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The gel-forming polysaccharide of psyllium husk (*Plantago ovata* Forsk)

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Abstract—The physiologically active, gel-forming fraction of the alkali-extractable polysaccharides of *Plantago ovata* Forsk seed husk (psyllium seed) and some derived partial hydrolysis products were studied by compositional and methylation analysis and NMR spectroscopy. Resolving the conflicting claims of previous investigators, the material was found to be a *neutral* arabinoxylan (arabinose 22.6%, xylose 74.6%, molar basis; only traces of other sugars). With about 35% of nonreducing terminal residues, the polysaccharide is highly branched. The data are compatible with a structure consisting of a densely substituted main chain of β-(1 → 4)-linked D-xylopyranosyl residues, some carrying single xylopyranosyl side chains at position 2, others bearing, at position 3, trisaccharide branches having the sequence L-Ara*f*-α-(1 → 3)-D-Xyl*p*-β-(1 → 3)-L-Ara*f*. The presence of this sequence is supported by methylation and NMR data, and by the isolation of the disaccharide 3-*O*-β-D-xylopyranosyl-L-arabinose as a product of partial acid hydrolysis of the polysaccharide.

Keywords: *Plantago ovata* mucilage; Psyllium seed polysaccharide; Arabinoxylan; 3-*O*-β-D-Xylopyranosyl-L-arabinose

1. Introduction

The seed husk of *Plantago ovata* Forsk has a long history of use as a dietary fiber supplement to promote the regulation of large bowel function,[1] and more recently it has been shown to lower blood cholesterol levels.[2] In early chemical studies Laidlaw and Percival[3,4] analyzed the polysaccharide mucilage extracted from whole seeds by first cold, then hot water. They secured evidence for two components, which they characterized as a polyuronide and a neutral arabinoxylan. Later Kennedy and co-workers[5,6] studied the mucilage obtained from *Plantago* seed husk by extraction with alkali and concluded that the preparation, although polydisperse, represented a single species of polysaccharide, a highly branched, acidic arabinoxylan.

In our laboratory we developed an efficient, reproducible process for the alkaline extraction and fractionation of the polysaccharide from the husk in order to obtain material for biological studies and to address issues relating to the composition and structure of the active substance.[7] Through animal and human feeding experiments, we could show that a gel-forming fraction, amounting to some 55–60% of the husk, is responsible for both the laxative and cholesterol-lowering activities.[7,8] Other viscous, non-nutrient polysaccharides, such as β-glucans and pectins, lower blood cholesterol levels by the same mechanism as psyllium,[9] but these substances have negligible effects on bowel function. They are rapidly and completely fermented in the gut, whereas

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psyllium husk largely survives, increasing stool output and imparting a gel-like consistency to the excreta.[8,10] In the present paper we present the results of chemical and physical studies of the active fraction of psyllium mucilage.

2. Experimental

2.1. Materials

Anion-exchange resin AG3-X4, 100–200 mesh and cation-exchange resin AG50W-X8, 100–200 mesh were supplied by the BioRad Corp., Richmond, CA. α-L-Arabinofuranosidase (EC 3.2.1.55) from *A. niger* was obtained from Megazyme, Wicklow, Ireland. Psyllium seed husk, identical with the principal component of the preparation marketed as Metamucil®, was provided by the Procter & Gamble Company, Cincinnati, Ohio.

2.2. Initial preparation

The procedure for the isolation of the gel-forming polysaccharide (fraction B) and two additional fractions (A and C) from the husk is described elsewhere.[11] Fraction A amounted to 17%, B 57.5%, and C 12.9% of the weight of the husk.[7] Thus the three fractions account for nearly all of the carbohydrate and about 90% of the mass of the starting material. Neutral sugars in the husk and fractions were measured by GLC as alditol acetate derivatives using the method of Kraus et al.,[12] as modified.[13] Uronic acids were measured by a colorimetric assay employing 3-phenylphenol (3-hydroxybiphenyl).[14] The results of these analyses are recorded in our previous paper.[7]

Purified fraction B was used for all structural studies. It was prepared by subjecting isolated fraction B sequentially to two further rounds of alkali solubilization followed by acidification, centrifugation, and dehydration, as in the original isolation procedure. The monosaccharide composition of this purified material appears in Table 1.

2.3. Partial hydrolysis

2.3.1. With acid. Two dry samples (50 mg) of fraction B were triturated with successive 0.3-mL portions of 0.005 M sulfuric acid to hydrate them, after which acid was added to final volumes of 10 mL. The samples were placed in a heating block at 95 °C and vortexed periodically to facilitate solution of the gel. One sample was removed from the heat source after 2 h and the other after 6 h. When the cooled solutions were examined in a Ubbelohde capillary viscometer the observed efflux times were 161 s for the 2 h sample, 108 s for the 6 h sample, and 93 s for the acid solvent. After measurement, the solutions were poured into ethanol to a final alcohol concentration of 80%, giving precipitates that could be recovered by centrifugation. These were washed with 80% ethanol, 95% ethanol, and ether, then dried for 2 d at 40 °C. The yields of partially hydrolyzed products, B-H^+, were 41 and 33 mg, respectively. When 0.17% solutions of the products were dialyzed against water, using a membrane having a cutoff at MW 6000–8000, B-H^+ (2 h) was 100% retained, and B-H^+ (6 h) was 95% retained. On examination by paper chromatography the supernatants from the alcohol precipitations were found to contain both arabinose and xylose; no oligosaccharides were detected. From the compositions of B-H^+ (2 h) and (6 h), recorded in Table 1, and the weights of these fractions, it could be calculated that the amounts of monosaccharides released were: after 2 h heating, Ara 3.6 mg, Xyl 5.3 mg; after 6 h, Ara 8.4 mg, Xyl 9.7 mg.

2.3.2. Isolation and characterization of a disaccharide. Sixteen 50-mg samples of fraction B were triturated with 0.125 M sulfuric acid, acid was added to a final volume of 5 mL per sample, and all were heated at 100 °C for 45 min.[15] The samples were combined, and the solution was neutralized with barium carbonate, then filtered. The filtrate was concentrated in vacuo and passed through AG50W-X8 cation-exchange resin. The effluent was reduced in volume to 20 mL, and 95% ethanol was added to a final concentration of 79%. The resulting precipitate was recovered by centrifugation, and the supernatant was concentrated to a syrup. On analytical

Table 1. Monosaccharide composition of selected fractions and hydrolysis products

Monosaccharide	Compositions (mol%)						
	Purified B	B-H^+ (2 h)	B-H^+ (6 h)	B-E1	B-E2	C-1	C-2
Arabinose	22.6	19.4	11.4	13.7	7.2	16.0	3.9
Xylose	74.6	78.7	87.0	84.8	91.5	79.9	11.9
Galactose	1.5	1.5	1.3	1.3	1.2	2.3	1.8
Glucose	0.3	0.2	0.2	0.2	0.1	0.9	0.6
Rhamnose	0.4	0.1	0.1	0	0	0.3	40.5
Uronic acid	0.7	nd[a]	nd[a]	nd[a]	nd[a]	0.6	41.4

[a] nd = not determined.

paper chromatography using butanol–benzene–pyridine–water (5:1:3:3, upper phase) as the solvent[15] and ammoniacal silver nitrate for detection, no mobile components were found in the precipitate, but the syrup yielded three major spots corresponding to xylose, arabinose, and a substance having the mobility (R_{Glc} 0.84) expected of a disaccharide. To isolate this unknown the main portion of the syrup was chromatographed on several sheets of Fisher thick paper, and the relevant areas were excised. The compound was eluted with water and recovered by lyophilization.

Characterization of the unknown product was accomplished by sugar analysis and by NMR spectroscopic examination. For analysis, a portion (15 mg) was first reduced with sodium borohydride (20 mg) overnight, excess borohydride was destroyed by the addition of acetic acid, and the solution was evaporated to dryness. A sample of the reduced material was subjected to the acid-hydrolysis step of the usual GLC sugar assay (see above), after the addition of *myo*-inositol as an internal standard. The $NaBH_4$ reduction step of the assay was omitted; instead sodium acetate was added, and the sample was dried and acetylated. A mixture of D-xylose and L-arabinitol was treated in the same manner. Like the authentic mixture, the reduced sample gave peaks corresponding to the acetates of α-xylopyranose, β-xylopyranose, and arabinitol. From the peak areas the ratio of xylose (α + β) to arabinitol was found to be 0.98, showing that the R_{Glc} 0.84 product must be a xylosylarabinose.

2.3.3. Enzyme hydrolysis. Dry fraction B (0.5 g) was wetted by the careful dropwise addition of 5 mL of sodium acetate buffer (0.05 M, pH 4.0). The resulting gel was suspended in additional buffer (95 mL), and α-L-arabinofuranosidase (150 units in 3.2 M ammonium sulfate) was added. The gel suspension was shaken at 40 °C for 30 h, with further additions of 150 units of enzyme after 6 and 24 h. At the end of the incubation, the gel mass was extremely well dispersed, giving the mixture a translucent appearance. The mixture was centrifuged for 20 min at 23,500*g*, and the gel pellet (fraction B-E1) was washed with 80% ethanol, 95% ethanol, absolute ethanol, and ether before drying at 40 °C (yield 282 mg). The supernatant was deionized by sequential passage over cation- and anion-exchange resins, concentrated, and poured into ethanol to a final alcohol concentration of 70%. The precipitate (fraction B-E2) was recovered, washed, and dried (118 mg). The compositions of fractions B-E1 and B-E2 are given in Table 1.

2.4. Isolation of the polyuronide fraction

Fraction C (200 mg) from the original treatment of the husk was dissolved in water (40 mL). The addition of a 5% solution of cetylpyridinium chloride in 0.02 M sodium sulfate (10 mL) produced a turbidity, but no precipitate. After 2 days, centrifugation of the mixture for 1 h at 39,000*g* gave a pellet. The clear supernatant was added with stirring to ethanol to yield a precipitate (fraction C-1). This fraction was recovered by centrifugation, washed successively with 95% ethanol and ether, and dried (yield 103 mg). The detergent-complexed material (initial pellet) was dissolved in 4 M magnesium chloride and shaken with ether. The aqueous phase was dialyzed against water, and the retentate was lyophilized to yield fraction C-2 (137 mg). The compositions of these fractions are reported in Table 1.

2.5. Methylation and reductive cleavage analyses

The purified fraction B and its 2 and 6 h hydrolysis products were methylated by a modification of the method of Ciucanu and Kerek.[16] The sample (3.2 mg) and a small stir bar were placed in a small flask and dried under high vacuum for 24 h. Dimethyl sulfoxide (4.0 mL) was then added, and the mixture was kept overnight at room temperature to yield a clear solution. This was treated with powdered NaOH (60 mg), and the mixture was stirred for 2 h, then placed in a small bath sonicator overnight. Iodomethane (0.4 mL) was added, and after being stirred for 6 h the mixture was again placed in the sonicator overnight. Chloroform (5 mL) was added to the brown solution, the mixture was stirred for 30 min, water (1.5 mL) was added, and stirring was continued for a few minutes. Then, after phase separation, the water layer was removed. The chloroform solution was extracted six times with 4-mL portions of dilute $Na_2S_2O_3$ to remove the brown color, three more times with water after adjustment to pH 4–6 with a few drops of 30% HOAc, and then dried over Na_2SO_4 and evaporated to dryness under a stream of nitrogen.

Reductive cleavage of the methylated polysaccharides was carried out in the presence of triethylsilane and a mixture of trimethylsilyl methanesulfonate and boron trifluoride etherate.[17] The products were acetylated in situ[17] and analyzed by GLC–MS on a DB-17 column programmed from 40 to 200 °C at 2 °C/min. The identification of the resulting substituted anhydroalditols was accomplished by comparing them with synthetically prepared anhydroxylitol,[18] anhydroarabinitol,[19] and anhydrogalactitol[20] derivatives.

For traditional analysis the methylated polysaccharide was dissolved in 3.2 mL of chloroform, and a 0.5-mL aliquot was removed and evaporated to dryness under a stream of dry nitrogen in a hydrolysis tube. Trifluoroacetic acid (0.5 mL of 4 M) was added, and the tube, capped and evacuated, was kept 4 h at 105 °C. After cooling, the mixture was subjected to several cycles of isopropyl alcohol addition and evaporation (N_2 stream) to remove the trifluoroacetic acid, then the residue was dissolved in 1 mL of water. Sodium borodeuteride

(10 mg) was added, and the mixture was kept at room temperature overnight, then treated with 10 μL of glacial HOAc to destroy excess reagent, and evaporated to dryness. Borate was removed by repeated additions and evaporations first of 9:1 methanol–acetic acid then methanol alone. The residue was dissolved in 1.5 mL of water, the solution was deionized with a few beads of Amberlite IR-120 (H^+), and after filtration again evaporated to dryness. Pyridine and acetic anhydride (0.4 mL each) were added, reaction was allowed to proceed overnight, and then the reagents were removed by evaporation under vacuum at 40 °C followed by two cycles of methanol addition and evaporation. The residue of partially methylated 1-deuteroalditol acetates was dissolved in 0.5 mL of chloroform and analyzed by GLC combined with CIMS and EIMS on a DB-5 column programmed from 80 to 180 °C at 1 °C/min, followed by a hold at 180 °C for 30 min.

2.6. NMR spectroscopy

Samples (15–30 mg) of fraction B and its partial hydrolysis products in DMSO-d_6 or D_2O (400 μL) were examined in a Bruker DRX-360 instrument fitted with a 5-mm ^1H/broad band gradient probe with inverse geometry (proton coils closest to the sample). We used the standard Bruker implementations of the traditional suite of 1D and 2D (gradient-COSY, TOCSY, ^1H-detected and gradient-selected HMQC, HMQC-TOCSY, HMBC) pulse sequences; only selected HMQC experiments, and the most informative HMQC-TOCSY, are shown and discussed here. The TOCSY mixing time was 80 ms; all other parameters were standard. It was not possible to prepare a D_2O solution of fraction B at a concentration sufficient for ^{13}C NMR, so DMSO-d_6 was employed as the solvent for this fraction. The 6 h acid hydrolysis product proved to be adequately soluble in D_2O, and useful results were obtained with the solution. The solutions were extremely viscous at 300 K, but were run at that temperature to permit comparison with chemical shift data from the literature. Relevant data for elevated temperatures are lacking. Referencing in DMSO-d_6 was from the solvent signals (δ_H 2.49 ppm; δ_C 39.5 ppm). For D_2O solutions, as is now common, acetone was used as the internal standard. The standard (2 μL) was added after acquisition of the required spectra and the reference back-applied to those spectra (δ_H 2.225 ppm; δ_C 31.07 ppm[†]).

The usual pulse sequences were employed in measuring the spectra of the isolated disaccharide and its reduction product in D_2O with a Bruker 750 MHz instrument. Assignments were made with the aid of COSY, HSQC, and DEPT spectra. Proton chemical shifts and coupling constants were accurately determined by full spectral simulation using gNMR 3.6 for MacOS (Cherwell Scientific Publishing, Oxford, UK). The data are recorded in Table 2.

[†] Comparisons with ^{13}C chemical shifts determined from dioxane, the other common reference for D_2O solutions (δ_C 67.40),[21] should be valid.

3. Results and discussion

3.1. Compositional analysis and characterization of hydrolysis products

As noted above, careful examination showed that both arabinose and xylose residues were cleaved from purified fraction B by mild acid treatment. According to the calculations outlined in Section 2.3.1, about 29% of the arabinose and 13% of the xylose residues were lost during the first 2 h, while after 6 h the figures were ~66% and ~23%, respectively. The *absolute amounts* of xylose released were nearly the same as for the arabinose, reflecting the predominance of the former sugar in the starting polysaccharide.

NMR spectra were used to complete the characterization of the disaccharide fragment obtained from fraction B by brief heating with moderately strong acid. As already described, compositional analysis showed the product to be a disaccharide of xylose and arabinose having the sequence Xyl → Ara. Since earlier work[3,4] established the xylose of psyllium polysaccharides as the D-isomer, and the arabinose as the L-form, we assume these are the isomers present in our fragment. The proton spectrum of the disaccharide in D_2O revealed the presence of two components in a 68:32 ratio. The vicinal H–H coupling constants derived from the spectrum (Table 2) show that the arabinose residue, in the pyranose ring form, adopts the *alternate* chair conformation ($^4C_1$ for L-sugars) in both components. Thus in the major component, where $J_{1,2}$ for Ara H-1 = 7.5 Hz, the arabinose unit is the α-anomer, and in the minor component ($J_{1,2}$ 2.4 Hz) the β-anomer. The β-anomeric configuration of the xylose residue is indicated by the value 8.1 Hz for $J_{1,2}$ shown by both species of the free disaccharide, and 7.7 Hz found for the derived alditol.

The 1 → 3 location of the interunit linkage was established by the ^{13}C spectrum, where the signals for Ara C-3 in the α- and β-anomers were found at δ 82.6 and 79.1, respectively, on the average ~11 ppm downfield of the signals for C-2 and C-4. This sizeable downward shift is a definitive indication of substitution at position 3; in unsubstituted arabinopyranose the differences between the δ values for C-3 and those for C-2 and C-4 are confined to the range 0–3.9 ppm.[22] Confirmation of our signal assignments, clearly distinguishing the disaccharide from its possible linkage isomers (β-Xyl attached to O-2 or O-5 of Ara, or α-Xyl at any position), was provided by comparison with litera-

Table 2. NMR data for β-D-xylopyranosyl-(1 → 3)-L-arabinose and its derived alditol

δ (ppm) or J (Hz)[a]	Positions											
	Xylose unit						Arabinose unit					
	5ax	5eq	4	3	2	1	5ax	5eq	4	3	2	1
Xylp-β-(1→3)-Arap-α												
δ_H	3.29	3.94	3.61[b]	3.44	3.35	4.60[c]	3.66	3.88	4.10[b]	3.77	3.64	4.54[c]
$J_{n-1,n}$	10.2	5.5	9.9	9.0	8.1		1.0	2.1	3.9	9.8	7.5	
	J_{gem} 11.3						J_{gem} 13.6					
δ_C	65.9		70.0	76.3	74.0	105.1	66.7		69.0	82.6	71.8	97.3
Xylp-β-(1→3)-Arap-β												
δ_H	3.30	3.93	3.61[b]	3.44	3.35	4.60[c]	3.64[c]	4.02	4.15	3.97	3.96	5.24[c]
$J_{n-1,n}$	10.2	5.5	9.9	9.0	8.1			0.8	2.7	7.5	2.4	
	J_{gem} 11.3						J_{gem} 13.9					
δ_C	65.9		70.0	76.3	74.0	105.0	62.9		69.3	79.1	68.2	93.3
Xylp-β-(1→3)-Ara-ol[f]												
							5a	5b				1a, 1b
δ_H	3.28	3.95	3.61[b]	3.42	3.31	4.40[c]	3.69	3.77	3.83[d]	3.81	3.97[e]	3.73[c]
$J_{n-1,n}$	10.6	5.2	9.3	9.4	7.7		6.71	3.4	6.66	2.3	6.3	
	J_{gem} 11.8						J_{gem} 12.3			$^4J_{2,4}$ 0.8		
δ_C	65.6		69.7	76.0	73.9	104.4	63.4		71.7	79.6	71.74	63.0

[a] ^{1}H signal multiplicities are dd except as indicated by footnote.
[b] ddd.
[c] d.
[d] dddd.
[e] tdd.
[f] The spectra of the disaccharide alditol were simpler than those of the anomeric mixture, but complete assignment of the ^{1}H signals could only be done on a sample prepared by reduction with $NaBD_4$, which differentiated positions 1 and 2 of the Ara-ol moiety from positions 4 and 5.

ture data for the related compounds Gal-β-(1 → 3)-Ara (for the arabinose moiety)[23] and the several xylobioses (for the xylose unit).[21] Thus the data as a whole identify the hydrolysis fragment as 3-*O*-β-D-xylopyranosyl-L-arabinose, a disaccharide previously characterized, without NMR analysis, as a hydrolysis product of the mucilage of *Opuntia ficus-indica* (prickly pear cactus).[24] The sequence D-Xyl*p*-(1 → 3)-L-Ara has been found in a number of plant polysaccharides, with the xylose occurring in both the α-anomeric and β-anomeric forms. The α form is apparently the more common one.

An examination of fraction C from the crude psyllium extract was of interest because of earlier conflicting ideas (see Section 1) about the relationship between the uronic acid present in the extract and its major, arabinoxylan component. Our results show that uronic acid and the associated sugar rhamnose segregate in fraction C; gel-forming fraction B is essentially devoid of these two saccharides (Table 1). As noted in Section 2, fraction C could be further separated into a detergent-precipitable polyuronide (C-2, 82% uronic acid + Rha) and a xylose-rich arabinoxylan (C-1). Interestingly, the latter formed a viscous solution in water, not a gel.

3.2. Methylation and reductive cleavage analysis

The application of reductive cleavage methodology to fraction B and its 2 and 6 h hydrolysis products provided an unequivocal determination of the ring forms of the glycosyl units.[25,26] All the xylose residues yielded exclusively 1,5-anhydroxylitol derivatives, and the arabinose residues gave only 1,4-anhydroarabinitol derivatives. Hence xylose is present only in the pyranose ring form, while arabinose is solely in the furanose form.

Compositional data were obtained from our analyses of the reductive cleavage mixtures, but the values are unreliable because of the significant volatility of the anhydropentitol derivatives. For purposes of linkage analysis we therefore look to the figures provided by the quantitation of the methylated alditol acetate mixtures resulting from the standard methylation procedure. The identities of the individual alditol acetates were confirmed by one-to-one correlation with the components of the anhydroalditol mixture from reductive cleavage analysis. (For example, the only 2,3,5-trimethylated anhydroalditol in this mixture was shown to be an anhydroarabinitol. Therefore the 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylpentitol found among the products of standard methylation must be an arabinitol derivative.) Table 3 presents the data from the methylation and reductive cleavage analyses.

The highly branched nature of the purified polysaccharide is evident from the methylation analysis data. Some 34% of the glycosyl residues of fraction B are in nonreducing terminal (T) positions. Since it is inherent in polysaccharide structure that in a molecule having substantial branching the number (n) of branch point (BP) units approximately equals the number ($n + 1$) of

Table 3. Glycosyl-linkage compositions of fractions B, B-H$^+$ (2 h), and B-H$^+$ (6 h) as deduced from standard methylation and reductive cleavage analysis

Residue linkage	Corresponding alditol and *anhydroalditol* derivatives	Amounts present (mol %)[a]		
		Purified B	B-H$^+$ (2 h)	B-H$^+$ (6 h)
T-Ara*f*	1,4-*O*-Ac_2-2,3,5-*O*-Me_3-arabinitol	**12.6**	**14.9**	**7.9**
	1,4-Anhydro-2,3,5-O-Me_3-arabinitol	*11.6*	*14.4*	*2.2*
T-Xyl*p*	1,5-*O*-Ac_2-2,3,4-*O*-Me_3-xylitol	**20.4**	**28.3**	**36.1**
	1,5-Anhydro-2,3,4-O-Me_3-xylitol	*23.7*	*40.9*	*26.5*
→3)-Ara*f*	1,3,4-*O*-Ac_3-2,5-*O*-Me_2-arabinitol	**12.6**	**10.0**	**7.2**
	3-O-Ac-1,4-anhydro-2,5-O-Me_2-arabinitol	*13.1*	*12.6*	*5.6*
→3)-Xyl*p*	1,3,5-*O*-Ac_3-2,4-*O*-Me_2-xylitol	**14.4**	**11.6**	**9.7**
	3-O-Ac-1,5-anhydro-2,4-O-Me_2-xylitol	*12.7*	*14.8*	*13.0*
→4)-Xyl*p*	1,4,5-*O*-Ac_3-2,3-*O*-Me_2-xylitol	**5.8**	**5.3**	**12.1**
	4-O-Ac-1,5-anhydro-2,3-O-Me_2-xylitol	*1.6*	*1.5*	*14.1*
→3,4)-Xyl*p*	1,3,4,5-*O*-Ac_4-2-*O*-Me-xylitol[b]	**12.8**	**9.7**	**6.6**
	3,4-O-Ac_2-1,5-anhydro-2-O-Me-xylitol	*7.2*	*2.3*	*0.8*
→2,4)-Xyl*p*	1,2,4,5-*O*-Ac_4-3-*O*-Me-xylitol	**17.4**	**16.8**	**17.3**
	2,4-O-Ac_2-1,5-anhydro-3-O-Me-xylitol	*27.8*	*10.6*	*32.2*
→2,3,4)-Xyl*p*	1,2,3,4,5-*O*-Ac_5-xylitol	**2.9**	**2.4**	**2.0**
	2,3,4-O-Ac_3-1,5-anhydroxylitol	*2.0*	*1.8*	*5.3*
T-Gal*p*	1,5-*O*-Ac_2-2,3,4,6-*O*-Me_4-galactitol	**1.1**	**1.0**	**1.1**
	1,5-Anhydro-2,3,4,6-O-Me_4-galactitol	*0.3*	*1.1*	*0.3*
Nonbranching interior	[→3)-Ara*f* + →3)-Xyl*p* + →4)Xyl*p*]	**32.8**	**26.9**	**29.0**
T:BP[c]	$\frac{[\text{T-Ara}f + \text{T-Xyl}p + \text{T-Gal}p]}{[\rightarrow 3,4)\text{Xyl}p + \rightarrow 2,4) \rightarrow \text{Xyl}p + \rightarrow 2,3,4)\text{Xyl}p]}$	1.0	1.5	1.7

[a] See text regarding the unreliability of the values for the derivatized anhydroalditols.

[b] The use of borodeuteride in the reduction to the alditol stage permitted the mass-spectroscopic identification of this component as the 2-*O*-methyl derivative. Without deuterium substitution it would be indistinguishable from the 4-*O*-methyl compound, except by a procedure capable of separating enantiomers.

[c] BP = branch point residues. The mol % →2,3,4)-Xyl*p* is included without multiplication on the supposition that the figure may represent single branch point residues on which the lone free OH group escaped methylation.

terminal units, one would expect about one-third of the residues of fraction B to be in branching positions. As may be seen from the table, the actual figure is 33%.

The methylation analysis of the partial acid hydrolyzates of fraction B gave strange results, in that the ratios of apparent terminal residues to apparent branch point units (T:BP) depart widely from the norm, reaching 1.5 for the 2-h sample and 1.7 for the 6-h sample. Independent methylation analyses of fraction B-H$^+$ (6 h), done in another laboratory, gave values agreeing within experimental error with the figures in Table 3.

If taken at face value, T:BP ratios $\gg 1$ indicate small numbers of branches per molecule, and if the molecule is 'highly branched' the number of ordinary interior residues (i) and thus the overall dp must also be small. Specifically, the values T:BP = 1.5 and I (= % i) = 27 suggest a dp in the heptasaccharide range (MW ~950) for fraction B-H$^+$ (2 h), and T:BP = 1.7, I = 29 suggest the penta- to hexasaccharide range (MW ~700–800) for the dp of fraction B-H$^+$ (6 h). These estimates are, however, inconsistent with the dialysis behavior of the two fractions, which were retained by membranes having MW cutoffs in the 6000–8000 range. Some of this discrepancy might be due to molecular association in solution, but the gap seems too large for sole attribution to this cause. The values in the B-H$^+$ (2 h) and B-H$^+$ (6 h) columns of Table 3 are thus subject to some question, but they may be regarded as indicative of trends in the way the linkage composition of fraction B changes during partial acid hydrolysis.

3.3. NMR spectroscopy of the polysaccharide

Representative HMQC and HMQC-TOCSY spectra are shown in Figure 1. In the plots each set of ^{1}H–^{13}C correlation peaks (identified as such from the usefully redundant HMQC-TOCSY correlations) has a different color, and the major sets are further defined by grid lines.

Turning first to panel A of the figure, it will be seen that the HMQC spectrum of fraction B in DMSO is deceptively clean. It shows well-resolved arabinofuranose peaks (blue), and a series of contours characteristic of xylopyranose residues, but lacks sufficient detail for linkage assignment. We suspect that the extreme viscosity of the solution and the consequently short t_2 relaxation times artificially enhanced the signals of the more mobile terminal units over those of the presumably constrained internal units.

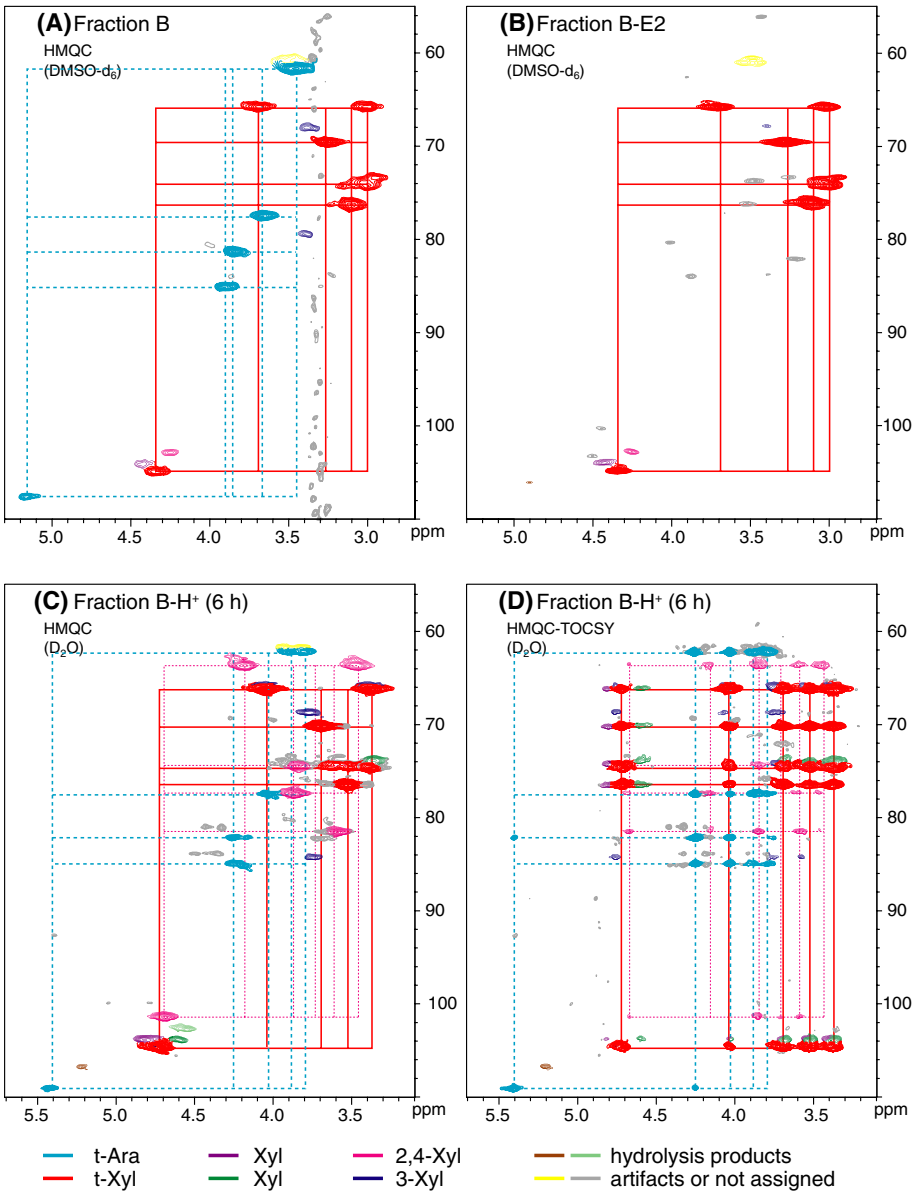

Figure 1. NMR spectra of psyllium polysaccharide preparations: fraction B, as isolated; fraction B-E2, enzyme treated; fraction B-H^+ (6 h), partially acid hydrolyzed. Note that coloring is approximate only; where contours overlap only the major component is colored. Color assignments are identified more fully in Table 4.

The spectrum of the fraction solubilized (B-E2) by the arabinofuranosidase treatment, shown in Figure 1B, differs from that of the starting material only by the complete disappearance of the arabinofuranose peaks. Since it is known from the compositional analysis (Table 1) that fraction B-E2 still contains a significant percentage of arabinose residues, the absence of Ara peaks from the spectrum presumably reflects the removal of most or all of the terminal Ara residues by the enzyme.

The 6-h acid hydrolysis product, which formed a low-viscosity solution, gave more detailed spectra (HMQC, Fig. 1C; HMQC-TOCSY, Fig. 1D), and the fact that these were determined in D_2O allowed us to use reference data from the literature in analyzing them. In Table 4 chemical shifts measured in the HMQC-TOCSY experiment are given for the six discernible ^1H–^{13}C correlation sets, along with comparable data from the literature indicating the identity and linkage arrangement of the glycosyl residues responsible for each pattern. Thus, the units having $\delta_{H\text{-}1}$ 5.40, $\delta_{C\text{-}1}$ 109.0 (cyan) can be identified as terminal α-arabinofuranose residues. Next, three sets of peaks have shifts characteristic of terminal β-xylopyranose units: $\delta_{H\text{-}1}$ 4.72, $\delta_{C\text{-}1}$ 104.6 (red); $\delta_{H\text{-}1}$ 4.81, $\delta_{C\text{-}1}$ 103.7 (purple); and $\delta_{H\text{-}1}$ 4.59, $\delta_{C\text{-}1}$ 103.8 (green). Units showing $\delta_{H\text{-}1}$ 4.75, $\delta_{C\text{-}1}$ 104.5 (dark blue) have shifts corresponding to those recorded for 3-linked β-xylopyranose [→3-Xyl*p*-β-(1→] residues, and those with $\delta_{H\text{-}1}$ 4.68, $\delta_{C\text{-}1}$ 101.3 (magenta) can be identified

Table 4. NMR chemical shifts shown by fraction B-H$^+$ (6 h) (Fig. 1D) and the literature values used for residue identification

Residue *literature model*	Chemical shift (δ)						Ref.
	H-1	C-1	C-2	C-3	C-4	C-5	
T-Ara*f* (cyan)	**5.40**	**109.0**	**82.0**	**77.4**	**85.0**	**61.8**	
L-*Ara*f-α-(1→3)-*2-O-R*[a]-L-*Ara*f-α-(1→	*5.25*	*107.8*	*82.0*	*77.5*	*84.8*	*62.0*	27
T-Xyl*p* (red)	**4.72**	**104.6**	**74.6**	**76.3**	**70.1**	**66.1**	
T-Xyl*p* (purple)	**4.81**	**103.7**	**74.2**	**75.5**	**70.1**	**66.2**	
T-Xyl*p* (green)	**4.59**	**103.8**	**73.7**	**76.4**	**70.0**	**66.0**	
D-*Xyl*p-β-(1→3)-D-*Xyl*p-β-(1→*OMe*		*104.8*	*74.6*	*76.9*	*70.4*	*66.4*	21
D-*Xyl*p-β-(1→4)-D-*Xyl*p-β-(1→*OMe*		*103.1*	*74.0*	*76.9*	*70.4*	*66.5*	21
→3)-D-Xyl*p*-β-(1→ (dark blue)	**4.75**	**104.5**	**~74.0**	**84.2**	**68.5**	**65.7**	
→3)-D-*Xyl*p-β-(1→4)-D-*Xyl*-β-(1→*OMe*		*103.0*	*73.8*	*84.9*	*69.0*	*66.2*	21
→2[→4]-D-Xyl*p*-β-(1→(magenta)	**4.68**	**101.3**	**81.4**	**74.3**	**77.3**	**63.5**	
→2[→4]-D-*Xyl*p-β-(1→*OMe*		*105.3*	*81.9*	*75.0*	*77.9*	*63.9*	21

[a] R = *trans*-feruloyl.

with 2→4-linked, branching xylopyranose [→4[→2]-Xyl*p*-β-(1→] units. In this case the ^{13}C shift of the anomeric carbon does not match well with the literature value of 105.3 ppm, but when account is taken of the fact that the relevant residue in the literature model is a methyl glycoside, this discrepancy can be tolerated.

In summary, the NMR data point to the presence, in molecules of psyllium fraction B and its partial hydrolysis products, of α-L-arabinofuranose end groups, and of β-D-xylopyranose residues in terminal, 3-linked interior, and 2→4-linked branch point locations. These findings corroborate the results of our methylation analyses, discussed above. In one respect the NMR analysis extends the methylation results, revealing three sets of correlation peaks characteristic of terminal xylopyranose units, which suggests that the xylose end groups of fraction B-H$^+$ (6 h) occupy a corresponding number of distinct microenvironments. This in turn supports the notion of a complex, highly branched structure for the polysaccharide.

Methylation analysis is of course not capable of distinguishing among the different surroundings of residues that have the same linkage mode. However, it may be seen from Table 3 that our methylation studies reveal three types of sugar residues not found in our NMR experiments, namely 3-linked interior arabinofuranose units, 4-linked interior xylopyranose units, and 3→4-linked branch point xylopyranose units. Our characterization of xylopyranosyl-β-(1→3)-arabinose as a degradation product validates the methylation finding in the case of 3-linked arabinose residues. In view of the fact, mentioned above, that an independent laboratory could fully confirm the methylation results we obtained on fraction B-H$^+$ (6 h), the argument for the additional xylose units also seems solid. We can offer no explanation, other than the apparent response of only the more mobile components, for the lack of NMR evidence for the three sets of residues.

4. Conclusions

The present investigation has focused on the unique, physiologically active, gel-forming component of psyllium seed husk, here designated fraction B. The data so far obtained by intensive examination of this fraction are not sufficient to fully characterize its molecular structure, but they do allow us to formulate a hypothesis. Accepting the highly branched arabinoxylan nature of the polysaccharide, one can visualize a main chain of densely substituted (1→4)-linked xylopyranose residues, some carrying single xylose units, others bearing trisaccharide branches having the structure Ara*f*-α-(1→3)-Xyl*p*-β-(1→3)-Ara*f*. The nearly equivalent mole percentages of T-Xyl and →2,4)-Xyl [including '→2,3,4)-Xyl'] residues (Table 3) suggest the attachment of the xylose single unit branches at position 2 of the underlying main chain residues. Similarly, the near equivalence of T-Ara, →3)-Xyl, →3)-Ara, and →3,4)-Xyl is compatible with the proposed trisaccharide structure and suggests the attachment of these branches at position 3 of the underlying main-chain xyloses. Because of the dense branching of the structure, the proportion of *unsubstituted* →4)-Xyl is relatively small. The presence of Xyl*p*-β-(1→3)-Ara sequences in the fraction B molecule is established by our isolation of the corresponding disaccharide as a hydrolysis product; the side chains would seem to be the most likely location for these sequences. Since the arabinose residues of the polysaccharide are in the furanose form, the linkages of the T-Ara units to side-chain xylose and of the internal Ara units to the main chain would be labile to acid. Thus on partial acid hydrolysis one might expect to see an increase in the mol percent of T-Xyl at the expense of T-Ara, and if the linkage of the Ara-Xyl-Ara side chain is to O-3 of the underlying xylose there would be a conversion of →3,4)-Xyl to →4)-Xyl. Such *trends* are indeed discernible in the data of Table 3. Cleavage from

the polymer by α-arabinofuranosidase shows the T-Ara units to be α-anomers, but the anomeric configuration of internal Ara remains undecided.

The structure just outlined differs from that suggested for psyllium mucilage by Kennedy and co-workers[5,6] on the basis of their methylation analysis of the unfractionated husk. The analysis revealed a substantial content of →3)-Xyl residues, which the British authors assigned without explanation to the main chain of the xylan core. This assignment does not seem to be required by their data. A result consistent with our proposed structure was presented at a recent symposium by Edwards et al.,[28] who worked with crude preparations from psyllium seed husk. These authors reported finding the tetrasaccharide L-Ara*f*-(1→3)-[D-Xyl*p*-(1→4)]$_2$-D-Xyl among the fragments produced by enzymic digestion of their extracts.

An important property of fraction B, as mentioned in Section 1, is its resistance to digestion by the intestinal microflora. This property implies some unique features of molecular structure, such as those proposed here. It can be assumed that the organisms of the flora will possess enzymes capable of cleaving off the terminal α-L-arabinofuranose residues. However, the loss of these residues does not affect the gelling ability of the polysaccharide. This is shown by our experiment with arabinofuranosidase, in which the major portion of the substrate persisted as a gel after a prolonged incubation that removed almost 50% of the starting arabinose, thereby reducing the T-Ara units to a level undetectable by NMR spectroscopy. Further digestion would require a complement of exoxylosidases and xylanases, which one would expect to be present in the gut. The resistance of the residual polysaccharide to the action of these enzymes must be a function of an unusual linkage pattern in the molecule and/or the high density of its branching. Since the present data do not permit a decision as to the relative importance of these two factors, the question will have to be left for examination by future investigators.

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