# Luminescence Characteristics of Lysergic Acid Diethylamide and Related Ergolines

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The fluorescence and phosphorescence characteristics of lysergic acid diethylamide and a number of related ergolines have been examined. Fluorescence decay times have been determined using a time-correlated single photon counting technique. Pronounced changes were observed in the fluorescence spectra and decay times with change of solvent. These effects have been attributed to the change in both the dielectric constant of the solvent and the extent of dipole–dipole interaction in the excited state.

In a previous paper [1] we described the measurement of the fluorescence and phosphorescence spectra of lysergic acid diethylamide (LSD) and its 2-bromo-derivative. The spectroscopic data obtained enabled us to characterise for the first time the lowest excited triplet states of these compounds, and to comment on the effect of the bromine atom on the intersystem crossing rate constant ($S_1 \longrightarrow T_1$) in LSD.

We now present further spectroscopic data on a series of ergoline derivatives. In particular we have determined fluorescence and phosphorescence parameters for LSD, lysergic acid, and the epimeric pairs ergometrine (I) and ergometrinine, ergotamine (II) and ergotaminine, ergocristine (III), and ergocristinine. In addition data have also been included on the non-conjugated ergoline agroclavine (IV). Spectroscopic parameters of these compounds are of particular interest in the forensic field at the present time for the identification of LSD itself, and for distinguishing LSD from other members of the ergoline family. A feature of our current work is the application of a time-correlated single photon counting technique [2] to the determination of fluorescence decay times with high precision.

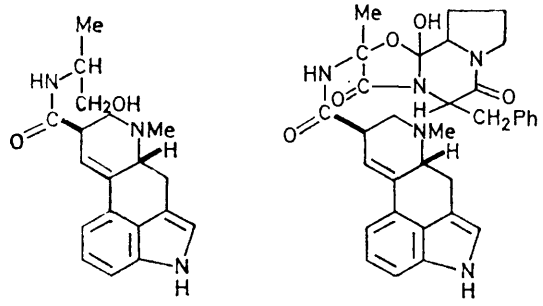
*Absorption Spectra.*—All the compounds studied, with the exception of agroclavine (IV), possessed the $\Delta^9$ ergoline structure. Absorption spectra, measured in ethanolic solution at 25 °C, showed the characteristic intense absorption band at $\lambda_{max.}$ 311—312 nm ($\varepsilon_{max.}$ 8000—9000), associated with the $\Delta^9$ conjugated indole chromophore. The absorption spectrum of ergometrine is typical. This band undergoes a small red shift with increasing dielectric constant of the solvent (Table 1), and it is assigned to a $\pi \longrightarrow \pi^*$ transition. Agroclavine possesses a weaker absorption, characteristic of the isolated indole nucleus with absorption bands at 281 and 292 nm ($\varepsilon_{max.}$ 7100) (Table 1).

*Fluorescence Spectra.*—Fluorescence spectra of the $\Delta^9$ ergolines were measured in ethanol and in water at 25 °C. The spectra of LSD are characteristic of this series of derivatives. In ethanol a single unresolved band is observed with a maximum at 400—405 nm. This band presumably represents the $\pi \longrightarrow \pi^*$ transition corresponding to the absorption maximum at 312 nm. The Stokes shift of the fluorescence peak has a value of 7280 $cm^{-1}$. The fluorescence spectrum in water as compared with ethanol, shows a marked displacement to the red (1730 $cm^{-1}$). This behaviour is normally attributed to dipole–dipole interaction between solvent and

[1] A. Bowd, J. B. Hudson, and J. H. Turnbull, *J. Chem. Soc.* (*B*), 1971, 1509.

[2] J. B. Birks, 'Photophysics of Aromatic Molecules,' Wiley–Interscience, New York, 1970, p. 95.

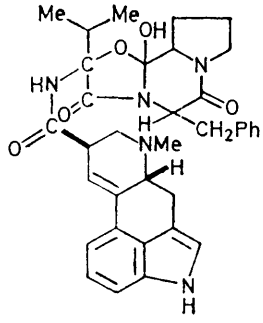
polarised solute molecules in the excited state.[3] The fact that the fluorescence maximum undergoes a much greater red shift with increasing dielectric constant than



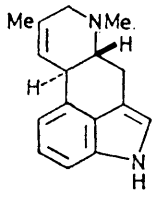
(I)



(II)



(III)



(IV)

the corresponding shift in the absorption maximum indicates that the excited singlet state is strongly

TABLE 1

U.v. absorption characteristics of LSD and some ergot alkaloids in ethanol and water at 25 °C

| | Ethanol | | Water | |
|---|---|---|---|---|
| | $\lambda_{max.}$/nm | $\varepsilon_{max.}$ | $\lambda_{max.}$/nm | $\varepsilon_{max.}$ |
| LSD (tartrate) | 311 | 16,600 | 312 | 13,800 |
| Ergometrine | 311 | 9200 | 311 | 8100 |
| Ergometrinine | 311 | 9200 | 312 | 7600 |
| Ergotamine | 311 | 8600 | 313 | 7700 |
| Ergotaminine | 313 | 8400 | | |
| Ergocristine | 312 | 8800 | 316 | 7700 |
| Ergocristinine | 314 | 8900 | 313 * | 7600 * |
| Lysergic acid | 310 | 9300 | 309 | 8300 |
| Agroclavine | 281, 292 | 7100 | 279, 289 * | 7000 * |

* In water–ethanol (95 : 5).

polarised relative to the ground state.[4] Since $\pi,\pi^*$ states tend to have ground states of lower polarity than $n,\pi^*$ states, and probably, in addition, provide greater delocalisation of charge in the excited state, the experimental data are consistent with the assignment of $\pi,\pi^*$ to this fluorescent state. Fluorescence spectra of the $\Delta^9$ ergolines were also measured at 77 K in ethanol. A typical fluorescence spectrum is that of ergometrine (Figure 1). This spectrum shows better resolution than the fluorescence spectrum at 25 °C, and there is some suggestion of fine structure. The low temperature spectra in general show a pronounced blue shift in the wavelength of the fluorescence peak as compared with room temperature. The displacement observed amounted to 1360 $cm^{-1}$ (1830 $cm^{-1}$ for ergometrine and ergometrinine). This displacement indicates that the excited singlet has moved to a higher energy configuration relative to the ground state. We attribute this effect to destabilisation of the excited state at these low temperatures due to decreased mobility of the polar solvent molecules in the rigid glass.

The fluorescence spectrum of agroclavine shows a fluorescence maximum at shorter wavelengths (Table 2) characteristic of the isolated indole fluorophore. The

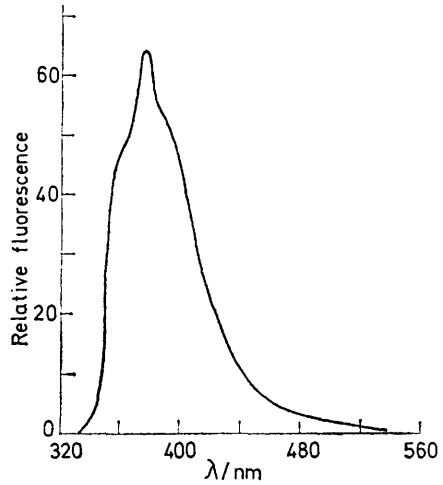



FIGURE 1 Corrected fluorescence spectrum of ergometrine in ethanol at 77 K ($\lambda_{ex}$ = 320 nm)

fluorescence shows very similar shifts with change of solvent and temperature to those observed in the $\Delta^9$ ergoline series.

*Fluorescence Quantum Yields and Lifetimes.*—Fluorescence quantum yields ($\phi_f$) were determined in ethanol and in water. In ethanol at 25 °C, values of $\phi_f$ of 0·69—0·79 were found for the $\Delta^9$ ergoline series (Table 2). The consistent, and relatively high values of $\phi_f$ observed, support the $\pi,\pi^*$ assignment for the first excited singlet state of the $\Delta^9$ ergolines. Fluorescence quantum yields in water were somewhat higher for LSD, lysergic acid, and ergometrine (Table 2). The ergotamine and ergocristine epimers, on the other hand, showed very low values of fluorescence quantum yield in water ($\phi_f$ 0·05—0·14). Agroclavine showed the lower quantum yields of fluorescence characteristic of the isolated indole nucleus.

Fluorescence lifetimes ($\tau_f$) were also measured in ethanol and water. The high precision of the technique employed provides values of $\tau_f$ within an experimental error of 0·1 ns. The values given by the $\Delta^9$ ergolines in ethanol at 25 °C (Table 2) were relatively long for singlet lifetimes ($\tau_f$ *ca.* 6 ns) consistent with the high quantum efficiencies of fluorescence observed. The values of $\tau_f$

[3] N. Mataga, Y. Kaifu, and M. Koizumi, *Bull. Chem. Soc., Japan*, 1956, **29**, 465.
[4] J. Eisinger and G. Navon, *J. Chem. Phys.*, 1969, **50**, 2069.

in fact have the magnitude associated with $\pi,\pi^*$ excited singlets. Lower values of $\tau_f$ have been quoted for $n,\pi^*$ transitions.[5,6]

Fluorescence lifetimes determined in ethanol at 77 K were significantly shorter ($\tau_f$ *ca.* 5 ns, Table 2). This result would indicate that the quantum yield of fluorescence is probably lower at 77 K than at room temperature. Experimental difficulties in measuring quantum yields at 77 K have prevented us from clarifying this point. If however one assumes that the quanta absorbed are unchanged at 77 K, the lower fluorescence efficiency observed might indicate increased intersystem crossing ($S_1 \longrightarrow T_1$). Phosphorescence is, in fact observed in all cases at 77 K. The values of the fluorescence lifetimes of LSD, lysergic acid, ergometrine, and ergometrinine in water (Table 2) were markedly increased ($\tau_f$ *ca.* 12 ns) compared with the lifetimes in ethanol. Since the absorption spectra of these compounds are almost identical in ethanol and water, the radiative lifetime ($\tau_r$) must remain substantially unchanged. One would therefore expect an increase in fluorescence decay time ($\tau_f$) to be associated with a corresponding increase in the quantum yield of fluorescence. In fact this is not observed. We have explored this phenomenon a little further in the case of LSD itself. Fluorescence spectra of LSD were measured in aqueous ethanol mixtures at 25 °C (Table 3). The fluorescence peak was displaced progressively towards longer wavelengths with decreasing ethanol content of the solvent. At the same time, the fluorescence lifetime increased from about 6·3 to 12·3 ns. These results are consistent with increasing stabilisation of the singlet by dipole interaction as the dielectric constant of the solvent rises to that of pure water.

The fluorescence lifetimes of ergotamine, ergocristine, and ergocristinine in water show a marked decrease compared with $\tau_f$ in ethanol. As noted earlier, this is accompanied by the predicted decrease in the fluorescence quantum yield. Similar behaviour was observed in the case of the non-conjugated agroclavine.

*Phosphorescence Spectra.*—The phosphorescence emission from the $\Delta^9$ ergolines is very weak, and can only be detected by the use of carefully purified solvent and a cooled photomultiplier. The phosphorescence spectra in ethanol at 77 K (Table 4) are very similar, showing three well-resolved peaks around 515, 555, and 610 nm. A typical spectrum (ergometrinine) is shown in Figure 2. The phosphorescence lifetimes are short, lying within the range 22·9—27·9 ms. These very short lifetimes would indicate that the emitting triplet state is $n,\pi^*$.[7]

The phosphorescence of agroclavine in ethanol at 77 K (Figure 2), showed the expected similarity to the phosphorescence of indole.[8] The emission occurs at shorter wavelengths than the phosphorescence of the $\Delta^9$ ergolines, and is much more intense. The phosphorescence of agroclavine has a lifetime of 6·0 s, comparable with that of indole (6·9 s), and on this basis we assign the triplet state to $\pi,\pi^*$.

TABLE 2

Fluorescence parameters of the lysergic acid derivatives at 25 °C and 77 K (excited at 320 nm)

| | 25 °C | | | | | | 77 K | |
|---|---|---|---|---|---|---|---|---|
| | Ethanol | | | Water | | | Ethanol | |
| | $\lambda_{max.}$/nm | $\phi_f$ | $\tau_f$/ns | $\lambda_{max.}$/nm | $\phi_f$ | $\tau_f$/ns | $\lambda_{max.}$/nm | $\tau_f$/ns |
| LSD (tartrate) | 402 | 0·73 | 6·3 | 432 | 0·84 | 12·3 | 383 | 5·0 |
| Ergometrine | 405 | 0·74 | 6·2 | 432 | 0·80 | 12·3 | 377 | 5·0 |
| Ergometrinine | 405 | 0·74 | 6·2 | 439 | 0·63 | 11·6 | 377 | 4·7 |
| Ergotamine | 404 | 0·70 | 6·2 | 432 | 0·05 | 2·9 | 383 | 5·3 |
| Ergotaminine | 405 | 0·75 | 6·3 | | | | 380 | 4·9 |
| Ergocristine | 405 | 0·69 | 6·3 | 434 | 0·14 | 2·8 | 380 | |
| Ergocristinine | 405 | 0·79 | 6·7 | 435 † | 0·10 † | 1·3 † | 382 | |
| Lysergic acid | 394 | 0·72 | 5·3 | 427 | 0·92 | 11·5 | 381 | |
| Agroclavine * | 336 | 0·37 | 4·1 | 350 † | 0·18 † | 2·5 † | 316 | |

* Excited at 280 nm. † In water–ethanol (95 : 5).

TABLE 3

The effect of solvent composition on the fluorescence of LSD at 25 °C

| % Ethanol (v : v) | $\tau_f$/ns | $\lambda_{max.}$/nm | $\phi_f$ |
|---|---|---|---|
| 100 | 6·3 | 402 | 0·74 |
| 95 | 7·5 | 404 | 0·77 |
| 90 | 7·7 | 405 | 0·81 |
| 75 | 9·4 | 409 | 0·86 |
| 50 | 10·7 | 416 | 0·90 |
| 25 | 11·8 | 424 | 0·82 |
| 0 | 12·3 | 432 | 0·84 |

TABLE 4

Phosphorescence of LSD and some ergoline derivatives in ethanol at 77 K

| | $\lambda_{max.}$/nm | | | | $\tau_p$/ms |
|---|---|---|---|---|---|
| LSD (tartrate) | 514 | 553 | 610 | | 23·1 |
| Ergometrine | 514 | 554 | 611 | | 23·9 |
| Ergometrinine | 512 | 552 | 611 | | 23·8 |
| Ergotamine | 516 | 558 | 613 | | 24·3 |
| Ergotaminine | 516 | 557 | 613 | | 22·9 |
| Ergocristine | 513 | 555 | 610 | | 27·1 |
| Ergocristinine | 515 | 557 | 608 | | 27·8 |
| Agroclavine | 420 | 432 | 449 | 478 | 6·0s |

[5] B. Cohen and L. Goodman, *J. Chem. Phys.*, 1967, **46**, 713.
[6] B. Cohen, H. Baba, and L. Goodman, *J. Chem. Phys.*, 1965, **43**, 2902.
[7] R. Shimada and L. Goodman, *J. Chem. Phys.*, 1965, **43**, 2027.
[8] O. Hutzinger and M. Zander, *Analyt. Biochem.*, 1969, **28**, 70.

EXPERIMENTAL

*Materials.*—LSD-25 (lysergic acid diethylamide tartrate, methanol solvated) was supplied by Sandoz Products. D-Lysergic acid (monohydrate) was supplied by Koch-Light and agroclavine by Ralph Emanuel. All other ergot alkaloids were supplied by Fluka. Ethanol (99·7—100%) was purified by distillation through a Widmer fractionating column at a reflux ratio of 20 : 1, discarding the first and last 20% of the charge. Water used as solvent was freshly distilled and passed through an ion exchange column (Elgastat B102).

*Instrumentation.*—The spectrofluorometer used has been described previously.[9] The system utilises a 2 kW high-pressure xenon arc lamp (Mazda XE/D), two 500 mm grating monochromators (Bausch and Lomb), and an EMI 9558QB photomultiplier tube. Slit widths of 6 mm (bandpass 10 nm) were used on the excitation monochromator, and 3 mm (bandpass 5 nm) on the emission monochromator. 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 the measurement of phosphorescence lifetimes 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. The lifetime was then calculated from a plot of log (intensity) against time. The photomultiplier tube was cooled to −70 °C for all phosphorescence measurements in order to reduce the dark current.

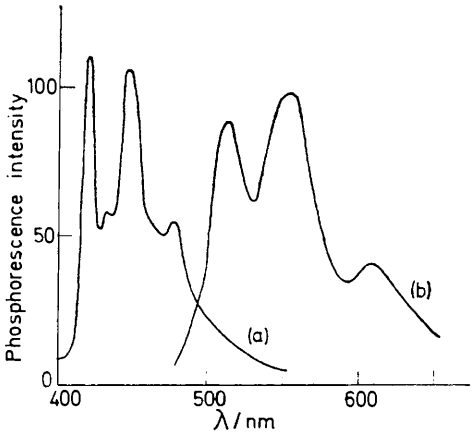



FIGURE 2 Phosphorescence spectra of (a) agroclavine and (b) ergometrinine in ethanol at 77 K (the spectrum of ergometrinine has been scaled up by a factor of 100)

Fluorescence decay time measurements were made using a time-correlated single photon counting system (Applied Photophysics). The excitation pulse had a width at half-height of *ca.* 4 ns and a repetition rate of 10 kHz. The determination of the decay time involves recording the spark profile, the fluorescence decay profile, and the calibration of the time base of the pulse height analyser (Northern Scientific model NS 606). The spark and fluorescence decay profiles are transferred from the memory of the analyser on to punched tape for computer analysis. The computer program evaluates the fluorescence decay time by synthesising the recorded fluorescence decay $F(t)$ at a time $t$ by means of a convolution integral (1) where $f(t)$ represents the

$$F(t) = \int_0^t I(t')f(t - t')dt' \qquad (1)$$

true fluorescence decay time which is modified by the instrumental response $I(t)$.

*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 a reference standard.[10] This method, outlined by Parker and Rees,[11] was modified to include the effect of refractive index.[12] The quantum yield ($\phi$) is calculated from the relation (2)

$$\phi_x = \phi_{st} \cdot \frac{A_x}{A_{st}} \cdot \frac{OD_{st}}{OD_x} \cdot \frac{\theta_{st}}{\theta_x} \cdot \frac{n_x^2}{n_{st}^2} \qquad (2)$$

where $A$ is the area under the emission, OD is the optical density at the exciting wavelength, $\theta$ is the relative photon output of the light source at the exciting wavelength, and $n$ is the refractive index; the subscripts refer to the standard (st) and the unknown (x).

*Correction of Spectra.*—All reported emission spectra are corrected for the spectral response of the emission monochromator and photomultiplier. Correction factors were obtained in the u.v. by the use of a Rhodamine B quantum counter.[13] For the 500—700 nm region a standard tungsten lamp was used.[11] Very good agreement was obtained between the two methods in the 450—550 nm region where both methods are applicable.

*Procedure.*—For fluorescence measurements solutions of concentration $10^{-5}$M were used. Phosphorescence measurements necessitated the use of more concentrated solutions ($10^{-4}$M). For low temperature measurements solutions in clear ethanolic glasses were used, which were deaerated on the vacuum line by the freeze–pump–thaw technique to exclude oxygen and minimise cracking of the solvent glass.

[3/083 *Received*, 15*th January*, 1973]

[9] A. Bowd, P. Byrom, J. B. Hudson, and J. H. Turnbull, *Photochem. Photobiol.*, 1968, **8**, 1.
[10] R. F. Chen, *Analyt. Letters*, 1967, **1**, 35.
[11] C. A. Parker and W. T. Rees, *Analyst*, 1960, **85**, 587.
[12] A. N. Fletcher, *J. Mol. Spectroscopy*, 1967, **23**, 221.
[13] W. H. Melhuish, *J. Optical Soc. Amer.*, 1962, **52**, 1256.