Journal of Fluorescence, Vol. 9, No. 4, 1999

Specific Fluorescence Properties and Picosecond Transient Absorption of 8-Azasteroids

A. A. Akhrem,[1] N. A. Borisevich,[2] O. V. Gulyakevich,[1] A. L. Mikhal'chuk,[1] T. F. Raichyonok,[2] S. A. Tikhomirov,[2] and G. B. Tolstorozhev[2]

Received August 7, 1998; accepted March 12, 1999

The specific fluorescence properties as well as picosecond transient absorption features have been studied for two 8-azasteroids. It is shown that at various excitation wavelengths the essentially different final excited electronic states are realized. Because of the multicenter character of 8-azasteroids the spectroscopic data obtained may be analyzed on a basis of the "mesomeric tautomerism" model taking into account the dynamic combination of cis- and trans-configurations. The dependence of fluorescence spectral characteristics on the solvent nature is a manifestation of intermolecular H-bond interactions.

KEY WORDS: 8-Azasteroids; α-acyl-β-aminovinylcarbonyl fragment; picosecond transient absorption.

INTRODUCTION

Chemical and biomedical research in the area of heterocyclic steroid analogues, in particular, of aza-analogues, has arisen in the last 50 years with the purpose of the separation of unfavorable hormonal and rather valuable physiological effects peculiar to steroids. Initial attempts in this area were directed to the synthesis of compounds similar to natural steroids where carbon atoms forming an ABCD-tetracyclic gonanic (cyclopentanoperhydrophenantrene) skeleton were substituted by heteroatoms (N, O, S, etc.). However, bioscreening has shown that the introduction of heteroatoms dramatically changes the biological properties of steroids. The biological characteristics of new compounds were so attractive that further investigations in this field became systematic, including two directions: (a) the modification of natural carbocyclic steroids to heterocyclic analogues [1] and (b) the "total synthesis" of heterocyclic compounds which are similar to their carbocyclic natural analogues [2].

Our investigations in this field have shown that 8-azasteroids [3–5] represent a new class of low-molecular nonantigenic agents modulating immune functions of the human organism and animals. Both stimulators and depressants of immune response were found among compounds of this type. It was shown that the structural and functional modification of 8-azasteroids could regulate both the level and the directionality of their immune action. These features are of theoretical and practical interest in the search for new effective and safe immune modulators.

The experimental data provide evidence that the pharmacological effect of 8-azasteroids is realized at the level of regulation of T cells and macrophages. These compounds control the migration of trunkal cells from the bone marrow to the central lymphoid organs and effect their proliferation in the presence of antigen factors.

The most significant stimulation effect on the initial immune response in the investigated 8-azasteroid series was noted for 8-aza-D-homogonanes [6–8]. These compounds at a dose from 1/20 to 1/10 of the LD_{50} increase

[1] Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus, Minsk, Belarus.

[2] Institute of Molecular & Atomic Physics, National Academy of Sciences of Belarus, Minsk, Belarus. e-mail: lirp@imaph.bas-net.by

1053-0509/99/1200-0357$16.00/0

the quantity of antibody forming cells in the spleen by 150–300% and the titers of antibody in the serum by 1.2–1.8 times. The immune stimulating effect of these compounds increases over a wide range of doses (up to 20% of the LD_{50}) and exceeds by 5–28 times the index value of the pharmacological action width (LD_{50}/ED_{50}) compared to the known Levamisol drug.

Biophysical studies of 8-azasteroids are important both for elucidation of the electronic structure of these substances and in the search of a correlation between their spectroscopic parameters and their physiological function such as immune modulation. The fluorescence of 8-azasteroid solutions was observed only recently.

Here the specific fluorescence properties as well as the picosecond transient absorption features are presented for two 8-azasteroids, **I**, and **II**, with the structures shown below.

RESULTS AND DISCUSSION

Compound **I** was prepared by the [2 + 4] cyclocondensation of 3,4-dihydroisoquinoline with 2-acetyl-1,3-cyclopentadione by the method in Ref. 9, compound **II** was prepared by the cyclocondensation of 6,7-dimetiloxy-3,4-dihydroisoquinoline with 2-[1-(metilamino)-ethylidene]dimedone hydrochloride according to Ref. 10. The samples of substances **I** and **II** were additionally purified by multiple recrystallization from ethanol.

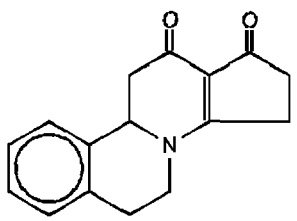
There are three points of view concerning the electronic structure of the donor–acceptor molecular fragment in 8-azasteroids such as N−C=(−C=O)−C=O α-acyl-β-aminovinylcarbonyl: (i) "cross-conjugation" conception (A) [11], (ii) "configurations of conjugation" (B) [12], and finally, (iii) dynamic "mesomeric tautomerism" (C), proposed by us [13]. The cross-conjugation and configurations of conjugations models describe the static electronic structure of the fragment. The mesomeric tautomerism model supposes the dynamic character of the electronic structure for the molecular fragment under consideration.

Figure 1 represents the absorption (1′) and fluorescence excitation (1) spectra as well as the fluorescence spectrum (2) of 8-azasteroid **I** in acetonitrile at room temperature. The molar decimal extinction coefficient for the short-wavelength absorption band at 265 nm is about 17,000 $cm^{-1}\,M^{-1}$. As can be seen from Fig. 1 the large Stokes spectral shift between the absorption and the fluorescence spectra is characteristic. No noticeable absorption in the 350-to 425-nm spectral range is observed. At the same time a fluorescence excitation spectrum band which is nearly symmetrical to the fluorescence spectral band is registered in this spectral region. In addition, the long-wavelength band intensity is more than one order of magnitude higher, at least, than that for the short-wavelength band.

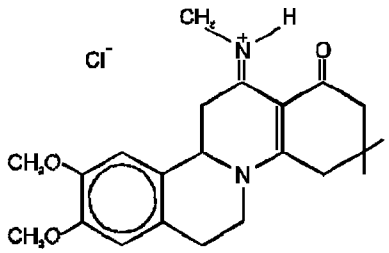
It should be noted that upon a change of the recording fluorescence wavelength within 450–600 nm, an essential spectral shift by ≈30 nm for the long-wavelength fluorescence excitation band is observed for 8-azasteroid **I** in acetonitrile (Fig. 2). In contrast, any noticeable change of the short-wavelength band in the fluorescence excitation spectrum in the 250-to 300-nm region is not observed. Finally, the position and form of the 8-azasteroid **I** fluorescence spectra also depend on the excitation wavelength.

Accordingly, the π–π* and n–π* nature of electronic transitions for the short- and long-wavelength excitation regions may be proposed as an explanation of the observed spectral behavior. We can suppose that the main reason for the fluorescence peculiarities of 8-azasteroids is connected with the multicenter character of the molecules due to the coexistence of various possible mesomeric forms.

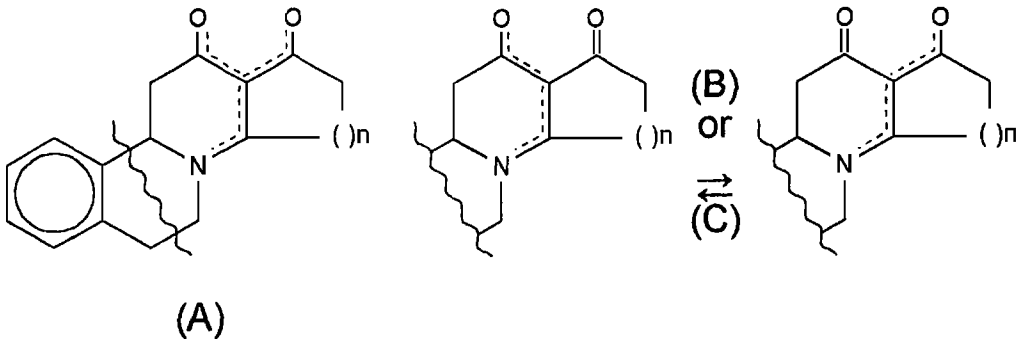
By the method of picosecond kinetic spectroscopy a transient absorption of 8-azasteroid **I** in acetonitrile in the 450-to 700-nm spectral range was observed (Fig. 3). The measurements were carried out with a picosecond



I



II



Scheme 1

double-beam spectrometer based on a passively mode-locked negative feedback-controlled Nd:glass laser. The pulse duration was about 3–5 ps. The experiments were of the pump-probe type. Pump pulses of the third (λ_{exc} = 352 nm) and fourth (λ_{exc} = 264 nm) harmonics of the fundamental frequency of Nd:glass laser correspond to the different absorption bands of 8-azasteroid I. The picosecond continuum generated in the cell containing D_2O was used as a probe pulse. The description of the spectrometer was given in [14].

Figure 3 presents the transient absorption spectra of 8-azasteroid **I** in acetonitrile at a 10-ps delay time after pump pulses. As one can see, at λ_{exc} = 264 nm widespread transient absorption over the whole registration spectral range is observed, while at λ_{exc}= 352 nm excitation a negative absorption band is observed, being spectrally coincident with the fluorescence band.

Optical density kinetics for probing pulse λ_{probe} = 520 nm at λ_{exc} = 264 nm are shown in Fig. 4. The solid line is the best-fitting curve for the relaxation time of τ_{rel} = 4 ps. Open circles are the instrumental response of the spectrometer. The Optical density kinetics at λ_{exc} = 352 nm are nearly the same as the instrumental response. These results suggest that at various excitation wavelengths, different relaxation processes and final states of a dissimilar electronic nature are realized.

Interesting observations were obtained for fluorescence spectra of 8-azasteroid **II**. Figure 5a represents the 8-azasteroid **I** fluorescence spectra in ethanol (curve 1) and acetonitrile (curve 2) at λ_{exc} = 350 nm. It is shown

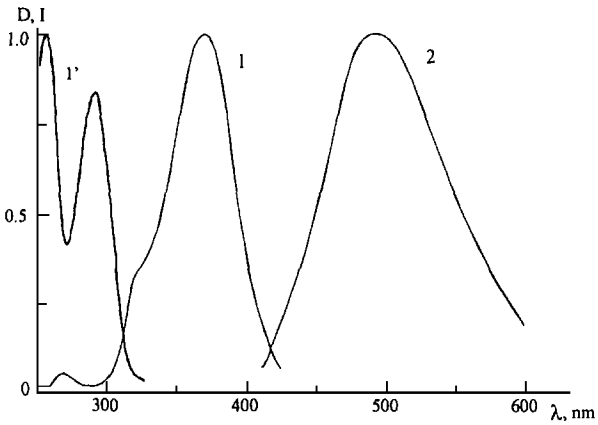


Fig. 1. Absorption (1'), fluorescence excitation (1), and fluorescence (2) spectra of 8-azasteroid **I** in acetonitrile (T = 293 K).

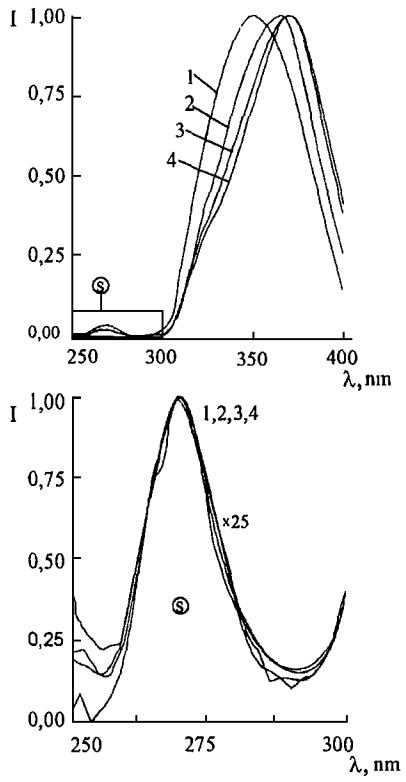


Fig. 2. Fluorescence excitation spectra of 8-azasteroid **I** in acetonitrile at different recording wavelengths (λ_{em}) in the emission spectrum: (1) λ_{em} = 450 nm; (2) λ_{em} = 500 nm; (3) λ_{em} = 550 nm; (4) λ_{em} = 600 nm.

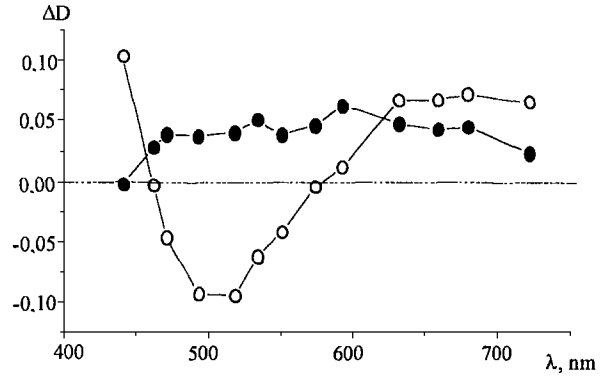


Fig. 3. Transient absorption spectra of 8-azasteroid **I** in acetonitrile at delay time Δt = 10 ps between excitation λ_{exc} = 264 nm (filled circles), λ_{exc} = 352 nm (open circles), and probe (picosecond continuum) pulses.

that a rather essential long-wavelength spectral shift by approximately 50 nm is observed on the transition from ethanol to acetonitrile. For 8-azasteroid **II** the opposite picture is observed (Fig. 5b, curves 3 and 4). The fluorescence spectrum is of a longer wavelength in the protonodonor ethanol. The addition of 0.1% HCl to ethanol leads to a short-wavelength fluorescence spectrum shift (curve 5).

The observed spectral shift for 8-azasteroid **I** in two solvents may be explained on the basis of the dipole–dipole interaction model. The refraction index n = 1.36, the static dielectric constant ε = 24.3, and the dipole moment μ = 1.69 D for ethanol; n = 1.34, ε = 36.2, and μ = 3.92 *D* for acetonitrile. According to the function of universal interaction [15] the fluorescence spectrum of the molecules under consideration should be of a longer wavelength in acetonitrile relative to ethanol. This effect was observed for 8-azasteroid **I**.

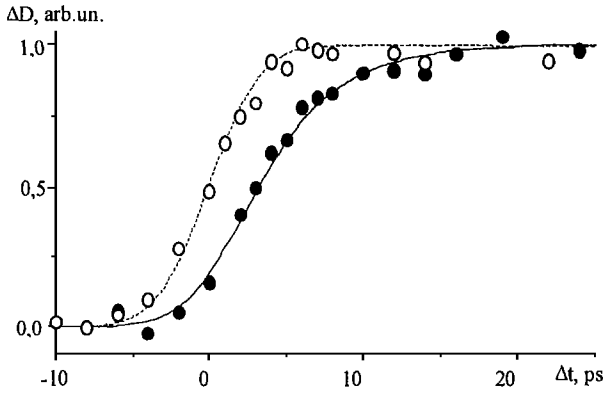


Fig. 4. Optical density kinetics of 8-azasteroid **I** in acetonitrile at λ_{exc} = 264 nm excitation (filled circles). Open circles are the instrumental response of the spectrometer. Solid and dashed lines are the best-fitting curves.

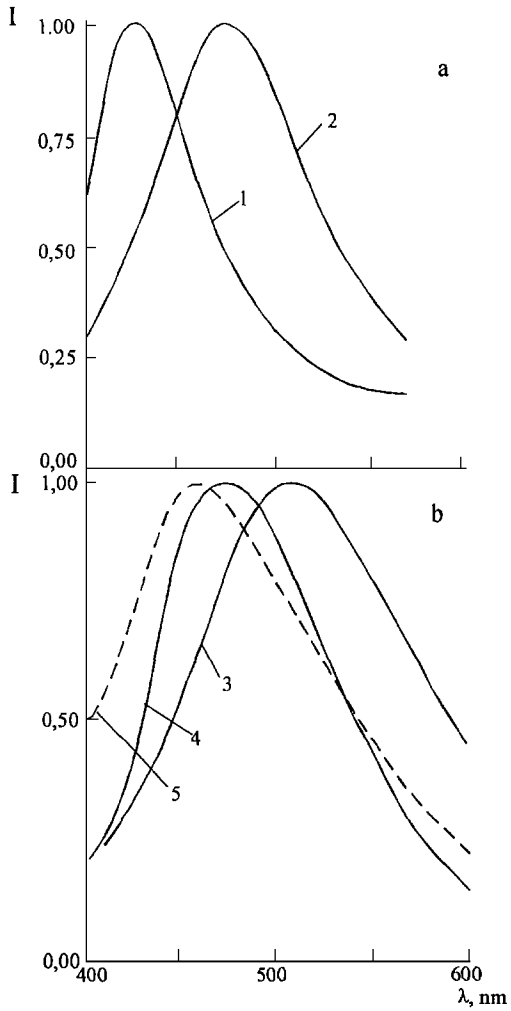


Fig. 5. Fluorescence spectra of 8-azasteroid **I** (a) and **II** (b) in different solvents: (1, 3) ethanol; (2, 4) acetonitrile; (5) ethanol + 0.1% HCl.

The reason for the observed spectral effects for 8-azasteroid **II** may be connected with donor–acceptor intermolecular interactions such as H-bonding. Ethanol is the more effective protonodonor solvent. It is known that HCl addition in a protonodonor solvent blocks H-bond formation. It should lead to a short-wavelength shift of fluorescence spectra as was observed in the experiment (Fig. 5b, curve 5).

CONCLUSION

The long-wavelength band position of fluorescence excitation spectra for the compounds depends strongly on the recording emission wavelength. Dependence of flourescence spectra on the excitation wavelength is also

found. The observed changes in the picosecond transient absorption spectra and kinetic dependences upon the variation of excitation wavelengths point out that different final states of a dissimilar electronic nature and geometric structure are formed. The mesomeric tautomerism model may be proposed for the interpretation of specific fluorescence properties of 8-azasteroid molecules. Intermolecular donor–acceptor interactions of the H-bond type may be responsible for the dependence of fluorescence spectra on the solvent nature.

REFERENCES

1. J. W. Morzycki (1995) *Pol. J. Chem.* **69**, 321–340.
2. F. A. Lakhvich, L. G. Lis, and A. A. Akhrem (1984) *Uspehi Khimii.* **53**, 1014–1044 (Russian).
3. O. V. Gulyakevich, A. L. Mikhal'chuk, A. I. Verenich *et al.* (1996) in *Enemines in Organic Synthesis*, UrO RAN, Ekaterinburg, pp. 11–132 (Russian).
4. A. L. Mikhal'chuk, O. V. Gulyakevich, A. A. Zenuk *et al.* (1991) *Dokl. Akad. Nauk SSSR* **317**, 1397–1399 (Russian).
5. A. L. Mikhal'chuk, O. V. Gulyakevich, and A. A. Akhrem (1993) *Khim. Geterotsicl. Soedin.* **1**, 86–93 (Russian).
6. B. B. Kuzmitskii, I. G. Dad'kov, Yu. L. Zhuravkov *et al.* (1989) *Vestsi Akad. Navuk BSSR. Ser. Chim.* **1**, 64–68 (Russian).
7. G. S. Lubin, I. G. Dad'kov, O. V. Gulyakevich *et al.* (1990) *Vestsi Akad. Navuk BSSR. Ser. Khim.* **5**, 93–97 (Russian).
8. N. A. Konoplya, O. V. Gulyakevich, A. L. Mikhal'chuk, and B. B. Kuzmitskii (1994) *Vestsi Akad. Nauk Belarusi Ser. Khim.* **3**, 91–96 (Russian).
9. A. A. Akhrem, A. M. Moiseenkov, V. A. Krivoruchko *et al.* (1972) *Izv. Akad. Nauk USSR. Ser. Khim.* **9**, 2078–2083 (Russian).
10. O. V. Gulyakevich, A. L. Mikhal'chuk, and V. A. Khripach (1991) *Zh. Org. Khim.* **27**, 213–214 (Russian).
11. H. J. O. Becker (1974) *Einfuhrung in due elektronentheorie organisch-chemischer reaktionen*, VEB Dentscher Verlag der Wissenschaften, Berlin.
12. V. Z. Kurbako (1990) *Dokl. Akad. Nauk BSSR* **34**, 716–719 (Russian).
13. A. L. Mikhal'chuk, O. V. Gulyakevich, and A. A. Akhrem (1991) in *Proceedings of V Conference on Chemistry of Nitrogen Containing Heterocyclic Compounds*, Czernogolovka, Pt. 1, p. 20 (Russian).
14. N. A. Lysak, S. V. Mel'nichuk, S. A. Tikhomirov, and G. B. Tolstorozhev (1987) *Zh. Prikl. Spectroscopii* **47**, 267–272 (Russian).
15. N. G. Bakhshiev (1972) *Spectr. Intermol. Interact. Nauka Leningrad* (Russian).