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Preliminary communication

Structure-dependent, molecular-motion parameters of branched-chain polysaccharides*

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Signal characteristics of ^{13}C-nuclear magnetic resonance (n.m.r.) spectra depend on the rate of motion of the solute molecules, a rate that modulates the spin–lattice relaxation time T_1, the spin–spin relaxation time T_2, and the nuclear Overhauser enhancement (n.O.e.) of the ^{13}C nucleus[1,2]. In solutions of polysaccharides which have long correlation times (δ_c) of $\geqslant$ 10 ns, the nuclei have low n.O.e. values which lead to decreased signal intensity, and short T_2 values resulting in wide signals[3–5]. The latter effect is aggravated in gels, so that signals of ^{13}C nuclei in the vicinity of junction zones disappear[4–6]. However, T_1 values should be short, so that frequent pulses can be applied, and data accumulation is rapid. Few T_1 values have been reported for polysaccharides, but those of bovine nasal cartilage and chondroitin 4-sulfate[4], and of gels and solutions of a β-D-(1→3)-linked D-glucopyranan from *Alcaligenes faecalis*[5] are from 0.06 to 0.09 s.

The segmental motion of different units in oligosaccharides can be compared through the T_1 values of their component nuclei, and interpreted in terms of chemical structure. Stachyose [*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside] contains a D-galactopyranosyl end-group having higher T_1 values and greater mobility than the (interior) D-galactopyranosyl residue[7]. By contrast, in a trisaccharide consisting of *N*-acetyl-β-neuraminic acid (2→3)-linked to the D-galactosyl group of a lactose residue, the T_1 values of resonances of the D-glucosyl and D-galactosyl residues were 0.11–0.26 s more than the 0.05 s reported for one of the nuclei of the highly solvated, nonreducing end-group, which acts as an anchor[8]. Such an effect also occurs in a glycoside consisting of a bulky strophanthidin aglycon attached to an *O*-β-D-glucopyranosyl-(1→6)-*O*-D-glucopyranosyl-(1→4)-β-D-cymaropyranosyl sequence. The T_1 values of the pyranose-ring nuclei increased[9] on going from the strophanthidin-linked cymarose unit (0.11–0.20 s) through the (internal) D-glucosyl residue to the D-glucosyl end-group (0.31–0.37 s). Thus, in branched-chain polysaccharides, segmental motion should be greater in side-chain units than in those of the main chain.

The present study is the first to be made on the determination of T_1 and n.O.e. values (25.2 MHz) of branched-chain polysaccharides. T_1 values were measured by using

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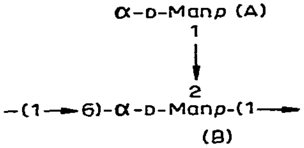
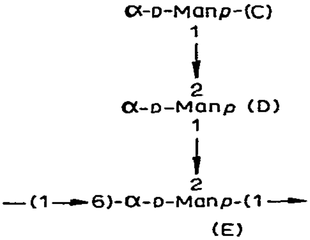
degassed, 20% solutions in D_2O, by the Freeman–Hill modification[10] of the inversion-recovery, Fourier-transform method, 20 τ-values being used to define large signals, and 10 for smaller ones. The minimum accuracy was within ±10%. Values of n.O.e. were determined by comparison of the signal sizes obtained in a conventional spectrum and in one obtained by the anti-gated technique[11,12], all other instrumentational parameters being the same. D-Mannose-containing polysaccharides having the principal repeating-structures **1**, **2**, **3**, **4** (ref. 13), **5**, and **6** (ref. 14) were investigated. As may be seen from Tables I and II,



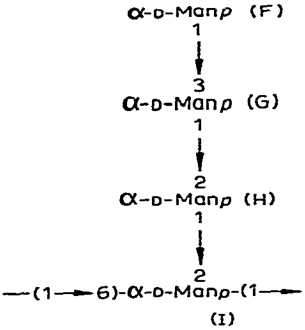
$$\begin{array}{c}\alpha\text{-D-Man}p\ (\text{A})\\ 1\\ \downarrow\\ 2\\ \text{—(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\\ (\text{B})\end{array}$$

1



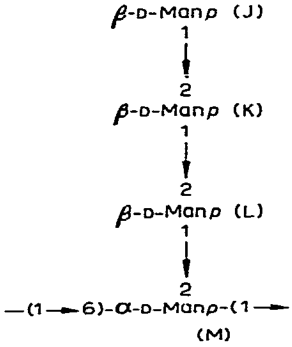
$$\begin{array}{c}\alpha\text{-D-Man}p\text{-(C)}\\ 1\\ \downarrow\\ 2\\ \alpha\text{-D-Man}p\ (\text{D})\\ 1\\ \downarrow\\ 2\\ \text{—(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\\ (\text{E})\end{array}$$

2



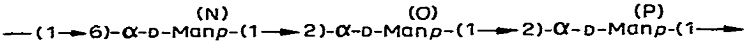
$$\begin{array}{c}\alpha\text{-D-Man}p\ (\text{F})\\ 1\\ \downarrow\\ 3\\ \alpha\text{-D-Man}p\ (\text{G})\\ 1\\ \downarrow\\ 2\\ \alpha\text{-D-Man}p\ (\text{H})\\ 1\\ \downarrow\\ 2\\ \text{—(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\\ (\text{I})\end{array}$$

3



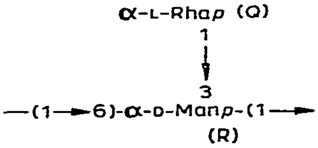
$$\begin{array}{c}\beta\text{-D-Man}p\ (\text{J})\\ 1\\ \downarrow\\ 2\\ \beta\text{-D-Man}p\ (\text{K})\\ 1\\ \downarrow\\ 2\\ \beta\text{-D-Man}p\ (\text{L})\\ 1\\ \downarrow\\ 2\\ \text{—(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\\ (\text{M})\end{array}$$

4



$$\text{—(1}\rightarrow\text{6)-}\underset{}{\overset{(\text{N})}{\alpha\text{-D-Man}p}}\text{-(1}\rightarrow\text{2)-}\overset{(\text{O})}{\alpha\text{-D-Man}p}\text{-(1}\rightarrow\text{2)-}\overset{(\text{P})}{\alpha\text{-D-Man}p}\text{-(1}\rightarrow$$

5



$$\begin{array}{c}\alpha\text{-L-Rha}p\ (\text{Q})\\ 1\\ \downarrow\\ 3\\ \text{—(1}\rightarrow\text{6)-}\alpha\text{-D-Man}p\text{-(1}\rightarrow\\ (\text{R})\end{array}$$

6

TABLE I

T_1 AND n.O.e. VALUES OF ^{13}C NUCLEI OF *Saccharomyces fragilis* MANNAN, AN α-D-(1→6)-LINKED D-MANNOPYRANAN, AND AN L-RHAMNO-D-MANNAN FROM *Sporothrix schenckii*, DETERMINED AT VARIOUS TEMPERATURES

Polysaccharide	*Signal shift* [a] *(assignment* [b]*)*	T_1 *(s) (n.O.e. values in parentheses)*		
		30°	*70°*	*90°*
Saccharomyces fragilis mannan (repeating unit 1)	103.7; C-1, A	0.11 (1.92)	0.17 (2.01)	0.22 (2.38)
	100.0; C-1, B	0.09 (1.58)	0.10 (1.92)	0.11 (2.31)
	80.1; C-2, B	0.09 (1.67)	0.09 (1.83)	0.12 (1.91)
	62.8; C-6, A	0.07 (2.18)	0.13 (2.35)	0.20 (2.59)
(1→6)-α-D-Mannopyranan	101.1; C-1	0.11 (2.74)	0.28	0.31 (2.91)
	71.7; C-3	0.12 (2.29)	0.19	0.27 (2.64)
	68.5; C-4	0.12 (2.54)	0.22	0.30 (2.68)
	67.5; C-6	0.07 (2.44)	0.17	0.20 (2.68)
Sporothrix schenckii L-Rhamno-D-mannan (repeating unit 6)	101.5; C-1, R	0.08 (1.91)	0.11 (2.18)	0.12
	98.3; C-1, Q	0.16 (1.73)	0.16 (2.10)	0.19
	77.0; C-3, R	0.09 (1.34)	0.13 (2.09)	0.12
	73.8; C-4, Q	0.11 (1.88)	0.14 (2.52)	0.18
	70.4; C-5, Q	0.12 (2.30)	0.12 (2.15)	0.15
	67.9; C-2, R	0.08 (2.08)	0.08 (2.20)	0.10
	66.5; C-4, R	0.08 (1.78)	0.08 (1.91)	0.11
	18.4; CH_3, Q	– (2.49)	0.41 (2.79)	0.44

[a] Chemical shifts, determined at 70°, relative to external tetramethylsilane. [b] A, B, Q, and R refer to units depicted.

TABLE II

T_1 AND n.O.e. VALUES OF VARIOUS ^{13}C NUCLEI OF MANNANS, DETERMINED AT 70°

Repeating unit	*Signal shift*	*Assignment*	T_1 *(s)*
Endomycopsis fibuliger 1	103.7	C-1, A	0.16[a]
	100.0	C-1, B	0.09
	80.1	C-2, B	0.11
	62.8	C-6, A	0.14[a]
Saccharomyces rouxii 2	103.7	C-1, C	0.20
	102.2	C-1, D	0.13
	99.1	C-1, E	0.09
Saccharomyces cerevisiae 3	103.7	C-1, F and G	0.18
	102.2	C-1, H	0.12
	99.1	C-1, I	0.08

Repeating unit	*Signal shift*	*Assignment*	T_1 *(s)*
Citeromyces matritensis 4	102.6	C-1, K and L	0.31
	101.6	C-1, M	0.11
	101.2	C-1, J	0.26
	80.6	C-2, M	0.21
	80.1	C-2, K and L	0.33
Hansenula capsulata[b] 5	103.7	C-1, N	0.14 (2.07)
	102.8	C-1, O	0.14 (2.21)
	100.3	C-1, P	0.14 (2.76)
	79.4	C-2, O and P	0.14 (2.13)

[a] Average value, due to the presence of minor *O*-α-D-mannopyranosyl-(1→3)-*O*-α-D-mannopyranosyl-(1→2) side-chains having two C-1 signals of δ_C 103.7. [b] n.O.e. value given in parentheses.

side-chain nuclei have higher T_1 values than those of the main chain. For structure **2**, the T_1 values of C-1 are in the order: nonreducing end-group C (0.20 s) > adjacent side-chain unit D (0.13 s) > main-chain unit E (0.09 s). (A similar order of T_1 values was observed for structure **3**, although the C-1 signals for units F and G are superimposed, and only an average value was obtainable.) Such values are analogous to those obtained by Levy *et al.*[15] for branched-chain poly(*n*-alkyl methacrylates) which were less segmentally mobile on going from the terminal CH_3 groups of the *n*-alkyl side-chain to the main chain.

Like the T_1 values, the n.O.e. values of branched-chain polysaccharides increase with temperature, but the magnitude of the latter has no apparent correlation with segmental motion (see Table I).

The ^{13}C-n.m.r. spectrum of **1**, determined at 30°, contained a C-1 signal (see Table I) of main-chain unit B, whose line width $\nu_{1/2}$ was 12.3 Hz, a magnitude appreciably different from that of side-chain unit A, which was 8.6 Hz. The difference was less pronounced at 70° (7.1 and 8.4 Hz) and at 90° (6.6 and 6.8 Hz, respectively).

The T_1 values of C-1 of a linear D-mannan (from *Hansenula capsulata*) which contains[16] consecutive (1→2)-, (1→2)-, and (1→6)-linked α-D-mannopyranosyl units N, O, and P (see **5**) were not distinguishable, being 0.14 s at 70° (see Table II).

High T_1 values of ~0.3 s were observed for C-1, C-3, C-4, and C-6 nuclei of an α-D-(1→6)-linked D-mannopyranan[14] at 70 and 90° (see Table I) and for C-1 atoms of β-D-linked side-chains J, K, and L of structure **4** at 70° (see Table II). Although such values do not pose problems for preparation of conventional ^{13}C-n.m.r. spectra, they indicate that determinations of T_1 are necessary prior to quantitation of signal intensities, in order to calculate a suitable delay in an anti-gated experiment[11,12]. Caution is suggested in the use of high-field spectrometers[17] for such experiments on polysaccharides, as T_1 values of nuclei in branched-chain lipids often increase with the magnetic field applied[15].

Measurements of T_1 can be useful in distinguishing resonances of side chains from those of main chains. The C-1 signal of the main chain M of **4** is at δ_C 101.6, and has T_1 = 0.11 s. It is distinguishable from side-chain, C-1 resonances, which have T_1 ~0.3 s. Similarly, the C-2 signal at δ_C 80.6 can be assigned to signals of *O*-substituted C-2, as its T_1 value is 0.21 s [rather than 0.33 s, the value obtained for the side-chain signal of *O*-substituted C-2, at δ_C 80.1 (see Table II)]. The signals of side-chain and main-chain nuclei in an L-rhamno-D-mannan having the repeating unit **6** may also be distinguished in the same way (see Table I).

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