p-Hydroxyphenacyl Phototriggers: The Reactive Excited State of Phosphate Photorelease

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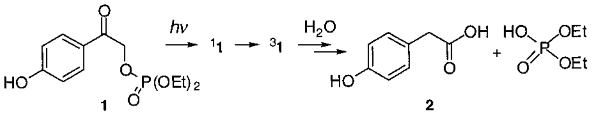
Fast release of biological stimulants is needed to monitor physiological response in real time.[1] *p*-Hydroxyphenacyl (*p*HP) phototriggers meet this requirement. Moreover, the side products are biologically compatible and transparent to excitation wavelengths >300 nm.[2] Knowledge of the release rate is essential to biophysical applications. Givens et al. identified a short-lived triplet state as the reactive excited state of *p*HP phosphates and carboxylates on the basis of quenching experiments.[2] In a recent study of *p*HP esters Zhang et al.[3a] questioned that evidence and favored a singlet mechanism. Here, we prove that the reactive excited state of *p*HP diethyl phosphate (**1**) is a very short-lived triplet state and we provide direct evidence for adiabatic tautomerization of *p*-hydroxyacetophenone and its derivatives in the triplet state.



p-Hydroxyphenylacetic acid (**2**) is the only product detected by GC−MS after irradiation of **1** in wet acetonitrile (AN, H_2O ≥ 5% by vol). Numerous products of higher molecular weight are formed in dry AN. Relative quantum yields of the photoreaction in different media were determined by the permanent bleaching of the absorption by **1** at 270 nm (pulsed laser excitation at 308 nm). Water (5%) accelerated the reaction about 5-fold, but saturation with air or addition of 0.5 mM piperylene slowed the conversion about 4-fold in dry AN. The absolute quantum yield in wet AN (5%) was determined as 0.94 (steady-state 313-nm irradiation, azobenzene actinometry); it was reduced to 0.37 by addition of 10 mM piperylene. Still higher quencher concentrations were required to reduce the quantum yield in solutions containing more water.[2c,3a]

Identification of the transients produced by nanosecond laser flash photolysis (LFP)[4] of **1** was aided by referring to *p*-hydroxyacetophenone (**3**) and *p*-methoxyacetophenone (**4**) as model compounds. Strong absorption was formed by LFP of **4** (1×10^{-4} M) in aqueous AN (50% H_2O, $\lambda_{max} \approx 395$ nm) or AN (385 nm). Admission of air reduced the lifetime of this transient from ~30 μs to 80 ns in AN, and it was identified as the triplet state of **4**, ^{3}**4**, by energy transfer to naphthalene ($E_T = 61$ kcal mol^{-1}),[5] $k_{et} \approx 1 \times 10^{10}$ M^{-1} s^{-1}. Pump−probe spectroscopy of **4** in AN (248-nm excitation, 1 mJ per pulse, 0.8 ps pulse length) showed that intersystem crossing (ISC), ^{1}**4** → ^{3}**4**, is very fast, $k_{ISC} = (3.1 \pm 0.2) \times 10^{11}$ s^{-1}.

Replacement of the methoxy group by an ionizable hydroxy group introduces new functionality (Scheme 1). The ionization constant of **3** is pK_a(**3**) = 7.9 ± 0.1.[6] The equilibrium constant for the isomerization of **3** to its quinonoid enol tautomer **3′**, pK_E = 16.4, was calculated using density functional theory (DFT).[7] A thermodynamic cycle then defines the acidity of enol **3′** to be very high, pK_a(**3′**) = −8.5.

Formation of ^{3}**3** is equally fast in aqueous AN solvent mixtures ($k_{ISC} = 2.7 \times 10^{11}$ s^{-1}, $\lambda_{max} = 370$ in dry AN, 395 nm with 50% H_2O). Energy transfer from ^{3}**3** to naphthalene was again observed.[5] The transient is acidic,[8] but does not ionize in dry AN. Adiabatic ionization, ^{3}**3** → ^{3}**3**$^-$ + H$^+$, takes place in aqueous AN (50% H_2O), $k_{ion} \approx 9 \times 10^6$ s^{-1}, and is too fast for detection by LFP in water, $k_{ion} > 3 \times 10^7$ s^{-1}. The spectra of ^{3}**3** and ^{3}**3**$^-$ appear to be quite similar; the absorption maximum of ^{3}**3**$^-$ is shifted to slightly longer wavelength (405 nm) and the weaker band in the visible, $\lambda_{max} \approx 500$ nm, is more pronounced. In a second process, $k = 1.9 \times 10^6$ s^{-1}, the intensity of the 405-nm band is reduced, and a new strong band appears at 350 nm.[3b] Excitation of the anion **3**$^-$ in aqueous NaOH (0.1 M) at 308 nm gave only the 405 and 500 nm bands, which shows that these are due to the triplet state of the anion, ^{3}**3**$^-$.

Apparently, ^{3}**3**$^-$ is re-protonated to form the 350-nm transient in acidic solutions. The first-order rate coefficient for reprotonation in dilute aqueous acid ([HClO$_4$] = 3−10 × 10^{-4} M) was proportional to acid concentration, $k_{H^+} \approx 4 \times 10^{10}$ M^{-1} s^{-1}. Spectrographic traces of the equilibrated triplet−triplet absorptions (delay relative to excitation 200 ns) formed by excitation of **3** in aqueous buffer solutions at pH 7.1 (phosphate, $I = 0.01$ M), 5.32, 4.66, 4.38, 4.04 (acetate, $I = 0.01$ M), and 2.0 (HClO$_4$, 10^{-2} M) are shown in Figure 1. Factor analysis indicated that two components sufficed to reproduce all spectra within experimental accuracy. Fitting of a titration function to the loading coefficients gave pK_a = 4.6 ± 0.2.

We attribute the protonated species observed at low pH ($\lambda_{max} \approx 350$ nm) to the quinonoid enol triplet, ^{3}**3′**. Clearly, ^{3}**3′** is an excited triplet—it is in equilibrium with ^{3}**3**$^-$ and the lifetimes of both are equally reduced by oxygen—but its properties are distinctly different from those of ^{3}**3**. The triplet energy of **3** (E_T = 70.5 kcal mol^{-1},[2c] DFT calculation:[7] E_T = 69.5 kcal mol^{-1}) is well above that of naphthalene. On the other hand, the excitation energy of the quinonoid enol **3′** is expected to be much lower

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[2] (a) Givens, R. S.; Park, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6259−6262. (b) Park, C.-H.; Givens, R. S. *J. Am. Chem. Soc.* **1997**, *119*, 2453−2463. (c) Givens, R. S.; Weber, J. F. W.; Conrad, P. G.; Orosz, G.; Donahue, S. L.; Thayer, S. A. *J. Am. Chem. Soc.* **2000**, *122*, 2687−2697.

[3] (a) Zhang, K.; Corrie, J. E. T.; Munasinghe, V. R. N.; Wan, P. *J. Am. Chem. Soc.* **1999**, *121*, 5625−5632. (b) Brousmiche, D. W.; Wan, P. *J. Photochem. Photobiol., A* **2000**, *130*, 113−118.

[4] Excimer laser operated on XeCl, 308 nm, ~100 mJ per pulse, pulse width 25 ns. Low concentrations of ketones **1**, **3**, and **4** were used to avoid radical formation by intermolecular hydrogen abstraction: Das, P. K.; Encinas, M. V.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 4154−4162. Canonica, S.; Hellrung, B.; Wirz, J. *J. Phys. Chem. A* **2000**, *104*, 1226−1232.

[5] Naphthalene concentrations ≤1 mM, selective excitation of the ketone at 351 nm (XeF excimer laser). The formation of triplet naphthalene was monitored at $\lambda_{max} = 415$ nm.

[6] Spectrophotometric titration in tris(hydroxymethyl)amine/HCl buffer (ionic strength $I = 0.1$ M). See also: Vandenbelt, J. M.; Henrich, C.; Vandenberg, S. G. *Anal. Chem.* **1954**, *26*, 726−727.

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[8] Bromophenol blue is protonated by ^{3}**3** at a diffusion-controlled rate ($\lambda_{obs} = 600$ nm), but the proton transfer is largely suppressed when ^{3}**3** is quenched by oxygen (1 atm).

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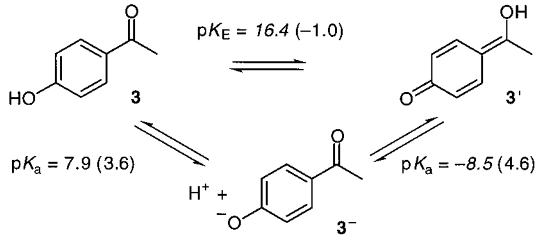
Scheme 1. Ionization and Tautomerization Equilibria of **3**[a]



[a] Values for the lowest triplet states are given in brackets. Values relying on DFT calculations[7] are in italic.

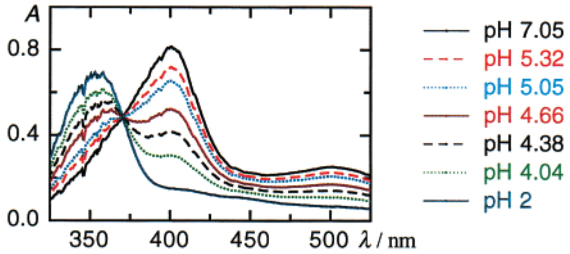


Figure 1. Transient absorption spectra obtained by LFP of **3** in water (10% AN) with various buffers.

(DFT calculation:[7] $E_{\mathrm{T}} = 38.4$ kcal mol^{-1}). Indeed, ^{3}**3′** fails to sensitize naphthalene,[5] $k_{\mathrm{et}} < 1 \times 10^{8}\ \mathrm{M^{-1}\ s^{-1}}$.

DFT calculations for the lowest triplet state correctly predict that enol ^{3}**3′** is favored over phenol ^{3}**3**, $\mathrm{p}K_{\mathrm{E}}^{*} = -7.0$, but the calculated value is too low. The triplet energy of **3**$^{-}$ is $E_{\mathrm{T}}(\mathbf{3}^{-}) = 64.7$ kcal mol^{-1}.[9] Ignoring entropy terms, the enolization constant of ^{3}**3** may be calculated as $\mathrm{p}K_{\mathrm{E}}^{*} = \mathrm{p}K_{\mathrm{a}}(\mathbf{3}) - \mathrm{p}K_{\mathrm{a}}^{*}(\mathbf{3'}) + \{E_{\mathrm{T}}(\mathbf{3}^{-}) - E_{\mathrm{T}}(\mathbf{3})\}/(2.3RT) = 7.9 - 4.6 + \{64.7 - 70.5\}/1.36 \approx -1.0$, and the acidity constant of ^{3}**3** as $\mathrm{p}K_{\mathrm{a}}^{*}(\mathbf{3}) = \mathrm{p}K_{\mathrm{E}}^{*} + \mathrm{p}K_{\mathrm{a}}^{*}(\mathbf{3'}) \approx 3.6$ (Scheme 1). This is consistent with the observed rate of ionization, $k_{\mathrm{ion}} \approx 9 \times 10^{6}\ \mathrm{s^{-1}}$ in aqueous AN (50% H_2O): $K_{\mathrm{a}}^{*}(\mathbf{3}) = k_{\mathrm{ion}}/k_{\mathrm{H^{+}}}$. The observed fast ionization of ^{3}**3**, followed by diffusional reprotonation of the resulting anion ^{3}**3**$^{-}$ to form enol ^{3}**3′**, excludes a concerted, "intramolecular"[3a] proton-transfer mechanism through water.

Yet another intermediate, $\lambda_{\max} \approx 325$ nm, is formed by LFP of **3** in neutral aqueous solutions. The yield and the lifetime of this transient are not affected by oxygen, and its absorption spectrum is identical with that of the anion **3**$^{-}$ in aqueous base, as noted by Wan and co-workers.[3] The main precursor of **3**$^{-}$ is the triplet anion ^{3}**3**$^{-}$: a resolved absorbance growth of **3**$^{-}$ at 325 nm, $k = 5 \times 10^{6}\ \mathrm{s^{-1}}$, matches the decay of ^{3}**3**$^{-}$ that is observed at $\lambda \geq 400$ nm in oxygen-saturated water. The subsequent decay of **3**$^{-}$ is not first order in the absence of buffers, because pH increases as **3**$^{-}$ is protonated to **3**.

LFP[4] of diethyl phosphate **1** in carefully dried, degassed AN showed a strong transient absorbance, $\lambda_{\max} = 395$ nm, which is assigned to ^{3}**1**. The decay rate of ^{3}**1** increased linearly with increasing concentration of **1** (0.1−1.5 mM, self-quenching rate constant $k_{\mathrm{sq}} = 8.5 \times 10^{8}\ \mathrm{M^{-1}\ s^{-1}}$, intercept $k_0 = 2 \times 10^{5}\ \mathrm{s^{-1}}$). Self-quenching is attributed to head-to-tail hydrogen abstraction,[4] which leads to the radical-derived photoproducts found in dry AN. Energy transfer to naphthalene,[5] $k_{\mathrm{et}} = 7.8 \times 10^{9}\ \mathrm{M^{-1}\ s^{-1}}$, identified ^{3}**1** as an excited triplet. The decay rate of ^{3}**1** increased upon admission of air or oxygen, $k_{\mathrm{q}} \approx 3 \times 10^{9}\ \mathrm{M^{-1}\ s^{-1}}$, and upon addition of piperylene ($0-1.25 \times 10^{-2}$ M), $k_{\mathrm{q}} = 4.5 \times 10^{9}\ \mathrm{M^{-1}\ s^{-1}}$. The rate constant increased in a parabolic fashion with increasing water content, reaching $2.8 \times 10^{7}\ \mathrm{s^{-1}}$ at 5% of water, and the transient escaped detection by nanosecond LFP at water contents exceeding 10%.

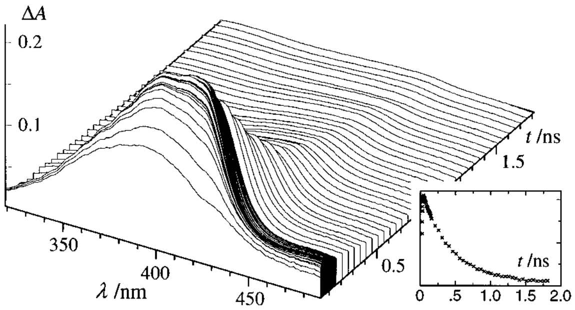


Figure 2. Pump-probe spectra of **1** in aqueous AN (1:1). The kinetic trace shows the temporal evolution of the loading coefficients of the first eigenspectrum obtained by factor analysis.

Excellent agreement between the effect of piperylene on the steady-state and time-resolved data establishes that the triplet state is the reactive excited state in the photorelease of diethyl phosphate from **1**: the ratio of the quantum yields obtained with (10 mM) and without piperylene in 5% aqueous AN, $\phi^{\mathrm{q}}/\phi^{0} = 0.39$ (vide supra), is equal, within the limits of error, to the corresponding ratio of the lifetimes of the transient intermediate ^{3}**1**, $\tau^{\mathrm{q}}/\tau^{0} = k_{\mathrm{obs}}/(k_{\mathrm{obs}} + k_{\mathrm{q}}[\mathrm{q}]) = 0.38$.

Addition of concentrated $HClO_4$ ($1-100 \times 10^{-4}$ M) to AN resulted in a titration plot similar to that shown in Figure 1. Half-protonation was reached at about 4×10^{-3} M acid. The acidic form of the transient is attributed to the quinonoid enol triplet ^{3}**1′**. Zhang et al. noted that the quantum yield of the photoreaction of *p*HP acetate drops in aqueous acid.[3a] We observe a similar, if less pronounced drop in acidic aqueous (5%) AN, ϕ ([$HClO_4$]/M): 0.94 (1×10^{-4}), 0.90 (0.05), 0.84 (0.2), 0.74 (1.0). This drop is attributed to protonation of ^{3}**1**$^{-}$ to the neutral enol ^{3}**1′**, which is less prone to the release of diethyl phosphate. We could not perform quantum yield measurements in aqueous base, because **1** is quickly hydrolyzed in the ground state. The drop observed at high pH with *p*HP esters[3] may be due to a low quantum yield of ISC. We found that excitation of **3**$^{-}$ gives low yields of ^{3}**3**$^{-}$, especially at excitation wavelengths of less than 350 nm.

The sharp increase of the decay rate of ^{3}**1** with increasing water content in AN is attributed to acceleration of heterolytic phosphate release by water. The spectral evolution seen by pump−probe spectroscopy of **1** in aqueous AN (1:1) is shown in Figure 2. Factor analysis of 79 spectra recorded with time delays of 5−2000 ps required 2 spectral components. Adequate fit to the loading factors was obtained with a dual exponential function, $k_1 = (3.8 \pm 0.2) \times 10^{11}\ \mathrm{s^{-1}}$ ($^{1}\mathbf{1} \rightarrow {}^{3}\mathbf{1}$) and $k_2 = (2.4 \pm 0.2) \times 10^{9}\ \mathrm{s^{-1}}$ (phosphate release). We believe that phosphate release occurs directly from the neutral ^{3}**1**, but rate-determining ionization to ^{3}**1**$^{-}$ followed by rapid release cannot be excluded.

In summary, the photorelease of diethyl phosphate from *p*-hydroxyphenacyl diethyl phosphate (**1**) in neutral aqueous media proceeds via the triplet state, ^{3}**1**, which is formed within a few ps and has a lifetime of 0.4 ns in 1:1 aqueous acetonitrile. The subsequent steps of the rearrangement and hydrolysis to *p*-hydroxyphenyl acetic acid (**2**) remain to be elucidated.

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[9] Phosphorescence of **3**$^{-}$ was measured at 77 K in glassy ethanol over solid Na_2CO_3, $\lambda_{\max}(0-0) = 442$ nm, $\tau_{\mathrm{Ph}} = 0.79$ s.