Deuterium-Induced Differential Isotope Shift ^{13}C NMR. 1. Resonance Reassignments of Mono- and Disaccharides[1]

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Abstract: Previous assignments of natural-abundance ^{13}C NMR chemical shifts of mono- and disaccharides have been reevaluated by use of a newly developed differential isotope shift (DIS) technique. Deuterium-induced ^{13}C isotope shifts were produced through rapid interchange of carbohydrate hydroxyl groups in a D_2O environment. The differential shift positions (D_2O vs. H_2O environments) were measured simultaneously in the magnetic field with a dual coaxial NMR cell. Each isotopic chemical shift position was sharply defined because of rapid OH and OD interchange in the separate, respective solvent environments. The largest induced upfield displacements due to deuterium substitution of OH were noted for those carbons bearing hydroxyl groups, β shifts (0.14 ppm). β shifts at C-1 were smaller (0.11 ppm) than all other β induced shifts. Shifts due to vicinal OD, γ shifts, were ~0.03–0.06 ppm and additive. Differences in induced γ shifts directed from cis vs. trans hydroxyl groups at C-1 were found to be statistically significant. Isotope shift parameters were calculated from a linear regression analysis of data compiled from 12 structurally different pyranose structures. These parameters were used to calculate the isotope shifts for other pyranose and furanose mono- and disaccharides. DIS analysis was also applied to different substituted carbohydrates in both aqueous and nonaqueous systems as well as α- and β-D-glucuronopyranoses.

^{13}C NMR spectroscopy is becoming more important as a tool for studying the structural interactions of low molecular weight carbohydrates,[2-4] oligosaccharides, polysaccharides,[5-9] and antigenic polysaccharides.[10,11] In all such studies it is imperative that the correct assignment of the ^{13}C resonances be unambiguous. Several strategies have been applied to assist in making unequivocal assignments.[12] Early studies[13-15] with continuous-wave instrumentation relied heavily on analogies to available data of model compounds. With the advent of pulsed Fourier transform instrumentation, techniques such as spin–lattice relaxation,[2] off-resonance decoupling, selective heteronuclear decoupling, and long-range ^{13}C–H coupling[12] became viable alternatives. Unfortunately these methods are in many cases difficult to perform, i.e., they require large

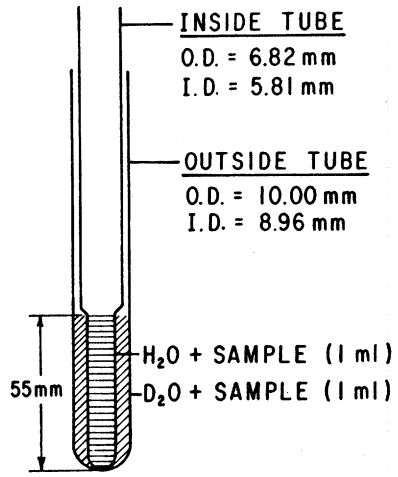


Figure 1. Dual NMR cell.

magnetic fields and precise knowledge of specific proton resonance frequencies. Also, many require position-specific isotope enrichment (^2H or ^{13}C) before shift identification can be established.[16-20] A recent advance in this area[21a,b] has simplified isotopic labeling to the extent that specific deuteration at hydroxyl-bearing carbons may be more easily performed, enabling ready identification of non-hydroxyl-bearing carbon with ^{13}C NMR. This technique simplifies the assignment process but still leaves uncertainties concerning the designation of other shifts. Consequently, many of the original ^{13}C resonance assignments made for a number of common carbohydrate molecules are still in question.

Feeney et al.[22] and shortly thereafter Ladner et al.[23] demonstrated that deuterium isotope effects could be transmitted to ^{13}C carbonyl resonances in peptides through slowly exchanging vicinal N-D bonds in a 50:50 H_2O-D_2O solution. The observed difference in chemical shift between the two $^{13}C{=}O$ resonances gave a direct measure of the isotope effect and allowed for differentiation between $^{13}C{=}O$ associated with rapidly exchanging OH and $^{13}C{=}O$ of slowly exchanging amide NH.[23] Subsequently, resolution of individual shifts for $^{13}C{=}O$ of amides corresponding to the species $O{=}CNH_2$, $O{=}CNHD$, and $O{=}CND_2$ was achieved in a slowly exchanging environment of dipolar aprotic solvents.[24] Similarly, Gagnaire and Vincendon[25] chose a dipolar aprotic environment and low temperature (14 °C) to limit the exchange rate of carbohydrate hydroxyl groups. Their ^{13}C NMR spectra taken of 50% deuterium exchanged mono- and disaccharides in dimethyl sulfoxide (Me_2SO) revealed a broadening in those resonances associated with carbons having directly bonded hydroxyl groups. This technique, while valuable for assigning non-OH-bearing carbon resonances, requires low temperature (limiting it to the study of small molecules) and high fields (62.8 MHz) and yields no information for assignment of C-OH resonances.

This paper describes a new approach to measuring the deuterium isotope shift of ^{13}C resonances resulting from rapid exchange with D_2O and the unique application of this measurement to spectral assignments. It also describes several ^{13}C resonance reassignments of some common carbohydrates, based on calculated isotope shifts derived from empirically obtained isotope shift parameters.

Experimental Section

Natural-abundance ^{13}C NMR spectra were obtained at 30 °C on a JEOL FX 60-Q spectrometer[26] operating at 15.04 MHz with proton noise decoupling. Differential spectra were obtained with a coaxial dual cell (Figure 1) purchased from Wilmad Glass Co. The inner tube contained 100 mg of sample dissolved in 1 mL of H_2O containing 1% *p*-dioxane. The outer tube contained the same concentration of materials dissolved in 1 mL of D_2O. The D_2O-dissolved sample was exchanged three times with D_2O prior to running the spectra. Except for compounds **15a** and **15b**, deionized water and commercial D_2O were used for all experiments without adjustment of pH. With the dissolved samples, the pH and pD (pD = observed pH meter reading + 0.4) of the H_2O and D_2O solutions were 6.75 and 7.35, respectively. Addition of 1 drop of commercial *p*-dioxane to each solution lowered the pH and pD to 3.0 and 3.6, respectively. When the pH of the H_2O solution was adjusted with 0.1 N NaOH to correspond to the D_2O solution, pH = pD = 3.6, the DIS values varied from the unadjusted solution with a mean of 0.006 and an average standard deviation of 0.005 ppm. Each spectrum was obtained after 1000 transients with a spectral width of 1000 Hz, a computer data memory size for the free induction decay of 16K, repetition rates of 8.3 s, and pulse angle of 58°. All chemical shifts are given in δ values and rounded off to the nearest 0.01 ppm and were measured relative to the internal standard *p*-dioxane assigned a shift of 67.40 ppm. Line widths for typical monosaccharides were approximately 1.0 Hz and for disaccharides 1.4 Hz. Magnetic susceptibility contributions were verified with spectra obtained from a Bruker WH-360/180 superconducting magnet NMR spectrometer. Spectral widths were 3000 Hz with 32K data points.

The reproducibility of the JEOL instrumentation was evaluated by five consecutive accumulations of the isotope shift data. The average standard error of the mean for these data was 0.002 ppm. Five independently exchanged samples were also run consecutively, and the DIS values had a standard error of the mean of 0.004 ppm.

The observed DIS values, i.e., the differences between the ^{13}C resonance position in D_2O and its shift in H_2O ($\delta^{13}C_{H_2O} - \delta^{13}C_{D_2O}$) for 12 D-gluco- and D-galactopyranoses and pyranosides were used to calculate the isotope shift parameters. Each of these empirically observed DIS values was set equal to the sum of all its β and γ parameters which contribute to the induced shift. A linear regression analysis of these equations led to a solution which could be readily fit to the experimental data. The calculated DIS values showed good agreement with the observed values, having a standard error of estimate of 0.009 ppm.

Results and Discussion

A deuterium-induced ^{13}C differential isotope shift (DIS) is defined as the chemical shift difference in parts per million between the ^{13}C shift as observed in H_2O and the upfield D_2O-induced ^{13}C shift ($\delta^{13}C_{H_2O} - \delta^{13}C_{D_2O}$). DIS values were measured at 15.04 MHz (conventional electromagnet instrument) with a coaxial dual NMR cell as pictured in Figure 1. The dimensions as illustrated allowed for equal volumes of solvent and equal sample concentrations in both compartments to be aligned in the probe simultaneously. To minimize bulk magnetic susceptibility contributions to the observed differential shifts, the H_2O solvent was placed in the inner tube and the D_2O in the outer tube.[27] To verify and quantify the magnitude of the shift contribution due to magnetic susceptibility, the following experiments were carried out. Dual samples having in one case H_2O outside and in the other D_2O outside, were examined at both 15 MHz (conventional electromagnet) and 45 MHz in a superconducting magnet. Since the polarizing magnetic field of a superconducting magnet is along the long axis of the NMR tube as opposed to being perpendicular in the former conventional magnet, the observed shift due to susceptibility is twice in magnitude and opposite in sign as found with a conventional electromagnet.[28] Induced shifts observed for H_2O outside in the supercon instrument were 0.024 ppm smaller than those found with the conventional magnet instrument, indicating a magnetic susceptibility contribution to the observed shifts of +0.012 ppm in the latter. No such difference in overall induced shifts was noted when the D_2O-outside sample was examined. Thus, the induced shifts as measured in this study (D_2O outside) reflect the solvent-induced isotope effect with no measurable contributions from bulk magnetic susceptibility.

To assess the generality of the DIS, we undertook a systematic investigation of D_2O-induced shifts in molecules of

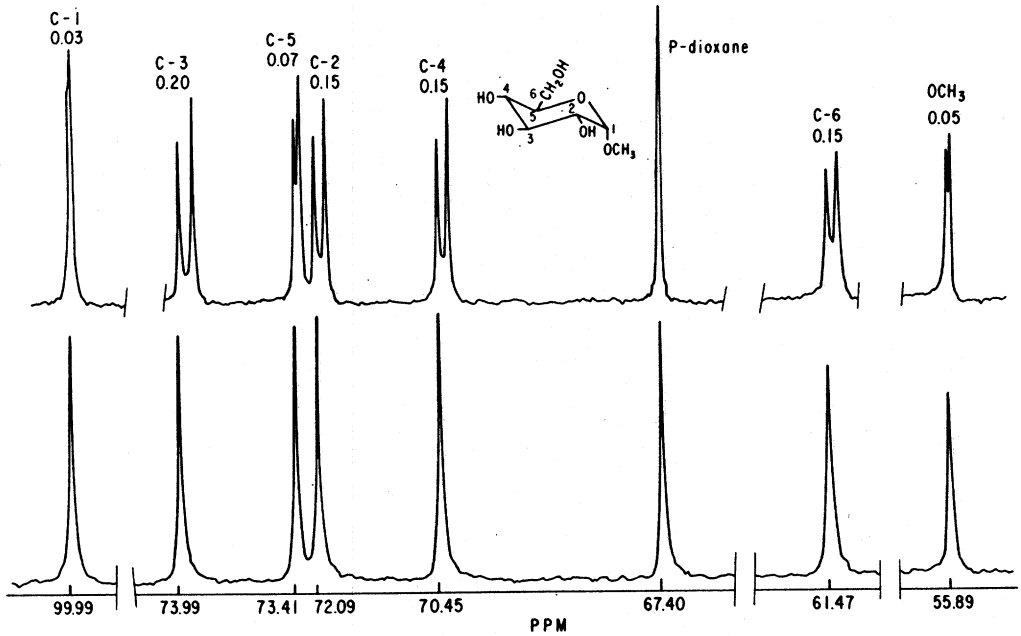


Figure 2. (a) 15.04-MHz proton noise decoupled spectrum of methyl α-D-glucopyranoside (**1a**) in H_2O; (b) DIS spectrum of **1a** taken with a dual coaxial tube containing **1a** in D_2O and H_2O. Each spectrum was obtained at 30 °C after 1000 transients with a displayed spectral width of 200 Hz and 16K data.

Table I. ^{13}C Chemical Shifts and DIS[a] of α- and β-Gluco- and Galactopyranoses and Pyranosides

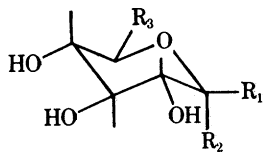
compd	chemical shift and DIS, ppm[b]						
	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3
methyl α-D-glucopyranoside **1a**	100.02 (0.01)	72.20 (0.15)	74.11 (0.20)	70.55 (0.15)	72.46 (0.07)	61.57 (0.15)	55.93 (0.06)
methyl β-D-glucopyranoside **1b**	103.95 (0.01)	74.07 (0.15)	76.79 (0.21)	70.64 (0.16)	76.79 (0.07)	61.77 (0.15)	58.09 (0.06)
α-D-glucose **1c**	92.94 (0.13)	72.47 (0.20)	73.75 (0.20)	70.56 (0.15)	72.28 (0.06)	61.59 (0.15)	
β-D-glucose **1d**	(96.74 (0.14)	75.14 (0.21)	76.71 (0.20)	70.60 (0.15)	76.78 (0.06)	61.74 (0.15)	
α-D-xylose **2a**	93.10 (0.15)	72.47 (0.20)	73.85 (0.21)	70.38 (0.17)	61.860 (0.07)		
β-D-xylose **2b**	97.49 (0.15)	75.06 (0.23)	76.83 (0.21)	70.21 (0.17)	66.08 (0.07)		
methyl α-D-galactopyranoside **3a**	100.13 (0.00)	69.17 (0.15)	70.47 (0.18)	70.20 (0.15)	71.59 (0.05)	62.21 (0.14)	55.95 (0.05)
methyl β-D-galactopyranoside **3b**	104.534 (0.00)	71.70 (0.17)	73.78 (0.18)	69.65 (0.15)	75.97 (0.05)	61.97 (0.15)	58.05 (0.04)
α-D-galactose **3c**	93.16 (0.14)	69.37 (0.20)	70.17 (0.19)	70.29 (0.16)	71.35 (0.07)	62.18 (0.16)	
β-D-galactose **3d**	97.32 (0.13)	72.93 (0.24)	73.79 (0.19)	69.73 (0.17)	76.00 (0.07)	61.98 (0.16)	
α-D-fucose **4a**	93.08 (0.13)	69.10 (0.20)	70.27 (0.18)	72.86 (0.16)	67.76 (0.03)	16.59 (0.07)	
β-D-fucose **4b**	97.10 (0.14)	72.71 (0.22)	73.92 (0.19)	72.42 (0.17)	71.70 (0.04)	16.59 (0.07)	

[a]Differential isotope shift given in parentheses. [b]All chemical shifts are relative to internal *p*-dioxane taken as 67.40 ppm and reported to the nearest 0.01 ppm. The average standard error of the mean for the reproduction of each chemical shift was 0.004 ppm.

well-defined stereochemistry. For an illustration of the technique of DIS, we consider first the spectrum of methyl α-D-glucopyranoside (**1a**), a molecule whose ^{13}C assignments have been made previously without ambiguity.[18,19] Figure 2a shows the ^{13}C spectrum of **1a** in H_2O. Figure 2b shows the resulting DIS spectrum taken with 100 mg of previously deuterium-exchanged **1a** dissolved in 1 mL of D_2O in the outer tube. Table I lists the chemical shifts for each carbon resonance and the observed DIS. The DIS values obtained from five independent experiments had a standard error of the mean of 0.004 ppm. Little measurable differences in DIS were observed for variations in temperature from 25 to 55 °C. Care was taken to have

Table II. Calculated Differential Isotope Shift Parameters

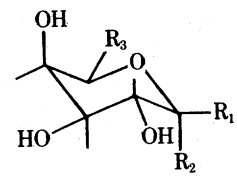
symbol	value	definition
β	0.14	β induced shift
β^1	0.11	β induced shift at C-1
β^6	0.15	β induced shift at C-6
γ	0.03	γ induced shift
$\gamma_t{}^1$	0.06	γ induced shift from a trans anomeric hydroxyl
$\gamma_c{}^1$	0.03	γ induced shift from a cis anomeric hydroxyl



1a, R_1 = H; R_2 = OCH_3; R_3 = CH_2OH
b, R_1 = OCH_3; R_2 = H; R_3 = CH_2OH
c, R_1 = H; R_2 = OH; R_3 = CH_2OH
d, R_1 = OH; R_2 = H; R_3 = CH_2OH
2a, R_1 = H; R_2 = OH; R_3 = H
b, R_1 = OH; R_2 = H; R_3 = H

equal concentrations of sample in both compartments; however, 10–15% differences in concentrations appeared to have little effect on the observed values. Measurable deuterium isotope shifts are principally induced on ^{13}C resonances from (a) directly bonded hydroxyl groups (β shifts) and (b) hydroxyl groups positioned on vicinal carbons (γ shifts). These shifts are about twice the size of those reported for the corresponding covalently deuterated derivatives, and, unlike the previous report, we do not observe any noticeable ^{13}C line broadening or ^{13}C–H coupling.[17] Also, with one exception, relaxation times (T_1) appear to be nearly the same in both solvents. From a casual inspection of the spectrum of **1a** or the DIS listed in Table I, it is obvious which resonances represent the hydroxyl bearing ring carbons C-2, C-3, and C-4. A clue to assignment is given by the additivity of each isotope shift contribution, e.g., C-3, 0.20 ppm ($\beta + 2\gamma$ shifts); C-2, 0.15 ppm ($\beta + \gamma$ shift); C-4, 0.15 ppm ($\beta + \gamma$ shift). Carbon 6 has a relatively larger shift, 0.15 ppm, considering that it has only a single β contribution. Conceivably this larger isotope effect could be accounted for by preferential hydration by D_2O between the ring oxygen and the C-6 OH as postulated by Czarniecki and Thornton.[3] Carbons 1 and 5 are easily identified by their characteristically smaller γ induced shifts (0.03 and 0.07 ppm) which originate from one adjacent and two adjacent vicinal carbon hydroxyl groups, respectively. Because of their characteristic field positions it is usually a trivial matter to assign resonances to C-1, C-4, and C-6. However, the assignments for closely spaced C-2, C-3, and C-5 resonances have been problematic.[13–15] Here we see a clear example of how DIS can directly differentiate between these three shift designations, enabling complete spectral assignments. It is noteworthy that we observe a 0.06-ppm DIS of the OCH_3 resonance, since this carbon has neither β or γ exchangeable hydrogens. Again we can only conjecture that preferential solvation is inducing changes in the field about this ether linkage.

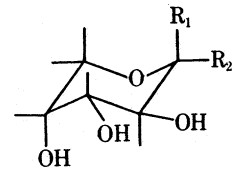
The DIS values for methyl β-D-glucopyranoside (**1b**) are substantially the same as those reported for **1a**, which indicates that a change in orientation of the glycosidic linkage has little perturbing influence on the isotope shift inducing mechanism. Examination of the reducing pyranoses α- and β-D-glucose **1c** and **1d** revealed the anticipated changes in DIS at the reducing center, C-1 and C-2. Induced shifts increased at C-1 to 0.13 and 0.14 ppm ($\beta + \gamma$) and at C-2 to 0.20 and 0.21 ppm ($\beta + 2\gamma$) for **1c** and **1d** relative to the respective pyranosides **1a** and **1b**. The spectrum of α- and β-D-xylose **2a** and **2b** displayed DIS values for C-1 and C-2 of the same order of magnitude as for **1c** and **1d**, yet the expected lowering of the shift at C-5 due to the absence of a γ shift from CH_2OH did not occur. In the D-galacto series methyl α,β-D-galactopyranosides, we observed the lowering in C-1 and C-2 DIS values with C-1 glycosidation. We also noted a small reduction in DIS at C-3 for all galacto compounds. The range in isotope shifts for the C-3 in the gluco series is 0.20–0.21 ppm, whereas in the galacto series it is 0.18–0.19 ppm. This diminished DIS at C-3 might be attributed to a smaller γ induced shift from the cis C-4 OH. Induced shifts were larger for C-2 resonances in β-pyranoses (0.21–0.24 ppm) than in the α-pyranoses (0.20 ppm). Again, orientation of OH at C-1 may be responsible for this effect. The resonance assignments for α- and β-D-galactose **3c** and **3d** and D-fucose **4a** and **4b** were based on recently reported assignments of these



3a, R_1 = H; R_2 = OCH_3; R_3 = CH_2OH
b, R_1 = OCH_3; R_2 = H; R_3 = CH_2OH
c, R_1 = H; R_2 = OH; R_3 = CH_2OH
d, R_1 = OH; R_2 = H; R_3 = CH_2OH
4a, R_1 = H; R_2 = OH; R_3 = CH_3
b, R_1 = OH; R_2 = H; R_3 = CH_3

compounds, which were elegantly carried out with ^2H and ^{13}C labeling.[18,19]

Shift parameters were calculated from the DIS data in Table I by use of linear regression analysis. The agreement between calculated and observed DIS values had a standard error of estimate of 0.01 ppm. Within this set no statistically significant difference was noted for a γ induced shift from an axial vs. an equatorial hydroxyl group with the exception of those induced from C-1 OH. The β induced shift at C-1 was also found to be smaller (0.11 vs. 0.14) than all other β induced shifts. A similar observation has been made for the β induced shifts in α,β-D-glucose-*2-*^2H.[18] Table II defines six derived shift parameters and lists their respective calculated values. Table III contains shift assignments for ten mono- and disaccharides and the observed and calculated DIS values. The observed DIS show relatively good agreement with the proposed shift assignments and the values calculated from the above set. While the D-arabinose compounds **5a–d** all exist in the $^1C_4$ configuration,



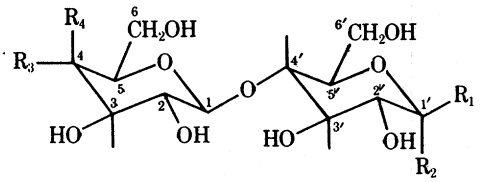
5a, R_1 = OCH_3; R_2 = H
b, R_1 = H; R_2 = OCH_3
c, R_1 = OH; R_2 = H
d, R_1 = H; R_2 = OH

their DIS appear to be essentially the same as their $^4C_1$ counterparts **3a–d** and **4c,d.** Removal of OCH_3 to yield α-D-arabinose **5c** increased the induced shift for C-1 and C-2 to 0.15 and 0.23 ppm, respectively, while C-3 remained at 0.19 ppm. Similar trends are observed for the remaining disaccharides listed in Table III. For example, a sharp increase in the DIS at C-1′ from 0.03 to 0.15 and 0.13 occurred after the transformation of **6a** to **6b** and **6c,** respectively. The C-4′ DIS values are much smaller for the disaccharides, with the exception of sucrose (no glucosidation is found at C-4′ in sucrose), than those of the corresponding monosaccharide (Table I). This decrease from approximately 0.16 to 0.03 ppm is directly at-

Table III. Chemical Shift Assignments and DIS[a] Values Observed (Calculated)[b] for Glucomono- and Galactomono- and -disaccharides

compd	chemical shift[b] and DIS, ppm											
	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
methyl α-D-arabinopyranoside **5a**	107.03	73.89	75.57	71.47	69.27							
	0.04	0.17	(0.19	0.17	0.06							
	(0.03)	(0.17)	(0.20)	(0.16)	(0.03)							
methyl β-D-arabinopyranoside **5b**	102.99	72.11	70.05	71.38	65.65							
	0.04	0.16	0.19	0.16	0.05							
	(0.03)	(0.17)	(0.20)	(0.17)	(0.03)							
α-D-arabinose **5c**	97.63	72.91	73.45	69.63	67.24							
	0.15	0.23	0.19	0.17	0.07							
	0.13)	(0.23)	(0.20)	(0.17)	(0.03)							
β-D-arabinose **5d**	93.44	69.46	69.46	69.46	63.36							
	0.14											
	(0.14)	(0.20)	(0.20)	(0.17)	(0.03)							
methyl β-D-lactopyranoside **6a**	103.75	71.92	73.51	69.52	76.19	62.03	103.75	73.76	75.34	79.27	75.57	61.10
	0.00	0.15	0.19	0.16	0.05	0.17	0.00	0.16	0.14	0.00	0.00	0.15
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.03)	(0.17)	(0.17)	(0.03)	(0.03)	(0.15)
α-D-lactose **6b**	103.63	71.95	73.54	69.54	76.20	62.03	92.71	72.18	72.37	79.32	70.98	60.98
	0.00	0.16	0.19	0.16	0.07	0.15	0.15	0.23	0.15	0.03	0.03	0.15
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.14)	(0.20)	(0.17)	(0.03)	(0.03)	(0.15)
β-D-lactose **6c**	103.69	71.95	73.54	69.54	76.20	62.03	96.62	74.84	75.34	79.21	75.63	61.11
							0.13	0.22	0.15	0.03	0.02	0.12
							(0.14)	(0.22)	(0.17)	(0.03)	(0.03)	(0.15)
α-D-cellobiose **7b**	103.27	74.12	76.52	70.44	76.83	61.59	92.69	72.25	72.30	79.60	70.96	61.09
	0.00	0.15	0.20	0.15	0.05	0.15	0.11	0.21	0.15	0.05	0.04	0.14
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.14)	(0.20)	(0.17)	(0.03)	(0.03)	(0.15)
β-D-cellobiose **7c**	103.27	74.12	76.52	70.44	76.83	61.59	96.61	74.92	75.25	79.48	75.59	61.09
							0.13	0.22	0.16	0.00	0.00	0.14
							(0.14)	(0.23)	(0.17)	(0.03)	(0.03)	(0.15)
α,α-trehalose **8a**	93.95	71.99	73.52	70.64	72.95	61.49						
	0.00	0.15	0.20	0.15	0.07	0.13						
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)						
α,β-trehalose **8b**	100.91	72.37	73.82	70.36	73.55	61.61						
α anomer	0.00	0.13	0.19	0.15	0.07	0.14						
	(0.03)	(0.17)	(0.20)	(0.17)	(0.05)	(0.15)						
β anomer	103.61	74.08	76.40	70.36	76.97	61.98						
	0.00	0.14	0.21	0.15	0.00	0.14						
	(0.03)	(0.14)	(0.20)	(0.17)	(0.05)	(0.15)						
sucrose **9**	92.94	72.03	73.58	70.19	73.27	61.12	63.34	104.41	77.39	74.96	82.21	63.35
	0.02	0.16	0.21	0.15	0.07	0.14	0.15	0.03	0.16	0.16	0.06	0.14
	(0.03)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)	(0.14)	(0.06)	(0.17)	(0.17)	(0.06)	(0.15)

[a]DIS—differential isotope shift. [b]The standard deviations in parts per million between observed and calculated values follow: **5a,** 0.012; **5b,** 0.012; **5c,** 0.014; **5d,** 0.020; **6a,** 0.021; **6b,** 0.011; **6c,** 0.012; **7b,** 0.015; **7c,** 0.015; **8,** 0.017; **9,** 0.012. [c]Chemical shifts are reported in H_2O relative to internal *p*-dioxane taken as 67.40 ppm.



6, R_3 = OH; R_4 = H
7, R_3 = H; R_4 = OH

a, R_1 = OCH_3; R_2 = H
b, R_1 = H; R_2 = OH
c, R_1 = OH; R_2 = H

tributable to glycosidation of C-4′ OH which effectively removes the β-isotope shift contribution. A concomitant lowering of DIS is also evident for the C-3′ resonance resulting from the loss of a γ isotope shift contribution from C-4′ OH. Resonances assigned to C-3 (galactopyranosyl ring) in compounds **6a–c** exhibit isotope shifts which appear to be smaller than those found for C-3 of the glucopyranosyl rings (0.19 vs. 0.20) of **7b, 7c, 8,** and **9**. However, the calculated DIS parameters fail to demonstrate that there is a statistically significant difference between these values. In general, the observed isotope shift for the C-2 resonance is, on the average, larger when C-1 OH is trans to C-2 OH as predicted from the calculated parameters. Also, the β induced shifts at C-1 are consistently smaller than all other β induced shifts.

The ^{13}C chemical shifts assembled in Table III were assigned in accordance with their anticipated DIS values. Agreement between calculated and observed DIS values is excellent in most cases. This new method for identifying ^{13}C shifts suggests several resonance reassignments taken from the current literature. All proposed ^{13}C resonance reassignments are listed in Table IV with the corresponding shifts (ppm) and the previously reported data. The spectra in Figure 3 display the normal and DIS shifts of methyl β-D-lactopyranoside **6a.**

Table IV. ^{13}C Shift Reassignments Based on DIS

		shift assignment, ppm				ref
methyl β-D-lactopyranoside **6a**			C-3′		C-5′	
			75.6		75.4	*a*
			(75.60[b] / 75.25[b])		(75.60[b] / 75.25[b])	*c*
			75.34		75.57	present study
	DIS values		0.14		0.00	
α-D-lactose **6b**		C-2′	C-3′		C-5′	
		71.20	(72.20[d] / 72.50[d])		(72.20[d] / 72.50[d])	*c*
		72.18	72.35		70.98	present study
	DIS values	0.23	0.15		0.03	
α,β-D-cellobiose **7b, 7c**			C-3		C-5	
			76.7		76.5	*e*
			76.52		76.83	present study
	DIS values		0.20		0.05	
α-D-cellobiose **7b**		C-2′	C-3′	C-4′		
		72.1[f]	72.1[f]	79.9[f]		*e*
		72.25	72.30	79.60		present study
	DIS values	0.21	0.15	0.05		
β-D-cellobiose **7c**		C-2′	C-3′	C-4′	C-5′	
		75.1[f]	75.5	79.9[f]	74.9[f]	*e*
		74.92	75.25	79.48	75.59	present study
	DIS values	0.22	0.16	0.00	0.00	
α,α-trehalose **8a**		C-2	C-3		C-5	
		72.9	73.4		71.8	*g*
		73.5	72.0		73.0	*h*
		71.99	73.52		72.95	present study
	DIS values	0.16	0.20		0.00	
α,β-trehalose **8b**		C-2	C-3		C-5	
α anomer		73.61	(74.21 / 73.91)		72.51	*g*
		72.37	73.82		73.55	present study
	DIS values	0.13	0.19		0.05	
β anomer		C-2	C-3		C-5	
		(74.21 / 73.91)	77.04		76.42	*g*
		74.08	76.04		76.97	present study
	DIS values	0.14	0.21		0.00	
sucrose **9**		C-2	C-3		C-5	
		73.6	(73.2 / 72.0)		(73.2 / 72.0)	*i*
		73.6	72.0		73.2	*h*
		72.03	73.58		73.27	present study
	DIS values	0.16	0.21		0.07	
			C-3′	C-4′	C-5′	
			(82.2 / 77.5)	(82.2 / 77.5)	75.0	*i*
			77.5	75.0	82.2	*h*
			77.39[j]	74.96[j]	82.21	present study
	DIS values		0.16	0.16	0.06	

[a]Data taken from ref 15. All shifts were converted from external CS_2 taken as 126.1 ppm to *p*-dioxane taken as reference at 67.40 ppm. [b]All shifts were converted from external reference Me_4Si to internal *p*-dioxane by adding 0.4 ppm to all reported shifts. [c]Data taken from ref 35. [d]To make all shifts consistent with the present study 1 ppm was added to all the previously reported values. [e]Data taken from ref 9; 0.5 ppm subtracted from all shifts. [f]Shifts were not resolved because of the large spectral width (5000 Hz) and small number of data points used (4K). [g]Data taken from ref 36; 1 ppm subtracted from all previously reported shifts in **8a** and 0.39 ppm from all previously reported shifts in **8b**. [h]Shift values estimated from schematic representation given in ref 21a. [i]Data taken from ref 37. To make all shifts consistent with the present study, 1.1 ppm was added to all the previously reported values. [j]May be reversed, although based on electronegative environments and assignment of fructose;[38] this is the most likely assignment.

The double-headed arrow indicates the assigned shifts that were interchanged. While it was thought in earlier studies[15] that C-3′ was at lower fields than C-5′, it is obvious from the difference in magnitude of these two DIS values (0.16 vs. ~0.00) that the C-5′ designation corresponds to the lower field absorption. Previous assignment[29] of the α-D-lactose spectrum had made no distinction between C-3′ or C-5′ shifts. The DIS spectrum clearly differentiates between the two resonances (DIS, 0.23 and 0.15) and shows that neither of these originally designated shifts corresponds to C-5′. In fact, we find that the originally assigned shift for C-2′ (DIS 0.03) corresponds to C-5′ and those previously[36] assigned to C-3′ and C-5′ corre-

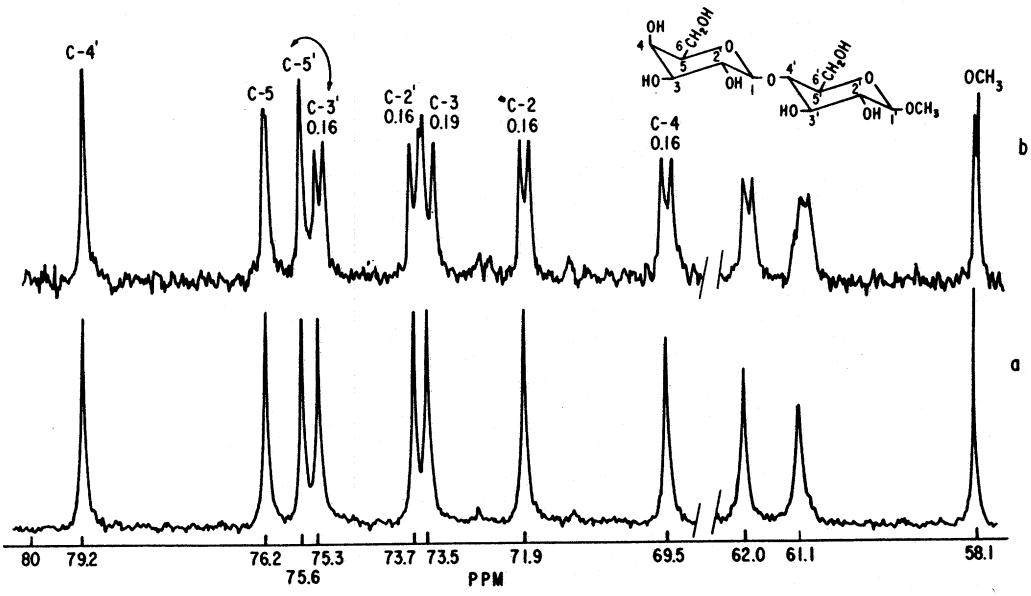
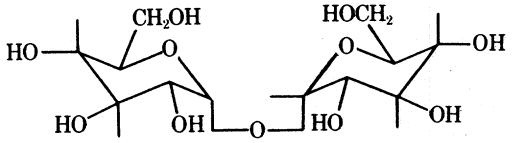
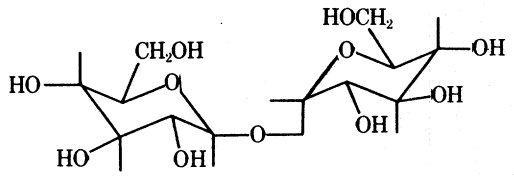


Figure 3. (a) 15.04-MHz proton noise decoupled spectrum of methyl β-D-lactopyranoside (**6a**) in H_2O; (b) DIS spectrum of **6a** taken with a dual coaxial tube containing **6a** in D_2O and H_2O. Each spectrum was obtained at 30 °C after 1000 transients with a displayed spectral width of 200 Hz and 16K data. Double-headed arrow indicates reversal of previous assignments.

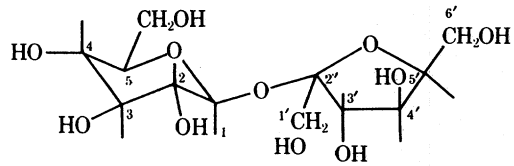
spond to C-3′ and C-2′. Reexamination of α,β-D-cellobiose **7b** and **7c** yielded assignment reversals on C-3, C-5, C-3′, and C-5′ of the α anomer (Table IV).[9] Also a better resolved spectrum (1000-Hz sweep width, 16K data vs. 5000-Hz sweep width and 4K data of the previous study[9]) allowed for separation of resonances corresponding to C-2′ and C-5′ of the β anomer and C-4′ and C-4′ of the α and β anomers, respectively. Both α,α-trehalose **8** and sucrose **9** show similar assignment dis-



8a



8b



9

crepancies in their respective ^{13}C pyranoside ring resonances, C-2, C-3, and C-5 under DIS examination. Although earlier studies[15,30,31] have relied on analogies for making these assignments, a recent report[21a] has indicated that the assignment of the C-3 resonance could be made on the basis of its sluggish rate of deuteration in the presence of Raney nickel. Our results show that the C-2 and C-3 resonance assignments for **8** and **9** should be interchanged (Table IV) from those reported in his latter study.[21a] Thus, it appears that C-2 may be the more sluggishly exchanging carbon. Figure 4 shows the DIS spectrum of sucrose **9** with the double-headed arrows indicating the shifts which have been transposed. Besides the reassignment described above, an interchange in resonances corresponding to C-4′ and C-5′ of the furanoside ring is also indicated (Table IV). We have used the previously established pyranose DIS parameters (Table I) to calculate the furanoside isotope shifts in sucrose **9**. These values are in excellent agreement with the observed isotope shifts (standard deviation 0.01 ppm) (Table III). Interchange of the C-5′ resonance with either the C-3′ or C-4′ is evident from an inspection of the DIS values. Although we cannot distinguish between C-3′ or C-4′ by the DIS method (both values are 0.16 ppm) (Table IV), it is obvious, based on previous assignments made for fructose with deuterium labeling,[32] that C-4′ and C-5′ shifts should be interchanged and the C-3′ assignment remain with its present designation. A study of α,β-trehalose **8b** has also uncovered a number of reassignments which are listed in Table IV.

It was of interest to us to examine the effects of the change in OH stereochemistry at carbons other than 4. Consequently, we made a study of the six manno sugars, methyl α,β-D-mannopyranoside, **10a,b**, α,β-D-mannose, **10c,d**, and α,β-L-rhamnose, **10e,f**. The table of chemical shifts and DIS values is available as supplementary material upon request. The chemical shift assignments for each of these materials have been previously assigned unambiguously by isotope labeling methods.[18] Calculated DIS values show good agreement with the observed shifts except for minor deviations exhibited by the C-2 resonance of **10c** and **10e**. Deviations such as these probably reflect differences brought on by a change in C-2 OH stereochemistry and the potential for increased intramolecular hydrogen bonding. Unlike the galacto series (Tables I and II) the C-3 resonances of the present set show no indication of a decrease in DIS as a result of the proximity of a cis hydroxyl group at C-2. Obviously the changes in stereochemistry of OH groups have very subtle and varying effects on the magnitude of the induced shift values. It is therefore unlikely that these small differences will be useful for distinguishing between isomeric structures. A similar conclusion was reached by Gorin and Mazurek[18] in their study on covalently bound axial and equatorial deuteriums.

Chemical shift assignments and DIS values for two furanose–pyranose equilibrating sugars are summarized in Table V. Shift designations corresponding to those established by Angyal and Bethell[32] are given for the three forms of fructose, **11a–c**. These materials are present in a mixture in the ratio of

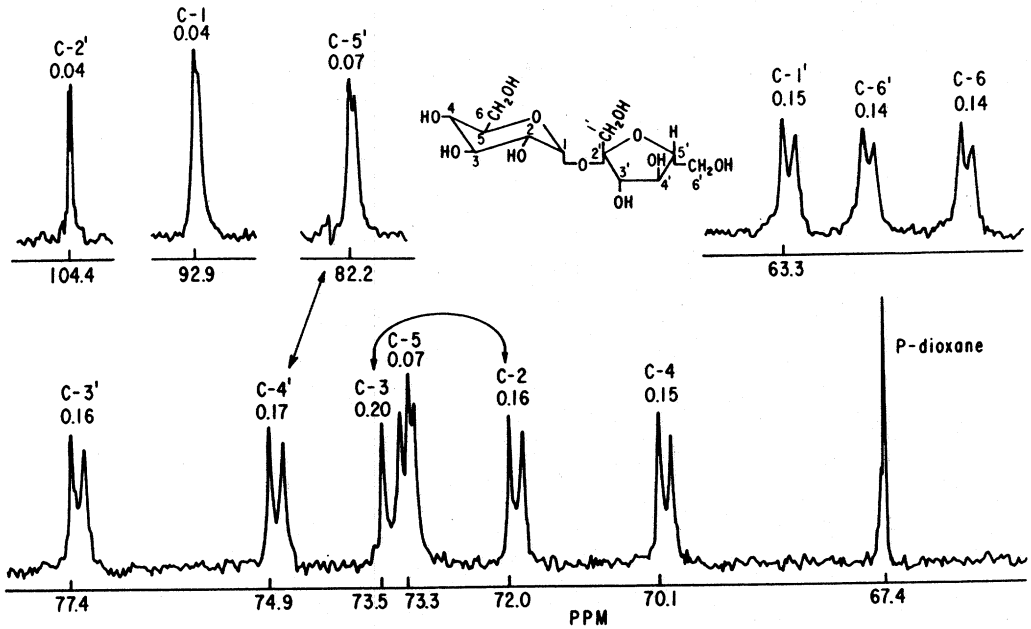
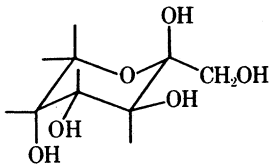


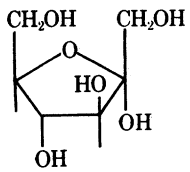
Figure 4. 15.04-MHz DIS proton noise decoupled spectrum of sucrose taken at 30 °C after 1000 transients with a displayed spectral width of 200 Hz and 16K data. Double-headed arrows indicate reversals of previous assignments.

approximately 75:5:20, respectively.[32] Small amounts of two other components were also observed in this mixture. These are presumably the α-pyranose form and possibly an open-chain species. Because of the low concentration of these components they were not examined further. Except for the significantly lower than anticipated DIS values observed for the C-2 resonances of **11a–c,** all isotope shifts gave good agreement



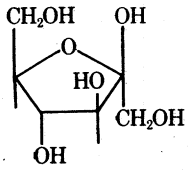
β-D-fructopyranose

11a



α-D-fructofuranose

11b

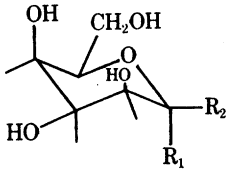


β-D-fructofuranose

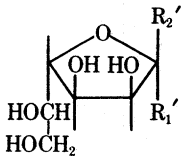
11c

(standard deviation 0.01 ppm) when compared with the calculated values derived from the parameters in Table II. Furthermore, the large difference in the DIS for C-1 and C-6 of the β-pyranose form **11a** makes clear the distinction between these closely separated resonances, thus confirming the results of Angyal and Bethell's isotope labeling assignment experiments.[32] The sharp differences in isotope shifts for C-4 and C-5 in **11b** and **11c** are in good agreement with the corresponding isotope shift differences found above for sucrose (see Tables III and IV). The ^{13}C spectrum of D-talose contains resonances ascribable to the α- and β-pyranose **12a** and **12b** and the α- and β-furanose forms **12c** and **12d.** Although a previous[33] ^{13}C study described only the three forms **12a,b,d** and a single shift of the α-anomeric carbon of **12c,** we observed all four anomeric components in the ratio of approximately 4:3:2:1 in accordance with the results of earlier ^{1}H NMR studies.[34] All but one of the 24 ^{13}C chemical shifts of this equilibrium mixture are found in Table V. Shift assignments are based on three criteria: (1) relative amount of component in the mixture (intensity of resonance), (2) resonance field position, and (3) DIS values. Aside from reporting the unreported shifts corresponding to **12c,** we propose two reassignments from the previous study.[33] Based on the established composition[34] and previous information about the ^{13}C field positions of α- and β-anomeric carbon resonances of furanoses,[35] we have exchanged the assignments for the C-1 resonances of **12c** and **12d.** Also, we designate C-4 of **12c** for the shift previously ascribed to C-3 of **12d** on the basis of intensity (integrated area) and the small magnitude of its exhibited DIS (Table V). The identity of resonances C-2 and C-3 in **12a** and **12b** is still in question, since the observed DIS values reported for the C-3 absorptions as assigned in Table VI are considerably lower than predicted. It is conceivable that this lower value is attributable to a diminished γ shift directed from two adjacent cis OH groups, analogous to the smaller γ shifts induced from the cis vs. the trans anomeric hydroxyl group mentioned earlier. Verification of this hypothesis will await more detailed studies.

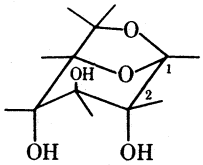
The DIS method can be useful for elucidating the structural features and substitution patterns of various carbohydrate derivatives. Table IV gives a compilation of data for a few selected examples of modified carbohydrate structures. The shift assignments for 1,6-anhydro-D-glucose **13,**[20] with the exception



12a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH



c, R_1' = OH; R_2' = H
d, R_1' = H; R_2' = OH



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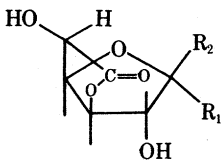


14a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH

Table V. Chemical Shift Assignments and Differential Isotope Shift (DIS) Values, Observed (Calculated), for Pyranose–Furanose Equilibrating Sugars

compd	chemical shifts[a] and DIS, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
fructose[b]						
β-pyranose **11a**	64.91	98.89	68.57	70.68	70.16	64.24
	0.18	0.12	0.18	0.19	0.16	0.06
	(0.18)	(0.17)	(0.20)	(0.20)	(0.17)	(0.03)
α-furanose **11b**	63.94	105.23	82.96	77.02	82.16	62.08
	c	0.10	0.22	0.18	0.07	0.13
	(0.18)	(0.17)	(0.23)	(0.17)	(0.06)	(0.15)
β-furanose **11c**	63.71	102.31	76.37	75.41	81.51	63.34
	0.18	0.11	0.17	0.15	0.06	0.14
	(0.18)	(0.17)	(0.17)	(0.17)	(0.06)	(0.15)
talose[d]						
α-pyranose **12a**	95.47	71.68[e]	70.59[e]	66.03	72.03	62.41
	0.14	0.20	0.16	0.16	0.06	0.15
	(0.14)	(0.20)	(0.20)	(0.17)	(0.07)	(0.15)
β-pyranose **12b**	94.97	72.50[f]	69.59[f]	69.36	76.47	62.17
	0.13	0.20	0.16	0.17	0.07	0.16
	(0.14)	(0.23)	(0.20)	(0.17)	(0.06)	(0.15)
α-furanose **12c**	101.75	76.11	72.73	82.69	71.55	63.71
	0.16	0.18	0.16	0.05	0.18	0.19
	(0.14)	(0.20)	(0.17)	(0.06)	(0.17)	(0.18)
β-furanose **12d**	97.34	71.55	71.97	83.25	*c*	63.82
	0.13	0.18	0.15	0.06		0.18
	(0.14)	(0.17)	(0.17)	(0.05)		(0.18)

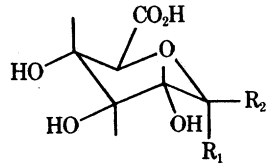
[a]Chemical shifts are reported in H_2O relative to internal *p*-dioxane taken as 67.40 ppm. [b]The standard deviation between calculated and observed DIS values, excluding the C_2 resonances in **11a–c**, was 0.008 ppm. [c]Not resolved. [d]The standard deviation between calculated and observed DIS values was 0.009. [e]These assignments may be interchanged. [f]These assignments may be interchanged.

Table VI. ^{13}C Chemical Shifts[a] and Differential Isotope Shifts (DIS) Values for Carbohydrate Derivatives

compd	chemical shifts and DIS, ppm					
	C-1	C-2	C-3	C-4	C-5	C-6
1,6-anhydro-D-glucose **13**	102.19	71.19	73.52	71.81	77.11	66.01
	0.05	0.17	0.21	0.17	0.03	0.00
α-D-glucofuranuronic acid δ-lactone **14a**	99.09	74.79	85.57	76.66	70.36	177.76
	0.13	0.12	0.00	0.00	0.11	0.00
β-D-glucofuranuronic acid δ-lactone **14b**	103.69	74.79	85.60	78.40	70.07	177.92
	0.16	0.14	0.04	0.00	0.11	0.00
α-D-glucopyranuronic acid[b] **15a** pH 1.8	93.17	72.03	73.36	72.42	71.39	172.91
	0.13	0.19	0.20	0.19	0.14	0.245
α-D-glucopyranuronate **15a** pH 7.8	92.94	72.23	73.52	73.00	76.93	176.85
	0.13	0.18	0.18	0.14	0.00	0.03
β-D-glucopyranuronic acid[b] **15b** pH 1.8	96.92	74.70	76.27	72.22	75.40	173.81
	0.13	0.20	0.21	0.19	0.14	0.23
β-D-glucopyranuronic acid **15b** pH 7.8	96.66	74.96	76.50	72.74	72.60	177.62
	0.13	0.20	0.19	0.14	0.00	0.03
methyl 6-*O*-benzoyl-α-D-glucopyranoside **16**	103.18	75.51	77.27	74.06	73.21	67.91
	0.00	0.16	0.20	0.14	0.00	0.00
methyl 6-bromo-4-*O*-benzoyl-α-D-glucopyranoside **17**	100.00	72.37	71.60	69.36	74.20	55.40
	0.00	0.15	0.12	0.00	0.00	0.00

[a]All shifts were referenced to internal standard *p*-dioxane at 67.40 ppm. [b]Shifts were assigned in accordance with ref 18 and confirmed by the CH_2OH to CO_2H displacements in the glucopyranose series.

of C-2 and C-4, were made in accordance with the expected isotope shifts. The identity of the C-3 resonance is readily apparent from its large DIS, whereas C-5 can be distinguished easily from all the other midfield resonances, i.e., C-2, C-3, and C-4, by its unusually small isotope shift. Since C-6 is isolated from any neighboring hydroxyl groups, it shows no induced shift. Similarly, the DIS spectrum of a 22:78 mixture of α- and β-D-glucofuranuronic acid γ-lactones **14a** and **14b** directly identifies the resonances of carbons 1, 2, and 5 as hydroxyl bearing (Table VI). Resonances corresponding to carbons 3, 4, and 6 undergo little or no isotope shifting and can be easily distinguished from one another by their characteristic field positions. Hydrolysis of **14a** and **14b** introduces a new kind of exchangeable site, the COOH group, into the glucose ring structure. Thus, examination of a 30:70 mixture of α- and β-D-glucopyranuronic acids **15a** and **15b** with the DIS technique at pH 1.8 gives the isotope shift values for a COOD group. As can be seen in Table VI, these β and γ induced shifts are approximately twice as large as those induced from C-OD ($\beta \simeq 0.25$, $\gamma \simeq 0.06$). These larger β values are in good agreement with those previously determined for the $-CO_2H$, $-CO_2D$ equilibrating system in Me_2SO.[23] Also, we observe a small increased δ shift at C-4 as well as an increase in the relaxation times, T_1, of the C=O resonances in D_2O, relative



15a, R_1 = OH; R_2 = H
b, R_1 = H; R_2 = OH

to their corresponding resonances in the H_2O environment (D_2O, **15a,** 21.9 s; H_2O, **15a,** 14.5 s; D_2O, **15b,** 20.4 s; H_2O, **15b,** 13.5 s). This is attributed to a stronger association of the deuterium with the carboxylate moiety which gives rise to a reduction in the dipolar ^{13}C relaxation rate. Raising the pH and pD (pD = pH reading + 0.4) of the systems to 7.8 and 7.4, respectively, allows us to examine the DIS spectrum without the contribution of the COOD isotope shift. It also facilitates the assignment of all other shifts, as described previously.[18] As anticipated, a large decrease in DIS is observed for C=O (~0.24, pH 1.8 to 0.03, pH 7.8), C-5 (0.14, pH 1.8 to 0.00, pH 7.8), and C-4 (0.19, pH 1.8 to 0.14, pH 7.8), while only minor differences were observed for other positions.

In some instances it becomes necessary to examine carbohydrates or other compounds which are only slightly water soluble. Methyl 6-*O*-benzoyl-α-D-glucopyranoside (**16**) and methyl 6-bromo-4-*O*-benzoyl-α-D-glucopyranoside (**17**) are two examples of compounds whose DIS spectra were examined in 75% aqueous and 16% aqueous acetone-d_6, respectively (Table VI). While the magnitude of the DIS values for the former compound **16** agrees well with those found for the parent compound **1a** in 100% aqueous media, the observed values appear to be somewhat lower for the hydroxy-bearing carbons (C-2 and C-5) of **17,** in the acetone-rich solvent system. Regardless of this small decline in isotopic shift, the two resonances representing the nonsubstituted C–OH carbons can be easily differentiated from all others.

Conclusions

While the DIS technique can be a very powerful tool for establishing the identity of carbohydrate ^{13}C resonances, it obviously cannot stand alone. In consort with other strategies it greatly simplifies and shortens the time required to make full structural assignments. This is an important point. Although almost all of the reliable ^{13}C assignment work[16-20] has relied heavily on isotopic labeling, the labeling method is restricted with one exception[36] to monosaccharides, primarily because of the inherent difficulties encountered in specifically labeling polysaccharides. As seen in the present study, DIS can greatly simplify the job of assignment by diminishing the number of unidentified shifts before having to resort to, if necessary, the more lengthy identification techniques.

As the ever-increasing number of sophisticated ^{13}C NMR methods for defining nuclear motion and interaction become more widely used, greater emphasis must be placed on establishing unambiguous resonance assignments. Two recent examples,[3,4] a T_1 and T_2 nuclear Overhauser enhancement (NOE) study, which relied entirely on previous literature results, were found in the present report to be using incorrect shift assignments. Although the misassignments were not necessarily critical to the interpretation of the data in these works, they certainly have the potential of being so in others.

Application of the DIS technique in the area of polysaccharide chemistry is in progress. Metal binding and its influence on proton- and deuterium-induced shifts are also presently being explored. We expect that the DIS will also find utility in the structural elucidation of other carbohydrates and noncarbohydrate-derived materials; however, the interpretation of the deuterium-induced shift changes must be approached with caution until empirical correlations are extended to a wider variety of systems and are better understood theoretically.

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Supplementary Material Available: Table of chemical shifts and DIS values for manno sugars (1 page). Ordering information is given on any current masthead page.

References and Notes

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