Reactivity of 4-vinylphenol radical cations in solution: implications for the biosynthesis of lignans

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Nanosecond laser flash photolysis studies of the radical cation of 4-hydroxy-3-methoxystyrene show that the radical cation reacts with neutral 4-hydroxy-3-methoxystyrene and non-phenolic styrenes with rate constants that range from 1×10^8 to 5×10^8 M^{-1} s^{-1}. Similar 4-vinylphenol radical cations such as the radical cations of isoeugenol and coniferyl alcohol display reduced reactivity, presumably due to the presence of β-alkyl substituents. Overall, the results show that the reactivity of 4-vinylphenol radical cations with neutral styrenes parallels the reactivity of non-phenolic styrene radical cations, which are known to undergo efficient radical cation mediated dimerization reactions to give lignan-like compounds. The possibility that the biosynthesis of some lignans may follow a radical cation mediated mechanism is discussed.

Introduction

Coupling of propenylphenol derivatives is an important reaction in a number of biosynthetic pathways, including the biosynthesis of medicinally important lignans found in many plants.[1] While several aspects of lignan biosynthesis are being actively investigated,[2–4] the coupling step for most lignans is widely accepted to involve initial enzymatic oxidation of two substrates containing a 4-hydroxystyrene (or 4-vinylphenol) framework to give a pair of 4-vinylphenoxyl radicals that then combine to produce a dimeric species.[5] This dimer is then further processed enzymatically to give the completed lignan.[2,3,6]

Some common lignans contain a carbon framework that can readily be generated by initial 8,8'-coupling of two 4-vinylphenoxyl radicals (Scheme 1). Interestingly, studies[7–15] of non-phenolic styrenes have shown that a similar 8,8'-coupling occurs upon addition of oxidized styrenes to their corresponding neutral form ($R_1 \neq OH$; Scheme 2). This radical cation mediated dimerization reaction may be significant because it gives rise to the possibility that the biosynthesis of lignans might, at least in some cases, follow a pathway characterized by initial oxidation of a 4-hydroxystyrene to its radical cation that then adds to a second, neutral precursor to give a distonic radical cation dimer that is subsequently further processed to give the lignan.
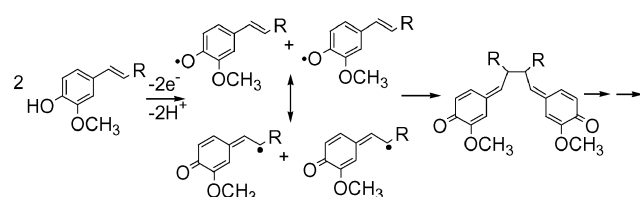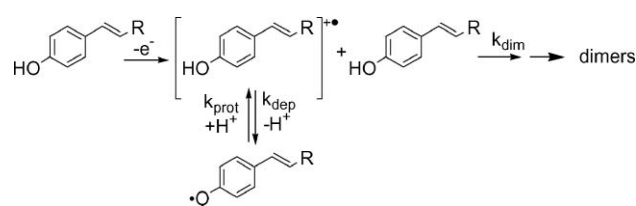

Scheme 1
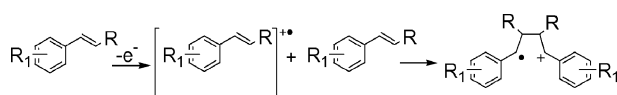
Scheme 2

The radical cation mediated dimerization reaction of non-phenolic styrenes ($R_1 \neq OH$; Scheme 2) has been firmly established using both a product study-based approach[8–10,12] and a kinetic approach whereby radical cation reactivity is examined directly.[13,15,16] Using a product study-based approach to study the same reactions of 4-vinylphenol radical cations is complicated

by the need to unambiguously rule out product formation *via* the conventional phenoxyl coupling pathway. On the other hand, the reactivity of 4-vinylphenol radical cations with neutral styrenes can be examined unambiguously using a technique like laser flash photolysis (LFP) where the radical cations can be observed directly. This approach can lead to information needed to determine if the radical cation mediated dimerization is, in principle, a feasible processs. In particular, measuring the lifetime of the 4-vinylphenol radical cations as a function of the concentration of neutral 4-vinylphenols or other styrenes can help to establish if a dimerization reaction is occurring. Absolute rate constants for the addition of 4-vinylphenol radical cations to styrenes can also be obtained, and these rate constants can be used to establish whether or not the addition reaction, k_{dim}, (Scheme 3) is competitive with the expected rapid deprotonation reaction, k_{dep}. In addition, by measuring rate constants for the formation of 4-vinylphenol radicals by protonation of 4-vinylphenoxyl radicals, k_{prot}, under acidic conditions, the possibility that radical cation mediated dimerization can be initiated by reprotonation of 4-vinylphenoxyl radicals can also be investigated.

Scheme 3

In the present work, we have examined the dynamics for the addition of radical cations of three 4-vinylphenol derivatives (coniferyl alcohol, isoeugenol and 2-methoxy-4-vinylphenol) to neutral styrenes, and have compared these results to those obtained for other previously studied styrene radical cations known to participate in dimerization reactions. Kinetic parameters for the protonation of 4-vinylphenoxyl radicals have also been determined to explore the reversibility of 4-vinylphenol radical cation deprotonation reactions.

Results

Generation and characterization of 4-vinylphenol radical cations

As described in previous work,[17] the radical cations of isoeugenol **2a** [1-(4-hydroxy-3-methoxyphenyl)propene, R = CH₃] and

coniferyl alcohol **2b** [1-(4-hydroxy-3-methoxyphenyl)propen-3-ol, R = CH₂OH] are readily generated by 308 nm laser-induced photoionization of isoeugenol **1a** and coniferyl alcohol **1b**, respectively, in acetonitrile [eqn. (1)]. Both radical cations have a strong absorption band at 380 nm and a slightly weaker band at 580 nm (Fig. 1a and 1b). For the isoeugenol radical cation, both bands decay in acetonitrile in a first-order manner with a rate constant of 9×10^5 s⁻¹ (all data were collected at 22 ± 1 °C) to give a new transient with an absorption maximum at 340 nm. The transient at 340 nm can be identified as the 4-(1-propenyl)-3-methoxyphenoxyl radical **3a** generated by loss of a proton from the 4-hydroxyl group of the isoeugenol radical cation[17] [eqn. (1)]. The radical cation of coniferyl alcohol also decays in a first-order manner with a rate constant of 8×10^5 s⁻¹ to give the 4-(3-hydroxy-1-propenyl)-3-methoxyphenoxyl radical **3b** with a maximum at 340 nm.
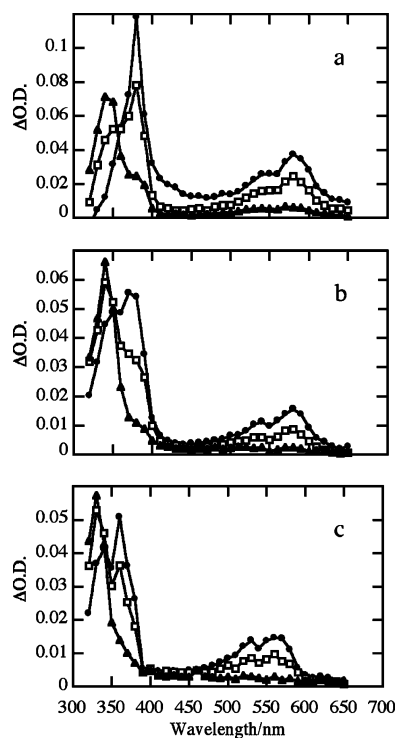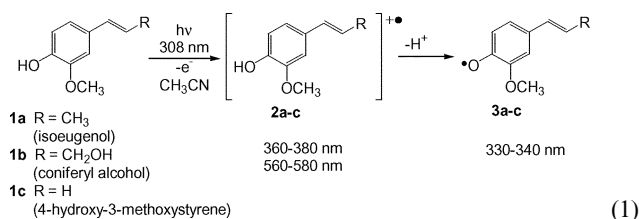

(1)

Fig. 1 Transient absorption spectra recorded (●) 0.2 µs, (□) 0.5 µs, and (▲) 3.0 µs after 308 nm laser irradiation of (a) isoeugenol, (b) coniferyl alcohol, and (c) 2-methoxy-4-vinylphenol in acetonitrile.

Since both the isoeugenol and coniferyl alcohol radical cations have β-alkyl groups that are known to substantially reduce the reactivity of styrene radical cations with alkene nucleophiles,[18] laser experiments were carried out to generate the radical cation of 4-hydroxy-3-methoxystyrene **2c** [eqn. (1); R = H] which is unsubstituted at the β-position. Laser irradiation of 4-hydroxy-3-methoxystyrene **1c** led to the formation of a transient species with absorption maxima at 360 and 560 nm. Both of these bands decayed with the same rate constant of 2×10^6 s⁻¹, giving rise to a new transient with an absorption at 330 nm. These observations are closely similar to those observed for the isoeugenol and coniferyl alcohol radical cations, leading to the identification of the transient at 360 and 560 nm as the radical cation of 4-

hydroxy-3-methoxystyrene, and the transient at 330 nm as the 4-vinyl-2-methoxyphenoxyl radical **3c**.

All three radical cations could also be generated by photoinduced electron-transfer according to the reaction sequence shown in eqn. (2). Laser irradiation (355 nm) of chloranil gave the chloranil triplet ($\lambda_{max} = 500$ nm),[19] which was quenched by the vinyl phenol derivatives with second-order rate constants near 1×10^{10} M⁻¹ s⁻¹. This rate constant is consistent with a diffusion-controlled electron-transfer reaction between the triplet chloranil and the vinylphenol derivatives. After complete quenching of the triplet, the absorption spectra were dominated by a band at 440 nm due to the chloranil radical anion,[19] as well as by bands in the 360–370 nm and 560–580 nm regions. These latter bands match well with the bands of the radical cations generated by photoionization, thus providing good evidence that electron-transfer did occur and led to the formation of the radical cations of the vinylphenol derivatives.

(2)
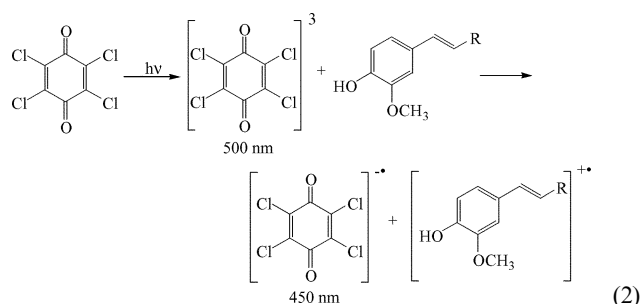
Reactivity of 4-vinylphenol radical cations toward neutral precursors

As mentioned in the Introduction, one goal of the present work was to determine if the radical cations of the 4-vinylphenol derivatives can undergo reactions with their neutral precursors as outlined in Scheme 3. This possibility can be probed kinetically by measuring the decay of the radical cations as a function of the concentration of the precursors to the radical cations. In these experiments, the 4-vinylphenol radical cations were generated by photoinduced electron-transfer using chloranil excited with 355 nm laser irradiation; this method is compatible with the use of relatively high concentrations of the precursors, while direct photoionization using 308 nm laser irradiation is more suitable for experiments using lower concentrations of the precursors.

The 4-hydroxy-3-methoxystyrene radical cation generated by photoinduced electron-transfer decayed in a first-order manner at all concentrations of 4-hydroxy-3-methoxystyrene used. As shown in Fig. 2 (closed circles), the observed rate constants increased as a function of increasing precursor concentration from 0.5 to 20 mM, indicating the presence of a reaction between the radical cation and its precursor. Linear least squares analysis of the relationship between observed first-order rate constants and the concentration of the substituted styrene over
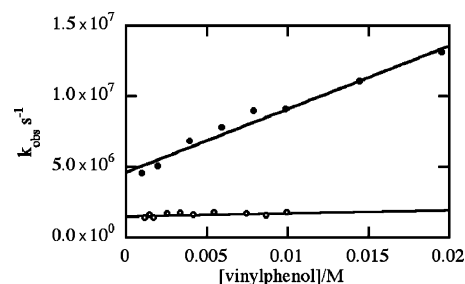

Fig. 2 (●) Relationship between observed rate constants for the decay of the 4-hydroxy-3-methoxystyrene radical cation and the concentration of 4-hydroxy-3-methoxystyrene in acetonitrile. (○) Relationship between the observed rate constants for the decay of the isoeugenol radical cation and the concentration of isoeugenol in acetonitrile.

the concentration range of 0.5 to 20 mM gave a second-order rate constant of 4.3×10^8 M^{-1} s^{-1}. Attempts were made to obtain data at even higher concentrations of 4-hydroxy-3-methoxystyrene. However, at these higher concentrations the solution became increasingly deeply coloured, and it was not possible to obtain accurate kinetic data.

Results from experiments carried out using isoeugenol are also shown in Fig. 2 (open circles). As can be seen, the rate constants for the decay of the isoeugenol radical cation remained unchanged at *ca.* 1.5×10^6 M^{-1} s^{-1} over the entire concentration range. As for the 4-hydroxy-3-methoxystyrene radical cation described above, higher concentrations could not be studied due to deep colouration of the solution. The same results were obtained for the coniferyl alcohol radical cation whose decay remained unchanged at all coniferyl alcohol concentrations studied (0.001 to 0.01 M).

Reactivity of 4-vinylphenol radical cations toward other styrenes

The decay of the 4-vinylphenol radical cations was also measured as a function of the concentration of styrene, 4-methylstyrene and α-methylstyrene. In this case, the radical cations were generated by photoionization using 308 nm laser excitation. This method is useful for these experiments since the three styrenes investigated have no detectable absorption at 308 nm and irradiation with 308 nm laser light selectively excites the 4-hydroxystyrene derivative.

The observed rate constants for the decay of the radical cation of 4-hydroxy-3-methoxystyrene in acetonitrile containing 0 to 10 mM 4-methylstyrene are shown in Fig. 3. As can be seen, the rate constants increase significantly from 1.8×10^6 s^{-1} to 3.9×10^6 s^{-1} over this concentration range. Linear least squares analysis gave a second-order rate constant of $(2.5 \pm 0.2) \times 10^8$ M^{-1} s^{-1} for the reaction of the radical cation with 4-methylstyrene. The addition of α-methylstyrene and styrene also increased the decay of the 4-hydroxy-3-methoxystyrene radical cation, but the increases were smaller than those observed for 4-methylstyrene. The relationships between the observed rate constants and concentrations of the styrenes were linear, with linear least squares analysis giving slopes of $(1.4 \pm 0.2) \times 10^8$ M^{-1} s^{-1} for quenching by styrene, and $(1.0 \pm 0.1) \times 10^8$ M^{-1} s^{-1} for quenching by α-methylstyrene.
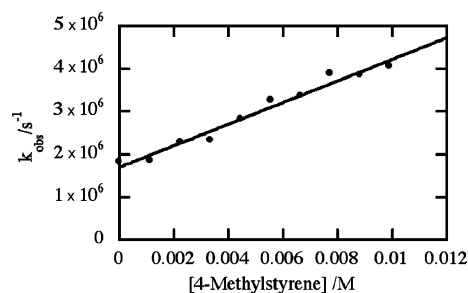

Fig. 3 Relationship between the observed rate constants for the decay of the radical cation of 4-vinyl-2-methoxyphenol and the concentration of 4-methylstyrene in acetonitrile.

Similar experiments were carried out to examine the reactivity of the radical cations of coniferyl alcohol and isoeugenol with the same three styrenes. The decays of both these radical cations remained unchanged at styrene concentrations up to 0.02 M.

Reactivity of 4-hydroxy-3-methoxystyrene radical cation toward a simple phenol

Quenching of the radical cation of 4-hydroxy-3-methoxystyrene using a simple phenol, 4-isopropylphenol, was also examined to determine the importance of a possible reaction between the radical cation and a phenolic OH. No measurable quenching was observed, even at concentrations as high 50 mM 4-

isopropylphenol. Given that the radical cation decays with a rate constant of 2×10^6 s^{-1} in neat acetonitrile, the lack of observable quenching at 50 mM phenol indicates that the potential reactivity of the phenol towards the radical cation must be less than 1×10^7 M^{-1} s^{-1}, which is greater than one order of magnitude smaller than the rate constant measured for the reaction of the same radical cation with 4-hydroxy-3-methoxystyrene.

Protonation of 4-vinylphenoxyl radicals

Laser irradiation (355 nm) of 10% di-*tert*-butyl peroxide in N_2-saturated acetonitrile rapidly induces the homolytic cleavage of the di-*tert*-butyl peroxide to give two *tert*-butoxyl radicals [eqn. (3)]. In the absence of added 4-vinylphenol, little change in the absorption upon laser irradiation was observed, which is consistent with the weak absorbance of the *t*-butoxyl radical in the 320–700 nm spectral region.[20] However, as shown in Fig. 4a, a new transient species at 340 nm grew in after laser irradiation of a di-*tert*-butyl peroxide/acetonitrile solution with added isoeugenol. The absorption at 340 nm grew in with a first-order rate constant that was dependent on the concentration of isoeugenol [Fig. 4a (inset)]. This behaviour is consistent with a hydrogen abstraction reaction in which the *tert*-butoxyl radical removes a hydrogen atom from the phenolic OH of the isoeugenol to give the vinylphenoxyl radical **3a** [eqn. (4)]. Linear least squares analysis of the relationship between the observed rate constant for growth of the 340 nm transient and the concentration of isoeugenol gives a second-order rate constant for this reaction of 1.4×10^8 M^{-1} s^{-1}.

$$t\text{BuO}-Ot\text{Bu} \xrightarrow[355\ nm]{h\nu} 2\ t\text{BuO}\bullet \qquad (3)$$

$$(4)$$
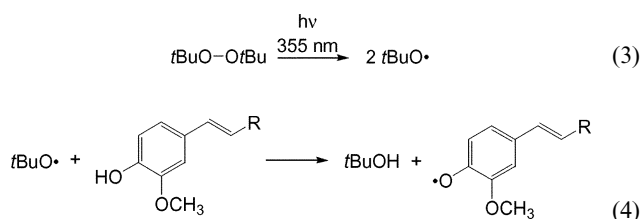
The magnitude of the rate constant is consistent with transfer of a hydrogen atom from phenolic O–H to the photogenerated *tert*-butoxyl.[21] In addition, the location of the absorption maximum is similar to those of other phenoxyl radicals.[20] Furthermore, the decay of the transient at 340 nm in O_2-saturated solution is the same as that in N_2-saturated solution. All of these observations are consistent with identification of the transient as the 2-methoxy-4-(1-propenyl)-phenoxyl radical generated by hydrogen atom transfer from the OH of isoeugenol to the photogenerated *tert*-butoxyl radical [eqn. (4)].

Similar results were observed using coniferyl alcohol or 4-hydroxy-3-methoxystyrene instead of isoeugenol. In both cases, 355 nm laser irradiation of 10% di-*tert*-butyl peroxide in acetonitrile with the added 4-vinylphenol derivative resulted in the formation of a new transient at 340 nm with properties consistent with identification as the corresponding vinylphenoxyl radicals **3b** and **3c**.

In acidic (1 mM HClO$_4$) acetonitrile with 10% di-*tert*-butylperoxide and 6 mM isoeugenol, the 2-methoxy-4-(1-propenyl)-phenoxyl radical still grew in at 340 nm with the same rate constant as that measured in the absence of acid. However, two additional absorption bands at 380 and 580 nm were also observed (Fig. 4b). The two absorption bands grew in with a rate constant of 4.5×10^5 s^{-1} that was slightly smaller than the rate constant for the growth of the radical cation at 340 nm. The location and relative intensities of these bands are very similar to those for the isoeugenol radical cation generated by photoinduced electron-transfer or by photoionization as described above. Thus, the additional transient generated under acidic conditions can be identified as the isoeugenol radical cation.
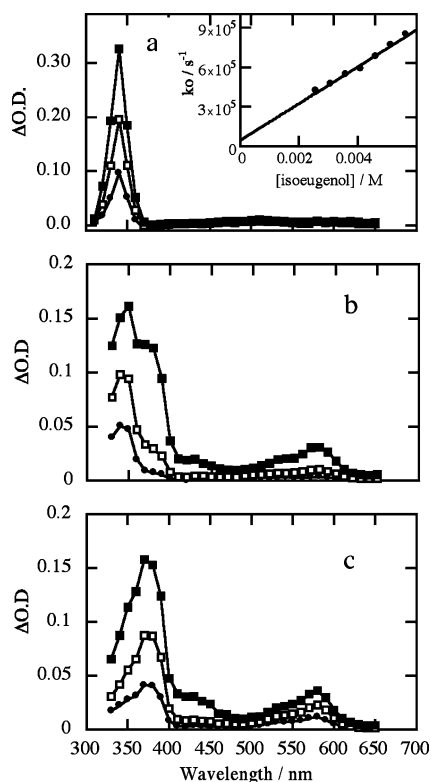
Fig. 4 Transient absorption spectra recorded (●) 0.18 μs, (□) 0.86 μs, and (■) 4.5 μs after 355 nm laser iradiation of 10% di-*tert*-butyl peroxide in acetonitrile with 6 mM isoeugenol and (a) 0 mM HClO$_4$, (b) 1 mM HClO$_4$, and (c) 10 mM HClO$_4$. The inset in (a) shows the relationship between the rate constants for the growth at 340 nm and the concentration of isoeugenol in 10% di-*tert*-butyl peroxide/acetonitrile (0 mM HClO$_4$).

As shown in Fig. 4c, the absorption bands due to the isoeugenol radical cation at 370 and 580 nm were more intense as the acid concentration increased to 10 mM, and the absorption band due to the phenoxyl radical was considerably reduced. The effect of increasing acid concentrations is more clearly illustrated in Fig. 5a, which shows the time-resolved absorption changes at 380 nm at several different acid concentrations. The growth curves clearly show that the magnitude of the absorption due to the radical cation increases dramatically as the concentration of
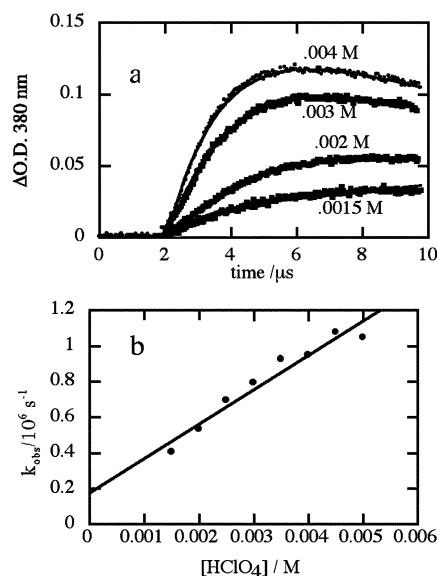

Fig. 5 Effect of concentration of HClO$_4$ on (a) the maximum optical density at 380 nm, and (b) the rate constant for growth at 580 nm upon 355 nm irradiation of di-*tert*-butyl peroxide in acetonitrile with 7 mM isoeugenol.

perchloric acid is increased from 1.0 to 7.0 mM. In addition, the rate constant for the growth of the radical cation increased with acid concentration over the concentration range of 1.0–5.0 mM (Fig. 5b). The relationship between the rate constants and acid concentrations over this range is linear, and linear least squares analysis gave a slope of $(1.9 \pm 0.2) \times 10^8$ M^{-1} s^{-1}.

At a concentration of 6 mM, isoeugenol in acetonitrile has a small absorption at 355 nm, the excitation wavelength used in these experiments. To remove any possibility that the isoeugenol radical cation at 370 and 580 nm might be generated by direct excitation of isoeugenol, a solution of acidic acetonitrile (10 mM HClO$_4$) with 6 mM isoeugenol but no di-*tert*-butyl peroxide was examined with 355 nm laser irradiation. No change in absorption was detected after the laser pulse, indicating that the radical cation was not generated in this way. Thus, the *tert*-butoxyl radical generated by photoinduced homolytic cleavage of di-*tert*-butyl peroxide is required to initiate radical cation formation under these conditions.

Similar experiments could not be carried out using coniferyl alcohol which decomposed in acidic acetonitrile. Presumably, coniferyl alcohol is prone to acid-catalyzed elimination of the β-OH group leading to formation of the unstable quinone methide and subsequent products derived from the quinone methide. Irradiation of *t*-butyl peroxide in the presence of 4-hydroxy-3-methoxystyrene under acidic conditions also failed to produce detectable amounts of the 4-hydroxy-3-methoxystyrene radical cation. This inability to detect the radical cation **3c** most likely originates from its rapid dimerization reaction with 4-hydroxy-3-methoxystyrene as described above. In particular, the rate constant of 4.3×10^8 M^{-1} s^{-1} for the addition of radical cation **3c** to its neutral precursor **3a** is greater than the rate constant of 1.4×10^8 M^{-1} s^{-1} for hydrogen abstraction from 4-hydroxy-3-methoxystyrene by the *t*-butyl peroxy radical. Thus, the disappearance of the radical cation would be faster than its formation due to the relatively slow rate constant for the generation of the vinylphenoxyl radical.

Discussion

Reactivity of 4-hydroxystyrene radical cations with neutral precursors

Of the three 4-hydroxystyrene derivatives examined in this work, only the 4-hydroxy-3-methoxystyrene radical cation clearly reacted with its neutral precursor, 4-hydroxy-3-methoxystyrene. The second-order rate constant for this reaction was measured to be 4.3×10^8 M^{-1} s^{-1}, which is about one order of magnitude slower than a diffusion-controlled process in acetonitrile. Unlike other styrenes that only contain a nucleophilic vinyl group and typically react with radical cations by 8,8′-coupling, 4-hydroxy-3-methoxystyrene contains an additional reactive phenolic OH group that could react as a nucleophile or a base with the 4-hydroxy-3-methoxystyrene radical cation. Direct participation of the phenolic OH can be ruled out upon consideration of the reactivity of 4-hydroxystyrene radical cations with other hydroxy-containing compounds. In particular, rate constants for the reaction of simple alcohols with 4-hydroxystyrene radical cations in acetonitrile[17] are about one order of magnitude smaller than the second-order rate constant of 4.3×10^8 M^{-1} s^{-1} measured in this work. Simple phenols are similarly less reactive, as demonstrated in the present work by the absence of a noticeable increase in the rate constant for the decay of the 4-hydroxy-3-methoxystyrene radical in the presence of 4-isopropylphenol.

The magnitude of the rate constant for the reaction between the 4-hydroxy-3-methoxystyrene radical cation and its neutral precursor, $k = 4.3 \times 10^8$ M^{-1} s^{-1}, is similar to the rate constant of 1.1×10^9 M^{-1} s^{-1} for the quenching of the 4-methoxystyrene radical cation by neutral 4-methoxystyrene, a reaction which is known to involve addition to give an 8,8′-coupling product. Thus, on the basis of these comparisons, it is reasonable to

Table 1 Second-order rate constants (M^{-1} s^{-1}) for the quenching of the 4-hydroxy-3-methoxystyrene and 4-methoxystyrene radical cations by substituted styrenes in acetonitrile ($22 \pm 1\,°C$)

	4-Hydroxy-3-methoxy styrene radical cation[a]	4-Methoxystyrene radical cation[b]
4-Methoxystyrene	—	1.1×10^9
4-Hydroxy-3-methoxystyrene	4.3×10^8	—
Styrene	1.4×10^8	1.3×10^7
4-Methylstyrene	2.5×10^8	7.8×10^7
α-Methylstyrene	1.0×10^8	1.2×10^8

[a] This work. [b] Refs. 16 and 18.

suggest that the quenching of the 4-hydroxy-3-methoxystyrene radical cation with 4-hydroxy-3-methoxystyrene is also due to a coupling reaction similar to that shown in Scheme 2.

Further evidence that the phenolic OH of 4-hydroxy-3-methoxystyrene is not responsible for the observed quenching of the 4-hydroxy-3-methoxystyrene radical cation comes from results obtained upon reacting the 4-hydroxy-3-methoxystyrene radical cation with non-phenolic styrenes. The 4-hydroxy-3-methoxystyrene radical cation reacted with styrene, 4-methylstyrene and α-methylstyrene with rate constants ranging from 1.0×10^8 M^{-1} s^{-1} for α-methylstyrene to 2.5×10^8 M^{-1} s^{-1} for 4-methylstyrene. Since these styrenes contain only the nucleophilic vinyl group, the second-order reaction must involve a dimerization reaction that presumably leads to an 8,8′-coupling product.

A comparison between the rate constants for the addition of styrenes to the 4-methoxystyrene radical cation[18] and the rate constants for the reaction of the 4-hydroxy-3-methoxystyrene radical cation with the same styrenes is shown in Table 1. The data in Table 1 indicate that the reactivities of both radical cations towards styrenes are overall quite similar, with the largest difference being a 10-fold greater reactivity of styrene toward the 4-hydroxy-3-methoxystyrene radical cation compared with the 4-methoxystyrene radical cation.

Coniferyl alcohol and isoeugenol contain an additional alkyl substituent at the β-position of the double bond, and the radical cations from these two substrates showed no measurable reactivity toward their neutral precursors, or towards the other styrenes used in this study. A similar observation had been made previously for the radical cation of anethole [1-(4-methoxyphenyl)propene] which showed no observable reactivity with anethole due to the presence of the additional β-methyl group.[13] While this lack of measurable reactivity may suggest no reaction between anethole and its radical cation, product studies have provided strong evidence that the addition reaction does occur to give 2,3-dimethyl-1,4-bis(4-methoxyphenyl)cyclobutane, but with a rate constant of 2×10^6 M^{-1} s^{-1} that is too slow to measure directly using LFP techniques.[13]

Protonation of vinylphenoxyl radicals

Increasing the acidity of the acetonitrile solution resulted in a decrease of the observed yield of the 2-methoxy-4-(1-propenyl)-phenoxyl radical, and a concomitant increase in the yield of the isoeugenol radical cation. In addition, the rate constant for the formation of the radical cation increased in a linear manner with respect to the concentration of the acid. These observations are consistent with the formation of the radical cation *via* protonation of the initially formed radical [eqn. (5)].

$$(5)$$
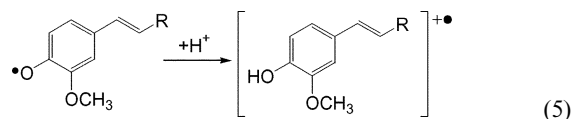
Arene radical cations are known to be produced upon direct irradiation of arenes under acidic conditions.[22,23] However, the isoeugenol radical cation was not generated upon 355 nm laser irradiation of isoeugenol in acidic acetonitrile when di-*tert*-butyl peroxide was withheld. This observation rules out the possibility that radical cation formation took place by a mechanism requiring direct excitation of isoeugenol.

According to the reaction in eqn. (5), the rate constant for the decay of the radical is expected to increase in the same manner as the rate constant for the formation of the radical cation. This was not observed, presumably due to the close similarity between the observed rate constant for the formation of the radical by H· atom abstraction (1.0×10^6 s^{-1} when [isoeugenol] = 7 mM) and the observed rate constant for protonation of the radical (0.2 to 1.0×10^6 s^{-1} for [H⁺] = 1.5 to 5 mM). In addition, the absorption spectra of the radical and the radical cation overlap strongly at 340 nm; as a result, any decrease in absorption intensity at 340 nm due to radical decay will at least be partially compensated by an increase in absorption intensity as the radical cation grows in. Nonetheless, despite our inability to observe a distinct change in the rate constant for the decay at 340 nm as a function of acid concentration, the formation of the radical cation under acidic conditions and the observations that the yield and the dynamics for the growth of the radical cation are affected by acid concentration provide strong evidence that the radical cation is produced by protonation of the radical.

The slope of $(1.9 \pm 0.2) \times 10^8$ M^{-1} s^{-1} determined from the relationship between the observed rate constants for the growth of the radical cation and acid concentration should represent the rate constant for protonation of the radical, k_{H^+}. This rate constant is large, and clearly shows that the isoeugenol radical is sufficiently basic to be rapidly converted into its conjugate acid under suitable conditions.

Implications for the biosynthesis of lignans

While definitive information about the mode of reaction between the 4-hydroxy-3-methoxystyrene radical cation and neutral styrenes cannot be obtained from kinetic studies alone, our results are consistent with an 8,8′-coupling reaction as observed previously for other styrenes. In particular, our results clearly demonstrate that the 4-hydroxy-3-methoxystyrene radical cation does undergo a rapid reaction with its neutral form, as well as with other non-phenolic styrenes. Furthermore, the magnitude of the rate constants are similar to those obtained with the 4-methoxystyrene radical cation, which is known to undergo initial 8,8′-coupling upon reaction with styrenes. The other 4-hydroxystyrene derivatives, isoeugenol and coniferyl alcohol, investigated in this study showed no observable increase in reactivity in the presence of neutral styrenes. However, these two substrates contain β-alkyl substituents, which are known to dramatically reduce the reactivity of styrene radical cations toward neutral styrenes. In fact, the lack of reactivity parallels that observed for other β-substituted styrene radical cations, such as the anethole radical cation, which does react with anethole to give an 8,8′-coupling product,[10] but with a second-order rate constant too slow to measure using LFP.

The 4-hydroxystyrene radical cations all undergo a rapid deprotonation reaction, but, as shown for the isoeugenol radical, rapid reprotonation also takes place under moderately acidic conditions in a non-protic, polar solvent. Thus, in a biological system, 4-vinylphenol radical cations need not undergo a dimerization event as soon as they are produced by enzymatic oxidation; instead, the radical cations can be 'stored' in their less reactive 4-vinylphenoxyl radical form, and then regenerated by enzymatic protonation when dimerization is ready to proceed.

We acknowledge that at the present time, there is little biochemical evidence to suggest that radical cations are involved in the biosynthesis of lignans. Nonetheless, our results indicate that radical cation mediated dimerization of 4-hydroxystyrenes is, in principle, a viable pathway, and that this mechanism may be helpful in understanding results as explorations into the mechanism of lignan formation continue.

Experimental

Materials

Coniferyl alcohol, isoeugenol and 4-hydroxy-3-methoxystyrene were purchased (Aldrich) and used as received. Acetonitrile was spectroscopic grade (Omnisolve, BDH). All other materials were the best available commercial grades, and used without further purification.

Laser flash photolysis

The nanosecond laser flash photolysis system at Dalhousie University has been described previously.[24] A Lambda Physiks excimer laser (308 nm, XeCl, 10 ns/pulse, <100 mJ/pulse) was used as the excitation source for most experiments. In experiments using chloranil or di-*tert*-butyl peroxide, a Continuum Nd:YAG NY-61 laser (355 nm; 8 ns/pulse; < 35 mJ/pulse) was used as the excitation source.

Solutions for kinetics experiments were prepared by the addition of small aliquots (*ca.* 10 µL) of stock solutions of the appropriate substituted 4-vinylphenol to 2.0 or 3.0 mL of acetonitrile in 7 × 7 mm laser cells made out of Suprasil quartz tubing. The absorption of the 4-vinylphenols in the laser cells was approximately 0.4 at 308 nm, corresponding to concentrations of *ca.* 0.1 mM. Measurements of quenching rate constants were made by adding small amounts (1–20 µL) of a stock solution with a known concentration of the quencher dissolved in acetonitrile. Solutions for the experiments carried out under acidic conditions were prepared by making a 0.010 M HClO$_4$ stock solution in acetonitrile, and then making appropriate dilutions of this solution using acetonitrile. To minimize the possibility of acid-catalyzed decomposition of coniferyl alcohol or isoeugenol, these materials were added to the acidic sample solutions just prior to performing the laser experiments. In the chloranil-sensitized experiments, acetonitrile solutions were prepared so that the absorption of chloranil at 355 nm was approximately 0.4. Appropriate amounts of stock solutions with

known concentrations of the 4-vinylphenols were then added to given final concentrations of 4-vinylphenols ranging from 0.1 mM to 1 mM.

For the measurement of absorption spectra, a flow cell system was used to ensure that only fresh solution was subjected to each laser pulse. Solutions were prepared as described above with the compound to be excited having an absorption at the excitation wavelength of 0.4.

Most laser experiments were carried out using aerated samples. When oxygen-free solutions were required, the samples were bubbled with a slow stream of dry nitrogen for 20 min prior to laser irradiation. All kinetics experiments were carried out at room temperature (22 ± 1 °C).

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