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Ground and singlet excited state hydrogen-bonding interactions between 1-azacarbazole and amides

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Abstract

In cyclohexane as the solvent, 1-azacarbazole (or α-carboline) (AC) forms fluorescent ground state 1:1 hydrogen-bonded complexes with *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMA) and hexamethylphosphoramide (HMPA). The absorption and fluorescence spectra of the complexes are red shifted with respect to those of the non-bonded AC, and their association constants increase as the hydrogen-bonding acceptor properties of the amides increase. Fluorescence of the AC–DMF and AC–DMA solutions show monoexponential or biexponential decays depending on the monitored emission wavelength. To aid the interpretation of these results, we have also studied the effect that triethylamine and methylethylketone addition produces on the absorption and fluorescence spectra of AC. Triethylamine does not significantly affect the absorption spectrum of AC, but it dynamically quenches its fluorescence. Conversely, methylethylketone behaves similarly as amides do. On the basis of the above results, we assume that, in the ground state, the hydrogen-bonding interaction takes place between the pyrrolic NH group of AC and the carbonyl group of the amide. Hydrogen bonded complexes and non-bonded AC behave in the singlet excited state as independent fluorophores. Singlet excited state of free AC is dynamically quenched by DMF and DMA. The quenching mechanism involves the hydrogen-bonding interaction of the pyrrolic NH group of AC and the lone electron pair of the amide nitrogen atom. For HMPA, probably due to geometrical restrictions, this quenching process is absent.

Keywords: 1-Azacarbazole; Amides; Hydrogen-bonding; Ground state; Excited state

1. Introduction

1-Azacarbazole (AC) or α-carboline is a representative member of the carboline family, a class of drug-binding alkaloids possessing interesting photophysical and biological properties [1]. Because of its structural relation with 7-azaindole (AI), a molecule whose dimer has long been recognized as a model for the DNA base pairs, the photophysics of AC has received much attention. Similarly to AI, AC forms doubly hydrogen-bonded dimers that, upon photoexcitation, undergo a concerted intermolecular excited state double proton transfer (ESDPT) reaction. Excited-state tautomerization reactions of AI and AC dimers have been intensively studied as model systems for understanding the photoinduced mutations of the DNA base pairs (for a recent review see [2]).

Owing to its bifunctional proton donor and acceptor properties, AC may form a variety of complexes with different hydrogen-bonding partners. Particularly, cyclic doubly hydrogen-bonded complexes of the sort illustrated in Fig. 1, have arisen great interest, since as the AI dimers, they are also able to undergo ESDPT reactions. While much work has been done on the photophysics of the cyclic complexes [3–12], single non-cyclic 1:1 hydrogen-bonded complexes involving only one of the hydrogen-bonding centers of the AC ring have received very scarce attention. However, the study of the nature of the last complexes and the dynamics of their hydrogen-bonded mediated proton transfer reactions is the key for the full understanding of the photophysics and photochemistry of AC. Thus, it would be of interest to study the photophysics of these hydrogen-bonded complexes of AC.

In this paper, we report a spectroscopic study (UV–visible, steady-state and time-resolved fluorescence) of the ground and singlet excited state hydrogen-bonding interactions of AC with different amides; namely, *N*,*N*-dimethylformamide (DMF), *N*,*N*-dimethylacetamide (DMA) and hexamethylphosphoramide (HMPA). These amides possess only proton accepting groups, hence they can only form hydrogen-bonds with the pyrrolic NH site of the AC. These studies have been conducted in cyclohexane, a solvent that due to its low

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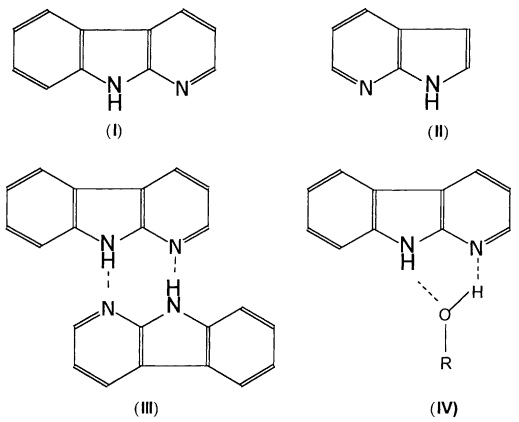


Fig. 1. Structural formulae of AC (I), 1-AI (II), the AC dimer (III) and the doubly hydrogen-bonded cyclic complexes of AC.

polarity and its negligible hydrogen-bonding properties can be considered inert.

2. Experimental

AC was synthesized and purified as described in the literature [13]. The hydrogen-bonding acceptors DMF, DMA, and HMPA, triethylamine, methylethylketone and the cyclohexane used as the solvent were commercial products (Sigma, Aldrich) of the best available quality, they were used without further purification and were stored on 4 Å molecular sieves.

The UV–visible absorption spectra were recorded on a Perkin-Elmer Lambda-5 spectrophotometer. Stationary fluorescence measurements were carried out in a Hitachi F-2500 spectrofluorimeter. Fluorescence lifetimes were measured with an Edinburgh Analytical Instruments CD-900 spectrofluorimeter employing the time correlated single photon counting technique. The source was a nanosecond flashlamp filled with H_2 (0.4 bar) operating at 6.8 kV with a repetition rate of 40.0 kHz. Fluorescence decays were acquired to 10^4 counts in the peak. The decays were repeated at least twice and were fitted by reference deconvolution to a sum of exponentials

$$I(t) = \sum A_i \exp\left(\frac{-t}{\tau_i}\right) \tag{1}$$

with amplitudes, A_i, and lifetimes, τ_i. Decay curves were both individually and globally analyzed by using single, double and triple exponentials. Goodness of the individual fits was judged by the magnitude of χ_r^2 and the shape of the autocorrelation function of the weighted residuals. The analysis of the lifetime data at different acceptor concentrations were performed with a global analysis program based on the Marquardt algorithm. These results were judged by the statistical fitting parameter χ_g^2. Fluorescence measurements were carried out with nondegassed solutions under temperature controlled conditions ($25 \pm 0.1\,^{\circ}C$).

3. Results and discussion

As stated in Section 1, AC is prone to dimerize in low polar solvents. To check this possibility, we previously studied the concentration dependent absorption and fluorescence spectra of AC in cyclohexane. In the AC concentration range used in this work, up to 10^{-4} M, we did not observe changes in the absorption spectrum attributable to dimer formation and the absorbances fitted acceptably the Lambert–Beer law at different absorption wavelengths. Neither the fluorescence spectrum of AC showed indication of dimer formation. The dimer would be clearly detected by the emission of its photoinduced tautomer at 500 nm, which was absent in the fluorescence spectra of AC in cyclohexane under our experimental conditions.

Figs. 2 and 3 show the changes produced in the UV–visible and fluorescence spectra of AC in cyclohexane upon adding HMPA. Similar changes are observed for DMA and DMF. As this figure shows, the UV–visible absorption spectrum of AC is shifted to the red as the amide concentration is increased. The presence of isosbestic points in these spectra indicated the formation of AC–amide stoichiometric complexes. Owing to the nature of the interacting substrates we will assume that these complexes are stabilized by hydrogen-bonding interactions involving the pyrrolic NH group of the AC ring. Unfortunately, the small magnitude of the absorbance changes precluded the determination of the association constants of the complexes from the absorption spectra.

As it is typically shown in Fig. 3 for the AC–HMPA system, the fluorescence spectra of the hydrogen bonded complexes of AC with DMA, DMF and HMPA are red shifted by 10–15 nm with respect to that of free AC. The fluorescence data, obtained at the maximum emission of the complexes, were analyzed using the Benesi–Hildebrand equation for 1:1 stoichiometric binding

$$\frac{1}{I - I_0} = \frac{1}{I_1 - I_0} + \frac{1}{K_G(I_1 - I_0)}\frac{1}{[\text{amide}]} \tag{2}$$

where I_0 is the initial fluorescence intensity of free AC at the titration wavelength, I_1 the fluorescence intensity of the AC–amide complex and I the observed fluorescence intensity of the corresponding AC–amide mixtures. As the inset in Fig. 3 shows, the linear dependence of $1/(I_0 - I)$ on the reciprocal of amide concentration, confirms the assumed 1:1 stoichiometry of the complexes. The slope and the intercept of these plots allowed us to calculate the values of the ground state formation constants reported in Table 1. The sequence of these association constants DMF < DMA < HMPA closely follows that of β hydrogen-bonding acceptor parameters of the amides [14]. On the other hand, the red shifts observed in the absorption and fluorescence spectra upon the formation of the complexes indicate that the hydrogen-bonding interaction is reinforced in the excited state. This is in agreement with the charge density decrease

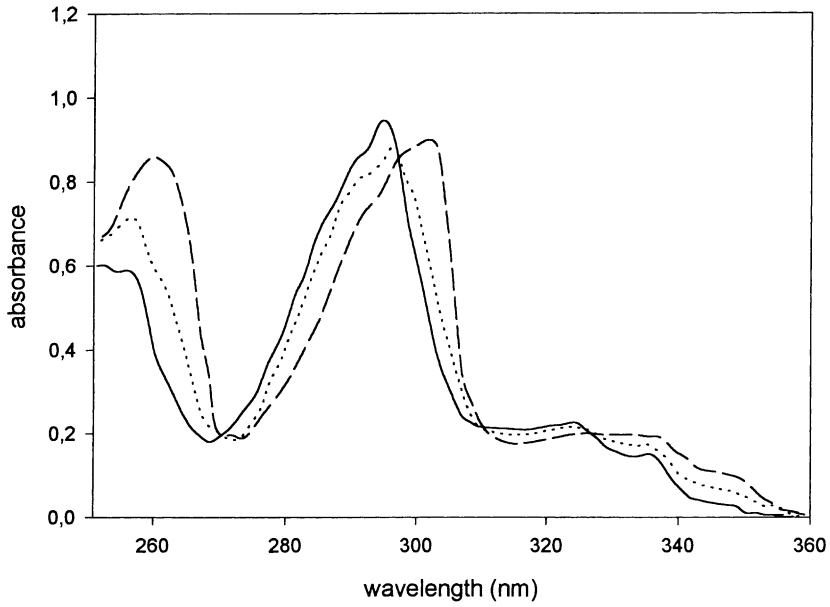


Fig. 2. Changes in the absorption spectrum of AC in cyclohexane upon the addition of increasing amounts of HMPA: (—) 0 M, (···) 1×10^{-3} M and (- - -) 9×10^{-2} M.

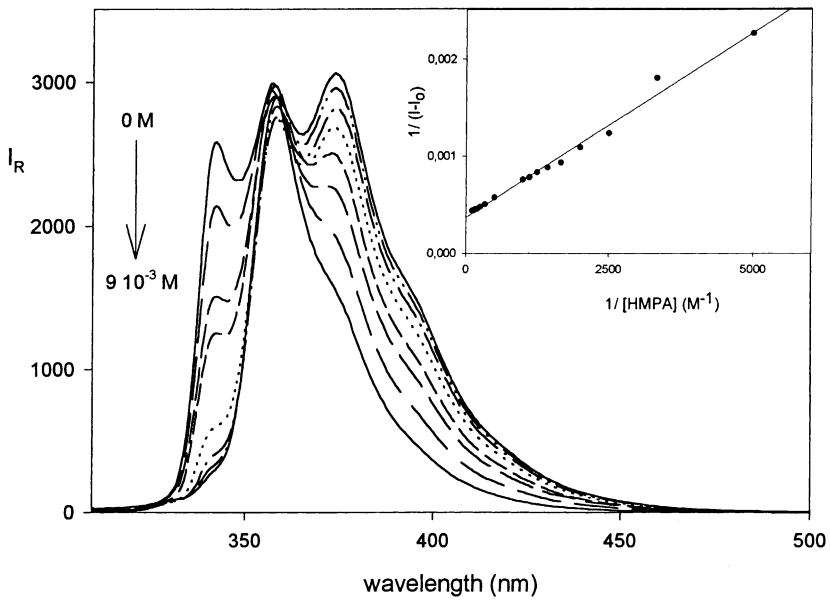


Fig. 3. Changes in the steady-state fluorescence spectrum of AC in cyclohexane upon the addition of increasing amounts of HMPA. In the inset, Benesi–Hildebrand plots of the fluorescence data according to Eq. (2).

Table 1
Ground state association constants, K_G, and dynamic quenching constants, k_q, for the hydrogen-bonding interactions of AC with amides in cyclohexane at 298 K

	β[a]	K_G (M^{-1})	k_q ($\times 10^{-9}\,M^{-1}\,s^{-1}$)
DMF	0.69	150	13 ± 1
DMA	0.76	250 ± 15	15 ± 1
HMPA	1.05	970 ± 30	–

[a] Hydrogen-bonding acceptor descriptor values according to Marcus [14].

experienced by the pyrrole nitrogen atom upon excitation of AC to its S_1 state [12].

In order to gain some insight into the dynamics of the hydrogen-bonding interaction in the singlet excited state, we carried out time-resolved fluorescence measurements of the AC–amide solutions in cyclohexane. We will discuss firstly the results obtained for the AC–DMF and AC–DMA systems, since they are somewhat different from those of the AC–HMPA system. The fluorescence decays of the AC–DMF and AC–DMA mixtures depended on the

Table 2
Analysis of the biexponential decays of the AC–DMA system in cyclohexane at 298 K ($\lambda_{exc} = 320$ nm, $\lambda_{em} = 340$ nm)

[DMA] ($\times 10^3$ M)	τ_1 (ns)[a]	τ_2 (ns)[a]
2	5.6	–
4	4.3 (0.062)	7.4 (0.021)
6	4.1 (0.063)	7.9 (0.016)
8	3.7 (0.056)	7.7 (0.018)
10	3.5 (0.052)	7.7 (0.018)
30	1.8 (0.046)	7.3 (0.033)
50	1.1 (0.036)	7.2 (0.036)

[a] The figures in the parenthesis are the preexponential factors A_i.

emission wavelength. As it is illustrated in Table 2 for the AC–DMA mixture, at 340 nm the fluorescence decays could be fairly well described by a linear combination of two exponentials

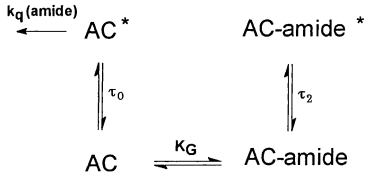
$$I(t) = A_1 \exp\left(\frac{-t}{\tau_1}\right) + A_2 \exp\left(\frac{-t}{\tau_2}\right) \quad (3)$$

where A_1 and A_2 stand for the contribution at zero time of the two components with lifetimes τ_1 and τ_2, respectively.

The shorter lifetimes, τ_1, decreased with the amide concentration, while the longer lifetime component, τ_2, was independent of the amide concentration and remained practically constant around 7.4 ns. As Fig. 4 typically shows the plots of the fluorescence rate constant k_1, $k_1 = 1/\tau_1$, against amide concentration according to equation

$$k_1 = k_0 + k_q[\text{amide}] \quad (4)$$

are linears with intercept values, k_0, very close to that of the reciprocal of the lifetime of non-bonded AC in cyclohexane, $\tau_0 = 6.4$ ns. The slopes of these plots, k_q, are reported



Scheme 1.

in Table 1. At the red end of the emission spectra, this short lifetime component disappears and the fluorescence decays could reasonably be fitted to monoexponential functions with lifetimes whose values, within the experimental error, are equal to those of τ_2.

The above results show that, in the excited state, the equilibrium between the hydrogen bonded complexes and the free AC is not established during the life span of these species. If this were the case, these species would be coupled in the excited state and, therefore, the fluorescence decays would be always biexponential, irrespective of the monitored emission wavelength. On the other hand, in the case of irreversible formation of the hydrogen-bonded complexes in the excited state, we would expect biexponential decays at the longest wavelengths and the appearance of a rising time, i.e. a negative preexponential factor. Since such behavior was not observed, it is quite unlikely that free AC is the excited state precursor of the hydrogen-bonded complexes. Therefore, we conclude that, as depicted in Scheme 1, the hydrogen-bonded complexes and the non-bonded AC behave as independent fluorophores. The short lifetime in Table 2, τ_1, corresponds to non-bonded AC species and the long lifetime, τ_2, which is independent on amide concentration, to the hydrogen bonded complex. It must be noted that similar behavior was

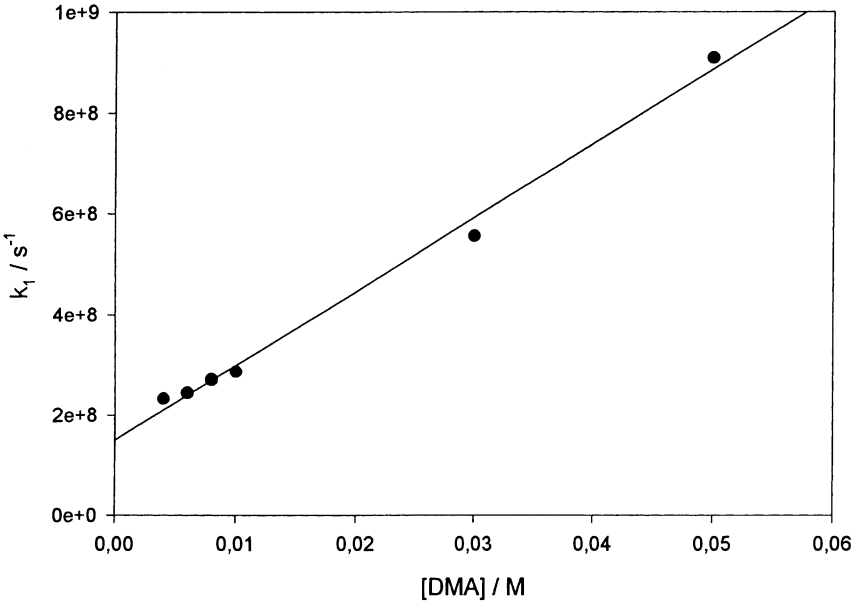


Fig. 4. Plots of the reciprocal of τ_1 lifetimes, k_1, against the DMA concentration.

previously observed in the hydrogen-bonding interaction of 2-azacarbazole (β-carboline) with amides [15].

To account for the dynamic quenching of the non-bonded AC species, we will assume that they interact in the singlet excited state with DMF and DMA differently as they do in the ground state. In this sense, it is interesting to realize that DMF and DMA molecules have two potential acceptor centers for hydrogen-bonding interactions, the oxygen atom of the carbonyl group and the lone electron pair on the nitrogen atom. Therefore, it is conceivable that AC could interact independently with each one of these centers in the amide molecule. Thus, to test this hypothesis, we have studied the interactions of AC with triethylamine and methylethylketone as model compounds.

The changes observed in the absorption and fluorescence spectra and in the fluorescence decays of AC upon the addition of methylethylketone entirely resemble those produced by the amides. Conversely, the addition of triethylamine had no significant effects on the absorption spectrum of AC, but, as Fig. 5 shows, it quenches the fluorescence intensity and decreases the fluorescence lifetime of AC. Stern–Volmer plots of the steady-state and time-resolved fluorescence data (inset of Fig. 5) reveal that the quenching has a dynamic nature; i.e. it is mainly due to the excited state interaction of AC with the amine. The Stern–Volmer plot of τ_0/τ versus triethylamine concentration gives a quenching constant k_q of $5 \times 10^9\,M^{-1}\,s^{-1}$. However, the slight upward deviation observed in the Stern–Volmer plot obtained from the fluorescence intensities could be taken as an indication of a very weak interaction between AC and triethylamine in the ground state.

At this point, it would be noted that the parent carbazole forms ground state 1:1 hydrogen bonded complexes with amines [16]. In the singlet excited state, these hydrogen-bonding interactions quench the carbazole fluorescence with an efficiency that depends on the nature of the amine and the solvent. In a low polar solvent as *n*-hexane, the quenching rate constants k_q ranged from 3×10^9 to $\sim 30 \times 10^9\,M^{-1}\,s^{-1}$. In particular, carbazole and triethylamine form a 1:1 hydrogen bonded complex whose association constant has a value of $5.5\,M^{-1}$ in *n*-hexane at 293 K. Moreover, in the singlet excited state this hydrogen-bonding interaction quenches the carbazole fluorescence with a quenching constant k_q of $6.5 \times 10^9\,M^{-1}\,s^{-1}$. This value is very close to that of $5 \times 10^9\,M^{-1}\,s^{-1}$ obtained for the quenching of AC fluorescence by triethylamine and similar to those of the k_q constants reported in Table 1 for the AC–DMF and AC–DMA systems.

On the basis of the above results, we conclude that, in the ground state, the pyrrolic NH group of AC interacts preferentially with the carbonyl groups of the amides and forms stoichiometric 1:1 hydrogen bonded complexes. These complexes are slightly more fluorescent than the free AC and their emissions are shifted to the red by 10–15 nm. Moreover, the pyrrolic NH group of non-complexed AC molecules interacts in the singlet excited state with the lone electron pair of the nitrogen atoms of the amides. This excited state hydrogen-bonding interaction efficiently quenches the fluorescence of the free AC molecules. Possibly, as in the carbazole–amine systems, this interaction involves the transfer of the pyrrolic proton to the amide and the formation of AC anions which are very weakly fluorescent [16].

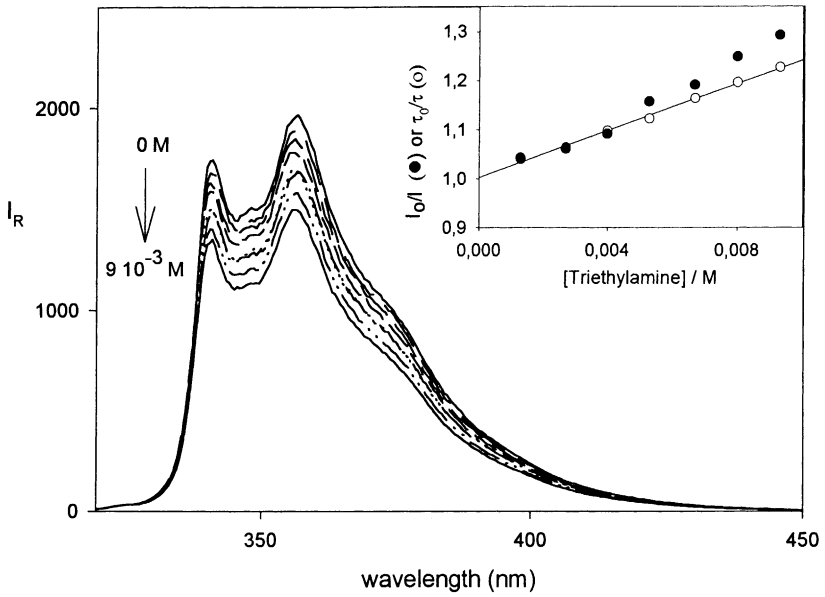


Fig. 5. Changes in the steady-state fluorescence spectrum of AC in cyclohexane upon the addition of increasing amounts of triethylamine. In the inset, Stern–Volmer plots of the fluorescence data.

Turning now to the AC–HMPA system, time-resolved fluorescence measurements showed that, independently of the emission wavelength, the fluorescence of the cyclohexane solutions of AC in the presence of HMPA decayed monoexponentially. The lifetimes of the decays were very close to that of no bonded AC, 6.4 ns, and slightly increased as the acceptor concentration increased. Double exponential analyses were also attempted, but neither χ_r^2 nor χ_g^2 improved. Furthermore, the second component of these analyses revealed at random and, occasionally, physically inexplicable negative contributions to the overall decay. We think that this conflicting result is an artifact of the fitting procedure which cannot satisfactorily resolve lifetimes whose values differ less than 1 ns [17]. Thus, the fluorescence decays of AC–HMPA solutions in cyclohexane could also be reasonably interpreted according to Scheme 1, assuming a system formed by a mixture of two independently absorbing and emitting species, the complex and the non-bonded AC, with very close fluorescence lifetime. Interestingly, HMPA, possibly due to geometrical constrains, does not appreciably quench the fluorescence of free AC.

Finally, it is interesting to note that the AC–amide hydrogen-bonded complexes have slightly larger fluorescence quantum yields, ϕ, and longer lifetimes, τ, than free AC. Therefore, because $\phi = k_r/(k_r + k_{nr})$ and $\tau = 1/(k_r + k_{nr})$, complexation apparently hinders the non-radiative process, k_{nr}. It has been reported that in the singlet manifold of the AC monomer, there is two coupled low-lying singlet excited states that are analogous to the ^1L_a and ^1L_b states of indole and other polyacenes [6,18,19]. Since the ^1L_b state is photoionizable, coupling of these states leads to an efficient non-radiative process involving the dissociation of the NH pyrrolic group. Possibly, the hydrogen-bonding interactions between the NH pyrrolic group of AC and the carbonyl group of the amides decouples the ^1L_a and ^1L_b states by stabilizing the more polar ^1L_a state.

Finally, it must be noted that hydrogen-bonding interactions of AC with alcohols and water are known to decrease the quantum yield and the fluorescence lifetime of AC [8]. Waluk et al. [8] have attributed this behavior to the enhancement of the internal conversion process caused by these hydrogen-bonding interactions. Alcohols and water are bifunctional donor–acceptor hydrogen-bonding molecules that can interact with both the pyrrolic and the pyridine nitrogen atoms of AC. Therefore, we suspect that the interaction through the pyridine nitrogen atom is responsible of such a behavior. In this case, a different mechanism of non-radiative deactivation of the lowest singlet excited state of AC could take place. In fact, Waluk et al. have also considered the possibility of a hydrogen-bonding mediated charge transfer process. We think that a systematic study of the hydrogen-bonding interactions of the pyridinic nitrogen atom of AC with selected hydrogen-bonded donors could help to clarify this question. This study is now in progress in our laboratory and it will be the subject of a forthcoming publication.

Acknowledgements

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