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CHARACTERIZATION OF POLYSACCHARIDES ISOLATED FROM MAPLE SYRUP*

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Abstract—Seven high molecular weight polysaccharides, including a dextran, four hydroxyprolyl-containing arabinogalactans, a rhamnogalacturonan, and another fraction rich in fucosyl, glucosyl, mannosyl and xylosyl residues, have been identified as components of maple syrup. The glycosyl-linkage compositions of several of these polysaccharides are similar to those of plant cell wall polysaccharides.

INTRODUCTION

Evidence has been obtained that the structures of non-cellulosic polysaccharides of primary cell walls of plants are complex [1] and include fragments called 'oligosaccharins,' which are oligosaccharides that can regulate various physiological processes in plants [2]. Tree sap, which rises in the spring as trees begin to flower and renew growth, is a logical source of oligosaccharins. To obtain information about this possibility, we have fractionated and partially characterized the polysaccharides present in maple syrup, a commercial concentrate of maple tree sap. It was already known that maple syrup contains a dextran [3] and two arabinogalactans [4, 5]. We confirmed these findings and now report the presence of several additional polysaccharides in maple syrup.

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RESULTS AND DISCUSSION

Dialysed, lyophilized maple syrup (1.0 g) was fractionated on a QAE-Sephadex anion-exchange column (Fig. 1). The carbohydrate-containing column fractions

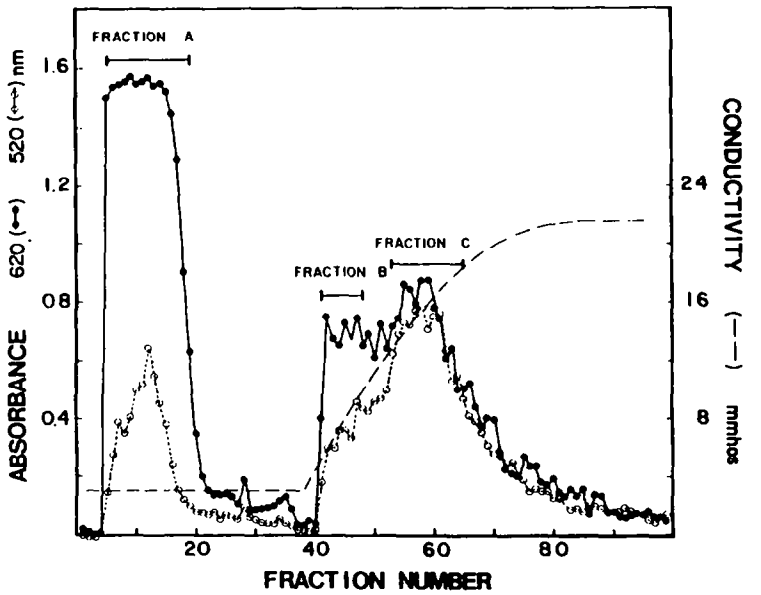

Fig. 1. Chromatography of maple syrup polysaccharides on QAE-Sephadex. The (2.6 × 18 cm, 100 ml) column was equilibrated with 10 mM imidazole–HCl, pH 7.0. The sample was loaded and then washed with 200 ml of the same buffer and then eluted with a 0.01–1.2 M logarithmic gradient of imidazole–HCl, pH 7.0. The glycosyl content (A_{620}) of the fractions was determined by the anthrone method [9], and the glycosyluronic acid content (A_{520}) by the *m*-hydroxydiphenyl method [10].

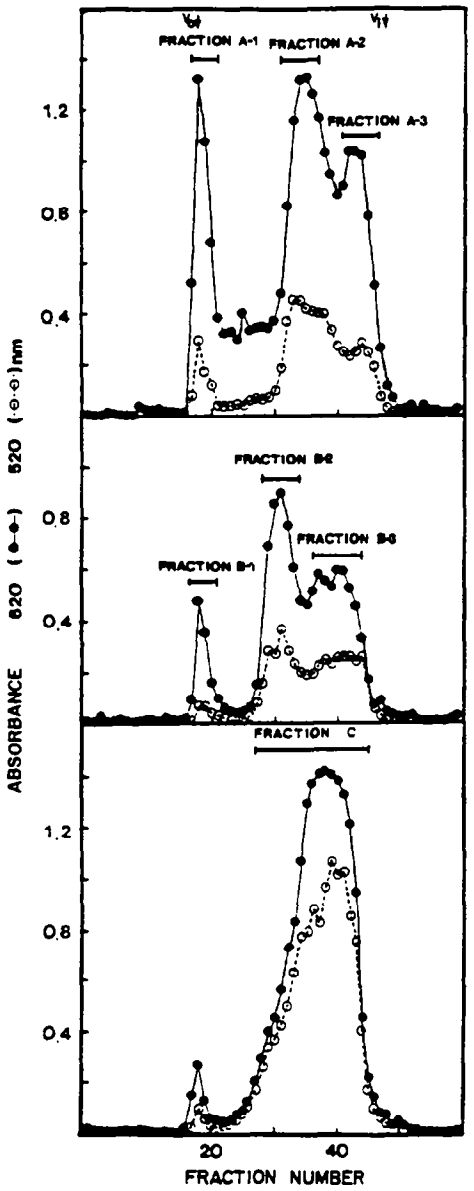

Fig. 2. Chromatography of QAE fractions A, B, and C on Agarose 5m. The 3 × 60-cm 500-ml column was equilibrated and eluted with 50 mM NaOAc, pH 5.2. The glycosyl (A_{620}) and glycosyluronic acid (A_{520}) content was assayed as described in Fig. 1.

were combined into three pooled fractions: fraction A, comprised of polysaccharides that did not bind to the column, and fractions B and C, two ionic polysaccharide fractions that did bind to the column. The two ionic fractions were distinguished from one another by their different glycosyluronic acid contents.

Fractions A, B, and C were dialysed and lyophilized and then applied separately to an Agarose 5m column eluted with 50 mM sodium acetate, pH 5.2 (Fig. 2). Fraction A was separated into the three components, labeled A-1, A-2 and A-3 (Fig. 2). Fraction B was also separated into three different-size components, labeled B-1, B-2 and B-3 (Fig. 2). Polysaccharide fraction C was not further resolved by the gel-filtration chromatography (Fig. 2). Each of the A-5m polysaccharide fractions were pooled as indicated (Fig. 2), dialysed and analysed for glycosyl and glycosyl-linkage compositions.

Glycosyl-composition analysis of polysaccharide fraction A-1 revealed predominantely glucosyl residues (~90%), also mannosyl (7%) and galactosyl (2%) residues, and smaller amounts of arabinosyl and xylosyl residues. Further characterization of fraction A-1 by glycosyl-linkage analysis established that the glucosyl residues were predominantly 6-linked, with small amounts of 3- and 4-linked glucosyl residues also present. A per-*O*-methylated aliquot of fraction A-1 was also analysed by ^{1}H NMR spectrometry. The resonance signals for the anomeric protons were at δ5.02 with a coupling constant of 2.5 Hz, which established that the glucosyl residues were α-linked [6]. The molecular weight of the glucan in fraction A-1 was estimated by gel-filtration chromatography on Agarose 150m in 50 mM sodium acetate, pH 5.2. The dispersal of the glucan across the entire elution volume of the column indicated a broad range of molecular weights greater than 5 000 000. Thus, the major component in fraction A-1 was a polydispersed, large molecular weight, α-6-linked glucan (dextran).

Fraction B-1 was similarly analysed and shown to be composed of mannosyl (30%), xylosyl (29%), glucosyl (16%) and fucosyl (12%) residues and smaller amounts of galactosyl (7%), arabinosyl (6%) and rhamnosyl (1%) residues. In addition, fraction B-1 contained 5% glucosyluronic acid residues. The glycosyl-linkage composition of fraction B-1 is given in Table 1. Gel-filtration chromatography on Agarose 150m established that the polysaccharide(s) in fraction B-1 had the same polydispersity and range of molecular weights as the dextran in fraction A-1. The A150m fractions containing polysaccharides of various sizes had the same glycosyl composition. Thus, the polysaccharide in fraction B-1 was either a single, polydispersed polysaccharide of unknown type or, more likely, two or more polysaccharides of types previously characterized in plants [1]. The small amount of this fraction that was available did not permit further purification or analysis.

Glycosyl-composition analysis of fractions A-2, A-3, B-2 and B-3 showed that all the polysaccharides in the

Table 1. Glycosyl-linkage composition of fraction B-1

Glycosyl residue	Deduced glycosidic linkage	Mole %
Arabinosyl	terminal	5
Fucosyl	terminal	11
Xylosyl	terminal	16
	2	4
	4	4
Mannosyl	terminal	3
	2	9
	3	12
	2, 3	11
	3, 4	2
	2, 3, 6	6
Galactosyl	terminal	3
	2, 6	8
Glucosyl	6	4
	2, 3	3

fractions were composed predominantly of arabinosyl and galactosyl residues. These polysaccharides were rechromatographed on Agarose 5m (Fig. 3). The glycosyl compositions of column fractions across the polysaccharide-containing peaks were very similar (Table 2), which was evidence that the polysaccharides in each fraction were essentially pure. Repeating this analysis three times established that the small variations observed could be accounted for by variations inherent in the analytical procedure, although some heterogeneity could not be ruled out. In fact, the presence of 13 mole % glucose in column fraction 42 established that fraction A-3 was contaminated by a glucose-rich polymer.

The glycosyl-linkage compositions of the rechromatographed and pooled polysaccharide fractions A-2, A-3, B-2 and B-3 are presented in Table 3. The polysaccharides in each of the four fractions were very similar. Terminal and 5-linked arabinofuranosyl residues and terminal, 3-, 6- and 3,6-linked galactopyranosyl residues were also major components of all fractions, although the ratio of these residues varied. The glycosyluronic acid composition of each fraction was determined by methanolysis and trimethylsilation. Galactosyluronic acid was not detected in any of the fractions, but glucosyluronic acid was present in the following percentages: fraction A-2, 3%; fraction A-3, 1%; fraction B-2, 6%; and fraction B-3, 8%. All four fractions also contained between 1% and 2% hydroxyproline. The terminal and 5-linked arabinosyl, 3,6-linked galactosyl, glucosyluronic acid and hydroxyprolyl residues of the fractions are characteristic of arabinogalactan glycoproteins [7]. However, we failed to obtain evidence for other amino acids in these fractions. Therefore, throughout this manuscript we refer to the arabinogalactans as polysaccharides rather than glycoproteins.

We obtained some insight into the structure of the arabinogalactan in fraction A-2 by subjecting the polymer to treatment with acid under conditions that hydrolysed two-thirds of the glycosidic linkages of the arabinofuranosyl residues without hydrolysing detectable amounts of the glycosidic linkages of the galactopyranosyl residues. Comparing the glycosyl-linkage analyses before and after partial acid hydrolysis indicated that the polymer has a backbone of 6-linked galactopyranosyl residues to which side chains of arabinofuranosyl residues are attached at O-3 of the galactopyranosyl residues (data not shown). The side chains consist predominantly of terminal and 5-linked arabinosyl residues. These structural features are characteristic of arabinogalactans isolated from intact plant tissues [7].

The polysaccharides in fraction C (Fig. 2) were composed of galactosyl (40%), rhamnosyl (18%), galactosyluronic acid (18%) and arabinosyl (10%) residues. Much smaller amounts of mannosyl and glucosyl residues were also present. A high content of rhamnosyl and galactosyluronic acid residues is characteristic of rhamnogalacturonans [8]; a high content of galactosyl and arabinosyl residues is characteristic of arabinogalactans [7]. Therefore, several techniques were used in an attempt to separate fraction C into two or more components. De-

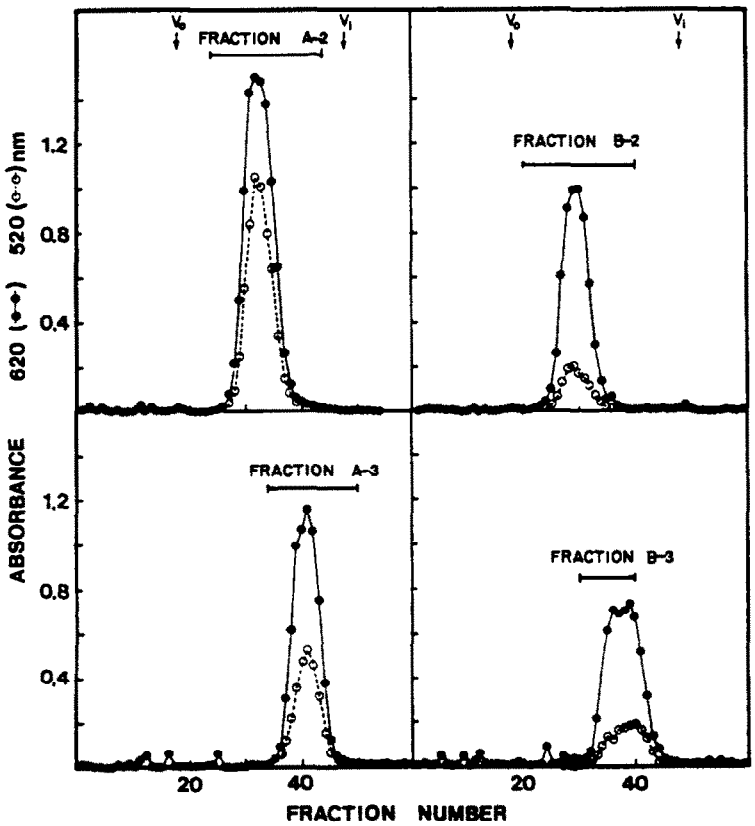

Fig. 3. Rechromatography of A5m fractions A-2, A-3, B-2 and B-3 on the same Agarose 5m column and with the same conditions as in Fig. 2.

Table 2. Glycosyl residue compositions of A 5 m column fractions of rechromatographed fractions A-2, A-3, B-2 and B-3 (see Fig. 3)

Glycosyl residue	Fractions containing A-2			Fractions containing A-3		
	29	33	36	38	42	44
	mole %			mole %		
Rhamnosyl	4	4	4	4	3	5
Fucosyl	0	0	0	2	4	6
Arabinosyl	43	44	37	39	35	37
Xylosyl	0	0	0	1	1	4
Mannosyl	5	1	4	4	4	5
Galactosyl	47	50	53	46	40	43
Glucosyl	2	1	1	4	13	0
Glycosyl residue	Fractions containing B-2			Fractions containing B-3		
	26	30	33	33	37	40
Rhamnosyl	7	7	8	9	8	10
Fucosyl	0	0	0	0	0	0
Arabinosyl	46	43	39	35	30	29
Xylosyl	0	0	0	0	0	3
Mannosyl	0	0	0	1	1	2
Galactosyl	47	50	53	55	60	54
Glucosyl	0	0	0	0	0	2

Table 3. Glycosyl-linkage composition* of rechromatographed fractions A-2, A-3, B-2 and B-3

Glycosyl residue	Deduced glycosidic linkage	Fraction A-2	A-3 (mole %)	B-2	B-3
Rhamnosyl	terminal	0	0	1	1
Arabinosyl	terminal	26	22	18	19
	2	2	4	1	3
	3	2	4	1	3
	5	18	10	12	12
	2, 5	1	2	2	1
	3, 5	1	1	3	1
Galactosyl	terminal	3	4	5	9
	3	10	7	9	13
	4	0	1	4	2
	6	7	9	5	7
	3, 4	0	0	3	1
	3, 6	26	17	11	20
	3, 4, 6	0	1	8	1

*Any derivative that comprised less than 1% of the fraction has been omitted.

Table 4. Glycosyl-linkage composition* of fraction C

Glycosyl residue	Deduced glycosidic linkage	Mole %
Rhamnosyl	terminal	1
	2	7
	4	5
	2, 4	9
Arabinosyl	terminal	12
	5	3
Xylosyl	terminal	2
Galactosyl	terminal	19
	3	6
	4	7
	6	4
	3, 6	6
Glucosyl	terminal	6
	4	3

*Any derivative that comprised less than 1% of the fraction has been omitted.

esterification and treatment with endo-α-1,4-polygalacturonase did not result in any separation by either QAE-Sephadex or Agarose 5m chromatography. Nor was separation of the polysaccharides achieved on an Agarose 5m column eluted with a high-salt buffer (0.5 M imidazole–HCl with 10 mmol EDTA, pH 7.0). The glycosyl-linkage composition of the polysaccharides in fraction C is given in Table 4. The high mole % of 3,6-galactosyl residues suggests the presence of an arabinogalactan. However, the high mole % of 2- and 2,4-linked rhamnosyl residues is characteristic of rhamnogalacturonan I, a pectic polysaccharide found in primary cell walls of higher plants [1, 8]. Fraction C was therefore considered to contain an arabinogalactan and a rhamnogalacturonan, covalently attached to each other.

We have shown that maple syrup contains at least seven large molecular weight polysaccharides. Analyses of the glycosyl and glycosyl-linkage compositions showed the polysaccharides to be an α-6-linked glucan (dextran), four

hydroxyprolyl-containing arabinogalactans, one pectic rhamnogalacturonan, and a fraction rich in fucosyl, xylosyl, mannosyl and glucosyl residues. Only the dextran [3] and two arabinogalactan polysaccharides [4, 5] had previously been reported. The glycosyl-linkage composition of the arabinogalactans and the rhamnogalacturonan are similar to those of polysaccharides isolated from primary cell walls of plants [1]. We cannot rule out the possibility that one or more of the polysaccharides, especially the dextran, are not products of the maple tree but, for example, could have resulted from bacterial contamination of the sap while the sap was being collected.

EXPERIMENTAL

Isolation of maple syrup polysaccharides. Pure, commercial maple syrup (MacDonald's Pure Maple Syrup, Safeway) was diluted with 5 vols H_2O and both filtered and concd to 1/3 of its initial volume with a Pellicon cassette system (Millipore) with a 10000 M_r cut-off cassette (PTGC). This procedure removed most of the sucrose and other low molecular weight oligosaccharides. The concentrate was dialysed extensively against distilled H_2O in 12000–14000 M_r cut-off tubing and then lyophilized.

Anion-exchange and gel-filtration chromatography. Anion-exchange fractionation of the maple syrup polysaccharides was carried out on a QAE-Sephadex column (2.6 × 18 cm, 100 ml) eluted with a 0.01–1.2 M logarithmic gradient of imidazole–HCl, pH 7.0. The polysaccharides were further fractionated by gel-filtration chromatography on Agarose 5m (3 × 60 cm, 500 ml) or 150m (2 × 30 cm, 70 ml) columns eluted with 50 mM NaOAc, pH 5.2.

Colorimetric assays. Neutral sugar concns were determined by the anthrone assay of ref. [9] and glycosyluronic acid concns by the *m*-hydroxydiphenyl assay of ref. [10]. The hydroxyproline content was determined by the *p*-methylaminobenzaldehyde assay of ref. [11].

Analysis of glycosyl composition. The neutral glycosyl compositions of the polysaccharides were determined by GC analysis [12] of alditol acetate derivatives obtained by 2-hr, 2 N TFA hydrolysis at 120°, reduction with $NaBD_4$ and acetylation [12].

The neutral glycosyl and glycosyluronic acid compositions of the polysaccharides were determined by GC analysis [13] of methylglycoside derivatives made by methanolysis with HCl–MeOH followed by silylation with Trisil (Pierce) silylating reagent [14].

Partial acid hydrolysis of arabinogalactan fraction A-2. Two samples of arabinogalactan fraction A-2 (2 mg each) were dissolved in 100 μl of 2 N TFA. One sample was heated to 60° for 70 min; the other was kept at 0° for 70 min. The 60° for 70 min hydrolysis conditions had been determined (see ref. [16] for method) to hydrolyse 68% of the arabinosyl residues and 0% of the galactosyl residues of arabinogalactan fraction A-2. The partially hydrolysed and unhydrolysed samples were dissolved in H_2O, dialysed extensively against distilled H_2O, and then lyophilized. The glycosyl-linkage composition of the hydrolysed samples was determined [15, 16]. This experiment was repeated twice.

Glycosyl-linkage-composition analysis. The glycosyl-linkage compositions of the polysaccharides were determined by the methylation procedure described in ref. [15] and modified in ref. [16]. The samples (250 μg) were dissolved in DMSO (250 μl) and treated × 3 with 4 M potassium dimethyl sulphinyl anion (20 μl) followed by addition 16 M MeI (5 μl). The per-*O*-methylated samples were separated from the reactants on Sep-Pak C_{18} cartridges (Waters Associates) [16] and then hydrolysed, reduced and per-*O*-acetylated. The partially *O*-methylated alditol acetates were analysed by GC and GC/MS [16].

^{1}H NMR spectroscopy. ^{1}H NMR spectra of per-*O*-methylated polysaccharides dissolved in hexadeuterioacetone (99.997% D) were recorded with a Bruker WM-250, Fourier-transform NMR spectrometer.

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REFERENCES

1. Darvill, A. G., McNeil, M. Albersheim, P. and Delmer, D. P. (1980) in *The Biochemistry of Plants, A Comprehensive Treatise* (Tolbert, N. E., ed.) pp. 91–162. Academic Press, New York.
2. Albersheim, P., Darvill, A. G., McNeil, M., Valent, B., Sharp, J. K., Nothnagel, E. A., Davis, K. E., Yamazaki, N., Gollin, D. J., York, W. S., Dudman, W. F., Darvill, J. E. and Dell, A. (1983) in *Structure and Function of Plant Genomes* (Ciferri, O. and Dure, L., III, eds) pp. 293–312. Plenum Press, New York.
3. Roberts, E. J. (1982) *Publ. Tech. Pap. Proc. Annu. Meet. Sugar Ind. Technol.* **14**, 298.
4. Adams, G. A. and Bishop, C. T. (1960) *Can. J. Chem.* **38**, 2380.
5. Lamport, D. T. A. (1977) in *Advances in Phytochemistry* (Loewus, F. A. and Runeckles, V. C., eds) Vol. 11, pp. 93–97. Plenum Press, New York.
6. Hall, L. D. (1964) in *Advances in Carbohydrate Chemistry* (Wolfrom, M. L., ed.) Vol. 19, pp. 51–93. Academic Press, New York.
7. Clarke, A. E., Anderson, R. L. and Stone, B. A. (1979) *Phytochemistry* **18**, 521.
8. McNeil, M., Darvill, A. G. and Albersheim, P. (1980) *Plant Physiol.* **66**, 1128.
9. Dische, Z. (1962) in *Methods in Carbohydrate Chemistry* (Whistler, R. and Wolfrom, M., eds) Vol. 1, pp. 478–512. Academic Press, New York.
10. Blumenkrantz, N. and Asboe-Hansen, G. (1973) *Analyt. Biochem.* **54**, 484.
11. Kivirikko, K. I. and Liesmaa, M. (1959) *Scand. J. Clin. Lab. Invest.* **11**, 128.
12. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A. (1967) *Carbohydr. Res.* **5**, 340.
13. Hahn, M. G., Darvill, A. G. and Albersheim, P. (1981) *Plant Physiol.* **68**, 1161.
14. Chambers, R. E. and Clamp, R. J. (1971) *Biochem. J.* **125**, 1009.
15. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
16. Waeghe, T. J., Darvill, A. G., McNeil, M. and Albersheim, P. (1983) *Carbohydr. Res.* **123**, 281.