Pergamon

PII: S0031-9422(97)00132-5

Phytochemistry, Vol. 45, No. 6, pp. 1123–1129, 1997

0031-9422/97 $17.00+0.00

DIGESTION BY FUNGAL GLYCANASES OF ARABINOXYLANS WITH DIFFERENT FERULOYLATED SIDE-CHAINS

GUNDOLF WENDE* and STEPHEN C. FRY†

ICMB, DBS, The University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Edinburgh EH9 3JH, U.K.

(*Received* 22 *September* 1996)

Key Word Index—*Festuca arundinacea*; tall fescue grass; *Zea mays*; maize; Gramineae; feruloyl esters; oligosaccharides; arabinoxylans; Driselase; plant cell walls; glycosylhydrolases.

Abstract—Alcohol-insoluble residues (AIRs) from *Festuca* and *Zea* cell cultures contained 7.4 and 35 nmol esterified ferulate mg^{-1}, respectively. Driselase solubilised 79% of the feruloylated material from both AIRs. Of the feruloyl esters solubilised from *Festuca* and *Zea* AIRs, 72 and 56% respectively were small enough to be mobile on paper chromatography. The major feruloylated product of *Zea* AIR was the known 5-*O*-feruloyl-α-L-Ara*f*-(1 → 3)-β-D-Xyl*p*-(1 → 4)-D-Xyl (Fer-Ara-Xyl-Xyl). In contrast, the smallest major feruloylated product of *Festuca* AIR was a feruloyl pentasaccharide (**3**) containing 3 Xyl, 1 Ara and 1 non-pentose residue (NPR). The Ara and two of the three Xyl groups of **3** were resistant to $NaIO_4$. Mild acid hydrolysis of **3** gave xylobiose, a feruloyl trisaccharide and β-D-Xyl*p*-(1 → 2)-(5-*O*-feruloyl)-L-Ara. Compound **3** was therefore NPR-(1 → 3)-β-D-Xyl*p*-(1 → 2)-(5-*O*-feruloyl)-α-L-Ara*f*-(1 → 3)-β-D-Xyl*p*-(1 → 4)-D-Xyl. We conclude that the complex feruloyl oligosaccharide side-chains of *Festuca* arabinoxylan do not protect the polysaccharide against hydrolysis by the fungal glycanases present in Driselase.

INTRODUCTION

The non-lignified, growing cell walls of plants are largely composed of polysaccharides with smaller amounts of glycoproteins; in addition, they often contain phenolic components, especially ferulate and *p*-coumarate [1–4]. These phenolics are ester-linked to polysaccharides, mainly arabinogalactans in the Chenopodiaceae [5, 6], and arabinoxylans in the Gramineae [7–11]. One well characterised site of feruloylation in many Gramineae is *O*-5 of an α-L-Ara*f* side chain attached to *O*-3 of a β-D-Xyl*p* residue in the xylan backbone [7–9].

Several biological roles have been proposed for the feruloylation of polysaccharides, including (a) providing sites through which the polysaccharides can be cross-linked *in vivo* by peroxidase-catalysed oxidative coupling [12, 13], or by photo-dimerisation [14], thus tightening the cell wall and limiting cell expansion [15, 16], or strengthening cell–cell adhesion [17], and (b) protecting the polysaccharides from hydrolysis by microbial enzymes, thus limiting the suitability of the plant as a host for phytopathogens or as fodder for animals [18].

The hypothesis that feruloyl (fer) esters protect polysaccharides against enzymic attack has been supported in some studies [18]. There is also counter-evidence: for example, the feruloylated pectin of spinach cell walls was efficiently hydrolysed by the fungal glycanase preparation 'Driselase', which lacks fer-esterase activity [5, 19], and by the micro-organisms present in the rat caecum [20]. Fungal enzymes also readily hydrolyse the fer-arabinoxylans of many Gramineae, such as *Zea*, efficiently releasing a fer-trisaccharide (Fer-Ara-Xyl-Xyl) [8, 9].

Recently it has been shown that the xylans of many Gramineae bear fer-oligosaccharide side-chains [21–25] in place of the simple side-chain 5-*O*-Fer-L-Ara*f* (Fer-Ara). The shortest of these, β-D-Xyl*p*-(1 → 2)-(5-*O*-feruloyl)-L-Ara [Xyl-(Fer)-Ara)], was not resistant to digestion by the microbes present in the rat gut [26]. However, it has not yet been reported whether the larger fer-oligosaccharide side-chains provide greater resistance to enzymic hydrolysis than the simple side-chains. Driselase, a mixture of endo- and exo-glycanases from the fungus *Irpex lacteus*, is a useful preparation with which to test this because it contains the enzyme activities needed to digest all the major polysaccharides of the plant cell wall to monosaccharides and disaccharides, and can therefore pro-

* Present address: Division of Biochemistry and Molecular Biology, Bower Building, University of Glasgow, Glasgow G12 8QQ, U.K.

† Author to whom correspondence should be addressed.

Table 1. Solubilisation, by Driselase, of saponifiable feruloyl compounds from *Festuca* and *Zea* AIR*

Sample	Saponifiable ferulate† (nmol per 10 mg AIR and/or 2.5 mg Driselase) in AIR of *Festuca*	*Zea*
(a) AIR + buffer	73.9	353
(b) AIR + Driselase + buffer: soluble products	103	362
(c) AIR + Driselase + buffer: insoluble products	16.3	88.4
(d) Driselase + buffer	39.6	
(e) Apparent recovery in digested AIR‡	79.7	411

* AIR was incubated with Driselase: soluble (b) and insoluble (c) products were then saponified and the A_{343} of a suitable dilution was measured. AIR in buffer alone (a) and Driselase alone (d) served as controls.

† As Na ferulate; $\varepsilon_{343} = 23\,500\ M^{-1}\ cm^{-1}$.

‡ (e) = (b) + (c) − (d).

vide information about the protective effect of phenolic groups.

We found that Driselase failed to give high yields of Fer-Ara-Xyl-Xyl from the fer-arabinoxylan of *Festuca* cells, suggesting that this fer-arabinoxylan might be unusually resistant to enzymic digestion. We have therefore compared the digestion of *Festuca* and *Zea* cell walls.

RESULTS

Quantitative analysis of feruloyl esters released by Driselase digestion of Festuca *and* Zea *AIR*

The total saponifiable ferulate content of AIR from cultured cells of *Festuca* and *Zea* was 7.4 and 35.3 nmol mg^{-1}, respectively (Table 1, row a). The ability of Driselase to solubilise these fer-esters was investigated. Driselase itself contained small amounts of material that, when saponified, gave fer-like material (Table 1, row d). Allowing for this artefact, the apparent recovery of the AIR-bound ferulate in the soluble plus insoluble fractions of the Driselase digest was 108 and 116% for *Festuca* and *Zea*, respectively. Of the total ferulate detected, the proportion that had been released in soluble form by Driselase from the two species was 79.5 and 78.5%, respectively.

Molecular size of soluble products released from Festuca *and* Zea *AIR by Driselase*

Since Driselase solubilised most of the AIR-bound feruloyl esters in both species, the solubilised material was next investigated qualitatively. The digestion products were subjected to preparative PC in butanol, acetic acid and water (Fig. 1). Several peaks of material were observed that, after saponification, absorbed at 343 nm, the λ_{max} of Na ferulate. The peaks of A_{343}-material generally coincided with zones on the chromatogram that exhibited the characteristic fluorescence of *O*-fer-esters (blue, turning intense blue-green in NH_3 vapour, under a 366 nm UV lamp). Little, if any, free ferulic acid was released by Driselase, confirming the absence of fer-esterase activity.

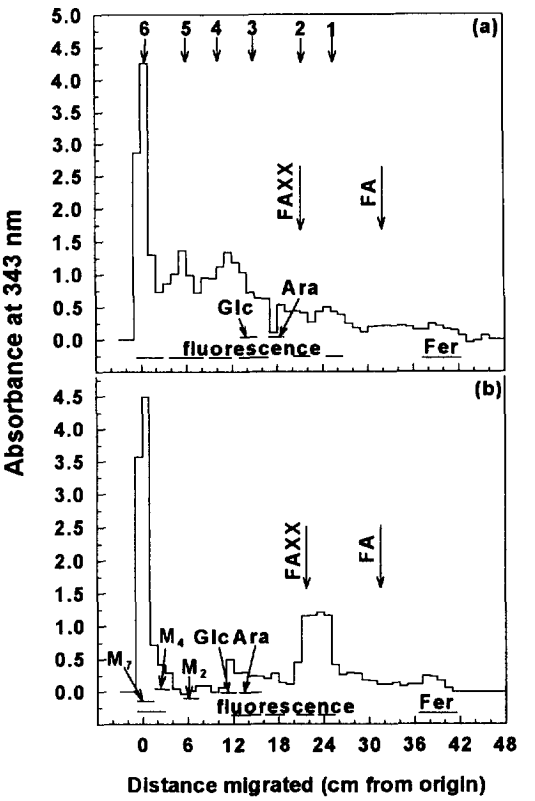

Fig. 1. PC in BAW of soluble Driselase-digestion products of AIR from *Festuca* (a) and *Zea* (b). Each loading contained the products of 36.4 mg AIR + 9.1 mg Driselase. Each strip of the chromatogram was treated with NaOH to hydrolyse *O*-feruloyl esters, and the A_{343} due to Na ferulate was measured. As compensation for Driselase autolysis products, the A_{343} from each corresponding strip of the control chromatogram (loaded with 9.1 mg Driselase only; data not shown) has been subtracted. Fer, Ara, Glc, maltose (M_2) and maltotetraose and -heptaose (M_4, M_7) were external markers. The approximate R_f values of Fer-Ara (FA) and Fer-Ara-Xyl-Xyl (FAXX) are marked (↓).

The distribution of A_{343}-material along the PC indicated that about 72% (*Festuca*) or 56% (*Zea*) of the Driselase-solubilised fer-material was oligomeric ($R_{Ara} > 0.15$) rather than polymeric (remaining at or

near the origin). However, there was a clear difference between the species in the pattern of oligomeric material.

There were two major fluorescent digestion products of *Zea* AIR, clearly resolved on visual inspection of the PC but included together in a single broad peak of UV-absorbing material in the histogram [Fig. 1(b)]. The slower-migrating of these two products was presumably the well-characterised [8, 9] fer-trisaccharide, Fer-Ara-Xyl-Xyl [Fig. 1(b)]. The faster-migrating product may have been a fer-disaccharide, perhaps related to the structure, 5-*O*-fer-β-L-Ara*f*-(1 → 2)-D-Xyl, proposed by Smith and Hartley [7], but later doubted [27].

In *Festuca*, in contrast, only small amounts of Fer-Ara-Xyl-Xyl were formed; the major feruloylated products had lower R_f values, suggesting that they possessed larger carbohydrate moieties [Fig. 1(a)]. The *Festuca* products were therefore investigated in more detail.

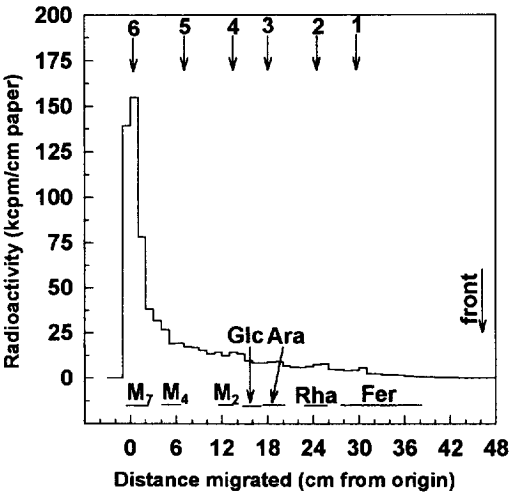

Fig. 2. PC in BEW of the aromatic products of Driselase digestion of ^{3}H-labelled *Festuca* AIR. Six zones with the fluorescence properties of *O*-feruloyl esters (**1**–**6**) were located. The arrows (↓) indicate the centre of each zone. M_7, M_4, M_2 (see Fig. 1), Glc, Ara, Rha and Fer were external markers.

Partial characterisation of the smallest feruloylated oligosaccharides from Festuca *AIR*

It was expected that Driselase would cleave the β-(1 → 4)-D-xylan backbone of arabinoxylan, but would not attack the fer-Ara*f* residue. A common core unit expected in Driselase digestion products was thus Fer-Ara-Xyl-Xyl. The large *Festuca* products could *a priori* include a larger portion of the xylan backbone than β-D-Xyl*p*-(1 → 4)-D-Xyl (Xyl-Xyl), or the backbone fragment could be multiply substituted, or additional sugar residues could be attached to the Ara*f* residue.

To facilitate analysis of the fer-oligosaccharides, we isolated them in radioactive form. Driselase digestion products of (*pentosyl*-^{3}H)-labelled *Festuca* AIR, after removal of [^{3}H]arabinose and [^{3}H]xylobiose by low pressure reversed-phase chromatography, were resolved into six fluorescent fer-ester zones (**1**–**6**) by PC in butanol, ethanol and water (Fig. 2). Each zone was associated with ^{3}H.

Complete acid hydrolysis of the three highest-R_f ^{3}H-labelled fer-ester zones (**1**–**3**) yielded [^{3}H]xylose and [^{3}H]arabinose [Fig. 3(a–c)]. The molar ratios Xyl:Ara (corrected for specific radioactivity [24]) for fractions **1**, **2** nd **3** were 1.05:1, 2.32:1 and 3.06:1, respectively. Reduction of fraction **2** followed by acid hydrolysis [Fig. 3(d)] gave xylose, xylitol and arabinose in the corrected molar ratio of 1.04:0.90:1, indicating a feruloylated trisaccharide (Fer · Ara · Xyl_2) with xylose as the reducing terminus.

Mild acid hydrolysis was used to cleave selectively the link between the Ara*f* residue and the fragment of the xylan backbone. Mild acid hydrolysis, under the conditions described, cleaved ~ 100% of Ara*f* bonds, ~ 60% of Xyl*p* bonds and ~ 10% of *O*-fer-ester bonds [24, 25]. Mild acid treatment of fraction **1** yielded ^{3}H-Fer-Ara and ^{3}H-monosaccharide(s) (xylose and/or arabinose, not resolved in BAW), in agreement with it being a fer-disaccharide (Fer · Ara · Xyl). The major ^{3}H-products of mild acid hydrolysis of fraction **2** were Fer-Ara, Xyl-(Fer)-Ara, xylobiose and monosaccharide(s); this is compatible only with fraction **2** being a mixture, possibly of Fer-Ara-Xyl-Xyl and β-D-Xyl*p*-(1 → 2)-(5-*O*-feruloyl)-α-L-Ara*f*-(1 → 3)-D-Xyl [Xyl-(Fer)-Ara-Xyl], both of which have the constitution Fer · Ara · Xyl_2 with Xyl as the reducing terminus.

Compound **3**, *a novel feruloylated pentasaccharide from* Festuca *AIR*

Compound **3** was present in larger amounts and could therefore be characterised in more detail. Mild acid hydrolysis of **3** gave radioactive products (Fig. 4) that co-migrated with Fer-Ara, Xyl-(Fer)-Ara, a fer-trisaccharide {co-migrating with β-D-Xyl*p*-(1 → 3)-β-D-Xyl*p*-(1 → 2)-(5-*O*-feruloyl)-L-Ara [Xyl-Xyl-(Fer)-Ara] [25]}, Xyl-Xyl, a putative trisaccharide and the monosaccharide(s). This fragmentation pattern is not compatible with **3** being the feruloylated tetrasaccharide, Xyl-(Fer)-Ara-Xyl-Xyl, which was found in enzymic digests of *Cynodon dactylon* polysaccharide [21]. Mild acid hydrolysis of Xyl-(Fer)-Ara-Xyl-Xyl would not give detectable yields of a trisaccharide or fer-trisaccharide since cleavage of the Ara*f* linkage, which occurs at ~ 100% efficiency, would generate only Xyl-Xyl, Xyl-(Fer)-Ara and smaller products. We conclude that compound **3** was a fer-pentasaccharide (Fer · Xylp_3 · Araf_1 · Unk_1), in which two of the xylose units were present as Xyl-Xyl and in which the Ara*f* residue acted as a readily-hydrolysed bridge between the Xyl-Xyl and the rest of the molecule; the Unk (unknown) residue was non-radioactive and therefore not L-Ara or D-Xyl. Mild acid hydrolysis of a

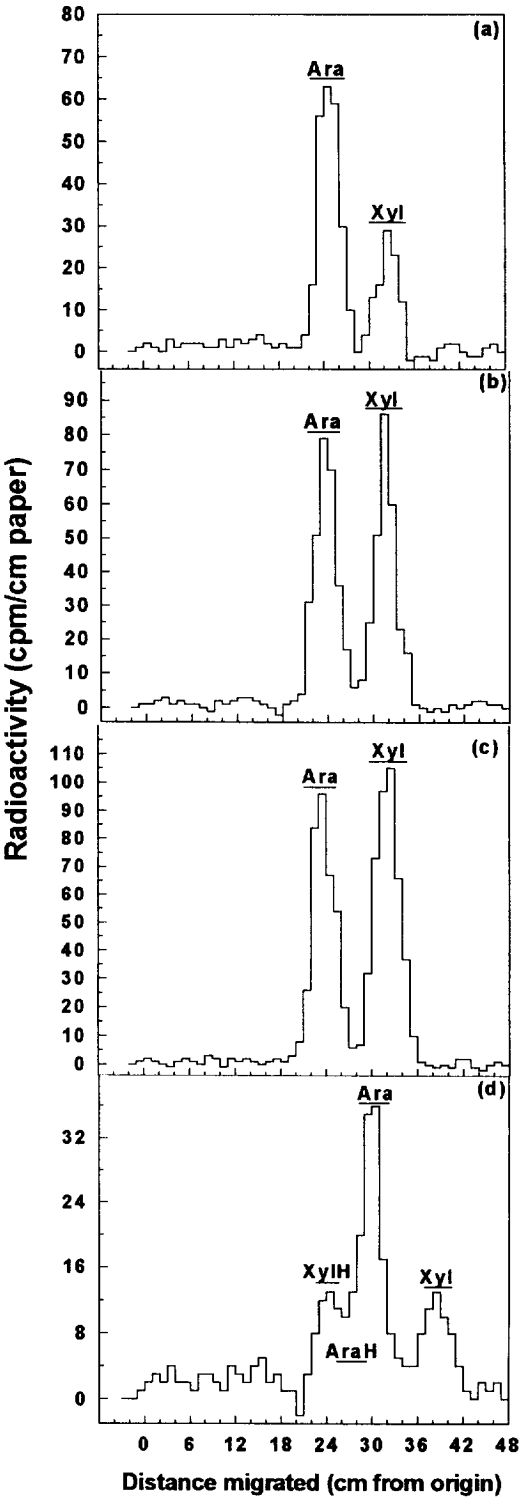

Fig. 3. PC in EPW of complete acid hydrolysis products of (*pentosyl*-1-^{3}H)-labelled feruloyl esters. (a) Fraction **1**; (b) fraction **2**; (c) fraction **3**; (d) $NaBH_4$-reduced fraction **2**. Ara and Xyl were present as internal markers; arabinitol (AraH) and xylitol (XylH) were external markers.

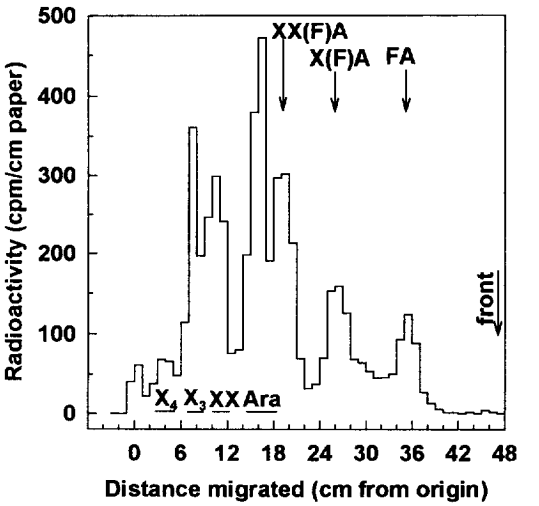

Fig. 4. PC in BAW of mild acid hydrolysis products of (*pentosyl*-1-^{3}H)-labelled compound **3**. Ara, Xyl-Xyl, xylotriose (X_3) and xylotetraose (X_4) served as external markers. Fer-Ara (FA). Xyl-(Fer)-Ara [X(F)A], and Xyl-Xyl-(Fer)-Ara [XX(F)A] show the positions of these compounds predicted from their known R_{Ara} values [24, 25].

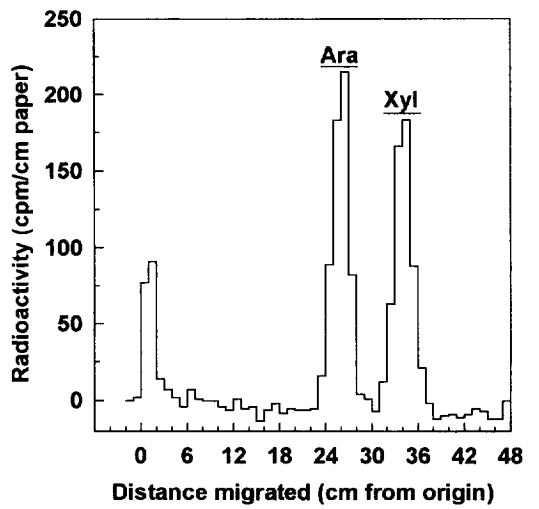

Fig. 5. PC in EPW of Smith degradation products of (*pentosyl*-1-^{3}H)-labelled compound **3**. Arabinose and xylose were internal markers.

fer-pentasaccharide (Fer·Xylp_3·Araf_1·Unk$_1$) could, depending on the inter-sugar linkages, efficiently generate Xyl-Xyl and a fer-trisaccharide (Fer·Xylp_1·Araf_1·Unk$_1$), as observed. Also in agreement with compound **3** being a fer-pentasaccharide, it was slightly less mobile on PC in BAW (R_{Ara} 0.88) than was a feruloylated, acetylated pentasaccharide (compound **4** of ref. [25]; R_{Ara} 0.95).

Smith degradation of (*pentosyl*-1-^{3}H)-labelled compound **3** gave [^{3}H]xylose and [^{3}H]arabinose in the molar ratio of 2.28:1 (Fig. 5). The reducing terminal, 4-linked xylose group of the Xyl-Xyl moiety would, if unsubstituted or if substituted at *O*-3, give [^{3}H]formic acid, which would be lost on PC; if substituted at *O*-2, it would give [^{3}H]xylitol. No [^{3}H]xylitol was observed

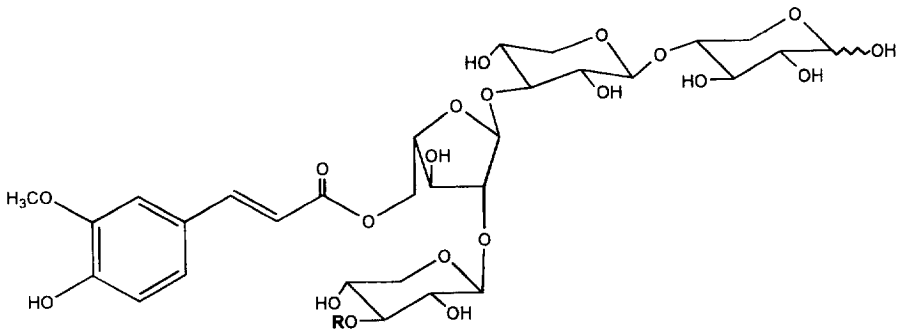

Fig. 6. Deduced partial structure of compound **3**, the smallest major feruloyl ester released by Driselase digestion of *Festuca* cell walls. **R** is a non-pentose residue.

(Fig. 5). For both the non-reducing Xyl*p* residues to be resistant to $NaIO_4$, each would have to be substituted either singly at *O*-3 or doubly. Since the Ara*f* residue was also $NaIO_4$-resistant, it too must be substituted (at *O*-2 and/or *O*-3). The only way for a pentasaccharide to meet all these criteria, and to give a fer-trisaccharide plus Xyl-Xyl on mild acid hydrolysis, is for it to be unbranched, with the Ara*f* residue in the middle and with each non-reducing Xyl*p* residue singly substituted at *O*-3:

Unk-(1 → 3)-Xyl*p*-(1 → 2/3)-Ara*f*-

(1 → 3)-Xyl*p*-(1 → 4)-Xyl.

The labelling of the pentose residues by exogenous [^{3}H]arabinose indicates that they were L-arabinose and D-xylose [28]. We have previously shown that a β-D-Xyl*p* residue is frequently linked to *O*-2 of an Fer-Ara group [24]. The linkage of L-Ara*f* in arabinoxylans has, with one exception [7], been reported to be α [9, 27, 29–31]; we therefore propose that the pentasaccharide core of compound **3** is:

Unk-(1 → 3)-β-D-Xyl*p*-(1 → 2)-α-L-Ara*f*-

(1 → 3)-β-D-Xyl*p*-(1 → 4)-D-Xyl.

The feruloyl group of compound **3** is presumably linked to the Ara*f* residue since one of the products obtained on mild acid hydrolysis co-migrated with authentic Fer-Ara. Thus, the proposed structure of compound **3** is as shown in Fig. 6.

The best known non-pentose sugar in the position shown for 'Unk' is β-D-Gal*p* [1, 30]. However, Driselase usually hydrolyses β-D-galactopyranosides [2, 5, 6]. Another possibility is that Unk is L-galactose: the sequence L-Gal*p*-(1 → 4)-D-Xyl*p*-(1 → 2)-L-Ara has been reported from *Zea* arabinoxylan [32] and Driselase lacks L-galactosidase activity [33].

DISCUSSION

Driselase is a mixture of fungal glycanases and is highly effective in digesting non-lignified plant cell walls to give mainly mono- and disaccharides, often in very high yield [2]. Driselase lacks fer-esterase activity, and generally releases much of the ferulate from graminaceous cell walls (e.g. *Zea*) in the form of a fer-trisaccharide (Fer-Ara-Xyl-Xyl) [8, 9, 27] and possibly also a fer-disaccharide [7]. However, Driselase failed to give high yields of fer- di- or trisaccharides from the arabinoxylan of *Festuca* cells. This suggested that the *Festuca* polysaccharide was unusually resistant to enzymic hydrolysis. Possible reasons included (a) a very high degree of feruloylation (and/or tight clustering of feruloyl groups), (b) a high degree of oxidative coupling to form diferulate and related cross-links, and (c) unusual inter-sugar linkages in the polysaccharide.

The present work showed that *Festuca* AIR was not particularly rich in ferulate, containing less than *Zea*, and that Driselase solubilised the fer-esters from *Festuca* AIR as effectively as from *Zea* (~79% of the total). Furthermore, a slightly higher proportion (72%) of the feruloylated fragments solubilised from *Festuca* walls were small enough to migrate on PC than of those from *Zea* (56%). Thus, the feruloylated polysaccharide of *Festuca* was not resistant to enzymic digestion.

To investigate the chemical nature of the major feruloylated fragments solubilised from *Festuca* AIR, we focused on compound **3**, the smallest such product obtained in useful yields. This was a fer-pentasaccharide, with the partial structure shown in Fig. 6.

Our results show that although *Festuca* arabinoxylan has complex oligosaccharide structures as feruloylated side-chains, these structures do not confer appreciable resistance to enzymic hydrolysis of the polysaccharide backbone. It is therefore interesting to consider alternative roles for the complex feruloylated side-chains. We suggest that they might (a) act as oligosaccharins (biologically-active oligosaccharides), with a role in intercellular signalling [34], or (b) direct the rate and detailed chemistry of the cross-linking of feruloyl residues, either by enzyme-catalysed oxidative coupling [13, 16] or by non-enzymic photo-dimerisation [14].

EXPERIMENTAL

Chemicals. Fer-Ara and Fer-Ara-Xyl-Xyl were obtained by hydrolysis of *Zea* AIR with mild acid [2]

and Driselase [9], respectively, followed by preparative PC in BAW. Xyl-(Fer)-Ara [24] and Xyl-Xyl-(Fer)-Ara [25] were prepd as before. L-[1-^{3}H]Arabinose (97 MBq μmol^{-1}) was from Amersham.

Plant material. Cell suspension cultures of tall fescue grass (*Festuca arundinacea* Schreber) were maintained as before [24]. For radiolabelling, a 4-day-old culture (100 ml) was supplied with 47 MBq of L-[1-^{3}H]arabinose for 5 hr. For prepn of AIR, the cells were packed into a column and washed for a least 24 hr with slowly-flowing 80% EtOH.

Driselase digestion of AIR. Driselase was de-salted as described [2, 5]. AIR (200 mg) was incubated with 50 mg of de-salted Driselase in 10 ml of buffer (pyridine–HOAc–1,1,1-trichloro-2-methylpropan-2-ol–H_2O, 2:2:1:195, pH 4.7) at 25° for 48 hr with gentle shaking. The suspension was then bench-centrifuged, the pellet was washed with H_2O, and the washings were combined with the supernatant. As a control, 25 mg of Driselase was incubated in 5 ml of the buffer for 48 hr.

For qualitative analysis, 2.00 ml of the AIR–Driselase-digest ($\equiv$ 36.4 mg AIR + 9.1 mg Driselase) or 1.82 ml of the Driselase-only soln ($\equiv$ 9.1 mg Driselase) was mixed with 0.5 ml HCO_2H to inactivate the Driselase, then applied as a 37-cm streak to Whatman 3MM for PC in BAW.

Saponification and assay of ferulate. Samples were suspended in 0.5 M NaOH and incubated at 25° for 1 hr to hydrolyse *O*-feruloyl esters; the A_{343} due to Na ferulate was then measured (ε_{343} = 23 500 M^{-1} cm^{-1} [5]).

For the assay of ferulate on PCs, the paper was cut into 1-cm strips, each of which was treated with 0.5 M NaOH as above. To compensate for Driselase-autolysis products, the A_{343} of each corresponding strip of a control PC (loaded with Driselase only) was subtracted.

Acid hydrolysis. Mild acid hydrolysis, to hydrolyse preferentially the furanosidic bonds, was performed with 0.1 M TFA (50–100 μl mg^{-1} dry wt) at 100° for 1 hr. 'Complete' acid hydrolysis was with 2 M TFA at 120° for 1 hr in a sealed glass tube.

Paper chromatography. PC was performed on Whatman 3MM paper by the descending method in the solvents BAW [*n*-BuOH–HOAc–H_2O (12:3:5 by vol.)], BEW [*n*-BuOH–EtOH–H_2O (20:5:11 by vol.)] and EPW (EtOAc–pyridine–H_2O (8:2:1 by vol.)].

Low-pressure reversed-phase chromatography. This was performed on 100 mg columns of C_{18}-substituted silica (BondElut, Varian, Analytichem, Harbor City, CA) that had been pre-treated with 3 ml MeOH followed by 3 ml H_2O. Aq. solns were passed through the column and rinsed with 5 ml H_2O to remove simple sugars; the retained feruloyl sugars were then eluted with 50% MeOH (10 ml) and dried *in vacuo*.

$NaBH_4$ *Reduction.* Samples were reduced with 0.5 M $NaBH_4$ in 1 M NH_4OH (two 0.2 ml portions added at 12 hr intervals) at 25°, acidified with HOAc, treated with Dowex 50, freed of H_3BO_3 [24], re-dissolved in H_2O, and subjected to complete acid hydrolysis.

Smith degradation. Dried compound **3** was incubated in 0.1 ml of 50 mM $NaIO_4$ in 0.25 M formate (Na^+) buffer, pH 3.7, for 6 days at 4° in the dark. Ethane-1,2-diol (20 μl) was then added and incubated for a further 1 hr to destroy excess $NaIO_4$. Oxidation products were reduced with $NaBH_4$, as above, re-dissolved in H_2O, passed through a 3-ml column of Dowex 1 (OAc^- form) to bind iodate, eluted with 8 ml H_2O and dried. The product was finally treated with 2 M TFA at 100° for 10 min.

Assay of radioactivity. Strips of chromatography paper were assayed for radioactivity as before [24]. The sp. act. of the [^{3}H]arabinose and [^{3}H]xylose residues in AIR was determined as in ref. [24].

Acknowledgements—We thank the EC for funding this work (contract AIR2-CT93-1661).

REFERENCES

1. McNeil, M., Darvill, A. G., Fry, S. C. and Albersheim, P., *Annual Review of Biochemistry*, 1984, **53**, 625.
2. Fry, S. C., *The Growing Plant Cell Wall: Chemical and Metabolic Analysis*. Longman, Harlow, 1988.
3. Brett, C. T. and Waldron, K., *Physiology and Biochemistry of Plant Cell Walls*, 2nd edn. Chapman and Hall, London, 1996.
4. Carpita, N. C. and Gibeaut, D. M., *Plant Journal*, 1993, **3**, 1.
5. Fry, S. C., *Biochemical Journal*, 1982, **203**, 493.
6. Fry, S. C., *Planta* 1983, **157**, 111.
7. Smith, M. M. and Hartley, R. D., *Carbohydrate Research*, 1983, **118**, 65.
8. Kato, A. Azuma, J. and Koshijima, T., *Chemistry Letters*, 1983, 137.
9. Kato, Y. and Nevins, D. J., *Carbohydrate Research*, 1985, **137**, 139.
10. Ahluwalia, B. and Fry, S. C., *Journal of Cereal Science*, 1986, **4**, 287.
11. Ishii, T., *Phytochemistry*, 1991, **30**, 2317.
12. Markwalder, H.-U. and Neukom, H., *Phytochemistry*, 1976, **15**, 836.
13. Ralph, J., Quideau, S., Grabber, J. H. and Hatfield, R. D., *Journal of the Chemical Society, Perkin Transactions I*, 1994, 3485.
14. Morrison, W. H., Hartley, R. D. and Himmelsbach, D. S., *Journal of Agricultural and Food Chemistry*, 1992, **40**, 768.
15. Fry, S. C., *Planta*, 1979, **146**, 343.
16. Fry, S. C., *Annual Review of Plant Physiology* 1986, **37**, 165.
17. Kato, Y., Tamanouchi, H., Hinata, K., Ohsumi, C. and Hayashi, T., *Plant Physiology*, 1994, **104**, 147.
18. Jung, H.-J. G. and Sahlu, T., *Journal of the Science of Food and Agriculture*, 1986, **37**, 659.

19. Fry, S. C., *Phytochemistry*, 1984, **23**, 59.
20. Buchanan, C. J., Wallace, G., Fry, S. C. and Eastwood, M. A., *Journal of the Science of Food and Agriculture*, 1996, **71**, 459.
21. Himmelsbach, D. S., Hartley, R. D., Borneman, W. S., Poppe, L. and van Halbeek, H., *Magnetic Resonance Chemistry*, 1994, **32**, 158.
22. Myton, K. E. and Fry, S. C., *Planta* 1994, **193**, 326.
23. Saulnier, L., Vigouroux, J. and Thibault, J.-F., *Carbohydrate Research*, 1995, **272**, 241.
24. Wende, G. and Fry, S. C. *Phytochemistry*, 1997, **44**, 1019.
25. Wende, G. and Fry, S. C. *Phytochemistry*, 1997, **44**, 1011.
26. Wende, G., Buchanan, C. J. and Fry, S. C. *Journal of the Science of Food and Agriculture* (in press).
27. Mueller-Harvey, I., Hartley, R. D., Harris, P. J. and Curzon, E. H., *Carbohydrate Research*, 1986, **148**, 71.
28. Fry, S. C. and Northcote, D. H., *Plant Physiology*, 1983, **73**, 1055.
29. Shibuya, N. and Misaki, A., *Agricultural and Biological Chemistry*, 1978, **42**, 2267.
30. Wilkie, K. C. B., *Advances in Carbohydrate Chemistry and Biochemistry*, 1979, **36**, 215.
31. Wood, T. M. and McCrae, S. I., *Carbohydrate Research*, 1986, **148**, 321.
32. Whistler, R. L. and Corbett, W. M., *Journal of the American Chemical Society*, 1995, **77**, 6328.
33. Baydoun, E. A.-H. and Fry, S. C., *Journal of Plant Physiology*, 1988, **132**, 484.
34. Ishii, T. and Saka, H., *Plant and Cell Physiology*, 1992, **33**, 321.