

A spectroscopic study of the molecular interactions of harmane with pyrimidine and other diazines

M.A. Muñoz, P. Guardado, M. Galán, C. Carmona, M. Balón*

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

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Abstract

FTIR, UV-vis, steady state and time-resolved fluorescence measurements show that harmane (1-methyl-9H-pyrido/3,4-*b*/indole) interacts with pyrimidine and its isomers pyrazine and pyridazine in its ground and lowest singlet states. The mechanisms of interaction are dependent on both the structure of the diazine and the nature of the solvent. Thus, in a low polar solvent such as toluene, harmane forms ground state 1:1 hydrogen-bonded complexes with all the diazines. These complexes quench the fluorescence of harmane and diminish its fluorescence lifetime. Conversely, in buffered (pH 8.7) aqueous solutions, pyrimidine behaves differently from the other diazines. Thus, whereas pyrimidine only interacts with harmane in its ground state, pyrazine and pyridazine also interact in the excited state. The harmane–pyrimidine ground state interaction is an entropic controlled process. Therefore, we propose the formation of π – π stacked 1:1 complexes between these substrates. Association constants for the different types of complexes and quenching parameters are reported. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Hydrogen-bonding; Hydrophobic interactions; π – π stacked complexes; Betacarbolines; Pyrimidine; Pyrazine; Pyridazine

1. Introduction

In the last years, the betacarboline alkaloids [1,2] (9H-pyrido/3,4-*b*/indoles) have attracted

much attention because of their interesting biochemical properties as cytotoxic and neuroregulatory agents [3–6]. It has been suggested that, as for many other drugs, these properties could be related to the formation of molecular association complexes between the betacarbolines and ADN or other biological substrates. In fact, the peculiar structures of the betacarbolines enable them to interact with a great variety of receptor

* Corresponding author. Tel.: +34-95-455-6733; fax: +34-95-455-7174.

E-mail address: balon@fajar.us.es (M. Balón)

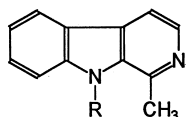
sites. Thus, on the one hand, their pyridinic and pyrrolic nitrogen atoms are donor and acceptor sites, respectively, for hydrogen-bonding interactions. On the other hand, the existence of strong electron donor centres in the betacarboline ring and its planarity [7], allow these compounds to form π – π stacked complexes with suitable substrates.

To get a deeper insight into the nature of their biochemical properties, we are carrying out studies on the interactions of betacarbolines with model heteroaromatic rings [8,9]. Within this context, we present now a spectroscopic study on the ground and singlet excited state interactions of harmane (1-methyl-9*H*-pyrido[3,4-*b*]indole) (HN), with pyrimidine (PM), pyrazine (PZ) and pyridazine (PD). These diazines, besides their intrinsic interest as prototypical heteroaromatic rings, also possess interesting biochemical properties. In particular, PM [10] is the parent heterocycle of a very important group of compounds that have been extensively studied due to their occurrence in living systems.

2. Experimental

HN, PM, PZ and PD were commercial products

STRUCTURAL FORMULAE



HN R = H
MHN R = CH₃



PM



PZ



PD

of the best available quality (Sigma-Aldrich) and were used as received. *N*₉-Methylharmane (MHN), was prepared according to literature methods [11]. Spectral grade toluene was stored on 4 Å molecular sieves. Other chemicals were reagent grade commercial products and were used without further purification.

Stationary fluorescence measurements were carried out in a Perkin Elmer 650-40 spectrofluorimeter equipped with a Perkin Elmer data processor 650-0178 to obtain corrected spectra (rhodamine-B as quantum counter). Fluorescence measurements were carried out with non-degassed solutions under temperature controlled conditions (25 ± 0.1°C). Dilute solutions of HN and MHN (~4 × 10⁻⁵ M) were used to avoid inner filter effects and reabsorption phenomena. Sometimes, when the absorption of the heteroaromatic base at the excitation wavelength was significant, a triangular quartz cell was used.

Fluorescence lifetimes were measured with an Edinburgh Analytical Instruments CD-900 spectrometer employing the time correlated single photon counting technique. The source was a 0.4-bar H₂ nanosecond flashlamp operating at 6.8 kV and with a repetition rate of 40.0 kHz. The decay functions were deconvoluted and the quality of the fits analysed by the randomness of the residuals and the reduced χ^2 (< 1.2).

The UV-visible absorption spectra were recorded on a Perkin Elmer Lambda-5 spectrophotometer. Absorbance measurements were made in quartz cells of 1 cm pathlength thermostated to within 25 ± 0.1°C. Infrared spectra were run on a Mattson FTIR Galaxy 2020 spectrometer, equipped with a mercury–cadmium–telluride detector with a resolution of 4 cm⁻¹. The spectra were recorded using a 0.1-cm pathlength liquid cell equipped with ZnSe windows. FTIR spectra were measured in toluene at room temperature by using solutions containing a constant amount of HN and at least a 10-fold excess of variable concentration of the corresponding diazine. Blank spectra of the diazine at the appropriate concentration were subtracted from those of the mixed solution.

UV-vis and fluorescence measurements in water were made at constant ionic strength (*I* = 0.2

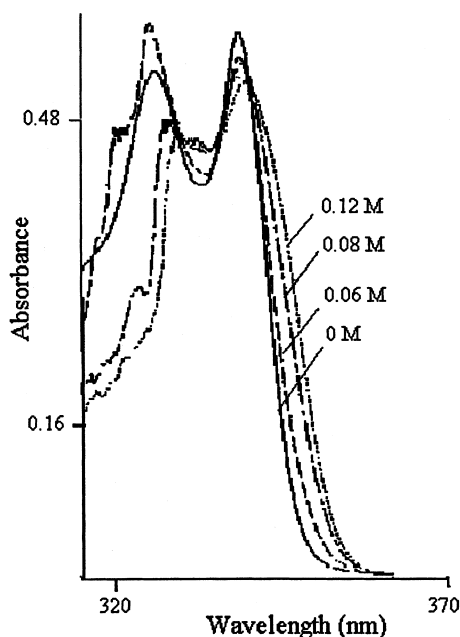


Fig. 1. Changes in the UV-vis absorption spectrum of HN ($\sim 10^{-4}$ M) in cyclohexane with the increase of PM concentration.

M) in $\text{NH}_3\text{--NH}_4\text{Cl}$ (pH 8.7) buffered solutions. Under these conditions, more than 95% of HN is present in the ground state as the neutral form ($\text{p}K_a = 7.34$) [12]. However, it should be noted that, neutral species protonate upon excitation to the lowest singlet state. Therefore, the emitting species of the fluorescence spectra in these media are the pyridinic protonated cations of HN.

3. Results and discussion

3.1. Interactions of harmane with diazines in toluene

3.1.1. UV-vis and FTIR absorption measurements

As shown typically for the HN–PM system in Fig. 1, the absorption spectrum of HN in cyclohexane is red-shifted and slightly broadened with the increase of the diazine concentrations. Similar spectral shifts are also observed in other low polar solvents such as toluene or chloroform.

These changes resemble those previously observed in the HN–pyridine system upon hydrogen-bonding complexation [8]. Therefore, to test this possibility we have studied the effect that the addition of the diazines produces on the fundamental stretching frequency of the pyrrolic NH bond of HN.

As shown in Fig. 2, in the presence of the diazines the N–H stretching band of HN in toluene at 3433 cm^{-1} decreases in intensity and, simultaneously, a new broad associate band appears in the $3400\text{--}3100\text{ cm}^{-1}$ region of the spectra. These results conclusively show that the pyrrolic NH group of HN is engaged in the formation of hydrogen-bonded complexes with the diazines. Furthermore, the broadness of the associate bands suggests an important degree of proton transfer within the hydrogen bond. As it is well known, hydrogen bonds involving proton transfer equilibria ($\text{AH}\dots\text{B} \leftrightarrow \text{A}^- \dots \text{H}^+\text{B}$) show large proton polarizabilities due to the proton motion. Because of the large polarizabilities, these hydrogen bonds interact very strongly with the environment so that continua instead of bands are observed in the infrared spectra [13].

3.1.2. Steady-state and time-resolved fluorescence measurements

When the toluene solutions of HN in the presence of increasing amounts of diazine are excited close to the isosbestic points of their absorption spectra, neither the shape nor the maxima of the spectra change. However, the fluorescence intensities progressively diminish, as typically shown in Fig. 3 for the HN–PM system. On the other hand, the fluorescence of these solutions decays monoexponentially, decreasing the lifetimes with the increase of the diazine concentration. It should be noted that the fluorescence of HN in toluene decays monoexponentially with a lifetime $\tau_0 = 2.7 \pm 0.1\text{ ns}$. These results reveal the simultaneous presence of static and dynamic components in the quenching mechanism. Therefore, quenching data were analysed by the model depicted in Scheme 1.

According to Scheme 1, photoexcited HN^* ei-

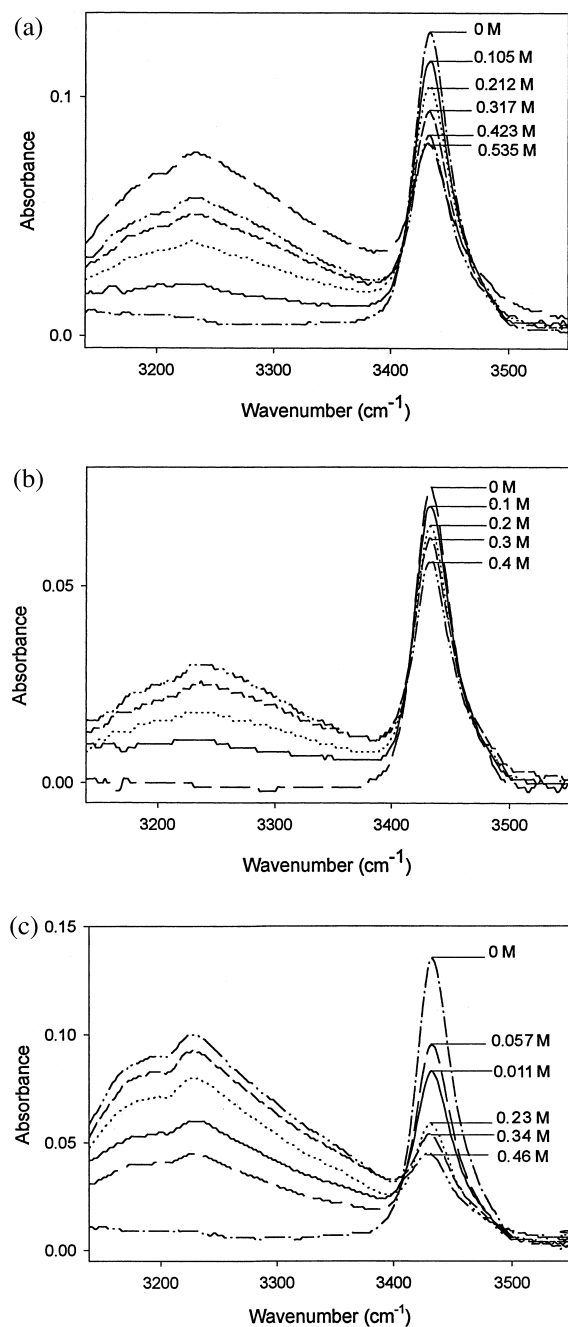


Fig. 2. Changes in the FTIR absorption spectrum of HN in toluene with the increase of (a) PM, (b) PZ and (c) PD concentrations. Concentration of HN: (a), (c) 0.01 M; (b) $6 \cdot 10^{-3}$ M.

ther fluoresces (rate constant k_f), undergoes radiationless transitions (rate constant k_i) or it is

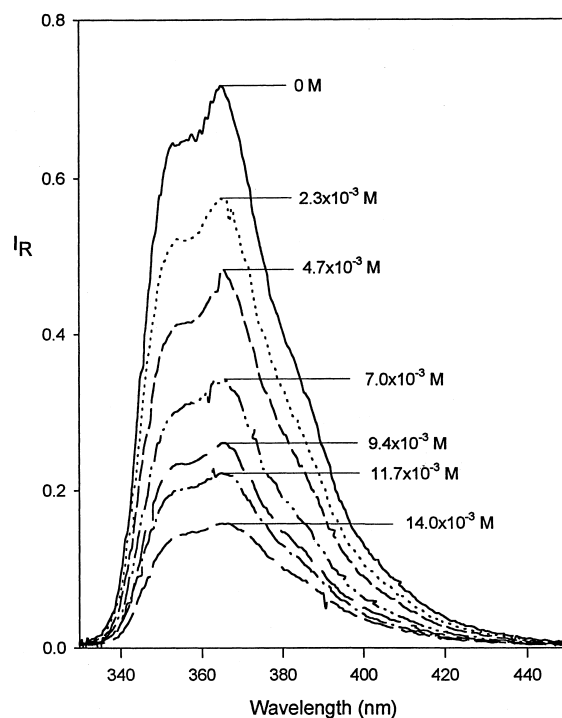
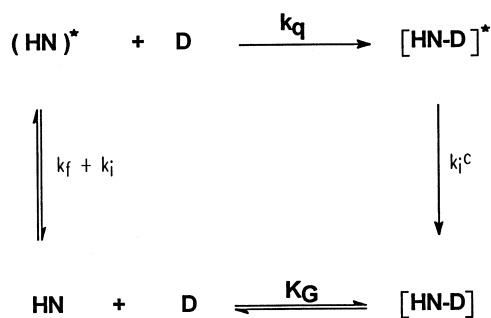


Fig. 3. Quenching of HN fluorescence in toluene with the increase of PM concentration.

quenched (rate constant k_q). The singlet excited complexes $[\text{HN-D}]^*$ are assumed to give no detectable emission at the monitoring wavelength (radiationless rate constant k_i^c). Under photostationary conditions, i.e. $d[\text{HN}^*]/dt = 0$, and for excitation at an isosbestic point, the model leads to the following Stern–Volmer equations [9]:

$$(I_0/I) = (1 + K_G \cdot [D])(1 + k_q \cdot \tau_0 \cdot [D]) \quad (1)$$



Scheme 1.

and

$$\frac{\tau_0}{\tau} = 1 + k_q \cdot \tau_0 \cdot [D]. \quad (2)$$

The combination of Eqs. (1) and (2) gives:

$$\frac{(I_0/I)}{(\tau_0/\tau)} = 1 + K_G \cdot [D]. \quad (3)$$

As shown in Figs. 4 and 5, the plots of the quenching data according to Eqs. (2) and (3) are reasonably linear. The values of K_G and k_q obtained from these equations are reported in Table 1. The K_G and k_q values in Table 1 are similar to those of related systems [8,9]. On the other hand, the values of the quenching constants, k_q , are rather close to those theoretically expected for a diffusion controlled reaction.

Interestingly, in the case of compound MHN, which has no NH group for hydrogen-bonding, the diazines neither quench its steady state fluorescence nor affect its fluorescence lifetime ($\tau_0 = 2.24$ ns). Therefore, these results show that hydrogen-bonding interactions operate in the ground and also in the excited state. This behaviour was previously observed in the HN–pyridine system [8]. In this case, we were able to observe the very weak emission from the hydrogen-bonded HN–pyridine complex in the singlet excited state. Thus, we presume that the formation of an excited state hydrogen bond is a rather general mechanism for the fluorescence quenching of HN by *N*-heteroaromatic bases. This dynamic quenching, as proposed by Mataga [14–16], could be

Table 1

Ground state association constants and quenching data for the interaction of harmane with diazines in toluene at 298 K

	Pyrimidine	Pyrazine	Pyridazine
β_2^{Ha}	0.65	0.62	0.81
$K_{G,FL} (M^{-1})$	4 ± 1	5.0 ± 0.8	— ^b
$k_q (10^9 M^{-1} s^{-1})$	5.1	5.4	9.9

^a Hydrogen bonding acceptor parameters taken from Abraham et al. [19].

^b Could not be measured due to the great magnitude of the inner filter and reabsorption effects.

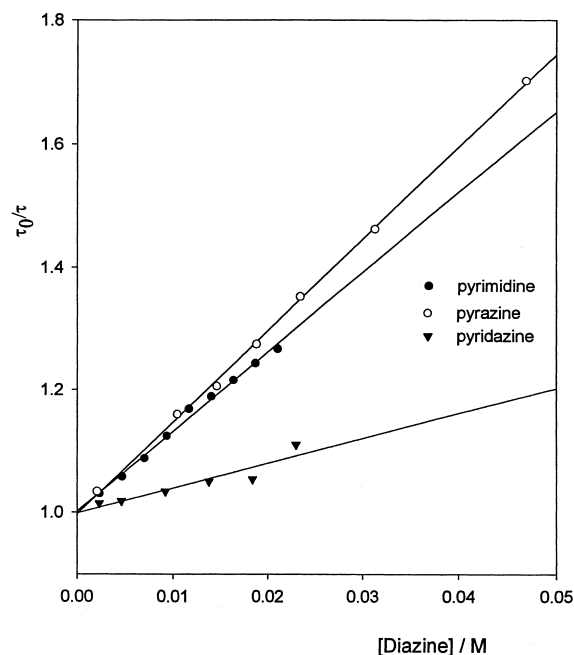


Fig. 4. Stern–Volmer plots [Eq. (2)] for the quenching of HN lifetime by PM ($10 \times [\text{Diazine}]$), PZ ($10 \times [\text{Diazine}]$) and PD ($10^2 \times [\text{Diazine}]$) in toluene at 298 K.

related with a charge transfer interaction between the hydrogen-bonded aromatic molecules.

3.2. Interactions of harmane with diazines in buffered (pH 8.7) aqueous solutions

3.2.1. UV-vis absorption measurements

In buffered (pH 8.7) aqueous solutions, the changes in the UV-visible absorption spectra of HN upon addition of diazines are clearly different to those observed in the low polar solvent toluene. Thus, the diazines produce a decrease of the absorbance of HN without any appreciable change in the position of the maxima. These changes are small for PZ and PD and more pronounced for PM.

The presence of isosbestic points in the spectra of the HN–PM mixtures (see Fig. 6) show the formation of a stoichiometric ground state complex between these substrates. The formation constant, K_G , was calculated at 298 K using the

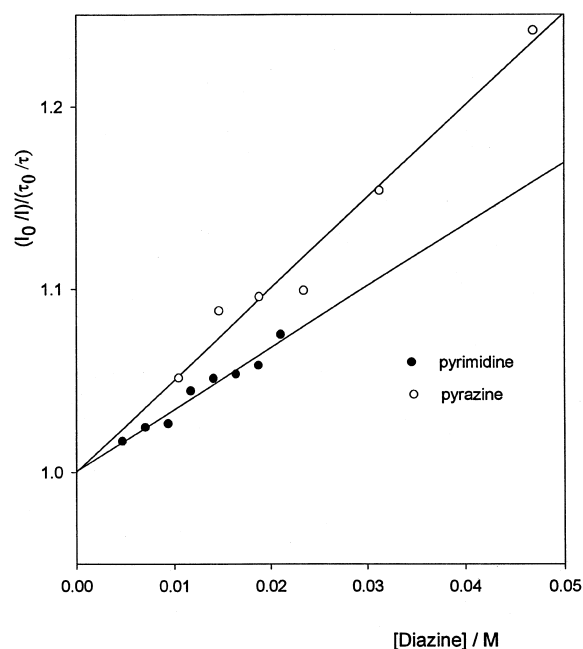


Fig. 5. Plots of the $(I_0/I)/(\tau_0/\tau)$ quenching ratio (Eq. (3)) of HN against PM and PZ concentrations in toluene at 298 K.

following Benesi–Hildebrand equation for 1:1 stoichiometric binding:

$$\frac{1}{\Delta A} = \frac{1}{(\varepsilon_c - \varepsilon)} \frac{1}{[HN]_0} + \frac{1}{K_G(\varepsilon_c - \varepsilon)[HN]_0} \frac{1}{[D]} \quad (4)$$

where ε_c and ε are the molar extinction coefficients at the titration wavelength of the complex and HN, respectively; ΔA is the absorbance change of the mixture relative to the completely free HN at this wavelength. As shown in the inset of Fig. 6, the appropriate plot of the experimental data according to Eq. (4) gives a straight line from whose slope and intercept we obtain the values of $270 \text{ M}^{-1}\text{cm}^{-1}$ and $13.5 \pm 0.1 \text{ M}^{-1}$ for ε_c and K_G , respectively.

In the case of compounds PZ and PD, the small changes of the absorption spectra of HN do not allow one to draw conclusions about the possible formation of ground state complexes. However, as we will see below, the existence of a small static component in the quenching of the

HN fluorescence by these diazines could be interpreted in this sense.

3.2.2. Steady-state and fluorescence measurements

At pH 8.7, the fluorescence spectrum of HN shows a band centred at 434 nm. This band, as stated in Section 2, corresponds to the emission of the pyridinic protonated cations of HN. As it is typically shown for the HN–PM solutions in Fig. 7, the diazines efficiently quench the fluorescence intensity of the HN cations. However, while PZ and PD diminish the fluorescence lifetime of the HN cations ($\tau_0 = 14.1 \pm 0.1 \text{ ns}$), PM has no effect on it. Therefore, PM quenches the fluorescence of HN differently than PZ and PD do.

In the case of compounds PZ and PD, the decrease of the fluorescence lifetime of HN shows that the quenching process has a dynamic component: i.e. the diazines PZ and PD interact with the HN cations in the excited state. Possibly, this quenching component has its origin, as we previously proposed for pyridine [8], in the excited state proton transfer reaction between the substrates. Since the Stern–Volmer plots of I_0/I smoothly deviate upward from linearity, the

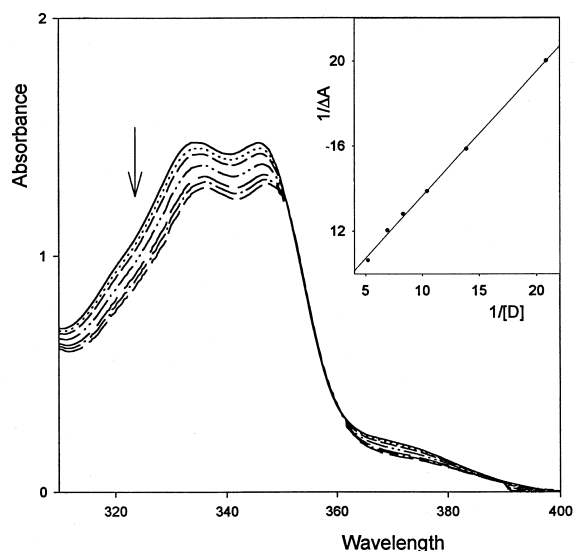


Fig. 6. UV-visible absorption spectra of HN ($4 \times 10^{-4} \text{ M}$) in buffered (pH 8.7) aqueous solutions containing different concentrations of PM. In the inset, Benesi–Hildebrand plot of the spectrophotometric titration data measured at 340 nm.

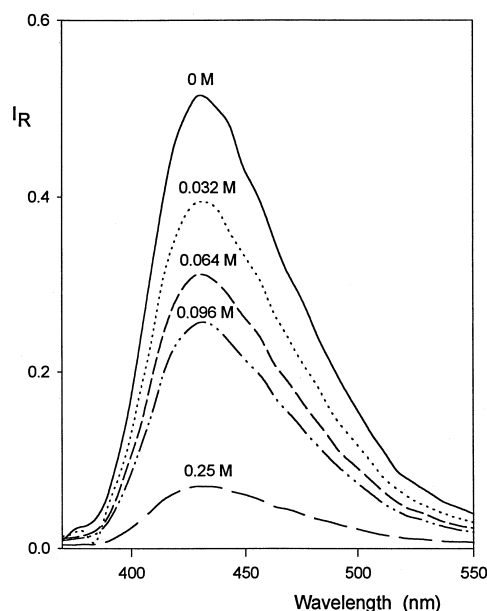


Fig. 7. Quenching of (HN) fluorescence in buffered (pH 8.7) aqueous solutions with the increase of PM concentration.

quenching data were satisfactorily fitted (see Fig. 8) to Eq. (5):

$$\frac{I_0}{I} = (1 + K_{SV}[D])\exp(K_A[D]) \quad (5)$$

where D represents either PZ or PD, $K_{SV} = k_q\tau_0$ is the Stern–Volmer dynamic quenching constant and K_A is a static quenching constant. The values of K_{SV} and K_A obtained from these fittings are reported in Table 2.

One conceivable explanation for the static component in these quenching mechanisms would be the formation of weak ground state complexes between the interacting substrates. Alternatively,

another possibility, which does not imply ground state interaction, would be the existence of the so called ‘active sphere’ effect [17]; the sphere within the fluorophore and the quencher are so close that there exists a high probability of quenching before the molecules diffuse apart. However, the radii of ~ 11 Å estimated for these active spheres from the K_A values, is much more greater than the ~ 6 Å distance of closer approach between the substrates estimated as the sum of their molecular radii. Therefore, we think the more likely explanation for the static quenching is the formation of weak ground state complexes.

Turning now to the HN–PM system, the exper-

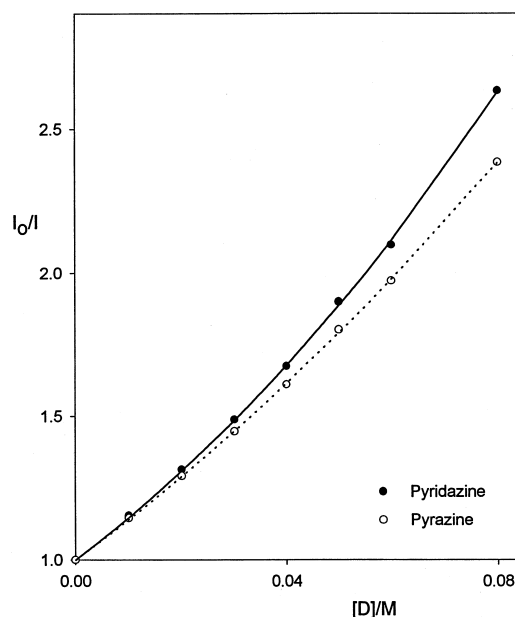


Fig. 8. Quenching plots for the HN–PZ and HN–PD system according to Eq. (6).

Table 2

Ground state association constants and quenching data for the interaction of harmane with diazines in buffered (pH 8.7) aqueous solutions

	T (K)	Pyrimidine	Pyrazine	Pridazine
K_{SV} (M^{-1})	288	14.4 ± 0.9		
	298	14.0 ± 0.4	10.5 ± 0.4	8.9 ± 0.6
	313	13.8 ± 0.9		
K_A (M^{-1})	298	–	3.3 ± 0.3	5.3 ± 0.4
	298	–	7.4	6.3
k_q ($10^8 M^{-1} s^{-1}$)	298	–		

imental data show that this diazine only interacts with neutral HN in the ground state, but not with the HN cations in the excited state. Moreover, quenching experiments carried out in acetic acid–acetate (pH 5.5) buffered solutions reveal that PM does not interact with the HN cations in the ground state. Thus, in these acidic media where HN is completely protonated in the ground state, PM does not quench the fluorescence of HN.

Thus, if we assume that the ground state HN–PM complexes are non-fluorescent, then the fraction of fluorescence of HN which remains upon complexation, I/I_0 , is given by the fraction of the total fluorophores that are not complexed $[HN]/[HN]_0$. Recalling that the total concentration of the fluorophore $[HN]_0$ is given by:

$$[HN]_0 = [HN] + [HN - PM] \quad (6)$$

and the formation constant of the HN–PM complexes is

$$K_G = \frac{[HN - PM]}{[HN][PM]}, \quad (7)$$

substitution of Eq. (6) into Eq. (7) yields:

$$K_G = \frac{[HN]_0 - [HN]}{[HN][PM]} = \frac{[HN]_0}{[HN][PM]} - \frac{1}{[PM]}. \quad (8)$$

Substituting the ratio of the fluorophore concentrations by the ratio of fluorescence intensities, and rearranging Eq. (8) we obtain

$$\frac{I_0}{I} = 1 + K_G[PM] \quad (9)$$

where the quenching constant, K_{SV} , is identified with the formation constant, K_G , of the HN–PM complex. Note that according to this model, the value of the quenching constant at 298 K, $14.0 \pm 0.4 \text{ M}^{-1}$, is in excellent agreement with that of $13.5 \pm 0.1 \text{ M}^{-1}$ obtained for K_G from spectrophotometric titration. It is also worth to note, that in aqueous media (pH 8.7), compound MHN behaves as HN. Thus, MHN interacts with PM in the ground state, but not in the excited state. The

formation constant of the MHN–PM complexes has been calculated from quenching fluorescence data as $12.8 \pm 0.1 \text{ M}^{-1}$. Therefore, the NH pyrrolic group plays only a very minor role in the ground state interaction of HN with PM.

Concerning the nature of the HN–PM complex, the data in Table 2 show the very small influence of the temperature on the formation constants K_G . Indeed, the differences are within the experimental error. This shows that the formation of the HN–PM complex is a very weak exothermic process. Thus, assuming that $\Delta H \approx 0$, we obtain a value of $\Delta S \approx 21.9 \text{ J mol}^{-1} \text{ K}^{-1}$ for the formation of the complex at 298 K. These data show that the formation of the HN–PM complex is an entropic controlled process. This situation is typical for associative processes governed by ‘hydrophobic interactions’ [18].

Hydrophobic interactions between non-polar molecules and water result in the formation of organic clusters. The positive entropic contribution arises from two main factors: the creation of the cavity to accommodate the organic solutes, and the release of the highly structured water molecules surrounding the individual organic molecules. Therefore, owing to the chemical nature of the interacting molecules, it is very likely that hydrophobic interactions could play an important role in the stabilisation of the HN–PM complexes in aqueous media. Then, if we consider the rigid planar geometries of the interacting substrates, it is reasonable to assume a π – π stacked structure for the complex. This assumption is further supported by the observation that methanol diminishes the associative interactions between HN and PM. Alcohols are known to significantly reduce π -stacked interactions because the solvent molecules effectively solvate the solute.

4. Conclusions

The results of the present study show the involvement of two different binding forces in the interaction of HN with diazines. One of them is conclusively characterised from the FTIR spectra

as hydrogen-bonding. These forces mainly operate in low polar solvents. The formation of these hydrogen-bonded complexes gives rise to new associate bands in the UV-vis and FTIR spectra of HN. Upon excitation to the singlet excited state, fluorescence intensity of HN is quenched and its lifetime is diminished. The dynamic quenching might be related to a general mechanism, previously proposed by Mataga [14–16], consisting of the charge transfer interaction between the hydrogen-bonded aromatic molecules.

In aqueous media, the diazines PZ and PD interact weakly with HN in its ground state, but they efficiently quench the fluorescence of the singlet excited state pyridinic protonated HN cations. This dynamic quenching is possibly due to an excited state proton transfer reaction between the substrates. Interestingly, in these aqueous media, the diazine PM behaves differently. Thus, PM does not interact with the excited HN cations, but it forms a non-fluorescent ground state complex with HN. Owing to the thermodynamical characteristics of this association process and the chemical structures of the interacting substrates, it seems very likely that HN and PM form π – π stacked complexes stabilised by hydrophobic interactions.

Acknowledgements

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