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Effect of Substitution on the 2+2 Cycloaddition Reaction of Phenylpropanoids

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Formation of the cyclobutane products truxillic and truxinic acid within the plant cell wall could occur by photocycloaddition or possibly by enzyme-mediated radical coupling. Model precursors were synthesized and the mode of formation of the cyclobutane products was investigated. Modeling studies suggested that all the model compounds with the exception of the 2-hydroxylated diester contained the correct geometry for photocyclization and this was confirmed by irradiation. Delocalization of the unpaired electron was measured by ESR and the photochemical reaction rates were compared with the partial atomic orbital population of the singly occupied molecular orbital. It was observed that the 3-hydroxylated derivative had almost no delocalization to C8 and the lowest reaction rate. The rate of reaction increased with increasing extent of methoxylation. Incubation with silver oxide or with peroxidase failed to induce intramolecular dimerization. These results suggest that the biological mode of formation of truxillic and truxinic acid is the most likely *via* light-catalyzed 2+2 cycloaddition.

INTRODUCTION

Photochemical cyclodimerization of crystalline cinnamic acid (**1**) (Fig. 1) has been extensively studied and the efficiency of the reaction in the solid state has been shown to be determined entirely by the dimensions of the crystal lattice and hence by the separation and relative orientation of the exocyclic double bonds (*1*). The upper limit for the separation of the double bonds is of the order of 4.0 Å and the relative orientation must be parallel (*2*). This has prompted interest in the mechanism of formation of the widely reported cyclobutane derivatives truxillic and truxinic acids (**7** and **8**) found within and contributing to the cross-linking of the polymer network of the plant cell wall (*3,4*).

Because of the strict steric requirements for photocyclization, irradiation of substituted cinnamic acids in solution produces only insignificant amounts of cycloaddition products and therefore topochemical assistance is required in order to probe the mechanism (*5–7*). It has been demonstrated that by varying the length of the methylene spacer group linking two cinnamic acid moieties, optimum separation of <4.0 Å with parallel alignment of the α–β unsaturated double bond can be satisfied by the

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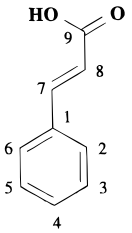

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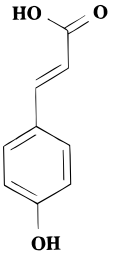

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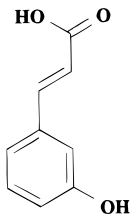

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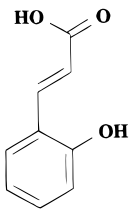

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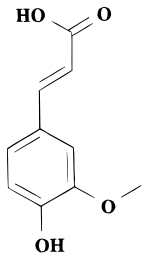

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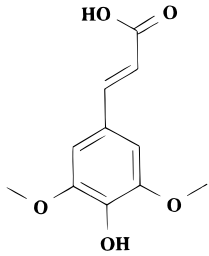

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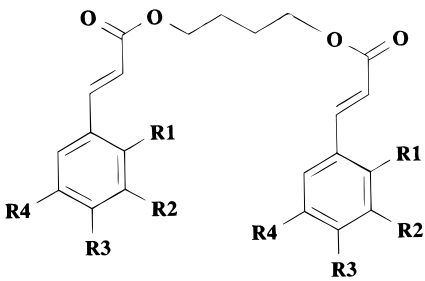

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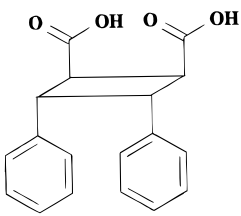

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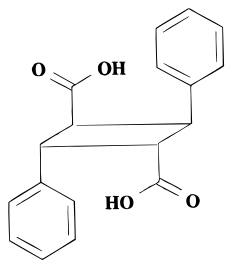

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preparation of dicinnamyl esters of propane 1,3-diol and butane 1,4-diol (*8*). In the case of a radical coupling mechanism, similar molecular separation and alignment of the exocyclic double bonds are required; however, in addition a 4-hydroxyl substituent is a prerequisite as it facilitates formation of the quinone methide and concomitant stabilization of the β alkenyl radical.

In this study butane 1,4-diol diesters have been used as model precursors of **7** and **8**. These were prepared by reacting butane-1,4-diol with 4-hydroxycinnamic acid (**2**), 4-hydroxy-3-methoxycinnamic acid (**5**), and 4-hydroxy-3, 5-dimethoxycinnamic acid (**6**). Although not naturally occurring within the plant cell wall, the 2- and 3-hydroxylated derivatives (**4** and **3**) have also been included. Of these latter two, delocalization of the unpaired electron to C8, facilitating cyclodimerization involving a β alkenyl radical, can be achieved only by the 2-hydroxycinnamate diester (**4a**). The 3-hydroxylated compound (**3a**) is unable to undergo cyclization by this mechanism but may photodimerize. Competing reactions, for example, the intramolecular bond formation found in coumarin biosynthesis (*9*) and also the steric effect of substitution at position 2, can be investigated by the 2-hydroxylated compound (**4a**). Another reaction competing with photocycloaddition is *E–Z* isomerization (*10*). However, since the favorable conformation for high quantum efficiency of cyclobutane formation is when both cinnamic moieties are in the *E* conformation, removal of the *E–E* confomer by dimerization should therefore shift the equilibrium in favor of further production of the *E* isomer (*11*).

The distribution of the unpaired electron has important consequences not only for the enzymatic formation of the cyclobutane derivative but also for the alternative mechanism viz. the photocycloaddition reaction (*12*). In general photochemical reactivity can be described reasonably on the basis of the partial atomic orbital population on the SOMO (singly occupied molecular orbital) (*13*) and it has been demonstrated that cycloaddition by direct irradiation can involve both the triplet (t_1) and the singlet (s_1) excited states (*14*). It should therefore be possible to correlate the substituent effect on reactivity for cycloaddition on the basis of unpaired electron density, as high efficiency is observed with high electron density of the lowest $\pi \rightarrow \pi^*$ excited state.

In this investigation we sought to determine the effects of ring substitution on the efficiency of photodimerization of the model compounds and to correlate this with unpaired electron spin density at the exocyclic double bond positions. Finally, by determining which mechanism is operating and by correlating that with the steric and electronic requirements, this study is designed to shed light on the likely mechanism of formation of truxillic and truxinic acids *in vivo* and thereby to contribute to the understanding of their role within the cell wall structure.

FIG. 1. Structures of cinnamic acid **1**, 4-hydroxycinnamic acid **2**, 3-hydroxycinnamic acid **3**, 2-hydroxycinnamic acid **4**, 4-hydroxy-3-methoxycinnamic acid **5**, and 3,5-dimethoxy-4-hydroxycinnamic acid **6** and butane-1,4-diol diesters containing 4-hydroxycinnamic acid **2a** (R3 = OH; R1, R2, and R4 = H), 3-hydroxycinnamic acid **3a** (R2 = OH; R1, R3, and R4 = H), 2-hydroxycinnamic acid **4a** (R1 = OH; R2, R3, and R4 = H), 4-hydroxy-3-methoxycinnamic acid **5a** (R3 = OH; R2 = OCH_3; R1 and R4 = H), and 3,5-dimethoxy-4-hydroxycinnamic acid **6a** (R3 = OH; R1 = H; R2 and R4 = OCH_3) and truxillic acid **7** and truxinic acid **8**.

RESULTS AND DISCUSSION

The distances between C7–C7′ and C8–C8′ for the energy-minimized structures of the various diesters calculated using the program Cerius[2] are shown in Table 1. In each case the distances are less than 4.0 Å with the exception of the 2-hydroxycinnamate diester (**4a**). In addition modeling suggests that the parallel orientation of the exocyclic double bonds can be achieved on minimization in all cases except that of **4a**.

ESR spectra were recorded following the one-electron oxidation of the substituted cinnamic acids (Fig. 2) and the hyperfine coupling constants were determined by computer simulation (Table 2, Fig. 2). Applying the McConnell (*15*) relationship with a *Q* value of 22.5 Gauss (considered the best estimate, assuming the value to be constant for all C–H bonds (*16*)), values were estimated for the unpaired electron spin density (Fig. 3). Polarization was accounted for by an Austin Model 1 (AM1) calculation on the energy-minimized structures (*12*). For all but 3-hydroxycinnamic acid (**3**) a significant amount of unpaired electron density was found on C8, indicating the possible participation of this position in radical-coupling reactions.

The predictions made on the basis of modeling were tested experimentally by irradiation at 3500 Å and the rate constants for the reaction calculated (Table 3, Fig. 4). As expected no product was obtained for the 2-hydroxy-substituted model (**4a**), although some *E–Z* isomerization was observed. All other diesters gave two products in varying concentrations (Table 4, A and B). It was not possible to characterize the exact stereochemistry of the products obtained, due to the attachment of the methylene side-chain (*17*). In an attempt to estimate the relative orientation of the cyclobutane substituents, dihedral angles were estimated from the observed cyclobutane proton coupling constants *via* the Karplus equation and these were compared to values obtained computationally from energy-minimized structures. The models constructed by head-to-tail dimerization, with the tetramethylene chain extending from the same side of the cyclobutane ring, generally gave minimized structures with the lowest energy. The structures containing the *E–E* isomers (dihedral angles of around 20° and 120°) and the *E–Z* isomers (dihedral angles of around 124° and 125°) gave the best comparison with the cycloaddition products A and B obtained on irradiation (Table 4).

TABLE 1

Distance (Å) between Positions C7–7′ and C8–8′ Calculated for Energy-Minimized Structures of the Cinnamate Diesters of (a) Cinnamic Acid **1**, (b) 4-Hydroxycinnamic Acid **2**, (c) 3-Hydroxycinnamic Acid **3**, (d) 2-Hydroxycinnamic Acid **4**, (e) 4-Hydroxy-3-Methoxycinnamic Acid **5**, and (f) 3,5-Dimethoxy-4-hydroxycinnamic Acid **6**

	C7–7′	C8–8′
a	3.550	3.552
b	3.564	3.561
c	3.544	3.564
d	4.127	3.390
e	3.596	3.561
f	3.614	3.590

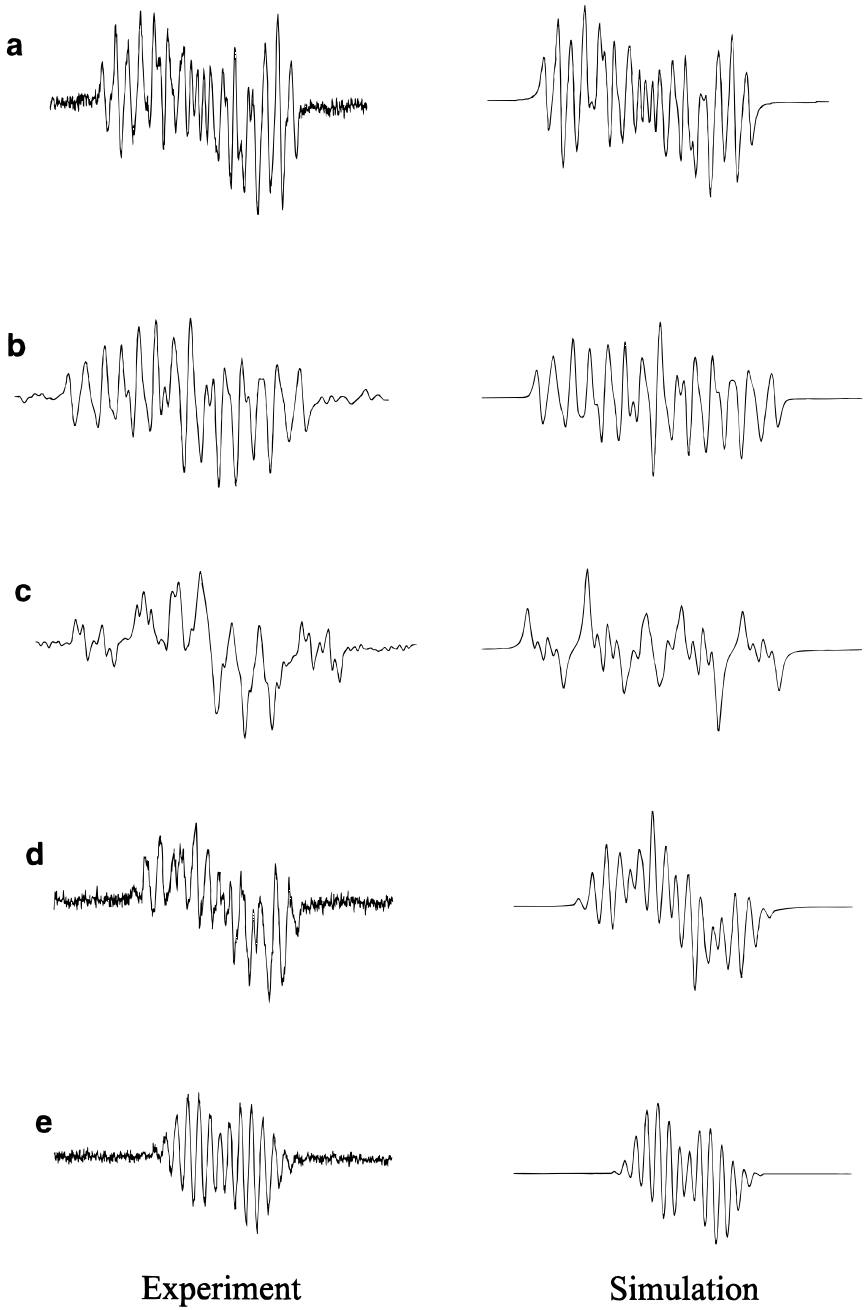

FIG. 2. ESR spectra and computer simulation of radicals obtained from (a) 4-hydroxycinnamic acid **2**, (b) 3-hydroxycinnamic acid **3**, (c) 2-hydroxycinnamic acid **4**, (d) 4-hydroxy-3-methoxycinnamic acid **5**, and (e) 3,5-dimethoxy-4-hydroxycinnamic acid **6**.

TABLE 2

ESR Hyperfine Coupling Constants (a_H) for the Radicals Generated from (a) 4-Hydroxycinnamic Acid **2**, (b) 3-Hydroxycinnamic Acid **3**, (c) 2-Hydroxycinnamic Acid **4**, (d) 4-Hydroxy-3-methoxycinnamic Acid **5**, and (e) 3,5-Dimethoxy-4-hydroxycinnamic Acid **6**

	$a_{H(2)}$	$a_{H(3)}$	$a_{H(4)}$	$a_{H(5)}$	$a_{H(6)}$	$a_{H(7)}$	$a_{H(8)}$	$a_{H(OCH3)}$
a	1.68	5.26	—	5.65	1.68	3.06	6.36	—
b	6.21	—	6.22	1.08	9.29	0.56	2.22	—
c	—	3.74	2.56	9.08	1.64	2.06	5.50	—
d	1.53	—	—	5.28	1.70	2.64	5.56	1.69
e	0.95	—	—	—	1.26	1.69	4.82	1.33

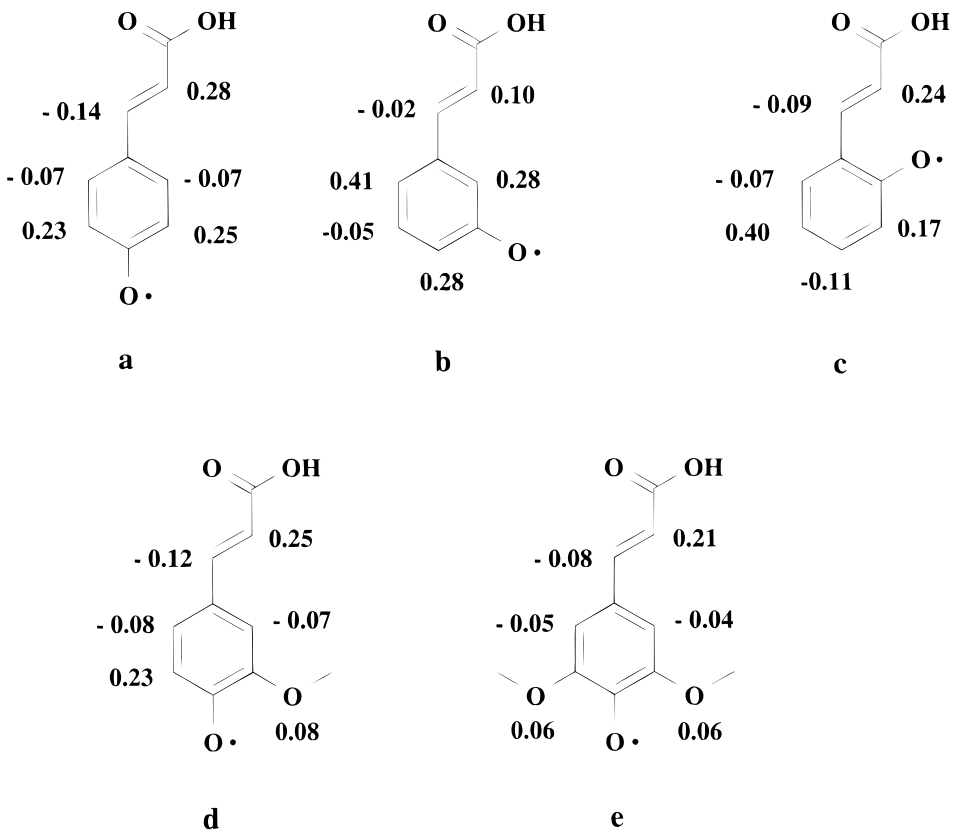

FIG. 3. Unpaired electron density calculated from ESR hyperfine splittings *via* the McConnell equation for (a) 4-hydroxycinnamic acid **2**, (b) 3-hydroxycinnamic acid **3**, (c) 2-hydroxycinnamic acid **4**, (d) 4-hydroxy-3-methoxycinnamic acid **5**, and (e) 3,5-dimethoxy-4-hydroxycinnamic acid **6**.

TABLE 3

Rate Constants (mol^{-1} dm^3 s^{-1}) and r^2 Values for the Diesters of (a) 4-Hydroxycinnamic Acid **2**, (b) 3-Hydroxycinnamic Acid **3**, (c) 2-Hydroxycinnamic Acid **4**, (d) 4-Hydroxy-3-methoxycinnamic Acid **5**, and (e) 3,5-Dimethoxy-4-hydroxycinnamic Acid **6**

	a	b	c	d	e
Rate constant	2.77×10^{-4}	0.81×10^{-4}	—	3.28×10^{-4}	7.06×10^{-4}
r^2	0.986	0.911	—	0.954	0.995

Cyclization of the 4-hydroxycinnamate diester (**2a**) had a significantly higher rate constant than the 3-hydroxylated model (**3a**) and the rate constant was further increased by methoxyl substitution (**5a** and **6a**) (Table 3). The limited delocalization of the unpaired electron to C8 for the 3-hydroxylated diester may explain the low efficiency of this reaction. The similar values obtained for spin density at C8 for the 4-hydroxylated, mono- and dimethoxylated models, however, could not explain the apparent increase in reaction rate with increasing methoxyl substitution on the basis of spin density at this position alone. A more likely explanation of the enhanced rate of photodimerization with methoxyl substitution is the overall π electron density observed to increase with increasing methoxylation.

Incubation of all the models with peroxidase at various pH values failed to yield

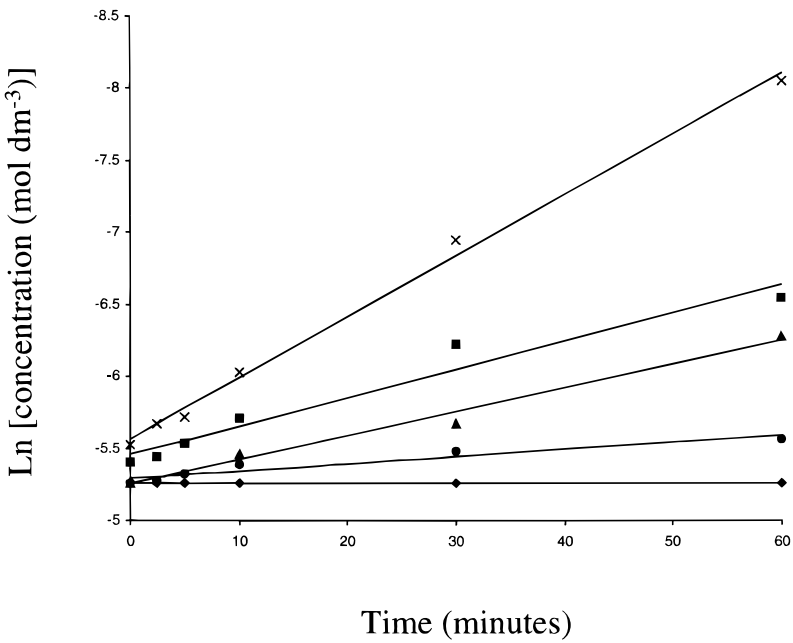

FIG. 4. Graph of ln [concentration (mol dm^{-3})] against time (minutes) with best fit line (Table 2) for the diesters of ▲, 4-hydroxycinnamic acid **2**; ●, 3-hydroxycinnamic acid **3**; ♦, 2-hydroxycinnamic acid **4**; ×, 4-hydroxy-3-methoxycinnamic acid **5**; and ×, 3,5-dimethoxy-4-hydroxycinnamic acid **6**.

TABLE 4

Chemical Shift, Coupling Constants, and Estimated Dihedral Angles for the Cyclobutane Protons of Products A and B for the Photodimerized Diesters of (a) 4-Hydroxycinnamic Acid **2**, (b) 3-Hydroxycinnamic Acid **3**, (c) 2-Hydroxycinnamic Acid **4**, (d) 4-Hydroxy-3-Methoxycinnamic Acid **5**, and (e) 3,5-Dimethoxy-4-Hydroxycinnamic Acid **6**

	Ratio A:B	Product A (δ 3.0 and 3.6 ppm)	Estimated φ	Product B (δ 3.0 and 3.6 ppm)	Estimated φ
a	1.5:1.0	(J = 10.1 and 3.8 Hz)	21° 123°	(J = 6.2 and 2.4 Hz)	133° 115°
b	0.8:1.0	(J = 9.8 and 3.7 Hz)	23° 122°	(J = 6.6 and 2.2 Hz)	135° 114°
c	—	—	—	—	—
d	1.0:1.0	(J = 10.1 and 3.8 Hz)	21° 123°	(J = 5.8 and 1.9 Hz)	131° 112°
e	1.0:1.0	(J = 10.4 and 4.0 Hz)	19° 124°	(J = 6.0 and 2.3 Hz)	132° 115°

cycloaddition products. This was as expected for the 3-hydroxylated model for which only starting material was recovered after incubation. Delocalization of the unpaired electron to C8 is a requirement for oxidative coupling of phenylpropanoids which was not possible in the case of the 3-hydroxylated compound (**3a**). The recovery of virtually all of the 2-hydroxylated derivative (**4a**) and most of the 4-hydroxylated model, normally considered substrates for peroxidase-catalyzed oxidation, was probably due to the decreased electron density on the phenolic hydroxyl, in comparison with the methoxylated compounds (*12*) and their decreased ability to reduce peroxidase compound I (*18*). Products of oxidative coupling, normally observed for both the mono- and the dimethoxylated substrates, were present as characterized previously (*12*). This indicates that the phenoxyl radical was generated. However, no signals indicative of cycloaddition products were detected for either substrate.

It is possible that an enzyme-catalyzed mechanism may operate *in vivo* and that the experimental conditions used in the incubations were inappropriate. Therefore, incubation of with silver(I) oxide, a known one-electron oxidant of phenylpropanoids, was performed. Again, there was no cyclodimer formation observed. These results and the recent discovery of a unsubstituted cyclobutane derivative from a plant source (*19*) appear to support the hypothesis that light-catalyzed cyclodimerization is the most likely mechanism for the formation of truxillic and truxinic acids in plant cell walls. This may argue for a role for cycloaddition products in the trophic response of the growing plant to light.

EXPERIMENTAL

General laboratory reagents and substituted 4-hydroxycinnamic acids were from Aldrich (UK). Evaporations were performed under reduced pressure at temperatures less than 40°C. NMR spectra were recorded using a Jeol LA-300 spectrometer, fitted with a 5-mm multinuclear, normal-geometry TH5 probe. Operating field for ^{1}H NMR was 300.4 MHz. Tetramethylsilane (0.03% v/v) was used as an internal reference and samples were run with a probe temperature of 25°C. J values are given in Hz.

Electron spin resonance and computer simulation. Radicals were generated in a two-stream ESR flow cell, positioned in the cavity of a Bruker E106 spectrometer,

via the continuous mixing of substrate (10^{-2} mol dm^{-3} in 50% v/v methanol) and ammonium cerium(IV) nitrate (10^{-3} mol dm^{-3} in 0.25 mol dm^{-3} sulfuric acid) solutions. Flow was maintained using a Watson–Marlow Flow 505S/RL pump placed before the cell which produced a combined flow rate of 4.8 cm^3 s^{-1}. Spectra (X-band) were recorded using the instrument settings modulation frequency 100 KHz, center field 3479 Gauss, sweep width 40 Gauss, time constant 41 ms, sweep time 90 s, and power 20 mW and a suitable receiver gain setting (typically 6.3×10^4). Computer simulations of spectra, giving the hydrogen hyperfine coupling constants (a_H), were performed using the SIMEPR program (*20*), which sequentially varies all the parameters for each radical species until a minimum in the error surface is located. Goodness of fit was determined by visual comparison and as a minimum in the sum of the squared residuals. The density of the unpaired electron (ρ) at each carbon atom was calculated from the hyperfine coupling constants using the McConnell relationship, $a_H = \rho Q$, in which Q is the proportionality factor. (*16*).

Modeling. Theoretical values for the spin density were obtained by the AM1 method using the HyperChem molecular simulation program. Compensation for the radical center was achieved by deleting the phenoxyl hydrogen and setting the specific charge to zero and spin multiplicity to 2. An Unrestricted Hartree-Fock wave function was then selected to allow for the unpaired electron. Once optimized, a single point calculation employing AM1 was performed to give distribution of spin density. Energy minimization calculations were performed using the Universal 1.01 force field using the program Cerius^2 developed by BIOSYM/Molecular Simulations on an SGI Indigo 2 workstation. Initially most molecular systems were minimized by the steepest-descent method for approximately 100 steps. Conjugate gradient minimization was then used until a convergence criteria of 0.1 kcal mol^{-1} Å^{-1} was reached.

Preparation of the methoxycarbonyloxycinnamic acids. To a solution of NaOH (3 g, 75 mmol) in water (80 cm^3), cooled to −10°C, the respective hydroxycinnamic acids (26 mmol) were added and vigorously stirred. $ClCO_2CH_3$ (4 g, 36 mmol) was then added dropwise and the mixture maintained at −5°C for a further 4 h. The pH was reduced to pH 5 with HCl/water (1/1) and the resulting precipitate was filtered. Crystallization from ethanol afforded the corresponding methoxycarbonyloxycinnamic acids as white needles. 4-Methoxy carbonyloxycinnamic acid (70%): mp 194–196°C, δ_H (CD_3OD) 3.88 (3H, s, OCH_3), 6.47 (1H, d, J = 16, C(8)H), 7.23 (2H, dt, J = 8.7 and 2.7, C(3,5)H), 7.64 (2H, dt, J = 8.7 and 2.7, C(2,6)H), 7.66 (1H, d, J = 16, C(7)H) ppm, *m/z* 221. 3-Methoxy carbonyloxycinnamic acid (72%): mp 138–141°C, δ_H (CD_3OD) 3.88 (3H, s, OCH_3), 6.51 (1H, d, J = 16, C(8)H), 7.22 (1H, ddd, J = 8 and 2.2, C(4)H), 7.44 (1H, t, J = 8, C(5)H), 7.44 (1H, t, J = 2.2, C(2)H), 7.51 (1H, ddd, J = 8 and 2.2, C(6)H), 7.65 (1H, d, J = 16 C(7)H) ppm, *m/z* 221. 2-Methoxy carbonyloxycinnamic acid (68%): mp 174–176°C, δ_H (CD_3OD) 3.89 (3H, s, OCH_3), 6.52 (1H, d, J = 16, C(8)H), 7.25 (1H, dd, J = 8 and 1.7 C(6)H), 7.32 (1H, dt, J = 8 and 1.7, C(5)H), 7.45 (1H, dt, J = 8 and 1.7, C(4)H), 7.74 (1H, d, J = 16, C(7)H), 7.8 (1H, dd, J = 8 and 1.7, C(3)H) ppm, *m/z* 221. 3-Methoxy-4-methoxy carbonyloxycinnamic acid (50%): mp 170–173°C, δ_H ($(CD_3)_2CO$) 3.87 (3H, s, OCH_3), 3.94 (3H, s, CO_2CH_3), 6.58 (1H, d, J = 16, C(8)H), 7.22 (1H, d, J = 8.2, C(2)H), 7.30 (1H, dd, J = 8.2 and 1.8, C(6)H), 7.51 (1H, d, J = 1.8, C(5)H), 7.67 (1H, d, J = 16, C(7)H) ppm, *m/z* 251. 3,5-Dimethoxy-4-methoxy carbonyloxycinnamic

acid (70%): mp 156–159°C, δ_H ($(CD_3)_2CO$) 3.86 (3H, s, OCH_3), 6.60 (1H, d, J = 15.8, C(8)H), 7.13 (2H, s, C(2,6)H), 7.65 (1H, d, J = 15.8, C(7)H) ppm, *m/z* 281.

Preparation of methoxycarbonyloxy protected substituted butane-1,4-dicinnamates. 4-Methoxycarbonyloxycinnamic acid (1.11 g, 5 mmol) was dissolved in toluene (150 cm^3) under reflux. $SOCl_2$ (0.41 cm^3, 5.5 mmol) was added and the mixture maintained under reflux for 90 min. The solution was cooled and the solvent removed under reduced pressure to yield a white solid. This product was suspended in pyridine (75 cm^3) under nitrogen, and butane-1,4-diol (0.2 cm^3, 2 mmol) was added. This mixture was left with stirring at room temperature for 24 h. The pyridine was removed by coevaporation with toluene under reduced pressure and the product redissolved in $CHCl_3$. This was extracted with $NaHCO_3$ (3% w/v in water) and the organic layer dried over anhydrous Na_2SO_4. The solvent was then removed *in vacuo*. Crystallization from chloroform afforded butane 1,4-di-(4-methoxycarbonyloxy) cinnamate as white crystals (35%): mp 171–173°C, δ_H ($CDCl_3$) 1.85 (4H, br signal, $C(CH_2)_2C$), 3.91 (6H, s, CO_2CH_3), 4.28 (4H, br signal, OCH_2), 6.4, (2H, d, *J*-16, C(8)H), 7.21 (4H dt, J = 8.8 and 1.9, C(2,6)H), 7.54 (4H, dt, J = 8.8 and 1.9, C(3,5)H), 7.66 (2H, d, J = 16, C(7)H) ppm, *m/z* 497. All other methoxycarbonyloxy-protected butane 1,4-diol dicinnamates were prepared by the same method. Butane 1,4-di-(3-methoxycarbonyloxy)cinnamate was crystallized from methanol as white plates (30%): mp 109–111°C, δ_H ($CDCl_3$) 1.84 (4H, br signal, $C(CH_2)_2C$), 3.91 (6H, s, CO_2CH_3), 4.28 (4H, br signal, OCH_2), 6.44, (2H d, J = 16, C(8)H), 7.2 (2H, t, J = 4.6, C(5)H), 7.3 (2H, d, J = 1.2, C(2)H), 7.4 (4H, dt, J = 4.6 and 1.2, C(4,6)H), 7.65 (1H, d, J = 16, C(7)H) ppm, *m/z* 497. Butane 1,4-di-(2-methoxycarbonyloxy)cinnamate was crystallized from a large volume of methanol as off-white plates (49%): mp 119–122°C, δ_H ($CDCl_3$) 1.8 (4H, br signal, $C(CH_2)_2C$), 3.90 (6H, s, CO_2CH_3), 4.3 (4H, br signal, OCH_2), 6.48, (2H, d, J = 16, C(8)H), 7.22 (2H, dd, J = 8.0 and 1.0, C(6)H), 7.28 (2H, dt, J = 8.0 and 1.0, C(5)H), 7.41 (2H, dt, J = 8.0 and 1.0, C(4)H), 7.63 (2H, dd, J = 8.0 and 1.0, C(3)H), 7.65 (2H, d, J = 16, C(7)H) ppm, *m/z* 497. Butane 1,4-di-(4-methoxycarbonyloxy-3-methoxy)cinnamate was obtained as an oil (65%): δ_H ($(CD_3)_2CO$) 1.83 (4H, br signal, $C(CH_2)_2C$), 3.88 (6H, s, OCH_3), 3.90 (6H, s, CO_2CH_3), 4.26 (4H, br signal, OCH_2), 6.75 (2H, d, J = 16, C(8)H), 7.28 (2H, d, J = 8.01, C(6)H), 7.34 (2H, d, J = 8.01, C(5)H), 7.58 (2H, s, C(2)H), 7.69 (2H, d, J = 16, C(8)H) ppm. Butane 1,4-di-(4-methoxycarbonyloxy-3,5-dimethoxy)cinnamate was obtained as a brown solid (60%): mp 87–88°C, δ_H ($(CD_3)_2CO$) 1.77 (4H, br signal, $C(CH_2)_2C$), 3.81 (6H, s, OCH_3), 3.84 (6H, s, CO_2CH_3), 4.26 (4H, br signal, OCH_2), 6.74 (2H, d, J = 16, C(8)H), 7.26 (4H, s, C(2,6)H), 7.62 (2H, d, J = 16, C(7)H) ppm.

Preparation of the substituted butane 1,4-dicinnamates. Butane 1,4-di-(4-methoxycarbonyloxy)cinnamate (0.65 g, 1.3 mmol) was suspended in ethanol (288 cm^3). Ammonia solution (112 cm^3, 35% w/v in water) was added and the reaction left for 16 h in which time the solution became clear. The solution was then neutralized by dropwise addition of acetic acid and the solvent removed under reduced pressure. The crude product was then dissolved in ethyl acetate and extracted with $NaHCO_3$ (3% w/v in water). The organic layer was then dried over anhydrous Na_2SO_4 and filtered and the solvent evaporated under reduced pressure. Crystallization from methanol afforded butane 1,4-di-(4-hydroxy)cinnamate as white crystals (71%): mp 176–177°C, δ_H (CD_3OD) 1.83 (4H, br signal, $C(CH_2)_2C$), 4.23 (4H, br signal, OCH_2),

6.32, (2H, d, J = 16, C(7)H), 6.78 (4H, d, J = 8.69, C(2,6)H), 7.44 (4H, d, J = 8.6, C(3,5)H), 7.60 (2H, d, J = 16, C(8)H) ppm, m/z 381. Calcd for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80%. Found: C, 68.65; H, 5.68%. All other substituted butane 1,4-dicinnamates were produced by the same method. Butane 1,4-di-(3-hydroxy)cinnamate was crystallized from methanol as brown crystals (98%): mp 172–174°C, δ_H (CD_3OD) 1.84 (4H, br signal, $C(CH_2)_2C$), 4.26 (4H, br signal, OCH_2), 6.45, (2H, d, J-16, C(8)H), 6.82 (2H, dd, J = 7.8 and 1.9, C(6)H), 6.99 (2H, d, J = 1.9, C(2)H), 7.03 (2H, dd, J = 7.9 and 1.9, C(4)H), 7.2 (2H, t, J = 7.8, C(5)H) 7.59 (2H, d, J = 16, C(7)H) ppm, m/z 381. Calcd for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80%. Found: C, 69.14; H, 5.85%. Butane 1,4-di-(2-hydroxy)cinnamate was crystallized from methanol as light brown crystals (89%): mp 200–202°C, δ_H (CD_3OD) 1.85 (4H, br signal, $C(CH_2)_2C$), 4.25 (4H, br signal, OCH_2), 6.60, (2H, d, J = 16, C(8)H), 6.82 (2H, dt, J = 8.1 and 1.4, C(5)H), 6.83 (2H, dd, J = 8.1 and 1.4, C(6)H), 7.28 (2H, dt, J = 8.1 and 1.4, C(4)H), 7.48 (2H, dd, J = 8.1 and 1.4, C(3)H), 7.65 (2H, d, J = 16, C(7)H) ppm, m/z 381. Calcd for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80%. Found: C, 68.45; H, 6.19%. Butane 1,4-di-(3-methoxy-4-hydroxy)cinnamate was crystallized from methanol/water as a yellow-brown crystalline solid (36%): mp 157–159°C, δ_H ($(CD_3)_2CO$) 1.82 (4H, br signal, $C(CH_2)_2C$), 3.91 (6H, s, OCH_3), 4.23 (4H, br signal, OCH_2), 6.42 (2H, d, J = 15.8, C(8)H), 6.87 (2H, d, J = 8.2, C(5)H), 7.15 (2H, d, J = 8.2 and 1.88, C(6)H), 7.35 (2H, d, J = 1.88, C(2)H), 7.61 (2H, d, J = 15.8, C(7)H) ppm, m/z 441. Calcd for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92%. Found: C, 64.71; H, 6.04%. Butane 1,4-di-(3,5-dimethoxy-4-hydroxy)cinnamate was crystallized from methanol/water as yellow crystals (34%): mp 155–156°C, δ_H ($(CD_3)_2CO$) 1.83 (4H, br signal, $C(CH_2)_2C$), 3.89 (6H, s, OCH_3), 4.23 (4H, br signal, OCH_2), 6.45 (2H, d, J = 16, C(8)H), 7.03 (4H, s, C(2,6)H), 7.60 (2H, d, J = 16, C(7)H) ppm, m/z 501. Calcd for $C_{26}H_{30}O_{10}$: C, 62.14; H, 6.02%. Found: C, 61.45; H, 6.12%.

Photodimerization. De-aerated solutions in $(CD_3)_2CO$ were irradiated in a Hanova irradiation cell at 3500 Å. Samples (0.5 cm^3) were taken at 2.5, 5.0, 10.0, 30.0, and 60.0 min and the products observed immediately by NMR.

Reaction with peroxidase. The substituted butane 1,4-dicinnamates (64 mmol) were dissolved in buffer/methanol (1:1) at pH 5, pH 7, and pH 9. Solutions were individually added with H_2O_2 (30 μl, 30%) in the same buffer to peroxidase (Type I ex horseradish; Sigma) (1 mg, 116 U) at a rate of 1 $cm^3\ h^{-1}$ under nitrogen and with light excluded. After 24 h the products were extracted into ethyl acetate. The organic layer was then washed with water and dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation at reduced pressure.

Reaction with silver(I) oxide. The substituted butane 1,4-dicinnamates (32 mmol) were dissolved in CH_2Cl_2 (25 cm^3) under nitrogen and light was excluded. Ag_2O (1.5 eq) was added. After 24 h the product was isolated by filtration through a bed of magnesium sulfate and removal of the solvent by evaporation at reduced pressure.

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