Photochemical Reaction of Harmaline. Part 1. Electronic Spectra

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Electronic spectra (absorption, excitation and fluorescence spectra) of harmaline in organic solvents have been recorded. The unusual behaviour of this alkaloid has been studied by excited state proton transfer from organic solvents. The effect of the presence of water in the organic media on the electronic spectra and on the prototropic equilibria in the ground and excited singlet states are discussed briefly. Harmine was selected to be used as a model compound.

As part of our study of the photochemical reactions of carbazole[1-3] and azacarbazoles (β-carbolines)[4,5] in organic solutions, we decided to examine the photochemical behaviour of a dihydro β-carboline, namely, harmaline (3,4-dihydro-7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole) in organic solvents. Harmaline is a naturally occurring alkaloid of the harmala series.[6-9] This substance, being hallucinogenic,[10] presents an extensive pharmacological activity since it inhibits the monoamine oxidase enzyme,[11] and is present in about 26 plant families, cells, animal tissues, human urine[12] and human lenses.[13] The mechanism by which the β-carbolines are formed constitutes an important area in the study of the chemistry of vision.[13] Thus a knowledge of the chemical and physical properties of these molecules in the ground and electronic excited states is very important. It is well-known that β-carbolines are markedly fluorescent[14] and they have been suggested for the fluorimetric measurement of small changes of acidity in the physiological range[15] and as fluorescence standards.[16] Like full aromatic β-carbolines,[17] dihydro β-carbolines are phototoxic[18-20] and they have been reported to produce singlet oxygen ($^1O_2$) and superoxide ($O_2^{-\bullet}$) with varying efficiency.[21] It has been demonstrated that they are also photoactive, *i.e.* capable of absorbing sunlight energy to increase their toxicity towards living organisms.[22]

The acid–base behaviour of harmaline in aqueous solution, in the ground (S_0) and in the electronic excited state (S_1 and T_1)[23-26] and the fluorescence lifetime[27] in ethanol–water mixtures has been investigated. The photoluminescence of harmaline in organic solvents (1,4-dioxane, acetonitrile and methanol) has been reported recently.[28]

As suggested by Dogra[28] and Wolfbeis,[29] there exists a controversy over the fluorescence emission of harmaline. Dogra[28] has shown that the fluorescence maximum of neutral harmaline is at 445 nm, whereas Tomas Vert[25] has reported the same to be at 377 nm and Camacho[27] at *ca.* 380 nm.* In previous work, Hadley[14] described that the fluorescence of aqueous harmaline originates at 401 nm with a shoulder or peak at 425 nm and a maximum at 470 nm and that apparently there was no change in those spectral features in either acid or base.

We initiated our study by re-examining the electronic spectroscopic properties of the substrates in organic solutions. In view of the paucity of data, it was of interest firstly to examine the electronic spectra (absorption, fluorescence excitation and emission) of harmaline in different organic solvents, exciting at different wavelength values to solve the above-mentioned discrepancy. Surprisingly, we observed neither a typical fluorescence for neutral harmaline species nor its corresponding excitation fluorescence spectra. The study was extended to provide an example of a full aromatic β-carboline, namely, harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), and to compare its behaviour with the unusual one observed for harmaline.

* In ref. 27 the total fluorescence spectrum of harmaline in a basic solution of ethanol in which neutral, cationic and zwitterionic species fluoresce, is shown. Only the λ_{max}^{fl} of the cationic species was assigned (490 nm).

Experimental

Harmaline and harmine were purchased from Fluka and Aldrich as free bases. The purity of the alkaloids was controlled by TLC, m.p. and MS. The corresponding hydrochlorides were prepared by bubbling gaseous HCl into an ethanolic solution of the alkaloid. The solutions of these compounds (1.0 mg, 100 cm^{-3}) were prepared in organic solvents in the dark and used immediately to avoid photoreactions.

Organic solvents (Mallinckrodt A.R.: hexane, acetonitrile, carbon tetrachloride, chloroform, dichloromethane, propan-2-ol and *tert*-butanol; E. Merck uvasol grade: methanol and ethanol; Carlo Erba A.R.: ethylene glycol) were purified as described in the literature,[30] while ethanol was also passed through a chromatography column filled with basic aluminium oxide (M. Woelm–Eschwege, activity I) just before use. Analytical grade sulfuric acid (E. Merck) and potassium hydroxide (E. Merck) were used to prepare solutions of various acidities.

Absorption spectra were recorded with stoppered quartz cells (quartz Suprasil Hellma) of length 1 cm in a Hewlett-Packard HP 8451A diode array spectrophotometer (25–28 °C) with the reference cell containing the solvent and using a filter wheel (HP 08451-60302, position 2).

The fluorescence and phosphorescence measurements were performed on a Perkin-Elmer LS 5 spectrofluorometer whose output is automatically corrected for instrumental response by means of a Rhodamine B quantum counter and equipped with a Hamamatsu R928 photomultiplier tube. The excitation spectra were performed on the same spectrometer. The fluorescence emission and excitation spectra of the solvent blanks were run in each case, to check that they showed negligible emission over the wavelength range monitored for emission and excitation experiments. The measurements at room temperature were recorded with stoppered quartz cells of 1 cm using the 90° mode. The spectra at 77 K were recorded in transparent matrices produced by freezing the ethanol solution contained in a round cell (2 mm pathlength) with liquid nitrogen. Using the Front Surface Accessory (FSA), measurements of the intensity of the fluorescence from powdered alkaloid samples and their hydrochlorides were made. The

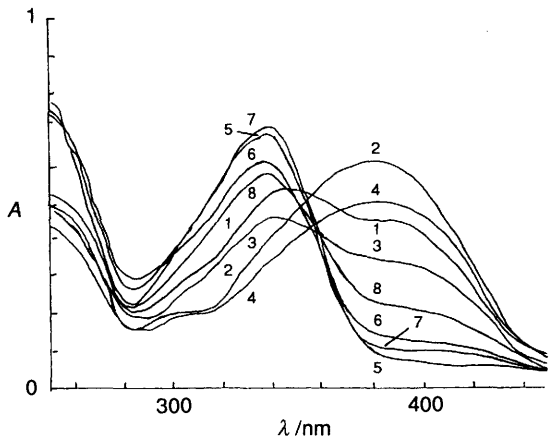


Fig. 1 Effect of water on the absorption spectra of harmaline in organic solvents: 1, methanol; 2, methanol + water; 3, ethanol; 4, ethanol + water; 5, *tert*-butanol; 6, *tert*-butanol + water; 7, propan-2-ol; 8, propan-2-ol + water

powdered samples were previously kept in a dried vacuum atmosphere for at least 24 h. FSA was also used to measure fluorescence from the adsorbed phase. Neutral alkaloids are adsorbed well by silica gel surfaces (TLC aluminium sheet silica gel 60, layer thickness 0.2 mm). These surfaces were treated as follows: the sheets were eluted with methanol, then irradiated with a 366 nm Hg lamp for 10 min, and were finally kept at 110 °C for 10 min. The alkaloids were placed on the sheet and the chromatograms were eluted as usual (ethyl acetate–ethanol). The dried strip of the sheet showing only one fluorescent spot was placed on the FSA and the fluorescence spectra were recorded.

Results and Discussion

Absorption Spectra.—The absorption spectra of harmaline in different solvents were recorded (Table 1). The absorption spectra of cationic harmaline in each organic medium was obtained by the addition of the appropriate quantity of sulfuric acid. In non-polar as well as in polar aprotic solvents, harmaline exists in the neutral form in the S_0 state. In polar protic solvents, like alcohols and water, the intensity and the wavelength value of the absorption maximum of the 0–0 band of harmaline depends dramatically on the acid ionization constant of the protic solvent. The measurements of the acidity of alcohols made by Long and Ballinger[31,32] and by Hine and Hine[33] using a conductivity method, show that the relative acidities of methanol, water, ethanol, *tert*-butanol and propan-2-ol are found to be in the ratio 4.0:1.2:0.95:0.2:0.076. Besides, the α[34] scale of solvents [hydrogen bond donor (HBD) acidities for organic solvents] gives the following values: acetonitrile 0.19, dichloromethane 0.30, chloroform 0.44, *tert*-butanol 0.68, propan-2-ol 0.76, ethanol 0.83, ethylene glycol 0.90, methanol 0.93, water 1.17. As shown in Table 1, in *tert*-butanol (λ_{max} = 334 nm) and in propan-2-ol solution (λ_{max} = 336 nm) harmaline exists in the neutral form in the S_0 state and in more acidic solvents like methanol (λ_{max} = 338 and 380 nm), ethanol (λ_{max} = 336 and 380 nm), ethylene glycol (λ_{max} = 336 and 378 nm), chloroform, methanol–water and ethanol–water (Fig. 1), harmaline exists in both neutral and cationic forms in the S_0 state. In aqueous solution, the neutral harmaline cationic form is the predominant species in the S_0 state. In conclusion, formation of a strongly hydrogen bonded complex or a cationic species is favoured in the presence of acidic alcohols and, as can be seen from Fig. 1, the presence of water in the solution modifies the absorption spectra of harmaline in propan-2-ol, as well as in ethanol and methanol solution because water behaves as an acidic solvent in the presence of harmaline. This behaviour is due to the high basicity of harmaline [pK_a (S_0) 9.6[28] and 10.0[25]]. By virtue of the unsaturation in the piperidine centre, harmaline is more basic than the parent alkaloid, harmine [pK_a (S_0) 8.0[35]]. The absorption data of this full aromatic β-carboline (Table 2), clearly indicate that harmine does not give cationic species in the ground state (S_0) in methanol, ethanol and alcohol–water media. The anomalous behaviour shown by harmaline in *tert*-butanol solution when water was added (Fig. 1) could be explained by taking into account the Swain–Grunwald mechanism.[36,37] It has been suggested that the rate of dissociation of pre-existing amine–alcohol complexes is primarily determined by the energy required to create a cavity prior to dissociation and that this energy increases with the size of the solvent (*tert*-butanol > propan-2-ol > methanol).[36,37]

The absorption maximum of the neutral harmaline is reported to be at 327–328 nm by Dogra[28] (solvent: 1,4-dioxane and acetonitrile) and at 330 nm by Tomas Vert[25] (solvent: aqueous solution pH 12.90). On the other hand, the absorption maximum of harmaline in spectrograde methanol is reported to be at 376 nm,[28] whereas the same absorption band is reported as a broad band between 330–375 nm,[27] in uvasol grade ethanol. Comparing these spectroscopic data with our data in methanol, methanol–water, ethanol and ethanol–water solution (Table 1 and Fig. 1), the presence of water in spectrograde ethanol (max. 5%)[38] or acidic impurities in alcohols could explain the absorption spectra described.[27,28]

Fluorescence Emission Spectra.—The fluorescence emission maxima of harmaline in different organic solvents are listed in Table 1. Comparing the emission spectrum in the organic neutral solvents with that in organic solvents properly acidified (Table 1, *tert*-butanol and *tert*-butanol + sulfuric acid; methanol and methanol + sulfuric acid; ethanol and ethanol + sulfuric acid; acetonitrile and acetonitrile + sulfuric acid), the 459–483 nm band is assigned to the emitting cationic species.

As is already known, harmaline in the S_1 state is more basic than in the S_0 state [pK_a(S_0) 10.0[25] and pK_a(S_1) 19.5[25]]. Thus, in going from non-polar to polar aprotic and polar protic solvents (Table 1, fluorescence emission spectra), the formation of a strongly-emitting hydrogen-bonded complex (N*···HR) and/or the excited cationic species (C*) occurs easily, even with those solvents in which S_0 harmaline is not protonated (Table 1, absorption spectra of harmaline in *tert*-butanol, in propan-2-ol and in dichloromethane).

As can be seen in Table 1 the intensity of the fluorescence maximum, which is proportional to the fluorophore concentration, depends on the protic character of the solvent[33,34] and on the value of the exciting wavelength. The effect of varying the exciting wavelength at which emission measurements were taken was checked in several media. For example, the absorption spectrum of harmaline in *tert*-butanol solution did not show the presence of the cationic species; meanwhile, we only observed the fluorescence band of the corresponding cationic species exciting at 270, 330 and 390 nm.

Our assignment of the cationic harmaline emission bands seems to be correct because the addition of acid to harmaline in *tert*-butanol solution leads to the formation of a species whose spectral characteristics (absorption and emission spectra) resemble those described by Tomas Vert,[25] Pardo[27] and by Dogra[23] in acidic aqueous solution. However, we were not able to observe, at any exciting wavelength, the 442, 445 or 446 nm fluorescence band of the emitting neutral species from neutral harmaline solutions (see Table 1) as has been reported by Dogra.[28] The 377–380 nm fluorescence band described by other authors[25,27] will be discussed later.

Protonation of neutral species of harmaline in the excited

Table 1 Electronic absorption and fluorescence emission and excitation spectral data for harmaline ($A = \varepsilon lc$, A, absorbance, ε in $dm^3\ mol^{-1}\ cm^{-1}$; λ in nm; RI, relative intensities which reflect the relative quantum yields; c, 4.67×10^{-5} mol dm^{-3})

	Absorption data			Emission data			Excitation data		
Solvent	λ_{max}	A[a]	log ε	λ_{exc}	λ_{max}	RI	λ_{emm}	λ_{max}	RI
MeOH	338	0.795		260	480	155	390	—	—
	380	0.413		300	480	19	480	260	150
				330	484	97		390	655
				390	480	665			
MeOH–AE[b]	338	0.824							
	380	0.422							
MeOH + SA[c]	380	1.268	4.43	260	480	500	390	—	—
				300	480	90	480	258	520
				330	480	450		358(sh)	690
								393	830
EtOH	336	0.812		260	479	23	390	—	—
	380	0.276		300	480	15	480	260	180
				330	480	65		389	640
				390	479	635			
EtOH–AE	336	0.782							
	380	0.290							
EtOH + SA	336	0.416		260	479	100	390	—	—
	380	1.171		300	477	76	480	260	64
				330	481	518		369(sh)	860
				390	480	650		392	1060
Pr^{i}OH	336	0.962	4.26	280	478	2	390	—	—
	382	0.002		300	—	—	480	257	160
				330	480	6		263	160
				390	482	96		390	800
Pr^{i}OH–AE	336	0.970							
	382	0.002							
Pr^{i}OH + SA	336	0.412		280	479	85	390	—	—
	380	1.092		300	471	72	480	257	480
				330	475	795		262	480
								360(sh)	860
								391	1060
Bu^{t}OH	334		4.16	270	480	2	390	—	—
				300			480	260	4
				330	480	7		390	28
				390	480	26			
Bu^{t}OH–AE	334	0.669							
	380	0.005							
Bu^{t}OH + SA	378		4.28	270	475	270	390	—	—
				300	475	60	480	260	430
				330	474	440		360(sh)	700
				390	475	670		390	890
EG[d]	336	0.648		280	476	93	390	—	—
	378	0.123		300	476	78	480	258	550
				330	481	420		360(sh)	820
				390	481	1000		390	1000
							520	260	300
								360(sh)	500
								390	620
EG + SA	378	0.813							
H_2O	258	0.380	3.91	300	473	76	380	314	3
	372	1.039	4.35	330	483	370	420	245	112
								316	60
							480	356	500
								391	600
CH_3CN	328	0.974	4.27	280	480	5	390	—	—
	380	0.003		300	480	1	480	262	28
				330	481	15		385	240
				390	481	220			
CH_3CN–AE	328	0.957							
	380	0.002							
CH_3CN + SA	378		4.39	280	477	75	390	—	—
				300	474	72	480	255	290
				330	477	450		262	300
								356(sh)	700
								390	850

Table 1 (*continued*)

Solvent	Absorption data			Emission data			Excitation data		
	λ_{max}	A[a]	log ε	λ_{exc}	λ_{max}	RI	λ_{emm}	λ_{max}	RI
CH_2Cl_2	330	0.675	4.17	280	475	1	390	—	—
	380	0.003		300	—	—			
				330	475	4			
				390	473	38			
CH_2Cl_2–AE	330	0.677							
	380	0.002							
CH_2Cl_2 + SA	380	0.892		280	474	8	390	—	—
				300	473	5	480	260	54
				330	481	30		399	160
$CHCl_3$	330	0.427		280	450	6	380	—	—
	382(sh)	0.235		300	450	5	480	260	24
				330	450	25		390	138
				390	450	176	520	258	30
								390	57

[a] Absorbances (*A*) are indicated when both the neutral and the cationic harmaline absorb. [b] AE, after excitation. The data were obtained after recording of fluorescence emission and excitation spectra on the same solution. [c] SA, with 1% sulfuric acid (0.5 mol dm^{-3}). [d] EG, ethylene glycol.

Table 2 Electronic absorption, fluorescence emission and excitation spectral data for harmine ($A = \varepsilon lc$, *A*, absorbance, ε in $dm^3\ mol^{-1}\ cm^{-1}$; λ in nm; RI, relative intensities which reflect the relative quantum yields; *c*, 4.71×10^{-5} mol dm^{-3})

Solvent	Absorption data		Emission data			Excitation data		
	λ_{max}	log ε	λ_{exc}	λ_{max}	RI	λ_{emm}	λ_{max}	RI
MeOH	300	4.24	260	355(sh)	330	390	362	300
	324	3.80		367	390		300	270
	336	3.73	300	355(sh)	360		322	195
				367	420		334	190
			322	355(sh)	250	440	262	110
				367	300		300	90
			340	368	380		324	68
			390	—	—		334	66
MeOH–AE[a]	300	4.25						
	324	3.82						
	336	3.72						
MeOH + SA[b]	324	4.28	260	420	660	390	240	300
	360(sh)	3.91	300	417	430		260	300
			322	419	630		330	280
						480	240	550
							260	550
							330	520
							360(sh)	510
EtOH	302	4.21	300	355	760	380	252	700
	324	3.76		367	910		300	660
	338	3.86	330	355(sh)	500		325	450
				367	570		338	440
						420	257	128
			340	368	410		300	330
			390	—	—		326	70
							338	50
EtOH + SA	324	4.25	300	415	550	380	242	95
	336(sh)	3.85	330	418	910		258	100
			350	416	730		330	90
						480	242	950
							258	1000
							330	900
							360(sh)	700
Pr^{i}OH	300	4.22	300	353	580	380	251	500
	324	3.91		367	670		300	500
	338	3.86	330	353	325		326	320
				367	390		338	310
						420	252	30
							300	30
							326	20
							336	19

Table 2 (*continued*)

Solvent	Absorption data		Emission data			Excitation data		
	λ_{max}	log ε	λ_{exc}	λ_{max}	RI	λ_{emm}	λ_{max}	RI
Pr^iOH + SA	324	4.29	300	416	520	380	263	82
	360(sh)	3.91	330	415	780		316	92
							326	92
						420	264	700
							332	780
Bu^tOH	300	4.30	300	353	730	380	257	550
	324	3.82		366	830		296	500
	338	3.77	330	353	520		324	410
				366	620		336	400
						420	257	60
							294	57
							325	44
							336	43
Bu^tOH + SA	324	4.36	300	411	620			
	360(sh)	3.97	330	414	820			
CH_3CN	298	4.36	300	350	330	390	298	122
	320(sh)	3.97		362	370		321	129
	332	3.91	340	365	390		334	190
			390	—	—	440	300	140
							320	140
							336	170
CH_2Cl_2	298	4.44	300	348	600	380	252	365
	318(sh)	4.10		360	720		297	330
	332	4.03	324	348	360		318(sh)	220
				360	440		332	200
			410	—	—	430	250	14
							297	13
							318	11
							330	10
$CHCl_3$			300	358	415	390	256	110
			326	359	235		298	130
			332	359	240		320	100
							332(sh)	92
						440	258	15
							325	20
							358(sh)	10
$CHCl_3$ + SA			300	403	225	390	256	330
			326	403	435		323	370
			332	404	415		350(sh)	250
						440	256	230
							326	220

[a] AE, after excitation. The data were obtained after recording of the fluorescence emission and excitation spectra on the same solution. [b] SA, with 1% sulfuric acid (0.5 mol dm^{-3}).

states was described as very rapid by Tomas Vert[25,26] and by Pardo[27] in aqueous alkaline and in aqueous–ethanolic alkaline solution but this behaviour was not described by Dogra[23] either in methanol or in acetonitrile solution.

A comparison of the absorption and fluorescence spectra of harmaline (Table 1) and harmine (Table 2) in methanol and in acidified methanol solution indicates that the electronic excited neutral harmine is less basic than the electronic excited neutral harmaline and that harmine does not form the emitting electronic excited cationic species either in methanol or in ethanol, dichloromethane, acetonitrile solution or in the corresponding aqueous mixtures. We also observed that the fluorescence bands of neutral and cationic harmine are not sensitive to the variation of the exciting wavelength value and that no fluorescence emission was observed exciting at 390 nm. It is interesting to mention that a different harmine behaviour was described by Tomas Vert.[35] This author stated that in alkaline aqueous solution the low intensity of the neutral emission and strong fluorescence from the cation suggests that protonation of the neutral species in the excited state is very rapid.

Fluorescence Excitation Spectra.—The excitation spectra of neutral harmaline in ethanol solution at λ_{em} = 380 nm is different from that observed at λ_{em} = 420 nm [Fig. 2(*b*), spectra 1 and 2]. The former (λ_{em} = 380 nm; λ_{max} = 240, 285, 300 nm) always gave low relative intensity values and resulted in similar excitation fluorescence spectra and absorption spectra to neutral harmine [Table 2 and Figs. 2(*a*) and 2(*b*)]. The latter (λ_{em} = 420 nm) agreed with both the excitation spectra of the cationic harmaline and with the excitation spectra of neutral harmine [Figs. 2(*a*) and 2(*b*)]. Furthermore, the excitation fluorescence spectrum of harmaline in an acidified ethanol solution observed at λ_{em} = 390 nm [Fig. 2(*b*), spectrum 3] agrees with that of harmine in an acidified ethanol solution [Fig. 2(*b*)]. When one drop of NaOH (10 mol dm^{-3}) was added to the harmaline ethanol solution the neutral harmaline-absorbing species was observed in the absorption spectrum [Fig. 2(*a*)] and the excitation and emission spectra were both similar to those in neutral ethanol solution. We observed only a modification in the intensity of the band corresponding to the cationic harmaline species

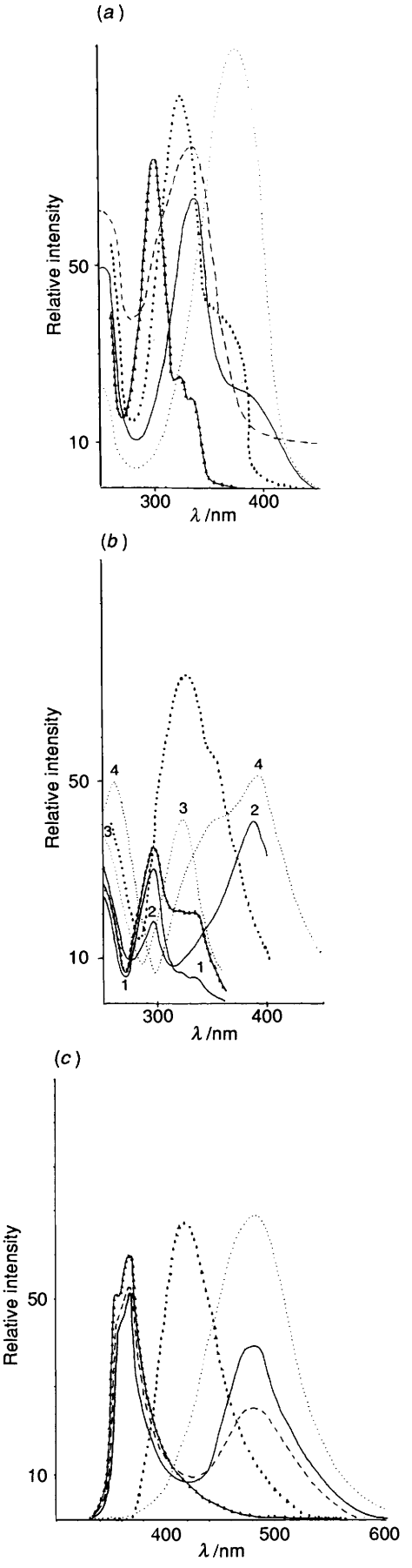


Fig. 2 (*a*) Electronic absorption spectra; (*b*) fluorescence excitation spectra; 1, harmaline (ha) in EtOH (——) at λ_{em} 380 nm; 2, ha in EtOH (——) at λ_{em} 420 nm; 3, ha in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (····) at λ_{em} 390 nm; 4, ha in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (····) at λ_{em} 480 nm; harmine in EtOH (—▲—▲—▲—) at λ_{em} 390 nm; harmine in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{em} 415 nm; (*c*) fluorescence emission spectra, ha in EtOH (——) at λ_{exc} 300 nm; ha in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (····) at λ_{exc} 300 nm; ha in EtOH + 1% NaOH (1 mol dm^{-3}) (– – – –) at λ_{exc} 300 nm; harmine in EtOH (▲—▲—▲) at λ_{exc} 300 nm; and harmine in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{exc} 300 nm.

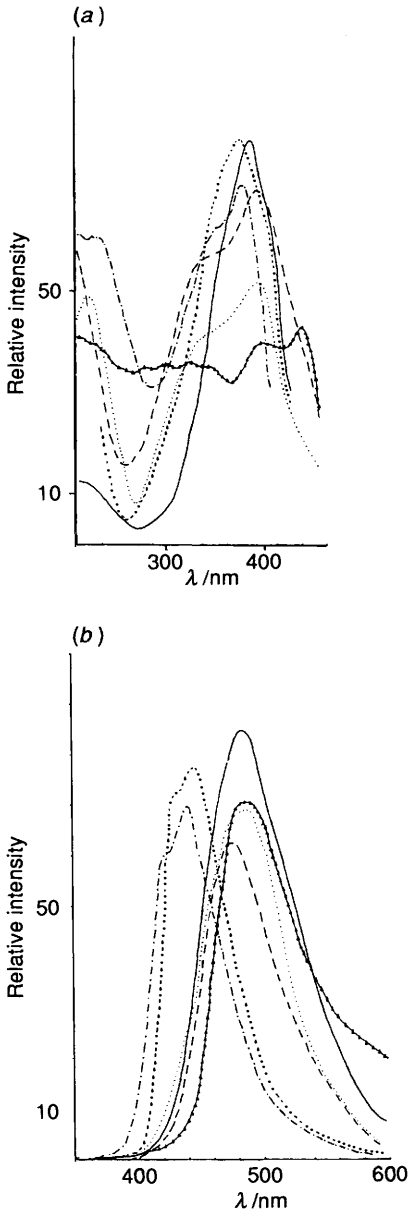


Fig. 3 (*a*) Fluorescence excitation spectra, harmaline (ha) in EtOH (——) at λ_{em} 480 nm; ha in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (····) at λ_{em} 480 nm; ha in EtOH at 77 K (– – –) at λ_{em} 440 nm; ha in the adsorbed phase (— — —) at λ_{em} 440 nm; ha as solid sample (—·—·—) at λ_{em} 440 nm; and ha · HCl as solid sample (—▲—▲—▲—) at λ_{em} 480 nm; (*b*) fluorescence emission spectra of harmaline as for 3(*a*) except λ_{exc} 330, 300, 330, 320, 300 and 300 nm, respectively

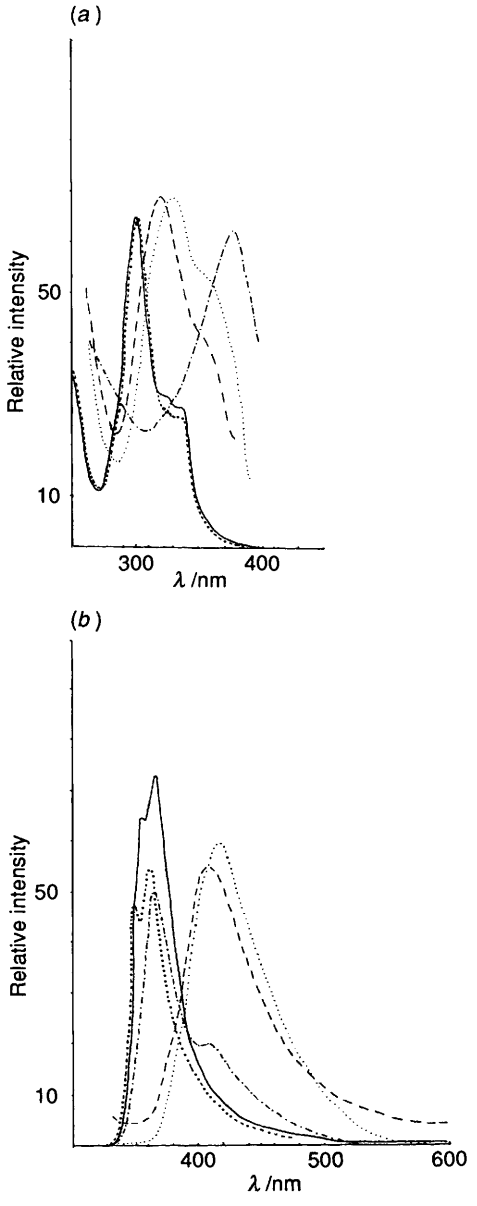


Fig. 4 (*a*) Fluorescence excitation spectra; harmine in EtOH (——) at λ_{em} 420 nm; harmine in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (····) at λ_{em} 415 nm; harmine in EtOH at 77 K (– – – –) at λ_{em} 420 nm; harmine in the adsorbed phase (— — — —) at λ_{em} 410 nm; and harmine as solid sample (—·—·—) at λ_{em} 440 nm; (*b*) fluorescence emission spectra, as for 4(*a*) but with λ_{exc} 300 nm for all samples.

[Figs. 2(*b*) and 2(*c*)]. Finally, when harmine was added to the harmaline ethanol solution, higher intensity at the excitation and emission fluorescence bands was observed at λ_{max} = 300 nm and λ_{max} = 368 nm, respectively.

We also observed that at λ_{em} = 390 nm the excitation spectra of neutral harmaline in *tert*-butanol, propan-2-ol dichloromethane and acetonitrile solution (λ_{max} = 300 nm) showed low relative intensity values and agreed with that of neutral harmine.

The results reported above allow us to conclude that harmaline in its neutral form does not show fluorescence emission and that its electronic excited state readily gives cationic harmaline which shows a high-efficiency fluorescent emission. The emission described in the literature as corresponding to neutral harmaline [Fig. 2(*c*): λ_{max} = 368 nm and lit.,[25–27]] is due to the harmine present as an impurity in commercial harmaline or due to the oxidation of harmaline to harmine. In order to confirm this assignment we have carried out additional experiments in the adsorbed phase.

Table 3 $\Delta pK_a'$[a] values for harmaline in organic solutions at 25 °C

$\Delta pK_a'$[a] for harmaline	Solvent
12.6	MeOH
13.0	EtOH
12.9	Pr^iOH
13.0	Bu^tOH
12.8	EG[b]
14.2	CH_3CN
13.9	CH_2Cl_2
12.8	$CHCl_3$

[a] Ref. 25, ΔpK_a and $\Delta pK_a'$ values in aqueous solution: 9.9 and 13.1, respectively, see text for definition. [b] EG: ethylene glycol.

When commercial harmaline was adsorbed on silica gel plates and eluted with ethyl acetate–ethanol two spots were observed under UV light (λ_{exc} = 366 nm; R_f 0.30 and 0.70). The emission fluorescence spectra obtained from the latter spot agreed with the emission spectra in the adsorbed phase of pure harmine [Fig. 4(*b*)] and that from the former would correspond to that of pure harmaline [Fig. 3(*b*)].

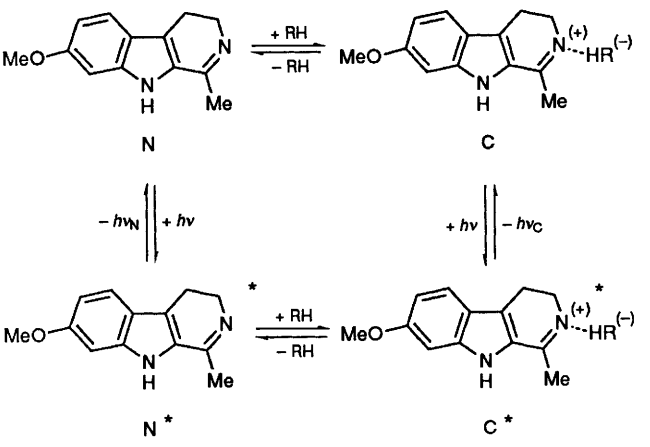
To identify the molecular species which are dominant in the organic solvents used, after we had recorded the absorption, emission and excitation fluorescence spectra of purified harmaline (Table 1) and harmine (Table 2) in the liquid phase at 25 °C we studied the effect of phase rigidity and low temperature on the fluorescence spectra. Thus, we recorded the fluorescence spectra both in ethanol solution at 77 K, at room temperature adsorbed on a dry surface (silica gel Merck), and at room temperature using a solid sample (Figs. 3 and 4).

The fluorescence spectra of harmaline in an ethanol solution at room temperature, in an ethanol solution at 77 K, and in the adsorbed phase and that of the hydrochloride harmaline in the solid state are similar to that recorded in acidified ethanol solution [Fig. 3(*b*)]. The resolution of the emission spectra in ethanol solution is better at 77 K than at room temperature and the former are blue shifted. The modifications of the molecular environment in the solid phase at 77 K relative to that in the ethanol liquid phase are the cause of the differences in the fluorescence spectra as has also been suggested elsewhere.[39] Thus, the rigidity of the vitreous phase retards the free relaxation of the molecular environment. Because the relaxation is restricted, the solvated form at 77 K is more energetic than at room temperature in the liquid phase where the solvent rearrangement is free, and consequently the fluorescence at 77 K should occur at shorter wavelengths. The improved resolution of the fluorescence spectra is due to the rigidity and the low temperature. A similar effect was observed in the fluorescence of solid neutral harmaline [Fig. 3(*b*)].

The spectra were recorded exciting at 300 and 330 nm, where the neutral species fundamentally absorb. In all the examples shown in Fig. 3(*b*) the emission of the cationic and/or the hydrogen-bonded harmaline species can be seen. These results fully support the idea that the excited-state proton transfer along a pre-existing hydrogen bond occurs very rapidly both in the liquid and in the solid state, most probably without activation energy.[40,41] Hydrogen-bonded dimers in the neutral solid state, hydrogen-bonded silica-gel complexes in the adsorbed phase and hydrogen-bonded alcohol complexes in an ethanol medium would be the pre-existing hydrogen-bonded species formed in each medium studied. The excitation spectra obtained agree with the above conclusion [Fig. 3(*a*)].

In contrast with the fluorescence emission of harmaline in ethanol solution, the fluorescence of harmine in ethanol solution at room temperature and at 77 K are different from that in acidified ethanol solution (Fig. 4). In agreement with our previous results (Tables 1 and 2) harmine is less basic than harmaline in both S_0 and S_1 states and the fluorescence emission of the cationic and/or hydrogen bonded exciplex are observed only if the cationic species exist in the ground state and are shown in the absorption and excitation spectra. The acid character of the silica gel plates used as adsorbing surfaces supports the above conclusion.

The high fluorescence emission observed at 480 nm from the neutral harmaline solutions with λ_{exc} = 390 nm (Table 1) suggests that similar emitting species are formed from neutral harmaline in each solution and that a forbidden n,π^* transition in neutral harmaline at 390 nm occurs. This low efficiency $n\pi^*$ transition would populate a more basic electronic excited state which yields the strongly emitting cationic and/or hydrogen-bonded harmaline (Scheme 1). The higher basicity would indicate the preferential localization of the lone pair of the



Scheme 1 Prototropic equilibria of harmaline in organic solvents

nitrogen piperidine atom on itself in this n,π^* state.[42,43] Additionally, an exceptionally high increase in fluorescence emission at 480 nm has been observed on going from λ_{exc} = 270 to 390 nm (Table 1). A higher concentration of the basic excited state would be obtained with longer wavelength values.

As shown in Table 2, no fluorescence emission was observed from harmine when the exciting wavelength was 390 nm and, in addition, the excitation spectra observed are the same from all the fluorescence maxima. Thus, under our experimental conditions the emitting species of harmine all had a common state precursor.

Finally, we can briefly comment on the rather small differences observed in the electronic absorption spectra of the harmaline solution on which the fluorescence emission and excitation spectra had been previously recorded (Table 1, data AE: after excitation). These results would indicate that after the spectral measurements the fluorophores are recovered unchanged.

Prototropic Equilibria.—The acid–base equilibria of harmaline in the S_0 and S_1 states are indicated in Scheme 1. In some organic media studied there is a ground-state equilibrium between neutral (N) and cationic (C) harmaline (Table 1, absorption data and Fig. 1). Also, according to our results, in all organic media studied there is an excited-state equilibrium between excited neutral (N*) and excited cation (C*) (Scheme 1, Table 1 absorption and emission data, Figs. 2 and 3). Thus, we concluded that harmaline in the electronic excited state (N*) is more basic than in the corresponding ground state (N) and that the prototropic process in the S_1 state is of a comparable rate to fluorescence decay, even in acetonitrile, dichloromethane and chloroform solution (Table 1, absorption and emission data).

To evaluate the modification of acidity constants (ΔpK_a) in the organic media studied, we used the Förster cycle method, eqn. (1),[44–46] where $\Delta\bar{\nu}$, which is expressed in reciprocal

$$\Delta pK_a = pK_a(S_1) - pK_a(S_0) = 0.625\,(\Delta\bar{\nu})/T \quad (1)$$

centimeters, is the difference between the energy of the 0–0 electronic transition in the free base (N) and the corresponding energy in the protonated molecule (C). In the present study we calculated ΔpK_a as follows: the energy for the free base was estimated from the least energetic maximum of the absorption spectrum and that for the protonated molecule was estimated by taking the average of the energy corresponding to the least energetic maximum of the absorption spectrum and the energy corresponding to the most energetic maximum of the emission spectrum. This procedure was used in all the examples studied. The $\Delta pK_a'$ values calculated are listed in Table 3 and by

Table 4 Effect of added water on the emission spectra of harmaline in organic solvents (λ in nm, λ_{max}; emission fluorescence wavelength of cation; RI, relative intensities, which reflect the relative quantum yields; RI_C and RI_O: RI of cation emission with and without added water; *c*, 4.67×10^{-5} mol dm^{-3})

Solvent	Added water (%)	λ_{exc}	λ_{max}	RI_C/RI_O
MeOH	—	336	480	1.00
	1.2			1.18
	3.7			1.64
	6.4			1.91
	8.7			2.18
EtOH	—	336	478	1.00
	0.8			1.15
	3.2			1.54
	5.2			1.69
	6.7			2.00
Pr^{i}OH	—	336	480	1.00
	1.4			1.25
	2.6			1.75
	3.6			4.50
	4.9			7.00
	6.2			13.25
Bu^{t}OH	—	336	478	1.00
	1.2			1.13
	2.1			1.20
	3.7			1.27
	7.7			1.67
	10.6			2.66
EG[a]	—	330	470	1.00
	1.0			1.06
	2.3			1.13
	4.9			1.23
	6.2			1.35
	7.4			1.48

[a] EG: ethylene glycol.

comparing with the ΔpK_a and $\Delta pK_a'$ obtained by Tomas Vert[25] (Table 3, see footnote) we can conclude that in polar hydroxylic solvents (R–OH: water,[25] methanol, ethanol, propan-2-ol, *tert*-butanol, ethylene glycol) and in chloroform (organic solvents with $\alpha \geqslant 0.44$[34]) the ΔpK_a values are similar and they are lower than those obtained in polar non-hydroxylic organic solvents (acetonitrile, $\alpha = 0.19$[34] and dichloromethane, $\alpha = 0.38$[34]).

Finally we also studied the effect of the presence of added water on the absorption (Fig. 1) and on the fluorescence emission spectra of harmaline and harmine in alcoholic solution (Table 4; $\lambda_{exc} = 336$ nm; dependence of the RI_C/RI_O ratio on the presence of water added, RI = relative intensity of the fluorescence emission, RI_O = RI of cation emission in the organic media without water added, RI_C = RI of cation emission in the organic media with water added). As shown in Table 4, the fluorescence emission of harmaline is very sensitive to the presence of water. The most modified spectra are those in propan-2-ol solution whereas those in *tert*-butanol solution are modified least. The anomalous behaviour in *tert*-butanol solution could be explained by taking into account the Swain–Grunwald mechanism previously discussed.[36,37] In contrast, no modifications were observed in the absorption and emission spectra of harmine when water was added to the alcoholic solutions.

Conclusions

In conclusion, the results presented here show the electronic spectra (absorption, fluorescence excitation and emission spectra) of harmaline in different organic solvents. Firstly, from these data, the effect of the presence of water in the organic media on the electronic spectra, and on the prototropic equilibria in the ground and excited singlet state (Scheme 1, Fig. 1 and Table 4) have been described showing the importance of the use of anhydrous organic solvents. Secondly, the unusual behaviour of electronically-excited harmaline in organic non-polar and in polar-aprotic solvents has been described for the first time as well as the behaviour of harmaline in the S_0 and S_1 states in alcoholic media. The results obtained have been compared with the electronic spectra of harmine in similar media. Finally, this behaviour has been compared with that previously described in water.[14–29]

Our results lead us to conclude that the Taft and Kamlet α-scale of solvent hydrogen-bond donation ability[34] and the acidity alcohol scale of Ballinger[32] and Hine[33] must be used together with the Swain–Grunwald model[36,37] in order to explain the prototropic equilibria of harmaline in the S_0 and S_1 states observed in organic solvents and in organic solvent–water mixtures.

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