Flavonols and Crown-Flavonols as Metal Cation Chelators. The Different Nature of Ba^{2+} and Mg^{2+} Complexes

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The derivatives of 3-hydroxyflavone exhibit excited-state intramolecular proton transfer (ESIPT) reaction with significant (60−80 nm) shifts of fluorescence spectra between normal and phototautomer forms. This fact makes these compounds attractive as fluorescence probes in analytical chemistry, biophysics, and molecular biology. Different flavonol derivatives, including 4′-(monoaza-15-crown-5)flavonol, were synthesized, and their absorption and fluorescent spectra were studied in acetonitrile in the presence of different concentrations of Mg^{2+} and Ba^{2+} ions. It was shown that the general feature of flavonols is the ability to form two types of complexes with alkaline-earth cations: the low-stability "external" and high-stability chelating complexes. On the formation of the complexes, parent flavonols and their 4′-dialkylamino derivatives undergo different perturbations of their electronic structures. 4′-(Monoaza-15-crown-5)flavonol forms two types of complexes with both Mg^{2+} and Ba^{2+} ions; the sequence of steps in formation of Ba^{2+} and Mg^{2+} complexes is different.

Introduction

The derivatives of 3-hydroxyflavone recently have attracted the attention of many researchers because of their specific spectroscopic properties. They exhibit excited-state intramolecular proton transfer (ESIPT) reaction,[1−3] which is accompanied by a very significant Stokes shift of about 9000 cm^{-1}. The observation of two forms of emission, normal (NF) and proton transfer (PT), separated by 60−80 nm and of variation of relative intensities of these forms under the influence of different factors makes these compounds very attractive fluorescence probes for analytical chemistry, biophysics, and cellular biology. Thus, the ratio of intensities of these forms exhibits strong dependence on solvent polarity[4] and hydrogen bonding with the solvent molecules.[2,4,5] The sensitivity of fluorescence spectra of flavonols to the properties of their environment suggests their use as the probes for formation of micelles[6,7] and as fluorochromic indicators for Sc^{3+}, Ga^{3+}, and Th^{3+} ions.[8]

The most prospective in this respect are 4′-dialkylaminoflavonols.[4,9] Their normal form of emission demonstrates significant charge-transfer character. Being very sensitive to perturbation by a dielectric environment, these and related aminoflavonols may serve the basis for creation of fluorescent indicators with high sensitivity and selectivity. The demand for compounds with such properties exists, in particular, in biochemical studies, where the nanomolar (10^{-7} M) concentrations of Ca^{2+} ions have to be determined in the presence of millimolar (10^{-3} M) concentrations of Mg^{2+} ions.

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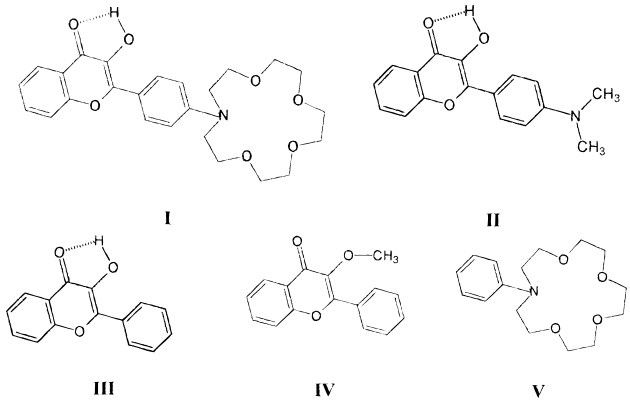
SCHEME 1



Recently we synthesized 4′-(15-azacrown-5)flavonol (**I**)[10,11] (see structure in Scheme 1), which has the prospect of highly selective binding with cations of different charges, radii, and electronic properties and which changes dramatically its properties on complex formation. This molecule possesses two ion chelator sites, which are coupled with the system of conjugated double bonds of the chromophore system: ortho-groups, hydroxyl 3-OH and carbonyl 4-C═O form one site, and a crown-macrocycle at another side of molecule may serve as the second site. It is known that phenolic ortho-groups have a high affinity to multivalent cations of small radii, such as Mg^{2+}, Al^{3+}, or Fe^{3+}, while the 15-member crown macrocycles have the highest affinity to alkaline and alkaline-earth metals of medium radius: Na^+, K^+, Ca^{2+}, and Sr^{2+}.[12] Since one of the chelation sites is situated close to the negative, and the other to the positive end of molecular dipole, the complex formation is expected to result in different, probably opposite, effects on absorption and

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fluorescence spectra of this flavonol. An additional effect, the disappearance of the PT fluorescence band, should be observed on substitution by a metal cation of a proton belonging to the hydrophylic group 3-OH.

In the present study we investigated the absorption and fluorescence spectra of crown-flavonol **I** in acetonitrile in the presence of different concentrations of ions Mg^{2+} and Ba^{2+}. These cations substantially differ by their ionic radius r ($r_{cryst}(Ba^{2+})/r_{cryst}(Mg^{2+}) \approx 1.9-2.2$[13]) and also by their ionization potential and electron affinity. The last property determines the difference in their electron-acceptor abilities and their abilities to polarize the π-electronic system of the chromophore. To understand in detail the processes that occur on complex formation of flavonol **I**, we studied also the interaction with the above-mentioned cations of other compounds which contain ion-binding centers of similar type: flavonols **II**−**IV** and phenyl-15-azacrown-5 (**V**). Since the spectra of flavonols are very sensitive to the presence of proton-donor impurities in the solvent, we studied also the influence of the addition of water on fluorescence spectra of flavonols **I**−**IV** in acetonitrile. Our results demonstrate that ion binding to flavonol **I** proceeds in two steps, and the sequence of these steps is not the same in the cases of Mg^{2+} and Ba^{2+} ions.

Experimental Section

Flavonol Synthesis. The flavonols **I**−**IV** have been synthesized from 2-hydroxyacetophenone and the corresponding benzaldehydes by the Algar−Flynn−Oyamada reaction[14,15] and purified by means of repeated recrystallization or column chromatography. All flavonols were homogeneous according to thin-layer chromatography (TLC) on Silufol UV-254 in chloroform−methanol (95:5, 9:1, or 85:15, v/v). Their structures have been confirmed by quantitative elemental analysis, proton magnetic resonance (PMR), and UV−visible and infrared (IR) spectrometry (see the next subsection). Compound **V**, 98% purity, was purchased from TCI American, Inc. (Portland, OR).

Spectroscopic Confirmation of Structure. PMR spectra were recorded on a Bruker WP-100 Fourier spectrometer at room temperature with tetramethylsilane as internal standard. The data presented below are on the δ-scale and ordered as follows: signal position in ppm, form of signal, (coupling constant in Hz), number of protons, and (their position in the molecule). Absorption spectra were recorded on a Hitachi U3210 instrument on 2×10^{-5} M solutions of flavonols in ethanol or in 0.01 M Tris buffer, pH 7.4. Infrared (IR) spectra were recorded in a Pye Unicam SP3-300 instrument in pellets of KBr. Data presented are in cm^{-1}.

*3-hydroxy-4′-N-(15-azacrown-5)flavone (**I**).* PMR spectrum in $CDCl_3$: 8.98s, 1H(OH-3); 8.25m, 1H(H-5); 7.38m, 1H(H-6); 7.63m, 1H(H-7); 7.55m, 1H(H-8); 8.17d(J=9), 2H(H-2′,6′); 6.80d(J=9), 2H(H-3′,5′); 3.68m, 20H(CH2−CH2). IR spectrum: 1090, 1120, 1170 − ν(C−O); 1580 − ν(C═O); 1505 − ν(C═C); 2860, 2920 − $\nu(CH_2)$. Absorption spectrum in ethanol: $\lambda_{max} = 408$ nm ($\epsilon = 39\,900$ $dm^3\ mol^{-1}\ cm^{-1}$).

*3-Hydroxy-4′-dimethylaminoflavone (**II**).* PMR spectrum in DMSO: 9.16s, 1H(OH-3); 8.08dd(J_1=8;J_2=2), 1H(H-5); 7.66−7.77m, 2H(H-6,7); 7.43m, 1H(H-8); 8.13d(J=9), 2H(H-2′,6′); 6.85d(J=9), 2H(H-3′,5′); 3.00c, 6H(NCH_3-4′). Absorption spectrum in ethanol: $\lambda_{max} = 405$ nm ($\epsilon = 39\,000$ $dm^3\ mol^{-1}\ cm^{-1}$).

Basic physical−chemical characteristics of flavonols **II**−**IV** correspond to those presented previously.[14,16]

Other Chemical Procedures. Acetonitrile, which was used as a solvent, was additionally purified, as described elsewhere,[17] because the 3-hydroxyflavone derivatives are sensitive to the presence of water. In the studies of complex formation we used the anhydrous barium perchlorate from Aldrich and dehydrated magnesium perchlorate obtained by roasting of trihydrate sample at 215 °C and 0.15 mmHg pressure during 3 h.[18] The quality of dehydration was controlled gravimetrically.

Spectroscopic Measurements. Absorption spectra were measured on a Hitachi U-3210 spectrophotometer. Fluorescence spectra were measured on a Hitachi F-4010 spectrofluorimeter at 20.0 ± 0.1 °C.

Ligand Concentrations, Methods of Titration. Affinity constants K_s and stoichiometric composition of the complexes were calculated from absorption spectra by iteration methods analogous to those described.[19,20]

Titrations were usually made by addition of small amounts of acetonitrile solution of investigated flavonol (1×10^{-5} to 5×10^{-5} mol/L) and alkali-earth metal perchlorate (2 mol/L) to the solution of the same flavonol in an initial concentration.

In the case of two complexes with similar K_s the dependency of extinction on metal concentration for one of the components was measured at isosbestic points produced by titration by the other component.[21,22]

Quantum-Chemical Calculations. Electronic structure of flavonols and their complexes with Mg^{2+} was calculated in full valent semiempirical approximation by the method PM3.[23] The degree of redistribution of electronic density between different fragments of the studied molecules on transition from the ground to the excited state (ΔQ) was determined as the difference of total charges on atoms which compose these fragments in the S_0 and S_1 states:

$$\Delta Q = \sum q_i S_1 - \sum q_i S_0$$

where q_i are the charges on atoms that are included in the particular fragment.

Results and Discussion

The spectroscopic properties of flavonol derivatives depend significantly on intramolecular hydrogen bonding in their molecules. On transition to the excited state, the basicity of the carbonyl group and the acidity of the hydroxy group increase. This fact favors the proton-transfer reaction, so the fluorescence spectra of flavonols consist of two well-resolved emission bands. The ratio of intensities of these forms depends not only on experimental conditions but also on the structure of the flavonol molecule. Thus in the spectrum of flavonol **III** in acetonitrile the emission intensity of the phototautomer form is higher by 1−2 orders of magnitude than the intensity of the normal form, while in the emission spectra of dialkylamino derivatives **I** and **II** the fluorescence intensities of the two forms are similar with even somewhat lower phototautomer emission. The latter result may be due to more significant increase of hydroxylic group 3-OH acidity in the excited state of dialkylamino derivatives as a result of the stabilizing influence of π-electron-donor substituents in the 4′ position.

Intermolecular Hydrogen Bonding with Water. In flavonol solutions in both proton-donor and proton-acceptor solvents the intramolecular hydrogen bonds may compete with intermolecular bonds with solvent molecules. This competition hampers the proton-transfer reaction and results in the decrease of phototautomer emission up to its complete disappearance (as it was observed for dimethylaminoflavonol in alcohols[24]). This fact and the possibility of significant influence of trace amounts

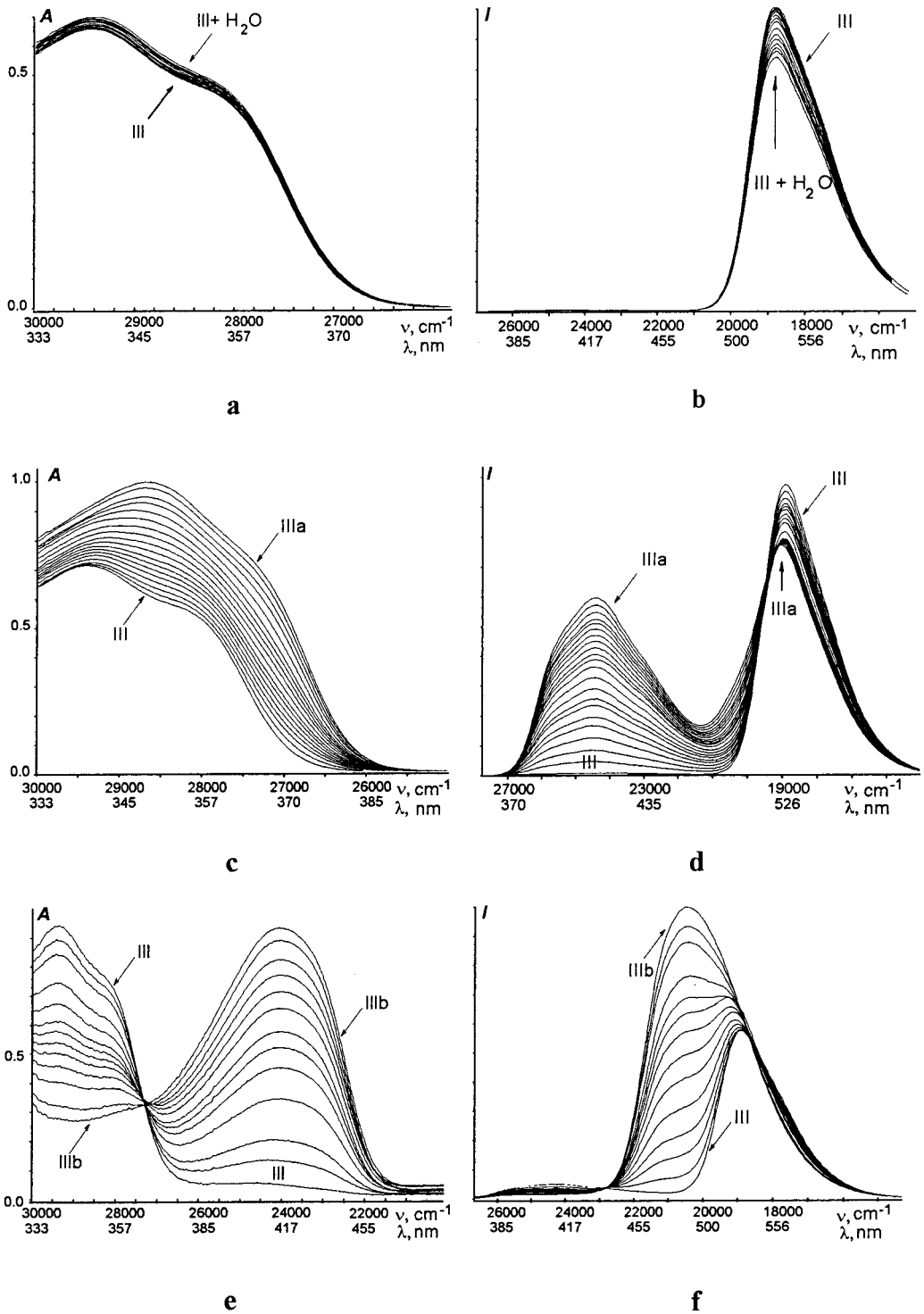


Figure 1. Changes in the absorption (a, c, e) and fluorescence spectra (b, d, f) of flavonol **III** on addition of water (a, b), barium perchlorate (c, d), or magnesium perchlorate (e, f). The symbols representing complexes are the same as those used in Scheme 2.

of water on ion titration results enabled us to perform studies on the effect of water on ESIPT reaction in our compounds.

In Figure 1a,b the absorption and fluorescence spectra of flavonol **III** are presented. The spectra were recorded in acetonitrile with the addition of water in the concentration range from 2.0×10^{-5} to 2.0 mol/L. We observe that opening of intramolecular hydrogen bonds and formation of intermolecular bonds with water molecules do not result in essential changes in absorption spectra: there occurs only a small bathochromic shift ($100-150$ cm^{-1}) and some redistribution of intensities between vibronic components of the long-wavelength absorption band. In the fluorescence spectrum the decrease of intensity of the phototautomer form by only 10−15% is observed. It should be noticed that these changes occur at relatively high water content, and the constant of complex formation is low ($\log K_S = 0.17$). The same observations take place in the experiments with flavonols **I** and **II** ($\log K_S = -1.26$ and -0.76, respectively).

Complex Formation with Ba and Mg Ions. "External" Complexes. On the addition of alkaline-earth cations to flavones **II**−**IV** in acetonitrile the changes in their absorption and fluorescence spectra are more noticeable (Figure 1c−f), and

TABLE 1: Spectroscopic Properties of Flavonols I−IV with Mg^{2+} and Ba^{2+} Cations

		absorption spectra			fluorescence spectra			
complex	ion	λ, nm	ν, cm^{-1}	log ϵ	λ, nm	ν, cm^{-1}	$\Delta\nu_{St}$, cm^{-1}	φ, %
I		399	25 060	4.58	522	19 150	5900	4.58
					576*[a]	17 370	7690	3.72
Ic	Ba^{2+}	356	28 080	4.64	522	19 150	5900	4.58
					576*	17 370	7690	3.72
Ica	Ba^{2+}	382	26 180	4.57	535	18 680	7500	46.8
Ib	Mg^{2+}	444	22 600	4.53	538	18 600	4000	79.3
Ibc	Mg^{2+}	419	29 840	4.43	503	19 880	3980	62.4
II		397	25 180	4.53	527	18 980	6200	6.48
					578*	17 300	7880	2.34
IIa	Ba^{2+}	428	23 340	4.73	548	18 220	5120	56.3
IIb	Mg^{2+}	448	22 340	4.59	548	18 240	4100	73.3
III		340	29 400	4.11	406	24 630	4810	0.09
					530*	18 860	10580	4.17
IIIa	Ba^{2+}	350	28 600	4.33	410	24 420	3980	4.23
IIIb	Mg^{2+}	420	23 840	4.11	488	20 480	3360	10.50
IV		296	33 820	4.16				
IVa	Mg^{2+}	300	33 280	4.56				
IVa	Ba^{2+}	304	32 940	4.29				

[a] * = spectroscopic properties of phototautomer.

together with the presence of isosbestic points, they provide evidence for complex formation. The basic spectroscopic properties of flavonols and their complexes are presented in Table 1. The studies of the dependence of absorption and emission spectra on ion concentration allow us to conclude that in all cases the flavonols **II**−**IV** exhibit 1:1 stoichiometry of binding.

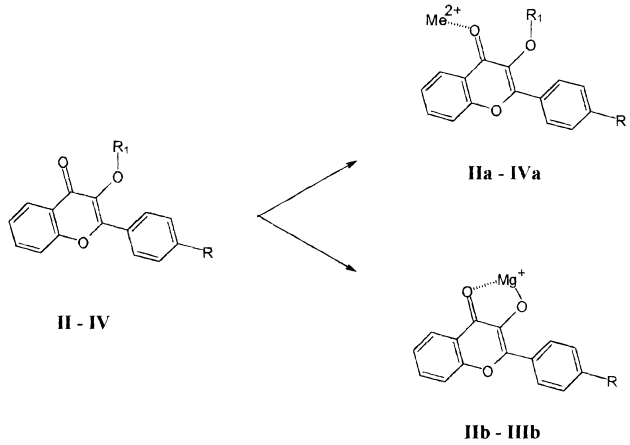
By the character of spectroscopic changes, the complexes with metal cations can be classified into two groups. The complexes of the first type (Figure 1c) are formed on interaction of Ba^{2+} ions with flavonols **II** and **III** and also of Ba^{2+} and Mg^{2+} ions with 3-methoxyflavone **IV**. Long-wavelength bands in their absorption spectra are more intensive than in the case of absence of metal cations. In addition, the small bathochromic shifts are observed both in absorption and in fluorescence spectra. Stokes shifts ($\Delta\nu_{St}$) for complexes of this type are somewhat smaller than those for correspondent-free flavonols **II**−**IV**.

The similarity of spectroscopic properties of the complexes of the first type for flavonols **II**−**III** and 3-methoxyflavone **IV** elucidates the fact that the flavonol 3-OH group does not participate in complex formation. This conclusion is supported by the retention of ESIPT effect (the presence of a long-wavelength phototautomer band) for the barium complex of flavonol **III**. In its fluorescence spectrum the phototautomer band is always present, up to the highest cation concentrations (Figure 1d). Thus it may be suggested that in the complexes of the first type (**IIa**−**IVa**) the proton of the flavonol hydroxylic group is not substituted by a metal cation. The metal cation forms a donor−acceptor linkage with the oxygen electron lone pair belonging to the C═O group (Scheme 2).

The data presented in Table 1 demonstrate that the fluorescence spectroscopic properties of the complexes of the first type are similar to the properties of flavonols without cations. Therefore we can suggest that in this case the cation binding does not cause essential changes in the flavonol electronic structure. The stability of the barium complexes depends very little on the nature of the substituent in flavonol at positions 3 and 4′. Whether it is a hydroxy group or a methoxy group in position 3, log K_S is within the range 0.72−0.89 (Table 2).

Thus, the 3-hydroxy group does not participate in the formation of complexes of the first type (Scheme 2). The metal cation forms a donor−acceptor bond with the "external" (not participating in the formation of intramolecular hydrogen bond)

SCHEME 2



electron pair of the oxygen atom of the flavonol carbonyl group. Since this electron pair is located in the plane of the molecule, the contribution of the π-electronic system to the cation binding is minimal. This explains both a small influence of the π-electronic system on complex stability and the relatively weak polarizing effect of ion charge on this system, which results in only small electrochromic effects in absorption and fluorescence spectra.

Chelating Complexes of 3-Hydroxyflavones. In contrast to the data presented above, interaction of flavonols **II** and **III** with Mg^{2+} cations results in significant bathochromic shifts of both absorption and emission spectra (Figure 1e,f). The complexes **Ib**−**IIIb**, which are formed in this case, may be of different origin. They are observed only for 3-hydroxy-, but not for 3-methoxyflavones. Their stability constant is higher by 1 order of magnitude than that for the "external" complexes and depends on the substituent in the flavonol phenyl ring (Table 2). On this complexation the tautomer fluorescence band disappears completely. Thus, we can suggest that the flavonol hydroxylic group participates in the formation of **Ib**−**IIIb** complexes.

For these complexes the $\Delta\nu_{St}$ values are approximately 1.5 times smaller than for the corresponding structures **IIa**−**IVa**. This fact points to the formation of a complex with more rigid molecular structure, whose conformation and interaction with the solvent do not change substantially in the excited state compared with the ground state.

TABLE 2: Logarithm of Stability Constants (K_S) of Flavonols I−IV with Ba^{2+} and Mg^{2+} Ions

type of complex	metal ion	I	II	III	IV	V
a	Ba^{2+}		0.72 ± 0.01	0.76 ± 0.10	0.75 ± 0.04	
	Mg^{2+}				0.32 ± 0.03	
b	Mg^{2+}	2.25 ± 0.07	2.07 ± 0.06	1.71 ± 0.02		
c	Ba^{2+}	2.40 ± 0.02				3.57 ± 0.02
	Mg^{2+}					$1.97^{*} \pm 0.09$
ca	Ba^{2+}	0.89 ± 0.04				
bc	Mg^{2+}	0.83 ± 0.06				

TABLE 3: Distribution of Charge on the Fragments and Interfragment Charge Transfer in Flavonol Molecules

	total charge on the molecule	charges on the fragments				transfer of charge, ΔQ (%)
		S_0		S_1		
		phenyl	benzopyrone + ion	phenyl	benzopyrone + ion	
I	0	+0.106	−0.106	+0.233	−0.233	12.7
Ia	+1	+0.207	+0.793	+0.595	+0.406	38.7
III	0	+0.052	−0.052	+0.048	−0.048	0
IIIa	+1	+0.168	+0.832	+0.334	+0.666	16.6

These data allow us to suggest that the complexes of the second type are characterized by cyclic arrangement of chelates with magnesium ions (Scheme 2). Due to its larger size ($r_{Ba^{2+}} = 1.39$ Å, compare with $r_{Mg^{2+}} = 0.60$ Å), the barium cation cannot be located in the cavity between flavonol oxygen atoms of C═O and OH groups (the radius of the cavity is approximated to be 0.80−0.90 Å) and therefore does not form chelates of this type.

The calculations of electronic structures of the complexes are in good agreement with this interpretation of experimental data. According to these calculations, the **IIIb** chelate formation with the Mg^{2+} ions results in substantial decrease of electronic density on the benzopyrone fragment. The distribution of charges on atoms of this bicyclic for the case of chelates in the S_0 and S_1 states is similar to the distribution for 2-phenylbenzopyrilic salts, especially for the salts of 3,4-dihydroxyflavilium, which are obtained on protonation of flavone **III** derivatives in media of high acidity. Thus, the transition $S_0 \rightarrow S_1$ in chelate **IIIb** occurs between the states that are qualitatively different from that of flavonol. According to calculations, the difference in energy of boundary orbitals—HOMO and LUMO in the case of the **IIIb** complex—is less by about 1.5 eV than for the corresponding orbitals of uncomplexed flavonol **III**. This explains the significant bathochromic shift of its long-wavelength absorption band on complex formation.

Introduction of a metal cation results not only in the increase of HOMO energy over that of LUMO but also in delocalization of the occupied orbital over the whole molecule (it is accounted that in the equation for the HOMO wave function there is a substantial contribution not only of the atomic orbitals of benzopyrone cycle atoms, as for flavonols, but also of atomic orbitals of the side phenyl ring). The wave function of the $S_0 \rightarrow S_1$ transition may be described by the single excited configuration $\chi_{1\rightarrow 1'}$. As a result, this transition is associated with rather significant charge transfer from the side phenyl ring to the benzopyrone bicyclic (Table 3).

Analysis of localization of molecular orbitals for 4′-*N*,*N*-dimethylamino derivatives of 3-hydroxyflavone demonstrates that in the presence of an amino group in the phenyl ring, the HOMO is essentially localized on this ring and on the nitrogen atom. The nature of the LUMO does not change: the orbital is localized on the benzopyrone fragment. Thus the presence of a dimethylamino group in flavonols **I** and **II** results in formation of an excited state of charge-transfer character even in the absence of complexation. It can be assumed that the similar nature of the $S_0 \rightarrow S_1$ transition is characteristic also for the complex of the first type, **IIa**, which is close to flavonol **II** by electronic structure.

Complex Formation by Crown-Flavonol. Crown-flavonol **I** and 4′-dimethylaminoflavonol **II** exhibit similar spectroscopic properties in acetonitrile solution. The absorption maximum of **I** is observed at 400 nm, while the fluorescence maxima are at 522 and 576 nm (Table 1). As in the case of **II**, the spectroscopic parameters of **I** are determined to a significant extent by electron donor characteristics of the dialkylamino group.

The presence of small amounts of magnesium ions in acetonitrile solution of crown-flavonol **I** results in the appearance of a long-wavelength absorption band at 444 nm (Figure 2a).

On the grounds of our data obtained for compounds **II**−**IV**, which do not contain a crown group, it can be suggested that flavonol **I** with Mg^{2+} cations initially forms the chelation complex of the type **Ib** with the participation of 3-OH and 4-C═O oxygen atoms (Scheme 3).

On increase of the Mg^{2+} ion concentration, there begins the complex formation with the crown cyclic, which is associated with partial shift of the electron density of the nitrogen atom toward the metal cation. This effect results in a short-wavelength shift of the absorption band up to 25 nm.

Similar behavior was typical for other azacrown ether dyes for which the complexation of the crown cyclic led to a decrease of electron donative ability of the azacrown group and also to a decrease of its influence on the main chromophoric fragment of studied molecules.[25−27] However, complete disconjugation of the nitrogen atom and the π-electronic system, which is observed for protonation in acidic media, does not occur. The absence of such a disconjugation in our case is in line with the observation that the absorption spectra of magnium complexes **Ibc** are situated at longer wavelengths compared with the spectra of protonated flavonol and have a higher intensity.

The absorption spectra of **Ib** and **Ibc** demonstrate also that the binding of the second cation does not disrupt the complex with the first cation. Thus, the complex **Ibc**, which is formed at high Mg^{2+} concentrations, is probably of the type 2:1.

In the fluorescence spectrum of flavonol **I** the increase of Mg^{2+} concentration and the formation of chelating complex **Ib** result in the disappearance of fluorescence emission bands of both its normal form and of the phototautomer. Instead of them a new intensive band appears at intermediate wavelengths. The complex formation by the crown cyclic is revealed at the short-wavelength shift of this band to 503 nm and is followed by a slight decrease of fluorescence intensity (Figure 2b). Since the

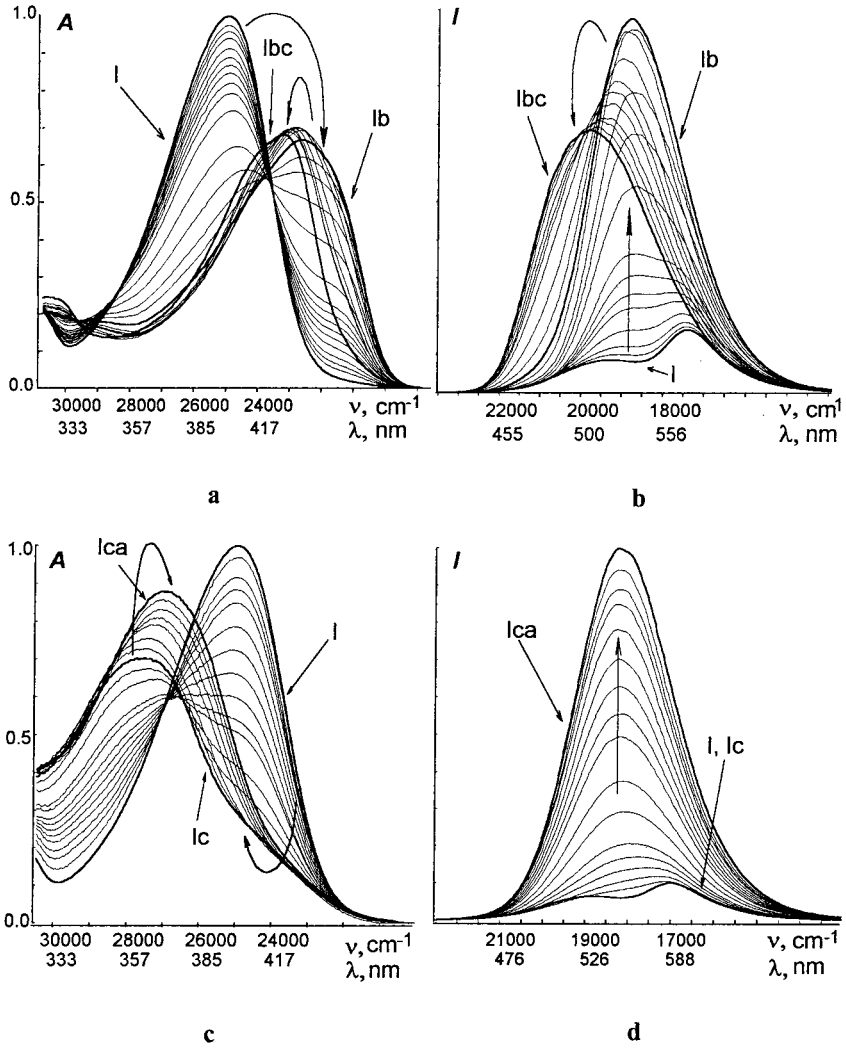


Figure 2. Changes in the absorption (a, c) and fluorescence spectra (b, d) of 4′-(15-azacrown-5)flavonol **I** on addition of magnesium perchlorate (a, b) or barium perchlorate (c, d). The symbols representing complexes are the same as those used in Scheme 3.

emission intensity in this case remains relatively high (by one order higher than that of a free flavonol **I**), it can be suggested that in the excited state the complex **Ibc** does not dissociate and exists with cation−flavonol stoichiometry of 2:1.

The complex formation of flavonol **I** with Ba^{2+} ions differs from the complex formation with Mg^{2+}.

In the absorption spectrum of **I** on increase of concentration of barium ions we observe initially the appearance of a short-wavelength band at 356 nm. This fact suggests that the first barium cation is bound to the crown group and the complex **Ic** is formed.

As in the case of complex **Ibc** formation with the Mg^{2+} ion, we could suppose that the significant hypsochromic shift of the long-wavelength absorption band of the **Ic** complex with barium ion is probably the result of the shift of electronic density from the nitrogen atom to the bound cation.

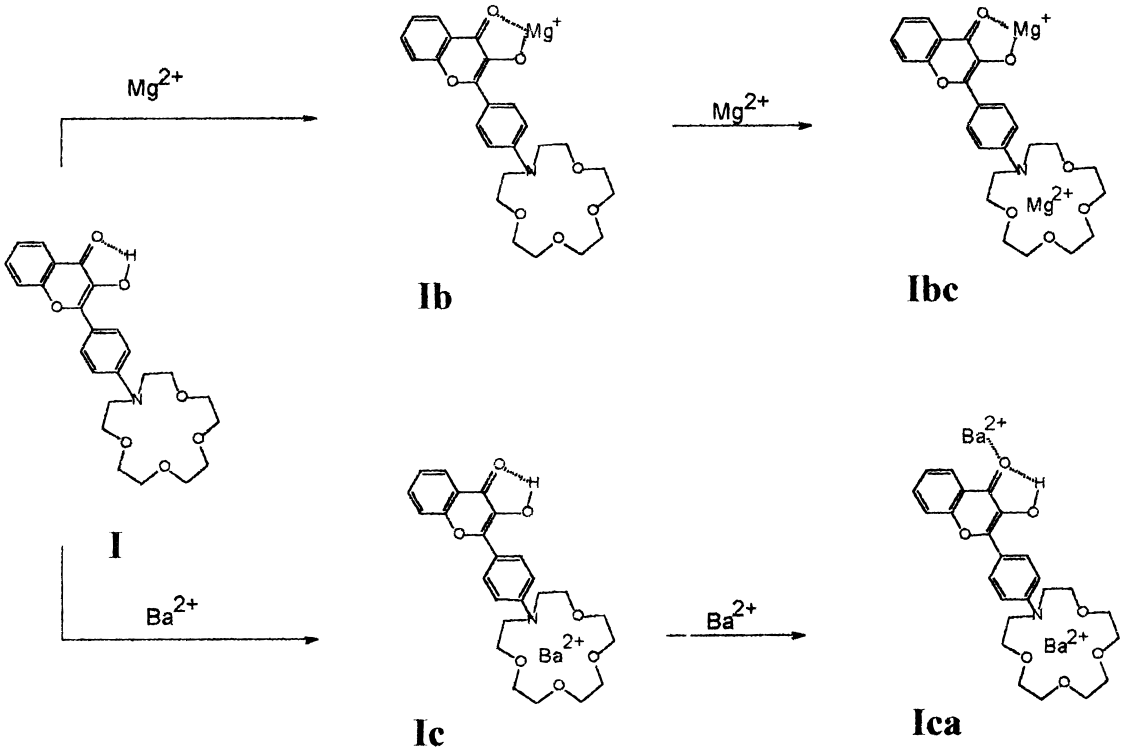
At higher Ba^{2+} ion concentrations the absorption maximum of complex **Ic** shifts to longer wavelength (to 385 nm). In this case the second cation binds to the carbonyl oxygen atom and the complex **Ica** is formed, with 2:1 stoichiometry. Regarding the magnitude of the bathochromic shift of the absorption band (26 nm) and low stability constant of this complex ($\log K_S = 0.89$), it may be suggested that the "external" complex with very small participation of the oxygen atom of the 3-OH hydroxylic group was formed (Scheme 3).

The fluorescence spectrum of the **Ic** complex does not differ from the spectrum of free flavonol **I**. Therefore we may conclude that the complex **Ic** after electronic excitation dissociates, and fluorescence emission occurs from the same S_1 state of flavonol **I**, which is characterized by substantial charge transfer from the side aromatic ring to the benzopyrone ring (Scheme 4). Similar dissociation of the complex in the excited state is observed also for other compounds that possess an azacrown group.[25,26,28]

More significant increase of Ba^{2+} ion concentration in solution results in the disappearance of the bands of flavonol **I** and the appearance of a new band of emission with high intensity and a maximum at 535 nm. In our opinion, this emission band corresponds to an "external" complex, which does not exist in a ground state. This "external" complex is similar in structure to complex IIa with metal−ligand stoichiometry of 1:1. It should be noted that in this case the Ba^{2+} ion is bound with a carbonyl group, whereas the crown-cyclic is free.

The suggestion that flavonol **I** gives a complex similar to **IIa** is based on comparison of decomposition rates of "external" and of crown ether complexes. According to refs 26 and 29, lifetimes of Li^+ and Ca^{2+} complexes with 5-azacrown-15 merocyanine are 2 and 30 ps, respectively. The excited-state lifetime of the "external" complex **IIIa** is 1.8 ns. So, it could be suggested that Ba^{2+} ion ejection from the crown-cyclic is approxi-

SCHEME 3



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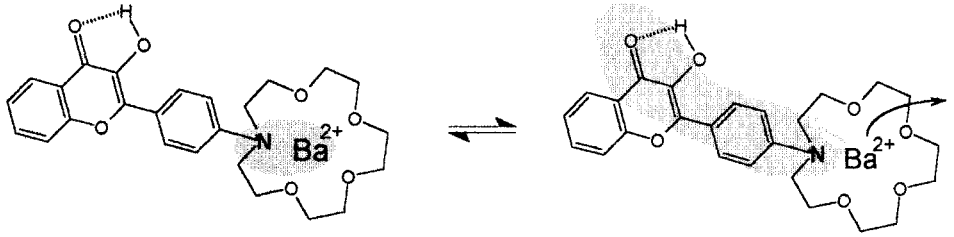

matively 1.5−2 orders faster than decomposition of the "external" complex of Ba^{2+} ion with the flavonol carbonyl group.

The difference in the ways of complexation of flavonol **I** with Mg^{2+} and Ba^{2+} ions (see Scheme 3) is determined not only by the difference between "external" and "internal" complex structures for these metals with 3-hydroxy and carbonyl groups but also by the stability of the crown-complexes formed.

It should be noted that the stability of a barium complex with model phenyl azacrown **V** is much higher than the stability of the corresponding magnesium complex (Table 2). This fact can be explained by better correspondence of the barium ion size than that of the magnesium ion to the diameter of a cavity of the crown cyclic. As a result, the interaction of a barium ion with crown cyclic oxygens is more effective.

The comparison of stability constants of barium complexes of crown-flavonol **I** and model azacrown **V** points out that the electronic state of nitrogen atoms of the crown cyclic influences the strength of complexes with metal cations. Thus, inclusion of an electron-accepting 3-hydroxybenzopyrone fragment into molecule **V** to give flavonol **I** results in a decrease of stability constant with metal cations by more than 1 order of magnitude (Table 2).

As was expected, the Ba^{2+} ion at first forms a more stable complex with the crown-cyclic and then forms a less stable "external" complex. In the case of the Mg^{2+} ion, the chelate complex is more stable and forms first.

Taking into account that "internal" and chelate complex stability nearly does not depend on flavonol type, it could be suggested that for ions of other metals the way of complexation with crown-flavonol would be determined by their ability to form chelating complexes and by the stability of the ion complexes with the crown-cyclic.

Conclusions

Flavonols and akaline-earth cations may form two types of complexes: low-stability "external" and high-stability chelating complexes. "Extenal" complexes are formed only by interaction with a metal cation of the sole electron pair of the oxygen atom belonging to the carbonyl group. In the formation of cyclic chelating complexes both the carbonyl group and the oxygen atom of of 3-hydroxy group participate.

Parent flavonols and 4′-dialkylamino flavonols behave differently on complex formation. In the former case the complex formation at the 3-OH and 4-C═O sites results in considerable perturbation of electronic structure: the charge is transferred substantially from phenyl ring to the chromone part of the molecule. In the case of 4′-dialkylamino flavonols the com-

plexes and free flavonols possess similar electronic composition both in the ground and in the excited states. In the excited state the charge transfer is observed for both free 4′-dialkylamino flavonol and its complexes with metal cations.

4′-(Aza-15-crown-5)-flavonol in acetonitrile forms two types of complexes with Mg^{2+} and Ba^{2+} cations with complex cation−flavonol stoichiometry of 1:1 or 1:2. The sequence of steps in complex formation is different. First the Mg^{2+} ion is bound in a chelating site formed by groups 3-OH and 4-C═O and then is bound by the crown cyclic. In contrast, first the Ba^{2+} ion is bound with the crown cyclic and then forms the complex with the oxygen of carbonyl 4-C═O. Magnesium complexes do not dissociate in the excited state substantially, while for barium ions the ejection of the cation from the crown complex is observed. The opposite sequence of interaction of the coordination centers of crown flavonol with Mg^{2+} and Ba^{2+} ions at complex formation leads to different changes in absorption and fluorescence spectra of this dye.

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