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5-HYDROXYFERULIC ACID IN *ZEA MAYS* AND *HORDEUM VULGARE* CELL WALLS

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Key Word Index—*Zea mays*; *Hordeum vulgare*; Gramineae; cell wall; GCMS; cinnamic acid derivatives; 5-hydroxyferulic acid; sinapic acid.

Abstract—Considerable amounts of esterified *E*-5-hydroxyferulic acid and very small amounts of esterified *E*-sinapic acid were detected and identified in cell walls of young *Zea mays* and *Hordeum vulgare*, in addition to known *E*-*p*-coumaric and ferulic acids. Their relative amounts were determined by peak areas using GC. The ratios of *E*-*p*-coumaric–5-hydroxyferulic–sinapic–ferulic acid were 440:46:2:100 in corn, and 37:26:3:100 in barley, respectively.

INTRODUCTION

It is well established that cinnamic acid derivatives, such as *p*-coumaric acid (*p*-CA), ferulic acid (FA) and diferulic acid are cell wall bound to vascular plants [1–10]. In the Gramineae, these acids are predominantly *p*-coumaryl and feruloyl esters linked to hemicelluloses. Recent studies with wheat bran [9], sugar cane bagasse [10] and maize [11] have shown ferulic acid to be esterified to arabinoglucuronoxylans.

The function of these cell wall bound esters is currently not well understood. It has long been proposed that they are involved in the process of lignification [4, 12, 13]. Some recent evidence tends to support this hypothesis as lignin fractions isolated from wheat straw contain ether linked phenolic acids [14]. However, this has yet to be demonstrated in the intact plant. Another role for these acids has also been proposed, namely that the *E*/*Z* photoisomerism, in UV-A, of cell wall ferulate and diferulate-carbohydrate esters is a possible mechanism for transduction of light energy leading to changes in wall structure and hence water flux, turgor pressure, and growth [15]. Quantitative changes in the level of cell wall FA and *p*-CA and the photoisomerism of these esterified cinnamic acid derivatives in barley sprouts have also been described [16].

Lignification is currently believed to occur *exclusively* via the random, dehydrogenative polymerization of the three *trans* (*E*) monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols. All of the enzymes leading to the *trans* (*E*) monolignols have been found following the recent isolation of ferulic acid-5-hydroxylase [17]. Sinapic acid (SA) is thus apparently formed via methylation of 5-hydroxyferulic acid and not 3,4,5-trihydroxycinnamic acid. Whilst previous work on the substrate specificity of angiosperm methyltransferases had suggested this result [18], 5-hydroxyferulic acid has not been isolated from any plant material.

RESULTS AND DISCUSSION

Homogenates of 19 days old corn (*Zea mays*) or barley (*Hordeum vulgare*) were thoroughly extracted with MeOH by Soxhlet extractors. Extractive-free cell walls were saponified under mild alkaline conditions and the hydrolysate was extracted with diethyl ether to remove neutral constituents. The remaining aqueous layer was acidified, extracted with ethyl acetate, and silylated [19]. The trimethylsilylated samples were submitted to GC/MS analyses and examined for cinnamic acid derivatives.

In addition to known *E*- and *Z*-*p*-CA and *E*-FA, considerable amounts of *E*-5-hydroxyferulic acid (5-HFA) and small amounts of *E*-sinapic acid (SA) were detected in these cell walls for the first time. They were identified by their GC retention times and by comparison of MS spectra with authentic specimens. We established that three-week-old *Zea mays* contains 4–8 mg ferulic acid per gram of dry cell walls. The ratios of the esterified cinnamic acid derivatives, relative to ferulic acid, are shown in Table 1. The amounts of the less abundant constituents, *Z*-FA, *Z*-5-HFA and *Z*-SA were not determined as their retention times coincided with other constituents.

In conclusion, 5-HFA is abundant in both plant tissues as a cell wall bound ester. Whilst the enzyme catalysing its formation has been found, this study represents the first example of the isolation of 5-HFA from the plant kingdom.

Table 1. Relative amounts of cinnamic acid derivatives in cell walls of 19 days old corn and barley*

Cinnamic acid derivatives	Corn	Barley
Z-*p*-Coumaric acid	90	6
E-*p*-Coumaric acid	440	37
E-Ferulic acid	100	100
E-5-Hydroxyferulic acid	46	26
E-Sinapic acid	2	3

*Calculated on the basis of peak area of *E*-ferulic acid.

EXPERIMENTAL

Fresh corn (*Zea mays* L. cv S259) or barley (*Hordeum vulgare* L. cv Conquest) tillers were homogenized in a mixture of 95% EtOH and C_6H_6 (2:1) in a Waring blender. The homogenate was filtered, the residue extracted with MeOH for 26 hr by a Soxhlet extractor and dried *in vacuo*.

Saponification and extraction of cinnamic acid derivatives. Cinnamic acids were isolated from dried extracted cell wall material (25 mg) as previously reported [16], except that the saponification solution was extracted with Et_2O (3 ml, 2 ×) prior to acidification.

Preparation of TMS of cinnamic acid derivatives. The dried residue was treated with 0.3 ml of bis(trimethylsilyl)trifluoroacetamide (BS/FTFA) at 125° for 10 min in a sealed vial. The reaction mixture was submitted to GC/MS analysis as described below. *E*-5-hydroxyferulic acid was synthesized according to the method of Neish [20].

GC/MS analysis of TMS derivatives of hydroxycinnamic acids. GC/MS analysis was performed on a Finnigan automated GC/MS 1020 equipped with a data acquisition system. GC/MS operation conditions were: trimethylsilylated samples were separated on a capillary column (SE-54, 30 m). The He carrier gas flow pressure was 1.4 kg/cm^2 and the injection and separator temps were maintained at 280°. Column temp. was programmed as follows: equilibration at 130°C for 4 min, then raised to 260°C at 27° per min, and held at 260°C for 10 min. EIMS were obtained with an ionization voltage of 70 eV. Retention time R_ts (min): *Z*-*p*-CA (7.8), *E*-*p*-CA (8.5), *Z*-FA (8.2), *E*-FA (9.5), *Z*-SA (9.1), *E*-SA (10.4), *Z*-5-HFA (10.1) and *E*-5-HFA (11.0). MS spectra *E*-5-HFA (TMS) *m/z* (%): 427 $[M+1]^+$ (37), 426 $[M]^+$ (69), 412 (16), 411 (42), 323 (12), 279 (10), 250 (24), 249 (87), 197 (10), 75 (19), 74 (27), 73 (100), 59 (13), 45 (46), 44 (32), and 43 (12). *E*-SA (TMS) *m/z* (%): 369 $[M+1]^+$ (40), 368 $[M]^+$ (85), 355 (13), 354 (30), 353 (69), 340 (23), 339 (25), 338 (100), 324 (13), 323 (48), 280 (12), 279 (51), 249 (18), 218 (10), 162 (24), 89 (14), 75 (24), 74 (25), 73 (99), 59 (25), 58 (16), 45 (75), and 43 (15).

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REFERENCES

1. Smith, D. C. C. (1985) *J. Chem. Soc.* 2347.
2. Stafford, H. A. (1962) *Plant Physiol.* **37**, 643.
3. El-Basyouni, S. and Towers, G. H. N. (1964) *Can. J. Biochem.* **42**, 203.
4. Harris, P. J. and Hartley, R. D. (1976) *Nature* **259**, 508.
5. Markwalder, H. U. and Newcom, K. (1976) *Phytochemistry* **15**, 836.
6. Hartley, R. D. and Harris, P. J. (1981) *Biochem. Syst. Ecol.* **9**, 189.
7. Kato, Y. and Nevins, D. J. (1983) *Suppl. Plant Physiol.* **72**, 74.
8. Fry, S. C. (1983) *Planta* **157**, 111.
9. Smith, M. M. and Hartley, R. D. (1983) *Carbohydr. Res.* **118**, 65.
10. Kato, A., Azuma, J. and Koshima, T. (1983) *Chem. Letters* 137.
11. Kato, Y. and Nevins, D. J. (1985) *Carbohydr. Res.* **137**, 139.
12. El-Basyouni, S. Z., Neish, A. C. and Towers, G. H. N. (1964) *Phytochemistry* **3**, 627.
13. Hartley, R. D. and Jones, E. C. (1976) *Phytochemistry* **15**, 1157.
14. Scalbert, A., Monties, B., Lallemand, J. Y., Guittet, E. and Rolando, C. (1985) *Phytochemistry* **24**, 1359.
15. Towers, G. H. N. and Abeysekera, B. (1984) *Phytochemistry* **23**, 951.
16. Yamamoto, E. and Towers, G. H. N. (1985) *J. Plant Physiol.* **117**, 441.
17. Grand, G. (1984) *FEBS Letters* **169**, 7.
18. Higuchi, T., Shimada, M., Nakatsubo, F. and Tanahashi, M. (1977) *Wood Sci. Technol.* **11**, 153.
19. Casteele, D. V., DePooter, H. and Van Sumere, C. F. (1976) *J. Chromatogr.* **121**, 49.
20. Neish, A. C. (1959) *Can. J. Biochem.* **87**, 1451.