KINETIC EVIDENCE OF ANIONIC INTERMEDIATES IN THE BASE-CATALYZED CLEAVAGE OF GLYCOSIDIC BONDS IN THE METHYL D-GLUCOPYRANOSIDES

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ABSTRACT

A general, kinetic expression is presented for the rate of alkaline cleavage of glycosides in terms of the formation of anionic species as reactive intermediates that undergo a slow, rate-dependent, intramolecular displacement of the aglycon Equilibrium and specific rate-constants have been determined for the degradation of the anomeric methyl D-glucopyranosides and methyl β-cellobioside The difference in reactivity of the anomers is primarily due to the relative acidities of the hydroxyl groups involved in the intramolecular displacement process The kinetic data confirm that these reactions of α- and β-D-glucopyranosides are facilitated by anchimeric assistance of the hydroxyl groups at C-6 and C-2, respectively

INTRODUCTION

Although the reaction of alkaline cleavage of glycosidic bonds in various glycosides is now reasonably well understood[1,2], it has not been satisfactorily rationalized on the basis of one single mechanism or of available kinetic data The mechanism for the reaction of the β anomer of D-glucopyranosides[3–5] has been repeatedly suggested as proceeding through the intermediate formation of a 1,2-anhydride, as illustrated in Fig 1, the reaction, as indicated, is facilitated by anchimeric assistance of an ionized hydroxyl group at C-2 Similarly, it has been proposed[3] that reaction of the α anomer proceeds through an intramolecular, nucleophilic attack at C-1 by the hydroxyl ion at C-6 (see Fig 1) However, the role of these anionic species has not been kinetically demonstrated for the reaction of glycosides

Recently, Best and Green[5] and Robins and Green[6] provided some pertinent data on the kinetics of alkaline cleavage of the anomeric methyl D-glucopyranosides and methyl β-cellobioside, but the original data were not kinetically interpreted in terms of formation of the anionic intermediates shown in Fig 1 The reaction rate

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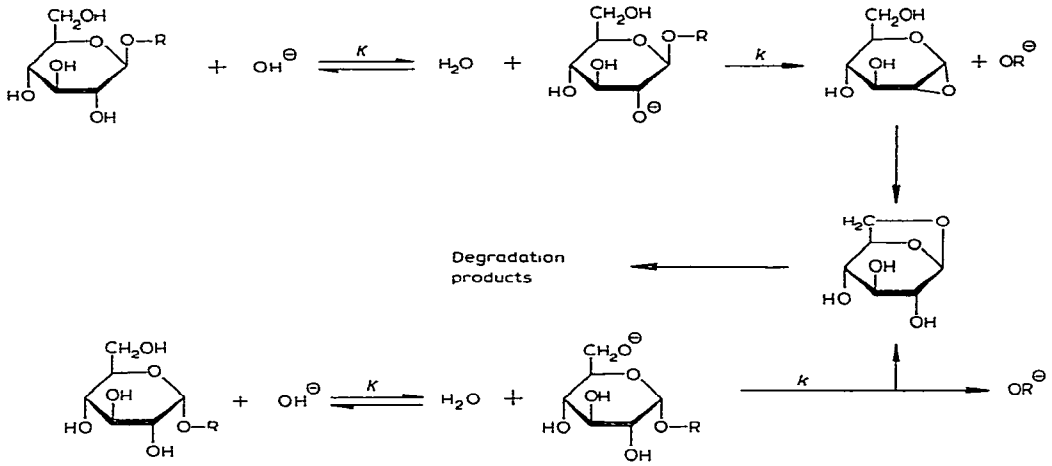

Fig 1 Possible mechanisms for the degradation of the α- and β-D-glucopyranosides

was observed to increase initially with increasing concentration of hydroxyl ion, but to level off to a constant value at a higher concentration (see Fig 2) This kinetic pattern, closely similar to that of end-wise depolymerization of amylose[7] and β-(1→3)-glucans[8], excludes the direct participation of the hydroxyl ion in the rate-determining step and supports the involvement of anionic species as reactive intermediates However, the original data were interpreted as first-order kinetics relative

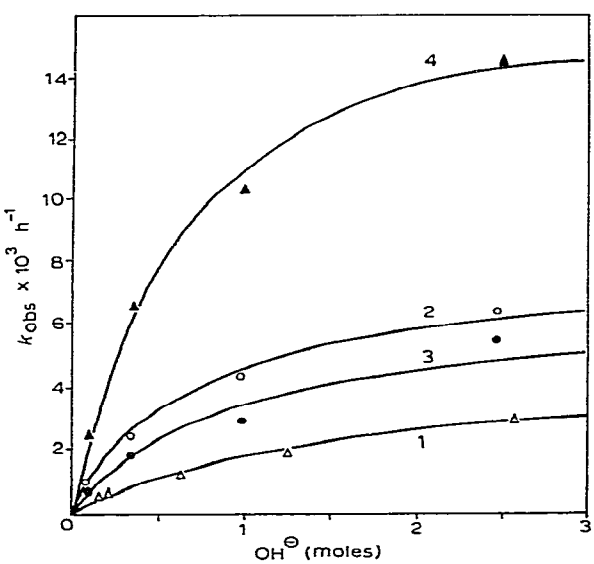

Fig 2 Comparison of the experimental rate-data with theoretical curves derived from eq 6 by use of the values of K and k at 170° shown in Table I [1, Methyl α-D-glucopyranoside, 2, methyl β-D-glucopyranoside, 3, methyl β-cellobioside (the "model" glycosidic bond), and 4, methyl β-cellobioside (the interior glycosidic bond), the value is half the actual value]

to the hydroxyl ion Thus, values of the reaction order with respect to base concentration were found to deviate appreciably from unity, and were also shown to decrease with an increase of base concentration For example, the value of the reaction order varies from 0 9 in 0 1M to 0 4 in 2 5M sodium hydroxide solution, for the reaction of methyl β-cellobioside to give methyl β-D-glucopyranoside Furthermore, the significant deviation from unity was inadequately claimed to be a deviation caused by the changes in activity coefficient and salt effects, although these have been reported to be negligible in the comparable reaction of glycosides[9]

The purpose of this article is to provide a general, kinetic expression valid for the entire range of base concentration used in the reaction of glycosides, with specific reference to the experimental data for the methyl D-glucopyranosides

THEORY

The generally accepted mechanisms for the base-catalysed cleavage of the anomers of methyl D-glucopyranoside are illustrated in Fig 1, and can also be represented by equations *1* and *2* for glycosides in general

$$\mathrm{GlcOR} + \mathrm{OH}^- \overset{K}{\rightleftarrows} \mathrm{GlcOR}^- + \mathrm{H_2O} \qquad (1)$$

$$\mathrm{GlcOR}^- \overset{k}{\rightarrow} \text{degradation products} \qquad (2)$$

where GlcOR is the glycoside, GlcOR^- is the anionic intermediate, K is the equilibrium constant between the neutral and ionized glycosides, and k is the specific rate-constant in the conversion of anionic intermediates to degradation products

In equation *2*, it is presumed that the reaction occurs only *via* the monanionic species, *i e*, an ionized hydroxyl group at C-2 in the β amoner, and at C-6 in the α anomer This assumption is valid because, as will be shown later, the ionization of hydroxyl groups at positions other than the reactive site has practically no effect on the reaction rate

A similar reaction-scheme was postulated by Gasman and Johnson[9] in a study of the alkaline cleavage of *p*-nitrophenyl β-D-galactopyranoside and α-D-mannopyranoside Their kinetic data on the hydrogen isotope-effect showed that the step involving the conversion of anionic intermediates into the reaction products (equation *2*) is the rate-determining step of the overall reaction The same conclusion may be valid for base-catalyzed degradation of glycosides in general Thus, the rate of alkaline degradation of glycosides is expressed in equation *3*

$$\frac{\mathrm{d}[P]}{\mathrm{d}t} = k[\mathrm{GlcOR}^-], \qquad (3)$$

where $[P]$ represents the mole fraction of glycosides reacted after time t, and $[\mathrm{GlcOR}^-]$ is the mole fraction of ionized, intermediate glycosides at time t

From equation *1*, it follows that ionization constant K can be represented by equation *4*

$$K = [\mathrm{GlcOR}^-]/\{[\mathrm{GlcOR}]_0 - [\mathrm{GlcOR}^-] - [P]\}\,[\mathrm{OH}^-] \tag{4}$$

where $[\mathrm{GlcOR}]_0$ is the mole fraction of glycosides at zero time Combination of equations *3* and *4* yields a rate equation which is integrated to give

$$\ln \frac{[\mathrm{GlcOR}]_0 - [P]}{[\mathrm{GlcOR}]_0} = -\frac{Kk[\mathrm{OH}^-]}{1 + K[\mathrm{OH}^-]}\,t \tag{5}$$

Then, if $k_{obs} = \dfrac{Kk[\mathrm{OH}^-]}{1 + K[\mathrm{OH}^-]}$ (6)

where k_{obs} is the pseudo-first-order constant of the reaction, equation *5* becomes

$$\ln \frac{[\mathrm{GlcOR}]_0 - [P]}{[\mathrm{GlcOR}]_0} = -k_{obs}\,t \tag{7}$$

Inversion of equation *6* gives

$$1/k_{obs} = 1/k + 1/kK\;1/[\mathrm{OH}^-] \tag{8}$$

DISCUSSION

The kinetics of alkaline cleavage both of methyl and phenyl glycosides have been the subject of many publications[3-6,9,10], and the rates of these reactions have all been found to conform with equation *7* The pseudo-first-order rate-constant, k_{obs}, has generally been reported, and no attempts have ever been made to determine

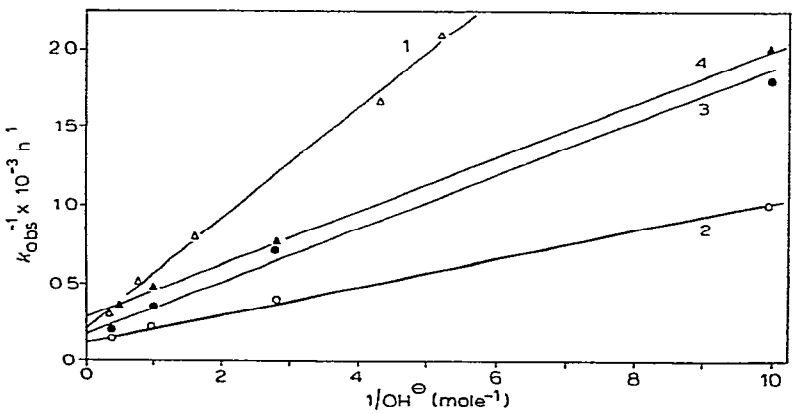

Fig 3 Plot of $1/k_{obs}$ *versus* $1/[\mathrm{OH}^-]$ for the reactions at 170° [1, Methyl α-D-glucopyranoside, 2, methyl β-D-glucopyranoside, 3, methyl β-cellobioside (the "model" glycosidic bond), 4, methyl β-cellobioside (the interior glycosidic bond), the value of $1/k_{obs}$ shown is ten times the actual value]

TABLE I

DETERMINED AND ESTIMATED EQUILIBRIUM CONSTANTS (K) AND SPECIFIC RATE CONSTANTS[a] (k) FOR REACTIONS OF VARIOUS METHYL D-GLUCOPYRANOSIDES

Glycoside	*170°*		*160°*		*150°*		*140°*		*25°*	
	K	k	K	k	K	k	K	k	K_l (*calculated*)	K_l (*Lit., ref 15*)
Methyl α-D glucopyranoside[b]	0 60	4 90	0 53	2 35	0 46	0 98	0 40	0 36	1.0×10^{-15}[c] 2.3×10^{-14}[d]	1.97×10^{-14}
Methyl β-D glucopyranoside	1 50	7 60	1 32	3 20	1 16	1 46	1,00	0 51	2.4×10^{-15}[c] 7.0×10^{-14}[d]	2.64×10^{-14}
Methyl β-cellobioside[e]										
(I) Model glycosidic bond	1 16	6 70	1 02	3 38	0 89	1 21	0.77	0 47		
(II) Interior bond	1 65	35 80	1 44	12 80	1 26	5 20	1 10	2 06		

[a]Units of k are 10^{-3} h^{-1} [b]According to the original data[6], the pseudo first order rate-constants of methyl α D glucopyranoside are in the order of 10^{-1} h^{-1} That is incorrect This is based on two reasons (i) the conclusion that the reaction rate of sodium (methyl α-D glucopyranosid)uronate is 280 times that of methyl α glucopyranoside, and the former has a rate constant in the order of 10^{-1} h^{-1}, (ii) data of Janson and Lindberg[21] indicate that methyl α D glucopyranoside has a rate constant of 2.3×10^{-3} h^{-1} under similar conditions [c]Calculated from K at 25° (by eq. *9*), which was first estimated from K at 170° [d]Estimated from K_l at 170°, which was first calculated from K at 170° (by eq *9*) [e]Glc—O—Glc—OMe

II I

the specific rate-constant k However, previous data on the methyl D-glucopyranosides[5,6] can be used for calculating the equilibrium rate-constant (K) and the specific rate-constant (k) for the reaction as depicted in equations *1* and *2* According to equation *8*, a plot of $1/k_{obs}$ *versus* $1/[OH^-]$ should give a straight line whose slope is $1/kK$ and intercept is $1/k$ The linear relationship observed (see Fig 3) supports the mechanism postulated, namely that both anomers react through the anionic intermediate

Table I summarizes the equilibrium rate-constants (K) and specific rate-constants (k) determined for the reactions of the anomers of methyl D-glucopyranoside and the β anomer of methyl cellobioside at 170° Values of k were calculated from the intercept of the plot (see Fig 3), and the constant K was then calculated from the value k and the slope of the plot (by equation *8*)

From the data in Table I, it is clear that the value of K for the β anomer is about three times that of the α anomer This result is consistent with the proposed mechanism shown in Fig 1, which illustrates that the reaction of α and β anomers is facilitated by anchimeric assistance of the ionized hydroxyl group at C-6 and C-2, respectively It is well known[11,12] that the hydroxyl group at C-2 is slightly more acidic than that at C-6 Moreover, the ratio of the dissociation constant for the 2- and 6-hydroxyl groups, namely 3, that is derived from the data for K conforms well with their relative reactivities observed in the base-catalyzed, etherification reactions of cellulose[12]

Furthermore, the ionization constant (K_i) for both anomers could be obtained through equation *9*

$$K_i = K K_w \qquad (9)$$

where K_w is the thermodynamic, ionic product of water It is noted that there are two methods for calculating K_i at 25° from the value K determined at 170°, depending on whether the value of K_w is used at 170° or 25° Table I records the values of K_i obtained by the two different methods Values of K_w at 170° were estimated from the data reported for lower temperatures[13] On the other hand, the heat of activation for the ionization reaction and proton-transfer reaction (see eq *1*) of glycosides were assumed to be the same as for D-glucose, values of which had previously been determined to be 10 and 5 kcal $mole^{-1}$, respectively That the K_i values estimated by the two methods are different is not surprising, in view of the uncertainty involved in determining K_w at high temperatures Therefore, the calculated dissociation constants for the β anomer are considered to be in satisfactory agreement with K_i values for the methyl D-glucopyranosides determined by Michaelis[15] (see Table I) and to give further support to the mechanism shown in Fig 1 It should be noted that, in a glycoside, the first ionization may be assumed to be largely associated with the 2-hydroxyl group

Furthermore, the magnitudes of K for the cleavage of the two glycosidic bonds in methyl β-cellobioside were, as expected, found to be similar to that of methyl β-D-glucopyranoside (see Table I), indicating that they proceeded through the same intermediate

On the other hand, the magnitude of specific rate-constants (k) is shown to

depend exclusively on the nature of the aglycon An almost identical value was obtained for the cleavage reaction of the α and β anomers of methyl D-glucopyranoside and the "model" glycosidic bond of methyl β-cellobioside, these reactions involve the same leaving-group, a methoxide ion However, this value was only one-fifth of that obtained for the cleavage of the interior glycosidic bond of methyl β-cellobioside It is thus clearly indicated that the methyl β-D-glucopyranoside anion is a much better leaving-group than the methoxide ion It may also be concluded that the difference in the reaction rate observed in the cleavage of the two glycosidic bonds in methyl β-cellobioside is due mainly to the leaving-group effect The different reactivity found for the anomers of methyl D-glucopyranoside is attributed to the different acidities of the hydroxyl group involved in the intramolecular displacement process (see Fig 1)

Fig 2 shows the effect of concentration of base upon the pseudo-first-order rate-constant (k_{obs}) for the reactions of the α and β anomers of methyl D-glucopyranoside and methyl β-cellobioside The rates experimentally determined for the reactions at 170° are shown, and are compared with a theoretical plot derived from equation *6* The values of K and k used in the calculation were obtained as already discussed (see Table I) It should be noted that there was no increase in the rate in the region of higher concentration of base, where the dianion species is the major anionic species Thus, it may be concluded that ionization of the hydroxyl group at positions other than that directly involved in the reaction has practically no effect on the reaction rate The excellent agreement between the experimental points and the theoretical curve confirms this assumption and the validity of the kinetic expression derived Furthermore, the activity coefficients of the species are not known with any accuracy However, the kinetic data discussed confirm a previous report[9] that salt effects are practically absent in the reaction of glycosides in an alkaline medium Also, the difference in the activity coefficient of sodium hydroxide in aqueous solution was reported to be less than 10% between the concentrations of 0 1 and 2 5M at a given temperature[16]

Similarly, the effect of temperature upon the specific rate-constant (k) obtained from the pseudo-first-order rate-constants previously reported[5,6] is also illustrated in Table I The value of K at a given temperature was estimated from the experimental value at 170° by assuming that the heat of activation for the proton-transfer reaction equals that of D-glucose[14], namely, 5 kcal mole^{-1} The activation energies for the reactions of the various glycosides (see Table II) were then calculated from the data in Table I As may be seen from equations *5* and *6*, the current values calculated from the specific rate-constant (k) are almost the same as those previously determined from the pseudo-first-order rate-data (k_{obs}) (see Table II)

Table II also records the thermodynamic activation-functions for these reactions, the values were calculated from the specific rate-data at 170° in 2 5M sodium hydroxide solution It is noticeable that values of the activation entropy and enthalpy functions for the β anomers, methyl β-D-glucopyranoside and methyl β-cellobioside, are very similar The entropy values (−6 8 and −10 4 eu) are also very close to those previously reported[9] (−7 3 and −8 1 eu) for the similar reaction of *p*-nitrophenyl β-D-galactopyranoside and *p*-nitrophenyl α-D-mannopyranoside In contrast,

TABLE II

THERMODYNAMIC ACTIVATION FUNCTIONS FOR REACTIONS OF METHYL α-D-GLUCOPYRANOSIDE (*A*), METHYL β-D-GLUCOPYRANOSIDE (*B*), AND THE MODEL GLYCOSIDIC BOND (*C*) AND THE INTERIOR BOND (*D*) OF METHYL β-CELLOBIOSIDE IN 2 5M SODIUM HYDROXIDE AT 170°

Function	A	B	C	D
E_a, kcal mol^{-1}	32 2 (33 3)[a]	35 6 (37 5)	37 5 (36 3)	34 5 (37 8)
ΔH, kcal mol^{-1}	31.3 (32 4)	34 7	33 6	33 6
ΔF, kcal mol^{-1}	40 3 (38 4)	37 7	36 8	38 2
ΔS, cal deg^{-1} mol^{-1}	−20 4 (−13 6)	−6 8	−7 2	−10 4

[a]Values inside the parentheses are previous data calculated from the observed rate-constants, K_{obs} (refs 5 and 6).

a significantly lower entropy (−20 4 eu) was found in the reaction of methyl α-D-glucopyranoside These data are also consistent with the proposed mechanism given in Fig 1, and thus, seem to indicate that the loss of rotational and vibrational degrees of freedom in forming the 1,6-anhydride (from the α anomer) is greater than that in the formation of the 1,2-anhydride (from the β anomer) As the intramolecular displacement process requires a coplanar arrangement of the atomic centers involved, both anomers must react through the *1C* (D) conformation

It should be pointed out that, although an identical rate-law of the form of equation *5* can also be obtained for an alternative mechanism in which the anion of the D-glycosides is an unreactive species (see equation *10*),

$$\mathrm{GlcOR^-} \overset{K}{\rightleftarrows} \mathrm{GlcOR + OH^-} \overset{k}{\rightarrow} \text{degradation products} \qquad (10)$$

this mechanism, involving a direct displacement by hydroxide ion, is excluded in the reaction of glycosides in alkali, because the major product isolated from both the β anomer of phenyl D-glucopyranoside and the α anomers of phenyl D-galactopyranoside[17] and phenyl 2-deoxy-D-*arabino*-hexopyranoside[18] is the 1,6-anhydride, and the formation of compounds of this type is consistent with a mechanism involving neighboring-group participation by an ionized hydroxyl group at C-2, or an intramolecular displacement process by the ionized 6-hydroxyl group (see Fig 1) In constrast, the expected product of a direct-displacement mechanism would be D-glucose, which would be degraded to products other than the 1,6-anhydride

Furthermore, a novel mechanism has recently been reported for the release of *p*-nitrophenoxide from *p*-nitrophenyl α-D-glucopyranoside in alkali[19], the reaction proceeds by a three-stage process and involves a base-catalyzed O-1→O-2 and O-2→O-3 migration of the *p*-nitrophenyl group Reaction of this type is also likely to involve the anionic species as the reactive intermediate, and the kinetics followed by the liberation of phenol should be very similar to that of the alkaline degradation of β-(1→3)-glucan[8]

The preceding kinetic data clearly indicae the nature and the mechanism of the reaction involved for the α and β anomers of methyl D-glucopyranoside, and lead to

the following general conclusions The current data confirm that such reactions of the α and β anomers of D-glycopyranosides are facilitated by anchimeric assistance of the hydroxyl groups at C-6 and C-2, respectively The data give strong support to the contention that both anomers have comparable reactivity in alkali

It is demonstrated that the relationship between the reaction rate and the concentration of base may serve as a clue for differentiating between a base-catalyzed, neighboring-group participation and an SN2 mechanism The kinetic pattern observed for the reaction of glycosides, similar to that of end-wise depolymerization of amylose[7] and of β-(1→3)-glucan[8], seems to be typical of base-catalyzed degradation of carbohydrates, which are shown to proceed through anionic species as reactive intermediates On the other hand, in the case of base-catalyzed cleavage of glycosidic bonds in cellulose[20], the reaction rates were found to be directly proportional to the base concentration This observation indicates that the mechanism involved is not a neighboring-group participation, but an SN2 process The deviation observed, however, may well be caused by the heterogeneous nature of the reaction or by the submicroscopic structure of cellulose, which has been demonstrated to have a dominating influence on the termination process of base-catalyzed, end-wise depolymerization of polysaccharides[7 20]

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