β-Carboline Photosensitizers. 3. Studies on Ground and Excited State Partitioning in AOT/Water/Cyclohexane Microemulsions

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The photophysical behavior of the β-carbolines norharmane, harmane, and harmine has been studied in water-in-oil (w/o) microemulsions in AOT/cyclohexane. With pure solvents, solubility and partitioning studies indicate that these molecules dissolve preferentially in the organic phase but that their solubility in the aqueous phase increases upon decreasing pH as a consequence of protonation. However, in w/o microemulsions these molecules appear to be located predominantly at the surfactant/water interface. Again, decreasing pH increases partitioning into the water region. From absorption and fluorescence spectral studies with the microemulsion system, differences are observed in the predominant species at any "pH" compared with that in pure water. From analysis of fluorescence decays, four kinetically distinguishable species have been observed in the excited state, namely, neutral species in cyclohexane, neutral species in the microemulsion droplets, cation, and zwitterion. For harmine, the effect of water/surfactant ratio (w/o) on the photophysical behavior and distribution of these species was studied. Partitioning was observed between aqueous and organic subphases. A study has been carried out on the effect of pH on these processes. In agreement with other studies on "pH" in microemulsions, the cation is observed up to apparent pH values much higher than those for pure aqueous solutions. The results are interpreted in terms of the compartmentalization of the hydroxide ions between the water pools. Effects of changing w/o on the decay of the various species are interpreted in terms of "quenching" of the neutral species in the cyclohexane subphase by its trapping within the microemulsion droplets. A kinetic model for this is presented. The effect of changing the polarity (by addition of methanol) upon the partitioning behavior has been studied, and it is shown that addition of methanol favors partitioning into the organic phase.

Introduction

The β-carboline alkaloids are a widely distributed group of naturally occurring compounds, linked by a common tricyclic system having adjacent indole and pyridine rings.1 These show photosensitizing activity toward a variety of systems, including bacteria, fungi, viruses, insects, etc.2-6 In addition, it is suggested that these compounds are formed as photoproducts from tryptophan in human lenses.7-9 A large number of photophysical studies have been carried out on these compounds,10-20 stimulated by both their application in areas, such as their use as fluorescence standards,21,22 and their photobiological relevance.

Particular interest has been shown in the acid-base behavior of the β-carbolines. Because of charge-density differences in the ground and excited states of the pyrrole and pyridine rings, interesting tautomeric behavior is observed, with neutral forms in the ground state being replaced by zwitterions and cations in the excited state in polar solvents.12,13,19 It is suggested that important factors in the phototoxicty of these compounds include their localization in relation to the target molecules2 and their diffusion across membranes.3 In spite of the importance of their solubility behavior in these areas, it is surprising that our knowledge of the partitioning of the various forms between water and nonpolar phases is very limited. Qualitative measurements have been made on the polarity of the molecules in the ground state using chromatography,6,21,22 and distribution coefficients between 1-octanol and water have been reported for alkaline solutions.22

In an earlier report,18 the photophysics of the excited singlet states of the β-carbolines norharmane, harmane, harmine, and, to a lesser extent, harmaline were considered in the solvents benzene and methanol, and both experimental measurements and theoretical calculations were used to assign the various species produced in the excited state. In nonpolar solvents, only a neutral species (N*) is observed upon excitation. However, in the protic solvent methanol, cationic (C*) and zwitterionic (Z*) species are also formed, in the complex equilibria, shown schematically:

Note that in the absence of a strong base (such as hydroxide) the interconversion of N* to C* or Z* is, effectively, irreversible. We extend this study to the photophysical behavior of β-carbolines in water-in-oil microemulsions formed by the anionic surfactant Aerosol-OT (sodium bis(2-ethylhexyl) sulfosuccinate, abbreviated AOT). The microemulsion droplets in these have a water pool of a few nanometers diameter surrounded by a surfactant layer and a continuous hydrocarbon phase. Because
of changes in the polarity of the β-carbolines upon excitation, it is anticipated that they can provide both dynamic and equilibrium information upon solute distribution in microemulsion systems. In addition to their relevance to our understanding of the behavior of microemulsions, these are expected to mimic certain facets of the behavior of β-carbolines in biological systems.

Experimental Section

Norharmane (1), harmane (2), and harmine (3) were used as purchased (Aldrich). The structures of these molecules and their ground and excited state pKₐ's are given in Figure 1. Benzene, methanol (Merck, P.A., Uvasol), 1-octanol (Merck, P.A.), and cyclohexane (Merck, P.A., Uvasol) were used without further purification. For some solutions, methanol was dried by distillation over magnesium and chromatographed on an A1203 column. The surfactant AOT, C₂₀H₃₇O₇SNa, from Sigma, was dissolved in methanol and filtered with alumina, and the solvent was evaporated (purification procedure adapted from ref 24). The aqueous solutions of β-carbolines were prepared with an AOT solution of the pulse profile and the sample emission were continued using a VAX 2000 computer using the modulation functions method with shift correction.

Results and Discussion

Solubility and Partition Behavior. Solubilities in water and 1-octanol/water partition coefficients of the compounds norharmane, harmane, and harmine are presented in Table 1. For all the conditions harmine presents the lowest solubility in water, while norharmane is the most soluble. This agrees with previous partitioning studies on these systems. The results also confirm the higher solubility in neutral solutions (pH 7) in water compared with basic media (pH 13) for all the compounds. This is associated with formation of the cation in the ground state, since the pKₐ values in all cases are in the region 7-8. With respect to partitioning of these compounds between organic and aqueous phases, literature values²⁹ for all the compounds at pH 13 show very large partition coefficients, indicating only very minor quantities of the β-carbolines pass to the aqueous phase, the rest being either in 1-octanol or at the interface. At pH 5.5, the results indicate more partitioning of the carbolines into the aqueous phase (Table 1), supporting the conclusions from the solubility studies. It is reasonable to conclude that the main partitioning is between cation in water and neutral species in 1-octanol.

Absorption and Emission Spectral Studies in Microemulsions. The β-carbolines norharmane, harmane, and harmine have been examined regarding absorption, emission, and excited-state equilibria in several classes of organic solvents. In hydrocarbon and nonprotic solvents, excitation results only in an excited-state neutral species. In neutral protic solvents (i.e., methanol), excitation of the neutral form results in multiple excited-state species—neutral, cation, and zwitterion—and emission. The excited cation and zwitterion are formed from the neutral species during the lifetime of its lowest (π,π*) singlet state. A kinetic model has been developed for the complex decays, and an analysis has been presented for neutral protic solvents and for aqueous solutions at different pH's. When ground state cation is directly excited, absorption, emission, and kinetic decay data show that only a single excited-state species exists and emits, independent of the solvent.

The absorption and fluorescence spectra of all the compounds were studied in w/o microemulsions, water/AOT/cyclohexane, for the water/surfactant molar ratio ([H₂O]/[AOT]) w₀ = 5 at

Table 1: Solubility (s) and Partition Coefficient (P) Values of the β-Carbolines Norharmane, Harmane, and Harmine as a Function of pH and Temperature (T = 20 °C)

<table>
<thead>
<tr>
<th>Compound</th>
<th>s/M (pH = 7)</th>
<th>s/M (pH = 13)</th>
<th>log P (pH = 7)</th>
<th>log P (pH = 13)</th>
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</thead>
<tbody>
<tr>
<td>norharmane</td>
<td>1.86 x 10⁻⁴</td>
<td>1.81 x 10⁻⁴</td>
<td>0.82 ± 0.04</td>
<td>3.17</td>
</tr>
<tr>
<td>harmane</td>
<td>1.61 x 10⁻⁴</td>
<td>7.86 x 10⁻²</td>
<td>0.70 ± 0.05</td>
<td>3.50</td>
</tr>
<tr>
<td>harmine</td>
<td>3.31 x 10⁻⁵</td>
<td>2.08 x 10⁻³</td>
<td>0.90 ± 0.07</td>
<td>3.56</td>
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</table>

* From ref 22.
different pH’s of added solution and are shown in Figures 2 and 3. Similar spectral behavior is observed for all of them, for pH values between 6.4 and 12.4, where only the cationic species show absorption (λ ~ 400–450 nm). However, at pH 12.8 a different absorption band is seen, which is characteristic of the neutral form (λ ~ 350–380 nm). Comparable changes occur in the emission spectra. For example, in the case of harmine, from pH 10.3 to pH 12.0, a decrease can be seen for the emission of the cationic species (λ = 403 nm), and a corresponding increase is observed for the emission of the neutral form (λ = 365 nm). For harmine and norharmane similar behavior is observed in the emission for the neutral form at λ = 361 nm, pH 12.5, and at λ = 365 nm, pH 12.3, respectively. Note that for norharmane, at pH 12.3, the emission spectrum is very similar to that observed in water with respect to the contribution of the N* and C* species. This can be related to the higher solubility of this compound in water, compared with the other carbolines.

Harmine was also studied over the same pH range at w_0 = 12.5, where better definition of the species is observed. Absorption and emission spectra are shown in Figures 4 and 5, respectively. In both cases, well-defined isosbestic points are observed, showing clean interconversion of C* to N* upon increasing pH. However, as will be discussed with the fluorescence decays, because of the compartmentalization of the hydroxide ions between the microemulsion droplets, care needs to be taken in the interpretation of pH in these systems.

**Effect of Water/Surfactant Mole Ratio on Photophysical Behavior.** Harmine was chosen as a representative β-carboline to study their spectral behavior and kinetic decays as a function of different water/surfactant ratios (w_0) and of pH. Absorption and fluorescence spectra of harmine are given in Figure 6 in microemulsions of different w_0 ratios. A change in the relative intensities of the neutral and cationic forms can be seen for different w_0 values. At w_0 = 2.5 the contributions of both species are approximately equal. However, a significant decrease in the contribution of the neutral form is observed with increasing w_0. This is followed by a corresponding increase in the absorbance of the cationic species (Figure 6a). The same is clearly observed in the emission spectra at 367 and 410 nm for the neutral and cation, respectively (Figure 6b). The
excitation spectra also confirm these observations. These results are in agreement with the effect of increasing volume fraction of microemulsion droplets on the predicted partitioning of harmine (and the other β-carbolines studied) between the organic and microemulsion droplet subphases. The kinetic behavior of harmine in the excited state was studied in the microemulsion system. The decay of its emission was studied following excitation of its solution (λ = 337 nm, [AOT] = 0.1 M, w₀ = 12.5) at various pH values. Emission was studied at the wavelengths 350, 410, and 500 nm, where the dominant excited species are N*, C*, and Z*, respectively. Data were analysed in terms of mono-, bi-, and triexponential decays to get the best possible statistical fit and are presented in Table 2. From the analysis, there appear to be four kinetically distinguishable components, whose lifetimes are independent of observation wavelength. These are assigned, on the basis of spectral studies and lifetimes in pure solvents,18,30 to the neutral species in the microemulsion droplets (τ₁), neutral species in cyclohexane (τ₂), cation (τ₃), and zwitterion (τ₄). A number of facts become apparent from these results. First, these four species are observed over the whole pH range studied, showing that there must be partitioning in the ground and/or excited state between aqueous and organic phases of some of the species. Second, in agreement with the spectral data, the cation is observed up to apparent pH values considerably in excess of the pKₐ of C in aqueous solutions. Variations of indicator pKₐ (or of pH) have previously been reported for microemulsion systems31 and have been associated with changes in the localization site. However, in the present system, using nonbuffered aqueous solution, the compartmentalization of hydroxide ions between the microemulsion droplets must also be considered. The statistical distribution of quencher molecules among micelles32,33 or microemulsion droplets34 has been treated in terms of a Poisson curve. If we assume that the water in the microemulsion droplets has a similar autoprotolysis constant to bulk water, then the distribution of hydroxide ions between the droplets can be

### Table 2: Fluorescence Decay Times (τᵢ) and Preexponential Factors (aᵢ) for Decay of Harmine Fluorescence in AOT/H₂O/Cyclohexane Microemulsions ([AOT] = 0.1 M, w₀ = 12.5) at 20 °C as a Function of pH

<table>
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<tr>
<th>pH</th>
<th>λ[nm]</th>
<th>τ₁/ns</th>
<th>τ₂/ns</th>
<th>τ₃/ns</th>
<th>τ₄/ns</th>
<th>a₁</th>
<th>a₂</th>
<th>a₃</th>
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<th>χ²</th>
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<td>5.5</td>
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<td>1.13</td>
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*a The separation of the Preexponential factors was not possible, but a₁ + a₂ = 1.0.
TABLE 3: Calculated “Quenching” Rates for the Decay of Harmine Excited Neutral Species in Cyclohexane in AOT/H$_2$O/Cyclohexane ([AOT] = 0.1 M) Microemulsions at Different $\omega_0$ Values

<table>
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<tr>
<th>$\omega_0$</th>
<th>$N^a$</th>
<th>$r_p$/nm$^b$</th>
<th>$k_q \times 10^9$/dm$^3$ (mol of micelle)$^{-1}$s$^{-1}$</th>
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<td>2.5</td>
<td>31.5$^c$</td>
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<td>10</td>
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<td>12.5</td>
<td>145.5$^d$</td>
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<td>15</td>
<td>175</td>
<td>3.14</td>
<td>16.64</td>
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</tbody>
</table>

$^a$ Aggregation number for microemulsions, using data from ref 42.
$^b$ Microemulsion radius from ref 42.
$^c$ By interpolation.
$^d$ From ref 43 for microemulsions in decane.

Figure 7. Poisson distribution of number of hydroxide ions per microemulsion droplet as a function of pH for the system AOT (0.1 M), water ($w_0 = 12.5$), cyclohexane. (a) $i = 0$, (b) $i = 1$, (c) $i = 2$, (d) $i = 3$, (e) $i = 4$. The fraction of microemulsion droplets (α) containing $i$ molecules of OH$^-$ is given by:

$$\alpha = [M_i]/[M] = n_{OH^{-}}^i \exp(-n_{OH^{-}})/i!$$

where $[M_i]$ is the concentration of droplets containing $i$ molecules of hydroxide, $[M]$ is the total concentration of droplets (given by [AOT]/$N$, where $N$ is the aggregation number), and $n_{OH^{-}}$ is the average number of OH$^-$ ions per droplet. For [AOT] = 0.1 M, $w_0 = 12.5$, and taking an aggregation number of 145.5 (Table 3), values of α have been calculated for occupation of droplets by between 0 and 4 hydroxide ions and are shown in Figure 7. The pH is that of the solution added. It is clear that, up to pH 11, the number of droplets containing hydroxide ions is very small. Further, for nonbuffered solutions, an overall pH of the microemulsion system does not have any clear meaning. For these solutions, assuming a droplet water radius of 2.84 nm (Table 3), the pH values for droplet with 1, 2, 3, and 4 hydroxide ions are 12.2, 12.5, 12.7, and 12.85, respectively. Thus, the observed effects of pH on the spectra and lifetimes and the observation of C at apparent pH values for microemulsions in decane. However, its value is greater than that of the neutral species in pure water, which has a value of 0.48 ± 0.03 ns over the pH range 7–12.30 It is suggested, therefore, that t1 corresponds to decay of the neutral species at the microemulsion interface. This result is in agreement with the spectral studies and follows from the low solubility of this species in water in the ground state. The longer lifetime compared with that of pure water is probably a result of differences in the structure of water at the droplet interface compared with bulk water, as has previously been shown by NMR,37 FTIR,38 and fluorescence probe39 studies. While the pH effects must be considered in terms of the distribution of OH$^-$ ions between droplets, the decrease in lifetime on decreasing pH may suggest that protonation of the neutral species at the interface is a significant reaction. Indicator studies in progress40 strongly suggest that there is a pH gradient both in these droplets and in AOT/water liquid crystals, possibly due to double-layer effects. From these results, even for systems whose bulk pH is close to neutrality, the pH at the interface may be considerably lower.

At 500 nm, where the neutral species in water does not emit, a similar kinetic component to that at 350 nm is observed as a rise time, whose amplitude, which is negative (as this corresponds to a growing-in of emission), also increases with increasing pH. The only probable candidates for this growing-in are Z$^+$ or C$^*$. However, it is hard to see why the yield of C$^*$ should increase with increasing pH, and it is tempting to assign this process to the interconversion of N$^*$ in this phase to Z$^+$. While this may not be the only possible assignment, some support for this comes from the effect of pH on the preexponential amplitude of the zwitterion (α2), as will be discussed in the next section.

Within experimental error, the lifetime of the neutral species in the cyclohexane phase (t2) is independent of the pH of the microemulsion droplets. However, its value (t2 = 3.2 ± 0.2 ns) is shorter than that in the pure solvent. A possible explanation will be presented after consideration of the effect of $w_0$ on the photophysics of harmine in these systems. It is noted, in addition, that the amplitude of this component (α2) shows a significant increase with increasing pH.

The lifetime of the zwitterion, t3, is intermediate between the values for pure water (14.1 ns39) and for methanol (28 ns40), and it is suggested that this might correspond to this species being localized at the interface. Comparison of the lifetimes of N$^*$ and Z$^+$ (t1 and t4) with values for various dioxane–water mixtures41 suggests that these species are in a region with polarity close to that of 20% dioxane–water, compatible with harmine being predominantly in the zone of the AOT headgroup.

If the interpretation of the pH behavior of the neutral species N$^*$ in the microemulsions is correct, and this correspond to interconversion of this species to the cation and zwitterion, the
The decay of excited harmine was studied at constant pH (5.5) in the microemulsions over the range 2.5 to 5.5. The rate constant for decay of N* in water is directly proportional to the water/surfactant mole ratio in the microemulsion droplets (k_l is a function of both hydroxide ion distribution between the bulk pH, increases are observed in both τ and the amplitude a_0 for the zwitterion emission at 500 nm (which can be taken as a measure of the quantum yield for Z* formation), supporting the above general scheme.

The radius of the water pool for AOT microemulsions is 12.5 nm, which is reasonable to assume that this is some function of the total aggregation number (number of monomers of AOT per microemulsion aggregate) in cyclohexane, the second-order rate constant for this process has been calculated for the various w_0 values (Table 3). The rates calculated for this process are high and are likely to be dominated by diffusion. For diffusion-controlled process, the rate constant is given by the Smoluchowski equation, k_diff = 4πσδDN, where σ is the particle radius and D is the diffusion coefficient. If reaction 5 is diffusion controlled, a plot of k_2 against the microemulsion droplet radius should be linear.

Since the lifetime of the excited neutral species in microemulsion droplets is less than that in cyclohexane, this process becomes rate determining. Using literature data for AOT microemulsion aggregation numbers (number of monomers of AOT per microemulsion aggregate) in cyclohexane, the second-order rate constant for this process has been calculated for the various w_0 values (Table 3). The rates calculated for this process are high and are likely to be dominated by diffusion. For diffusion-controlled process, the rate constant is given by the Smoluchowski equation, k_diff = 4πσδDN, where σ is the particle radius and D is the diffusion coefficient. If reaction 5 is diffusion controlled, a plot of k_2 against the microemulsion droplet radius should be linear. Using data from Table 3, a reasonable linear relationship is observed (Figure 8). Further, from the slope of this graph it is possible to estimate a diffusion coefficient D = 1.5 × 10^-4 cm^2 s^-1 for cyclohexane solution. Diffusion coefficients can also be calculated from solvent viscosity (η) using the Stokes–Einstein relationship, D = kT/6πησD. Because harmine is much smaller than the microemulsion droplets, we can assume, as a first approximation, that this is the main diffusing species. Using a typical value of k_0 = 5 Å, gives a diffusion coefficient 4.2 × 10^-4 cm^2 s^-1 for cyclohexane solutions at 20 °C. Given the somewhat drastic approximations involved in these calculations, the order of magnitude agreement is encouraging and supports the basic suggestion that the decrease in the lifetime of the neutral species in the cyclohexane phase with increasing w_0 is associated with diffusion of N^* of the microemulsion droplets.

**Partitioning of Harmine between Cyclohexane and Microemulsion Droplets.** Considering the preexponential amplitudes of the various components in Table 4, the values

### Table 4: Fluorescence Decay Times (τ) and Preexponential Factors (a_0) for Decay of Harmine Fluorescence in AOT/H_2O/Cyclohexane Microemulsions ([AOT] = 0.1 M, pH = 5.5) at 20 °C as a Function of w_0

<table>
<thead>
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<th>w_0</th>
<th>a_0 λ_{cm}/nm</th>
<th>τ_1/ns</th>
<th>τ_2/ns</th>
<th>τ_3/ns</th>
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<td>0.91</td>
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The lifetimes of the neutral species in the microemulsion droplets (τ_1 = 1.45 ± 0.14 ns), the cation (τ_2 = 6.1 ± 0.2 ns), and the zwitterion (τ_3 = 20.2 ± 3.1 ns) appear to be effectively independent of w_0. However, the lifetime of the neutral species in cyclohexane decreases with increasing w_0. Further, up to w_0 = 12.5, 1/τ_3 is a linear function of w_0 (correlation coefficient r = 0.996). Extrapolation to w_0 = 0 gives a lifetime (4.75 ns) which is close to that of the neutral species in pure cyclohexane. A possible explanation is that the neutral species in cyclohexane is effectively quenched by the microemulsion droplet in the process N^* + μE → N^*E.
Figure 8. Plot of quenching of excited neutral form of harmine in cyclohexane phase as a function of radius of microemulsion droplets.

Figure 9. Plot of amplitudes of neutral form of excited harmine in microemulsion and cyclohexane phases as a function of their volume ratio.

obtained at 350 nm for $a_1$ are seen to increase and for $a_2$ to decrease on increasing $w_0$. A likely explanation is that we are getting partitioning of the neutral species between cyclohexane and the microemulsion droplets. If this is due to partitioning in the ground state (i.e., before excitation), the amplitudes should be proportional to the product of the number of the molecules in each phase ($n_i$) and the extinction coefficient ($c_i$) in that phase at the excitation wavelength ($a_i = n_i c_i$). We can define a distribution coefficient

$$K = \frac{[Hi]_{Cx}}{[Hi]_{E}} = \frac{n_{Cx}}{n_{E}} \frac{q V}{(1 - q)V}$$

where $n_{Cx}$ and $n_{E}$ are the number of molecules in each phase, $q$ is the volume fraction of the microemulsion droplets, and $V$ is the molar volume of the solution. We can thus suggest that a plot of the relationship of the amplitudes in microemulsion droplet and bulk cyclohexane phases against the volume fraction ratio should be linear. Although uncertainties are inherent in the values of $a_1$ and $a_2$ as a result of the separation of multieponential decays, the experimental data can be fitted to straight line (Figure 9). From the slope and the values of the extinction coefficients at the excitation wavelength of 337 nm ($\epsilon_{Cx} = 1.07 \times 10^4$ M$^{-1}$ cm$^{-1}$, $\epsilon_{E}$(water) = $5.05 \times 10^3$ M$^{-1}$ cm$^{-1}$, $\epsilon_{E}$(cyclohexane) = $1.82 \times 10^3$ M$^{-1}$ cm$^{-1}$), a value of $K = 0.12 \pm 0.02$ was estimated. Even considering the approximations involved in this analysis, the results clearly show a partition coefficient much lower than that between octanol and water under similar conditions and show a higher concentration in the microemulsion droplet than expected just from partitioning between cyclohexane and water. The results support a model in which the neutral form of harmine partitions between cyclohexane and the AOT/water interface, while the cation is predominantly in the aqueous phase.

Effect of Polarity on Acid–Base Behavior in Microemulsions. From the above results, a general picture emerges, where the neutral $\beta$-carbolines in their ground states are distributed between the cyclohexane phase, and the interfacial region between surfactant and water, while the cation is predominantly in a water-rich region. Upon excitation, changes in the distribution of the neutral species may be occurring, due to differences in electron density, and the results suggest that partitioning can occur during the lifetimes of the excited singlet states. While excited state partitioning has been observed for singlet oxygen in microemulsion systems, we are not aware of other examples of partitioning of excited species with such short lifetimes as these. Both distribution studies on octanol–water systems and photophysical studies in the microemulsions suggest that there is partitioning between cation in the aqueous subphase and neutral and/or zwitterion in the interfacial region. Further, addition of methanol to the microemulsion system, which from both octanol–water partition coefficients and extrapolation of partitioning data for short chain length alcohols between water and phospholipid membranes is expected to be predominantly in the aqueous phase, will be expected to reduce the polarity of the water pool and push the above
equilibria in the direction of neutral/zwitterion. As can be seen in Figure 10, this prediction is verified experimentally from changes in both absorption and fluorescence spectra observed upon addition of methanol to the system AOT/water/cyclohexane ($[\text{AOT}] = 0.1 \text{ M}, \text{pH} = 5.5$). Good isosbestic points are observed for changes in both absorption and fluorescence spectra, suggesting that we are just seeing the effect of changing polarity on a simple equilibria between C and NZ in both ground and excited states.

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References and Notes

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