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Short Communication

Fluorescence characteristics of Rauwolfia alkaloids in highly concentrated sulphuric acid solutions. Fluorimetric determination of reserpiline

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Introduction

During an investigation [1] of the protonation equilibria of the indole ring of some pharmacologically active Rauwolfia alkaloids in concentrated acid solutions, the fluorescence characteristics of the protonated species were noted. Although the native fluorescence of these alkaloids has been thoroughly reported [2–6], to our knowledge, no study appears to have been carried out on their fluorescence properties in strongly acidic solutions.

Hence, the principal purpose of this study was to examine the fluorescence behaviour of Rauwolfia alkaloids in strongly acidic solutions, to determine whether it might form the basis of an analytical method [7–12]. Quantitative procedures for these alkaloids usually involve measurements of their native fluorescence or fluorescence induced by chemical reactions. Rauwolfia alkaloids have long attracted interest because of their pharmacological properties [13] as anti-hypertensive, tranquillizer and sedative agents.

Experimental

Apparatus

Fluorescent measurements were made on a Perkin–Elmer Model 650-40 spectrophotofluorometer. Spectra were obtained using a Perkin–Elmer Model 057 X–Y recorder. A Perkin–Elmer Data Processor 650-0178 was used to obtain corrected spectra. Sensitivity and stability were checked by using the Raman band of distilled water. The wavelengths

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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. UV absorbance measurements were made with a Perkin–Elmer Model Lambda 5 spectrophotometer.

Chemicals

Alkaloids and all the other chemicals were of the best available quality and were used without further purification. The alkaloids were shown to be pure by T.L.C. analysis performed as previously described [14], using Kieselgel GF (Merck) with *n*-butanol–glacial acetic acid–water as developing solvent and UV detection.

Standard solutions and procedures

Stock solutions, 1×10^{-3} M, of the alkaloids in methanol were freshly prepared prior to use. An aliquot of 50 μl of each stock solution was pipetted into 5 ml of either methanol or 80% sulphuric acid solution and, after thoroughly mixing, the fluorescence spectra were registered. Working standard solutions for the fluorimetric assay of reserpiline were prepared by suitable dilutions of the stock solution of reserpiline with methanol. After mixing with 5 ml of 60% sulphuric acid solution, their fluorescences were measured at λ_{exc} of 320 nm and λ_{em} of 530 nm. The slit widths were 4 and 6 nm for excitation and emission respectively. These measurements were made at room temperature.

Results and Discussion

The alkaloid solutions in concentrated sulphuric acid solutions were usually sufficiently stable for UV spectrophotometric measurements to be made because the UV-spectra changed only slightly over 5–10 min periods. However, their relative intensities of fluorescence were dependent on time although the excitation and emission maxima did not change. The time required to obtain a stable intensity of fluorescence was dependent on the sulphuric acid concentration. At least 4 h was required for stabilization in 80–90% sulphuric acid solution whilst only 10–20 min was required with 60% sulphuric acid solution (Fig. 1). After stable intensities of fluorescence were attained, longer times did not affect the fluorescence spectra.

As stated previously [1], Rauwolfia alkaloids undergo protonation equilibria on their indole nucleus and display their basic properties only in highly acidic solutions. The first column of Table 1 shows the pKa values which were calculated with reference to the H_I acidity function for indole protonation established by Hinman and Lang [15], as well as the approximate sulphuric acid concentrations necessary for half neutralization in parentheses. Protonation causes important changes in the UV spectra of the alkaloids [1]. Unprotonated alkaloids showed two main absorption bands with maxima around 200–220 nm and 270–290 nm, these latter bands possessing in some instances secondary peaks or inflections. Upon protonation, the first band lost intensity or disappeared while the second band shifted bathochromically to 305–320 nm. Moreover, bands around 266 nm, which are typical of indolenines, appeared upon protonation.

The results obtained in this study indicate that protonation also causes important changes in the fluorescence spectra. Table 1 summarizes the wavelengths of excitation and emission maxima of these spectra. The native fluorescence spectra are also reported for comparison. Examination of the data in Table 1 indicates similarities when the spectra of the different alkaloids are compared. Thus yohimbine, coryanthine and

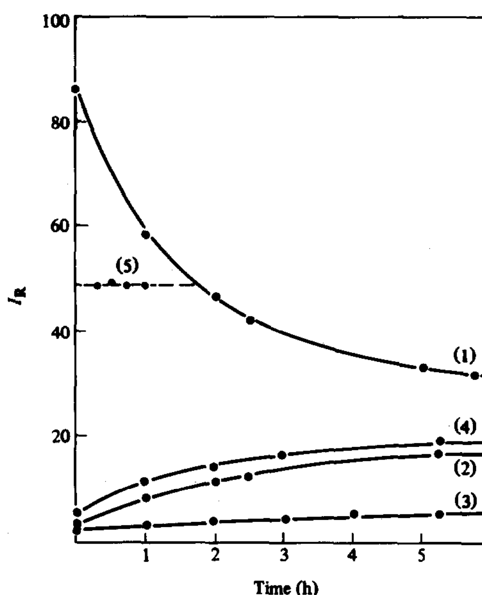


Figure 1
Variation of relative fluorescence intensity (I_R) with time. (1) Reserpiline–80% H_2SO_4, (2) reserpine–80% H_2SO_4, (3) yohimbine–80% H_2SO_4, (4) rescinnamine–80% H_2SO_4, (5) reserpiline–60% H_2SO_4.

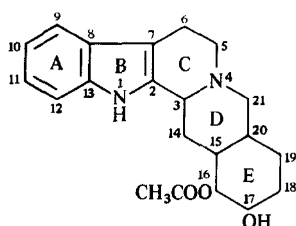
Table 1
pK_a values, excitation and fluorescence maxima (in nm) and fluorescence quantum yields (Q) at 25°C of Rauwolfia alkaloids

		Fluorescence spectra				
		Native*		80% H_2SO_4		
Compound	pK_a	λ_{exc}†	λ_{flu}†	λ_{exc}	λ_{flu}	Q‡
Yohimbine	−8.30 (12.4M)	282	352	265, 305	357	
Corynanthine	—	282	351	265, 305	356	
Ajmalicine	−8.31 (12.4M)	282	352	265, 305	355	
Reserpine	−9.26 (13.7M)	271, 295sh	360	265, 317	520	0.01
Rescinnamine	—	303	436	266, 318	525	
Reserpiline	−7.26 (10.8M)	303	335	270, 320	530	0.07

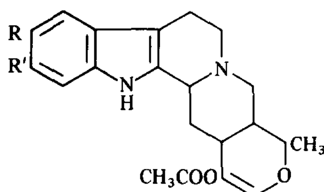
*Ref. [6].
†In methanol.
‡In 60% H_2SO_4. Calculated as in Ref. [6].

ajmalicine, which are not methoxy-substituted on the indole ring, show fluorescence spectra in 80% sulphuric acid that are practically identical. The ester alkaloids reserpine and rescinnamine which possess a C_{11} methoxy group also have closely related spectra.

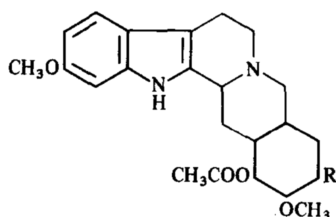
As can be seen from Scheme 1, the structures of the alkaloids differ principally in the substitution on the C_{10} and C_{11} positions and in the *cis-trans* conformation of C/D and D/E rings. Apparently, only methoxy substitution on the indole ring is important because the C/D and D/E conformations and other substituents in the E ring do not influence the fluorescence properties. This clearly indicates that the nucleus of the electronic transitions leading to fluorescence emission is situated in the indole ring nucleus. Substitution of methoxy groups on the indole ring [16, 17] results in an



	C_{16}	C/D	D/E	Conformation
Yohimbine	α	*trans*	*trans*	
Corynanthine	β	*trans*	*trans*	



	C_{10}	C_{11}	C/D	D/E
Ajmalicine	H	H	*trans*	*cis*
Reserpiline	OCH_3	OCH_3	*cis*	*cis*



	R	C/D	D/E
Reserpine	TMB	*cis*	*cis*
Rescinnamine	TMC	*cis*	*cis*

TMB —OOC (ring with OCH_3, OCH_3, OCH_3)

TMC —OOC—CH=CH (ring with OCH_3, OCH_3, OCH_3)

alteration of the fluorescent properties of the molecule by directly influencing the π-electrons of the aromatic system.

On the other hand protonation usually weakened the relative intensities of fluorescence. Only reserpiline fluoresced more intensively in concentrated sulphuric acid solutions than in methanol solutions. The fluorescence quantum yields (Q) of reserpine and reserpiline, taken as model compounds, are reported in Table 1. This means that the analytical value of the fluorescence emission of Rauwolfia alkaloids in strongly sulphuric acid solutions is limited, except for reserpiline. This alkaloid has weak native fluorescence [6] that is increased in these media.

To assess the utility of this fluorimetric method, reserpiline standards were prepared and their fluorescences were measured as described previously. A plot of fluorescence intensity against concentration in the range (1×10^{-6}–10×10^{-6} M) was linear and passed through the origin. Under these conditions the detection limit was calculated to be 0.3 ng ml^{-1}.

The precision of the proposed method was determined as the variability obtained upon repeated analyses of a reserpiline standard. A relative standard deviation of a 5×10^{-6} M reserpiline standard in replicate analysis was less than 4%. Standard mixtures of reserpiline and each of the other alkaloids were prepared and analysed for reserpiline. No interference was noted.

Conclusion

The results indicate that reserpiline can be assayed by fluorescence in highly acidic solutions. The method is simple, sensitive, and has a high degree of specificity. Reserpiline is a common contaminant of commercial reserpine–rescinnamine preparations, increasing the apparent reserpine–rescinnamine content determined by the U.S.P. [18] or A.O.A.C. [19] method. This method can be employed for the determination of reserpiline in those preparations.

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