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Magnesium and calcium chelation by a bis-spiropyran

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Abstract

A bis-benzospiropyranindoline was prepared by a simple two-step procedure. The magnesium and calcium chelating ability of this photochromic spiropyran was investigated and compared to simple mono-spiropyrans. Kinetic binding constants were measured. Moderately strong metal binding occurs in acetone solution (K=40 000 M^{-1} for Mg, K=13 000 M^{-1} for Ca) when the bis-spiropyran is irradiated with light at 365 nm. This binding is eight times higher than the binding of the mono-spiropyrans studied. The color of the merocyanine form of the bis-spiropyran ($\lambda_{\max}$=548 nm) is strongly influenced by the metal, blue-shifting the maximum absorbance 43 nm (Mg) and 22 nm (Ca). Strong fluorescence is observed when the bis-spiropyran complexed to either metal is irradiated at 365 nm, with emission maxima of 586 nm (Mg) and 606 nm (Ca). The strength of the binding is inversely correlated to the unimolecular decomposition rate constant of the spiropyran–metal complex. The fluorescence emission maxima become increasingly blue-shifted as the strength of the binding increases. The fluorescence is compared to the metal-free spiropyran, as well as to simple mono-spiropyrans coordinated to calcium. The mechanism of decoloration of the bis-spiropyran with and without metals present is discussed. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Spiropyran; Magnesium; Calcium; Chelation; Kinetics; Fluorescence

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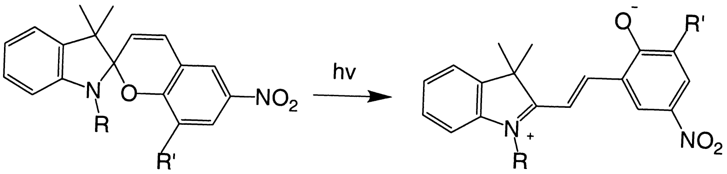
1. Introduction

The appealing nature of photochromic spiropyrans has prompted a number of metal binding studies of derivatives of these molecules [1,2]. The spiropyrans are photoconverted between the closed (spiro) form and the colored open (merocyanine) form, as depicted in Eq. (1).



(1)

For metal binding applications, the negatively charged phenolic oxygen in the zwitterionic open form may bind to a metal in cooperation with other ligating groups, attached at R and R′ in Eq. (1). In such compounds, metal binding can be influenced by light; normally, ultraviolet light produces high concentrations of the open form, and enhances metal binding; visible light produces high concentrations of the spiro form, and hinders metal binding. In fact, a number of interesting systems have been developed, displaying photodynamic transport [3], cation extraction [4], and alkali metal recognition [5]. More complicated systems, with multiple metal binding sites [6] and two spiropyrans attached to a crown ether [7], have been prepared. Other work has focused on equilibrium binding of copper [8], and the binding of other divalent metals to hetero-substituted spiropyrans [9–11].

Most of these systems contain only one spiropyran unit per molecule, meaning only one additional atom is made available for metal binding when the molecule is opened up.

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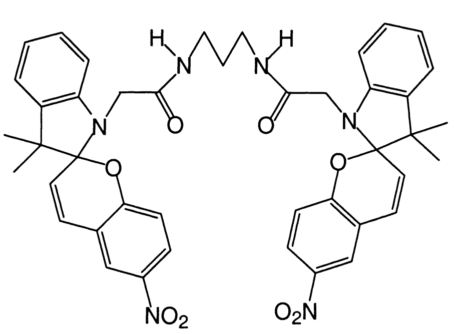
This could have two consequences: first, the molecule could show only a small additional ability to bind a particular metal ion when opened, and second, the unopened molecule, because it has all but one of its ligating atoms intact, will display high 'dark' binding. Thus, the possible high binding of the closed form will make it difficult to detect enhanced binding in the open form, if it is there. Even in the case of the bis-spiropyran featured in [7], the crown ether part of the molecule still displays high dark binding, although the presence of two spiropyrans imparts unusual photoswitchable selectivity to the molecule. No system examined to date has attempted to reveal the intrinsic binding of an open spiropyran to a metal, that is, binding that is not influenced in large part by ancillary ligands.

In order to develop photochromic ligands for removing metals such as calcium from waste streams, we have endeavored to study the intrinsic binding of photochromic molecules to metal ions by producing molecules that contain two or more spiropyran units attached to a flexible backbone, such that the phenolic oxygen atoms of the open form can converge on a single metal atom and bond to it. Such a molecule would be expected to be indifferent toward a metal in the closed form (low dark binding), so that if binding occurs in the open form, even to a small extent, such binding should be well above the background, and thus easy to detect. Other, better spiropyran-based chelators can then be designed, once the factors that control spiropyran metal binding are understood. Herein we report our first efforts at putting this idea into practice: the synthesis of bis-spiropyran-bis-amide **1** and its unusual chelating ability toward magnesium and calcium. For comparative purposes, we have also examined the binding of two mono-spiropyrans, methyl spiropyran (R=methyl, R′=H in Eq. (1)) and the spiropyran-amide **2**. We also describe the strong fluorescence displayed by these spiropyrans in the presence of magnesium and calcium ions in acetone solution.



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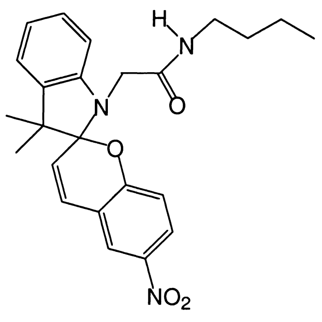

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2. Experimental

Reagents were obtained from commercial suppliers and used without further purification. UV-Vis spectra were recorded on an Hewlett–Packard 8453 diode array spectrometer and fluorescence spectra were recorded on a Spex Fluorolog 1680 spectrometer. Kinetic experiments were performed at 25°C. Repeated measurements of k_1 and the other rate constants gave values with a standard deviation of 3%; the reported binding constants have an accuracy estimated at ±10%. UV light (365 nm) for inducing coloration of the spiropyrans was supplied by a lamp (Spectroline model ENF 280-C) held **1** in from the cuvettes immediately before recording the spectra of the solutions or measuring their decoloration kinetics. Irradiation times of 2 min for all samples gave maximum absorbance values at the λ_{max} of the merocyanine form of the spiropyrans. All solutions were colorless (A<0.01) at infinite time (greater than 20 half-lives). The metals were introduced into acetone solution as $Mg(NO_3)_2 \cdot 6H_2O$ or $Ca(NO_3)_2 \cdot 4H_2O$. Methyl spiropyran (R=methyl, R′=H in Eq. (1)) was prepared by refluxing 2-methylene-1,3,3-trimethylindoline and 5-nitrosalicylaldehyde in ethanol and recrystallizing from this solvent. The other spiropyrans were made from the corresponding chloroacetamides, by a method adapted from Inouye et. al. [5]. Chloroacetamides were made by the Schotten–Baumann procedure [12].

2.1. *Bis-spiropyran-bis-amide* 1

A mixture of 1.02 g (6.4 mmole) 2,3,3-trimethylindolenine, 0.73 g (3.2 mmole) 1,3-bischloroacetamidopropane, 0.96 g (6.4 mmole) NaI, and 30 ml acetonitrile was refluxed overnight under N_2. The dark mixture was poured into 6 g NaOH dissolved in 50 ml water, and was allowed to stir for 10 min. The orange organic phase was separated and treated with 1.1 g (6.4 mmole) of 5-nitrosalicylaldehyde and an additional 15 ml acetonitrile, and distilled. After 20 ml of distillate was collected (to remove water), the mixture was refluxed for 5 h. The solvent was then removed by rotary evaporation, and the residue was taken up in ethyl acetate (10 ml), filtered, and loaded onto 30 g silica gel. The first colored band that eluted with ethyl acetate was collected, the solvent was removed, and the residue was taken up in hot anhydrous ethanol (5 ml). After standing overnight, 1.2 g of solid was deposited, which could be recrystallized from anhydrous ethanol to give 0.36 g (14.7%) of yellow microcrystals. Melting point 178–183, IR (thin film) 2961, 2952, 2856, 1656, 1607, 1581, 1513, 1479, 1334, 1274, 1092, 950, 818, 748 cm^{-1}, ^{1}H NMR ($CDCl_3$, 300 MHz) d: 1.26 (6H, s,CCH_3), 1.29 (6H, s,CCH_3), 3.15 (4H, d of d, *J*6, N$\mathit{CH_2}$$CH_2CH_2$N), 3.58 (2H, d, *J* 17, NCH_2CO) 3.70 (2H, m, *J* 7, $NCH_2$$\mathit{CH_2}$$CH_2N$), 3.83 (2H, d, *J* 17, NCH_2CO), 5.94 (2H, d, *J* 10, vinyl-H), 6.46–8.03 (18H, m, ArH, vinyl-H, NH). Electrospray MS (chloroform/methanol, 50/50) *m/z* 771 (M$^+$, 100%). Anal. $C_{43}H_{42}N_6O_8$: C 67.00, H 5.49, N 10.90. Found C 65.91, H 5.44, N 10.81.

2.2. *Spiropyran-amide* **2**

A mixture of 0.95 g (6.4 mmole) *N*-butylchloroacetamide and 1.0 g (6.3 mmole) 2,3,3-trimethylindolenine and 0.96 g (6.4 mmole) NaI was refluxed overnight. The reaction mixture was treated in a fashion analogous to the procedure used for compound **1**, to give 0.14 g (5%) of **2**. Melting point 197–198, IR (thin film) 2961, 2921, 2850, 1657, 1591, 1511, 1475, 1338, 1272, 1089, 954, 798, 747 cm^{-1}, ^{1}H NMR ($CDCl_3$, 300 MHz) d: 0.852 (3H, t, *J*7, $\mathit{CH_3}$$CH_2CH_2CH_2N$), 1.17–1.45 (4H, m, $CH_3$$\mathit{CH_2CH_2}$$CH_2N$), 1.23 (3H, s, CCH_3), 1.31 (3H, s, CCH_3), 3.23 (2H, d of d, *J* 6, $CH_3CH_2CH_2$$\mathit{CH_2}$N), 3.67 (1H, d, *J* 17, NCH_2CO), 3.86 (1H, d, *J* 17, NCH_2CO), 5.80 (1H, d, *J* 10, vinyl-H), 6.41 (1H, bt, *J* 5, NH), 6.5–8.0 (8H, m, ArH, vinyl-H). Anal. $C_{24}H_{27}N_3O_4$: C 68.39, H 6.46 N 9.97. Found C 68.54, H 7.05 N 9.97.

3. Results and discussion

The syntheses of spiropyrans **1** and **2** proceed from the readily accessible chloroacetamide precursors. The low yields of the final products reflect un-optimized conditions, and the low yields reported for these reactions [5].

Acetone solutions of compounds **1** and **2** are photochromic. Thus, after irradiation at 365 nm, the bis-amide-bis-spiropyran turns purple; in the presence of calcium ion, it turns pinkish red. The spiropyran–amide appears blue purple after irradiation; in the presence of calcium it turns red-purple. Similar color changes were seen for the spiropyrans in the presence of magnesium. The visible spectra for the bis-spiropyran alone and in the presence of the metals are shown in Fig. 1, and the spectral features for all the compounds and complexes in this study are summarized in Table 1. The spiropyrans in the presence of either magnesium or calcium displayed strong orange fluorescence under UV (365 nm) irradiation, which grew in intensity as the solutions became more colored, suggesting that the fluorescent entity was the open form of the spiropyran. It also appeared that the metals were important for this unusual emission (vide infra).

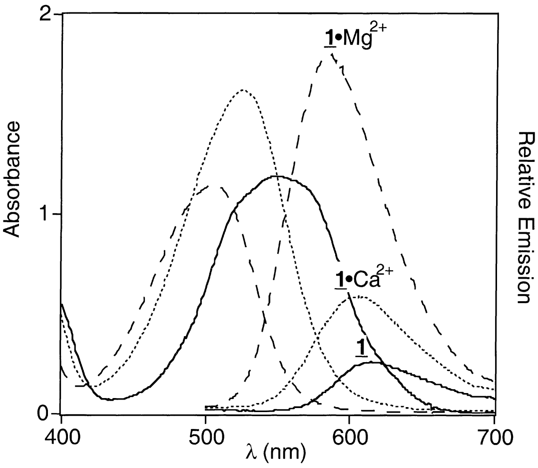


Fig. 1. Visible spectra and fluorescence emission spectra (λ_{ex}=365 nm) for the bis-spiropyran-bis amide **1** (solid line) and its complexes with magnesium (dashed line) and calcium (dotted line). Absorption spectra: [**1**]=0.05 mM, [Ca^{2+}]=[Mg^{2+}]=3.0 mM. Emission spectra: [**1**]=1.3 μM, [Ca^{2+}]=[Mg^{2+}]=100 μM.

In order to characterize the spiropyran–metal complexes, detailed kinetic binding constants were measured, using the method of Song [11]. Eqs. (2)–(4) (where Sp_c and Sp_o represent the closed and open spiropyran, respectively), can be used to derive Eq. (5),

$$Sp_c \underset{k_1}{\overset{UV}{\rightleftharpoons}} SP_o \tag{2}$$

$$Sp_o + M \overset{K_{eq}}{\rightleftharpoons} Sp_o \cdot M \tag{3}$$

$$Sp_o \cdot M \xrightarrow{k_2} SP_c + M \tag{4}$$

Table 1

Visible spectral features, corrected fluorescence emission maxima (excitation at 365 nm), metal binding constants (K_{eq}) and decomposition rate constants (k_2) of compounds **1** and **2** and their metal complexes in acetone solution

Spiropyran	λ_{max} (nm)	$\varepsilon_{apparent}$ (cm^{-1} M^{-1})[a]	$\lambda_{max\ (emission)}$ (nm)	$K_{eq}\times10^3$ (M^{-1})	$k_2\times10^{-4}$ (s^{-1})
1	548	23 000	615	–	–
1˙Ca^{2+}	526	31 000	606	13	6.3
1˙Mg^{2+}	505	21 000	586	40	0.91
2	573	13 000	653	–	–
2˙Ca^{2+}	535	12 000	620	1.6	25
2˙Mg^{2+}	506	6400	596	5.3	16
Methylspiropyran Ca^{2+}	–	–	–	1.4	14

[a] Absolute extinction coefficients were not measured. See [2].

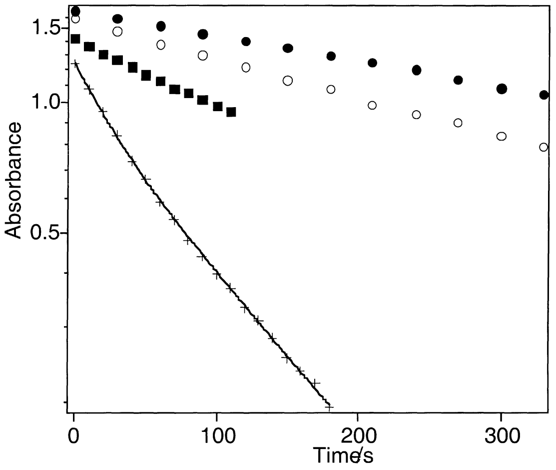


Fig. 2. Decay plot for the bis-spiropyran-bis-amide in acetone without added Ca^{2+} (+), $[Ca^{2+}]$=0.922 mM (■), $[Ca^{2+}]$=1.84 mM (○), $[Ca^{2+}]$= 3.69 mM (●), and the fit to the curved line: $A = 0.94\exp(-0.0087t) + 0.30\exp(-0.035t)$.

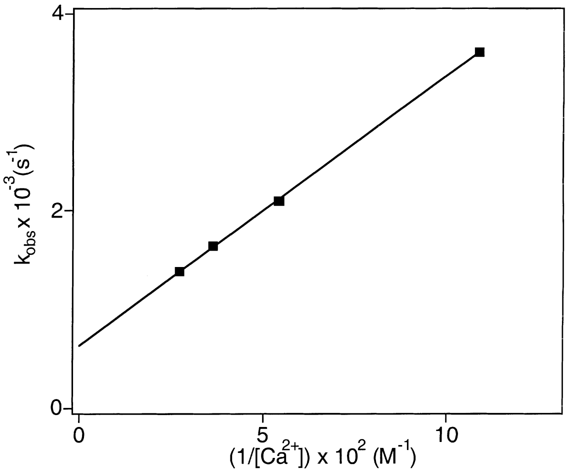


Fig. 3. Plot of the observed decoloration rate constant vs. 1/[Ca] for 0.05 mM 1 and $[Ca^{2+}]$ ranging from 0.922 to 3.69 mM in acetone.

$$k_{obs} = \frac{k_1}{K_{eq}[M]} + k_2 \tag{5}$$

which gives the relationship between the equilibrium constant in Eq. (3) and the observed rate constant, k_{obs}, of decoloration of the spiropyran–metal complex. In order to determine K_{eq}, it is first necessary to measure the thermal rate of decoloration in the absence of metal (k_1). This was done in pure acetone, and the results are shown in Fig. 2. Unlike normal spiropyrans, which display first-order decoloration kinetics in nonalcoholic solvents [2], the bis-spiropyran-bis-amide displays a curved falloff in a plot of ln(A) vs. time. This behavior may be due to two decoloration processes, each with its own rate constant. Consider the bis-spiropyran with both its spiropyrans in the open form: if one closes first at a fast rate, and then the newly closed spiropyran intramolecularly stabilizes the remaining open spiropyran (perhaps by dipole–dipole interactions), this could cause the second spiropyran to close more slowly. In this case, double exponential behavior might be expected. To determine the two rate constants, the data were fit to two exponentials. The calculated fit is shown in Fig. 2. We are interested in the effects of the metals on the rate of closure of the first spiropyran, so we chose the fast rate of decoloration to use as k_1 in Eq. (5).

Good first-order kinetics were obtained when the rate of decoloration of the bis-spiropyran-bis-amide was measured in the presence of magnesium or calcium (see Fig. 2 and Table 2). These data can be used to construct plots such as that shown in Fig. 3, from which binding constants can be calculated. Similar experiments with magnesium, calcium, and the other spiropyrans were also performed. The measured binding constants are shown in Table 1.

The kinetic binding data in Table 1 indicate that the bis-spiropyran has an enhanced ability to bind magnesium and calcium in acetone solvent compared to the simple spiropyrans. That the binding is not higher may be a result of the poor geometry of the chelate that is formed between **1** and a divalent metal, because the chain connecting the two spiropyrans is long. However, the amide carbonyls can come into bonding proximity to the metal, as shown in complex **3**. The spiropyran–amide could also form a chelate (complex **4**) with analogous carbonyl-metal bonding. Because the presence of the amide group in the spiropyran-amide does not appear to enhance binding of calcium compared to the methyl spiropyran, we surmise that the modest gain in

Table 2

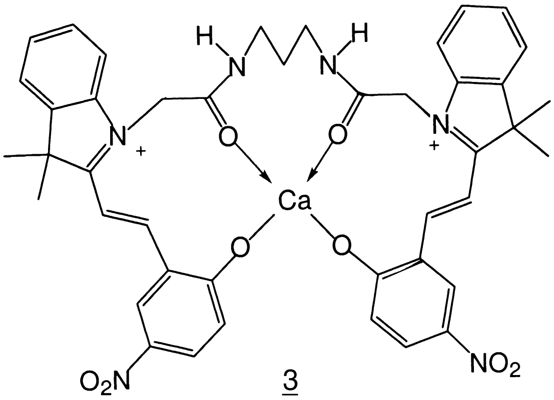
Observed rate constants for the thermal decoloration of **1** and **2** at various calcium or magnesium concentrations

Cmpound **1**				Compound **2**			
$[Ca^{2+}]\times10^{-3}$ (M)	$k_{obs}\times10^{-2}$ (s^{-1})	$[Mg^{2+}]\times10^{-3}$ (M)	$k_{obs}\times10^{-2}$ (s^{-1})	$[Ca^{2+}]\times10^{-3}$ (M)	$k_{obs}\times10^{-2}$ (s^{-1})	$[Mg^{2+}]\times10^{-3}$ (M)	$k_{obs}\times10^{-2}$ (s^{-1})
0	3.5[a]	0	3.5	0	2.8	0	2.8
0.922	0.361	0.842	0.113	1.95	1.21	1.85	0.469
1.84	0.210	1.68	0.0628	3.9	0.795	3.7	0.305
2.76	0.163	2.52	0.0447	5.85	0.597	5.55	0.271
3.69	0.138	3.37	0.0337	7.8	0.448	7.4	0.233

[a] The value at zero metal concentration is k_1 in Eqs. (2) and (5), determined for compound **1** from the fit in Fig. 2.

binding ability of the bis-spiropyran by a factor of about 8 over the mono-spiropyrans for both magnesium and calcium does not involve these carbonyls, and is instead a manifestation of the chelate effect involving only the phenolate oxygen atoms.

We note the values of k_2 in Table 1 for the amide containing spiropyrans increase going from the **1**-magnesium complex to the **2**-calcium complex, which is the reverse of the trend observed for the binding constants. The trend in k_2 suggests that the **1**-magnesium complex is also the most stable with respect to unimolecular decomposition to the closed form and free metal (Eq. (4)), and again may be a manifestation of the chelate effect as well as the higher intrinsic affinity of the ligand for magnesium.



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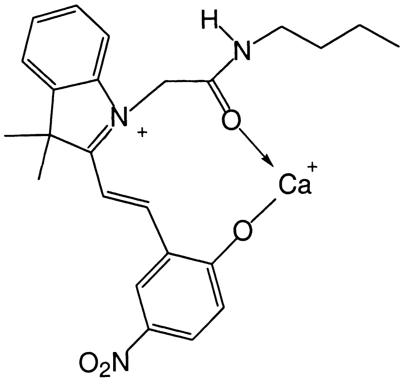

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The mono-spiropyrans display significant binding of magnesium and calcium, which may be expected because the metals are hard acids and phenolate anion is a relatively hard base. The mono-spiropyran-amide also displays a new absorption band in the presence of the metals, and the shift in absorbance is in the same direction and of the same magnitude as for the bis-spiropyran (Table 1). Thus, the metals apparently exert the same dramatic influence on the electronic structure of the mono- and bis-spiropyrans: ionic bonding with the phenolate anion may stabilize the non-bonding anionic n-orbitals in the ground state, increasing the energy of the n to π^* transition, and causing the blue shift observed in the UV-VIS spectrum. The blue-shifted magnesium spectral maxima relative to the calcium spectra would be expected on the grounds of the greater hardness of the former ion. Similar behavior has been observed for spiropyrans bound to transition and rare-earth metals [13].

The kinetic behavior of the spiropyrans in the presence of magnesium and calcium is worthy of further comment. First, the rate constants for decoloration are smaller than k_1 by a factor of up to 100 (Table 2). This shows that the metal binds to the open spiropyran and causes the dramatic drop in the decoloration rate. The fact that the mono-spiropyrans do not show the same drop in decoloration rates, at even higher metal concentrations, shows that the rates are not changed by ionic strength effects alone. Second, the absorbance vs. time plots for the bis-spiropyran in the presence of calcium fit a single exponential, which can be compared to the double exponential behavior seen for the bis-spiropyran in the absence of calcium (Fig. 2). This change suggests that the metal influences the mechanism of closure: at high metal concentrations, k_2 becomes a larger part of k_{obs} in Eq. (5), resulting in good first-order kinetics. But even at low metal concentrations (when k_2 is a small fraction of k_{obs}), the decoloration must also be influenced by the metal, as good first-order kinetics are observed in this regime as well (Fig. 2). We do not offer a detailed explanation for this, but point out that other intermediates and equilibria could be involved. For example, the complex between calcium and the bis-spiropyran with both spiropyrans open ($Sp_{oo}\cdot M$) may close to a complex with one spiropyran closed ($Sp_{oc}\cdot M$), and this complex is in equilibrium with the half-open spiropyran and metal ($Sp_{oc}+M$). The unimolecular decomposition of the latter complex might account for a large part of the observed rates at low metal concentrations, and give first-order kinetics. Undoubtedly, a more complicated reaction sequence would model the reaction better, but would also suffer from the inclusion of entities that are difficult to observe and assumptions that are difficult to test. Nevertheless, the metals clearly influence the closure of the bis-spiropyran dramatically, as a result of specific ligand–metal interactions.

The investigation of the fluorescence of the spiropyrans and their metal complexes was limited to measurements of the spectra and to recording relative emission intensities. The changes in the emission maxima of the complexes follow a similar trend as was observed in the absorbance spectra (Table 1), becoming increasingly blue-shifted going from the free ligand to the magnesium complex. The emission spectra of the bis-spiropyran and its metal complexes are shown in Fig. 1, where the excitation was at 365 nm. Note that the starting concentration of spiropyran for each curve was the same, suggesting that the highest emission occurs from the magnesium complex. Quantum yields were not measured, however, since merocyanine forms of spiropyrans are closed by visible light, and stationary states are produced by UV light [2]. Hence, the absorbances of the complexes are not accurately predetermined (however, see

[14] for flash spectroscopic fluorescence measurements of the methyl spiropyran). These observations suggest that the strength of the binding of the spiropyran to the metal is important in blue-shifting the emission maxima as well as in enhancing the fluorescence.

4. Conclusions

An unusual bis-spiropyran-bis-amide has been prepared that binds magnesium ions (K=40 000 M^{-1}) and calcium ions (K=13 000 M^{-1}) in acetone solution. Comparison of the binding of the spiropyran-amide to the binding of structurally similar methyl spiropyran allows us to conclude that the amide part of the molecule is a poor ligand for the metals, and that the observed binding is a reflection of the inherent affinity of the phenolate moiety of the open spiropyrans for the metals. The presence of two spiropyrans in the same molecule increases the binding constant by a factor of about eight over the mono-spiropyrans. This enhanced binding is attributed to the chelate effect. The decoloration kinetics of the bis-spiropyran can be described by two first-order reactions, where the second, slower reaction is explained by the intramolecular stabilization of one open spiropyran by the other closed spiropyran. Magnesium and calcium ions have a dramatic influence on the kinetics of the decoloration, causing the double exponential behavior to disappear. Finally, the pronounced fluorescence of the open spiropyrans bound to magnesium and calcium ions in acetone is described, where the fluorescence emission maxima are more blue-shifted as the metal binding constant increases.

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