Fingerprints of Molecular Structure and Hydrogen Bonding Effects in the ^{13}C NMR Spectra of Monosaccharides with Partially Deuterated Hydroxyls[†1]

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Abstract: This paper presents a new NMR approach to structure elucidation of carbohydrates in solution. Examined in detail are the isotopic multiplets in ^{13}C NMR spectra that result from partial deuteration of the hydroxyls for a series of monosaccharides and some of their deoxy and methyl glycoside derivatives in Me_2SO-d_6 solutions. Chemical shift and isotope effect data are presented for the pyranose and furanose forms of aldopentoses, aldohexoses, and ketohexoses. The results show that the magnitude of the γ effect resulting from deuteration of a hydroxyl on a vicinal carbon atom is sensitive to the relative geometric relationship, cis or trans, of the hydroxyls in vicinal diol arrays. Thus, the multiplet pattern for carbons 3 and 4 of the pyranose ring can serve as a fingerprint of molecular structure at the pentopyranose level. The aldopentoses and ketohexoses are amenable to structural analysis by this simple approach. Ambiguity will arise for pairs of aldohexoses related to each other by epimerization at C5. Intramolecular hydrogen bonding between the hydroxyls at C2 and C4 in α-D-talopyranose gives rise to some unusual effects. A mechanism involving isotopic perturbation of the equilibrium between the hydrogen-bonded structures O4-H···O2-H and O2-H···O4-H is suggested as the possible source of these effects. Similarly, the extra splittings observed in the ^{13}C resonance of C3 of β-D-fucofuranose are rationalized in terms of an equilibrium between the hydrogen-bonded structures C5-O5-H···O3-H and C1-O1-H···O3-H. The approach of isotopic multiplets appears to be uniquely suited for the study of such structures.

Carbon-13 NMR spectroscopy is one of the main tools for structure elucidation of carbohydrates in solution.[2-5] The two outstanding features in the ^{13}C NMR spectra of carbohydrates are the anomeric carbon atoms in the 90–110 ppm spectral region and the CH_2OH groups in the 60–64 ppm region. Methine groups bonded to an oxygen resonate in the 65–85 ppm region. Pentoses and hexoses, have, respectively, three or four such atoms. Unfortunately the assignment of their resonances is not always a straightforward task. In fact, some of the assignments in the series of simple monosaccharides are still incomplete,[2] while others are being disputed in the literature.[6-8] Particularly difficult appears to be the assignment of carbons 3 and 5 of hexopyranoses. Thus, e.g., the assignment of these atoms in the reducing end of cellobiose has been the subject of considerable controversy in the literature.[9]

Recently concurrent communications from two laboratories described an approach to spectral assignment of carbohydrates based on the presence of isotopic multiplets in the spectra of Me_2SO solutions of materials with partially deuterated hydroxyls.[10,11] These multiplets are due to upfield deuterium isotope effects on the carbon-13 chemical shifts: 0.09–0.12 ppm for directly bonded hydroxyls (β effect, Δ_β) and 0.07 ppm or less for hydroxyls on vicinal carbons (γ effect, Δ_γ).[8,10-14] Thus, when only part of the molecules are deuterated, the resonance of a hydroxylated carbon is split into a doublet with a spacing of Δ_β. In partially deuterated vicinal diols, arrays of which are a characteristic feature of carbohydrates, the ^{13}C resonance of each hydroxylated carbon atom appears as a quartet with spacings of Δ_β and Δ_γ. For hydroxylated carbon atoms flanked by two hydroxylated carbons the isotopic multiplet can be an octet since the permutation of two isotopes among three positions results in eight isotopic isomers.[10,11] The discovery that in cyclic vicinal diol systems Δ_γ depends on the relative orientation, cis or trans, of the hydroxyls has the potential of rendering the phenomenon of isotopic multiplets as a tool for structure elucidation.[1]

This article reports results on isotopic multiplets in a series of monosaccharides and some of their deoxy and methyl glycoside derivatives. The structural traits emerging from the results show that the multiplet patterns can serve as fingerprints of molecular structure. A detailed discussion of the effects of intramolecular hydrogen bonding is also presented.

Experimental Section

Carbohydrates were obtained from commercial sources. Solutions of ca. 10% w/v in Me_2SO-d_6 were prepared. A calculated amount of D_2O was added to each solution. It was then treated with $CaSO_4$ (nonindicating Drierite) and filtered into the NMR tube. In most cases the H/D ratio was close to unity. Carbon-13 NMR spectra were recorded at 90.56 MHz and 24 ± 1 °C on a Nicolet 360WB NMR spectrometer operating in the pulsed Fourier transform mode. Low-power broad-band proton decoupling was achieved by using the MLEV-64 pulse sequence.[15] When necessary, the spectral resolution was enhanced by using apodization routines supplied by the instrument manufacturer. The central peak of the solvent resonance was used as an internal reference with a chemical shift of 41.105 ppm relative to TSP. Results for more than one anomeric, or ring, form of a given sugar were obtained from the spectrum of a mutarotated mixture (not necessarily at equilibrium).

Results and Discussion

The resonances of carbon atoms in the vicinity of partially deuterated hydroxyl groups exhibit multiplicities analogous to those due to spin–spin couplings.[1,10] Five general types of isotopic multiplets have been demonstrated.[10,11] The most complex multiplet structures occur for hydroxylated carbon atoms flanked by two hydroxylated carbons. The resonance of such a carbon atom may appear as an octet ($\Delta_\beta > \Delta_\gamma > \Delta_{\gamma'}$), septet ($\Delta_\beta = \Delta_\gamma$

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Table I. Carbon-13 Chemical Shifts[a] and Deuterium Isotope Effects[b] for Some Aldopyranoses and Related Derivatives

compound	C1	C2	C3	C4	C5	C6
β-D-ribopyranose, **1a**	96.06	73.50	70.03	69.65	64.88	
	104, 14	104, 57, 22	109, 12, 12	*c*	26	
β-D-allopyranose, **1b**	95.62	73.56	73.08	69.33	75.76	63.17
	103, 23	107, 68, 19	107, 27, 27	107, 17	30, 30	117
α-D-talopyranose, **2**	96.28	73.71	66.68	71.36	72.67	62.18
	104, −13	103, 38, 19, 19[d]	107, 19, 19	102, 27, 14[d]	30, *c*	115
methyl β-D-arabinopyranoside, **3b**	102.22	70.23	70.66	69.88	64.49[e]	
	13	110, 36	107, 41, 16	111, 19	35	
α-L-arabinopyranose, **4a**	99.03	73.57	74.49	69.44	66.80	
	114, 23	106, 72, 36	103, 47, 16	*f*	29	
β-L-arabinopyranose, **4b**	94.45	71.19	71.01	69.32	64.35	
	99, *c*	114, 37, 37	109, 44, 15	115, 15	35	
α-D-fucopyranose, **4c**	94.26	70.16	71.23	73.54	66.84	18.35
	97, 10	109, 40, 40	108, 44, 15	114, 18	21	
β-D-fucopyranose, **4d**	98.94	73.52	75.31	72.91	71.35	18.47
	106, 21	*f*	107, 46, 16	110, 16	22	
α-D-galactopyranose, **4e**	94.22	70.43	71.10	70.59	72.02	62.27
	97, 12	108, 40, 40	106, 44, 13	109, 21	26, 26	117
α-D-glucopyranose, **5a**	93.83	73.99	74.70	72.21	73.58	62.84
	97, *c*	108, 39, 39	99, 43, 43	104, 36	23, 23	117
β-D-glucopyranose, **5b**	98.50	76.45	78.38	71.91	78.35	62.84
	104, 17	101, 67, 34	100, 45, 45	100, 31	23, 23	117
methyl α-D-glucopyranoside, **5c**	101.37	73.72	75.15	72.08	74.29	62.75[g]
	17	107, 35	102, 44, 44	104, 36	25, 25	117
methyl β-D-glucopyranoside, **5d**	105.52	75.02	78.28	71.71	78.45	62.72[h]
	19	100, 34	102, 46, 46	103, 36	25, 25	115
α-D-xylopyranose, **5e**	94.19	74.02	74.88	71.85	63.22	
	98, 10	107, 39, 39	105, 45, 45	107, 38	27	
α-D-lyxopyranose, **7a**	96.03	72.35	72.66	69.08	64.73	
	103, 24	107, 52, 18	109, 45, 21	106, 40	33	
α-D-mannopyranose, **7b**	95.61	73.05	72.23	69.01	74.81	63.17
	102, 25	107, 40, 15	110, 45, 20	104, 40	26, 26	116
methyl α-D-mannopyranoside, **7c**	102.54	71.79	72.62	68.62	75.46	62.88[i]
	24	105, 18	107, 43, 22	106, 42	26, 26	117
α-L-rhamnopyranose, **8**	95.77	73.27	72.16	74.15	69.46	19.79
	103, 26	109, 34, 20	108, 45, 20	108, 41	27	

[a] In ppm downfield from TSP. [b] Upfield shifts (spacings of multiplets) in ppb ± 3. [c] Line(s) too broad. [d] Long-range effect (see text). [e] Methyl at 56.43 ppm. [f] Obscured by more intense signals. [g] Methyl at 56.10 ppm. [h] Methyl at 57.63 ppm is a doublet with a 18-ppb spacing. [i] Methyl at 55.54 ppm.

Table II. Carbon-13 Chemical Shifts[a] and Deuterium Isotope Effects[b] for Some Ketoses

compound	C1	C2	C3	C4	C5	C6
β-D-fructopyranose, **3a**	66.08	99.74	69.51	71.62	70.92	64.79
	117, 47	81, *c*	106, 33, 33	107, 41, 10	112, 18	36
α-L-sorbopyranose, **6**	65.68	99.23	72.32	75.98	71.82	63.72
	106, 46	83	103, 27, 27	107, 46, 46	105, 37	28
α-D-tagatopyranose, **7d**	66.18	99.47	72.28	73.06	68.31	64.48
	118, 51	76	115, 35, 15	111, 49, 13	108, 42	28
α-D-fructofuranose, **11a**	65.44	105.84	84.62	77.41	82.60	62.79
	108, 51	86, 10, 10	104, 71, 48	107, 45	33, 33	103
β-D-fructofuranose, **11b**	64.65	103.68	77.50	77.03	83.57	64.65
	112, 37	79, 12, 12	~100, *c*	105, 53	31, 31	98

[a] In ppm downfield from TSP. [b] Upfield shifts (spacings of multiplets) in ppb ± 3. [c] Broad line(s).

Table III. Summary of γ-Isotope Effects in Vicinal Diol Arrays of Pyranoses

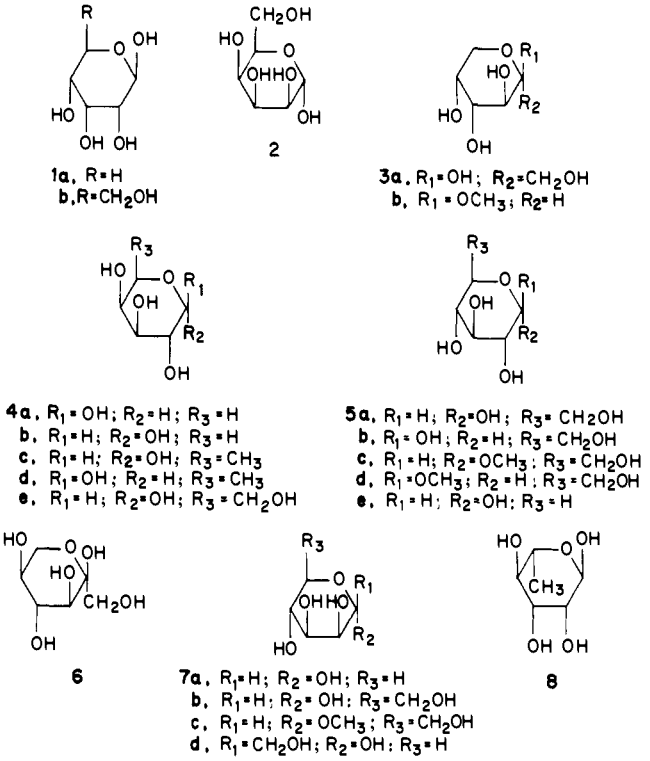
affected C, by OH on C	trans range, ppb	cis, range ppb
Δ_γ(C1,2)[a]	14–26[b]	0–14
Δ_γ(C2,1)[a]	34–72[c]	37–40
Δ_γ(C2,3)[d]	27–40	15–22
Δ_γ(C3,2)[d]	41–47	12–27
Δ_γ(C3,4)[d]	43–49	0–27
Δ_γ(C4,3)[d]	31–42	14–21

[a] Excluding ketoses. [b] Excluding α-D-talopyranose (**2**). [c] The range is 34–52 ppb for diaxial and 57–72 ppb for diequatorial conformations of the hydroxyls. [d] In ketopyranoses carbons 3, 4, and 5 correspond to 2, 3, and 4 of aldopyranoses.

+ $\Delta_{\gamma'}$; $\Delta_\gamma > \Delta_{\gamma'}$), sextet ($\Delta_\beta > \Delta_\gamma + \Delta_{\gamma'}$; $\Delta_\gamma = \Delta_{\gamma'}$), or quintet ($\Delta_\beta = \Delta_\gamma + \Delta_{\gamma'}$; $\Delta_\gamma = \Delta_{\gamma'}$), depending on the relatiosnhip between the magnitude of the isotope effects. Thus, the multiplet structure should be apparent from the relative values of the isotope effects tabulated in this paper (two entries are given even when $\Delta_\gamma = \Delta_{\gamma'}$).

Pyranoses. The chemical shift and isotope effect data for the 18 aldopyranoses studied are summarized in Table I. The data for the three ketopyranoses (along with those for two ketofuranoses) can be found in Table II. This separation was deemed convenient since the enumeration of the ring carbons in ketopyranoses starts with C2 for the anomeric carbon and also since the latter is quaternary. A summary of the ranges of the γ effects for individual atoms of the pyranose ring is given in Table III.

An examination of the data reveals a number of important structural trends. In general the magnitudes of the isotope effects, both Δ_β and Δ_γ, depend on the degree of substitution of the carbon atom, decreasing in the order primary > secondary > tertiary > quaternary. Thus, e.g., for the anomeric carbon, Δ_β(C2) of ketoses is in the range 76–86 ppb, whereas in aldoses Δ_β(C1) is in the range 96–114 ppb. Other relationships emerging from the data are related to vicinal interactions. As noted earlier,[1] the values of Δ_γ in vicinal diol systems are not necessarily the same for both carbon atoms. The following inequalities hold for Δ_γ (trans) and



to a lesser extent for Δ_γ (cis):

$$\Delta_\gamma(C2,1) > \Delta_\gamma(C1,2) \quad (1)$$

$$\Delta_\gamma(C3,2) > \Delta_\gamma(C2,3) \quad (2)$$

$$\Delta_\gamma(C3,4) > \Delta_\gamma(C4,3) \quad (3)$$

One of the most important structural features of the isotope effects is the cis–trans relationship of Δ_γ, viz.

$$\Delta_\gamma(\text{trans}) > \Delta_\gamma(\text{cis}) \quad (4)$$

In order to establish structural traits in the isotopic multiplets, it is instructive to consider the spectral appearance of each individual carbon atom of the pyranose ring. In the following discussion the conventional numbering of the aldopyranoses is used.

C1. Examples of C1 resonances of pyranoses with partially deuterated hydroxyls are shown in Figure 1. The spectral band is usually a doublet with a spacing (Δ_β) of 106 ± 9 ppb in aldopyranoses and 81 ± 5 ppb (for C2) in ketopyranoses. Each of the doublet components may exhibit an additional splitting of up to 26 ppb due to the γ effect. For a cis relationship of the hydroxyls on C1 and C2, Δ_γ(C1,2) is often too small to cause an observable splitting. Also, no additional splitting is observed for the doublet of the quaternary anomeric carbon of ketopyranoses (Figure 1D). The cis–trans difference for Δ_γ seems to persist in the methyl aldosides. Thus, in the absence of complicating factors, the magnitude of the γ effect at C1 can be used as an indicator of the relative configuration at this carbon atom.

The only downfield isotope effect observed in this work was found with Δ_γ(C1) of α-D-talopyranose (**2**). This anomaly is probably due to the particular spatial disposition of the OH bond of the hydroxyl on C2, arising from the intramolecular hydrogen bond with the hydroxyl on C4. Other unusual spectral consequences observed with α-D-talopyranose can also be attributed to this hydrogen bond. They are discussed in detail later on.

C2. Examples of isotopic multiplets for C2 of pyranoses are shown in Figure 1 alongside those for C1. Carbon 2 is part of the C2–C3–C4 moiety, which is the key structural feature of the pyranose ring and distinguishes one monosaccharide from another. However, the spectral appearance of C2 is gretaly influenced by the configuration at the anomeric carbon as well. The spectral type[10] of C2 is $\beta\gamma\gamma'$, indicating a β effect and two γ effects. C2 can be an octet, a septet, or a sextet (vide supra) depending upon the relationship between the magnitudes of Δ_β, Δ_γ, and $\Delta_{\gamma'}$. The

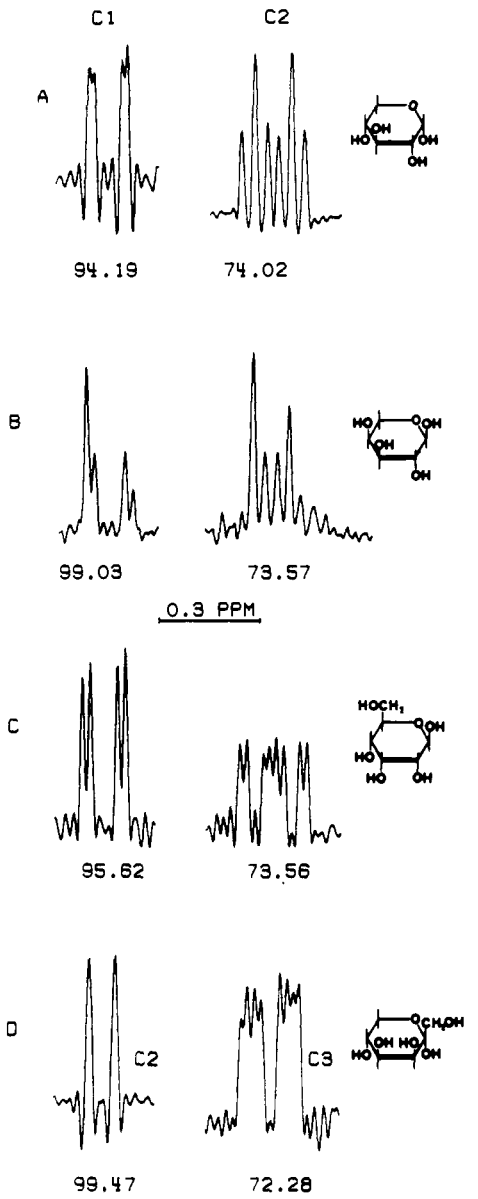


Figure 1. Isotopic multiplets in the ^{13}C resonances of C1 and C2 of pyranoses with partially deuterated hydroxyls: (A) α-D-xylopyranose (**5e**); (B) α-L-arabinopyranose (**4a**); (C) β-D-allopyranose (**1b**); (D) C2 and C3 of the ketose α-D-tagatopyranose (**7d**).

values of Δ_β(C2) span the narrow range of 107 ± 7 ppb. Thus, it is the relationship between Δ_γ(C2,1) and Δ_γ(C2,3) which dominates the spectral appearance. Note that $\Delta_\gamma(C2,1)_{cis} \approx \Delta_\gamma(C2,3)_{trans}$. As a result, for a cis–trans configuration centered at C2, the multiplet will be of the degenerate $\beta\gamma_2$ type. This is a sextet (doublet of triplets) as shown in Figure 1A for the C2 resonance of α-D-xylopyranose (**5e**). For a trans–trans configuration, $\Delta_\gamma(C2,1) + \Delta_\gamma(C2,3) \approx \Delta_\beta$ and the resonance is a septet as shown in Figure 1B for C2 of α-L-arabinopyranose (**4a**). Note that, for the sample of Figure 1B, the H/D ratio was much different than unity (ca. 2.0), and, as a result, the relative intensities of the multiplet components are different than expected for the simpler multiplets of spin–spin coupling. A calculation[10,11] with H/D = 2.0 shows that the relative intensities of the C1 quartet should be 4:2:2:1, and those of the C2 septet should be 8:4:4:6:2:2:1. The experimental intensities in Figure 1B are in good agreement with these estimates. For a trans–cis configuration, $\Delta_\gamma(C2,1)_{trans} > \Delta_\gamma(C2,3)_{cis}$ and an octet (double doublet of doublets) is observed as shown in Figure 1C,D for the C2 resonance of β-D-allopyranose (**1b**) and the C3 resonance of α-D-tagatopyranose (**7d**). It should be pointed out that for a trans-diaxial conformation Δ_γ(C2,1) is smaller than that for a

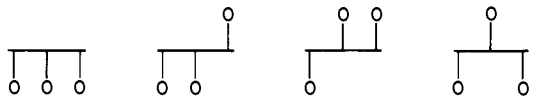
Table IV. Carbon-13 Chemical Shifts[a] and Deuterium Isotope Effects[b] for Some Aldofuranoses

compound	C1	C2	C3	C4	C5	C6
β-D-ribofuranose, **9**	103.04	77.13	72.28	84.48	64.71	
	107, 36	108, 47, 13	107, *c*	58, 28	106	
α-D-talofuranose, **10**	102.85	77.16	71.82	83.30	72.49	64.64
	99, 36	109, 43, 11	106, 11	54, 13	102, 32	117, 33
α-L-arabinofuranose, **12a**	103.58	84.55	78.46	84.72	63.38	
	114, 30	104, 49, 49	105, 42	35, *c*	114	
β-L-arabinofuranose, **12b**	97.44	78.94	76.94	84.43	64.87	
	99, 12	105, 36, 36	101, 54	*d*	110	
α-D-fucofuranose, **12c**	96.99	79.34	76.88	87.36	69.11	21.40
	96, *c*	108, 48, 29	103, 52	*d*	95	57
β-D-fucofuranose, **12d**	103.29	84.62	78.64	87.39	67.87	21.54
	111, 28	101, 51, 51	105, 39, 15[e]	43, 25	106	62
β-D-galactofuranose, **12e**	103.30	84.18	77.73	82.87	73.77	64.53
	113, 26	100, 55, 48	100, 40	*c*	110, 44	111, 36

[a] In ppm downfield from TSP. [b] Upfield shifts (spacings of multiplets) in ppb ± 3. [c] Line(s) too broad. [d] Obscured by more intense signals. [e] Long-range effect (see text).

trans-diequatorial conformation and has values similar to and even smaller than those of $\Delta_\gamma(C2,1)_{cis}$. Thus, e.g., $\Delta_\gamma(C2,1)$ is 38 ppb for α-D-talopyranose (**2**) but is 40 ppb for α-D-galactopyranose (**4e**), which is "obtained" by epimerization at C2. This anomaly has been observed earlier,[1] when comparing α-L-rhamnopyranose (**8**) ($\Delta_\gamma(C2,1)_{trans}$ = 34 ppb) and α-D-fucopyranose (**4c**) ($\Delta_\gamma(C2,1)_{cis}$ = 40 ppb), and also by Pfeffer et al.[8] in relation to the relatively small isotope shifts at C2 of α-D-mannopyranose (**7b**) and α-L-rhamnopyranose (**8**). Note also that the γ effect due to a tertiary hydroxyl, such as the one on the anomeric carbon of ketopyranoses, is usually smaller than that due to a similarly situated secondary hydroxyl. The complicating effect of the interaction with the anomeric hydroxyl is removed upon glycosidation. It is important to note that this has little or no effect on the value of $\Delta_\gamma(C2,3)$.

C3 and C4. Examples of the isotopic multiplets observed for carbons 3 and 4 of pyranoses are shown in Figure 2. Carbon 3 is flanked by two hydroxylated carbons and its resonance is either an octet or a sextet. With one hydroxyl on a vicinal carbon, the resonance of C4 is a doublet of doublets. The large spacings in these multiplets, i.e., the values of Δ_β span the relatively narrow ranges of 107 ± 4 and 109 ± 6 ppb for C3 and C4, respectively. As will become clear from the following discussion, the cis–trans effect on the value of Δ_γ renders the *multiplet pattern of C3 and C4 as a fingerprint of monosaccharide structure.*



Carbon 3 is at the pivotal point of the pyranose ring. In the *ribo* structure (**1** and **2**) C3 is at the center of a cis–cis array of hydroxyls. In such a case $\Delta_\gamma(C3,2)_{cis} = \Delta_\gamma(C3,4)_{cis}$ and the multiplet is a doublet of triplets (sextet) as shown in Figure 2A for β-D-allopyranose (**1b**). Carbon 4 is a doublet of doublets with relatively small spacings. In the *arabino* structure (**3** and **4**) the configuration at C3 is trans–cis. Since $\Delta_\gamma(C3,2)_{trans} > \Delta_\gamma(C3,4)_{cis}$, C3 is now an octet, while C4 is still a doublet of closely spaced doublets. This case is depicted in Figure 2B with the C3 and C4 resonances of α-D-galactopyrnose (**4e**). Also shown in Figure 2B is the isotopic multiplet of C2, which is adjacent to that of C4. The markedly different multiplet structures of C2 and C4 permit a ready spectral assignment of these two closely spaced resonances (0.16 ppm between the protio forms). In the *lyxo* structure (**7** and **8**) the configuration at C3 is reversed to trans–cis. The multiplet of C3 remains similar in its appearance to that for the *arabino* structure. However, the C4 multiplet exhibits now the larger spacings due to $\Delta_\gamma(C4,3)_{trans}$. This situation is illustrated in Figure 2C with the C3 and C4 resonances of α-D-lyxopyranose (**7a**). The *xylo* structure (**5** and **6**) has a trans–trans configuration at C3. Both multiplets exhibit now larger spacings. As an example, shown in Figure 2D are the C3 and C4 resonances of α-D-xylopyranose (**5e**). Note that here, as well as for the *ribo* structure, $\Delta_\gamma(C3,2) = \Delta_\gamma(C3,4)$.

Table V. Summary of γ-Isotope Effects in Vicinal Diol Arrays of Furanoses

affected C, by OH on C	trans range, ppb	cis, ppb
$\Delta_\gamma(C1,2)$[a]	26–36	12
$\Delta_\gamma(C2,1)$[a]	43–71	29, 36
$\Delta_\gamma(C2,3)$[b]	36–50	11, 13
$\Delta_\gamma(C3,2)$[b]	39–54	11

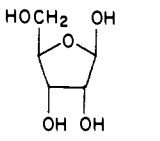
[a] Excluding ketoses. [b] In ketofuranoses carbons 3 and 4 correspond to carbons 2 and 3 of aldofuranoses.

It is noteworthy that, because $\Delta_\gamma(C2,3)_{cis} \approx \Delta_\gamma(C4,3)_{cis}$ and $\Delta_\gamma(C2,3)_{trans} \approx \Delta_\gamma(C4,3)_{trans}$, the multiplets of C2 and C4 in glycosides will be identical twins. In such cases and in the absence of other means of spectral assignment, it will be impossible to distinguish between the symmetry-related *arabino* and *lyxo* structures.

C5. Carbon 5 lacks a directly attached hydroxyl and therefore can be readily identified in the ^{13}C NMR spectra of pyranoses with partially deuterated hydroxyls. Its resonance is a closely spaced doublet for aldopentoses, 6-deoxyaldohexoses, and ketohexoses (C6) and a triplet for aldohexoses (except for α-D-talopyranose, vide infra). The magnitude of the γ effect at C5 is in the range 28 ± 7 ppb. No structural trends are apparent in these values.

C6. Carbon 6, hydroxylated itself but lacking hydroxylated neighbors, appears as a widely spaced doublet. The values of Δ_β are very constant at 116 ± 1 ppb and are larger than the values for tertiary and quaternary carbons.

Furanoses. The chemical shift and isotope effect data for the seven aldofuranoses examined in this work are summarized in Table IV. The data for the two ketofuranoses are in Table II.



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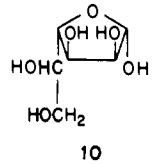

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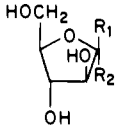
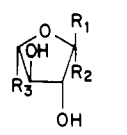


11a, R_1 = CH_2OH; R_2 = OH
b, R_1 = OH; R_2 = CH_2OH



12a, R_1 = OH; R_2 = H; R_3 = CH_2OH
b, R_1 = H; R_2 = OH; R_3 = CH_2OH
c, R_1 = H; R_2 = OH; R_3 = $CHOHCH_3$
d, R_1 = OH; R_2 = H; R_3 = $CHOHCH_3$
e, R_1 = OH; R_2 = H; R_3 = $CHOHCH_2OH$

A summary of the γ effects for individual carbon atoms of the furanose ring is presented in Table V. The effect of cis–trans isomerism on the values of Δ_γ (see eq 4) is manifested in the

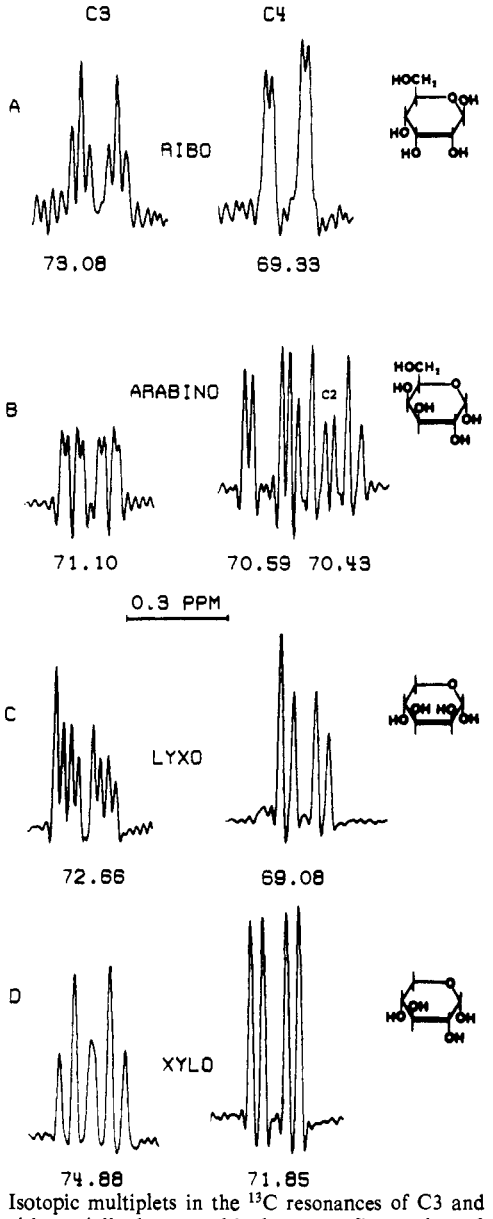


Figure 2. Isotopic multiplets in the ^{13}C resonances of C3 and C4 of pyranoses with partially deuterated hydroxyls as fingerprints of monosaccharide structure: (A) β-D-allopyranose (**1b**); (B) α-D-galactopyranose (**4e**), with C2 also shown; (C) α-D-lyxopyranose (**7a**); (D) α-D-xylopyranose (**5e**).

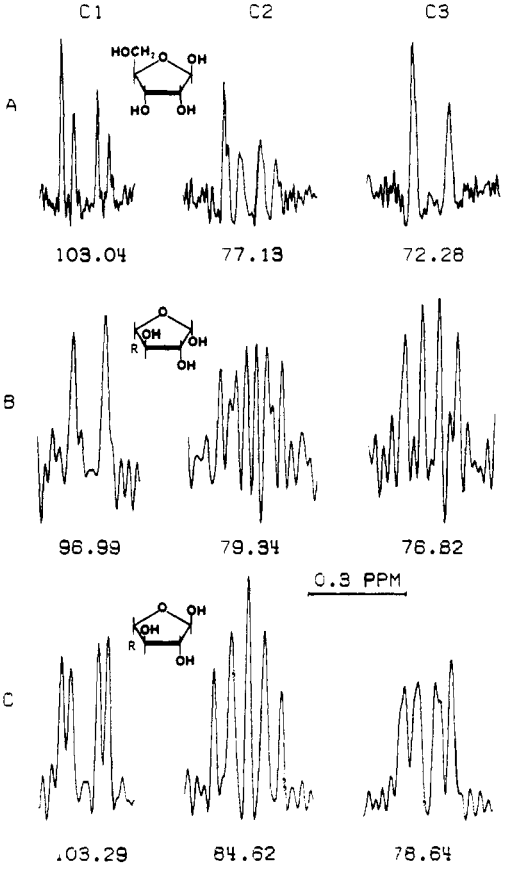


Figure 3. Isotopic multiplets in the ^{13}C resonances of C1, C2, and C3 of furanoses with partially deuterated hydroxyls: (A) β-D-ribofuranose (**9**); (B) α-D-fucofuranose (**12c**), R = $CHOHCH_3$; (C) β-D-fucofuranose (**12d**), R = $CHOHCH_3$.

furanose data as well. Examples of the isotopic multiplets for carbons 1, 2, and 3 of furanoses are shown in Figure 3. One can determine the relative configuration of the two fused diol fragments (on C1 and C2, and C2 and C3) from the magnitude of the Δ_γ splittings in a fashion similar to that described above for the pyranoses.

C1. The inequality of the γ effect on C1 and C2 (see eq 1) is found here as well. In addition, a comparison with the pyranose data shows that

$$\Delta_\gamma(C1,2)_{fur} > \Delta_\gamma(C1,2)_{pyr} \quad (5)$$

Thus, for the quaternary anomeric carbon of ketoses (C2), the small splittings due to Δ_γ are observed in the furanose form but not in the pyranose form. The inequality of eq 5 should be useful for the assignment of anomeric carbons of ketoses.

C2. The isotopic multiplets for C2 of aldofuranoses are very similar to those of the pyranoses. A quintet is observed for C2 of β-D-fucofuranose (**12d**) (Figure 3C), where $\Delta_\gamma + \Delta_{\gamma'} \approx \Delta_\beta$ and $\Delta_\gamma = \Delta_{\gamma'}$.

C3. In aldofuranoses, the oxygen atom on C4 is part of the ring. As a result C3 has only one hydroxylated neighbor (C2) and its isotopic multiplet is usually a doublet of doublets, similar to C4 of pyranoses. For β-D-ribofuranose (**9**), however, the small splitting due to Δ_γ(C3,2) could not be revealed even with resolution enhancement (Figure 3A). On the other hand, extra splittings are observed for β-D-fucofuranose (**12d**) (Figure 3C). The origin of this effect is discussed in the next section.

C4. The hydroxylic environment of C4 in aldofuranoses is analogous to that of C5 in pyranoses. In the aldofuranoses one finds $\Delta_\gamma(C4,3) = \Delta_\gamma(C4,5)$. However, an assessment of the precise origin of the γ effect at C4 could not be made on the basis of the present data.

The remaining carbon atoms are exocyclic. Note that the methyls of α- and β-fucofuranose (**12c,d**) exhibit the relatively large γ effects (57 and 62 ppb, respectively) appropriate for primary carbons. Also noteworthy is the appearance as a βγ quartet of the C6 resonance of aldohexofuranoses.

Hydrogen Bonding Effects. The multiplicity, magnitude, and direction of splitting of some of the ^{13}C resonances of α-D-talopyranose (**2**) with partially deuterated hydroxyls were found to be unusual. The isotopic multiplets of **2** are shown in Figure 4. The assignments indicated in the figure (see also Table I) are in agreement with those of Angyal and Tran.[7] For comparison, also shown in Figure 4 is the normal multiplet structure observed for α-D-mannopyranose (**7b**), which is epimeric with **2** at C4. The structural formula of **2** was drawn to indicate the intramolecular hydrogen bond C2–O2–H···O_4–H. This hydrogen bond is known from the crystal structure.[16] For solutions, the alternative C4–O4–H···O2–H must also be considered. The condition for such hydrogen bonding is axial hydroxyl groups on alternate carbons. Of the 21 pyranoses examined in this work only **2** satisfies this condition. The three unusual effects believed to be a consequence of intramolecular hydrogen bonding are (i) extra splittings on the

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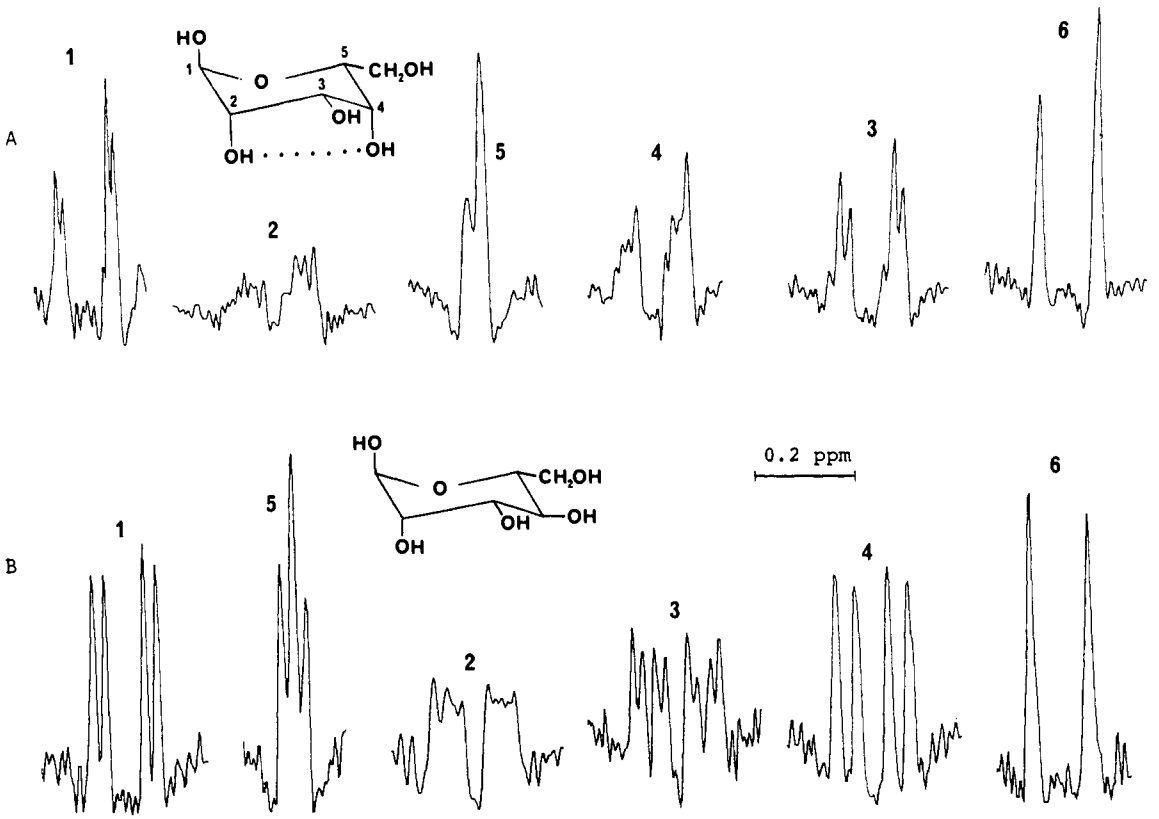

Figure 4. Carbon-13 NMR spectra (resolution enhanced) showing isotopic multiplets of (A) α-D-talopyranose (**2**) and (B) α-D-mannopyranose (**7b**). Note that, for convenience, the structural formulas have been rotated.

resonances of C2 and C4, (ii) Δ_γ(C5,4) smaller than the line width resulting in a doublet for C5, and (iii), as mentioned above, a negative Δ_γ(C1,2). The last two effects can be explained by a dihedral angular dependence of the isotope shift, since a particular spatial orientation of the O–H bond will be brought about by the intramolecular hydrogen bond. Similar effects have been observed in hydrocarbon fragments.[17] Influences of intramolecular hydrogen bonding on the deuterium isotope effects in the ^{13}C NMR spectra of oligosaccharides have been observed by Bock and Lemieux[18] and by Christofides and Davies.[11] Both groups have assumed that this is an effect transmitted through a hydrogen bond of a given and fixed spatial orientation. However, as will be shown below, an alternative mechanism is also possible.

The two general types of deuterium isotope effects in ^{13}C NMR spectroscopy are the "intrinsic" shift, which involves a single species, and the "equilibrium" isotope shift caused by perturbations of the relative populations of several equilibrating species.[19,20] Intrinsic isotope shifts have usually been discussed in terms of contributions from intramolecular electric field effects, changes in bond hybridization, and isotopic perturbation of the thermal distribution of molecules over excited vibrational states.[21] The latter two mechanisms stipulate transmittal of the isotope effect through the relatively rigid framework of covalent bonds. Only the short-range electric field effects operate through space. The importance of spatial proximity of interacting atoms in causing longer range (in terms of chemical bonds) isotope shifts has been recognized.[17,20] Note, however, that for the observation of isotope effects transmitted through intramolecular hydrogen bonds, the lifetime of these bonds must be at least 0.3 s (for a shift of 1 Hz). Such long lifetimes seem to be rather improbable, in particular when several hydrogen bonded structures are possible. On the other hand, the observation of shifts arising from the isotopic perturbation of chemical equilibria does not rely on the magnitude of rate constants.

As isotope effect will be observed if there is an isotopic perturbation on the rapid equilibrium of the type

$$\underset{a}{\text{C4–O4–H···O2–H}} \overset{K}{\rightleftharpoons} \underset{b}{\text{C2–O2–H···O4–H}} \qquad (6)$$

provided the chemical shift of the carbon of interest is different in a and b. The term "flip-flop hydrogen bond" has been coined to describe equilibrium situations of this kind.[22] The presence of such equilibria in the crystalline hydrates of β-cyclodextrin has been inferred from neutron diffraction results, indicating extremely short (~1 Å) distances between half-occupied hydrogen atom sites.[22] The probability of occurrence of flip-flop hydrogen bonds should be even higher in solutions, where the participating hydroxyls are free from the constraints and rigidity of the crystalline state. The average resonance position of a carbon nucleus, the chemical shift of which is influenced by the equilibrium of eq 6, is given by

$$\delta = f_a\delta_a + f_b\delta_b \qquad (7)$$

where f_a and f_b are the corresponding mole fractions. With the equilibrium constant defined as $K = f_a/f_b$ one obtains

$$\delta = (K\delta_a + \delta_b)/(1 + K) \qquad (8)$$

The effect of isotopic substitution at one of the hydroxyls will be to modify the equilibrium constant by a factor k, the magnitude of the equilibrium isotope effect. The average resonance position becomes now

$$\delta_D = (kK\delta_a + \delta_b)/(1 + kK) \qquad (9)$$

The isotope effect on the chemical shift is defined as

$$\Delta = \delta - \delta_D \qquad (10)$$

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Substituting eq 8 and 9 into eq 10 one obtains

$$\Delta = K(k - 1)(\delta_b - \delta_a)/[(1 + K)(1 + kK)] \quad (11)$$

It can be shown that a maximum in Δ is otained when $K = k^{-1/2}$. Since k is expected to be close to unity, this means that a maximum isotope effect on the chemical shift will be observed when the equilibrium constant itself is close to unity. In such a case

$$\Delta \approx (k - 1)(\delta_b - \delta_a)/4 \quad (12)$$

Assuming $k = 1.01$ and $\Delta = 0.02$ ppm, one obtains $\delta_b - \delta_a = 8$ ppm, a value within the known limits of hydrogen bonding effects on ^{13}C chemical shifts.[23]

It has been suggested that flip-flop bridges are entropically favored over normal hydrogen bonds because the two states a and b (see eq 6) are energetically almost equivalent.[22] This will lead to equilibrium constants close to unity, i.e., to the condition of maximum probability for the observation of the appropriate effects in the isotopic multiplets. However, with a practical resolution limit of 0.01 ppm, one must also have $\delta_b - \delta_a > 4$ ppm in order to observe these effects. Thus, if this latter requirement is not met, additional splittings due to the flip-flop hydrogen bonds may not be observed in the isotopic multiplets.

The extra splittings in the isotopic multiplet of C3 of β-D-fucofuranose (**12d**) (see Figure 3C) also seem to be due to an equilibrium between two hydrogen bonded structures. A hydrogen bonding interaction with the anomeric hydroxyl, viz. C1–O1–H···O3–H, is quite probable. However, according to a large body of crystallographic evidence regarding the participation in hydrogen bonds of hydroxyls attached to anomeric carbons, the occurrence of the reversed bond, C3–O3–H···O1–H, seems to be rather unlikely.[24] Thus, a flip-flop hydrogen bond can be ruled out. Note also that no extra splittings are observed for β-D-galactofuranose (**12e**), in which a cis configuration of hydroxyls at C1 and C3 is also present. Another possibility for hydrogen bonding interaction in **12d** is C5–O5–H···O3-H. In this case, however, a similar effect should have been observed for the α-anomer, α-D-fucofuranose (**12c**). One is thus led to the conclusion that the most likely origin of the extra splittings in the resonance C3 of **12d** is an equilibrium of the type

$$\text{C1–O1–H···O3–H} \rightleftarrows \text{C5–O5–H···O3–H} \quad (13)$$

In this scheme a pivotal motion about the C3–O3 bond is required, which is analogous to the one for a flip-flop bridge involving O3–H.

Molecular Structure from Isotopic Multiplets. Finally, it is instructive to attempt a structural interpretation of a spectrum with isotopic multiplets. The spectrum of α-mannopyranose (**7b**) given in Figure 4B will be used for this purpose. The interpretation proceeds along the following sequence of logical steps:

(i) The signal at the highest field, a methylene, is a widely spaced doublet and not a doublet of doublets nor a closely spaced doublet. This indicates either an aldopyranose or a ketofuranose. The latter is ruled out by the absence of signals (e.g., a doublet of doublets for C5) at about 65 ppm.

(ii) The large γ effect on the anomeric carbon, which resonates at the lowest field, indicates a trans configuration of the hydroxyls at C1 and C2.

(iii) Carbons 5 and 4 are easily identified from their multiplcities: a triplet and a doublet of doublets, respectively. The relatively large γ effect on the latter indicates a trans configuration of the hydroxyls at C4 and C3.

(iv) The remaining two resonances are due to C2 and C3. Both multiplets exhibit a larger and a smaller γ effect. Since the resonances of C1 and C4 have already indicated that C2 and C3 are parts of *trans*-diol systems, the smaller γ effect indicates a cis configuration of the hydroxyls on these two carbons.

(v) In summary, a trans–cis–trans array of four hydroxyls is clearly identified from the pattern of isotopic multiplets. Only the structures of α-mannopyranose (**7b**) and β-gulopyranose are consistent with this arrangement of hydroxyl groups. These two sugars are related to each other by epimerization at C5.

Conclusions

The pattern of isotopic multiplets in the ^{13}C NMR spectra of monosaccharides with partially deuterated hydroxyls is governed by the mutual geometric relationship, cis or trans, of vicinal hydroxyl groups. As a result, the sequence of such groups on a pyranose (or a furanose) ring can be established from the multiplet pattern by mere examination. Thus, molecular structure at the pentopyranose level can be readily determined. The aldopentoses and ketohexoses are amenable to such analysis. In the aldohexose series, pairs of sugars related to each other by epimerization at C5 (e.g., mannose and gulose) will give rise to similar multiplet patterns and will be indistinguishable by this simple approach. Rapidly equilibrating hydrogen-bonded structures give rise to extra splittings and to other unusual effects. Thus, the approach of isotopic multiplets appears to be uniquely suited for the study of such structures.

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Registry No. 1a, 7296-60-8; **1b**, 7283-09-2; **2**, 7282-81-7; **3a**, 7660-25-5; **3b**, 5328-63-2; **4a**, 7296-55-1; **4b**, 7296-56-2; **4c**, 6189-71-5; **4d**, 28161-52-6; **4e**, 3646-73-9; **5a**, 492-62-6; **5b**, 492-61-5; **5c**, 97-30-3; **5d**, 709-50-2; **5e**, 6763-34-4; **6**, 470-15-5; **7a**, 608-46-8; **7b**, 7296-15-3; **7c**, 617-04-9; **7d**, 512-20-9; **8**, 6014-42-2; **9**, 36468-53-8; **10**, 51076-04-1; **11a**, 10489-79-9; **11b**, 470-23-5; **12a**, 38029-69-5; **12b**, 20074-49-1; **12c**, 91443-98-0; **12d**, 91443-99-1; **12e**, 7045-51-4.

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