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Spectroscopic Study of a Symmetrical Bis-crown Fluoroionophore of the Diphenylpentadienone Series

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A new fluoroionophore bearing two crown ethers on the conjugated system, 1,5-bis[4*N*-(aza-15-crown-5)-phenyl]-1,4-pentadien-3-one (**II**), was synthesized together with model compound **I** where the crown ethers are replaced by diethylamino groups. The behavior of molecules **I** and **II** in the presence of alkali and alkaline-earth perchlorates was investigated in acetonitrile solution by absorption and emission spectroscopy (including picosecond techniques), mass spectrometry, and NMR spectroscopy. Two types of interaction must be distinguished: on the one hand, the expected complexation of the crown ether moieties, which generated strong spectroscopic effects with Na^+, Li^+, Ba^{2+}, and Ca^{2+} ions, and on the other hand, an interaction between the unsaturated ligand core and the cation, particularly noticeable with magnesium. The absorption data were correctly fitted by taking into account three different stoichiometries and the corresponding association constants were calculated. The emissive properties of the species involved were discussed.

1. Introduction

Crown-ether derivatives incorporating a fluorescent moiety are attractive tools for optical sensing of metal ions. During the past decade, numerous fluoroionophores have been built to this pattern. This abundance of structures allowed a classification of the compounds to be established according to the photophysical mechanisms involved: photoinduced electron transfer, alteration of the conjugated system, excimer formation, etc.[1−7] However, very little information is available about molecules in which two ionophore groups are directly linked to a conjugated electron system.

Few compounds of this type are mentioned in the literature. Most have been designed so that the two crown-ether moieties are spatially close and display a synergistic effect. Crowns incorporating biphenanthryl[8] or anthracenoyl[9,10] residues have been used as fluorescent probes for chiral molecular recognition and lipid phase-transition sensing, respectively, but their behavior in the presence of ions has not been reported. As far as cation recognition is concerned, several bis-crown-ether derivatives have been synthesized in the aim of favoring intramolecular sandwich structures. In fact, according to Pedersen and Frensdorff,[11] the stability of the cation/crown-ether complex depends on how the cation matches the crown cavity. It has been noticed that when the cation is too big to be accommodated by the crown, sandwich structures are formed where two ligands coordinate the same cation. In order to regulate the relative positions of the two crown-ether moieties, a photochromic group has been introduced between them. Such molecules are modified upon light irradiation which results in bringing the crown ethers close together or in moving them apart, and thus modulates their affinity for cations. Examples are Lindsten's stilbenic derivatives,[12] Shinkai's azoic compound,[13] and the malachite green derivative of Kimura.[14] These systems are potentially useful for light-driven cation pumps, although they have not been extensively investigated from a photophysical viewpoint.

Actually, it seems that fluoroionophores bearing two crown ethers remaining far apart in space have been very rarely studied until now. Exceptions are the phenanthroline derivative of Schmittel[15] whose spectral properties have not been reported, and the red-emitting squaraine dye studied by Das et al.[16] and Akkaya.[17] The latter compound, symmetrically substituted by two crown ethers, undergoes a moderate decrease of the fluorescence quantum yield attributed to complexation and has been proposed for the analysis of alkali cations in solution.

These considerations prompted us to conceive and study an original bis-crown-ether fluoroionophore devoid of any possibility of forming a sandwich-type intramolecular complex with cations. We retained the idea of a symmetrical structure, but with a bis-chromophoric dye where each chromophore contains a cation chelator. The chromophores are conjugated and thus interact with one another.[18,19] It is expected that binding to a cation breaks the dye symmetry, which could result in striking changes in the spectroscopic properties. In this case, the dye may become a sensor for the analysis of cations in solution.

1,5-Bis[4-(diethylamino)phenyl]-1,4-pentadien-3-one (**I**) (Scheme 1) was therefore chosen as the fluorophore because the dyes in

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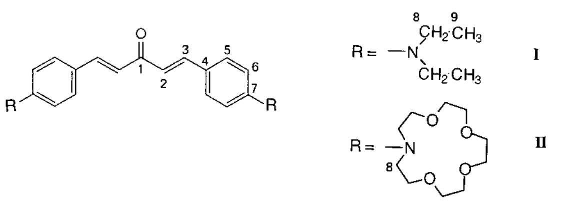
SCHEME 1



this series show interesting spectral properties. Moreover, they are directly obtained as trans,trans isomers and are fairly stable since they display a surprising absence of photochemical isomerization.[20,21] (Note, however, that rotational isomerism also exists: compounds of this class differ by the spatial orientation of the propenonic C=C and C=O bonds. It was shown that the *s-cis,cis* form in Scheme 1 is the most stable.[22−25]). In order to install an ionophore in this molecule, the diethylamino group was replaced by an aza-15-crown-5 residue, a crown ether selected for its ability to complex alkali and alkaline-earth cations. Consequently, *trans,trans*-1,5-bis[4*N*-(aza-15-crown-5)phenyl]-1,4-pentadien-3-one (**II**) was synthesized (Scheme 1).

The influence of alkali and alkaline-earth cations upon the absorption and emission characteristics of **II** was investigated. The observed effects were interpreted by comparison with the behavior of **I** studied in identical conditions, with the help of additional techniques such as mass spectrometry and NMR spectroscopy. Measurements were carried out at room temperature in acetonitrile. Perchlorate salts were used both because of their solubility in this solvent and because the perchlorate anion did not generate any shift in the absorption or emission bands.

2. Experimental Section

2.1. Materials. Spectroscopic grade acetonitrile (Merck) was used for absorption and fluorescence measurements. Alkali and alkaline-earth perchlorates ($NaClO_4 \cdot H_2O$; $LiClO_4$; $Ca(ClO_4)_2 \cdot 4H_2O$; $Mg(ClO_4)_2 \cdot 6H_2O$; $Ba(ClO_4)_2$) from Acros and Aldrich were used as received.

2.2. Synthesis of the Compounds. Samples of 1,5-bis[4-(diethylamino)phenyl]-1,4-pentadien-3-one (**I**) were prepared from condensation in alkaline medium of 4-(diethylamino)-benzaldehyde (4.43 g, 2.5×10^{-2} mol, Aldrich) with acetone (0.92 mL, 1.25×10^{-2} mol) by the method of Olomucki and Le Gall.[26] The orange precipitate was rinsed with ethanol and then purified by plate chromatography on silica gel with diethyl ether−hexane (60:40) as the eluent. Yield 60%; mp 167−168 °C; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.19 (t, $J = 7$ Hz, 12 H, −CH3−), 3.40 (q, $J = 7$ Hz, 8 H, −CH2−), 6.65 and 7.49 (2×d, $J = 8.8$ Hz, 8 H, Ph); 6.87 and 7.68 (2×d, $J = 15.8$ Hz, 4 H, −CH=CH−); ^{13}C NMR ($CDCl_3$) δ (ppm): 188.6 (C, C1), 150.2 (C, C7), 142.9 (CH, C3), 130.9 (CH, C5), 122.4 (C, C4), 121.3 (CH, C2), 112.0 (CH, C6), 44.8 (CH_2, C8), 12.5 (CH_3, C9); IR (KBr) ν (cm^{-1}): 1650 (conjugated CO), 1585 (C=C), 1180 (C−N), 1000 (trans CH=CH), 840 (para-substituted Ph); mass spectrometry (electrospray, positive mode) $m/z = 377.2$ (M+1).

1,5-Bis[4*N*-(aza-15-crown-5)phenyl]-1,4-pentadien-3-one (**II**) was prepared as follows. *N*-Phenylaza-15-crown-5 (1 g, 3.7×10^{-3} mol, Acros) was formylated by reacting with an equimolar amount of $POCl_3$ in DMF according to the method of Dix and Vögtle.[27] The crowned aldehyde was then condensed with acetone as previously described. The oily product obtained was purified on preparative silica plates, using 95% ethanol as eluent. After extraction with dichloromethane and drying, a red waxy solid was isolated with a 10% yield. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.6 (m, 32 H, H crown), 3.7 (t, $J = 6.6$ Hz, 8 H, CH_2−N), 6.66 and 7.48 (2×d, $J = 8.8$ Hz, 8 H, Ph), 6.86 and 7.66 (2×d, $J = 15.7$ Hz, 4 H, −CH=CH−); ^{13}C NMR ($CDCl_3$) δ (ppm): 189.1 (C, C1), 150.7 (C, C7), 143.2 (CH, C3), 131.1 (CH, C5), 123.5 (C, C4), 122.1 (CH, C2), 112.8 (CH, C6), 71.6 (CH_2, C crown), 71.0 (CH2, C crown), 70.4 (CH2, C crown), 69.1 (CH2, C crown), 53.5 (CH_2, C8); IR (KBr) ν (cm^{-1}): 1585 (C=C), 1170 (C−N), 1120 (C−O−C), 1000 (CH=CH trans), 840 (para-substituted Ph); mass spectrometry (electrospray, positive mode): $m/z = 669.4$ (M+1).

2.3. Apparatus. Absorbance spectra were recorded on a Hewlett-Packard 8452 A diode array spectrophotometer. It was checked that the absorption spectra did not vary over a period of 48 h. Cuvettes of 5 cm optical path length were used. This allowed the use of the same concentrations for absorbance measurements and for fluorescence measurements, so the results were directly comparable. Steady state fluorescence work was performed on a Photon Technology International (PTI) Quanta Master 1 spectrofluorometer. All fluorescence spectra were corrected. The fluorescence quantum yields were determined using coumarin 6 in ethanol ($\Phi_F = 0.78$) as standard.[28] The measurements were conducted at 20 °C in a thermostated cell. Fluorescence decay was obtained by the single-photon-counting technique. The excitation source was the second harmonic ($\lambda_{ex} = 300$ nm) of a dye laser (Rhodamine 6G) synchronously pumped by a mode-locked Nd:Yag laser. A Glan-Thomson prism, forming an angle of 54.7° with the polarization direction of the exciting beam, was positioned in front of the monochromator. The detector was a microchannel plate (R1564 U Hamamatsu) providing an instrumental response function of 60 ps (fwhm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers operating at 200 and 400 MHz, respectively. The mass spectrum was obtained on a simple quadrupole mass spectrometer (Perkin-Elmer Sciex API 100), using electrospray as the ionization mode. The infusion rate was 5 μL/min. Positive fast atom bombardment spectra were acquired on a Nermag R10-10H mass spectrometer. A 9 kV xenon atom beam was used to desorb samples from the 3-nitrobenzyl alcohol matrix. Infrared spectra were recorded on a 683 Perkin-Elmer spectrophotometer. The melting point was measured on a Buchi precision apparatus and was left uncorrected.

2.4. Data Analysis. The absorption experimental data were processed on a HP 9000 series 710 workstation. Absorbance A was related to concentrations C_i using Beer−Lambert's law: $A = l\sum(\epsilon_i C_i)$, where ϵ_i is the molar absorption coefficient of species i and l is the optical path length. The system of three equilibrium equations with three independent variables was numerically solved by an iterative method. The sum of the squares of the differences between the experimental values and those of the numerical calculation was minimized by a Powell nonlinear minimization algorithm. The method used has been extensively described in a previous paper.[29] For dynamic fluorescence, the decay times were fitted without using the iterative reconvolution procedure on a HP 9000 series 710 workstation.

3. Results

Absorption Spectra. Special attention has been paid in the literature to the diphenylpentadienone chromophore. In particular, the spectroscopic and photophysical properties of the dimethylamino derivative have been reported[20,21,30] and were useful for the interpretation of our data.

In the absence of cations, **I** and **II** in acetonitrile gave intensely colored orange-yellow solutions. The absorption

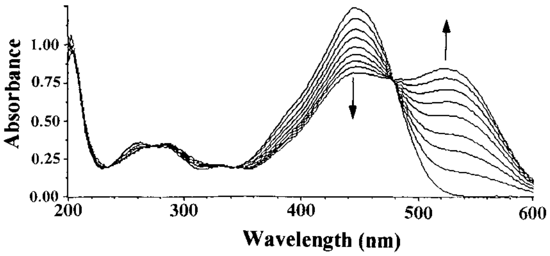


Figure 1. Absorption spectra of **I** (5.1×10^{-6} M) in acetonitrile before and after addition of magnesium perchlorate. From top to bottom at 420 nm: [$Mg(ClO_4)_2 \cdot 6H_2O$]: 0; 6.5×10^{-4} M; 1.3×10^{-3} M; 2.0×10^{-3} M; 3.3×10^{-3} M; 4.5×10^{-3} M; 6.5×10^{-3} M; 9.1×10^{-3} M; 1.3×10^{-2} M. Arrows indicate increasing salt concentration.

spectrum (Figure 1) showed two characteristic bands: a band of weak intensity, with a maximum around 262 nm and an intense long-wavelength band ($\lambda_{max} = 446$ and 438 nm, for **I** and **II** respectively). According to Khalaf et al.,[30] the first band can be ascribed to a $\pi \rightarrow \pi^*$ transition. The second one can be identified as a charge-transfer (CT) band resulting from the displacement of the electron density from the donor amino group toward the acceptor carbonyl group. Actually, it seems that both bands have a more complex nature as denoted by the shoulders at 280 and 380 nm. They could result from the existence of a two-chromophore system in the molecule.[18,19] Transitions of the $n \rightarrow \pi^*$ type are probably masked under these two absorption bands.

Beer–Lambert's law was satisfied in the range of concentration studied (10^{-6}–10^{-5} M), showing the absence of association in the ground state, which is in agreement with the literature.[20] The molar extinction coefficient was measured at the CT band maximum. For compound **I**, it was found to be equal to 50 000 M^{-1} cm^{-1}, which is quite close to the value given in the literature for 1,5-bis[4-(dimethylamino)phenyl]-1,4-pentadien-3-one.[20,30] It was equal to 28 000 M^{-1} cm^{-1} for **II**. This low molar absorption coefficient together with the blue shift in absorption wavelength observed for the crown derivative by comparison with the uncrowned one may be attributed to the electron-withdrawing effect of the crown oxygen atoms, which decreases the donor strength of the nitrogen atom and therefore the CT character of the compound.

Then, **I** and **II** were studied in the presence of alkali and alkaline-earth cations. Absorption spectra were recorded after adding known amounts of metal perchlorates to a solution of **I** or **II** in acetonitrile. The absorption characteristics are collected in Table 1.

Compound **I** bears no ionophore group. However, the structure of this molecule does not rule out the possibility of an interaction with ions. Actually, a reddening of the solution was observed in the presence of magnesium and calcium perchlorate salts. This variation corresponds to a strong modification of the spectrum (Figure 1). In this case, the CT band situated at 446 nm decreased whereas a new intense band appeared at higher wavelengths. This new band peaked at 518 nm ($\epsilon = 43\,000$ M^{-1} cm^{-1}) for 5×10^{-2} M magnesium perchlorate and 516 nm ($\epsilon = 50\,100$ M^{-1} cm^{-1}) for the same concentration of calcium. The presence of isosbestic points is consistent with the fact that only one equilibrium is involved. It is likely to assume that two species are concerned, the free dye and the dye interacting with the cation, since the ligand displays only one site of possible interaction. It can be noted that very high concentrations (about 1 M) of lithium, sodium, and barium perchlorates induced a similar effect, although far weaker.

TABLE 1: Spectral Characteristics of Reference Compound I in the Absence of Salts and of the Free and Complexed Ligand II: Maximum Absorption Wavelength of the CT Band (λ_{abs}), Apparent Molar Extinction Coefficient at the Absorption Maximum (ϵ), Maximum Emission Wavelength (λ_{em}), Quantum Yield Φ_f[a]

	λ_{abs}, nm	ϵ, M^{-1} cm^{-1}	λ_{em}, nm	Φ_f
I	446	50 000	574	0.15
II	438	28 000	566	0.16
II + Li^+	422	23 050	574	0.09
II + Na^+	408	18 815	586	0.09
II + Mg^{2+}	518		586	0.08
	shoulder: 432	18 390		
II + Ba^{2+}	368	14 700	584	0.09
II + Ca^{2+}	344		594	0.04
	shoulder: 412	9 770		

[a] Ligand concentration: 5×10^{-6} to 10^{-5} M. Salt concentration for absorption measurements: [Li] = 1.7×10^{-2} M, [Na] = 8.3×10^{-2} M, [Mg] = 1.0×10^{-2} M, [Ba] = 8.0×10^{-3} M, [Ca] = 3.8×10^{-3} M; for emission measurements: [Li] = 5.3×10^{-3} M, [Na] = 2.5×10^{-2} M, [Mg] = 1.6×10^{-2} M, [Ba] = 8.1×10^{-4} M, [Ca] = 6.5×10^{-4} M. Excitation wavelength: Li 300 nm; Na 254 nm; Mg 346 nm; Ba 364 nm; Ca 250 nm.

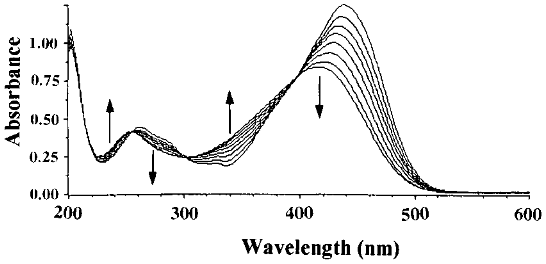


Figure 2. Absorption spectra of **II** (9.1×10^{-6} M) in acetonitrile before and after addition of sodium perchlorate. From top to bottom at 420 nm: [$Na(ClO_4) \cdot H_2O$]: 0; 1.3×10^{-3} M; 3.5×10^{-3} M; 5.8×10^{-3} M; 9.3×10^{-3} M; 1.1×10^{-2} M; 2.3×10^{-2} M; 3.4×10^{-2} M.

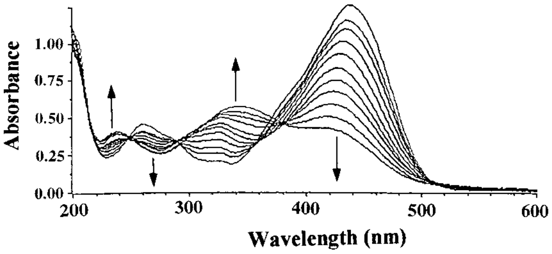


Figure 3. Absorption spectra of **II** (9.4×10^{-6} M) in acetonitrile before and after addition of calcium perchlorate. From top to bottom at 420 nm: [$Ca(ClO_4)_2 \cdot 4H_2O$]: 0; 3.2×10^{-5} M; 6.5×10^{-5} M; 1.6×10^{-4} M; 3.2×10^{-4} M; 6.4×10^{-4} M; 9.5×10^{-4} M; 1.6×10^{-3} M; 1.8×10^{-3} M; 2.4×10^{-3} M; 3.8×10^{-3} M.

In contrast, upon addition of cation perchlorates, the orange-yellow solution of **II** was discolored. Sodium and lithium salts induced a hypochromic and hypsochromic shift on the CT band (Figure 2). Isosbestic points were noted at 300 and 408 nm with lithium and at 250 and 398 nm with sodium at low salt concentrations. At high salt concentrations these points were lost. A stronger effect was observed with calcium (Figure 3) and barium perchlorates, since the intensity of the CT band was drastically reduced while a band appeared at around 330 nm. No tight isosbestic points were observed in this case. In the presence of magnesium perchlorate (Figure 4), an additional effect was generated, i.e., the appearance of a strong new band at 518 nm, reminiscent of the effect observed in **I** with the same salt.

Steady-State Fluorescence Spectra. The emission characteristics are gathered in Table 1. In the absence of salts, the emission spectra of **I** and **II** (9×10^{-6} M) in acetonitrile showed

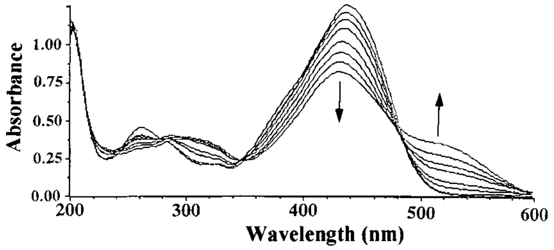


Figure 4. Absorption spectra of **II** (1×10^{-5} M) in acetonitrile before and after addition of magnesium perchlorate. From top to bottom at 420 nm: [$Mg(ClO_4)_2{\cdot}6H_2O$]: 0; 1.4×10^{-5} M; 2.3×10^{-5} M; 3.7×10^{-4} M; 1.1×10^{-3} M; 2.0×10^{-3} M; 3.7×10^{-3} M; 7.4×10^{-3} M.

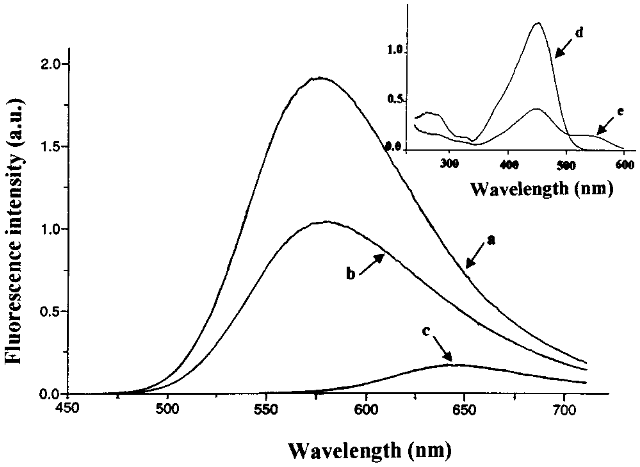


Figure 5. Influence of the excitation wavelength on the emission spectra of **II** (2×10^{-6} M) in acetonitrile before (curve a, $\lambda_{ex} = 438$ nm) and after addition of 1×10^{-3} M magnesium perchlorate (curve b: $\lambda_{ex} = 438$ nm; curve c: $\lambda_{ex} = 530$ nm). Inset: Excitation spectrum of **II** in the absence (curve d, $\lambda_{em} = 610$ nm) and in the presence (curve e, $\lambda_{em} = 650$ nm) of magnesium salt.

only one band with maxima situated at around 574 and 566 nm, respectively, whatever the excitation wavelength. The Stokes shifts were 5000 and 5160 cm^{-1}, respectively, so the absorption and emission spectra did not overlap. Both compounds exhibited similar quantum yields (0.15 and 0.16 respectively).

In the presence of salts, **I** and **II** (9×10^{-6} M) were excited at the wavelengths which correspond to isosbestic points (alkali cations) or loose pseudo-isosbestic points (alkaline-earth cations), in order to minimize the effect of absorption variations. For **I**, the presence of cations resulted in a red shift of the emission band and a moderate decrease in fluorescence intensity. So, obviously, this red shift is related to the fluorophore interacting with cations, independently of the presence of the crowns.

As far as **II** is concerned, it was surprising to see that a red shift of the emission spectrum was also observed in the presence of cations. This shift reached 28 nm with calcium perchlorate (Table 1). Meanwhile, the fluorescence intensity collapsed, the fluorescence quantum yield being as low as 0.04 with calcium. It was checked that the excitation spectra were very close to the corrresponding absorption spectra (inset in Figure 5). It was also shown in compounds **I** and **II** that exciting in the red part of the CT band induced the emission spectrum to shift toward the red (Figure 5).

Fluorescence Lifetimes. Fluorescence decays of reference compound **I** and crowned compound **II** in the absence and in the presence of cations were measured at different excitation and emission wavelengths. For both free ligands, the fluorescence decay was a single-exponential yielding an identical fluorescence lifetime τ of 800 ps (Table 2). In the presence of lithium and sodium, the fluorescence decay of both compounds was again found to be monoexponential though the corresponding lifetime was slightly weaker than that of the free ligands. In contrast, upon addition of calcium and magnesium perchlorates, the data could not be fitted with one only exponential: **I** and **II** showed two distinct lifetimes. The first one was very close to that of the free ligand (and arbitrarily set at 800 ps when necessary to improve the fitting) and the second one was markedly shorter, between 300 and 500 ps. This indicates the presence of two distinct emitting species, at least. The fractional intensity of the short-lived species increased with the amount of salt added. It also depended on the observation wavelength as shown with calcium: the short-lived component passed from 13% at $\lambda_{em} = 560$ nm to 63% at $\lambda_{em} = 650$ nm. Conversely, it was the only one observed when excitation was at 560 nm in the red part of the absorption CT band. So, obviously, the short lifetime was related to the species responsible for the red shift of the absorption spectrum and for the high-wavelength emission, that is, the species resulting from the direct interaction of ligands **I** and **II** with magnesium and calcium cations.

TABLE 2: Fluorescence Lifetime of I (1.7×10^{-6} M) and II (1×10^{-5} M) in the Presence or Absence of Metal Perchlorates: Cation Concentration, Fluorescence Lifetime, and Fractional Intensities ($\alpha_1 = A_1\tau_1/(A_1\tau_1 + A_2\tau_2)$ and $\alpha_2 = A_2\tau_2/(A_1\tau_1 + A_2\tau_2)$) in Parentheses[a]

		τ, ps			
		compound **I**		compound **II**	
cation	[cation], M				
Ca^{2+}	4.6×10^{-3}	800 (37%)	340 (63%)	800 (32%)	420 (68%)
	1.4×10^{-2}	800 (16%)	330 (84%)		
	1.4×10^{-3} [b]	800 (87%)	390 (13%)	800 (71%)	390 (29%)
Mg^{2+}	3.9×10^{-3}	800 (57%)	330 (43%)	800 (73%)	490 (27%)
Ba^{2+}	1.9×10^{-3}	720		800 (49%)	480 (51%)
Li^+	1.2×10^{-2}	760		790	
Na^+	7.4×10^{-2}	570		710	
none	0	800		800	

[a] Excitation wavelength: 295 nm. Emission wavelength: 650 nm unless specified. [b] $\lambda_{em} = 560$ nm.

In the case of barium, two lifetimes were found for **II**. Only one was found for **I**, but the correct fit obtained using only one exponential does not rule out the involvement of a short-lived component but which would be present as traces and impossible to analyze. This is probably the case also for ligands **I** and **II** in the presence of alkaline salts.

Interaction Model and Treatment of the Spectroscopic Data. The absorbances were analyzed versus cation concentration. Since the shape of the absorption spectrum strongly varied in the presence of cations, absorbance was recorded at four different wavelengths chosen to obtain maximum information. Among the different models which were investigated, the following resulted in the most satisfactory fit:

$$A + M \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} AM \qquad K_1 = k_1/k_{-1} \tag{I}$$

$$AM + A \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} A_2M \qquad K_2 = k_2/k_{-2} \tag{II}$$

$$AM + M \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} AM_2 \qquad K_3 = k_3/k_{-3} \tag{III}$$

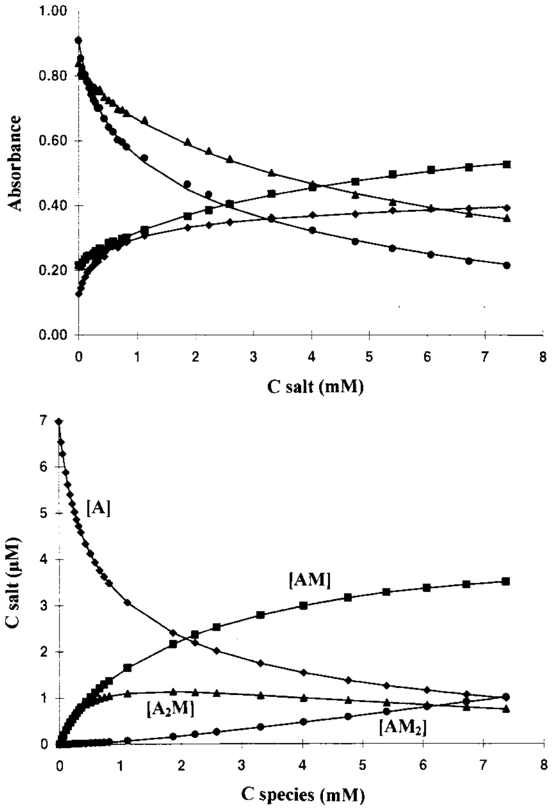


Figure 6. Absorption data processing for **II** in acetonitrile in the presence of barium perchlorate. Top: Absorbance versus the cation concentration. $\lambda_{abs} = 330$ (diamonds), 356 (squares), 420 (circles), and 450 nm (triangles). The points are experimental and the curves were calculated by fitting the data. Bottom: Corresponding calculated concentrations of the different species: [A] (diamonds), [AM] (squares), $[A_2M]$ (triangles) and $[AM_2]$ (circles) versus the cation concentration.

where A is ligand **II** and M the metal cation. AM, A_2M, and AM_2 represent the three complexes of different stoichiometries which may reasonably be considered. The equilibrium equations are as follows:

$$K_1[A][M] - [AM] = 0 \tag{1}$$

$$K_2[AM][A] - [A_2M] = 0 \tag{2}$$

$$K_3[AM][M] - [AM_2] = 0 \tag{3}$$

The two concentrations [M] and $[AM_2]$ can be written as a linear combination of the other concentrations with the help of the mass conservation equations:

$$[M] = [M]_0 - [AM] - [A_2M] - 2[AM_2] \tag{4}$$

$$[AM_2] = [A]_0 - [A] - [AM] - 2[A_2M] \tag{5}$$

in which subscript 0 refers to initial concentrations. Therefore, only three independent variables [A], [AM], and $[A_2M]$ remain in eqs 1, 2, and 3 which can then be solved using an iterative method.

For each salt, fitting was performed *simultaneously* on the four curves obtained by absorption spectroscopy (see Experimental Section). The values of the parameters corresponding to equilibrium constants and molar extinction coefficients were refined to improve the fit between the model and the experimental data. Good fits were obtained, as illustrated in Figure 6 with the barium salt. The association constants are reported in Table 3 (the calculated molar extinction coefficients are given in the Supporting Information).

TABLE 3: Association Constants Related to Species AM, A_2M, and AM_2 for Ligands I and II with Cations in Acetonitrile, Determined by Processing the Absorption Data and Cation Diameter (from Ref 43)

	AM K_1, M^{-1}	A_2M K_2, M^{-1}	AM_2 K_3, M^{-1}	D_{ion}, Å
II + Li^+	3.6×10^2		3.3	1.36
II + Na^+	1.8×10^2	5.2×10^4		1.94
II + Ba^{2+}	4.8×10^2	2.2×10^5	3.9×10^1	2.68
II + Mg^{2+}	1.4×10^3		2.6×10^1	1.32
II + Ca^{2+}	1.5×10^4	1.1×10^4	3.2×10^2	1.98
I + Mg^{2+}	2.7×10^2[a]			
I + Ca^{2+}	2.2×10^2[a]			

[a] K'_1, M^{-1}.

It may be noticed that in all cases, the model provided an excellent fit. The association constants strongly varied with the nature of the cation investigated. However, some general trends do appear. First, association constants K_1 for the AM complex increased in the order Na < Li < Ba < Mg < Ca. Second, the association constant related to the A_2M complex was particularly high with barium and decreased in the order Ba ≫ Na > Ca. Finally, constant K_3 was always weaker than constant K_1. Note that with lithium and magnesium, the fit can be satisfactorily achieved by only taking into account the AM and AM_2 complexes.

Processing the data as above involved the analysis of the absorbance variations at several wavelengths. In the case of magnesium, the band which appears at 520 nm on the red side of the absorption spectrum was included in the analysis. However, it is obvious that this band was not dependent on complexation of the crown-ether moieties since it also appeared with **I** in the presence of magnesium salts. So, this means that the association constants K_1 obtained for **II** in the presence of salt correspond to the overall situation of complexation with the crown and direct interaction with the fluorophore.

This latter phenomenon was studied on compound **I**. In order to quantify the strength of this interaction, a calculation similar to that previously used was applied to process the variations of the absorption spectrum of **I** versus the concentration of calcium and magnesium salts. The absorption data were exploited using two wavelengths, 440 and 520 nm, the latter being characteristic of the interaction. The fit obtained was totally satisfying when considering only equilibrium I. The fitted association constants K'_1 are reported in Table 3. It is shown that when alkaline-earth cations interact directly with the fluorophore, the association constant is about 2×10^2 M^{-1} and formation of A_2M species can be ruled out.

The association constants given above were established from absorption spectroscopy data. It seemed worthwhile to compare these values with those obtained using the fluorescence data. Actually, it was not reasonable to process only the emission data which contain not enough information according to the large number of unknown parameters. Therefore, the emission and absorption data were treated *simultaneously*. The fit was excellent. The association constants obtained for Ba^{2+}, Na^+, and Ca^{2+} were not significantly different from those given in Table 3. For Mg^{2+} and Li^+, there was a 5-fold variation in the constant K_3. This suggests that the excited state interactions do not markedly modify the ground state equilibrium.

Mass Spectrometry. Until now, the term “complexation” was used by analogy with what is known about crown ethers from the literature, where numerous crown-ether complexes have been characterized by X-ray analysis.[31] In this work, another type of interaction taking place between ligand and cation is also strongly suspected. We wanted experimental proof of the existence of complexes and possibly of other interacting forms through additional techniques.

First, the mass spectrum was recorded using the electrospray technique of positive ionization, from an acetonitrile solution of free ligand **II** (10^{-6} M). As expected, the molecular peak, corresponding to ionization by a proton, was observed at $m/z = 669$. Then the effect of magnesium and calcium salts was investigated by adding the corresponding perchlorates to the solution (salt concentration: 10^{-4} M). Peaks at $m/z = 354$ and $m/z = 346$ were observed with calcium and magnesium, respectively, and assigned to the *doubly charged* AM species. The complexes observed correspond to the most stable ones, as indicated by the fit of the spectrophotometric data. This corroborates the model chosen for the optimization of the parameters. Finally, the positive FAB technique using a MNBA matrix allowed a peak at 499 to be observed for ligand **I** in the presence of magnesium, showing that a strong interaction indeed exists between the molecule and $[MgClO_4]^+$.

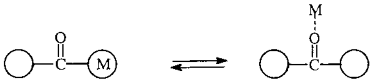
NMR Spectroscopy. In the literature, NMR spectroscopy has been used to study dibenzylideneacetone (dba) coordinated by metal atoms. In the case of $(dba)_2Pt$ or $(dba)_2Pd$, the ^1H NMR spectrum of did not vary with respect to that of the free ligand leading to the conclusion that the metal atom was bound to the carbonyl group.[32] In the case of $(dba)_3Pd_2$ (CH_3Cl_3), the olefinic proton resonances were shifted in coordinated dba, implying an influencial role of the double bonds.[33] Therefore, in this work, the ^1H and ^{13}C NMR spectra of **I** (1.8×10^{-2} M) in the absence and in the presence of magnesium perchlorate (3.6×10^{-2} M) were carried out with the aim to characterize the interaction between the ligand and the cation. On the ^1H NMR spectrum, the only notable variation was that of the signal attributed to H3, that is, the olefinic proton close to the phenyl ring: the doublet centered at 7.59 ppm for the free ligand was shifted to 7.76 ppm in the presence of salt. In ^{13}C NMR spectroscopy, carbon atoms 1, 2, and 3 (see Scheme 1) resonances which were at 188.61, 121.27, and 142.91 ppm, respectively, without salt moved to 190.56, 119.71, and 147.01 ppm, respectively upon addition of magnesium perchlorate. The signals of the aromatic carbon atoms also underwent a weak shift. These results strongly suggest that in our case the unsaturated core of the molecule directly interacted with the cation. It is also noticeable that the ligand symmetry was not broken during the interaction.

4. Discussion

In the absence of cations, compounds **I** and **II** display very similar spectral properties. In the presence of cations, these compounds behave differently, which allowed two types of phenomena to be distinguished.

At the concentrations used, the first phenomenon is neatly observed with magnesium on both the crown and the noncrown compounds. It can be attributed to the direct interaction of one cation with one unsaturated ligand core, as supported by mass spectrometry and NMR data. It must be recalled that cross-conjugated dibenzylideneacetone is a ligand commonly used for stabilizing various organometallic compounds[32−36] and a weaker interaction could very well take place with alkali and alkaline-earth cations. This is evidenced by a red shift in the absorption spectra, which indicates that the interaction takes place in the ground state. One explanation for this red shift is that the cation interacting with the carbonyl group withdraws the oxygen atom free pairs, reinforcing the electron-withdrawing character of this group and therefore charge transfer over the entire molecule. Consequently, a bathochromic effect is generated. This interpretation is in line with the fact that protonation in acidic medium of the benzylideneacetone carbonyl group leads to a red shift very similar to that observed in this work.[37]

SCHEME 2



This interaction is also visible on the emission spectra of compounds **I** and **II**. The newly formed species had a fluorescence lifetime much shorter than that of the free ligand and was revealed by a red shift of the emission spectra in the presence of cations. The strongest effect was observed with doubly charged cations, but it must be noted that an effect was detected with every salt. In contrast, such an effect was not observed on the absorption spectrum recorded in the presence of Na^+, Li^+, or Ba^{2+}, although the salt and ligand concentrations were identical to those used for emission. Therefore, the interaction seems to be stronger for the excited state ligand than for the ground state ligand. Logically, the interaction should be favored by an increase of the charge on the carbonyl group in the excited state, which is very likely to happen considering the positive solvatochromic behavior reported for the compounds of this series[20,21,30] and also observed by us for compound **I**.

The second phenomenon only concerns compound **II** and can be assigned to the expected complexation on the crown-ether moiety. It is revealed by a strong blue shift and hypochromic effect in the CT absorption band as observed with cations Ca^{2+}, Li^+, Ba^{2+}, and Na^+ (in the case of magnesium, this effect is altered by the second phenomenon). This shift may be interpreted as follows: the cation complexed by the crown-ether withdraws the free electron pair of nitrogen, reducing the participation of the latter in the conjugated system. Similar effects have been reported for chromo- and fluoroionophores based on the same photophysical mechanism.[1−6,38,39] It can also be noted that protonation of the nitrogen atom on *p*-(dimethylamino)benzylideneacetone leads to a hypsochromic shift of the absorption CT band.[40] In the emission spectrum, complexation in the crowns results in a drastic collapse of the fluorescence intensity at 566 nm due to the disappearance of the free ligand. The complexed species seems to be very weakly emissive if not totally unemissive at all, since no variation was observed in the position of the emission spectrum nor in the fluorescence lifetime. This is reminiscent of dibenzylideneacetone behavior, the fluorescence of which was not observed by Harvey et al. at room temperature.[41] A comparison can also be attempted with *p*-(diethylamino)benzylideneacetone, which can serve as a model for **II** complexed in the crown, and therefore deprived of one donor group. The quantum yield of *p*-(diethylamino)benzylideneacetone was measured by us and also found to be very low (9×10^{-3} in acetonitrile). Work is presently underway to study the excited state properties of related compounds, to explain why complexed compound **II** exhibits such a low fluorescence, if any.

Competition therefore takes place between two sites of interaction, according to the nature of the cation involved. For the ground state ligand, lithium and sodium salts only lead to complexation in the crown ether. With calcium and barium, complexation in the crown predominates but an interaction occurs on the unsaturated ligand core. The latter interaction becomes particularly important in the case of magnesium (Scheme 2).

The association constants were calculated to allow the interaction strength between ligands and cations to be measured. Evidence was given for the occurrence of three stoichiometries: AM, A_2M, and AM_2.

The most stable AM complex was formed with calcium, as expected for an aza-15-crown-5.[42] In effect, the calcium ion diameter[43] (1.98 Å) closely fits the crown-ether cavity,[11,42] which is 1.7−2.2 Å and its double charge increases the strength of the interaction.

The most stable A_2M complex is the barium one, whereas this type of complex was not observed with the small ions lithium and magnesium. The barium ion size[43] (2.68 Å), which by far exceeds that of the crown cavity, favors complexation between the metal and two molecules of ligand whereas the lithium and magnesium cations (1.36 and 1.32 Å, respectively[43]) only partially fill one crown-ether cavity and do not allow such sandwich type interactions. Quite high values of the association constants K_2 were found for sodium and calcium ions, which have intermediate diameters.

As far as the AM_2 complexes are considered, it must be emphasized that the corresponding association constants K_3 are low. Complexation is very unlikely to occur in both crowns at the same time due to the symmetrical structure of the ligand. In fact, when one of the crowns is complexed by a cation, its electron-donating effect is strongly reduced. So, the electron-withdrawing effect of the carbonyl group now mainly applies to the second crown, which is consequently deactivated with respect to complexation. Logically, in AM_2 complexes, one cation is accommodated by the crown while the second cation interacts with the unsaturated ligand core.

Actually, the model used to calculate the association constants does not make any distinction between the two sites of interaction, crown-ether and unsaturated ligand system. However, comparing the value of the association constants obtained for **I** with those obtained for **II** allows an estimation to be made of the competition which takes place between the two sites of interaction. In the case of magnesium, the association constant K_1 obtained with ligand **II** is only 5 times higher than constant K'_1 obtained with ligand **I**. Therefore, cation interaction on the unsaturated ligand core strongly competes with complexation on the crown ether and both phenomena are simultaneously visible in the absorption spectra. The high affinity of magnesium for the unsaturated system could be explained by the high charge density of this cation. In contrast, for calcium, constant K_1 is 100 times higher than constant K'_1, suggesting the preferential complexation of the crown ether. This explains that, with calcium, the crown ether is almost totally complexed before the interaction with the carbonyl group is observable in the spectra.

5. Conclusion

It was shown in this work that probe **II** is sensitive to the presence of cations. The spectroscopic properties of this dye are in good agreement with the theory of interaction of chromophores in symmetrical bis-chromophoric molecules: upon complexation with an alkali or alkaline-earth cation, the symmetry of crown dye **II** is broken resulting in substantial changes in the absorption and emission spectra. It seems that these variations are stronger than in the case of the squaraine dye reported by Das[16] and Akkaya[17] although the experimental conditions are different and therefore not comparable. The response of compound **II** is useful both in absorption and emission spectroscopy: the strong wavelength shifts observed in the presence of cations make the dye suitable for dual-wavelength analysis in self-calibrating measurements.

Molecule **II** is the first probe of a series designed with the purpose to measure cation concentration at the surface of biological membranes. The interactions with membranes will be the topic of further investigations, as will the behavior of the probe in solid matrices for optode preparation.

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Supporting Information Available: Table 4 displays the calculated molar extinction coefficients at the wavelengths used for data fitting, for complexes AM, A_2M, and AM_2 formed with each salt. This material is available free of charge via the Internet at http://pubs.acs.org.

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