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Structure identification of feruloylated oligosaccharides from sugar-beet pulp by NMR spectroscopy ☆

Ian J. Colquhoun [a], Marie-Christine Ralet [b,1], Jean-François Thibault [b,*], Craig B. Faulds [a], Gary Williamson [a]

[a] *Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA, United Kingdom*

[b] *Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, BP 527, 44026 Nantes, France*

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Abstract

1D NMR (^{1}H and ^{13}C) and 2D NMR spectroscopy have been used to determine the structure of feruloylated oligosaccharides obtained by enzymic degradation or mild acid hydrolysis of sugar-beet pulp. Feruloylated oligosaccharides derived from pectic neutral side-chains containing arabinose or galactose residues were identified. In the feruloylated arabinose oligosaccharides, feruloyl groups were linked to O-2 of L-Ara*f* residues. The structure of the feruloylated arabinose disaccharide was identified as *O*-[2-*O*-(*trans*-feruloyl)-α-L-Ara*f*]-(1 → 5)-L-Ara*f* and that of the feruloylated arabinose trisaccharide as *O*-α-L-Ara*f*-(1 → 3)-*O*-[2-*O*-(*trans*-feruloyl)-α-L-Ara*f*]-(1 → 5)-L-Ara*f*. The structure of the feruloylated galactose disaccharide was identified as *O*-[6-*O*-(*trans*-feruloyl)-β-D-Gal*p*]-(1 → 4)-D-Gal*p*. From our results, we suggest that the feruloyl groups present in sugar-beet pulp are linked to the arabinofuranosyl residues of the main core of α-(1 → 5)-linked arabinan chains and to the galactopyranosyl residues of the main core of β-(1 → 4)-linked type I galactan chains.

Keywords: Sugar-beet pulp; Ferulic acid; Structure; NMR

☆ Feruloylated oligosaccharides from cell-wall polysaccharides, Part II. For Part I, see Ref. [17].

* Corresponding author.

[1] Present address: CBAI, Chaire de Biochimie, INA-PG, F-78850 Thiverval-Grignon, France.

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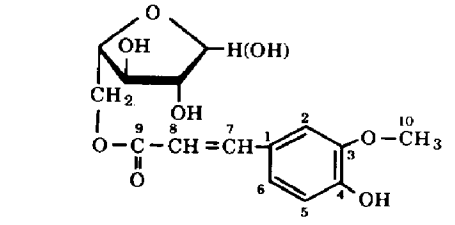
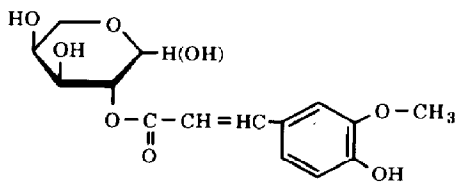
1. Introduction

Ferulic acid is a component of some plant families where it can be esterified to cell-wall polysaccharides. The determination of the type and site of linkage between ferulic acid and polysaccharides is based on the analysis of low molecular weight carbohydrate esters of ferulic acid obtained from enzymic or chemical hydrolysates of cell walls. Numerous studies have been carried out on isolated ferulic and *p*-coumaric esters of hemicellulosic oligosaccharides from *Gramineae*



Ara$_1$F(W)



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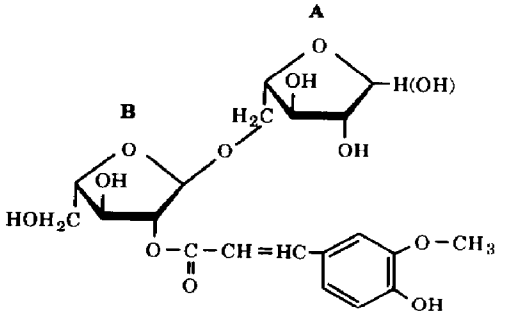
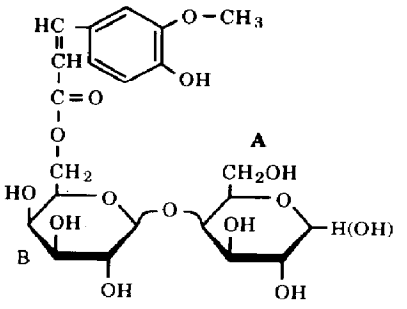
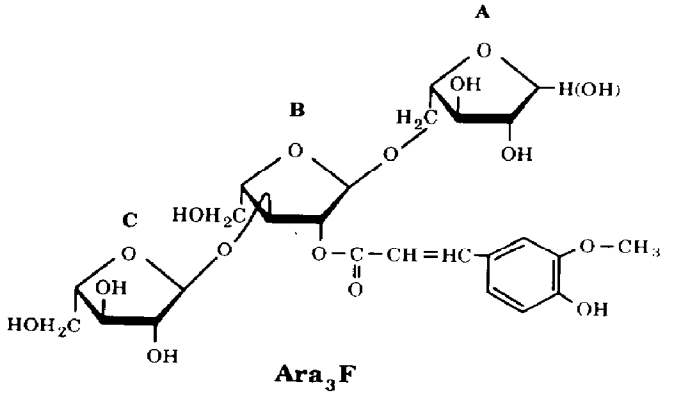


Ara$_2$F



Gal$_2$F



Ara$_3$F

Fig. 1. Structures of feruloylated mono-, di-, and tri-saccharides. Ara$_1$F(W) was isolated from wheat bran; all other compounds are from sugar-beet pulp.

cell walls. The trisaccharide *O*-[5-*O*-(*trans*-feruloyl)-α-L-Ara*f*]-(1 → 3)-*O*-β-D-Xyl*p*-(1 → 4)-D-Xyl*p* and the corresponding *trans*-*p*-coumaroyl compound have been isolated from many such sources [1–7]. The disaccharides *O*-[5-*O*-(*trans*-feruloyl)-β-L-Ara*f*]-(1 → 2)-D-Xyl*p* and *O*-[4-*O*-(*trans*-feruloyl)-α-D-Xyl*p*]-(1 → 6)-D-Glc*p* have been isolated from wheat bran [8] and bamboo shoots [6], respectively. Reports on the detailed structure of feruloylated oligosaccharides from dicotyledons are less frequent. Ferulic acid present in dicots was found associated with the pectic fraction of the cell walls [9–16]. Fry [9] identified two feruloylated disaccharides from suspension-cultured spinach cell walls digested with Driselase. Radioactive compounds were isolated from uniformly ^{14}C-labelled cell walls, and purified by thin layer chromatography and electrophoresis. It was deduced that the feruloylated disaccharides were *O*-[6-*O*-(*trans*-feruloyl)-β-D-Gal*p*]-(1 → 4)-D-Gal*p* and *O*-[3-*O*-(*trans*-feruloyl)-α-L-Ara*p*]-(1 → ?3)-L-Ara*f*, and suggested that ferulic acid residues were bound to the non-reducing termini of arabinose- and/or galactose-containing domains of pectins from spinach. The arabinose residues of pectins are usually in the furanoid form, but a few Ara*p* residues are also present. Fry [9] showed that ferulic acid was linked to such Ara*p* residues in spinach cell walls.

We have isolated various feruloylated oligosaccharides from sugar-beet pulp digested with Driselase or treated by mild acid. Among these, feruloylated arabinose mono-, di-, tri-, hexa-, hepta-, and octa-saccharides (Ara_1F, Ara_2F, Ara_3F, Ara_6F, Ara_7F, and Ara_8F, respectively) as well as feruloylated galactose disaccharides (Gal_2F) were recovered and purified [17]. We now report ^1H and ^{13}C NMR data for these feruloylated oligosaccharides derived from sugar-beet pulp and for a related monosaccharide $Ara_1F(W)$ derived from wheat bran [18]. The structures of the mono-, di-, and tri-saccharides were deduced from the NMR data and are shown in Fig. 1.

2. Results

Ferulic acid ester groups.—The ^1H and ^{13}C NMR spectra of the ferulic acid moiety in Ara_3F are shown in Fig. 2. The spectra are typical of those obtained for the mono-, di-, and tri-saccharides. The intensity of the signals relative to those in the carbohydrate region showed that each molecule contained a single ferulic acid group. The ^1H and ^{13}C chemical shifts are given in Tables 1 and 2. Proton coupling constants within the ferulic acid group were the same for all compounds and were $J_{7,8}$ 15.9, $J_{2,6}$ 1.8, and $J_{5,6}$ 8.2 Hz (the numbering scheme is given in Fig. 1). The value of $J_{7,8}$ shows that the double bond has the *trans* configuration and the data agree well with previous NMR studies of *trans*-feruloyl groups linked to arabinose in arabinoxylan oligosaccharides [1,3,4]. The assignments in Tables 1 and 2 are largely based on literature values [1,3,4,19] although the assignment of C-5(F) and C-8(F) has been reversed from that given previously [1,4]. This is based on the observation that in the monosaccharides two resonances appear at ca. 114.8 ppm (arising from the α and β anomers of Ara) but only a single resonance at 116.4

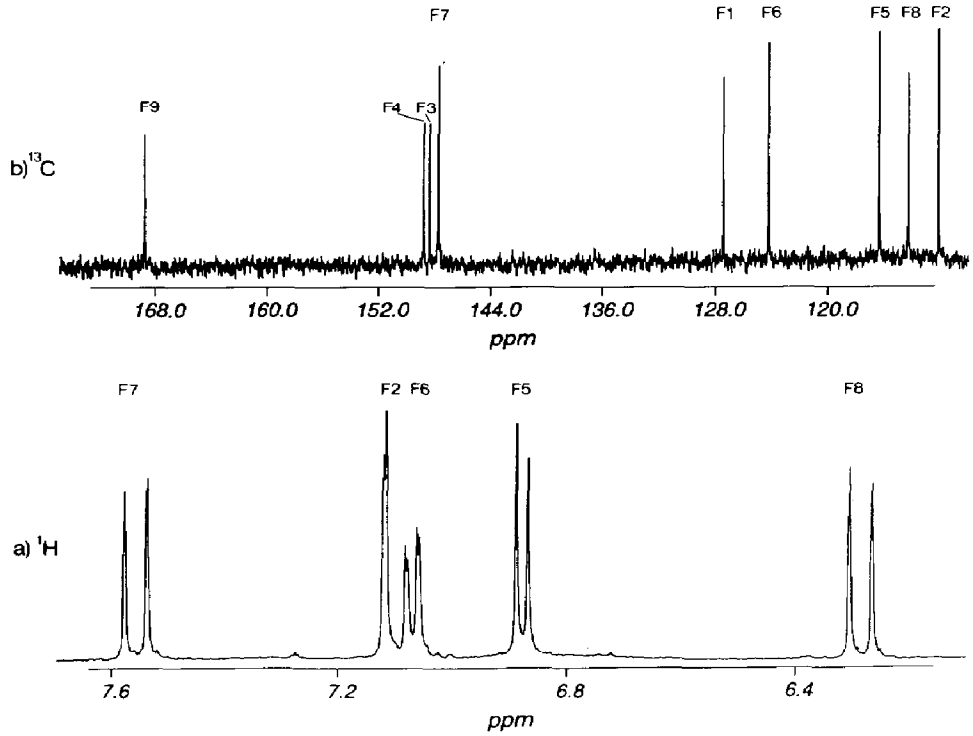


Fig. 2. (a) ^{1}H (400 MHz) and (b) ^{13}C (100 MHz) NMR spectra of the ferulic acid group in Ara_3F (F10 not shown).

ppm. The H-8(F) resonance is doubled in the monosaccharides, but that of H-5(F) is not. It is probable that the proximity of C-8(F) to the sugar ring makes the anomerisation effect greater for this carbon than for C-5(F). Other "doublets", resolved for the same reason, are indicated in the Tables but their chemical shifts are not listed separately, since the differences were generally small. NMR parameters for all the feruloyl groups are rather similar, but there are slight differences between the ^{1}H chemical shifts of ferulic acid linked to Ara*f* and Ara*p*, and between the ^{13}C chemical shifts of ferulic acid in the arabinose compounds and in the galactose disaccharide.

Feruloylated arabinose mono-, di-, and tri-saccharides.—2D NMR methods (COSY and C–H shift correlation) have been used to assign the regions of the ^{1}H and ^{13}C spectra attributable to carbohydrate units. The positions of the feruloyl groups and of the glycosidic linkages were determined from internal evidence and by comparison with literature data or reference compounds. The general effects of acyl group substitution in carbohydrates are to increase the chemical shift of the sugar proton α to the substitution site by 0.5–1 ppm and to increase the shifts of β-protons by smaller amounts. The chemical shift of the α-carbon is increased, typically by 2–3 ppm, but the shifts of β-carbons are decreased, usually also by 2–3 ppm [20]. The ^{13}C chemical shifts of some relevant model compounds are given in Table 3. The sugar residues are designated A for the reducing end units, B for the

Table 1
Chemical shifts [a] (ppm) of the ^{1}H resonances for feruloylated arabinose mono-, di-, and tri-saccharides and galactose disaccharide (see Fig. 1)

Fraction	Unit		H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-10
$Ara_1F(W)$ [b]	Ara*f*	α-A	5.30	4.08	4.08	4.35	4.32, 4.44				
		β-A	5.33	4.13	4.17	4.05	4.29, 4.46				
	Ferulic acid	F		7.17 [c]			6.89	7.10	7.60 [c]	6.36 [c]	3.86
Ara_1F	Ara*p*	α-A	4.74	5.02	3.94	4.03	3.74, 3.97				
		β-A	5.45	5.09	4.17	4.10	3.72, 4.11				
	Ferulic acid	F		7.26 [c]			6.92	7.17	7.71 [c]	6.44 [c]	3.88
Ara_2F	Ara*f*	α-A	5.27	4.04	4.05	4.26	3.80, 3.88				
		β-A	5.30	4.10	4.10	3.96	3.79, ?				
	α-Ara*f*	B	5.25	5.08	4.19	4.19	3.76, 3.87				
	Ferulic acid	F		7.17			6.90	7.10	7.60	6.33	3.86
Ara_3F	Ara*f*	α-A	5.27	4.05	4.08	4.25	3.80, 3.88				
		β-A	5.31	4.11	4.13	3.97	3.81, 3.84				
	α-Ara*f*	B	5.30	5.16	4.22	4.27	3.78, 3.88				
	α-Ara*f*	C	5.25	4.18	3.95	4.04	3.71, 3.88				
	Ferulic acid	F		7.12			6.88	7.07	7.56	6.29	3.84
Gal_2F	Gal*p*	α-A	5.24	3.84	3.89	4.10	4.05	3.70, 3.70			
		β-A	4.55	3.53	3.68	4.04	3.64	3.72, 3.72			
	β-Gal*p*	B	4.52 [c]	3.57	3.65	3.93	3.85	4.28, 4.41			
	Ferulic acid	F		7.18			6.81	7.11	7.62	6.30	3.84

[a] ±0.01 ppm.
[b] From wheat bran. All other compounds from sugar beet.
[c] Peaks "doubled" by the presence of anomers in the neighbouring reducing residue.

Table 2
Chemical shifts [a] (ppm) of the ^{13}C resonances for feruloylated arabinose mono-, di-, and tri-saccharides and galactose disaccharide (see Fig. 1)

Fraction	Unit		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
$Ara_1F(W)$ [b]	Ara*f*	α-A	102.07	82.08	76.83	81.52	64.76					
		β-A	96.14	76.74	75.12	79.45	65.85					
	Ferulic acid	F	127.70 [c]	112.12 [c]	148.4	148.6 [c]	116.37	124.17	147.23 [c]	114.91 [c]	170.0 [c]	56.68
Ara_1F	Ara*p*	α-A	95.88	74.39	71.61	69.24	67.26					
		β-A	91.06	72.12	67.32	69.57	63.26					
	Ferulic acid	F	127.53	112.26 [c]	148.56	149.06	116.53	124.33 [c]	147.65 [c]	114.82 [c]	169.64	56.74
Ara_2F	Ara*f*	α-A	101.98	82.17	76.69	82.17	67.48					
		β-A	96.16	76.84	75.24	80.19	68.88					
	α-Ara*f*	B	106.34 [c]	84.3 [c]	75.95 [c]	85.24 [c]	61.77					
	Ferulic acid	F	127.46	112.18	148.49	148.96	116.43	124.31	147.70	114.43	169.02	56.68
Ara_3F	Ara*f*	α-A	101.92	82.22	76.58	82.02	66.95					
		β-A	96.13	76.89	75.22	80.19	68.62					
	α-Ara*f*	B	106.23 [c]	82.33 [c]	80.97 [c]	84.51 [c]	61.51					
	α-Ara*f*	C	107.83 [c]	82.02 [c]	77.51	84.78	61.97					
	Ferulic acid	F	127.46	112.13	148.40	148.86	116.35	124.31	147.78	114.27	168.78	56.65
Gal_2F	Gal*p*	α-A	93.12	69.69	70.41	79.88	70.64	62.03				
		β-A	97.20	73.08	74.00	78.79	75.17	61.86				
	β-Gal*p*	B	105.33 [c]	72.14 [c]	73.47	69.40	73.55	64.33				
	Ferulic acid	F	124.25	111.90	150.21	155.23	117.77	125.45	148.05	112.49	170.27	56.64

[a] ±0.01 ppm.
[b] From wheat bran. All other compounds from sugar beet.
[c] Peaks "doubled" by the presence of anomers in the neighbouring reducing residue.

Table 3
^{13}C chemical shifts (ppm) of arabinose model compounds

Unit	C-1	C-2	C-3	C-4	C-5	Ref
Ara*f* α	101.9	82.3	76.5	83.8	62.0	21
β	96.0	77.1	75.1	82.2	62.0	21
Ara*p* α	97.6	72.9	73.5	69.6	67.2	21
β	93.4	69.5	69.5	69.5	63.4	21
(1 → 5)-α-Ara*f*	108.4	81.7	77.7	83.2	67.8	22,29
Terminal Ara*f* [a]	108.9	82.2	77.4	84.7	62.0	1

[a] In arabinoxylan oligosaccharide; values corrected to our reference system for which δ (^{13}C) of 1,4-dioxane is 67.4 ppm.

non-reducing terminus in the disaccharides, and C for the non-reducing terminus in Ara_3F (Tables 1 and 2).

The ^{13}C NMR spectrum of the arabinose monosaccharide from wheat bran, $Ara_1F(W)$, is shown in Fig. 3a. The signals fall into two groups with an intensity ratio of 60:40, corresponding to α and β anomers, respectively, and the C-1 chemical shifts are close to those for Ara*f* (Table 3), showing that in $Ara_1F(W)$ the ring is in the furanose form. The ^1H spectra could be assigned (Table 1) from the COSY spectrum starting from the anomeric signals, H-1(βA) at 5.33 ppm (d, $J_{1,2}$ 4.6 Hz) and H-1(αA) at 5.30 ppm (t, $|J_{1,2}+J_{1,3}|$ 3.1 Hz). The latter resonance is a

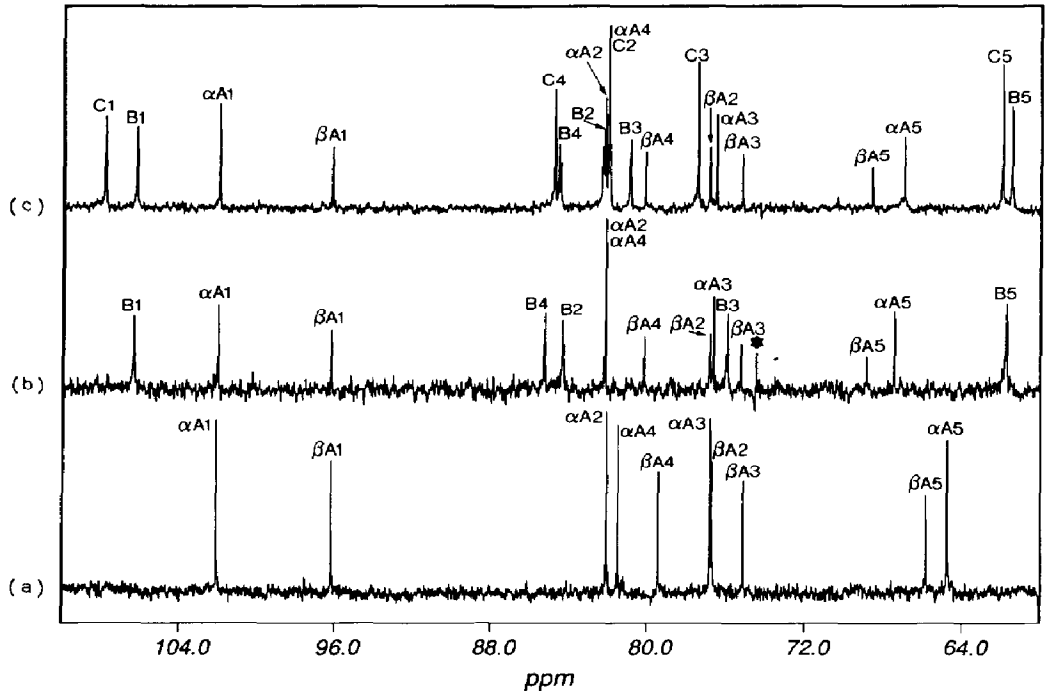


Fig. 3. 100-MHz ^{13}C NMR spectra (carbohydrate region) of (a) $Ara_1F(W)$, (b) Ara_2F, and (c) Ara_3F (* = artifact).

triplet because of the near-coincidence of the chemical shifts for H-2 and H-3(αA). The ^{13}C assignments (Table 2) can then be made from the C–H correlation spectrum. It was found that the chemical shifts of C-1, C-2, and C-3 for Ara$_1$F(W) were very close to those of Ara$f$ (Table 3), but C-5 was shifted downfield by $\sim$3 ppm and C-4 was shifted upfield by $\sim$2.5 ppm. Also, the H-5(α,βA) chemical shift is 4.4 ppm for Ara$_1$F(W), considerably downfield from the value of $\sim$3.8 ppm expected for a free $-CH_2OH$ group on an Ara$f$ ring. These data indicate that the sugar unit is Ara$f$, ester linked at O-5 to ferulic acid (Fig. 1). There is good agreement between the chemical shift data for H-5 and C-5(αA) in Ara$_1$F(W) and those found for α-Ara$f$, linked to ferulic acid at O-5 in arabinoxylan oligosaccharides from various sources. The preparation and ^{1}H NMR spectrum of Ara$_1$F from wheat bran were reported previously [23], but the ^{1}H spectra were recorded in $CDCl_3$ and the chemical shift values given differ somewhat from those reported here.

The NMR spectra for Ara$_1$F from sugar-beet pectin were quite different from those of Ara$_1$F(W). In the ^{13}C spectrum, the anomeric region contained two peaks of equal intensity at 95.9 and 91.1 ppm. There were three multiplets in the ^{1}H spectrum with chemical shifts between 5 and 5.5 ppm. The C–H shift correlation spectrum showed that there were two CH_2OH groups with ^{13}C shifts of 67.3 and 63.3 ppm. These shifts are very close to the literature values for C-5(α) and C-5(β) in the pyranose form of arabinose [21]. The C–H shift correlation also revealed that the anomeric ^{13}C resonance at 95.9 ppm was correlated to a ^{1}H resonance at 4.74 ppm which was obscured by the residual HDO peak in the ^{1}H spectrum. The other ^{13}C anomeric peak (91.1 ppm) was correlated to the H-doublet at 5.45 ppm ($J_{1,2}$ 3.4 Hz). The remaining resonances of this ring were assignable from COSY and included the CH_2 protons which correlated with the C-5(β) resonance at 63.3 ppm. The resonances for the α anomer were established similarly although the cross-peak between H-1(αA) and H-2(αA) could not be observed because of the saturating irradiation at the water resonance frequency. However, the coupling constant $J_{1,2}$(αA) was found to be 7.9 Hz from the H-2(αA) multiplet, consistent with expectations for an Ara$p$ ring [21]. The two resonances at 5.02 and 5.09 ppm were shown to arise from H-2(αA) and H-2(βA), respectively, i.e., both at higher chemical shifts than in Ara$p$ itself, and comparison of C-2(α,βA) (Table 2) for Ara$_1$F with values for Ara$p$ (Table 3) show that both are shifted downfield in the feruloylated molecule whilst C-1(α,βA) and C-3(α,βA) are shifted upfield. Thus, in Ara$_1$F, the feruloyl group is located at O-2 and the Ara unit is in the pyranose form. There was evidence from the ^{13}C spectrum for a small population (ca. 10%) of another species, probably the furanose form, but this was not investigated further.

The ^{13}C spectrum of Ara$_2$F is shown in Fig. 3b. The similarity of the chemical shifts of C-1, C-2, and C-3(α,βA) to those for Ara$_1$F(W) (Fig. 3a) and Ara$f$ itself (Table 3) indicate that the reducing end unit of Ara$_2$F is an arabinofuranose residue. COSY and C–H shift correlation experiments confirm that the resonance at 67.5 ppm is from C-5(αA), this being the value expected for a 5-linked α-Ara$f$ unit (Table 3). The anomeric resonances of the non-reducing residues are at 5.25

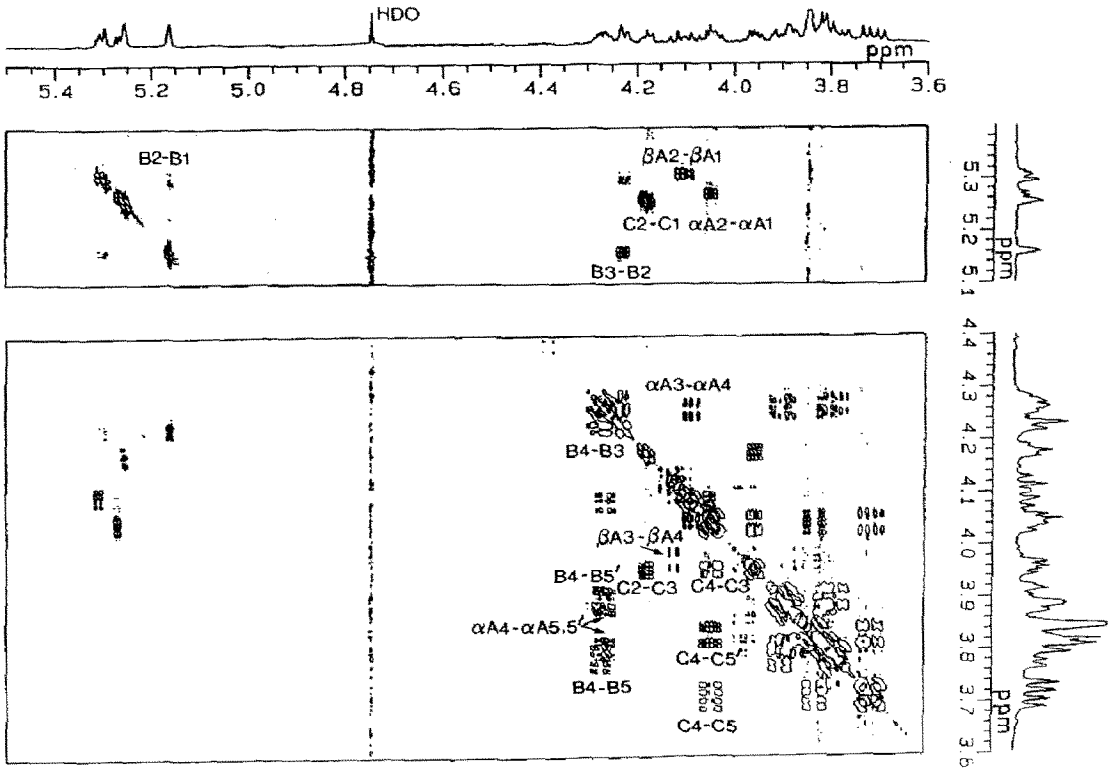


Fig. 4. 400-MHz COSY spectrum of Ara_3F. For each cross-peak, the first proton designated has the chemical shift given by the position on the horizontal axis. The upper segment has been plotted at a lower contour level in order to display the weak B2–B1 cross-peak.

ppm for H-1(B) and 106.34 ppm for C-1(B), with C-5(B) at 61.8 ppm. The ^{13}C data show that B is an α-Ara*f* unit (Table 3). The H-1(B) resonance has a cross-peak in the COSY spectrum with a signal at 5.08 ppm which must arise from H-2(B). This cross-peak can only be observed at low contour levels of the COSY spectrum because $J_{1,2}$(B) is small, ca. 1 Hz. The chemical shift of H-2(B) is ca. 1 ppm higher than that normally expected for an unsubstituted Ara*f* unit, indicating that the feruloyl group is located at O-2 of the non-reducing residue. The downfield shift of C-2(B) and upfield shifts of C-1(B) and C-3(B) in comparison with a terminal α-Ara*f* group (Table 3) are in agreement with this interpretation. Ara_2F therefore has the structure shown in Fig. 1.

The ^{13}C spectrum of Ara_3F is shown in Fig. 3c, and the ^{1}H and COSY spectra in Fig. 4. The assignment of the anomeric ^{13}C resonances for the reducing end unit is obvious, and it is clear from further comparison of Figs. 3b and 3c that the reducing end units of Ara_2F and Ara_3F are identical. Of the two remaining anomeric resonances, heteronuclear shift correlation showed that C-1(C) was correlated with the ^{1}H signal at 5.25 ppm. Starting from this point, assignment of the other ^{1}H and ^{13}C resonances for ring C could be made from COSY and C–H correlation spectra. The resulting ^{13}C chemical shifts (Table 2) show that ring C is

an unsubstituted terminal α-Ara*f* unit. The same procedure can be used to assign the signals of ring B which had anomeric resonances at 5.30 (^{1}H) and 106.2 (^{13}C) ppm. As was found for Ara_2F, the feruloyl group is located at O-2 of ring B, shown by the high-frequency chemical shift of H-2(B) at 5.16 ppm. However, the downfield shift of C-3(B) and upfield shifts of C-2(B) and C-4(B), when compared with the same carbon atoms in ring B of Ara_2F, establish that ring B in Ara_3F is glycosidically linked at O-3. Ara_3F therefore has the structure shown in Fig. 1.

Feruloylated galactose disaccharide.—The spectra for Gal_2F were assigned from 2D NMR experiments and from the intensities of the ^{13}C NMR signals, the anomeric ratio being 60:40 in favour of the β form of ring A. The chemical shifts of C-1(B) and C-4(α,βA) (Table 2), together with published data [22] for β-(1 → 4)-galactan chains, showed that the disaccharide was β-Gal*p*-(1 → 4)-Gal*p*. The high frequency shifts of C-6(B) and H-6a,6b(B), compared with the values normally expected for the $-CH_2OH$ group (e.g., those shown by ring A), indicate that the non-reducing sugar unit is feruloylated at O-6 (Fig. 1).

Feruloylated arabinose hexa-, hepta-, and octa-saccharides.—Because of the small amounts available, only 1D ^{1}H and ^{13}C NMR spectra of the higher arabinose oligosaccharides were recorded. The chromatographic fractions were designated Ara_6F, Ara_7F, and Ara_8F on the basis of the ferulic acid:arabinose ratios measured in Part I [17]. The NMR spectra showed that each of these fractions contained a mixture of molecular species.

The ^{1}H spectra of the three oligosaccharides contained a broad envelope of resonances in the anomeric region extending from ~5 to 5.3 ppm, and were not informative. The olefinic resonances in the ferulic acid region of the spectrum showed three locations for the ferulic acid group in Ara_6F, but apparently only one major site in Ara_8F. The coupling constant between the olefinic protons was again characteristic of the *trans* configuration, but the chemical shifts of the protons (~6.48 and 7.73 ppm) were closer to those found for ferulic acid linked to the Ara*p* residue of Ara_1F than to the Ara*f* residues of Ara_2F and Ara_3F. However, no evidence was found in the ^{13}C spectra for the presence of Ara*p* residues in the three higher arabinose oligosaccharides.

Some additional conclusions on the structure of the oligosaccharides could be drawn from the ^{13}C spectra in the carbohydrate region. The ^{13}C spectra of Ara_6F, Ara_7F, and Ara_8F have similar features and only that of Ara_6F will be discussed in detail. There were five groups of anomeric resonances at 108.2 (3.9), 107.8 (5.1), 107.1 (2.2), 106.2 (2.4), and 101.98/96.14 ppm (total 3). The integrated intensity for each group is given in brackets. The final pair corresponds to C-1(α,β) of the reducing end unit. Apart from the signal at 108.2 ppm, each group contained at least two or three resonances. Signals at the first three positions have previously been observed in spectra of arabinans from *Rosa glauca* [24], and in an arabinose-rich polysaccharide, MHR, from modified hairy regions of apple pectin [25]. The resonances at 108.2 and 107.8 ppm can be assigned to C-1 of α-Ara*f* residues linked to O-5 and O-3, respectively, of neighbouring Ara*f* residues. The resonances at 106.2 ppm can be assigned to C-1 of α-Ara*f*, substituted with ferulic acid at O-2, and linked to O-5 of another Ara*f* unit, as discussed above for Ara_2F

and Ara_3F. Arabinan side-chains of pectic polysaccharides, including those from sugar-beet [14], are known to contain 2-, (2,5)-, and (2,3,5)-linked Ara*f* residues in addition to the predominant terminal, 5-, and (3,5)-linked units [24,25]. Although the resonances at 107.1 ppm have not been definitively assigned, it is likely that their intensity corresponds to the number of residues with a linkage at O-2. The signal could arise from the C-1 of a terminal Ara*f* unit linked to O-2 of a main-chain residue, or from C-1 of a main-chain residue, substituted at O-2.

Major signals in the non-anomeric region (integrals given in brackets) are at 84.85 (8.2), 82.1 (13.2), 77.4 (7.6), 67.0 (5.6), and 61.9 ppm (11.1). Apart from the sharp resonance at 61.9 ppm, these groups all consist of three or four partly resolved signals. The resonances listed above are assigned to C-4, C-2, C-3, C-5 (glycosidically linked at O-5), and C-5 (not glycosidically linked) of α-Ara*f* units. The relative intensities of the two C-5 signals show that the ratio of free $-CH_2OH$ to $-CH_2OR$ groups is ca. 2:1 and the major C-4 signal (84.85 ppm) is typical of a terminal and not of a 5-linked α-Ara*f* residue (Table 3). The general conclusion is that the Ara_6F, Ara_7F, and Ara_8F fractions contain mixtures of oligosaccharides in which the residues originating from the main $(1 \rightarrow 5)$-α-Ara*f* chain are highly substituted by terminal Ara*f* units. The feruloyl groups are linked to O-2 of Ara*f* residues, as in the smaller oligosaccharides studied here. Without further purification of the fractions, it is not possible at present to give more specific information on the structures.

3. Discussion

Cell walls isolated from sugar-beet yield feruloylated oligosaccharides of various dp after treatment with Driselase [17]. The results presented here show that feruloyl groups were linked on O-2 of L-Ara*f* residues in the arabinose oligosaccharides and on O-6 of the non-reducing β-D-Gal*p* residue in the galactose disaccharide. After treatment of the sugar-beet pulp with dilute trifluoroacetic acid, feruloylated arabinose monomers were isolated [17] and identified as 2-*O*-(*trans*-feruloyl)-L-Ara*p*. The acidic conditions obviously led to a recyclisation of the arabinofuranose ring as pointed out by Fry [26]. This rearrangement cannot occur with the monomer from wheat bran because the feruloyl group is linked to O-5.

From the results obtained previously [16], it appeared that $\sim 50\%$ of the feruloyl groups were linked to galactose residues in the sugar-beet-pulp cell walls. The structural analysis of the isolated feruloylated galactose disaccharides is compatible with a feruloylated type I β-galactan as already shown by Fry for spinach cell walls [9].

In contrast, the structural features observed for arabinose-containing feruloylated oligosaccharides differ considerably from those previously observed by Fry [9] in terms of the site of feruloylation and the type of linkage between arabinose residues. We have shown unambiguously that feruloyl groups were linked at the O-2 position of Ara*f* residues in sugar-beet-pulp cell walls while Fry [9] found

feruloyl groups linked at the O-3 position of Ara*p* residues in spinach cell walls. Furthermore, the linkage between the Ara*f* residues in the feruloylated arabinose disaccharide was shown to be α-(1 → 5) and not α-(1 → 3) as in spinach cell walls [9].

Arabinans are composed of α-L-Ara*f* residues which are (1 → 5)-linked, a varying number of them being substituted with single unit or short side-chains of other α-L-Ara*f* residues at the O-2 and/or O-3 position [14,15,27–30]. In the particular case of sugar-beet pulp, the core of α-L-(1 → 5)-Ara*f* residues is partly substituted at the O-3 position, probably mainly by single-unit side-chains and, to a more limited extent, by short α-L-(1 → 3)-Ara*f* side-chains [14,15]. The isolation of feruloylated oligosaccharides in which feruloyl groups were exclusively linked to α-L-(1 → 5)-linked Ara*f* suggests that feruloyl groups are directly linked to the arabinan main core in the sugar-beet pectins. Furthermore, the only feruloylated trisaccharide isolated exhibited an Ara*f* unit linked at the O-3 position of the Ara*f* residue carrying the feruloyl group at the O-2 position. Feruloyl groups could therefore be preferentially linked to the branching points of the arabinan main chain.

From our results, it appears that the feruloyl groups present in sugar-beet pulp are linked to the Ara*f* residues of the main core of α-(1 → 5)-linked arabinan chains and to the Gal*p* residues of the main core of β-(1 → 4)-linked type I galactan chains, leading to a low accessibility of the feruloyl groups in the pectins. The low accessibility to ferulic acid esterases of feruloyl groups in sugar-beet pectins was indicated by Faulds and Williamson [31]. Guillon and Thibault [16] also pointed out that feruloyl groups carried by the arabinose side-chains were not directly accessible for cross-linking reactions in the presence of persulfate ions.

A modified [15] tentative structure for the sugar-beet pectin "hairy" fragments, including the feruloylation sites, is shown in Fig. 5.

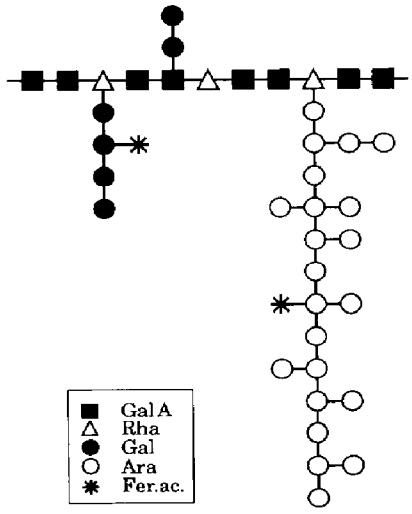


Fig. 5. Tentative model of the "hairy" fragments of sugar-beet pectin, showing the feruloylation sites.

4. Experimental

Sugar-beet-pulp feruloylated oligosaccharides.—Feruloylated arabinose di-, tri-, hexa-, hepta-, and octa-saccharides (Ara_2F, Ara_3F, Ara_6F, Ara_7F, and Ara_8F, respectively) as well as the feruloylated galactose disaccharide (Gal_2F) were obtained after hydrolysis of sugar-beet pulp with Driselase [17]. Feruloylated arabinose monosaccharides (Ara_1F) were obtained by mild acid hydrolysis [17,18].

NMR spectroscopy.—Samples (between 2 and 10 mg) were dissolved in D_2O, and ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded at 27°C with a Jeol GX-400 spectrometer. Double quantum filtered COSY [32] and $^{13}C-^1H$ shift-correlation experiments [33] were carried out in the phase-sensitive mode using the method of States et al. [34]. COSY spectra were measured with spectral widths of 2500 or 1000 Hz (carbohydrate region only) and a 2048 (t_2) × 256 (t_1) × 2 data matrix. $^{13}C-^1H$ shift correlation spectra were obtained with spectral widths of 8000 Hz (^{13}C) × 1000 Hz (^1H, carbohydrate region only) and a data matrix of 4096 × 64 × 2. The t_1 dimension was zero-filled to 128 data points before Fourier transformation. The chemical shifts for the methyl group of internal acetone were taken to be 2.217 (^1H) and 31.07 ppm (^{13}C) with respect to the signals for Me_4Si (δ 0).

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