Phosphorescence of 8-Azasteroid in Solid Solution

S. A. Bagnich,[1,3] **N. N. Khropik,**[1] **V. N. Knyukshto,**[1] **and A. L. Mikhalchuk**[2]

This paper presents the results of the investigation of delayed luminescence of 2,3-methoxy-8-azagona-1,3,5(10),13-tetraene-12,17-dione classed with 8-azasteroids in ethanol solution at 77 K. Dual phosphorescence with maxima in the region of 400 and 500 nm, depending on the excitation wavelength, has been discovered. It is shown that the short-wavelength luminescence is due to the radiation transition from second triplet state. The possible mechanisms of emergence of dual phosphorescence in the compound investigated are considered.

Systematic studies in the field of bio-organic chemistry of 8-azasteroids have shown that compounds of this class are modulators of human and animal immunity [1]. By now effective methods of synthesis of 8-azasteroids have been developed, and recommendation on their selection as immunity stimulators and depressants have been worked out. The study of the photophysical properties of biologically active molecules is important for both establishing the electronic structure of these compounds and revealing the possible correlation between their spectroscopic characteristics and the physiological action on living organisms. Spectral-luminescent studies of the compounds of the class of 8-azasteroids were begun comparatively recently [2]. However, practically all these works presented the results of the investigation of the fluorescence of the above compounds.

This paper presents the results of the investigation of phosphorescence of solid solution in ethanol of one of the compounds of the class of 8-azasteroids. The distinguishing feature of 8-azasteroids is the presence in their structure of a conformationally limited heptatomic cross-linked α-acyl-β-aminovinylcarbonyl fragment $N^8-C^{14}=C^{13}(-C^{12}=O)-C^{17}=O$. Fig. 1 shows the structure of 2,3-methoxy-8-azagona-1,3,5(10),13-tetraene-12,17-dione (hereinafter referred to as 8-azasteroid), which was the object of our investigations. This compound is one of the few compounds of the class of 8-azasteroids exhibiting phosphorescence.

Figure 2 shows the spectra of absorption, fluorescence, and excitation of fluorescence of 8-azasteroid in ethanol at room temperature. The absorption spectrum has two absorption bands whose extinction coefficients are of the order 20,000 cm^{-1} M^{-1} ($\lambda = 260$ nm) and 16,000 cm^{-1}M^{-1} ($\lambda = 290$ nm). In the long-wavelength region, no pronounced absorption bands are observed. At the same time, in the 300–400 nm range a fluorescence excitation spectrum registered. A similar behavior was observed in [3] for 8-azagona-12,17-dione in acetonitryl. The authors of [3] have shown that the observed spectral features are due to the fact that in the 250–300 nm range the $\pi \rightarrow \pi^*$ type transitions are responsible for the absorption and in the 300–400 nm range − the n$\rightarrow \pi^*$ type transitions with an extinction coefficient of less than 100 cm^{-1}M^{-1}. And fluorescence is emitted from the nπ^*-level.

It should be noted that for both compounds the fluorescence excitation spectrum appreciably differs from the absorption one. Unlike in the absorption spectrum in the

[1] Institute of Molecular and Atomic Physics, F. Skaryna Ave. 70, 220072 Minsk, Belarus.
[2] Institute of Bio-Organic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus.
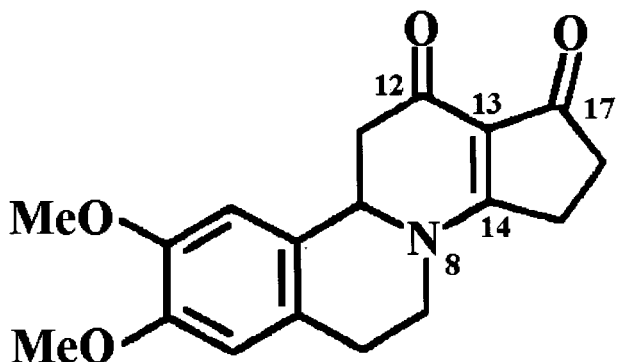[3] To whom correspondence should be addressed. E-mail: bagnich@imaph.bas-net.by

Fig. 1. Structure formula of 2,3-methoxy-8-azagona-1,3,5(10),13-tetraene-12,17-dione.

400–290 nm range, there is a monotonic increase in the extinction coefficient, and in the fluorescence excitation spectrum a maximum in the region of 340 nm is observed. The concentration of substance in the solution and the extinction coefficient value in this spectral region are low enough that only small fraction of the incident excitation light is absorbed by a sample. Thus, we can expect a good agreement between the fluorescence excitation spectrum and the normal absorption spectrum.

The decrease in the efficiency of fluorescence excitation in the region of wavelengths <350 nm is indicative of the appearance of a new, more effective (compared with the internal conversion to the S_1-state) channel for electronic excitation energy relaxation. In our opinion, the investigation of the 8-azasteroid phosphorescence makes it possible to determine the nature of this channel.

Figure 3 shows the spectra of delayed luminescence and the spectra of its excitation obtained for various solid
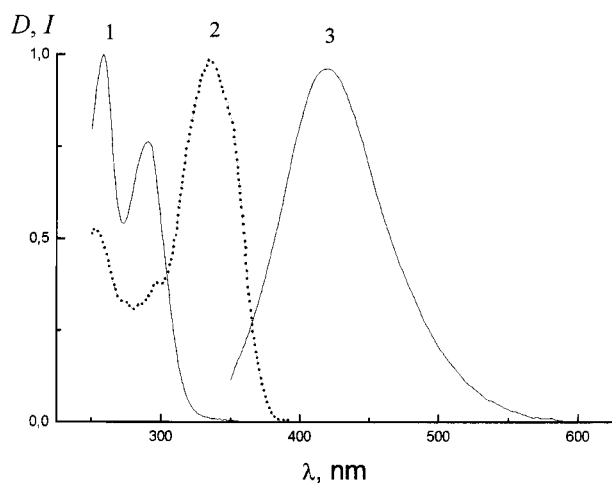

Fig. 2. Normolized absorption (1), excitation fluorescence (2), and fluorescence (3) spectra of 8-azasteroid in ethanol at 293 K. D: Optical density; I: intensity.
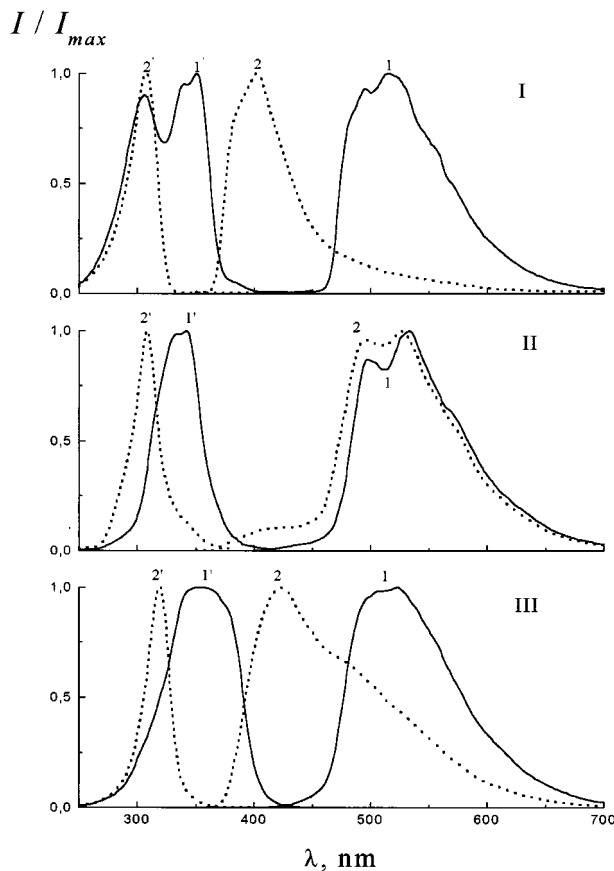

Fig. 3. Uncorrected spectra of phosphorescence (1, 2) and its excitation (1′, 2′) of 8-azasteroid in solid matrices at 77 K. λ_{exc}: 1 − 350; 2 − 310 nm. λ_{reg}: 1′ − 500; 2′ − 400 nm. Top to bottom: ethanol, polymethyl methacrylate, and porous silicate glass.

matrices at liquid nitrogen temperature. For the given compound, two types of luminescence with maxima in the region of 400 and 500 nm whose excitation spectra lie in the region of 310 and 350 nm are observed. There is a coincidence of the spectra of excitation of fluorescence and long-wavelength luminescence.

Long-wavelength luminescence is characterized by exponential decay with $\tau = 1.5$ s. Short-wavelength luminescence decays with $\tau = 0.18$ s.

The first impression that arises at observation of the two types of delayed luminescence is the presence of impurity in the solution. But the method of synthesis and purification of 8-azasteroid ensures the purity of substrate is no less than 99%. At the same time, the short-wavelength luminescence is more intensive that the long-wavelength one. All these reasons prompt consideration of the other possible origins of the dual luminescence observed in this paper.

Similarity of the fluorescence and short-wavelength delayed luminescence spectra can lead to the conclusion

that the second is a delayed fluorescence caused by 1-photon photoionization or 2-photon photoionization via the triplet state, with subsequent delayed recombination. In this case we should observe strong nonexponetial decay, but we observed exponential decay for both types of luminescence.

It is obvious that the dual luminescence observed cannot be due to an emitting of two different species of the same compound in the solution. The two spectral components can be attributed to neither free molecules and molecules forming hydrogen bonds with the solvent molecules [4] nor two tautomeric forms [5]. The effects in these cases are too small. A more formidable effect was observed for benzil in polyhydric solvent that had at least two hydroxylic hydrogen atoms per molecule, for example, in an ethylene glycol glass at 77 K [6]. In such matrices, benzil shows dual phosphorescence. The short-lived phosphorescence at 504 nm is assigned to near-planar transoid geometry. This type of phosphorescence does not depend on the type of solvent. The long-lived one at 402 nm is due to medium imposed near-planar cisoid geometry. It is obvious that the 8-azasteroid molecule has a sufficienty rigid structure. This means we can exclude the possibility that two spectral components observed for 8-azasteroid are attributed to two isomeric excited states.

The observed types of delayed luminescence can be interpreted as phosphorescence emitted from the triplet states T_1 and T_2. From this point of view of the Kasha's rule [7], emission from the T_2 state is an anomalous event.

Situations similar to that discussed here situation were observed in [8,9] for 10,11-dihydro-5H-dibenzo-[a,d]cycloheptene-10-11-dione (bridged benzil). This compound exhibits dual phosphorescence depending on the excitation wavelength, i.e., phosphorescence from the T_1 ($n\pi^*$) and T_2 ($\pi\pi^*$) states with maxima in the region of 530 and 400 nm, respectively. The relatively weak phosphorescence from a lower triplet state proceeds on excitation to the S_1 ($n\pi^*$) state (440 nm), whereas excita-

tion to the S_2 state with a $\pi\pi^*$-configuration (273 nm) causes phosphorescence emission over a much shorter-wavelength region with a high emission efficiency. The authors explain the presence of phosphorescence from the second triplet state for the given compound by the large energy gap between the T_1 and T_2 levels that lead to unusually low efficiency of the internal conversion by analogy with the energy-gap law, which is applied to the anomalous S_2 fluorescence of azulene and other compounds (for example [10]).

The conclusion that the two types of delayed luminescence observed for 8-azasteroid are dual phosphorescence from two triplet states is consistent with the results of the theoretical analysis of the electronic structure and spectroscopic characteristics of 8-azagona-12,17-dione performed in [3]. Some parameters of the calculation are presented in Table I. These investigations have shown that the first two singlet states are of the $n\pi^*$-type. The state with $\pi\pi^*$-configuration lies higher. The investigation of the spectrum fluorescence and the spectrum of its excitation suggests that the lower singlet state of the compound under investigation in ethanol is in the region of 27,800 cm^{-1} (360 nm). All these states are linked by a strong spin-orbital interaction with the triplet states. For the S_1 state, the channel of intersystem crossing is dominant ($k_{ST} \approx 3*10^9$ s^{-1}). Calculations yield the following energy values for the two lower triplet states: T_1 ($n\pi^*$-state) − 21241 cm^{-1} (471 nm) and T_2 ($\pi\pi^*$-state) − 25424 cm^{-1} (393 nm). As seen, the experimental results agree with the calculated data.

However, despite the presence of certain common features, we should also note the existing difference in dual phosphorescence between 8-azasteroid and bridged benzil. This concerns the disposition of energy levels. Unlike bridged benzil, in the 8-azasteroid molecule both triplet levels lie lower than the S_1 level. At the same time, it is well known [11]-that the intersystem crossing of the $^1n\pi^* \rightsquigarrow\rightarrow {}^3\pi\pi^*$ type is more effective than that of the $^1n\pi^* \rightsquigarrow\rightarrow {}^3n\pi^*$ type. The above mentioned value of k_{ST}

Table I. Results of Theoretical Analysis of the Electronic Structure and Spectroscopic Characteristics of 8-Azagona-12,17-dione Performed in [3]

State	S_1	S_2	S_3	S_4	S_5	S_6
Type of state	$n\pi^*$	$n\pi^* + \pi\pi^*$	$\pi\pi^* + n\pi^*$	$\pi\pi^* + n\pi^*$	$\pi\pi^*$	$\pi\pi^* + \pi\sigma^*$
E, nm/E, cm^{-1}	328/30522	317/31584	313/31952	297/33719	288/34733	251/39904
f	0.0002	0.029	0.026	0.095	0.530	0.141
k, s^{-1}	1.2*10^5	2.1*10^6	1.9*10^7	7.7*10^7	4.5*10^8	1.6*10^8
State	T_1	T_2	T_3	T_4	T_5	T_6
Type of state	$\pi\pi^* + n\pi^*$	$\pi\pi^*$	$\pi\pi^*$	$n\pi^* + \pi\pi^*$	$n\pi^* + \pi\pi^*$	$\pi\pi^*$
E, nm/E, cm^{-1}	471/21241	393/25424	345/28968	344/29051	336/29787	319/31389

E: Energy of state; f: transition oscillator strength; k: radiation rate constant.

corresponds to $S_1 \sim\to T_2$ transition. In our case however, only the $^3n\pi^*$ level is populated at excitation of the lower singlet state. The relatively weak phosphorescence at 500 nm points to a low efficiency of $S_1 \sim\to T_1$ transition. This can be explained with regard to the data for the dibenzil molecule [12]. As in our case, the dibenzil molecule has two emitting triplet levels lying lower than the singlet S_1 level. Figure 4 presents the scheme of levels and transitions in dibenzil molecule, which perhaps we can use, and for the 8-azasteroid molecule. The emission from the second triplet state is due to the forbiddingness of internal conversion $T_2 \sim\to T_1$ as to the symmetry. At a low-level energy excitation (λ = 365 nm) only the lower triplet state is populated (for 8-azasteroid with low efficiency). Intersystem crossing $S_1 \sim\to T_2$ is only possible from very high vibrational levels, i.e., at a higher-energy excitation (λ = 302 nm), just as it takes place for the compound under investigation. As mentioned above, the efficiency of intersystem crossing to the T_2 state for 8-azasteroid should be much higher than to the lower triplet level. The appearance, with increasing excitation wavelength, of a more effective deactivation channel of the singlet state leads to the observed difference between the fluorescence excitation spectrum and the absorption spectrum.

Thus, the results obtained in this work demonstrate the presence of delayed luminescence in the molecules of the class of 8-azasteroids. The low probability of the presence of impurities in the substance studied, the existence of a molecule with close spectroscopic properties (bridged benzil), and the good agreement of experimental data with theoretical investigation allow us to make the conclusion that observed dual luminescence is associated with the radiative transitions from the first and second triplet states. The phosphorescence from the second triplet state is associated with the forbiddingness of internal conversion $T_2 \sim\to T_1$, which is due to either a large (over 4000 cm^{-1}) energy gap between the levels or the symmetry of the state. Despite the fact that both triplet states lie below the singlet level, the second triplet state is only populated at more short-wavelength excitation than is the case for the T_1 state. Different efficiencies of intersystem crossing into the triplet states, which are due to their molecular-orbital nature, lead to a radical difference between the fluorescence excitation spectrum and the absorption spectrum.
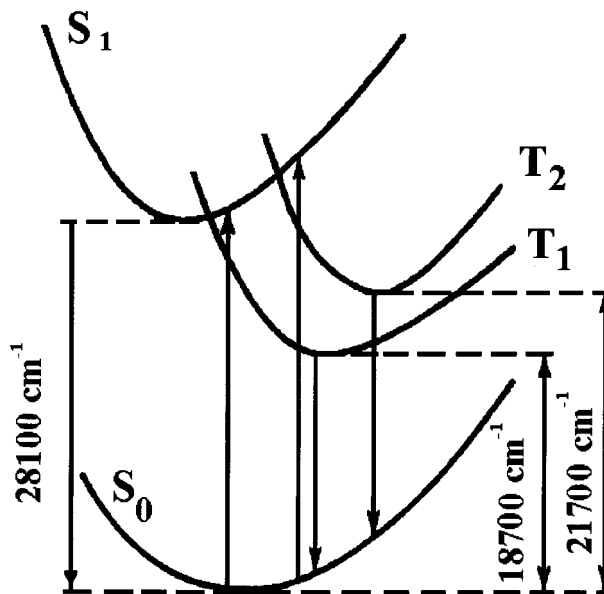

Fig. 4. Scheme of levels and transitions in dibenzil molecule. [12]

ACKNOWLEDGMENT

REFERENCES

1. N. A. Konoplya, O. V. Gulyakevich, A. L. Mikhalchuk, and B. B. Kuzmickiy (1994) *Vesti Akad Nauk Belarusi. Seriya khim.nauk.* 3 91 (in Russian).
2. A. A. Akhrem, N. A. Borisevich, O. V. Gulyakevich, A. L. Mikhal-chuk, T. F. Raichenok, S. A. Tikhomirov, and G. B. Tolstorozhev (1999) *J. Fluoresc* **9**, 357.
3. V. Y. Artyukhov, A. A. Akhrem, N. A. Borisevich, V. A. Ksenzov, V. G. Mayer, A. L. Mikhalchuk, and G. B. Tolstorozhev (2001) *Zhurnal Prikladnoy Spektroskopii* **68**, 5 (in Russian).
4. T. Nakayma, K. Sakurai, K. Ushida, K. Kawatsura, and K. Hama-nou, (1991) *J. Chem. Faraday Trans.* **87**, 449.
5. B. Nickel, K. H. Grellmann, J. S. Stephan, and P. J. Walla (1998) *Chem. Physics* **102**, 436.
6. H. Inoue, T. Mori, and T. Sakurai (1980) *Chem. Soc. Jap., Chem. Lett.* 683.
7. M. Kasha (1950) *Discuss. Faraday Soc.* **9**, 14.
8. H. Inoue and (1983) T. Sakurai *J. Chem. Soc. Chem. Commun.* 314.
9. H. Inoue, T. Sakurai, T. Hoshi, J. Okubo, and T. Kawashima (1986) *J. Chem. Soc. Faraday Trans.* **82**, 523.
10. S. Ghosh and M. Chowdhury (1982) *Chem. Phys. Lett.* **85**, 233.
11. M. A. El-Sayed (1963) *J. Chem. Phys.* **38**, 2834.
12. N. D. Zhevandrov, A. G. Vitukhnovski, Y. V. Voronov (1975) *Izvestiya Akademii Nauk USSR, Seriya fizicheskaya* **39**, 1868.