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NMR characterization of a water-soluble 1,4-linked β-D-glucan having ether groups from yellow mustard (*Sinapis alba* L.) mucilage

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A water soluble 1,4-linked β-D-glucan isolated from yellow mustard mucilage was characterized by one- and two-dimensional NMR spectroscopy. The complete assignment of the ^{1}H and ^{13}C resonances was obtained with the assistance of a heteronuclear shift correlated experiment. The connectivities within the residue were established through homonuclear correlation (COSY) while NOE correlation confirmed the 1,4-linked backbone chain structure. In addition, *O*-ethyl and *O*-propyl groups were found linked at C2, C3 and C6 positions in some of the glucosyl residues along the cellulose-like backbone chain.

INTRODUCTION

A water-soluble 1,4-linked β-D-glucan (WSCS-I) isolated from yellow mustard (*Sinapis alba* L.) mucilage exhibited pronounced shear thinning behavior in aqueous solution at 0·5% (Cui *et al.*, 1994). Natural cellulose of high molecular weight is usually insoluble in water. Introduction of ether or ester groups on the cellulose backbone chain could improve the physical properties such as solubility, crystallization, gel formation, liquid-crystal formation and resistance to enzymatic degradation (Kondo & Gray, 1991). There is little information available on the chemical structure of naturally occurring water-soluble cellulose. An examination of the chemical structure of this water-soluble cellulose-like polysaccharide from yellow mustard mucilage will be useful in understanding its physical properties, particularly the solubility and rheological properties of its aqueous solutions/dispersions. This paper focuses on the structural characterization of this unusual cellulosic polysaccharide by one- and two-dimensional NMR spectroscopy.

MATERIALS AND METHODS

Materials

WSCS-I was isolated by ion exchange chromatography as described previously (Cui *et al.*, 1994). The linkage pattern in this polysaccharide was determined by methylation analysis as reported previously (Cui *et al.*, 1994). All chemicals were reagent grade unless otherwise specified.

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NMR Spectroscopy

NMR spectra were recorded on a Bruker AMX500 spectrometer in 4% polymer solutions in D_2O (5 and 10 mm tube). Internal *p*-dioxane was used as a chemical shift reference for ^{13}C spectra. Reported values have been converted to the TMS (Tetra Methyl Silane) scale. Sample temperature was controlled at 65°C for all spectra.

Homonuclear correlation (COSY) spectra (Aue *et al.*, 1976), and NOE (nuclear Overhauser effect) correlation (NOESY) spectra (Bodenhausen *et al.*, 1985) were recorded with F2 time domains of 1024 points and F1 time domains of 256 points. Zero filling in F1 yielded a 512 (real) matrix after transformation. A 90° mixing pulse was employed for the COSY spectra. A 100 ms mixing time was employed for the NOESY spectra. COSY spectra were recorded in the magnitude mode while NOESY spectra were recorded in the phase sensitive mode employing time proportional phase increments for F1 quadrature detection. Heteronuclear correlation spectra were recorded with the proton detected single quantum coherence (HSQC) experiment (Bodenhausen & Ruben, 1980), with an F2 time domain of 4096 points and F1 time domain of 256 points. Zero

filling in F1 and F2 resulted in a 4096 (real) by 512 (real) matrix after transformation.

RESULTS AND DISCUSSION

^{1}H and ^{13}C NMR analyses of WSCS-I

The ^{1}H and ^{13}C NMR spectra of WSCS-I are shown in Fig. 1. Six major signals were observed for the ^{13}C NMR spectrum which correspond to the 6 carbons of the repeating 1,4-linked β-D-glucosyl residues: 103·08 ppm, C1; 73·84 ppm, C2; 74·95 ppm, C3: 79·36 ppm, C4; 75·67 ppm, C5; and 60·96 ppm, C6 (Defaye *et al.*, 1983; Bock *et al.*, 1984). In addition to the sugar carbon signals there were a number of minor resonances in the regions of 9–21, 45–60 and 65–70 ppm. This observation suggests that WSCS-I may have some branches. According to the literature, the observed non-sugar resonances are probably caused by ether, ester or cyclic acetal derivatives of the cellulose which are not common in naturally occurring cellulosics (Tezuka *et al.*, 1991; Kondo & Gray, 1991; Iwata *et al.*, 1992; Isogai *et al.*, 1993; Tezuka, 1993). The absence of signals in the region 150–190 ppm suggested that those non-sugar signals (Fig. 1A) were caused by ethers of the cellulose-like polymer because esters should have strong carbonyl signals in this region. Resonances in the ^{1}H NMR spectrum of WSCS-I appeared to be difficult to assign due to poor resolution and heavy overlap in the resonance region for sugars (3–4·5 ppm, Fig. 1B). Three strong signals at 1–1·5 ppm, however, can be attributed to $-CH_3$ groups of the ethers (Fig. 1B).

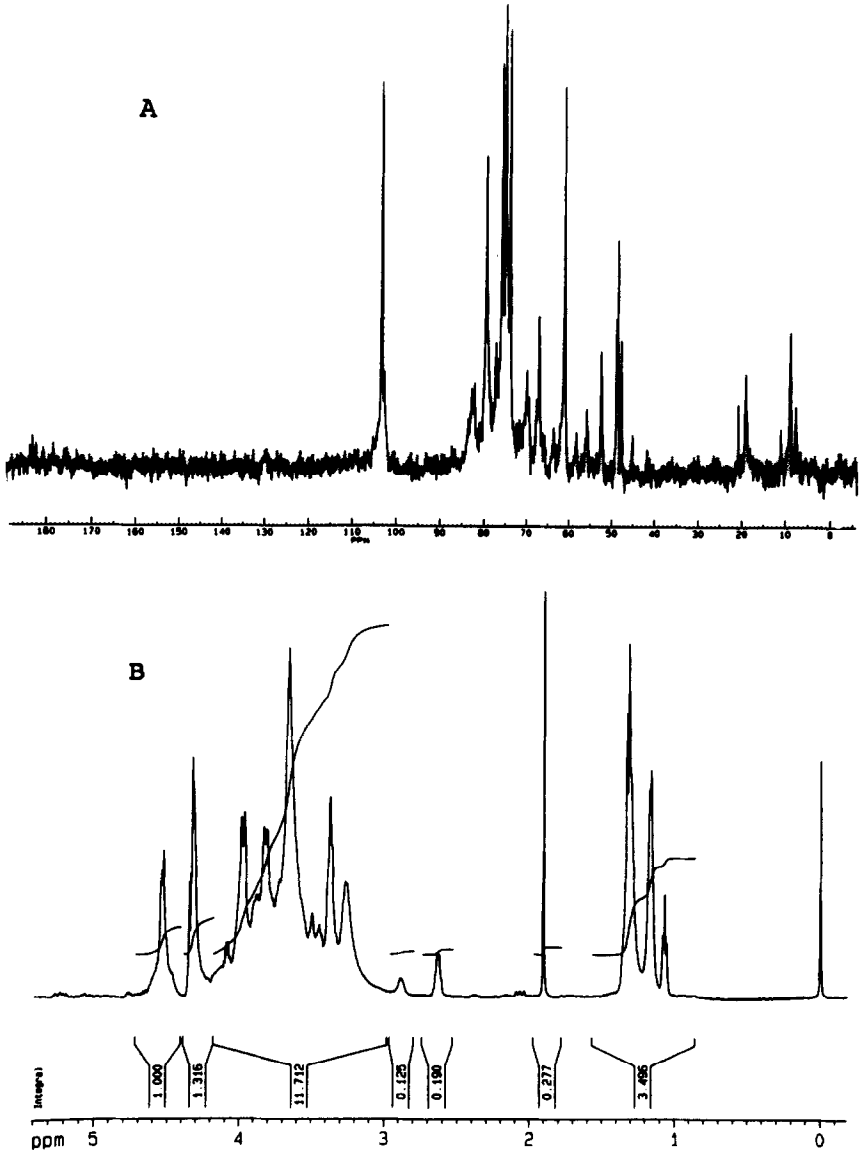

Fig. 1. ^{13}C (A) and ^{1}H (B) spectra of a water-soluble 1,4-linked β-D-glucan from yellow mustard mucilage (4% polymer in D_2O, 65°C).

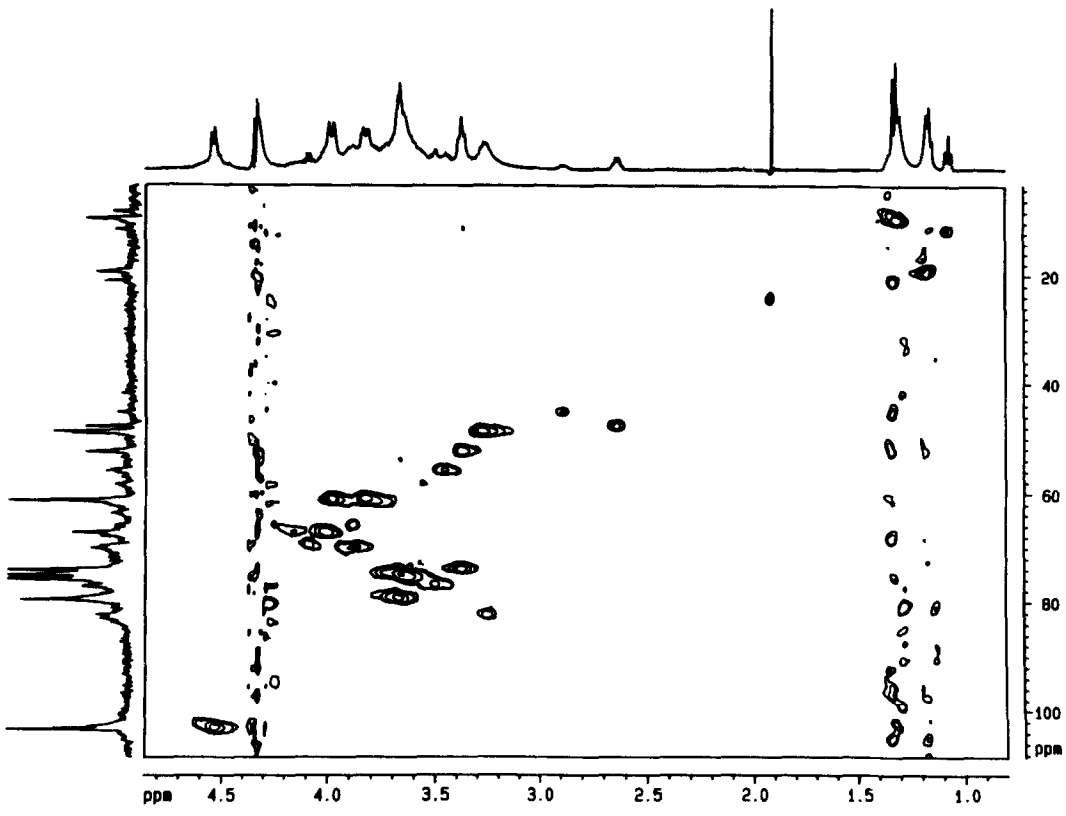

Fig. 2. H/C heteronuclear correlation NMR spectrum of a water-soluble 1,4-linked β-D-glucan from yellow mustard mucilage (4% polymer in D_2O, 65°C).

H/C correlation of WSCS-I

A $^1H/^{13}C$ correlation experiment was done to resolve the overlap problem, and the result is shown in Fig. 2. Spectrum resolution was significantly improved in Fig. 2 compared to Fig. 1B because the signals were spread out over the two-dimensional domain. The chemical shift at 4·54 ppm in the ^1H spectrum correlated with a signal at 103·08 ppm of the ^{13}C spectrum and, therefore, can be attributed to the anomeric proton at the C1 position. In a similar manner, all the signals in the ^1H spectrum can be assigned to their corresponding counterparts in the ^{13}C spectrum. A complete assignment of the resonances of the ^1H and ^{13}C NMR spectra was obtained as shown in Table 1 (sugar ring ^1H and ^{13}C resonances) and Table 2(non-sugar ^1H and ^{13}C resonances), respectively. The $^1H/^1H$ coupling constant of the anomeric proton was 7·5 Hz (Table 1) which confirmed that the anomeric carbon was in β configuration (Iwata *et al.*, 1992). As shown in Table 2, non-sugar signals were classified into three groups according to their chemical shift (Group 1: ^1H 1·07–1·32 ppm, ^{13}C 9–21 ppm; Group 2: ^1H 2·65–3·4 ppm, ^{13}C 44–55 ppm; Group 3: ^1H 3·2–4·1 ppm, ^{13}C 63–70 ppm). The signals in the first group were attributed to $-CH_3$ of the ether moiety, signals in the second group could be caused by $-CH_2-$ in the propyl and/or $-CH_2-O$ of ethyl at position 3 of some of the glucosyl residues. Signals in the third group could arise from $-CH_2-O$ of the ethyl and propyl moieties (Kondo & Gray, 1991; Tezuka *et al.* 1992; Isogai *et al.*, 1993).

COSY Spectrum of WSCS-I

COSY was carried out to establish the connectivity of the signals. The intraresidue connectivities of the sugar

Table 1. Complete assignment of the major resonances of ^1H and ^{13}C spectra of WSCS-I

	1(C, H)	2(C, H)	3(C, H)	4(C, H)	5(C, H)	6(C, H)
C (ppm)	103·08	73·84	74·95	79·36	75·67	60·96
H (ppm)	4·54	3·38	3·65	3·66	3·52	3·82, 3·98
	$J_{1,2}$ 7·5 Hz					

$J_{1,2}$, coupling constant of protons and 1 and 2 positions.

Table 2. C/H correlations and assignment of non-sugar resonances

^{1}H (ppm)	^{13}C (ppm)	Assignment[a]	Position assigned on glucose ring
1·07	11·0	a: **CH_3**-CH_2-O	3
1·17	19·22	b: **CH_3**-CH_2-O	2
1·32	21·0	c: **CH_3**-CH_2-O	6
1·32	9·04	d: **CH_3**-CH_2-CH_2-O	6
2·65	47·65	a: CH_3-**CH_2**-O	3
3·27	48·50	d: CH_3-**CH_2**-CH_2-O	6
3·98	66·96	b: CH_3-**CH_2**-O	2
4·05	69·50	c: CH_3-**CH_2**-O	6
3·88	69·79	d: CH_3-CH_2-**CH_2**-O	6

[a]a, b, c and d correspond to different ether groups while the groups in bold indicate the proton and carbon which are responsible for the assigned resonances.

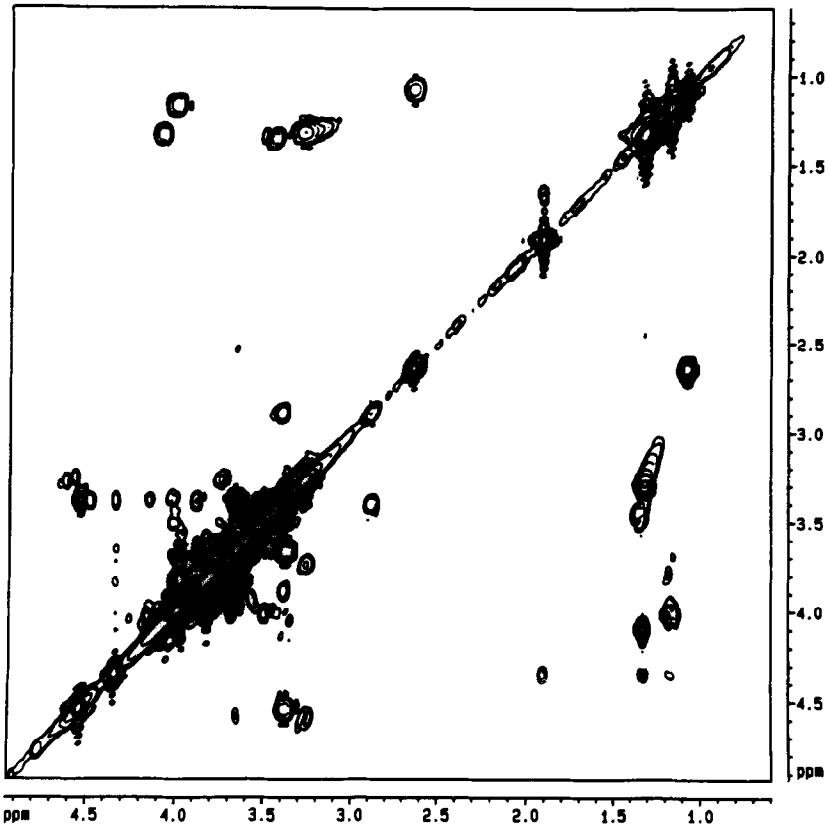

Fig. 3. Homonuclear shift correlated spectrum (COSY) of a water-soluble 1,4-linked β-D-glucan from yellow mustard mucilage (4% polymer in D_2O, 65°C).

ring start from H1->H2->H3->H4->H5->H6, as shown in Fig. 3. The connectivity of H3 and H4 cannot be seen because the two signals are too close (3·65 and 3·66 ppm, respectively). Four ether groups were identified and their connectivity established for the non-sugar signals as shown in Table 2. Ether groups a, b and c (Table 2) have the same chemical formula (CH_3CH_2-O-) but are different in chemical shifts in both ^{1}H and ^{13}C NMR spectra. These changes in chemical shift of the ethyl groups may suggest that they are attached to different sites on the glucose residues (i.e. C2, C3, C6).

NOESY of WSCS-I

The NOESY spectrum of WSCS-I, shown in Fig. 4, provides information on the intra- and inter-residue connectivities based on dipole correlation (through

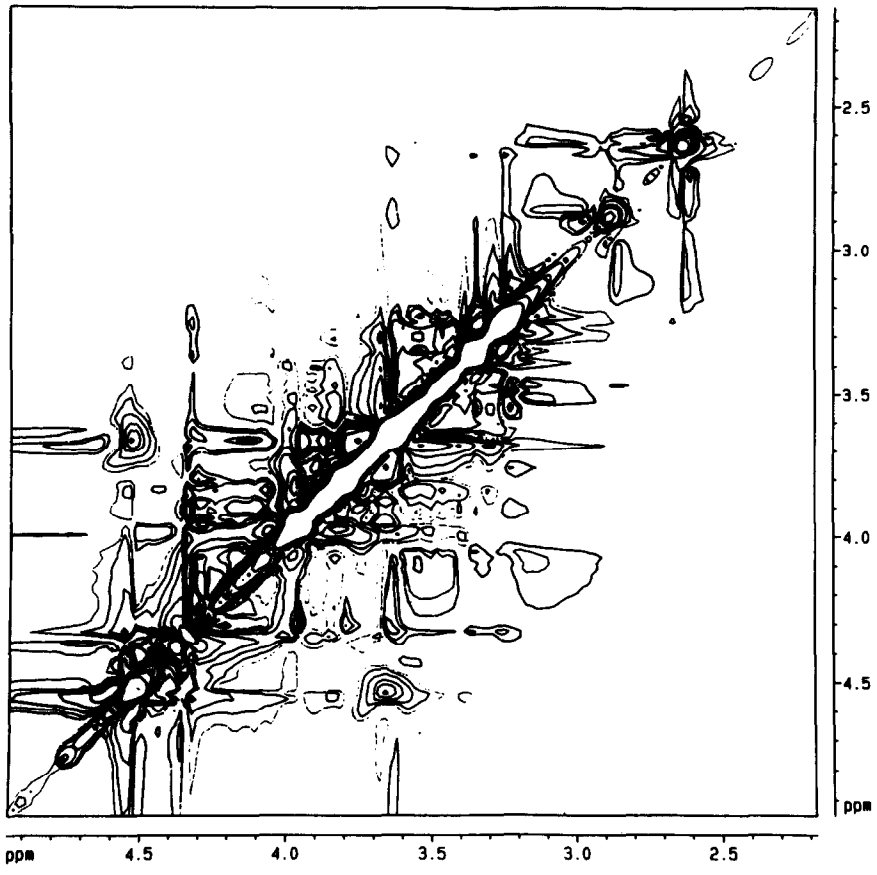

Fig. 4. NOESY of a water-soluble 1,4-linked β-D-glucan from yellow mustard mucilage (4% polymer in D_2O, 65°C).

space) (Dabrowski, 1987; Martin & Zektzer, 1988). Strong correlation was observed between ^{1}H1 and ^{1}H4. This is an inter-residue correlation which confirms that the β-D-glucose was 1,4-linked in the polymer (Cui *et al.*, 1994). From the NOESY spectrum correlations were also observed between ethyl a and ^{1}H3; ethyl b and ^{1}H2; ethyl c and ^{1}H6; and propyl ether d and ^{1}H6. This observation suggests that the ethyls were linked at positions 2, 3 and 6 while the propyl only could be found at position 6 of the glucosyl residues.

In summary, WSCS-I is composed of a 1,4-β-D-glucose backbone chain. Some of the hydroxyl groups at C2, 3 and 6 are occasionally substituted by ether groups (ethyl and propyl). The ethyl group was randomly distributed in the C2, 3 and 6 positions while the propyl ether was predominant at the 6 position (Fig. 4 and Table 2). These ether groups along the cellulose-like backbone chain of WSCS-I may act as 'kinks'. These 'kinks' can alter the conformational regularity of the 1,4-linked β-D-glucose backbone chain favouring the solubilization of the polymer in an aqueous medium. Alternatively, for steric reasons, these groups will hinder interchain associations among the cellulose chains, thus enhancing solubility of the polysaccharide.

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