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Short Communications

On the Mutarotation Equilibria of D-Glucose and D-Mannose and the Assignment of Configurations to their α- and β-Anomers

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As well known the interpretation of Roentgen-diffraction-spectra of α-D-glucose [1] seem to have decided the assignment of the two possible configurations at C_1 to the α- and β-anomers as follows: In the Haworth structural picture drawn as usual (C_5,C_4,C_3,C_2,C_1, 12345 clockwise) the configuration at C_1 is for the α-anomer: H above OH, and for the β-anomer: OH above H, where the system of nomenclature of α- and β-anomers is that of Hudson [2].

As shown by one of us in several papers [3] many chemical facts become much easier to understand, when it is assumed that the assignment of configurations to the α- and β-anomers is the reverse of that found roentgenographically. It may be remarked in this connection, that the former assignment has until recently been considered as proved chemically by Böeseken's interpretation of his investigations on the complex-formation between aldoses and boric acid [4]. However, as shown by one of us [5], serious doubt concerning the validity of this interpretation arises when the newer aspects of the conformation of aldohexoses, notably by Hassel and Ottar [6] and by Reeves [7] are taken into account. Whether or not it be permissible to doubt the correctness of the current assignment, it may be of interest to try to use the facts concerning the mutarotation equilibria between the α- and β-anomers to arrive at the assignment in question. For that purpose we have chosen D-glucose and D-mannose because at equilibrium and during their transformations their solutions seem to contain only their α- and β-pyranose modifications [8,9].

In accordance with Hassel-Ottar and Reeves we shall assume, that their most stable structure is the C1 chair conformation, carrying the bulky group at C_5 and the hydroxyl groups at C_4 and C_3 in equatorial positions.

D-*Glucose*. According to the authors mentioned above, D-glucose has one of the two configurations E or A defined as E: 4e, 3e, 2e, 1e; A: 4e, 3e, 2e, 1a.

We inquire into the equilibrium distribution of the forms E and A. We remark that the quotient $q(\mathrm{E/A})$ of the equilibrium concentrations of molecules in the E-state and in the A-state respectively is

$$q(\mathrm{E/A}) = (W_\mathrm{E}/W_\mathrm{A})\exp[(H_\mathrm{A}-H_\mathrm{E})/RT + (S_\mathrm{E}-S_\mathrm{A})/R] \quad (1)$$

where the W's are the "statistical weights" of the states, while the H's and the S's mean enthalpy and entropy, respectively, of the molecules in the states indicated by subscripts.

Inspection of models of the two forms show that the two differences must be small. We therefore expect that approximately

$$q(\mathrm{E/A}) = W_\mathrm{E}/W_\mathrm{A} \quad (2)$$

The problem is thus reduced to estimating the ratio $W_\mathrm{E}/W_\mathrm{a}$.

At each of the carbon-atoms 1 to 4 we have evidently a vector represented by H–OH. These four vectors are equal in magnitude and are in planes through the vertical axis of the pyranose ring. Their projection on this axis are also equal in magnitude but are either parallel or antiparallel to each other. The statistical weight w of a certain state means the number of ways in which this state can be realised. Let us take as an example a pair of vectors which are represented by their distances

from the center of a benzene-ring and their directions either in the *ortho-* or in the *para*-position. We first fix one of the vectors and then add the other in the *para*-position. This can only be done in one way and we therefore ascribe the weight unity to the *para*-state. Next we fix one vector and add the other one in the *ortho*-position. This can be done in two ways, and we therefore ascribe the weight 2 to the ortho-state. In other words, if the energies of the molecule in the two states are equal, there must be two parts of the molecules in the *ortho*-state for each part in the *para*-state at equilibrium.

In the *ortho*-state the two vectors form an angle of 60° with each other, but if the angle were decreased to say 10° the situation would be the same as far as the statistical weight is concerned, and this must remain true even if the angle were decreased to zero. Therefore to a pair of vectors of equal length the weight 2 must be ascribed if they are parallel but the weight unity if they are antiparallel. To avoid confusion with the locations in the pyranosering we shall denote the two possible orientations, not as *ortho* and *para*, but as *syn-* and *anti-*, respectively.

In the hexoses there are evidently three pairs of H—OH vectors which include the one at C_1. There are also three pairs not including the one at C_1. The latter ones are obviously equal in the E- and A-forms and may therefore be neglected. For the E-form we denote the former type of pairs by (E2), (E3) and (E4), respectively. Inspection of the model or of the Haworth picture corresponding to D-glucose shows that, the pairs (E2) and (E4) are *anti-*, while (E3) is *syn-*.
Consequently

$$w(\mathrm{E2}) = w(\mathrm{E4}) = 1; w(\mathrm{E3}) = 2. \quad (3)$$

We get the value of W for the whole molecule by combination of the w's for the three pairs or

$$W_\mathrm{E} = w(\mathrm{E2})\cdot w(\mathrm{E3})\cdot w(\mathrm{E4}) = 1\times 2\times 1 = 2 \quad (4)$$

Similarly the pairs (A2), (A3) and (A4) are, respectively, *syn-*, *anti-*, and *syn-*, and therefore

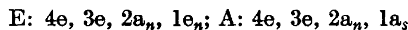
$$W_\mathrm{A} = w(\mathrm{A2})\cdot w(\mathrm{A3})\cdot w(\mathrm{A4}) = 2\times 1\times 2 = 4 \quad (5)$$

The equlibrium quotient $q(\mathrm{E/A})$ thus approximates ½. It should be added, that $q(\mathrm{E/A}) = K$, where K is the equilibrium constant for the reaction A ⇌ E.

As well known experiments show that at equilibrium the concentration of β-glucose is about twice that of the α-anomer [8,9]. Consequently comparison of theory and experiment shows that, in the α-form of D-glucose the OH at C_1 is located equatorially »northwards» and in the β-form axially »southwards».

Thus we arrive by this argument to an assignment which is the opposite one of that arrived at by interpretation of the Roentgen, diffractionspectra, while it agrees with the one arrived at by means of chemical arguments by one of us [3,5].

D-*Mannose*. Assuming as we did that the arrangement in D-glucose at C_4, C_3 and C_2 is 4e, 3e, 2e, the two anomers of D-mannose must be



$$\mathrm{E{:}}\ 4\mathrm{e}, 3\mathrm{e}, 2\mathrm{a}_n, 1\mathrm{e}_n;\ \mathrm{A{:}}\ 4\mathrm{e}, 3\mathrm{e}, 2\mathrm{a}_n, 1\mathrm{a}_s$$

where the two subscripts n and s mean »pointing northwards» and »pointing southwards», respectively. The model or the Haworth picture then shows, that in this case the pairs (E2), (E3), and (E4) are, respectively, *syn-*, *syn-* and *anti-* while the pairs (A2), (A3), and (A4) are, respectively, *anti-*, *anti-* and *syn-*.

Consequently we get for D-mannose:

$$W_\mathrm{E} = 2\times 2\times 1 = 4 \quad (6)$$

$$W_\mathrm{A} = 1\times 1\times 2 = 2 \quad (7)$$

Therefore $q(\mathrm{E/A})$ approximates 2 for D-mannose. On the other hand, it is known from experiments [8,9] that there is in the equilibrium solution nearly twice as much of the α-anomer as of the β-anomer. Consequently, by this argument, we find that the OH at C_1 is located equatorially »northwards» in α-D-mannose and axially »southwards» in the β-anomer. In other words, we refind the assignment arrived at in the case of D-glucose.

It might be argued against the considerations above, that equilibrium constants for mutarotation-equilibria are different in different solvents. The answer to this objection is that water is the only solvent, whose molecules have the same composition as the vectors H—OH in question, and that therefore its use as a solvent must lead to the simplest results.

A detailed treatment of some published experimental results concerning mutarotation of glucose and mannose in the light of eqn. (1) will be given by the latter author.

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A Modification for the Determination of Sulphate in Mucopolysaccharides by the Benzidine Method

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The methods for determination of microgram quantities of sulphate in mucopolysaccharides have been studied. Gravimetric methods have been found to be unsuitable for small amounts of sulphate, while certain colorimetric methods have been found to be generally more suitable. One of the most frequently used techniques is to precipitate the sulphate with benzidine and then to determine the amount of benzidine-sulphate spectrophotometrically.

When using a modification of this procedure described by Kent and Whitehouse [1] difficulties were encountered, particularly in filtering the benzidine-sulphate precipitate through sintred glass to remove the excess benzidine. The process is slow and the results were found to be inconsistent.

A modification of this method in which the benzidine sulphate was separated by centrifugation was also tried [2] but the results obtained were unsatisfactory. With additional modifications of the above methods, more consistent results were obtained with standard solutions as well as with mucopolysaccharides after hydrolysis, and these modifications are described here.

Reagents.

98–100 % Formic acid.
95 % Ethanol.
Benzidine reagent: 0.5 % solution benzidine in 95 % ethanol. Kept in the dark in a refrigerator and renewed every 3 weeks.

Acetone-ethanol mixture, 1:1 (v/v).
1.0 N Hydrochloric acid.
0.5 % (w/v) Thymol in 2 N NaOH.
0.1 N sodium nitrite.

The mucopolysaccharides are hydrolyzed in sealed tubes with 25 % formic acid on a boiling water bath. The hydrolysis curve (Fig. 1) shows that 24 h are sufficiént to liberate all the sulphate. Usually 0.75 ml of a mucopolysaccharide solution containing 10–80 μg of sulphate is mixed with 0.25 ml of formic acid.

Standard solutions. 10, 20, 40 and 50 μg SO_4/ml in 25 % formic acid.

Procedure. 0.3 ml of the hydrolysate is placed in a conical centrifuge tube (15 ml) to which 0.5 ml 95 % ethanol, 0.2 ml benzidine reagent and 0.5 ml amyl alcohol are added. The standard solutions are treated in the same way using 0.3 ml aliquots. The tubes are shaken and allowed to stand 60 min at 0°C. They are then centrifuged for 6 min in an ordinary laboratory centrifuge with "swing out" head at 4000 r.p.m. and the supernatant is carefully decanted and discarded. 1 ml acetone-ethanol and 0.5 ml amyl alcohol are added to each tube, the tubes are shaken to disperse the precipitate and then recentrifuged for 6 min. The supernatant is decanted as before, after which 1 ml acetone-ethanol is added, the tubes are shaken and centrifuged again. The supernatant is again decanted.

The precipitate is dissolved in 3 ml of 1.0 N hydrochloric acid and 2 ml of distilled water. The benzidine solutions are diazotized at room temperature by the addition of 1 ml of 0.1 N sodium nitrite. After 3 min, 5 ml of the alkaline thymol solution is added and the contents of the tubes are mixed.

The optical density is measured at 505 mμ in 1 cm cells. The colour is stable for at least 4 h. The amount of sulphate is calculated from a standard curve.