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Note

P.m.r. spectra and conformation of the pyranose amino sugars, 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose, and 2-acetamido-2-deoxy-β-D-mannopyranose

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INTRODUCTION

There has been interest in the proton magnetic resonance of carbohydrates for more than a decade[1,2]. Most of the work has centered on monosaccharide derivatives, because of the complexity of spectra of larger molecules, and because the proton resonances of monomers must be assigned in order to study oligomers. The introduction of high-field n.m.r. spectrometers equipped with superconducting solenoid magnets makes it now possible to assign completely the spectra of naturally occurring monosaccharides. This paper presents p.m.r. data for solutions of 2-acetamido-2-deoxy-α-D-glucopyranose, 2-acetamido-2-deoxy-α-D-galactopyranose, and 2-acetamido-2-deoxy-β-D-mannopyranose in dimethyl sulfoxide and 1,1,1,3,3,3-hexafluoro-2-propanol.

EXPERIMENTAL

Materials and methods. — The amino sugars (Pierce, 99% pure) were used without further purification. The dimethyl sulfoxide-d_6 (Me_2SO) was dried over molecular sieves. The 1,1,1,3,3,3-hexafluoro-2-propanol-d_2 (HFP) was prepared as previously described[3].

The p.m.r. spectra were obtained by using the Bruker HX-270 spectrometer of the Department of Chemistry, University of Chicago. The solutions were ~0.2M and were prepared just prior to use. All resonances were unambiguously assigned in both solvents by their spin-coupling patterns and/or spin-decoupling experiments.

Spectral interpretations. — As shown in Fig. 1A for 2-acetamido-2-deoxy-β-D-mannopyranose in HFP, irradiation of the partially exchanged doublet at δ 6.62 resulted in sharpening of the doublet at δ 4.46. Irradiation of the peak at δ 4.46 resulted in sharpening the peaks at δ 6.62 and 3.90. The peak at δ 4.46 was identified as the H-2 resonance from its downfield position, due to the amide group on C-2, and also from its coupling with the partially exchanged amide proton at

δ 6.62. Amide protons have been shown to exchange slowly in HFP in peptides[3]. The peak at δ 3.90 was assigned to either H-1 or H-3 from its coupling to H-2.

Irradiation of the peak at δ 3.45 resulted in sharpening the peaks at δ 4.02 and 3.91, and collapsed the triplet at δ 3.71 to a doublet. Irradiation of the peak at δ 3.71 sharpened the peaks at δ 3.90 and 3.45. As the resonance at δ 3.90 was coupled to two CH groups, it was identified as that of H-3. The H-4 resonance was identified at δ 3.71 from its coupling to H-3. Similarly, the H-5 signal was identified at δ 3.45 from its coupling to H-4. The H-6 resonances were identified as the peaks at δ 4.02 and 3.91 because of their coupling to H-5.

The singlet at δ 5.04 was assigned to H-1 because of its downfield position, and also because H-2 was noticeably coupled only to H-3 and the NH group, which was consistent with H-1 resonating as a singlet.

In Me_2SO (Fig. 1B), the doublet at δ 7.19 was observed to be coupled to the quartet at δ 4.16. Irradiation of the doublet at δ 6.59 indicated coupling to the doublet at δ 4.68. Irradiation of the quartet at δ 4.16 collapsed the doublet at δ 7.19, sharpened the doublet at δ 4.68 slightly, and shifted the positions of the exposed peaks on either side of the water peak. This indicated that the δ 4.68 resonance was that of H-1 and δ 4.16 that of H-2, because of their downfield positions and coupling patterns; δ 6.59 and 7.19 were then the HO-1 and HN-2 resonances, respectively.

The doublet at δ 4.82 was coupled to the peak at δ 3.23, and the doublet at δ 4.71 was coupled to the peak under the water peak at δ 3.42. The quartet at δ 4.42 was coupled to peaks at δ 3.69 and 3.52. Irradiation of the peak at δ 3.07 sharpened the peaks at δ 3.69, 3.52, and 3.23.

The H-3 resonance was identified at δ 3.42 from its coupling to H-2, therefore, δ 4.71 was the HO-3 signal. The HO-6 signal was that at δ 4.42 because it was coupled to two carbon-bonded protons and, therefore, the signals at δ 3.69 and 3.52 are the H-6 resonances. The H-4 signal was identified at δ 3.23 because it was the only other carbon-bonded proton to be coupled to an OH group (HO-4 at δ 4.82). The H-5 resonance was the peak at δ 3.07, because it was not coupled to an OH or NH group, but was coupled to the H-6 protons and to H-4.

RESULTS AND DISCUSSION

Fig. 1 shows representative spectra in both solvents. Table I presents the p.m.r. data collected.

The hydroxyl and amide protons did not exchange rapidly in dimethyl sulfoxide, so that H–O–C–H and H–N–C–H coupling constants were observable. In hexafluoro-2-propanol-d_2, all of the acidic protons exchanged with the solvent in less than 5 min, except for the amide proton of 2-acetamido-2-deoxy-β-D-mannopyranose, which was completely exchanged after 1 h.

No significant change in ring H–C–C–H coupling-constants occurs in any of the compounds upon changing from the hydrogen-bond proton acceptor, dimethyl sulfoxide, to the hydrogen-bond proton donor, hexafluoro-2-propanol. Model

TABLE I

PROTON CHEMICAL SHIFTS AND FIRST-ORDER COUPLING CONSTANTS[a]

Solvent		*H-1*		*H-2*		*H-3*		*H-4*		*H-5*		*H-6*	
		δ	J[b]	δ	J[c]	δ	J[b]	δ	J[b]	δ	J[b]	δ	J[d]
2-acetamido-2-deoxy-α-D-glucopyranose													
Me_2SO	CH	4.92	3.3	3.58	10.4	3.51	8.7	3.12	9.3	3.59	[e]	3.62/3.46	
	OH/NH	6.45	4.2	7.69	8.0	4.67	5.2	4.94	5.4			4.46	5.7/5.8
HFP	CH	5.24	3.8	4.06	10.4	3.9[f]	9.4	3.64	9.4	3.9[f]	[e]	4.0[f]	
2-acetamido-2-deoxy-α-D-galactopyranose													
Me_2SO	CH	4.92	3.2	3.97	11.0	3.61	3.0	3.71	<0.5	3.80	6.0/5.0	3.53/3.41	11.5
	OH/NH	6.37	4.2	7.58	8.5	4.35	7.2	4.47	4.2			4.35	6.8/4.6
HFP	CH	5.30	3.5	4.32	10.5	3.96	2.5	4.08	<0.5	4.19	6.0/3.7	4.01/3.89	12.0
2-acetamido-2-deoxy-β-D-mannopyranose													
Me_2SO	CH	4.68	<1.0	4.16	4.0	3.42	9.3	3.23	9.3	3.07	1.0/2.0	3.69/3.52	12.0
	OH/NH	6.59	6.6	7.19	9.5	4.71	5.1	4.82	5.1			4.42	4.7/6.9
HFP	CH	5.04	<0.5	4.46	3.0	3.90	9.6	3.71	9.6	3.45	1.5/4.5	4.02/3.91	12.0
	NH			6.62[g]	6.9								

[a]Coupling constants are in Hz and chemical shifts are p.p.m. downfield from internal tetramethylsilane. The carbohydrate concentration was 30–40 mg/ml and the temperature 19°. Samples were prepared just prior to use. Coupling constants were obtained by first-order analysis of the spectra. [b]Coupling constants are given for H-*n*–H-(*n*+1) and H–O–C–H. [c]Coupling constants are given for H-2–H-3 and HN-2-H. [d]Coupling constants are given for H-6-H and HO-6-H. [e]Coupling constants unobtainable because of spectral complexity. [f]Precise chemical shift position obscured by overlap. [g]Partially exchanged.

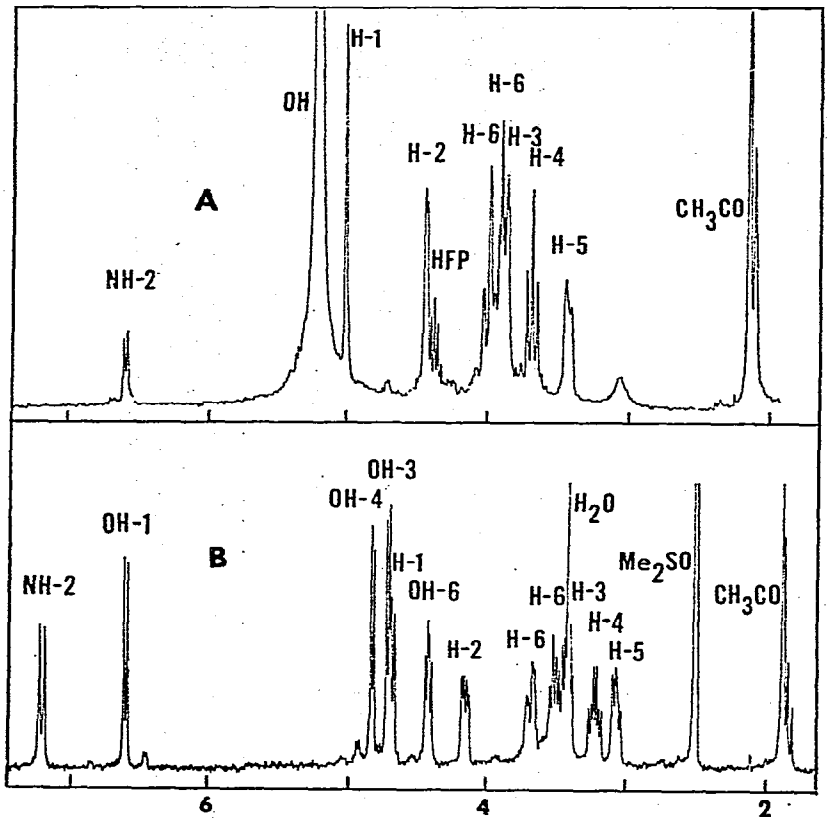

Fig. 1. 270 MHz spectra of 2-acetamido-2-deoxy-β-D-mannopyranose in (A) hexafluoro-2-propanol (HFP) and (B) dimethyl sulfoxide (Me_2SO). The scale given is p.p.m. downfield from internal tetramethylsilane. The concentrations were 30–40 mg/ml and the temperature was 19°. H-*i* indicates the resonance position of the *i*th carbon-bonded hydrogen atom and HO-*i* is that of the corresponding oxygen-bonded hydrogen atom. Extraneous peaks, most visible upfield of the acetyl singlet, probably indicate the presence of the α anomer and/or impurities.

building, using dihedral angles derived by applying the Karplus relationship[4] to the ring CH coupling constants, indicates that all three carbohydrates exist as $^4C_1$(D) chair forms, as was previously suggested for 2-acetamido-2-deoxy-α-D-galactopyranose in water[5]. Published reviews[1,2] indicate that most acetylated D-aldopyranose derivatives also exist as $^4C_1$ chairs.

It has long been known that the resonances of equatorially oriented hydrogen atoms appear at lower field than those of chemically similar but axially oriented hydrogens. The chemical shifts of the equatorial H-1 protons are identical in 2-acetamido-2-deoxy-α-D-glucopyranose and 2-acetamido-2-deoxy-α-D-galactopyranose; the axial H-1 proton of 2-acetamido-2-deoxy-β-D-mannopyranose resonates at higher field. The resonance of the equatorial H-2 proton of 2-acetamido-2-deoxy-β-D-mannopyranose is shifted downfield relative to the resonances of the axial protons at H-2 of the other two compounds, but the two axial protons do not have

identical chemical shifts. The axial HO-4 resonance is in proximity to that of H-2 in 2-acetamido-2-deoxy-α-D-galactopyranose; this brings about a considerable downfield shift relative to H-2 of 2-acetamido-2-deoxy-α-D-glucopyranose, where HO-4 is in the more distant equatorial disposition. All three axial H-3 protons display only slight chemical-shift differences. The resonance of the equatorial H-4 proton of 2-acetamido-2-deoxy-α-D-galactopyranose is again shifted downfield relative to the resonances of the axial H-4 protons in the other two compounds.

The differences in the chemical shift of the three axial H-5 protons are related to the variation of the H-5–6-OH angle that is evidenced by the variation in the H-5–H-6 coupling constants, that is, to the proximity of H-5 and HO-6. There is no appreciable chemical-shift difference associated with the sidechain H-6 protons.

The H-3–H-4 and H-4–H-5 coupling constants are not equal in 2-acetamido-2-deoxy-α-D-galactopyranose, whereas in the other two compounds the coupling constants about H-4 are equal. This indicates that, in 2-acetamido-2-deoxy-α-D-galactopyranose, there is asymmetry in the positioning of H-3 and H-5 about a line joining C-1 and C-4.

The amide coupling-constants of the acetamido sugars do not differ significantly from those found by Cerezo[6], and indicate a nearly *trans* H–N–C–H relationship[7]. No definite conclusions can be drawn from the H–O–C–H coupling constants. Their intermediate values probably arise from rotation about the C–O bond. Barfield and Karplus[8] calculated an average value of the H–O–C–H coupling constant, assuming free rotation, of 4.0 Hz. An experimental relation[9] for $J_{H-C-O-H}$

$$J_{H-C-O-H} = 10.4 \cos^2 \phi - 1.5 \cos \phi + 0.2$$

yields an average value of H–O–C–H of 5.4 Hz. The values found in this study range between 4.2 and 7.2 Hz.

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