Studies on Vegetables. Investigation of Water, Oxalate, and Sodium Hydroxide Soluble Celery (*Apium graveolens*) Polysaccharides: Pectic Polysaccharides[†]

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Pectin fractions were isolated from celery, by extraction with water and aqueous ammonium oxalate. The former fraction had $[\alpha]^{25}_D$ +219° and contained L-arabinose, D-galactose, D-glucose, and L-rhamnose in the molar proportion 0.67:1.00:0.12:0.27 and 65.1% galacturonic acid residues having a degree of esterification of 18.4%. The major oxalate soluble pectinic acid (A_2: $[\alpha]^{25}_D$ +207°; arabinose, galactose, glucose, rhamnose, xylose ratio, 0.69:1.00:0.10:0.67:0.08; galacturonic acid, 70.2%; degree of esterification, 1.65%) was quite similar to the water fraction, with a small difference in the uronic acid content and degree of esterification of the uronate residues and L-rhamnose content but wide variations in specific optical rotation and uronic acid contents compared to pectinic acids A_1 and A_2. All four pectinic acids were homogeneous on the ultracentrifuge. The pectinic acid A_0 from the water fraction was reduced and subjected to methylation analysis, and its general structural features are discussed. Evidence is also presented for the presence of an amyloid and a 4-*O*-methylglucuronoxylan in the sodium hydroxide soluble fraction of celery.

In the context of dietary fiber, the structural elucidation of component polysaccharides is important. Structural identities, which influence their physiological action, cannot be determined by simple procedures of sugar analysis. Their diversity and complexity therefore require detailed and complex methods of analysis.

The pectic polysaccharides are quite widely distributed in edible plants, and a number of polysaccharides of this nature have been studied in detail in the past (Barret and Northcote, 1965; Aspinall et al., 1967, 1968; Aspinall and Jiang, 1974; Rees and Wight, 1969; Siddiqui and Wood, 1976; Talmadge et al., 1973). In recent years, in view of their physiological significance, renewed attention has been focused on the pectic polysaccharides of fruits and vegetables. The noteworthy studies in this connection are those carried out on apples (Aspinall et al., 1983; Aspinall and Fanous, 1983; Stevens and Selvendran, 1984a; Varies et al., 1981, 1983), carrots (Stevens and Selvendran, 1984b; Aspinall et al., 1983), onions (Makarios et al., 1980; Redgwell and Selvendran, 1986), dwarf french and runner beans (O'Neill and Selvendran, 1980), and cabbage (Stevens and Selvendran, 1984c).

Pectins contribute to many physiological effects such as lowering of cholesterol and binding of bile acids, polyvalent cations, and water, etc. (Furda, 1979; Miettinen and Tarpilla, 1977). The cation- and water-binding properties originate from the ability of component sugars especially the uronic acids in the pectic polysaccharides to form hydrated coordination complexes with the mono-, di-, and trivalent ions (Gould et al., 1975; Cook and Bugg, 1977; Allen, 1980). The viscous or gelling fibers (pectin, guar, and tragacanth gums, etc.) appear to be most effective in producing lower blood lipid levels and a flatter glycemic curve (Jenkins et al., 1985).

In previous papers, the isolation and characterization of an arabinogalactan were described (Siddiqui, 1989). We now report on pectic polysaccharides of celery stalks.

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EXPERIMENTAL SECTION

The general experimental methods have been reported previously (Siddiqui, 1989).

Analysis of Celery Pectinic Acid (Water Fraction). The pectinic acid from the water fraction (0.87 g; Siddiqui, 1989), now designated A_0, had $[\alpha]^{25}_D$ +219° (c 1, 0.05 M sodium hydroxide) (found: uronic anhydride, 65.1%; OME, methyl ester content, 1.96%). Sedimentation analysis (Svedberg and Pederson, 1940) of a 0.5% solution in 0.02 M sodium acetate at 52 000 rpm using a synthetic boundary cell showed a single symmetrical peak.

Pectic polysaccharide (5 mg) was hydrolyzed with chilled 72% sulfuric acid (0.12 mL) at 5 °C for 2 h and, following dilution, 1 M acid for 2 h at 100 °C. After neutralization ($BaCO_3$), filtration, deionization with Rexyn-101 (H^+) resin, and concentration, the mixture of acidic and neutral sugars was fractionated on a column of Dowex 1X2 (CO_3^{2-}) resin. The mixture of neutral sugars (eluted with water) was reduced with sodium borohydride, and the resulting mixture of glycitols was acetylated and examined by GLC, giving arabinose, galactose, glucose, and rhamnose in the molar ratio 0.67:1.00:0.12:0.27.

Carboxyl Reduction of Pectinic Acid from Water Fraction. The major pectinic acid A_0 (500 mg) from the water fraction was dissolved in water (75 mL) and the resultant mixture adjusted to pH 4.75. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (5 g) was added over a period of 1 h, and the solution was maintained at pH 4.75 by the addition of 0.1 M hydrochloric acid with a pH stat (New Brunswick Scientific, Edison, NJ). After an additional 1 h, 2 M sodium borodeuteride (50 mL) was added slowly over a period of 1 h to the stirred reaction mixture maintained at pH 7 by the automatic addition of 4 M hydrochloric acid. After an additional 1 h, the mixture was dialyzed (Spectrapore membrane tubing, molecular weight cutoff 6000–8000) against running tap water for 64 h, then concentrated, and freeze-dried to a carboxyl-reduced product (450 mg). A second reduction was performed as above, except that the solution was maintained for 3 h instead of 2 h, and yielded a product: 225 mg; uronic anhydride, 27.8%. Two further reductions yielded a product: 119 mg; $[\alpha]^{25}_D$ +156° (c 0.4, water); uronic anhydride, 12.4%; galactose ratio (unreduced polysaccharide to reduced polysaccharide), 1:5.7 following hydrolysis, reduction, acetylation, and GLC of the samples.

Methylation of Reduced Pectinic Acid. The reduced polysaccharide (26 mg) was dissolved in dry methyl sulfoxide (1.5 mL) by stirring at 50 °C. The solution was flushed with nitrogen, and a 2 M solution (0.3 mL) of methylsulfonyl car-

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Table I. Methylation Data for the Celery Water Soluble Pectinic Acid

acetate	ret time, min (DB-225)	molar ratio
2,3,5-tri-*O*-methylarabinitol	0.75	6
3,4-di-*O*-methylrhamnitol	0.92	1
2,3-di-*O*-methylarabinitol[c]	0.94	4
2,3,4,6-tetra-*O*-methylglucitol[a,c,d]	0.96	2
2,3,5,6-tetra-*O*-methylgalactitol	0.98	3
2,3,4,6-tetra-*O*-methylgalactitol	1.00	2
3-*O*-methylrhamnitol	1.10	1
2-*O*-methylrhamnitol	1.13	2
2,3,6-tri-*O*-methylglucitol	1.14	2
2,3,6-tri-*O*-methylgalactitol[a,b]	1.16	52
arabinitol	1.19	4
2,3,4-tri-*O*-methylgalactitol	1.21	3
2,6-di-*O*-methylgalactitol[a]	1.23	1
3,6-di-*O*-methylgalactitol[a]	1.27	1
2,4-di-*O*-methylgalactitol	1.34	5

[a] Deuterated at C-6. [b] Contains ≈1 mol of 2,3,6-tri-*O*-methylgalactitol of non uronic acid origin. [c] These components were separated by changing the initial column temperature (General Methods, Siddiqui, 1989) to 100 °C with a hold time of 1 min, programmed to 225 °C at a rate of 4 °C/min. [d] RRT to 2,3,4,6-tetra-*O*-methylgalactitol.

banion (Hakomori, 1965) was added dropwise. The viscous solution was stirred magnetically and periodically on a Vortex mixer for 6 h. Methyl iodide (0.2 mL) was added dropwise with external cooling (ice–water). Following two such treatments, the sample was poured in water (5 mL) and the mixture was then dialyzed (Spectrapore tubing, molecular weight cutoff 6000–8000) for 18 h, concentrated, and extracted continuously with chloroform. The extract was dried (sodium sulfate) and concentrated to a syrup: 26 mg; $[\alpha]^{25}_{D}$ +117.7° (*c* 1, chloroform); no IR absorption for hydroxyl.

Analysis of Methyl Sugars. The methylated polysaccharide (25 mg) was boiled, under reflux, with 3% methanolic hydrogen chloride for 24 h, neutralized (Ag_2CO_3), filtered, and concentrated and the product hydrolyzed [0.5 M H_2SO_4 (2.5 mL), 30 h, 100 °C]. The hydrolysate was neutralized ($BaCO_3$), filtered, and concentrated to a syrup (20 mg).

PC of a solution of methyl sugars in solvent C showed at least eight components: R_f 0.05, 0.15, 0.19, 0.35, 0.44, 0.47, 0.66, 0.83. A portion (3 mg) of methyl sugars was reduced with sodium borohydride; and the products were acetylated and examined by GLC. The identity of the components (Table I) was confirmed from the GLC, GLC–EI-MS, and GLC–CI-MS results. Although no standard for 2,3,5,6-tetra-*O*methylgalactitol diacetate was available, the presence of primary fragments m/z 45, 89, 117, and 205 (Bjorndal et al., 1967) left no doubt as to its identity.

Analysis of Pectinic Acids (Oxalate Fraction). A water-eluted fraction W_1 (0.13 g) and three pectinic acids (A_1, 0.071 g; A_2, 0.71 g; A_3, 0.09 g) were similarly fractionated and recovered from the oxalate fraction, (Siddiqui, 1989). Fraction A_1 on analysis as above showed the following: $[\alpha]^{25}_{D}$ +102.7° (*c* 1, 0.10 M sodium hydroxide); uronic anhydride, 34.9% (Siddiqui and Morris, 1979); arabinose, galactose, glucose, rhamnose, and xylose ratio, 0.67:1.00:0.11:0.32:0.06; symmetrical peak on sedimentation analysis with a tiny shoulder ratio of 98:2.

Major pectinic acid A_2 similarly analyzed showed the following: $[\alpha]^{25}_{D}$ +207° (*c* 1, 0.1 M sodium hydroxide); uronic anhydride, 70.2%; OME, 0.19; arabinose, galactose, glucose, rhamnose, and xylose ratio, 0.69:1.00:0.1:0.67:0.08; single symmetrical peak on sedimentation analysis.

Pectinic acid A_3 showed the following: $[\alpha]^{25}_{D}$ +267° (*c* 1, 0.1 M sodium hydroxide); uronic anhydride, 84%; arabinose, galactose, glucose, and rhamnose ratio, 0.69:1.00:0.25:0.72; symmetrical, rapidly diffusing peak on sedimentation analysis.

Characterization and Proportions of Neutral and Acidic Sugars. Pectic polysaccharide A_2 (0.6 g) was hydrolyzed with chilled 72% sulfuric acid (32.4 mL) for 2 h at 5 °C and following dilution to 400 mL for 6 h at 100 °C. The hydrolysate was neutralized ($BaCO_3$), filtered, and concentrated to a solid, which

was dissolved in water, and, following decationization with Rexyn-101 (H^+), was fractionated on a Column of Dowex 1X2 (CO_3^{2-}) resin. Washing with water yielded neutral sugars (96 mg), and elution with 0.25 M ammonium carbonate yielded the acidic material (112 mg), which was recovered by removing the bulk of ammonium carbonate in vacuo at 65–70 °C and the remainder with Rexyn-101 (H^+) resin.

The mixture of neutral sugars was separated by PC (solvent A), giving the following fractions: 1, 37 mg; 2, 28 mg; 3, 32 mg. Fractions 1 and 2 on crystallization and recrystallization from 90–95% aqueous ethanol gave, respectively, D-galactose [mp and mixture mp 161–162 °C; $[\alpha]^{24}_{D}$ +83° (*c* 0.5, water)] and L-arabinose [mp and mixture mp 147–148 °C; $[\alpha]^{25}_{D}$ +105° (*c* 0.3, water)]. Fraction 3 (L-rhamnose) was identified by its $[\alpha]^{26}_{D}$ +7.7° (*c* 0.5, water) value. Glucose and xylose were recovered in amounts too small to permit further characterization.

The acidic sugar was identified by its behavior in PC solvent B and its M_{GLC} value (0.78) in buffer C (Haug and Larsen, 1961) to be D-galacturonic acid. It (100 mg) was dissolved in water (5 mL), and the solution was stirred with Rexyn-101 (H^+) resin (5 mL). Filtration yielded a solution (10 mL) that was heated with brucine (210 mg) for 10 min at 100 °C. Removal of excess brucine by extraction with chloroform (3 × 10 mL) and concentration of the aqueous fraction to dryness yielded a solid that was crystallized from aqueous methanol to yield brucine D-galacturonate, mp 173–174 °C, undepressed in admixture with an authentic sample, $[\alpha]^{28}_{D}$ −8.6° (*c* 1, water).

Fractionation of Hemicellullose B Fraction. A solution of the hemicellulose B fraction (2.6 g) in water (30 mL) was stirred with Rexyn-101 (H^+) resin. The insoluble residue was collected by filtration, and the soluble portion, following concentration to 20 mL, was fractionated on DEAE–cellulose (Siddiqui, 1989), yielding a neutral fraction W_2 (352 mg) and an acidic fraction A_4 (80 mg).

The insoluble residue was washed from the resin with 0.5 M sodium hydroxide and the solution acidified with glacial acetic acid, concentrated, and mixed with ethanol (4 volumes). The precipitate was washed with aqueous 80% ethanol, dissolved in water, and freeze-dried to yield a third fraction, A_5 (296 mg).

The neutral fraction W_2 showed $[\alpha]^{25}_{D}$ +4.62° (*c* 0.3, water). Acid hydrolysis of a portion (5 mg) with 72% sulfuric acid (Siddiqui, 1989), reduction of the resulting sugars ($NaBH_4$), acetylation, and GLC of glycitols revealed derivatives of arabinose–galactose–glucose–rhamnose–xylose in the ratio 0.69:1.00:2.00:0.40:1.97. Fractions A_4 and A_5 analyzed similarly showed $[\alpha]^{25}_{D}$ +25.3° (*c* 0.1 M, sodium hydroxide) and arabinose–galactose–glucose–rhamnose–xylose ratio 1.10:1.00:0.15:0.35:0.20 in fraction A_4 and $[\alpha]^{25}_{D}$ −12.9° (*c* 0.1 M, sodium hydroxide) and arabinose–galactose–glucose–rhamnose–xylose ratio 0.52:1.00:3.91:0.28:6.42 in fraction A_5.

PC and PE of the hydrolysates revealed traces of uronic acid in W_2 and minor amounts in A_4 and A_5.

Fractionation of Combined Fraction W_2 and A_5. The combined fraction (638 mg) in water (25 mL) was added to a column (2.5 × 35 cm) of DEAE–cellulose (borate form). Elution with water (400 mL), followed by gradient elution 0–0.5 M sodium metaborate (1.5 L) yielded five fractions. The water-eluted fractions were concentrated, acidified with acetic acid, dialyzed (16 h, tap water; 4 h, distilled water), and freeze-dried. Borate fractions B_1, B_2, and B_3 were similarly recovered.

The analytical data are summarized in Table II.

Further Fractionation of Borate-Fractionated Fractions. (a) *With Fehling's Solution.* Attempts to fractionate fractions W_3 and W_4 (350 mg) and fractions B_1, B_2, and B_3 (139 mg) with Cetavlon (cetyltrimethylammonium bromide) were unsuccessful. The material was recovered, and a portion of combined fractions (110 mg) in 0.5 M sodium hydroxide was centrifuged to yield insoluble fraction S (21.3 mg) recovered following neutralization (50% acetic acid) and washing with 80% aqueous ethanol, ethanol, and acetone. The clear supernatant was mixed with a freshly prepared Fehling's solution (2 mL). The gelatinous precipitate was removed by centrifugation, washed with water, suspended in cold water, acidified with 5% hydrochloric acid, and recovered, as above, yielding the copper complexing fraction (23 mg). The supernatant (copper noncomplexing fraction) solution was acidified with acetic acid, mixed

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Table II. Results of Borate Fractionation

	yield,	$[\alpha]^{25}_D$,	neutral sugar components						
fraction	mg	deg	Ara	Gal	Glc	Man	Rha	Xyl	uronic acid
W3	95	+9.7	0.30	1.00	3.18	0.11	0.56	3.14	tr
W4	265	-3.8	0.53	1.00	2.15	tr	0.52	3.83	tr
B1	19		0.11	1.00	4.04	0.34	0.59	8.44	major
B2	37		1.03	1.00	1.38	0.25	0.27	6.40	minor
B3	59		0.42	1.00	4.57	1.46	0.43	2.92	minor

with ethanol (4 volumes), and centrifuged. The precipitate was suspended in water, dialyzed for 40 h against running tap water and for 4 h against distilled water, and freezed-dried to yield and acidic xylan: 29 mg; $[\alpha]^{25}_D$ -43.8° (c 0.49, 0.25 M sodium hydroxide). A portion (5 mg) was hydrolyzed with 72% sulfuric acid (general methods) and the mixture of acidic and neutral sugars separated on Dowex 1X2 (CO_3^{2-}) as stated earlier. The mixture of neutral sugars was reduced and following acetylation and GLC showed arabinose, galactose, mannose, and xylose in the molar ratio 1.2:1.0:3.7:19.5; traces of glucose and rhamnose were also detected. Paper electrophoresis (Haug and Larsen, 1961) ofthe acidic fraction showed a major and minor component, having M_{GLC} values identical with those of authentic samples of galacturonic acid and 4-O-methylglucuronic acid, respectively.

(b) *With Barium Hydroxide.* The sodium hydroxide insoluble fraction S (15 mg) and the copper-complexing fraction (11 mg) were combined and dissolved in 2 M sodium hydroxide (3 mL), and the solution was mixed with saturated barium hydroxide solution (2 mL). A negligible amount of precipitate was removed by centrifugation and discarded. The supernatant or barium hydroxide noncomplexing fraction was acidified with acetic acid and the solution mixed with ethanol (4 volumes). The precipitae was washed with 80% aqueous ethanol, ethanol, and acetone to yield a product (11 mg) that was discarded. The barium hydroxide noncomplexing fraction on analysis showed the presence of an amyloid: $[\alpha]^{25}_D$ +24.7° (c 0.44, 0.25 M sodium hydroxide); ν_{max} (KBr) 895 cm^{-1}; glucose, xylose, galactose, and fucose ratio, 8.3:6.0:1.8:1.0; trace of mannose also detected. The scarcity of material and impure nature of the xylan and the amyloid precluded further work.

RESULTS AND DISCUSSION

Fractionation of water soluble polysaccharides (Siddiqui, 1989) of celery on DEAE–cellulose (CO_3^{2-}) yielded a minor neutral polysaccharide fraction in a yield of 10.5% and a major acidic fraction in a yield of 43.5% of the water-soluble polysaccharide. The former fraction, from its $[\alpha]_D$ value and hydrolysis results, appeared to be a mixture of neutral polysaccharides but was not analyzed further. The oxalate soluble polysaccharides, fractionated similarly, yielded a neutral fraction (6.5%) and the three pectinic acids A_1 (3.5%), A_2 (35.5%), and A_3 (4.5%). The characterization of an arabinogalactan from the neutral fraction has been described previously (Siddiqui, 1989).

Hydrolysis of pectinic acid A_2, with fractionation of the hydrolysis products on an anion-exchange resin and subsequently on paper chromatogram, gave crystalline D-galactose and L-arabinose. Rhamnose was identified by its specific optical rotation to be the L isomer. Traces of an aldobiuronic acid were tentatively identified as the commonly occurring (1→2)-linked galactosyluronic acid–rhamnose. The major hexuronic acid was characterized by the isolation of crystalline brucine D-galacturonate.

The pectinic acid from the water soluble fraction A_0 was reduced (Taylor and Conrad, 1972) with sodium borodeuteride. The twice-reduced product (uronic anhydride, 27.8%) was reduced two more times to yield a reduced polysaccharide that was methylated by the Hakomori method (Hakomori, 1964) and following methanolysis, hydrolysis, reduction, and acetylation was analyzed by GLC, GLC–EI-MS (Bjorndal et al., 1967), and GLC–

Table III. Structural Data for the Celery Water Soluble Pectinic Acid

component	molar ratio	mode of linkage
2,3,5-tri-O-methyl-L-arabinose	6	L-Ara$_f$-(1→
3,4-di-O-methyl-L-rhamnose	1	→2)-L-Rha$_p$-(1→
2,3-di-O-methyl-L-arabinose	4	→5)-L-Ara$_f$-(1→
2,3,4-tri-O-methyl-D-glucuronic acid	2	D-Glc$_p$A-(1→
2,3,5,6-tetra-O-methyl-D-galactose	3	D-Gal$_f$-(1→
2,3,4,6-tetra-O-methyl-D-galactose	2	D-Gal$_p$-(1→
3-O-methyl-L-rhamnose	1	→2,4)-L-Rha$_p$-(1→
2-O-methyl-L-rhamnose	2	→3,4)-L-Rha$_p$-(1→
2,3,6-tri-O-methyl-D-glucose	2	→4)-D-Glc$_p$-(1→
2,3-di-O-methyl-D-galacturonic acid	51	→4)-D-Gal$_p$A-(1→
2,3,6-tri-O-methyl-D-galactose	1	→4)-D-Gal$_p$-(1→
L-arabinose	4	→2,3,5)-L-Ara-(1→
2,3,4-tri-O-methyl-D-galactose	3	→6)-D-Gal$_p$-(1→
2-O-methyl-D-galacturonic acid	1	→3,4)-D-Gal$_p$A-(1→
3-O-methyl-D-galacturonic acid	1	→2,4)-D-Gal$_p$A-(1→
2,4-di-O-methyl-D-galactose	5	→3,6)-D-Gal$_p$-(1→

CI-MS (Horton et al., 1974). The analytical data translated in terms of unreduced polysaccharide are summarized in Table III. From the methylation results, the overall quantitative ratio of methyl ethers (forming the terminal, nonreducing end groups) to those involved in branching (1:1.08) instead of the required (1:4), on account of four doubly linked arabinose units, was indicative of a preferential loss of the more volatile arabinose methyl ethers and/or undermethylation of some of the arabinose residues in the polysaccharide. The molar ratio of arabinose, galactose, glucose, and rhamnose (1.00:1.00:0.14:0.27) calculated from the proportion of methylated sugars was in fairly good agreement with that (0.67:1.00:0.12:0.26) found for the unmethylated polysaccharide. The proportion of uronic acid residues calculated from the proportion of methylated reduced uronic acid residues was 61.8% compared to 65.1% for the unmethylated polysaccharide. The difference (3%) in the uronic acid content indicated that the reduction of the pectic polysaccharide was essentially complete.

Results in Table III are consistent with an (α-1→4)-linked galacturonan chain, containing 2-linked L-rhamnose residues commonly found in pectic polysaccharides. The main types of branching are through O-3 and -6 of D-galactose and O-2, -3, and -5 of L-arabinose. Other branching occurs through O-3 and -4 and O-2 and -4 of D-galacturonic acid and L-rhamnose.

Celery pectinic acid, like the tobacco leaf lamina (Siddiqui et al., 1984), rapeseed (Siddiqui and Wood, 1976; Aspinall and Jiang, 1974), and mustard (Rees and Wight, 1969) seed pectins do not contain chains of (1→4)-linked D-galactopyranosyl units as have been reported in soybean (Aspinall et al., 1967). The primary structure of cellery pectin appears to be similar to those from rapeseed and mustard seed but differs from them in having no xylose residues. There is also a strikingly close resemblance to the apple pectin (Aspinall and Fanous, 1983).

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Table IV. Comparative Structural Features of Some Pectic Polysaccharides from Vegetables by Methylation Analysis of Carboxyl-Reduced Derivatives

| | relative proportion of methyl sugars | | | |
sugar	apple (Vares et al., 1983)	apple (Aspinall and Fannous, 1984)	carrot (Stevens and Selvendran, 1984)	celery (present study)
2,3,5-Me$_3$Ara	23.8	8	14.7	6
2,5-Me$_2$Ara	4.1			
3,5-Me$_2$Ara			0.4	
2,3-Me$_2$Ara	18.3	7	13.5	4
2-MeAra	11.8	5	8.3	
3-MeAra	2.7			
Ara	8.3	4	3.3	4
3,4-Me$_2$Rha	3.1	2	6.1	1
2,4-Me$_2$Rha	2.7			
2-MeRha			0.5	2
3-MeRha	2.3	1	4.2	1
2,3,5,6-Me$_4$Gal				3
2,3,4,6-Me$_4$Gal	4.8	3	7.5	2
2,4,6-Me$_3$Gal				2
2,3,6-Me$_3$Gal	10.7	40[a]	28.2	52[b]
2,3,4-Me$_3$Gal	0.6		1.1	3
2,6-Me$_2$Gal	0.6[c]	3	0.5	1[c]
3,6-Me$_2$Gal	0.7[c]			1[c]
2,4-Me$_2$Gal	3.8		1.3	5
2,3-Me$_2$Gal			2.1	
2,3,4,6-Me$_4$Glc				2[d]
2,3,6-Me$_3$Glc	0.8			
2,3,4-Me$_3$Xyl	2.1		1.4	
2,3-Me$_2$Xyl			4.2	
3,4-Me$_2$Xyl			0.5	
hexitol			2.2	

[a] Contains 2 mol of galactose of nonuronic acid origin. [b] Contains 1 mol of galactose of nonuronic acid origin. [c] Originates from galacturonic acid residues. [d] Originates from glucuronic acid residues.

Glucose is not considered to be (Rees and Wight, 1969) a genuine constituent of pectic polysaccharides, but the occurrence of such residues has been reported in a variety of pectins including the present study, and such units have been interpreted as evidence in support of a covalent linkage between the amyloid (xyloglucans) and pectic materials (Albersheim, 1972). Glucuronic acid residues have been reported in many pectic polysaccharides. Their presence in the present study was clearly demonstrated by the presence of a fragment ion m/z 207 instead of m/z 205. The observed shift demonstrated the presence of two deuterium atoms at C-6 in the EI-MS of 1,5-di-O-acetyl-2,3,4,6-tetra-Omethyl-D-glucitol derived from glucuronic acid end group in the sodium borodeuteride reduced polysaccharide. Similarly, D-galacturonic acid on reduction and methylation yielded 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-galactitol-6,6-d_2, 1,3,4,5-tetra-O-acetyl-2,6-di-O-methyl-D-galactitol-6,6-d_2, and 1,2,4,5-tetra-O-acetyl-3,6-di-O-methyl-D-galactitol-6,6-d_2 corresponding to 2,3-di-O-methyl-, 2-O-methyl-, and 3-O-methyl-D-galacturonic acid residues. The relative proportions (51:1) between the 2,3,6-tri-O-methyl-D-galactose of galacturonic and non-galacturonic acid origin were determined by comparing the relative abundance of appropriate m/z values in the MS of the corresponding tri-O-methylgalactitol acetate. Galactose in the furanose form has not been reported in pectic polysaccharides. In the present study, its presence was unequivocally confirmed by the identification of 2,3,5,6-tetra-O-methyl-D-galactose in the hydrolysis product, from the reduced methylated polysaccharide by GLC-EI-MS.

The comparable structural data for a few pectic polysaccharides studied in recent years are presented in Table IV.

In vitro studies on vegetable fiber from carrot, cabbage, broccoli, and onion have indicated that binding of bile acids to fiber largely occurs through calcium salt linkages to the calcium pectate residues in the cell wall. Such binding (Hoagland and Pfeffer, 1987) results in an increase in the HDL to LDL ratio. Similar results were noted by in vivo studies on Sprague–Dawley rats (Mongeau et al., 1989) using parsnip and rutabaga fiber. The binding of certain other dietary anions (fatty acids, phytate, oxalate) to calcium pectate (Hoagland, private communication) seems beneficial, since elimination of these substances is very desirable from a physiological standpoint.

In conclusion, the major non-cellulose polysaccharides of celery consists of pectic substances. Among the minor polysaccharides present in the sodium hydroxide soluble fraction, the presence of a 4-O-methylglucuron-oxylan and a fucoamyloid was unequivocally established from their specific optical rotation and sugar analysis data. The presence of the amyloid was further confirmed by a characteristic absorption at 895 cm^{-1} in the infrared. The results also suggested that the purity of both the minor components ranged ≈80%. The xylan appeared to be contaminated with the pectinic acids and the amyloid with the pectic arabinogalactan (Siddiqui, 1989).

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