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Fluorescent symmetric phenazines from naphthoquinones 4. Solvent effect on time-resolved fluorescence☆

Carlos E.M. Carvalho[a], Nanci C. de Lucas[b], Juan O.M. Herrera[c], Antonio V. Pinto[d], Maria C.F.R. Pinto[d], Ira M. Brinn[b,*]

[a] *Laboratório de Fotoquímica, Instituto de Química, Universidade Federal Fluminense, Campus Valonginho, Centro, Niterói 24.020-150, RJ, Brazil*
[b] *Laboratório de Espectroscopia Resolvida no Tempo, Instituto de Química Universidade Federal do Rio de Janeiro, Ilha do Fundão, C.P. 68.563, Rio de Janeiro 21.945-970, RJ, Brazil*
[c] *Instituto de Química, Universidade Federal do Rio de Janeiro, Ilha do Fundão, C.P. 68.563, Rio de Janeiro 21.945-970, RJ, Brazil*
[d] *Núcleo de Pesquisas de Produtos Naturais, Universidade Federal do Rio de Janeiro, Ilha do Fundão, C.P. 68.563, Rio de Janeiro 21.945-970, RJ, Brazil*

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Abstract

The time-resolved fluorescence spectroscopy of seven strongly fluorescent phenazine derivatives (two which can be associated approximately to the C_{2h} point group, two to the C_{2v} point group and three to the C_s point group) in organic solvents of varying polarity have been reported for the first time. An increase in excited singlet lifetime and fluorescence quantum yield was observed as the polarity of the solvent increases for the C_{2h} and the C_s compounds, however the C_{2v} compounds presented no appreciable solvent effect. In all five cases where there is a change in lifetime, solvent polarity has almost no effect on the radiative rate constants, whereas the rate constants for non-radiative decay can vary by an order of magnitude, or more. These results are explained on the basis of decreased intersystem crossing as solvent polarity is increased. This model is supported by the results of transient absorption spectroscopy and semi-empirical molecular orbital calculations on the excited states of some of the compounds studied.

The large solvent effect on the time-resolved fluorescence observed for the compounds belonging to the C_{2h} and C_s point groups, coupled with their extremely high fluorescence quantum yields, indicate these compounds as potentially very important molecular polarity probes. The strong correlation between the experimentally observed solvent effects and the theoretically calculated excited state energy levels for each point group, as well as an obvious symmetry dependence of the observed behavior is discussed.

Keywords: Solvent effect; Time-resolved fluorescence; Symmetry effect; Molecular probe

1. Introduction

High fluorescence quantum yield compounds are of great interest as probes because they require lower concentrations to be detected, thus causing smaller perturbations in the system under study. They are also obvious candidates for lasing media. Although phenazine itself is weakly fluorescent, with a fluorescence quantum yield reported [2,3] as $\phi_{\mathrm{Fl}} = 3 \times 10^{-5}$, there have been reports [4,5] of reasonably strong fluorescence from naturally occurring phenazine derivatives isolated from *Pseudomonas* sp. In a previous article [6], the preliminary results on three new fluorescent phenazine derivatives prepared from β-lapachone and nor-β-lapachone were presented. (The lapachones, in turn, were both prepared from the natural product lapachol.) More recently the spectral characteristics, solvent effects and fluorescence quantum yields of seven phenazine derivatives (see Fig. 1) prepared from β-lapachone analogs were reported [1]. All are strongly fluorescent, some especially so in polar solvents, and would seem to be good candidates as fluorescent probes for monitoring, in vivo, the anti-cancer activity [7] and anti-malarial [8] of drugs with structures similar to that of β-lapachone.

What follows are the previously unpublished lifetimes, radiative and non-radiative rate constants as a function of solvent polarity, for the first excited singlet state of these seven phenazine derivatives and the transient absorption spectra of the triplet state of three of these derivatives, one representative of each symmetry group. The experimental, as well

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* Corresponding author. Tel.: +55-21-2562-7251; fax: +55-21-2562-7559.
E-mail address: irabrinn@iq.ufrj.br (I.M. Brinn).

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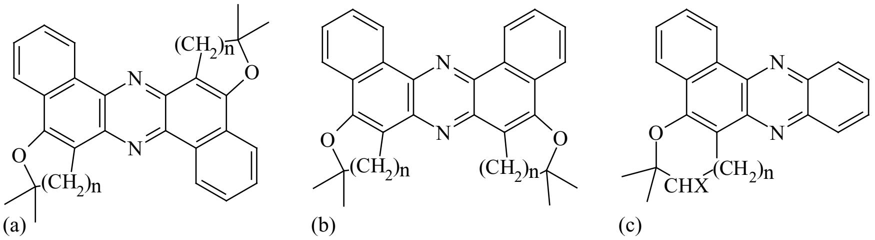

Fig. 1. Molecular structures: (a) DPZ5t ($n = 1$) and DPZ6t ($n = 2$); (b) DPZ5c ($n = 1$) and DPZ6c ($n = 2$); and (c) PZ5 ($n = 0$, X = H), PZ6 ($n = 1$, X = H) and PZ6Br ($n = 1$, X = Br).

as the calculational, evidence for a strong solvent effect on the non-radiative rate constant of the two centro-symmetric and the three less symmetric phenazines studied here is discussed and an explanation is given for the observed difference in solvent effect as a function of molecular symmetry.

2. Experimental

All seven of the phenazine derivatives reported here were prepared as previously [1] described. The purity of each compound was checked by thin layer chromatography and the structure verified by NMR spectroscopy and mass spectrometry. All were recrystallized immediately beforehand.

The following spectroquality grade solvents were used for spectroscopy: acetonitrile, benzene, chloroform, 1,4-dioxane, *n*-hexane, methanol and 2-propanol (Grupo Química/Brasil), dimethylformamide (DMF) and tetrahydrofuran (THF, Merck/Brasil) and cyclohexane (Vetec/Brasil).

Fluorescence decay profiles were taken on a custom made Edinburgh Analytical Instruments CD-900 Time Resolved Spectrophotometer which already has been described [9] in greater detail. A hydrogen excitation lamp was used with 4.1×10^4 Pa pressure and electrodes spaced by 0.70 mm. The time profiles were analyzed using software obtained from the manufacturer, which is based on the Newton–Gauss–Marquardt [10] algorithm. This program minimizes the weighted differences between the raw decay profile and the fitted exponential function which has been convoluted by the lamp profile. The latter is determined separately by a non-fluorescent scattering solution. All lifetime values reported reflect data taken with at least 2×10^3 pulses in the maximum channel. It has been suggested [11] that 185 total pulses are sufficient to characterize a mono-exponential decay with an error $\leq$10%. As a measure of goodness of fit, it was found $0.98 \leq \chi^2 \leq 1.2$, considered to be quite satisfactory. Fluorescence lifetimes were compared to those of a deaerated solution of anthracene in cyclohexane [12] as the standard, with a $\tau_{\mathrm{Fl}} \equiv 5.24$ ns.

Transient absorption spectra were done on an Edinburgh Analytical Instruments model LP900 Time Resolved Spectrophotometer, which has been described [13] previously. Excitation is accomplished by a Continuum Surelite Nd-YAG laser, model II-10, using the tripled fundamental frequency ($\approx$5 ns fwha and $\geq$50 mJ/pulse). The probe source is a 500 W, pulsed, Xenon lamp. Detection was by a Hamamatsu R955 photomultiplier tube, setup to use only 4–6 dynode stages and the signal read into a Tektronix model TDS 520 A digital storage oscilloscope. The solutions used were prepared with a high enough concentration to have an absorbance of $\approx$0.3 (concentration $\approx 10^{-4}$ M) at 355 nm and were placed in a 10 mm × 10 mm Pyrex tubing cell. The origin of the signal observed was tested by comparing the results of an aerated system to those of a system flushed with oxygen-free N_2 for 20 min and those of a system to which β-carotene was added to the solution. Kinetic traces were taken every 5 nm, in the range starting from approximately 400 nm through the termination of observed signal (approximately 700 nm), using fifty contiguous temporal windows, of 320 ns width, from 4 to 20 μs (in the cases of DPZ5c and PZ6Br) and widths of 640 ns, from 8 to 40 μs (in the case of DPZ5t). Each point represents the average of five laser pulses. The kinetic data were used to construct the transient spectra, utilizing a commercial package, supplied by the manufacturer, which fits the data by reconvolution. A first order fit was found to be satisfactory in most cases.

The ORIGIN 7.5, MATLAB 5.3 and the Excel-2002 programs were used for data fitting and plotting. A locally written program [14] based on the self-modeling factor analysis method [15] was used to determine the number of components contributing to transient absorption and their spectra.

Molecular orbital calculations on the excited states of model systems representing the seven phenazine derivatives studied here were done with the AMSOL-Version 6.7.2 program [16]. When the calculations were done on the complete molecular structure, the aliphatic rings were found to distort the symmetry of the system, decreasing it appreciably and not permitting the assignment of a representation to each orbital. This caused considerable mixing of π–π* and n–π* states in some cases. The model systems chosen simply substituted the aliphatic rings with terminal hydrogen atoms. With this simplification, the calculations did not differentiate between derivatives of the same point group, however the experimentally observed similarities in photochemical properties, within each of the point groups clearly justifies this choice. Coordinates were optimized for S_0 using the AM1 method [17]. These same coordinates were then frozen and the excited states calculated using the ZINDO method [18] employing configuration interaction involving all 225 singly excited configurations generated from excitations between HOMO-14 through HOMO → LUMO through LUMO + 14.

3. Results

3.1. Solvent effect

Tables 1–4 show the fluorescence lifetimes, natural (k_N), radiative (k_{Fl}) and non-radiative (k_{nr}) rate constants for the phenazine derivatives studied here for various solvents of different polarity. The k_N values are calculated [19] by integrating over the lowest energy band of the experimental absorption spectrum:

$$k_N = 2.88 \times 10^{-9} n^2 \int \{[2\bar{\upsilon}_0 - \bar{\upsilon}]^3/\bar{\upsilon}\}\varepsilon(\bar{\upsilon})\,\mathrm{d}\bar{\upsilon} \qquad (1)$$

where n is the mean refractive index of the solvent over the absorption band and $\bar{\upsilon}_0$ the wavenumber of the mirror symmetry point of the absorption and fluorescence spectra. The k_{Fl} and k_{nr} values are calculated from the experimentally determined fluorescence lifetime and fluorescence quantum yields [1], i.e.:

$$k_{Fl} \equiv \frac{\phi_{Fl}}{\tau} \qquad (2)$$

$$k_{nr} \equiv (\tau)^{-1} - k_{Fl} \qquad (3)$$

Table 1
Solvent dependence of the photophysics of the singlet manifold (C_{2h})

Solvent	DPZ5t				DPZ6t			
	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})
Cyclohexane	4.96	8.9 ± 0.9	8.5 ± 0.9	12 ± 1	1.57	24 ± 2	24 ± 2	40 ± 2
n-Hexane	4.89	8.7 ± 0.9	5.5 ± 0.6	15 ± 1	1.58	22 ± 2	20 ± 2	43 ± 2
Benzene	6.66	12 ± 1	7.8 ± 0.8	7.2 ± 0.8	2.19	13 ± 1	19 ± 2	27 ± 2
1,4-Dioxane	8.16	9.2 ± 0.9	5.8 ± 0.6	6.5 ± 0.6	2.45	11 ± 1	11 ± 1	30 ± 1
Chloroform	7.97	8.3 ± 0.8	6.6 ± 0.7	5.9 ± 0.7	2.50	10 ± 1	17 ± 2	23 ± 2
THF	–	–	–	–	2.22	11 ± 1	15 ± 2	30 ± 2
DMF	–	–	–	–	2.76	6.9 ± 0.7	21 ± 2	15 ± 2
2-Propanol	8.56	10 ± 1	12 ± 1	≤1.2	2.56	7.8 ± 0.8	21 ± 2	18 ± 2
Acetonitrile	8.47	6.8 ± 0.7	12 ± 1	≤1.2	2.68	8.4 ± 0.8	23 ± 2	14 ± 2
Methanol	8.50	4.6 ± 0.5	12 ± 1	≤1.2	3.28	6.0 ± 0.6	19 ± 2	11 ± 2

Lifetime errors ± 0.01. Errors given as most probable error.

Table 2
Solvent dependence of the photophysics of the singlet manifold (C_{2v})

Solvent	DPZ5c				DPZ6c			
	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})
Cyclohexane	3.69	40 ± 4	12 ± 1	15 ± 1	2.40	50 ± 5	14 ± 1	28 ± 1
n-Hexane	3.71	37 ± 4	11 ± 1	16 ± 1	2.45	33 ± 3	15 ± 2	26 ± 2
Benzene	3.68	24 ± 2	11 ± 1	16 ± 1	2.34	25 ± 3	12 ± 1	31 ± 1
1,4-Dioxane	2.65	33 ± 3	20 ± 2	18 ± 2	2.47	24 ± 2	19 ± 2	21 ± 2
Chloroform	3.74	19 ± 2	11 ± 1	16 ± 1	2.44	19 ± 2	17 ± 2	24 ± 2
2-Propanol	3.92	16 ± 2	15 ± 1	11 ± 1	2.63	18 ± 2	15 ± 2	23 ± 2
Acetonitrile	3.69	16 ± 2	11 ± 1	16 ± 1	2.55	13 ± 1	11 ± 1	28 ± 1

Lifetime errors ± 0.01. Errors given as most probable error.

Table 3
Solvent dependence of the photophysics of the singlet manifold (C_s)

Solvent	PZ5				PZ6			
	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})	τ (ns)	k_N (10^7 s^{-1})	k_{Fl} (10^7 s^{-1})	k_{nr} (10^7 s^{-1})
Cyclohexane	0.77 ± 0.01	11 ± 1	11 ± 1	120 ± 1	0.36 ± 0.01	14 ± 1	14 ± 2	260 ± 2
Benzene	2.09 ± 0.01	10 ± 1	10 ± 1	38 ± 1	0.73 ± 0.01	17 ± 2	16 ± 2	120 ± 2
Chloroform	3.22 ± 0.01	9.3 ± 0.9	8.1 ± 0.8	23 ± 1	1.22 ± 0.01	14 ± 1	14 ± 1	68 ± 1
THF	3.25 ± 0.01	8.2 ± 0.8	4.0 ± 0.4	26.8 ± 0.4	0.93 ± 0.01	13 ± 1	11 ± 1	97 ± 1
DMF	6.95 ± 0.02	7.7 ± 0.8	6.3 ± 0.6	8.1 ± 0.6	1.89 ± 0.01	12 ± 1	6.9 ± 0.7	46 ± 7
Methanol	8.21 ± 0.02	7.2 ± 0.7	4.5 ± 0.5	7.7 ± 0.5	3.53 ± 0.01	8.2 ± 0.8	6.5 ± 0.7	21.8 ± 0.7

Errors given as most probable error.

Table 4
Solvent dependence of the photophysics of the singlet manifold (PZ6Br)

Solvent	τ (ns)	k_N ($10^7\,s^{-1}$)	k_{Fl} ($10^7\,s^{-1}$)	k_{nr} ($10^7\,s^{-1}$)
Cyclohexane	0.34	20 ± 2	83 ± 9	210 ± 10
n-Hexane	0.34	22 ± 2	73 ± 7	220 ± 10
Benzene	0.56	19 ± 2	50 ± 5	130 ± 10
1,4-Dioxane	0.72	17 ± 2	61 ± 6	77 ± 6
THF	0.91	24 ± 2	43 ± 4	67 ± 4
DMF	0.91	12 ± 1	37 ± 4	73 ± 4
2-Propanol	0.95	9.3 ± 0.9	41 ± 4	64 ± 4
Acetonitrile	0.79	16 ± 2	62 ± 6	65 ± 6
Methanol	1.47	12 ± 1	39 ± 4	29 ± 4

Lifetime errors ± 0.01. Errors given as most probable error.

The k_N values vary a bit from compound to compound, attaining their highest values in the case of the two C_{2v} compounds. For any particular phenazine derivative, as solvent polarity is increased, an almost generalized decrease in k_N is observed, which varies from a factor of 2 to a factor of 4. Basically all of the k_{Fl} values fall within the range $(10 \pm 5) \times 10^7\,s^{-1}$, i.e., they are almost independent of solvent, the size of the aliphatic ring and the arrangement of the aromatic rings, for the three basic structures. (The one obvious exception being PZ6Br, whose values are approximately an order of magnitude greater.) This result is interpreted as indicating that the aliphatic ring does not participate in the electronic redistribution upon excitation (only having an effect to the extent that it distorts the aromatic rings) and supports the assumption [1] that the excitation in S_1 is localized on only part of the conjugated section of the molecule. Also, there is basically no solvent effect on k_{Fl}, within a factor of 2, for any of the seven structures studied here. This suggests that there is no strong specific interaction between any of the solvents and the part of the conjugated system that participates in the excitation to S_1, in the phenazine derivatives studied here.

In comparison, k_{nr} for the compounds studied here varied from basically zero to more than $10^9\,s^{-1}$, the variation for any given phenazine derivative as a function of solvent generally was found to be a function of point group. Small, trend less variations were noted for the two C_{2v} structures, whereas almost monotonic decrease, of an order of magnitude, with increasing solvent polarity were observed for the three C_s structures. The two C_{2h} structures showed the same trend as the C_s structures, however on a smaller scale.

3.2. Transient absorption spectra

Considering that the parent compound, phenazine, is known [20] to undergo intersystem crossing with a very high quantum yield ($\phi_{isc} = 0.96$), a single compound of each point group studied here was selected in order to test these phenazine derivatives for intersystem crossing under the conditions used to study fluorescence. The three compounds chosen were DPZ5c (C_{2v}), DPZ5t (C_{2h}) and PZ6Br (C_s), all in *n*-hexane. All of the above showed a transient absorption spectrum. Some typical time-resolved spectra are shown in Fig. 2 and kinetic traces for the lowest energy band of each of the three compounds are shown in Fig. 3. Factor analysis was applied to the first 20 spectra, i.e. 8–20.8 μs for DPZ5t and 4–10.4 μs for DPZ5c and PZ6Br, after these times the normalized spectra being identical.

It was found that the experimental spectra of all three compounds could be described quite adequately by only two calculated spectra, each one well represented by a single component. The first band, at the blue edge of the region studied, had considerable overlap with the normal absorption band in the case of DPZ5t and some slight overlap in the cases of the other two compounds. In all three cases this first transient absorption band had considerable overlap with the fluorescence band. The second transient absorption band, with a maximum above 500 nm, overlapped with the

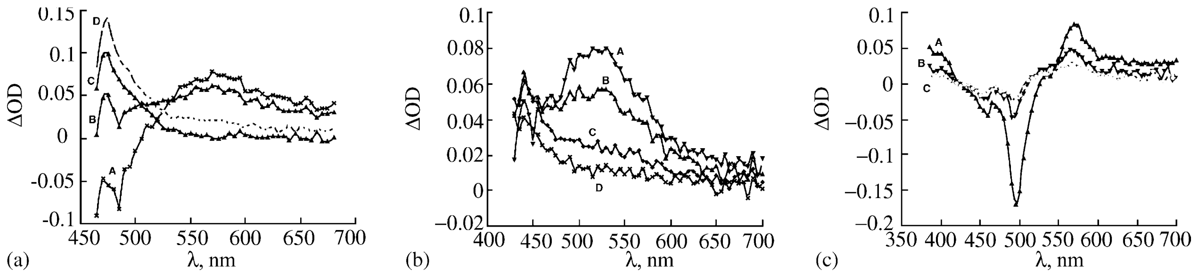

Fig. 2. Time-resolved transient absorption spectra measured at various times after 355 nm laser pulse excitation of a degassed *n*-hexane solution. (a) DPZ5c. Times (in μs) after laser pulse: A, 4.16; B, 4.80; C, 7.36; D, 18.88. (b) PZ6Br. Times (in μs) after laser pulse: A, 4.80; B, 5.76; C, 8.64; D, 15.04. (c) DPZ5t. Times (in μs) after laser pulse: A, 8.32; B, 20.48; C, 30.08.

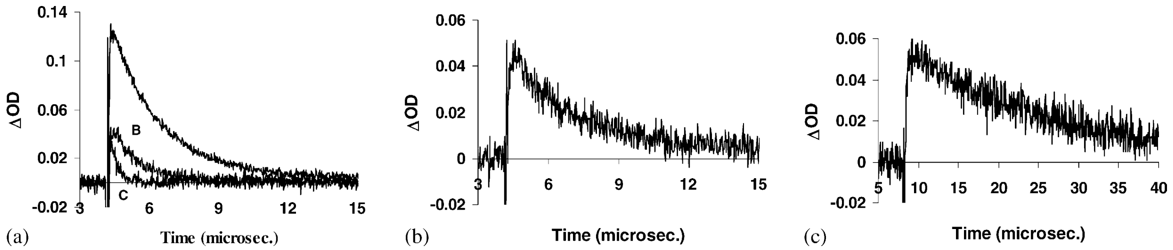

Fig. 3. Kinetic traces obtained after excitation of a degassed hexane solution of (a) DPZ5c, $\lambda_{abs} = 575$ nm (B: with air; C: with β-carotene); (b) PZ6Br, $\lambda_{abs} = 530$ nm; and (c) DPZ5t, $\lambda_{abs} = 570$ nm.

fluorescence band, but not the ground state absorption band, in all three cases.

Some time-resolved transient absorption spectra DPZ5c are shown in Fig. 2a. The first band, with $\lambda_{max} = 475$ nm, showed a bi-exponential behavior, with a rise time of $\tau = 1.7$ μs and a decay of $\tau = 33$ μs. The second band, whose kinetic trace at its maximum of 570 nm is shown in Fig. 3a, is already present in the first time window at 4 μs after the laser flash. It exhibited a mono-exponential decay time of $\tau = 2.7$ μs. It is suggested that the species responsible for the band at 475 nm is derived from the species which absorbs at 570 nm. The difference in the τ values of the decay of the latter and the appearance of the former are interpreted as suggestive of the errors involved in these values, i.e., in the order of 30%, or more. When this solution was aerated (profile B of Fig. 3a) both the OD and the lifetime of the second band decreased, the latter to 0.3 μs. As shown in time profile C of Fig. 3a, the presence of β-carotene, an often used [21] triplet state quencher because of its low triplet energy, also diminished the OD and the lifetime (to 1.2 μs) of the second transient absorption band observed. Assuming that the β-carotene did not undergo appreciable degradation during the experiment, the measured lifetime generates a quenching rate constant of $k_Q = (2 \pm 1) \times 10^{10}$ M^{-1} s^{-1}, considered to be at the diffusion controlled limit. This evidence strongly suggests that intersystem crossing contributes significantly to non-radiative deactivation of S_1.

Fig. 2b shows some transient absorption spectra of PZ6Br. The blue band, with $\lambda_{max} = 440$ nm, again showed a bi-exponential behavior, with a rise time of $\tau = 1.1$ μs and a decay time of $\tau = 15$ μs. The second band, whose kinetic trace at its maximum of 530 nm, is shown in Fig. 3b, is already present in the first time window at 4 μs after the laser flash. It also exhibited a mono-exponential decay time, of $\tau = 5.1$ μs. The significant difference in the decay time of the red band and the rise time of the blue band would seem to suggest that coupling of the two species is not occurring. It is best said that the kinetics of this system is not well understood at this point. When the solution was aerated (not shown) the OD of the second band decreased appreciably, and the lifetime of the mono-exponential decay was calculated to be 0.5 μs. When β-carotene was added to the solution (not shown) once again the OD decreased significantly, but the poor quality of the decay profile did not permit a reasonable calculation of the lifetime. These results again support the assertion that intersystem crossing contributes significantly to non-radiative deactivation of S_1.

The transient absorption spectrum (Fig. 2c) and, therefore, the excited state kinetics of DPZ5t are even more complex. In addition to the two transient absorption bands, with maxima at 380 and 570 nm, there is an obvious negative absorption region at $\lambda_{max} = 495$ nm. (This value of the wavelength of the minimum can be associated with the 483 nm absorption maximum reported [1] previously, the difference in wavelengths being rationalized by the fact that the LP900 spectrometer does not generate corrected spectra.) The two positive absorption bands were associated with mono-exponential decays, the 380 nm band with $\tau = 17$ μs and the 570 nm band, whose kinetic trace is shown in Fig. 3c, with $\tau = 19$ μs. These τ values are understood to be equal, suggesting that the two transitions originate from the same state, T_1. Because the first absorption band of the ground state of DPZ5t is further red shifted than that of the other two compounds, there is significant overlapping of this band with the transient absorption spectrum. Appreciable population of the triplet manifold, coupled with non-overlapping absorption bands between T_1 and S_0, could cause the negative difference spectrum observed in Fig. 3b. This bleaching of the absorption spectrum decreased mono-exponentially, with $\tau = 11$ μs. These three τ values are considered equal, within the error of ±30% already cited.

The bleaching of the S_0 absorption band was used to calculate the fraction of DPZ5t molecules which are not in the S_0 state when the measurements were initiated, approximately 40%. The short lifetime of S_1, the lack of observable photo-degradation of the solution and the factor analyses indicating a single contribution to each transient absorption band effectively eliminates the possibility of appreciable accumulation of photoproduct. Thus the 40% quoted above is basically all in the T_1 state. If one assumes appreciable cancellation of the effect of multiple excitations of the same molecule due to the non-negligible temporal width of the exciting laser (which would lead one to conclude that $\varphi_{isc} < 0.4$) by the effect of extrapolating the $[T_1]$ to 0 μs (which would indicate $\varphi_{isc} > 0.4$), one arrives at the approximate $\varphi_{isc} \approx 0.4$. This value is quite consistent with the previously published [1] value of $\varphi_{Fl} = 0.27$. Supporting this interpretation, when this solution was aerated, the lifetime

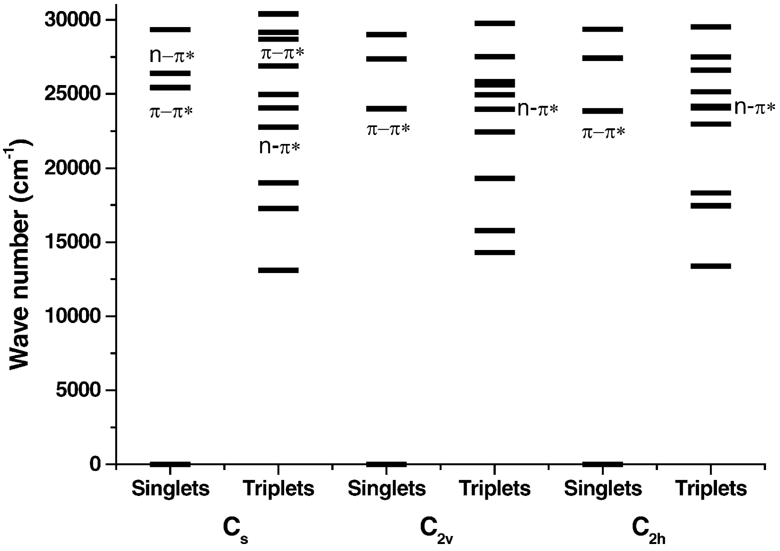

Fig. 4. Theoretically calculated energies of low-lying excited singlet and triplet states of three model compounds.

of the species responsible for the second band decreased significantly, to 0.3 μs. β-Carotene also caused a significant deterioration in the strength of the signal, but it was not possible to abstract a reasonable value for the lifetime of the species responsible for the second band. It is suggested that all of this evidence points conclusively to intersystem crossing contributing significantly to non-radiative deactivation of S_1 in all three systems in which the transient absorption spectrum was determined and by extension, to all seven phenazine derivatives studied here.

3.3. Molecular orbital calculations

The most important results of the molecular orbital calculations are summarized in Fig. 4, which shows, the calculated singlet and triplet energy manifolds in the lower energy region for the model compounds. For each of the three model compounds the representations and energies of the states calculated to be involved in intersystem crossing ($S_1 \rightarrow T_n$) are identified explicitly in Table 5.

In Fig. 4 one notes the following differences, in the theoretically calculated energies, between the compounds of the different point groups:

(a) In the C_s compounds the first and second excited singlet states are quite close in energy, the same not occurring in the case of the more symmetric compounds.

(b) In the case of the C_{2h} compounds there is an n–π* triplet state of slightly higher energy than the lowest excited singlet state, whereas in the case of the C_{2v} compounds the corresponding n–π* triplet state is lower in energy than the lowest excited singlet state.

4. Discussion

The results presented here are intuitively surprising for three reasons:

(1) The increase in fluorescence lifetime observed with increasing solvent polarity seems to go against the argument that increasing solvent polarity should lead to increasing solute–solvent interactions which, in turn, should facilitate energy transfer from the excited state of the solute to the solvent, and increase the non-radiative rate constant.

(2) The C_{2h} derivatives are more sensitive to solvent polarity than the C_{2v} derivatives, in spite of the fact that the former, because of their (quasi) center of symmetry, should have basically a zero dipole moment and therefore their dipole–dipole interaction with the solvents should be trivial.

(3) PZ6Br is the only one of the seven derivatives which contains an atom “heavier” than oxygen. Due to the

Table 5
Theoretically calculated excited state properties—intersystem crossing

Point group	Singlet		Triplet		Transition	
	State	$\bar{\upsilon}$ (cm^{-1})	State	$\bar{\upsilon}$ (cm^{-1})	Representation	$\Delta\bar{\upsilon}$ (cm^{-1})
C_s	$S_1(A', \pi–\pi^*)$	25424	$T_4(A'', n–\pi^*)$	22760	A''	−2664
	$S_2(A'', n–\pi^*)$	26409	$T_7(A', \pi–\pi^*)$	26885	A''	476
C_{2v}	$S_1(B_2, \pi–\pi^*)$	24013	$T_5(B_1, n–\pi^*)$	23973	A_2	−40
C_{2h}	$S_1(B_u, \pi–\pi^*)$	23850	$T_5(A_u, n–\pi^*)$	24055	B_g	205

"heavy atom effect", one would expect this derivative to be much more susceptible to intersystem crossing than any of the other derivatives studied here. This difference should manifest itself in a much shorter fluorescence lifetime and a lower fluorescence quantum yield for PZ6Br, as compared to the other compounds. Although the shortest fluorescence lifetimes are observed for PZ6Br, a lower fluorescence quantum yield is simply not observed.

The solvent effect observed here has been reported [22] for other *N*-heterocycles and explained [23] considering the following model. The generally small variations in k_{Fl} from one compound to another, as well as with variation of solvent for any given compound, suggest that the striking differences observed are due to the differences in the k_{nr} values. The latter can be expressed as the sum of the contributions of internal conversion (ic), intersystem crossing (isc) and irreversible reactions (rx), i.e.:

$$k_{\mathrm{nr}} = k_{\mathrm{ic}} + k_{\mathrm{isc}} + k_{\mathrm{rx}} \tag{4}$$

As no deterioration of the solutions was observed and the fluorescence signal was stable for long periods of time, k_{rx} can be assumed to be negligible. On the other hand, the detection of a transient absorption spectrum, which is almost totally quenched when oxygen is not previously removed from the system and is partially quenched by the presence of β-carotene, is interpreted as very strong evidence for the presence of triplet–triplet absorption, which implies that intersystem crossing is important. It can be assumed initially, without loss of generality, that k_{ic} is also non-negligible.

The two remaining terms on the right side of Eq. (4) can be broken down even further. Thus k_{isc} can be separated [24,25] as the sum of a "first order" term (k_{so}), due to spin–orbit coupling and a "second order" term (k_{vib}), due to vibronic coupling. It has been shown [26,27] that spin–orbit coupling is heavily favored between states with different configurations. S_1 is a π–π* state for all seven phenazine derivatives studied here, as shown by the significant bathochromic fluorescence shifts, as well as the theoretical calculations on the excited states. Thus, it is reasonable to assume that, in general, the fraction of intersystem crossing via k_{so} will be preferentially to T_n's which are n–π* states. Because the dipole moment of n–π* states is normally considerably smaller than that of π–π* states, to a reasonable approximation the energy variation with increasing solvent polarity of T_n can be ignored compared to the variation in the energy of the S_1 state, which decreases. If the T_n state involved in intersystem crossing has a slightly higher energy than the S_1 state, there will exist an energy barrier to k_{so} which will increase with increasing solvent polarity, thus rapidly decreasing k_{so} and k_{nr} and increasing ϕ_{Fl}. If, on the other hand, T_n has a lower energy than S_1, this barrier will tend to disappear and a much smaller dependence of k_{so} on solvent polarity would be expected.

The possibility of second order vibronic contributions to intersystem crossing also should be considered. The presence of vibronic coupling has been observed [24] in both the phosphorescence spectrum of the parent phenazine and the second derivative of the absorption and fluorescence spectra [1] of the phenazine derivatives studied here. In the case of the parent compound, 18 vibrational progressions were reported [25], including $\bar{\upsilon}= 1404$ and $1475\,\mathrm{cm}^{-1}$. In the absorption and fluorescence spectra of the phenazine derivatives studied here only progressions at $\bar{\upsilon} \cong 1450 \pm 50\,\mathrm{cm}^{-1}$ were observed [1], at a much lower spectral resolution. Fundamental vibrational frequencies in this region have been attributed [28] previously to a stretching frequency of a heterocycle with three, or more, rings. The observation of this vibrational fine structure suggests that vibronic coupling can be important in intersystem crossing for these phenazine derivatives as well, in addition to its contribution to internal conversion. If so, the restriction that T_n be an n–π* state will be relaxed. In addition, one would expect to observe only a slight increase in the vibronic coupling rate with increasing solvent polarity. The tentative suggestion given here is that intersystem crossing may occur via various channels, being largely the determining factor in the non-radiative decay observed in non-polar solvents.

This suggestion is consistent with the general decrease of k_{nr} and increase in ϕ_{Fl} in the C_{2h} derivatives, as solvent polarity is increased, if one assumes that in these cases spin–orbit coupling is heavily involved in intersystem crossing in non-polar solvents and that there is an increasing energy barrier as the solvent polarity increases. On the other hand, in the case of the two C_{2v} derivatives, the relative solvent independence of the observed k_{nr} values suggests the absence of any such barrier. Both of these observations are consistent with the molecular orbital calculation results which indicate that in intersystem crossing from the S_1(π–π*) state to the T_5(n–π*) state, in the case of the C_{2h} derivatives, there will be a small energy barrier ($205\,\mathrm{cm}^{-1}$) whereas in the case of the C_{2v} derivatives, T_5 is actually slightly lower in energy ($40\,\mathrm{cm}^{-1}$) than S_1. The molecular orbital calculation results for the C_s derivatives lead to a more complicated rationalization of the experimentally observed behavior. The S_1(π–π*) → T_4(n–π*) transition is calculated to be so exoergic ($2664\,\mathrm{cm}^{-1}$) that considerable vibrational coupling would be expected to be necessary for this to be the major intersystem crossing pathway. The close proximity of S_2(n–π*) to S_1(π–π*) suggests that the above transition may well mix with, or be dominated by, the S_2(n–π*) → T_7(π–π*) transition (both transitions belong to the A″ representation). If so, an energy barrier $\leq 476\,\mathrm{cm}^{-1}$ is to be expected, according to the calculations. One notes from Fig. 4 that this proximity of singlet excited states is not encountered in the calculations for the compounds of the other two point groups.

The contribution of intersystem crossing to non-radiative deactivation of the S_1 state is consistent with both the general solvent effect observed and also the difference between the behavior of the C_{2v} and C_{2h} derivatives. The contribution of internal conversion, in which dipole–dipole solvent–solute interactions would be expected to have a strong influence, to this deactivation is not capable of explaining either of these observed trends. This strongly suggests that intersystem crossing, which was shown by transient absorption spectroscopy to be present in these systems, totally dominates internal conversion as the principal contributor to non-radiative deactivation.

The very surprising apparent lack of a heavy atom effect on the fluorescence quantum yield of PZ6Br, as compared to the results for PZ6 and PZ5, is consistent with the observed change in the NMR band, due to the six hydrogen atoms of the two methyl groups, of PZ6Br as compared to PZ6. The substitution of a single bromine atom for a hydrogen atom in the aliphatic ring, causes the singlet band at $\delta = 1.58$ to become a doublet at $\delta = 1.83$. This difference is attributed to a more rigid structure in the aliphatic ring of PZ6Br, which allows a separation of the two methyl group peaks. The fact that in PZ6 a coalescence of the bands is observed, demonstrates that they are exchanging positions too rapidly to be followed by the NMR instrument used (200 MHz) on a time scale which is comparable to the nanosecond lifetime of S_1. Although these results apply to the electronic ground state, it is not unreasonable to assume that the aliphatic ring of PZ6Br is also more rigid in the S_1 state, as compared to PZ6. The former would then be expected to have higher ring stretching frequencies (exactly those which were observed in the absorption and fluorescence spectra), which would make it more efficient in vibronic coupling. This increased vibronic coupling would be expected to turn transitions originating from the (calculated to be in the C_s derivatives) close-lying $S_2(n–\pi^*)$ to become much more important. This, in turn, could manifest itself either as an increased φ_{isc} (which we are able to detect, however, whose value we are not able to measure) and/or as an increased k_{Fl}, which was observed, as shown in Table 4. It is to be noted that either one of these effects would shorten τ of S_1, as is also shown in Table 4. It can also be pointed out that the above argument is consistent with the conclusion previously published [1] which was arrived at by considering the fluorescence quantum yields, "that the aliphatic ring does not participate in the electronic redistribution upon excitation, however, acts as a sink for the degradation of electronic energy via internal conversion and/or intersystem crossing".

As a closing remark, it is worth noting that these results support the idea that selection rules for the $S_1 \rightarrow T_n$ transition play no role here. In the C_s point group transitions of both possible representations are allowed. In the C_{2v} point group both possible representations of an $S(\pi–\pi^*) \rightarrow T(n–\pi^*)$ transition are allowed. In the C_{2h} point group this transition can be of either the A_u or B_g representations, only the latter being allowed. (The operators in the integrals for $S_1(\pi–\pi^*) \rightarrow T_n(n–\pi^*)$ intersystem crossing transform as the rotation operators.) As can be seen in Table 5, the theoretically calculated closest T_n (n–π^*) state for the C_{2h} compounds belongs to the A_u representation whereas the $S_1(\pi–\pi^*)$ state belongs to the B_u representation, generating the symmetry permitted B_g transition.

5. Conclusions

Of the seven phenazine derivatives studied here, all showed mono-exponential fluorescence decay. Five of them show appreciable variations in fluorescence lifetime with variations in solvent polarity, attaining an order of magnitude difference in the derivatives of lower symmetry. This difference, combined with the extremely high fluorescence quantum yields, is sufficient to recommend using these compounds as polarity probes. The lifetimes of the two derivatives that belong to the C_{2v} point group are not particularly sensitive to solvent polarity.

The transient absorption results are interpreted as indicating that intersystem crossing is predominant in the quenching of the phenazine derivatives studied here, in non-polar solvents. The differences in behavior between the various derivatives can be rationalized on the basis of the theoretically calculated energy separations of the states, which presumably would have a strong influence on intersystem crossing.

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