New Insights on the Excited-State Proton-Transfer Reactions of Betacarbolines: Cationic Exciplex Formation

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Received: July 9, 2003; In Final Form: January 6, 2004

The photoinduced proton-transfer reactions of two betacarbolines, 9-methyl-9*H*-pyrido[3,4-*b*]indole, MBC, and 1,9-dimethyl-9*H*-pyrido[3,4-*b*]indole, MHN, in the presence of the proton donor 1,1,1,3,3,3-hexafluoro-2-propanol, HFIP, are compared. Although MBC is more reactive than MHN, the behavior of both substrates is similar which indicates that a general mechanism in the exciplex formation of these derivatives operates. Three reactive excited-state species have been identified: a 1:2 hydrogen-bonded proton-transfer complex, PTC, between the pyridinic nitrogen of the substrate and the proton donor, a cation-like exciplex, CL, formed by the interaction of excited PTC with a new HFIP molecule, and finally, excited-state cations, C, which can be formed by an excited-state reaction from the CL exciplexes and/or simply by direct excitation of ground-state cations. With the help of the steady-state and time-resolved fluorescence results, the mechanism of these excited-state reactions has been established and the kinetic parameters determined.

Introduction

The betacarboline ring, 9*H*-pyrido[3,4-*b*]indole, BC, is a prototype molecule to study the spectroscopy and dynamics of hydrogen-bonding excited-state proton-transfer reactions. The simultaneous presence of proton-donating and proton-accepting sites in the betacarboline derivatives, pyrrolic and pyridinic nitrogens, respectively, has revealed interesting photophysical properties. Thus, upon excitation by light absorption, a drastic change in the acid−base properties of these nitrogen atoms has been observed. As a consequence, the existence of different phototautomers and/or exciplexes has been reported.[1−15]

For betacarboline proton donor systems in low-polarity solvents, such as cyclohexane, it now seems generally accepted that the proper forms for the excited-state reactions are not, as previously reported,[2,3] the neutral species but ground-state hydrogen-bonded complexes.[12−15] The structure and stoichiometry of these complexes could depend on the system under investigation.

Thus, in the formation of zwiterionic complexes of BC, Z*, with acetic acid in cyclohexane Chou et al.[14] suggest that the excited 1:2 BC/acetic acid cyclic complex, with a structure of triple hydrogen-bonding formation, is responsible for the excited-state proton-transfer tautomerism in this solvent. According to these authors, once this cyclic complex is excited only a small geometry adjustment is necessary for the triple proton transfer to occur, and Z* species are observed; that is, a concerted mechanism works for the exciplex formation of this derivative.

However, we have recently demonstrated that in the case of harmane, 1-methyl-9*H*-pyrido[3,4-*b*]indole, HN, the formation of exciplexes occurs through a different mechanism. Thus, in the interaction of 1,9-dimethyl-9*H*-pyrido[3,4-*b*]indole, MHN,[13] where the formation of the cyclic complex is blocked, and HN[15] with the proton donor hexafluoro-2-propanol, HFIP, exciplexes were formed through a stepwise mechanism. Undoubtedly, a prerequisite for exciplexes to be observed was the formation of ground-state hydrogen-bonded complexes with the donor through the pyridinic nitrogen atom of the substrate: first, a 1:1 hydrogen-bonded complex HBC and second, its 1:2 proton-transfer complex, PTC. The 1:2 stoichiometry of the PTC indicates the specific solvation of the oxygen atom of the HBC by a second donor molecule. Upon excitation of the ground-state PTC complexes, the excited PTC molecules react with another proton donor molecule to give a hydrogen-bonded cation-like exciplex, CL*. Because a new donor molecule assists this reaction, the stoichiometry of this exciplex was postulated to be at least 1:3. In the case of HN, further reaction of CL* with another HFIP molecule produces Z*, $\lambda_{em} \approx 500$ nm.[15]

Although the Chou et al. mechanism[14] for the exciplex formation of the unsubstituted derivative, BC, seems plausible, we believe that a stepwise mechanism, as proposed for HN, could also act for BC. In this sense, and as a continuation of our previous paper,[15] we have undertaken the study of exciplex formation of BC and HFIP. Because of the low hydrogen bond acceptor capability of HFIP, the cyclic complex is unfavorable with this donor. Therefore, this study will allow us to check whether the stepwise mechanism observed for HN is a general mechanism for betacarboline exciplex formation.

Thus, trying to separate the different excited-state reactions, we have started this study with the analysis of the capability of BC to form the CL* exciplex. To do this, we have synthesized 9-methyl-9*H*-pyrido[3,4-*b*]indole, MBC, and studied its reactivity with HFIP in cyclohexane. As mentioned, a similar study carried out previously for MHN showed the appearance of CL* exciplex in equilibrium with excited ground-state PTC.[13] (Structures of HN, MHN, BC, and MBC are shown in Chart 1.)

Surprisingly, the MBC/HFIP system in cyclohexane behaves differently than the MHN/HFIP system does. As the HFIP concentration is increased up to its solubility limit in cyclohexane, the absorption spectrum of MHN does not change, but small changes in the long wavelength region of the spectra are observed for MBC. Steady-state and time-resolved emission spectroscopy clearly show the appearance of a new species,

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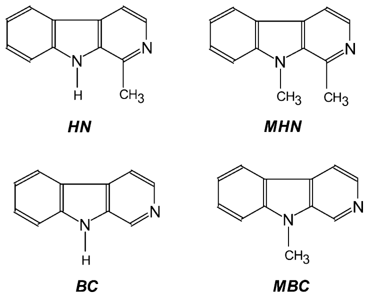
10.1021/jp030829a CCC: $27.50

CHART 1: Structural Formulas



different from the CL* exciplex. The study of the MBC/HFIP system in mixtures of toluene/cyclohexane, 20% vol/vol, where HFIP solubility is higher, undoubtedly shows that the modifications observed in the absorption and emission spectra are due to the formation of cationic species. Furthermore, these studies also show that excited-state cations, C*, can be formed simply by direct excitation of ground-state cations and/or by an excited-state reaction.

Until now, when cationic exciplexes have been observed they have been supposed to be formed from the excited neutral molecule. Thus, upon excitation the neutral molecule suffers a complete proton transfer and emission from the betacarboline cation is observed. However, the previously mentioned results point to a much more complex mechanism for the cation exciplex formation.

Because of our interest in understanding the complex photophysics of these betacarboline donor systems, this behavior has prompted us to start with the detailed analysis of this cationic species formation. Thus, this paper presents an absorption, steady-state, and time-resolved fluorescence study of the MBC/HFIP system in cyclohexane (CY) and cyclohexane/toluene mixtures (CY/TL). Also, and with the aim of comparison, a similar study on the MHN/HFIP system in the same cyclohexane/toluene mixtures has been carried out. Finally, to analyze the influence of isotopic substitution on the mechanism of exciplex formation, we have repeated the study of MBC in pure CY using deuterated HFIP, HFIP-*d*. All these results will allow us to establish the mechanism of cation exciplex formation and check if the stepwise mechanism proposed for Z* formation also holds for the cations. Furthermore, the comparison between MHN and MBC will permit us to know if the stepwise mechanism can be considered a general mechanism for the betacarboline family.

Materials and Methods

MBC and MHN were prepared as described elsewhere.[16] The complexing agents, HFIP and deuterated HFIP, HFIP-*d*, and the solvents, spectral grade cyclohexane and toluene, were stored on molecular sieves. Absorption and fluorescence spectra of the reagents and the solvents did not show any indication of impurities.

The UV−vis absorption spectra were recorded on a Perkin-Elmer Lambda-5 spectrophotometer. The spectra were recorded under controlled 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. Dilute solutions of MBC and MHN (10^{-5} M) were used to avoid inner filter effects and reabsorption phenomena. Resolution of the fluorescence profiles of MBC and MHN/HFIP emission spectra into their individual components, PTC*, CL*, and C*, has been performed using the Peak Fit Jandel Scientific program. Thus, the experimental spectra were fitted to logistic asymmetric functions, optimizing the spectral parameters (amplitude, center, width, and shape) by means of the Marquardt algorithm. The goodness of the fits was judged by the correlation coefficient. As will be seen later, depending on the experimental conditions, PTC* and CL*, CL* and C* or PTC*, CL* and C* are the observed emitting species. While the CL* and C* spectra consist of a unique band system, with maxima around 415−420 and 445−450 nm, respectively, the PTC spectra must be deconvolved in a three-band system. In all the deconvolved spectra, the emission maxima calculated for the PTC*, CL*, and C* bands were the same within 3−4 nm. Only the intensities of these bands changed with HFIP concentration. Moreover, the emission maxima of the PTC* bands agree with those obtained when PTC* is the only emitting species.[12] Also, the emission maxima calculated for the CL* and the C* complexes were in excellent agreement with the values previously reported for the CL* of MHN[13] and for betacarboline cations in different media.[2,17] The measurement of the area under the spectrum of each species allows us to calculate the relative quantum yields at different HFIP concentrations.

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 the excitation side (rhodamine B) and on the emission side (cell diffuser). Fluorescence lifetimes were measured with an Edinburgh Analytical Instruments FL900CD spectrophotometer employing the time-correlated single-photon counting technique.[18] The decay curves were deconvolved and the quality of the fits analyzed by the randomness of the residuals and the reduced chi-squares. Because quenching by molecular oxygen was found to be negligible,[13,15] the measurements were carried out with nondegassed solutions under temperature-controlled conditions (25 °C). Each decay curve has been obtained at different emission wavelengths at two or three fixed excitation wavelengths. Global analysis of these decay curves, at fixed excitation wavelengths and HFIP concentrations, were performed using the standard program Level 2 based on the tried and tested Marquardt−Levenberg algorithm and supplied by Edinburg Analytical Instruments.

Results

The changes in the UV−vis spectra of MBC in pure cyclohexane upon the addition of HFIP, in the very low concentration range of the donor (from 10^{-5} to 10^{-4} M), are completely similar to those previously observed for MHN.[13] Thus, MBC forms a 1:1 ground-state hydrogen-bonded complex, HBC, through the pyridinic nitrogen atom with HFIP. The slight changes observed in the absorption spectra upon the formation of this complex make the determination, from absorbance measurements, of the association constant for this equilibrium difficult. As will be seen later, we can estimate these equilibrium constants from time-resolved data.

In the low concentration range of the donor, between 10^{-4} and 10^{-3} M, once the HBC complex has been completely formed the UV−vis absorption spectra of MBC/HFIP in pure cyclohexane changes with the addition of HFIP. Figure 1a shows, as previously reported for MHN,[13] bathochromic shifts and isosbestic points indicating the formation of a second ground-state complex. This complex, formed from HBC and with a stoichiometry of 1:2, is called a proton-transfer complex, PTC. In the inset of Figure 1a, the Benesi−Hildebrand plot of the absorbance data for PTC formation versus the reciprocal of HFIP concentration allows the association constant for the HBC/

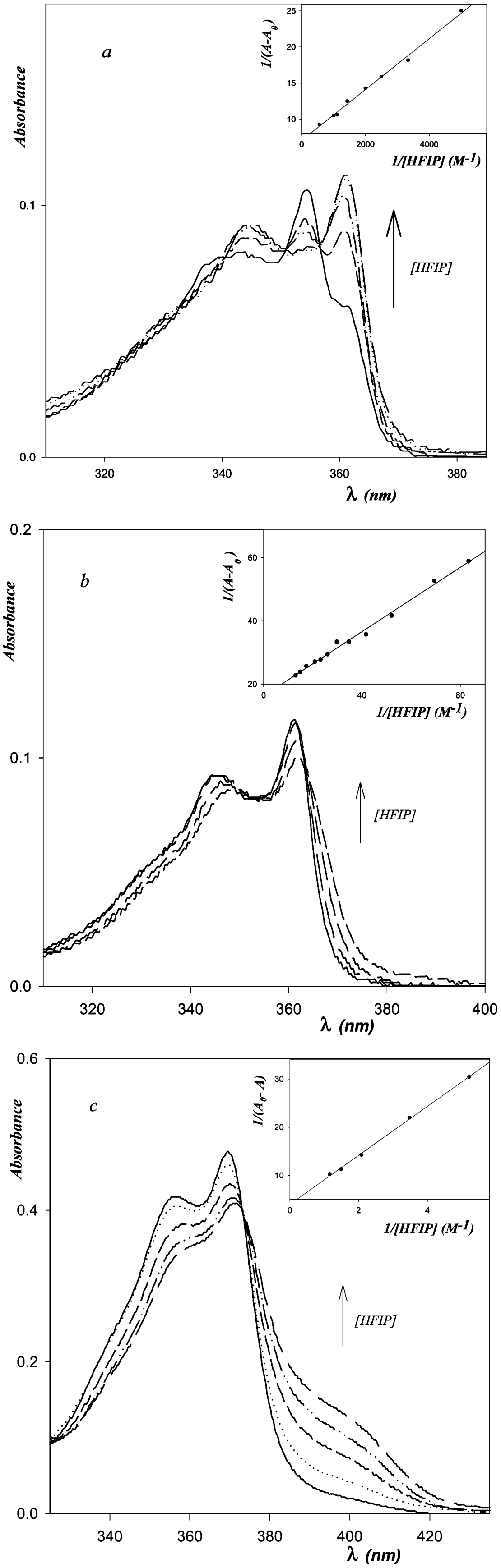


Figure 1. Changes in the absorption spectra of the MBC/HFIP system upon increasing HFIP concentration: (a) formation of PTC complexes in pure cyclohexane; (b) formation of C species in pure cyclohexane; (c) formation of C species in the cyclohexane/toluene mixtures. Insets: Benesi−Hildebrand plots for the corresponding equilibria. [MBC] = 2 × 10^{-5} M.

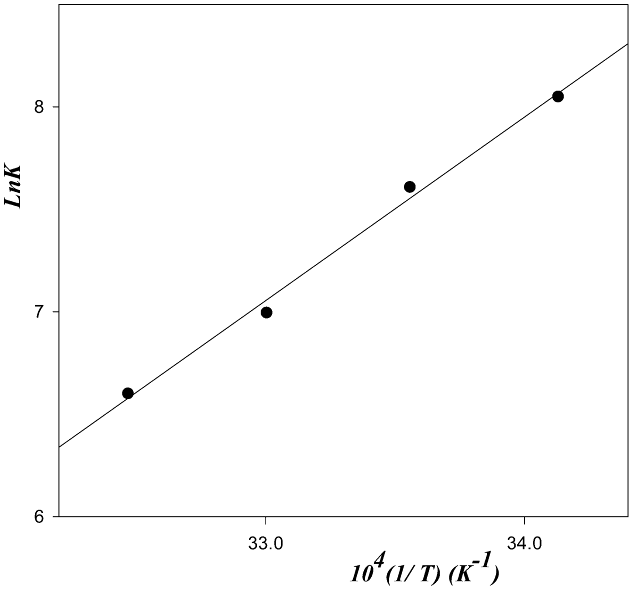


Figure 2. Van't Hoff plot of the association constants for the HBC/PTC equilibrium of MBC/HFIP in pure cyclohexane.

TABLE 1: Apparent Association Constants and Thermodynamic Parameters for the Formation of the Ground-State Hydrogen-Bonded Complexes, HBC, PTC, and C of the MBC/HFIP and MHN/HFIP Systems in Pure Cyclohexane and in Cyclohexane/Toluene Mixtures[a]

	MBC			MHN		
	HBC	PTC	C	HBC	PTC	C
		Pure Cyclohexane				
K_{20} /M^{-1}		3134	24		3533	
K_{25} /M^{-1}	> 8000	2016	32	>4000[b]	2488[b]	
K_{30} /M^{-1}		1092	61		1866	
K_{35} /M^{-1}		736	100		1265	
K_{40} /M^{-1}			125		1073	
ΔH^0 /kJ mol^{-1}		−74	68		−47	
ΔS^0 /J $mol^{-1}K^{-1}$		−187	258		−92	
		HFIP-*d* in Pure Cyclohexane				
K_{25} /M^{-1}		2564				
		Cyclohexane/Toluene				
K_{25} /M^{-1}		1760	0.78		1873	0.84

[a] [MBC/MHN] = 2 × 10^{-5} M. [b] Taken from ref 12.

PTC equilibria to be obtained (see Table 1). Also, the influence of temperature on these association constants has been studied. The thermodynamic parameters, ΔH^0 and ΔS^0, obtained from a van't Hoff plot (see Figure 2) have been recorded in Table 1.

However, for MBC/HFIP in pure cyclohexane the increase of HFIP concentration up to its solubility limit in cyclohexane, ≈9 × 10^{-2} M, gives rise to the appearance of an important difference between the MHN/HFIP and MBC/HFIP systems. Thus, while the absorption spectrum of MHN does not change, small changes are observed in the long wavelength side of the absorption spectra of MBC (Figure 1b). As can be seen immediately, these modifications, clearly observed in cyclohexane/toluene mixtures, are due to the formation of the ground-state cationic species C. From the corresponding Benesi−Hildebrand plot (see the inset in Figure 1b) the value reported in Table 1 can be estimated for the ground-state PTC/C equilibrium constant of the MBC/HFIP system in cyclohexane. The thermodynamic parameters, ΔH^0 and ΔS^0, obtained from a van't Hoff plot for this equilibrium have been also recorded in Table 1. As can be seen in this table, different from the behavior observed for HBC/PTC, the PTC/C equilibrium is an entropy-controlled process. This uncommon behavior might be related to the complete proton transfer necessary for the cation formation.

As mentioned, the kinetic isotope effect has only been studied for MBC in pure cyclohexane. The behavior observed in the presence of HFIP-*d* is completely similar to that described for the non-deuterated donor. From the changes of the absorption spectra of MBC upon the addition of HFIP-*d*, the corresponding association constant for the HBC/PTC equilibrium has been calculated (see Table 1).

In the cyclohexane/toluene mixtures the spectral changes observed for the MBC and MHN/HFIP systems are completely similar to those described for MBC/HFIP in pure cyclohexane. Ground-state HBC and PTC complexes are formed. Again, the formation of HBC complexes only produces slight changes in the absorption spectrum, and therefore, the ground-state MBC/HBC equilibrium constant cannot be calculated from these measurements. From the changes in the absorption spectra upon PTC formation, the HBC/PTC equilibrium constants for both systems are calculated (Table 1). The increase of HFIP concentration up to 0.9 M, its solubility limit in cyclohexane/toluene mixtures, produces clear changes in the absorption spectra. These changes are shown for the MBC/HFIP system in Figure 1c. A new absorption band centered around 380–390 nm, which can be safely ascribed to the formation of cationic species C, grows up with increasing donor concentration. From the corresponding Benesi–Hildebrand plot (see the inset in Figure 1c) the values in Table 1 for the PTC/C equilibrium constants in this medium can be calculated.

The changes in the fluorescence emission spectra upon changing the donor concentration are completely similar for MBC/HFIP and HFIP-*d* in cyclohexane and the MBC(MHN)/HFIP systems in the cyclohexane/toluene mixtures. These modifications are shown in Figure 3 for MBC/HFIP in pure cyclohexane. For the sake of clarity, the spectral changes have been independently analyzed at different ranges of donor concentrations. The region of the lowest HFIP concentration range, from 10^{-5} to 10^{-4} M, where ground-state MBC(MHN)/HBC equilibrium is established, has been omitted because, as will be seen later, these species behave as independent fluorophores and, therefore, do not participate in the excited-state reactions. Figure 3a shows the changes of the emission spectra, $\lambda_{exc} = 335$ nm, in the low range of HFIP concentrations, where ground-state PTC complexes are being formed. As can be seen in this figure, an isoemissive point and a shoulder around 415 nm clearly appear in the spectra. In a higher HFIP concentration range, where ground-state cationic species are not still being formed (Figure 3b) the spectra at $\lambda_{exc} = 355$ nm show a neat isoemissive point and the development of a band centered at 430 nm. This band, although widened and shifted to the red, is reminiscent, at least at the lowest HFIP concentrations in this figure, of the band previously observed for MHN/HFIP in pure cyclohexane. In the latter systems, this band was ascribed to the formation of CL exciplexes from excited PTC. Finally, in the highest HFIP concentration range, from 10^{-2} to 10^{-1} M, the emission spectra in Figure 3c have been obtained at $\lambda_{exc} =$ 380 nm. As can be seen in the absorption spectra (Figure 1b,c) at this wavelength only ground-state cations absorb. In the recorded emission spectra (Figure 3c) the growth of a very broad emission band centered at 440–450 nm can be clearly observed.

In all the cases, the corrected excitation spectra, at different emission wavelengths and HFIP concentrations, agree well with the observed absorption spectra.

Time-resolved fluorescence studies have been also carried out for MBC/HFIP and HFIP-*d* in cyclohexane and for MBC(MHN)/HFIP in the cyclohexane/toluene mixtures. In the lowest HFIP concentration range, from 10^{-5} to 10^{-4} M, these studies have only been performed for MBC/HFIP in pure cyclohexane just to check whether this system behaves as MHN/HFIP does. We found that, as previously reported for MHN,[13,15] MBC and its HBC behave as independent fluorophores, that is, as mentioned before, they do not participate in the excited-state reactions. The lifetimes measured for MBC and its corresponding HBC are 2.5 and 4.5 ns, respectively. These results are consistent with the lifetimes measured for free MHN and its HBC with HFIP in pure cyclohexane, 2.1 and 3.7 ns, respectively, and with the value obtained for free MBC in cyclohexane, 2.5 ns.

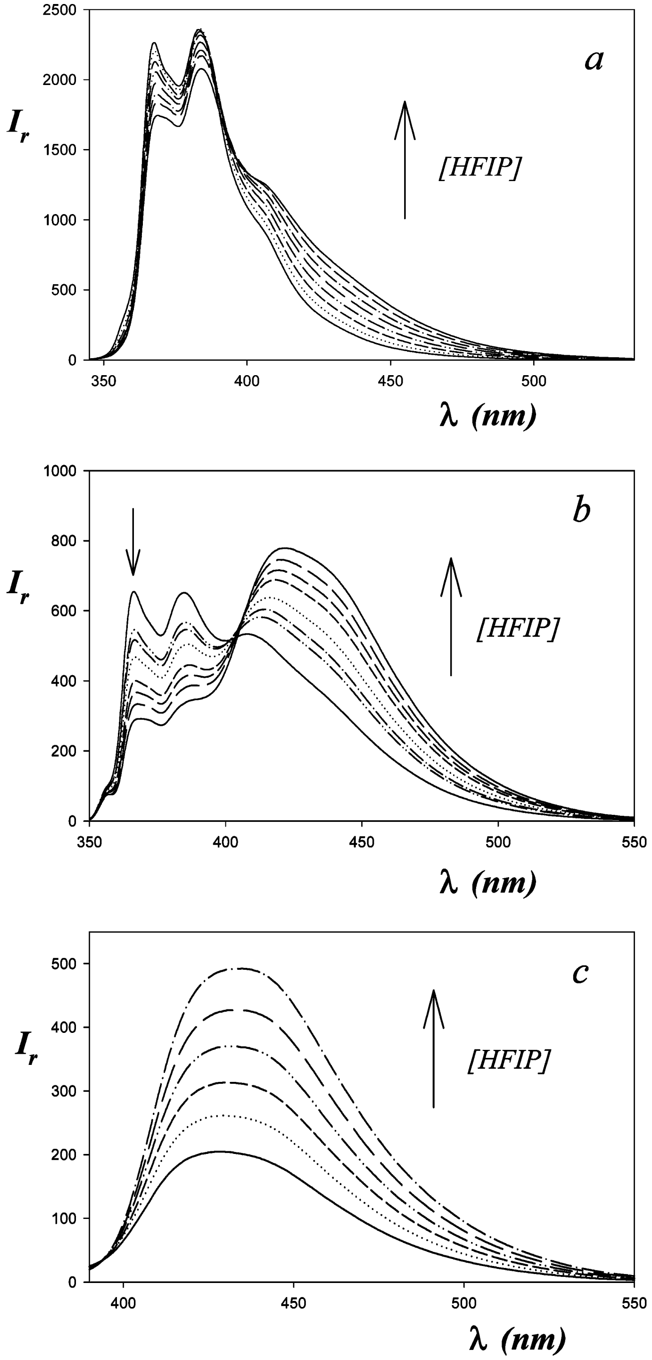


Figure 3. Changes in the emission spectra of the MBC/HFIP system in pure cyclohexane upon increasing HFIP concentration: (a) low concentration range of HFIP, $\lambda_{exc} = 335$ nm; (b) medium concentration range, $\lambda_{exc} = 355$ nm; (c) high concentration range, $\lambda_{exc} = 380$ nm. [MBC] = 2×10^{-5} M.

TABLE 2: Lifetimes and Preexponential Factors (in Parentheses) Obtained by Global Analysis of the Fluorescence Decays, at Different Observation Wavelengths, of MBC/HFIP in the Cyclohexane/Toluene Mixtures[a]

λ_{em} /nm	τ_1 /ns	τ_2 /ns	τ_3 /ns
420[b]	15 ± 3 (0.009)	7.2 ± 0.6 (0.216)	3.7 ± 0.7 (0.211)
420[c]	15 ± 2 (0.007)	6.5 ± 0.9 (0.087)	3 ±1 (0.054)
430[c]	17 ± 2 (0.009)	6.8 ± 0.9 (0.098)	2 ±1 (0.043)
440[c]	15 ± 2 (0.019)	6 ± 1 (0.096)	2 ± 1 (0.032)
450[c]	16 ± 2 (0.022)	7 ± 1 (0.095)	2 ± 2 (0.024)

[a] [HFIP] = 1.92×10^{-3} M, $\lambda_{exc} = 325$ nm, $\chi_g^2 = 1.093$. [MBC] = 2×10^{-5} M. [b] Decay curve with 3×10^4 counts at the maximum. [c] Decay curves with 1×10^4 counts at the maximum.

Because MBC and its HBC in cyclohexane behave as independent fluorophores, the preexponential factors, α_i, of their lifetimes, τ_i, contributing to the signal at zero time can be used to evaluate the ground-state formation constant of the HBC complex. Thus, when both components MBC and HBC are irradiated with a light source of the same intensity and the fluorescence is collected for the same amount of time the preexponential factors can be written as[8]

$$\alpha_i \propto c_i\, \epsilon_i\, \phi_i\, \tau_i^{-1} \tag{1}$$

where ϕ_i is the quantum yield of the corresponding species and the other symbols have their usual meaning. Furthermore, when the system is excited at the wavelength of the isosbestic point the ratio of the preexponential factors is expressed by

$$\frac{\alpha_{HBC}}{\alpha_{MBC}} = K \frac{\phi_{HBC}}{\phi_{MBC}} \frac{\tau_{MBC}}{\tau_{HBC}} [\mathrm{HFIP}] \tag{2}$$

Assuming that $\phi_{HBC}/\phi_{MBC} \sim 1$, the changes of the preexponential factors of MBC and HBC with the changes of HFIP concentration allow us to estimate the MBC/HBC ground-state equilibrium constant from eq 2. This value together with the value for MHN/HFIP system, taken from a previous work,[12] have been recorded in Table 1.

As the HFIP concentration is increased, the dynamics of the systems studied in the present work also show an interesting and unexpected behavior different from that previously reported for the MHN/HFIP system in pure cyclohexane. Thus, while for the latter system the fluorescence decays were always biexponential, for these systems the decays must be fitted to triexponential functions. As will be seen later, the only exceptions to this assertion are found when the decays are followed in selected HFIP concentration ranges at selected excitation or emission wavelengths. In these cases, biexponential decays nicely fit the experimental results.

As is well-known, the resolution of multiexponential decays becomes more difficult as the number of decay times increases.[19] One way to improve the resolution is to perform measurements at additional wavelengths and to do a global analysis. In this sense, we have carried out measurements at different emission and excitation wavelengths and fixed HFIP concentrations. This procedure has been repeated at different donor concentrations. Although the data support acceptance of three decay times, the values of the preexponential factors and the lifetimes are not well determined. This is illustrated in Tables 2 and 3 where the global analysis of the decays for MBC/HFIP at different emission wavelengths and fixed [HFIP] are reported in cyclohexane/toluene mixtures and in pure cyclohexane, respectively. As mentioned, this suggests that the actual uncertainties in the recovered lifetimes are larger than those expected from the asymptotic standard errors, this behavior being more evident when closely spaced lifetimes are involved. Furthermore, it has been reported that in the case of biexponential decays recovery of accurate lifetimes is extremely difficult if the values differ by less than 2-fold.[19] This is even worse for a three-fluorophore system. As the lifetimes become closer together the parameter values become more highly correlated and it is difficult to know the true uncertainties.

Thus, although the quantitative analysis of the dynamic results is intractable, some significant features, which can help in the understanding of the mechanism of the excited-state processes, can be inferred from the results. As shown in Table 2 for the MBC/HFIP system, in the low HFIP concentration range, where ground-state PTC is being formed, a short and a medium lifetime around 3−4 ns and 6−7 ns and a long lifetime, 15−16 ns, are observed. On the basis of previous results, the shortest lifetime can be tentatively assigned to the HBC species, the medium lifetime to the PTC species, and the longest lifetime to CL exciplexes. In fact, a lifetime of 16 ns was previously reported for the CL exciplex of the MHN/HFIP system in pure cyclohexane.[13]

Furthermore, at the shortest wavelengths, 360−380 nm, where mainly HBC and PTC complexes emit, the long lifetime component is not observed and the decays are biexponential. Thus, for MBC/HFIP in the cyclohexane/toluene mixtures and a [HFIP] of 1.92×10^{-3} M, we get biexponential decays with $\chi^2 = 1.147$ and lifetimes around 3 and 6 ns. Although the medium lifetime seems to decrease with the increase of HFIP concentration, the uncertainties in the results preclude reaching any conclusion. For the above HFIP concentration, at sufficiently long emission wavelengths, $\lambda_{em} > 460$ nm, the decays are also clearly biexponential, $\chi^2 = 1.119$, with a long lifetime term around 16 ns ($\alpha_1 = 0.150$) and the medium lifetime, 6 ns, appearing as a rise time ($\alpha_2 = -0.051$). The appearance of the negative preexponential factors and the fact that at the shortest wavelengths the long lifetime term in the decays is not observed point to CL exciplexes being formed in an irreversible step from excited PTC species. Unfortunately, the small intensity obtained at the longest wavelength precludes any quantitative measurements on this transformation.

In the medium HFIP concentration range, where the HBC complexes no longer exist and ground-state cations are not still being formed, the decays were always triexponential functions, independent of excitation or emission wavelength (see Table 3). Thus, apart from excited PTC and CL complexes another

TABLE 3: Lifetimes and Preexponential Factors (in Parentheses) Obtained by Global Analysis of the Fluorescence Decays, at Different Excitation and Observation Wavelengths, of MBC/HFIP in Pure Cyclohexane[a]

λ_{exc} /nm	λ_{em} /nm	τ_1 /ns	τ_2 /ns	τ_3 /ns
350	420	18.1 ± 0.5 (0.091)	2.4 ± 0.9 (0.075)	0.6 ± 0.5 (−0.052)
325	430	18.0 ± 0.4 (0.111)	3 ± 1 (0.047)	0.6 ± 0.4 (−0.073)
360	430	18.1 ± 0.4 (0.114)	2.0 ± 0.9 (0.070)	0.9 ± 0.6 (−0.088)
350	430	17.9 ± 0.4 (0.110)	3 ± 1 (0.037)	0.7 ± 0.9 (−0.031)
350	450	18.0 ± 0.4 (0.132)	2 ± 3 (0.023)	1.2 ± 0.8 (−0.066)
350	470	18.1 ± 0.3 (0.145)	3 ± 2 (0.015)	1.1 ± 0.7 (−0.053)

[a] [HFIP] = 0.0864 M, $\chi_g^2 = 1.198$. [MBC] = 2×10^{-5} M.

TABLE 4: Lifetimes and Preexponential Factors (in Parentheses) Obtained by Global Analysis of the Fluorescence Decays, at Different Excitation and Observation Wavelengths, of MHN/HFIP in the Cyclohexane/Toluene Mixtures[a]

λ_{exc} /nm	λ_{em} /nm	τ_1 /ns	τ_2 /ns
340	415	18.9 ± 0.4 (0.122)	1.1 ± 0.9 (0.049)
360	415	18.9 ± 0.5 (0.116)	1.2 ± 0.8 (0.062)
370	415	19.0 ± 0.5 (0.122)	0.9 ± 0.6 (0.063)
340	420	18.9 ± 0.4 (0.123)	1 ± 1 (0.046)
360	430	19.0 ± 0.4 (0.130)	2 ± 2 (0.020)
340	430	18.8 ± 0.4 (0.126)	3 ± 3 (0.018)
360	440	19.0 ± 0.4 (0.132)	2 ± 2 (0.016)

[a] [HFIP] = 0.864 M, $\chi_g^2 = 1.177$. [MHN] = 2×10^{-5} M.

TABLE 5: Lifetimes and Preexponential Factors (in Parentheses) Obtained by Global Analysis of the Fluorescence Decays, at Different Excitation and Observation Wavelengths, of MBC/HFIP in the Cyclohexane/Toluene Mixtures[a]

λ_{exc} /nm	λ_{em} /nm	τ_1 /ns	τ_2 /ns
300	430	21.4 ± 0.4 (0.113)	2 ± 2 (0.026)
300	450	21.4 ± 0.5 (0.116)	3 ± 3 (0.012)
300	470	21.5 ± 0.5 (0.118)	3 ± 2 (0.008)
375	440	21.5 ± 0.4 (0.115)	2 ± 2 (0.023)

[a] [HFIP] = 0.864 M, $\chi_g^2 = 1.200$. [MBC] = 2×10^{-5} M.

excited-state species must be considered to account for the experimental results. Under these conditions two short lifetime terms randomly changing with [HFIP] are observed with the shortest lifetime always appearing as a rise time. The long lifetime term increases upon increasing the donor concentration.

Finally, at the highest [HFIP], up to 0.9 M, obtained only in the cyclohexane/toluene mixtures, the decays are again biexponential (see Tables 4 and 5). As can be seen in these tables, because of the small contribution, 1%, of the short lifetime terms to the total emission, the uncertainties in their values are high.[15] The long lifetime component achieves, in the case of the MBC/HFIP system, a value of 21 ns which is very similar to the value previously reported for betacarboline cations.[2,13] Also, the unreported absorption and emission spectra of MHN in pure HFIP correspond to the spectra of the cationic species,[2,17] and the MHN lifetime is 21.2 ns.

Thus, the first conclusions we can draw, at least from a qualitative point of view, are the following: The reactivity of MBC, in both the ground and excited states, is similar, although higher than the reactivity observed for MHN. Thus, MBC forms ground-state 1:1, HBC, and 1:2, PTC, complexes. Moreover, the formation of CL exciplexes from excited PTC is also observed. Furthermore, it seems that even in pure cyclohexane CL exciplexes of MBC react with another donor molecule to produce cationic exciplexes.

These experimental results prompted us to go back and analyze in detail the emission fluorescence spectra in order to search for the contribution of the different species at the different HFIP concentration ranges. Thus, these emission spectra have been deconvolved as shown in Figure 4. As can be seen, in the low concentration range (Figure 4a) the experimental profiles are well reproduced with the three bands corresponding to the PTC complex and the band of the CL exciplex. This is probably so because the contribution of HBC emission can be considered negligible at these HFIP concentrations. The obtained emission band for CL exciplexes of MBC, ~420 nm, has a maximum wavelength similar to that previously observed for the CL of MHN, 415 nm.[13]

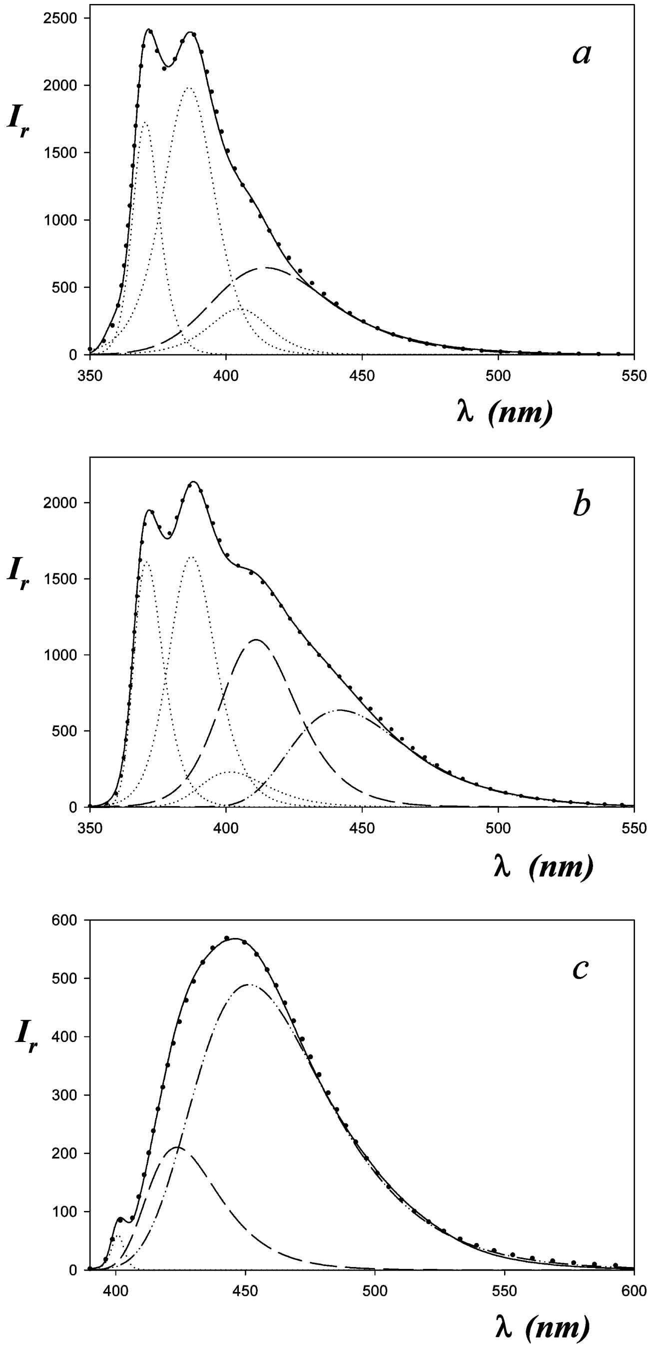
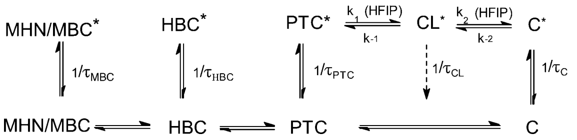


Figure 4. Deconvolved spectra of the MBC/HFIP system in the cyclohexane/toluene mixtures: (a) [HFIP] = 3.84×10^{-3} M, λ_{exc} = 355 nm, PTC (dotted curve), and CL (dashed curve); (b) [HFIP] = 9.6×10^{-3} M, λ_{exc} = 365 nm, PTC (dotted curve), CL (dashed curve), and C (dotted solid curve); (c) [HFIP] = 4.32×10^{-1} M, λ_{exc} = 400 nm, CL (dashed curve), and C (dotted solid curve).

In the medium range of donor concentration (Figure 4b) the fitting process clearly shows that a band at 450 nm, different from PTC and CL bands, must be taken into account to reproduce the experimental fluorescence profiles. This new band corresponds to betacarboline cationic species.[17] Thus, as mentioned, emission from the cations appears even in a HFIP concentration range where ground-state cations are not still observed; that is, they are formed in an excited-state reaction.

Finally, at the highest concentrations used, and when the solutions are excited at a wavelength where only cations absorb, emission from CL and C species is observed (Figure 4c). Although mentioned previously, it is worth remembering that the maximum and the shape corresponding to the deconvolved bands are always the same independent of the HFIP concentration range analyzed. This fact confirms the goodness of the

SCHEME 1



fitting procedure and also confirms that, despite the system being complex, PTC, CL, and C are the only reactive excited-state species emitting in these media.

Discussion

All these results led us to propose the general kinetic mechanism in Scheme 1 for the reactivity of betacarbolines with HFIP and its deuterated derivative in the ground and the first singlet excited state. In this scheme, τ_{MBC}, τ_{HBC}, and τ_{PTC} are the fluorescence lifetimes of the free MBC(MHN) and their HBC and PTC complexes, respectively; τ_{CL} and τ_{C} are the lifetimes of CL and C, k_1 and k_2 are the bimolecular rate constants for the PTC interaction with HFIP to give the CL and that of CL to produce C, respectively. k_{-1} and k_{-2} are the unimolecular rate constants for the back-reactions of CL and C, respectively.

To model the kinetics of the excited-state processes presented in Scheme 1, the following set of differential equations are needed,

$$-\int_{\alpha_{\mathrm{PTC}}}^{0} \mathrm{d}[\mathrm{PTC}]^* = \int_0^\infty \left(\frac{1}{\tau^0_{\mathrm{PTC}}} + k_1[\mathrm{HFIP}]\right)[\mathrm{PTC}]^*\,\mathrm{d}t - \int_0^\infty k_{-1}[\mathrm{CL}]^*\,\mathrm{d}t \tag{3}$$

$$-\int_{\alpha_{\mathrm{CL}}}^{0} \mathrm{d}[\mathrm{CL}]^* = \int_0^\infty \left(\frac{1}{\tau^0_{\mathrm{CL}}} + k_{-1} + k_2[\mathrm{HFIP}]\right)[\mathrm{CL}]^*\,\mathrm{d}t - \int_0^\infty k_1[\mathrm{HFIP}][\mathrm{PTC}]^*\,\mathrm{d}t - \int_0^\infty k_{-2}[\mathrm{C}]^*\,\mathrm{d}t \tag{4}$$

$$-\int_{\alpha_{\mathrm{C}}}^{0} \mathrm{d}[\mathrm{C}]^* = \int_0^\infty \left(\frac{1}{\tau^0_{\mathrm{C}}} + k_{-2}\right)[\mathrm{C}]^*\,\mathrm{d}t - \int_0^\infty k_2[\mathrm{HFIP}][\mathrm{CL}]^*\,\mathrm{d}t \tag{5}$$

where α represents the light fraction absorbed by the corresponding species and the other parameters have the usual meaning. Taking into account that [PTC]*, [CL]*, and [C]* can be expressed by $(\varphi_{\mathrm{PTC}}/\varphi^0_{\mathrm{PTC}})\tau^0_{\mathrm{PTC}}$, $(\varphi_{\mathrm{CL}}/\varphi^0_{\mathrm{CL}})\tau^0_{\mathrm{CL}}$, and $(\varphi_{\mathrm{C}}/\varphi^0_{\mathrm{C}})\tau^0_{\mathrm{C}}$, integration of the above equations gives

$$\alpha_{\mathrm{PTC}} = \left[\frac{1}{\tau^0_{\mathrm{PTC}}} + k_1[\mathrm{HFIP}]\right]\frac{\varphi_{\mathrm{PTC}}}{\varphi^0_{\mathrm{PTC}}}\tau^0_{\mathrm{PTC}} - k_{-1}\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} \tag{6}$$

$$\alpha_{\mathrm{CL}} = 0 = \left[\frac{1}{\tau^0_{\mathrm{CL}}} + k_{-1} + k_2[\mathrm{HFIP}]\right]\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} - k_1[\mathrm{HFIP}]\frac{\varphi_{\mathrm{PTC}}}{\varphi^0_{\mathrm{PTC}}}\tau^0_{\mathrm{PTC}} - k_{-2}\frac{\varphi_{\mathrm{C}}}{\varphi^0_{\mathrm{C}}}\tau^0_{\mathrm{C}} \tag{7}$$

$$\alpha_{\mathrm{C}} = \left[\frac{1}{\tau^0_{\mathrm{C}}} + k_{-2}\right]\frac{\varphi_{\mathrm{C}}}{\varphi^0_{\mathrm{C}}}\tau^0_{\mathrm{C}} - k_2[\mathrm{HFIP}]\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} \tag{8}$$

Thus, to get quantitative information on the kinetic parameters involved in these excited-state processes, we have calculated the relative quantum yields of PTC, CL, and C complexes at different donor concentrations by measuring the area under the deconvolved spectra of each species. The experimental conditions have been chosen so as to ensure that, under photostationary conditions, only one of the possible reactive excited-state species, PTC or C, absorb, and therefore, eqs 6−8 can be simplified. With this in mind, we have first analyzed the HFIP concentration region where PTC absorbs and only the PTC/CL reaction is observed in the excited state (Figure 3a), that is, the region where C exciplexes are not formed. Under these conditions, the following relations should hold,

$$\alpha_{\mathrm{PTC}} = 1 = \left[\frac{1}{\tau^0_{\mathrm{PTC}}} + k_1[\mathrm{HFIP}]\right]\frac{\varphi_{\mathrm{PTC}}}{\varphi^0_{\mathrm{PTC}}}\tau^0_{\mathrm{PTC}} - k_{-1}\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} \tag{9}$$

$$\alpha_{\mathrm{CL}} = 0 = \left[\frac{1}{\tau^0_{\mathrm{CL}}} + k_{-1}\right]\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} - k_1[\mathrm{HFIP}]\frac{\varphi_{\mathrm{PTC}}}{\varphi^0_{\mathrm{PTC}}}\tau^0_{\mathrm{PTC}} \tag{10}$$

According to these relations, the dependence of the relative quantum yields of PTC and CL on HFIP concentration is given by the following equations:

$$\frac{\varphi^0_{\mathrm{PTC}}}{\varphi_{\mathrm{PTC}}} = 1 + \frac{k_1\tau^0_{\mathrm{PTC}}[\mathrm{HFIP}]}{1 + k_{-1}\tau^0_{\mathrm{CL}}} \tag{11}$$

and

$$\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}} = \frac{k_1\tau^0_{\mathrm{PTC}}[\mathrm{HFIP}]}{1 + k_{-1}\tau^0_{\mathrm{CL}} + k_1\tau^0_{\mathrm{PTC}}[\mathrm{HFIP}]} \tag{12}$$

Thus, the plot of the PTC quantum yields versus HFIP concentration should be linear with an intercept of unity (eq 11). As shown in Figure 5a for the MBC/HFIP system in CY/TL mixtures, the experimental results hold for this equation. Also, the calculated relative quantum yields of the CL exciplexes have been fitted by an iterative Levenberg−Marquardt nonlinear global fitting to eq 12 (Figure 5b). This latter fit clearly shows that the term $k_{-1}\ \tau^0_{\mathrm{CL}}$ must be considered negligible. That is, the back-reaction from CL to PTC, the k_{-1} step, is not operative under these experimental conditions. As can be seen in Table 6, the values of $k_1\ \tau^0_{\mathrm{PTC}}$, calculated for all the systems studied using both equations, are in excellent agreement.

In the highest concentration range of the donor (Figure 1c) when the excitation wavelength is selected so that only the cationic species absorb, the following relations should hold,

$$\alpha_{\mathrm{CL}} = 0 = -k_{-2}\frac{\varphi_{\mathrm{C}}}{\varphi^0_{\mathrm{C}}}\tau^0_{\mathrm{C}} + \left[k_2[\mathrm{HFIP}] + \frac{1}{\tau^0_{\mathrm{CL}}}\right]\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} \tag{13}$$

$$\alpha_{\mathrm{C}} = 1 = \left[\frac{1}{\tau^0_{\mathrm{C}}} + k_{-2}\right]\frac{\varphi_{\mathrm{C}}}{\varphi^0_{\mathrm{C}}}\tau^0_{\mathrm{C}} - k_2[\mathrm{HFIP}]\frac{\varphi_{\mathrm{CL}}}{\varphi^0_{\mathrm{CL}}}\tau^0_{\mathrm{CL}} \tag{14}$$

From these relations, the relative quantum yields for the CL and C species are given by eqs 15 and 16,

$$\frac{\varphi^0_{\mathrm{CL}}}{\varphi_{\mathrm{CL}}} = \left[1 + \frac{1}{k_{-2}\tau^0_{\mathrm{C}}}\right] + \frac{k_2\tau^0_{\mathrm{CL}}}{k_{-2}\tau^0_{\mathrm{C}}}[\mathrm{HFIP}] \tag{15}$$

and

$$\frac{\varphi_{\mathrm{C}}}{\varphi^0_{\mathrm{C}}} = \frac{1 + k_2\tau^0_{\mathrm{CL}}[\mathrm{HFIP}]}{1 + k_{-2}\tau^0_{\mathrm{C}} + k_2\tau^0_{\mathrm{CL}}[\mathrm{HFIP}]} \tag{16}$$

As shown in Figure 6a for the MBC/HFIP system in cyclohex-

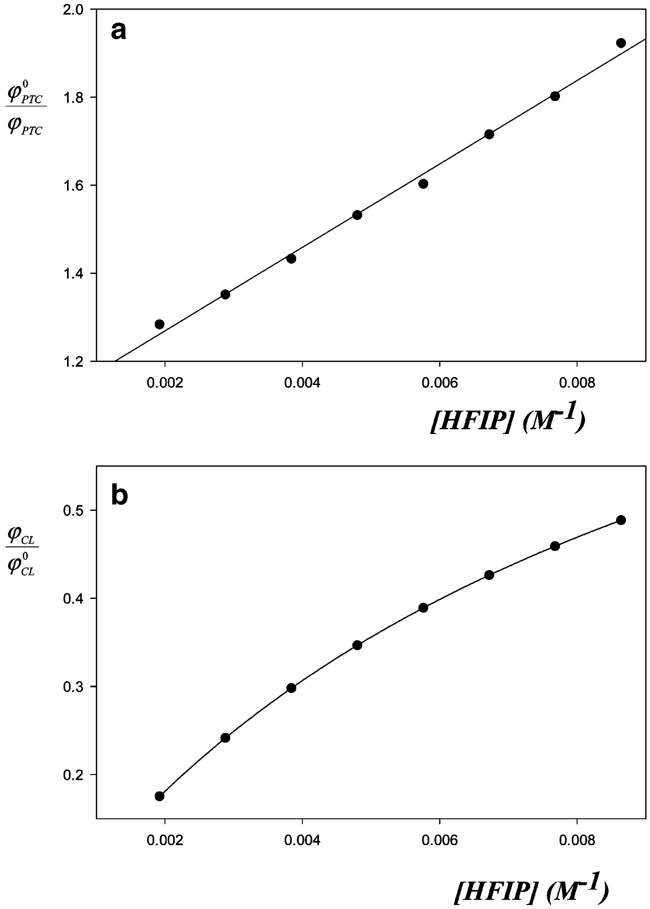


Figure 5. Plots of the relative quantum yields vs HFIP concentration for the MBC/HFIP system in the cyclohexane/toluene mixtures according to (a) eq 11 and (b) eq 12.

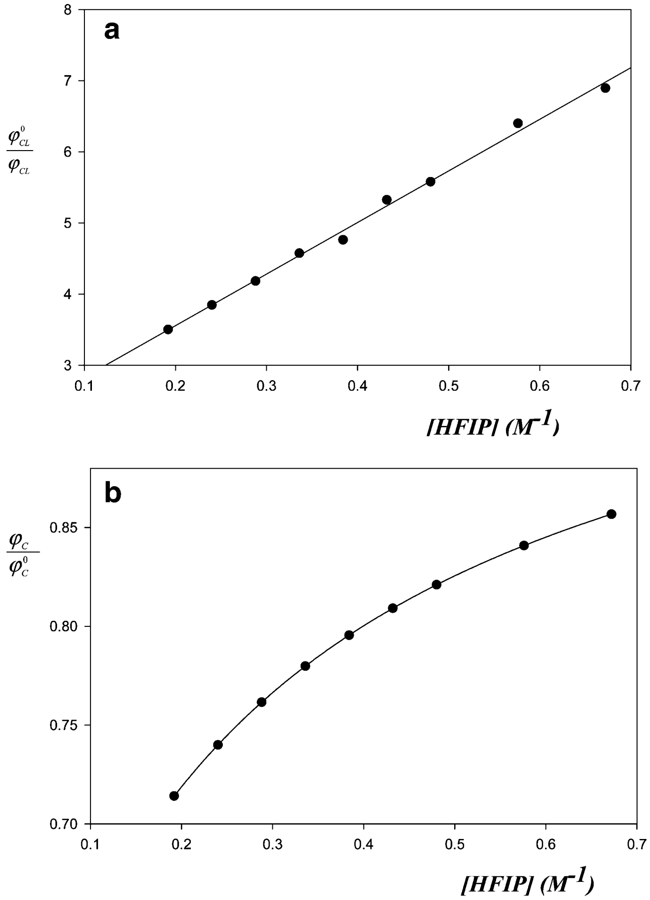


Figure 6. Plots of the relative quantum yields vs HFIP concentration for the MBC/HFIP system in the cyclohexane/toluene mixtures according to (a) eq 15 and (b) eq 16.

ane/toluene mixtures, the calculated relative quantum yields of the CL exciplexes satisfactorily fit eq 15. In these cases both the direct and reverse steps in the mechanism in Scheme 1 must be considered to account for the experimental results. From the slopes and intercepts of these plots the kinetic parameters in Table 6 have been obtained. Also, the relative quantum yields of the C species have been independently analyzed by an iterative Levenberg−Marquardt nonlinear global fitting to eq 16 (Figure 6b). The results have also been collected in Table 6. As can be seen in this table, there is an excellent agreement between the parameters calculated from both equations for all the systems studied.

The fulfillment of the above equations confirms that the mechanism in Scheme 1 operates for MBC and MHN. Furthermore, time-resolved data are also in agreement with Scheme 1 and the results in Table 6. As these results made clear, in the low range of HFIP concentration CL should be formed from PTC in an irreversible step, that is, k_{-1} must be nonoperative. This is so because, as mentioned, at these short wavelengths where CL species do not emit, the CL lifetime does not appear in the decays. As the [HFIP] is increased, once a significant concentration of CL is formed it preferably reacts to produce cationic exciplexes, that is, $k_2 \gg k_{-1}$. This explains the impossibility of finding an adequate concentration range of HFIP to independently study the PTC/CL transformation by dynamic measurements. Also, the triexponential behavior of the decays, in the medium HFIP concentration range (Table 3) where no ground-state cations are observed, is in agreement with the formation of cationic exciplexes. So, as shown in Scheme 1, excited cations of MBC and MHN can be formed in an excited-

TABLE 6: Excited-State Kinetic Parameters for the MBC/HFIP and MHN/HFIP Systems in Pure Cyclohexane and in the Cyclohexane/Toluene Mixtures

MBC			MHN		
$k_1\tau^0_{PTC}$ /M^{-1}	$k_2\tau^0_{CL}$ /M^{-1}	$k_{-2}\tau^0_{C}$	$k_1\tau^0_{PTC}$ /M^{-1}	$k_2\tau^0_{CL}$ /M^{-1}	$k_{-2}\tau^0_{C}$
		Pure Cyclohexane			
73 ± 3[b]	28 ± 2[d]	1.4 ± 0.1[d]	33[a]		
77 ± 2[c]	29 ± 3[e]	1.4 ± 0.1[e]			
		HFIP-*d* in Pure Cyclohexane			
67 ± 3[b]	139 ± 31[d]	5.0 ± 1.0[d]			
80 ± 2[c]	142 ± 30[e]	5.0 ± 0.9[e]			
		Cyclohexane/Toluene			
95 ± 3[b]	6.6 ± 0.6[d]	0.90 ± 0.06[d]	46 ± 1[b]	1.7 ± 0.1[d]	0.90±0.01[d]
110 ± 3[c]	6.6 ± 0.4[e]	0.90 ± 0.04[e]	60 ± 3[c]	1.7 ± 0.1[e]	0.90±0.01[e]

[a] Taken from ref 13. [b] Calculated from eq 9. [c] Calculated from eq 10. [d] Calculated from eq 13. [e] Calculated from eq14.

state reaction from CL complexes and/or, when possible, by direct excitation of ground-state cations. In any case, a CL/C equilibria is established in the excited state. Dynamic results are also in accordance with this observation. Thus, the facts that the long lifetime term in the decays increases on increasing HFIP concentration and that the decays shown in Tables 4 and 5 are biexponential can be only explained by assuming the reversibility of the CL/C step. Otherwise, a constant cation lifetime, around 22 ns, should have been observed independent of HFIP concentration.

The results in Table 6 also confirm that, as mentioned, the reactivity of MBC, although similar, is higher than that of MHN. Thus, in cyclohexane/toluene mixtures, where all the kinetic parameters for both substrates can be determined, and assuming that the lifetimes of the different species do not change with the media, the formation of excited-state CL and C species is favored in the case of MBC. Only the C to CL back-step is apparently of the same order of magnitude for both substrates. The results also show that the kinetic isotope effect is negligible in the formation of the CL exciplexes, but it increases the rate of the step for the formation of cationic species. This fact can be taken as an indication that the mechanisms for both steps must be significantly different. Thus, while in the formation of the C exciplexes proton movement can be the rate-controlling step, in the case of the CL exciplexes it should play a secondary role. In this sense, it can be advanced that these preliminary results seem to indicate that in the formation of CL exciplexes a tautomerization process, which requires a strong geometrical redistribution, can be involved.

So, two important contributions to the knowledge of the ground and excited-state proton-transfer equilibria of betacarbolines stand out from these results. First, although the reactivity of MBC is higher than that of MHN, we have demonstrated that the stepwise mechanism proposed for the exciplex formation of MHN also operates in the case of MBC. Thus, for the MBC/HFIP system, the ground-state 1:1, HBC, and 1:2, PTC, complexes are formed. Moreover, as in the case of MHN, the PTC complex is the excited-state CL precursor too. Once the PTC complex is excited, it interacts with another HFIP molecule to produce CL exciplexes.

Second, the novel contribution of these results is the characterization of cationic exciplexes of MHN and MBC and the establishment of the mechanism of their formations. Thus, as mentioned, although emission from C exciplexes has been previously reported, the corresponding neutral forms have been supposed to be their precursors.[2] In this paper we have shown that C exciplexes are only observed when ground-state PTC and CL exciplexes have been formed. Under the appropriate experimental conditions these CL complexes, assisted by another donor molecule, give the cation exciplexes.

We can, therefore, conclude that the stepwise mechanism proposed for MHN can be considered a general mechanism for the betacarboline family. Moreover, this mechanism, previously postulated for the formation of CL exciplexes of MHN and CL and Z exciplexes of HN, must be extended to account for the formation of cationic species.

Finally, it should be remembered that the original purpose of this work was to demonstrate that zwiterionic exciplexes of the non-methylated derivative, BC, can also be formed, as those of HN, in a stepwise mechanism with the corresponding CL exciplexes as their precursors. Although in light of the present results such a mechanism most likely works for the formation of BC zwiterions too, the detailed study of such a process seems intractable. In the BC/HFIP system, PTC, CL, C, and Z complexes can simultaneously coexist even in pure cyclohexane. As mentioned, to quantitatively solve a system formed by four fluorophores capable of interacting in the excited state is extremely complex. Thus, unless the experimental conditions could be carefully selected so as to reduce the number of exciplexes, the results of steady-state and time-resolved fluorescence measurements on this system will be worthless.

Acknowledgment. We are gratefully thankful for financial support from the Dirección General de Investigación Científica y Técnica, BQU2002-01582 and Junta de Andalucía.

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