Kinetic Study of Hydrogen Bonded Exciplex Formation of N_9-methyl Harmane†

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Received: February 7, 2001; In Final Form: September 28, 2001

The kinetics of exciplex formation of N_9-methyl-1-methyl-9H-pyrido[3,4-b]indole, MHN, in the presence of the proton donor hexafluoro2-propanol, HFIP, in cyclohexane has been studied by UV-vis, steady-state, and time-resolved fluorescence measurements. The results conclusively show the formation of a 1:2 ground-state proton-transfer hydrogen bonded complex, PTC, between the pyridinic nitrogen of the substrate and the proton donor. The formation of these complexes is a necessary prerequisite for the exciplex to be observed. Thus, upon excitation of PTC, an excited-state equilibrium is established between PTC* and a cation like exciplex, CL*, λ_{em}. 410 nm. This excited-state reaction is assisted by a proton donor molecule. From the analysis of the multiexponential decays, measured at different emission wavelengths and as a function of HFIP concentration, the excited-state kinetics of this phototautomeric process has been analyzed in detail.

Introduction

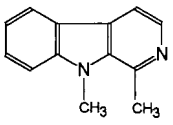
The dynamics of intermolecular excited-state proton transfer in hydrogen bonded systems has been attracting considerable attention due to its complexity and its great interest in the photochemistry field.[1] In solution, a change of solute dipole moment induced by electronic excitation initiates a complex process: Hydrogen bonds can be affected where proton transfer often happens and/or where the hydrogen bonds may be broken. Therefore, for a deeper understanding of the dynamics of these processes it is necessary to know not only the nature of the ground-state precursors, but also the molecular mechanism of the excited-state relaxation induced by intermolecular hydrogen bond formation.

One of the most challenging problems in this field is the study of excited state proton transfer reactions in molecules possessing both proton acceptor and donor sites. When either the acidic or the basic moieties of the same molecule become stronger acids or bases in the excited state, proton transfer may occur rapidly to form exciplexes. Thus, the dynamics of these excited state reactions is usually complicated by the fact that multiple equilibria and different species can appear.

Among the great variety of substrates possessing potential hydrogen bond donor/acceptor sites, the beta-carboline ring, 9H-pyrido[3,4-b]indole, BC, has been widely studied.[2−11] These rings are the structural units of numerous naturally ocurring alkaloids, which possess a wide range of biological and pharmacological properties.[12] Also, some of them have been proposed as fluorescence standards,[13,14] their fluorescence activity being highly sensitive to the solvent.[15,16] In these molecules, upon excitation by light absorption, the charge density on the nitrogen atoms changes considerably, the pyridinic nitrogen becoming more basic and the pyrrolic nitrogen more acidic in the first singlet excited state than in the ground state.[2] As a consequence, the prototropic equilibria of these molecules are considerably modified in the first singlet excited-state giving rise to the formation of prototropic species under the appropriate conditions.

Depending on the media, the simultaneous presence of different species can be observed. As an example, for beta-carboline in benzene, only the neutral species emit, N*, λ_{em}. 360 nm, whereas N*, cation, C*, λ_{em}. 450 nm, and zwitterion, Z*, λ_{em}. 500 nm, have been observed in pure methanol by Dias et al.[6] Also, for BC in dichloromethane-acetic acid mixtures, Reyman et al.[4] observed the emission of N*, C*, Z*, and a novel species, which they call a phototautomer, P*, λ_{em}. 400 nm. Thus, the simultaneous presence of several species, which in principle can be formed from the same or different precursors, makes it difficult to separate the different equilibria. This is the main reason there is not a complete description of the dynamics of these processes and even controversy between different authors.[17,18]

To broach the study of the mechanism of these excited-state proton-transfer reactions, we have selected as the substrate N_9-methyl-1-methyl-9H-pyrido[3,4-b]indole, MHN. In this molecule, the pyrrolic nitrogen is blocked, and therefore, the system is simplified because zwitterions cannot be formed.



MHN

Previous spectroscopic studies on the interactions of MHN with trifluoroethanol in cyclohexane, carried out in our laboratory,[8] provided evidences for the formation, through the pyridine nitrogen, of two different ground state hydrogen bonded complexes, at low and high trifluoroethanol concentrations, respectively. The results were interpreted by assuming an equilibrium between a 1:1 hydrogen bonded complex, HBC, and its 1:2 proton transfer complex, PTC, formed through the pyridinic nitrogen atom. The 1:2 stoichiometry assumed for the PTC indicates the specific solvation of the oxygen atom of the HBC by a second trifluoroethanol molecule. Upon excitation, only HBC* and PTC* emissions were observed.

Recently, in the study of the interaction of MHN with hexafluoro2-propanol, HFIP, in cyclohexane,[19] we detected the

† This work was presented at the PP2000 in Costa do Estoril, Portugal, honoring Professor Ralph Becker's contributions.

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10.1021/jp0104942 CCC: $20.00
Published on Web 10/24/2001

appearance of a new species not observed in the MHN-trifluoroethanol system.[8] This species, created during the lifetime of the first singlet excited state, appeared in a high concentration range of HFIP, that is, once the PTC complex had been formed. It showed a fluorescence emission band at λ_{em}. 410 nm similar to that reported by Reyman et al. for the phototautomer P*. Thus, although we agree with these authors on this species being a hydrogen bonded complex, we differ with them in its nature. According to these previous results, this complex should have an important extent of the proton shift along the pyridinic hydrogen bond. This conclusion is consistent with the red shift of its emission band, with respect to that of the PTC complex, and with the long lifetime estimated for this species, around 15 ns. Therefore, hereafter, we will call it a cation like species, CL*.

Within our interest to separate the different excited-state reactions, the main goal of this work is to perform a detailed study on the mechanism of the formation of the CL* exciplex and to discern without ambiguity the nature of its precursor. To this end, we have carried out steady state and time-resolved fluorescence measurements for the MHN−HFIP system in cyclohexane at different HFIP concentrations and different emission wavelengths.

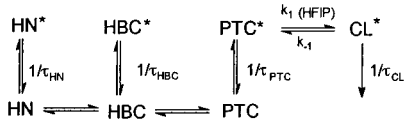
Experimental Section

MHN, was prepared as described elsewhere.[20] The complexing agent, HFIP, and the solvent, spectral grade cyclohexane, were stored on 4 Å molecular sieves. Absorption and fluorescence spectra of the reagents and the solvents did not show indication of impurities.

The UV−vis absorption spectra were recorded on a Perkin-Elmer Lambda-5 spectrophotometer. The spectra were recorded at room temperature using a 1 cm path length cell.

Stationary fluorescence measurements were carried out in a Hitachi F-2500 fluorescence spectrofluorometer interfaced to a PC for the recording and handling of the spectra. The excitation spectra were recorded in a Perkin-Elmer spectrofluorometer 650−40 equipped with a data processor 650−0178. The spectra were corrected by measuring the instrumental response on excitation side (rhodamine B) and on emission side (cell diffuser). Fluorescence lifetimes were measured with an Edinburgh Analytical Instruments FL900CD spectrometer employing the time correlated single photon counting technique.[21] The decay curves with $(1-2) \times 10^4$ counts at the maximum were deconvoluted and the quality of the fits analyzed by the randomness of the residuals and the reduced χ^2_r (<1.2). Each decay has been repeated at least twice to get a mean lifetime value. The standard deviations were always less than ±0.2 ns. When necessary, global analyses of the decay curves were performed using the standard program Level 2 supplied by Edinburgh Analytical Instruments based on the tried and tested Marquardt−Levenberg algorithm. Dilute solutions of MHN (-10^{-5} M) were used to avoid inner filter effects and reabsorption phenomena. We have also checked the effect of molecular oxygen on lifetime measurements. In principle, it could be expected an efficient oxygen quenching on the long lifetime component of the decays. However, the differences in lifetime values, obtained in aerated solutions and after bubling N_2, were within the experimental errors. Thus, the measurements has been carried out in aerated solutions under temperature controlled conditions (25 ± 0.1 °C). This unusual behavior has already been observed for cationic derivatives of betacarbolines[13−15] and other aromatic compounds.[22] In fact, an explanation for the inefficiency of oxygen quenching on acridinium ions, can be found in the literature.[22]

SCHEME 1



Results and Discussion

In a previous work,[19] we showed that, upon the addition of HFIP in a low concentration range, around 10^{-4} M, MHN in cyclohexane forms a ground-state HBC complex. From time-resolved fluorescence experiments, we found that free MHN and HBC behave as independent fluorophores with lifetimes of 2.1 and 3.7 ns for free MHN and HBC, respectively. These results are consistent with values of 1.99 and 3.3 ns obtained for MHN and HBC with the proton donor trifluoroethanol and with the 2.15 ns lifetime measured for free MHN in pure cyclohexane. On increasing proton donor concentration, a second ground-state complex, PTC, was observed. The photoinduced reaction of this second complex seems, as we highlighted in that paper, to produce a novel species, CL*, which only appears in the highest concentration range, $10^{-3}-10^{-2}$ M, of HFIP. This species, was not observed in the MHN-trifluoroethanol system in cyclohexane.[8] We now describe results obtained from the study of this species and discuss the kinetics and mechanism of its formation.

As it will be shown later, the excited state formation of the CL* exciplex can be interpreted according to the general kinetics scheme shown in Scheme 1. For the sake of clarity, the scheme corresponding to the low concentration range, where the ground-state hydrogen bonded complexes, HBC and PTC, have already been formed, is also included.

In this Scheme, τ_{HN}, τ_{HBC}, and τ_{PTC} are the fluorescence lifetimes of the free and of the two hydrogen bonded complexes of the substrate MHN; τ_{CL} is the lifetime of the CL* exciplex, k_1 is the bimolecular rate constants for the PTC* interaction with HFIP to give CL* and k_{-1} is the unimolecular rate constant for the back reaction of CL*.

The time evolution of the excited-state concentrations of PTC* and CL* upon pulse excitation are defined by a well-known set of differential equations[6] with the general solutions

$$[\mathrm{PTC}^*] = \sum n_i e^{-\lambda_i t} \; (i = 1,2) \tag{1}$$

$$[\mathrm{CL}^*] = \sum m_i e^{-\lambda_i t} \; (i = 1,2) \tag{2}$$

where the reciprocal lifetimes are

$$\lambda_{1,2} = \frac{(A+B) \mp \sqrt{(A+B)^2 + 4k_1 k_{-1}[\mathrm{HFIP}]}}{2} \tag{3}$$

with

$$A = k_1[\mathrm{HFIP}] + 1/\tau_{\mathrm{PTC}} \; B = k_{-1} + 1/\tau_{\mathrm{CL}} \tag{4}$$

According to the kinetics model in Scheme 1, for PTC* and CL*, the decays should be biexponential. Furthermore, taking into account the boundary conditions for CL*, preexponential factors of the same magnitude but different sign should be observed for this species.

Figure 1 shows the changes in the emission spectra of MHN in cyclohexane upon addition of HFIP in the high concentration

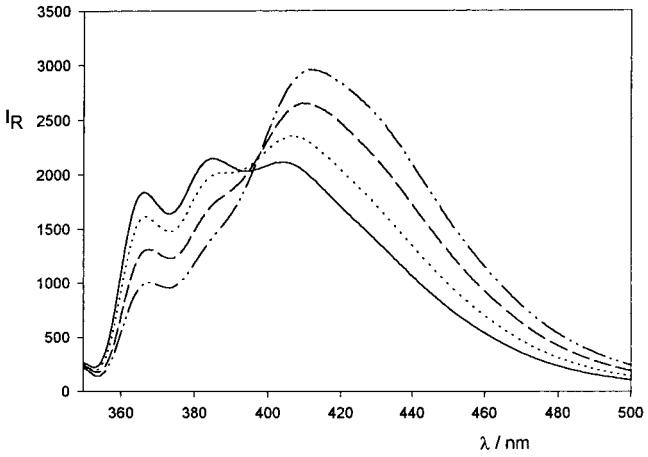


Figure 1. Emission spectra of MHN−HFIP mixtures in cyclohexane, $\lambda_{exc} = 347$ nm, [HFIP] from 9 10^{-3} (−) to 5 10^{-2} (−•••−) M.

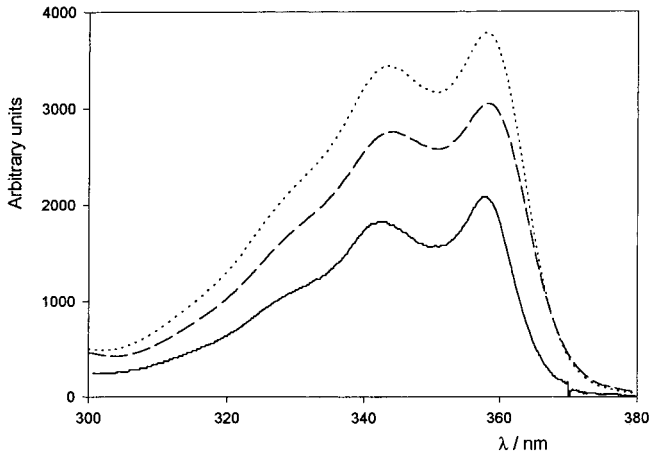


Figure 2. Absorption (−) and excitation, at λ_{em}= 390 nm (••••) and 410 nm (− −) nm, spectra of MHN−HFIP mixtures in cyclohexane, [HFIP] = 0.01 M.

range of the proton donor. As can be seen in Figure 1, the intensity of the band corresponding to the emission of the PTC* species, λ_{em} around 365 and 380 nm, decreases on increasing donor concentration. Simultaneously, a new band, λ_{em} around 410 nm, is observed. The intensity of this band increases on increasing HFIP concentration. At this point, it is worth pointing out that this behavior is not unique to this particular system. Thus, completely similar changes in the emission spectra are observed for MHN−HFIP in toluene and for MHN in dichloromethane upon the addition of acetic acid.

It has been also found that under the experimental conditions used, no changes are observed in the absorption spectra. The corrected excitation spectra have been recorded at 390 and 410 nm emission wavelengths, where PTC* and CL* mainly fluoresce, and compared with the absorption spectra. As it is typically shown in Figure 2, no appreciable changes are observed among these spectra.

Fluorescence decays of the MHN−HFIP system in cyclohexane have been recorded at two different wavelengths, 380 and 440 nm, λ_{exc}= 340 nm, and different HFIP concentrations. These emission wavelengths have been selected in order to minimize the overlap of the emission spectra of the precursor and the exciplex, together with the condition of having enough intensity for the measurements to be carried out efficiently. For both wavelengths, the decays were biexponential with a long lifetime component increasing and a short lifetime component decreasing on increasing HFIP concentration. Moreover, at the

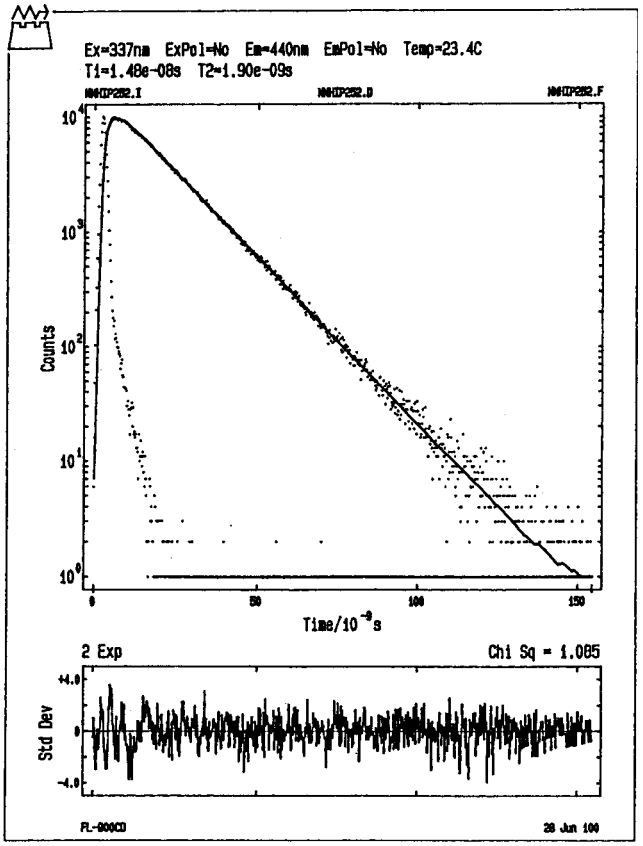


Figure 3. Fluorescence decay of MHN in cyclohexane, weighted residuals and autocorrelation function for double exponential analysis of the decay. $\lambda_{em} = 440$ nm, $\lambda_{exc} = 340$ nm and [HFIP] = 0.05 M.

TABLE 1: Fluorescence Lifetimes and Preexponential Factors, into Brackets, at 440 nm Emission Wavelength and Different HFIP Concentrations for MHN in Cyclohexane, λ_{exc}= 340 nm

10^2 [HFIP]/M	τ_1/ns	τ_2/ns	χ^2_r
2.0	14.15 (0.121)	2.82 (−0.043)	1.262
3.0	14.31 (0.117)	2.53 (−0.062)	1.222
4.0	14.47 (0.116)	1.98 (−0.081)	1.150
5.0	14.78 (0.151)	1.89 (−0.084)	1.168
6.0	14.91 (0.144)	1.66 (−0.083)	1.180
7.0	15.05 (0.137)	1.44 (−0.085)	1.157

longest wavelength, the short component appears as a rise time, i.e., with a negative preexponential. Figure 3 shows a typical fluorescence decay of MHN in cyclohexane at $\lambda_{em} = 440$ nm. The lifetimes measured at different HFIP concentrations and 440 nm emission wavelength are recorded in Table 1. It will be shown below that the results obtained at 380 nm are the same, within experimental error, and since they give the same kinetics constants, they have not been included in this Table.

According to the mechanism in Scheme 1, the preexponential factors should have, as observed, different sign, but also similar magnitude. The differences in their magnitudes, shown in Table 1, are due to the strong overlap of PTC* and CL* emission bands. In fact, before selecting 440 nm as the wavelength to carry out the studies, other wavelengths were tested. Negative preexponential factors begin to appear around 420 nm when the percent of contribution of CL* to the total emission light is higher than 90%. As the emission wavelength is increased, the preexponential factors become more and more similar. Unfortunately, the small intensity of the emission from the samples at the longest wavelengths precludes the acquisition of precise

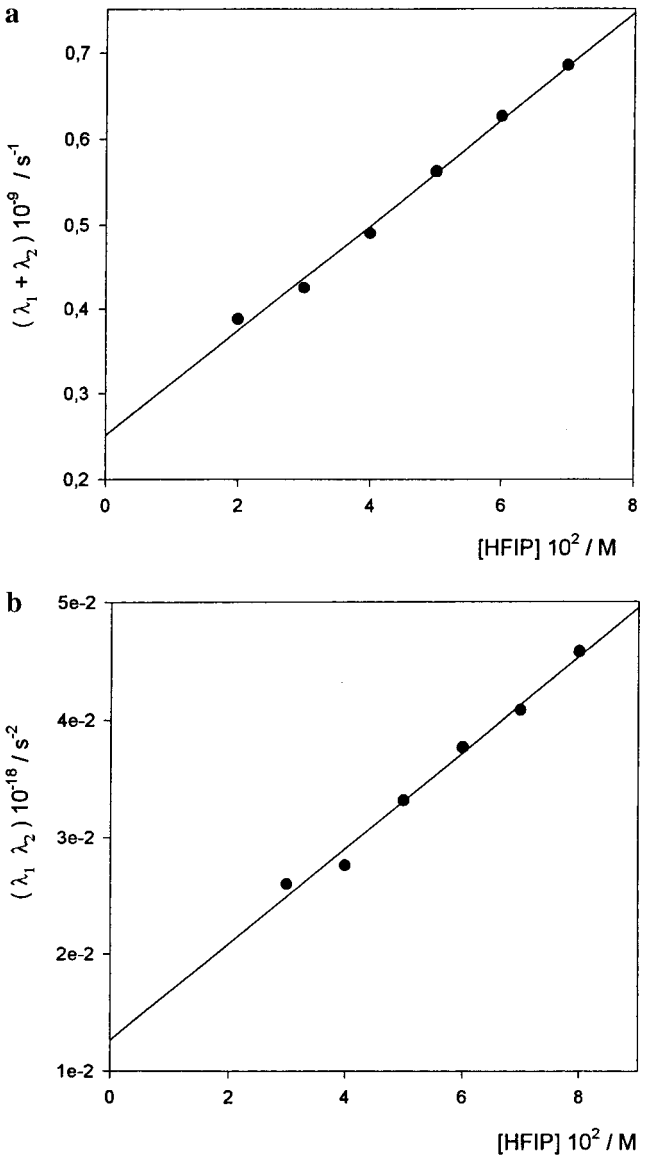


Figure 4. Plots of a: $\lambda_1 + \lambda_2$ and b: $\lambda_1\ \lambda_2$ versus [HFIP] for MHN−HFIP system in cyclohexane. Rate constants measured at 380 nm emission wavelength, λ_{exc}= 340 nm.

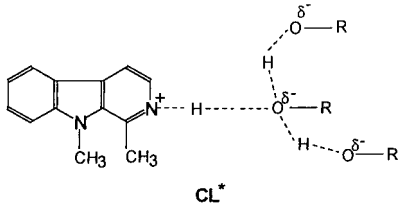
measurements and for this reason they were carried out at 440 nm.

As mentioned before, the appearance of the band around 410 nm depends on HFIP concentration. Thus, it is obvious that upon excitation, PTC* interacts with another HFIP molecule to produce the exciplex. The appearance of negative pre-exponential factors indicates that this exciplex is created during the lifetime of the lowest excited singlet state. As mentioned, we believe this band is due to a hydrogen bonded exciplex in which the extent of the proton shift is still greater than in the PTC complex, i.e., a cation like species, CL*. This assumption is in agreement with the red shift of its emission band and with the long lifetime component decays measured at these high HFIP concentrations, see Table 1. It should be noted that the shift of the fluorescence maxima brought about by the addition of HFIP is quite large but still smaller than that of the cationic species upon ionic dissociation in aqueous solutions.[17] The same occurrences are observed when cyclohexane, toluene, or dichloromethane are used as the solvents. Moreover, the long lifetime measured for this exciplex is also smaller than that of the cation. Thus, this exciplex is not the cationic species of MHN in which the proton transfer has been completed. Furthermore,

TABLE 2: Rate Constants and Lifetimes for MHN−HFIP System in Cyclohexane, Obtained by Fitting the Kinetic Parameters of eqs 5 and 6 to the Experimental Results

k_1	$6.6 \times 10^9\ M^{-1}\ s^{-1}$
k_{-1}	$1.5 \times 10^7\ s^{-1}$
τ_{CL}	16 ns
τ_{PTC}	5 ns

SCHEME 2



CL*

the N-pyrido methyl derivative of beta-carboline, which is a model of the beta-carboline cation, emits around 450 nm in cyclohexane, a wavelength characteristic of the cation emission. Similarly, the cation of MHN, which has been observed either in pure methanol, acidic aqueous solvents, or in pure HFIP, also emits around 450 nm[16] and has a lifetime around 21−22 ns.[2]

For the mechanism in Scheme 1, the reciprocal lifetimes should satisfy the following equations

$$\lambda_1 + \lambda_2 = + k_1[\mathrm{KFIP}]_+ + 1/\tau_{PTC} + 1/\tau_{CL} + k_{-1} \quad (5)$$

and

$$\lambda_1 + \lambda_2 = + k_1(1/\tau_{CL})\,[\mathrm{KFIP}] + 1/\tau_{PTC}\,(k_{-1} + 1/\tau_{CL}) \quad (6)$$

thus, both the sum and the product of the reciprocal of the measured lifetimes, should vary linearly with HFIP concentration. As shown in Figure 4, for the data measured at 380 nm emission wavelength, the experimental results fulfill the predictions of eqs 5 and 6. The same behavior is observed at 440 nm. From the plots of $\lambda_1 + \lambda_2$ versus HFIP concentration, slopes of $(7 \pm 1)\ 10^9$ and $(6.2 \pm 0.6)\ 10^9\ M^{-1}\ s^{-1}$ and intercepts of $(0.28 \pm 0.06)\ 10^9$ and $(0.25 \pm 0.03)\ 10^9\ s^{-1}$ are obtained at 440 and 380 nm, respectively. From the $\lambda_1\ \lambda_2$ against HFIP concentration plots, the corresponding values are: $(0.41 \pm 0.09)\ 10^{18}$ and $(0.41 \pm 0.06)\ 10^{18}\ M^{-1}\ s^{-2}$ for the slopes and $(0.016 \pm 0.004)\ 10^{18}$ and $(0.013 \pm 0.003)\ 10^{18}\ s^{-2}$ for the intercepts at the origin, respectively. As can be seen, the agreement between the values obtained at both wavelengths is excellent. Using the mean values of the slopes and intercepts of the plots of $\lambda_1 + \lambda_2$ and $\lambda_1\ \lambda_2$ versus HFIP concentrations at the two different wavelengths, the rate constants for this system were calculated, Table 2. The calculated PTC* lifetime in Table 2 is in excellent agreement with the value estimated for this species in the low range of HFIP concentrations, around 5−6 ns and also with the value determined in the MHN-trifluoroethanol system.[19]

According to the results obtained in this study, the novel species, i.e., the CL* exciplex, is formed by the reaction of PTC* with a new molecule of HFIP. Therefore, the stoichiometry of this excited state hydrogen bonded complex should be 1:3 or higher. According to this, we can tentatively assign a structure for this exciplex as depicted in Scheme 2. In this structure, the formation of a 1:3 hydrogen bonded complex may be related to the so-called cooperative effect between hydrogen bonds.[23,24] The oxygen atom in the N···H···O bridge of the PTC* should be a stronger proton acceptor than the corresponding atom in the 1:1 hydrogen bonded complex, HBC, and also stronger than the oxygen atom in the free alcohol molecule. Thus, the

assistance of the third HFIP molecule allows the negative charge to be distributed among the three oxygen atoms of the donor molecules. This weakens the O···H bond strength considerably allowing proton transfer to occur, during the fluorescence lifetime, along the reaction coordinate shifting the equilibrium from PTC* to CL*.

Therefore, the results obtained in this paper conclusively show that the precursor of the beta-carboline exciplex emitting at 410 nm, CL*, is not, as previously suggested,[7] the ground-state neutral species, but the 1:2 hydrogen bonded complex, PTC*.

The appearance of the spectrum of CL* species of MHN depends on the proton donor capability and the solvent polarity. In cyclohexane, it is only observed when the strong proton donor HFIP is added, but it does not appear with a weaker proton donor such as trifluoroethanol. However, in solvents of higher polarity than cyclohexane, CL* species of MHN are observed with proton donors of different proton donor capabilities.

Finally, we would like to advance the following fact. For the formation of zwitterionic species, a strong positive charge density has to be created on the pyridine nitrogen atom of the betacarboline ring. Thus, in a previous paper, we postulated that PTC was the ground-state precursor of Z*. However, in the light of the results obtained in this paper, we ask the following question: Are Z* and CL* independently formed via parallel reactions of the photoexcited PTC, or are these species coupled, with CL* being the precursor of Z*?. Further results from our laboratory will allow distinction between these possibilities.

Acknowledgment. We gratefully thank financial support from the Dirección General de Investigación Científica y Técnica, PB98-1160, and Junta de Andalucía.

References and Notes

(1) Waluk, J. *Conformational Analysis of Molecules in Excited States*; Wiley−VCH, Inc.: New York, 2000; Chapter 2.

(2) Dias, A.; Varela, A. P.; Miguel, M. G.; Maçanita, A. L.; Becker, R. S. *J. Phys. Chem.* **1992**, *96*, 10 290.

(3) Draxler, S.; Lippitsch, M. E. *J. Phys. Chem.* **1993**, *97*, 11 493.

(4) Reyman, D.; Pardo, A.; Poyato, J. M. L. *J. Phys. Chem.* **1994**, *98*, 10 408.

(5) Balón, M.; Muñoz, M. A.; Guardado, P.; Carmona, C. *Photochem. Photobiol.* **1996**, *64* (3), 531.

(6) Dias, A.; Varela, A. P.; Miguel, M. G.; Becker, R. S.; Burrows, H. D.; Maçanita, A. L. *J. Phys. Chem.* **1996**, *100*, 17 970.

(7) Reyman, D.; Viñas, M. H.; Poyato, J. M. L.; Pardo, A. *J. Phys Chem.* A **1997**, *101*, 768.

(8) Balón, M.; Carmona, C.; Guardado, P.; Muñoz, M. A. *Photochem. Photobiol.* **1998**, *67* (4), 414.

(9) Biondic, M. C.; Erra-Basells, R. *J. Chem Res. (S)*, **1998**, 114.

(10) Reyman, D.; Viñas, M. H.; Camacho, J. J. *J. Photochem. Photobiol. A: Chemistry*, **1999**, *120*, 85.

(11) Reyman, D.; Viñas, M. H. *Chem. Phys. Lett.* **1999**, *301*, 551.

(12) Allen, J. R. F.; Holmsted, B. R. *Phytochemistry* **1980**, *19*, 1573.

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

(14) Pardo, A.; Reyman, D.; Poyato, J. M. L.; Medina, F. *J. Luminesc.* **1992**, *51*, 269.

(15) Sakuros, R.; Ghiggino, K. P. *J. Photochem.* **1982**, *18*, 1.

(16) Balón, M.; Hidalgo, J.; Guardado, P.; Muñoz, M. A.; Carmona, C. *J. Chem. Soc., Perkin Trans. 2* **1993**, 99.

(17) Varela, A. P.; Dias, A.; Miguel, M. G.; Becker, R. S.; Maçanita, A. L. *J. Phys. Chem.* **1995**, *99*, 2239.

(18) Draxler, S.; Lippitsch, M. E. *J. Phys. Chem.* **1995**, *99*, 2241.

(19) Carmona, C.; Galán, M.; Angulo, G.; Muñoz, M. A.; Guardado, P.; Balón, M. *Phys Chem. Chem. Phys.* **2000**, *2*, 5076.

(20) Doig, G. G.; Loudon, J. D.; McCloskey, P. *J. Chem. Soc.* **1952**, 3912.

(21) O'Connor, D. V.; Phillips, D. *Time-correlated Single Photon Counting*, Academic Press Inc., London, 1984.

(22) Kikuchi, K.; Sato, C.; Watabe, M.; Ikeda, H.; Takahashi, Y.; Miyashi, T. *J. Am. Chem. Soc.* **1993**, *115*, 5180.

(23) Frank, H. S.; Wen, W. Y. *Discss. Faraday Soc.* **1957**, *24*, 133.

(24) Gutmann, V. *Struct. Bonding (Berlin)*, **1973**, *15*, 141.