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Isolation and purification of feruloylated oligosaccharides from cell walls of sugar-beet pulp ☆

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Abstract

Cell walls from sugar-beet pulp contain some feruloyl groups linked to the pectic neutral side-chains. Enzymic as well as chemical hydrolysis of the pulp yielded a series of feruloylated oligosaccharides, which have been purified by Sephadex LH-20 and Biogel P-2 chromatography in aqueous solvents. Feruloylated arabinose di-, tri-, hexa-, hepta-, and octa-saccharides as well as feruloylated galactose disaccharides were obtained after hydrolysis of the pulp with a mixture of fungal carbohydrases (Driselase). Feruloylated arabinose and galactose monosaccharides were obtained through mild acid hydrolyses. Both arabinose and galactose residues in the side-chains are feruloylated, 50–55% of the feruloyl groups being linked to arabinose residues and 45–50% to galactose residues. It is concluded that 1 out of 56 arabinose residues and 1 out of 16 galactose residues present as pectic side-chains in sugar-beet pulp carry a feruloyl group.

Keywords: Beet pulp; Pectins; Ferulic acid; Arabinans; Galactans

1. Introduction

Ferulic acid is now well known to be one of the major phenolic acids in ester linkages to carbohydrates in the cell walls of several plants. These wall-bound

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phenolic acids may play an important structural role by cross-linking the polysaccharides to which they are attached. Such cross-linking, mediated by oxidation in the presence of peroxidases and hydrogen peroxide, may contribute to the control of cell growth [1–4] and restrict cell-wall digestibility [4–5]. Ferulic and *p*-coumaric esters of arabinoxylan oligosaccharides have been isolated from numerous graminaceous cell walls and the nature of the linkages between phenolic acids and carbohydrates has been extensively studied in monocots. Isolated feruloylated oligosaccharides from *Graminae* cell walls showed ferulic acid attached at the C-5 position of L-Ara*f* side-chains of arabinoxylans [6–12]. In dicots, ferulic acid seems to be restricted to the Caryophyllales (*Centrospermae*) [13]. The cell walls of spinach [1,2], sugar-beet [13–17], and glasswort [18] have been reported to contain ferulic acid. Fry [1] isolated two feruloylated disaccharides from suspension-cultured spinach cell walls digested with Driselase and showed that ferulic acid residues were linked to the pectic arabinose and galactose residues in the side-chains. It has been shown that ferulic acid residues in sugar-beet pulp were linked to the pectic side-chains [14–17]; however, the exact location of feruloyl groups has not been studied.

The aim of our study was to isolate, purify, and characterise feruloylated oligosaccharides of various degrees of polymerisation (dp) from sugar-beet cell-wall polysaccharides in order to gain a better understanding of the structure of feruloylated pectic side-chains. Moreover, ferulic acid esterases showing different activities on methyl ester derivatives of cinnamic and benzoic acids have been isolated [19,20]. The specificity of these enzymes on substrates from plant cell walls is presently unknown. The isolation of feruloylated mono- and oligo-saccharides of known structure from sugar-beet-pulp cell walls would provide adequate substrates for the ferulic acid esterases and permit the study of the requirements of these enzymes for the nature and the length of the sugar moiety to which ferulic acid is attached.

In the present paper, we report on the isolation of feruloylated oligosaccharides of various dp from sugar-beet pulp through enzymic and mild acid hydrolyses.

2. Results

(*a*) *Enzymic hydrolysis of the sugar-beet pulp.—Composition of the starting material and hydrolysis kinetics.* In agreement with previously published data [21,22], the alcohol-insoluble residue (AIR) from sugar-beet pulp was composed mainly of arabinose (23.5%), glucose (23.3%), galacturonic acid (19.8%), and galactose (5.8%). Rhamnose (2.1%), xylose (1.7%), mannose (1.3%), and fucose (0.2%) were detected as minor constituents. Proteins (10.2%) and ash (4.7%) were present as well as methanol (1.8%) and acetic acid (3.9%). Assuming that galacturonic acid is the only residue carrying methanol or acetic acid groups, a degree of methylation and a degree of acetylation (number of methyl or acetyl groups per 100 moles of galacturonic acid) of 50 and 59, respectively, have been calculated. As ferulic acid is the major (> 99%) phenolic acid present in sugar-beet pulp [13,15],

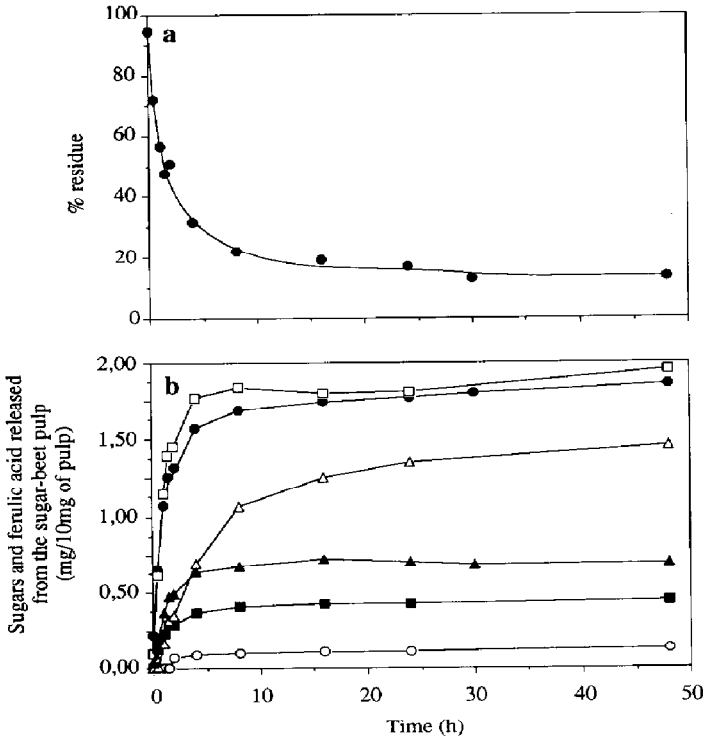

Fig. 1. Kinetics of hydrolysis of sugar-beet pulp by Driselase: (a) ●, residue; (b) ●, galacturonic acid; □, arabinose; △, glucose; ▲, ferulic acid×10; ■, galactose; ○, rhamnose.

it is accurately measured by a spectrophotometric method [15,17,22]. Sugar-beet pulp contained 0.8% of ferulic acid.

Driselase from Basidiomycetes contains various exo- and endo-carbohydrases and glycosidases, including arabinanase, cellulase, xylanase, galactanase, and polygalacturonase [21], but is devoid of ferulic acid esterase activity [1,22]. Analytical hydrolyses of sugar-beet pulp (100 mg) in distilled water (9 mL) by Driselase (1 mL containing 10 mg of protein) were performed for 0–48 h at 37°C. As shown in Fig. 1, sugar-beet pulp was very rapidly hydrolysed by the Driselase mixture, more than 75% of the pulp being solubilised after 8 h of hydrolysis. Approximately 50% of the arabinose, galacturonic acid, galactose, and ferulic acid were solubilised within the first hour and ~95–100% after 8 h. Rhamnose, xylose, mannose, and glucose were solubilised to a lesser extent and 65, 50, 76, and 74%, respectively, of these components were recovered in the soluble fraction after 48 h of hydrolysis. The UV absorption spectrum at pH 10 of the soluble fractions revealed a peak with a λ_{max} of 375 nm showing [1,2,13] that ferulic acid was linked through its carboxyl group.

Preparative hydrolysis of sugar-beet pulp (10 g) was then performed at 37°C. The isolation procedure for obtaining feruloylated oligosaccharides is summarised in Fig. 2. Hydrolysates were concentrated to 100 mL by vacuum evaporation at 35°C and 4 volumes of absolute ethanol were added in order to eliminate

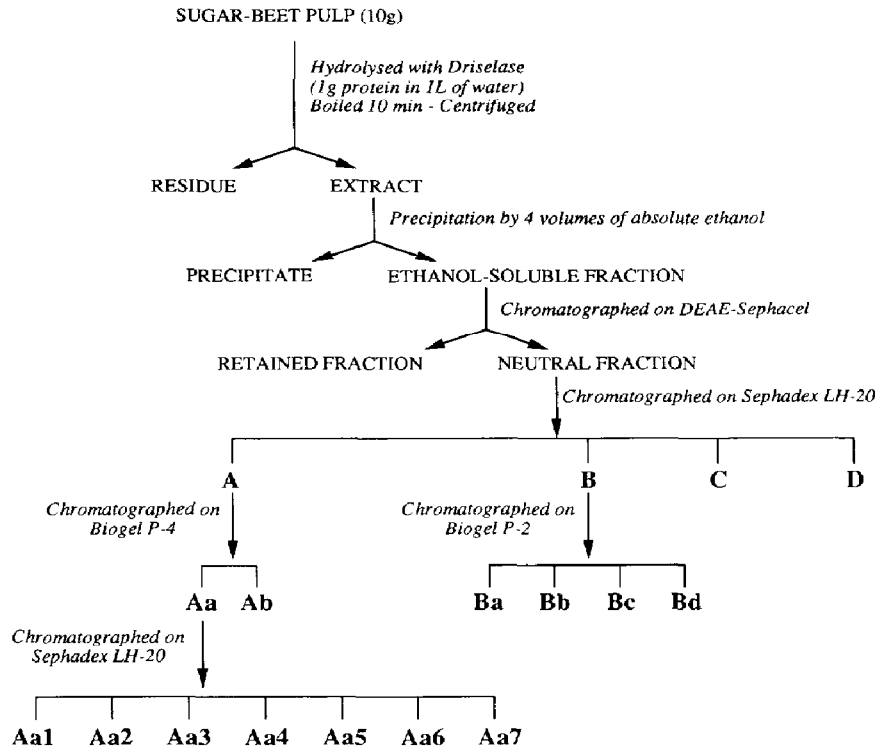

Fig. 2. Scheme of isolation procedures for feruloylated oligosaccharides from sugar-beet pulp hydrolysed with Driselase.

polymeric fragments. After 16 h of hydrolysis, the recoveries in the precipitate (calculated from the amount in the pulp) were 82.5% of the galacturonic acid, 24.8% of the neutral sugars, and 35.9% of the ferulic acid, whereas these recoveries were 83.6% of the galacturonic acid, 19.8% of the neutral sugars, and 22.3% of the ferulic acid after 48 h of hydrolysis. The 70% ethanol-soluble products were concentrated and injected on a column of DEAE-Sephacel in order to eliminate the residual galacturonic acid-containing material (chromatograms not shown). All the galacturonic acid residues were eluted by the buffer gradient together with only minor amounts of ferulic acid ($\sim 2\%$ of the total ferulic acid injected) and neutral sugars ($\sim 1\%$ of the total neutral sugars injected). For both hydrolysis times, the material that was not bound to the gel was concentrated and injected on a Sephadex LH-20 column, since this gel is known to retard aromatic compounds. In order to get a better separation of the various feruloylated oligomers, this chromatography was performed in distilled water.

Sephadex LH-20 chromatography. The recoveries of neutral sugars and ferulic acid after the chromatography were close to 100%. After 16 h of hydrolysis, 70% of the injected ferulic acid was eluted between the void and the total volume of the column, which revealed the presence of feruloylated oligomers of high dp (chromatogram not shown). After 48 h of hydrolysis, only 24% of the injected ferulic

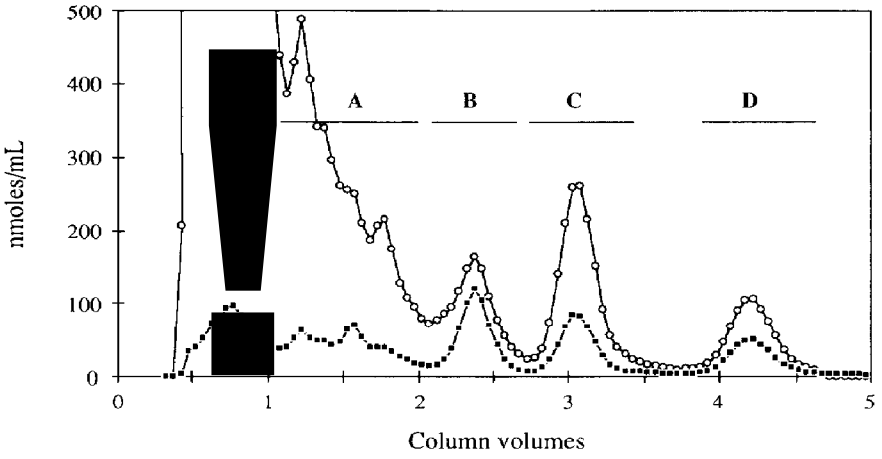

Fig. 3. Chromatography on Sephadex LH-20 of the ethanol extract after 48 h of hydrolysis with Driselase: ○, neutral sugars; ■, ferulic acid.

acid was eluted between the void and the total volume of the column; this fraction was not further studied. The elution profile (Fig. 3) showed the occurrence of a series of retained peaks. Unresolved peaks (fraction A) eluted between 1 and 2 column volumes contained 2.4% of the injected neutral sugars and 24.5% of the injected ferulic acid. Three more defined peaks eluted at 2.35 (fraction B), 3.1 (fraction C), and 4.2 column volumes (fraction D) representing 0.6, 0.9, and 0.4% of the neutral sugars injected, and 20.1, 16.5, and 12.0% of the ferulic acid injected, respectively. Compositional analysis (Table 1) showed that fraction A was composed mainly of arabinose residues with lower quantities of galactose residues and ferulic acid. A molar ratio (arabinose + galactose):ferulic acid of 7.4 was found. Fraction B contained mainly galactose residues, and the molar ratio (arabinose + galactose):ferulic acid of 2.8 suggested that a mixture of feruloylated dimers and trimers could be present in this fraction. Fractions C and D were composed of arabinose and ferulic acid together with only traces of galactose, rhamnose, and glucose. The high purity and the molar ratios (arabinose + galactose):ferulic acid of these two fractions (3.1 and 2.1, respectively) indicated that fractions C and D consisted of feruloylated arabinose tri- and di-saccharides, respectively. Approximately 12 mg of feruloylated arabinose disaccharides (Ara_2F)

Table 1
Composition (mol%) of the Sephadex LH-20 fractions of the extract of sugar-beet pulp hydrolysed by Driselase for 48 h

Fraction	Rha	Ara	Gal	Glc	Ferulic acid
A	1.0	72.3	12.0	3.4	11.4
B	0.8	18.4	50.4	5.2	24.5
C		73.5	1.0	1.2	24.2
D	0.2	67.8	0.3		31.7

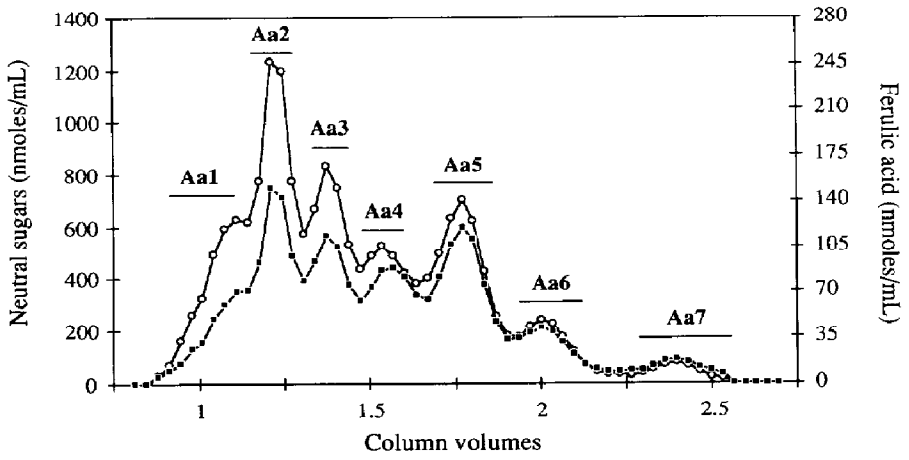

Fig. 4. Chromatography on Sephadex LH-20 of fraction A: ○, neutral sugars; ■, ferulic acid.

and 23 mg of feruloylated arabinose trisaccharides (Ara_3F) were recovered, corresponding to 10 and 13%, respectively, of the ferulic acid initially present in the pulp. Fractions A and B were further purified.

Purification of fraction A. When chromatographed on Biogel P-4, fraction A revealed two main peaks; one (fraction Aa), containing all the ferulic acid and ~ 50% of the neutral sugars injected, was eluted as a narrow peak at K_{av} 0.55, and the other, consisting of non-feruloylated oligosaccharides, at the total volume of the column (chromatogram not shown). Fraction Aa was injected on Sephadex LH-20 (recovery: neutral sugars, 95%; ferulic acid, 94%), using a lower flow-rate (20 instead of 40 mL/h) in order to achieve a better separation. The chromatogram of fraction Aa (Fig. 4) showed seven different peaks eluted between 1 and 2.5 column volumes. As shown in Table 2, fractions Aa2, Aa3, Aa5, and Aa6 were almost exclusively composed of arabinose and ferulic acid in molar ratios of 8.0, 6.9, 5.8, and 5.4, respectively, indicating the occurrence of feruloylated arabinose octa-, hepta-, hexa-, and penta-saccharides. The feruloylated arabinose pentasaccharides were recovered in small amounts (< 1 mg) and are probably contaminated by some feruloylated arabinose hexasaccharides, indicated by the

Table 2
Composition (mol%) of the Sephadex LH-20 fractions of fraction A

Fraction	Ara	Gal	Glc	Ferulic acid
Aa1	62.6	2.8	27.1	6.5
Aa2	87.3	1.8		10.9
Aa3	84.3	3.5		12.2
Aa4	78.3	8.8		12.9
Aa5	83.8	1.7		14.5
Aa6	82.9	1.7		15.4
Aa7	66.9	1.6	12.3	19.2

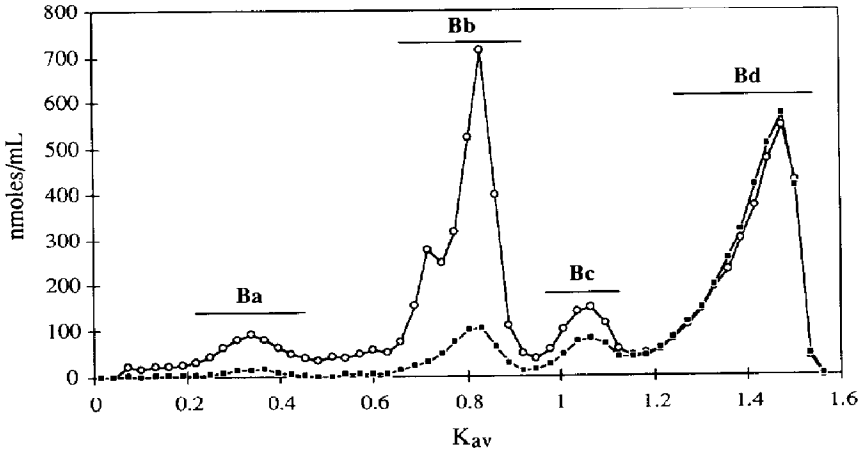

Fig. 5. Chromatography on Biogel P-2 of fraction B: ○, neutral sugars; ■, ferulic acid.

molar ratio of 5.4 of arabinose and ferulic acid. Approximately 3.5 mg of each of the feruloylated arabinose octa-, hepta-, and hexa-saccharides (Ara_8F, Ara_7F, and Ara_6F, respectively) were recovered, each corresponding to ~ 1% of the feruloyl groups initially present in the pulp. Although arabinose was still the major component, the other fractions were contaminated with galactose and glucose residues and were not further purified.

Purification of fraction B. Chromatography (recovery: neutral sugars, 100%; ferulic acid, 97%) on Biogel P-2 (Fig. 5) of fraction B yielded four distinct peaks containing feruloyl groups. The first (Ba) and the third (Bc) peaks, eluted at K_{av} 0.35 and 1.05, respectively, were present in very small quantities. A large peak (Bb) eluted at K_{av} 0.83 contained arabinose as the major sugar with a fairly high proportion of glucose and some ferulic acid (Table 3). It is very likely that the non-feruloylated arabinose and glucose monomers are present in this peak together with small amounts of feruloylated oligosaccharides. Another large peak (Bd), containing a high proportion of ferulic acid, was eluted at K_{av} 1.47. As already pointed out [15], the material containing feruloyl groups was retarded on Biogel P-2, probably because of interactions between the phenolic compounds and the polyacrylamide matrix. The sugar analysis revealed that galactose and ferulic acid were the major components of this fraction together with traces of arabinose and glucose. The molar ratio of 2.0 for galactose and ferulic acid was consistent

Table 3
Composition (mol%) of the Biogel P-2 fractions of fraction B

Fraction	Ara	Gal	Glc	Ferulic acid
Ba	83.1	2.6		14.4
Bb	61.2	2.6	23.6	12.5
Bc	23.5	52.5	1.6	22.4
Bd	2.0	64.0	0.7	33.3

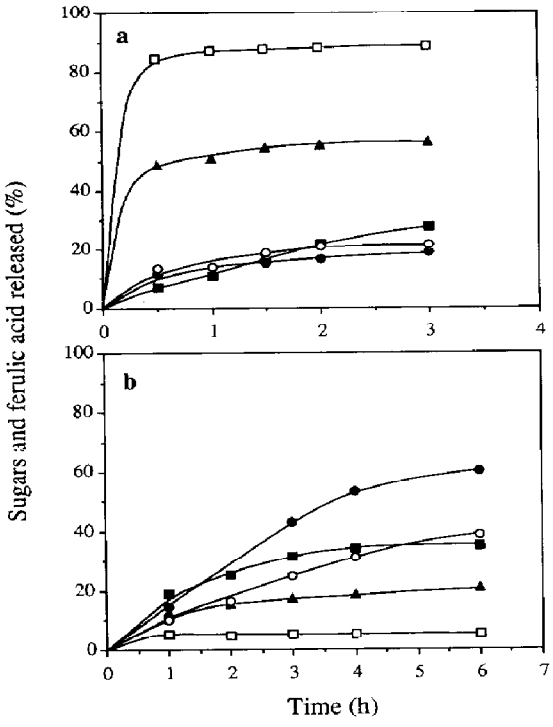

Fig. 6. Kinetics of hydrolysis of sugar-beet pulp by trifluoroacetic acid. (a) 70% Ethanol-soluble neutral sugars, galacturonic acid, and ferulic acid from sugar-beet pulp hydrolysed with 0.05 M trifluoroacetic acid at 100°C as a function of time. (b) 70% Ethanol-soluble neutral sugars, galacturonic acid, and ferulic acid from the first-step ethanol precipitate hydrolysed with 0.1 M trifluoroacetic acid at 100°C as a function of time. Values (%) are expressed relative to the initial amount of the corresponding parent residues in the pulp: ●, galacturonic acid; □, arabinose; ▲, ferulic acid; ■, galactose; ○, rhamnose.

with the presence of feruloylated galactose disaccharides (Gal_2F). An apparent molar ratio of ~1 for the neutral sugars and the ferulic acid could be deduced from the chromatogram; arabinose was used as standard. Approximately 12 mg of this component were recovered, corresponding to ~6% of the feruloyl groups initially present in the pulp.

Enzymic hydrolysis of the pulp by Driselase yielded various feruloylated oligosaccharides, but no feruloylated monosaccharides. Mild acid hydrolyses were performed in order to obtain feruloylated arabinose and galactose monosaccharides from sugar-beet pulp.

(*b*) *Mild acid hydrolysis.*—Sugar-beet pulp (100 mg) was hydrolysed by 0.05 M trifluoroacetic acid (10 mL) at 100°C for various times, and the ethanol-soluble extracts were analysed for their content of neutral sugars, and galacturonic and ferulic acids. As shown in Fig. 6a, galacturonic acid was recovered in the ethanol extract up to a limit of ca. 18% after 3 h of hydrolysis. The degrees of hydrolysis of rhamnose and galactose (21 and 27%, respectively) were close to that of galacturonic acid. Arabinose was released more rapidly and to a much greater extent since ca. 84% was recovered in the ethanol extract within 30 min and 92% after 3 h of

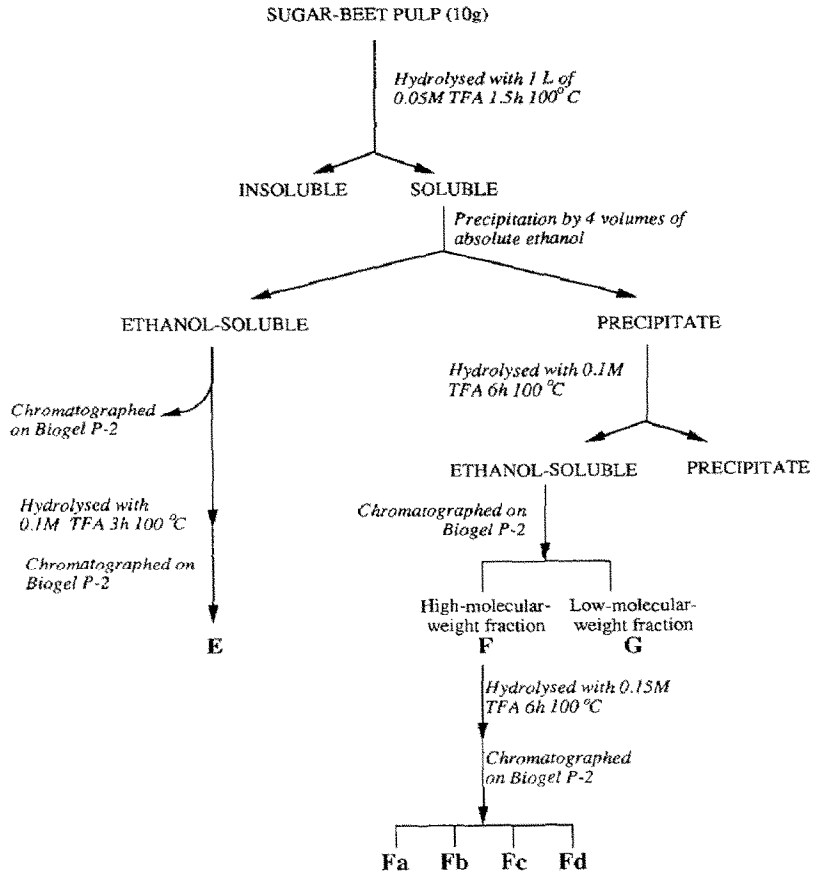

Fig. 7. Scheme of isolation procedures for feruloylated oligosaccharides from sugar-beet pulp by mild acid hydrolyses.

hydrolysis. More than 50% of the feruloyl groups were released in a similar manner. It is therefore very likely that the feruloyl groups released are linked to arabinose residues.

The isolation procedure for obtaining significant amounts of feruloylated oligosaccharides by hydrolysis of the pulp with 0.05 M trifluoroacetic acid is summarised in Fig. 7.

Study of the 70% ethanol-soluble products of the hydrolysate. The composition of the ethanol-soluble products obtained after 1.5 h of hydrolysis of the sugar-beet pulp by 0.05 M trifluoroacetic acid is presented in Table 4. The elution pattern (Fig. 8) on Biogel P-2 (FPLC) revealed a small peak eluted at the void volume and a large zone, containing the major proportion of ferulic acid, eluted between 0.2 and 2.5 column volumes. Two peaks could be distinguished at 1.5 and 2.1 column volumes. By analogy to the preceding Biogel P-2 chromatography (cf. Fig. 5), it was assumed that these two peaks consisted of feruloylated di- and mono-saccharides, respectively. In order to obtain more easily the feruloylated monomers, the

Table 4
Composition (mol%) of the trifluoroacetic acid hydrolysates of sugar-beet pulp

Fraction	Rha	Ara	Gal	Glc	GalA	Ferulic acid
Extract 1 [a]	1.8	77.3	3.4	1.4	14.3	1.3
Extract 2 [b]	3.5	6.2	10.0	1.6	77.3	0.7
Fraction E		49.8	2.2	2.6		45.3
Fraction Fc		15.4	36.7			47.9
Fraction Fd	3.1	7.9	6.9	1.3		80.8

[a] Ethanol extract after 1.5 h of hydrolysis of the sugar-beet pulp with 0.05 M trifluoroacetic acid at 100°C.
[b] Ethanol extract after 6 h of hydrolysis by 0.1 M trifluoroacetic acid at 100°C of the first-step ethanol precipitate.

ethanol-soluble products were further hydrolysed by 0.1 M trifluoroacetic acid for 3 h at 100°C. When chromatographed on Biogel P-2 (FPLC) (Fig. 8), this hydrolysate revealed a single peak (fraction E) containing feruloyl groups at 2.1 column volumes. Fraction E was composed of arabinose and ferulic acid in a molar ratio of 1.1, galactose and glucose being detected as trace components (Table 4). This

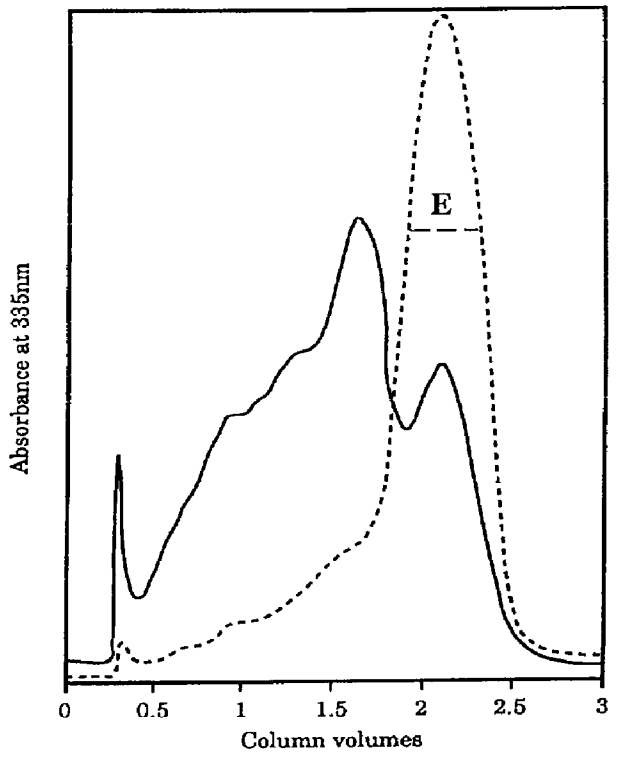

Fig. 8. Chromatography on Biogel P-2 of the trifluoroacetic acid hydrolysates: (———), ethanol-soluble fraction obtained from sugar-beet pulp hydrolysed with 0.05 M trifluoroacetic acid at 100°C for 1.5 h; (— — —), ethanol-soluble fraction hydrolysed with 0.1 M trifluoroacetic acid at 100°C for 3 h.

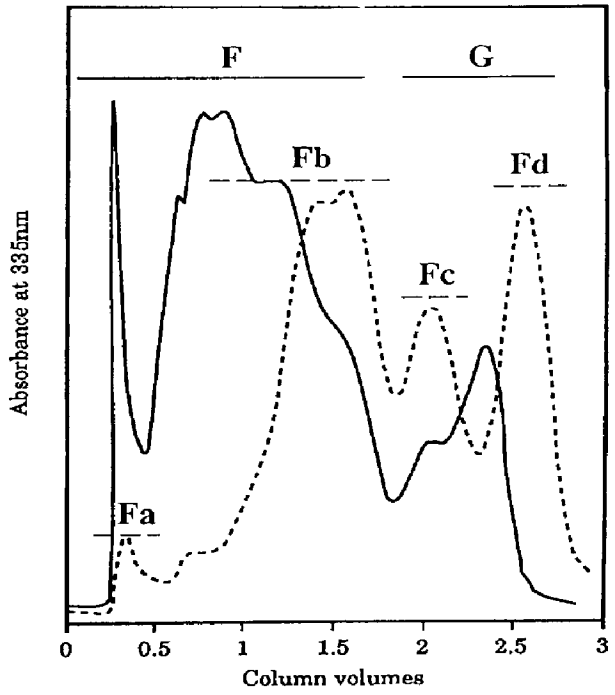

Fig. 9. Chromatography on Biogel P-2 of the second-step mild acid hydrolysates: (———), ethanol-soluble fraction obtained from the first-step ethanol precipitate hydrolysed with 0.1 M trifluoroacetic acid at 100°C for 6 h; (— — —), fraction F hydrolysed with 0.15 M trifluoroacetic acid at 100°C for 6 h.

result was consistent with the isolation of feruloylated arabinose monosaccharides (Ara_1F).

Study of the 70% ethanol-insoluble products of the hydrolysate. The products obtained after 1.5 h of hydrolysis of the sugar-beet pulp by 0.05 M trifluoroacetic acid, and insoluble in 70% ethanol, contained more than 80% of the galactose, galacturonic acid, and rhamnose, 46% of the ferulic acid, and 11% of the arabinose initially present in the pulp. This precipitate (2.5 g) was dissolved in distilled water (500 mL), and aliquots (4 mL) were further hydrolysed by 0.1 M trifluoroacetic acid for various times at 100°C and submitted to 70% ethanol precipitation. As shown in Fig. 6b, approximately 60% of the galacturonic acid, 38% of the rhamnose, 35% of the galactose, 21% of the ferulic acid, and 5% of the arabinose initially present in the sugar-beet pulp are soluble in 70% ethanol after 6 h of hydrolysis. Cumulating the two hydrolysis steps, 94% of the arabinose, 75% of the galacturonic and ferulic acids, 57% of the rhamnose, and 53% of the galactose initially present in the pulp were recovered in the ethanol extracts.

The ethanol-soluble products obtained after 6 h of hydrolysis by 0.1 M trifluoroacetic acid (cf. Fig. 7) were analysed (Table 4) and injected on Biogel P-2 (FPLC). The elution pattern (Fig. 9) revealed a narrow peak eluted at the void volume; a large zone, containing the major proportion of ferulic acid, eluted between 0.2 and 1.7 column volumes (fraction F); and a shouldered peak eluted at

2.4 column volumes (fraction G). The high molecular weight fractions (fraction F) were further hydrolysed by 0.15 M trifluoroacetic acid for 6 h at 100°C. When chromatographed on Biogel P-2 (Fig. 9), this hydrolysate yielded 4 distinguishable peaks containing feruloyl groups: a minor one at the void volume (Fa), a large peak with a shoulder eluted between 1 and 1.8 column volumes (Fb), and two narrow, fairly well separated peaks eluted at 2.1 (Fc) and 2.6 (Fd) column volumes, respectively. As previously pointed out, it can be assumed that the peak eluted at 2.1 column volumes (Fc) was composed of feruloylated monomers. This peak was mainly composed of ferulic acid (47.9 mol%), galactose (36.7 mol%), and arabinose (15.4 mol%) (Table 4), which is consistent with the presence of feruloylated galactose monosaccharides (Gal_1F) contaminated with some feruloylated arabinose monosaccharides. This fraction was recovered in very small quantity (300 μg). The peak eluted at 2.6 column volumes (Fd) showed a maximum absorbance of 350 nm at pH 10 and 310 nm at pH 6. Furthermore, the sugar analysis revealed only very small amounts of arabinose and galactose (Table 4). These results suggested that this fraction was mainly composed of free ferulic acid.

The resistance to acid of the linkages between galactose residues did not allow the recovery of large amounts of feruloylated galactose monomers, and the attempted use of more concentrated (1 M) trifluoroacetic acid led to release of free ferulic acid rather than feruloylated monomers.

3. Discussion

Hydrolysis of the sugar-beet pulp with Driselase.—As already reported [21], sugar-beet pulp was readily degraded, up to 80%, by Driselase which is devoid of ferulic acid esterases [1,22]. Although extensive solubilisation of the pectic polysaccharides was observed, long hydrolysis times were required to obtain a large release of feruloylated side-chains from the pectic backbone. Even after extensive hydrolysis, 22.3% of the total ferulic acid was recovered in the ethanol precipitate and was therefore still attached to polymeric fragments. A further 18.6% of the total ferulic acid was linked to oligosaccharides of relatively high dp and was eluted between the void and total volumes of the Sephadex LH-20 column. It was previously pointed out [15,17] that type I galactans in sugar-beet pectins had a low dp and could be degraded to only a very limited extent by endo-galactanase. This would explain why a large part of the galactose residues (71%) were still present as part of polymeric fragments after an extensive hydrolysis step. Assuming that all feruloyl groups recovered in the ethanol precipitate and eluted before the Sephadex LH-20 total volume were linked to galactose residues, it appears that $\sim 50\%$ of the ferulic acid was linked to galactose residues and $\sim 50\%$ to arabinose residues. Feruloylated arabinose di- and tri-saccharides were well separated and could be easily recovered as pure components from the Sephadex LH-20 fractionation. Approximately 13, 10, and 6% of the ferulic acid initially present in the sugar-beet pulp was recovered in the feruloylated arabinose trisaccharides, arabinose disaccharides, and galactose disaccharides, respectively.

Mild acid hydrolysis of the sugar-beet pulp.—Over the initial 1.5-h incubation period with 0.05 M trifluoroacetic acid, arabinose residues were mostly removed ($\sim 90\%$) together with 54% of the ferulic acid. The resulting ethanol precipitate was rich in galacturonic acid, galactose, and rhamnose. It is well known that peripheral Ara*f* residues are the most sensitive to acid hydrolysis and that mild acid hydrolysis of highly branched arabinans results in a rapid linearisation of the molecule followed by a slower breakdown of the α-(1 → 5)-linked main chain [3,16]. However, after 1.5 h of hydrolysis with 0.05 M trifluoroacetic acid, the ethanol extract contained only a small amount of feruloylated monomers. This suggests that feruloyl groups could either be linked to the α-(1 → 5)-linked arabinose residues or, as shown by Fry [1] in spinach cell walls, to terminal non-reducing ends of arabinose in the pyranoid form. The ethanol extract, after being further hydrolysed, yielded almost exclusively monomers, the feruloylated ones consisting of $> 98\%$ pure arabinose monomers. It therefore appears that, as already pointed out above, $\sim 54\%$ of the feruloyl groups were linked to arabinose residues. It was reported [15,17] that $\sim 30\%$ of the feruloyl groups were linked to arabinose residues of acid-extracted sugar-beet pectins. It is likely that some of the feruloylated arabinose residues have been lost during the acid extraction of the pectic substances. This hypothesis is supported by the large difference in the (arabinose : galactose) ratio between sugar-beet pulp and sugar-beet pectins. In agreement with previously published data [13,17,23–26], a weight ratio of 4.1 between arabinose and galactose was found for sugar-beet pulp while ratios of 1.7–2.8 were found for acid-extracted sugar-beet pectins.

From the enzymic and acid hydrolysis results, it appeared therefore that approximately 50–55% of the ferulic acid was linked to arabinose residues and the remaining 45–50% to galactose residues. It can therefore be concluded that 1 out of 56 arabinose residues and 1 out of 16 galactose residues present as neutral pectic side-chains in sugar-beet pulp carry a feruloyl group. Some feruloylated oligomers, namely the feruloylated arabinose mono-, di-, tri-, hexa-, hepta-, and octa-saccharides (Ara_1F, Ara_2F, Ara_3F, Ara_6F, Ara_7F, and Ara_8F) and the feruloylated galactose disaccharides (Gal_2F), were recovered in sufficiant quantities to be analysed by NMR spectroscopy in order to determine their structure. This is described in the following paper [27].

4. Experimental

Materials.—Sugar-beet pulp from the Générale Sucrière factory in Artenay (France) was ground with a hammer mill (Culati) at a linear velocity of 100 m/s to pass a 2-mm mesh screen. Particles between 80 and 2000 μm (yield $\sim 93\%$) were boiled in aq 70% EtOH and then extensively rinsed with 70% EtOH at room temperature. Alcohol-insoluble residue (AIR) (yield $\sim 90\%$) was recovered by filtration on a G-4 sintered glass, dried by solvent exchange, and then air-dried overnight at 40°C.

Driselase was obtained from Sigma. Driselase (1 g) was dissolved in distilled water (100 mL) and centrifuged. The supernatant solution was used.

Analytical methods.—All values were calculated on a moisture-free basis. Protein content (N × 6.25) was determined by the Kjeldahl procedure. Ash was measured after incineration overnight at 550°C, then for 1 h at 900°C. Galacturonic acid and neutral sugars (expressed as arabinose) were determined by the automated *m*-phenylphenol [28] and orcinol [29] methods, respectively, the latter being corrected for interfering galacturonic acid. Soluble fractions were hydrolysed in 2 M trifluoroacetic acid (2 h, 121°C) while insoluble fractions were prehydrolysed by 72% sulphuric acid (20 min, 25°C) [30] diluted to M and heated (2 h, 100°C). The individual sugars were reduced, acetylated, and analysed by GLC [30]. Methanol and acetic acid were determined by HPLC on an Aminex HPX 87 H column [32]. Feruloyl groups were determined spectrophotometrically at 375 nm on solutions in 0.04 M glycine–NaOH (pH 10) buffer, using [1] a molar extinction coefficient of 31600 M^{-1} cm^{-1}.

Chromatography.—Chromatography on DEAE-Sephacel was performed on a column (20 × 1.6 cm) equilibrated with distilled water at a flow-rate of 50 mL/h. The sample (20 mL) was loaded onto the column and the gel was washed with 100 mL of distilled water. The bound material was eluted with a linear sodium acetate buffer gradient at pH 4.8 (0 to 1 M; 100 mL). Fractions (5 mL) were collected.

Chromatography on a column (74 × 2.1 cm) of Sephadex LH-20 was carried out at a flow-rate of 40 or 20 mL/h with distilled water. Fractions (12 or 7 mL) were collected.

Chromatography on a column (80 × 3 cm) of Biogel P-4 and on a column (80 × 3 cm) of Biogel P-2 was carried out at 40°C and at a flow-rate of 25 mL/h with distilled water. Fractions (5 mL) were collected. Chromatography on Biogel P-2 was also performed by "fast protein liquid chromatography" (FPLC, Pharmacia) on a column (8.5 × 1 cm) eluted by distilled water at a flow-rate of 45 mL/h. The eluate was continuously analysed for its absorbance at 335 nm; 0.5-mL fractions were collected.

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