FLUORESCENCE LIFETIMES OF SOME HARMALA AND RAUWOLFIA ALKALOIDS

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Summary

Quantum yields and lifetimes of several derivatives of the Peganum Harmala and Rauwolfia alkaloids have been measured. The natural lifetimes of these alkaloids have been calculated from a modified form of the Strickler–Berg equation and the results are compared with the observed lifetimes.

1. Introduction

The Harmala and Rauwolfia alkaloids are an indole class of alkaloids structurally related to β-carboline (9*H*-pyrido[3,4-*b*]indole) (Scheme 1).

They are of theoretical and pharmacological interest. The pharmacological properties of the Harmala alkaloids include hallucinogenic effects, inhibition of monoamine oxidase and cardiovascular alterations [1 - 4]. Rauwolfia alkaloids have achieved significance as sympatholytic, antihypertensive and sedative agents [5]. Moreover, serpentine selectively inhibits cancer cell DNA synthesis *in vitro* [6, 7].

As part of a study in which the spectrofluorometric characteristics and stability of various indole alkaloids are being investigated, we report in this paper the measurements of the actual lifetimes, τ, of four Harmala alkaloids (norharman, harmane, harmalol and harmol) and those of four Rauwolfia alkaloids (yohimbine, corynanthine, serpentine and ajmaline) in ethanol. This parameter is of particular interest because of its numerous applications and it permits the completion of the fluorometric data for these pharmacologically and biologically important alkaloids. We determined also the quantum yields, ϕ, of the Harmala alkaloids; those corresponding to the Rauwolfia alkaloids have been determined previously [8, 9]. The fluorescence natural lifetimes, τ_0, were calculated by using a modified form of the Strickler–Berg equation, proposed by Birks and Dyson. In a previous com-

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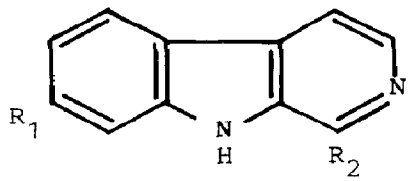
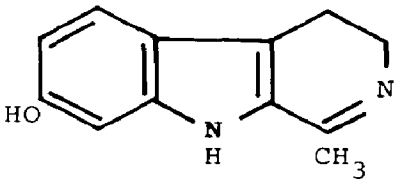
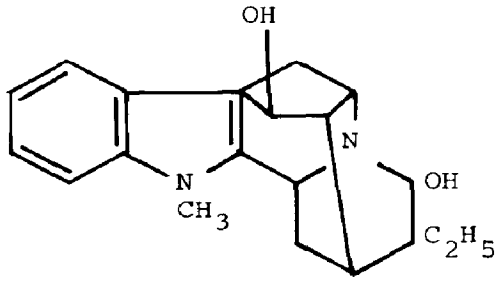


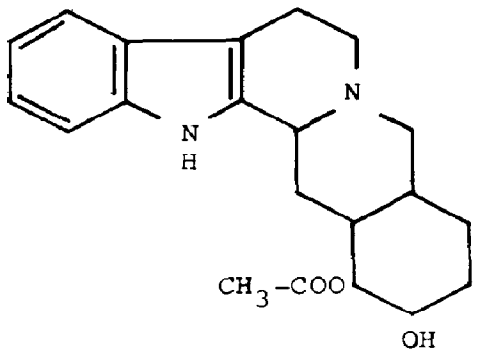
Norharman, $R_1 \equiv R_2 \equiv H$
Harmane, $R_1 \equiv H$; $R_2 \equiv CH_3$
Harmol, $R_1 \equiv OH$; $R_2 \equiv CH_3$



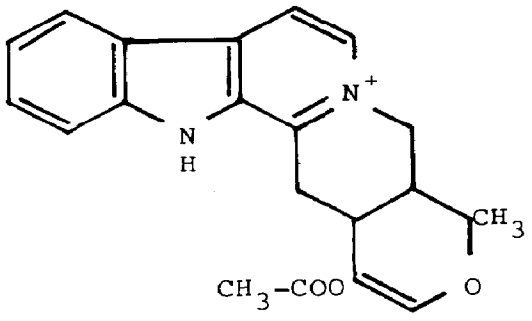
Harmalol



Ajmaline



Yohimbine
Corynanthine (cis isomer of yohimbine)



Serpentine

Scheme 1.

munication [10] we have reported the calculated τ_0 values of some Rauwolfia alkaloids. A comparison between these values and the values measured in the present work has been made.

2. Experimental details

2.1. Materials

Harmala alkaloids were purchased from EGA-Chemie and the Rauwolfia alkaloids were kindly supplied by C. H. Boehringer Sohn, Ingelheim. All the alkaloids were of the best available quality and were used as received. Ethanol

(Merck) was used as solvent for fluorometry. The other chemicals were reagent grade sulphuric acid and U.S.P. quinine bisulphate.

2.2. Apparatus

Absorption spectra were recorded using a Perkin–Elmer Lambda 5 spectrophotometer. Fluorescence spectra were recorded using a Perkin–Elmer 650-40 spectrophotofluorometer. A Perkin–Elmer Data Processor 650-0178 was used to obtain corrected spectra. The wavelengths of excitation and emission were calibrated against the xenon line emission spectrum. The sensitivity and stability were checked by using the Raman band of distilled water. Fluorescence decay curves were measured with a computerized Applied Photophysics nanosecond spectrometer. The system for lifetime measurements consists of the nanosecond flashlamp, an excitation monochromator and a photomultiplier. The electronic processing equipment for lifetime measurements uses NIM modules in conjunction with a multichannel analyser and the system has a digital computer for data processing.

All measurements were made at 25 °C and fresh nitrogen-bubbled dilute solutions were employed.

3. Results and discussion

3.1. Determination of quantum yields

The corrected excitation and emission spectra of the Harmala alkaloids were recorded as the relative number of quanta per unit wavelength interval *vs.* wavelength. Rhodamine B was the standard employed for the quantum counter. The fluorescence quantum yields at 25 °C were determined by comparing the corrected emission spectra with the spectrum of a fluorescence standard (quinine bisulphate) in optically dilute solutions [10, 11] using the following equation:

$$\phi_x = \phi_r \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \frac{I(\lambda_r)}{I(\lambda_x)} \frac{D_x}{D_r} \frac{n_x^2}{n_r^2} \quad (1)$$

where the subscripts r and x refer to the reference and the unknown solutions, ϕ is the quantum yield, $A(\lambda)$ is the absorbance per centimetre of the solution at the excitation wavelength, $I(\lambda)$ is the relative intensity of the exciting light at the wavelength λ, D is the integrated area under the corrected emission spectrum and n is the average refractive index of the solution at the maximum luminescence.

The areas under the corrected emission spectra were calculated by connecting a microprocessor-based data acquisition system to the spectrofluorometer using an especially written program. In order to diminish errors due to re-absorption and re-emission, dilute solutions of the standard and of the alkaloids with comparable absorbances of about 0.01 cm^{-1} were used. Also, the solutions were bubbled with nitrogen to avoid quenching by dissolved oxygen.

TABLE 1

Comparison of experimental and calculated values of natural lifetimes in ethanol

Compound	$10^{-3}\int\epsilon(\bar{\nu})\,d\bar{\nu}/\bar{\nu}$	$10^{13}\langle\bar{\nu}_f^{-3}\rangle_{av}$	n_a	n_f	$(\tau_0)_{cal}$ (ns)	τ (ns)	ϕ	$(\tau_0)_{exp}$ (ns)	$\alpha = \frac{(\tau_0)_{cal}}{(\tau_0)_{exp}}$
Norharman	0.59	0.57	1.38	1.38	18.0	3.45	0.16	21.5	0.84
Harmane	0.60	0.56	1.38	1.38	17.2	3.08	0.17	18.1	0.95
Harmalol	2.70	1.26	1.38	1.37	8.7	4.51	0.39	11.6	0.75
Harmol	2.80	0.83	1.38	1.37	5.5	4.14	0.49	8.5	0.65
Yohimbine	1.06	0.46	1.40	1.38	8.1[c]	5.70	0.40[a]	14.2	0.57
Corynanthine	1.06	0.46	1.40	1.38	8.1[c]	5.40	0.34[a]	15.8	0.52
Serpentine	0.57	0.95	1.38	1.37	31.0[c]	15.1	0.63[b]	24.0	1.29
Ajmaline	0.27	0.48	1.39	1.38	33.1[c]	4.10	0.10[a]	41.0	0.81

[a] From ref. 8.
[b] From ref. 9.
[c] From ref. 10.

The fluorescence emission spectra of the standard and the unknown solutions were measured with the same slit arrangement and at the same excitation wavelength (350 nm). Quinine bisulphate in 0.1 N sulphuric acid appears to be an appropriate standard [12]. Melhuish's value [13] of 0.546 at 25 °C was used for the absolute quantum yield of quinine bisulphate. The values of the refractive index were taken from ref. 14. The quantum yields are reported in Table 1.

3.2. Lifetime measurements

When the decay time is short (less than 15 ns) and a nanosecond flash-lamp is used, the experimental data are significantly distorted by the finite decay time of the lamp pulse. Since the measured decay function is a convolution of the true fluorescence decay, it is necessary to analyse the data by deconvolution to extract the fluorescence lifetime [15].

In the analysis of data, statistical criteria have been applied, the values of reduced χ^2 and Durbin–Watson parameters being the most important [16, 17].

For validation of the system, fluorescence standards such as quinine and fluorescein were utilized. Typical values obtained are 18.5 ns and 4.6 ns for the lifetime of quinine in 0.1 N H_2SO_4 and fluorescein in ethanol respectively. The measured actual lifetimes are shown in Table 1.

3.3. Fluorescence natural lifetime calculations

The natural or radiative lifetimes, τ_0, are related theoretically to the absorption band area [18]. Strickler and Berg [19] have developed a theory and proposed an equation which is applicable to polyatomic molecules under certain conditions. Birks and Dyson [20] have derived a modified form of the Strickler–Berg equation:

$$\frac{1}{\tau_0} = 2.88 \times 10^{-9} \frac{n_f^3}{n_a} \langle \bar{\nu}_f^{-3} \rangle_{av}^{-1} \int \epsilon(\bar{\nu}) \frac{d\bar{\nu}}{\bar{\nu}} \tag{2}$$

where

$$\langle \bar{\nu}_f^{-3} \rangle_{av}^{-1} = \frac{\int F(\bar{\nu})\, d\bar{\nu}}{\int F(\bar{\nu}) \bar{\nu}^{-3}\, d\bar{\nu}} \tag{3}$$

$F(\bar{\nu})$ is the fluorescence intensity in units of relative numbers of quanta at each frequency, n_f is the mean refractive index of the solvent over the fluorescence band, n_a is the mean refractive index of the solvent over the absorption band and $\epsilon(\bar{\nu})$ is the molar absorption coefficient.

The integral was calculated by using Simpson's method applied to specific bands. Previously the spectra were deconvoluted into individual bands by a least-squares program, assuming a log-normal shape [21]. In Figs. 1 and 2 the corrected excitation and emission spectra for harmane and yohimbine are shown as examples. The refractive index values, n_f and n_a in eqn. (2), for ethanol were taken from International Critical Tables [14].

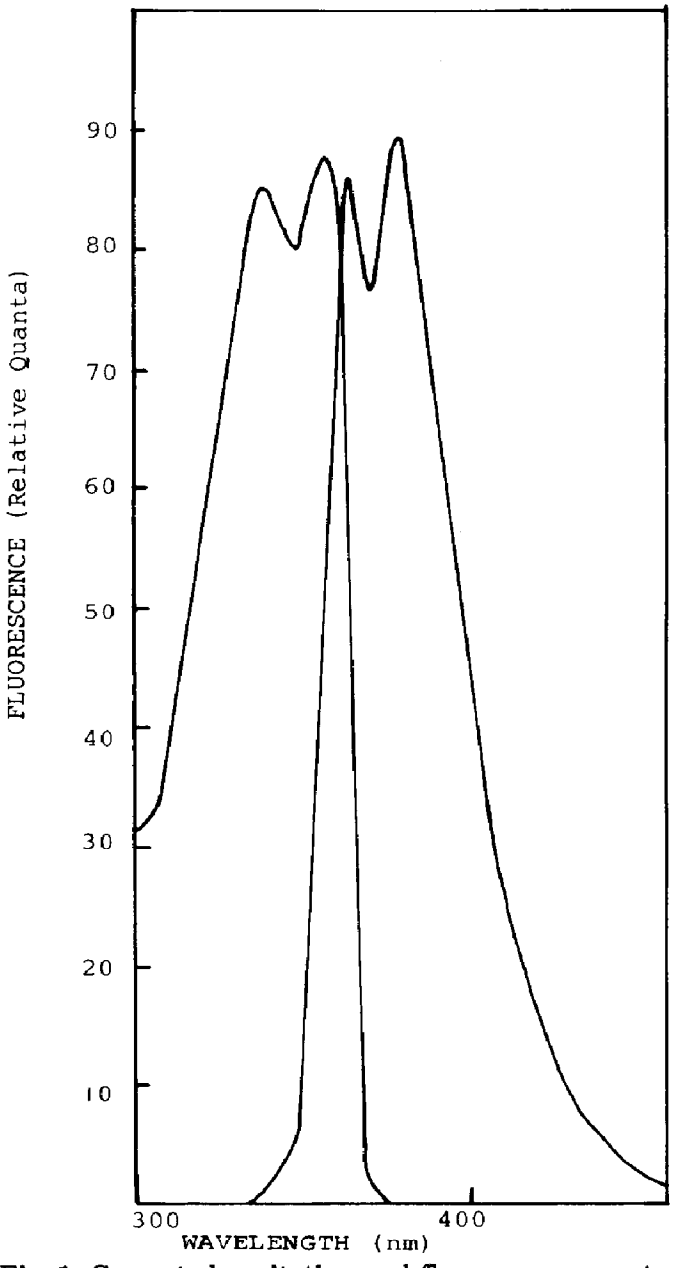


Fig. 1. Corrected excitation and fluorescence spectra of harmane in ethanol.

The results are reported in Table 1. Columns 2 - 5 list the quantities used to determine $(\tau_0)_{cal}$, calculated from eqn. (2). Columns 7 and 8 give the measured values of τ and ϕ used to evaluate the radiative lifetime $(\tau_0)_{exp}$. Finally, column 10 lists the values of $\alpha = (\tau_0)_{cal}/(\tau_0)_{exp}$.

The radiative lifetime $(\tau_0)_{exp}$ was determined from the most important parameters which characterize the fluorescence emission, *i.e.* the lifetime τ and the quantum yield ϕ:

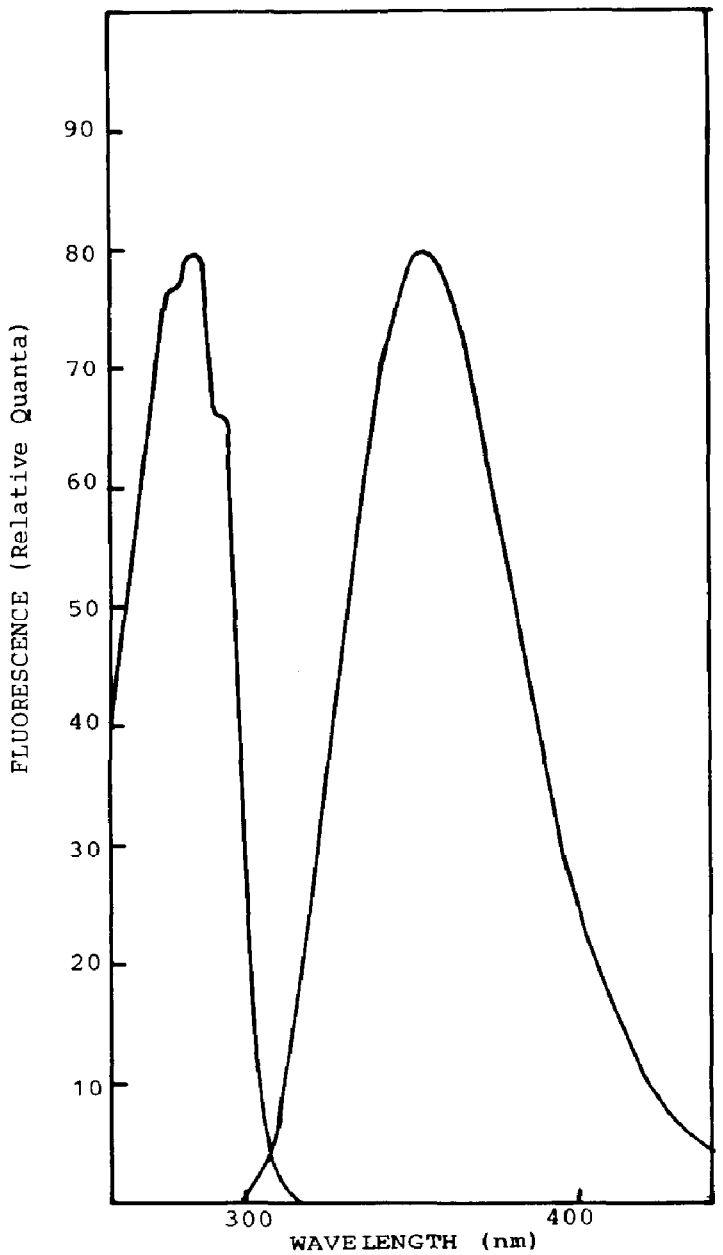


Fig. 2. Corrected excitation and fluorescence spectra of yohimbine in ethanol.

$$(\tau_0)_{exp} = \frac{\tau}{\phi} \tag{4}$$

The calculated $(\tau_0)_{cal}$ value is observed to be smaller than the experimental $(\tau_0)_{exp}$ value except for serpentine ($\alpha = 1.29$). For norharman, harmane and ajmaline, which satisfy the mirror-image relation (see Fig. 1 as an example for harmane), the difference is within the experimental error. However, the α values for the other alkaloids differ greatly from unity. This

could be due to the fact that the mirror-image relation breaks down as shown for yohimbine in Fig. 2.

A straightforward comparison between our lifetime measurements and those reported in the literature cannot be made. The only data for the β-carboline (norharman) and for the 2-methyl-β-carboline have been obtained in aqueous solutions at different pH and they oscillate between 1.32 and 22.9 ns [22]. As can be seen in Table 1, our values change from 3 to 6 ns, except for serpentine (15 ns) which has also a high fluorescence. As is well known, the lifetime values depend on the solvent and media acidity. In fact, a change from 6 ns (ethanol) to 12 ns (water) has been observed for other indole derivatives.

However, the lifetimes of lysergic acid and some related ergolines, which are also indole alkaloids, are between 4 and 6 ns in ethanol [23]. In spite of the different structural characteristics, the values agree with those obtained in the present work.

Acknowledgments

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