The Mutarotation of D-Glucose and Its Dependence on Solvent*

I. Studies of Reaction and Equilibrium in the Mutarotation Catalyzed by Water

FREDERICK GRAM, JOHAN A. HVEDING and ANDREAS REINE

Department of Chemistry, University of Oslo, Oslo 3, Norway

The rate of anomerization and the equilibrium of D-glucose at 20, 30, and 40°C were determined in water and in water-DMF mixtures of high water content. Only in such mixtures was the mutarotation found to be simple. The specific rotations of the pyranoses were determined also for mixtures with lower concentration of water. GLC technique was used to correct for anomeric impurity. For the temperature interval $20-30°C$ ΔH for the $\alpha \rightarrow \beta$ conversion in water was found to be about -280 cal mol^{-1}. This value agrees better with earlier thermochemical results than with ΔH values earlier determined polarimetrically.

Equations for a two-step reversible path give an expression for the "mutarotation constant" k as a function of the elementary rate constants k_{12} and k_{32}. The experimental data found for the change in k with water concentration in the water/DMF system, and also those found by other authors in the water/dioxane and water/methanol systems, seem to indicate that the ring opening reaction steps are second order in water. The results give some support to the Lowry idea of a concerted acid-base mechanism for the special case of catalytic action of water.

Quantitative investigations of the reactions[1] involved in the mutarotation** of free aldoses have usually been carried out with aqueous solutions. The intention of the present investigation has been primarily to study the influence of the water concentration upon the anomerization reaction of D-glucose. To this end we have employed the classical method of varying the concentration of the catalytic solvent by dilution with another liquid. The diluent we have chosen, N,N-dimethylformamide (DMF), was assumed

---

* Presented in part at the 11th National Meeting of the Norwegian Chemical Society, June 1970, and a short abstract was printed in *Tidsskr. Kjemi, Bergvesen Met.* **30** No. 6 (1970) 8.

** The term mutarotation is here throughout used in its original meaning to denote a change in the optical rotation of a solution.

to have, in both the pure state and in mixtures, very little catalytic influence on the anomerization.

The mechanism of the acid-base catalysis in mutarotation reactions and in other proton transfer reactions has been a subject of great interest. The idea of a concerted push-pull mechanism was first proposed by Lowry [2] to explain the result on tetramethylglucose in nonaqueous solvents.[3] According to this mechanism the reaction should be trimolecular with the substrate, an acid, and a base, giving for the total rate of reaction

$$v = \{S\} \sum_i \sum_j k_{ij} \{A_i\} \{B_j\}$$

and resulting in second order catalyst terms. According to Pedersen [4] the reactions showing acid-base catalysis proceed in two bimolecular steps. The rate expression in this case should give first order catalyst terms:

$$v = \{S\} \sum (k_A \{A\} + k_B \{B\})$$

Dawson and Spivey,[5] examining the enolization of acetone in acetic acid/acetate ion buffered aqueous solutions, found a small catalytic product term containing $\{AcOH\}\{AcO^-\}$. Pedersen, however, has held this term to be much smaller than could be predicted on the basis of a concerted mechanism. In 1950 it was shown by Swain [6] that Pedersen's argument is not valid since it ignores the experimental equivalence of some kinetic terms. Some more recent experiments by different workers have brought Swain and his collaborators [7] to doubt the general validity of Lowry's concept, especially the catalysis of the anomerization of tetramethylglucose by pyridine and pyridinium ion in aqueous acetone. No second order catalyst term was detected in this reaction. Another example mentioned in which such terms are said to be conspicuously missing is the anomerization of D-glucose in buffered aqueous solutions of varying methanol content, studied by Hill and Thumm.[8] We believe, however, that the way in which these authors tested the kinetic order of water is somewhat unsatisfactory. The amphiprotic properties of methanol ought not to have been neglected in testing the *third* order kinetics as combined action of water and methanol may well be of importance (see Discussion).

The conversion of α-D-glucopyranose (α-gl$p$) to the β-pyranose form (β-gl$p$) and *vice versa* is now generally accepted to pass through the aldehyde form (μ), the ring openings being the slow, rate-determining reaction steps. The reaction scheme may thus be written

$$\alpha\text{-gl}p \underset{k_{21}}{\overset{k_{12}}{\rightleftarrows}} \mu \underset{k_{32}}{\overset{k_{23}}{\rightleftarrows}} \beta\text{-gl}p$$
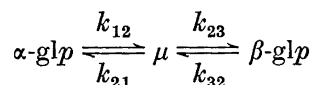
The solution [9] of the differential equations for this scheme leads, when k_{21}, $k_{23} \gg k_{12}$, k_{32},[10] to the following expression for the rotation at the time t: $\gamma_t = \gamma_\infty + (\gamma_0 - \gamma_\infty) \exp(-kt)$, where

$$k = (k_{12}k_{23} + k_{32}k_{21})/(k_{21} + k_{23}) \tag{1}$$

The β/α equilibrium constant (K) is given by

$$K = k_{12}k_{23}/k_{32}k_{21} \tag{2}$$

Using the numerical values obtained by polarography,[10] the maximum concentration of μ[11] is found to be less than 0.01 % of the total sugar concentration. Under such conditions K can be determined experimentally from the well known formula

$$K = ([\gamma_\alpha]_\lambda^T - [\gamma_\infty]_\lambda^T)/([\gamma_\infty]_\lambda^T - [\gamma_\beta]_\lambda^T) \tag{3}$$

Combining eqns. (1) and (2), we arrive at

$$k = [(K+1)/K]/(1/Kk_{32} + 1/k_{12}) \tag{4}$$

Eqn. (4) relates the rate constants k_{12} and k_{32} of the ring opening reaction steps to the "mutarotation constant" (k) and equilibrium constant (K) found experimentally (see Discussion).

EXPERIMENTAL

Materials. α-D-Glucose (Eastman Kodak Co., *anhydrous*) and β-D-glucose (Koch-Light, *pure*) showed both $[\gamma_\infty]_D^{19.99} = +52.67 \pm 0.04°$ (c 2.998, water), in accordance with literature values. The anomeric purity was tested by GLC. We used a method of trimethylsilylation similar to that described by Sweeley *et al.*[12] To be quite sure that no anomerization takes place before the silylation the reagents, hexamethyldisilazane and trimethylchlorosilane, were added to the solvent, DMF, previous to the addition of the sugar. The "α-D-glucose" was found to contain as a mean 4.3 % (4.2, 4.4, 4.2, 4.4) of β-D-glucose and the "β-D-glucose" as a mean 1.26 % (1.21, 1.27, 1.31) of α-D-glucose. However, instead of using purification procedures, which according to literature are assumed to leave some uncertainty as to the anomeric purity, we preferred to use the commercial glucose products and correct for the contents of the opposite anomer.

N,N-Dimethylformamide (E. Merck AG, *Uvasol*) was found by GLC to contain *ca.* 0.05 wt. % water. The liquid was stored in a dark, tightly stoppered bottle in a refrigerator. Distilled water was used in the mutarotation experiments.

Instruments. A Perkin-Elmer 141 digital polarimeter, with a glass standard cell of 5 ml volume and 1 dm length, was used for the measurements of optical rotation. All measurements were made at 589 nm and the γ values for the equilibrium solutions were estimated to be correct to $\pm 0.001°$.

Two Colora ultrathermostats were used for temperature control: (a) A Colora TS of water volume 22 l and pumping speed 16 l/min was used to supply the water jacket of the polarimeter cell through an isolated rubber tube. The tolerance quoted for this instrument was $\pm 0.005°C$. (b) A Colora KT 10 was used as a fore-thermostat to circulate water through the cooling spiral of the precision thermostat.

The thermometer (Thermo-Schneider, 0.1°C division) was compared with a calibrated platinum resistance thermometer and from several readings was found to show a one hundredth of a degree higher temperature. We judge the temperature data given in this paper to be the true temperatures to within $\pm 0.01°C$.

For the analysis of anomeric purity we used a Varian Aerograph 1200, equipped with a flame ionization detector. The column was stainless steel, 150 cm × 3 mm, containing 5 % SE-30 on Chromosorb W, and kept at 160°C.

Procedures. A careful experimental technique, especially in order to find correct initial rotations, was required. The calculated weight quantities of water and DMF to give a certain composition were mixed in a glass-stoppered flask and brought, in the thermostat, to the desired temperature. A quantity of sugar to give a concentration of 3 g per 100 ml solution was then added. With agitation of the flask in the thermostat the dissolution took only about 15 sec in the case of water and some longer time in the case of mixed solvents. The preheated polarimeter cell was quickly filled with the solution and readings began. Time was measured with a stop watch. The initial rota-

tions, γ_0, found by extrapolation to zero time of course have some limit to their accuracy caused by the time required for dissolution. To enable an accurate calculation of the concentrations in weight/volume units the densities of the various sugar solutions at the experimental temperatures were determined separately.

Measurements of mutarotation were carried out at three temperatures, 19.99, 29.99, and 39.99°C, with water, DMF, and 12 water-DMF mixtures as solvents. The "mutarotation constant" (k) was calculated from $\ln(\gamma_t - \gamma_\infty) = -kt + \ln(\gamma_0 - \gamma_\infty)$ by a least squares method. The values of the variable $\ln(\gamma_t - \gamma_\infty)$ were given weight according to $(\gamma_t - \gamma_\infty)$.[13] Calculations of the k values and their standard deviations were performed on a CDC 3300 computer.

RESULTS

The calculations showed that the mutarotation follows the simple logarithmic law only when the mol fraction (x_w) of water in the solvent was higher than about 0.7. Mixtures with lower water content showed an important deviation from simple linearity. The mutarotation in DMF-rich solvents has been subject to further investigations.[14]

Specific rotations. Knowledge of the anomeric composition of both species used in our experiments made it possible to calculate the specific rotations, $[\gamma_\alpha]_D{}^T$ and $[\gamma_\beta]_D{}^T$, of pure α-D-glucose and pure β-D-glucose from polarimetrically determined initial specific rotations S_α and S_β. Assuming the measured rotations to be linear functions of the composition, the equations $0.957 [\gamma_\alpha]_D{}^T + 0.043[\gamma_\beta]_D{}^T = S_\alpha$ and $0.0126 [\gamma_\alpha]_D{}^T + 0.9874[\gamma_\beta]_D{}^T = S_\beta$ are valid. The S values are obtained ($S = \gamma_0/\varepsilon d$) from the initial rotations γ_0 determined polarimetrically, the weight fraction ε of the sugar, and the solution density d. Table 1 contains $[\gamma_\alpha]_D{}^T$ (columns 2, 3, and 4) and $[\gamma_\beta]_D{}^T$ (columns 5, 6, and 7) at the different mol fractions and temperatures. $[\gamma_\infty]_D{}^T$ for the solutions showing simple mutarotation are given in Table 2.

Table 1. Specific rotations of α- and β-D-glucose in water-DMF mixtures.

x_w	$[\gamma_\alpha]_D{}^{19.99}$	$[\gamma_\alpha]_D{}^{29.99}$	$[\gamma_\alpha]_D{}^{39.99}$	$[\gamma_\beta]_D{}^{19.99}$	$[\gamma_\beta]_D{}^{29.99}$	$[\gamma_\beta]_D{}^{39.99}$
1	112.9	112.8	112.7	17.37	17.25	17.11
0.976	113.7	113.4	113.2	17.06	16.98	16.69
0.950	114.4	114.1	113.8	16.89	16.65	16.17
0.897	116.0	115.6	115.1	16.46	16.37	16.06
0.849	117.1	116.6	116.1	16.22	16.19	15.85
0.750	120.0	118.8	118.6	15.84	15.69	15.43
0.650	122.8	121.9	121.2	15.79	15.66	15.53
0.500	126.3	125.5	124.9	16.08	15.94	15.74
0.350	130.0	129.2	128.3	16.80	16.60	16.35
0.256	132.3	131.6	130.4	17.49	17.23	16.89
0.156	134.6	133.9	132.6	18.38	17.97	17.55
0.100	135.6	134.9	134.0	18.90	18.46	17.95
0.0504	136.8	136.0	135.1	19.39	18.92	18.38
0	137.8	137.0	136.1	20.00	19.45	18.87

Table 2. Equilibrium specific rotations of D-glucose in water-DMF mixtures.

x_w	$[\gamma\infty]_D^{19.99}$	$[\gamma\infty]_D^{29.99}$	$[\gamma\infty]_D^{39.99}$
1	52.67	52.89	53.17
0.976	53.37	53.52	53.71
0.950	53.87	54.00	54.17
0.897	54.69	54.88	55.05
0.849	55.31	55.51	55.72
0.750	56.36	56.59	56.85

Without correction for anomeric impurity our specific rotation data for α-D-glucose would have been from 3.6 to 3.7 % lower, *e.g.* for water at 19.99°C, 108.8° instead of 112.9°. The highest value quoted in the literature seems to be 113.4° at 20°C, found by Hudson and Yanovsky.[15] For the β form the lowest value given in the literature (for water at 20°C) seems to be 17.5°, found by Nelson and Beegle.[16] This agrees fairly well with our corrected value, 17.37°. We believe that the variety of specific rotations of the D-glucose anomers quoted in the literature may be partly due to the presence of varying amounts of the opposite anomer.

Table 3. β/α ratio (K) in equilibrium solutions. The molar enthalpies (ΔH) calculated from the K values.

x_w	K(19.99°C)	K(29.99°C)	K(39.99°C)	ΔH(20−30°C) cal mol⁻¹	ΔH(30−40°C) cal mol⁻¹
1	1.708	1.681	1.652	− 280	− 330
0.976	1.662	1.640	1.607	− 230	− 390
0.950	1.636	1.608	1.569	− 310	− 460
0.897	1.603	1.576	1.539	− 300	− 440
0.849	1.579	1.553	1.516	− 290	− 460
0.750	1.571	1.522	1.491	− 560	− 390

The equilibrium constant. Table 3 contains the K values, calculated by eqn. (3), for the solutions showing simple mutarotation. It is interesting to note that the excess of β- over α-D-glucose in the equilibrium is diminished with increasing fraction of the organic solvent. The temperature coefficient is clearly negative. In this table are also included the molar enthalpies of the α→β transformation calculated using the van't Hoff equation. From repeated experiments at $x_w = 1$ the uncertainty in K is calculated to be 0.15, 0.35, and 0.85 % at 20, 30, and 40°C, respectively. Calculations based on K values of so nearly the same magnitude give, of course, very large relative errors in ΔH (*e.g.* 25 % or 70 cal for the interval 20−30°C). The very good agreement of our value of − 280 cal mol⁻¹ in water with ΔH determined calorimetrically by Sturtevant[17] and by Kabayama *et al.*[18] may therefore be casual.

Table 4. The "mutarotation constant" (k) at different solvent compositions and temperatures.

x_w	$k \times 10^2(19.99°C)$ min^{-1}	$k \times 10^2(29.99°C)$ min^{-1}	$k \times 10^2(39.99°C)$ min^{-1}
1	1.48	3.88	9.34
0.976	1.24	3.19	7.67
0.950	0.978	2.52	6.38
0.897	0.632	1.72	4.11
0.849	0.418	1.13	2.88
0.750	0.182	0.492	1.28

Kinetic results. Table 4 contains the "mutarotation constant" (k) at the different mol fractions and temperatures. The standard deviation in k in single runs were never greater than 0.11 %. Duplicate runs were made in a number of cases, *e.g.* four runs in water at 39.99°C gave $k \times 10^2 = 9.398$, 9.285 starting with "α-D-glucose" and $k \times 10^2 = 9.346$, 9.312 starting with "β-D-glucose". The reproducibility in k was good enough to make the uncertainty in all cases less than 1 %.

Table 5. $k/c_w{}^n$ data, calculated from the k values in Table 4, with $n = 1$, 2, and 3. Sugar concentration: About 3 g in 100 ml solution. $k' = k/c_w$, $k'' = k/c_w^2$, and $k''' = k/c_w^3$.

x_w	c_w mol l^{-1}	$k' \times 10^4$ l mol^{-1} min^{-1}	$k'' \times 10^6$ l^2 mol^{-2} min^{-1}	$k''' \times 10^8$ l^3 mol^{-3} min^{-1}
		19.99°C		
1	54.30	2.73	5.02	9.24
0.976	49.34	2.51	5.09	10.3
0.950	44.71	2.19	4.89	10.9
0.897	37.12	1.70	4.59	12.4
0.849	31.54	1.33	4.20	13.3
0.750	23.05	0.790	3.43	14.9
		29.99°C		
1	54.18	7.16	13.2	24.4
0.976	49.21	6.48	12.2	26.8
0.950	44.57	5.65	12.7	28.5
0.897	36.96	4.65	12.6	34.1
0.849	31.35	3.60	11.5	36.7
0.750	22.87	2.15	9.40	41.1
		39.99°C		
1	53.99	17.3	32.0	59.4
0.976	49.00	15.7	32.0	65.2
0.950	44.35	14.4	32.4	73.2
0.897	36.70	11.2	30.5	83.1
0.849	31.12	9.25	29.7	95.5
0.750	22.68	5.64	24.9	109.8

Table 6. $k/c_w{}^n$ data for mutarotation of D-glucose in water/dioxane solvents at 25°C. Sugar concentration: About 4 g in 100 ml solution. k', k'' and k''', see Table 5.

x_w	c_w mol l^{-1}	$k \times 10^2$ min^{-1}	$k' \times 10^4$ l mol^{-1} min^{-1}	$k'' \times 10^6$ l^2 mol^{-2} min^{-1}	$k''' \times 10^8$ l^3 mol^{-3} min^{-1}
1	54.0	2.42	4.48	8.29	15.3
0.950	43.6	1.47	3.36	7.72	17.7
0.876	32.9	0.838	2.55	7.74	23.5
0.826	27.5	0.596	2.17	7.87	28.6
0.759	22.0	0.387	1.76	8.01	36.4

Table 7. $k/c_w{}^n$ data for mutarotation of D-glucose in water/methanol solvents at 20°C. k', k'' and k''', see Table 5.

x_w	Sugar conc. %	c_w mol l^{-1}	$k \times 10^2$ min^{-1}	$k' \times 10^4$ l mol^{-1} min^{-1}	$k'' \times 10^6$ l^2 mol^{-2} min^{-1}	$k''' \times 10^8$ l^3 mol^{-3} min^{-1}
1	5	53.7	1.46	2.72	5.07	9.45
0.941	3	48.1	1.18	2.45	5.10	10.6
0.910	3	45.1	1.05	2.33	5.16	11.4
0.807	3	36.4	0.77	2.11	5.81	16.0
0.727	5	30.2	0.625	2.07	6.86	22.7
0.633	3	24.5	0.48	1.96	7.99	32.6

Dependence of k *on water concentration.* In order to examine how k depends on the molar concentration of water, c_w, when DMF is the diluent, we have listed in Table 5 the "water constants" found by division of the k values by different powers of c_w. The effect of two other diluents, dioxane and methanol, is represented in the same way in Tables 6 and 7. The k data for the dioxane and methanol systems are taken from Rowley and Hubbard [19] and Richards, Faulkner, and Lowry,[20] respectively. To find the water concentrations it was necessary to determine the densities of the sugar solutions used by these authors. From the tables can be seen that the second power relation clearly is preferred when dioxane is the diluent. This is, at first sight, less obvious for the two other solvent systems. Evidently the three systems differ in catalytic properties.

DISCUSSION

The reduction of the surplus of β form in aqueous equilibrium solutions by the addition of DMF may have a connection with the anomeric effect and its dependence on the dielectric properties and solvation.

The question of whether acid-base catalyses generally are concerted or not will be a matter of dispute so long no conclusive experimental proof or disproof can be delivered. In the case of highly aqueous solutions without stronger ionic catalysts there seems, however, to be some evidence that water molecules obtain their relatively high catalytic power from a con-

certed mechanism. A support to this view is the relatively high isotope effect, k_{H_2O}/k_{D_2O} being from 3.5 to 4.0 (*cf.* Ref. 1b, p. 34).

A concerted mechanism should give second or higher order in water for the ring opening reaction steps (*e.g.* $k_{12} = k_{12}''c_w^2$). Assuming that the ring opening reaction steps are both nth order in water, it can be derived from eqn. (4) that k must be nearly proportional to c_w^n. The known variations in K and the approximate magnitude expected for the k_{12}/k_{32} ratio [10] implies that the water/DMF and water/methanol systems in none of the mixtures examined should deviate more than a few per cent from this proportionality. In the case of water/dioxane it has been difficult to find enough rotation [19] or K data to calculate the deviation from proportionality, but the error factor for the lowest water concentration may be estimated to be greater than 0.9.

In view of the above considerations the "water constant" data for the water/dioxane system indicate that the ring opening reaction steps are essentially second order in water. In the water/DMF system these reaction steps also seem to be preferentially of this order, but a downward drift of the k'' values is apparent, especially at the lowest water concentrations. The drift in this case may be understood when considering the thermodynamic excess properties of water-DMF mixtures. Dimethylformamide is known extensively to form hydrogen-bonded complexes $HCON(CH_3)_2.nH_2O$, where $n = 2 - 4$.[21] Determination of partial vapour pressures has given that the activity coefficients of water in water-DMF mixtures at our solvent compositions are below unity.[22] This ought to reduce k more than by an ideal dilution. Table 5 shows that the decrease in k'' is less at higher temperature, and this may be expected if the decrease results from water-DMF complexing. It is of interest in this connection to note that in the water-dioxane and water-methanol mixtures the activity coefficients of water are found to be above unity.[23,24] We believe that in the water/methanol system the amphiprotic properties of methanol should give a substantial increase in k'' caused by a combined action of water and methanol in a concerted mechanism. The effect on the rate constants k_{12} and k_{32} by $c_{MeOH}c_w$ terms, and consequently an increase in k'', should be most visible at the lowest water concentrations here examined (see k'' column in Table 7).

The polarimetric rate data we have considered here, even if indicating the existence of second order catalyst terms, cannot of course be regarded as a conclusive proof of a concerted bimolecular water catalysis. For a further approach more experimental material, not least from polarographic studies on the elementary rate constants in mixed solvents, is desirable.

REFERENCES

1. a. Pigman, W. and Isbell, H. S. *Advan. Carbohydrate Chem.* **23** (1968) 11; b. Isbell, H. S. and Pigman, W. *Advan. Carbohydrate Chem. Biochem.* **24** (1969) 14.
2. Lowry, T. M. *J. Chem. Soc.* **129** (1927) 2554.
3. Lowry, T. M. and Faulkner, I. J. *J. Chem. Soc.* **127** (1925) 2883.
4. Pedersen, K. J. *J. Phys. Chem.* **38** (1934) 581.
5. Dawson, H. M. and Spivey, E. *J. Chem. Soc.* **1930** 2180.
6. Swain, C. G. *J. Am. Chem. Soc.* **72** (1950) 4578.

7. Swain, C. G., Di Milo, A. J. and Cordner, J. P. *J. Am. Chem. Soc.* **80** (1958) 5983.
8. Hill, D. G. and Thumm, B. A. *J. Am. Chem. Soc.* **74** (1952) 1380.
9. Rakowski, A. *Z. physik. Chem.* **57** (1906) 321.
10. a. Los, J. M. and Wiesner, K. *J. Am. Chem. Soc.* **75** (1953) 6346; b. Los, J. M., Simpson, L. B. and Wiesner, K. *J. Am. Chem. Soc.* **78** (1956) 1564.
11. Lowry, T. M. and John, W. T. *J. Chem. Soc.* **97** (1910) 2634, p. 2637.
12. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Am. Chem. Soc.* **85** (1963) 2497.
13. Schmid, H. and Bauer, G. *Z. Naturforsch.* **21b** (1966) 1009.
14. Hveding, J. A., Kjølberg, O. and Reine, A. *To be published.*
15. Hudson, C. S. and Yanovsky, E. *J. Am. Chem. Soc.* **39** (1917) 1013.
16. Nelson, J. M. and Beegle, F. M. *J. Am. Chem. Soc.* **41** (1919) 559.
17. Sturtevant, J. M. *J. Phys. Chem.* **45** (1941) 127.
18. Kabayama, M. A., Patterson, D. and Piche, L. *Can. J. Chem.* **36** (1958) 557.
19. Rowley, H. H. and Hubbard, W. R. *J. Am. Chem. Soc.* **64** (1942) 1010.
20. Richards, E. M., Faulkner, I. J. and Lowry, T. M. *J. Chem. Soc.* **129** (1927) 1733.
21. Geller, B. E. *Russ. J. Phys. Chem.* **35** (1961) 542.
22. Jones, J. *M. S. Thesis*, University of Oklahoma, Norman, Oklahoma 1969.
23. Goates, J. R. and Sullivan, R. J. *J. Phys. Chem.* **62** (1958) 188.
24. Gölles, F. *Monatsh. Chem.* **92** (1961) 981.