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AROMATIC ALDEHYDE CONSTITUENTS OF GRAMINACEOUS CELL WALLS

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Key Word Index—*Zea mays*; *Hordeum vulgare*, *Triticum aestivum*; Gramineae; cell walls; *p*-hydroxybenzaldehyde; vanillin, syringaldehyde; lignin.

Abstract—*p*-Hydroxybenzaldehyde, vanillin and syringaldehyde were released as their sodium salts from graminaceous cell walls by treatment with sodium hydroxide. Treatment of the walls with 'cellulase' having both cellulase and hemicellulase activity released the aldehydes in bound form apparently linked at their phenolic groups to the wall polysaccharides. These findings are discussed in relation to tests for lignin using phloroglucinol-HCl and alkaline nitrobenzene reagents.

INTRODUCTION

Low molecular weight phenolic compounds including *trans*-ferulic, *trans*-*p*-coumaric and *trans*,*trans*-diferulic acids are linked to the polysaccharide constituents of graminaceous cell walls [1–7]. The amounts of phenolic compounds released from the walls of graminaceous forages by treatment with alkali, are positively correlated with increases in the amounts of forages digested by ruminants [8, 9]. Forage digestibility is an important factor in determining rates of animal production. Phenolic constituents of cell walls might also influence the decomposition of organic matter in soil and act as growth inhibitors of plant pathogens [10]. We report here that *p*-hydroxybenzaldehyde, vanillin and syringaldehyde also occur in the cell walls of maize stem, barley stem and wheat bran and are apparently linked at their phenolic groups to polysaccharides.

RESULTS AND DISCUSSION

The yields of cell walls isolated from the maize stem, barley stem and wheat bran and their contents of carbon, hydrogen and nitrogen are shown in Table 1.

p-Hydroxybenzaldehyde, vanillin and syringaldehyde were isolated by treating the maize cell walls with NaOH, then separated by preparative HPLC. They were characterised by TLC, UV absorption spectroscopy and mass spectrometry (Table 2). Analytical HPLC of alkaline extracts of the barley walls indicated that the same aldehydes were present while only *p*-hydroxybenzaldehyde and vanillin were detected in similar extracts of the bran walls. Amounts of the aldehydes and of *p*-coumaric, ferulic and diferulic acids found (Table 3) represent the minimum bound to the walls as the alkaline treatment employed is unlikely to give quantitative recoveries. For example, if some of the phenolic compounds are ether-linked to sugar units of the wall polysaccharides, such linkages are likely to be resistant to alkaline hydrolysis.

When the maize, barley or bran walls were treated with a commercial 'cellulase' preparation and the water-soluble compounds separated by HPLC, none of the three aldehydes could be detected, indicating that they were not present in the free form. The aldehydes were released as their sodium salts from the water-soluble compounds by treatment with 1 M NaOH at 20°, and separation using HPLC. Hence the aldehydes are apparently linked at their phenolic groups to the wall polysaccharides, probably as phenolic glycosides or ethers. It is of interest that Hayashi [11] suggested that 'lignin carbohydrate complexes' from wheat straw contain phenolic glycoside linkages.

p-Hydroxybenzaldehyde and vanillin, which arise from treatment of the walls with sodium hydroxide, are not artefacts that arise by oxidation of the *p*-coumaric and ferulic acid constituents. Submission of similar amounts of the acids (and ten times the amounts) to those present in the walls to the same isolation and analytical procedures failed to produce any of the aldehydes. It is noteworthy that sinapic acid, from which syringaldehyde might be produced by oxidation, has not been detected in graminaceous cell walls [12].

The findings of the three aldehydes attached to plant cell walls raise doubt about two commonly used tests for lignin which has been defined as a polymeric natural product arising from an enzyme-initiated dehydrogenative polymerisation of the *trans* isomers of *p*-coumaryl, coniferyl and sinapyl alcohols [13]. First, the phloroglucinol-HCl histochemical test gives positive colour reactions with the three aldehydes and with their corresponding methyl ethers [14, 15]. Hence this test may give a positive reaction in the absence of lignin. Secondly, the production of any of the three aldehydes by alkaline nitrobenzene oxidation of plant cell walls at high temperature and pressure, is often used as an indicator for lignin but, under these conditions, at least some of the aldehydes linked to wall polysaccharides would be released. Moreover, oxidation of the *p*-coumaric and ferulic constituents of cell walls could also lead to the production of *p*-hydroxybenzaldehyde and vanillin.

The cell walls of other graminaceous plant parts

Table 1. Cell wall contents of maize stem, barley stem and wheat bran and elemental analysis of their walls (mean of six analyses)

	Cell walls (% dry matter)	Elemental analysis of cell walls (% cell walls) Carbon	Hydrogen	Nitrogen	Lignin (ash-free, % cell walls)
Maize stem (mature)	66.2*	46.4 (±0.01)	5.87 (±0.04)	0.08 (±0.2)	11.4*
Barley stem (from straw)	87.0 (±0.2)	45.9 (±0.9)	6 05 (±0 17)	0 16 (±0 02)	10.2*
Wheat bran	33.8*	45.4*	6 62*	0.30*	8 1*

*Duplicate analysis.

Table 2. Characterisation of aldehydes released from cell walls of maize stem by treatment with NaOH

	HPLC Retention time (min)	TLC Solvent 1 (R_f)	TLC Solvent 2 (R_f)	UV absorption Methanol λ_{max} (nm)	UV absorption Methanol log ε	UV absorption 0.1 M NaOH λ_{max} (nm)	Mass spectrum (*m/z*, rel int.)
p-Hydroxybenzaldehyde	11.7	0.72	0.26	220	4.11	240	122 (M, 88), 121 (100), 93 (38),
				284	4 20	330	65 (40), 39 (40)
Vanillin	21.2	0.68	0.78	230	4.19	250	152 (M, 98), 151 (100), 123 (18),
				278	4.03	346	109 (19), 81 (28), 53 (18)
				308	4.01		
Syringaldehyde	46.0	0 65	0.71	214	4 29	218	182 (M, 100), 181 (57), 111 (21),
				230	4 23	250	96 (18), 65 (22), 51 (20),
				306	4 29	364	39 (33)

Experimental details of HPLC, TLC, UV absorption spectroscopy and mass spectrometry given in text

Table 3 Phenolic aldehydes and acids released from cell walls of maize stem, barley stem and wheat bran by treatment with NaOH

Phenolic acid or aldehyde	Cell walls: Maize stem (mature) (mg/g wall)	Barley stem (from straw) (mg/g wall)	Wheat bran (mg/g wall)
p-Hydroxybenzaldehyde	0.26	0 02	0.06
Vanillin	0 22	0.23	0 10
Syringaldehyde	0.22	0.10	zero
Trans-*p*-coumaric acid + *cis*-*p*-coumaric acid	33 05	3.42	0 07
Trans-ferulic acid	3 78	2.26	3.27
Cis-ferulic acid	zero	zero	2 33
Trans,trans-diferulic acid	0.11	0.13	0 18

Duplicate analyses.

examined, including the shoots of Italian ryegrass and wheat, and the leaf, sheath and seed of maize, all contained the aldehydes. Preliminary results suggest that the walls of the more mature tissues have higher contents of syringaldehyde than younger tissues. The results may be compared with those from alkaline nitrobenzene oxidation in which walls of the more mature tissues also give greater yields of this aldehyde [16].

EXPERIMENTAL

Plant materials. Maize (*Zea mays* L, cv Troyer Reid) was grown from seed in a glasshouse in soil taken from the Institute farm. The plants were harvested when the cobs were mature The stems were cut at the 14th node above the root and the lower stem ('mature stem') taken after removal of leaves and sheaths. Barley (*Hordeum vulgare* L, cv. Julia) was grown to maturity in the field

at the Institute and the stem fraction of the straw was separated by hand. Wheat bran (*Triticum aestivum* L.) obtained from the American Association of Cereal Chemists ('AACC Certified Food Grade Wheat Bran RO7-3691'), was derived from a commerical blend of white wheats; analytical data are available from AACC The plant materials were freeze-dried and ground to pass a 0.8 mm sieve. The dry matter content of the materials was determined by drying at 100° for 16 hr.

Isolation of plant cell walls. Cell walls were isolated by a modification [17] of the neutral detergent procedure of Van Soest and Wine [18]. C, H and N contents were determined by a micro-Dumas combustion method (Carlo Erba Elemental Analyser 1106) (Table 1).

Estimation of lignin. The lignin content of the cell walls was estimated by the method of Goering and Van Soest [19] using cell walls (0 5 g) instead of acid-detergent fibre; no asbestos was added before treatment with 72% H_2SO_4.

Treatment of cell walls with NaOH *(for preparative HPLC).* Cell walls (100 mg) were treated with 1 M NaOH (5 ml) under N_2 (containing < 5 ppm O_2) at 20° for 20 hr. The suspension was filtered (No. 1 porosity glass sinter), and the residue washed with H_2O (5 ml). The filtrate and washings were acidified with 6 M HCl to pH 2.5 and extracted with CH_2Cl_2 (3 × 10 ml). The CH_2Cl_2 extract was dried over dry Na_2SO_4 and the solvent removed in a stream of N_2.

Preparative HPLC of the residue from CH_2Cl_2 *extraction.* The residue was dissolved in the HPLC solvent (1 ml of 0.01 M NaOH containing 0 4 M NaCl) and separated preparatively by HPLC on a column (25 × 0 46 cm i.d , packed with PRP-1 resin, 10 μm, Hamilton) with a flow rate of 0.8 ml/min and 3.5 × 10^6 Pa pres. (Laboratory Data Control Constametric III pump) A loop injector (Reodyne 7125) was employed to inject samples (10–100 μl) and detection was by UV absorption at 340 nm (LDC Spectromonitor III, 10μl flow cell). Separations were monitored with a recorder (Houston Instrument Omniscribe). The separated aldehydes (*p*-hydroxybenzaldehyde, vanillin and syringaldehyde) were acidified to pH 2.5 with 6 M HCl and extracted with CH_2Cl_2 (3 × 1 vol) The organic layer was dried over dry Na_2SO_4 and the solvent removed

TLC of the residue from CH_2Cl_2 *extraction before HPLC separation and of separated aldehydes.* Cellulose plates (Schleicher and Schull, F1400) were employed with HCO_2H–H_2O (1:24) and PhMe–HCO_2H–H_2O (8·9:3, upper phase) as previously described [20] The aldehydes gave yellow colorations with 2,4-dinitrophenylhydrazine reagent [21]

MS of separated aldehydes. A Finnigan Automated Gas Chromatograph 4021 E_1C_1 was employed Samples were introduced by direct probe (temp. of ion source 250°).

Estimation of phenolic aldehydes and acids released from cell walls by treatment with NaOH Cell walls (30 mg) were shaken with 1 M NaOH (2 ml) under N_2 (containing < 5 ppm O_2) at 20° or 20 hr. The suspension was filtered, the residue washed with H_2O and the filtrate diluted to 5 ml. The phenolic aldehydes and diferulic acid were determined analytically by HPLC using the method described above for preparative HPLC. *p*-Coumaric and ferulic acids were separated similarly but with 0 1 M $NaHCO_3$–MeOH (19 1) as solvent: for analysis, an aliquot of the filtrate was adjusted to the pH of the solvent (8.6) using 6 M HCl then 40 μl injected on to the chromatographic column. The amounts of aldehydes and acids present in the extracts were determined by comparison with calibration curves of the reference compounds [5, 22].

Treatment of cell walls with 'cellulase'. Cell walls (30 mg) were treated at 37° for 20 hr with an aq. soln of cellulase (E. Merck, Darmstadt; *Oxyporus* sp., a Basidiomycete, cat. no. 2312, activity 20 mU/mg, 11.25 mg in 2 ml buffer of pH 4.8) as described previously [17]. The suspension was filtered (No. 1 porosity glass sinter) and the filtrate submitted to analytical HPLC as above using a reverse-phase column of Spherisorb 50DS as described previously [6]. An aliquot of the filtrate (1 ml) was treated with 10 M NaOH (0 11 ml) under N_2 at 20° for 20 hr and submitted to analytical HPLC using the method described above for preparative HPLC

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