Interactions between Betacarboline and Benzenoid π Bases: FTIR Evidence for the Formation of NH−π Hydrogen Bonds

María A. Muñoz,* Octavio Sama, Manuel Galán, Pilar Guardado, Carmen Carmona, and Manuel Balón[†]

Departamento de Química Física, Facultad de Farmacia, Universidad de Sevilla, 41012 Sevilla, Spain

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In the presence of benzene, naphthalene, or phenanthrene, the NH stretching band of betacarboline in tetrachloroethane diminishes in intensity while a new red-shifted band grows. These changes are attributed to the formation of molecular association complexes between betacarboline and the benzenoid compounds. Deconvolution of these IR bands allowed us to calculate their association constants, which increase linearly with the number of π electrons of the benzenoid substrates. The shifts of the associated bands were, however, independent of the bases. These results suggest that the complexes are stabilized by the hydrogen-bonding interaction between the pyrrolic NH group of betacarboline and the π-delocalized electrons of the benzenoid substrates. Low-level semiempirical calculations (AM1/MOPAC) also predict T-shaped structures as the most stable. On the other hand, experiments using harmane, the 1-methylated betacarboline, proved steric hindrance for naphthalene and phenanthrene, which should be a consequence of the geometry of the complexes.

Introduction

Betacarbolines or 9*H*-pyrido[3,4-*b*]indoles are a class of indole alkaloids which have interesting biochemical properties.[1,2] The betacarboline ring possesses potential donor and acceptor hydrogen-bonding sites, as well as strong electron donor centers.[3,4] Therefore, betacarbolines can interact with their biochemical receptors through a variety of cooperative binding forces whose relevance will markedly depend on the structure of the receptor sites and the nature of the medium.

In recent years, we have studied the interactions between harmane, HN, the 1-methyl derivative of betacarboline, and different substrates with the aim of characterizing the forces responsible for the biochemical action of these drugs. Thus, in previous works,[5,6] we have reported that HN forms 1:1 stoichiometric hydrogen-bonded complexes with pyridine, PY, and its benzene derivatives, quinoline, QN, and phenanthridine, PD, in solvents of different nature. These studies showed that, as the number of rings of the benzopyridines and the solvent polarity increases, the NH−N hydrogen-bonding interactions weaken, whereas other weaker forces, which we attributed to π−π stacking interactions,[7] became apparent.

However, bearing in mind the structural characteristics of the interacting substrates, other feasible interactions could also be operative. Thus, in recent years, clear evidence for the formation of non conventional NH−π hydrogen bonds has come from experimental[8−11] and theoretical[10−12] studies. In particular, interactions between nonaromatic molecules having NH groups and benzene derivatives have long been characterized by IR study.[13,14]

Weak hydrogen-bonding and face-to-face or edge-to-face stacking interactions are also known to exist as hybride structures or sometimes in competition.[15−18] They seem to play a key role in the conformational stability of a variety of biochemical systems, influencing the binding properties of
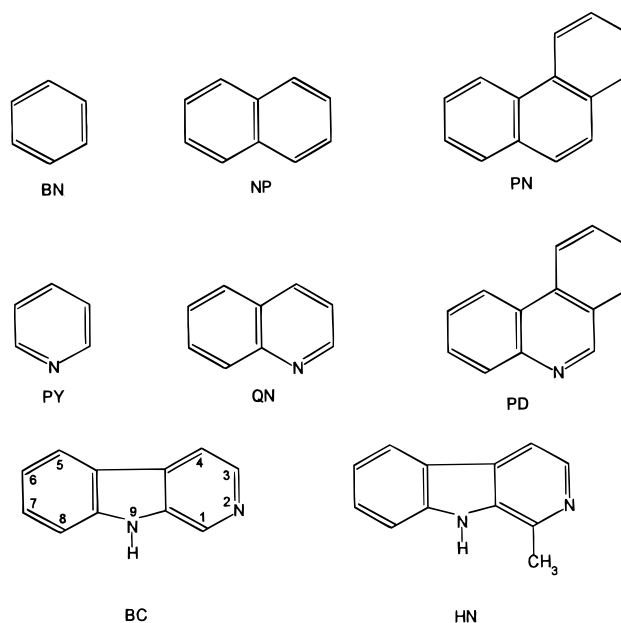
nucleic acids, the stability of proteins, etc. Taking into account the weakness of such bindings, the problem arises in the experimental separation of both contributions, which is nowadays an important matter to be elucidated.

To gain further insight into the binding ability of the betacarboline ring, we have carried out a UV−vis and FTIR study of the interactions of betacarboline, BC, and HN with benzene, BN, naphthalene, NP, and phenanthrene, PN. These molecules are similar in size and geometry to PY, QN, and PD, but lacking their nitrogen heteroatoms. Therefore, they are very adequate substrates to study π-stacking and NH−π interactions of betacarbolines without the interference of the NH−N hydrogen bonding. To carry out this study, we have selected tetrachloroethane as solvent. For comparison purposes, we have also studied the hydrogen-bonding NH−N interactions of these

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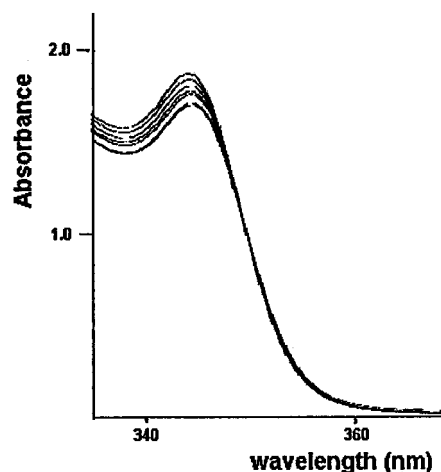*J. Phys. Chem. B, Vol. 103, No. 41, 1999* **8795**

Figure 1. Hypochromic effects in the UV−vis absorption spectrum of BC (8.4×10^{-3} M) with the increase of NP from 0 to 0.705 M. Path length 0.5 mm.

betacarbolines with the heteroaromatic bases PY, QN, and PD in this solvent.

Experimental Section

BC, HN, BN, NP, PN, PY, QN, and PD were commercially available products (Fluka, Aldrich, >98%), and they were used as received. Tetrachloroethane (Fluka, ≥98%) was stored on 4 Å molecular sieves.

The UV−vis absorption spectra were made on a Perkin-Elmer Lambda 5 spectrophotometer. To carry out these measurements in the same concentration range used in infrared experiments, quartz microcells of 0.1 or 0.5 mm path length were employed.

IR spectra were recorded on a Mattson FTIR Galaxy 2020 spectrometer, equipped with a mercury−cadmium−telluride detector. The spectral resolution was 2 cm^{-1}, the spectral range was 4000−700 cm^{-1}, and 400 scans were co-added to obtain each spectrum. A liquid cell, with SnZn windows and 0.1 cm path length, was used. All measurements were made at room temperature.

The solutions contained a fixed concentration of carboline (ca. 8×10^{-3} M) and varying concentrations, in at least 10-fold excess, of the acceptors. Blank spectra of the last compounds, at the appropriate concentrations, were always subtracted from those of the mixed solutions.

When the IR spectra of the donor and the complex over-lapped, the bands were deconvoluted by using the Peakfit program, v. 4.0, of Jandel Scientific. The hidden peaks were detected by the second-derivative method, and fitted to an asymmetric logistic peak function given by the program. Deconvolution analysis was achieved using the parameters of the monomer NH band (center, width, and shape) as the reference. Then, after the addition of each acceptor concentration, averages of the above parameters were calculated for the associated band. Finally, the fits were repeated with the height of the bands as floating parameters. The goodness of the deconvolution fits was always excellent ($r^2 > 0.99$).

Results

1. BC−Benzenoid Systems. The addition of increasing amounts of BN or NP produces only a slight hypochromic effect on the UV−vis spectrum of BC, as is shown for the BC−NP system in Figure 1. Unfortunately, spectral overlapping pre-cluded these measurements in the case of PN. Hypochromic

effects on the UV−vis spectra have often been imputed to the molecular pairing caused by $\pi-\pi$ stacking interactions.[19]

However, the changes observed in the IR spectrum of BC point to a different class of action. Thus, stacking interactions are usually revealed by small high-frequency shifts of stretching fundamentals.[20,21] Conversely, as Figure 2 shows, upon the addition of increasing concentrations of BN, NP, or PN, the NH stretching band of BC at 3461 cm^{-1} decreases in intensity and, simultaneously, a new red-shifted band appears. Decon-volution analyses, as illustrated in Figure 3 for the BC−PN system, show that the associated bands lay 36−39 cm^{-1} to the red side of the main band; see Table 1. The other bands in the IR spectrum of BC only experience minor changes.

It should be mentioned that we also observed similar spectral shifts with other benzene derivatives. Thus, the NH stretching band of BC in toluene, *m*-xylene, and mesitylene, as the solvents is shifted to the red by 43, 50, and 59 cm^{-1}, respectively, with respect to that in tetrachloroethane.

The appearance of new bands and the presence of isosbestic points in the IR spectra show the formation of association complexes between the benzene derivatives and BC in which the NH pyrrolic group is engaged. The association constants, K_{asc}, and the stoichiometry of the complexes, n, were obtained from the following Benesi−Hildebrand equation:

$$\log(A_M^0/A_M - 1) = \log K_{asc} + n \log c_A \qquad (1)$$

A_M^0 and A_M being the absorbances of BC at 3461 cm^{-1}, initially, and after the addition of variable concentrations, c_A, of the benzene derivative, respectively.

As Figure 4 shows, plots according to eq 1 are linear with slopes close to unity; see Table 1. Therefore, BC forms 1:1 stoichiometric complexes with BN and its polycyclic derivatives NP and PN. The association constants, K_{asc}, obtained from the intercepts of these plots are reported in Table 1. These data show that the interaction increases almost linearly with the number of benzene rings of the substrates.

On the other hand, the molar extinction coefficients of the complexes, ϵ_c, reported in Table 1, were obtained from the equation

$$\log\left(\frac{A_c}{A_M}\right) = \log\left(K_{asc}\frac{\epsilon_c}{\epsilon_N} + n \log c_A\right) \qquad (2)$$

where A_c, represents the absorbances, at the maximum wave-length of the associated bands, obtained from the deconvolution analyses. The plots according to this equation are shown in Figure 5. As the data in Table 1 show, the extinction coefficients of the complexes are of the same order of magnitude as that of the free BC (225 ± 7 M^{-1} cm^{-1}).

At this point, it should be noticed that the absorbances of the BC solutions measured at the NH band obey Beer's law up to at least 10^{-2} M. Thus, self-association of BC can be neglected in this range of concentration. Furthermore, despite the well-known ability of the benzenoid substrates to form dimers in solution,[15] our results show that self-associations of these substrates are also negligible. This is supported by the excellent linearity of the plots in Figure 4.

2. BC−Benzopyridine Systems. The changes produced by the addition of PY and its benzoderivatives, QN and PD, in the UV−vis and FTIR spectra of BC are typically shown for the BC−QN system in Figures 6 and 7, respectively. These changes are expected for the formation of NH−N hydrogen-bonded complexes between these substrates.[5,6] Thus, the UV−vis spectrum of BC is broadened and bathochromically shifted as the concentration of the benzopyridine increases. Likewise, the
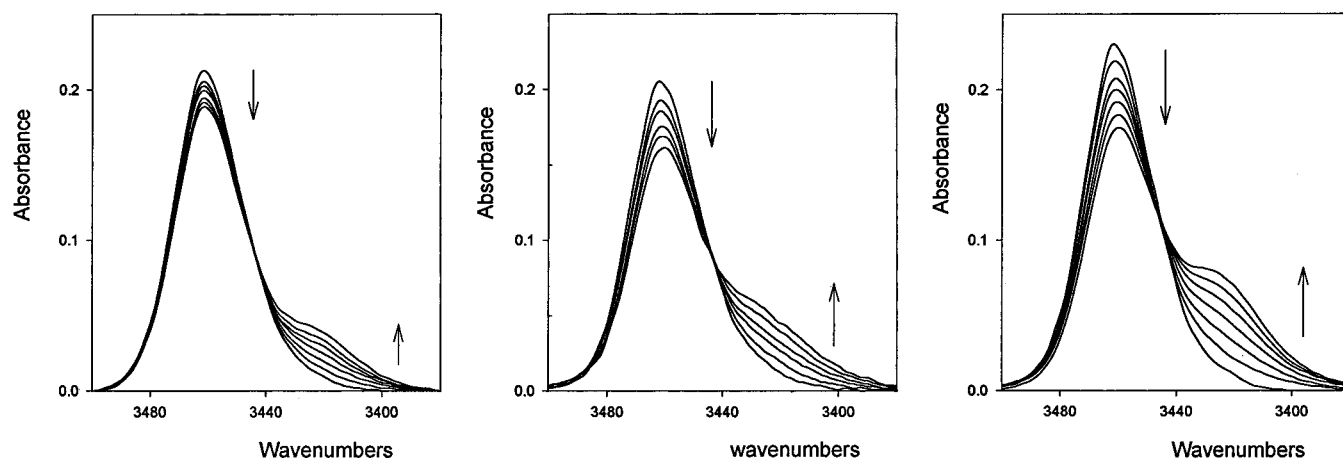
Figure 2. Changes in the IR spectrum of BC with the increase of (a, left) BN (0.141−0.846 M), [BC] = 8.16 × 10^{-3} M, (b, middle) NP (0.141−0.705 M), [BC] = 8.44 × 10^{-3} M, and (c, right) PN (0.094−0.567 M), [BC] = 8.49 × 10^{-3} M.
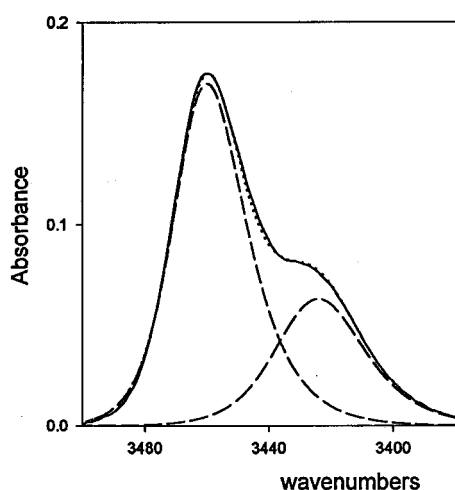

Figure 3. Deconvolution fit for the BC−PN system: experimental spectrum (full line), deconvoluted bands (dashed lines), summation (dotted line).

Figure 4. Equation 1 plots for BC−BN (●), BC−NP (○), and BC−PN (▼) systems (λ = 3461 cm^{-1}).

NH stretching band of BC diminishes in intensity while a broad new band grows around 3100−3200 cm^{-1}. These frequency shifts ($\Delta\nu$) are reported in Table 1. As can be observed in Figure 7, the associated bands show the continuum absorption due to the large polarizability of the protons in these NH−N hydrogen bonds.[22]

The association constants and the extinction coefficients of the hydrogen-bonded BC−benzopyridine complexes, estimated as previously from eqs 1 and 2, are reported in Table 1. As these data show, the strength of the hydrogen-bonding interaction does not appreciably change when benzene rings are added to the active pyridine center. This behavior is expected taking
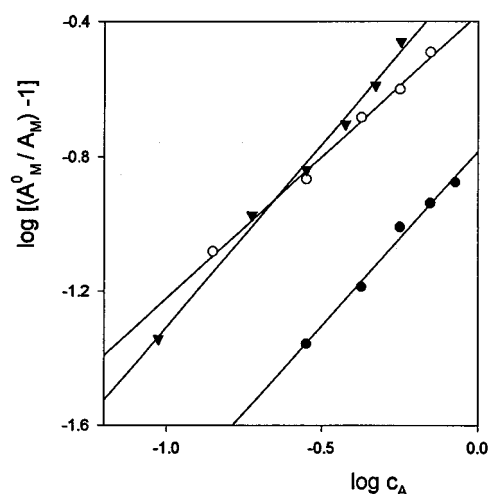
into account the similar hydrogen-bonding acceptor properties reported for PY, QN, and PD.[23]

3. HN−Benzenoid and HN−Benzopyridine Systems. To obtain additional information on the nature of the BC−benzenoid complexes, we were interested in studying the influence exerted by the methyl group of HN on the formation of each type of complex. The results of this study are summarized in Table 1. These data show that HN interacts with benzene and pyridine derivatives similarly to BC. However, whereas the 1-methylation of BC has an important destabilizing effect on the benzenoid complexes, it has only a minor effect on the benzopyridinic ones. These results suggest that both type of complexes should have different geometrical constraints.

TABLE 1: Association Constants and Spectral Characteristics of the BC and HN Complexes[a]

	BC				HN			
	K_{asc} (M^{-1})	$\Delta\upsilon$ (cm^{-1})	n	$\epsilon_{asc} \times 10^{-1}$ (M^{-1} cm^{-1})	K_{asc} (M^{-1})	$\Delta\upsilon$ (cm^{-1})	n	$\epsilon_{as} \times 10^{-1}$ (M^{-1} cm^{-1})
BN	0.16 ± 0.06	39	0.96 ± 0.09	26 ± 1	0.145 ± 0.006	30	1.06 ± 0.04	21 ± 1
NP	0.42 ± 0.06	36	0.9 ± 0.1	21 ± 1	0.20 ± 0.01	29	0.98 ± 0.02	23 ± 1
PN	0.65 ± 0.04	37	1.16 ± 0.04	25 ± 1	0.23 ± 0.01	28	1.51 ± 0.04	22 ± 1
PY	1.86 ± 0.03	320	1.06 ± 0.01	12 ± 2	2.5 ± 0.2	320	1.09 ± 0.08	14 ± 2
QN	1.86 ± 0.09	303	1.12 ± 0.04	9 ± 2	1.86 ± 0.09	322	1.0 ± 0.1	13 ± 1
PD	2.04 ± 0.09	314	1.16 ± 0.04		1.86 ± 0.09	318	1.03 ± 0.04	14 ± 1

[a] $\epsilon_{BC}^{3461cm^{-1}}$ = 225 ± 7 M^{-1} cm^{-1}; $\epsilon_{HN}^{3463cm^{-1}}$ = 233 ± 9 M^{-1} cm^{-1}.

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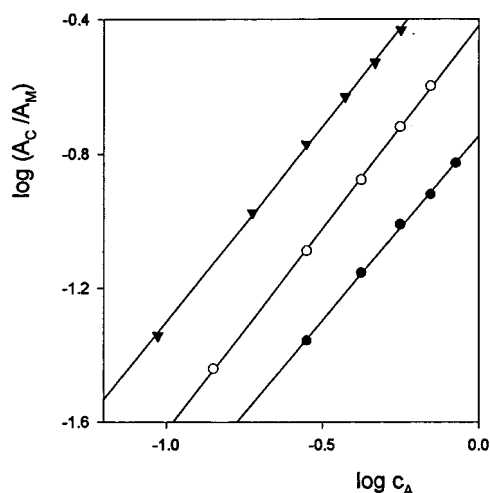*J. Phys. Chem. B, Vol. 103, No. 41, 1999* **8797**

Figure 5. Equation 2 plots for BC−BN (●), BC−NP (○), and BC−PN (▼) systems (A_c at 3422, 3425, and 3424 cm^{-1} for BN, NP, and PN, respectively).
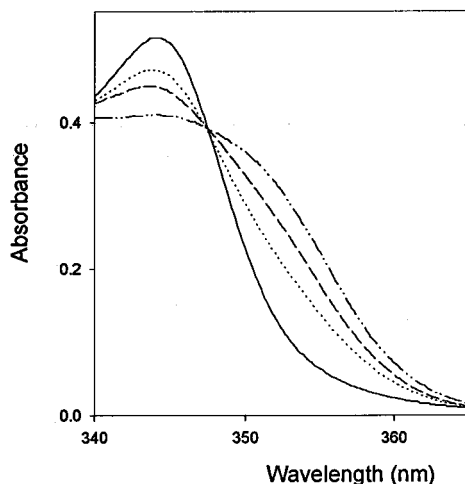

Figure 6. Changes in the UV−vis absorption spectrum of BC (8.4 × 10^{-3} M) with the increase of QN from 0 M (full line) to 0.846 M (-·-·-). Path length 0.1 mm.
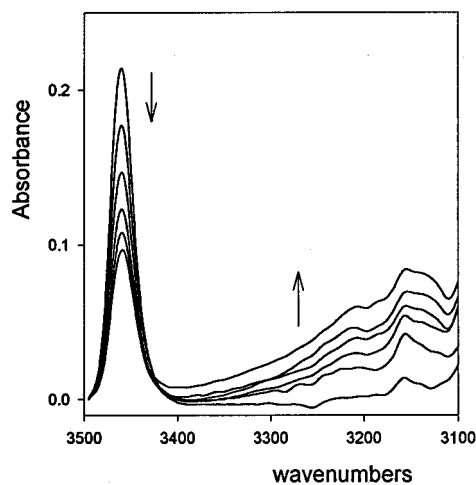

Figure 7. Changes in the IR spectrum of BC (8.3 × 10^{-3} M) with the increase of QN: 0.141−0.705 M.

Discussion

The results obtained in this work prove that BC forms 1:1 stoichiometric complexes with benzene derivatives. However, it seems rather unlikely that they have a stacked $\pi-\pi$ geometry.

Thus, π-stacking interactions would produce shifts in the fingerprint region of the IR spectrum of BC. Nevertheless, our FTIR experiments clearly show that only the NH vibration band of BC is significantly modified upon interaction with the benzenoid substrates. Therefore, the NH pyrrolic group must play an essential role in these interactions.

Interestingly, the red shift and the broadening experienced by the NH stretching band of BC and HN upon complexation are the features expected for NH hydrogen-bonding interactions. In fact, these changes are similar to, but less pronounced than those observed for the BC−benzopyridine and HN−benzopyridine systems, which have been used in this work as the prototype of NH−N hydrogen-bonding interactions. Therefore, it seems reasonable to assume that hydrogen bonds are the forces responsible for the stabilization of the BC−benzenoid complexes.

Thus, considering the structural characteristics of the interacting substrates, these complexes should be formed by the hydrogen-bonding interaction between the pyrrolic NH group of the BC and the π-electron cloud of the benzenoid substrates. This hypothesis is further supported by the experimental results obtained for the HN−benzenoid systems. Thus, the steric hindrance of the 1-methyl group of HN would have, as it is experimentally observed, a destabilizing influence on these NH−π interactions. Conversely, owing to their different geometries, this group has a smaller influence on the stability of the HN−benzopyridine complexes.

As stated in the Introduction, the literature provides several references on benzene derivatives acting as π-acceptors of hydrogen bonds against NH pyrrolic groups.[24] Thus, in a much earlier study, Josien et al.[25] measured a shift in the NH frequency of pyrrole in Cl$_4$C of around 35 cm^{-1} upon the addition of BN. More recently, an indole−BN hydrogen-bonded complex has been found by threshold ion (MATI) spectroscopy.[26] Also, in a recent study on the quenching of indole fluorescence by monosubstituted benzenes,[27] a ground-state quenching constant of 0.19 M^{-1} for the indole−BN pair in cyclohexane has been reported. Although this quenching was ascribed to a sphere of action effect, we think a more likely explanation is the formation of a ground-state NH−π complex.

As has been observed by other authors,[8,14] the red shifts of the vibrational NH−π bands depend on the π electron density of the benzenoid acceptor. In our experiments, the spectral shifts are independent of the acceptor employed, which is consistent with the similar π electron density of BN, NP, and PN.[28] Moreover, as our results using methylbenzene derivatives as the solvents show, the NH band of BC undergoes larger displacements when the π-electron density of the acceptor is enhanced by methyl substitution on the benzene ring. These results support our assumption on the formation of NH−π hydrogen bonds.

Quite simple semiempirical calculations (AM1/MOPAC)[29] allowed us a plausible rationalization of the experimental results. Thus, in all the cases, these calculations showed a minimum energy (~1.2 kcal mol^{-1}) at equilibrium distances of ~2.3 Å for the T-shaped hydrogen-bonded complexes. Conversely, no minima were found for stacking structures. Furthermore, this starting geometry always gave a T-shaped structure as the most stable configuration. Additionally, these calculations also showed that the interaction energy of the pyrrolic hydrogen with the different benzene rings of NP and PN is the same.

On this basis, the higher constant values experimentally obtained for these substrates should be ascribed to an enhancement of the entropic contribution to the free energy on increasing

the number of benzene rings in the molecule. Thus, the rise of the experimental association constant values from BN to PN is an effect related to the chance of interaction as the number of rings increases, rather than a strengthening of the binding forces. This assumption, on the other hand, could reasonably explain the smaller values of the association constants obtained for the NH$-\pi$ hydrogen-bonded complexes of HN. Due to steric hindrance of its 1-methyl group, the ability of the NH group of HN to interact with the different benzene rings of the polycyclic molecules would be strongly reduced. Thus, the suppression of this entropic factor should have, as experimentally observed, a leveling effect on the values of the association constants of the complexes.

We can, therefore, conclude that the betacarboline ring forms nonconventional NH$-\pi$ hydrogen bonds through its pyrrolic nitrogen atom, and the strength of the interaction increases as the π-electron density of the benzene acceptor increases. IR spectra provide a useful tool to identify and quantify the parameters involved in this type of hydrogen bond.

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