

## FLUORESCENCE CHARACTERISTICS OF $\beta$ -CARBOLINE ALKALOIDS IN HIGHLY CONCENTRATED HYDROXIDE SOLUTIONS

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### Summary

The room temperature electronic absorption and fluorescence spectra of the pharmacologically active  $\beta$ -carboline alkaloids norharman, harmane, harmine, harmaline, reserpine and yohimbine were measured in moderately and highly concentrated hydroxide solutions. The appearance of a new fluorescence emission band in the latter media for all the compounds under study was ascribed to anions in which the NH group of the indole ring is deprotonated. The differences between the acidities of their excited singlet states and their ground states were estimated by means of the Förster-Weller cycle.

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### 1. Introduction

Because of the theoretical and pharmacological interest in harmala alkaloids [1 - 7], an indole class of alkaloids structurally related to  $\beta$ -carboline (9H-pyrido[3,4-b]indole), their fluorescent properties have been extensively studied and recently a number of papers have been published concerning the effect of pH on their absorption and fluorescence spectra [8 - 16].

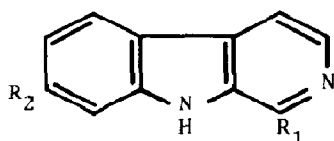
Owing to the acidic NH indolic group and the basic non-indolic nitrogen atom on the tricyclic ring,  $\beta$ -carboline derivatives can exist in aqueous solutions as differently charged species (cationic, anionic and neutral). Moreover, additional zwitterionic species have been detected in the singlet excited state of the fully aromatic  $\beta$ -carboline derivatives [9 - 12].

Whereas the spectral characteristics of the cationic, neutral and zwitterionic species have been assigned unambiguously, controversial results have been obtained concerning the fluorescent properties of the anions. Thus, although some researchers have claimed that no emission of the anions can be detected and that excitation of the anions produces zwitterion emission [9, 12, 15], other workers have observed emission fluorescence from some harmala alkaloid anions in very concentrated hydroxide solutions [10, 11].

Recently, we have investigated the deprotonation equilibria of the NH indolic group of several  $\beta$ -carboline alkaloids [17, 18]. The  $pK_a$  of these equilibria ranged between 15 and 17 on the  $H_-$  acidity scale [19], depending on the hydrogenation state of the pyridyl moiety of the  $\beta$ -carboline ring. During the course of these investigations we have re-examined the fluorescent properties of these alkaloids in highly concentrated hydroxide solutions in order to provide some insight into the acidity of the excited states of  $\beta$ -carbolines. This paper presents the results of this study.

In our experiments we have also included the Rauwolfia alkaloids yohimbine and reserpine which are tetrahydro- $\beta$ -carboline derivatives of pharmacological significance [3, 20].

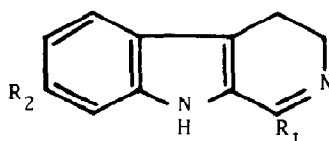
The  $\beta$ -carboline alkaloids studied have the following chemical structures:



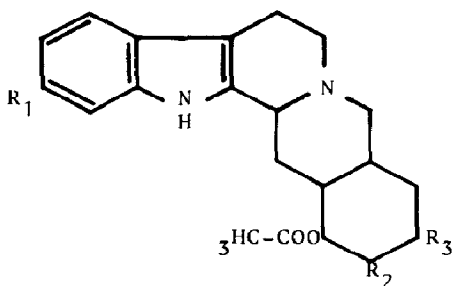
Norharman  $R_1-R_2 = H$

Harmane  $R_1 = CH_3$ ;  $R_2 = H$

Harmine  $R_1 = CH_3$ ;  $R_2 = OCH_3$



Harmaline  $R_1 = CH_3$ ;  $R_2 = OCH_3$



Reserpine

$R_1-R_2 = OCH_3$ ;  $R_3 = 3,4,5(OCH_3)C_6H_2COO$

Yohimbine

$R_1=R_3 = H$ ;  $R_2 = OH$

## 2. Experimental details

### 2.1. Chemicals and solutions

The harmala alkaloids norharman ( $\beta$ -carboline), harmane, harmine hydrochloride hydrate and harmaline hydrochloride dihydrate were purchased from EGA-Chemie. The Rauwolfia alkaloids yohimbine and reserpine were the kind gift of Boehringer und Sohn. All the alkaloids were of the best available quality and were used as received.

Stock solutions of each alkaloid ( $1 \times 10^{-3}$  M) were prepared in methanol (for fluorometry, Merck) and stored in the dark. The hydroxide solutions were prepared from Merck R.A. potassium hydroxide as described by Yagil [19] and were standardized against standard sulphuric acid solutions.

The solutions for absorption and fluorescence measurements were prepared by pipetting aliquots of the alkaloid solutions into hydroxide solutions. They were usually stable although, in some cases, warming in hot water was necessary to bring about complete dissolution. Only reserpine solutions were unstable and difficulty was encountered in obtaining reliable data in this case.

## 2.2. Apparatus and procedures

Fluorescence measurements were made on a Perkin-Elmer 650-40 spectrophotofluorometer. A Perkin-Elmer Data Processor 650-0178 was used to obtain corrected spectra. The sensitivity and the stability were checked by using the Raman band of distilled water; likewise, the wavelengths of excitation and emission were checked by using the xenon lines at 450.1 and 467.1 nm of the xenon lamp and also by USP quinine bisulphate solutions. Fluorescence quantum yields were calculated as in previous work [21].

Absorbance measurements were carried out on a Perkin-Elmer Lambda-5 spectrophotometer. Since the hydroxide solutions appreciably absorb at the wavelengths used for the measurements, these solutions were usually used as references.

All the absorption and fluorescence spectra were obtained at room temperature with solutions of similar concentrations (about  $2 \times 10^{-5}$  M) and with the same instrument settings.

## 3. Results and discussion

### 3.1. Absorption spectra

The absorption spectra of harmala alkaloids in weak and highly concentrated KOH solutions are shown in Figs. 1 - 4 and their respective maxima are summarized in Table 1. According to the  $pK_a$  values of these alkaloids [8, 16 - 18], in these media neutral and anionic species are the only species to absorb light.

It was observed that dissociation of the NH indolic group is accompanied by a marked decrease in the intensities of the absorption band maxima of the neutral species and, in some instances, by the appearance of a new band (norharman and harmane) or broad shoulder (harmine) at longer wavelengths. It should be noted that these systems showed clear isosbestic points.

In the case of Rauwolfia alkaloids, the absorption peaks of the neutral forms decrease in intensity and shift about 20 nm to longer wavelengths on conversion to base. Moreover, an additional shoulder appears at 310 - 320 nm (Figs. 5 and 6). Their respective maxima are summarized in Table 1.

### 3.2. Fluorescence emission spectra

The fluorescence emission spectra for the excitation wavelengths of the absorption maxima of both neutral and anionic species of harmala and

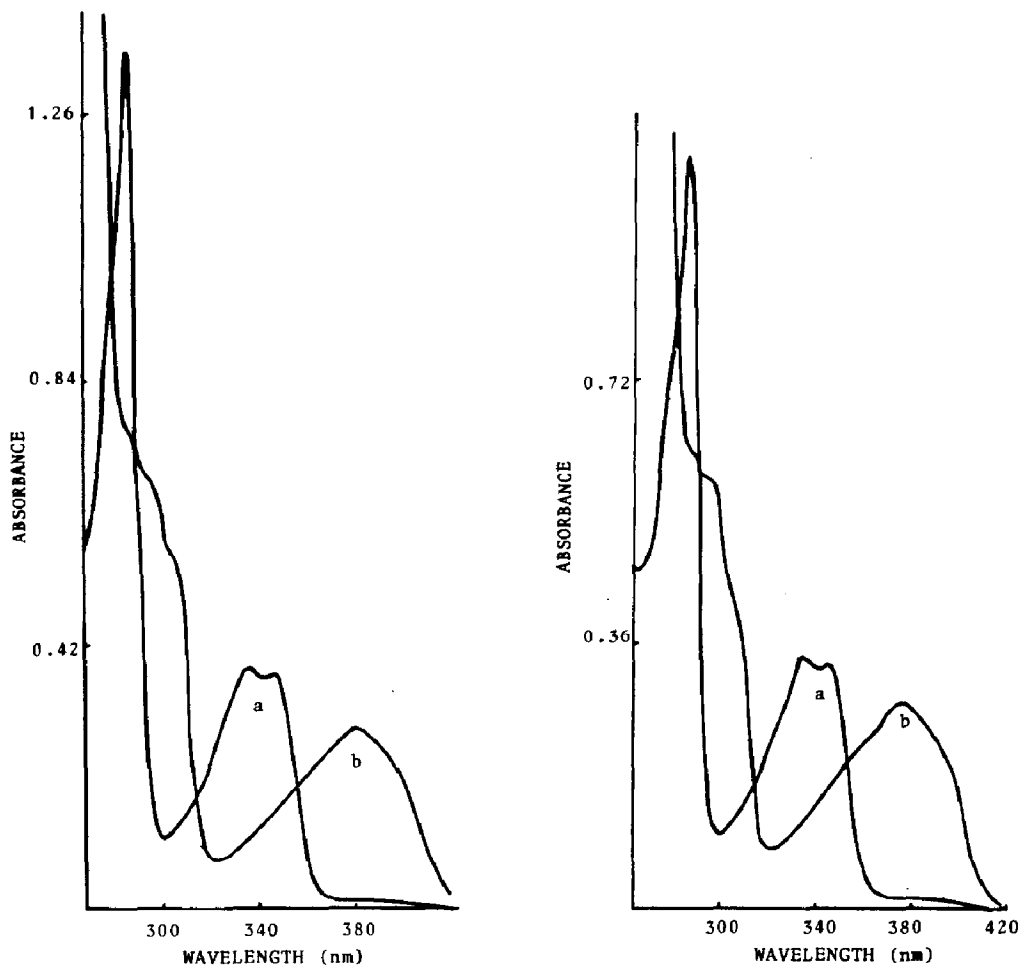


Fig. 1. Absorption spectra of norharman in 0.1 M KOH (curve a) and 6 M KOH (curve b).

Fig. 2. Absorption spectra of harmane in 0.1 M. KOH (curve a) and 6 M KOH (curve b).

Rauwolfia alkaloids are shown in Figs. 7 - 12, and their maxima are listed in Table 1.

Excitation of the neutral forms of norharman, harmane and harmine produces two emission bands with maxima around 380 nm and 500 nm, which are in good agreement with literature values [9 - 12]; these maxima have previously been assigned to fluorescence from neutral and zwitterionic species respectively. The latter species are thought to be formed by proton transfer reactions during the lifetime of the excited states.

The emission spectrum of neutral harmaline shows a very weak shoulder near 375 nm and a more intense fluorescence band maximum near 480 nm. According to the literature data [14, 15], these maxima correspond to fluorescence emission from neutral and cationic species of harmaline.

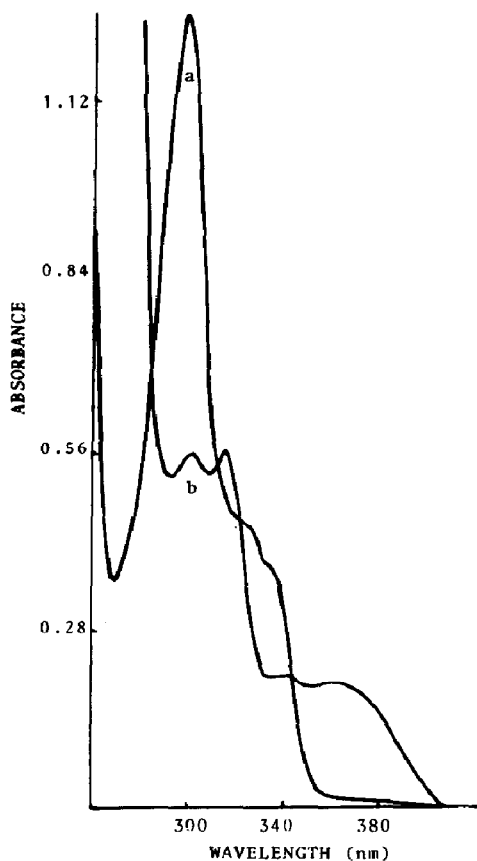


Fig. 3. Absorption spectra of harmine in 0.1 M KOH (curve a) and 6 M KOH (curve b).

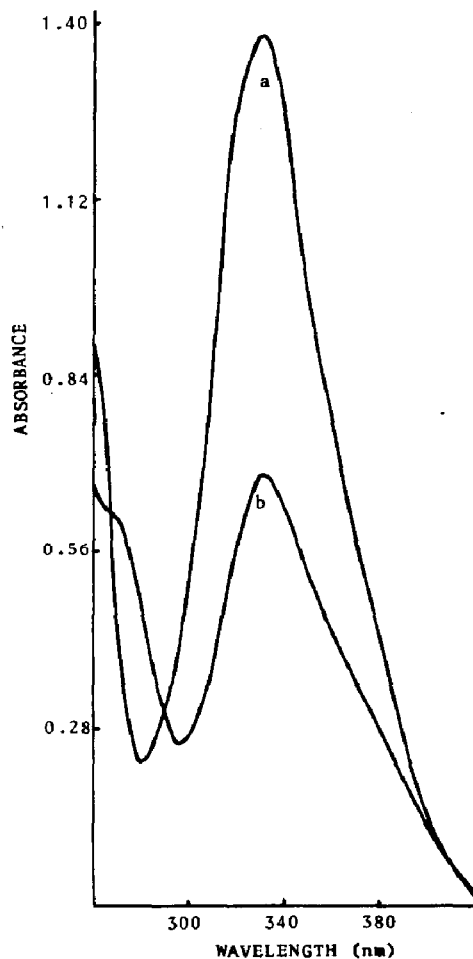


Fig. 4. Absorption spectra of harmaline in 0.1 M KOH (curve a) and 6.5 M KOH (curve b).

The Rauwolfia alkaloids displayed fluorescence spectra in 0.1 M KOH arising from the parent compounds indole and 6-methoxyindole, as has been reported recently [13].

The fluorescences of  $\beta$ -carboline alkaloids were found to diminish with increasing hydroxide concentration; concurrently a new emission band appeared near to 440 nm for harmala alkaloids and at shorter wavelengths for Rauwolfia alkaloids. The fluorescence emission spectra registered in hydroxide solutions of  $H_+$  greater than 16, where the anions are the only species to absorb light [17, 18], show only these new emission bands, which may be attributed to fluorescence emission from the indolate anions. Moreover, under these conditions fluorescence excitation spectra and absorption spectra were in good agreement.

TABLE 1

Absorption and fluorescence maxima (sh, shoulder) of  $\beta$ -carboline alkaloids together with quantum yields  $\phi$  in 6.8 M KOH at 25 °C

Compound	$\lambda_{\text{max}}^{\text{abs}}$ (nm)		$\lambda_{\text{max}}^{\text{flu}}$ (nm)		$\phi$
	AH	A <sup>-</sup>	AH	A <sup>-</sup>	
Norharman	287 336 348	380	385	450	0.057
Harmane	286 335 348	375	380	440	0.056
Harmine	298 335sh	300	372	435	0.034
Harmaline	330	330	380	435	<0.001
Reserpine	280 295sh	303 316	375	402	—
Yohimbine	279	295 312sh	365	410	—

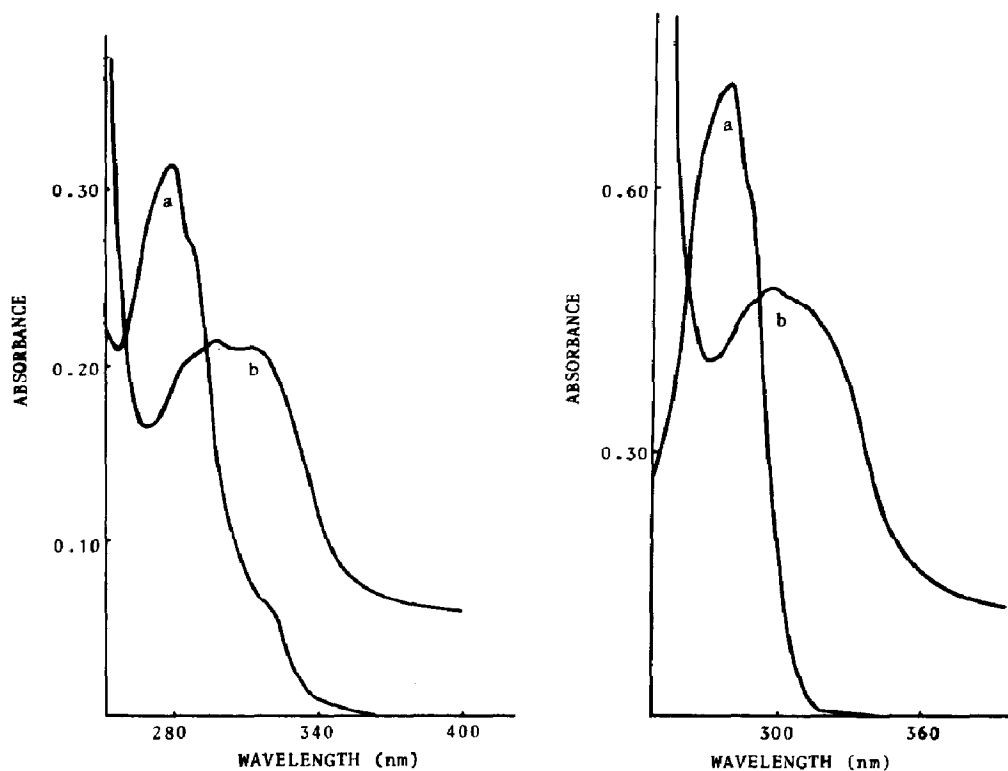


Fig. 5. Absorption spectra of reserpine in 0.3 M KOH (curve a) and 14 M KOH (curve b).

Fig. 6. Absorption spectra of yohimbine in 0.3 M KOH (curve a) and 15 M KOH (curve b).

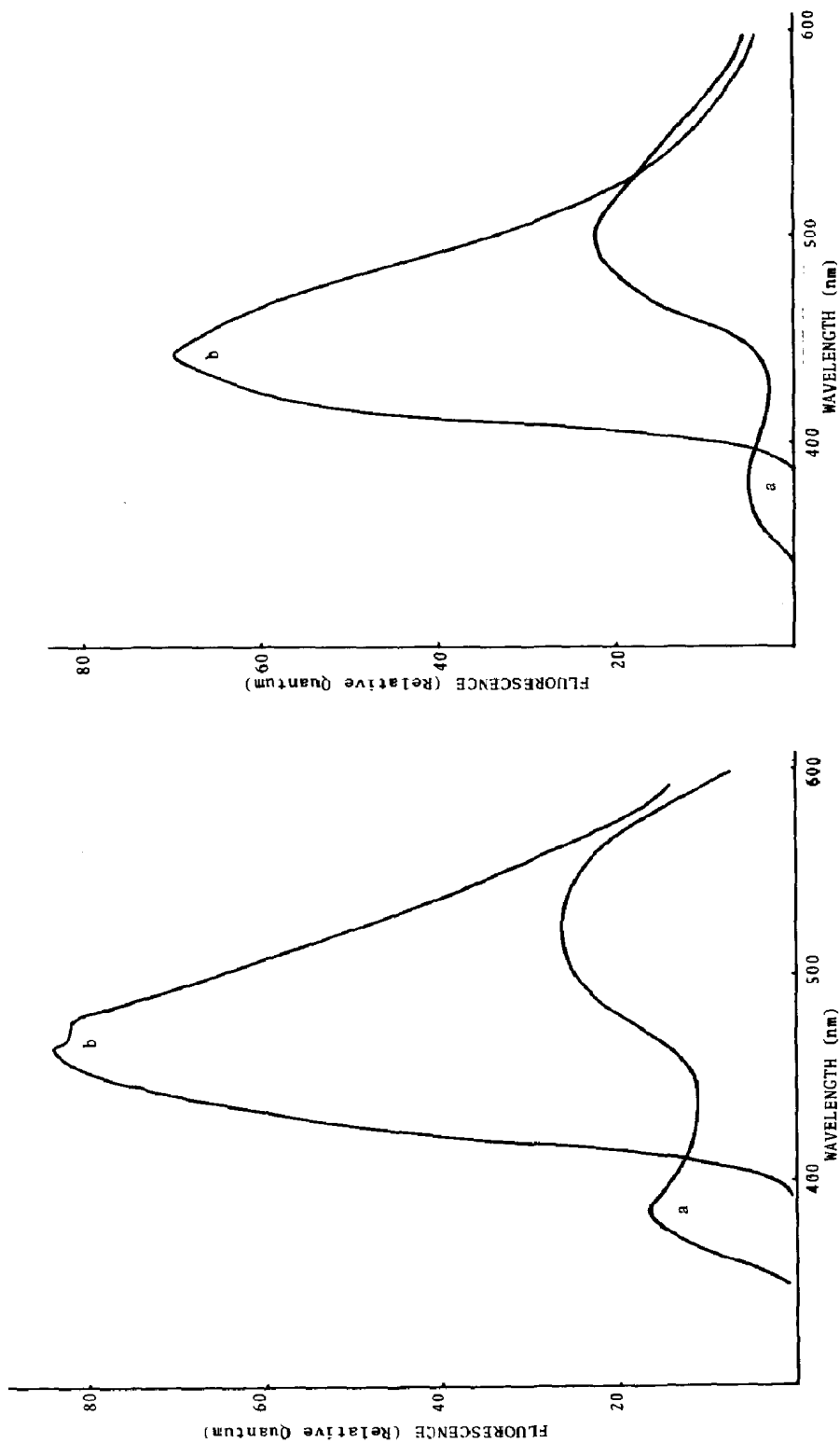


Fig. 7. Fluorescence spectra of norharman in 0.1 M KOH (curve a) and 6.8 M KOH (curve b).

Fig. 8. Fluorescence spectra of harmaine in 0.1 M KOH (curve a) and 6.8 M KOH (curve b).

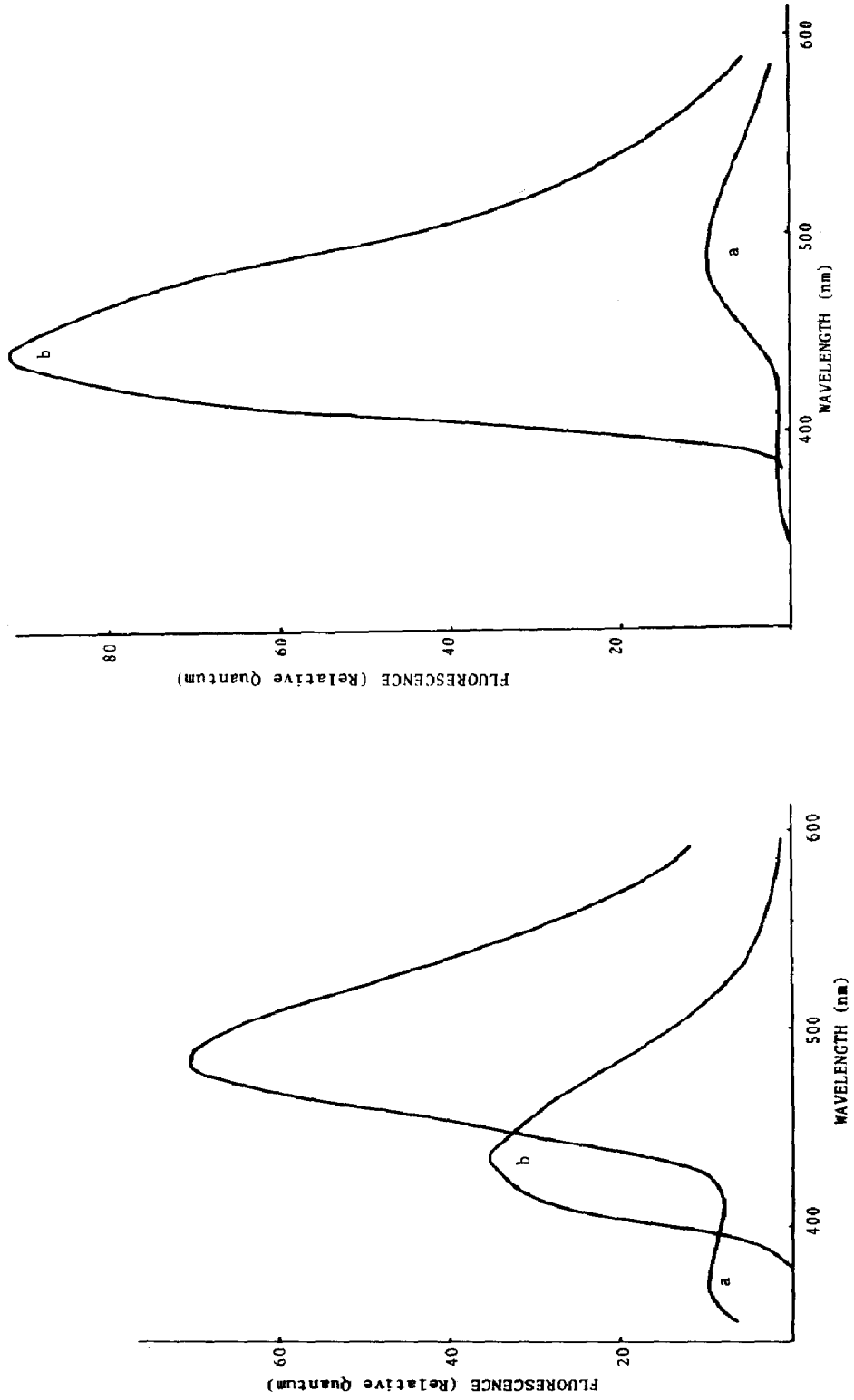


Fig. 9. Fluorescence spectra of harmaline in 0.1 M KOH (curve a) and 6.8 M KOH (curve b).

Fig. 10. Fluorescence spectra of harmaline in 0.1 M KOH (curve a) and 6.8 M KOH (curve b).



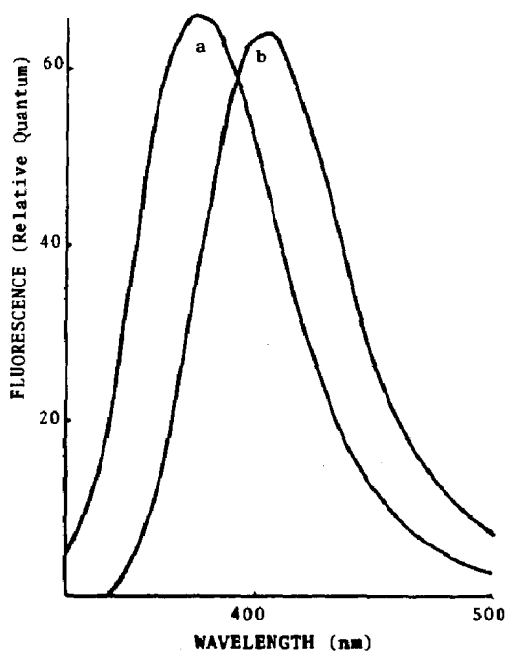


Fig. 11. Fluorescence spectra of reserpine in 0.1 M KOH (curve a) and 14 M KOH (curve b).

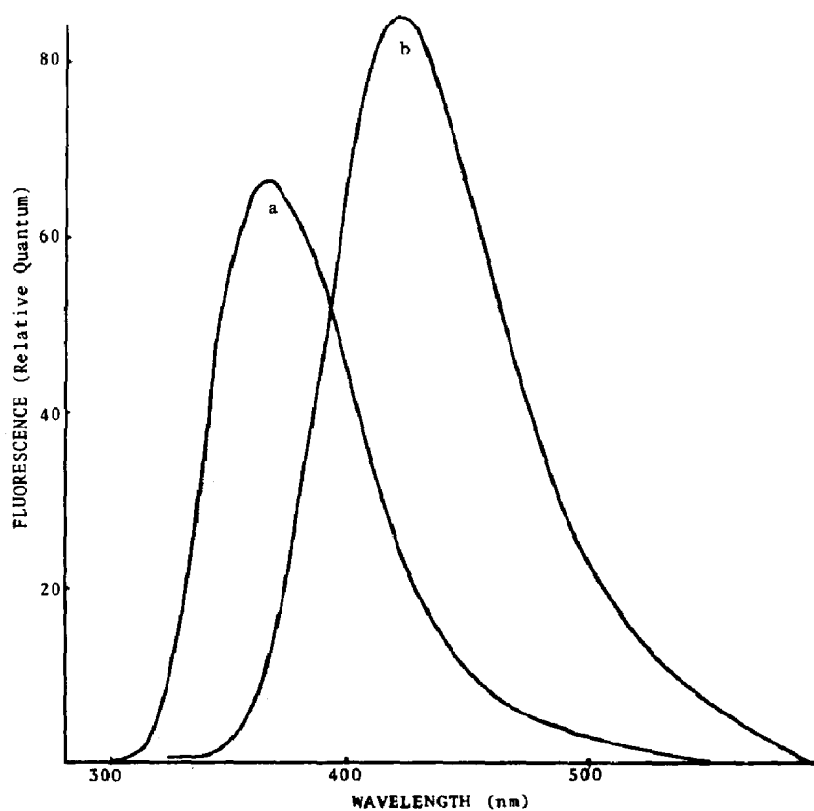


Fig. 12. Fluorescence spectra of yohimbine in 0.1 M KOH (curve a) and 14 M KOH (curve b).

It should be noted that the fluorescence intensity of harmaline anions changed during exposure to the exciting radiation. However, the nature of this photochemical process has not been investigated.

The spectra of  $\beta$ -carboline alkaloids in highly concentrated hydroxide solutions show the expected close similarity to the spectra of indole itself and other indole derivatives such as methylindoles and tryptophan [22 - 28], which display the same prototropic process in concentrated hydroxide solutions [19, 29]. As in the case of these indoles, the emission bands of  $\beta$ -carboline anions are red shifted by about 20 - 60 nm with respect to those of the neutral molecules, and both indoles and  $\beta$ -carbolines yield similar fluorescence quantum yields ( $\phi_F$  of indole in 5 M KOH, 0.034) [27].

The foregoing results provide information on the acidities of  $\beta$ -carboline alkaloids in their lowest singlet states. It is well known that differences between excited singlet state  $pK_a(S_1)$  and ground state  $pK_a(S_0)$  can be estimated from the equation

$$pK_a(S_1) - pK_a(S_0) = \frac{0.625}{T} (\bar{\nu}_{AH} - \bar{\nu}_{A^-}) \quad (1)$$

derived from the Förster-Weller cycle [24, 30, 31], where  $\bar{\nu}_{AH}$  and  $\bar{\nu}_{A^-}$  are the averages of the absorption and the fluorescence maxima (in reciprocal centimetres) of the acids and their conjugated bases (taken as 0-0 transitions) and  $T$  is the absolute temperature.

The ground state and singlet state  $pK_a$  values ascribed to the equilibria between the neutral and the anionic species of  $\beta$ -carboline alkaloids are listed in Table 2. The  $pK_a(S_1)$  values are in the range 8 - 12 depending on the degree of aromaticity of the  $\beta$ -carboline ring. It should be noted that the  $pK_a$  values of tetrahydro- $\beta$ -carboline alkaloids are of the same order of magnitude as those of indoles [23, 28] ( $pK_a$  of indole, 12.3).

It can be seen that  $\beta$ -carboline alkaloids are stronger acids in their singlet excited states than in their ground states by about 6  $pK_a$  units. This

TABLE 2

Experimental ground state and calculated lowest excited singlet state  $pK_a$  of  $\beta$ -carboline alkaloids at 25 °C

Compound	$pK_a(S_0)$	$\bar{\nu}_{abs} (cm^{-1})$		$\bar{\nu}_{em} (cm^{-1})$		0-0 transition		$pK_a(S_1)$
		AH	A <sup>-</sup>	AH	A <sup>-</sup>	AH	A <sup>-</sup>	
Norharman	14.49 ± 0.07 <sup>a</sup>	28736	26316	25974	22222	27355	24269	8.0
Harmane	14.50 ± 0.07 <sup>a</sup>	28736	26667	26316	22727	27526	24697	8.6
Harmine	14.43 ± 0.02 <sup>a</sup>	29851	27624	26882	22989	28367	25306	8.0
Harmaline	15.34 ± 0.03 <sup>a</sup>	30303	30303	26316	22936	28310	26620	11.8
Reserpine	15.5 ± 0.5 <sup>b</sup>	33898	31646	26667	24876	30283	28261	11.3
Yohimbine	16.09 ± 0.04 <sup>b</sup>	35842	33898	27397	24390	31620	29144	10.9

<sup>a</sup>From ref. 17.

<sup>b</sup>From ref. 18.

behaviour is reminiscent of indoles and other nitrogen heterocycles [30] in which the heteronitrogen is involved in the formation of three  $\sigma$  bonds. In the case of indoles this has been rationalized on the basis of a substantial loss of electron density on the nitrogen atom in the first excited singlet state, in agreement with theoretical calculations [32 - 35].

#### 4. Conclusions

The absorption and fluorescence spectra of neutral  $\beta$ -carboline alkaloids are markedly affected as the hydroxide concentration is increased, owing to the deprotonation of the NH indolic group.

For all the  $\beta$ -carboline alkaloids investigated in this study, the appearance of a new fluorescence emission band in hydroxide solutions of  $H_-$  greater than 16 may be ascribed to fluorescence emission from anions.

Differences between singlet excited state and ground state  $pK_a$  values for the neutral-anion prototropic process, estimated by means of the Förster-Weller cycle, indicate that  $\beta$ -carboline alkaloids are stronger acids in their singlet excited states by about 6  $pK_a$  units.

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