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THE ^{13}C-N.M.R. SPECTRA OF THE XYLO- AND CELLO-OLIGOSACCHARIDES

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ABSTRACT

The ^{13}C-n.m.r. spectra have been recorded and assigned for the xylo- and cello-oligosaccharides, the former up to xylopentaose, and the latter up to cellotetraose. A spectrum of a low-d.p. cellulose in dimethyl sulfoxide-d_6 was also assigned. In every instance, the spectra of the higher oligosaccharides closely parallel those of the corresponding disaccharides. Variations in line intensities permitted assignment of peaks to both terminal groups and internal residues. A particularly important difference was observed between the chemical shifts at the internal C-4 atoms for the two series of oligomers. This difference has been interpreted as evidence for significant differences in average linkage-orientation or solvation, which is related to the absence of C-6 in the xylo-oligosaccharides.

INTRODUCTION

Assignment of the ^{13}C-n.m.r. spectra of various oligosaccharides has proven useful for evaluating the composition, configuration, and sequence of soluble polysaccharides[1–3]. The usual method of assignment involves comparison of the spectrum of the oligosaccharide with those of the constituent monosaccharides[4–5] or closely related disaccharides[2,6].

For homologous oligosaccharides, it is also possible to use the variation of peak intensities with chain length to aid in assignment[1]. The similarity of chemical shifts for equivalent carbon atoms of the internal residues may be used to distinguish peaks associated with them from those assigned to terminal groups. Signals from carbon atoms several bonds removed from the linkage in a disaccharide should become relatively less intense in the spectrum of the corresponding oligosaccharide, whereas carbon atoms close to the linkage should appear more intense. In this way, lines of similar chemical shift may be differentiated on the basis of peak intensities, as this feature can be related to proximity to the glycosidic linkage.

In a study of factors that affect the β-(1→4) linkage-conformation of the xylo- and cello-oligosaccharides, it was necessary to obtain and assign their ^{13}C-n.m.r. spectra. The xylo-oligosaccharides were studied up to xylopentaose, and the cello-oligosaccharides up to cellotetraose. The spectra of these compounds have been considered on the basis of the corresponding mono- and di-saccharides, as well as

on the basis of variations in peak intensity. Intensity variations have been used to verify some of the previous assignments of cellobiose[5] in the case of the cello-oligosaccharides, and to assist in developing assignments for the previously unassigned xylobiose*. Manifestations of differences in linkage-conformation or solvation between the xylo- and cello-oligosaccharides have also been explored from these ^{13}C-n.m.r. assignments.

RESULTS AND DISCUSSION

The spectral assignments for the cello-oligosaccharides in D_2O and a low-d.p. cellulose in dimethyl sulfoxide-d_6 are given in Table I. In order to avoid contaminating

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS[a] OF THE CELLO-OLIGOSACCHARIDES IN D_2O SOLUTION

Compound	*Residue or group*		*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Cellobiose	Reducing end-group	α	92.6	72.2[b]	72.3[b]	79.7	70.9	61.0
		β	96.6	75.1[b,c]	74.8[b,c]	79.5	75.6[b]	61.1
	Nonreducing end-group		103.4	74.0	76.4[b]	70.3	76.8[b]	61.5
Cellotriose	Reducing end-group	α	92.7	72.1	72.1	79.5[d]	70.9	60.8
		β	96.6	75.1[b,c]	74.8[b,c]	79.5[b]	75.7[b]	60.8
	Internal residues		103.2	73.8[d]	74.9[b,c]	79.3[d]	75.7[b]	60.8
	Nonreducing end-group		103.4	74.0[d]	76.4[b]	70.4	76.8[b]	61.5
Cellotetraose	Reducing end-group	α	92.7	72.2	72.2	79.4[d,f]	71.0	60.9
		β	96.6	75.2[b,c]	75.0[b,c,e]	79.4[d,f]	75.7[b]	60.9
	Internal residues		103.2	73.8[d]	75.0[b,c,e]	79.3[d,g]	75.7[b]	60.9
	Nonreducing end-group		103.4	74.1[d]	76.5[b]	70.4	76.9[b]	61.6
	In Me$_2$SO-d$_6$							
Cellulose	Reducing end-group	α	92.0	s[h]	s	80.7[f]	s	60.6
		β	96.8	s	74.9[b]	80.7[f]	75.1[b]	60.6
	Internal residues		102.7	73.2	74.9[b]	80.1	75.1[b]	60.6
	Nonreducing end-group		s		76.7[b]	70.3	76.9[b]	61.2

[a]Chemical shifts in p.p.m. relative to Me_4Si by setting the shift of the terminal, nonreducing end-group C-1 atom equal to the value observed in cellobiose. The shifts for cellobiose are relative to dilute 1,4-dioxane at δ 67.4. The cellulose is referenced to Me_2SO-d_6 at 39.5 p.p.m. from Me_4Si. [b]Some authors have reversed these assignments[4–6], with the most recent assignment appearing here (see footnote *c*)[8–10,19,20]. [c]The assignments for C-2 and C-3 are different from those found in the literature during revision of the article[19,20]. The reasons for the assignments here are explained in the text. The alternative assignments[19,20] are based on homonuclear decoupling[19] and[20] a "differential isotope effect". The decoupling experiment relies on the assignment of H-2 in β-cellobiose in the skeletal-proton region, which is difficult to interpret. The assignment resulting from the "differential isotope effect" does not consider the possible existence of the 3-OH···O-5′ intramolecular hydrogen-bond†. [d]Assignments confirmed by the spectrum of low-d.p. cellulose. [e]Resolved in cellotriose only. [f]Observed as shoulders on the peak of internal C-4. [g]Actually two distinct peaks are resolved. [h]Peaks observed as a weak shoulder on a larger peak or as a very weak peak are marked s.

*A paper being prepared develops assignments for xylobiose from xylose, cellobiose, and various derivatives[7].

†See note added in proof, p. 145.

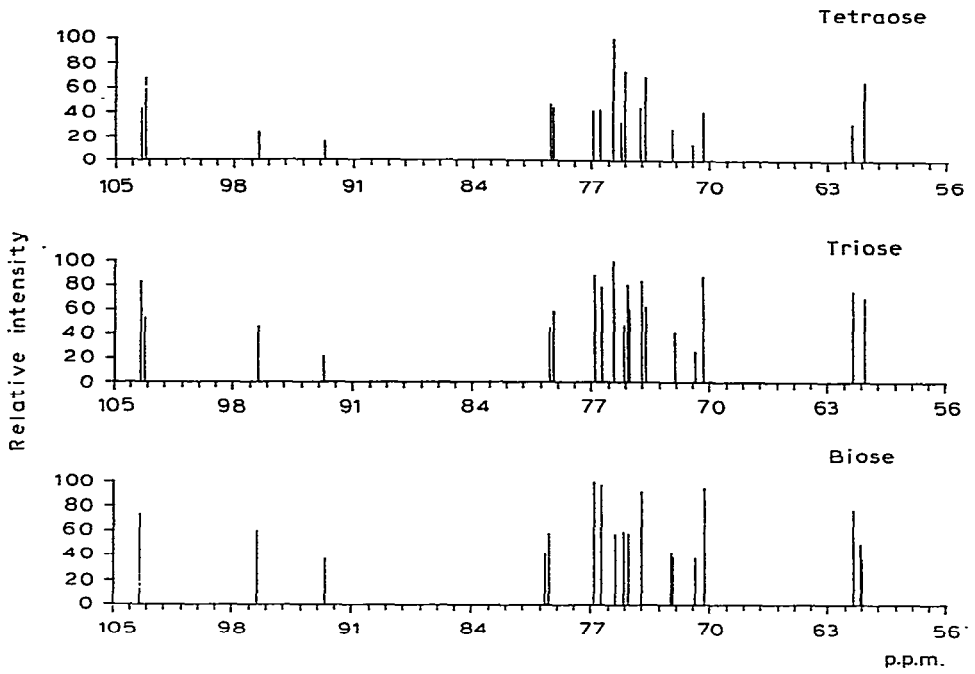

Fig. 1. Comparison of ^{13}C-n.m.r. spectra of cello-biose, -triose, and -tetraose.

our meager supply of the cello-oligomers with a reference material, we chose to set C-1† of the nonreducing end-group to the value of the equivalent carbon atom of cellobiose. A close correspondence of peak locations is observed throughout the series from biose to tetraose, permitting ready assignment of the triose and tetraose spectra. The assignments agree in general with those made by Inoue and Chujo[6] on cello-oligosaccharide fractions having $\overline{\text{d.p.}}$ 3.7 and 5.3, the exceptions being C-3 and C-5. Several different groups[8–10] have recently presented evidence to support reversal of the earlier assignments of C-3 and C-5. The new assignments have been adopted in the present study.

Examination of the assignments listed in Table I, as well as the line spectra displayed in Fig. 1, reveals that differences from the chemical shifts of cellobiose are 0.2 p.p.m. or less for the equivalent carbon atoms of the cello-oligosaccharides. The largest variations are at C-1, C-2, and C-4 of the internal residues. These small changes may be a result of the influence of an additional substituent many bonds away (the next glucosyl ring) or may result from the effects of slight differences in linkage-conformation or solvation between the exterior and interior linkages. The influence of linkage conformation was suggested by Colson *et al.*[11] to explain the relatively large changes noted when the C-1 and C-4 chemical shifts of the malto-oligosaccharides were compared with those of the cycloamyloses.

†The nonreducing group of the disaccharide is primed.

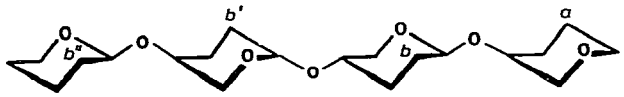

Fig. 2. An illustration of the nearly equivalent carbon atoms of a homologous oligosaccharide.

The spectra shown in Fig. 1 also illustrate the differences in peak intensities that appear within the cello-oligomer series. The variations in intensity are related to the location of a carbon atom on the reducing end-group, on the internal residues, or on the nonreducing end-group. This relation follows from the equivalence or near equivalence of chemical shifts for carbon atoms that occupy the same positions relative to the glycosidic linkage. The variation in environment is illustrated in Fig. 2, where the carbon atoms at position 2 are taken as representative. In the disaccharide there are two types (*a* and *b*), whereas four types (*a*, *b*, *b'*, and *b''*) exist in the tetrasaccharide. However, those marked *b*, *b'*, and *b''* are essentially equivalent with respect to their position relative to a glycosidic linkage. The C-2 atom of the reducing group, in contrast, has an environment that differs considerably. The intensities would approximate a 1:2:1 or 3:1 pattern, depending on whether or not *b''* can be resolved from *b* and *b'*, which are expected to be coincident. Assignments of peaks that are slightly shifted in the internal residues are based on these variations in intensity.

The lowest-field peak of cellobiose, the C-1' resonance at 103.4 p.p.m., is representative of the difference expected between *b''* and *b'* in Fig. 2. In the spectra of the higher oligomers, two peaks are observed in this region, at 103.2 and 103.4 p.p.m. Because the peak at 103.2 p.p.m. increases in intensity, relative to the 103.4-p.p.m. peak, as the number of pyranose residues increases, it may be assigned to the

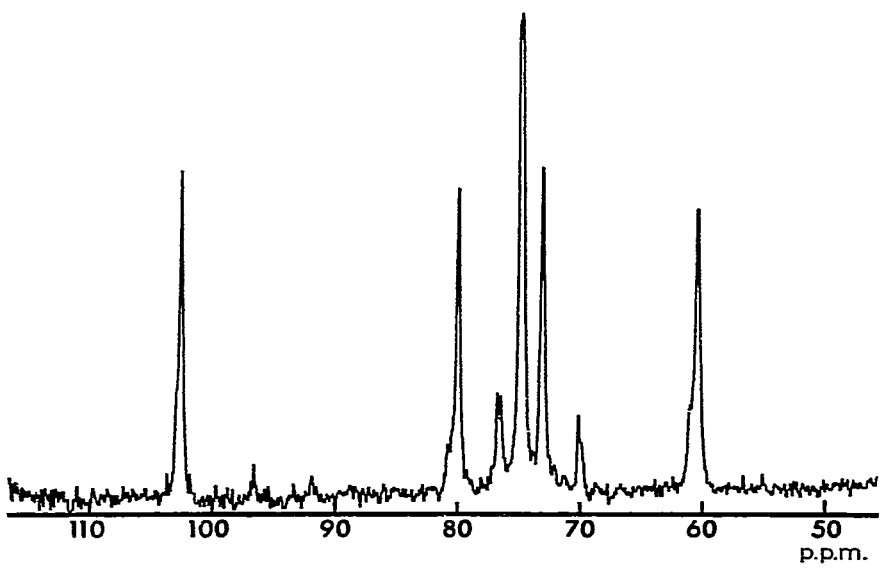

Fig. 3. The ^{13}C-n.m.r. spectrum of a low-d.p. cellulose.

internal-linkage carbon atoms (C-1), and the 103.4-p.p.m. peak may be assigned to the nonreducing, terminal group. The spectrum of a low-$\overline{\text{d.p.}}$ cellulose (Fig. 3) shows a small, downfield shoulder corresponding to C-1 of the nonreducing end-group. Following a similar analysis, the less-intense of the pairs of lines centered at 73.9 and 79.4 p.p.m. may be assigned to C-2 and C-4 of the oligomer terminal-groups, respectively.

In a reciprocal manner, the spectra of the higher cello-oligosaccharides (Fig. 1) may be used to confirm some of the assignments for cellobiose. An obvious example corresponding to the difference between *a* and *b* in Fig. 2 is the assignment of the 61.5-p.p.m. resonance to C-6 of the nonreducing end-group; this peak declines steadily in the spectra of the higher oligomers. The 61.0-p.p.m. peak, which corresponds to C-6 of the internal residues and reducing-end groups, increases, as expected, in the higher cello-oligosaccharides. Thus, the differences in position of the C-6 atoms, relative to the glycosidic linkage, appear to result in the changes observed in chemical shift.

A pattern of variation similar to the one reported here suggests the assignment of the peak at 75.1 p.p.m. to C-2 in β-cellobiose*. While previous authors[4-6] have reported two peaks in this region and have viewed the 75.1-p.p.m. peak as a coincidence of signals for C-2 and either C-3 or C-5, three distinct peaks are resolved in the present report, the others appearing at 74.8 and 75.6 p.p.m. As the 75.1-p.p.m. peak declines steadily, relative to the other two peaks, with increasing chain-length, the latter must contain contributions from carbon atoms on internal residues; differentiation of C-3 and C-5 is not possible on the basis of these spectra.

The spectrum of a low-$\overline{\text{d.p.}}$ cellulose fraction in dimethyl sulfoxide-d_6 is shown in Fig. 3. The appearance of the spectrum is similar to that reported by Gagnaire, Mancier, and Vincendon[10,12] for a degraded cellulose of $\overline{\text{d.p.}}$ 10. In the present work, several reducing-end-group peaks were also observed. The assignments for the low-$\overline{\text{d.p.}}$ cellulose are consistent with those of the cello-oligosaccharides.

Previous reports[6] have suggested that the intensities of the C-1 peaks may be used to estimate the number-average $\overline{\text{d.p.}}$ for oligosaccharides of low molecular weight. Comparison of these intensities for cellotriose, cellotetraose, and the low-$\overline{\text{d.p.}}$ cellulose suggests that this correlation is not generally true under all conditions. The spectrum of cellotriose, in particular, shows a significant difference in the intensity for C-1 in the nonreducing group and the internal residue, suggesting that the two carbon atoms possibly have different nuclear Overhauser enhancements or that, with the present degree of resolution of these signals, the peak heights are not a true indication of peak intensity.

The ^{13}C-n.m.r. spectra of the xylo-oligosaccharides are graphically depicted in Fig. 4, with the corresponding assignments in Table II. Assignments for xylobiose have been developed from comparisons with assignments for xylose[8,13,14] and cellobiose. Again, as in the cello-oligosaccharides, intensity variations confirm these

*See note added in Proof, p. 145.

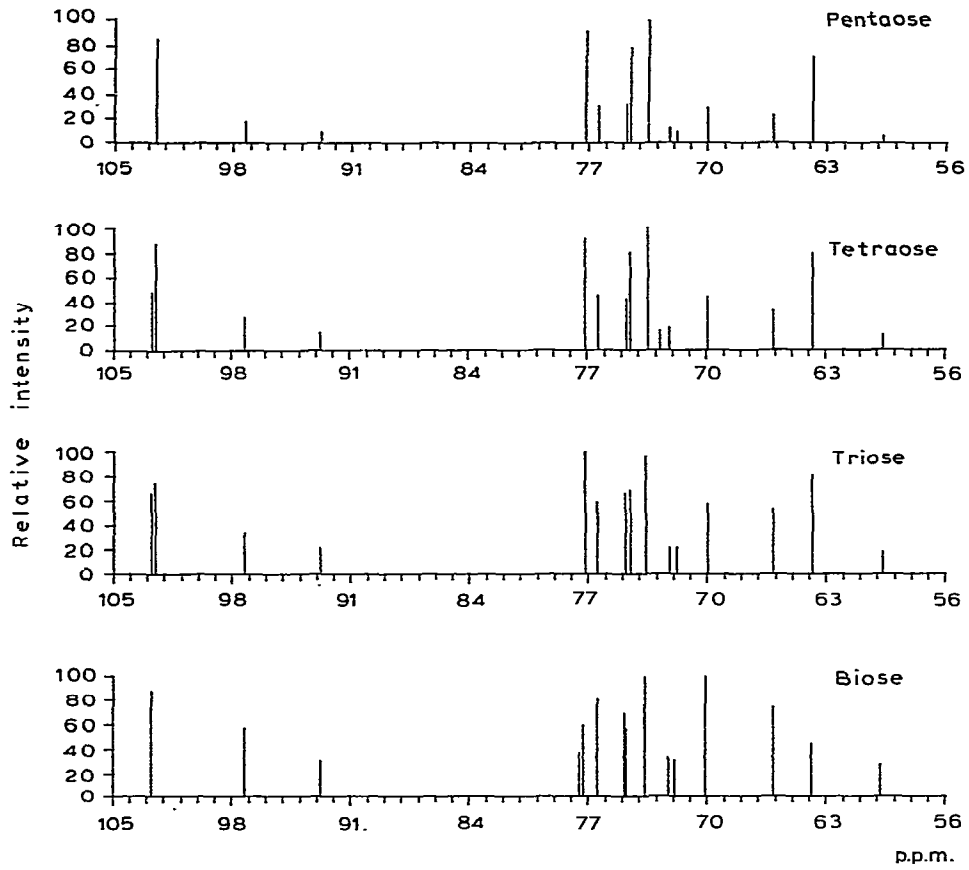

Fig. 4. Comparison of the ^{13}C-n.m.r. spectra of xylo-biose, -triose, -tetraose. and -pentaose.

assignments. For example, changes in the intensities of the lines at 66.1, 63.9, and 59.8 p.p.m. support assignment of the 63.9-p.p.m. line to C-5 of β-xylobiose and the internal C-5 resonances of the higher oligomers.

For the xylo-oligosaccharides, the chemical shifts are reported relative to C-1′ of the nonreducing group of xylobiose. In comparing the series xylobiose–xylopentaose, the shifts of C-2 and C-5 do not vary by more than 0.2 p.p.m. from the values anticipated from the spectrum of xylobiose. C-1 and C-4, next to the glycosidic linkage, vary to a slightly greater extent, suggesting that slightly different conformations might exist at the interior linkages[2,11]. Internal C-3 atoms undergo upfield shifts of the same order of magnitude, indicating the sensitivity to conformational change at the linkage. As for the cello-oligosaccharides, the small shifts may also arise from the substitution in the internal residues of a monosaccharide group for a proton.

The contrasts between the spectra of the xylo- and cello-oligosaccharides suggest significant differences between the two series in the constraints on the linkage.

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS[a] OF THE XYLO-OLIGOSACCHARIDES IN D_2O SOLUTION

Compound	*Residue or group*		*C-1*	*C-2*	*C-3*	*C-4*	*C-5*
Xylobiose	Reducing end-group	α	92.8	72.3[b]	71.9[b]	77.5	59.8
		β	97.3	74.9[c]	74.9[c]	77.3	63.9
	Nonreducing end-group		102.7	73.7	76.5	70.1	66.1
Xylotriose	Reducing end-group	α	92.8	72.2[b]	71.8[b]	77.2	59.7
		β	97.3	74.8	74.8	77.2	63.8
	Internal residues		102.5	73.6	74.5	77.2	63.8
	Nonreducing end-group		102.7	73.6	76.5	70.0	66.1
Xylotetraose	Reducing end-group	α	92.8	72.2[b]	71.8[b]	77.2	59.7
		β	97.3	74.7	74.7	77.2	63.8
	Internal residues		102.5	73.5	74.5	77.2	63.8
	Nonreducing end-group		102.7	73.5	76.4	70.0	66.1
Xylopentaose	Reducing end-group	α	92.8	72.2[b]	71.8[b]	77.2	59.7
		β	97.3	74.7	74.7	77.2	63.8
	Internal residues		102.5	73.5	74.5	77.2	63.8
	Nonreducing end-group		s[d]	73.5	76.4	70.0	66.1

[a]Chemical shifts in p.p.m. relative to Me_4Si by setting the shift of the terminal, nonreducing C-1 atom equal to the value observed in xylobiose. The spectrum of xylobiose is referenced to dilute 1,4-dioxane given a value of 67.4 p.p.m. [b]These resonances may be exchanged. [c]These resonances are slightly resolved in xylobiose. [d]A shoulder on the 102.5-p.p.m. peak.

While the chemical shift of C-4 differs only slightly between glucose and xylose[18], indicating that presence of a hydroxymethyl group (C-6) at C-5 in glucose has a relatively small effect, the difference between the linkage C-4 chemical shifts of the xylo- and cello-oligosaccharides is ~1.8 p.p.m. in D_2O*. Such a large difference would not be expected on the basis of the effect of mere replacement of a xylosyl by a glucosyl residue, as those two substituents, which differ only by the absence of C-6 for xylose, would be perceived[3] as nearly equivalent by the C-4 atom. It appears most likely that this difference is a manifestation of significant differences in the average conformation, or in the solvation environment, of the β-(1→4) linkages in the two, homologous, oligosaccharide series. Such an interpretation is consistent with model studies of the linkage conformation of the disaccharide[15]. Differences in average linkage-conformation or in accessibility to the linkage may explain differences in reactivity and solubility of the two types of oligosaccharide.

Colson *et al.*[11], in comparing the chemical shifts of the linkage C-1 and C-4 atoms in maltotriose with those of the cycloamyloses, found downfield shifts of 1.8 and 4.0 p.p.m., respectively, for these atoms in the (more constrained) cycloamyloses. The comparable shifts for the cello-oligosaccharides relative to the xylo-oligosaccharides are downfield by 0.7 and 1.8 p.p.m.

The greater constraints at the linkage for the cello-oligosaccharides appear to

*In dimethyl sulfoxide-d_6, the difference becomes 5.2 p.p.m.

TABLE III

CHEMICAL SHIFTS OF THE LINKAGE C-4 ATOM FOR SEVERAL β-(1→4)-LINKED DISACCHARIDES

Compound	*Chemical shift of C-4*	*Chemical-shift change relative to monosaccharide*
β-Xylobiose	77.3	7.1
4-*O*-β-D-Galactosyl-D-xylose	77.7[a]	7.5
4-*O*-β-D-Glucosyl-D-xylose	77.4	7.2
β-Cellobiose	79.5	8.9
β-Mannobiose	77.5	9.9
4-*O*-β-D-Glucosyl-β-D-mannose	77.3	9.7
β-Xylose	70.2[b]	
β-Glucose	70.6[b]	
β-Mannose	67.6[b]	

[a]Relative[17] to external Me_4Si. [b]See ref. 18.

result from the presence of C-6. The role of C-6 is suggested by the pattern of chemical shifts of the disaccharides, recorded in Table III. The chemical shifts of the linkage C-4 atom of xylobiose and cellobiose, relative to those of the appropriate monosaccharides, are compared with data for several other β-(1→4)-linked disaccharides. Both mannobiose and 4-*O*-β-D-glucosyl-D-mannose, which possess reducing-end C-6 groups constituted similarly to that of cellobiose, have chemical shifts, relative to the respective monosaccharides, comparable with that of cellobiose. In contrast, the chemical shifts of C-4 for 4-*O*-β-D-galactosyl-D-xylose[17] and 4-*O*-β-D-glucosyl-D-xylose, which do not have a C-6 atom in the reducing ring, are nearly identical to that for xylobiose.

SUMMARY

Internally consistent assignments have been developed for the ^{13}C-n.m.r. spectra of the xylo- and cello-oligosaccharides. Variations of peak intensity with chain length made it possible to distinguish between the resonances of terminal groups and internal monosaccharide residues. These intensity variations also permitted verifications of previously published assignments for cellobiose.

While differences between the chemical shifts of equivalent carbon atoms on internal residues and terminal groups are slight, larger changes are observed for the linkage carbon atoms (C-1 and C-4). This difference may result from variations in linkage conformation along the chain. A more significant observation is the difference between the chemical shifts of C-4 for xylobiose and cellobiose. Comparison with several disaccharide-models suggests that the absence of C-6 at the reducing end makes the linkage more accessible to the solvent and probably allows a much wider range of conformations for the glycosidic linkage. Thus the β-(1→4) linkage appears to be more flexible in the xylo-oligosaccharides than in the cello-oligosaccharides.

EXPERIMENTAL

Spectra. — ^{13}C-N.m.r. spectra were recorded with a Jeol FX-100 n.m.r. spectrometer operating at 25.05 MHz in the noise-decoupled mode. Spectra were accumulated for 50,000–100,000 pulses for the trioses, tetraoses, mannose disaccharides, and xylopentaose by using a 5000-Hz spectral width and a 1-sec pulse-interval, and 8192 data-points. All spectra were recorded at room temperature, except for cellotetraose (44°) and low-$\overline{\text{d.p.}}$ cellulose (75°). A Jeol microprobe was used to obtain the spectra of the trioses, tetraoses, xylopentaose, mannobiose, and of 4-*O*-β-D-glucosyl-D-mannose, because of the limited amount of sample available. Concentrations of 2–8 mg in 75 μL of D_2O were used. The spectra of cellobiose, xylobiose, and 4-*O*-β-D-glucosyl-D-xylose were recorded with a 5-mm probe at equivalent concentrations, with internal 1,4-dioxane as a reference. All other chemical shifts were referenced to the value of the internal, anomeric carbon atom of either the relevant disaccharide or mannose. This calibration procedure was used to avoid potential contamination of the small amounts of available sample with any internal reference. The spectrum of low-$\overline{\text{d.p.}}$ cellulose was recorded by using a 10-mm probe with dimethyl sulfoxide-d_6 as the solvent.

Samples. — The xylo-oligosaccharides were obtained from the collection of carbohydrates available at The Institute of Paper Chemistry, as were the mannose-containing disaccharides. The cello-oligosaccharides were prepared by acid hydrolysis of cellulose, followed by column chromatography on carbon–celite[16]. The low-$\overline{\text{d.p.}}$ cellulose was obtained from the methanol-treated filtrate of phosphoric acid-hydrolyzed, Whatman CF-1 cellulose powder. Xylobiose and 4-*O*-β-D-glucosyl-D-xylose were synthesized; the synthetic sequence is reported elsewhere[7]. Cellobiose was purchased (Matheson, Coleman, and Bell).

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NOTE ADDED IN PROOF

While the present report was in the editorial process, L. D. Hall, G. A. Morris, and S. Sukumar, [*J. Am. Chem. Soc.*, 102 (1980) 1745–1747], have demonstrated, utilizing the techniques of homo- and hetero-nuclear two-dimensional n.m.r. spectroscopy, that the C-2 atom resonates at 74.8 p.p.m. while the C-3 atom resonates at 75.1 p.p.m., in β-cellobiose. Since it is the 74.8 p.p.m. peak that increases in intensity

in the higher oligomers it must be the signal for the C-3 atoms in the internal residues that is shifted upfield relative to C-3 in β-cellobiose. This upfield shift may result from variations in the energies of the 3-OH···O-5′ intramolecular hydrogen-bonds at the internal linkages.

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