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Complex formation between azacrown derivatives of dibenzylidenecyclopentanone and alkali-earth metal ions

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The complex formation of 2,5-bis[4-(1,7,10,13-tetraoxa-4-azacyclopentadec-4-yl)benzylidene]cyclopentanone and several model compounds, prospective metal-sensitive fluorescent probes, with Mg^{2+}, Ca^{2+}, and Ba^{2+} ions in acetonitrile was studied. The azacrown derivatives of dibenzylidenecyclopentanone have two complex formation centers, azacrown cycle and carbonyl group. The sequence of binding to these sites is different for different ions. The efficient ejection of the Ca^{2+} and Ba^{2+} ions from their complexes with azacoronands was observed in the excited state, whereas in the case of the Mg^{2+} ion, this process occurred only partially.

Key words: bis(azacrown) derivatives of dibenzylidenecyclopentanone, ketocyanine dyes, electronic spectra, sequence of complex formation, fluorescent probes, carbonyl group, crown ethers, Kiprianov effect.

The problem of development of ion-selective fluorescent sensors for medical biological research has become urgent due to an increasing need for studying processes related to a change in the concentration of alkali and alkali-earth metal ions in biological objects.[1,2] Crown-containing organic luminophores are promising in this respect, and their spectral parameters change substantially when a metal binds to the macrocycle.[3—5] The related probes sensitive to the ion concentration allow monitoring of the microparameters of a biological object associated with the polarity of the microenvironment of the probe and also the microelectric parameters (for example, the surface potential of biomembranes related to the concentration of ions in the near-surface layer).[6]

Organic aminocarbonyl compounds have long ago and widely been used as fluorescent probes. Since their molecules contain the electron-donating (alkyl)amino group and electron-withdrawing carbonyl group, the electron density redistribution between these groups during electron excitation results in a considerable increase in the dipole moment in the S_1 state. This determines the observed substantial solvatochromic and solvatofluorochromic properties[7] of similar molecules.

We assumed that symmetric dyes with two similar interacting chromophores and two complex formation centers can provide somewhat higher spectral sensitivity to metal ions. According to the Kiprianov theory,[8] when the chromophoric fragments interact, the long-wavelength absorption band of similar compounds is split to a doublet, whose components are arranged at both sides from the position of this band in the initial monochromophoric compound. Binding of one ion should break the symmetry of the dye molecule. According to the theory of interaction of chromophores, this should result in a noticeable hypsochromic shift of the absorption band. Symmetric bis(azacrown)ketocyanine dyes based on dibenzylidenecyclopentanone are appropriate objects to check this hypothesis. The nitrogen atoms of the azacrown fragments in these compounds participate in conjugation with the π-electron system of the molecule, which suggests a noticeable dependence of the spectral parameters on the coordination of the metal ions with the crown ether groups.

The following compounds were chosen as objects for the study: 2,5-bis[4-(1,7,10,13-tetraoxa-4-azacyclopentadec-4-yl)benzylidene]cyclopentan-1-one (**1**), 2-[4-(1,7,10,13-tetraoxa-4-azacyclopentadec-4-yl)benzylidene]-5-(4-diethylaminobenzylidene)cyclopentan-1-one (**2**), 2,5-bis[(4-dimethylamino)benzylidene]cyclopentan-1-one (**3**), 1,5-bis(4-dimethylaminophenyl)penta-

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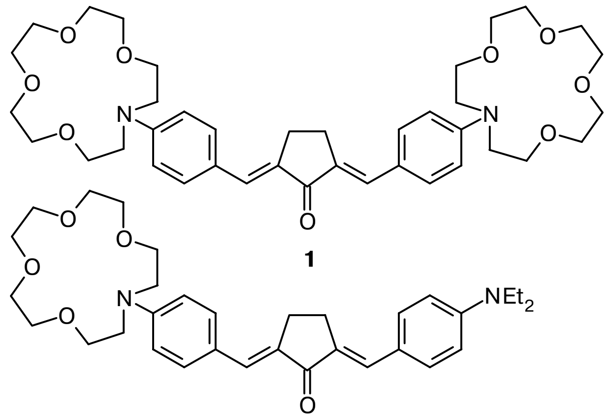
1,4-dien-3-one (**4**), 4,4′-bis(dimethylamino)benzophenone (**5**), and 2-(4-diethylaminobenzylidene)cyclopentan-1-one (**6**).



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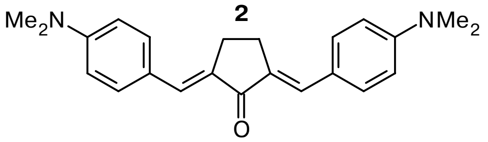

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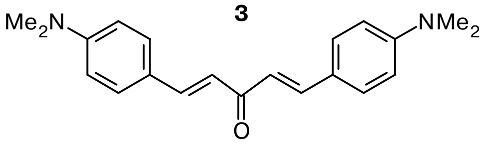

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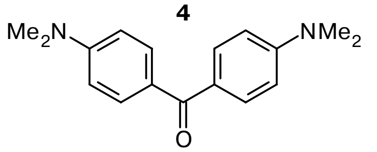

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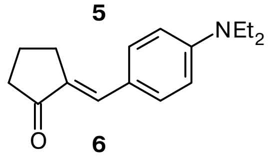

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Experimental

Compounds **1—4** and **6** were synthesized by a general procedure[9,10] that included the condensation of the corresponding *N*-alkylated amino derivatives of benzaldehyde (15-azacrown-5 derivative formally belongs to them) with cyclopentanone or acetone under base catalysis conditions.

15-Azacrown-5-substituted benzaldehyde, which is the semiproduct in the synthesis of compounds **1** and **2**, was synthesized according to a previously described procedure.[11] Commercial reagents (Aldrich) and distilled solvents were used for syntheses. Compound **5** was purchased from Aldrich. The structure of compounds **1—4** and **6** was proved by ^{1}H NMR and IR spectroscopy, and their purity was monitored by TLC on Silufol UV-254 plates 50 × 150 mm in size in the $CHCl_3$—MeOH system with a volume ratio of the components of 98 : 2, 95 : 5, 9 : 1, or 85 : 15. ^{1}H NMR spectra were recorded on a Bruker WP-100 spectrometer at 20 °C using Me_4Si as an internal standard. IR spectra were recorded on a Pye Unicam SP3-300 instrument in pellets with KBr. The physicochemical parameters of the synthesized compounds are presented in Tables 1 and 2.

Table 1. Parameters of synthesized dibenzalcyclopentanone derivatives

Compound	Yield (%)	M.p. /°C	M	$\frac{\text{Found}}{\text{Calculated}}$ (%) N	Molecular formula
1	70	81—82	694.9	4.30 4.03	$C_{39}H_{54}N_2O_9$
2	20	—*	548.7	5.70 5.10	$C_{33}H_{44}N_2O_5$
3	95	>320 (>300)[9]	346.5	—	$C_{23}H_{26}N_2O$
4	80	193—194 (192)[12]	320.4	—	$C_{21}H_{24}N_2O$
6	42	78—80	243.4	—	$C_{16}H_{21}NO$

* Glassy amorphous substance (homogeneous according to the TLC data). The melting point was not determined.

Acetonitrile, a solvent for studying complex formation, was purified and dried using a known procedure.[13] Magnesium, calcium, and barium perchlorates were prepared by calcination of the corresponding crystal hydrates at 215 °C and 0.15 Torr.[14]

Absorption spectra were recorded on a Hitachi U-3210 spectrophotometer. Spectra and quantum yields of fluorescence (φ_f) were obtained on a Hitachi F-4010 spectrofluorimeter relatively to a solution of fluorescein in the carbonate buffer ($\varphi_f = 0.85$)[15] and a solution of quinine in 0.05 *M* sulfuric acid ($\varphi_f = 0.55$).[16] Measurements were carried out in an isothermic cell at 20±0.1 °C.

Complex formation was studied at the same initial concentration of compounds **1—6** (~1—$2 \cdot 10^{-5}$ mol L^{-1}) and in a wide range of concentrations of Mg, Ca, and Ba perchlorates ($1 \cdot 10^{-5}$—1 mol L^{-1}). A low and unchanged concentration of the electroneutral organic ligand allowed us to exclude from consideration the theoretically possible formation of biligand complexes (according to the equilibrium $2\ L + M^{2+} \rightleftharpoons (ML_2)^{2+}$), whose detection, under our experimental conditions, requires the complex formation constant to be higher than 10^{10}—10^{15} mol^{-2} L^2.[17]

The effective complex formation constants (ECC) presented in Table 3 were calculated by the nonlinear least-squares method using the Fletcher—Pawell algorithm in the specially developed program[18] minimizing the sum of squared deviations of the experimental and calculated absorption (*A*) by the formula

$$A = \frac{A_0[M]^3 + A_1K_1[M]^2 + A_2K_1K_2[M] + A_3K_1K_2K_3}{[M]^3 + K_1[M]^2 + K_1K_2[M] + K_1K_2K_3}, \qquad (1)$$

where [M] is the concentration of metal ions; K_1, K_2, and K_3 are the equilibrium constants of the successive addition to the ligand of one, two, and three metal ions, respectively (stability constants of the corresponding M_nL complexes).* A_0—A_3 are the absorbance values of the initial solution containing no metal ions and hypothetical solutions containing only the ML, M_2L, and M_3L species, respectively, at a specified analytical wavelength.[19] For analysis, we chose 20—25 wavelengths within the

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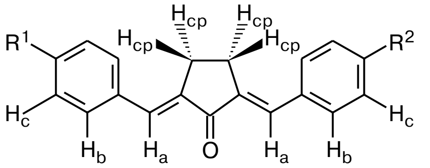
* If two ions were added, the terms containing the K_3 constant were excluded from formula (1); if one ion was added, the terms containing the K_2 and K_3 constants were excluded (as well as the corresponding powers of [M] were canceled out).

Table 2. IR data and ^{1}H NMR spectra of synthesized dibenzalcyclopentanone derivatives



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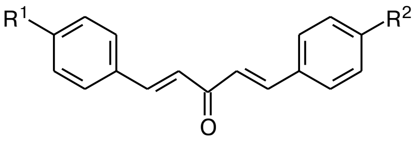

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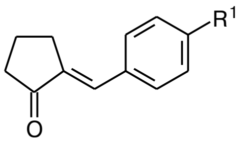

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Compound	R^1, R^2	IR, ν/cm^{-1}	Solvent	^{1}H NMR, δ (J/Hz)				
				H_a	H_b	H_c	H_{cp}	R^1, R^2
1	$R^1 = R^2 =$ = 15-azacrown-5	2850 (ν_{C-H}) 1670 ($\nu_{C=O}$) 1115 (ν_{C-O})	CD_3OD— DMSO-d_6	7.27 (s, 2 H)	7.43 (d, 4 H, J = 8)	6.71 (d, 4 H, J = 8)	3.06 (s, 4 H)	4.22 (m, 8 H); 3.55—3.65 (m, 32 H)
2	R^1 = 15-azacrown-5, $R^2 = NEt_2$	2840 (ν_{C-H}) 1670 ($\nu_{C=O}$) 1122 (ν_{C-O})	DMSO-d_6	7.52 (s, 2 H)	7.52 (dd, 4 H, J = 9, J = 2)	6.72 (dd, 4 H, J = 9, J = 2)	3.06 (s, 4 H)	3.67 (m, 20 H); 3.42 (q, 4 H, J = 6); 1.20 (t, 6 H, J = 6)
3	$R^1 = R^2 = NMe_2$	1665 ($\nu_{C=O}$)	DMSO-d_6	7.31 (s, 2 H)	7.51 (dd, 4 H, J = 9, J = 2)	6.71 (dd, 4 H, J = 9, J = 2)	3.00	3.00 (s, 16 H, H_{cp} + R^1, R^2)
4	$R^1 = R^2 = NMe_2$	—	$CDCl_3$	7.68* (d, 2 H, J = 16)	7.50 (dd, 4 H, J = 9, J = 2)	6.67 (dd, 4 H, J = 9, J = 2)	6.86** (d, 2 H, J = 16)	3.01 (s, 12 H)
6	$R^1 = NEt_2$	—	$CDCl_3$	7.35 (s, 1 H)	7.43 (dd, 2 H, J_1 = 9, J_2 = 2)	6.70 (dd, 2 H, J_1 = 9, J_2 = 2)	2.96 (t, 2 H, J = 7); 2.35 (m, 2 H); 2.00 (t, 2 H, J = 7)	3.41 (q, 4 H, J = 6); 1.20 (t, 6 H, J = 6)

* Olefinic protons in the β-position to the carbonyl group.

** Olefinic protons in the α-position to the carbonyl group.

long-wavelength absorption (or fluorescence) band. The obtained values of stability constants were averaged by the formula of weighted average taking into account mean-square-root errors of the estimated K_i values for each wavelength. The equilibriums involving counterions ($M^{2+} + ClO_4^-$; $LM^{2+} + ClO_4^-$) were ignored in calculation because their detection by spectrophotometric methods is difficult.

Deconvolution of spectra into individual components was effected by the specially developed program, which uses the calculation algorithm of the nonlinear least-squares method and approximates the shape of the individual band by the asymmetric log-normal function similar to that proposed in Ref. 20.

Results and Discussion

The complex formation of compounds **1—6** with the Mg^{2+}, Ca^{2+}, and Ba^{2+} ions was studied in solutions of acetonitrile (Table 3), which provided a sufficient solubility of the reacting components. However, in several cases and especially for compound **1**, complex formation with metal ions does not occur completely because the entire shift of equilibrium toward the formed metallocomplexes requires the concentrations of the used perchlorates exceeding the concentrations of their saturated solutions in acetonitrile. In these cases, extrapolation methods were used to calculate ECC. The spectral parameters of the corresponding complexes were also determined using extrapolation (these values are presented in parentheses in Table 3).

Compounds **1** and **3—5** contain two similar chromophoric fragments, whose interaction[8] results in splitting of the long-wavelength absorption band into a doublet. In compound **2**, these fragments are also related to the same chemotype. The maxima and relative intensities of individual components also depend on the extent of interaction and mutual steric orientation of the chromophoric fragments of the molecule. When the metal ion binds to the nitrogen atom during complex

Table 3. Complex formation of compounds **1—6** with the Mg^{2+}, Ca^{2+}, and Ba^{2+} ions in acetonitrile

Compound	Ion	Type of complex	Absorbance			Fluorescence			
			ν_{abs}/cm^{-1}	ε_a	logK_{abs}	ν_{fl}/cm^{-1}	$\Delta\nu_{ST}$/cm^{-1}	φ_{fl}	logK_{fl}
1	—	—	21440	77000	—	17380	4080	0.330	—
	Mg^{2+}/1	Crown	22080	(71000)	2.77±0.03	—	—	—	3.32±0.09
	Mg^{2+}/2	CO	19040	(58000)	1.17±0.05	15900	3140	0.029	1.82±0.01
	Mg^{2+}/3	Crown	26780	(90000)	0.14±0.08	24400	2380	0.039	1.05±0.01
	Ca^{2+}/1	Crown	22120	(54640)	3.10±0.02	—	—	—	3.67±0.13
	Ca^{2+}/2	Crown	26560	(50360)	2.07±0.02	21860	4700	0.001	2.90±0.01
	Ca^{2+}/3	CO	23800	(55770)	1.56±0.05	16000	7800	0.089	
	Ba^{2+}/1	Crown	22320	(49200)	3.52±0.02	—	—	—	3.53±0.02
	Ba^{2+}/2	Crown	25000	(48100)	1.75±0.04	16030	8950	0.052	1.20±0.03
	Ba^{2+}/3	CO	23000	(49800)	1.02±0.02	~22000	~1000	0.023	
2	—	—	21380	68200	—	17260	4120	0.230	—
	Mg^{2+}/1	Crown	21760	(51700)	2.12±0.04	—	—	—	2.31±0.06
	Mg^{2+}/2	CO	18860	(75900)	1.70±0.06	15650	3210	0.020	1.96±0.03
	Ca^{2+}/1	Crown	21760	(51500)	2.05±0.02	—	—	—	2.85±0.04
	Ca^{2+}/2	CO	19360	(63100)	1.43±0.03	15400	3960	0.020	2.05±0.06
	Ba^{2+}/1	Crown	21760	(52000)	2.83±0.05	—	—	—	2.62±0.02
	Ba^{2+}/2	CO	20080	(69000)	1.35±0.01	15760	4320	0.030	1.52±0.06
3	—	—	21860	65000	—	17220	4640	0.230	—
	Mg^{2+}	CO	18880	(90000)	1.35±0.05	15860	3020	0.022	1.66±0.01
	Ca^{2+}	CO	19040	(80200)	1.87±0.02	15900	3140	0.032	1.80±0.01
	Ba^{2+}	CO	19660	(88600)	1.54±0.03	16140	3520	0.045	1.51±0.01
4	—	—	23000	58700	—	16880	6100	0.200	—
	Mg^{2+}	CO	19600	(77600)	1.30±0.08	15520	4080	0.030	1.85±0.01
	Ca^{2+}	CO	19540	(84000)	1.81±0.02	15540	4000	0.138	2.26±0.01
	Ba^{2+}	CO	20280	(68000)	1.52±0.02	15820	4460	0.060	1.75±0.01
5	—	—	28300	45600	—	17880	10420	0.006	—
	Mg^{2+}	CO	24800	(62000)	0.52±0.02	21440	3370	0.009	—
	Ca^{2+}	CO	24920	(59000)	1.69±0.01	21760	3160	0.001	—
	Ba^{2+}	CO	25740	(51000)	1.14±0.06	22400	3340	0.020	—
6	—	—	25900	39200	—	20520	5380	0.011	—
	Mg^{2+}	CO	22880	(41600)	1.07±0.02	19220	3660	0.007	—
	Ca^{2+}	CO	23340	(35300)	1.20±0.03	19540	3800	0.008	—
	Ba^{2+}	CO	24340	(39300)	0.92±0.03	19300	5040	0.006	—

Note. The molar extinction coefficients obtained by extrapolation with somewhat higher error of determination are presented in parentheses.

formation at the crown group, the splitting mentioned above should disappear* because the parameters of the corresponding chromophoric fragment in **1—5** change substantially. After binding of the metal ion to one of the azacrown groups, the asymmetric absorption band of the dye takes a form of the "regular" nonstructured band with a maximum localized between the maxima of the picked out components of the band of the free (unbound in a complex) ligand (Fig. 1). The disappearance of the Kiprianov splitting of the long-wavelength absorption band was detected for the addition of a proton at the nitrogen atoms of the azacrown cycles/dialkylamino

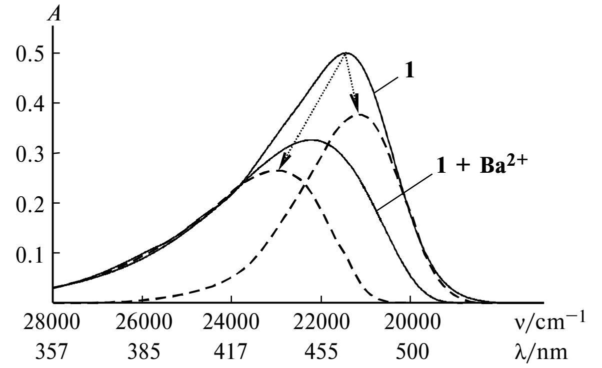

Fig. 1. Disappearance of the Kiprianov splitting in the absorption spectrum when the Ba^{2+} ion is added in MeCN for compound **1** (the spectrum of the complex was measured at the concentration of $Ba(ClO_4)_2$ ~10^{-2} mol L^{-1}). The deconvoluted components of the long-wave absorption band in the spectrum of compound **1** are shown by dotted line.

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* Undoubtedly, the crown cycle contains still more four oxygen atoms, whose unpaired electron pairs participate in the formation of coordination bonds with metal ions. However, their binding to the oxygen centers are manifested to a much lesser extent in the absorption and fluorescence spectra.

groups of the ketocyanines under study.[21,22] We succeeded to identify and determine the close protonation constants of two nitrogen atoms, which differed less than by 1.0—1.2 pK units, only due to an insignificant change (related to this effect) in the shape of the long-wavelength band.[22] Similar changes in the absorption spectra were also observed for complex formation with the metal ions studied in this work.

First we expected that changes in the absorption and fluorescence spectra appear only for azacoronands **1** and **2**, whose molecules bear rather efficient centers of complex formation. However, it turned out that all studied compounds in acetonitrile are capable of coordinating ions of alkali-earth metals.

In the case of compounds **3—6**, the addition of the salts listed above to a solution of the dye resulted in the long-wavelength shift of the absorption spectra, being 2000—4000 cm^{-1}. The difference in the shift value due to the interaction with the Mg^{2+}, Ca^{2+}, and Ba^{2+} ions ranged from 500 to 2000 cm^{-1}, which is probably related to a decrease in their effective ion radii in the series Ba^{2+} < Ca^{2+} < Mg^{2+}.[23] This determines, most likely, a more substantial influence of the magnesium ions on the spectral parameters of the studied ketocyanines.

The observed changes in the spectra of **3—6** with an increase in the concentration of alkali-earth metal ions can be interpreted as a result of the formation of donor-acceptor complexes involving lone electron pairs of the oxygen atom of the carbonyl group. In our opinion, there is an analogy between complex formation with the metal ions described in this work and the formation of complexes with the hydrogen bond, for example, in alcohol solutions of the studied compounds. In the last case, some long-wavelength shift of the absorption and fluorescence spectra is also observed as compared to that of a solution in MeCN. This shift exceeds insignificantly 1000 cm^{-1} and is accompanied by a 1.5—2-fold decrease in the quantum yield of fluorescence. For the formation of donor-acceptor complexes with the alkali-earth metal ions, φ_f decreases 8—10-fold. In the most cases, complex formation is accompanied by a decrease in the Stokes shift of fluorescence, which indicates somewhat greater rigidity of the structure of the excited complexes compared to the free ligands.

Compounds **3—6** exhibit the addition of only one metal ion to one ligand molecule, and the formation of some other complexes (for example, biligand complexes) was not detected. Based on the general views about the properties of the carbonyl group, we could not expect a high efficiency of the complex formation of this group with the alkali-earth metal ions. Indeed, the stability constants of similar complexes of compounds **3—6** estimated by us were rather low ($\log K \approx 1.0$—2.0).

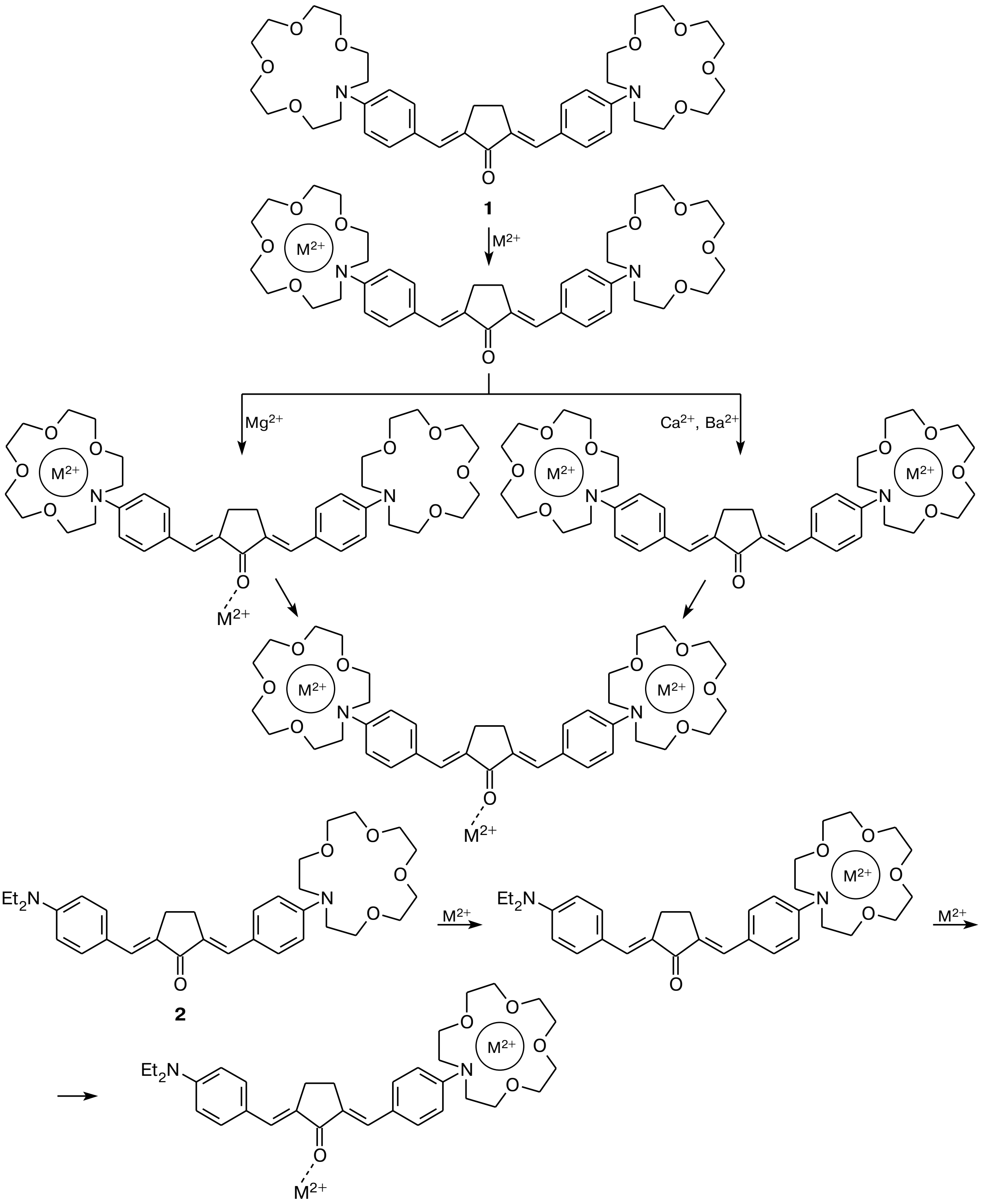
Somewhat higher efficiency of complex formation with the Ba^{2+} and Ca^{2+} ions than that with Mg^{2+} is unexpected to some extent because a stronger interaction of Mg^{2+} with the ligand can be concluded from more substantial changes in its spectral parameters. It is most probably that the degree of changing the spectrum of the ligand during complex formation depends mainly on the surface charge density at the cation and on the distance between the central ion and ligand, which are finally determined by the ion radius. The stability of solvate complexes formed by the ions of alkali-earth metals with acetonitrile molecules also depends on the ion size: we may expect a greater stability of the Mg^{2+} solvates than that of the Ba^{2+} solvates. Since the formation of a complex involving azacrown groups of the ketocyanines under study requires the complete or partial decomposition of the solvate shell of the cation, it is clear why the formation of complexes **1** and **2** with the Ba^{2+} ions is more efficient than that with the Mg^{2+} ions. The complexes with the carbonyl group also exhibit a similar dependence, although it is less pronounced.

In the curves of fluorimetric titration of compound **4**, the inflection point lies in the region of lower concentrations of the metal than that in the curves of spectrophotometric titration. This effect is less pronounced for other compounds. Although the application of identical formulas for processing of the spectrophotometric and fluorimetric titration data is not completely correct,[24] in this case, we can suggest somewhat higher efficiency of complex formation at the carbonyl group in the excited state than that in the ground state. This agrees with the results of quantum-chemical calculations indicating an increase in the electron density at the oxygen atom of the carbonyl group in the S$_1$ state.[21,22,25]

As should be expected, in the case of compounds **1** and **2** containing reaction centers of two different types, the interaction with the alkali-earth metal ions occurs in two or three stages, depending on the number of complex formation centers. For example, for the nonsymmetric compound **2** with all studied ions, we detected two stages of complex formation, and the first ion adds to the azacrown group, whereas the second ion adds to the carbonyl group. A different picture is observed for compound **1** because it has two identical azacrown cycles. The sequence of complex formation stages of this compound with different ions is different (Scheme 1).

All ions demonstrate the common feature: first, in the region of low concentrations, the metal ion adds to one crown group. This is followed by an insignificant short-wave shift of the maximum ($\Delta\nu \approx 500$—1000 cm^{-1}) accompanied by a decrease in the intensity and a specific change in the shape of the long-wave absorption band due to the disappearance of the Kiprianov splitting.[8] This indicates that a nitrogen atom is excluded from conjugation with the π-system of the molecule due to the formation of a complex. This effect is not too high, however, it is quite noticeable (Figs. 2 and 3, arrow ***a***). Probably, more substantial changes in the spectra of similar asymmetric dyes can be achieved for the systems with a more substantial interaction of chromophoric fragments than that in the studied derivatives of dibenzalcyclopentanone and dibenzalacetone.

Scheme 1



Further complex formation with various ions occurs *via* different routes. For example, when the concentration of the Ba^{2+} and Ca^{2+} ions in a solution increases (Fig. 2), a considerable short-wave shift of the absorption band ($\Delta\nu \approx 3000$ cm^{-1}, Fig. 2, arrow ***b***) is observed, which is also characteristic of complex formation at the azacrown fragment accompanied by the complete "exclusion" of its nitrogen atom from conjugation with the

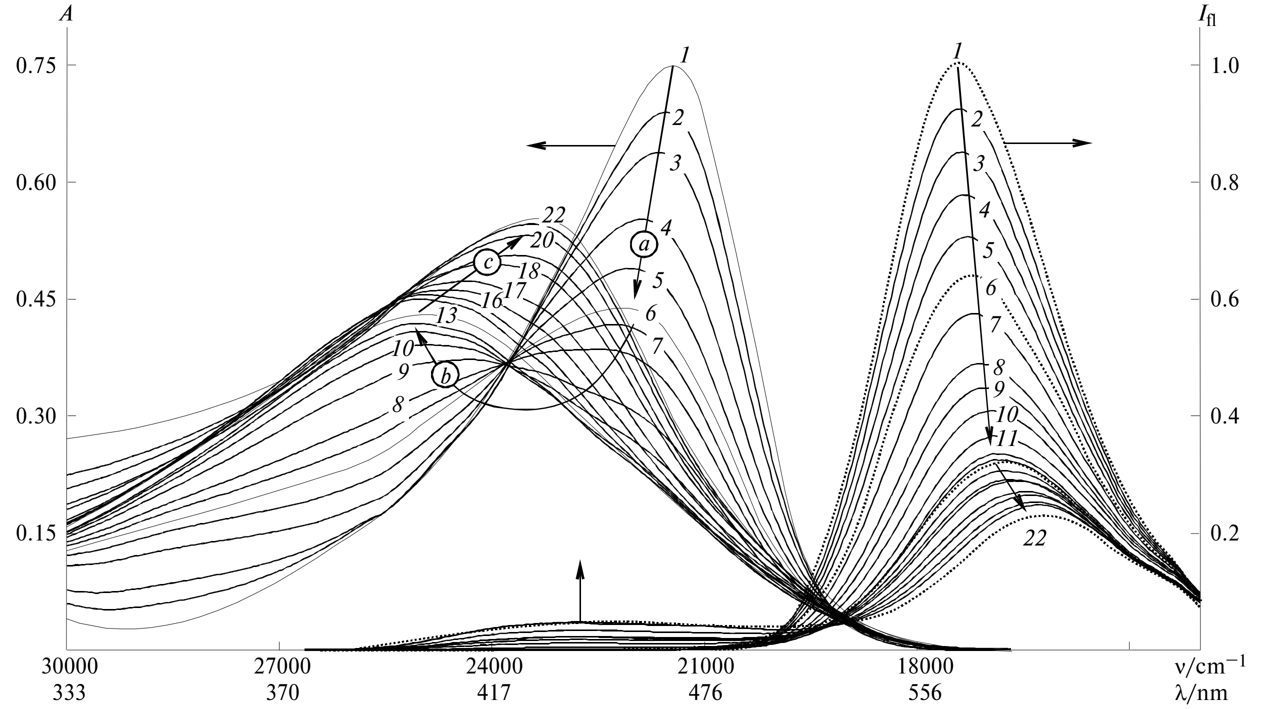


Fig. 2. Changes in the absorption and fluorescence spectra (the latter are reduced to the same absorbance at the excitation wavelength of 370 nm) during the complex formation of compound **1** (~$9.7 \cdot 10^{-4}$ mol L^{-1}) with the Ba^{2+} ions in MeCN. Arrows indicate a change in the absorbance (*A*) and fluorescence intensity (I_{fl}) with an increase in the concentration of Ba^{2+}/mol L^{-1}: 0 (*1*), $2.5 \cdot 10^{-4}$ (*2*), $5.6 \cdot 10^{-4}$ (*3*), $2.1 \cdot 10^{-3}$ (*4*), $4.2 \cdot 10^{-3}$ (*5*), $6.3 \cdot 10^{-3}$ (*6*), $7.9 \cdot 10^{-3}$ (*7*), 0.011 (*8*), 0.016 (*9*), 0.021 (*10*), 0.026 (*11*), 0.031 (*12*), 0.038 (*13*), 0.049 (*14*), 0.063 (*15*), 0.089 (*16*), 0.11 (*17*), 0.17 (*18*), 0.22 (*19*), 0.29 (*20*), 0.36 (*21*), and 0.51 (*22*).

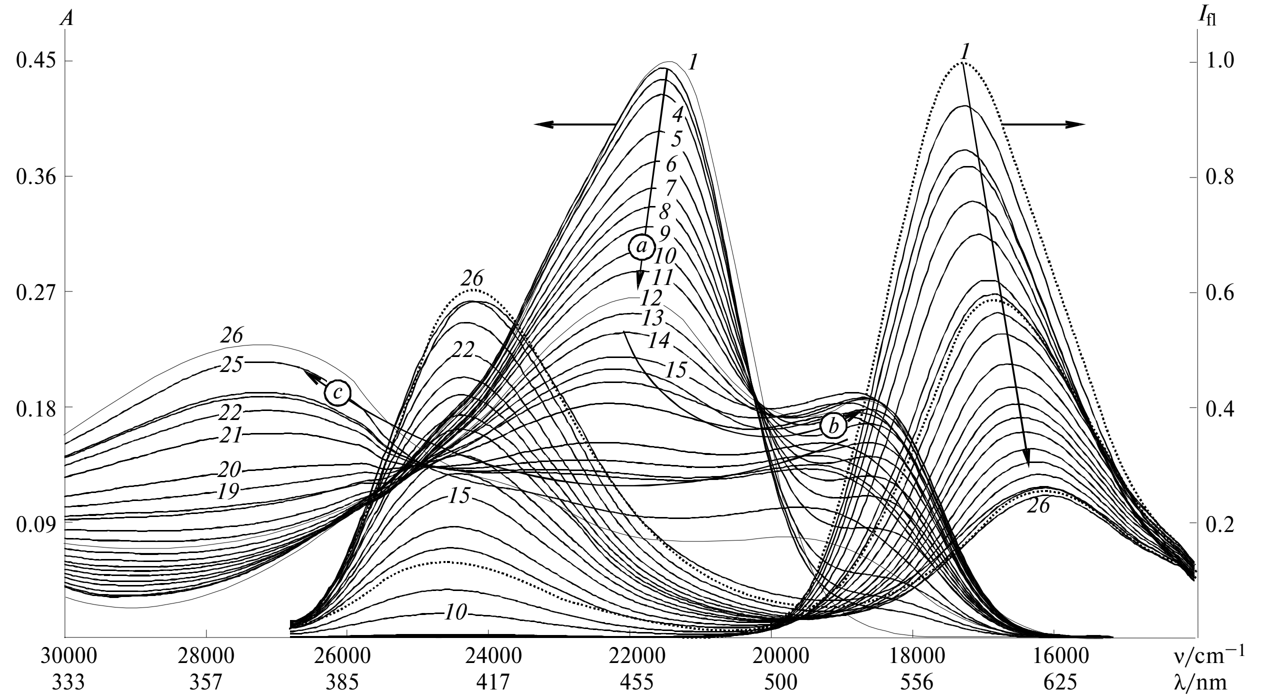


Fig. 3. Changes in the absorption and fluorescence spectra (the latter are reduced to the same absorbance at the excitation wavelength of 370 nm, shown by dotted lines) during the complex formation of compound **1** (~$5.8 \cdot 10^{-4}$ mol L^{-1}) with the Mg^{2+} ions in MeCN. Arrows indicate a change in the absorbance (*A*) and fluorescence intensity (I_{fl}) with an increase in the concentration of Mg^{2+}/mol L^{-1}: 0 (*1*), $9.2 \cdot 10^{-5}$ (*2*), $4.2 \cdot 10^{-4}$ (*3*), $1.9 \cdot 10^{-3}$ (*4*), $5.5 \cdot 10^{-3}$ (*5*), 0.011 (*6*), 0.020 (*7*), 0.030 (*8*), 0.043 (*9*), 0.059 (*10*), 0.087 (*11*), 0.13 (*12*), 0.17 (*13*), 0.20 (*14*), 0.23 (*15*), 0.26 (*16*), 0.32 (*17*), 0.37 (*18*), 0.46 (*19*), 0.53 (*20*), 0.59 (*21*), 0.63 (*22*), 0.69 (*23*), 0.80 (*24*), 0.89 (*25*), and 1.26 (*26*).

π-system of the molecule. The latter is somewhat similar to the "exclusion" of the amino group during protonation. The interaction with the magnesium ions, which occurs *via* somewhat different scheme, will be considered below.

Unlike the absorption spectra characterized, as a whole, by a substantial short-wavelength shift, the maximum in the fluorescence spectrum is insignificantly shifted, by contrast, to the long-wavelength region (by at most 1000 cm^{-1}) when the concentration of the Ba^{2+} and Ca^{2+} ions increases. The Stokes shift of fluorescence, counted as a difference in the positions of maxima of the absorption and fluorescence bands in the spectra of solutions with a specified concentration of the added metal, increases to 7000—8000 cm^{-1}. Thus, the distinction between the nature of the complex absorbed a photon and that of the fluorescent species is evident. The azacrown derivatives with electron-withdrawing substituents in the direct polar conjugation with the complex formation center are characterized by the effect of "ejection" of the metal ion on going to the excited state, which occurs with a rate much higher than the fluorescence rate of the complex.[26,27] As a result, the fluorescence spectrum of the free ligand is observed instead of the emission spectrum of the metallocomplex. A similar phenomenon also takes place, most likely, for the azacrown-substituted ketocyanines considered in this work.

At the same time, the position of the fluorescence spectrum of an acetonitrile solution of compound **1** with a content of the Ca^{2+} and Ba^{2+} ions of ~0.1 mol L^{-1} is close to that of the spectra of the calcium and barium donor-acceptor complexes formed due to binding to the carbonyl group of compound **3**. Thus, we can assume the formation of the complex at the CO group in the S_1 state in the case of **1** (as well as in the case of **2**) after the metal ion was released from the crown cycle. It is most likely that a similar phenomenon of "transcomplexation" is favored by some enhancement (found for the studied model molecules) of the efficiency of formation of donor-acceptor complexes at the carbonyl group in the excited state as compared to that in the ground state.

The further increase in the metal concentration results in a small long-wavelength shift of the absorption spectrum (~2000 cm^{-1}) corresponding to the coordination of the Ba^{2+} and Ca^{2+} ions at the carbonyl group already in the ground state (Fig. 2, arrow ***c***). The short-wavelength region of the fluorescence spectrum at a high concentration of metal ions exhibits a low-intensity band at 21500—23000 cm^{-1}, which represents, most likely, a superposition of the fluorescence spectra of the complexes at the crown cycles and bound in a complex and/or free carbonyl group. The fluorescence of these complexes is characterized by the standard values of the Stokes shift.

In the case of the complex formation of **1** and **2** with the Mg^{2+} ions (Fig. 3), after the described above primary formation of the complex at one crown ether group, which appeared as a small short-wavelength shift of the absorption spectrum (Fig. 3, arrow ***a***), we can see a noticeable long-wavelength shift of the absorption and fluorescence spectra corresponding to binding of the metal ion to the carbonyl group (Fig. 3, arrow ***b***). With the further increase in the concentration of Mg^{2+}, compound **1** manifests a tendency for the short-wavelength shift in the absorption spectrum corresponding to the inclusion of the metal ions into the still free second azacrown cycle: the shift of the long-wavelength band of the absorption spectrum is up to 6000 cm^{-1} relatively to the position of the corresponding band in the spectrum of compound **1** unbound in a complex (Fig. 3, arrow ***c***). Note that we failed to shift completely the equilibrium toward the 1 : 3 complexes with simultaneous metal coordination at both azacrown cycles and carbonyl group due to the limited solubility of the perchlorates under study (especially magnesium salt) in acetonitrile. Therefore, the last complex, which absorbs at ~27000 cm^{-1}, is present in a solution in a relatively low concentration.

It is of interest that, in the case of the magnesium ion, the inclusion of the metal into the azacrown cycle is accompanied by the appearance of a sufficiently intense short-wavelength fluorescence with the Stokes shift barely exceeding 2000 cm^{-1}. Thus, we can assume that, in the case of the magnesium complex, either the metal ion is not ejected from the crown cycle at all, or its rate is much lower than the fluorescence rate of the corresponding complex.

Our studies show that the complex formation of the (15-azacrown-5) derivatives of dibenzalcyclopentanone **1** and **2** with the alkali-earth metal ions Mg^{2+}, Ca^{2+}, and Ba^{2+} involves both the carbonyl group and azacrown fragments. For the polyfunctional derivatives, the sequences of binding of these metal ions to the listed complex formation centers are different. In the excited state, the Ca^{2+} and Ba^{2+} ions coordinated to the azacrown fragment are efficiently "ejected" from the macroheterocycle, whereas either a similar ejection of the Mg^{2+} ion does not occur at all, or its efficiency is much lower than that in the case of the barium and calcium ions. Due to rather efficient complex formation, which changes noticeably the spectral parameters, the bis(azacrown)-containing derivatives of dibenzalcyclopentanone and dibenzalacetone are promising for the development of related fluorescent sensors for alkali-earth metal ions and, probably, as fluorescent probes appropriate for the estimation of the surface potential of biomembranes.

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