

# Phosphorescence of Enaminodicarbonyl-8-Azasteroids and Related Compounds

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We present the results of the investigation of the spectral-luminescent properties of some 8-azasteroids and model compounds in ethanol solution. The absorption and luminescence spectra of these molecules have been investigated. It is shown that all the compounds under investigation have common absorption and phosphorescence spectra that are close to the corresponding spectra of the related enaminodicarbonyl compounds. The results obtained allow to conclude that the main channel of singlet state deactivation is the intersystem crossing. It has been found that the 8-azasteroids are unstable under laser irradiation.

**KEY WORDS:** 8-Azasteroid; absorption; luminescence; solution.

## INTRODUCTION

Steroids comprise a large group of natural substances that must frequently be monitored in various biological materials. The steroid chemistry has made rapid progress since A. Butenandt isolated and determined the structure of the first hormones in 1929 (Estrone) and in 1931 (Androsterone). This is evidenced by the innumerable steroids that have been synthesized in the research laboratories of the most important universities, as well as in the pharmaceutical industry, whose pharmacological effectiveness is sometimes much higher than that of natural steroids. However, the problem of their structure determination has become even more detailed and specific. This is particularly true in the correlations between certain IR and UV absorption bands and the chemical structure [1].

Accumulation of steroids in biological membranes gives rise to a series of phenomena, that are due to either

the changes in the membrane structure or the steroid microcompartmentation within the cell. Depending on the functional groups and the general structure of the steroid skeleton, steroids in the biomembranes are more or less effective modulators of the membrane structure and fluidity leading to a change in the ion permeability, membrane conductivity, enzyme-enzyme coupling, lateral rotation mobility of proteins, and the capacity of further accumulation of ligands. Fluorescent steroids have often been used to probe the membrane structure and function [2–4], steroid-protein interaction [5–8] as well as steroid inclusion into cyclodextrins [9,10].

The fluorescent properties of steroids are widely used for their analysis and determination by different analytical techniques such as high-performance liquid chromatography [11], capillary electrophoresis with laser-induced fluorescence detection [12,13], thin-layer chromatography [14,15], and fluorescent immunoassay [16,17]. This is why the investigation of the steroids luminescence attract the attention of a wide circle of researchers. Naturally fluorescing steroids have been found [2,18,19] and numerous steroids have been synthesized [2,20,21]. However, it should be noted that the intrinsic fluorescence of most steroids is too weak and cannot be used as a diagnostic tool. In this connection, a highly fluorescent material has

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been obtained by denaturation (treatment by strong acid) [22,23]. Another way is to attach different fluorescent fluorophores to steroids [24].

At the same time there is lack of information about the phosphorescence of steroids. This is most likely to be due to difficulty of using this type of luminescence for analytical purposes. Nevertheless, the investigation of the steroid phosphorescence seems to be an important and topical problem of the current biophysical and photobiological research, since the excited states of steroids can be of importance in understanding their biological activity.

Studies in the field of heterocyclic analogs of steroids, in particular, azaanalogues were begun in the 1950s with the aim to separate hormonal (mineral and carbohydrate metabolism) and highly valuable therapeutic (antiinflammatory, antiallergic, antishock, etc.) actions typical of steroids. The first efforts in this field were to synthesize of compounds similar to natural ones, in which one or more carbon atoms forming the tetracyclic gonane (cyclopentanoperhydrophenanthrene) skeleton [25–27] were replaced by heteroatoms (N, O, and S).

This paper presents the results of the investigations of the spectral–luminescent properties of molecules of the 8-azasteroid series and some related compounds. 8-azasteroids, namely 8-azagona-1,3,5(10),13-tetraen-12,17-diones and their D-homoanalogues [28,29] represent a class of low–molecular immune–modulating compounds, which, depending on the structure, produce either an immunopotentiating (–stimulating) or an immunosuppressive (–depressive) effect on man and animals [28–30]. This makes them highly promising objects in developing immunity regulating and controlling means, especially taking into account the steady growth of immune disorders and their associated diseases.

## MATERIALS AND METHODS

The methods for synthesizing the compounds under investigation (see Fig. 1) are described in [31–33].

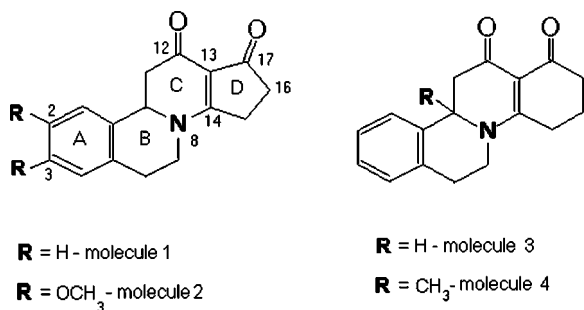


Fig. 1. Structural formulas of 8-azasteroid molecules.

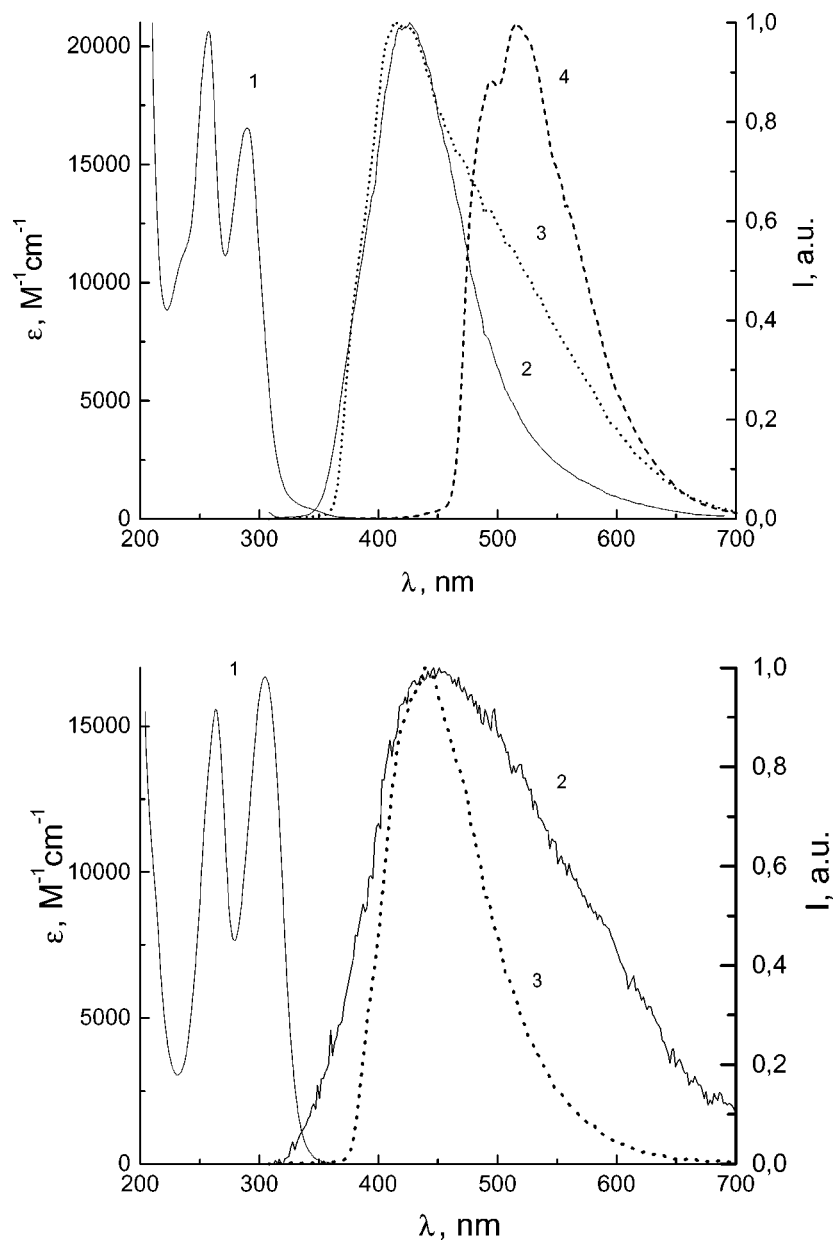
The absorption spectrum was measured on a Perkin-Elmer Lambda 9 spectrophotometer. The fluorescence and phosphorescence spectra were recorded by an optical multichannel analyser (PAR, model 1460) with a time-gated intensified diode array detector the, and gate width thereby was varied from 100 ns to 10 ms with a delay from 0 to 10 ms. The specially designed mechanical shutter enabled us to increase the decay time to seconds. The third harmonic of a Q-switched Nd: YAG – laser ( $\lambda = 355$  nm, repetition rate of 10 Hz), the Lambda Physik XeCl-excimer laser ( $\lambda = 308$  nm, repetition rate of 10 Hz) and the Lambda Physik FL 2001 dye laser were used for excitation.

## RESULTS AND DISCUSSION

Studies of the luminescent properties of compounds of this series were begun relatively recently [34–38]. However, practically all these works except [35] presented the results of the investigation of the fluorescence of the above compounds. It has been shown [35] that compound 2 (2,3-dimethoxy-8-azagona-1,3,5(10),13-tetraene-12,17-dione) in acetonitrile at 77 K manifests delayed luminescence in the region of 520 nm a decay time of 1 s. This luminescence was interpreted as a phosphorescence of this compound. However, it was found later [39] that there were two types of delayed luminescence for compound 2 with different spectra of excitation (see Fig. 2). The investigation of the spectral-kinetic properties of both types of luminescence was carried out in various solid matrices, namely, in low-molecular solvents, polymers, and porous glass [40]. Using porous glass as a matrix, experiments on the quenching of the delayed luminescence of compound 2 by molecular oxygen have been performed. As in the case of solid solutions, both types of delayed luminescence have been observed at low temperatures in a dry porous matrix in vacuum, while in air delayed luminescence was absent [40]. This result permits concluding that both types of delayed luminescence are the phosphorescence. Also the assumption that exactly the short-wavelength luminescence is the phosphorescence of the compound under investigation was made in [41].

The results of the investigation of various enaminodi-carbonyl compounds that enable us to draw conclusions concerning the phosphorescence of this type of molecules are presented below.

Figure 2 shows the absorption spectra of the solutions of compounds 2 and 4 in ethanol. It is seen that the spectra of both molecules have two bands. The change from the five-atomic ring D to a six-atomic one leads, respectively, to a 15 and 8 nm red shift and a change in the relative



**Fig. 2.** Absorption (1), fluorescence (2), and phosphorescence (3, 4) spectra of the ethanol solutions of compound 2 (top) and 4 (bottom). The concentration of 8-azasteroids in the solution was  $C = 10^{-5}$  M.  $\lambda_{exc}$ : 2, 3—308; 4—355 nm. Temperature: 1, 2—293; 3, 4—77 K.

intensity of the bands. In the case of compounds 1 and 3, the absorption spectra are close in position and form to the absorption spectra of compounds 2 and 4 respectively. The spectra obtained are close in position, form, and values of absorption coefficients to the data for similar compounds obtained in [42,43].

For compound 2, a strong fluorescence (the quantum yield is 0.15) has been observed for the solution in ace-

tonitrile [38] and in ethanol [41]. At the same time, there is a wide difference in both shape and position between the fluorescence excitation spectrum and the absorption spectrum [38,41]. In contrast to compound 2, the ethanol solutions of compounds 1, 3 and 4 demonstrate a very weak fluorescence (the quantum yields is about  $10^{-3}$ ) with a wide structureless spectrum. At liquid nitrogen temperature the excitation of the solid solutions of all compounds

under investigation in the first absorption band gives rise to strong blue phosphorescence (see Fig. 2). The quantum yield of this phosphorescence is 0.3–0.7 depending on the molecules and the decay time is 0.2 s. Unlike the results obtained for compound 2 [41], no new phosphorescence has been observed for compound 4 at excitation by light with a wavelength of 355 nm. In the case of molecules 1 and 3, some long-wavelength phosphorescence has been observed, but its intensity is about two orders of magnitude lower than for compound 2.

A distinguishing feature of the series of 8-azasteroids under investigation is the presence of a conformationally restricted cross-linked enaminodicarbonyl seven-atomic fragment  $N^8-C^{14}=C^{13}(-C^{12}=O)-C^{17}=O$ . Such fragments as well as their isostructural and isoelectronic analogies are widely distributed in natural and synthetic biologically active compounds. It is obvious that the similarity in the structure of these compounds should lead to a similarity in their spectral-luminescent properties. And this is actually observed for the absorption spectra. The shift of the absorption spectra and the change in the relative intensity of the bands observed on passing from compounds with a five-atomic ring D to compounds with a six-atomic ring are explained in [42,43] and do not break this commonness. Weak fluorescence with a quantum yield of about  $10^{-3}$  is observed for all compounds except for molecule 2. For the latter, strong fluorescence with a quantum yield of 0.15 has been observed [41]. At the same time, all compounds manifest blue phosphorescence with similar spectra, decay time (200 ms), and values of the quantum yield. The value of the phosphorescence spectrum maximum shift observed on passing from compounds with the five-atomic ring D (1 and 2) to the compounds with a six-atomic ring (3 and 4) agrees with that for the corresponding absorption spectra. In our opinion, these data permit concluding that the short-wavelength phosphorescence observed for all compounds under investigation is the real phosphorescence of the enaminodicarbonyl-8-azasteroids.

This conclusion agrees with the results of the quantum-chemical calculations of the electronic structure of molecules 1 and 2 [44,45]. It has been shown [44,45] that a deviation of the fragments B, C and D of the molecule skeleton from the plane of the aromatic ring A leads to a mixing of singly excited configurations of the  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$ -types when electron-excited states  $S_1$  and  $S_2$  of molecules 1 and 2 are formed. Since the contribution of the  $n \rightarrow \pi^*$  configuration is greater, the nature of the first two electron transitions is assigned to the  $n\pi^*$ -type. It is necessary to note that no weak band corresponding to the  $n\pi^*$ -transition has been observed in the absorption spectra of all compounds except for substance 2. In accordance with the results of the quantum-chemical

calculations, this  $n\pi^*$ -transition should be hidden under the band corresponding to the effective transition to the  $\pi\pi^*$ -states lying close to the lower excited state. The rate constants of the intramolecular photophysical processes (internal and singlet-triplet conversion) have also been calculated [45]. It follows from the calculation that the main deactivation channel of the fluorescent state in both molecules is the intersystem crossing. The calculated value of the quantum yield of fluorescence is  $10^{-2}$ – $10^{-3}$ . This result agrees with the experimental data for molecules 1, 3 and 4.

The above conclusion is supported by the data obtained for molecules that are not steroids but have the same cross-linked enaminodicarbonyl seven-atomic fragment (see Fig. 3). The absorption, fluorescence and phosphorescence spectra of the solution of these compounds in ethanol are presented in Fig. 4. It is seen that the absorption spectrum of molecule 5 is close to the spectra for molecules 3 and 4 in form, position and values of the extinction coefficient. The fluorescence spectrum is similar to the corresponding spectrum for compound 4 but the quantum yield is less. The same can be said about the phosphorescence of this compound. But in this case the quantum yield is higher by a factor of 1.5. A different picture is observed for substance 6. It is seen from Fig. 3 that the absorption spectrum of this substance differs considerably from the spectra of the other compounds under investigation. The form of this spectrum is similar to the spectra of enaminodicarbonyl compounds of the 8-azasteroid series [46]. These compounds have only one carbonyl group. This likeness can be due to the ability of one carbonyl group to rotate which leads to a non-planar structure of molecule 6 and to the going out of this group of conjugation in the enaminodicarbonyl seven-atomic fragment. The fluorescence spectrum of molecule 6 and its quantum yield are similar to the corresponding features of molecule 5. This suggests that the nature of the lower singlet states of these compounds is the same. At the same time the solid solution of molecule 6 does not manifest any delayed luminescence. This means that the structure of enaminodicarbonyl compounds should be almost planar for their phosphorescence to be observed.

Nevertheless the nature of the long-wavelength phosphorescence observed for compound 2 is unclear. In our opinion, the photoproduct can be one of the possible origins of these types of luminescence. Our investigations have shown that the 8-azasteroids are photounstable. Their luminescent properties change under a laser irradiation. Therefore, the irradiation of solid solutions of the compounds under investigation at 77 K by an XeCl-excimer laser for 30 min ( $\lambda = 308$  nm, pulse repetition rate of 10 Hz, pulse energy of 2  $\mu$ J) decreases the

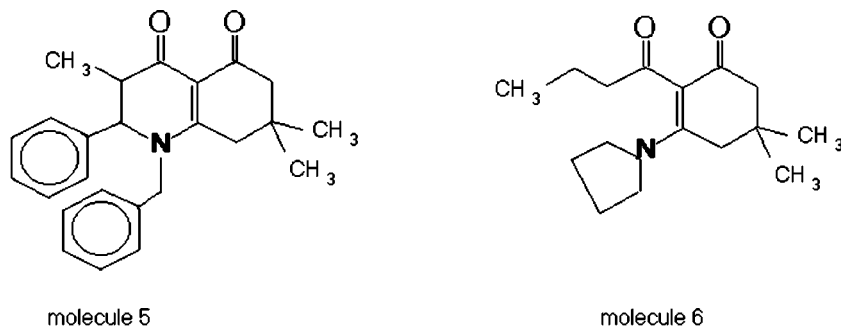


Fig. 3. Structural formulas of compounds 5 and 6.

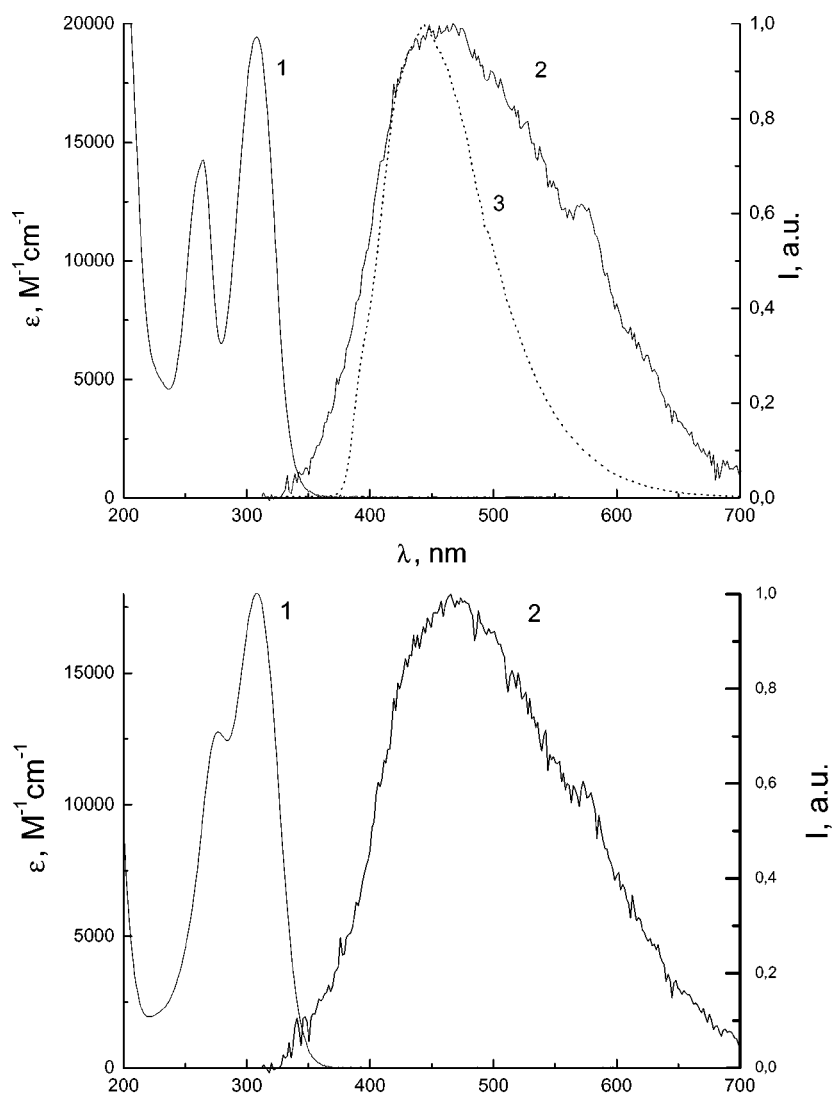
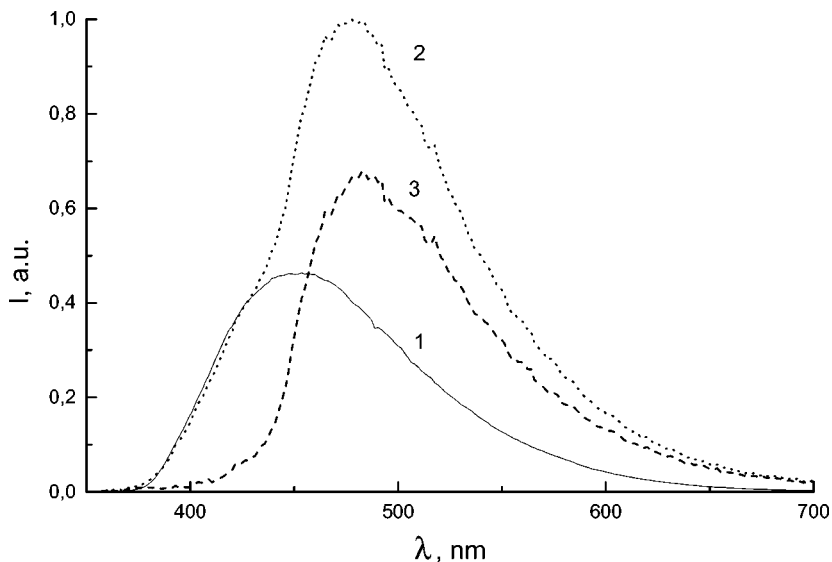


Fig. 4. Absorption (1), fluorescence (2), and phosphorescence (3) spectra of the ethanol solutions of compounds 5 and 6.  $C = 10^{-5}$  M.  $\lambda_{exc} = 308$  nm. Temperature: 1, 2—293; 3—77 K.



**Fig. 5.** Phosphorescence spectra of the fresh ethanol solution of compound 4 (1) and the solution after 30 min irradiation by excimer laser at 77 K (2).  $\lambda_{\text{exc}} = 355$  nm.  $T = 77$  K. Spectrum 3 is the difference between spectra 2 and 1 taking into account the decrease in the phosphorescence intensity of compound 4 as a result of the irradiation.

phosphorescence intensity by a factor from 1.5 time for molecule 4 to 3 times for molecule 1. As is seen from Fig. 5, the action of light leads not only to a decrease in the phosphorescence intensity but also to the appearance of new green luminescence with a decay time of about 1 s at the solution excitation at 355 nm. This effect is more pronounced if the solution is irradiated at room temperature. The exposure to the laser beam of the compound 1 solution at 293 K for about 1 min leads to a rise of new luminescent species that exhibit phosphorescence, as does the solution of compound 2. In our opinion, it is necessary to take into account the tendency of the  $\text{N}=\text{C}=\text{C}-\text{C}=\text{O}$  group to photochemical transformations, in particular, to a  $\gamma$ -pyridone system [47]. Our next step is to synthesize and investigate such compounds.

## CONCLUSION

Thus, from the above data the following conclusions can be drawn. The enamino-dicarbonyl compounds of the 8-azasteroid series have a two-band absorption spectrum in the ultraviolet region with extinction coefficients at the maxima from 10000 to 20000  $\text{M}^{-1} \text{cm}^{-1}$ . The change from the five-atomic ring D to a six-atomic ring leads to a red shift of the spectrum and an absorption intensity redistribution in the bands. All the investigated compounds (except compound 2) in solutions exhibit a very weak flu-

orescence, which points to the  $n \rightarrow \pi^*$ -nature of the lower excited singlet state. The high values of the quantum yield of phosphorescence observed at lower temperatures point to the fact that the main deactivation channel of the excited singlet state is the intersystem crossing. The 8-azasteroids are photounstable compounds. The irradiation of the solution by laser light leads to a rise of new species with strong fluorescence and phosphorescence. The tendency of the  $\text{N}=\text{C}=\text{C}-\text{C}=\text{O}$  group to photochemical transformations, in particular, to a  $\gamma$ -pyridone system can be considered as the possible reason for effects observed.

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