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^{13}C-NMR Investigations of Synthetic Branched Polysaccharides

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SUMMARY

^{13}C-NMR spectra of glucose-branched polysaccharides synthesized by the reaction of 3,4,6tri-O-acetyl-(1,2-O-ethylortho-acetyl)-α-D-glucopyranose with curdlan or cellulose acetate followed by deacetylation were measured and assigned. Although most of the branches are β-(1 → 6) linked, a small amount of α-(1 → 6)linked branches were found.

INTRODUCTION

In our previous papers (Matsuzaki *et al.*, 1985, 1986), we described the synthesis of water-soluble branched polysaccharides from cellulose and curdlan (β-(1 → 3)-glucan) and discussed their antitumor activity. In this paper we wish to report ^{13}C-NMR investigations of those polysaccharides.

Several branched β-(1 → 3)-glucans, such as lentinan and scleroglucan, are found in nature. ^{13}C-NMR spectra of curdlan and lentinan (Saito *et al.*, 1978, 1979) and scleroglucan (Rinaudo & Vincendon, 1982) have previously been reported. There have also been ^{13}C-NMR studies of oligosaccharides of cellulose (Heyraud *et al.*, 1979; Dudley

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et al., 1983) and cellulose dissolved in organic solvents (Gagnaire *et al.*, 1980; El-Kafrawy, 1982). Recently, the CP/MAS (cross-polarization/magic angle spinning) technique has been applied to solid cellulose (e.g. Atalla *et al.*, 1980; Earl & Vanderhart, 1980), but ^{13}C-NMR spectra of branched polysaccharides of cellulose have not been reported.

EXPERIMENTAL

Materials

Water-soluble polysaccharides were synthesized by the reaction of 3,4,6-tri-O-acetyl-(1,2-O-ethylorthoacetyl)-α-D-glucopyranose with curdlan or with cellulose acetate in chlorobenzene at 140°C with dimethyl pyridinium perchlorate as catalyst.

PSCEL-1 and -2 are polysaccharides derived from cellulose acetate with degrees of branching (number of glucose branches per 100 glucosidic residues in the main chain) of 29·7 and 16%, respectively. The degree of branching was determined by the titration of acetyl groups in partly acetylated branched polysaccharides. Methylation analysis revealed that the branches attach to C6, C3 and C2 carbons in a ratio of 78 : 8 : 14 for PSCEL-1 and only to C6 carbons for PSCEL-2, since the polymer was synthesized from 2,3-di-O-acetyl cellulose. 2,3-Di-O-acetyl cellulose was synthesized by acetylation of 6-O-trityl cellulose (Green, 1963) followed by deacetylation.

PSCUR-1 and -2 are polysaccharides derived from curdlan with 63·7% and 27·1% of degree of branching, respectively. The methylation analysis revealed that the position of branches is at C6/C4 = 94·4/5·6 for PSCUR-1. For PSCUR-2, it is deduced that the branches mainly attach to C6 carbons of glucosidic residues in the main chain as well as PSCUR-1.

The structures of these two types of branched polysaccharides are shown in Figs 1 and 2. Letters are assigned to the sugar units to facilitate the later discussion of the origin of the chemical shifts.

Methods

NMR spectra were determined on DMSO-d_6 solutions (conc. *c.* 10 wt%) with a JEOL-GX-400 FT spectrometer at 100·40 MHz using

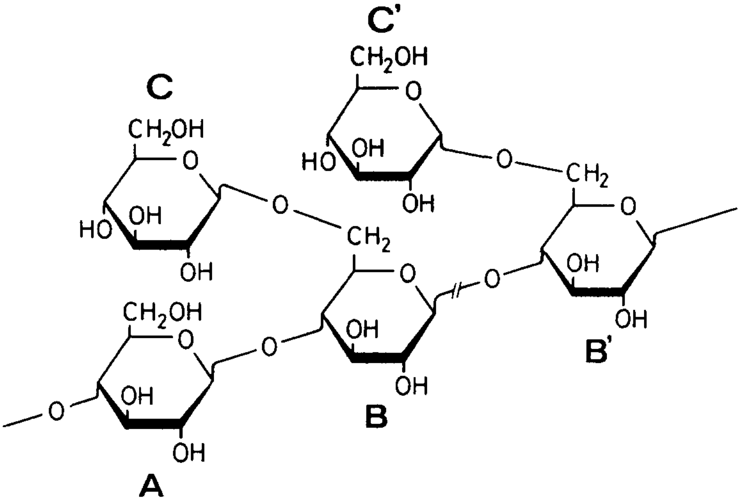

Fig. 1. Structure of branched polysaccharides synthesized from cellulose acetate.

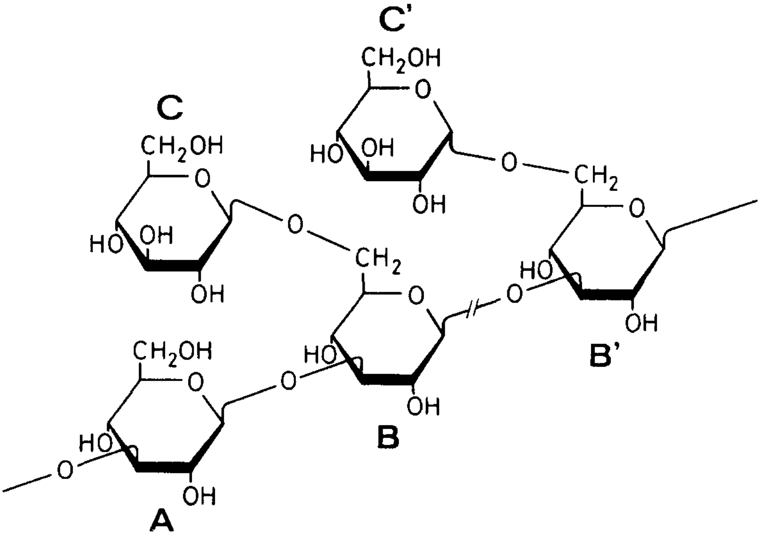

Fig. 2. Structure of branched polysaccharides synthesized from curdlan.

the following conditions: pulse angle 45°, pulse length 5 ms, with single pulse bimodal complete noise decoupling, data points 32 768 and apodisation at 90%, with 10 000–50 000 scans. Since the concentration of polymer was low (*c*. 5%) in the case of PSCEL-1, considerable noise is seen in the spectrum.

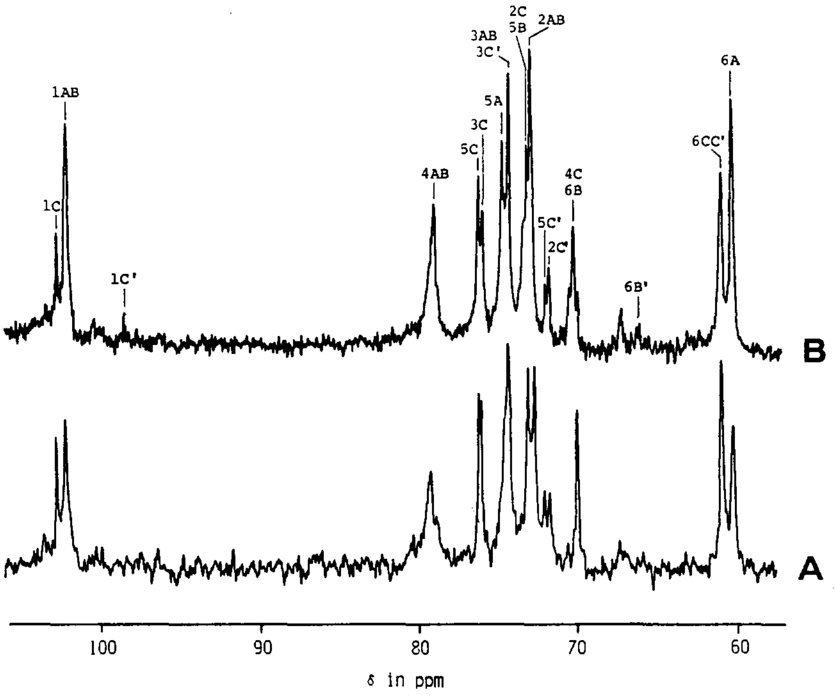

Fig. 3. ^{13}C-NMR spectra of branched polysaccharides synthesized from cellulose acetate, measured in DMSO-d_6 at 140°C (DMSO-d_6 = 39·54 ppm): A, PSCEL-1; B, PSCEL-2. Assignments for the lower spectrum are the same as the upper one.

The chemical shifts in the figures were obtained using a reference value for DMSO-d_6 of 39·54 ppm. When the NMR measurements were carried out at 140°C or 70°C, the chemical shifts were corrected by adding 0·97 or 0·35 ppm, respectively, as the temperature coefficient is 0·088 ppm °C^{-1} (K. Hatada, Faculty of Engineering Science, Osako University, pers. comm.).

RESULTS AND DISCUSSION

^{13}C-NMR spectra of the branched polysaccharides obtained from cellulose acetate

Figure 3 shows ^{13}C-NMR spectra of PSCEL-1 and -2. The assignments were carried out by comparison of the spectra of PSCEL-1 and

TABLE 1

^{13}C-NMR Chemical Shifts of Branched Polysaccharides Synthesized from Cellulose Acetate[a]

	Methyl glucoside[b]		*Cellohexaose*[c]	*PSCEL-1*	*(1→6)-Glucan*[d]
	Alpha	*Beta*			
C1 AB			103·2	102·99	
C		104·0	103·4	103·60	102·78
C′	100·0			99·5	99·25
C2 AB			73·7	73·02	
C		74·1	73·9	74·15	
C′	72·2			73·02	
C3 AB			74·8	75·34	
C		76·8	76·3	77·01	
C′	74·1			75·34	
C4 AB			79·0	80·00	
C	70·6	70·6	70·3	71·23	
C5 AB			75·6	75·74	
C		76·8	76·8	77·28	
C′	72·5			72·81	
C6 A			60·4	61·34	
B				71·23	68·31
B′				67·3	66·7
C		61·8	61·4	62·07	
C′	61·6			62·07	

[a] C and C′ indicate carbons in β- and α-(1→6)-linked glucose branch units, respectively.

[b] Cf. Bock & Pedersen (1983).

[c] Chemical shifts in lines AB indicate those of internal units and those in lines C are those of non-reducing units of cellohexaose.

[d] The chemical shifts of C1 C and C6 B are those of C1 and C6 carbons of β-(1→6)-glucan (pustulan) (authors' data) and those of C1 C′ and C6 B′ are those of α-(1→6)-glucan (Colson *et al.*, 1974).

-2 and the chemical shifts of oligosaccharides of cellulose (Dudley *et al.*, 1983) and are shown in Table 1. It is seen in Fig. 3 that the intensity of absorptions of carbons (denoted by C and C′) in the glucose units belonging to the side chains increased as the degree of branching increased from PSCEL-2 to PSCEL-1. The chemical shifts of carbons

(1C–6C of PSCEL) in β-glycosidically linked glucose branches coincide well with those in the non-reducing units of cellohexaose.

Small absorptions at 66·3 and 98·5 ppm in Fig. 3 (corrected values are 67·3 and 99·5 ppm, respectively) are assigned to α-(1→6) linked 6B′ carbons in the main chain units and 1C′ carbons in side chain units, respectively, by comparison with the chemical shifts of α-(1→6)-glucan (Colson *et al.*, 1974). Therefore, the absorptions at 71·84 and 72·05 ppm (corrected values are 72·81 and 73·02 ppm, respectively) are assigned to 2C′ and 5C′ carbons in α-glycosidically linked glucose branches, respectively, by comparison with the chemical shifts of α-methyl glucoside, as shown in Table 1. Although glycosidation by α-orthoesters generally gives β-glycosides (Wulff & Röhle, 1974), a small amount of α-glycoside is formed in this reaction. The absorption at 67·5 ppm may be related to that of 100·4 ppm, but assignments of those absorptions could not be made.

^{13}C-NMR spectra of the branched polysaccharides obtained from curdlan

Figure 4 shows a ^{13}C-NMR spectrum of PSCUR-1. The assignments were carried out by comparison of the spectra of PSCUR-1 and -2 and scleroglucan. Table 2 lists the chemical shifts. They are mostly the same as those reported by Rinaudo & Vincendon (1982), except the absorption at 74·33 ppm (corrected value 74·68 ppm) which was assigned to 5B carbon in the glucose units with branches at C6 carbon. The reason is that this assignment accords the rule that the shift to higher field occurs with the glycosidation of the neighboring carbon, that is, C6. In addition, this absorption increased together with 6C, 4C and 2C carbons as the degree of branching increased.

Small absorptions at 66·0 and 98·53 ppm (corrected values are 66·35 and 98·88 ppm, respectively) are also ascribed to 6B′ and 1C′ as well as PSCEL-1 and -2. The absorptions of carbons (2C′–5C′) in α-linked glucose branches may be overlapped with absorptions of 2C and 5C carbons (73·5–75 ppm), except 4C′ which may be overlapped with absorption of 4C.

A feature of the spectrum is the singlet absorption of C-3-O-Glc (3AB carbon), which is contrary to the triplet absorptions observed in scleroglucan. This is due to the random distribution of branches and clearly indicates that the natural polysaccharide (scleroglucan) has a regular structure as Rinaudo & Vincendon proposed.

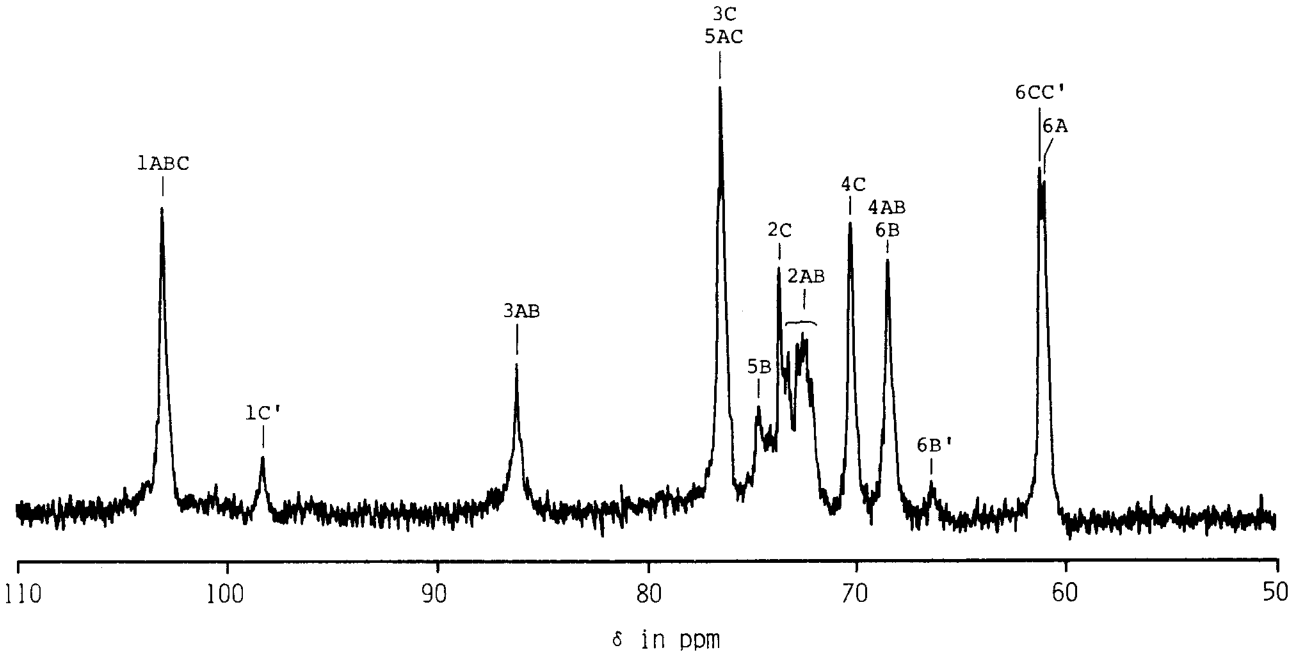

Fig. 4. ^{13}C-NMR spectrum of branched polysaccharide synthesized from curdlan, measured in DMSO-d_6 at 70°C. Sample: PSCUR-1.

TABLE 2
^{13}C-NMR Chemical Shifts of Branched Polysaccharide Synthesized from Curdlan

	Pustulan[a]	*PSCUR-1*[b]	*β-(1→3)-glucan*[c]
C1 AB		103·54	103·7
C	103·75	103·54	
C′		98·88	
C2 AB		73·0–73·5	73·6
C	74·11	74·18	
C3 AB		86·75	86·9
C	77·28	76·47	
C4 AB		69·01	69·2
C	71·13	70·76	
C5 A		76·47	77·1
B		74·68	
C	76·07	76·47	
C6 A		61·46	61·7
B	69·28	69·01	
B′		66·35	
C		61·69	
C′		61·69	

[a] Author's data.
[b] C and C′ indicate carbons in β- and α-(1→6)-linked glucose branch units, respectively.
[c] Cf. Rinaudo & Vincendon (1982).

In Table 2, the chemical shifts of pustulan are also given, in which absorptions at 77·28 and 76·07 ppm were assigned to C3 and C5 carbons, respectively, since this assignment is also consistent with the general rule that the substitution of neighboring carbon (C6) with glucose units gives a shift to higher field.

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