A New Family of Luminescent Sensors for Alkaline Earth Metal Ions

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Abstract: The effects on the photophysical properties of three crown ethers (**1a–c**) containing the 1,8-dioxyxanthone residue caused by the complexation with Ba^{2+}, Sr^{2+}, and Ca^{2+} metal ions are reported. The strong enhancement of the fluorescence, the appearance of a long-lived delayed fluorescence, and a good selectivity towards Ba^{2+} ions prove that **1c** is an efficient fluorescent chemosensor for this ion. In addition, the range of selectivities shown by this family of crown ethers towards the alkaline earth metal ions together with their favorable photophysical properties, make them suitable for the construction of (multi)sensory devices. The differences in the photophysical properties between the uncomplexed and complexed crown ethers can be accounted for by the stabilization of a different ground state conformer of the dioxyxanthone moiety by the metal ion within the cavity of the crown ether.

Keywords: alkaline earth metals · fluorescence spectroscopy · luminescence · sensors · supramolecular chemistry

Introduction

The development of new chemosensors represents one of the more attractive challenges that chemists have to face nowadays. The synthesis of efficient chemosensors requires a thorough knowledge of the principles governing the processes of molecular recognition and signal transduction, that is the mechanism by which the complexation of the sensor with the analyte causes a change in the properties of the sensor itself.[1–6]

One of the properties normally required of a chemosensor is the selectivity towards a specific analyte. This feature is evidently important, and may be necessary for real-space applications. However, multisensory systems can be created with differing sensors which can recognize a specific family of analytes, each one with a different association constant. The signals from the whole set of sensors are then mathematically treated to give simultaneously the concentrations of all the analytes of interest. These devices, in analogy to the mammalian sensory systems, have been called electronic *noses* or *tongues* and represent a promising solution to complex analytical problems.[7, 8]

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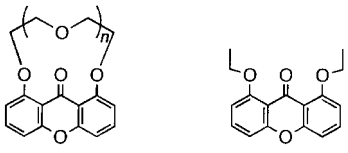
Of the various kinds of chemosensors, the luminescent variety present many advantages since luminescence measurements are usually very sensitive, are not costly, are easily performed, and are versatile.[1, 2] With this idea in mind, we have studied the luminescence response of a family of crown ethers incorporating the 1,8-dioxyxanthone chromophore upon complexation with alkaline earth metal ions.[9] We show here that these compounds are very promising for the construction of (multi)sensory devices.

Experimental Section

Materials: The solvents used were methanol and ethanol UVASOL from Merck without further purification. The crowns **1a–c** and an acyclic model **2** (Scheme 1) were available from earlier work.[9] The spectroscopic and chromatographic methods used, including ^1H and ^{13}C NMR and HPLC, established the purity of **1a–c** and of **2** as $>99{,}95\,\%$, a level adequate to eliminate the possibility of the effect described herein as being due to impurities.



Scheme 1. Crowns **1** (**a**: $n=3$; **b**: $n=4$; **c**: $n=5$) and acyclic model **2**.

Instrumentation: Absorption spectra were recorded with a Perkin–Elmer λ16 spectrophotometer. Uncorrected emission, corrected excitation spectra, and phosphorescence lifetimes were obtained with a Perkin–Elmer LS50 spectrofluorimeter.

The fluorescence lifetimes (uncertainty $\pm 5\,\%$) were obtained with an Edinburgh single-photon counting apparatus, in which the flash lamp was filled with D_2. Luminescence quantum yields (uncertainty $\pm 15\,\%$) were determined with quinine sulfate in aqueous 1N H_2SO_4 ($\Phi = 0.546$[10]) as a reference. In order to allow comparison of the emission intensities, corrections for instrumental response, inner filter effects, and phototube

0947-6539/98/0406-1090 $ 17.50+.50/0 *Chem. Eur. J.* **1998**, *4*, No. 6

sensitivity were performed.[11] A correction for differences in the refraction index was introduced when necessary. Degassed solutions were obtained with the freeze-thaw-pump method. Emission spectra at 77 K were obtained in a MeOH/EtOH (4:1, v/v) rigid, transparent matrix in quartz tubes immersed in a quartz Dewar filled with liquid N_2.

Metal-binding studies: UV and emission spectra were run on a solution of the crown ether in methanol (3 mL, 1×10^{-4} M). Aliquots (10 μL) of the salt solution (1×10^{-2} M) were then added with a micro syringe and the spectra recorded. The emission spectra were obtained by excitation of the solution at an isobestic point. The intensity, read at the maximum of the adduct band, was fitted to $I_{corr} = \phi_{cr}C_{cr} + \phi_{cm}C_{cm}$, where C_{cr} and C_{cm} are the concentrations of uncomplexed and complexed crown, respectively, and ϕ_{cr} and ϕ_{cm} are the proportionality constant between the corrected emission intensity (in arbitrary units) and the concentration of the uncomplexed and complexed crown ether, respectively. C_{cm} satisfies the usual binding expression in Equation (1) where M_{tot} is the total concentration of added metal ion.

$$C_{cm}^2 - [(C_{cm} + C_{cr}) + M_{tot} + 1/K]C_{cm} + M_{tot}(C_{cm} + C_{cr}) = 0 \quad (1)$$

Values for the equilibrium constant, K, were then obtained by simulation of the data with both K and φ_{cm} as adjustable parameters, using a Newton–Raphson procedure to minimize the sum of squares of residuals.

Results

Absorption spectra: The absorption spectra of the crown ethers **1a–c** in methanol (Table 1 and Figure 1) are similar to the absorption spectrum of the model compound **2**.

As already observed,[9] addition of Ca^{2+}, Sr^{2+}, and Ba^{2+} metal ions induces profound changes in the absorption spectra of the crown ethers (Figure 1 for **1c** and Ba^{2+}), while little or no change was observed on addition of Mg^{2+} and the alkali metal ions. Neither were changes observed when metal ions were added to a solution of **2**. The changes observed were dependent on the crown ether and on the metal ion added in all cases (Table 1).

Table 1. Relevant absorption data of the crown ethers **1a–c** and their complexes in solution in methanol.

	λ_{max} [nm]	ε ([$M^{-1}cm^{-1}$])
1a	305	12000
	339	5900
1a·Ba^{2+}	308	11400
	347	5600
1a·Sr^{2+}	308	11400
	343	5600
1a·Ca^{2+}	306	11600
	340	5500
1b	305	11700
	341	5600
1b·Ba^{2+}	313	12200
	352	5400
1b·Sr^{2+}	314	12500
	351	5500
1b·Ca^{2+}	308	10900
	340	5500
1c	305	12500
	341	6000
1c·Ba^{2+}	313	12400
	349	5900
1c·Sr^{2+}	309	11200
	347	5350
1c·Ca^{2+}	[a]	[a]

[a] Association constant too low to obtain complete association.

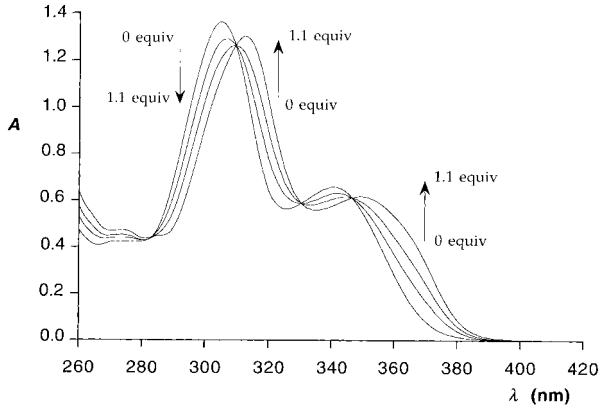


Figure 1. Absorption spectra of a solution of **1c** in methanol (1.10×10^{-4} M) upon addition of an increasing amount (0, 0.34, 0,67, and 1.1 molar equivalents) of $BaCl_2$.

Room-temperature luminescence spectra: In aerated methanol solution at room temperature, the crown ethers **1a–c** and the parent chromophore **2** show only a very weak fluorescence ($\Phi < 10^{-4}$, Table 2). While no changes were observed for **2**, the addition of Ca^{2+}, Sr^{2+}, and Ba^{2+} metal ions to a crown ether solution produced a strong increase in the fluorescence band and changed its peak (see Figure 2 for **1c** and Ba^{2+}). As can be seen from Table 2, the position of the peak, the luminescence quantum yield, and the excited state lifetime depend slightly on the crown ether and on the added metal ion. The excitation spectra parallel the changes observed for the absorption spectra. No luminescence increase was observed on addition of Mg^{2+} or alkali metal ions.

Table 2. Room-temperature luminescence properties of **2**, of the crown ethers **1a–c**, and of their complexes with the alkaline earth metal ions in solution in methanol.

	λ_{max} [nm]	Φ	τ_1[ns]	τ_2 [μs][a]	K_{ass} [M^{-1}]
2	439	$< 10^{-4}$	< 0.5	–	–
1a	440	$< 10^{-4}$	< 0.5		
1a·Ca^{2+}	434	0.12	9	20	1.7×10^2
1a·Sr^{2+}	437	0.033	1.5	11	4.0×10^2
1a·Ba^{2+}	443	0.017	1.1	18	1.9×10^3
1b	440	$< 10^{-4}$	< 0.5	–	–
1b·Ca^{2+}	435	0.17	11.8	45	1.7×10^3
1b·Sr^{2+}	424	0.019	1.1	52	$3{,}8 \times 10^4$
1b·Ba^{2+}	434	0.029	1.3	54	$6{,}5 \times 10^4$
1c	441	$< 10^{-4}$	< 0.5	–	–
1c·Ca^{2+}	445	0.17	12.0	32	7.0×10^1
1c·Sr^{2+}	435	0.086	5.9	38	1.2×10^3
1c·Ba^{2+}	430	0.0083	1.0	20	8.9×10^4

[a] Lifetime of the delayed luminescence. See the text for more details.

It is of note that in some cases, the fluorescence quantum yield increases more than a 1000-fold in going from the crown

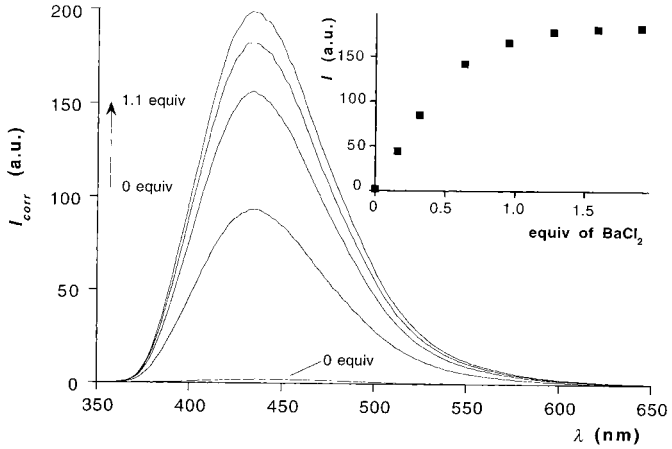


Figure 2. Luminescence spectra ($\lambda_{exc} = 309$ nm) of an aerated methanol solution of **1c** upon addition of an increasing amount (0, 0.40, 0,67, 0,90, and 1.1 molar equivalents) of $BaCl_2$. Inset: The corrected value of the intensity read at 430 nm versus the equivalents of added $BaCl_2$.

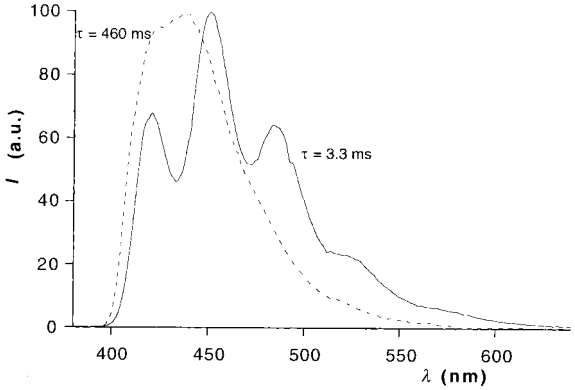


Figure 3. Phosphorescence spectra of **2** ($\lambda_{exc} = 340$ nm) in a MeOH/EtOH (4:1, v/v) rigid matrix at 77 K recorded with a delay time of 5 μs and a gate time of 500 μs (- - - -) and with a delay time of 100 ms and a gate time of 80 ms (—).

ether to its complexed form. Therefore association constants between the crown ether and the metal ion can be determined by monitoring the changes in the luminescence intensity, even if the changes in the absorption spectra are very small. Table 2 presents the association constants determined by this method; these values are in good agreement with those previously determined for Sr^{2+} and Ba^{2+} by a spectrophotometric method.[9] The complexation process does not depend on the pH of the solution, except in very strongly acidic conditions.[9]

Interestingly, degassed solutions of all the complexes studied show, together with the above reported fluorescence, a delayed fluorescence with a lifetime in the range 10–60 μs (Table 2), which is 10^4 times longer than that of the prompt fluorescence. Time-resolved spectra show that the delayed luminescence has the same band shape and excitation spectra as the fluorescence observed in aerated solution. No delayed luminescence was observed for deaerated solutions of **2** and of the uncomplexed crowns **1a–c**.

Low-temperature luminescence spectra: The luminescence spectra of **1a–c** and **2** at 77 K show interesting behavior. While only weak fluorescence was observed, the parent chromophore and the crown ethers show two relatively intense, long-lived phosphorescence bands (Table 3). Thanks to the large differences in their lifetimes, the two bands can easily be time-resolved. As can be seen in Figure 3 for **1c**, the two bands lie in the same spectral region and, in particular, the highest energy features of the two bands peak at similar wavelengths. The structures of the two bands, however, differ, showing that the emissions originate from different excited states. The excitation spectra are also different, as seen in Figure 4.

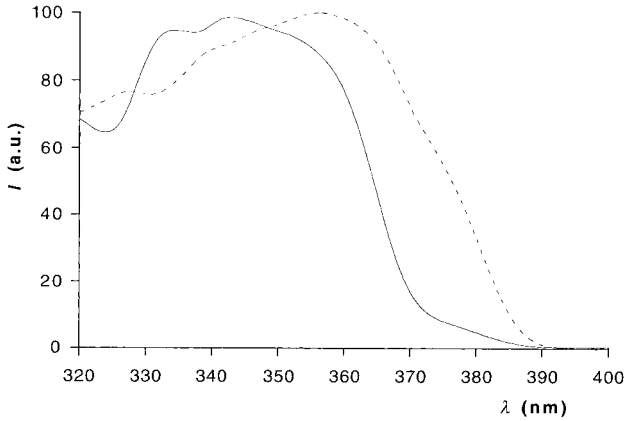


Figure 4. Excitation spectra of **2** ($\lambda_{em} = 450$ nm) in MeOH/EtOH (4:1, v/v) rigid matrix at 77 K recorded with a delay time of 5 μs and a gate time of 500 μs (- - - -) and with a delay time of 100 ms and a gate time of 80 ms (—).

Table 3. Phosphorescence data of **2**, **1c**, and **1c**·Ba^{2+} (77 K, MeOH/EtOH 4:1).

	λ_{max} [nm]	τ [ms]
2	428, 457, 491	3.0
	435	740
1c	421, 451, 484	3.3
	422(s), 438	460
1c·Ba^{2+}	427	300

If alkaline earth metal ions (except for Be^{2+} and Mg^{2+}) are added in excess to a solution of one of the crown ethers, however, only one phosphorescence band is observed (Figure 5), and the luminescence decay profile can be fitted with a single exponential. The band shape, the energy, the lifetime, and excitation spectra are, in all cases, very similar to the longer lived component of the free crown ethers (Table 3).

Discussion

The very low fluorescence quantum yield at room temperature of **2** and **1a–c** closely parallels the behavior of xanthone itself, for which a very efficient intersystem crossing (>99%) was reported.[12–14] As discussed above, the luminescence enhancement on addition of alkaline earth metal ions to the crown ethers **1a–c** is dramatic. The fluorescence quantum yield increases in going from the crown ether to the complexed species by more than one hundred times, and, if

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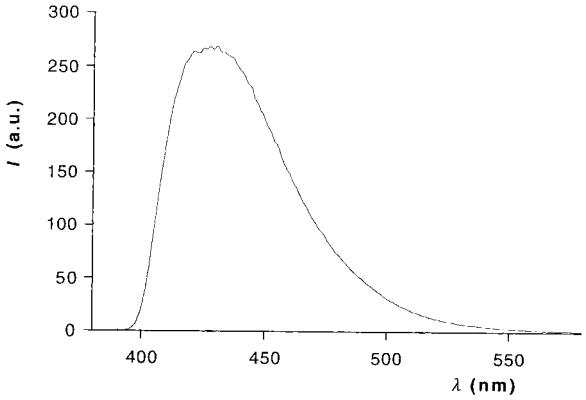


Figure 5. Phosphorescence spectra of **1c**·Ba^{2+} (λ_{exc} = 340 nm) in MeOH/EtOH (4:1, v/v) rigid matrix at 77 K recorded with a delay time of 100 ms and a gate time of 80 ms.

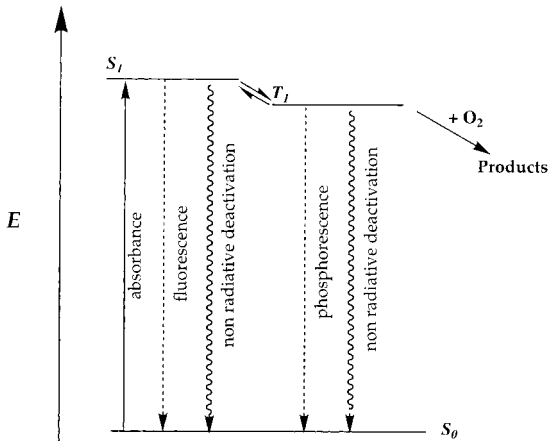


Figure 6. Schematic representation of the lowest energy levels and corresponding photophysical processes at room temperature for a complexed species.

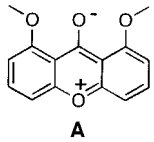
excited in the 370–380 nm region where only the complexed species absorbs, the enhancement factor for the observed fluorescence is even larger. Since all the measured quantum yields for the complexed species are rather high (from 0.008 to 0.2, Table 2), the system shows an effective off/on response to the complexation of alkaline earth metal ions. This kind of response is one of the most desirable properties that a luminescent sensor should have. Furthermore, **1c** shows good selectivity towards Ba^{2+} ions (see K_{ass} values in Table 2), so that this crown ether can act as a sensor for Ba^{2+} in the presence of the alkaline and the alkaline earth metal ions Na^+, K^+, Cs^+, Mg^{2+}, Sr^{2+} (10-fold excess) and Ca^{2+} (100-fold excess), even though the Ba^{2+} complex has the lowest quantum yield.

The whole family of crown ethers reported here can be seen as an array of chemical sensors with partial specificity for the different alkaline earth metal ions, and can therefore be used for the construction of an *electronic tongue*[7] which can, in principle, determine simultaneously the concentrations of Ba^{+2}, Sr^{2+}, and Ca^{2+} as a function of time. An additional relevant property for physiological applications is that the complexation does not strongly depend on the pH of the solution.

The delayed fluorescence observed in degassed solutions can be accounted for by the presence of an equilibrium between the fluorescent singlet excited state and a lower lying and longer living triplet state (see Figure 6 and below). The fact that in all cases an identical bandshape was observed for prompt and delayed fluorescence rules out the possibility of an involvement of different luminescent excited states. A delayed fluorescence due to an equilibrium between the singlet and a triplet excited state was also observed for other aromatic ketones, such as benzophenone.[15] The presence of molecular oxygen is most probably responsible for the fast deactivation of the triplet excited state in solutions which had not been degassed.

The phenomenon of delayed fluorescence has some importance in terms of applications. One of the main problems in the analysis of the fluorescence of very complicated systems is the background signal arising from scattered light and from the luminescence of other fluorophores (in biological samples, for example, from amino acids). This noise is usually very short-lived (usually it lasts less than 10 ns), and time-resolved spectroscopy can help considerably to increase of the signal-to-noise ratio if the analyte shows a longer lifetime.[16] In particular, a lifetime in the microsecond region, as shown by the complexed species, can allow a very efficient time resolution with commercially available pulsed-lamp spectrofluorimeters, so that a very high sensitivity is possible. The main drawback is that the complexed species may not come into contact with molecular oxygen.

As far as the spectra recorded at 77 K are concerned, the double phosphorescence shown by **2** and **1a–c** is clearly reminiscent of the behavior of the xanthone itself, for which a double phosphorescence is also observed.[12–14] One of the most plausible explanations for this was the presence of two different conformers, interconverting at room temperature, with their own set of ground and excited states.[12] While the shorter lived component was assigned to a configuration in which the dihedral angle between the two benzene rings deviates from planarity, the longer lived component was assigned to a planar conformer in which the form **A** can be accommodated.



A

This explanation would also account for the different excitation spectra obtained for the two phosphorescence bands. The 1,8-dialkoxyxanthone chromophore seems to behave in a similar way. X-ray structures and molecular mechanics calculations show that the most stable conformation of uncomplexed 1,8-dialkoxyxanthones is nonplanar; there is a fold about the central ring such that the angles between the planes of the benzene rings are between 172 and 163°.[9] The disappearance of the short-lived component in **1a–c** on addition of the metal ion, which stabilizes the planar conformer, is additional evidence for the presence of two different ground state conformers in the uncomplexed species. We believe that the stabilization of the planar isomer at room temperature is the reason for the much higher fluorescence quantum yield shown by the complexed species.

Conclusion

We have demonstrated that one of the crown ethers studied, namely **1c**, has all the necessary characteristics for an efficient fluorescent chemosensor for Ba^{2+} ions: good selectivity towards this analyte, a very large fluorescence enhancement factor, and a substantial independence of the pH of the solution. In addition, if molecular oxygen is not allowed to interact with the complexed species, the observed delayed luminescence can be used to improve the signal-to-noise ratio and, in this way, the sensitivity of the sensor. The whole family of the crown ethers could also be used to produce multisensory devices, such as the so-called electronic tongues, in order to monitor simultaneously the concentrations of Ba^{2+}, Sr^{2+}, and Ca^{2+} metal ions. The results obtained can be explained in terms of a stabilization of one of two possible isomers caused by the complexation of the ion.

Acknowledgements: This work was supported by MURST, CNR, University of Bologna (Funds for Selected Research Topics) in Italy, and by EPSRC and Zeneca FCMO in the UK.

Received: September 8, 1997 [F815]

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