# The Mutarotation of Tetramethylglucose in Water

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Kinetic and equilibrium data are reported for the mutarotation of tetramethylglucose in aqueous solution. Specific optical rotations for the two optical forms are found to be very similar to those for glucose, whereas the equilibrium constants for mutarotation of the two sugars are different presumably due to various degrees of hydration. The kinetic data for hydroxide and hydronium ion catalysis may suggest that tetramethylglucose is a weaker acid as well as a weaker base compared to glucose, but in this context anomalies are observed for the catalysis by weak bases.

Tetramethylglucose (TMG) has often been used as a substrate for studies of detailed mechanisms of mutarotation of cyclic sugars in solution, mainly because of its relatively high solubility in many non-aqueous solvents[1,2] and because secondary isotope effects are avoided.[3,4] Recently, TMG was used by us to study mutarotation in mixed solvents[5] and we decided also to investigate the reaction in water in more detail as only relatively few and rather scattered data have been reported for such experiments. Results are presented in the following.

## EXPERIMENTAL

*Materials.* 2,3,4,6-α-D-tetramethylglucose was synthesized according to West and Holden.[6] To obtain the pure α-form the sugar was recrystallized twice from light petroleum ether[7] (Petri, b.p. <50 °C) and the product stored over silica gel, m.p. 93 °C. The NMR spectrum (Varian A-60 D instrument) of the substance dissolved in DMSO,[8] where no mutarotation takes place, indicated optical purity as only one signal from the 1-OH$_\alpha$ proton was observed. DMSO (*purum*, Fluka) was distilled *in vacuo* from calcium hydride before use. All other chemicals were of Analar grade and redistilled water was used throughout.

*Equilibrium composition.* Earlier reports on the equilibrium constant for the mutarotation of TMG based on NMR measurements[5] are of preliminary nature and for D$_2$O solutions as described by Lemieux and Stevens.[9] We, therefore, developed a method to determine this constant in H$_2$O. 100 mg of α-TMG was dissolved in 5 ml H$_2$O and equilibrated at 25 °C for about 24 h. Most of the water was then evaporated by a stream of dry nitrogen and the remaining sugar was finally dried *in vacuo* over P$_2$O$_5$. The crystalline material, now expected to consist of an equilibrium mixture of α- and β-TMG, was then dissolved in 1 cm³ of DMSO and investigated by NMR.

*Kinetic measurements.* Rates of mutarotation were measured by a Perkin-Elmer 141 polarimeter fitted with recorder and a 10 cm thermostatted reaction cell. Well-defined solutions of TMG (0.05 M) in buffers were made up in standard flasks and quickly transferred to the cell. All measurements were carried out at 25.0 °C and usually at a wavelength of 365 nm where relatively large specific optical rotations are observed.

## RESULTS AND DISCUSSION

*Equilibrium composition.* As shown by Casu et al.[8] the NMR spectrum of a sugar dissolved in DMSO may give quantitative information about the anomeric composition. Thus for the anomerized samples of TMG — obtained by evaporation of water (*cf.* experimental section) — dissolved in DMSO we found well-defined 1-OH$_\alpha$ and 1-OH$_\beta$ doublets at δ 6.35 and 6.75, respectively. If the areas of the signals were compared we found, from four experiments, an equilibrium composition corresponding to $(66.1 \pm 0.6)\%$ α-form, i.e. $K_\alpha = [\beta\text{-form}]_{eq}/[\alpha\text{-form}]_{eq} = 0.51 \pm 0.01$ (25 °C). It was checked that the sugar actually did represent an equilibrium mixture. A small sample quickly dis-

solved in water and transferred to the polarimeter cell gave no indication of mutarotation. $K_\alpha$ is in disagreement with our previously reported value $[K_\alpha = 0.92 \, (30 \, °C)]$[5] and the statement that the rate constants of the forward and reverse reactions for TMG are practically equal.[1] However, it is worthwhile pointing out that the uncertainty in $K_\alpha$ given $(s/\sqrt{n})$ does not include possible systematic errors in $K_\alpha$ (see later). Reliable data for the specific optical rotation of pure $\alpha$-TMG in water have not been quoted in the literature and we were able to determine this quantity from extrapolation to zero time of kinetic plots obtained by following the mutarotation of pure $\alpha$-TMG. Thus, $[\alpha_\alpha]_{5893 \, A}^{25.0}$ was found to be $+110.9$ and $+111.5$ degrees, respectively, from independent experiments. Eastham et al.[10] report $[\alpha]_D^{25} = +112°$ (water) for a sample of TMG, which was expected but not proved to consist of pure $\alpha$-form. By observing the infinity optical rotation of the same curves, $[\alpha_\infty]_{5893 \, A}^{25.0}$ was found to be $+79.6$ and $+79.8$ degrees, respectively, in good agreement with literature values.[5,6] It is striking that $[\alpha_\alpha]_{5893 \, A}^{25.0}$ is practically identical with the corresponding value for $\alpha$-D-glucose (G), e.g. $+110.0$ degrees given by Kendrew and Moelwyn-Hughes[11] or $+112.2$ degrees by Isbell and Pigman[12] both at $20 \, °C$. $K_\alpha$ for a given sugar can be found from $K_\alpha = ([\alpha_\alpha] - [\alpha_\infty])/([\alpha_\infty] - [\alpha_\beta])$, where $[\alpha_\alpha]$ and $[\alpha_\beta]$ are the specific rotations of the pure $\alpha$- and $\beta$-form, respectively, and $[\alpha_\infty]$ the specific rotation of the equilibrium mixture. If we insert $K_\alpha = 0.51$ and the mean values of $[\alpha_\alpha]$ and $[\alpha_\infty]$ from above, $[\alpha_\beta]_{5893 \, A}^{25.0}$, is found to be $+17.9$ degrees, which is again very close to literature values $(+18.4$ and $+18.7$ degrees)[12] for $\beta$-D-glucose.

Specific optical rotations for $\alpha$- and $\beta$-TMG, respectively, (water) may be obtained from optical data for the corresponding methyl tetramethylglucosides $([\alpha_\alpha]_{5893 \, A} = 147.4;$[13] $[\alpha_\beta]_{5893 \, A} = -11.6,$[13] $-17.3,$[14,15] and $-18.2$[16]) according to the method described by Freudenberg.[17,*] If displacements in molar optical rotations for glucose/ methyl glucoside ($\alpha$-form: $105°$ and $\beta$-form: $100°$)[17] are applied to the specific optical rotations for $\alpha$-methyl tetramethylglucoside ($147.4°$) and $\beta$-methyl tetramethylglucoside ($-18.2°$) given above, the following values are obtained for TMG: $[\alpha_\alpha]_{5893 \, A} = 111.7°$ and $[\alpha_\beta]_{5893 \, A} = 23.1°$. The first value is in excellent agreement with our experimental

findings whereas the last is higher and gives a $K_\alpha$ of 0.56. Whether this discrepancy is caused by minor errors in our $K_\alpha$ or in $[\alpha_\beta]_{5893 \, A}$ for $\beta$-methyl tetramethylglucoside, the reported values of which seem rather scattered, or in both, is not clear, but there is no doubt that $K_\alpha = 0.51$ is more correct than our previously reported value,[5] which is clearly in error.

Thus, the relative stability of the $\alpha$- and $\beta$-forms of glucose in water $(K_\alpha = 1.71)$[5] is drastically affected in favour of the $\alpha$-form when the substance is methylated to give TMG. This may be expected, since the relatively high stability of $\beta$-D-glucose in water is due to a considerable interaction between the equatorial $C_1 - OH$ hydroxyl group and the solvent.[5] This interaction may be lower for TMG, because of the pronounced hydrophobic character of this compound, e.g. reflected in a much lower solubility in water compared to glucose, but a comprehensive discussion will also have to take into consideration conformational changes which might affect the stability of the anomers.

*Kinetics.* All kinetic curves were of first order and $k_{obs}$, equal to the sum of the first-order rate constants for the forward and the reverse reaction, were obtained from plots of $\ln [\alpha_t - \alpha_\infty]$ against time.

From three series of acetate buffer (pH = 3.62, 4.59 and 5.63) − results from one series described in detail in Table 1 − and three solutions of acetate (pH adjusted to 6.53, 7.01 and 8.2 by addition of small amounts of concentrated hydrocloric acid), $k_{obs}^°$, which is the linearly extrapolated value (to zero catalyst concentration) of $k_{obs}$, were found as follows:

| pH | 3.62 | 4.59 | 5.63 | 6.53 | 7.01 | 8.20 |
|---|---|---|---|---|---|---|
| $10^4 \times k_{obs}^° \, (s^{-1})$ | 3.2 | 3.1 | 3.5 | 3.2 | 3.8 | 3.5 |

The mean value of $k_{obs}^°$ is in good agreement with $k_{obs}^° \approx 3.44 \times 10^{-4} \, s^{-1}$ found for the mutarotation of TMG in very dilute acetate buffer (pH = 4.7) by Challis et al.;[18] $k_{obs}^°$ is the rate constant referring to catalysis by pure solvent (water).

The catalytic constant for the acetate ion, $k_{Ac}$, was determined by the usual procedure[19] to be $(20 \pm 1) \times 10^{-4} \, dm^3 \, mol^{-1} \, s^{-1}$, which agrees with $20.5 \times 10^{-4} \, dm^3 \, mol^{-1} \, s^{-1}$ given by Huang et al.[3]

So far only very few data have been reported for the mutarotation of TMG in alkaline solution. From two series of runs in ammonia buffer (pH measured to 9.32 and 9.64, $I = 0.1$) $k_{obs}$ was found to be $(14 \pm 1) \times 10^{-4} \, s^{-1}$ and $(21 \pm 1) \times 10^{-4} \, s^{-1}$,

Table 1. Observed first-order rate constants as a function of total acetate buffer concentration ([Acetate]/[Acetic acid] = 10.00, I = 0.10); pH (measured): 5.63, (calculated): 5.65.

| $10^4 \times$ [total buffer]/mol dm$^{-3}$ | 0 | 200 | 400 | 600 | 800 | 1000 |
|---|---|---|---|---|---|---|
| $10^4 \times k_{obs}$/s$^{-1}$ measured | – | 3.8 | 4.5 | 4.7 | 5.1 | 5.4 |
| calculated | 3.5 | 3.9 | 4.3 | 4.6 | 5.0 | 5.4 |

respectively. The data gave a value of $(0.23 \pm 0.02)$ dm$^3$ mol$^{-1}$ s$^{-1}$ for $k_{NH_3}$ and $k_{NH_4^+}$ was found to be negligible as for glucose.[23] Similarly, linear extrapolation of observed rate constants to zero borate buffer concentration at pH = 9.19 gave $k_{obs}^{\circ}$ equal to $(10 \pm 1) \times 10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$.

If the $k_{obs}^{\circ}$-values given above are plotted against [HO$^-$], calculated from the respective pH-values, a fairly good straight line is obtained and the catalytic constant for the hydroxyl ion, $k_{HO^-}$, is calculated from the slope to be $(31 \pm 2)$ dm$^3$ mol$^{-1}$ s$^{-1}$. However this is only an apparent value since catalysis by sugar anions[20] has not been taken into account. The contribution to catalysis from these species is unknown for TMG but for glucose it amounts to up to 40 % of the total catalytic constant at ordinary sugar concentrations.[21] Lowry and Wilson[22] determined the apparent $k_{HO^-}$-(TMG)
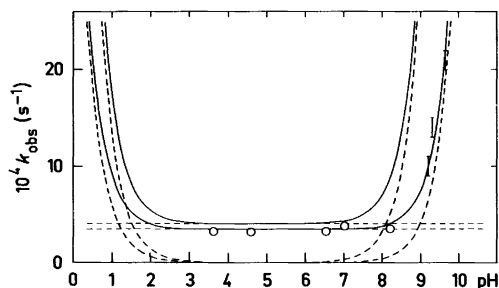


Fig. 1. pH-profiles for mutarotation of tetramethyl-glucose (TMG; lower curves) and glucose (G; upper curves) in water at 25.0 °C (solid curves); $k_{obs} = k_{obs}^{\circ} + k_H + [H^+] + k_{HO^-} [HO^-]$ or $k_{obs} = k_{obs}^{\circ} + k_{H^+} \times 10^{-pH}/f_{H^+} + k_{HO^-} \times 10^{pH-14.00}/f_{HO^-}$; the individual terms are shown separately (broken curves). The following constants have been applied for constructing the curves (Ref. in parentheses): TMG: $k_{obs}^{\circ} = 3.44 \times 10^{-4}$ s$^{-1}$ (18); $k_{H^+} = 48.3 \times 10^{-4}$ dm$^3$ mol$^{-1}$ s$^{-1}$ (18); $k_{HO^-} = 31$ dm$^3$ mol$^{-1}$ s$^{-1}$ (this paper). G: $k_{obs}^{\circ} = 4.01 \times 10^{-4}$ s$^{-1}$ (11); $k_{H^+} = 11.1 \times 10^{-3}$ dm$^3$ mol$^{-1}$ s$^{-1}$ (26), $k_{HO^-} = 230$ dm$^3$ mol$^{-1}$ s$^{-1}$ (23). $f_{H^+} = 0.83$ (27), $f_{HO^-} = 0.76$ (27). The indicated points are results from the present investigation.

to be 61 dm$^3$ mol$^{-1}$ s$^{-1}$ at 20 °C from mutarotation in unbuffered alkaline solution, but it is doubtful to what extent their method gives correct results because they also determined a very high value for $k_{HO^-}$-(G) at 20 °C: 307 dm$^3$ mol$^{-1}$ s$^{-1}$ compared to 230 dm$^3$ mol$^{-1}$ s$^{-1}$ for this constant at 25 °C reported by Brønsted and Guggenheim.[23]

Fig. 1 shows $k_{obs}$/pH-profiles for D-glucose and TMG where only catalysis by the solvent and the species H$^+$ and HO$^-$ has been taken into account. If the mutarotation of a cyclic sugar is described as a process where the observed rate is mainly determined by the rate of ring opening of either the protonated or the deprotonated sugar, the relative position of the two profiles may readily be explained by saying that the TMG molecule has a less acidic anomeric hydroxyl group as well as a less basic ring oxygen atom compared to glucose. Unfortunately the acid-base properties of TMG are unknown, but the explanation may well hold for two, or more, closely related sugars. In general, however, the rates of mutarotation are not only dependent on the acid-base properties as described but also on the strength of the bond between $C_1$ and the ring ocygen atom, so that a simple correlation between catalytic constants and acid-base strengths of the sugars may not be expected in all cases.

Some catalytic data for the mutarotation of glucose and TMG are given in Table 2. We believe that the value 0.19 dm$^3$ mol$^{-1}$ s$^{-1}$ for $k_{NH_3}$(G), derived from some very careful measurements by

Table 2. Some catalytic constants (dm$^3$ mol$^{-1}$ s$^{-1}$) at 25 °C.

| | G | TMG | |
|---|---|---|---|
| $10^3 k_{Ac}$ | 2.22[21] | 2.0$\pm$0.1<br>2.05[3] | This work |
| $k_{NH_3}$ | 0.123[23]<br>0.14[24]<br>0.19[25] | 0.23$\pm$0.02 | This work |

Kilde and Wynne-Jones,[25] is the most reliable, and therefore, within the limits of uncertainty, the two catalytic constants show practically no dependence upon the change of substrate from glucose to TMG. At first sight, this disagrees with our observations for $k_{HO^-}$ but before further conclusions can be drawn more extensive experimental work is needed.

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