FLUORESCENCE PROPERTIES OF ERGOSTEROL

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Summary

Ergosterol is fluorescent in low temperature matrices as are several molecules with a 1,3-diene structure. The fluorescence lifetime of 3.3 ns and the fluorescence quantum yield of 0.19 have been obtained in a methyl-cyclohexane–isopentane matrix at 77 K. The non-radiative transition rate constant k_{nr} has been determined to be $10^{13.6} \exp(-11.1/RT)$ where RT is in kilojoules per mole. The activation energy can be ascribed to a solvent-dependent viscosity barrier because no detectable fluorescence is observed under supersonic jet conditions.

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1. Introduction

There is very limited information regarding fluorescence from molecules with a 1,3-diene structure. Emission from butadiene has not been detected. However, ergosterol (ergosta-5,7,22-triene-3β-ol, provitamin D_2 (I)) and its related compounds have a *cis*-1,3-diene structure and are fluorescent in an ether–isopentane–ethanol (EPA) matrix [1].

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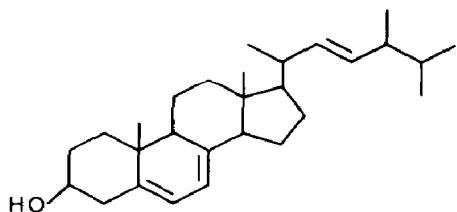
The photochemistry and thermochemistry of the ergosterol–vitamin D_2 system have been investigated thoroughly [2, 3] but the system continues to attract interest. Hence an improved product distribution and yield of vitamin D_2 has recently been obtained by a two-stage photolysis using lasers [4]. The system is also of interest from the viewpoint of the nature of the non-radiative transition in the S_1 state of molecules with a 1,3-diene structure.

As far as the photophysics of ergosterol is concerned, only the fluorescence spectrum has been reported to date [1]. In this paper more detailed studies are reported.

2. Experimental details

Fluorescence lifetimes were measured using the time-correlated single-photon counting technique, the excitation source being a Spectra Physics synchronously pumped cavity-pumped dye laser at 296 nm. This system has been described elsewhere [5]. Fluorescence measurements were made using Hitachi MPH 4 and Shimadzu 502F fluorometers, the sample temperature being continuously monitored using a thermocouple attached to the sample cell in an Oxford Instruments Type 704 cryostat with an accuracy to ± 2 K. The fluorescence quantum yield was determined by a relative method which involved the comparison of the corrected areas of the fluorescence spectra of ergosterol with that of a calibrant. In order to correct fluorescence spectra for the spectral response of fluorometers MgO was used as a reflection material and the absolute fluorescence spectra of 2-aminopyridine and quinine bisulphate in 1 N H_2SO_4 solution were used as standards [6]. The fluorescence quantum yield of 9,10-diphenylanthracene (DA) at 77 K in EPA and 3-methylpentane matrices has been determined to be 0.95 ± 0.05 [7]. The quantum yield of fluorescence of DA in a 1:1 methylcyclohexane–isopentane (MP) matrix was assumed to be 0.9 ± 0.05 in this report [8, 9]. Two other standards were used: phenanthracene (quantum yield, 0.13) [7, 10] and 2,5-diphenyl oxazole (quantum yield, 0.9 ± 0.05, this work) in MP (1:1) matrices at 77 K, because the extinction coefficients of DA are lower in the excitation region used here (280 - 295 nm) where those of ergosterol are high. Spectral resolutions were 1 nm or better for excitation and 1.5 nm or better for fluorescence observation. Degassed samples were used with an optical density less than 0.1 (about 7×10^{-6} M). A search for the fluorescence of ergosterol was carried out in supersonic jet conditions using a continuous wave slit nozzle apparatus, exciting at a range of wavelengths around 300 nm [11]. Ergosterol (Sigma) was recrystallized from freshly distilled 2-methyltetrahydrofuran–methanol solution.

3. Results

Absorption and fluorescence spectra in an MP matrix at 77 K are shown in Fig. 1. These spectra are in good agreement with those reported by Havinga *et al.* [1], except that fluorescence intensities in the region below 30 000 cm^{-1} are low in our spectra, presumably because of correction for the spectral response. The fluorescence lifetime $\tau_f^{77\,K}$ and the fluorescence quantum yield $\phi_f^{77\,K}$ in an MP matrix at 77 K have been determined as
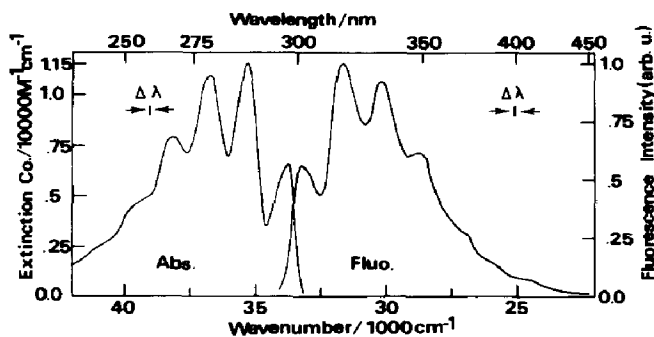
Fig. 1. Absorption and fluorescence spectra of ergosterol in an MP matrix at 77 K. Molar extinction coefficients have been corrected for the volume shrinkage due to cooling (see Appendix A). The fluorescence spectrum has been corrected for the spectral response of the detector.

$$\tau_f^{77\,K} \approx 3.3 \pm 0.1 \text{ ns}$$

and

$$\phi_f^{77\,K} \approx 0.19 \pm 0.03$$

Similar lifetimes have been obtained in 2-methyltetrahydrofuran (3.2 ns) and methylcyclohexane–butanol (4:1, 3.1 ns) matrices at 77 K. The fact that there was no detectable fluorescence intensity at room temperature ($\phi_f \leqslant$ 0.005) indicates that the fluorescence yield depends strongly on temperature or on the rigidity of the medium.

The fluorescence intensity in an MP matrix has a constant value in the temperature region below 89 K and decreases with increase in temperature to approximately 1/20 of the constant level at 150 K. The temperature dependence has been analysed in terms of the simplest kinetics:

$$S_0 \xrightarrow{h\nu} S_1 \qquad S_1 \xrightarrow{k_r} S_0 + h\nu_f \tag{1}$$

$$S_1 \xrightarrow{k_{nr}} S_0, T, \text{ products} \tag{2}$$

The following relation is derived immediately, where the non-radiative rate constant $k_{nr} = (k_{nr}^0 + k_{nr}(T))$ and the radiative rate constant k_r is assumed to be independent of temperature.

$$\ln\left(\frac{I_f^0}{I_f(T)}\right) - 1 = \ln(\tau_f^0 k_{nr}(T))$$

where I_f^0 is the fluorescence intensity at temperatures lower than 89 K and $I_f(T)$ is that at T K. The Arrhenius plot of $\ln\{I_f^0/I_f(T) - 1\}$ versus $1/T$ is linear as shown in Fig. 2. The slope gives the activation energy E associated with k_{nr} using $\tau_f^{77\,K}$ as τ_f^0 and the intercept at $\tau_f = \infty$ gives a pre-exponential factor k_0 of the non-radiative rates as follows from eqn. (3).
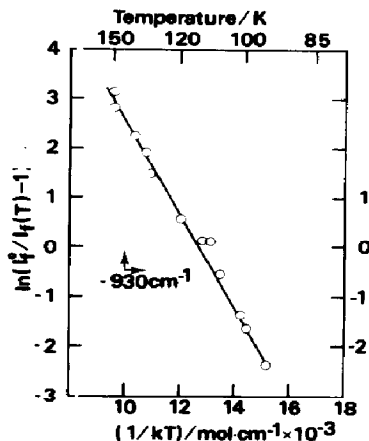
Fig. 2. Arrhenius plots for $\ln\{I_f^0/I_f(T)-1\}$ vs. kT^{-1}. I_f^0 is an average of fluorescence intensities in the range between 85 and 89 K; $I_f(T)$ is the fluorescence intensity at temperature T K. The slope of the plot, -930 cm^{-1} as shown, gives an activation energy of 11.1 kJ mol^{-1} (see text).

$$k_{\mathrm{nr}}(T) = k^0 \exp(-E/RT) \qquad (3)$$

where

$$k^0 = 10^{13.6 \pm 2}$$

$$E = 11.1 \pm 0.15 \text{ kJ mol}^{-1}$$

4. Discussion

4.1. Activation energy

Any measured activation energy E in solution generally involves an inherent thermal barrier E_t and a solvent-dependent viscosity barrier E_v. An activation energy measured in isolated molecule conditions in a supersonic jet gives E_t as has already been indicated by elegant experiments [12]. Ergosterol is thermally stable with a vapour pressure of several torr at 540 K [2, 13] and thus the beam experiment could be performed. However, we did not detect fluorescence, which suggests that the fluorescence quantum yield in beam conditions was less than 0.05 (based on results obtained with acenaphthene in slit beam conditions [11]). The failure to observe fluorescence in the supersonic jet, where cooling to translational and rotational temperatures of less than 7 K and vibrational temperatures of less than 50 K is commonly realized, deserves comment. If the intrinsic barrier to twisting motion in the molecule is indeed small, it may be that the molecules were insufficiently cold to freeze out the non-radiative decay process. Results on other molecules, however, suggest that cooling under the conditions used in these experiments should be extensive with the majority of molecules residing in the rotationally cold zero-point vibrational level of the ground state, which should give rise to a fluorescent singlet excited state on excitation in the 0–0 transition. A possible explanation of the absence of fluorescence

might be that the emission in the solid matrix actually occurs from an underlying forbidden state (as is seen commonly in conjugated olefins) populated by internal conversion from the initially pumped singlet state. In the jet, in isolated molecule conditions, this internal conversion process could provide enough energy to surmount any small barrier to the twisting motion which promotes non-radiative decay, and hence the absence of fluorescence.

The moderately high quantum yield of fluorescence exhibited by the ergosterol molecule in solution at temperatures below 89 K appears to be due to the rigidity of the solvent matrix. The observed activation energy E can be ascribed solely to the solvent-dependent viscosity barrier ($E_v = 11.1$ kJ mol^{-1} and $E_t \approx 0$).

Saltiel and D'Agostino have analysed in detail E_t and E_v for several systems by measuring fluorescence intensities at different viscosities and varying temperatures [14].

For stilbene in methylcyclohexane [14] an E_t value of 2.7 kcal mol^{-1} has been obtained and this has been confirmed by a beam experiment [12]. In some systems E_t has a value close to zero. A simple relation between the pre-exponential factor k^0 and E_v was proposed [14]:

$$\log k^0 = 11.7 + 0.71 E_v \tag{4}$$

where E_v is in kilocalories per mole. The present value of 11.1 kJ mol^{-1} (2.66 kcal mol^{-1}) satisfies excellently the above relationship. Equation (3) can be rewritten as follows

$$k_{nr} = k_t^0 k_v^0 \exp\left\{ -\frac{(E_v + E_t)}{RT} \right\}$$

$$k_t^0 = 10^{11.7} \qquad k_v^0 = 10^{1.9}$$

$$E_v = 11.1 \text{ kJ mol}^{-1} \qquad E_t \approx 0$$

4.2. Radiative lifetime

A value for the radiative lifetime τ_r of 17.4 ns can be evaluated from $\tau_f^{77\,K}$ and $\phi_f^{77\,K}$. τ_r is calculated to be 4.25 ns from the absorption and fluorescence spectra in Fig. 1 (see Appendix A). The discrepancy requires comment. Although the fluorescence spectrum is a good mirror image of the absorption spectrum, a forbidden A_g state may be hidden under the allowed transition $^1B_u \leftarrow {}^1A_g$ as in the case of large linear polyenes [15]. The alternative explanation that the geometry in the S_1 state changes before fluorescence even in an MP matrix, giving a longer fluorescence lifetime as observed for styrene derivatives in the gas phase [16], is not borne out by the mirror symmetry relationship between absorption and emission.

4.3. Reaction in matrices

The ergosterol fluorescence intensity distribution changes slowly in an MP matrix at 77 K on irradiation. Fluorescence intensities longer than 400

nm increase as those around 300 nm decrease. In a low viscosity matrix of isopentane—cyclopentane (4:1), irradiation for 4 min at 283 nm with a 4 nm slit width in a Hitachi MPF 4 fluorometer leads to a doubling of the initial fluorescence intensity at 400 nm.

The fluorescent molecule tachysterol, which is formed by isomerization of the primary product provitamin D (non-fluorescent) [2], is probably produced. Tachysterol shows a fluorescence peak around 400 nm and has a short wavelength onset around 330 nm which has been confirmed in the excitation spectrum. Even in the hard matrix, ethanol at 80 K, reactions have been observed [17]. This high reactivity suggests that deformation with a very low activation energy leading to chemical reactions is one of the main routes for the non-radiative transition.

4.4. Related compounds

7-dehydrocholesterol (provitamin D_3) is also fluorescent in matrices at 77 K. However, we did not detect any fluorescence from such relatively rigid conjugated molecules as cholesterilene, cyclopentadiene and 1,3-cyclohexadiene, although fluorescence of cholesterilene has been reported on edge excitation of the absorption spectrum [18]. Subpicosecond relaxation processes involving twisting motion are the reason for non-fluorescence in open-chain flexible conjugated molecules such as butadiene [19]. The absence of fluorescence from rigid cyclic dienes with low chemical reactivities such as cyclopentadiene or cholesterilene is perhaps surprising. One of the notable differences between these compounds and the provitamins D is the energy of the 0–0 transitions. The wavelengths of the 0–0 bands in ergosterol and 7-dehydrocholesterol are 293 nm in MP at room temperature, for cyclopentadiene the value is 263 nm and for cholesterilene it is 243 nm. Thus it may be that a state above 4.25 eV may strongly couple with the S_1 state in the non-fluorescent compounds and may induce non-radiative transitions.

5. Conclusion

It has been demonstrated in this paper that even in fluorescent molecules with a 1,3-diene structure deformation with negligibly small intrinsic activation energy induces a rapid non-radiative transition.

Acknowledgments

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Appendix A

In order to calculate the radiative rate constant k_r from eqn. (A1) [A1], the molar extinction coefficients $E(\nu)$ and the refractive indices at the mean frequency of absorption and emission, n_a and n_f respectively, are required at 77 K.

$$k_r = 2.880 \times 10^{-9} \frac{n_f{}^3(340 \text{ nm})}{n_a(280 \text{ nm})} \langle \bar{\nu}_f{}^{-3} \rangle_{av}{}^{-1} \int \frac{E(\nu) \, d\nu}{\nu} \tag{A1}$$

The volume shrinkage of the methylcyclohexane–isopentane (1:1) solvent owing to cooling to 77 K has been estimated to be $\nu(77 \text{ K})/\nu(293 \text{ K}) = 0.79$ by extrapolating several values of methylcyclohexane–isopentane mixtures [A1]. The refractive indices have been calculated from [7]

$$n(T)^2 = n_0{}^2 \left\{ 1 + \frac{C}{n_0} \alpha(T - T_0) \right\}^2 \tag{A2}$$

where $n(T)$ and n_0 are the refractive indices at temperatures T and T_0 respectively, $C = 0.502$ and $\alpha = -9.72 \times 10^{-4}$, using the following single term Sellmeier equation for evaluation of the indices n_0 at $T_0 = 293$ K

$$n_0{}^2 = 1.9006 + \frac{9.1088 \times 10^{-3}}{\lambda^2 - 0.01310} \, d \qquad\qquad (A3)$$

where the wavelength of emission λ is in micrometres. In eqns. (A2) and (A3), C, d and n_0 have been estimated by weighting the individual values of the densities and refractive indices of the two components.

References for Appendix A

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