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An experimental and theoretical approach to the acid–base and photophysical properties of 3-substituted β-carbolines in aqueous solutions

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Abstract

The photophysical properties and the acid–base equilibrium of β-carboline-3-carboxylic acid *N*-methylamide (βCMAM) in aqueous solution have been investigated. The pK_a values in the ground and the first excited state have been spectrophotometrically determined using the Förster cycle for the excited state. This compound and methyl-β-carboline-3-carboxylate (βCCM) are the most acidic β-carboline derivatives studied till now both in the ground and the first excited state. The acidity of these compounds is explained in terms of the optimised structure of the solvated molecules using the density functional method's (DFT) computational method and the polarized continuum model's (PCM) solvatation model. In contrast with most of the gas phase calculation published on β-carbolines, in this work, we have included solvatation effects in order to reproduce the relative experimental pyridinic nitrogen proton affinities of these 3-subtituted β-carbolines and Norharmane. The gas phase calculations are insufficient and the inclusion of these solvatation effects in the calculations is vital. Potential energy barrier when dihedral angle between the Norharmane skeleton and the substituent varies is also calculated in order to elucidate the importance of steric hindrance in these 3-substituted β-carboline derivatives.

The different photophysical properties of the neutral and cationic forms of βCCM and βCMAM are explained in terms of their optimised structures.

Keywords: β-Carbolines; Photophysic; Acid–base behaviour

1. Introduction

β-Carbolines are a group of molecules widely studied because of their presence in human tissues [1–3], foods [4,5], their pharmacological applications [6–9] or their photophysical properties as fluorescence standards [10,11] or as indicators for the fluorimetric determination of small acidities in the physiological pH range [12].

Numerous experimental studies have been carried out for the determination of β-carbolines pK_a values both in the ground and the first excited singlet state [12–20]. From these studies, it can be concluded that β-carbolines become more basic after excitation. The pK_a values of most of them are around 7 for the ground state and around 13 for the first excited state [13]. This is theoretically explained by the increase of the pyridinic nitrogen basicity upon excitation [21,22] which is affected by the substituents [23]. The proton affinities of β-carbolines in gas phase calculated by semi-empirical calculations have been supposed to be a good basis for the discussion of the protonation process in solution because good agreements between the theoretical and the experimental trends had been reached [23].

However, we have recently found that the pK_a values of methyl-β-carboline-3-carboxylate (βCCM, Fig. 1) in aqueous solution in the ground and the first excited singlet state, 4.4 and 6.0, respectively, are quite a bit lower than the average pK_a values of the other β-carboline derivatives [24]. These unexpected experimental results could not be explained with the preliminary semi-empirical calculations carried out in gas phase.

We are now interested in studying the pK_a values in aqueous solution of other β-carboline derivatives with structures close to that of βCCM to see if their pK_a values are also lower than the average pK_a values of most β-carbolines. The first aim of this work is therefore to study the acid–base

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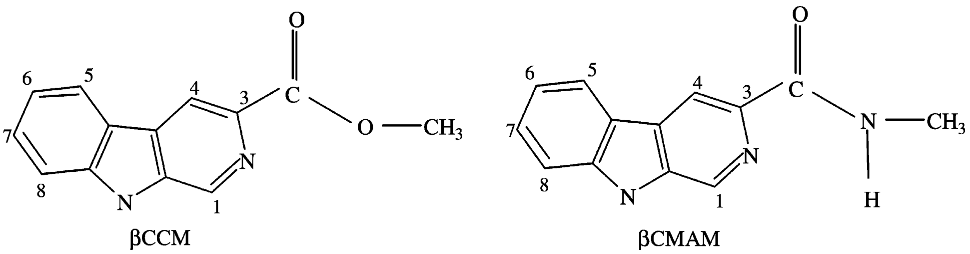

Fig. 1. Methyl-β-carboline-3-carboxylate (βCCM) and β-carboline-3-carboxylic acid *N*-methylamide (βCMAM).

properties of β-carboline-3-carboxylic acid *N*-methylamide (βCMAM, Fig. 1) in aqueous solution. It also has a voluminous substituent in position 3, close to the pyridinic nitrogen and similar results to those obtained with βCCM can be expected.

The second goal of this work is to carry out more accurate theoretical calculations on βCCM and βCMAM and the non-substituted Norharmane (βC) not only in gas phase but also taking into account the solvent in order to explain the acid base properties of these three compounds.

The last aim of this work is to determine lifetimes, quantum yield and kinetic constants of radiative and non-radiative processes of βCMAM neutral and cationic forms in aqueous solution since this has not yet been studied in water. The only photophysical study found on this compound was carried out in organic solvents of variable polarity [25].

2. Experimental

2.1. Materials

Norharmane (βC) and β-carboline-3-carboxylic acid *N*-methylamide (βCMAM) were purchased from Sigma, potassium chloride from Aldrich, glacial acetic acid (AcH) from Riedel-deHaën, benzene (uvasol grade solvent) and sulfuric acid from Fluka. All the reagents were used without further purification and the solvents were degassed bubbling nitrogen. Diluted solutions of βCMAM in water were prepared stirring the solution over 24 h and filtering the solutions with a paper filter to remove the undissolved fraction. pK_a measurements were carried out varying pH with drop addition of a 1.5×10^{-2} M aqueous solution of sulfuric acid and keeping constant the ionic strength (0.1 M KCl). Solutions were also acidified adding acetic acid (0–0.34% v/v).

2.2. Experimental and theoretical methods

Absorption spectra were obtained using a Hewlett Packard spectrophotometer HP 8453. The steady-state fluorescence spectra were measured with a Shimadzu spectrofluorimeter RF-5301 PC with a 3 nm bandwidth in excitation and 1.5 nm bandwidth in emission. Fluorescence decays were obtained using the time-correlated single-photon-counting method (Edinburgh Analytical Instruments). The excitation source was a hydrogen nanosecond flash lamp: repetition rate 40 kHz and excitation pulse width less than 1 ns. Fluorescence decays were analysed with the method of non-linear least squares iterative deconvolution and the quality of the fits was judged by the value of the reduced Chi-square (χ^2) and the autocorrelation function of the residuals. All experiments were carried out at 25 °C.

Calculations of proton affinities were carried out by means ab initio calculations using density functional method (DFT). The optimised geometries for neutral and cationic species were obtained using a B3LYP functional and 3-21G* basis set. With these geometries, single point calculations were made, also using a B3LYP functional but with a more complete 6-311G^{++}(d,p) extended basis set. In order to include the solvatation effects, a polarized continuum model (PCM) was used. In this model, the solvent is considered as a continuous medium of uniform dielectric constant. The solute occupied a spherical cavity of radius a_0 within it. A dipole in the molecule will induce a dipole in the medium, and the electric field applied by the solvent dipole will in turn interact with the molecular dipole leading to net stabilization. In this context, we used the modification of the original model proposed by Miertus and Tomasi [26]. Finally, the proton affinities were obtained as the energy difference between neutral and cationic species. All calculations were carried out using the GAUSSIAN98 (A-9 version) commercial programme.

3. Experimental results

The *absorption spectra* of βCMAM at several pH are shown in Fig. 2. The solutions were acidified with dropping addition of sulfuric acid solution (1.5×10^{-2} M) and the ionic strength was kept constant (0.1 M KCl). At 6.1 pH, in the range between 320 and 420 nm, only the neutral form absorbs with two maxima at 335 and 347 nm. The absorption of the protonated form at 376 nm increases by decreasing pH. An isosbestic point at 353 nm indicates that both species are in equilibrium. From the absorption spectra changes, the βCCM pK_a in the ground state, $pK_a(S_0)$, can be calculated [27].

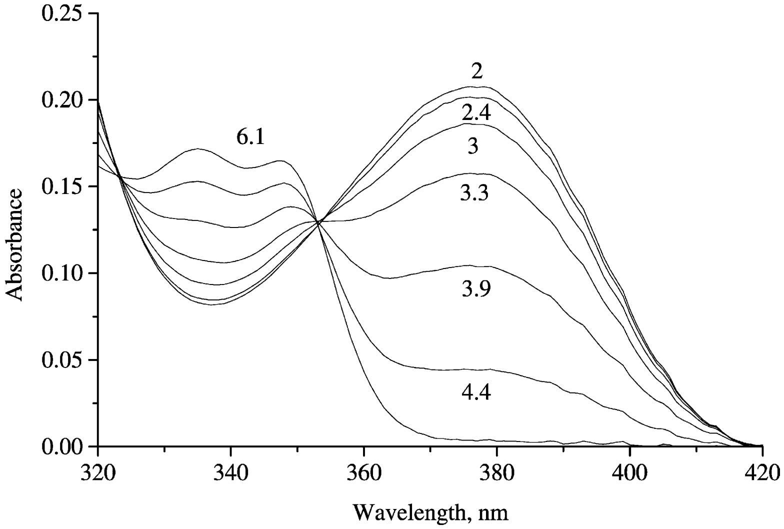

Fig. 2. Absorption spectra of βCMAM in aqueous solution at several pH (ionic strength 0.1 M KCl).

The average $pK_a(S_0)$ calculated at 335 and 377 nm is 3.85 ± 0.03. Similar experiments carried out by acidifying with acetic acid, AcH, and without KCl give a $pK_a(S_0)$ value 3.65 ± 0.02.

The *emission spectra* of βCMAM at different pH are shown in Fig. 3. By decreasing pH, the neutral form emission intensity at 387 nm diminishes and the cationic form emission at 456 nm increases. An isosbestic point at 425 nm is indicative of the equilibrium between both forms.

The pK_a of the first excited state, $pK_a(S_1)$, can be calculated from the Förster cycle [28].

The average calculated value of $pK_a(S_1)$ was 5.4 ± 0.5. Similar calculations done on the samples acidified adding AcH gave a $pK_a(S_1)$ of 5.1 which is below the experimental error.

The *quantum yields* (ϕ) of the neutral and the cationic forms of βCMAM in aqueous solution were calculated using Norharmane in benzene ($\phi = 0.30$) [21] and Norharmane in 0.05 M H_2SO_4 ($\phi = 0.60$) as standards [11], respectively. The calculated quantum yields were 0.47 ± 0.02 and 0.54 ± 0.02 for the βCMAM neutral and cationic forms, respectively. If AcH is used to diminish the pH, the cationic form quantum yield is 0.48 ± 0.03, slightly lower than the calculated one when sulfuric acid is used.

Lifetime measurements of βCMAM have been carried out for solutions with several pH (7, 4.5, 3.9, 3.3 and 2.1), acidifying with sulfuric acid, and for solutions with variable percentages of AcH (0, 0.004, 0.008, 0.07 and 0.9%). Excitation wavelengths (330 or 370 nm) and emission wavelengths (387, 456 and 520 nm) were followed. Recording the neutral form emission at 387 nm the decays were mono-exponential with a lifetime of 4.6 ± 0.3 ns. Recording the cationic form emission (456 or 520 nm) mono-exponential decays were also obtained with long lifetimes, 15.5 ± 0.3 ns.

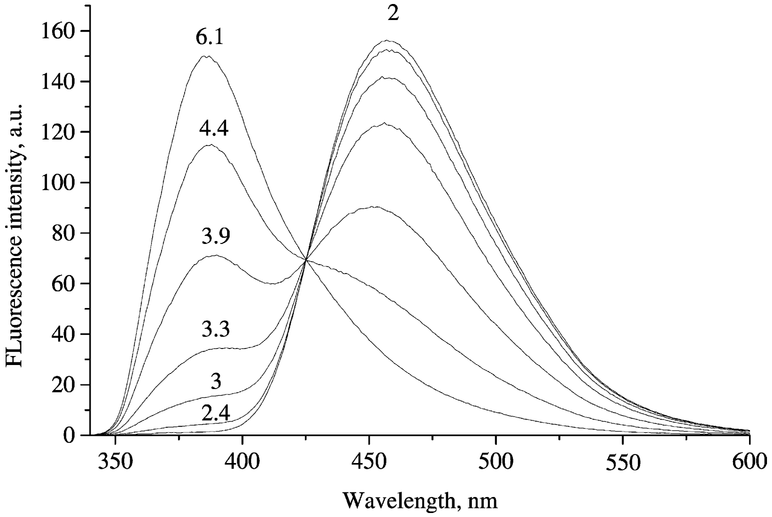

Fig. 3. Emission spectra of βCMAM in aqueous solution at several pH (ionic strength 0.1 M KCl).

From the lifetime and quantum yield of the neutral and cationic forms of βCMAM, the *radiative rate constant* (k_r) and the *non-radiative rate constant* (k_{nr}) of both forms were calculated. k_r, values are 1.02×10^8 and $3.48 \times 10^7\,s^{-1}$ for the neutral and for the cationic form, respectively, and the non-radiative rate constants, k_{nr}, were 1.15×10^8 and $2.97 \times 10^7\,s^{-1}$, for the same two forms, respectively.

4. Theoretical results and discussion

A theoretical magnitude that can be correlated to the pK_a experimental values is the proton affinity. This value is obtained as the energy difference between the neutral and the cationic species (see Section 2.2), and it has been calculated for Norharmane, βCCM and βCMAM in gas phase and in aqueous solution. We have exclusively considered the protonation on the pyridinic nitrogen because this is the most basic site of this kind of compounds [23]. The obtained results are shown in Table 1.

The proton affinity of βC shown in Table 1 is around 20 kcal/mol higher than the value obtained by Hidalgo et al. [23] with less accurate semi-empirical calculations at the AM1 level. As for the relative acidity of the three compounds, it can be said that it follows the sequence βCMAM > βC > βCCM, if only the results in gas phase are taken into account. This sequence is different from the experimental acidity sequence which is: βCMAM > βCCM > βC. Only when solvent effects are considered, the experimental results are reproduced by the calculated proton affinities.

Hidalgo et al., on the basis of AM1 calculations concluded that the gas phase is a good approach to the protonation process in solution because the proton affinity is retained in solutions [23]. However, it is proved that pK_a values of βCMAM, βCCM and βC are only explained carrying out more refined calculations in which solvent effects have to be considered. This is an important contribution because there are not many theoretical studies published on β-carbolines, no one on βCMAM and βCCM, as far as we know, and most of them do not include polarized continuum models to consider the solvent effects [21,23,29]. Although, more recent works on structurally related molecules to β-carbolines include PCM calculations or other ones to reproduce solvent effects [30–32].

The geometrical parameters of the neutral and cationic form of βCCM and βCMAM are quite similar both in the ground and the first excited state but there are two noticeable differences between them. The first one is related to the angle formed between the Norharmane skeleton and the substituent planes. Both planes are twisted an angle of 19.58° (α) for both, ground and excited states of the βCMAM protonated form. They are coplanar for βCCM neutral and cationic forms in the ground and excited state (Fig. 4, $\alpha = 0$) and for βCMAM neutral form in S_0 and S_1. The second difference is the rotational potential energy barriers when the dihedral angle between Norharmane skeleton and substituent is varied. These barriers for both compounds in the ground state are shown in Fig. 5. As it is shown in this figure, the rotation of the substituent plane with respect to the Norharmane skeleton increases the potential energy reaching a maximum when both planes are more or less perpendicular. This is quite acceptable since the π interactions between the Norharmane skeleton and the substituent carbonile group is favoured in a coplanar disposition of the skeleton and substituent. Apart from that, the rotation approximates the negatively charged pyridinic nitrogen atom and the carbonilic oxygen that reduces the structure stability. These effects are common both for βCCM and βCMAM. However, there should be other factors to take into account since the protonation increases the barriers height for βCCM and drastically decreases the rotational barriers for βCMAM. The increases in the βCCM rotation barriers could be explained by the steric hindrance produced after pyridinic nitrogen atom protonation. But, why is the same effect not observed with βCMAM? The reason could be in the N–H of the *N*-methylamide group with the hydrogen atom orientated towards the pyridinic nitrogen probably shared up to a certain point, by the two nitrogen atoms, although without forming a hydrogen bridge. This new factor for βCMAM could explain the higher rotation barrier for βCMAM that increases for rotational angles beyond 90 grades. Once that the pyridinic nitrogen atom is blocked by protonation the N–H bond of *N*-Methylamide group is not compelled to be orientated towards the pyridinic nitrogen and the rotational barriers are drastically reduced.

The information on the first excited state of βCMAM and βCCM comes from the fluorescence experiments. The main photophysical parameters of βCMAM and βCCM (neutral and cationic species) in aqueous solutions are summed up in Table 2. It can be seen that quantum yields, lifetimes and radiative rate constants of βCMAM species are higher than the same parameters for βCCM and the non-radiative rate constants of the latter are higher than the non-radiative constants of βCMAM. These results are consistent with a more hindered rotation of the *N*-methylamide group of βCMAM with respect to the methyl ester group of βCCM in the first excited state since these substituents in position 3 constitute the main structural difference between the two compounds.

Table 1
Proton affinities and pK_a values in the ground state, pK_a(S_0), of Norharmane (bC), methyl-b-carboline-3-carboxylate (bCCM) and b-carboline-3-carboxylic acid *N*-methylamide (bCMAM)

Compound	Proton affinity		pK_a(S_0)
	Gas phase (kcal/mol)	Aqueous solution (kcal/mol)	
βC	243.2	294.8	6.8 [13]
βCCM	249.5	280.3	4.4 [24]
βCMAM	231.5	271.6	3.8

Pyridinic nitrogen is the protonation site.

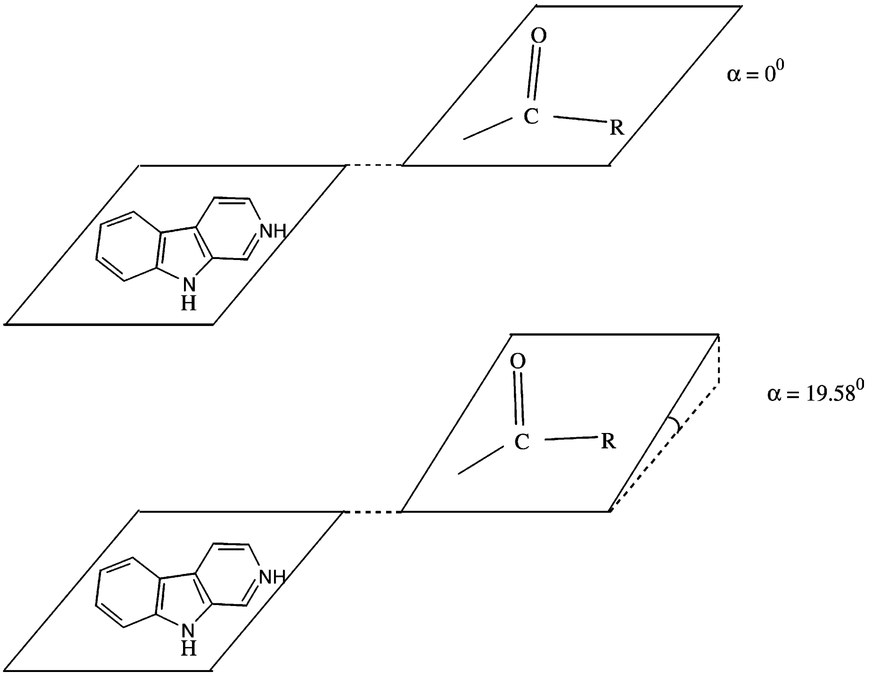

Fig. 4. Coplanar structure ($\alpha = 0^\circ$) and twisted structure ($\alpha = 19.58^\circ$).

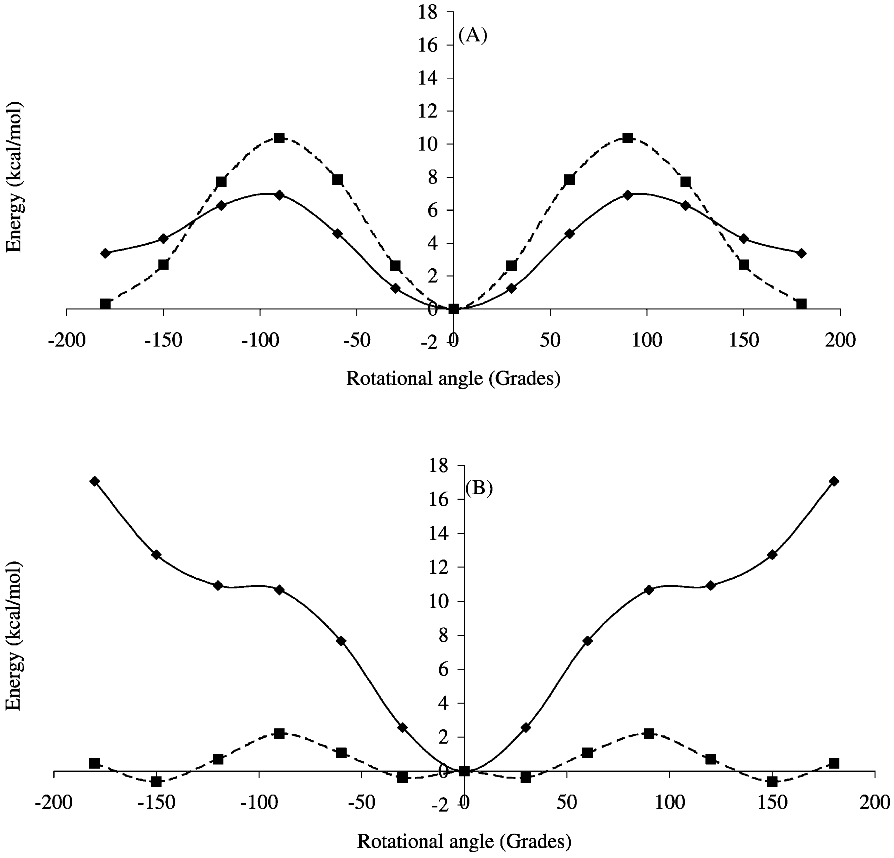

Fig. 5. Potential energy barriers for the substituent rotation in the ground state of βCCM (A) and βCMAM (B). Neutral form (continuous line), cationic form (dashed line).

Table 2
Photophysical properties of βCMAM and βCCM [24] in aqueous solution: quantum yield (ϕ), lifetimes (τ), radiative (k_r) and non-radiative (k_{nr}) constants

	ϕ^n	ϕ^c	τ^n (ns)	τ^c (ns)	$k_r^n \times 10^7$ (s^{-1})	k_r^c (s^{-1}) $\times 10^7$	k_{nr}^n (s^{-1}) $\times 10^7$	k_{nr}^c (s^{-1}) $\times 10^7$
βCMAM	0.47	0.54	4.6	15.5	10.2	3.5	11	2.9
βCCM	0.21	0.26	3.2	14.5	6.6	1.8	25	5.1

The superscripts n and c stand for the neutral and cationic forms, respectively.

For cationic form of βCMAM the optimised first excited state structure with the twist angle shown in Fig. 4 is a theoretical probe that in some cases βCMAM shows more steric hindrance.

Finally, it is important to underline that the acid–base properties both in the ground and the first excited state of βCMAM and βCCM are quite different to the other β-carboline derivatives previously studied. On the one hand their acidity, both in the ground and the first excited state, is higher than for any other β-carboline derivatives [13,17,33] that can make them useful probes in more acidic environments than other β-carbolines. On the other hand, although as for the other β-carbolines, these compounds become more basic upon excitation, the increment in the pK_a values is less than a pH unit while the average increments for the other derivatives are around 7 units in aqueous solution [17,33]. Only for Harmine, this increment is also lower than one. It is well known that β-carbolines become more basic upon excitation as a consequence of the electron charge density migration towards the pyridinic nitrogen [21]. This effect is also observed in the studied β-carboline derivatives comparing the HOMO (highest occupied molecular orbital) and LUMO (lower unoccupied molecular orbital) of βC, βCCM and βCMAM (Fig. 6). However, the electron charge density migration towards the pyridinic nitrogen is reduced in the case of βCMAM and βCCM in comparison to βC

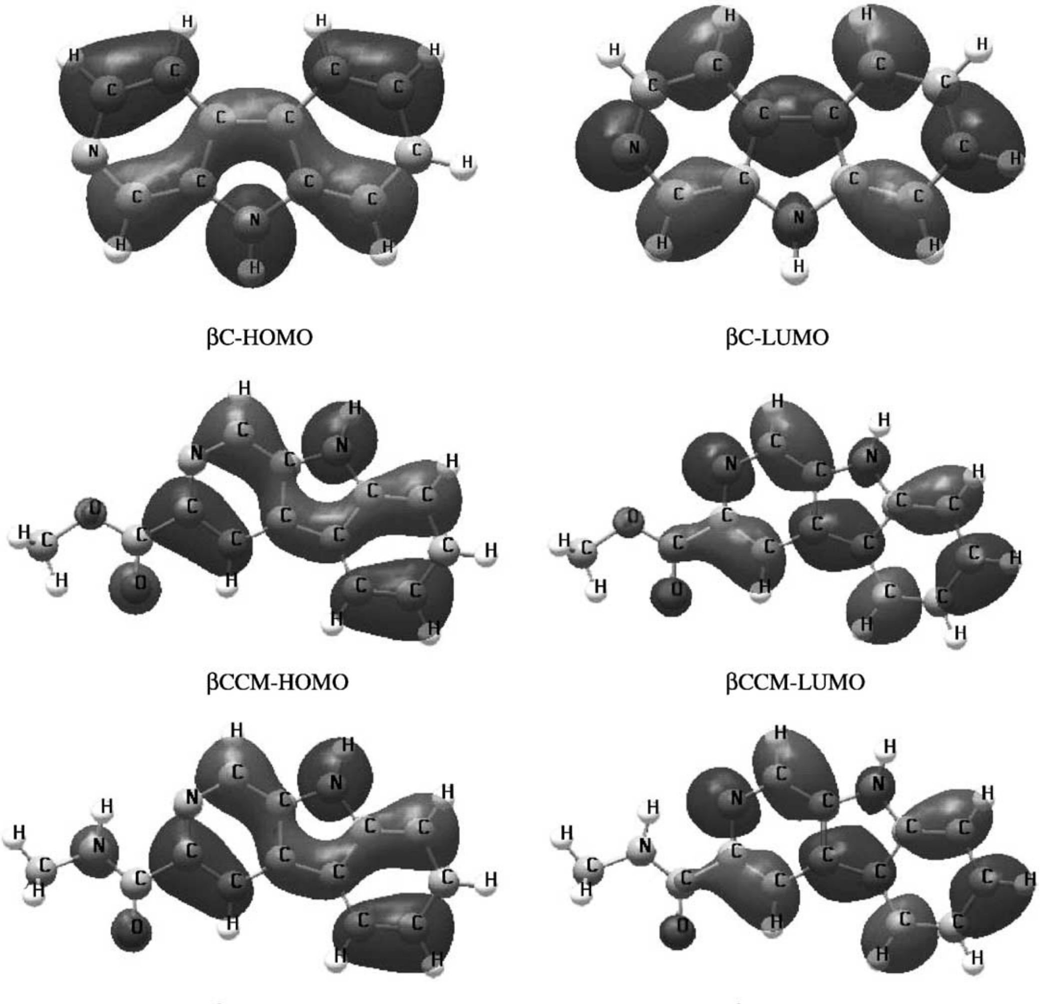
βC-HOMO βC-LUMO
βCCM-HOMO βCCM-LUMO
βCMAM-HOMO βCMAM-LUMO

Fig. 6. HOMO and LUMO orbitals of βC, βCCM and βCMAM.

due to a partially electron charge density shift towards the substituents (Fig. 6) what explains the lower increment in the pK_a values for these 3-substituted β-carbolines.

5. Conclusions

β-Carboline-3-carboxylic acid *N*-methylamide (βCMAM), as the previously studied methyl-β-carboline-3-carboxylate (βCCM), has pK_a values in the ground and the first excited electronic state lower than the average pK_a values of most of β-carboline derivatives in aqueous solutions.

The acid−base behaviour of these 3-substituted β-carbolines is explained in terms of the calculated proton affinities only when the solvent effects are taken into account in the model. This proves that the gas phase results are not always enough to explain the protonation in solution as other authors had previously suggested.

The pK_a increments between S_1 and S_0 are lower for these 3-substituted β-carbolines than for Norharmane what it is explained by the electron charge density shift towards the substituents of βCCM and βCMAM.

βCMAM differs from βCCM because it is a more acidic compound and it has higher quantum yields, longer lifetimes and longer radiative constants. These facts are explained by the structural characteristics of the susbstituents of both compounds. The substituent group at the position 3 of the protonated form of βCMAM in the first excited state shows more steric hindrance than that of the protonated βCCM in the same state.

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