Fractionation, Structural Analysis, and Rheological Properties of Water-Soluble Yellow Mustard (*Sinapis alba* L.) Polysaccharides

Wuwei Cui,† N. A. Michael Eskin,*,† and Costas G. Biliaderis‡,§

Department of Foods and Nutrition and Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

The water-soluble yellow mustard (*Sinapis alba* L.) polysaccharides were separated into CTAB-precipitated and CTAB-soluble polysaccharide fractions by precipitation with 5% CTAB. Each of these materials was subsequently fractionated into five fractions by an ion-exchange (DEAE-cellulose) column eluted with a stepwise gradient of NaCl (0.1–1.0 M) in NaAc buffer (pH 5, 25 mM). Of the 10 subfractions obtained, 2 neutral polysaccharide fractions (WSCP-I, WSCS-I) and an acidic fraction (WSCP-III) were identified as the major components responsible for the pronounced shear thinning behavior of yellow mustard mucilage solutions. The WSCP-I and WSCS-I fractions were mainly composed of a 1,4-linked β-D-glucan, although WSCP-I appeared to be more heterogeneous than WSCS-I. The WSCP-III fraction consisted of nonreducing end glucuronic acid (13.3%), 1,4-linked galacturonic acid (13.6%), 1,6-linked galactose (22.9%), and 1,2-linked (11%) and 1,2,4-linked (17%) rhamnose. Gel filtration chromatography revealed differences in the molecular size of the constituent polysaccharides among the fractions.

INTRODUCTION

Interesting rheological and interfacial properties of yellow mustard (*Sinapis alba* L.) mucilage and its fractions were previously reported (Cui et al., 1993a). Under optimized conditions, the water-soluble polysaccharides, responsible for most of the rheological properties of yellow mustard mucilage, were separated into a CTAB-precipitated fraction (WSCP) and a CTAB-soluble fraction (WSCS) (Cui et al., 1993b). The WSCP fraction was found to be the major component (52.0%), exhibiting more pronounced "weak gel" characteristics compared to WSCS, the minor fraction (34.0%) (Cui et al., 1993b). The primary structures of WSCP and WSCS were examined by ^{13}C NMR spectroscopy and methylation analysis. The WSCP was composed of two major polysaccharides: an acidic pectic-like polysaccharide containing nonreducing end glucuronic acid, 1,4-linked galacturonic acid, 1,2- and 1,2,4-linked rhamnose, and 1,2- and 1,6-linked β-galactose; and a neutral polysaccharide with a predominant 1,4-linked β-D-glucose backbone. The WSCS also consisted of a similar 1,4-linked β-D-glucose based neutral polysaccharide; unlike WSCP, it contained only glucuronic acid (Cui et al., 1993b). Both WSCP and WSCS are thus heterogeneous mixtures of polysaccharides so that their structures and the influence of structure on physical properties remain unclear. This paper reports on further fractionation and purification of the WSCP and WSCS polysaccharides by DEAE high-capacity cellulose ion-exchange chromatography, the rheological properties, and the structural characteristics of the purified subfractions as revealed by methylation analysis and ^{13}C NMR spectroscopy.

MATERIALS AND METHODS

Fractionation. Yellow mustard mucilage water-soluble CTAB-precipitated (WSCP) and CTAB-soluble (WSCS) fractions were separated as described earlier (Cui et al., 1993b). All chemicals were of reagent grade unless otherwise specified.

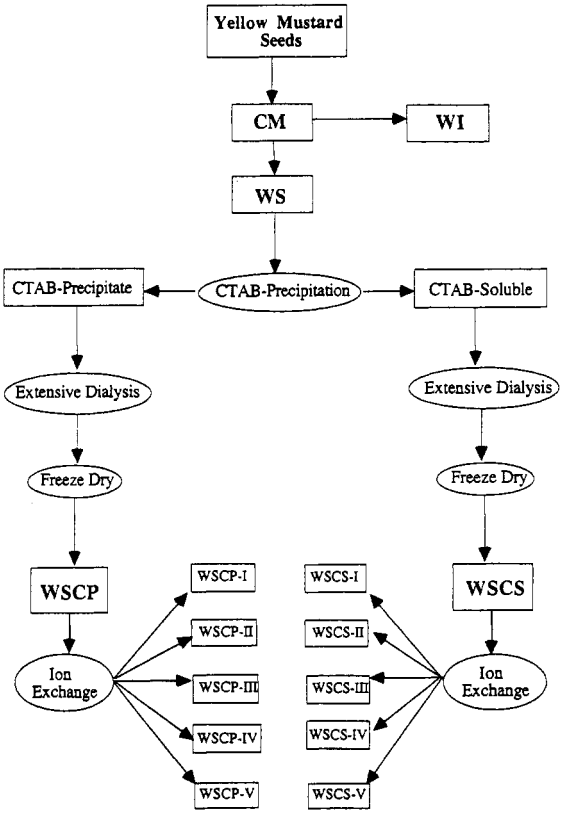

Figure 1. Flow chart of isolation and fractionation of yellow mustard mucilage and its fractions.

Ion-Exchange Chromatography. Ion-exchange chromatography was carried out on a DEAE high-capacity ion-exchange cellulose (Pierce Chemical Co., Rockford, IL) column (2.6 × 50 cm). The resin was pre-equilibrated with 25 mM NaAc buffer (pH 5.0) prior to use (Kato et al., 1992). A 240-mg sample was dissolved in 500 mL of distilled water and then adjusted to the same buffer concentration (25 mM NaAc buffer, pH 5.0) and loaded onto the column. The column was first eluted (flow rate 60mL/h, 25 °C) with buffer (25 mM NaAc, pH 5.0), followed by

* Author to whom correspondence should be addressed.
† Department of Foods and Nutrition.
‡ Department of Food Science.
§ Present address: Department of Food Science and Technology, School of Agriculture, Box 256, Aristotle University, Thessaloniki, Greece 54006.

0021-8561/94/1442-0657$04.50/0

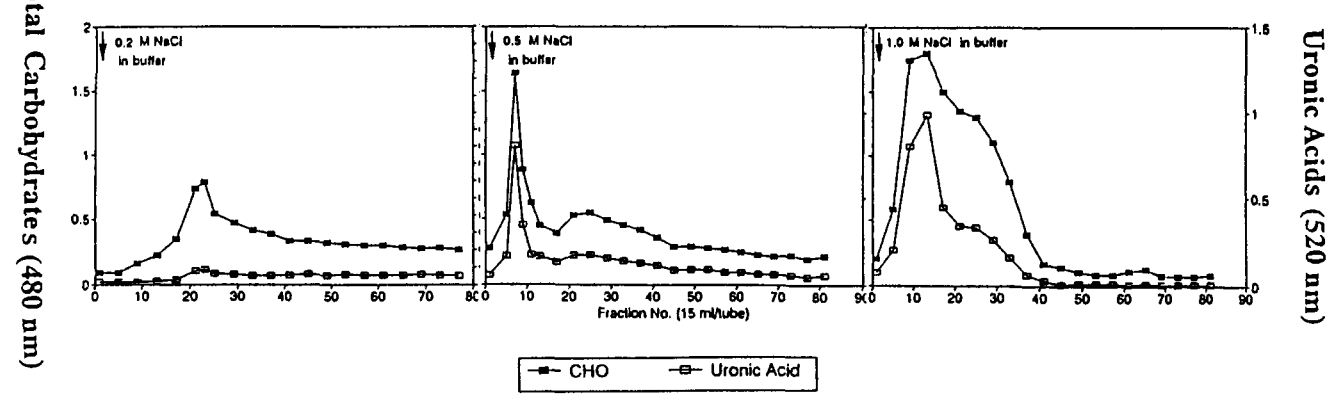

Figure 2. Ion-exchange chromatographic profiles of WSCP fractions.

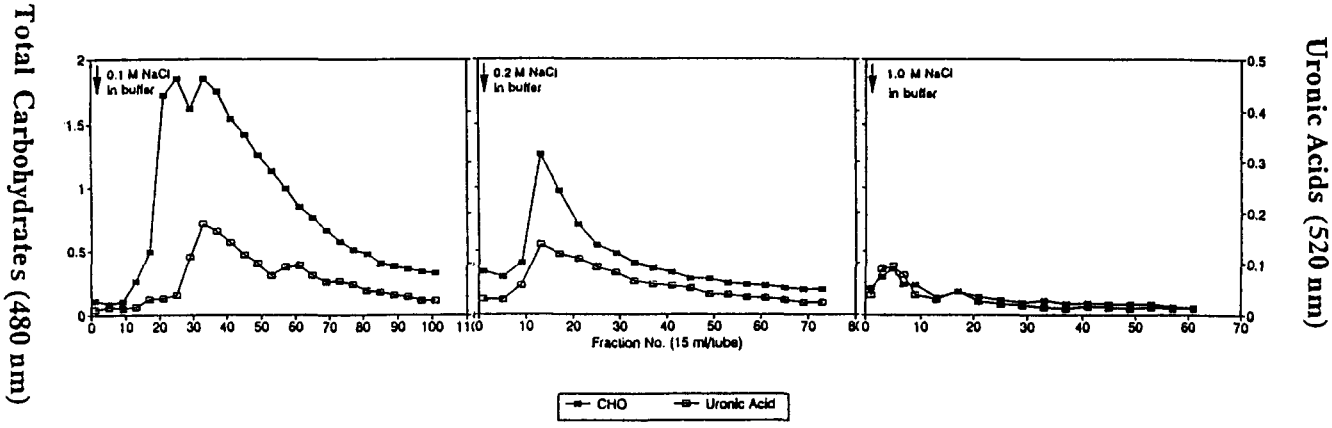

Figure 3. Ion-exchange chromatographic profiles of WSCS fractions.

stepwise increase of ionic strength: 0.2, 0.5, and 1.0 M NaCl for WSCP and 0.1, 0.2, and 1.0 M NaCl for WSCS, in the same buffer, respectively. Finally, the column was eluted with 6.0 M urea. Carbohydrates and uronic acids were monitored using the methods described by Dubois et al. (1956) and Blumenkrantz and Asboe-Hansen (1973), respectively. Following ion-exchange chromatography, the samples were concentrated in a rotary evaporator (40 °C), exhaustively dialyzed against distilled water, and freeze-dried.

Rheological Measurements. Rheological properties were determined on a Bohlin VOR rheometer (Bohlin Reologi, Sweden). A concentric cylinder geometry, with a cylinder height of 30 mm and radii of the inner and outer cylinders of 14 and 15.4 mm, respectively, was used for the rheological measurements. In steady shear tests, samples at 0.5% (w/w) concentration were subjected to shear sweeps between 0.01 and 1164 s^{-1}.

Gel Filtration Chromatography. Gel filtration chromatography was conducted on a Sephacryl S-500 (HR, Pharmacia Ltd., Montreal, PQ) column (2.6 × 98 cm) which eluted with 0.1 M NaCl solution (60 mL/h, 25 °C). Samples dissolved in the same solution (1 mg/mL) were applied onto the column and 5-mL fractions collected. D-Glucose was used to determine the total volume, while linear dextrans T-500 (MW 466 000) and T-70 (MW 69 000, Pharmacia Ltd.) were used as relative molecular weight markers. Carbohydrate and uronic acids were also monitored (Dubois et al., 1956; Blumenkrantz and Asboe-Hansen, 1973).

Monosaccharide Analysis and ^{13}C NMR Spectroscopy. All samples were treated with 72% H_2SO_4 at 35 °C for 30 min prior to hydrolysis with 2 M H_2SO_4. Neutral monosaccharides in the hydrolysates were determined following the procedure described by Englyst et al. (1982) on a SP-2330 glass capillary column (30 m × 0.75 mm i.d.). The ^{13}C NMR spectra of the polysaccharide fractions were recorded on a Bruker AMX 500 spectrometer at 65 °C, at approximately 5% (w/w) solutions in D_2O with 5-mm NMR test tubes.

Methylation Analysis. Methylation analysis was carried out as described by Ciucanu and Kerek (1984). The reduction of carboxyl groups after methylation was performed according to the procedure of O'Neill et al. (1990). Qualitative and quantitative measurements of partially methylated alditol acetates were performed as described previously (Cui et al., 1993b).

RESULTS AND DISCUSSION

Fractionation and Ion-Exchange Chromatography. Figure 1 shows the general fractionation scheme followed to separate yellow mustard mucilage into its subfractions. Extracted yellow mustard mucilage was dialyzed and fractionated by centrifugation into a water-soluble fraction and a water-insoluble fraction as described earlier (Cui et al., 1993a). The water-soluble fraction, the major component of yellow mustard mucilage exhibiting strong shear thinning behavior in solutions, was further separated into a CTAB-precipitated fraction (WSCP) and a CTAB-soluble fraction (WSCS) by complexation of CTAB (hexadecyltrimethylammonium bromide) with the acidic polysaccharides (Cui et al., 1993b). The WSCP and WSCS fractions were further fractionated by ion-exchange chromatography on a DEAE high-capacity cellulose column into five subfractions respectively (Figure 1). The ion-exchange chromatography profiles are presented in Figures 2 and 3, respectively. The DEAE-cellulose column was initially equilibrated with NaAc buffer (25 mM, pH 5.0) prior to use. Samples were first dissolved in water and then adjusted to the appropriate ionic strength with NaAc buffer before being introduced onto the column. The column was eluted with the same buffer until eluted carbohydrates were no longer detected by the phenol–sulfuric acid method (Dubois et al., 1956). Subsequently, a stepwise increase in ionic strength (0.2, 0.5, and 1.0 M NaCl, Figure 2) of the elution buffer (pH 5.0) was applied for WSCP on the basis of results of preliminary experi-

Table 1. Recovery of Fractions from the DEAE High-Capacity Cellulose Ion-Exchange Column

	recovery (%)		recovery (%)
WSCP-I[a]	15.2	WSCS-I	9.2
WSCP-II	22.4	WSCS-II	28.3
WSCP-III	14.5	WSCS-III	12.2
WSCP-IV	6.1	WSCS-IV	22.9
WSCP-V	44.2	WSCS-V	31.6

[a] WSCP: I, eluted with buffer (50 mM NaAc, pH 5.0); II, eluted with 0.2 M NaCl in buffer; III, eluted with 0.5 M NaCl in buffer; IV, eluted with 1.0 M NaCl in buffer; V, eluted with 6.0 M urea. WSCS: I, eluted with buffer (50 mM NaAc, pH 5.0); II, eluted with 0.1 M NaCl in buffer; III, eluted with 0.2 M NaCl in buffer; IV, eluted with 1.0 M NaCl in buffer; V, eluted with 6.0 M urea.

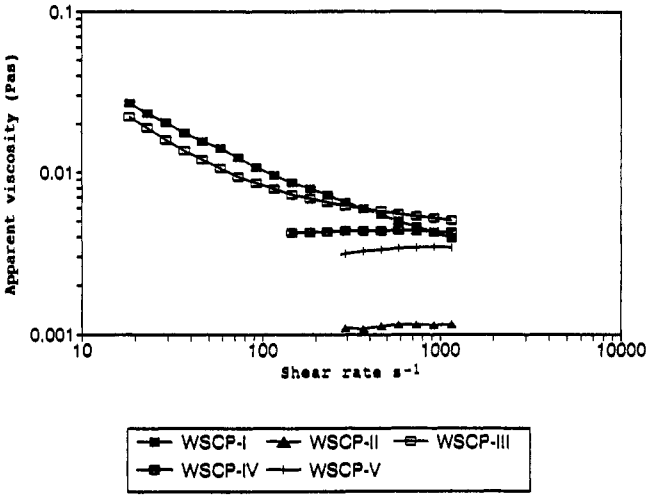

Figure 4. Steady shear flow curves of WSCP fractions at 0.5% (w/w) polymer concentration, 22 °C.

ments before the column was finally eluted with 6.0 M urea solution. Five subfractions were thus obtained for WSCP including WSCP-I (eluted with the buffer pH 5.0), WSCP-II (0.2 M NaCl buffer elution), WSCP-III (0.5 M NaCl buffer elution), WSCP-IV (1.0 M NaCl buffer elution), and WSCP-V (6.0 M urea elution) (Figure 1). In a similar manner, WSCS was also separated into five fractions with a stepwise increase of the ionic strength (0.1, 0.2, and 1.0 M NaCl) using the same buffer system (Figure 3). As a result, five fractions were obtained: WSCS-I (eluted with buffer pH 5.0), WSCS-II (0.1 M NaCl elution), WSCS-III (0.2 M NaCl elution), WSCS-IV (1.0 M NaCl elution), and WSCS-V (6.0 M urea elution) as shown in Figure 1. The approximate recovery of these fractions is shown in Table 1.

Rheological Properties. The results of steady shear rheological tests for the WSCP and WSCS subfractions are presented in Figures 4 and 5, respectively. At 0.5% (w/w) fractions WSCP-I and WSCP-III (Figure 4) exhibited typical shear thinning behavior where the apparent viscosity decreased, increasing shear rate (Morris et al., 1981). The shear thinning ability was greater for WSCP-I compared to WSCP-III, exhibited by higher viscosity at low shear rates and lower viscosity at higher shear rates. WSCP-III exhibited slightly more shear thinning behavior at lower shear rates, while the reverse was observed at higher shear rates, as shown in Figure 4. This may indicate that the flow curve of WSCP-III solutions under the test conditions was close to the second Newtonian-like plateau at the high shear rate region (Morris et al., 1981). The strong shear thinning behavior of WSCP-I dispersion may be in part related to the partial insolubility of this material, as it was not possible to completely redissolve it in aqueous solution. The rest of the WSCP fractions (WSCP-II, WSCP-IV, and WSCP-V) exhibited typical Newtonian behavior under the test conditions (0.5% w/w); the apparent viscosity was independent of the shear rate. The rank of the apparent viscosity among these fractions was in the order WSCP-IV > WSCP-V > WSCP-II, as shown in Figure 4.

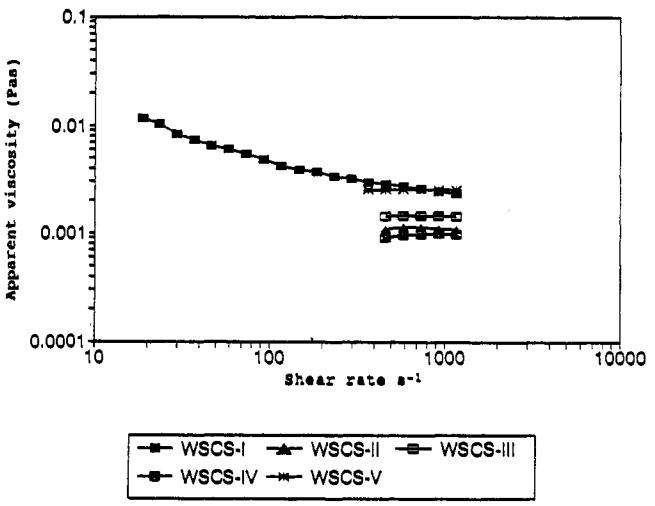

Figure 5. Steady shear flow curves of WSCS fractions at 0.5% (w/w) polymer concentration, 22 °C.

Among the WSCS fractions, only WSCS-I was found to exhibit shear thinning behavior at 0.5% (w/w) concentration (Figure 5). The shear thinning behavior of WSCS-I was weaker compared to that of WSCP-I and WSCP-III, as evidenced by the lower apparent viscosity over the entire shear rate range investigated. All other fractions of the WSCS showed Newtonian behavior, and their apparent viscosity followed the order WSCS-V > WSCS-III > WSCS-II > WSCS-IV. The observations for the rheological properties of the isolated fractions were in agreement with previous data (Cui et al., 1993b) and indicate that WSCP contributes more to the pronounced shear thinning rheological behavior of water-soluble yellow mustard polysaccharide solutions/dispersions than WSCS.

Gel Filtration Chromatography. Figures 6 and 7 show the molecular weight distributions of the isolated WSCP and WSCS subfractions, respectively. In Figure 6, the WSCP-I profile was not presented due to the partial insolubility of this fraction. Among the rest of the WSCP fractions, WSCP-II contained a lower amount of uronic acids compared to WSCP-III and WSCP-IV. The curve for uronic acids coincides with that for carbohydrates in WSCP-III, indicative of relative homogeneity. Additional evidence for the chemical homogeneity of this fraction was provided by monosaccharide analysis, methylation, and ^{13}C NMR spectroscopy (Tables 2 and 3; Figure 8). WSCP-IV contained the second largest amount of uronic acids, although the eluting peak position for carbohydrates came later than the uronic acids, which may suggest a heterogeneous mixture of polysaccharide species in this fraction. In Figure 6, WSCP-V was almost free of uronic acids (eluted from the ion-exchange column by 6.0 M urea solution). The peak elution number was highest for WSCS-II, followed by WSCP-V, WSCP-IV, and WSCP-III. This order of the elution volume coincides with the increase in apparent viscosity (Figure 4), suggesting that constituent polysaccharides of higher molecular size contribute to the higher apparent viscosity for some of these fractions. This is in agreement with the findings of Izydorczyk and Biliaderis (1992) on wheat endosperm water-soluble arabinoxylans that higher molecular weight

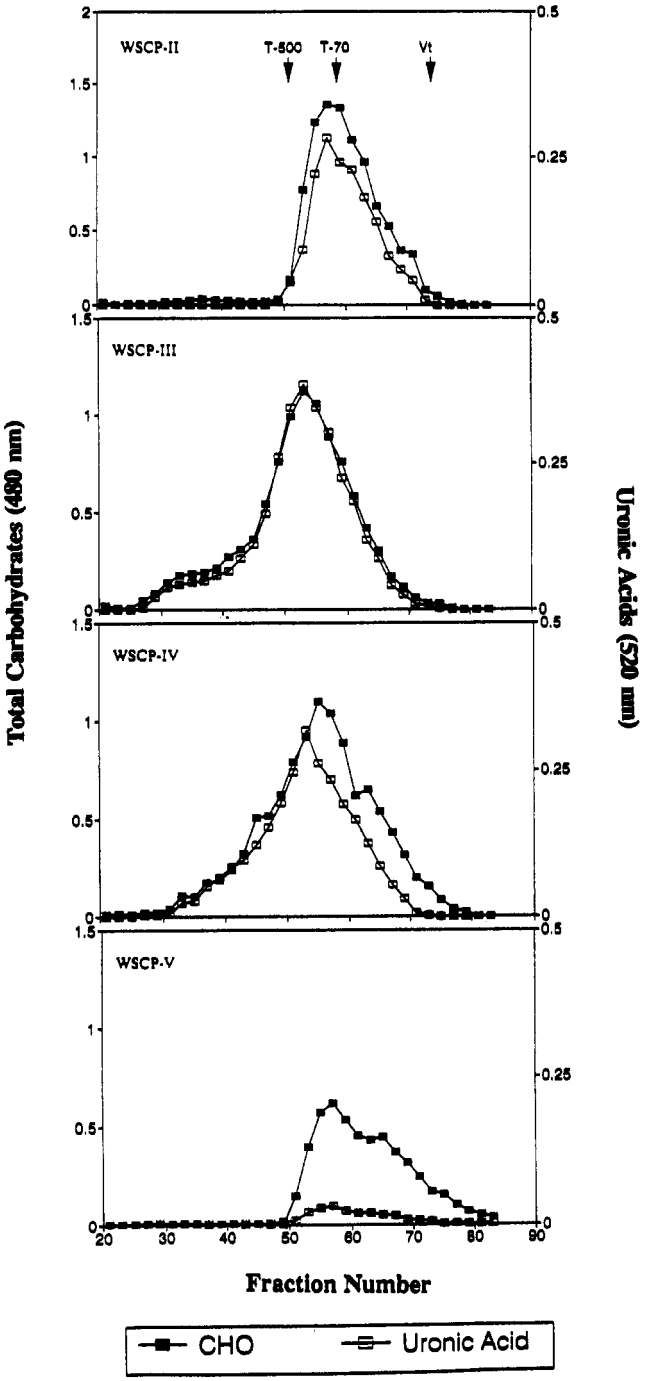

Figure 6. Gel filtration chromatographic profiles of WSCP fractions on Sephacryl S-500 (2.6 × 98 cm, 60 mL/h at 25 °C, 6 mL/tube).

polysaccharides contribute to a higher viscosity and shear thinning behavior.

In Figure 7, the WSCS-I fraction appeared to consist of neutral polysaccharides (no detectable uronic acids). The uronic acid level was higher in WSCS-III and WSCS-IV compared to WSCS-II, although WSCS-IV exhibited a wider elution peak. There were two major eluting components in the WSCS-V profile: peak a, fractions 35–45; peak b, fractions 50–66. The high molecular size component (lower elution number) present in WSCS-V may account for the high apparent solution viscosity of this fraction, similar to that of WSCS-I, in the high shear rate region (Figure 5).

Monosaccharide Analysis. Table 2 shows the molar ratios of neutral monosaccharide constituents of the WSCP and WSCS fractions. In the WSCP series, WSCP-I

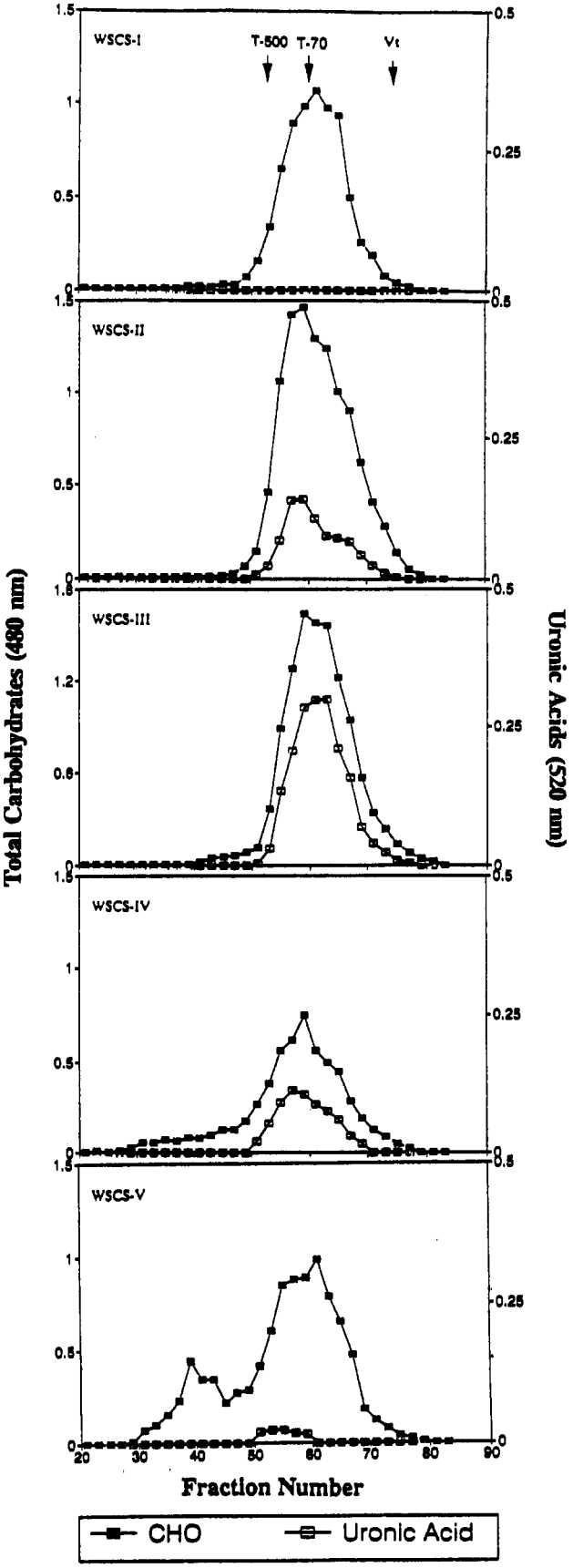

Figure 7. Gel filtration chromatographic profiles of WSCS fractions on Sephacryl S-500 (2.6 × 98 cm, 60 mL/h at 25 °C, 6 mL/tube).

contained high amounts of glucose and rhamnose, followed by galactose, mannose, a small amount of arabinose, and a trace amount of xylose. With increased ionic strength of the eluting buffer, the acidic fractions eluted from the

Table 2. Monosaccharide Molar Ratios of Yellow Mustard Mucilage Water-Soluble CTAB-Precipitated (WSCP) and Water-Soluble CTAB-Soluble (WSCS) Fractions Isolated by Ion-Exchange Chromatography

	WSCP					WSCS				
	I[a]	II	III	IV	V	I	II	III	IV	V
Rha	2.0	0.3	0.4	1.1	2.3	3.5	0.4	0.5	0.6	1.1
Ara	0.3	tr[b]	tr	tr	0.1	tr	0.1	0.3	0.3	1.1
Xyl	tr	0	0	0	0	tr	0.2	0.2	tr	1.1
Man	0.5	0.1	tr	0.2	0.7	tr	0.6	0.3	0.4	1.5
Gal	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Glc	2.7	0.2	tr	0.2	6.7	16.4	0.5	0.3	0.6	12.8

[a] I–V were isolated by ion-exchange chromatography as described in Table 1. [b] tr, trace amount, less than 0.05.

ion-exchange column and were found to contain mainly galactose and rhamnose as neutral monosaccharides. WSCP-III was also found to contain mainly galactose and rhamnose with trace amounts of glucose, mannose, and arabinose. In contrast, WSCP-II and WSCP-IV were less homogeneous, containing a fair amount of glucose and mannose. The monosaccharide composition of WSCP-V is predominated by glucose, followed by rhamnose, galactose, mannose, and arabinose. In the WSCS series, WSCS-I contained much greater amounts of glucose than fraction WSCP-I (glucose:galactose ratio 16.4:1 and 2.4:1, respectively). With increasing ionic strength the order of elution through the column was WSCS-II, WSCS-III, and WSCS-IV, respectively (Figure 3). These three fractions were composed mainly of galactose, rhamnose, mannose, glucose, xylose, and arabinose. WSCS-III contained smaller amounts of glucose and mannose than the other two fractions. WSCS-V (fraction eluted with 6.0 M urea) was similar to WSCS-I by being composed mainly of glucose with smaller amounts of mannose, xylose, arabinose, rhamnose, and galactose.

Methylation Analysis. The methylation analysis of WSCP-I–V is summarized in Table 3. WSCP-I contained 46% glucose, primarily as 1,4 linkage (44.5%) together with a small amount of nonreducing end glucose (1.6%) and trace amounts of 1,4,6 linkages. This is in agreement with the monosaccharide analysis that glucose was the predominant sugar. The second largest component of WSCP-I, xylose, was mainly present as 1,4-linked xylopyranose (18.7%) and a small amount of all substituted xylose (3.9%). In addition, 5.9% of 1,6-linked galactose and 3% of 1,2-linked galactose together with a trace amount of 1,3,6-linked galactose were found in the WSCP-I fraction.

The methylation results in Table 3 show that WSCP-III is a pectic polysaccharide, confirming our earlier findings that WSCP contained an acidic polysaccharide (Cui et al., 1993b). Its major neutral monosaccharide components were 1,6-linked galactose (22.9%), 1,2-linked (11%), and 1,2,4-linked (17%) rhamnoses. The 1,4-linked galacturonic acid content was 13.6%, and an almost equal amount of nonreducing end glucuronic acid (13.3%) was also found.

Compared to WSCP-III, WSCP-II and WSCP-IV were less homogeneous, containing fairly high amounts of methyl ethers of glucose (20.8% for WSCP-II and 10.2% for WSCP-IV). In addition, the 1,4-linked galacturonic acid contents of WSCP-II and WSCP-IV were much lower than that for WSCP-III. The nonreducing end glucuronic acid content of the fractions decreased as the ionic strength of the eluting buffer increased from 0.2 to 1.0 M NaCl (WSCP-II, 15.9%; WSCP-III, 13.3%; WSCP-IV, nd). This may suggest that polysaccharides containing nonreducing end glucuronic acid have a lower capacity to bind on the DEAE column, thus being eluted more easily with relatively low ionic strength buffer. The methylation analysis also confirmed that WSCP-V was composed solely of 1,4-linked β-D-glucan.

Table 4 represents the molar ratios of partially permethylated acetyl alditols through fractions WSCS-I–V. Both WSCS-I and WSCS-V were neutral polysaccharides, composed primarily of 1,4-linked β-D-glucan (66 and 72%,

Table 3. Molar Ratios of Partially Permethylated Acetyl Alditols of the Water-Soluble CTAB-Precipitated (WSCP) Fractions of Yellow Mustard Mucilage

	molar ratio[a]				
	WSCP-I	WSCP-II[b]	WSCP-III	WSCP-IV	WSCP-V
2,3,5-Me_3-Ara	2.8	0.8	0.4	3.6	0
2,3-Me_2-Ara	4.3	2.7	2.5	4.0	0
total methyl ethers of Ara	7.1	3.5	2.9	7.6	0
2,3-Me_2-Xyl	18.7	4.0	1.3	1.8	0
Xyl $(acet)_5$	3.9	0.6	tr[d]	15.3	0
total methyl ethers of Xyl	22.6	4.6	1.3	17.1	0
2,3,4,6-Me_4-Glc	1.6	1.4	0	4.2	1.8
2,3,6-Me_3-Glc	44.5	13.9	1.0	4.3	81.3
2,3-Me_2-Glc	tr	5.5	0	1.7	0
total methyl ethers of Glc	46.1	20.8	1.0	10.2	83.1
2,3,4-Me_3-Glc (6D_2)	nd[c]	15.9	13.3	nd	nd
2,3,4,6-Me_4-Gal	nd	5.5	2.3	4.0	0
3,4,6-Me_3-Gal	3.0	0	2.4	4.6	0
2,3,4-Me_3-Gal	5.9	23.8	22.9	39.4	0
2,4-Me_2-Gal	tr	0.8	0.6	tr	0
total methyl ethers of Gal	8.9	30.1	28.2	48.0	0
2,3-Me_2-Gal (6D_2)	nd	5.4	13.6	5.6	nd
2,3,6-Me_3-Man	4.5	5.3	0.2	nd	nd
3,4-Me_2-Rham	1.2	2.1	11.0	0	0
3-Me-Rham	tr	5.6	17.7	4.9	0
total methyl ethers of Rham	1.2	7.7	28.7	4.9	0

[a] Relative molar ratio calculated from the ratio of peak heights. [b] WSCP-II, -III, and -IV were carboxyl reduced (O'Neill, 1990). [c] nd, not determined. [d] tr, trace amount.

Table 4. Molar Ratios of Partially Permethylated Acetyl Alditols of the Water-Soluble CTAB-Soluble (WSCS) Fractions of Yellow Mustard Mucilage

	molar ratio[a]				
	WSCS-I	WSCS-II[b]	WSCS-III	WSCS-IV	WSCS-V
2,3,5-Me_3-Ara	1.8	1.0	0.6	tr[d]	tr
2,3-Me_2-Ara	1.6	5.8	6.2	tr	1.2
total methyl ethers of Ara	3.4	6.8	6.8		1.2
2,3-Me_2-Xyl	tr	2.0	5.2	tr	1.0
Xyl $(acet)_5$	2.3	0.3	0.3	tr	2.0
total methyl ethers of Xyl	2.3	2.3	5.5		3.0
2,3,4,6-Me_4-Glc	3.2	2.4	4.2	0	1.8
2,3,6-Me_3-Glc	66.0	17.5	4.9	tr	71.6
2,3-Me_2-Glc	4.1	8.4	4.9	0	5.2
total methyl ethers of Glc	73.3	27.9	14.0		78.6
2,3,4-Me_3-Glc (6D_2)	nd[c]	9.0	9.7	6.0	nd
2,3,4,6-Me_4-Gal	1.6	2.0	4.0	tr	0.4
3,4,6-Me_3-Gal	0.8	8.8	2.1	tr	0.5
2,3,4-Me_3-Gal	1.4	12.4	12.1	21.8	1.5
2,4-Me_2-Gal	6.9	0.4	2.8	tr	7.6
total methyl ethers of Gal	10.7	23.6	21.0	21.8	10.0
2,3-Me_2-Gal (6D_2)	nd	tr	3.3	2.4	nd
2,3,6-Me_3-Man	1.2	8.7	1.3	0	1.0
3,4-Me_2-Rham	0.6	1.2	3.4	tr	0
3-Me-Rham	tr	0.4	4.7	tr	1.1
total methyl ethers of Rham	0.6	1.6	8.1		1.1

[a] Relative molar ratio calculated from the ratio of peak heights. [b] WSCS-II, -III, and -IV were carboxyl reduced (O'Neill, 1990). [c] nd, not determined. [d] tr, trace amount.

respectively) with small amount of branches (4 and 5%, respectively). Other components were present in a similar ratio between the two fractions. These data are in agreement with the monosaccharide analysis (Table 2) in which both WSCS-I and WSCS-V were found to contain mainly glucose and traces of other sugars. By increasing the ionic strength during ion-exchange chromatography, the eluted WSCS-II and WSCS-III fractions had similar contents of nonreducing end glucuronic acid (9.0 and 9.3%, respectively), while the 1,4-linked galacturonic acid increased from trace amounts in WSCS-II to about 3% in WSCS-III. The increase in galacturonic acid with increasing ionic strength suggested that the presence of galacturonic acid in the polysaccharides favors their binding to the DEAE-cellulose. This is in agreement with the DEAE-cellulose ion-exchange chromatographic results for WSCP series that less glucuronic acid and more galacturonic acid favored retention of the polymers on the column (Table 3; Figure 2). In addition, the presence of 1,4-linked mannose was greater in WSCS-II (8.7%) than in WSCS-III (3%), while the amount of 1,4-linked xylopyranose was lower in WSCS-II (2%) than in WSCS-III (5.2%). Neither of these fractions appeared to be homogeneous since they both contained 14–28% glucose, in agreement with the results of monosaccharide analysis (Table 2).

NMR Spectroscopy. ^{13}C NMR spectra of WSCP-II–V are presented in Figure 8. The spectrum of WSCP-I was not determined because it was partially insoluble. In Figure 8, two uronic acid peaks were identified with almost equal intensity and could be attributed to nonreducing end glucuronic acid (δ 176.1) and 1,4-linked galacturonic acid (δ 175.2), respectively. This is consistent with the methylation data, in which glucuronic acid and 1,4-linked galacturonic acid contents for the WSCP-III fraction accounted for 13.3 and 13.6%, respectively (Table 3). A resonance at δ 17.8 is due to the C-6 methyl group of rhamnose. In the anomeric carbon resonance region (δ 90–110), two strong peaks were identified at δ 104.1 and 103.4 as were two medium-intensity peaks with chemical shifts of δ 99.17 and 98.55. The weak resonances at chemical shifts δ 108–110 could be due to trace amounts of arabinose (Table 3), which is commonly present in pectic polysaccharides (Gould et al., 1965). As shown in Figure 8, both WSCP-II and WSCP-IV exhibited spectral patterns similar to that of WSCP-III; however, their anomeric resonance region was more complex. This is in agreement with the monosaccharide and methylation analyses, implying that WSCP-III is more homogeneous than WSCP-II and WSCP-IV; WSCP-III contains only trace amounts of glucose. As shown in Figure 8, the ^{13}C NMR spectrum of WSCP-V is rather simple. There is only one single major resonance in the anomeric region which can be attributed to the C-1 of 1,4-linked β-D-glucose residue, while the resonance at δ 60.97 is due to the C-6 of the same sugar. The C-4 resonance is located at δ 79.37, while chemical shifts of δ 75.69, 74.97, and 73.84 could be attributed to C-5, C-3, and C-2 of the same residue, respectively (Bock et al., 1984; Grimmecke et al., 1991). Other resonances over the δ 47–55 region could be due to the presence of methyl or ethyl ethers (Tezuka et al., 1991). The presence of these groups could account for the solubility of this cellulose-like material in aqueous solution.

The ^{13}C NMR spectra of WSCS-I–V are presented in Figure 9. The spectra of WSCS-I and WSCS-V are similar to that of WSCP-V, typical of cellulose-like structure. It appeared that the relative resonance intensity of the methyl ether groups (δ 45–55) is higher for WSCS-V as compared to WSCS-I. These structural differences could be related to the flow behavior of these fractions in solutions; WSCS-I contained fewer methyl groups and exhibited shear thinning properties, while WSCS-V contained a relatively higher amount of methyl groups and showed Newtonian flow behavior at the same polymer concentration. A higher content of methyl groups may favor the solubilization of the cellulose-like material, while a lower content of methyl ether groups may favor network development (solid-like character) in the solutions of this

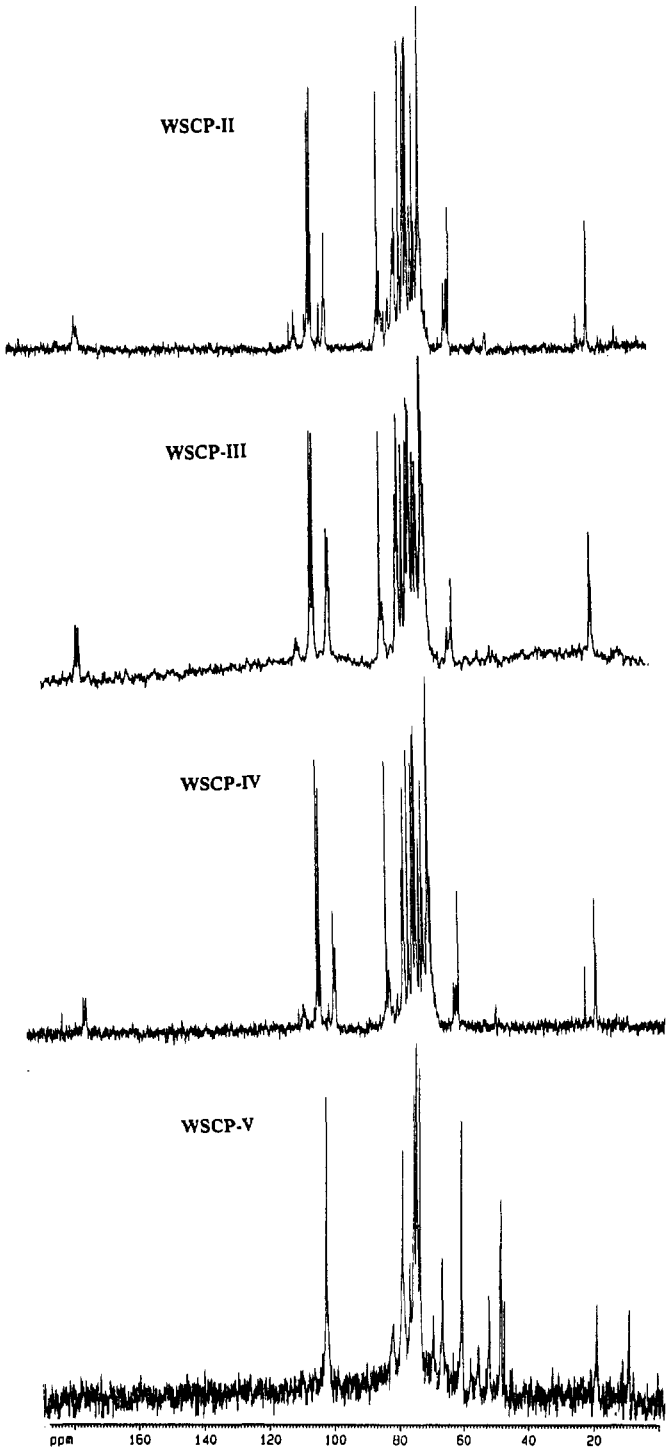

Figure 8. Comparison of ^{13}C NMR spectra of WSCP-II–V.

material. Only the nonreducing end glucuronic acid resonance was identified for WSCS-II (Figure 9). This is in agreement with the methylation analysis, where only trace amounts of 1,4-linked galacturonic acid were found (Table 4). The content of 1,4-linked galacturonic acid increased from WSCS-II to WSCS-IV, consistent with the methylation data, thus supporting the view that the DEAE-cellulose column selectively retained polysaccharides containing 1,4-linked galacturonic acid. The anomeric carbon regions of WSCS-II–IV are quite complicated, although some reductions in resonance intensities were observed. This resonance diversity in the anomeric region reflects the nonhomogeneity of these fractions, as confirmed by monosaccharide and methylation analyses (Tables 2 and 4).

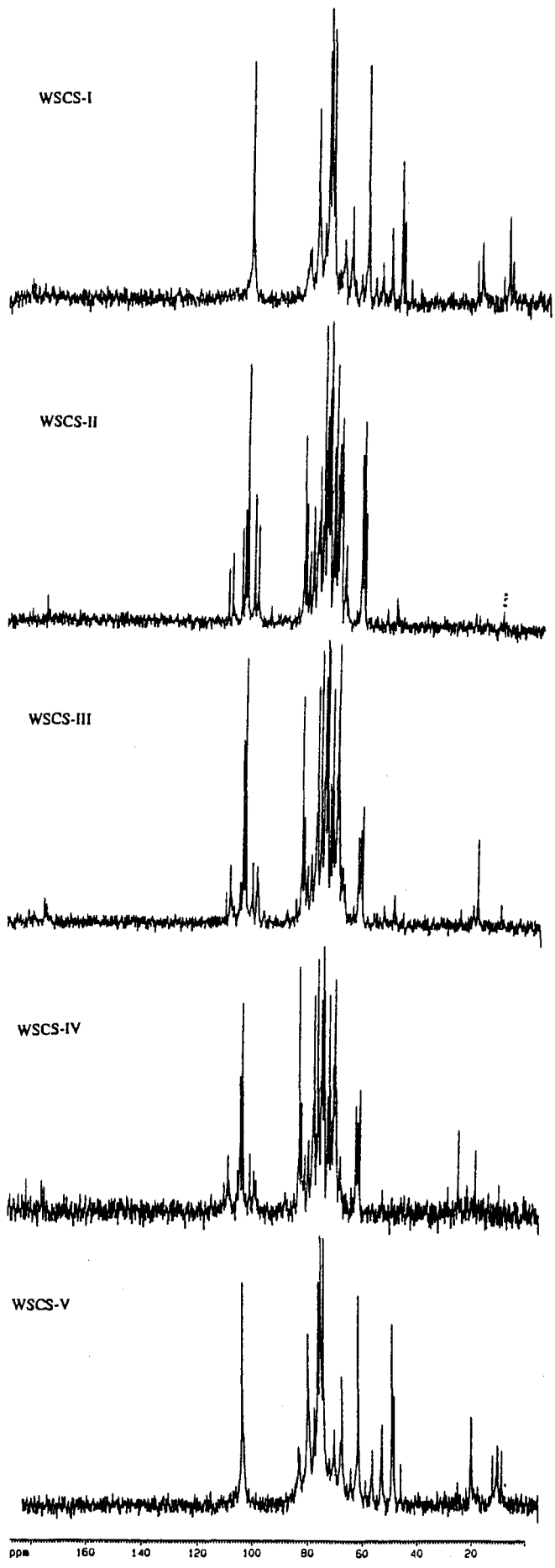

Figure 9. Comparison of ^{13}C NMR spectra of WSCS-I–V.

Conclusions. The water-soluble yellow mustard polysaccharides were fractionated into 10 subfractions by DEAE high-capacity ion-exchange chromatography. Of the isolated fractions, WSCP-I, WSCP-III, and WSCS-I were identified as those responsible for the pronounced shear thinning properties of yellow mustard mucilage solutions (Cui et al., 1993a,b). All of the other fractions exhibited Newtonian-like behavior in solution under the conditions investigated. Methylation analysis revealed that WSCP-I was mainly composed of 1,4-linked β-D-glucose together with a small amount of 1,4-linked xylopyranose and all substituted xylose. WSCP-III was a relatively homogeneous pectic polysaccharide containing 1,6-linked galactose (22.9%) and 1,2-linked (11%) and 1,2,4-linked (17%) rhamnose together with 13.6% nonreducing end glucuronic acid and 13.3% 1,4-linked galacturonic acid as shown by methylation analysis and ^{13}C NMR spectrum. The structure of WSCS-I is rather simple, containing primarily 1,4-linked β-D-glucose. This study revealed, for the first time, the relationship between the internal structure of some purified yellow mustard mucilage polysaccharide fractions and their rheological properties, particularly those exhibiting strong shear thinning behavior in aqueous solutions.

ACKNOWLEDGMENT

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.

Bock, K.; Pedersen, C.; Pedersen, H. Carbon-13 nuclear magnetic resonance data for oligosaccharides. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 192–226.

Ciucanu, I.; Kerek, F. A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.* **1984**, *131*, 209–217.

Cui, W.; Eskin, N. A. M.; Biliaderis, C. G. Physical and chemical properties of yellow mustard (Sinapis alba, L) mucilage. *Food Chem.* **1993a**, *46*, 169–176.

Cui, W.; Eskin, N. A. M.; Biliaderis, C. G. Water-soluble yellow mustard (Sinapis alba L.) polysaccharides: partial characterization, molecular size distribution and rheological properties. *Carbohydr. Polym.* **1993b**, *20*, 215–225.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

Englyst, H.; Wiggins, H. S.; Cummings, J. H. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1982**, *107*, 307–318.

Gould, S. E. B.; Rees, D. A.; Richardson, N. G.; Steele, I. W. Pectic polysaccharides in the growth of plant cells: molecular structural factors and their role in the germination of white mustard. *Nature* **1965**, *208*, 876–878.

Grimmecke, H. D.; Mamat, U.; Voges, M.; Lauk, W.; Shashkov, A. S.; Knirel, Y. A. Structure of the extracellular polysaccharide of Acetobacter methanolicus MB 58/4 (IMET 10945). *Carbohydr. Res.* **1991**, *218*, 247–251.

Izydorczyk, M. S.; Biliaderis, C. G. Effect of molecular size on physical properties of wheat arabinoxylan. *J. Agric. Food Chem.* **1992**, *40*, 561–568.

Kato, Y.; Nevins, D. J. Structural characterization of an arabinoxylan-rhamnose-galacturonan complex from cell walls of *Zea* shoots. *Carbohydr. Res.* **1992**, *227*, 315–329.

Morris, E. R.; Cutler, A. N.; Ross-Murrphy, S. B.; Rees, D. A. Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydr. Polym.* **1981**, *1*, 5–21.

O'Neill, M. A.; Darvill, A. G.; Albersheim, P. Structural analysis of an acidic polysaccharide secreted by *Xanthobacter* sp CATC (53272). *Carbohydr. Res.* **1990**, *206*, 289–296.

Tezuka, Y.; Imai, K.; Ohhima, M.; Ito, K. ^{13}C NMR structural study on an enteric pharmaceutical coating cellulose derivative having ether and ester substituents. *Carbohydr. Res.* **1991**, *222*, 250–259.

Received for review July 19, 1993. Accepted November 29, 1993.*

* Abstract published in *Advance ACS Abstracts*, January 15, 1994.