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4,4′-DIHYDROXYTRUXILLIC ACID AS A COMPONENT OF THE CELL WALLS OF THE BAMBOO *PHYLLOSTACHYS EDULIS*

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Key Word Index—*Phyllostachys edulis*; Poaceae; bamboo; cell walls; GC-MS; dihydroxytruxillic acid; photodimer of *p*-coumaric acid.

Abstract—4,4′-Dihydroxytruxillic acid, a photodimer of *p*-coumaric acid, as well as *p*-coumaric and ferulic acids, were identified from alkaline hydrolysates of the cell walls of stems, leaves and shoots of bamboo (*Phyllostachys edulis*). The truxillic acid content was highest in leaves but was only about 1/1000th that of wall bound *p*-coumarate. Truxillic acid levels increased on grinding in a Wiley mill suggesting that it can be generated in cell walls by mechanochemical energy.

INTRODUCTION

p-Coumaric acid (CA) and ferulic acid (FA), as well as other phenolic acids, are cell wall components of various monocots [1–6]. They are present as esters, covalently linked to carbohydrates and easily released by cold alkaline hydrolysis. Cyclobutane dimers of CA and FA, 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 be expected to affect the digestibility of grasses by ruminants and insects.

Bamboos, which are large perennial grasses with woody stalks, are placed in a tribe of about 45 genera, widely distributed in the Tropics but extending their range to sub-frigid zones [10]. There are only a few studies of the heteroxylans of bamboo cell walls and two compounds have been identified from this source. These are *O*-(4-*O*-*trans*-feruloyl-α-D-xylopyranosyl)-(1→6)-D-glucopyranose and *O*-[5-*O*-(*trans*-*p*-coumaroyl)-α-L-arabinofuranosyl]-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose [11, 12]. Hitherto there have been no reports of the presence of cyclobutane dimers of hydroxycinnamic acids in bamboo cell walls. We describe the presence of a cyclobutane dimer of *p*-coumaric acid, namely 4,4′-dihydroxytruxillic acid, in the cell walls of the bamboo, *Phyllostachys edulis*.

RESULTS AND DISCUSSION

The tetra-TMS derivative of 4,4′-dihydroxytruxillic acid, synthesized from *E*-*p*-coumaric acid by photodimerization, had a R_t (GS-MS) of 13.2 min compared to 4.0 min for the bis-TMS derivative of *E*-*p*-coumaric acid and 5.1 min for the bis-TMS derivative of *E*-ferulic acid. The mass spectrum of the tetra-TMS derivative of 4,4′-dihydroxytruxillic acid contained the same major ions as the mass spectrum of the bis-TMS derivative of *E*-*p*-coumaric acid indicating that the cyclobutane dimer was split symmetrically to form two equal fragments on electron impact. Eleven isomers of dihydroxy-truxillic and -truxinic acids may be formed when *E*-*p*-coumaric acid undergoes photodimerization [13]. The mass spectra of all the tetra-TMS derivatives of the head to tail dimers (isomers of 4,4′-truxillic acid) are similar [9, 14, 15] so that this method of analysis is not useful in distinguishing them. Our cell wall isolate, its dimethyl ether and its diacetate were found to have the same melting points, i.e. >349°, 261–263° and 245–246°, as those reported for a known, chemically synthesized photodimer of *p*-coumaric acid and its derivatives. The same photodimer and its derivatives were prepared by the methods of Cohen *et al.* [16] and Homas *et al.* [17] and no depressions of mixed melting points were observed. The synthesized as well as the isolated cyclobutane dimer from *Phyllostachys* is, therefore, [2α,4β-bis(4-hydroxyphenylcyclobutane)-1α, 3β-dicarboxylic acid] or 4,4′-dihydroxytruxillic acid (Fig. 1).

Extractive-free cell walls were saponified under mild alkaline conditions and the hydrolysate was 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 CA, FA and truxillic acid.

The amounts of esterified acids obtained are shown in Table 1. The existence of other photodimers of CA and FA was apparent in selective ion monitoring of the

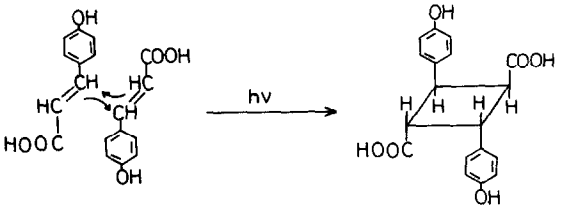

Fig. 1. Formation of 4,4′-dihydroxytruxillic acid from *trans*-*p*-coumaric acid by light.

Table 1. Amounts of *p*-coumaric acid (CA), ferulic acid (FA) and 4,4'-dihydroxytruxillic acid (DTA) recovered from cell wall preparations of *Phyllostachys edulis*

Sample		CA ($mg\,g^{-1}$ wall)	FA ($mg\,g^{-1}$ wall)	DTA ($\mu g\,g^{-1}$ wall)
Stems	outer 3 cm	13.8 (16.5)	0.81 (1.25)	33.6 (44.2)
	middle 3 cm	16.3 (17.1)	0.88 (1.18)	16.4 (19.6)
	inner 3 cm	15.5 (16.7)	0.91 (1.38)	11.3 (13.8)
Leaves		16.6	1.72	172.0
Shoots		15.1	1.58	3.39

Numbers in parentheses are the contents of acids recovered from duplicate samples after milling. Amounts of CA and FA include *E*- and *Z*-isomers and are the means of duplicate analyses. Yields of cell walls for each fraction are shown in the Experimental.

characteristic fragment ions at *m/z* 308 and 338, cleaved from the TMS derivatives, but the low amounts found and the absence of standards precluded their identification.

4,4'-Dihydroxytruxillic acid was present in all parts of the bamboo samples. Analyses were repeated twice for each sample and a centripetal decrease in the amount of the cyclobutane dimer was observed in each case. This suggests that, in bamboo stems, the degree of dimerization is a function of the increasing attenuation of light as one proceeds to the centre of the stem [18].

There was a 20–30% increase of wall-bound truxillic acid in stem tissues when these were re-ground in a Wiley mill (Table 1), an increase much higher than the increases in CA and FA under the same conditions. This shows that a significant dimerization of CA by mechanochemical energy occurs during milling. Coniferyl alcohol methyl ether has been shown to dimerize during milling forming a truxillic acid type of dimer [19] and according to Heinicke [20] mechanochemical reactions of this type proceed by a radical mechanism resembling photochemical reactions.

Our results indicate that juxtapositions of some *p*-coumaroyl residues in the carbohydrate chains are ideally suited either for photodimerization or mechanochemical reaction. It also emphasizes that care has to be taken in the procedures for grinding cell walls of grasses, prior to chemical analysis, because of the possibility of producing cyclobutane dimers by mechanical means.

EXPERIMENTAL

Plant material. Five-year-old bamboo (*Phyllostachys edulis*) stems, 1.5 m above ground level, leaves and young shoots were collected in Tobe, Ehime Prefecture, in October and May 1990.

Preparation of cell walls. Cell walls of stems were prepared as follows: 10 cm lengths of stem (9 mm thickness in x-section) were equally divided into three fractions (each of 3 mm thickness) designated as outer, middle and inner fractions proceeding towards the centre of the stem. Small pieces of each fraction were frozen in liq. N_2, ground with pestle and mortar and extracted thoroughly with $CHCl_3$–MeOH (1 : 1) and MeOH to remove extractives. Yields of cell walls from each fraction was 85% (outer fractions), 82% (middle fraction) and 79% (inner fraction) respectively of starting materials. Cell walls of leaves, prepared by the above method gave a yield of 80%. Cell walls of shoots, prepared by the method of ref. [11] gave a yield of 71%. In addition, ground cell walls, prepd as above, were first pulverized using a Wiley mill (× 3, 2 mm mesh size) at ambient temp. for 30 min, removed and frozen in liq. N_2 and then ground with pestle and mortar. This was followed by extraction with $CHCl_3$–MeOH (1 : 1) and MeOH as mentioned above. Yields of cell walls by this increased grinding procedure were 81% (outer fraction), 79% (middle fraction) and 75% (inner fraction). All wall reparations were refrigerated in dark until used.

Alkaline hydrolysis of cell walls. Each wall preparation (90 mg., o.d. in duplicate) was shaken with 15 ml of 1 M NaOH for 20 hr at 25° under N_2. The alkaline hydrolysate, after centrifugation, was extracted with EtOEt, acidified (pH 2.0) with dil. HCl and re-extracted with Et_2O. The ethereal soln was dried with dry Na_2SO_4 and evapd to dryness.

TMS derivatives of alkaline hydrolysate components. The dried acids and 0.2 mg of *E*-sinapic acid as int. standard 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 mixture was submitted to GC-MS analysis.

GC-MS analyses of TMS derivatives of acids. GC-MS operation conditions were as follows: TMS samples were sepd on a capillary column (OV-101, 25 m). Carrier gas flow pressure was $1.3\,kg\,cm^{-2}$ and the injection and separator temps were maintained at 280° and 250° respectively. Column temp. was programmed as follows: equilibration at 200° for 2 min, then raised to 290° at $10°\,min^{-1}$ and held at 290° for 10 min. EIMS were obtained with an ionization voltage of 70 eV. R_ts in min were: *E*-CA (4.0), *E*-FA (5.1), *E*-SA (int. standard) (6.1), DTA (13.2). MS of the truxillic acid TMS was *m/z* (%): 308 $[M-308]^+$ (87), 293 (86), 249 (40), 219 (73), 75 (20), 73 (100).

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