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One- and Two-Photon Excitation of *β*-Carbolines in Aqueous Solution: pH-Dependent Spectroscopy, Photochemistry, and Photophysics

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β-Carboline (*β*C) alkaloids are present in a wide range of biological systems and play a variety of significant photodependent roles. In this work, a study of the aqueous solution-phase photochemistry, photophysics, and spectroscopy of three important *β*Cs [norharmane (nHo), harmane (Ho), and harmine (Ha)] and two *β*C derivatives [*N*-methylnorharmane (*N*-Me-nHo) and *N*-methylharmane (*N*-Me-Ho)] upon one- and two-photon excitation is presented. The results obtained depend significantly on pH, the ambient oxygen concentration, and the *β*C substituent and provide unique insight into a variety of fundamental photophysical phenomena. The data reported herein should not only help to understand the roles played by *β*C alkaloids in biological systems but should also help in the development of methods by which the photoinduced behavior of these important compounds can be controlled.

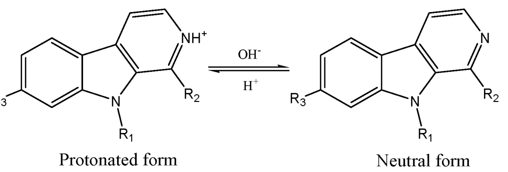
Introduction

β-Carboline (*β*C) alkaloids are a group of heterocyclic compounds with the structural unit 9*H*-pyrido[3,4-*b*]indole. The structures of three *β*C alkaloids and two derivatives are shown in Scheme 1. These alkaloids are widespread in biological systems. They are found in many tropical plants as a normal constituent of the seeds,[1] roots,[2] stems,[3] and leaves.[4−6] Consequently, they also occur as minor constituents in foods, alcoholic drinks, cigarettes/tobacco smoke, etc.[7] In mammals, *β*Cs occur normally in plasma, platelets, and urine.[8] Moreover, it seems that some are formed in the human body after alcohol intake and smoking.[9]

It has been suggested that *β*Cs participate in relevant biological processes and pathologies,[12,13] some of them induced by light. Under UV-A irradiation, norharmane and harmane are able to induce chromosome damage in mammalian cells,[14,15] and to inactivate bacteria[16,17] and viruses.[18] In addition, it has been proposed that the biological role of some *β*Cs in plants could be related with the defense response (i.e., phototoxic effects) against insects, webworms, etc.[19] Despite their acknowledged importance, the main phytological role of these alkaloids and the mechanisms involved in these processes are, to date, nevertheless still poorly understood.

Although the photochemistry[20−27] and photophysics[10,28−37] of these alkaloids have been extensively studied over the past two decades, only a few studies have been performed in aqueous media. In particular, only the photostability of norharmane in aqueous solution has been described in detail.[38] Adding to the potential complexity of an aqueous study is the fact that, over the pH-range 4−11, these alkaloids show an acid−base equilibrium characterized by a pK_a value around 7 (Scheme 1).[10,11] Thus, these molecules are sensitive to physiological pH conditions. Furthermore, although it has been proposed that, upon irradiation, these alkaloids produce singlet molecular oxygen ($^1O_2$),[39] the superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), only sparse and/or qualitative studies have been done.[40]

SCHEME 1: Structures of the Five *β*Cs Studied and the Acid−Base Equilibrium with pK_a ∼ 7 in Aqueous Solution[10,11]



	R_1[a]	R_2	R_3
norHarmane (**nHo**)	-H	-H	-H
Harmane (**Ho**)	-H	-CH_3	-H
N-Methylnorharmane (**N-Me-nHo**)	-CH_3	-H	-H
N-Methylharmane (**N-Me-Ho**)	-CH_3	-CH_3	-H
Harmine (**Ha**)	-H	-CH_3	-OCH_3

[a] The nitrogen of the indolic ring (i.e., R_1 = H; nHo, Ho, and Ha) has a pK_a value greater than 12.

In the present work, we set out to systematically examine the pH- and oxygen-dependent aqueous solution-phase photochemical and photophysical behavior of three *β*Cs [norharmane (nHo), harmane (Ho) and harmine (Ha)] and two *β*C derivatives [*N*-methylnorharmane (*N*-Me-nHo) and *N*-methylharmane (*N*-Me-Ho)], upon one- and two-photon excitation (Scheme 2). To our knowledge, the two-photon behavior of these compounds has yet to be examined. Quantifying the nonlinear two-photon optical behavior of these compounds can have significant ramifications in mechanistic studies where the desire is to selectively excite a *β*C alkaloid in a given biological system at

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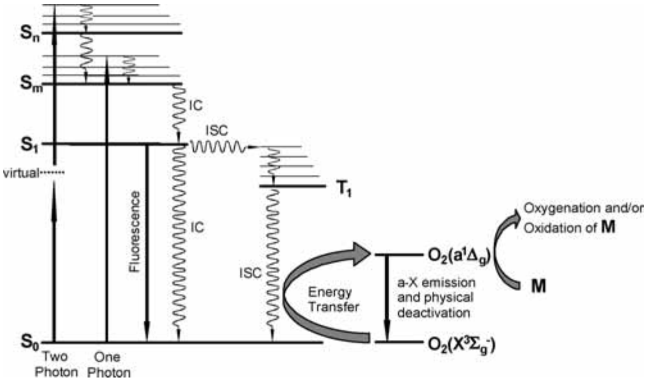
SCHEME 2: Diagram of Pertinent Photoinduced Processes[a]



[a] IC and ISC denote internal conversion and intersystem crossing, respectively. Depending on the sensitizer and its symmetry, the simultaneous absorption of two photons may or may not populate the same state as that created upon the absorption of a single higher-energy photon.[69,72]

the exclusion of other endogenous chromophores (i.e., in a system of multiple chromophores, one can often achieve better discrimination when using two-photon excitation).

Experimental Section

Materials. Norharmane (nHo), harmane (Ho), and harmine (Ha) (Sigma-Aldrich) were of the highest purity available (>98%) and were used without further purification. The method used to synthesize and purify *N*-methylnorharmane (*N*-Me-nHo) and *N*-methylharmane (*N*-Me-Ho) has been published elsewhere.[41] All solvents were purchased from Aldrich (spectroscopic grade) and were also used as received.

The pH of the aqueous solutions was adjusted by adding drops of HCl or NaOH solutions from a micropipet. For experiments in which oxygen was removed by bubbling with Ar or N_2, the pH was adjusted after an initial period of bubbling to account for any changes in pH associated with CO_2 loss. The concentrations of the acid and/or base used for this purpose ranged from 0.1 to 2 M.

The ionic strength was approximately 10^{-3} M in all the experiments. In experiments using D_2O as solvent, D_2O (>99.9%; Euriso-top or Aldrich), DCl (99.5%; Aldrich) in D_2O, and NaOD (Aldrich) in D_2O were employed.

Instrumentation and Approaches for Steady-State Experiments. ***Elapsed Irradiation.*** Aqueous βC solutions were irradiated at 350 nm in 1 cm quartz cells at room temperature with a Rayonet RPR lamp (bandwidth ~ 20 nm, Southern N.E. Ultraviolet Co.). Experiments were performed in the presence and absence of air, and oxygen-free solutions were obtained by bubbling with Ar or N_2 gas for 20 min.

UV/Vis Analysis. Electronic absorption spectra were recorded on a Shimadzu PC2101 spectrophotometer. Measurements were made using 1 cm path length quartz cuvettes.

High-Performance Liquid Chromatography (HPLC). A Waters 600E Pump Controller with a UV/vis photoarray detector was used to monitor and quantify the photochemical reactions. Aqueous solutions of commercial standards were employed for the calibration curves. A Supelco-C18 column (250 × 4.6 mm, 5 mm) was used for product separation, the elution being achieved with a solution of 80% MeOH and 20% H_2O (pH = 3).

Quantum Yield Determinations. The quantum yields of reactant disappearance (Φ_R) were obtained using the following equation:

$$\Phi_R = -\frac{(d[R]/dt)_0}{P_a} \qquad (1)$$

where $(d[R]/dt)_0$ is the initial rate of reactant consumption and P_a is the photon flux absorbed by the reactant. For determining the consumption rate, experiments were carried out using solutions with an initial reactant concentration of 250 μM (except for Ha at pH 10.0 where ~30 μM was used). Under these conditions, the time evolution of the reactant concentration followed a zero-order rate law over a period of time within which the change of P_a was negligible. In our experiments, this condition was fulfilled at irradiation times less than 150 min. The initial rates were obtained from the slope of the corresponding plots of concentration vs irradiation time within this time window.

Aberchrome 540 (Aberchromics Ltd.) was used as an actinometer for the measurements of the incident photon flux (P_0) at the excitation wavelength. The general approach used is described in detail elsewhere.[42] Values of the photon flux absorbed (P_a), were calculated from P_0 according to the Lambert−Beer law:

$$P_a = P_0(1 - 10^{-A}) \qquad (2)$$

where A is the absorbance of the sensitizer at the excitation wavelength.

Detection and Quantification of H_2O_2. For the determination of H_2O_2, a Cholesterol Kit (Wiener Laboratorios SAIC) was used. H_2O_2 was quantified after reaction with 4-aminophenazone and phenol.[43,44] Briefly, 400 μL of irradiated solution (UV-A, 350 nm) was added to 2 mL of reagent. The absorbance at 505 nm of the resulting mixture was measured after 30 min at room temperature, using the reagent as a blank. Aqueous H_2O_2 solutions prepared from commercial standards were employed for obtaining the corresponding calibration curves.

Fluorescence Measurements. Steady-state fluorescence measurements were performed using a Horiba Jobin Yvon spectrofluorometer. Corrected fluorescence spectra were recorded in a 1 cm path length quartz cell at room temperature.

Fluorescence quantum yields were determined from the corrected fluorescence spectra, integrated over the entire emission profile, using both 9,10-diphenylanthracene (9,10-DPA) in cyclohexane and quinine sulfate in 0.1 M H_2SO_4 as references ($\Phi_F = 0.95$[45] and 0.577,[46] respectively). To avoid inner filter effects, the absorbance of the solutions, at the excitation wavelength, was kept below 0.10. Φ_F values obtained were independent of the standard used.

Nanosecond Time-Resolved Experiments. Quantum yields of photosensitized singlet oxygen production, Φ_Δ, were obtained using a pulsed Nd:YAG laser as the excitation source (λ_{EXC} = 355 nm), looking at the 1270 nm $^1O_2$ phosphorescence with a cooled germanium detector.[47] Experiments were performed by comparing the magnitude of the integrated time-resolved $^1O_2$ phosphorescence signal produced upon one-photon irradiation of the molecule under study to that obtained upon one-photon irradiation of a standard sensitizer (perinaphthenone-2-sulfonic acid (PNS) in deuterated water with $\Phi_\Delta = 0.97 \pm 0.05$[48]).[47,49]

Fluorescence lifetimes, τ_F, were recorded using a photodiode (Thorlabs DET10A, 1 ns risetime) and monitored on a Tektronix TDS5032B digital oscilloscope. The fluorescence decays were extracted using a commercial deconvolution routine (Sound Analysis 1.50D, Quantum Northwest). All lifetimes showed single exponential decay kinetics.

Femtosecond Time-Resolved Experiments. Details of the system used have been published previously.[47] Briefly, femtosecond laser pulses were generated in a Ti:sapphire oscillator (Tsunami, Spectra Physics) and amplified using a regenerative amplifier (Spitfire, Spectra Physics). The output of the latter was then used to pump an optical parametric amplifier (OPA-800CF, Spectra Physics). The resulting pulses, approximately 130 fs fwhm[47] and 15 nm spectral width, were tuned to cover the spectral range from 540−780 nm. The laser power was controlled using a Glan-Taylor polarizer and neutral density filters, and the light was focused and collimated prior to sample illumination. The sample was contained in 1 × 1 cm quartz cuvette mounted in a light-tight housing connected to a liquid-nitrogen-cooled VIS/near-IR PMT (Hamamatsu R5509-42). Sample luminescence was collected and spectrally isolated using different interference filters. The PMT output was amplified and sent to a photon counter (Stanford Research Systems model 400) operated using a program written in LabView (National Instruments).[47]

Determination of the Two-Photon Absorption Cross Section (δ). The general approach used to quantify two-photon absorption cross sections, δ, has been described previously.[47] Briefly, for a given sample, δ values were determined relative to a reference compound for which δ-values are known over a large spectral range. As standards, we used both 2,5-dibromo-1,4-bis(2-(4-(diphenylamino)phenyl)vinyl)benzene in toluene,[47] and 1,4-bis(2-methylstyryl)benzene in cyclohexane.[50,51]

In all cases, fluorescence emission was used as the spectroscopic probe. For a given excitation wavelength, values of δ were quantified according to eq 3.[52] For each sample, excitation was performed at a number of different wavelengths to yield a two-photon excitation spectrum (vide infra).

$$\delta_S = \delta_R \frac{I_S \cdot \Phi_{F,R} \cdot \eta_R P_R^2 \cdot C_R}{I_R \cdot \Phi_{F,S} \cdot \eta_S P_S^2 \cdot C_S} \qquad (3)$$

In eq 3, the subscripts S and R refer to the sample and the reference, respectively. I is the observed fluorescence intensity, Φ_F is the fluorescence quantum yield (all under air-saturated conditions), P is the laser power, and C is the solute concentration. Finally, η is a parameter that reflects the relative collection efficiency of the fluorescence, as calculated from the spectral overlap of the optics used, the fluorescence spectrum, the refractive index of the solvent, and the response of the PMT.[52]

Results and Discussion

Stability of βCs under UV-A Steady-State Irradiation. The photostability of the five βCs under steady-state irradiation with UV-A light (350 ± 20 nm) was studied. Experiments were carried out with solutions of βCs at pH 4.8 and 10.0, where each βC is present at more than 99% in the protonated and neutral forms, respectively (Scheme 1). The absorption spectra shown in Figure 1 indicate that the βCs have appreciable absorption at 350 nm under both pH conditions.

HPLC analysis of the air-equilibrated solutions showed a small decrease in βC concentration as a function of elapsed irradiation time. The decrease followed zero-order kinetics during the first 120 min of irradiation for all compounds under both pH conditions (see Figure 2), except for the protonated form of Ha (i.e., the pH 4.8 experiment) where zero-order kinetics was followed only during the first 45 min of irradiation. Taking into account the incident photon flux and the absorbance of each βC at 350 nm, the values of the quantum yields of βC disappearance (Φ_R) were calculated according to eq 1. Results listed in Table 1 and 2 show that all compounds studied are reasonably photostable in aqueous solution. For three of the compounds (nHo, Ho, and Ha), Φ_R depends noticeably on pH with the neutral form being more stable than the protonated form.

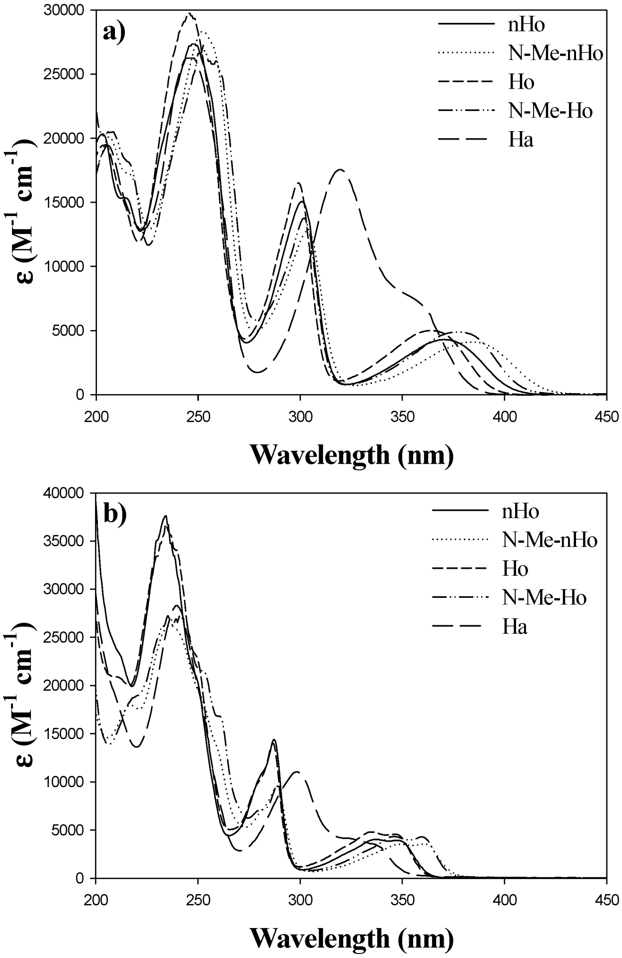


Figure 1. Absorption spectra of the five βCs studied recorded in aqueous solutions: (a) pH = 4.8; (b) pH = 10.0.

In contrast, no significant changes in the absorption spectra of these βC derivatives were observed after 120 min irradiation of oxygen-free aqueous solutions under both acidic (pH 4.8) and alkaline (pH 10.0) conditions (results not shown). These data were corroborated by the results of an HPLC analysis (see data in the Supporting Information) that also showed the absence of product formation. These results are consistent with the reasonable expectation that, although extremely inefficient, the photoinitiated degradation of these βC derivatives nevertheless depends on the presence of oxygen.

Fluorescence of βCs. Fluorescence spectra of both the protonated and neutral forms of each βC were recorded (exemplified using nHo and *N*-Me-Ho in Figure 3; additional spectra can be found in the Supporting Information). The corresponding quantum yields of fluorescence (Φ_F), are listed in Tables 1 and 2. In each case, values of Φ_F obtained using excitation wavelengths over the entire range of the lowest-energy absorption band were the same within the experimental error (results not shown). Moreover, recall that independent data sets determined in separate experiments against different standards were indistinguishable from each other (see Experimental Section).

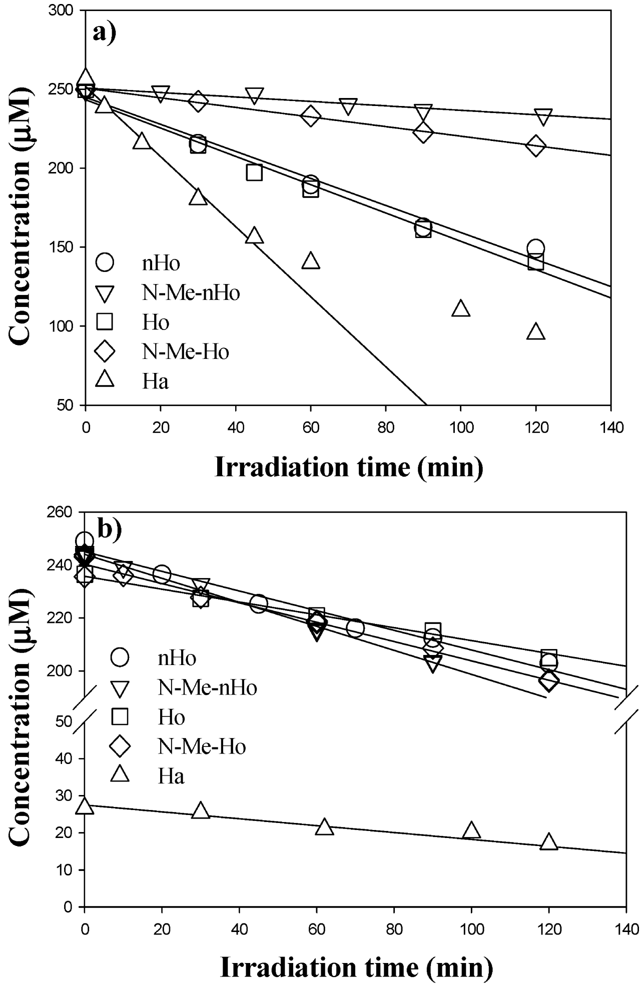


Figure 2. Evolution of βC concentration in air-equilibrated aqueous solutions as a function of the elapsed 350 nm irradiation time. Concentrations were assessed using HPLC analysis (Absorption wavelength used for detection = 370 nm). Experiments were performed at (a) pH = 4.8 and (b) pH = 10.0.

The results show that the protonated form of each alkaloid has a rather large Φ_F value (Table 1). The data obtained are consistent with the correspondingly low values for the quantum yield of βC consumption (Φ_R).

Values of Φ_F that we obtain for Ho and Ha are in good agreement with those previously published.[53,54] In the case of nHo, however, the Φ_F value listed in Table 1 for an aerated solution (0.70 ± 0.05) is slightly larger than a published value (0.60 ± 0.02) that has been suggested for use as a fluorescence standard.[28] To the best of our knowledge, emission spectra and Φ_F values of *N*-methylated βC derivatives dissolved in aqueous solution have not previously been reported.

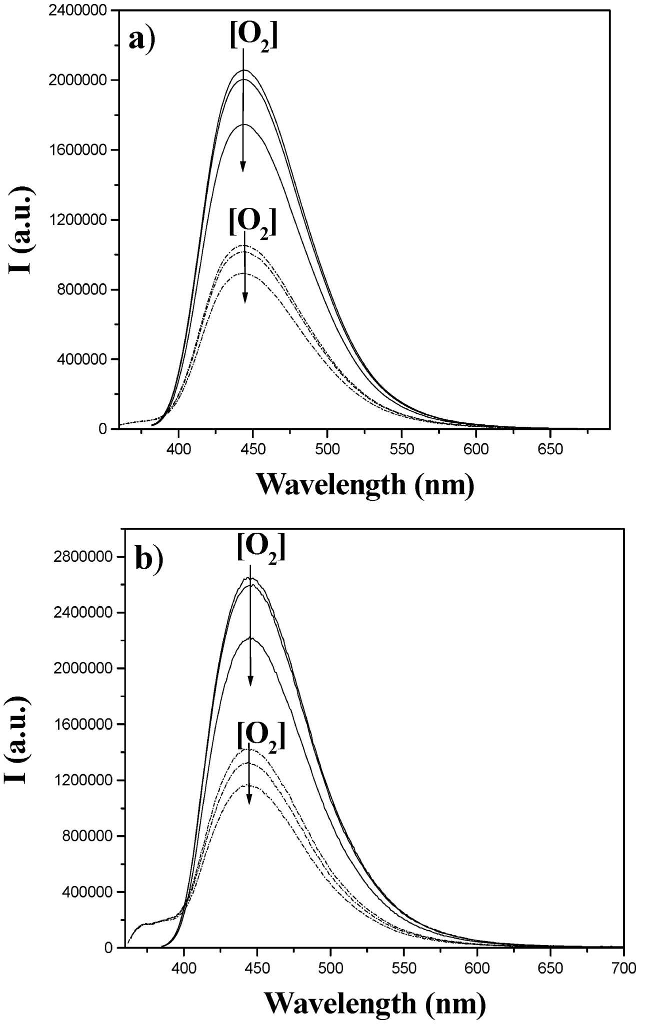


Figure 3. Corrected fluorescence spectra of (a) nHo and (b) *N*-Me-Ho in aqueous solution as a function of pH and O_2 concentration. For a given compound, spectra were obtained for three oxygen concentrations (i.e., N_2-saturated, aerated, and O_2-saturated) under acidic (group of more intense spectra, solid lines) and alkaline (group of less intense spectra, dotted lines) conditions. The excitation wavelengths were 370 and 348 nm for the pH 4.8 and 10.0 experiments, respectively. Spectra of the other βCs are shown in the Supporting Information.

Fluorescence spectra in alkaline media (pH 10.0) were also recorded (Figure 3). Under these conditions, the presence of an extra pH-dependent emission band is particularly evident in the spectra of *N*-Me-Ho, Ho, and Ha. The more intense of the two bands has a maximum at the same wavelength as the band observed from pH 4.8 solutions (i.e., at ~440 nm for the data in Figure 3). Accordingly, this band is assigned to the protonated form of the alkaloid. The less intense band, with a maximum at ~350 nm, can be assigned to the neutral form of the βC.[55]

TABLE 1: Photochemical and Photophysical Parameters Determined upon One-Photon Excitation of βCs in Aqueous Solution, at pH 4.8

	pH = 4.8						
	$10^3\Phi_R{}^a$ (air)	$\Phi_F{}^b$ (N_2)	$\Phi_F{}^b$ (air)	$\Phi_F{}^b$ (O_2)	$\Phi_\Delta{}^c$ (air)	$\Phi_\Delta{}^c$ (O_2)	$10^3\Phi_{H_2O_2}{}^a$ (air)
nHo	1.82	0.72	0.70	0.61	0.10	0.14	0.46
Ho	1.82	0.91	0.85	0.75	0.09	0.13	0.37
Ha	3.73	0.47	0.49	0.47	0.22	0.24	0.84
N-Me-nHo	0.66	0.79	0.75	0.66	0.10	0.14	0.38
N-Me-Ho	0.68	0.95	0.93	0.79	0.09	0.11	0.30

[a] Error of ±0.05 on the mantissa shown. [b] Error of ±0.05. [c] Error of ±10%.

TABLE 2: Photochemical and Photophysical Parameters Determined upon One-Photon Excitation of βCs in Aqueous Solution at pH 10.0

	pH = 10.0						
	$10^3\Phi_R$[a] (air)	Φ_F[b] (N_2)	Φ_F[b] (air)	Φ_F[b] (O_2)	Φ_Δ[c] (air)	Φ_Δ[c] (O_2)	$10^3\Phi_{H_2O_2}$[a] (air)
nHo	0.51	0.22	0.21	0.18	0.08	0.10	0.12
Ho	0.53	0.49	0.47	0.41	0.10	0.13	0.14
Ha	1.63	0.39	0.38	0.36	0.13	0.13	0.37
N-Me-nHo	0.83	0.71	0.68	0.65	0.08	0.10	0.57
N-Me-Ho	0.67	0.74	0.68	0.61	0.10	0.10	0.47

[a] Error of ±0.05 on the mantissa shown. [b] Error of ±0.05. [c] Error of ±10%.

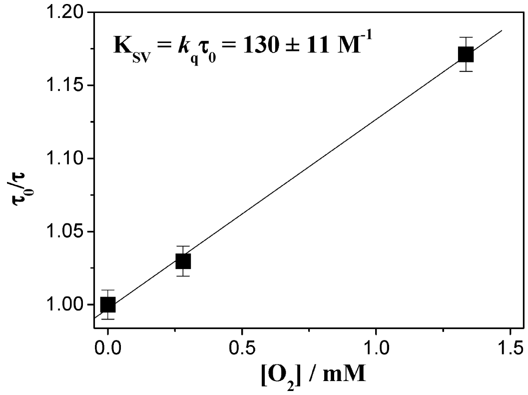


Figure 4. Dynamic Stern−Volmer study of the quenching of nHo fluorescence by oxygen at pH = 4.8.

These results are consistent with the suggestion that, upon excitation, the basicity of the βC pyridinic nitrogen is greatly enhanced.[55,56] As such, even in pH 10.0 water, the excited neutral βC species is readily protonated during the lifetime of its S_1 state. In turn, the predominant emitting species is the excited-state pyridinic protonated βC cation ($[\beta CH^+]^*$), not the excited-state neutral form.

It is also important to note that the intensity of fluorescence observed upon excitation of the neutral βC (i.e., the pH 10.0 experiment) is significantly lower than that of the respective protonated form (i.e., the pH 4.8 experiment), as manifested in a considerable difference in Φ_F (Tables 1 and 2). This suggests that only a fraction of the excited-state neutral form is protonated to yield $[\beta CH^+]^*$, which then emits. The remaining excited states formed apparently deactivate via internal conversion. This is consistent with the singlet oxygen data independently obtained (vide infra) as well as the pH-dependent data on reactive consumption (vide supra).

Quenching of βC Fluorescence by O_2. The fluorescence intensity observed from all βCs studied depended on the ambient oxygen partial pressure to which the aqueous solution was exposed (Figure 3, Tables 1 and 2). The data clearly show that, with an increase in ambient oxygen partial pressure, the quantum yield of fluorescence decreases. This decrease was quantified by steady-state Stern−Volmer plots (see Supporting Information) of the Φ_F data as a function of the three oxygen concentrations used (i.e., nitrogen-, air-, and oxygen-saturated water, where $[O_2]_{air} = 0.28$ mM and $[O_2]_{oxygen} = 1.33$ mM), yielding slopes of $k_q\tau_0 \sim 1.4 \times 10^2\ M^{-1}$ (with the exception of Ha where smaller $k_q\tau_0$ values were obtained;[57] see Supporting Information). This observation is consistent with the diffusion-limited quenching of S_1 states, a phenomenon that is entirely in keeping with oxygen-induced deactivation of fluorescence.[58]

To further substantiate the dependence of βC fluorescence on the dissolved oxygen concentration, we investigated nHo in a time-resolved Stern−Volmer quenching study performed at pH = 4.8 (Figure 4). Under air-saturated conditions, τ_F(nHo) = 21.0 ± 1 ns, which is in excellent agreement with published values obtained under similar conditions (22.0 ± 0.1 ns).[29] The Stern−Volmer constant obtained from this exercise (130 ± 11 M^{-1}) is entirely consistent with that obtained from the independent steady-state experiment (138 ± 7 M^{-1}, see Supporting Information) and yields a quenching rate constant of $k_q = (6.0 \pm 0.6) \times 10^9\ M^{-1}\ s^{-1}$, which is reasonable for a diffusion-limited process in water.[59]

It is interesting to note that for *N*-Me-Ho at pH 4.8, in particular, the oxygen-concentration-dependent decrease in Φ_F was not accompanied by a corresponding increase in the quantum yield of singlet oxygen production, Φ_Δ (vide infra and Tables 1 and 2). For this compound, Φ_F is comparatively large and $\Phi_F(\text{air}) + \Phi_\Delta \sim 1$. Under the reasonable assumptions that (1) the βC T_1 state is the principal precursor to $^1O_2$ and (2) the fraction of T_1 states quenched by oxygen that produce $^1O_2$ is comparatively large (i.e., $S_\Delta \rightarrow 1$), these data suggest that oxygen-induced S_1-T_1 intersystem crossing must not be a significant process. Rather, it appears that oxygen-induced S_1-S_0 internal conversion may play a competing role.

It is well-documented that, in organic molecules, oxygen-induced deactivation of the S_1 state proceeds via S_1-T_1 intersystem crossing.[60] To our knowledge, only one exception to this general rule has thus far been suggested: 9,10-diphenylanthracene.[61] As such, these βC systems may provide an interesting and important perspective on the coupling of spin states in the oxygen−organic molecule photosystem. Clearly, more work needs to be done to properly elucidate this aspect of our present results.

Our results are also significant because these βC molecules have been proposed as fluorescence standards.[28,53] Specifically, given that the βC fluorescence intensity can depend significantly on the ambient oxygen concentration, care must be exercised when fluorescence data are obtained against these molecules. Otherwise, data may be incorrectly interpreted and erroneous conclusions may be drawn.

Quantum Yield of βC Sensitized $^1O_2$ Production (Φ_Δ) in Aqueous Solution. It has been proposed that βC alkaloids are excellent $^1O_2$ photosensitizers.[19,62] Becker et al.[23] measured values of Φ_Δ for some βCs (nHo, Ho, and Ha) in nonprotic organic solvents, and found Φ_Δ values for the neutral form of these alkaloids that ranged from 0.31 to 0.40. To the best of our knowledge, data in aqueous solution have only been recorded for Ho; a Φ_Δ value of ~0.03 was obtained at pH 7, where a mixture of both acid−base forms of Ho is present.[39,63,64] This value was subsequently corrected to yield $\Phi_\Delta \sim 0.08$.[65]

We determined quantum yields for the photosensitized production of $^1O_2$ for the five βCs studied in aqueous solution. The data were recorded as a function of both pH and ambient oxygen partial pressure (i.e., exposure to air and oxygen). The results, shown in Tables 1 and 2, indicate that both acidic and

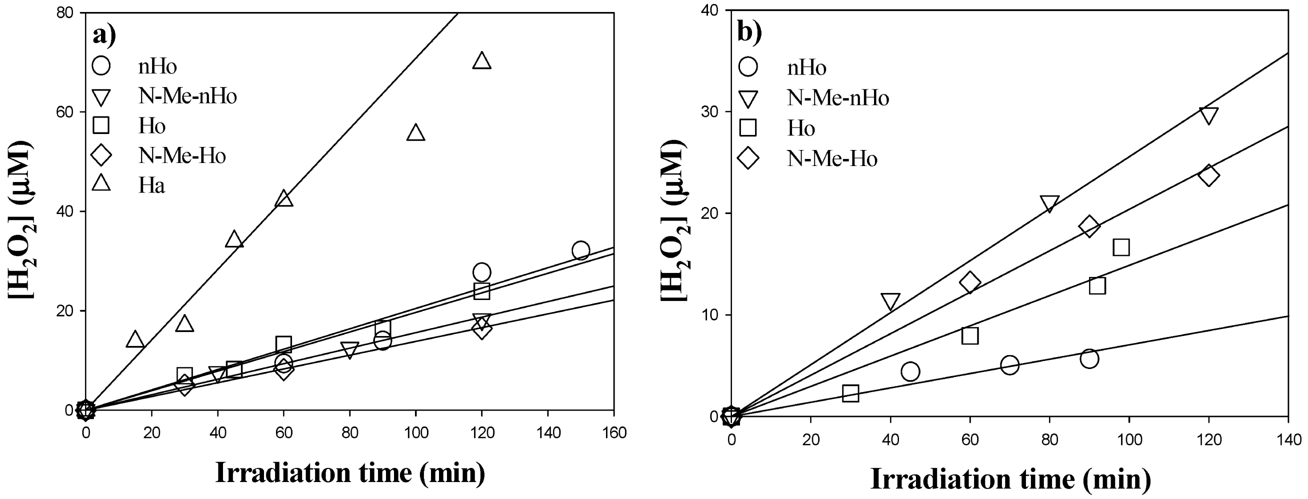


Figure 5. Evolution of the H_2O_2 concentration in irradiated solutions of βC (250 μM) as a function of irradiation time. Experiments performed at (a) pH 4.8 and (b) pH 10.0.

basic forms of these βCs are rather poor $^1O_2$ photosensitizers in aqueous solution. In the case of Ho, the Φ_Δ values listed in Tables 1 and 2 (~0.1) are similar to the corrected value reported in the literature (~0.08).

It is important to note that, on one hand, the values of Φ_Δ obtained are entirely consistent with the values of Φ_F that were recorded at the same oxygen concentration in independent experiments (i.e., the sum of Φ_Δ and Φ_F does not exceed 1.0). On the other hand, and as discussed in the preceding section, some of the molecules in this βC system appear to show unique oxygen-concentration-dependent behavior. Specifically, with an increase in the oxygen concentration, the observed decrease in Φ_F is not accompanied by a corresponding increase in Φ_Δ. Thus, we conclude that oxygen-induced S_1-S_0 internal conversion may play a role in these systems.

Hydrogen Peroxide Production under UV-A Steady-State Irradiation. The presence of H_2O_2 was detected upon irradiation of air-equilibrated solutions of all the βCs studied, both at pH 4.8 and 10.0. Figure 5 shows the evolution of the concentration of H_2O_2 as a function of elapsed irradiation time ($[\beta C]_0 = 250$ μM). Because of the low solubility of Ha in water at pH 10.0, the experiments with this βC were performed using a 25 μM solution (see Supporting Information). All H_2O_2 experiments were carried out under the same experimental conditions as those used for the determination of the quantum yield of βC disappearance, Φ_R (vide supra).

The production of H_2O_2 upon UV-A irradiation of βCs has been reported.[38] In an earlier study of nHo,[38] it was proposed that H_2O_2 is formed by electron transfer from S_1 to O_2 yielding the superoxide anion ($O_2^{\bullet -}$) and, as a result of the spontaneous disproportionation of $O_2^{\bullet -}$, H_2O_2 is produced. This may indeed be one source of H_2O_2 that is applicable in our present work.

Quantum yields of H_2O_2 formation listed in Tables 1 and 2 show that all compounds studied have a very low efficiency of H_2O_2 production upon irradiation. For three of the compounds (nHo, Ho, and Ha), $\Phi_{H_2O_2}$ depends noticeably on pH with the neutral form being less efficient than the protonated form. For these three compounds, this pH-dependent effect also correlates with the pH-dependent response of Φ_R, suggesting that H_2O_2, or compounds related to its formation (e.g., $HOO^\bullet$ or $O_2^{\bullet -}$), may be involved in the photoinitiated degradation of these βCs.

***βC* Photophysics upon Two-Photon Excitation.** Two-photon excitation of a given chromophore offers a number of unique features as compared with one-photon excitation. With respect to imaging and/or initiating reactions in localized domains, two-photon excitation in the small focal volume of a focused laser beam can impart remarkable spatial resolution.[66−68] In media that consist of multiple chromophores (e.g., a biological cell), two-photon excitation of a chromophore with a comparatively large transition probability (i.e., the so-called two-photon absorption cross section, δ) can impart remarkable selectivity to the process of light absorption. With these points in mind, we wanted to characterize two-photon light absorption by βCs and to quantify the wavelength-dependent magnitudes of δ.

(a) Two-Photon Excitation Spectra and Absorption Cross Sections of nHo, Ho, and Ha. Experiments were performed using three βCs (nHo, Ho, and Ha) and, with these molecules dissolved in water at pH 4.8 and 10.0, it was indeed possible to observe two-photon induced fluorescence signals in photon counting experiments performed at several irradiation wavelengths. In each case, the fact that excitation was achieved via a two-photon process was verified in a plot of the observed signal against the laser power that showed a quadratic dependence (e.g., Figure 6a).

The two-photon fluorescence excitation spectrum and the one-photon absorption spectrum for nHo at pH = 4.8 and at pH = 10.0 are shown in Figure 6b,c, respectively. To facilitate comparison between the one- and two-photon spectra, the *x*-axis shows the total transition energy. As such, it is important to keep in mind that the actual two-photon experiments are performed at twice the wavelength shown, in a spectral region where there is no competing one-photon absorption.

Upon examination of the data in Figure 6b,c, we note the striking similarities between the one- and two-photon spectra. Such similarity was seen for all three βCs under both acidic and basic conditions (see Supporting Information for the spectra obtained from the other two molecules). This observation is arguably expected since these molecules are noncentrosymmetric (i.e., they do not possess a center of inversion).[69] In this circumstance, selection rules dictate that the same states will be populated upon both one- and two-photon absorption.[70]

The δ-values, as quantified according to eq 3, are collected in Table 3. In general, the βCs studied are comparatively poor two-photon absorbers, with δ-values around 1 GM. Nevertheless, a rather large substituent-dependent change is observed, both in the apparent λ_{max} and in the magnitude of δ at this λ_{max}. Moreover, for a given compound, the acidic form generally appears to be a better two-photon absorber than the basic form.

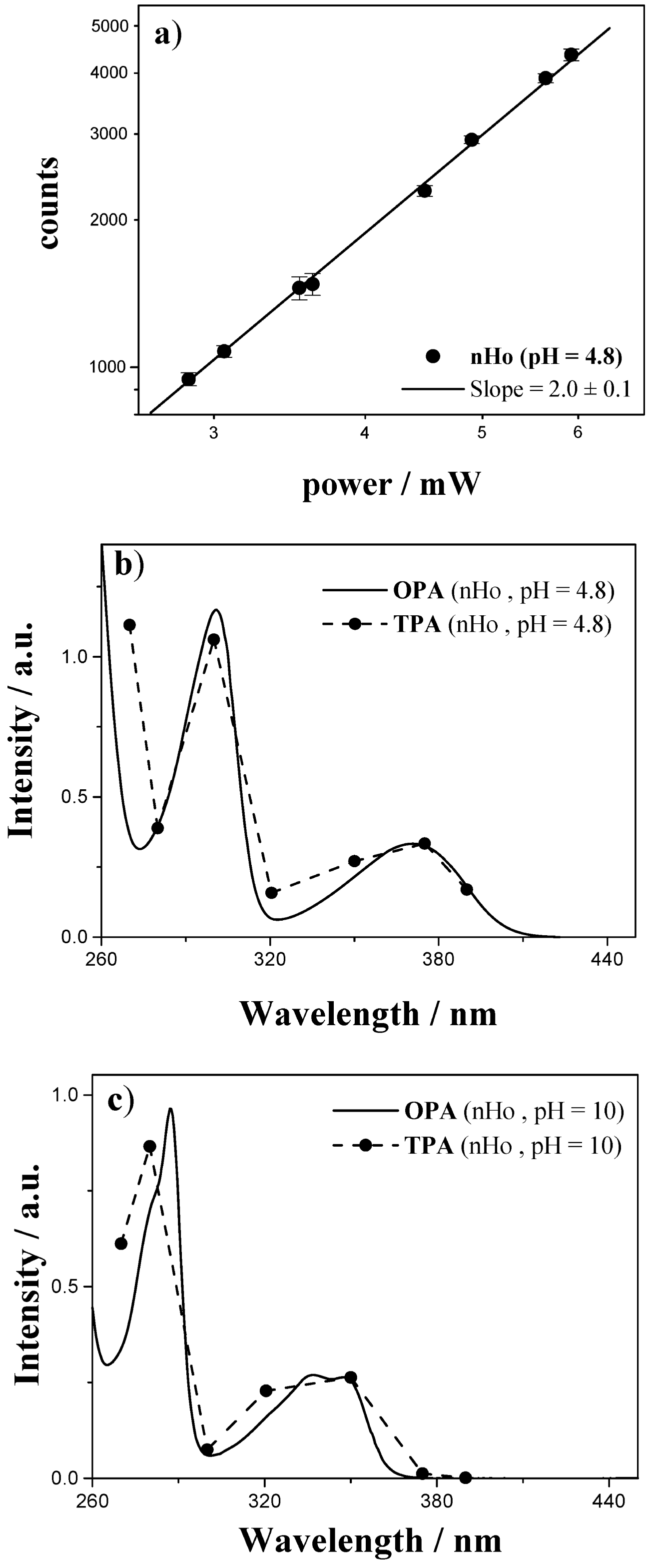


Figure 6. (a) Double logarithmic plot of the fluorescence intensity against the incident laser power upon 641 nm irradiation of a pH = 4.8 aqueous nHo solution. A least-squares fit to the data yield a slope of 2.0 ± 0.1. A slope of 2 is characteristic of two-photon excitation. (b) One-photon absorption spectrum (solid line) and two-photon excitation spectrum (filled circles) for nHo at pH = 4.8. (c) One-photon absorption spectrum (solid line) and two-photon excitation spectrum (filled circles) for nHo at pH = 10.0. In both panels b and c, a dotted line connects the two-photon data points to help guide the eye. The *x*-axis denotes the total transition energy (i.e., the two-photon experiments were performed using excitation wavelengths two times larger than those shown on the axis). Corresponding data for the other βCs studied are shown in the Supporting Information.

This latter phenomenon could reflect a pronounced increase in the extent of internal charge-transfer (CT) character in the protonated form. It is known that internal CT character can enhance the transition dipole moment and, in turn, be reflected in a larger value of δ.[71]

Finally, we note that the short-wavelength limit for these two-photon spectra (540 nm) was determined by the calibrated spectrum of the reference molecule used. We nevertheless expect that, as the excitation wavelength is decreased below 540 nm, these βCs will have pronounced two-photon absorption that progressively gets larger. Such a wavelength-dependent increase in δ is expected as a consequence of the phenomenon of resonance enhancement.[72−74] Specifically, at these shorter wavelengths, the two-photon transition will borrow intensity from a near-resonant one-photon transition and, thereby, gain intensity. Resonance enhancement is expected to be pertinent down to around 400 nm, at which point the onset of one-photon absorption generally begins for these compounds.

(b) Singlet Oxygen Production by the Protonated Form of Ha upon Two-Photon Excitation. Despite comparatively small values of both Φ_Δ and δ (vide supra), we wanted to establish, as a proof of principle, if it was possible to produce and optically detect singlet oxygen following two-photon absorption of a βC. The success of such an experiment could have significant ramifications in mechanistic studies of the roles played by βCs in biological systems.

Clearly, a salient factor in this experiment is the product of the quantum yield of singlet oxygen production and the two-photon absorption cross section. As such, we opted to excite a pH = 4.8 solution of Ha at ~660 nm; these are conditions that should yield the largest two-photon induced singlet oxygen phosphorescence signal (Tables 1 and 3). Moreover, the experiment was performed in D_2O to take advantage of the well-known solvent isotope effect to increase the singlet oxygen phosphorescence efficiency.[75]

As shown in Figure 7, we indeed succeeded in obtaining a singlet oxygen signal that increased quadratically with the laser power. As expected, the intensity of this signal increased with an increase in the partial pressure of ambient oxygen (Figure 7) and the magnitude of this increase is, within the errors afforded by the experiment, in keeping with the singlet oxygen quantum yield data in Table 1. For the sample saturated with nitrogen gas, the signal observed was comparable to our background signal.

The data in Figure 7 demonstrate that an appreciably large and readily detectable singlet oxygen phosphorescence signal can indeed be generated upon two-photon excitation of a βC.

Conclusions

Data have been presented to show that studies of β-carboline (βC) alkaloids in aqueous media provide a unique opportunity to examine a variety of fundamental photophysical phenomena. Moreover, the information gained facilitates efforts to further elucidate the roles played by these important compounds in a range of biological systems.

When considering all the material provided in this report, four key points arguably rise to the surface:

(i) The photochemical, photophysical, and spectroscopic behavior of βCs in aqueous solution clearly depend on the βC molecular structure, the pH, and the concentration of dissolved oxygen. This is an important point to keep in mind when considering the roles played by βCs in natural systems.

(ii) A given βC can have a non-negligible two-photon absorption cross section, δ, in a spectral domain that is

TABLE 3: pH-Dependent Two-Photon Absorption Cross Sections (δ) of three βCs at Seven Different Wavelengths

	δ^{540nm} (GM)[a]	δ^{560nm} (GM)	δ^{600nm} (GM)	δ^{641nm} (GM)	δ^{700nm} (GM)	δ^{750nm} (GM)	δ^{780nm} (GM)
nHo (pH 4.8)	1.42	0.70	1.35	0.20	0.35	0.43	0.22
nHo (pH 10.0)	0.60	0.85	0.073	0.22	0.26	0.012	1.1×10^{-3}
Ho (pH 4.8)	1.21	0.63	1.30	0.18	0.39	0.35	0.093
Ho (pH 10.0)	0.48	0.47	0.054	0.14	0.12	3.6×10^{-3}	1.3×10^{-3}
Ha (pH 4.8)	1.92	0.55	2.83	8.22	2.61	0.54	0.039
Ha (pH 10.0)		1.03	1.76	0.71	0.44	2.7×10^{-3}	

[a] 1 GM = 10^{-50} cm^4 s. Error on each number is ±20%.

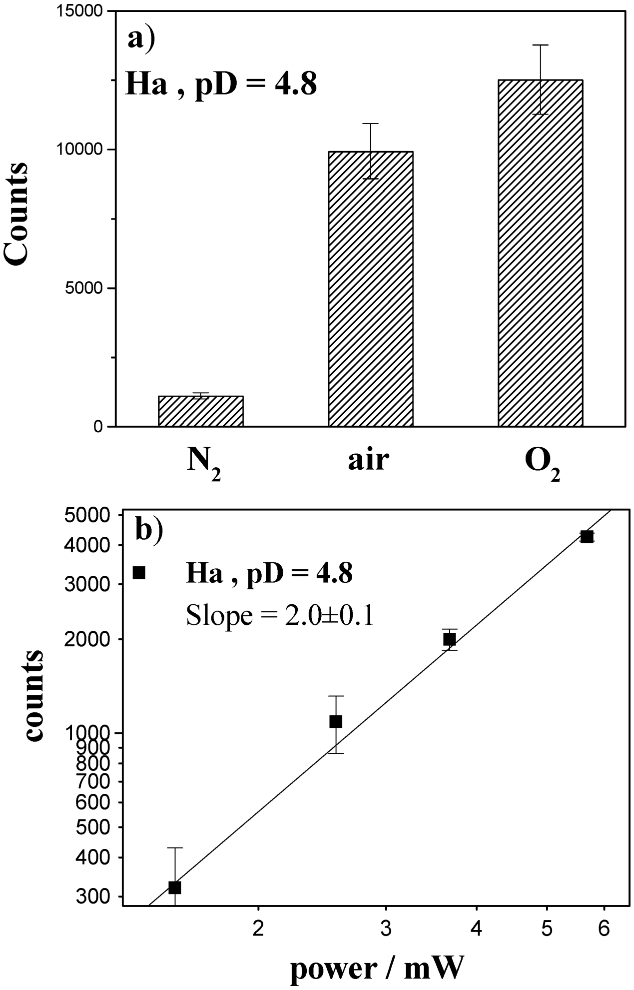


Figure 7. (a) Singlet oxygen phosphorescence counts obtained from a D_2O solution of 180 μM Ha at pD 4.8 upon two-photon excitation (λ_{exc} = 660 nm) under different atmosphere conditions. The data reflect an integral of the time-resolved $^1O_2$ signal. (b) Double logarithmic plot of the $^1O_2$-phosphorescence counts versus the energy of the laser.

significantly red-shifted relative to that for one-photon absorption. Although the magnitude of this δ depends on the solution pH and on the βC substituent, this phenomenon could nevertheless provide added flexibility and control in mechanistic studies of βC behavior in complex systems.

(iii) Although the efficiency of sensitized singlet oxygen production upon two-photon excitation of a given βC is comparatively small, as expressed by the product of δ and Φ_Δ, the singlet oxygen formed under these conditions can nevertheless be spectroscopically observed by its weak 1270 nm phosphorescence. This likewise provides added flexibility to a mechanistic study of βC behavior in complex systems.

(iv) Selected βCs may provide an exception to the rule that oxygen-induced deactivation of the S_1 state in organic molecules proceeds via S_1-T_1 intersystem crossing. Data provide herein indicate that, for some βCs, oxygen-induced S_1 deactivation may proceed by S_1-S_0 internal conversion. This observation may provide important insight into features that facilitate the coupling of spin states in the oxygen−organic molecule photosystem and, in turn, contribute to a better understanding of induced spin-forbidden transitions in general.

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Supporting Information Available: Evolution of βC concentration in irradiated oxygen-free solutions, corrected fluorescence spectra, and Stern−Volmer plots of βCs in aqueous solution as a function of pH and O_2 concentration, evolution of the H_2O_2 concentration in irradiated solutions of Ha as a function of irradiation time and two-photon excitation spectra for Ho and Ha. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Hemmateenejad, B.; Abbaspour, A.; Maghami, H.; Miri, R.; Panjehshahin, M. R. *Anal. Chim. Acta* **2006**, *575*, 290–299.

(2) Bais, H. P.; Park, S.-W.; Stermitz, F. R.; Halligan, K. M.; Vivanco, J. M. *Phytochemistry* **2002**, *61*, 539–543.

(3) Kam, T.-S.; Sim, K.-M.; Koyano, T.; Komiyama, K. *Phytochemistry* **1999**, *50*, 75–79.

(4) Kam, T.-S.; Sim, K.-M. *Phytochemistry* **1998**, *47*, 145–147.

(5) Bourke, C. A.; Stevens, G. R.; Carrigan, M. J. *Aust. Vet J.* **1992**, *69*, 163–165.

(6) Abourashed, E. A.; Vanderplank, J.; Khan, I. A. *Pharm. Biol.* **2003**, *41*, 100–106.

(7) de Meester, C. *Mutat. Res.* **1995**, *339*, 139–153.

(8) Torreilles, J.; Guerin, M. C.; Previero, A. *Biochimie* **1985**, *67*, 929–947.

(9) Breyer-Pfaff, U.; Wiatr, G.; Stevens, I.; Gaertner, H.; Mundle, G.; Mann, K. *Life Sci.* **1996**, *58*, 1425–1432.

(10) Vert, F. T.; Zabala Sanchez, I.; Olba Torrent, A. *J. Photochem.* **1983**, *23*, 355–368.

(11) Vert, F. T.; Zabala, I.; Olba, A. *J. Photochem.* **1985**, *31*, 253–263.

(12) Kuhn, W.; Muller, T.; Grose, H.; Rommelspacher, H. *J. Neural Transm.* **1996**, *103*, 1435–1440.

(13) Hiroaki, N. *Mutat. Res.* **2003**, *541*, 123–136.

(14) Mori, T.; Nakagawa, A.; Kobayashi, N.; Hashimoto, M. W.; Wakabayashi, K.; Shimoi, K.; Kinae, N. *J. Radiat. Res.* **1998**, *39*, 21–33.

(15) Shimoi, K.; Kawabata, H.; Tomita, I. *Mutat. Res.* **1992**, *268*, 287–295.

(16) Chang, C.; Castellazzi, M.; Glover, T.; Trosko, J. *Cancer Res.* **1978**, *38*, 4527–4533.

(17) Shimoi, K.; Miyamura, R.; Mori, T.; Todo, T.; Ohtsuka, E.; Wakabayashi, K.; Kinae, N. *Carcinogenesis* **1996**, *17*, 1279–1283.

(18) Hudson, J. B.; Graham, E. A.; Towers, G. H. N. *Photochem. Photobiol.* **1986**, *43*, 21–26.

(19) Downum, K. R. *New Phytol.* **1992**, *122*, 401–420.

(20) Varela, A.; Burrows, H.; Douglas, P.; da Graça, M. J. *Photochem. Photobiol. A: Chem.* **2001**, *146*, 29–36.

(21) Reyman, D.; Tapia, M.; Caicedo, C.; Viñas, M. *Biophys. Chem.* **2003**, *104*, 683–696.

(22) Reyman, D.; Vi, M.; Poyato, J.; Pardo, A. *J. Phys. Chem. A* **1997**, *101*, 768–775.

(23) Becker, R. S.; Ferreira, L. F. V.; Elisei, F.; Machado, I.; Latterini, L. *Photochem. Photobiol.* **2005**, *81*, 1195–1204.

(24) Biondic, M. C.; Erra-Balsells, R. *J. Photochem. Photobiol. A* **1994**, *77*, 149–159.

(25) Biondic, M. C.; Erra-Balsells, R. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1049–1058.

(26) Biondic, M. C.; Erra-Balsells, R. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1323–1327.

(27) Olba Torrent, A.; Tomas Vert, F.; Zabala Sanchez, I.; Medina Casamayor, P. *J. Photochem.* **1987**, *37*, 109–116.

(28) Ghiggino, K. P.; Skilton, P. F.; Thistlethwaite, P. J. *J. Photochem.* **1985**, *31*, 113–121.

(29) Sakurovs, R.; Ghiggino, K. P. *J. Photochem.* **1982**, *18*, 1–8.

(30) Tapia, M.; Reyman, D.; Vinas, M.; Arroyo, A.; Poyato, J. *J. Photochem. Photobiol. A: Chem.* **2003**, *156*, 1–7.

(31) Balón, M.; Carmona, C.; Muñoz, M. *Chem. Phys.* **2004**, *302*, 13–20.

(32) Balón, M.; Angulo, G.; Carmona, C.; Muñoz, M.; Guardado, P.; Galán, M. *Chem. Phys.* **2002**, *276*, 155–165.

(33) Carmona, C.; Balón, M.; Galán, M.; Angulo, G.; Guardado, P.; Muñoz, M. *J. Phys. Chem. A* **2001**, *105*, 10334–10338.

(34) Wolfbeis, O. S.; Fürlinger, E. *Z. Phys. Chem. Neue Folge* **1982**, *129*, 171–183.

(35) Muñoz, M.; Balón, M.; Hidalgo, J.; Carmona, C.; Pappalardo, R. R.; Sanchez Marcos, E. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1729–1734.

(36) Krishnamurthy, M.; Kumar Dogra, S. *J. Chem. Soc. Perkin Trans. 2* **1986**, 1247–1251.

(37) Vert, F. T.; Zabala Sanchez, I.; Olba Torrent, A. *J. Photochem.* **1984**, *26*, 285.

(38) Gonzalez, M. M.; Salum, M. L.; Gholipour, Y.; Cabrerizo, F. M.; Erra-Balsells, R. *Photochem. Photobiol. Sci.* **2009**, DOI: 10.1039/b822173a.

(39) Yu Egorov, S.; Babizhayev, M. A.; Krasnovsky, A. A., Jr.; Shvedova, A. A. *Biophysics* **1987**, *32*, 184–186.

(40) Chae, K. H.; Ham, H. S. *Bull. Korean Chem. Soc.* **1986**, *7*, 478–479.

(41) Nonami, H.; Orcoyen, M.; Fukuyama, Y.; Biondic, M. C.; Erra-Balsells, R. *An. Asoc. Quim. Argent.* **1998**, *86*, 81–89.

(42) Braun, A. M.; Maurette, M. T.; Oliveros, E. *Photochemical Technology*; John Wiley & Sons: Chichester, U.K., 1991; Chapter 2.

(43) Allain, C. C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; Fu, P. C. *Clin. Chem.* **1974**, *20*, 470–475.

(44) Flegg, H. M. *Ann. Clin. Biochem.* **1973**, *10*, 79–84.

(45) Mardelli, M.; Olmsted, J. *J. Photochem.* **1977**, *7*, 277–285.

(46) Eastman, J. W. *Photochem. Photobiol.* **1967**, *6*, 55–72.

(47) Arnbjerg, J.; Johnsen, M.; Frederiksen, P. K.; Braslavsky, S. E.; Ogilby, P. R. *J. Phys. Chem. A* **2006**, *110*, 7375–7385.

(48) Marti, C.; Jürgens, O.; Cuenca, O.; Casals, M.; Nonell, S. *J. Photochem. Photobiol., A: Chem.* **1996**, *97*, 11–18.

(49) Scurlock, R. D.; Mártire, D. O.; Ogilby, P. R.; Taylor, V. L.; Clough, R. L. *Macromolecules* **1994**, *27*, 4787–4794.

(50) Kennedy, S. M.; Lytle, F. E. *Anal. Chem.* **1986**, *58*, 2643–2647.

(51) Xu, C.; Webb, W. W. *J. Opt. Soc. Am. B.* **1996**, *13*, 481–491.

(52) Rumi, M.; Ehrlich, J. E.; Heikal, A. A.; Perry, J. W.; Barlow, S.; Hu, Z.; McCord-Maughon, D.; Parker, T. C.; Röckel, H.; Thayumanavan, S.; Marder, S. R.; Beljonne, D.; Bredas, J.-L. *J. Am. Chem. Soc.* **2000**, *122*, 9500–9510.

(53) Pardo, A.; Reyman, D.; Poyato, J. M. L.; Medina, F. *J. Lumin.* **1992**, *51*, 269–274.

(54) Wolfweis, O.; Furlinger, E.; Wintersteiger, R. *Monatsh. Chem.* **1982**, *113*, 509–517.

(55) Draxler, S.; Lippitsch, M. E. *J. Phys. Chem.* **1993**, *97*, 11493–11496.

(56) Balon, M.; Munoz, M. A.; Guardado, P.; Carmona, C. *Photochem. Photobiol.* **1996**, *64*, 531.

(57) In the case of Ha, the steady-state Stern−Volmer plots show that, under both pH conditions, the slope ($k_q\tau_0$) is 1 order of magnitude lower than those observed for the other four βC alkaloids studied (see Supporting Information). However, taking into account the fluorescence lifetime of Ha reported in the literature ($\tau_0 = 6.6 \pm 0.2$ ns),[53] a quenching rate constant of $k_q \sim 10^9$ M^{-1} s^{-1} was also obtained, which is quite similar to those observed for the other βCs studied in the present work.

(58) Montaldi, M.; Credi, A.; Prodi, L.; Gandolfi, M. T. *Handbook of photochemistry*, 3rd ed.; CRC Taylor & Francis Group: Boca Raton, London, NY, 2006; Chapter 6.

(59) Note that the diffusion coefficient of oxygen in 25 °C water is roughly 2.5 times smaller than that in a typical hydrocarbon solvent. Thus, although one typically associates rate constants of $\sim(2-3) \times 10^{10}$ M^{-1} s^{-1} with S_1 quenching by oxygen in hydrocarbon solvents, a value of $\sim 6 \times 10^9$ M^{-1} s^{-1} is reasonable for water.

(60) Turro, N. J. *Modern Molecular Photochemistry*; The Benjamin/Cummings Publishing Co., Inc.: Menlo Park, CA, 1978; Chapter 6.

(61) Darmanyan, A. P. *Chem. Phys. Lett.* **1982**, *91*, 396–400.

(62) Berenbaum, M. R.; Lee, K. *Chemoecology* **1990**, *1*, 81–85.

(63) Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1993**, *22* (1), 113–262.

(64) Table 1, pp 160, entry 1.226 in ref 63 indicate that the data are for Ho, not nHo. This appears to be a typographical error.

(65) Egorov, S. Y.; Krasnovsky, A. A., Jr. *SPIE Proceedings* **1990**, *1403*, 611–621.

(66) Skovsen, E.; Snyder, J. W.; Ogilby, P. R. *Photochem. Photobiol.* **2006**, *82*, 1187–1197.

(67) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248*, 73–76.

(68) Sun, H.-B.; Tanaka, T.; Kawata, S. *Appl. Phys. Lett.* **2002**, *80*, 3673–3675.

(69) Arnbjerg, J.; Paterson, M. J.; Nielsen, C. B.; Jørgensen, M.; Christiansen, O.; Ogilby, P. R. *J. Phys. Chem. A* **2007**, *111*, 5756–5767.

(70) McClain, W. M. *Acc. Chem. Res.* **1974**, *7*, 129–135.

(71) Albota, M.; Beljonne, D.; Bredas, J. L.; Ehrlich, J. E.; Fu, J. Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M. D.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Rockel, H.; Rumi, M.; Subramaniam, C.; Webb, W. W.; Wu, X. L.; Xu, C. *Science* **1998**, *281*, 1653–1656.

(72) Arnbjerg, J.; Jimenez-Banzo, A.; Paterson, M. J.; Nonell, S.; Borrell, J. I.; Christiansen, O.; Ogilby, P. R. *J. Am. Chem. Soc.* **2007**, *129*, 5188–5199.

(73) Drobizhev, M.; Karotki, A.; Kruk, M.; Rebane, A. *Chem. Phys. Lett.* **2002**, *355*, 175–182.

(74) Johnsen, M.; Paterson, M. J.; Arnbjerg, J.; Christiansen, O.; Nielsen, C. B.; Jørgensen, M.; Ogilby, P. R. *Phys. Chem. Chem. Phys.* **2008**, *10*, 1177–1191.

(75) Ogilby, P. R.; Foote, C. S. *J. Am. Chem. Soc.* **1983**, *105*, 3423–3430.

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