Carbon-13 Nuclear Magnetic Resonance Spectroscopy of [1-^{13}C] Enriched Monosaccharides. Signal Assignments and Orientational Dependence of Geminal and Vicinal Carbon–Carbon and Carbon–Hydrogen Spin–Spin Coupling Constants

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Abstract: Early assignments of the ^{13}C resonances in the natural abundance ^{13}C NMR spectra of monosaccharides have been reevaluated in light of recent coupling data from the spectra of ^{13}C-1 labeled sugars. The technique of specific ^{13}C enrichment not only identifies the labeled carbon unambiguously but can be used to assign more remote carbon resonances due to scalar carbon–carbon coupling. The pattern of carbon-carbon coupling observed in all of the sugars thus far studied is remarkably constant. In addition to the large (~46 Hz) one-bond coupling between C-1 and C-2, C-3 exhibits a coupling to C-1 only in the β anomer (~4 Hz) while C-5 is coupled to C-1 only in the α anomer (~2 Hz). In addition, C-6 is coupled to C-1 in both anomers and C-4 shows no evidence of coupling to C-1 in any of the sugars examined. These couplings have been used to reassign several resonances and the original assignments are discussed in terms of the predictive rules used for resonance assignments in carbohydrates. The vicinal couplings of C-6 and C-4 to C-1 appear to obey a Karplus-type relationship. The geminal $^2J_{CCC}$ and $^2J_{COC}$ couplings are discussed in terms of a dihedral angle dependence where the angle is defined by the relative orientations of C-3 or C-5 and the electronegative oxygen substituents on C-1. Additional data on $^2J_{CCH}$ couplings involving C-1 and H-2 are also readily obtained with the C-1 labeled sugars.

Carbon-13 NMR spectroscopy is a promising technique for studying the structures of oligosaccharides,[2-7] antigenic determinants,[8,9] glycoside–protein complexes,[10,11] and perhaps even intact cell walls.[12] The prime requirement in these studies is the proper assignment of the ^{13}C resonances of simple sugar derivatives and this requirement is a stringent one, because structurally significant perturbations may have only a small and unpredictable effect on the ^{13}C chemical shift values and spin–lattice relaxation times. The assignment of the closely spaced C-2, C-3, and C-5 ^{13}C resonances of glucose provides an instructive case history. The assignments of the C-2 and C-3 resonances (Table I) originally made by Dorman and Roberts[13] were revised by Koch and Perlin[14] who obtained the ^{13}C spectrum of glucose specifically labeled with deuterium at C-3. The original assignments were based to a large extent on the pattern (i.e., predictability) of the ^{13}C shifts of a large number of glycoside derivatives.

Although this assignment procedure was shown to be unreliable in the case of glucose, until quite recently,[5,15-18] there has been no systematic reassessment of the ^{13}C assignments of other simple sugars. In this study, we take advantage of the one-bond and two-bond carbon-13 spin–spin coupling interactions in the determination of the C-2, C-3, and C-5 ^{13}C resonance positions in derivatives of [1-^{13}C]glucose, [1-^{13}C]mannose, [1-^{13}C]fucose, and [1-^{13}C]galactose. The use of sugars specifically labeled with carbon-13 in ^{13}C NMR studies offers the additional advantage of permitting the facile determination of geminal and vicinal carbon–carbon and carbon–hydrogen spin–spin coupling constants. We discuss in this report also the considerable potential that these coupling constants have in the conformational analysis of sugar derivatives, a potential that has been unexploited, with a few significant exceptions,[19-23] due largely to the difficulty in obtaining many of the coupling constants from the ^{1}H NMR or ^{13}C NMR spectra of saccharides containing ^{13}C at natural abundance. Conversely, although saccharides uniformly enriched to high levels of ^{13}C by biosynthetic methods have become available recently, the complexity of their nuclear magnetic resonance spectra precludes an evaluation even of certain one-bond coupling constants.[19,24]

Materials and Methods

Published procedures were used for the preparation[25] and purification[26] of [1-^{13}C]glucose (**1**) and [1-^{13}C]mannose (**3**) and for the preparation[27] and purification[28] of the corresponding methyl glycosides. The procedures used for the preparation of [1-^{13}C]galactose (**2a, 2b**) and [1-^{13}C]fucose (**2c, 2d**) will be published elsewhere.

Pulse ^{13}C NMR spectra were obtained with a Varian XL-100 spectrometer (25.2 MHz) interfaced to a Nova 1210 computer. All spectra were obtained for aqueous solutions at 50 ± 5 °C and the spectrometer was locked to the resonance of D_2O contained in a capillary. Spectra were recorded with a spectral width of 1000 Hz and 2K spectral points at an acquisition time of 2.05 s. For the examination of the high field natural abundance ^{13}C resonances, the carrier was placed just upfield of the C-6 resonance with the filter set at 500 Hz to minimize the intensity of the ^{13}C-1 resonances in enriched compounds. For examining the intense ^{13}C-1 resonance, a spectral width of 500 Hz with a filter setting of 650 Hz and 2K spectral points were used. Peak positions were determined by computer examination of the final Fourier transformed spectrum. All chemical shifts are given relative to external tetramethylsilane and are accurate to within ±0.1 ppm.

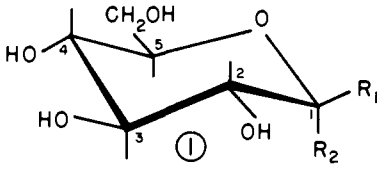
Results

^{13}C–^{13}C Couplings in Hexopyranoses. The C-2 resonance of a ^{13}C-1 labeled sugar can be unequivocally identified due to a large coupling with ^{13}C-1. In the spectra of a mixture of 90% enriched α- and β-D[1-^{13}C]glucose (**1a, 1b**) and the α-methyl glycoside shown (**1c**) in Figure 1, these couplings are clearly apparent. In the spectrum of the α- and β-glucopyranose anomers (Figure 1b), C-2β occurs as a doublet ($J_{1,2}$ = 46.0 Hz) with a small singlet due to residual ^{12}C at C-1. For the α anomer, a singlet due to C-2 is found downfield from C-5α. In contrast for methyl α-D-glucopyranoside (Figure 1a), the C-2 singlet is upfield of C-5α. Although C-2α and C-5α are not widely separated, it is nevertheless possible to assign

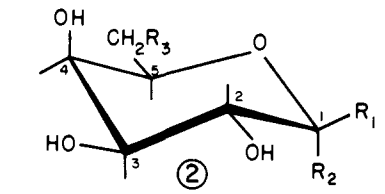
Table I. Carbon-13 Chemical Shifts of Labeled Monosaccharides

Compd	Carbon position, ppm[a] C-1	C-2	C-3	C-4	C-5	C-6	OMe
α-D-[1-^{13}C]Glucose (**1a**)	93.6	73.2 d	74.5	71.4	73.0 br	62.3 d	
β-D-[1-^{13}C]Glucose (**1b**)	97.4	75.9 d	77.5 d	71.3	77.4	62.5 d	
Methyl α-D-[1-^{13}C]glucopyranoside (**1c**)	100.6	72.7 d	74.7	71.2	73.0 br	62.2 d	56.5
Methyl β-D-[1-^{13}C]glucopyranoside (**1d**)	104.6	74.6 d	77.4 d	71.2	77.3	62.4 d	58.5
α-D-[1-^{13}C]Mannose (**3a**)	95.5	72.3 d	71.9	68.5	73.9 br	62.6 d	
β-D-[1-^{13}C]Mannose (**3b**)	95.2	72.8 d	74.8 d	68.3	77.6	62.6 d	
Methyl α-D-[1-^{13}C]mannopyranoside (**3c**)	102.2	71.4 d	72.1	68.3	73.9 br	62.5 d	56.1
Methyl β-D-[1-^{13}C]mannopyranoside (**3d**)	102.3	71.7 d	74.5 d	68.4	77.6	62.6 d	
α-D-[1-^{13}C]Galactose (**2a**)	93.8	70.0 d	70.8	70.9	72.0 br	62.8 d	
β-D-[1-^{13}C]Galactose (**2b**)	98.0	73.6 d	74.4 d	70.4	76.6	62.6 d	
α-L-[1-^{13}C]Fucose (**2c**)	93.8	69.8 d	71.0	73.5	67.8 br	17.2 d	
β-L-[1-^{13}C]Fucose (**2d**)	97.8	73.4 d	74.6 d	73.1	72.3	17.2 d	

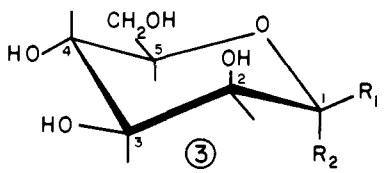
[a] All chemical shifts are relative to a TMS external standard. Those peaks designated by "d" were observed as doublets; those peaks designated by "br" were broadened.



1-a R_1 = H, R_2 = OH
1-b R_1 = OH, R_2 = H
1-c R_1 = H, R_2 = OCH_3
1-d R_1 = OCH_3, R_2 = H



2-a R_1 = H, R_2 = OH, R_3 = OH
2-b R_1 = OH, R_2 = H, R_3 = OH
2-c R_1 = H, R_2 = OH, R_3 = H
2-d R_1 = OH, R_2 = H, R_3 = H



3-a R_1 = H, R_2 = OH
3-b R_1 = OH, R_2 = H
3-c R_1 = H, R_2 = OCH_3
3-d R_1 = OCH_3, R_2 = H

the peaks unequivocally on the basis of ^{13}C-1 coupling even when one shifts upfield of the other after derivatization. The splitting patterns and intensities found for C-2 are consistent with the enrichment of C-1 (ca. 90% for glucose and galactose and their derivatives and ca. 60% for mannose).

Although two- and three-bond ^{13}C–^{13}C couplings are much smaller and less predictable than the one-bond couplings, consistent trends were observed in the series of hexopyranoses studied here. In the CW ^{13}C NMR spectrum of uniformly labeled glucose reported by Perlin and Casu,[19] no resolved long-range couplings are apparent in spite of a stated resolution of 0.5–1.0 Hz, nor were London et al.[24] able to resolve any long-range couplings in uniformly labeled galactose using FT ^{13}C NMR. In both cases,[19,24] however, all resonances were somewhat broadened and the small, very weak signals which were observed in the C-1 region could be due to unresolved geminal C–C–C coupling. On the other hand, these splittings are resolvable (Table II) with ^{13}C-1 labeled carbohydrates and exhibit a strong conformational dependence. Apparently, these splittings are resolvable in the specifically labeled sugars because there are fewer possible interactions than in the uniformly labeled sugars, e.g., each carbon will be coupled only to ^{13}C-1.

All of the ^{13}C-1 sugars examined show a consistent pattern of C-1–C-3, C-1–C-5, and C-1–C-6 coupling with no indication of C-1–C-4 coupling. In the β-D-[1-^{13}C]pyranose sugars, C-3 is coupled to C-1 by 2–5 Hz, whereas C-5 shows no indication of coupling. In the α anomers, C-3 is not affected while C-5 is broadened. Both α- and β-pyranose sugars show C-1–C-6 couplings.

The long-range couplings are illustrated in Figures 1–3. For example, in the natural abundance ^{13}C NMR spectrum of α- and β-D-galactopyranose (Figure 2b), all of the carbon atoms in the C-2–C-6 region of both anomers appear as single peaks. In the spectrum of α- and β-D-[1-^{13}C]galactose (Figure 2a), the C-3β splitting can be observed, although the downfield resonance of that doublet overlaps with the downfield resonance of the C-2β doublet. For the α anomer, C-3 is not split and the left portion of the C-2α doublet is superimposed on C-4α. In addition, C-5α is broadened while C-5β is not affected.

For most of the hexoses, the C-6α and -β signals are separate as observed in the spectrum of galactose (Figure 2b) and the enriched compound gives a three-peak multiplet for C-6 similar to that shown in Figure 2a. In the methyl glycosides (Table II) C-6 of both α and β anomers is coupled to C-1 with coupling constants of about 3.2 and 4.3 Hz, respectively. The complexity of the resonances in the enriched free sugars suggests that C-6 is split for both anomers in these cases also.

The ^{13}C-1 enriched carbohydrates are particularly useful for obtaining C-1–H couplings which are difficult to measure in unlabeled molecules due to the lack of a Nuclear Overhauser Enhancement in proton coupled spectra. In particular, the proton coupled ^{13}C NMR spectrum of β-D-glucose (Figure 4a) shows a 5.5 Hz coupling to H-2 and an additional small proton coupling previously unobserved. Since this resonance does not appear as a simple multiplet, a coupling constant cannot be obtained nor is it possible to predict its origin. The proton coupled ^{13}C NMR spectra of both α- and β-D-mannose (Figure 4b) show no resolvable vicinal or geminal proton coupling, although the β C-1 resonance is broadened relative to the α C-1 resonance.

Carbon-13 Resonance Assignments. The large one-bond couplings and the smaller two-bond C–C couplings make it

Table II. Carbon-13 Coupling Constants of Labeled Monosaccharides

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

[a] Those couplings designated by an asterisk were observed but not measured; those couplings with no entry refer to no observable coupling. [b] FT spectra were obtained with a spectral resolution of ±0.5 Hz. [c] A broadening of ~1.6 Hz was observed in the proton coupled spectrum which could be due to C-1–H-2 coupling.

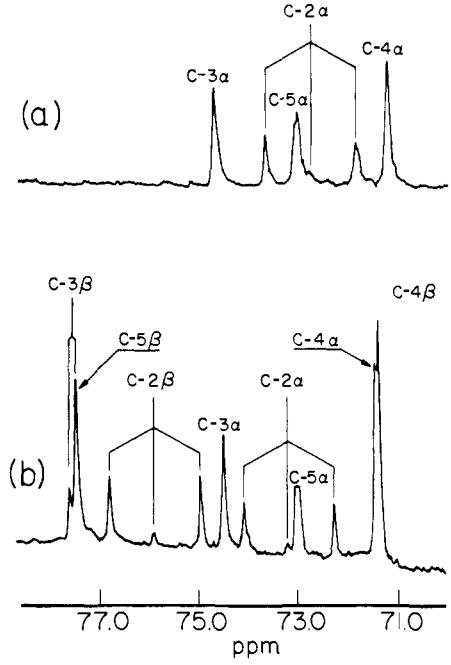


Figure 1. The proton decoupled ^{13}C NMR spectrum of the C-2–C-5 region of (a) methyl α-D[1-^{13}C]glucopyranoside (**1c**) and (b) α- and β-D-[1-^{13}C]glucopyranose (**1a, 1b**).

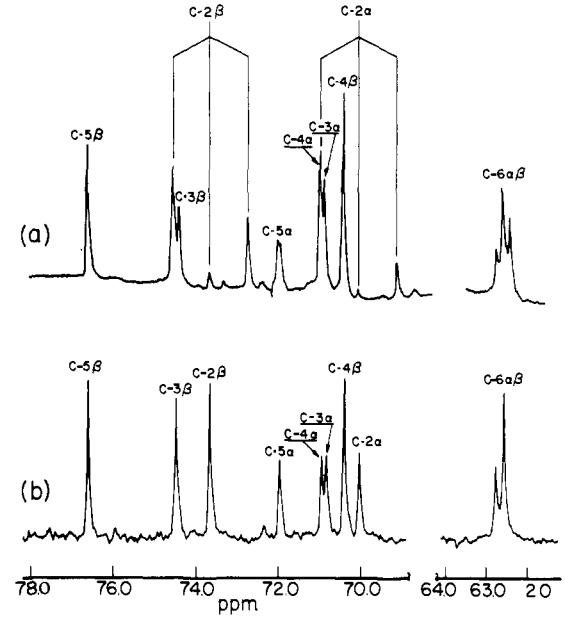


Figure 2. The proton decoupled ^{13}C NMR spectrum of the C-2–C-6 region of (a) α- and β-D-[1-^{13}C]galactopyranose (**2a, 2b**) and (b) α- and β-D-galactopyranose (**2a, 2b**).

possible to make unequivocal assignments of most of the resonances in a sugar, even those which are very closely spaced. For example, in D-glucose, C-2α is shifted upfield of C-5α in the methyl glycoside (Figure 1). This upfield shift (0.5 ppm) for C-2, however, was not predicted in the early work and C-2α and C-5α were incorrectly assigned.[13] Using the ^{13}C-1 coupling technique we have reassigned three other resonances (Table III), in agreement with the results of Gorin[17,18] who examined specifically deuterated compounds. The spectrum of methyl α-D-mannopyranoside (Figure 3a) illustrates another case where C-2 shifted upfield in the methyl glycoside, although a downfield shift was predicted. In addition, C-2 and C-3 in α-D-galactose have been incorrectly assigned (Table III). In our spectra (Figure 2a), the upfield resonance in the 70 ppm region which was assigned to C-3[13] is split and must be C-2. In a separate study,[29] the upfield resonance was incorrectly assigned to C-4 (Table III). As expected, a similar incorrect assignment[13] was found for α-L-fucose (**2c**) where C-2 and C-3 have chemical shifts similar to those for galactose.

Discussion

The Relationship of Geminal and Vicinal ^{13}C Couplings to Conformation Factors. It is apparent from the couplings listed in Table II that the magnitude of the geminal and vicinal carbon–carbon and carbon–hydrogen couplings varies with the configuration about C-1 of the monosaccharides. An increased understanding of the relationship between scalar coupling and the configuration or conformation of the monosaccharides will be extremely useful for analyzing spectra of more complex compounds and making ^{13}C assignments of carbons two and three bonds from the labeled position. In this section the empirical relationships of the J_{CCH}, J_{CCC}, and J_{COC} couplings to conformation are discussed in terms of observations which have been made on geminal J_{CCH} couplings in carbohydrates and inositol derivatives. In all of the following discussions it is assumed that the hexopyranoses exist in the $^4C_1$ conformation.

The sign and magnitude of two-bond C–H couplings in carbohydrates have been studied by Perlin and co-workers[19,20,30,31] and were found to be related to the dihedral angle between H-2 and the electronegative oxygen substituents on

Table III. ^{13}C NMR Resonance Reassignments

Compd	FT resonance	Correct assignment	CW resonance	Early assignment
α-D-Galactose	70.0[a]	C-2	69.4[c]	C-3
	70.8[a]	C-3	70.2[c]	C-2
α-D-Galactose	70.0[a]	C-2	69.9[d]	C-4
	70.9[a]	C-4	70.2[d]	C-2
α-L-Fucose	69.8[a]	C-2	69.2[c]	C-3
	71.0[a]	C-3	70.4[c]	C-2
Methyl α-D-glucopyranoside	72.7[a]	C-2	72.3[c]	C-5
	73.0[a]	C-5	72.5[c]	C-2
Methyl α-D-mannopyranoside	71.4[a]	C-2	71.0[d]	C-3
	72.1[a]	C-3	71.7[d]	C-2
Methyl α-D-galactopyranoside	69.4[b]	C-2	69.8[d]	C-4
	70.4[b]	C-4	70.8[d]	C-2
α,β-D-Arabinose	67.5[b]	C-5	67.1[c]	C-2
	69.6[b]	C-2	69.6[c]	C-5
	69.8[b]	C-3	67.1[c]	C-3
β-D-Allose	71.9[b]	C-2	72.3[c]	C-5
	74.2[b]	C-5	74.4[c]	C-2
α-D-Xylose	72.5[b]	C-2	72.5[c]	C-3
	73.9[b]	C-3	73.9[c]	C-2

[a] Data taken from Table I. [b] Data taken from ref 17 and 18. [c] Data taken from ref 13. [d] Data taken from ref 29.

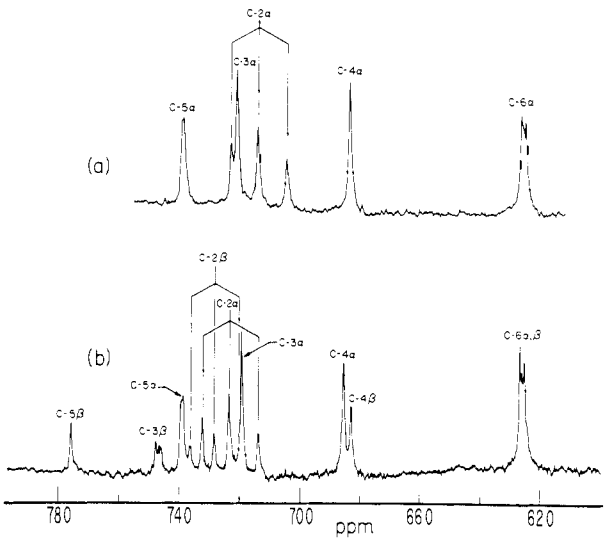


Figure 3. The proton decoupled ^{13}C NMR spectrum of the C-2–C-6 region of (a) methyl α-D-[1-^{13}C]mannopyranoside (**3c**) and (b) α- and β-D-[1-^{13}C]mannopyranose (**3a, 3b**).

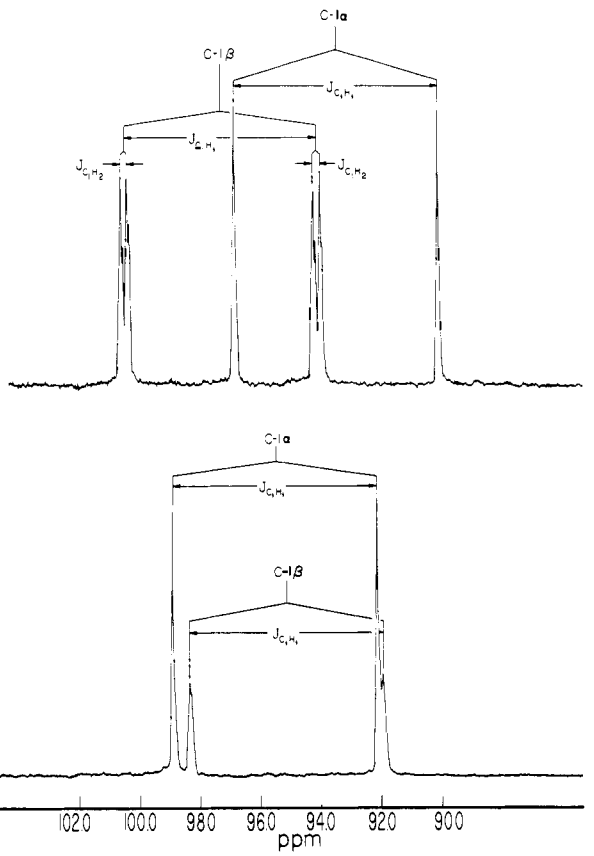


Figure 4. The proton coupled ^{13}C NMR spectrum of the C-1 region of (a) α- and β-D-[1-^{13}C]glucopyranose (**1a, 1b**) and (b) α- and β-D-[1-^{13}C] mannopyranose (**3a, 3b**).

C-1, as is illustrated in C-1–C-2 rotamer projections in Figure 5. Since there are two oxygens bonded to C-1, two dihedral angles must be considered. In general, the results are summarized by a "dihedral angle rule" which states that an oxygen anti to the coupled proton makes a positive contribution to the coupling, whereas a gauche oxygen makes a negative contribution.[20] Thus, in β-D-allose a coupling constant of −5.0 Hz ($^2J_{CCH}$) has been measured for the conformation in which both O-1 and O-5 are gauche to H-2 (Figure 5a), whereas negligible coupling was observed for the α anomer corresponding to O-5 gauche and O-1 anti to H-2 (Figure 5b).[31] This general trend in the dihedral angle dependence of the coupling constants is qualitatively consistent with theoretical calculations on ethane and ethanediol.[31]

Dorman, in studies of proton coupled ^{13}C spectra of inositol derivatives,[22] pointed out that carbons with axial hydroxyl groups showed no evidence of geminal or vicinal coupling to protons (the "axial rule") whereas carbons with equatorial hydroxyl groups did exhibit such coupling. For the case of 1,4-di-*O*-methyl-*myo*-inositol considered by Dorman, however, the observed geminal C–H couplings can also be related to the dihedral angles between the proton and the hydroxyl group on the carbon to which it is coupled. Thus, for C-2 which bears an axial hydroxyl the dihedral angles between OH-2 and the geminal protons H-3 and H-1 are both 180° and no coupling is observed between C-2 and H-3 or H-1 (Figure 6), as predicted from the rules deduced by Schwarcz and Perlin.[20] The fact that qualitatively similar results are obtained with the

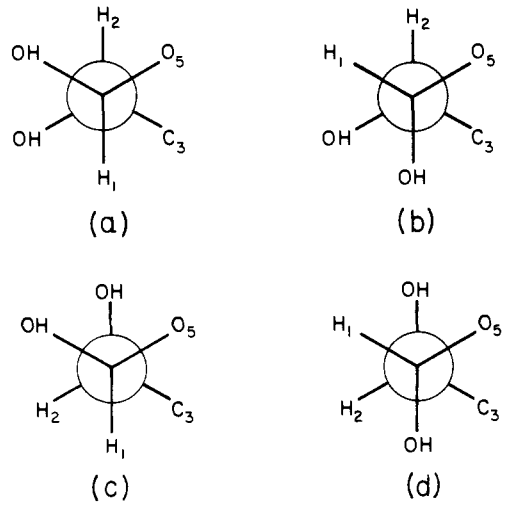


Figure 5. The rotomer projections along the C-1-C-2 bond for the $^4C_1$ conformation of (a) β-D-glucopyranose ($^1J_{CH}$ = 161.2 Hz, $^2J_{CCH}$ = 5.5 Hz), (b) α-D-glucopyranose ($^1J_{CH}$ = 169.8 Hz), (c) β-D-mannopyranose ($^1J_{CH}$ = 160.7 Hz), and (d) α-D-mannopyranose ($^1J_{CH}$ = 170.4 Hz).

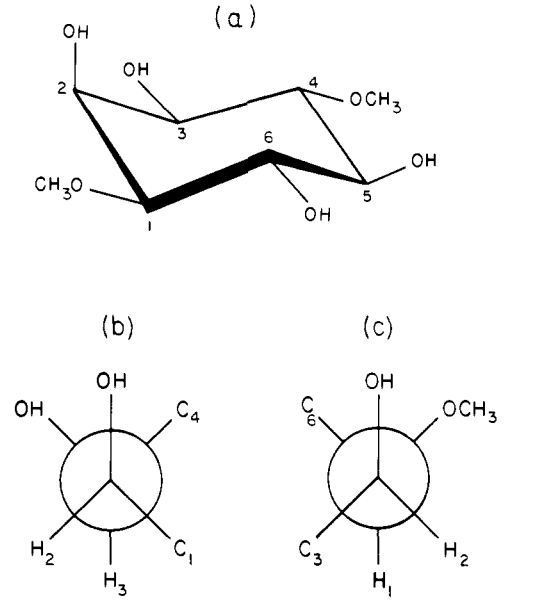


Figure 6. (a) 1,4-Di-*O*-methyl-*myo*-inositol, (b) C-2-C-3 rotomer projection, and (c) C-2-C-C-1 rotomer projection.

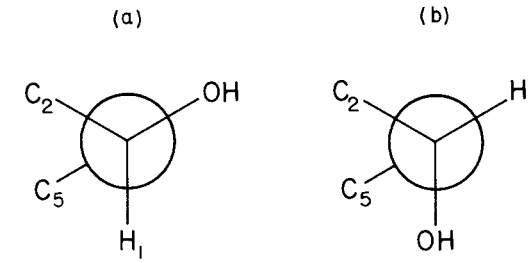


Figure 7. The rotomer projections along the C-1-C-5 bond for the $^4C_1$ conformation of (a) β-D-glycopyranose and (b) α-D-glycopyranose.

inositol and the glucose C-1 carbon indicates that a ring carbon can substitute for the ring oxygen for this type of behavior.

The C-1-H-2 coupling observed in D-mannose is particularly useful for judging the merits for the dihedral angle and the "axial rule" analyses of the geminal C-H coupling. Thus, in both anomers O-5 is trans and O-1 gauche relative to H-2 (Figures 5c and 5d) and the dihedral angle rules would predict no difference in the coupling. Only a small difference is, in fact, observed (Table II). Alternatively, based on the "axial rule" we would predict a significantly smaller coupling between C-1 and H-2 for the α anomer than for the β anomer. A comparison of the C-1-H-2 couplings observed in both anomers of D-glucose and D-mannose (Figure 4) indicates that the dihedral angle dependence predicts a larger coupling only in β-D-glucose and fits the observed coupling data well. However, a small additional coupling (1.6 Hz) for the β-D-mannose peak is observed. In every case thus far examined, the carbons with axial hydroxyl groups exhibit J_{CCH} couplings very close to 0 suggesting that additional physical factors must be operative to reduce the magnitude of the coupling in these cases. Finally, we note that the geminal C-1-H-2 coupling parallels the vicinal H-1-H-2[32] coupling which should be maximal for conformation a in Figure 5 and considerably smaller for conformations b, c, and d.

Anomeric differences in the geminal carbon-carbon coupling constants (Table II) suggest that these depend on the same factors as the $^2J_{CCH}$ couplings. Consider the C-1-C-5 coupling through the O-5 oxygen atom, in terms of the "dihedral angle rule".[20] As noted above, a ring oxygen and a ring carbon appear to contribute similarly to the observed coupling. The conformations available are depicted in Figure 7 as projections along the C_1-O_5 bond. The C-2 gauche and the O-1 trans to C-5 in the β anomer correspond to no coupling; the C-2 and O-1 both gauche in the α anomer correspond to small couplings. The opposing effects of the gauche carbon and trans oxygen in the β anomers would thus be expected to lead to a smaller coupling than the additive effects of both C-2 and O-1 gauche relative to C-5 in the α anomers (Figure 7). These predictions are borne out as shown by the data in Table II. Furthermore, if the effects of the electronegative substituents on the sign of the coupling constants can be extrapolated to the $^2J_{COC}$ case, the coupling constants should be negative. Unfortunately, we have not been able to determine these signs.

In contrast to the cases discussed above, the magnitude of the $^2J_{CCC}$ coupling between C-1 and C-3 exhibits a different dependence on the dihedral angle subtended by C-3 and the electronegative oxygens on C-1. In the β anomers with O-1 trans and O-5 gauche to C-3, the magnitude of the coupling is larger than in the α anomers with both oxygens gauche to C-3 (Figure 5). This apparent contradiction to the general rules outlined above may reflect differences in the magnitude of the contributions of gauche and trans oxygens. Thus, the contribution of a trans oxygen may be large and positive and the contribution of a gauche oxygen small and negative. The net effect might be a positive coupling constant for a trans and a gauche but a very small negative one for two gauche oxygens. Thus, if the generalization of the effect of electronegative substituents extends to the $^2J_{CCC}$ case, the sign of the C-1-C-3 coupling constant should be positive. Studies are currently in progress to obtain the signs of these geminal coupling constants.

On the other hand, there may be a fundamental difference between the $^2J_{CCH}$ and $^2J_{CCC}$ couplings. In the latter case, two dihedral angles should be considered: one about C-1-C-2 and the other about C-2-C-3. Thus, the orientation of O-3 relative to C-1 may be as important a consideration as the orientation of O-1 relative to C-3 which was considered above. In all of the examples considered in this paper, the former dihedral angle is fixed at 180° so that no data are as yet available on the effects of a configurational change at C-3. It seems reasonable, however, to predict that in β-D-allose, which bears an axial hydroxyl group at C-3, the C-1-C-3 coupling should be considerably smaller in magnitude than that observed for all of the β sugars reported here.

In addition to the geminal couplings discussed above, vicinal coupling has been observed between C-1 and C-6 but not be-

tween C-1 and C-4. The former case reflects a three-bond coupling where C-1 is anti to C-6 while in the latter case C-1 is a gauche relative to C-4. Thus, the vicinal $^{13}C-^{13}C$ coupling is consistent with a Karplus or modified Karplus-type behavior as has recently been reported by Marshall and Miiller.[33] It is interesting to note that the C-1–C-6 coupling shows an anomeric dependence, being somewhat larger for the β anomer (Table II).

The anomeric differences for all of the carbon–carbon and carbon–hydrogen couplings observed in this study can best be analyzed in terms of the dihedral angle as described by Perlin and co-workers.[20] Alternatively, the "axial rule" appears to be somewhat poorer as a predictive tool. This is especially true for the $^2J_{CCH}$ coupling in the D-mannose case discussed above. In the case of $^2J_{COC}$ coupling, the larger magnitude of the coupling is found with the α anomer in direct opposition to the prediction of the "axial rule". However, the magnitude of the $^2J_{CCC}$ and $^3J_{COCC}$ couplings are smaller for the conformations in which OH-1 is axial. It is somewhat surprising that the $^2J_{CCH}$ and $^2J_{CCC}$ couplings appear to be very close to zero where the coupled carbon bears an axial hydroxyl group. Even in the case of the C-1–C-5 coupling where this is not true the magnitude of the $^2J_{COC}$ coupling is considerably smaller than for the $^2J_{CCC}$ case. The physical basis for the "axial rule" has yet to be determined; however, there do appear to be factors which lead to a reduction in the magnitude of the coupling for carbons with axial hydroxyl groups.

We conclude that the generalization of the dihedral angle dependence of the $^2J_{CCH}$ case to the $^2J_{COC}$ case appears reasonable; however, the validity of the generalization to the $^2J_{CCC}$ case awaits a determination of the signs of the coupling constants as well as theoretical calculations. Recent calculations of $^2J_{CCC}$ coupling in 2-butanol[34] indicate a negative sign for the C-1–C-3 coupling constant. Unfortunately, the C-2–C-4 coupling which would be most relevant to the observations on the C-1 labeled sugars was not calculated.

^{13}C Resonance Assignments

The enrichment of a specific carbon of a sugar with ^{13}C permits the unequivocal assignment of resonances to the adjacent carbon and, as discussed above, to the geminal carbons. The data presented in Table III demonstrate that earlier workers have wrongly assigned resonances in several of the common monosaccharides. The incorrect assignments were also found by Gorin[17,18] who examined a series of specifically deuterated compounds. Gorin found several others which are also listed in the table. The deuteration technique allows some discrimination between two closely spaced resonances which cannot be assigned by ^{13}C-1 labeling. For example, C-3 and C-4 of α-D-galactose give resonances separated by only 0.07 ppm which cannot be distinguished by ^{13}C-1 coupling (i.e., neither C-3 nor C-4 are split); however, the spectrum of α-D-[4-^2H]galactose shows a small upfield shift for C-3 and C-5 (β-isotope effect) while C-4 disappears. It should be noted that the very small upfield shift (0.1 ppm) observed for the β-isotope effect requires that the spectrum of a mixture of the labeled and unlabeled compounds be recorded. In contrast, the ^{13}C coupling technique described here does not require such accurate shift measurements; it only requires that splitting resulting from ^{13}C enrichment be observable and predictable. In addition, labeled compounds such as ^{13}C-1 sugars require less synthetic effort than specifically deuterated compounds.

Considering the substantial impact that ^{13}C NMR spectroscopy has had on structural carbohydrate chemistry, it is useful to explore in more detail the origins of the errors in assignments that have been made: (1) errors in assignments in one spectrum have led to the development of a rule which was applied to other spectra; (2) rules which predict the effect of derivatization on chemical shifts are not always reliable; (3) CW data may be inadequate to demonstrate small chemical shift differences between spectra. For example, the early assignments of monosaccharides were based on a study of inositol derivatives and comparison with β-D-glucose[35] in which all of the hydroxyls are equatorial. The early assignments of C-2 and C-3 for α-D-glucose were later shown to be incorrect by Koch and Perlin[14] who prepared glucose specifically deuterated at C-2. Although the early procedures were proven unreliable in this case, until recently there has been no systematic reassessment of the additional carbohydrate ^{13}C assignments. In addition, the original α-D-galactose assignments[13] were based on the observation that the conversion of glucose from the β to the α anomer resulted in nearly equivalent upfield shifts for C-3 and C-5 and a smaller upfield shift for C-2. Since α-D-galactose assignments were based on a rule which was deduced from an incorrect assignment, the α-D-galactose C-2 and C-3 assignments were also incorrect. Indeed, the correct rule found for glucose and galactose indicates nearly equivalent upfield shifts for C-2 and C-3 and a larger upfield shift for C-5.

The early resonance assignments for the methyl glycosides[13,29] were made by comparing spectra of the methylated and free sugars. According to the original assignments for α-D-glucose, α-D-mannose, and α-D-galactose, C-2 shifted downfield after methylation at C-1 while C-3–C-6 were virtually unaffected. In contrast, labeling studies[17,18] indicate that C-2 shifts upfield after methylation at C-1, an observation which is verified by the present data (Table I). These incorrect assignments reflect two problems: (1) the inability of theory to predict an upfield shift for C-2 after methylation, and (2) the difficulty inherent in obtaining accurate chemical shift determinations from CW spectrum with relatively low signal to noise and broad peaks. Although the data from a single spectrum might be very reasonable, a comparison of spectra to obtain small chemical shift differences particularly without the use of an internal standard requires data obtained under identical conditions. It is clear that empirical chemical shift rules for the effect of derivatization must be reassessed carefully, especially in view of the rapidly expanding literature on di- and polysaccharides.[2-7] The elegant work of Gorin[5,16-18] is a model in this regard.

Conclusion

The studies reported here, and the work of Gorin and coworkers,[5,16-18] Bundle et al.,[15] and Koch and Perlin,[14] demonstrate the utility of isotopically labeled compounds in making unambiguous assignments of carbohydrate ^{13}C resonances. As noted in the text, specific deuterium or carbon-13 labels are both useful but not equally so. In the general case, the following considerations would tend to favor carbon-13 as the label of choice: (a) carbon-13 coupling constants, which should prove to be useful in conformational analyses, can be obtained conveniently only for the carbon-13 enriched material; (b) deuterium labeling frequently "obliterates" the ^{13}C resonance of the C-D moiety; (c) line widths in the spectra of deuterated oligosaccharides may preclude the observation of a β-isotope shift for these compounds;[5] (d) the potential exchange of a deuterium label with solvent protons is always a consideration in the introduction of a specific label[5] and the later manipulation of the labeled material in chemical or enzymic systems; (e) it is unnecessary with a carbon-13 label to compare closely spaced chemical shifts derived from different compounds. Although the relative costs of the isotopes is an important factor in the favor of deuterium as a label, carbon-13 has become much less expensive in recent years,[36] and perhaps more important, the syntheses of ^{13}C-1 enriched carbohydrates requires less synthetic effort.

There is a substantial and growing body of literature illustrating the great potential of ^{13}C NMR as a nondestructive tool

for analysis of structure and dynamics of carbohydrates and their protein complexes. For example, Lemiuex and coworkers[8] have recently executed a brilliant tour de force in the synthesis of the blood group antigenic determinants. However, certain of the resonances in their ^{13}C NMR spectra have been assigned by comparison with the incorrect fucose and galactose assignments,[13,29] and it is not entirely clear what the correct assignments should be, given the demonstrated current uncertainty about the effects of derivatization upon ^{13}C chemical shifts. Carbon-13 labeling would be a decided advantage here and by extension in ^{13}C NMR studies of antigen–antibody reactions.

We have discussed the carbohydrate assignment problem at some length in order to prevent further propagation of errors and to introduce a cautionary note about the general reliability of the assignment methods which have been used. Although, in retrospect, the assignment difficulties have been evident since the initial reassignment of α-D-glucose in 1970,[14] there has been little recognition of this fact. Thus, relaxation studies of the interaction of methyl glycosides with conconavalin A[10] have been based on incorrect assignments, although it is unlikely that the particular incorrect assignment has led to significant error. Correct assignments of the ^{13}C resonances are the foundation upon which all further ^{13}C NMR work is based; uncertainties which exist in these assignments must be emphasized to avoid propagation of errors.

Acknowledgment. This work was performed under the auspices of the U.S. Energy Research and Development Administration and was supported in part by Grant GM 21731 (R.B.) and the National Institutes of Health Research Grant 1P07 RR-00962-01 (NAM) from the Division of Research Resources, DHEW. T. E. Walker gratefully acknowledges a Postdoctoral Fellowship (1 F22 CA00971-01) from the National Cancer Institute.

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Metal–Metal Interactions Involving Metalloporphyrins. 4. A New Antiferromagnetic Phase of Tetraphenylporphinatoiron(III) Fluoride

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Abstract: Tetraphenylporphinatoiron(III) fluoride (TPPFeF) has been found to exist in two different solid phases. The α-phase is a normal $S = 5/2$ paramagnet and is the solid which has been studied earlier. The isomorphic β-phase is an antiferromagnet which shows a sharp room temperature Mössbauer spectrum. The two solids dissolve to produce solutions containing monomeric, paramagnetic TPPFeF but the original α- or β-phase re-form upon precipitation. The presence of trace amounts of excess HF in solution is required for formation of the β-phase. Solid $(TPPFe)_2FBF_4$ has been prepared and is also antiferromagnetic. Molecular weight and conductivity studies indicate that a stable fluoride bridged dimer does not exist in solution.

Hemin halides (porphyrin $Fe^{III}X$) have been the subject of extensive study. Structural work[1] has shown them to be monomeric five-coordinate Fe(III) complexes and magnetic[2] studies have found them to be normal $S = 5/2$ systems. Their Mössbauer spectra are characteristically broad above liquid He temperatures due to spin relaxation effects at the isolated high spin Fe(III) centers.[3,4] On the other hand, the presence of potentially bridging axial ligands has allowed the formation