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Carbon-13 as a Tool for the Study of Carbohydrate Structures, Conformations and Interactions

H. A. Nunez, T. E. Walker, R. Fuentes, J. O'Connor, A. Serianni, and R. Barker

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824

The application of ^{13}C-NMR spectroscopy to problems involving the structures and interactions of carbohydrates is described. Both ^{13}C-enriched and natural abundance compounds were used and some advantages of the use of the stable isotope are described. Carbon-carbon and carbon-proton coupling constants obtained from 1-^{13}C enriched carbohydrates were employed in the assignment of their chemical shifts and to establish solution conformation. In all cases studied thus far, C-3 couples to C-1 only in the β-anomers while C-5 couples to C-1 only in the α-anomers. C-6 and C-2 always couple to C-1 in both anomeric species. The alkaline degradation of glucose [1-^{13}C] to saccharinic acids was followed by ^{13}C-NMR. The conversion of glucose [1-^{13}C] to fructose-1,6-bisphosphate [1,6-^{13}C] by enzymes of the glycolytic pathway was shown as an example of the use of ^{13}C-enriched carbohydrates to elucidate biochemical pathways. In a large number of glycosyl phosphates the ^{31}P to H-1 and ^{31}P to C-2 coupling constants demonstrate that in the preferred conformation **the phosphate group lies between the O-5 and the H-1 of the pyranose ring. The** influence of paramagnetic Mn^{2+} ions on the proton decoupled ^{13}C-NMR spectra of uridine diphosphate N-acetylglucosamine indicates that the Mn^{2+} interacts strongly with the pyrophosphate moiety and with the carbonyl groups of the uracil and N-acetyl groups.

Key words: ^{13}C-enriched carbohydrates, glycosyl phosphates, ^{13}C-NMR, carbohydrate conformations

Since carbon-13 has a low natural abundance of 1.1% and a small magnetic moment it is 6,000 times more difficult than hydrogen to detect by nuclear magnetic resonance methods. Recent technical developments (1) have greatly facilitated the observation of ^{13}C in natural abundance samples, and the use of isotopic enrichment with ^{13}C can further improve observation so that carbon magnetic resonance spectroscopy (^{13}C-NMR) rivals proton magnetic resonance spectroscopy (^{1}H-NMR) as a tool for application to chemical and biochemical problems (2–5). Spectrometers utilizing pulsed Fourier transformation, broad-band decoupling of protons, and computers for accumulation of time-averaged data

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T. E. Walker's present address is Los Alamos Scientific Laboratory, University of California, Los Alamos, New Mexico 87545

make it possible to obtain a spectrum of a 0.5 M solution in a few minutes and that of a 10^{-3} M solution in a few hours. With isotopic enrichment, spectra can be obtained using 2 ml of a 10^{-2} M solution with a single scan (typically < 2 sec) at 14.1 kilogauss, the magnetic field used in the least expensive commercial spectrometers. The use of higher magnetic fields and larger samples make the technique applicable to systems with compounds at those concentration levels encountered in biological systems (5, 6).

In this article we present examples of some of the ways in which ^{13}C-NMR can be applied to studies of carbohydrates of biological importance. We describe applications using enriched compounds and compounds at natural abundance. The applications include: the assignment of chemical shifts to specific carbons of the common carbohydrates; the evaluation of the factors that determine the intensity of spin-spin coupling between nuclei within a monosaccharide; the effects of structural modifications and pH changes on the spectra of carbohydrates such as glycosyl phosphates; the effects of interactions between carbohydrates and metal ions; and the use of enriched compounds to follow chemical and biochemical conversions.

^{13}C-ENRICHED CARBOHYDRATES: STRUCTURE AND CONFORMATION

Hexoses containing ^{13}C at 90% enrichment in C-1 can be prepared from the corresponding pentoses by modifications of the methods used to prepare ^{14}C-labeled compounds (7, 8). The ^{13}C-NMR spectrum D-galactose [1-^{13}C] is shown on Fig. 1A. In this spectrum only 2 strong resonances are observed due to the α and β forms of the sugar. The intensities of the 2 resonances are roughly proportional to the ratio of the 2 forms in the sample. The positions of these 2 resonances unequivocally establish the chemical shifts for C-1 in the α and β forms of D-galactopyranose. The spectrum of the C-2, C-3, C-4, C-5, and C-6 carbons in D-galactose [1-^{13}C], shown on Fig. 1B, can be compared to the same region of the spectrum of unenriched D-galactose on Fig. 1C. Several significant differences exist between the spectrum of the enriched and unenriched compound. The resonances due to C-2 in the latter have virtually disappeared. The small residual resonances are due to the 10% of the enriched sample that has ^{12}C at C-1. Ninety percent of the molecules in the enriched sample give a spectrum in which the signal due to C-2 is split into 2 resonances due to spin-spin coupling to the ^{13}C at C-1. This splitting permits the unequivocal identification of the resonance due to C-2. In addition, much smaller splittings are observed in the signals for C-3 in the β form and C-5 in the α form. Both anomers show splitting of the C-6 resonance. Splittings of this type are characteristic and can be used to identify the resonances due to specific carbons.

Unequivocal assignment of ^{13}C chemical shifts in unenriched carbohydrates is often difficult. This is illustrated by incorrect assignments made by early workers (9, 10). The use of ^{13}C- and ^{2}H-enriched derivatives has permitted correct assignments to be made (11–14). These assignments are given in Table I. They can be accepted as correct and are principally due to the extensive investigations of Gorin and co-workers (11, 12, 14).

Correct assignment of chemical shifts to specific carbons is an essential prerequisite to all studies of structure and interaction. Without correct assignments the molecular meaning of observed spectral changes will be misinterpreted.

When the chemical shifts of individual carbons of monosaccharides are known it is possible to examine more complex structures such as oligosaccharides and to assign anomeric configuration, linkage position, and conformation about the glycosidic bond. The ^{13}C-NMR spectra of galactose, N-acetylglucosamine, and N-acetyllactosamine are shown on Fig. 2. The position of the linkage in the N-acetylglucosamine moiety of the

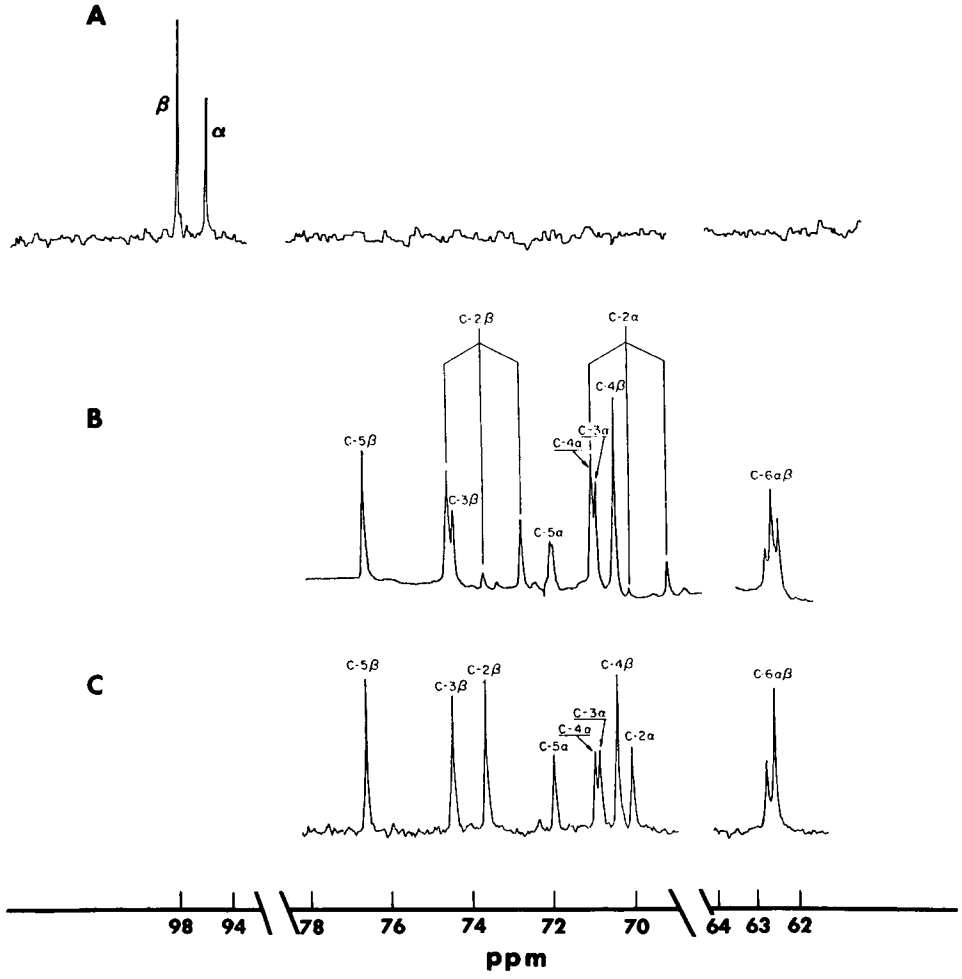

Fig. 1. A) The proton-decoupled, 25 MHz, ^{13}C-NMR spectrum of 0.2 M α and β mixture of 90%-enriched D-galactopyranose [1-^{13}C] obtained in one scan. B) The natural abundance peaks C-2 to C-6 of the enriched compound after 5,000 scans at 2.05 sec per scan. C) The C-2 to C-6 region of the ^{13}C-NMR spectrum of natural abundance α- and β-D-galactopyranose. For interpretation of the spectra see text.

latter is apparent from the shift of the resonance due to C-4. The configuration of the glycosidic linkage is easily established from the chemical shift of the C-1 of D-galactose which is characteristic of the β anomer. The conformation about the glycosidic bond was established using D-galactose [1-^{13}C] to form UDP galactose [1-^{13}C] and from it, enzymatically, N-acetyllactosamine with ^{13}C at C-1 of the galactosyl moiety (15). As described below, coupling between C-1 and C-5 of 1-^{13}C-enriched monosaccharides depends on the conformation of the substituents at C-1. Assuming that this dependence holds for the coupling between C-1 of galactose and the C-4 of N-acetylglucosamine through the glycosidic oxygen, the small coupling constant (less than 1 Hz) indicates that the preferred

TABLE I. ^{13}C-NMR Resonance Assignments

	Glucopyranoses[a]						
	C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-glucose	92.7	72.14[b]	73.4	70.4	72.10	61.3	
β-D-glucose	96.5	74.8	76.4	70.3	76.6	61.5	
Methyl α-D-glucopyranoside	100.3	72.5[b]	74.2[b]	70.6	72.7	61.7	56.2
Methyl β-D-glucopyranoside	104.3	74.2	76.9	70.8	76.9	61.9	58.3
6-Deoxy-α-D-glucose	93.1	72.9	73.6	76.4	68.6	18.0	
6-Deoxy-β-D-glucose	96.8	75.6	76.6	76.1	73.0	18.0	
Methyl 6-deoxy-α-D-glucopyranoside	100.3	72.6	73.9	76.2	68.7	17.6	56.2
Methyl 6-deoxy-β-D-glucopyranoside	104.3	74.5	76.7	76.2	73.0	17.8	58.3
Methyl α-D-glucopyranosiduronic acid	100.7	71.9	73.8	72.5	71.9	[d]	56.7
Methyl β-D-glucopyranosiduronic acid	104.3	73.8	76.5	72.3	75.6	[d]	58.5
Methyl (methyl α-D-glucopyranosid) uronate	100.8	71.94	73.7	72.4	71.87	[d]	56.8
Methyl (methyl β-D-glucopyranosid) uronate	104.6	73.7	76.3	72.4	75.7	[d]	58.7
	Mannopyranoses[a]						
α-D-Mannose	95.0	71.7	71.3	68.0	73.4	62.1	
β-D-Mannose	94.6	72.3	74.1	67.8	77.2	62.1	
Methyl α-D-mannopyranoside	101.9	71.2[c]	71.8[c]	68.0	73.7	62.1	55.9
Methyl β-D-mannopyranoside[d]	102.0	71.4	74.2	68.1	77.3	62.1	[d]
α-L-rhamnose	95.0	71.9	71.1	73.3	69.4	18.0	
β-L-rhamnose	94.6	72.4	73.8	72.9	73.1	18.0	
Methyl α-L-rhamnopyranoside	101.9	71.0	71.3	73.1	69.4	17.7	55.8
	Galactopyranoses[a]						
α-D-galactose	93.6	69.8[b,c]	70.56[b]	70.63[c]	71.7	62.5	
β-D-galactose	97.7	73.3	74.2	70.1	76.3	62.3	
Methyl α-D-galactopyranoside	100.5	69.4[c]	70.6[c]	70.4	71.8	62.3	56.3
Methyl β-D-galactopyranoside	104.9	71.8	73.9	69.8	76.2	62.1	58.3
α-D-fucose	93.3	69.2[b]	70.4[b]	73.0	67.4	16.7	
β-D-fucose	97.3	72.8	74.0	72.5	71.9	16.7	
Methyl α-D-fucopyranoside	100.5	69.0	70.6	72.9	67.5	16.5	56.3
Methyl β-D-fucopyranoside	104.8	71.5	74.1	72.4	71.9	16.5	58.3
	Allopyranoses[a]						
α-D-allose	93.4	67.6	72.3	66.7	67.5	61.3	
β-D-allose	94.0	71.9[2]	71.8	67.4	74.2[b]	61.8	

	Amino sugars[f]						
α-D-glucosamine•HCl	90.7	56.0	71.2	71.2	73.1	62.0	
β-D-glucosamine•HCl	94.3	58.5	73.6	71.2	77.6	62.0	
α-D-mannosamine•HCl	91.8	56.0	68.3	67.6	73.1	61.7	
β-D-mannosamine•HCl	92.4	57.1	70.9	67.4	77.4	61.8	
	Pentopyranoses[a]						
α-D-xylose	93.3	72.5[b]	73.9[b]	70.4	62.1		
β-D-xylose	97.6	75.1	76.9	70.3	66.3		
Methyl α-D-xylopyranoside	100.6	72.3	74.3	70.4	62.0		56.0
Methyl β-D-xylopyranoside	105.1	74.0	76.9	70.4	66.3		58.3
α-L-arabinose[a,b]	97.8	73.0	73.5	69.6	67.5		
β-L-arabinose[a,b]	93.7	69.6	69.8	69.8	63.6		
Methyl α-L-arabinopyranoside	105.1	71.8	73.4	69.4	67.3		58.1
Methyl β-L-arabinopyranoside	101.0	69.4	69.92	69.96	63.8		56.3
	Furanosides[g]						
Methyl α-D-galactofuranoside	103.1	77.4	75.5	82.3	73.7	63.4	56.1
Methyl β-D-galactofuranoside	109.2	81.9	77.8	84.0	72.0	63.9	56.1
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Methyl α-D-arabinofuranoside	109.3	81.9	77.5	84.9	62.4		56.1
Methyl β-D-arabinofuranoside	103.2	77.5	75.7	83.1	64.2		56.3
Methyl α-D-lyxofuranoside	109.1	77.0	72.0	81.3	61.2		56.9
Methyl β-D-lyxofuranoside	103.2	72.9	70.7	81.9	62.4		56.5
Methyl α-D-xylofuranoside	103.0	77.7	76.0	79.3	61.5		56.6
Methyl β-D-xylofuranoside	109.6	80.9	76.0	83.5	62.1		56.2
Methyl α-D-ribofuranoside	104.2	72.1	70.8	85.5	62.6		56.5
Methyl β-D-ribofuranoside	109.0	75.3	71.9	83.9	63.9		56.3

[a]Assignments made by Gorin and Mazurek (14). Spectra were obtained on D_2O solutions at 33°C and are referenced to external TMS = 0 ppm.

[b]Assignments reversed from that reported by Dorman and Roberts (9).

[c]Assignments reversed from that reported by Perlin et al. (10).

[d]The position of this resonance was not reported.

[e]Assignments by Walker et al. (13) and the shifts corrected to 33°C to fit with the shifts reported by Gorin and Mazurek (12).

[f]Assignments made by Walker et al. (13). Spectra were obtained on H_2O solutions at ambient probe temperature (~ 40°C) and are referenced to external TMS = 0 ppm.

[g]Assignments made by Gorin and Mazurek (12). Spectra were obtained on D_2O solutions at 33°C and are referenced to external TMS = 0 ppm.

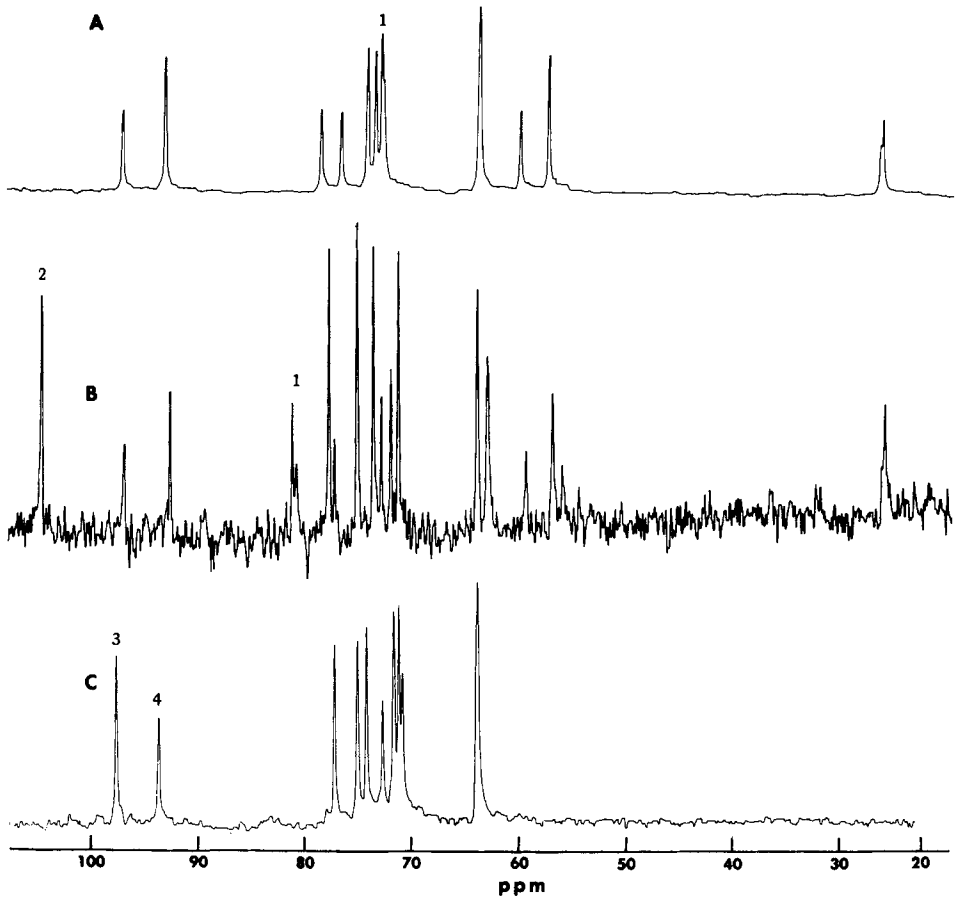

Fig. 2. A) The natural abundance proton-decoupled ^{13}C-NMR spectrum of α- and β-D-acetylglucosamine (15.08 MHz). B) The natural abundance spectrum of α- and β-N-acetyllactosamine. C) The natural abundance spectrum of α- and β-D-galactose. The peaks at 71 ppm in A and the peaks at 80 ppm in B are due to C-4; both are labelled 1. In both spectra the peak is a doublet due to the presence of α- and β-anomers. The peak at 104.1 ppm in B, labelled 2, is assigned to the β-D-galactopyranosyl linkage. The peaks at 98 and 93.8 ppm, labelled 3 and 4 in C, are the β- and α- carbon-1 resonances of D-galactose, respectively.

conformer has the C-4 of N-acetylglucosamine trans to the C-2 of the galactosyl moiety. Smith and his co-workers (16, 17) have applied ^{13}C-NMR spectroscopy to the elucidation of several important polysaccahride structures. Perlin (18), Lemieux (19), and Gorin (20) have each made important contributions to this field.

As the spectra on Fig. 1 demonstrate, ^{13}C enrichment at a specific carbon (C-1) permits the coupling between that carbon and other carbons within the structure to be observed. When this coupling involves a single bond, such as between C-1 and C-2, it does not vary greatly with structural changes and at present no attempt has been made to interpret the changes observed in carbohydrates. Coupling involving 2 or more bonds, however, seems to be sensitive to conformational changes and to depend on the geometry

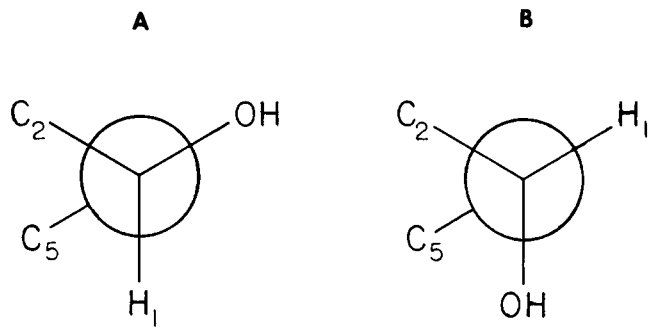

Fig. 3. The Newman projection along the C-1 to O-5 bond for the $^4C_1$ conformation of: A) β-D-glucopyranose and B) α-D-glucopyranose.

and nature of the substituent groups (21–23). Thus, when coupling between C-1 and C-5 in the hexoses is considered, the positions of the substituent groups on C-1 appear to influence the magnitude of the interaction. A "dihedral angle rule" has been proposed (24) which states that C or O substituents gauche to the coupled atom make a negative contribution to coupling (i.e., that coupling is present but has a negative sign). On the other hand, a trans substituent makes a positive contribution. When one gauche and one trans substituent are present, as on Fig. 3A, no coupling is observed since the 2 contributions cancel. When 2 gauche substituents are present, as on Fig. 3B, coupling is observed and the sign of the coupling should be negative. Although the sign of coupling has not been determined, all carbohydrates observed to date show coupling between C-1 and C-5 the α-D forms, which have the arrangement shown on Fig. 3B in the $^4C_1$ chair conformation. The data are presented in Table II.

A similar dependence of the magnitude of coupling on the position of substituents is shown in the case of coupling between C-1 and H-2 in relationship to the oxygens attached to C-1. Thus, in the cases studied, coupling is large (~ 5 Hz) only when H-2 is gauche to both oxygens at C-1. This is the case in sugars with H-2 axial and 1-OH equatorial as in β-D-glucopyranose (Table II). In the case of β-D-allose this coupling has been shown to be negative (24).

Empirical rules such as the one discussed above are helpful if used with caution. They are probably not universally applicable as can be seen by examining coupling between C-1 and C-3 in the D-hexopyranoses. Here the β-D- forms should show significant coupling if the "rule" concerning gauche and trans substituents holds. Clearly, it does not since only α-D-anomers show coupling. Although the lack of universality complicates the interpretation of coupling constants, there is ample evidence that changes in geometry affect the magnitude of coupling. Also, the values of the coupling constants in unperturbed systems are essential for studies where perturbations can be expected, as in binding to metals or proteins and in the conversion to derivatives such as phosphate esters.

TABLE II. Carbon-13 Coupling Constants of Labeled Monosaccharides

Compound	Coupling constant (Hz)[a]					
	C-1,2	C-1,3	C-1,5	C-1,6	C-1, H-1	C-1, H-2
α-D-[1-^{13}C] Glucose	46.0	–	~ 1.8	*	169.8	–
β-D-[1-^{13}C] Glucose	46.0	~ 3.5	–	*	161.2	5.5
Methyl α-D-[1-^{13}C] Glucopyranoside	46.4	–	~ 1.7	3.2	*	–
Methyl β-D-[1-^{13}C] Glucopyranoside	46.8	~ 4.1	–	4.3	*	4.1 ± 1.0
α-D-[1-^{13}C] Mannose	46.8	–	1.7	*	170.4	–
β-D-[1-^{13}C] Mannose	42.4	4.3	–	*	160.7	b
Methyl α-D-[1-^{13}C] Mannopyranoside	47.0	–	2.3	3.0	*	*
Methyl β-D-[1-^{13}C] Mannopyranoside	43.8	3.4	–	4.0	*	*
α-D-[1-^{13}C] Galactose	46.6	–	2.1	*	168.6	–
β-D-[1-^{13}C] Galactose	46.0	3.7	–	*	162.7	5.7
α-L-[1-^{13}C] Fucose	45.8	–	2.3	3.5	*	*
β-L-[1-^{13}C] Fucose	46.0	4.1	–	3.5	*	*

[a]Those couplings designated by * were not measured; those designated by – refer to no observable coupling.
[b]A broadening of ~ 1.6 Hz was observed in the proton coupled spectrum which could be due to C-1–H-2 coupling.

^{13}C-ENRICHED CARBOHYDRATES: CHEMICAL AND BIOCHEMICAL CONVERSIONS

In certain applications, ^{13}C has a great advantage over ^{14}C in permitting the evaluation of the transformations that occur during a chemical reaction or in a biochemical conversion. This is illustrated on Fig. 4 where the conversion of D-glucose to saccharinic acids under anaerobic conditions in 2.4 M NaOH is followed using D glucose [1-^{13}C]. The starting compound is 94% ^{13}C enriched at C-1, and other carbons are at natural abundance. As the reaction proceeds an intermediate substance is formed in which the enriched carbon resonates at a position characteristic of the 1-CH_2OH group of fructose. With time the ^{13}C label is distributed into 3 resonances characteristic of carboxylate anions and one resonance characteristic of $-CH_3$ groups. The time-course of the transformation is readily followed since a spectrum with good signal-to-noise characteristics can be obtained in 5 to 10 min. The changes in the location of the ^{13}C label with time are shown on Fig. 5. The data of Fig. 5 were obtained in the time taken to carry out the transformation. To obtain the same data using ^{14}C would have required extensive work-up at each time of sampling and chemical degradation to locate the isotope. The advantage of ^{13}C in this circumstance is clear.

Similar studies can be made of enzymatically catalyzed conversions. An example is presented on Fig. 6 which shows the conversion of glucose [1-^{13}C] to fructose-6-phosphate [1-^{13}C], to fructose-1,6-bisphosphate [1-^{13}C], and finally to fructose-1,6-phosphate [1,6-^{13}C] using the enzymes of the glycolytic pathway. The conversion of the latter back to glucose [1,6-^{13}C] can be followed using the appropriate phosphatases.

Other examples of the use of ^{13}C-enriched compounds to elucidate biochemical

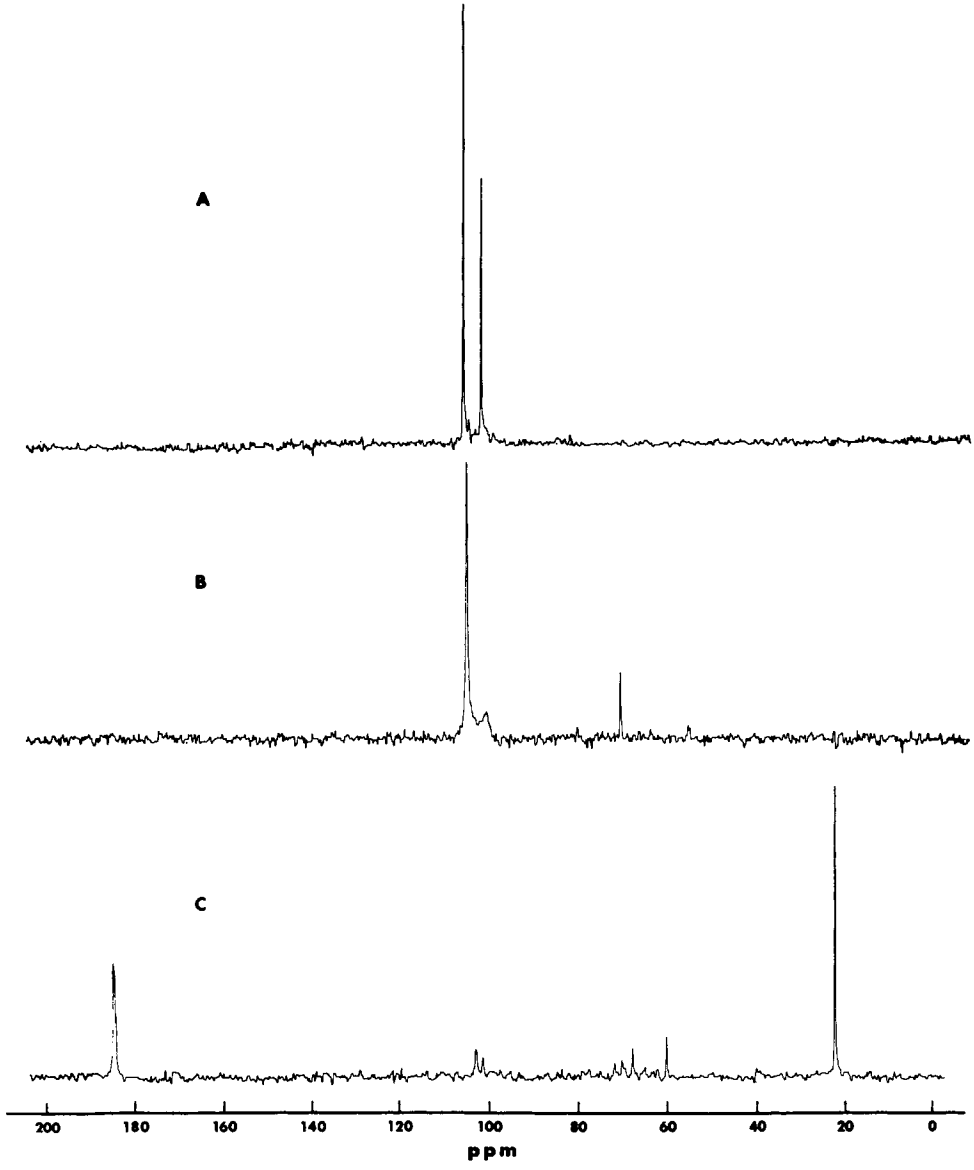

Fig. 4. The rearrangement of 0.1 M D-glucose [1-^{13}C] in 2.4 M aqueous sodium hydroxide at 37°C. A) β- and α-D-glucopyranose [1-^{13}C] in water at 37°C. B) D-glucose in 2.4 M aqueous sodium hydroxide at 37°C after 14 min of reaction, the signal for the α form is greatly broadened and the 1-CH_2OH of fructose is apparent. C) Reaction B after 5.5 h, resonances of 3 carboxylic acids and a methyl carbon are predominant. Small peaks due to a small proportion of D-mannose and some scrambling can be seen.

pathways are abundant. For example, the incorporation of enriched glucose into living Candida utilis cells has been followed (25). This and other examples are given in several reviews (4, 26–28).

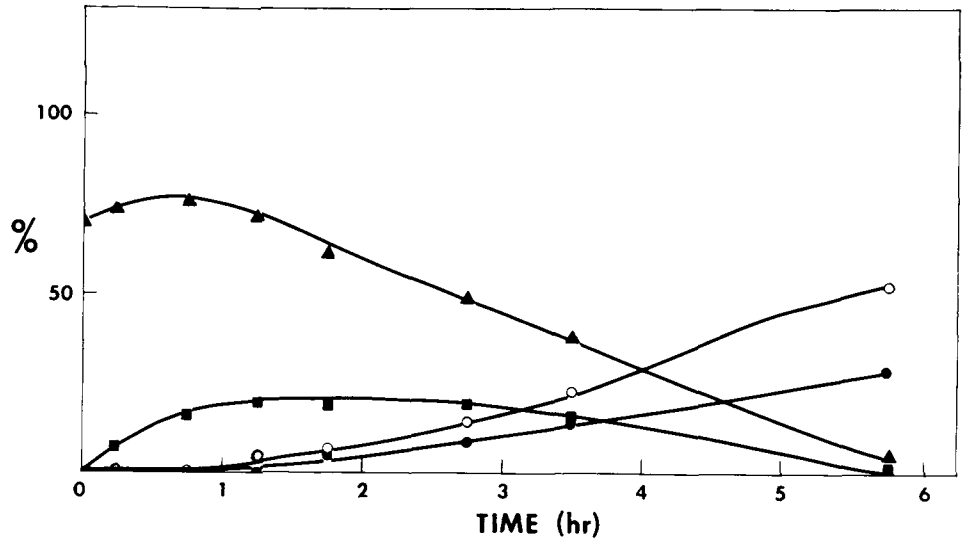

Fig. 5. The rearrangement of D-glucose [1-^{13}C] in 2.4 M aqueous sodium hydroxide at 37°C. Curves show the percentage of the total ^{13}C from the enriched carbon appearing in: ▲) β-D-glucopyranose C-1, ■) fructose C-1, ○) methyl group of saccharinic acids, ●) carboxyl group of saccharinic acids.

NATURAL ABUNDANCE CARBOHYDRATES: GLYCOSYL PHOSPHATES.

The glycosyl phosphates are important intermediates in the formation and degradation of oligo- and polysaccharides. In addition, they are structural elements in nucleoside diphosphate sugars in some polysaccharides and in glycosyl phosphate dolichol derivatives. The conformation of these glycosyl phosphates in solution has been established by a combination of proton (29) and carbon magnetic resonance spectroscopy.

Phosphorus (^{31}P) has spin equal to ½ and couples to carbon and hydrogen in the same way that these couple to each other, with the magnitude of the coupling dependent on conformation and number of bonds involved. Lee and Sarma (29) examined the proton spectra of a number of α-D-glycosyl phosphates and were able to conclude that the phosphorus atom resided in the angle subtended by the O-5 and H-1 or in the angle between H-1 and C-2 (Fig. 7). Examination of the carbon spectra of the same compounds has permitted us to conclude that the phosphorus resides between the O-5 and H-1 in all of the α-D-glycosyl phosphates examined (Table III). This conclusion arises from the magnitude of the coupling between C-2 and P (~ 8 Hz), characteristic of a trans relationship for these 2 atoms around the glycosidic bond.

The β-anomers of the glycosyl phosphates show coupling between C-2 and P of 5–7 Hz, which also can be characteristic of a trans arrangement about the glycosidic bond. This geometry is supported by the $^{3}J_{P\text{-}H_1}$ coupling constant of 7–9 Hz (Table III).

Examination of α-D-galactopyranosyl phosphate [1-^{13}C] indicates that the coupling and thus the conformation of the pyranosyl ring is not significantly influenced by the

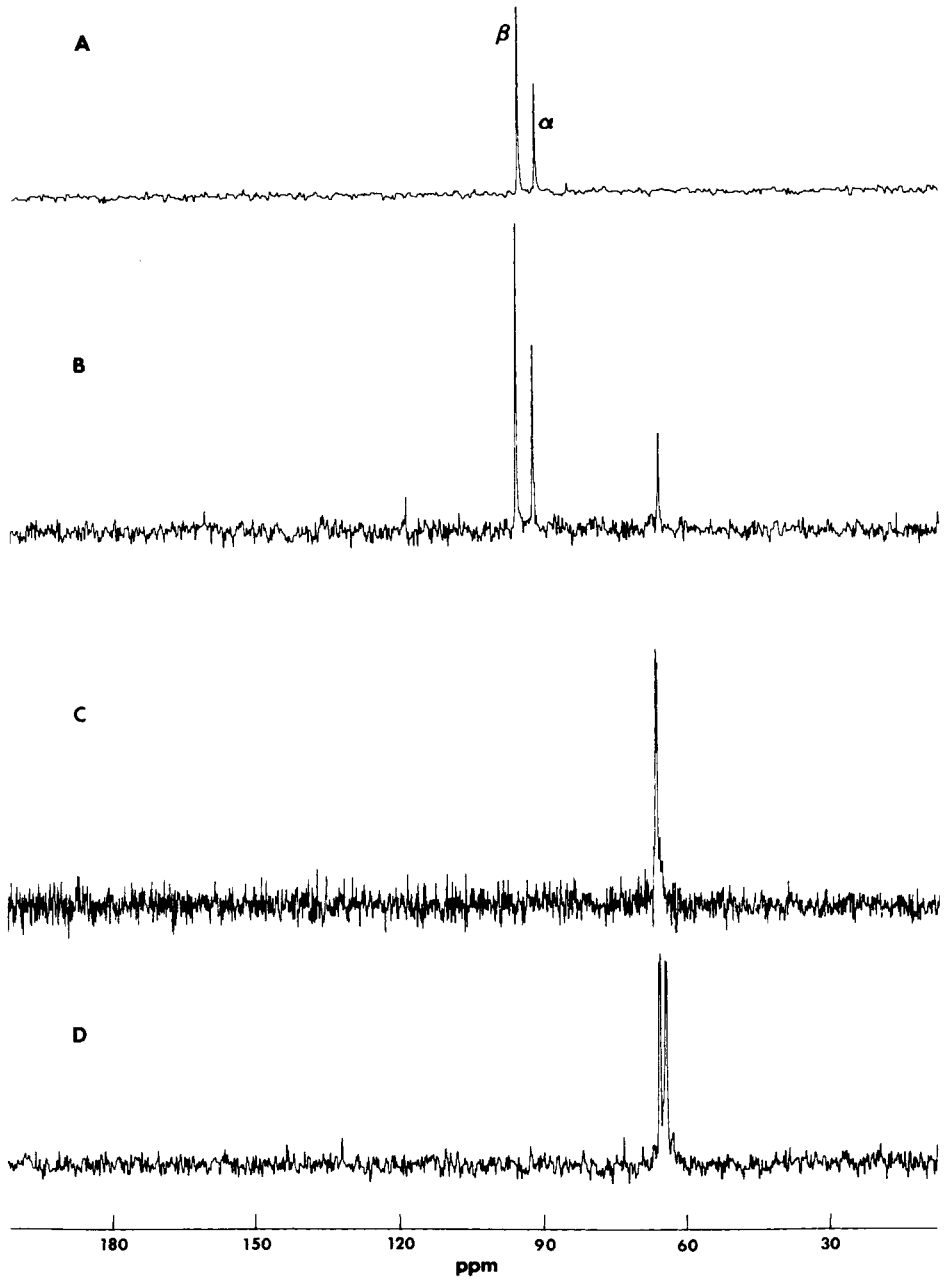

Fig. 6. The 15.08 MHz carbon-13 NMR spectra of glycolytic products obtained from an in vitro enzymatic conversion of D-glucopyranose [^{13}C-1] at an initial concentration of 50 mM and pH 7.4. ATP and $MgCl_2$ were added in 1:1 cofactor/substrate ratios. Spectra C and D show splitting due to carbon-phosphorus coupling. A) D-glucopyranose [^{13}C-1]. B) D-glucose-6-phosphate (97.4 ppm and 93.6 ppm) and D-fructose-6-phosphate [^{13}C-1] (64.2 ppm); C) D-fructose-1,6-bisphosphate [^{13}C-1]. D) D-fructose-1,6-bisphosphate [^{13}C-1,6].

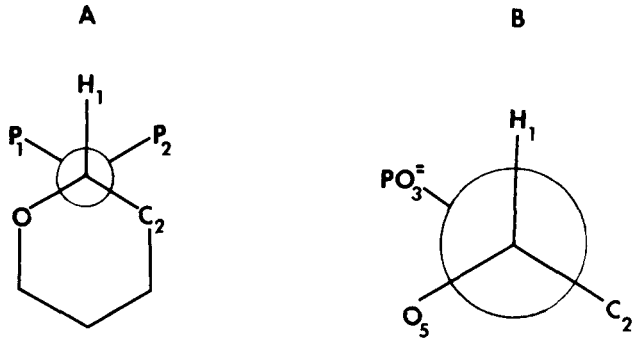

Fig. 7. The conformation of the phosphate group in α-D-glycosyl phosphates. A) The β-D glycosyl phosphate viewed from above. P_1 and P_2 represent possible orientation of phosphorus as determined by ^{1}H-NMR (29) where $^3J_{P-H-I} = 7.5$ Hz. The ^{13}C-NMR spectra show $^3J_{P-O-C-C} = 8$ Hz indicating that the conformation is that represented by P_1. B) A Newman projection along the glycosidic bond showing the preferred orientation of the phosphate group.

TABLE III. Phosphorus Coupling Constants for Glycosyl Phosphates

Compound	Coupling constant (Hz)[a]		
	P-C_1	P-C_2	P-H_1
α-D-glucopyranosyl-1-phosphate	5.3	7.5	7.2[b]
β-D-glucopyranosyl-1-phosphate	4.4	6.6	7.7
α-D-galactopyranosyl-1-phosphate	5.3	7.4	7.2[b]
β-D-galactopyranosyl-1-phosphate	3.7	5.1	7.6
α-D-mannopyranosyl-1-phosphate	5.3	7.5	7.7–8.6[b]
β-D-mannopyranosyl-1-phosphate	2.9	5.1	8.8
α-L-fucopyranosyl-1-phosphate	5.9	6.6	7.2
β-L-fucopyranosyl-1-phosphate	4.4	5.1	7.5

[a] All measurements were done in D_2O at pD 7.4.
[b] Taken from Lee and Sarma (29).

phosphate group. The magnitude of the coupling constants $^2J_{C-1,C-5}$ and $^3J_{C-1,C-6}$ are similar to those for α-D-galactopyranose.

The state of ionization of the phosphate group of a glycosyl phosphate markedly affects the chemical shifts of most carbons. These shifts are large enough to change the relative positions of resonances in some cases. The spectra of β-L-fucopyranosyl phosphate at pH 8 and pH 4 are shown on Fig. 8 to illustrate this point. In general, the coupling of phosphorus to C-2 is greater than coupling to C-1, a characteristic of phosphorus-carbon coupling. The resonances of those carbons which shift significantly with pH can be plotted

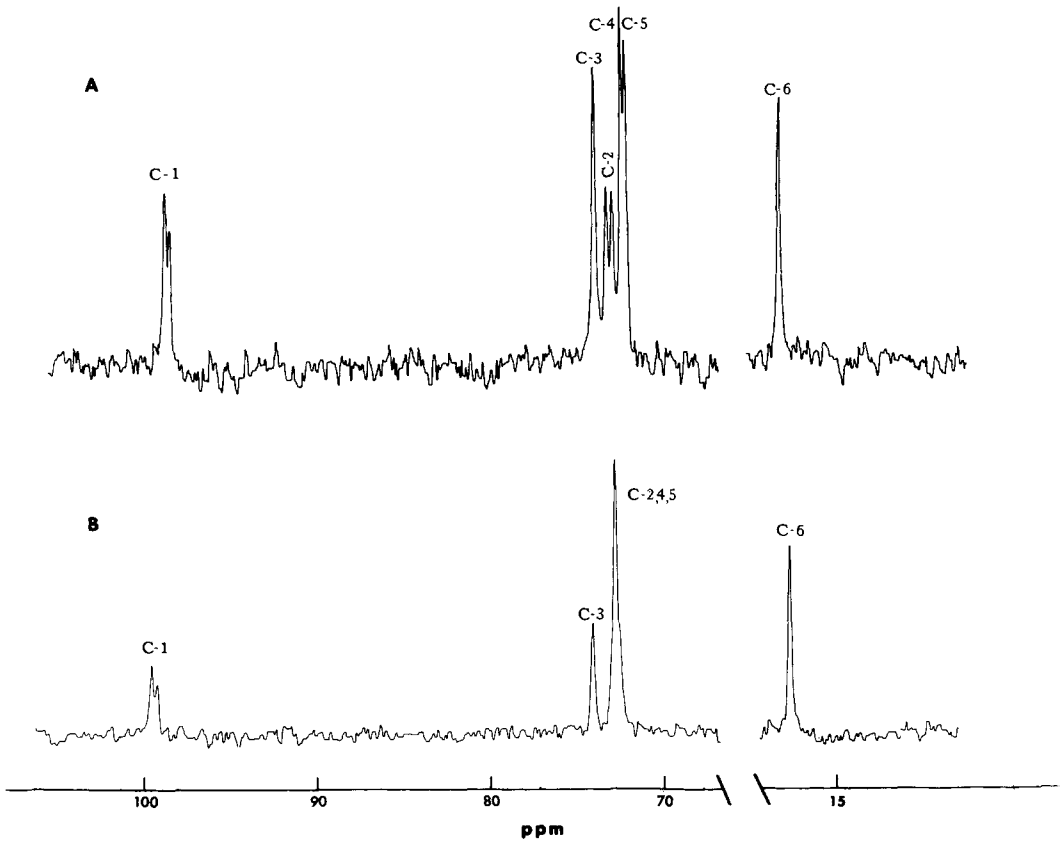

Fig. 8. The effect of pH on the 15.08 MHz proton decoupled ^{13}C-NMR spectrum of β-L-fucopyranosyl-1-phosphate in water. A) At pH 8, all 6 of the carbon resonances are resolved. The C-1 and C-2 resonances are split by the phosphorus atom through $^{2}J_{P\text{-}O\text{-}C}$ and $^{3}J_{P\text{-}O\text{-}C\text{-}C}$ coupling. B) At pH 4, the C-1, C-3 and C-6 resonances are shifted downfield about 0.5, 0.2, and 0.1 ppm respectively. C-4 and C-5 are shifted downfield slightly and C-2 is shifted upfield to the same chemical shift causing one broadened peak to appear.

as a function of pH to give titration curves that accurately reflect the ionization state of the phosphate group and allow estimation of its pK_a value. The values of the coupling constants between P and C-1 or C-2 change with pH, but at present no pattern can be discerned in these changes.

NATURAL ABUNDANCE CARBOHYDRATES: INTERACTIONS WITH METAL IONS.

Nuclear magnetic resonance spectroscopy provides a powerful tool for examining the interactions that occur between proteins and their ligands. This potential has been realized in several cases (30–34) and it is possible under ideal conditions to create a 3-dimensional map of the ligands bound to a protein (35, 36). This mapping requires the

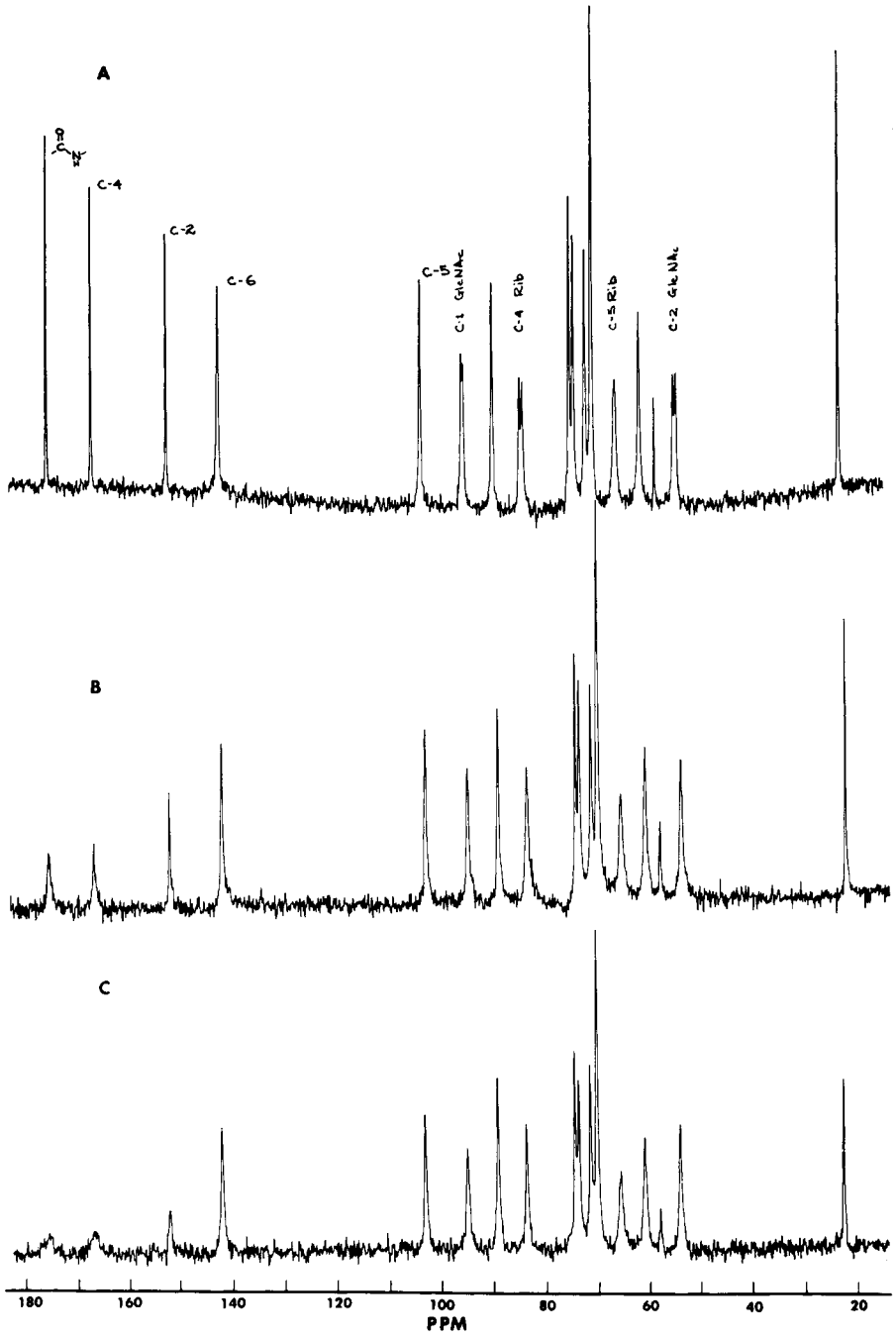

Fig. 9. The effect of Mn^{+2} ions on the proton- decoupled, ^{13}C-NMR spectra of 0.6 M UDP N-acetyl-glucosamine in D_2O (pD 7.0) and 37°C. A) Metal free solution. B) 5×10^{-4} M Mn^{2+}. C) 1×10^{-3} M Mn^{2+}. The spectra are discussed in the text.

presence of a paramagnetic ion, which acts to increase the rate of relaxation of magnetically susceptible nuclei, such as ^{13}C and ^{1}H, which come within its domain. The effect of the close association of a paramagnetic ion such as Mn^{2+} with a specific carbon is to broaden the resonance due to that carbon and in the extreme cause it to become unobservable.

The effect of Mn^{2+} on UDP N-acetylglucosamine is shown on Fig. 9. Very low concentrations of Mn^{2+} cause the resonances dur to the carbonyl groups of the acetyl and uracil moieties to broaden. In addition, the splitting due to P-C coupling observable in the resonances due to C-4 and C-5 of ribose and C-1 and C-2 of N-acetylglucosamine is abolished at 5×10^{-4} M Mn^{2+}. In contrast, resonances due to other carbons are affected little at 1×10^{-3} M Mn^{2+}. These observations indicate that Mn^{2+} interacts strongly with pyrophosphate moiety and with the carbonyl groups in uracil and of the acetyl group. Although no precise structure for the UDP N-acetylglucosamine/Mn^{2+} complex can be deduced from these data, they do permit the conclusions made above. Precise evaluation of the relaxation times of the carbons in the presence and absence of Mn^{2+}, together with knowledge of the reaction stoichiometry, would permit a refined model for the complex to be developed. Such studies are in progress. Since similar complexes are probably involved in biochemical conversions, and are certainly involved in Mn^{2+}-catalyzed chemical degradations (37), the evaluation of their structures is of value.

CONCLUSIONS

A number of applications of NMR spectroscopy have been described which show the kinds of information that can be obtained about carbohydrate structures, conformations, and interactions. The value of ^{13}C enrichment as a tool in structure elucidation and in following chemical and biochemical conversions has been demonstrated. The applications of these approaches to the study of more complex systems is inevitable and requires the kind of base-line studies described here.

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