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Photoionization and Photosensitized Electron-Transfer Reactions of Psoralens and Coumarins[1]

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The radical cations of several psoralens (furocoumarins) and coumarins have been generated via direct excitation ($\lambda_{ex} = 355$ nm) in aqueous solution and/or via photosensitized electron transfer in acetonitrile and characterized using transient absorption spectroscopy. Significant photoionization yields were observed for 8-methoxypsoralen (8-MOP) and three methoxy-substituted coumarins in aqueous buffer and for 4,5′,8-trimethylpsoralen in aqueous acetonitrile. Of those, 6,7-dimethoxycoumarin (67-DMC) was found to have the highest quantum (0.2 ± 0.03) yield for photoionization in aqueous buffer following direct excitation at both 355 and 308 nm. Laser energy dependence plots for the photoionization process are linear and pass through (0,0) for $\lambda_{ex} =$ 355, 308, and 266 nm, providing strong evidence for a monophotonic process. The photoionization results are compared with both the one-electron oxidation potentials of the compounds (obtained via cyclic voltammetry) and the fluorescence quantum yields and lifetimes (obtained via single photon counting). The results demonstrate that photoionization is of general importance in psoralen and coumarin photochemistry and varies substantially in efficiency, depending on the structure, photophysical parameters, and environment of the substrate. The observation of photoionization upon low-intensity UVA irradiation is clearly significant with respect to the clinical use of these compounds in psoralen UVA therapy.

Introduction

Psoralens (furocoumarins) have been used in conjunction with UVA irradiation as a clinical treatment, known as PUVA therapy, for dermatological disorders for more than 20 years. More recently, psoralens have been employed in the photoinactivation of lymphocytes and hence therapy of cutaneous T-cell lymphoma and graft-versus-host disease (for reviews see refs 2−4 and references therein) as well as for virus inactivation.[5] There is considerable current interest in the development of new psoralen derivatives for a variety of therapeutic applications. However, questions regarding the long-term safety of PUVA therapy have also been raised; for example, there is some evidence linking long-term PUVA therapy with an increased probability of skin cancer which is clearly of critical importance for a dermatological therapy.[3,6,7]

Many dermatological applications have focused on 8-methoxypsoralen (8-MOP) which is generally believed to exert its phototherapeutic activity via inhibition of DNA replication. This is accomplished by intercalation of the psoralen between adjacent base pairs in the DNA duplex followed by two successive photocycloaddition reactions that generate a psoralen diadduct that cross-links the two DNA strands. Considerable research efforts have been directed toward identifying the excited-state intermediates involved in the photocycloaddition chemistry of psoralens that are used in PUVA. Early attention focused on characterization of the triplet states of 8-MOP and other psoralens by transient absorption spectroscopy[8] and the measurement of singlet oxygen yields.[9−16] A number of coumarin photosensitizers have also attracted attention since they do not form diadducts and can serve as models for the initial furan monoadduct that is the precursor to DNA cross-links.[17−19] More recently, the importance of binding to proteins,[20] cycloaddition reactions with unsaturated fatty acids,[21,22] and electron-transfer reactions[19,23−27] in psoralen photochemistry has been examined. A detailed understanding of the molecular basis for both the therapeutic and harmful effects of PUVA therapy is obviously required to aid in the further development of potential new therapeutic agents.

We recently reported that the radical cation of 8-MOP (8-MOP$^{\bullet+}$) is generated in aqueous solution via monophotonic photoionization ($\Phi = 0.015$) under conditions relevant to those employed in PUVA therapy: UVA irradiation (355 nm), unfocused, low-intensity source.[28] By contrast, photoionization of 5-methoxypsoralen (5-MOP) and psoralen was not observed. 8-MOP$^{\bullet+}$ was found to react with guanosine monophosphate (GMP) with a bimolecular rate constant, $k_{bm} = 2.5 \times 10^9$ M^{-1} s^{-1}, to produce a GMP-derived radical. Adenosine monophosphate behaved similarly but with a much lower efficiency ($k_{bm} = 3.4 \times 10^7$ M^{-1} s^{-1}). These results may explain the recent observations of both DNA cleavage and cycloaddition reactions for DNA-intercalated psoralens,[29] and similar processes in vivo would obviously be of considerable significance. As part of our general interest in elucidating the role of electron transfer in psoralen photochemistry, we have generated the radical cations of a series of structurally related psoralen derivatives by photoinduced electron transfer. The structures of these compounds are given in Scheme 1. We have also used transient absorption spectroscopy to investigate the ease of photoionization for the same compounds in aqueous solution and find that radical cation formation is of general importance in psoralen and coumarin photochemistry. Oxidation potentials, fluorescence yields and lifetimes, and solvent effects provide insight into the factors that determine photoionization efficiencies for these substrates.

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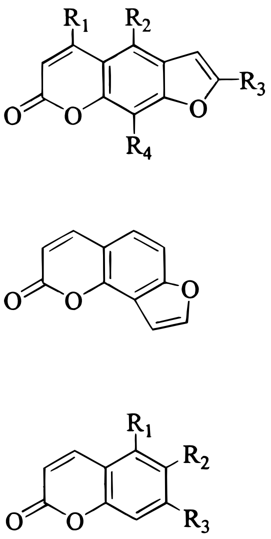
SCHEME 1



Psoralen	(R_1=R_2=R_3=R_4=H)
5-Methoxypsoralen (5-MOP)	(R_2=OCH_3)
8-Methoxypsoralen (8-MOP)	(R_4=OCH_3)
4,5',8-Trimethylpsoralen (458-TMP)	(R_1=R_3=R_4=CH_3)
Angelicin	
Coumarin	(R_1=R_2=R_3=H)
7-Methylcoumarin	(R_3=CH_3)
7-Methoxycoumarin	(R_3=OCH_3)
5,7-Dimethoxycoumarin (57-DMC)	(R_1=R_3=OCH_3)
6,7-Dimethoxycoumarin (67-DMC)	(R_2=R_3=OCH_3)

Experimental Section

Materials. Coumarin, 7-methylcoumarin, 7-methoxycoumarin, 5,7-dimethoxycoumarin, 6,7-dimethoxycoumarin, 4,5′,8-trimethylpsoralen, 5-methoxypsoralen, 8-methoxypsoralen, psoralen, angelicin, benzophenone, 9,10-diphenylanthracene, ferrocene (all Aldrich), and anthracene (BDH) were commercial samples and were used as received. Chloranil (tetrachloro-1,4-benzoquinone, Aldrich) was recrystallized from ethanol. All solvents were of the highest available commercial grade (Omnisolv). For electrochemical measurements, acetonitrile was distilled over calcium hydride under argon, prior to use. Tetrabutylammonium tetrafluoroborate was recrystallized three times from ethyl acetate/hexane (9:1) and dried prior to use.

Laser Flash Photolysis. The nanosecond laser system has been described in detail elsewhere.[30] The excitation source was either a Lumonics HY750 Nd:YAG laser (266 nm, 10 ns/20 mJ pulses and 355 nm, 10 ns/40 mJ pulses), a Lumonics EX-530 excimer laser (XeCl, 308 nm, 6 ns/40 mJ pulses), or a Nd:YAG-pumped Lumonics HD500 dye laser (420 nm, 10 ns/12 mJ pulses). All solutions were made up in potassium phosphate buffer (pH = 7), in acetonitrile, or in mixtures of the two. Samples were contained in a 7 × 7 mm^2 quartz cell and were prepared with absorbances, A, of 0.4 ± 0.02 at the excitation wavelength unless otherwise stated. UV−visible absorption spectra were recorded on a Varian CARY 3 spectrophotometer. Samples were purged with nitrogen, oxygen, or nitrous oxide for a minimum of 2 min per milliliter.

Cyclic Voltammetry. Electrochemical measurements were made using a standard three-electrode single-cell arrangement and an E.G.&G. Princeton Research model 173 potentiostat interfaced to a Tektronix TDS620 digital oscilloscope. The reference electrode was silver wire in electrolyte solution, the indicating electrode was a glassy carbon disk (0.5 mm), and the counter electrode was platinum wire. Prior to use, samples were purged with argon while stirring. Measurements were made with 2.5 ± 0.2 mM samples in acetonitrile containing 0.1 M tetrabutylammonium tetrafluoroborate as the supporting electrode. Peak potentials are reported vs SCE and were determined by reference to the ferrocene/ferrocinium couple ($E°$ = 0.44 V versus SCE).

TABLE 1: Bimolecular Rate Constants for Reaction of Coumarin and Psoralen Donors with Triplet Chloranil and λ_{max} Values for the Respective Radical Cations in Acetonitrile

compound	$k_{bm}(^3Chl^*)/M^{-1}\,s^{-1}$	$R^{•+}$ λ_{max}/nm
coumarin	$<3.0 \times 10^7$	
7-methylcoumarin	1.7×10^9	610
angelicin	$\sim 9 \times 10^8$ [a]	
7-methoxycoumarin	1.6×10^{10}	630
psoralen	1.7×10^{10}	600[b]
57-DMC	1.2×10^{10}	620
8-MOP	1.6×10^{10} [b]	650[b]
5-MOP	2.5×10^{10}	550[b]
458-TMP	1.6×10^{10}	640
67-DMC	1.4×10^{10}	590

[a] Radical cation was not formed. [b] Reference 28.

Fluorescence. Experiments were carried out using a Photon Technology International fluorescence spectrometer with “Time Master” single photon counting capability. Samples were prepared immediately prior to use and contained in a 10 × 10 mm^2 quartz cell. For steady-state fluorescence spectra and quantum yields, aerated or nitrogen-purged solutions were optically matched at the excitation wavelength of 320 nm with absorbances less than 0.1. For the lifetime measurements, nitrogen-purged samples were optically matched at the excitation wavelength of 320 nm with A = 0.11 ± 0.01.

Results

Generation of Radical Cations via Photosensitized Electron Transfer. Previous work has shown that triplet chloranil is an efficient photosensitizer for the generation of 8-MOP, 5-MOP, and psoralen radical cations (Table 1).[28] A similar approach has been used here to attempt to generate the radical cations of 4,5′,8-trimethylpsoralen (458-TMP), angelicin, and five coumarins. Direct excitation at 420 nm (to avoid competing ground-state absorption by the psoralen) of a nitrogen-purged acetonitrile solution of chloranil (A_{420} = 0.4) generates the chloranil triplet state which has an absorption maximum at 510 nm. The chloranil triplet state reacts with most of the compounds studied with a diffusion-controlled rate constant in excess of 1 × 10^{10} M^{-1} s^{-1} (Table 1). The only exceptions

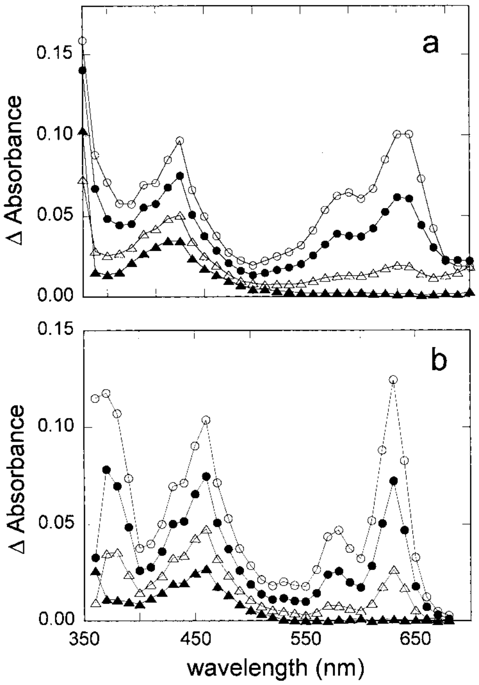
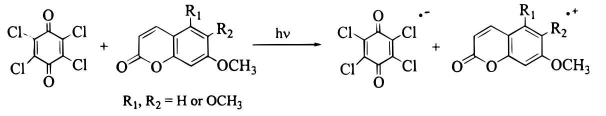


Figure 1. Transient absorption spectra obtained via direct excitation at 420 nm of nitrogen-saturated acetonitrile containing chloranil (OD_{420} = 0.5) and: (a) 4,5′,8-trimethylpsoralen (5.6×10^{-3} M) at delays of 280 ns (○), 680 ns (●), 2.1 μs (△), and 6.8 μs (▲) after the laser pulse; (b) 7-methoxycoumarin (5.5×10^{-3} M) at delays of 280 ns (○), 760 ns (●), 2.1 μs (△), and 6.8 μs (▲).

are 7-methylcoumarin and angelicin, for which the rate constants were approximately an order of magnitude slower, and coumarin, for which only an upper limit of 3×10^7 M^{-1} s^{-1} could be estimated. Transient absorption spectra are consistent with electron transfer from the psoralen or coumarin donor to triplet chloranil to give chloranil radical anion with λ_{max} at 450 nm and the donor radical cation, eq 1.



(1)

Figures 1 and 2a show examples of the generation of the radical cations of 458-TMP, 7-methoxycoumarin, and 67-DMC with λ_{max} at 640, 630, and 590 nm, respectively. In each case the radical cation has a shoulder at shorter wavelength and is similar to spectra obtained previously for related psoralen radical cations.[28] Similar results were obtained for 57-DMC and 7-methylcoumarin, and the data are summarized in Table 1. Although angelicin did react with the triplet chloranil, there was no evidence for the formation of either the chloranil radical anion or a transient that could be assigned to angelicin radical cation.

The decays of some of the psoralen and coumarin radical cations were dependent on the precursor concentration. For 57-DMC and 458-TMP rate constants of ~10^7 and ~2×10^7 M^{-1} s^{-1} were estimated from the observed radical cation decays at two different precursor concentrations. The combination of slow rate constants and relatively low solubility of the precursors made more accurate determination of the rate constants impractical. However, these estimates are in line with previously measured rate constants of 1.5×10^7 and 3.8×10^6 M^{-1} s^{-1} for the reaction of 8-MOP and 5-MOP radical cations with their precursors in acetonitrile.[28] By contrast, the decays for the 7-methoxycoumarin, 67-DMC, 7-methylcoumarin, and psoralen radical cations were not affected by the precursor coumarin or psoralen concentration over the limited range studied, suggesting an upper limit of 5×10^6 M^{-1} s^{-1}. For these substrates the radical cations decay predominantly by back electron transfer from chloranil radical anion, as illustrated in Figure 2A for 67-DMC. The decay of all the psoralen and coumarin radical cations was unaffected by the presence of oxygen.

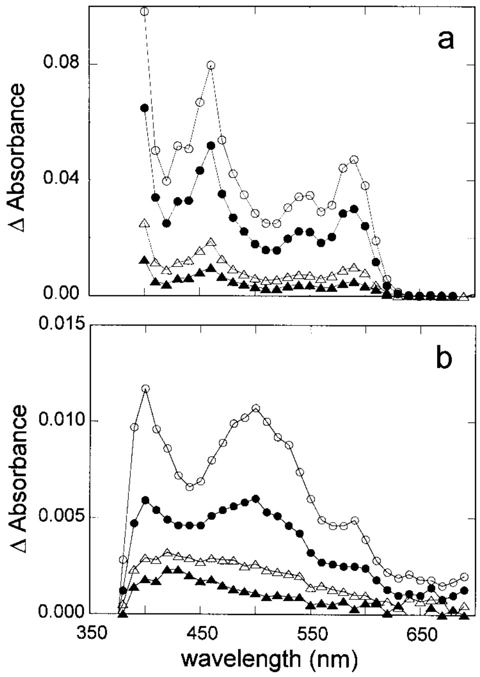


Figure 2. (a) Transient absorption spectra obtained via direct excitation at 420 nm of nitrogen-saturated acetonitrile containing chloranil and 6,7-dimethoxycoumarin (5.6×10^{-3} M) at delays of 720 ns (○), 1.5 μs (●), 6.3 μs (△), and 14 μs (▲) after the laser pulse. (b) Transient absorption spectra obtained via direct excitation at 355 nm of nitrogen-saturated acetonitrile containing 6,7-dimethoxycoumarin (OD_{355} = 0.4) at delays of 1.8 μs (○), 7.0 μs (●), 16 μs (△), and 35 μs (▲) after the laser pulse.

Direct Excitation in Acetonitrile. Direct 355 nm excitation of the four psoralens and angelicin in nitrogen-saturated acetonitrile gave rise to one major transient absorption between 400 and 500 nm that decayed via first-order kinetics and that was quenched efficiently by oxygen. These absorptions are attributed to the respective triplet states; in all cases, the absorption maxima are consistent with those previously determined in a variety of organic solvents.[31,32] Excitation of coumarin and 7-methylcoumarin under the same conditions did not result in any significant absorption in the 350−700 nm region, consistent with literature reports of low triplet yields for related compounds.[33] However, the three methoxy-substituted coumarins yielded transient absorption spectra with λ_{max} values between 470 and 500 nm, as shown in Figure 2b for 67-DMC. These transients reacted with oxygen and are assigned to the triplet−triplet absorption spectra in good agreement with literature reports for a variety of coumarins, including 57-DMC, in water and organic solvents.[19,34−36] The spectra in Figure 2b also show weak absorptions at longer wavelength that are not removed by oxygen and are similar to the radical cation spectra obtained by chloranil photosensitization. Similarly, weak absorptions consistent with radical cation formation were also observed for 57-DMC.

Direct Excitation in Aqueous Phosphate Buffer (pH = 7.0). Direct excitation at 355 nm was carried out for all the compounds (A_{355} = 0.4) in either aqueous phosphate buffer (pH

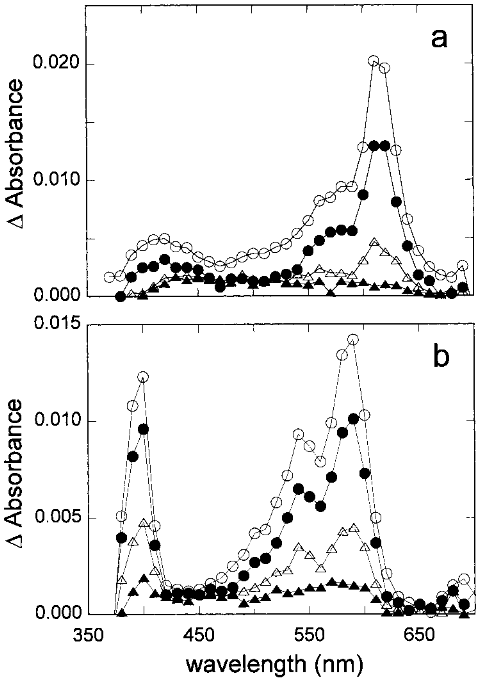
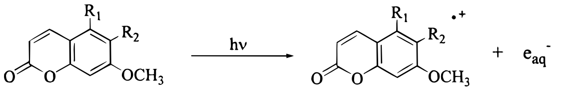


Figure 3. Transient absorption spectra obtained via direct excitation at 355 nm of oxygen-saturated aqueous phosphate buffer (pH = 7.0) containing: (a) 5,7-dimethoxycoumarin (OD_{355} = 0.4) at delays of 1.4 μs (○), 3.4 μs (●), 13 μs (△), and 35 μs (▲) after the laser pulse; (b) 6,7-dimethoxycoumarin (OD_{355} = 0.4) at delays of 1.0 μs (○), 3.8 μs (●), 13 μs (△), and 34 μs (▲) after the laser pulse.

= 7) or 1:1 buffer:acetonitrile, depending on solubility. Excitation of 57-DMC, 67-DMC, and 7-methoxycoumarin in oxygen-purged aqueous buffer generated transient absorptions with λ_{max} values of 620, 590, and 630 nm, respectively, that are essentially identical to the radical cation spectra obtained in the unambiguous electron-transfer experiments. Representative spectra for 57-DMC and 67-DMC radical cations produced by photoionization in oxygen-saturated aqueous buffer are shown in Figure 3. Note that the use of a shorter excitation wavelength than in the sensitized experiments makes it possible to detect a second strong absorption band at 400 nm for 67-DMC$^{•+}$. A similar UV band (λ_{max} < 370 nm) is also evident for 7-methoxycoumarin. The radical cation absorptions were observed "instantaneously" following the laser pulse (i.e., within the rise time of the apparatus, ~10 ns). Excitation of the same compounds in nitrogen-saturated buffer showed the characteristic long wavelength absorption due to the solvated electron in addition to the radical cation, eq 2, further confirming that photoionization of the coumarins occurs. Triplet absorptions between 450 and 500 nm were also observed for nitrogen-saturated solutions; in each case the triplet absorption was shifted to shorter wavelength, relative to the corresponding absorptions in acetonitrile.



(2)

R_1, R_2 = H or OCH_3

Direct excitation of 458-TMP, angelicin, and 7-methylcoumarin was only practical in 1:1 buffer:acetonitrile. Under these conditions only triplet-state absorption was observed for angelicin (under nitrogen), while 7-methylcoumarin failed to yield any transients. However, radical cation formation was observed for 458-TMP in 1:1 aqueous buffer.

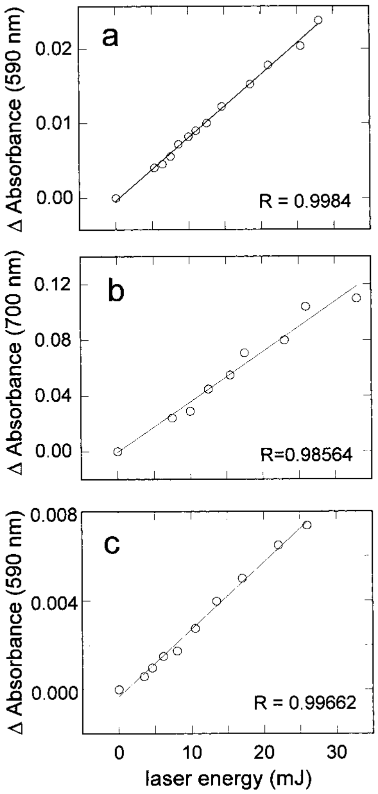


Figure 4. Plots of transient absorbance versus incident laser energy obtained via direct excitation of 67-DMC in aqueous phosphate buffer: (a) 355 nm excitation (O_2-saturated solution, radical cation absorption); (b) 308 nm excitation (N_2-saturated solution, solvated electron absorption; (c) 266 nm excitation (O_2-saturated solution, radical cation absorption).

The radical cations generated by photoionization decayed via mixed kinetics with lifetimes of ~2−8 μs at the concentrations required for 355 nm excitation (A_{355} = 0.4). The decays were dependent on the precursor psoralen or coumarin concentration as noted above in acetonitrile, although the limited solubility of the compounds in aqueous solutions prevented accurate measurement of this dependence.

Laser Energy Dependencies and Quantum Yields for Photoionization. Oxygen-saturated solutions of 57-DMC, 67-DMC, and 7-methoxycoumarin in aqueous buffer and 458-TMP in 1:1 aqueous acetonitrile were excited (λ_{ex} = 355 nm) over a range of laser energies, and the absorptions of $R^{•+}$ at the respective maxima were extrapolated back to "zero time", t_0. The amplitude of absorption at t_0, ΔA_0, was plotted versus the laser energy, as shown in Figure 4 for 67-DMC. These plots were found to be linear and to pass through (0,0) for all four compounds. The photoionization of 67-DMC was also examined using both 266 and 308 nm excitation. In both cases the spectrum was identical to that obtained using 355 nm excitation. Plots of ΔA_0 for the radical cation at 590 nm (266 nm) and for the solvated electron at 700 nm (308 nm, nitrogen-saturated solution) versus the laser energy were straight lines that passed through (0,0) (Figure 4).

The quantum yield of the 8-MOP photoionization process, Φ_{pi}, was determined previously to be 0.015[28] in a comparative actinometry experiment using benzophenone in nitrogen-saturated benzene as the actinometer (Φ_T = 1; ϵ_T (525 nm) = 7870 M^{-1} cm^{-1} [37]). The same method was used here to measure the quantum yield for 67-DMC photoionization in aqueous buffer. The laser energy dependence of the benzophenone triplet absorption at 525 nm was compared to ΔA_0 (700 nm) for the

TABLE 2: Oxidation Potentials (Acetonitrile), Fluorescence Maxima, Quantum Yields and Lifetimes (Aqueous Buffer), and Photoionization Yields (Aqueous Buffer) for Psoralens and Coumarins at Room Temperature

compound	E_p^{ox} (V)[a]	Φ_f	fluor λ_{max} (nm)	τ_f (ns)	Φ_{pi}
psoralen	1.89	0.016	456		
8-MOP	1.67	0.003[b]	510[b]	1.0[b]	0.015[c]
5-MOP	1.63	0.001[b]	505[b]	0.3[b]	
458-TMP	1.60	0.030	468		0.020[d]
angelicin	2.15	0.0096	470		
coumarin	2.31	0.0032	363		
7-methylcoumarin	2.17	0.0066	383		
7-methoxycoumarin	1.91	0.34	392	1.5	0.10
57-DMC	1.73	0.52	452	7.3	0.10
67-DMC	1.53	0.51	434	4.2	0.20

[a] vs SCE. [b] Reference 42. [c] Reference 28. [d] In 1:1 aqueous acetonitrile.

solvated electron for an optically matched (at the excitation wavelength) solution of 67-DMC. The slopes of the two laser energy dependence plots and a value of 18 000 M^{-1} cm^{-1} for $\epsilon(e^-(aq))$[38] were substituted in eq 3

$$\Phi_{pi} = \Phi_{e^-(aq)} = (\text{slope }(e^-(aq))/\text{slope (Bp)})(\epsilon_T^{Bp}/\epsilon_{e^-(aq)})\Phi_T^{Bp} \quad (3)$$

to give a value of 0.2 ± 0.03 (Table 2) for $\Phi(e^-(aq))$, and hence Φ_{pi}. This value is an average of six independent experiments using both 308 and 355 nm excitation. Photoionization yields of 0.10 for both 57-DMC and 7-methoxycoumarin in aqueous buffer were estimated using the same procedure for single determinations using 355 nm excitation (Table 2).

The photoionization yield for 458-TMP in 1:1 aqueous buffer was also measured. Since the solvated electron is not observed in this solvent, the yield of the radical cation at 640 nm was plotted as a function of laser energy and compared to a matched sample of benzophenone as actinometer. The molar absorption coefficient for TMP$^{\bullet+}$ was estimated by generating the radical cation by photosensitized electron transfer using triplet chloranil. By making the reasonable assumption that the yields of the two radical ions are equal, the relative signal intensities for TMP$^{\bullet+}$ and the chloranil radical anion and the known molar absorption coefficient for the latter[39] give an estimate of 11 800 M^{-1} cm^{-1} for ϵ(TMP$^{\bullet+}$) in acetonitrile. This value was then used to obtain a photoionization quantum yield of 0.02 for TMP in aqueous acetonitrile (Table 2).

The photoionization yield for 67-DMC was also estimated in several solvents. In these experiments optically matched samples of 67-DMC in buffer, 1:1 aqueous acetonitrile, and pure acetonitrile were directly excited at 355 nm. This gave rise to radical cation absorptions in the ratio of 4.3:2.8:1, respectively, which provides an indication of the relative ease of photoionization of 67-DMC in the three solvent systems, assuming that the molar absorption coefficient of the radical cation is unaffected by changes in the solvent. Note that these experiments cannot be based on electron yields since in acetonitrile the electron is trapped by solvent to give a dimer radical anion that does not absorb appreciably below 750 nm.[40]

Determination of Oxidation Potentials via Cyclic Voltammetry. The one-electron oxidation potentials of the 10 compounds in acetonitrile were determined via cyclic voltammetry using a standard three-electrode, single-cell arrangement. The oxidation was irreversible in all cases. The results are summarized in Table 2 and are reported as peak potentials vs SCE.

Fluorescence Studies. The low absorbance ($A < 0.1$) required for fluorescence measurements requires low concentrations of ground state (typically $<10^{-4}$ M). Such concentrations were achievable in water for all the compounds studied here, allowing fluorescence spectra to be obtained and fluorescence quantum yields, Φ_f, to be determined. Two fluorescence standards[41] were employed: anthracene in ethanol ($\Phi_f = 0.27$) and 9,10-diphenylanthracene in cyclohexane ($\Phi_f = 0.90$). The values of Φ_f for the compounds studied were determined using eq 4

$$\Phi_f^u = \Phi_f^s(I^u/I^s)(n^u/n^s)^2 \quad (4)$$

in which I is the fluorescence intensity and n is the refractive index of the solvent. The superscripts "s" and "u" refer to standard and unknown, respectively. The values of Φ_f (an average of determinations in aerated and N$_2$-saturated solution) and the fluorescence maxima are summarized in Table 2, along with literature data for 8-MOP and 5-MOP.[42] Fluorescence lifetimes were also measured for the three methoxy-substituted coumarins using the single photon counting technique. The data are summarized in Table 2 and are in reasonable agreement with literature data for 7-methoxycoumarin and 57-DMC, as summarized in ref 43.

Discussion

The radical cations of four psoralens and four related coumarins have been generated by photoinduced electron transfer using triplet chloranil as the sensitizer and characterized in acetonitrile. In most cases quenching of the chloranil triplet occurs with a diffusion-controlled rate constant. However, 7-methylcoumarin is substantially less reactive with a rate constant of 1.7×109 M^{-1} s^{-1}, and coumarin is even less reactive. These results are completely consistent with the measured oxidation peak potentials, which are substantially higher for coumarin and 7-methylcoumarin than for the other substrates. Triplet chloranil should reduce any donor with an oxidation potential less than 2.25 V with a diffusion-controlled rate constant.[44] Our measured value of 2.31 V for coumarin is in good agreement with the fact that the rate constant for electron transfer is too slow to measure in this case, particularly given the potential error associated with using irreversible peak potentials to estimate oxidation potentials. The results for angelicin are somewhat anomalous; although reaction with triplet chloranil is observed, there is no transient evidence for the expected electron-transfer products. It is likely that reactions other than electron transfer occur in this case, as observed in earlier attempts to use triplet chloranil to generate alkene radical cations.[45]

The spectra for the psoralen and coumarin radical cations are all quite similar with a visible absorption between 550 and 650 nm. The variation in the λ_{max} value for the psoralen radical cations is considerably larger than that observed for the coumarins. In several cases a UV band with $\lambda_{max} < 400$ nm is also detected. These results are analogous to those obtained previously for a number of arylalkene radical cations.[46] The reaction of the radical cations with their precursors and their insensitivity toward oxygen are also typical of the behavior of arylalkene radical cations.

Direct excitation in acetonitrile of most of the compounds studied here gives rise to the triplet state as the major transient, in agreement with earlier studies in other organic solvents. The triplet-state parameters for many psoralens in a variety of solvents have been quantified in some detail over the past 25 years and have been summarized by Bensasson et al.[8] Data on triplet coumarins are also available.[19,33−36] In contrast to the results in acetonitrile, direct excitation of two psoralens (8-MOP and 458-TMP) and the three methoxy-substituted coumarins in aqueous media results in significant yields of photoionization, in addition to triplet formation. The generation of the same radical cations via photosensitized electron transfer and the detection of the solvated electron in aqueous solution provide unequivocal proof for photoionization of these substrates.[47] Photoionization of easily oxidized aromatic substrates has been frequently observed upon laser excitation and often, although by no means always, occurs via a biphotonic process involving absorption of a second photon by either the first excited singlet or triplet state of the substrate.[40] The question of monophotonic versus biphotonic ionization of the psoralens and coumarins studied herein is obviously of key importance in determining the biological relevance of this reaction. Clinical use of these compounds for PUVA involves lamp irradiation under which conditions two-photon processes involving short-lived intermediates are highly improbable.

Our earlier work on 8-MOP had demonstrated that the radical cation yield in aqueous buffer depended linearly on the laser energy for both 308 and 355 nm excitation, strongly suggesting that photoionization is monophotonic.[28] Similar experiments indicate that the radical cation yields for 458-TMP and the three methoxy-substituted coumarins also increase linearly with laser energy. There are circumstances under which linear energy plots can be observed for processes that require more than one photon.[48] However, a linear energy dependence for formation of 67-DMC$^{•+}$ is observed for three excitation wavelengths that cover an approximately 100 nm range. This makes it extremely unlikely that the observed linear dependence arises from some fortuitous combination of absorption coefficients and concentrations for ground and excited states at the excitation wavelengths and reinforces the conclusion that photoionization is a monophotonic process. Photoionization is assumed to occur from the singlet manifold since radical cation absorption is observed within 10 ns of the laser pulse while the triplet-state lifetimes are of the order of microseconds for nitrogen-purged solutions.

The observed photoionization efficiencies for the psoralens and coumarins may be compared to literature results for other aromatic substrates. For example, monophotonic photoionization of anthracene, 9-phenylanthracene, and 9,10-diphenylanthracene has been observed for several different excitation wavelengths in acetonitrile.[49−51] The quantum yields for photoionization range from 0.03 to 0.12 and are comparable to those reported herein for the psoralens and coumarins. Similarly, monophotonic ionization of diphenylamine and several related amines in acetonitrile, alcohols, and aqueous mixtures has been reported.[52−54] In contrast to our recent conclusions of monophotonic ionization of 8-MOP,[28] several earlier transient absorption studies had reported that 8-MOP undergoes biphotonic photoionization.[25,26,55] The most detailed of these studies was carried out using picosecond laser irradiation, conditions that would favor multiphotonic processes.[25]

There is substantial variation in photoionization efficiency among the compounds studied. Some of the variation is consistent with trends in the ease of oxidation of the substrates. For example, psoralen has a higher oxidation peak potential than its substituted derivatives and does not photoionize under our experimental conditions. However, other factors are clearly important since the oxidation peak potentials are almost the same for 8-MOP, 5-MOP, and 458-TMP, yet 5-MOP shows no evidence for photoionization. This is obviously not due to the fact that 5-MOP could only be studied in aqueous acetonitrile since 458-TMP is photoionized in this solvent with a comparable yield to 8-MOP in aqueous buffer. For the coumarin series the more easily oxidized methoxy-substituted derivatives show efficient photoionization; 7-methoxycoumarin and 67-DMC which differ in oxidation peak potentials by almost 0.4 V have photoionization efficiencies that differ by a factor of 2. The fluorescence data suggest that a longer-lived singlet state favors photoionization, since the three methoxy-substituted coumarins all have considerably longer-lived singlets (and higher photoionization yields) than any of the other substrates. The slightly higher fluorescence yield for 458-TMP may also explain its higher photoionization yield; however, neither oxidation potentials nor fluorescence data appear to account for the fact that 5-MOP does not photoionize under our experimental conditions.

The variation in photoionization yield for 67-DMC decreases by a factor of 4.3 upon changing the solvent from aqueous buffer to acetonitrile, with a 1:1 mixture of the two giving intermediate results. Interestingly, the fluorescence yield also decreases similarly in changing the solvent from aqueous buffer to acetonitrile. The higher photoionization yield in aqueous solvent is well-precedented[40,52,56] and clearly emphasizes the importance of the microenvironment on the photochemistry of these compounds. Since many psoralens are soluble to some extent in both organic and aqueous media, the actual in vivo localization site will play a significant role in determining which photophysical processes occur from the singlet manifold.

Conclusions

A combination of photosensitized electron transfer using triplet chloranil and direct laser photoionization has been used to generate a number of psoralen and coumarin radical cations. The radical cations have been characterized using transient absorption spectroscopy and have absorption maxima between 550 and 650 nm and kinetic behavior typical of other arylalkene radical cations. Significant photoionization yields are observed for two psoralens and three coumarins in aqueous buffer or aqueous acetonitrile, in contrast to the results in acetonitrile where triplet formation predominates. Linear energy dependence plots for radical cation or solvated electron formation under a variety of conditions demonstrate that photoionization is monophotonic. The correlation of photoionization yields with oxidation potentials and fluorescence parameters indicates that the photoionization efficiency increases with increasing ease of oxidation and longer singlet lifetimes, although these general trends do not explain all the available data. The results clearly demonstrate the importance and generality of photoionization in psoralen and coumarin photochemistry. This is of particular significance in light of our earlier observations of electron-transfer reactions of 8-MOP radical cations with purine bases[28] and literature reports of DNA cleavage initiated by psoralen irradiation.[29] It is clear that electron transfer may be of more general significance than has previously been considered and may play a role in both the phototherapeutic and phototoxic effects of psoralens and related substrates that are used in photochemotherapy. Our results are also of interest in the context of the photodecomposition of psoralens in aqueous solution and the recent observations of the biological activity of psoralen decomposition products.[57,58]

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References and Notes

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