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A Stopped-flow Polarimetric Study of the Hydroxide Ion Catalyzed Mutarotation of a Series of Glucopyranoses in Water

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The construction and use of a polarimeter unit for a Durrum stopped-flow spectrophotometer is described. It is demonstrated that the apparatus is a good tool for investigating kinetic details of fast optical rotatory phenomena such as the hydroxide ion catalyzed mutarotation of sugars. In a series of glucopyranoses, glucose differs remarkably from its substituted analogues, probably due to the dissociation of two hydroxyl groups as the hydroxide ion concentration is increased. Acidity constants for the sugars are determined conductometrically, but the changes in pK with substituents are too small to justify any quantitative conclusions from Brønsted plots. However, the experimental data do suggest coupled proton transfer in water catalysis, whereas for the hydroxide ion a stepwise mechanism, involving formation of the sugar anion – and for glucose also to some extent the dianion – as an intermediate, is more likely. First order rate constants for the mutarotation of some of the sugar anions and apparent catalytic constants for the hydroxide ion are presented. For glucose, k_{G^-} (25 °C)=2.2 s^{-1} and k_{HO^-} (25 °C)=90±10 dm^3 mol^{-1} s^{-1}. Our kinetic data were best explained by assuming the second dissociation constants of glucose to be no larger than $10^{-14.6}$ and those for the substituted compounds to be considerably smaller.

The mutarotation of monosaccharides such as glucose and its various analogues is no doubt one of the most studied types of reaction in all physical organic chemistry. Change in optical rotation with time for freshly prepared solutions of glucose in water was reported for the first time by Dubrunfaut in 1846,[1] and the mutarotation reaction has played a major role in chemical kinetics ever since, in particular with respect to studies of mechanism in acid-base catalysis. The strong development in this area is obvious from a number of monographs and review articles from the early part of the century and from more recent contributions by Isbell and Pigman.[2] However, it is interesting to note that although an enormous amount of experimental work has indeed been done, kinetic investigations are almost totally limited by the rate capacity of relatively slow techniques such as conventional polarimetry, dilatometry, *etc.*, and the behaviour of relatively strong basic catalysts in mutarotation – requiring faster techniques – has not yet been subject to direct systematic studies. This may seem surprising because stopped-flow polarimeters have now been available for quite some time and have been relatively widely applied in chemical kinetics, though mainly to biological systems. The proper function of a stopped-flow polarimeter is often checked by measuring the rate of glucose mutarotation in *e.g.* 0.5 M aqueous sodium hydroxide, but usually no further attention is paid to the kinetics of the reaction itself. Thus, in testing their stopped-flow polarimeter, Goodall and Cross[3] correctly report a decreasing catalytic constant for the hydroxide ion with increasing concentration of this catalyst, but, as we shall see later, interpret this effect in an ambiguous way as being due to gradual conversion of glucose to its *less* reactive anionic form.

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Extended kinetic studies of sugar mutarotation in strong basic media are of interest for several reasons: (1) Catalytic constants for hydroxide ions and similar species are predominantly known from conventional studies and exhibit large scatter. More direct determinations using a stopped-flow technique are likely to produce more precise values for this constant. (2) Catalytic constants for hydroxide seem to vary with hydroxide ion concentration at high pH.[3] This effect may be reasonably explained by the conversion of the sugar to its anion. (3) In strong alkaline solution several sugar hydroxyl groups are likely to dissociate and thereby affect kinetics. The qualitative and quantitative extent of this behaviour is practically unknown. (4) Considerable extensions of the existing Brønsted plots for base-catalyzed mutarotation of glucose[4] and other sugars are possible if catalytic constants for stronger bases are determined. Brønsted plots covering large ΔpK-ranges often exhibit curvature and thereby reveal important information about reaction mechanisms such as changes in coupling between relevant degrees of freedom or shifts in rate-determining steps. (5) The diagnostic importance of kinetic deuterium isotope effects as a function of ΔpK in studies of mechanisms in acid-base catalysis is well-known, and no reliable data have so far been reported for mutarotation catalyzed by relatively strong bases.

In the present paper we report kinetic data primarily concerning the hydroxide ion catalyzed mutarotation of various glucoses (1–3 in the previous paragraph). A paper dealing with base catalysis in general of these reactions and corresponding kinetic deuterium isotope effects (4–5 in the previous paragraph) will be published shortly.[5]

EXPERIMENTAL

Materials. The following glucopyranoses were used without further purification: α-D-(+)-glucopyranose (α-glucose)(BDH Chemicals, Analar); 2-amino-2-deoxy-α-glucose·HCl (α-glucosamine·HCl)(BDH Biochemicals); 2-acetamino-2-deoxy-α-glucose (*N*-acetyl-α-glucosamine)(BDH Biochemicals); 2-benzamido-2-deoxy-α-glucose (*N*-benzoyl-α-glucosamine) (Sigma Chemical Company); 4,6-*O*-ethylidene-α-glucose (EGA-CHEMIE); 2-*O*-methyl-β- and 3-*O*-methyl-α-glucose were synthesized at the Institute of Organic Chemistry, Technical University of Denmark, and kindly supplied by Professor Chr. Pedersen (the compounds had m.p. 152–153 and 161–162 °C, respectively, and were identified by NMR spectroscopy); 2,3,4,6-tetra-*O*-methyl-α-glucose (TMG) was prepared and tested as described earlier.[6] Other chemicals were of Analar grade, and millipore water from a Milli-Q2 system was used throughout.

Acidity constants. The pK_a values of the anomeric hydroxyl group of the various sugars apart from α-glucosamine·HCl, were determined by a conductivity method described by Bell and Onwood.[7] The electrode unit (CDC104) of a

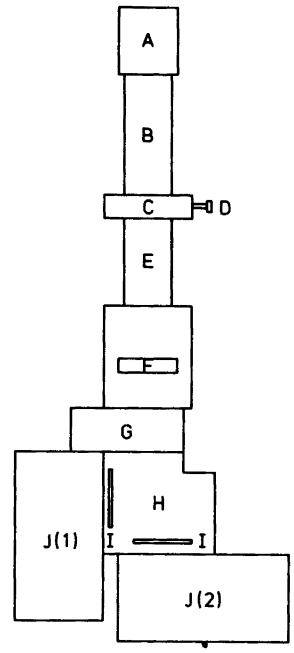


Fig. 1. Schematic drawing of the polarimeter unit fitted on a Durrum stopped-flow apparatus. A. Lamp house with mercury lamp (Hanau, st. 48). B+E. Light-tubes with lenses (Spindler and Hoyer, type 311115 biconvex, 50 mm focal length). C + I. Polarizers (B. Halle, type Glan-Thomsen-II, 7.5 mm aperture). D+I. Polarizer adjustment. F. Interchangable mercury line interference filter (Oriel Corp., type G-522-4358 (435.8 nm) or G-522-5461 (548.1 nm)). G. Reaction cell housing of the Durrum stopped-flow spectrophotometer (20 mm light path). H. Beam splitter (Spindler and Hoyer, type 344161). J. Photodetector houses provided with lenses (Spindler and Hoyer, type 314811, 40 mm focal length) and detectors (EMI, type 9660B).

Radiometer CDM201 conductivity meter was placed in a thermostated solution of the sugar (in excess) in sodium hydroxide, and the conductivity was measured. The solution was carefully protected from atmospheric carbon dioxide by a stream of nitrogen.

Kinetic measurements. Mutarotation rates in pure water were measured by a Perkin-Elmer 141 polarimeter fitted with a 10 cm thermostated reaction cell and data acquisition system. The typical concentration of sugar was 0.01 M, and the 365 nm filter was used in most cases for the polarized light, resulting in relatively large changes in optical rotations.

Mutarotation rates of sugars in alkaline media (pH > *ca.* 10) could conveniently be measured by mixing freshly prepared solutions of the sugar in water (usually 0.08 M) with aqueous sodium hydroxide in a Durrum stopped-flow apparatus equipped with a polarimeter unit built in our laboratory. This unit is shown schematically in Fig. 1, where G denotes the original reaction cell housing of the Durrum instrument. Several stopped-flow polarimeters have been reported in the literature[3,8,9] and may now be regarded as a standard type of apparatus. The application of a dual detector system (Fig. 1) yields enhanced signals when the ratio of the two detector outputs is analyzed. With the polarizers adjusted according to well-defined rules[10] the output voltage from each detector (v_1 and v_2) is closely proportional to the angle of the optical rotation of the solution in the reaction cell. The performance of the apparatus is demonstrated in Fig. 2, where *ca.* two hundred measurements of $(v_1-v_2)/v_1$ – recorded by a data acquisition system – are plotted against time for a reacting solution of glucose. The solid line (hardly visible) is a computer-fitted (least squares) exponential.

A Radiometer pH-meter 28 equipped with calomel electrode K401 and glass electrode G202 B was used for pH-measurements. Standard borate buffer and saturated aqueous calcium hydroxide (25 °C)[11] were used for calibration and pH-values were corrected for errors due to sodium ion where necessary.

All experiments were carried out at 25.0± 0.2 °C and ionic strengths were adjusted to 0.2 by sodium chloride where possible in kinetic runs.

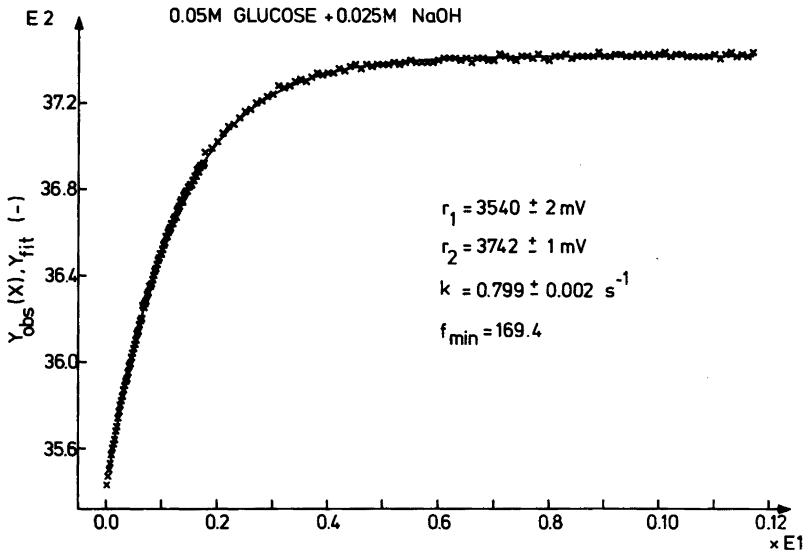


Fig. 2. Plot of output signal ($(v_1-v_2)/v_1$ from J(1) and J(2) in Fig. 1) against time, obtained by mixing 0.05 M glucose with 0.025 M NaOH (0.4 M in NaCl) in the stopped-flow polarimeter at 25 °C and λ=435.8 nm. The observed total change in voltage (~200 mV) correspond to a change of approximately .04 degrees in optical rotation of the sugar solution in the cell. The solid curve represents a computed function (exponential) $r'_t=(r_1-r_2)\exp(-kt)+r_2$ where r_1 (initial voltage), r_2 (final voltage) and k (first order rate constant) are determined by an iterative procedure to give the best fit with the observed points (r_t, t), which means minimization of $f=\Sigma(r_t'-r_t)^2$. The calculated values of r_1, r_2, k and f_{min} in the present case are given in the plot.

Table 1. Dependence of conductivity on sugar concentration in a 2–3×10^{-3} M solution of sodium hydroxide at 25.0 °C. I. Glucose. II. 3-*O*-Methylglucose. III. 2-*O*-Methylglucose. IV. *N*-Acetylglucosamine. V. *N*-Benzoylglucosamine. VI. 4,6-*O*-Ethylideneglucose. VII. TMG.

[Sugar]/ mol dm^{-3}	$10^6 \times \kappa$/ohm^{-1}cm^{-1} I	II	III	IV	V	VI	VII
0.0000	607.6	637.0	646.8	612.5	597.8	399.8	612.5
0.0110	448.8	459.6	461.1	395.9	383.2	242.9	431.2
0.0125	434.4	441.0	450.8	381.2	370.4	232.6	406.7
0.0143	419.4	424.3	433.2	363.6	352.8	221.2	395.9
0.0167	400.8	409.6	411.6	347.9	335.2	210.6	374.4
0.0200	377.3	385.1	392.0	328.3	318.5	198.9	354.8
0.0250	354.8	360.6	364.6	304.8	299.9	185.6	330.3
0.0333	332.2	333.2	342.0	284.2	277.3	170.5	305.8
0.0500	297.9	302.8	307.7	254.8	254.8	157.4	273.4
0.1000	250.9	254.8	259.7	220.5	205.8	135.7	227.4
K_1/K_w [a]	50(5)	60(5)	60(5)	88(5)	93(5)	109(5)	63(5)
pK_1	12.30(4)	12.22(3)	12.22(3)	12.06(3)	12.03(2)	11.96(2)	12.20(3)

[a] Negative intercept in eqn. (1) (Fig. 3).

Table 2. Observed first-order rate constants for mutarotation of some of the sugars in Table 1 in sodium hydroxide solution [Sugar]=4×10^{-2} M, *I* adjusted to 0.20 (NaCl) where [HO^-] <0.20 M, temp.=25.0±0.2 °C, λ=435.8 nm. Each constant is an average from at least 3 single runs (7 for glucose). Numbering of the sugars refer to Table 1.

$10^2 \times$[HO^-]/[a] mol dm^{-3}	$10^2 \times k_{obs}$/ s^{-1}	$10^2 \times$[HO^-]/[a] mol dm^{-2}	$10^2 \times k_{obs}$/ s^{-1}
Glucose (I)			
0.038	3.61(9)	20	420(5)
0.067	6.63(6)	22	475(6)
0.098	9.90(6)	24	510(8)
0.600	35.0(4)	25	500(7)
1.01	70.1(4)	27	500(10)
2.01	120(4)	29	595(11)
3.0	150(3)	30	550(5)
4.0	200(8)	32	605(6)
6.2	215(7)	34	645(5)
8.0	240(6)	35	600(7)
8.4	250(5)	37	650(5)
9.6	300(6)	39	700(6)
11.0	315(4)	40	700(5)
13.2	360(8)	42	725(9)
13.8	362(8)	44	875(15)
15	375(9)	45	750(9)
17	395(4)	48	770(12)
18	400(6)	50	850(9)

Table 2. Continued.

N-Acetylglucosamine (IV)			
0.172	26.5(5)	36	119(1)
0.600	56(1)	46	122(3)
1.78	84.7(4)	56	121(2)
5.12	108(2)	66	125(1)
6.0	106(1)	76	128(6)
16	114(1)	86	114(2)
26	116(1)	96	116(2)
2-*O*-Methylglucose (III) [b]			
0.229	14(2)	26	138(10)
0.389	27(4)	36	148(15)
0.954	54(3)	46	198(34)
2.24	74(3)	56	152(32)
5.3	116(5)		
11.7	138(11)		
3-*O*-Methylglucose (II)			
0.229	26(1)	12.6	229(8)
0.407	45(1)	26	240(2)
0.976	89(1)	36	247(3)
2.09	135(3)	46	265(3)
5.12	196(1)	56	277(1)
4,6-*O*-Ethylideneglucose (VI)			
0.051	950(30)	0.544	6900(400)
0.105	1500(100)	1.74	10700(500)
0.166	3700(700)	4.49	17700(1600)

[a] Below 0.2 M, hydroxide ion concentrations were obtained from the pH of the reacting solutions and an activity coefficient (f_{HO^-}) calculated according to Kielland.[16] The higher values are derived by subtracting 0.04 M, due to neutralization by the sugar, from the actual hydroxide ion concentration. There was good agreement between calculations from either method in the transition area and the uncertainties are in all cases believed to be less than 5–10 %. [b] At low hydroxide ion concentrations this compound behaves "normally" but the relatively large errors associated with k_{obs} at higher [HO^-] reflect a substantial decrease in total change of optical rotation under these conditions, the reason for which is not understood.

RESULTS AND DISCUSSION

Acidity constants. It has been shown that the acidity constant of a weak acid such as formaldehyde hydrate can be determined fairly accurately by measuring the conductivity of solutions where the acid is partly neutralized by sodium hydroxide.[7] Under circumstances where less than a few percent neutralization has taken place the following expression holds:

$$1/a = \frac{K_1 K_w^{-1}(l_{HO^-} - l_{A^-})}{l_{Na^+} + l_{HO^-}} \cdot \frac{\kappa_{NaOH}}{\kappa_{NaOH} - \kappa} - K_1 K_w^{-1} \quad (1)$$

where a denotes the total concentration of acid; l_{OH^-}, l_{A^-} and l_{Na^+} are specific conductivities (mobilities) of the various ions in solution (A^- is the acid anion); κ_{NaOH} and κ are conductivities of the aqueous sodium hydroxide before and after addition of acid, and K_1 the acidity constant for HA. Eqn. (1) predicts linearity between $1/a$ and $\kappa_{NaOH}/(\kappa_{NaOH} - \kappa)$ where $K_1 K_w^{-1}$ is given by the intercept. Bell and Onwood [7] found the pK_1 of formaldehyde hydrate to be 13.27 at 25 °C. By repetition of their experiment we obtained a value of 13.26 and, furthermore, found that the method is also readily applicable to sugars such as the glucoses investigated in this paper. Table 1 contains conductivity measurements for these compounds.

Fig. 3 shows plots * according to eqn. (1) for these measurements and the resulting pK_1 values are given in Table 1. Because of the relatively alkaline conditions under which the pK_a values are determined – leading to equilibrated solutions in all cases – these are weighted means of pK_1 (α-form) and pK_1 (β-form) in each case.

The acidity constant K_1 for glucose was determined as early as 1900 by Osaka,[12] who found pK_1=11.89 at 25 °C. Our value of 12.30 for glucose is in excellent agreement with pK_1=12.28 found as a weighted average of pK_1 (α-form)=12.47 and pK_1 (β-form)=12.17 by Los and Simpson [13] as the result of reliable potentiometric studies. For TMG Wit *et al.*[14] have recently reported a pK_1 of 12.2 determined by ^{13}C NMR ([TMG]=0.53 M, temp. 3–5 °C) compared with our value of 12.20 at 25 °C for this compound.

For the remaining sugars in Table 3, only the pK_1 of glucosamine is available in the literature (12.66 and 12.34 for α- and β-forms, respectively, at 5 °C).[15] We were not able to investigate this species by the conductivity method, due to the presence of HCl, but a pK_1 value of approximately 12.0 (mean) is obtained at 25 °C from the data at 5 °C by the van't Hoff equation if ΔH for the acid dissociation is taken as 42 kJ mol^{-1}, which is the experimental value found for glucose.[13] This result seems reasonable compared to $pK \sim 12.05$ for the two substituted glucosamines.

Kinetics. Kinetic results from measurements of the spontaneous (water catalyzed) mutarotation of the sugars by conventional polarimetry are shown in Table 4 and those for hydroxide solutions by stopped-flow technique in Table 2.

All data from Table 2 are plotted in Fig. 4, from which the non-linearity of each plot is

* For some of the sugars considerable curvature of the plots were observed – especially at higher sugar concentrations – and we discovered this to be caused by small amounts of conducting impurities in the sugar, which could be corrected.

Table 3. Statistical fitting of data for glucose in Table 2 to expression (3). Values and standard deviations of k'_β and k''_β determined for varying K_2. Sum of squares refers to deviations along the k_{obs}-axis.

pK_2	k'_β/s^{-1}	k''_β/s^{-1}	Sum of squares/s^{-2}
13.7	0.3(2)	3.4(4)	4.93
14.0	0.4(2)	4.2(5)	3.74
14.2	0.4(1)	6.6(8)	2.90
14.3	0.5(1)	7.8(9)	2.76
14.6	0.5(1)	14(2)	2.54
14.9	0.5(1)	25(3)	2.47
15.2	0.5(1)	48(6)	2.44
15.5	0.5(1)	94(12)	2.43
15.8	0.5(1)	187(24)	2.43
16.5	0.5(1)	925(121)	2.43

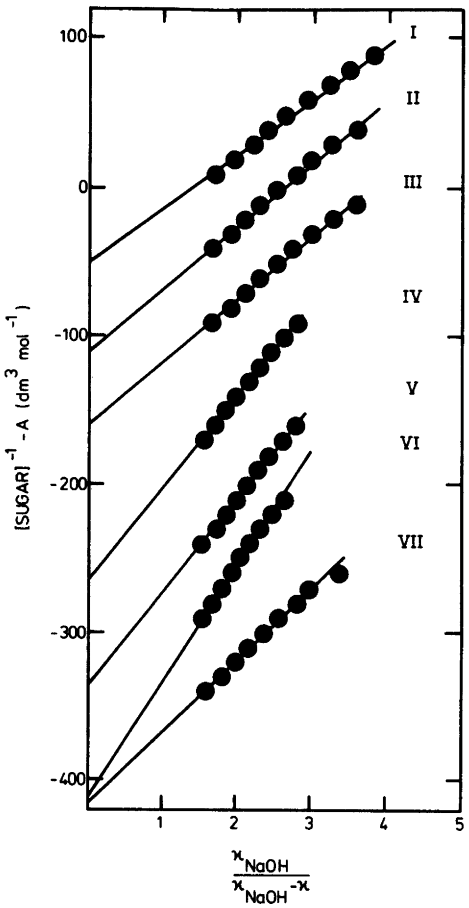


Fig. 3. Plots according to Table 1 and eqn. (1) and from which pK_1 of the various sugars can be evaluated. I. Glucose (A=0). II. 3-*O*-Methylglucose (A=50). III. 2-*O*-Methylglucose (A=100). IV. *N*-Acetylglucosamine (A=180). V. *N*-Benzoylglucosamine (A=50). VI. 4,6-*O*-Ethylideneglucose (A=300). VII. TMG (A=350).

Table 4. Collected thermodynamic and kinetic data for the various sugars investigated. I=0.20 (NaCl), temp.=25.0±0.2°C. Numbering of the sugars refer to Table 1.

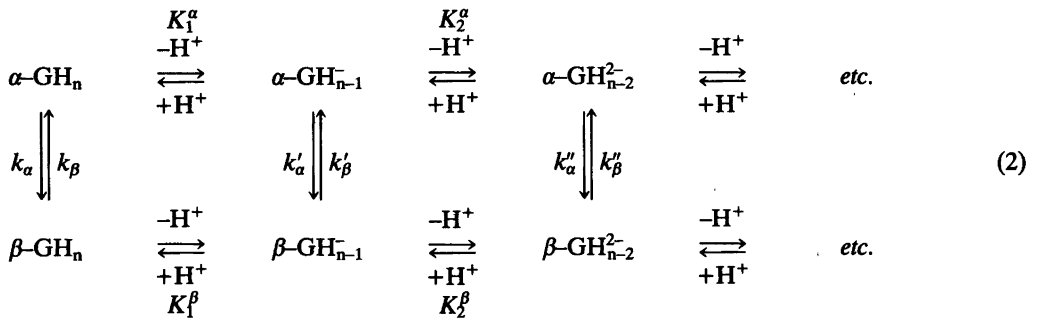
Sugar	pK_1[a]	$10^4 \times k_G$/[b] s^{-1}	$k_{G^-} = k'_\alpha + k'_\beta$/[c] s^{-1}	$k_{G^-} = k'_\alpha + k'_\beta$/[d] s^{-1}	k_{HO^-}/[e] $dm^3\ mol^{-1}\ s^{-1}$
Glucose (I)	12.30(4)	4.00(3)	2.2[f]	2.2[f]	91[g]
2-*O*-Methyl-glucose (III)	12.22(3)	5.02(2)	1.7	1.2	72
3-*O*-Methyl-glucose (II)	12.22(3)	3.53(3)	2.8	2.1	127
TMG (VII)	12.20(3)	3.68(8)	–	0.5[h]	31[i]
N-Acetyl-glucosamine (IV)	12.06(3)	5.17(3)	1.2	2.3	200
N-Benzoyl-glucosamine (V)	12.03(2)	5.08(5)	–	–	–
4,6-*O*-Ethyl-ideneglucose (VI)	11.96(2)	6.4(1)	215	172	19000

[a] From Table 1. [b] Rate constants for mutarotation in pure water, averages from at least four runs. [c] From intercepts in Fig. 5. [d] From slopes of Fig. 5 and pK_1 values in Table 1 (see expression (5)). [e] Calculated from expression (7). [f] From $k'_\alpha + k'_\beta = 3.42\ k'_\beta + k'_\beta = 4.42\ k'_\beta$, where $k'_\beta = 0.5$ according to Table 3. [g] Calculated from expression (6). [h] Calculated from k_{HO^-} and pK_1 according to expression (7). [i] Taken from Ref. 6.

obvious as well as certain differences in individual shapes of these. Previously obtained stopped-flow data for glucose [3] are also indicated for comparison.

The first order mutarotation rate constants in Table 2 were all obtained at a constant and relatively low sugar concentration (0.04 M) to suppress catalysis by the sugar anion. As shown as early as 1925 by v. Euler and Ölander [17] the catalytic properties of this species must also be taken into account in alkaline solution, although the effect is strongly masked by the hydroxide ion. As catalysis of mutarotation by bases in general is dealt with in another paper [5,18] this subject will not be treated further here.

Rate expressions. The fact that the plots of observed rate constants in Table 2 (Fig. 4) do not exhibit a linear increase with hydroxide ion concentration but level off at more or less the same value of $[HO^-]$ – corresponding to 50 % "titration" of the anomeric hydroxyl group – may suggest a preequilibrium mechanism in alkaline solution *i.e.* rapid formation of the glucose anion, which then mutarotates in a second rate-determining step. In the case of glucose the deprotonation of more than one hydroxyl group, leading to



(2)

Scheme 1.

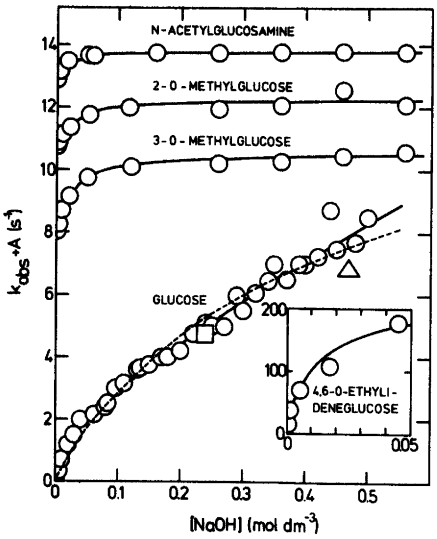


Fig. 4. Dependence of observed first order rate constants for the mutarotation of some glucoses on hydroxide ion concentration. The plots contain all data from Table 2 except a few which are omitted for clarity. The solid curves are drawn according to Eqns. (4) and (5) and figures from Table 4 for the substituted glucoses, whereas eqn. (3) is employed in the case of glucose as described in the text. For glucose pK_2 was chosen as 14.6, and higher values (Table 3) do not change the position or shape of the fitted curve substantially. However, when lower pK_2 values are taken bad fits are obtained as seen from the broken curve which represents pK_2=13.7. *N*-Acetylglucosamine (A=12.6), 3-*O*-methylglucose (A=7.8), 2-*O*-methylglucose (A=10.6), Glucose (A=0), 4,6-*O*-Ethylideneglucose (A=0). △,□ Ref. 3.

anions more reactive than the monoanion, can readily explain the steady increase of the curve for this compound with hydroxide ion concentration.

This mechanism now results in a general scheme for the mutarotation process (Scheme 1),* where mutarotation rate constants for the various protolytic forms of the sugar are indicated. K_1 and K_2 are acid dissociation constants and, as we shall see later, the experimental data can only justify the consideration of two successive dissociative steps, *i.e.* in the case of glucose.

If it is assumed that the protolytic reactions in eqn. (2) are always at equilibrium it can be shown that the observed first order rate constant from the rate expression for mutarotation, $v=|d\alpha/dt|=k_{obs}([\alpha_\alpha]_\lambda^{25}[\alpha\text{-G}]+[\alpha_\beta]_\lambda^{25}[\beta\text{-G}])\cdot l$ $[\alpha_\alpha]_\lambda^{25}$ and $[\alpha_\beta]_\lambda^{25}$ being specific optical rotations and l the light path, is given by expression (3):

$$k_{obs}=\frac{k_\alpha K_w^2+k'_\alpha K_1^\alpha K_w[HO^-]+k''_\alpha K_1^\alpha K_2^\alpha[HO^-]^2}{K_w^2+K_1^\alpha K_w[HO^-]+K_1^\alpha K_2{}^\alpha[HO^-]^2} + \frac{k_\beta K_w^2+k'_\beta K_1^\beta K_w[HO^-]+k''_\beta K_1^\beta K_2^\beta[HO^-]^2}{K_w^2+K_1^\beta K_w[HO^-]+K_1^\beta K_2^\beta[HO^-]^2} \quad (3)$$

It is now appropriate to divide further treatment of the experimental data in Table 2 (Fig. 4) into two groups: (1) glucose and (2) other sugars.

Glucose. The behaviour of this substance clearly deviates from the other sugars and it is necessary to apply eqn. (3) in full. In this expression the constants k_α, k_β, K_w, K_1^α and K_1^β are known already* for glucose and can be inserted. The remaining parameters k'_α, k'_β,k''_α, $k''_\beta K_2^\alpha$ and K_2^β may now be determined by a statistical analysis where the data in Table 2 (Fig. 4) are fitted to expression (3). However, although the data set for glucose in Table 2 are rather extensive – at least compared to the other sugars – it is clearly not sufficiently detailed and accurate for an unambiguous determination of all these constants, not even when further limitations are introduced, *e.g.* that

$k'_\alpha=k'_\beta\times k_\alpha K_1^\beta/k_\beta K_1^\alpha=3.42k'_\beta$, $k''_\alpha=3.42k''_\beta$ (assumed) and $K_2^\alpha\approx K_2^\beta\approx K_2$ (mean value). We have tried to vary K_2 systematically over a range of realistic values (pK_2=13.7–16.5) and then see how the calculated values and accuracies of the fitted k'_β and k''_β 's are affected. The results are given in Table 3 and it is clear from these that k'_β stabilizes at a value of $k'_\beta=0.5\pm0.1$ s^{-1} for pK_2 values higher than 14.2.

The second rate constant k''_β is, however, very uncertain and no clear minimum is reached in the

* The excellent first order behaviour always observed experimentally suggests that the consideration of a more complicated kinetic scheme, involving a consecutive reaction with the chain form of the sugar as an intermediate,[2b] is unnecessary under the present conditions.

* $k_G=k_\alpha+k_\beta$ = 4.00×10^{-4} s^{-1} (Table 4) and k_α/k_β = 1.71 [2] leading to $k_\alpha=2.52\times10^{-4}$ s^{-1} and k_β = 1.48×10^{-4} s^{-1}. K_w is taken as $10^{-14.00}$ while K_1^α and K_1^β are equal to $10^{-12.47}$ and $10^{-12.17}$, respectively.[13]

sum of squares even when an unreasonably high pK_2 value is employed. The only way to overcome this problem is to choose a likely value for pK_2 of glucose – apparently higher than 14.2 – and then accept the fitted value for k''_β. Two estimates of pK_2 for glucose have been reported in the literature: 13.8 [19a] and 13.9 [19b] both at 25 °C and determined by combined measurements of conductivities and hydrogen ion concentrations of alkaline glucose solutions. These rather qualitative values are lower than suggested by our results, but the discrepancy seems to be systematic, since the same authors also found pK_1=12.11 and 12.09, respectively, *vs.* our pK_1=12.30. We think that a pK_2 similar to pK_1 for ethylene glycol (14.77) [20] or rather glycerol (14.40) [20] is not unreasonable, although more specific experimental work is needed to verify this (see also next paragraph).

Other sugars. There is a pronounced difference in kinetic behaviour of glucose and its substituted analogues. Apart from 4,6-*O*-ethylideneglucose, the mutarotation of which is too fast to be followed at high hydroxide concentrations, all the substituted glucoses clearly exhibit almost completely $[HO^-]$-independent k_{obs} values at high $[HO^-]$. This situation can be accounted for by omitting the third terms of the numerators in eqn. (3) and probably also of the denominators as this would otherwise ultimately lead to decreasing k_{obs} for increasing $[HO^-]$ which is not observed. We see no immediate reason why k''_α and k''_β should be considerably smaller for substituted glucose than for unsubstituted with no distinction between different substituent sites and we therefore tend to believe that the explanation is to be sought in a gradually decreasing K_2 as glucose is successively converted into mono-, di-, *etc.*, substituted derivatives, qualitatively like the trend of K_1 in the series glycerol, ethylene glycol and propanol. If this is the case expression (3) reduces to eqn. (4):

$$k_{obs} = \frac{k_\alpha K_w + k'_\alpha K_1^\alpha [HO^-]}{K_w + K_1^\alpha [HO^-]} + \frac{k_\beta K_w + k'_\beta K_1^\beta [HO^-]}{K_w + K_1^\beta [HO^-]} \quad (4)$$

The hydroxide ion concentrations applied in the stopped-flow measurements are always high enough to justify neglect of $k_\alpha K_w$ and $k_\beta K_w$ (deriving from water catalysis) in eqn. (4), and if K_1^α and K_1^β are both replaced by K_1, which is the usual weighted average value that has been found experimentally, expression (4) can be further reduced to a double reciprocal linear equation as follows:

$$1/k_{obs} = \frac{K_w}{(k'_\alpha + k'_\beta)K_1} \times 1/[OH^-] + 1/(k'_\alpha + k'_\beta) \quad (5)$$

from which a plot of $1/k_{obs}$ *vs.* $1/[HO^-]$ should give $k'_\alpha + k'_\beta$ as well as K_1.* Such plots are shown for the various sugars in Fig. 5 ** and the respective values of $k'_\alpha + k'_\beta$ calculated from the intercepts and from the slopes – using experimentally determined K_1 values from Table 1 – are given in Table 4 together with other relevant data. This sum, which is actually the observed rate constant for mutarotation of purely monoanionic sugars, seems to be somewhat dependent on the method of calculation, although obviously not in a uniform way. We believe that the values deduced from the *slopes* of eqn. (5) are the most reliable and these are therefore used in subsequent calculations of k_{HO^-} (next paragraph).

Apparent catalytic constants for the hydroxide ion (k_{HO^-}) can be deduced from eqn. (3) by considering conditions under which $[HO^-]$ is low, *i.e.* eqn. (3) reduces to eqn. (6):

$$k_{obs} = (k'_\alpha K_1^\alpha + k'_\beta K_1^\beta) \times [HO^-] = k_{HO^-}[HO^-] \quad (6)$$

where again terms referring to water catalysis are omitted. If K_1 is only known as an averaged value, eqn. (6) becomes eqn. (7):

$$k_{obs} = (k'_\alpha + k'_\beta) K_1 K_w^{-1} \times [HO^-] = k_{HO^-}[HO^-] \quad (7)$$

A value for k_{HO^-} of glucose equal to 91 dm^3 mol^{-1} s^{-1} is determined from eqn. (6), and should be preferred to k_{HO^-}=110 dm^3 mol^{-1} s^{-1} according to eqn. (7). As mentioned earlier in this paper

* As expected the deviation from linearity for glucose at high hydroxide ion concentrations is pronounced.

** In principle, K_1 can be calculated from the kinetic results as K_1=intercept×K_w/slope according to eqn. (5). The following values are obtained for pK_1: II(12.34), III(12.38), IV(11.78) and VI(12.05) (for numbering see Table 1). Qualitatively these figures are of the right order of magnitude although presumably much more inaccurate than the constants derived more directly from conductivity measurements.

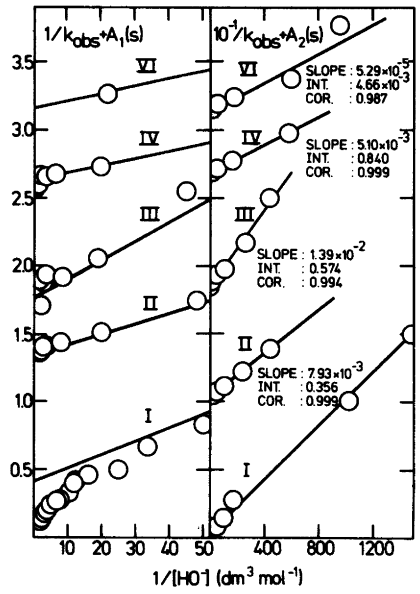


Fig. 5. Plots according to eqn. (5) for the sugars in Table 2 (a few points are omitted for clarity). I. Glucose. II. 3-*O*-Methylglucose (A_1=1.0, A_2=1.0). III. 2-*O*-Methylglucose (A_1=1.2, A_2=1.8). IV. *N*-Acetylglucosamine (A_1=1.8, A_2=2.6). VI. 4,6-*O*-Ethylideneglucose (A_1=2.7, A_2=3.1, 10^{-2} k_{obs} on Y-axis) (notations follow Table 1). Results from linear regression treatments are given in the plots. It is clear that glucose fails to obey eqn. (5) as expected (deviation from linearity at large hydroxide ion concentrations). The plot is divided into two parts to allow different scalings. A_1 and A_2 are constants chosen arbitrarily so as to arrange the plots conveniently in the diagram.

a number of different values of k_{HO^-} have been presented in the literature throughout the years: $k_{HO^-}^{25°C}$ (dm^3 mol^{-1} s^{-1})=374,[21] 359,[22] 165,[23] 83,[13] 91,[24] 80,[25] 148,[26] 230,[27] demonstrates this. Taking into account the uncertainties due to neglect of possible catalytic effects by the sugar anion we think that a value of k_{HO^-} for glucose at 25 °C in the range 80–100 dm^3 mol^{-1} s^{-1} ($k_{HO^-}^{25°C}$=90±10 dm^3 mol^{-1} s^{-1}) has now been rendered most probable.

The data in Table 4 represent a case not often met with in general acid-base catalysis, namely a situation where the catalyst (H_2O or HO^-) is kept constant in a series of reactions where the acid-base strength of the *substrate* is changed systematically by substitution. However, the change in p*K*-value of the sugars is far too small to permit any quantitative conclusions to be drawn from Brønsted plots. Nevertheless, an interesting qualitative difference between water and hydroxide ion catalysis may be noticed, *i.e.* for water catalysis the data seem to obey a usual Brønsted relationship with $0<\alpha<1$, indicating at least partly rate-determining proton transfer possibly in a coupled or concerted mechanism, while the situation is quite different for hydroxide ion catalysis, where the large increase in k_{HO^-} as glucose is converted into the 4,6-*O*-ethylidene derivative is incompatible with rate-determining proton transfer and rather suggests, as pointed out earlier, a stepwise mechanism with diffusion controlled proton transfer and rate-determining ring opening of the glucose anion. The fast mutarotation of 4,6-*O*-ethylideneglucose compared to glucose is presumably due to a weakening of the hemiacetal C–O bond in the ring imposed by the strain creating ethylidene bridge.

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