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Cell Wall Esterified Phenolic Dimers: Identification and Quantification by Reverse Phase High Performance Liquid Chromatography and Diode Array Detection

Keith W. Waldron,†* Adrian J. Parr,† Annie Ng† and John Ralph‡

† Department of Food Biophysics, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK
‡ US Dairy Forage Research Center, Agricultural Research Service, US Department of Agriculture, 1925 Linden Drive West, Madison, Wisconsin 53706, USA

An optimized high performance liquid chromatography (HPLC) procedure has been developed for the analysis and quantification of all of the known ferulic acid dehydrodimers, and the principle phenolic aldehydes and acids, found in the cell walls of higher plants. The HPLC system uses an ODS2 reverse phase column (5 μm particle size) eluted with a methanol, acetonitrile and water gradient with detection at 280 nm. In addition to providing baseline resolution of most components, the method employs a spectrometric detector which enables the precise identification of eluted components through the analysis of their spectral properties. Analysis of the cell wall phenolics of wheat straw stem (*Triticum vulgare*) was carried out using this method which is highly versatile and, for certain components, more sensitive than the current gas chromatography–mass spectrometry methodology.

Keywords: High performance liquid chromatography; diode array detection; cell walls; phenolics; dehydrodimers; ferulic acid.

INTRODUCTION

Plant cell walls contain a range of alkali labile phenolic moieties. These components, particularly *trans*-ferulic acid (*trans*-FA), are found in significant quantities in monocotyledonous plants of the order Commelinales (e.g. grasses) and dicotyledonous plants of the order Caryophyllales (e.g. beets). In grasses, FA is esterified to cell wall arabinoxylans at the C-5 position of arabinose (Ara*f*) residues (Hartley and Ford, 1989; Jung and Deetz, 1993; Ralph and Helm, 1993; Yamamoto *et al.*, 1989). In sugar beet, FA is esterified to the neutral sugar side chains of the pectin polysaccharides, particularly the C-2 position of Ara*f* and C-6 position of galactose (Gal*p*; Colquhoun *et al.*, 1994; Ralet *et al.*, 1994).

Phenolic esters have attracted considerable interest due to the potential which they provide for peroxidase catalysed cross-linking of cell wall polysaccharides (Biggs and Fry, 1987; Brett and Waldron, 1996). Until recently, the only FA dehydrodimer to have been identified was 5,5′-diferulic acid (5,5′-DiFA; **1**). This was first isolated from cell walls of grass by saponification (Markwaulder and Neukom, 1976) and later shown to cross link arabinoxylans in bamboo (Ishii, 1991). Such cross linking provides a biochemical mechanism for controlling the mechanical properties of cell walls (Biggs and Fry, 1987; Parker and Waldron, 1995) either towards the end of, or after, the extension phase (Carpita and Gibeaut, 1993). This has been supported by the observation that light inhibition of *Oryza* and *Avena* coleoptile extension results in an increase in the cell wall content of FA and 5,5′-DiFA esterified to the hermicellulosic Ara*f* residues, and that this is correlated with an increase in stress relaxation parameters (Tan *et al.*, 1992; Miyamoto *et al.*, 1994). The high content of FA in the cell walls of grass stems has also been associated with a reduction in the ease of grass tissue digestibility in ruminants (Besle *et al.*, 1994).

Recent investigations (Ralph *et al.*, 1994) have led to the identification and characterization of a number of additional FA dehydrodimers in grass stems (compounds **1–6**). Furthermore, the yields of these compounds indicate that phenolic dehydrodimer cross linking of cell wall polysaccharides is much greater than was previously thought. Some of these new dimers have also been identified in the cell walls of other monocotyledons, including the parenchyma of Chinese water chestnut, in which they confer thermal stability of texture (Parker and Waldron, 1995; Parr *et al.*, 1996) and of dicotyledons such as sugar beet (Waldron *et al.*, 1996). Hence, there is considerable interest in rapid and accurate methods for their analysis.

To date, the analysis and quantification of the newly discovered FA dehydrodimers has relied on gas chromatography (GC) and GC coupled with mass spectrometry (MS) (Ralph *et al.*, 1994). The use of GC presents a number of difficulties, including the relatively low sensitivity of flame ionization detectors (FID), the instability of the derivatized (silylated) phenolic compounds, and the potential for further chemical modification of the dimers during derivitization (Ralph *et al.*, 1994). Reverse phase high performance liquid chromatography (HPLC) with ultra violet (UV) detection has been used successfully in the separation and determination of mainly monomeric phenolic acids and aldehydes in plant cell walls (e.g. Hartley, 1987). The identification of the different components by HPLC usually relies on the comparison of their retention times (R_t) with those of defined standards. This technique does not, however, discriminate between co-eluting UV-absorbing compounds.

* Author to whom correspondence should be addressed.

However, unlike GC methods, HPLC does not require derivatization in specialist solvents, and involves fewer steps.

The aim of the present work has been to develop a reverse phase HPLC method for the separation, unambiguous identification and quantification of FA dehydrodimers, monomeric cell wall phenolic acids and aldehydes. This approach exploits the diagnostic UV absorbance spectra of the phenolic acids of interest with the use of a diode array spectometric detector (DAD).

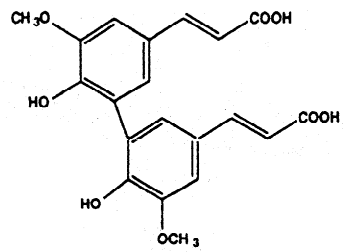
EXPERIMENTAL

Preparation of standards. 5,5′-Diferulic acid (**1**) ((E,E)-4, 4′-dihydroxy-5,5′-dimethoxy-3′-bicinnamic acid; 5,5′-DiFA)—the method of Baumgartner and Neukom (1972) was employed to synthesize 5,5′-dehydrodivanillin from vanillin. This was then converted into 5,5′-DiFA (**1**) by reaction for several hours at mildly elevated temperatures with malonic acid in pyridine containing one drop of piperidine until all substrate had disappeared (confirmed by HPLC).

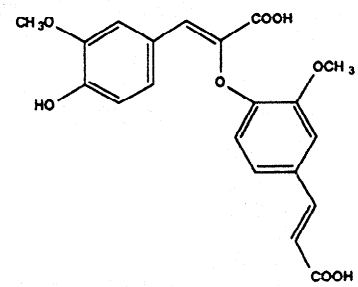
8-0-4′-Diferulic acid (**2**) ((Z)-β-(4-((E)-2-carboxyvinyl)-2-methoxyphenoxy)-4-hydroxy-3-methoxycinnamic acid; 8-0-4′-DiFA), 8,5′-diferulic acid open form (**3**) ((E,E)-4,4′dihydroxy-3,5′-dimethoxy-β,3′-bicinammic acid; 8,5′-DiFA), 8,8′-diferulic acid open form (**5**) (4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid; 8,8′-DiFA), and 8,8′-diferulic acid aryltetralin form (**6**) (*trans*-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl) - 6 - methoxy - 1,2 - dihydronaphthalene - 2,3 - dicarboxylic acid)—were prepared by the method of Ralph *et al.* (1994).

8,5′-Diferulic acid benzofuran form (**4**) (*trans*-5-((E)-2-carboxyvinyl) - 2 - (4 - hydroxy - 3 - methoxyphenyl) - 7 - methoxy-2,3 - dihydrobenzofuran - 3 - carboxylic acid) — was prepared from ethyl ferulate using the method of Ralph *et al.* (1994). Alkaline hydrolysis of the diethyl 8,5′-DiFA produced as an intermediate generated several products, and **4** was identified by spectral similarity to the diethyl ester.

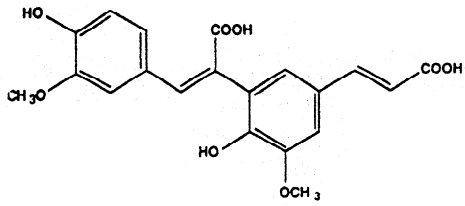
FA glucose ester and caffeic acid glucose esters were extracted from placental tissue of *Capsicum annuum* fruits (Winter and Herrman, 1986). *cis*-FA and *cis-p*-coumaric acid (CA) were prepared by dissolving known quantities of the pure *trans*- isomers and *trans*-cinnamic acid (internal standard; IS) in dilute ammonia and exposing to UV light (350 nm) for 3 h. The ammonia was removed by rotary evaporation and the samples dissolved in 50% (v/v) aqueous methanol prior to analysis by HPLC. A range of truxillic acids were prepared from *trans*-FA and *trans-p*-CA (Hartley *et al.*, 1990) by exposing thin layers of the *trans*- isomers to UV light (350 nm) for 30 min. The products were dissolved in 50% (v/v) aqueous methanol prior to analysis by HPLC. All other phenolic standards were of analytical reagent quality.



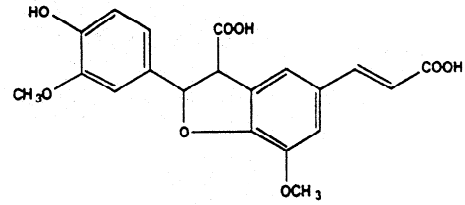
(1) 5,5'-Diferulic acid



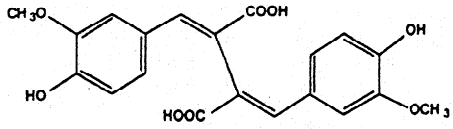
(2) 8-0-4'-Diferulic acid



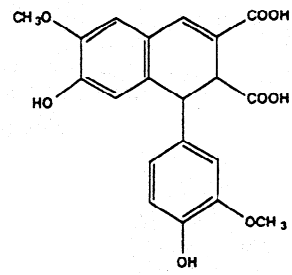
(3) 8,5'-Diferulic acid



(4) 8,5'-Diferulic acid benzofuran form



(5) 8,8'-Diferulic acid



(6) 8,8'-Diferulic acid aryltetralin form

Structures

Preparation of purified cell wall fractions. Wheat straw (*Triticum vulgare*) was freeze-milled in liquid nitrogen (10 s, speed 5) in a Spex freeze-mill (Spex Industries, Edison NJ, USA). The milled material (300 mg) was blended in 1.5% aqueous sodium lauryl sulphate containing 5 mM $Na_2S_2O_5$ with an Ystral homogenizer (Ystral GmbH, Dottingen, Germany) for 2 min. A few drops of octanol were added to reduce foaming. The homogenate was filtered through a 35 μm nylon mesh (John Stannier and Co., Manchester, UK), and the residue was washed exhaustively in water, filtering each time. The cell wall residue was confirmed to be free of starch granules by light microscopy and staining with iodine/potassium iodide. The cell wall material (CWM) was stored as a frozen suspension at −20 °C. Prior to extraction of cell wall phenolics, the CWM was further extracted with hot ethanol, to remove any alcohol soluble phenolics, washed three times with acetone and then air dried.

Extraction of wall bound ester linked phenolics. Wall bound phenolics were released by sequential alkaline hydrolysis of isolated cell wall material under progressively more vigorous conditions as described by Hartley and Morrison (1991). Cell wall material (30 mg) was extracted with 0.1 M NaOH for 1 h at 25 °C under nitrogen. The suspension was filtered through glass fibre paper, and the residue retained for futher extraction with, sequentially, 0.1 M NaOH for 24 h, 1 M NaOH for 24 h, and 2 M NaOH for 24 h, each at 25 °C. *trans*-Cinnamic acid (1.4 μg) was added to all filtrates as IS, and the solutions were then acidified to pH <2 with concentrated HCl and extracted three times with ethyl acetate (3 volumes). The phenolic ethyl acetate extracts were evaporated to dryness under a stream of nitrogen, and the samples redissolved in 200 μL 50% (v/v) aqueous methanol prior to analysis by HPLC. During the alkali stages of the extraction, care was taken to shield the samples from light.

Analysis of phenols by HPLC. Phenolic acid standards (monomers and dimers) were dissolved in 50% (v/v) aqueous methanol; *trans*-cinnamic acid was added to the mixtures as IS.

Phenolics were quantified by HPLC using an Inertpak ODS2 reverse phase column (25 cm × 5 mm i.d., 5 μm; Capital HPLC Ltd, Broxburn, West Lothian, UK). Elution was performed using a gradient system which increased the relative amounts of methanol and acetonitrile present in aqueous 1 mM trifluoroacetic acid (TFA). The optimized gradient profile for the separation of wall bound phenolic monomers and dimers was formed using solvent A (10% (v/v) aqueous acetonitrile plus TFA to 1 mM), solvent B (80% (v/v) aqueous methanol plus TFA to 1 mM), and solvent C (80% (v/v) aqueous acetonitrile plus TFA to 1 mM) in the following programme: initially A 90%, B 5%, C 5%; linear gradient over 25 min to A 25%, B 37%, C 37%; exponential gradient over 5 min to A0%, B 50%, C 50%; exponential gradient over 15 min to A 90%, B 5%, C 5%; held isocratically at A 90%, B 5%, C 5% for a further 10 min. The flow-rate was maintained at 1 mL/min; all three solvents were sparged with helium prior to use. Since solvents B and C were used in constant proportions, they may be pre-mixed if a binary gradient pump system is being employed.

Phenolics were detected using a Perkin Elmer Model 235C DAD. Quantitation was by integration of peak-areas at 280 nm, with reference to calibrations made using known amounts of pure compounds.

Unless otherwise stated, all chemicals were of far UV quality HPLC grade purity where available.

RESULTS AND DISCUSSION

Separation of standards by reverse phase HPLC

Phenolic monomers (commercially available and previously found in plant cell walls), dehydrodimers, truxillic acid type cyclodimers and selected phenolic sugar esters were applied to the HPLC column and eluted with an acetonitrile and/or methanol gradient. Initial attempts to separate the phenolic monomers and dehydrodimers involved a simple methanol gradient. However, whilst separation of the early eluting

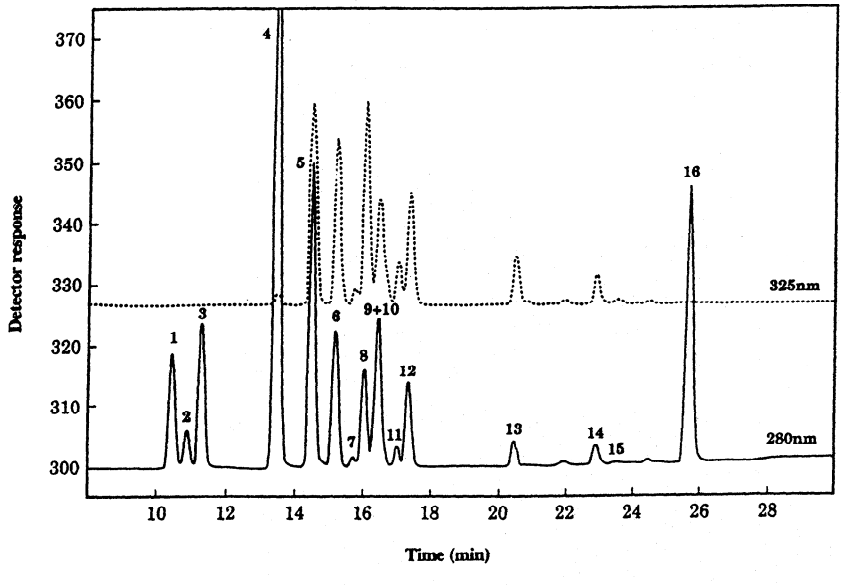


Figure 1. HPLC elution profile of selected phenolic standards with detection at 280 nm (——) and at 325 nm (- - -). Key to peak identity: **1**, *p-OH*-benzoic acid; **2**, *p-OH*-phenylacetic acid; **3**, vanillic acid; **4**, *p-OH*-benzaldehyde; **5**, vanillin; **6**, *trans-p*-coumaric acid; **7**, 8,8′-DiFA aryltetralin form; **8**, *trans*-ferulic acid, **9+10**, *cis-p*-coumaric acid+8,8′-DiFA; **11**, 8,5′-DiFA; **12** *cis*-ferulic acid; **13**, 5,5′-DiFA; **14**, 8-0-4′-DiFA; **15**, 8,5′-DiFA benzofuran form; **16**, *trans*-cinnamic acid. (For chromatographic protocol see Experimental section.)

Table 1. Diagnostic absorption wavelengths of peaks (λ_{max}) and of troughs (λ_{min}), response factors (RF) in relation to *trans*-cinnamic acid, and retention times (R_t) of phenolic monomers, dimers, selected glycosides and truxillic acids

Compound	λ_{max} (nm)	λ_{min} (nm)	RF	R_t (min)
p-OH-Benzaldehyde	220, 284	239	0.82	13.4
p-OH-Benzoic acid	255	225	0.18	10.4
trans-Cinnamic acid	276.5	232	1.00	25.7
o-Coumaric acid	276, 326	245.3, 305	–	15.2
trans-p-Coumaric acid	310	249	0.65	15.2
cis-p-Coumaric acid	298	246	0.54	16.4
trans-Ferulic acid	324.6	262	0.36	16.0
cis-Ferulic acid	313	259.7	0.32	17.3
Caffeic acid glucose ester	218, 236, 331	230, 256.5	–	5.9
Ferulic-acid glucose ester	217, 239, 331	228, 265	–	9.4
p-OH-Phenylacetic acid	276.1	251.3	0.18	10.9
Protocatechuic acid	260, 295.4	236, 281	0.22	6.8
Protocatechuic aldehyde	280.5, 311.7	250.2, 299.8	0.60	9.5
FA truxillic acid[a]	230, 282	257	–	13.9
CA truxillic acid[a]	226, 279	254.7	–	13.8
Vanillic acid	260.7, 292.8	236, 281	0.23	11.3
Vanillin	280, 310	249, 296	0.5	14.5
Divanillin	233.6, 292.8	267	–	22.5
5,5′-DiFA (1)	245.3, 324.8	272.8	0.21	20.5
8-0-4′-DiFA (2)	234.5, 326.7	260.2	0.14	22.9
8,5′-DiFA (3)	324	264.4	0.18	17.0
8,5′-DiFA benzofuran form (4)	229.2, 325.5	266.3	0.12	23.5
8,8′-DiFA (5)	326.9	263.5	0.17	16.4
8,8′-DiFA aryltetralin form (6)	337.2	278.4	0.04	15.7

[a] These are representative of the respective truxillic acid families (see text for details).

monomers was possible, separation of the more slowly eluting FA dehydrodimers either from each other, or from some of the late running monomers, was not. In contrast, an acetonitrile gradient enabled separation of the late running FA dehydrodimers, but not of the earlier eluting monomers. The use of a methanol:acetonitrile mixture in the primary solvents facilitated separation of all of the main monomer and dimer standards from each other except 8,8′-DiFA (5) and *cis*-coumaric acid which co-chromatographed (Fig. 1). The presence of both of the compounds can be assessed through analysis of the spectral properties (see below). Also, their separation may be facilitated by rerunning a modified gradient. Typical retention times of these and other phenolic compounds are shown in Table 1.

Spectral properties of phenolic compounds

The individual absorption spectra of the pure compounds were recorded under conditions pertaining to the HPLC analysis procedure (Figs 2 and 3). In all cases, the spectra were significantly different, as confirmed by the differences in the absorbance maxima (λ_{max}) and minima (λ_{min}) (Table 1). The spectra of all but one of the FA dehydrodimers exhibited ferulic acid-like profiles, with a broad, often double, peak between 280 and 340 nm. The exception was 8,8′-DiFA aryltetralin form (Fig. 3) in which the double peak had become reduced. The truxillic acid type cyclodimers of *trans*-FA and *trans*-CA had profoundly different spectra from their parent monomers and dehydrodimers, allowing the identification of this class of dimer in tissues which also contain the latter. Hence, the absorption profiles are diagnostic of the individual compounds. In conjunction with the separative methods above, the spectral data allow precise identification of cell wall derived phenolic aldehydes, acids and FA dimers.

Choice of solvents

The use of far UV-quality HPLC grade solvents in the procedure described provided a virtually flat baseline above 220 nm as demonstrated by a 3-dimensional elution profile of selected phenolic monomers (Fig. 4). Such a baseline allows the spectral characteristics of the eluted components to be assessed accurately. Spectral quality of phenolics extracted from cell walls, and the elution profile, was optimized by the use of HPLC grade NaOH and ethyl acetate in the saponification and partitioning procedures. Use of analytical reagent quality or lower quality chemicals resulted in the concentration of unidentified compounds in the final sample which absorbed in the UV region and gave spurious peaks.

Detection wavelength

Because even the highest purity methanol results in a considerable absorbance below 210 nm, the most useful area of the spectrum for component detection lies above 220 nm. In this study, 280 nm was chosen as the most effective wavelength for data capture, even though it was by no means the most efficient for all components. For example, the λ_{max} of many of the phenolics, particularly the FA-derived compounds, is approximately 325 nm. Data capture at such a wavelength will not detect *trans*-cinnamic acid (used here as IS), protocatechuic acid, *p*-hydroxyphenylacetic acid, *p*-hydroxybenzoic acid and vanillic acid

(Fig. 1). However, the differences in spectral properties can be advantageous. Component detection at different wavelengths permits discrimination between co-eluting compounds which exhibit significantly different absorption

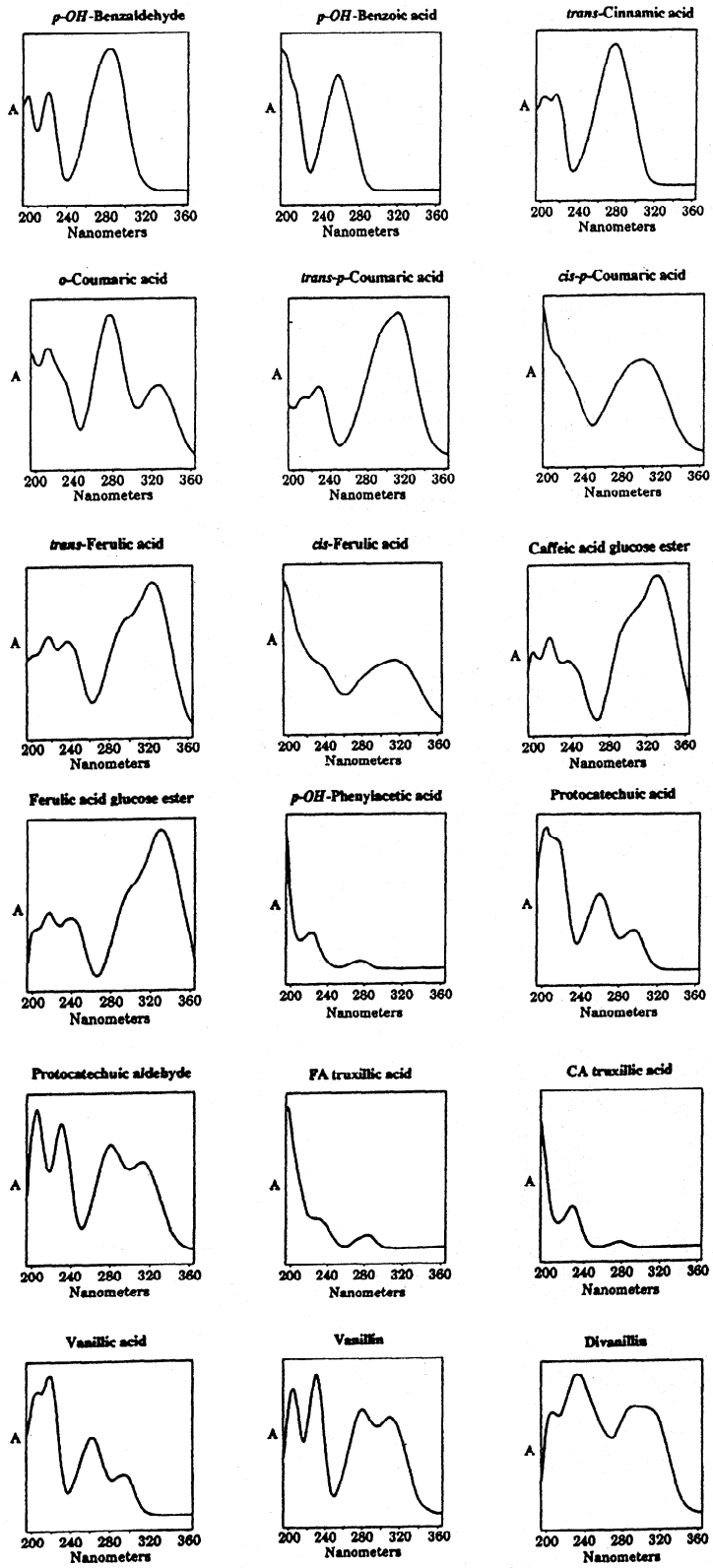


Figure 2. Absorption spectra of standard monomeric phenolics occurring in plant cell walls recorded under conditions pertaining to the HPLC analysis procedure described in the Experimental section.

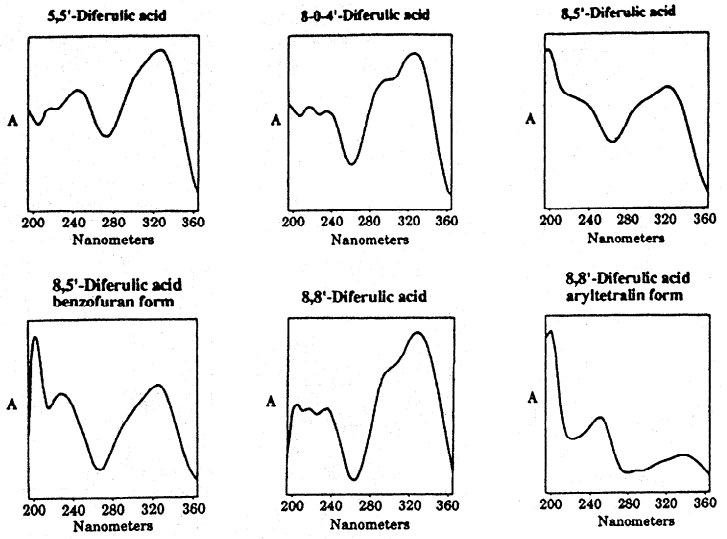


Figure 3. Absorption spectra of standard FA dehydrodimers occurring in plant cell walls recorded under conditions pertaining to the HPLC analysis procedure described in the Experimental section.

spectra. For example, *cis*-CA has a very low absorbance at 350 nm, whilst the co-eluting 8,8′–DiFA (**5**) absorbs at approximately 50% of its λ_{max} value.

Response factors (at 280 nm)

The response factors (RF) have been based on gravimetric quantitation of the very small quantities of virtually pure standards. These are presented in Table 1. The RF values of all the cell wall phenolics, in comparison with the IS (*trans*-cinnamic acid), are generally less than 0.4. This reflects the different absorption maxima of the compounds (Figs 2 and 3).

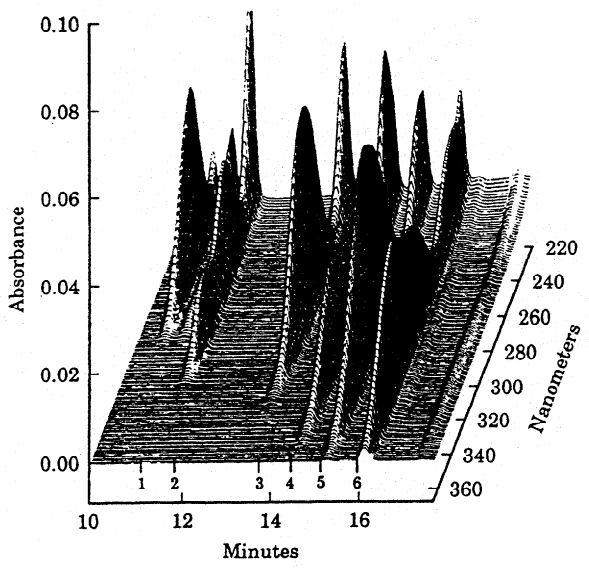


Figure 4. Three-dimensional elution profile of selected monomeric phenolics detected by DAD scanning between 220 and 360 nm. Key to component identity: **1**, *p-OH*-benzoic acid; **2**, vanillic acid; **3**, *p-OH*-benzaldehyde; **4**, vanillin; **5**, *trans-p*-coumaric acid; **6**, *trans*-ferulic acid. (For chromatographic protocol see Experimental section.)

Phenolic acids in the cell walls of wheat straw

As an example, the HPLC method has been applied to the quantification of phenolic esters from the cell walls of wheat straw. CWM was prepared from fresh, dry stem and gave a yield of 61% w/w. The CWM consisted mainly of carbohydrate (70%) which comprised the following sugars: Ara*f*, 25.8; xylose (Xyl*p*), 243.7; mannose (Man*p*), 1.7; Gal*p*, 3.6; glucose (Glc*p*), 353.9 and uronic acid, 74.6 μg/mg. The CWM was extracted for phenolic esters; in all of the extracts all but a few minor components in the HPLC profile were identified and quantified (Fig. 5). FA and CA were the major compounds and the majority of these were recovered in the 0.1 M NaOH (24 h) and 1.0 M NaOH (24 h) extracts. Only very small quantities of the *cis* forms were detected. The levels of FA and CA in the wheat stem (approximately 2 mg/g CWM) are of the same order as in barley straw stem (Hartley, 1987). Several of the newly discovered FA dehydrodimers were also detected for the first time in this tissue. The most prominent were 8-0-4′-DiFA (**2**) and 8,5′-DiFA benzofuran form (**4**). In contrast, a relatively small quantity of the commonly known 5,5′-DiFA (**1**) was detected, supporting the observations of Ralph *et al.* (1994) and Parr *et al.* (1996). The dimers comprised 25% of the total FA complement, indicating a high degree of cell wall cross linking. It is likely that arabinoxylan hemicelluloses are the main polymers cross linked (Brett and Waldron, 1996). Several small peaks with truxillic acid like spectra were detected, mainly in the 0.1 NaOH (1 h) extact. Since no characterized standards were available, precise identification was not possible. Significant quantities of substituted truxillic and truxinic acids have been reported in the cell walls of bermudagrass (Hartley *et al.*, 1990) and Italian ryegrass (Eraso and Hartley, 1990).

CONCLUSIONS

Cell wall esterified phenolic monomers and dimers are an exciting class of compounds, important in determining the mechanical properties and biodegradability of plant cell walls. Interest in these compounds has been enhanced by

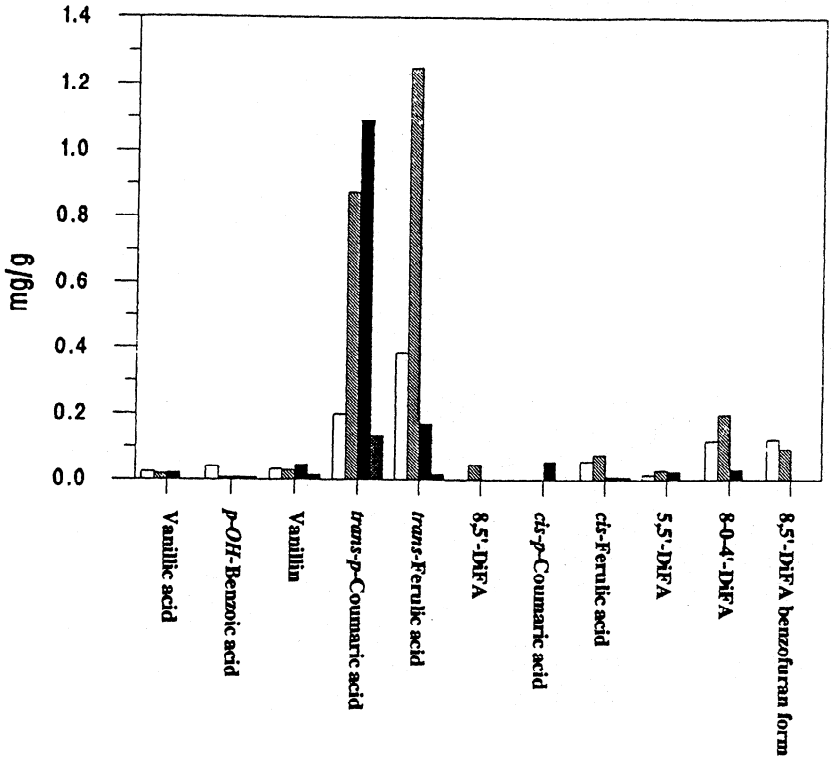


Figure 5. Phenolic acids released from the cell walls of wheat straw by saponification (as described in Experimental section) by: □ 0.1 M NaOH for 1 h; ▨ 0.1 M NaOH for 24 h; ■ 1 M NaOH for 24 h; ■ 2 M NaOH for 24 h.

the identification of a number of additional FA dehydrodimers in the cell walls of several monocotyledon and dicotyledon species. This paper reports the development of a quick and easy reverse phase HPLC method to separate FA dehydrodimers, phenolic acid and aldehyde monomers, and cyclodimers. By using a diode array detector, the method exploits the diagnostic spectral properties of the different compounds, thus ensuring their unambiguous identification; such precision is not usually possible with GC-MS without authentic standards, since different compounds may show the same fragment ions/similar fragmentation patterns.

The spectral properties potentially offer considerable information about the chemistry of unknown moieties, and may aid in their identification. The results above show that small changes in linkage are accompanied by changes in spectral properties. For example, virtually all of the dehydrodimers and the glucose ester of FA display spectra which exhibit subtle differences compared with that of *trans*-FA; cyclodimerization results in radical changes to the spectra. Clearly, further work using chemically synthesized compounds is likely to facilitate much greater interpretation of the spectra in relation to the chemical structures, enhancing the diagnostic capability of this approach.

Finally, this method is user friendly and relatively inexpensive, and lends itself to routine analysis.

Acknowledgements

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REFERENCES

Baumgartner, J. and Neukon, H. (1972). Enzymatische oxydation von vanillin. *Chimia* **26**, 366–368.

Besle, J-M., Cornu, A. and Jouany, J-P. (1994). Roles of structural phenylpropanoids in forage cell wall digestion. *J. Sci. Food Agric.* **64**, 171–190.

Brett, C. T. and Waldron, K. W. (1996). Cell walls in diet and health, In *Physiology and Biochemistry of Plant Cell Walls.* (Black, M. and Charlwood, B. V. Ser. Eds.) pp. 222–238. Chapman and Hall, London.

Biggs, K. J. and Fry, S. C. (1987). Phenolic cross-linking in the plant cell wall. In *Physiology of Expansion During Plant Growth: Proceedings of the Second Annual Pennsylvania State Symposium in Plant Physiology* (Cosgrove, D. J. and Knieval, D. P. eds), pp. 46–57. American Society of Plant Physiologists, Rockville, The Pennsylvania Sta. A.

Colquhoun, I. J., Ralet M-C., Thibault, J-F., Faulds, C. B. and Williamson, G. (1994). Structure identification of feruloylated oligosaccharides from sugar-beet pulp by NMR spectroscopy. *Carbohydr. Res.* **263**, 243–256.

Carpita, N. C. and Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* **3**, 1–30.

Eraso, F. and Hartley, R. D. (1990). Monomeric and dimeric phenolic constituents of plant cell walls—possible factors influencing wall biodegradability. *J. Sci. Food. Agric.* **51**, 163–170.

Hartley, R. D. (1987). HPLC for the separation and determination of phenolic compounds in plant cell walls. *Modern Methods Plant Anal.* **5**, 92–103.

Hartley, R. D. and Ford, C. W. (1989). Phenolic constituents of plant cell walls and biodegradability, In *Plant Cell Wall Polymers, Biogenesis and Biodegradation* (Lewis, N. G. and Paice, M. G., eds), pp. 137–145. American Chemical Society, Washington.

Hartley, R. D. and Morrison, W. H. (1991). Monomeric and dimeric phenolic acids released from cell walls of grasses by sequential treatment with sodium hydroxide. *J. Sci. Food Agric.* **55**, 365–375.

Hartley, R. D., Morrison, W. H., Balza, P. and Towers, G. H. N. (1990). Substituted truxillic and truxinic acids in cell walls of *Cynodon dactylon. Phytochemistry* **29**, 3699–3703.

Ishii, T. (1991). Isolation and characterisation of a diferuloyl arabinoxylan hexasaccharide from bamboo shoot cell walls. *Carbohydr. Res.* **219**, 15–22.

Jung, H. G. and Deetz, D. A. (1993). Cell wall lignification and degradability, In *Forage Cell Wall Structure and Digestibility* (Jung, H. G., Buxton, D. R., Hatafield, R. D. and Ralph, J., eds), pp. 315–346. ASA-CSSA-SSSA, Madison.

Markwalder, H. U. and Neukom. H. (1976). Diferulic acid as a possible cross-link in hemicelluloses from wheat germ. *Phytochemistry* **15**, 836–837.

Miyamoto, K., Ueda, J., Takeda, S., Ida, K., Hoson, T., Masuda, Y. and Kamisaka, S. (1994). Light-induced increase in the contents of ferulic and diferulic acids in cell walls of *Avena* coleoptiles—its relationship to growth inhibition by light. *Physiol. Plant.* **92**, 350–355.

Parker, M. L. and Waldron, K. W. (1995). Texture of Chinese water chestnut: inovlvement of cell wall phenolics. *J. Sci. Food Agric.* **68**, 337–346.

Parr, A. J., Waldron, K. W., Ng, A. and Parker, M. L. (1996). The wall-bound phenolics of Chinese water chestnut (*Eleocharis dulcis*, Cyperaceae). *J. Sci. Food Agric.* **71**, 501–507.

Ralet, M-C., Thibault, J-F., Faulds, C. B. and Williamson, G. (1994). Isolation and purification of feruloylated oligosaccharides from cell walls of sugar-beet pulp. *Carbohydr. Res.* **263**, 227–241.

Ralph, J. and Helm, R. F. (1993). The lignin-hydroxycinnamic acid–polysaccharide complex: synthetic models for regiochemical characterisation, In *Forage Cell Wall Structure and Digestibility* (Jung, H. G., Buxton, D. R., Hatfield, R. D. and Ralph, J., eds), pp. 201–246. ASA-CSSA-SSSA, Madison.

Ralph, J., Quideau, S., Grabber, J. H. and Hatfield, R. D. (1994). Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. *J. Chem. Soc. Perkin Trans-1*, 3485–3498.

Tan, K-S., Hoson, T., Masuda, Y. and Kamisaka, S. (1992). Involvement of cell wall-bound diferulic acid in light-induced decrease in growth rate and cell-wall extensibility of *Oryza* coleoptiles. *Plant Cell Physiol.* **33**, 103–108.

Waldron, K. W., Ng, A., Parker, M. L. and Parr, A. J. (1996). Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. *J. Sci. Food Agric.* (in press).

Winter, M. and Herrman, K. (1986). Esters and glucosides of hydroxycinnamic acids in vegetables. *J. Agric. Food Chem.* **34**, 616–620.

Yamamoto, E., Bokelman, G. H. and Lewis, N. G. (1989). Phenylpropanoid metabolism in cell walls: an overview, In *Plant Cell Wall Polymers, Biogenesis and Biodegradation* (Lewis, N. G. and Paice, M. G., eds), pp. 68–88. American Chemical Society, Washington.