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LUMINESCENCE SPECTRA OF YOHIMBINE, RESERPINE AND RELATED ALKALOIDS

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Summary

Fluorescence and phosphorescence spectra of yohimbine, reserpine, rescinnamine, corynanthine, ajmalicine, reserpic acid and 6-methoxyindole were measured in solution at 298 and 77 K. The data obtained provide a basis for the analytical determination of yohimbine and reserpine in low concentrations. Insight is afforded into quenching processes which degrade emission in reserpine and rescinnamine molecules. The lowest triplet state of reserpine is located and characterized.

1. Introduction

The indole alkaloids yohimbine and reserpine have long attracted interest on account of their pharmacological properties. Yohimbine is an adrenergic blocking agent which has been used medically in the treatment of angina pectoris and arteriosclerosis, and as a veterinary aphrodisiac. Reserpine is well known as an antihypertensive, tranquillizer and sedative. The drug has acquired some notoriety in recent years on the turf where it has been used illicitly to affect the performance of race horses.

In the work now described we have made a systematic study of the luminescence spectra of yohimbine, reserpine, rescinnamine and some of their derivatives, together with the related alkaloids corynanthine and ajmalicine. The spectral data obtained have afforded excited state parameters which are of value for the identification and determination of these compounds in very low concentrations in solution. This investigation has also afforded insight into intramolecular quenching processes which are operative in excited reserpine and rescinnamine molecules. In addition we have been able to locate and characterize the emitting triplet state of reserpine.

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2. Experimental details

The techniques, spectroscopic equipment and spectral corrections employed have been described elsewhere [1, 2].

3. Quantum yield determinations and procedure

The spectral quantum yields of the luminescence were determined as described previously [2].

4. Results and discussion

Spectral measurements were made on ethanolic solutions of yohimbine (I) (Fig. 1), corynanthine (II) (Fig. 1), ajmalicine (III) (Fig. 1), reserpine (Va) (Fig. 2), rescinnamine (Vb) (Fig. 2) and reserpic acid (Vc) (Fig. 2). The spectra of indole (IV) (Fig. 1) and 6-methoxyindole (VII) (Fig. 2) were used as a basis for comparison with their respective parent alkaloids.

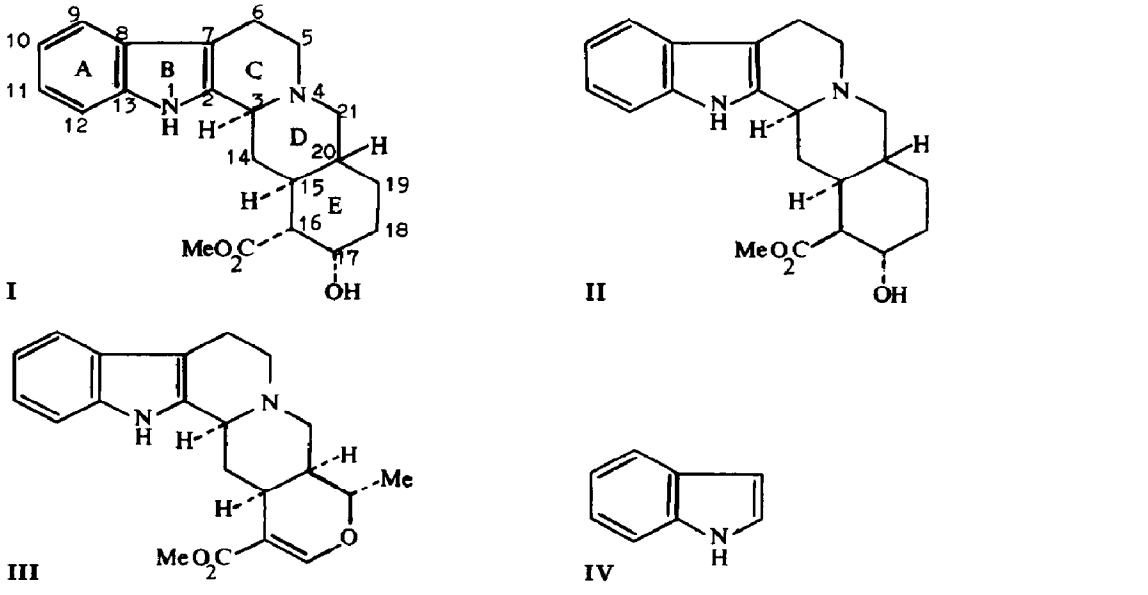

Fig. 1. Chemical structures of yohimbine (I), corynanthine (II), ajmalicine (III) and indole (IV).

4.1. Stereochemistry

The compounds studied fall into two major conformational series.

The yohimbine series, here comprising yohimbine, corynanthine and ajmalicine, is derived from the unsubstituted indole nucleus and possesses

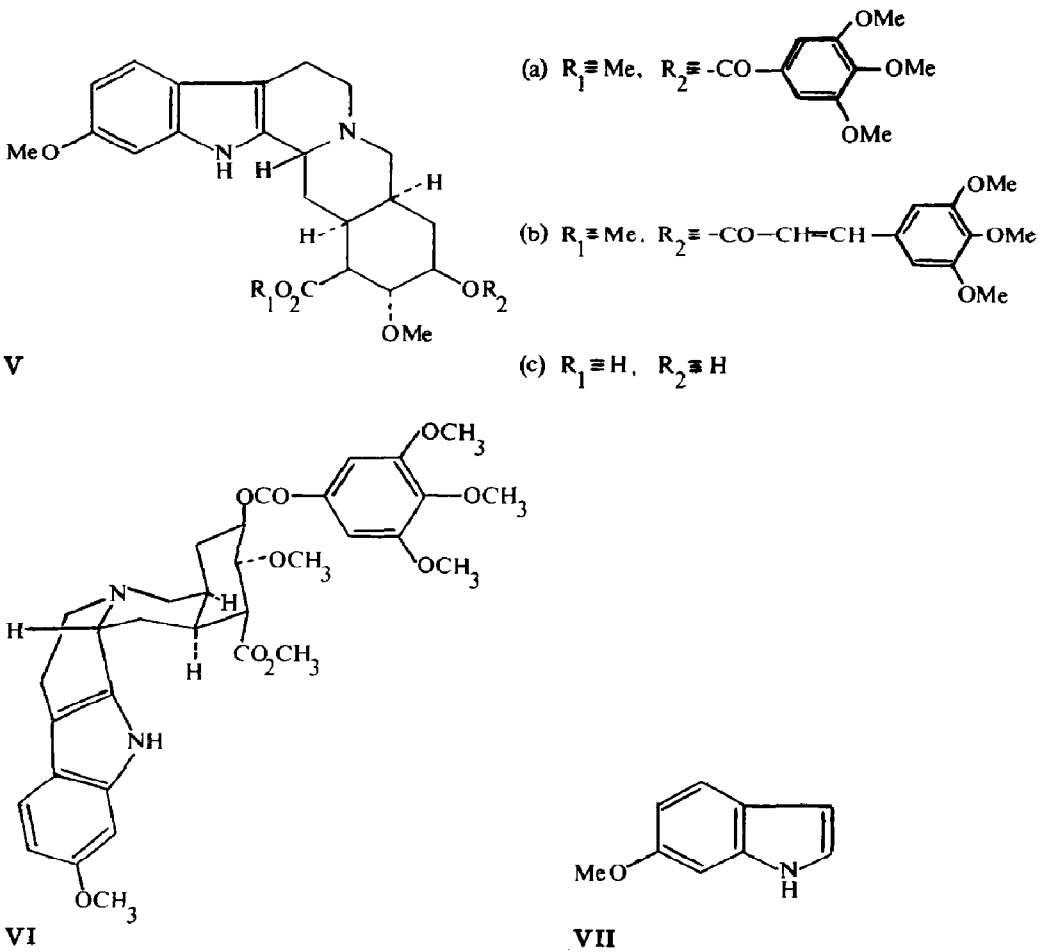

Fig. 2. Chemical structures of reserpine (Va), rescinnamine (Vb), reserpic acid (Vc), the preferred conformation of reserpine (VI) and 6-methoxyindole (VII).

the common structural feature of *trans*-fused chair D and E rings (ajmalicine is anomalous as it has a *cis*-fused D–E ring junction [3]). This conformation has the effect of extending the molecule. A further feature of significance lies in the stereochemistry of the hydrogen atom at position 3. This hydrogen atom lies below the plane of the ring system in the three foregoing alkaloids, and in consequence it adopts the α axial configuration.

The reserpine series includes reserpine itself, rescinnamine and reserpic acid. All three of these molecules derive from the 6-methoxyindole nucleus and possess *cis*-fused D and E rings. Significantly, this conformation folds the molecule about the C(15)–C(20) axis. The hydrogen atom at position 3 now lies above the ring plane in the β equatorial configuration. Stereochemical differences also arise in respect of the substituents in ring E, but these are not relevant in the context of this study.

4.2. Absorption spectra [4]

4.2.1. Yohimbine series

In the yohimbine series the absorption spectra reveal in each case the characteristic spectrum of the unsubstituted indole nucleus. The absorption spectrum of yohimbine (Fig. 3, curve A) is typical. The moderately intense peaks at λ_{max} = 273 nm (36 630 cm^{-1}) (ϵ = 8606 dm^3 mol^{-1} cm^{-1}), λ_{max} = 279 nm (35 842 cm^{-1}) (ϵ = 8555 dm^3 mol^{-1} cm^{-1}) and λ_{max} = 289 nm (34 602 cm^{-1}) (ϵ = 7153 dm^3 mol^{-1} cm^{-1}) closely resemble those of indole. The corresponding absorption bands at around 37 000 cm^{-1} and 35 000 cm^{-1} in the indole molecule have been assigned to ^1L_a and ^1L_b transitions respectively by analogy with the isoelectronic molecules naphthalene and quinoline and also with styrene [5].

The absorption spectra of corynanthine (λ_{max} = 283 nm, ϵ = 7468 dm^3 mol^{-1} cm^{-1}; λ_{max} = 290 nm, ϵ = 6311 dm^3 mol^{-1} cm^{-1}) and ajmalicine (λ_{max} = 283 nm, ϵ = 9460 dm^3 mol^{-1} cm^{-1}; λ_{max} = 291 nm, ϵ = 8043 dm^3 mol^{-1} cm^{-1}) were almost identical with that of yohimbine.

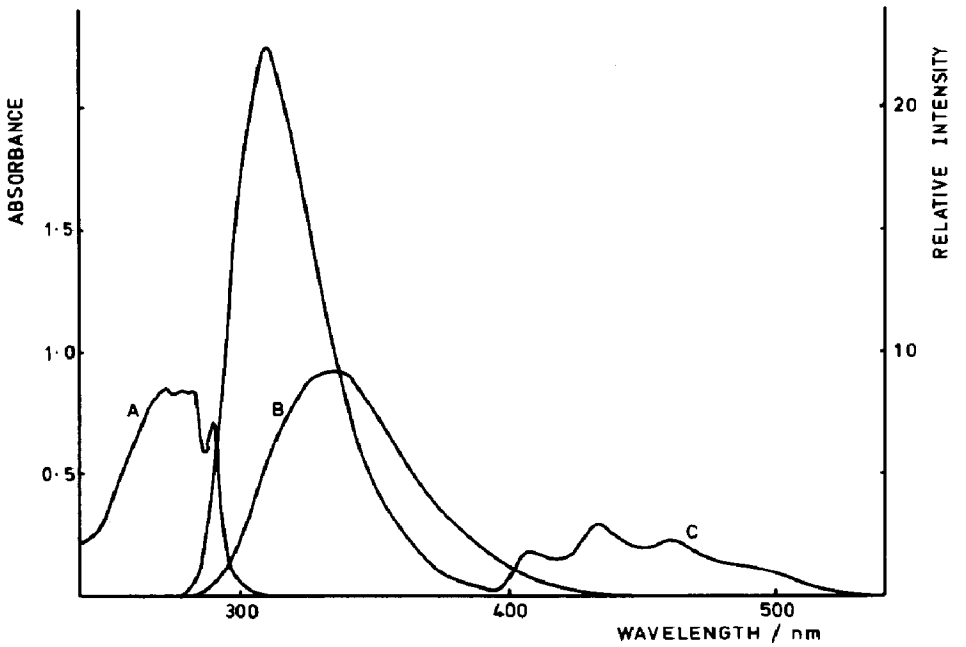

Fig. 3. Absorption spectrum at 298 K (curve A), fluorescence spectrum at 298 K (curve B) and fluorescence and phosphorescence spectrum at 77 K (curve C) of yohimbine in 95% aqueous ethanol.

4.2.2. Reserpine series

In the reserpine series the absorption spectra embody the spectral characteristics of the parent 6-methoxyindole system which is modified in reserpine and rescinnamine by the trimethoxybenzoyl and trimethoxycinnamoyl ester substituents at position 18. The absorption spectrum of 6-methoxyindole (Fig. 4) displays the three characteristic indole bands

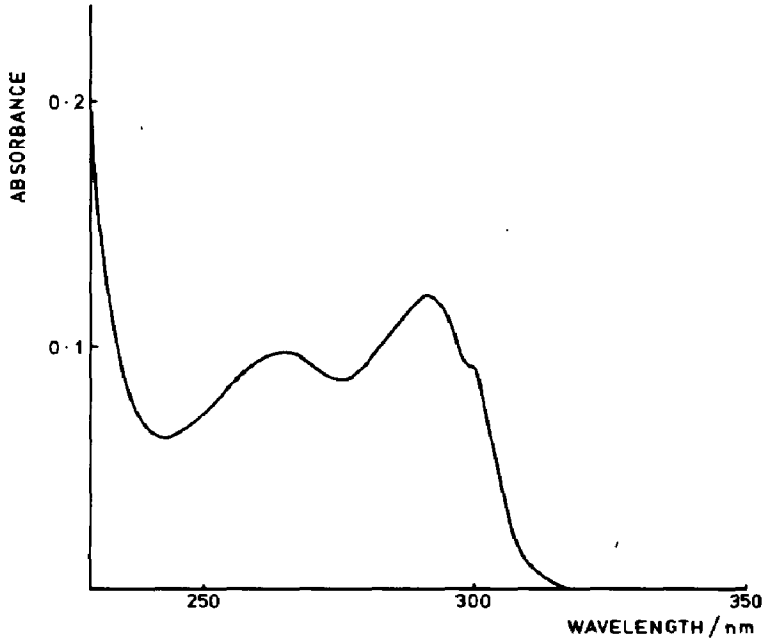

Fig. 4. Absorption spectrum of 6-methoxyindole in 95% aqueous ethanol.

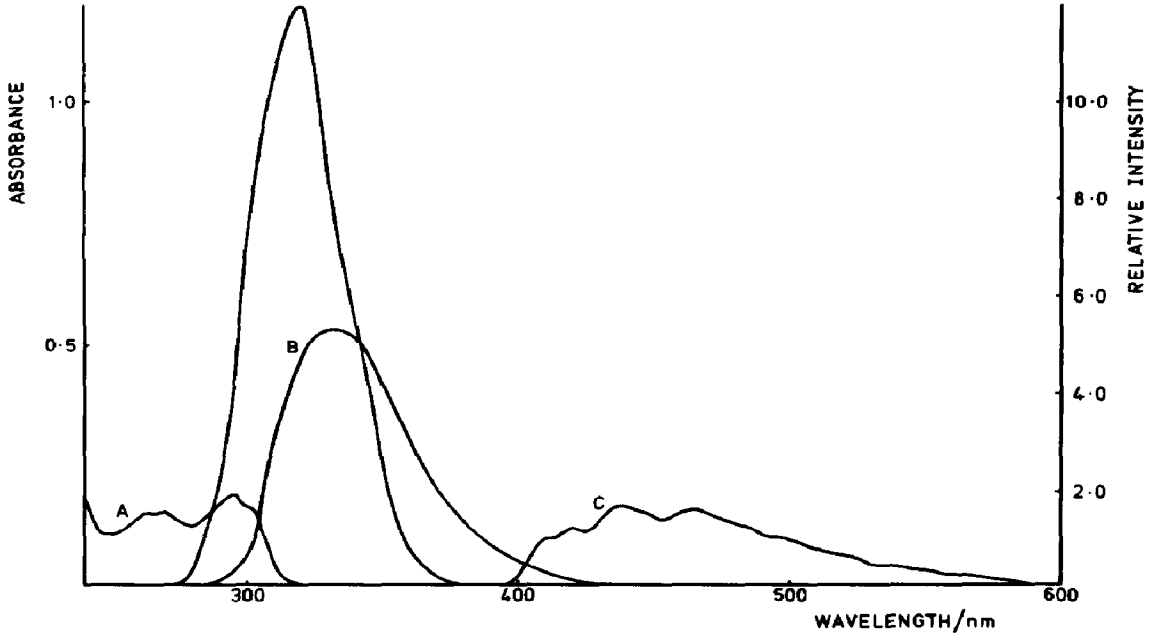

Fig. 5. Absorption spectrum at 298 K (curve A), fluorescence spectrum at 298 K (curve B) and fluorescence and phosphorescence spectrum at 77 K (curve C) of reserpic acid in 95% aqueous ethanol.

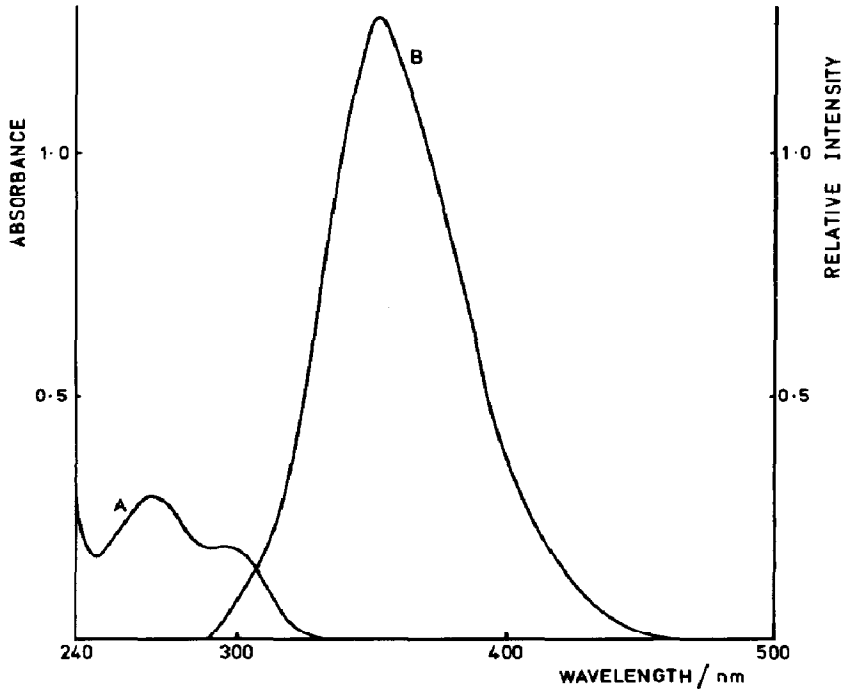

Fig. 6. Absorption spectrum (curve A) and fluorescence spectrum (curve B) at 298 K of reserpine in 95% aqueous ethanol.

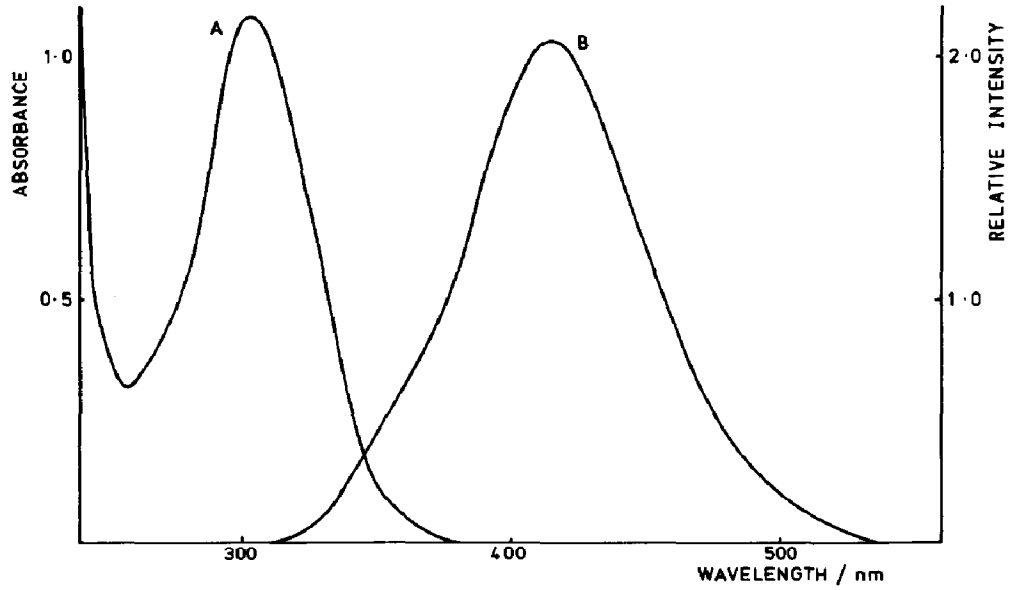

Fig. 7. Absorption spectrum (curve A) and fluorescence spectrum (curve B) at 298 K of rescinnamine in 95% aqueous ethanol.

shifted to the red by the 6-methoxy substituent (λ_{max} = 274 nm, ϵ = 3486 $dm^3\ mol^{-1}\ cm^{-1}$; λ_{max} = 292 nm, ϵ = 4531 $dm^3\ mol^{-1}\ cm^{-1}$; λ_{max} = 301 nm, ϵ = 3331 $dm^3\ mol^{-1}\ cm^{-1}$). These features are evident in the absorption

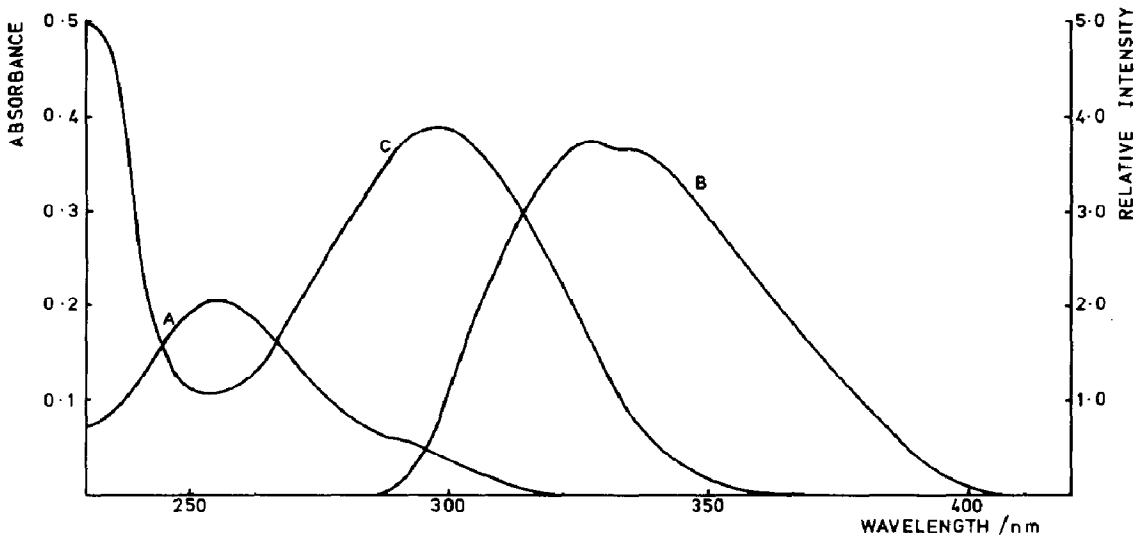

Fig. 8. Absorption spectrum of 3,4,5-trimethoxybenzoic acid (curve A), emission spectrum of 6-methoxyindole (curve B) and absorption spectrum of 3,4,5-trimethoxycinnamic acid (curve C) at 298 K in 95% aqueous ethanol.

spectrum of reserpic acid (Fig. 5, curve A) which shows peaks at λ_{max} = 271 nm (ϵ = 5949 dm^3 mol^{-1} cm^{-1}), λ_{max} = 295 nm (ϵ = 7465 dm^3 mol^{-1} cm^{-1}) and λ_{max} = 303 nm (ϵ = 6143 dm^3 mol^{-1} cm^{-1}). However, in the absorption spectrum of reserpine (Fig. 6, curve A), which shows peaks at λ_{max} = 269 nm (ϵ = 14 612 dm^3 mol^{-1} cm^{-1}) and λ_{max} = 297 nm (ϵ = 9488 dm^3 mol^{-1} cm^{-1}), the intense absorption of the trimethoxybenzoyl ester group at C(18) submerges the weaker 6-methoxyindole spectrum. A similar situation obtains in rescinnamine (Fig. 7, curve A), which shows a peak at λ_{max} = 304 nm (ϵ = 26 438 dm^3 mol^{-1} cm^{-1}), where the intense absorption of the trimethoxycinnamoyl group is predominant. The absorption spectra of 3,4,5-trimethoxybenzoic acid (λ_{max} = 256 nm, ϵ = 6664 dm^3 mol^{-1} cm^{-1}) and 3,4,5-trimethoxycinnamic acid (λ_{max} = 297 nm, ϵ = 14 852 dm^3 mol^{-1} cm^{-1}) are shown in Fig. 8.

4.3. Emission spectra [6]

4.3.1. Yohimbine series

The emission spectra of yohimbine, corynanthine and ajmalicine (Table 1) display similar features to the emission spectra of indole itself. The fluorescence spectrum of yohimbine at 298 K (Fig. 3, curve B) is typical. This singlet emission, which is excited at 279 nm, appears as a single peak of moderate intensity (λ_{max} = 330 nm, ϕ_f = 0.29) corresponding to a Stokes shift of 5539 cm^{-1} in the ethanolic solution employed. The emission is intensified at 77 K (λ_{max} = 312 nm, ϕ_f = 0.43). The blue shift of 1748 cm^{-1} on cooling to this temperature is normal for indole derivatives in ethanol. The phosphorescence spectrum of yohimbine [7] (Fig. 3, curve C) displays well-resolved bands characteristic of the indole nucleus (λ_{max} values of 407,

TABLE 1

Fluorescence and phosphorescence emission characteristics of yohimbine alkaloids in 95% aqueous ethanol

Compound	*Fluorescence (298 K)*		*Fluorescence (77 K)*		*Phosphorescence (77 K)*		
	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_p	τ_p (s)
Yohimbine (HCl) λ_{ex} = 279 nm	330	0.29	312	0.43	407, 432, 461	0.05	6.8
Corynanthine λ_{ex} = 290 nm	348	0.36	320	0.58	409, 435, 464	0.06	7.3
Ajmalicine λ_{ex} = 291 nm	347	0.06	320	0.91	411, 437	—	5.5

432 and 461 nm). The quantum yield of phosphorescence (ϕ_p = 0.05) is somewhat less than that of indole itself, but the triplet lifetime (τ_p = 6.8 s) and the singlet–triplet splitting ($\Delta\nu_{S_1-T}$ = 7255 cm^{-1}) lie very close to the values for indole. Similar data (Table 1) were obtained for the stereoisomer corynanthine and also for ajmalicine. We conclude that the excited states of the indole nucleus are not significantly modified by the heterocyclic ring structure CDE. The fact that the quantum yield of fluorescence and the lifetime of the triplet state of the indole nucleus are virtually unchanged is indicative of minimal non-radiative–vibrational deactivation in the rigid cyclic framework present in these molecules.

4.3.2. Reserpine series

The emission spectra of reserpic acid and 6-methoxyindole are very similar (Table 2). The fluorescence spectrum of reserpic acid at 298 K (Fig. 5, curve B) displays a single peak of high intensity (λ_{max} = 331 nm, ϕ_f = 0.68) which is characteristic of the 6-methoxyindole system. The effect of the methoxy substituent is to enhance the fluorescence intensity of the indole nucleus. This emission is intensified at 77 K (λ_{max} = 320 nm, ϕ_f = 0.95) and reveals the blue shift of fluorescence on cooling ($\Delta\nu$ = 1039 cm^{-1}) associated with indole derivatives. The phosphorescence spectrum of reserpic acid [8] (Fig. 5, curve C) displays bands (λ_{max} values of 412, 437 and 464 nm) characteristic of the triplet emission of the 6-methoxyindole nucleus. The quantum yield of phosphorescence (ϕ_p = 0.14) lies quite close to that of 6-methoxyindole (ϕ_p = 0.21) and the values of ϕ_p/ϕ_f are of the same order (about 0.2). The phosphorescence lifetimes of reserpic acid (τ_p = 3.7 s) and 6-methoxyindole (τ_p = 3.4 s) are also very similar. These long lifetimes, together with the large singlet–triplet splitting $\Delta\nu_{S_1-T} \approx 10\,000$ cm^{-1}), are consistent with the $\pi\pi^*$ nature of the emitting triplet states. The close similarity between the excited state parameters of reserpic acid and 6-methoxyindole leads us to conclude that the ring structure CDE in reserpic

TABLE 2

Fluorescence and phosphorescence emission characteristics of reserpine and some related compounds in 95% aqueous ethanol

Compound	*Fluorescence (298 K)*		*Fluorescence (77 K)*		*Phosphorescence (77 K)*		
	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_p	τ_p (s)
6-methoxyindole	332	0.95	317	0.79	408, 434, 463	0.21	3.4
Reserpic acid	331	0.68	320	0.95	412, 437, 464	0.14	3.7
Reserpine	353	0.08	331	0.33	452	0.05	3.2
Rescinnamine	416	2.0×10^{-3}	382	5.6×10^{-3}	—	—	—
3,4,5-trimethoxybenzoic acid	345	0.09	322	0.23	445 (broad, very weak)	—	—
3,4,5-trimethoxycinnamic acid	407	4.0×10^{-3}	371	0.35	—	—	—

acid exerts no significant effect on the excited states of the 6-methoxyindole nucleus.

In reserpine and rescinnamine, however, the emission characteristics of the 6-methoxyindole nucleus are considerably modified by the presence of the trimethoxybenzoyl and cinnamoyl ester substituents at position 18. The fluorescence spectrum of reserpine (Fig. 6, curve B) at 298 K (λ_{max} = 353 nm) is weak ($\phi_f = 0.08$). This emission is strengthened on cooling to 77 K (λ_{max} = 331 nm, $\phi_f = 0.33$). The phosphorescence spectrum of reserpine is also of low intensity (λ_{max} = 452 nm, $\phi_p = 0.05$). This emission afforded a triplet lifetime of $\tau_p = 3.2$ s for the T_1–S transition.

The fluorescence spectrum of rescinnamine (Fig. 7, curve B) at 298 K (λ_{max} = 416 nm) appears at a longer wavelength than that of reserpine, and the emission is considerably weaker ($\phi_f = 2.0 \times 10^{-3}$). This emission is enhanced at 77 K but it is still weak (λ_{max} = 382 nm, $\phi_f = 5.6 \times 10^{-3}$). No phosphorescence was detectable.

It is evident from these emission parameters that quenching of the emitting excited states of the 6-methoxyindole nucleus occurs in reserpine and rescinnamine. This quenching is attributable to deactivation by the trimethoxybenzoyl and trimethoxycinnamoyl ester side chains. In reserpine and rescinnamine the *cis* configuration about the C(15)–C(20) axis (Fig. 2, (VI)) will favour proximity between the 6-methoxyindole nucleus and the trimethoxyphenyl groups. As a result efficient intramolecular energy transfer of the dipole–dipole type can arise provided that there is sufficient overlap between the absorption and emission bands of the chromophores concerned. Figure 8 shows that in reserpine the amount of overlap between the absorption band envelope of the trimethoxybenzoyl group (curve A) and the emission spectrum of the 6-methoxyindole nucleus (curve B) is small. Dipole–dipole energy transfer should therefore be minimal, and the reserpine molecule as a whole will display the spectral features and emission charac-

TABLE 3

Luminescence data illustrating intermolecular quenching in reserpic acid–trimethoxycinnamic acid solutions in 95% aqueous ethanol

Compound	*Concentration* ($\times 10^{-5}$ M)	*Fluorescence (298 K)*		*Phosphorescence (77 K)*	
		λ_{max} (nm)	*Relative intensity*	λ_{max} (nm)	*Relative intensity*
3,4,5-trimethoxycinnamic acid	5.02	408	0.8	—	—
Reserpic acid + 3,4,5-trimethoxycinnamic acid	5.01 + 5.02	346	14.9	437	0.13
Reserpic acid	5.01	332	57.4	438	0.38
Rescinnamine	5.36	416	1.2	—	—

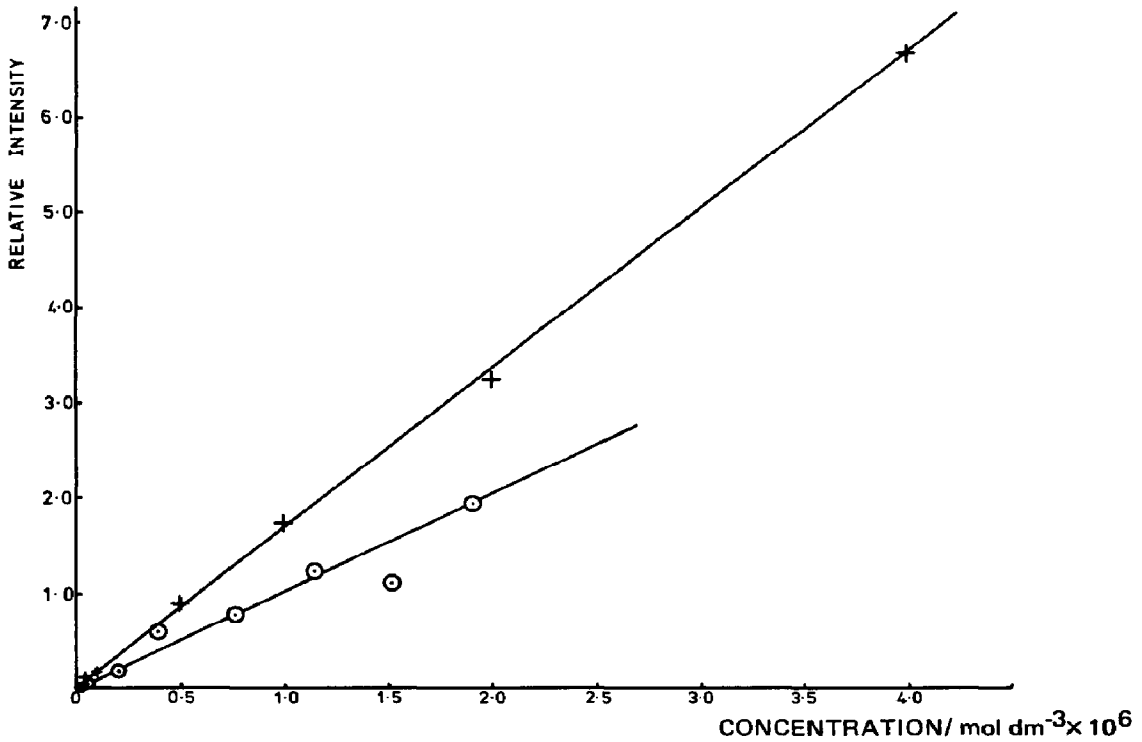

Fig. 9. Fluorescence intensity of reserpine (⊙) and yohimbine (+) *vs.* the concentration in 95% aqueous ethanol at 298 K.

teristics of 6-methoxyindole, modified somewhat by vibrational deactivation by the C(18) side chain.

In rescinnamine, however, the extent of band overlap is much greater (Fig. 8). In consequence excitation energy from the excited 6-methoxyindole nucleus can be transferred to the trimethoxycinnamoyl group, causing

quenching of the emission of the indole system. In the event the only detectable emission is a weak fluorescence deriving from the trimethoxycinnamoyl group itself. The degree of vibrational deactivation of the singlet state in this case is probably small because the fluorescence remains weak at 77 K. Luminescence measurements on equimolecular mixtures of reserpic acid and trimethoxycinnamic acid in solution also reveal some degree of quenching (Table 3), indicating that similar quenching to that observed in the rescinnamine molecule can also occur by *inter*molecular energy transfer, albeit less efficiently.

4.4. Analysis

Although reserpine shows relatively weak emission, its fluorescence at 298 K can be utilized as a means of analysis (Fig. 9). In solution in 95% ethanol, without further treatment, the minimum concentration of reserpine estimable was of the order of 1.6×10^{-8} mol dm^{-3} (*i.e.* 10 ng ml^{-1}). Yohimbine can also be estimated by this technique in somewhat higher concentrations, *i.e.* 5.1×10^{-8} mol dm^{-3} (20 ng ml^{-1}).

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