IONISATION CONSTANTS OF 2-AMINO-2-DEOXY-D-GLUCOSE AND THE ANOMERIC EFFECT

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ABSTRACT

It is pointed out that in the protonated and *N*-acetylated derivatives of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose the proportion of α anomer at equilibrium is markedly greater than in the parent sugar or in the non-protonated amino sugar base. This increased anomeric effect is interpreted as being caused by increased electrostatic interaction with dipoles on C-1. This explanation is also used to rationalize the differences observed between the ionisation constants of the two protonated anomers of 2-amino-2-deoxy-D-glucose. In mannose derivatives, different findings were obtained; these are also interpreted in terms of ion–dipole or dipole–dipole interaction on C-1 and C-2.

In addition, the second ionisation constants of the two anomers of 2-amino-2-deoxy-D-glucose have been measured, and these are compared with the corresponding values for D-glucose. Anomer equilibria for the two glycosate ions were calculated; it appears that in these ions the anomeric effect has almost disappeared. Approximate calculations are also given for the proportion of zwitterions present in solutions of 2-amino-2-deoxy-D-glucose.

INTRODUCTION

In 1969[1], it was reported by us that the two pyranoid anomers of the protonated forms of 2-amino-2-deoxy-D-glucose have different ionisation constants. The equilibrium constants for the two anomers measured at various temperatures between 5 and 25° differed by a factor of 2.5 to 4.0, with the α anomer being the stronger base. Various, possible reasons for this difference were discussed, but no completely satisfactory explanation was advanced.

The present paper is concerned, in the first place, with an explanation of this difference in ionisation constants in terms of the anomeric effect. Secondly, we wish to report measurements concerned with the second ionisation of 2-amino-2-deoxy-D-glucose, and thirdly we wish to discuss the possible presence of a zwitterionic or dipolar form in solutions of the amino sugar base.

Anomeric equilibria of 2-amino sugar and ionisation. — It was pointed out in the earlier paper[1] that the ionic dissociation constants of the two anomers of the protonated form of 2-amino-2-deoxy-D-glucose are related quantitatively to the mutarotation equilibrium constants in the following manner: AH^+ and BH^+ are the

protonated forms corresponding to the free base forms of the α anomer and the β anomer, respectively. Thus, the two ionisation constants are defined by the following equations:

$$K_{1\alpha} = [A]\,[H^+]/[AH^+], \text{ and } K_{1\beta} = [B]\,[H^+]/[BH^+];$$

if $K_{M1} = [AH^+]/[BH^+]$ and $K_{M2} = [A]/[B]$, it follows that $K_{1\alpha}/K_{1\beta} = K_{M2}/K_{M1}$.

In other words, the difference in dissociation constants between the two anomers can be interpreted as resulting from the fact that the mutarotation equilibria of the amino sugar cation and the amino sugar free base are different.

The anomeric composition at equilibrium, which varies for different reducing hexoses and pentoses, has been much discussed. On the basis of general information available about equilibria between equatorial and axial hydroxyl groups in cyclohexane and similar structures, it has been calculated[2] that the free energy of α-D-glucopyranose should be $\sim$900 cal/mole higher than that of β-D-glucopyranose. The equilibrium mixture, instead of consisting of 85% of β-D-anomer and 15% of α-D-anomer as postulated, has a distribution of 64% of β- and 35% of α-D anomer, corresponding to a free-energy difference of 300 to 350 cal/mole.

The difference of approximately 550 cal is ascribed[3] to the operation of the "anomeric effect". The nature of this effect was satisfactorily explained in terms of dipole–dipole interaction[4].

In the α-D pyranose, where the C-1 hydroxyl group is axial, the dipole moment of the bond between the anomeric carbon atom and the glycosidic oxygen atom form a large angle with the vector of the dipole moment centred on the free orbitals of the ring oxygen atom, and this is the reason why the energy of the α-D anomer is lowered relative to that of the β-D anomer where these two dipoles form a small angle. As Table I shows, D-galactose resembles D-glucose in the anomer ratio at equilibrium and so does 2-amino-2-deoxy-D-glucose. However, a different behaviour is shown by the two protonated forms of the amino sugars. With both the hydrochlorides, the amount of the α-D anomer exceeds that of the β-D anomer at equilibrium. Thus, both in the glucose and galactose series, the free energy of the α-D anomer of the protonated 2-amino sugars, relative to that of the corresponding derivative of the β-D anomer, is lowered by a further 500 to 600 cal/mole, if compared with glucose and galactose. The 2-amino-2-deoxy-D-glucose base, on the other hand, resembles D-glucose with respect to anomer equilibrium and not the hydrochloride. It thus appears that the electrostatic interaction between the dipoles mentioned and a positively charged group at C-2 is considerably greater, at least in the glucose and galactose series, than it is in derivatives in which the substituent at C-2 is a hydroxyl or uncharged amino group. The same argument can be put in another way, at least in the case of 2-amino-2-deoxy-D-glucose, where data on the ionisation of the anomers are available. In the protonated β-D anomer, the two dipoles, *i.e.*, the C-1–OH and that due to the ring oxygen atom, reinforce one another and thus facilitate loss of a proton from the NH_3^+ group. In the α-D anomer, the effects of the two dipoles cancel to a significant extent, and thus the tendency to ionisation is diminished. Thus, the cationic α-D

anomer is the weaker acid. In view of the relationship discussed above between mutarotation and ionisation equilibria, it follows that the mutarotation equilibria of the cationic species and of the free bases must differ in a quantitatively predictable manner.

2-Acetamido-2-deoxy-D-glucose closely resembles the cationic 2-amino-2-deoxy-D-glucose species in its anomer distribution at equilibrium, and the same applies to 2-acetamido-2-deoxy-D-galactose if compared with D-galactose or the corresponding 2-amino-2-deoxy-D-galactose hydrochloride. The three-dimensional structure of both acetamido sugars in solution is unknown, but the crystal structure of 2-acetamido-2-deoxy-α-D-glucose has been elucidated by Johnson[5], and the oxygen atom of the amide group appears to be almost as far removed from the anomeric oxygen atom as possible (~5 Å). If this conformation is the one which is preferred in solution, unfavourable electrostatic interaction is reduced to a minimum in the α-D anomer. Optical rotatory dispersion studies[6] and measurements of dichroism[7] of 2-acetamido-2-deoxy-D-glucose have also led to the conclusion that C-5 of the pyranose ring and the amide group are in the same plane, and that the latter is in a *cis* relationship to the axial hydrogen atom on C-2.

Whilst the conformation of the β-D anomer is not known, it is likely that the glycosidic oxygen and the amide oxygen atoms are closer to each other than in the α-D anomer, and that unfavourable coulombic interaction is greater in the β-D anomer. If, in addition, the contribution of the dipole moment of the glycosidic oxygen atom is taken into account, the energy of the β-D anomer is thus increased and its concentration at equilibrium is lower.

In α-D-mannose, the dipole vectors of the axial, anomeric hydroxyl group and of the axial HO-2 group are at an angle of 180°, and this obviously reduces the energy of the α-D anomer as compared with the β-D anomer, accounting for the fact that the α-D anomer is favoured to a greater extent than in D-glucose. In mannose, the effect of the dipole moment of the ring oxygen atom appears to be of less importance than the directions of the dipole moments of C-1–OH and C-2–OH or C-2–NHR, respectively.

In protonated 2-amino-2-deoxy-α-D-mannose, the glycosidic oxygen atom, which carries a partial negative charge, is ~0.8 Å further away from the positively charged nitrogen atom than in the β-D anomer, and this change in electrostatic energy reduces the anomer ratio to a value near unity. Similar explanations may apply to the two 2-acetamido-2-deoxy-D-mannosides. The explanations may be an over-simplification, since we have neglected steric interaction and possible internal hydrogen-bond formation, which in any case appears to be of no great importance, as judged from the results of X-ray crystallography. However, a rational explanation for a number of experimental findings is provided.

The second dissociation constant of 2-amino-2-deoxy-D-glucose. — Like all other reducing sugars, 2-amino-2-deoxy-D-glucose in aqueous solution shows an ionisation in the very alkaline range. When 2-amino-2-deoxy-α-D-glucopyranose hydrochloride is dissolved in an amount of sodium hydroxide equivalent to 1.05 to

TABLE I

ANOMER EQUILIBRIA[a] FOR VARIOUS AMINO SUGARS AND THEIR DERIVATIVES

Hexose	$[\alpha]_D$ *(degrees)*				*Anomer ratio* (α/β)		*Ref. for n.m.r.*	ΔG^{20} *(Cal/mole)*
	α-D Anomer	*β-D Anomer*	*Equil.*	*Ref.*	*From rotation*	*From n.m.r.*		
D-Glucose	+112.2	+18.7	+52.7	20	36.3:63.7	36:64	25	+320
2-Amino-2-deoxy-D-glucose	+100	+14	+47.5	1	39.0:61.0	— —	—	+260
2-Amino-2-deoxy-D-glucose·HCl	+100	+25	+72.5	20	63.3:36.7	63:37	26	−310
2-Acetamido-2-deoxy-D-glucose	+82	−21.5	+46.6	5,21	65.8:34.2	68:32	26	−380
D-Mannose	+29.3	−17.0	+14.2	20	67.4:32.6	67:33	25	−420
2-Amino-2-deoxy-D-mannose·HCl	—	—	—	—	—	43:57	14	+170
2-Acetamido-2-deoxy-D-mannose	—	—	—	—	—	57:43	26	−160
D-Galactose	+150.7	+52.8	+80.2	20	28:72	27:73	25	+550
2-Amino-2-deoxy-D-galactose·HCl	+135.0	+39	+93	22	56.3:43.7	47:53	26	−145; +80
	+121.0	+44.5	+80	23	53.5:46.5	—	—	−80
2-Acetamido-2-deoxy-D-galactose	+115	—	+80	24	—	65:35	26	−360

[a]The values of equilibrium ratios of the anomers derived from measurement of optical rotation and from n.m.r. data agree in general very well, except for 2-amino-2-deoxy-D-galactose hydrochloride.

1.08 moles of the amine hydrochloride, the pH falls in the period between one minute after dissolution and ten minutes by ~0.1 to 0.12 unit, and then remains fairly constant. If the β-D amine (free base) is dissolved in about 0.07 equivalent of alkali, the pH rises in the first ten minutes by approximately 0.06 unit. Exact extrapolation to zero time is difficult, because reaction is fast at that pH, especially at the beginning, but we may assume that the pK_2 values of the two anomers at the temperatures chosen differ by at least 0.25, but possibly by 0.3 unit. The pH measurements were done at temperatures between 0 and 15°, and the initial pH values were between 11.14 and 11.37. This range was chosen so as to exclude significant interference from pk_1. At these pH ranges, however, the rate of mutarotation is quite fast, even at temperatures of 0–10°, and this makes an exact extrapolation to zero time uncertain. This difficulty does not arise with the anomers of D-glucose[8], where a very low degree of neutralisation and consequently a lower pH can be used, resulting in a relatively slow rate of mutarotation. On the other hand, the pH was low enough to avoid decomposition of the amino sugar by alkali[9]. At a higher pH or at a higher temperature, 2-amino-2-deoxy-D-glucose decomposes to give ammonia and/or pyrazines, but under the conditions used no significant amount of decomposition is likely to take place, as shown by the fact that the pH remains essentially constant for many hours after mutarotation is completed. Electrometric titration of a mutarotated solution of 2-amino-2-deoxy-D-glucose up to pH 12.5 at a temperature of 15° is also completely reversible. Measurements were carried out at 0.4, 5, and 15°, and at ionic strengths between 0.007 and 0.1. At 10°, values extrapolated to zero ionic strength for the α-D anomer varied between 12.54 and 12.59 and for the β-D anomer between 12.29 and 12.35. Observed pH values obtained at identical times after dissolving the amine under identical conditions differed sometimes by as much as 0.04, and values calculated for zero ionic strengths at given temperatures varied for the two anomers by as much as 0.07. However at 5°, pK_2^T of the α-D anomer was found to be 12.66 ±0.05, and pK_2^T for the β-D anomer 12.34 ±0.05. The data were not sufficiently accurate to calculate heats of ionisation.

2-Amino-2-deoxy-D-glucose appears to exist in aqueous solution almost entirely in the pyranoid form and is assumed to be present in the normal (*N*) chair conformation. Furanoid forms appear to be practically absent, and the contribution of the open-chain form is quantitatively very small and is neglected in this discussion. It may also be assumed that the pK_1 corresponds mainly to the addition of a proton to the amino group of the reducing sugar. This is shown, for example, by the fact that pK_1 of 2-amino-2-deoxy-α-D-glucose and p*K* of ethyl 2-amino-2-deoxy-α-D-glucopyranoside are practically identical, being 7.82 at 20°, and 7.81 at 22.5°, respectively (unpublished data, Dr. R. D. Marshall).

The second p*K* may be assumed to correspond with the ionisation of HO-1 on the following reasoning. An isolated, aliphatic hydroxyl-group may be assumed to have a p*K* of ~16; the p*K* of ethanol is estimated to be 16.2. If a second oxygen atom is linked to the carbon atom carrying the primary hydroxyl group, the p*K* is lowered by about 2.7 units. Thus, the hydrated form of acetaldehyde has a p*K* of 13.52[10]. A

similar difference is seen if the pK of trichloroethanol (12.24 at 25°)[11] is compared with the pK of chloral hydrate (9.77 at 25°)[12]. The other hydroxyl groups in a hexose may also be expected to further increase the acidity of HO-1. Thus, the pK of ethylene glycol has been reported[11] to be 15.1, and that of glycerol[13] as 14.15 at 15°. We may thus predict that the pK of HO-1 will lie between 12 and 13. In fact, all reducing hexoses that exist almost entirely in the pyranoid form have been found to have pK values in this range[13,14]. The pK values of the two anomers of D-glucose have been determined by Los and Simpson[8] at three temperatures, 0, 15, and 25°. They obtained, at 15°, a pK of the α-D anomer of 12.69, and for the β-D anomer a value of 12.44. These figures are very close to those obtained for the two anomers of the 2-amino-2-deoxy-D-glucose, indicating that substitution of HO-2 by an amino group has little effect on the acidity of the anomeric hydroxyl group. In both cases, the hydroxyl group of the β-D anomer was more acidic, and this has been explained[8] as being due to the equatorial locations of the hydroxyl groups in the β-D anomer which facilitate solvation of the ion.

The anomer equilibria for the glycosate ions can be calculated if the relevant ionisation constants of the two anomers have been measured and if the anomeric equilibrium constant for the neutral form is known (see above). Such calculations have been made (Table II) for both D-glucose and 2-amino-2-deoxy-D-glucose. It appears that, with both sugars, the glycosate ion at equilibrium has a greater preponderance of the β-D anomer than has the neutral sugar, the difference in ΔG being about 300 cal/mole. This means that the anomeric effect in these ions has almost disappeared. Ionisation in C-1 changes the electrostatic interaction, and we are now not concerned with the effect of two vectorial dipoles, but with one dipole and a point charge. It is also likely that the ion derived from the β-D anomer, which has the charge in an equatorial position, is stabilised in relation to the α-D anomer which has the charge in an axial position, where solvation is somewhat more difficult for steric reasons.

Equilibrium between uncharged and dipolar forms of 2-amino-2-deoxy-D-glucose. — The next problem to be considered is the possible presence of the dipolar form (3) in aqueous solutions of 2-amino-2-deoxy-D-glucose. The ionisation of the α-D anomer can be represented as follows:

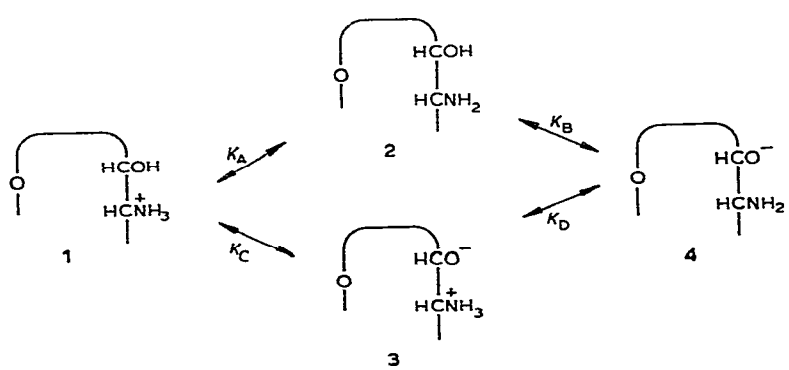

TABLE II

ANOMER EQUILIBRIA FOR THE GLYCOSATE IONS OF D-GLUCOSE[a] AND 2-AMINO-2-DEOXY-D-GLUCOSE

Hexose	*Anomeric equilibrium for neutral form* (α/β)	*Dissociation constants of anomers*		*Calc. anomeric equilibria for anion*	$\Delta G^{+15°}$ $\alpha \rightarrow \beta$ (*cal/mole*)
		α	β		
D-Glucose	36.3:63.7	2.05×10^{-13}	3.66×10^{-13}	23.9:76.1	+650
2-Amino-2-deoxy-D-glucose	39.0:61.0	2.2×10^{-13}	4.6×10^{-13}	25:75	+530

[a]The data for D-glucose were taken from Los and Simpson (1956)[8] and those for 2-amino-2-deoxy-D-glucose from the present and earlier paper by Neuberger and Fletcher[1].

As K_C is likely to be much smaller than K_A, we may equate pK_A with $pK_{1\alpha}$ of our earlier paper[1]. This was found to be about 8.1 at 10°. Similarly, we may equate pK_B with pK_2^T determined in this work. At 10°, this is approximately 12.5. K_C and K_D cannot be determined experimentally, nor can these constants be calculated with any great precision. However, an approximate estimate can be made on the following basis. A positively charged amino group on C-2 will obviously facilitate ionisation of HO-1, and the effect should be similar to that of the positive charge in the ionisation of the carboxyl group in glycine. The distance between the charges in the latter case has been calculated[15] to be 2.8 Å, and in 2-amino-2-deoxy-D-glucose the corresponding distance[16] is ~2.9 Å. As with glycine, we may assume that the positive charge reduces the pK by 2.0 units[17], and this makes pK_C ~10.5. A check on this calculation can be made in the following manner. It has been found (unpublished data) that mutarotated 2-acetamido-2-deoxy-D-glucose has a pK of 11.65. The effect on the acidity of HO-1 in 2-amino-2-deoxy-D-glucose of substituting the acetamido group by a charged amino group is likely to be similar to that shown in the difference between the pK of *N*-acetylglycine and the pK of glycine, with respect to the pK of the carboxyl group. The pK of *N*-acetylglycine is 3.6, whereas that of glycine[17] is 2.35. We may thus expect pK_C to be 10.4. A similar calculation can be made for K_D by comparing the pK of glycine ester (7.7) with the pK_2 of glycine (9.7). This leads to a value of 10.1 for the pK_D. Following the reasoning of Wegscheider[18] and Ebert[19], it can be seen that $K_A/K_C = K_B/K_D = 2/3$. It would thus seem that the ratio of the uncharged form to that of the dipolar form is 300:1 at 15°. The analogy between glycine and 2-amino-2-deoxy-D-glucose postulated here is unlikely to be fully correct quantitatively, and the estimate given is therefore not precise. But if this calculation is approximately correct, the difference in free energy of the two forms is of the order of 3 kcal/mole.

EXPERIMENTAL

All pH measurements were made with a Beckman Research pH Meter in a specially constructed cell with the temperature controlled within 0.02°. Further details are as given in our earlier paper[1]. The electrodes had been specially selected by Beckman to give a very small correction at alkaline pH. No correction was in fact made for alkaline error, but this is thought not to exceed 0.01 of a pH unit. Special care was taken to exclude atmospheric carbon dioxide.

The samples of 2-amino-2-deoxy-α-D-glucose hydrochloride and of 2-amino-2-deoxy-β-D-glucose were prepared as described earlier[1]. The samples of β-D anomer probably contained ~4% of the α-D anomer.

Accurately weighed samples of the amino sugar were dissolved in measured volumes of sodium hydroxide of known concentration which had been cooled to the desired temperature. Dissolution was generally complete after 15 sec, and the first readings were made at 30 sec. Readings were continued for 30 or 45 min, and the curve (pH *versus* time) was extrapolated to zero time, either by freehand or by

assuming that the reaction was first order. Since all the readings were made over pH ranges at which hydrolysis was important, hydroxyl-ion concentration had to be calculated from the pH. This was done by using the following equation: $K_w = m_H \cdot m_{OH} \cdot \gamma_{\pm}^2$. K_w values for different temperatures were taken from Bates[27]. Ionic activity coefficients (γ) were calculated from the equation $-\log \gamma = (AI^{0.5})/(1+\rho I^{0.5})$, using the values for A and ρ given by Bates for sodium chloride. The constant pK_a, which varies with ionic strength, was calculated from the equation $pK_a = pH + \log(b-[OH]) - \log(a-b+[OH])$, where a is the molar concentration of 2-amino-2-deoxy-D-glucose, b is the concentration of sodium hydroxide, after the amount required to neutralise the amino sugar had been subtracted, and [OH] is the molar concentration of hydroxyl ions; pK^T was calculated from the equation: $pK^T = pK_a + \log \gamma_{\pm}$.

The following representative experiments are given. 2-Amino-2-deoxy-α-D-glucose hydrochloride (201 mg) in 10.0 ml of 0.1 M NaOH at 10.0°:

t (min): 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 15.0, 20.0.
pH: 11.250, 11.222, 11.206, 11.193, 11.183, 11.173, 11.165, 11.162, 11.161.

2-Amino-2-deoxy-β-D-glucose (179.0 mg) in 9.3 ml of H_2O + 0.7 ml of M NaOH at 10.0°:

t (min): 0.5, 1.0, 1.5, 2.0, 3.0, 10.0.
pH: 11.198, 11.234, 11.244, 11.248, 11.252, 11.252.

2-Amino-2-deoxy-α-D-glucose hydrochloride (210.0 mg) in 10.0 ml of 0.10 M NaOH at 5.0°:

t (min): 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 10.0, 15.0, 25.0, 30.0.
pH: 11.270, 11.254, 11.245, 11.239, 11.230, 11.220, 11.200, 11.189, 11.183, 11.183.

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