Fluorescence Spectral Study of 9-Acridinecarboxylic Acid and Its Methyl Ester. Understanding the Unusual Fluorescence Behavior of 9-Anthroic Acid

Joykrishna Dey, Judson L. Haynes III, and Isiah M. Warner*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

Asit K. Chandra

Laboratorie De Materiaux Catalytiques Et Catalyse En Chimie Organique, UMR 5618 ENSCM-CNRS, Montpellier, Cedex 1, France

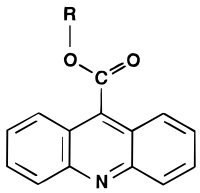
Received: October 21, 1996; In Final Form: January 20, 1997[⊗]

The absorption and fluorescence spectral characteristics of 9-acridinecarboxylic acid (9-ACA) and 9-(methoxycarbonyl)acridine (9-MCA) were studied in a series of organic solvents and in aqueous solutions. Fluorescence quantum yields (Φ_f) and lifetimes (τ_f) of the compounds were measured in these solvents. Unlike 9-anthroic acid (9-AA), as reported in the literature, no large Stokes-shifted fluorescence emission band was observed for 9-ACA and 9-MCA in neutral organic solvents or water. The absence of large Stokes-shifted emission in the case of 9-ACA and 9-MCA suggests the existence of a charge-transfer emitting state in 9-AA in which the carboxyl group is nearly coplanar with the aromatic ring. The Φ_f values for both compounds increase as a function of hydrogen-bonding capacity of the solvents. In near neutral to slightly acidic solutions, 9-ACA exists mainly in the zwitterionic form. Both 9-ACA and 9-MCA form monoprotonated species in moderately concentrated acid solutions. The acidium cation of 9-AA formed in the excited state in moderately concentrated acid solution reorganizes to produce a carbocation centered at the carbon atom of the carboxyl group. However, there was no indication of the formation of such acidium cations in the case of 9-ACA and 9-MCA even in concentrated perchloric acid medium. The pK_as of various prototropic equilibria involved in the ground electronic state of the compounds were estimated. Semiempirical AM1 calculations were performed to obtain the energies of the various configurations of 9-AA and 9-ACA in the ground (S_0) as well as in the lowest excited singlet (S_1) electronic state. The results suggest that the COOH group is oriented at an angle of ∼55° with respect to the aromatic ring in the S_0 state in both the molecules. However, in the S_1 state, it approaches coplanarity with the aromatic ring. The calculated bond lengths, charge densities, and dipole moments suggest that the resonance charge transfer from the aromatic ring to the COOH group increases in the S_1 state of 9-AA. However, despite the decrease of twist angle of the COOH group, no significant charge transfer was observed in 9-ACA. The charge density data indicate that the ring nitrogen and the carbonyl oxygen of the COOH group become more basic upon electronic excitation.

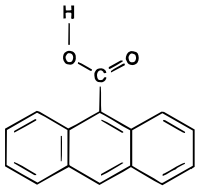
Introduction

Fluorescence studies of 9-substituted anthracene derivatives[1−4] have shown that at high concentrations these molecules display a new broad emission band on the long-wavelength side of the normal emission. The appearance of the large Stokes-shifted emission has been ascribed to excimer formation in the excited state. However, the long-wavelength fluorescence emission of 9-anthroic acid (9-AA) and its ester has generated some controversy over its origin, and different interpretations have been offered in the literature.[5−8] It has been reported that a dilute solution (10^{-5} M) of 9-AA in hydroxylic solvents exhibits only one fluorescence band corresponding to either a molecular or a ionic form. However, when the concentration of 9-AA was raised above 10^{-3} M or the solution was acidified, a broad and large Stokes-shifted emission appeared. Similar spectra were also observed for dilute solutions in nonpolar solvents. Upon addition of base, however, this band disappeared. The methyl ester of 9-AA also showed similar fluorescence behavior in organic solvents.[8,9] On the basis of these observations, Cherkasov et al.[5,6] attributed the large Stokes-shifted emission to a dimer (excimer) formed through intermolecular hydrogen bonding between an excited molecule and a ground state molecule. While some researchers have also proposed solute−solvent complex formation in the excited state,[7] Werner and co-workers[8] argued that the long-wavelength emission is due to a rigid planar structure, **I** (Scheme 1), of the molecule formed through rotation of the carboxyl group in the excited emitting

SCHEME 1



I R = H (9-ACA)
II R = CH_3 (9-MCA)



(9-AA)

* To whom correspondence should be addressed.
[⊗] Abstract published in *Advance ACS Abstracts,* March 1, 1997.

S1089-5639(96)03263-X CCC: $14.00

state. In the planar configuration, charge-transfer interaction between the carboxyl group and the aromatic ring results in a broad Stokes-shifted fluorescence spectrum. Recently, one of us has also investigated the effect of cyclodextrins on the fluorescence properties of 9-AA and concluded that the anomalous fluorescence emission in organic solvents is not due to any dimeric species but rather due to the neutral molecule.[10]

In continuation of our previous studies to understand the origin of the anomalous fluorescence behavior of 9-AA and to verify the proposed mechanism of the process, we have chosen 9-acridinecarboxylic acid (9-ACA) and its methyl ester, 9-(methoxycarbonyl)acridine (9-MCA), for this study to investigate their fluorescence properties in solution. Acridine is a heteroaromatic molecule differing from its parent hydrocarbon, anthracene, in the replacement of a C−H group in the central ring by a nitrogen atom. It is generally found and also predicted by theory[11,12] that the introduction of a nitrogen atom into an alternant aromatic hydrocarbon produces only a small change in the π electronic energy levels, resulting in similar absorption spectra for the heteroaromatic molecule and its parent hydrocarbon. The fluorescence spectral characteristics of 9-ACA and 9-MCA are also expected to be similar to their respective anthracene analogs. The focus of the present study is (i) to investigate the electronic absorption and fluorescence spectral characteristics of 9-ACA and 9-MCA in organic solvents and aqueous media, (ii) to measure the fluorescence quantum yields and lifetimes, (iii) to compare the pH dependence of the absorption and fluorescence spectral characteristics of the molecules with that of 9-AA, and (iv) to estimate the acidity constants of the acid−base equilibria involved in the ground and excited electronic states. We also present the results of semiempirical MO calculations to aid in interpretation of the experimental results.

Experimental Details

Materials. The 9-acridinecarboxylic acid and 9-anthroic acid were obtained from Aldrich and used directly from the bottle. The 9-(methoxycarbonyl)acridine was synthesized and purified according to the procedure reported in the literature.[13] The compound was identified by its melting point and ^{1}H NMR spectrum [O(CH_3), 4.15 (s, 3 H); Ar−H, 8.32, 8.33 (d, 2 H); 7.97, 7.94 (d, 2 H); 7.81−7.75 (t, 2 H); 7.57−7.54 (t, 2 H)]. The purity of the compounds was checked by TLC and by fluorescence excitation spectra and found to be satisfactory. Anhydrous spectroscopic grade methanol (ACS), acetonitrile (Aldrich), 1,4-dioxane (Aldrich), 1,2-dimethoxyethane (Aldrich), tetrahydrofuran (EM), ethanol (EM), 1-propanol (Mallinckrodt), n-butanol (Mallinckrodt), n-pentanol (Mallinckrodt), ethylene glycol (Aldrich), dichloromethane (EM), and chloroform (Mallinckrodt) were used as received. Analytical grade $HClO_4$ (ACS), NaH_2PO_4, Na_2HPO_4 (Fischer), and NaOH (Becker) were directly used from the bottle. Megapore deionized distilled water was used for making aqueous solutions. All measurements were performed at 25 °C unless otherwise mentioned.

Methods. Aqueous solutions in the pH range 2−9 were made by mixing appropriate amounts of 100 mM NaH_2PO_4 and Na_2HPO_4 or H_3PO_4 solutions. Total buffer concentration and ionic strength were respectively maintained at 50 mM and 0.4. Hammett's acidity scale (H_0)[14] was followed to make acid solutions in the range of pH 1.0 to H_0 −7.7 using perchloric acid. Because of the poor aqueous solubility, a saturated solution of the compound in water was used. For pH titrations, 1 mL of this stock solution was diluted to 5 mL by appropriate pH solutions. Fluorimetric titrations were performed by exciting the solutions at the isosbestic point of the absorption spectra. The pK_a values were estimated from the plot of the Henderson−Hasselbalch equation. Fluorescence quantum yields were determined according to the method of Parker and Rees[15] in reference to quinine sulfate solution in 0.1 N H_2SO_4 (Φ_f = 0.545).[16] The absorbance of the solutions at the wavelength of excitation (350 nm) was maintained at <0.05.

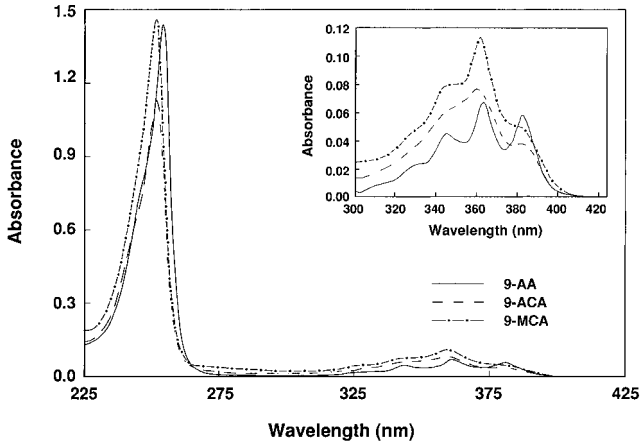


Figure 1. Absorption spectra of 9-AA, 9-ACA, and 9-MCA in tetrahydrofuran. Insert: long-wavelength band on expanded scale; c = ~10^{-5} M.

Apparatus. The absorption spectra were recorded on a double-beam Shimadzu UV-3101PC UV−vis−near-IR scanning spectrophotometer equipped with a constant temperature circulator. The fluorescence spectra were measured on a Spex-Fluorolog Model F2T21I spectrofluorometer with a cell compartment thermostated by a VWR Model 1160 constant temperature circulator. The band-pass of the excitation and emission slits was maintained at 5.1 and 1.7, respectively. Fluorescence intensity was measured at right angle configuration. The ^{1}H NMR spectra were measured in a Brucker 250 MHz instrument. TMS was used as internal reference in $CDCl_3$ solvent. The pH of the aqueous solutions was measured by a Orion Model 420A pH meter using single probe glass electrode.

Fluorescence lifetimes were measured by using a PTI Inc. LS-100 luminescence spectrometer. A N_2 and He gas mixture was used in the discharge tube for excitation. A dilute solution of colloidal starch was used as a scatterer to determine the exciting lamp flash profile. The 358 nm emission of N_2 was used for sample excitation. The decay curves were obtained by use of time-correlated single-photon counting (TC-SPC). To obtain fluorescence decay curves, (2−5) × 10^4 counts were collected at the peak channel. Each data set was collected in 512 channels. The data were analyzed by use of a multiexponential decay analysis program. The goodness of fit between experimental and computed decay curves was evaluated by use of the reduced χ^2 (0.9−1.3) and by the randomness of the plot of weighted residuals and the autocorrelation functions. The measurements were repeated more than once to obtain the best data set.

Results and Discussion

Solvent Effects on Absorption and Fluorescence Spectra. The representative absorption spectra of 9-ACA, 9-MCA, and 9-AA in tetrahydrofuran (THF) solvent are displayed in Figure 1. The spectral data of 9-ACA and 9-MCA are respectively summarized in Tables 1 and 2. Although the long-wavelength absorption bands of 9-ACA and 9-MCA are less structured and red-shifted as compared to that of 9-AA, the overall feature of the spectra is very similar. The spectra resemble the spectrum of the respective parent molecule, acridine or anthracene.[17] The long-wavelength absorption band of 9-MCA is red-shifted relative to that of 9-ACA. The absorption spectra are characterized by two band systems: a strong band at ~250 nm and a

TABLE 1: Absorption Maxima (λ_{max}(abs)), Molar Absorptivity (ϵ_{max}), Fluorescence Maxima (λ_{max}(flu)), Fluorescence Quantum Yield (Φ_f), Fluorescence Lifetime (τ_f), and Radiative (k_r) and Nonradiative (k_{nr}) Rate Constants of 9-ACA in Organic Solvents

solvent	λ_{max}(abs) (nm)	ϵ_{max} (L mol^{-1} cm^{-1})	λ_{max}(flu) (nm)	$\Phi_f \times 10^2$ [a]	τ_f (ns)[b]	$k_r \times 10^{-7}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
1,4-dioxane	362	7 265	421	0.3	0.9	0.3	1.1
	257	93 662					
1,2-dimethoxyethane	360	7 090	419	0.6	1.5	0.4	0.7
	252	100 578					
tetrahydrofuran	360	6 828	418	0.2	1.1	1.8	0.9
	252	97 339					
chloroform	362	8 228	436	2.3	0.6	3.8	1.6
	254	101 016					
dichloromethane	362		422	0.8	0.9	0.9	1.1
	254						
acetonitrile	354	6 740	421	1.3	0.8	1.6	1.2
	254	86 130					
ethanol	369	5 340	421	2.8	0.6	4.6	1.6
	354	8 316					
	252	111 432					
methanol	356	8 053	421	3.1	0.8	3.9	1.2
	252	107 405					
ethylene glycol	356	8 578	425	3.7	0.8	4.6	1.2
	254	110 294					
1-propanol	368	5 427	422	3.4	0.7	4.8	1.4
	355	8 490					
	253	112 132					
1-butanol	370	5 602	422	3.6	0.8	4.5	1.2
	350	8 666					
	253	113 796					
1-pentanol	370	5 252	422	3.6	0.7	5.1	1.5
	357	8 053					
	253	110 294					

[a] Average deviation = 0.001. [b] Average deviation = 0.1 ns.

TABLE 2: Absorption Maxima (λ_{max}(abs)), Molar Absorptivity (ϵ_{max}), Fluorescence Maxima (λ_{max}(flu)), Fluorescence Quantum Yield (Φ_f), Fluorescence Lifetime (τ_f), and Radiative (k_r) and Nonradiative (k_{nr}) Rate Constants of 9-MCA in Organic Solvents

solvent	λ_{max}(abs) (nm)	ϵ_{max} (L mol^{-1} cm^{-1})	λ_{max}(flu) (nm)	$\Phi_f \times 10^2$ [a]	τ_f (ns)[b]	$k_r \times 10^{-7}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
1,4-dioxane	361	10 496	420	0.4	0.9	0.4	1.1
	251	107 836					
1,2-dimethoxyethane	361	10 460	420	0.4	1.3	0.3	0.8
	251	115 852					
tetrahydrofuran	361	10 424	418	0.3	1.1	0.3	0.9
	251	115 672					
chloroform	361	12 078	428	2.9	0.9	3.2	1.1
	254	145 363					
dichloromethane	361	12 006	422	1.4	1.3	1.1	0.8
	253	148 310					
acetonitrile	360	11 029	422	1.1	0.9	1.2	1.1
	250	137 836					
ethanol	360	11 215	432	3.0	0.8	3.8	1.2
	251	111 143					
methanol	360	10 819	432	3.2	1.2	2.7	0.8
	249	116 858					
ethylene glycol	361	10 927	418	6.1	1.1	5.7	0.9
	253	148 260					
1-propanol	361	11 503	430	4.1	0.7	5.8	1.3
	251	149 461					
1-butanol	360	11 559	431	4.2	0.8	5.3	1.2
	252	146 441					
1-pentanol	361	11 936	432	3.0	0.7	4.3	1.3
	252	145 625					

[a] Average deviation = 0.001. [b] Average deviation = 0.1 ns.

structured band centered at ~360 nm. In analogy with anthracene and as predicted by theoretical calculations,[18−20] the long-wavelength band involves two transitions, $1^1A_g \rightarrow 1^1B_{1u}$ and $1^1A_g \rightarrow 1^1B_{2u}$ (^1L_a and ^1L_b, respectively, in Platt's notation[18]). The ^1L_b transition is 2 orders of magnitude weaker than the ^1L_a transition and appears on the long-wavelength side. Thus, the ^1L_b band remains hidden under the stronger ^1L_a band. The intense band at ~250 nm is assigned to the $1^1A_g \rightarrow 2^1B_{2u}$ (^1B_b) transition. Since the ^1L_a transition has a transition moment along the short axis of the molecule, it is subjected to a greater influence than the ^1L_b transition by the introduction of a substituent group at the 9-position. Indeed, the ^1L_a band in 9-aminoanthracene[21] and 9-aminoacridine[22] is displaced to the red, leaving the ^1L_b band in its original position. However, since the COOH (or COOMe) group is sterically hindered by the *peri*-hydrogens at the 1- and 8-positions, the resonance interaction with the aromatic ring is reduced, and there is only an inductive effect of the substituent. The molar absorptivity data in Tables 1 and 2 suggest that the electronic transitions in both 9-ACA and 9-MCA are π,π^* type.

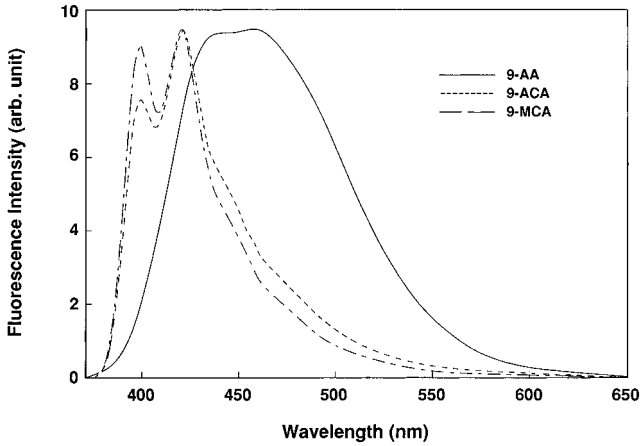


Figure 2. Fluorescence spectra of 9-AA, 9-ACA, and 9-MCA in tetrahydrofuran. $c = \sim 10^{-5}$ M, $\lambda_{ex} = 350$ nm.

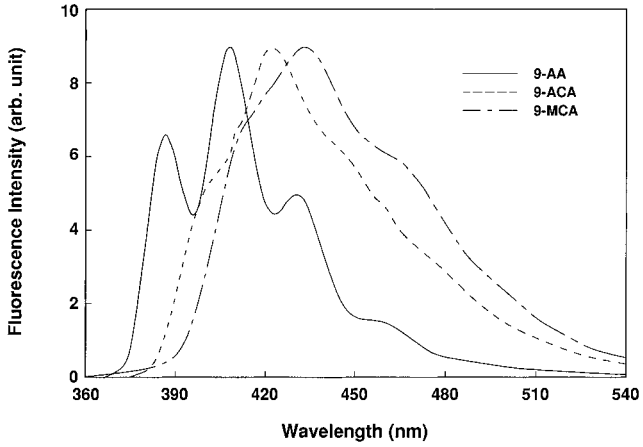


Figure 3. Fluorescence spectra of 9-AA, 9-ACA, and 9-MCA in methanol. $c = \sim 10^{-5}$ M, $\lambda_{ex} = 350$ nm.

The spectral data show that the long-wavelength absorption band of 9-ACA is blue-shifted in protic solvents. However, no significant change in the position of the corresponding absorption band of 9-MCA was observed in going from nonpolar to polar solvent. Therefore, the blue shift in the long-wavelength absorption band of 9-ACA can be ascribed to strong hydrogen-bonding interaction with the solvent molecules, which results in partial deprotonation of the carboxylic proton. The COOH group is so strongly solvated that it is out of plane of the aromatic ring. As a result, the resonance interaction of the functional group with the π-electrons of the aromatic ring is reduced, which causes the blue shift of the transition. Similar changes were also observed when the alcoholic solution of 9-ACA was made alkaline. The absence of solvent-induced shifts in the absorption spectra suggests that the dipole moments of both the molecules are low in the S_0 state.

The fluorescence spectra (Figure 2) of 9-ACA and 9-MCA in THF are structured and are similar to each other; also, they bear a mirror-image relationship with the respective absorption spectrum in the same solvent. It is interesting to note that although the absorption spectra of 9-ACA and 9-MCA are similar to that of 9-AA, the fluorescence spectrum of the latter is far more red-shifted relative to those of the former. In methanol, however, the fluorescence spectra (Figure 3) of the former molecules are red-shifted relative to that of the latter, and no large Stokes-shifted fluorescence emission was observed for any of these molecules. In contrast, the methyl ester of 9-AA is reported to exhibit a large Stokes-shifted fluorescence band as observed for 9-AA in nonpolar solvents.[9] This led to the conclusion that partial ionization of the COOH group in protic solvents produces an out-of-plane configuration and thus reduces

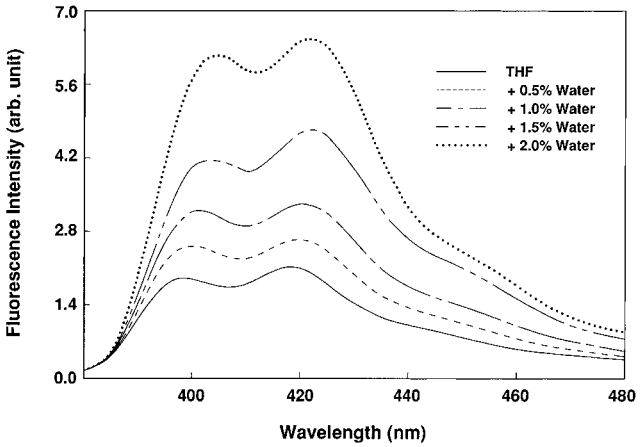


Figure 4. Fluorescence spectra of 9-MCA in tetrahydrofuran in the presence of varying percentage of water.

the resonance interaction with the π-electrons of the aromatic ring. However, if the coplanarity of the COOH group was the only reason for this observation, then one would have expected similar large Stokes-shifted emission in the case of 9-MCA also, at least in nonpolar solvents. This suggests that the excited state structures of 9-ACA and 9-MCA are different from that of 9-AA. It seems that the resonance charge transfer from the aromatic ring to the COOH (or COOMe) group is important for such anomalous fluorescence behavior of 9-AA. In the case of 9-ACA or 9-MCA, such resonance interaction seems to be opposed by the electron-withdrawing character of the ring nitrogen, which is known to become more basic in the excited state.[23] However, in methanol, the COOH group of 9-AA and 9-ACA being partially ionized are highly solvated and thus restricted from the plane of the aromatic ring. This results in less resonance interaction and hence the normal Stokes-shifted emission. For the same reason, the fluorescence spectrum of 9-ACA is blue-shifted relative to that of 9-MCA. The red shift of the fluorescence spectra of 9-ACA and 9-MCA in hydrogen-bonding solvents, e.g., methanol as compared to 9-AA, can be attributed to hydrogen-bonding interaction with the lone-pair electrons on the ring nitrogen and the solvent molecules.

Solvent Dependence of Fluorescence Quantum Yields and Lifetimes. As can be seen in Tables 1 and 2, the fluorescence quantum yields (Φ_f) of both 9-ACA and 9-MCA are strongly affected by the solvent properties. In hydrogen-bonding solvents, the Φ_f is much higher compared to that in non-hydrogen-bonding solvents. To demonstrate the role of hydrogen bonding in enhancing the fluorescence quantum yield, we have measured the fluorescence spectra of 9-MCA in THF containing various percentages of water. Figure 4 shows that the fluorescence intensity increases with an increase in the percentage of water in THF. The increase of fluorescence quantum yield of many heterocyclic organic molecules, e.g., quinoline and isoquinoline including acridine, in protic solvents is reported in the literature.[24−26] In hydrocarbon solvents, these molecules are nonfluorescent or weakly fluorescent. However, in protic solvents they become fluorescent. In acridine, there is a higher energy $^1(n,\pi^*)$ state very close to the S_1 (π,π^*) state.[27] In hydrocarbon solvents, due to vibronic interaction between these states, the lowest energy $^1(\pi,\pi^*)$ state gains some n,π^* character and consequently becomes less fluorescent.[28,29] In protic solvents, hydrogen-bonding interaction between the lone-pair electrons on the nitrogen atom and the solvent molecules destabilizes the $^1(n,\pi^*)$ state and stabilizes the $^1(\pi,\pi^*)$ state, thus increasing the energy gap and thereby reducing the vibronic interaction between the states. This results in an increase of fluorescence quantum yield. The high Φ_f values of 9-ACA and 9-MCA in ethylene glycol could be due to higher

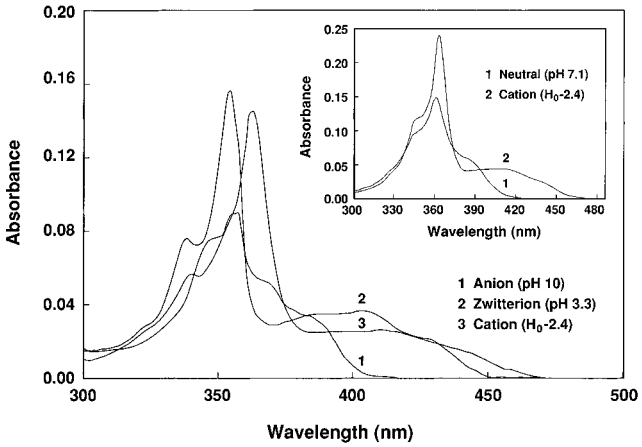


Figure 5. Absorption spectra (long-wavelength band) of the prototropic species of 9-ACA and 9-MCA (insert). $c = \sim 1 \times 10^{-5}$ M.

viscosity of the medium. Since in fluid solution the COOH or COOMe group can rotate freely, they approach coplanarity with the aromatic ring upon electronic excitation which increases the charge-transfer character of the S_1 state. Consequently, the fluorescence quantum yield is low in fluid solvents. However, in viscous solvents, the rotation of the COOH group is hindered and kept out of plane of the aromatic ring, thus reducing the charge-transfer character of the emitting state and thereby increasing the fluorescence quantum yield.

The fluorescence lifetimes of 9-ACA and 9-MCA in the organic solvents obtained by the TC-SPC method are listed in Tables 1 and 2, respectively. The data in Tables 1 and 2 also include the radiative (k_r) and nonradiative (k_{nr}) rate constants calculated from the measured Φ_f and τ_f values using equations $k_r = \Phi_f/\tau_f$ and $k_{nr} = (1 - \Phi_f)/\tau_f$, respectively. The fluorescence of both molecules exhibits single-exponential decay in the solvents employed. The measured τ_f values, although very low, are slightly higher than those reported for acridine.[30,31] The fluorescence lifetime of 9-MCA is also higher than that of 9-ACA in any solvent. Unlike Φ_f values, the τ_f values of the compounds do not show any clear trend in going from nonpolar to polar solvents. However, a slight decrease of τ_f is observed in hydrogen-bonding solvents. This is in contrast to what is expected because the difference in nonradiative deactivation is ascribed to the variation in energy gap between the π,π^* and n,π^* states. This suggests that, regardless of hydrogen bonding, there may be another mechanism for nonradiative deactivation of the molecule. However, it can be seen from Tables 1 and 2 that the nonradiative decay rates, within the limit of experimental error, remain almost constant while radiative rate constant increases in hydrogen-bonding solvents. This is consistent with the high molar absorptivity of the lowest energy transition of the molecules in these solvents as discussed earlier. Thus, the decrease of τ_f in hydrogen-bonding solvents may be attributed to increase of radiative rate constant.

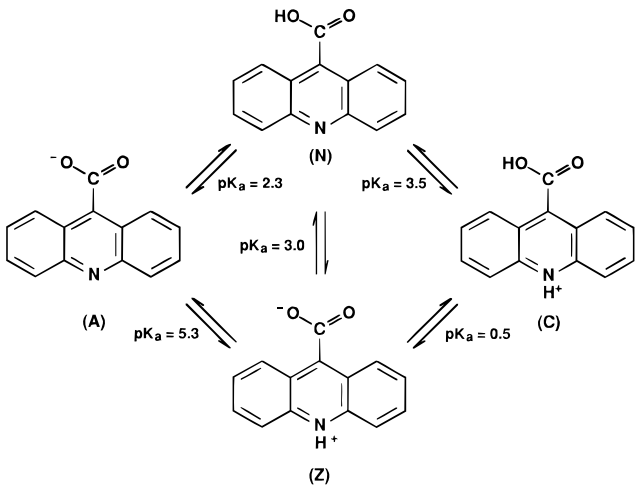
Effect of Acidity on Absorption Spectra and Ground State Ionization Processes. The absorption spectra of the different prototropic species of 9-ACA and 9-MCA are depicted in Figure 5. The absorption and emission maxima as well as the fluorescence quantum yields and lifetimes of 9-ACA and 9-MCA at various pHs are presented in Table 3. In alkaline solution, 9-ACA shows an absorption spectrum similar in appearance to that in methanol. Since the dissociation of the carboxylic proton is not expected to change the spectrum of the neutral molecule much, the absorption spectrum in alkaline solution can be associated with the anionic form (A). In dilute acid solutions (pH 3), a new absorption band develops at 387 nm with sharp isosbestic points. The splitting of the long-wavelength absorption band is also reported for the acridinium cation.[22] Accordingly, the long- and short-wavelength bands are respectively assigned to the ^1L_b and ^1L_a transitions. It is important to note that the position of the ^1L_a band did not change. However, its intensity increased. Similar spectral changes were also observed in the case of 9-MCA in acidic solution. Because of the acridinium-like appearance of the absorption spectrum of 9-MCA at pH 3 and its lack of identity with that of uncharged 9-MCA, this protonation is believed to occur at the ring nitrogen atom, resulting in the formation of a zwitterion (Z). Upon further decrease of pH the ^1L_a band shifts to red. The position of the ^1L_a band is almost equal to that of the cation (C) of 9-MCA. Therefore, in this pH range (3 to -1.5), the protonation occurs at the carboxylate group of the zwitterion to form the cation. The red shift of the ^1L_a band of the cation compared to that of the zwitterion suggests that the neutral carboxylic acid group of 9-ACA or the carbomethoxy group of 9-MCA is conjugated and nearly coplanar with the aromatic ring in the ground state. Similar changes in spectral features with changes in acid concentration have also been reported for quinoline-4-carboxylic acid.[32] No further spectral change was observed for the cations of either 9-ACA or 9−MCA in concentrated acid solutions, although aromatic carboxylic acids and esters are known to undergo protonation

TABLE 3: Absorption Maxima (λ_{max}(abs)), Molar Absorptivity (ϵ_{max}), Fluorescence Maxima (λ_{max}(flu)), Fluorescence Quantum Yield (Φ_f), Fluorescence Lifetime (τ_f), Radiative (k_r) and Nonradiative (k_{nr}) Rate Constants of the Prototropic Forms of 9-ACA and 9-MCA

species	λ_{max}(abs) (nm)	ϵ_{max} (L mol^{-1} cm^{-1})	λ_{max}(flu) (nm)	$\Phi_f \times 10^2$ [a]	τ_f (ns)[b]	$k_r \times 10^{-7}$ (s^{-1})	$k_{nr} \times 10^{-8}$ (s^{-1})
9-ACA							
anion (pH 10)	357	8 666	429	24.7	6.7	3.7	1.1
	251	115 021					
zwitterion (pH 3.3)	387	3 064	480	6.5	3.2	2.0	2.9
	354	13 655					
	338	6 565					
	256	87 010					
cation (H_0 −2.4)	407	2 451	512	10.5	9.7	1.1	0.92
	361	13 043					
	257	83 158					
9-MCA							
neutral (pH 7.1)	361	10 856	440	21.9	11.2	1.95	0.70
	251	134 004					
cation (H_0 −2.4)	411	3 235	514	11.4	10.1	1.12	0.88
	395	3 235					
	362	17 326					
	258	105 392					

[a] Average deviation = 0.001. [b] Average deviation = 0.2.

SCHEME 2



at the carbonyl oxygen near H_0 −8.[33] The absorption spectrum of 9-AA also does not show any change in the above acidity region, except in concentrated $HClO_4$ (H_0 −7.7) where it is reported to undergo decarboxylation to produce anthracene.[34]

The prototropic equilibria involved in the ground electronic state of 9-ACA in the pH/H_0 range 10 to −3.0 are represented in Scheme 2. The pK_a values of the corresponding equilibria are also indicated in the scheme. The pK_a value of the cation of 9-MCA is estimated to be equal to 3.5, which is very close to the reported value (3.45).[35] Since the electronic structure of 9-ACA is similar to that of 9-MCA, the pK_a value of the cationic species of the former can be assumed to be equal to that of the latter. On the basis of this assumption and applying the principle of microscopic reversibility, we are able to estimate the pK_as of the zwitterion−neutral and neutral−anion equilibria of 9-ACA. The acid dissociation constant of 9-ACA (2.3) thus obtained is less than that of 9-AA (3.0).[36] Also, the pK_a of the cationic species of 9-MCA (or 9-ACA) is low as compared to unsubstituted acridine (pK_a = 5.45).[36] This suggests that the COOH group is electronically connected to the ring nitrogen atom in the ground state. In other words, the COOH or the COOMe groups, if not coplanar with the aromatic ring, are close enough to interact with the π-electrons of the aromatic ring through resonance. However, the COO^- group, as noted before, is out of plane of the aromatic ring. This is indicated by the pK_a value (5.3) of the acid dissociation of the zwitterion, which is very close to that of the unsubstituted acridinium ion.

Effect of Acidity on the Fluorescence Spectra and Excited State Processes. The fluorescence spectra of the prototropic species of 9-ACA and 9-MCA are portrayed in Figure 6. In alkaline solution, the anion derived from the 9-ACA has a strong fluorescence at ∼440 nm. This fluorescence is stronger than that of the methyl ester. This suggests that the anion fluorescence is stronger than that of the neutral molecular form of 9-ACA as indicated by the Φ_f values (Table 3). This can be explained by the fact that the intramolecular hydrogen bonding in the neutral molecule with the *peri*-hydrogens at the 1- and 8-positions forms a rigid and more planar structure which enhances the resonance interaction of the COOH group with the aromatic ring and thus increases the CT character of the S_1 state. The intramolecular hydrogen bonding and the CT character of the S_1 state are known to play an active role as a nonradiative decay channel in many organic molecules.

As the pH is lowered, the fluorescence intensity of the anion decreases and a new band appears at ∼475 nm. The conversion of the 430 nm fluorescence into the 475 nm fluorescence with decreasing pH occurs with the same titration characteristics as the absorption spectrum changes in the pH range 7−2. In

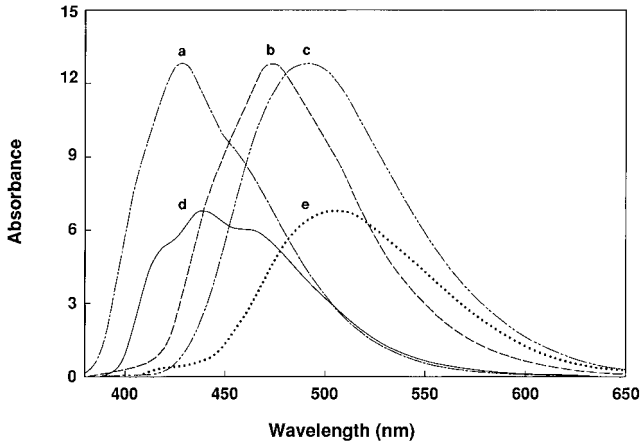


Figure 6. Fluorescence spectra of the prototropic species of 9-ACA: (a) anion (pH 10), (b) zwitterion (pH 3.3), (c) cation (H_0 −2.4) and 9-MCA, (d) neutral (pH 7.1), (e) cation (H_0 −2.4). c =1 × 10^{-5} M, λ_{ex} = 350 nm.

alkaline to neutral solutions, 9-MCA fluoresces with the maximum at ∼440 nm (Figure 6). Since the methyl ester is electronically similar to the neutral carboxylic acid and fluoresces at a different wavelength than does the protonation product of the anion, it can be concluded that the emission at 475 nm arises from the excited zwitterion. The identity between the fluorometric and absorptiometric titrations for the interconversion between the anion and zwitterion indicates that the equilibrium does not occur during the lifetime of the excited state. However, since the fluorescence of the zwitterion and the methyl ester appears at longer wavelengths than that of the anion, both the COOH group and the ring nitrogen atom become more basic in the S_1 state than in the ground state. Also, because the zwitterion fluoresces at longer wavelength than the methyl ester, the ring nitrogen seems to be more basic than the carboxylate group in the excited state.

At pH <2 the fluorescence spectrum of 9-ACA shows a further red shift. The red-shifted fluorescence (λ_{max} = 508 nm) at H_0 −2 resembles the spectrum of the protonated species of 9-MCA and therefore can be associated with the cation formed as a result of protonation at the carboxylate group of the zwitterion. The large red shift of the fluorescence spectrum of the cation relative to that of the zwitterion is due to the approach of the COOH group toward coplanarity with the aromatic ring as a result of less solvation as compared to the COO^- group. The conversion of the zwitterion to the cation in the excited state follows an identical titration curve as observed in the ground state, which implies that equilibrium is not established in the excited state. This is supported by the low fluorescence lifetime (Table 3) of the zwitterionic species as compared to that of cation or anion. According to the proposed theory of the effect of hydrogen bonding on the fluorescence quantum yield of acridine derivatives, one should expect an increase in Φ_f value for the cationic species of both 9-ACA and 9-MCA, because protonation is an extreme case of hydrogen bonding. However, the data in Table 3 show a decrease in Φ_f of the cationic species compared to the neutral form. This is possibly due to a difference in the nature of the emitting states in the two forms of the molecule. In the neutral molecule, the emitting state is ^1L_a whereas in the cation, it is ^1L_b. The transition probability of the $^1A \rightarrow {}^1L_b$ transition is much lower as compared to that of $^1A \rightarrow {}^1L_a$ transition in the neutral molecule as evidenced by the molar absorptivities. Therefore, the radiative rate constant of the former transition is higher than that of the latter. Also, the large stabilization of the S_1 state of the cation reduces the S_1-T_1 energy gap and thus increases the intersystem crossing rate. This is clearly indicated by the

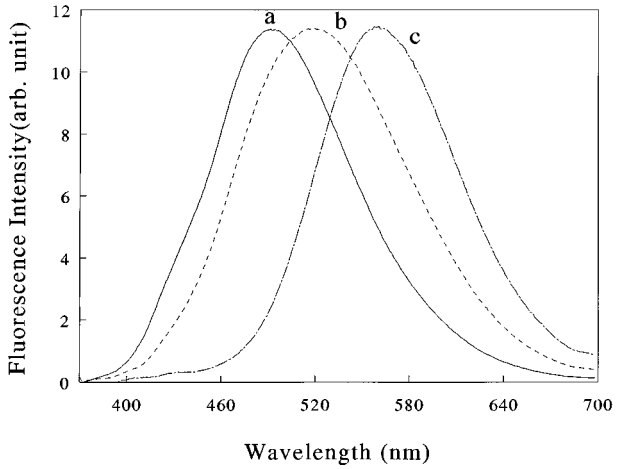
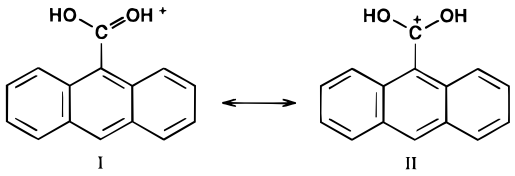


Figure 7. Fluorescence spectra of the various proton-transfer forms of 9-AA: (a) neutral, (b) acidium cation, **I**, (c) carbocation, **II**. $c = \sim 5 \times 10^{-6}$ M, $\lambda_{ex} = 360$ nm.

SCHEME 3



respective nonradiative decay rates of the prototropic species of 9-MCA. The low Φ_f value of the zwitterionic species of 9-ACA can also be explained along the same lines.

Like absorption spectra, the fluorescence spectra of 9-ACA and 9-MCA in concentrated acid solution do not show any change for the same reason as discussed in the previous section. This is indicative of the fact that no protonation at the carbonyl oxygen occurs even in the excited state. However, the fluorescence spectrum of 9-AA below pH 0.5 shows a red shift (Figure 7) which increases with an increase in acid concentration. In this acidity region (pH 0.5 to H_0 −3.5), the absorption spectra of the molecule did not show any change, which suggests that the above fluorescence spectral change is due to excited state reaction. It has been reported that the COOH group of 9-AA becomes more basic in the S_1 state.[37] Therefore, the red shift in the fluorescence spectrum of the neutral molecule can be attributed to the formation of the acidium cation, **I** (Scheme 3), as a result of protonation at the carbonyl oxygen of the COOH group. The difference in the prototropic behavior of 9-ACA or 9-MCA from 9-AA in concentrated acid is likely due to the positive charge on the ring nitrogen atom of the cations, which reduces the basicity of the carboxyl group. The protonation at the ring nitrogen completely destroys the resonance interaction of the -COOH group with the aromatic ring because this makes the carbon atom at the 9-position highly π-electron-deficient.[38] However, in the case of 9-AA, the resonance charge transfer from the aromatic ring stabilizes the acidium cation, **I**, as well as the carbocation, **II**, in the excited state. The acidity-dependent red shift of the cation, **I**, is possibly due to its resonance stabilization to form the carbocation-like species, **II**. Because of the high solvation requirement, the acidium cation is less stable in concentrated acid in which the activity of water is low.[14] The generation of such carbocation in strong H_2SO_4 medium is also reported for benzoic and naphthoic acids.[39]

Quantum Chemical MO Calculations. To verify the proposed explanation of the appearance of the large Stokes-shifted emission in 9-AA and its methyl ester, i.e., the coplanarity of the COOH or COOMe group upon electronic

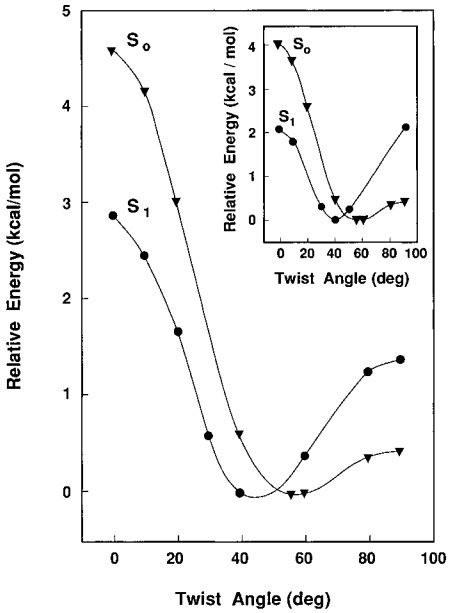
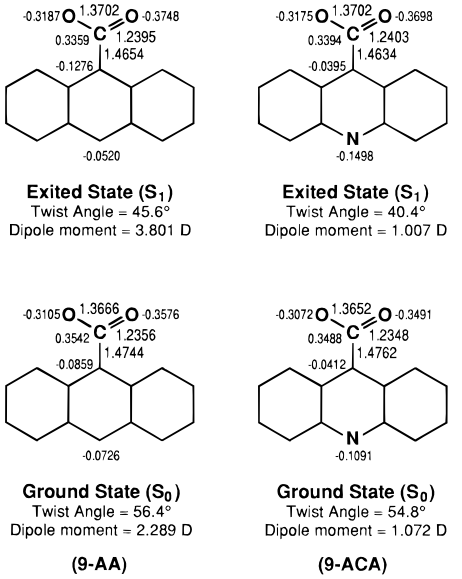


Figure 8. Plot of relative energy vs twist angle of the carboxylic acid group of 9-AA and 9-ACA (insert).

excitation, we have performed semiempirical AM1 calculations[40] to obtain relative ground (S_0) and excited state (S_1) energies of the various configurations of 9-AA and 9-ACA. This was done by calculating the total energy for various conformers by fixing the twist angle of the COOH group with respect to the plane of the aromatic ring followed by optimizing all bond lengths and angles. The twist angle was varied from 0° in which the COOH group is coplanar with the aromatic ring to an angle of 90°. A plot of relative energy versus the twist angle for 9-AA and 9-ACA is shown in Figure 8. As can be seen, the twist angle at the minimum energy is ∼55° for both molecules in the ground electronic state. This angle is much higher than that in 1-naphthoic or 1-anthroic acid (11°),[41] which indicates large steric hindrance due to the *peri*-hydrogens at the 1- and 8-positions of the molecule. However, in the S_1 state, the twist angle is 40.4° in the case of 9-ACA and 45.6° in the case of 9-AA. This suggests an approach of the COOH group toward coplanarity in the excited state. The energy barrier for such rotation in the S_0 state is higher (∼5 kcal/mol) compared to that in the S_1 state (∼2−3 kcal/mol). Therefore, it is not impossible to obtain a significant population of the planar configuration in the excited state. Further, it can be seen from Scheme 4 that the bond length between the carboxylic carbon and the C-9 atom decreased and that of the C═O bond increased in the S_1 state, showing an increase in bond order. This means that the resonance interaction increases in the excited state. The enhancement of resonance interaction of the COOH group with the aromatic ring is also indicated by a change in electron densities of the atoms in going from S_0 to the S_1 state. Although the twist angle of the -COOH group is less in 9-ACA as compared to that in 9-AA, the charge density data suggest that the resonance interaction in the former molecule is less. This is indicated by the low dipole moment (1.007 D) of the molecule in the S_1 state. In fact, the dipole moment of 9-ACA decreased upon excitation. However, in the case of 9-AA, the dipole moment increased from 2.289 to 3.801 D upon excitation. Since the resonance interaction is proportional to $\cos^2 \theta$, θ being the twist angle, a considerable amount of (almost one-half of full resonance energy) interaction is possible even at an angle of 45°.[42] Although hydrogen bond formation with the *peri*-hydrogens is difficult in this configuration, such a possibility cannot be ruled out because of electrostatic nature of hydrogen bonding. The charge densities on the ring nitrogen and the

SCHEME 4



carbonyl oxygen atoms also suggest that they become more basic upon electronic excitation of the molecule to the S_1 state.

Conclusion

We have studied the absorption and fluorescence spectra of 9-ACA and 9-MCA and their various prototropic species in 12 organic solvents and in aqueous solutions. The spectral behavior of the molecules have been elucidated and compared with that of 9-AA. The increase in fluorescence quantum yields of 9-ACA and 9-MCA in protic solvents indicates the presence of a S_2 (n,π^*) state close to the S_1 (π,π^*) state. Although 9-ACA and 9-MCA are structurally similar to that 9-AA, they do not exhibit any large Stokes-shifted fluorescence emission in organic solvents. The results suggest that the cooperative resonance charge transfer from the anthryl ring in 9-AA mediated by the intramolecular hydrogen-bonding formation with the *peri*-hydrogens results in stabilization of the S_1 state. However, intramolecular hydrogen bonding is not a sufficient condition for the appearance of the large Stokes-shifted fluorescence. The introduction of an electron-attracting nitrogen atom at the *para* position of the COOH (or COOMe) group inhibits resonance charge transfer from the aromatic ring in 9-ACA and 9-MCA. The results of MO calculations clearly suggest that the resonance interaction is the important factor for the appearance of the large Stokes-shifted fluorescence emission of 9-AA. The resonance interaction of the COOH group with the aromatic ring is less in the case of 9-ACA or 9-MCA although the twist angle is small compared to that in 9-AA. The 9-ACA is mainly present in the zwitterionic form in slightly acidic to neutral solution. The COO^- group of the anion is out of plane with the aromatic ring in contrast to the COOH or COOMe group which is coplanar as suggested by the pK_a values of the neutral molecule (2.3) and the cation (3.5) which are respectively lower than that of 9-AA (3.0) and unsubstituted acridine (5.45). Unlike 9-AA, protonation at the carbonyl oxygen does not occur in the excited state of 9-ACA and 9-MCA to form acidium cations even in strong acid medium.

Acknowledgment. The authors acknowledge NSF (CHE 9224177) and DOE (DE-FG05-93ER-14367) for support of this research.

References and Notes

(1) Vember, T. M; Cherkasov, A. S. *Opt. Spektrosk.* **1959**, *6*, 232; *Opt. Spectrosc.* **1959**, *6*, 148.
(2) Trischenco, G. A.; Sveshnikov, B. Ya; Cherkasov, A. S. *Opt. i Spektrosk.* **1958**, *4*, 631.
(3) Birks, J. B.; Aladekomo, J. B. *Photochem. Photobiol*. **1963**, *2*, 416.
(4) Forster, Th.; Kasper, K. *Z. Phys. Chem. (Munich)* **1954**, *1*, 275; *Z. Elektrochem.* **1955**, *59*, 976.
(5) Bazilevskaya, N. S.; Cherkasov, A. S. *Opt. Spectrosc.* **1965**, *18*, 30.
(6) Bazilevskaya, N. S.; Cherkasov, A. S. *Zh. Prikl. Spectrosk. Akad. Nauk Belorussk. SSR* **1965**, 548.
(7) Walker, M. S.; Bednar, T. W.; Lumry, R. *J. Chem. Phys*. **1967**, *47*, 1020.
(8) Werner, T. C.; Hercules, D. M. *J. Phys. Chem*. **1969**, *73*, 2005; **1970**, *74*, 1030.
(9) Werner, T. C.; Hoffman, R. M. *J. Phys. Chem*. **1973**, *77*, 1611.
(10) Agbaria, R. A.; Butterfield, M. T.; Warner, I. M. *J. Phys. Chem.* **1996**, *100*, 17133.
(11) Craig, D. P.; Short, L. N. *J. Chem. Soc*. **1945**, 419.
(12) Murrell, J. N. *The Theory of the Electronic Spectra of Organic Molecules*; Methuen: London, 1963; p 197.
(13) Rapaport, E.; Malcom, W. C.; White, E. H. *J. Am. Chem. Soc*. **1972**, *94*, 3153.
(14) Yates, K.; Wai, H. *J. Am. Chem. Soc*. **1964**, *86*, 5408.
(15) Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587.
(16) (a) Melhuish, W. H. *J. Phys. Chem*. **1961**, *65*, 229. (b) Meach, S. R.; Phillips, D. *J. Photochem*. **1983**, *23*, 193.
(17) Berlman, I. B. *Handbook of Fluorescence Spectra of Aromatic Molecules*; Academic Press: New York, 1971; p 355.
(18) Platt, J. R. *J. Chem. Phys*. **1949**, *17*, 484.
(19) (a) Clar, E. *Spectrochim. Acta* **1950**, *4*, 116. (b) Klevens, H. B.; Platt, J. R. *J. Chem. Phys*. **1949**, *17*, 470.
(20) Mulliken, R. S. *J. Chem. Phys*. **1955**, *23*, 1997.
(21) Grabowski, Z. R.; Rotkiewicz, K.; Sadlej, A. J. *Proc. Int. Conf. Lumin*. **1966**, 310.
(22) (a) Weller, A. *Z. Elektrochem*. **1957**, *61*, 956. (b) Weller, A. *Prog. React. Kinet*. **1961**, *1*, 187.
(23) Schulman, S. G. *Rev. Anal. Chem*. **1972**, *1*, 85.
(24) El-Sayed, M. *J. Chem. Phys*. **1963**, *38*, 2834; **1962**, *36*, 573.
(25) Fischer, G.; Naaman, R. *Chem. Phys*. **1976**, *12*, 367.
(26) Schulman, S. G. *Fluorescence and Phosphorescence Spectroscopy: Physicochemical Principles and Practice*; Pergamon: New York, 1977; p 60.
(27) Ladner, S.; Becker, R. S. *J. Phys. Chem*. **1963**, *67*, 2481.
(28) Lim, E. C. In *Excited States*; Lim, E. C., Ed.; Academic Press: New York, 1977; Vol. 3, p 305.
(29) Hochstrasser, R. M.; Marzzaco, C. A. In *Molecular Luminescence*; Lim, E. C., Ed.; W. A. Benjamin: New York, 1967; p 631.
(30) Lin, H. B.; Topp, M. *Chem. Phys*. **1979**, *36*, 365.
(31) Barbara, P. F.; Brus, L. E.; Rentzepis, P. M. *Chem. Phys. Lett.* **1980**, *69*, 447.
(32) Zalis, B.; Capomacchia, A. C.; Jackman, D.; Schulman, S. G. *Talanta* **1973**, *20*, 33.
(33) Yates, K.; Wai, H. *Can. J. Chem*. **1965**, *43*, 2131.
(34) Schenkel, H.; Schenkel-Rudin, M. *Helv. Chim. Acta* **1948**, *31*, 514.
(35) Albert, A. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; 1963; Vol. 1, p 72.
(36) Ireland, J. F.; Wyatt, P. A. *Adv. Phys. Org. Chem*. **1976**, *12*, 131.
(37) Pace, I.; Schulman, S. G. *J. Phys. Chem*. **1972**, *76*, 1996.
(38) Baily, M. L.; Burnstall, M. L.; Jefford, C. W.; Sansom, B. F. *J. Chem. Soc*. **1954**, 3742.
(39) (a) Hosoya, H.; Nagakura, S. *Spectochim. Acta* **1961**, *17*, 324. (b) Stewart, R.; Yates, K. *J. Am. Chem. Soc*. **1960**, *82*, 4059. (c) Norman, R. O. C.; Ralph, P. D. *J. Am. Chem. Soc*. **1942**, *64*, 900. (d) Vander Donckt, E.; Porter, G. *Trans. Faraday Soc*. **1968**, *64*, 3215, 3218.
(40) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc*. **1985**, *107*, 3902.
(41) Trotter, J. *Acta Crystallogr*. **1962**, *13*, 732.
(42) Jaffe, H. H.; Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy*; John Wiley & Sons: New York, 1964; p 389.