

Fluorescence behavior of isoflavones: pH-dependent effects

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Fluorescence spectra of isoflavones are studied in the pH/H_0 range 12 to -10 . The assignment of the observed emission bands to acid-base forms of isoflavones is done, the pH/H_0 ranges of the existence of each of forms are determined. In 7-methoxyisoflavone solutions, only fluorescence of the cationic form is detected. In the case of 7-hydroxyisoflavones, the fluorescence of the cationic, anionic and phototautomeric forms are found. The emission band in the range 400–420 nm characteristic for all isoflavones is assigned to their photodestruction products. The dissociation and protonation constants of isoflavones in the ground and excited states are evaluated.

Изучены спектры флуоресценции изофлавонов в интервале pH/H_0 от 12 до -10 . Проведено отнесение наблюдаемых полос испускания к кислотно-основным формам изофлавонов в возбужденном состоянии, определены интервалы pH/H_0 существования каждой из форм. В растворах 7-метоксиизофлавонов обнаружена флуоресценция только катионной формы. В случае 7-гидроксиизофлавонов обнаружена флуоресценция катионной, анионной и фототаутомерной форм. Полоса испускания в области 400–420 нм, характерная для всех изофлавонов, отнесена к продуктам их фотораспада. Рассчитаны константы диссоциации и протонирования изофлавонов в основном и возбужденном состояниях.

Natural and synthetic 3-phenylchromones (isoflavones) having significant antioxidant properties are often used as food and drugs additives [1]. Furthermore, isoflavones are also promising photoadsorbents, which now begin to find application as anti-reflecting components in laser lithography [2]. Since many chromone derivatives are characterized by intense fluorescence, they attract the attention as laser dyes and fluorescent labels [3].

In contrast to isomeric 2-phenylchromones (flavones), an electronic structure, acid-base and spectral properties of isoflavones have been studied insufficiently. Recently, we have calculated the electronic structure of isoflavones [4, 5], that allowed us to determine types of electronic transi-

tions in isoflavone molecules and to assign these transitions to bands observed in experimental absorption spectra. Acid-base properties of some natural isoflavones have also been studied [6, 7]. It has been shown that intense bands in absorption spectra of neutral and anionic forms of isoflavones are caused by transitions localized on *ortho*-oxybenzaldehyde and chromone moieties [5]. The bands in absorption spectra of cations are caused by the presence of a benzopyrrolic moiety [8]. Fluorescent properties of isoflavones are least studied. It is known that the number of emission bands in fluorescence spectra of these compounds is found to exceed the number of acid-base forms determined in the ground-state [9]. The nature of bands is studied insufficiently, and no

Table 1. Dissociation and protonation constants of isoflavones in the ground and excited states (*)

	pK_a	pK_a^*	ΔpK_a^*	pK_b	pK_b^*	ΔpK_b^*
I	9.03±0.01	1.1	-7.9	-2.37±0.09	5.5	+7.9
II	-	-	-	-2.31±0.10	5.8	+8.1
III	8.50±0.01	0.9	-7.6	-2.86±0.06	7.6	+10.5
IV	9.56±0.01	2.0	-7.6	-2.38±0.03	7.9	+10.3
V	-	-	-	-2.47±0.04	7.7	+9.5
VI	8.90±0.01	1.0	-7.9	-3.60±0.04	3.8	+7.4
VII	-	-	-	-3.52±0.06	3.6	+7.1

—* pK_a , pK_a^* — values of the dissociation constant in the ground and the excited states, pK_b , pK_b^* — values of the protonation constant in the ground and the excited states, ΔpK_a^* , ΔpK_b^* — the changes of the dissociation and protonation constants of isoflavones upon the excitation. pK^*IV_a and pK^*_b values evaluated by the Foerster method.

reliable band assignment to certain molecular forms of isoflavones is proposed. The purpose of this work is to study fluorescence properties of isoflavones and to examine the nature and interconversion of their forms in the excited state.

Isoflavones used for this study (Chart 1) were synthesized as described in [10]. Purity of the studied compounds was controlled chromatographically. The structure of synthesized compounds was confirmed by proton magnetic resonance (PMR) and infrared (IR) spectroscopy. The absorption and fluorescence spectra were recorded using a Hitachi U3210 spectrophotometer and a Hitachi F4010 spectrofluorimeter, respectively. The lifetimes of fluorescent forms were measured by the time-correlated single photon counting technique [11]. A quinine bisulphate solution in 1N H₂SO₄ was used as a standard ($\phi = 54.6\%$) to estimate the fluorescence quantum yield. The study of acid-base forms of thiazolylchromones in the pH range 1–12 was done in water-methanol solutions (the water:methanol ratio 2:8 v/v), using the NaOH and HCl solutions in the same solvent as titrants. Measurements in the H_0 range 0 to -10 were done in H₂SO₄ aqueous solutions. The solvents were purified

using procedures described in [12]. The presence of spectrally active impurities during the purification and distillation was controlled by spectrophotometry and spectrofluorimetry. The analysis of spectral data obtained by the photometric titration and the evaluation of equilibrium constants were done using the Spectra Data Lab program package developed at the Institute of Chemistry, Kharkiv National University [13]. Judging from the structure of isoflavones, they are able in the ground state to manifest both weak acidic and weak basic properties. In the first case, the isoflavonolate anion (A⁻) is formed due to dissociation of the 7-hydroxy group; in the second case, protonation of the carboxylic group results in formation of the isoflavylum cation (C⁺), Chart 2. The protonation and dissociation constants for the studied isoflavones are presented in Table 1. As it is seen from the data obtained, the dissociation pK of the 7-hydroxyflavones is found to be in the range 8.5–9.5, whereas it seems to be impossible to establish a clear dependence between the isoflavone acidity and the nature

Chart 1. The structure of studied isoflavones.

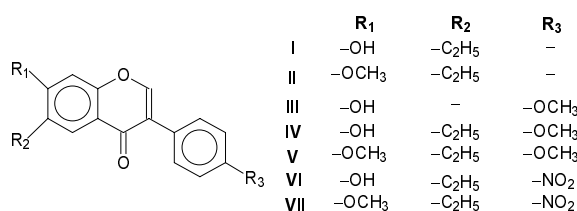
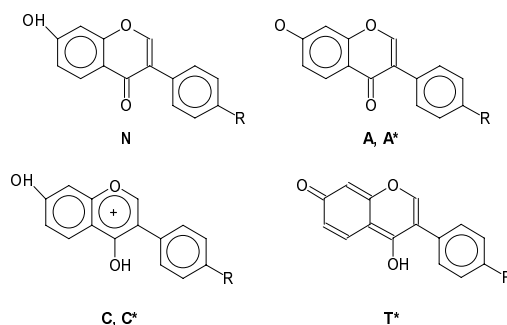


Chart 2. Acid-base forms of 7-hydroxyisoflavone in the ground and excited states.



and localization of the substituents. The protonation pK of compounds *I–V* is found to be in a range -2.4 to -2.9 ; the introduction of a nitro group into the side phenyl ring results in a decrease of pK_b to $-3.5 - -3.6$.

Considering the positions of absorption band maxima of acid-base forms of isoflavones, it is possible to draw a set of conclusions concerning their fluorescence properties. Thus, in absorption spectra of isoflavones, the presence of the band caused by the $n \rightarrow \pi^*$ transition with $\lambda_{max} = 330$ nm [14] and localized on the carboxylic group rules out the neutral form fluorescence. The presence of transitions similar in nature at 360 nm [15] and 500 nm [16] in the nitro-compounds causes the absence of fluorescence of the anionic and cationic forms of 4'-nitroisoflavones, the long-wavelength $\pi \rightarrow \pi^*$ transitions of which are found to be at 340–350 nm (*VI*) and 340–370 nm (*VI–VII*), respectively.

Consequently, in emission spectra of 7-methoxyisoflavones (*II* and *V*), the fluorescence due to only one, the cationic form is expected to be observed; 7-hydroxyisoflavones (*I*, *III* and *IV*) should demonstrate the emission of the anionic and cationic forms; 4'-nitroisoflavones (*VI*, *VII*) should not fluoresce at all. The analysis of the experimental spectra shows the presence of three or four emission bands for compounds *I*, *III* and *IV*, of two bands for *II* and *V*, of one emission band for *VI* and *VII*. In order to assign each of emission bands to corresponding acid-base forms, we have investigated the behavior of these bands upon changing pH of solutions.

The weak fluorescence in the 400–420 nm range, which is observed in all studied isoflavones, was assigned by authors [9] to the neutral form of isoflavones. In our opinion, this is impossible because the S_1 -state of the neutral form of isoflavones has the $n\pi^*$ nature. The authors [17] have assumed that the observed fluorescence could be caused by some impurities formed during destruction of isoflavones. Moreover, an optical density decrease of the *III* solution under UV irradiation was noted [6], that might also point to some photodestruction processes. In order to verify this assumption, we irradiated the solutions of *I*, *III* and *IV* by monochromatic light ($\lambda = 313$ nm). The exposure of solutions with the quantum intensity of $3.8 \cdot 10^9$ E/s during 5 min resulted in the appearance of the sought emission bands with a maximum at 400–430 nm. The same exposure applied for 10 h resulted in the almost total photodestruction of isofla-

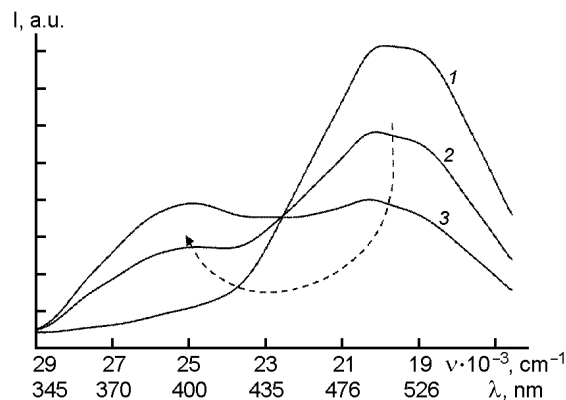


Fig. 1. Fluorescence spectra of isoflavone *III* after 5 min (1), 30 min (2) and 90 min (3) of UV-irradiation at 313 nm with the light energy of $3.8 \cdot 10^9$ E/s.

vones, Fig. 1. Thus, the emission in the mentioned spectral range should be assigned to some destruction products of the compounds under study formed during measurements of the fluorescence spectra.

The emission band at 485–495 nm (Table 1) is observed in the spectra of isoflavones *I*, *III* and *IV* in the acidity range 12–0 of pH units, Fig. 2a. Since this band is found to be characteristic only for spectra of the isoflavones containing the hydroxy group, and also it is the most intense in solutions with the high pH values, we assigned this band to the anionic form. The acidity range where the anion absorption is observed is found to be significantly lower than the pH range where its fluorescence is detected, Fig. 3a and 3b. This points to an essential decrease of the hydroxy group acidity in isoflavones upon the electronic excitation that might be caused by significant differences between the electronic structure of anions in the ground and the excited states. The anomalous high Stokes shifts of the fluorescence ($8400\text{--}8900$ cm^{-1}) is also a confirmation of the essential changes of the electronic structure of the anion upon excitation. At the same time, the spectral characteristics of the anions of all the studied isoflavones, their radiative and nonradiative deactivation rate constants are found to be very similar, independently on the substituent nature in both molecular moieties. It should also be noted that the emission bands of the anion in the neutral and acidic solutions are characterized by a larger half-width than in solutions with higher pH values. The difference of the half-width values varies from 340 cm^{-1} (*I*)

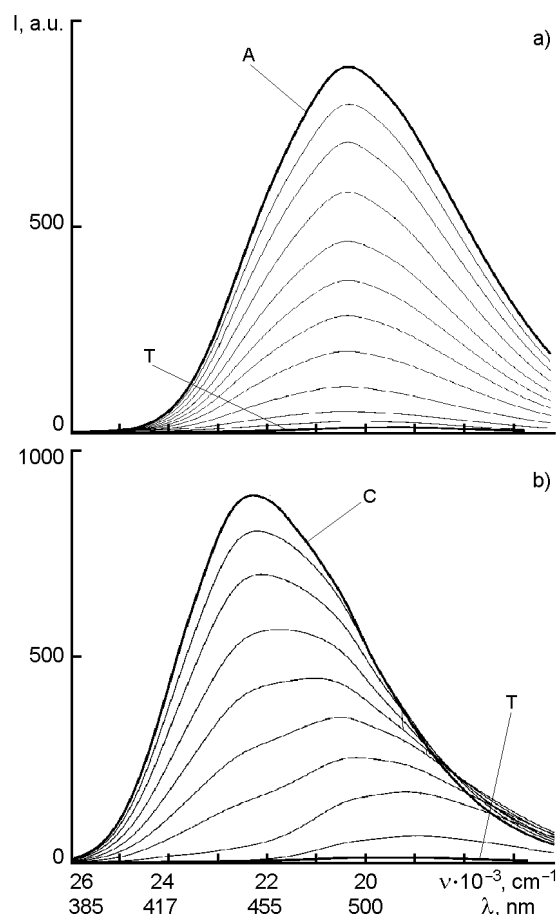


Fig. 2. Fluorescence spectra of isoflavone *I* in the acidity range from pH = 12.0 to 0.8 (a), from pH = 0.8 to $H_0 = -5.1$ (b).

to 430 cm^{-1} (*III*). We interpret such a broadening as being due to changes in the nature of intramolecular interaction between the anion and solvent molecules.

In the high acidity region (H_0 varying from -7.1 to 0), the absorption and emission bands of the cationic form are observed in electronic spectra of isoflavones, Fig. 2b. The fluorescence band maxima of isoflavylium are found to be in the range $440\text{--}465\text{ nm}$; the fluorescence Stokes shift amounts 5900 to 6400 cm^{-1} . The Stokes shift values for the cation are lower than those of the anionic form, thus pointing to a smaller energy loss needed for structural and solvation relaxations of the cations in the excited-state. The absorption and emission bands of the cations appear approximately in the same H_0 range, Fig. 3a and 3b. The fluorescence intensity of isoflavylium cations depends strongly on the nature of substituents in the side phenyl ring. So, introduction of a methoxy group into $4'$ po-

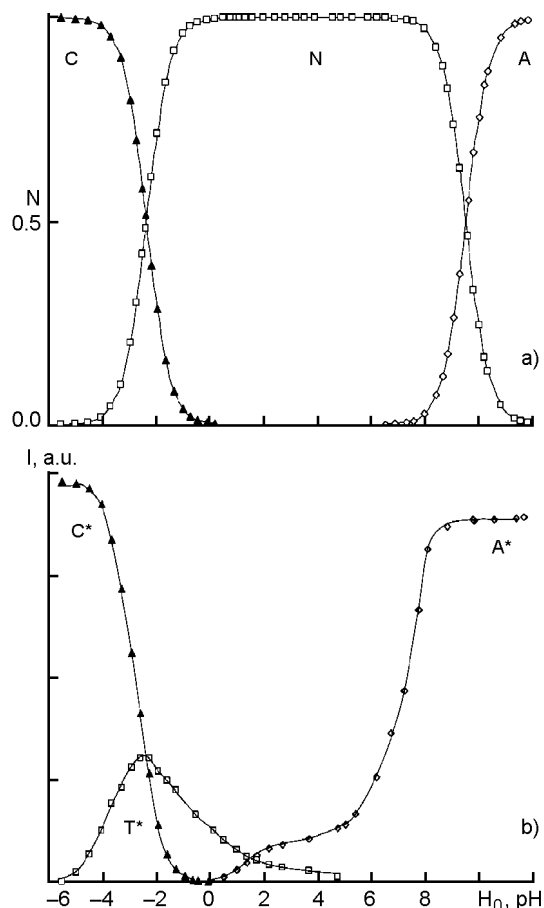


Fig. 3. Acid-base forms of isoflavones *I* in the ground (a) and the excited (b) states. The yields of the forms in the ground state are given in the mole fractions (n), whereas in the excited state, in terms of the relative fluorescence intensity. *C* and C^* are the cationic forms, *A* and A^* are the anionic forms, *N* — the neutral form, T^* — phototautomer.

sition results in a significant increase of the nonradiative deactivation rate constant and in a decrease of the emission intensity. The changes in spectral properties due to introduction of a donor substituent can, in our opinion, be explained by the fact that in the case of flavylium and isoflavylium cations, the electronic transition to S_1 state is accompanied by some charge transfer between the phenyl and benzopyrilium moieties [8]. In the presence of donor fragments in the side phenyl ring as well as a result of the nucleophilic solvation by solvent [18], the significant increase of the interfragment charge transfer occurs that causes the formation of a non-fluorescent or a weakly fluorescent TICT-conformation of the cation [19].

Table 2. Spectral properties of isoflavones*

	ν_{abs} , cm^{-1}	λ_{abs} , nm	ν_{fl} , cm^{-1}	λ_{fl} , nm	$\Delta\nu_{St}$, cm^{-1}	ϕ , %	τ , ns	k_f , s^{-1}	k_d , s^{-1}
neutral form (absorption), phototautomer (fluorescence)**									
<i>I</i>	32480	308	18900	529	13580	4.3	0.9	$4.8 \cdot 10^7$	$1.1 \cdot 10^9$
<i>II</i>	32600	307	–	–	–	–	–	–	–
<i>III</i>	33000	303	16030	624	16030	0.2	~0.4	$\sim 5.0 \cdot 10^6$	$\sim 2.5 \cdot 10^9$
<i>IV</i>	32320	309	17920	558	14400	0.2	~0.7	$\sim 2.9 \cdot 10^6$	$\sim 1.4 \cdot 10^9$
<i>V</i>	32420	308	–	–	–	–	–	–	–
<i>VI</i>	32770	305	–	–	–	–	–	–	–
<i>VII</i>	32060	312	–	–	–	–	–	–	–
anion									
<i>I</i>	28740	348	20320	492	8420	12.1	2.2	$5.5 \cdot 10^7$	4,0108
<i>III</i>	29360	341	20480	488	8880	17.5	3.4	$5.2 \cdot 10^7$	$2.4 \cdot 10^8$
<i>IV</i>	28740	348	20380	491	8360	18.4	1.7	1.1·108	$4.8 \cdot 10^8$
<i>VI</i>	28980	345	–	–	–	–	–	–	–
cation									
<i>I</i>	28680	349	22360	447	6320	15.3	3.8	$4.0 \cdot 10^7$	$2.2 \cdot 10^8$
<i>II</i>	28740	348	22740	440	6000	11.4	3.3	$3.5 \cdot 10^7$	$2.7 \cdot 10^8$
<i>III</i>	28000	357	21600	463	6400	0.1	~4.8	$< 1 \cdot 10^6$	$\sim 2.1 \cdot 10^8$
<i>IV</i>	27400	365	23260	430	4140	< 0.1	~2.2	$< 1 \cdot 10^6$	$\sim 4.5 \cdot 10^8$
<i>V</i>	27920	358	22620	442	5300	< 0.1	weak fluorescence		
<i>VI</i>	29220	342	–	–	–	–	–	–	–
<i>VII</i>	28660	349	–	–	–	–	–	–	–

—* ν_{abs} , ν_{fl} , λ_{abs} , λ_{fl} — positions of absorption and emission maxima given in wave number and wavelength units, $\Delta\nu_{St}$ — fluorescence Stokes shift, ϕ — fluorescence quantum yield, τ — fluorescence lifetime of a molecule in the excited state, k_f , k_d — radiative and non-radiative deactivation rate constants of isoflavone forms in the excited state.

—** since the rate constant of the phototautomer formation in the excited state is unknown, uncorrected values of its spectral and kinetic parameters ϕ , k_f and k_d are given in the Table.

In order to explain the excited state behavior of the anionic and cationic forms of isoflavones, we have estimated the acid-base equilibrium constant values using the Foerster method, Table 2. Since the neutral form has no fluorescence, the maxima of the long-wavelength absorption band of the neutral molecule, cation and anion of isoflavones were used to evaluate the corresponding rate constants instead of the energy of purely electronic transition ν_{0-0} [20]. As it is seen from the presented data, the pK_a values of isoflavones are decreased by approximately eight orders of magnitude, and as a consequence, the estimated pK_a^* values are found to be within 1–2. The strong increase of the acidity of isoflavones in the excited state causes the photodissociation thereof and explains the appearance of the

anionic form fluorescence of the studied compounds in acidic media up to H_0 equal to -0.5 (calculated value). Similar estimations of the basicity for isoflavones in the excited state predict the increase of pK_b by seven to ten orders of magnitude up to the values of 3.5 – 10.5 . Thus, inversion of the pK_a^* and pK_b^* values is observed. This allows evaluating the pH range where the zwitterionic form or a phototautomer T^* will exist.

Consideration of the experimental fluorescence spectra has allowed to distinguish in the acidity range from pH of 5 to H_0 of -2 the emission bands located in the 530 – 630 nm range, the positions of which depend essentially on the nature of substituents in both chromone and phenyl molecular fragments of isoflavones. The fluorescence Stokes shift with respect to the long-

wavelength absorption band of the neutral form varies from 13500 to 17000 cm^{-1} , that points unambiguously to the occurrence of a chemical process in the excited state and allows assigning the observed band to tautomeric form. The fluorescence excitation spectra of isoflavones, recorded in solutions with pH values at which the phototautomer dominates in the excited state, correspond to the neutral form absorption spectra. Thus, the precursor of the phototautomer in the excited state is the neutral form, rather than the anion or the cation. These latter participate as intermediates during the formation of the tautomer from the neutral form in the excited state.

Thus, the presented study has shown that three fluorescence bands are observed in the fluorescence spectra of isoflavones, that are assigned to the emission of anionic, cationic and phototautomeric forms. When the 7-hydroxyl group is absent, the emission of only one, cationic form is observed in acidic media. The fluorescence bands in the range 400–430 nm have been assigned to the emission of photodestruction products of the studied compounds. The assignment of the emission bands to certain acid-base forms of isoflavones and the establishment of a dependence of the fluorescence properties of isoflavones on the structure and the medium acidity can be used for making new fluorescent labels based on both 3-phenylchromone and its heterocyclic analogues.

References

1. A.L.Kazakov, V.P.Khilya, V.V.Mezherickiy, Yu.Litkei, Natural and Modified Isoflavonoids, Rostov, Rostov University (1985) [in Russian].
2. K.D.Ahn, D.K.Han, J.Y.Kim et al., *J. Photopolymer Sci. Technol.*, **14**(3), 475 (2001).
3. O.Wolfbeis, A.Knierzinger, *Z. Naturforsch.*, **24a**, 510 (1979).
4. A.D.Roshal, A.A.Sukhorukov, V.D.Orlov, A.O.Ponomariov, *Functional Materials*, **4**, 114 (1997).
5. A.D.Roshal, A.A.Sukhorukov, V.D.Orlov, A.O.Ponomariov, *Functional Materials*, **4**, 121 (1997).
6. C.L.Dunford, G.J.Smith, E.E.Swinny, K.R.Markham, *Photochem. Photobiol. Sci.*, **2**, 611 (2003).
7. E. de Rijke, H.C.Joshi, H.R.Sanderse et al., *Anal. Chim. Acta*, **468**, 3 (2002).
8. A.D.Roshal, A.V.Grigorovich, D.Yu.Minayev, S.I.Egorova, *Functional Materials*, **4**, 584 (1997).
9. O.S.Wolfbeis, E.Furlinger, H.C.Yha, F.Zilliken, *Z. Naturforsch. B. Anorg. Chem. Org. Chem.*, **39**, 238 (1984).
10. V.P.Khilya, M.S.Lukiyanchikov, A.L.Kazakov, N.B.Gorbuleiko, *Ukr. Khim. Zh.*, **50**, 1301 (1984).
11. O.A.Ponomaryov, A.O.Doroshenko, V.G.Mitina, *Khim. Fizika*, **8**, 1369 (1989).
12. A.Vaisberg, E.Proskauer, J.Piddik, E.Tups, *Organicheskie Rastvoriteli (Organic Solvents)*, Moscow, Int. Lit. (1958), p.522.
13. A.O.Doroshenko, "Spectral Data Lab", Program Package, Research Institute of Chemistry at Kharkov National University (1999).
14. A.A.Efimov, R.N.Nurmukhametov, A.I.Tolmachev, *Opt. Spekt.*, **29**, 11 (1970).
15. R.N.Nurmukhametov, V.G.Tischenko, *Opt. Spekt.*, **23**, 83 (1967).
16. E.Lippert, *Z. Phys. Chem (N.F.)*, **2**, 328 (1954).
17. A.A.Efimov, R.N.Nurmukhametov, A.I.Tolmachev, *Opt. Spekt.*, **30**, 622 (1971).
18. A.D.Roshal, D.Y.Minayev, V.L.Koval, A.I.Novikov, *Opt. Spectr.*, **85**, 705 (1998).
19. A.D.Roshal, *Kharkov Univ. Bull. Chem. Sci.*, **4**, 125 (1999).
20. I.Yu.Martynov, A.B.Demyashkevich, B.M.Uzhinov, M.G.Kuz'min, *Usp. Khimii*, **46**, 3 (1977).

Флуоресцентні властивості ізофлавонів: залежність від кислотності середовища

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Досліджено спектри флуоресценції ізофлавонів в інтервалі pH/H_0 від 12 до -10 . Проведено віднесення смуг випромінювання, що спостерігаються, до кислотно-основних форм ізофлавонів у збудженому стані, визначено інтервали pH/H_0 існування кожної з форм у розчинах. В розчинах 7-метоксиізофлавонів знайдено флуоресценцію тільки однієї — катіонної форми. Для 7-гідроксиізофлавонів зареєстровано флуоресценцію катіонної, аніонної та фототаутомерної форм. Смуга випромінювання в області 400–420 нм, що є характерною для всіх ізофлавонів, віднесена до продуктів їх фоторозкладу. Розраховано константи дисоціації та протонування ізофлавонів в основному та збудженому станах.