The Luminescence of Bisbenzyltetrahydroisoquinoline Alkaloids. The Berbamine and Oxyacanthine Alkaloids

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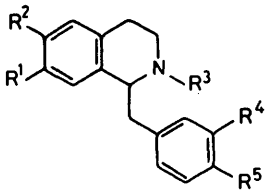
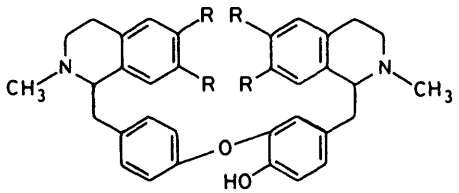
A detailed study of the fluorescence and phosphorescence characteristics of some bisbenzyltetrahydroisoquinoline alkaloids has been undertaken. The emission parameters have been found to depend upon the absolute configuration of the alkaloids. In only one case, hernandezine, has exciplex emission been observed.

THE largest group of isoquinoline alkaloids is the bisbenzylisoquinolines, which are currently under study because of their potential pharmacological value. Tetrandrine (VIII) has been shown to have tumour-inhibiting properties,[1] strong tuberculostatic activity *in vitro* against a number of strains of *Mycobacterium tuboculosis*, and to prolong the life expectancy of mice infected with various tuberculosis strains.[2] Tetrandrine and dauricine (V) exhibit anti-inflammatory and anaesthetic properties,[3] and obamegine (X) has been shown to possess antitubercular activity against *Mycobacterium smegmatis* ATCC 607.[4] Tomita *et al.*,[5] having



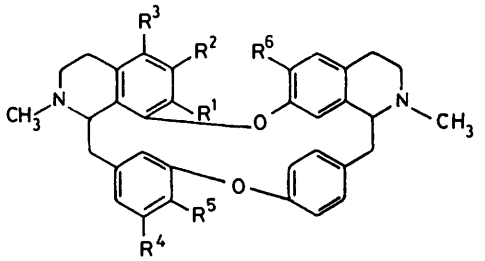
(I) $R^1 = R^2 = R^4 = R^5 = OCH_3$, $R^3 = H$
(II) $R^1 = R^2 = R^4 = R^5 = OH$, $R^3 = CH_3$
(III) $R^1 = R^2 = OH$, $R^4 = R^5 = OCH_3$, $R^3 = CH_3$
(IV) $R^1 = R^2 = R^4 = R^5 = OCH_3$, $R^3 = CH_3$



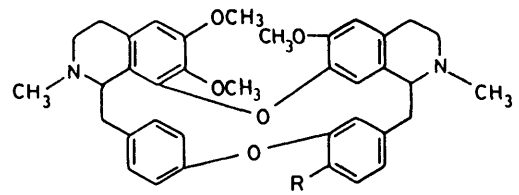
(V) R = OCH_3

studied 23 bisbenzylisoquinoline alkaloids, have tentatively proposed that those which have *S,S* configuration at C-1 and -1′ would have antitumour activity *in vivo*. It has been shown that the head-to-tail arrangement of the bisbenzylisoquinoline alkaloids (*e.g.* tubocurarine) seems to confer greater pharmacological activity than the head-to-head arrangement (*e.g.* berbamine).[6]

The different arrangements of aromatic residues in the bisbenzyltetrahydroisoquinoline alkaloids provide a series of molecules in which to examine the effect of configuration upon photophysical parameters. Previous communications have described the use of photophysical techniques for the determination [7] and stereochemical analysis [8] of some of these alkaloids. We now present a study of the emission properties of some bisbenzylisoquinolines of the berbamine (VI) and oxyacanthine (XV) groups.



(VI) (*R,S*) $R^1 = R^2 = R^6 = OCH_3$, $R^5 = OH$, $R^3 = R^4 = H$
(VII) (*R,R*), (VIII) (*S,S*), (IX) (*R,S*) $R^1 = R^2 = R^5 = R^6 = OCH_3$, $R^3 = R^4 = H$
(X) (*R,S*) $R^1 = R^5 = OH$, $R^2 = R^6 = OCH_3$, $R^3 = R^4 = H$
(XI) (*S,S*) $R^1 = OH$, $R^2 = R^5 = R^6 = OCH_3$, $R^3 = R^4 = H$
(XII) (*S,S*) $R^1 = R^2 = R^3 = R^5 = R^6 = OCH_3$, $R^4 = H$
(XIII) (*S,S*) $R^1 = OH$, $R^2 = R^6 = OCH_3$, $R^3 = H$, $R^4,R^5 = -O-CH_2-O-$
(XIV) $R^1 = R^2 = R^6 = OCH_3$, $R^3 = H$, $R^4,R^5 = -O-CH_2-O-$



(XV) (*S,R*), (XVI) (*S,S*) R = OH
(XVII) (*S,R*), (XVIII) (*S,S*) R = OCH_3

RESULTS AND DISCUSSION

U.v. Absorption Data.—The u.v. absorption spectra of the compounds under study are broad structureless bands with maxima at *ca.* 283 nm (Table 1). The molar extinction coefficients of the benzylisoquinolines (I)—

(IV) are at least twice that of 3,4-dimethoxytoluene [9] ($\varepsilon_{max.}$ 2 730 dm^3 mol^{-1} cm^{-1}). With the noticeable exception of dauricine (V), the extinction coefficients of the bisbenzyltetrahydroisoquinolines are of the same order of magnitude as those of the benzyltetrahydroisoquinolines, whereas one would expect that the extinction coefficients of the dimeric alkaloids would be approximately twice those of the monomers. Although little work has been done before this study, our absorption data are in good agreement with published values. Absorption maxima and extinction coefficients for phaeanthine (VII), isotetrandrine (IX), oxyacanthine (XV), and *O*-methylrepandine (XVIII) in methanolic solution have been reported [10] (our values in parentheses) as $\lambda_{max.}$ 282 nm, ε 8 100 dm^3 mol^{-1} cm^{-1} (283 nm, 8 100); 282 nm, 7 050 (283 nm, —); 282 nm, 8 400 (284 nm, 7 600) and 282 nm, 6 500 (283 nm, 7 500), respectively. Additionally the absorption maximum and extinction coefficient for tetrandrine in ethanolic solution have been reported [11] as 282 nm and 7 800 dm^3 mol^{-1} cm^{-1} (our values 283 nm and 8 400 dm^3 mol^{-1} cm^{-1}).

TABLE 1

U.v. absorption data of some bisbenzylisoquinoline alkaloids and model compounds in ethanolic solution at 298 K

Compound	$\lambda_{max.}$/nm	$\varepsilon_{max.}$/dm^3 mol^{-1} cm^{-1}
(I)	283	8 700
(II)	287	7 700
(III)	284	6 700
(IV)	283	6 400
(V)	284	11 800
(VI)	284	9 300
(VII)	283	8 100
(VIII)	283	8 400
(IX)	283	
(X)	285	8 400
(XI)	283	9 300
(XII)	283	9 800
(XIII)	284	8 400
(XIV)	283	7 600
(XV)	284	7 600
(XVI)	284	7 300
(XVII)	284	8 100
(XVIII)	283	7 500

Emission Characteristics.—The fluorescence emissions of the model benzyltetrahydroisoquinoline compounds at room temperature (Table 2) are single bands with maxima at *ca.* 320 nm and quantum yields of the order of 0.1. Upon cooling to 77 K the fluorescence maxima shift to *ca.* 310 nm and the quantum yields and the fluorescence lifetimes increase by a factor of *ca.* 5. Single structureless phosphorescence bands with maxima between 415 and 452 nm, and quantum yields between 6.4×10^{-4} and 3.1×10^{-2}, are found in all four cases. The phosphorescence of laudanosine (IV) was too weak to measure its lifetime and the phosphorescence lifetimes of the other three benzyltetrahydroisoquinolines are of the order of 0.8 s.

The fluorescence maxima of laudanosoline (II), laudanosoline 3′,4′-dimethyl ether (III), and laudanosine (IV) shift to higher energy upon progressive *O*-methylation of the hydroxy-groups. At 298 K *O*-methylation causes a decrease in the fluorescence quantum yield and lifetime, whereas the reverse is found at 77 K; *O*-methylation also decreases the phosphorescence quantum yields. Hence the methoxy-group is more efficient than the hydroxy-group in promoting radiationless transitions, probably internal conversion rather than intersystem crossing. Tetrahydropapaverine (I) and laudanosine (IV) have similar emission spectra, but the luminescence quantum yields of tetrahydropapaverine are greater than those of laudanosine. This suggests that the *N*-methyl group of laudanosine has an important role in promoting radiationless decay from the excited states.

The large differences in the phosphorescence characteristics between laudanosine and tetrahydropapaverine, on the one hand, and laudanosoline and laudanosoline 3′,4′-dimethyl ether, on the other, allow us to assign the phosphorescing state of the former pair of the 6,7-dimethoxytetrahydroisoquinoline moiety. The phosphorescing moieties of laudanosoline 3′,4′-dimethyl ether and laudanosine are therefore the 3′,4′-dimethoxybenzyl and 3′,4′-dihydroxybenzyl moieties, respectively.

TABLE 2

Emission characteristics of some model compounds and some bisbenzylisoquinoline alkaloids in ethanol excited at 285 nm

	Fluorescence (298 K)			Fluorescence (77 K)			Phosphorescence (77 K)		
Compound	$\lambda_{max.}$/nm	ϕ_f	τ_f/ns	$\lambda_{max.}$/nm	ϕ_f	τ_f/ns	$\lambda_{max.}$/nm	ϕ_p	τ_p/s
(I)	317	1.54×10^{-1}	1.0	306	0.864	5.7	436	9.26×10^{-4}	0.80
(II)	321	1.27×10^{-1}	2.5	313	0.314	6.2	416	9.36×10^{-2}	0.87
(III)	319	7.64×10^{-2}	1.5	310	0.333	7.0	415	3.13×10^{-2}	0.75
(IV)	315	5.12×10^{-2}	0.9	306	0.501	8.5	452	6.4×10^{-4}	
(V)	316	2.05×10^{-2}		307	0.211	1.1	435	6.92×10^{-2}	0.83
(XV)	316	6.70×10^{-3}		310	0.185	1.9	439	1.79×10^{-2}	0.98
(XVI)	319	2.40×10^{-2}		306	0.139	1.0	450	2.12×10^{-2}	1.08
(XVII)	314	6.85×10^{-3}		310	0.138	1.2	413	4.66×10^{-2}	2.33
(XVIII)	313	1.97×10^{-2}		305	0.265	0.8	445	4.31×10^{-2}	0.77

The fluorescence emission of the berbamine alkaloids (VI)—(XIV) at 298 K (Table 3) consists of single structureless bands with maxima at *ca.* 315 nm and quantum yields of the order of 0.01, with the notable exception of hernandezine (XII) which has a long wavelength emission band with a maximum at 398 nm in addition to the normal fluorescence maximum at 316 nm (see Figure). The fluorescence lifetimes at 298 K were found to be too short to be measured by the

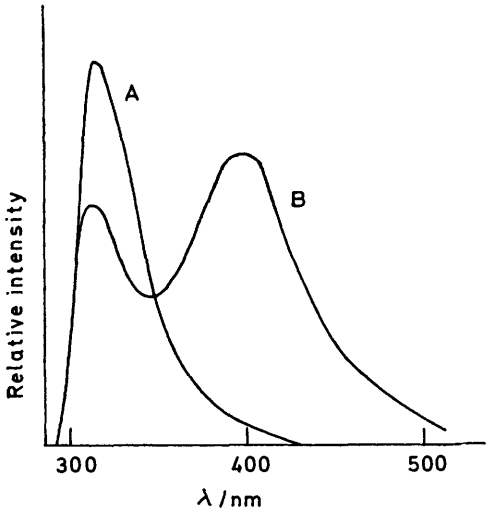
single photon counting technique, but comparison with those measured at 77 K show that they are *ca.* 0.1 ns at 298 K. At 77 K the fluorescence maxima shift to shorter wavelengths, the fluorescence quantum yields increase to between 0.35 and 0.08 and the fluorescence lifetimes lie between 0.5 and 1.2 ns. The long wavelength fluorescence of hernandezine at room temperature was not observed at 77 K. The phosphorescence emission at 77 K consists of single structureless bands with maxima between 420 and 465 nm, with quantum yields lying between 0.1 and 3×10^{-5}, and lifetimes between 2.3 and 0.3 s.

TABLE 3

Emission characteristics of some berbamine alkaloids in ethanol excited at 285 nm

	Fluorescence (298 K)		Fluorescence (77 K)			Phosphorescence (77 K)		
Compound	$\lambda_{max.}$/nm	ϕ_f	$\lambda_{max.}$/nm	ϕ_f	τ_f/ns	$\lambda_{max.}$/nm	ϕ_p	τ_p/s
(VI)	318	1.44×10^{-2}	312	0.177	1.1	424	4.85×10^{-2}	1.35
(VII)	312	1.46×10^{-2}	307	0.349	0.6	434	4.39×10^{-4}	2.20
(VIII)	312	1.46×10^{-2}	307	0.300	0.5	438	3.36×10^{-4}	2.30
(IX)	312	8.55×10^{-3}	308	0.137	0.8	426	3.02×10^{-2}	1.22
(X)	317	1.32×10^{-2}	302	0.231	1.2	420	1.34×10^{-1}	0.87
(XI)	314	1.10×10^{-2}	309	0.105	0.9	465	9.81×10^{-3}	1.17
(XII)	316	4.12×10^{-3}	305	0.083	1.2	424	3.63×10^{-5}	
	398	4.23×10^{-3}						
(XIII)	315	1.75×10^{-2}	309	0.192	0.9	452	7.43×10^{-2}	0.34
(XIV)	312	1.49×10^{-2}	306	0.234	1.3	449	9.49×10^{-2}	0.34

The fluorescence maxima of the berbamine alkaloids show that methylation of the hydroxy-groups causes a hypsochromic shift, in common with the benzylisoquinolines. In the alkaloids obamegine (X) and berbamine (VI), which have the *R,S* configuration at C-1 and -1′ the fluorescence quantum yield at 298 K increases upon *O*-methylation in the isoquinoline moiety, but at 77 K decreases. In the alkaloids fangchinoline (XI) and tetrandrine (VIII), which have the *S,S* configuration at C-1 and -1′, ϕ_f increases at both 298 and 77 K upon *O*-methylation. Furthermore, a comparison of the three stereoisomers, (*R,R*)-phaeanthine (VII), (*R,S*)-isotetrandrine (IX), and (*S,S*)-tetrandrine (VIII), reveals that, at 298 K, ϕ_f of phaeanthine and tetrandrine are equal and greater than that of isotetrandrine, and at 77 K, ϕ_f increases in the order isotetrandrine < tetrandrine < phaeanthine, whilst ϕ_p decreases in that order. It is therefore evident that the photophysical properties of these complex alkaloids are sensitive to their stereochemistry,[8] even the enantiomers, pheaeanthine and tetrandrine, having significant differences.



Fluorescence emission spectrum of (A) berbamine and (B) hernandezine excited at 285 nm in ethanolic solution at 298 K

The excitation spectrum of the long wavelength fluorescence of hernandezine (XII) at 298 K was identical to that of the short wavelength fluorescence. As the temperature was decreased both emission bands increased in intensity, until at 150 K the intensity of the long wavelength band began to decrease and at 77 K it was undetectable. As the presence of the long wavelength emission band is independent of concentration in the range 10^{-5}—5×10^{-3}M it is concluded that it is due to an intramolecular exciplex. At 77 K only very weak phosphorescence could be detected, and it was not possible to measure its lifetime.

Nortenuipine (XIII) and repandinine (XIV) have identical phosphorescence lifetimes and similar phosphorescence quantum yields. They have the same structure in the isoquinoline system as fangchinoline and obamegine respectively. It is apparent that the phosphorescent chromophore is common to nortenuipine and repandine, but not to fangchinoline and obamegine. Thus in repandinine and fangchinoline the phosphorescent chromophore is the methylenedioxyphenyl ester.

At 298 K the fluorescence emission of dauricine (V) and the oxycanthine alkaloids (XV)—(XVIII) (Table 2) consists of single structureless bands with maxima at *ca.* 315 nm and quantum yields of the order of 10^{-2}. Upon cooling to 77 K the emission maxima are shifted to higher energy and increased in intensity *ca.* 10-fold. At longer wavelengths broad structureless phosphorescence is detectable with maxima between 413 and 450 nm, quantum yields between 2×10^{-2} and 7×10^{-2}, and lifetimes of the order of 1 s.

The fluorescence maxima of dauricine, oxyacanthine, and repandine at 298 K are at longer wavelengths than those of the *O*-methyl esters, obaberine and *O*-methylrepandine. Significantly the *S,S*-stereoisomers have greater values of ϕ_f than their *S,R*-enantiomers, and the ϕ_f of the (*R,R*)-dauricine is similar to that of (*S,S*)-

repandine. The effect of *O*-methylation in the benzyl moiety upon ϕ_f is to cause an increase in the *S,R*-configuration, and a decrease in the *S,S*-configuration; these changes are reversed at 77 K. It is interesting to compare these changes in ϕ_f with those observed upon *O*-methylation of (*R,S*)-berbamine to produce (*R,S*)-isotetrandrine; in this case ϕ_f decreases both at 298 and 77 K.

The phosphorescence data of the oxyacanthine alkaloids also show differences due to the variation of stereochemistry, the phosphorescence maxima of the *S,S*-stereoisomers are at shorter wavelength than those of the *S,R*-stereoisomers. The phosphorescence maxima shift to higher energy upon *O*-methylation, and the quantum yields increase, although the shift of the phosphorescence maximum and the increase in ϕ_p are greater in the *S,R*-stereoisomer than in the *S,S*-stereoisomer.

Conclusions.—The emission characteristics of the benzylisoquinolines and the bisbenzyltetrahydroisoquinoline alkaloids allow the assignment of the emissive singlet and triplet states as (π, π^*). A survey of the absorption data has shown that *O*-methylation usually causes a hypsochromic shift of the absorption maxima, which is reflexed in a similar shift in the fluorescence maxima. It has been shown that there are differences in the emission characteristics of stereoisomers in the oxyacanthine and berbamine alkaloids. Furthermore, the effect of *O*-methylation upon the photophysical parameters depends upon the absolute configurations of the asymmetric carbon atoms.

It has also been shown that (*S,S*)-hernandezine forms an intramolecular exciplex; this result is surprising bearing in mind the rigidity of the molecule, as found in molecular models and as shown by the crystal structure of the 5-demethoxy-compound, (*S,S*)-tetrandrine.[12] As exciplex formation was not observed in any other bisbenzyltetrahydroisoquinoline alkaloid it is proposed that the exciplex is formed between the 5-methoxy-substituent of the 5,6,7-trimethoxytetrahydroisoquinoline system and the aromatic ring of the other tetrahydroisoquinoline moiety.

EXPERIMENTAL

Tetrahydropapaverine was supplied by Maybridge Chemical Company Limited and recrystallised before use, laudanosoline and laudanosine by Aldrich Chemical Company Limited, and laudanosoline 3′,4′-dimethyl ether, berbamine, and phaeanthine were supplied by Alfred Bader Chemicals. All other alkaloids were kindly donated (see acknowledgements). Ethanol used as a solvent was purified by distilling through a Widmer fractionating column at a reflux ratio of 20 : 1 (the first and last 20% of the charge were discarded). It was found that ethanolic solutions underwent degradation upon standing; therefore all measurements were performed on freshly made up solutions and all operations were conducted under red ' safe-lights '.

The spectrofluorimeter used has been described previously.[13] The system utilises a 2 kW xenon arc lamp (Mazda XE/D), two 500 mm grating monochromators (Bausch and Lomb), and an EMI 9558 QB photomultiplier tube in a housing cooled to 203 K. For ambient temperature measurements samples were mounted in a thermostatically controlled cell block at 298 K. Low temperature measurements were made at 77 K by use of a cylindrical quartz sample cell mounted in a quartz Dewar flask containing liquid nitrogen. At intermediate temperatures the cylindrical quartz sample cell was inserted into an Oxford Instruments Ltd. DN 704 liquid nitrogen cryostat with an Oxford Instruments Ltd. DTC 2 temperature programmer. A rotating can phosphoroscope attachment inserted around the Dewar flask was used to eliminate fluorescence when phosphorescence emission was being studied. Phosphorescence lifetimes were determined by photographing the cathode ray oscilloscope trace of the phosphorescence decay at the wavelength of maximum intensity.

Fluorescence lifetime measurements were made using a time-correlated single photon counting system employing a thyratron-controlled gated lamp. The excitation pulse had a typical width at half-height of 4 ns and a repetition rate of 20—50 kHz. The method of calculating the decay time from the experimental data has been described previously.[14]

Quantum Yield Determination.—Luminescence quantum yields were determined by the comparative method [15] using tryptophan in water (ϕ_f 0.13 [16]) and benzophenone (ϕ_p 0.74 [17]) as reference standards at 298 and 77 K, respectively. Additionally it was assumed that the change in optical density with temperature was not significant between different samples. The quantum yield (ϕ) is calculated from the relation (1), where I is the area under the corrected emission curve, A the absorbance at the exciting wavelength, θ the relative photon output of the excitation system at the exciting wavelength, and n the refractive index, the subscripts refer to the standard (st) and the unknown (x).

$$\phi_x = \phi_{st} \cdot \frac{I_x}{I_{st}} \cdot \frac{A_{st}}{A_x} \cdot \frac{\theta_{st}}{\theta_x} \cdot \frac{n^2_x}{n^2_{st}} \qquad (1)$$

Procedure.—For fluorescence and phosphorescence measurements solutions of 10^{-5}M concentration were used in order to eliminate distortion caused by inner filter effects. For low temperature measurements solutions in clear ethanolic glasses were used, which were degassed on a vacuum line by the cyclic freeze–pump–thaw technique to minimise oxygen quenching and to reduce the frequency of cracking of the glass. The spectra were corrected for the spectral response of the emission monochromator and photomultiplier by the method of Melhuish.[18]

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