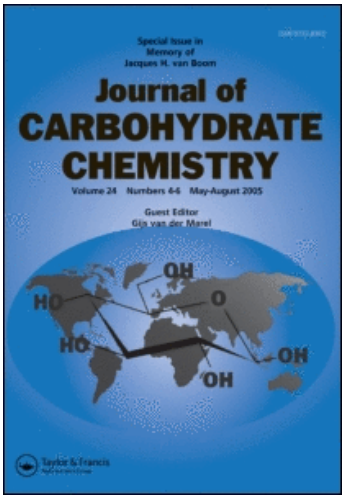

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Complete Solid State ^{13}C NMR Chemical Shift Assignments for α-D-Glucose, α-D-Glucose-H_2O and β-D-Glucose

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COMPLETE SOLID STATE ^{13}C NMR CHEMICAL SHIFT ASSIGNMENTS FOR α-D-GLUCOSE, α-D-GLUCOSE·H_2O AND β-D-GLUCOSE[1]

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ABSTRACT

NMR spectra of crystalline α-D-glucose·H_2O (1), α-D-glucose (2), and β-D-glucose (3) were examined by ^{13}C cross polarization magic angle spinning (CPMAS) methods. Each of the three forms of glucose exhibited a distinctly different spectrum. Chemical interconversion of 2 and 3 as well as the *in situ* dehydration of 1 during the course of the CPMAS NMR experiment was monitored in the ^{13}C spectra. Samples of 1, 2, and 3 specifically enriched at C-1 and C-6 with ^{13}C yielded ^{13}C spectra in which the resonances corresponding to the adjacent C-2 and C-5 carbons were not visible due to strong homonuclear ^{13}C dipolar interactions with the high

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abundance label. Spectra of these analogues as well as the C-2 and C-3 labeled materials provided the complete ^{13}C chemical shift assignments of crystalline 1, 2, and 3. A comparison of the solid state and solution ^{13}C spectra revealed substantial resonance shifts for each of the three structures examined.

INTRODUCTION

^{13}C solid state cross polarization magic angle spinning (CPMAS) NMR spectroscopy has advanced rapidly within the past 5 years.[2] Likewise, the application of this methodology has found its way into the area of carbohydrate chemistry in structural studies of crystalline carbohydrates[3-6] as well as insoluble polysaccharides.[7-15] Solid state ^{13}C NMR complements both solution NMR and X-ray crystallography since it can provide important configurational and conformational information on crystalline powders without the necessity of growing single crystals to perform high precision crystal structure analysis.[5] However, before making comparisons between the observed solution and solid state spectra e.g., relative positions of chemical shifts, it is essential that the resonance assignments be well established in each state. This is especially important if CPMAS methodology is to be used to assess the dependence of solid state chemical shift positions on either intramolecular interactions, which are largely dependent on conformational preferences, or intermolecular interactions (crystal field effects), which are associated with the close proximity of neighboring molecules in the restrictive crystalline matrix. Clearly, the latter should have a dominant effect in the solid state and may often outweigh or mask the subtle shift perturbations attributable to the structural variations in the monomeric species. Contributions to chemical shifts from both sources must be quantified if the information derived from solid-state NMR and X-ray crystallographic analysis is to be correlated with the well-established solution data.

Resemblance in chemical shift patterns often make it tempting to make corresponding assignments from solution to solid state

spectra.[3,12,16,17] As we have shown,[4,18] these extrapolations can in some instances be misleading. Some solid-state ^{13}C resonance assignments are possible based on differences in CH dipolar interactions between carbons with and without directly bonded protons using dipolar dephasing (Selpen) experiments,[19] the splitting and broadening of resonances from carbon directly bonded to nitrogen,[20] and selections derived from proton-coupled multiplicities.[21] Unfortunately, there are many carbon sites that these methods cannot differentiate, either because they have the same number of attached protons or are not bonded to nitrogen. Such is often the case in NMR studies of sugars and polysaccharides. Consequently, isotopic enrichment is often needed to facilitate complete spectral assignments. ^{13}C enrichment at specific sites readily identifies the corresponding resonance by its increased intensity. Furthermore, in ^{13}C solution spectra neighboring carbon resonances can be assigned by their ^{13}C-^{13}C spin-spin interactions.[22] For example, Walker et al.[22] have shown that in carbohydrate solution spectra the one-bond (40 Hz) and two-bond (4 Hz) ^{13}C-^{13}C J couplings could be used to assign the resonances for the labeled site, its nearest neighbor, and its next nearest neighbor. We[23] have recently described the observation of a phenomenon in a study of ^{13}C labeled α-D-glucose (2) in the solid state, in which the assignments of resonances corresponding to labeled sites and sites adjacent to the labeled position could be readily identified. The present study examines the ^{13}C solid state spectra of specifically labeled α-D-glucose·H_2O (1), α-D-glucose (2), and β-D-glucose (3) and establishes the complete unequivocal ^{13}C resonance assignments for each of these structures.

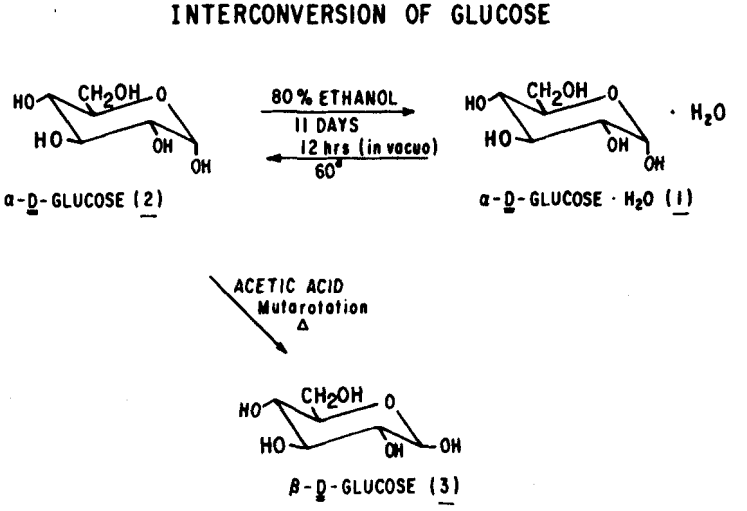
RESULTS AND DISCUSSION

Interconversion and Isomerization of Glucose. In order to study the chemistry of ^{13}C labeled glucose molecules, we had to develop a means for interconverting the three distinct forms, and

at the same time, develop a method for monitoring these changes. CPMAS ^{13}C NMR is an especially valuable tool for differentiating between hydrated and nonhydrated crystalline carbohydrates,[4,6] determining the proportions of crystalline and amorphous forms of polysaccharides,[7,13,14,15] establishing the multiplicity of isomeric forms in a crystal,[3-6] and defining unit cell inequivalences.[4,14,15] Therefore, it is no surprise that ^{13}C solid state NMR is well suited for evaluating the hydration-dehydration and isomerization chemistry of glucose.

Scheme I outlines the general procedures used to convert α-D-glucose (2) to α-D-glucose·H_2O (1) or β-D-glucose (3). We observed that room temperature treatment of 2 with 80% ethanol[24] (see Experimental) for 11 days gives the crystalline α-monohydrate 1. This material can be converted back to the anhydrous α form, 2, by *in vacuo* heating for 12 h at 60 °C. Figure 1 shows the ^{13}C solid state spectra of 1 (A) and 2 (B) prepared by these procedures.

While evaluating the purity of a preparation of 1, we observed that during long periods of CPMAS signal averaging, which were sometimes necessary to obtain high signal-to-noise ratios, 1 was partially transformed into the anhydrous form 2. This phenomenon appears to depend on the duration of ^{1}H irradiation and rapid sample spinning. For example, an experiment requiring 2,000 scans with an acquisition time of 256 ms, repetition rate of 280 seconds, 11 Gauss decoupling, and spinning at 2.2 KHz converted 40% of 1 to 2. Neither ^{1}H irradiation or rapid magic angle spinning alone, for comparable periods of time produced this structural modification. It appears that a combination of centrifugation (12,000-15,000 G of force acting on some portion of the sample) and the radiative heating produced by the decoupling coil around the sample rotor have a synergistic effect on the liberation of water from the crystalline state of 1. Ultimately, this water must diffuse to the walls of the rotor whereby it may escape through the vapor-permeable threads of the rotor cap. The conversion seems to be limited to approximately 40%, which is probably a consequence of the drop off in the intensity of radi-



Scheme I

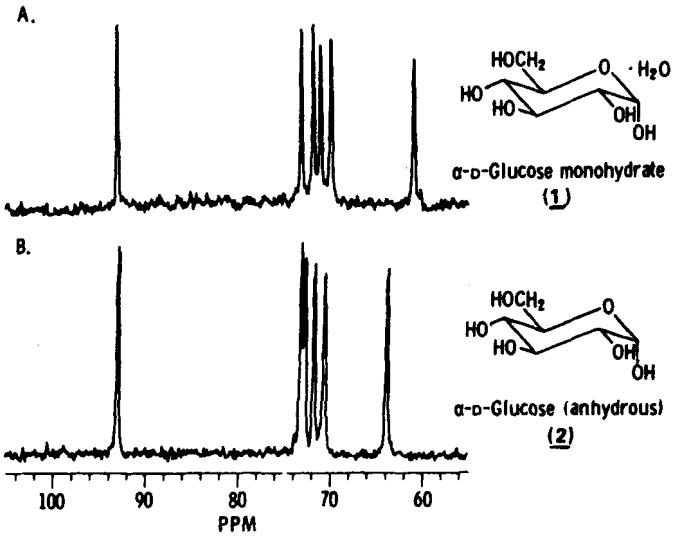


Fig. 1. A) 37.8 MHz CPMAS ^{13}C spectrum of α-D-glucose·H_2O (1); B) α-D-glucose (2). Both spectra were obtained with 64 transients, a spectral width of 5,000 Hz, and 8 K data points.

ated heat from the coils to the inner sections of the sample. Dehydration of 1 might be anticipated since water is known to be loosely bound to 1.[25] With this in mind we have been careful not to exceed 256 transients, using short acquisition times to obtain spectra of the monohydrate 1.

Although some experimental details for the conversion of α-D-glucose (2) to β-D-glucose (3) were previously outlined, no information concerning times and temperature were mentioned.[26] Figure 2 shows the somewhat lower resolution 15 MHz spectra of the crystalline product obtained after different periods of heating 2 in acetic acid and subsequent seeding with 3. We used the C-1 and C-6 resonance areas to follow the progress of the isomerization and establish the purity of the final product. A relatively quantitative conversion to 3 is achieved after a concentrated aqueous solution (syrup) of 2 is mixed with glacial acetic acid at 100 °C, heated for 1 h, and then allowed to crystallize overnight with seeding (see Experimental). Presumably it is critical to effect mutarotation at high temperature in acetic acid prior to slow crystallization to obtain pure crystals of 3.

Solid State ^{13}C Resonance Assignments for D-Glucose. Based on earlier ^{13}C NMR solution studies,[22] it could be anticipated that the ring carbon resonances in solid state spectra could be identified based on the scalar J coupling (40 Hz one bond, ~4 Hz two bond) interaction of a nearby ^{13}C labeled site. However, as we have recently shown, strong homonuclear dipole-dipole broadening in the solid state precludes the observation of resonances from adjacent carbons.[23] In effect, the "disappearance" of those resonances from carbons adjacent to a labeled position provided us with the identity of two resonances with each label, and ultimately the complete ^{13}C assignment of all the ring carbon resonances.

In order to establish the identity of the ring carbon resonances in D-glucose, we chose to examine the ^{13}C spectra of glucose structures containing the ^{13}C label in the C-1 and C-6 positions. These

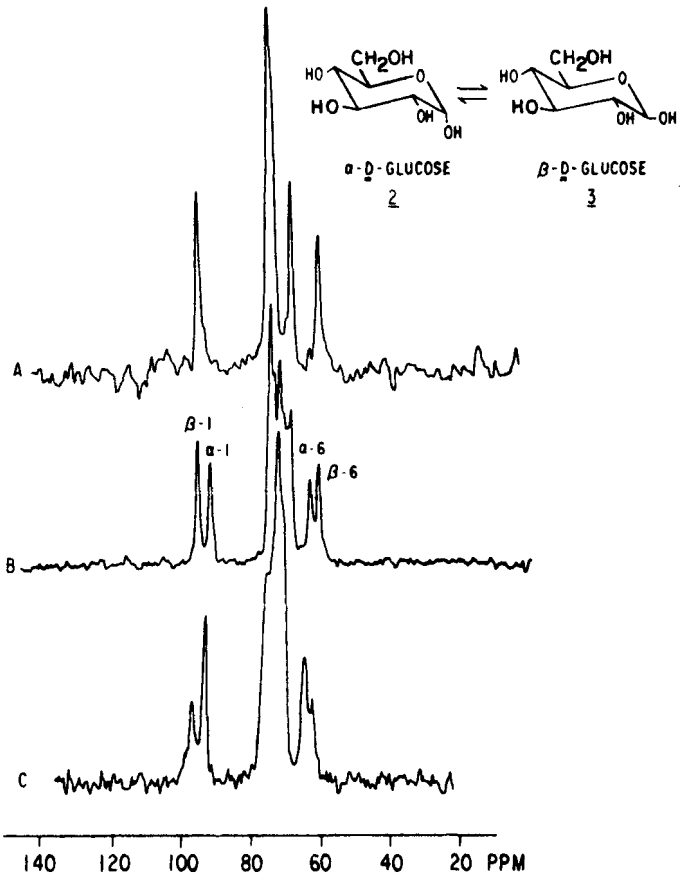


FIG. 2. A) 15.0 MHz CPMAS ^{13}C spectrum of the product obtained after heating a concentrated D-glucose solution for 1 h in glacial acetic acid at 100 °C, seeding with β-glucose, and crystallizing overnight; B) product from hot, seeded acetic acid without 1 h of prior heating; C) product from 60 °C acetic acid no prior heating. All spectra were obtained with 200 transients, a 2-ms contact time, and 15-second pulse delay.

molecules were particularly useful as starting points since a) each of these labeled resonance positions (C-1 and C-6) were well established; b) the positions of the natural abundance resonances corresponding to the ring carbons adjacent to the labeled sites were sufficiently separated from C-1 and C-6 to be clearly examined. The assignment of the remaining carbons was completed with the aid of the C-2 and C-3 enriched counterparts. The C-1 and C-6 labeled glucose samples in the α-monohydrate 1, α-anhydrous 2, and β-anhydrous 3 forms were appropriately crystallized (see Experimental) and evaluated for purity based on a comparison of their ^{13}C spectra and melting points of the unlabeled materials.

Figure 3A shows the spectrum of the unlabeled α-D-glucose·H_2O (1); 3B and 3C show the C-1 and C-6 labeled counterparts, respectively (natural abundance lines have been kept on scale while the resonances of the enriched carbons are cut off for clarity). In 3B the "absence" of the resonance at 70.91 ppm assigns this position to C-2. Likewise in 3C the loss of the line at 72.98 ppm confirms that this position corresponds to C-5. Examination of the C-2 labeled 1 verified that the missing 70.91 ppm shift was indeed C-2. Unfortunately, the position of the C-2 resonance, which is located in close proximity to the other ring carbons, precluded its use in assigning the adjacent C-3 resonance (i.e., it is difficult to make accurate observations about a 1% peak within 1 ppm of the 100% peak). The subsequent identification of C-3 at 72.98 ppm was made with the C-3 enriched 1. In addition, the absence of the line at 70.46 ppm, which is well separated from C-3 in the natural abundance spectrum, confirmed that this position corresponded to C-4.

Figure 4 shows the results of the comparable experiment utilizing the C-1 and C-6 labeled analogues of 2. In Figure 4B we see a loss of signal at 70.48 ppm for C-2 and in 4C the disappearence of the 71.62 ppm resonance designated as C-5. The spectrum of the C-2 enriched 2 could only be used to verify the C-2 position as 70.48 ppm since this resonance overlapped and

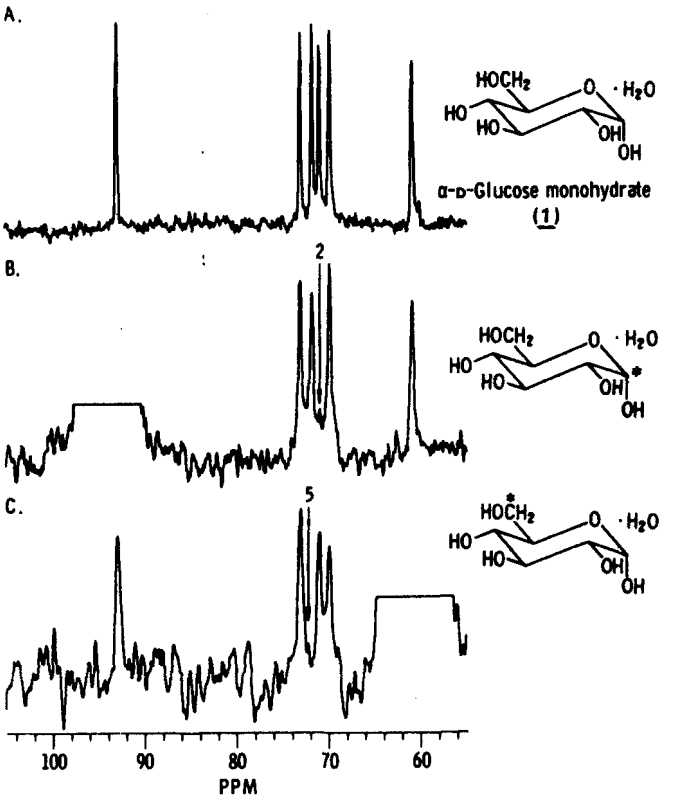


FIG. 3. 37.8 MHz CPMAS ^{13}C spectra of: A) unlabeled α-D-glucose $\cdot H_2O$ (1); B) α-D-[1-^{13}C]-glucose$\cdot H_2O$; C) α-D-[6-^{13}C]-glucose$\cdot H_2O$.

masked the other ring carbon positions. In the spectrum of the C-3 labeled 2, the C-3 resonance also dominated the center lines concealing the identity of the natural abundance ring carbons. In spite of this, the position of C-3 was clearly demonstrated to be at 73.04 ppm based on its position relative to the C-1 and C-6 natural abundance lines.

The assignment of the spectra for 3 was somewhat more challenging because of the closeness of the low field ring carbon lines (Fig. 5). It is clear from 5C that the 74.37 ppm line must

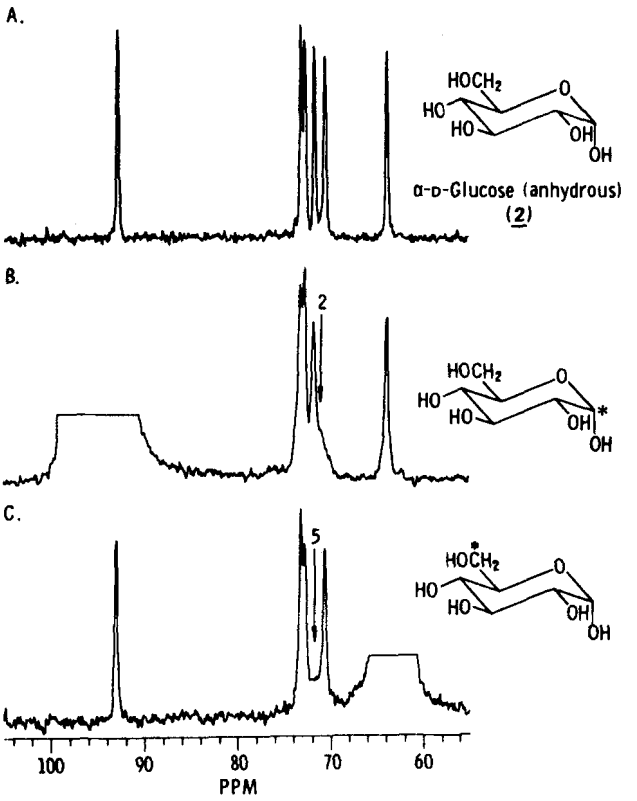


FIG. 4. 37.8 MHz ^{13}C spectra of: A) unlabeled α-D-glucose (2); B) α-D-[1-^{13}C]-glucose; C) α-D-[6-^{13}C]-glucose.

represent C-5; however, the lack of sufficient resolution in spectrum 5B does not allow for a definite assignment of the C-2 position. Alternatively, the examination of C-2 labeled 3 pinpointed the C-2 resonance at 75.71 ppm. Furthermore, a persistent line at 69.57 ppm was tentatively identified as C-4. The C-3 labeled 3 identified the 75.50 ppm line as C-3 (overlapping closely with the C-2 resonance at 75.71). In addition, the absence of the well-separated resonance at 69.57 ppm confirmed that this line was C-4 in accord with the observation made from the spectrum of the C-2 labeled 3 described above.

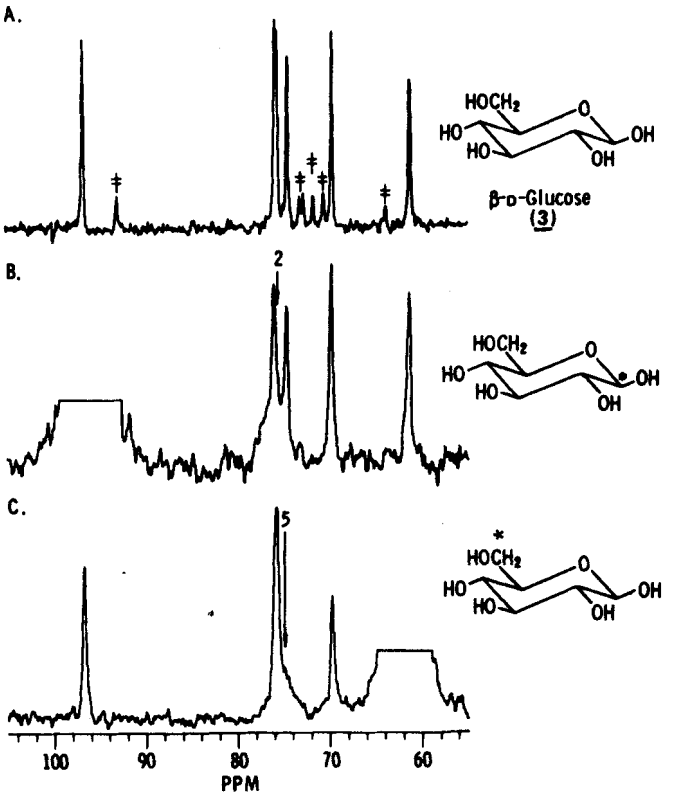


FIG. 5. 37.8 MHz CPMAS ^{13}C spectra of: A) unlabeled β-D-glucose (3); B) β-D-[1-^{13}C]-glucose; C) β-D-[6-^{13}C]-glucose. ≠ Resonances attributed to contamination from α-D-glucose.

Comparison of Solid State Spectra of α-D-Glucose·H_2O (1) and α-D-Glucose (2). The largest shifts of ~3 ppm between α-D-glucose and the monohydrate are for C-4 and C-6 (See Table 1). While it is tempting to try to rationalize these shifts with the presence of the water molecules in the crystal, the crystal structures[27-29] provide no obvious basis for doing so. C-6 is, in fact, the closest carbon atom to one of the water hydrogens at 2.98 Å, but C-2 and C-3 are closer to the other hydrogen at 2.86 and 2.95 Å, respectively,

TABLE 1

CPMAS ^{13}C Shifts for α-D-Glucose·H_2O (1) and α-D-Glucose (2)

	Chemical Shifts 9 ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
α-D-glucose·H_2O (1)	92.89	70.91	72.98	69.79	71.70	60.85
α-D-glucose (2)	92.89	70.48	73.04	72.61	71.62	63.74
Δδ (2-1)	0.00	-0.43	0.06	2.82	-0.08	2.89

while C-4 is greater than 3.5 Å from either of the water hydrogens. The water molecule is hydrogen bonded to four different glucose molecules, by donation to O-3 and O-4 of different molecules, and by accepting hydrogen bonds from O-2-H and O-3-H of the other two molecules.

At the intramolecular level, the differences between C-4-O-4 and C-6-O-6 in the two crystal structures are 0.009 Å and 0.022 Å, respectively. The differences in the angles are also very small.

Comparison of ^{13}C Chemical Shifts in the Solid State and in Solution. As discussed above, intermolecular forces may play a role in determining the isotropic chemical shift. Therefore, chemical shift changes might be significant when going from a solid (in which intermolecular solute-solute lattice interactions are severe) to a dilute solution in which such interactions play a relatively minor role. Table 2 compares the ^{13}C CPMAS chemical shift positions of the glucose structures 1, 2, and 3 and their corresponding shifts in D_2O.[30]

As in the case of the solid state spectra of 1 and 2 we observed a large difference in chemical shift for C-4 and C-6 when comparing the respective solution and solid state spectra of 2 (Table 2). Again the presence of water appears to be associated with a greater than 2 ppm upfield shift of C-4 and C-6 found in the solution vs solid state spectrum. In addition, C-2 is observed

TABLE 2

CPMAS ^{13}C Shifts in Solution[a] and Solid State[b] for α-D-Glucose·H_2O (1), α-D-Glucose (2), and β-D-Glucose (3)

	Chemical Shifts (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
α-D-glucose (D_2O)	92.84	72.25	73.60	70.46	72.16	61.50
α-D-glucose (2) solid	92.89	70.48	73.04	72.61	71.62	63.74
Δδ(solid (2)-D_2O)	0.05	-1.77	-0.56	2.15	-0.54	2.25
α-D-glucose·H_2O (1) solid	92.89	70.91	72.98	69.79	71.70	60.85
Δδ(solid (1)-D_2O)	0.05	-1.34	-0.62	-0.67	-0.46	-0.65
β-D-glucose (D_2O)	96.66	74.91	76.53	70.38	76.62	61.50
β-D-glucose (3) solid	96.59	75.71	75.50	69.57	74.37	61.15
Δδ(solid (3)-D_2O)	-0.07	0.80	-1.03	-0.81	-2.25	-0.35

a. Shifts given in D_2O relative to internal p-dioxane 67.4 ppm

b. Shifts relative to p-dioxane 67.4 ppm

at lower field in the solution spectrum of α-D-glucose when compared with the solid state spectrum of 2. The C-2 is also observed at a lower field in the monohydrate 1 relative to the anhydrous spectrum 2 in the solid state, although the magnitude of the downfield shift is smaller. The overall displacement of shifts in the solid vs the solution spectrum of 2 results in a transposition of the C-2 and C-4 resonances (Table 3).

Somewhat smaller differences in resonance positions were noted in the comparison of the monohydrate 1 in the solid state

TABLE 3

Relative Order of ^{13}C Shifts for 1, 2, and 3 in Solution[a] and Solid State[b]

	Chemical shift
α-D-Glucose·H_2O (1)	
Solution	C-1 > C-3 > C-2 > C-5 > C-4 > C-6
Solid	C-1 > C-3 > C-4 > C-5 > C-2 > C-6
α-D-Glucose (2)	
Solution	C-1 > C-3 > C-2 > C-5 > C-4 > C-6
Solid	C-1 > C-3 > C-5 > C-2 > C-4 > C-6
β-D-Glucose (3)	
Solution	C-1 > C-5 > C-3 > C-2 > C-4 > C-6
Solid	C-1 > C-2 > C-3 > C-5 > C-4 > C-6

a. In D_2O relative to internal p-dioxane 67.4 ppm

b. relative to p-dioxane 67.4 ppm

and the solution spectra of α-D-glucose. This is consistent with the fact that both states of glucose should be intramolecularly associated with water molecules. The only large difference that we observe in 1 is seen for C-2 which is shifted upfield in the solid by 1.3 ppm, relative to the solution spectrum. This movement has the effect of exchanging the relative positions of C-2 and C-5 from the solution to the solid spectrum (Table 3).

A recent report[12] based on the direct correspondence of chemical shifts of β-D-glucose (3) in solution and solid state has asserted that intermolecular oxygen distances for hydrogen-bonded pairs[31a, 31b] O_5-O_3', O_6-O_2' and O_2'-O_3" have little effect on the solid state chemical shift positions of the corresponding carbons.

Although this statement may be correct, the authors'[12] assumption concerning the correspondence of solution and solid state ^{13}C spectra is not. Clearly, the 2.3 ppm upfield displacement of C-5 in 3 places it in a relatively different position than is observed in the corresponding solution spectrum (Table 3). Furthermore, the downfield movement of C-2 and upfield shift of C-3 positions C-2 as the lowest field ring carbon in the spectrum. In contrast, C-2 is observed as the second highest field ring carbon in the solution spectrum.

It is becoming increasingly clear that extreme care should be taken in comparing small chemical shifts between solid and solution.[32] The chemical shifts reported in this work are reported to four significant figures because we are interested in relative shifts within a given compound. It should be noted that absolute shifts in carbohydrates are not accurate to this degree either in solution or in solids. Solution NMR shifts are expected to be a function of hydrogen bonding which is a function of the exact nature of the solvent, including pH and degree of deuterium substitution. Shifts measured in solids are also sensitive to the exact nature of sample preparation and to the method of referencing.[33] We do not expect to find differences in relative shifts in crystalline carbohydrates because we can reasonably assume that the molecular environment is the same in a crystal independent of the method of crystallization unless it has a different crystalline form. Methods of referencing and the shift standard used can produce significant variations in the absolute shift measured.

CONCLUSIONS

The processes of chemical interconversion as well as in situ dehydration of sugars such as D-glucose, can be monitored effectively with ^{13}C CPMAS NMR.

The use of ^{13}C labeling can aid in the assignment of ^{13}C resonance lines in solid state NMR. The label not only provides

unequivocal identification of the labeled position by virtue of its increase in intensity, but in the case where the compound is synthesized with the label at near 100% abundance, the strong homonuclear dipole-dipole coupling to adjacent carbons in the molecule leads to line broadening which can be used to assign those carbons. This technique is particularly useful for carbohydrates because there is little intermolecular ^{13}C-^{13}C dipole-dipole coupling due to the hydroxyl groups which effectively hold the molecules apart. The dipolar interaction is proportional to r^{-6} (where r is the internuclear distance), and thus small differences in internuclear distance can make large differences in the strength of the interaction. In favorable cases, it should be possible to assess intermolecular interactions through the ^{13}C-^{13}C dipolar interaction and proper ^{13}C labeling.

There may be significant changes in chemical shift upon going from the solution to solid.[32] This effect can be seen in Table 2, but it is more clearly pointed out in Table 3, where it is obvious that there are changes in the relative positions of the resonance lines from solid to solution. These relative changes will make it difficult to assign the resonance positions of crystalline carbohydrates by simple comparison with the solution spectra. In fact, carbohydrates are perhaps a worst case for comparing solid and solution spectra because they are highly hydrogen bonded, and one would frequently like to compare resonances of ring carbons where the chemical shift differences are limited to only 1 or 2 ppm.

In principle, the detailed configuration as well as hydrogen bonding and crystal field effects determine the exact shift positions of ^{13}C NMR resonance lines both in solution and solid state. One of the results of this work is to point out that we do not know which of these effects contributes most to the observed shifts. This conclusion is most easily noted in the case of C-1 of α-D-glucose and α-D-glucose·H_2O for which there are significant changes in bond angle, yet there are no changes in chemical shift. Chemical shifts in solids can offer unique

information about multiplicities in the unit cell and qualitative information about structure, but until more is known about the detailed mechanism of chemical shifts, they will not provide detailed structural information by themselves.

EXPERIMENTAL

NMR Spectrum. The high-resolution CPMAS ^{13}C NMR spectra were recorded with a home-built, wide-bore 150-MHz instrument operating at 37.8 MHz with an ^{1}H decoupling field of 25 Gauss, spinning rate of 3.0 KHz, and contact time of 1 ms. The pulse delay time used to obtain spectra with each of these spectrometers was at least 45 seconds due to the extremely long proton T_1 values (α-D-glucose 44 seconds, α-D-glucose·H_2O 77 seconds at 50 MHz) observed.

The somewhat lower resolution spectra used to monitor the conversion of D-glucose were recorded with a JEOL FX60QS-NMR spectrometer operating at 15 MHz with an ^{1}H decoupling field of 11 Gauss, spinning rate of 2.0 KHz, and a contact time of 1 ms.

All solid state spectra were referenced to the low field peak of adamantane at 38.49 ppm which was established relative to the position of p-dioxane taken as 67.4 ppm.

Purification and Crystallization of α-D-[2-^{13}C] Glucose·H_2O.

A sample (2.5 g) of D-[2-^{13}C] glucose, prepared by a published procedure,[24] was initially too impure to crystallize from ethanol/water. HPLC of this sample (Zorbax-NH_2 column, 77/23 acetonitrile/H_2O mobile phase) revealed the presence of three additional components, two of which had retention times identical to those of D-mannose and D-fructose. Hence, the D-[2-^{13}C] glucose was purified on a preparative (9 mm x 60 cm) HPLC column[34] packed with Aminex Q-15-S [Ca^{++}] form. Fractions containing pure D-glucose were pooled and evaporated; yield 1.0 g chromatograpically pure D-glucose. This material was dissolved in 2.0 mL of H_2O, and warmed to 50 °C. Slowly, while holding the 50 °C temperature, 6 mL of

ethanol was added, and then the solution was allowed to slowly cool to room temperature. Over the next 4 days, additional (2 mL) ethanol was added, bringing the total ethanol concentration to approximately 80%. Under these conditions, the crystalline α-D-glucose that was isolated (0.5 g) after 11 days was the monohydrate form, mp 82-90 °C. Failure to allow the crystals to sit in the 80% ethanol solution for this approximate length of time led to a mixture of both monohydrate and anhydrous crystals.

Preparation of α-D-[1-^{13}C] Glucose·H_2O, α-D-[3-^{13}C]-Glucose·H_2O. These products were crystallized from 80% ethanol, as described above for 2-^{13}C-labelled D-glucose. The 6-^{13}C-labelled product was first purified by preparative HPLC, the 1-^{13}C-labelled compound was crystallized directly.

Synthesis of D-[3-^{13}C]-Glucose. Pure commercial D-glucose was converted to D-erythrose by the procedure of Perlin.[35] This erythrose was then treated with K^{13}CN and reduced with $Pd/BaSO_4$ (conditions) to yield a 50:50 mixture of D-[1-^{13}C]-arabinose and D-[1-^{13}C]-ribose.[36] Separation of the arabinose was effected on a Dowex 50 x 8 [Ca^{++}] form column,[37] and the purified ribose was treated with molybdate at 90 °C according to the procedure of Barker[38] to yield a mixture containing 55% D-[2-^{13}C] arabinose and 23% D-[1-^{13}C]-ribose. Separation again on a Dowex 50 x 8 [Ca^{++}] form column[37] gave pure D-[2-^{13}C]-arabinose. A second treatment[34] with natural abundance KCN and reduction gave a mixture of 35% D-[3-^{13}C]-glucose and 65% D-[3-^{13}C]-mannose. Reaction of this mixture with molybdate[36] effected the isomerization again to give 70% D-[3-^{13}C]-glucose and 30% D-[3-^{13}C]-mannose. Subsequent separation on a Dowex 50 x 8 [Ca^{++}] form column gave pure D-[3-^{13}C]-glucose.

Preparation of ^{13}C Labeled β-D-Glucose. This procedure is a modification of the one reported by Hudson and Dale.[26] A concentrated

solution of isotopically labeled glucose was prepared by dissolving 0.5 g of α-D-glucose in 50 μL of water in a small test tube with a loose rubber stopper. The tube was submerged in boiling water and left for 1 h. After this period the tube was removed and the top of the tube was "rolled" over a hot plate (temperature approximately 160 °C) until the solution (syrup) was clear. Care should be taken to roll the tube rapidly to prevent caramelization of the sample. Immediately 0.6 mL of preheated acetic acid was added and stirred until a homogeneous solution was formed. The sample was left in boiling water for 1 h, seeded with a few crystals of β-D-glucose and allowed to cool in the slowly cooling water bath (heat source turned off) overnight. The crystalline product (0.46 g) was collected with suction filtration, washed with ethanol, and dried in a stream of nitrogen.

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