ORIGINAL PAPER

Spectral-Luminescent Properties of 12-Oximino Derivatives of 8-AZA-*D*-Homogona-12,17*a*-Diones and their Concentration Dependence

S. A. Bagnich · O. V. Gulyakevich · A. L. Mikhalchuk

Abstract This paper presents the results of the investigation of the spectral-luminescent characteristics of 12-oximino derivative of 8-aza-*D*-homogona-12,17*a*-dion, its hydrochloride, and their dependences on the concentration. It has been shown that the form and position of the absorption spectrums of 16,16-dimethyl-12-oximino-8-aza-*D*-homogona-1,3,5(10),13-tetraene-17*a*-one in all solvents used are independent of its concentration. At the same time, for its hydrochloride in ethanol and water, a strong dependence of the absorption spectrum on its concentration has been revealed. On the basis of the experimental data it has been concluded that in these solvents dimers of investigated substance, whose bonding force is chelate hydrogen bonds, are formed. The investigation of the luminescence has shown that these substances practically do not fluoresce. Only in ethanol and water we managed to register a very weak luminescence, whose excitation spectrum is close to the absorption spectrum.

Keywords 8-azasteroid · Monomers · Dimer · Organic solvents · Hydrogen bond · Tautomerism · Absorption · Luminescence · Fluorescence

S. A. Bagnich (✉)
Institute of Molecular and Atomic Physics,
National Academy of Sciences of Belarus,
F. Skaryna Ave. 70,
220072 Minsk, Belarus
e-mail: bagnich@imaph.bas-net.by

O. V. Gulyakevich · A. L. Mikhalchuk
Institute of Bio-Organic Chemistry,
National Academy of Sciences of Belarus,
Acad. Kuprievich str. 5/2,
220141 Minsk, Belarus

Introduction

Along with the π–π- and Coulomb complexes, whose forming force is, as a rule, Van der Waals interactions between individual molecules or fragments of macromolecules, the attention of researchers is attracted by the structure and dynamics of molecular aggregates that are based on hydrogen bonds, since the latter play an extremely important part in chemistry and biology [1, 2]. The hydrogen bonds forming complexes of low-molecular compounds are of great importance, since they, first, largely determine the physico-chemical characteristics and biological properties of these compounds and, second, serve as models for investigating the interactions between pairs of bases in the DNA, separate fragments of protein molecules, and lipids. Moreover, the proton transfer is one of the most fundamental and general natural processes realized through the intermediate formation of hydrogen bonds [3–6]. The data set of laser-spectroscopic investigations and quantum-mechanical calculations has contributed to the progress made in recent years in understanding the functioning of the most important model and biological systems: NH_3 [7], H_2O [8], CH_3COOH [9], and pairs of DNA bases [10].

The simplest and well-studied representatives of chelates with a hydrogen bond are dimers of carbonic acids [11–14]. The energy of the hydrogen bond of the benzoic acid dimer is of the order of 6,000 cm^{-1} [15, 16]. As a result, benzoic acid in aprotic non-polar solvents exists only in the form of dimers [17, 18], and in crystals—in the form of equilibrium of prototropic tautomers bonded by hydrogen bonds, that is due to the proton dynamics [19].

This paper presents the results of the investigation of the spectral-luminescent properties and their concentration dependence of solutions of 16,16-dimethyl-12-

oximino-8-aza-*D*-homogona-1,3,5 (10),13-tetraene-17 *a*-one *1* and its hydrochloride *2* (Fig. 1).

Experiment

The derivatives of 8-azasteroids (3,3-dimethyl-13-hydroximino-2,3,4,6,7,11*b*,12,13-octahydro-1H-isoquino[2,1-*a*] quinoline-1-one and its hydrochloride) *1* and *2*, respectively, have been obtained by the previously developed methods [20], and, as their synthetic predecessor 16,16-dymethyl-8-aza-*D*-homogona-1,3,5(10),13-tetraene-12,17*a*-dion (3,3-dimethyl-2,3,4,6,7,11*b*,12,13-octahydro-1H-izoquino[2,1-*a*] quinoline-1,13-dion) [2], they exhibit immune modulating properties [22].

The electronic absorption spectra of derivatives *1*, *2* were recorded on a Cary 500 Scan (Varian) spectropho-

tometer. The luminescence spectra were recorded on an SDL-2 spectrometer. All solutions were sonicated 30 min to ensure proper dissolution. In some cases it was done at temperature of 60 C.

Results and discussion

The structure of hydrochloride *2* given in Fig. 1 reflects only the salt nature of this compound distinguishing it from derivative *1* and does not reflect the features of its structure. Theoretically, for hydrochloride *2* four probable structures may be supposed. A structure with a proton localized at the N^8–H^+ atom *3*. A structure with a proton located at the oxygen atom of the C^{17a}-carbonyl group *2* (OH) and a structure with a proton located at the nitrogen atom of the C^{12}-oximino group, which can be represented by boundary
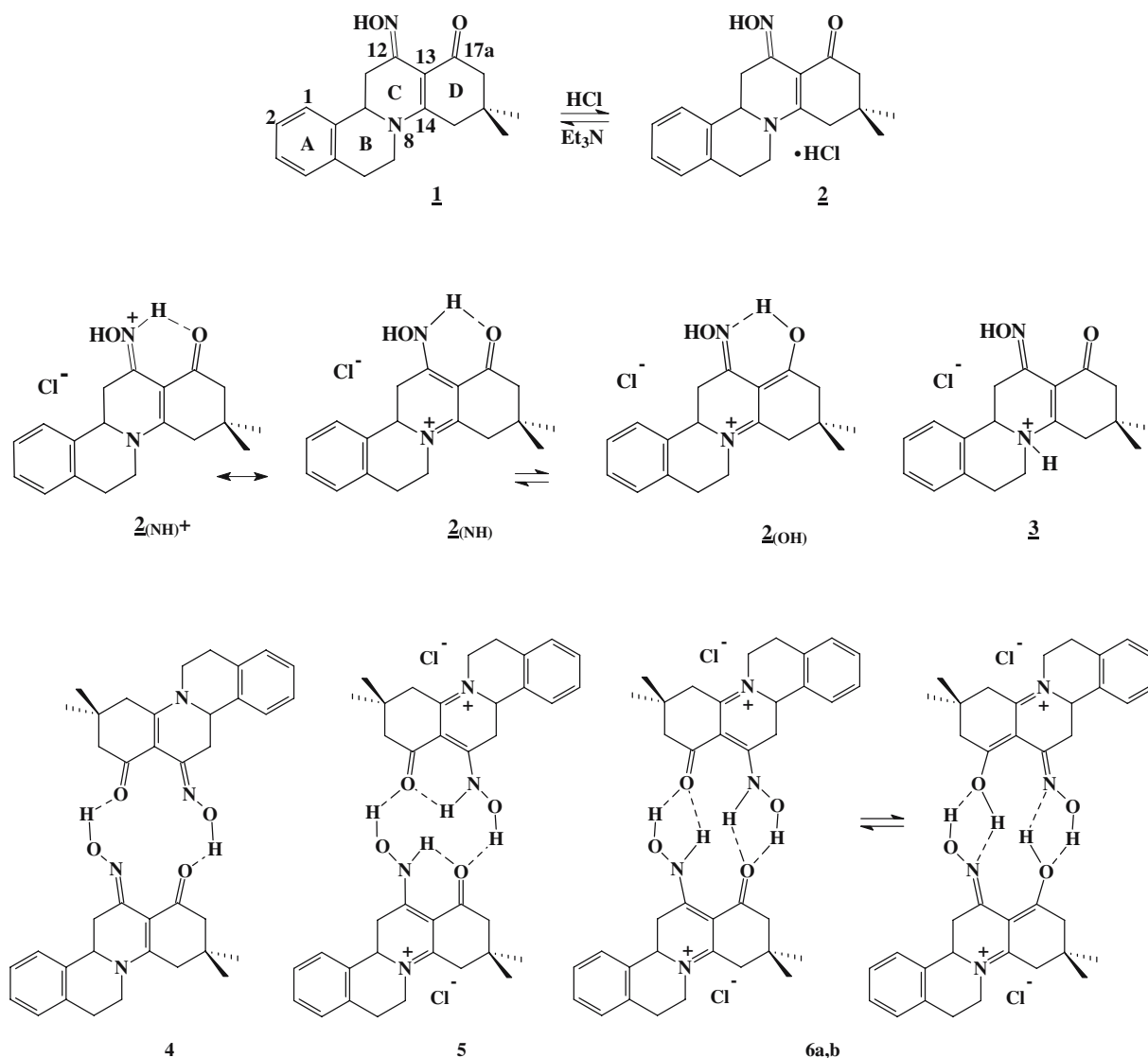

Fig. 1 Structural formulas of 16,16-dimethyl-12-oximino-8-aza-*D*-homogone-1,3,5 (10),13-tetraene-17a-one *1*, its hydrochloride *2*, and their possible dimers

structures $2_{(NH)}^+$ and $2_{(NH)}$. Analysis of these structures and the X-ray data for contiguous 12-oxo- and 12-imino derivatives of 8-azasteroids [23–26] permit stating that the most populated structures for hydrochloride 2 are the structures $2_{(NH)}^+$, $2_{(NH)}$ and $2_{(OH)}$ with the dominating population of exactly the mesomeric oximino state $2_{(NH)}^+$.

Absorption spectra

As was shown earlier [20], the absorption spectra of ethanol solutions of derivatives 1 and 2, unlike their ketonic predecessors characterized by the presence of two absorption bands at $\lambda \sim 265$ nm and $\lambda \sim 300$ nm [20, 21], are characterized by an asymmetric absorption band in the 280–345 nm range with a shoulder on the short-wave slope of the band. In general, the present investigation of the absorption spectra of derivatives 1 and 2 has confirmed these data and revealed new distinguishing features that show up as a different dependence of the spectra on the concentration. Figure 2 shows the normalized absorption spectra of derivatives 1 and 2 in various solvents with a concentration of the investigated substances $C = 10^{-5}$ M. Extinction coefficient at maximum of the long wavelength band is of around 19,000 $M^{-1}cm^{-1}$ for all solvents used. It is well seen that the spectra of compounds 1 and 2 coincide, on the whole, in case of using water, ethanol or dimethyl formamide (DMF) as a solvent. However, there is a considerable difference for solutions in dichlorethane (DCE). Unlike compound 1, whose absorption spectrum in DCE is characterized by one band with a maximum at $\lambda \sim 330$ nm, the spectrum of compound 2 in DCE demonstrates two bands with maxima at $\lambda \sim 280$ nm and $\lambda \sim 327$ nm. It should be noted thereby that the spectral form remains unaltered with a concentration in the 10^{-5}–10^{-3} M range. The observed difference becomes clear from the results of the investigation of the concentration dependences of the absorption spectra obtained for solutions in other solvents.

Figure 3 shows the spectra of derivative 2 in ethanol at room temperature for its various concentrations. The investigation has shown that in the 10^{-6}–10^{-5} M range of concentrations the optical density of the derivative 2 solution linearly increases with increasing concentration without changing the form. In so doing, unlike 8-azasteroid 12,17a-dions [21, 27], the spectrum has only one intensive band with a shoulder on the short-wave side which is bathochromically shifted by 3,000 cm^{-1} from the long-wavelength band of 12,17a-dioxo derivatives. A further increase in the concentration of 8-azasteroid 2 leads to a change in the absorption spectrum, and this process occurs in a narrow range of concentrations (see Fig. 3). The process of spectrum transformation ceases in the region of concentrations of 10^{-3} M and a further increase in the
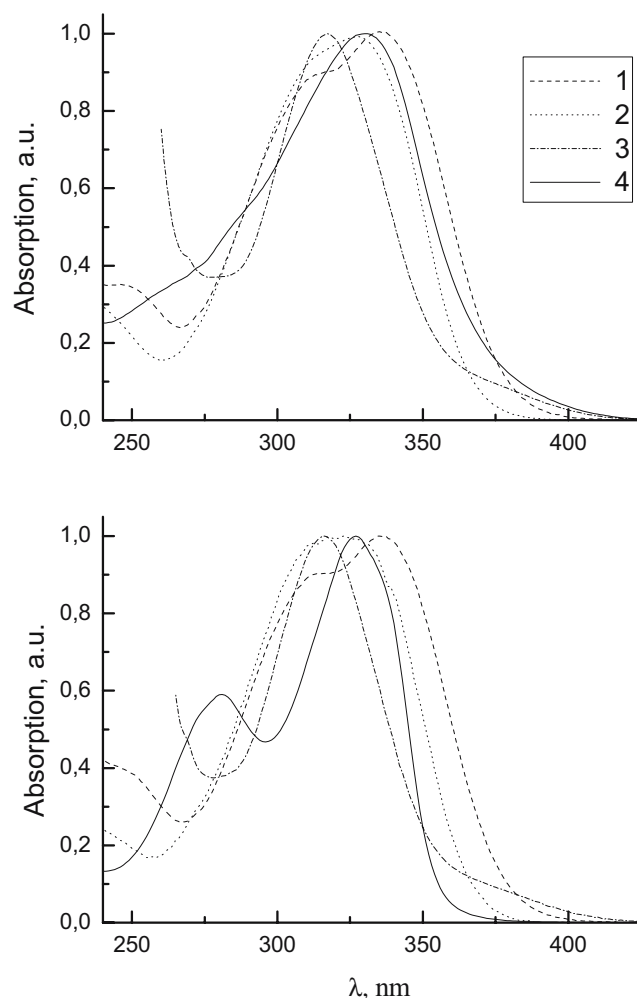

Fig. 2 Absorption spectra of the solutions of compounds 1 (*upper figure*) and 2 (*lower figure*) in ethanol (1), water (2), dimethyl formamide (3) and dichlorethane (4) at room temperature. Substance concentration in solutions $C = 10^{-5}$ M

concentration no longer leads to any further changes. Importantly, subsequent dilution of the solution returns the spectrum to its initial form passing through all stages of changes given in Fig. 3 in the reverse order. This gives reason to assume that this process is associated with formation of intermolecular complexes of the compound being investigated. It should be noted thereby that in the region of concentrations of $> 10^{-3}$ M in the solution already only complexes are likely to exist. Analysis of the absorption spectrum of the thin amorphous film obtained from the solution by the spin-coating process also indicates that in this case too hydrochloride 2 exists in the form of complexes (see Fig. 4). A similar behavior is also observed for the solution in water. But in the latter case the spectrum transformation begins at a concentration an order of magnitude larger than in the case of the solution in ethanol.

The most probable motive force for the formation of such complexes can be the hydrogen bonds and Coulomb or π–π interactions of derivative 2. The coulomb or π–π
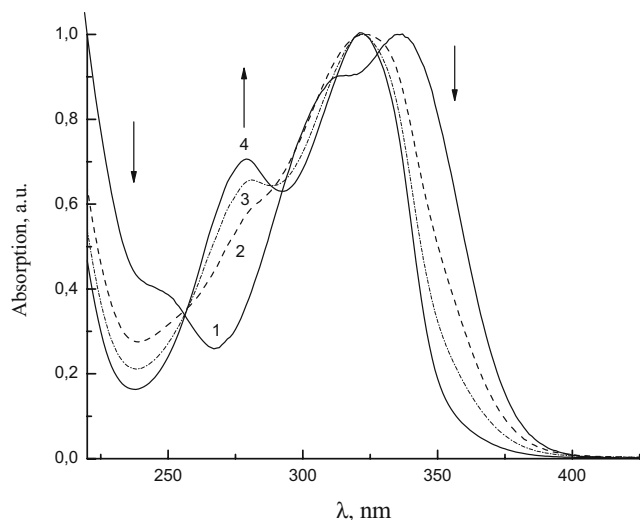
Fig. 3 Absorption spectra of the solution of compound *2* in ethanol at room temperature for its various concentrations: $1-10^{-5}$; $2-3 \cdot 10^{-5}$; $3-10^{-4}$; $4-10^{-3}$ M

interactions as the cause of the observed spectral dependences are excluded, since they should have also appeared in the case of derivative *1*. Consequently, the reason for the observed spectral manifestations is still the hydrogen bonds, and the most probable structure of the complexes is dimers, of the type carbonic acid dimers [11–18].

From Figs. 3 and 4 it may be concluded that in this case dimers are a kind of an intermolecular compound on which the association of molecules stops. This points to the specificity of the forces binding two molecules and excluding the addition to them of other molecules. Additive dipole-dipole and dispersion forces do not exhibit such a property [28]. This gives reason to assume that dimers, as in the case of benzoic acid [15, 16], result from the formation of hydrogen bonds between the $C=N(H)OH^{+}$ group of one molecule and the C^{17a}-carbonyl group of the

other molecule. In principle, for the base of *1*, too, one may admit the formation of intermolecular complexes bound by hydrogen bonds, e.g. in the form *4*. However, the investigations have shown that in the case of compound *1* the absorption spectrum in ethanol coincides in form and position with the absorption spectra of low concentrated solutions of compound *2*. In this case, the spectrum form is independent of the concentration in the $10^{-6}-10^{-3}$ M range, and the optical density of solutions linearly changes with the substance concentration. Moreover, the absorption spectra of amorphous films of compound *1*, unlike those for compound *2*, demonstrate only one asymmetric absorption band (see Fig. 4). These data point to the fact that in the case of compound *1* the formation of dimers of *4* or any other molecular aggregates does not occur. Probably, the energy of such hydrogen bonds is small, and if even dimers *4* are formed, their population is extremely low and does not show up in the absorption spectra of either solutions or films.

Taking into account the structure of derivative *2*, it may be suggested that its dimers, as in the case of benzoic acid, arise as a result of the formation of hydrogen bonds between the $C=N(H)OH^{+}$group of one molecule and the $C=0$; group of another molecule, e.g., of *5*. And, as in the case of the hypothetical dimer *4*, for each pair of dimer molecules a pair of such bonds can be formed. However, it is unlikely that the energy of hydrogen bonds of the dimers of *5* is higher than in the dimers of *4*. Consequently, such dimers are also unlikely. The most probable for the investigation conditions seem to be chelate hydrogen-bonded tautomeric dimers of *6a,b*. In this case, the dimer complex *5* cannot be excluded from the consideration, assuming it as an intermediate stage in the formation of
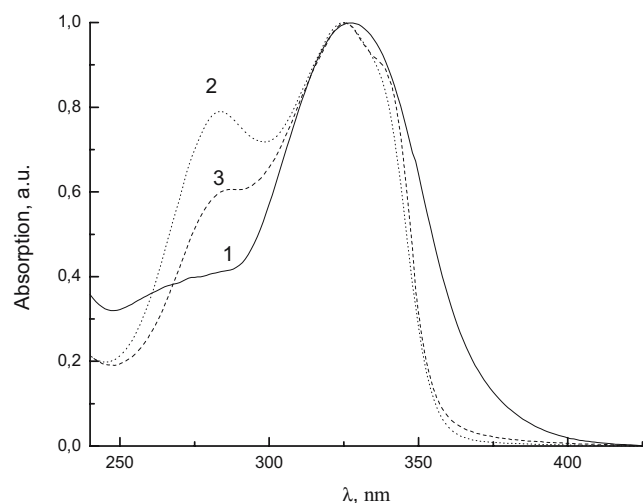

Fig. 4 Absorption spectra of amorphous films of compounds *1* (1) and *2* (2, 3) obtained from the solution in ethanol (1, 2) and dimethyl formamide (3)
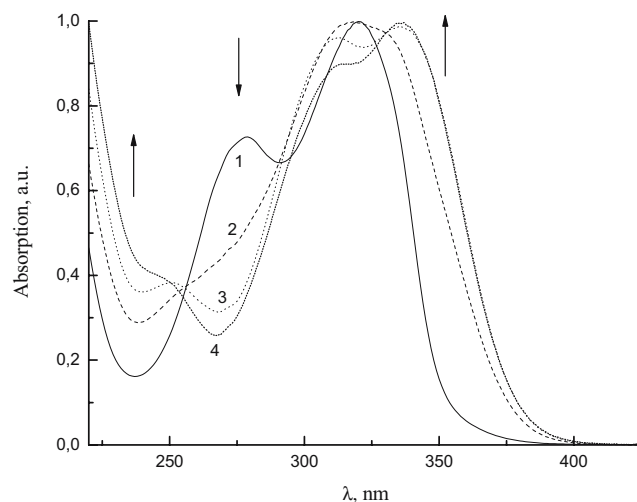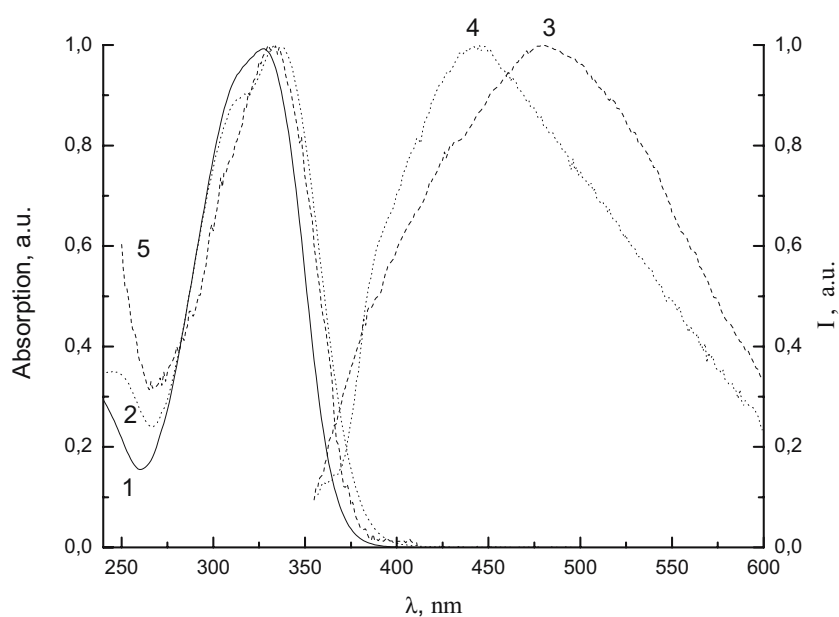

Fig. 5 Absorption spectra of the solution of compound *2* in ethanol with a concentration $C=10^{-3}$ M at room temperature for various concentrations of triethylamine: $1-0$; $2-8 \cdot 10^{-4}$; $3-5 \cdot 10^{-4}$ M. Spectrum 4 corresponds to the solution with a concentration of compound *2* $C=10^{-5}$ M

complexes *6a,b*. The data given in Fig. 5 additionally confirm the formation of dimers of *6a,b*. This figure demonstrates the influence of triethylamine (TEA) on the absorption spectra of the solution in ethanol of derivative *2* with a concentration of 10^{-3} M. TEA is a stronger base than derivative *1* and, as a consequence, it destroys the salt *2*, regenerating derivative *1*. From Fig. 5 it is seen that as TEA is added to the solution of hydrochloride 12-oximino derivative *2* the change of the two-band spectrum characteristic of dimers into a single-band spectrum characteristic of nonaggregated molecules is observed. As the TEA concentration reaches 5–10^{-3} M, the absorption spectrum

of the investigated solution practically coincides with the absorption spectrum of the low concentrated solution, which point to the decomposition of both the *6a,b* dimers and the *2* salt.

The next important point is the data obtained for solutions of derivatives *1* and *2* in DMF. In this case, the form of the absorption spectra for both compounds is practically the same and remains unaltered in the range of concentrations of 10^{-6}–10^{-3} M, and the optical density of the solutions linearly depends on the concentration. This argues in favor of the fact that in DMF dimers are not formed. In all probability, this is due to the polarity and the

Fig. 7 Fluorescence spectra of ethanol solutions of compounds *1* (1) and *2* (2, 3) with a concentration C=10^{-3} M at room temperature. Concentration of triethylamine: 1, 2–0; 3–5–10^{-3} M
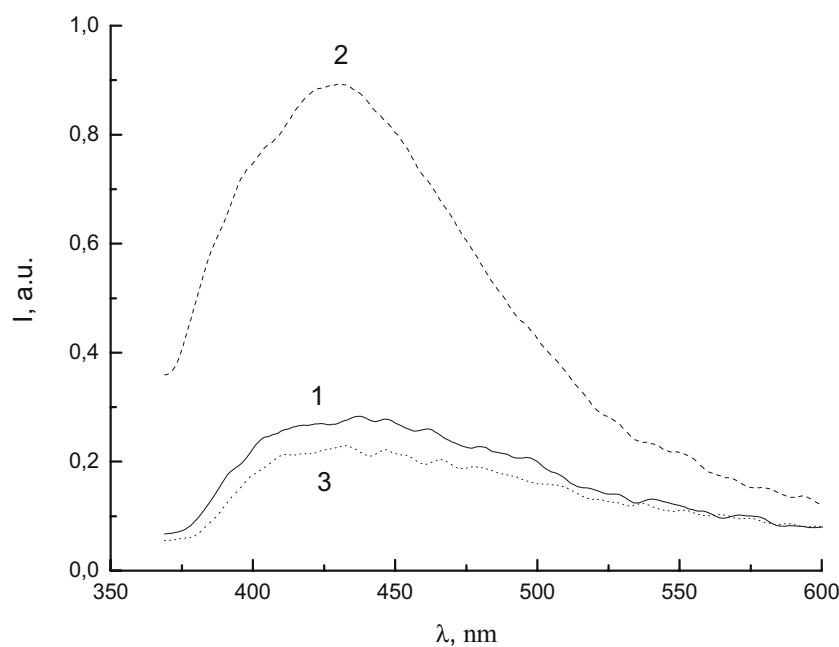
proton-acceptor or electrodonor properties of DMF [1] capable to solvate both cations H^+ and anions Cl^- and destroy the *6a,b* salt complexes. At the same time, it is seen from Fig. 4 that the absorption spectrum of the compound *2* film, obtained from the solution in DMF exhibit two bands and is close in form and position to the absorption spectrum of the film obtained from the solution in ethanol, which points to the formation of *6a,b* dimers when the solution of *2* in DMF is supersaturated. On the basis of the data presented it may be concluded that exactly the chelate hydrogen bonds in *6a,b* are dimer-forming.

While the mechanisms of dimer formation of in the case of benzoic acid and the 8-azasteroid being investigated are similar, the properties of the dimers differ considerably. The benzoic acid dimers, despite the fairly large value of the molecular binding bond energy in them, represent the limiting case of a weak exciton interaction that is realized when the electron interaction between the monomeric units is a small [14, 29]. In this case, the absorption spectrum of the dimer can be considered as the spectrum of a slightly perturbed monomer. Therefore, it is believed that the two halves of the dimer absorb light independently of one another and the electron excitation is initially localized on one monomeric unit. As a result, the spectral-luminescent properties of the dimers do not differ very widely from those for the monomers forming them [17, 30]. But in the case of compound *2*, the dimer formation leads to a considerable change in the absorption spectrum.

Luminescence spectra

The investigation of the luminescence of diluted solutions of compound *1* has shown, that in DMF this substance does not exhibit any fluorescence. In the ethanol solution, compound *1* demonstrates a very weak fluorescence (Fig. 6), which is registered as an excess luminescence in comparison to the luminescence of the solvent. Unfortunately, the relative fluorescence intensity is small for recording its excitation spectrum. However, on passing to the solution in water the fluorescence intensity of compound *1* increases several times, which permits obtaining not only the spectrum of fluorescence, but also a spectrum of its excitation. From Fig. 6 it is seen that, in general, the fluorescence excitation spectrum coincides with the absorption spectrum.

To elucidate the influence of "dimerization" on the luminescent characteristics of derivative *2*, we have investigated the luminescence of ethanol solutions of compounds *1* and *2* with a concentration of 10^{-3} M at which, as was shown above (Fig. 3), derivative *2* exists mainly in the form of dimers. From Fig. 7 it is seen that the passing from compound *1* to compound *2* is followed by a marked increase in the fluorescence intensity of the

solution. Upon addition to the solution being investigated of TЭA in a concentration of $5 \cdot 10^{-3}$ M at which dimers are destroyed (Fig. 5), the fluorescence of the compound *2* solution becomes close to the fluorescence of the compound *1* solution in both intensity and form of its spectra.

Conclusions

The results of the present investigation show that unlike 8-azasteroids with an $N^8-C^{14}=C^{13}$ $(-C^{12}=O)-C^{17a}=O$ enaminodicarbonyl fragment, 8-azasteroid *1* with an $N^8-C^{14}=C^{13}$ $(-C^{12}=NOH)-C^{17a}=O$ fragment has an asymmetric absorption band with a maximum in the 310–340 nm range depending on the solvent. The absorption spectrum of 8-azasteroid *1* remains unaltered as the concentration changes from 10^{-6} to 10^{-3} M. In the case of its hydrochloride *2*, analogous spectral characteristics are observed for low-concentration solutions in protolytic solvents (alcohol, water, DMF). At the same time, in DCE a two-band spectrum is observed. And the maxima position of both bands thereby differs from the maximum position of the absorption band of compound *1*. A similar spectrum takes place for concentrated solutions in ethanol and water, as well as for amorphous films. Analysis of the experimental data permits the conclusion about the equilibrium formation of dimer molecular complexes *6a,b* bound by chelate hydrogen bonds. The investigation of the luminescence has shown that the investigated complexes feature small quantum yields of fluorescence. We managed to register a weak fluorescence whose excitation spectrum is close to the absorption spectrum, only in ethanol and in water. The formation of *6a,b* dimers leads to an increase in the fluorescence of the solutions.

References

1. Reichardt C (1988) Solvents and solvent effects in organic chemistry. VCH, Weinheim
2. Kuz'mickiy BB, Dad'kov IG, Zhuravkov YL, Konoplya NA, Lyubin GS, Mashkovich AE, Nasek BM, Gulyakevich OV, Pshenichniy VN, Khripach VA (1989) Proc of the BSSR Acad of Sci, Ser Chem Sci (1):64
3. Hammes-Schiffer Sh (1996) Multiconfigurational molecular dynamics with quantum transitions: multiple proton transfer reactions. J Chem Phys 105:2236
4. Kohen A, Klinman JP (1999) Hydrogen tunneling in biology. Chem Biol 6:R191
5. Scheiner S (2000) Calculation of isotope effects from first principles. BBA Bioenergetics 1458:28
6. Bell RP (1973) The proton in chemistry. Chapman and Hall, London
7. Nelson DD Jr, Fraser GT, Klemperer W (1988) Does ammonia hydrogen bond? Science 238:1670

8. Liu K, Gruzan JD, Saykally RS (1996) Water clusters. Science 271:929
9. Guthrie JP (1993) Energetics of acetic acid enol in aqueous solution. A new method for thermodynamic estimation. Can J Chem 71(12):2123–2128
10. Nir E, Kleinermanns K, de Vries MS (2000) Pairing of isolated nucleic-acid bases in the absence of the DNA backbone. Nature (London) 408:949
11. Madeja F, Havenith M (2002) High resolution spectroscopy of carboxylic acid in the gas phase: observation of proton transfer in $(DCOOH)_2$. J Chem Phys 117:7162
12. Nandi CK, Chakraborty T (2004) Hydrogen bond-induced vibronic mode mixing in benzoic acid dimer: a laser-induced fluorescence study. J Chem Phys 120:8521
13. Remmers K, Meerts WL, Ozier I (2000) Proton tunnelling in the benzoic acid dimer studied by high resolution ultraviolet spectroscopy. J Chem Phys 112:10890
14. Poeltl DE, McVey JK (1984) Excited-state dynamics of hydrogen-bonded dimers of benzoic acid. J Chem Phys 80:1801
15. Allen G, Wathinson JG, Webb KH (1966) An infra-red study of the association of benzoic acid in the vapour phase, and in dilute solution in non-polar solvents. Spectrochim Acta 22:807
16. Nagaoka S, Hirota N, Matsushita T, Nishimoto K (1982) An ab initio calculation on the proton transfer in the benzoic acid dimer. Chem Phys Lett 92:498
17. Baum JC, McClure DS (1979) The ultraviolet transitions of benzoic acid. 1. Interpretation of the singlet absorption spectrum. J Am Chem Soc 101:2335
18. Acree WE, Bertrand GL (1981) Thermochemical investigations of nearly ideal binary solvents. 7. Monomer and dimer models for the solubility of benzoic acid in simple binary and ternary solvents. J Pharm Sci 70:1033
19. Neumann MA, Crasinn S, Corval A, Johnson MR, Horsewill AJ, Benderskii VF, Trommsdorff HP (1998) Proton dynamics and the tautomerization potential in benzoic acid crystals. Ber Bunsen-Ges Phys Chem 102(3):325–334
20. Gulyakevich OV (1989) Synthesis and properties of enaminodicarbonyl compounds of 8-aza-D-homogonane series. Thesis on Cand. of Chem. Sci. Degree Competition. Minsk. pp 198
21. Garbuz NI, Kurbako VZ, Lakhvich FA, Lis LG (1982) Investigation of enaminodicarbonyl compounds of 8-azasteroids series by the IR spectroscopy method. Zhurnal Prikladnoy Spektroskopii 36:269 (in Russian)
22. Kuz'mickiy BB, Lyubin GS, Dad'kov IG, Gulyakevich OV (1990) Proc of the BSSR Acad of Sci, Ser Chem Sci (5):93
23. Verenich AI, Galitskii NM, Govorova AA, Gulyakevich OV, Mikhal'chuk AL, Akhrem AA (1991) VII All-Union. Conf. Chemistry of Dicarbonyl Compounds: Abstracts. Riga, 67, (in Russian)
24. Verenich AI, Mikhal'chuk AL, Gulyakevich OV, Akhrem AA (1991) VI All-Union. Conf. on Organic Crystallochemistry: Abstracts. Kiev, 145, (in Russian)
25. Verenich AI, Galitskii NM, Gulyakevich OV, Govorova AA, Mikhal'chuk AL, Akhrem AA (1991) V All-Union. Conf. on Chemistry of Nitrogen Heterocyclic Compounds: Abstracts. AN USSR: Chernogolovka, Part 1. P. 56 (in Russian)
26. Mikhal'chuk AL, Verenich AI, Gulyakevich OV, Govorova AA, Akhrem AA (1992) "14-ECM": Abstracts, Enschede, The Netherland, P. 319
27. Kurbako VZ, Garbuz NI, Lis LG, Lakhvich FA, Solov'ev KN (1983) Electron absorption spectra of enaminodicarbonyl compounds of the 8-azasteroids series. Zhurnal Prikladnoy Spektroskopii 38:407 (in Russian)
28. Terenin AN (1967) Photonics of dyes molecules. Nauka, Leningrad (in Russian)
29. Baum JC, McClure DS (1980) The ultraviolet transitions of benzoic acid. 4. High-resolution spectral studies of hydrogen bonding in the excited states of the benzoic acid dimer. J Am Chem Soc 102:720
30. Baum JC (1980) The ultraviolet transitions of benzoic acid. 3. Effects of hydrogen bonding on the emission properties. J Am Chem Soc 102:716