

Note

¹³C-N.m.r. resonance assignments of psicose by the differential isotope-shift technique*

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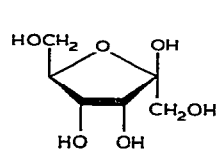
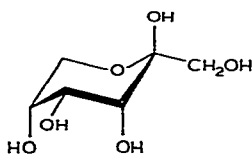
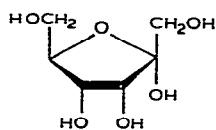
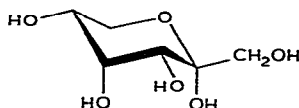
INTRODUCTION

Exchangeable-site deuteration in molecules has provided a mechanism for the assignment of ¹³C nuclear magnetic resonances^{1–7}. Exchange of hydroxyl protons with deuterons in carbohydrates causes a change in the chemical shifts, which can be used to identify resonances from empirically derived isotope-parameters¹. As these parameters (β and α shifts) are additive, the calculated isotope-shifts may be readily compared to the observed shifts from each differential isotope-shift (DIS) spectrum and assignments may be made without the need for isotope labeling and/or decoupling experiments¹.

The resonance assignments of the ¹³C spectrum of D-psicose pose a complex problem. This ketohexose in aqueous solution exists as a mixture of furanose and both pyranose tautomers. The proton-decoupled ¹³C spectrum displays 20 individual signals for the 24 carbon atoms to be assigned. The ratio of tautomers is 45:14:20:20 (α -f: β -f: α -p: β -p) at 31°, as determined by the integrated intensities of the anomeric carbon atoms. This ratio is helpful in distinguishing the furanose resonances, but the equal contribution of pyranose forms may cause confusion. Assignments of the ¹³C resonances of D-psicose were made by duPenhoat and Perlin⁸ using derivatives of psicose and specifically deuterated D-psicose, and by Que and Gray⁹ using comparison to related compounds. Their assignments differ for several carbon resonances. Angyal and Bethell¹⁰ later made several reassignments of the D-psicose spectrum using the foregoing methods, plus varying temperature and addition of calcium chloride. In the present study, we applied the DIS technique to the ¹³C spectrum of D-psicose to clarify the identity of some of the uncertain resonance assignments.

*Deuterium-Induced, Differential Isotope-Shift ¹³C N.M.R., Part 3. For Parts 1 and 2, see ref. 1.

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 β -D-f β -D-p α -D-f α -D-p

EXPERIMENTAL

The DIS technique¹ involves a differential spectrum taken in equal volume, coaxial n.m.r. tubes. The inner tube contains a M solution of the compound in 100% H₂O containing 1% of 1,4-dioxane as the internal standard. The outer tube contains an equimolar concentration of the same compound in 99.8% D₂O plus 1% of 1,4-dioxane. The compound in D₂O is previously exchanged three times with D₂O to ensure total deuterium exchange of the hydroxyl-group protons. The resulting spectra include two lines for each ¹³C resonance when the deuterium perturbation produced is >0.03 p.p.m. (limit of the instrumental resolution obtainable).

The DIS spectra were taken with JEOL FX60Q* and Bruker WH-180 n.m.r. spectrometers operating at 15 and 45 MHz, respectively, with proton-noise decoupling. For each spectrum, 2000 scans were taken with a 90° pulse-angle and a 10-s delay between pulses.

D-Psicose was prepared according to the method of Doner¹¹. The purity of the compound was >95%, as indicated by l.c.¹¹ and by the absence of additional ¹³C resonance signals above the noise level. On being kept in solution for more than 24 h, D-psicose decomposes and impurities appear. Integration of the spectra was performed before measurable decomposition occurred.

DISCUSSION

In previous work¹, we empirically determined deuterium isotope-shift parameters using as a basis six monosaccharide pairs whose ¹³C resonance assignments

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had been unambiguously established. We derived the shift parameters of Table I from a linear-regression analysis of the ^{13}C resonance data of DIS spectra^{1a}. The agreement between calculated and observed DIS values had a standard error of estimate of 0.01 p.p.m.

TABLE I

CALCULATED DIFFERENTIAL ISOTOPE-SHIFT PARAMETERS^a

Symbol	Value	Definition
β	0.14	Shift induced on ring carbon atoms by directly bonded OD
β_{an}	0.11	Shift induced on anomeric carbon atoms by directly bonded OD
β_{pr}	0.15	Shift induced on primary carbon atoms (CH_2OH) by directly bonded OD
γ	0.03	Shift induced on ring carbon atoms by OD bonded to adjacent carbon

^aCalculated from least-squares treatment; see ref. 1.

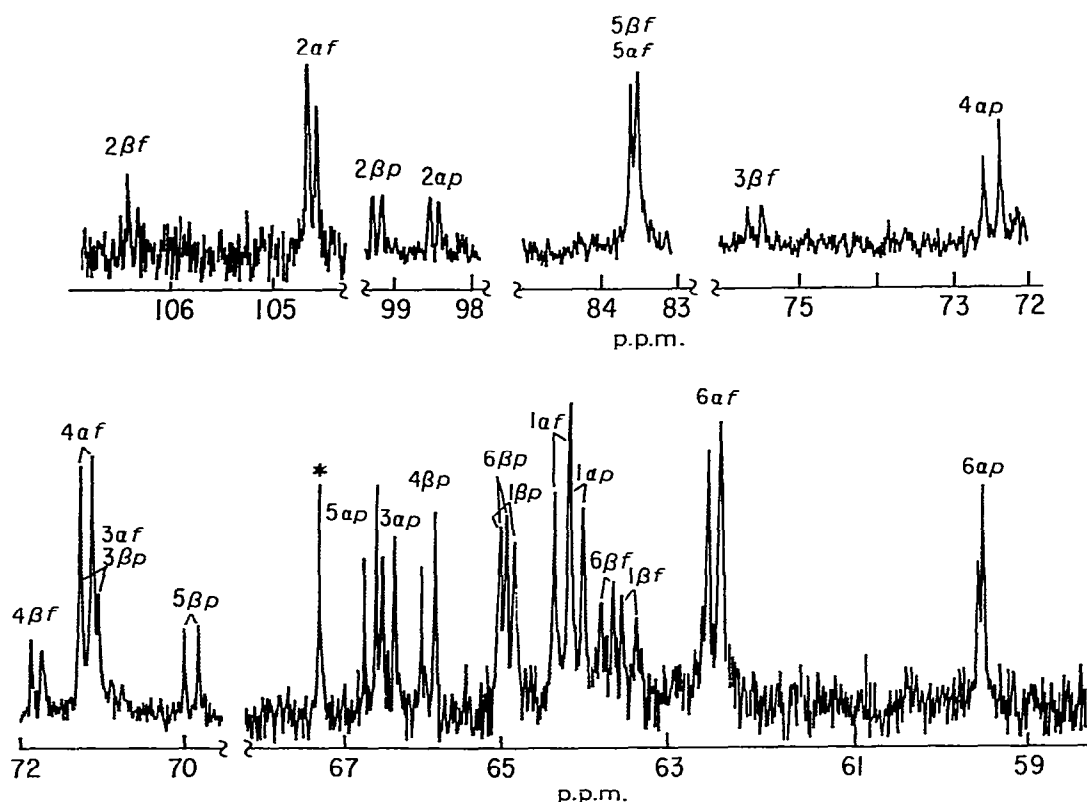


Fig. 1. 45.6-MHz DIS proton-noise-decoupled spectrum of D-psicose taken at 30° after 2300 transients with 16K data points.

TABLE II

CHEMICAL-SHIFT ASSIGNMENTS AND DIS^a VALUES FOR D-PSICOSE

<i>Tautomer</i>	<i>Chemical shifts^b and observed DIS (calculated DIS)</i>					
	<i>C-1</i>	<i>C-2</i>	<i>C-3</i>	<i>C-4</i>	<i>C-5</i>	<i>C-6</i>
α -Furanose	64.34	104.13	71.30	71.30	83.61	62.34
	0.19	0.13	0.14	0.14	0.09	0.16
	(0.18)	(0.17)	(0.20)	(0.17)	(0.06)	(0.15)
β -Furanose	63.46	106.46	75.70	71.93	83.61	63.73
	0.20	0.13	0.18	0.14	0.09	0.16
	(0.17)	(0.18)	(0.20)	(0.17)	(0.06)	(0.15)
α -Pyranose	64.14	98.54	66.58	72.67	66.82	58.86
	0.17	0.13	0.17	0.19	0.16	0.06
	(0.18)	(0.17)	(0.20)	(0.20)	(0.17)	(0.03)
β -Pyranose	65.02	99.27	71.30	66.06	69.96	65.02
	0.17	0.11	0.21	0.16	0.18	0.07
	(0.18)	(0.17)	(0.20)	(0.20)	(0.17)	(0.03)

^aDIS = Differential isotope-shift. ^bChemical shifts in p.p.m. are reported in H₂O, relative to internal 1,4-dioxane taken as 67.40 p.p.m.

TABLE III

¹³C-SHIFT ASSIGNMENTS BASED ON DIS

<i>Compound</i>	<i>Shift assignment, p.p.m.</i>						<i>Ref.</i>
	<i>C-1</i>	<i>C-2</i>	<i>C-3</i>	<i>C-4</i>	<i>C-5</i>	<i>C-6</i>	
α -D-Psicofuranose				72.80		64.40	8 ^a
				72.64			9 ^a
				71.30		62.34	10 and present study
				0.14		0.16	
β -D-Psicofuranose	63.72						
	63.46					63.40	9
	DIS obs.					63.73	10 and present study
	DIS calc.					0.16	
α -D-Psicopyranose	65.2	99.4		66.1	70.1	62.40	8
	66.1		71.4	66.6			9
	64.14	98.54	66.58	72.67	66.82	58.86	10 and present study
	DIS obs.	0.17	0.13	0.17	0.19	0.16	
β -D-Psicopyranose	DIS calc.	(0.18)	(0.17)	(0.20)	(0.17)	(0.03)	
		98.7		71.4	66.9	59.1	8
				64.2			9
		99.27		66.06	69.96	65.02	10 and present study
DIS obs.		0.11		0.16	0.18	0.07	
	DIS calc.	(0.17)		(0.20)	(0.17)	(0.03)	

^aAll shifts were converted from external CS₂ taken as 126.1 p.p.m. to 1,4-dioxane taken as reference at 67.4 p.p.m.

The calculated DIS values for D-psicose were directly derived from the parameters in Table I. To be more general, we have redefined the earlier β^1 and β^6 parameters (Table II, ref. 1a) to β^{an} (β isotope shift at the anomeric center) and β^{pr} (β isotope shift at the primary carbon atom), respectively. This same parameter set was successfully applied to the determination of the DIS values for the pyranose-furanose equilibrating sugars fructose and talose^{1a}. From the DIS spectra of D-psicose (Fig. 1), agreement between the calculated and observed DIS values for the "corrected" ¹³C resonance assignments of D-psicose was found to be ± 0.02 p.p.m. (see Table II).

The shift assignments based on DIS are given in Table III. Que and Gray⁹ based their assignments for the furanose forms of psicose on comparisons with the fructofuranosyl resonances of sucrose¹² and on the analogous 1,5-anhydrohexitol. We have shown^{1a} that several of the original assignments for sucrose were incorrect, namely, the C-3', C-4' and C-5' resonances of the fructofuranosyl ring. The DIS technique reveals a similar adjustment in the C-3 and C-4 carbon resonances designated initially^{8,9} for α -D-psicofuranose and β -D-psicopyranose, confirming the reassignments of Angyal and Bethell¹⁰.

When the temperature of D-psicose is increased, the proportion of the α -furanose tautomer increases¹⁰. The resulting increased intensity of the resonance at 71.3 p.p.m. indicates the assignment of two α -furanose carbon atoms to this resonance. The calculated DIS value for C-4 in α -D-psicofuranose is 0.17 p.p.m. The assignment of C-4 to this resonance indicates a DIS value of 0.14 p.p.m. for this atom and also satisfies the increased integration of the 71.3 p.p.m. resonance at high temperatures¹⁰. The resonance for C-6 of α -D-psicofuranose at 64.40 p.p.m. designated by duPenhoat and Perlin⁸ exhibits a DIS value of 0.19 p.p.m., whereas the calculated DIS should be only 0.15 p.p.m. With assignment of the resonance at 62.34 p.p.m. to C-6 of α -D-psicofuranose, the DIS value of 0.16 p.p.m. concurs. Furthermore, the intensity of this resonance substantiates its assignment to an α -furanose carbon atom.

The assignments for β -D-psicofuranose selected by Que and Gray⁹ have DIS values of 0.16 p.p.m. for C-1 and 0.20 p.p.m. for C-6. The calculated DIS values for these atoms are 0.17 and 0.15 p.p.m., respectively. This confirms the reassignment by Angyal and Bethell¹⁰ of 63.72 p.p.m. to C-1 of β -D-psicofuranose and 63.40 p.p.m. to C-6 of β -D-psicofuranose. This distinction is not obvious using other identification techniques, such as those which require off-resonance or proton-coupled spectra.

The resonance at 62.40 p.p.m. assigned by duPenhoat and Perlin⁸ to C-6 of α -D-psicopyranose has a DIS value of 0.16 p.p.m. The calculated DIS value of this atom is 0.03 p.p.m. With assignment of the resonance at 58.86 p.p.m. to this atom, the DIS value of 0.06 p.p.m. agrees well with the calculated value. The integrated intensity of the resonance clearly confirms this assignment. The remaining reassignments of α -D-psicopyranose were convincingly determined by Angyal and Bethell¹⁰ using assignments for 3-O-methyl-D-psicose for comparison. The observed DIS values substantiate these reassignments.

It is a simple matter to distinguish between C-6 and C-1 resonances in β -D-psicopyranose because of the large difference in isotope shift. The calculated DIS value for C-1 is 0.18 p.p.m. and the observed value for this resonance is 0.17 p.p.m. The resonance at 65.01 p.p.m., which both Que and Gray⁹ and duPenhoat and Perlin⁸ assigned to C-1 of β -D-psicopyranose, remains for the assignment to C-6 of β -D-psicopyranose. The small calculated DIS value of 0.03 p.p.m. for C-6 and the observed value of 0.07 p.p.m. for this resonance substantiates this interchange.

In the present study, several assignments for psicose have been firmly established with the DIS method. A few additional assignments (Table II) that cannot be made unambiguously with DIS have been determined by a combination of methods¹⁰. The DIS technique, however, provides a facile way to monitor ambiguities inherent in comparison of spectra of related compounds.

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