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

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An arabinogalactan isolated from celery (*Apium graveolens*) consists of L-arabinose, D-galactose, and D-glucose residues in the molar ratio of 2.53:1.00:0.12. Sedimentation analysis data indicate that the polysaccharide is homogeneous to the extent of 96%. Methylation studies show a statistical unit of 78 sugar residues with 27 terminal, nonreducing end groups (20 L-arabinosyl, 7 D-galactosyl). There are also 19 residues of L-arabinose involved in branching including 12 through positions 3, 5, and 7 through positions 2, 3, and 5. The remaining 32 nonterminal residues consist of eighteen (1→5)-linked L-arabinosyl residues, nine (1→4)-linked D-galactosyl residues, and five (1→3)-linked D-galactosyl residues.

Although advocated since Biblical times, the importance of dietary fiber was largely overlooked in view of its being a negligible source of energy and a general lack of discernible deficiency symptoms associated with its absence in diet. The current interest in fiber has been aroused by a hypothesis (Burkitt, 1973; Trowell, 1976) associating a lack of fiber in diet with many diseases prevalent in the western world.

Of the three major sources of fiber, the cereal fibers, being available in a relatively concentrated form, have received the most attention. The importance of fruits and vegetables as good sources of dietary fibers has been realized during the past decade as shown by the per capita increase (Bingham, 1986) in the consumption of these commodities. The chemical, compositional, and structural elucidation of carbohydrates from vegetables is therefore important to the food industry. It would also improve their promotion in view of health benefits and possibly also lead to value added processed products from high-fiber raw materials.

In previous papers, the fiber content and chemical composition of ethanol-insoluble and -soluble residues (Siddiqui, 1989) from four Canadian vegetables and the effect of fiber from celery, parsnip, and rutabaga on serum cholesterol and blood glucose response in rats (Mongeau et al., in press) were described. We now report on the nature of water, oxalate soluble, and sodium hydroxide soluble fractions of celery and the characterization of an arabinogalactan from the oxalate fraction.

EXPERIMENTAL SECTION

General Method. IR spectra were recorded with a Beckman IR 4250 spectrophotometer. Gas–liquid chromatography (GLC) was performed with a Varian Vista 6000 gas chromatograph, with a flame ionization detector: DB-225 fused silica capillary column (0.25-μm film, 30 m × 0.32 mm (i.d.), cold-column injection); initial temperature, 50 °C with a hold time of 2.5 min, programmed to 250 °C at a rate of 5 °C/min; helium flow rate, 1.5 mL/min; nitrogen makeup gas (30 mL/min); injector temperature program, 180 °C/min from 30 to 220 °C. Peak areas were evaluated with a Vista 402 data system. Combined gas–liquid chromatography mass–spectrometry (GLC–MS; Bjorndal et al., 1967) and gas–liquid chromatography—chemical ionization mass spectrometry (GLC–CI-MS; Horton

et al., 1974) were performed on the same column and under the same GLC conditions with a Finnigan Incos MAT-312 system. Operational details for EI-MS: 70 eV; separator temperature, 250 °C; transfer line temperature, 230 °C; source temperature, 250 °C. Operational details for CI-MS: 250 eV; reagent gas butane; ion source temperature, 150 °C; filament emission, 0.5 mA; accelerating voltage, 3 kV; electron multiplier, 2 kV; scanning range, 60–750 at 2.5 s/scan. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

Descending paper chromatography (PC) was performed on Whatman No. 1 paper with the organic phases of (A) ethyl acetate–pyridine–water (8:2:1), (B) 1-butanol–acetic acid–water (4:1:5), and (C) butanone saturated with water containing 2% ammonia. Paper electrophoresis (PE) (Foster, 1957) was performed on Whatman No. 3MM paper in (A) 0.2 M borate buffer (pH 10), (B) 0.2 M acetate buffer (pH 5), and (C) borate–calcium chloride buffer (pH 9.2) (Haug and Larsen, 1961) at a potential gradient of 14.0 V/cm. Detection was effected with (A) aniline hydrogen phthalate, (B) alkaline silver nitrate, and (C) naphthoresorcinol–phosphoric acid spray reagents. Concentrations were carried out at 35 °C on a rotary evaporator.

Routine hydrolyses of the polysaccharide samples (2–3 mg) were performed with 1 M sulfuric acid (0.3 mL) for 3 h at 100 °C. When necessary, polysaccharide samples (5 mg) were hydrolyzed with aqueous 72% sulfuric acid (0.12 mL) at 5 °C for 1–2 h, following dilution to 1 M acid for 2–3 h at 100 °C. The hydrolysates were neutralized with barium carbonate. The sugar sample (5 mg or less) was reduced with borohydride, and the alditol acetates were prepared essentially as described by Bjorndal et al. (1967) but for 1 h at 100 °C followed by storage overnight at room temperature. Demethylations (Allen et al., 1958) were carried out at –40 °C with boron trichloride. Uronic acid determinations were carried out (Siddiqui and Morris, 1979) by decarboxylation of samples (≈20 mg) with 57% HI at 145 °C for 1 h. Melting points were determined on an electrothermal apparatus and are uncorrected.

Isolation of Polysaccharides. (*a*) *With Hot Water.* The aqueous 80% ethanol insoluble residue (20 g) of celery [N, 1.56%; ash, 8.83; uronic anhydride, 25.5% (Siddiqui, 1989)] was stirred with hot water (2 L) at 72 ± 2 °C for 6 h. The insoluble residue was collected by filtration and extracted two more times by the same procedure. The combined filtrate and washings (6.5 L) were concentrated, centrifuged, and then freeze-dried to yield the water soluble fraction (4.1 g). The hot water insoluble residue was also recovered after washing with ethanol and acetone.

(*b*) *With Ammonium Oxalate.* The hot water insoluble residue was stirred with aqueous 0.5% ammonium oxalate at 92 ± 3 °C. The combined filtrate and washings (6.0 L) from three extractions were concentrated and clarified by centrifugation, and the solution was dialyzed for 48 h against running tap water

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Table I. GLC Data for Sugars following Hydrolysis, Reduction, and Acetylation

| acetate | ret rel to galactitol | hot water fraction | | ammonium oxalate fraction | | hemicellulose | | | | | | cellulose residue: 72% |
| | | | | | | A | | B | | C | | |
		M	72%	M	72%	M	72%	M	72%	M	72%	
glucitol	1.04	0.17	0.71	0.09	0.11	tr	tr	3.5	5.21	2.07	1.37	98.00
galactitol	1.00	1.00	1.00	1.00	1.00	tr	tr	1.00	1.00	1.00	1.00	tr
mannitol	0.95	tr	0.17					1.66	1.61	0.19	0.59	tr
xylitol	0.73	0.01	0.11	tr	0.07	tr	tr	3.63	2.46	0.45	0.38	tr
arabinitol	0.68	1.13	0.95	1.40	1.18	tr	tr	0.58	0.58	1.67	1.99	tr
ribitol	0.66	tr	0.08							tr	tr	
fucitol	0.63	tr	tr	0.20						tr	tr	
rhamnitol	0.62	0.46	0.36	0.48	0.46			0.18		0.37	0.12	tr

and for 20 h against three changes of distilled water. The solution was stirred with Rexyn-101 (H$^+$) resin, giving a clear filtrate that was mixed with ethanol (4 volumes), and the precipitate was recovered by centrifugation, washed with ethanol and acetone, and then dried to yield the oxalate soluble fraction (2.72 g).

(c) *With 10% Sodium Hydroxide.* The oxalate insoluble residue was stirred with aqueous 10% sodium hydroxide (2.0 L) at 25 °C for 24 h under nitrogen. The filtrate and washings (2.5 L) were adjusted to pH 4.5 with glacial acetic acid, and the precipitate was collected by centrifugation, washed with aqueous 80% ethanol, ethanol, and acetone, and then dried to yield the hemicellulose A fraction (0.39 g; N, 11.27%).

Ethanol (3 volumes) was added to the supernatant solution to yield the hemicellulose B fraction (2.67 g). The aqueous 75% ethanolic supernatant was concentrated, dialyzed, and freeze-dried to yield the hemicellulose C fraction (137 mg).

The sodium hydroxide insoluble material was neutralized with glacial acetic acid, collected by centrifugation, washed with aqueous 80% ethanol, ethanol, and acetone, and then dried to yield the cellulose residue (6.40 g).

The sugar analysis data on the water, oxalate, sodium hydroxide soluble fractions and the final cellulosic residue are shown in Table I.

Fractionation of the Polysaccharides. *Water Fraction.* A solution of the water soluble fraction (2.0 g) in water (25 mL) was added to a column (2.5 × 40 cm) of DEAE–cellulose (CO$_3$$^{2-}$ form) (Siddiqui and Wood, 1971). Elution with water (500 mL) removed a neutral fraction: 0.21 g; $[\alpha]_D^{25}$ +0.4° (c 1, water). Gradient elution with 0–0.5 M ammonium carbonate (2.0 L) yielded an acidic fraction (1.2 g). The acidic fraction was dissolved in water (25 mL), and the solution was deionized with Rexyn-101 (H$^+$) resin, filtered, centrifuged, concentrated, and mixed with ethanol (4 volumes). The precipitate was collected by centrifugation, washed with ethanol and acetone, and then dried to yield the acidic polysaccharide: 0.87 g; $[\alpha]_D^{25}$ +219° (c 1, 0.05 M sodium hydroxide); uronic anhydride, 65.1%.

PC of the hydrolysate (1 M sulfuric acid, 100 °C, 3 h) of the water-eluted fraction gave mainly glucose with small proportions of arabinose, mannose, and galactose, and GLC of the hydrolysate (72% sulfuric acid) of the acidic fraction, following reduction and acetylation, showed arabinose, galactose, glucose, and rhamnose in a molar ratio 0.62:1.00:0.12:0.26.

Oxalate Fraction. A portion (2.0 g) of the oxalate soluble fraction was similarly fractionated on a column of DEAE–cellulose (CO$_3$$^{2-}$), yielding a water-eluted neutral fraction W (0.13 g) and carbonate-eluted acidic fractions A$_1$ (0.071 g), A$_2$ (0.71 g), and A$_3$ (0.09 g).

PC and PE of the hydrolysates W–A$_3$ revealed mainly arabinose and galactose with traces of glucose in W; arabinose, galactose, and rhamnose with traces of glucose, xylose, and uronic acid in A$_1$; arabinose, galactose, and rhamnose with traces of glucose, xylose, and relatively higher proportions of uronic acid in A$_2$; arabinose, galactose, and rhamnose with traces of glucose in A$_3$.

Analysis of Celery Arabinogalactan (W). The celery arabinogalactan had $[\alpha]_D^{25}$ −83° (c 1, water). Sedimentation analysis (Svedberg and Pederson, 1960) (0.3% Na$_2$B$_4$O$_7$, 10H$_2$O solution; synthetic boundry cell; 52 000 rpm) gave a single, symmetrical peak with a tiny shoulder. The areas under the peaks showed a ratio of 96:4. The arabinogalactan (50 mg) was hydrolyzed

(1 M sulfuric acid), and the hydrolysate was neutralized (BaCO$_3$), filtered, and concentrated to dryness. A portion (2 mg) of the residue was reduced with sodium borohydride and then acetylated. GLC of glycitol acetates revealed derivatives of galactose, arabinose, and glucose in the ratio 1.00:2.53:0.12. Preparative PC of the remainder yielded L-arabinose and D-galactose (ratio 2.3:1.0 by weight). The former sugar had mp 150–152 °C and $[\alpha]_D^{24}$ +103° (c 0.5, water), and the latter had mp 161–162 °C and $[\alpha]_D^{24}$ +84° (c 0.5, water).

Methylation Analysis of Arabinogalactan. The dried arabinogalactan (48 mg) in dry methyl sulfoxide (2.5 mL) was methylated (Hakomori, 1964) with use of methylsulfinyl carbanion (0.5 mL; reaction time, 6 h) and methyl iodide (1 mL; reaction time, 30 min). The product (42 mg), recovered by dialysis, was methylated again by the above procedure, and the sample was recovered by continuous extraction with chloroform and, following dialysis, yielded a fully methylated product [38 mg; $[\alpha]_D^{25}$ −96.5° (c 0.4, chloroform)], showing no IR absorption for hydroxyl.

The methylated polysaccharide (30 mg) was methanolyzed (methanolic 3% hydrogen chloride, 12 mL; 24 h, reflux; neutralization with Ag$_2$CO$_3$) and hydrolyzed (MH$_2$SO$_4$, 3 mL; 30 h, 100 °C; neutralization with BaCO$_3$). PC (solvent C) of the syrupy product (18 mg) showed seven components: R_f 0.92, 0.87, 0.80, 0.43, 0.27, 0.23, 0.19.

A portion (5 mg) of the mixture was reduced (NaBH$_4$) and then acelytated, and the products were examined by GLC, GLC-EI-MS (Bjorndal et al., 1967), and GLC–CI-MS (Horton et al., 1974), confirming the identity of the components including the respective [MH]$^+$ ions.

RESULTS AND DISCUSSION

A sample of celery stalks [*Apium graveolens* (variety Vantura; origin, Deniel Inc., St. Isidore Co., Laprairie, P.Q.)] yielded an aqueous 80% ethanol soluble residue (1.6%), corresponding to a fiber content of 1.1% (Siddiqui, 1989). The residue on extraction with hot water, hot ammonium oxalate, and 10% sodium hydroxide yielded fractions amounting to 20.9%, 13.6%, and 16.0%, respectively, and a cellulosic residue 32.0% based on the aqueous 80% ethanol insoluble residue.

Fractionation of the water soluble polysaccharides on DEAE–cellulose (CO$_3$$^{2-}$) yielded a water-eluted neutral polysaccharide and an acidic polysaccharide (10% and 60%, respectively) of the water soluble fraction. The water-eluted fraction on the basis of its specific optical rotation and sugar composition appeared to be a mixture of polysaccharides and was not investigated any further. The oxalate soluble polysaccharides similarly fractionated yielded a water-eluted polysaccharide W (6.5%) and a series of acidic polysaccharides (pectinic acids) A$_1$, A$_2$, and A$_3$ (3.5%, 35.5%, and 4.5%, respectively). The major pectinic acid appeared to be similar to the major acidic polysaccharide from the water soluble fraction and will be described later. The neutral polysaccharide fraction W on further chromatographic examination appeared to be an arabinogalactan.

The arabinogalactan, $[\alpha]_D^{25}$ −83°, gave a major, sym-

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Table II. GLC and CI-MS Data for Sugars from Methylated Arabinogalactan

acetate	ret time, min	CI-MS data [MH]$^+$: m/z (relative abundance, %)
2,3,5-tri-O-methylarabinitol	0.87	279 (100), 280 (16), 247 (24), 219 (20), 177 (10)
2,3-di-O-methylarabinitol	0.97	307 (100), 308 (16), 248 (11), 247 (88)
2,3,4,6-tetra-O-methylgalactitol	1.00	323 (82), 291 (22), 264 (13), 263 (100)
2-O-methylarabinitol	1.07	335 (63), 276 (13), 275 (100)
2,3,6-tri-O-methylgalactitol	1.10	351 (100), 352 (15), 319 (51), 291 (43)
2,4,6-tri-O-methylgalactitol	1.11	351 (29), 292 (15), 291 (100)
arabinitol	1.15	303 (100), 304 (15), 185 (8)

Table III. Analytical Data ($[\alpha]_D$ Values, Arabinose and Galactose Ratios, and Methylated Glycitol Composition) of Some Arabinogalactans

component	rapeseed (B. campestris)[a]	rapeseed (B. napus)[b]	soybean[c]	celery[d]
2,3,5-trimethylarabinitol	31.1	46	6.1	25.6
2,3,4-trimethylarabinitol		2	1.0	
2,3-dimethylarabinitol	11.1	3	13.2	23.1
2-methylarabinitol	4.4			15.4
3-methylarabinitol	2.2	41		
arabinitol				9.0
2,3,4,6-tetramethylgalactitol	2.2			9.0
2,3,6-trimethylgalactitol			70.1	11.5
2,3,4-trimethylgalactitol	4.4			
2,4,6-trimethylgalactitol	8.8			6.4
2,6-dimethylgalactitol			9.5	
2,4-dimethylgalactitol	28.6	8		
2,3,4-trimethylglucuronic acid	6.6			
arabinose to galactose ratio	1:1.05	10:1	1:2.8	2.5:1
$[\alpha]_D$, deg	−49.2 (−80.6)	−112	+51 (−17)	−83 (−96.5)

[a] Siddiqui and Wood (1972). [b] Larm et al. (1976). [c] Aspinall and Cottrell, 1971. [d] Present study. $[\alpha]_D$ for methylated polysaccharides in parentheses.

metrical peak on sedimentation analysis, and the minor corresponded to ca. 4% of the total. Thus, the polysaccharide appeared to be homogeneous to the extent of ca. 96%. The detection of 3.4% glucose in the arabinogalactan suggested that the minor peak might have originated from a glucan contaminant.

The arabinogalactan yielded on hydrolysis L-arabinose, D-galactose, and D-glucose in the molar ratio 2.53:1.00:0.12. Preparative PC of the mixture gave crystalline L-arabinose and D-galactose. Methylation of the arabinogalactan gave a product with $[\alpha]_D^{25}$ −96.5° that was methanolyzed and then hydrolyzed. GLC, GLC-EI-MS, and GLC-CI-MS of the derived glycitols (Table II) indicated the molar proportions of the parent sugars to be as follows: 2,3,5-tri-O-methyl-L-arabinose, 20; 2,3-di-O-methyl-L-arabinose, 18; 2,3,4,6-tetra-O-methyl-D-galactose, 7; 2-O-methyl-L-arabinose, 12; 2,3,6-tri-O-methyl-D-galactose, 9; 2,4,6-tri-O-methyl-D-galactose, 5; L-arabinose, 7. The quantitative ratio of methyl ethers (forming the terminal, nonreducing end group) to those involved in branching (1:0.96) was in excellent agreement. The molar ratio of arabinose to galactose (2.43:1) calculated from the proportion of methylated sugars was also in excellent agreement with that (2.53:1) found for the original polysaccharide.

Methylation analysis showed that the molecule has a highly branched structure. A statistical unit of 78 sugar residues consists of 27 terminal nonreducing end groups (20 L-arabinosyl and 7 D-galactosyl). There are 12 residues of L-arabinose involved in branching, through positions 3 and 5, and 7 residues of L-arabinose involved in branching, through positions 2, 3, and 5. The remaining 32 nonterminal residues consist of 18 (1→5)-linked L-arabinosyl residues, 9 (1→4)-linked D-galactosyl residues, and 5 (1→3)-linked D-galactosyl residues.

The highly negative $[\alpha]_D$ values (−83° and −96.5°, respectively) for the unmethylated and methylated polysaccharide strongly indicated that the galactosidic bonds were predominantly of the β-D type and the arabinosidic were predominantly of the α-L type.

The nonstarchy polysaccharide of plant origin belongs (Aspinall et al., 1983) to a few structural groups, and included among them are the pectic substances. To this subgroup inter alia belong the arabinogalactans, which in turn fall into two types: type I, 4-linked; type II, 3,6-linked. The arabinogalactan from celery (Table III) has structural features in common with both types but differs from them in having doubly branched L-arabinose residues, a characteristic typical of arabinans. The possibility of the presence of both an arabinan and arabinogalactan are ruled out in view of the methylation results and a perfectly symmetrical sedimentation pattern in the ultracentrifuge.

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A Kinetic Model for Equilibration of Isomeric β-Carotenes[†]

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The equilibration kinetics and spontaneous isomerization of β-carotenes were studied. Carotene samples were stored at 25 or 45 °C in the dark for varied lengths of time. Isomerization reactions were monitored by HPLC with diode array detection. Two major cis isomers of β-carotene were separated and detected. Results indicated that the extent of isomerization and the proportions of cis isomers formed depended upon the solvent system in which the carotenoid was dissolved. An equilibrium mixture of β-carotenes contained 66.7 ± 3.9% *all-trans-*, 8.2 ± 3.1% 9-*cis-*, and 25.1 ± 2.9% 13-*cis-*β-carotene.

Advances in carotenoid analysis procedures have allowed collection of new information about trans and cis isomers of food carotenes. Determining an exact carotenoid profile, including amounts of various isomers of individual carotenoids in a food, is important for several reasons. The possibility that certain carotenes or their isomers have anticancer potential continues to be investigated (Peto et al., 1981; Watson and Moriguchi, 1985; Watson, 1986; Hennekens, 1986; Beems, 1987). The vitamin A potentials of cis isomers of β-carotene and others are less than those of their all-trans counterparts (Sweeney and Marsh, 1971); therefore, from a bioavailability standpoint it is important to be able to accurately distinguish and quantify the various forms. Also, the colorant properties of carotenoids may be changed significantly with the formation of cis isomers (Zechmeister, 1944). Work by Beadle and Zscheile (1942) demonstrated the conversion of *all-trans-* to *cis-*β-carotene in hexane at 30 °C.

Advances in chromatography have made it possible to separate isomeric forms of carotenoids, and several high-performance liquid chromatography (HPLC) procedures dealing with separation of isomeric β-carotenes have been published (Bushway, 1985, 1986; Khachik et al., 1986; Chandler and Schwartz, 1987; Quackenbush, 1987; Tan, 1988).

Processing and storage conditions can cause isomerization or degradation of carotenoids in foods (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Ogunlesi and Lee, 1979; Chandler and Schwartz, 1987). However, no information on the exact kinetics of the isomerization reactions in model or food systems has been published.

One objective of this study was to examine the influence of solvent system on spontaneous isomerization of β-carotene with use of common extraction and HPLC mobile-phase solvents. A second objective was to determine the equilibration kinetics of isomerization of *all-trans-*β-carotene.

METHODS

Evaluation of Spontaneous Isomerization. The spontaneous isomerization of β-carotene was evaluated with a working solution of 34 µg/mL β-carotene (type IV) (Sigma Chemical Co., St. Louis, MO) in petroleum ether–acetone (50:50, v/v). Aliquots of 3 mL were evaporated to dryness with nitrogen. Then, the β-carotene was resuspended in 3.0 mL of one of the following solvents: acetonitrile, methanol, tetrahydrofuran (THF, stabilized with 0.025% BHT), chloroform, methylene chloride, or the HPLC mobile phase (acetonitrile–methanol–THF, 42:58:1, v/v/v). The acetonitrile, chloroform, and methanol used were HPLC-grade solvents. Other solvents used in this study were analytical reagent grade.