Mutarotation of 2-Amino-2-deoxy-D-glucose

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The rate of mutarotation of 2-amino-2-deoxy-D-glucose hydrochloride at temperatures between 15 and 35 °C was found to be very similar to that of D-glucose. The reaction was found to be first order and there was no marked catalysis by the amino-sugar cation. In strongly acid solution acid catalysis was observed but the hydronium ion was considerably less effective in catalysing the mutarotation of the amino-sugar cation than it is in in its effect on the mutarotation of glucose. This difference is explained in terms of Coulombic interaction. On the other hand catalysis by acetate was found to be quantitatively closely similar with 2-amino-2-deoxyglucose and glucose. The rate of mutarotation between pH 5·5 and 8·3 was found to be greatly affected by the concentration of amino-sugar. Over this pH range several species of the amino-sugar exist in the aqueous solution and this fact and the presence of several catalytic species renders a quantitative interpretation of the results difficult. However, it appears that the rate of mutarotation over this neutral pH range is mainly affected by the concentration by the free amino-sugar base.

THE mutarotation of sugars in solution which has been intensively investigated for more than a hundred years has been recently comprehensively reviewed by Pigman and Isbell [1] and Isbell and Pigman.[2] However, little is known about the mutarotation of amino-sugars. The present paper is concerned with the changes of optical rotation which occur when a pure anomer of 2-amino-2-deoxy-D-glucose is dissolved in water at different pH values.

Mutarotation in Moderately Acid Solution.—The ionisation constants corresponding to the protonation of the amino-group have recently been determined; [3] they were found to be $1{\cdot}95 \times 10^{-8}$ at 25 °C for the α-anomer and $5{\cdot}37 \times 10^{-8}$ at 25 °C for the β-anomer.

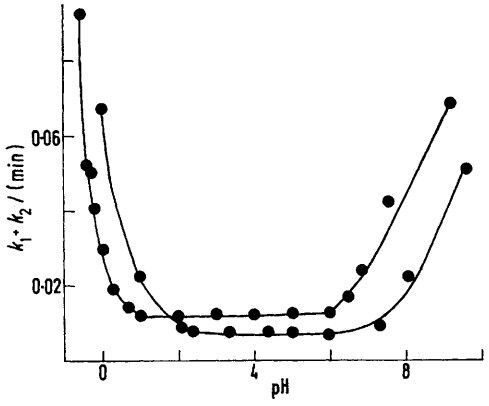


FIGURE 1 Variation of $k_1 + k_2$ with pH for α-D-glucose (lower curve) and 2-amino-2-deoxy-α-D-glucose (upper curve). The glucose values are for a temperature of 20 °C and those for 2-amino-2-deoxyglucose are for 25 °C. The values for glucose are taken from a variety of sources, but most of them are from H. S. Isbell and C. W. R. Wade, *J. Res. Nat. Bur. Stand.*, 1967, **71A**, 137. The values for 2-amino-2-deoxyglucose are taken from this paper and refer to a 0·1M-solution. At very low concentrations at pH values above 3·0 and at identical temperatures the figure for glucose and for 2-amino-2-deoxy-glucose would largely coincide

Thus at a pH of 5·5 only *ca.* 2% or less of the amino-sugar present in solution will be in the form of the free base. On the other hand, as will be discussed later, acids are considerably less effective as catalysts of the mutarotation of 2-amino-2-deoxy-D-glucose than they are of the corresponding reaction of the parent sugar, glucose (Figure 1). Thus, over the range of pH of 1·0—5·7 no significant change of the rate of mutarotation of

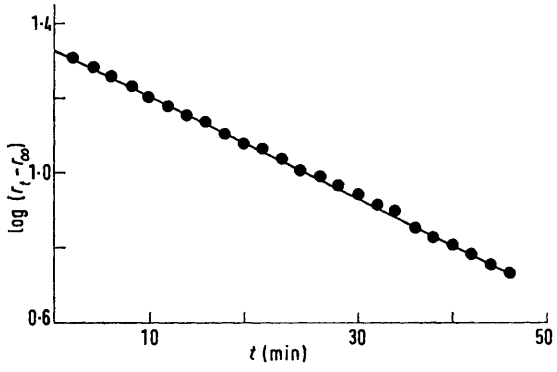


FIGURE 2 Changes of rotation of a 0·2M-solution of 2-amino-2-deoxy-α-D-glucose hydrochloride in water at 25 °C

2-amino-2-deoxyglucose with pH was observed. In addition, it is suggested that the very small amounts of furanose anomers and of the acyclic forms which are undoubtedly present in an aqueous solution of the amino-sugar can be neglected. This is supported by measurements of n.m.r. of solutions of 2-amino-2-deoxyglucose hydrochloride in D_2O.[4] In other words, it is assumed that mutarotation in moderately acid solution, as defined above, can be entirely interpreted as being a reversible conversion of the two protonated anomers of the pyranose form of the amino-sugar. This assumption is in accord with the observation that the mutarotation of 2-amino-2-deoxyglucose hydrochloride follows first-order kinetics (Figure 2). Thus, the amino-sugar resembles in this respect other compounds having *gluco-* or *manno*-conformation. It also seems that the protonated amino-sugar has no marked catalytic effect on its own mutarotation. This follows from the observations that the rate of mutarotation of the amino-sugar hydro-

[1] W. Pigman and H. S. Isbell, *Adv. Carbohydrate Chem.*, 1968, **23**, 11.
[2] H. S. Isbell and W. Pigman, *Adv. Carbohydrate Chem.*, 1969, **24**, 14.
[3] A. Neuberger and A. P. Fletcher, *J. Chem. Soc.* (*B*), 1969, 178.
[4] D. Horton, J. S. Jewell, and K. D. Philips, *J. Org. Chem.*, 1966, **31**, 4022.

chloride is, more or less, independent of its concentration. This is not surprising as the mutarotation of at least the cationic form of this amino-sugar is relatively insensitive to catalysis by acids. Thus, at 25° the value of the mutarotation constant $k_1 + k_2$ for a 0·2-molar solution of the amino-sugar hydrochloride was found to be 0·0126, whilst a 0·05-molar solution gave a value of 0·0130.

As with other reducing sugars the mutarotation constant $k_1 + k_2$ increases markedly with temperature. Following the convention used by sugar chemists the mutarotation constant is expressed in minutes with logarithm to the base ten and is calculated from the equation:

$$k_1 + k_2 = (1/t) \log [(r_0 - r_\infty)/(r_t - r_\infty)] \quad (1)$$

The mutarotation constant of 2-amino-2-deoxyglucose hydrochloride at the temperatures measured does not differ from that of glucose by more than 10—15% (Table 1). It is thus lower than the values found for almost all other hexoses and pentoses. It seems reasonable to conclude that the rate of anomerisation depends mainly on the conformation of the ring and that the substitution of a charged ammonium group for a hydroxy-group in C-2 has only a slight effect on the rate of mutarotation. The temperature coefficient of the mutarotation of the amino-sugar hydrochloride in water is similar to that of glucose and corresponds to an Arrhenius heat of activation of *ca.* 17·1 kcal mol^{-1}, at least over the temperature measured. Values of this order are characteristic for α ⟶ β pyranose conversion and are higher than for the pyranose–furanose interconversion for which activation energies of 10·7—15·8 kcal mol^{-1} have been reported.[2,5] However, it appears that, whilst the equilibria between the two anomeric forms of the pyranose structures of most non-amino-hexoses are not greatly affected by temperature, this does not appear to apply to 2-amino-2-deoxyglucose hydrochloride. Thus in the earlier paper [3] the equilibrium constants at temperatures between 10 and 25 °C for the anomerisation of the amino-sugar hydrochloride have been calculated from the ionisation constants of the two anomers and the pH at equilibrium of half neutralised solutions. The values obtained cannot be claimed to be very accurate, but they indicate that the equilibrium ratio of β- to α-anomer changes between 15 to 25 °C from 0·49 to 0·88. As the equilibrium constant K equals k_1/k_2, it follows that the temperature coefficients of the two reactions α ⟶ β and β ⟶ α are also markedly different. By using the values of the constants of the anomerisation equilibrium calculated earlier and the values of $k_1 + k_2$ of Table 1 average energies of activation were calculated for temperatures between 10 and 35 °C for the two reversible reactions characterised by k_1 and k_2 respectively. The possibility of using the equilibrium rotations at the relevant temperatures as indications of the α : β ratio was considered, but owing to the difficulty, due to rapid mutarotation, of measuring accurately the specific rotations of the un-ionised anomers at higher temperatures, this was not further pursued.

The Arrhenius activation energy E was calculated in the usual way. It was found that for k_1 (conversion of α- into β-anomer) the energies of activation were 21—23 kcal mol^{-1}, whilst for k_2 (conversion of β- to α-anomer) they were *ca.* 14—16 kcal mol^{-1}. These values are likely to be only approximations and work is in progress to measure the equilibrium concentrations of the two anomers at different temperatures directly by n.m.r. spectroscopy.

Mutarotation in Strongly Acid Solution.—In strongly acid solution the mutarotation constant $k = k_1 + k_2$ can be expressed by equation (2), where k_{H_2O} and $k_{H_3O^+}$ are

$$k = k_{H_2O} + k_{H_3O^+} \times [H_3O^+] \quad (2)$$

the mutarotation constants respectively of the uncatalysed reaction and of the reaction catalysed by the hydronium ion.

We have obtained no indication that in strongly acid solution the amino-sugar cation, at least up to concentrations of 0·2 mol l^{-1}, has any appreciable catalytic activity. The values obtained with hydrochloric acid solutions of concentrations of up to 4 mol l^{-1} (Table 2) show that at the molarity of acid of 0·1 there is no

TABLE 1

Values of the mutarotation coefficient of 2-amino-2-deoxy-α-D-glucose hydrochloride at various temperatures in comparison with those of α-D-glucose: $k = k_1 + k_2$ in min^{-1}

Temp.	Glucose [a]	2-Amino-2-deoxyglucose
0°	0·000780	
10	0·00231	
15	0·00390	0·00398
20	0·00639	0·00648
25	0·01043	0·01250
30		0·0188
35	0·02578	0·0304

[a] Values from G. F. Smith and M. C. Smith, *J. Chem. Soc.*, 1937, 1413.

TABLE 2

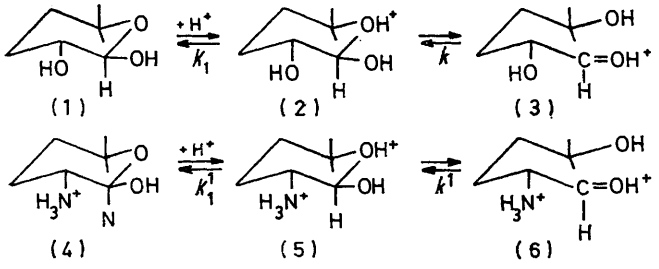
Rates of mutarotation of 2-amino-2-deoxy-α-D-glucose hydrochloride in solutions of hydrochloric acid of different concentrations. Concentration of amino-sugar 0·1M; temperature 25°. Values are for $k_1 + k_2$ (min^{-1}). Value of k_{H_2O} is taken as 0·0121

Concentration of HCl (M)	$k_1 + k_2$	$k_1 + k_2 - k_{H_2O}$
0·001	0·0121	0
0·01	0·0114	−0·0007
0·1	0·0114	−0·0007
0·2	0·0142	+0·0021
0·5	0·0187	+0·0066
1·0	0·0298	+0·0177
1·5	0·0408	+0·0287
2·0	0·0497	+0·0376
2·5	0·0519	+0·0398
3·0	0·0720	+0·0599
4·0	0·0922	+0·0801

[5] H. S. Isbell and W. W. Pigman, *J. Res. Nat. Bur. Stand.*, 1938, **20**, 773.

significant catalysis by the hydronium ion. As the acidity increases to 4-molar acid the rate of mutarotation rises to reach a value of 0·08, *i.e.* is *ca.* 7 times faster than in water. The rates are not exactly proportional to the acid concentration C_{H^+}, but the data fit better a proportionality to c_{H^+} than to $-H_0$. It appears that at higher concentrations of acid, *i.e.* above 1 mol l^{-1}, there is an additional medium or salt effect. If the values given in Table 2 for $k_{H_3O^+}$ $[H_3O]$ are divided by $[H_3O^+]$ and averaged, a mean value for $k_{H_3O^+}$ of $17{\cdot}8 \times 10^{-3}$ is obtained, compared with k_{H_2O} of $12{\cdot}0 \times 10^{-3}$. The corresponding figures for the mutarotation of glucose at the same temperature are 260×10^{-3} for hydrochloric acid catalysis and $10{\cdot}6 \times 10^{-3}$ for catalysis by water.[2,6] Thus, strong acids are *ca.* 15 times less effective in catalysing the mutarotation of 2-amino-2-deoxyglucose than that of glucose.

There is still no unanimity about the detailed mechanism of the acid-catalysed mutarotation of glucose and the different types of interpretation have been fully discussed by Isbell and Pigman.[2] According to Challis, Long, and Pocker [7] and Long and Bigeleisen [8] the rate of protonation of the ring oxygen is fast and the concentration of the protonated form is defined by an ionisation equilibrium constant K_1. This protonation is followed by a series of electron shifts and the rupture of a bond resulting in an acyclic intermediate. We may assume that protonation on the ring oxygen which is the postulated first step in the acid-catalysed mutarotation is more difficult in the cation of 2-amino-2-deoxyglucose than in glucose. In other words, the equilibrium constant K_1 is considerably greater for the already protonated aminosugar than for the uncharged parent hexose. The distance between the ring oxygen atom and the positively charged nitrogen atom in the protonated 2-amino-2-deoxyglucose is *ca.* 4 Å. It is thus reasonable to assume that the substitution of the NH_3^+ group for a hydroxy-group will markedly hinder protonation on the ring oxygen atom. Other workers [9,10] have



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assumed protonation to be slow and to coincide with the opening of the pyranose ring. With this interpretation too we may assume that further protonation of the pyranose ring cation of 2-amino-2-deoxyglucose is slower than in the uncharged glucose molecule.

[6] C. S. Hudson, *J. Amer. Chem. Soc.*, 1907, **29**, 1571.
[7] B. C. Challis, F. A. Long, and Y. Pocker, *J. Chem. Soc.*, 1957, 4679.
[8] F. A. Long and J. Bigeleisen, *Trans. Faraday Soc.*, 1959, **55**, 2077.

Catalysis of Mutarotation by Acetate.—At a pH of 4—5 and at a temperature of 25 °C mutarotation of 2-amino-2-deoxyglucose hydrochloride is increased by acetate in the same way as that of glucose. Indeed, the rates of mutarotation increase quantitatively in the same manner with rising molarity of the sodium acetate–acetic acid buffer as with glucose (Table 3). The catalysis in this

TABLE 3

Rate of mutarotation of 2-amino-2-deoxy-α-D-glucose and of α-D-glucose in acetate buffers at 25°. The acetate buffer was prepared by half neutralising a solution of AnalaR acetic acid in water with sodium hydroxide.

Concentration of acetate (M)	$k_1 + k_2$ 2-Amino-2-deoxyglucose	glucose
0·4	0·0365	0·0362
0·6	0·0467	0·0480
0·8	0·0560	0·0585
1·0	0·0611	0·0618

system may be described by equation (3) where HA is acetic acid and A^- is acetate ion.

$$k = k_{H_2O} + k_{HA}[HA] + k_{A^-}[A^-] \qquad (3)$$

The mutarotation coefficient k_{HA}, due to the undissociated acid was estimated in a series of experiments in which the mutarotation of solutions 2-amino-2-deoxyglucose hydrochloride was measured in the presence of varying amounts of acetic acid (Table 4). It can be seen

TABLE 4

Rate of mutarotation of 2-amino-2-deoxy-D-α-glucose hydrochloride in the presence of varying amounts of acetic acid at 25°. Mutarotation rates were calculated from the observed changes in rotation by the method of Guggenheim * and the slopes of the best lines calculated by the method of least squares using a Varian Data 620/i computer programmed in Basic.†

Glucosamine (M)	Acetic acid (M)	k_1+k_2
0·096	0	0·117
0·088	0·1	0·0122
0·088	0·5	0·0125
0·095	1	0·0137
1·096	2	0·0142
0·090	5	0·0176

* E. A. Guggenheim, *Phil. Mag.*, 1926, **2**, 538.
† Average of two or three determinations.

that k_{HA} is small being *ca.* $1{\cdot}2 \times 10^{-3}$, compared with a value of 61×10^{-3} for k_{A^-}.

The almost negligible effect of undissociated acetic acid means that the rising rate of mutarotation of the aminosugar with increasing concentration of the acetate buffer shown in Table 3 must be entirely due to base catalysis by the acetate ion. From the ionisation constants of the amino-groups of the two anomers of 2-amino-2-deoxyglucose [3] it can be calculated that at the pH of the acetate experiments, which is *ca.* 4·6, *ca.* 99·9% of the

[9] R. P. Bell, ' Acid-Base Catalysis,' Clarendon Press, Oxford, ch. IV and V.
[10] L. P. Hammett, Physical Organic Chemistry, 1940, McGraw-Hill, New York and London, p. 337.

amino-hexose is in the protonated form and we may thus assume that the mutarotating species in these experiments is the pyranose form of the cation in the normal chair conformation.

The finding that base catalysis by acetate of mutarotation of the 2-amino-2-deoxyglucose cation is no greater than that of the uncharged glucose seems, at first, surprising. One might have expected to find the reverse of what was reported above about acid catalysis of the amino-sugar cation by hydronium ion.

Further work is being carried out on the effects of charged and uncharged catalysts of both acid and base type on the mutarotation of glucose and of 2-amino-2-deoxyglucose respectively. This we hope may lead to a rational interpretation of the various findings.

Mutarotation Under Neutral or Slightly Alkaline Conditions.—As the pH of an aqueous solution of 2-amino-2-deoxy-D-glucose hydrochloride is raised above 6·0, the free base, the cationic and the zwitterionic forms of the pyranose conformation of the amino-sugar will be present in significant amounts. The relative quantities of both pyranose anomers of these forms present at a given temperature can be calculated from the known ionisation constants.[3,11] All these species will have their own mutarotation coefficients. In addition, as mutarotation is an example of general acid-base catalysis, the catalytic effects of the hydroxide ion, of the free bases and of the two glucosate ions have to be considered. The difficulties in the exact interpretation of experimental results may thus be expected to be formidable.

Measurements were made of changes of rotation with time of various concentrations of the amino-sugar hydrochloride neutralised to different degrees and with pH values ranging from 6·0 to *ca.* 8·4. Some measurements

TABLE 5

Rate of mutarotation of 2-amino-2-deoxy-D-α-glucose hydrochloride neutralised with sodium hydroxide to varying extent at 25 °C. The figures are values of $k_1 + k_2$ in min^{-1}

Concentration of amino-sugar (M)	Degree of neutralisation (%) 5	10	20	50	80
0·0025		0·0110	0·0137	0·0174	0·0222
0·0050		0·0127	0·0156	0·0206	0·0276
0·01		0·0136	0·0161	0·0221	0·0303
0·02				0·0239	
0·05				0·0319	
0·10	0·012	0·0157	0·0231	0·0410	0·0682
0·20	0·014	0·0189	0·0302	0·0704	0·0904

were made with 2-amino-2-deoxy-β-D-glucose and the results obtained agreed well with those done under identical conditions with the α-anomer. The results shown in Table 5 indicate that the rate of mutarotation increases as the concentration of the amino-sugar (and hence free-base form) rises. At the lowest concentration of the amino-sugar tested, which was 0·0025M, the rate doubles as the pH is raised from 4·5 to 8·2. At the highest concentration examined the rate of mutarotation increases *ca.* seven-fold over the same pH range. In moderately acid solution the rate of mutarotation of the protonated 2-amino-2-deoxy-D-glucose differs very little from that of D-glucose; the same applies to the rate of mutarotation as catalysed by the base acetate (see above). It seems, therefore, reasonable to assume that the positive charge of the amino-sugar cation does not greatly affect the rate of mutarotation as catalysed by water or bases. It is thus justified, at least as an approximation, to assume that the rates of mutarotation of the cation of the amino-sugar and of the free base are identical. The small amount of zwitterions will also be neglected and we shall treat the experimental results as if they represented the mutarotation of one molecular species.

Making this simplifying approximation the changes of rotation with time over the pH range of 6—8·5 should be represented by the following equation:

$$k = k_1 + k_2 = k_{H_2O} + k_{OH^-}[OH^-] + k_{G^-}[G^-] + k_G[G] \quad (4)$$

The first term is constant; k_{OH^-} is unknown, but the hydroxide ion concentration can be calculated from the degree of neutralisation and the ionisation constants. Both these terms are independent of the amino-sugar concentration and could, in principle, be calculated if the results in Table 5 were extrapolated to zero amino-sugar concentration. From the few data available this cannot be done with any accuracy, but the values are compatible with the assumption that k_{OH^-} for 2-amino-2-deoxy-D-glucose is *ca.* 5000, the most reliable value obtained for D-glucose.[12] During mutarotation of a partially neutralised solution of 2-amino-2-deoxyglucose hydrochloride, the pH changes by *ca.* 0·2 units [3] and thus the hydroxide ion concentration is not constant during experiments carried out over the pH range 6—8·5. However, this change is not sufficiently large to be detected in these experiments and the values obtained indicated that the mutarotation was approximately of first order.

The last two terms of the equation, $k_{G^-}[G^-]$ and $k_G[G]$, where k_{G^-} represents the two mutarotation coefficients due to the catalytic effects of the two glycosate ions G^- and k_G represents the coefficients due to the catalytic effects of the two anomers of the free base G, are both dependent on the concentrations of the amino-sugar. Whilst the mutarotation coefficients of the two anomers of both the base and the glycosate ion are bound to be different, these differences can be neglected in this semiquantitative treatment as being relatively small. It may be further suggested that the term due to the glycosate ions is relatively small. Thus at the highest concentration of the amino-sugar (0·2M) and at the highest degree of neutralisation the concentration of the glycosate ions can be calculated to be between 1·0 and $2·1 \times 10^{-5}$M. We may also assume that the mutarotation coefficients reflecting the catalysis by the two glycosate ions of 2-amino-2-deoxy-D-glucose are similar to those of D-glucose calculated by Los and Simpson.[12]

[11] A. Neuberger and A. P. Fletcher, *Carbohydrate Res.*, 1971, **17**, 79.

[12] J. M. Los and L. B. Simpson, *Rec. Trav. chim.*, 1957, **76**, 267.

These values are unlikely to be higher than 300. It is thus possible to calculate at least a minimum value for $k_G[G]$. In the example just quoted (80% neutralisation and 0·2M) the following holds:

$$k_G[G] = 0{\cdot}0904 - 0{\cdot}0122 - 0{\cdot}0063 - 0{\cdot}0060 = 0{\cdot}0659$$
$$(H_2O) \quad (OH^-) \quad (G)$$

It thus appears that the major factor responsible for the increased rate of mutarotation, as the concentration of the amino-sugar is raised, is catalysis by the free-base forms of the amino-sugar. Calculating k_G from this example gives a value of 0·45. Similar estimates can be made from other kinetic experiments given in Table 4; they show a considerable scatter, but they all lie between 0·42 and 1·61. Bell [9] has modified the well-known Brönsted equation which relates the dissociation constants of acids and bases to their catalytic effects in specific reactions. For the base catalysis of the mutarotation of glucose at 18 °C $k_B = q \times 3{\cdot}3 \times 10^{-4}[p/q \times K_A]^{0{\cdot}4}$ where p is the number of protons bound equally firmly and q is the number of points to which a proton can be attached. Applying this equation to the two anomeric bases and correcting for the temperature difference, values of 1·3 and 0·8 for the α- and β-anomer respectively are obtained. This supports the belief that the order of magnitude calculated for k_G is correct.

Further evidence for the general conclusion that the increased rate of mutarotation at higher amino-sugar concentrations is mainly due to the base catalysis by the free amino-group of the sugar was obtained in the following experiment. Mutarotation was measured at 25° of a solution containing 2-amino-2-deoxy-α-D-glucose hydrochloride (0·005M), methyl 2-amino-2-deoxy-β-D-glucoside (0·045M), and hydrochloric acid (0·020M) in 0·2M-KCl. The value for k obtained was 0·0477. A half neutralised solution of the amino-sugar hydrochloride at an identical concentration, but without the glycoside, gave a value for k of 0·0206. Since approximately half the glycoside is ionised, the catalytic coefficient of the glycoside can be calculated to be 1·2. The ionisation constants of the β-anomer of the amino-sugar and of the β-glycoside are more or less identical and the catalytic coefficient of 2-amino-2-deoxy-β-D-glucose must thus also be about 1·2. The general validity of the argument can be further tested if the range of observations is extended to pH values of 8·5—10·5. Under such conditions the relative contribution of the free base is more of less independent of pH, whilst the contributions of hydroxide ions and of glycosate ions change with pH in a predictable fashion.

It has been assumed in this discussion that no covalent bond is formed between various species of amino-sugar under the experimental conditions. This may not be correct; indeed it is not impossible that Schiff's bases are produced between the free base of a pyranose anomer and an open-chain aldehylic form and this is likely to be reversible. The occurrence of such a reaction may possibly be the reason why k_G values calculated from higher concentrations of amino-sugar are smaller than those calculated from experiments using lower concentrations.

It appears from these observations that in order to obtain the specific rotation of a pure base anomer of an amino-sugar, measurements should be made at the lowest concentration practicable.

EXPERIMENTAL

All measurements of optical rotation were made with a Perkin-Elmer model 141 polarimeter. In most experiments a water-jacketted cell of 1 cm path-length was used, but in some cases where low concentrations of the sugar were necessary, a similar cell of 10-cm path-length was used. Some measurements were made at a wavelength of 589 nm, whilst others were made at wavelength of 436 and 365 nm. Temperature was kept constant to within 0·02 °C with a water-bath and cooling coil (Grant Instruments, Cambridge). Care was taken to exclude atmospheric carbon dioxide from all neutral and alkaline solutions. Solutions contained KCl in amounts to make the ionic strength 0·2.

Methyl-2-amino-2-deoxy-β-D-glucoside, prepared by the method of Neuberger and Wilson [13] was given by Dr. B. M. G. Wilson. 2-Amino-2-deoxy-α-D-glucose hydrochloride (Thomas Kerfoot & Sons Ltd.) was used as supplied, but in some cases was recrystallised from ethanol–water to achieve an acceptable value for initial $[\alpha]_D$. 2-Amino-2-deoxy-β-D-glucose was prepared from the α-anomer by the method of Westphal and Holzmann.[14]

We thank Miss J. Cossill for technical assistance and Mr. D. M. L. Morgan for carrying out the mutarotation experiments with acetic acid.

[1/154 *Received, February 18th,* 1971]

[13] A. Neuberger and B. M. G. Wilson, *Carbohydrate Res.*, 1971, **17**, 89.
[14] O. Westphal and H. Holzmann, *Ber.*, 1942, **75**, 1274.