Determination of acid dissociation constants of anomers of amino sugars by ^{1}H NMR spectroscopy

Andrei Blaskó [a], Clifford A. Bunton [a,*], Sergio Bunel [b], Carmen Ibarra [b], Exiquiel Moraga [b]

[a] *Department of Chemistry, University of California, Santa Barbara, CA 93106, USA*
[b] *Departamento de Quimica Inorganica y Analitica, Facultad de Ciencias Quimicas y Farmaceuticas, Universidad de Chile, Santiago, Casilla 233, Chile*

Abstract

Acid dissociation constants of α- and β-D-glucos-, mannos-, and galactos-ammonium ions have been determined from ^{1}H NMR chemical shifts of the individual anomers in D_2O. Values of $pK_a(D)$ for the α- and β-ammonium ions are, respectively: glucosamine, 8.12 and 7.87, mannosamine, 7.78 and 8.50, galactosamine, 8.49 and 8.02. The differences are ascribed largely to differences in the hydration requirements of ammonium and amino groups in the axial and equatorial positions and hydration at upper and lower faces of the sugars. Acid dissociation constants of the 1-hydroxyl group of nonionic D-glucosamine and D-glucose are higher for the β than the α anomer. © 1997 Elsevier Science Ltd. All rights reserved.

*Keywords:* NMR; Dissociation constants; Amino sugars

Many sugar reactions involve proton transfers, but most values of acid dissociation constants are for mixtures of tautomers [1,2]. Sugars are more acidic at anomeric hydroxyl groups than secondary aliphatic alcohols due to inductive electron withdrawal by endocyclic oxygen. Acid dissociation constants of sugars are similar to those of aldehyde hydrates and 2-trifluoroethanol with $pK_a \equiv 12$ [1,2]. Amino sugars are weaker bases than simple aliphatic amines due to inductive effects, and here also dissociation constants are often apparent values for mixtures of tautomers. Solvation has major effects on tautomeric equilibria of sugars [3] and equatorial hydroxyl groups of pyranoses interact with water without disrupting its structure [4–6]. Hydrogen exchange between water and 1-OH is faster for β- than for α-D-glucose [5,6], which is consistent with differences in hydration of equatorial and axial groups which also make β-D-glucose a stronger acid than the α anomer [7]. Acid dissociation constants of amino sugars depend upon inductive effects of nearby groups and hydration of ammonium and amino groups, which should be sensitive to conformation.

We are interested in complexation of sugars and their amino derivatives with such transition metal

---

* Corresponding author.
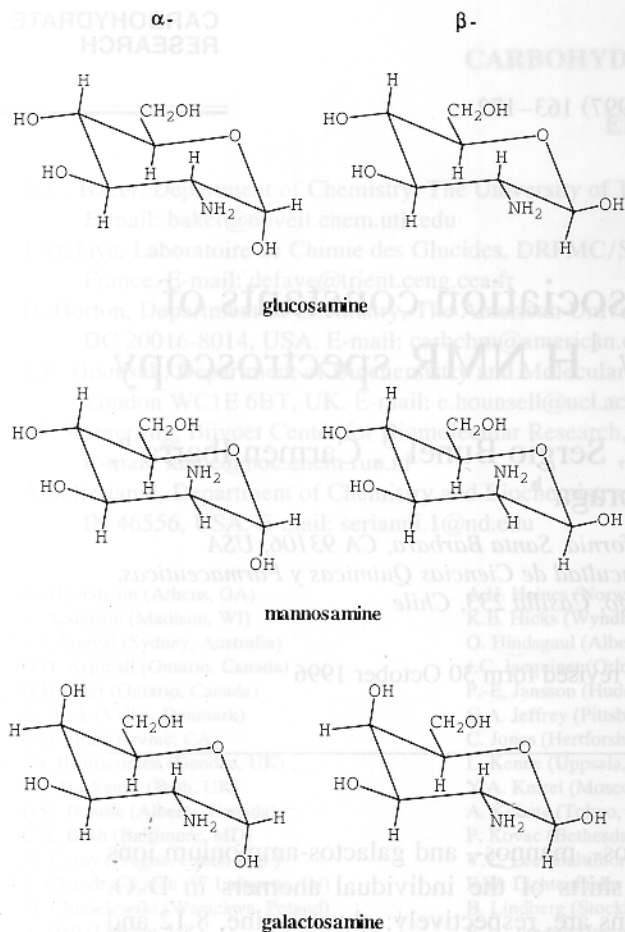
Scheme 1.

ions as Co(III) [8–11].[1] D-Glucosamine [8,9], D-mannosamine, and D-galactosamine[1] complex with metal ions, and mixed complexes with diamines or ammonia and Co(III) have been isolated or characterized. The nonionic amino sugars are shown in Scheme 1. Complexation of the amino sugars involves the 2-amino and 1-alkoxide groups, and so deprotonation is a key step. Neuberger and Fletcher concluded that differences in reported values of dissociation constants of D-glucosammonium ions were probably due to differences in anomeric composition [12]. They used variations of optical rotation with pH to calculate equilibrium constants involving the anomers and estimated $pK_a = 7.71$ and 7.24 (at 25 °C) for the α- and β-ammonium ions, respectively. This method requires isolation of pure anomers, which interconvert relatively slowly in aqueous solution, and so there may be problems at high pH or in the presence

of catalytic acids or bases. We planned to monitor acid–base equilibria by following ^{1}H chemical shifts of the individual anomers in D_2O as a function of pD, because ^{1}H chemical shifts at positions 1 and 2 are readily measured [3,13–15] and chose amino sugars for which there should be differences in hydration of the ammonium and amino groups.

Provided that proton transfers are fast and anomerization is slow on the NMR time scale chemical shifts are weighted averages, eqs (1) and (2):

$$\alpha \text{ anomer, } \delta_\alpha = \delta_\alpha^o \chi_\alpha^o + \delta_\alpha^+ \chi_\alpha^+, \tag{1}$$

$$\beta \text{ anomer, } \delta_\beta = \delta_\beta^o \chi_\beta^o + \delta_\beta^+ \chi_\beta^+, \tag{2}$$

where superscripts o and + denote nonionic and cationic species respectively, and χ is a mole fraction. Similar equations apply to acid dissociations of the nonionic species [2]. The classical acid dissociation constant in D_2O is given by:

$$pK_a(D) = pD - \log(\chi^o/\chi^+) \tag{3}$$

for dissociation of the ammonium ion (Experimental section). A similar equation applies to dissociation of the hydroxyl group and $pD = pH + 0.38$ [16].

We did not use buffers, or constant ionic strength, in order to minimize electrolyte effects on the NMR spectra. We worked with dilute electrolyte for dissociation of the ammonium ions, but for dissociation of the hydroxyl groups we had to use relatively high, and variable, amounts of KOD. However, ionic strength effects on dissociation of the ammonium ions are small and constant because we used 0.01 M amino sugar. There are solvent hydrogen isotope effects on our dissociation constants [17–20] but for a variety of oxyacids and ammonium ions isotope effects, values of $K_a(H)/K_a(D)$ increase similarly with increasing pK_a.

Neuberger and Fletcher ascribed differences in acid dissociation constants of α- and β-glucosammonium ions to differences in hydration [12]. They noted that the equatorial 2-NH_3^+ (NH_2) group is located below the horizontal plane of the pyranose ring and postulated that an axial, 1-OH group will increase hydration in this region and therefore stabilize NH_3^+, relative to NH_2, resulting in $K_a(\beta) > K_a(\alpha)$. On this hypothesis galactosammonium ions, with equatorial NH_3^+, should behave like the glucosammonium ions, but mannosammonium ions, with axial NH_3^+, should behave differently. We estimated values of $pK_a(D)$ for the three ammonium ion sugars from changes of ^{1}H chemical shifts as a function of pD (eqs (1)–(3)), and attempted to use this general

---

[1] S. Bunel, C. Ibarra, and E. Moraga, unpublished results.

Table 1
[1]H chemical shifts and coupling constants of glucosamine (pD 10.6) and its conjugate acid

Chemical shifts, δ (ppm)		H-1	H-2	H-3	H-4	H-5	H-6	H-6'
Glucosammonium ion	α	5.48	3.33	3.92	3.52	3.89	3.88	3.82
	β	4.98	3.05	3.77	3.54	a	a	a
Glucosamine	α	5.22	2.72	3.57	3.42	3.44	3.86	3.78
	β	4.58	2.60	3.38	3.40	3.48	3.92	3.74
Coupling constants, ^3J (Hz)		$H_{1,2}$	$H_{2,3}$	$H_{3,4}$	$H_{4,5}$	$H_{5,6}$	$H_{5,6'}$	$H_{6,6'}$
Glucosammonium ion	α	3.8	11	10	9	2.3	5.5	12.5
	β	8.5	11.5	9				
Glucosamine	α	3.5	10	10	m			12.5
	β	8	9 [b]	bm	m			12

b – broad, m – multiplet.
[a] Overlapped with signals of α.
[b] $^4J_{2,4} = 1.5$ Hz.

method to estimate dissociation constants of the anomeric 1-hydroxyl groups. We examined the ^{1}H NMR spectra of the amino sugars and their conjugate acids in order to identify signals which could easily be monitored in D_2O over a range of pD.

1. Results and discussion

^{1}H NMR spectra.—The ^{1}H chemical shifts of the α- and β-glucosammonium ions are given in Table 1. They differ numerically from earlier values [9] which were referred to $\delta_{HDO} = 4.63$ ppm, rather than to the revised value of $\delta_{HDO} = 4.80$ ppm in conformity with $\delta = 0$ for TSP (Experimental section). Earlier values for glucosamine were measured in 0.03 M KOD where there was probably some formation of alkoxide ion, and values relative to the new reference and at pD = 10.6 are in Table 1. Some corresponding data for the mannosammonium and galactosammo-

nium ions and limited data for some of the amines are in Tables 2 and 3, respectively. Coupling constants are as expected for pyranose rings [13–15] (Table 1). For nonionic mannosamine and galactosamine we did not assign signals of hydrogen atoms distant from the NH_2 and OH groups. Chemical shifts of α- and β-glucose are, respectively: H-1, 5.23 and 4.65; H-2, 3.54 and 3.25 ppm and $J_{1,2}$ are 4 and 8 Hz and $J_{2,3}$ are 10 and 9.5 Hz, respectively. These δ values agree with those in the literature where available [14,21], and coupling constants are as expected for pyranoses [13,14]. The H-1 signal of β-mannosamine was too broad for determination of $J_{1,2}$ (Experimental section), but we did not have this problem with the α anomer or with any of the other ammonium or amino sugars. Chemical shifts of the nonionic amino sugars were measured at pD $\cong$ 10 where solutions contain small amounts of the ammonium and alkoxide ions. These chemical shifts are therefore less reliable than those of the ammonium ions.

Table 2
[1]H chemical shifts and coupling constants of mannosamine (pD 9.7) and its conjugate acid
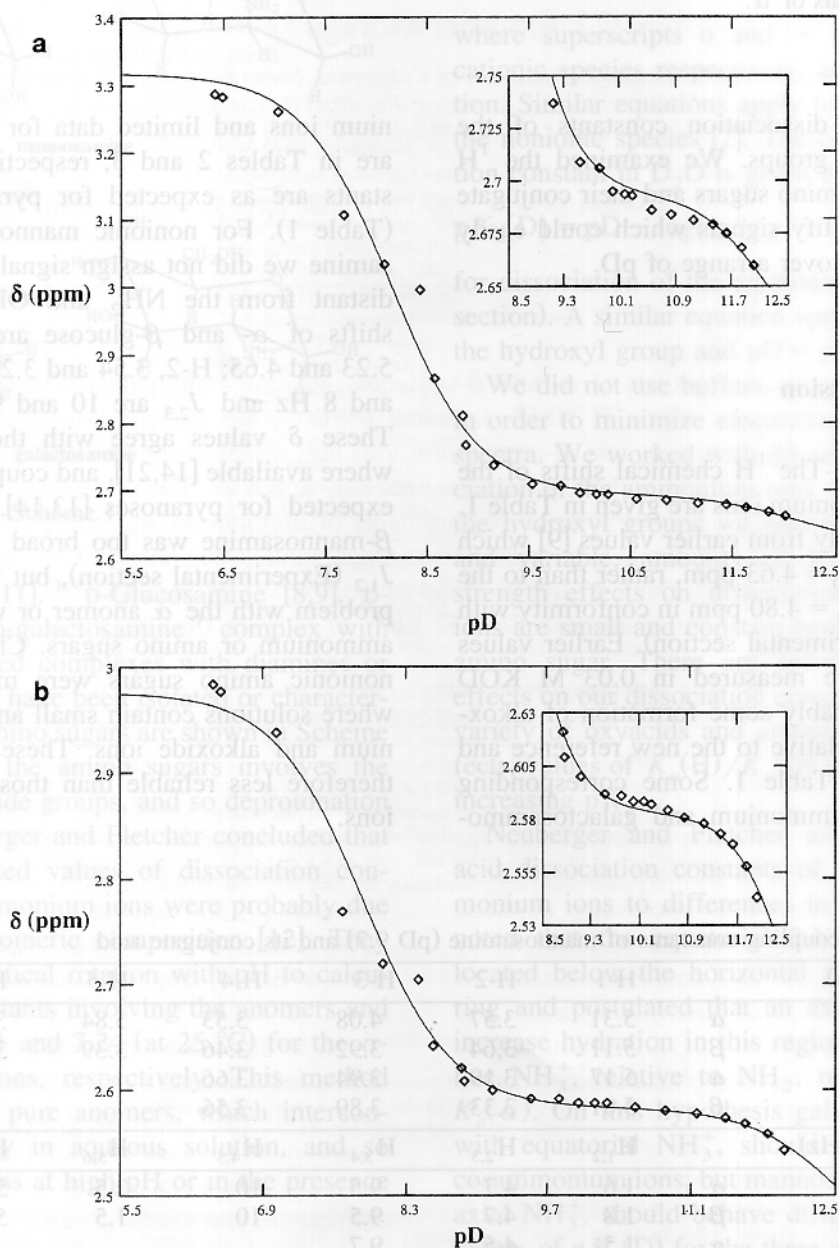
Chemical shifts, δ (ppm)		H-1	H-2	H-3	H-4	H-5	H-6	H-6'
Mannosammonium ion	α	5.31	3.57	4.08	3.53	3.84	3.77	3.73
	β	5.11	3.64	3.92	3.46	3.39	3.82	3.67
Mannosamine	α	5.17	3.19	3.94	3.66			
	β	5.01	3.33	3.89	3.56			
Coupling constants, ^3J (Hz)		$H_{1,2}$	$H_{2,3}$	$H_{3,4}$	$H_{4,5}$	$H_{5,6}$	$H_{5,6'}$	$H_{6,6'}$
Mannosammonium ion	α	1.6	4.7	9.5	10	2.5	5.5	12.5
	β	1.8	4.7	9.5	10	1.5	5.5	12.3
Mannosamine	α	1.5	4.5	9.7				
	β	a	2.5	11				

[a] Broad signal.

Table 3
^{1}H chemical shifts and coupling constants of galactosamine (pD 10) and its conjugate acid

Chemical shifts, δ (ppm)		H-1	H-2	H-3	H-4	H-5	H-6	H-6'
Galactosammonium ion	α	5.48	3.48	4.07	4.01	4.15	3.80	3.75
	β	4.88	3.18	3.89	3.97	3.75	3.78	3.76
Galactosamine	α	5.28	3.04	3.77	4.10			
	β	4.53	2.85	3.57	3.87			

Coupling constants, ^3J (Hz)		$H_{1,2}$	$H_{2,3}$	$H_{3,4}$	$H_{4,5}$	$H_{5,6}$	$H_{5,6'}$
Galactosammonium ion	α	3.7	10.5	3.5	3.5	6	a
	β	8.5	11	3.5	3		
Galactosamine	α	4	11	6	6		
	β	8	10.5	3.5	3.5		

^{a} Small.

Fig. 1. Variations of ^{1}H NMR chemical shifts with pD at H-2 for α- and β-glucosammonium ion (a and b, respectively) and for the nonionic sugars (inserts).
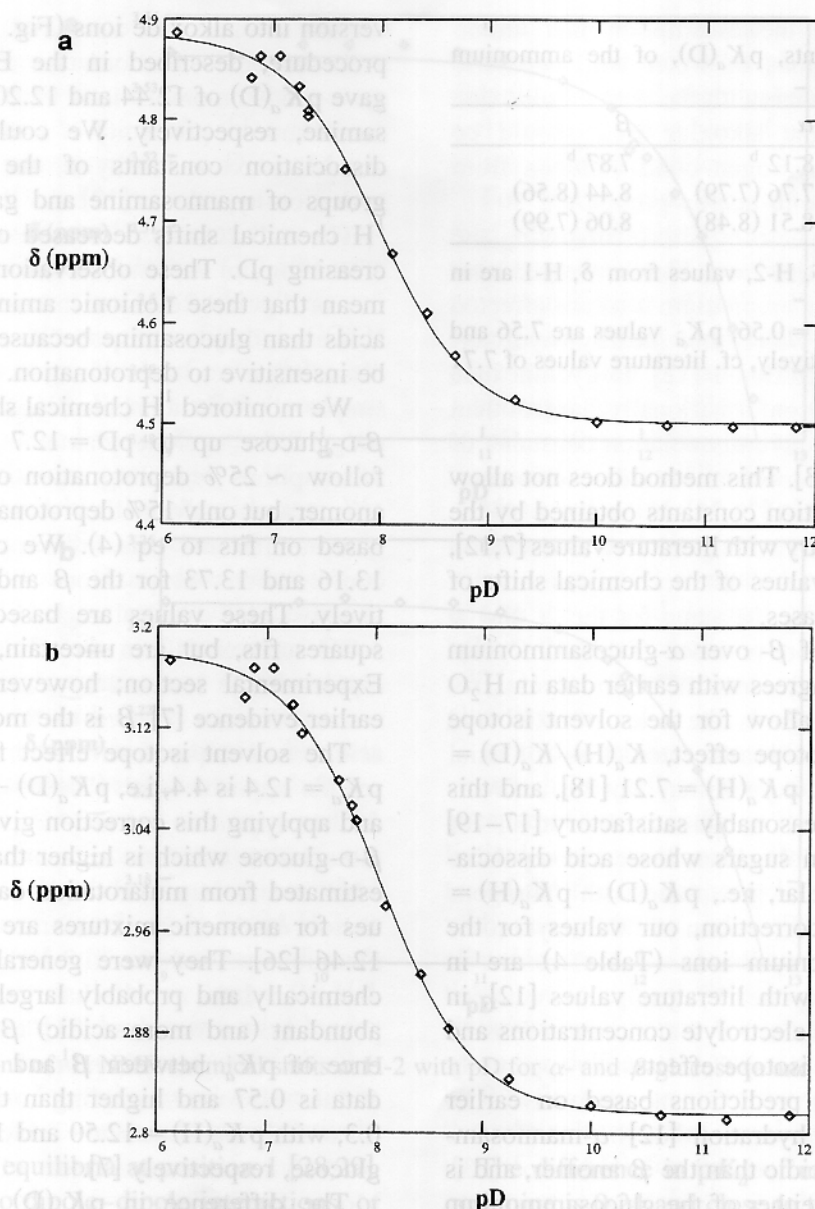
Fig. 2. Variations of ^{1}H NMR chemical shifts at H-1 and H-2 with pD for β-galactosammonium ion (a and b, respectively).

Acid dissociation of the ammonium ions.—Fitting of chemical shifts to changes in pH(pD) has been discussed [2,22]. If only one acid dissociation is involved the chemical shift, δ, of a given anomer is related to $[D^+] = 10^{-pD}$, by:

$$\delta = \frac{\delta^o + \delta^+ [D^+]/K_a}{1 + [D^+]/K_a}. \tag{4}$$

If two equilibria are involved the relation becomes [22]:

$$\delta = \frac{\delta^o + \delta^+ [D^+]/K_{a_1} + \delta^- K_{a_2}/[D^+]}{1 + [D^+]/K_{a_1} + K_{a_2}/[D^+]}, \tag{5}$$

where superscript $^-$ denotes alkoxide ion and K_{a_1} and K_{a_2} are, respectively, acid dissociation constants of the ammonium ion and the nonionic sugar, respectively. The fitting procedure is described in the Experimental section, and examples are shown in Figs. 1 and 2. There are significant decreases in δ at H-1 and H-2 in going from an ammonium to an amino sugar and then to the alkoxide ion. Values of pK_a of the ammonium ions in D_2O are given in Table 4, based on changes of chemical shifts of H-2 or H-1. In an investigation of the so-called reverse anomeric effect in 1-amino sugars and in aminocyclohexanes, Perrin showed that chemical-shift data can be used to estimate relative dissociation constants without mea-

Table 4
Acid dissociation constants, $pK_a(D)$, of the ammonium ions [a]

	α	β
Glucosammonium	8.12 [b]	7.87 [b]
Mannosammonium	7.76 (7.79)	8.44 (8.56)
Galactosammonium	8.51 (8.48)	8.06 (7.99)

[a] Values at 25 °C from δ, H-2, values from δ, H-1 are in parentheses.
[b] With $pK_a(D) - pK_a(H) = 0.56$, pK_a values are 7.56 and 7.31 for α and β, respectively, cf. literature values of 7.71 and 7.27 [12].

surement of pH(pD) [23]. This method does not allow comparison of dissociation constants obtained by the use of NMR spectrometry with literature values [7,12], and requires accurate values of the chemical shifts of the related acids and bases.

The higher acidity of β- over α-glucosammonium ion in D_2O (Table 4) agrees with earlier data in H_2O [12], but we have to allow for the solvent isotope effect. The solvent isotope effect, $K_a(H)/K_a(D) = 3.67$ for p-nitrophenol, $pK_a(H) = 7.21$ [18], and this correction should be reasonably satisfactory [17–19] for our ammonium ion sugars whose acid dissociation constants are similar, i.e., $pK_a(D) - pK_a(H) = 0.56$. Based on this correction, our values for the anomeric glucosammonium ions (Table 4) are in reasonable agreement with literature values [12], in view of differences in electrolyte concentrations and assumptions regarding isotope effects.

In agreement with predictions based on earlier hypotheses regarding hydration [12] α-mannosammonium ion is more acidic than the β anomer, and is also more acidic than either of the glucosammonium ions (Table 4), but for the galactosammonium ions the β is more acidic than the α anomer, as for glucosammonium ions.

Dissociation of anomeric hydroxyl groups.—Unless one tautomer is dominant, as with sorbose [24], acid dissociation constants determined electrochemically, for example, are apparent values [1,2,25], and these methods cannot readily be applied to acid dissociation of nonionic amino sugars. Changes in NMR chemical shifts give information on acidities of individual anomers, but a weakness of this method is that the relation between the pH and pD scales [16] becomes less reliable in alkaline solutions, and the solvent isotope-effect compounds the problem [17–20].

We monitored ^1H chemical shifts of H-2 of α- and β-glucosamine at pD > 10 and followed ∼ 40% con-

version into alkoxide ions (Fig. 1, insert). The fitting procedure, described in the Experimental section, gave $pK_a(D)$ of 12.44 and 12.20 for α- and β-glucosamine, respectively. We could not estimate acid dissociation constants of the anomeric hydroxyl groups of mannosamine and galactosamine because ^1H chemical shifts decreased only slightly with increasing pD. These observations do not necessarily mean that these nonionic amino sugars are weaker acids than glucosamine because chemical shifts may be insensitive to deprotonation.

We monitored ^1H chemical shifts of H-2 of α- and β-D-glucose up to pD = 12.7 (Fig. 3). We could follow ∼ 25% deprotonation of the more acidic β anomer, but only 15% deprotonation of the α anomer, based on fits to eq (4). We calculated $pK_a(D)$ of 13.16 and 13.73 for the β and α anomers, respectively. These values are based on objective least-squares fits, but are uncertain, as discussed in the Experimental section; however, in agreement with earlier evidence [7] β is the more acidic anomer.

The solvent isotope effect for 2-trifluoroethanol, $pK_a = 12.4$ is 4.4, i.e, $pK_a(D) - pK_a(H) = 0.64$ [17], and applying this correction gives $pK_a(H) \cong 12.5$ for β-D-glucose which is higher than the value of 12.20 estimated from mutarotation data [7]. Literature values for anomeric mixtures are in the range 12.33–12.46 [26]. They were generally measured electrochemically and probably largely represent the more abundant (and more acidic) β anomer. The difference of pK_a between β and α anomers from our data is 0.57 and higher than the literature value of 0.3, with $pK_a(H) = 12.50$ and 12.20 for α- and β-D-glucose, respectively [7].

The difference in $pK_a(D)$ of nonionic α- and β-glucosamine is 0.24 which is similar to the difference in $pK_a(H)$ of 0.3 for glucose [7]. If we assume that the isotope effect on deprotonation of α- and β-glucosamine is the same as that upon 2-trifluoroethanol we calculate $pK_a(H) = 11.8$ and 11.56 for α- and β-glucosamine, respectively.

Structural effects on acid dissociation.—Hydration stabilizes ammonium and alkoxide ions relative to the nonionic precursors. Therefore, equatorial should be more acidic than axial hydroxyl [4–6], and β-glucose [7] and β-glucosamine are more acidic at HO-1 than the α anomers. These differences are also important in acid dissociation of cyclohexane derivatives [23,27].

The anomeric effect, which favors α over β anomers due to interactions between 1-OH and the endocyclic oxygen, must also be considered in dis-
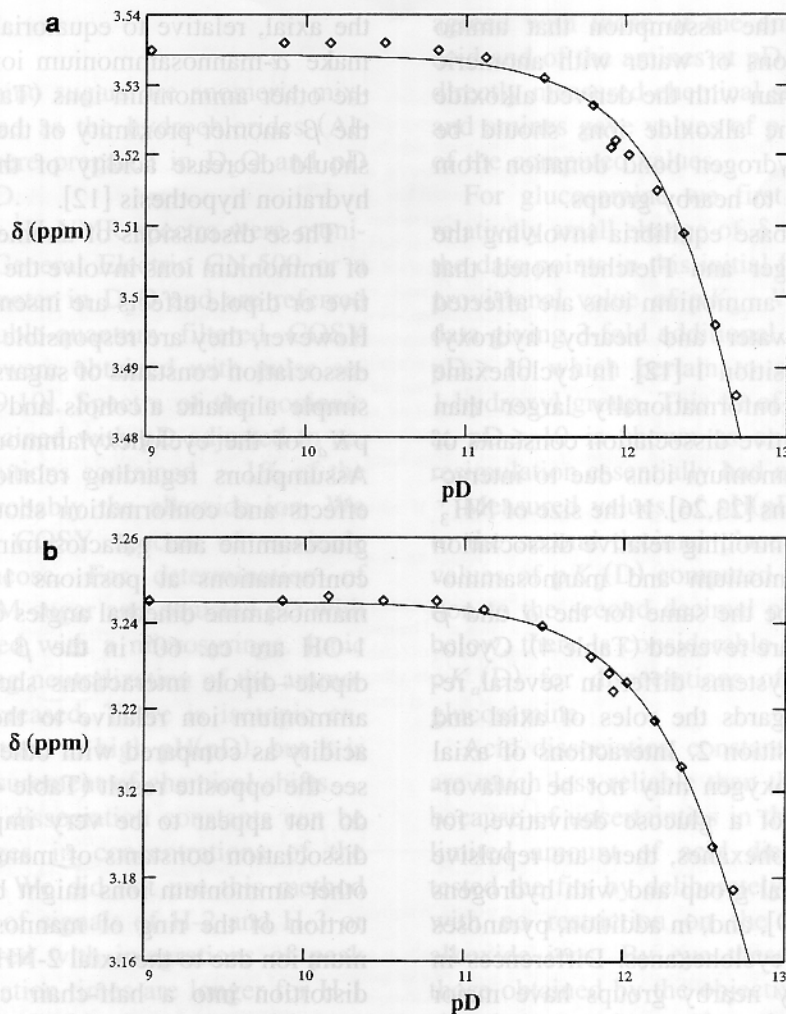
a

δ (ppm)

3.54
3.53
3.52
3.51
3.5
3.49
3.48

9   10   11   12   13

pD

b

δ (ppm)

3.26
3.24
3.22
3.2
3.18
3.16

9   10   11   12   13

pD

Fig. 3. Variations of ^{1}H NMR chemical shifts at H-2 with pD for α- and β-glucose (a and b, respectively).

cussion of acid–base equilibria at position 1 [28,29]. It has been ascribed to dipole–dipole interactions, or to 'no-bond' resonance in the α, but not in the β anomer (Scheme 2). Delocalization should be relatively unimportant in an alkoxide ion because it involves a classical structure with dinegative oxygen. Therefore, delocalization and hydration combine to make β more acidic than α anomers. If the anomeric effect is dependent on dipole–dipole, or dipole–ion, interactions it should increase the acidity of the α anomer and oppose the hydration effect. Interactions between an alkoxide residue and the dipole between C-1 and the endocyclic oxygen should be stronger than the dipole–dipole interactions, which should increase the acidity of the α relative to the β anomer. Perrin and Armstrong have discussed the roles of ion–dipole interactions and steric preferences of nonionic and ionic groups in determining anomeric effects [29].

The difference in pK_a of nonionic α- and β-glucosamine is 0.24 and close to the literature values for α- and β-glucose [7]. The higher acidities of the anomeric hydroxyl groups in glucosamine, relative to glucose, are probably due to differences in hydration, rather than to inductive effects of groups in the sugar. It is postulated that water binds to sugar OH groups by both donating and accepting hydrogen bounds without necessarily disrupting water structure [4–6], and these interactions should be affected by replacement of OH by NH_2 at position 2. The higher acidities of α- and β-glucosamine relative to glucose
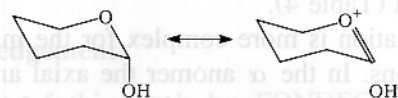
Scheme 2.

are understandable on the assumption that amino groups disrupt interactions of water with anomeric hydroxyl groups more than with the derived alkoxide ions. Interactions of the alkoxide ions should be dominated by strong hydrogen bond donation from water and be insensitive to nearby groups.

In considering acid–base equilibria involving the 2-amino group Neuberger and Fletcher noted that acid dissociations of the ammonium ions are affected by interactions with water and nearby hydroxyl groups, especially at position 1 [12]. In cyclohexane derivatives, NH_3^+ is conformationally larger than NH_2, which affects relative dissociation constants of axial and equatorial ammonium ions due to interactions with axial hydrogens [23,26]. If the size of NH_3^+ and NH_2 alone were controlling relative dissociation constants of glucosammonium and mannosammonium ions they should be the same for the α and β anomers, whereas they are reversed (Table 4). Cyclohexane and pyranose systems differ in several respects, especially as regards the roles of axial and equatorial groups at position 2. Interactions of axial NH_3^+ with endocyclic oxygen may not be unfavorable, whereas at C-1, of a glucose derivative, for example, as in the cyclohexanes, there are repulsive interactions between axial group and with hydrogens at C-3 and C-5 [23,28,29], and, in addition, pyranoses are more flexible than cyclohexanes. Differences in hydration influenced by nearby groups have major effects on acid dissociation of 2-ammonium ions of amino sugars [12], and size differences are apparently less important than in cyclohexane derivatives, or with anomeric amino groups, although the effective size of a group should be related to its solvation [23]. However, conformational preferences are clearly related to protonation equilibria in cyclohexanes and 1-amino sugars provided that groups are too far apart to interact either directly or through solvent [23,27,26].

The amino groups of both glucosamine and galactosamine are equatorial in the $^4C_1$ conformation (Scheme 1), and the explanation of the role of hydration in making β- more acidic than α-glucosammonium ion [12] is also applicable to the galactosammonium ions. The axial 4-OH group in the galactosammonium ions should also, on this hypothesis, make them less acidic than the glucosammonium ion, as is found (Table 4).

The situation is more complex for the mannosammonium ions. In the α anomer the axial ammonium and 1-hydroxyl groups should be too far apart to affect each other's hydration and weaker hydration of

the axial, relative to equatorial, NH_3^+ (ND_3^+) should make α-mannosammonium ion a stronger acid than the other ammonium ions (Table 4). However, with the β anomer proximity of the OH and NH_3^+ groups should decrease acidity of the latter based on the hydration hypothesis [12].

These discussions of anomeric effects on acidities of ammonium ions involve the assumption that inductive or dipole effects are insensitive to conformation. However, they are responsible for differences in acid dissociation constants of sugars and amino sugars and simple aliphatic alcohols and amines [1,2], e.g., the pK_a of the cyclohexylammonium ion is 10.7 [30]. Assumptions regarding relations between inductive effects and conformation should be satisfactory for glucosamine and galactosamine, which have similar conformations at positions 1 and 2. However, in mannosamine dihedral angles of bonds to 2-NH_2 and 1-OH are ca. 60° in the β anomer and therefore dipole–dipole interactions should destabilize the β-ammonium ion relative to the amine, i.e., increase acidity as compared with other ammonium ions. We see the opposite result (Table 4) so these interactions do not appear to be very important. Differences in dissociation constants of mannosammonium and the other ammonium ions might be associated with distortion of the ring of mannosamine and its ammonium ion due to the axial 2-NH_2, NH_3^+ group. Such a distortion into a half-chair conformation has been postulated for mannose [31], and it could affect relative free energies of anomeric ammonium ions and amines. However, coupling constants are as expected for a chair conformation (Table 2), although they are not very sensitive indicators of dihedral angles [32]. More significantly it is not obvious how such a conformational change would explain the marked anomeric effects on dissociation constants of the mannosammonium as compared with the glucosammonium and galactosammonium ions (Table 4), which are consistent with the earlier hydration hypothesis [12].

Comparison of our dissociation constants in D_2O with literature values in H_2O [1,7,12,25] depends on $K_a(H)/K_a(D)$ and its relation to pK_a [17–20]. Solvent hydrogen isotope effects depend upon interactions with water, which are related to acid strengths of alcohols, phenols, or aromatic ammonium ions. However, hydration requirements of those indicators are different from those of sugars, and our estimates of $pK_a(H)$ are therefore approximations, but these uncertainties should not affect relative dissociation constants of anomers.

2. Experimental

Materials.—The amino sugars are anomeric mixtures and were obtained as the hydrochlorides (Aldrich). The samples were prepared in D_2O and pD was adjusted with KOD.

NMR spectra.—The ^1H NMR spectra were monitored at 25 °C on a General Electric GN-500 or a Varian U-500 spectrometer in D_2O and are referred to TSP ($\delta = 0$). Double-quantum filtered COSY (DQF-COSY) spectra were obtained with pulse sequences used earlier [9,10]. Spectra of the nonionic amino sugars were obtained with pD adjusted to ca. 10 with KOD, and solutions contained ~ 1% of the ammonium ion and probably the alkoxide ion. We did not examine the COSY spectra of nonionic galactosamine or glucose. For determination of $pK_a(D)$ we used 0.01 M sugar and adjusted pD with 1 or 0.1 M KOD added with a microsyringe. Ionic strength was 0.01 during neutralization of the ammonium ion and then increased. There is isotopic exchange of H-2 after a time at high pH(pD), but it is too slow to affect measurement of chemical shifts.

In principle relative dissociation constants can be calculated from changes in concentrations of the anomers with pH(pD). We did not use this method because the proximity of signals of H-2 and H-3 or H-1 and HDO interfered with integrations of peak areas. In addition relaxation times are longer for H-1 than for the other protons and when we used longer interpulse delays drift in pD of our unbuffered solutions became a problem. In particular relaxation times of H-1 and H-2 of β-mannosamine are relatively long, which complicates measurements of peak areas, but not ^1H chemical shifts.

Determination of pK_a of sugars by variations of ^1H or ^{13}C NMR chemical shifts with pH or pD [2,22] does not require isolation of the individual anomers and is unaffected by their interconversion or decomposition, provided that these reactions are slow on the NMR time scale. However, if ^1H chemical shifts are monitored it is convenient to use deuterated solvents, e.g., D_2O, and dissociation constants are then based on the pD, rather than the pH scale [16].

Calculation of dissociation constants.—Variations of δ, ^1H, of α and β anomers with pD were fitted to eqs (4) or (5) by using the Mathcad 5.0 Plus program (MathSoft, Cambridge, MA) with minimization of errors and without specifying ^1H chemical shifts of any of the species. For mannosamine and galactosamine we considered only equilibria between ammonium ions and amines. The predicted chemical shifts agreed with those of the ammonium ions in dilute acid and of the amines at pD $\cong$ 10. Calculations with directly measured chemical shifts of ammonium ions and amines gave values of $pK_a(D)$ within 0.05 units of the computed values.

For glucosamine we first used eq (5), with the relatively small change of δ at pD > 10. We used all the data points in this initial fit that gave pK_{a_1}, and a provisional value of pK_{a_2}. We then resimulated the data giving 3-fold additional weight to data points at pD > 10 which pertain to acid dissociation of the 1-hydroxyl group. This fit of data for β-glucosamine at pD > 10 is shown as an insert in Fig. 1. This resimulation essentially had no effect on pK_{a_1}.

Measured values of pH(pD) are usually specified to the second decimal place and we therefore give values of $pK_a(D)$ computed by an objective simulation to the second decimal place, although, as noted below, there is considerable uncertainty in values of $pK_a(D)$ for dissociations of glucose and nonionic glucosamine.

Acid dissociation constants of α- and β-glucose are much less reliable than those of the other sugars because of uncertainties in the pD scale [16] and the limited amount of acid dissociation (Fig. 3). We tested the fits by deliberately varying pK_a by ± 0.3 with no restriction on the chemical shifts of the alkoxide ions. By eye these fits were worse than those obtained by the objective least-squares fit. The situation was better for glucosamine at pD > 10, when we deliberately varied pK_{a_2} of α-glucosamine by 0.1, fits were worse to the eye than those given by the original computer simulation. However, our values of $pK_a(D)$ for dissociations of the 1-hydroxyl groups are uncertain due to the unreliabilities of pH and pD scales in alkaline solutions. These uncertainties do not affect conclusions regarding relative acidities of α and β anomers, and the pH–pD relation is reliable in the range used for study of the ammonium ions [16].

Our fitting procedure at pD > 10 predicts ^1H chemical shifts at H-2 of the conjugate bases of glucosamine and glucose. Values of δ, H-2 are: for α and β anomers, respectively, glucosamine 2.60 and 2.47; glucose 2.91 and 2.96 ppm. These predicted values are uncertain, especially for α-glucose, for the reasons already noted.

Acknowledgements

References

[1] B. Capon and W.G. Overend, *Adv. Carbohydr. Chem.*, 15 (1960) 11–51.

[2] A. Albert and E.P. Serjeant, *The Determination of Ionization Constants*, 3rd ed., Chapman and Hall, London, 1984.

[3] P. Dais and A.S. Perlin, *Carbohydr. Res.*, 136 (1985) 215–223; M. Jasea, A.S. Perlin, and P. Dais, *Mag. Reson. Chem.*, 28 (1990) 283–289.

[4] M.A. Kabayama and D. Patterson, *Can. J. Chem.*, 36 (1958) 568–573.

[5] J.M. Harvey and M.J.R. Symons, *J. Solution Chem.*, 7 (1978) 571–586.

[6] F. Franks, *Pure Appl. Chem.*, 59 (1987) 1189–1202; S. Bociek and F. Franks, *J. Chem. Soc., Faraday Trans. 1*, 75 (1979) 262–270.

[7] J.M. Losand and L.B. Simpson, *Rec. Trav. Chim.*, 73 (1954) 941–958; 75 (1956) 267–285.

[8] S. Bunel, C. Ibarra, E. Moraga, V. Calvo, A. Blaskó, and C.A. Bunton, *Carbohydr. Res.*, 239 (1992) 185–196; A. Blaskó, C.A. Bunton, S. Bunel, E. Moraga, C. Ibarra, and J. Parada, *Bol. Soc. Chil. Quim.*, 40 (1995) 449–454.

[9] S. Bunel, C. Ibarra, E. Moraga, A. Blaskó, and C.A. Bunton, *Carbohydr. Res.*, 244 (1993) 1–14.

[10] E. Moraga, S. Bunel, C. Ibarra, A. Blaskó, and C.A. Bunton, *Carbohydr. Res.*, 268 (1995) 1–15; A. Blaskó, C.A. Bunton, E. Moraga, S. Bunel, and C. Ibarra, *Carbohydr. Res.*, 278 (1995) 315–328.

[11] D.M. Whitfield, S. Stojkovski, and B. Sarkar, *Cord. Chem. Rev.*, 122 (1993) 171–225; Kozlowski, P. Decock, I. Oliver, G., Micera, A. Pusino, and L.D. Petitt, *Carbohydr. Res.*, 197 (1990) 109–117, and references therein.

[12] A. Neuberger and A.P. Fletcher, *J. Chem. Soc., B* (1969) 178–181.

[13] J. Keeler, *Chem. Soc. Rev.*, 19 (1990) 381–406.

[14] D. Horton, J.S. Jewell, and K.D. Philips, *J. Org. Chem.*, 31 (1966) 4022–4025.

[15] G.E. Martin and A.S. Zektzer, *Two-Dimensional NMR Methods for Establishing Connectivity*, VCH, New York, 1988, pp 99–101.

[16] T.H. Fife and T.C. Bruice, *J. Phys. Chem.*, 65 (1961) 1079–1079.

[17] P. Ballinger and F.A. Long, *J. Am. Chem. Soc.*, 81 (1959) 1050–1053, 2347–2352.

[18] D.C. Martin and J.A.V. Butler, *J. Chem. Soc.*, (1939) 1366–1369.

[19] E. Högfeldt and J. Bigeleisen, *J. Am. Chem. Soc.*, 82 (1960) 15–20.

[20] C.A. Bunton and V.J. Shiner, *J. Am. Chem. Soc.*, 83 (1961) 42–47.

[21] A. De Bruyn, M. Anteunis, and P. Kovác, *Coll. Czech. Chem. Comm.*, 42 (1977) 3057–3068.

[22] S.J. Berners-Price, T.A. Frenkiel, U. Frey, J.D. Ranford, and P.J. Sadler, *J. Chem. Soc., Chem. Commun.*, (1992) 789–791.

[23] C.L. Perrin, *Pure Appl. Chem.*, 67 (1995) 719–728.

[24] E.L. Martell and R.M. Smith, *Critical Stability Constants*, Vol. 3, Plenum, New York, 1977, p 274.

[25] A. Albert and E.P. Serjeant, *The Determination of Ionization Constants*, 3rd ed., Chapman and Hall, London, 1984, p 143; C.A. Bunton and H. Chaimovich, *J. Am. Chem. Soc.*, 88 (1966) 4082–4089.

[26] J.G. Batchelor, *J. Chem. Soc., Perkin Trans. 2*, (1976) 1585–1590; H. Booth and M.L. Jozefowicz, *J. Chem. Soc., Perkin Trans. 2*, (1976) 895–901.

[27] E.L. Eliel, *Stereochemistry of Carbon Compounds*, McGraw-Hill, New York, 1962, ch. 8.

[28] A.J. Kirby, *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*, Springer, New York, 1983; J.-P. Praly and R.U. Lemieux, *Can. J. Chem.*, 65 (1987) 213–223; E. Juaristi and G. Cuevas, *Tetrahedron*, 48 (1992) 5019–5085.

[29] C.L. Perrin and K.B. Armstrong, *J. Am. Chem. Soc.*, 115 (1993) 6825–6834.

[30] *Handbook of Chemistry and Physics*, 52 ed., R.C. Weast, Chemical Rubber Co., Cleveland, 1971, p D-117.

[31] R.W. Lenz and J.P. Heeschen, *J. Polym. Sci.*, 51 (1961) 247–261.

[32] P.D.E. Pretsch, T. Clerc, J. Seibl, and W. Simon, *Tables of Spectral Data for Determination of Organic Compounds*, 2nd ed., Springer-Verlag, New York, 1989, p H25.