

CINNAMIC ACID DERIVATIVES IN CELL WALLS OF BAMBOO AND BAMBOO GRASS

SANRO TACHIBANA, KATSUMI OHKUBO and G. H. NEIL TOWERS*

Department of Bio-Resource Science and Biotechnology, Faculty of Agriculture, Ehime University, Ehime 790, Japan;

*Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 2B1

(Received in revised form 9 January 1992)

Key Word Index—*Phyllostachys edulis*; bamboo; *Sasa kurilensis*; bamboo grass; Gramineae; cell walls; *p*-coumaric, ferulic, caffeic, 5-hydroxyferulic and sinapic acids; truxillic acid derivatives of *p*-coumarate and ferulate.

Abstract—4,4'-Dihydroxytruxillic acid (DHTA) and 4,4'-dihydroxy-3,3'-dimethoxytruxillic acid (DHMTA), photodimers of *p*-coumaric and ferulic acids, respectively, as well as *p*-coumaric, ferulic, caffeic, hydroxyferulic and sinapic acids, were identified as components of the cell walls of stems and leaves of bamboo (*Phyllostachys edulis*) and bamboo grass (*Sasa kurilensis*). The amounts were determined by measurement of peak areas using GC-MS with 1-octadecanol as internal standard.

INTRODUCTION

Alkaline hydrolysis of grass cell walls releases various amounts of cinnamic acid derivatives including dimers and cyclobutane dimers [1–9, 25, 26]. Wall-bound *p*-coumaric acid (CA) and ferulic acid (FA), as well as other phenolic acids, are known to be esterified to heteroxylans [10–12]. The significance of this phenomenon has not been determined, although it has been suggested that the esterified acids form a type of anchor for lignin [13–15] or that they are important in the transduction of light energy to mechanical changes in the wall, e.g. *E/Z* photoisomerism of ferulic acid esters [16].

Cyclobutane dimers of CA and FA, i.e. analogues of truxillic acid, also occur in cell walls of tropical and temperate grasses and are presumed to be formed photochemically [7–9]. The presence of these phenolic esters would also be expected to affect the digestibility of grasses by ruminants and insects. Although there are *ca* 45 genera of bamboos [17], there are only a few studies of the cell wall heteroxylans of bamboo cell walls [18, 19].

We have previously described the presence of a cyclobutane dimer of *p*-coumaric acid in the cell walls of *Phyllostachys edulis*. In the present study we have identified a truxillic acid of ferulic acid as well as the rare phenolic acid, hydroxyferulic acid, in the cell walls of this species as well as in the walls of *Sasa kurilensis*, a smaller species of bamboo.

RESULTS AND DISCUSSION

The tetra-TMSi derivative of 4,4'-dihydroxy-3,3'-dimethoxytruxillic acid, synthesized from *E*-ferulic acid by photodimerization, had an R_t (GC-MS) of 19.4 min compared to 7.5 min for the bis-TMSi derivative of *E*-ferulic acid. The mass spectrum of the tetra-TMSi derivative of this compound contained the same major ions as the mass spectrum of the bis-TMSi derivative of *E*-ferulic acid. This is similar to that observed in the mass spectra of

4,4'-dihydroxy truxillic acid (DHTA) and *E*-*p*-coumaric acid [7, 20]. Theoretically, 11 isomers of truxillic and truxinic acids can be formed from the photodimerization of ferulic acid [21]. The mass spectra of the tetra-TMSi derivatives of the head to tail dimers (truxillates) are similar [9, 22, 23] and the stereochemistry of these cyclobutane dimers cannot be determined by this method of analysis. Our cell wall isolate, its dimethyl ether and its diacetate were found to have the same melting points as those reported for a known, photochemically synthesized dimer of ferulic acid and its corresponding derivatives [24]. The photodimer, as well as its derivatives, prepared by the method of Lee *et al.* [24], showed no depression of mixed melting points. Therefore, the isolate from *Phyllostachys* and *Sasa* is 2 α ,4 β -bis(4-hydroxy-3-methoxyphenyl cyclobutane)-1 α ,3 β -dicarboxylic acid or 4,4'-dihydroxy-3,3'-dimethoxytruxillic acid (Fig. 1).

Extractive-free cell walls were saponified under mild alkaline conditions and the hydrolysate extracted with ether to remove neutral compounds. The remaining aqueous layer was acidified, extracted with ether, silylated and subjected to quantitative GC-MS analysis for phenolic acids.

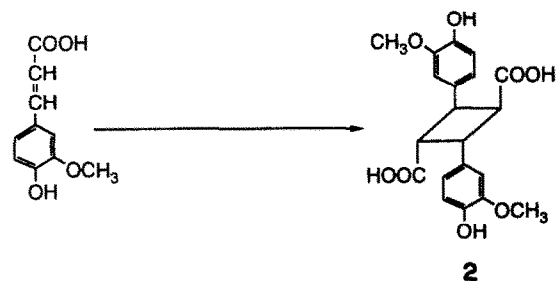


Fig. 1. Photodimerization of *E*-ferulic acid (1) to 4,4'-dihydroxy-3,3'-dimethoxytruxillic acid (2).

Table 1. Amounts of *p*-coumaric acid (CA), ferulic acid (FA), caffeic acid (CFA), sinapic acid (SA), 5-hydroxyferulic acid (HFA) and the truxillic acids, DHTA and DHMTA, in cell walls of *Phyllostachys edulis* (P) and *Sasa kurilensis* (S)

Sample	CA	FA	CFA	HFA	SA	DHTA	DHMTA
	(mg g ⁻¹ cell walls)			(μg g ⁻¹ cell walls)			
P Stems	15.4	0.9	13.9	12.2	36.7	22.0	1.7
P Leaves	16.1	1.8	35.3	20.0	42.2	169.4	17.1
S Stems	14.2	0.7	20.8	15.0	41.7	15.6	1.7
S Leaves	15.7	0.8	29.6	16.5	87.2	291.7	31.9

The amounts of esterified acids obtained are shown in Table 1. The existence of other photodimers of CA and FA was apparent following selective ion monitoring of the characteristic fragment ions at *m/z* 308 and 338, cleaved from the TMSi derivatives, but the very low amounts found and the absence of standards precluded their identification.

Small amounts of caffeic (CFA), sinapic (SA) and 5-hydroxyferulic acids (HFA) were detected for the first time in bamboo cell walls. Leaves contained 10–20 times the amounts of the two truxillic acids compared to stems, suggesting the role of photodimerization in sunlight [20].

EXPERIMENTAL

Plant material. Five year-old bamboo (*P. edulis*) stems, 1.5 m above ground level, and leaves were collected in Tobe, Ehime Prefecture, in October and May 1990. Two-year-old bamboo grass (*Sasa kurilensis*) stems and leaves were collected on the campus of the University of British Columbia, B.C., Canada in November 1990.

Preparation of cell wall extracts. Cell walls of stems were prep and saponified as reported previously [20]. Cell wall preps were refrigerated in the dark until analysed.

TMSi derivatives of alkaline hydrolysate components. Dried hydrolysis products and 0.1 mg of 1-octadecanol, as int. std, were treated with 20 μl of bis(trimethylsilyl)trifluoroacetamide (BSTFA) in pyridine (20 μl) at 25° for 30 min in a sealed vial. The reaction mixt. was submitted to GC-MS analysis. DHTA (mp > 340°) was synthesized according to the method of ref. [27]. DHMTA (mp 279–280°) and its dimethyl ester (mp 181–182°) were synthesized according to the method of ref. [24]. The diacetate of DHMTA (mp 172–173°) was synthesized according to the method of ref. [28].

GC-MS analyses of TMSi derivatives. Operation conditions were as follows. TMSi samples were sep on a capillary column (OV-101, 25 m). Carrier gas flow pressure was 1.4 kg cm⁻² and the injection and separation temps were maintained at 280° and 250°, respectively. The column temp. was programmed at 185° for 1.5 min, then raised to 215° at 3° min⁻¹ and held at 215° for 0.5 min, and finally raised to 290° at 30° min⁻¹ and held at this temp. for 6 min. EIMS were obtained with an ionization voltage of 70 eV. *R_s* in min were: E-CA (5.4), E-FA (7.5), CFA (8.7), 1-octadecanol (int. std; 9.1), E-SA (10.3), HFA (11.4), DHTA (17.8) and DHMTA (19.4). The mass spectrum of the TMSi derivative of DHMTA was *m/z* (%): 338 [M–338]⁺ (53), 323 (15), 308 (8), 279 (1), 249 (18), 75 (33), 73 (100).

Acknowledgements—We thank Mr Maturu Hino for the gift of bamboo samples, Dr Masashi Sumimoto (Kyushu University)

for the use of the GC-MS equipment and Mr Felipe Balza (UBC) for valuable advice. We gratefully acknowledge the Department of Energy, U.S.A. (Grant No. De-FG06-91ER20022 to Dr Norman Lewis of Washington State University) and to the Natural Sciences and Engineering Research Council, Canada, for support of this research.

REFERENCES

1. El-Basyouni, S. and Towers, G. H. N. (1964) *Can. J. Biochem.* **42**, 203.
2. Markwalder, H. U. and Neukom, H. (1976) *Phytochemistry* **15**, 836.
3. Hartley, R. D. and Harris, P. J. (1981) *Biochem. Syst. Ecol.* **9**, 189.
4. Smith, M. M. and Hartley, R. D. (1983) *Carbohydr. Res.* **118**, 65.
5. Ohashi, H., Yamamoto, E., Lewis, N. G. and Towers, G. H. N. (1987) *Phytochemistry* **26**, 1915.
6. Ford, C. W. and Hartley, R. D. (1988) *J. Chromatogr.* **436**, 484.
7. Hartley, R. D., Whately, F. R. and Harris, P. J. (1988) *Phytochemistry* **27**, 349.
8. Ford, C. W. and Hartley, R. D. (1990) *J. Sci. Food Agric.* **50**, 29.
9. Hartley, R. D., Morrison, W. H., Balza, F. and Towers, G. H. N. (1990) *Phytochemistry* **29**, 3699.
10. Kato, A. and Nevins, R. (1985) *Carbohydr. Res.* **137**, 139.
11. Harvey, M. and Hartley, R. D. (1986) *Carbohydr. Res.* **148**, 71.
12. Kato, A., Azuma, J. and Koshima, T. (1983) *Chem. Letters* 137.
13. Harris, P. J. and Hartley, R. D. (1976) *Nature* **259**, 508.
14. El-Basyouni, S. Z., Neish, A. C. and Towers, G. H. N. (1964) *Phytochemistry* **3**, 627.
15. Hartley, R. D. and Jones, E. C. (1976) *Phytochemistry* **24**, 1359.
16. Towers, G. H. N. and Abeysekera, B. (1984) *Phytochemistry* **23**, 951.
17. Porter, C. L. (1967) in *Taxonomy of Flowering Plants*, 2nd edn, p. 202. W. H. Freeman, San Francisco.
18. Ishi, T., Hiroi, T. and Thomas, J. R. (1990) *Phytochemistry* **29**, 1999.
19. Ishi, T. and Hiroi, T. (1990) *Carbohydr. Res.* **206**, 297.
20. Tachibana, S., Ohkubo, K. and Towers, G. H. N. (1992) *Phytochemistry* **31**, 81.
21. Moore, J. M., Cooper, D. A., Lurie, I. S., Kram, T. C., Carr, S., Harper, C. and Yeh, J. (1987) *J. Chromatogr.* **410**, 297.
22. Caccamese, S., Montaudo, G. and Przybylski, M. (1974) *Org. Mass Spectrom.* **9**, 1114.

23. Egerton, P. L., Hyde, E. M. Trigg, J., Payne, A. Benyon, P. Mijovic, M. V. and Reiser, A. (1981) *J. Am. Chem. Soc.* **103**, 3859.
24. Lee, D. Y., Tachibana, S. and Sumimoto, M. (1988) *Cell. Chem. Technol.* **22**, 201.
25. Castele, D. V., DePooter, H. and Van Sumere, C. F. (1976) *J. Chromatogr.* **121**, 49.
26. Higuchi, T. (1975) *Kagaku to Seibutsu* **13**, 206.
27. Cohen, M. D., Schmidt, G. M. J. and Sonntag, F. I. (1964) *J. Chem. Soc.* 2000.
28. Homas, J., Stelzner, R. and Zukow, A. (1891) *Chem. Ber.* **24**, 2589.