

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. © 1999

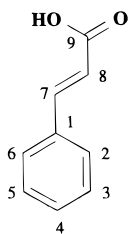
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INTRODUCTION

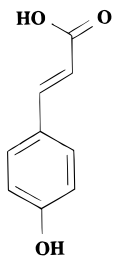
Photochemical cyclodimerization of crystalline cinnamic acid (**1**) (Fig. 1) has been extensively studied and the efficiency of the reaction in the solid state has been shown to be determined entirely by the dimensions of the crystal lattice and hence by the separation and relative orientation of the exocyclic double bonds (*J*). 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

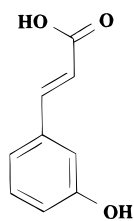




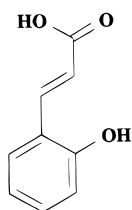
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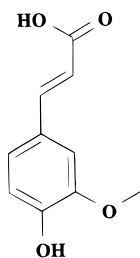
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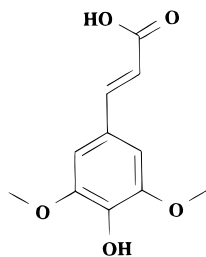
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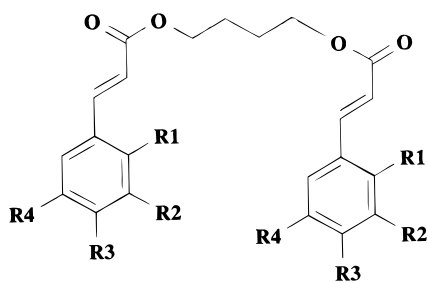
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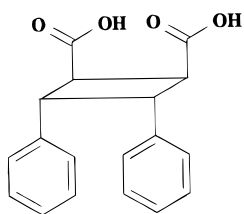
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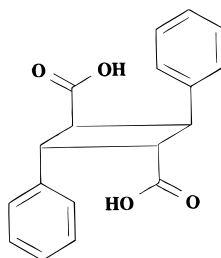
6



2a - 6a



7



8

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* conformer 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 = OCH₃; R1 and R4 = H), and 3,5-dimethoxy-4-hydroxycinnamic acid **6a** (R3 = OH; R1 = H; R2 and R4 = OCH₃) 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 Cerius² 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

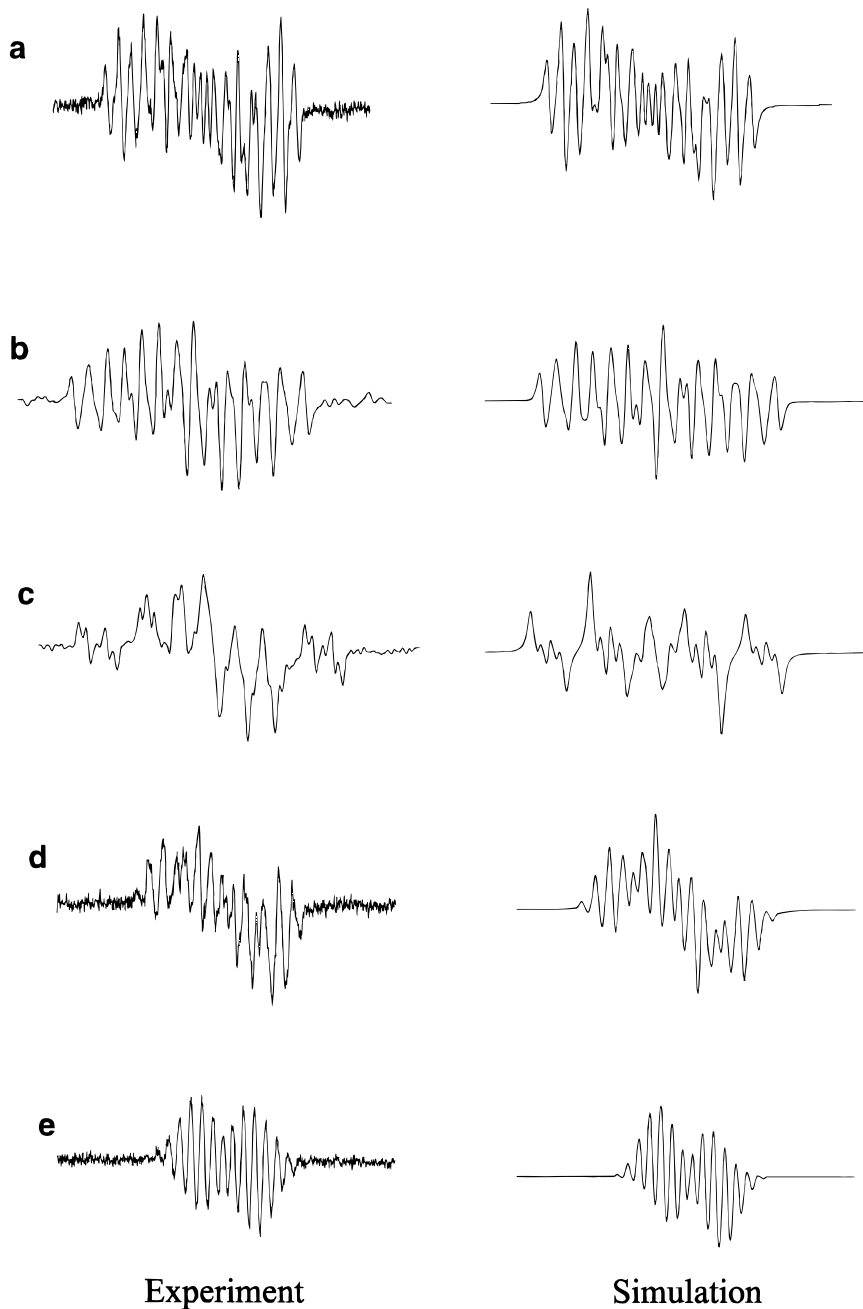


FIG. 2. ESR spectra and computer simulation of radicals obtained from (a) 4-hydroxycinnamic acid **2**, (b) 3-hydroxycinnamic acid **3**, (c) 2-hydroxycinnamic acid **4**, (d) 4-hydroxy-3-methoxycinnamic acid **5**, and (e) 3,5-dimethoxy-4-hydroxycinnamic acid **6**.

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**

	$a_{H(2)}$	$a_{H(3)}$	$a_{H(4)}$	$a_{H(5)}$	$a_{H(6)}$	$a_{H(7)}$	$a_{H(8)}$	$a_{H(OCH_3)}$
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

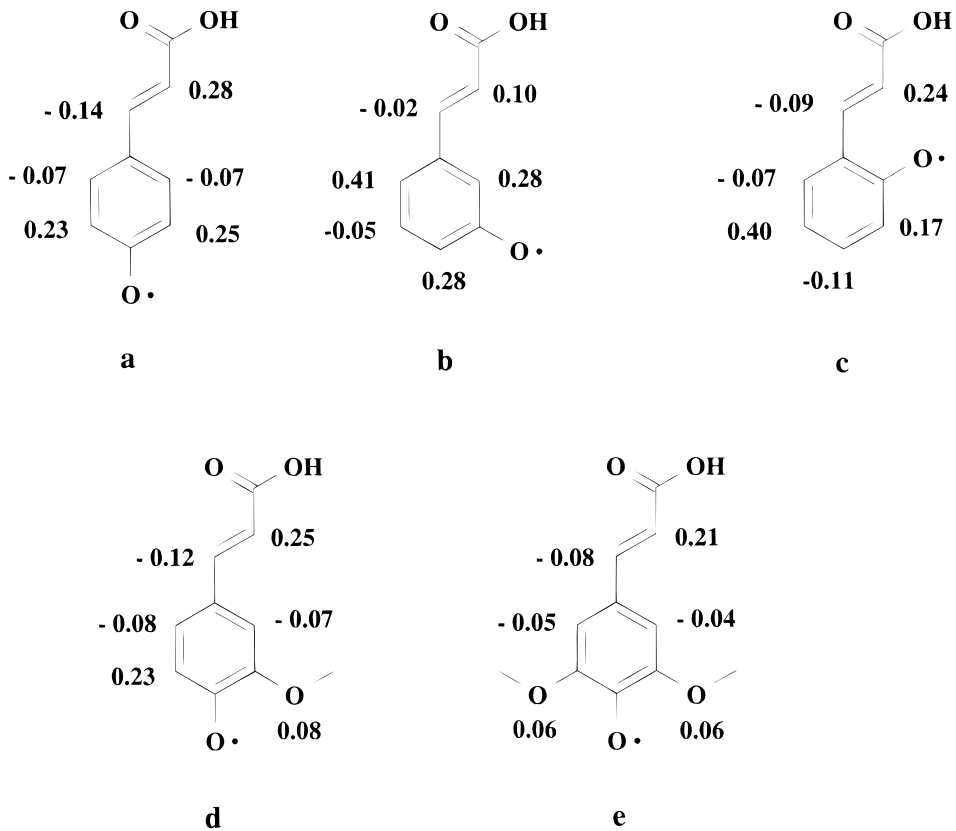


FIG. 3. Unpaired electron density calculated from ESR hyperfine splittings *via* the McConnell equation for (a) 4-hydroxycinnamic acid **2**, (b) 3-hydroxycinnamic acid **3**, (c) 2-hydroxycinnamic acid **4**, (d) 4-hydroxy-3-methoxycinnamic acid **5**, and (e) 3,5-dimethoxy-4-hydroxycinnamic acid **6**.