

NATURE OF ELECTRONICALLY EXCITED STATES OF FUROCOUMARINS

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UDC 539.194:535.37

A comprehensive study of spectral-luminescent and photophysical properties of coumarin sensitizers is performed using the methods of quantum chemistry, electron spectroscopy, and fluorescence. A comparative study of photophysical processes occurring in 8-methoxypsoralen (8-MOP) and 4,9-dimethoxy-7-methylfuro [3,2-g][1] benzofuran-5-1 (khellin) is performed using the quantum-chemical software package based on the semi-empirical method of intermediate neglect of differential overlap with original spectroscopic parameterization. The dependences of the fluorescent properties and reactivity of the compounds on the geometry and composition of the solvation shell of molecules are established. It is shown that incorporation of additional methoxy groups in the structure of molecules has the strongest effect on the spectral-luminescent characteristics of khellin compared with 8-MOP.

Keywords: psoralen, furocoumarin, photosensitizer, absorption, fluorescence, photophysical processes, quantum-chemical calculation.

INTRODUCTION

Furocoumarins are photosensitizers of plant origin that increase the sensitivity of biological tissues to light. Furocoumarins form a group of drugs with photosensitizer properties for radiation with wavelengths from 320 to 400 nm (UV-A range). In combination with UV-A radiation, they are successfully used to treat many skin (vitiligo, mycosis, and, especially, psoriasis) and autoimmune diseases using phototherapy and photophoresis. PUFA therapy (P means psoralen, UV means ultraviolet flux, and A means long-wavelength electromagnetic rays [1]) or, in other words, phototherapy is the treatment method based on irradiating a patient with dosed ultraviolet rays. Various cancers are treated using phototherapy. 8-methoxypsoralen (8-MOP) is still most commonly used today for such treatment; 5-methoxypsoralen (5-MOP), 4,5',8-trimethylpsoralen, and 4,9-dimethoxy-7-methylfuro [3,2-g][1] benzofuran-5-1 (khellin) are less commonly used [1, 2]. 5-MOP does not directly act on the skin infected by psoriasis and becomes active after long-wavelength ultraviolet irradiation of the body reacting with epidermis cells and suppressing excessive DNA synthesis without inhibiting cellular functions for 1.5 h [3]. 8-MOP currently has the highest photosensitizing activity compared with its analogs; however, it has a more pronounced side effects compared with 5-MOP. The high photosensitizing activity of 8-MOP can hinder its use in patients with delicate skin and vitiligo due to the risk of skin burn [4].

The search for compounds with antioxidant and protective properties is the main problem of researchers dealing with clinical medicine. Electron spectroscopy and quantum-chemical calculations allow the dependence of the properties of chemical compounds and of their reactivity on the compound composition to be studied, as well as the behavior of the examined organic molecules in biological systems to be predicted. In the present work, a comparative

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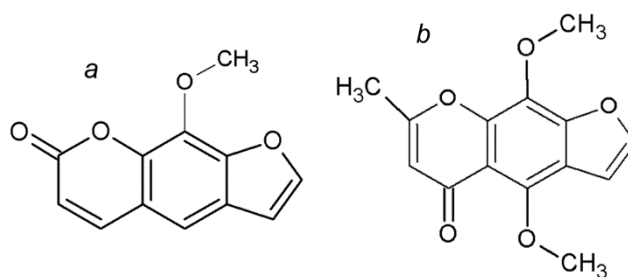


Fig. 1. Structural formulas of the examined molecules: *a* is for 8-methoxypsoralen and *b* is for 4,9-dimethoxy-7-methylfuro [3,2-g][1] benzofuran-5-1.

experimental and theoretical study of photophysical processes occurring in 8-methoxypsoralen and 4,9-dimethoxy-7-methylfuro [3,2-g][1] benzofuran-5-1 is presented. The experimental results are compared with the data of quantum chemical calculations.

OBJECTS AND METHODS OF RESEARCH

In the present work, coumarin substituted compounds 8-methoxypsoralen (8-MOP) (Fig. 1*a*) and 4,9-dimethoxy-7-methylfuro [3,2-g][1] benzofuran-5-1 (Fig. 1*b*) (Sigma-Aldrich) were used.

The examined compounds are well soluble in ethanol and poorly soluble in weakly polar solvents. Hence, the spectral and luminescent properties of these substances were studied in ethanol solutions. Ethyl alcohol with a purity of 99.6% (Sigma-Aldrich) was used as a solvent [5]. The spectral-luminescent characteristics of the solutions were recorded with a SM-2203 spectrofluorometer (SOLAR, Belarus).

The theoretical studies were performed using the software package based on the semi-empirical INDO/S method. To solve the spectroscopy problems, quantum-chemical programs and methods for estimating the rate constants of photoprocesses have been developed by Professor G. V. Mayer, Professor V. Ya. Artyukhov, Associate Professor A. I. Galejeva, and Associate Professor V. A. Pomogaev [6]. The software package allows the following characteristics of the electronic states of polyatomic molecules to be determined: energy and nature of molecular orbitals, energy and wave functions of the singlet and triplet electronically excited states, oscillator strengths and polarization of electronic transitions, distribution of electron density on atoms and bonds of the molecule, dipole moments in the ground and excited states, as well as absorption and fluorescence spectra. The geometry was optimized using the AM1 and PM3 quantum-chemical methods [7]: AM1 means AustinModel1 (Austin model No. 1) and PM3 means ParameterModel3 (parametric model No. 3). In the AM1 method certain overestimation of the long-range interaction between atoms leading to overestimation of the internal rotation barriers in the molecules has been eliminated compared with the MNDO method. As a result, it became possible to correctly calculate the hydrogen bonds calculated in the MNDO method only within the framework of special modifications. The PM3 method only improves the set of the parameters of the AM1 method and as a whole, has the same advantages and disadvantages. The methods considered in the present work allow a large spectrum of molecular characteristics to be calculated [8–11].

RESULTS AND DISCUSSION

1. Spectral characteristics of 8-MOP and khellin dissolved in ethanol

The first stage of the work was a comparative analysis of the absorption and fluorescence spectra at concentrations corresponding to linear segments of the curve. To calculate the fluorescence quantum yield by the relative method, the concentration of the examined compounds was chosen to be equal to $5 \cdot 10^{-5}$ mol/L. A solution of

TABLE 1. Spectroscopic Characteristics of the Examined Compounds Dissolved in Ethanol

Substance	C, mol/l	λ_{abs} , nm	$\Delta\nu_{1/2}$, cm^{-1}	λ_{fl} , nm	φ	Stokes shift	
						$\Delta\lambda_{\text{St}}$, nm	$\Delta\nu_{\text{St}}$, nm^{-1}
8-MOP	$5 \cdot 10^{-5}$	300	4668	480	0.0013	180	12500
Khellin		331	5892	489	0.0036	158	9762

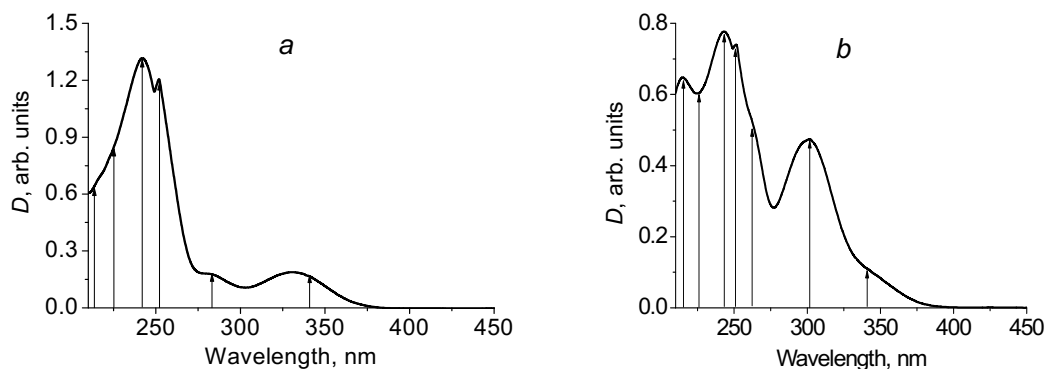


Fig. 2. Absorption spectra of khellin (a) and 8-MOP (b) dissolved in ethanol. Arrows indicate electronic transitions.

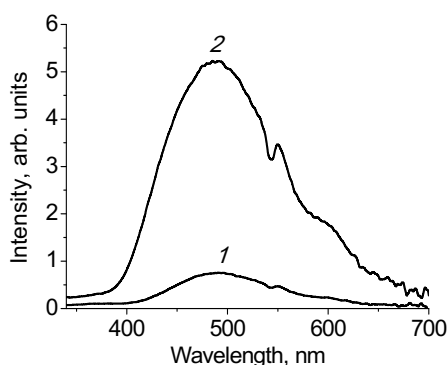


Fig. 3. Fluorescence spectrum of 8-MOP (curve 1) and khellin (curve 2) dissolved in ethanol.

phenol in ethanol ($\varphi = 0.23$) was used as an etalon. Figures 2 and 3 show the absorption and fluorescence spectra of 8-MOP and khellin dissolved in ethanol.

The 8-MOP absorption spectrum consists of 300, 263, and 245 nm bands. The maximum fluorescence band of the examined compound is at 485 nm (Table 1). The khellin absorption spectrum consists of 331, 284, and 242 nm bands; the maximum of the fluorescence spectrum is at 487 nm. Transitions manifested only as hidden maxima and fuzzy kinks in the absorption spectrum were obtained using the method of derivative spectrophotometry. This method is based on the same principles as conventional spectrophotometry; however, the analytical signal is not the optical density, but its derivative of the n th order (usually, with respect to the wavelength). Spectrum differentiation allows the position of the maximum absorption band to be determined more accurately, and also narrows down the bands and allows substances to be detected that absorb at close wavelengths and the initial spectra of which are partially overlapped. According to this technique, it was possible to distinguish electronic transitions (indicated by arrows in Fig. 2) in the absorption spectra of the examined molecules.

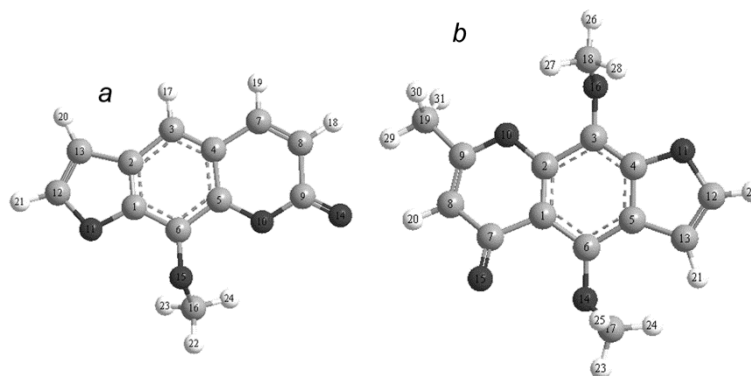


Fig. 4. Structures of the 8-MOP (a) and khellin (b) molecules optimized by the PM3 method.

It was established that the fluorescence intensity of 8-MOP was approximately 7 times less than the fluorescence intensity of khellin (Fig. 3). This behavior is caused by a change in the structure of the khellin molecule, namely, the incorporation of substituents – the additional methoxy group (position 6 in Fig. 4b) and the methyl group in position 9 and the change of the position of carbonyl oxygen compared to 8-MOP from position 9 (Fig. 4a) to 7 (Fig. 4b).

From the data obtained it is clear that the Stokes shifts of 8-MOP and khellin have large values [12]. This can be caused not only by the change of the geometry of molecules in the excited states, but also by interaction with the solvent. From the analysis of the 8-MOP geometry it follows that the structure of the molecule is flat, and the oxygen atom of the carbonyl group (No. 14 in Fig. 4a) leaves the plane together with two hydrogen atoms of the methoxy group (Nos. 23–24 in Fig. 4a). In contrast to the 8-MOP structure, the khellin molecule changes as follows: hydrogen atoms of the methoxy group (Nos. 27–28 and 24–25 in Fig. 4b) and hydrogen atoms of the methyl group (Nos. 30–31 in Fig. 4b), which additionally remove carbon atoms (Nos. 8–9 in Fig. 4b) from the plane, leave the plane of the furocoumarin rings.

2. Quantum-chemical study of spectral-luminescent properties of the complex of the 8-MOP and khellin molecules with a solvent

Let us consider a complex of molecules with a solvent. The place of the most probable approach of the solvent molecule (Table 2) was determined using effective charges on atoms in the state S_0 [13–15]. The analysis of the effective charge distribution on atoms in the molecule upon excitation showed that in the states S_0 and S_1 , the oxygen atom of the carbonyl group (No. 14 in Table 2) is the most electronegative one in 8-MOP regardless of the geometry optimization method. For khellin in the state S_0 , the oxygen atom of the carbonyl group (No. 15 in Table 2) is also an electronegative atom. This suggests that for the examined molecules, the solvation center in the ground state is most likely the oxygen atom of the carbonyl group. However, delocalization of the electron density is observed for khellin upon excitation: redistribution between the oxygen atoms of the methoxy- and carbonyl groups and the pyrone ring (Nos. 11 and 14–16 in Table 2). Thus, in the excited state the formation of intermolecular interaction is possible for these atoms with the same probabilities.

In the present work, a complex with a hydrogen bond on the oxygen atom of the carbonyl group (Fig. 5 and Table 3) was modeled: the oxygen atom of the 8-MOP carbonyl group with the H-bond (No. 14 in Fig. 5a) leaves the plane. In contrast to 8-MOP, the structure of the khellin complex changes as follows: the hydrogen atoms of methyl group (Nos. 30–31 in Fig. 5b) and methoxy group (Nos. 27–28 and 24–25 in Fig. 5b) leave the plane of the furocoumarin rings, and hydrogen atoms Nos. 30–31 unfold to the oxygen atom of the carbonyl group (No. 19 in Fig. 5b), thereby returning it into the plane of the furocoumarin rings compared to the isolated molecule (Fig. 4b and Table 3).

TABLE 2. Effective Charges on Atoms in the Complexes of 8-MOP and Khellin in the States S_0 and S_1

Atom No.	Effective charges on atoms							
	State S_0				State S_1			
	8-MOP		Khellin		8-MOP		Khellin	
	AM1	PM3	AM1	PM3	AM1	PM3	AM1	PM3
1	-0.038	0.173	-0.096	-0.071	-0.064	0.179	-0.056	-0.090
2	0.104	-0.047	0.161	0.135	0.237	-0.002	0.141	0.040
3	0.163	-0.051	0.121	0.134	0.182	-0.090	0.064	0.097
4	0.158	-0.041	0.130	0.153	0.153	-0.060	0.063	0.151
5	-0.044	0.108	-0.081	-0.092	-0.016	0.240	-0.087	-0.138
6	-0.055	0.175	0.205	0.493	-0.076	0.199	0.104	0.109
7	-0.225	-0.033	0.439	0.445	-0.205	-0.158	0.383	0.356
8	0.130	-0.051	-0.113	-0.111	0.135	-0.132	-0.049	0.006
9	-0.064	0.646	0.185	0.183	-0.053	0.566	0.117	0.170
10	-0.036	-0.185	-0.169	-0.172	-0.160	-0.144	-0.161	-0.159
11	-0.048	-0.210	-0.234	-0.232	-0.136	-0.187	-0.231	-0.229
12	-0.167	0.131	0.140	0.139	-0.122	0.166	0.109	0.091
13	0.644	-0.071	-0.085	-0.080	0.576	-0.044	-0.110	-0.102
14	-0.611	-0.601	-0.238	-0.274	-0.629	-0.624	-0.234	-0.267
15	-0.287	-0.324	-0.558	-0.554	-0.211	-0.297	-0.242	-0.351
16	0.178	0.179	-0.324	-0.323	0.181	0.181	-0.299	-0.294
17	0.037	0.037	0.187	0.189	0.037	0.037	0.180	0.189
18	0.039	0.032	0.190	0.189	0.039	0.032	0.181	0.187
19	0.020	0.036	-0.008	-0.011	0.020	0.036	-0.003	-0.007
20	0.036	0.038	0.027	0.029	0.036	0.038	0.027	0.025
21	0.033	0.021	0.039	0.045	0.033	0.021	0.039	0.042
22	0.013	0.017	0.014	0.016	0.013	0.017	0.012	0.016
23	0.011	0.007	0.012	0.012	0.014	0.007	0.010	0.011
24	0.011	0.016	0.003	0.001	0.014	0.016	0.000	0.002
25			-0.001	0.002			-0.004	0.002
26			0.008	0.012			0.004	0.010
27			-0.005	-0.001			-0.007	0.000
28			0.013	0.004			0.012	0.005
29			0.009	0.009			0.008	0.009
30			0.012	0.017			0.012	0.020
31			0.019	0.018			0.017	0.020

An analysis of the data presented in Table 3 demonstrated that for the khellin complex, in contrast to the 8-MOP, a pronounced minimum of the electrostatic potential appears near the oxygen atom of the methoxy group (No. 15). This fact indicates that intermolecular interaction with the solvent molecule is possible through this center, and a stronger dependence of the khellin spectral properties on the solvent should be expected in an experiment.

After geometry optimization using the AM1 and PM3 methods (see Fig. 4), the energy level diagrams of electronically excited states were constructed, and the rate constants of photophysical processes were estimated. Based on the results obtained, we can say that there is good agreement between the experimental and calculated data (Table 4). An analysis of the energy level diagrams of the electronically excited state (Fig. 6) demonstrated that for the examined compounds, the lower singlet electronically excited state S_1 is of the $\pi\pi^*$ -type, and the state S_2 is of the $n\pi^*$ -type. The electronic $\pi\pi^*$ -type transitions in the lower singlet excited state S_1 and in the state S_3 are formed mainly by carbon atoms, whereas the $n\pi^*$ -type transitions in 8-MOP and khellin are formed by oxygen atoms.

TABLE 3. Bond Lengths Together with Valence and Torsion Angles for the Geometries of the Examined Molecules Optimized by the PM3 Method

8-MOP					Khellin				
Bond between atoms		Bond length	Valence Angle	Torsion angle	Bond between atoms		Bond length	Valence Angle	Torsion angle
1	2	1.28	79.8	94.6	1	2	1.33	84.4	89.5
2	3	1.49	123.8	264.6	2	3	1.44	121.8	275.4
3	4	1.33	114.9	0.3	3	4	1.42	114.8	358.8
4	5	1.45	123	359.6	4	5	1.42	123.5	3.1
5	6	1.44	119.9	359	5	6	1.33	120.9	359.1
4	7	1.50	121.7	179.4	1	7	1.50	97.7	209.5
7	8	1.34	119.7	180.5	7	8	1.50	110	278.8
8	9	1.52	121.1	0.05	8	9	1.34	121.3	354.5
9	10	1.35	119.7	0.01	9	10	1.35	125	357.8
1	11	1.37	88.7	207.2	4	11	1.43	124.5	181.1
11	12	1.43	104.9	281.4	11	12	1.43	102.1	183.2
12	13	1.33	111	0.1	12	13	1.34	115.1	359.2
9	14	1.21	120.4	179.4	6	14	1.35	125	172.9
6	15	1.38	122.7	184.2	3	15	1.39	123.9	177.4
15	16	1.41	115.6	277.2	15	16	1.41	115.4	93.3
3	17	1.10	122.5	180.4	9	17	1.49	122.7	178.5
8	18	1.10	119.5	180	14	18	1.41	116.7	42.8
7	19	1.10	120.2	0.5	7	19	1.21	124.6	108
13	20	1.10	128.1	180	8	20	1.09	117.9	172.7
12	21	1.09	118.5	180.1	13	21	1.09	127	180
16	22	1.09	106.7	180.5	12	22	1.10	116.5	179.2
16	23	1.09	106.7	296.9	16	23	1.09	106.7	181.3
16	24	1.09	106.7	64	16	24	1.09	112.1	299.4
14	25	1.00	180	180.0	16	25	1.09	112.2	63.1
25	26	1.33	180	0	17	26	1.09	111	1.9
26	27	1.33	120.2	0	17	27	1.09	110.5	122.1
					17	28	1.09	111	241.4
					18	29	1.09	106.7	175.2
					18	30	1.10	112.2	293.2
					18	31	1.09	112.1	57
					19	32	1.38	180	180
					32	33	1.33	180	0
					33	34	1.33	120.2	0

The rate constants of the photophysical processes occurring in the examined compounds are presented in Table 5. Such a significant difference between the experimental and calculated data can be caused by the need to construct a complex with participation of several reaction centers.

The process of intersystem crossing is the main channel of degradation of the excitation energy ($k_{ST} \sim 10^{10} \text{ s}^{-1}$) in 8-MOP and khellin. The high value of the constant is caused by the close location of the singlet $S_3 \pi\pi^*$ -type and triplet $T_8 n\pi^*$ -type states of 8-MOP ($T_7 n\pi^*$ -type in khellin) (Fig. 6). It should be noted that the state S_2 is located near S_3 of 8-MOP, whereas the state S_2 is close to S_1 of khellin. From Fig. 6 it can be seen that the degradation of the excitation energy in the 8-MOP complex has one main channel through the system of triplet states:

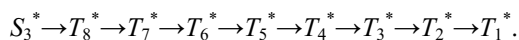


TABLE 4. Positions of Electronic Transitions of 8-MOP and Khellin Dissolved in Ethanol

Electronically Excited States, nm							
8-MOP				Khellin			
Transition	Experiment	Calculation		Transition	Experiment	Calculation	
		AM1	PM3			AM1	PM3
$S_0 \rightarrow S_1$	342	316	310	$S_0 \rightarrow S_1$	337	336	330
$S_0 \rightarrow S_2$	302	297	296	$S_0 \rightarrow S_2$	284	290	300
$S_0 \rightarrow S_3$	265	260	264	$S_0 \rightarrow S_3$	252	251	250
$S_0 \rightarrow S_4$	252	250	252	$S_0 \rightarrow S_4$	237	238	237
$S_0 \rightarrow S_5$	244	244	245	$S_0 \rightarrow S_5$	223	223	223
$S_0 \rightarrow S_6$	230	236	233	$S_0 \rightarrow S_6$	212	213	216
$S_0 \rightarrow S_7$	214	216	217	–	–	–	–

TABLE 5. Calculated Rate Constants of Photophysical Processes of Lower Singlet Excited States and Quantum Yields of the Examined Compounds

Compound	PM3 method				Experiment, ϕ
	k_r, s^{-1}	k_{IC}, s^{-1}	k_{ST}, s^{-1}	ϕ	
8-MOP	$7.3 \cdot 10^7$	$1.2 \cdot 10^4$	$7.7 \cdot 10^{10}$	0.0009	0.0013
Khellin	$4.2 \cdot 10^7$	$7.8 \cdot 10^2$	$3.1 \cdot 10^{10}$	0.0014	0.0036

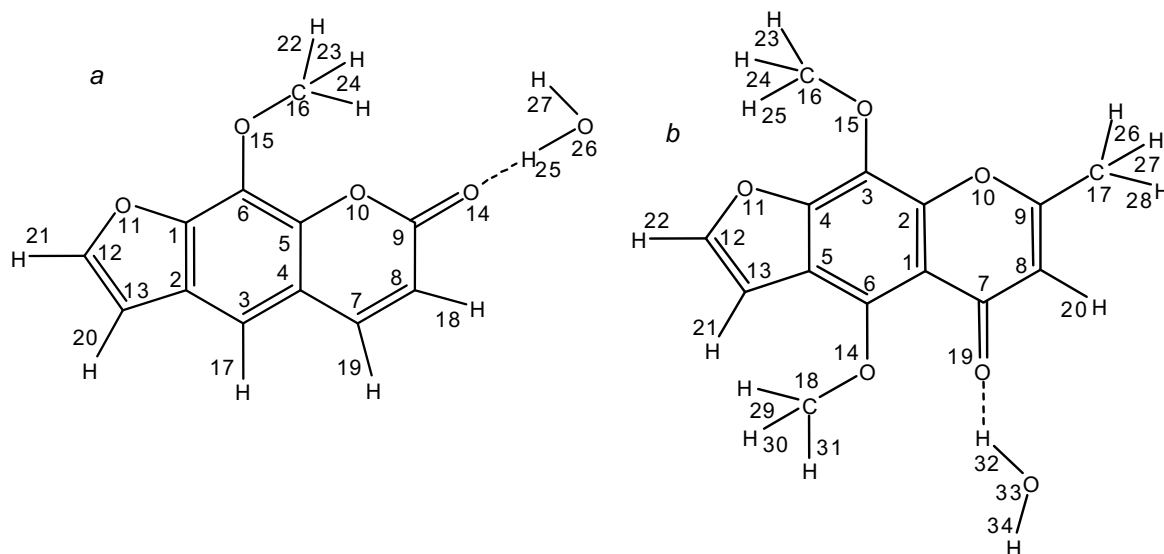
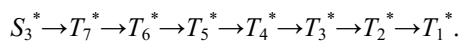


Fig. 5. Structures of complexes of the examined 8-MOP (a) and khellin (b) molecules with a solvent.

Khellin has also one main channel of degradation of the excitation energy:



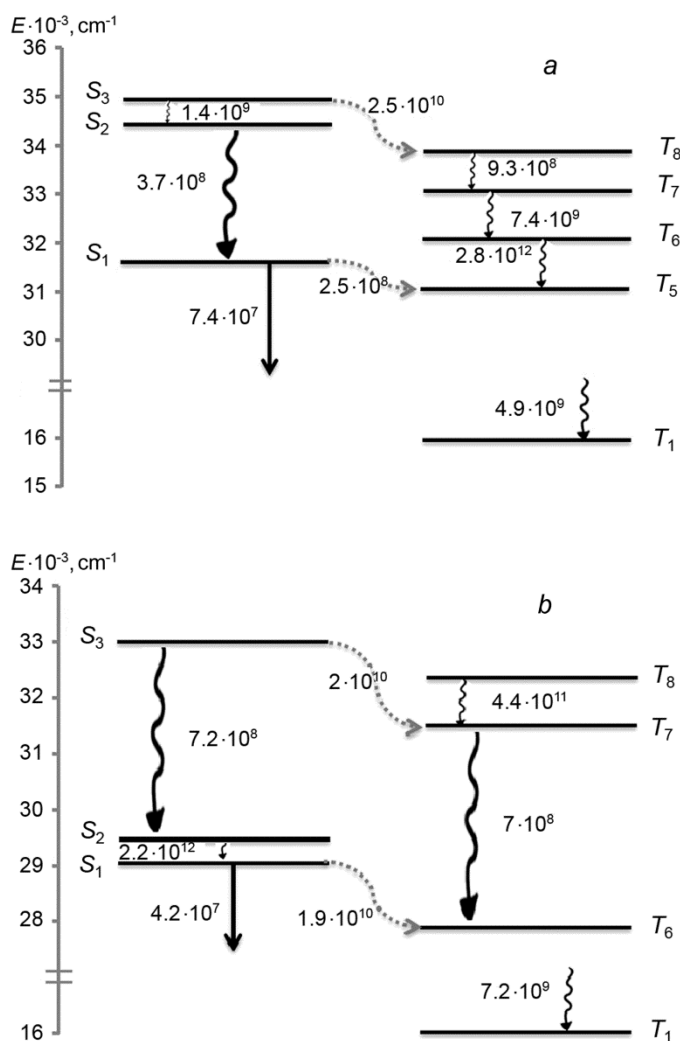


Fig. 6. Energy level diagrams of the 8-MOP (a) and khellin (b) molecules with the attached H₂O molecule calculated by the PM3 geometry optimization method.

CONCLUSIONS

As a result of the present work, it was established experimentally and theoretically that:

1. The geometry of the examined molecules optimized by the PM3 method better describes the spectral data obtained in the experiment.
2. The fluorescence quantum yield of khellin dissolved in ethanol was $\varphi = 0.0014$, which was determined by high rate constants of non-radiative processes compared with the radiative decay ($k_{ST} \sim 10^{10} \text{ s}^{-1}$).
3. The main channel of degradation of the excitation energy in the complexes of the examined molecules with a solvent is the decay through the system of triplet states: $S_3^* \rightarrow T_8^* \rightarrow T_n^* \dots \rightarrow T_1^*$ in 8-MOP and $S_3^* \rightarrow T_7^* \rightarrow T_n^* \dots \rightarrow T_1^*$ in khellin.

This work was supported in part by the Ministry of Education and Science of the Russian Federation within the framework of State Assignment No. 4.6027.2017/8.9 and by the Program of Enhancement of Tomsk State University Competitiveness among the World's Leading Research and Educational Centers.

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