ELSEVIER

Carbohydrate Research 292 (1996) 173–183

CARBOHYDRATE RESEARCH

Note

NMR characterization of a 4-*O*-methyl-β-D-glucuronic acid-containing rhamnogalacturonan from yellow mustard (*Sinapis alba* L.) mucilage

Wuwei Cui [a,*,1], Michael N.A. Eskin [a], Costas G. Biliaderis [b], Kirk Marat [c]

[a] *Department of Foods and Nutrition, University of Manitoba, Winnipeg, MB, Canada R3T 2N2*
[b] *Department of Food Science and Technology, School of Agriculture, Box 256, Aristotle University, Thessaloniki, 54006, Greece*
[c] *Department of Chemistry, University of Manitoba, Winnipeg, MB, Canada R3T 2N2*

Received 22 May 1995; accepted 17 August 1995

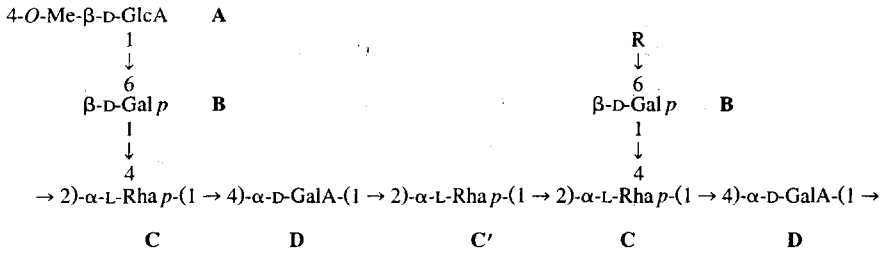
Keywords: Yellow mustard mucilage; Pectic polysaccharides; NMR spectra; Structure

Water-soluble yellow mustard mucilage is a heterogeneous mixture of neutral and acidic polysaccharides [1–4]. Recent interest in the mucilage of yellow mustard seed is attributed to its unique rheological behaviour in solutions/dispersions and its ability to interact with galactomannans synergistically, which may have commercial potential in the food hydrocolloids industry [3–5]. In previous studies, the water-soluble yellow mustard mucilage (WS-YMM) was fractionated into ten fractions by a combination of cationic-detergent precipitation and DEAE-cellulose ion-exchange chromatography [4]. Of the ten fractions obtained, two neutral (WSCP-I and WSCS-I) and one acidic (WSCP-III) polysaccharide fractions appeared to be responsible for the pronounced shear thinning flow behaviour of the WS-YMM in aqueous solutions/dispersions [4]. Methylation analysis and NMR spectroscopy revealed that WSCS-I contained a 4-linked β-D-glucose backbone chain with occasional substitutions of ethyl and/or propyl groups at the 2, 3, or 6 positions of the glucose residue [6]. The results of a preliminary study

* Corresponding author.
[1] Current address: Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada K1A 0C6.

PII S0008-6215(96)00158-9

suggested that the WSCP-III fraction was a pectic polysaccharide composed of 2,4-linked and 2-linked L-rhamnose, 6-linked D-galactose, terminal non-reducing D-glucuronic acid, and 4-linked D-galacturonic acid [4]. This paper reports the elucidation of the structure of the WSCP-III fraction by methylation analysis, NMR spectroscopy, and partial hydrolysis. As a result, a possible average repeating unit is proposed for WSCP-III as follows.



where R = mostly 4-*O*-Me-β-D-GlcA-(1 → 6)-β-D-Gal *p*-(1; occasionally 4-O-Me-β-D-GlcA(1 → 2)-β-D-Gal *p*-(1.

Methylation analysis revealed that the purified WSCP-III was composed of terminal non-reducing D-glucuronic acid, 4-linked-D-galacturonic acid, 6-linked-D-galactose, and 2,4-linked and 2-linked L-rhamnose in the ratio of 0.9:0.8:1.5:1:0.5 together with a trace amount of 2-linked D-galactose. This result is in general agreement with earlier studies on WSCP-III in a less purified form [4].

Four signals were observed in the anomeric region of the ^{13}C NMR spectrum of WSCP-III, which suggests the presence of at least four anomeric carbons in the polymer (Fig. 1). This observation is in agreement with the result from methylation analysis in which two neutral sugars and two uronic acids were identified. Signals at δ 176.22 and 175.39 originate from the carbonyl groups of the terminal non-reducing D-glucuronic acid and 4-linked D-galacturonic acid, respectively. The signal at δ 60.35 can be attributed to an *O*-methyl group attached at the 4 position of the D-glucuronic acid. Identification of the 4-*O*-methyl group was achieved by comparison of the observed spectrum against literature values [7,8]. Two signals at high field, δ 17.85 and 17.50, are in the approximate intensity ratio of 2:1, which can be attributed to the $-CH_3$ (C-6) group of the 2,4-linked and 2-linked rhamnose, respectively. This ratio is in good agreement with the result of methylation analysis of the two residues, as described earlier. A complete assignment of the $^1H-^{13}C$ spectra of the WSCP-III was achieved by $^1H-^{13}C$ heteronuclear-correlated spectroscopy and COSY, as well as by comparison of the observed data against literature values (Table 1).

A full assignment of the ^1H and ^{13}C signals of the 4-*O*-methyl-β-D-glucuronic acid (**A**) is shown in Table 1. The H-1 (4.48 ppm), C-1 (103.40 ppm) signals and the $J_{H1,2} \sim 8$ Hz established that it has the β configuration. A signal at δ 60.35 in the ^{13}C spectrum correlated with a strong single resonance at δ 3.50 in the proton spectrum, which is assigned to the *O*-methyl group. The connectivity from H-1 to H-5 was clearly established from the COSY spectrum. The resonance of C-4 at δ 82.80 indicated that

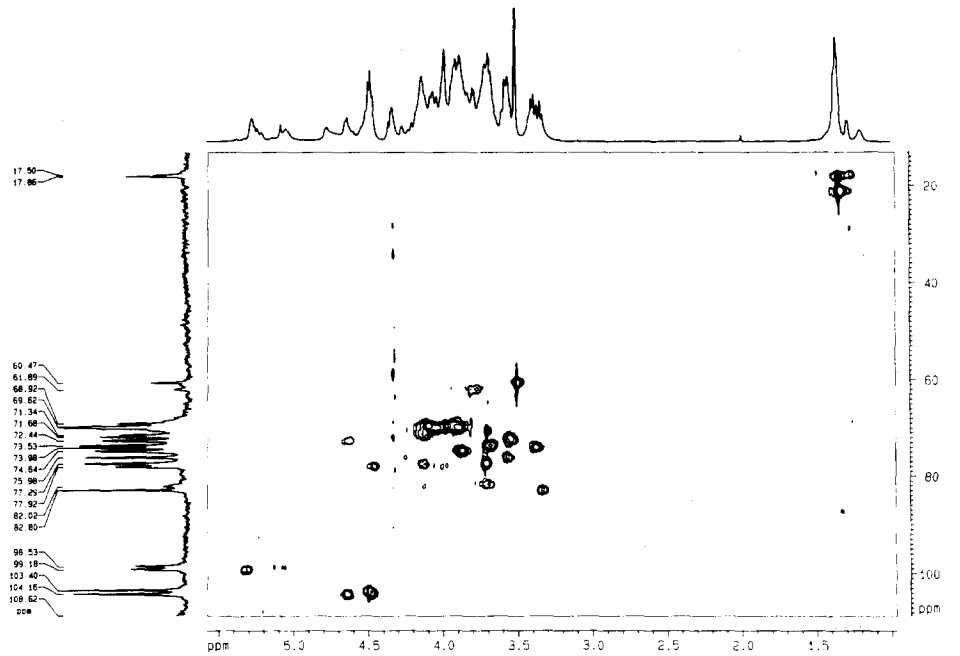


Fig. 1. H/C correlation spectrum of WSCP-III. The spectrum was recorded at 65 °C with 4% polymer concentration in D_2O.

the *O*-methyl group was linked at this position, which otherwise would be in the range of 72 to 74 ppm [8,9]. All of these assignments are in agreement with literature values reported for a 4-*O*-methyl or other of 4-substituted β-D-glucuronic acid [7–9].

A complete assignment of the NMR signals of the 6-linked β-D-galactose (**B**) is also summarised in Table 1. The β configuration at position 1 was evidenced by the signals at 4.64 (H-1), 104.16 ppm (C-1), and $J_{H1,2} \sim 8$ Hz. The connectivity from H-1 and H-6 was established from the COSY analysis. All signals assigned are in agreement with literature values of 6-linked β-D-galactose [10,11]. A signal at δ 3.78 (^{1}H) and 61.89 (^{13}C) was assigned to H-6 of 2-linked D-galactose, although other resonances originating from this residue could not be assigned due to overlap with signals of the 6-linked residue. Signals originating from 2,4-linked α-L-rhamnose (**C**) are assigned as shown in Table 1. Signals at δ 5.31 (H-1) and 99.18 (C-1) confirmed the α configuration, while the pyranosyl ring connectivity was established by COSY analysis. These assignments agreed well with literature values for 2,4-linked α-L-rhamnose [7,12–15]. The complete assignments of signals from the 2-linked α-L-rhamnose were obtained by COSY analysis, starting from the H-6 (δ 1.28) which correlated to H-5 (δ 3.84). This assignment is also in agreement with literature values [8,14,15].

Finally, a complete assignment of the signals originating from the 4-linked α-D-galacturonic acid was obtained by comparison of the observed chemical shifts against literature values for this residue [8,12]. The α configuration was established by signals at δ 5.05 (H-1) and 98.53 (C-1). The correlations between H-1 signals of the

Table 1
^{1}H and ^{13}C NMR data and assignments for polysaccharide WSCP-III

Residue		Chemical shift (δ /ppm)				
		Proton	Reference		Carbon-13	Reference
4-*O*-Me-β-D-Glc*p*A-(1 →	H-1	4.48		C-1	103.40	
	H-2	3.38	[7]	C-2	73.98	[7]
A	H-3	3.57	[8]	C-3	75.98	[8]
	H-4	3.33		C-4	82.80	
	H-5	3.70		C-5	77.29	
				C-6	176.22	
	H-4-*O*-Me	3.50		C-4-*O*-Me	60.48	
→ 6)-β-D-Gal*p*-(1 →	H-1	4.64		C-1	104.16	
	H-2	3.54	[10]	C-2	72.44	[10]
B	H-3	3.66	[11]	C-3	73.53	[11]
	H-4	3.91		C-4	69.72	
	H-5	3.88		C-5	74.64	
	H-6	3.98		C-6	69.62	
		3.76				
↓	H-1	5.31		C-1	99.16	
4	H-2	4.12	[12]	C-2	77.29	[12]
→ 2)-α-L-Rha*p*-(1 →	H-3	3.98		C-3	69.62	
	H-4	3.68		C-4	82.02	
C	H-5	4.10		C-5	71.48	
	H-6	1.30		C-6	17.75	
→ 2)-α-L-Rha*p*-(1 →	H-1	5.29		C-1	99.18	
	H-2	4.12	[11]	C-2	77.29	[15]
C′	H-3	3.98	[15]	C-3	69.62	
	H-4	3.68		C-4	73.98	
	H-5	4.10		C-5	69.62	
	H-6	1.28		C-6	17.50	
→ 4)-α-D-Gal*p*A-(1 →	H-1	5.05		C-1	98.53	
	H-2	3.95	[12]	C-2	69.62	[12]
D	H-3	4.12	[13]	C-3	71.34	[13]
	H-4	4.44		C-4	77.92	
	H-5	4.62		C-5	72.49	
				C-6	175.39	

α-L-rhamnose and the α-D-galacturonic acid (δ 5.31 and 5.05 respectively) and the H-2 signals (δ 4.12 and 3.68, respectively) were not observed in the COSY spectrum of WSCP-III, which created difficulties during the assignment. However, this problem was overcome by comparing the chemical shifts with literature values, and by NOESY analysis (Fig. 2 and Table 2), in which clear correlations were observed for those anomeric resonances.

2D NOESY is a dipole correlated experiment concerning through-space coupling. By examination of the anomeric region of the NOESY spectrum of WSCP-III (Fig. 2), linkage sites and sequence of the identified residues were determined, as shown in Table

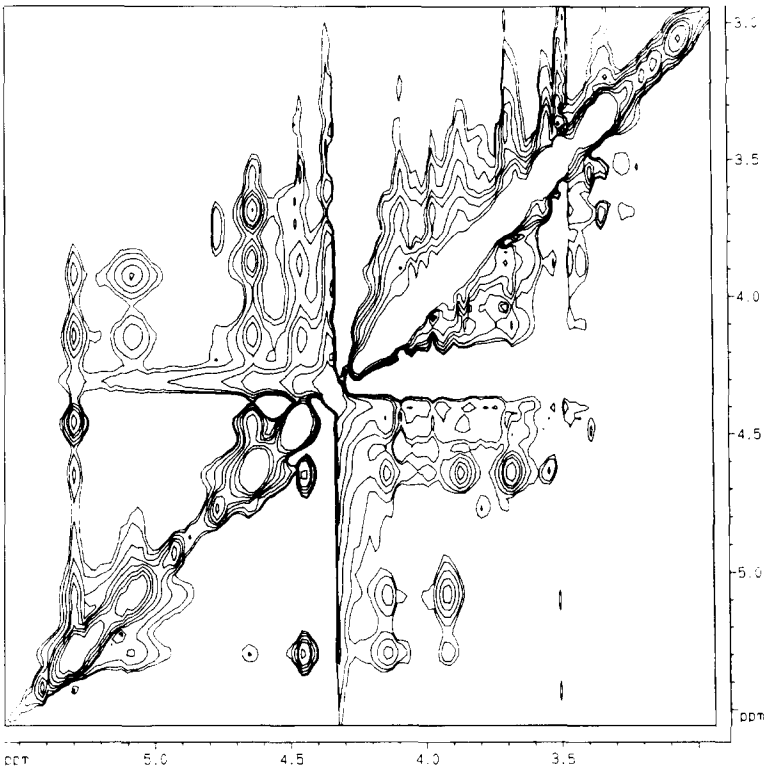


Fig. 2. NOESY spectrum of WSCP-III recorded at 65 °C in D_2O.

2. The anomeric resonance (δ 4.48) of the terminal non-reducing 4-*O*-Me-β-D-Glc*p*A (residue **A** in Tables 1 and 2) correlated to the H-6 (δ 3.98) of the 6-linked β-D-Gal*p* (residue **B** in Tables 1 and 2). NOE correlations were observed between H-1 (δ 4.64) of

Table 2
Observed NOE contacts from anomeric protons of polysaccharide WSCP-III

Anomeric proton (ppm)			NOE contacts to(ppm)		
4.48	**A**	4-*O*-Me-β-D-Glc*p*A-(1 →	3.98	**B**	H-6
4.64	**B**	→ 6)-β-D-Gal*p*-(1 →	3.68	**C**	H-4
5.31	**C** [a]	R ↓ 4 → 2)-α-L-Rha*p*-(1 →	4.12 4.44	**C** **D**	H-2 H-4
5.05	**D**	→ 4)-α-D-Gal*p*A-(1 →	4.12	**C**	H-2

[a] R = H, the structure is **C′**, without the 4-linkage. R = **B**, the structure is **C**, with the 4-linkage.

Table 3
^{1}H and ^{13}C NMR data and assignment of three oligosaccharide alditols derived from polysaccharide WSCP-III

Residue		Chemical Shift (δ/ppm)						
		F-I ($J_{1,2}$)	F-II ($J_{1,2}$)	F-III ($J_{1,2}$)		F-I	F-II	F-III
4-*O*-Me-β-D-Glc *p*A-(1 →	H-1	4.49 (8 Hz)	4.48 (8 Hz)	4.46 (8 Hz)	C-1	103.60	103.60	103.00
	H-2	3.38	3.39	3.38	C-2	73.98	73.80	73.80
	H-3	3.57	3.56	3.57	C-3	76.00	76.00	76.00
	H-4	3.34	3.34	3.33	C-4	82.69	83.00	83.00
	H-5	3.70	3.71	3.72	C-5	77.29	77.35	77.30
					C-6	(176.22)	(176.22)	(176.22) [a]
	H-4-*O*-Me	3.50			C-4-*O*-Me	60.54	60.52	60.35
→ 6)-β-D-Gal *p*-(1 → (F-I)	H-1	4.64 (8 Hz)	3.70	3.74	C-1	104.16	64.16	63.47
	H-2	3.54	3.95	4.00	C-2	72.44	72.50	72.40 [b]
→ 6)-D-Galactitol (F-II)	H-3	3.66	3.96	3.70	C-3	73.53	74.04	71.71
	H-4	3.91	3.72	3.68	C-4	69.72	71.00	71.90
→ 2)-D-Galactitol (F-III) [b]	H-5	3.88	4.15	3.84	C-5	74.64	74.00	72.00
	H-6	3.98	3.98	3.65	C-6	69.62	69.50	63.47
		3.76	3.68					
→ 4)L-Rhamnitol	H-1	3.73			C-1	64.17		
	H-2	3.94			C-2	70.71		
	H-3	3.98			C-3	69.62		
	H-4	3.84			C-4	75.95		
	H-5	4.14			C-5	69.90		
	H-6	1.29			C-6	17.50		

[a] Resonance was not determined, because the ^{13}C resonances were obtained from proton detected spectrum. Data presented were from the polysaccharide.
[b] Tentative assignment.

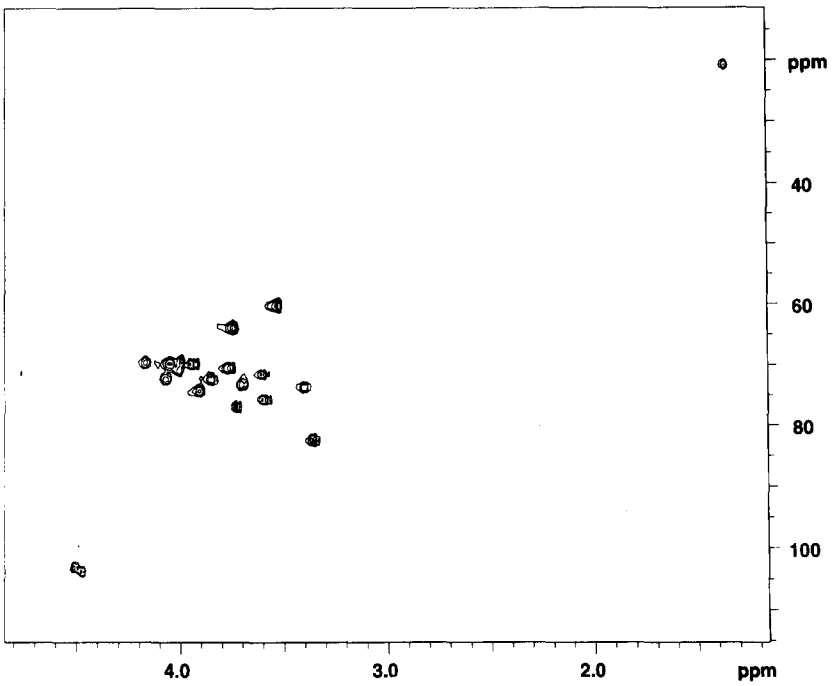


Fig. 3. H/C correlation spectra of oligosaccharide alditol (F-I) isolated from the partial hydrolysis of WSCP-III (65 °C, in D_2O).

B and H-4 (δ 3.68) of residue **C** and H-6 (δ 3.98) of residue **B**, respectively. This observation suggests that in some cases, two or more **B** residues were linked together before being linked at the 4 position of the α-L-rhamnose residue of the backbone chain. H-1 of **C** or **C'** (δ 5.29 & 5.31) correlated with H-2 (δ 4.12) of **C** or **C'**, which also correlated with H-4 (δ 4.44) of residue **D**, the 4-linked α-D-Gal*p*A.

As described in the experimental section, three oligosaccharide-containing fractions (F-I, F-II, and F-III) were isolated and purified from a partial hydrolysate of WSCP-III. The oligosaccharides were reduced to alditols by borohydride to simplify their NMR spectra [16]. In the region of anomeric protons (4.4–4.5) of the ^{1}H NMR spectra, two doublets were observed for F-I, while only one doublet was found for both F-II and F-III. The presence of two doublets suggested a trisaccharide for F-I, while the single doublet indicated that both F-II and F-III were disaccharides since the reducing terminal C-1 groups were reduced to alditols by borohydride [16]. 2D NMR spectroscopy was carried out to establish the connectivities and linkage sites of those oligosaccharides. As shown in Table 3, the full assignments of all resonances were obtained by comparing the chemical shifts against literature values and with the assistance of homonuclear shift-correlated spectroscopy. The COSY experiments established the scalar coupling connectivities of the oligosaccharides, which supported the complete assignment of the

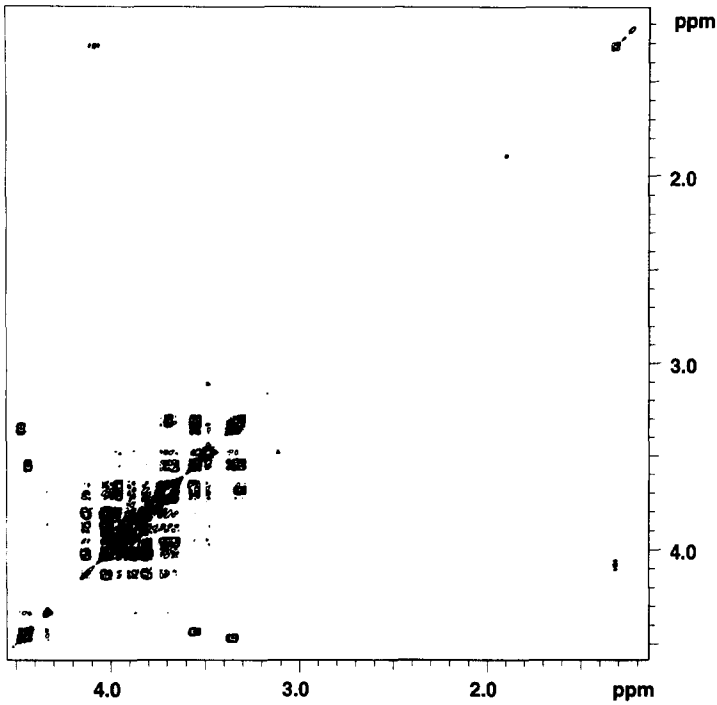


Fig. 4. COSY spectrum of oligosaccharide alditol (F-I) isolated from the partial hydrolysis of WSCP-III (65 °C, in D_2O).

resonances (Table 3). Examples of the heteronuclear correlation and COSY spectra of the oligosaccharides are shown in Figs. 3 and 4, respectively.

F-I was established as a disaccharide alditol containing terminal non-reducing 4-*O*-Me-β-D-Glc*p*A, 6-linked β-D-Gal*p*, and 4-linked rhamnitol, as shown in Structure 1.

4-*O*-Me-β-D-Glc*p*A-(1 → 6)-β-D-Gal*p*-(1 → 4)-L-rhamnitol

Structure 1 (F-I)

The β configurations were apparent for the 4-*O*-Me-D-Glc*p*A and the 6-linked D-Gal*p* by $J_{H1,2}$ (~ 8 Hz) as well as by the δ values of the anomeric protons and carbons (Table 3). The rhamnitol residue was evidenced by δ 1.29 (^1H) and 17.85 (^{13}C) which are caused by the CH_3 group in deoxy sugars. The sequence and site of linkage of F-I was established by a difference NOE experiment and the result is shown in Table 4.

Both F-II and F-III were monosaccharide alditols which contained 4-*O*-Me-β-D-Glc*p*A as the terminal non-reducing groups. The two alditols were identified as D-galactitol. The difference NOE experiment suggested that the 4-*O*-Me-β-D-Glc*p*A was

Table 4
Difference NOE inter-residue connectivities of oligosaccharide alditols derived from polysaccharide WSCP-III

		Anomeric proton(ppm)	NOE inter-residue connectivity(ppm)			
F-I						
4-*O*-Me-β-D-Glc*p*A-(1 →	**a**	4.49	3.98	H-6	of	**b**
→ 6)-β-D-Gal*p*-(1 →	**b**	4.46	3.84	H-4	of	**c**
→ 4)-L-rhamnitol	**c**					
F-II						
4-*O*-Me-β-D-Glc*p*A-(1 →	**a′**	4.48	3.98	H-6	of	**b′**
→ 6)-D-galactitol	**b′**					
F-III						
4-*O*-Me-β-D-GlcpA-(1 →	**a″**	4.46	4.00	H-2	of	**b″**
→ 6)-D-galactitol	**b″**					

linked to the 6 position in F-II, while it linked to the 2 position in F-III (Table 4). The two structures are shown here.

4-*O*-Me-β-D-Glc*p*A-(1 → 6)-D-Gal-ol

Structure 2 (F-II)

4-*O*-Me-β-D-Glc*p*A-(1 → 2)-D-Gal-ol

Structure 3 (F-III)

The structure and linkage patterns identified for the three oligosaccharides are in agreement with the results from 2D NMR spectroscopy of the polysaccharide.

In summary, WSCP-III was identified as a pectic polysaccharide composed of disaccharide backbone repeating-unit:

→ 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1 →

Oligosaccharide side chains are attached to the 4 position of the 2-linked α-L-rhamnose residue. The ratio of the 4-substituted and unsubstituted 2-linked α-L-rhamnose is 2:1. The side chains are composed of a terminal non-reducing end 4-*O*-Me-β-D-Glc*p*A which is attached to the 4 position of the 2-linked α-L-Rha*p* in the backbone chain mainly through 6-linked (a small portion through 2-linked) β-D-Gal*p*.

The rhamnogalacturonan structure elucidated in this paper is common in the plant kingdom [17]. The identified → 2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*-(1 → backbone chain is typical of pectic polysaccharides [7,14,18–20]. The terminal non-reducing 4-*O*-Me-β-D-Glc*p*A linked through 6-linked and/or 2-linked β-D-Gal*p* at the 4 position of the 2-linked α-L-Rha*p* in the rhamnogalacturonan backbone chain is also present in pectic polysaccharides [7,17]. However, this type of structure has not been reported before for yellow mustard mucilage.

1. Experimental

Materials.—A pectic polysaccharide (WSCP-III) was prepared from yellow mustard mucilage by anion-exchange chromatography and purified by size-exclusion chromatography, as described earlier [4]. All chemicals were reagent grade unless otherwise specified.

Methylation analysis.—Methylation analysis was carried out as previously described [21]. Carboxyl reduction after methylation was performed according to O'Neill et al. [22]. Qualitative and quantitative determinations of partially methylated alditol acetates were performed as described previously [3].

Partial hydrolysis.—The purified WSCP-III (~ 30 mg) was dissolved in 20 mL of boiling water, and adjusted to 0.4 M trifluoroacetic acid. Hydrolysis was performed for 2.5 h at 100 °C [22]. The hydrolysate was concentrated to dryness by rotary evaporation under vacuum at 40 °C. The residue was dissolved in 25 mM NaOAc buffer (pH 5.0) and applied to a DEAE-Sephadex A-25 column (1.6 × 20 cm) equilibrated with the same buffer [23]. The column was sequentially eluted (1 mL/min) with the buffer and buffer containing 0.1, 0.3, and 0.5 M NaCl, respectively. The neutral fraction (eluted with buffer alone) was monitored for sugars by the phenol-H_2SO_4 method [24]. Following stepwise changes in ionic strength (NaCl) the eluted acidic fractions were detected using the method as described [25]. Appropriate fractions were combined and concentrated on a rotary evaporator at 40 °C (F-I: fractions 22–26; F-II: fractions 29–33; F-III: fractions 35–39). The fractions obtained by ion-exchange chromatography were applied to a BioGel P-2 column (2.5 × 100 cm) for further purification. The column was eluted with 25 mM NaOAc buffer and monitored for total and acidic carbohydrates by the methods described previously [24,25]. The oligosaccharide fractions collected were reduced with borohydride [22] and then desalted on a BioGel P-2 column (1.6 × 90 cm) by elution with distilled water.

NMR spectroscopy.—NMR spectra were recorded with a Bruker AMX500 spectrometer using 4% carbohydrate solutions in D_2O (5 and 10 mm tube). Internal 1,4-dioxane was used as an internal chemical-shift reference for ^{13}C spectra. Sample temperature was controlled at 65 °C for all spectra. Homonuclear correlation (COSY) spectra [26], and NOE correlation (NOESY) spectra [27] were recorded with F2 time domains of 1024 points and F1 time domains of 256 points. Zero filling in F1 yielded a 512 × 512 (real) matrix after transformation. 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 [28], 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. Difference NOE experiments of the oligosaccharides were performed with a spectral width of ~ 4000 Hz and a real frequency-domain data size of 32 K points, resulting in a digital resolution of 0.12 Hz per point. Multiplets were irradiated by stepping the decoupler frequency between each line of the multiplet at 200 ms intervals [29] and each multiplet was irradiated for a total of 5 s.

Acknowledgements

This work was supported by a grant from the Natural Science and Engineering Research Council (NSERC) of Canada. The authors wish to thank Dr. Gerald O. Aspinall (Department of Chemistry, York University, Canada) for his valuable discussion during the early stage of this study.

References

[1] E.L. Hirst, D.A. Rees, and N.G. Richardson, *Biochem. J.*, 95 (1965) 453–458.
[2] D.A. Rees and I.W. Steele, *Biochemistry*, 5 (1966) 3108–3110.
[3] W. Cui, N.A.M. Eskin, and C.G. Biliaderis, *Carbohydr. Polym.*, 20 (1993) 215–225.
[4] W. Cui, N.A.M. Eskin, and C.G. Biliaderis, *J. Agric. Food Chem.*, 42 (1994) 657–664.
[5] W. Cui, N.A.M. Eskin, and C.G. Biliaderis, *Carbohydr. Polym.*, 27 (1995) 123–127.
[6] W. Cui, N.A.M. Eskin, and C.G. Biliaderis, *Carbohydr. Polym.*, 27 (1995) 117–122.
[7] J. An, M.A. O'Neill, P. Albersheim, and A.G. Darvill, *Carbohydr. Res.*, 252 (1994) 235–243.
[8] J. An, L. Zhang, M.A. O'Neill, P. Albersheim, and A.G. Darvill, *Carbohydr. Res.*, 264 (1994) 83–96.
[9] P. Edebrink, P.E. Jansson, G. Widmalm, and W. Nimmich, *Carbohydr. Res.*, 257 (1994) 107–117.
[10] B. Jann, A.S. Shashkov, M. Hahne, H. Kochanowski, and K. Jann, *Carbohydr.Res.*, 261 (1994) 215–222.
[11] P.E. Jansson, L. Kenne, and I. Kolare, *Carbohydr. Res.*, 257 (1994) 163–174.
[12] I.J. Colquhoun, G.A. Ruiter, H.A. Schols, and A.G.J. Voragen, *Carbohydr. Res.*, 206 (1990) 131–144.
[13] H. Parolis and L.A.S. Parolis, *Carbohydr. Res.*, 267 (1995) 263–269.
[14] H.A. Schols, A.G.J. Voragen, and I.J. Colquhoun, *Carbohydr. Res.*, 256 (1994) 97–111.
[15] A.M. Winn, A.W. Miller, and S.G. Wilkinson, *Carbohydr. Res.*, 267 (1995) 127–133.
[16] L.L. Kiefer, W.S. York, P. Albersheim, and A.G. Darvill, *Carbohydr. Res.*, 197 (1990) 139–158.
[17] G.O. Aspinall, *Polysaccharides*, Pergamon Press, Toronto, Canada, 1970; pp 13–144.
[18] G.O. Aspinall, E.L. Hirst, and N.K. Matheson, *J. Chem., Soc.*, (1956) 989.
[19] G.O. Aspinall, M.J. Johnston, and A.M. Stephen, *J. Chem. Soc.*, (1960) 4918.
[20] Q.P. Gao, H. Kiyohara, and H. Yamada, *Carbohydr. Res.*, 198 (1990) 111–125.
[21] I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[22] M.A. O'Neill, A.G. Darvill, and P. Albersheim, *Carbohydr. Res.*, 206 (1990) 289–296.
[23] Y. Kato and D.J. Nevins, *Carbohydr. Res.*, 227 (1992) 315–329.
[24] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[25] N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
[26] W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229.
[27] G. Bodenhausen, H. Kogler, and R.R. Ernst, *J. Magn. Reson.*, 58 (1985) 370.
[28] G. Bodenhausen and D.J. Ruben, *Chem. Phys. Lett.*, 69 (1980) 185.
[29] M. Kinns and J.K.M. Sanders, *J. Magn. Reson.*, 56 (1984) 518.