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Fluorescence Reactions of "Crowned" Benzothiazolylphenols with Alkali and Alkaline Earth Metal Ions and Their Analytical Applications

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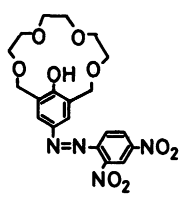
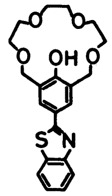
The fluorescence behavior by a complexation of "crowned" benzothiazolylphenols (CBTP and CBBTP) with alkali and alkaline earth metal ions in the presence of triethylamine was examined in various organic solvents. The fluorescence spectra of these CBTP- and CBBTP-metal ion complexes were measured in order to compare mutually both their excitation and emission maxima together with the relative fluorescence intensities (RFI). Both the Li-CBTP and Li-CBBTP complexes showed considerably higher RFI than the other metal complexes. The stability of the fluorescence intensity and various conditions for the determination of Li$^+$ or Ca^{2+} were fully examined. On the basis of these experiments, fluorometric methods were developed for a sensitive determination of trace amounts of Li or Ca by using the crown compounds (CBTP and/or CBBTP). Moreover, a thin-layer chromatographic method for the separation of Li from other metal ions was examined in order to selectively determine Li by using CBBTP.

New applications of crown compounds as reagents for chemical analyses are becoming of interest in analytical chemistry.[1] A great many improvements and further applications in this field have been extensively carried out. For example, several crown compounds have been studied for their usefulness as neutral carriers of Li$^+$-selective electrodes.[2] However, it seems that their sensitivity is as yet quite unsatisfactory for applications to the determination of lithium, as compared with atomic and light absorption spectrometry. Recently, a new method has been proposed[3] for the spectrophotometric determination of Li$^+$ in the concentration range of 25—250 ng ml^{-1} with a "crowned" dinitrophenylazophenol (CDPA) in chloroform-dimethyl sulfoxide-triethylamine (TEA). This was the first example of a colorimetric determination of Li$^+$ with a crown ether. Succeedingly, a complexation of CDPA with alkali and alkaline earth metal ions in chloroform-MeOH-TEA was studied by means of their absorption spectra; two kinds of linear relationships were found between the ionic radii of these ions, both with the wavenumbers of the absorption maxima and with the molar absorption coefficients of the resulting complexes except for Be^{2+}.[4,5] Moreover, a new sensitive and simple method for the spectrophotometric determination of alkylamine with a CDPA-Ba^{2+} complex in MeOH was developed.[6]

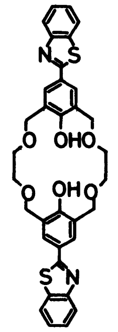
The incorporation of a fluorescent moiety in the place of the dinitrophenylazo group of CDPA should greatly improve the sensitivity in the determination of Li$^+$. The new fluorescent "crowned" benzothiazolylphenols (CBTP and its bis-type compound, CBBTP) have now been synthesized and show characteristic fluorescence with alkali and alkaline earth metal ions.[7] In this paper, we report the fluorescence reactions of CBTP and CBBTP with alkali and alkaline earth metal ions, and a highly sensitive fluorometric determination of Li$^+$ or Ca^{2+}. This is based on a complex formation with CBTP and/or CBBTP, followed by a measurement of the fluorescence of the phenolate of the complex obtained by a treatment with TEA. Attempts to improve the selectivity for lithium were made and then fluorometry of Li$^+$ combined with a thin-layer silica-gel chromatography (TLC) was proposed.



CDPA



CBTP



CBBTP

Experimental

Reagents. Metal chlorides and TEA were of analytical grade. Organic solvents (MeOH, EtOH, benzene, and CH$_2$Cl$_2$) were used after distillation. Commercially available silica-gel plates (Kieselgel 60, Merck) were used.

Apparatus. The fluorescence spectra and intensities were measured with a Shimadzu RF-540 spectrofluorometer in 10×10 mm quartz cells.

General Procedures. a)[8] Determination of Li$^+$ by CBTP: To a 50-μl portion of a LiCl solution in MeOH in a 10-ml standard volumetric flask, 5.0 ml of 10^{-4} M (1 M=1 mol dm^{-3}) CBTP in benzene were added and mixed. After the addition of TEA (50 μl) the mixture was diluted to the mark with benzene and mixed well; then the relative fluorescence intensity (RFI) was measured at 410 nm (emission, λ_{em}) with excitation (λ_{ex}) at 375 nm (cf. Fig. 2).

b) Determination of Li^+ by CBBTP: To a 50-μl portion of LiCl solution in MeOH, 5 ml of CH_2Cl_2 and a solution of CBBTP in CH_2Cl_2 (2×10^{-4} M, 100 μl) were added successively and mixed. Then TEA (20 μl) was added and the whole was finally adjusted to 10 ml with CH_2Cl_2 and mixed well. RFI was measured at 410 nm (λ_{em}) with λ_{ex} at 360 nm.

c) Determination of Ca^{2+} by CBBTP: For a 100-μl portion of a $CaCl_2$ solution in MeOH, the same operation as b) was carried out and then RFI was measured at 400 nm with λ_{ex} at 360 nm.

TLC Method. a) Procedure: A 1 wt% aq solution (10 μl) of metal ion (Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr, or Ba) was spotted on a TLC plate. The plate spotted was developed up to 12 cm by using EtOH–acetic acid (100:1), and dried at room temperature. After being sprayed with violuric acid (1.5 wt%, in EtOH), the plate was dried for 20 min at 110 °C. b) Li^+-determination: The spot for Li^+ developed according to the above procedure was thoroughly scratched and transferred into a 10-ml glass-stoppered tube, to which MeOH (2 ml) was added, vortexed for 2 min, and then centrifuged at 2000 g for 10 min. The supernatant (100 μl) was taken into a 10-ml volumetric flask; to this, CH_2Cl_2 (5 ml) and a solution of CBBTP in CH_2Cl_2 (10.8×10^{-4} M, 250 μl), and then TEA (20 μl) were added successively. The mixture was diluted to the mark with CH_2Cl_2 and mixed well; the RFI was measured at λ_{ex} and λ_{em}. For the blank, the methanol eluate of the part of the corresponding Li-spot on the TLC, which was free from Li salt, was used.

Results and Discussion

Fluorescence Reactions of CBTP and CBBTP with Alkali and Alkaline Earth Metal Ions. The crown rings of CBTP and CBBTP easily form complexes with alkali and alkaline earth metal ions in various solvents such as benzene, chloroform, and dichloromethane. Their fluorescence spectra have been shown to be almost identical with those of the corresponding ligands. By an addition of TEA, the spectra exhibited red shifts as well as the enhanced relative fluorescence intensities (RFI) due to the formation of phenolates. It has been demonstrated that neither CBTP nor CBBTP dissociates in the aprotic solvents, even in the presence of excess TEA.[7] In the present experiments, since MeOH, even in small amounts, should be used for the preparation of a standard solution (homogeneous) of metal salts, a dissociation of the phenols is, more or less, unavoidable. However, the spectra of the phenolates complexed with metal ions have been clearly shown to be different from those derived from the corresponding ligands themselves (Table 1 and cf. Figs. 2 and 3). Thus, it was interestingly observed on the complexes that both excitation and emission maxima were shifted to the longer wavelengths with

Table 1. Fluorescence Spectral Data for the Complexes of CBTP and CBBTP with Alkali and Alkaline Earth Metal Ions

Metal ion	Ligand	
	CBTP	CBBTP
	λ_{em}(λ_{ex}: 375 nm)	λ_{em}(λ_{ex}: 345 nm)
Li^+	410 nm	411 nm
Na^+	414	411
K^+	419	379
Rb^+	421	378
Cs^+	424	380
Be^{2+}	408	381
Mg^{2+}	410	408
Ca^{2+}	412	410
Sr^{2+}	411	413
Ba^{2+}	414	379

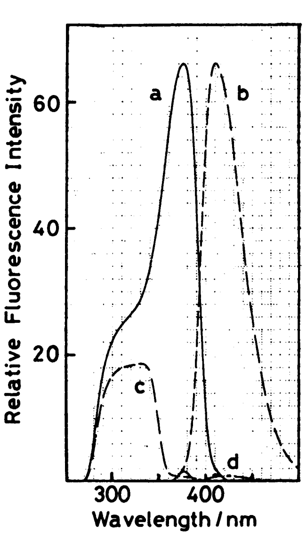


Fig. 1. Fluorescence spectra of the CBTP–Li^+ complex in benzene (Li^+: 1×10^{-6}M).
Excitation spectra: a, the complex; c, CBTP. Emission spectra: b, the complex; d, CBTP (λ_{ex}: 330 nm).

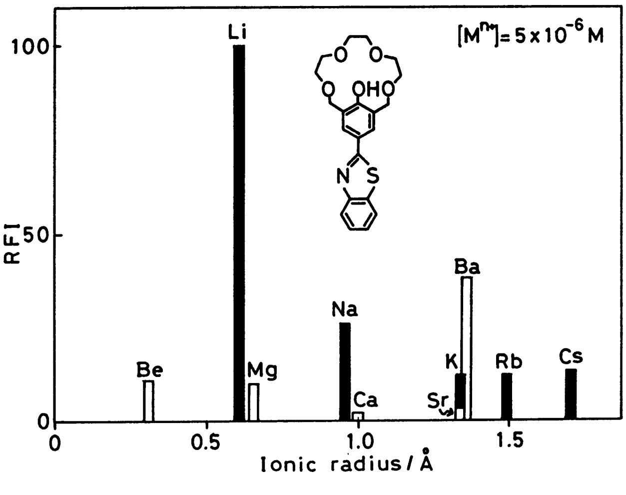


Fig. 2. RFI of the CBTP complexes vs. ionic radius.

an accompanying increase of RFI.

a) CBTP. The fluorescence reaction of CBTP with alkali and alkaline earth metal ions was examined in benzene. The fluorescence of the Li^+-complex is fairly unstable in the light, even though it is the most stable among all the complexes examined in the dark. The fluorescence spectral data are listed in Table 1. Figure 1 shows the excitation spectra of the CBTP-Li^+ complex (a) and a reagent blank (c) and the emission spectra of the complex (b) and the reagent blank (d) excited at 375 nm. The excitation maximum for the reagent blank is at 330 nm, and the emission intensity of the reagent blank is negligible compared with that of the complex when an excitation wavelength (λ_{ex}) of 375 nm is used. The emission maximum of the blank is at 382 nm if λ_{ex} is 330 nm. This choice of λ_{ex} in the above measurement is important for a determination of Li^+ stated later. The relation of the CBTP-M^{n+} complex (M^{n+}=alkali or alkaline earth metal ion) between RFI and ionic radius is shown in Fig. 2 (RFI=100 to the CBTP-Li^+ complex). The order of decreasing RFI is as follows:

$Li^+ > Ba^{2+} > (Na^+, Sr^{2+}) \gg (K^+, Rb^+, Cs^+, Be^{2+}, Mg^{2+}, Ca^{2+})$.

In this series the intensity of Li^+ was extremely high and its ratios of Li^+ to Ba^{2+}, Na^+, and K^+ were about 2, 4, and 10, respectively. It is interesting to note that the RFI of the Na^+, Be^{2+}, Ba^{2+}, and Sr^{2+} complexes, which would interfere in the determination of Li^+, decrease remarkably within 30 min, even in the dark. In the experiment carried out on the Ba^{2+}-complex, it was observed that the RFI decreased promptly and then recovered temporarily, upon adding the standard Ba^{2+} methanolic solution. However, the addition of barium chloride powder into the resulting solution caused an increased RFI which persisted even in the light. We have no idea yet of how to account for this anomalous behavior, which seems to be related to changes in the composition of the host to the guest ion in the complexes yielded in the presence of trace amounts of metal ions.

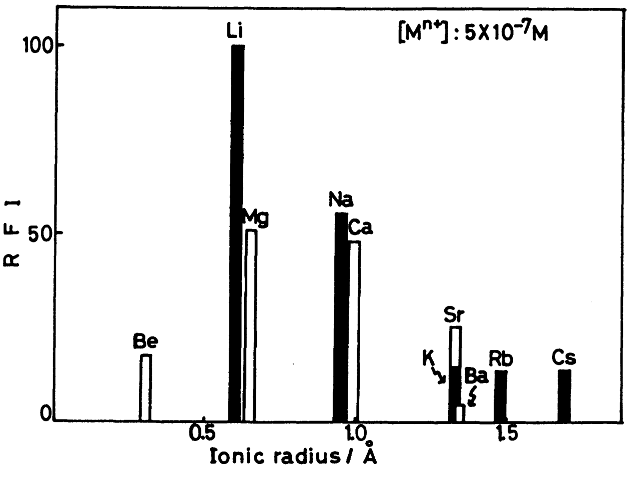


Fig. 3. RFI of the CBBTP complexes vs. ionic radius.

b) CBBTP. In the case of the fluorescence reaction of CBBTP with the metal ions, dichloromethane was particularly chosen as a solvent in order to obtain optimum conditions (Table 1). It is noted that the fluorescence resulting from complexation in the presence of TEA is quite stable relative to the CBTP-complexes, even in the light. In a correlation with the

Table 2. The Mole Ratio of Metal Ion to CBBTP[a)]

Ion	Mole ratio
Li^+	2 : 1
Na^+	2 : 1
K^+	2 : 1
Rb^+	2 : 1
Cs^+	2 : 1
Be^{2+}	4 : 1
Mg^{2+}	2 : 1
Ca^{2+}	1 : 1
Sr^{2+}	1 : 1
Ba^{2+}	1 : 1

a) RFI was measured at λ_{em} described in Table 1, and ([CBBTP]+[M^{n+}])/mol dm^{-3} in which square blankets denote amount-of-substance concentration, corresponds to $(1-2)\times10^{-6}$ M.

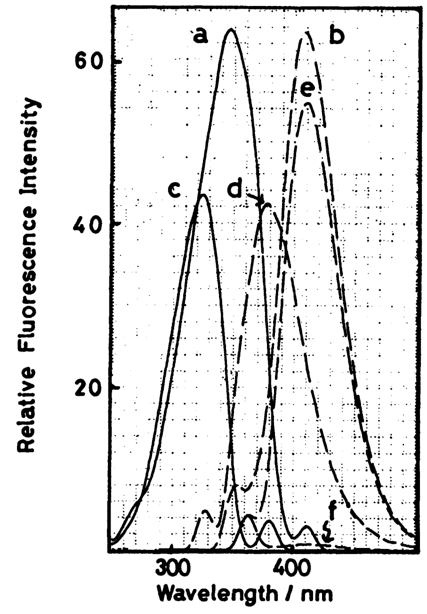


Fig. 4. Fluorescence spectra of the CBBTP-Li^+ complex in CH_2Cl_2 (Li^+: $5X10^{-7}$M).
Excitation spectra: a, the complex; c, CBBTP. Emission spectra: b, the complex at λ_{ex} 345 nm; d, CBBTP; e, the complex at λ_{ex} 360 nm; f, CBBTP at λ_{ex} 360 nm.

ionic radii of the metals, Fig. 3 shows the RFI (the Li$^+$-complex, 100) of the CBBTP complexes, with a decreasing order of:

$$\mathrm{Li^+} \gg \mathrm{Na^+} > (\mathrm{Mg^{2+}}, \mathrm{Ca^{2+}}) \gg \mathrm{Sr^{2+}} > (\mathrm{Be^{2+}}, \mathrm{K^+}, \mathrm{Rb^+}, \mathrm{Cs^+}) \gg \mathrm{Ba^{2+}}.$$

In this connection, it is interesting to examine the composition ratios of the complexes. The mole ratio of metal to CBBTP were examined spectrofluorometrically by the continuous variation method (Table 2). The anomalous behavior of Be^{2+} seems to be largely caused by its extremely small ionic size. As typical representative spectra, the case of the CBBTP–Li$^+$ complex is shown with the blank (CBBTP free from Li$^+$) in Fig. 4, in which the selection of 360 nm as the λ_{ex} can minimize the influence of the blank. This selection is very important for a highly sensitive determination of Li$^+$.

Determination of Li$^+$ or Ca^{2+}. Before establishing a procedure for the determination of Li$^+$ using CBTP, the CBTP, TEA, and MeOH concentrations were examined. It was shown that at least a 10-fold molar excess of reagent was necessary in the final concentration. The maximal RFI of the complex was obtained with final concentrations of 0.5—1.1 vol% TEA and 0.3—0.5 vol% MeOH. The fluorescence developed immediately and its RFI was constant for at least 120 min or more when the solution was kept in the dark. The calibration curve obtained by the standard method passed through the origin and was linear from 0.39 to 39 ng ml^{-1}. The relative standard deviation (RSD) for five replicates at the 24-ng ml^{-1} was 2.2%. The use of CBBTP instead of CBTP resulted in about a three-fold increase in the sensitivity for Li. The calibration graph obtained by the established standard procedure was linear from 0.14—7.0 ng ml^{-1} [RSD, 2.7% at 2.1-ng ml^{-1} (n=8)].

Moreover, in connection with an assay of Ca^{2+} in biological products, CBBTP was examined and was found to be useful for a sensitive determination of trace amounts of Ca^{2+}. After examining the fluorometric conditions, the maximal FRI of the Ca-complex was obtained with a final concentration of 0.2 vol% TEA and 2×10^{-6} M CBBTP. The calibration curve for Ca^{2+} was obtained in the concentration range 0.8—20.0 ng ml^{-1} [RSD, 3.8% at 22-ng ml^{-1} (n=4)].

Separation and Determination of Lithium by TLC. Lithium-selective reagents should be very important for monitoring the Li$^+$ activity in biological systems. The realization of a simple and sensitive Li$^+$-determination method is expected for the therapy of maniacal psychosis.[9] In our present studies, it has become apparent that an improvement in the selectivity is inevitable for a Li$^+$-determination. We preliminarily examined the separation and determination of Li$^+$ by a TLC method.[10] According to the chromatographic conditions described in Experimental, the spots due to metal salts can first be clearly visualized by drying at 110 °C after spraying a solution of violuric acid in EtOH as a potential color-producing reagent:[11] Li$^+$, reddish pink; Na$^+$, reddish orange; K$^+$, bluish violet; Rb$^+$, reddish violet; Cs$^+$, blue; Be^{2+}, pale blue; Mg^{2+}, yellowish orange; Ca^{2+}, orange; Sr^{2+}, reddish violet; Ba^{2+}, reddish violet. Li$^+$, Be^{2+}, and Mg^{2+} showed their R_f-values at 0.35, 0.17, and 0.15 respectively, and other metal spots stayed at the origins. For a determination the Li$^+$-spot colored was completely scratched and eluted with MeOH. The eluate was treated according to b) in Experimental. The fluorometric measurements of the Li$^+$–CBBTP complex showed that the calibration graph was linear from 3.3 to 16.5 μg/10 ml. The lower limit of the determination was 0.82 μg/10 ml (S/N>2). Aoyama reported that the range of the effective content of Li in normal human blood is generally 0.6—1.2 mEq l^{-1}.[12] Since 0.6 mEq of Li corresponds to 0.1 μg/0.1 ml blood, the TLC method examined here cannot be applicable to the determination of Li in normal blood. This TLC method, which has preliminarily been carried out, may prove to be useful in both its simplicity and selectivity. However, regarding sensitivity, the development of stronger color-producing reagents than violuric acid for trace amounts of Li should intensively be carried out next.

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