ISSN 0030-400X, Optics and Spectroscopy, 2012, Vol. 112, No. 5, pp. 671–678. © Pleiades Publishing, Ltd., 2012.
Original Russian Text © E.A. Slyusareva, F.N. Tomilin, A.G. Sizykh, E.Yu. Tankevich, A.A. Kuzubov, S.G. Ovchinnikov, 2012, published in Optika i Spektroskopiya, 2012, Vol. 112, No. 5, pp. 729–737.

SPECTROSCOPY OF ATOMS AND MOLECULES

The Effect of Halogen Substitution on the Structure and Electronic Spectra of Fluorone Dyes

E. A. Slyusareva[a], **F. N. Tomilin**[a, b], **A. G. Sizykh**[a], **E. Yu. Tankevich**[a],
A. A. Kuzubov[a], **and S. G. Ovchinnikov**[a, b]
[a] *Siberian Federal University, Krasnoyarsk, 660041 Russia*
[b] *Kirensky Institute of Physics, Siberian Branch, Russian Academy of Sciences, Krasnoyarsk, 660036 Russia*
e-mail: ESlyusareva@sfu-kras.ru

Received June 21, 2011

Abstract—By means of the B3LYP density functional method with the use of the polarized continuum model PCM, we have performed quantum-chemical computations of the electronic absorption and fluorescence spectra of fluorone dianions: fluorescein, dibromofluorescein, eosin, erythrosine, and Rose Bengal in vacuum and methanol. We have revealed conformational features of the structure of fluorone dianions (charge redistribution, changes in the bond lengths and angles between bonds) second by the halogen substitution, the transition from the ground state to an excited state, and the change of the solvent (vacuum-methanol). Absorption and fluorescence wavelengths, constant dipole moments, transition dipole moments, and oscillator strengths have been calculated. We have showed that, upon halogenation of fluorones, the absorption spectra are redshifted and the Stokes shift decreases, which is qualitatively consistent with experimental results.

DOI: 10.1134/S0030400X12040194

1. INTRODUCTION

Fluorone dyes form a homologous series of compounds based on fluorescein with gradual replacement of hydrogen atoms by atoms of halogens (bromine, chlorine, iodine) (Fig. 1a). The interest in studies of solutions of fluorone dyes is explained by their application as active media of tunable lasers [1], media for information optical recording [2], and fluorescent labels for studying biological objects [3]. Replacement of hydrogen atoms by heavier atoms in the series of fluorone dyes leads to a redistribution of probabilities of radiative and nonradiative transitions, which ensures additional research possibilities for their use as fluorescent probes [4–6].

At present, a large volume of experimental data on spectral and photophysical properties of liquid and

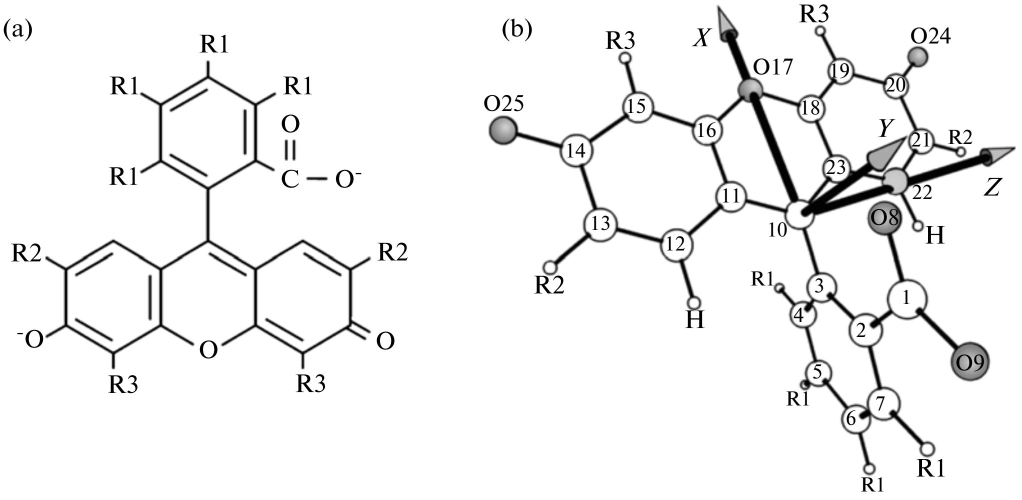

Fig. 1. Structural formulas and spatial structures of fluorones: (a) fluorescein (R1 = R2 = R3 = H), 4'5'-dibromofluorescein (R1 = R2 = H, R3 = Br), eosin (R1 = H, R2 = R3 = Br), erythrosine (R1 = H, R2 = R3 = I), Rose Bengal (R1 = Cl, R2 = R3 = I); (b) conformer 1.

Table 1. Spectral characteristics of fluorone dyes in a basic methanol solution

Parameter \ Dye	Fl	Br_2–Fl	Br_4–Fl	I_4–Fl	I_4Cl_4–Fl
$\lambda_{a,\,\max}$, nm	496 ± 1	513 ± 1	523 ± 1	530 ± 1	557 ± 1
$\lambda_{F,\,\max}$, nm	520 ± 1	539 ± 1	544 ± 1	551 ± 2	575 ± 3
$\tilde{\nu}_{00}$, cm^{-1}	19696	19023	18751	18508	17672
$\sigma_{a,\,00}$, 10^{-16} cm^2	1.79*	1.37	1.98	3.63	2.31
$\Delta\tilde{\nu}_{St}$, cm^{-1}	930 ± 78	940 ± 72	738 ± 70	719 ± 101	562 ± 123

*Refined compared to the data from [10].

solid solutions of fluorone dyes have been accumulated. A considerable number of works have been devoted to the investigation of the influence of a solvent on their electronic spectra [7–10]. The solvatochromic effect leads to a small red shift of electronic spectra. A more significant factor that affects the spectral properties of these dyes is a change in their ionic form [11–13]. For the majority of fluorone dyes, the dianionic form possesses the most pronounced absorption and luminescence properties, which are less pronounced in anionic and neutral forms. This makes it possible to use fluorone dyes as ionic indicators [14].

It is well known that halogen substitution of fluorones results in a considerable decrease in their fluorescence quantum yield and in an increase in the rate constants of their nonradiative transitions [7, 10, 15–24]. This effect is explained by the development of intersystem crossing due to enhancing the spin–orbit interaction in molecules that contain heavy atoms. The halogen substitution causes also a red shift of absorption bands of fluorone dyes. Previous works [25, 26] empirically describe the red shift of the long-wavelength absorption band of fluorones caused by the gradual replacement of hydrogen atoms by halogen atoms in the monocycle and tricycle of fluorones. A detailed analysis of reasons for the red shift has not been performed.

Quantum-chemical calculations are an informative tool in the description of the electronic structure of molecules. An analysis of electronic states and electronic absorption spectra of fluorescein dianion and monoanion by the Hartree–Fock and DFT B3PW91/6-311G methods made it possible to reveal differences in their geometric and electronic structures [27]. The authors of [28], using the SCF-MO-CI semiempirical calculation method in the Pariser–Parr–Pople approximation, determined the energy structure, transition energies, oscillator strengths, and polarization of transitions of dianions such as uranin and eosin. The majority of works have been devoted to fluorescein and its derivatives that do not contain heavy atoms [29, 30], whereas its halogen-substituted derivatives (dibromofluorescein, eosin, erythrosine, and Rose Bengal) have been less studied. The possibility of performing calculations for large organic molecules that contain heavy atoms is related to the progress in computation achieved in recent years.

In this work, by comparing experimental results obtained by the electronic spectroscopy methods [10] with results of quantum-chemical calculations (charge distribution), we revealed the effect of halogen substituents on the spatial structure and electronic spectra of dianions of fluorone dyes.

2. EXPERIMENTAL RESULTS

In this work, we used data on the electronic absorption and fluorescence spectra of fluorescein (Fl), dibromofluorescein (Br_2–Fl), eosin (Br_4–Fl), erythrosine (I_4–Fl), and Rose Bengal (I_4Cl_4–Fl) that were obtained in [10, 24]. As was shown in [11–13], in a basic solvent, dyes predominantly exist in a dianionic form.

Table 1 presents measured spectral characteristics of fluorone dyes dissolved in basic methanol, such as the positions of the maxima of long-wavelength absorption bands ($\lambda_{a,\max}$) and fluorescence bands ($\lambda_{F,\max}$), the wavenumbers of electronic transitions ($\tilde{\nu}_{00} = (\lambda^{-1}_{a,\max} + \lambda^{-1}_{F,\max})/2$), the absorption cross sections at the electronic transition frequency ($\sigma_{a,00}$), and the Stokes shift values ($\Delta\tilde{\nu}_{St} = \lambda^{-1}_{F,\max} - \lambda^{-1}_{a,\max}$).

It should be noted that the positions of the maxima of the absorption and fluorescence spectra presented in Table 1 agree well with the results of [31]. Phosphorescence in liquid solutions does not occur due to efficient quenching processes [32]. From the data of this table, certain regularities in the series of fluorones (Fl–I_4Cl_4–Fl) should be noted, namely, red shifts of the spectra and a decrease in the Stokes shift. These characteristics change most upon passage from I_4–Fl to I_4Cl_4–Fl. These, as well as some other spectroscopic phenomena, will be discussed using the results of quantum-chemical calculations.

3. QUANTUM-CHEMICAL CALCULATIONS

3.1. Methods of Quantum-Chemical Calculations

Quantum-chemical calculations of characteristics of fluorone dianions were performed by the B3LYP density functional method [33, 34] in the 6-311** and 6-31** basis sets using the program GAMESS [35]. The influence of the solvent (methanol) was taken into account using the Tomasi polarized continuum model (PCM) [36–39], in accordance with which a dissolved molecule is located in a cavity bounded by the space of atomic spheres of the solvent molecule. The transition wavelengths in the absorption spectrum were calculated by the TD-B3LYP method [40, 41]. Partial electron charges were presented according to Mulliken in units of the absolute electron charge e^-. Calculations were performed for singlet states of molecules with the charge $-2e^-$.

To find a stable configuration of a dye molecule in the ground state in vacuum (solvent), which corresponds to a minimum of the potential surface, the Γ of the molecule was optimized by the B3LYP/6-311** and PCM//B3LYP/6-311** methods. Then, the energies of transitions and their oscillator strengths were calculated by the TD-B3LYP/6-311** and PCM//TD-B3LYP/6-311** methods. The program GAMESS ensures the finding of the geometry of a molecule in an excited state only under conditions of vacuum. Therefore, initially, we optimized the configuration of the molecule in the excited state without taking into account the solvent. Then, transition energies of fluorones in the solvent were found by the PCM//TD-B3LYP/6-31** method.

The choice of the B3LYP method was determined by the high effectiveness of density functional methods, which is comparable with the possibilities of most complicated post-Hartree–Fock approximations (e.g., the configurational interaction method).

3.2. Conformation of Fluorones in the Ground and Excited States

Our calculations showed that, in the ground state, molecules of fluorone dyes can occur in the form of two spatial conformers. The geometry of conformer 1 (Fig. 1b), in which the COO$^-$ group lies in the plane of the monocycle and is most favorable energetically for all dyes except for I_4Cl_4–Fl, for which conformation 2 proved to be most favorable (the plane of the COO$^-$ group is perpendicular to the plane of the monocycle). The energy difference between two conformers of different fluorones is in the range 8–20 kJ/mol. The two conformers belong to the C_S symmetry group. In connection with this, data for half of monocycle atoms will be presented and discussed.

The halogen substitution leads to a redistribution of charges on the majority of atoms of fluorone molecules. Upon passage from Fl to I_4Cl_4–Fl, partial charges on oxygen atoms in the position O(24) decrease from –0.5 to –0.41 e^-. For all the substituted fluorones, the charge on heteroatom O(17) varies little, being in the range 0.35–0.38. The influence of the solvent (methanol) on the partial charges of oxygen atoms results in an increase in the negative charge on atoms O(8), O(9), and O(24) by 0.04–0.06 e^-, whereas, for heteroatom O(17), the charge remains the same.

A comparison of the partial charges on carbon atoms of the Br_2–Fl and Br_4–Fl molecules (the same substituent Br), as well as of the I_4–Fl and I_4Cl_4–Fl molecules (the same substituent I), showed the following. For atoms C(18), C(19), C(20), and C(21) of the I_4–Fl and I_4Cl_4–Fl molecules, the partial electron charges are the same. Due to the replacement by bromine atoms, the partial charges for the Br_2–Fl and Br_4–Fl molecules are close only for the C(18) and C(19) atoms. The partial charges on atoms C(1), C(2), C(3), C(4), C(5), C(6), C(7), C(10), and C(23), for molecules Fl, Br_2–Fl, Br_4–Fl, and I_4–Fl, have close values for each carbon atom in the series of fluorones. Different charges on the same carbon atoms of the I_4Cl_4–Fl molecule are caused by the presence of chlorine atoms in the monocycle. The partial charges on atom C(22) are different for all fluorones (the range of variation is from –0.01 to 0.17 e^-), because this atom experiences the influence of substituent R2 and is spatially close to the COO$^-$ group. For all carbon atoms, except for C(1), the values of partial charges in vacuum and in methanol proved to be close to each other (the difference is no more than 0.02 e^-). For all the dyes, the charge on this atom proved to be smaller by 0.04–0.05 e^-.

The charges on hydrogen atoms bonded to C(19) and C(21) of Fl are 0.04 and 0.03 e^-, respectively. For halogen-substituted Fl, the charges of halogen atoms differ from the charges on hydrogen atoms that they replace. Thus, the partial charges on atoms R3 are –0.1 e^- for Br_2–Fl and –0.07 e^- for Br_4–Fl, and the charge on substituent R2 of the Br_4–Fl molecule is –0.12 e^-. The charges of atoms R3 and R2 of the I_4–Fl molecule (–0.02 e^- and –0.08 e^-) are greater than for the I_4Cl_4–Fl molecule (0.01 e^- and –0.05 e^-, respectively). The values of the charges on chlorine atoms lie in the range –0.02–0.04 e^-. For molecules in the solution, charges on atoms of substituents increase approximately by 0.02–0.06 e^-.

Upon passage from Fl to I_4Cl_4–Fl, the spatial structure of fluorones undergoes cable changes. In the series C–H, C–Cl, C–Br, and C–I, the bond lengths increase (1.09, 1.74, 1.93, and 2.14 Å, respectively). This is consistent with experimental data [42]. Cable changes are observed for angles C(2)–C(3)–C(10), O(8)–C(1)–O(9), and C(1)–C(2)–C(7) (Fig. 2). Changes in the charge distribution and spatial struc-

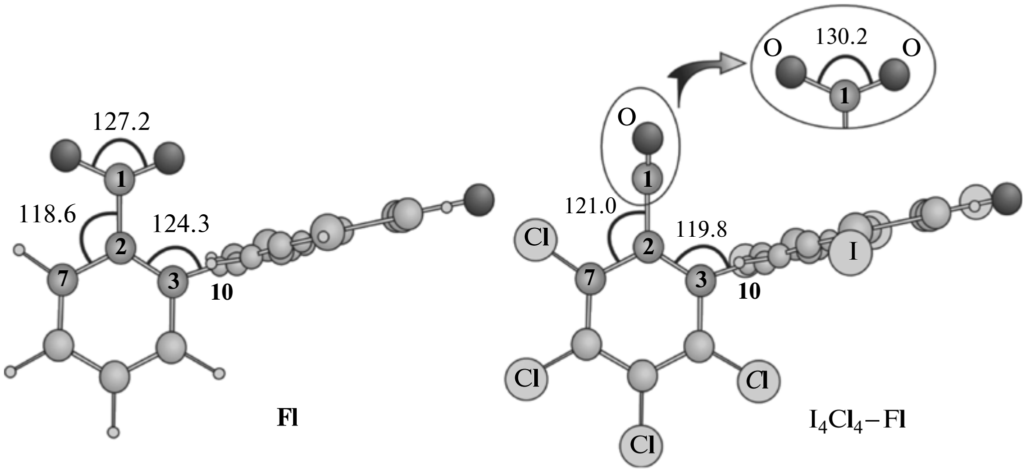

Fig. 2. Changes of the spatial structures of fluorones in methanol in the ground electronic state.

ture of fluorone molecules are the reason for changes in their energy structure and spectral characteristics.

Let us consider differences in the geometry of fluorone molecules in methanol for the ground and excited states (Table 2). Our calculations show that the atoms of the tricycle do not lie in one and the same plane and its two approximately planar fragments form a dihedral angle α with the edge C(10)–O(17). The meaning of the angles β and γ is seen from Fig. 3, where, for the Br_4–Fl molecule, the mutual arrangement of approximately planar fragments of the tricycle is shown, as well as of the planes of the monocycle and COO^- group. In the series Fl–I_4Cl_4–Fl, there is no systematic change in the angles. It should be noted that, in I_4Cl_4–Fl, conformational changes due to the transition to the excited state are smaller than in the remaining dyes.

Table 2. Geometric characteristics of dianions of fluorone dyes in vacuum in the ground and excited electronic states

Dye (state)	Angle	α, deg	β, deg	γ, deg
Fl	Ground	182.4	94.3	0
	Excited	166.1	79.4	0
Br_2–Fl	Ground	181.2	94.9	0
	Excited	159.1	75.5	0
Br_4–Fl	Ground	180.2	95.2	0
	Excited	181.3	55.2	32.7
I_4–Fl	Ground	178.2	95.2	0
	Excited	184.6	97.7	41.8
I_4Cl_4–Fl	Ground	181.2	90.3	90.1
	Excited	191.2	94.2	83.4

3.3. Dipole Moments, Transition Probabilities, and Electronic Spectra of Fluorones

The permanent dipole moments μ^0 and μ^* of fluorones of the ground and excited states are presented in the Table 3. Except for the case of the ground state of all the fluorones and excited states of Fl and Br_2–Fl in vacuum, the resultant dipole moment is formed by summation of three components. The *Y*-component is the largest for all fluorones. The values of the permanent dipole moments of fluorones in different electronic states (ground and excited) and in each of the situations of the intermolecular interaction (vacuum, methanol) are close to each other. The exception is I_4Cl_4–Fl, for which the permanent dipole moments are lower than the dipole moments of the remaining fluorones approximately by 1–2 D because of a lower value of the *X*-component. Characteristically, due to the electronic excitation, the dipole moments increase.

As distinct from the permanent dipole moments, dipole moments of absorption electronic transitions (d^a) and fluorescence (d^F) are directed along the *z* axis, which approximately lies in the plane of the fluorone tricycle. This result is consistent with the data of calculations for uranin and eosin [27], as well as for the fluorescein dianion and monoanion [28], which provide evidence that the highest occupied and the lowest unoccupied molecular orbitals belong to the tricycle atoms of the dyes under consideration. The orientation of the absorption and fluorescence transition dipole moments in the tricycle plane of eosin *Y* and erythrosine *B* was also confirmed by experiments on the angle-resolved fluorescence depolarization [23].

The calculated oscillator strengths of absorption transitions (f_a) confirmed the existence of intense bands. The values of the oscillator strengths for long-wavelength transitions in the absorption spectra of fluorones lie in the range 0.7–0.8. The wavelengths of

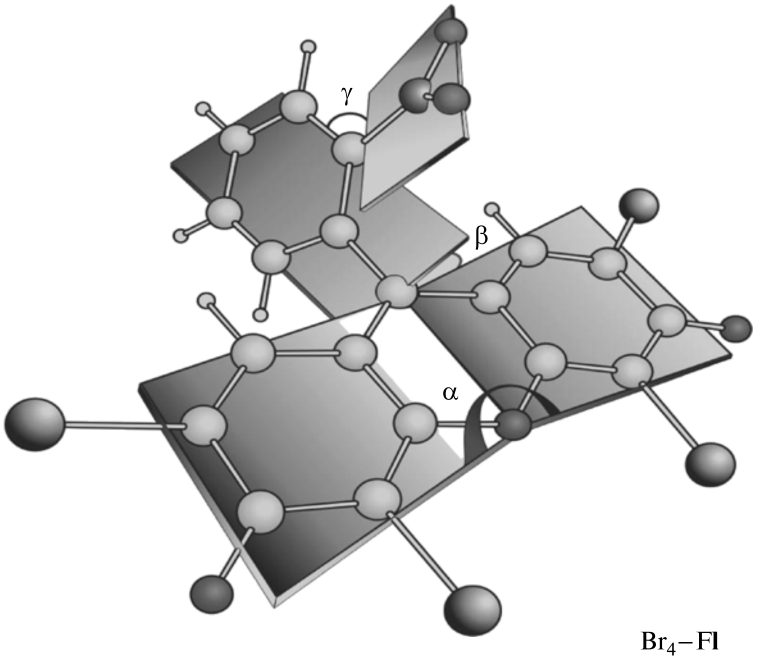

Fig. 3. Mutual arrangement of the planes of the tricycle, monocycle, and COO$^-$ group.

light absorption transitions (λ_a) experience a red shift, the value of which in the series Fl–I$_4$Cl$_4$–Fl is 2380 cm^{-1}.

The wavenumber of the electronic transition in the fluorescence spectrum in methanol for fluorophores Fl–I$_4$–Fl almost does not change (21086 ± 116 cm^{-1}) and, only for I$_4$Cl$_4$–Fl, becomes equal to 20576 cm^{-1}. The Stokes shift $\Delta\tilde{\nu}_{St}$ expressed as a difference between the wavenumbers of absorption and fluorescence transitions decreases upon halogenation of fluorones and reaches a minimal value for I$_4$Cl$_4$–Fl (in methanol).

Upon change of the environment of fluorone molecules (vacuum–methanol), the permanent dipole moments, the transition moments, and the oscillator strengths increase. For the excited (fluorescence) state, the permanent dipole moments increase and the transition dipole moments and the oscillator strengths change to a lesser degree. Upon passage to methanol, the solvatochromic red shift in the absorption and fluorescence spectra in the series of fluorones changes nonmonotonically. With increasing solvent polarity, the direction of the shift is typical of π–π^* transitions [43].

4. COMPARISON OF EXPERIMENTAL RESULTS WITH RESULTS OF QUANTUM-CHEMICAL CALCULATIONS

It should be noted that the values of the permanent dipole moments of fluorescein in the methanol solution that were calculated in this work and in [44] considerably exceed those that were experimentally determined by the Guggenheim–Smith method (for the ground state) and from the spectral shifts (for the excited state); however, the inequality $\mu^0 < \mu^*$ is fulfilled.

The calculated and experimentally determined absorption spectra are compared in Fig. 4. It can be seen from this figure that all the calculated absorption spectra are shifted toward the short-wavelength range compared to the position of the measured spectra. In the calculated absorption and fluorescence spectra, the intense long-wavelength bands are shifted by 3100–3200 cm^{-1}. Seemingly, this can be explained by a systematic calculation error, which is related to the fact that the effect of intermolecular interactions was insufficiently taken into account and the role played by vibronic states was neglected. A similar short-wavelength detuning of calculated and experimental data was obtained in [28] for uranin (2200 cm^{-1}) and eosin *Y* (1670 cm^{-1}).

Nevertheless, upon passage from Fl to I$_4$Cl$_4$–Fl, the calculated absorption spectra of fluorones show a systematic red shift (2380 cm^{-1}), which correlates well with the experimental result (2200 cm^{-1}). This effect is most pronounced upon passage from I$_4$–Fl to I$_4$Cl$_4$–Fl (the differences of the wavenumbers of transitions in the experimental and calculated absorption spectra of these fluorones are 836 and 1345 cm^{-1}, respectively).

Table 3. Characteristics of dianions of fluorone dyes in vacuum and methanol

Dye Parameter	Fl	Br_2–Fl	Br_4–Fl	I_4–Fl	I_4Cl_4–Fl
$(\mu_x^0; \mu_y^0; \mu_z^0)$	(4.1; 7.7; 0.0)	(4.7; 7.6; 0.0)	(2.6; 7.9; 0.0)	(4.7; 7.9; 0.0)	(1.5; 7.3; 0.0)
μ^0, D (vacuum)	8.74	8.92	8.3	9.2	7.5
$(\mu_x^0; \mu_y^0; \mu_z^0)$	(6.6; 11.6; 0.0)	(2.3; 11.4; 0.06)	(1.1; 11.9; 0.02)	(3.2; 11.3; 0.06)	(0.7; 11.3; 0.06)
μ^0, D (methanol)	13.3	11.6	12.0	11.8	11.3
$(\mu_x^*; \mu_y^*; \mu_z^*)$	(3.6; 10.4; 0.0)	(4.8; 9.7; 0.0)	(4.2; 9.4; 2.9)	(7.9; 8.5; 0.2)	(1.4; 9.0; 0.0)
μ^*, D (vacuum)	11.0	10.8	10.7	11.6	9.1
$(\mu_x^*; \mu_y^*; \mu_z^*)$	(5.8; 14.2; 0.0)	(2.2; 13.8; 0.04)	(2.5; 13.2; 4.9)	(8.2; 12.7; 0.3)	(1.0; 13.0; 0.07)
μ^*, D (methanol)	15.4	14.0	14.3	15.2	13.0
$(d_x^a; d_y^a; d_z^a)$, D (vacuum)	(0.0; 0.0; 2.7)	(0.0; 0.0; 2.5)	(0.0; 0.0; 2.3)	(0.0; 0.0; 2.4)	(0.0; 0.0; 2.6)
$(d_x^a; d_y^a; d_z^a)$, D (methanol)	(0.0; 0.0; 3.3)	(0.0; 0.0; 3.1)	(0.0; 0.0; 3.4)	(0.0; 0.0; 3.5)	(0.0; 0.0; 3.5)
$(d_x^F; d_y^F; d_z^F)$, D (vacuum)	(0.0; 0.0; 2.9)	(0.0; 0.0; 2.8)	(0.1; 0.2; 3.0)	(0.1; 0.0; 3.1)	(0.0; 0.0; 3.2)
$(d_x^F; d_y^F; d_z^F)$, D (methanol)	(0.0; 0.0; 2.6)	(0.0; 0.0; 2.5)	(0.0; 0.3; 3.4)	(0.1; 0.0; 3.5)	(0.0; 0.0; 3.2)
λ_a (vacuum), nm	422	430	443	447	461
λ_a (methanol), nm	432	438	447	450	479
f_a (vacuum)	0.54	0.45	0.38	0.39	0.46
f_a (methanol)	0.75	0.68	0.78	0.81	0.78
$\Delta\tilde{\nu}_{St}$ (methanol), cm^{-1}	1961	1866	1362	1035	300

Based on analysis of the data on the absorption cross section on the electronic transition frequency (Table 1) and the values of the calculated oscillator strengths of fluorones in methanol (Table 3), we can conclude that, in the series of the investigated dyes, these values correlate between each other and increase in the sequence Br_2–Fl, Fl, Br_4–Fl, I_4Cl_4–Fl, and I_4–Fl.

As can be seen from Tables 1 and 3 (experiment and calculation, respectively), the electronic spectra of fluorones in methanol show a Stokes shift, which decreases in the series Fl–I_4Cl_4–Fl. The experimentally observed Stokes shift is a result of several dynamical processes. They include energy losses due to the intramolecular vibrational relaxation and a change in the field of intramolecular forces upon transition from the ground to excited electronic state. The Stokes shift obtained from quantum-chemical calculations is determined only by changes in the shape of the potential surface in the excited state of the molecule due to the redistribution of partial charges and changes in the bond lengths and angles between bonds. A decrease in the Stokes shift upon sequential replacement of hydrogen atoms in fluorophore molecules with heavier halogens can be related to a gradual decay of conformational changes of molecules upon electronic excitation, which increases the similarity between the potential curves in the ground and excited states. Although the interpretation of the Stokes shift in terms of quantum-chemical calculations is very limited, as the degree of halogenation of fluorones increases, the calculated shift decreases, which is qualitatively consistent with experimental data.

The charge distribution on oxygen atoms determines the acidic–basic properties of fluorones. Dianions of fluorone dyes in basic solutions are formed due to the appearance of charges on two groups of atoms: O^- (in the tricycle) and COO^- (in the monocycle). An anion of fluorones can be represented by two tautomers, which are formed by binding of hydrogen to one of the listed fragments of molecules. Numerous experiments on potentiometric and spectrophotometric titration [13] showed that the anion of fluorescein in a solution is dissociated in the carboxyl group, whereas anions of halogen-substituted derivatives (Br_4–Fl, I_4Cl_4–Fl, I_4–Fl) are dissociated on the atom O^- of the tricycle. This result can be explained by comparing partial charges on oxygen atoms O(24) and O(9): in fluorescein, the negative charge on O(24) ($-0.50\ e^-$) is greater than on O(9) ($-0.48\ e^-$), whereas, for the remaining halogen-substituted derivatives, this relation is reversed. Thus, for Br_2–Fl, Br_4–Fl, I_4–Fl,

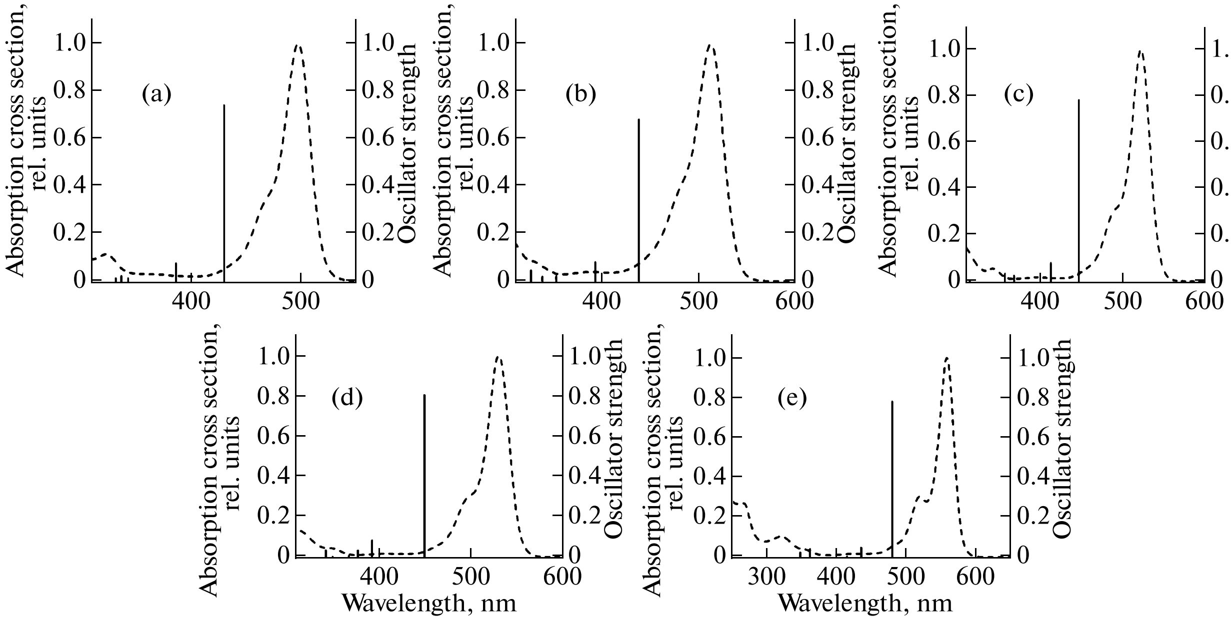

Fig. 4. Absorption spectra fluorones in methanol: (a) Fl, (b) Br_2–Fl, (c) Br_4–Fl, (d) I_4–Fl, and (e) I_4Cl_4–Fl. The experimental absorption cross sections are shown by dashed curves, and the calculated oscillator strengths are indicated by solid vertical lines.

and I_4Cl_4–Fl, the charge on O(24) is lower than the charge on O(9) by 0.05–0.07 e^-.

5. CONCLUSIONS

Using methods of quantum-chemical calculations, we revealed conformational changes in the structure of molecules of fluorones in the series from fluorescein to Rose Bengal that are related to the redistribution of partial charges, which cause changes in bond lengths and angles between bonds. We determined the values of the permanent dipole moments, the transition dipole moments, and the oscillator strengths. We revealed the shift of electronic absorption spectra (red shift), which is caused both by halogenation of fluorones and by the "switching-on" of the intermolecular interaction. The close values of the calculated (2380 cm^{-1}) and experimentally determined (2200 cm^{-1}) red shift of the position of the electronic transition in the absorption spectra of fluorones upon halogen substitution show that this effect can be explained by the redistribution of partial charges. The experimentally observed decrease in the Stokes shift in the series of fluorones from fluorescein to Rose Bengal was qualitatively confirmed by quantum-chemical calculations. In methanol, the calculated permanent dipole moments of fluorones in the ground and excited electronic states proved to be overestimated compared to the experimental values of these parameters; however, the increase in the dipole moment upon electronic excitation has been confirmed experimentally. The acidic–basic properties of fluorone dianions correspond to the distribution of partial charges on oxygen atoms. In general, the results of our quantum-chemical calculations of characteristics of electronic states of fluorones yield a correct pattern and, by extending the informative capabilities of theoretical models and methods, a more adequate description of experimental data can be reached.

ACKNOWLEDGMENTS

This work was supported by the FTsP Kadry GK-P333 and by the Siberian Branch of the Russian Academy of Sciences (Integration project no. 2). We are grateful to the Joint Supercomputer Center of the Russian Academy of Sciences (Moscow) and to the Complex of High-Performance Computations of the IKIT of Siberian Federal University. E.A. Slyusareva acknowledges support from the German Academic Exchange Service and the Ministry of Education and Science of the Russian Federation (the program "Mikhail Lomonosov II") of scientific research at the University of Regensburg and in the Helmholtz Center in Berlin (Germany).

REFERENCES

1. *Dye Lasers*, Ed. by F. P. Schafer (Springer, New York, 1974; Mir, Moscow, 1976).
2. M. E. Dailey, E. Manders, D. R. Soll, and M. Terasaki, in *Handbook on Biological Confocal Microscopy*, Ed. by J. B. Pawley (Springer Science + Business Media, New York, 2006), pp. 381–403.