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Fluoroionophores with Phenanthridinyl Units

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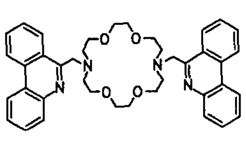
Abstract: Novel fluoroionophores bearing two (1) and one (2) fluorophoric 6-phenanthridinyl units attached by a short methylene spacer onto nitrogen atoms of 7,16-diaza- and aza-18-crown-6 rings have been prepared in high yields. The fluoroionophores exhibited the ability to signal binding of certain metal cations through the specific complexation induced shifts (CIS) of phenanthridine protons in ^1H NMR spectra as well as by the large intensity enhancements in fluorescence spectra. The X-ray structure analysis of [1, $K^+(C_2H_4Cl_2)$] picrate complex revealed that K^+ is nine-coordinated, using all donors from the diazacrown ring, one chlorine atom from 1,2-dichloroethane solvent molecule and both nitrogen donors from *syn*-oriented phenanthridine units.

The attachment of fluorophoric units onto crown ethers gives fluoroionophores, molecules capable of signaling the binding of certain metal cations through the specific fluorescence response[1-9]. Fluorophoric units used to date in combination with crown ethers include naphthalene[1], tryptophane[2], pyrene[2,3], benzene[4], flavin[5], 1,4-benzoxazin-2-one derivative[6], the laser dye coumarin 153[7] and, the most frequently, anthracene[8,9]. Tsien's fluoroionophore, bearing benzofuran isophthalate as the fluorophoric unit, is of biological importance, exhibiting sensitivity to cytosolic concentrations of free Na^+ and Na^+/K^+ selectivity of about 20[10]. Among the crown type of fluoroionophores those bearing pendant fluorophoric arms with additional cation binding site showed enhanced cation binding ability together with strong dependence of their photophysical properties on the charge density of the bound cation[7,10]. It appears that the major properties of a fluoroionophore such as cation recognition and signaling through specific fluorescence response could be modulated by correct selection of the fluorophoric pendant arm carrying additional cation binding functionality. In such a case the recognition of cations by their size and also by their charge density could be achieved. With this in mind we have prepared the first phenanthridine containing fluoroionophores **1** and **2**, whose phenanthridine nitrogens are expected to participate in cation binding and whose fluorescence response should be affected by the same process. In the present communication we report on the synthesis of **1** and **2** and their Na- and K-picrate and iodide complexes. We also present evidence that **1** and **2** are able to signal the binding of Na^+ and K^+ by specific complexation induced shifts (CIS) of



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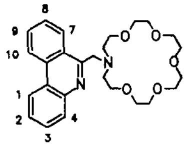

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phenanthridine protons in ^{1}H-NMR spectra, that **1** binds K^+ with participation of phenanthridine nitrogens, and that the large intensity enhancements in fluorescence spectra are produced in the presence of alkaline earth cations and not with alkaline ones presumably due to the greater charge densities of the former.

The ionophores have been prepared by *N*-alkylation of 7,16-diaza- and aza-18-crown-6 with 6-chloromethylphenanthridine[11] (acetonitrile, Na_2CO_3 /NaI) giving [**1**, Na^+] I^- and [**2**, Na^+] I^- complexes in 93 and 92 % yield, respectively. Sodium iodide was removed from the complexes by water extraction of their CH_2Cl_2 solutions, yielding, after evaporation of the solvent, free **1** and **2**. The Na- and K-picrate and KI complexes of **1** and **2** have been prepared by extraction of the aqueous solution of the respective salts with CH_2Cl_2 solution of the ligand[12].

In the ^{1}H-NMR spectra of **1** and **2** complexed with Na- or K-iodides or picrates strong complexation induced shifts (CIS) have been observed, giving considerably different phenanthridine signal patterns for Na- and K-complexes with the same ligand[13]. The CIS values [CIS(ppm) = $\delta_{H(i)compl.} - \delta_{H(i)lig.}$] for each of the phenanthridine protons in the complexes of **1** and **2** with Na-, K-iodides and picrates are shown graphically in Figure 1. The CIS values for [**1**, Na^+] I^- and [**1**, Na^+] picrate are very close, giving almost identical curves, the same also being the case for [**1**, K^+] I^- and [**1**, K^+] picrate complexes. On the other hand, the CIS values for **1** complexed with Na^+ and K^+ are clearly distinct, showing that phenanthridine H-2, H-3 and H-4 are more shielded in the complexes with K^+. This result suggests that the observed CIS values for Na- and K-complexes of **1** depend mostly on the cation being complexed in the diazacrown ring and not on the present anion, excluding possible picrate-phenanthridine stacking interactions. Consequently, the difference between CIS values observed for Na- and K-complexes of **1** originate most probably from slightly different mutual positions of two *syn*-oriented phenanthridines, resulting in shielding or deshielding of some of their protons relative to free **1**.

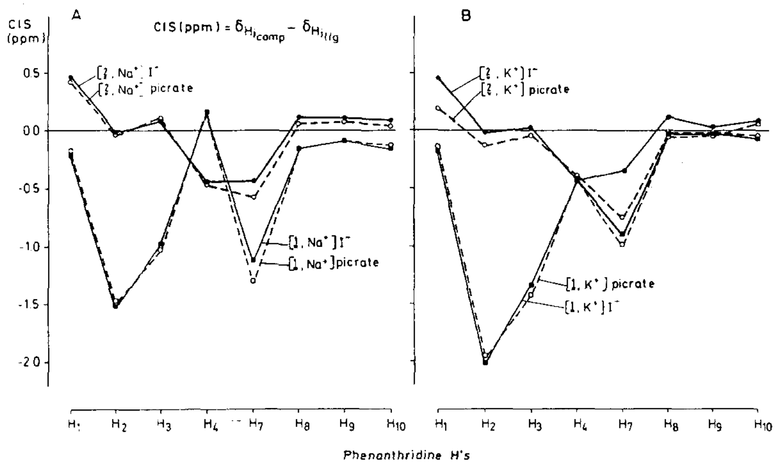

Figure 1. The graphical representation of the complexation induced shifts (CIS) of phenanthridine protons in ^{1}H NMR spectra of complexes of **1** and **2** with Na- and K-iodides and picrates.

The CIS values observed for the complexes of **2** (Figure 1) are generally less negative than those observed for complexes of **1** with the same salts. At variance to the complexes of **1** where the mutual aromatic shielding of two phenanthridine units presents the major contribution to CIS values, in the complexes of **2** bearing one phenanthridine unit the CIS values are most likely the consequence of the complexation induced conformational changes and/or "through bond" electronic effects. While the CIS values for [**2**, Na^+] I^- and [**2**, Na^+] picrate are very close (Figure 1A), those for KI and K-picrate complexes are clearly distinct with the phenanthridine H-7 being the most strongly shielded in the picrate complex (Figure 1B). This, together with the fact that picrate H's are also shifted upfield in the spectrum of the latter complex ($\delta_{picrate}$ 7.99 ppm; $\delta_{picrate}$ in [**1**, Na^+], [**1**, K^+], [**2**, Na^+] picrate and in [7,16-diaza-18-crown-6,K^+] picrate complexes is around 8.6 ppm) strongly indicate the stacking interactions between the picrate anion and the phenanthridine unit of **2** resulting in the mutual aromatic shielding of their protons.

The basic structural features of complexes of **1**, revealed from ^{1}H-NMR study, have been confirmed by the X-ray crystal structure of [**1**, K^+ ($C_2H_4Cl_2$)] picrate 1:1 complex[14] (Figure 2). The structure reveals the nine-coordinated K^+: four oxygen atoms (O1, O4, O10, O13), two nitrogen atoms from the crown (N7,N16), two more nitrogens from the *syn* positioned phenanthridine units (N51, N52) and one chlorine atom (Cl2) of a solvent molecule - dichloroethane coordinated to K^+. The crown exhibits a noncrystallographic two-fold symmetry with the phenanthridine units being antiparallel and approximately C_2 symmetric; the dihedral angle between the least-squares planes of phenanthridine units is 1.8(1)° with an interplane distance of 3.51(1) Å, showing that the phenanthridine units are practically stacked together. The structure nicely explains the differences of CIS values measured for Na^+ and K^+ complexes of **1**. It is reasonable to assume that both cations bind with **1** in the same way, engaging all of the crown and phenanthridine nitrogen donors. Consequently, **1** must adapt itself to their different ionic sizes by changing the conformation of the azacrown ring and the mutual position of the phenanthridine units, which results in stronger or weaker shielding of some of the phenanthridine protons.

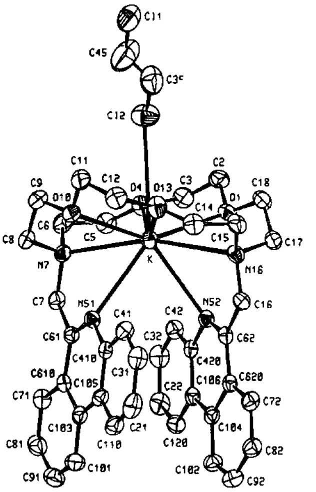

Figure 2. Molecular structure of [**1**,($C_2H_4Cl_2$)K^+] cation with coordinated solvent molecule (ORTEP thermal ellipsoids at the 30% probability level). For clarity all hydrogen atoms are omitted.

The fluoroionophores **1** and **2** when excited at 240 nm exhibited weak emission at 350 - 370 nm with the intensity of fluorescence (I_f) depending on solvent. In 10^{-6} M ethanolic solution the emission intensity of **2** (I_f 100; $\lambda_{emiss.}$ 365 nm) having one phenanthridine unit is approximately two times higher than that of **1** (I_f 55; $\lambda_{emiss.}$ 367) bearing two phenanthridine units, indicating the possible self-quenching of the *syn*-positioned phenanthridines in **1**. While fluorescence spectra of both **1** and **2** are strongly affected by addition of various amounts of alkaline earth cations the alkaline cations produced only minor effects (Figure 3). In the presence of Sr^{2+}, Ca^{2+} and Ba^{2+}, the I_f values of both **1** and **2** are enhanced, whereas Na^+ and K^+ have a small opposite effect, decreasing slightly the emission intensity of **1** and **2**. The presence of Mg^{2+} has no detectable influence. The effects of alkaline earth cations are much stronger for **2** than for **1**, being in both cases dependent on cation to ligand ratio. The cations also produced remarkable shifts of the emission band maxima, the largest from 365 and 367 nm for free **1** and **2** to 382 and 376 nm, respectively, being observed in the presence of Ca^{2+} ions.

In conclusion, the preliminary fluorescence studies revealed the sensitive and specific response of **1** and especially **2** to alkaline earth cations, indicating their potency as fluorescent sensor molecules or reagents for such cations.

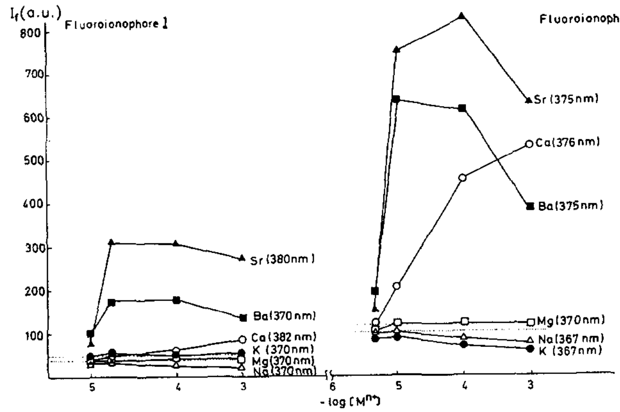

Figure 3. The influence of various concentrations of metal cations (added as nitrate salts) (M^{n+}, n=1,2; ▲, Sr^{2+}; ○, Ca^{2+}; ■, Ba^{2+}; □, Mg^{2+}; △, Na^+; and ●, K^+) on intensity of fluorescence (I_f) of fluoroionophores **1** and **2** in ethanol; dotted lines indicate the range of I_f increase for free **1** and **2** during the total measurement time; [**1**]=5.12 x 10^{-6} M; [**2**]=5.76 x 10^{-6} M; excitation 240 nm; emission 367-382 nm; the wavelengths of the emission maxima in the presence of cations are given in parentheses.

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12. The complexes [**1**,Na^+] picrate, [**1**, K^+] I^-, [**1**,K^+] picrate, [**2**, Na^+] picrate, [**2**, K^+] I^- and [**2**, K^+] picrate have been prepared by extraction of the aqueous solution of the respective salt with CH_2Cl_2 solution of **1** or **2** followed by evaporation of CH_2Cl_2 and recrystallization of the complex formed. All of the complexes prepared as well as **1** and **2** had correct spectroscopic and elemental analysis data.
13. To determine the CIS values the assignment of all phenanthridine protons of **1** and **2**, free and complexed was necessary. This was accomplished by COSY and NOESY experiments performed for free **1** and **2** and each of their complexes. In each NOESY spectrum the NOE interactions between the phenanthridine H-4 and H-7 and crown -CH_2O- and phenanthridine 6-CH_2, respectively, have been observed. This enabled then the full assignement of phenanthridine protons from COSY spectra. All one- and two-dimensional ^1H-NMR spectra were recorded at 298 K with a Varian Gemini 300 spectrometer. COSY and NOESY experiments were performed using standard sequence of the Varian Gemini software package; mixing time for NOESY spectra were 0.4 s.
14. Suitable crystals for the X-ray structure analysis were obtained by crystallization from ethyl acetate/ethanol/dichloroethane solvent mixture at 298 K. The final X-ray data are deposited at the Cambridge Crystallografic Centre.

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