Effect of cyclodextrin complexation on the photochemistry of the lignin model α-guaiacoxyacetoveratrone

L.T. Okano, R. Ovans, V. Zunic, J.N. Moorthy, and C. Bohne

Abstract: The photodecomposition of α-guaiacoxyacetoveratrone (GAV) in homogeneous solvents and in aqueous cyclodextrin solutions was studied by following the fluorescence emission of the photoproducts formed. The same qualitative behavior as previously observed for the photodegradation quantum yield measurements was reproduced in the fluorescence studies in dry or wet methanol and acetonitrile. The yield for GAV photodegradation in water is smaller than in the organic solvents. Complexation of GAV to β- and γ-cyclodextrins leads to an increase in the photodecomposition yield, especially during the early part of the photodecomposition kinetics. The transients formed in the photolysis of GAV in water are similar to those observed in acetonitrile, and the lifetime of triplet GAV in water is 130 ns. In addition, solvated electrons were formed when GAV was photolyzed in water. Complexation of GAV to cyclodextrins leads to an increase in the triplet yield compared to the radical yield and a moderate shortening of the triplet lifetime.

Key words: α-guaiacoxyacetoveratrone, cyclodextrin, excited singlet states, excited triplet states, radicals.

Résumé : Utilisant l'émission de fluorescence des photoproduits formés, on a étudié la photodécomposition de l'α-guaïacoxyacétovératrone (GAV) dans des solvants homogènes et dans des solutions aqueuses de cyclodextrine. Dans les études de fluorescence dans le méthanol sec ou aqueux ainsi que dans l'acétonitrile, on a reproduit le même comportement qualitatif que celui observé antérieurement lors de mesures de rendements quantiques pour la photodégradation. Le rendement pour la photodégradation de la GAV dans l'eau est inférieur à celui observé dans les solvants organiques. La complexation de la GAV par les β- et γ-cyclodextrines provoque une augmentation du rendement de photodécomposition, particulièrement au début de la cinétique de photodécomposition. Les espèces transitoires qui se forment lors de la photolyse de la GAV dans l'eau sont semblables à celles observées dans l'acétonitrile et le temps de vie de l'état triplet de la GAV dans l'eau est de 130 ns. De plus, il se forme des électrons solvatés lors de la photolyse de la GAV dans l'eau. La complexation de la GAV par les cyclodextrines provoque une augmentation du rendement de l'état triplet par rapport au rendement en radical ainsi qu'à une diminution modérée du temps de vie du triplet.

Mots clés : α-guaïacoxyacétovératrone, cyclodextrine, états singulets excités, états triplets excités, radicaux.

[Traduit par la Rédaction]

Introduction

The photodecomposition of lignin is responsible for the yellowing observed in paper manufactured from mechanical pulp. In recent years, a significant effort has been made to find efficient inhibition strategies to eliminate or considerably slow down the yellowing of lignin-containing paper. The mechanisms for lignin photodegradation are complex involving several reactive intermediates (1, 2). Most mechanistic studies have been performed in solution employing model compounds because lignin is a high molecular weight polymer that is difficult to handle in solution. Solution studies were important to determine the decomposition pathways, the multiplicity of the excited states involved, and to characterize spectroscopically the reactive intermediates involved (3–13). α-Guaiacoxyacetoveratrone (GAV) has frequently been used as a model compound (8, 9, 13, 14), because it contains key features of lignin. In contrast to other aryl ketones, it was shown that α-phenoxyacetophenones, such as GAV, react from their excited singlet state (8, 11–14) to form the corresponding phenacyl and phenoxy radicals (Scheme 1). This reactivity suggests that the β-cleavage process from the excited singlet state readily competes with intersystem crossing, leading to quantum yields for the latter process that are significantly smaller than unity (13). Compared to α-phenoxyacetophenone (15), the triplet reactivity of GAV is diminished because its lowest triplet state has a π,π^* configuration (9). The triplet lifetime is determined by the efficiency for intramolecular deactivation through β-phenyl quenching, as was observed for β-arylpropiophenone and α-(aryloxy)acetophenones (15–21), and the rate constant for β-cleavage (14). The photodecomposition quantum yields and triplet lifetimes of GAV vary significantly in different solvents (8, 14). This complex behavior is

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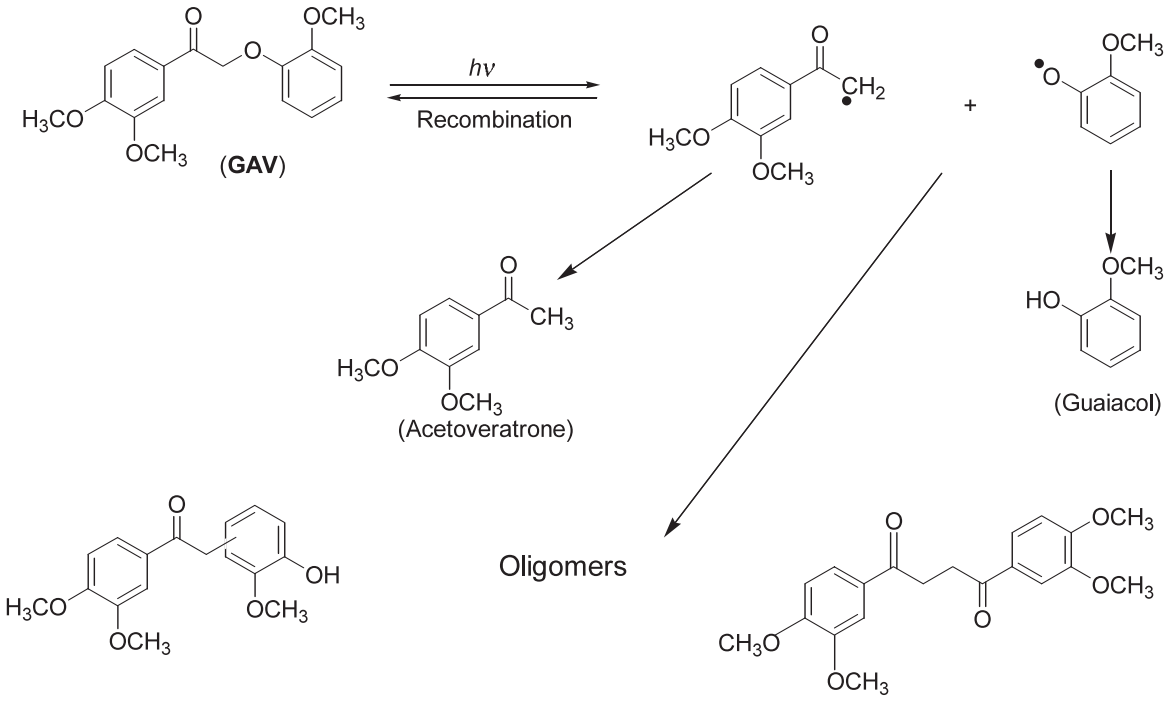
Scheme 1.



related to the reactivity from the GAV singlet excited state and a solvent effect on the intramolecular quenching in the triplet state.

Lignin is located in a constrained environment in the cell wall of wood fibers that influences to a different extent the deactivation pathways of the excited singlet and triplet states when compared to these processes in solution. There are a limited number of studies on the photoreactivity of lignin model compounds in constrained environments or on solid supports. The photodegradation of GAV has been shown to decrease when this compound is adsorbed on paper fibers (9). In addition, GAV is stable in the crystalline state, a fact that was attributed to the formation of excimer-like arrangements in the solid state (6). The photochemistry of α-(aryloxy)acetoveratrones was studied on solid silica gel, Na-X zeolites, and cellulose (4). In the case of cellulose, an inefficient photodecomposition and an enhanced triplet lifetime were observed. These experiments show that the confinement of lignin in the fibers of paper can alter the lignin photoreactivity. Furthermore, the mobility of oxygen through the paper fiber may also be important, since yellow products are only formed in the presence of oxygen (2). In this respect, it has been shown that the quenching of reactive intermediates on cellulose or paper by oxygen is very inefficient (3, 4, 22–24), suggesting that the reaction of reactive intermediates with oxygen may be the rate-limiting step in the photoyellowing process.

The objective of this work is to establish how constrained environments can affect the photochemistry of GAV and the mobility of the radicals formed in its photodecomposition. Since the work on solids has proven to be difficult, we decided to investigate the effect of a constrained environment on the photochemistry of GAV in solution. Cyclodextrins (CDs) were chosen, since they provide a chemical environment similar to cellulose, and the effect of CD inclusion on the photochemistry of β-phenylpropiophenone (25, 26) and α-phenoxyacetophenone (27) have been previously reported. In addition, we chose to work in water, since paper has some moisture (5–10% water), and lignin is probably exposed to a hydroxylic environment.

Experimental

The β- (lot F6080–191) and γ-CDs (lot E 8056) were a kind gift from Cerestar and were used as received. Guaiacol (98%, Aldrich) and 3,4-dimethoxyacetophenone (acetoveratrone) (98%, Aldrich) were distilled under reduced pressure, methanol (ACP, spectrograde) was distilled from CaH_2, and acetonitrile (ACP, HPLC grade) was distilled under N_2 from $LiAlH_4$. Deionized water (SYBRON, Barnstead deionizing system) was used for all aqueous samples. Whatman filter papers (number 1001 125) were used as received.

The α-guaiacoxyacetoveratrone (3′,4′-dimethoxy-α-(2-methoxyphenoxy)acetophenone, GAV) was synthesized by the method previously reported (28). The required synthesis of 3,4-dimethoxy-α-bromoacetophenone was modified from that previously reported in that dimethoxyacetophenone was reacted with pyridinium bromide perbromide in chloroform and under nitrogen (29–33). GAV was purified by repeated recrystallization from ethanol (95%), and its purity was checked by mp (77–80°C) and ^1H NMR measurements.

UV-vis absorption spectra were measured on Cary 1 or Cary 5 spectrometers from Varian. Induced circular dichroism spectra were measured on a Jasco J-720 spectropolarimeter. Fluorescence was measured with a PTI QM-2 fluorimeter at 20.0 ± 0.5°C. Most samples were excited at 280 nm, however, when necessary the excitation wavelength was shortened to avoid the Raman emission from the solvents. Emission spectra were collected before and after the photodecomposition kinetics to monitor prod-

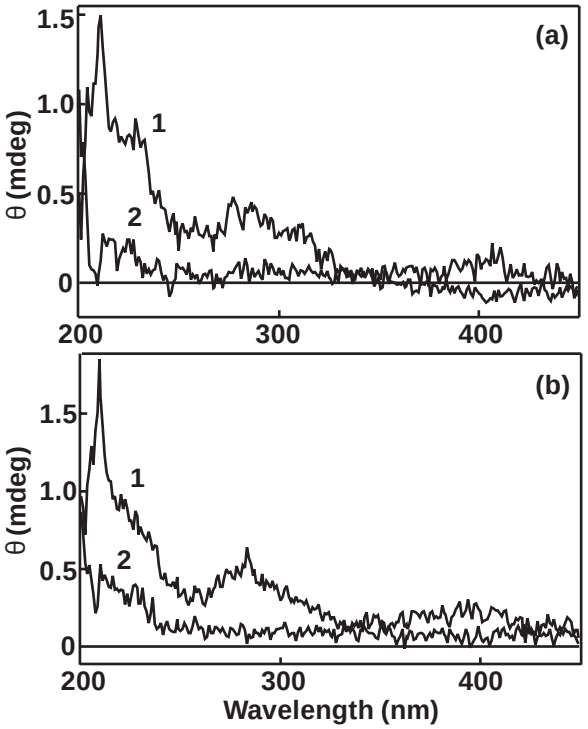
uct formation. Narrow slits (bandwidth 1–2 nm) were employed to measure the emission spectra to minimize the photodecomposition of GAV. The photodecomposition kinetics of GAV was monitored simultaneously by the product emission at 310 and 350 nm. The rate of decomposition was dependent on the excitation intensity and bandwidth (5 or 7 nm) employed for the excitation slits. Solutions were contained in 10 × 10 mm quartz cells and were purged with N_2 or O_2 for 30 min before the fluorescence measurements were performed. Solid samples were either mounted on a triangular metal support or were suspended within the sample holder. In both cases, the excitation beam and detector were in a 90° arrangement, and for the solid samples, the surface of the paper was placed at a 45° angle with respect to the excitation beam.

The laser flash photolysis system used for the transient studies has been previously described (34). Samples at 20 ± 2°C were excited either with a Lumonics excimer laser at 308 nm (<30 mJ/pulse, 5 ns pulse width) or a Spectra Physics Nd:YAG laser at 266 nm (<20 mJ/pulse, 6 ns pulse width). A program written with Labview 4.0™ (National Instruments) was used to control the laser flash photolysis systems, acquire the data, and for initial analysis of the decay curves. A flow cell (7 × 7 mm Suprasil) was employed for solutions purged with N_2 and O_2 (30–40 min) so that a fresh portion of the solution was irradiated at each laser pulse. The solution in the reservoir before entering the flow cell was covered with aluminum foil to avoid photodecomposition by ambient light. A 7 × 7 mm Suprasil cell stoppered with a rubber septum was used for N_2O purged (20 min) solutions. The photodecomposition in the static samples was followed by UV-vis absorption, and the sample was subjected to a small number of laser shots so that only limited decomposition of GAV occurred.

Solutions of GAV in all solvents except water were prepared by dissolving the solid in the respective solvent. Aqueous solutions were prepared by the injection of a small amount (<1%) of the methanolic GAV solution into water. In all cases, the concentration of GAV was 15.8 μM. The CD solutions were prepared by dissolving, in the aqueous GAV solution, the appropriate amounts of β- and γ-CD to reach final concentrations of 10 and 30 mM, respectively. These solutions were warmed in hot water for 5 min and were left stirring for at least 12 h. Some insoluble material was removed by filtering the solutions through 40 μm Millipore filters. For this reason, it is likely that the GAV and CD concentrations quoted are somewhat lower than stated. During preparation and storage, all solutions were shielded as much as possible from ambient light.

Filter paper samples (5 × 1.1 cm) containing GAV were prepared by using either the drop or immersion methods (35). For the drop method, 0.15–0.5 mL of a methanolic or methanol–water solution of GAV (≤0.01 M) was slowly dropped with a microsyringe on samples mounted in Delrin frames. Two samples were always prepared from the same GAV solutions. After application of GAV, the samples were protected from light and were dried for at least 12 h. In the case of the immersion method, two samples were simultaneously placed for 20 min in Petri dishes containing GAV solutions (≤0.01 M). After the immersion period, the paper samples were mounted on Delrin frames and were dried as described above. The immersion method leads to a more homogeneous distribution of additives in paper (35). The amount of GAV added was established by comparing the weight of each paper sample before and after the application of GAV. Filter papers without any treatment were employed as a reference. The samples were irradiated for 1 h in front of a 75W Xe lamp (PTI).

Fig. 1. Induced circular dichroism of 15.8 μM GAV in the presence of 5 mM β-CD (*a*, 1) and 10 mM γ-CD (*b*, 1). The spectra for CDs (2) are also shown.

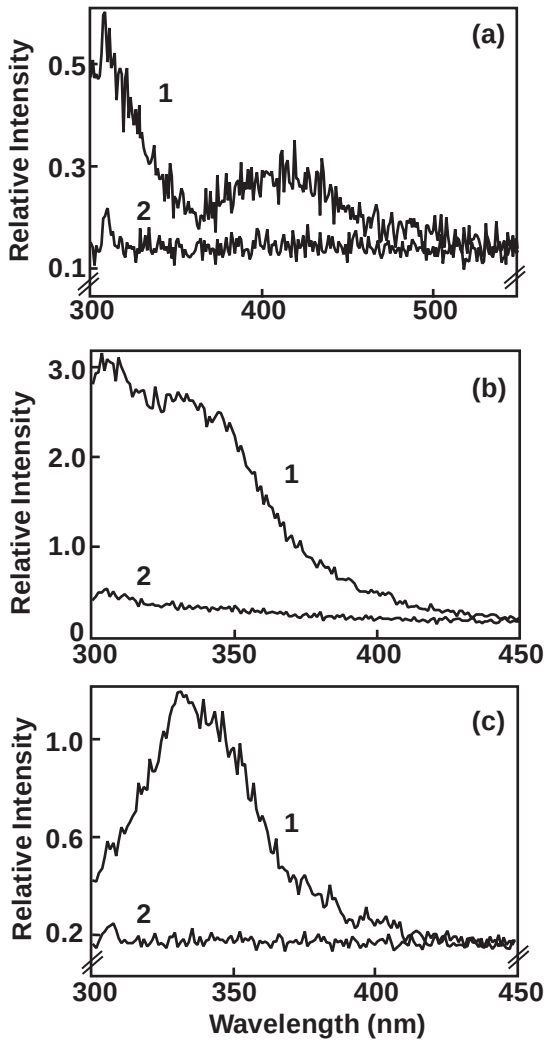


Results

Several spectroscopic techniques can be employed to establish the complexation of guest molecules with CDs. The primary consideration in choosing a technique is that a change of a spectroscopic property has to be observed for the free and complexed guest molecule. The formation of the GAV–CD complexes was observed by circular dichroism spectroscopy. GAV in water does not show a circular dichroism signal because it is achiral. In the presence of β- or γ-CD, induced circular dichroism signals were observed in the spectral region where GAV absorbs (Fig. 1). These experiments clearly show that GAV is incorporated to some extent into both CD cavities. Unfortunately, circular dichroism could not be employed to determine the equilibrium constants values and the complexation stoichiometries due to the poor signal-to-noise ratio observed and the fact that some photodestruction (<10%) occurs upon irradiation in the spectrometer. Likewise, ^1H NMR could not be employed to determine the complexation efficiency because of the low solubility of GAV in water (<20 μM).

Fluorescence is, in principle, an ideal technique to establish the complexation of guest molecules with CDs, because fluorescence properties, such as the emission quantum yields

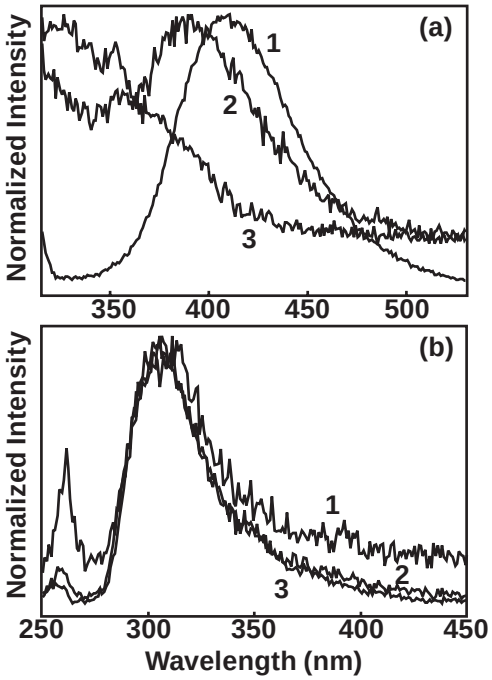
Fig. 2. Emission spectra before (2) and after (1) irradiation of 15.8 μM GAV for 1 h at 280 nm in (*a*) water, (*b*) methanol, and (*c*) acetonitrile.



and lifetimes, can be very sensitive to the environment around the fluorophore. The first approach tried to establish the complexation of GAV to β and γ-CD was fluorimetry, since an emission assigned to the fluorescence of GAV in methanol had been previously reported (6). Unfortunately, we established that GAV does not fluoresce when the samples are shielded from the exposure to ambient light (Fig. 2). Appreciable fluorescence is only observed after the samples are exposed to the excitation beam in the fluorimeter (Fig. 2). This emission was assigned to the fluorescence of photoproducts. The spectral distribution of the emission is solvent dependent. The emission observed in water and methanol between 310 and 320 nm, after exposure of the sample to the excitation beam, is not due to Raman emission of the solvent because the spectra are the same at different excitation wavelengths.

Guaiacol and acetoveratrone are likely products in the photolysis of GAV (Scheme 1). For this reason, their fluorescence spectra in various solvents was measured (Fig. 3). Guaiacol has a higher fluorescence quantum yield by about one order of magnitude than acetoveratrone. In addition, the

Fig. 3. Fluorescence spectra of 15.8 μM acetoveratrone (*a*, λ_{ex} = 280 nm) and guaiacol (*b*, λ_{ex} = 240 nm) in water (1), methanol (2), and acetonitrile (3). The sharp peak at 260 nm (*b*) corresponds to the Raman emission of the solvent.



fluorescence spectra for guaiacol were the same for all three solvents, and its emission efficiency in acetonitrile and methanol was somewhat higher (ca. 2) than in water. In contrast, the acetoveratrone emission is very solvent dependent with emission maxima at 420, 390, and ca. 330 nm, respectively, in water, methanol, and acetonitrile, and the emission quantum yield is higher in water than in the organic solvents.

The decomposition quantum yields of GAV in homogeneous solution are very dependent on the nature of the solvent and the presence of water (8). For this reason, we used the appearance of the photoproduct emission to measure relative photodecomposition yields of GAV in different solvents. The kinetic traces for the appearance of photoproducts were measured at 310 and 350 nm (Fig. 4). The rate of appearance was faster in methanol than in acetonitrile. At 310 nm, the photoproduct formation rate follows a fairly linear relationship, whereas at 350 nm, a considerable slowdown is observed as the photodecomposition of GAV proceeds. Most kinetic traces showed initially a fast increase in the fluorescence intensity. This burst kinetics appears as an initial jump in the traces shown in Fig. 4. The initial jump also incorporates any product formed when spectra were collected with narrow slits before the kinetic run and any product formed due to exposure to ambient light during sample preparation and handling.

Relative yields for the formation of products were estimated from the initial slopes of the photodecomposition kinetics after the burst phase (Table 1). In the absence of oxygen, the rate for appearance of photoproduct emission at 310 nm is highest in methanol and comparable in acetonitrile and water. At 350 nm, the photodecomposition

Table 1. Relative rates for the appearance of the photoproduct emission at 310 or 350 nm, estimated from the initial slopes and normalized to the rate at 310 nm in water in the presence of nitrogen.

	Relative rates			
Solvent	310 nm/N_2	310 nm/O_2	350 nm/N_2	350 nm/O_2
H_2O	1	1	0.3	0.5
CH_3OH	8	1	22	3
CH_3OH/H_2O (60/40)	9	3	2	1
CH_3OH/H_2O (40/60)	2	2	12	6
CH_3CN	1	0.2	3	1
CH_3CN/H_2O (60/40)	3	2	5	3
CH_3CN/H_2O (40/60)	4	4	11	2

Fig. 4. Kinetics for the appearance of photoproduct emission for the photolysis (λ_{ex} = 280 nm) of 15.8 μM GAV monitored at 310 (*a*) and 350 (*b*) nm in methanol (1), acetonitrile (2), and water (3). The opening of the shutter leads to the initial jump in the emission intensities.

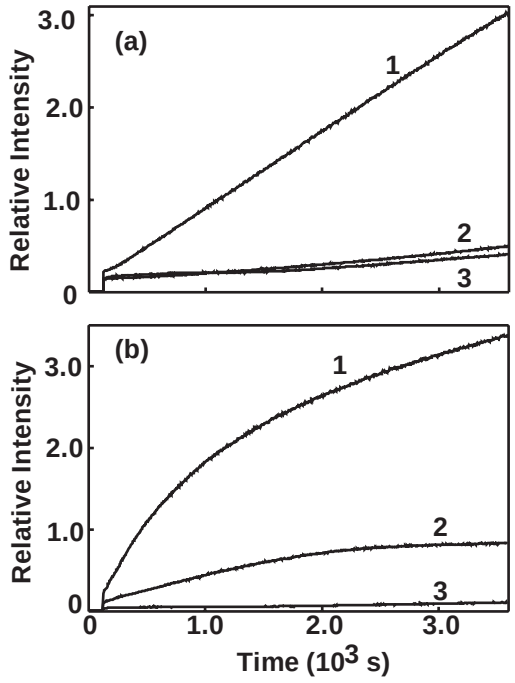
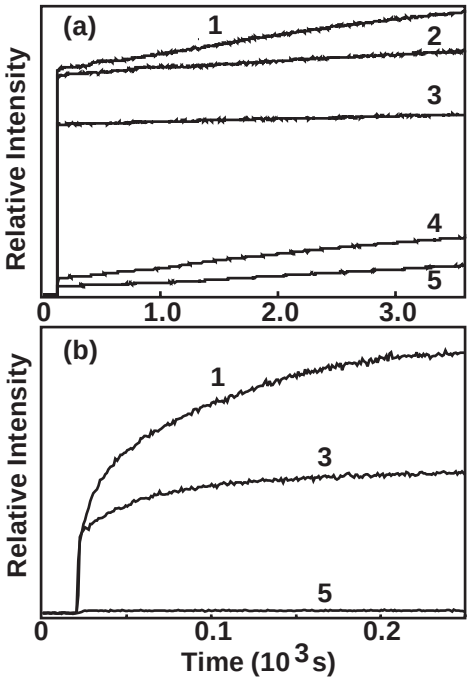


Fig. 5. Kinetics for the appearance of photoproduct emission for the photolysis (λ_{ex} = 280 nm) of 15.8 μM GAV monitored at 310 (*a*) and 345 (*b*) nm in the presence of 10 mM β-CD (1), 30 mM γ-CD (2), 10 mM γ-CD (3), 5 mM β-CD (4), and in water (5). The traces in (*a*) show the kinetics after the initial jump, while the experiments in (*b*) show the burst kinetics at short times.



rate is highest in methanol and lowest in water (Fig. 4). The presence of oxygen has only a small effect on the rate of product appearance in water, due to the low solubility of oxygen in this solvent (1.4 mM) (36). In methanol and acetonitrile, the presence of oxygen led to a marked decrease in the rate of emission of the photoproducts, suggesting that oxygen efficiently quenched the excited state responsible for product formation.

The presence of water in methanol was shown to decrease the degradation quantum yield of GAV, whereas the opposite effect was reported for the photolysis in acetonitrile (8). The relative rates for photoproduct emission showed a similar trend. The emission rate at 310 nm in methanol was only decreased at a high water content (60%), whereas a more significant decrease of the rate was measured at 350 nm (Table 1). In the case of GAV photolysis in acetonitrile, an increase of the photoproduct formation was observed in the presence of water (Table 1).

The emission spectrum of GAV in the presence of β- and γ-CD is similar to that observed for GAV in water. However, the contribution of the emission at 450 nm is higher in the presence of CDs. The complexation of GAV to β- and γ-CD led to a slight increase in the rate of appearance of the photoproduct emission when monitored at 310 and 350 nm (Fig. 5, Table 2). Some of this enhancement is quenched in the presence of oxygen. A more significant effect of the CD complexation is observed on the initial jump corresponding to the burst phase (Table 3). The enhancement of the initial jump increases when the β-CD concentration is raised, and this enhancement is not sensitive to the presence of oxygen. In contrast, the enhancement in the presence of γ-CD is sensitive to the presence of oxygen (Table 3).

Table 2. Relative rate ratio for the estimated appearance of the GAV photoproduct emission in the presence of β- and γ-CD with respect to the appearance rate in water.

	Relative rate ratio (CD/H_2O)	
CDs	310 nm/N_2	350 nm/N_2
5 mM β-	3	2
10 mM β-	4	6
10 mM γ-	1	2
30 mM γ-	3	4

Table 3. Ratios for the intensities of the initial jump in the presence of β- and γ-CD with respect to the intensity in water.

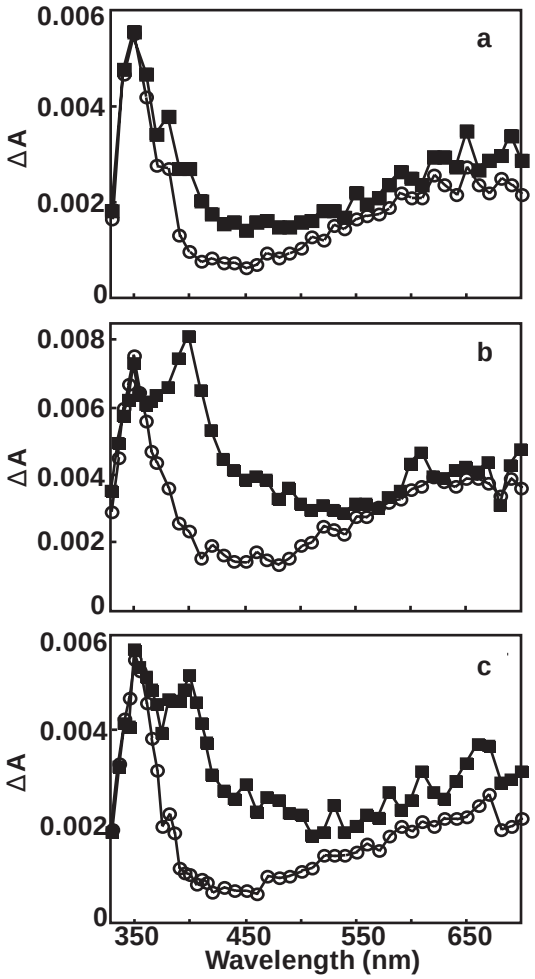
	Relative rate ratio (CD/H_2O)			
CDs	310 nm/N_2	310 nm/O_2	350 nm/N_2	350 nm/O_2
5 mM β-	2	2	3	2
10 mM β-	29	24	15	13
10 mM γ-	22	5	13	5
30 mM γ-	28	6	20	14

The emission of filter paper with and without GAV was measured to determine whether fluorescence spectroscopy could be employed to monitor the photodecomposition of GAV absorbed into the paper matrix. The filter paper without GAV showed some background emission. In the presence of GAV (≤3% w/w), the same emission spectra were observed when the sample was excited at 270, 280, or 310 nm. The emission spectra were also measured after the reference and paper sample containing GAV were irradiated for 1 h. The emission intensities measured for the reference and GAV samples were nonreproducible. In some cases, an increase of the emission intensity was observed, whereas for duplicate samples prepared from the same GAV solution a decrease of the intensity was measured. The same pattern was observed for samples prepared either by the drop or the immersion methods. Although the contradictory results on the emission intensity are difficult to explain at this point, it was observed that the magnitude of the enhancement or the decrease is dependent on the amount of GAV adsorbed into the paper and the duration of the irradiation of the sample.

Laser flash photolysis was employed to study the transient species formed in the photolysis of GAV. All transient studies, with the exception of those in the presence of N_2O, were performed using flow cells. When static cells were employed, a higher yield of radicals compared to the triplet yield was observed. This is particularly noticeable when GAV is excited in the presence of CDs. The higher yield of radicals is probably due to the accumulation of long-lived radicals after subsequent laser shots.

The transient phenomena observed after excitation at 308 nm of GAV in acetonitrile were similar to those previously reported (5, 7, 8, 13). The short-lived triplet (τ = 254 ns) was observed around 400 nm, and the weak transient absorption in the 400–500 nm region of the phenacyl radical was also present. Both these transients were efficiently quenched by oxygen. The remaining transient absorption in the 300–400 nm region in the presence of oxygen is due to the guaiacoxy radical.

Fig. 6. Transient absorption spectra of 15.8 μM GAV/N_2 after excitation at 308 nm in water (*a*, (■) 90 and (○) 570 ns delays), in the presence of 10 mM β-CD (*b*, (■) 70 and (○) 360 ns delays), and in the presence of 10 mM γ-CD (*c*, (■) 80 and (○) 640 ns delays).



The transient phenomena of GAV in water have not been previously described. The same transients as observed in acetonitrile were also present in water (Fig. 6*a*). In addition, in water we observed the formation of solvated electrons that absorb above 600 nm. The solvated electrons are probably formed in a two-photon process leading to the photoionization of GAV. The presence of solvated electrons was established by the disappearance, in the presence of N_2O, of the short-lived absorption measured at 640 nm. The triplet lifetime of GAV in water was (130 ± 10) ns, and it is shortened to (76 ± 3) ns in oxygen saturated solutions. This result is consistent with a diffusional quenching rate constant by oxygen, taking into account the oxygen solubility in water (1.4 mM).

Complexation of GAV to 10 mM β- or γ-CD leads to a larger yield of triplets when compared to the radical yield measured for the photolysis of GAV in water (Fig. 6). The triplet GAV lifetime in the presence of β-CD is (110 ± 3) ns and (104 ± 4) ns in the absence and presence of oxygen, whereas in the presence of γ-CD the respective triplet lifetimes are (97 ± 5) and (88 ± 7) ns. The residual absorption

Table 4. Average ratios for the solvated electron yield formed in the photolysis at 308 nm of GAV in the presence of CDs and in water. The number in parentheses corresponds to the number of experiments averaged.

CD	Solvated electron ratio (CD/H_2O)
10 mM β-	1.4 ± 0.2 (5)
10 mM γ-	1.2 ± 0.1 (5)
30 mM γ-	1.3 ± 0.2 (2)

observed after the decay of the triplet corresponds to the guaiacoxy radical, which is very long lived (>100 μs).

In the presence of CDs, an increase was observed for the yield of solvated electrons. The amount of solvated electrons formed was estimated by subtracting the ΔA values at 640 nm in the presence of N_2O, where all solvated electrons are quenched, from the value determined in the presence of N_2. Since the photoionization of GAV is likely to be a two-photon process, the absolute yield for solvated electron formation is very dependent on the laser energy that excites the sample. For this reason, the yields for solvated electron formation have to be compared for measurements performed for the same experimental conditions. Although the absolute values for these yields changed between experiments performed on different days, it was always observed that the yields in the presence of β- and γ-CD were higher than those measured in water (Table 4). Experiments performed by exciting GAV at 266 nm in the presence of 10 mM of both CDs led to the same ratios for solvated electron formation as observed for the excitation at 308 nm.

Discussion

The photodecomposition of GAV in homogeneous solution leads to the formation several products (Scheme 1) (8, 13, 14). Some of these photoproducts fluoresce, and since fluorescence spectroscopy is a very sensitive technique, a small amount of product formation can be detected before any decomposition is visible by other spectroscopic techniques, such as UV-vis absorption and NMR. The spectral distribution for the photoproduct emission following the photolysis of GAV is very dependent on the nature of the solvent. This difference could be due to changes in the product distribution or different emission quantum yields for each product in the different solvents. Since the emission quantum yields of guaiacol or acetoveratrone were shown not to vary by more than a factor of 2, we explain the different spectra observed to be due to different product distributions.

The products from the photolysis of GAV will have different fluorescence spectra as exemplified for guaiacol and acetoveratrone. The coupling products shown in Scheme 1 are expected to have a fluorescence emission that is similar to that of acetoveratrone, since the chromophore of the latter is present in these compounds. The emission between 300 and 320 nm observed for the photoproduct formation of GAV in methanol and water is assigned to the formation of guaiacol. This spectral feature is absent for the photolysis of GAV in acetonitrile, suggesting that guaiacol is not formed in this solvent. The lack of detection of guaiacol is not due to a sensitivity problem, since the guaiacol emission quantum yield is about one order of magnitude higher than that for acetoveratrone. The emission observed at 420 nm in water and between 320 and 340 nm in methanol and acetonitrile is assigned to products with the acetoveratrone moiety. This blue shift of the emission maxima was also observed when the fluorescence of acetoveratrone was measured in these organic solvents. The larger blue shift observed for the photoproduct emission of GAV could be due to a more significant solvent effect on the emission spectra of the coupling products when compared to the shift for acetoveratrone.

A brief comment on the fact that acetoveratrone fluoresces is justified because it has implications on why GAV is reactive from the excited singlet state. The fluorescence of some methoxy substituted aryl ketones in water has been previously reported (37), but in most cases, ketones do not fluoresce because their intersystem crossing quantum yield is close to unity (38). The intersystem crossing efficiency depends on the configurations and the energy difference between the singlet and triplet states involved in the transition. The configuration of the excited states of ketones can be influenced by the presence of electron-donating substituents that stabilize π,π^* states compared to n,π^* states (39). In the case of acetoveratrone, the presence of two methoxy substituents will stabilize the π,π^* states in the singlet and triplet manifold, leading to a decrease of the intersystem crossing rate constant by either switching the configuration of S_1 to a π,π^* one or by influencing the S_1–T_n energy gap involved in the intersystem crossing process. This decrease of the intersystem crossing quantum yield leads to lengthening of the excited singlet lifetime and the observation of fluorescence. This stabilization in the case of GAV leads to a less reactive triplet state when compared to ketones with n,π^* triplets (13). Furthermore, the GAV photophysics will primarily be determined by the acetoveratrone moiety. This ensures that the rate constant for intersystem crossing of GAV is lower than for other ketones (13) and that the GAV excited singlet state can undergo a β-cleavage reaction. In contrast to acetoveratrone, GAV does not fluoresce in solution, suggesting that the rate constant for β-cleavage is larger than the radiative rate constant responsible for fluorescence. The emission of the photoproducts of GAV in methanol was previously assigned incorrectly to the fluorescence of GAV (6), and based on this assignment, the authors explained the crystalline GAV emission with a maximum at 450 nm to be due to an excimer formed from two adjacent acetoveratrone moieties. The emission observed in the solid state may be due to the emission of GAV, since this emission occurs in the spectral region where acetoveratrone fluoresces in water. However, other evidence previously reported (6) supports this emission to be due to excimer formation. A definite assignment of this emission is problematic, since the emission spectra of acetoveratrone was shown to depend on the solvent polarity, and a similar effect is expected to be observed for GAV.

The objective of the kinetic studies for photoproduct appearance was to investigate if the relative rates observed in the fluorescence measurements can be correlated to the reported photodegradation quantum yields (8). It is important to take into account that the fluorescence intensity is directly proportional to the emission quantum yield of the excited

fluorophores. For this reason, the presence in solution of any substance that quenches the emission of the fluorophore will lead to a decrease of the measured fluorescence intensity. For example, if during the photolysis of GAV one of the products is an efficient quencher of the fluorescence from a second product, the emission intensity of the latter will be decreased. Due to this possible quenching effect, any analysis of the shape of the kinetic trace (i.e., linear at 310 nm and curved at 350 nm) was not attempted. Furthermore, the photolysis of GAV leads to a mixture of products that can fluoresce. At each wavelength, the intensity measured is a function of the concentration of each product, its absorption coefficient at the excitation wavelength, and its emission quantum yield at the wavelength where the kinetics is being monitored; we did not expect to observe the same relative rates when the photoproduct formation was monitored at 310 and 350 nm. Clearly, no quantitative information is available from the kinetic studies performed. However, the relative ordering of the photodegradation yields in methanol and acetonitrile as well as the effect of water addition to these solvents were reproduced. This adequate qualitative correspondence of the photodegradation quantum yield with the relative fluorescence rates shows that fluorescence can be used as a first screening technique to determine the photodecomposition yields of lignin model compounds. The advantage of using fluorescence for preliminary studies is that the experiments are much quicker to perform than the determination of quantum yields, and fluorescence can be employed for the screening of different experimental conditions before performing quantum yield measurements.

The fluorescence experiments were employed to estimate the GAV photodecomposition quantum yield in water, since this value has not been previously reported. The quantum yield in water is much smaller than in methanol and is probably also smaller than in acetonitrile. The emission spectra for the photoproducts indicate that the product distribution observed in water is closer to that in methanol than the product distribution observed in acetonitrile. These experiments show that the photodecomposition pattern of GAV in water, and probably other lignin model molecules, cannot be extrapolated from studies in organic solvents. This is an important point when trying to understand the photodegradation of lignin because the paper matrix contains some water.

We tried preliminary experiments to explore the fluorescence of product formation for the decomposition of GAV when incorporated into paper. These experiments were inconclusive, suggesting that the decomposition rate and (or) the emission properties of the photodecomposition products are affected by the incorporation in the paper matrix. Moreover, these experiments show the complexity of the behavior of fluorophores in paper and that degradation of lignin may have a quenching effect when fluorescent brightners are employed to counteract the yellowing of paper made from mechanical pulp.

The complexation of GAV to β- and γ-CD was established by the appearance of an induced circular dichroism signal when GAV was solubilized in the presence of CD. The direction and strengths of the induced circular dichroism signals of an achiral guest in the CD cavity can be related to the position of the guest within the CD cavity (40–42). The fact that the same direction was observed for the induced circular dichroism signals of GAV in β- and γ-CD suggests that the guest is incorporated in a similar environment in both cavities. No detailed information on the complexation environment could be obtained because the signal-to-noise ratio of the spectra is too poor to warrant any further analysis. The complexation of α-phenoxyacetophenone with β-CD has been previously reported (27), and a 1:1 complex in which the benzoyl moiety is encapsulated was proposed. GAV is too large to completely fit within the β-CD cavity, and for 1:1 complexes, part of the molecule will be exposed to the aqueous phase. However, the possibility exists that at higher β-CD concentrations, a 1:2 (GAV:CD) complex is formed that protects the guest from the aqueous environment. In the case of the larger γ-CD, the benzoyl and phenoxy moieties could be included in the same cavity as was previously proposed for the inclusion of β-phenyl-*p*-methoxypropiophenone (25).

In principle, the complexation of GAV to CDs could lead to either a decrease or increase of the photodegradation rate. In the case of a 1:2 (GAV:CD) complex, we would expect a decrease of the photodegradation rate, since separation of the radicals would be inhibited, leading to an increase in the probability for GAV regeneration. Since such an increase was not observed, it is likely that 1:2 complexes are not present to an appreciable amount. In contrast, the entrapment of one of the radical moieties within the CD cavity and efficient release of the second radical could lead to an enhancement of the GAV photodecomposition quantum yield. Surprisingly, most of the enhancement effect is observed on the initial jump and not on the relative rates at longer times. The relative rates are increased moderately (2–6) by the presence of CDs, indicating that complexation of the radicals to the CD cavity does not affect significantly the rate of product formation after the burst phase (initial jump). In the laser flash photolysis studies, we established that at least the phenoxy radicals are very long lived (>100 μs). Since the exit rate constants of guests from CD cavities are of the order of 10^5–10^6 s^{-1} (43, 44), the long radical lifetimes ensure that radicals formed from the GAV photoreaction can exit and enter CD cavities for many times before they are involved in recombination reactions. Consequently, the entrapment of the radicals does not lead to a significant alteration of the rate for product formation.

The most pronounced effect of CD complexation on the GAV photodecomposition was observed on the enhancement of the amount of product formed during the burst phase. The magnitude of this effect depends on the CD concentration, indicating that this enhancement is due to the complexation of GAV to CDs. This increase is not due to an inhibition of the recombination probability of the phenacyl and guaiacoxy radicals to regenerate GAV, since this inhibition would mean that the exit of one of the radicals from the CD cavity is faster than the separation of the radicals in the solvent cage formed in water. This is an unlikely scenario because it was shown that polar molecules such as triplet xanthone, which are not completely included in the CD cavity (45), have an exit constant of 8.4×10^6 s^{-1} (46), which is much slower than the separation of encounter complexes of neutral molecules in aqueous solution. For this reason, it is likely that the phenacyl and guaiacoxy radicals remain in close contact within the CD cavity for a longer period of time than an en-

counter complex in the aqueous phase. In this case, the enhanced emission intensity is due to a larger yield of coupling product formation in the CD cavities when compared to the reaction in the bulk phase.

An increase of the intersystem crossing quantum yield of GAV when this molecule is complexed to the CDs could also be responsible for the increase of products formed during the burst phase. The laser flash photolysis data suggest that the intersystem crossing quantum yield increased, since a higher triplet to radical concentration is observed for GAV in the presence of CDs than in water. The triplet lifetimes of GAV in β- (110 ns) and γ-CD (97 ns) are short enough that some β-cleavage will occur within the CD cavity. The radical pair formed from the cleavage of a triplet excited state has triplet multiplicity, and an intersystem crossing process has to occur before these radicals can react. For this reason, it is likely that one of the radicals will have to escape the CD cavity before any of the recombination or coupling reactions can occur. In this respect, the increased intersystem crossing for GAV leads to a decrease of the probability for the recombination of GAV and an enhancement of the product formation.

A puzzling effect was observed when oxygen was added to the solutions containing GAV and CDs. The initial jump for the fluorescence experiments decreased when GAV was complexed to γ-CD, but no effect was observed in the presence of β-CD or for GAV solubilized in the aqueous phase. Since the excited triplet state of GAV is quenched in the aqueous phase, the lack of oxygen quenching in the homogeneous solution suggests that the products formed in the initial burst are mainly due to the reactivity from the excited singlet GAV. The same explanation can be employed for the results with β-CD. In the case of γ-CD, the quenching by oxygen indicates that some of the products formed during the burst phase involve a long-lived transient that can react with oxygen. The triplet lifetime of GAV when complexed to γ-CD is decreased by a factor of 1.1 when oxygen is present in solution. This decrease is much smaller than observed for the quenching of triplet GAV in water, and it suggests that the triplet GAV complexed to γ-CD is mainly protected from the interaction with oxygen. This eliminates the triplet as the candidate for the transient being intercepted by oxygen. The phenacyl radical is the other transient in the GAV photolysis that reacts readily with oxygen (7). Thus, the oxygen effect in the presence of γ-CD could be explained by the trapping of this radical when it exits the CD cavity. Although we cannot fully explain the oxygen result, it is worth noting that the GAV complexes with β- and γ-CD must have sufficiently different geometries to lead to the photochemical behavior observed.

The transients formed in the photolysis of GAV in water were studied by laser flash photolysis. As already emphasized above, the comparison of the photochemistry of GAV in water to the previously reported reactions in organic solvents is relevant, since lignin will be exposed to an aqueous like environment in paper. The same transients, i.e., excited triplet state, and phenacyl and guaiacoxy radicals as observed in acetonitrile were also detected in water. The triplet lifetime of GAV in water is shorter than observed in the organic solvents (8). This result explains why the GAV triplet lifetimes in alcohols containing water are shorter than in the pure alcohols leading to a decrease of the photodecomposition quantum yield of GAV (8). The triplet lifetime of GAV in water is also shorter than in pure acetonitrile. In contrast to the results in alcohols, a slight increase (ca. 1.25) (8) was observed for the triplet lifetime in acetonitrile containing 40% water, which is accompanied with a significant increase (2.6) of the photodecomposition quantum yield (14). Our qualitative fluorescence results indicate that the photodegradation rate is smaller in water than in acetonitrile, which is in line with the shorter triplet lifetimes. The fluorescence results also show the same trend as previously reported (8) for the GAV degradation in acetonitrile–water mixtures. These results show that it is not easy to predict the photochemistry of GAV in mixed solvents.

In addition to the formation of the excited triplet states and radicals, we also observed that GAV in water is photoionized, leading to the observation of solvated electrons in the laser flash photolysis experiments. This photoionization reaction is common for ketones in aqueous solution and is augmented when the ketone is complexed to supramolecular structures, such as SDS micelles and bile salt aggregates (47). The same effect was observed for GAV when complexed to both CDs, suggesting that incorporation of GAV into the CD cavity leads to a more efficient separation of the solvated electron and the GAV radical cation. No transient signals were observed for the GAV radical cation probably because its decomposition is very fast. A similar effect has been reported for other ketones (47). Since the photoionization process involves the sequential absorption of two photons, this process can only contribute to the yellowing of paper made from mechanical pulp when very high irradiation intensities are employed. In most applications this reaction will not be relevant.

In summary, complexation of GAV to β- and γ-CD leads to an increase of the initial product formation but has only a small effect on the rate following the burst kinetics. This result indicates that the constrained environment around lignin within the paper matrix could determine the ratio between coupling of the radicals at different positions of the phenol ring and recombination to reform GAV, which will influence the degree of photoyellowing of the paper. The small effect observed for the relative rates after the initial burst are a consequence of the fast entry and exit dynamics of the radicals compared to the time domain for the radical–radical reactions. A more viscous environment will have to be employed to study how the decrease of the mobility of radicals by encapsulation into constrained environments affects the photochemistry of GAV. Finally, we report that GAV does not fluoresce in homogeneous solution, but the fluorescence of the products of GAV photolysis can be employed to gain qualitative information on the photodegradation kinetics.

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