Prototropic Equilibria of Some *Harmala* Alkaloids in Acid Solutions: Proton-induced Fluorescence Quenching of the Monocations of Harmaline and Harmalol

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Absorption and fluorescence spectra of harman homologues in acid solutions have shown the presence of mono-, di-, and tri-cations. Fluorescence spectra for the various cations at 77 K and their quantum yields at 298 K are reported. The acidity constants indicate that these alkaloids are more basic in the excited singlet state than in the ground state. The proton-induced fluorescence quenching rate constant for the monovalent cations is observed to be of the order of 10^9 dm^3 mol^{-1} s^{-1} for harmaline and harmalol and considerably less for harman, harmine, and harmol.

Harman (1-methyl-9*H*-pyrido[3,4-*b*]indole), harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol), harmaline (3,4-dihydro-7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), and harmalol (3,4-dihydro-1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol) are naturally occurring alkaloids of the *harmala* series[1] and are pharmaceutically potent substances, being hallucinogens.[2] Like norharman,[3] harman homologues are markedly fluorescent[4] and their use has been suggested for the fluorimetric measurement of small changes of acidities in the physiological range.[5]

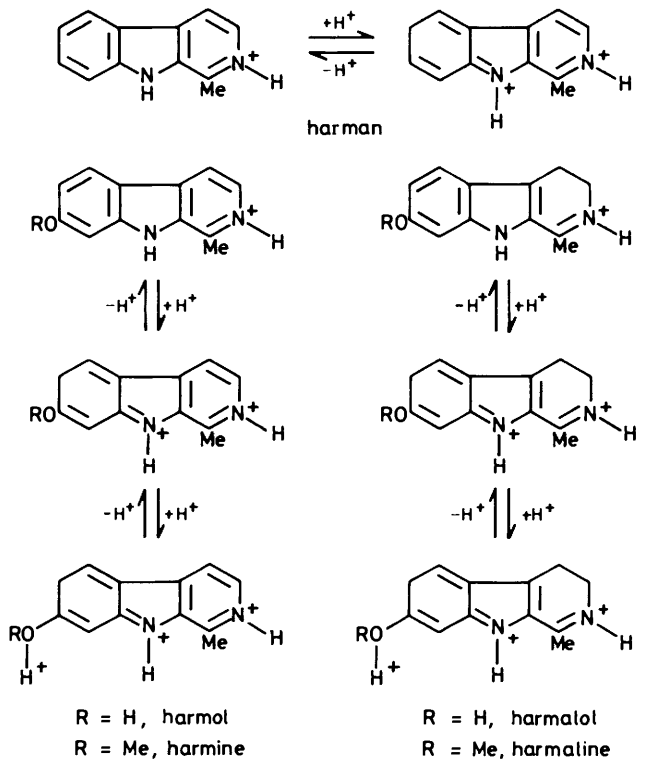
The presence of monocations of these alkaloids in neutral aqueous solutions has been reported recently.[4-8] Since these compounds are composites of pyrrole, pyridine, and phenol (except in the case of harman) rings, we thought it interesting to study their prototropic behaviour in dilute and in strong acid solutions, in both S_0 and S_1 states. We have established the existence of dications (by absorption and fluorescence spectra) and the trications (by fluorescence spectra). The quantum yields (φ_f) for the mono- and di-cations are reported here, and also the proton-induced fluorescence quenching constants for the monocations of harmaline and harmalol.

Experimental

The alkaloids were purchased from Aldrich Chemical Company, as their hydrochlorides. Their aqueous solutions were made alkaline with dilute aqueous sodium hydrogen carbonate. The solids obtained were filtered off and recrystallised twice from acetone. The purity of the alkaloids was shown by the fact that the same fluorescence maxima were obtained by excitation at different wavelengths. A modified Hammett acidity scale[9] was followed for the preparation of solutions in the H_0 range. Analytical grade sulphuric acid (B.D.H.) and triply distilled water were used to prepare solutions of various acidities. All the solutions for absorptiometric and fluorimetric titrations were prepared just before measurement. Stock solutions of the alkaloids were prepared in methanol–water and were diluted each time with a solution of known acidity (H_0 or pH) so as to contain finally not more than 1% (v/v) methanol. Absorption spectra were recorded with a Shimadzu UV-190 spectrophotometer attached to a U-135 recorder. The fluorescence spectra were recorded with a laboratory-made scanning spectrofluorimeter[10] and were corrected according to the procedure mentioned earlier.[10] Spectra at 77 K were recorded with an Aminco-Bowman low-temperature accessory in our fluorimeter. In the fluorimetric titrations, the relative fluorescence intensities were measured at the analytical wavelength as a function of H_0/pH; the isosbestic wavelengths (365 nm for harman, 360 nm for harmine, 366 nm for harmol, 335 nm for harmaline, and 344 nm for harmalol) were used for excitation. The φ_f values were calculated from the corrected spectra by taking quinine sulphate in sulphuric acid (1 mol dm^{-3}) as standard.[11]

Results and Discussion

Absorption Spectra.—The absorption spectra of the alkaloids in neutral solutions do not show much change up to H_0 −6 for harman, harmine, and harmol and H_0 −5 for harmaline and harmalol. These spectra are due to the monocations, as suggested earlier.[5,8] In solutions of high acid concentration the spectra show slightly blue shifts, attributed to the formation of dications. The *harmala* alkaloids are structurally similar to



Scheme. Prototropic equilibria of harman homologues in aqueous acidic solutions

Table 1. Profiles of absorption and fluorescence spectra of the cations of harman, harmine, harmol, harmaline, and harmalol in aqueous acidic solutions at 298 K (λ and ε in nm and $dm^3\ mol^{-1}\ cm^{-1}$, respectively)

	Monocation at pH 2				Dication at H_0 −8.5				Trication at H_0 −10
Alkaloid	$\lambda^{ab}_{max.}$	log ε	$\lambda^{fl\ a}_{max.}$	φ_f	$\lambda^{ab}_{max.}$	log ε	$\lambda^{fl\ a}_{max.}$	φ_f	$\lambda^{fl\ a}_{max.}$
Harman	365	3.66	424	0.88	360	3.71	398	0.72	
	297	4.18	(392)		297	4.30	(374)		
	246	4.44			250	4.60			
	204	4.26			202	4.15			
Harmine	361	3.90	415	0.51	359	3.94	394	0.30	385
	319	4.29	(383)		318	4.29	(369)		(361)
	246	4.56			253	4.60			
	204	4.33			203	4.26			
Harmol	360	3.82	413	0.44	357	3.92	390	0.28	379
	320	4.26	(382)		315	4.20	(367)		(359)
	247	4.53			252	4.55			
	204	4.31			203	4.26			
Harmaline	373	4.35	484	0.54	317	4.34	441	0.38	437
	260	3.86	(443)		275	4.00	(420)		(411)
	213	4.36			213	4.30			
	203	4.38			196	4.26			
Harmalol	368	4.19	482	0.45	367	4.20	436	0.34	429
	258	3.73	(440)		276	3.87	(418)		(408)
	215	4.21			215	4.21			
	203	4.26			197	4.16			

[a] Values in parentheses are at 77 K.

Table 2. Values of pK_a and pK_a^* for the equilibrium between mono- and di-cations of harman homologues in aqueous acidic solution at 298 K

	Photometric titration	Förster cycle method				Fluorimetric titration
Alkaloid	pK_a	pK_a^*(ab)[a]	pK_a^*(fl)[b]	pK_a^*(av)[c]	pK_a^*(fl)[d]	pK_a
Harman	−7.8	−7.0	−4.6	−5.8	−5.2	−6.9
Harmine	−7.5	−7.2	−4.8	−6.0	−5.4	−6.5
Harmol	−7.6	−7.2	−4.8	−6.0	−5.4	−6.7
Harmaline	−6.4	−6.1	−2.2	−4.2	−3.8	−5.5
Harmalol	−6.5	−6.3	−2.0	−4.2	−4.0	−5.9

[a] From absorption maxima. [b] From fluorescence maxima. [c] Average of values from absorption and fluorescence maxima. [d] From fluorescence maxima at 77 K.

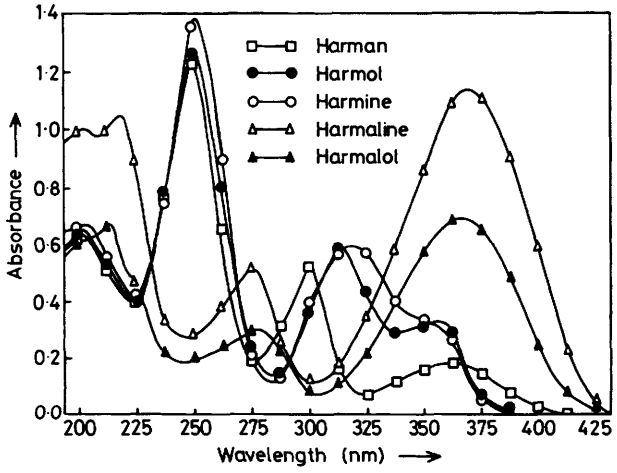


Figure 1. Absorption spectra of the dications of harman homologues in aqueous acidic solutions at 298 K

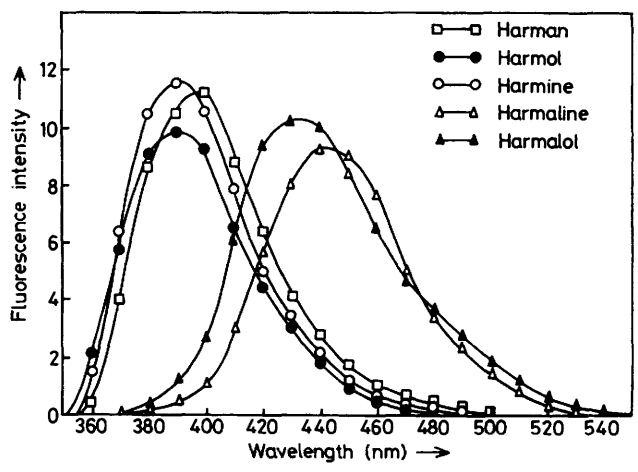


Figure 2. Fluorescence spectra of the dications of harman homologues in aqueous acidic solutions at 298 K

carbazole, for which charge migration from N to C-3 or C-6 in acid solutions has been reported.[12] Hence, charge migration to C-6 seems probable. To satisfy the negative charge, in order to overcome the destablisation of the electron density of the molecules, a proton can be accommodated on C-6. Protonation of carbocyclic rings are reported to be associated with red-shifted absorption bands.[13] The blue shift observed in the present case could be due to the loss in aromatic character, as illustrated in the Scheme. This observation is similar to the formation of monocations of carbazole and indoles.[14] The absorption spectra of the dications of the present alkaloids are shown in Figure 1; the absorption maxima and molar absorptivity values for both mono- and di-cations are recorded in Table 1. No further change in absorption pattern is observed up to H_0 −10, the highest acid concentration studied.

Fluorescence Spectra.—The fluorescence maxima of the monocations show no change in neutral and dilute acid solutions, but are blue shifted in solutions of H_0 −7. This is expected, as a result of the *C*-protonation, giving a positive charge on the pyrrole nitrogen atom,[15] and leading to the polarised nature of the dications. The fluorescence spectra of the dications are shown in Figure 2; the fluorescence maxima are listed in Table 1. At H_0 −10, though no change is observed in absorption spectra, the fluorescence maxima (except for harman) are further blue shifted, indicating further protonation. This change is presumably due to the acceptance of a proton by the phenolic oxygen, leading to the formation of a trication. This behaviour is not observed in the case of harman, where no phenolic oxygen is present.

Fluorescence maxima of all the cations at 77 K are also listed in Table 1. Solvent-induced relaxation is minimal at low temperatures for all the cations. Furthermore, the change in the polarity of the monocations upon excitation is large, particularly for those of the harmaline and harmolol (the blue shift for these cations is larger than for the others).

Values of φ_f for both mono- and di-cations are also listed in Table 1. All the cations are markedly fluorescent. Further, the fluorescence bands of the cations of methoxy compounds are more intense than for the corresponding hydroxy compounds. This observation is reflected in the absorption data, and is in analogy with results for phenols and their ethers.[16]

Prototropic Equilibria and Acidity Constants.—The acidity constants for the ground state governing the prototropic equilibria between mono- and di-cations (Scheme) have been determined spectrophotometrically; the values are listed in Table 2. These values suggest that (i) compounds containing phenolic oxygen are more basic than the non-phenolic compounds, (ii) the methoxy compounds are less acidic than the corresponding hydroxy compounds, and (iii) the dihydro alkaloids are more basic than the aromatic alkaloids. The first observation is presumably due to the electron-releasing nature of the hydroxy and methoxy groups; the second arises from the more basic nature of the methoxy group than of the hydroxy group. Resonance interactions predominate over inductive effects, thus increasing charge densities at the protonation sites. The third observation would be expected because charge migration from the imino group towards the carbocyclic ring will be more than towards the heterocyclic ring of the dihydro alkaloids, whereas in the case of the aromatic alkaloids, charge will be delocalised over the complete molecule.

The acidity constants for the excited singlet state governing the equilibrium between mono- and di-cations were estimated from the fluorimetric titration curves of Figure 3. These curves intersect at a φ/φ_0 value of about 0.4 for harman, harmine, and harmol and of almost zero for harmaline and harmalol. The lack of correspondence of these titration curves, especially for the

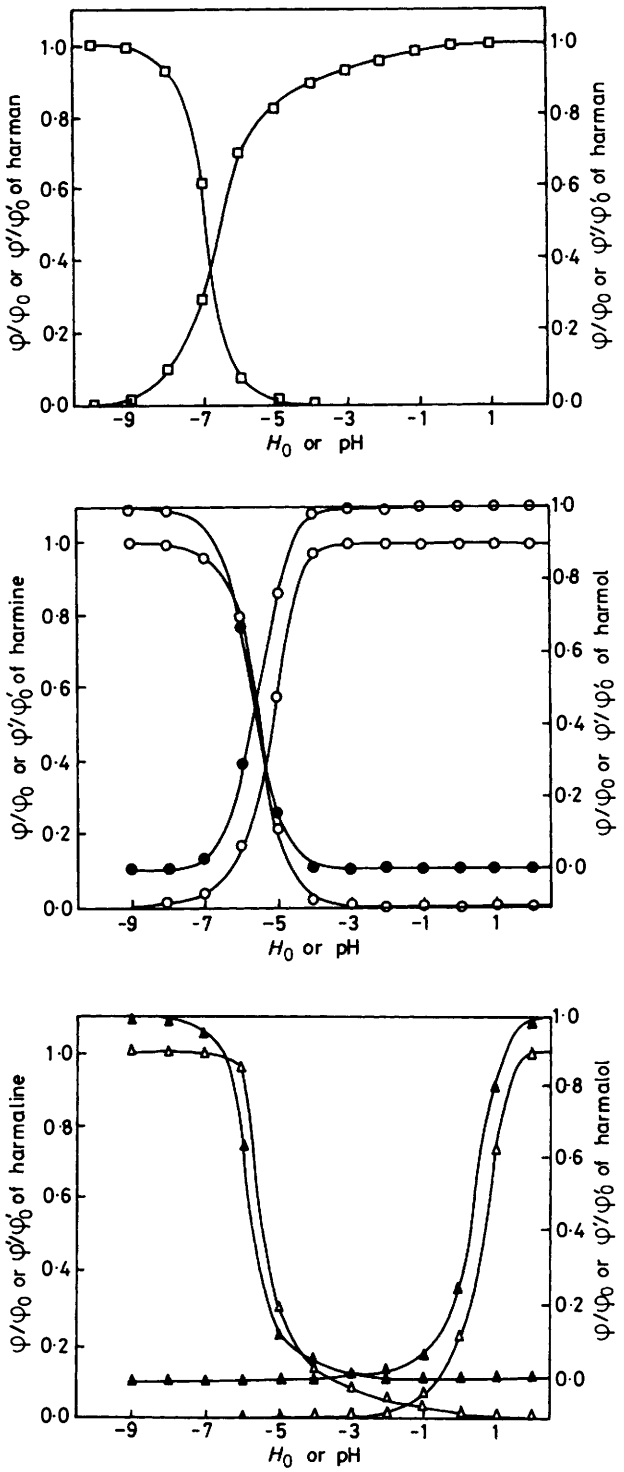


Figure 3. Plots of the relative fluorescence efficiencies of the monovalent and di-cations of harman (□), harmine (○), harmol (●), harmaline (△), and harmalol (▲) *versus* pH or H_0

latter compounds, argues against a simple acid–base equilibrium in the S_1 state, and indicates fluorescence quenching of the monocations. Therefore the pK_a^* values were calculated from the formation curves of the dications and not from the decrease in monocation fluorescence efficiencies. The protonation constants of the excited singlet state (Table 2) are higher than the corresponding ground-state values, indicating that these

alkaloids are more basic in the S_1 than in the S_0 state. This observation corroborates earlier results for nitrogen heterocycles.[17,18] Acidity constants for the equilibrium between di- and tri-cations cannot be calculated because change is observed only at H_0 −10 and the formation of the trications is incomplete.

The acidity constants for the excited singlet state were also calculated by the Förster cycle method,[19] at 298 K, from equation (1), where $\bar{\nu}_A$ and $\bar{\nu}_B$ are the band maxima of the acid and its conjugate base, respectively.

$$pK_a^* = pK_a - 2.1 \times 10^{-3}(\bar{\nu}_A - \bar{\nu}_B) \quad (1)$$

The differences between pK_a^* values of harman, harmine, and harmol obtained from averages of absorption and fluorescence maxima and measured by fluorimetric titration are not large. This could be due either to matching 0–0 bands of respective species or to cancelling of errors in relaxation due to solvents. However, the corresponding difference is large in the cases of harmaline and harmalol. This difference could be due to (i) change in the entropy of the species, or (ii) change in the geometry of the molecules upon excitation, or (iii) change in solvent-induced relaxation of the species involved upon excitation to the S_1 state. It is generally observed that factor (i) does not contribute much to differences in protonation constants; in this case in particular the molecules are rigid and planar and thus entropy change will be negligible. Since the molecules are rigid aromatic systems, change in the geometry upon excitation [factor (ii)] will also not contribute much to the pK_a^* values. Thus, the large difference may be attributed to a change in solvent-induced relaxation of the conjugate acid–base species in both S_0 and S_1 states. Large blue shifts observed in the fluorescence maxima of the monovalent cations of harmaline and harmalol at 77 K support this postulate. A small difference remains between the pK_a^* values calculated from absorption data and the fluoresence data at 77 K. This could be attributed to the use of band maxima rather than 0–0 transitions.

Proton-induced Fluorescence Quenching of Monocations.— The lack of correspondence in the fluorimetric titration curves of the cations (Figure 3) suggests that fluorescence quenching of the monocations takes place before further protonation; this quenching is quite large for harmaline and harmalol. The role of nucleophiles like HSO_4^- and SO_4^{2-} in the fluorescence quenching of the monocations may be tested by measuring the fluorescence efficiencies of the cations in the presence of these nucleophiles (introduced by adding appropriate amounts of $KHSO_4$ and K_2SO_4 to solutions containing the cations at pH 2). The fluorescence efficiencies of the cations are found to be insensitive to the nucleophiles, indicating that quenching is induced by protons. Further, the fluorescence efficiencies remain unchanged at liquid nitrogen temperature even in the presence of high acid concentrations (*e.g.* H_0 −4 and −5). This proves that the observed fluorescence quenching is dynamic in nature.

The kinetic model proposed by Shizuka *et al.*[20] can be applied to the present system; the steady-state model can be simplified to a Stern–Volmer relation [equation (2)] if the concentration of the hydrogen ion is small enough for the rate of formation of the dication to be negligible in comparison with its rate of decomposition, and the lifetime (τ) of the monocation is larger than that of dication. In equation (2), φ_0 and φ are the

$$\varphi_0/\varphi = 1 + k_q\tau[H^+] \quad (2)$$

fluorescence efficiencies of the monocations in the absence and in the presence of the quencher, respectively, and k_q is the proton-induced fluorescence quenching rate constant. The τ values for the monocations of harmaline and harmalol may be calculated by Strickler and Berg's integrated absorption method[21] from the φ_f values. The respective values were found to be 2.4×10^{-9} and 3.0×10^{-9} s for the cations of harmaline and harmalol. Plots of $(\varphi_0 - \varphi)/\varphi$ for the monocations of harmaline and harmalol *versus* hydrogen ion concentration (Figure 4) yielded straight lines with slopes ($k_q\tau$) 3.2 and 2.3 $dm^3\ mol^{-1}$ for harmaline and harmalol, respectively. The values of k_q are thus 1.3×10^9 and 8×10^8 $dm^3\ mol^{-1}\ s^{-1}$ for harmaline and harmalol respectively. The non-linearity of the Stern–Volmer plots at $[H^+] > 0.1$ mol dm^{-3} could be due to the non-ideal behaviour of the solutions at high acid concentrations; activities deviate markedly from proton concentration at these acidities.

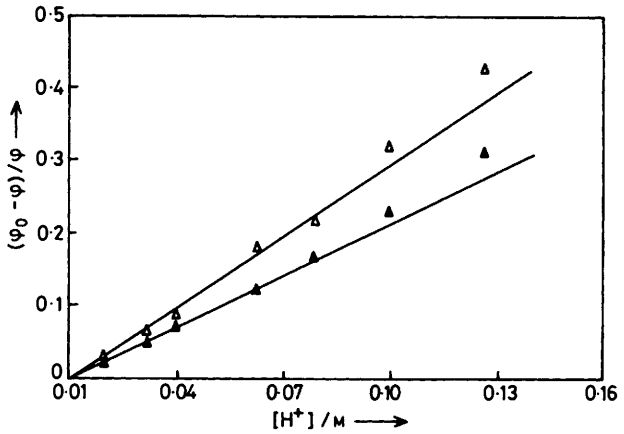


Figure 4. Plots of $(\varphi_0 - \varphi)/\varphi$ of the monovalent cations of harmaline (△) and harmalol (▲) *versus* hydrogen ion concentration

The proton-induced fluorescence quenching is suggested to arise from deactivation of the fluorescence intensity of the aromatic amines by proton-transfer reactions in the S_1 state,[22] and from ring protonation.[22] However, Schulman[23] suggests that quenching is due to the formation of a stoicheiometric excited-state complex. Nevertheless, the relaxed fluorescence state with intramolecular charge-transfer of the charge density from the nitrogen atom of the amino group as suggested by Shizuka *et al.*[20,24] seems more probable and acceptable.[25,26] In the case of the present alkaloids, it appears that charge migration may take place from the pyrrole nitrogen atom to the carbon atom of the homocyclic ring, as the lone pair of the nitrogen atom of the pyridine ring is involved in protonation leading to the formation of monocations. The value of k_q for the fluorescence quenching of neutral amines[20,24,27–30] is reported to be in the order of 10^8—10^9 $dm^3\ mol^{-1}\ s^{-1}$, and is similar for the cations of harmaline and harmalol. The nearly negligible fluorescence quenching of the monocations of harman, harmine, and harmol as compared with those of harmaline and harmalol is due to the complete delocalisation of the charge distribution of the pyrrole nitrogen over the aromatic system rather than its localisation at any one of the carbon atom. Similar behaviour has been observed in the case of 3-aminofluoranthene[30] and 2-phenylbenzimidazoles.[31]

Acknowledgements

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References

1 L. Marion, 'The Alkaloids,' ed. R. H. F. Manske and H. L. Holmes, Academic Press, New York, 1952, vol. II, p. 394.
2 A. Hoffer and H. Osmond, 'The Hallucinogens,' Academic Press, New York, 1967.
3 R. A. Abramovitch and L. D. Spenser, *Adv. Heterocycl. Chem.*, 1964, **3**, 79.
4 S. G. Hadley, A. S. Muraki, and K. Spitzer, *J. Forensic Sci.*, 1974, **19**, 657.
5 O. S. Wolfbeis and E. Furlinger, *Z. Phys. Chem., Neue Folge*, 1982, **129**, 171; *Monatsh. Chem.*, 1982, **113**, 509.
6 G. Ekhalil and A. Mayouf, *Can. J. Spectrosc.*, 1984, **29**, 104.
7 B. Savory and J. H. Turnbull, *J. Photochem.*, 1984, **24**, 355.
8 F. T. Vert, I. Z. Sanchez, and A. O. Torrent, *J. Photochem.*, 1983, **23**, 355; 1984, **26**, 285.
9 M. J. Jorgensen and D. R. Hartter, *J. Am. Chem. Soc.*, 1963, **85**, 878.
10 M. Swaminathan and S. K. Dogra, *Indian J. Chem.*, 1983, **22A**, 853.
11 C. A. Parker, 'Photoluminescence in Solutions,' Elsevier, Amsterdam, 1968, p. 267.
12 H. H. Jaffé and M. Orchin, 'Theory and Applications of Ultraviolet Spectroscopy,' Wiley, New York, 1962, p. 356.
13 S. F. Mason and B. E. Smith, *J. Chem. Soc. A*, 1969, 325.
14 O. S. Wolfbeis, in 'Molecular Luminescence Spectroscopy: Methods and Applications—Part 1,' ed. S. G. Schulman, Wiley, New York, 1985, pp. 199—203.
15 R. Klein, I. Tatischeff, M. Bezin, and R. Sautus, *J. Phys. Chem.*, 1981, **85**, 670.
16 G. Kohler, G. Kittle, and N. Getoff, *J. Photochem.*, 1982, **18**, 19.
17 A. Weller, *Prog. React. Kinet.*, 1961, **1**, 187.
18 A. Albert, 'Heterocyclic Chemistry,' ed. A. R. Katritzky, Academic Press, New York, 1963, vol. I, p. 48.
19 T. Förster, *Z. Elektrochem.*, 1950, **54**, 42.
20 K. Tsutsumi, S. Sekiguchi, and H. Shizuka, *J. Chem. Soc., Faraday Trans. 1*, 1982, **78**, 1087.
21 S. J. Strickler and R. A. Berg, *J. Chem. Phys.*, 1962, **37**, 814.
22 Th. Förster, *Chem. Phys. Lett.*, 1972, **17**, 309.
23 S. G. Schulman and P. Liedke, *Z. Phys. Chem., Neue Folge*, 1973, **84**, 317.
24 K. Tsutsumi and H. Shizuka, *Chem. Phys. Lett.*, 1977, **52**, 485.
25 H. B. Ambroz, K. R. Bulter and T. J. Kemp, *Faraday Discuss. Chem. Soc.*, 1984, **78**, 107.
26 S. Hagopian and L. A. Singer, *J. Am. Chem. Soc.*, 1985, **107**, 1874.
27 C. M. Harris and B. K. Sellinger, *J. Phys. Chem.*, 1980, **84**, 891, 1366.
28 A. K. Mishra and S. K. Dogra, *J. Photochem.*, 1983, **23**, 163.
29 M. Swaminathan and S. K. Dogra, *Can. J. Chem.*, 1983, **61**, 1064.
30 A. K. Mishra and S. K. Dogra, *J. Chem. Soc., Perkin Trans. 2*, 1984, 943.
31 M. Krishnamurthy, P. Phaniraj, and S. K. Dogra, *J. Chem. Soc., Perkin Trans 2*, in the press.

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