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Solvent- and *pH*-Dependence of the Absorption and Fluorescence Spectra of Harman: Detection of Three Ground State and Four Excited State Species*

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The *pH*-dependence of the absorption and fluorescence spectra of the alkaloid harman** has been investigated. Three species, namely the cation, the neutral molecule and the anion have been found in absorption, whilst four species, namely the cation, the neutral molecule, the anion and the zwitterion were detected by fluorimetry. The zwitterion must be formed by a double proton transfer during lifetime of the excited state. Fluorescence quantum yields are entirely different for the various species, being highest for the cation (φ_f in 1 *N* sulfuric acid 0.89).

Unlike quinine sulfate the fluorescence of harman cation is not quenched by chloride ion, which suggests its use as a fluorescence standard superior to quinine.

The ground state pK_a's of harman are 7.37 and 14.6, the excieted state pK_a's, as calculated from the *Förster-Weller*-equation, are 12.0 and 8.65. Thus the observed zwitterion fluorescence is predicted from the calculations.

Die Lösungsmittel- und pH-Abhängigkeit der Absorptions- und Fluoreszenzspektren des Harmans: Nachweis von drei Formen im Grundzustand und von vier Formen im angeregten Zustand

Die *pH*-Abhängigkeit des Absorptions- und Fluoreszenzspektren des Alkaloids Harman wurde untersucht. Drei Spezies, nämlich das Kation, das Neutralmolekül und das Anion, wurden in Absorption gefunden, während vier Formen, nämlich das Kation, das Neutralmolekül, das Anion und das Zwit-

* Dedicated to Prof. Dr. Dr. h.c. *O. Kratky* on the occasion of his 80th birthday.

** The IUPAC name for harman is 1-methyl-9*H*-pyrido[3,4—b]indol; the trivial name will be used throughout this paper.

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terion, durch Fluoreszenz nachgewiesen wurden. Das Zwitterion wird durch einen doppelten Protonentransfer während der Lebenszeit des angeregten Zustandes gebildet. Die Fluoreszenzquantenausbeuten der einzelnen Formen sind deutlich verschieden und für das Kation am höchsten ($\varphi_f = 0{,}89$ in $1\,N$ Schwefelsäure). Im Gegensatz zum Chininsulfat wird Harman durch Chloridion nicht gelöscht, was seine Verwendung als Fluoreszenzstandard nahelegt.

Die Grundzustands-pK-Werte des Harmans betragen 7,37 und 14,6, die pK-Werte des angeregten Zustandes, wie sie aus der *Förster-Weller*-Gleichung erhalten werden, betragen 12,0 und 8,65. Die beobachtete Fluoreszenz aus der zwitterionischen Form wird somit auch von den Berechnungen vorausgesagt.

(Keywords: Alkaloid; Dissociation constants; Fluorescence; Proton transfer; Tautomerism)

Introduction

Harman is one of a number of related compounds, the harmala alkaloids. Most of these compounds are potent inhibitors of the enzyme monoamine oxidase[1]. They are now mainly used for experimental studies in animals, although they had been used clinically in the past. The harmala alkaloids are known to be highly fluorescent and can be extracted from plant tissues. Harman itself has been isolated from various plants[2], but also from cigarette smoke[3].

We have focussed our interest on this compound in continuation of our studies on the solvent- and pH-dependence of the fluorescence spectra of natural products, but also because of an early report, that harman is a useful indicator for the fluorimetric determination of pH's in the physiological range[4,5].

Experimental

Harman was purchased from Fluka (Buchs, Switzerland) and was purified by preparative thin layer chromatography to remove a fluorescent impurity present in the commercial product. Stock solutions were prepared in methanol and were diluted with either triple distilled water or buffer solutions to contain finally not more than 10% methanol. The absorption spectra were run on an UVICON 810 spectrophotometer (Kontron, Switzerland), in buffered solutions (for solvents and buffers, see[6]). The fluorescence spectra, recorded on an Aminco SPF 500 spectrofluorimeter in rectangular quartz cells at room temperature are uncorrected. The pH of the unbuffered, nondegassed solutions was adjusted by addition of either sulfuric acid or sodium hydroxide to avoid fluorescence quenching effects by buffer ions. The pK_a's were obtained by the spectrophotometric method in phosphate buffer at ionic strength $J < 0.05$.

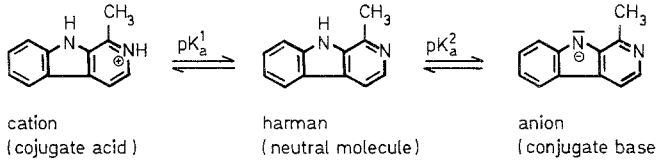
Fluorescence quantum yields were determined with quinine sulfate as a reference standard ($\varphi_f = 0.546$)[7].

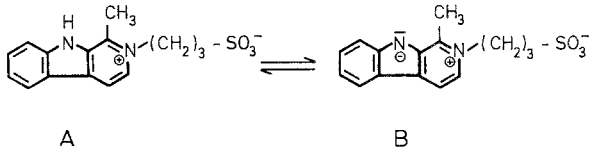
Results and Discussion

Due to the acidic NH-function and the basic nitrogen atom in the pyridine nucleus harman can exist in three differently charged ground

state species. Their respective equilibria, shown in Scheme 1, are governed by two pK_a's.

Scheme 1





According to the absorption spectra the cation is present in the pH 6 to 0 range, whereas the neutral molecule is present in the pH 8 to 13 range. In more alkaline solutions anion absorption is apparent having λ_{max} at 374 nm.

The pH-dependent absorption and fluorescence spectra of harman are compiled in more detail in Table 1.

There are four species evident in the fluorescence spectra having maxima at 433.5 nm (cation), 381 nm (neutral molecule), 490.5 nm and 445 nm (anion). The assignement of the three shortwave bands at 433.5, 381 and 445 nm to the cation, to the neutral molecule and to the anion can be made unambiguously. In benzene and methanol solution the neutral molecule's fluorescence lies in the UV too.

In order to assign the 490 nm band we studied the behaviour of model compound **A**, the structure of which is given in Scheme 1. **A** was considered in neutral solution to be a fixed derivative of harman cation, and its anion (**B**) a fixed derivative of a suspected harman zwitterion.

The pK_a value of **A** was determined to be 11.0 ± 0.1 at 25 °C. In neutral solution it shows maximum absorption at 367 nm and maximum fluorescence at 441 nm, which agrees favourably with the data of harman cation. The respective maxima in pH 14 solution (i.e. of zwitterion **B**) are at 408 and 505 nm. A comparison of the absorption

Table 1. *Absorption data as well as excitation and fluorescence maxima (in nm) of harman in aqueous solutions of various acidity, in benzene and in methanol at 25 °C.* Conc. 42.5 to 63.8 μM. Isosbestic points for the system harman/harman conjugate acid are observed in absorption at 268, 279, 314 and 351.5 nm

Solvent	λ_{max}^{abs}	log ε (M^{-1} cm^{-1})	λ_{min}^{abs}	log ε (M^{-1} cm^{-1})	λ_{max}^{exc}	λ_{max}^{flu}	fluorescent species[c]
water							
pH 0.0	364.4	3.873	320.1	3.332	368.5	433.5	C
(1 *N*-H_2SO_4)	299.1	4.387	273.2	3.867	309		
	245.0	4.618			~300 (sh)		
pH 7.1	349.2	3.701	316.3	3.178	368	430	C
	299.7	4.106	293.6	4.040	355		
	287.7	4.151	271.3	3.627	302		
	239.9	4.558					
pH 10.0	346.8	3.780	341.6	3.764	347	431	C
	334.9	3.794	300.0	2.988	337	~480 (sh)	Z
	286.8	4.284	265.1	3.719	287	~370 (br. sh)	N
	233.9	4.633					
pH 14.0	~375 (br, sh)	~3.04	343	3.274	~390	490.5 (*pH* 13)	Z
(1 *N*-NaOH)	349	3.738	315	3.440		384 (*pH* 13)[a]	N
	336	3.734					
H_- 15.8	374	3.700	318	3.32	379	445	A
	385 (sh)				393 (sh)		
	355 (sh)				360 (sh)		
benzene	343	3.851	337	3.754	349	367.5	N
	330	3.830	298		337	355[b]	
	321 (sh)	3.655			318	385 (sh)[b]	
	288				295 (sh)		
methanol[d]	348	3.834	342	3.721	349	374	N
	335	3.790	300	3.212	337	360[b]	
	288	4.386	264	3.833	289	390 (sh)[b]	
	234	4.664					

[a] This emission, originating from the excited neutral molecule, is observed at exciting wavelengths smaller than 350. When excited at 390 nm only the zwitterion fluorescence is evident.

[b] Vibrational level.

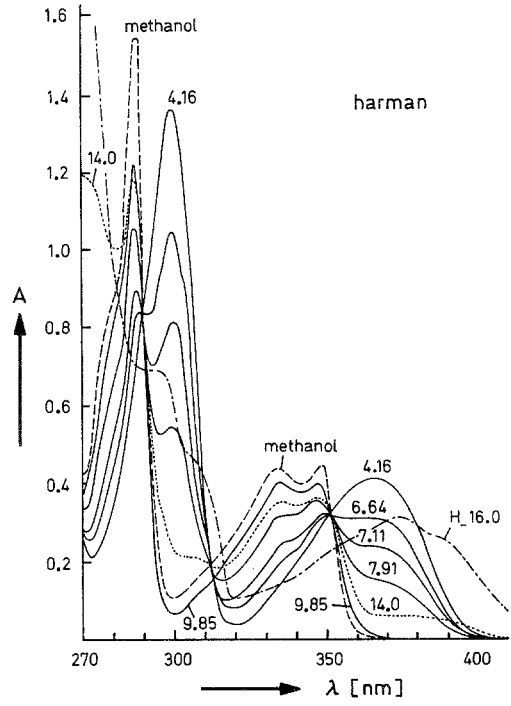
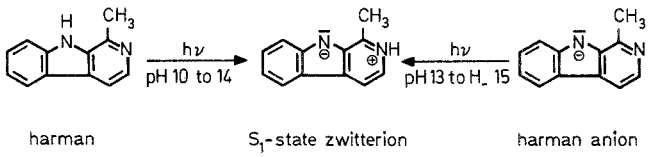


Fig. 1. Absorption spectra of harman in methanol and in aqueous solutions of various acidity. Conc. 63.8 μM

and fluorescence maxima, together with an unusual large *Stokes* shift for harman in *pH* 14 solution indicates, that the 490 nm emission of harman arises from the zwitterion.

The formation of harman zwitterion as a result of photoexcitation is sketched in Scheme 2.

Scheme 2



The fluorescence quantum yields of the various species are entirely different and are highest for the cation. The latter has φ_f 0.89 ± 0.07 in 1 N sulfuric acid in the 345 to 360 nm excitation wavelength range.

Unlike quinine, the fluorescence of harman is not quenched by chloride ions. This, together with its high fluorescence quantum yields, the invariability of the fluorescence intensity in the *pH* 0 to 5 range, its stability and the availability of the compound suggests its use as a reference indicator in fluorescence intensity measurements.

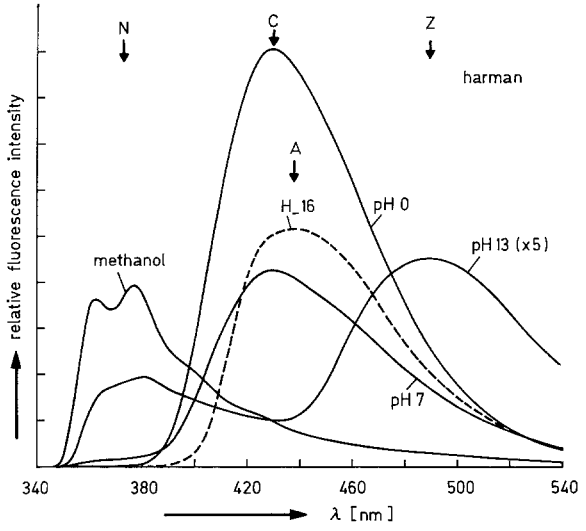


Fig. 2. Fluorescence spectra of harman in aqueous solutions of various acidity and in methanol. Conc. 50.5 μM

The fluorescence intensity of the neutral molecule is rather weak. From *pH* 8 up to *pH* 12 the 381 nm band can be recognized only as a shoulder beside the intense emission band of the cation, but at *pH* 13 it forms a distinct maximum. However, in *pH* 14 solution the zwitterion fluorescence is the only one to be observed at excitation wavelength 350 nm, despite of considerable amounts of neutral molecule being present according to the absorption spectrum. It is likely that zwitterion fluorescence is already present at *pH*'s lower than 13, as predicted from the results of the *Förster*-cycle calculations. If so, this fluorescence is overridden by the strong fluorescence band of the cation present up to *pH* 12. Anion fluorescence, maximizing at 445 nm, can only be found in solutions of H_- greater than 15. Following excitation at 380 nm, where the anion is the only species to absorb light, luminescence of the zwitterion is fairly strong in the *pH* 13 to H_- 15 range, thus indicating a proton gain of harman anion in its S_1-state (Scheme 2).

Ground and First Excited Singlet State pK_a's

The ground state pK_a of harman conjugate acid (pK_a^1 in Scheme 1) was determined photometrically to be 7.37 ± 0.04 (cf. a pK_a of 7.93 for 1,6-Diazainden[8]). The pK_a^2 has been determined in potassium hydroxide solution of various basicity[9] to be 14.6 (cf. pK_a of pyrrol: 16.5[10] and a pK_a 16.97 for indol[11]).

The pK_a's for the S_1-state have been calculated applying the *Förster-Weller*-equation[12]

$$pK(S_1) = pK(S_0) + \frac{0.625}{T}(\tilde{\nu}_B - \tilde{\nu}_{BH})$$

The means of frequencies of the absorption and fluorescence maxima have been taken as 0-0-transitions. The results are given in Table 2, together with the pK_a's obtained by fluorescence titration. They show harman in its S_1-state to become a stronger acid by 5.95 units, and harman conjugate acid to become a stronger base by 4.63 units thus having $pK(S_1) = 12.0$.

Two pK_a's have been obtained for harman conjugate acid by fluorescence titration at excitation wavelength 351.5 nm (which is the iosbestic wavelength in absorption) and at emission wavelength 433.5 nm. The one (7.4) corresponds to the ground state pK_a of harman cation, the second (11.4) corresponds to the excites state pK_a of harman conjugate acid and is in good agreement with the calculated value (12.00; cf. Table 2).

The formation of harman conjugate acid following photoexcitation in the pH 8 to 12 range results from the high basicity of harman in its S_1-state and is thought to proceed according to the following pseudo first order reaction:

$$\text{harman}(S_1) + H_2O \rightarrow \text{harman} \cdot H^-(S_1) + OH^-$$

The results from the *Förster-Weller* calculations predict harman to form a zwitterion in its S_1-state from pH 8.65 to 12.00. If, however, harman is excited in pH 12 solution, where the UV-spectrum shows it to exist as the neutral molecule only, fluorescence is observed from the excited cation, the neutral molecule and from the zwitterion altogether. This in our eyes demonstrates, that all the prototropic processes in the S_1-state are of comparable rate with fluorescence decay.

Excited state prototropic processes have been demonstrated to occur with both indole[13] and carbazole[14]. Carbazole has an electronic structure and shows an absorption spectrum very similar to that of harman. But lacking a basic nitrogen atom carbazol shows blue fluorescence in alkaline solution as a result of photodissociation[14],

Table 2. *Ground and first excited singlet state dissociation constants of harman at 295 K*. The calculated pK_a's (S_1) were obtained from the *Förster-Weller*-equation[12]

Species	pK_a (S_0)	ν_{max}^{abs} (cm^{-1})	ν_{max}^{flu} (cm^{-1})	0-0-transition (cm^{-1})	Calculated pK_a (S_1)	pK_a by fluorescence titration
anion	—	26738	22472	24604	—	—
neutral molecule	14.6 ± 0.1	28835	26042	27439	8.65	—
cation	7.37 ± 0.04	27442	23068	25255	12.00	7.4 11.4

whilst harman can form both a greenish fluorescent zwitterion and a blue fluorescent anion.

Finally, with respect to an analytical application of these results both harman and norharman* (the luminescent compound in the fluorimetric determination of both tryptophane and tryptamine[15]) can evidently be assayed best in pH 5 to 0 solution, where fluorescence intensity is highest and variation of the intensity with pH is practically zero. The zwitterion fluorescence at 490.5 nm may be another useful emission, but its strong variation in intensity with pH requires careful adjustment of alkalinity. On the other hand, harman shows a pK_a that can make it a useful fluorescence indicator in measuring physiological pH's.

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* Norharman differs from harman in having no methyl group in the 1-position.

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