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Note

The ^{13}C-n.m.r. spectra of (1→4)-linked β-D-gluco-oligosaccharides

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^{13}C-N.m.r. spectroscopy is of great value for the elucidation of the composition, sequence, and conformation of carbohydrates in solution[1–10]. ^{13}C-N.m.r. and ^{1}H-n.m.r. spectra of D-gluco-saccharides may be used as fingerprints for identification and classification of the naturally occurring sugars, provided that the complete assignments of the spectra of relevant model compounds are established and the dependence of chemical shifts on the structure is clarified. There are many natural oligo- and poly-saccharides which contain (1 → 4)-linked β-D-glucopyranose residues, for instance, cellobiose (4-*O*-β-D-glucopyranosyl-D-glucopyranose), cellulose, lichenin and isolichenin isolated from Iceland moss (*Cetraria islandica*)[11], and xanthan gum produced by *Xanthomonas campestris* NRRL B-1459[12]. Although the ^{13}C-n.m.r. spectra of cellobiose[2,4], methyl cellobioside[2,4,13], cellobiose octa-acetate[2,9], and cellulose triacetate[2,9] have been analysed, the ^{13}C-chemical shift data of unmodified, internal, (1 → 4)-linked β-D-glucopyranose residues of oligo- and poly-saccharides in aqueous solution have not been reported. Such data may be useful for the ^{13}C-n.m.r. study of structures and properties of water-soluble natural polysaccharides, polysaccharide–protein complexes, and glycoproteins.

We now describe the ^{13}C-n.m.r. spectra of unmodified, (1 → 4)-linked β-D-gluco-oligosaccharides obtained by acetolysis of cellulose.

The ^{13}C-n.m.r. spectrum (D_2O, 90°) of cellulose oligomer G-3.7 (average d.p. = 3.7) is illustrated in Fig. 1. Assignments were readily made on the basis of comparison with the spectra of D-glucose and cellobiose[2,4], and the results are shown in Table I. For reference, the chemical shifts of cellobiose measured in D_2O solution at 90° are also tabulated. Except for relative intensities, the number and the chemical shifts of peaks appearing in the spectra of the oligomers G-3.7 and G-5.3 (average d.p. = 5.3) coincide with each other. Thus, it is concluded that the chemical shifts of the resonances of the central D-glucosyl residues are independent of the chain length. A similar independence of ^{13}C-chemical shifts has been found for homologous series of oligosaccharides having (1 → 4)- and (1 → 6)-linked α-D-glucopyranose residues[8], and (1 → 2)-linked α-D-mannopyranose residues[3].

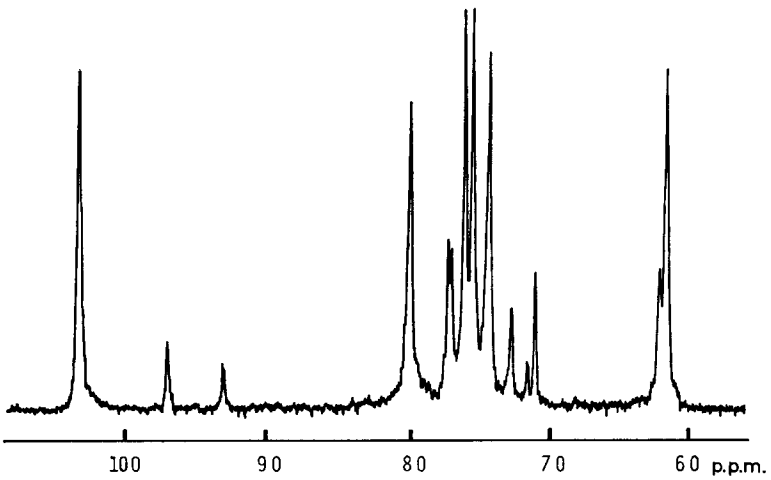

Fig. 1. ^{13}C-N.m.r. spectrum of a 5% solution of cellulose oligomer G-3.7 (average d.p. 3.7) in D_2O at 25 MHz and 90°: spectral width 5 kHz, 4096 data points, cycle time 1 sec, pulse angle 90°, 2000 transients.

TABLE I

^{13}C-N.M.R. CHEMICAL SHIFTS[a] OF CELLULOSE OLIGOMERS IN D_2O SOLUTION

			C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Cellobiose	Reducing end-unit	α-	93.0	72.6	72.6	80.4	71.4	61.6
		β-	97.0	75.6	76.0	80.4	75.6	61.6
	Non-reducing end-unit	β-	103.7	74.5	77.2[b]	70.9	77.0[b]	62.1
Oligomer G-3.7	Reducing end-unit	α-	93.0	72.6	72.6	80.0	71.6	61.5
		β-	97.0	75.4	76.0	80.0	75.4	61.5
	Internal unit	β-	103.4	74.3	76.0	80.0	75.4	61.5
	Non-reducing end-unit	β-	103.4	74.3	77.2[b]	70.9	77.0[b]	62.0
Oligomer G-5.3	Reducing end-unit	α-	93.2	72.7	72.7	79.9	71.5	61.5
		β-	97.2	75.4	76.1	79.9	75.4	61.5
	Internal unit	β-	103.4	74.3	76.1	79.9	75.4	61.5
	Non-reducing end-unit	β-	103.4	74.3	77.3[b]	71.1	77.0[b]	62.0

[a]Chemical shifts in p.p.m. downfield from external tetramethylsilane. [b]Assignments which may be interchanged.

The chemical shifts of the carbons of the reducing and non-reducing end-units are also not affected by a change in the chain length. The chemical shifts of C-1α and C-1β of the reducing end-units in the oligomers, including cellobiose, coincide with those of D-glucopyranose[4]. Similar results are also found for the chemical shifts of C-4 of the non-reducing end-unit. These results correspond well with those of Colson and King[6], who measured the ^{13}C-n.m.r. spectra of a few disaccharides

having β-D-glucosidic linkages. They showed that changing the nature of the reducing D-glucopyranose ring has no important influence on the chemical shifts of the non-reducing D-glucopyranose ring, and, conversely, that the chemical shifts of the carbons of the reducing glucopyranose ring are not noticeably affected by changing the non-reducing unit.

The ratio of the C-1 peak-heights for non-reducing units (103.4 p.p.m.) and the sum of the C-1α and C-1β peak-heights for the reducing unit (93.0 and 97.0 p.p.m.) coincides well with the value expected from the degree of polymerization (d.p.). This result implies that the number-average molecular weight of the polysaccharide can be calculated by measuring the ratio of resonances for C-1 of internal and non-reducing units to those of reducing units, if the d.p. is not too large and if the individual carbons do not have markedly different spin–lattice relaxation times and nuclear Overhauser enhancement factors[14].

The signals for anomeric carbons are, in general, readily distinguished and have been used for the detection and quantification of α and β anomers, and various types of glycosidic linkage, in mono-, oligo-, and poly-saccharides[4,5]. The chemical shifts of C-1 in a (1 → 4)-linked β-D-glucopyranosyl residue (103.4–103.7 p.p.m.) are very close to those of C-1 in a (1 → 3)-β-D-glucan (103.8 p.p.m.)[5] and β-(1 → 6)-linked D-glucopyranose oligomers (103.7–103.8 p.p.m.)[4]. The signal for C-4 in a (1 → 4)-linked β-D-glucopyranose residue is likely to be the most informative for the detection of a β-(1 → 4)-linked unit in a glucan, as it occurs in a region (~80 p.p.m.) that is free from other signals[4,5].

EXPERIMENTAL

^{13}C-N.m.r. spectra. — The spectra of oligosaccharides were obtained on a JEOL JNM PS-100 NMR spectrometer (25.14 MHz) equipped with a PFT-100 Fourier-transform system, using a glass tube of outside diameter 8 mm. The spectra were obtained for 5% solutions in D_2O at 90°, and the solvent deuterium resonance was used as a field-frequency lock. The spectra incorporated 4096 data points with a sweep width of 5000 Hz. Chemical shifts were measured with respect to that of internal *p*-dioxane, which was taken as 67.7 p.p.m. downfield from tetramethylsilane.

Materials. — Cellulose oligosaccharides were prepared by acetolysis of cellulose (commercially available filter paper), followed by fractionation and saponification of the acetolysate[15]. The filter paper (90 g) was treated with a mixture of glacial acetic acid (340 ml), acetic anhydride (340 ml), and conc. sulfuric acid (36 ml) at room temperature for 6.3 h. A methanol-soluble fraction of the acetolysate was further fractionated by fractional precipitation from solution in *p*-dioxane with isopropyl ether. The average degree of polymerization (d.p.) of each fraction of the acetolysate was determined for a solution in *p*-dioxane with a Hewlett-Packard 302D vapor-pressure osmometer at 37°. The fractions of the acetolysate were deacetylated with methanolic sodium methoxide, and thus unmodified cellulose oligomers G-3.7 (d.p. = 3.7) and G-5.3 (d.p. = 5.3) were obtained. These products showed no C=O

absorption band in their infrared spectra. T.l.c. on silica gel (2-propanol–water, 85:15) showed the absence of glucose and cellobiose in G-3.7 and G-5.3. A mixture of anisaldehyde (1 ml), ethyl alcohol (20 ml), sulfuric acid (1 ml), and acetic acid (several drops) was used as the detecting reagent.

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