Luminescence Characteristics of Morphine Derivatives

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A detailed study of the fluorescence and phosphorescence characteristics of morphine, diamorphine (heroin), and a number of morphine derivatives has been undertaken. Fluorescence decay times have been determined where appropriate by using a time-correlated single photon counting technique. The effect on intersystem crossing rate of a heavy atom substituent in the chromophore has been investigated.

EARLY studies on fluorescence of alkaloids have produced several qualitative observations on the fluorescence of morphine,[1-4] but there appeared to be little agreement on the spectral region of the emission. The first definitive report is probably that of Bowman *et al.*[5] Brandt *et al.*[6] subsequently carried out a detailed study of the fluorescence of morphine and codeine in acidic and in alkaline media and observed that, although both compounds fluoresce in 0.05M-sulphuric acid, only codeine fluoresces in 0.1M-sodium hydroxide. They concluded that for morphine the fluorescent species is the undissociated form. These results have recently been used as the basis of an analytical method for determining morphine and codeine in mixtures.[7]

The phosphorescence emission spectra and decay times of morphine, codeine, and thebaine were reported by Hollifield and Winefordner,[8] who suggested that the method was more sensitive than fluorescence as an analytical technique. In more recent studies,[9] phase resolved phosphorimetry has been applied to the

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detection of simple morphine derivatives in the presence of other alkaloids.

We now present comprehensive luminescence data on morphine and a series of morphine derivatives. Of these, diacetylmorphine (diamorphine, heroin) is of particular interest because its triplet level had not previously been located. We have also investigated the effect of internal heavy atom perturbation of singlet–triplet transitions in this series, using 1-bromomorphine derivatives.

Absorption Spectra.—The lowest energy absorption band of the simple morphine derivatives, measured in 100% ethanol at 298 K, appears as a single unresolved peak between 260 and 310 nm. The maximum for compounds which possess an isolated double bond in ring C and no halogen substituent in ring A occurs between 282 and 288 nm, with fairly low values of molar extinction coefficient ($\varepsilon_{max.}$ 1 500—1 900), typical of the

TABLE 1

Effect of solvent on the absorption and fluorescence spectra of morphine

Solvent	ε	$\lambda_{max.}$ (absorption)/ nm	$\lambda_{max.}$ (fluorescence)/ nm
Dioxan	2.2	288	341
Chloroform	4.8	288	
Ethanol	24.3	288	340
Water	78.5	285.5	345

undissociated phenolic chromophore (phenol $\varepsilon_{max.}$ 1 900). Thebaine (6), which contains a conjugated diene structure in ring C, has a much higher molar extinction coefficient ($\lambda_{max.}$ 286 nm, $\varepsilon_{max.}$ 7 580). The introduction of a 1-bromine atom in codeine and neopine causes the expected bathochromic shift in the absorption band of anomalous small blue shift of $\lambda_{max.}$ observed in water as compared with less polar solvents (Table 1), a phenomenon often attributed to $n \longrightarrow \pi^*$ transitions.

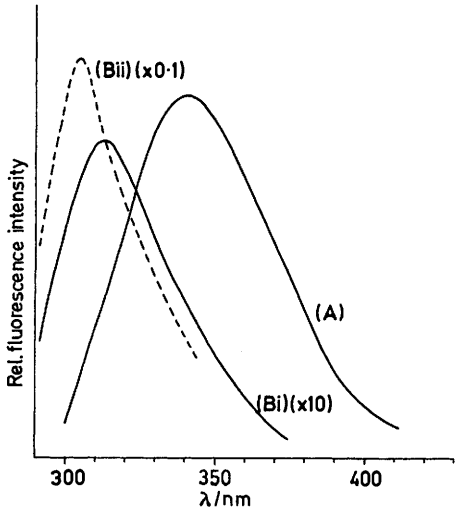


FIGURE 1 Corrected fluorescence emission spectra of (A) morphine at 298 K and (B) diamorphine at (i) 298 and (ii) 77 K in ethanol ($\lambda_{exc.}$ 280 nm)

Luminescence Characteristics.—Fluorescence spectra, quantum yields, and decay times were measured in 100% ethanol at 298 ± 0.2 K. Fluorescence and phosphorescence spectra and phosphorescence lifetimes were measured in clear ethanolic glasses at 77 K under anaerobic conditions. The spectral data are summarised in Tables 2 and 3.

The fluorescence emission spectra of morphine, codeine, ethylmorphine, and dihydromorphine at 298 K are very similar, consisting of a single low intensity

TABLE 2

U.v. absorption and fluorescence characteristics of morphine and some morphine derivatives in ethanol at 298 K

	Absorption		Fluorescence			
				Stokes shift		
	$\lambda_{max.}$/nm	$\varepsilon_{max.}$	$\lambda_{max.}$/nm	(cm^{-1})	ϕ_f	τ_f/ns
Morphine	288	1 710	340	5 310	0.030	0.6
Codeine	287	1 540	336	5 080	0.045	1.0
Ethylmorphine	287	1 660	342	5 600	0.050	0.8
Dihydromorphine	286	1 580	338	5 380	0.021	0.8
Neopine (HBr) *	286	1 590	320	3 710	0.032	0.4
Thebaine	286	7 580				
Diacetylmorphine	282	1 890	312	3 410	0.002 5	0.7
1-Bromocodeine	294	2 240				
1-Bromoneopine	293	2 320				
Guaiacol	277	2 570	306	3 421	0.22	2.4
Guaiacol acetate	270	1 710	296	3·253	0.001 3	
Etorphine (HCl) *	291	1 570	352	5 950	0.036	1.3
Diprenorphine (HCl) *	289	1 650	349	5 950	0.047	1.6
Buprenorphine (HCl) *	289	1 650	351	6 110	0.039	1.5
Cyclopropyletorphine (HCl) *	290	1 600	350	5 910	0.036	1.4

* Measured in ethanol–water (95 : 5 v/v).

between 600 and 830 cm^{-1} and also a slight increase in the intensity of the band in comparison with the unsubstituted compounds.

On the basis of its relatively high intensity and short wavelength, we assign the absorption band in this series of compounds to a $\pi \longrightarrow \pi^*$ transition, in spite of the band in the region 300—450 nm corresponding to the emission $S_1 \longrightarrow S_0$ (Figure 1). Slight but significant differences in $\lambda_{max.}$ are, however, evident amongst the members of this group. The fluorescence quantum yields show much greater variation, ranging from 0.021 for dihydromorphine to 0.050 for ethylmorphine. The

fluorescence decay times are very similar (0.6—1.0 ns), and in view of the difficulties encountered in measuring such short lifetimes the slight differences observed cannot be considered significant. The efficiency of fluorescence emission in these four compounds is a factor of 20 less than for the simple substituted phenol guaiacol (Table 2), indicating the importance of rings B and C in influencing the relative rates of the singlet deactivation process. From the phosphorescence data (Table 3) it appears that this effect can only be partly due to an increase in intersystem crossing rate ($S_1 \longrightarrow T_1$) since guaiacol shows only slightly reduced phosphorescence emission in comparison with morphine, codeine, and ethylmorphine. The major contributing factor must therefore be an increase in the non-radiative deactivation process $S_1 \longrightarrow S_0$.

TABLE 3

Fluorescence and phosphorescence characteristics of morphine and some morphine derivatives in ethanol at 77 K

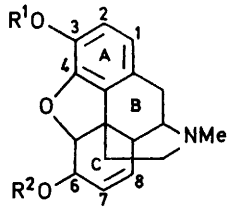
	Fluorescence	Phosphorescence		
	$\lambda_{max.}$/nm	$\lambda_{max.}$/nm	τ_p/s	Rel. intensity
Morphine	328	522	0.020	1
Codeine	325	525	0.035	1.1
Ethylmorphine	328	525	0.034	1
Dihydromorphine	323	489	0.28	6
Neopine (HBr) *	312	482	0.30	35
Thebaine		456	0.84	20
Diacetylmorphine	305	445		0.1
1-Bromocodeine		500	2.4	0.1
1-Bromoneopine		464	1.7	0.1
Guaiacol	298	441		0.5
Guaiacol acetate	287	394	1.4	10
Etorphine (HCl) *	343	520	0.10	15
Diprenorphine (HCl) *	338	519	0.10	20
Buprenorphine (HCl) *	339	514	0.17	15
Cyclopropyletorphine (HCl) *	344	512	0.10	15

* Measured in ethanol–water (95 : 5 v/v).

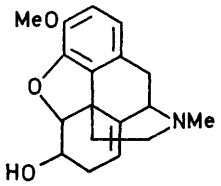
Thebaine (6) shows no detectable fluorescence emission at 298 K in ethanol. This indicates a high degree of thermal depopulation of the singlet by non-radiative $S_1 \longrightarrow S_0$ transitions and by intersystem crossing, $S_1 \longrightarrow T_1$. The contribution of the non-radiative process is not known, but an increase in $S_1 \longrightarrow T_1$ is consistent with the marked enhancement of phosphorescence observed for the triplet emission $T_1 \longrightarrow S_0$.

In all the above cases the intensity of fluorescence emission is increased slightly, and the maximum moved to higher energy, in spectra recorded in ethanolic glass at 77 K. The magnitude of the blue shift (*ca.* 1 000 cm^{-1}) is due to the usual restriction of solvent reorientation processes in the highly viscous matrix, allowing emission from the Franck–Condon state.

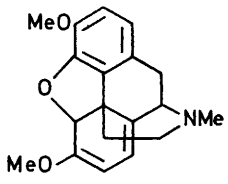
Acetylation of morphine to diamorphine (heroin) reduces the fluorescence emission to a very low level and moves the maximum to higher energy (Figure 1). An analogous effect is observed between guaiacol and its acetate. It is generally assumed that this phenomenon is due to an enhancement of the rate constant for intersystem crossing (k_{isc}), as is observed when a carbonyl group is attached directly to the ring, *i.e.* when the triplet state is $n\pi^*$ in character. This certainly appears to be the case for guaiacol acetate, which exhibits a fairly intense phosphorescence emission in comparison with guaiacol itself, with a long phosphorescence lifetime (1.4 s). Diamorphine, however, shows an extremely weak phosphorescence at 77 K (Figure 2), indicating either that k_{isc} is not increased, or that non-radiative decay of the triplet is the major route of deactivation at this temperature. We have not been able to measure precisely the phosphorescence lifetime of diamorphine owing to the very low intensity of emission observed. Our studies indicate, however, that the lifetime is short, in the region 10—100 ms.



(1) $R^1=R^2=H$ (morphine)
(2) $R^1=Me, R^2=H$ (codeine)
(3) $R^1=Et, R^2=H$ (ethylmorphine)
(4) $R^1=R^2=Ac$ (diamorphine)



(5) (neopine)



(6) (thebaine)

Neopine (5) shows surprisingly large differences in its emission properties from the isomeric codeine, in spite of its almost identical absorption characteristics. The fluorescence maximum is at shorter wavelength (320 nm) indicating that the change in position of the double bond has moved the *O–O* transition to higher energy. The fluorescence decay time and quantum yield are both less than for codeine, suggesting that an alternative singlet deactivation process is more competitive than

Diamorphine and guaiacol acetate both show a much greater increase in fluorescence intensity than is normal on lowering the temperature to 77 K. It is widely assumed that non-radiative $S_1 \longrightarrow S_0$ transitions are of negligible importance in rigid media, and thus the anomalous behaviour of the acetates indicates that for these two compounds non-radiative deactivation, $S_1 \longrightarrow S_0$, is a much more efficient process at 298 K than is the case with the other morphine derivatives.

The introduction of a bromine atom into the chromophore causes a complete quenching of the fluorescence emission both at 298 and 77 K. This internal heavy atom perturbation is attributed to a high degree of singlet–triplet mixing in the wave function of the electronic state, mainly by a spin–orbit interaction, and

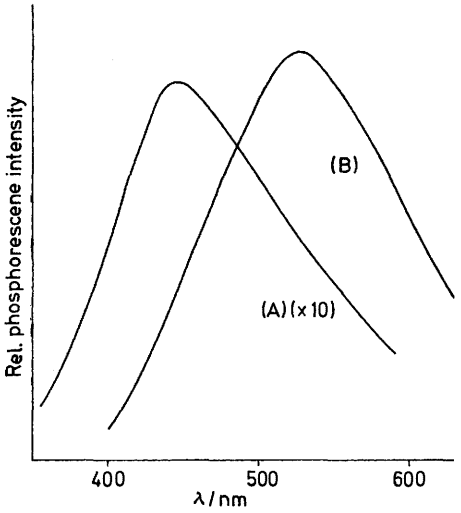


FIGURE 2 Corrected phosphorescence emission spectra of (A) diamorphine and (B) codeine in ethanol at 77 K ($\lambda_{exc.}$ 280 nm)

generally results in increased $S_1 \longrightarrow T_1$ intersystem crossing and a reduction in the $S_1 \longrightarrow S_0$ radiative transition. The increase in k_{isc} normally results in an enhanced intensity of phosphorescence as compared with the unsubstituted molecule. The fact that this is not observed (Table 3) points to an increase in the non-radiative deactivation of the triplet state which supervenes over the increase in k_{isc}.

The etorphine series of compounds show spectral properties similar to those of the simple morphine derivatives. In general, however, the fluorescence emission at 298 K is at lower energy with a significantly longer decay time (1.3—1.6 ns). Phosphorescence characteristics resemble those of dihydromorphine in having higher intensities and longer lifetimes than those of morphine itself.

EXPERIMENTAL

Morphine, ethylmorphine, and diacetylmorphine were supplied by Macfarlan Smith Ltd., Edinburgh; codeine and thebaine by May and Baker Ltd., Dagenham; dihydromorphine and the etorphine derivatives by Reckitt and Colman, Kingston-upon-Hull. Neopine, 1-bromoneopine, and 1-bromocodeine were kindly provided by Professor K. W. Bentley, University of Technology, Loughborough. Guaiacol acetate was prepared by acetylation of guaiacol with acetic anhydride, and purified by preparative scale g.l.c. Ethanol used as a solvent for phosphorescence was purified by distillation through a Widmer fractionating column at a reflux ratio of 20 : 1 (the first and last 20% of the charge were discarded).

The spectrofluorimeter used has been described previously.[10] 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 cooled housing at 203 K. An excitation wavelength of 280 nm was used throughout. For ambient temperature measurements samples were mounted in a thermostatically controlled cell block at 298 K and slit widths of 3 mm (bandpass 5 nm) were used on both excitation and emission monochromators. Low temperature measurements were made at 77 K by use of a cylindrical quartz sample tube mounted in a quartz Dewar flask containing liquid nitrogen. A rotating can phosphoroscope attachment inserted around the Dewar flask was used to eliminate fluorescence when phosphorescence emission was being studied. For measurement of phosphorescence lifetimes less than 0.5 s the phosphoroscope was rotated at slow speed and the cathode ray oscilloscope trace of the phosphorescence decay at the wavelength of maximum intensity was photographed. Longer lifetimes were measured by manual operation of the excitation monochromator shutter.

Fluorescence decay time measurements were made by using a time-correlated single photon counting system (Applied Photophysics Ltd.) employing either a high-pressure free running lamp or a thyratron-controlled gated lamp. The excitation pulse had a typical width at half-height of 2—5 ns and a repetition rate of 5—10 kHz. The method of calculating the decay time from the experimental data has been described previously.[11]

Quantum Yield Determinations.—Fluorescence quantum yields were determined by the comparative method, using Chen's value of 0.13 for L-tryptophan in water as reference standard.[12] The quantum yield (ϕ) is calculated from the relation (i), where I is the area under the corrected emission

$$\phi_x = \phi_{st}\left(\frac{I_x}{I_{st}}\frac{A_{st}}{A_x}\frac{\theta_{st}}{\theta_x}\frac{n_x^2}{n_{st}^2}\right) \quad \text{(i)}$$

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).

Correction of Spectra.—All reported emission data are corrected for the spectral response of the emission monochromator and photomultiplier. Correction factors were obtained by the method of Melhuish,[13] by using a Rhodamine B quantum counter.

Procedure.—For fluorescence measurements, solutions of less than 10^{-4}M concentration were used in order to eliminate distortion caused by inner filter effects. Observation of

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the very weak phosphorescence emissions encountered with these compounds required in some cases the use of solutions up to 10^{-3}M concentration. For low temperature measurements solutions in clear ethanolic glasses were used, which were deaerated on a vacuum line by the cyclic freeze–pump–thaw technique to minimise oxygen quenching of the triplet state, and to reduce the frequency of cracking of the solvent glass.

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