Absorptiometric and Fluorimetric Study of Solvent Dependence and Prototropism of Benzimidazole Homologues

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Electronic absorption and fluorescence spectra of benzimidazole and six of its derivatives in different solvents and at various pH values have been studied. The dual fluorescence observed in dilute acid solutions and the similarity of the fluorescence excitation spectra to the absorption spectra indicate the existence of several excited states of benzimidazoles. The emitting transition of neutral benzimidazoles is $\pi \longleftarrow \pi^*$; in the case of the monocations the short-wavelength fluorescence band is from the $\pi \longleftarrow \pi^*$ transition and the long-wavelength band arises from charge transfer. The pK_a values for various prototropic reactions in aqueous solutions in the S_0 and S_1 states have been calculated, and the proton-induced fluorescence quenching of the monocations is discussed.

Benzimidazole (BI) and its methyl derivatives are reported to be useful both biologically[1] and commercially.[2] 5,6-Dimethylbenzimidazole is an integral part of the structure of vitamin B_{12}.[3] Detailed studies of the u.v. spectra of BI and its homologues[4–12] show that the absorption pattern of BI resembles that of a substituted benzene derivative; the short- and the long-wavelength absorption bands correspond to transitions in the imidazole and aryl rings, respectively. Prototropism of BI as a function of pH is reported,[10–18] but there is a lack of agreement among the various reports with respect to the large Stokes shift of the monocations. Borrensen[19] explained the large red shift in the fluorescence spectrum of the cation (in comparison with that of neutral BI) in terms of a stoicheiometric excited state complex with the solvent. Kondo and Kuwano[15] attributed this observation to the reversal of L_a and L_b electronic states in the S_1 state, and Tway and Love[17] point to the appearance of a new charge-transfer band in the fluorescence spectra. The extent of the charge-transfer transition[20] and the distinction between L_a and L_b electronic states[21] are frequently attributed to substitutent effects, because of the greater sensitivity of the excited states of the benzenoid molecules to substituents. Further, it has been proposed that the monocations of BI derivatives are excited by the absorption of light to a range of excited singlet states; one has predominantly $\pi \longleftarrow \pi^*$ character, and the other has charge-transfer characteristics.[10] Which state predominates depends on the substituents and on the solvent system. In view of this we have undertaken the present study as a continuation of our recent studies on the photophysical properties of heterocyclic compounds bearing multifunctional groups.[11,12,18,22–26]

Experimental

Materials.—Benzimidazole (BI), prepared by Pillai's method,[27] was recrystallised from water. 2-Methylbenzimidazole (2MBI), 5,6-dimethylbenzimidazole (DMBI), 2,5,6-trimethylbenzimidazole (TMBI), and 1-methyl-2-phenylbenzimidazole (MPBI) were all obtained from Aldrich Chemical Co. and were purified by recrystallisation from methanol. 1-Ethyl-2-methylbenzimidazole (EMBI) was also obtained from Aldrich and was used as such. 1-Methylbenzimidazole (1MBI) was prepared and purified by the method of Pilarski.[28] The purity of the compounds was checked by comparing fluorescence maxima obtained on exciting at different wavelengths. B.D.H. spectrograde methanol, analytical grade sulphuric acid and sodium hydroxide, and Fluka puriss grade trifluoroacetic acid were used as such. Analytical grade cyclohexane, ether, and acetonitrile (E. Merck) were further purified by standard methods.[29] Triply distilled water was used for the preparation of aqueous solutions.

Instrumentation.—A Toshniwal CL 44 pH meter was used for measuring pH values between 1 and 13. All the absorption measurements were made with a Shimadzu UV-190 spectrophotometer with U-135 recorder, and fluorescence spectra were recorded with a laboratory-constructed scanning spectrofluorimeter described earlier.[30] Spectra at 77 K were recorded with an Aminco-Bowman low-temperature accessory in the fluorimeter.

Methods.—The acidity of solutions of high acid and base concentrations were measured on a modified Hammett acidity scale[31] (H_0 0 to -10) and on Yagil's basicity scale[32] (H_- 14 to 16), respectively. Stock solutions of benzimidazoles were prepared in water–methanol mixtures and diluted appropriately to obtain a final concentration of *ca.* 10^{-5} mol dm^{-3}; the overall concentration of methanol in each solution was about 1% (v/v). For fluorimetric titrations the solutions were excited at the isosbestic point. Fluorescence quantum yield (φ_f) values were determined from solutions having absorbances less than 0.1. The fluorescence spectra were corrected[33] and compared with those of reference standards, *e.g.* BI[14] and anthracene.[34]

Results

Absorption Spectra.—The absorption maxima of benzimidazoles in various solvents and the molar extinction coefficients at band maxima are given in Table 1. All the band maxima are slightly blue-shifted on going from a non-polar solvent (cyclohexane) to a polar solvent (water). A blue shift is observed in the formation of monocations of all substituted benzimidazoles, similar to that observed for the parent BI;[7] protonation occurs at the tertiary N-3. Deprotonation leading to anions is associated with red-shifted absorption bands in 2MBI, DMBI, and TMBI (dissociation of the NH group). Anion formation is not observed in the case of 1-substituted benzimidazoles, as there is no dissociative imino group. The absorption spectra of the prototropic species are given in Figure 1; profiles are listed in Table 1.

Fluorescence Spectra.—Fluorescence maxima of the substituted benzimidazoles in various solvents are listed in Table 2. As for the absorption spectra, a small blue shift (or near constancy) in the emission spectra is observed for all the benzimidazoles with increasing polarity and hydrogen-bonding ability of the solvent. The fluorescence spectra of MPBI in cyclohexane is

Table 1. Absorption maxima [λ/nm (log ε[a])] of benzimidazole homologues at 298 K

Species (Solvent)	BI	1MBI	2MBI	DMBI	TMBI	EMBI	MPBI
Neutral		294 (3.67)	282	288	290	284 (3.76)	292 (4.20)
(Cyclohexane)		284 (3.84)	274	282	285	278 (3.69)	232 (4.01)
		277 (3.77)	241	278	278	253 (3.84)	211 (4.39)
		254 (3.74)	210	245	246	214 (4.19)	
		211 (4.65)		210	210		
Neutral		294 (3.90)	282 (3.93)	289 (3.81)	290 (4.04)	284 (3.83)	291 (4.27)
(Ether)		283 (4.07)	274 (3.90)	282 (3.77)	284 (4.04)	277 (3.77)	232 (4.04)
		277 (3.97)	244 (3.93)	278 (3.76)	278 (4.02)	253 (3.90)	218 (4.31)
		248 (4.15)	214 (4.02)	247 (3.79)	246 (4.04)	216 (4.01)	
		217 (4.46)		214 (4.11)	216 (4.28)		
Neutral		291 (3.73)	281 (3.79)	286 (3.74)	289 (3.90)	283 (3.69)	290 (4.27)
(Acetonitrile)		281 (3.94)	274 (3.68)	282 (3.72)	284 (3.91)	276 (3.68)	234 (4.09)
		275 (3.90)	244 (3.80)	278 (3.76)	278 (3.89)	253 (3.77)	208 (4.43)
		247 (4.10)	208 (4.42)	247 (3.72)	246 (3.89)	212 (4.26)	
		207 (4.76)		207 (4.44)	209 (4.54)		
Neutral	278[b]	289 (3.74)	279 (3.88)	286 (3.79)	288 (3.79)	281 (3.74)	288 (4.19)
(Methanol)	271	280 (3.96)	272 (3.87)	281 (3.76)	282 (3.78)	275 (3.71)	233 (4.07)
		274 (3.93)	242 (3.82)	277 (3.76)	277 (3.77)	252 (3.77)	209 (4.43)
		247 (4.08)	207 (4.36)	247 (3.69)	246 (3.67)	212 (4.13)	
		207 (4.79)		207 (4.40)	208 (4.40)		
Neutral	274[c]	277 (3.92)	279 (3.80)	285 (3.77)	286 (3.67)	280 (3.63)	287 (4.21)
(Water, pH 8)		270 (3.98)	272 (3.82)	281 (3.77)	282 (3.68)	274 (3.74)	233 (4.11)
		252 (3.89)	241 (3.76)	276 (3.78)	277 (3.68)	250 (3.73)	203 (4.58)
		202 (4.59)	202 (4.49)	247 (3.62)	245 (3.42)	207 (4.28)	
				202 (4.49)	201 (4.40)		
Monoanion	273[c]		286 (3.74)	289 (3.79)	289 (3.99)		
(NaOH, H_- 15)			279 (3.89)	285 (3.83)	286 (3.95)		
			257 (3.63)	263 (3.61)	263 (3.78)		
			226 (3.93)	226 (3.94)	227 (4.06)		
Monocation	274[b]	274 (3.93)	274 (3.89)	281 (3.83)	282 (3.97)	276 (3.92)	285 (4.27)
(Water, pH 2)	267	266 (3.98)	266 (3.88)	274 (3.82)	273 (3.94)	268 (3.89)	237 (4.25)
		226 (4.26)	231 (3.55)	219 (3.86)	219 (3.97)	240 (3.60)	197 (4.59)
		198 (4.55)	198 (4.52)	196 (4.43)	197 (4.53)	203 (4.27)	

[a] In $dm^3\ mol^{-1}\ cm^{-1}$. [b] Ref. 14. [c] Ref. 10.

clearly structured; the structure is lost with increase in the polarity of the solvent as expected for polar molecules. Similar behaviour is observed with other substituted benzimidazoles, except those having substituents on the six-membered ring.

In basic solution the fluorescence maxima of 2MBI, DMBI, and TMBI are red-shifted relative to the neutral species. This is as expected, owing to the dissociation of the NH group leading to anions; the effect is similar to that noticed in the absorption spectra. Fluorescence maxima are largely red-shifted for the formation of cations. Besides the long-wavelength fluorescence band of the cations, a slightly blue-shifted fluorescence band is observed for these cations (as compared with neutral species), except for MPBI. The dependence of the long-wavelength fluorescence spectra of the cations was on solvent polarity verified by using trifluoroacetic acid in non-aqueous media; the results are compiled in Table 3. The excitation spectra of the cations recorded at both the wavelengths are similar to the absorption spectra.

In solutions of H_0 −10, though there is no change in the absorption spectra, changes are observed in the fluorescence spectra. This is as expected, owing to the formation of dications. The fluorescence spectra of the various prototropic forms of the substituted benzimidazoles are depicted in Figure 2; the fluorescence maxima are in Table 2.

Acidity Constants.—From the observations of the shifts in the absorption and fluorescence spectra, the proton-transfer reactions of the substituted benzimidazoles in aqueous solutions may be deduced (see Scheme). The acidity constants in the ground state can be calculated from the absorption spectra; in the excited singlet state these are derived from the fluorimetric titration curves and by using the Forster cycle method.[35,36] These values are listed in Table 4.

The deprotonation constants of 2-methylbenzimidazoles indicate that the NH group of these compounds is slightly more basic than in the parent BI. Although the pK_a values in the S_1 state for protonation are almost same as the pK_a values in the S_0 state, the pK_a values are less in the S_1 state for deprotonation.

Protonation constants for the formation of dications cannot be calculated, as the change in fluorescence spectra is observed only at H_0 −10, the highest acid concentration studied.

Fluorescence Quantum Yields and Lifetimes.—Fluorescence quantum yields of the substituted benzimidazoles are listed in Table 2. The values indicate that the fluorescence intensities are high, relative to the parent BI. Quantum yields for the different prototropic forms are also listed in Table 2. In general these values follow the trend observed for the parent molecule,[10,14] *i.e.* the fluorescence quantum yields of the monocations are less than those of the neutral molecules. The quantum yield for the shorter wavelength band of the monocation is smaller than that observed for the longer wavelength band, except for TMBI.

Lifetimes for the neutral molecules and the monocations are reported to be of the order of 1 ns.[10] However, increased stabilisation can be attained for the cations in the S_1 states, thereby enhancing the lifetimes. Radiative lifetimes (τ_{FM}) of the monocations for the shorter wavelength band can be calculated from the corrected fluorescence spectra and absorption data as suggested by Strickler and Berg's relation;[37] this equation is only valid for similar absorbing and emitting states. Actual

Table 2. Fluorescence maxima [λ/nm (quantum yield)] of benzimidazole homologues at 298 K

Species (Solvent)	BI	1MBI	2MBI	DMBI	TMBI	EMBI	MPBI
Neutral (Cyclohexane)	293[a]	304 (0.63) 297	292 (0.16) 281	302 (0.37)	304 (0.34)	296 (0.83) 285	357 (0.61) 341 325
Neutral (Ether)		303 (0.68) 296	292 (0.19) 281	302 (0.46)	303 (0.41)	295 (0.88) 283	355 (0.62) 340 325
Neutral (Acetonitrile)	292[a]	302 (0.67) 296	291 (0.26) 281	301 (0.48)	302 (0.44)	295 (0.85) 283	355 (0.69) 347
Neutral (Methanol)	299[a] 291	301 (0.7) 295sh	290 (0.44) 280sh	300 (0.67)	302 (0.55)	293 (0.68) 282	352 (0.73) 346
Neutral (Water, pH 8)	290[b] (0.67)	301 (0.74)	290 (0.69) 280sh	300 (0.74)	302 (0.86)	292 (0.76) 282sh	352 (0.74)
Monoanion (NaOH, H_- 15)	310[b] (0.02)		311 (0.02)	317 (0.03)	322 (0.02)		
Monocation (Water, pH 2)	360[b] (0.06)	360 (0.25) 292 (0.07)	368 (0.27) 284 (0.20)	380 (0.30) 298 (0.09)	380 (0.03) 298 (0.13)	361 (0.14) 286 (0.04)	359 (0.80)
Dication (H_2SO_4, H_0 −10)		302	313	322	325	292	348

[a] Ref. 10. [b] Ref. 14.

Table 3. Fluorescence maxima (λ/nm) of the monocation of 5,6-dimethylbenzimidazole in different solvents, and charge-transfer transition energies (eV)

Solvent	Fluorescence maxima: Charge transfer	Fluorescence maxima: π–π*	Charge-transfer transition energy
Cyclohexane	352	297	3.521
Ether	366	297	3.386
Acetonitrile	374	298	3.314
Methanol	378	298	3.279
Water	380	298	3.262

Table 4. Protonation and deprotonation constants in the S_0 and S_1 states

Benzimidazole substituent	$pK_a(S_0)$[a]	$pK_a(S_1)$[b]	$pK_a(S_1)$[c]	$pK_a(S_1)$[d]
Equilibrium between monocation and neutral				
None	5.5[e]			5.6[f]
1-Me	5.7	4.60	3.55	6.0
2-Me	5.9	4.74	4.37	6.1
5,6-Me_2	5.4	4.35	4.92	5.6
2,5,6-Me_3	6.0	4.94	5.07	6.1
1-Et-2-Me	6.3	5.22	5.00	6.4
1-Me-2-Ph	5.2	4.69	6.19	5.3
Equilibrium between neutral and monoanion				
None	13.2[g]			
2-Me	13.4	11.56	8.51	12.6
5,6-Me_2	13.2	12.18	9.45	12.5
2,5,6-Me_3	13.4	12.64	9.08	12.7

[a] From spectrophotometric data. [b,c] By Förster cycle method using absorption and fluorescence data, respectively. [d] From fluorimetric titration. [e] Ref. 17. [f] Ref. 18. [g] Ref. 52.

lifetimes or molecular lifetimes (τ) were calculated from the equation $\tau = \tau_{FM}\varphi_f$ and are listed in Table 5.

Proton-induced Fluorescence Quenching of Monocations.— The fluorescence intensities of the monocations are constant in dilute acid solutions up to pH 2, and decrease at greater acidities, with the exception of 2-phenylbenzimidazoles. The decrease in the fluorescence intensity can be attributed to proton-induced fluorescence quenching,[38–44] since no decrease in fluorescence intensity is observed for solutions containing monocations (pH 3) and SO_4^{2-} ions up to 1 mol dm^{-3} (adjusted by adding Na_2SO_4). The quenching can be explained by a simple Stern-Volmer plot [equation (i)].[40] A plot of $(\varphi_0 - \varphi)/\varphi$

$$\frac{\varphi_0}{\varphi} - 1 = k_q\tau[H^+] \qquad \text{(i)}$$

vs. $[H^+]$ gives a slope equal to $k_q\tau$ (Figure 3), where φ_0 and φ are the fluorescence intensities in the absence and in the presence of the quencher, k_q is the proton-induced quenching constant, and τ is the molecular lifetime of the cation. A non-linear trend is observed in these plots with proton concentrations greater than 0.1 mol dm^{-3}; this could be due to use of concentrations rather than activities. Values of $k_q\tau$ for both fluorescence bands and of $k_q^{\pi-\pi^*}$ for the shorter wavelength band are listed in Table 5. These results indicate that the values of $k_q^{\pi-\pi^*}$ are nearly of the same order of magnitude as observed for the neutral amino compounds [40–44] (10^8—10^9 dm^3 mol^{-1} s^{-1}). Further, the fluorescence intensities of the monocations at 77 K are nearly constant in the range from pH 4 to H_0 −8.

Discussion

In the benzimidazole molecule, three kinds of transition are possible: (i) $n \longrightarrow \pi^*$, (ii) $\pi \longrightarrow \pi^*$, and (iii) charge-transfer (CT). The nature of the transition depends upon the type of solvent used and the nature of the substituents. There are well known procedures for establishing the nature of the transition. The data of Tables 1 and 2 (*i.e.* large values of the molecular extinction coefficient, very small change in the absorption spectra in strong polar and hydrogen-bonding solvents, and high fluorescence quantum yields) as well as theoretical calculations have clearly shown that the $n \longrightarrow \pi^*$ transitions are not observed in neutral, cationic, or anionic benzimidazole molecules. Similar conclusions have been arrived at by Tway and Love,[10] on the basis of a similar study, and from phosphorescence quantum yields and the increase in the lifetime of the singlet state in more polar solvents. Distinction between $\pi \longrightarrow \pi^*$ and charge-transfer bands can be made from a correlation of the magnitude of the Stokes shift with the nature of the substituent and solvent. In general, a large Stokes shift and greater solvent dependence are observed for the charge-transfer band. Our data (Tables 1 and 2) clearly show that the

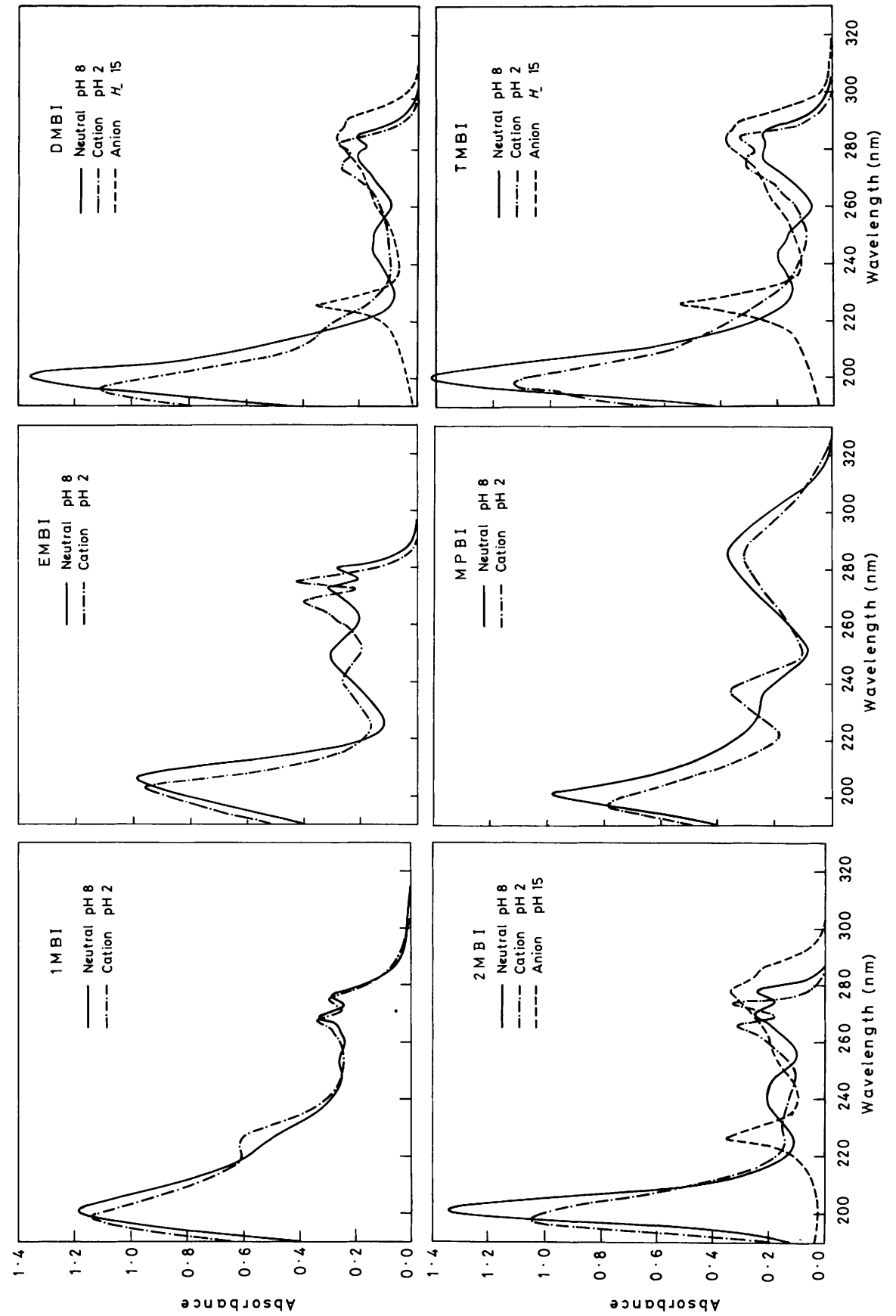


Figure 1. Absorption spectra of the prototropic forms of benzimidazole homologues at 298 K

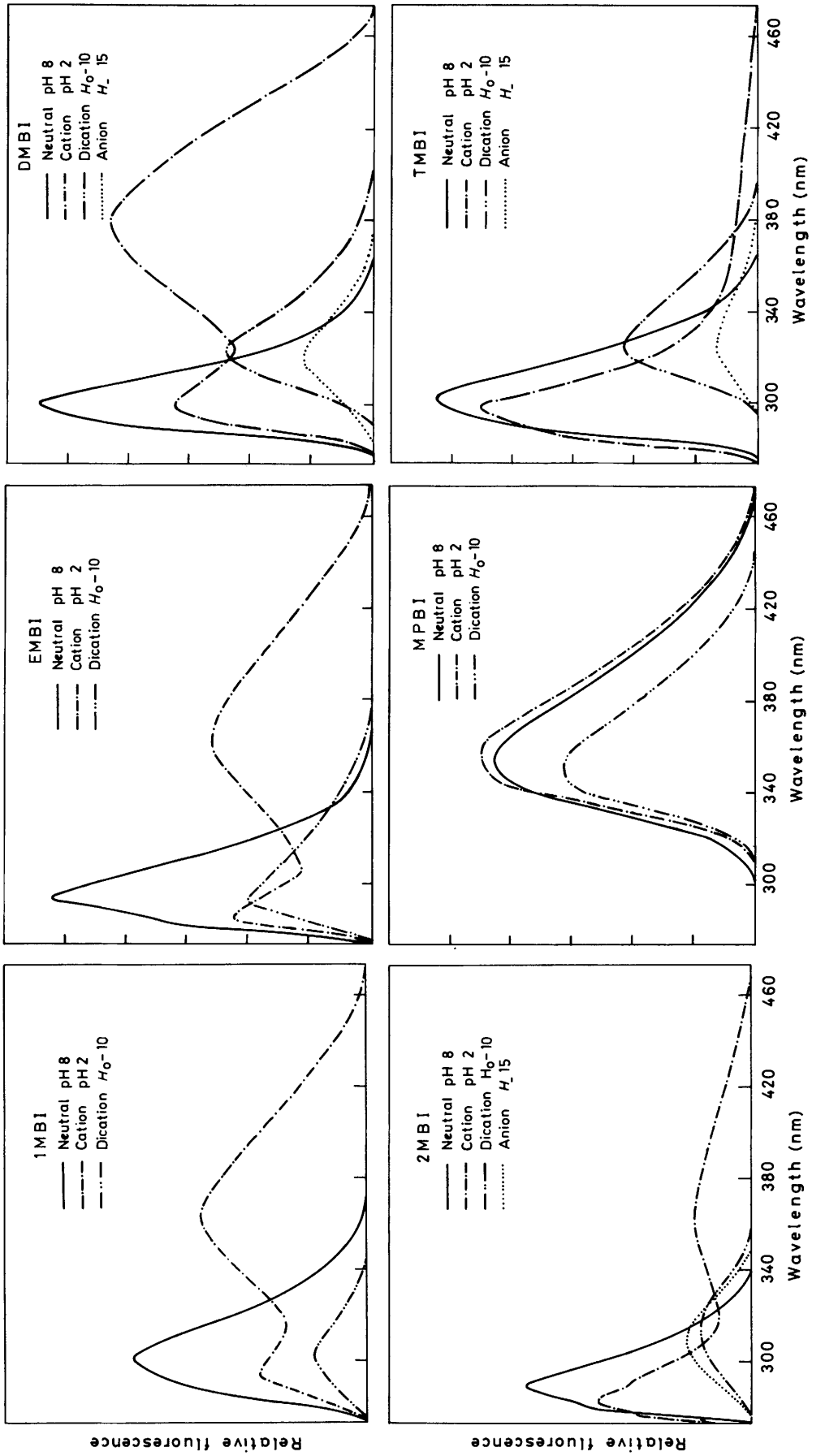
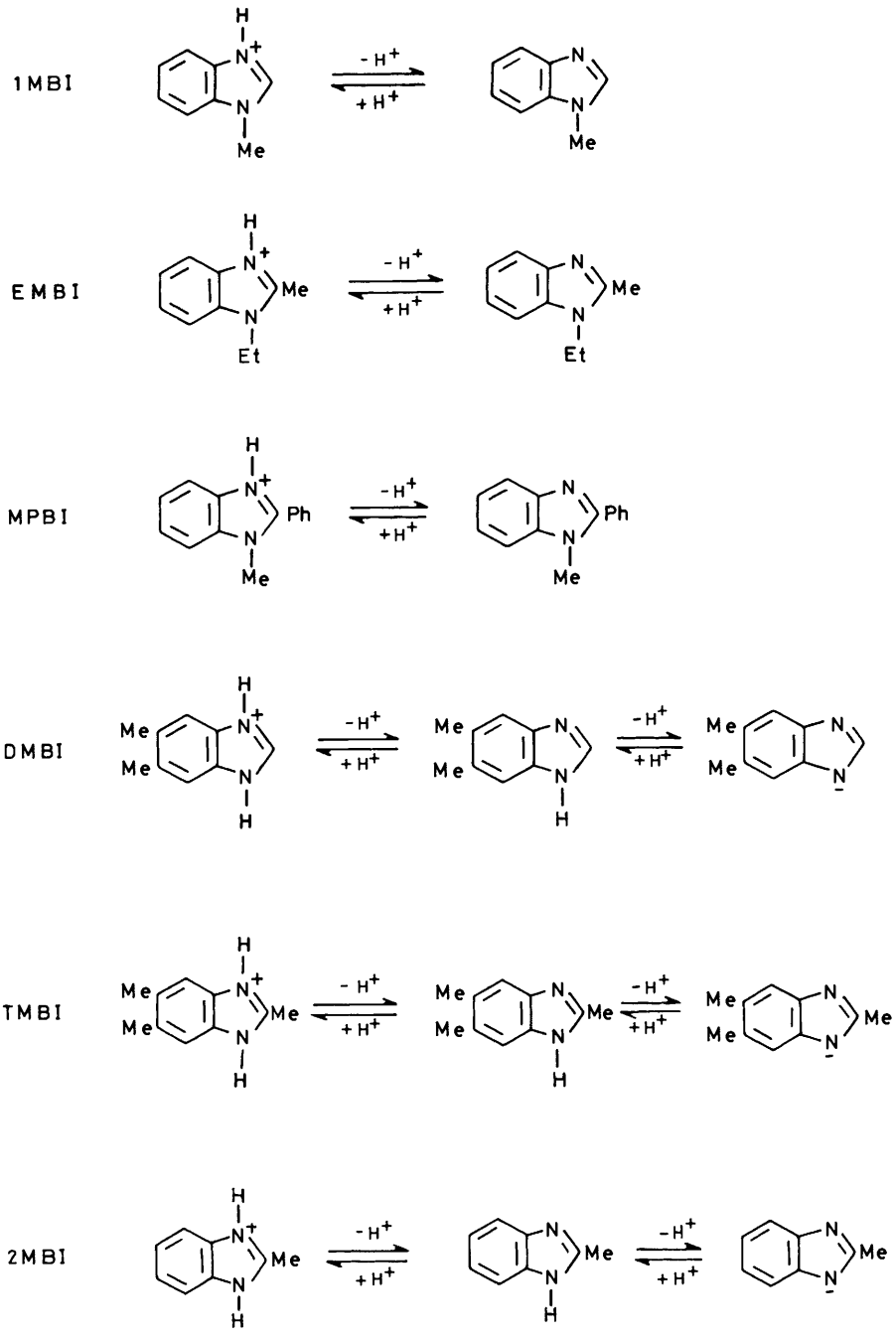


Figure 2. Fluorescence spectra of the prototropic forms of benzimidazole homologues at 298 K



Scheme. Prototropic equilibria of benzimidazoles

lowest energy transition in all the neutral benzimidazoles is of $\pi \longrightarrow \pi^*$ character: the Stokes shifts observed in all cases are *ca.* 20—30 nm (*ca.* 2 000 cm^{-1} except for MPBI) and neither absorption nor fluorescence bands depend markedly on the nature of the solvent.

The data in Table 1 show that the structure of the long-wavelength band is retained for all the substituted benzimidazoles except MPBI. This indicates that, unlike the case of 2-phenylbenzimidazole (2PBI),[11] the phenyl ring in MPBI is not conjugated with the benzimidazole moiety in the ground state (the long-wavelength band of 2PBI is at 317 nm; *cf.* 287 nm for MPBI). The non-coplanarity of the phenyl ring with the benzimidazole moiety in MPBI is due to the methyl substituent at position 1, which hinders rotation of phenyl ring. The effect on the long-wavelength band of BI is greater, if substituents are present in the homocyclic ring rather than in the imidazole ring, and at position 2 than position 1; the middle band (*ca.* 240 nm) is more affected if the substituent is on the imidazole moiety and at position 1. This is consistent with earlier observations that the long-wavelength band is localised more on the benzene ring and long-axis polarised, whereas the middle band is localised more on the imidazole moiety and short-axis polarised.[7]

The fluorescence spectra of all the substituted benzimidazoles are similar to that of the parent BI, except that the vibrational structure is lost with increasing substitution on BI, particularly on the homocyclic ring. This is expected because of the increase in vibrational degrees of freedom attained by the presence of the methyl group, resulting in an increase in vibronic coupling in the S_1 as compared with the S_0 state. However, in cyclohexane, the fluorescence spectrum of MPBI is more structured than its

Table 5. Values of τ_{rad}, τ, $k_q\tau$, and $k_q^{\pi-\pi^*}$ for the monocations of benzimidazole homologues

	τ_{rad} / ns	$\Phi^{\pi-\pi^*}$	τ/ns	$k_q\tau^{\pi-\pi^*}$ / dm^3 mol^{-1}	$k_q^{\pi-\pi^*}$ / dm^3 mol^{-1} s^{-1}	τk_q^{CT} / dm^3 mol^{-1}
BI						0.10
1MBI	5.4	0.07	0.38	0.18	0.47×10^9	0.18
2MBI	3.2	0.20	0.64	0.51	0.78	0.45
DMBI	6.8	0.09	0.62	0.68	1.02	0.90
TMBI	6.2	0.13	0.80	0.44	0.55	0.58
EMBI	5.9	0.04	0.24	0.18	0.75	0.26
MPBI	13.9	0.8	11.11			
2PBI	14.0	0.14	1.97			

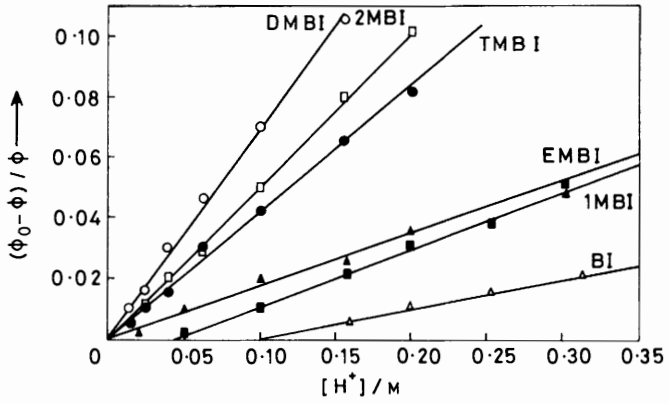


Figure 3. Plot of $(\varphi_0 - \varphi)/\varphi$ *versus* hydrogen ion concentration

absorption spectrum, but less structured than that of 2PBI,[11] and the Stokes shift observed in water is quite large (*ca.* 6 500 cm^{-1}) compared with the other benzimidazoles (*ca.* 2 000 cm^{-1}). This suggests that the phenyl ring of MPBI is closer to coplanarity in the S_1 than in the S_0 state, and that the large Stokes shift is due to a change in the geometry of the molecule. The structure in the fluorescence spectrum of MPBI can be explained in terms of the vibrational frequency of *ca.* 1 400 cm^{-1}, similar to that observed for 2PBI.[11] The similarity in geometry of MPBI and 2PBI in the S_1 state can be seen from the similar fluorescence maxima (325 nm for MPBI and 321 nm for 2PBI in cyclohexane).

The small blue shift observed in the long-wavelength absorption band as well as in the fluorescence maxima of all the benzimidazoles with increase in polarity or hydrogen-bonding ability of the solvent suggests that the benzimidazoles are acting as proton acceptors. This behaviour can be explained, as noted earlier, by assuming that the long-wavelength band is localised on the benzene ring and that the lone pair of the tertiary nitrogen atom is perturbing this transition in the same manner as an amino group, but to a smaller extent. Thus in hydrogen-bonding solvents protonation at the tertiary nitrogen atom would lead to the blue shift in absorption and fluorescence maxima. Though theoretical calculations have not been carried out, it seems from the structure of the molecules that this perturbation will be achieved through the inductive effect, rather than the resonance effect, because the lone pair of the tertiary nitrogen atom is perpendicular to the plane of the π-cloud. This also proves that the emitting and absorbing states in neutral benzimidazoles are the same. Similar behaviour has been observed in the case of phenanthro[9,10-*d*]imidazole.[45]

The red shift of both absorption and fluroescence spectra of 2-MBI, DMBI, and TMBI in high basic solutions is due to dissociation of the imino group, leading to monoanions. These results are consistent with data for similar groups,[30,46] if $\pi \longrightarrow \pi^*$ is the lowest energy transition.

The absorption maxima of all the benzimidazoles are slightly blue-shifted in dilute acidic aqueous solution, indicating the formation of monocations by protonation of the tertiary nitrogen atom. This can be explained on the same lines as for the absorption spectra of the neutral molecules in strong hydrogen-bonding solvents; protonation is the extreme case of hydrogen bonding.

In contrast to the fluorescence spectra of neutral benzimidazoles, two fluorescence bands are observed (except for MPBI) in acidic solutions; one is largely red-shifted and the other is slightly blue-shifted, relative to the neutral molecules. The similarity of the excitation spectra, observed at both fluorescence maxima, to the absorption spectra supports the existence of a multiplicity of excited singlet states of BI, *i.e.* $\pi \longrightarrow \pi^*$ and charge-transfer states. The latter is more stabilised in polar media and in solutes which contain electron-donating groups. Thus the large Stokes-shifted weak and broad fluorescence band is assigned to the charge-transfer transition. Though this band is of lower energy than the $\pi \longrightarrow \pi^*$ band, it has a low quantum yield. The broadness is due to loss of vibrational quantisation in the ground states because nuclear adjustments take place in the charge-transfer state.[21] The main reason for the stabilisation of this transition is the transfer of charge from the homocyclic to the heterocyclic ring when protonation has occurred at the tertiary nitrogen atom. This is further supported by the following facts. (i) The large Stokes-shifted band of the monocation of DMBI (Table 3) is stabilised in more polar solvents whereas the blue-shifted band is insensitive to solvent polarity. This is consistent with earlier results.[47–49] (ii) The Stokes shift increases when methyl groups are substituted in the homocyclic ring rather than in the heterocyclic ring. This kind of behaviour is observed in substituted quinolines.[50] (iii) The monocation of DMBI has its fluorescence maximum at 348 nm at 77 K, whereas the maximum of the $\pi \longrightarrow \pi^*$ state remains unchanged at 77 K, indicating that solvent relaxation of the charge-transfer state is minimal at low temperature.

A mirror-image relationship exists between the absorption and the short-wavelength fluorescence bands of the monocations of benzimidazoles. This observation and the data of Tables 2 and 3 clearly indicate that this band belongs to the $\pi \longrightarrow \pi^*$ transition. It can be concluded from the foregoing that benzimidazoles (except MPBI) in acidic media exhibit both $\pi \longrightarrow \pi^*$ and charge-transfer transitions, although the latter is the main path of deactivation. The existence of a multiplicity of singlet states has been proposed earlier to explain the fluorescence properties of indole-type molecules.[51–53]

The postulate of Kondo and Kuwano [15] concerning the dependence of the reversal of L_a and L_b states on the energy difference between these states could apply to the molecules studied by these workers, but cannot be generalised to other substituted derivatives of benzimidazole. For example, the energy difference between the L_a (shorter-axis polarised, at *ca.*

240 nm) and L_b (longer-axis polarised, at *ca.* 277 nm) states of the monocations of 1MBI, 2MBI, and EMBI are nearly the same as in the neutral molecules, but still both fluorescence bands are observed for the cations of these molecules. On the other hand the L_a and L_b states of the monocations of DMBI and TMBI are nearly degenerate, but a very weak fluorescence is observed at long wavelength. Further, similar excitation spectra are observed for the monocations of all the molecules when recorded at shorter and longer fluorescence wavelengths. From these results it can be concluded that multiple electron states ($\pi \longrightarrow \pi^*$ and CT) of nearly similar energy exist for the monocations of methyl-substituted benzimidazoles. The stability of the states depends upon the nature and position of substitution as well as on the nature of solvents, and fluorescence is observed from both states. MPBI, in contrast to other benzimidazoles, undergoes only a $\pi \longrightarrow \pi^*$ transition in its neutral as well as in the monocation form. The fluorescence quantum yield is not very sensitive to the polarity of the solvent for MPBI, suggesting that there is no change in the mechanism on excitation. The positive charge of the singly protonated MPBI is distributed over a large area by virtue of the conjugation in the S_1 state, thus confirming that the energies of the two states depend upon the nature and position of the substituent.

The change at H_0 −10 in the fluorescence maxima could be attributed to the presence of the dication, formed by protonation of the carbon centre of the homocyclic ring, as in aromatic compounds.[54]

The results in Table 4 indicate that substituents on position 2 have a considerable effect on the protonation constant of BI. A methyl group at position 2 enhances the basicity of the BI, because of its electron-releasing nature and its proximity to the protonation site. A phenyl group at position 2 increases the acidity of BI, consistent with the view that the phenyl group is electron-withdrawing. The effect of substituents on the homocyclic ring on the protonation constant of BI is almost negligible. Fluorimetric titrations give pK_a^* values for the protonation of the tertiary nitrogen atom which are similar to the ground-state values, indicating that this equilibrium is not established in the S_1 state. This is expected because of the short lifetimes of the species involved in the equilibria.[17]

In contrast to the protonation constant behaviour, a methyl group at position 2 enhances the basicity of the NH group of benzimidazoles to a slight extent only. Deprotonation constants in the S_1 state indicate that the NH group becomes more acidic upon excitation; this is consistent with the behaviour of other heterocyclic molecules.[21–23,44–46,50]

The Förster cycle method cannot be applied to calculate pK_a^* values for the monocation–neutral species equilibrium from data for the long-wavelength fluorescence band, as the electronic transitions involved in the two species are different, *i.e.* charge-transfer in the former and $\pi \longrightarrow \pi^*$ in the latter. On the other hand application of the Förster method to the absorption and the short-wavelength fluorescence bands (Table 4) indicates that the tertiary nitrogen atom becomes slightly less basic on excitation to the S_1 state; this is opposite to the normal trend, but similar to that observed in phenanthro[9,10-*d*]-imidazole.[45] This behaviour can be explained on the same lines as suggested earlier. However, the deprotonation constant can be calculated by the method of Förster; the data of Table 4 show that the imino group becomes more acidic upon excitation to the S_1 state. Similar results have been obtained for imidazoles,[46] pyrazoles,[30] and indazoles.[51] The difference between pK_a^* values obtained from absorption and fluorescence data could be due to differences in solvent relaxation of these species in the two states.

Tustsumi *et al.*[40] suggested that proton-induced fluorescence quenching arises from migration of the electrons from the lone pair to any one carbon centre of the ring, rather than complete delocalisation over the whole ring. The values of the quenching constants for the benzimidazole monocations are rather low in comparison with those of the neutral amines. This is expected, since protonation has already occurred on the tertiary nitrogen atom. In the cations of 2PBI and MPBI no quenching is observed; this may be due to charge migration over a larger area instead of migration to a particular carbon atom. The value of the quenching constant increases with the availability of more electron-donating groups. In solutions with H_0 more negative than −6, complete quenching of the fluorescence is observed at 298 K. However, at 77 K no fluorescence quenching is observed under the similar conditions. This suggests that the quenching of the monocations in acid solutions is purely dynamic.

Conclusions

(i) The excited singlet states of benzimidazoles are not well separated. The nature of the emitting state depends upon the nature of the substituents and on the environment. For example, in neutral benzimidazoles, the emission corresponds to the $\pi \longrightarrow \pi^*$ transition, whereas in case of the monocations emission is observed from both $\pi \longrightarrow \pi^*$ (shorter wavelength) and charge-transfer (longer wavelength) states. (ii) Methyl substitution on the homocyclic ring lowers the energy of the charge-transfer state of the monocations more than substitution on the heterocyclic ring. The driving force behind charge transfer from the carbocyclic ring to the heterocyclic ring is the positive charge on the tertiary nitrogen atom. (iii) Methyl substitution at position 2 increases the electron density at position 3 more than substitution at position 1. A methyl group on the homocyclic ring has a negligible effect on protonation and deprotonation constants. (iv) Proton-induced fluorescence quenching of the monocations is observed for all the benzimidazoles, except the 2-phenyl derivative; the explanation follows that offered by Shizuka *et al.*[40,41]

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