Spectroscopic Investigation of Fluorescence Quenching Agents. Part III: Effect of Solvent Polarity on the Selectivity of Nitromethane for Discriminating Between Alternant Versus Nonalternant Polycyclic Aromatic Hydrocarbons

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To better assess the applicability of nitromethane as a selective quenching agent for alternant versus nonalternant polycyclic aromatic hydrocarbons in HPLC, TLC, and HPTLC analysis, we measured the effect that it has on the fluorescence emission behavior of 96 different polycyclic aromatic hydrocarbons dissolved in binary toluene/acetonitrile solvent mixtures. Results of these measurements revealed that the "selective quenching" rule is obeyed for the vast majority of PAHs, with the coronene derivatives being the only major exceptions. Fluorescence emission spectra are also reported for benzo[g]chrysene, naphtho[2,3g]chrysene, 4*H*-benzo[c]cyclopenta[mno]chrysene, dibenzo[ghi,mno]fluoranthene (commonly called corannulene), rubicene, diacenaphtho[1,2j:1′,2′l]fluoranthene, 10-methylbenzo[b]fluoranthene, 3-methoxybenzo[k]fluoranthene, and 3-hydroxybenzo[k]fluoranthene in organic nonelectrolyte solvents of varying polarity. Calculated emission intensity ratios failed to vary systematically with solvent polarity, and all nine of the aforementioned solutes were thus classified as nonprobe molecules.

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

INTRODUCTION

Polycyclic aromatic compounds (PACs) have received considerable attention in the recent chemical literature, in part because of the widely varying carcinogenic and mutagenic properties of closely related isomers. Carcinogenic properties of select isomers, combined with an increasing awareness of environmental pollution and toxic material disposal, have prompted research to develop analytical methods specific for the different aromatic compounds. Current analytical methods generally employ high-resolution chromatographic techniques combined with pre-concentration via supercritical fluid[1-5] or Soxhlet extraction,[5-7] relatively sophisticated or lengthy isolation, or group separation schemes to obtain fractions from environmental or biological samples suitable for chromatographic analysis. Gas chromatographic separations utilizing liquid crystalline[8-11] and/or polymeric liquid crystalline stationary phases,[12-15] or using specially deactivated and stabilized thin-film open-tubular columns,[16,17] enable concentrations of several (though by no means all) of the aromatic constituents to be determined experimentally. Unfortunately, even these high-efficiency columns cannot completely resolve all polycyclic aromatic compound isomers found in environmental samples, and because of volatility constraints, quantification of the higher-molecular-weight PACs is not generally possible through gas chromatographic measurements.

Mixtures containing larger, nonvolatile polycyclic aromatic hydrocarbons can be conveniently analyzed by high-performance liquid chromatography (HPLC). Conventional chemically bonded monomeric C_{18} stationary phases[18-22] (i.e., phases prepared with monofunctional silanes) can generally separate the various PAH molecules present according to overall size and number of aromatic rings. Monomeric C_{18} phases possess only limited inherent ability to separate PAH isomers on the basis of molecular shape. Enhanced shape recognition is achieved on either a "charge transfer"[23] or a polymeric C_{18} stationary phase[18-22,24,25] (i.e., phase prepared with either difunctional or trifunctional silanes in the presence of water), the latter of which is believed to be molecularly ordered to some extent. Relative retention values for similar PAH structures of increasing size (i.e., naphthalene, pyrene, benzo[ghi]perylene, benzo[pqr]naphtho[8,1,2bcd]perylene, benzo[rst]dinaphtho[2,1,8,7defg; 2′,1′,8′7′ijkl]pentaphene, and tetrabenzo[def,lm,qrs,yz]-pyranthene) with the use of different polymeric C_{18} stationary and mobile phase combinations, along with the elution strengths of the eleven common HPLC solvents, are reviewed in detail elsewhere.[25]

Conventional thin-layer chromatographic[26-32] (TLC) and high-performance thin-layer chromatographic[33-37] (HPTLC) methods have been employed with limited success in the analysis of unknown PAC samples. In many of the published studies, the various polycyclic aromatic compounds were separated on alumina, silica gel, 3-cyanopropylsilanized silica gel, 3-aminopropylsilanized silica gel, octadecylsilanized silica gel, or partially acetylated cellulose plates. The spots or zones corresponding to expected PACs were then excised, and the concentrations of the individual PAC isomers determined by elu-

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tion and solution fluorometry. As in the case of HPLC, resolution in thin-layer chromatography is determined to a large extent by the stationary and mobile phase combination used. Even under optimum experimental conditions, it is very difficult (if not impossible) to completely separate all the different polycyclic aromatic compounds present in the environmental/biological samples commonly encountered.

Problems associated with co-eluting components in HPLC and TLC can be overcome in part by making the detector selective for each given PAC subclass. Of the four most common HPLC detectors on the market, fluorescence affords the most selectivity in that the excitation and emission wavelengths can be varied independently. While several polycyclic aromatic compounds may absorb at the same excitation wavelength, not all will emit at the wavelength(s) monitored by the detector. Utilization of selective fluorescence quenching agents further simplifies observed emission spectra by eliminating signals from undesired chemical interferences having only slightly different molecular structures. On the basis of limited fluorescence measurements for perylene, dibenzo[b,k]chrysene, dibenzo[h,rst]pentaphene, naphtho[1,2b]fluoranthene, indeno[1,2,3cd]pyrene, and 12,11-(peri-naphthylene)fluoranthene dissolved in a binary aqueous/acetonitrile mixture (20:80 percent by volume), Blümer and Zander[38] noted that nitrobenzene and nitromethane selectively quenched fluorescence emission of the so-called "alternant" polycyclic aromatic hydrocarbons (PAHs). Emission intensities of the three nonalternant PAHs (e.g., naphtho[1,2b]fluoranthene, indeno[1,2,3cd]pyrene, and 12,11-(perinaphthylene)-fluoranthene) were unaffected. Published studies[39-44] involving over 63 PAHs have identified dibenzo-[hi,wx]heptacene, benzo[k]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. More recent measurements[45] revealed that nitromethane quenched fluorescence emission of all nine acenaphthylene-derivatives studied thus far, which is completely contrary to what would be expected on the basis of the fact that the nine solutes are nonalternant PAH molecules. Despite the aforementioned exceptions, nitromethane remains a very useful, selective quenching reagent in fluorescence analysis of unknown mixtures of polycyclic aromatic compounds.

For the most part previous nitromethane quenching investigations have been confined to more polar solvents such as neat acetonitrile or binary aqueous/acetonitrile mixtures. Both systems are used routinely as mobile phases in HPLC analysis of small PAHs having six rings or less. However, larger six- to ten-ring PAH molecules may require an aromatic hydrocarbon or a dichloromethane mobile phase cosolvent in order for the solutes to elute from the chromatographic column (or to dissolve from the TLC/HPTLC plate) in timely fashion.[25] To better assess the applicability of nitromethane as a selective quenching agent for HPLC, TLC, and HPTLC analysis, we report the effect that nitromethane has on the fluorescence emission behavior of 96 different alternant and nonalternant PAHs dissolved in binary toluene/acetonitrile solvent mixtures. Also, as a continuation of our fluorescence studies we have measured the fluorescence behavior of benzo[g]chrysene, naphtho-[2,3g]chrysene, 4*H*-benzo[c]cyclopenta[mno]chrysene, dibenzo[ghi,mno]fluoranthene (commonly called corannulene), rubicene, diacenaphtho[1,2j:1′,2′l]fluoranthene, 10-methylbenzo[b]fluoranthene, 3-methoxybenzo[k]fluoranthene, and 3-hydroxybenzo[k]fluoranthene (see Fig. 1 for molecular structures) dissolved in nonelectrolyte organic solvents of varying solvent polarity.

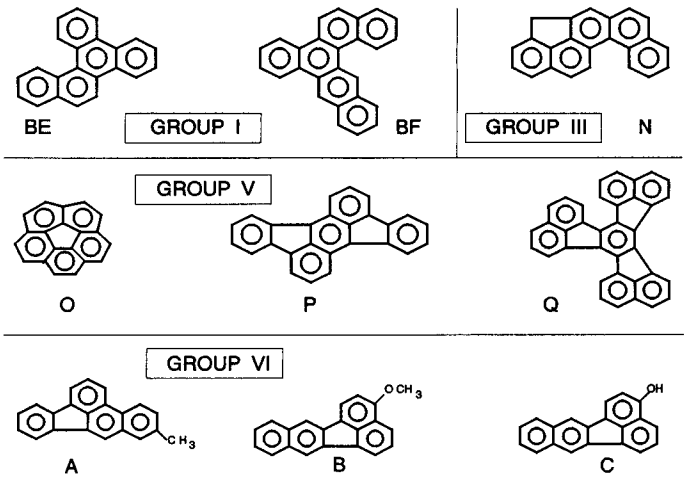

FIG. 1. Molecular structures of the polycyclic aromatic hydrocarbons: (**BE**) benzo[g]chrysene; (**BF**) naphtho[2,3g]chrysene; (**N**) 4*H*-benzo[c]cyclopenta[mno]chrysene; (**O**) dibenzo[ghi,mno]fluoranthene (corannulene); (**P**) rubicene; (**Q**) diacenaphtho[1,2j:1′,2′l]fluoranthene; (**A**) 10-methylbenzo[b]fluoranthene; (**B**) 3-methoxybenzo[k]fluoranthene; (**C**) 3-hydroxybenzo[k]fluoranthene. See Table I for group definitions.

MATERIALS AND METHODS

The nine new PAH solutes were synthesized and purified by procedures described in the chemical literature.[46-53] 10-Methylbenzo[b]fluoranthene and 3-hydroxybenzo[k]fluoranthene are also available from the NCI Chemical Carcinogen Reference Standard Repository.[54] Synthetical references and/or commercial suppliers for the remaining PAH solutes contained in Table I are listed in our earlier papers. 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 wavelengths, except for the nitromethane 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 1-cm^2 quartz cuvette at 19°C, ambient room

TABLE I. Summary of excitation wavelength, probe character and fluorescence quenching (by nitromethane) data for alternant and nonalternant PAHs.

Letter[a]	Chemical name	Probe	λ_{ex} (nm)	λ_{em}[b] (nm)	Quenched ACN	Quenched ACN-TOLN[e]
	Group I: Alternant PAHs					
A	Coronene	Yes	334	425	Yes[d]	No
B	Benzo[a]coronene	Yes	316	429	Yes[d]	No
C	Naphtho[2,3a]coronene	Yes	333	442	Yes[d]	No
D	Dibenzo[a,j]coronene	Yes	324	431	Yes[d]	No
E	Naphtho[8,1,2abc]coronene	Yes	322	458	Yes[d]	No
F	Naphtho[2′,1′,8′,7′:4,10,5]anthra[1,9,8abcd]coronene	No	425			
G	Benzo[1,2,3bc;4,5,6b′c′]dicoronene	No	440			
H	Dibenzo[bc,ef]coronene	No	366			
I	Tetrabenzo[de,hi,mn,qr]naphthacene	No	318	418	Yes	Yes
J	Dinaphtho[8,1,2abc;2′,1′,8′klm]coronene	Yes	425			
K	Dinaphtho[8,1,2abc;2′,1′,8′jkl]coronene	NA[c]	370	481	Yes	No
L	Anthra[2,3a]coronene	Yes	357	488	Yes[d]	No
M	Pyrene	Yes	338	371	Yes	Yes
N	Perylene	No	403	437	Yes	Yes
O	Benzo[ghi]perylene	Yes	380	405	Yes	Yes
P	Ovalene	Yes	445			
Q	Naphtho[1,2,3,4ghi]perylene	No	316	416	Yes	Yes
R	Benzo[pqr]naphtho[8,1,2bcd]perylene	No	325	418	Yes[d]	Yes
S	Dibenzo[cd,lm]perylene	No	324			
T	Benzo[a]pyrene	No	350	402	Yes	Yes
U	Benzo[e]pyrene	Yes	335	386	Yes	Yes
V	Dibenzo[a,e]pyrene	No	360	394	Yes	Yes
W	Dibenzo[fg,ij]phenanthro[2,1,10,9,8,7pqrstuv]pentaphene	Yes	393			
X	Tribenzo[de,kl,rst]pentaphene	No	510			
Y	Dinaphtho[2,1,8,7defg;2′,1′,8′,7′ijkl]pentaphene	NA[c]	420			
Z	Benz[rst]anthra[9,1,2cde]pentaphene	NA[c]	400			
AA	Benz[4,10]anthra[1,9,8abcd]coronene	No	370			
AB	Benzo[cd]chryseno[4,5,6,7fghijk]perylene	No	330			
AC	Benzo[b]perylene	No	312	437	Yes[d]	Yes
AD	Dibenzo[e,ghi]perylene	NA[c]	330			
AE	Phenanthro[5,4,3,2efghi]perylene	Yes	365			
AF	Dibenzo[cd,k]naphtho[8,1,2fghi]perylene	No	321	451	Yes[d]	Yes
AG	Benzo[a]naphtho[8,1,2cde]naphthacene	No	411	446	Yes[d]	Yes
AH	Dibenzo[def,p]chrysene	Yes	310	419	Yes	Yes
AI	Anthranthrene	No	306	430	Yes	Yes
AJ	Benzo[rst]pentaphene	Yes	307	432	Yes	Yes
AK	Benzo[rst]dinaphtho[2,1,8,7defg;2′,1′,8′,7′ijkl]pentaphene	No	315	438	Yes	No
AL	Dibenzo[de,kl]naphtho[1,2,3,4rst]pentaphene	No	375			
AM	Dibenzo[h,rst]pentaphene	Yes	366	416	Yes[d]	Yes
AN	Dibenzo[a,rst]naphtho[8,1,2cde]pentaphene	No	300	413	Yes	Yes
AO	Dibenzo[fg,ij]pentaphene	No	300			
AP	Tetrabenzo[de,hi,op,st]pentacene	No	375			
AQ	Dinaphtho[2,1,8,7defg;2′,1′,8′,7′opqr]pentacene	No	410	484	Yes[d]	Yes
AR	Pyranthene	NA[c]	300	467	Yes	Yes
AS	Dibenzo[de,qr]naphthacene	Yes	340			
AT	Dibenzo[de,st]pentacene	No	408	470	Yes	Yes
AU	Dibenzo[fg,qr]pentacene	No	400	435	Yes	Yes
AV	Naphtho[8,1,2hij]hexaphene	No	360	446	Yes	Yes
AW	Benzo[vwx]hexaphene	Yes	360	477	Yes	Yes
AX	Benzo[1,2,3cd;4,5,6c′d′]diperylene	No	400	437	Yes	Yes
AY	Dibenzo[hi,wx]heptacene	No	400	485	No	No
AZ	Benzo[b]triphenylene	No	300	375	Yes	Yes
BA	Anthracene	No	340	377	Yes	Yes
BB	Triphenylene	No	300	354	Yes	Yes
BC	Phenanthrene	No	300	363	Yes	Yes
BD	Chrysene	No	320	380	Yes	Yes
BE	Benzo[g]chrysene	No	320	392	Yes	Yes
BF	Naphtho[2,3g]chrysene	No	350	424	Yes	Yes
	Group II: Alternant PAH derivatives					
A	1-Methylpyrene	No	330	375	Yes[d]	Yes
B	2-Methylpyrene	No	333	374	Yes[d]	Yes
C	4-Methylpyrene	No	320	375	Yes[d]	Yes
D	1,5-Dimethylpyrene	No	320	379	Yes[d]	Yes
E	1-Butylpyrene	No	320	375	Yes[d]	Yes
F	1-Decylpyrene	No	320	375	Yes[d]	Yes
G	4-Methylchrysene	No	320	368	Yes	Yes
H	5-Methylchrysene	No	320	370	Yes	Yes
I	5-Methyldibenzo[b,def]chrysene	No	300	460	Yes[d]	Yes
J	3,4,5-Trihydrobenzo[cd]pyrene	No	340	377	Yes	Yes

TABLE I. Continued.

Letter[a]	Chemical name	Probe	λ_{ex} (nm)	λ_{em}[b] (nm)	Quenched ACN	Quenched ACN-TOLN[e]
K	3,4-Dihydrobenzo[ghi]perylene	Yes	334	381	Yes	Yes
L	5,6,7,8,9,10-Hexahydrobenzo[ghi]perylene	No	350	384	Yes	Yes
	Group III: Alternant cyclopenta-PAH derivatives					
A	11*H*-benzo[bc]aceanthrylene	Yes	350	389	Yes	Yes
B	4*H*-cyclopenta[def]phenanthrene	No	320	362	Yes	Yes
C	4*H*-cyclopenta[def]chrysene	Yes	320	360	Yes	Yes
D	13*H*-dibenzo[a,g]fluorene	No	340	381	Yes	Yes
E	13*H*-dibenzo[a,i]fluorene	No	320	384	Yes	Yes
F	4*H*-benzo[b]cyclopenta[mno]chrysene	Yes	330	398	Yes	Yes
G	4*H*-cyclopenta[pqr]picene	Yes	330	395	Yes	Yes
H	13*H*-dibenz[bc,l]aceanthrylene	Yes	320	398	Yes	Yes
I	13*H*-dibenz[bc,k]aceanthrylene	No	400	460	Yes	Yes
J	4*H*-benzo[b]cyclopenta[jkl]triphenylene	No	300	397	Yes	Yes
K	7*H*-dibenzo[c,g]fluorene	No	300	386	Yes	Yes
L	9*H*-benz[6,7]indeno[1,2l]phenanthrene	No	345	394	Yes	Yes
M	4*H*-benzo[def]cyclopenta[mno]chrysene	No	320	400	Yes	Yes
N	4*H*-Benzo[c]cyclopenta[mno]chrysene	No	300	383	Yes	Yes
	Group IV: Nonalternant acenaphthylene derivatives					
A	Acenaphthylene	No	288	336	Yes	Yes
B	Aceanthrylene	No	360	427	Yes	Yes
C	Acephenanthrylene	No	300	355	Yes	Yes
D	Benz[e]aceanthrylene	No	360	402	Yes	Yes
E	3-Methylbenz[j]aceanthrylene	No	300	397	Yes	Yes
F	6-Methylbenz[j]aceanthrylene	No	300	426	Yes	Yes
G	Benzo[def]cyclopenta[hi]chrysene	No	300	410	Yes	Yes
H	Cyclopenta[cd]pyrene	No	336	377	Yes	Yes
I	Acenaphth[1,2a]acenaphthylene	No	406	435	Yes	Yes
	Group V: Nonalternant fluoranthenoids and fluorenoids					
A	Benz[def]indeno[1,2,3hi]chrysene	No	406	474	No[d]	No
B	Fluoreno[2,3,4,9defg]chrysene	No	315	478	No[d]	No
C	Benz[def]indeno[1,2,3qr]chrysene	No	408	490	No	No
D	Benzo[k]fluoranthene	Yes	306	408	Yes[d]	Yes
E	Dibenzo[a,e]fluoranthene	No	390	482	No[d]	No
F	Indeno[1,2,3cd]pyrene	No	326	474	No[d]	No
G	Naphtho[1,2b]fluoranthene	No	350	438	No[d]	No
H	Benzo[b]fluoranthene	No	346	446	No[d]	No
I	Benzo[j]fluoranthene	No	315	508	No[d]	No
J	Fluoranthene	No	300	461	No[d]	No
K	Benzo[ghi]fluoranthene	No	340	447	No	No
L	Naphtho[2,1a]fluoranthene	No	400	466	No[d]	No
M	Naphtho[2,3b]fluoranthene	Yes	316	422	Yes[d]	No
N	Benzo[a]fluoranthene	No	406	488	No	No
O	Dibenzo[ghi,mno]fluoranthene	No	290	433	No	No
P	Rubicene	No	370	542	No	No
				402	Yes	Yes
Q	Diacenaphtho[1,2j:1′,2′l]flouranthene	No	374	502	No	No
	Group VI: Nonalternant fluoranthenoid derivatives					
A	10-Methylbenzo[b]fluoranthene	No	347	445	No	No
B	3-Methoxybenzo[k]fluoranthene	No	312	456	Yes	Yes
C	3-Hydroxybenzo[k]fluoranthene	No	310	459	Yes	Yes

[a] Molecular structures are given in Refs. 39–41, 45, 57, and 63.
[b] Emission wavelength used in the nitromethane quenching studies.
[c] NA indicates that the PAC is unacceptable as a solvent polarity probe molecule because a common set of bands could not be assigned in all solvents studied. The molecule may exhibit selective enhancement of one (or more) fluorescence emission band(s).
[d] Quenching studies performed in a binary aqueous/acetonitrile solvent mixture (20:80 percent by volume). Quenching of coronene and benzo[a]coronene fluorescence emission, after correcting for primary inner-filtering and dilution, amounted to about a 20% reduction in signal intensity. Much larger reductions were observed for many of the other altenrant PAHs studied.
[e] Quenching studies performed in a binary acetonitrile/toluene solvent mixture (60:40 percent by volume).

temperature, with excitation and emission slit width settings of 15 nm and 3 nm, respectively. For solvent polarity determinations involving pyrene, the emission slit width was reduced to 1.5 nm so that the calculated intensity ratios could be compared directly with literature values.[55] Street and Acree[56] had previously shown that emission intensity ratios vary with emission slit setting. The fluorescence spectra, depicted in Figs. 2 and 3, 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. Many of the PAHs have excitation wavelengths in the 300–320 nm spectral region, and a few drops of nitromethane gave solutions having appreciable absorbances. Mathematical

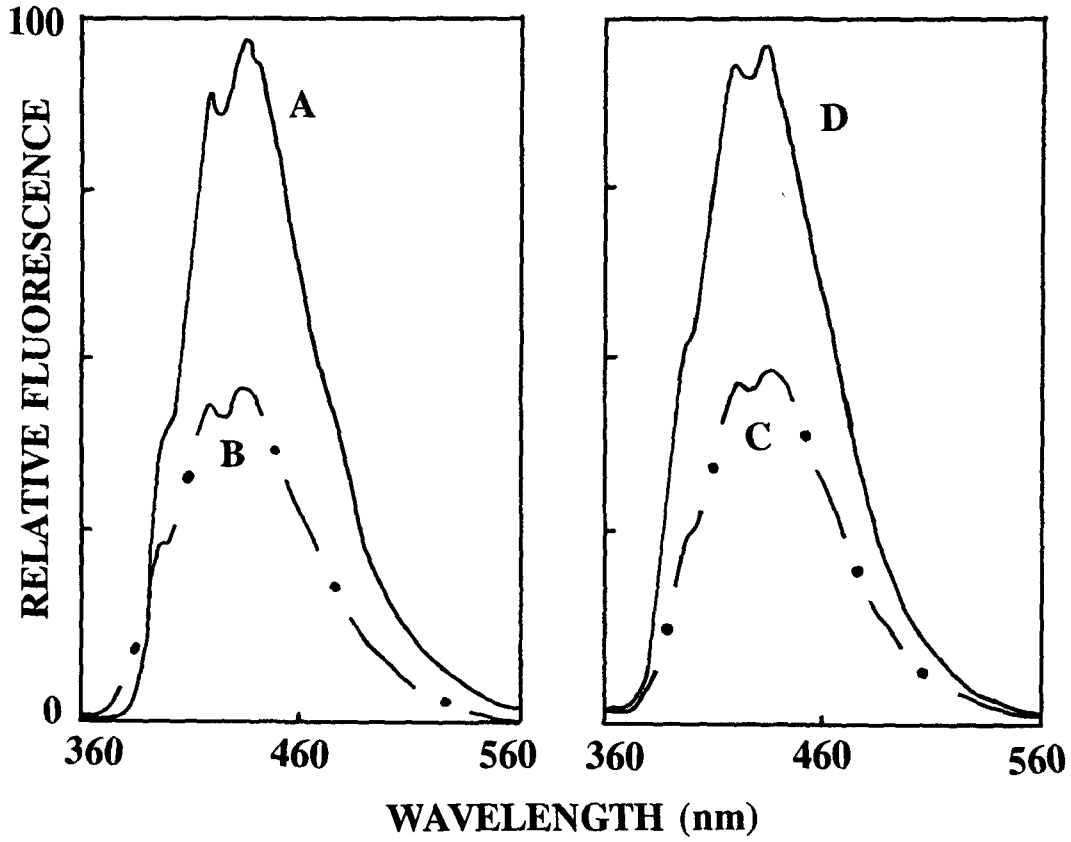

FIG. 2. Fluorescence emission spectra of corannulene dissolved in [*A* (——)] *n*-hexadecane; [*B* (— · —)] butyl acetate; [*C* (— · —)] dichloromethane; and [*D* (——)] dimethyl sulfoxide. In butyl acetate emission bands occur at about s-395, 419, and 434 nm.

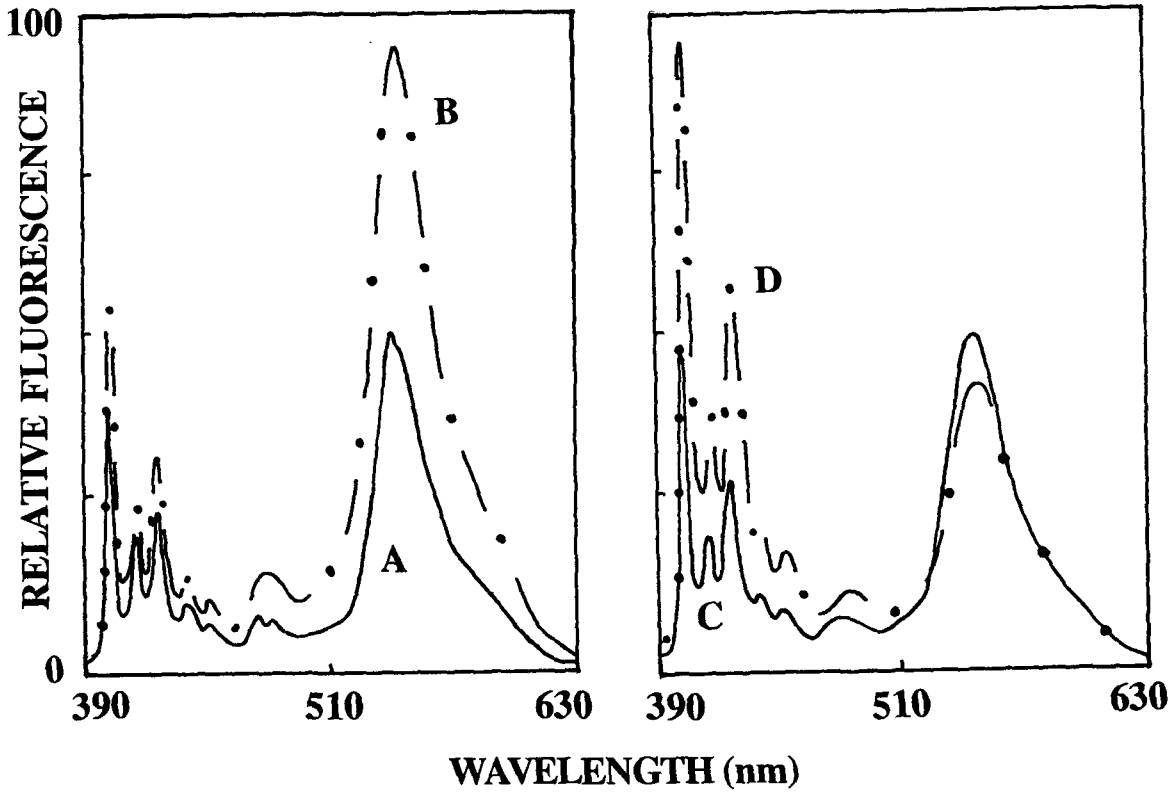

FIG. 3. Fluorescence emission spectra of rubicene dissolved in [*A* (——)] *n*-hexadecane; [*B* (— · —)] butyl acetate; [*C* (——)] dichloromethane; and [*D* (— · —)] dimethyl sulfoxide. In butyl acetate emission bands occur at about 402, 415, 425, 439, 450, 478, and 541 nm.

expressions, computational procedures, and interrogation zone dimensions are given elsewhere.[39-41,57,53] Every effort was made to work at solution absorbances below 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 nitromethane is "optically transparent" in most of these PAHs' emission ranges.

RESULTS AND DISCUSSION

Representative fluorescence emission spectra of corannulene (dibenzo[ghi,mno]fluoranthene) and rubicene dissolved in *n*-hexadecane, butyl acetate, dichloromethane, and dimethyl sulfoxide are depicted in Figs. 2 and 3. These four 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 solvents to the very polar dimethyl sulfoxide, which is the most polar solvent considered in this present study. The five-membered ring in the center of corannulene introduces curvature, giving it the shape of a bowl. The geometry of corannulene is reminiscent of the carbon cage molecules called fullerenes. Recent studies by Scott *et al.* suggest that its bowl inverts more than 200,000 times per second at room temperature.[59] The fluorescence lifetime for most organic molecules is about 10^{-7} to 10^{-9} seconds; therefore, corannulene should not have time to complete a full inversion during the fluorophore's lifetime. The fluorescence spectra of corannulene in Fig. 2 are thought to be an average of the constantly inverting molecule. Although it is completely different in its molecular geometry than any other fluoranthenoid studied to date, its fluorescence emission spectra are typical for a member of this PAH subclass. Fluorescence emission spectra showed little or no fine structure and consisted primarily of 1–3 very broad bands for most of the fluoranthenoid and fluorenoid compounds studied.

Table II documents that within each group (see Table I for Group definitions) the newly studied PAHs' behavior is indicative of their respective subclasses, with the exception of rubicene. As with the previously studied pyrene derivatives, the substituted benzo[b]fluoranthene and benzo[k]fluoranthene fluorophores have fluorescence emission spectra similar to their parent compound, but with reduced fine structure.[57] Closer examination of Fig. 3 reveals that rubicene initially showed signs of probe character as evidenced by variation of emission intensity ratios with solvent polarity. Calculated emission intensities for rubicene (RUB; I @ 403 nm/VII @ 544 nm) ranged from RUB = 0.80 for *n*-hexadecane, RUB = 0.58 butyl acetate, and RUB = 0.96 for dichloromethane to RUB = 2.16 for dimethyl sulfoxide. The emission intensity ratios, however, do not vary systematically with solvent polarity regardless of how the emission peak ratios are defined. All of the solutes listed in Table II are thus classified as nonprobes. For a compound in the fluoranthenoid subclass, rubicene shows very unusual behavior. To date, we have not found any compound in this particular subclass to have more than three emission peaks in its fluorescence spectra. Rubicene has seven and sometimes eight emission peaks, and most are very well resolved. In light of nitromethane quenching behavior (see below), the fluorescence characteristics of rubicene will be examined in greater detail.

TABLE II. Summary of excitation and emission wavelengths in butyl acetate for newly reported PAHs contained in Fig. 1.

Letter	Chemical name	λ_{ex} (nm)	λ_{em} (nm)
	Group I		
BE	Benzo[g]chrysene	320	379 392 s-413[a]
BF	Naphtho[2,3g]chrysene	350	403 422
	Group III		
N	4*H*-Benzo[c]cyclopenta[mno]-chrysene	300	384 s-397 406 429[a]
	Group V		
O	Dibenzo[ghi,mno]fluoranthene	290	s-395 419 434[a]
P	Rubicene	370	402 415 425 439 450 478 541
Q	Diacenaphtho[1,2j:1′,2′l]-fluoranthene	374	474 504
	Group VI		
A	10-Methylbenzo[b]fluoranthene	347	443
B	3-Methoxybenzo[k]fluoranthene	312	430 453
C	3-Hydroxybenzo[k]fluoranthene	310	459

[a] The s- denotes a shoulder.

Previous studies involving nitromethane as a selective quenching agent for discriminating between alternant vs. nonalternant PAHs utilized either neat acetonitrile or a binary aqueous/acetonitrile mixture (20:80 percent by volume). Such solvents work well as mobile phases in HPLC separations for those PAHs that contain six rings or less, but different solvent strengths must be employed for larger PAHs having six to ten rings. Toluene/acetonitrile or ethyl acetate/acetonitrile mixtures have served well in the past as HPLC mobile-phase solvents for the separation of the larger PAHs.[25,60] Table I compares the experimental results regarding the ability of nitromethane to act as a selective quenching agent for alternant vs. nonalternant PAHs in the aqueous/acetonitrile mixture utilized earlier and a toluene/acetonitrile mixture (40:60 percent by volume). While a published paper[38] has alluded to the fact that solvent polarity affects nitromethane's ability to act as a selective quenching agent, there was no in-depth study ever reported. Examination of Table I reveals that the toluene/acetonitrile mixture (Py = 1.49), although less polar than the aqueous/acetonitrile mixture (observed Py = 1.60[61]; Py = 1.80 after correction for slit width effects[56]) or pure acetonitrile (Py = 1.79[55]), can effectively be used as an HPLC solvent when nitromethane is employed to selectively quench the fluorescence emission signals of alternant vs. nonalternant PAHs. Of the 96 compounds examined, only nine behave differently in the toluene/acetonitrile solvent mixture as compared to the aqueous/acetonitrile solvent mixture or neat acetonitrile. Interestingly, seven of the nine exceptions are coronene derivatives. Earlier studies utilized the aqueous/acetonitrile mixtures, but we have found that for all 96 compounds, except for the aforementioned coronene derivatives, the selective quenching rule is obeyed in neat acetonitrile as well. Since then, we have employed acetonitrile, rather than the aqueous/

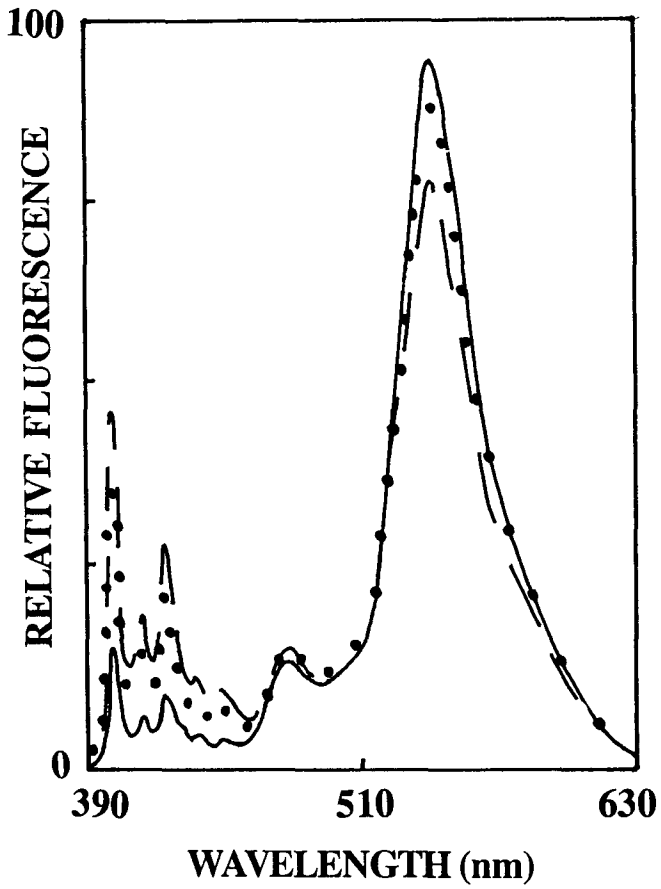

FIG. 4. Corrected fluorescence emission spectra of rubicene dissolved in acetonitrile at various nitromethane concentrations. From top to bottom in the 390–460 nm spectral range, the curves correspond to 0, 1, and 5 Pasteur pipet drops of nitromethane.

acetonitrile mixture, to enhance the solubility of the larger PAHs.

One of the unexpected "selective quenching" rule exceptions listed in Table I is rubicene. Examination of Fig. 4 reveals that the quenching of rubicene is very different. Rubicene is a nonalternant fluoranthenoid; therefore, it should not be quenched by nitromethane. The first five emission peaks (403, 416, 426, 441, and 453 nm) that are narrow and well defined are quenched, while the two broad peaks (482 and 541 nm) remain unquenched. The slight increase in intensity of the 541-nm emission peak via addition of nitromethane may be attributed to enhanced solubility, viscosity, or solvent polarity. As mentioned earlier, the number of peaks and the surprising amount of detailed fine structure of the first five peaks are not indicative of this subclass. The fluorescence emission spectra and the nitromethane quenching behavior suggest that rubicene acts as if it had two electronic centers, the first corresponding to an alternant moiety that would be quenched and the second corresponding to an unquenched nonalternant moiety. The molecular structure would also suggest this possibility since rubicene has an alternant anthracene moiety through the center of the compound as well as two nonalternant fluoranthene moieties on each end. The alternant moiety would explain the fine structure of the first five bands in the spectra, and the nonalternant moiety could explain the last two broad, unstructured bands. Another plausible explanation of the broad red-shifted fluorescence emission peak at 541 nm in the rubicene spectra is excimer formation. To eliminate this possibility, we undertook a concentration study. It was found that even when the solute's concentration was so low that it gave only very weak fluorescence emission intensities, all seven or eight bands still remained, including the band at 541 nm. The change in the excitation spectra via changing emission bands was also examined. In differing solvents the excitation spectra contained the same wavelengths at the various emission peak settings, but the ratios of the bands were significantly different. Also, the excitation spectrum with the emission collected at 541 nm was completely different than it was with the emission setting at 403 nm. In dimethyl sulfoxide and *n*-hexadecane, the excitation spectra did not change when the emission setting was at any of five well-defined peaks (DMSO: 403, 416, 426, 441, and 453 nm), but the excitation spectra were not the same as the spectrum obtained at 541 nm. The small broad peak at 482 nm gave an excitation spectrum that appeared to be the addition of the two spectra obtained for the broad band at 541 nm and well-defined bands at 403, 416, 426, 441, or 453 nm, since it was not identical to either one, but contained peaks belonging to both. Experimental data from the concentration and excitation studies, combined with the nitromethane quenching phenomena, as well as the molecular structure arguments, support the idea that rubicene acts like two electronic centers. A technique such as ultraviolet or fluorescence spectroscopy with polarized light in organized solvent media could perhaps determine whether this is true.[62]

Even in light of the few mentioned exceptions, nitromethane remains very useful as a selective quenching reagent. This work further provides a clear indication that it promises to be useful in actual HPLC separations. Future studies will examine several other HPLC solvents to verify that the "selective quenching" rule is obeyed.

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