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FLUORESCENCE LIFETIME MEASUREMENTS OF PSEUDOAZULENES USING PICOSECOND-RESOLVED SINGLE PHOTON COUNTING

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ABSTRACT

The very rapidly decaying fluorescence of three different pseudoazulenes was measured, enabling to check simultaneously the time resolution and accuracy of the set-up with mode-locked and synchronously pumped lasers and time correlated single photon counting. Instrumental details are given to reach optimum time resolution and accuracy. The fluorescence decay of a widely used fluorescent standard was used in the deconvolution procedure.

INTRODUCTION

The anomalous fluorescence from the higher singlet excited state found for the first time in azulene (ref. 1) has been observed in numerous compounds (refs. 2-4). In some azulene compounds and in some pseudoazulenes the $S_2 - S_0$ fluorescence is distinguishable for its relatively high quantum yield. Pseudoazulenes are the heterocyclic azulene compounds in which a heteroatom replaces a -CH=CH- group in the heptagonal ring of azulene. Spectroscopic properties of these compounds are similar to those of azulenes (refs. 5,6). Absorption bands due to $S_0 - S_2$ and higher transitions are located in the UV region <330 nm. Fluorescence appears in the region 360-450 nm with quantum yields of the order 10^{-5} to 10^{-3}, depending on the type of derivatives (ref. 7).

Pseudoazulenes do not exhibit any detectable emission which can be related to the $S_1 - S_0$ transition. Radiationless processes are rapid in these molecules with rate constants several orders of magnitude higher than in aromatics or heteroaromatics, probably related to the nonalternant character of pseudoazulenes. The rate constant of the radiationless decay of S_1 is larger than 10^{12} s^{-1}, and of the same order for the S_2 state.

Estimates from the Jablonski relation for the high polarisation of emission in low viscosity solutions give the limits for the fluorescence decay time to be less than 0.1 ns. Measurements of the fluorescence lifetime of the

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S_2 state performed with picosecond laser techniques confirmed this range of lifetimes (ref. 8). Precise measurements of the fluorescence decay in pseudoazulenes were up to now very difficult. In this paper we describe the determination of fluorescence lifetimes of pseudoazulenes and discuss the experimental limits.

MATERIALS AND METHODS

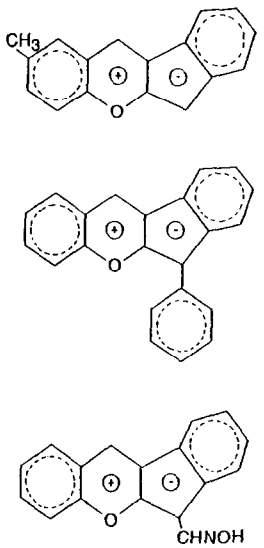

Fig. 1 Chemical structures of CH_3-DBO (1), Phe-DBO (2) and CHNOH-DBO (3).

The pseudoazulenes, derivatives of 1,2-5,6 dibenzooxalene (DBO, systematic name benzo/b/indeno/1,2-e/piran) were purified as described prevously (ref. 6). N-hexane, fluorescence grade (Merck) was used to prepare solutions with a concentration corresponding to 0.2 OD at 300 nm. We used the pseudoazulenes CH_3-DBO (1), Phe-DBO (2) and CHNOH-DBO (3), see Fig. 1. The absorption and fluorescence maxima were around 330 and 400 nm respectively. The quantum yield was $1.2.10^{-3}$ for CH_3-DBO, 6.10^{-3} for Phe-DBO and $1.2.10^{-3}$ for CHNOH-DBO.

The quantum counter p-bis[2-(5-phenyloxazolyl)]benzene (POPOP), purchased from Eastman Kodak (scintillation grade), was dissolved in fluorescence grade ethanol (Merck), concentration corresponding to an OD of about 0.1 at 300 nm.

The experimental set-up was as described earlier (ref. 9). Excitation was from the frequency doubled output of a synchronously pumped CW dye laser, supplying pulses of several picoseconds at 300 nm. An electro-optic modulator was used to reduce the rate of excitation pulses (ref. 10). Fluorescence detection was at 90° with respect to the excitation direction and a 402 nm interference filter (Baird Atomic) was used for scatter rejection. All measurements were carried out at 20°C.

A sheet type polarizer (Polaroid type HNP'B) could be rotated from parallel to perpendicular position or, depending on experimental requirements, set under magic angle with respect to the direction of excitation polarization. The photomultiplier was a Hamamatsu R1645U-01 microchannel plate type, detecting fluorescence photons at maximum 30 kHz at a rate of excitation pulses of 600 kHz. The time of arrival of fluorescence photons was repeatedly registrated by conventional start-stop equipment and gathered in a multichannel analyzer. The width of the overall instrumental response curve was 140 ps.

DATA ANALYSIS

Data analysis was as earlier described (ref. 9). A reference method was used in the deconvolution procedure. In addition to the fluorescence decay of the unknown sample also that of a well known reference compound is recorded and used for deconvolution purposes. In the deconvolution procedure the experimental data were fitted in an iteration process, minimizing the weighted sum of squared residuals (reduced χ^2). In that way fluorescence lifetimes much shorter than the full width at half maximum (FWHM) of the instrumental response were precisely determined. In the present case the relatively long decay time of the quantum counter is exactly known. The lifetimes of the pseudoazulenes were estimated by systematic variation of the (ps) reference lifetime until the reduced χ^2 was minimized.

INSTRUMENTAL REQUIREMENTS

The time correlated single photon counting technique is a very sensitive tool in fluorescence spectroscopy (ref. 11). Using the short pulses of CW synchronously pumped dye lasers for excitation, the time resolution is limited by the photomultiplier (ref. 12). Because of the very short decay times to be detected in this study, special care was taken to avoid artefacts.

For instance, when a filter is placed somewhere in the exciting beam or fluorescence light path an extra time delay (around 1 ps per mm substrate) is introduced due to the difference of the index of refraction of the filter and air. That delay should introduce a shift in time of the recorded decay. These small time shifts would introduce an artificial short component when the shifts occur between the registration of data from unknown and reference compound. In principle these shifts, when known, can be corrected for during data analysis. When adjusting the energy of excitation pulses a variable neutral density filter with metallic coating was used or neutral density filters with metallic coating and equal thickness were exchanged.

Another source of errors can be the time drift due to variation of the ambient temperature during the measurements. All electronic components in the set-up (discriminators, delay-line, etc.) have temperature dependent transit times. This dependency is for instance +10 to 20 ps/°C for discriminators and - 20 ps/°C for a 100 ns delay line (50 ohm type RG-58 C/U). Therefore for picosecond measurements the ambient temperature should be very stable. Correction for this kind of shifts is hardly possible because they also will distort the decays due to the time shift during the registration of data. Not only a stable ambient temperature but also a good balancing of components in start and stop line of the set-up will minimize these temperature dependent shifts.

RESULTS

In the analysis of fluorescence decay of short lived fluorescence the instrumental response function of the exciting pulse must be taken into account. However, when the pulse was measured with a scatterer at the wavelength of excitation (300 nm) the pulse was delayed with respect to the fluorescence response of either compound (1), (2) or (3), measured at 402 nm. Although less such a reverse shift can still be observed at 363 nm. This effect must be due to a wavelength dependent response of the microchannel plate detector. The presence of this effect calls for the reference convolution method as recently described by us (ref. 9). By noting that the FWHM of the pulse and the fluorescence profiles are approximately identical an important conclusion can be drawn. The fluorescence lifetimes of the pseudoazulenes are extremely short (< 50 ps), as otherwise some broadening of the fluorescence response must be observed.

In Fig. 2 an example of a decay analysis of POPOP is shown, in which reference compound (1) was measured under identical conditions. Both the fluorescence lifetimes of POPOP and of compound (1) were treated as free parameters and were optimized until a minimum in reduced χ^2 was obtained. The lifetime of POPOP was recovered at high precision: 1294 ± 2 ps. However, the lifetime of (1) turned out to yield a negative value: -7 ± 2 ps. This negative value must imply that an additional process in POPOP competes with the extremely rapid fluorescence decay of (1). The latter process is most likely internal conversion from excited vibronic states to the emitting S_1 state of the dye molecule. The rate constant of such radiationless transitions is estimated to be in the THz range yielding a time constant of a few picoseconds.

In order to estimate the lifetime of (1) an analysis of POPOP according to a biexponential fluorescence decay model consisting of a ps lifetime component and the long lifetime of POPOP with a negative preexponential factor was carried out. The positive preexponential factor can be accounted for by the extremely rapid growing in of fluorescent species. The reference compound was given discrete values between 1 and 30 ps and the fit was calculated with the same fixed decay parameters throughout. For short reference lifetimes we obtained fits of similar quality as the one presented in Fig. 2. For longer reference lifetimes, however, there is a clear deviation between calculated and experimental fluorescence responses. In Fig. 3 we have plotted the reduced χ^2 against the reference lifetime for the particular experiment described in Fig. 2. It can be clearly observed that there is a distinct minimum in χ^2 for a certain reference lifetime.

We have analyzed other POPOP fluorescence decays with all reference compounds in the same way and data have been collected in Table 1. In all experi-

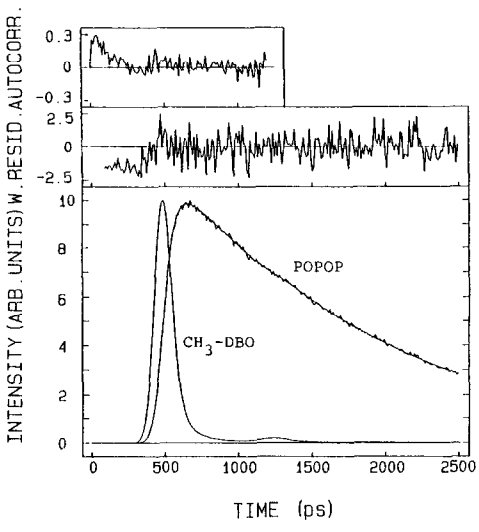

Fig. 2 Example of decay analysis of POPOP, shown are the decay curves of POPOP and CH_3-DBO.

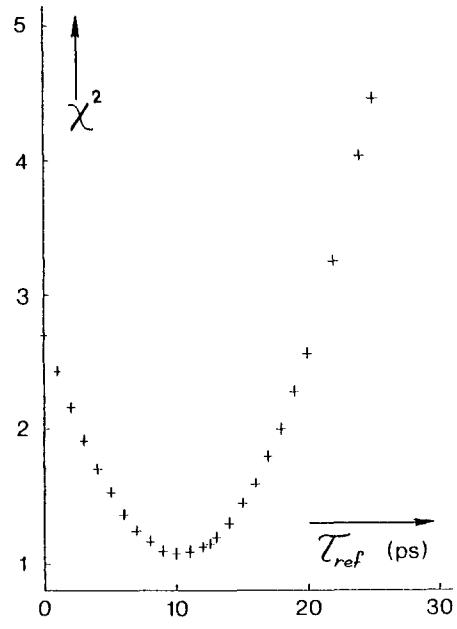

Fig. 3 Plot of χ^2 as a function of τ_{ref}, all other parameters were fixed.

ments it is evident that the pseudoazulenes have approximately the same fluorescence lifetimes. The spread in values is quite large and only a range between 1 and 10 ps can be indicated. One source of the lack of precision is electronic drift from one experiment to another as explained in the preceding section. Also, the exact value of the time constant of internal conversion is an uncertain factor.

TABLE 1

Fluorescence lifetime and initial and time-averaged fluorescence anisotropy of three pseudoazulenes.

Compound (no.)	τ [ps]	r_0 [-]	<r> [-]
1	1-10	0.290	0.286
2	1-6	0.171	0.170
3	1-11	0.191	0.189

Another line of evidence for extremely rapid fluorescence of the pseudoazulenes comes from time-resolved polarized fluorescence spectroscopy. In Table 1 the initial (at zero time) and the time-averaged fluorescence anisotropy are given. Within experimental error the fundamental and the steady-state anisotropies are similar. Such a lack of depolarization of the fluorescence can only be observed when the reorientation time of the pseudoazulenes is longer than the fluorescence lifetime. Otherwise a distinct difference between fundamental and average anisotropies will be predicted from the Perrin equation. An estimate of the reorientation or rotational correlation time would be in the range of 50-100 ps in hexane and it suggests a fluorescence lifetime of pseudoazulenes an order of magnitude shorter.

CONCLUSION

From the experiments described we have unambiguously shown that the fluorescence lifetimes of several pseudoazulenes are in the range of a few picoseconds. It is believed that this lifetime is the lowest limit of fluorescence lifetime determinations with sync-pumped lasers and time-correlated single photon counting with microchannel plate detectors under stable measuring conditions.

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