ARTICLES

Lifetime, Reduction Potential and Base-Induced Fragmentation of the Veratryl Alcohol Radical Cation in Aqueous Solution. Pulse Radiolysis Studies on a Ligninase "Mediator"

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The radical cation of veratryl alcohol (3,4-dimethoxybenzyl alcohol), $VA^{\bullet+}$, was produced in aqueous solution, mainly by oxidation with the radiation chemically generated $SO_4^{\bullet-}$ or Tl^{2+}. By electron-transfer equilibration with thioanisole as a redox standard, the reduction potential of $VA^{\bullet+}$ was determined to be 1.36 ± 0.01 V/NHE. On the basis of product analysis results, the radical cation undergoes a C−H deprotonation reaction from the side chain, leading to an α-hydroxybenzyl-type radical, with $k = (17 \pm 1)\ s^{-1}$ at pH ≤ 5, as determined by time-resolved conductance. The α-hydroxybenzyl-type radical was also produced by reduction of veratryl aldehyde with the hydrated electron, and the pK_a value of this radical was determined to be 10.0. The deprotonation of $VA^{\bullet+}$ is enhanced by bases such as OH^-, with the rate constant being $1.3 \times 10^9\ M^{-1}\ s^{-1}$. In contrast, the corresponding rate constant for reaction of OH^- with the radical cation of veratryl alcohol *methyl ether,* whose reduction potential is also 1.36 V/NHE, is only $2 \times 10^7\ M^{-1}\ s^{-1}$. With the veratryl alcohol derivative, $3,4\text{-}(MeO)_2C_6H_3CH(OH)CMe_3$, the radical cation undergoes both a proton loss from the benzylic position and a $C_\alpha-C_\beta$ fragmentation with the ratio, at pH ≤ 5, of ca. 1:2. The decay of the radical cation is strongly enhanced by OH^- ($k = 8.3 \times 10^9\ M^{-1}\ s^{-1}$), with the base induction favoring the C−C fragmentation relative to the proton loss from the benzylic position. The possible bearing of these results with respect to the role of VA in the lignin peroxidase-catalyzed decomposition of lignin is discussed.

Introduction

Second only to cellulose, lignin is the most abundant biopolymer on earth. It is estimated that the planet currently contains 3×10^{11} metric tons of lignin with an annual biosynthetic rate of $\sim 2 \times 10^{10}$ tons. Lignin constitutes ∼30% of the dry weight of softwoods and about 20% of the weight of hardwoods. The presence of lignin within the cellulosic fiber wall, mixed with hemicelluloses, creates a composite material, which imparts strength and rigidity to trees and plants.

Chemically, lignin is built from phenylpropane units linked together by different bonds to give an amorphous, 3D-cross-linked network polymer, formed via a random free-radical polymerization process and characterized by the presence of several different bonding patterns. Among these, the most abundant (50−60% of the linkages) are the aryl ether β-O-4 interunit linkages, connecting a phenoxy oxygen with the β carbon of the side chain. Benzyl alcohol groups are also common structures in lignin, accounting for more than 30% of the phenylpropane units.[2]

The chemical separation of lignin from cellulose has been termed "delignification" and is one of the complex processes of the pulp and paper industry. The process of delignification results in the production of vast amounts of lignin whose properties may vary depending on which delignification process was employed and at which stage of delignification the lignin was isolated.

The oxidative degradation of lignins to lower molecular weight aromatic compounds has been intensively studied,[2,3] due to the mechanistic interest and commercial importance. In this respect, several oxidizing systems have been employed, such as chlorine dioxide,[4] air in alkaline solution at high temperature,[5] or nitroaromatics.[6] However, chemical pulping processes are low yield and require significant waste treatment and chemical recycling operations. Moreover, the effluents from these processes cannot be recycled and contain large amounts of compounds that are known to have toxic, mutagenic, and carcinogenic effects. Thus, increased public concern about the environmental effects of the pulp and paper industry is rapidly moving it toward alternative technologies.

In this respect, biopulping, defined as the treatment of wood chips with lignin-degrading fungi prior to pulping, is attracting growing attention thanks to the reduction in environmental impact and energy consumption it allows.[2] Among these fungi, particularly interesting is the white rot fungus *Phanerochaete chrysosporium*, which secretes a ferric hemoprotein, lignin peroxidase (LiP), that is able to cleave oxidatively the side chain $C_\alpha-C_\beta$ bond in both lignin and lignin model compounds.[7] For a mechanistic explanation, one-electron oxidation of lignin by LiP to form the corresponding radical cation, which subsequently undergoes C−C bond cleavage, was initially proposed.[7c−d,8] However, it was observed that lignin degradation by LiP requires the presence of veratryl alcohol (VA, 3,4-dimethoxybenzyl

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alcohol),[9] a secondary metabolite of *Phanerochaete chrysosporium*, and moreover that the geometry of the active site of the enzyme prevents direct interaction with the lignin polymer but not with VA.[10]

Different mechanistic roles have thus been proposed for the action of VA on lignin degradation catalyzed by LiP, forexample, it could act as a redox mediator, get oxidized by LiP to yield $VA^{\bullet+}$ as a diffusible oxidant able to abstract one electron from lignin,[11,12] protect the enzyme from inactivation by H_2O_2,[13] or donate electrons to LiP in order to complete the catalytic cycle.[14] More recently, it has been proposed that $VA^{\bullet+}$ reacts with a nucleophile in the active-site channel and is subsequently regenerated outside the channel where it can interact with the lignin polymer.[15]

Thus, VA, although it does *not* contain a C_β in the side chain (that is, C−C cleavage is not possible), has been intensively studied with respect to the properties and reactions of its radical cation.[16−19] However, there are still unsolved questions, relating, in particular, to the spectroscopic properties and to the lifetime of this radical cation. For example, whereas Candeias reports $k(\text{decay}) = 17\ s^{-1}$,[16] the number measured by Aust (with a different method of $VA^{\bullet+}$ production) is $1.2 \times 10^3\ s^{-1}$.[19] This difference is important in conjunction with the question of whether $VA^{\bullet+}$ is able to act as a diffusible oxidant in the enzyme system.[20] Also, there is so far no information on the susceptibility of $VA^{\bullet+}$ toward catalytic decomposition, especially with respect to bases. However, this is a likely reaction, as judged by the behavior of model systems.[21] Thus, to obtain a more complete and consistent picture, we have performed the experiments described in the following.

Results and Discussion

1. Reduction Potential and Absorption Spectrum of $VA^{\bullet+}$. In Figure 1 is presented the absorption spectrum (triangles) of the radical cation of veratryl alcohol (VA) as measured in an aqueous solution containing 0.05 mM VA, 1 mM thioanisole (TA), 0.1 M 2-methyl-2-propanol (to scavenge $^\bullet OH$, eq 2), and 5 mM $K_2S_2O_8$ (to scavenge e^-_{aq} to produce the oxidant $SO_4^{\bullet-}$, eq 3).

The generation of $VA^{\bullet+}$, whose absorption spectrum (Figure 1, triangles) is essentially the same as that described by Candeias and Harvey[16] but *not* as that of Khindaria et al.,[17] occurs via the following reactions (eqs 1−4):

$$H_2O \rightsquigarrow H^+, {}^\bullet OH, e^-_{aq} \tag{1}$$

$${}^\bullet OH + CH_3C(CH_3)_2OH \rightarrow H_2O + {}^\bullet CH_2C(CH_3)_2OH \tag{2}$$

$$e^-_{aq} + S_2O_8^{2-} \rightarrow SO_4^{2-} + SO_4^{\bullet-} \tag{3}$$

$$SO_4^{\bullet-} + VA\ (TA) \rightarrow SO_4^{2-} + VA^{\bullet+}\ (TA^{\bullet+}) \tag{4}$$

Alternatively, it was produced via eqs 5 and 6:

$${}^\bullet OH + Tl^+ + H^+ \rightarrow Tl^{2+} + H_2O \tag{5}$$

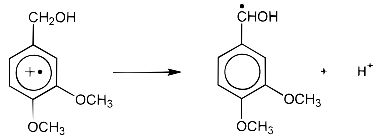
$$Tl^{2+} + VA\ (TA) \rightarrow Tl^+ + VA^{\bullet+}\ (TA^{\bullet+}) \tag{6}$$

TA was added to the VA solutions as a "redox standard" (the reduction potential of $TA^{\bullet+}$, whose maximum absorption values are at 310 and 530 nm,[22] has been determined[23] to be 1.49 V/NHE), and it was found that a reversible electron transfer takes place between VA and $TA^{\bullet+}$, according to the equation

$$TA^{\bullet+} + VA \underset{k_r}{\overset{k_f}{\rightleftharpoons}} TA + VA^{\bullet+} \qquad K = k_f/k_r = \frac{[TA][VA^{\bullet+}]}{[TA^{\bullet+}][VA]} \tag{7}$$

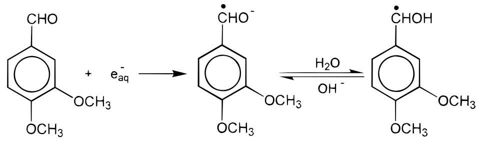
This is visible in the insets of Figure 1, where the (first-order) buildup of $VA^{\bullet+}$ at 430 nm and the decay of $TA^{\bullet+}$ at 530 nm are presented. The equilibrium constant for reaction 7 can be obtained by measuring the concentration ratio $[VA^{\bullet+}]/[TA^{\bullet+}]$, via the absorbances at equilibrium, as a function of [TA]/[VA]. A second way to obtain K is from a kinetic analysis, that is, via the rate of approach to equilibrium. The result of this analysis is shown in the first (from the left) inset in Figure 1; the numbers obtained were: $K = 1.3 \times 10^2$, $k_f = 3.3 \times 10^9\ M^{-1}\ s^{-1}$, $k_r = 2.6 \times 10^7\ M^{-1}\ s^{-1}$, to be compared with $K = 1.8 \times 10^2$ obtained from the absorbances at equilibrium.[24] The constant K reflects the difference between the reduction potentials of $VA^{\bullet+}$ and $TA^{\bullet+}$, and with the Nernst equation, it can be converted into a potential. When 1.49 V/NHE is taken as the absolute potential for $TA^{\bullet+}$,[23] the reduction potentials $E(VA^{\bullet+} + e^- \rightarrow VA)$ determined with these two methods, absorbances at equilibrium and kinetic analysis, result as 1.356 and 1.365 V, respectively, leading to an averaged value of 1.36 ± 0.01 V/NHE.[25] This number, which refers to aqueous solution, is the same as that (1.36 V)[19] measured in acetone solution using cyclic voltammetry. On this basis, a number can be attached to the oxidizing power of LiP. Obviously, the oxidizing power has to be >1.36 V/NHE for the reaction to be exothermic. This is within the range of a recently published estimate (≥1.2 V/NHE),[26] which is based on the ability of LiP to oxidize a series of methoxybenzenes with known oxidation potentials.[27]

2. Decay of the Radical Cation. *a. In the Absence of Bases*. In the absence of electron-transfer partners, $VA^{\bullet+}$ can have a very long lifetime. In practice, the decay by (bimolecular) radical−radical reactions is an important path limiting the lifetime of $VA^{\bullet+}$. However, at $VA^{\bullet+}$ concentrations of ≤1 μM, the radical cation is seen to disappear in a reaction that is predominantly first-order and that is assigned, on the basis of steady-state γ-radiolysis studies showing the formation of veratryl aldehyde as the main reaction product, to C−H deprotonation from the benzylic carbon of the side chain,[28] which is shown in eq 8, with $k = (17 \pm 1)\ s^{-1}$.



(8)

The α-hydroxybenzyl-type radical resulting from the deprotonation, eq 8, was also produced in an alternative way, that is, by reaction of veratrylaldehyde (VCHO) with e^-_{aq}, as shown in eq 9



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By variation of the concentration of veratrylaldehyde, $k(e^-_{aq} + VCHO)$ was determined to be $1.6 \times 10^{10}\ M^{-1}\ s^{-1}$. The absorption spectrum of the radical is shown in Figure 2 at two pH values: at pH 12 (triangles), where the ketyl radical anion predominates, and at pH 6 (circles), where the neutral form (the conjugate acid) is present. By measurement of the absorption

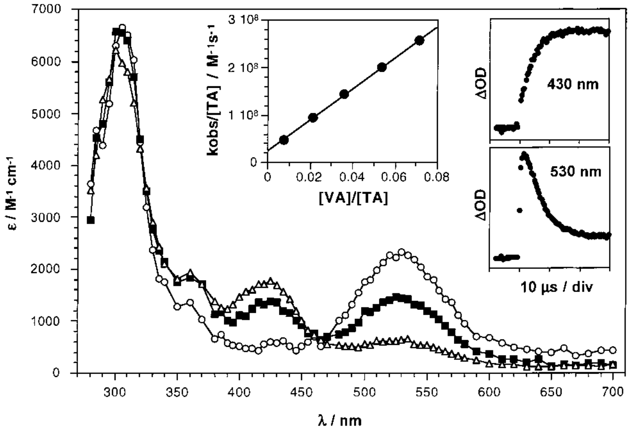


Figure 1. Time-resolved absorption spectra for the reaction of thioanisole radical cation (TA$^{•+}$) with veratryl alcohol (VA) obtained by pulse radiolysis in an Ar-saturated aqueous solution, recorded 1 μs (circles), 6 μs (filled squares), and 20 μs (triangles) after the 3 MeV, 300 ns pulse. The insets on the right show the decay of TA$^{•+}$ monitored at 530 nm and the corresponding buildup of VA$^{•+}$ at 430 nm. The left inset shows the kinetic analysis of the electron-transfer equilibrium (see text).

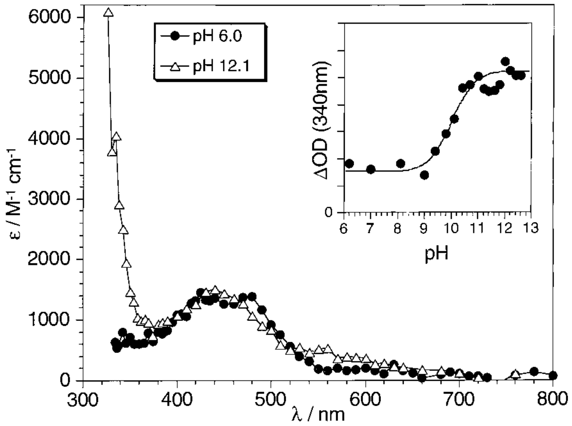
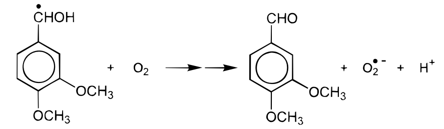


Figure 2. Absorption spectra recorded at 8 μs after the pulse on irradiating deoxygenated 0.2 mM solutions of veratrylaldehyde containing 0.1 M 2-methyl-2-propanol at pH 6.0 (circles) and 12.1 (triangles). For obtaining the ε-values, it is assumed that G(radical) = $G(e^-_{aq})$ = 2.7.

at 340 nm as a function of pH, the pK_a value of the neutral radical was determined (see inset in Figure 2) to be 10.0, which is higher (as expected) than that (pK_a = 9.3, measured in an analogous way) of the corresponding radical from 4-methoxybenzaldehyde (whereby $k(e^-_{aq})$ + 4-methoxybenzaldehyde = 3.2×10^{10} M^{-1} s^{-1}).[29] Oxidation, for example, with oxygen, of the α-hydroxybenzyl-type radical produced in eq 8 leads to the final product, veratrylaldehyde. By observation of the decay rate of the radical as a function of [O$_2$], the rate constant for reaction 10,



(10)

which probably proceeds by an addition/elimination mechanism,[30] was determined to be 1.6×10^9 M^{-1} s^{-1}.

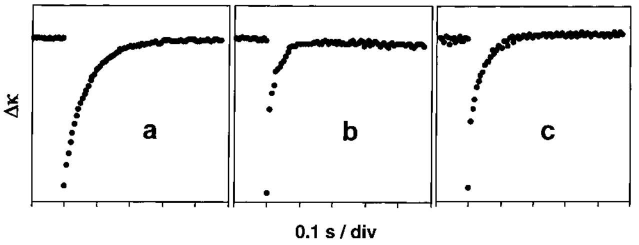
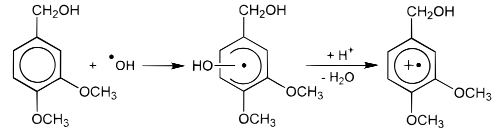


Figure 3. (a) Conductance increase initiated by a 1 Gy pulse in an N$_2$O-saturated aqueous solution at pH 3.4 containing 0.5 mM Tl$_2$SO$_4$ and 0.1 mM VA at 25 °C. (b) 8.3 mM 2-methyl-2-propanol added to the solution in (a). (c) The solution in (a) saturated with the mixture N$_2$O/O$_2$ = 1:1.

To resolve the discrepancy in the literature[16,19] on the rate of reaction 8, we measured this reaction by using the time-resolved ac-conductance technique, monitoring the production of H$^+$ at pH ≈ 3 (see Figure 3). The radical cation was produced in three different ways: (i) with SO$_4^{•-}$ (eq 4); (ii) with Tl^{2+} (eq 6); (iii) via $^•$OH, by reaction of H$^+$ with the OH adduct of VA (eq 11), analogous to reactions previously studied.[31] The latter method provides an internal standard for the concentration changes of H$^+$, since the production of the radical cation is accompanied by the loss of H$^+$ (eq 11) and the decay of the radical cation (eq 8) by the same amount of gain of H$^+$



(11)

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It was found that the rate of conductance increase resulting from the disappearance of VA$^{•+}$ depended on the method of its generation; with methods i and iii, the measured rate was (1−2) × 10^2 s^{-1}, whereas with method ii it was 17 ± 1 s^{-1}. This value is the same as that of Candeias and Harvey,[16] and we suggest that this is the correct one on the basis of the following considerations. In system iii, $^•$OH is likely to react with VA not only with the nucleus but also with the side chain, in which case neutral radicals are formed,[32] which may react rapidly with VA$^{•+}$ and thus limit its lifetime. Along this line, in system i, the lifetime of VA$^{•+}$ would be limited by the *tert*-butyl alcohol radicals produced according to eq 2. To check this idea, in system ii *tert*-butyl alcohol was added in a concentration sufficient to scavenge 50% of the initially produced OH radicals, leading to the production of Me$_2$C(OH)CH$_2^•$, thus preventing $^•$OH from producing Tl^{2+} and thus VA$^{•+}$ (via eqs 5 and 6). In fact, as seen in Figure 3b, addition of *tert*-butyl alcohol led to a drastic increase in the rate of proton formation, indicating the enhanced disappearance of VA$^{•+}$. This would be understandable if by radical−radical decay of a radical cation and a neutral radical a *carbo*cation was produced, which typically has a much higher reactivity with water (to give H$^+$) than a radical cation.[33] Thus, we suggest that the high value (1.2×10^3 s^{-1})[19,34] reported by Aust for the decay rate of VA$^{•+}$ is an artifact indicating the presence of radicals other than VA$^{•+}$ under his experimental conditions. Aust also reports[19] that there is an effect of oxygen on the lifetime of VA$^{•+}$. We have now checked this, using method ii to generate the radical cation and monitoring its decay by the conductance technique. From the very small enhancing effect of O$_2$ on the rate of conductance regeneration (compare Figure 3 parts a and c), the value we obtain is k(O$_2$ + VA$^{•+}$) ≤ 8×10^3 M^{-1} s^{-1}.

As a further model compound for lignin, an alcohol was studied that is derived from VA by substitution of one hydrogen at the benzylic position by a *tert*-butyl group, that is, 3,4-(MeO)$_2$C$_6$H$_3$CH(OH)CMe$_3$ (VA*t*Bu). On reaction with SO$_4^{•-}$

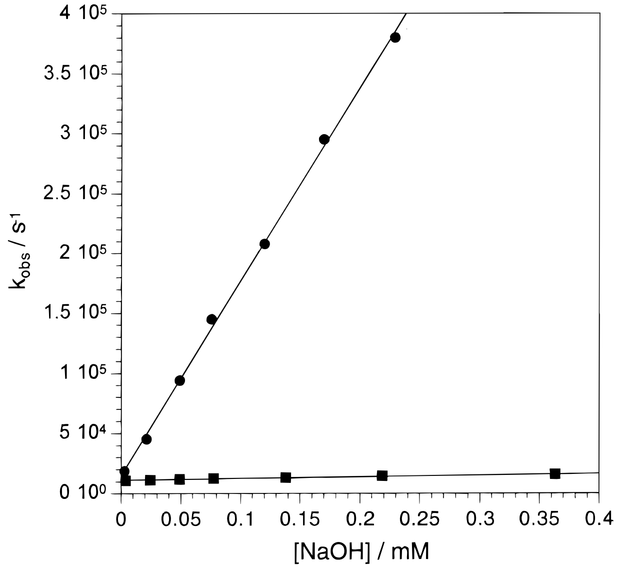
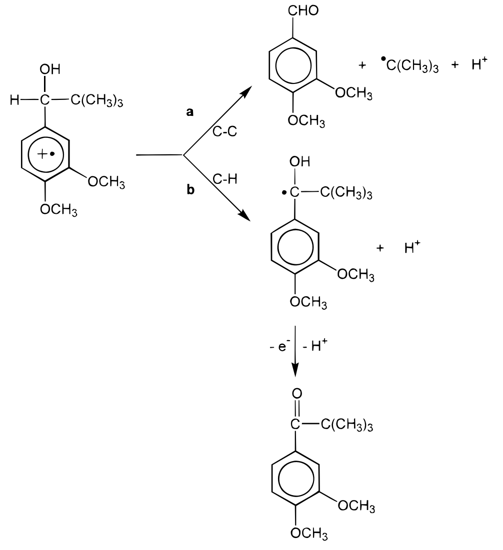


Figure 4. Dependence on [OH$^-$] of k_{obs} for decay of VA$^{\bullet+}$ (circles) and VAME$^{\bullet+}$ (squares). The O_2-saturated aqueous solutions contained 0.1 M 2-methyl-2-propanol (to scavenge $^{\bullet}$OH), 10 mM $K_2S_2O_8$ (to scavenge e^-_{aq}), 1 mM $Na_2B_4O_7$, and 0.5 mM of VA (VAME).

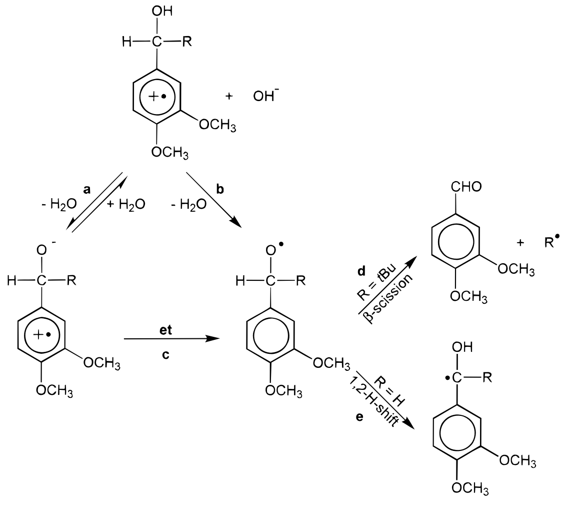
SCHEME 1



or Tl^{2+}, this compound gave a spectrum very similar to that of VA$^{\bullet+}$, which, at pH ≤ 5 and low dose, decayed in a first-order reaction with the averaged[35] rate constant[36] $k = (24 \pm 1)\ s^{-1}$. On the basis of product analysis studies performed after γ-irradiation, the decay at pH = 4−5 leads to two products: veratrylaldehyde and the ketone $3,4\text{-}(MeO)_2C_6H_3C(O)CMe_3$, with the ratio aldehyde/ketone = 2. The production of veratrylaldehyde clearly indicates that the radical cation undergoes a C−C fragmentation of the side chain (Scheme 1, path a),[37] whereas the ketone is the product of a C−H deprotonation reaction (path b)[38] followed by oxidation of the resulting α-hydroxybenzyl-type radical.[39]

b. In the Presence of Bases. Monitoring the absorption of VA$^{\bullet+}$ at 430 nm, we found that its decay rate can be considerably increased by adding OH$^-$ to the solution.[40] In fact, the rate of decay of VA$^{\bullet+}$ increases linearly with [OH$^-$] (see Figure 4), and, from the slope of this dependence, the second-order rate constant for reaction of OH$^-$ with VA$^{\bullet+}$, which leads to the formation of the α-hydroxybenzyl-type radical,[28] results as $(1.3 \pm 0.3) \times 10^9\ M^{-1}\ s^{-1}$. To elucidate the mechanism of this reaction, the OH function of VA was methylated, thus producing veratryl alcohol methyl ether (VAME), and this compound was oxidized with $SO_4^{\bullet-}$ or Tl^{2+}, analogous to reaction 4 or 6. The absorption spectrum of the resulting radical cation, VAME$^{\bullet+}$, was very similar to that of VA$^{\bullet+}$, showing absorption bands at 295 and 425 nm, and at low dose (≤1 Gy/pulse) the radical cation (as produced with Tl^{2+}) decayed at pH ≤ 5 predominantly by first-order kinetics with[36] $k = 20 \pm 1\ s^{-1}$, a value very similar to that of VA$^{\bullet+}$. The reduction potential of VAME$^{\bullet+}$ was measured by a method analogous to that described above for VA$^{\bullet+}$, and the value 1.36 V/NHE was obtained. In contrast to the situation with VA$^{\bullet+}$, the decay of VAME$^{\bullet+}$ increased only slightly in the presence of OH$^-$. From the slope of the k_{obs}(decay) vs [OH$^-$] plot (see Figure 4), the rate constant for decay of the radical cation by reaction with OH$^-$ is only $2 \times 10^7\ M^{-1}\ s^{-1}$, that is, 65 times *lower* than that for the case of the alcohol, VA, and not much higher than that ($2.1 \times 10^6\ M^{-1}\ s^{-1}$) we measured for the reaction of the radical cation of 3,4-dimethoxy*toluene* with OH$^-$, where deprotonation involves necessarily the C−H bond of the methyl group.[41] This indicates that the interaction of OH$^-$ with VAME$^{\bullet+}$ should also involve base attack at the C−H bond,[42] in contrast to the case of VA$^{\bullet+}$ where it is the O−H bond that is involved.

SCHEME 2



We found that also the rate of fragmentation of the radical cation of $3,4\text{-}(MeO)_2C_6H_3CH(OH)CMe_3$ increases strongly with increasing [OH$^-$]. From the slope of the corresponding plot, the second-order rate constant for the OH$^-$-induced fragmentation (which now almost exclusively involves the C−C bond)[38] is $8.3 \times 10^9\ M^{-1}\ s^{-1}$, a value somewhat larger than that for VA$^{\bullet+}$ (see above)[43] and close to that ($(1{-}2) \times 10^{10}\ M^{-1}\ s^{-1}$) for diffusion-controlled OH$^-$ reactions with protons bonded to hetero atoms (the rate constants for reaction of OH$^-$ with C−H protons are typically up to several orders of magnitude *lower*).[44,45] The similar rate constants for reactions of VA$^{\bullet+}$ and VA*t*Bu$^{\bullet+}$, with OH$^-$ despite the different bonds cleaved in the two processes, make it therefore reasonable to assume that OH$^-$ deprotonates in both cases the OH function in the side chain of the radical cations.[46] The result would be a radical zwitterion (Scheme 2). However, the radical zwitterion, being *less* electron deficient than the radical *cation*, should be *less* prone to undergo C−C or C−H fragmentation reactions. Thus, the experimentally observed positive “catalysis” of OH$^-$ cannot be explained by the formation of the radical zwitterion as such. However, if it is assumed that in the radical zwitterion an intramolecular electron transfer occurs from the oxyanion in the side chain to the electron “hole” in the aromatic π-system, an oxyl radical is the result, and radicals of this type are known to undergo β-C−C fragmentation (Scheme 2, step d) and, also, in aqueous solution, 1,2-H-atom shift reactions (step e),[47] thus, the mechanism accounts for all the experimental observations. Formation of the benzyloxyl radical may also be concerted[48] with base-induced deprotonation of the radical cation (Scheme 2, path b). The same type of mechanism has recently been proposed[21] for the case of 4-(mono)methoxybenzyl alcohol radical cations.[49] This indicates that this mechanism may be a general one for radical cations with an OH-function at the α-position[50] of the side chain.

Summary and Conclusions

The reduction potential of the radical cation of veratryl alcohol, $VA^{\bullet+}$, as produced in aqueous solution with $SO_4^{\bullet-}$, was determined by electron-transfer equilibration with thioanisole as a redox standard to be 1.36 V/NHE. The radical cation undergoes a C−H deprotonation reaction from the side chain with $k = (17 \pm 1)\ s^{-1}$ at pH $\leq$ 5. This value confirms a previous one[16] and indicates that $VA^{\bullet+}$ should in fact be able to act as a diffusible mediator[11,12,16] in the degradation of lignin by LiP. The deprotonation reaction of $VA^{\bullet+}$ is induced by OH^-, with the "catalytic" rate constant being $1.3 \times 10^9\ M^{-1}\ s^{-1}$. In contrast, the corresponding rate constant for reaction of OH^- with the radical cation of veratryl alcohol methyl ether, $VAME^{\bullet+}$, whose reduction potential is also 1.36 V/NHE, is only $2 \times 10^7\ M^{-1}\ s^{-1}$. From this large difference in reactivity, which cannot (as derived from the redox potentials) be due to a difference in acidity, it is concluded that in the former case (the alcohol) the reaction of OH^- involves the deprotonation from the alcohol function to yield (in a stepwise or concerted fashion) a benzyloxyl radical followed by a 1,2 H-atom shift, which constitutes the loss of H from the carbon. In the case of the ether, however, interaction of OH^- is with the hydrogen on the benzylic carbon. In the case of VA, an important consequence of the "catalysis" by OH^- is that the lifetime of the radical cation at pH $\geq$ 7 is limited by this reaction (for example, k(pseudo first order) at pH = 8 is $1.6 \times 10^3\ s^{-1}$), rather than by the uncatalyzed decay. With the veratryl alcohol derivative, 3,4-$(MeO)_2C_6H_3CH(OH)CMe_3$, the radical cation undergoes both a proton loss from the benzylic position and a $C_\alpha-C_\beta$ fragmentation with the ratio, at pH $\leq$ 5, of ca. 1:2. The decay of the radical cation is strongly enhanced by OH^- ($k = 8.3 \times 10^9\ M^{-1}\ s^{-1}$), with the base induction favoring the C−C fragmentation relative to the proton loss from the benzylic position.[43] The conclusion to be drawn from this for the biological decomposition of lignin is that in view of an optimal functioning of VA as a diffusible mediator in the oxidation of lignin by LiP it is clearly advantageous for the biological system to avoid the enhancement of the $VA^{\bullet+}$ decay by OH^-, that is, it is important to keep the pH of the enzyme environment as *low* as possible. On the other hand, to take advantage of the OH^- effect of radical cation decomposition, the (local) pH in the vicinity of the lignin macromolecule should be *high*. A conclusion of relevance to practical or industrial mechanistic applications is that for all processes of lignin degradation based on one-electron oxidation, basic media should be superior to acidic media.

Supporting Information Available: Complete experimental section with details on pulse radiolysis and γ-radiolysis experiments (3 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) Università "Tor Vergata". (b) Università "La Sapienza" (c) Max-Planck-Institut.

(2) *Biotechnology in the Pulp and Paper Industry*; Eriksson, K.-E. L., Ed.; Springer-Verlag: Berlin Heidelberg, 1997.

(3) Pardini, V. L.; Smith, C. Z.; Utley, J. H. P.; Vargas, R. R.; Viertler, H. *J. Org. Chem.* **1991**, *56*, 7305.

(4) Gunnarsson, P.-I.; Ljunggren, S. *Acta Chem. Scand.* **1996**, *50*, 422.

(5) Chum, H. L.; Baizer, M. M. *The Electrochemistry of Biomass and Derived Materials*; ACS Monograph 183; American Chemical Society: Washington, DC, 1985; p 244.

(6) Othmer, K. *Encyclopedia of Chemical Technology*, 3rd ed.; J. Wiley: New York, 1983; vol. 23, p 704.

(7) (a) Tien, M.; Kirk, T. K. *Science* **1983**, *221*, 661. (b) Glenn, J. K.; Morgan, M. A.; Mayfield, M. B.; Kuwahara, M.; Gold, M. H. *Biochem. Biophys. Res. Commun.* **1983**, *114*, 1077. (c) Schoemaker, H. E.; Harvey, P. J.; Bowen, R. M.; Palmer, J. M. *FEBS Lett*. **1985**, *183*, 7. (d) Harvey, P. J.; Schoemaker, H. E.; Bowen, R. M.; Palmer, J. M. *FEBS Lett*. **1985**, *183*, 13. (e) Hammel, K. E.; Kalyanaraman, B.; Kirk, T. K. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3708. (f) Hammel, K. E.; Jensen, K. A.; Mozuch, M. D.; Landucci, L. L.; Tien, M.; Pease, E. A. *J. Biol. Chem.* **1993**, *268*, 12274.

(8) Kersten, P. J.; Tien, M.; Kalyanaraman, B.; Kirk, T. K. *J. Biol. Chem.* **1985**, *260*, 2609.

(9) Hammel, K. E.; Moen, M. A. *Enzyme Microb. Technol.* **1991**, *13*, 5.

(10) (a) Piontek, K.; Glumoff, T.; Winterhalter, K. *FEBS Lett.* **1993**, *315*, 119. (b) Poulos, T. L.; Edwards, S. L.; Wariishi, H.; Gold, M. H. *J. Biol. Chem.* **1993**, *268*, 4429.

(11) Harvey, P. J.; Schoemaker, H. E.; Palmer, J. M. *FEBS Lett.* **1986**, *195*, 242.

(12) Goodwin, D. C.; Aust, S. D.; Grover, T. A. *Biochemistry* **1995**, *34*, 5060.

(13) Valli, K.; Wariishi, H.; Gold, M. H. *Biochemistry* **1990**, *29*, 8535.

(14) Koduri, R. S.; Tien, M. *Biochemistry* **1994**, *33*, 4225.

(15) Schoemaker, H. E.; Piontek, K. *Pure Appl. Chem.* **1996**, *68*, 2089.

(16) Candeias, L. P.; Harvey, P. J. *J. Biol. Chem.* **1995**, *270*, 16745.

(17) Khindaria, A.; Grover, T. A.; Aust, S. D. *Biochemistry* **1995**, *34*, 6020.

(18) Khindaria, A.; Yamazaki, I.; Aust, S. D. *Biochemistry* **1995**, *34*, 16860.

(19) Khindaria, A.; Yamazaki, I.; Aust, S. D. *Biochemistry* **1996**, *35*, 6418.

(20) There is also the problem of the "stabilization" of $VA^{\bullet+}$ by LiP; k(decay) of $VA^{\bullet+}$, as generated by LiP, was reported[19] to be $1.8\ s^{-1}$.

(21) Baciocchi, E.; Bietti, M.; Steenken, S. *J. Am. Chem. Soc.* **1997**, *119*, 4078.

(22) Ioele, M.; Steenken, S.; Baciocchi, E. *J. Phys. Chem. A* **1997**, *101*, 2979.

(23) This, our number, is from electron-transfer equilibration between $TA^{\bullet+}$ and 3-(2-methoxyphenoxyl)-1,2-propanediol, for which the potential was reported to be 1.40 V/NHE (a) Jonsson, M.; Lind, J.; Merenyi, G.; Eriksen, T. E. *J. Chem. Soc., Perkin Trans. 2*, **1995**, 67.

(24) For details on this technique, see the following. Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637. Steenken, S.; Neta, P. *J. Phys. Chem.* **1982**, *86*, 3661. Reference 23a. Typically, the *K* from the absorbances is more reliable than that from the rates.

(25) To check this number, we have equilibrated $VA^{\bullet+}$ also with guanosine, whose potential relative to that (1.13 V)[23a] of 1,2,4-trimethoxybenzene has been measured to be 1.26 V/NHE (Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc*. **1997**, *119*, 617). To improve the reliability of the potential for 1,2,4-trimethoxybenzene, we have now measured this value by cyclic voltammetry in aqueous solution, pH 3−5, with 0.1 M KCl as the electrolyte, to be 1.14 V/NHE, which is in excellent agreement with the pulse radiolysis value from ref 23a. Then, from the measured difference in the potential between guanosine and VA, $E(VA^{\bullet+})$ results as 1.37 V/NHE, a number which is practically the same as that from the comparison with $TA^{\bullet+}$ (see text).

(26) Kersten, P. J.; Kalyanaraman, B.; Hammel, K. E.; Reinhammar, B.; Kirk, T. K. *Biochem. J.* **1990**, *268*, 475.

(27) A similar value was found for manganese peroxidase (see: Popp, J. L.; Kirk, T. K. *Arch. Biochem. Biophys.* **1991**, *288*, 145).

(28) Since the lifetime of the $VA^{\bullet+}$ *deprotonation product* (which decays by radical−radical reaction) is considerably shorter than that of its precursor, $VA^{\bullet+}$, the spectrum of the 3,4-dimethoxy-α-hydroxybenzyl radical cannot be seen at pH $\leq$ 7. However, by speeding up the deprotonation reaction with base (see Scheme 2, steps a−c, and e), the spectrum (which turns out to be the same as that from eq 9; see Figure 2) can be easily measured.

(29) Baciocchi, E.; Bietti, M.; Steenken, S. Unpublished results.

(30) Steenken, S. In *Free Radicals: Chemistry, Pathology and Medicine*; Rice-Evans, C., Dormandy, T., Eds.; Richelieu Press: London, 1988, p 51.

(31) O'Neill, P.; Steenken, S.; Schulte-Frohlinde, D. *J. Phys. Chem.* **1975**, *79*, 2773.

(32) The yield of $VA^{\bullet+}$ from eq 11 compared to that (≡100%) from the reaction with Tl^{2+} (eq 6) was found to be 35%. The remaining 65% is assigned to H-abstraction from the substituents by $^{\bullet}OH$ and/or to ipso-addition at the MeO groups followed by elimination of MeOH to give phenoxyl-type radicals. See the following. Steenken, S.; O'Neill, P. *J. Phys. Chem*. **1977**, *81*, 505. O'Neill, P.; Steenken, S. *Ber. Bunsen-Ges. Phys. Chem.* **1977**, *81*, 550. The yield of $VA^{\bullet+}$ from eq 4 was the same as that from eq 6, which indicates that H-abstraction by $SO_4^{\bullet-}$ from the side chain is not important.

(33) Reaction of a radical with the radical cation could proceed via dimerization to yield a (delocalized) cyclohexadienyl-type carbocation or via (electron transfer) disproportionation giving, again, a carbocation.

(34) This value was determined in air or oxygen saturated solution, whereas that of ref 16 was measured in the absence of O_2.

(35) In the conductance traces, there were two first-order components visible, with the ratio of the rate constants being 2:1. On the basis of the product analysis results (see text), the higher value is assigned to the C−C fragmentation process (Scheme 1, path a) and the lower one to the C−H deprotonation reaction (Scheme 1, path b). The "averaged" rate constant, $24 \pm 1\ s^{-1}$, is similar to that of $VA^{\bullet+}$.

(36) Value obtained by the conductance technique in the dose range 0.5−2 Gy/pulse.

(37) On the basis of the redox potentials (see: Wayner, D. D. M.; Sim, B. A.; Dannenberg, J. J. *J. Org. Chem*. **1991**, *56*, 4835. Wayner, D. D. M.; McPhee, D. J.; Griller, D. *J. Am. Chem. Soc*. **1988**.) of the pertinent fragments, the conceivable *inverse* C−C fragmentation, that is, that leading to the *tert*-butyl cation, is thermodynamically much less favorable. Also, with the 4-methoxy analogue (see: Baciocchi, E.; Bietti, M.; Putignani, L.; Steenken, S. *J. Am. Chem. Soc.* **1996**, *118*, 5952), the *tert*-butyl radical was actually detected by product analysis.

(38) It was found that in the presence of phosphate buffer at pH = 6.9 (10 mM KH_2PO_4 and 10 mM Na_2HPO_4), the veratryl aldehyde/ketone ratio increased to 50 (aldehyde 98%, ketone 2%), that is, the C−C bond cleavage pathway is strongly assisted by the base phosphate, whereas C−H deprotonation apparently profits much less from the base (the details of this mechanism are presently under investigation: Baciocchi, E.; Bietti, M.; Steenken, S. To be published). At the same time, the total yield increased by a factor of 4 as compared to that obtained at pH 4−5. In addition to $SO_4^{\bullet-}/S_2O_8^{2-}$, the oxidant $Co(III)W_{12}O_{40}^{5-}$ (see: Baciocchi, E.; Bietti, M.; Steenken, S. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1261 and references therein) was used for the oxidation of this substrate at 25 °C. In this case, the only observed product was veratrylaldehyde; no ketone was detected.

(39) $S_2O_8^{2-}$ oxidizes the α-hydroxybenzyl-type radical yielding the ketone, H^+, and $SO_4^{\bullet-}$ which continues the chain.

(40) This dependence of lifetime on pH constitutes a correction of the report of Aust[19] in which it is stated that the lifetime of the radical cation is *independent* of pH.

(41) At pH 4−5, the rate constant for the deprotonation of the radical cation of 3,4-dimethoxy*toluene* was measured (by conductance) to be 1.1 s^{-1}.

(42) Less likely is a change of mechanism, for example, toward OH^- addition at the ring.

(43) The mechanistic implications of these findings are presently under investigation: Baciocchi, E.; Bietti, M.; Steenken, S. To be published.

(44) Eigen, M. *Angew. Chem.* **1963**, *75*, 489; *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1.

(45) In acetonitrile solvent, however, rate constants for base-induced radical cation deprotonations of up to $10^{10}\ M^{-1}\ s^{-1}$ have been measured (Anne, A.; Hapiot, P.; Moiroux, J.; Neta, P.; Saveant, J.-M. *J. Am. Chem. Soc.* **1992**, *114*, 4694 and references therein. See also Sehested, K.; Holcman, J. *J. Phys. Chem*. **1978**, *82*, 651 for rate constants in *aqueous* solution of up to $10^{10}\ M^{-1}\ s^{-1}$).

(46) In comparison with the strong base OH^-, the efficiency of the weaker base HPO_4^{2-} is much lower, that is, $k(HPO_4^{2-} + \text{radical cation}) \leq 5 \times 10^4\ M^{-1}\ s^{-1}$ (this value relates to both VA and VA*t*Bu).

(47) Gilbert, B. C.; Laue, H. A. H.; Norman, R. O. C.; Sealy, R. C. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1040. Dobbs, A. J.; Gilbert, B. C.; Laue, H. A. H.; Norman, R. O. C. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1044. Gilbert, B. C.; Holmes, R. G. G.; Laue, H. A. H.; Norman, R. O. C. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1047. Gilbert, B. C.; Warren, C. J. *Res. Chem. Intermed.* **1989**, *11*, 1. The 1,2-H shift also takes place in alcoholic solvents. See: Elford, P. E.; Roberts, B. P. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2247.

(48) If the rate of the (hypothetical) electron transfer, step c, is (much) larger than that of the protonation of the radical zwitterion, the reverse of step a, the formation of the benzyloxyl radical, would *appear* to be concerted with the reaction of OH^- with the radical cation.

(49) A similar mechanism involving the formation of a benzyloxyl radical has also been proposed for the oxidation of $3,4\text{-}(MeO)_2C_6H_3CH(OH)Me$ with ClO_2 (see ref 4).

(50) We have recently obtained evidence that this mechanism is not restricted to the alcohol function at the α-position.