The Mutarotation of D-Glucose and Its Dependence on Solvent. III. The Existence of α- and β-D-Glucofuranose in *N,N*-Dimethylformamide

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Few authors have hitherto been concerned with the behaviour of D-glucose in *N,N*-dimethylformamide (DMF). Kuhn and Grassner [1] assume that the mutarotation almost exclusively is due to a pyranose-furanose conversion, while Jacin *et al.*[2] from their GLC investigations conclude that no anomerization had occurred during their experimental time. Our polarimetric observations are not compatible with any of the above statements. The plot of ln $([\gamma_t]-[\gamma\infty])$ against time always showed a significant deviation from a straight line at the experimental temperatures 20, 30, and 40 °C.[3] The rate of rotational change in thoroughly purified DMF (see Experimental) is extremely low, but was found to be very sensitive to the degree of purity. The shape of the mutarotation curves for both α- and β-D-glucose in DMF is similar to that described for the corresponding anomers of D-galactose in water.[4] Also the "thermal" mutarotation was found to be complex in analogy with that found for D-galactose in water.[5,6]

The observed deviation from a simple logarithmic law can be explained by the formation of the furanose anomers. In a preliminary communication [7] we have described the presence of D-glucofuranose, detected as trimethylsilyl

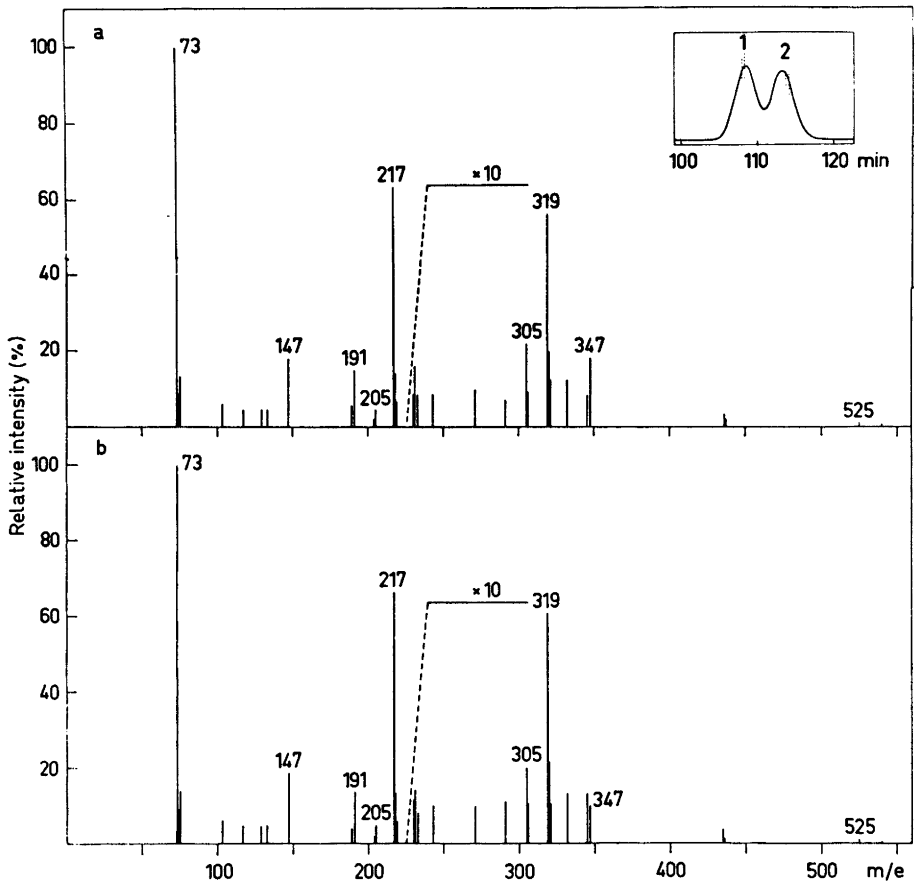

Fig. 1. Gas chromatographic separation and mass spectra of the two fastest moving persilylated tautomers of D-glucose. The partial mass spectra a and b are taken from peaks 1 and 2, respectively. The scan periods are indicated by broken lines in the chromatogram.

(TMS) derivative, in the equilibrium mixture (70 °C). We now report the existence of both α- and β-D-glucofuranose in this mixture. Using OV-17 as the stationary phase the previously described mixture of TMS derivatives was resolved into two minor peaks (1 and 2) and two major peaks (3 and 4). The relative retention times of these peaks were 0.76, 0.79, 1.00, and 1.65, respectively. The peaks 3 and 4 were, by the same methods as described earlier,[7] found to represent the α- and β-pyranosides, respectively. The gas chromatographic separation of peaks 1 and 2 in the GCMS analysis is shown in Fig. 1 along with partial mass spectra of these components. The two spectra (M^+ 540, 0.1 %) are nearly identical and show the characteristic differences from those of the pyranoses.[8,9] To establish which of peaks 1 and 2 represents the α- and which the β-anomer a separation was made by GLC on a preparative scale. To avoid a large α-pyranoid peak a mixture obtained by tautomerization of β-D-glucose at 70 °C for 1.5 min was used for this purpose. As the retention times of the two furanoid forms differ very little, they appeared as one broad peak under the preparative conditions. An NMR spectrum of a fraction collected from the first half of this peak showed a strong singlet at δ 5.04 and a weak doublet at δ 5.34, both being in the region ascribed to the anomeric protons. A fraction collected from the last half of the peak showed the same singlet and doublet, but both had approximately the same intensity. With the possible dihedral angles between neighbouring *cis*-hydrogens and between neighbouring *trans*-hydrogens in furanoid rings [10] the Karplus equation leads to the conclusion that the singlet only can be attributed to a β-furanoside. The α-furanoside should give a doublet. We judge our observations to indicate that peaks 1 and 2 represent the β- and α-furanoid form of TMS-glucose, respectively.

The total amount of furanose at 70 °C is calculated to be about 4.5 % of the total sugar content. This furanose proportion was found to develope in the first minutes of the mutarotation. This is in agreement with the many observations that in the ring formation of a sugar a five-membered ring is kinetically favourable while normally the six-membered ring is thermodynamically most stable. The mutarotation of D-glucose may have a complex character also in other organic solvents. It is of interest in this connection to note that the behaviour of D-glucose in ethylene glycol has been found to be "anormal".[11]

Experimental. The D-glucose products and *N*,*N*-dimethylformamide were those described earlier.[3] Purification of the DMF was obtained by filtration through Molecular Sieves (Union Carbide, 4A and 5A).

The polarimeter and the thermostats were as described in part I.[3] For the analytical studies a Hewlett Packard 5700A gas chromatograph equipped with a flame-ionization detector was used, while a Varian Aerograph 200 with a thermal conductivity detector was used for the preparative separation. The GLC-mass spectrometry was carried out on an LKB 9000 mass spectrometer. The column packing material was 10 % OV-17 on Chromosorb W (60 – 80 mesh, HMDS-treated for GLC and AW-DMCS for GCMS). For the analytical GLC an aluminium column (3 m × 3 mm) was maintained at 145 °C. The preparative GLC was carried out on a stainless steel column (1.5 m × 6 mm) at 150 °C and the separation for the mass spectrometer on a glass column (4 m × 6 mm) at 150 °C. The ion source block was maintained at 250 °C, the molecular separator at 190 °C, and the ionizing energy was 70 eV.

The NMR spectra ($CDCl_3$) were recorded on a Varian HA-100-15-D spectrometer operating at 98 MHz. Tetramethylsilane (TMS) was used as internal standard.

The mutarotation experiments were carried out as described previously.[3] For the preparation of the TMS derivatives the solutions (1 g/100 ml), still kept at the mutarotation temperature, were treated with trimethylchlorosilane and hexamethyldisilazane in the proportions described by Sweeley *et al.*[12] The reaction mixtures were stored for 8 h at room temperature in order to obtain full silylation, as short silylation time gave extra peaks in the chromatogram. The precipitated salts were removed by decantation, and the solutions were concentrated *in vacuo*. The sugar derivatives were extracted with hexane prior to injections into the gas chromatograph.

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