Photochemical Reaction of Harmalol. Part 2. Electronic Spectra†

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Electronic spectra (absorption, emission and excitation fluorescence spectra) of harmalol 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, as well as those owing to the presence of acidic and basic impurities in the organic media on the electronic spectra. Harmol was selected to be used as a model compound. Emission and excitation spectra of both alkaloids in an adsorbed phase and in the solid state are also described.

As part of our study of the photochemical reactions of carbazole[1-3] and azacarbazoles (β-carbolines)[4,5] in organic solution, we decided to examine the photochemical behaviour of dihydro β-carbolines, 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), in organic solvents. These compounds are markedly fluorescent alkaloids, whose physiological and pharmacological properties have been described elsewhere.[6-10] In a previous work we have revised the electronic spectra (absorption, emission and excitation fluorescence and phosphorescence spectra) of harmaline in different organic media (in organic solvents at 25 °C and at 77 K, in dispersed phase and in the solid state) as well as its acid–base behaviour in the first excited singlet state (S_1) in the same media.[10] Harmine (7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole) was selected to be used as a model compound in that study.[10]

The subject of this paper is to investigate the electronic spectra of harmalol in organic solvents (RH) at 25 °C and in frozen solution (at 77 K), in dispersed phase and in the solid state. For the present study we selected harmol (1-methyl-9*H*-pyrido[3,4-*b*]indol-7-ol), a fully aromatic β-carboline, to be used as a model compound.

The acid–base behaviour of harmalol in aqueous solution, in the ground (S_0) and in the electronic excited states (S_1 and T_1),[11,12] the photoluminescence in water,[13] in some organic solvents (EPA,[13] 1,4-dioxane, acetonitrile and methanol),[14] in adsorbed phase (cellulose),[15] and the fluorescence lifetime in ethanol–water mixtures,[16] have been investigated. However, there exists a controversy over the fluorescence of harmalol[14,17] and information about the fluorescence emission of the neutral harmalol is very scanty. It must be noted that Tomas Vert[11] assigned, 'by analogy with neutral harmaline' at λ_{max} = 377 nm, the fluorescence band of neutral harmalol because the authors say that 'no fluorescence of neutral harmalol could be detected in aqueous solutions'. Camacho[16] only shows the total fluorescence spectrum of harmalol in ethanol solution‡ and Dogra[14] has reported the same to be at 444 nm for the neutral harmalol species. It is noteworthy that Olba *et al.*[15] pointed out that it should be noted that they had not obtained any solution where the only emitting species was neutral harmalol as it had been in the case of harmol in methanol solution.[18] Hadley[13] shows that the fluorescence emission of aqueous harmalol at pH 6.40 originates at 376 nm with two peaks of equal intensity at 420 nm and 465 nm.

In view of the paucity of data it was of interest to examine the electronic spectra (absorption, fluorescence excitation and emission) of harmalol in different organic solvents, exciting at different wavelength values to solve the above mentioned discrepancy. Surprisingly, we observed neither a typical fluorescence emission spectrum nor an excitation spectrum of neutral harmalol in organic solutions, at room temperature, at 77 K, in dispersed phase and in solid state. This result agrees with that previously observed for harmaline[10] and with the suggestions of Tomas Vert[11] and Olba.[15] The study was extended to provide an example of a fully aromatic β-carboline, harmol, and to compare its behaviour with the unusual one observed for harmalol.

Finally, some additional electronic spectra of both alkaloids were run to show that the absorption spectra of harmalol are, not only a good tool to detect the presence of water and acidic impurities in organic media, as those of harmaline are,[10] but are also useful to detect the presence of basic impurities in organic solvents.

Experimental

Harmol and harmalol were purchased from Fluka. Harmalol was prepared from its hydrochloride, obtained from Fluka. The aqueous hydrochloride solution was made alkaline with sodium carbonate and cooled. The solid obtained was filtered off and (i) used without further purification (commercial harmalol) and (ii) recrystallized from water. The purity of the alkaloids was determined by TLC, m.p. and MS. 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 were purified as described elsewhere.[10,19-22] Immediately before use, the solvents were (i) dried and then distilled: DD solvent; (ii) distilled and then dried over molecular sieves (4 Å): MSD solvent; or (iii) distilled and then percolated through a chromatography column filled with basic aluminium oxide (M. Woelm-Eschwege, activity I): AD solvent. Anhydrous methanol was obtained by treatment with magnesium methoxide.[19] Analytical grade sulfuric acid (E. Merck) and potassium hydroxide (E. Merck) were used to prepare solutions of various acidities. Water of very low conductivity (purified by MilliQ method) was used.

Absorption spectra were recorded in a Hewlett Packard HP 8451A diode array spectrophotometer (25–28 °C) using a filter wheel (HP 08451-60302, position 2). Corrected excitation fluorescence and phosphorescence spectra were recorded using

† Part 1: see ref. 10.

‡ In ref. 16, the total fluorescence spectrum of neutral harmalol in ethanol solution (λ_{exc} = 310 nm) is shown. Only the λ_{max} of the cationic species was assigned (480 nm).

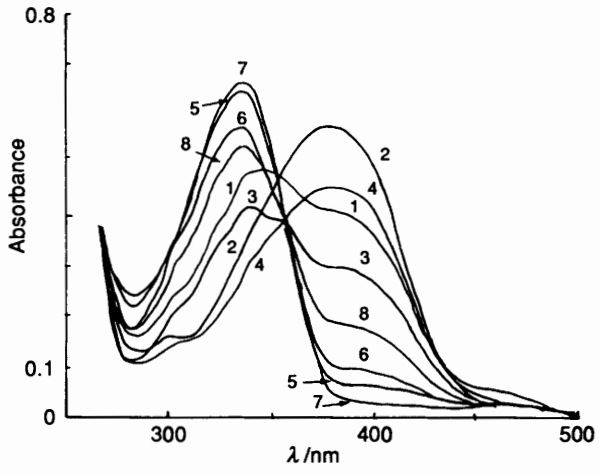


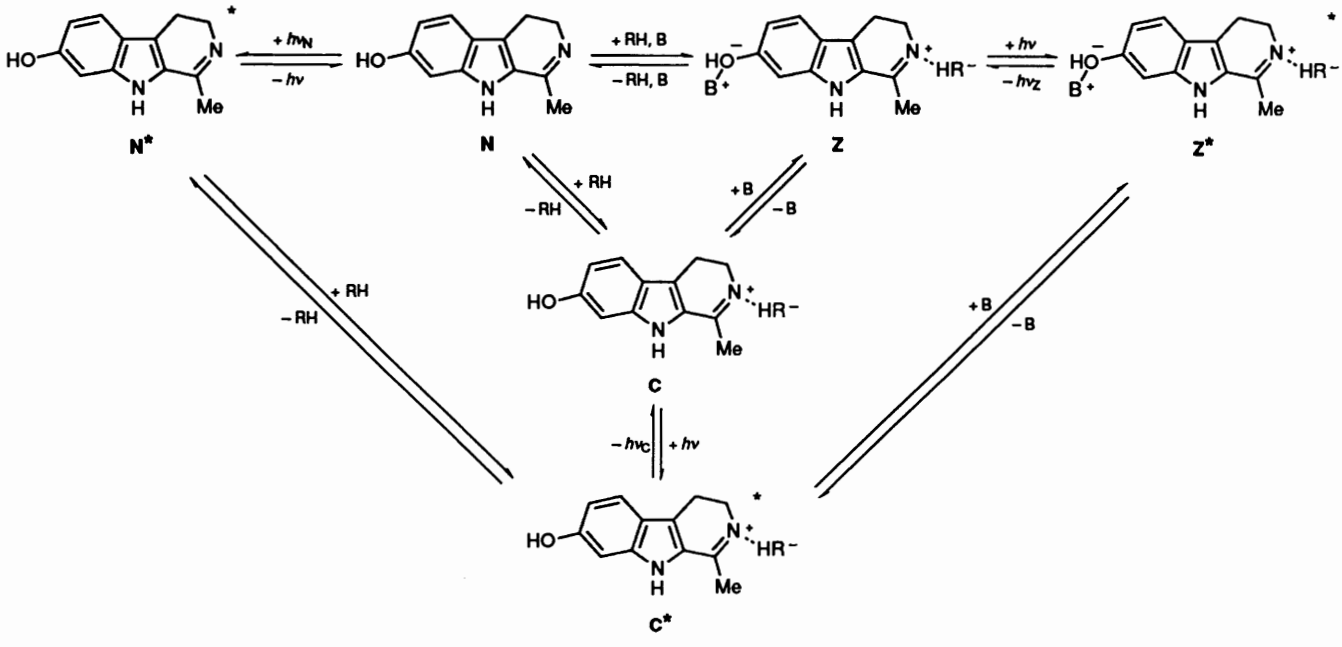
Fig. 1 Effect of added water on the absorption spectra of harmalol in organic solvents: 1, methanol; 2, methanol + water; 3, ethanol; 4, ethanol + water; 5, *tert*-butanol; 6, *tert*-butanol + water; 7, propan-2-ol; 8, propan-2-ol + water.

a Perkin-Elmer LS-5 spectrofluorimeter, whose output is automatically corrected for instrumental response by means of a Rhodamine B quantum counter, with low temperature, phosphorescence, and Front Surface (FSA) accessories according to methods previously described.[10] The spectra at 77 K were recorded in transparent matrices produced by freezing ethanol solution contained in a round cell (2 mm path length) with liquid nitrogen, as described elsewhere.[10] Using the FSA, measurements of the intensity of the fluorescence and phosphorescence from powdered alkaloid samples and their hydrochlorides, as well as from the adsorbed phase (TLC aluminium sheet silica gel 60 and paper Whatman no. 41) were made.

Results and Discussion

Absorption Spectra.—The absorption spectra of harmalol in different organic solvents were recorded (Table 1). The acidified organic solutions (Table 1, RH + SA: organic solvent with 1% 0.5 mol dm^{-3} sulfuric acid) of harmalol show absorption spectra (λ_{max} = 370–384 nm) very similar to those previously described[11,15,16] recorded in aqueous and ethanolic media at pH 1. As shown in Table 1, in non-polar as well as in polar aprotic solvents, harmalol exists predominantly in the neutral form in the S_0 state (acetonitrile λ_{max} = 332 nm and dichloromethane λ_{max} = 330 nm). In polar protic solvents, such as water and alcohols, the absorbance and the wavelength value of the absorption maximum of the 0–0 band of harmalol depend on the hydrogen-bond donor ability of the solvent. The acidities of alcohols and organic solvents have been measured by conductivity[23–25] and by solvatochromic shift (α-scale of solvents)[26,27] methods. As shown in Table 1, in *tert*-butyl alcohol and in propan-2-ol solution harmalol exists predominantly in the neutral form in the S_0 state (λ_{max} = 340 nm), and in more acidic solvents like methanol (λ_{max} = 342 and 382 nm), ethanol (λ_{max} = 342 and 384 nm), chloroform (λ_{max} = 332 and 376 nm), methanol–water and ethanol–water (Fig. 1) harmalol exists in both neutral (N) and cationic (C) forms in the S_0 state (Scheme 1). In conclusion, formation of a strongly hydrogen bonded complex or a cationic species is favoured in the presence of acidic alcohols, as can be seen from Fig. 1; the presence of water in the solution modifies the absorption spectra of harmalol in propan-2-ol as well as in ethanol and methanol solution because water behaves as an acidic solvent in the presence of harmalol. This behaviour is due to the high basicity of harmalol in the S_0 state [pK_a(S_0) 10[11,15] 9.8 and 11.6].[14] The absorption data of the fully aromatic parent β-carboline, harmol (Table 2), clearly indicate that harmol is not basic enough to give the cationic species in the ground state [pK_a (S_0) 8.0][15,28] in alcoholic media (methanol, ethanol, methanol–water and ethanol–water media). By virtue of the unsaturation in the piperidine centre, harmalol is more basic than harmol. The absorption spectra of cationic harmol in each organic medium were obtained by the addition of appropriate amounts of sulfuric acid.

It is interesting to mention that Tomas Vert *et al.*[28] could not see the absorption spectrum of isolated neutral harmol species in aqueous media (pH 1.05–13.00). In order to assign this maxima the spectrum of neutral harmol in tetrahydrofuran solution was recorded.[28] The anomalous behaviour shown by harmalol in *tert*-butyl alcohol solution when water was added (Fig. 1) could be explained by taking into account the Swain–Grunwald mechanism,[29,30] which has been discussed in our



Scheme 1 Prototropic equilibria of harmalol as detected from the ground state (S_0) and the excited state (S_1)

Table 1 Electronic absorption and fluorescence emission and excitation spectral data for harmalol in organic solution at 25 °C ($A = \varepsilon lc$: A, absorbance; ε in $dm^3\ mol^{-1}\ cm^{-1}$; λ in nm: RI, relative intensities: c. 3.93×10^{-5} mol dm^{-3})

	Absorption data			Emission data			Excitation data		
Solvent	λ_{max}	A^a	log ε	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
MeOH	342	0.470		280	482	42	390	—	—
	382	0.365			532	32	430	392	23
				300	467	39	480	260	150
				330	485	110		392	530
				390	483	530			
MeOH – AE[b]	342	0.486							
	382	0.382							
MeOH + SA[c]	382	0.814	4.32	280	480	90	390	—	—
				300	470	50	480	260	400
				330	473	300		360	600
								392	800
EtOH	342	0.531		260	482	135	390	—	—
	384	0.352		300	480	18	480	260	130
				330	479	70		391	530
				390	483	530			
EtOH – AE	342	0.521							
	384	0.352							
EtOH + SA	384	0.764	4.29	260	475	430	390	—	—
				300	469	45	480	260	44
				330	475	315		360(sh)	680
								390	900
Pr^{i}OH	340	0.702	4.25	260	477	14	390	—	—
				300	477	3	480	266	16
				330	477	8		396	100
				390	477	100			
Pr^{i}OH + SA	386	0.846	4.33	260	474	430	390	—	—
				300	472	52	480	263	440
				330	476	360		360(sh)	780
								391	1000
Bu^{t}OH	340	0.644	4.21	280	476	13	390	—	—
				300	470	6	480	265	55
				330	478	20		394	270
				390	478	220			
Bu^{t}OH + SA	384	0.686	4.24	280	478	140	380	—	—
				300	476	50	500	260	400
				340	475	550		360(sh)	600
								390	800
CH_3CN	332	0.603		280	476	26	390	—	—
	380	0.165		300	472	14	480	265	95
				340	473	120		364(sh)	300
				380	470	430		390	440
				450	—	—	560	265	14
								364(sh)	40
								390	65
CH_3CN + SA	378	0.744	4.28	280	475	120	390	—	—
				300	463	73	480	263	220
				340	474	530		360(sh)	600
								392	750
CH_2Cl_2	330	0.368	3.97	260	470	4	390	—	—
				300	—	—	480	260	5
				330	470	3		360(sh)	16
				390	480	20		385	22
CH_2Cl_2 + SA	370	0.419	4.03	260	481	13	390	—	—
				300	464	2	480	259	12
				330	472	7		395	40
				390	481	38			
$CHCl_3$	332	0.127		280	450	2	380	—	—
	376	0.103		300	—	—	450	260	15
				340	449	21		379	48
				380	446	46	550	260	2
				450	—	—		386	5
								430(sh)	1

[a] Absorbances (A) are indicated when both the neutral and the cationic harmalol absorb, square 1 cm path quartz cells were used. [b] AE, after excitation, the data were obtained after recording a fluorescence emission and excitation spectra on the same solution. [c] SA, with 1% sulfuric acid (0.5 mol dm^{-3}).

previous work.[10] The absorption maximum of the neutral harmalol is reported to be at 329–330 nm by Dogra[14] in 1,4-dioxane and in acetonitrile, and at 340 nm in aqueous solution at pH 13.98 by Tomas Vert.[11] On the other hand, the absorption maximum of harmalol in spectrograde methanol is reported to be at 375 nm,[14] whereas the same absorption band

Table 2 Electronic absorption and fluorescence emission and excitation spectral data for harmol in organic solution at 25 °C ($A = \varepsilon lc$; A, absorbance, ε in $dm^3\ mol^{-1}\ cm^{-1}$; λ in nm; RI, relative intensities: c, 5.04×10^{-5} mol dm^{-3})

	Absorption data		Emission data			Excitation data		
Solvent	λ_{max}	log ε[a]	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
MeOH	302	4.27	280	352(sh)	210	380	256	310
	324(sh)	3.87		366	270		300	280
	338	3.77		410(sh)	140		324	230
				456	90		336	210
			300	352(sh)	320	420	256	215
				365	400		304	200
				410(sh)	210		324	180
				456(sh)	140		330	170
			330	352(sh)	240	460	256	160
				365	310		304	140
				410(sh)	180		324	125
				456(sh)	120		336	120
			380	460	12			
MeOH + SA[b]	330	4.17	280	416	105	380	254	64
			300	419	300		330	60
			330	417	700	420	254	730
			380	416	200		332	700
EtOH	304	4.21	260	353(sh)	700	380	255	620
	326(sh)	3.86		367	800		302	580
	338	3.77	300	353(sh)	525		337	370
				366	700			
EtOH + SA	330	4.29	300	419	420	380	257	60
			330	420	750		335	60
						440	258	700
							336	700
Pr^{i}OH	304	4.21	260	352	770	380	254	580
	324(sh)	3.80		365	820		303	550
	338	3.74	340	352	450		323(sh)	420
				365	580		336	370
			300	352	670	420	256	70
				365	900		304	66
			330	352	480		323(sh)	50
				366	600		335	45
						440	254	25
							302	23
							323(sh)	19
							335	18
Pr^{i}OH + SA	332	4.25	300	417	400	380	265	63
			330	417	830		334	65
						420	264	700
							332	700
						440	264	570
							332	600
Bu^{t}OH	304	4.22	300	351	720	380	258	550
	326	3.82		364	900		300	500
	338	3.69	330	351	530		324	400
				364	700		336	350
						420	258	65
							300	57
							326	48
							338	45
Bu^{t}OH + SA	330	4.32	300	414	400	380	262	100
			330	415	750		327	110
						420	258	750
							336	740
CH_3CN	300	4.18	300	348(sh)	530	380	252	350
	322(sh)	3.77		359	660		296	340
	334	3.65	330	349(sh)	335		320	230
				360	410		332	215
						420	256	35
							295	30
							318	25
							330	24
CH_3CN + SA	326	4.30	300	421	400	380	258	50
			330	423	680		336	53
						420	258	650
							332	710
CH_2Cl_2	298	3.74	280	345	260	380	296	260
	320	3.35		356	350		318	140
	334	3.28	300	345	470		330	125
				356	570	420	296	18
			330	356	280		320	11
			370	—	—		332	10

Table 2 (*continued*)

Solvent	Absorption data		Emission data			Excitation data		
	λ_{max}	log ε[a]	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
$CHCl_3$	326	3.60	280	395	7	380	251	65
			300	395	40		322	65
			330	394	70		350(sh)	35
			380	449	4	420	251	53
							322	55
							350(sh)	30
						450	251	20
							322	21
							350(sh)	13
							390(sh)	4
						550	—	—

[a] Square 1 cm path quartz cells were used. [b] SA, with 1% sulfuric acid (0.5 mol dm^{-3}).

is reported as a broad band between 320–410 nm in Uvasol grade ethanol.[16] Comparing these reported spectroscopic data with our data in organic solvents (Table 1) and in organic solvent–water mixtures (Fig. 1) we conclude that the presence of water in spectrograde ethanol (max. 5%)[31] or acidic impurities in alcohols could explain the absorption spectra described.[11,14,16]

Fluorescence Emission Spectra.—The fluorescence emission maxima of harmalol in different organic solvents are listed in Table 1. Comparing the fluorescent spectrum in the neutral organic solvents with that in acidified organic solvents (Table 1, methanol and methanol + sulfuric acid, ethanol and ethanol + sulfuric acid, *tert*-butyl alcohol and *tert*-butyl alcohol + sulfuric acid, and the others), the 470–483 nm band is assigned to the emitting cationic species. Our assignment of the cationic harmalol emission bands seems to be correct because the addition of acid to harmalol solutions, mentioned above, leads to the formation of a species, whose spectral characteristics (absorption and emission spectra) resemble those described by Tomas Vert,[11] by Olba,[15] by Pardo,[16] and by Dogra[12,14] in acidic aqueous solution.

As is known, harmalol in the S_1 state is more basic than in the S_0 state [pK_a (S_0): 10.0[11,15] and pK_a (S_1): 22.6[15]]. Thus, in going from non-polar to polar aprotic solvents 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 organic solvents in which S_0 harmalol is not protonated (Table 1, absorption spectra of harmalol in *tert*-butyl alcohol, in propan-2-ol, in acetonitrile and in dichloromethane).

As can be seen in Table 1 the intensity of the fluorescence maximum depends on the protic character of the solvent and on the exciting wavelength value. The effect of varying the exciting wavelength at which emission measurements were taken was checked in each medium (Table 1). For example, the absorption spectrum of harmalol in propan-2-ol solution [Table 1 and Fig. 2(*a*)] did not show the presence of the cationic species; meanwhile we only observed the fluorescence band of the corresponding cationic species on exciting at 260, 300, 330 and 390 nm.

On the other hand we were not able to observe, at any exciting wavelength value, the 444 nm fluorescence band of the emitting neutral species from neutral harmalol solutions, as has been reported by Dogra.[14] The 352, 368 and 377 nm fluorescence bands described by other authors[11,16] will be discussed later (Fluorescence excitation spectra section).

Protonation of neutral species of harmalol in the excited state was described as very rapid by Tomas Vert[11] and by Pardo[16] in aqueous alkaline and in aqueous–ethanolic alkaline solution, but this behaviour was not described by Dogra in acetonitrile solution.[14] As shown in Table 3, in aqueous harmalol solution the fluorescence emission band corresponding to the excited cationic harmalol is observed.

A comparison of the absorption and fluorescence spectra of harmalol (Table 1) and harmol (Table 2) in methanol, in acidified methanol solution, in ethanol and in acidified ethanol solution indicates that the electronically excited harmol is less basic than the electronically excited harmalol and that the harmol does not form the emitting electronically excited cationic harmol either in methanol or in ethanol, dichloromethane or acetonitrile solution.

We also observed that the fluorescence bands of neutral and cationic harmol are less sensitive to the variation of the exciting wavelength value (Table 2) and that harmol is a more efficient fluorophore species. It is interesting to mention that a different harmol behaviour was described by Pardo.[16] This author stated that in ethanol solution the fluorescence emission spectrum, which was recorded on exciting at a wavelength where the neutral species can only absorb (313 nm), corresponds to both neutral and cationic species, and that he believes there is a proton transfer in the excited state in alcoholic solution. We think that the presence of water and/or acidic impurities in the ethanol solution would explain the results described by this author (see ref. 16, harmol absorption and fluorescence spectra in ethanol solution).

Fluorescence Excitation Spectra.—In our first experience we realized that the excitation spectra of commercial neutral harmalol in propan-2-ol solution at λ_{em} = 380 nm was different from that observed at 440 nm [Fig. 2(*b*)]. The former (λ_{em} = 380 nm, λ_{max} = 303 nm) was similar to the excitation fluorescence spectra and also to the absorption spectrum of neutral harmol [Table 2 and Figs. 2(*a*) and 2(*b*)]. The latter (λ_{em} = 440 nm) agreed with both the excitation spectra of the cationic harmalol (harmalol in propan-2-ol + sulfuric acid) and with the excitation spectra of neutral harmol [Figs. 2(*a*) and 2(*b*)]. Besides, when harmol was added to the harmalol–propan-2-ol solution a higher relative intensity at λ_{max} = 303 nm [Fig. 2(*b*)] and at λ_{max} = 365 nm [Fig. 2(*c*)] was observed in the excitation and emission fluorescence bands respectively.

We also observed that at λ_{em} = 380 nm the excitation spectra of neutral harmalol in methanol, ethanol, *tert*-butyl alcohol, dichloromethane, chloroform and acetonitrile solution (λ_{max} = 300 nm) showed low relative intensity values and agreed with that of neutral harmol.

The results reported above allow us to conclude that harmalol in organic media does not show typical excitation and fluorescence emission spectra owing to its neutral form. Its electronic excited state (N*) readily gives cationic excited

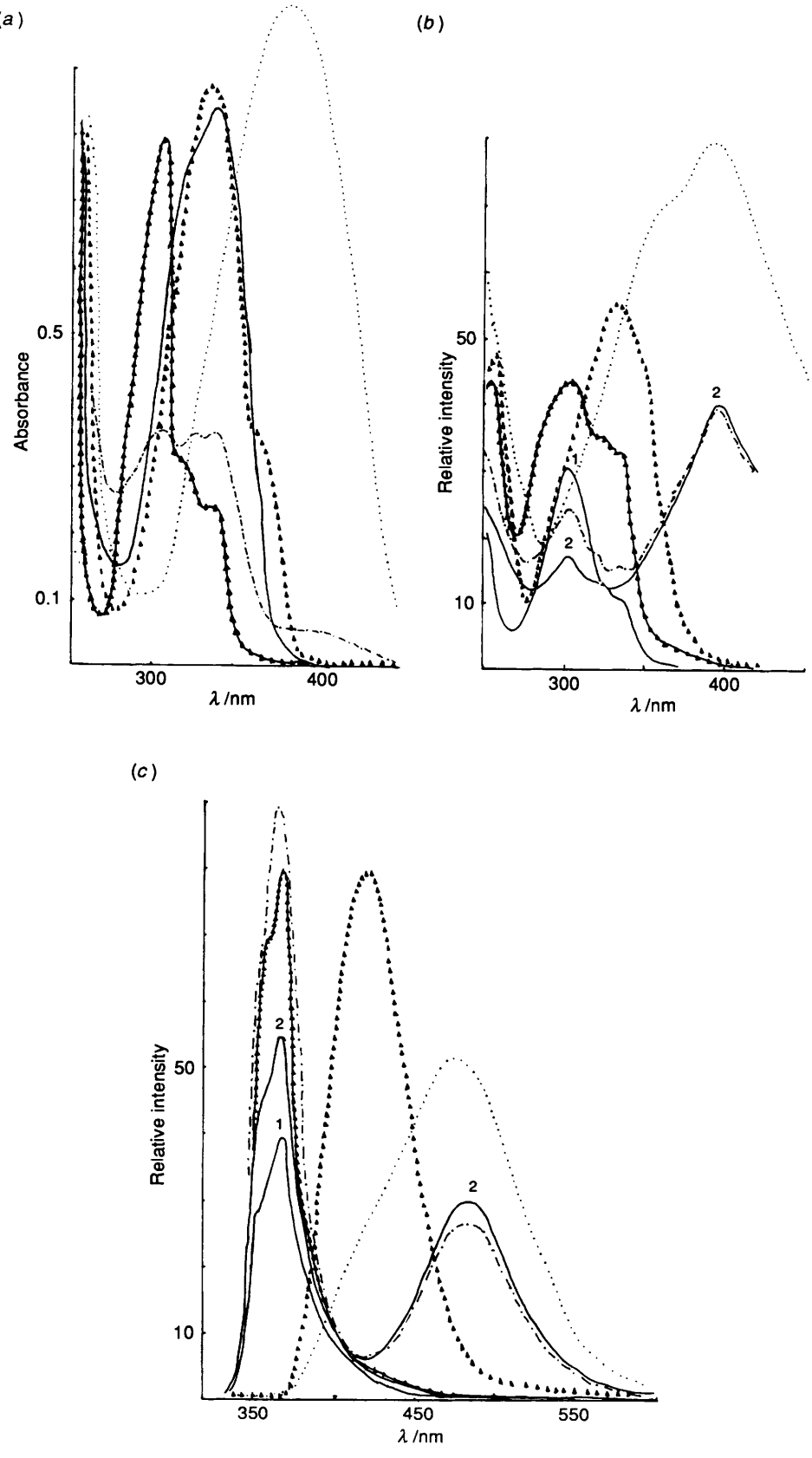


Fig. 2 (*a*) Electronic absorption spectra: (*b*) fluorescence excitation spectra: 1, harmalol (hl) in Pr^iOH (——) at λ_{em} 380 nm; 2, hl in Pr^iOH (——) at λ_{em} 440 nm: hl in Pr^iOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (· · · ·) at λ_{em} 480 nm: harmol (Hl) in Pr^iOH (-▲-▲-▲-▲-) at λ_{em} 440 nm: Hl in Pr^iOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{em} 440 nm; hl + Hl in Pr^iOH (— · — · —) at λ_{em} 440 nm; (*c*) fluorescence emission spectra: 1, hl in Pr^iOH (——) at λ_{exc} 300. 2, hl in Pr^iOH (——) at λ_{exc} 330 nm, hl in Pr^iOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (· · · ·) at λ_{exc} 300 nm: Hl in Pr^iOH (-▲-▲-▲-▲-) at λ_{exc} 330 nm: Hl in Pr^iOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{exc} 300 nm: hl + Hl in Pr^iOH (— · — · —) at λ_{exc} 330 nm.

harmalol (C*, Scheme 1) which shows a highly efficient fluorescent emission (Table 1). The emission described in literature as corresponding to neutral harmalol [Fig. 2(*c*) λ_{max} = 365 nm and ref. 16) would be due to the harmol present as impurity in commercial harmalol or due to the oxidation of harmalol to harmol.

Table 3 Electronic fluorescence and phosphorescence emission and excitation spectral data for harmalol and harmol in ethanol solution (round cell,[a] 298 K), frozen solution (77 K), in adsorbed phase and in solid state (λ in nm: RI, relative intensities: *c*. harmalol: 3.93×10^{-5} mol dm^{-3}, harmol: 5.04×10^{-5} mol dm^{-3})

Method	Temperature	Emission data			Excitation data		
		λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
Harmalol							
Fluorescence							
EtOH solution	298 K	280	478	25	380	—	—
		300	478	10	480	262	130
		340	475	115		391	395
		380	479	330	550	262	38
		470	547	15		391	100
						465	20
	77 K	280	445	75	380	—	—
			484	150	430	370	315
		340	447	330		390	365
			468	325	480	370(sh)	500
		380	447	600		395	680
			468	630		412(sh)	620
		450	489	390		440(sh)	350
					510	370(sh)	260
						392	350
						411	355
						440	300
Absolute EtOH solution	298 K	280	476	10	380	—	—
		300	476	4	480	262	50
		340	476	40		392	165
		380	475	130	550	262	16
		470	548	13		392	40
						470	16
	77 K	280	445	32	380	—	—
			489	57	430	352	100
		340	447	130		368	110
			460(sh)	110		386	115
		380	447	225	450	350(sh)	165
			466	220		370(sh)	220
		400	448	225		389	265
			470	225	480	350(sh)	160
		420	448	110		393	235
			487	180		408(sh)	215
		450	493	160	510	393(sh)	125
		470	497	7		411	135
						450	130
EtOH + SA[b] solution	298 K	280	477	56	380	—	—
		300	473	21	480	260	330
		340	480	325		258(sh)	600
		380	482	770		388	870
		450	480	33	550	260	80
		470	—	—		358(sh)	150
						388	205
	77 K	280	450	25	380	—	—
		300	442	76	430	>950	
			460(sh)	71	480	>950	
		340	>950		510	360(sh)	440
		380	>950			390	620
		450	465(sh)	16			
Adsorbed phase: silica gel	298 K	380	460	150	460	370	140
						392	170
					500	360(sh)	105
						390	130
Paper Whatman no. 41	298 K	280	—	—	450	396	5
		300	—	—	550	396	1
		340	458	3			
		380	458	5			
Powdered hydrochloride	298 K	280	511	355	380	—	—
		300	511	340	480	282	260
		380	510	320		392	260
		440	511	380		438	260
					510	283	360
						393	360
						438	360

Table 3 (*continued*)

Method	Temperature	Emission data			Excitation data		
		λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
Harmol							
Fluorescence							
EtOH solution	298 K	300	352	770	380	302	450
			366	950		326	210
		330	352	300		338	170
			366	370	420	303	100
		380	420	5		326	70
		400	—	—		338	60
						370	15
					480	303	10
						328	10
						370	3
	77 K	280	350	380	400	308	370
			365	430		328	340
			383	210		340	310
		330	365	770	420	310	150
			383	550		330	180
			405(sh)	330		340(sh)	175
		370	404	68		370	50
		400	—	—	480	330	10
						370	4
Phosphorescence	77 K	310	422	195	430	308	205
			434	215		330(sh)	115
			450	235		340	95
		330	423	95	480	310	125
			435	100		330(sh)	90
			453	115		340	80
		370	474	10		375	10
			494(sh)	8			
Fluorescence							
Absolute EtOH solution	298 K	280	351	215	380	302	410
			365	260		325(sh)	200
		300	351	620		338	170
			365	880	420	302	82
		330	351	285		325(sh)	38
			365	350		338	30
		380	462	1	480	302	4.5
		400	470	1.5		325	3
						338	3
						400	1.5
	77 K	280	350	420	380	sat	
			365	460	400	306	350
			382(sh)	210		328	190
		300	sat			340	170
		330	365	410	420	306	105
			383	420		328	55
		380	—	—		338	48
		400	483	2	460	306	11
						328	7
						338	6
						390	1
					480	306	5
						328	3
						340	3
						390	2
Phosphorescence							
	77 K	300	422	95	430	308	110
			434	110		330(sh)	70
			450	120		342	55
		330	422	46			
			434	50			
			450	56			
		370	—	—			
Fluorescence							
EtOH + SA solution	298 K	280	415	79	380	246	170
		300	416	220		330	100
		330	415	930	420	250	sat
		380	416	35		330	950
		400	415	5	480	246	290
						330	180

Table 3 (*continued*)

Method	Temperature	Emission data			Excitation data		
		λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
	77 K	280	384	100	430	242	sat
			404	115		334	900
		300	384	400		365(sh)	350
			402	440	480	334	78
		360	384	630		365(sh)	32
			403	690			
		380	410	70			
		400	—	—			
Phosphorescence							
	77 K	310	474	64	430	—	—
			495(sh)	52	480	252	240
		330	474	130		264	220
			495(sh)	100		336	170
		370	473	35		370(sh)	75
			495(sh)	30			
Fluorescence							
Adsorbed phase: silica gel	298 K	280	408	43	420	244	135
		300	408	74		325	115
		330	413	100		360(sh)	88
		380	414	47	500	325	15
		400	—	—			
Paper Whatman no. 41	298 K	280	406	3	410	250	23
		300	409	7		330	16
		330	410	7	450	250	10
		370	410	16		330	6
Powdered hydrochloride	298 K	280	463	200	380	—	—
		300	468	190	480	393	235
		340	466	180		430(sh)	90
		400	474	230	500	395	200
		440	500	100		430(sh)	110
					550	397	75
						440	64

[a] 2 mm round quartz cells were used. [b] SA, with 1% sulfuric acid (0.5 mol dm^{-3}).

In order to confirm this assignment we have carried out additional experiments in the adsorbed phase. When commercial harmalol 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 0.25 and 0.60). The emission fluorescence spectra obtained from the latter spot (R_f 0.60) agreed with the emission spectra in the adsorbed phase of pure harmol [Fig. 3(*b*)] and that from the former (R_f 0.25) would correspond to that of the pure harmalol in the adsorbed phase [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 harmalol (Table 1) and harmol (Table 2) in the liquid phase at 25 °C, we studied the effect of phase rigidity and low temperature (77 K) on the fluorescence spectra. Thus, we recorded the fluorescence spectra both in ethanol solution at 77 K (Table 3), at room temperature adsorbed on a dry surface (Table 3, silica gel, Merck; paper Whatman no. 41), and at room temperature using solid sample (Fig. 3, powdered alkaloid and Table 3, powdered alkaloid hydrochloride).

The fluorescence spectra of harmalol in ethanol solution at room temperature (Table 1, λ_{exc} = 260–390 nm), in the adsorbed phase [Fig. 3(*b*), silica gel Merck and Table 3, paper Whatman no. 41] and that of the harmalol hydrochloride in the solid state (Table 3) are similar to those recorded in acidified ethanol solution at room temperature (Table 1) and at 77 K (Table 3 and Fig. 4). The spectra were recorded on exciting, among others, at 280, 300 and 340 nm, where the neutral species fundamentally absorbs. In all the examples shown in Fig. 3(*b*) and in Table 3 the emission of the cationic and/or the hydrogen-bonded harmalol 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 adsorbed phase, most probably without activation energy.[32,33] Hydrogen-bonded silica gel and cellulose 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 [Figs. 3(*a*) and 4(*b*) and Tables 1 and 3]. Although harmalol in ethanol solution shows only one emission band, the same ethanol solution at 77 K shows two emission bands [Fig. 4(*c*), Table 3]. By comparing them with the fluorescence spectra of harmalol in acidified ethanol solution at 77 K and with that in AD and in MSD ethanol solution* (Table 4 and Fig. 5), we conclude that these emissions correspond to the cationic form (λ_{max} = 476–480 nm) and the zwitterionic form (λ_{max} = 545–548 nm) respectively. This result could be explained by taking into account that, simultaneously, the ring nitrogen atom is more basic and the hydroxy group is more acidic for harmalol in the excited state. The modifications of the molecular environment in the solid phase at 77 K relative to that in the

* See the definition of AD and MSD in the Experimental section and the characterization of the zwitterion in the following section.

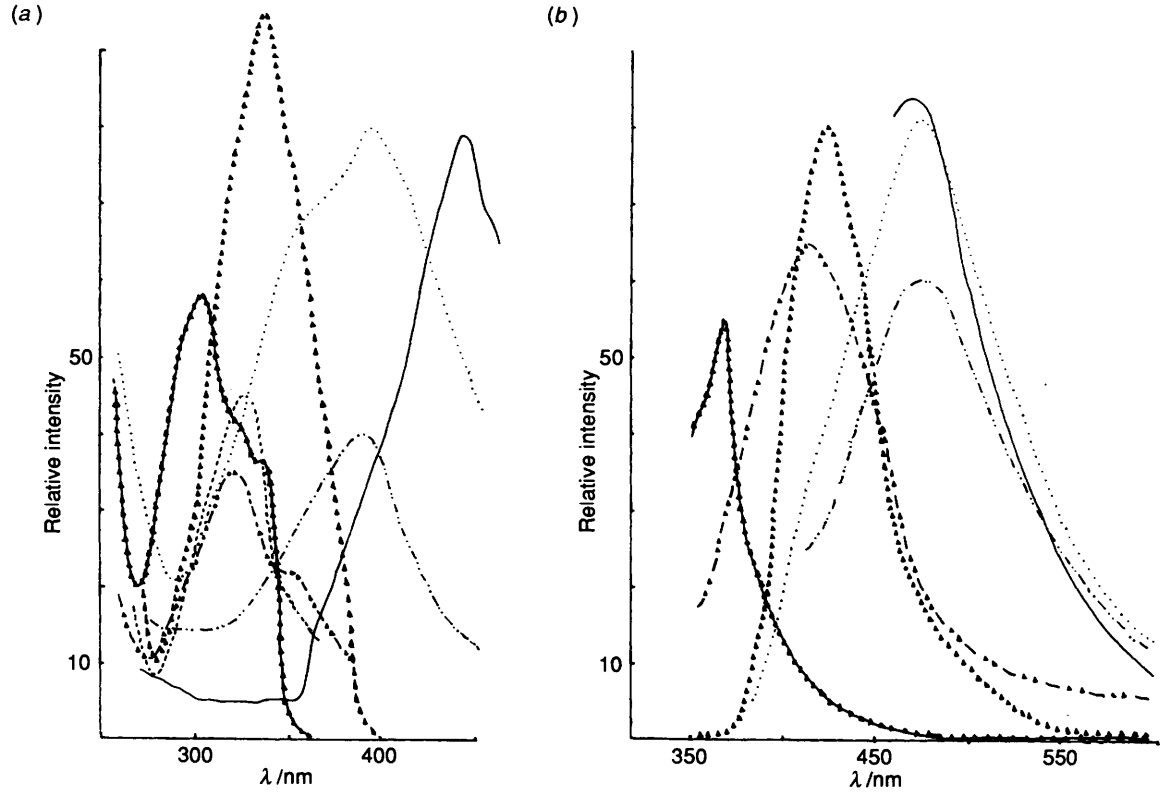


Fig. 3 (*a*) Fluorescence excitation spectra; hl in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (- - - -) at λ_{em} 390 nm; hl in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (· · · ·) at λ_{em} 480 nm; hl in adsorbed phase (silica gel) (- · · · · -) at λ_{em} 460 nm: Hl in EtOH (-▲-▲-▲-▲-▲-) at λ_{em} 380 nm; Hl in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{em} 440 nm: Hl in adsorbed phase (silica gel) (—▲▲—▲▲) at λ_{em} 410 nm: powdered Hl (solid sample) (——) at λ_{em} 500 nm; (*b*) fluorescence emission spectra; hl in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (· · · ·) at λ_{exc} 330 nm: hl in adsorbed phase (silica gel) (— · · — · ·) at λ_{exc} 380 nm: Hl in EtOH (-▲-▲-▲-▲-) at λ_{exc} 330 nm; Hl in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (▲▲▲▲) at λ_{exc} 330 nm; Hl in adsorbed phase (silica gel) (—▲▲—▲▲) at λ_{exc} 330 nm; powdered Hl (solid sample) (——) at λ_{exc} 430 nm

ethanol liquid phase are the cause of the differences observed in the fluorescence spectra. Besides, the resolution of the emission spectra in ethanol solution is better at 77 K than at room temperature and the former are blue shifted, as has also been suggested elsewhere.[10,34]

In contrast to the fluorescence emission of harmalol in ethanol solution, the fluorescence of harmol in ethanol solution at room temperature (Table 2) and at 77 K (Table 3 λ_{exc} = 280, 300 and 340 nm) are different from that in acidified ethanol solution [Fig. 3(*b*) and Table 3]. In agreement with our previous results (Tables 1 and 2) harmol is less basic than harmalol 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 conclusions as well as the fluorescence emission of the harmol hydrochloride in the solid state (Table 3). As shown in Table 3, the phosphorescence emission of harmol in ethanol solution at 77 K is different from that in acidified ethanol solution at 77 K. In agreement with the above mentioned results the phosphorescence at 77 K in ethanol solution corresponds to the neutral harmol species.

Although no fluorescence of harmalol could be detected in the solid state, that of harmol (Fig. 3) clearly shows the absorption maximum of the harmol zwitterionic species at 470 nm according to Wolfbeis[18] and Vert[28] previous assignments for harmol in aqueous solution. The former result could be due to the overlap of cationic harmalol fluorescence band with the harmalol zwitterion absorption band at around 450–470 nm, leading to the fluorescence reabsorption.

Effect of Drying Agents used for Organic Solvents on the Electronic Spectra of Harmalol and Harmol.—In order to remove small amounts of water from organic solvents we used both neutral alumina (AD solvents) as a column through which liquid was percolated and molecular sieves (4 Å) (MSD solvent). When immediately before use, the solvents were first distilled and then dried as it was described above and according to literature[22] instead of dried first and then distilled (DD solvent) some unexpected results were observed. As shown in Table 4, harmalol in AD methanol shows absorption spectrum very similar to that recorded in DD methanol (Table 1) but a new band at λ_{max} = 450 nm is observed [Fig. 5(*a*)]. A similar absorption spectrum was observed using MSD methanol. This absorption maximum at λ_{max} = 450–460 nm has also been detected in AD and in MSD ethanol, in water and in ethylene glycol solution (Table 4). Besides, the fluorescence spectra of harmalol in the above mentioned AD and MSD solvents show, at different λ_{exc} values, an intense lower energy emission band around 540 nm (532–550 nm) [Table 4 and Fig. 5(*c*)]. This emission band is also detected in the fluorescence emission spectra of harmalol in water and in ethylene glycol (Table 4).

By comparing these results with those described by Dogra[14] (methanol solution, pH 10.5–11.4; absorption: λ_{max} = 431 nm; fluorescence: λ_{max} = 522 nm) and Olba[15] (NaOH–ethanol solution, [NaOH] = 0.26 mol dm^{-3}, λ_{max} = 547 nm) the new absorption and fluorescence maxima can be assigned to the zwitterionic harmalol species (Scheme 1, Z and Z*).

We also observed that the excitation spectra of neutral harmalol at λ_{em} = 540 and 550 nm in AD and in MSD ethanol respectively, show a high intensity band at λ_{max} = 468 and 467 nm [Fig. 5(*b*)], together with the band corresponding to the cationic harmalol species (λ_{max} = 390 and 394 nm).

It is noteworthy that no zwitterionic species was observed in the electronic spectra of harmalol in AD and in MSD propan-

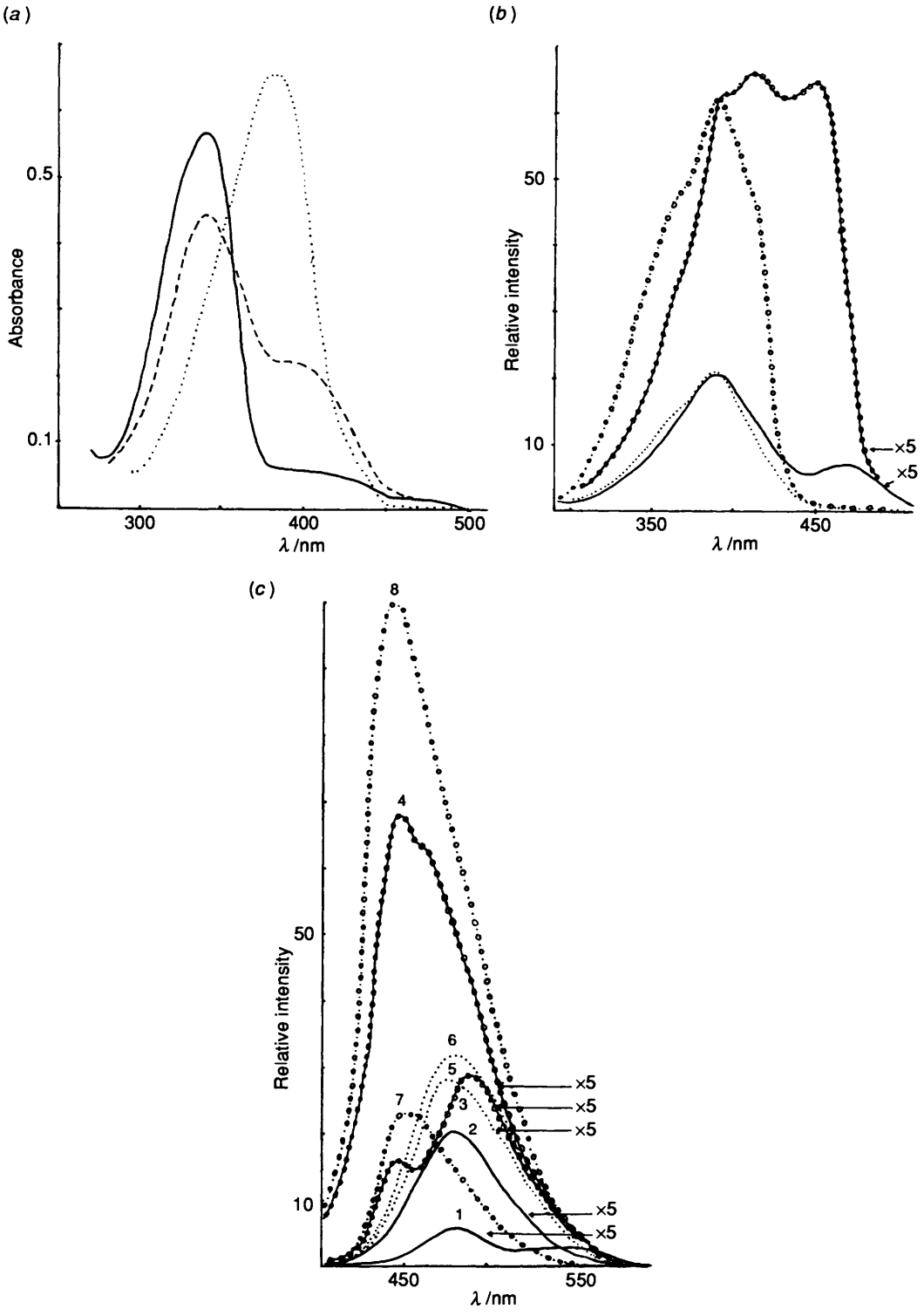


Fig. 4 Harmalol: (*a*) Absorption spectra: in absolute EtOH (——); in EtOH 96% (— — —); in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}) (· · · ·); (*b*) Fluorescence excitation spectra: in absolute EtOH: at 298 K (——) at λ_{em} 550 nm, at 77 K (o o o o) at λ_{em} 510 nm; in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}): at 298 K (· · · ·) at λ_{em} 550 nm, at 77K (· o · o · o ·) at λ_{em} 510 nm; (*c*) Fluorescence emission spectra: in absolute EtOH: 1, at 298 K (——) at λ_{exc} 280 nm; 2, at 298 K (——) at λ_{exc} 340 nm; 3, at 77 K (o o o o) at λ_{exc} 280 nm; 4, at 77 K (o o o o) at λ_{exc} 340 nm; in EtOH + 1% H_2SO_4 (0.5 mol dm^{-3}): 5, at 298 K (· · · ·) at λ_{exc} 280 nm; 6, at 298 K (· · · ·) at λ_{exc} 340 nm; 7, at 77 K (· o · o · o ·) at λ_{exc} 280 nm; 8, at 77 K (· o · o · o ·) at λ_{exc} 340 nm.

2-ol solution, while only the emission fluorescence of this species was observed in AD *tert*-butyl alcohol solution (Table 4).

Concerning the absorption and fluorescence spectra of harmol in AD and in MSD organic solvents there is evidence of the zwitterionic species only in the latter spectrum. The results of these measurements are compiled in Table 5 and show clearly that the absorption fluorescence maximum at λ_{max} = 443–472 nm agrees within a few nanometers with the emission fluorescence band found for zwitterionic harmol in basic aqueous (pH 9.5, λ_{max} = 440 nm)[28] and in basic methanol (pH 10 and 14, λ_{max} = 460 and 465 nm)[18] solutions. It is noteworthy that no fluorescence of zwitterionic harmol could be detected in aqueous solution (Table 5) while in ethylene glycol and in AD, MSD and DD methanol, and in commercial anhydrous methanol it was always observed (Table 5). As

Table 4 Effect of drying agents on the electronic spectra of harmalol in organic solvents at 298 K ($A = \varepsilon lc$; A, absorbance; ε in $dm^3\ mol^{-1}\ cm^{-1}$; λ in nm; RI, relative intensities; c, 3.93×10^{-5} mol dm^{-3})

	Absorption data		Emission data			Excitation data		
Solvent	λ_{max}	A^a	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
AD[b] MeOH	338	0.592	280	470	16	380	—	—
	456	0.091		545	40	480	252	50
			300	462	27		388	160
				535	17	550	390	100
			340	480	36		463	175
				528(sh)	28			
			380	485	130			
				530(sh)	106			
			450	545				
MSD[c] MeOH	342	0.512	280	480	130	380	—	—
	384(sh)	0.331	300	476	72	480	260	500
	456(sh)	0.070	340	476	300		360(sh)	700
			380	464(sh)	520		393	920
				480	600	550	260	115
			450	543	120		360(sh)	170
							390	220
							456(sh)	10
						600	260	21
							360(sh)	30
							392	39
							456(sh)	2
AD EtOH	340	0.501	280	480	10	380	—	—
	400(sh)	0.045		543	13	480	260	100
	450(sh)	0.016	300	476	6		390	500
				540	4	540	260	50
			330	478	18		391	200
			340	479	29		468	150
			380	480	146			
			470	545	60			
MSD EtOH	340	0.489	280	480	3	380	—	—
				550	5	480	260	7
			300	—	—		394	43
			340	480	6	550	260	5
			380	480	33		394	18
				548(sh)	15		467	33
			470	547	32			
AD Bu'OH	340	0.516	280	478	12	380	—	—
				546	6	480	260	50
			300	478	6		391	230
			340	475	45	540	260	20
			380	478	210		392	90
			460	478	8		475	18
				550	20			
MSD Bu'OH	340	0.565	280	476	6	380	—	—
			300	474	3	480	265	20
			340	476	12		393	118
			380	474	92			
			470	—	—			
EG[d]	340	0.418	280	470	20	370	—	—
	450	0.248		540	90	390	—	—
			300	470	44	410	—	—
				540	44	480	244	80
			330	470	49		387	88
				540	49	540	280	90
			390	470(sh)	70		390(sh)	180
				537	190		452	370
			450	541	370			
EG + SA[e]	380	0.805	280	479	156	380	—	—
			300	467	65	480	262	500
			340	475	600		360	770
			460	476	60		391	1000
						540	262	150
							360	250
							390	300

Table 4 (*continued*)

Solvent	Absorption data		Emission data			Excitation data		
	λ_{max}	A^a	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
H_2O	372	0.502	280	531	110	380	—	—
	450(sh)	0.219	300	425(sh)	26	470	258	160
				480(sh)	38		362(sh)	250
				519	44		386	280
			340	429	240	530	258	170
			380	496	330		362(sh)	250
			430	533	360		400	340
							432	360

[a] Absorbances are indicated when more than one species absorb, square 1 cm path quartz cells were used. [b] Solvent distilled and then percolated through a chromatography column filled with basic aluminium oxide. [c] Solvent distilled and then dried over molecular sieves. [d] EG, ethylene glycol. [e] SA, with 1% sulfuric acid (0.5 mol dm^{-3}).

shown in Figs. 1–5 and in Tables 1–5, the absorption and especially the fluorescence emission of harmalol are more sensitive to the presence of water, acidic impurities and basic impurities than those of harmol. Besides, harmalol fluorescence is more sensitive to basic impurities than harmaline is.[10] The presence of the hydroxy group (phenolic group) in the harmalol structure and the interaction of the phenolic proton of the harmalol cation with a basic compound B in the ground and in the S_1 state (Scheme 1) accounts for the results obtained.

Prototropic Equilibria in Organic Solvents.—Some of the acid–base equilibria of harmalol in S_0 and S_1 states are indicated in Scheme 1. In a few organic media there is a ground state equilibrium between neutral (N) and cationic (C) harmalol (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*), emitting only the C* species (Scheme 1, Table 1, absorption and emission data and Figs. 2 and 3). Thus we concluded that harmalol 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 comparable rate with fluorescence decay even in acetonitrile, dichloromethane and chloroform solution (Scheme 1, and Table 1, absorption and emission data).

As has been previously discussed when the alcohols used as solvents were percolated through a basic aluminium oxide chromatographic column or dried over molecular sieves a third absorbing species could be observed in the absorption, excitation and emission fluorescence spectra (Tables 4 and 5, Fig. 5). As is shown in Scheme 1, the presence of traces of a base such as aluminium oxide or molecular sieves (B in Scheme 1) in the alcoholic media would explain this behaviour. The results mentioned above allow us to conclude that the harmalol phenol moiety in the electronically excited state is more acid than in the ground state and that the process to give Z* is of comparable rate with fluorescence decay in AD and MSD alcoholic media (Tables 4 and 5).

The above result also suggests that the formation of zwitterion occurs only by a kind of dissociation of the hydroxy group of harmalol and is not due to tautomerism. The red shift is in good agreement with those reported for the dissociation of the hydroxy group of aromatic hydrocarbons.[35]

To evaluate the modification of acidity constants (ΔpK_a) in the organic media studied, we used the modified [10] Forster cycle method.[36–39]

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

In eqn. (1), $\Delta\bar{\nu}$, which is expressed in reciprocal centimetres, 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 $\Delta pK_a'$ 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 6 and by comparing with the ΔpK_a and $\Delta pK_a'$ for harmalol obtained by Tomas Vert[11] (Table 6, see footnote) we can conclude that in polar hydroxylic solvents (organic solvents with $\alpha > 0.44$)[26,27] the $\Delta pK_{a(NC)}'$ values are lower than those obtained in non-hydroxylic organic solvents (acetonitrile $\alpha = 0.19$, dichloromethane $\alpha = 0.30$ and chloroform $= 0.44$).[26,27] Similar behaviour of $\Delta pK_{a(NC)}'$ of harmaline had been described elsewhere.[10]

Finally we also studied the effect of the presence of added water on the absorption (Fig. 1) and on the fluorescence emission spectra of harmalol and harmol in alcoholic solution (Table 7; $\lambda_{exc} = 330$ 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 added water; RI_c: RI of cation emission in the organic media with added water). As shown in Table 7, harmalol is less sensitive to the presence of water than harmaline is (see ref. 10). The presence of the hydroxy group in the harmalol structure would account for this behaviour. In agreement with results previously obtained with harmaline the most modified spectra are those in propan-2-ol solution (Table 7) whereas those in *tert*-butyl alcohol solution are modified least. This anomalous behaviour could be explained by taking into account the Swain–Grunwald mechanism previously discussed.[29,30] In contrast, slight modifications were observed in the absorption and emission spectra of harmol when water was added to the alcoholic solutions.

These results, and those previously reported,[10] lead us to conclude that the Taft and Kamlet α scale of solvent hydrogen-bond donation ability[26,27] and the acidity alcohol scale of Ballinger[24] and Hine[25] must be used together with the Swain–Grunwald model[29,30] and the donor strength scale (D_s) of Sandström and Persson[39] in order to explain the prototropic equilibria of harmalol in the S_0 and S_1 states observed in organic solvents and in organic solvent–water mixtures.

Conclusions

The results presented here show the electronic spectra (absorption, fluorescence excitation and emission spectra) of harmalol in different organic solvents, in adsorbed phase and in

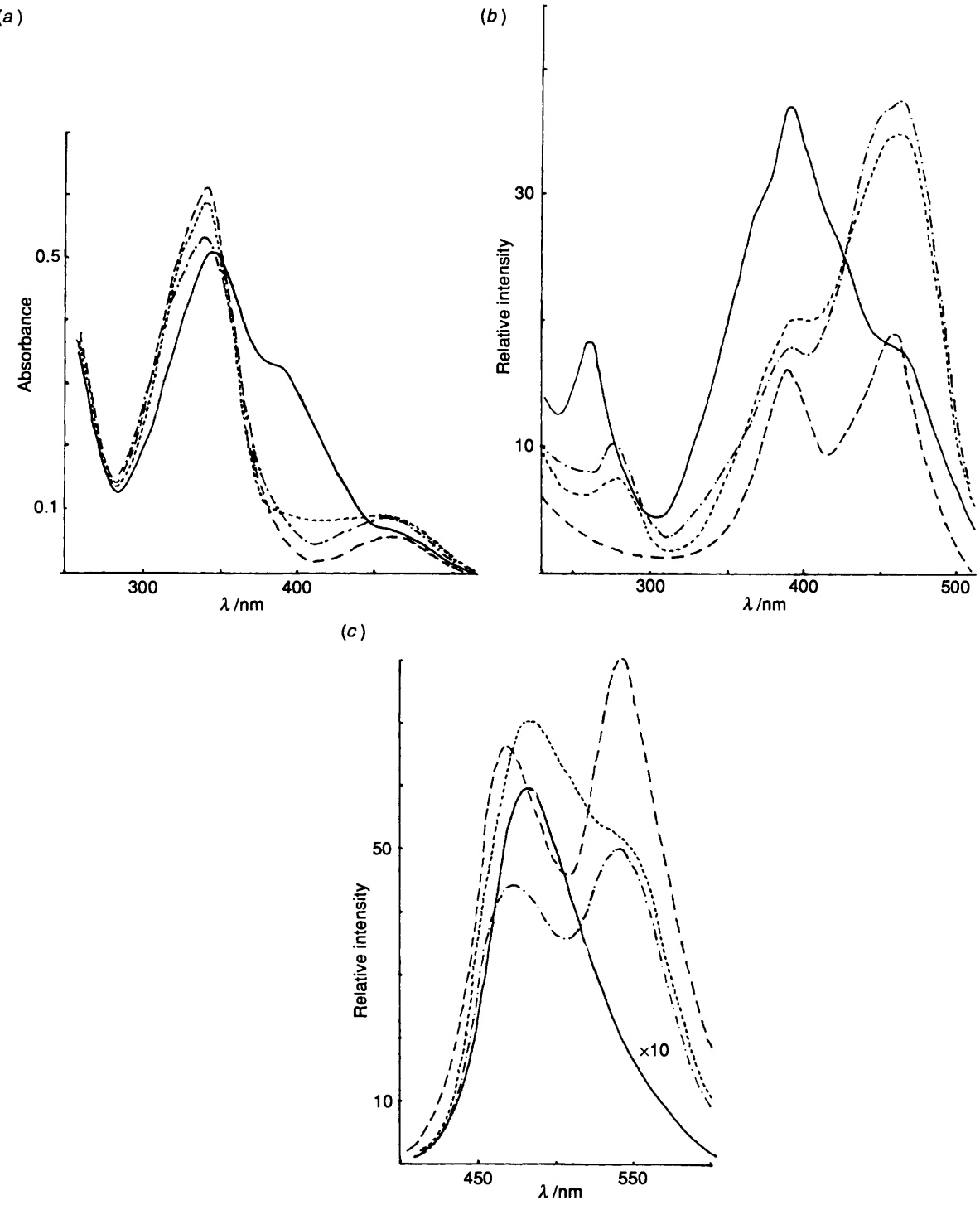


Fig. 5 Harmalol. (*a*) Absorption spectra; commercial anhydrous MeOH (——), AD MeOH (- - - -), MSD MeOH (— — —) MSD MeOH + 10% water (— · — · —); (*b*) fluorescence excitation spectra, λ_{em} 550 nm; (*c*) fluorescence emission spectra, λ_{exc} 340 nm.

the solid state. Firstly from these data, the effect of the presence of water in the organic media on the electronic spectra (Fig. 1) and on the prototropic equilibria in the ground and excited singlet state (Scheme 1, Fig. 1 and Table 7) have been described, showing the importance of the use of anhydrous organic solvents. Secondly, the effect of the presence of traces of aluminium oxide and molecular sieves in alcoholic media on the prototropic equilibria shown in Scheme 1 has been discussed. Thirdly, the unusual behaviour of electronically excited harmalol in organic solvents has been described for the first time. The results obtained have been compared with the electronic spectra of harmol run in similar media. Finally, this behaviour has been compared with that previously described in water.[11–16]

Acknowledgements

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Table 5 Effect of drying agents on the electronic spectra of harmol in organic solvents at 298 K ($A = \varepsilon lc$: A. absorbance: ε in $dm^3\ mol^{-1}\ cm^{-1}$: λ in nm: RI. relative intensities: c. 3.93×10^{-5} mol dm^{-3})

	Absorption data		Emission data			Excitation data		
Solvent	λ_{max}	log ε[a]	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
AD[b] MeOH	302	4.26	280	351(sh)	200	380	258	320
	324(sh)	3.87		365	260		300	300
	338	3.77		410(sh)	130		324	240
				456	90		336	220
			300	351(sh)	320	420	258	210
				365	420		300	190
				410(sh)	220		324	160
				456(sh)	140		336	140
			330	351(sh)	220	460	255	160
				366	310		300	145
				410(sh)	160		324	130
				456	130		336	128
			380	460	14		390	18
MSD[c] MeOH	304	4.17	280	353(sh)	150	380	254	260
	326	3.90		365	210		300	260
	338	3.86		454	110		324(sh)	190
			300	353(sh)	260		338	170
				366	390	420	254	200
				410(sh)	200		300	200
				454	160		324(sh)	140
			330	353	160		338	125
				365	230	460	254	240
				454	230		300(sh)	170
			390	465	19		334	250
							390	25
AD EtOH	304	4.20	280	353(sh)	325	380	253	600
	324	3.81		366	370		300	530
	338	3.69		462	20		322	390
			300	353(sh)	630		334	340
				366	690	420	253	112
				462	30		300	100
			330	353(sh)	400		322	76
				365	540		334	66
				462	30	480	253	30
			380	467	6		305	24
							327	21
							338	21
							396	10
MSD EtOH	304	4.13	280	352	250	380	254	540
	324	3.69		365	320		302	570
	338	3.54	300	352(sh)	650		324(sh)	410
				365	850		338	350
			330	352	440	420	255	75
				366	550		302	76
			380	430	2		324(sh)	57
				472	4		338	51
			400	472	7	460	256	11
							301	9
							324(sh)	9
							338	10
							402	6
EG[d]	304	4.23	280	370	150	380	258	240
	328	3.96		415	200		300	250
	338	3.89		455(sh)	150		324	170
			300	370	260		338	155
				414	350	420	258	460
				455(sh)	250		296(sh)	340
			330	370	170		306(sh)	360
				418	430		327	410
			380	443	68		365(sh)	150
						460	258	300
							300	260
							326	250
							338	240
							365(sh)	100
EG + SA[e]	328	4.33	280	419	170	380	262	740
	370(sh)	3.83	300	418	440		328	750
			330	418	750	420	264	58
							326	60

Table 5 (*continued*)

	Absorption data		Emission data			Excitation data		
	λ_{max}	log ε^a	λ_{exc}	λ_{max}	RI	λ_{em}	λ_{max}	RI
H_2O	322	4.05	280	421	120	380	323	65
	358(sh)	3.73	300	420	380		355	35
			330	420	700	420	246	700
			410	—	—		320	610
							355(sh)	350
						450	246	530
							324	430
							360(sh)	310
						550	238	40
							300	10
							326	11
							360	8

[a] 1 cm path quartz cells were used. [b] Solvent distilled and then percolated through a chromatography column filled with basic aluminium oxide. [c] Solvent distilled and then dried over molecular sieves. [d] EG, ethylene glycol. [e] SA, with 1% sulfuric acid (0.5 mol dm $^{-3}$).

Table 6 $\Delta pK_a'$ Values for harmalol in organic solutions at 25 °C[a]

Solvent	$\Delta pK_a'$
MeOH	11.7
EtOH	11.9
Pr^{i}OH	12.5
Bu^{t}OH	12.3
CH_3CN	13.3
CH_2Cl_2	13.4

[a] Ref. 11. ΔpK_a and $\Delta pK_a'$ values in aqueous solution: 10.0 and 11.3 respectively, see text for definition.

Table 7 Effect of added water on the emission spectra of harmalol in organic solvents (λ in nm; λ_{max} emission fluorescence wavelength of cation: RI: relative intensities, they reflect the relative quantum yields: RI_c and RI_o: RI of cation emission with and without added water; c: 3.93×10^{-5} mol dm^{-3})

Solvent	Added water (%)	λ_{exc}	λ_{max}	RI_c/RI_o
MeOH	—	330	478	1.00
	1.35			1.03
	7.09			1.41
	10.78			1.65
	14.30			1.81
EtOH	—	330	480	1.00
	2.08			1.06
	4.78			1.20
	8.78			1.43
	11.37			1.58
Pr^{i}OH	—	330	480	1.00
	0.86			1.06
	3.12			1.24
	5.79			1.76
	8.24			2.47
	10.44			2.82
Bu^{t}OH	—	330	475	1.00
	0.96			1.00
	2.90			1.00
	5.06			1.00
	8.73			1.00

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