Polycyclic Aromatic Nitrogen Heterocycles. Part IV: Effect of Solvent Polarity, Solvent Acidity, Nitromethane and 1,2,4-Trimethoxybenzene on the Fluorescence Emission Behavior of Select Monoaza- and Diazaarenes

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Fluorescence emission spectra are reported for naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline, benzo[lmn][3,8]phenanthroline (also called 2,7-diazapyrene), benz[4,10]anthra[1,9,8cdef]cinnoline, naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine, acenaphtho[1,2b]pyridine, benzo[a]phenazine, indeno[1,2,3ij][2,7]naphthyridine, and indeno-[1,2,3ij]isoquinoline dissolved in organic nonelectrolyte solvents of varying polarity and acidity. Results of these measurements indicate that naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline exhibits some signs of probe character as evidenced by changing emission intensity ratios; however, numerical values did not vary systematically with solvent polarity. The effect of nitromethane and 1,2,4-trimethoxybenzene as selective quenching agents on both the unprotonated and protonated PANHs was also examined. Nitromethane was found to quench fluorescence emission of roughly two-thirds of the alternant unprotonated PANHs studied to date. Emission intensities of the protonated PANHs remained essentially constant and were not affected by nitromethane. 1,2,4-Trimethoxybenzene, on the other hand, quenched the fluorescence emission of several unprotonated and all protonated PANHs examined.

Index Headings: **Fluorescence; Molecular structure; Spectroscopic techniques.**

INTRODUCTION

Identification and quantification of unknown polycyclic aromatic hydrocarbon (PAH) mixtures require accurate fluorescence emission intensity measurements and availability of a large spectral data file for comparing the unknown's spectrum against PAH standards. Mixtures of environmental/industrial importance rarely contain a single component. The majority of mixtures commonly encountered contain several isomeric pairs or structurally similar PAHs, which emit in approximately the same spectral regions. Kalman filtering and Gaussian or other curve-fitting techniques,[1–6] alone or in combination with phase-resolved[7–9] or synchronous scanning[10–12] fluorescence spectroscopy, theoretically allow uncoupling of overlapped spectra. Such methods become less reliable, however, as the number of mixture components increases. High-performance liquid chromatographic (HPLC) separation prior to fluorimetric analysis affords a viable alternative, but again the method is extremely time-consuming whenever large numbers of isomeric compounds are present. Blümer and Zander[13] recommended that nitromethane and/or nitrobenzene could be added to an aqueous-acetonitrile (20:80 percent by volume) binary mobile phase to selectively suppress fluorescence signals of alternant PAHs. Emission intensities of nonalternant PAHs would remain unchanged. Published studies[14–19] involving over 63 PAHs have identified dibenzo[hi,wx]-heptacene, benzo[k]fluoranthene, fluoranthene, and naphtho[2,3b]fluoranthene as among the few exceptions to the so-called nitromethane selective quenching rule in the PAH6 benzenoid, fluorenoid, fluoranthenoid, and "methylene-bridged" cyclopenta-PAH subclasses.

Quenching behavioral differences between alternant and nonalternant PAHs can be rationalized in terms of processes originating from the vibrationally relaxed first electronic excited singlet state, ^{1}PAH*. Deactivation from the ^{1}PAH* state is governed by the competition between radiative and nonradiative processes. Rate constants of fluorescence decay, $k_{fluores}$, for PAH fluorophores are generally insensitive to molecular environment. Efficiencies of nonradiative processes, on the other hand, depend to a large extent upon external pertubations resulting from interactions involving PAH solutes with solvent/quenching molecules. Three possible mechanisms include reduction of fluorescence emission intensities through: (1) intersystem crossing processes involving external "heavy atom" quenchers:

$$^1\text{PAH}^* + {}^1\text{Quencher} \rightarrow {}^3\text{PAH}^* + {}^1\text{Quencher};$$

(2) intermolecular transfer (or partial transfer, $\delta+/\delta-$) of a single electron to form free ions (or charge transfer complex):

$$^1\text{PAH}^* + {}^1\text{Quencher} \rightleftharpoons (\text{PAH}^{\pm}\text{---Quencher}^{\mp}) \rightarrow {}^2\text{PAH}^{\pm} + {}^2\text{Quencher}^{\mp};$$

and (3) intermolecular electronic energy transfer:

$$^1\text{PAH}^* + {}^1\text{Quencher} \rightarrow {}^1\text{PAH} + {}^1\text{Quencher}^*$$

with the quenching agent promoted from a ground singlet state to an excited triplet state, requiring Energy (^{1}PAH*) > Energy (^{1}Quencher*).

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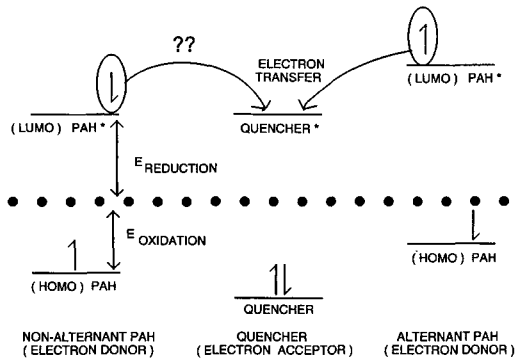

FIG. 1. Simplified molecular orbital diagram indicating favorable conditions for electron transfer between an electron donor alternant PAH and an electron acceptor quenching agent. The quencher's LUMO and nonalternant PAH's LUMO were placed at energies so as to discourage electron transfer. The dotted line represents the potential of a reference electrode. Nitromethane quenches only fluorescence emission of alternant PAHs. Intensities of nonalternant PAHs remain unchanged for the most part; however, a few exceptions are known. More detailed molecular orbital diagrams are given elsewhere.[18]

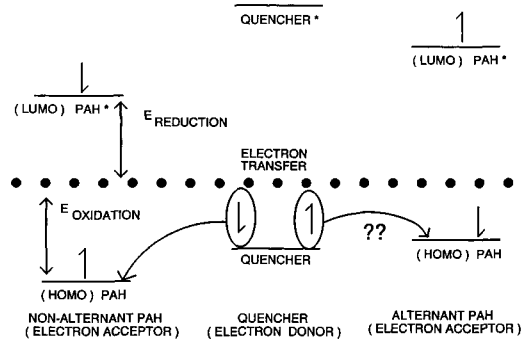

FIG. 2. Simplified molecular orbital diagram indicating favorable conditions for electron transfer between an electron acceptor nonalternant PAH and an electron donor quenching agent, such as 1,2,4-trimethoxybenzene. More detailed molecular orbital diagrams are given elsewhere.[18]

Of the aforementioned mechanisms, only the second could discriminate between alternant and nonalternant PAHs. Breymann *et al.*[18] attributed nitromethane's selectivity to an electron/charge transfer reaction. As argued by the authors, reduction potentials of nonalternant PAHs are generally 0.4 eV more positive than those of alternant PAHs. Quantum mechanical computations show the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies of nonalternant PAHs to be lowered against those of alternant PAHs of equal HOMO-LUMO separation. For the electron transfer reaction

$$^1\text{PAH}^* + {}^1\text{Quencher} \rightarrow {}^2\text{PAH}^+ + {}^2\text{Quencher}^-$$

the change in free energy[18]

$$\begin{aligned}\Delta G &\approx \text{Energy } ({}^1\text{PAH}^*/{}^2\text{PAH}^+) \\ &\quad - \text{Energy } ({}^2\text{Quencher}^-/{}^1\text{Quencher}) \\ &\quad + \text{Other Term(s)} \\ &\approx \text{Energy } ({}^1\text{PAH}/{}^2\text{PAH}^+) \\ &\quad - \text{Energy } ({}^1\text{PAH}/{}^1\text{PAH}^*) \\ &\quad - \text{Energy } ({}^2\text{Quencher}^-/{}^1\text{Quencher}) \\ &\quad + \text{Other Term(s)} \qquad (1)\end{aligned}$$

is expected to be more negative in the case of alternant PAHs (see Fig. 1). Assuming that one is in a Marcus-type region, a more favorable electron transfer process results in larger quenching rate constants, which, if comparable to (or larger than) $k_{fluores}$, would cause a significant reduction in PAH emission intensity. Slow electron transfer reactions (i.e., $k_{quench} \ll k_{fluores}$) are not expected to affect fluorescence intensities, since the photon is emitted long before electron transfer from the PAH donor to the quencher acceptor can occur. A similar argument could be made for electron donor quenching agents. Here, the quenching agent is more likely to affect fluorescence emission of the nonalternant PAHs (see Fig. 2).

Oxidation-reduction electrochemical potentials and HOMO-LUMO energies play major roles in controlling the selectivity of an electron transfer quenching agent for a particular fluorophore subclass. Our preliminary study[20] reported that nitromethane does quench the fluorescence emission of several polycyclic aromatic nitrogen hetero-atoms (PANHs); however, the percentage of PANH exceptions was considerably larger than what was found in the case of PAH6 benzenoids. Nitrogen atoms possess a lone electron pair. It is conceivable that the relative order of the π-bonding HOMO and n-nonbonding HOMO may be reversed, depending upon the PANH solute being examined. Fluorescence emission signals may correspond to either $\pi^* \rightarrow \pi$ or $\pi^* \rightarrow n$ electronic transitions. To more fully investigate the photophysical properties of PANHs, we have measured the fluorescence behavior of naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]-cinnoline, benzo[lmn][3,8]phenanthroline (also called 2,7-diazapyrene), benz[4,10]anthra[1,9,8cdef]cinnoline, naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine, acenaphtho[1,2b]pyridine, benzo[a]phenazine, indeno-[1,2,3ij][2,7]naphthyridine, and indeno[1,2,3ij]isoquinoline in organic solvents of varying polarity and acidity. The various molecular structures are depicted in Figs. 3 and 4, along with twelve other monoaza- and two diaza-PANHs studied previously.[20–23] Also included are nitromethane and limited 1,2,4-trimethoxybenzene quenching studies on both the protonated and unprotonated forms of the PANH molecules.

MATERIALS AND METHODS

PANH solutes **E**, **F**, **O**, **P**, **Q**, **R**, **T**, and **V** were synthesized and purified by procedures described in the chemical literature.[24–31] Sources of the remaining 14 PANHs are identified in earlier papers.[20–23] Stock solutions were prepared by dissolving the solutes in dichloromethane. Small aliquots of the stock solutions were transferred into test tubes, allowed to evaporate, and diluted with the solvent of interest. Final solute concentrations were sufficiently dilute to minimize inner-filtering artifacts. Solvents were of HPLC, spectroquality or AR grade, purchased commercially from either Aldrich or Fisher Scientific, and the resulting solutions were optically dilute (absorbances $cm^{-1} < 0.01$) at all wave-

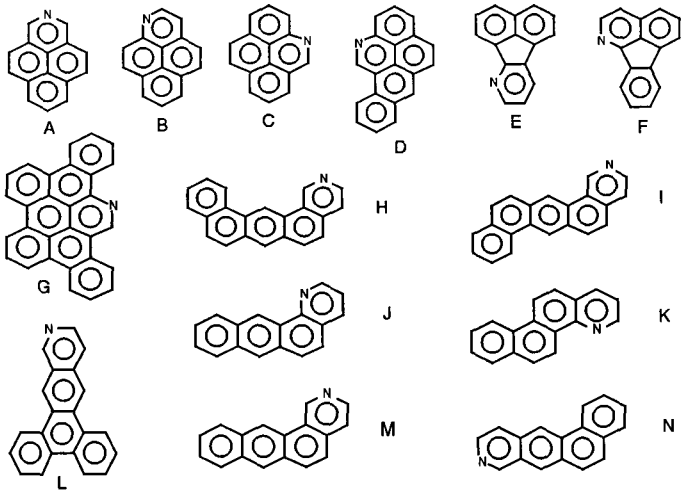

FIG. 3. Molecular structures of the various monoaza-PANH solutes: (**A**) 2-azapyrene; (**B**) 1-azapyrene; (**C**) 4-azapyrene; (**D**) 12-azabenzo[a]pyrene; (**E**) acenaphtho[1,2b]pyridine; (**F**) indeno[1,2,3ij]isoquinoline; (**G**) dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine; (**H**) phenanthro[2,3h]isoquinoline; (**I**) phenanthro[3,2h]isoquinoline; (**J**) 1-azabenz[a]anthracene; (**K**) 4-azachrysene; (**L**) phenanthro[9,10g]isoquinoline; (**M**) 2-azabenz[a]anthracene; and (**N**) 9-azabenz[a]anthracene.

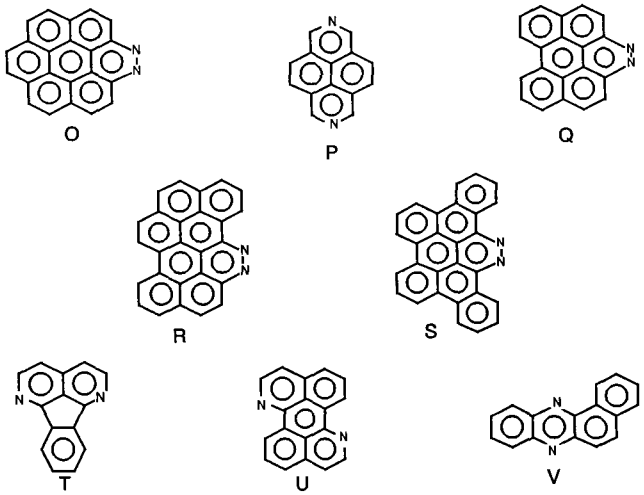

FIG. 4. Molecular structures of various diaza-PANH solutes: (**O**) naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline; (**P**) benzo[lmn][3,8]phenanthroline; (**Q**) benz[4,10]anthra[1,9,8cdef]cinnoline; (**R**) naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine; (**S**) diphenanthro[9,10,1def:1′,10′,9′hij]phthalazine; (**T**) indeno[1,2,3ij][2,7]naphthyridine; (**U**) benz[de]isoquino[1,8gh]quinoline; and (**V**) benzo[a]phenazine.

lengths, except for the nitromethane and 1,2,4-trimethoxybenzene quenching studies.

Absorption spectra were recorded on a Bausch and Lomb Spectronic 2000 and a Hewlett-Packard 8450A photodiode array spectrophotometer in the usual manner. The fluorescence spectra were run on a Shimadzu RF-5000U spectrofluorometer with the detector set at high sensitivity. Solutions were excited at the wavelengths listed in Table I. Fluorescence data were accumulated in a quartz 1-cm^2 cuvette at 19°C, ambient room temperature, with excitation and emission slit width settings of 15 nm and 3 nm, respectively. The PANH fluorescence spectra, depicted in Figs. 5–7, represent a single scan which was then solvent blank corrected and verified by repetitive measurements.

Emission intensities associated with the quenching study were corrected for primary inner-filtering artifacts arising from the absorption of excitation radiation. Several of the PANHs listed in Table I have excitation wavelengths in the 300–320 nm spectral region, and a few drops of nitromethane or 1,2,4-trimethoxybenzene gave solutions having appreciable absorbances. Mathematical expressions, computational procedures, and interrogation zone dimensions are given elsewhere.[14–16,20,32] Every effort was made to work at solution absorbances below

TABLE I. Summary of excitation wavelengths, probe character, and fluorescence quenching data for monoaza- and diaza-PANHs.

Compound	Chemical name	λ_{ex}	λ_{em}[a]	Probe	Quenched
	Monoaza-PANHs				
A	2-Azapyrene	338	376	No	Yes
B	1-Azapyrene	332	369	No	Yes
C	4-Azapyrene	331	371	No	Yes
D	12-Azabenzo[a]pyrene	364	407	Yes	Yes
E	Acenaphtho[1,2b]pyridine	340	460	No	No
F	Indeno[1,2,3ij]isoquinoline	360	467	No	No
G	Dibenzo[c,i]phenanthro[1,10,9,8anmlk]phenanthridine	350	397	No	No
H	Phenanthro[2,3h]isoquinoline	300		Yes	
I	Phenanthro[3,2h]isoquinoline	330	391	Yes	Yes
J	1-Azabenz[a]anthracene	340	394	Yes	Yes
K	4-Azachrysene	300	363	No	Yes
L	Phenanthro[9,10g]isoquinoline	300	382	No	Yes
M	2-Azabenz[a]anthracene	340	408	Yes	Yes
N	9-Azabenz[a]anthracene	300		Yes	
	Diaza-PANHs				
O	Naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline	317	436	No	No
P	Benzo[lmn][3,8]phenanthroline	328	419	No	Yes
Q	Benz[4,10]anthra[1,9,8cdef]cinnoline	374	430	No	No
R	Naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine	395	445	No	No
S	Diphenanthro[9,10,1def:1′,10′,9′hij]phthalazine	400	486	No	Yes
T	Indeno[1,2,3ij][2,7]naphthyridine	354	476	No	No
U	Benz[de]isoquino[1,8gh]quinoline	410	440	No	No
V	Benzo[a]phenazine	370	432	No	No

[a] Emission wavelengths used in the nitromethane quenching study.

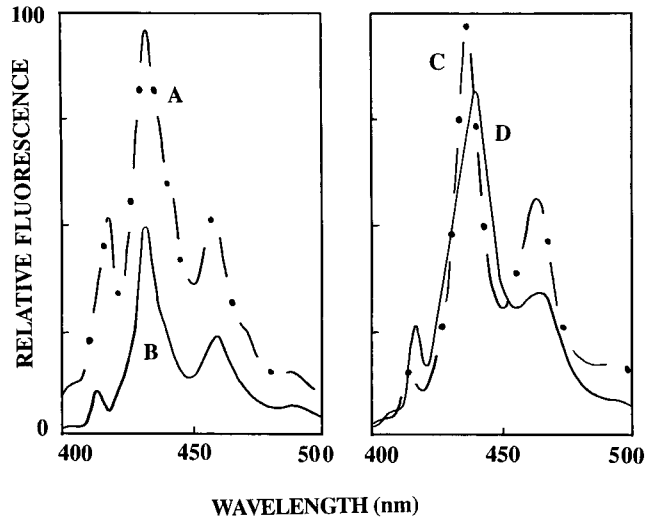

FIG. 5. Fluorescence emission spectra of naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline dissolved in [*A* (—●—)] *n*-hexadecane; [*B* (——)] butyl acetate; [*C* (—●—)] dichloromethane; and [*D* (——)] dimethyl sulfoxide. In butyl acetate emission bands occur at about 414, 434, 461, and 490 nm.

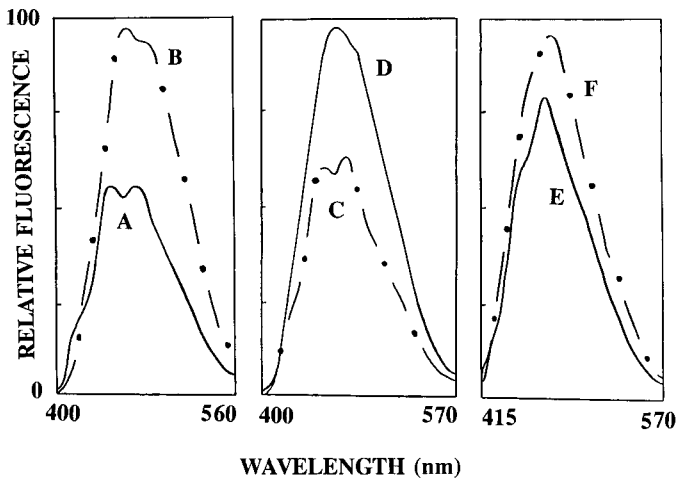

FIG. 7. Fluorescence emission spectra of acenaphtho[1,2b]pyridine dissolved in [*A* (——)] *n*-hexadecane and in [*B* (—●—)] dimethyl sulfoxide, of indeno[1,2,3ij]isoquinoline dissolved in [*C* (—●—)] *n*-hexadecane and in [*D* (——)] dimethyl sulfoxide, and of indeno-[1,2,3ij][2,7]naphthyridine dissolved in [*E* (——)] *n*-hexadecane and in [*F* (—●—)] dimethyl sulfoxide.

A cm^{-1} $\leq$ 0.95 (f_{prim} $\leq$ 3.00) where the inner-filtering correction equation is valid. Secondary inner-filtering corrections were not necessary in the present study since both nitromethane and 1,2,4-trimethoxybenzene are "optically transparent" in most of these PANHs' emission ranges.

RESULTS AND DISCUSSION

Representative fluorescence emission spectra of naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline (also called 1,2-diazacoronene), benzo[lmn][3,8]phenanthroline, acenaphtho[1,2b]pyridine, indeno[1,2,3ij]-isoquinoline, and indeno[1,2,3ij][2,7]naphthyridine dissolved in *n*-hexadecane, butyl acetate, dichloromethane, and dimethyl sulfoxide are shown in Figs. 5–7. Nonelectrolyte solvents were judiciously selected so as to encompass the entire range of solvent polarity, from the nonpolar *n*-hexadecane to the moderately polar butyl acetate and dichloromethane to the very polar dimethyl sulfoxide. Examination of the spectral data reveals that only 1,2-diazacoronene showed some signs of probe character, as evidenced by variation of emission intensity ratios with solvent polarity. Calculated emission intensities for diazacoronene (DAzCo; II/III) ranged from DAzCo = 1.41 for dibutyl ether to DAzCo = 2.39 for dimethyl sulfoxide. Actual numerical values for the fourteen solvents examined are tabulated in Table II. Estimated uncertainties in the measured intensity ratios are believed to be on the order of ±0.05 (or less) on the basis of replicate measurements. Although 1,2-diazacoronene does show some signs of probe character, the emission intensity ratios do not vary systematically with solvent polarity, as the numerical DAzCo values for dibutyl ether, chloroform, and dichloromethane are far too small. Even if the dibutyl ether value is ignored, the dynamic range of 0.68 is significantly smaller than those of pyrene (1.37), benzo[ghi]perylene (0.98), and ovalene (2.08).[34,35] To be consistent with our past classification scheme, 1,2-diazacoronene is labeled as a solvent polarity nonprobe molecule because of the unexplained, abnormally low DAzCo value measured for dibutyl ether and the unsystematic variation of DAzCo values with solvent polarity.

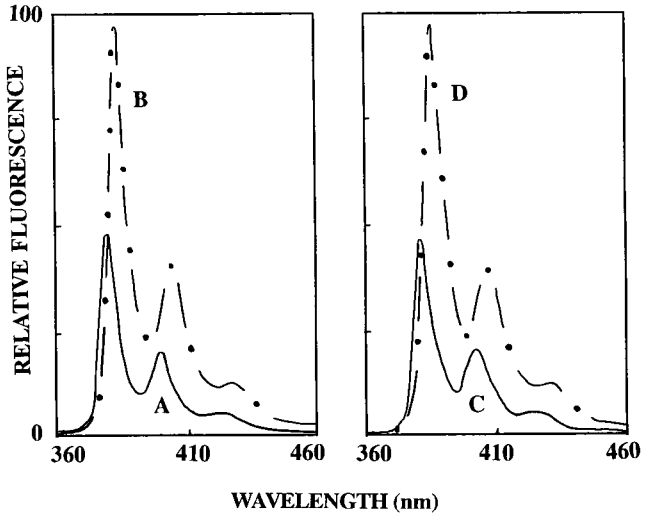

FIG. 6. Fluorescence emission spectra of benzo[lmn][3,8]-phenanthroline dissolved in [*A* (——)] *n*-hexadecane; [*B* (—●—)] dichloromethane; [*C* (——)] butyl acetate; and [*D* (—●—)] dimethyl sulfoxide. In butyl acetate emission bands occur at about 382, 403, and 426 nm.

Naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine also appeared to exhibit signs of probe character until it was noticed that the compound had only one fairly weak emission in several of the nonelectrolyte solvents. Fluorescence spectra of benzo[a]phenazine and the three nonalternant PANH solutes (acenaphtho[1,2b]pyridine, indeno[1,2,3ij]isoquinoline, and indeno[1,2,3ij][2,7]-naphthyridine; see Fig. 7) showed very little structured emission. Depending upon the solute and solvent combination being studied, one observed either one unsymmetrical band or two poorly resolved peaks. Emission intensity ratios for benzo[lmn][3,8]phenanthroline and benz[4,10]anthra[1,9,8cdef]cinnoline varied only slightly

TABLE II. Ratios of fluorescence emission intensities of 1,2-diazacoronene in various organic solvents.

Organic solvent	Py[a]	DAzCo[b]
Cyclohexane	0.58	1.86
2,2,4-Trimethylpentane	0.59	1.87
n-Hexadecane	0.60	1.88
Carbon tetrachloride		2.02
Dibutyl ether	0.84	1.41
Benzene	1.05	2.16
2-Propanol	1.09	2.24
Chloroform	1.25	1.78
Butyl acetate	1.35	2.03
Methanol	1.35	2.23
Dichloromethane	1.35	1.71
Acetonitrile	1.79	2.23
Dimethyl formamide	1.81	2.30
Dimethyl sulfoxide	1.95	2.39
Dynamic range	1.37	

[a] Experimental Py values are taken from Dong and Winnik[33] and are based upon the properties of pyrene.

[b] Defined as the ratio of band II (at 431–439 nm)/band III (at 458–466 nm) for 1,2-diazacoronene (also called naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline).

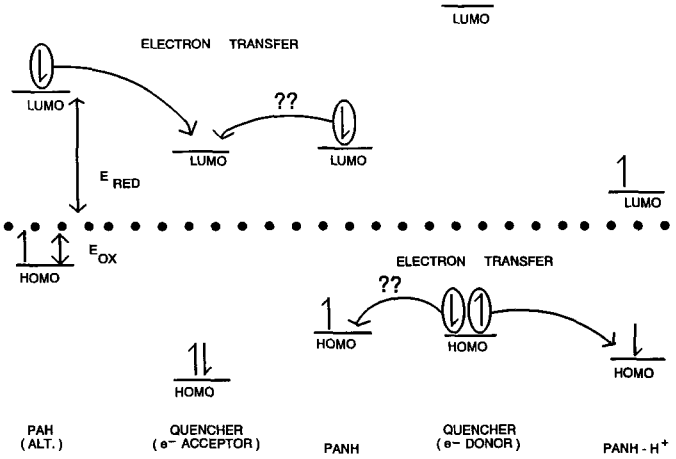

FIG. 8. Plausible molecular orbital diagram to explain observed quenching of unprotonated and protonated PANH fluorescence emission by nitromethane (electron acceptor) and 1,2,4-trimethoxybenzene (electron donor).

with solvent polarity. All eight PANHs examined for possible solvent polarity probe behavior in the present study are thus classified as nonprobe molecules.

Polycyclic aromatic nitrogen heterocycles have also served as solvent acidity probes. Lloyd[36] and Burnell and Hurturbise[37–39] previously reported fluorescence emission intensification whenever neutral solutions of acridine, benzo[f]quinoline, benzo[h]quinoline, and benz[c]acridine were passed through or adsorbed onto silica gel chromatographic columns. The excitation remained essentially identical, while the emission spectra were red-shifted to much longer wavelengths because of protonation of the nitrogen lone electron pair by a hydrogen ion coming from the acidic silanol group. Degree of protonation should be reflected by both solvent acidity and PANH basicity. 2,2,2-Trifluoroethanol was found to partially protonate all of the new PANH solutes studied except for acenaphtho[1,2b]pyridine (Compd. **E**), as evidenced by a decrease in the first band emission and the appearance of a new band at longer wavelengths. Titration of trifluoroethanolic solutions of benzo[lmn][3,8]phenanthroline, benzo[a]phenazine, and indeno[1,2,3ij][2,7]naphthyridine with extremely small amounts of perchloric acid yielded two different fluorescence spectra, which we attribute to emissions from the monoprotonated (PANH-H^+) and diprotonated (PANH-H_2^{+2}) forms of the PANHs. In the case of naphth[2′,1′,8′,7′:4,10,5]anthra[1,9,8cdef]cinnoline, benz[4,10]anthra[1,9,8cdef]cinnoline, and naphtho[8,1,2hij]pyreno[9,10,1def]phthalazine, only a single protonated species was observed. Close proximity of the two nitrogen heteroatoms in this latter set of diazaarenes should discourage the formation of PANH-H_2^{+2}, since this would require that a positive charge be placed on adjacent nitrogen atoms. The fluorescence emission spectra of acenaphtho[1,2b]pyridine showed no indication of the molecule being protonated upon addition of perchloric acid, and we are unable to explain this particular observation at the present time.

The effect of nitromethane on the fluorescence properties of select monoaza- and diaza-PANHs is summarized in the last column of Table I. Careful examination of the table reveals that nitromethane quenches the fluorescence emission of approximately two-thirds of the seventeen alternant PANHs considered. Corrected emission intensities of the three nonalternant PANHs (Compds. **E**, **F**, and **T**) remained essentially constant upon the nitromethane addition. Failure of nitromethane to quench fluorescence emission of many of the PANHs studied is not unexpected. As argued by Breymann *et al.*,[18] nitromethane quenches the fluorescence emission of alternant PAH6 benzenoids via an electron transfer process. The rate of electron transfer depends upon the change in the free energy, with the more favorable electron transfer process proceeding at a much faster reaction rate. The more positive reduction potentials of nonalternant PAHs result in less favorable electron transfer conditions and much smaller quenching rate constants. Emission intensities of nonalternant PAHs are for the most part unaffected by nitromethane, though a few exceptions are known. While electrochemical information on PANH vs. PAH reduction potentials in common solvents is relatively scarce in the chemical literature, Fox[40] noted that the reduction of acridine of $E = -1.24$ eV in acetonitrile was significantly more positive than the corresponding value of $E = -1.92$ eV for the anthracene counterpart. We would expect other PANHs to behave in similar fashion. With approximately one-third of the alternant PANHs being exceptions to the nitromethane selective quenching rule, we must conclude that the reduction potential range is fairly broad among the PANH solutes. Depending upon the PANH under examination, the PANH LUMO falls slightly above (or slightly below) the electron acceptor's LUMO, thereby encouraging (or discouraging) electron transfer.

Knowing that nitromethane reportedly functions as an electron acceptor, we decided to investigate whether nitromethane quenched the fluorescence emission of protonated PANHs. From simple coulombic considerations, it should be considerably more difficult to remove an electron from the cationic PANH-H^+ ion than from the neutral PANH molecule. As expected, fluorescence emission intensities of the twenty-one protonated PANHs

(all except acenaphtho[1,2b]pyridine, which would not protonate) were unaffected by nitromethane after the observed values were corrected for primary inner-filtering. The coulombic argument is placed on a more theoretical basis by observation that protonation does cause a decrease in the HOMO-LUMO energy separation, as evidenced by a sizeable red shift in the fluorescence emission wavelength. Of the five possible ways to decrease the HOMO-LUMO energy gap, nitromethane quenching data on the protonated PANHs suggest that the LUMO must be moved to give a more positive reduction potential (see Fig. 8 for a plausible molecular orbital diagram). This seems reasonable in light of recently published electrochemical data for various quaternized azaarene compounds. Reduction potential of 4,4′-bipyridine shifted to a more positive value with addition of the CH_3^+ group to the nitrogen hetero-atom, from −2.394 eV for 4,4′-bipyridine to −1.248 eV for the N-methyl-4,4′-bipyridinium$^+$ cation. A much larger shift was observed for the N,N-dimethyl-4,4′-bipyridinium^{+2} cation (−0.580 eV); however, the authors had to change solvents since the perchlorate salt was insoluble in dimethylformamide.[41]

The relative location of the unexcited PANH's and PANH-H$^+$'s highest occupied molecular orbital can be ascertained by studying the solute's fluorescence behavior in the presence of an electron donor quenching agent, such as 1,2,4-trimethoxybenzene. Preliminary measurements on benz[de]isoquino[1,8gh]quinoline, benzo[a]phenazine, 12-azabenzo[a]pyrene, and diphenanthro[9,10,1def;1′,10′,9′hij]phthalazine reveal that trimethoxybenzene quenches the fluorescence emission of the protonated forms of these four PANH solutes. Only benz[de]isoquino[1,8gh]quinoline and 12-azabenzo[a]pyrene were significantly quenched as neutral PANH molecules. Strong absorbance by trimethoxybenzene in the 300–350 nm spectral region gave primary inner-filtering corrections of $f_{prim} \geq 3.0$ under the experimental conditions used. To minimize primary inner-filtering corrections, we restricted our studies to select solutes having their major excitation wavelength greater than 350 nm. The 1,2,4-trimethoxybenzene quenching data suggest that upon protonation the PANH's HOMO must move to give a larger oxidation potential.

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