent molecules still remains.

1.5 Mechanisms of mutarotation

One of the main problems associated with general acid and general base catalysis is the problem of distinguishing between kinetically indistinguishable mechanisms. Perhaps the first obstacle to determining the mechanism of catalysis in these reactions is to decide at what site the catalyst is acting. Mutarotation being a classical example of general acid-base catalysis is, not surprisingly, subject to these mechanistic problems. This is illustrated below in equations (7) and (8) which show two possible mechanisms for the general acid catalysed mutarotation of α-D-glucose.



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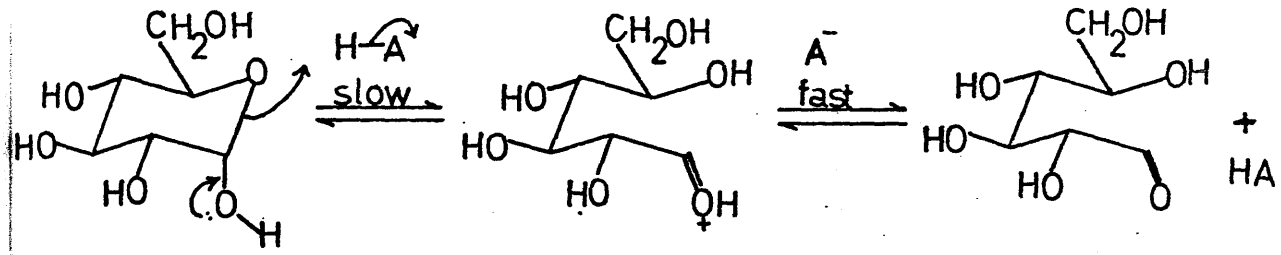


(8)

The evidence for the mechanism proceeding through an acyclic aldehydo form has already been discussed (section 1.2) and the mechanisms shown illustrate the reaction path only as far as this acyclic intermediate. The ring closure to give the β-anomer is a fast step and will occur by the same mechanistic pathway shown for the reverse reaction in the above mechanisms.

Clearly these two mechanisms differ in the site of the catalyst in the rate determining step and in the type of catalysis occurring in the rate determining step. In reaction (7) a fast pre-equilibrium proton transfer occurs and the rate determining step involves attack of the conjugate base of the catalyst on the conjugate acid of the sugar with simultaneous ring opening. Such

catalysis amounts to specific acid catalysis-general base catalysis of the reaction in the forward direction, and the catalyst is situated beside the C-1 hydroxy-group in the rate determining step.

The rate law for reaction (7) is

Rate = $k_s(SH^+)\ (A^-)$ ...(9) (where SH^+ is the conjugate acid of the sugar S. The equilibrium constants for dissociation of the general acid and of the conjugate acid of the sugar are

$$K_{HA} = \frac{(H^+)(A^-)}{(HA)} \quad \text{and} \quad K_{SH^+} = \frac{(H^+)(S)}{(SH^+)} \quad \ldots\ldots (10)$$

Substituting these in equation (9) one obtains

$$\text{Rate} = k_s \cdot \frac{K_{HA}}{K_{SH^+}} \times (S)\ (HA) \quad \ldots\ldots\ldots (11)$$

In reaction (8) the proton transfer from the general acid to the sugar occurs in the rate determining step with simultaneous ring opening. Such catalysis is true general acid catalysis and in this case the catalyst is situated near the sugar ring oxygen atom.

The rate law for reaction (8) is

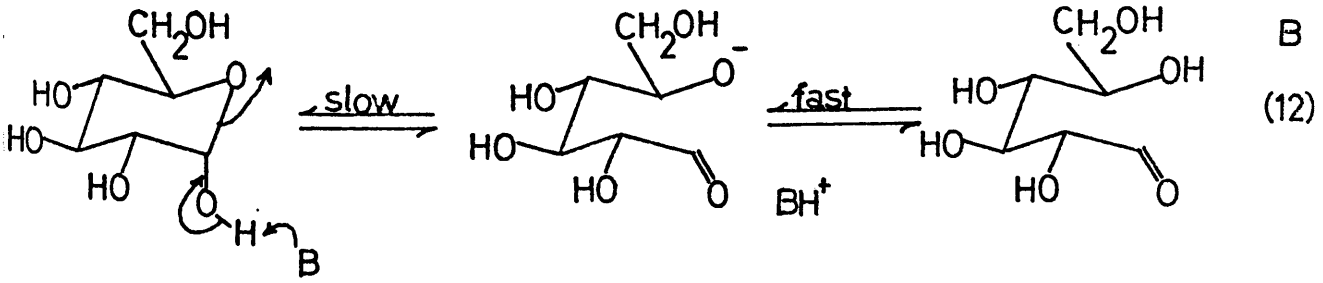
$$\text{Rate} = k'_s\ (S)(HA)$$

which is identical to the rate law for reaction (7) shown in equation (11) except that $k'_s = k_s \cdot \frac{K_{HA}}{K_{SH^+}}$

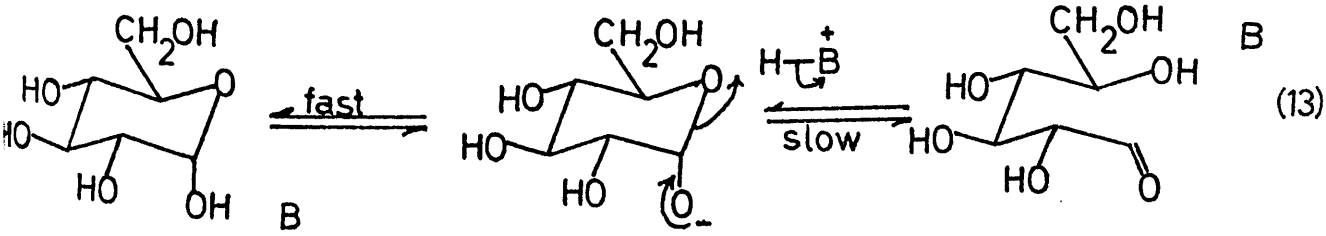
Thus despite the differences in mechanism and in the site of

the catalyst in the rate determining step both reactions follow identical rate laws and are indistinguishable by kinetic tests. It is interesting to note that the reaction (7) which is specific acid-general base catalysed in the forward direction is general acid catalysed in the reverse direction, while reaction (8) general acid catalysed in the forward direction is specific acid-general base catalysed in the reverse reaction. This is really a consequence of the law of microscopic reversibility whereby both the forward and reverse reactions must pass through the same transition state.

The same ambiguity of mechanism exists for general base catalysis. Equations (12) and (13) show two possible mechanisms for the general base catalysed mutarctation of α-D-glucose.



(12)



(13)

Reaction (12) represents true general base catalysis in the forward direction while reaction (13) represents specific base-general acid catalysis in the forward direction. The rate law for reaction (12) will be

$$\text{Rate} = k\,(S)(B) \quad \ldots\ldots\ldots\ (14)$$

while that for reaction (13) will be

$$\text{Rate} = k'(S^-)\,(BH^+)$$

$$= k' \frac{K_s}{K_{BH^+}} \cdot (S)\,(B) \quad \ldots\ldots\ldots\ (15)$$

where K_s is the dissociation constant of the sugar and K_{BH^+} the dissociation constant of the conjugate acid of the base catalyst. Thus again it can be seen that the two mechanisms are kinetically indistinguishable.

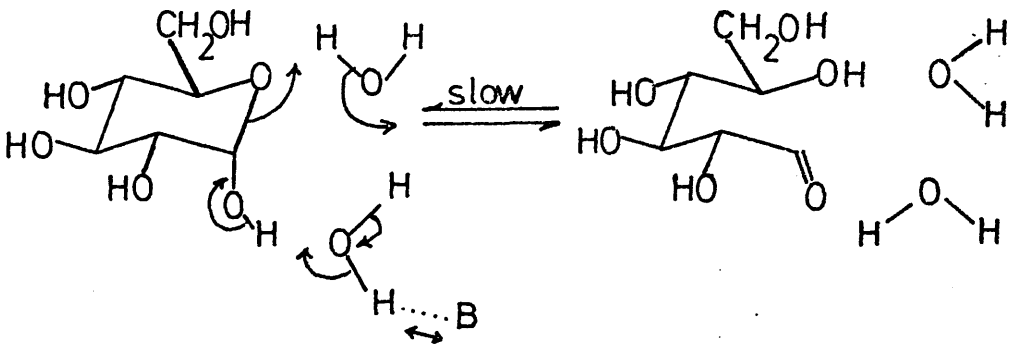
There are of course several techniques for resolving this kinetic indistinguishability for some reactions [69(a)]. Perhaps the most relevant one in the case of the mutarotation reaction is that of inspecting the reaction mechanism for thermodynamically unstable reaction intermediates and calculating whether or not this intermediate would react with another reactant at a rate greater than the diffusion controlled limit. Thus Eigen[70] calculates that for the dehydration of acetaldehyde hydrate a mechanism such as that depicted in equation (7) cannot hold for water catalysis since then the reaction of the conjugate base of the catalyst (HO^- for water as catalyst) with the conjugate acid

of the adlehyde hydrate would require to be faster than diffusion controlled. Since the dehydration of an aldehyde hydrate is a reaction highly analogous[67] to the mutarotation of an aldose this must cast some doubt upon a mechanism such as (7) for acid catalysed mutarotation. Jencks[69(b)] points out the possibility that catalysis by water may be an example not of acid catalysis but of base catalysis, whence a mechanism such as (7) would not be anticipated. He also calculates that for the dehydration reaction, the reverse of the rate determining step of mechanism (8) - reaction of the conjugate base of the catalyst with the conjugate acid of the aldehyde - would require to be faster than diffusion controlled. Thus by analogy, mechanism (8) would appear to be prohibited for acid catalysed mutarotation. Consideration of mechanisms (12) and (13) may lead to similar conclusions about the viability of these mechanisms. A detailed quantitative description of these calculations with respect to the mutarotation of α-D-glucose via all these mechanisms will be given in the discussion section.

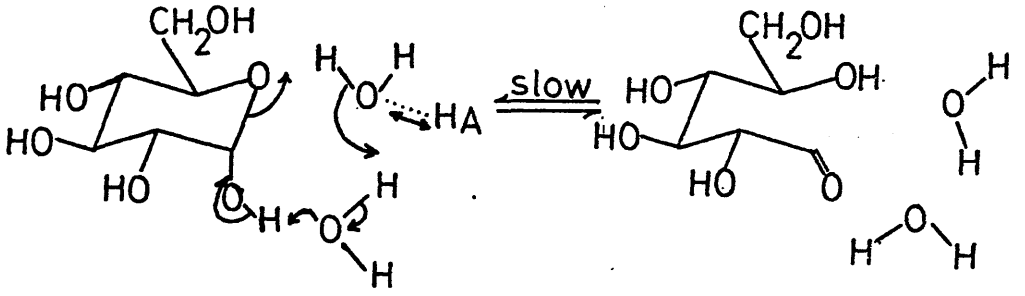
Due to the doubts cast on the acid and base catalysed mechanisms by such calculations as those above of Eigen and Jencks, other postulates have been made regarding the mechanism of such reactions. The "one-encounter" mechanism[69(b)] is exactly analogous to the mechanisms already postulated except that in each case the catalyst molecule brings about the fast proton transfer

steps required in mechanisms (7), (8), (12) and (13) in the same "encounter" with the sugar reactant as that in which the rate determining step occurs. The diffusion controlled limit is thus removed and any of the mechanisms may then occur.

A possibly more attractive way round the difficulties of the stepwise mechanisms (7), (8), (12) and (13) is the postulate of concerted mechanisms. The term "concerted" has been defined[70] as a correspondence between the motions of the protons involved in the reaction within times less than 10^{-10} seconds. To postulate that the reaction is simultaneously acid and base catalysed is clearly a way round the limiting values for diffusion controlled reactions which are possible stumbling blocks in the stepwise mechanisms. In aqueous solution it is an even more attractive postulate since one can easily formulate a mechanism of concerted acid-base catalysis with water acting either as the acid or as the base, thus utilising its amphoteric property. Such mechanisms could possibly include several water molecules in a cyclic concerted mechanism. The catalyst "triggers" the reaction and remains unchanged itself sitting outside the ring of water molecules. Two such mechanisms for the mutarotation of glucose might be envisaged as in equations (16) and (17)

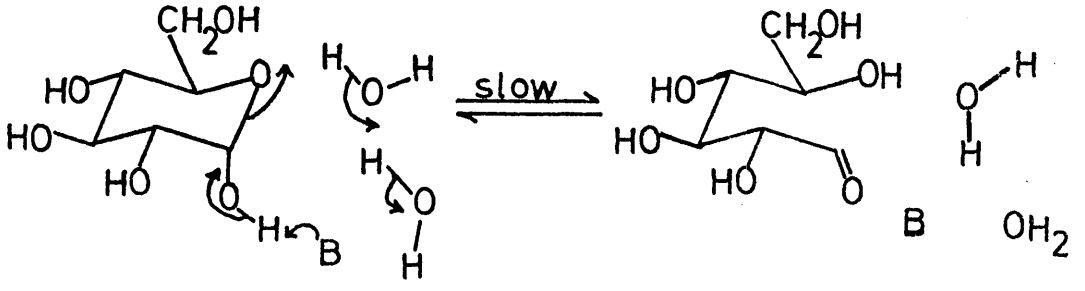


(16)



(17)

However it is difficult to reconcile this mechanism at least for general base catalysis with the previously observed (and further corroborated in the work reported here) steric hindrance to general base catalysis. The existence of this steric hindrance suggests that the catalyst base must be fairly close to the reaction centre so that some kind of cyclic mechanism such as (18) might be preferred.



(18)

Such mechanisms where the catalytic molecule sits outside a 'water shell' and acts as a catalyst by lining up the dipoles of solvent water to a greater or lesser degree depending on the strength of the catalyst, has received support from the work of Schmid[29]. He notes that the enthalpies of activation of the water, H_3O^+, formate, acetate, formic acid, acetic acid, glucosate and hydroxide ion catalysed reactions were all very similar and that differences in the catalytic coefficients of these species are in general due to differences in the entropies of activation. For example the catalytic coefficients for the glucosate ion and for water were 1.0 and 4.9×10^{-6} l.mol.$^{-1}$sec.$^{-1}$ respectively and their enthalpies of activation were 17.34 and 17.24 kcal/mol respectively. Clearly the difference of over 10^5 in the rate constants cannot be explained on the basis of activation enthalpies. Schmid proposes that the catalyses by these species are essentially catalyses by water - presumably proceeding by the same mechanism as the water catalysed mutarotation - with the catalyst lining up the water molecules' dipoles to a greater or lesser degree which reflects itself solely as an entropy factor. The same may not be true for amine bases since it is interesting to note that a similar analysis for ammonia as the catalytic species reveals that the increase in catalytic coefficient for ammonia over that for water (a factor of about 3×10^4) is due rather to a more favourable

enthalpy difference than to an entropy factor alone.

Thus the mechanistic problem still very much unsolved for the mutarotation reaction is the exact timing of the three processes occurring, namely - addition of a proton to the ring oxygen, breaking of the bond between C(1) and the ring oxygen (ring opening) and removal of a proton from the C(1) hydroxy group. Definite proof regarding this will not be easily obtained but an attempt to shine some light on this question of the timing of these processes using structure - reactivity relationships will be described in the work reported here.

The mechanism of mutarotation in non aqueous solvents has been fairly thoroughly outlined in section 1.3 and further reference will be made to it in the discussion section.

1.6 Mutarotase

The mutarotation of certain sugars is known to be catalysed by an enzyme which is found in the tissues of certain animals such as the kidney and liver of rats. A similar enzyme was isolated from penicillium notatum in preparations of D-glucose oxidase. This enzyme has been named 'mutarotase' and a small amount of work has been carried out on mutarotations catalysed by this enzyme.

From this work, mainly of Bentley and Bhate[73], it appears that the enzyme catalysed reaction is essentially similar to the spontaneous or acid-base catalysed mutarotation. Similar isotope effects were observed and by use of suitably labelled sugars, a

single displacement mechanism on C(1) was eliminated. The enzyme clearly has some manner of specificity in that it apparently acts as a catalyst in the mutarotation of sugars with the D-gluco-or D-galacto-configurations[74].

However meaningful work on this enzyme, which would appear to have a proton-transferring function, must await the easy availability of electrophoretically pure enzyme and ideally the three dimensional structure as well.

The foregoing discussion on aspects of mutarotation makes no attempt to be a comprehensive review of the vast amount of work which has been carried out on the subject. It merely highlights some of the more interesting aspects of mutarotation and the main points which are pertinent to the work reported here.

As has already been mentioned structure-reactivity relationships form the basis of some of the work in this thesis and it might therefore be useful to review briefly some of the pertinent work in the area of free energy relationships.

1.7 Free Energy Relationships

One of the most commonly used techniques of probing into the mechanistic details of a particular reaction is to systematically vary the structure of one of the reactants, keeping the other

variables of the reaction constant, and to study the kinetics of this reaction as such structural changes are made. The kinetic data are then expressed by one of many empirical equations, which enable the effects of structure change to be rationalised and predicted, and which are often used as an aid to distinguishing between possible mechanisms for the reaction. These structure-reactivity studies have given rise to the subject of "free energy relationships" which for the most part take the form of linear equations. The subject of linear free energy relationships has been fairly extensively reviewed[78-80] and only those points which provide a relevant background to the studies presented in this thesis will be included in this brief survey.

It has been realised since their inception that the theories on structure-reactivity are concerned with the correlation of potential energies of reacting systems. However real systems are governed by both potential and kinetic energies and so it is not entirely expected that the theories should give reliable predictions of chemical reactivity. Yet the success of linear free energy relationships in correlating reactivity with structural changes has been very considerable. Such correlations are of great value in storing and predicting rate and equilibrium data.

The variations of the standard free-energy change with a variable x can be expressed by

$$d\Delta G = \left(\frac{\delta \Delta G}{\delta x}\right)_T dx \quad \ldots\ldots\ldots (19)$$ at constant temperature.

For a finite change in x from some abitrary standard value x_o to x_1, the free energy change will be given by

$$\Delta G_1 - \Delta G_o = \left(\frac{\delta \Delta G}{\delta x}\right)_T (x_1 - x_o) \quad \ldots\ldots\ldots (20)$$

This represents a linear relationship between G and x (and hence between log k and x) provided $\left(\frac{\delta \Delta G}{\delta x}\right)_T = g_x^o$. remains constant within the range of variation of x.

Since the transition state theory gives the relationship between the rate constant (k) and the standard free energy of activation (ΔG^*).

$$\log k = \log \frac{RT}{Nh} - \frac{\Delta G^*}{2.3RT} \quad \ldots\ldots\ldots (21)$$

then combining equation (20) and equation (21) then for the same change in the variable x on a particular reaction A

$$\log (k_1/k_0)_A = g_x^A . (x_0 - x_1) \,/\, 2.3\, RT_A \quad \ldots\ldots\ldots (22)$$

where g_x^A is the susceptibility of reaction A to changes in x. Clearly for another reaction B a similar equation can be written:-

$$\log (k_1/k_0)_B = g_x^B . (x_0 - x_1) \,/\, 2.3\, RT_B \quad \ldots\ldots\ldots (23)$$

Combination of equations (22) and (23) leads to a linear free energy correlation between the two reaction series A and B.

$$\log (k_1/k_0)_B = \frac{g_x^B . T_{A_o}}{g_x^A . T_B} \log (k_1/k_0)_A \quad \ldots\ldots\ldots (24)$$

From eq. (24) can be readily seen the well quoted fact that all linear free energy relationships are based on the criterion that the free energy changes produced by a systematic variation of the

reactant structure of a particular reaction are related in a linear fashion to the free energy changes that the same variations bring about in another reaction.

Clearly for the two reactions A and B being carried out at the same temperature, equation (24) predicts a linear relationship between the free energy changes created in the two reactions by variation of x provided g_x^B/g_x^A remains constant throughout the range of variation of x. If one of the reactions, say A, is selected as a standard, equation (24) may be considered as a two parameter equation

$$\log (k_1/k_0)_B = CX_1 \quad \ldots\ldots\ldots\ldots\ldots\ldots (25)$$

where $C = g_x^B/g_x^A$ = the susceptibility of reaction B to changes in x relative to the standard reaction A, and $X_1 = \log (k_1/k_0)_A$ dependent only on the change in the variable x.

The foregoing is the derivation of a generalised relationship for linear free energy relationshops set up by Wells in his review.[78] One of the necessary conditions for a linear relationship to hold is that g_x^B/g_x^A be a constant, and the relative standard free energy change can be factored into relative enthalpy and entropy changes, we can say:-

$$\Delta G = \Delta H - T\Delta S$$

$$\therefore \quad (\delta\Delta G/\delta x)_T = (\delta\Delta H/\delta x)_T - T\,(\delta\Delta S/\delta x)_T$$

Since $g_x^B = (\delta\Delta G^B/\delta x)_T$ and $g_x^A = (\delta\Delta G^A/\delta x)_T$

then the requirement that, for a linear free energy relationship to

hold, g_x^B/g_x^A should be constant then implies[78] that the reactions should fall under one of the following categories:-

a) $(\delta\Delta S/\delta x)_T = 0$ whence the series are isoentropic.

b) $(\delta\Delta H/\delta x)_T = 0$ whence the series are isoenthalpic.

c) $(\delta\Delta S/\delta x)_T$ and $(\delta\Delta H/\delta x)_T$ are linearly related and the series are isokinetic.

As a measure of the lack of understanding of linear free energy relationships it can be noted that the reaction chosen as the standard reaction for Hammett relationships (see later) is not isoentropic and yet there are many reactions which correlate with this standard reaction in a linear free energy relationship.

Further, it has been pointed out[81] that a linear relationship between enthalpy and entropy although held by some[82] to exist, may in fact arise from experimental errors in determining the enthalpy. In any case examples are known[83] of reactions which exhibit linear free energy relationships but whose entropies and enthalpies cannot be correlated by the isokinetic relationship. And in taking this one stage further, Ritchie and Sager[79] have examined much of the available literature data to see whether many reactions can be classified as obeying an isokinetic relationship and simultaneously be correlated with a particular linear free energy relationship - namely the Hammett Equation. They found no reactions simultaneously obeying these two relationships, but found that there were many series of reactions which obeyed one of the relationships but not the other.

Thus it appears that there are reaction series which will obey a linear free energy relationship but are not isoentropic or isokinetic (for example the ionisation of substituted benzoic and acetic acids). Thus understanding of why the empirical free energy relationships, which are really potential energy models, can correlate rate and equilibrium data in reactions where clearly kinetic energies must also be taken into consideration is still lacking.

Perhaps the statistical mechanical treatment of Ritchie and Sager[79] holds at least part of the explanation. They found that the relationship between entropy and enthalpy changes was temperature dependent leading to an appreciable cancellation in $\Delta H - T\Delta S$ at all temperatures. They also claim to have shows that the free energy change of a system is a better measure of potential energy changes than the enthalpy change.

1.8 The Hammett and Taft Equations

The Hammett Equation was the first linear free energy relationship proposed, and it correlated the rate data obtained for the side-chain reactions of meta - or para - substituted derivatives of benzene[84]. The Hammett equation takes the form for rate data :-

$$\log (k/k_o) = \sigma\rho \qquad \ldots\ldots\ldots\ldots\ldots (26)$$

where k is the rate constant for the side chain reaction of a meta - or para - substituted benzene derivative, and k_o is the rate constant for the same reaction of the unsubstituted (parent) benzene compound. The relationship between equation (26) and the generalised equation (25)

can be seen readily, and it can be appreciated that σ corresponding to $\log (k_1/k_0)_A$ in eq. 25, measures the effect of changing the substituent, an effect which was considered to be purely a polar effect in meta - and para - derivatives; ρ corresponding to g_x^B/g_x^A in the generalised equation measures the susceptibility of the reactions to changes in σ, relative to the standard reaction A. Hammett chose the ionisation of benzoic acids in water at 25°C as this standard reaction for which ρ was then defined as unity. The value of $\log (K/K_o)$, (where K = ionisation constant of the substituted benzoic acid and K_o - ionisation constant of benzoic acid itself) then defined σ the variable corresponding to x in the generalised treatment. By 1953, several hundred reaction series[85] could be correlated with a fair degree of accuracy using this equation. However, certain deviations from this linear relationship were noted primarily in cases where the substituent and the reaction centre could interact mesomerically. Thus the σ values for certain para - substituents were found to be unsuitable for some reactions. This led to a mass of work in which new substituent constants such as σ^+, σ^-, σ^n, σ_R^o and σ_I to name but a few were defined and used in modified Hammett equations by various workers.[86-88]

It was found that linear free energy relationships such as these simple Hammett equations did not hold in aliphatic and ortho-substituted aromatic systems, an observation which was attributed to the existence of steric effects in addition to the polar effects

previously noted. The separation of the polar and steric effects in some of these systems was achieved by Taft[89]. Since this work is of most relevance to the work reported in this thesis it will be useful to summarise the main points briefly.

Taft chose as his field of study the hydrolysis of aliphatic esters, and derived the following equation for evaluating the polar effects of substituents R in the rates of hydrolysis of esters RCO_2R^1 :-

$$\sigma^* = {}^1/2.48 \left(\log (k/k_o)_B - \log(k/k_o)_A\right) \quad \ldots\ldots\ldots\ldots (27)$$

σ^* is a substituent constant indicating the net <u>polar</u> effect of the substituent (corresponding to the rate constant k) relative to that for the standard of comparison (k_o, R = CH_3). The subscripts B and A refer to otherwise identical alkaline and acidic hydrolysis. The factor 2.48 is a constant introduced in an attempt to put the polar effects so obtained on about the same scale as for the Hammett σ values.

The relationship (27) is based on three assumptions:-

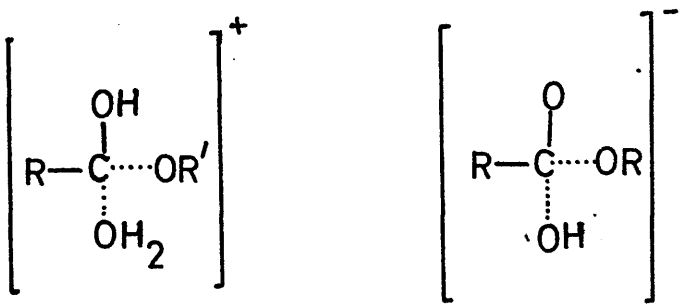
a) The relative free energy of activation may be treated as the sum of independent contributions from polar, steric and resonance effects.

b) In corresponding acidic and basic reactions, the steric and resonance effects are the same.

c) The polar effects of substituents are markedly greater in the basic than in the acidic series.

Certainly the first two assumptions are open to some doubt. The effects may not be independent of one another. e.g. there may be steric inhibition of resonance. If the effects interact linear analysis is unsuitable. However unless this simplifying assumption is made, no great progress is possible. The validity of the assumption is tested by the results obtained.

Assumption (b) lies at the heart of Taft's analysis. Taft justified it by pointing out that the transition states for the acidic and alkaline hydrolysis of esters only differed in the positions of two protons (Fig. 3). Since the size of these protons is small, he reasoned that the steric interaction in reaching the transition state should be the same.



Acidic Transition State Basic Transition State

Fig. 3

However the assumption of these constant steric and resonance effects has been criticised by Shorter.[90] Some of the work carried out by Shorter and his colleagues on the acid and base catalysed hydrolysis of methyl trans - and cis - 4 - t - butylcyclohexane

carboxylates in 1:3 dioxan - water[91] led them to suggest a difference in the steric effects in the acidic and basic reactions thus casting some doubt on Taft's assumption (b). Further work mainly on ortho - substituted benzene derivatives (e.g. hydrolysis of benzoates[92] and esterification of benzoic acids and phenylacetic acids[93]) led to the criticism that assumption (b) ignored the possible role of solvent. "Since the transition states of the acidic and basic reactions carry opposite charges it is unlikely that the solvation patterns will be so similar that the steric interactions in both systems will be the same". The same conclusion was reached by Noyes[94] whose studies showed that extreme differences in solvent structure existed around ions of opposite charge. Further references on the question of the validity of assumption (b) are given in Shorter's review[90].

However Taft considers that the validity of the assumptions (a) - (c) can be judged by the final results he obtains and the success he had in correlating the reactivity of aliphatic esters and ortho - substituted benzoates was undeniably considerable.

Assumption (c) receives support from the Hammett ρ constants for the hydrolysis of m - and p - substituted benzoates. The values for base catalysed hydrolysis are very much larger than those for acid catalysed hydrolysis where they lie close to zero in the range -0.2 to +0.5.[85] Taft uses this assumption, namely that the susceptibility of ester hydrolysis to polar effects is virtually zero, to postulate that the relative rate constants for acidic hydrolysis of

a substituted ester to the standard ester are near quantitative measures of the net steric effects between the two esters. He defines a steric substituent E_s by

$$E_s = \log (k/k_o)_A \quad \text{.....(28)}$$

E_s is a measure of the total steric effect associated with a given substituent relative to the standard of comparison which in Taft's work is the CH_3 group. Combining (27) and (28) gives the equation

$$\log (k/k_o)_B = E_s + 2.48\sigma^* \quad \text{.....(29)}$$

Once again the general validity of this definition of E_s is in some doubt with the observations[93,95] that polar effects sometimes make significant contributions to the term $\log(k/k_o)_A$.

However Taft was able, using the parameters σ^* and E_s derived as above, to correlate the rate constants for a large variety of reactions[89] with the equation

$$\log (k/k_o) = \sigma^* . \rho^* \quad \text{.....(30)}$$

ρ^* being the reaction constant analogous to the Hammett ρ constant. Conformity to equation (30) implies that all effects other than polar effects remain nearly constant throughout the reaction series. Indeed equation (30) is not really a linear free energy relationship for as defined σ^* measures "polar energy" which has been separated out from free energies. This of course means that steric effects of substituents must either be absent or constant within the range considered. Clearly substituents which can resonate with the reaction centre will cause breakdown of equation (30) as well. Thus equation

(30) represents a linear free energy - polar energy relationship and if one accepts the postulate that polar, steric and resonance effects are completely different, unrelated functions of structure, then the demonstration of a simple proportionality between a series of log k/k_o values and the corresponding Taft polar substituent constants σ^* implies that only one variable, the polar effect, is in operation. Deviations from this proportionality are taken by Taft to be indicative of variable steric and/or resonance effects, where the deviations are outwith the precision of equation (30) ($\pm$ 10%). This is not to say that because equation (30) is found to hold for a reaction series, there are no steric interactions between the substituent and functional group. These steric interactions may be present but because they make the same contributions to the free energies of both the reactants and the transition state the free energy difference between the two states will receive no contribution from steric interactions. The number of reaction series which can be correlated with equation (30) confirm the existence of this cancellation of steric effects.

Taft[89] proposed that some reactions could be correlated with the equation

$$\log (k/k_o) = \delta E_s \quad \ldots\ldots\ldots\ldots (31)$$

where δ = steric susceptibility constant. Clearly in such reactions polar effects of substituents are taken to be negligible and $\log (k/k_o)$ is a measure of the steric effect of a substituent relative to the standard of comparison. Such reactions - e.g. acid

catalysed methanolysis of β-napthyl esters - normally give a good correlation with equation (31) over a very limited range of substituents. Indeed in nearly every case the substituents are all alkyl groups of varying size. It is possible that the susceptibility of these reactions to polar effects is zero, but it is interesting to note Ritchie and Sager's suggestion[79] that the substituent constant for all alkyl groups be the same - on Taft's scale they would all be zero. This would of course also explain why a relationship such as (31) could hold for the limited range of alkyl substituents. This linear free energy - steric energy relationship (31) is thus clearly of rather limited applicability and it was suggested[97] that the equation

$$\log (k/ko) = \sigma^*.\rho^* + \delta.E_s \quad \ldots\ldots\ldots\ldots (32)$$

would have a wider range of applicability than either (30) or (31). The equation implies that the relative free energy of activation may be regarded as a sum of independent contributions from polar and steric effects. This follows also from the generalised equations of Wells[78] given in section 1.7. In particular he notes that equation (25) can be extended to correlate rate data of reactions in which more than one variable does not remain constant. This gives the generalised equation

$\log (k_1/k_0)_B = CX_1 + DY_1$ where in addition to the symbols used in equation (25) $D = g_y^B/g_y^A$ and measures the susceptibility of the reaction B to changes in y relative to the standard reaction A and $Y_1 = \log (k_1/k_0)_A$ dependent only on the change in the second

variable y.

Equation (32) still depends on equations (28) and (31) as accurate estimates of steric effects and behaviour. It has been found to fit well the rate data for the alkaline transesterification of 1-menthyl esters[97], and gives a poorer fit for the rate data for the base catalysed hydrolysis of anilides.[98] But Taft's prediction[89] that equation (32) was liable to be useful in correlating the effect of structure on reactivity in other series has had disappointingly little corroboration since it was made. An attempt to use this Taft separation of polar and steric effects on the reactivity of the series of sugars studied in this thesis will be described in the discussion. That resonance effects have not been discussed in this brief survey of the Taft and Hammett equations is because they are not relevant to the discussion of the studies reported herin. Full accounts on resonance effects can be readily obtained in existing reviews[78,89,90].

1.9 The Brönsted Equation

The Brönsted equation was really the first linear free energy relationship known, although at the time of its discovery it was not appreciated as such. It was developed empirically to correlate the acid and base strength of compounds with their effectiveness as catalysts in general acid-base catalysed reactions (e.g. mutarotation previously mentioned). It takes the form

$$k_a = G_A . K_a^{\alpha} \quad ; \quad k_b = G_B . K_b^{\beta} \quad ;$$

where k_a and k_b are the rate constants of an acid or base catalysed reaction respectively, K_a & K_b are the dissociation constants of the

acid and base respectively, G is a constant for the reaction and α and β are proportionality constants for the acid and base catalysed reactions respectively.

These equations are more often written :-

$$\left.\begin{aligned} \log k_a &= \log G_A + \alpha \log K_a \\ \log k_b &= \log G_B - \beta \log K_a \end{aligned}\right\} \quad \ldots\ldots\ldots (33)$$

It can be readily shown[99] for many reactions that the existence of a Brönsted relationship implies the existence of a free energy relationship between the rate constant for one of the individual steps of the reaction and the equilibrium constant for this same step (rate determining step). The proportionality constants α and β are equivalent to the term g_x^B/g_x^A in the generalised quation (25) and measure the susceptibility of the reaction to acid or base catalysis.

Another view of the Brönsted relationship is that it compares the free energy of an equilibrium for complete proton transfer to the free energy of a transition state which involves partial proton transfer (equations (34) and (35).

$$B + HA \rightleftharpoons BH^+ + A^- \quad \ldots\ldots\ldots\ldots (34)$$

$$S + HA \rightleftharpoons S^{\delta+} \ldots H \ldots A^{\delta-} \quad \ldots\ldots\ldots\ldots (35)$$

Much debate exists as to the interpretation of the magnitude of α or β in a reaction correlated by the Bronsted relation. In a kinetically specific acid catalysed reaction the observed rate is proportional to only the hydroxonium ion and catalysis by other acids is almost undetectable. This results in an α value of 1.0. At the other extreme when the observed rate is independent of all acidic

species including the hydroxonium ion (i.e. spontaneous reaction) α will be 0. For a reaction of this latter type the proton will be very much on the proton donor, whereas with specific acid catalysis the proton will be completely transferred. In general therefore as we go from reactions which are not acid catalysed at all to reactions which are general acid catalysed and finally to the specific acid catalysed reactions one would expect α to increase from 0 to 1.0. Clearly it is a great temptation to correlate the α value with the exact degree of proton transfer in the reaction, and indeed one finds authors[100,101,111] who make just that interpretation.

In considering the validity of such interpretations it is worthwhile considering the related Hammond postulate[102]. This postulate states that "if two states as for example, a transition state and an unstable intermediate occur consecutively during a reaction process and have nearly the same energy content, their interconversion will involve only a small reorganisation of the molecular structures". The most obvious deductions to be taken from this postulate are that endothermic processes should have transition states resembling the products of the reaction while exothermic processes should have transition states which bear a closer resemblance to the reactants. In the large majority of reactions a situation intermediate to this pertains. These processes are shown in Fig 4. A represents the endothermic reaction, B the exothermic and C the more usual thermally balanced reaction.

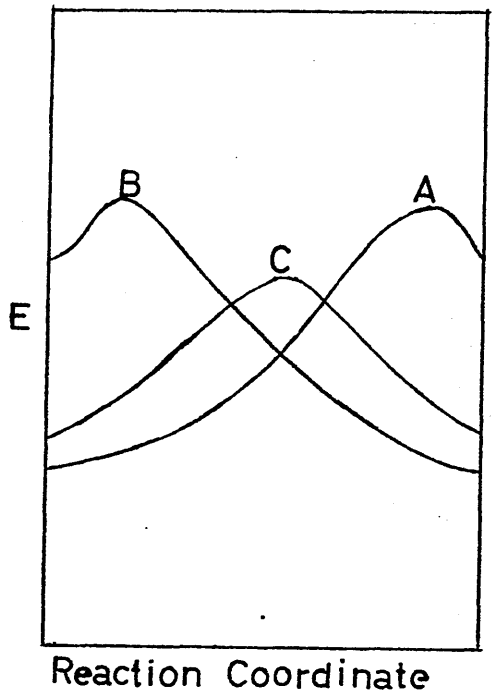


Fig. 4

To translate this into the sort of proton transfer reactions which correlate with the Brönsted equation, we might consider that A represented a reaction such as $B + H_2O \rightleftharpoons BH^+ + OH^-$ when B is a very much weaker base than hydroxide ion. Similarly curve B might represent a reaction such as $H_3O^+ + B \rightleftharpoons BH^{\oplus} + H_2O$ when $BH^{\oplus}$ is a very much weaker acid than H_3O^+. In the former reaction the Hammond postulate would predict that the transition state would be "product-like" and hence proton transfer would be considerably advanced. In the latter reaction the transition state would be "reactant-like" and proton transfer would not be far advanced. This corresponds to the often quoted statement that if a proton is being transferred from one basic site to another the transition state will be such that the proton lies nearer the weaker base. Clearly

then on this basis, as the pK difference between the donor and acceptor varied, the Hammond postulate would predict the position of the proton to change. If one accepts this postulate, then clearly it is contradictory to at the same time hold that the Brönsted α or β value, obtained by plotting rate constants for acid catalysis (say) against the pK_a of a systematically varied proton donor, is representative of an exact degree of proton transfer in the transition state of that set of reactions.

Kresgee[103] points out that the Brönsted exponent α is the ratio of substituent effects on the free energy of activation of a proton transfer process to substituent effects on its overall free energy change. i.e. ratio of substituent effects on ΔG^* to those on ΔG^o for the particular proton transfer process.. Although it is reasonable that the substituent effects on ΔG^* be less than those on ΔG^o there is no real reason for supposing that the relationship between these substituent effects be exactly linear - a condition which is required if α is to accurately reflect the degree of proton transfer.

Eigen has calculated that for proton transfer reactions extending over a wide range of pK differences between donor and acceptor, that the Brönsted exponent should vary between 0 and 1. This is readily understood when one first considers reactions of the hydrated proton with bases - a reaction which is well documented.[104,105]. These studies show that in most cases of the reaction of H_3O^+ with a base the proton transfer is diffusion controlled and hence the proton transfer is not

rate limiting α must be zero. Normally the pK_a of the acid formed by the transfer of a proton from H_3O^+ to a base is much higher than the $pK_{H_3O^+}$, but when the opposite is true (e.g. protonation of acetals or carbonyls) then the proton transfer is normally subject to specific acid catalysis with an α value of 1.0. Intermediate to these two extremes, when the pK difference between the donor and acceptor is small, the rate of proton transfer will depend on this difference and α will vary between 0 and 1.0. Such variations in α as the pKa difference between donor and acceptor changes have been observed,[106,107] but in addition there have been several reported cases of proton transfers where the data are correlated by a linear Brönsted plot with a unique α value over a very wide range of pKa.[108-110] As previously mentioned mutarotation falls into this latter class and it is this observation which prompts Eigen to propose a cyclic synchronous mechanism involving solvent molecules.

The preceeding arguments regarding the position of the proton in a proton transfer reaction are in conflict with the "solvation rule" of Swain et. al.[111] which in fact proposes that "a proton being transferred from one oxygen (or nitrogen) to another should lie in an entirely stable potential at the transition state and be closer to the more basic oxygen atom ....." This is in fact completely in contradiction to the Hammond postulate and all the previous considerations of this section. The solvation rule

appears to be based on the rather weak experimental evidence of a three point Brönsted plot for the base catalysed formation of tetrahydrofuran from 4-chlorobutanol. The points used in the plot are the catalytic constants for water, borate and hydroxide ion catalysis - just those points which are most frequently found to deviate from more conventional Brönsted plots! The Brönsted exponent is 0.25 which is taken by the authors to mean "that the transfer of the alcoholic proton is about 25% complete at the transition state." This being so - with the proviso that the statement is probably erroneous anyway (on the basis of this section's discussion) - the solvation rule if correct would require that the alcoholic oxygen be more basic than a hydroxide ion at the transition state. Since this seems also to be highly unlikely, it would appear that if the solvation rule does have any theoretical basis it is not the reaction of hydroxide ion with 4-chlorobutanol.

As a final note of warning on the interpretation of Brönsted exponents it should be noted that Bordwell et. al.[112] have reported that the base catalysed abstraction of a proton from substituted aryl nitro-alkanes yields Brönsted exponents of 1.31 and 1.61, the reverse reactions having the corresponding slopes less than zero -0.31 and -0.61. Thus this reaction demonstrates a greater sensitivity to structural change in its rates than in the position of equilibrium. Quite obviously the Brönsted exponent cannot be interpreted as the degree of proton transfer in the proton transfer reactions of these carbon acids. Further recent work on these nitroalkanes have corroborated

this conclusion.[113]

2. Experimental (Preparative)

General

Melting points were measured on a Kofler-Reichert hot stage melting point apparatus and are uncorrected.

I.R. spectra were measured using a Perkin Elmer 237 spectrometer and were calibrated with a polystyrene film.

N.M.R. spectra were determined as approximately 10% solutions on a 60 MHz Varian A-60 spectrometer. Chemical shifts were measured downfield from internal T.M.S. and are quoted in Hz. The chemical shift quoted for multiplets is normally the centre of that multiplet.

Elemental analyses were determined by Mr. J. Cameron, University of Glasgow, and are quoted as percentages.

Preparation of 1,2-O-isopropylidene-α-D-glucofuranose (I)

The acid catalysed condensation of glucose and acetone was carried out by the method of Mehltretter et. al.,[114] with the modification that after neutralisation of the reaction mixture, filtration to remove sodium sulphate and removal of the solvent in vacuo, the resulting red syrup was dissolved in ethyl acetate and acidified with concentrated nitric acid (10 ml). On standing at 0° for several days the 1,2-O-isopropylidene glucofuranose crystallised out in 30% yield. The crude product was recrystallised from ethyl acetate to give the pure product with m.p. 159° (lit.[114] 161°).

N.M.R. (d-5 pyridine)

2 singlets - 79, 90 Hz (6), isopropylidene methyls;

multiplet - 257 Hz (2);

multiplet - 184 Hz (3);

2 doublets - 292, 376 Hz (2), J_{ab} = 4Hz (H-2 and H-1);

singlet - 391 Hz (3), hydroxy-protons.

I.R. (Nujol Mull)

3440(s), 3320(s) (both OH stretch), 1282(m), 1270(m), 1245(m) 1225(s), 1160(m), 1114(w), 1085(s), 1062(s), 1038(s), 1004(s) 958(s) (C-O stretches), 881(m), 865(m), 852(m), 790 cm^{-1}

Preparation of 1,2-O-isopropylidene-6-O-(p-toluenesulphonyl)-α-D-glucofuranose (II).

Treatment of 1,2-O-isopropylidene-α-D-glucofuranose with p-toluenesulphonyl chloride in pyridine using the method of Ohle and Dickhauser[115] yielded the desired product in 33% yield. Recrystallisation from benzene gave the pure product with m.p. 106-107° (lit.[115] 108°).

N.M.R. (Deuterochloroform)

2 singlets - 78, 88 Hz (6), isopropylidene methyls;

singlet - 149 Hz (3), aromatic methyl;

broad singlet - 189 Hz (2), hydroxy-protons;

multiplet - 254 Hz (5), (H-3, H-4, H-5, H-6);

2 doublets - 271, 355 Hz (2), (H-2, H-1);

AA' BB' system - 437, 445, 465, 473 Hz (4), (p-substituted aromatic ring protons)

I.R. (Nujol Mull)

3525 (OH stretch), 1600 (aromatic double bond str.), 1330,

1172 (S = O stretch), 1220, 1102, 1067, 1022, 964, 905, 870, 829, 809, 791, 708 $cm.^{-1}$

Preparation of 6-azido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (III).

Displacement of the sulphonic ester of 1,2-O-isopropylidene-6-O-(p-toluenesulphonyl)-α-D-glucofuranose with the azide ion using the method of Cramer[116] gave the desired product in 70% yield. Recrystallisation from benzene yielded pure material with m.p. 105° (lit.[116] 104°).

N.M.R. (Deuterochloroform)

2 singlets - 79,90 Hz (6), isopropylidene methyls;

broad singlet - 205 Hz (2), hydroxy-protons;

doublet - 214 Hz (2), J_{ab} = 4Hz, (H-6);

multiplet - 244 Hz (2), (H-5, H-3 or H-4);

doublet - 261 Hz (1), J_{ab} = 2Hz, (H-4 or H-3);

2 doublets - 271, 356 Hz (2), J_{ab} = 4Hz (H-2, H-1);

I.R. (Nujol Mull)

3540 (OH stretch), 2190 (N N stretch), 1225, 1170, 1085, 970, 910, 872, 805, 692 cm^{-1}.

Analysis

Found C 43.88; H 5.98; N 16.89;

$C_9H_{15}N_2O_5$ requires C 44.08; H 6.17; N 17.13%

Preparation of 3, 5 di-O-acetyl-6-azido-6deoxy-1,2-O isopropylidene-α-D- glucofuranose (IV)

III was acetylated in the usual manner using pyridine and acetic anhydride to yield the desired product (IV) in 90% yield after recrystallisation from 50% methanol/water. m.p. 65-66°. (lit.[116] 66-67°).

N.M.R. (Deuterochloroform)

2 singlets - 79, 92 Hz (6), isopropylidene methyls;

singlet - 123 Hz (6), acetyl methyls;

multiplet - 214 Hz (2), (H-6);

2 multiplets - 267, 308 Hz (2), (1), (H-3, H-4, H-5);

2 doublets - 322, 355 Hz (2), J_{ab} = 4Hz, (H-2, H-1);

I.R. (Nujol Mull)

2130 (N ≡ N stretch), 1740 (carbonyl stretch of acetates), 1240, 1080, 1030 (C-O stretch), 970, 905, 875 cm^{-1}.

Preparation of 6-acetamido-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (V)

IV was hydrogenated using Raney Nickel (W-2 grade[117]) according to Cramer's[116] procedure. After recrystallisation from ethyl acetate the product (V) was obtained in 70% yield with m.p. 164° (lit[116] 164-5°).

N.M.R. (d-5-pyridine)

2 singlets - 74, 86 Hz (6), isopropylidene methyls;

singlet - 116 Hz (3), acetamido-methyl;

complex multiplets - 194 Hz - 279 Hz (7), (H-2, H-3, H-4, H-5, H-6 and N-H);

doublet - 355 Hz (1), J_{ab} = 3.5 Hz, (H-1);

broad peak - 392 Hz (2), hydroxy-protons;

I.R. (Nujol Mull)

3450 (OH stretch), 3340 (NH stretch), 1643, (amide carbonyl stretch), 1540 (NH bend), 1220, 1160, 1090, 1015, 955, 910, 865, 797 cm^{-1}.

Preparation of 6-acetamido-6-deoxy-β-D-glucose (VI)

Removal of the isopropylidene protecting group from V was accomplished using the mild method of Goodman[118] for the general removal of acetal and ketal groups.

V (2.5 g) was dissolved in 90% (v:v) trifluoroacetic acid - water (25 ml) and left for 15 minutes at room temperature. T.l.c. in ethyl acetate/ethanol/water (8/2/1) revealed quantitative conversion in this time. The solvent was then evaporated in vacuo ($< 45^{o}$) and traces of water and trifluoroacetic acid were entrained out by several coevaporations with absolute ethanol. The resulting syrup was dissolved in absolute ethanol, ether added to incipient turbidity and crystalline 6-acetamido-6-deoxy-glucose (1.6 g) was obtained on leaving overnight at 0^{o}. Recrystallisation from ethanol yields pure material of m.p. 200 - 202^{o}. Unlike the 6-acetamido-6-deoxy-glucose prepared by Cramer[116] (m.p. 182-83^{o}), compound VI would appear to be the "β" anomer. (N.M.R. and direction of mutarotation confirm this).

Analysis

Found	C 43.20;	H 6.76;	N 6.27;
$C_8H_{15}NO_6$ requires	C 43.44;	H 6.83;	N 6.33%

N.M.R. (d-6-dimethylsulphoxide)

singlet - 100 Hz (3), acetamido-methyl;

complex multiplet - 166 - 206 Hz (6), hydroxy-protons and H-6;

2 multiplets - 254, 287 Hz (4), ring protons;

doublet - 389 Hz, (1), J_{ab} = 6 Hz, (H-1);

broad singlet - 463 Hz, (1), (N-H);

I.R. (Nujol Mull)

broad 3500 - 3100 (OH, NH stretch), 1630 (amide carbonyl stretch) 1580 (N-H bend), 1170, 1075, 1035 (C-O stretches).

Preparation of 6-cyano-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (VII)

1,2-O-isopropylidene-6-O-(p-toluenesulphonyl)-α-D-glucofuranose (10.0 g, 0.026 mol) was dissolved in acetone (80 ml) and potassium cyanide (3.9 g, 0.06 mol) in water (70 ml) was added dropwise. The mixture was refluxed for 90 minutes, during which the reaction was followed closely by t.l.c. in ethyl acetate. At the end of 90 minutes the starting ester had reacted quantitatively and the acetone was removed under reduced pressure. The remaining aqueous solution was thoroughly extracted with ethyl acetate (6 x 80 ml). The combined extracts were washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure to give a syrup which crystallised overnight at 0°. Recrystallisation from benzene gave pure 6-cyano-6-deoxy-1,2-O-isopropylidene-α-D-glucofuranose (4.5g; 75%) m.p. 109° (lit.[119] 111-112°) R_f = 0.7 (ethyl acetate).

N.M.R. (deuterochloroform)

2 singlets - 79, 89 Hz (6), isopropylidene methyls;

multiplet - 167 Hz (2), (H-6);

broad singlet - 222 Hz (2), hydroxy-protons;

complex multiplets - 232-262 Hz (3), ring protons;

doublet - 271 Hz (1), J_{ab} = 4 Hz, (H-2);

doublet - 354 Hz (1), J_{ab} = 4 Hz, (H-1);

I.R. (Nujol Mull)

3500(s) (OH stretch), 2300(w) (C≡ N stretch), 1215, 1170, 1080, 1030 (C - O stretches), 900, 870, 790cm.$^{-1}$

Preparation of 6-cyano-6-deoxy-β- D-glucose (VIII)

The isopropylidene group of VII was removed by the usual treatment with 90% trifluoroacetic acid.[118] Coevaporation with ethanol yielded white crystalline material which can be recrystallised from absolute ethanol to give the required product. (β anomer) m.p. 148-149° (lit.[120] 147°) yield 80%.

Analysis

Found	:	C 44.40;	H 5.77;	N 7.40;
$C_7H_{11}NO_5$ requires	:	C 44.45;	H 5.86;	N 7.40%

N.M.R. (d-5 pyridine)

broad singlet - 193 Hz (2), (H-6);

complex multiplets - 225 - 195 Hz (4), ring protons H-2,H-3,H-4,H-5;

doublet - 318 Hz (1), J_{ab} = 6Hz, anomeric proton (β);

very broad singlet - 435 Hz (4), hydroxy-protons;

I.R. (Nujol Mull)

3440, 3300(s) (OH stretch), 2250(w) (C ≡ N stretch), 1310(m), 1155(m), 1110(s), 1085(s), 1055(s), 1030(m), 1015(m), (C-O stretches), 880 cm.$^{-1}$

Preparation of 6-deoxy-1,2-O-isopropylidene-α-D-gluco-hepturonic acid (IX)

Following the procedure of Prey and Szabolcs[119] the hydrolysis of the nitrile group of VII was achieved:-

VII (5g, 0.022 mol) was dissolved in a solution of barium hydroxide (3.95g in 50 ml), and heated at 90° for 4 hours. The solution was reduced in vacuo to approximately 10 ml and ethanol (200 ml) was added. The solution was again reduced in vacuo to approximately 20 ml and then diluted with ethanol (250 ml). The barium salt (5.8g ; 84%) of IX was thus precipitated, collected by filtration, washed with ethanol and ether and dried in air.

Preparation of 6-deoxy-α-D-gluco-hepturonic acid (X)

The isopropylidene group of IX was removed using sulphuric acid,[119] to yield crude 6-deoxy-α-D-gluco-hepturonic acid (3.2g) which on recrystallisation from absolute ethanol gave the pure product (mixed anomers) yield 2.8 g, 73% overall. m.p. 175-177° (lit.[119] 175-176°).

Analysis

Found	:	C 40.21;	H 5.94;
$C_7H_{12}O_7$ requires	:	C 40.39;	H 5.81%

N.M.R. (d-5 pyridine)

complex series of multiplets between 167 and 328 Hz;

doublet - 349 Hz ($\approx {}^{4}/_{5}$H), J_{ab} = 3.5 Hz, anomeric proton (α);

singlet - 500 Hz (5), hydroxy-and carboxy-protons;

The N.M.R. is difficult to interpret in detail at this level of resolution without further decoupling studies. However it appears that a mixture of α and β isomers is present in the ratio of approximately 4/1 (α /β).

I.R. (Nujol Mull)

3520(m) (weakly H bonded OH stretch), 3320(s) 3200(s) (strongly H bonded OH stretch), 1715(s) (carbonyl stretch), 1155(m), 1110(m), 1055(s), 1015(s) (C-O stretches), also carboxylic acid OH stretching 3000 - 2500 $cm.^{-1}$

Preparation of 6-chloro-6-deoxy-methyl-α-D-glucoside (XI)

This compound was prepared by the improved method of Evans, Long and Parrish.[121]

Methyl sulphonyl chloride (7.95 ml) was added dropwise to a stirred solution ofα-D-methyl glucoside in dry dimethyl formamide (9.0g in 60 ml) at 65°. After 16 hours at this temperature the mixture was concentrated to a syrup and after dissolving in methanol was treated with sodium methoxide to destroy formate esters. Chromatography on silica eluted with ethyl acetate/ethanol/water (45/5/3) yielded the desired product. Recrystallisation from n-propanol gave white needles in 65% yield. m.p. 114° (lit.[121] 113 - 114°).

N.M.R. (d-5 pyridine)

singlet - 207 Hz (3), glucoside methyl protons;

complex multiplets - 227 - 277 Hz (6), (H-6 and ring protons);

doublet - 306 Hz (1), J_{ab} = 3.5 Hz, (H-1);

broad singlet - 407 Hz (3), (hydroxy-protons);

I.R. (Nujol Mull)

3480 (shoulder), 3420(s), broad absorption 3500 - 3100 (OH stretch), 1346(m), 1337(m), 1287(m), 1232(w), 1184(w), 1142(m), 1104(m), 1086(m), 1042(s), 1017(s), 951(m), 757(m), 748(m), 712(w), 667(m) $cm.^{-1}$

Preparation of 6-chloro-6-deoxy-α-D-glucose (XII)

The glucoside XI was hydrolysed to the corresponding free sugar by a modification of the method of Helferich and Bredereck.[122]

6-Chloro-6-deoxy-methyl-α-D-glucoside (3.5 g) was dissolved in hydrochloric acid (10% by weight; 30 ml) and heated for three hours on a steam bath. The dark brown solution was treated with animal charcoal, filtered and the filtrate neutralised with barium carbonate. The aqueous solution was lyophilised and the resulting residue extracted with boiling ethanol. The insoluble salts are filtered off and the filtrate reduced in vacuo to yield a syrup which was crystallised with difficulty from acetone to give the desired product (α-anomer) m.p. 138° (lit.[122] 135 - 136°) yield 60%.

N.M.R. (d-5-pyridine)

complex multiplets - 232 - 292 Hz (6), (H-6 and ring protons);

doublet - 346 Hz (1), Jab = 3.5 Hz, (H-1);

very broad peak - 300 - 470 Hz (4), hydroxy-protons;

I.R. (Nujol Mull)

broad absorption 3600-3100(s), 1306(w), 1250(w), 1138(s), 1093(s), 1049(s), 1002(s), 953(m), 907(m), 839(w), 818(w), 763(m), 712(m), 660(m) $cm.^{-1}$

Preparation of 6-O-methyl-α-D-glucose (XIII)

1,2-O-Isopropylidene-6-O-(p-toluenesulphonyl)-α-D-glucofuranose (7.48 g, 0.02 mol) was dissolved in absolute methanol and to this solution was added sodium methoxide (3.24g, 0.06 mol) in absolute methanol (25 ml). The reaction was left overnight at room temperature when t.l.c. in ethyl acetate showed that the starting material (R_f = 0.85) was quantitatively converted into a main product (R_f = 0.5) with two minor contaminants. The methanol was evaporated in vacuo and the residue was extracted with dry acetone. The insoluble salts were filtered off and the filtrate neutralised with hydrochloric acid. The precipitated salts were again filtered off and the solvent evaporated in vacuo. The residue was chromatographed on silica, eluted with ethyl acetate, to give the compound with R_f = 0.5 pure. I.R. and N.M.R. spectra indicated that this compound was 1,2-O-isopropylidene-6-O-methyl-α-D-glucofuranose but all attempts to crystallise the compound failed. Accordingly the isopropylidene group was removed in the usual way to give crude 6-O-methyl-α-D-glucose (1.8g, 46%). Recrystallisation from ethanol gave pure product (1.5g) m.p. 141-2° (lit.[123] 142-3°).

Analysis

Found	:	C 43.21;	H 7.14;
$C_7H_{14}O_6$ requires	:	C 43.30;	H 7.27%

N.M.R. (d-5 pyridine)

singlet - 206 Hz (3), methoxy protons;

complex multiplet - 246 Hz (4), (H-6, 2 ring protons);

complex multiplet - 284 Hz (2), (2 remaining ring protons);

doublet - 353 Hz (1), J_{ab} = 3 Hz, anomeric proton;

broad singlet - 408 Hz (4), hydroxy-protons;

I.R. (Nujol Mull)

3420(s) (OH stretch), 1145(s), 1065(s), 1020(m) (C-O stretches), 870(w).

Preparation of 6-O-phenyl-α-D-glucose (XIV)

Phenol (3.76g, 0.04 mol) was dissolved in dry dimethylformamide (10 ml) and added dropwise to a cooled, stirred suspension of sodium hydride (0.96g, 0.04 mol) in dry dimethylformamide (20 ml) in a flask equipped with an efficient reflux condenser. After evolution of hydrogen has ceased, 1,2-O-isopropylidene-6-O-(p-toluenesulphonyl)-α-D-glucofuranose (7.5g, 0.02 mol) in dry DMF (10 ml) is added dropwise. The solution is allowed to come to room temperature slowly and then heated in a water bath at 45° for four hours.

The reaction mixture is diluted with water (200 ml) and thoroughly extracted with ethyl acetate (6 x 100 ml). The combined extracts were washed with 0.5N sodium hydroxide to remove the phenol present, then washed with water (2 x 100 ml), dried over anhydrous sodium sulphate to yield a pale yellow oil. T.l.c. in ethyl acetate reveals that the oil is homogeneous. (yield 3.8g, 62%). As previously experienced,[124] this oil proved extremely difficult to crystallise, and so was used for the next

stage of the reaction without isolation as a crystalline solid. Both I.R. and N.M.R. spectra indicated the oil to be 1,2-O-isopropylidene-6-O-phenyl-α-D-glucafuranose.

The isopropylidene group was removed in the usual way to yield crude 6-O-phenyl glucose (3.0g, 90%) as a white crystalline material. This was recrystallised from absolute ethanol to give 2.4g of pure 6-O-phenyl-α-D-glucose. m.p. 161 - 162° (lit.[124] 180°).

Analysis

Found : C 56.40; H 6.31;

$C_{12}H_{16}O_6$ requires : C 56.25; H 6.29%

N.M.R. (d-5-pyridine)

complex multiplets - 252 Hz, 285 Hz (6), (H-6 and ring protons);

doublet - 351 Hz (1), J_{ab} = 3.5 Hz, (H-1 proton);

multiplet - 384-444 Hz (5), (aromatic protons);

singlet - 430 Hz (4), (hydroxy-protons);

I.R. (Nujol Mull)

3390(s) (O-H stretch), 1600, 1588 (aromatic skeletal stretch), 1498(m), 1235(s), 1143(m), 1120(s), 1079(m), 1069(m), 1052(m), 1035(s), 1018(m), 1000(m), 940(w), 885(m), 860(m), 812(m), 752(s) (mono-substituted aromatic), 690(m) $cm.^{-1}$

Preparation of 6-O-(o-hydroxyphenyl)-β-D-glucose (XV)

6-O-(o-Hydroxyphenyl) glucose was prepared using the same method as was used to prepare 6-O-phenyl-α-D-glucose with the following alterations. Removal of catechol from the reaction products was not effected by alkali washing since the desired product was also soluble in

alkali. Instead, chromatography on silica eluted with 50% chloroform/ethyl acetate yielded pure 1,2-O-isopropylidene-6-O-(o-hydroxyphenyl)-α-D-glucofuranose in 55% yield as a brown-red gum which could not be induced to crystallise. Chromatography is also necessary since a few minor by-products are formed in the reaction. N.M.R. and I.R. spectra confirmed the identity and purity of the isolated material which was used as a gum in the next stage in which the isopropylidene group was removed in the usual manner. The resulting crude 6-O-(o-hydroxyphenyl)-β-D-glucose was recrystallised from ethanol/ether with difficulty to yield a white crystalline material with m.p. 153 - 154°. Overall yield = 45%.

Analysis

Found : C 53.03; H 5.91;

$C_{12}H_{16}O_7$ requires: C 52.94; H 5.92%

N.M.R. (d-5-pyridine)

broad singlet - 247 Hz (3), (ring protons);

complex multiplet - 283 Hz (3), (ring protons);

doublet - 309 Hz (1), Jab = 6.5 Hz, (anomeric proton);

complex multiplets - 393-441 Hz (4), (aromatic protons);

broad singlet - 400 - 550 Hz (5), (hydroxy protons);

I.R. (Nujol Mull)

3500 (shoulder), 3430(s), 3325(s) (OH stretch, H bonded), 1609, 1597, (C=C stretch), 1503(s), 1409(m), 1269(s), 1204(s), 1142(m), 1115(m), 1070(s), 1018(s), 927(m), 734(m) $cm.^{-1}$

Preparation of 6-deoxy-6-thioethyl-glucose (XVI)

1,2-O-isopropylidene-6-O-(p-toluenesulphonyl)-glucofuranose (7.5g, 0.02 mol) was dissolved in absolute ethanol (40 ml) and sodium thioethoxide (3.4g, 0.04 mol) in absolute ethanol (30 ml) was added dropwise with stirring. The solution was stirred overnight at room temperature and the resultant precipitate of sodium tosylate filtered off and the filtrate evaporated in vacuo. The residue is thoroughly extracted with anhydrous ether, the precipitated excess sodium ethoxide being filtered off. The combined ether extracts are washed with water, dried over anhydrous sodium sulphate and the ether evaporated in vacuo leaving a pale brown glass (5.0g, 94%). The N.M.R. (a) below shows clearly that this compound is 1,2-O-isopropylidene-6-deoxy-6-thioethyl-α-D-glucofuranose.

N.M.R.(a) (Deuterochloroform)

triplet	-	76 Hz (3), J=7.5 Hz, (methyl protons of C-5 side chain);
2 singlets	-	79, 89 Hz (6), isopropylidene methyls;
quartet	-	156 Hz (2), J=7.5 Hz, (methylene of thioethyl group);
doublet	-	172 Hz (2), J=4 Hz, (H-6);
complex multiplet	-	240 Hz (2), (ring protons);
broad singlet	-	261 Hz (1), (ring proton);
doublet	-	269 Hz (1), J_{ab} = 3.5 Hz, (H-2);
doublet	-	355 Hz (1), J_{ab} = 3.5 Hz, (H-1);
broad peak	-	368 Hz (2), (hydroxy-protons);

However all attempts to crystallise this glass failed and so

the isopropylidene group was removed using sulphuric acid[119] yielding 6-deoxy-6-thioethyl glucose (4.0 g, 80%) as an oil which defied all attempts to crystallise it over a period of eighteen months. The N.M.R.((b)below) showed the presence of both the "α" and "β" anomers.

N.M.R. (b) (d-5 pyridine)

triplet - 72 Hz (3), Jab = 8 Hz, (methyl protons);

complex series of multiplets between 140 Hz and 304 Hz which correspond to the ring and methylene protons of both isomers present;

doublet - 315 Hz ($\frac{2}{3}$), Jab = 6 Hz, (β anomeric proton);

doublet - 348 Hz ($\frac{1}{3}$), Jab = 3.5 Hz (α anomeric proton);

broad peak - 399 Hz (4), (hydroxy-protons);

Preparation of 2-O-methyl-β-D-glucose (XVII)

This compound was prepared by the four stage synthetic sequence of Hodge and Rist[125] in an overall 7% yield compared with the authors' 10%. The only alteration in the experimental procedure was that in the final stage, the preparation of 2-O-methyl-β-D-glucose from N-(2-O-methyl-D-glucosyl)-piperidine, the desired product could not be obtained crystalline unless the reaction mixture was purified by column chromatography on silica eluted with ethyl acetate/ethanol/water (8:2:1). The sugar then crystallised readily from ethanol-ethyl acetate to give the desired product in 7% yield (based on penta-acetyl-β-D-glucose) m.p. 159-60° (lit[125] 160°). Despite several recrystallisations the β-anomer could not be obtained pure but cocrystallised with the α-anomer.

Analysis

Found : C 43.10; H 7.24;

$C_7H_{14}O_6$ requires : C 43.30; H 7.27%

N.M.R. (d-5 pyridine)

The N.M.R. spectrum is complicated by the fact that two isomers are present. The main features are the two doublets corresponding to the α and β anomers.

α:- 354 Hz, Jab = 3.5 Hz; β:- 314 Hz, Jab = 7.5 Hz;

Ratio $\alpha/\beta = \frac{1}{2}$

I.R. (Nujol Mull)

3540, 3325 (s), (OH stretch), 1365(s), 1330(w), 1278(w), 1263(w), 1220(m), 1194(w), 1163(s), 1128(s), 1094(s), 1073(s), 1033(s), 971(s), 933(m), 921(m), 878(m), 789(m), 756(m), 720(m), 664(m) $cm.^{-1}$

Preparation of 2-deoxy-2-(p-toluenesulphonamido)glucose (XVIII)

The procedure of Takiura and Nakaniski[126] yielded the desired product as follows:-

2-amino-2-deoxy-glucose hydrochloride (6.3 g, 0.03 mol) and p-toluenesulphonyl chloride (5.8 g, 0.03 mol) were stirred together in a mixture of 50% aqueous acetone (40 ml). Sodium bicarbonate (5.2 g) was added and the mixture stirred overnight at room temperature. The reaction mixture was then evaporated in vacuo and the residue extracted with absolute ethanol. The insoluble salts were filtered off leaving approximately 8 grams of an oily residue which on t.l.c. (solvent - ethyl acetate/ethanol/water; 45/5/3) is very nearly homogeneous with R_f = 0.5. Due to difficulty experienced in removing completely the inorganic salts, this crude reaction product was chromatographed on a

silica column eluted with ethyl acetate/ethanol/water (40/10/3), whence pure crystalline material was obtained. This was recrystallised from ethyl acetate/ethanol to give very fine needle crystals with m.p. 168 - 69° (lit[126] 168-69°) yield (3.0 g, 29%).

Analysis

Found - C 45.24; H 5.91; N 3.88

$C_{13}H_{19}NSO_7$ requires C 46.85; H 5.71; N 4.20%

Despite prolonged drying at 120° in vacuo, the elements of water could not be removed from this compound.

N.M.R. (d-5 pyridine)

singlet - 128 Hz (3), (aryl methyl);

broad complex multiplets - 230 - 300 Hz (6) (ring protons);

very broad absorption - 230 - 440 Hz, (5), (hydroxy and N-H protons);

AA' BB' system - 420, 429, 482, 491 Hz (4), (p-substituted aromatic ring protons);

broad singlet - 337 Hz (1), (H-1).

I.R. (Nujol Mull)

3600 (shoulder) 3450(s), 1600(w), 1328(s), 1155(s), 1125(s), 1090(s), 1065(s), 1029(s), 1005(s), 956(w), 920(m), 857(m), 826(s), 790(m), 685(s) $cm.^{-1}$

3. Results and Kinetic Details

3.1 Kinetic Experimental

(a) Solutions and Buffers

All chemicals used for the preparation of buffered and other solutions were of the highest grade commercially available. Analar pyridine was dried by refluxing over potassium hydroxide and fractionally distilling twice with a Fenske column. 4-methylpyridine obtained from Koch-Light Ltd. was similarly purified. 4-acetylpyridine (Koch-Light Ltd.) was fractionally distilled several times and 4-ethoxypyridine was prepared by refluxing 4-chloropyridine hydrochloride (Koch-Light Ltd.) with three equivalents of sodium ethoxide in ethanol, the product again being purified by several fractional distillations. All these 4-substituted pyridines were subjected to vapor phase chromatography to check their purity. All were found to be homogeneous - details in Table 222. 2,6-lutidine was purified and checked in the same way as pyridine. Morpholine and diethanolamine (B.D.H. Ltd.) were purified by refluxing over potassium hydroxide and fractionally distilling. Tris obtained from B.D.H. Ltd. was used without further purification. For all buffer solutions only degassed distilled water was used and the ionic strength was maintained at a particular ionic strength with potassium chloride.

D_2O and 20% DCl were purchased from Koch-Light, and the purity of the solutions checked by adding dioxan as a standard and measuring the quantity of water by N.M.R. This was always less than 0.3%.

(b) pH measurements

The pH of all buffer solutions was measured at the temperature of the kinetic experiment with a Radiometer model 26 pH meter, with an

external temperature compensator. A Radiometer type G 202C glass electrode was used together with a type K401 calomel electrode. The pH meter was standardised against commercial standard buffers complying to BS, 1647, 1961.

(c) Polarimetric Rate Determinations

All of the reported rate constants were determined on a Perkin Elmer 141 polarimeter fitted with a transmitting potentiometer which allowed the optical rotation to be continuously monitored on a strip chart recorder. The reaction cell of the polarimeter was equipped with a water jacket through which water at a constant temperature was circulated from a Lauda electronic thermostatting bath. The temperature difference between the reaction cell and the bath was measured using a thermocouple and was found to be less than 0.1° at 25°. The temperature in the bath was measured with a National Physical Laboratory calibrated thermometer.

The output from the polarimeter was fed into a Solartron Compact Data Logger which digitised the optical rotation reading. The latter was then transferred on to 5-channel paper tape, via a Creed punch, at convenient time intervals. Normally between 100 and 700 values were punched but for kinetic measurements read off the chart recorder between 30 and 50 values were taken.

The first order rate constants were determined using a generalised least squares program, written by Dr. B. Capon following the procedure of Wentworth[127] and Deming[128]. This program was written to fit the

function

$$k = (1/t)\ln (r_o - r_\infty) / (r_t - r_\infty)$$

where t and r_t are variables and k, r_o and r_∞ are constants r_o = initial rotation, r_∞ = equilibrium rotation, r_t is the rotation at time t. Evaluation was performed on an English Electric KDF 9 computer. The slopes and intercepts of plots of $k_{obsd.}$ against buffer concentration were also determined by a generalised least squares procedure.

(d) Non Aqueous Solvents

The dioxan used was Merck 'spectrograde' dioxan and was used without further purification. Analar sodium dried benzene was further dried by refluxing with phosphorus pentoxide and fractional distillation.

(e) Substrates

The sugars not prepared as detailed in the experimental section, were obtained commercially from Koch Light Ltd. and B.D.H. Ltd. They were further purified by recrystallisation from aqueous alcohol.

(f) Procedure

The buffer was equilibrated in the thermostatting temperature bath for half an hour before use. Approximately 1 ml was pipetted into an ampoule containing approximately 0.02 moles of the sugar which was quickly dissolved. The solution was immediately pipetted with a fine pipette into the reaction cell whose volume was just less than 1 ml. Any air bubbles trapped in the cell were removed by shaking. The optical rotation changes were normally followed at a wavelength of

365 nm (mercury vapor lamp) to obtain the largest possible rotational change, but where the solution of substrate absorbed in that region of the U.V. spectrum, a higher wavelength had to be used.

(g) N.B. In all the following results tables, the units of $k_{obsd.}$ and $k_{calc.}$ are sec^{-1} unless otherwise stated.

3.2. Results

Table 1

The HCl catalysed mutarotation of 6-deoxyα-D-glucose at 25° in water, I = 0.1M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	0.98	2.57, 2.77	2.72
0.080	1.09	2.31, 2.36	2.36
0.060	1.21	2.04, 1.88	1.99
0.040	1.40	1.66, 1.68	1.63
0.020	1.68	1.28, 1.31	1.27
0.010	1.98	1.08, 1.08	1.09

$k_{H_3O^+}$ = 1.808×10^{-2} M^{-1} sec^{-1} ; S. D. = 0.65%

k_{int} = 9.09×10^{-4} sec^{-1} ; S. D. = 0.35%

Table 2

The HCl catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	0.98	4.78, 4.81	4.79
0.080	1.09	4.10, 4.11	4.10
0.060	1.21	3.34, 3.36	3.41
0.040	1.40	2.73, 2.76	2.72
0.020	1.68	2.04, 2.04	2.03
0.010	1.98	1.67, 1.63	1.68

$k_{H_3O^+}$ = 3.459×10^{-2} M^{-1} sec^{-1} ; S. D. = 0.49%

k_{int} = 1.33×10^{-3} sec^{-1} ; S. D. = 0.32%

Table 3

The HCl catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	15.91, 14.81	15.07
0.080	1.09	12.85, 12.62	12.85
0.060	1.21	10.74, 10.70	10.64
0.040	1.40	8.41, 8.02	8.42
0.020	1.68	6.34, 6.24	6.21
0.010	1.98	4.95, 5.25	5.11

$k_{H_3O^+}$ = 1.106×10^{-2} M^{-1} sec^{-1} ; S. D. = 0.86%

k_{int} = 4.00×10^{-4} sec^{-1} ; S. D. = 0.87%

Table 4

The HCl catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.1M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	11.91, 12.15	12.10
0.080	1.09	10.53, 10.21	10.36
0.060	1.21	8.76, 8.71	8.63
0.040	1.40	6.97, 6.74	6.90
0.020	1.68	5.20, 5.20	5.17
0.010	1.98	4.28, 4.31	4.31

$k_{H_3O^+}$ = 8.66×10^{-3} M^{-1} sec^{-1} ; S. D. = 0.68%

k_{int} = 3.44×10^{-4} sec^{-1} ; S. D. = 0.70%

Table 5

The HCl catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	10.02	10.23
0.080	1.09	8.80	8.69
0.060	1.21	7.16	7.15
0.040	1.40	5.63	5.61
0.020	1.68	3.95	4.06
0.010	1.98	3.30	3.28

$k_{H_3O^+}$ = 7.72×10^{-3} M^{-1} sec^{-1} ; S. D. = 1.40%

$k_{int.}$ = 2.51×10^{-4} sec^{-1} ; S. D. = 1.67%

Table 6

The HCl catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	11.17, 11.07	11.05
0.080	1.09	9.77, 9.45	9.46
0.060	1.21	7.62, 7.93	7.87
0.040	1.40	6.26, 6.14	6.29
0.020	1.68	4.85, 4.77	4.70
0.010	1.98	3.91, 3.86	3.90

$k_{H_3O^+}$ = 7.94×10^{-3} M^{-1} sec^{-1} ; S. D. = 0.57%

$k_{int.}$ = 3.11×10^{-4} sec^{-1} ; S. D. = 0.51%

Table 7

The HCl catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	8.57, 8.49	8.56
0.080	1.09	7.28, 7.22	7.34
0.060	1.21	6.11, 6.25	6.13
0.040	1.40	4.88, 4.97	4.91
0.020	1.68	3.77, 3.68	3.70
0.010	1.98	3.04, 3.16	3.09

$k_{H_3O^+}$ = 6.07×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.84%

$k_{int.}$ = 2.48×10^{-4} sec^{-1} ; S.D. = 0.55%

Table 8

The HCl catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 0.10M

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	7.49, 7.61	7.43
0.080	1.09	6.48, 6.58	6.40
0.060	1.21	5.36, 5.36	5.37
0.040	1.40	4.13, 4.38	4.34
0.020	1.68	3.16, 3.26	3.31
0.010	1.98	2.85, 2.83	2.79

$k_{H_3O^+}$ = 5.16×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.61%

$k_{int.}$ = 2.28×10^{-4} sec^{-1} ; S.D. = 0.43%

Table 9

The HCl catalysed mutarotation of 6-cyano-6 deoxy-β-D-glucose at 25° in water, I = 0.10M.

(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	4.33, 4.31	4.32
0.080	1.09	3.68, 3.72	3.72
0.060	1.21	3.23, 3.17	3.13
0.040	1.40	2.48, 2.54	2.53
0.020	1.68	1.88, 1.92	1.93
0.010	1.98	1.60, 1.66	1.63

$k_{H_3O^+}$ = 2.98×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.70%

$k_{int.}$ = 1.34×10^{-4} sec^{-1} ; S.D. = 0.49%

Table 10

The HCl catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M

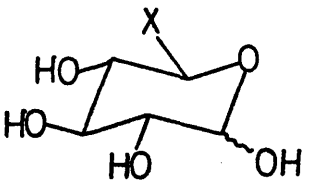
(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	13.43, 13.27	13.49
0.080	1.09	11.46, 11.84	11.53
0.060	1.21	9.59, 9.65	9.57
0.040	1.40	7.58, 7.65	7.60
0.020	1.68	5.69, 5.45	5.64
0.010	1.98	4.77, 4.54	4.66

$k_{H_3O^+}$ = 9.82×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.73%

$k_{int.}$ = 3.68×10^{-4} sec^{-1} ; S.D. = 0.61%

Table 11

Comparison of $k_{H_3O^+}$ and k_{H_2O} for the series of substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CO_2H$	$-CH_2CN$
$k_{H_3O^+} \times 10^3$ (M^{-1} sec^{-1})	18.08	34.59	11.06	8.66	7.72	7.94	6.07	5.16	2.98
$k_{H_2O} \times 10^6$ (M^{-1} sec^{-1})	16.38	24.03	7.21	6.20	4.53	5.60	4.48	4.10	2.41
σ	-0.05	0.00	0.05	0.07	0.07	0.11*	0.15	0.17	0.18

* estimated from the data of Charton.[129]

Table 12

The Tris catalysed mutarotation of α-D-glucose at 25° in water,

I = 0.10M. (Tris) : (Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.08	13.67, 13.75	13.64
0.048	8.10	11.96	11.92
0.036	8.10	10.24, 10.29	10.20
0.024	8.11	8.50, 8.49	8.48
0.012	8.10	6.76, 6.74	6.76
0.006	8.11	5.91	5.90

k_{Tris} = 1.43×10^{-2} M^{-1} sec^{-1} ; S.D. = 0.84%

$k_{int.}$ = 5.04×10^{-4} sec^{-1} ; S.D. = 0.47%

Table 13

The Tris catalysed mutarotation of α-D-glucose at 25° in water,

I = 0.10M. (Tris) : ($TrisH^+$) = 1.0.

(Tris) M	p_H25	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.33	14.57	14.57
0.050	8.33	13.05	13.09
0.040	8.32	11.61	11.61
0.030	8.34	10.16	10.14
0.010	8.34	7.18	7.18

k_{Tris} = 1.48×10^{-2} M^{-1} sec^{-1} ; S.D. = 1.2%

$k_{int.}$ = 5.70×10^{-4} sec^{-1} ; S.D. = 0.68%

Table 14

The Tris catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (Tris) : (Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.068	8.56	16.85	16.83
0.051	8.57	14.36	14.38
0.034	8.57	11.94	11.94
0.017	8.57	9.50	9.49
0.0085	8.55	8.19	8.19

$k_{Tris} = 1.44 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.39%

$k_{int.} = 7.04 \times 10^{-4}\ sec^{-1}$; S.D. = 0.68%

Table 15

The Tris catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (Tris) : (Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	2.47, 2.52	2.48
0.085	8.87	2.22, 2.23	2.23
0.068	8.87	1.99, 2.00	1.98
0.051	8.87	1.68, 1.74	1.73
0.034	8.875	1.49, 1.48	1.48
0.017	8.88	1.23, 1.22	1.23

$k_{Tris} = 1.48 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.21%

$k_{int.} = 9.75 \times 10^{-4}\ sec^{-1}$; S.D. = 0.87%

Table 16

The Tris catalysed mutarotation of α-D-glucose at 25° in water,

I = 0.10M. (Tris) : (Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	3.85, 3.48	3.63
0.106	9.17	3.09, 3.14	3.19
0.079	9.17	2.69, 2.72	2.73
0.053	9.16	2.32, 2.26	2.29
0.026	9.16	1.89, 1.88	1.83
0.013	9.14	1.58, 1.59	1.61

$k_{Tris} = 1.69 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.81%

$k_{int.} = 1.39 \times 10^{-3}\ sec^{-1}$; S.D. = 0.52%

Table 17

The Tris catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water,

I = 0.10M. (Tris) : (Tris) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.08	1.56	1.57
0.048	8.10	1.45	1.44
0.036	8.10	1.32	1.31
0.024	8.11	1.17	1.18
0.012	8.10	1.06	1.05
0.006	8.11	0.99	0.99

$k_{Tris} = 1.07 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.95%

$k_{int.} = 9.23 \times 10^{-4}\ sec^{-1}$; S.D. = 0.15%

Table 18

The Tris catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.1 M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	1.63	1.61
0.050	8.33	1.50	1.51
0.040	8.32	1.39	1.40
0.030	8.34	1.27	1.29
0.010	8.34	1.08	1.08

$k_{Tris} = 1.07 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.09%

$k_{int.} = 9.71 \times 10^{-4}\ sec^{-1}$; S.D. = 0.32%

Table 19

The Tris catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	1.71	1.76
0.051	8.57	1.58, 1.57	1.57
0.034	8.57	1.40, 1.41	1.38
0.017	8.57	1.20	1.20
0.0085	8.55	1.10, 1.09	1.10

$k_{Tris} = 1.10 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.97%

$k_{int} = 1.01 \times 10^{-3}\ sec^{-1}$; S.D. = 0.28%

Table 20

The Tris catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	2.26	2.27
0.085	8.87	2.07	2.08
0.068	8.87	1.90	1.89
0.051	8.87	1.72	1.70
0.034	8.875	1.52	1.51
0.017	8.88	1.32	1.32

$k_{Tris} = 1.12 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.15%

$k_{int.} = 1.13 \times 10^{-3}\ sec^{-1}$; S.D. = 0.43%

Table 21

The Tris catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.1M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	2.90	2.86
0.106	9.17	2.51	2.56
0.079	9.17	2.25	2.25
0.053	9.16	1.95	1.95
0.026	9.16	1.67	1.65
0.013	9.14	1.48	1.50

$k_{Tris} = 1.14 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.03%

$k_{int.} = 1.35 \times 10^{-3}\ sec^{-1}$; S.D. = 0.44%

Table 22

The Tris catalysed mutarotation of α-D-xylose at 25°in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.08	6.84, 6.92	6.87
0.048	8.10	5.84, 5.87	5.91
0.036	8.10	4.93, 5.03	4.95
0.024	8.11	4.03, 4.00	3.99
0.012	8.10	3.00, 3.04	3.03
0.006	8.11	2.54, 2.57	2.54

$k_{Tris} = 8.01 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.62%

$k_{int.} = 2.06 \times 10^{-3}\ sec^{-1}$; S.D. = 0.49%

Table 23

The Tris catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	7.22, 7.19	7.39
0.050	8.33	6.57, 6.61	6.56
0.040	8.32	5.82, 5.76	5.73
0.030	8.34	5.01, 4.92	4.90
0.010	8.34	3.28, 3.16	3.25

$k_{Tris} = 8.29 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.73%

$k_{int.} = 2.42 \times 10^{-3}\ sec^{-1}$; S.D. = 0.62%

Table 24

The Tris catalysed mutarotation of α-D-xyclose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	8.63, 8.69	8.68
0.051	8.57	7.28, 7.31	7.31
0.034	8.57	5.95, 5.94	5.95
0.017	8.57	4.57, 4.67	4.58
0.0085	8.55	3.88, 3.80	3.85

$k_{Tris} = 8.04 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.77%

$k_{int.} = 3.21 \times 10^{-3}\ sec^{-1}$; S.D. = 0.37%

Table 25

The Tris catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	13.70, 13.47, 12.88	13.61
0.085	8.87	11.96, 12.28, 12.22	12.16
0.068	8.87	10.69, 10.65, 11.10	10.70
0.051	8.87	9.34, 9.30	9.25
0.034	8.875	7.79, 7.99, 7.71	7.79
0.017	8.88	6.42, 6.40, 6.12	6.33

$k_{Tris} = 8.56 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.73%.

$k_{int.} = 4.88 \times 10^{-3}\ sec^{-1}$; S.D. = 0.59%.

Table 26

The Tris catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.079	9.17	14.47 14.62	14.91
0.053	9.16	12.63, 12.43, 12.23	12.65
0.026	9.16	10.69, 10.68	10.30
0.013	9.14	8.97, 8.81	9.17

$k_{Tris} = 8.69 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.91%.

$k_{int.} = 8.04 \times 10^{-3}\ sec^{-1}$; S.D. = 0.62%

Table 27

The Tris catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.08	13.62	13.39
0.048	8.10	11.44	11.52
0.036	8.10	9.67	9.64
0.024	8.11	7.59	7.76
0.012	8.10	6.06	5.89
0.006	8.11	4.91	4.95

$k_{Tris} = 1.56 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.92%

$k_{int.} = 4.01 \times 10^{-4}\ sec^{-1}$; S.D. = 0.88%.

Table 28

The Tris catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.33	13.83	13.90
0.050	8.33	12.32	12.31
0.040	8.32	10.82	10.72
0.030	8.34	9.03	9.13
0.010	8.34	5.95	5.94

$k_{Tris} = 1.59 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.99%

$k_{int.} = 4.35 \times 10^{-4}\ sec^{-1}$; S.D. = 1.01%

Table 29

The Tris catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.068	8.56	15.67	15.85
0.051	8.57	13.07	13.16
0.034	8.57	10.60	10.46
0.017	8.57	7.81	7.77
0.0085	8.55	6.30	6.34

$k_{Tris} = 1.59 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.99%

$k_{int.} = 5.07 \times 10^{-4}\ sec^{-1}$; S.D. = 0.76%.

Table 30

The Tris catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	2.28	2.29
0.085	8.87	2.02	2.03
0.068	8.87	1.77	1.76
0.051	8.87	1.48	1.49
0.034	8.875	1.24	1.22
0.017	8.88	0.94	0.96

$k_{Tris} = 1.57 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.26%.

$k_{int.} = 6.88 \times 10^{-4}\ sec^{-1}$; S.D. = 1.31%.

Table 31

The Tris catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	3.22, 3.17	3.20
0.106	9.17	2.75	2.78
0.079	9.17	2.38	2.34
0.026	9.16	1.50	1.48
0.013	9.14	1.26	1.27

$k_{Tris} = 1.62 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.89%

$k_{int.} = 1.06 \times 10^{-3}\ sec^{-1}$; S.D. = 0.72%.

Table 32

The Tris catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	1.56	1.58
0.050	8.33	1.43	1.42
0.040	8.32	1.27	1.26
0.030	8.34	1.10	1.10
0.010	8.34	0.77	0.78

$k_{Tris} = 1.60 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.63%

$k_{int.} = 6.19 \times 10^{-4}\ sec^{-1}$; S.D. = 2.55%

Table 33

The Tris catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	1.86	1.90
0.051	8.57	1.67	1.60
0.034	8.57	1.30	1.31
0.017	8.57	0.97	1.02
0.0085	8.55	0.90	0.87

$k_{Tris} = 1.72 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.38%.

$k_{int.} = 7.28 \times 10^{-4}\ sec^{-1}$; S.D. = 1.56%.

Table 34

The Tris catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	2.70, 2.79	2.75
0.085	8.87	2.42	2.45
0.051	8.87	1.90	1.86
0.034	8.875	1.58	1.56
0.017	8.88	1.26	1.27

$k_{Tris} = 1.75 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.74%

$k_{int.} = 9.71 \times 10^{-4}\ sec^{-1}$; S.D. = 1.34%.

Table 35

The Tris catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	4.71	4.78
0.106	9.17	4.31	4.30
0.079	9.17	4.11	3.81
0.053	9.16	3.33	3.33
0.026	9.16	2.92	2.83
0.013	9.14	2.49	2.59

$k_{Tris} = 1.84 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 3.54%

$k_{int.} = 2.35 \times 10^{-3}\ sec^{-1}$; S.D. = 2.19%.

Table 36

The Tris catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.08	23.13, 22.92	22.70
0.048	8.10	18.45, 18.37	18.97
0.036	8.10	15.61, 15.47	15.24
0.024	8.11	11.59, 11.93	11.51
0.012	8.10	7.44, 7.47	7.78
0.006	8.11	5.94, 5.96	5.91

k_{Tris} = 3.11×10^{-2} M^{-1} sec^{-1} ; S.D. = 0.54%

$k_{int.}$ = 4.05×10^{-4} sec^{-1} ; S.D. = 0.67%.

Table 37

The Tris catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.33	24.26, 24.25	24.78
0.050	8.33	21.69, 21.48	21.43
0.040	8.32	17.97, 18.08	18.08
0.030	8.34	14.95, 15.05	14.73
0.010	8.34	7.93, 8.04	8.03

k_{Tris} = 3.35×10^{-2} M^{-1} sec^{-1} ; S.D. = 0.83%

$k_{int.}$ = 4.69×10^{-4} sec^{-1} ; S.D. = 1.55%.

Table 38

The Tris catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I - 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	2.91, 2.86	2.89
0.051	8.57	2.33, 2.29	2.32
0.034	8.57	1.75, 1.73	1.75
0.017	8.57	1.17, 1.18	1.17
0.0085	8.55	0.86, 0.88	0.87

$k_{Tris} = 3.38 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.74%.

$k_{int.} = 5.98 \times 10^{-4}\ sec^{-1}$; S.D. = 0.74%.

Table 39

The Tris catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	4.43, 4.41	4.43
0.085	8.87	3.79, 3.81	3.84
0.068	8.87	3.29, 3.32	3.25
0.051	8.87	2.68, 2.64	2.65
0.034	8.875	2.13, 1.99	2.05
0.017	8.88	1.47, 1.46	1.46

$k_{Tris} = 3.50 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.68%.

$k_{int.} = 8.68 \times 10^{-4}\ sec^{-1}$; S.D. = 1.07%.

Table 40.

The Tris catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	5.99, 6.11	6.17
0.106	9.17	5.33, 5.35	5.26
0.079	9.17	4.48, 4.40	4.32
0.053	9.16	3.38, 3.39	3.41
0.026	9.16	2.49, 2.43	2.46
0.013	9.14	2.04, 1.98	2.01.

$k_{Tris} = 3.50 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.81%.

$k_{int.} = 1.55 \times 10^{-3}\ sec^{-1}$; S.D. = 0.79%.

Table 41

The Tris catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.08	3.68, 3.65	3.61
0.048	8.10	2.97, 2.98	3.03
0.036	8.10	2.49, 2.48	2.45
0.024	8.11	1.85, 1.87	1.87
0.012	8.10	1.28, 1.30	1.29
0.006	8.11	1.00, 0.99	0.99

$k_{Tris} = 4.85 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.81%.

$k_{int.} = 7.03 \times 10^{-4}\ sec^{-1}$; S.D. = 0.72%.

Table 42

The Tris catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	3.98, 3.89	3.99
0.050	8.33	3.53, 3.47	3.49
0.040	8.32	2.98, 2.98	2.99
0.030	8.34	2.49, 2.56	2.49
0.020	8.34	1.48, 1.48	1.48

$k_{Tris} = 5.02 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.83%.

$k_{int.} = 9.81 \times 10^{-4}\ sec^{-1}$; S.D. = 0.96%.

Table 43

The Tris catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I - 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	5.13, 5.19	5.15
0.051	8.57	4.16, 4.15	4.21
0.034	8.57	3.31, 3.32	3.26
0.017	8.57	2.18, 2.33	2.32
0.0085	8.55	1.83, 1.80	1.82

$k_{Tris} = 5.55 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.72%.

$k_{int.} = 1.38 \times 10^{-3}\ sec^{-1}$; S.D. = 0.60%.

Table 44.

The Tris catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	8.25, 8.07	8.25
0.085	8.87	7.22, 7.20	7.35
0.068	8.87	6.44, 6.59	6.45
0.051	8.87	5.61, 5.68	5.55
0.034	8.875	4.70, 4.71	4.65
0.017	8.88	3.73, 3.69	3.75

$k_{Tris} = 5.30 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.90%.

$k_{int.} = 2.85 \times 10^{-3}\ sec^{-1}$; S.D. = 0.70%.

Table 45.

The Tris catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	12.26, 12.24	12.85
0.106	9.17	11.09, 10.90	11.28
0.079	9.17	9.56, 9.79	9.64
0.053	9.16	8.43, 8.33	8.08
0.026	9.16	6.84, 6.61	6.43
0.013	9.14	5.70, 5.41	5.65

$k_{Tris} = 6.05 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.98%.

$k_{int.} = 4.86 \times 10^{-3}\ sec^{-1}$; S.D. = 0.55%.

Table 46.

The Tris catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.08	6.60, 6.52, 6.72	6.75
0.048	8.10	5.62, 5.79	5.80
0.036	8.10	4.92, 4.97	4.85
0.024	8.11	3.91, 3.95, 4.03	3.90
0.012	8.10	2.93, 3.01	2.95
0.006	8.11	2.38, 2:37	2.48

$k_{Tris} = 7.92 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.20%.

$k_{int.} = 2.00 \times 10^{-3}\ sec^{-1}$; S.D. = 1.31%.

Table 47

The Tris catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	8.10, 7.98	8.21
0.050	8.33	7.09, 7.46	7.36
0.040	8.32	6.61, 6.46	6.50
0.030	8.34	5.88, 5.82	5.64
0.010	8.34	3.89, 3.98	3.93

$k_{Tris.} = 8.56 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.47%.

$k_{int.} = 3.08 \times 10^{-4}\ sec^{-1}$; S.D. = 1.17%,

Table 48

The Tris catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	11.15, 11.23	11.32
0.051	8.57	9.35, 9.71	9.72
0.034	8.57	8.16, 8.53	8.12
0.017	8.57	6.74, 6.65	6.53
0.0085	8.55	5.54, 5.76	5.68

$k_{Tris} = 9.39 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.25%.

$k_{int.} = 4.93 \times 10^{-3}\ sec^{-1}$; S.D. = 1.05%.

Table 49

The Tris catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.051	8.87	15.72, 16.04, 15.92 16.00	15.76
0.042	8.87	14.93, 14.99, 14.20	14.65
0.034	8.875	13.84, 13.40, 13.57 13.00	13.67
0.017	8.88	11.23, 11.77, 11.87 11.70	11.57
0.0085	8.88	10.77, 10.32	10.47

$k_{Tris} = 12.31 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.78%.

$k_{int.} = 9.48 \times 10^{-3}\ sec^{-1}$; S.D. = 1.08%.

Table 50

The Tris catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 0.6.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	8.08	25.74	25.87
0.048	8.10	21.83	21.96
0.036	8.10	18.43	18.04
0.024	8.11	14.13	14.12
0.012	8.10	10.21	10.20
0.006	8.11	8.24	8.24

$k_{Tris} = 3.26 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.41%.

$k_{int.} = 6.28 \times 10^{-4}\ sec^{-1}$; S.D. = 0.91%.

Table 51

The Tris catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.0.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	8.33	2.80	2.83
0.050	8.33	2.54	2.49
0.040	8.32	2.13	2.15
0.030	8.34	1.83	1.81
0.010	8.34	1.14	1.14

$k_{Tris} = 3.39 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.38%.

$k_{int.} = 7.96 \times 10^{-4}\ sec^{-1}$; S.D. = 1.04%.

Table 52

The Tris catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 1.7.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.068	8.56	3.32	3.39
0.051	8.57	2.84	2.80
0.034	8.57	2.26	2.21
0.017	8.57	1.61	1.62
0.0085	8.55	1.31	1.31

$k_{Tris} = 3.47 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.52%.

$k_{int.} = 1.03 \times 10^{-3}\ sec^{-1}$; S.D. = 1.46%.

Table 53

The Tris catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 3.4.

(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.102	8.87	5.39	5.41
0.085	8.87	4.79	4.81
0.068	8.87	4.23	4.21
0.051	8.87	3.66	3.61
0.034	8.875	2.96	3.00
0.017	8.88	2.38	2.40

$k_{Tris} = 3.54 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.75%.

$k_{int.} = 1.80 \times 10^{-3}\ sec^{-1}$; S.D. = 3.45%.

Table 54

The Tris catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Tris):(Tris H^+) = 6.6.

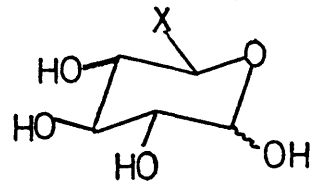
(Tris)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.132	9.17	7.73, 7.67	7.75
0.106	9.17	7.00, 6.95	6.88
0.079	9.17	5.80, 5.99	5.98
0.053	9.16	5.10, 5.02	5.12
0.026	9.16	4.24	4.22

$k_{Tris} = 3.33 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.20%.

$k_{int.} = 3.36 \times 10^{-3}\ sec^{-1}$; S.D. = 1.65%.

Table 55

Comparison of catalytic coefficients for catalysis by Tris for the series of sugars



	σ	-0.05	0.00	0.05	0.07	0.07	0.11*	0.15	0.18	
pH	X	$-CH_3$	-H	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
8.10	$10^2k^{**}_{Tris}$	1.07	8.01	1.43	1.56	-	3.11	4.85	7.92	3.26
8.33	"	1.07	8.29	1.48	1.59	1.60	3.35	5.02	8.56	3.39
8.56	"	1.10	8.04	1.44	1.59	1.72	3.38	5.55	9.39	3.47
8.87	"	1.12	8.56	1.48	1.57	1.75	3.50	5.30	12.31	3.54
9.16	"	1.14	8.69	1.69	1.62	1.84	3.50	6.05	-	3.33
Mean[a]	$10^2k^{**}_{Tris}$	1.08	8.11	1.45	1.58	1.66	3.28	5.14	7.92[b]	3.37

a. the average of the values at pH's 8.10, 8.33, 8.56 (see discussion).

b. single value at pH 8.10.

* estimated from data of Charton[129]

** units are M^{-1} sec^{-1}

Table 56

The hydroxide ion catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	1.35	1.36
8.87	7.41	1.13	1.13
8.56	3.63	1.01	1.01
8.33	2.14	0.97	0.96
8.10	1.26	0.92	0.93

$k_{OH^-} = 32.7\ M^{-1}\ sec^{-1}$; S.D. = 1.61%.

$k'_{int.} = 0.89 \times 10^{-3}\ sec^{-1}$; S.D. = 0.25%.

Table 57

The hydroxide ion catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	8.04	8.14
8.87	7.41	4.88	4.89
8.56	3.63	3.21	3.15
8.33	2.14	2.42	2.46
8.10	1.26	2.06	2.06

$k_{OH^-} = 4.6 \times 10^2\ M^{-1}\ sec^{-1}$; S.D. = 1.01%.

$k'_{int.} = 1.48 \times 10^{-3}\ sec^{-1}$; S.D. = 0.91%.

Table 58

The hydroxide ion catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
8.87	7.41	0.98	0.99
8.56	3.63	0.70	0.69
8.33	2.14	0.57	0.57
8.10	1.26	0.50	0.50

$k_{OH^-} = 79.9\ M^{-1}\ sec^{-1}$; S.D. = 1.88%.

$k'_{int.} = 4.00 \times 10^{-4}\ sec$; S.D. = 0.90%.

Table 59

The hydroxide ion catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	1.06	1.05
8.87	7.41	0.69	0.70
8.56	3.63	0.51	0.51
8.33	2.14	0.44	0.44
8.10	1.26	0.40	0.40

$k_{OH^-} = 49.5\ M^{-1}\ sec^{-1}$; S.D. = 1.55%

$k'_{int.} = 3.34 \times 10^{-4}\ sec^{-1}$; S.D. = 0.97%.

Table 60

The hydroxide ion catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	2.35	2.54
8.87	7.41	0.97	1.11
8.56	3.63	0.73	0.71
8.33	2.14	0.62	0.46

k_{OH^-} = 103.3 M^{-1} sec^{-1} S.D. = 3.30%.

k_{int} = 3.48×10^{-4} sec^{-1} S.D. = 4.86%.

Table 61

The hydroxide ion catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	1.55	1.50
8.87	7.41	0.87	0.91
8.56	3.63	0.60	0.60
8.33	2.14	0.47	0.47
8.10	1.26	0.40	0.40

k_{OH^-} = 83.9 M^{-1} sec^{-1} ; S.D. = 1.26%.

$k_{int.}$ = 2.93×10^{-4} sec^{-1} ; S.D. = 1.15%.

Table 62

The hydroxide ion catalysed mutarotation of 6-chlcro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	4.86	4.93
8.87	7.41	2.85	2.67
8.56	3.63	1.38	1.45
8.33	2.14	0.98	0.98
8.10	1.26	0.70	0.69

$k_{OH^-} = 3.21 \times 10^2\ M^{-1}\ sec^{-1}$; S.D. = 0.94%.

$k'_{int.} = 2.88 \times 10^{-4}\ sec^{-1}$; S.D. = 2.78%.

Table 63

The hydroxide ion catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M.

pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
8.87	7.41	9.48	9.52
8.56	3.63	4.93	4.90
8.33	2.14	3.08	3.08
8.10	1.26	2.00	2.00

$k_{OH^-} = 1.22 \times 10^3\ M^{-1}\ sec^{-1}$; S.D. = 1.56%.

$k'_{int.} = 4.63 \times 10^{-4}\ sec^{-1}$; S.D. = 9.50%.

Table 64

The hydroxide ion catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M.

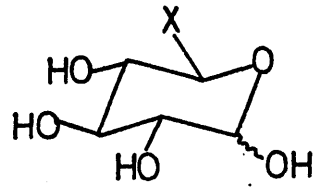
pH_{25}	$(OH^-) \times 10^6$	$k_{int.} \times 10^3$	$k_{calc.} \times 10^3$
9.16	14.45	3.36	3.21
8.87	7.41	1.80	1.83
8.56	3.63	1.03	1.09
8.-	2.14	0.80	0.80
8.10	1.26	0.63	0.62

k_{OH^-} = 1.96×10^2 M^{-1} sec^{-1} ; S.D. = 1.93%.

$k'_{int.}$ = 3.78×10^{-4} sec^{-1} ; S.D. = 2.25%.

Table 65

Comparison of the catalytic coefficients for catalysis by hydroxide ion for the series of sugars



σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11[a]	+0.15	+0.18	+x
X	$-CH_3$	-H	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_{OH^-} \times 10^{-2}$ (M^{-1} sec^{-1})	0.33	4.61	0.80	0.50	-	0.84	3.21	12.2	1.96
$k^*_{H_2O} \times 10^{6}$ (M^{-1} sec^{-1})	16.01	26.66	7.19	6.01	-	5.27	5.18	8.33	6.80

* derived from the $k'_{int.}$ from tables 56 - 64 divided by 55.6.

a. estimated from data of Charton.[129]

Table 66

The pyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.95	1.45, 1.46	1.46
0.080	5.93	1.29, 1.32	1.34
0.060	5.94	1.23, 1.28	1.23
0.040	5.92	1.14, 1.08	1.11
0.020	5.92	1.02, 0.95	0.99
0.010	5.94	0.90, 0.94	0.93

$k_{Py} = 5.84 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.79%.

$k_{int.} = 8.75 \times 10^{-4}\ sec^{-1}$; S.D. = 0.68%.

Table 67

The pyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.68	1.46, 1.45	1.45
0.080	5.70	1.34, 1.32	1.34
0.060	5.72	1.22, 1.23	1.23
0.040	5.72	1.12, 1.11	1.12
0.020	5.72	1.00	1.00
0.010	5.73	0.94, 0.97	0.95

$k_{Py} = 5.50 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.09%.

$k_{int.} = 8.96 \times 10^{-4}\ sec^{-1}$; S.D. = 0.66%.

Table 68

The pyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.33	1.47, 1.45	1.45
0.080	5.33	1.30, 1.34	1.34
0.060	5.34	1.22, 1.19	1.22
0.040	5.36	1.13, 1.14	1.11
0.020	5.37	1.00, 0.98	0.99
0.010	5.38	0.92, 0.91	0.94

$k_{Py} = 5.73 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.70%.

$k_{int.} = 8.79 \times 10^{-4}\ sec^{-1}$; S.D. = 0.61%

Table 69

The pyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	4.96	1.16, 1.18	1.18
0.040	4.99	1.13, 1.14	1.12
0.030	4.99	1.02, 1.10	1.06
0.020	5.03	1.01, 0.97	0.99
0.010	5.03	0.96, 0.94	0.94
0.005	5.03	0.90, 0.91	0.91

$k_{Py} = 6.07 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 3.15%.

$k_{int.} = 8.78 \times 10^{-4}\ sec^{-1}$; S.D. = 0.62%.

Table 70

The pyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.95	2.88, 2.76	2.82
0.080	5.93	2.51, 2.54	2.53
0.060	5.94	2.23, 2.23	2.23
0.040	5.92	1.92, 1.94	1.93
0.020	5.92	1.69, 1.60	1.63
0.010	5.94	1.49, 1.46	1.49

$k_{Py} = 1.49 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.51%.

$k_{int.} = 1.34 \times 10^{-3}\ sec^{-1}$; S.D. = 0.71%.

Table 71

The pyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.68	2.88, 2.83	2.84
0.080	5.70	2.54, 2.54	2.54
0.060	5.72	2.18, 2.29	2.24
0.040	5.72	1.88, 1.96	1.94
0.020	5.72	1.65, 1.64	1.64
0.010	5.73	1.48, 1.49	1.48

$k_{Py} = 1.50 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.40%.

$k_{int.} = 1.34 \times 10^{-3}\ sec^{-1}$; S.D. = 0.66%.

Table 72

The pyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.33	2.86, 2.87	2.89
0.080	5.33	2.55, 2.56	2.57
0.060	5.34	2.28, 2.29	2.25
0.040	5.36	1.99, 1.97	1.94
0.020	5.37	1.59, 1.58	1.62
0.010	5.38	1.45, 1.48	1.46

$k_{Py} = 1.60 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.27%.

$k_{int.} = 1.30 \times 10^{-3}\ sec^{-1}$; S.D. = 0.60%.

Table 73

The pyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	4.96	2.04, 2.03	2.06
0.040	4.99	1.89	1.91
0.030	4.99	1.79	1.75
0.020	5.03	1.61	1.60
0.010	5.03	1.46	1.45
0.005	5.03	1.31	1.38

$k_{Py} = 1.52 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.16%.

$k_{int.} = 1.30 \times 10^{-3}\ sec^{-1}$; S.D. = 0.33%.

Table 74

The pyridine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.1C<u>M</u>. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)<u>M</u>	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	10.07, 9.89	10.05
0.080	5.70	8.85, 8.86	8.87
0.060	5.72	7.49, 7.70	7.69
0.040	5.72	6.61, 6.58	6.51
0.020	5.72	5.27, 5.32	5.33
0.010	5.73	4.68	4.73

$k_{Py} = 5.91 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.19%.

$k_{int.} = 4.14 \times 10^{-4}\ sec^{-1}$; S.D. = 0.76%.

Table 75

The pyridine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.1C<u>M</u>. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)<u>M</u>	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	10.04, 10.17	10.12
0.080	5.33	8.84, 8.90	8.92
0.060	5.34	7.74, 7.77	7.72
0.040	5.36	6.38, 6.54	6.52
0.020	5.37	5.37, 5.41	5.32
0.010	5.38	4.64, 4.66	4.72

$k_{Py} = 6.00 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.04%.

$k_{int.} = 4.12 \times 10^{-4}\ sec^{-1}$; S.D. = 0.58%.

Table 76

The pyridine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	7.15, 7.14	7.12
0.040	4.99	6.53, 6.44	6.52
0.030	4.99	5.93, 5.97	5.92
0.020	5.03	5.33, 5.29	5.32
0.010	5.03	4.81, 4.65	4.72
0.005	5.03	4.50, 4.34	4.41

k_{Py} = 6.02 x 10^{-3} M^{-1} sec^{-1} ; S.D. = 1.59%.

$k_{int.}$ = 4.11 x 10^{-4} sec^{-1} ; S.D. = 0.66%.

Table 77

The pyridine catalysed mutarotation of 6-O-methyl -α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.93	9.19	8.92
0.060	5.94	7.53	7.56
0.040	5.92	6.12	6.21
0.020	5.92	4.87	4.85
0.010	5.94	4.18	4.18

k_{Py} = 6.77 x 10^{-3} M^{-1} sec^{-1} ; S.D. = 1.31%.

$k_{int.}$ = 3.50 x 10^{-4} sec^{-1} ; S.D. = 0.52%.

Table 78

The pyridine catalysed mutarotation of 6-C-methyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	10.89	10.91
0.080	5.70	9.55	9.41
0.060	5.72	7.56	7.91
0.040	5.72	6.52	6.42
0.020	5.72	4.92	4.92
0.010	5.73	4.17	4.17

$k_{Py} = 7.48 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.80%.

$k_{int.} = 3.42 \times 10^{-4}\ sec^{-1}$; S.D. = 0.42%.

Table 79

The pyridine catalysed mutarotation of 6-C-methyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	11.39	11.29
0.060	5.34	8.11	8.16
0.040	5.36	6.55	6.59
0.020	5.37	5.03	5.02
0.010	5.38	4.25	4.24

$k_{Py} = 7.84 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.16%.

$k_{int.} = 3.46 \times 10^{-4}\ sec^{-1}$; S.D. = 0.91%.

Table 80

The pyridine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	4.96	7.07	7.24
0.040	4.99	6.32	6.51
0.030	4.99	6.02	5.77
0.020	5.03	5.20	5.03
0.010	5.03	4.18	4.30
0.005	5.03	3.89	3.94

$k_{Py} = 7.35 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.37%.

$k_{int.} = 3.57 \times 10^{-4}\ sec^{-1}$; S.D. = 0.65%.

Table 81

The pyridine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.93	8.29	8.20
0.060	5.94	7.08	6.75
0.040	5.92	5.00	5.31
0.020	5.92	3.72	3.87
0.010	5.94	3.40	3.15

$k_{Py} = 7.21 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.68%.

$k_{int.} = 2.43 \times 10^{-4}\ sec^{-1}$; S.D. = 4.17%.

Table 82

The pyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium $^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	19.68, 18.36	18.71
0.080	5.70	15.74	15.67
0.060	5.72	12.89, 11.79	12.62
0.040	5.72	9.62, 9.37	9.57
0.020	5.72	6.52, 6.37	6.53
0.010	5.73	5.15, 4.87	5.00

$k_{Py} = 1.52 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.69%.

$k_{int.} = 3.48 \times 10^{-4}\ sec^{-1}$; S.D. = 0.77%.

Table 83

The pyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	19.55	19.62
0.080	5.33	16.32	16.28
0.060	5.34	12.90	12.94
0.040	5.36	9.69	9.61
0.020	5.37	6.27	6.27
0.010	5.38	4.60	4.60

$k_{Py} = 1.67 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.80%.

$k_{int.} = 2.93 \times 10^{-4}\ sec^{-1}$; S.D. = 1.17%.

Table 84

The pyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	12.20	12.13
0.040	4.99	9.83	10.34
0.030	4.99	8.92	8.56
0.020	5.03	6.90	6.77
0.010	5.03	5.21	4.98
0.005	5.03	4.01	4.09

$k_{Py} = 1.79 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.95%.

$k_{int.} = 3.20 \times 10^{-4}\ sec^{-1}$; S.D. = 0.82%.

Table 85

The pyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.95	13.32, 13.11	13.42
0.080	5.93	11.31, 11.42	11.25
0.060	5.94	9.14, 8.91	9.07
0.040	5.92	6.95, 6.81	6.90
0.020	5.92	4.65, 4.82	4.73
0.010	5.94	3.60	3.64

$k_{Py} = 1.09 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.91%.

$k_{int.} = 2.55 \times 10^{-4}\ sec^{-1}$; S.D. = 1.40%.

Table 86

The pyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	13.20, 13.13	13.10
0.080	5.70	11.01, 10.66	11.00
0.060	5.72	8.80, 8.66	8.89
0.040	5.72	6.93, 6.67	6.78
0.020	5.72	4.63, 5.03	4.68
0.010	5.73	3.47, 3.63	3.62

$k_{Py} = 1.05 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.81%.

$k_{int.} = 2.57 \times 10^{-4}\ sec^{-1}$; S.D. = 1.02%.

Table 87

The pyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M.	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	12.41, 12.56	12.83
0.080	5.33	10.84, 10.71	10.79
0.060	5.34	8.78, 8.85	8.74
0.040	5.36	6.72, 6.78	6.70
0.020	5.37	4.80, 4.78	4.66
0.010	5.38	3.57, 3.51	3.64

$k_{Py} = 1.02 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.78%.

$k_{int.} = 2.62 \times 10^{-4}\ sec^{-1}$; S.D. = 1.04%.

Table 88

The pyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M.	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	8.08	7.65
0.040	4.99	6.64	6.64
0.030	4.99	5.49	5.62
0.020	5.03	4.54	4.61
0.010	5.03	3.68	3.59
0.005	5.03	3.08	3.09

$k_{Py} = 1.01 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.40%.

$k_{int.} = 2.58 \times 10^{-4}\ sec^{-1}$; S.D. = 0.81%.

Table 89

The pyridine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.95	11.16, 11.58	11.58
0.080	5.93	9.57, 9.43	9.58
0.060	5.94	7.59, 7.56	7.58
0.040	5.92	5.87, 5.62	5.57
0.020	5.92	3.57, 3.60	3.57
0.010	5.94	2.57, 2.54	2.57

$k_{Py} = 1.00 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.71%.

$k_{int.} = 1.57 \times 10^{-4}\ sec^{-1}$; S.D. = 1.22%.

Table 90

The pyridine catalysed mutarotaticn of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	11.60, 11.02	11.26
0.080	5.70	9.35, 9.35	9.29
0.060	5.72	7.36, 6.95	7.32
0.040	5.72	5.40, 5.51	5.35
0.020	5.72	3.48, 3.30	3.38
0.010	5.73	2.36, 2.48	2.40

$k_{Py} = 9.84 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.67%.

$k_{int.} = 1.41 \times 10^{-4}\ sec^{-1}$; S.D. = 1.26%.

Table 91

The pyridine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	10.84, 10.90	10.96
0.080	5.33	8.85, 8.95	9.06
0.060	5.34	7.39, 7.39	7.16
0.040	5.36	5.32, 5.48	5.26
0.020	5.37	3.34, 3.50	3.37
0.010	5.38	2.36, 2.41	2.42

$k_{Py} = 9.49 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.69%.

$k_{int.} = 1.47 \times 10^{-4}\ sec^{-1}$; S.D. = 1.28%.

Table 92

The pyridine catalysed mutarctation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.1CM. (Pyridine):(Pyridinium$^+$) = 1:2.

(Pyridine)M.	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	5.71	5.95
0.040	4.99	5.16	5.04
0.030	4.99	4.19	4.12
0.020	5.03	3.29	3.21
0.010	5.03	2.30	2.30
0.005	5.03	1.83	1.83

$k_{Py} = 9.12 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.07%.

$k_{int.} = 1.39 \times 10^{-4}\ sec^{-1}$; S.D. = 1.51%.

Table 93

The pyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.1CM. (Pyridine):(Pyridinium$^+$) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.93	12.13	12.02
0.060	5.94	9.95	9.98
0.040	5.92	7.88	7.94
0.020	5.92	5.76	5.89
0.010	5.94	4,89	4.87

$k_{Py} = 10.22 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.43%.

$k_{int.} = 3.85 \times 10^{-4}\ sec^{-1}$; S.D. = 1.23%.

Table 94

The pyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.68	13.80	13.82
0.080	5.70	11.99	11.87
0.060	5.72	9.75	9.91
0.040	5.72	7.88	7.96
0.020	5.72	6.29	6.00
0.010	5.73	4.84	5.02

$k_{Py} = 9.78 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.25%.

$k_{int.} = 4.04 \times 10^{-4}\ sec^{-1}$; S.D. = 1.30%.

Table 95

The pyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	5.33	13.90	13.98
0.080	5.33	11.76	11.94
0.060	5.34	9.98	9.90
0.040	5.36	7.91	7.86
0.020	5.37	5.84	5.82
0.010	5.38	4.77	4.80

$k_{Py} = 10.21 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.33%.

$k_{int.} = 3.78 \times 10^{-4}\ sec^{-1}$; S.D. = 1.24%.

Table 96

The pyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium$^+$) = 1:2.

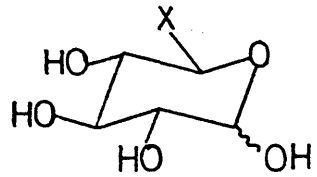
(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	8.45	8.50
0.040	4.99	7.49	7.53
0.030	4.99	6.50	6.55
0.020	5.03	5.63	5.57
0.010	5.03	4.63	4.60
0.005	5.03	4.09	4.11

$k_{Py} = 9.75 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.03%.

$k_{int.} = 3.63 \times 10^{-4}\ sec^{-1}$; S.D. = 0.48%.

Table 97

Comparison of catalytic coefficients for catalysis by pyridine for the series of sugars



	σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+x
pH	X	$-CH_3$	-H	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
5.93	$k^a_{Py} \times 10^3$	5.84	14.88	-	6.78	7.21	-	10.87	10.00	10.22
5.72	"	5.50	15.04	5.91	7.48	-	15.23	10.53	9.84	9.78
5.35	"	5.73	15.95	6.00	7.84	-	16.68	10.21	9.48	10.21
5.00	"	6.07	15.19	6.02	7.35	-	17.85	10.14	9.12	9.75
average	$k^a_{Py} \times 10^3$	5.79	15.27	5.98	7.36	7.21	16.59	10.44	9.60	9.99

pH	X	$-CH_3$	-H	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
5.93	$k^a_{H_2O} \times 10^6$	15.77	24.09	-	6.31	4.38	-	4.59	2.83	6.94
5.72	"	16.14	24.11	7.46	6.16	-	6.27	4.63	2.53	7.22
5.35	"	15.84	23.39	7.42	6.23	-	5.28	4.72	2.63	6.81
5.00	"	15.84	24.42	7.41	6.42	-	5.76	4.64	2.50	6.55
average	$k^a_{H_2O} \times 10^6$	15.90	23.75	7.33	6.31	4.38	5.77	4.65	2.65	6.88

* Estimated from data of Charton[129]

a units are M^{-1} sec^{-1}

Table 98

The morpholine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.025	8.18	3.25	3.23
0.020	8.18	2.76	2.76
0.015	8.17	2.18	2.30
0.010	8.22	1.90	1.84
0.005	8.21	1.39	1.37
0.0025	8.22	1.13	1.14

$k_{Morph.} = 9.27 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.75%.

$k_{int.} = 9.08 \times 10^{-4}\ sec^{-1}$; S.D. = 0.59%.

Table 99

The morpholine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.18	12.02	11.79
0.015	8.17	9.44, 9.45	9.45
0.010	8.22	7.13, 7.11	7.11
0.005	8.21	4.73, 4.66	4.77
0.0025	8.22	3.80, 3.85	3.60

$k_{Morph.} = 46.82 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.60%.

$k_{int.} = 2.43 \times 10^{-3}\ sec^{-1}$; S.D. = 0.57%.

Table 100

The morpholine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.025	8.18	3.26	3.22
0.020	8.18	2.64	2.67
0.015	8.17	2.04	2.12
0.010	8.22	1.63	1.57
0.005	8.21	1.02	1.02
0.0025	8.22	0.75	0.75

$k_{Morph.} = 10.98 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.6%.

$k_{int.} = 4.75 \times 10^{-4}\ sec^{-1}$; S.D. = 0.87%.

Table 101

The morpholine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.025	8.18	3.88	3.67
0.020	8.18	2.88	3.01
0.015	8.17	2.28	2.34
0.010	8.22	1.72	1.68
0.005	8.21	0.99	1.01
0.0025	8.22	0.70	0.68

$k_{Morph.} = 13.28 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.83%.

$k_{int.} = 3.51 \times 10^{-4}\ sec^{-1}$; S.D. = 2.54%.

Table 102

The morpholine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M.(Morpholine):(Morpholinium$^+$) = 1:2.

(Morpholine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	8.40	5.42	5.54
0.040	8.40	4.57	4.58
0.030	8.45	3.68	3.62
0.020	8.44	2.70	2.66
0.010	8.44	1.67	1.70
0.005	8.47	1.23	1.22

$k_{Morph.} = 9.58 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.88%.

$k_{int.} = 7.45 \times 10^{-4}\ sec^{-1}$; S.D. = 1.89%.

Table 103

The morpholine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.025	8.18	8.89, 8.57	8.94
0.020	8.18	7.39, 6.96	7.23
0.015	8.17	5.63, 5.39	5.52
0.010	8.22	3.88, 3.91	3.81
0.005	8.21	2.19, 2.14	2.10
0.0025	8.22	1.20	1.24

$k_{Morph.} = 34.23 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.63%.

$k_{int.} = 3.86 \times 10^{-4}\ sec^{-1}$; S.D. = 3.88%.

Table 104

The morpholine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.1C$\underline{M}$. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)$\underline{M}$	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.18	9.65, 9.65	9.65
0.015	8.17	7.39, 7.38	7.51
0.010	8.22	5.55, 5.51	5.37
0.005	8.21	3.18, 3.21	3.23
0.0025	8.22	2.05, 2.20	2.15

$k_{Morph.} = 42.85 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.71%.

$k_{int.} = 1.08 \times 10^{-3}\ sec^{-1}$; S.D. = 1.74%.

Table 105

The morpholine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10$\underline{M}$. (Morpholine):(Morpholinium$^+$) = 1:4.

(Morpholine)$\underline{M}$	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.18	12.96	12.81
0.015	8.17	10.28, 10.12	10.24
0.010	8.22	7.69, 7.73	7.66
0.005	8.21	5.06, 4.99	5.09
0.0025	8.22	3.80, 3.85	3.81

$k_{Morph.} = 51.44 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.97%.

$k_{int.} = 2.52 \times 10^{-3}\ sec^{-1}$; S.D. = 1.05%.

Table 106

The morpholine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (Morpholine):(Morpholinium$^+$) = 1:4.

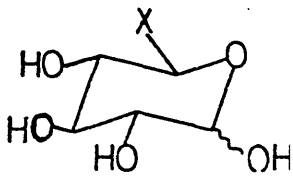
(Morpholine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.025	8.18	6.66	6.47
0.020	8.18	5.25	5.29
0.015	8.17	3.98	4.10
0.010	8.22	2.99	2.92
0.005	8.21	1.71	1.73
0.0025	8.22	1.14	1.14

$k_{Morph.} = 23.71 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.78%.

$k_{int.} = 5.46 \times 10^{-4}\ sec^{-1}$; S.D. = 2.40%.

Table 107

Comparison of catalytic coefficients for catalysis by morpholine for the series of sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_{Morph} \times 10^2$ (M^{-1} sec^{-1})	9.27	46.81	10.98	13.28	9.58	34.23	42.85	51.44	23.71
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+x

* derived from data of Charton[129]

Table 108

The diethanolamine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B) :(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	1.79, 1.77	1.77
0.016	8.34	1.63, 1.65	1.62
0.012	8.35	1.47, 1.45	1.47
0.008	8.36	1.30, 1.33	1.32
0.004	8.37	1.16, 1.13	1.17
0.002	8.37	1.10, 1.10	1.09

$k_B = 3.76 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.66%.

$k_{int.} = 1.02 \times 10^{-3}\ sec^{-1}$; S.D. = 0.17%.

Table 109

The diethanolamine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	8.24, 8.09	8.29
0.016	8.34	7.18, 7.25	7.24
0.012	8.35	6.36, 6.32	6.19
0.008	8.36	5.31, 5.28	5.14
0.004	8.37	3.91, 3.94	4.09
0.002	8.37	3.62, 3.57	3.57

$k_B = 26.21 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.50%.

$k_{int.} = 3.05 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.21%.

Table 110

The diethanolamine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	1.80	1.82
0.016	8.34	1.51	1.57
0.012	8.35	1.33	1.33
0.008	8.36	1.14	1.09
0.004	8.37	0.84	0.84
0.002	8.37	0.72	0.72

$k_B = 6.07 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.80%.

$k_{int.} = 6.02 \times 10^{-4}\ sec^{-1}$; S.D. = 0.57%.

Table 111.

The diethanolamine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	1.85	1.89
0.016	8.34	1.56	1.59
0.012	8.35	1.28	1.29
0.008	8.36	1.04	1.00
0.004	8.37	0.70	0.70
0.002	8.37	0.55	0.55

$k_B = 7.40 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.02%.

$k_{int.} = 4.05 \times 10^{-4}\ sec^{-1}$; S.D. = 0.98%.

Table 112

The diethanolamine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	1.96	1.97
0.016	8.34	1.65	1.68
0.012	8.35	1.42	1.39
0.008	8.36	1.10	1.11
0.004	8.37	0.84	0.82
0.002	8.37	0.67	0.68

$k_B = 7.19 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.99%,

$k_{int.} = 5.31 \times 10^{-4}\ sec^{-1}$; S.D. = 0.90%.

Table 113

The diethanolamine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	3.81	3.72
0.016	8.34	2.90	3.08
0.012	8.35	2.60	2.44
0.008	8.36	1.79	1.79
0.004	8.37	1.13	1.15
0.002	8.37	0.83	0.83

$k_B = 16.02 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.74%.

$k_{int.} = 5.13 \times 10^{-4}\ sec^{-1}$; S.D. = 0.93%.

Table 114

The diethanolamine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	5.73, 5.76	5.85
0.016	8.34	4.76, 4.68	4.93
0.012	8.35	4.14, 4.20	4.02
0.008	8.36	3.18, 3.23	3.11
0.004	8.37	2.09, 2.16	2.19
0.002	8.37	1.76	1.74

$k_B = 22.83 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.64%.

$k_{int.} = 1.28 \times 10^{-3}\ sec^{-1}$; S.D. = 0.78%.

Table 115

The diethanolamine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

(Diethanolamine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	12.01, 11.76	11.57
0.016	8.34	9.89	10.18
0.012	8.35	8.73, 9.08	8.79
0.008	8.36	7.49, 7.19	7.40
0.004	8.37	6.06	6.01
0.002	8.37	5.31	5.32

$k_B = 34.72 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.06%.

$k_{int.} = 4.63 \times 10^{-3}\ sec^{-1}$; S.D. = 0.53%.

Table 116

The diethanolamine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (B):(BH^+) = 1:5.

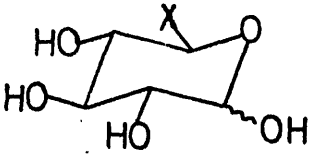
(Diethanolamine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.020	8.34	3.24	3.24
0.016	8.34	2.77	2.77
0.012	8.35	2.29	2.29
0.008	8.36	1.82	1.82
0.004	8.37	1.35	1.35
0.002	8.37	1.11	1.11

$k_B = 11.80 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.20%.

$k_{int.} = 8.79 \times 10^{-4}\ sec^{-1}$; S.D. = 1.37%.

Table 117

Comparison of catalytic coefficients for catalysis by diethanolamine in the series of sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_B \times 10^2$ (M^{-1} sec^{-1})	3.76	26.21	6.07	7.40	7.19	16.02	22.83	34.72	11.80
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x

* estimated from the data of Charton.[129]

Table 118

The DCl catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD^a_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	19.12	19.31
0.080	1.19	15.73	15.96
0.060	1.27	12.47	12.61
0.040	1.40	9.44	9.25
0.020	1.65	6.02	5.90
0.010	2.00	4.18	4.22

$k_{D_3O^+}$ = 16.77×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.66%.

$k_{int.}$ = 2.54×10^{-4} sec^{-1} ; S.D. = 0.97%.

a. PD = pH meter glass electrode reading +0.40 (ref. 196)

Table 119

The DCl catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	1.08	3.40	3.45
0.080	1.19	2.80	2.84
0.060	1.27	2.21	2.23
0.040	1.40	1.64	1.62
0.020	1.65	1.04	1.01
0.010	2.00	0.70	0.70

$k_{D_3O^+}$ = 30.47×10^{-3} M^{-1} sec^{-1} ; S.D. = 0.63%.

$k_{int.}$ = 4.00×10^{-4} sec^{-1} ; S.D. = 1.02%.

Table 120

The DCl catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	10.83	10.75
0.080	1.19	8.62	8.83
0.060	1.27	6.80	6.90
0.040	1.40	5.06	4.97
0.020	1.65	3.10	3.05
0.010	2.00	2.06	2.08

$k_{D_3O^+} = 9.63 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.65%.

$k_{int.} = 1.12 \times 10^{-4}\ sec^{-1}$; S.D. = 1.34%.

Table 121

The DCl catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	8.34	8.38
0.080	1.19	6.79	6.90
0.060	1.27	5.39	5.42
0.040	1.40	4.00	3.95
0.020	1.65	2.51	2.47
0.010	2.00	1.72	1.73

$k_{D_3O^+} = 7.38 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.68%.

$k_{int.} = 9.96 \times 10^{-5}\ sec^{-1}$; S.D. = 1.18%.

Table 122

The DCl catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	7.36	7.48
0.060	1.27	4.76	4.79
0.040	1.40	3.47	3.45
0.020	1.65	2.15	2.10
0.010	2.00	1.42	1.43

$k_{D_3O^+} = 6.73 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.87%.

$k_{int.} = 7.57 \times 10^{-5}\ sec^{-1}$; S.D. = 1.42%.

Table 123

The DCl catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	7.64	7.63
0.080	1.19	6.18	6.27
0.040	1.40	3.61	3.55
0.020	1.65	2.19	2.19
0.010	2.00	1.51	1.51

$k_{D_3O^+} = 6.80 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.78%.

$k_{int.} = 8.33 \times 10^{-5}\ sec^{-1}$; S.D. = 1.49%.

Table 124

The DCl catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	6.08	5.94
0.080	1.19	4.80	4.88
0.060	1.27	3.78	3.82
0.040	1.40	2.75	2.77
0.020	1.65	1.73	1.71
0.010	2.00	1.17	1.18

$k_{D_3O^+} = 5.30 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.69%.

$k_{int.} = 6.47 \times 10^{-5}\ sec^{-1}$; S.D. = 1.52%.

Table 125

The DCl catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	5.46	5.39
0.080	1.19	4.42	4.44
0.060	1.27	3.43	3.48
0.040	1.40	2.50	2.52
0.020	1.65	1.61	1.57
0.010	2.00	1.08	1.09

$k_{D_3O^+} = 4.78 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.70%.

$k_{int.} = 6.14 \times 10^{-5}\ sec^{-1}$; S.D. = 1.56%.

Table 126

The DCl catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M.

(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	3.02	3.00
0.080	1.19	2.43	2.47
0.060	1.27	1.91	1.94
0.040	1.40	1.41	1.40
0.020	1.65	0.89	0.87
0.010	2.00	0.59	0.60

$k_{D_3O^+} = 2.67 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.68%.

$k_{int.} = 3.34 \times 10^{-5}\ sec^{-1}$; S.D. = 1.39%.

Table 127

The DCl catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M.

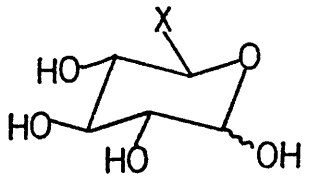
(D_3O^+)M	pD_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	1.08	9.84	9.70
0.080	1.19	7.80	7.94
0.060	1.27	6.13	6.18
0.040	1.40	4.38	4.41
0.020	1.65	2.72	2.65
0.010	2.00	1.75	1.78

$k_{D_3O^+} = 8.81 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.72%.

$k_{int.} = 8.92 \times 10^{-5}\ sec^{-1}$; S.D. = 1.92%.

Table 128

Comparison of $k_{D_3O^+}$ and k_{D_2O} for the series of substituted sugars



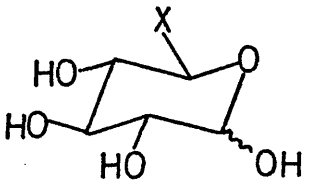
X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CO_2H$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_{D_3O^+} \times 10^3$ (M^{-1} sec^{-1})	16.77	30.47	9.63	7.38	6.73	6.80	5.30	4.78	2.67	8.81
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.17	+0.18	+x
$k^a_{D_2O} \times 10^6$ (M^{-1} sec^{-1})	4.58	7.20	2.13	1.79	1.36	1.50	1.16	1.11	0.60	1.61

* estimated from the data of Charton[129].

a. k_{D_2O} calculated by dividing the $k_{int.}$ for each sugar (tables 118 - 127) by 55.5 - the molarity of D_2O.

Table 129

Isotope effects for the series of substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CO_2H$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$\frac{k_{H_3O^+}}{k_{D_3O^+}}$	1.08	1.14	1.15	1.17	1.15	1.17	1.15	1.08	1.11	1.11
$\frac{k_{D_2O}}{k_{H_2O}}$	3.58	3.34	3.38	3.46	3.33	3.73	3.86	3.69	4.00	4.11

Table 130

The 4-methylpyridine catalysed mutarctation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B) : (EH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.080	6.10	1.87	1.89
0.060	6.12	1.65	1.64
0.040	6.13	1.40	1.39
0.020	6.16	1.13	1.14
0.010	6.18	1.02	1.02

$k_B = 1.24 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.94%.

$k_{int.} = 8.96 \times 10^{-4}\ sec^{-1}$; S.D. = 0.37%.

Table 131

The 4-methylpyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (B) : (EH^+) = 1:1.

(4-methylpyridine)	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	6.08	4.66, 4.65	4.79
0.080	6.10	4.05, 4.06	4.10
0.060	6.12	3.47, 3.40	3.42
0.040	6.13	2.80, 2.73	2.73
0.020	6.16	2.10, 2.08	2.04
0.010	6.18	1.67, 1.70	1.70

$k_B = 3.43 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.65%.

$k_{int.} = 1.28 \times 10^{-3}\ sec^{-1}$; S.D. = 0.52%.

Table 132

The 4-methylpryidine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	6.08	1.80	1.85
0.080	6.10	1.57	1.57
0.060	6.12	1.30	1.28
0.040	6.13	1.00	1.00
0.020	6.16	0.72	0.71
0.010	6.18	0.57	0.57

$k_B = 1.42 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.65%.

$k_{int.} = 4.28 \times 10^{-4}\ sec^{-1}$; S.D. = 0.52%.

Table 133

The 4-methylpyridine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	6.08	21.07	21.72
0.080	6.10	18.20	18.11
0.060	6.12	14.30	14.51
0.040	6.13	11.13	10.91
0.020	6.16	7.36	7.30
0.010	6.18	5.47	5.50

$k_B = 1.80 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.77%.

$k_{int.} = 3.70 \times 10^{-4}\ sec^{-1}$; S.D. = 0.88%.

Table 134

The 4-methylpyridine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	6.12	15.84	15.92
0.040	6.13	11.78	11.75
0.020	6.16	7.64	7.57
0.010	6.18	5.45	5.48

$k_B = 2.09 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.54%.

$k_{int.} = 3.39 \times 10^{-4}\ sec^{-1}$; S.D. = 2.26%.

Table 135

The 4-methylpyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	6.12	3.41	3.38
0.040	6.13	2.34	2.35
0.030	6.14	1.86	1.83
0.020	6.16	1.35	1.31
0.010	6.16	0.76	0.80
0.005	6.17	0.57	0.54

$k_B = 5.17 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.87%.

$k_{int.} = 2.80 \times 10^{-4}\ sec^{-1}$; S.D. = 2.31%.

Table 136

The 4-methylpyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	6.12	2.16	2.20
0.040	6.13	1.62	1.56
0.030	6.14	1.22	1.23
0.020	6.16	0.92	0.91
0.010	6.16	0.59	0.59
0.005	6.17	0.42	0.42

$k_B = 3.24 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.81%.

$k_{int.} = 2.62 \times 10^{-4}\ sec^{-1}$; S.D. = 0.91%.

Table 137

The 4-methylpyridine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.040	6.13	15.12, 15.14	15.15
0.030	6.14	11.84	11.84
0.020	6.16	8.52	8.52
0.010	6.16	5.22	5.20
0.005	6.17	3.53	3.54

$k_B = 3.32 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.76%.

$k_{int.} = 1.88 \times 10^{-4}\ sec^{-1}$; S.D. = 1.64%.

Table 138

The 4-methylpyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. $(B):(BH^{+})$ = 1:1.

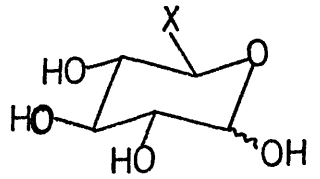
(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^{4}$	$k_{calc.} \times 10^{4}$
0.060	6.12	18.08	19.06
0.040	6.13	13.76	13.89
0.030	6.14	11.50	11.29
0.020	6.16	9.02	8.71
0.010	6.16	6.24	6.12
0.005	6.17	4.78	4.83

$k_{B} = 2.59 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.85%.

$k_{int.} = 3.53 \times 10^{-4}\ sec^{-1}$; S.D. = 0.74%.

Table 139

Comparison of the catalytic coefficients for catalysis by 4-methylpyridine for the series of substituted sugars



X	$-CH_3$	-H	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_B \times 10^2$ (M^{-1} sec^{-1})	1.24	3.43	1.42	1.80	2.09	5.17	3.24	3.32	2.59
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x

* estimated from data of Charton[129].

Table 140

The 4-ethoxypyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I - 0.10M. (E):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	1.86	1.87
0.040	6.74	1.64	1.67
0.030	6.73	1.49	1.48
0.020	6.73	1.30	1.29
0.010	6.75	1.10	1.09
0.005	6.75	1.00	1.00

$k_B = 1.93 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.75%.

$k_{int.} = 9.01 \times 10^{-4}\ sec^{-1}$; S.D. = 0.20%.

Table 141

The 4-ethoxypyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	3.98, 4.04	4.06
0.040	6.74	3.50, 3.48	3.52
0.030	6.73	2.95, 2.99	2.99
0.020	6.73	2.49, 2.48	2.46
0.010	6.75	1.96, 1.95	1.93
0.005	6.75	1.65, 1.64	1.66

$k_B = 5.33 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.49%.

$k_{int.} = 1.39 \times 10^{-3}\ sec^{-1}$; S.D. = 0.24%.

Table 142

The 4-ethoxypyridine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k'_{obsd.} \times 10^4$	$k'_{calc.} \times 10^4$
0.050	6.73	15.13	15.39
0.040	6.74	13.13	13.19
0.030	6.73	10.84	10.99
0.020	6.73	8.97	8.79
0.010	6.75	6.66	6.58
0.005	6.75	5.44	5.48

$k_B = 2.20 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.70%.

$k_{int.} = 4.38 \times 10^{-4}\ sec^{-1}$; S.D. = 0.46%.

Table 143

The 4-ethoxypyridine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k'_{obsd.} \times 10^4$	$k'_{calc.} \times 10^4$
0.050	6.73	16.48	17.09
0.040	6.74	13.61	14.42
0.030	6.73	11.97	11.75
0.020	6.73	9.38	9.09
0.010	6.75	6.73	6.42
0.005	6.75	4.97	5.08

$k_B = 2.67 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.74%.

$k_{int.} = 3.75 \times 10^{-4}\ sec^{-1}$; S.D. = 0.62%.

Table 144

The 4-ethoxypyridine catalysed mutarotation of 6-acetamido-6-decxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.73	20.43	20.93
0.040	6.74	16.53	17.34
0.030	6.73	13.51	13.75
0.020	6.73	10.43	10.16
0.010	6.75	6.80	6.57
0.005	6.75	4.64	4.78

$k_B = 3.59 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.99%.

$k_{int.} = 2.98 \times 10^{-4}\ sec^{-1}$; S.D. = 1.40%.

Table 145

The 4-ethoxypyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (B):(EH^+) = 1:1.

(4-ethoxypyridine)M.	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	4.30	4.36
0.040	6.74	3.56	3.56
0.030	6.73	2.76	2.77
0.020	6.73	1.99	1.97
0.010	6.75	1.19	1.18
0.005	6.75	0.78	0.78

$k_B = 7.94 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.69%.

$k_{int.} = 3.86 \times 10^{-4}\ sec^{-1}$; S.D. = 1.58%.

Table 146

The 4-ethoxypyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	2.97	3.07
0.040	6.74	2.47	2.51
0.030	6.73	1.95	1.96
0.020	6.73	1.43	1.41
0.010	6.75	0.90	0.85
0.005	6.75	0.56	0.57

$k_B = 5.55 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.63%.

$k_{int.} = 2.96 \times 10^{-4}\ sec^{-1}$; S.D. = 1.25%.

Table 147

The 4-ethoxypyridine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	3.51	3.47
0.040	6.74	2.71	2.84
0.030	6.73	2.19	2.20
0.020	6.73	1.59	1.57
0.010	6.75	1.01	0.93
0.005	6.75	0.60	0.61

$k_B = 6.35 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.73%.

$k_{int.} = 2.97 \times 10^{-4}\ sec^{-1}$; S.D. = 1.66%.

Table 148

The 4-ethoxypyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

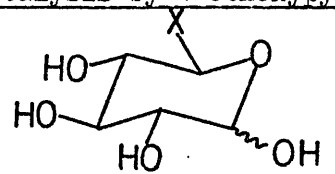
(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.73	23.25	23.61
0.040	6.74	19.26	19.68
0.030	6.73	15.79	15.74
0.020	6.73	12.07	11.81
0.010	6.75	8.07	7.87
0.005	6.75	5.84	5.91

$k_B = 3.93 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.74%.

$k_{int.} = 3.94 \times 10^{-4}\ sec^{-1}$; S.D. = 0.91%.

Table 149

Comparison of the catalytic coefficients for catalysis by 4-ethoxypyridine for the series of substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_B \times 10^2$ (M^{-1} sec^{-1})	1.93	5.33	2.20	2.67	3.59	7.94	5.55	6.35	3.93
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x

* estimated from data of Charton [129].

Table 150

The 4-acetylpyridine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M.	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	10.34	10.32
0.150	4.58	10.01	9.96
0.100	4.61	9.44	9.60
0.060	4.63	9.42	9.31
0.020	4.65	9.02	9.02

$k_B = 7.20 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 2.83%.

$k_{int.} = 8.88 \times 10^{-4}\ sec^{-1}$; S.D. = 0.17%.

Table 151

The 4-Acetylpyridine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	16.86	16.83
0.150	4.58	16.13	15.87
0.100	4.61	14.73	14.91
0.060	4.63	14.28	14.15
0.020	4.65	13.39	13.39

$k_B = 19.14 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.31%.

$k_{int} = 1.30 \times 10^{-3}\ sec^{-1}$; S.D. = 0.17%.

Table 152

The 4-Acetylpyridine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	5.27	5.31
0.150	4.58	4.93	4.98
0.100	4.61	4.72	4.65
0.060	4.63	4.39	4.39
0.020	4.65	4.10	4.13

$k_B = 6.55 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.52%.

$k_{int.} = 4.00 \times 10^{-4}\ sec^{-1}$; S.D. = 0.24%.

Table 153

The 4-Acetylpyridine catalysed mutarotation of 6-O-methly-α-D- glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	4.94	4.93
0.150	4.58	4.55	4.59
0.100	4.61	4.28	4.26
0.060	4.63	3.96	3.99
0.020	4.65	3.73	3.72

$k_B = 6.68 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.43%.

$k_{int.} = 3.59 \times 10^{-4}\ sec^{-1}$; S.D. = 0.21%.

Table 154

The 4-Acetylpyridine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	3.79	3.78
0.150	4.58	3.54	3.50
0.100	4.61	3.17	3.23
0.060	4.63	3.04	3.00

$k_B = 5.54 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 10.65%

$k_{int.} = 2.67 \times 10^{-4}\ sec^{-1}$; S.D. = 2.68%.

Table 155

The 4-acetylpyridine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	7.35	7.59
0.150	4.58	6.37	6.46
0.100	4.61	5.44	5.36
0.060	4.63	4.57	4.47
0.020	4.65	3.56	3.58

$k_B = 22.22 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.25%.

$k_{int.} = 3.14 \times 10^{-4}\ sec^{-1}$; S.D. = 0.48%.

Table 156

The 4-acetylpyridine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	4.14	4.17
0.150	4.58	3.74	3.72
0.100	4.61	3.26	3.27
0.060	4.63	2.93	2.91
0.020	4.65	2.55	2.55

$k_B = 8.99 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.33%.

$k_{int.} = 2.37 \times 10^{-4}\ sec^{-1}$; S.D. = 0.37%.

Table 157

The 4-acetylpyridine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	2.41	2.43
0.150	4.58	2.18	2.17
0.100	4.61	1.94	1.92
0.060	4.63	1.73	1.72
0.020	4.65	1.50	1.51

$k_B = 5.10 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.68%.

$k_{int.} = 1.41 \times 10^{-4}\ sec^{-1}$; S.D. = 0.63%.

Table 158

The 4-Acetylpyridine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

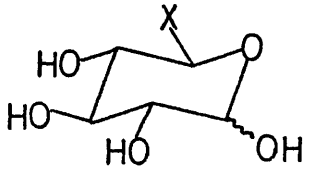
(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	5.53	5.63
0.150	4.58	5.22	5.12
0.100	4.61	4.51	4.61
0.060	4.63	4.34	4.21
0.020	4.65	3.76	3.80

$k_B = 10.19 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 3.08%.

$k_{int.} = 3.59 \times 10^{-4}\ sec^{-1}$; S.D. = 0.84%.

Table 159

Comparison of the catalytic coefficients for catalysis by 4-acetylpyridine in the series of substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_B \times 10^4$ $(M^{-1}\ sec^{-1})$	7.20	19.14	6.55	6.68	5.54	22.22	8.99	5.10	10.19
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x
$k^a_{H_2O} \times 10^6$ $(M^{-1}\ sec^{-1})$	16.00	23.52	7.20	6.47	4.81	5.65	4.28	2.54	6.48

* estimated from data of Charton[129].

a. calculated by dividing the $k_{int.}$ for each sugar (tables 150 - 158) by 55.6 - the molarity of water.

Table 160

The 2,6-lutidine catalysed mutarotation of 6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1.1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	9.65	9.62
0.040	6.87	9.32	9.38
0.030	6.89	9.12	9.15
0.020	6.90	8.93	8.92
0.005	6.92	8.58	8.57

$k_B = 2.32 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 3.14%.

$k_{int.} = 8.45 \times 10^{-4}\ sec^{-1}$; S.D. = 0.27%.

Table 161

The 2,6-lutidine catalysed mutarotation of α-D-xylose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.87	1.74	1.73
0.040	6.87	1.65	1.66
0.030	6.89	1.58	1.58
0.020	6.90	1.51	1.51
0.010	6.89	1.44	1.44
0.005	6.92	1.38	1.40

$k_B = 7.40 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.24%.

$k_{int.} = 1.36 \times 10^{-3}\ sec^{-1}$; S.D. = 0.15%.

Table 162

The 2,6-lutidine catalysed mutarotation of α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	4.86	4.89
0.040	6.87	4.65	4.71
0.030	6.89	4.57	4.53
0.020	6.90	4.32	4.35
0.010	6.89	4.22	4.17
0.005	6.92	4.05	4.08

$k_B = 1.80 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.33%.

$k_{int.} = 4.00 \times 10^{-4}\ sec^{-1}$; S.D. = 0.29%.

Table 163

The 2,6-lutidine catalysed mutarotation of 6-O-methyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	4.73	4.73
0.040	6.87	4.51	4.50
0.030	6.89	4.26	4.26
0.020	6.90	4.06	4.03
0.010	6.89	3.75	3.79
0.005	6.92	3.70	3.67

$k_B = 2.36 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.64%.

$k_{int.} = 3.55 \times 10^{-4}\ sec^{-1}$; S.D. = 0.40%.

Table 164

The 2,6-lutidine catalysed mutarotation of 6-acetamido-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	5.25	5.25
0.040	6.87	4.70	4.72
0.030	6.89	4.18	4.19
0.020	6.90	3.70	3.67
0.010	6.89	3.13	3.14

$k_B = 5.27 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.98%.

$k_{int.} = 2.61 \times 10^{-4}\ sec^{-1}$; S.D. = 1.56%.

Table 165

The 2,6-lutidine catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	8.97	9.34
0.040	6.87	7.98	8.09
0.030	6.89	6.97	6.83
0.020	6.90	5.76	5.58
0.010	6.89	4.30	4.32
0.005	6.92	3.69	3.70

$k_B = 12.55 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.87%.

$k_{int.} = 3.07 \times 10^{-4}\ sec^{-1}$; S.D. = 0.44%.

Table 166

The 2,6-lutidine catalysed mutarotation of 6-chloro-6-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	5.60	5.59
0.040	6.87	5.08	5.03
0.030	6.89	4.40	4.46
0.020	6.90	3.89	3.89
0.010	6.89	3.33	3.33
0.005	6.92	3.05	3.05

$k_B = 5.65 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.12%.

$k_{int.} = 2.76 \times 10^{-4}\ sec^{-1}$; S.D. = 0.40%.

Table 167

The 2,6-lutidine catalysed mutarotation of 6-cyano-6-deoxy-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	7.83	7.94
0.040	6.87	7.05	6.98
0.030	6.89	6.13	6.01
0.020	6.90	4.98	5.05
0.010	6.89	4.01	4.08
0.005	6.92	3.62	3.60

$k_B = 9.66 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.90%.

$k_{int.} = 3.11 \times 10^{-4}\ sec^{-1}$; S.D. = 0.41%.

Table 168

The 2,6-lutidine catalysed mutarotation of β-Gentiobiose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

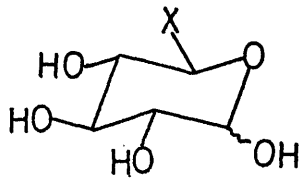
(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	5.15	5.18
0.040	6.87	4.92	4.90
0.030	6.89	4.65	4.61
0.020	6.90	4.30	4.33
0.010	6.89	4.05	4.05
0.005	6.92	3.91	3.91

$k_B = 2.81 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.99%.

$k_{int.} = 3.77 \times 10^{-4}\ sec^{-1}$; S.D. = 0.37%.

Table 169

Comparison of the catalytic coefficients for catalysis by 2,6-lutidine for the series of substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
$k_B \times 10^3$ (M^{-1} sec^{-1})	2.32	7.40	1.80	2.36	5.27	12.55	5.65	9.66	2.81
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x

* estimated from data of Charton.[129]

Table 170

Brönsted Plots for catalysis by 4-substituted pyridines.

(a) 6-deoxy-α-D-glucose

pK_a	$6 + \log k_B$(obsd.)	$6 + \log k_B$(calc.)
6.67	4.29	4.38
5.98	4.09	4.05
5.22	3.76	3.70
3.48	2.86	2.88

β = 0.47 ; S.D. = 2.47%.

(b) α-D-xylose

pK_a	$6 + \log k_B$ (obsd.)	$6 + \log k_B$ (calc)
6.67	4.73	4.82
5.98	4.54	4.49
5.22	4.18	4.13
3.48	3.28	3.30

β = 0.48 ; S.D. = 2.36%.

(c) α-D-glucose

pK_a	$6 + \log k_B$ (obsd.)	$6 + \log k_B$ (calc.)
6.67	4.34	4.44
5.98	4.15	4.10
5.22	3.78	3.72
3.48	2.82	2.84

β = 0.50 ; S.D. = 2.40%.

Table 170 (cont.)

(d) 6-O-methyl-α-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.43	4.57
5.98	4.26	4.19
5.22	3.87	3.78
3.48	2.82	2.84

β = 0.54 ; S.D. = 2.36%.

(e) 6-acetamido-6-deoxy-β-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.56	4.66
5.98	4.32	4.26
5.22	3.86	3.81
3.48	2.78	2.79

β = 0.58 ; S.D. = 2.30%.

(f) 6-O-phenyl-α-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.90	4.97
5.98	4.71	4.61
5.22	4.22	4.24
3.48	3.35	3.35

β = 0.51 ; S.D. = 2.54%.

Table 170 (cont.)

(g) 6-chloro-6-deoxy-α-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.74	4.84
5.98	4.51	4.43
5.22	4.02	4.00
3.48	2.95	2.96

β = 0.59 ; S.D. = 2.33%.

(h) 6-cyano-6-deoxy-β-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.80	4.92
5.98	4.52	4.44
5.22	3.98	3.92
3.48	2.71	2.73

β = 0.69 ; S.D. = 2.21%.

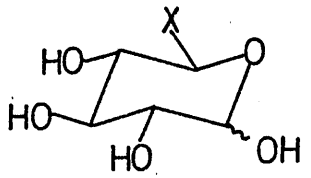
(i) β-Gentiobiose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.59	4.70
5.98	4.41	4.34
5.22	4.00	3.94
3.48	3.01	3.03

β = 0.525 ; S.D. = 2.42%.

Table 171

Brönsted exponents and Steric Hindrance Factors for the series of 5-substituted sugars



X	$-CH_3$	$-H$	$-CH_2OH$	$-CH_2OCH_3$	$-CH_2NHAc$	$-CH_2OPh$	$-CH_2Cl$	$-CH_2CN$	$-CH_2OC_6H_{11}O_5$
β	0.47	0.48	0.50	0.54	0.58	0.51	0.59	0.69	0.525
obsd. $k_{lut} \times 10^3$ (M^{-1} sec^{-1})	2.32	7.40	1.80	2.36	5.27	12.55	5.65	9.66	2.81
calc.[a] $k_{lut.} \times 10^3$ (M^{-1} sec^{-1})	25.06	69.34	29.31	39.08	49.1	99.00	73.96	90.16	53.58
S. H. F.	10.8	9.4	15.7	16.6	9.3	7.9	13.1	9.3	19.1
σ	-0.05	0.00	+0.05	+0.07	+0.07	+0.11*	+0.15	+0.18	+ x

a. calculated from the Bronsted plot. i.e. k_B for a base with $pK_a = 6.72$.

* estimated from data of Charton[129].

Table 172

The formic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(HCO_2H):(HCO_2^-)$ = 5:1.

(HCO_2H)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
1.00	2.71	7.53	7.39
0.80	2.72	6.77	6.71
0.60	2.73	5.94	6.03
0.40	2.76	5.35	5.35
0.20	2.80	4.69	4.67

$k_{slope} = 3.40 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.76%.

$k_{int.} = 3.99 \times 10^{-4}\ sec^{-1}$; S.D. = 0.73%.

Table 173

The formic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(HCO_2H):(HCO_2^-)$ = 2:1.

(HCO_2H)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
1.00	3.10	12.36	12.44
0.80	3.12	11.40	11.11
0.60	3.13	9.56	9.79
0.40	3.15	8.56	8.46
0.20	3.16	7.11	7.13

$k_{slope} = 6.64 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.49%.

$k_{int.} = 5.80 \times 10^{-4}\ sec^{-1}$; S.D. = 0.91%.

Table 174

The acetic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(CH_3Co_2H):(CH_3Co_2^-)$ = 5:1.

(CH_3Co_2H)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
1.00	3.85	1.93, 1.96	1.94
0.80	3.84	1.86, 1.84	1.83
0.60	3.85	1.75, 1.68	1.71
0.40	3.85	1.64, 1.66	1.60

$k_{slope} = 5.63 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 3.74%.

$k_{int.} = 1.38 \times 10^{-3}\ sec^{-1}$; S.D. = 1.13%.

Table 175

The acetic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(CH_3Co_2H):(CH_3Co_2^-)$ = 2:1.

(CH_3Co_2H)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
1.00	4.28	3.21, 3.23	3.22
0.80	4.27	3.02, 3.06	3.05
0.60	4.27	2.89, 2.87	2.88
0.40	4.27	2.72, 2.69	2.70

$k_{slope} = 8.61 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 4.19%.

$k_{int.} = 2.36 \times 10^{-3}\ sec^{-1}$; S.D. = 1.12%.

Table 176

The acetic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(CH_3Co_2H):(CH_3Co_2^-)$ = 1:1.

(CH_3Co_2H)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
1.00	4.63	4.40, 4.33	4.41
0.80	4.63	4.15, 4.16	4.15
0.60	4.61	3.91, 3.90	3.89
0.40	4.59	3.59, 3.65	3.62

$k_{slope} = 1.32 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.75%.

$k_{int.} = 3.09 \times 10^{-3}\ sec^{-1}$; S.D. = 0.78%.

Table 177

The acetic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(CH_3Co_2H):(CH_3Co_2^-)$ = 1:2.

(CH_3Co_2H)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.50	4.94	4.72, 4.74	4.79
0.40	4.93	4.56, 4.62	4.54
0.30	4.90	4.35, 4.27	4.28
0.20	4.89	4.02, 4.00	4.03

$k_{slope} = 2.54 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 3.49%.

$k_{int.} = 3.52 \times 10^{-3}\ sec^{-1}$; S.D. = 0.79%.

Table 178

The acetic acid catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M. $(CH_3Co_2H):(CH_3Co_2^-)$ = 1:5.

(CH_3Co_2H)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.200	5.32	4.84, 4.90	4.89
0.160	5.31	4.71, 4.74	4.71
0.120	5.30	4.60, 4.60	4.53
0.080	5.29	4.33, 4.33	4.35

$k_{slope} = 4.50 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 4.7%.

$k_{int.} = 3.99 \times 10^{-3}\ sec^{-1}$; S.D. = 0.69%.

Table 179

The pyridine catalysed mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 0.30M. (Pyridine):(Pyridinium) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.500	5.90	6.71	6.76
0.400	5.91	6.38, 6.29	6.30
0.300	5.92	5.84, 5.77	5.83
0.200	5.93	5.58, 5.35	5.37
0.100	5.93	4.88, 4.90	4.90

$k_{slope} = 4.67 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 5.65%.

$k_{int.} = 4.43 \times 10^{-3}\ sec^{-1}$; S.D. = 1.67%.

Table 180

The pH rate profile of the spontaneous mutarotation of 6-deoxy-α-D-gluco-hepturonic acid at 25° in water, I = 1.0M.

pH_{25}	observed spontaneous rate	($k_{int.}$)	calculated spontaneous rate *	
2.00	2.28×10^{-4}	sec^{-1}	2.69×10^{-4}	sec^{-1}
2.75	3.98 "	"	3.66 "	"
3.13	5.80 "	"	5.20 "	"
3.85	1.38×10^{-3}	"	1.37×10^{-3}	"
4.27	2.36 "	"	2.31 "	"
4.61	3.09 "	"	3.11 "	"
4.91	3.52 "	"	3.67 "	"
5.30	3.99 "	"	4.11 "	"
5.92	4.43 "	"	4.39 "	"

* The pH rate profile was calculated using a program written by Dr. B. Capon using a generalised least squares procedure according to the method of Wentworth[127] and Deming[128] to fit the expression

$$k_o = (k_{SH} \cdot 10^{-pH}/K_a + k_s)/(1 + 10^{-pH}/K_a)$$

where k_{SH} and k_s are the rate constants for the spontaneous mutarotation of the unionised and ionised forms of the acid and K_a is the dissociation constant of the acid.

Table 181

The HCl catalysed mutarotation of 6-O-(c-hydroxyphenyl)-β-D-glucose at 25° in water, I = 0.10M.

(H_3O^+)	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	12.17	12.59
0.080	1.09	10.82	10.72
0.060	1.21	8.77	8.85
0.040	1.40	7.14	6.97
0.020	1.68	5.06	5.10
0.010	1.98	4.17	4.16

$k_{H_3O^+} = 9.37 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.07%.

$k_{int.} = 3.23 \times 10^{-4}\ sec^{-1}$; S.D. = 0.94%.

Table 182

The pyridine catalysed mutarotation of 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium) = 1:2.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	4.96	14.25	14.24
0.040	4.99	12.65	12.54
0.030	4.99	10.71	10.84
0.020	5.03	9.10	9.15
0.010	5.03	7.58	7.45
0.005	5.03	6.50	6.60

$k_B = 16.97 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.36%.

$k_{int.} = 5.75 \times 10^{-4}\ sec^{-1}$; S.D. = 0.73%.

Table 183

The pyridine catalysed mutarotation of 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.400	5.89	7.66	7.75
0.300	5.90	6.29	6.37
0.200	5.93	5.01	4.99
0.100	5.93	3.71	3.61
0.050	5.94	2.84	2.91

$k_B = 1.38 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.21%.

$k_{int.} = 2.23 \times 10^{-3}\ sec^{-1}$; S.D. = 1.11%.

Table 184

The 4-methylpyridine catalysed mutarotation of 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	6.12	5.92	5.87
0.040	6.13	4.98	5.04
0.030	6.14	4.65	4.63
0.020	6.16	4.25	4.21
0.010	6.16	3.78	3.80

$k_B = 4.15 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.13%.

$k_{int.} = 3.38 \times 10^{-3}\ sec^{-1}$; S.D. = 0.70%.

Table 185

The 2,6-lutidine catalysed mutarotation of 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(Lutidine)M	pH_{25}	$k_{obsd.} \times 10^2$	$k_{calc.} \times 10^2$
0.030	6.89	1.18, 1.21	-
0.020	6.90	1.16, 1.03	-
0.010	6.89	1.15, 1.14	-
0.005	6.92	1.07, 1.16	-

As can be seen from the above results, general base catalysis is not readily detectable since the rates of mutarotation are faster than the maximum rate which the Polarimeter 141 can accurately measure ($k = 10^{-2}$). In addition, catalysis by 2,6-lutidine is small in comparison with the spontaneous rate and so falls within experimental error at this rate.

From the above data we can say that the spontaneous rate of mutarotation of 6-O-(o-hydroxyphenyl)-β-D-glucose at pH 6.90 is greater than 1.0×10^{-2} sec^{-1}.

Table 186

Comparison of the spontaneous rates of mutarotation of 6-O-phenyl-α-D-glucose and 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° in water.

	Spontaneous Rate at 25° of	
pH_{25}	6-O-phenyl- -D-glucose	6-O-(o-hydroxyphenyl)glucose
2.00	3.1×10^{-4} sec^{-1}	3.2×10^{-4} sec^{-1}
5.00	2.9 " "	5.8×10^{-4} "
5.92	3.2 " "	2.2×10^{-3} "
6.14	2.8 " "	3.4×10^{-3} "
6.90	3.1 " "	> 1.0×10^{-2} "

A plot of the spontaneous rates of hydrolysis (excluding the last value) for 6-O-(o-hydroxyphenly)-glucose against (OH^-) gave a straight line of slope 2.28×10^5 (see discussion section 4.8).

Table 187

Tests for intermolecular catalysis in the mutarotation of :-

a) 6-deoxy-α-D-gluco-hepturonic acid.

(Sugar)M	Buffer	pH_{25}	$k_{obsd.} \times 10^3$
0.082	0.4M Pyridine	5.9	6.20
0.115	"	"	6.24, 6.25
0.255	"	"	5.98
0.264	"	"	5.82
0.346	"	"	6.09
0.356	"	"	6.01
0.466	"	"	6.07
0.500	"	"	5.89

(b) 6-O-(o-hydroxyphenyl)-β-D-glucose

(Sugar)M	Buffer	pH_{25}	$k_{obsd.} \times 10^3$
0.026	0.1M pyridine	5.9	3.59
0.048	"	"	3.65
0.074	"	"	3.52
0.079	"	"	3.50
0.128	"	"	3.66, 3.47

Table 188

The phenclate ion catalysed mutarotation of 6-O-phenyl-α-D-glucose at 25° in water, I = 0.10M. (Phenol):(Phenolate) = 10:1.

(Phenolate)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.006	9.12	8.78	8.88
0.004	9.13	6.43	6.41
0.003	9.13	5.26	5.18
0.002	9.14	3.92	3.94

$k_{Pho^-} = 1.24\ M^{-1}\ sec^{-1}$; S.D. = 1.85%.

$k_{int.} = 1.47 \times 10^{-3}\ sec^{-1}$; S.D. = 4.48%.

Table 189

The dissociation constant of 6-O-(o-hydroxyphenyl)-β-D-glucose at 25° was determined spectrophotometrically by measurement of the optical density at zero time at 290 nm in 0.1 M HCl, 0.1M NaOH and at seven pH's around pH 10

$$pK_a = 9.67.$$

Adjusting this spectrophotometrically determined pK_a by the procedure recommended by Albert and Sergeant[130] gives a thermodynamic pK_a of 9.78.

Table 190

The pyridine catalysed mutarotation of 2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridiniun) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.080	5.33	4.07	4.03
0.060	5.34	3.42	3.37
0.040	5.36	2.70	2.71
0.020	5.37	2.00	2.04
0.010	5.38	1.73	1.71

$k_B = 3.32 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.49%.

$k_{int.} = 1.38 \times 10^{-3}\ sec^{-1}$; S.D. = 0.87%.

Table 191

The pyridine catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.33	11.96	12.09
0.060	5.34	10.53	10.35
0.040	5.36	8.59	8.62
0.020	5.37	6.83	6.88
0.010	5.38	6.04	6.02

$k_B = 8.67 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.89%.

$k_{int} = 5.15 \times 10^{-4}\ sec^{-1}$; S.D. = 0.39%.

Table 192

The pyridine catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (Pyridine):(Pyridinium) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.33	8.77	8.81
0.060	5.34	7.86	7.84
0.040	5.36	6.87	6.86
0.020	5.37	5.89	5.88
0.010	5.38	5.39	5.39

$k_B = 4.88 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.93%.

$k_{int.} = 4.91 \times 10^{-4}\ sec^{-1}$; S.D. = 0.22%.

Table 193

The pyridine catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M. $(B):(BH^+) = 1:1$.

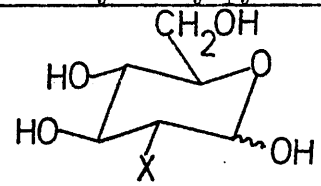
(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.080	5.33	9.01	8.97
0.060	5.34	8.01	7.91
0.040	5.36	6.76	6.85
0.020	5.37	5.74	5.79
0.010	5.38	5.30	5.26

$k_B = 5.30 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.99%.

$k_{int.} = 4.73 \times 10^{-4}\ sec^{-1}$; S.D. = 0.33%.

Table 194

Comparison of the catalytic coefficients for catalysis by pyridine for the series of 2- substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
observed $k_B \times 10^3$ (M^{-1} sec^{-1})	33.16	5.98	8.67	4.88	5.30
* $k_B' \times 10^3$ (M^{-1} sec^{-1})	14.94	3.83	4.34	1.56	1.96
σ	0.00	+0.25	+0.28	+0.28	+0.60
observed $k^a_{H_2O} \times 10^6$ (M^{-1} sec^{-1})	24.82	7.33	9.26	8.81	8.45

a. :- obtained by dividing the $k_{int.}$ of tables 190 - 193 by the molarity of water.

* $k_B' = \frac{k_B}{1+K}$ where K = equilibrium constant for the mutarotation of the different sugars. - see discussion section 4.3.B.

Table 195

The 4-methylpyridine catalysed mutarotation of 2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.060	6.12	6.86	6.95
0.040	6.13	5.14	5.19
0.030	6.14	4.29	4.30
0.020	6.16	3.53	3.42
0.010	6.18	2.51	2.54

$k_B = 8.84 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.11%.

$k_{int.} = 1.65 \times 10^{-3}\ sec^{-1}$; S.D. = 1.26%.

Table 196

The 4-methylpyridine catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	6.12	17.72	18.18
0.040	6.13	13.81	13.84
0.030	6.14	11.86	11.67
0.020	6.16	9.70	9.50
0.010	6.18	7.24	7.33
0.005	6.18	6.27	6.24

$k_B = 2.17 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.73%.

$k_{int.} = 5.15 \times 10^{-4}\ sec^{-1}$; S.D. = 0.42%.

Table 197

The 4-methylpyridine catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	6.12	12.11	12.45
0.040	6.13	9.78	9.91
0.030	6.14	8.74	8.65
0.020	6.16	7.48	7.38
0.010	6.16	6.20	6.11
0.005	6.18	5.45	5.48

$k_B = 1.267 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.93%.

$k_{int.} = 4.84 \times 10^{-4}\ sec^{-1}$; S.D. = 0.22%.

Table 198

The 4-methylpyridine catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

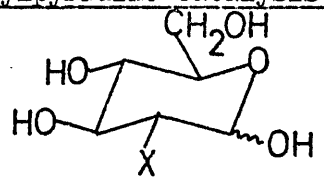
(4-methylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.060	6.12	12.49	12.92
0.040	6.13	10.22	10.19
0.030	6.14	8.86	8.83
0.020	6.16	7.52	7.46
0.010	6.16	6.18	6.10
0.005	6.18	5.35	5.41

$k_B = 1.365 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.78%.

$k_{int.} = 4.73 \times 10^{-4}\ sec^{-1}$; S.D. = 0.34%.

Table 199

Comparision of the catalytic coefficients for 4-methylpyridine catalysis in the series of 2- substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
observed $k_B \times 10^2$ $(M^{-1} sec^{-1})$	8.84	1.42	2.17	1.27	1.37
* $k_B' \times 10^2$ $(M^{-1} sec^{-1})$	3.98	0.91	1.09	0.41	0.51
σ	0.00	+0.25	+0.28	+0.28	+0.60

* see footnote table 194.

Table 200

The 4-ethoxypyridine catalysed mutarotation of 2-deoxy-α-D-glucose at 25° in water, I = 0.10M (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.040	6.74	8.16, 8.22	8.18
0.030	6.73	6.46, 6.84	6.79
0.020	6.73	5.40, 5.40	5.39
0.010	6.75	4.11, 4.15	4.00
0.005	6.75	3.14, 3.36	3.30

k_B = 13.95 x 10^{-2} M^{-1} sec^{-1} ; S.D. = 0.73%.

$k_{int.}$ = 2.60 x 10^{-3} sec^{-1} ; S.D. = 0.49%.

Table 201

The 4-ethoxypyridine catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M. (B):(EH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.050	6.73	2.26	2.29
0.040	6.74	1.99	1.93
0.030	6.73	1.52	1.57
0.020	6.73	1.22	1.21
0.010	6.75	0.90	0.86
0.005	6.75	0.67	0.68

k_B = 3.58 x 10^{-2} M^{-1} sec^{-1} ; S.D. = 0.78%.

$k_{int.}$ = 4.98 x 10^{-4} sec^{-1} ; S.D. = 0.66%.

Table 202

The 4-ethoxypyridine catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.73	13.66	14.12
0.040	6.74	12.32	12.33
0.030	6.73	10.69	10.55
0.020	6.73	8.74	8.76
0.010	6.75	7.13	6.97
0.005	6.75	6.04	6.08

$k_B = 1.79 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.70%.

$k_{int.} = 5.19 \times 10^{-4}\ sec^{-1}$; S.D. = 0.28%.

Table 203

The 4-ethoxypyridine catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

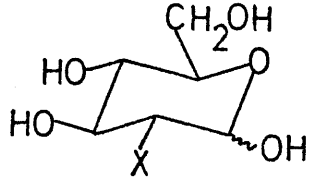
(4-ethoxypyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.73	14.30	14.51
0.040	6.74	12.57	12.57
0.030	6.73	10.90	10.63
0.020	6.73	9.05	8.69
0.010	6.75	6.58	6.75
0.005	6.75	5.84	5.78

$k_B = 1.94 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 0.80%.

$k_{int.} = 4.81 \times 10^{-4}$; S.D. = 0.38%.

Table 204

Comparison of the catalytic coefficients for catalysis by 4-ethoxypyridine for the series of 2- substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
observed $k_B \times 10^2$ (M^{-1} sec^{-1})	13.95	2.20	3.58	1.79	1.94
* $k_B' \times 10^2$ (M^{-1} sec^{-1})	6.28	1.41	1.79	0.57	0.72
σ	0.00	+0.25	+0.28	+0.28	+0.60

* see footnote table 194.

Table 205

The 4-acetylpyridine catalysed mutarotation of 2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.200	4.55	2.19	2.20
0.150	4.58	1.95	1.98
0.100	4.61	1.78	1.76
0.060	4.63	1.62	1.58
0.020	4.65	1.40	1.41

$k_B = 4.43 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.93%.

$k_{int.} = 1.32 \times 10^{-3}\ sec^{-1}$; S.D. = 0.37%.

Table 206

The 4-acetylpyridine catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	7.17	7.21
0.150	4.58	6.67	6.65
0.100	4.61	6.18	6.10
0.060	4.63	5.61	5.66
0.020	4.65	5.21	5.21

$k_B = 1.11 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.27%.

$k_{int.} = 4.99 \times 10^{-4}\ sec^{-1}$; S.D. = 0.23%.

Table 207

The 4-acetylpyridine catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	6.10	6.05
0.150	4.58	5.69	5.76
0.100	4.61	5.52	5.47
0.060	4.63	5.27	5.23
0.020	4.65	4.99	5.00

$k_B = 5.83 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 1.30%.

$k_{int.} = 4.88 \times 10^{-4}\ sec^{-1}$; S.D. = 0.15%.

Table 208

The 4-acetylpyridine catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M. (B):(BH^+) = 10:1.

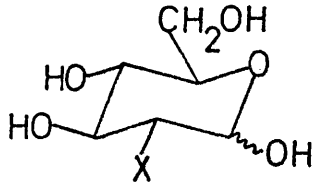
(4-acetylpyridine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.200	4.55	6.00	6.04
0.150	4.58	5.73	5.70
0.100	4.61	5.38	5.37
0.060	4.63	5.12	5.11
0.020	4.65	4.79	4.85

$k_B = 6.61 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 2.65%.

$k_{int.} = 4.71 \times 10^{-4}\ sec^{-1}$; S.D. = 0.48%.

Table 209

Comparison of the catalytic coefficients for catalysis by 4-acetylpyridine for the series of 2- substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
observed $k_B \times 10^3$ $(M^{-1}\ sec^{-1})$	4.43	0.66	1.11	0.58	0.66
* $k_B' \times 10^3$ $(M^{-1}\ sec^{-1})$	2.00	0.42	0.56	0.19	0.25
σ	0.00	+0.25	+0.28	+0.28	+0.60

* see footnote table 194.

Table 210

The 2,6-lutidine catalysed mutarotation of 2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	6.84	3.46, 3.46	3.45
0.080	6.84	3.38, 3.31	3.34
0.060	6.84	3.20	3.23
0.040	6.87	3.16, 3.13	3.12
0.030	6.89	3.06	3.06
0.020	6.90	3.02	3.00
0.010	6.89	2.93	2.95

$k_B = 5.65 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 3.02%.

$k_{int.} = 2.89 \times 10^{-3}\ sec^{-1}$; S.D. = 0.41%.

Table 211

The 2,6-lutidine catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	6.54	6.66
0.040	6.87	6.40	6.36
0.030	6.89	6.02	6.07
0.020	6.90	5.88	5.78
0.010	6.89	5.40	5.48
0.005	6.92	5.33	5.34

$k_B = 2.94 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 1.48%.

$k_{int.} = 5.19 \times 10^{-4}\ sec^{-1}$; S.D. = 0.18%.

Table 212

The 2,6-lutidine catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M. (B):(BH^+) = 1:1.

(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	5.65	5.65
0.040	6.87	5.53	5.52
0.030	6.89	5.37	5.38
0.020	6.90	5.23	5.25
0.010	6.89	5.16	5.12
0.005	6.92	5.07	5.05

$k_B = 1.34 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.44%.

$k_{int.} = 4.98 \times 10^{-4}\ sec^{-1}$; S.D. = 0.21%.

Table 213

The 2,6-lutidine catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M. (B):(BH^+) - 1:1.

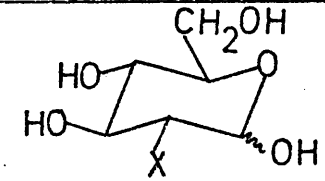
(2,6-lutidine)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.050	6.87	8.31	8.23
0.040	6.87	7.53	7.60
0.030	6.89	6.95	6.97
0.020	6.90	6.38	6.34
0.010	6.89	5.60	5.71

$k_B = 6.47 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 2.12%.

$k_{int.} = 5.01 \times 10^{-4}\ sec^{-1}$; S.D. = 0.79%.

Table 214

Comparison of the catalytic coefficients for catalysis by 2,6-lutidine for the series of 2- substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
observed $k_B \times 10^3$ (M^{-1} sec^{-1})	5.65	1.80	2.94	1.34	6.47
* $k_B' \times 10^3$ (M^{-1} sec^{-1})	2.55	1.22	1.47	0.43	2.40
σ	0.00	+0.25	+0.28	+0.28	+0.60

* see footnote - table 194.

Table 215

Brönsted Plots for catalysis by 4-substituted pyridines.

(a) 2-deoxy-α-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	5.145	5.217
5.98	4.946	4.880
5.22	4.521	4.508
3.48	3.646	3.655

β = 0.49 ; S.D. = 2.65%.

(b) 2-O-methyl-β-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.554	4.624
5.98	4.336	4.284
5.22	3.938	3.911
3.48	3.045	3.055

β = 0.49 ; S.D. = 2.48%.

Table 215 (cont.)

(c) 2-acetamido-2-deoxy-α-D-glucose

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.252	4.293
5.98	4.103	3.960
5.22	3.488	3.593
3.48	2.766	2.753

β = 0.48 ; S.D. = 2.42%.

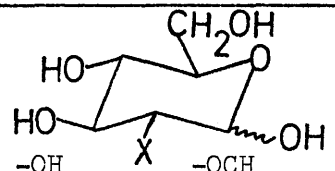
(d) 2-amino-2-deoxy-α-D-glucose hydrochloride

pK_a	6 + log k_B (obsd.)	6 + log k_B (calc.)
6.67	4.288	4.394
5.98	4.135	4.056
5.22	3.724	3.685
3.48	2.820	2.835

β = 0.49 ; S.D. = 2.42%.

Table 216

Brönsted exponents and Steric Hindrance Factors for the series of 2-substituted sugars



X	-H	-OH	$-OCH_3$	$-NHCOCH_3$	$-NH_3^+$
β	0.49	0.50	0.49	0.48	0.49
observed $k_{lut} \times 10^3$ (M^{-1} sec^{-1})	5.65	1.80	2.94	1.34	6.47
calculated [a.] $k_{lut.} \times 10^3$ (M^{-1} sec^{-1})	174.0	29.3	44.6	20.8	26.2
S. H. F.	30.8	15.4	15.2	15.5	4.1
σ	0.00	+0.25	+0.28	+0.28	+0.60

a. calculated from the Bronsted plot i.e. k_B for a base with $pK_a = 6.72$.

Table 217

The HCl catalysed mutarotation of 2-O-methyl-β-D-glucose at 25° in water, I = 0.10M.

$(H_3O^+)M$	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	14.60	14.46
0.080	1.09	12.66	12.58
0.060	1.21	10.68	10.67
0.040	1.40	8.67	8.77
0.020	1.68	6.82	6.88
0.010	1.98	5.95	5.93

$k_{H_3O^+} = 9.49 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.71%.

$k_{int.} = 4.98 \times 10^{-4}\ sec^{-1}$; S.D. = 0.33%.

Table 218

The HCl catalysed mutarotation of 2-acetamido-2-deoxy-α-D-glucose at 25° in water, I = 0.10M.

$(H_3O^+)M$	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	10.89	11.09
0.080	1.09	9.82	9.83
0.060	1.21	8.64	8.58
0.040	1.40	7.43	7.33
0.020	1.68	6.07	6.07
0.010	1.98	5.44	5.45

$k_{H_3O^+} = 6.27 \times 10^{-3}\ M^{-1}\ sec^{-1}$; S.D. = 0.69%.

$k_{int.} = 4.82 \times 10^{-4}\ sec^{-1}$; S.D. = 0.23%.

Table 219

The HCl catalysed mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride at 25° in water, I = 0.10M.

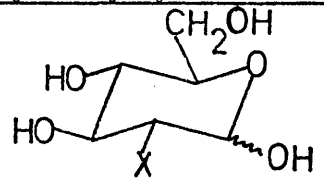
(H_3O^+)M	pH_{25}	$k_{obsd.} \times 10^4$	$k_{calc.} \times 10^4$
0.100	0.98	5.03	5.03
0.080	1.09	4.96	4.96
0.060	1.21	4.90	4.89
0.040	1.40	4.80	4.81
0.020	1.68	4.75	4.74
0.010	1.98	4.70	4.70

$k_{H_3O^+} = 3.65 \times 10^{-4}\ M^{-1}\ sec^{-1}$; S.D. = 3.57%.

$k_{int.} = 4.67 \times 10^{-4}\ sec^{-1}$; S.D. = 0.16%.

Table 220

Comparison of the catalytic coefficients for catalysis by hydronium ion for the series of 2-substituted sugars



X	-H	-OH	-O CH_3	-NHCOCH$_3$	-NH_3^+
$k_{H_3O^+} \times 10^3$ (M^{-1} sec^{-1})	> 1000	11.06	9.49	6.27	0.36
$k_{H_2O} \times 10^6$ (M^{-1} sec^{-1})	≈ 25	7.21	8.97	8.67	8.40
σ	0.00	+0.25	+0.28	+0.28	+0.60

Table 221

The Equilibrium Proportions of the Sugars studied

Sugar	%α observed		%β observed	%α literature (ref.)		%β literature (ref.)
6-deoxy-D-glucose	31		69			
D-xylose	37		63	35	(6)	65
D-glucose	37		63	36	(6)	64
6-O-methyl-D-glucose	35		65			
6-acetamido-6-deoxy-D-glucose	32	*	68			
D-gentiobiose	31	*	69			
6-O-phenyl-D-glucose	32	*	68			
6-chloro-6-deoxy-D-glucose	33	*	67			
6-deoxy-D-glucohepturonic acid	36		64			
6-cyano-6-deoxy-D-glucose	39		61			
D-glucose-6-phosphate				31	(32)	69
2-deoxy-D-glucose	55		45			
2-amino-2-deoxy-D-glucose HCl	61		39	63	(131)	37
2-acetamido-2-deoxy-D-glucose		*		66	(131)	34
2-O-methyl-D-glucose	50		50			

* The spinning side bands of the D-OH peak render these results less accurate.

Table 222

Vapor Phase Chromatography of substituted pyridines

Instrument:- Pye Argon Chromatograph

Conditions:- Column A - 10% 20M PEG

Column B - 1% SE30

temperature - 50°; argon flow rate - 44 ml/min.

Compound	Column	Retention Time (minutes)
pyridine	A	13
4-methylpyridine	A	28
4-ethoxypyridine	B	9.5
4-acetylpyridine	B	7.5
2,6-lutidine	A	21

Level of detection 0.5%.

Table 223

Mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in benzene at 25°

Catalyst	Initial catalyst concentration M	Initial TMG concentration M	$k_{obsd.} \times 10^5$ / sec^{-1}
2-pyridone	0.100	0.102	211.3
	0.100	0.101	218.4
	0.100	0.102	230.2
	0.010	0.099	79.6
	0.010	0.102	77.8
	0.010	0.100	76.3
	0.001	0.100	13.4
	0.001	0.100	13.6
	0.001	0.099	13.7
6-methoxy-2-pyridone	0.100	0.100	239.3
	0.100	0.102	247.2
	0.100	0.101	248.8
	0.010	0.100	49.9
	0.010	0.102	51.1
	0.010	0.102	50.1
	0.001	0.097	7.4
	0.001	0.098	7.9
	0.001	0.100	8.0
blank in benzene	-	0.100	1.5
	-	0.101	1.6
	-	0.100	1.8

Table 224

Mutarotation of 2,3,4,6-tetramethyl-α-D-glucose in dioxan at 25°

Catalyst	Initial catalyst concentration M	Initial TMG concentration M	$k_{obsd.} \times 10^6$ / sec^{-1}
2-pyridone	0.100	0.100	277.5
	0.100	0.098	282.9
	0.100	0.101	287.7
	0.010	0.100	5.0
	0.010	0.100	4.8
	0.010	0.101	4.9
	0.001	0.100	< 1.0
6-methoxy-2-pyridone	0.100	0.100	39.4
	0.100	0.101	34.5
	0.100	0.100	37.5
	0.010	0.100	3.8
	0.010	0.099	4.1
	0.001	0.101	1.2
pyridine/phenol	0.100 in both catalysts	0.101	≈ 0.1
6,7-dimethoxy-isoquinol-3(2H)-one	0.010	0.101	75.2
	0.010	0.100	72.1

Key for tables 225 - 227

The following conventions are used in the tables 225, 226 and 227 which follow:-

The results show the correlation coefficients of linear plots of certain functions - either log k_{cat} or the difference between two log k_{cat}'s - against the inductive substituent constant σ of the various substituents. The catalytic coefficients are denoted by "$k_{cat.}$" where the apprcpriate catalyst is subscripted. i.e. k_{H_2O} is the catalytic coefficient for catalysis by water. The abbreviations used in the tables are:-

H_3O^+	-	hydronium ion	;	D_3O^+	-	hydrated deuteron
H_2O	-	water	;	D_2O	-	deuterium oxide
OH^-	-	hydroxide ion	;	lut.	-	2,6-lutidine
Py	-	pyridine	;	MePy	-	4-methylpyridine
EtOPy	-	4-ethoxypyridine	;	AcPy	-	4-acetylpyridine
Morph.	-	morpholine	;	Tris.	-	Tris
Dieth.	-	diethanolamine	;			

The results are tabulated giving in the first row, the correlation coefficient when the results for all eight sugars are included in the calculations. Subsequently the appropriate result for each sugar is omitted in turn from the linear fit calculations, and the correlation coefficient listed.

The values given in the final row, are the slopes of the plots wher all the compounds are included in the calculations i.e. they correspond to the correlation coefficients given in the first row of the tables.

Table 225

Correlation Coefficients of Linear Free Energy Relationships

Substituent of compound omitted / Function plotted against σ	$\log k_{H_3O^+}$	$\log k_{H_2O}$	$\log k_{D_3O^+}$	$\log k_{D_2O}$	$\log k_{OH^-}$	$\log k_{lut.}$
NONE	0.887	0.896	0.894	0.911	0.597	0.545
$-CH_3$	0.926	0.883	0.922	0.914	0.399	0.398
$-H$	0.944	0.943	0.953	0.960	0.866	0.718
$-CH_2OH$	0.887	0.899	0.894	0.913	0.589	0.570
$-CH_2OCH_3$	0.889	0.898	0.897	0.912	0.638	0.590
$-CH_2NHCOCH_3$	0.894	0.921	0.902	0.928	-	0.547
$-CH_2OPh$	0.890	0.898	0.895	0.911	0.664	0.530
$-CH_2Cl$	0.890	0.903	0.897	0.915	0.560	0.559
$-CH_2CN$	0.826	0.844	0.837	0.865	0.332	0.421
ρ	-3.75	-3.84	-3.79	-4.12	4.24	2.23

/cont.

Table 225 (continued)

Correlation Coefficients of Linear Free Energy Relationships

Substituent of compound omitted / Function plotted against σ	log k_{Py}	log k_{MePy}	log k_{EtOPy}	log k_{AcPy}	log $k_{Morph.}$	log $k_{Tris.}$	log $k_{Dieth.}$
NONE	0.317	0.591	0.664	0.156	0.569	0.522	0.655
$-CH_3$	0.294	0.363	0.479	0.343	0.410	0.307	0.447
-H	0.661	0.790	0.838	0.852	0.867	0.924	0.956
$-CH_2OH$	0.296	0.603	0.688	0.189	0.567	0.516	0.658
$-CH_2OCH_3$	0.321	0.607	0.692	0.163	0.585	0.543	0.671
$-CH_2NHCOCH_3$	0.323	0.594	0.664	0.168	0.623	0.539	0.674
$-CH_2OPh$	0.251	0.604	0.672	0.409	0.547	0.520	0.645
$-CH_2Cl$	0.288	0.560	0.633	0.179	0.493	0.460	0.604
$-CH_2CN$	0.350	0.565	0.597	0.101	0.418	0.324	0.501
ρ	0.73	1.69	2.01	-	2.51	2.43	3.04

Table 226

Correlation Coefficients of Linear Free Energy Relationships

Substituent of compound omitted / Function plotted against σ	$\log(k_{EtoPy}/k_{Py})$	$\log(k_{MePy}/k_{Py})$	$\log(k_{AcPy}/k_{Py})$	$\log(k_{EtoPy}/k_{H_3O^+})$	$\log(k_{MePy}/k_{H_3O^+})$
NONE	0.904	0.943	0.698	0.960	0.961
$-CH_3$	0.903	0.933	0.687	0.959	0.956
-H	0.887	0.933	0.652	0.959	0.955
$-CH_2OH$	0.910	0.951	0.693	0.960	0.960
$-CH_2OCH_3$	0.943	0.961	0.702	0.960	0.961
$-CH_2NHCOCH_3$	0.931	0.959	0.717	0.960	0.961
$-CH_2CPh$	0.902	0.947	0.893	0.961	0.963
$-CH_2Cl$	0.892	0.939	0.698	0.960	0.963
$-CH_2CN$	0.853	0.914	0.485	0.960	0.961
ρ	1.29	0.95.	-1.22	5.73	5.44

/cont.

Table 226 (continued)

Correlation Coefficients of Linear Free Energy Relationships

Substituent of compound omitted / Function plotted against σ	$\log(k_{Py}/k_{H_3O^+})$	$\log(k_{AcPy}/k_{H_3O^+})$	$\log(k_{Morph.}/k_{H_3O^+})$	$\log(k_{Tris}/k_{H_3O^+})$	$\log(k_{Dieth.}/k_{H_3O^+})$
NONE	0.958	0.848	0.929	0.839	0.930
$-CH_3$	0.951	0.823	0.921	0.831	0.929
$-H$	0.957	0.825	0.931	0.836	0.932
$-CH_2OH$	0.958	0.833	0.932	0.842	0.934
$-CH_2OCH_3$	0.958	0.837	0.933	0.847	0.936
$-CH_2NHCOCH_3$	0.960	0.840	0.935	0.851	0.937
$-CH_2OPh$	0.960	0.853	0.934	0.855	0.937
$-CH_2Cl$	0.960	0.851	0.932	0.856	0.937
$-CH_2CN$	0.958	0.848	0.929	0.858	0.937
ρ	4.49	3.25	6.24	6.23	6.81

Table 227

Correlation Coefficients of Linear Free Energy Relationships

Substituent of compound omitted / Function plotted against σ	$\log(k_{Morph.}/k_{Tris})$	$\log(k_{Dieth.}/k_{Tris})$	$\log(k_{Morph.}/k_{Dieth.})$	$\log(k_{Morph.}/k_{Py})$	$\log(k_{Morph.}/k_{Me}$
NONE	0.068	0.745	0.480	0.639	0.344
$-CH_3$	0.322	0.633	0.089	0.595	0.320
-H	0.172	0.725	0.531	0.795	0.600
$-CH_2OH$	0.070	0.752	0.483	0.632	0.334
$-CH_2OCH_3$	0.072	0.793	0.480	0.651	0.347
$-CH_2NHCOCH_3$	0.070	0.751	0.637	0.722	0.423
$-CH_2OPh$	0.078	0.748	0.600	0.677	0.426
$-CH_2Cl$	0.015	0.750	0.568	0.554	0.210
$-CH_2CN$	0.304	0.818	0.322	0.419	0.052

/cont.

Table 227 (continued)

Substituent of compound omitted \ Function plotted against σ	$\log(k_{H_3O^+}/k_{D_3O^+})$	$\log(k_{H_2O}/k_{D_2O})$	$\log(k_{OH^-}/k_{Py})$	$\log(k_{OH^-}/k_{H_3O^+})$	$\log(k_{OH^-}/k_{H_2O})$
NONE	0.073	0.719	0.555	0.846	0.858
$-CH_3$	0.558	0.861	0.425	0.774	0.772
$-H$	0.050	0.679	0.721	0.903	0.922
$-CH_2OH$	0.063	0.715	0.552	0.844	0.856
$-CH_2OCH_3$	0.048	0.729	0.583	0.870	0.880
$-CH_2NHCOCH_3$	0.060	0.760	-	-	-
$-CH_2OPh$	0.136	0.715	0.730	0.888	0.890
$-CH_2Cl$	0.101	0.679	0.516	0.830	0.843
$-CH_2CN$	0.249	0.618	0.209	0.791	0.801
ρ	-	-	-	8.00	8.04

Table 228

The pyridine catalysed mutarotation of α-D-mannose at 25° in water, I = 0.25M, (Pyridine):(Pyridinium) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.150	6.02	4.17, 4.08	4.14
0.100	6.03	2.93, 3.20	3.14
0.050	6.05	2.20, 2.21	2.13
0.025	6.06	1.62, 1.61	1.63

$k_{Py} = 2.00 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.20%.

$k_{H_2O}(H_2O) = 1.13 \times 10^{-3}\ sec^{-1}$; S.D. = 1.11%.

Table 229

The pyridine catalysed mutarotation of α-D-mannose at 25° in water, I = 0.25M, (Pyridine):(Pyridinium $^+$) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.150	5.62	3.74, 3.99	4.06
0.100	5.64	3.10, 3.10	3.06
0.050	5.66	2.11, 2.03	2.06
0.025	5.67	1.58, 1.54	1.56

$k_{Py} = 2.00 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.52%.

$k_{H_2O}(H_2O) = 1.06 \times 10^{-3}\ sec^{-1}$; S.D. = 1.33%.

Table 230

The pyridine catalysed mutarotation of α-D-mannose at 25° in water, I = 0.25M, (Pyridine):(Pyridinium) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.150	5.28	4.28, 4.03	4.01
0.125	5.32	3.51, 3.35	3.53
0.100	5.34	3.03, 2.92	3.04
0.075	5.36	2.63, 2.63	2.56
0.050	5.37	2.10, 2.08	2.08
0.025	5.39	1.57, 1.60	1.60
0.012	5.39	1.37, 1.32	1.35

$k_{Py} = 1.93 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.07%.

$k_{H_2O}(H_2O) = 1.11 \times 10^{-3}\ sec^{-1}$; S.D. = 0.93%.

Table 231

The pyridine catalysed mutarotation of α-L-rhamnose at 25° in water, I = 0.25M, (Pyridine):(Pyridinium) = 4:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.150	6.02	5.44, 5.34	5.47
0.100	6.03	4.61, 4.65	4.54
0.050	6.05	3.71, 3.66	3.61
0.025	6.06	3.06, 3.11	3.14

$k_{Py} = 1.87 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.68%.

$k_{H_2O}(H_2O) = 2.67 \times 10^{-3}\ sec^{-1}$; S.D. = 1.29%.

Table 232

The pyridine catalysed mutarotation of α-L-rhamnose at 25° in water I = 0.25, (Pyridine):(Pyridinium) = 2:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.150	5.62	5.31, 5.37	5.38
0.100	5.64	4.45, 4.54	4.48
0.050	5.66	3.56, 3.59	3.57
0.025	5.67	3.05, 3.18	3.12

$k_{Py} = 1.81 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 1.99%.

$k_{H_2O}(H_2O) = 2.67 \times 10^{-3}\ sec^{-1}$; S.D. = 0.93%.

Table 233

The pyridine catalysed mutarotation of α-L-rhamnose at 25° in water, I = 0.25, (Pyridine):(Pyridinium) = 1:1.

(Pyridine)M	pH_{25}	$k_{obsd.} \times 10^3$	$k_{calc.} \times 10^3$
0.100	5.34	4.63, 4.60	4.71
0.075	5.36	4.18, 4.33	4.19
0.050	5.37	3.66, 3.67	3.68
0.025	5.39	3.18, 3.18	3.17
0.012	5.39	2.93, 2.90	2.91

$k_{Py} = 2.05 \times 10^{-2}\ M^{-1}\ sec^{-1}$; S.D. = 2.66%.

$k_{H_2O}(H_2O) = 2.66 \times 10^{-3}\ sec^{-1}$; S.D. = 0.96%.

Key to Comparitive Tables

a) 5-substituted sugars (i.e. Series 1)

b) 2-substituted sugars (i.e. Series 2)

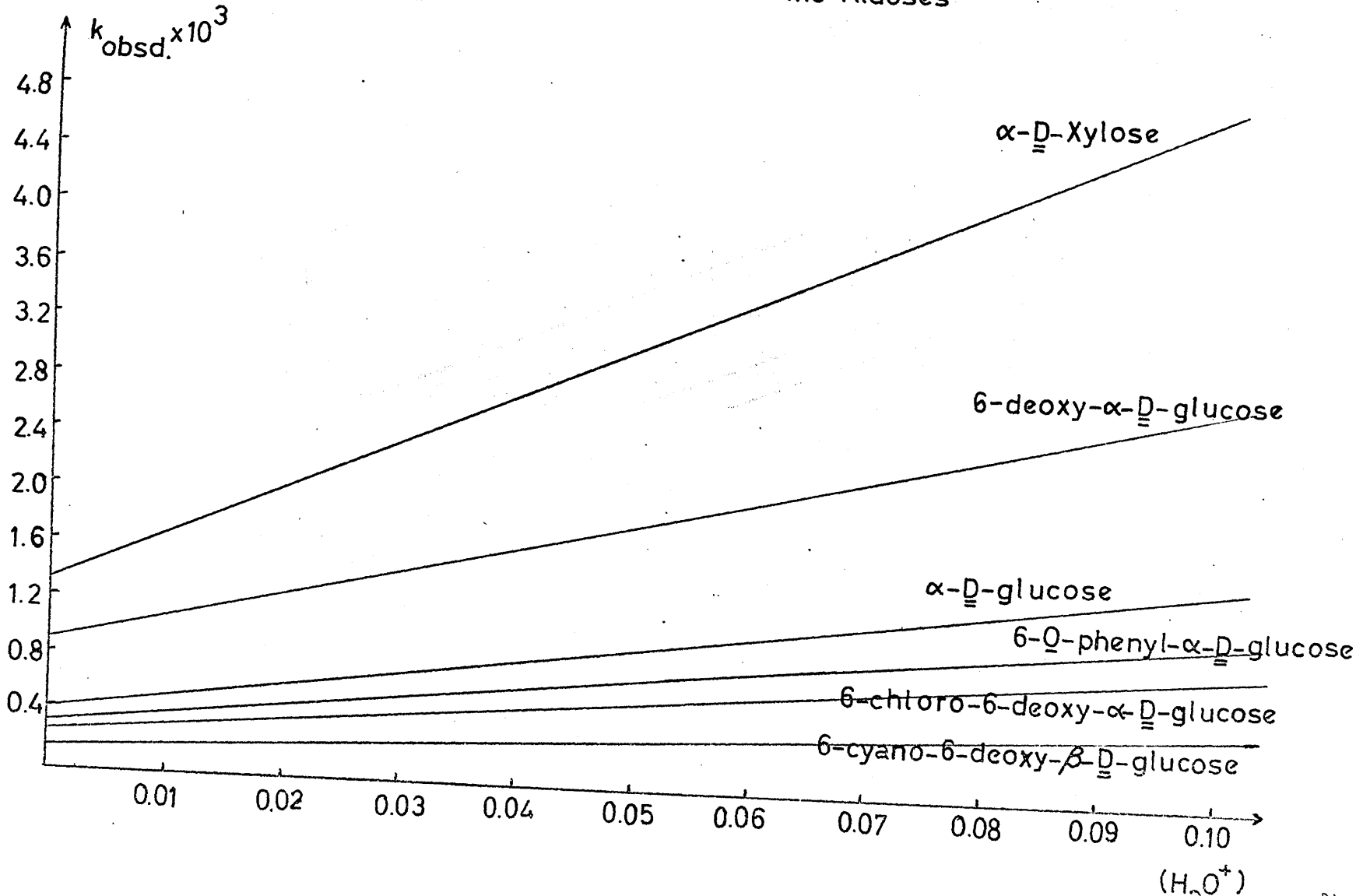

$k_{obsd.} \times 10^3$
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
α-D-Xylose
6-deoxy-α-D-glucose
α-D-glucose
6-O-phenyl-α-D-glucose
6-chloro-6-deoxy-α-D-glucose
6-cyano-6-deoxy-β-D-glucose
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
(H_3O^+)

Fig. 5

The Tris Catalysed Mutarotation of α-D-xylose
$k_{obsd.} \times 10^3$
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
pH 9.16
pH 8.87
pH 8.56
pH 8.33
pH 8.10
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
(Tris)

Fig. 6

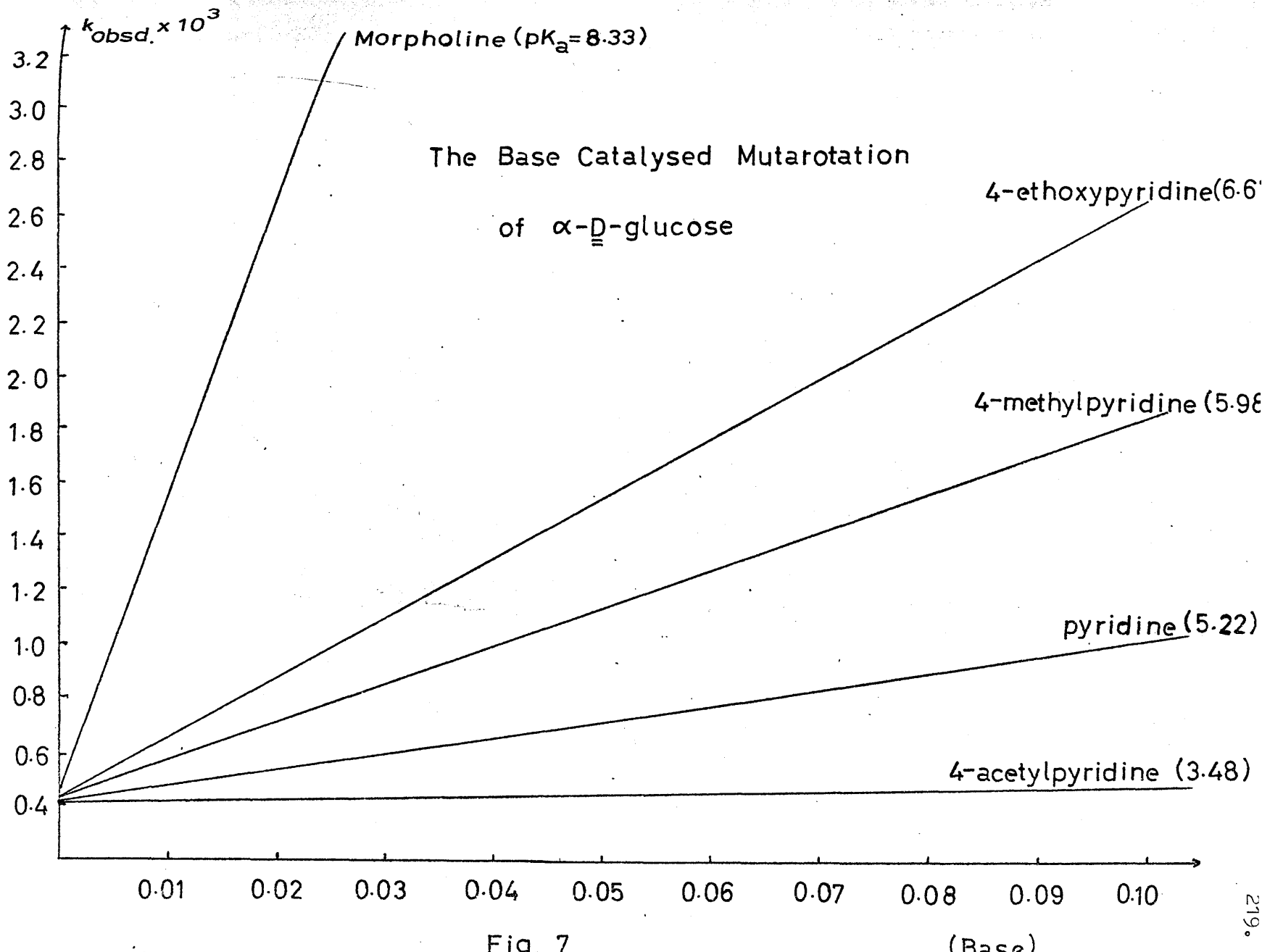

$k_{obsd.} \times 10^3$
Morpholine (pK_a=8.33)
The Base Catalysed Mutarotation
of α-D-glucose
4-ethoxypyridine(6.6
4-methylpyridine (5.98
pyridine (5.22)
4-acetylpyridine (3.48)
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
(Base)

Fig. 7

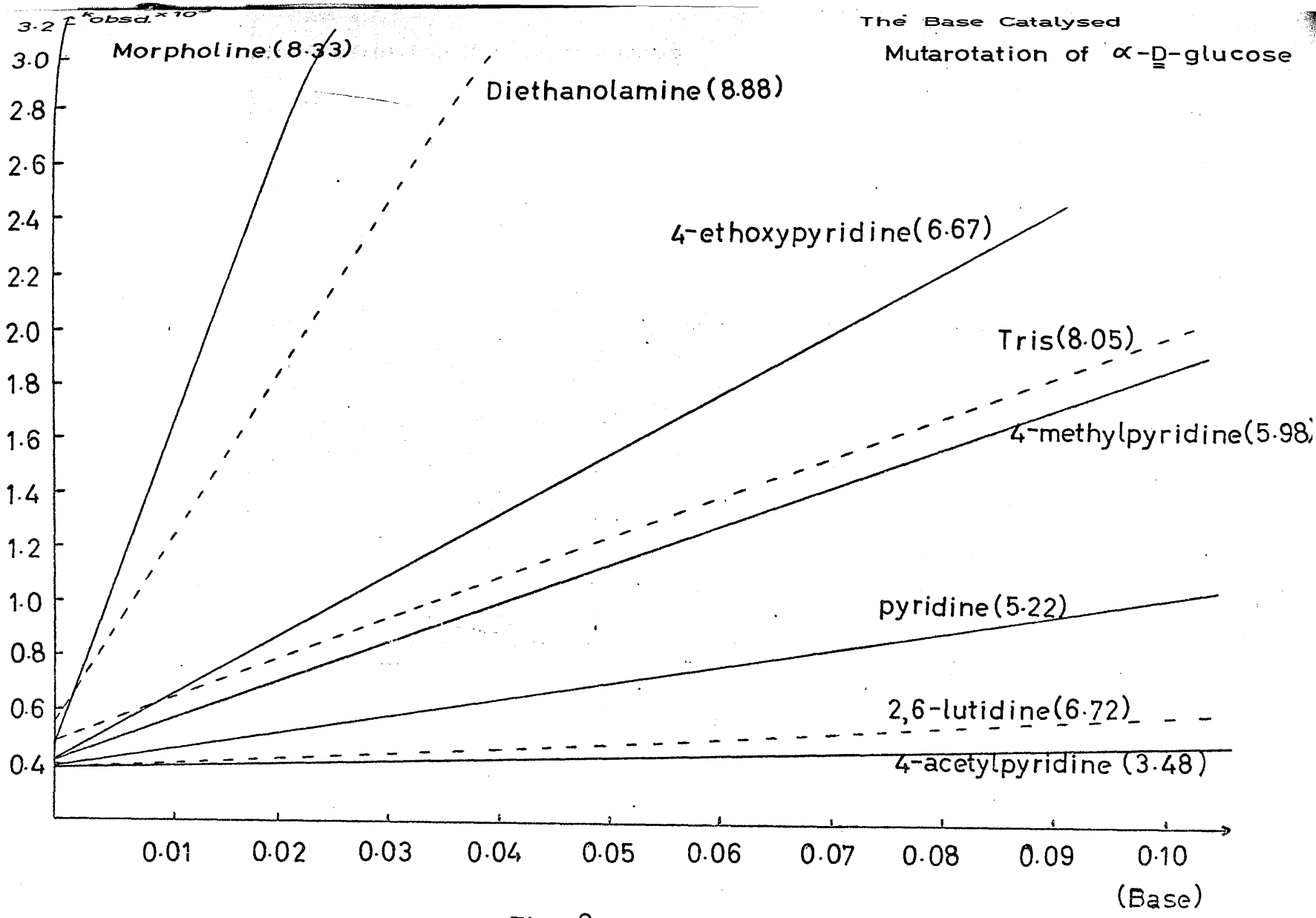

3·2
$k_{obsd.} \times 10^{-3}$
3·0
2·8
2·6
2·4
2·2
2·0
1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
Morpholine (8·33)
Diethanolamine (8·88)
4-ethoxypyridine (6·67)
Tris (8·05)
4-methylpyridine (5·98)
pyridine (5·22)
2,6-lutidine (6·72)
4-acetylpyridine (3·48)
0·01
0·02
0·03
0·04
0·05
0·06
0·07
0·08
0·09
0·10
(Base)

Fig. 8

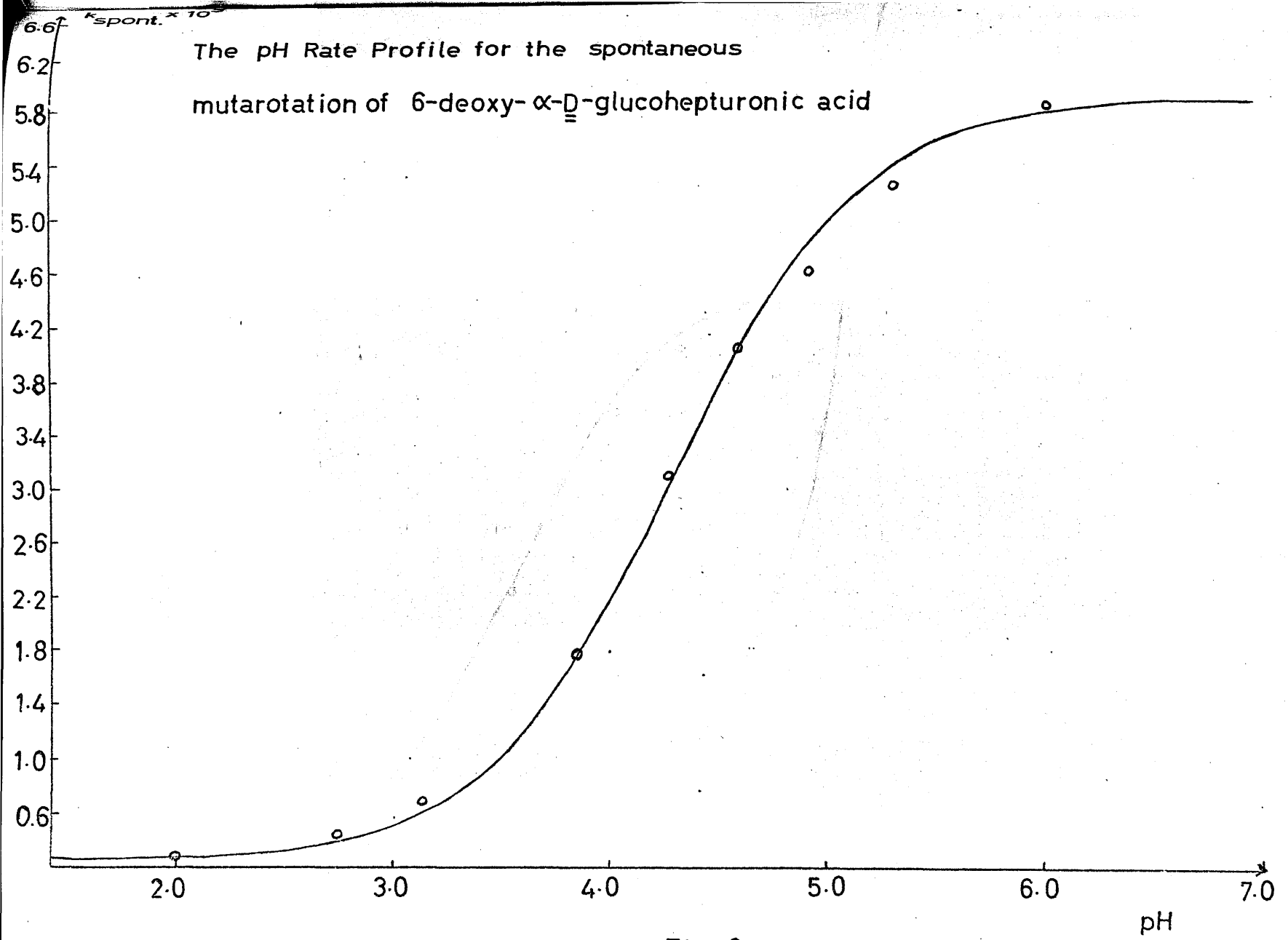

The pH Rate Profile for the spontaneous
mutarotation of 6-deoxy-α-D-glucohepturonic acid
$k_{spont.} \times 10^{-5}$
6.6
6.2
5.8
5.4
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.0
0.6
2.0
3.0
4.0
5.0
6.0
7.0
pH

Fig. 9

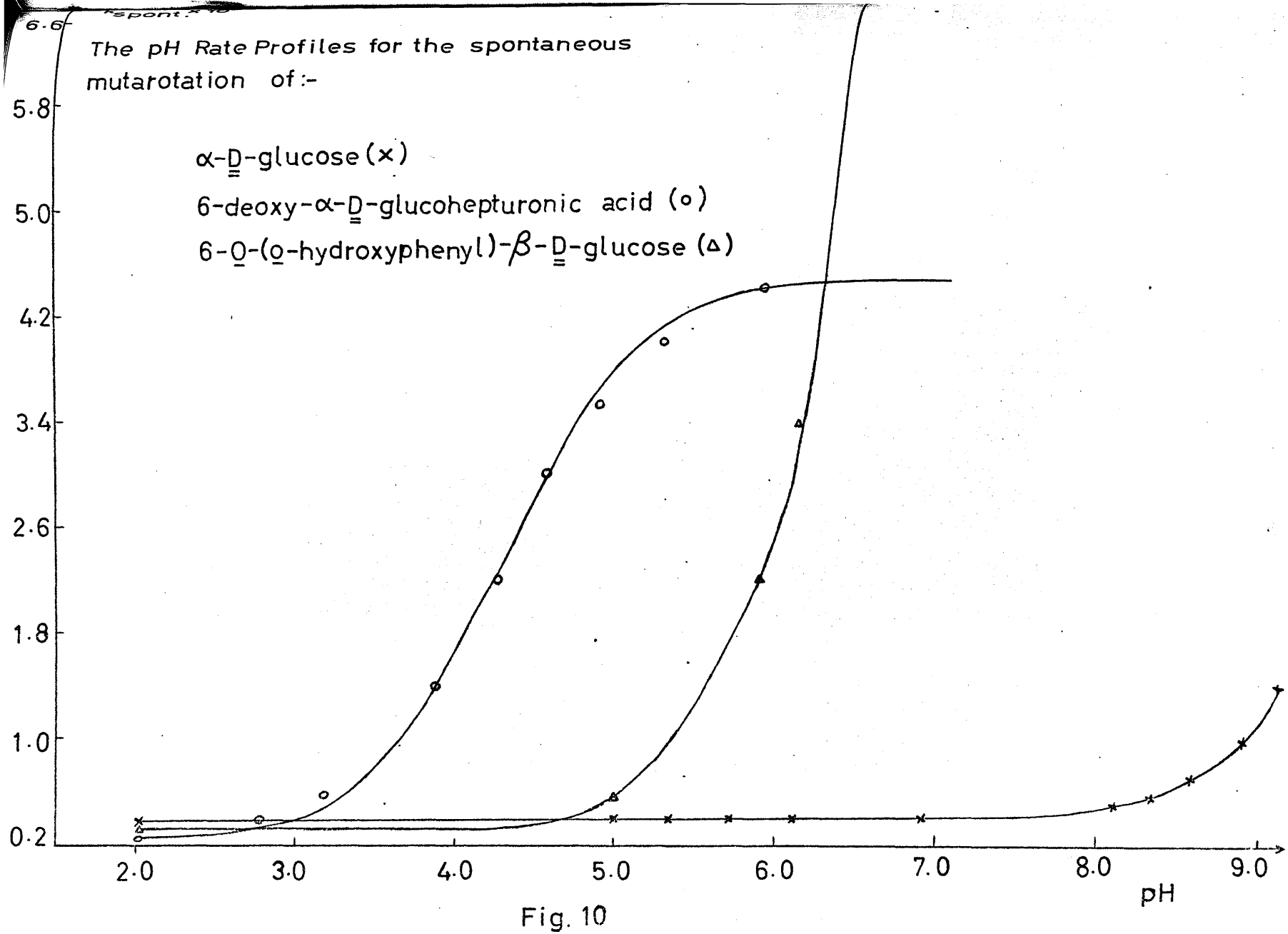
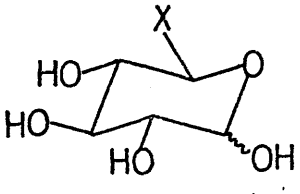

The pH Rate Profiles for the spontaneous mutarotation of:-
α-D-glucose (×)
6-deoxy-α-D-glucohepturonic acid (o)
6-O-(o-hydroxyphenyl)-β-D-glucose (Δ)
6.6
5.8
5.0
4.2
3.4
2.6
1.8
1.0
0.2
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
pH

Fig. 10

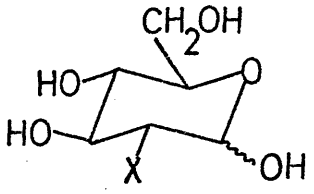
Discussion

4.1 General

It is clear that despite the mass of work which has been carried out on the mutarotation of glucose, much doubt still exists as to the mechanism of the reaction. In particular the timing of the two proton transfers and the ring opening are in doubt. It was therefore thought that the study of the kinetics of mutarotation of a series of sugars of structures 1 and 2 could perhaps elucidate, at least in part, the timing of the various steps in the mechanism.



1



2

In particular one might be able to comment upon the suggestion of Schowen et. al.[145] that proton transfer is not concerted with the rate determining elimination of the alkoxide ion from the carbonyl group.

The study of the kinetics of mutarotation of sugars of structure 1 and 2 as X is systematically varied is essentially a structure reactivity study in an aliphatic system. Since very few aliphatic reactions, outside Taft's work on the hydrolysis of esters, have been successfully correlated with linear free energy relationships, it was thought that it would be of interest to investigate what factors required to be considered in correlating the structure with the reactivity of the

sugars, and to see what degree of correlation could be achieved.

Furthermore, by suitable choice of the substituent X in series 1, it was thought that intramolecular catalysis of mutarotation might be demonstrated, confirming the only previously reported case of such intramolecular catalysis of mutarotation.

4.2 Kinetics of Mutarotation

Consider first a simplified representation of the mutarotation of α-D-glucose, where the reaction is regarded as a first order reversible reaction between the α and β forms of the sugar.

$$\alpha \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \beta \qquad \ldots\ldots\ldots\ldots\ldots \quad (36)$$

Initially at time t = o, the concentration of the α anomer is a ; if after time t, x moles per litre of the α anomer have been transformed into the β anomer, then the differential rate equation is

$$dx/dt = k_1 (a - x) - k_{-1} x$$

$$\text{i.e. } dx/dt = (k_1 + k_{-1})(c - x) \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (37)$$

where $c = k_1 a/(k_1 + k_{-1})$

Integration of (37) yields the equation

$$\ln (c/(c - x)) = (k_1 + k_{-1})t$$

$$\therefore k_1 + k_{-1} = (1/t).\ \ln(k_1 a/(k_1 a - (k_1 + k_{-1})x))$$

$$\text{i.e. } k_1 + k_{-1} = (1/t).\ \ln(Ka/(Ka - (1 + K)x)) \qquad \ldots\ldots\ldots\ldots \quad (38)$$

where $K = k_1/k_{-1}$

By use of the equations

$$r_o = S_\alpha \cdot a$$

$$r_t = S_\alpha.(a - x) + S_\beta \cdot x$$

$$r_\infty = S_\alpha (a - x_{eq.}) + S_\beta \cdot x_{eq.}$$

$$K = k_1/k_{-1} = x_{eq.}/(a - x_{eq.})$$

(where r_o = initial rotation, r_t = rotation at time t, r_∞ = rotation at final equilibrium, S_α and S_β are the specific rotations of the α and β anomers respectively, and $x_{eq.}$ is the quantity of the α anomer which has been transformed into the β anomer when the final equilibrium has been reached.)

equation (38) becomes

$$k_1 + k_{-1} = (1/t). \ln ((r_o - r_\infty)/(r_t - r_\infty)) \quad \ldots\ldots\ldots (39)$$

which is the expression to which all the observed rate data in this thesis have been fitted (see section 3.1.).

Thus the observed rate constant is in fact the sum of the rate constants for the forward and reverse reactions of equation (36).

As mentioned above, this is a somewhat simplified representation of the mutarotation reaction, which is known to proceed through an acyclic aldehydo-form (see section 1.2). A more accurate representation of the mutarotation is the three component equilibrium:-

$$\alpha \underset{k_{-\alpha}}{\overset{k_\alpha}{\rightleftharpoons}} A \underset{k_\beta}{\overset{k_{-\beta}}{\rightleftharpoons}} \beta \quad \ldots\ldots\ldots\ldots (40)$$

where A represents the acyclic aldehydo-intermediate.

It is known (see section 1.2) that the concentration of this acyclic form is very small and so if one applies the steady state hypothesis to the reaction one has :-

Rate of formation of acyclic aldehyde = Rate of reaction of acyclic aldehyde

$$\text{i.e. } k_{\alpha}(\alpha) + k_{\beta}(\beta) = k_{-\alpha}(A) + k_{-\beta}(A)$$

$$\therefore (A) = \frac{k_{\alpha}(\alpha) + k_{\beta}(\beta)}{k_{-\alpha} + k_{-\beta}} \quad \ldots\ldots\ldots\ldots (41)$$

The rate equation for mutarotation would then be given by :-

$$\text{Rate} = d\beta/dt = k_{-\beta}(A) - k_{\beta}(\beta)$$

$$= k_{-\beta}\left\{\frac{k_{\alpha}(\alpha) + k_{\beta}(\beta)}{k_{-\alpha} + k_{-\beta}}\right\} - k_{\beta}(\beta) \ldots\ldots (42)$$

$$= \frac{k_{\alpha}(\alpha) + k_{\beta}(\beta)}{1 + p} - k_{\beta}(\beta) \ldots\ldots\ldots\ldots (43)$$

where $p = k_{-\alpha}/k_{-\beta}$ = partition ratio of the acyclic aldehyde

$$\therefore d\beta/dt = (k_{\alpha}(\alpha) - p.\, k_{\beta}(\beta))/(1 + p) \ldots\ldots\ldots\ldots (44)$$

$$= k_{\alpha}(\alpha)/(1 + p) - p.\, k_{\beta}(\beta)/(1 + p) \ldots\ldots\ldots\ldots (45)$$

In treating the rate data obtained in this work by the simpler treatment of equations (36) - (39), we ignore the possible effect which the partitioning of the acyclic aldehyde (as represented by p) could have on the kinetics. Further consideration will be given to these kinetic analyses when the observed rate data are fitted to linear free energy relationships (see section 4.7).

4.3 Substituent Effects in Mutarotation

A. substituents in the 5-position of the sugar ring.

(i) substituent effects in acid catalysed mutarotation

The results for the hydronium ion catalysed mutarotation of the series of substituted sugars (1) are given in tables 1 - 10. The

comparitive table, table 11, shows the catalytic coefficient for hydronium ion catalysis, $k_{H_3O^+}$, for each sugar in the series together with the catalytic coefficient for spontaneous (water catalysed) mutarotation, k_{H_2O}. Also listed in table 11 are the σ values for each substituent X. These σ values are the inductive substituent constants of the substituents, i.e. they represent the polar effect of each substituent. The values themselves are taken from the work of Charton[129] whose σ_I values are used in preference to the σ^* values calculated by Taft[132].

From table 11 a quite discernible trend can be seen. As the substituent X becomes more electron withdrawing (i.e. σ increases in value), the value of $k_{H_3O^+}$ decreases. (See also Fig. 5). An almost identical trend through the series is observed for k_{H_2O}. In both cases the most notable deviation from this trend occurs in the catalytic coefficient of α-D-xylose (X = H). For this sugar both $k_{H_3O^+}$ and k_{H_2O} are higher (by a factor of approximately 2) than would be expected on the sole consideration of the polar effect of the substituent. This positive deviation is interpreted as indicating that the polar effect of the substituent X is not the only factor which influences the rate of mutarotation but that steric effects must also come into consideration. (Clearly resonance effects need not be considered for any of the sugars studied). Steric effects throughout the series may not be too readily discerned since the rate differences we are dealing with are small and the size of many of the substituents is quite similar (e.g. $-CH_3$, $-CH_2OH$, $-CH_2Cl$, $-CH_2CN$). The biggest difference in size is of course on

going from a $-CH_2Y$ group to -H (i.e. α-D-xylose), and it is therefore not surprising that an easily discernible deviation in the trend of rate constants should occur with α-D-xylose, since any steric effects would be expected to be most noticable with this compound. Indeed it will be found that this positive deviation for α-D-xylose is found in all the sets of catalytic coefficients reported in this thesis. The implications of both polar and steric effects influencing the kinetics of mutarotation will be discussed further in section 4.7 with regard to linear free energy relationships.

It is interesting to note that the variation in the catalytic coefficients for water catalysis follows the same trend as the $k_{H_3O^+}$ values as σ is varied. Pigman and Isbell[7] have calculated that in neutral solutions, the basic catalytic function of the water molecule predominates over the acidic catalytic function in the mutarotation of sugars. Were this the case in the work reported here, it would be expected that the catalytic coefficients for water catalysis should parallel the trend observed for the catalytic coefficients observed for catalysis by bases, which as will be seen is in fact exactly opposite to that observed for $k_{H_3O^+}$. Instead the values of k_{H_2O} parallel the trend for $k_{H_3O^+}$. The conclusion must be that the water catalysed mutarotation of these sugars must proceed by a mechanism which bears more resemblance to the mechanism of acid-catalysed mutarotation than to that for base catalysed mutarotation.

(ii) <u>substituent effects in base catalysed mutarotation</u>

The determination of the catalytic coefficient for catalysis by

hydroxide ion is difficult because mutarotation becomes too fast for accurate measurement when the concentration of hydroxide ions rises much above 10^{-5}M, due to the extraordinary magnitude of k_{OH^-}. By studying the kinetics of mutarotation of the sugars in Tris buffers at five different pH's, it was possible to extract the catalytic coefficients for catalysis by Tris and by hydroxide ion.

The rate constant in these conditions may be given by :-

$$k_{obsd.} = k_{H_2O}(H_2O) + k_{Tris}(Tris) + k_{TrisH^+}(TrisH^+) + k_{OH^-}(OH^-) + k_{G^-}(G^-) \quad \ldots\ (46)$$

where G^- represents the glucosate ion which is also catalytically active, and $TrisH^+$ represents the conjugate acid of the Tris molecule.

From subsequent reported data in this thesis (see page 233) we can say that k_{TrisH^+} approaches zero closely and that therefore

$$k_{obsd.} = k_{H_2O}(H_2O) + k_{Tris}(Tris) + k_{OH^-}(OH^-) + k_{G^-}(G^-) \quad \ldots\ (47)$$

Since the concentration of glucosate ion is proportional to the hydroxide concentration equation (47) can be written

$$k_{obsd.} = k_{H_2O}(H_2O) + k_{Tris}(Tris) + k_{OH^-}(OH^-) + k_{G^-}(GH)(OH^-).\ K_s/K_w \quad \ldots\ (48)$$

where K_s refers to the equilibrium $GH \rightleftharpoons G^- + H^+$ and K_w is the ionisation constant of water.

Thus plotting $k_{obsd.}$ against (Tris) should give a straight line of slope k_{Tris} and intercept $k_{int.}$.

where $k_{int.} = k_{H_2O}(H_2O) + k_{OH^-}(OH^-) + k_{G^-}(GH)(OH^-).\ K_s/K_w$

By carrying out the kinetics at five different buffer ratios and hence five different pH's, one obtains five values of intercept (see for example Fig. 6), which when plotted against the concentration of

hydroxide ion should give a straight line of slope $k_{OH^-} + k_G\text{-}(GH).K_s/K_w$ and intercept $k_{H_2O}(H_2O)$.

The contributions to the slope are factorised into that due to catalysis by hydroxide ion and that due to catalysis by the glucosate ion. Some idea of the importance of this latter quantity can be obtained by comparing the relative magnitudes of the terms k_{OH^-} and $k_G\text{-}(GH).\ K_s/K_w$. From the work of Smith[30] and Wynne-Jones[31] it appears that k_{G^-} is approximately 1/200th the magnitude of k_{OH^-} for α-D-glucose; in all of the studies reported in this thesis, the concentration of sugar used is approximately 0.02M; at 25°, $K_w = 10^{-14}$ (ref. 135); at 25°, K_s for D-glucose $= 4.6 \times 10^{-13}$ (ref. 31).

∴ at 25° for glucose,

$$k_{OH^-}/k_G\text{-}.(GH).(K_s/K_w) = 1/0.005 \times 0.02 \times (4.6 \times 10^{-13}/10^{-14})$$

$$= 1/4.6 \times 10^{-3}$$

i.e. the contribution made to the slope obtained by plotting the values of $k_{int.}$ against (OH^-), by glucosate ion catalysis, is $< 0.5\%$ for D-glucose.

In this work therefore, this contribution has been ignored, and the plot of $k_{int.}$ (from Tris catalysis) against the concentration of hydroxide ion gives a straight line whose slope has been equated with k_{OH^-}. It must be remembered that there is a very small error in this assumption due to the catalysis by glucosate ion just described. It does not appear likely that this error will be greater than the experimental error for the k_{OH^-} values of any of the sugars studied.

Tables 12 - 54 give the results for the Tris catalysed mutarotation

for all the sugars in the series 1. The comparitive table, table 55, lists the values for k_{Tris} for each sugar at the five pH's studied. As can be seen, there is some slight evidence for a small increase in k_{Tris} as the pH increases. However in most cases the error is little more than the experimental reproducibility (approx. 3 - 4%.) The trend apparently is more important in 6-chloro-6-deoxy-glucose and 6-cyano-6-deoxy-glucose, in particular the latter. To minimise any errors resulting from this increase, the average catalytic coefficient for Tris catalysis has been obtained by averaging the three values of k_{Tris} obtained at the three lowest pH's.

As can be seen from table 55, this increase in k_{Tris} is most significant for 6-cyano-6-deoxy-glucose. The possibility existed that at these alkaline pH's the sugar was not merely mutarotating but was undergoing some other change such as hydrolysis of the nitrile group. Such hydrolysis was tested for by heating some 6-cyano-6-deoxy-glucose at 60° in the most alkaline Tris buffer studied (pH 9.16) for three hours. At the end of this time t.l.c. showed that the sugar was still unchanged, a fact which was further corroborated by spectroscopic evidence (I.R.), which showed the unchanged C≡N stretch of the sugar and the lack of any carbonyl stretches which would result from hydrolysis of the nitrile.

Again, the figures for Tris catalysis show a quite obvious trend. As the substituent X becomes more electron withdrawing the value of k_{Tris} increases. This trend is directly opposite to the trend which was

observed in the acid catalysis of the same series of sugars (see table 11). Once more the sugar α-D-xylose exhibits a large positive deviation from the trend, a fact which has already been commented upon in section 4.3.A(i).

When the intercepts listed in tables 12 - 54 are plotted for each sugar against the concentration of hydroxide ion, the catalytic coefficient for catalysis by hydroxide ion is given by the slope of this linear plot (see above). These results for the series of sugars 1, are given in tables 56 - 64 and comparitive table 65.

Once more the trend is similar to that observed in the k_{Tris} values, namely k_{OH^-} increasing as the substituent X becomes more electron withdrawing.

The catalytic coefficients for catalysis by pyridine, detailed in tables 66 - 96 and comparitive table 97, were determined at four different buffer ratios to test whether the pyridinium ion was a strong enough acid to catalyse the mutarotation reaction. In pyridine buffers the observed rate constant is given by the expression

$$k_{obsd.} = k_{H_2O}(H_2O) + k_{Py}(Py) + k_{Py^+}(Py^+) + k_{H_3O^+}(H_3O^+) + k_{OH^-}(OH^-) \quad \ldots\ldots\ (49)$$

where Py^+ represents the pyridinium ion and k_{Py^+} is the catalytic coefficient for catalysis by this ion.

At pH's between 3 and 6 the terms due to H_3O^+ and OH^- catalysis are negligible[134], and so expression (49) simplifies to

$$k_{obsd.} = k_{H_2O}(H_2O) + k_{Py}(Py) + k_{Py^+}(Py^+)$$

$$= k_{H_2O}(H_2O) + k_{Py}(Py) + k_{Py^+}(Py)(H^+)/K_a \quad \ldots\ldots\ (50)$$

where K_a = acid dissociation constant of the conjugate acid of pyridine.

Hence a plot of $k_{obsd.}$ against the concentration of pyridine should give a linear plot of slope $(k_{Py} + k_{Py^+}.(H^+)/K_a)$.

Since, as can be seen from table 97, the slopes of these plots are constant at the four pH's studied, it can be concluded that k_{Py^+} is negligibly small and hence that catalysis by the pyridinium ion is negligible. The slopes therefore represent the catalytic coefficient for pyridine catalysis. Obviously the same situation will hold for all bases stronger than pyridine, although as the pK_a of the base (and hence the pH of the buffer) rises the catalysis by hydroxide ion will become significant. (It is because of these observations that in the expressions for factorising the observed rate constant for mutarotation into contributions for catalysis by the various species in the buffer - e.g. equation (47) no term is ever included for catalysis by the conjugate acid of a base stronger than pyridine.

The trend observed in the catalytic coefficients for catalysis by pyridine (table 97) is much the same as for the catalytic coefficients for catalysis by Tris and by hydroxide ion already discussed. Indeed this general pattern in which the catalytic coefficient for catalysis by a base increases as the electron withdrawing power of the substituent X increases is aquite general one for all base catalysts studied and is further explified by catalysis by 4-methylpyridine (tables 130 - 139), 4-ethoxypyridine (tables 140 - 149), 4-acetylpyridine (tables 150 - 159) 2,6-lutidine (tables 160 - 169), morpholine (tables 98 - 107), and

diethanolamine (tables 108 - 117) (N.B. the last table in each set is the comparitive table).

Certain deviations in these results are worth mentioning at this stage:-

(a) As already pointed out, the sugar α-D-xylose (X = H) in series 1, exhibits a large positive deviation in the comparitive tables for all the catalysts studied (both acidic and basic). This is taken to indicate that not only does the polar effect of the substituent influence the kinetics of mutarotation but also the size of the substituent (i.e. steric effect) is important.

(b) the catalysis of the mutarotation of 6-O-phenyl-α-D-glucose by amines reveals an interesting anomaly. When the catalyst is an aliphatic amine (e.g. morpoline, diethanolamine and tris), the magnitude of the catalytic coefficient is in good agreement with that expected from the inductive substituent constant of the group X (X = $-CH_2OPh$, see tables 107, 117 and 55). However if the catalyst is an aromatic amine, the value of the catalytic coefficient is very much higher than is anticipated on the basis of the inductive effect of the substituent. (cf. tables 97, 139, 149, 159 and 169). The catalytic coefficients for catalysis of 6-O-phenyl-α-D-glucose by aromatic amines are normally about twice as large as would have been expected - a deviation as big as that observed in α-D-xylose which was attributed to a steric effect. A possible explanation of this unexpected greater efficiency of aromatic amine catalysts will be discussed in section

4.5(iii)which deals with the significance of substituent effects on the mechanism of mutarotation.

(c) examination of the catalytic coefficients for catalyses by 4-acetylpyridine (table 159), while revealing both the deviations (a) and (b) outlined above, seems to indicate another deviation in that the k_B for 6-cyano-6-deoxy-β-D-glucose is lower than expected from the general trend observed in general base catalysed mutarotation. The explanation of this apparent deviation will be given in section 4.6 which describes the application and interpretation of the Brönsted catalysis law to these mutarotation studies.

(d) the k_{OH^-} values for 6-O-methyl-α-D-glucose and 6-O-phenyl-α-D-glucose appear to exhibit a negative deviation from the expected values (table 65) and the k_{H_2O} value for 6-acetamido-6-deoxy-β-D-glucose seems much lower than expected (table 11).

4.3.B substituents on the 2-position of the ring.

The kinetics of mutarotation have been summarised in section 4.2. It was pointed out there that the observed catalytic constants were in fact composite quantities consisting of the sum of the rate constants of the forward and reverse reactions of equation (36)

$$\alpha \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \beta \quad \ldots\ldots\ldots (36)$$

$K = k_{-1}/k_1 = (\alpha)/(\beta)$ (since at equilibrium $k_1(\alpha) = k_{-1}(\beta)$)

now $k_{obsd.} = k_1 + k_{-1} = k_1 + k_1K$ $\therefore$ $k_1 = k_{obsd}/(1 + K)$ .....(51)

Hence by measurement of the equilibrium anomeric proportions of

each sugar, the equilibrium constant K of reaction (36) can be measured and the observed catalytic constants can be factorised into k_1 and k_{-1}. This factorisation is unnecessary for 5-substituted sugars since the equilibrium anomeric proportions of these sugars are nearly constant within experimental error. Table 221 shows that the proportions of all 5-substituted sugars fall in the range:-

α = 35% ± 4%, β = 65% ± 4%. Thus comparisons may be made directly on the observed values for the catalytic coefficients of 5-substituted sugars.

However, for 2-substituted sugars, the equilibrium anomeric proportions vary wildly (see Table 221) and in order to compare a series of catalytic constants, the observed catalytic constants must be factorised into k_1 and k_{-1} using equation (51) whence comparison can be made on the factorised catalytic coefficients k_1. This in the comparitive tables 194, 199, 204, 209 and 214 the observed catalytic coefficients k_B are given and then the factorised catalytic coefficient k_B' (which is equivalent to the k_1 used in equation (36)).

The mutarotation of five 2-substituted sugars catalysed by pyridine (Tables 190 - 194), 4-methylpyridine (tables 195-199), 4-ethoxypyridine (tables 200 - 204), 4-acetylpyridine (tables 205 - 209) and 2,6-lutidine (tables 210 - 214) was studied. Inspection of the comparitive tables 194, 199, 204, 209 and 214 shows that no real discernible trend exists in the catalytic coefficients for base catalysis as the electronegativity of the substituent is increased. The values of the observed catalytic

coefficients, k_B, appear to vary quite randomly. Factorisation as described above provides no improvement.

In addition, the hydronium ion catalysed mutarotation of these five 2-substituted sugars was studied and the results are given in tables 217 - 220. The mutarotation of 2-deoxy-glucose was too fast for accurate measurement in the buffers used, but an estimate of the $k_{H_3O^+}$ for this sugar is given in the comparitive table 220. Although the observed $k_{H_3O^+}$ decreases as the electron withdrawing power of the substituent increases, this is of doubtful significance since several special factors may be in operation. Certainly no trend is observed in the k_{H_2O} values. When the substituent X = H, (2-deoxy glucose) a large increase in $k_{cat.}$ is observed for all catalysts in a similar fashion to that described in section 4.3.A for α-D-xylose. Presumably the cause of this effect is the same also. Apart from this, no similarity exists between the two series of results.

The results outlined in section 4.3.A and 4.3.B indicate that the mutarotation reaction is more susceptible to substituent effects in the 5-position of the sugar ring than in the 2-position. Reasons for this are advanced in section 4.6.

4.4. Steric Hindrance to General Base Catalysis of Mutarotation

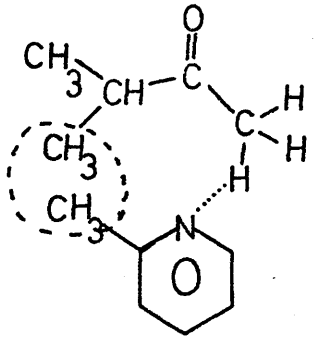
For any sugar in the series 1, the stronger the catalysing base, the larger the value of k_B as is to be expected from a general base catalysed reaction. This is illustrated in Fig. 7 for the sugar α-D-glucose. However, it is clear from Fig. 8 that for catalysis

by 2,6-lutidine, diethanolamine and tris, this simple rule (Brönsted catalysis law) does not hold. These three bases are much poorer catalysts than expected on the basis of their pK_a's (see Fig. 8). This appears to be due to steric hindrance of general base catalysis - a phenomenon which has been noted several times before.[49,68,136,137] The substituted pyridines have appeared to be particularly susceptible to steric hindrance, and this is corroborated in that, as can be seen from Fig. 8 and tables 97 and 169, 2,6-lutidine with a pK_a of 6.72[49] is a poorer catalyst than pyridine itself (pK_a = 5.22[49]). Further examples of such steric hindrance appear to occur in catalysis by the aliphatic amines diethanolamine (pK_a = 8.88[139]) which is a poorer catalyst than morpholine (pK_a = 8.33[140]), and tris (pK_a = 8.05[139]) which is a poorer catalyst than 4-ethoxypyridine (pK_a = 6.67[168]). Since diethanolamine and tris are much more sterically hindered bases than morpholine, it seems reasonable to equate the decrease in catalytic power with the increased steric hindrance.

However, although several authors[49,68,137] have described such steric hindrance to general base catalysis, there is a strange reticence when it comes to an explanation of such steric hindrance. The dissociation constants of methylpyridines[138] in aqueous solutions do not suggest that steric hindrance to ionisation is important, and so the low catalytic activity of α-methyl substituted pyridines must be associated with an unusual effect in the transition state of the reaction and cannot be attributed to an anomalous thermodynamic base strength. There are really

two probable sources from which the observed steric hindrance can arise. It could arise from an intermolecular steric strain caused by interactions between non bonding atoms on the molecules as they approach one another. That is to say, that as the substituted pyridine approaches the sugar to effect the proton transfer to or from that sugar, the α-alkyl groups interact with the sugar molecule, thus destabilising the transition state with respect to the reactants making the α-alkylpyridine a poorer catalyst than expected. This is the situation which apparently exists in the catalysis of the iodination of ketones by substituted pyridines studied by Feather and Gold[136]. This reaction is known to involve a rate determining proton transfer from the α-carbon atom of the ketone to the catalysing base. In studying the kinetics of iodination of a series of ketones catalysed by eleven alkyl substituted pyridines, Gold found that the degree of steric hindrance of abstraction of the proton from the ketone by the pyridine base - caused by α-alkyl substitution on the pyridine base - was readily correlated with the "increasing encumbrance" of the ketone. For a particular base - say 2-methylpyridine - the degree of steric hindrance of abstraction of an α-proton from the ketone increased along the series:- acetone < isopropyl methyl ketone < pinacolone.

Gold deduced that the explanation for the low catalytic power of the α-alkyl pyridines is that steric interaction occurs between the α-substituents on the base and the groups α to the carbonyl of the ketone as shown overleaf in the illustration for isopropyl methyl ketone.



Gold also draws attention to the stereochemistry which he considers the existence of such steric interactions imposes on both catalytic base and ketone. Firstly the breaking C-H bond and the carbonyl group are in a planar trans-conformation in the transition state so that the non reacting alkyl group of the ketone may come close to the approaching base and interact with the α-substitutents on that base. In any other conformation the base could not interact with the non reacting alkyl group of the ketone and so varying steric effects would not be anticipated. Furthermore, the heteroaromatic ring of the base must lie in the same plane as the carbonyl group and the breaking C-H bond of the ketone, since the observed steric hindrance by α-substituents on the base could otherwise be simply relieved by rotation of the pyridine ring relative to the plane of the carbonyl group. This implies some kind of "conjugative interaction" between the carbonyl group and the pyridine ring which is not easily explained. It may be related to the postulated conjugative interaction

between the heteroaromatic ring and the adjacent carbonyl group in 1-acetylpyridinium ions[141].

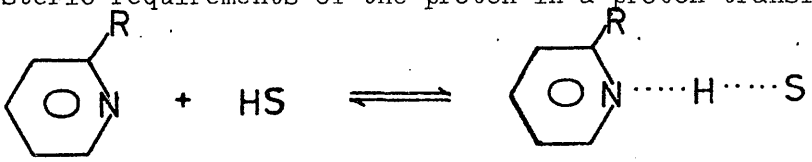
Hine[188] however calculates that although the steric hindrance observed by Gold in these studies is indeed due to intermolecular interactions between the α-methyl groups of the pyridine bases and the groups α to the carbonyl of the ketone, it is unnecessary to invoke that the breaking C-H bond be planar with the carbonyl and that therefore it is unnecessary to postulate that there exists the "conjugative interaction" between the carbonyl group and the pyridine ring (described above). His calculations he claims, show that the observed steric hindrance is anticipated even when the mechanism of hydrogen abstraction is the more commonly accepted one where the breaking C-H bond lies roughly parallel to the π -orbital of the carbonyl carbon atom, i.e. is nearly perpindicular to the plane described by the carbonyl group and the atoms attached directly to it.

The other possible source of the observed steric hindrance could be "steric hindrance of solvation".[142, 189] This could be envisaged as arising from the following:-

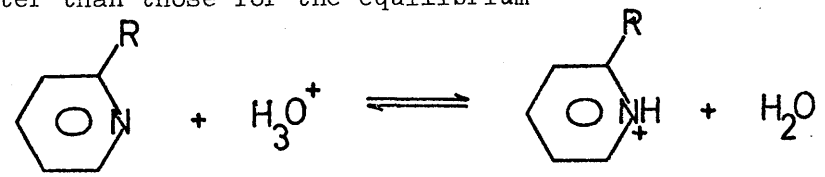
When the two neutral molecules, for example the ketone and pyridine base dealt with above, approach one another and the proton transfer between them proceeds, a certain amount of charge develops on the two reacting molecules. Naturally in a polar solvent the solvent molecules will solvate these developing charges, thereby stabilising them. It

is possible in 2-substituted pyridines that the α-substituents prevent solvent molecules from solvating the developing ionic charges, thereby destabilising the transition state relative to that of a reaction in which steric inhibition was impossible. Gold[136] however eliminates this as a possible explanation of the observed steric hindrance, on the basis that the ionic charge to be stabilised in the transition state of a proton transfer reaction is smaller than that of the pyridinium ion, and since no steric hindrance to ionisation (which could have arisen from steric hindrance of solvation) has been observed then no such explanation can be applied to proton transfer reactions.

The only remaining possible explanation is the rather dubious one that the steric requirements of the proton in a proton transfer reaction.



are greater than those for the equilibrium



That this should be so is by no means obvious.

Thus the most likely explanation for the observed steric hindrance in the general base catalysis of mutarotation is that there exists an intermolecular interaction between the 2,6-dimethyl-pyridine and the non reacting atoms of the sugar molecule, analogous to that found by Gold in the iodination of ketones.

Attempts to prove this and to use the observed steric hindrance of general base catalysis as a tool in distinguishing between kinetically indistinguishable mechanisms are described in section 4.5. (iii).

The cause of the observed steric hindrance of general base catalysis by diethanolamine and tris (Fig. 8) must be much the same as that discussed above for the substituted pyridines. The rigidity of the pyridine ring allowed the problem of the alkylpyridines to be discussed without reference to the change in bond angles of the nitrogen valencies which generally occurs when an amine is protonated. But with diethanolamine the occurrence of this structural change causes the groups bound to nitrogen to move closer to one another with the possibility of steric strain between these groups. However this is a steric effect which will also manifest itself in the ionisation of the amine and be reflected in the pK_a of the base. Hence the reason why the order of base strength in the following is :-

ammonia < methylamine > dimethylamine > trimethylamine,

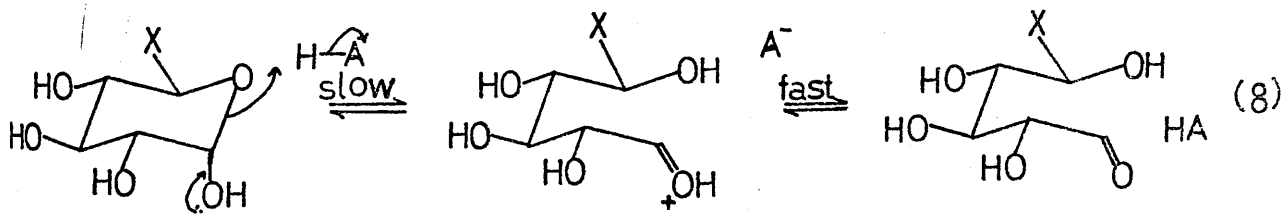
when on a purely electronic basis trimethylamine would have been expected to be the strongest base. Similarly the order of base strength in the ethanolamine series is :-

ethanolamine > diethanolamine > triethanolamine.

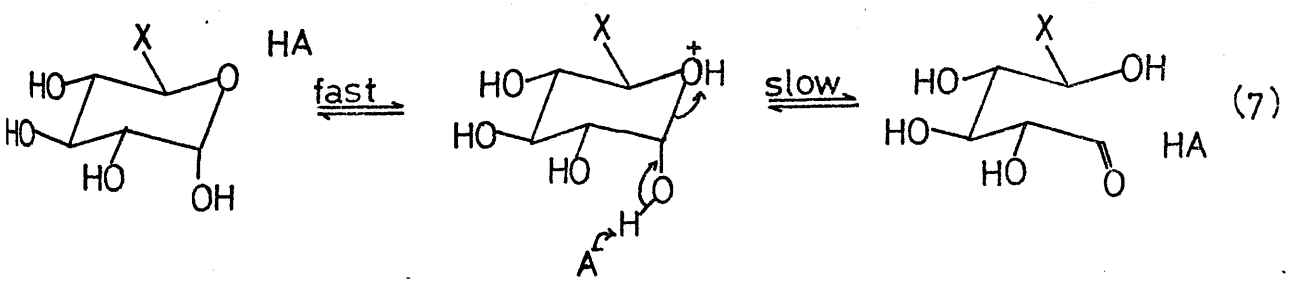
Since therefore this "B-steric strain"[143] is reflected in the pK_a of the base, one is left to conclude that the steric hindrance of general base catalysis of mutarotation by diethanolamine is also due to interactions between the nitrogen bound groups of the base and the non reacting atoms or groups of the substrate sugars.

4.5 (i) Substituent Effects and the Mechanism of Mutarotation

As was outlined in section 1.5 there is an unresolved problem in the mutarotation reaction, as in many general acid and general base catalysed reactions of distinguishing between kinetically indistinguishable mechanisms. For general acid catalysis of mutarotation the problem is to distinguish between the mechanisms depicted in equations (7) and (8). In considering the effect of substituents in the series 1, it has been assumed throughout that the substituent's effect on the kinetics arises because of its influence on the ether oxygen of the sugar ring rather than any effect which the substituent might have on the acidity of the anomeric hydroxy-group. For convenience equations (7) and (8) are reproduced here using the general substituent X rather than the specific formula for glucose.



(8)



(7)

As outlined in section 4.3.A(i), these studies have shown that

as the group X becomes more electronegative, then the catalytic coefficient for catalysis by hydronium ion, $k_{H_3O^+}$ decreases (see table 11).

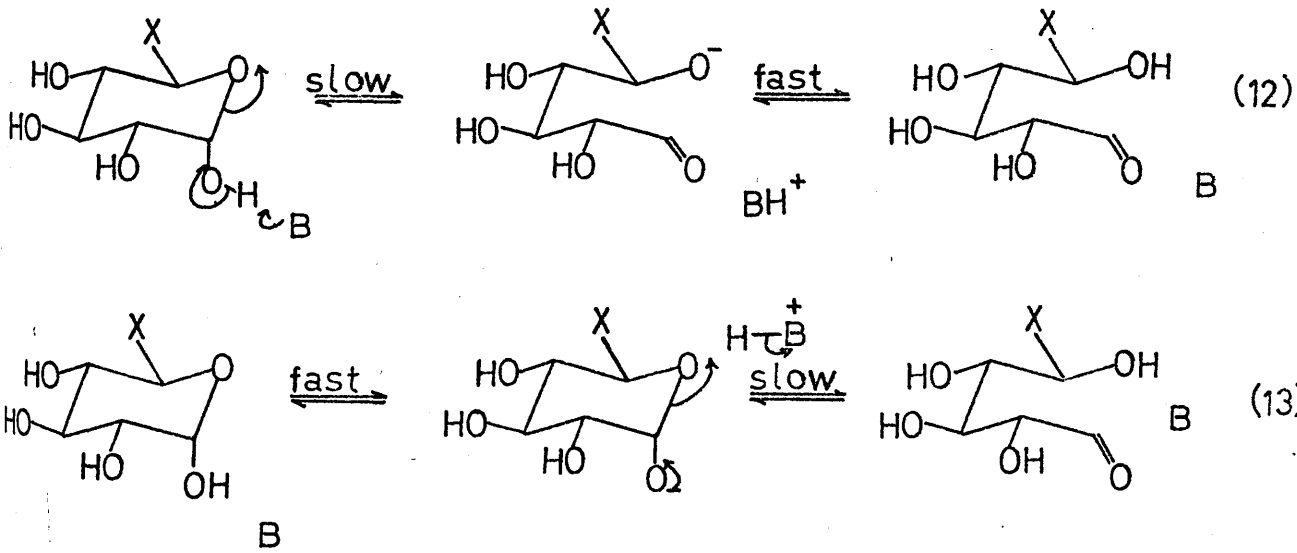
This observation unfortunately does not permit us to distinguish between mechanisms (7) and (8). The observed trend in $k_{H_3O^+}$ with increasing electronegativity of X can be explained almost equally well on the basis of either mechanism (7) or mechanism (8).

In mechanism (7) the rate determining step is the second step shown in which removal of the proton from the anomeric hydroxy-group by the conjugate base of the catalysing acid leads to simultaneous ring opening to give the open chain aldehydo-form of the sugar. In this step, the initial sugar species is the conjugate acid of the sugar which carries a full positive charge on the ether oxygen atom. This positive charge is neutralised by the ring opening to give the product of the rate determining step which is the neutral open chain aldehyde shown. Thus the transition state of this rate determining step must carry a partial positive charge on the ring oxygen, and one would therefore expect that increasing the electronegativity of the substituent at C-5 of the ring would lead to a destabilisation of this transition state and hence a decrease in the values of $k_{H_3O^+}$ as observed.

In mechanism (8) it is by no means evident that such a partial positive charge would have developed on the ether oxygen in the transition state of the rate determining step. However, if bond formation between the ether (ring) oxygen of the sugar and the proton of the acid

catalyst runs ahead of the breaking of the C(1)-ring oxygen bond (i.e. ring opening), then such a partial positive charge would develop on the ring oxygen and a decrease in $k_{H_3O^+}$ as the electronegativity of X increased would be anticipated on the same grounds as before. It is worth noting that such a situation would mean that two partial positive charges would be forming in the same molecule in the transition state. Although this situation seems intuitively less probable than that existing in mechanism (7) which is the mechanism favoured by several groups of workers,[62, 63, 144] it certainly cannot be discounted on the basis of these substituent effects, and so no definite and unambiguous choice between mechanisms (7) and (8) is possible.

The same mechanistic problem exists in base catalysed mutarotation where the choice of mechanisms lies between those depicted in equations (12) and (13). These mechanisms are again given below for convenience.



In mechanism (12), the rate determining step involves concerted removal of a proton from the anomeric hydroxy-group, formation of the aldehydo-carbonyl bond and ring opening, with expulsion of the ring oxygen as an alkoxide ion. Clearly then the transition state of this rate determining step will carry a partial negative charge on the ring oxygen atom, and one would expect electronegative substituents X to stabilise such a transition state. This would result in an increase in the catalytic coefficients for catalysis by bases as the electronegativity of X increased. This is the trend observed for all base catalysts (see section 4.3.A.ii).

It is more difficult to predict what effect the substituent X would have on the catalytic coefficients for base catalysis were mechanism (13) the correct mechanism. Again, using an argument similar to that used in trying to distinguish between the acid catalysed mechanisms, one can say that if bond formation between the ring oxygen and the proton of the conjugate acid of the catalysing base runs behind the bond breaking of the C(1)-ring oxygen bond then the ring oxygen will carry a partial negative charge in the transition state of the rate determining step. Thus the observed trend for base catalysis would also be explicable in terms of mechanism (13). One might however object to such an argument by pointing out that were it true, this would necessitate the existence of a transition state which contained two partial negative charges in the same sugar molecule. Again, however, no unambiguous assignation of mechanism is possible since we have no way of

ascertaining whether bond making or bond breaking is occurring ahead of the other.

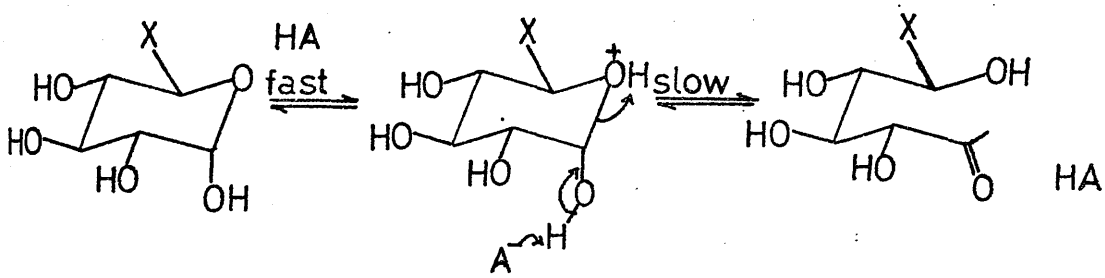
Owing to the amphoteric nature of water, mechanisms (7), (8), (12) and (13) are all possible mechanisms for the water catalysed reaction. Since the trend in k_{H_2O} with increasing electronegativity of X is identical to that for $k_{H_3O^+}$ and opposite to that for the catalytic coefficients for base catalysis, it seems likely that the mechanism for water catalysed mutarotation bears more resemblance to mechanisms (7) or (8) than to mechanisms (12) or (13). A mechanism such as (12) must be eliminated for water catalysis.

Obviously the substituent effects in these studies do not allow us to distinguish between the stepwise mechanisms (7), (8), (12) and (13) and the concerted mechanisms such as (16), (17) and (18). These latter mechanisms could also accommodate the observed substituent effects by similar arguments as above regarding the relative amounts of bond making or bond breaking in the transition state.

4.5(ii) The diffusion controlled limit and the mechanism of mutarotation.

As was discussed in section 1.5, one of the most common ways of trying to distinguish between mechanisms which are kinetically indistinguishable, is to inspect the mechanism for thermodynamically unstable reaction intermediates and to calculate whether or not this intermediate would react with another reactant at a rate greater than the diffusion controlled limit. It was pointed out in section 1.5 that Eigen[70] had

calculated that in the dehydration of acetaldehyde hydrate (a reaction which is highly analogous to the mutarotation of glucose) a mechanism such as (7) would require that the rate determining step be faster than diffusion controlled for catalysis by water. This clearly casts some doubt on the validity of mechanism (7) for the mutarotation reaction. It would therefore be useful to carry out the detailed calculation for mechanism (7) to see if Eigen's doubts are applicable to mutarotation.



(7)

If the sugar is denoted by S, and the conjugate acid (protonated at the ring oxygen) of the sugar is denoted by SH^+ then the rate determining step of mechanism (7) follows the rate equation:-

Rate = k_s (SH^+) (A^-) where k_s is the rate constant for the rate determining step in the forward direction.

i.e. Rate = $\dfrac{k_s\ (S)\ (H^+)}{K_{SH^+}} \circ \dfrac{K_{HA}\ (HA)}{(H^+)}$

where K_{SH^+} = dissociation constant of the conjugate acid of the sugar and K_{HA} = " " " " catalytic acid.

$\therefore$ Rate = $k_s \circ \dfrac{K_{HA}}{K_{SH^+}} \cdot (S)\ (HA)$ ..... (52)

Thus the observed rate constant for acid catalysis k_{HA} is related to

the rate constant for the rate determining step by the expression

$$k_{HA} = k_s \circ \frac{K_{HA}}{K_{SH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (53)$$

$$\therefore k_s = k_{HA} \cdot \frac{K_{SH^+}}{K_{HA}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (54)$$

The value which can be given to K_{SH^+} is doubtful but it should according to Hammett[146] be somewhat larger than the dissociation constant of a monohydric alcohol which is 10^2. More accurately one would think that the ether ring oxygen would be less basic than the ether oxygen of diethyl ether (pK_a[147] = -3.6) and hence have a lower pK_a. Thus the value of K_{SH^+} should be in the range 10^4 - 10^6.

To test whether k_s lies over the diffusion controlled limit we can evaluate expression (54) for certain catalysts.

e.g.1 for α-D-glucose at 25°, $k_{H_3O^+} = 1.106 \times 10^{-2}\ M^{-1}\ sec^{-1}$ (see table 11)

$$\therefore k_s = \frac{1.106 \times 10^{-2} \times K_{SH^+}}{55.6} = 1.99 \times 10^{-4} \circ K_{SH^+}\ M^{-1}\ sec^{-1}$$

Thus for catalysis by H_3O^+, for the expected range of values of K_{SH^+} ($10^4 \longrightarrow 10^6$), k_s is well within the diffusion controlled limit.

e.g.2 for α-D-glucose at 25°, $k_{HOAc} = 1.23 \times 10^{-4}\ M^{-1}\ sec^{-1}$ (ref. 29)

$$K_{HOAc} = 1.7 \times 10^{-5}$$

$$\therefore k_s = \frac{1.23 \times 10^{-4} \times K_{SH^+}}{1.7 \times 10^{-5}} = 8 \times K_{SH^+}\ M^{-1}\ sec^{-1}$$

Again, for catalysis by acetic acid, k_s is well within the diffusion controlled limit for the expected range of values of K_{SH^+}.

e.g.3 for α-D-glucose at 25°, k_{H_2O} = 7.21 x 10^{-6} M^{-1} sec^{-1} (see table 11)

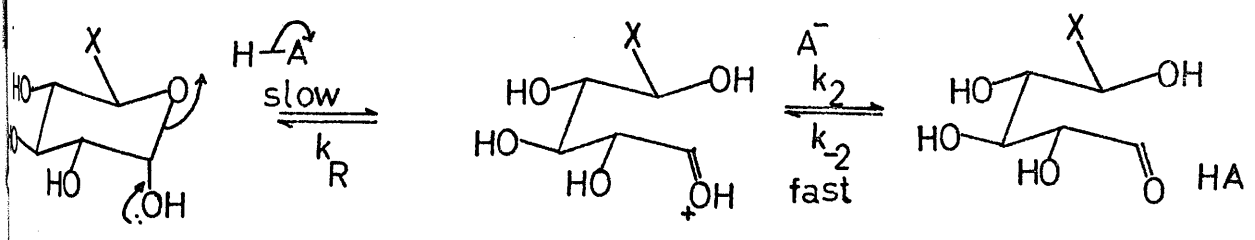
$$\therefore \quad k_s = \frac{7.21 \times 10^{-6} \times K_{SH^+}}{10^{-14}}$$

$$= 7.21 \times 10^{8} \, K_{SH^+} \; M^{-1} \; sec^{-1}$$

Now for catalysis by water, k_s would be greater than diffusion controlled for the expected range of values of K_{SH^+}, thus corroborating Eigen's calculations for the dehydration of acetaldehyde hydrate.

Thus mechanism (7) although apparently quite tenable for normal acid catalysts does not appear tenable for water catalysis. Jencks[69(b)] suggests that since water may actually be a base catalyst this objection to mechanism (7) might be irrelevant. However our studies have shown that water catalysis is subject to the same substituent effects as acid catalysis not base catalysis, and so mechanism (7) must be considered doubtful as atrue representation of the mechanism of acid catalysed mutarotation.

Jencks [69(b)] puts forward a similar objection to mechanism (8) for the dehydration of acetaldehyde hydrate, by calculating that the reverse reaction of the rate determining step must be faster than diffusion controlled. The calculation for the mutarotation reaction is as follows:-



(8)

S AldH$^+$ Ald.

Rate of reverse of R.D.S. $= k_R(AldH^+).(A^-)$

$$= \frac{k_R(Ald)(H^+)}{K_{AldH^+}} \cdot \frac{K_{HA}(HA)}{(H^+)}$$

where K_{AldH^+} = dissociation constant of conjugate acid of acyclic aldehyde and K_{HA} = " " " catalytic acid.

i.e. Rate of reverse of R.D.S. $= k_R \cdot \frac{K_{HA}}{K_{AldH^+}} \cdot (Ald)(HA)$ ..... (55)

Since the detectable amount of acyclic aldehyde in a glucose solution is of the order of 0.002%[52], then $(Ald)/(S) = 2 \times 10^{-5}$

∴ Rate of reverse R.D.S. $= k_R \cdot \frac{K_{HA}}{K_{AldH^+}} \cdot 2 \times 10^{-5} (S)(HA)$ ..... (56)

However, the rate constant for acid catalysis, k_{HA} is not simply related to the rate constant, k_R, for the reverse of the rate determining step. Instead the expression relating the two is :-

$$k_{HA}(S)(HA) + k_{-2}(Ald)(HA) = k_R \cdot \frac{K_{HA}}{K_{AldH^+}} \cdot 2 \times 10^{-5}(S)(HA) + k_2(AldH^+)(A^-) \quad \text{.....(57)}$$

which by use of the relationships above becomes

$$k_R = 5 \times 10^4 \cdot k_{HA} \cdot \frac{K_{AldH^+}}{K_{HA}} + k_{-2} \cdot \frac{K_{AldH^+}}{K_{HA}} - k_2 \quad \text{.....(58)}$$

where k_2 and k_{-2} are the rate constants in the forward and reverse directions of the fast (second) step of mechanism (8).

The first term of equation (58) can be evaluated from the known values of k_{HA}, K_{HA} and an estimated value for K_{AldH^+}. The dissociation constant for the conjugate acid of the open chain aldehydo-form of glucose is not known, but evaluations of the pK_a's of aldehydes are often within

the range - 6.0 to -10.0. A recent determination of the pK_a of acetaldehyde by Levy[146] gave a value of -10.2. If one therefore uses a value of 10^{10} for K_{AldH^+} (this may be on the low side) one can evaluate the first term of expression (58) for various catalysts.

e.g.1 for α-D-glucose, $k_{H_3O^+} = 1.106 \times 10^{-2}\ M^{-1}sec^{-1}$ (see table 11)

$$\therefore k_R = \frac{5 \times 10^4 . 1.106 \times 10^{-2} . 10^{10}}{55.6} + k_{-2} . \frac{10^{10}}{55.6} - k_2$$

$$= (10^{11} - k_2 + 1.8 \times 10^8 . k_{-2})\ M^{-1}\ sec^{-1}$$

Now k_2 must be very close to the diffusion controlled limit i.e. $10^9 - 10^{10}$ M^{-1} sec^{-1}. Thus it can be seen that although we cannot evalueate k_{-2}, its value will have no bearing on the conclusion that for the H_3O^+ catalysed mutarotation of D-glucose, mechanism (8) requires that the reverse of the rate determining step proceeds with an estimated rate constant greater than the diffusion controlled limit.

Similar calculations for catalysis by acetic acid and water, using the same catalytic coefficients as were used in the calculations for mechanism (7) give expressions for k_R as follows :-

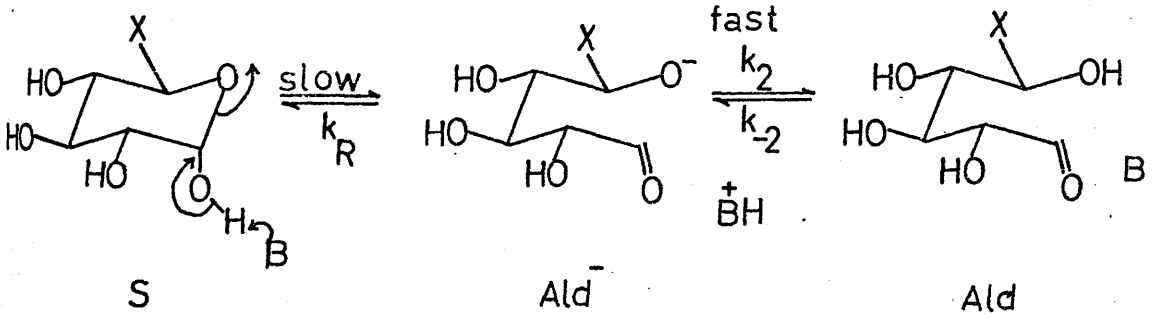
for acetic acid catalysis :- $k_R = (3.6 \times 10^{15} - k_2 + 6 \times 10^{14} . k_{-2})\ M^{-1}\ sec^{-1}$

for water catalysis :- $k_R = (3.6 \times 10^{23} - k_2 + 10^{24}\ k_{-2})\ M^{-1}\ sec^{-1}$

Clearly the values of k_R will be very much greater than diffusion controlled no matter what the value of k_{-2} is. Thus it would appear that Jencks' objection to mechanism (8) is not only valid for water catalysis but would eliminate mechanism (8) as a possible mechanism for all acid catalysts. It would therefore seem that mechanism (7) is the preferable one for acid catalysed mutarotation, although it too has

some doubt cast upon it, by the calculations herein, for catalysis by water. It could well be that some sort of concerted mechanism is required to account satisfactorily for water catalysed mutarotation.

It might also help to distinguish between mechanisms (12) and (13) if a similar set of calculations were carried out for base catalysed mutarotation. Consider first mechanism (12) :-



(12)

Rate of reverse R.D.S. $= k_R\,(Ald^-)(BH^+)$

$$= k_R \frac{(Ald)}{(H^+)} \cdot K_{Ald} \frac{(B)(H^+)}{K_{BH^+}}$$

$$= k_R \cdot \frac{K_{Ald.}}{K_{BH^+}} (Ald)(B) \quad \ldots\ldots\ldots\ldots\ldots (59)$$

where K_{Ald} = the dissociation constant of the C(5) hydroxy-group of the aldehyde

and K_{BH^+} = " " " " " conjugate acid of the catalytic base.

As before $(Ald)/(S) = 2 \times 10^{-5}$

$\therefore$ Rate of reverse of R.D.S. $= k_R(K_{Ald}/K_{BH^+}) \cdot 2 \times 10^{-5}(S)(B)$ ....(59a)

As in the previous discussion of mechanism (8) the expression relating k_R to the observed rate constant (for base catalysis), k_B, is a complex one:-

For a steady state approximation of the species "Ald^-" we have:-

$$k_B(B)(S) + k_{-2}(Ald)(B) = k_R(K_{Ald}/K_{BH^+}) \cdot 2 \times 10^{-5}(S)(B) + k_2(Ald^-)(BH^+) \quad \ldots\ldots\ldots\ldots\ldots\ldots(60)$$

By the usual calculations this gives

$$k_R = 5 \times 10^4 \cdot k_B \cdot \frac{K_{BH^+}}{K_{Ald}} + k_{-2} \cdot \frac{K_{BH^+}}{K_{Ald}} - k_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots(61)$$

where k_2 and k_{-2} are the rate constants of the forward and reverse reactions of the fast (second) step of mechanism (12).

Since the macroscopic dissociation constant of D-mannitol has been reported[148] as 4.38×10^{-14}, one may say with a fair degree of accuracy (certainly to well within an order of magnitude) that K_{Ald} will be approximately 10^{-13}.

Thus using this value of $K_{Ald.}$ and the catalytic coefficients for the mutarotation of glucose obtained in this work one can evaluate the first term of expression (61) for catalysis by several bases :-

e.g.1. for α-D-glucose at 25°, k_B for pyridine catalysis = 6×10^{-3} $M^{-1}sec^{-1}$
and $K_{BH^+} = 6.03 \times 10^{-6}$

∴ For the pyridine catalysed reaction:-

$$k_R = 5 \times 10^4 \cdot 6 \times 10^{-3} \cdot \frac{6.03 \times 10^{-6}}{10^{-13}} + k_{-2} \cdot \frac{6.03 \times 10^{-6}}{10^{-13}} - k_2$$

$$= (1.8 \times 10^{10} + 6.03 \times 10^7 \cdot k_{-2} - k_2)\ M^{-1}\ sec^{-1}$$

Again k_2 will be very close to the diffusion controlled limit, 10^{10} M^{-1} sec^{-1}, and so whether or not k_R is greater than the diffusion controlled limit may well depend on the size of k_{-2} which is unknown.

This may be seen more clearly for catalysis by morpholine:-

e.g.2. For α-D-glucose at 25°, k_B for morpholine catalysis

$= 1.1 \times 10^{-1}\ M^{-1}\ sec^{-1}$ and $K_{BH^+} = 4.7 \times 10^{-9}$.

$$\therefore k_R = \frac{5 \times 10^4 . 1.1 \times 10^{-1} . 4.7 \times 10^{-9}}{10^{-13}} + k_{-2} . \frac{4.7 \times 10^{-9}}{10^{-13}} - k_2$$

$$= (2.6 \times 10^8 + 4.7 \times 10^4 . k_{-2} - k_2)\ M^{-1}\ sec^{-1}.$$

e.g. 3. For α-D-glucose at 25°, k_B for acetylpyridine catalysis

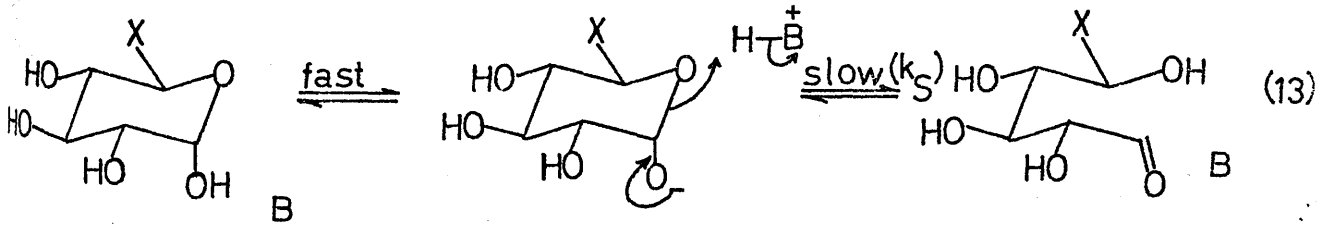
$= 6.55 \times 10^{-4}\ M^{-1}\ sec^{-1}$

$$\therefore k_R = 6.55 \times 10^{-4} . 5 \times 10^4 . \frac{3.3 \times 10^{-4}}{10^{-13}} + k_{-2} . \frac{3.3 \times 10^{-4}}{10^{-13}} - k_2$$

$$\therefore k_R = (1.1 \times 10^{11} + 3.3 \times 10^9 . k_{-2} - k_2)\ M^{-1}\ sec^{-1}.$$

Since $k_2 \approx 10^{10}$ it can be seen that k_R will be close to or over the diffusion controlled limit for pyridine and acetylpyridine catalysis no matter what the value of k_{-2} is. But for stronger bases such as morpholine the value of k_R is dependent on the value of k_{-2}. Since the value of k_R is greater than the diffusion controlled limit for some bases, (and perhaps also for others), this must raise some doubts about the validity of mechanism (12) for general base catalysed mutarotation.

Consider now mechanism (13) :-



(13)

S S⁻ Ald.

$$\text{Rate of R.D.S.} = k_s (S^-).(HB^+)$$

$$= k_s \cdot K_S \frac{(S)}{(H^+)} \cdot \frac{(B)\ (H^+)}{K_{BH^+}}$$

$$= k_s \cdot \frac{K_S}{K_{BH^+}} \cdot (S)\ (B) \quad \ldots\ldots\ldots\ldots (62)$$

where K_S = dissociation constant of the sugar (for α-D-glucose $= 4.6 \times 10^{-13}$ at 25°)

and K_{BH^+} = " " " " " conjugate acid of the catalytic base.

Thus the observed catalytic coefficient for base catalysis of mutarotation, k_B, is related to k_s by the following expression :-

$$k_B = k_s \cdot \frac{K_S}{K_{BH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (63)$$

$$\therefore k_s = k_B \cdot \frac{K_{BH^+}}{K_S} \quad \ldots\ldots\ldots\ldots\ldots\ldots (64)$$

We can now evaluate expression (64) for several base catalysts:-

eg. 1. For α-D-glucose at 25°, $k_{Pyridine} = 6 \times 10^{-3}\ M^{-1}\ sec^{-1}$ (see table 97)

$$\therefore k_s = \frac{6 \times 10^{-3} \cdot 6.03 \times 10^{-6}}{4.6 \times 10^{-13}} = 7.86 \times 10^4\ M^{-1}\ sec^{-1}.$$

eg. 2. For α-D-glucose at 25°, $k_{4\text{-Acetylpyridine}} = 6.55 \times 10^{-4}\ M^{-1} sec^{-1}$ (see table 159)

$$\therefore k_s = \frac{6.55 \times 10^{-4}\ 3.3 \times 10^{-4}}{4.6 \times 10^{-13}} = 4.7 \times 10^5\ M^{-1}\ sec^{-1}$$

eg. 3 For α-D-glucose at 25°, $k_{Morph} = 1.1 \times 10^{-1}\ M^{-1}\ sec^{-1}$ (see table 107)

$$\therefore k_s = \frac{1.1 \times 10^{-1} \times 4.7 \times 10^{-9}}{4.6 \times 10^{-13}} = 1.1 \times 10^3\ M^{-1}\ sec^{-1}$$

Thus as can be seen from these calculations mechanism (13) is quite compatible with the diffusion controlled limit.

To summarise what these calculations on the diffusion controlled limit with respect to mutarotation indicate:-

a) It appears that mechanism (7) is a more acceptable stepwise mechanism for acid catalysed mutarotation than mechanism (8). However some doubts also exist about mechanism (7) due to its inability to describe the water catalysed reaction satisfactorily.

b) For the water catalysed mutarotation of sugars, substituent effects indicate the mechanism is similar to that for acid catalysed mutarotation, but calculations show that neither equation (7) nor (8) is tenable for water catalysis since they both involve a step (either in the forward or reverse direction) which would require to be faster than diffusion controlled. This would imply that a concerted mechanism must exist for water catalysed mutarotation.

c) For base catalysed mutarotation mechanism (12) appears doubtful on the basis that the reverse of the rate determining step would be very close to and by the calculations herein often greater than the diffusion controlled limit. Mechanism (13) appears to be quite compatible with the observed data for general base catalysis.

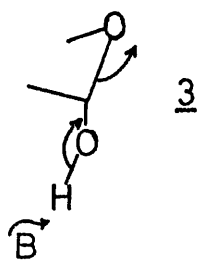
4.5.(iii) steric hindrance of general base catalysis and the mechanism of mutarotation

The mechanisms (12) and (13) differ principally in the site of the catalyst and the type of catalysis occurring in the rate determining step. In mechanism (12) the catalytic base functions as such in the

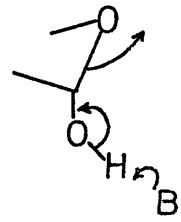
rate determining step and is situated beside the C(1)-anomeric hydroxy-group. In mechanism (13) the conjugate acid of the base functions as a general acid in the rate determining step and is situated beside the ring oxygen of the sugar. It has already been pointed out (section 4.4) that 2,6-lutidine is a much poorer catalyst than is expected on the basis of its basicity and that this has been widely ascribed to steric hindrance, by the α-methyl groups of the aromatic base, of the general base catalysis. The most likely cause of this steric hindrance as was discussed in section 4.4 is an intermolecular interaction between the methyl groups of the lutidine and non reacting atoms or groups of the sugar.

It might therefore appear possible that mechanisms (12) and (13) could be distinguished if one could correlate the degree of steric hindrance with the size of the group X either in series 1 or in series 2.

Clearly if the catalytic base is acting as in mechanism (12) it is conceivable that it will interact with substituents in the 2-position of the sugar ring. Not only is it conceivable, it seems highly likely since one would expect the removal of the proton from the anomeric hydroxy-group to occur in a trans-fashion as in 3 rather than in a cis fashion as in 4.



3



4

This would surely place the catalytic base closer to the substituent on the 2-position. Similarly if the catalyst is sited near the ether oxygen, one might justifiably postulate that the observed steric hindrance to lutidine catalysis occurred because of interactions between the catalyst and the substituent on the 5-position of the ring.

Accordingly an attempt to correlate the degree of steric hindrance to general base catalysis of mutarotation with the size of the substituents on the 2- and 5-positions of the ring was made.

Studies of the kinetics of mutarotation of series 1 sugars catalysed by pyridine (tables 66 - 97), 4-acetylpyridine (tables 150 - 159), 4-methylpyridine (tables 130 - 139) and 4-ethoxypyridine (tables 140 - 149) yielded the catalytic coefficients for catalysis by these bases, for each sugar in the series. This enabled Brönsted plots for catalysis by 4-substituted pyridine bases to be made (table 170) from which could be calculated the catalytic coefficient which a pyridine base with the pK_a of 2,6-lutidine would be expected to have. Dividing this calculated catalytic coefficient for "lutidine catalysis" by the observed catalytic coefficient for lutidine catalysis (table 169) yielded a steric hindrance factor (S.H.F. - see table 171). If the source of the steric hindrance were interaction between the lutidine molecule and the group X on the 5-position of the ring, then one would expect this S.H.F. to increase with increasing size of X.

Similar studies with 2-substituted sugars (tables 190 - 214) led to similar Brönsted plots (table 215) and identically calculated steric

hindrance factors (table 216).

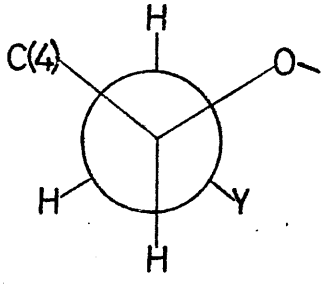
As can be seen from tables 171 and 216 the correlation between the degree of steric hindrance and the size of the substituents is very disappointing and inconclusive. With the 2-substituted sugars (table 216) there is no correlation with substituent size whatsoever, the smallest substituent (H) having the largest S.H.F. and the remaining substituents showing no correlation with size at all.

With the 5-substituted sugars, the correlation with size is poor but considerably better than with 2-substituted sugars. For example small substituents like H and CH_3 have S.H.F.'s at the low end of the scale, while the largest substituent X = $C_6H_{11}O_5$(Gentiobiose) has the largest S.H.F. But the range of these steric hindrance factors is covered by a factor of about 2.5 which is rather small, and there are several deviations from the expected trend of values (eg. cf. values of S.H.F. obtained for X = -$NHCOCH_3$ and X = CH_2OPh).

It is obvious that the simple idea of molecular interactions between these substituents and the catalyst is too naive for this situation. It is conceivable that factors such as the solvation of the group X or differences in the conformation around the C(5) - C(6) bond could combine to make the original model too simple.

For example let us consider briefly the steric implications of steric hindrance to general base catalysis in mechanism (13). It would appear, from models, that the existence of such steric hindrance would require that the plane of the lutidine base be approximately the

same as that of the sugar. Otherwise steric hindrance (assuming that this arises from interactions of the methyl groups with the sugar) could be relieved by rotating the lutidine ring through 90°. In this situation the maximum interaction between the substituent X and the catalyst would occur when the conformation of the C(5)-C(6) bond was as shown in 5 rather than the other two most stable, staggered conformers 6 and 7. This has been illustrated for the general substituent $X = CH_2Y$



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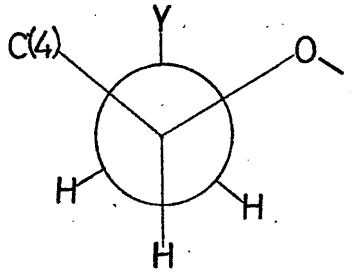

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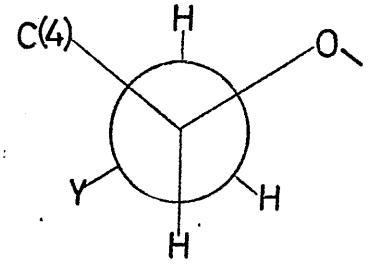

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Since there is no information available on the conformation around the C(5)-C(6) bond for the sugars in series 1 in aqueous solution, it can only be surmised that differences in this conformation from sugar to sugar could render the test for steric hindrance factors too simplified.

If anything can be drawn from the observed steric hindrance factors regarding the mechanisms (12) and (13), it is that there exists a bit more evidence to suggest mechanism (13) is correct than there is for mechanism (12). However a definite distinction between the mechanisms

on the basis of these steric hindrance factors is out of the question.

If the mechanism of base catalysed mutarotation is a concerted one such as (16) with the catalyst sitting outside a chain of water molecules, triggering off the reaction, it is harder to rationalise the observed steric hindrance of general base catalysis. The existence of such steric hindrance certainly seems to suggest a close interaction of sugar and catalyst.

A further piece of evidence which might suggest that the site of the catalyst is that depicted in mechanism (13) is the already mentioned observation (see section 4.3 A(ii)) in this work, that aromatic amines are much better catalysts relatively speaking for the base catalysis of the mutarotation of 6-O-phenyl-α-D-glucose than are aliphatic amines (see tables 97, 139, 149, 159, 169, 107, 117 and 55). It seems unlikely to be coincidence that the catalysis by aromatic amine catalysts should be enhanced in the only sugar containing an aromatic residue. Such an enhancement of catalytic effect is similar to that observed by Schneider[149] for the hydrolysis of p-nitrophenyl acetate catalysed by amine bases. He found that aromatic amines were up to twice as effective catalysts as aliphatic amines of the same pK_a, and he ascribed this to intermolecular forces between the aromatic residues of the substrate and the catalyst. One might visualise such an interaction occurring more readily if catalysis by bases occurred by mechanism (13) where the catalyst is situated near the ring oxygen. A hydrophobic substituent such as $-CH_2OPh$ might be envisaged as destroying

the solvation sheath of the sugar in that vicinity, perhaps creating a "pocket" or region where another hydrophobic molecule such as the aromatic amine catalyst might be more easily accommodated. Such considerations are largely speculation, but the enhanced catalytic activity of aromatic amines when X contains an aromatic residue is indeed interesting. Hydrophobic interactions between substrates and catalysts in aqueous solution are not unknown, and have been recently postulated[169] as a means of endowing catalytic specificity in the hydrolysis of long chain alkyl-p-nitrophenyl esters catalysed by long chain alkyl amines and long chain N-alkyl imidazoles.

4.6. The Brönsted Catalysis Law and Mutarotation

Mutarotation, being one of the classic examples of a general acid-general base catalysed reaction, obeys the Brönsted catalysis law. Indeed mutarotation was one of the first reactions to which Brönsted[26] applied his law, and found it to hold successfully. It is well known that the Brönsted relation is very often found to correlate successfully the catalytic coefficients for catalysis by acids or bases and the pK_a's of these acids or bases, provided that the acids or bases fall into a series of closely similar structure.[150,188] Thus in the reaction for which the Brönsted relation was first proposed, namely the decomposition of nitramide,[151] the Brönsted law is well correlated within several different groups of bases such as bases with one negative charge, bases with two negative charges, neutral amine bases and bases with two positive charges. Within each group the value of

the Brönsted exponent, β , was constant but appreciably different for each group. Such differences are detectable because the decomposition of nitramide obeys the Brönsted relationship with a high degree of accuracy. Other reactions where such accuracy within groups of bases is not observed, include mutarotation, and in these reactions it is possible to give a single equation which is approximately valid for all classes of catalyst.

In the studies reported in this work however, the Brönsted relationship was only derived for a single class of bases - the 4-substituted pyridines. The Brönsted exponents β, for all the sugars studied can be found in table 171 (for 5-substituted sugars) and table 216 (for 2-substituted sugars). These are of course obtained by linear plots least squares treatment) of the catalytic coefficients for catalysis by the substituted pyridines against the pK_a's of the bases. The β values themselves are accurate to $\pm$ 0.01. The results in tables 171 and 216 are interesting. For sugars with substituents in the 2-position, the Bronsted exponent β remains constant at 0.49 $\pm$ 0.01, while for sugars with substituents in the 5-position the β value ranges from 0.47 to 0.69 as the electronegativity of the substituent increases.

Such findings are interesting for several reasons. If one assumes for the moment, that such changes in the Brönsted exponent occur because of a change in the pK_a of the sugar molecule, then it is surprising that the β values for the 2-substituted sugars are constant since

surely substituents in the 2-position with σ values ranging from $0 \longrightarrow 0.6$ would affect the acidity of the anomeric hydroxy-group to a considerably greater extent than substituents in the 5-position (one bond further away) with σ values ranging from $-0.05 \longrightarrow 0.18$. Indeed one would think that the effect of the substituents in either position on the pK_a of the sugar would not be sufficient to cause a change in Brönsted exponents. Neuberger and Fletcher[131] estimate the pK_a's of 2-acetamido-2-deoxy-glucose and 2-amino-2-deoxy-glucose hydrochloride to be 11.65 and 10.5 respectively. This is much the same size of pK_a change (glucose pK_a = 12.4 approx.) as was present in the substrates in Bordwell's[113] study of arylnitroethanes where almost no variation of the Brönsted exponent was found as the substrate was changed. Indeed Bordwell further indicates that over a ΔpK range of almost 20 units the β value for deprotonation of nitroalkanes and ketones shows little variation and no consistent trend.

It seems most unlikely then, that the very small changes in the pK_a of the sugar caused by substituents in the 5-position of the ring, could account for the increase in β values shown in table 171. Indeed one can actually reason as follows that any trend due to this factor would be expected to be in an opposite sense to that actually observed:-

A proton being transferred from one base to another will (as discussed in section 1.9) in the transition state lie closer to the weaker base. The effect of putting increasingly electronegative

substituents in the 5-position will, if anything, cause the sugar pK_a to fall, i.e. the glucosate anion will become a weaker base.

Thus if we consider mechanism (12), the effect of increasingly electronegative substituents in the sugar on the degree of transfer of the anomeric hydroxy-proton to the catalytic base will be to make the proton lie relatively nearer to the sugar in the transition state - thereby decreasing β. This is of course exactly the opposite of the observed results. Precisely the same conclusion is reached in applying the same considerations to the alternative mechanism (13) for general base catalysis, where the proton transfer is actually the transfer of a proton from the conjugate acid of the catalytic base to the ring oxygen of the sugar.

It is therefore quite obvious that in the mutarotation reaction, a reaction which involves "heavy atom reorganisation"[152] as well as proton transfer in the rate determining step, the value of the Brönsted exponent β cannot be regarded as a measure of the degree of proton transfer in the transition state.

For the mutarotation reaction, the Brönsted β measures the susceptibility of the reaction to base catalysis, and by far the most important factor in determining this susceptibility is the ease with which the sugar ring can break to form the open chain aldehydo-form. The basicity of the proton involved in the accompanying proton transfer has apparently little or no effect in determining this susceptibility to base catalysis. This is of course why the reaction is also much

more susceptible to substituents in the 5-position of the ring than in the 2-position (cf. 4.3.B).

Since a very large proportion of acid-base catalysed processes consist of such proton transfers combined in the overall transformation with the reorganisation of a heavy atom framework, caution is urged in interpreting Brönsted exponents as measures of the degree of proton transfer in the transition state of such reactions.

In the list of Brönsted exponents in table 171, there are two sugars whose β values deviate from the observed trend. 6-acetamido-6-deoxy glucose and 6-O-phenyl glucose appear to have Brönsted β values which are higher and lower respectively than expected from their positions in the series.

Recently Kresge[153] has postulated that deviant Brönsted relations ($>$ 1.0 or $<$ zero) can be explained by an intermolecular effect between the substituent in the substrate and the catalyst. Such an effect would only be present in the transition state of the reaction, not in the initial or final states, and as such would explain the situation whereby a reaction can show greater sensitivity to structural change in its rate than in the position of equilibrium of the reaction. Kresge points out that Brönsted β values greater than 1.0 will be expected when the intermolecular effect between catalyst and substituent is greater than the intramolecular effect of the substituent within the substrate. This sort of situation he maintains, will exist in pseudoacids like the nitroalkanes studied by Bordwell[112,113]. With

more conventional catalysts, the intermolecular effect will be small but not totally absent. Its presence according to Kresge may still significantly affect the Brönsted exponent.

Whether considerations such as these could account for the deviations in β noted for 6-acetamido-6-deoxy-glucose and 6-O-phenyl glucose is doubtful, but it is interesting to note that certain intermolecular interactions between the $-CH_2OPh$ substituent and aromatic amines may exist on the basis of the enhanced catalytic activity of the latter (see section 4.5. (iii)) and it is not unreasonable to point out that a substituent such as $-CH_2NHCOCH_3$, containing as it does a dipolar carbonyl group, might also interact with a catalyst molecule more readily than the substituents in the other sugars studied.

It must however be pointed out that the anomalous Brönsted exponents ($>$ 1.0 and $<$ zero) have also been explained on a totally different basis by Marcus.[154]

4.7 Linear Free Energy Relationships and Mutarotation

As was outlined in section 4.1, the study of the mutarotation of the sugars in series 1 and 2 is a structure - reactivity study in an aliphatic system. This section discusses the fit of the observed data to linear free energy relationships, which is of interest due to the relatively few aliphatic reactions which have been so correlated.

Inspection of the data for the kinetics of mutarotation of 2-substituted sugars showed no trends and since only five such compounds were studied no attempts were made to fit the data to free energy

relationships (other than the Brönsted law for each individual sugar). The work of this section was restricted to the 5-substituted sugars (series 1).

One of the assumptions implicit in searching for linear free energy relationships in reactions of a series of compounds is that the mechanism of a reaction throughout the series is the same. One way of checking this is to observe a constancy of kinetic isotope effects throughout the series. Thus in order to determine these, the mutarotation of all the sugars of series 1 catalysed by D_3O^+ in deuterium oxide was studied (tables 118 - 128). This enabled the isotope effects $k_{H_3O^+}/k_{D_3O^+}$ and k_{H_2O}/k_{D_2O} to be calculated (see table 129). The near constancy of both these isotope effects was taken as an indication that the mechanism of mutarotation is constant throughout the series. The observed values are in close agreement with those previously observed (see section 1.4).

The first linear free energy relationship to which attempts were made to fit the observed kinetic data was equation (30) (see section 1.8).

$$\log (k/k_o) = \sigma^* \cdot \rho^* \quad \ldots\ldots\ldots\ldots\ldots (30)$$

where k = rate constant for the substituted compound and

k_o = " " " " standard of comparison.

In these studies the σ_I values of Charton[129] have been used instead of the σ^* values of Taft and equation (30) becomes

$$\log k = \sigma\rho - \log k_o \quad \ldots\ldots\ldots\ldots\ldots (65)$$

where $\sigma \equiv \sigma_I$, k = the catalytic coefficient for catalysis by a particular catalyst on the substituted sugar, k_o = the catalytic coefficient for catalysis by the same catalyst on the standard of comparison which is α-D-xylose, ρ is the susceptibility of the reaction to changes in σ .

Table 225 illustrates the result of fitting the observed rate constants for catalysis by most of the catalysts studied, to equation (65). The results are quoted in the form of a correlation coefficient "r" which can be taken as a measure of the degree to which the observed $k_{cat.}$ values fit a linear relationship when plotted against σ . The table quotes the correlation coefficient obtained for these linear plots, firstly when the $k_{cat.}$ values for all the eight sugars studied are included in the plot and then when one $k_{cat.}$ value is successively omitted. Wells[155] and Jaffe[85] describe those relationships with a correlation coefficient $>$ 0.99 as excellent, $>$ 0.95 as satisfactory and $>$ 0.90 as fair.

As can readily be seen from the first row of table 225, only one of the plots give correlation coefficients $>$ 0.90, the majority of them giving such low correlation coefficients as to make nonsense of a suggested linear relationship. In every case the correlation coefficients are most improved by omission of the $k_{cat.}$ value for α-D-xylose (X = H). Inspection of row 3 of Table 225 shows that the correlation coefficient increases, often dramatically, by omitting this point from the plots.

This suggested that equation (65), which assumes that only the polar effect of the substituent influences the free energy of activation of mutarotation, is not adequate to explain the effect of structure on the mutarotation reaction. The most obvious refinement of equation (65) is to not only consider the polar effect of the substituent but also to allow for the steric effect of the substituent.

It is interesting to note that the best fits to equation (65) is obtained for the catalysts H_2O, D_2O, H_3O^+, D_3O^+. These catalysts have the least steric requirements.

Thus the equation (66) which contains terms for both polar and steric effects was the next equation tried :-

$$\log (k/k_o) = \sigma\rho + \delta E_s \quad \ldots\ldots\ldots\ldots\ldots\ldots (66)$$

where k = catalytic coefficient for mutarotation of the substituted sugar

k_o = " " " " " " standard of comparison

σ = inductive substituent constant

E_s = steric substituent constant (see section 1.8)

ρ = susceptibility of the reaction to change in σ

δ = " " " " " " " E_s.

Taft, by assuming that the inductive effect of the substituents in acid catalysed ester hydrolysis was negligible was able to evaluate E_s for his substituents. Such an assumption for the mutarotation is patently false (see table 11) and so no evaluation of the steric

substituent constants is possible. Instead consider the relationship for two catalysts A and B :-

Then for catalyst A $\log (k^A/k_o^A) = \rho^A\sigma + \delta^A E_s$ ..... (67)

and for catalyst B $\log (k^B/k_o^B) = \rho^B\sigma + \delta^B E_s$ ..... (68)

Combination of (67(and (68) gives

$$\log (k^A/k_o^A) - \log (k^B/k_o^B) = (\rho^A - \rho^B)\sigma + (\delta^A - \delta^B) E_s \quad \ldots\ldots (69)$$

If we now make the assumption that the susceptibilities of the two reactions (differing only in the type of catalyst) to the steric effects of the substituent are the same, i.e. $\delta^A = \delta^B$, then equation (69) becomes

$$\log (k^A/k_o^A) - \log (k^B/k_o^B) = (\rho^A - \rho^B)\sigma \quad \ldots\ldots\ldots\ldots\ldots\ldots (70)$$

i.e. $\log (k^A/k^B) = (\rho^A - \rho^B)\sigma + \log (k_o^A/k_o^B)$ ..... (71)

Thus by plotting the differences in the logarithms of the $k_{cat.}$ values for two different catalysts we should eliminate the steric effect of the substituent provided $\delta^A = \delta^B$. This is of course the most important assumption in Taft's analysis of this problem (see section 1.8). The success which he had in correlating structure and reactivity in ester hydrolysis in his opinion justifies this assumption.

Table 226 shows the results obtained from fitting the function $\log (k^A_{cat.}/k^B_{cat.})$ to a linear relationship with σ.

As can be seen by comparing Row 1 of table 226 with the relevant values in Row 1 of table 225, a substantial improvement in the correlation coefficients indicates that equation (71) is a better representation of the factors which require to be taken into account in explaining

the relationship between the structure of the sugar and its rate of mutarotation. For example the correlation coefficients of plots of the catalytic coefficients for catalysis by pyridine and 4-methylpyridine are 0.317 and 0.591 respectively. When the data for these two catalysts is plotted in the form of equation (71) the correlation coefficient rises to 0.943.

However the correlation coefficients are still not particularly good, only a few of them falling into the category of a "fair" correlation described by Wells and Jaffe (see earlier). As would be expected after refinement of equation (65) to equation (71) to try to allow for substituent steric effects, the correlation coefficients are not consistently improved by the omission of any one sugar from the calculations and are not significantly improved by the omission of any of the sugars from the calculations.

Furthermore the functions used in table 226 have been selected from the possible combinations of catalysts, as those which give the best linear relationships with σ. There are other functions $\log (k^A/k^B)$ which when plotted against σ give exceedingly poor linear correlations. A selection of these are shown in Table 227.

So clearly although equation (71) brings about an improvement in the correlation of some of the catalytic coefficients with σ (table 226), there are others for which little improvement is achieved (table 227). It is not easy to see why this is so. If for example the reason was that for some catalysts the assumption of constant steric effects

($\delta^A = \delta^B$) was true, but that for other catalysts it was not, then surely it would be expected that δ^A would equal δ^B when A and B were catalysts of the same family. e.g. pyridine, 4-methylpyridine, 4-ethoxypyridine etc. But table 226 shows that for example, the correlation of $\log (k_{MePy}/k_{Py})$ with σ is poorer than that of $\log (k_{MePy}/k_{H_3O^+})$ with σ (where MePy is an abbreviation for 4-methylpyridine and Py for pyridine). Similarly $\log (k_{H_3O^+}/k_{D_3O^+})$ gives an extremely poor linear correlation (see table 227). Thus this explanation seems an unlikely one.

What seems more likely is that equation (71) is in need of further refinement to take into account some of the other factors which may be influencing free energy relationships. A few possible factors are as follows:-

(a) The rate constants which are being used in the correlations are composite quantities. On the simplified basis of equation (36) (see section 4.2)

$$\alpha \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \beta \quad \ldots\ldots\ldots\ldots (36)$$

$$k_{obsd.} = k_1 + k_{-1}$$

Only if $K = k_{-1}/k_1$ is constant throughout the series of sugars is it valid to use $k_{obsd.}$ values in the linear free energy plots (see section 4.3. B). That this is so for 5-substituted sugars within a small error has been verified in this thesis. However it is possible that such a kinetic treatment is too simplified and that as

detailed in section 4.2, the rate equation describing the equilibrium (40)

$$\alpha \underset{k_{-\alpha}}{\overset{k_{\alpha}}{\rightleftharpoons}} A \underset{k_{\beta}}{\overset{k_{-\beta}}{\rightleftharpoons}} \beta \quad \ldots\ldots\ldots\ldots (40)$$

is $$\text{Rate} = \frac{k_{\alpha}.(\alpha)}{1+p} - \frac{p.k_{\beta}.(\beta)}{1+p} \quad \ldots\ldots\ldots\ldots (45)$$

where p = partitioning ratio of the open chain aldehyde i.e. $p = k_{-\alpha}/k_{-\beta}$

In the more simplified treatment (equilibrium (36)) the rate equation is:-

$$\text{Rate} = k_1(\alpha) - k_{-1}(\beta) \quad \ldots\ldots\ldots\ldots\ldots (45^1)$$

If we compare equations (45) and (45^1) at zero time in an experiment in which the mutarotation of a pure α anomer is being studied, then we have

$$(\text{Rate})_o = k_1(\alpha)_o = k_{\alpha}(\alpha)_o/(1+p)$$

$$\therefore k_1 = k_{\alpha}/(1+p)$$

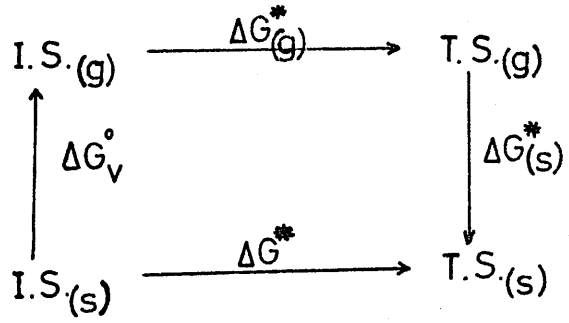
and similarly $k_{-1} = p.k_{\beta}/(1+p)$

It is possible that one should in fact be using k_{α} (or k_{β}) values instead of $k_{obsd.}$ values in these structure - reactivity studies. Since no information exists on the partitioning ratios of all the sugars in series 1, the rate constants k_{α}, k_{β} etc. cannot be extracted from the observed rate data. The use of the observed rate constants will only be equivalent to the use of k_{α} constants if p is constant throughout the series of sugars. By assuming that this is so, a source of error in the linear free-energy relationships may have been introduced.

(b) The assumption that $\delta^A = \delta^B$ may not be at all valid. This will patently mean that linear free-energy relationships based on equation (71) will have errors built into them due to this invalid assumption. Although the assumption of constant steric effects in acid and base catalysed hydrolysis of esters was one of the pillars of Taft's treatment, it may not be true for the mutarotation of glucose. (Section 1.8 gives some of the objections to this assumption). Certainly one of Taft's other assumptions - namely that the polar effect of substituents can be ignored in acid catalysed hydrolysis of esters (see section 1.8 - equation (28)) - is not true for acid catalysed mutarotation (see table 11).

(c) An assumption implicit in our treatment (and Taft's) of free energy relationships is that the relative free energy of activation may be treated as the sum of independent contributions from polar, steric and resonance effects of the substituent. This of course may not be true if there was also a contribution to the relative free energy of activation from differences in the solvation energies of the sugars.

This may be illustrated by the following thermodynamic cycle:-



where I.S. and T.S. represent the initial and the transition states respectively. ΔG^* the free energy of activation in solution differs from that in the gas phase $\Delta G^*(g)$, by the sum of $\Delta G^*(s)$ the free energy of solution of the transition state and ΔG_v^o the free energy of vaporisation of the initial state from the solvent. Now for the linear free energy relationships being studied one is concerned with the changes in the free energy of activation along the series of sugars. These changes can then be factorised as follows:-

$$d\Delta G^* = d\Delta G^*_{(g)} + d\Delta G^*_{(s)} + d\Delta G^o_v \quad \ldots\ldots\ldots (72)$$

It is quite possible that thecontributions to $d\Delta G^*$ from the solvation of the transition state and the vapourisation of the initial state from the solvent, will vary with the substituent on the sugar. Any analysis of the solvation of a solute in aqueous solution must include solvent-solvent interactions, solute-solvent interactions and perhaps solute-solute interactions.

Information on such interactions is scant, since even the solvent-solvent interactions are unknown. Several theories of the structure of water exist and these have been reviewed by Wicke.[157] Basically these theories are of two types. One which regards liquid water as a homogeneous extensively hydrogen bonded system,[161,162] and the other which regards water as consisting of different molecular aggregates ranging from "clusters"[158], of perhaps up to 100 water molecules extensively hydrogen bonded with a resultant fairly rigid structure, through smaller entities of two to six molecules ("the third

state"[157, 160]) to single free, non-hydrogen bonded water molecules in between the other molecular aggregates.

Opinion is just as divided on the nature of solute-solvent interactions. Some authors[158] prefer a model where the solute sits inside a solvent shell - a clathrate type model where the solute wears the solvent "like a suit of armour" while others[163] prefer a model where the small water molecules pack closely round the solute - "more like a tight fitting sweater" - with a resultant deformation of the normal hydrogen bonded structure of the solvent. Grunwald[163] concludes from some of his studies "that the interaction energy to be gained by the close approach of water molecules to the solute molecule is very substantial and that the necessary deformation of the normal water structure does take place.

Unfortunately, little or no information is available on the sugar-water interactions which can be expected to occur in the aqueous studies reported in this thesis. Kabayama and Patterson[156] discussed the solvation of sugars in aqueous solution on the assumption of the "continuum" model of the structure of water. However it is doubtful whether the tridymite structure which they assume is a true representation of the structure of liquid water at room temperature, and therefore their conclusions as to the mode of hydration of sugars are doubtful.

What seems possible is that if the solute-solvent interactions are of the form envisaged by Grunwald,[163] then in the series of sugars studied, where the substituent varies considerably in its polarity,

the differences in the free energy of solvation may have a considerable effect on the relative free energies of activation and hence lead to unpredictable deviations from linearity when rate data are fitted to free energy relationships such as equation (71).

One could argue that such solute-solvent interactions might lead to no net free energy change due to a concomitant decrease (due to the solute-solvent interactions) in the enthalpy and the entropy. That such changes in entropy and enthalpy should be of the same order of magnitude or linearly related is by no means obvious.

(d) The free energy change of a reaction at temperatures above absolute zero is related to the heat capacity changes by the equation

$$\Delta G^{o} = \Delta H^{o} + \int_{o}^{T} \Delta C_p dT - T \int_{o}^{T} \Delta C_p d\ln T \quad \ldots\ldots\ldots\ldots (73)$$

where ΔH^{o} = enthalpy change at $0^{o}K$ and the two integral terms represent the excess enthalpy and excess entropy at the temperature T. Then the change in the free energy brought about by varying the structure of the substrate is related to the heat capacity changes by

$$d\Delta G^{o} = d\Delta H + \int_{o}^{T} d\Delta C_p dT - T \int_{o}^{T} d\Delta C_p d\ln T \quad \ldots\ldots\ldots (74)$$

A linear free energy relationship would require that the heat capacity changes in a series of compounds to be zero, constants or to vary systematically. These heat capacity changes have often been assumed to be negligible[164] or constant in a series of similar, related compounds.

Recent studies[165, 166, 167] have shown that the heat capacities of

some organic compounds in solution are not zero or constant in series of similar compounds. Significantly, one of the classes of compounds studied was the low molecular weight alcohols[165] which would be expected to be highly solvated in aqueous solution. Previous examples of significant heat capacities were restricted to cases where solvation is known to be particularly strong - namely the creation or neutralisation of ionic charge, i.e. the reactions of ions (e.g. the ionisation of cyanoacetic acid in water[168a]) One might reasonably conclude therefore that the heat capacities of sugars in aqueous solutions, (where presumably the importance of solvation is intermediate to that of monohydric alcohols and ions) will not be zero or constant, and that in a series of sugars such as 1 where substitutents are both hydrophobic and hydrophilic, the heat capacities of solution may well vary erratically. This will in turn lead to unpredictable and possibly significant variations in the free energy of activation of the studied mutarotation reactions, which could also lead to deviations from linearity in free energy relationships.

Summary The studies reported in this thesis have amongst other things shown that the changes in free energy of activation for the mutarotation of a series of sugars cannot be factorised simply into polar and steric potential energy terms. Several possible reasons for this have been discussed in this section and perhaps the most likely explanations of the failure of linear free energy relationships are those outlined in (c) and (d) above. After the studies carried out in this thesis one tends to have sympathy with Arnett's view that

it is hazardous to draw conclusions from small differences in rate or equilibrium constants as to the inductive, steric and resonance contributions to these rate differences. Had the free energy relationships studied in this work proved to be rigorously linear, one might well have overlooked the pitfalls in dividing small reactivity differences into stereoelectronic factors whose theoretical development applies to the gas phase at absolute zero.

4.8. Intramolecular Catalysis and Mutarotation

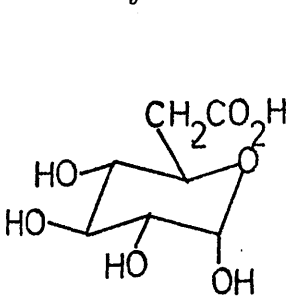
The field of intramolecular catalysis is a widely studied one, because of the large rate enhancements which are very often found in intramolecularly catalysed reactions and the analogy which is often drawn between such rate enhancements and the much larger rate enhancements observed in enzymic catalysis. As with intermolecular catalysis, nucleophilic, electrophilic and basic catalysis are possible, the most frequently encountered mode of catalysis being the first named. Much of the earlier work on intramolecular catalysis has been reviewed by Capon[170].

Many reactions have been shown to be intramolecularly catalysed. The hydrolysis of glycosides[171], acetals[171, 172]., esters[173, 174] and amides[173], substitution reactions at saturated carbon[170] and many solvolysis reactions[170] are among the examples of such reactions. Until recently there was no reported case of intramolecular catalysis of mutarotation.

However the observation of Salas et. al[175] that glucose-6-phosphate undergoes spontaneous mutarotation over 100 times faster than glucose

itself, prompted Bailey et. al.[32] to investigate the mutarotation of glucose-6-phosphate more closely. They confirmed the earlier observation by finding that the mutarotation of glucose-6-phosphate was 240 times faster than that of glucose under the same conditions. Further studies led them to postulate that this rate enhancement was due to intramolecular general base catalysis of the mutarotation reaction by the phosphate group on C-6. Although the most common form of intramolecular catalysis is nucleophilic catalysis some examples of intramolecular general base catalysis have been reported.[176-179] In the studies reported in this thesis the catalytic groups participating in intramolecular general base catalysis are the carboxy-group and a phenolic hydroxy-group.

It was therefore considered that by suitable choice of substituent in the 5 position of the sugars of series I, one might be able to confirm or reject the postulate of intramolecular general base catalysis in the mutarotation reaction. The compounds 8 and 9 were synthesised and the kinetics of their mutarotation studied.



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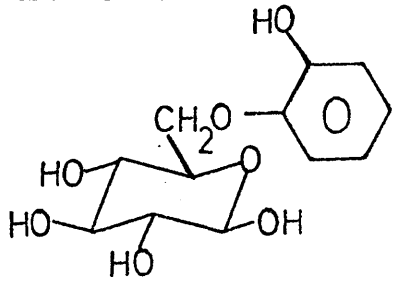

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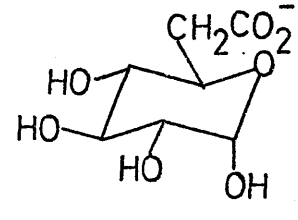


10

Tables 172 - 179 give the results for the mutarotation of 8 in a series of formate, acetate and pyridine buffers in the pH range

2 - 6. The results were extrapolated to zero buffer concentration and the resultant rate constants for spontaneous mutarotation were plotted against the pH of the buffer. The result as can be seen from Fig. 9 is a sigmoidal pH rate profile (data in table 180).

Now it is to be expected on purely inductive grounds that the spontaneous rate of mutarotation of the ionised form of 8 (i.e. compound 10) would be greater than that of the unionised form (i.e. compound 8) since the inductive substituent constant of the substituent - $CH_2Co_2^-$ is + 0.01 while the corresponding value for the unionised substituent $-CH_2CO_2H$ must be approximately 0.17 (see data of Charton[129]). This decrease in the electronegativity of the substituent as the acid becomes ionised would lead us to expect (by interpolation in table 11) an approximate rate constant for spontaneous mutarotation of 6×10^{-4}, for the fully ionised acid. The observed value of about 4.5×10^{-3} suggests that there may be a moderate contribution to the spontaneous rate from intramolecular general base catalysis of the reaction by the carboxylate group.

The kinetics of the spontaneous mutarotation of 8 can be expressed by :-

$$k_{obs.}\ (S_t) = k_{SH}\ (SH) + k_s(S) \quad \ldots\ldots\ldots\ldots\ (75)$$

where the rate constant $k_{obs.}$ refers to the observed rate of spontaneous mutarotation of the sugar, k_{SH} = rate constant for spontaneous mutarotation of the unionised form of the sugar which is denoted by SH, and k_s = rate constant for the spontaneous mutarotation of the ionised form of

the sugar which is denoted by S, and S_t = total sugar concentration.

Now $(S_t) = (SH) + (S)$ ..... (76)

Combining (75) and (76) gives

$$k_{obs.}\ (\ (SH) + (S)\ ) = k_{SH}\ (SH) + k_s\ (S)$$

$$\therefore k_{obs.} = \left\{k_{SH} \cdot \frac{(H^+)}{K_a} + k_s\right\} / \left\{1 + \frac{(H^+)}{K_a}\right\} \quad \ldots\ldots\ldots\ (77)$$

where K_a = dissociation constant of the acid SH.

i.e. $k_{obs.} = (\ (k_{SH} \cdot 10^{-pH}/K_a) + k_s) / (1 + 10^{-pH}/K_a)$ ..... (78)

The observed rate data were fitted to equation (78) by a generalised least squares procedure whence the best fit of the data was given when

$k_{SH} = 2.48 \times 10^{-4}$ sec^{-1} and $k_s = 4.49 \times 10^{-3}$ sec^{-1}

$K_a = 5.08 \times 10^{-5}$ (approximate experimentally determined $K_a = 4.0 \times 10^{-5}$)

Hence the solid line shown in Fig. 9 represents the equation

$$k_{obs} = ((2.48 \times 10^{-4} \cdot 10^{-pH}/5.08 \times 10^{-5}) + 4.49 \times 10^{-3})/(1+10^{-pH}/5.08 \times 10^{-5}) \quad \ldots\ldots\ldots\ (79)$$

The value of the k_{SH} is just under twice the rate constant for the spontaneous mutarotation of 6-cyano-6-deoxy-D-glucose (σ = 0.18, see table 11 where $k_{H_2O}(H_2O)$ for 6-cyano-6-deoxy-D-glucose = 1.34×10^{-4}). The value of k_s is about eleven times greater than the rate constant for the spontaneous mutarotation of glucose (σ = 0.05, table 11 shows $k_{H_2O}(H_2O) = 4.00 \times 10^{-4}$) and five times greater than the same figure for the spontaneous mutarotation of 6-deoxy-D-glucose (σ = 0.05, $k_{H_2O}(H_2O) = 9.09 \times 10^{-4}$ sec^{-1} (from table 11)).

It is often difficult to calculate the magnitude of the intramolecular catalysis, i.e. to compare the rate of the intramolecular

reaction with the rate of the equivalent intermolecular reaction. One of the most common ways of comparing the inter and intramolecular reactions is to compare the first-order rate constant for the intramolecular reaction (say k_1), with the second-order rate constant (say k_2) for the intermolecular reaction catalysed by the equivalent catalyst to that acting in the intramolecular reaction.

Then k_1/k_2 gives the hypothetical concentration of catalyst which would be required in the intermolecular reaction to give a pseudo first-order rate constant equal to the measured first-order rate constant of the intramolecular reaction. These hypothetical concentrations (or "effective molarities") are often very high and in practice unattainable.

To try to calculate the extent of the intramolecular catalysis occurring in both the unionised and ionised forms of 6-deoxy-D-glucoheptturonic acid we require the second order rate constant for the mutarotation of the species 8 catalysed by an acid (closely akin to acetic acid) with a pK_a of 4.3 ($K_a = 5.08 \times 10^{-5}$), and the second order rate constant for the mutarotation of the species 10 by the conjugate base of such an acid. We can only estimate these, but the estimates are of reasonable accuracy and will give a good approximation to the "effective molarity" of the carboxy-group in the intramolecular catalysis observed in 6-deoxy-D-glucoheptturonic acid.

From Schmid's work[29] we have that the second order rate constants for the acetic acid and formic acid catalysed mutarotation of glucose at 25° are 1.23×10^{-4} and 2.96×10^{-4} M^{-1} sec^{-1} respectively. Thus

the corresponding figure for an acid of pK_a = 4.3 would be 2.0×10^{-4} M^{-1} sec^{-1}. Due to the increased electronegativity of the $-CH_2CO_2H$ group compared with the $-CH_2OH$ group of glucose and the observed trend for the catalytic coefficients for acid catalysis as the electronegativity of the 5-substituent changes (see table 11), a second order rate constant of approximately 0.75×10^{-4} M^{-1} sec^{-1} for the catalysis of the mutarotation of 8 by an acid of pK_a 4.3 is reasonable.

∴ The effective molarity of the unionised carboxy-group in 8 is given by $k_{SH}/0.75 \times 10^{-4}$ M $= 2.48 \times 10^{-4}/0.75 \times 10^{-4}$ M

$\approx$ 3M.

Similarly the data of Schmid give the second order rate constants for the acetate and formate ion catalysed mutarotation of glucose at 25° as 1.48×10^{-3} and 8.63×10^{-4} M^{-1} sec^{-1} respectively. Thus the corresponding rate constant for catalysis by the conjugate base of an acid with pK_a = 4.3 is 1.2×10^{-3} M^{-1} sec^{-1}. Since the electronegativity of the $-CH_2OH$ group and the $-CH_2CO_2^-$ group of the ionised acid are similar this rate constant will be in fairly close agreement with the required rate constant for the catalysis of the mutarotation of the ionised form of 6-deoxy-D-glucohepturonic acid (i.e. 10) by the conjugate base of an acid of pK_a = 4.3.

∴ The effective molarity of the ionised carboxy-group in the mutarotation of 10 is given by $k_s/1.2 \times 10^{-3}$ M

i.e. effective molarity = $4.49 \times 10^{-3}/1.2 \times 10^{-3}$ M

$\approx$ 4M.

Thus in both species 8 and 10 the degree of intramolecular catalysis

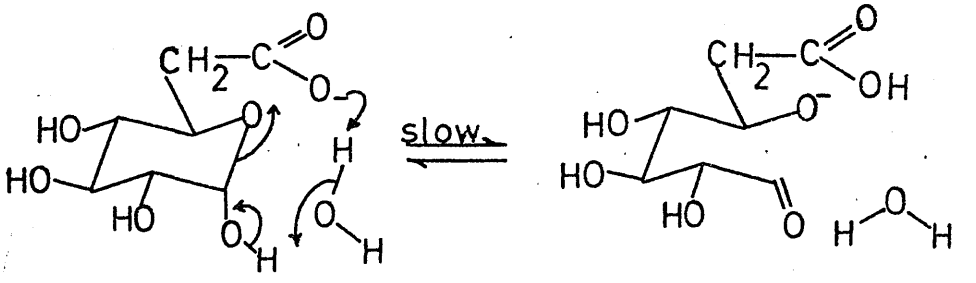
is represented by an "effective molarity" of the catalyst of about 3-4M.

Similar considerations by Bailey et. al.[32] gave a figure of 2.2M for the intramolecular catalysis observed in glucose-6-phosphate. These "effective molarities" are very much smaller than those often observed in intramolecular reactions,[182] and is indicative of fairly moderate intramolecular catalysis. That the mutarotation of glucose-6-phosphate is too fast for polarimetric measurement whereas that of 6-deoxy-D-glucohepturonic acid is not, is a result of the relative base strengths of the phosphate ion and the carboxylate ion.

Thus it would appear there is evidence of intramolecular general acid and general base catalysis in the mutarotation of 6-deoxy-D-glucohepturonic acid to support that postulated by Bailey et. al.[32] in the mutarotation of glucose-6-phosphate. However some doubt must exist as to the detailed mechanism of such catalysis.

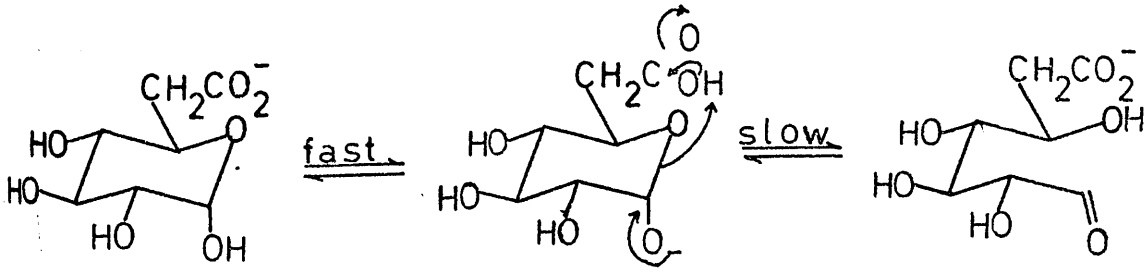
Bailey and coworkers claim that in a scale model of glucose-6-phosphate, "the freely rotating esterified primary hydroxy-group at C-6 allows ready contact between the oxygen of the phosphate group and the anomeric hydroxy-group of the glucose molecule whether this be in the α or β configuration". One must have reservations about this statement, particularly if one considers that, if base catalysis occurs by removal of the anomeric hydroxy-group's proton by the base with simultaneous ring opening (i.e. mechanism (12)), then the proton should be removed in a trans-fashion as in 3 (see section 4.5 (iii)) not in a cis-fashion as in 4. Such trans-removal would make it exceedingly

unlikely that the phosphate group on C-6 of glucose-6-phosphate could be in a position to act directly on the anomeric hydroxy-group as a general base. What is absolutely certain is that the ionised carboxy-group in 10, by no stretch of the imagination could be in a position to act as a base directly on the proton of the anomeric hydroxy- group. It is conceivable that if the above remarks regarding the trans-fashion of proton removal are erroneous, then one could visualise the ionised carboxy-group acting as a general base catalyst via a water molecule as in equation (79) in a manner somewhat analogous to the mechanism of aspirin hydrolysis.[177]



(79)

Another, possibly more probable, explanation is that the observed intramolecular general base catalysis of the mutarotation of 10 by the ionised carboxy-group, may in fact be intramolecular general acid catalysis of the mutarotation of the anion 11 by the unionised carboxy-group as depicted in equation (80).



(80)

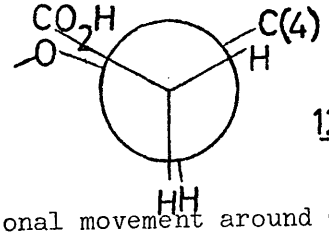
11

This is equivalent of course to mechanism (13) for general base catalysis. Clearly it may also be invoked in the case of glucose-6-phosphate, thereby overcoming any reservations about th ability of the phosphate group to act as a general base directl the anomeric hydroxy-group.

Such a mechanism may also explain the rather moderate valu obtained for the "effective molarities" by the following consid

If the mechanism of intramolecular base catalysis of the mu of 6-deoxy-D-glucohepturonic acid is as depicted in equation (8 it would be expected that the carboxy-group's proton (i.e. in st 11) would sit as close as possible to the ring oxygen of the su since bond formation will occur between these two atoms in the r determining step. The approach of this proton to the ring oxy closest when the sugar is in probably the least favoured confor This is true for three reasons.

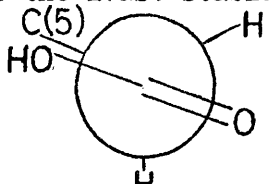
(a) when the carboxy-group's proton is closest to the ri oxygen of the sugar, the conformation around the C(5) - C(6) bon the sugar, is fully eclipsed (see structure 12)



12

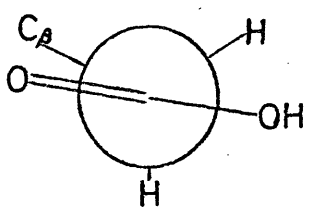
Any rotational movement around C(5) - C(6) to relieve this eclipsed conformation and form a more stable (staggered) confor results in moving the O - H of the carboxy-group further away f ring oxygen atom.

(b) In order that the proton of the carboxy-group may sit anywhere near the ring oxygen at all the conformation around the C(6) - C(7) bond is the least stable fully eclipsed conformation 13



13

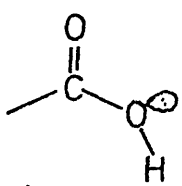
Although there is not a great deal of information concerning the conformation of carboxylic acids, X-ray studies[180] and circular dichroism spectra[181] seem to indicate that the most energetically favourable conformation about the $C(sp^3)$ - $C(sp^2)$ bond of carboxylic acids is that in which the carbonyl group is eclipsed with the β carbon atom as in 14.



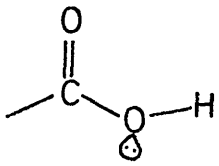
14

If one accepts Pauling's conception of the "banana" bond for a double bond, then 14 is equivalent to the fully staggered conformation about a $C(sp^3)$ - $C(sp^3)$ bond.

(c) the conformation within the carboxy-group itself is of necessity the cis-form 15 rather than the more stable trans-form 16[183,187]



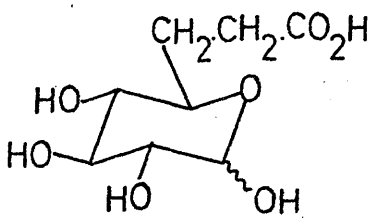
15



16

Thus when one considers these three factors, it is not surprising that the intramolecular catalysis observed is only moderate. It would be most interesting to study the kinetics of spontaneous mutarotation of the compound 17 to see if any increased intramolecular catalysis

could be observed.



(17)

Certainly, inspection of a model of 17 shows that the unfavourable interactions (a) and (b) detailed above, would be eliminated and substantially relieved respectively in compound 17 and hence one might expect greater intramolecular catalysis to exist in the spontaneous mutarotation of 17.

As has already been indicated, the mutarotation of glucose 6-phosphate is too fast for polarimetric measurement due to the strength of the base-phosphate ion HPO_4^{2-}. Thus when the kinetics of mutarotation of 6-O-(o-hydroxyphenyl)-D-glucose, 9, were studied, it came as no surprise that only a small fraction of the pH rate profile could be observed polarimetrically, since the potential intramolecular base catalyst was a phenoxide ion - a much stronger base than even the ion HPO_4^{2-}. Tables 181 - 185 give the results for the HCl, pyridine, 4-methylpyridine and 2,6-lutidine catalysed mutarotation of 9. Extrapolation to zero buffer concentration for each buffer gave the rate constant for the spontaneous mutarotation of 9 at the pH of that buffer. These rate constants for spontaneous mutarotation are listed in table 186 and Fig. 10 shows the pH rate profile of 9 (so far as can be studied) and that of glucose and 6-deoxy-D-glucohepturonic acid (8) for comparison.

Clearly, if the rate enhancement is due to rapid mutarotation of

the ionised form of 9 then the pH-rate profile of 9 will be of the same type as that for 8 with the kinetic expression (75) also describing the kinetics of the spontaneous mutarotation of 9. However due to the strong intramolecular catalysis observed in compound 9 we are only able to study a very small faction of the pH rate profile when the following expression holds $(S_t) \approx (SH)$ where S_t = total concentration of substrate in solution, SH = unionised substrate and S = ionised substrate.

$\therefore$ From (75) $k_{obs}(SH) = k_{SH}(SH) + k_s(S)$ ..... (81)

Now for the sugar equilibrium $SH \rightleftharpoons S + H^+$

$$K_a = \frac{(S)(H^+)}{(SH)}$$ where K_a = dissociation constant of the sugar.

$\therefore$ (81) becomes

$$k_{obs}(SH) = k_{SH}(SH) + k_s\frac{(SH)}{(H^+)} \cdot K_a \quad \text{.....(82)}$$

$$\therefore k_{obs}(SH) = k_{SH}(SH) + k_s(SH).\frac{K_a}{K_w} \cdot (OH^-)$$

$$\text{i.e. } k_{obs} = k_{SH} + k_s.\frac{K_a}{K_w}.(OH^-) \quad \text{..... (83)}$$

Thus the observed data for the spontaneous mutarotation of 6-O-(o-hydroxyphenyl)-D-glucose should obey the rate equation (83). When the data of table 186 are plotted against the concentration of hydroxide ion a linear plot of slope 2.28×10^5 is obtained. This is 2,700 times the value of k_{OH^-} for 6-O-phenyl-D-glucose and is presumably due to the rapid rate of mutarotation of the ionised form of 9.

Therefore $k_s \cdot \frac{K_a}{K_w} = 2.28 \times 10^5$

$$\therefore k_s = \frac{2.28 \times 10^5 \times 10^{-14}}{K_a} \quad sec^{-1}$$

Now the pK_a of 6-O-(o-hydroxyphenyl)-D-glucose was measured spectrophotometrically by the method of Albert and Sergeant[130] and = 9.78 (see table 189) $\therefore K_a = 1.66 \times 10^{-10}$

$$\therefore k_s = \frac{2.28 \times 10^{-9}}{1.66 \times 10^{-10}} \quad sec^{-1} \qquad 14.0 \quad sec^{-1}$$

This is the first-order rate constant for the mutarotation of the ionised form of 6-O-(o-hydroxyphenyl)-D-glucose (9).

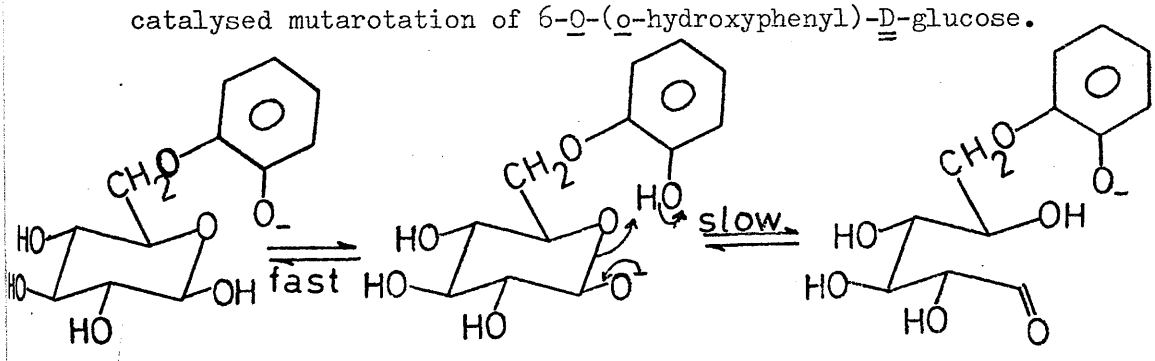
To calculate the effective molarity of the phenolate group in this intramolecular catalysis we require the second-order rate constant for the mutarotation of 9 catalysed by a phenolate base of pK_a 9.78. The closest which we can obtain to this figure is the second-order rate constant for the mutarotation of 6-O-phenyl-D-glucose catalysed by the phenolate ion itself (see table 188) which is 1.24 M^{-1} sec^{-1}. Since the catalytic coefficients for base catalysis of the mutarotation of 6-O-(o-hydroxyphenyl)-D-glucose are slightly lower than those for the mutarotation of 6-O-phenylglucose (cf. tables 182 - 185 with relevant tables for 6-O-phenylglucose) due presumably to the difference in the electronegativity of the groups, and since the phenolate ion is a slightly stronger base than the phenolate ion acting as the catalyst in the intramolecular reaction, then a more accurate second-order rate constant for comparison with the first-order intramolecular rate constant would be 1.0 M^{-1} sec^{-1}.

Hence the effective molarity of the phenolate group in the mutarotation of the ionised form of 9 is approximately equal to $k_s/1.0M$

$\approx$ 14M. (It should be repeated that all the "effective molarities" calculated in this section are approximate, owing to the need to estimate the second-order rate constants for the corresponding intermolecular reaction. However the studies reported in this thesis enable these estimates to be made with a fair degree of confidence, and so the effective molarities calculated for the mutarotation reactions herein reported give a good idea of the effectiveness of the intramolecular catalysis occurring.).

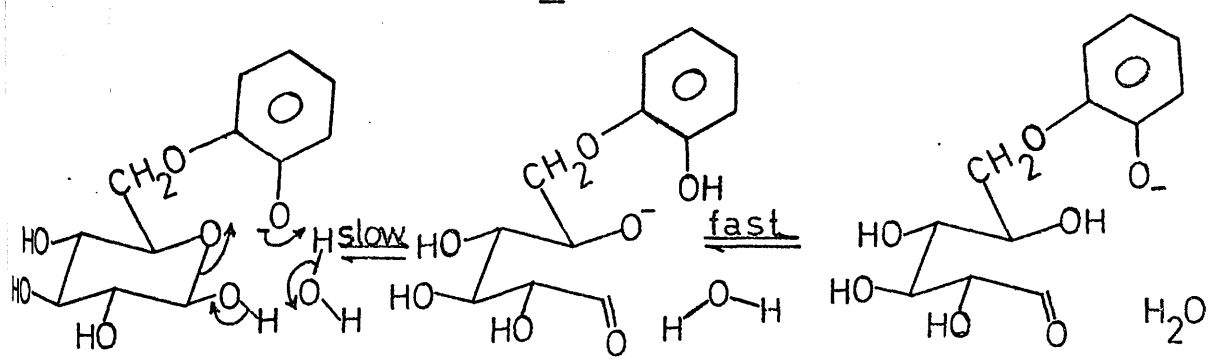
Again, the mechanism for intramolecular general base catalysis can be discussed in terms of analogies to the intermolecular mechanisms (12) and (13) (see sections 1.5 and 4.5).

Equations (84) and (85) illustrate the two types of mechanism (analogous to (12) and (13)) which may describe the intramolecularly catalysed mutarotation of 6-O-(o-hydroxyphenyl)-D-glucose.



(85)

18



(84)

Whereas with the compound 6-deoxy-D-glucohepturonic acid it was necessary if true general base catalysis were the mechanism (i.e. equation (79)) to invoke the presence of a water molecule in the mechanism, with 6-O-(o-hydroxyphenyl)-D-glucose (9) an inspection of models shows that a mechanism such as that depicted in equation (84) may not require a water molecule in the mechanism although it has been included in the illustration. i.e. it may be geometrically possible for the phenolate ion to remove a proton directrly from the anomeric hydroxy-group. However the same objection as before still remains - namely that the intramolecular base (whether via a water molecule or not) cannot be in a position to effect trans-removal of the proton. Therefore again one might prefer mechanism (85) where the rate determining step is in fact general acid catalysis of the sugar anion 18 by the phenol.

The increase in the degree of intramolecular catalysis (as measured by the effective molarities) is significant but disappointing in view of the fact that the unfavourable conformational interactions (a), (b) and (c) outlined previously for 6-deoxy-D-glucohepturonic acid are not present in 6-O-(o-hydroxyphenyl)-D-glucose.

Certainly, from table 187, it can be seen that the observed rate enhancements in the spontaneous mutarotation of 6-deoxy-D-glucohepturonic acid (8) and 6-O-(o-hydroxyphenyl)-D-glucose are not due to intermolecular catalysis by the sugar but appear to be examples of intramolecular general base catalysis in mutarotation thus confirming the first reported example of such catalysis[32] - namely the mutarotation of

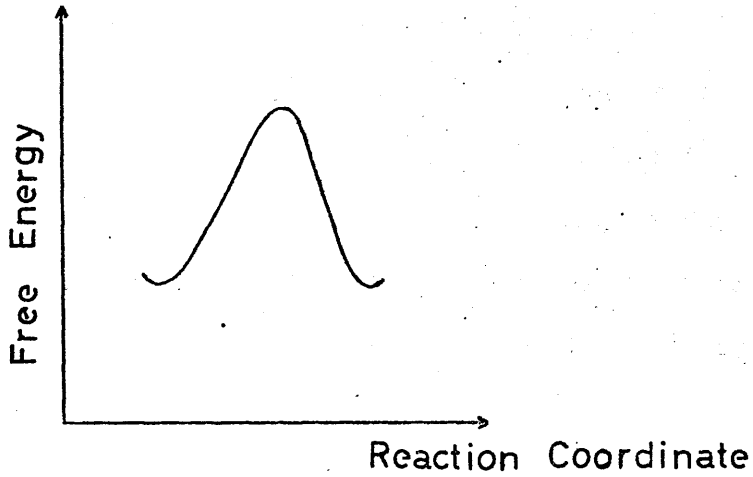
glucose-6-phosphate.

4.9. "Tautomeric Catalysis" and Mutarotation

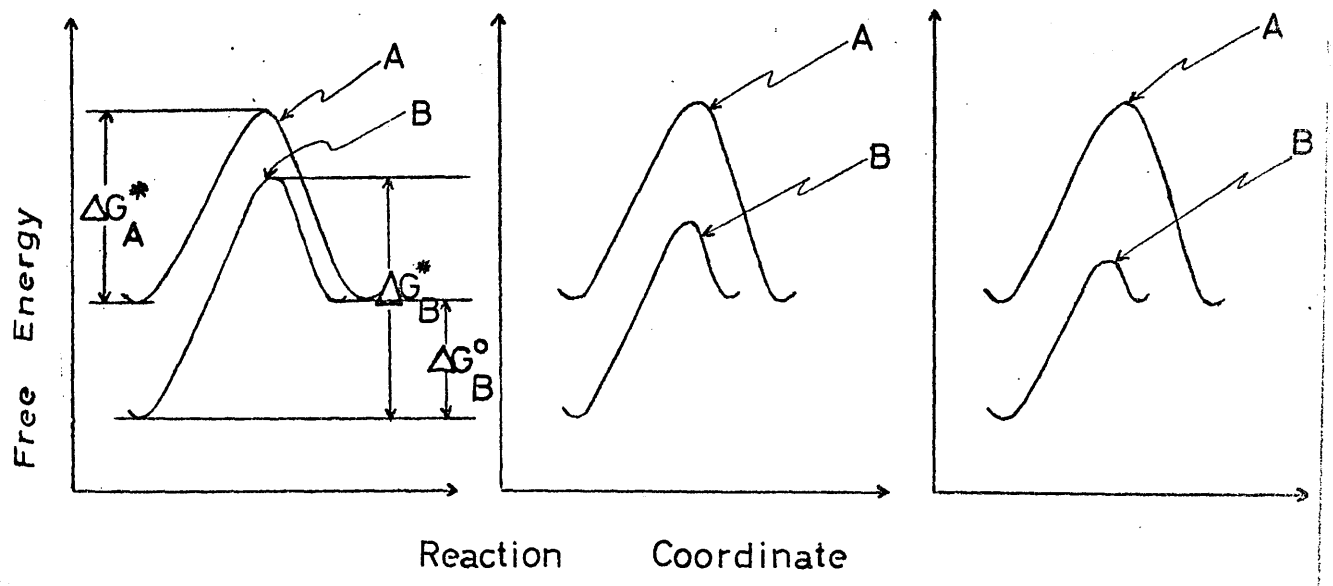
In section 1.3 the controversy over whether concerted general acid - general base catalysis existed in mutarotation reactions was discussed. It was pointed out that the most convincing examples of such catalysis appeared to exist in non-aqueous solution, in particular in the classical work of Swain and Brown[40,41]. Their work on the catalysis of the mutarotation of 2,3,4,6-tetramethyl-α-D-glucose by 2-pyridone in dry benzene first established the theory of concerted general acid-base catalysis which continues to enjoy considerable popularity[184-186].

However as outlined in section 1.3 several authors have disagreed with the concept of concerted general acid-base catalysis and recently Rony[50] has proposed that catalysts which exhibit bifunctional catalysis do so not because they can act as concerted general acid-base catalysts but because they are all tautomeric catalysts which derive their catalytic power from their ability to exchange two protons without forming high-energy dipolar ions. If this concept of tautomeric catalysis is valid, then one should be able to correlate the effectiveness of a catalyst with the free energy of activation of tautomerism of the molecule. Unfortunately little is known about the free energies of activation of tautomerism. Nearly all the data in the literature pertains to the equilibrium proportions of tautomeric mixtures in solutions i.e. the known data relate to the relative standard free energies of the tautomers.

Consider a tautomeric catalyst A, where at equilibrium both tautomers are present in equal proportions in solution. Thus the energy profile for the tautomerisation of A is



Now consider a closely related tautomeric catalyst B, where at equilibrium the tautomeric composition in solution is 99:1. For the sake of simpler illustration let the less stable isomer have the same free energy as the tautomers of compound A. Then there will exist three relative free energy profiles for tautomeric catalysts A and B.



In Fig. 11 (i) $\Delta G^*_B > \Delta G^*_A$

" " 11 (ii) $\Delta G^*_B = \Delta G^*_A$

" " 11 (iii) $\Delta G^*_B < \Delta G^*_A$

where ΔG^* = free energy of activation for relevant catalyst. If Fig. 11(i) represents the true situation then catalyst B will be a poorer catalyst than catalyst A, if Fig. 11(ii) were correct catalyst B would have the same catalytic power as A, and if Fig. 11(iii) were correct catalyst B would be expected to be a better catalyst than catalyst A.

On the assumption that Fig. 11(i) represented the most likely situation and that a favourable change in the free energy difference in the tautomeric forms of a catalyst would reflect itself also in a favourable change in the free energies of activation of the tautomerism, a search was made to find catalysts as similar as possible to 2-pyridone which had a lower ΔG^o in a suitable non aqueous solution. 2-pyridone is well known to exist premominantly as the "keto-tautomer" under nearly all conditions.[190] In terms of figure 11, we were looking for a catalyst which approached catalyst A, rather than B which might well represent the molecule 2-pyridone $\rightleftharpoons$ 2-hydroxypyridine.

A recent paper of Spinner and Yeoh[191] on pyridone-pyridol tautomerism in substituted 2-hydroxypyridines, suggested that the compound 2-hydroxy-6-methoxypyridine although existing predominantly as the pyridone in aqueous solution, existed very much more as both tautomers in non aqueous solvents. As the polarity of the solvent decreased the percentage of the pyridol form increased dramatically.

This does not occur with the compound 2-pyridone itself. Molecular orbital calculations of Dewar[192] have also indicated that the free energy difference between the tautomers of isoquinol-3(2H)-one is considerably less than that between the tautomers of 2-pyridone.

Thus if the assumption regarding Fig. 11 (i) is correct and Rony's theory as to the mode of catalysis being tautomeric catalysis is correct, then one would expect 2-hydroxy-6-methoxypyridine and isoquinol-3-ones to be better catalysts than 2-pyridone in the mutarotation of 2,3,4,6-tetramethylglucose in non aqueous solvents.

2-hydroxy-6-methoxypyridine was prepared from commercially obtained 2,6-dihydroxypyridinium hydrogen sulphate by Spinner's procedure[191], and a sample of 6,7-dimethoxy-isoquinol-3(2H)-one[193] was kindly donated to us by Dr. N.J. McCorkindale of this department.

Unfortunately, the solubility of this latter compound is poor, and studies with it were thus very restricted. The results listed in tables 223 and 224 show the observed rate constants for the mutarotation of 2,3,4,6-tetramethylglucose in dry benzene and dioxan respectively, catalysed by 2-pyridone and 6-methoxy-2-pyridone. The concentration of the sugar was kept constant throughout since it has been shown[194] that k_{obsd} varies with sugar concentration.

Dioxan was used as a solvent for these studies (in addition to the more commonly used benzene) since Spinner[191] had determined the tautomeric proportions of 6-methoxy-2-pyridone in this solvent but had not done so for benzene. Of all the solvents used in Spinner's work the solvent with the closest dielectric constant to benzene was

dioxan. Indeed in dioxan, 6-methoxy-2-pyridone, according to Spinner, exists in the pyridol form to the extent of about 95%. No attempt has been made, with such scanty data, to analyse the kinetics of the systems, kinetics which are fairly complex.[194] A mere comparison of the effectiveness of the two catalysts was all that could be obtained in the studies reported here.

As can be seen from tables 223 and 224, there is very little evidence indeed to suggest that 6-methoxy-2-pyridone is any more effective a catalyst for the mutarotation of tetramethylglucose either in benzene or in dioxan. In general the observed rates for the mutarotations catalysed by 6-methoxy-2-pyridone are lower than the corresponding mutarotations catalysed by 2-pyridone.

This could be due to the fact that Rony's theory, i.e. that it is the ability to tautomerise which gives catalysts such as 2-pyridone and 6-methoxy-2-pyridone their large catalytic activity, may be erroneous, or because the assumption that a favourable change in the free energy differences of the tautomers of such a molecule will reflect itself in a favourable change in the free energies of activation of tautomerism (i.e. Fig 11(i)) may be erroneous.

Clearly there is scope for future study here. A much more definitive test of the hypothesis of tautomeric catalysis could be made if evidence as to the relative free energies of activation of tautomerism of 2-pyridone and 6-methoxy-2-pyridone could be obtained - perhaps by N.M.R. or relaxation spectrometry studies. Indeed one can also regard the pyridone catalysed mutarotation of tetramethylglucose

as the tetramethylglucose catalysed tautomerism of 2-pyridone, and if one could find a method for following the kinetics of tautomerism of such molecules, then the kinetic results obtained should exactly parallel those obtained for the relevant mutarotation studies.

One interesting fact which emerges from the data of Tables 223 and 224 is that the rate enhancement in the 2-pyridone catalysed mutarotation of tetramethylglucose in dioxan over the corresponding pyridine/phenol catalysed reaction is considerably greater than that observed by Swain and Brown in benzene solution.[41] These authors found that 2-pyridone in 0.100M concentration gave an observed rate of mutarotation of tetramethylglucose more than twenty-two times the total rate with a mixture of 0.100M pyridine and 0.100M phenol. At concentrations of 0.05M for the catalysts, this rate enhancement rose to fifty while at concentrations of 0.001M, the observed rate enhancement is seven thousand.

From table 224 we can see that 2-pyridone in 0.100M concentration in dioxan gives an observed rate of mutarotation of tetramethylglucose of between 2000 and 3000 times more than the total rate with a mixture of 0.100M pyridine and 0.100M phenol. This is about two orders of magnitude greater than the corresponding rate enhancement in benzene solution. Due to the exceedingly low rate of mutarotation in pyridine/phenol catalysed reactions in dioxan it is not possible to calculate the rate enhancement at lower concentrations (e.g. 0.001M), when the enhancement would be expected to be much greater.

(N.B. - It should perhaps be pointed out that although the foregoing

observations, such as they are, do not provide any evidence to support Rony's theory of tautomeric catalysis, they also do not support the more accepted postulate of general acid-base catalysis. The effect of substitution of the 6-methoxy-group into 2-pyridine is to make it a three hundred fold stronger acid and a three fold stronger base (6-methoxy-2-pyridone :- pK_1 = 1.14, pK_2 = 9.47 (ref. 195); 2-pyridone :- pK_1 = 1.25, pK_2 = 11.99 (ref. 41)) and one might therefore expect on this basis that 6-methoxy-2-pyridone should be a slightly better catalyst than 2-pyridone. This might indicate that the ability of the catalyst to form a catalyst-substrate complex - which is thought to occur with 2-pyridone and tetramethylglucose[194] - also plays an important part in determining the relative catalytic activity of two catalysts).

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Appendix

Appendix

5.1 The understanding of enzyme action is as yet an unclimbed peak in the range of "biochemical mountains" currently under assault by biochemists and chemists alike. A tremendous amount of effort and activity is currently being directed towards the elucidation of the mechanism of enzyme action, principally because of the importance attached to enzymes as catalysts of biochemical reactions in vivo, and also because of the great efficiency with which they do catalyse chemical and biochemical reactions. Typically, enzymically catalysed reactions are often nine or ten powers of ten more efficient than the corresponding acid or base catalysed reactions. Of almost equal interest is the extraordinary specificity which many enzymes exhibit.

Several theories of enzyme action have been proposed. Theories such as the proximity effect[1,4,61], general acid or general base catalysis[2], concerted general acid-base catalysis[3], orientation of catalytic groups[4], rack and strain effects[5,1] and others[1] have all been suggested as possible explanations of enzymic catalytic efficiency. However as Koshland[1] has pointed out, none of these theories alone, nor even when the effects are summed together, can account for the efficiency of enzymic catalysis. More recently concepts such as orbital steering[6] and stereopopulation control[7] have been proposed to account for the phenomenon but these have been criticised by various authors.[8,17,28] Interestingly, Page[8] has recently

pointed out that standard physical organic principles can explain a large fraction of enzymic rate accelerations "by virtue of an enzyme's ability to utilise substrate binding forces to act as an 'entropy trap'". It will indeed be encouraging for physical organic chemists, if, as seems more and more likely, the catalytic power of enzymes - often termed "miraculous" - can eventually be completely defined within the classical principles of physical organic chemistry.

5.2 Ribonuclease

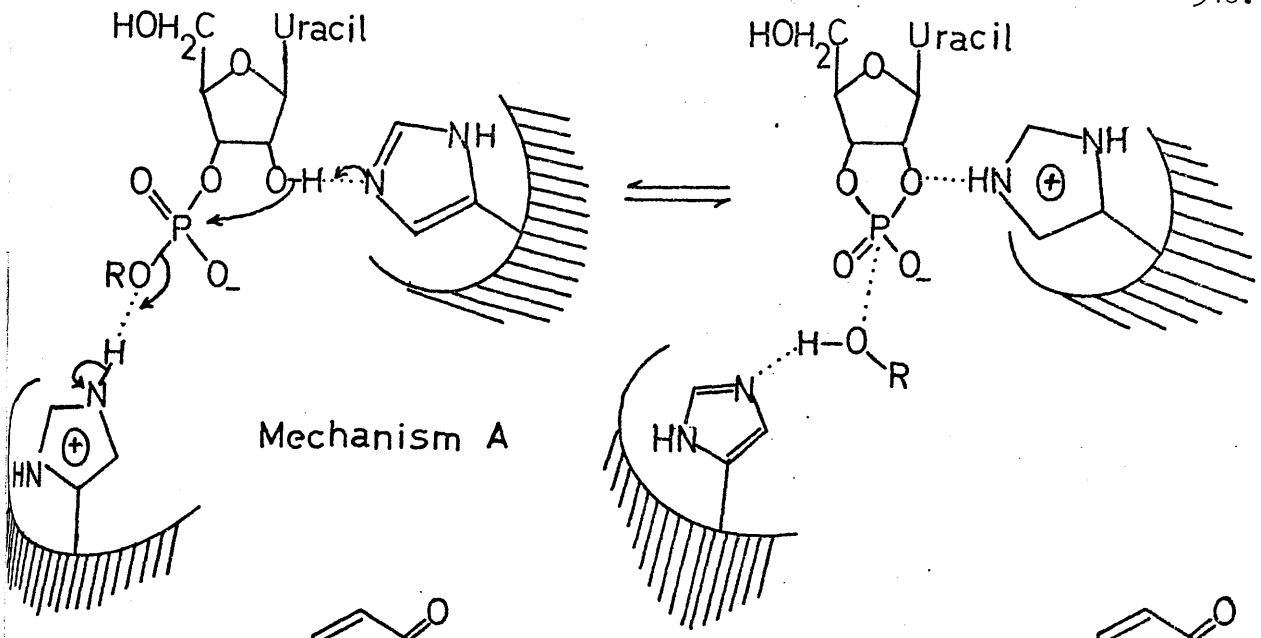
One of the most extensively studied enzymes is bovine pancreatic ribonuclease A, and several reviews have described much of the work on it[9-13]. Ribonuclease (RNase) A is a phosphotransferase of a much larger class of enzymes - the RNA depolymerases - which have recently been reviewed by Barnard[14]. Its unusually low molecular weight, easy availability and the lack of cofactor requirements have led to the determination of its amino-acid sequence,[15-17] the accumulation of evidence converning the active site,[18-24] and most significantly the elucidation of the complete three dimensional conformation of the enzyme by X-ray structural studies.[25, 26] Ribonuclease has thus provided one of the first examples of an enzyme where the indirect methods of determining the active site structure and protein structure have been corroborated fully by the direct determination of the enzyme structure by X-ray analysis. Obviously if enzyme kinetics are to be really meaningful, it is most desirable to have the three dimensional structure of the enzyme. Ribonuclease is also a landmark in the field

of enzyme chemistry since it was the first, and at the time of writing the only, enzyme to be fully synthesised by chemists[29,30].

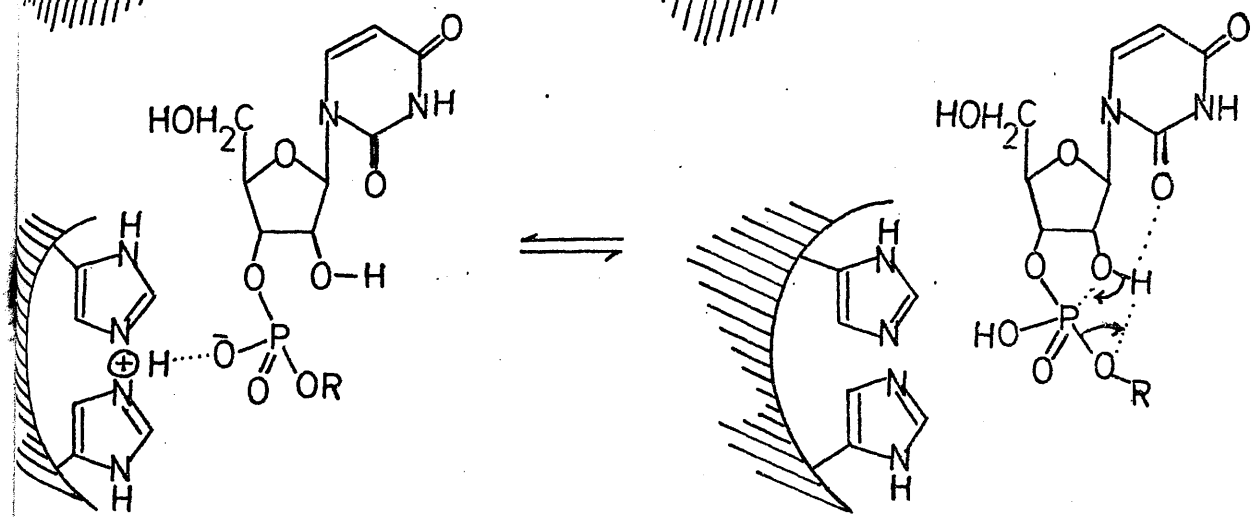
During the course of the very large amount of work on ribonuclease several mechanisms have been proposed.[31-37] A brief summary of the proposals so far will serve as a useful background to consider some of the objectives of this piece of work:-

5.3 Mechanism of Ribonuclease

Certain features were universally accepted regarding the broader aspects of the mechanism. The hydrolysis of the 3',5'-phosphodiester linkage of RNA was recognised to proceed in two stages, the first being a transphosphorylation to give an oligonucleotide terminating in a 2',3'-cyclic phosphate. The second stage was the hydrolysis of this cyclic phosphate to give the product - a terminal 3'-phosphate. Also recognised were the enzyme's specificity in only hydrolysing 3'-pyrimidine nucleoside phosphodiester linkages and the implication of histidine-12, histidine-119 and lysine-41 as groups involved in the catalytic step at the active site. However considerable differences arose in the proposals for how this two stage reaction was actually accomplished by the enzyme. The two early proposals by Rabin[31] and Witzel[32] differ radically and are depicted schematically in Fig.1. As can be seen, these mechanisms differ on several points. In Rabin's mechanism the imidazole moieties of histidine-12 and histidine-119 are used as catalytic species in a concerted general acid - general



Mechanism A



Mechanism B

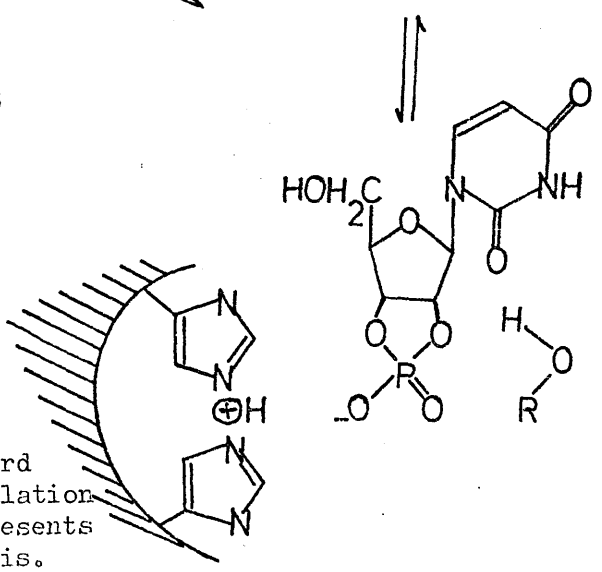


Fig.1.

Mechanisms proposed for RNase by Rabin (A) and Witzel (B). R represents a 5' - linked nucleoside and the forward reaction represents the transphosphorylation step. The reverse action (R now represents H) is the second stage of the hydrolysis.

base mechanism, while in Witzel's mechanism the imidazole groups are utilised only for binding the substrate to the enzyme. Rabin postulates that there are binding sites for the bases on either side of the phosphodiester grouping while Witzel maintains that the function of the pyrimidine base is to act specifically as a basic catalyst.

Subsequent mechanisms have tended to be modifications of either of these two or hybrids of them. Laidler's[33] mechanism is really a hybrid of Witzel's and Rabin's original suggestions, in which the imidazole groups of histidine-12 act to bind the substrate and not to act as a catalytic species. The carbonyl group of the pyrimidine ring is again held to take part in the catalytic step in conjunction with the imidazole group of histidine-119.

Hammes'[34] mechanism is a modification of Rabin's mechanism, in which he proposes that one of the imidazole groups is used only for binding the substrate, while the other imidazole group performs all the catalytic functions - acting first as a general base in removal of the proton from the 2'-OH and also as a general acid in protonating the leaving group. It will also act as a general base to water in the second stage. The pyrimidine base plays no part in the catalytic step.

Later workers[35,37] have tried to rationalise the original mechanisms with the additional geometrical restrictions imposed by the mechanism for phosphate ester hydrolysis proposed by Westheimer[38].

The mechanism of Roberts et al. is really a modification of Rabin's mechanism, the main difference being that Roberts postulates that the imidazole of histidine-119 acts principally to bind the phosphate group of the substrate rather than solely acting as a general acid catalyst as Rabin suggests. Roberts postulates a possible dual role for the histidine-119, since he maintains that it can simultaneously act as a general acid catalyst protonating the alkoxy-leaving group.

Whether this proton transfer is concerted with the departure of the -OR group is doubtful, and in my opinion unlikely to be so. This infers that the leaving group would be expelled from the pentavalent intermediate as the anion RO^-. Roberts also points out that two mechanisms are possible; one in which attack by the 2'-hydroxy-group is linear with expulsion of the leaving group (Fig.2A) and one in which pseudorotation of the pentacovalent intermediate is necessary before expulsion of the leaving group takes place (Fig. 2B). This latter mechanism according to Westheimer's "rules"[38] will be unfavoured and this is the type of mechanism necessary to rationalise Hammes' suggestion that one histidine group carried out all the catalytic functions. Indeed most of the current theories on the mechanism of ribonuclease require this pseudorotation mechanism. Usher[37] calls this an "adjacent" mechanism whereas he uses the term "in line" in place of the "linear" mechanism of Roberts. Clearly any mechanism involving an intermediate in which pseudorotation must occur

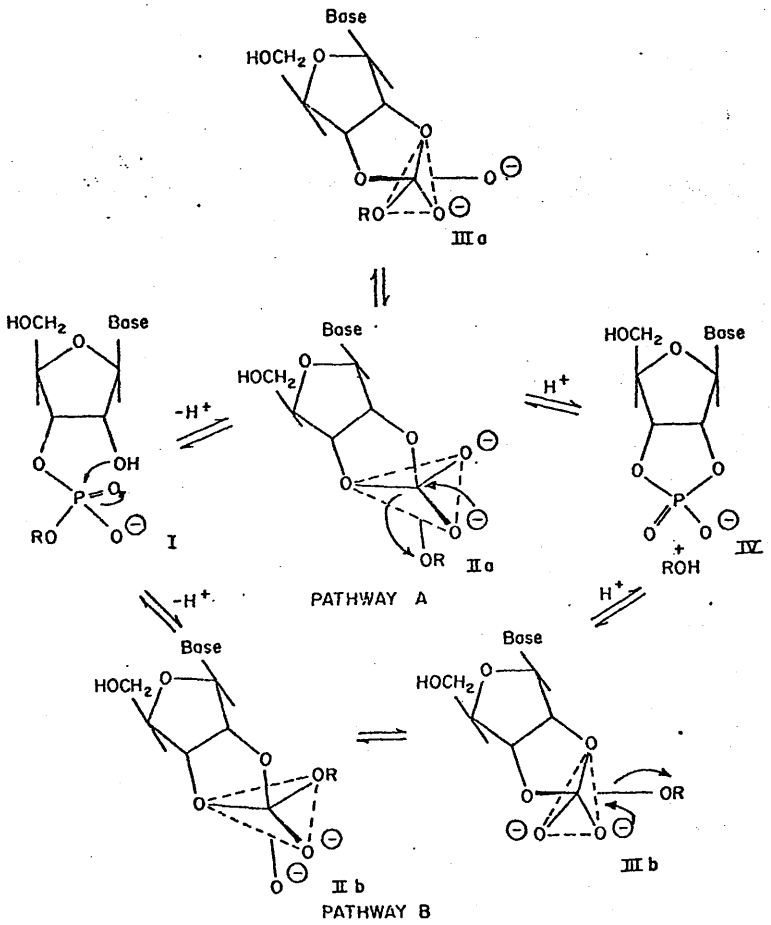
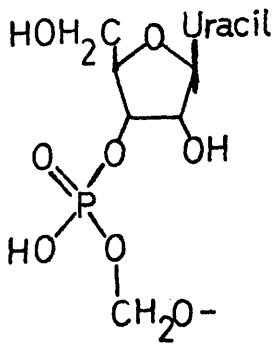


Fig.2 - Possible mechanisms for the reaction catalyzed by RNase. The second step is the reverse of the reactions shown, with R = H. In the pentacovalent intermediates, the phosphorus atom in the centre of the trigonal bipyramid is not specifically lettered.

before the leaving group is expelled must be considered doubtful until evidence has been found that the rate of pseudorotation of a pentaoxyphosphorane is high enough to satisfy the observed enzymic rate. There is only a little information available on this subject at the moment,[37] but it is clear that slowly, bit by bit, the picture of the mechanism of ribonuclease action is becoming more complete.

5.4 Objectives

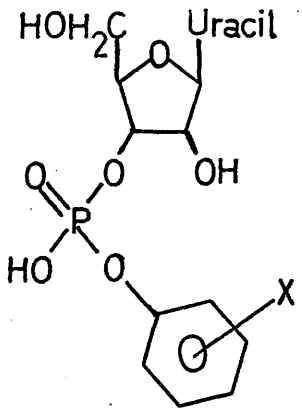
Peculiarly enough amongst all the work on ribonuclease A, no detailed kinetic investigation of the effect of substrate structure on the enzymic reaction has been studied. Indeed only one model reaction has been analysed in detail.[39] In this paper Usher draws attention to the importance of knowing whether the leaving group of a phosphodiester such as (1) is protonated or not in the reaction in which attack by the alkoxide ion takes place to form the cyclic phosphate product.



(1)

Much of the current controversy about the mechanism of rubonuclease action centres on the possible existence of a pentacoordinate intermediate which can form, pseudorotate if necessary and breakdown fast enough to satisfy the overall rate of enzyme action.

It seemed therefore that if one could synthesise a series of compounds of structure (2), a considerable amount of information could be derived from the acid catalysed, base catalysed and enzyme catalysed hydrolysis of these substrates - information which might shed some light on the possibilities of pseudorotation in ribonuclease action.



(2)

(a) X= p-NO_2

It should certainly shed some light on the state of protonation of the leaving group.

5.5 Nucleotide Synthesis

(a) General Considerations.

One of the principal reasons why so few investigations of this

type have been undertaken is the difficulty in the synthesis of model substrates for ribonucleic acid - the natural substrate for ribonuclease. Clearly dinucleotides which are commercially available now are good substrates, but lack the chromophoric group necessary for spectrophotometric measurement of the reactions. There have been many examples in the literature of the synthesis of alkyl-aryl phosphodiesters,[40,41] but very few examples indeed of synthesis of aryl esters of cis-1,2-diol phosphates.[36,42] The problem caused by the neighbouring hydroxy-group is one which has received a lot of attention[43-45] in the synthesis of oligonucleotides - a field which has received a considerable impetus from the work of Khorana. Clearly one of the critical factors in planning the synthesis of compounds such as (2), is the choice of a protecting group for the 2'-OH and 5'-OH functions. This protecting group must be stable under the reaction conditions of each stage of the synthesis, and must also be removable under conditions which are mild enough to avoid isomerisation or degradation of the desired phosphodiester. In the course of this study several protecting groups were used to protect the hydroxy-functions.

One of the generally recognised methods of synthesis of phosphate diesters is the condensation between an alcohol and a phosphate monoester. The most efficient condensing agents have been found[43] to be the carbodiimides, and in the majority of the present work the reagent of choice was N,N'-dicyclohexylcarbodiimide(DCCI). This is the reagent which

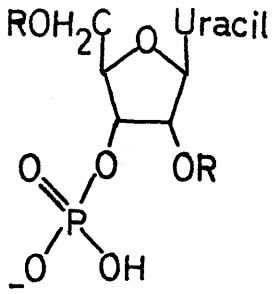
is used extensively in the preparation of oligonucleotides[46] and nucleotide aryl esters[40]. Two general approaches are available. A suitably protected nucleoside can be condensed with an aryl phosphate or a protected nucleotide could be condensed with the corresponding phenol. In this study both these methods were attempted. In addition, attempts were made to phosphorylate a suitably protected nucleoside with various phosphorylating agents.

A useful survey of some of the synthetic developments in this field of chemistry can be found in Khorana's[47] book.

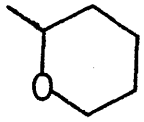
(b) Discussion

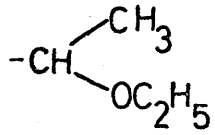
The first approach employed was the condensation between a suitably protected nucleotide and a phenol. It has long been known that in the preparation of phenolic esters of carboxylic acids using DCCI, the presence of a p-nitro group in the aromatic nucleus promotes the formation of the ester.[48] Whether this is due to the p-nitrophenol being a stronger acid than other phenols or to there being a higher concentration of the more nucleophic phenolate species present depends on the mechanism of ester formation.[49] It seemed therefore that the best phenol to use in the condensation reactions would be p-nitrophenol. One of the most widely used protecting groups in oligonucleotide synthesis has been the tetrahydropyranyl group which fulfils the requirements previously listed. Thus the nucleotide 2',5'-di-O-tetrahydropyranyluridine-3'-phosphate (3a) was

prepared by a method similar to a procedure used by Smrt.[43]



(3)

(a) R = 

(b) R = 

The problem was to ensure that the phosphate functional group is exclusively attached to the 3'-position of the ring since ribonuclease will hydrolyse only 3'-phosphodiesters of pyrimidine bases. The customary method of ensuring this is to treat the mixed uridine-2'(3')-phosphate isomers with DCCI to obtain the cyclic phosphate which can then be digested with RNase to give quantitative conversion to the 3'-phosphate. The resulting uridine-3'-phosphate was then treated with dihydropyran in the presence of a catalyst to yield the desired protected nucleotide (3a). It had already been shown that during the pyranylation stage no detectable isomerisation of the 3'-phosphate to the 2'-phosphate had occurred.[43]

It had been noted from previous studies[40] that the p-nitrophenyl esters of nucleotides, as well as showing a λ_{max} at approximately 260 nm in their U.V. spectrum, due to the

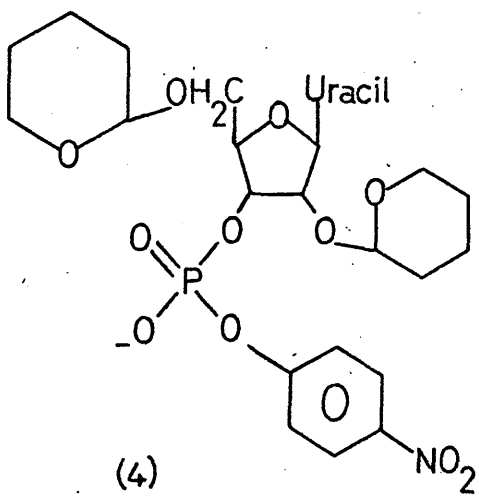
pyrimidine base, also show an inflexion at about 290 nm. This provided a criterion for judging whether in any particular reaction any of the desired p-nitrophenyl esters had been formed.

Condensation of the protected nucleotide (3a) with p-nitrophenol using DCCI in anhydrous pyridine yielded very small quantities of the desired esters, as judged from the U.V. criterion described above.

A typical U.V. spectrum of the crude worked up product is shown in Fig.3. This shows the very small absorption at approximately 300 nm which corresponds to the p-nitrophenyl ester. Clearly the yield of the desired product is very low. Despite many variations in the reaction conditions such as change of solvent,[40] addition of anhydrous cation exchanger, carrying out the reaction in the dark,[40] variations in relative reactant concentrations, change in reacting phenol, and variation of the reaction time, no improvement upon the yield could be obtained and in many cases no p-nitrophenyl ester at all was formed.

To check that the low absorption at 300 nm was indeed due to the formation of a small amount of the desired p-nitrophenyl ester, some hydrolysis studies were carried out on the crude reaction mixture. Fig. 4 represents the observed behaviour of the mixture. It can be seen that on addition of base to the reaction mixture a small amount of p-nitrophenoxide is released (absorption at 400 nm). This most probably arises from traces of p-nitrophenol remaining in the reaction mixture. However if, before base is added, the reaction mixture is

acidified to pH 1.0 with concentrated hydrochloric acid and allowed to sit for ten minutes, then when the reaction mixture is made alkaline with sodium hydroxide there is a large release of p-nitrophenoxide and a disappearance of the absorption at 300 nm which was ascribed to the desired p-nitrophenyl ester. This is precisely what would be expected from a compound of structure (4) since the alkaline hydrolysis of a phosphodiester of such a structure would be expected to be slow,[39] while after removal of the acid labile protecting group R to give a phosphodiester of structure (2), the alkaline hydrolysis would be expected to be very rapid,[39] due to anchimeric assistance from the 2'-hydroxy-group.



(4)

Although the best yield (estimated spectrophotometrically) was about 10%, the crude reaction mixture was chromatographed on a column of diethylaminoethyl cellulose (40 x 3 cm diameter), twice, to yield the desired 2',5'-di-O-tetrahydropyranyl-uridine-3'-p-nitrophenyl phosphate (4) as the triethylammonium salt contaminated with some triethylammonium p-nitrophenoxide. The contaminating

p-nitrophenoxide was removed by ether extraction after acidification to pH 5.5. The U.V. spectrum of the required 2',5'-di-O-tetrahydropyranyl-uridine-3'-p-nitrophenyl phosphate is shown in Fig.5.

The remaining problem was the removal of the tetrahydropyranyl protecting groups which are acid labile. A previous study[50] of the hydrolysis of tetrahydropyranyl derivatives found that 2-methoxy-tetrahydropyran hydrolysed at pH 3.10 with a half life of approximately 50 minutes. When a solution of (4) in water was acidified to approx. pH 2.0 either with hydrochloric acid or by addition of Dowex 50W-X8 ion exchanger, the hydrolysis of the tetrahydropyranyl protecting groups proceeded and was apparently complete after 2.5 hours. The removal of the 2'-tetrahydropyranyl group could be followed by taking aliquots of the reaction mixture at various time intervals, adjusting the pH to 10 with sodium hydroxide and following the release of p-nitrophenoxide at 405 nm and the disappearance of the absorption at 300 nm corresponding to the p-nitrophenyl phosphate ester. However, under the conditions used to remove the protecting groups the resulting product - the ester (2a) - was degraded to uridine-2'(3') phosphate and p-nitrophenol. Evidence for this is shown in Fig.6 where an aliquot removed after 30 minutes at pH 2.0 had its U.V. spectrum measured (a) after extraction with ether and (b) after adjustment to pH 10 subsequent to ether extraction. The significance of these measurements can be seen if one measures the ratio of the absorbance

at λ max to that at 300 nm. Fig.5 shows that in the pure 2',5'-di-O-tetrahydropyranyl uridine-3'-phosphate the ratio is approx. 1.5, whereas in Fig. 6(a) the ratio is 3.0. The change in this ratio could be due to changes in the extinction coefficients of the two absorptions on removal of the protecting groups, but in the author's opinion is almost certainly due to the hydrolysis of the desired product under the reaction conditions to form p-nitrophenol (removed by ether extraction) and uridine-2'(3')-phosphate. Clearly this would increase the ratio as found. Fig.6(b) demonstrates the existence of some of the desired p-nitrophenyl ester of type (2) - i.e. the product is not hydrolysing quite as fast as it is being formed. The addition of base (Fig. 6(b)) results in the release of a quantity of p-nitrophenoxide corresponding to the concentration of the desired product (2a) present in the solution at that time.

The removal of the protecting groups was carried out under varying conditions but the same situation resulted, the only difference being in the time scales which depend on the pH used to remove the tetrahydropyranyl groups. Some very crude kinetics were carried out on the substrate (2a) in the following way :-

If a solution of 2',5'-di-O-tetrahydropyranyl-uridine-3'-p-nitrophenyl phosphate is acidified to pH 0.8 with hydrochloric acid and allowed to sit for 10 minutes, the tetrahydropyranyl groups are completely removed.* A considerable amount of the desired substrate

* checked by addition of potassium hydroxide and subsequent complete disappearance of the absorption at 300 nm.

(2a) which results will also be hydrolysed in this time, but as noted above this hydrolysis appears to be slower in acid media than the removal of the protecting groups. Thus some of the desired substrate (2a) will remain. If after ether extraction, a few microlitres of this solution (pH 1.0) are injected into buffers of various pH's then the kinetics of hydrolysis of substrate (2a) in these buffers can be followed spectrophotometrically.

It was found that at all pH's studied, the substrate hydrolysed in the buffer spontaneously. The results, which are not of high accuracy owing to the method of obtaining the substrate, are shown in Table 1.

The approximate nature of the results in table 1 must be stressed. Several factors combine to lower the accuracy of the results.

Firstly as has been indicated, the uridine-3'-p-nitrophenyl phosphate as prepared in situ will be contaminated with uridine 3'-phosphate resulting from the degradation of the diester. Secondly, the results given in table 1 have not been extrapolated to zero buffer concentration and so the observed rates will depend on the varying concentrations of buffering species which themselves will vary greatly in catalytic strength. Clearly then the quoted half lives can only give an approximate idea of the stability of the uridine-3'-p-nitrophenyl phosphate at the respective pH's.

However the results will give an indication of the extent of

Table 1

The Rates of Hydrolysis of Uridine 3'-p-nitrophenyl Phosphate* at 30°C

Buffer	pH	I	half life
0.2M HCl	0.81	1.0	30 minutes
1.0M chloroacetic	2.02	1.0	160 "
1.0M formic	3.10	1.0	240 "
1.0M acetic	4.28	1.0	500 "
1.0M acetic	4.65	1.0	400 "
0.2M acetic	5.32	1.0	250 "
0.4M Na_2HPO_4) 0.8M NaH_2PO_4)	6.05	2.0	33 "
0.5M Na_2HPO_4) 0.5M NaH_2PO_4)	6.40	2.0	17 "
0.5M Na_2HPO_4) 0.25M NaH_2PO_4)	6.80	2.0	9.5 "
0.2M tris	7.50	1.0	100 seconds
0.4M tris	7.90	1.0	30 "

* prepared in situ as detailed in the text.

hydrolysis of the desired substrate which will occur during the removal of the protecting groups from the 2',5'-di-O-tetrahydropyranyl-uridine-3'-p-nitrophenyl phosphate.

For example, the hydrolysis of tetrahydro-2-methoxypyran[50] has a half life of 50 minutes at pH 3.10 and 30°. Thus hydrolysis will be 99% complete after 6 hours. Reference to table 1 shows that in 1.0M formic acid buffer at pH 3.1, the required substrate would be 65% hydrolysed in six hours.

Tetrahydro-2methoxypyran is not an ideal model for the present system though, and it is interesting to note that the hydrolysis of 2'-O-tetrahydropyranyluridine in 0.01M hydrochloric acid and temperature 20°, has a half life of 80 minutes.[51] (This seems larger than might have been anticipated in the light of our observation (see p. 329) that the hydrolysis of the tetrahydropyranyl protecting groups of (4) are apparently completely removed at pH 2.0 in 2.5 hours).

Thus the protecting group would be removed completely in about 9 hours. Again reference to table 1 shows that at pH 2 in 1.0M chloroacetate buffer the desired product would have hydrolysed to an extent of about 90%. As stated above, our experience is that the tetrahydropyranyl groups are removed from (4) more easily than this but even after 2.5 hours 50% hydrolysis of the desired product would have occurred at pH 2.0.

Thus these studies show how difficult it will be to remove tetrahydropyranyl groups from compound (4) without appreciable hydrolysis

of the resulting desired product.

Table 1 also shows that the compound (4) is susceptible to both acid and base catalysis. From pH 0.8 - pH 4.3 the rate of hydrolysis falls as the concentration of H_3O^+ falls. At pH's above approximately 4.4 the concentration of hydroxide ion becomes significant and the rate of hydrolysis rises rapidly. The hydrolysis of (4) is of course expected to be highly sensitive to base catalysis due to participation of the 2'-hydroxy group.

Throughout the discussion of this first approach, it has been assumed that the compounds isolated have been the required 3'-phosphate diesters which act as substrates for RNase A. It is therefore pleasing to note that when RNase A is added to a solution of substrate (2a) in acetate buffer at pH 4.85, very rapid hydrolysis of the substrate occurs compared with the spontaneous hydrolysis of the substrate in acetate buffer alone. This clearly indicates that our structure assignment to date has been correct and that uridine-3'-p-nitrophenyl-phosphate has been prepared in situ, although the problem of its isolation has not been overcome. That none of the corresponding 2'-isomer was present was shown by carrying out a RNase catalysed hydrolysis at pH 4.85 (acetate buffer). The p-nitrophenol released was extracted thoroughly with ether and the residual aqueous solution made alkaline with potassium hydroxode. No p-nitrophenoxide was released, thus showing that the substrate consisted purely of uridine-3'-p-nitrophenyl phosphate.

Clearly the removal of tetrahydropyranyl protecting groups is

going to lead to some degree of hydrolysis of the desired product, no matter what conditions are used to remove the groups. This will therefore lead to the need of a subsequent purification step to separate the desired phosphate diester from uridine-2'(3')-phosphate. Such techniques as are available for the separation of phosphates - e.g. paper chromatography, DEAE cellulose chromatography, ion exchange chromatography - all involve a lengthy procedure and solvents in which the desired uridine-3'-p-nitrophenyl phosphate is not stable. It would appear therefore that for this particular substrate the tetrahydropyranyl group is not suitable as a protecting group.

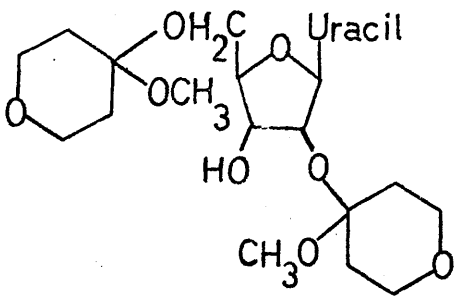
Two alternative remedies can be attempted. Either a phenol with poorer leaving group characteristics (e.g. phenol or 1-napthol) could be used, or a protecting group which is much more acid labile should be used. Both these remedies were attempted. When phenol or 1-napthol were used in condensations with (3a) no detectable phosphate diester was formed (determined as before using U.V. spectra and hydrolytic behaviour). This is perhaps not so surprising in the light of the very low yield obtained with p-nitrophenol. Many changes in reaction conditions (see before) were attempted but proved fruitless.

In their studies Smrt and Chladek[45] found the need of a protecting group which was more easily removed than the tetrahydropyranyl group. They found that the 1-ethoxyethyl group (derived from ethyl vinyl ether was about three times more labile than the tetrahydropyranyl group. Accordingly 2',5'-di-O-(1-ethoxyethyl) uridine-3'-phosphate (3b) was

synthesised and used in condensations as before with p-nitrophenol. However, using nucleotide 3(b) the yields of desired diester were very much poorer than the poor 10% maximum yield obtained using nucleotide 3(a). In many instances the yield was zero. This may well be due to the lability of the 1-ethoxyethyl protecting groups which tended to hydrolyse more easily than was desired. (See experimental). Again many variations of reaction conditions proved fruitless and because of the very low yields of phosphate diesters formed ($\approx$ 1 - 2%) and the difficulty experienced in obtaining nucleotide (3b) pure this synthetic approach was abandoned.

An alternative mode of synthesis would be the reaction of a suitably protected nucleoside and an aryl phosphate. Since tetrahydropyranyl protecting groups had not proved labile enough in the previous approach, it was decided to seek a more labile protecting group. The preparation of 2',5'-di-O-(1-ethoxyethyl) uridine by Smrt's method[45] of enzymatic dephosphorylation of the corresponding nucleotide was not successful, presumably due to impurities in the nucleotide inactivating the enzyme.

However Reese et al. have described[51,52] the use of a methoxytetrahydropyranyl protecting group which is two to three times more labile than the tetrahydropyranyl group. Thus the protected nucleoside 2',5'-di-O-methoxytetrahydropyranyluridine(5) was prepared by Reese's method[51,52].



(5)

Condensations with (5) and p-nitrophenyl phosphate were carried out using methods of both Borden and Smith[40] and Tigerstrom and Smith.[53] The only products isolated in all these condensations were unchanged nucleoside and P^1,P^2-di-p-nitrophenyl pyrophosphate. The problem here is to have the nucleoside in sufficient excess to make it compete effectively against the more nucleophilic phosphate group in the carbodiimide mechanism. Clearly this difficulty has not been overcome. A change of condensing agent to trichloroacetonitrile and change of phosphate to phenyl di-hydrogen phosphate as in Usher's method[39] gave no improvement, probably for the same reason.

The remaining synthetic approach was to attempt to phosphorylate the 3'-hydroxy-group of (5) with other phosphorylating agents. Among those tried were diphenylphosphorochloridate[54], tetra-p-nitrophenyl pyrophosphate[55] and o-phenylene phosphorochloridate.[56] All these reagents when reacted with (5) gave very complex reaction mixtures

on tlc, and even after extensive chromatography no compounds with the characteristics of a nucleotide aryl diester could be detected.

The difficulty experienced in forming a phosphodiester bond in condensations between nucleotides of type (3) and phenols, and also in phosphorylating nucleosides of type (5) was not expected on the basis of previous synthetic studies on oligonucleotides. There is no immediately apparent explanation for the difficulty experienced. In addition these studies have shown that even were condensation successfully achieved, the removal of the protecting groups from compounds of type (4), and the subsequent isolation of the required substrates of type (2) in a pure state, is a procedure which will not readily be achieved.

5.6 Experimental

All U.V. spectra were recorded on a Unicam SP800 spectrophotometer. N.M.R. spectra were measured at 60 MHz with a Varian A-60. Thin layer cellulose chromatography was used routinely as a check on reactions and product homogeneity, and was carried out on 0.25 mm plates using Whatman CC41 microgranular cellulose powder, using solvent A (isopropyl alcohol: ammonia: water - 7:1:2) as eluant. Ion exchange cellulose chromatography was carried out using Whatman microgranular preswollen DE52 diethylaminoethyl cellulose eluted with a linear gradient of water and 0.1M triethyl-ammonium bicarbonate.

Tetrahydro-4-pyrone was prepared from butane-1,3-diol[57]. Uridine 3'-acetate was prepared by the method of Reese et al.[58] using

triethyl orthoacetate instead of the trimethyl analogue. Ribonuclease A was obtained as a lyophilized dry powder of approximate activity - 40 Kunitz units/mg from the Boehringer Corporation (London) Ltd. Uridine-3'(2')-monophosphate was also obtained as the crystalline free acid from Boehringer Ltd. Uridine was obtained from Koch Light Laboratories Ltd.; p-nitrophenyl phosphate was obtained from B.D.H. Ltd. as the disodium salt. All these commercially obtained chemicals were used without further purification. Anhydrous pyridine was prepared by refluxing Analar pyridine over potassium hydroxide and fractionally distilling through a Fenskii column.

Preparation of 2',5'-di-O-tetrahydropyranyluridine 3'-phosphate (3a)

Uridine-3'(2')-menophosphate (972 mg, 3.0 mmol) was dissolved in aqueous ammonia (2N; 7.5 ml), the solution diluted with dimethyl formamide (15 ml) and DCCI (4.5g, 22 mmol) in tertiary butanol (18ml) added. After refluxing for 2.5 hours, quantitative conversion to uridine-2',3' cyclic phosphate has occurred. (R_f of monophosphate = 0.08; R_f of cyclic phosphate = 0.40). The butanol is then evaporated in vacuo (temperature 35°), the residue diluted with water (60ml) and extracted with ether (4 x 60ml). The aqueous solution is reduced in vacuo to 50 ml, the pH of the solution adjusted to 8.0 with dilute ammonia and the solution filtered clear by filtering through a plug of cotton wool. A solution of RNase A (12 mg in 5 ml water) was added and the reaction mixture incubated 15 hours at 37°. The

cyclic phosphate was quantitatively cleaved to uridine-3'-phosphate in this time (check by chromatography). The pH of the solution was adjusted to 7.5 by triethylamine, and then concentrated to small volume (10 ml) in vacuo. Ethanol (50 ml) was added, the solution left to stand for 30 minutes and any resultant turbidity filtered off. A solution of calcium chloride (3g) in ethanol (100 ml) was added, and after 15 hours at 0^{o} the resulting precipitate of the calcium salt of uridine-3'-phosphate was filtered off, washed with ethanol (3 x 100 ml) and ether (3 x 100 ml) and dried in air. (0.89g, 82%).

Uridine-3'-phosphate (calcium salt, 2 mmol) was suspended in a mixture of dimethylformamide (20 ml) and redistilled dihydropyran (5 ml), the mixture cooled to -30^{o} and a solution of hydrogen chloride (4.5 mmol) in dioxan (20 ml) was added dropwise with vigorous stirring over 30 minutes. The resulting clear solution was allowed to stand at room temperature whence quantitative conversion into (3a) occurred after approximately 90 hours (R_f of (3a) = 0.38). Triethylamine (1.5 ml) was added and the reaction mixture diluted by dropping into anhydrous ether (250 ml). After 30 minutes at 0^{o}, the resultant precipitate is filtered off by suction and washed with dry ether and repeatedly with chloroform. The remaining dried precipitate is the calcium salt of 2',5'-di-O-tetrahydropyranyluridine-3'-phosphate (0.91g, 85%).

For all condensations the calcium salt required to be converted into the pyridinium salt. This was done by dissolving the calcium salt in 50% aqueous pyridine, filtering through a column of Dowex 50W X8

cation exchanger (pyridinium form) and evaporating in vacuo. Many coevaporations with anhydrous pyridine were effected to entrain out any remaining traces of water. The remaining gum was the required pyridinium salt, and was homogeneous on t.l.c. (R_f = 0.40).

Preparation of 2',5'-di-O-tetrahydropyranyluridine-3'-p-nitrophenyl-phosphate (4)

2',5'-di-O-tetrahydropyranyluridine-3'-phosphate (1 mmol of the pyridinium salt) was dissolved in dry pyridine (10 ml) and p-nitrophenol (10mmol) and dicyclohexylcarbodiimide (10 mmol) were added. Anhydrous Dowex 50W X8 cation exchanger (pyridinium form, 200 mg) was added and the whole mixture stirred in a stoppered flask for 5 days. The pyridine was then removed under reduced pressure, the residue suspended in water (25 ml) and ether (20 ml), and the insoluble dicyclohexylurea and Dowex filtered off. The aqueous layer was extracted with ether (3 x 50 ml), concentrated under reduced pressure and chromatographed on a column of diethylaminoethylcellulose (40 x 3 cm diameter) in the carbonate form. Products were eluted using a linear gradient system with water (3ℓ) in the mixing chamber and 0.1M triethylammonium bicarbonate (3ℓ) in the reservoir. Fractions (12 ml) were collected at 7 minute intervals. The desired product (4) was eluted in fractions 211 - 310 as the triethylammonium salt, together with some other contaminating nucleotidic material and some triethylammonium p-nitrophenoxide. Fractions 211 - 310 were pooled, concentrated under reduced pressure

and rechromatographed on a similar column giving the product (4) free of other nucleotides but still contaminated with the p-nitrophenoxide. Acidification of a solution of this material to pH 5.5 and extraction with ether leaves an aqueous solution of the triethylammonium salt of (4). This is the material used in all the hydrolytic studies described in the discussion. (Yield is approx. 0.06 m mol).

Preparation of 2',5'-di-O-(1-ethoxyethyl) uridine-3'-phosphate (3b)

The barium salt of (3b) was prepared by the method of Smrt[46]. However it was found that even at -70°, using redistilled ethyl vinyl ether and pure reagents that a large amount of high colored polymeric material was formed as well as the desired nucleotide. This polymeric material coprecipitated with the protected nucleotide and could not be removed in the way recommended by Smrt. Only washing with methanol would remove the impurity completely and this also dissolved away a considerable quantity of the nucleotide. However even impure nucleotide was t.l.c. homogeneous with an R_f of 0.65. The yield of pure (3b) obtainable was approximately 20% (cf. Smrt:-80%), which was very wasteful of expensive nucleotide reagents.

As with (3a) all condensations with (3b) were carried out with the pyridinium salt which was obtained by dissolving the barium salt in pyridine at 0°, shaking with Dowex exchanger (pyridinium form) at 0°, and after filtering, removing the pyridine in vacuo (at low temperature). Thus chromatographically homogeneous pyridinium 2',5'-di-O-(1-ethoxyethyl) uridine-3'-phosphate was obtained (R_f = 0.70). If the procedure used

for converting (3a) to its pyridinium salt were used, the nucleotide partially decomposed to a mono-1-ethoxyethyl derivative and uridine-2'(3')-phosphate. This shows the increased lability of the ethoxyethyl protecting group.

Attempted preparation of 2',5'-di-O-(1-ethoxyethyl)uridine-3'-p-nitrophenyl phosphate

Many attempts at this synthesis were made along the same lines as the preparation of (4). Variations in the procedure such as change of solvent, absence of Dowex exchanger, different reaction times, different reactant ratios and carrying out the reaction in the dark, were attempted but in all attempts no desired phosphate anyl diester was formed (as determined spectrophotometrically). Products included the starting nucleotide, monoethoxyethyl nucleotides and uridine-3'(2')-phosphate as well as probably various pyrophosphates.

Preparation of 2',5'-di-O-methoxytetrahydropyranyluridine (5)

The enol ether from which the methoxytetrahydropyranyl protecting group is derived, 4-methoxy-5,6-dihydro-2H-pyran, was prepared by the method of Reese[52,59]. The protected nucleoside (5) was prepared by reacting uridine-3'-acetate with this enol ether to form 3'-O-acetyl-2',5'-di-O-methoxytetrahydropyranyluridine as prepared by Reese.[52,60] Removal of the acetate group gave pure the desired nucleoside (5) as a colorless glass.

Attempted preparation of 2',5'-di-O-methoxytetrahydropyranyluridine-3'-p-nitrophenyl phosphate.

p-Nitrophenyl phosphate, as the anhydrous pyridinium* salt (2 mmol) was dissolved in dimethylformamide (10 ml) and pyridine (5 ml) containing dry Dowex 50W-X8 (pyridinium form), followed by 2',5'-di-O-methoxytetrahydropyranyluridine (1 mmol) and dicyclohexylcarbodiimide (5 mmol). The mixture was stirred for 3 days, more dicyclohexylcarbodiimide added (2 mmol) and the mixture stirred for 3 more days. Water (1 ml) was then added. After a further 24 hours, the solution was concentrated by evaporation to remove pyridine. T.l.c. showed that no p-nitrophenylphosphate (R_f = 0.4) remained. However the normal spectrophotometric and hydrolytic studies (see discussion) showed no indication of formation of the desired p-nitrophenyl diester. Water was added to the residue and the aqueous solution extracted with chloroform (3 x 10 ml). T.l.c. and U.V. studies revealved that all the nucleosidic material was found in chloroform layer as the starting nucleoside. The water soluble product was identified as di-p-nitrophenyl pyrophosphate by comparison with the products of a "blank" reaction carried out identically but without inclusion of the nucleoside (5).

Several variations of this attempted synthesis were tried. The phosphate was added slowly to the nucleoside over a period of several hours to minimise pyrophosphate formation. The nucleoside/phosphate ratio was changed from 1:2 to 2:1, despite the waste of

* see earlier experimental for general method for conversion to pyridinium salts.

valuable nucleosidic material which this involved, in an effort to get the nucleoside to act more competitively as a nucleophile against the phosphate. In addition solvent changes, absence of Dowex etc. were tried. In no case was any of the desired product detected.

Attempted phosphorylations of 2',5'-di-O-methoxytetrahydropyranyluridine.

The details of these attempts will not be listed here. The required experimental details may be found in the references for each phosphorylating reagent. The reagents used were diphenyl-phosphorochloridate,[54] tetra-p-nitrophenyl pyrophosphate[55] and o-phenylene phosphorochloridate.[56] Suffice to say highly complex mixtures of products were obtained which gave no indication (by usual studies on crude reaction mixture) of containing desired products. These procedures were not pursued further.

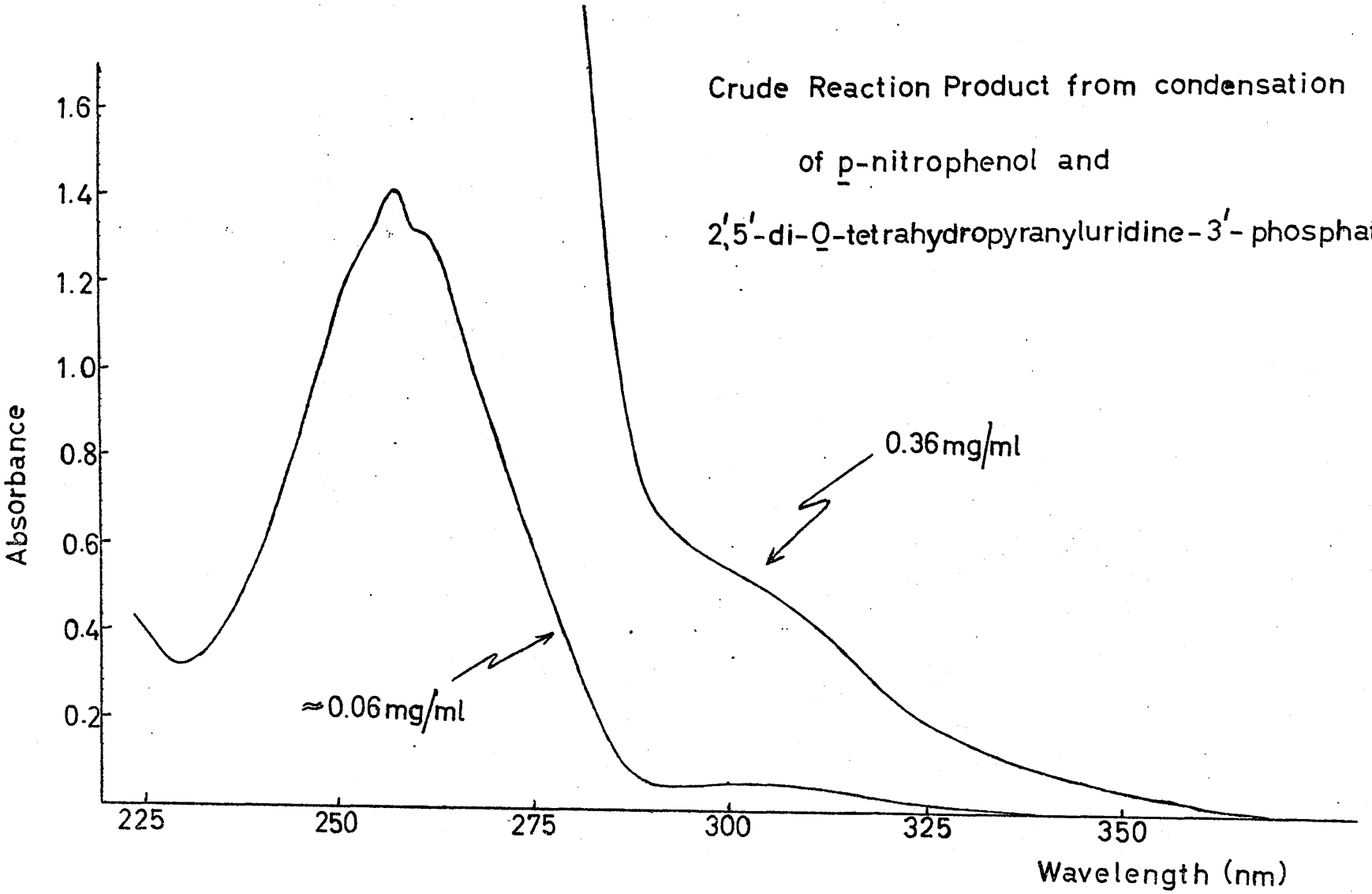

Crude Reaction Product from condensation
of p-nitrophenol and
2',5'-di-O-tetrahydropyranyluridine-3'-phosphate
0.36mg/ml
≈0.06mg/ml
Absorbance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
225
250
275
300
325
350
Wavelength (nm)

Fig. 3

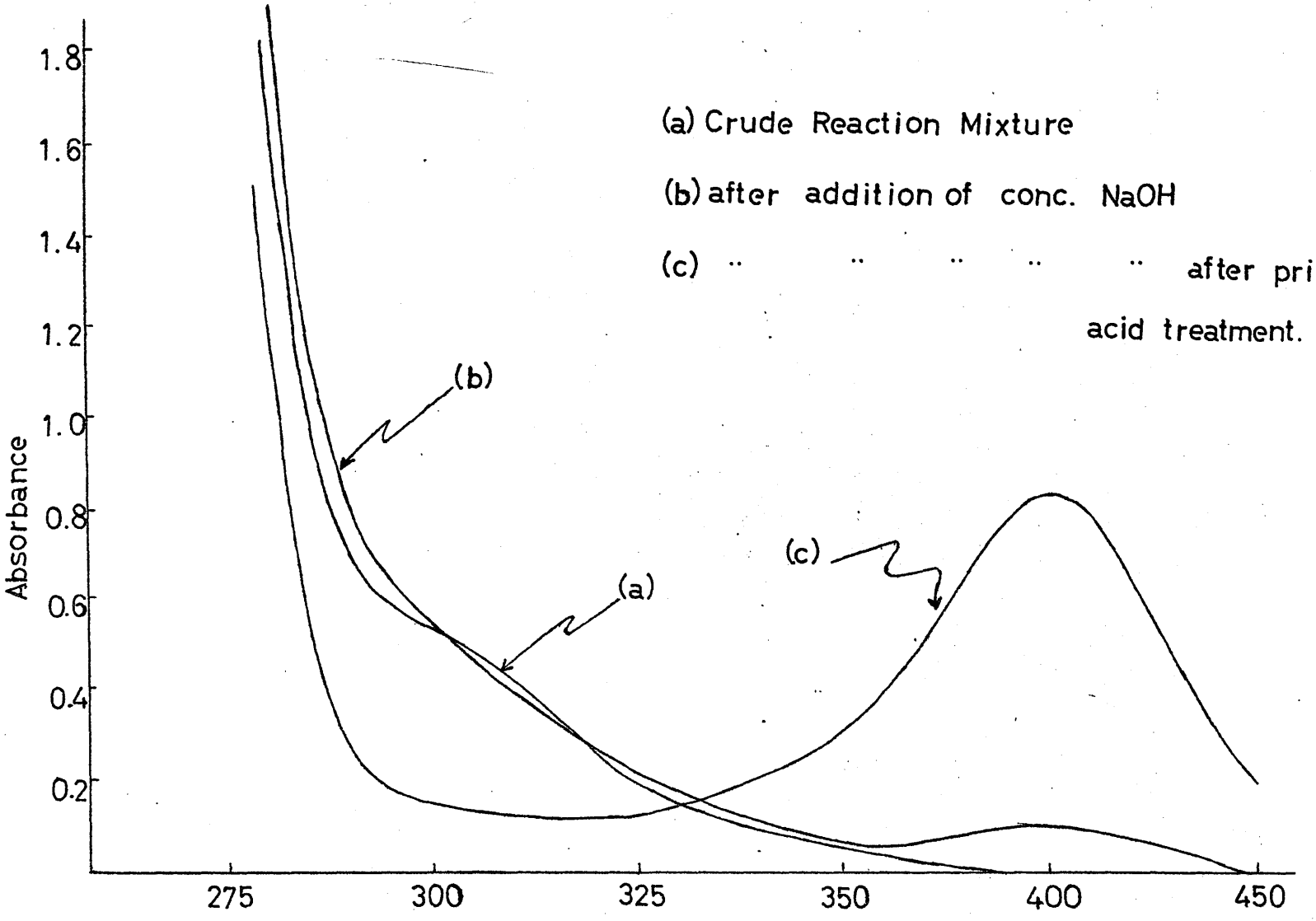

(a) Crude Reaction Mixture
(b) after addition of conc. NaOH
(c) .. .. after prior acid treatment.
(b)
(a)
(c)
Absorbance
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
275
300
325
350
400
450
Wavelength (nm)

Fig. 4

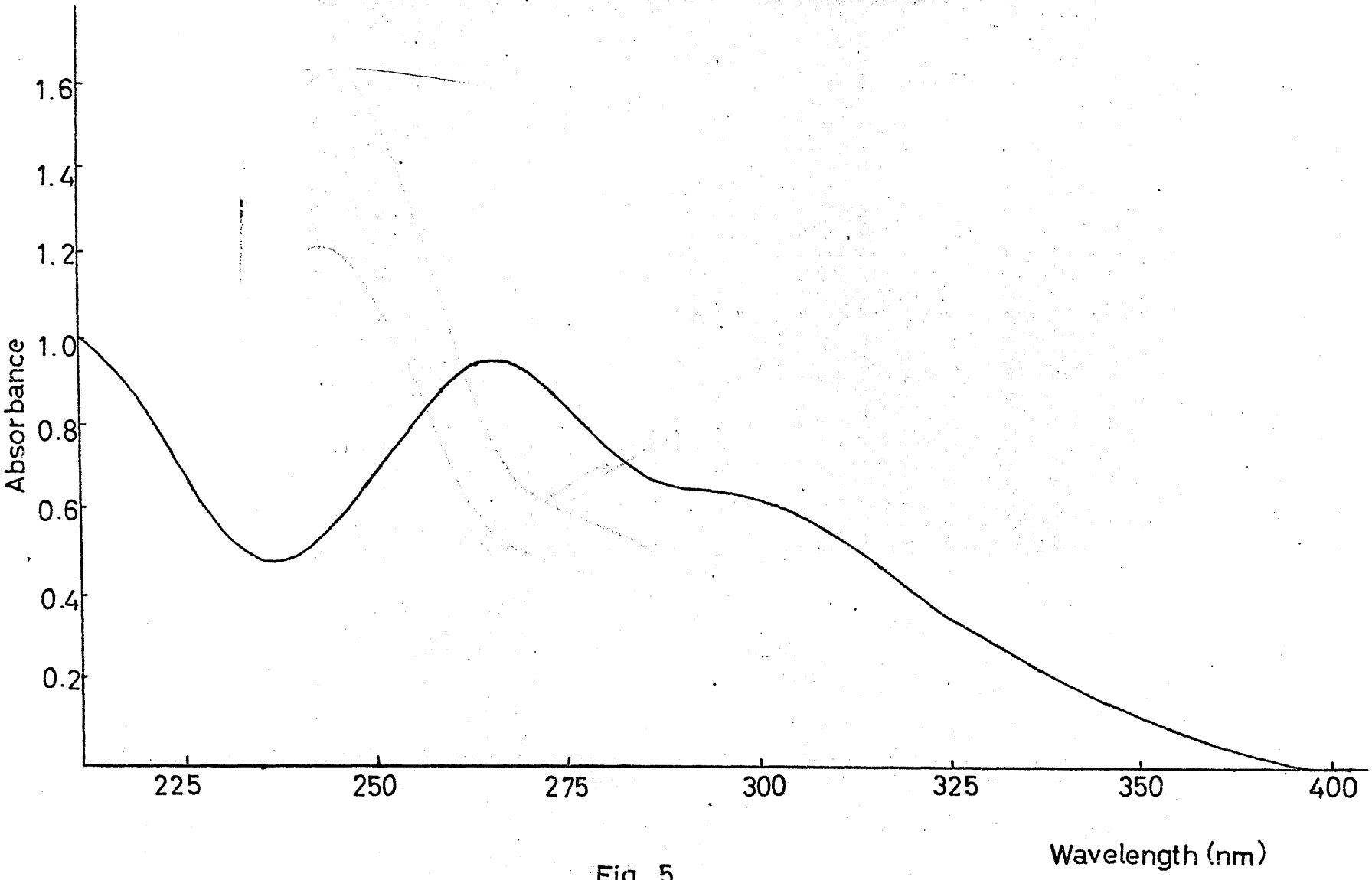

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Absorbance
225
250
275
300
325
350
400
Wavelength (nm)

Fig. 5

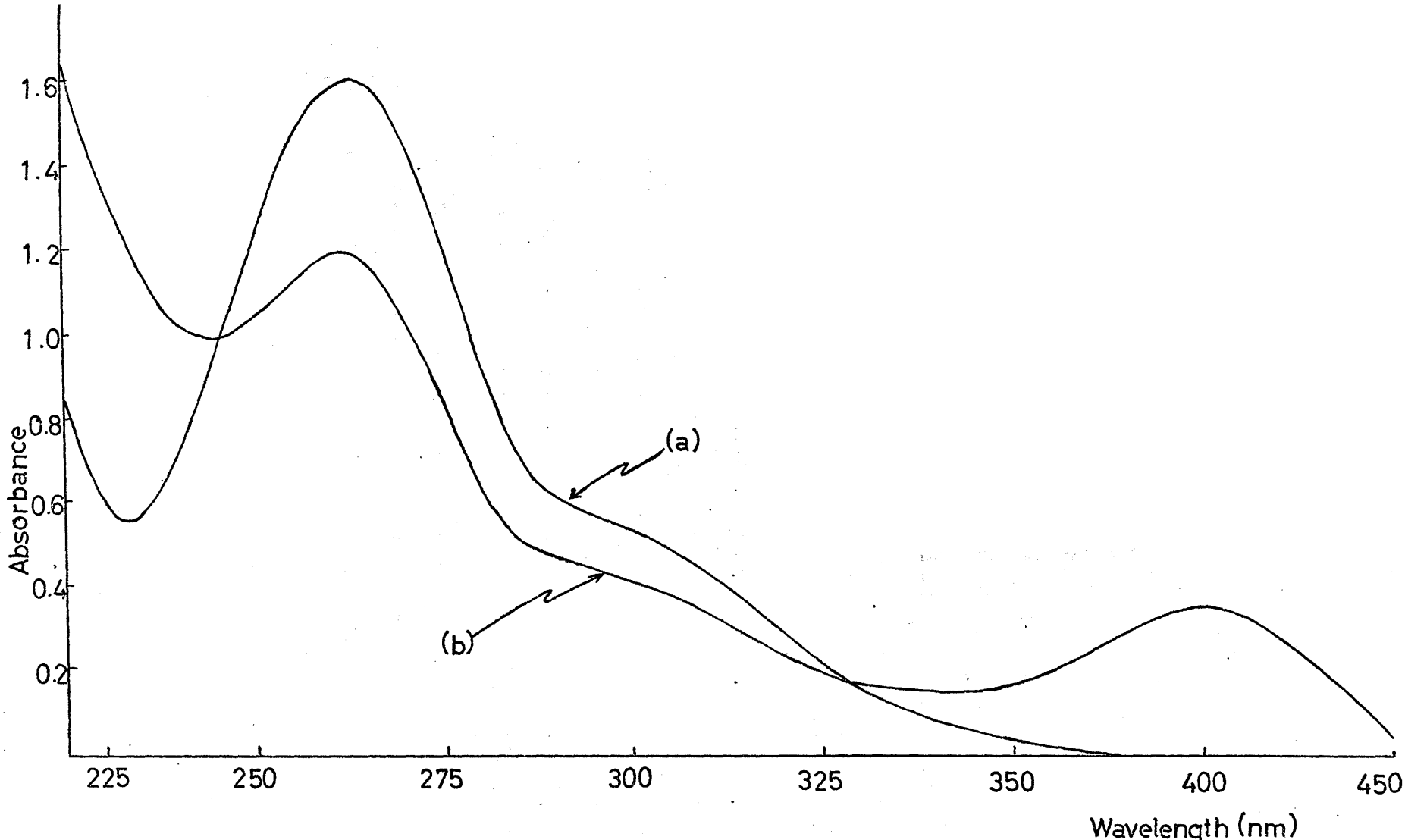

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Absorbance
(a)
(b)
225
250
275
300
325
350
400
450
Wavelength (nm)

Fig. 6

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