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ENOLISATION AND ISOMERISATION OF MONOSACCHARIDES IN AQUEOUS, ALKALINE SOLUTION

G. DE WIT, A. P. G. KIEBOOM, AND H. VAN BEKKUM

Laboratory of Organic Chemistry, Delft University of Technology, Julianalaan 136, 2628 BL Delft (The Netherlands)

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ABSTRACT

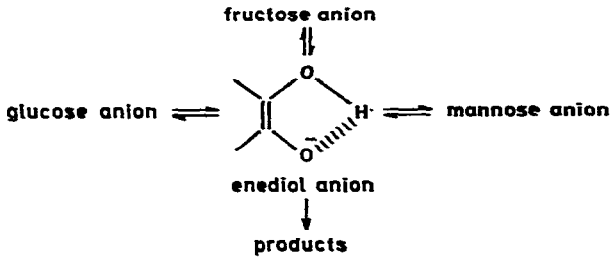
The initial behaviour of a series of monosaccharides in aqueous, alkaline medium has been studied by using u.v. spectroscopy. An absorption band at 310 nm is observed which may be due to the enediol-anion species as well as to β-elimination intermediates. Comparison of u.v. kinetic data with data from both H/D exchange and degradation reactions shows that the 310-nm band is mainly due to the enediol-anion species. Reaction rate constants have been determined for both the formation and the conversion of the enediol anion by using the overall reaction scheme:

sugar + $^{-}$OH ⇌ sugar anion ⇌ enediol anion → β-elimination intermediates → products.

Calcium (II) and carbonate ions promote the formation of the 310-nm band. Traces of oxygen cause an induction period, due to a rapid oxidation of the enediol anion. The rate of enediol-anion formation is strongly dependent on the configuration of the sugar, whereas the overall rates of conversion of the enediol anion are comparable for the different sugars. For the enolisation and the isomerisation reactions, a molecular picture is presented on the basis of the principle of least motion. It is proposed that the enediol anion is formed through a rate-determining, intramolecular proton-shift.

INTRODUCTION

The enolisation reaction is generally accepted to be an important step in the reactions of carbohydrates in alkaline solution[1–6]. Relatively simple isomerisation reactions, as well as the concomitant degradation reactions, *e.g.*, β–C–O or –C–C bond fission, of monosaccharides are thought to proceed through enediol-anion intermediates. For example, both the interconversion of glucose, fructose, and mannose (Lobry de Bruyn–Alberda van Ekenstein transformation) and their reaction to give hydroxycarboxylates (products) may be summarised according to Scheme 1.



Scheme 1.

The enediol-anion species is intuitively considered to exist predominantly in the (*Z*)-configuration with rapid transfer of the hydrogen between the two oxygens, whereas the transformation of the sugar anions into the enediol anion is assumed to proceed through open-chair (pseudo-cyclic) structures.

Initial processes undergone by carbohydrates in alkaline medium include ionisation[7], mutarotation[7], and enolisation. The last process has been studied by H/D and H/T exchange reactions[1-4] and by u.v. spectroscopy[5,6]. Several aspects remain to be clarified: for example, the kinetics of formation of the enediol anion, its reaction path to other products, and the effect of changing the hexose configuration. We now report on a kinetic study of enediol-anion formation for a series of twelve monosaccharides, using u.v. spectroscopy as the analytical tool. The experiments were performed in aqueous solution at low temperature in order to suppress degradation reactions. Additional data were obtained from H/D exchange and isomerisation experiments.

EXPERIMENTAL

General. — The monosaccharides were commercial products. 1,5-Anhydro-D-mannitol was prepared by the literature[8] procedure. N.m.r. spectra were recorded on a Varian XL-100 spectrometer at 5°. Mass-spectral measurements were performed on a Varian-Mat 311 mass spectrometer. The u.v. spectra were recorded with a Cary spectrometer Model 15, and the i.r. spectra with a Beckman IR-4210 spectrophotometer. G.l.c. was performed with a Becker Model 409 gas chromatograph equipped with a flame-ionisation detector and an Infotronics CRS 304 integrator.

Kinetic measurements. — Unless otherwise stated, all experiments were carried out under nitrogen at low temperature (10.4°), in order to suppress undesired alkaline oxidation and degradation reactions. The aqueous, alkaline solutions of the monosaccharides were obtained by mixing aqueous sugar and metal hydroxide solutions, both at the required temperature. Within 1 min, the mixed solution was transferred into a thermostatted u.v.-cuvette (5-cm path-length). Subsequently, the absorption at 310 nm was measured as a function of time.

G.l.c. — The alkaline sugar solutions were neutralised with a weakly acidic cation-exchanger (Amberlite IRC-50-H-AG) at 0–4°, and freeze-dried, and the residues were trimethylsilylated with a solution of bis(trimethylsilyl)trifluoroacetamide

and chlorotrimethylsilane in pyridine (2 : 1 : 8 by volume)[9]. The trimethylsilyl derivatives were analysed on a 3-m glass column containing 5% of QF-1 on Chromosorb W-HP (80–100 mesh), using temperature programming (2.5°/min, from 70 to 200°).

H/D exchange experiments. — Solutions of D-fructose and D-glucose (540 mg) in alkaline deuterium oxide (30 ml, 1.32M NaOD) were kept at 5° under N_2 for 19 and 72 h, respectively. Each solution was neutralised and freeze-dried, after which part (~ 10 mg) of the residue was trimethylsilylated and analysed. The remainder of the residue was transformed into the di-*O*-isopropylidene derivatives of D-glucose[10] and D-fructose[3], respectively. The deuterium incorporated into these derivatives was determined by mass spectrometry, by comparing the intensity of the isotope peaks of *m/e* 245 (M–15) with the isotope peaks of the unlabelled compounds. The deuterium incorporation was in agreement with ^{1}H-n.m.r. spectral data.

Peroxide determination. — Peroxides were measured iodometrically, in a spectroscopic way, by measuring the absorption at 350 nm[11]. To 0.5 ml of the alkaline sugar solution (0.1M D-glucose in 1.22M KOH) was added 2.5 ml of 0.125M H_2SO_4, 0.125 ml of a solution of 1 g of NaOH, 33 g of KI, and 0.1 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 500 ml of H_2O, 0.125 ml of a solution of 10 g of potassium hydrogenphthalate in 500 ml of H_2O, and 16.75 ml of H_2O. After ~3 h, the absorption reached its maximum.

Isolation of Ca(OH)$_2$–D-glucose complex[12]. — D-Glucose (5.0 g) was added to a stirred suspension of $Ca(OH)_2$ (5.0 g) in 200 ml of H_2O at 0° under N_2. After 2 h, undissolved $Ca(OH)_2$ was filtered off, and acetone (300 ml) was added to the filtrate. The white complex was filtered off, washed (100 ml of 80% acetone and 100 ml of dry acetone), dried (vacuum, P_2O_5), and stored under N_2 at 4°.

Isomerisation and degradation reactions. — Solutions of D-fructose and D-glucose (0.1M) in 1.22M KOH were kept under N_2 at 5° for 3.5 months. From time to time, samples were taken, and analysed by g.l.c. The compounds were identified by g.l.c.–m.s.

RESULTS AND DISCUSSION

U.v. spectral phenomena. — The u.v. spectra of monosaccharides in aqueous, alkaline media generally show major absorption bands at 263 and 310 nm. It is observed that the appearance of these two bands is dependent on the conditions applied, as illustrated in Fig. 1 for D-glucose: under O_2, a band at 263 nm is observed (curve 1), whereas under N_2, a maximum appears at 310 nm (curve 2); in the presence of Ca(II), the latter band shifts to 342 nm (curve 3; in view of the limited solubility of calcium hydroxide, different concentrations had to be chosen).

The absorption band at 263 nm is observed particularly when the alkaline, aqueous solution of sugar is presaturated with oxygen. In the literature, peroxide formation has been found upon oxygenation of aqueous, alkaline solutions of sugars[13–15]. In accordance with these results, we observed that oxygenation (for 15 min at 10°) of D-glucose (0.1 M in 1.22M KOH) causes peroxide formation (0.4mM

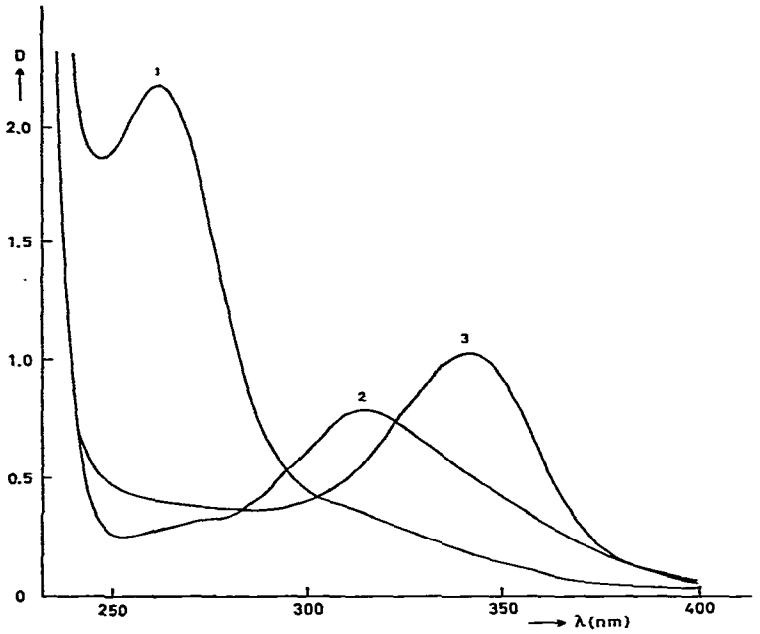


Fig. 1. U.v. spectra of D-glucose in aqueous, alkaline solution. 1, 0.1M D-glucose, 1.22M KOH, 10°, O_2; 2, 0.1M D-glucose, 1.22M KOH, 10°, N_2; 3, 0.5M D-glucose, 0.1M $CaCl_2$, 0.25M KOH, 10°, N_2.

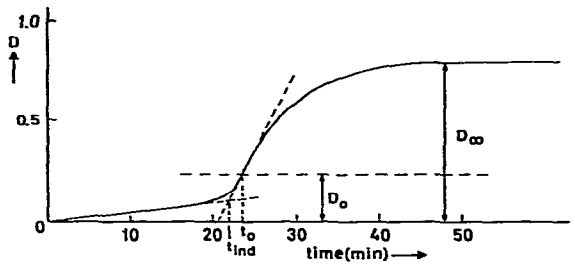


Fig. 2. Appearance of the 310-nm band of D-glucose; 0.1M D-glucose, 1.5M KOH, 10.4°, N_2, 5-cm cuvette.

after 2 h at 10°), together with the absorption at 263 nm. Therefore, either organic peroxides or some intermediate oxidation products[14-16] are responsible for the 263-nm absorption band. It may be mentioned that oxygenation of non-enolisable carbohydrates, known to give peroxides[15], also gives rise to a 263-nm band (*e.g.*, 0.1M 1,5-anhydro-D-mannitol, 1.22M KOH, O_2, 1 h, 100°).

The oxygen-induced appearance of the absorption band at 263 nm is connected with a retardation of the formation of the absorption band at 310 nm (Fig. 2). This retardation, denoted as the time of induction (t_{ind}), increases with the initial, total amount of oxygen present. As shown in Fig. 3, t_{ind} is also strongly dependent on such other variables as [sugar], [KOH], T, and [$Ca(OH)_2$]. Reaction conditions that favour formation of the 310-nm band reduce the time of induction. These results point to

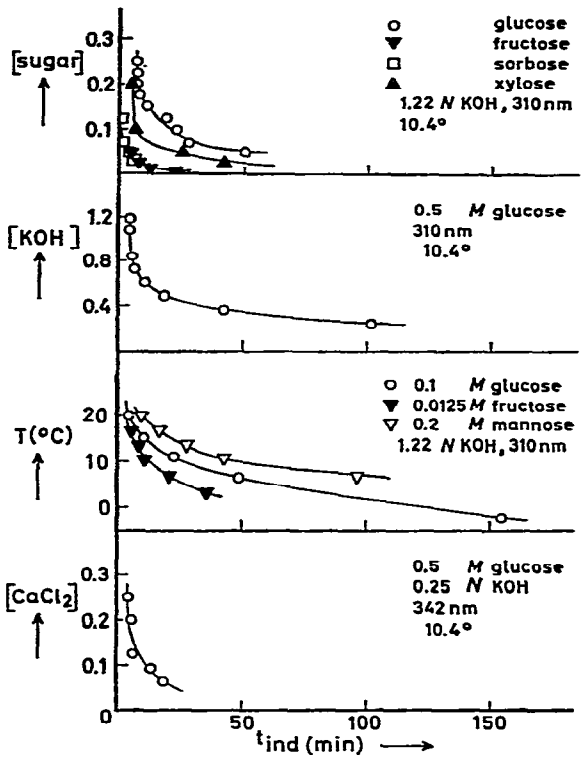


Fig. 3. Influence of several variables on the time of induction.

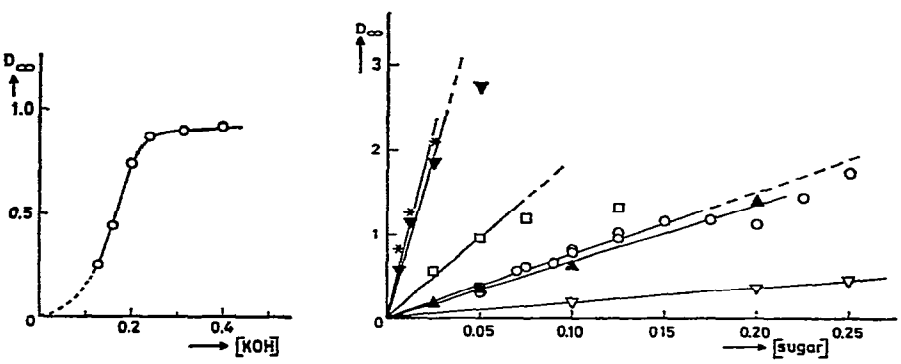


Fig. 4 (left). Absorbance (D_∞) *versus* hydroxide concentration; 0.125M D-glucose, 10.4°, N_2, 310 nm, 5-cm cuvette.

Fig. 5 (right). Absorbance (D_∞) *versus* sugar concentration for D-glucose (○), D-xylose (▲), L-sorbose (□), D-fructose (▼), D-tagatose (*), and D-mannose (▽); 1.22M KOH, 10.4°, N_2, 310 nm, 5-cm cuvette.

rapid reaction of the absorbing species with oxygen. In this connection, it should be noted that Gleason and Barker[17] concluded that an enediol anion is the species attacked by oxygen.

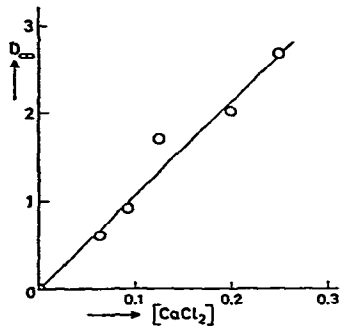


Fig. 6. Absorbance (D_∞) *versus* Ca(II) concentration; 0.5M D-glucose, 0.25M KOH, 10.4°, 342 nm, N_2, 5-cm cuvette.

TABLE I

H/D EXCHANGE OF D-GLUCOSE AND D-FRUCTOSE[a]

	Mol %	
Product	D-*Glucose (72 h)*	D-*Fructose (19 h)*
Glucose	82	
Glucose deuterated at C-2	3	8.5
Fructose		50.5
Fructose deuterated at C-1	11	31
Degradation products	4	10

[a]0.1M sugar, 1.32M NaOD in D_2O, 5°, N_2.

The ultimate absorbance (D_∞) at 310 nm reaches its maximum value at complete ionisation of the sugar, as shown for D-glucose at different hydroxyl-concentrations in Fig. 4. Under conditions where the sugars are completely ionised, the correlations of D_∞ with the amount of sugar anion are shown in Fig. 5. Deviations from linearity at high concentration of sugar are accompanied by relatively substantial degradation and isomerisation during the u.v. spectral measurements, as was observed by the colour of the solution and by g.l.c. analysis of the reaction mixture.

The shift of the 310-nm band to 342 nm by calcium(II) suggests complex formation of calcium(II) with the 310-nm species. This is supported by the fact that the absorbance at 342 nm is linearly related to the Ca(II) concentration (Fig. 6). On the other hand, the influence of the cations Sr(II), Ba(II), and Mg(II) (added as their chlorides to the alkaline, aqueous solution of D-glucose) on the absorption of 310 nm is negligible. Recently, Ziemecki *et al.*[12] isolated a $Ca(OH)_2$–D-glucose complex, for which we have observed a broad i.r. band (KBr disc) at 1550–1750 cm^{-1} containing detectable components at 1600, 1650 (the most intense band), and 1700 cm^{-1}. Addition of the solid complex to a solution of Tillmans' reagent[18] (at 100°) gives a positive

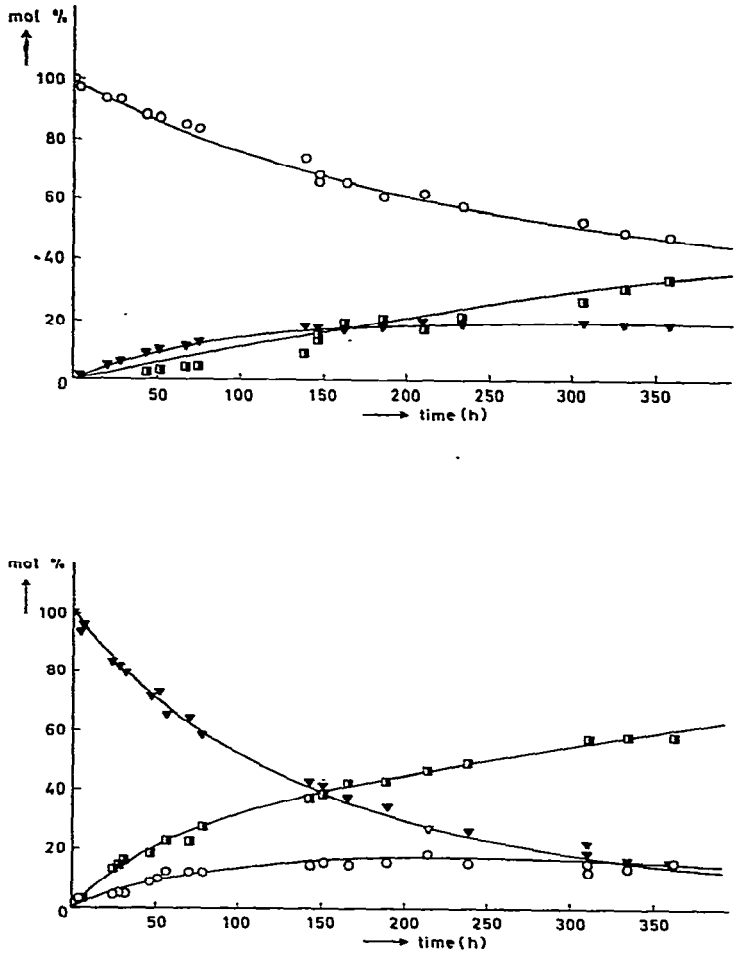


Fig. 7. Reaction of D-glucose (○) and D-fructose (▼); 0.1M in 1.22M KOH, 5°, N_2 (◨ = degradation products).

reaction for enediolic structures. D-Glucose or D-fructose gave no such reaction. These results indicate the presence of an enediolic structure in the complex.

The absorptions at 310 and 342 nm described above disappear upon acidification of the solution, and no other absorptions appear instead. Subsequent addition of base results in the reappearance of these absorptions (as in Fig. 2).

In order to compare rates of formation of the 310-nm species, as obtained from u.v. spectral data, with rates of isomerisation, we have determined the deuterium incorporation in D-fructose and D-glucose at 5°, using alkaline deuterium oxide as the reaction medium (Table I).

Also, the degradation reactions of D-glucose and of D-fructose at 5° were followed for a long period (Fig. 7; the mannose content was always less than 3 mol %). The composition of the reaction mixture after 3.5 months is summarised in Table II.

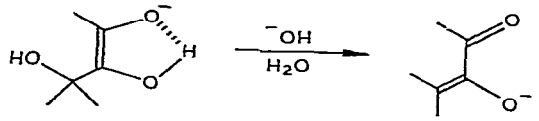
TABLE II

REACTION PRODUCTS OF D-GLUCOSE AND D-FRUCTOSE[a]

Product	*Mol %*	
	D-*Fructose*	D-*Glucose*
Lactic acid	79	73
Glycolic acid	1	< 0.5
2-Methylglyceric acid	< 0.5	< 0.5
Glyceric acid	1	1
2,4-Dihydroxybutyric acid	3	3
3-Deoxypentonic acid	2	1
Metasaccharinic acid	8	10
Fructose	3	5
Glucose	3	7
Unknown	< 0.5	< 0.5

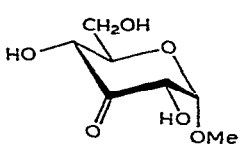
[a]0.1M Sugar, 1.22M KOH, 5°, N_2, 3.5 months.

Assignment of the 310-nm band. — The rather slow appearance of the 310-nm band excludes the possibility that the sugar anion itself is responsible for this absorption. Furthermore, in view of the formation of a steady-state absorption, the 310-nm band cannot be due to a final reaction product, but arises from an intermediate species. Consequently, two types of species remain to be considered, namely, the enediol anion (E^-) and the consecutively formed, ionised, β-elimination product (β^-)*.



E^- β^-

Both the enediol anion and the β-elimination anion will occur as reaction intermediates in an aqueous, alkaline solution of a sugar. First, it was ascertained whether such species result in an absorption in the 310-nm region. Some model compounds, *e.g.*, methyl α-D-*ribo*-hexopyranosid-3-ulose (**1**), (4*R*,5*S*,6*R*)-2,4,5,6-tetrahydroxy-2-cyclohexen-1-one (**2**), and α-hydroxycamphor (**3**), show an absorption band at 310–320 nm in aqueous, alkaline media[19].



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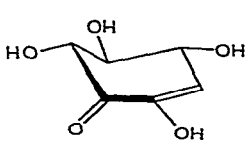

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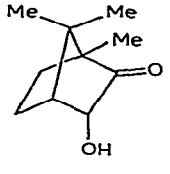


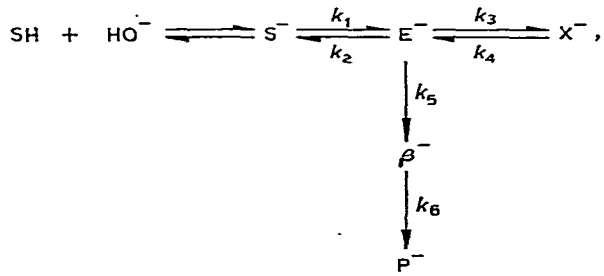
3

*The occurrence of substantial amounts of enediol dianion or undissociated enediol under the conditions applied, as suggested in the literature[5,6], may be rejected in view of the acidities expected for these species. The same applies to β-elimination intermediates.

This observation accords with expectation for **2**, but is, perhaps, surprising for **1** and **3**. Although **1** might undergo some β-elimination during the u.v. spectral measurements, the camphor derivative **3** should lead unequivocally to enediol-anion formation. This has been proved by Coulombeau and Rassat[20], who found that interconversion of the various α-hydroxy ketones of **3** in alkaline medium occurred without by-product formation. The rapid appearance of a 303-nm band upon dissolution of glycolaldehyde in aqueous, alkaline media further establishes the possible absorption of enediolic-anion species in the >300-nm region. In conclusion, the 310-nm band of alkaline solutions of sugars may apply equally well to both the enediol anion and the β-elimination anion.

Part of the u.v.-spectral phenomena observed are considered to be in favour of a significant contribution of the enediol-anion species. Thus, (*a*) the strong influence of Ca(II) on the 310-nm band as well as the enediolic nature of the glucose–Ca(II) complex are consistent with the catalytic effect of Ca(II) on both the Lobry de Bruyn–Alberda van Ekenstein transformation[21] and the formose reaction[12], reactions in which the enediol anion is generally considered to be the key-intermediate; and (*b*) the behaviour of the 310-nm band is consistent with Gleason and Barker's finding that the rate of oxygen uptake reflects the rate of enolisation of the corresponding sugar in aqueous, alkaline solution, *i.e.*, the formation of the enediol anion is the rate-determining step in the oxygenation process[17]. Quantitative evaluation will be necessary to establish from which of the two species the 310-nm band predominantly arises. Hence, a quantitative comparison has been made for D-glucose (see *Kinetics* section) by using u.v. kinetic data, data from H/D exchange, and degradation reactions. This approach leads to the conclusion that the 310-nm band is mainly due to the enediol anion.

Kinetics. — The processes in alkaline medium may be formulated as follows:



where SH = sugar, S^- = sugar anion, E^- = enediol anion, X^- = isomerisation products, β^- = β-elimination anion, and P^- = degradation products.

Under the experimental reaction conditions, $[S^-]$, $[HO^-] \gg [E^-]$, $[\beta^-]$, whereas enediol-anion formation from X^- may be neglected since $[S^-] \gg [X^-]$ during the kinetic experiments.

So, for the enediol-anion species,

$$\frac{d[E^-]}{dt} = k_1[S^-] - (k_2 + k_3 + k_5)[E^-], \tag{1}$$

in which $[S^-]$ may be taken as constant, so that the integrated form becomes

$$\ln \frac{k_1[S^-] - (k_2 + k_3 + k_5)[E^-]}{k_1[S^-]} = -(k_2 + k_3 + k_5)t. \tag{2}$$

At time $t = 0$,

$$\frac{d[E^-]}{dt} = k_1[S^-], \tag{3}$$

whereas at the time that $[E^-]$ has reached its pseudo-steady state $[E^-]_\infty$,

$$\frac{d[E^-]}{dt} = 0 = k_1[S^-] - (k_2 + k_3 + k_5)[E^-]_\infty. \tag{4}$$

Combination of equations (2) and (4) gives

$$\ln \frac{[E^-]_\infty - [E^-]}{[E^-]_\infty} = -(k_2 + k_3 + k_5)t. \tag{5}$$

For the β-elimination anion,

$$\frac{d[\beta^-]}{dt} = k_5[E^-] - k_6[\beta^-], \tag{6}$$

which, by analogy with (5), leads to

$$\ln \frac{[\beta^-]_\infty - [\beta^-]}{[\beta^-]_\infty} = -k_6t, \tag{7}$$

while in the pseudo-steady state,

$$k_5[E^-]_\infty = k_6[\beta^-]_\infty. \tag{8}$$

The concentration of E^- or β^- is given by $D/\varepsilon l$, in which D is the absorbance at 310 nm, ε is the molar extinction coefficient (litres. mol^{-1}. cm^{-1}) of E^- or β^-, and l is the path length of the absorption cell (cm). Using the definitions $t = 0 = t_0$ and $t = "t_\infty"$, with the respective absorbances D_0 and D_∞ as illustrated in Fig. 2, the equations (3) and (5) may be converted (in the case where E^- is responsible for the 310-nm band) into

$$\left(\frac{d(D - D_0)}{dt}\right)_{t_0} = k_1\varepsilon l[S^-] \tag{9}$$

and

$$\ln \left(\frac{D_\infty - D}{D_\infty - D_0}\right) = -(k_2 + k_3 + k_5)t, \tag{10}$$

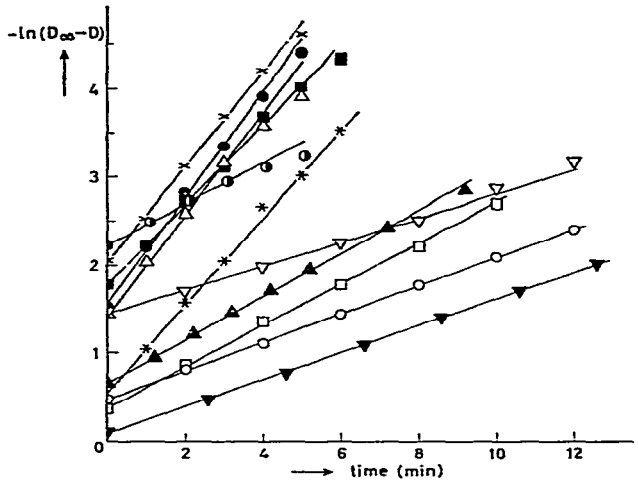


Fig. 8. Plots of ln (D_∞ – D) *versus* time for 0.2M D-ribose (△), 0.2M D-arabinose (●), 0.1M D-xylose (▲), 0.2M D-allose (◑), 0.1M D-glucose (○), 0.2M D-mannose (▽), 0.2M D-galactose (■), 0.2M D-talose (X), 0.0125M D-fructose (▼), 0.05M L-sorbose (□), and 0.006M D-tagatose (*); 1.22M KOH, 10.4°, N_2, 310 nm, 5-cm cuvette.

respectively, as the ultimate expressions for the formation and the subsequent conversion of the enediol anion; for the case where the 310-nm band is completely due to β^-, equation (7) becomes

$$\ln\left(\frac{D_\infty - D}{D_\infty - D_0}\right) = -k_6 t \qquad (11)$$

for the conversion of β-elimination anions into final products.

Now the rate of formation of degradation products allows a choice between (*10*) and (*11*). As an example, the data for D-glucose will be considered. U.v. spectral measurements show that either $k_2 + k_3 + k_5$ or k_6 is 0.15 min^{-1} (*cf.* Table IV). If we assume that the 310-nm band is solely due to β^- with $\varepsilon = 2000$–6000[19], then 20–50% of degradation products would be expected instead of the 4% found under the conditions applied (Table I). This discrepancy therefore shows E^- to be the major species giving rise to the 310-nm band. Some contribution of β^- to the 310-nm absorption cannot be completely excluded. However, equations (6) and (8) both show a good proportionality between E^- and β^-, *i.e.*, the subsequent kinetic and mechanistic treatments based only on the enediol-anion species seem to be justified.

The validity of (*10*) is illustrated in Fig. 8 for a number of sugars investigated.

Equation (*4*) indicates that the degree of ionisation of the sugar (at lower [HO^-] than applied above) should be linearly correlated with $[E^-]_\infty$. The dependence of $[E^-]_\infty$ (or D_∞) on [HO^-] should reflect the ionisation curve of the sugar. This, indeed, is found, as shown in Fig. 4 for D-glucose. In other words, the 310-nm absorption acts as an u.v.-indicator (at 310 nm) for the relative amount of sugar anion present. The apparent ionisation constants thus measured by u.v. spectroscopy, together with

TABLE III

pK_a-VALUES OF D-GLUCOSE IN WATER

[D-*Glucose*] (M)	*T* (*degrees*)	pK_a	*Source*
0.01	10	12.7	Ref. 23
0.01[a]	5	12.7	^{13}C n.m.r.[23]
0.125	10.4	13.5	u.v.
0.5	10.4	13.8	u.v.
1.1	5	14.0	^{13}C n.m.r.[22]

[a]Using ^{13}C-enriched D-glucose.

TABLE IV

REACTION RATE CONSTANTS FOR ENEDIOL-ANION FORMATION AND CONVERSION[a]

Sugar	*Conc.* (M)	D_0	D_∞	$k_1\varepsilon$ (*litres.min^{-1}.mol^{-1}.cm^{-1}*)	k_1[b] (*10^{-4} min^{-1}*)	$k_2 + k_3 + k_5$ (*min^{-1}*)
D-Ribose	0.2	0.25	0.50	0.13	0.4	0.58
D-Arabinose	0.2	0.28	0.50	0.18	0.6	0.61
D-Xylose	0.1	0.20	0.66	0.20	0.7	0.25
D-Allose	0.2	0.08	0.19	0.03	0.1	0.23
D-Glucose	0.1	0.20	0.8	0.15	0.5	0.15
D-Mannose	0.2	0.07	0.19	0.03	0.1	0.13
D-Galactose	0.2	0.16	0.33	0.08	0.3	0.48
D-Fucose	0.2	0.11	0.22	0.03	0.1	0.38
D-Talose	0.2	0.10	0.23	0.05	0.2	0.54
D-Fructose	0.0125	0.21	1.12	1.7	6	0.14
L-Sorbose	0.025	0.12	0.54	0.7	2.5	0.20
D-Tagatose	0.006	0.24	0.82	8.1	27	0.51

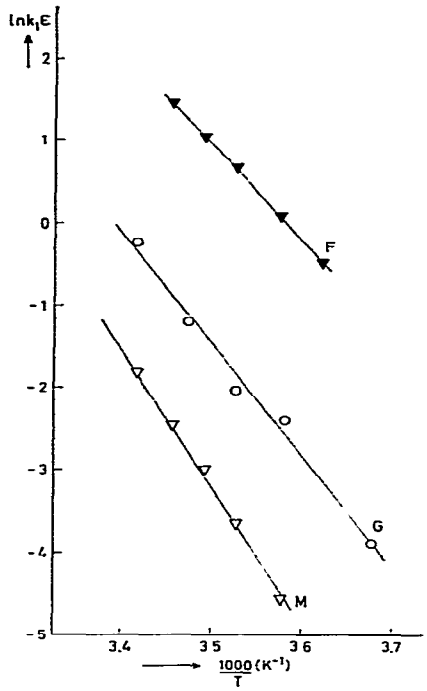
[a]1.22M KOH, 10.4°, N_2, 310 nm. [b]Using $\varepsilon_{\text{enediol anion}} = 3000$ (see text).

TABLE V

ACTIVATION PARAMETERS FOR ENEDIOL-ANION FORMATION

Hexose	$\Delta H^{\ddagger}$ (*kcal.mol^{-1}*)	$\Delta S^{\ddagger a}$ (*cal.K^{-1}.mol^{-1}*)	$\Delta G^{\ddagger a}$ (*kcal.mol^{-1}*)
D-Glucose	26.5	15	22
D-Fructose	22.5	3	21.5
D-Mannose	33.5	38	22.5

[a]Using $\varepsilon_{\text{enediol anion}} = 3000$ (see text).



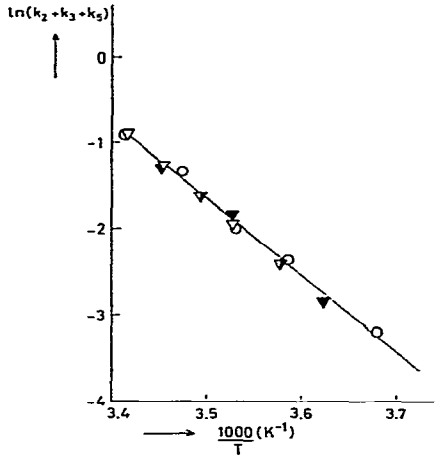


Fig. 9 (left). Arrhenius plots for enediol-anion formation from 0.1M D-glucose (○), 0.0125M D-fructose (▼), and 0.2M D-mannose (▽); 1.22M KOH, N_2, 310 nm, 5-cm cuvette.

Fig. 10 (right). "Arrhenius plot" for enediol-anion conversion for 0.1M D-glucose (○), 0.0125M D-fructose (▼), and 0.2M D-mannose (▽); 1.22M KOH, N_2, 310 nm, 5-cm cuvette.

those determined by ^{13}C-n.m.r. spectroscopy[22] and by a calorimetric method[23], are given in Table III. Apart from the expected variation of the apparent ionisation constant at different ionic strengths, the agreement in the values is quite reasonable.

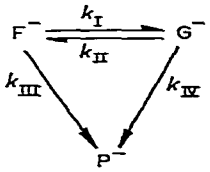
Using equations (*9*) and (*10*), the reaction rate constants for the formation of the enediol anion from the sugar anion (expressed as k_1 as well as $k_1\varepsilon$, because of the uncertainty of ε) and for the conversion of the enediol anion into X^- and β^- as well as into S^- ($k_2 + k_3 + k_5$) have been calculated from the u.v. spectral data. The results for k_1 and ($k_2 + k_3 + k_5$) are summarised in Table IV.

U.v. measurements for D-glucose, D-fructose, and D-mannose between 0–20° give the activation parameters for the enediol-anion formation (Table V), which have been calculated from the Arrhenius plots presented in Fig. 9.

A similar approach has been applied to the overall conversion of the enediol-anion species ($k_2 + k_3 + k_5$). This leads to one common "Arrhenius plot" for these three interconvertible monosaccharides with $\Delta H^{\ddagger} = 17.5$ kcal.mol^{-1} and $\Delta S^{\ddagger} = 0$ e.u. (Fig. 10). In kinetic terms, the interconversion of these sugars may be considered to proceed through one and the same intermediate (enediol anion).

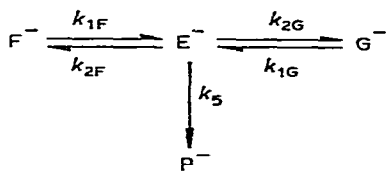
In order to compare the data for the degradation reactions with the literature

and with the present u.v. measurements, some computer simulations have been carried out. Firstly, the use of the overall kinetic model

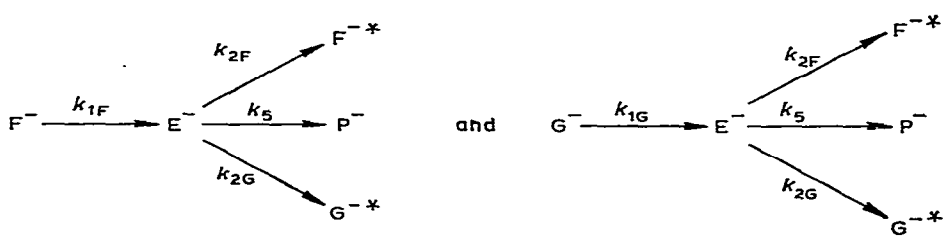


provides the following rate constants: $k_I = 4.7\times10^{-5}$ min^{-1}, $k_{II} = 3.8\times10^{-1}$ min^{-5}, $k_{III} = 6.7\times10^{-5}$ min^{-1},and $k_{IV} = 1.3\times10^{-5}$ min^{-1}. These rate constants fit within Vellenga's compilation of rate data[24].

Such an overall kinetic model would imply that the degradation products arise mainly from D-fructose. However, the fact that both D-glucose and D-fructose yield essentially the same product mixture, as shown in Table II, enables the model to be refined according to



Here, both the β-elimination anion and k_6 have not been included, since the rate-determining step in the product formation is considered to be the conversion of E^- into β^-. In the initial stage of the reaction, as in the H/D exchange experiments, the kinetic model may be considered as:



Here, F^-* and G^-* are D-fructose and D-glucose formed from the enediol anion; these species contain deuterium when the reaction is carried out in D_2O.

From the H/D exchange experiments and from the degradation reactions, we can obtain values of k_{1F}, k_{1G}, k_{2F}, k_{2G}, and k_5 (Table VI). The agreement between these values is reasonable.

TABLE VI

RATE CONSTANTS FROM H/D EXCHANGE AND DEGRADATION

Rate constant	*H/D exchange*	*Degradation*[a]
k_{1F}	5.9×10^{-4} min^{-1}	4.2×10^{-4} min^{-1}
k_{1G}	0.5×10^{-4} min^{-1}	0.5×10^{-4} min^{-1}
k_{2F}	0.09[b] min^{-1}	0.12 min^{-1}
k_{2G}	0.02[b] min^{-1}	0.01 min^{-1}
k_5	0.03[b] min^{-1}	0.03 min^{-1}

[a]Obtained by computer optimalisation on the basis of the data of Fig. 7, using the refined kinetic model.
[b]Calculated from both the k_{2F}/k_5 and k_{2G}/k_5 ratios from H/D exchange (Table I), and the sum ($k_{2F} + k_{2G} + k_5$) from u.v. data (Table IV).

TABLE VII

RELATIVE RATES OF ENOLISATION

Sugar	*T-uptake*[a] (*0.5M Na_2CO_3, 25°*)	*u.v.* (*1.22M KOH, 10.4°*)
D-Mannose	0.5	0.2
D-Allose	0.8	0.2
D-Talose	4.8	0.3
D-Galactose	0.9	0.5
D-Ribose	28.5	0.9
D-Glucose	1.0	1.0
D-Arabinose	4.1	1.2
D-Xylose	7.0	1.3
L-Sorbose	9.3	4.9
D-Fructose	10.7	11.3
D-Tagatose	25	54

[a]Taken from Ref. 1.

Finally, combination of the k values with those obtained from u.v. spectral data gives (*a*) a molar extinction coefficient of the enediol-anion species of $\varepsilon = 3000$ litres. mol^{-1}.cm^{-1}, as derived by dividing the $k_1\varepsilon$ values of D-fructose and D-glucose (Table IV) by k_{1F} and k_{1G}, respectively; and (*b*) the presence of enediol-anion species of the sugar anions amounting to 0.2–0.6%, as indicated by the ratios k_{1F}/k_{2F} and k_{1G}/k_{2G}.

Promoting effect of carbonate ions. — In Table VII, the relative initial rates of enolisation, obtained by Isbell *et al.*[1] by measuring the H/T exchange in T_2O–0.5M Na_2CO_3, are compared with the k_1 values obtained in this study (*cf.* Table IV). The agreement is poor, which may be due, in part, to the different media. In this connection, it may be noted that Gleason and Barker[17] found the rate of enolisation of ribose, measured as the initial rate of oxygen uptake, to be medium-dependent. Their

relative rate of enolisation for ribose in 0.5M Na_2CO_3 agrees with the value of Isbell, whereas the rate in 0.8M KOH agrees with our value. Using Na_2CO_3 as the base (0.5M, pH 11.6), we observed that glucose and ribose show absorption bands at 310 and 270 nm, whereas no absorption could be detected at pH 11.6 with KOH as the base; furthermore, the rate of appearance of these absorptions is $\sim$8 times higher for ribose than for glucose. The nature of this promoting effect of carbonate anions on the enolisation reaction is under further investigation.

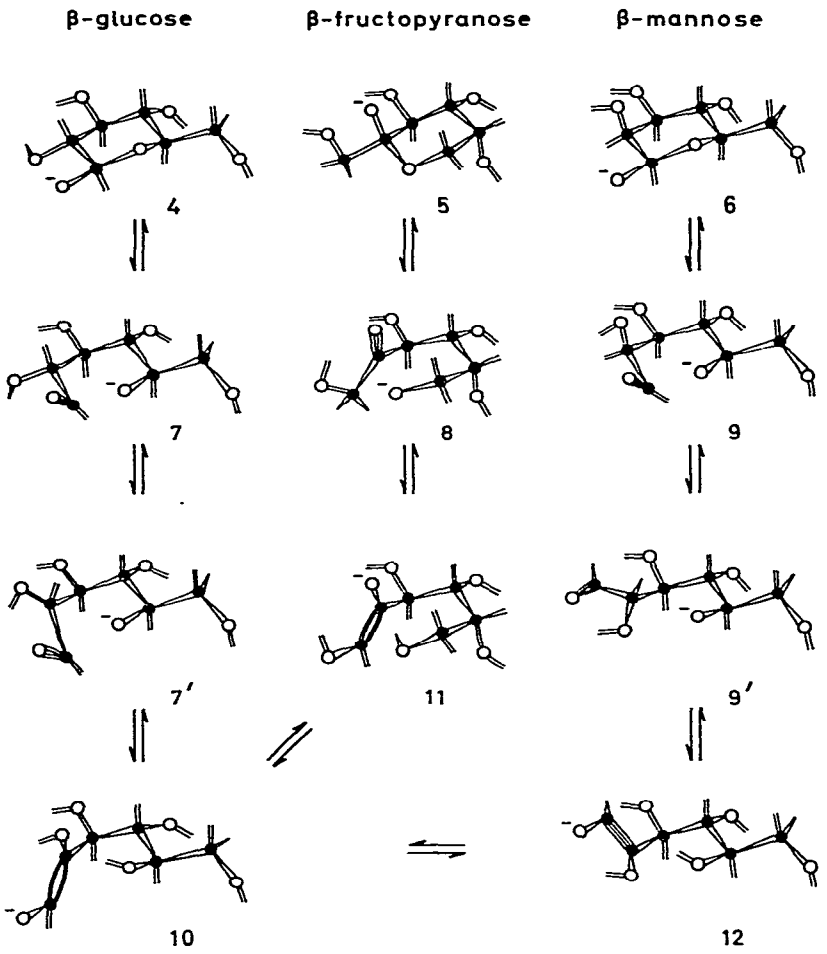
MECHANISTIC CONSIDERATIONS

Isbell *et al.*[1] suggested that one of the factors controlling the rate of enolisation is the concentration of an intermediate carbonyl form of the sugar. Furthermore, it was suggested that the interconversion of epimeric sugars proceeds through pseudo-acyclic intermediates that possess some characteristics of the respective ring forms. These concepts lead to the possible formation of (*Z*)- and (*E*)-enediols, the proportions of which may differ from sugar to sugar.

The occurrence of pseudo-(a)cyclic carbonyl intermediates was confirmed by ^{13}C-n.m.r. spectroscopy[7]. It was shown that the formation of these species is very fast and that a rapid equilibrium exists with the cyclic sugar anion. Glucose, fructose, and mannose show $\sim$5% carbonyl-bond character of their anions, which may be interpreted as the occurrence of $\sim$5% pseudo-cyclic carbonyl structure[7]. In view of these results, the large divergence in rate of enolisation between these sugars (Table IV) cannot be ascribed to differences in concentration of such intermediates. In this respect, the essentially equal ($k_2 + k_3 + k_5$) values of Table IV for such interconvertible sugars as fructose, glucose, and mannose (0.13–0.15), ribose and arabinose (0.58 and 0.61), and galactose, talose, and tagatose (0.48–0.54) support the formation of similar enediol-anion species from these interconvertible sugar anions. Therefore, the mode of formation of the enediol anion from the pseudo-cyclic carbonyl intermediate, rather than the occurrence of (*E*/*Z*)-isomeric enediol-anion structures[1,27,28], will be responsible for the differences in rate of enolisation.

The above considerations led us to modify the molecular picture for the enolisation and isomerisation processes. The interconversion of fructose, glucose, and mannose is taken as an example (Scheme 2).

For simplicity, the α-gluco- and α-manno-pyranose and the α- and β-fructofuranose anomers have been omitted from Scheme 2. Their anions will react through pseudo-cyclic intermediates similar to those depicted for the respective β-pyranose anomers. The present mechanism is based on the principle of least motion and comprises the following main features: (*a*) fast equilibrium between cyclic sugar anions and their pseudo-cyclic carbonyl structures; (*b*) formation of the enediol anion by a rate-determining, intramolecular proton-shift from C-2 (or C-1 for fructose) to the original ring oxygen, leading to pseudo-cyclic (*Z*)-enediol anions; and (*c*) reversal of process (*b*), leading, after conformational changes of the enediol-anion species, to isomerisation.



Scheme 2. Molecular picture of enolisation and isomerisation.

The key step (*b*) of the enolisation process is postulated on the basis of the following arguments. Firstly, the ring oxygen will become negatively charged during the ring opening and is (potentially) suited, in distance and character, to accept H-2 (H-1 for fructose). Co-operation of solvent molecules in this intramolecular proton-shift does not alter the process principally. Secondly, irrespective of whether α or β anomers are involved, a conformation having the carbonyl and the C-2–OH bond (C-1–OH for fructose) nearly eclipsed seems to be preferred, because of stabilising 1,2-hydrogen-bonding[25]. Such a conformation is required during the proton abstraction, in order to delocalise the negative charge on the developing carbanion (*cf.* the selective enediol formation from 1,6-anhydro-β-D-hexopyranosuloses[26]). This situation leads to the 1,2-hydrogen-bonded (*Z*)-enediol-anion species.

Inspection of atomic models shows that the formation of the enediol anion from β-fructopyranose according to the present molecular picture needs hardly any change in the geometry of the pseudo-cyclic species. This conclusion is in agreement with the

relatively low entropy of activation (3 e.u.), whereas the favourable position of the original ring oxygen for the transfer of the proton explains the relatively low enthalpy of activation (Table V). Glucose and mannose require a conformational change of the pseudo-cyclic carbonyl structure by rotation around the C-2–C-3 bond, in order to permit hydrogen transfer from C-2 towards O-5. In this respect, it may be noted that rotations need a substantial reorganisation of the water mantle[29,30]. This conformational change is particularly required for mannose, which is in agreement with its low rate of enediol-anion formation*. The same holds, of course, for the conversion of the enediol anions **10**, **11**, and **12** into the different sugar anions, so that the expected order in k_2 is fructose > glucose > mannose. This conclusion is in agreement with the experimental results from both H/D exchange and degradation reactions. Scheme 2 makes clear that mannose formation from glucose or fructose will be slow compared to the glucose–fructose interconversion. Finally, it may be noted that the α- and β-fructofuranose may be formed from **10** and **12** by similar processes with a minimum of geometrical change.

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*Alternatively, mannose may change from the $^4C_1$ to the less-favourable $^1C_4$ conformation, after which it can give the enediol anion as depicted above for glucose.

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