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EFFECTS OF CARBOHYDRATE-STRUCTURE CHANGES ON INDUCED SHIFTS IN DIFFERENTIAL ISOTOPE-SHIFT ^{13}C-N.M.R.*

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ABSTRACT

Deuterium-induced, ^{13}C-isotope shifts are shown to vary considerably from the initially predicted values calculated for ordinary pyranose and furanose sugars, when minor structural changes are introduced into the carbohydrate ring. Both substitution of C-O*H* groups or reduction of C-*O*H to CH_2 permitted the evaluation of γ effects of OD without the contribution of β-OD-induced shifting. The observed γ-shift values for these modified structures were twice as large as those previously noted. This difference is most probably due to favored solvation. Substitution of O*H* at C-6 led to the predicted loss of differential isotope-shift (d.i.s.) at C-6 because of its isolation from all β and γ O*D* groups. The ^{31}P resonances of D-glucose 6-phosphate show downfield deuterium shifts. Based on d.i.s. values, new ^{13}C-shift assignments are proposed for isomaltose and 2-amino-2-deoxy-α-D-glucose. A study of acidic carbohydrates has demonstrated that isotope shifts are somewhat larger for sp^2-hybridized carbon atoms whose OH groups are acidic. Relaxation times for sp^2 carbon atoms isolated from dipolar interaction with protons were very long in D_2O relative to their relaxation time in the H_2O environment.

INTRODUCTION

Deuterium has been known for some time to be able to induce chemical shifts of ^{13}C resonance signals through rapidly exchangeable OD groups in dimethyl sulfoxide $(CH_3)_2SO$ (ref. 3) or $CDCl_3$ (ref. 4), or through such slowly exchangeable sites as N–D in D_2O (ref. 5) as well as in $(CH_3)_2SO$ (ref. 6). In a recent report[1], we described a new technique we call differential-isotope shift (d.i.s.) ^{13}C n.m.r., which facilitates the simultaneous measurement of deuterium-induced ^{13}C shifts associated with rapidly exchangeable OD and OH groups in D_2O and H_2O environments. At about the same time as our initial disclosure[7] of this method, a report describing a similar phenomenon appeared[8]. The principle of our method relies on

*Deuterium-Induced, Differential Isotope-Shift ^{13}C-N.M.R., Part 2. For Part 1, see ref. 1. For a preliminary report, see ref. 2.
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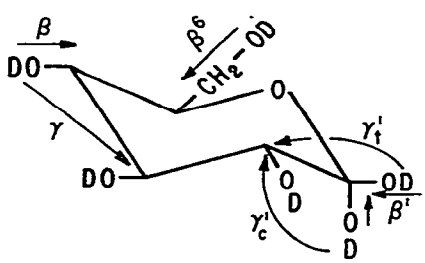
the ability of an OD group to induce a predictable upfield shift on the natural-abundance resonances of directly β- and γ-disposed, ^{13}C carbon atoms[6]. Simultaneous examination (by use of dual n.m.r. cells) of the spectra of carbohydrates in both D_2O and H_2O environments (d.i.s. spectra) furnishes the values of the isotope shifts and thereby the assignments of the ^{13}C resonances. Table I gives the corresponding shifts and d.i.s. values for each compound. The d.i.s. is defined as ($\delta H_2O - \delta D_2O$). There is a clear additivity for all β-induced (directly bound OD), as well as γ-induced shifts[1]. In our earlier study[1], we evaluated the additivity of the β- and γ-positioned OD group on the degree of upfield ^{13}C shifting and the magnetic-susceptibility contributions to the magnitude of these displacements. We concluded from the linear-regression analysis of shift data from twelve unambiguously assigned carbohydrate spectra that β and γ parameters could be established. These parameters were in turn used to predict the d.i.s. spectra for carbohydrates whose shifts were not firmly established[1]. Fig. 1 is a schematic representation of the defined ^2H-isotope-shift parameters and their designated contributions, in p.p.m., to the perturbed resonance-positions. New resonance-assignments for eight common mono- and di-saccharides have been readily delineated by appropriate application of the calculated d.i.s. values[1]. The standard deviation between the calculated and observed d.i.s. was less than 0.02 p.p.m. Differences in longitudinal relaxation-times (T_1) and nuclear Overhauser enhancement (n.O.e.) of ^{13}C signals derived from the D_2O and H_2O solutions were insignificant, as seen by careful comparison of integrated areas. Concentration differences (up to 20%) likewise showed little or no effect on the d.i.s. phenomenon.

To establish the generality of this technique for the assignment of resonance positions as well as for examination of the effects of solvation, we undertook a study of various substituted and modified carbohydrate molecules. The present paper describes the results of these experiments, with an emphasis on the observed deviations of the induced shifts from the previously established "predicted" values. Furthermore, the effects of concentration and relaxation differences found in specific systems are illustrated.



$\beta = 0.14$ $\gamma = 0.03$
$\beta' = 0.11$ $\gamma'_t = 0.06$
$\beta^6 = 0.15$ $\gamma'_c = 0.03$

Fig. 1. Induced-shift contributions.

TABLE I

CHEMICAL-SHIFT ASSIGNMENTS AND CORRESPONDING D.I.S. VALUES FOR SUBSTITUTED CARBOHYDRATES

Compound	*Anomer*	*Chemical shift and d.i.s. (p.p.m.)*[a]											
		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-glucopyranoside (**1**)								100.02 (0.01)	72.20 (0.15)	74.11 (0.20)	70.55 (0.15)	72.46 (0.07)	61.57 (0.15)
Isomaltose	**2a** α	99.40 (0.00)	73.10 (0.23)	74.70 (0.24)	71.10 0.18	73.30 0.07	62.10 0.16	93.70 (0.17)	73.10 (0.21)	74.71 (0.23)	71.01 (0.18)	71.62 (0.07)	67.20 (0.00)
	2a β	99.40 (0.00)	73.10 (0.23)	74.70 (0.24)	71.10 0.18	73.30 0.07	62.10 0.16	97.61 (0.17)	75.60 (0.23)	77.62 (0.23)	71.05 (0.18)	75.80 (0.07)	67.21 (0.00)
2-Amino-2-deoxy-D-glucopyranose	**6a** α							89.99 (0.22)	55.47 0.34	70.62 0.27	70.55 (0.15)	72.44 (0.10)	61.32 (0.17)
	6b β							93.57 (0.22)	57.92 (0.37)	72.98 (0.26)	70.62 (0.15)	76.96 (0.08)	61.46 (0.14)
3-Deoxy-D-*ribo*-hexopyranose[b]	**7a** α							91.79 (0.15)	67.44 (0.17)	34.69 (0.16)	65.18 (0.17)	73.05 (0.08)	61.63 (0.17)
	7b β							98.78 (0.15)	69.68 (0.19)	39.27 (0.17)	65.32 (0.14)	82.83 (0.07)	61.87 0.16
3-Deoxy-D-*ribo*-hexofuranose	**7c** α							97.35 (0.15)	73.85 (0.15)	31.92 (0.07)	77.57 (0.00)	71.59 (0.14)	63.45 (0.19)
3-Deoxy-D-*ribo*-hexofuranose	**7d** β							102.61 (0.18)	76.53 (0.17)	33.65 (0.07)	79.96 (0.05)	73.94 (0.14)	63.81 (0.20)
L-Ascorbic acid (**13**), pH = 2.5								174.05 (0.12)	118.72 (0.17)	156.51 (0.32)	77.07 (0.10)	69.89 (0.20)	63.08 (0.20)
Sodium L-ascorbate, pH = 6.5								178.00 (0.00)	114.08 (0.14)	176.15 (0.13)	79.20 (0.03)	70.50 (0.16)	63.6 (0.20)

[a]All shifts are relative to internal 1,4-dioxane referenced as 67.40 p.p.m. Primed numbers refer to the non-reducing sugar in disaccharides. D.i.s. values in parentheses. [b]Spectra obtained from a mixture of 51% β-pyranose, 26% α-pyranose, 17% β-furanose, and 6% α-furanose.

EXPERIMENTAL

Natural-abundance ^{13}C-n.m.r. spectra were obtained at 30° with a Jeol FX 60-Q spectrometer* operating at 15.04 MHz with proton-noise decoupling. Differential spectra (Fig. 1) were obtained with a coaxial, dual cell purchased from Wilmad Glass Co. The dimensions of the tubes were: outer tube o.d. = 10.00 mm, i.d. = 8.96 mm; and inner tube (55-mm lower section) o.d. = 6.82 mm, i.d. = 5.81 mm. The inner tube contained 100 mg of sample dissolved in 1 mL of H_2O containing 1% of 1,4-dioxane. The outer tube contained the same amount of material dissolved in 1 mL of D_2O. The D_2O-dissolved sample was exchanged three times with D_2O before the spectra were recorded. Except for compounds **13** and **14**, 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 one drop of commercial 1,4-dioxane to each solution lowered the pH and pD values to 3.0 and 3.6, respectively. When the pH of the H_2O solution was adjusted with 0.1M sodium hydroxide to correspond to the D_2O solution, pH = pD = 3.6, the d.i.s. values varied from the unadjusted solution with a mean of 0.006 and an average standard-deviation of 0.005 p.p.m. For the acidic sugars **13** and **14**, the hydrogen-ion concentration was carefully adjusted at each pH, that is, pD = observed pH meter reading +0.4. 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, rounded off to the nearest 0.01 p.p.m. and were measured relative to the internal standard (1,4-dioxane) assigned a shift of 67.40 p.p.m. relative to external tetramethylsilane in D_2O solution. Line widths for typical monosaccharides were ~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 n.m.r. 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 p.p.m. Five independently exchanged samples were also recorded consecutively, and the d.i.s. values had a standard error of the mean of 0.004 p.p.m.

The observed d.i.s., that is, the difference 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 twelve D-gluco- and D-galacto-pyranoses and -pyranosides was used to calculate the isotope-shift parameters. Each of these empirically observed d.i.s. values was set equal to the sum of all its β and γ parameters that contribute to the induced shift. A linear-regression analysis of these equations led to a solution that could be readily fitted to the experi-

*Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

mental data. The calculated d.i.s. values showed good agreement with the observed values, having a standard error of estimate of 0.009 p.p.m.

Relaxation times (T_1) were measured by the inversion-recovery 180° — τ — 90° method[9,10]. The standard error for these experiments was ~7%. N.O.e. values were established by comparing spectra of samples in both D_2O and H_2O independently. Values for n.O.e. were given as the ratio of the integrated area obtained for each line with full proton-decoupling to the integrated area of each corresponding line obtained by gated decoupling (proton irradiation only during r.f. pulsing and acquisition). A delay time (T) of approximately three T_1 values was used, as a pulse angle of 30° was employed throughout. The reported values were reproducible to within ±8%.

PREPARATIONS

2-Deoxy-D-*erythro*-pentose (**10**) and 3-deoxy-D-*ribo*-hexose (**11**) were prepared by published procedures[11,12]. The corresponding glycoside of **11** was prepared by the method of Hughes *et al.*[13].

RESULTS AND DISCUSSION

The d.i.s. method is particularly useful for discerning and elucidating changes in substitution patterns in carbohydrates. We have shown[1], for example, that glycosylation gives a predictable decrease in isotope shift of the resonance of the glycosylated carbon atom commensurate with the loss of a β-OD group. Thus, the ^{13}C resonances of 4-linked disaccharides exhibit d.i.s. values of 0.03 p.p.m., whereas the corresponding resonances of the monosaccharides are shifted by 0.16 p.p.m. Similarly, glycosylation of C-6, as in isomaltose (**2**), produces a marked lowering in the d.i.s. for the C-6 resonance consistent with this carbon atom's lack of interaction with directly bound or adjacent OD groups. In particular, the d.i.s. readily distinguish between the C-5α and C-4α resonances, whose assignments were previously not distinguishable[14]. Here it is evident that the resonance at δ 71.62, with an isotope shift of 0.07, and not the resonance at δ 71.01 (d.i.s. = 0.18), must correspond to the C-5α carbon atom. Also, the C-6′ resonance is clearly observed as two lines in the d.i.s. spectrum, with an isotope shift of 0.16 p.p.m., consistent with its free OD group.

The d.i.s. spectra in the shift-insensitive regions[15] of pH 8 and 3 of sugars having such ionizable groups at C-6 as phosphate and sulfate show no induced shift at C-6 in the ionic state at pH 8. The same is true of the C-1 resonance of α-D-glucosyl phosphate. At pH 3.0, only a minimal indication of an isotope shift at C-6 (0.03 p.p.m.) for the 6-phosphate is found. This is not surprising, as the deuterium atom of the P-OD group is 4 bonds removed from C-6. A large *downfield* deuterium-induced shift, (of 0.12 p.p.m.) is observed, however, for the ^{31}P resonance. Such a deuterium-induced shift on a ^{31}P resonance has never been reported before this study. The ^{13}C spectrum of D-glucose 6-sulfate could not be examined in the lower pH range because of its rapid decomposition.

Alkylation or acylation of OH sites other than at C-6 diminishes the d.i.s., although the decrease is not so large as predicted by calculations based on the previously established shift-parameters[1]. Calculation based on the values found in Fig. 2 predicts an induced shift of a substituted C–O adjacent to two hydroxyl-bearing carbon atoms to be $(2 \times \gamma)$ or $2 \times 0.03 = 0.06$ p.p.m. Alkylation or acylation at either C-2 or C-3 (Table II) produces d.i.s. values for these carbon resonances twice as large as expected (~ 0.12 p.p.m.). In contrast, the carbon atoms adjacent to the substituted ones have d.i.s. values in accord with prediction. Similarly, such C-1 substituted sugars as methyl glycosides show C-1 d.i.s. resonances having diminished values relative to their corresponding reducing-sugar counterparts, which are consistent with our calculations (Table I and ref. 1). The unusually large upfield shift observed for the CH resonances in compounds **3**, **4**, and **5** appears to be strictly characteristic of the specific atomic arrangement. A much smaller induced-shift is seen on such intervening methine carbon atoms as C-5 of aldohexoses[1,3a]. We cannot yet explain this dichotomy except to say that a difference in steric factors in these two arrangements may be responsible. Substitution of an amino for an OH group at C-2, as in 2-amino-2-deoxy-D-glucopyranose (**6**), gives additional shifting because of the multiple exchangeable sites for deuterium ($ND_3^+Cl^-$). Therefore, rapid identification of both the C-2 (d.i.s. = 0.34) and its adjacent C-3 (d.i.s. = 0.27) resonances is possible without resorting to decoupling or labeling experiments. More importantly,

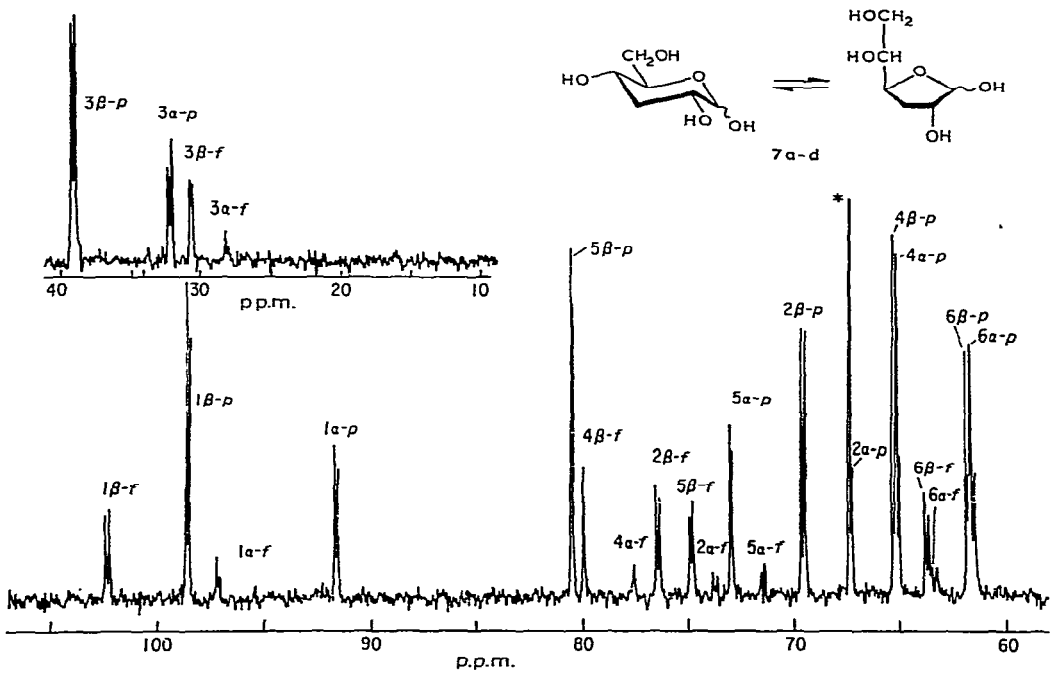


Fig. 2. Proton-noise decoupled, 15.04-MHz, d.i.s. spectrum of 3-deoxy-D-*ribo*-hexopyranose **7a–d**. Spectrum obtained with 170-mg samples of **7**, each in 1 mL of H_2O and D_2O, after 1,000 transients by use of 8K data points. The displayed spectral-width is 750 Hz.

TABLE II

OBSERVED AND CALCULATED[a] D.I.S. VALUES OF 2- AND 3-SUBSTITUTED GLUCOPYRANOSES

Compound	*Anomer*	*D.i.s. (p.p.m.)*[b]			
		C-1	*C-2*	*C-3*	*C-4*
2-Amino-2-deoxy-D-glucopyranose hydrochloride (pH = 2.4)	6a α	0.22	0.34	0.27	
	6b β	0.22		0.26	
3-*O*-Methyl-D-glucopyranose	3a α		0.18	0.12	0.13
			(0.17)	(0.06)	(0.14)
	3b β		0.18	0.12	0.12
			(0.20)	(0.06)	(0.14)
Nigerose (1→3 linkage)	4a α		0.13	0.12	0.13
			(0.17)	(0.06)	(0.14)
	4b β		0.15	0.13	0.13
			(0.20)	(0.06)	(0.14)
2,6-Di-*O*-(3-nitropropanoyl)-D-glucopyranose)[c]	5a α		0.13	0.17	
			(0.06)	(0.17)	
	5b β		0.15	0.17	
			(0.09)	(0.17)	
D-Glucose	α	0.15	0.20	0.20	0.15
		(0.17)	(0.20)	(0.23)	(0.17)
	β	0.15	0.21	0.20	0.15
		(0.17)	(0.23)	(0.23)	(0.17)

[a]Calculated from previously established parameter-set for glycopyranose sugars[1]. D.i.s. values in parentheses. [b]Each value is the average of three determinations having a standard error of the mean of 0.004 p.p.m. [c]Compound pre-exchanged in D_2O–acetone and spectra taken in 16% acetone–D_2O and 16% acetone–H_2O.

closeness of the chemical shifts for compound **6a** indicates that it would be difficult to differentiate readily between the C-3 (δ 70.62) and the C-4 (δ 70.55) resonances without the aid of the isotope shift (Table I). Previous assignment of comparable shifts in 2-acetamido-2-deoxy-α-D-glucopyranose could be established only with the aid of specific deuterium isotope-labeling[16]. Table I gives the full spectral assignments, d.i.s. values, and shift positions for the α and β forms of compound **6**.

In order to understand more fully and evaluate the isotope effect of OD groups on adjacent carbon resonances (γ shifts), we examined the d.i.s. spectra of several deoxy sugars. These compounds were considered especially good models, as the γ shifts on the methylene carbon atoms could be measured without the interference of other bonded alkyl or oxygen atoms. Also, many of these deoxy sugars, existing in both the pyranose as well as the furanose form, provided an opportunity to examine γ shifts in many structurally different environments. Table I contains the shift assignments and d.i.s. values for one of the model sugars, 3-deoxy-D-*ribo*-hexopyranose **7a–d** (α and β pyranose and furanose forms). Consistent with the foregoing observations reported for 2- and 3-substituted sugars, the C-3 resonances of the pyranose forms, **7a** and **7b**, are isotopically shifted upfield 2.6 times as much as predicted based

on previously evaluated γ-OD effects[1]. The spectra of the corresponding furanose forms, **7c** and **7d**, consistently yield smaller d.i.s. (0.07 p.p.m.) because of the loss of the C-4 OD group upon ring contraction of **7a** and **7b**. Fig. 2 presents the complete d.i.s. spectrum of **7a–d** and assignments for each of the 24 resonances in structures **7a–d** as depicted above the spectrum. Table III lists the d.i.s. values of deoxy carbon resonances observed for other deoxy sugars studied in various tautomeric and anomeric forms. Except for 3-deoxy-D-*ribo*-hexose (**7**), the d.i.s. of the methylene carbon atoms were 0.05–0.06 p.p.m. smaller in the furanose relative to the pyranose tautomers. Methylation of 1-OH also diminished the value of the CH_2 d.i.s. in 2-deoxy-D-*arabino*-hexose (methyl α- and β-glycosides, **9a** and **9b**) to 0.04–0.05 p.p.m. All other isotope-shift values for the remaining carbon atoms in sugars **8–12** were in the normal range, as predicted. Large d.i.s. values observed for intervening methylene-group resonances are not restricted to closed-ring sugar structures only. Examination of the d.i.s. spectrum of a simple 1,3-diol (1,3-propanediol) revealed similarly large (0.16 p.p.m.) γ-isotope shifts for the CH_2 resonance. However, unlike the sugars studied, this simple aliphatic diol also showed apparent changes in d.i.s. values with change in concentration. A plot of the ratio of $[HO(CH_2)_3OH]$ in $H_2O/[HO(CH_2)_3OH]$ in D_2O *vs.* apparent d.i.s. (Fig. 3) shows a linear concentration-dependence of the CH_2 resonance, that is, a downfield shift of the CH_2 resonance in H_2O (relative to the shift in D_2O) occurred with increasing $[HO(CH_2)_3OH]$ in H_2O. The opposite effect was noted when $[HO(CH_2)_3OH]$ was increased in D_2O, namely, a linear decrease in apparent d.i.s. of the CH_2 resonance with increasing $[HO(CH_2)_3OH]$ in D_2O. It is clear that these effects are a result of differing solute–

TABLE III

SUMMARY OF D.I.S. VALUES[a] OF METHYLENE CARBON RESONANCES IN DEOXY SUGARS

Compound	*D.i.s. (CH_2)*	*δ*	*Composition of mixture (%)*
2-Deoxy-α-D-*arabino*-hexopyranose (**8a**)	0.15	38.32	47
2-Deoxy-β-D-*arabino*-hexopyranose (**8b**)	0.16	40.57	53
Methyl 2-deoxy-α-D-*arabino*-hexopyranoside (**9a**)	0.10	37.59	
Methyl 2-deoxy-β-D-*arabino*-hexopyranoside (**9b**)	0.10	39.15	
2-Deoxy-α-D-*erythro*-pentopyranose (**10a**)	0.16	38.51	40
2-Deoxy-β-D-*erythro*-pentopyranose (**10b**)	0.16	39.86	35
2-Deoxy-α-D-*erythro*-pentofuranose (**10c**)	0.10	47.73	13
2-Deoxy-β-D-*erythro*-pentofuranose (**10d**)	0.10	45.90	12
3-Deoxy-α-D-*ribo*-hexopyranose (**11a**)	0.16	34.69	26
3-Deoxy-β-D-*ribo*-hexopyranose (**11b**)	0.16	39.27	51
3-Deoxy-α-D-*ribo*-hexofuranose (**11c**)	0.07	31.92	6
3-Deoxy-β-D-*ribo*-hexofuranose (**11d**)	0.07	33.65	17
2-Deoxy-α-D-*lyxo*-hexopyranose (**12a**)	0.15	33.01	38
2-Deoxy-β-D-*lyxo*-hexopyranose (**12b**)	0.16	35.88	37
2-Deoxy-α-D-*lyxo*-hexofuranose (**12c**)	0.10	42.25	13
2-Deoxy-β-D-*lyxo*-hexofuranose (**12d**)	0.10	42.16	12

[a]Expressed in p.p.m. from internal 1,4-dioxane taken as 67.4 p.p.m.

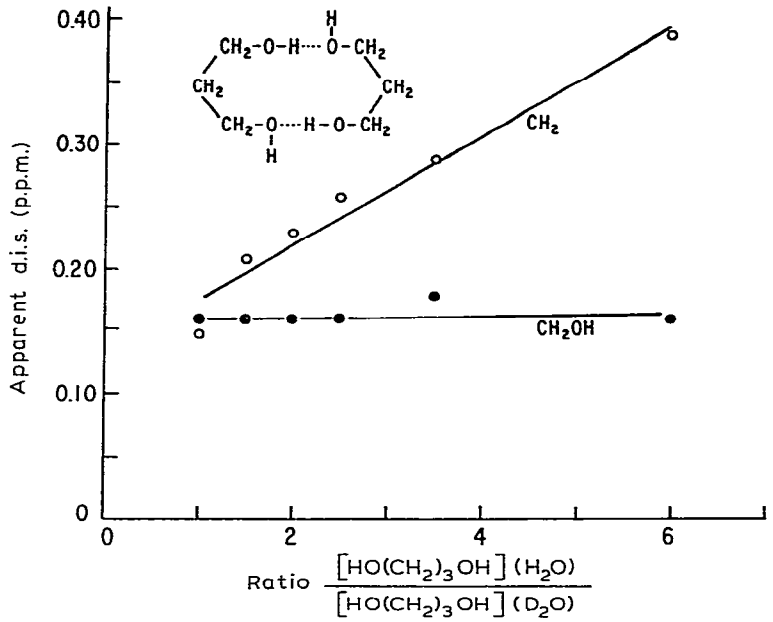
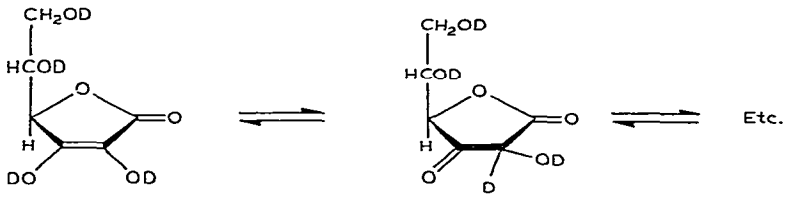


Fig. 3. Plot of apparent d.i.s. at 30° *vs.* ratio of 1,3-propanediol (H_2O)/1,3-propanediol (D_2O).

solute interactions resulting from a variation in concentration in the respective solutions of the two coaxial cells, and are not a consequence of specific D_2O *vs.* H_2O interaction with solute*. It appears that intermolecular hydrogen-bonding between solute molecules has a profound effect on the chemical shift of the methylene carbon resonance, whereas the CH_2OH shifts are essentially unaffected by this phenomenon. Although we have not noted any concentration effects of this magnitude with sugars studied thus far, it is always preferable to make the d.i.s. measurements with concentrations in both cells as close to equal as possible. This can be achieved in the specially constructed cells having equal inside and outside volumes (see Experimental section).

The acidic carbohydrates, such as ascorbic acid (**13**), offer an interesting opportunity to study the effects of isotope-induced shifts on sp^2 C–OD hybridized carbon resonances. We measured the induced deuterium ^{13}C-shifts of the acidic carbohydrates ascorbic acid (**13**) and α-D-glucopyranuronic acid (**14**) in both the ionic and acidic states, keeping in mind that pH-dependent shifts must be eliminated by careful regulation of the H^+ concentration in both tube compartments. Previous ^{13}C-n.m.r. studies of L-ascorbic acid (**13**) have suggested that this molecule exists essentially completely as the enediol reductone (**13***a*) with no detectable amounts of the corresponding keto forms present in solution[17]. Our results are consistent with these conclusions.

*The same shifting-phenomenon can be demonstrated when one solvent (either D_2O or H_2O) is used simultaneously in the inner and outer cells.



13

In earlier experiments in both $(CH_3)_2SO$ (refs. 1, 5) and D_2O–H_2O (ref. 1) solvent-systems, acidic C–OD groups yielded spectra showing relatively large deuterium-induced shifts compared with those of their alcohol C-OH counterparts. Likewise, the C-3 resonance of **13** displayed a large d.i.s. of 0.32 p.p.m. at pH 2.5 (Tables I and IV). A rise in pH to 6.5 removes this perturbation at the resonance corresponding to C-3, as we have observed[1] for compound **14**. Simultaneously, the d.i.s. at C-4 decreases from 0.1 to 0.03 p.p.m., verifying the ionization of 3-OD and the loss of a γ-induced shift from this position. As is obvious from the spectrum (Fig. 4), the intensity of the C-2 resonance emanating from the D_2O $(C\text{-}2)_{(D_2O)}$ solution is much diminished relative to that from the $C\text{-}2_{(H_2O)}$ solution (Table IV) resulting from the isolation of this sp^2 carbon atom from any proximate dipolar interacting protons. Similarly, n.O.e. values at $C\text{-}2_{(H_2O)}$ show a decrease, consistent with loss of neighboring, covalently bound protons. Carbon-13 relaxation had only a modest increase (14 s H_2O to 21 s D_2O), as dipolar interaction may still be obtained from H-4 in either solvent.

TABLE IV

D.I.S., T_1, AND N.O.E. VALUES FOR ACIDIC ^{13}C-OH RESONANCES

Compound	*D.i.s. C-6*[a]	T_1 *(s)*		*N.O.e.*	
		H_2O	*D_2O*	*H_2O*	*D_2O*
α-D-Glucopyranuronic acid (**14**), pH 1.80	0.25	14.4	21.9		
β-D-Glucopyranuronic acid, pH 1.80	0.23	13.5	20.4		
α-D-Glucopyranuronate, pH 7.80	0.03				
β-D-Glucopyranuronate, pH 7.80	0.03				
	C-3				
L-Ascorbic acid (**13**), pH 2.50	0.32	13.2	21.9	2.6	2.6
Sodium L-ascorbate, pH 6.50	0.03				
	C-2				
L-Ascorbic acid pH 2.50	0.17	19.4	66.0	2.8	1.9

[a]P.p.m.

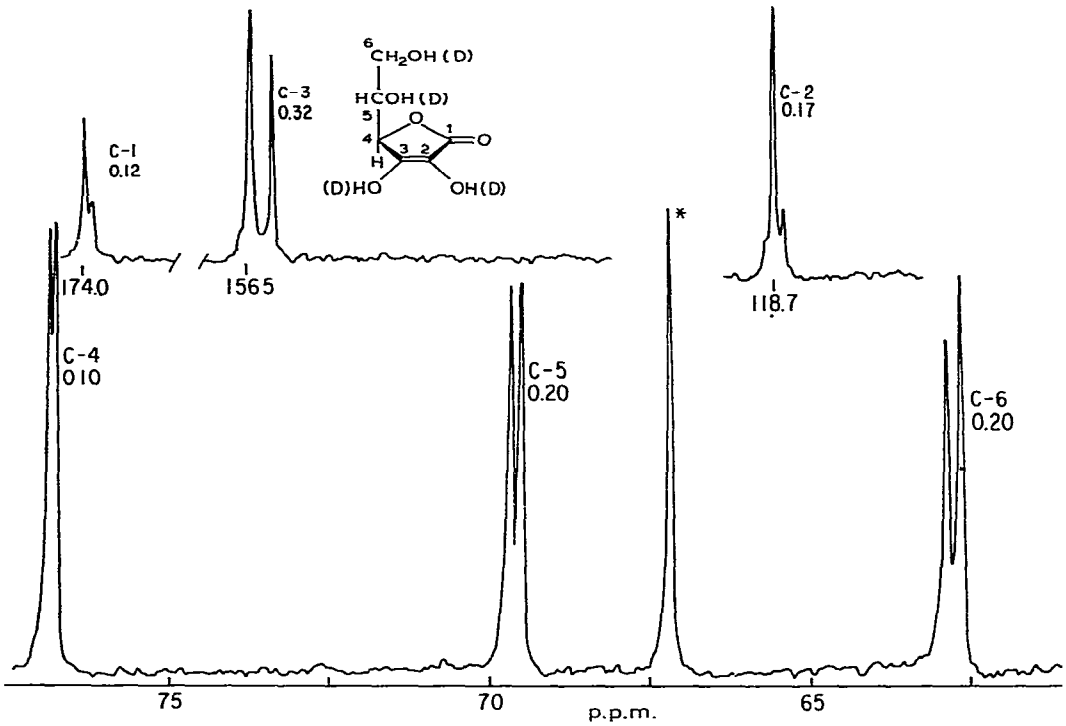


Fig. 4. Proton-noise decoupled, 15.04-MHz, d.i.s. spectrum of L-ascorbic acid (pH = 2.5, pD = 2.1). Spectrum obtained with 100-mg samples of L-ascorbic acid, each in 1 mL of H_2O and D_2O, after 3,000 transients by use of 8K data points. The displayed spectral-width is 250 Hz.

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