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The Complexation of "Crowned" 4-(2,4-Dinitrophenylazo)phenol with Alkali and Alkaline Earth Metal Ions, and Its Application to the Determination of Li(I) in a Pharmaceutical Preparation

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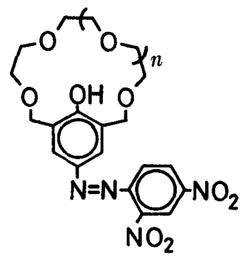
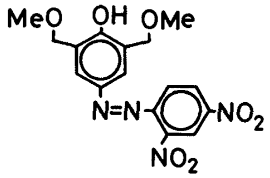
To characterize "crowned" 4-(2,4-dinitrophenylazo)phenol (CDPA), the acid dissociation constant (pK_a) was measured together with those of related compounds. The complexation behavior of CDPA was examined in various nonaqueous solvent systems, and it was found that in the $CHCl_3$–MeOH–triethylamine (TEA) system, the ionic radii of the alkali and alkaline earth metal ions are linearly correlated both with the wave numbers of the absorption maxima and with the molar absorptivities of the resulting complexes except for Be(II). The molar ratios of metal ions to CDPA in the complexes were determined by means of the continuous variation method. Next, the complexation of CDPA with Li(I) was fully examined in another solvent system ($CHCl_3$–dimethyl sulfoxide–TEA) and a sensitive colorimetric assay method for Li(I) was established in the range of 25—250 ppb of Li(I). This method was applied to the analysis of a commercial pharmaceutical preparation, lithium carbonate (tablet).

Keywords——crowned dinitrophenylazophenol; crown ether; complexation; lithium ion; lithium carbonate; colorimetry

Recently, it has been observed that crown ether molecules carrying chromophoric or colored residues can show changes in the absorption spectra upon interaction with metal ions.[1)] Some "crowned" 4-(2,4-dinitrophenylazo)phenols (**1**$_n$, $n=1$ (CDPA) and 2) have been synthesized, and CDPA was found to show a characteristic coloration only with lithium among various alkali metal ions in $CHCl_3$–pyridine.[2)] This coloration was accounted for by a facilitation of the dissociation of the phenol moiety, with delocalization of the negative charge. Based on the above behavior, we have investigated the utilization of CDPA as an analytical reagent for metal ions.



1$_n$
$n=1$: CDPA
$n=2$: **1**$_2$



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Initially, the acid dissociation constant (pK_a) of CDPA (closely related to the complexation behavior) was measured together with those of the related compounds (**1**$_2$ and **2**) by a spectrophotometric method.

The absorption spectra of the complexes of CDPA with alkali and alkaline earth metal ions were measured in $CHCl_3$–MeOH–triethylamine (TEA).[3,4)] Interestingly, both the wave numbers of the absorption maxima and the molar absorptivities of those complexes exhibited a linear relation with the ionic radius of the metal ions for both the alkali and the alkaline earth groups. In this solvent system, the stoichiometric ratios of these complexes were determined by means of the continuous variation method.

The coloration reaction of CDPA in other solvent systems was fully examined, and it was found that CDPA–Li(I) complex is formed quantitatively in $CHCl_3$–dimethyl sulfoxide (DMSO) (volume ratio, 95:5), and then on adding TEA, the solution turns reddish-purple from light yellow.[5] By using this coloration, a method for the determination of trace amounts of Li(I) was developed and this new method was successfully applied to an analysis of a commercial pharmaceutical preparation of lithium carbonate (tablet), which is used for the therapy of maniacal psychosis.[6,7]

We are currently investigating a thin layer chromatographic separations of Li(I) from mixed metal ions solutions or bioproducts following complexation of Li by CDPA.

Experimental

Reagents——Metal chlorides, DMSO, and TEA were of analytical grade. Lithium carbonate (tablet) used was a pharmaceutical preparation. Organic solvents (MeOH and $CHCl_3$) were used after distillation.

Apparatus——A Shimadzu 150 double-beam spectrophotometer was used for measurements of absorbance at a fixed wavelength. Absorbances at various wavelength were recorded with a Hitachi 200-10 recording spectrophotometer. Matched quartz cells of 10 mm path length were used.

General Procedures——a) Determination of Li(I): A solution of CDPA in $CHCl_3$ (2×10^{-4} M, 1 M = 1 mol dm^{-3}, 2.5 ml) was added to a solution of lithium chloride ($\leqq 2.5\ \mu g$ as Li) in DMSO (0.5 ml) in a 10 ml volumetric flask, and the mixture was diluted to *ca.* 9 ml with $CHCl_3$. Then TEA (0.05 ml) was added and the whole was adjusted finally to 10 ml with $CHCl_3$. This solution was mixed and allowed to stand for 10 min, then the absorbance was measured at 565 nm against a reagent blank prepared similarly.

b) Determination of Li(I) in Lithium Carbonate: A commercial tablet containing 200 mg as Li_2CO_3 was dissolved in conc. HCl (5 ml), and the solution was diluted with water, then filtered. The sample solution was prepared by diluting the filtrate with a definite volume of water, then a 1 ml aliquot was taken and evaporated *in vacuo*. After drying up under a vacuum (at 0.2 mmHg, 1 mmHg = 133.322 Pa), the residue obtained was dissolved by adding DMSO (1 ml), and 0.5 ml of this solution was used for the determination of Li(I) according to general procedure a).

Results and Discussion

Acid Dissociation Constants of CDPA and Related Compounds ($\mathbf{1}_2$ and 2)[8]

The absorption spectra of CDPA were recorded as a function of pH to determine the acid dissociation constant (pK_a). An isosbestic point was observed at 465 nm (Fig. 1). The pK_a values of CDPA, $\mathbf{1}_2$, and **2** were determined by using the absorbance at λ_{max}. The order of acidity was $\mathbf{2} > \mathbf{1}_2 >$ CDPA, as shown in Table I.

Complexation of CDPA with Alkali and Alkaline Earth Metal Ions in the $CHCl_3$–MeOH–TEA System

Characteristic Spectral Properties of the Complexes——The absorption spectra of the complexes of CDPA with the alkali and alkaline earth metal ions were measured in $CHCl_3$–MeOH–TEA. The longest-wavelength peaks (λ_{max}) and the molar absorptivities of the complexes are shown in Table II. As shown in Figs. 2 and 3, both $\tilde{\nu}_{max}$ ($=1/\lambda_{max}$) and ε are linearly dependent on the ionic radius of the metal ion in the cases of the alkali and alkaline earth groups, Be(II) being an exception. The alkaline earth metal ions behave as a separate group. It is interesting that a red shift and a hyperchromic effect are recognized with increase in ionic size. This behavior is presumably correlated with the structure of the complexes in solution. Relatively small ions such as Li(I) or Mg(II) fit well into the cavity. Measurements on space-filling models of CDPA indicate that the shortest transannular distance between the

TABLE I. Acid Dissociation Constants[a]

Compound	pK_a	λ_{max} (nm)
CDPA	7.05 ± 0.02[b]	565
1_2	6.69 ± 0.06	550
2	6.23 ± 0.05	545

a) Concentration, 1.5×10^{-5} M, 1 M = 1 mol dm^{-3}; ionic strength, 0.09—0.24 M; 25 °C. *b*) Mean deviation of three replicates.

TABLE II. Spectral Data of the Complexes[a]

Ion	λ_{max} (nm)	$\varepsilon \times 10^{-4}$ (mol^{-1} dm^3 cm^{-1})	Ionic radius (Å)[b]
Li^+	547	3.41	0.60
Na^+	562	4.27	0.95
K^+	573	4.18	1.33
Rb^+	575	4.26	1.48
Cs^+	580	4.66	1.69
Be^{2+}	560	0.51	0.31
Mg^{2+}	542	5.04	0.65
Ca^{2+}	556	6.31	0.99
Sr^{2+}	560	7.49	1.13
Ba^{2+}	563	8.91	1.35

a) The measurement of the absorbance: A solution of CDPA in $CHCl_3$ (2.5×10^{-4} M, 3.0 ml) was added to a solution of the alkali or alkaline earth metal salt (25—30 μg as metal ion) in MeOH (0.5 ml) in a 10 ml volumetric flask, and the mixture was diluted to *ca.* 9 ml with $CHCl_3$. Then TEA (0.05 ml) was added, and the total volume was adjusted to 10 ml with $CHCl_3$. The solution was mixed and allowed to stand for 10 min, then the absorbance was measured at the absorption maximum for each metal ion–CDPA complex against a reagent blank prepared similarly but without any metal ion. *b*) L. Pauling, "The Nature of the Chemical Bond," 3rd ed., Cornell Univ. Press, Ithaca, New York, 1960, p. 260.

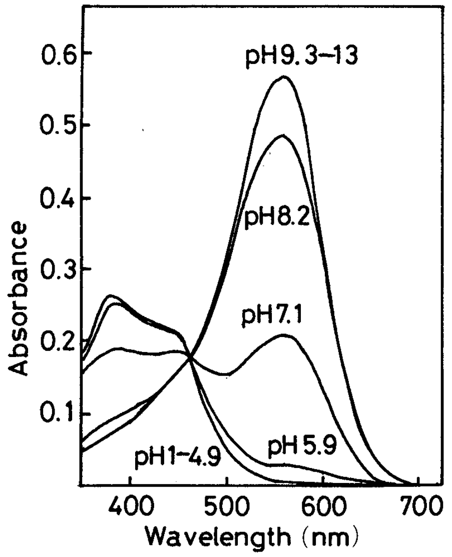

Fig. 1. Absorption Curves of CDPA as a Function of pH (25 °C)

A dioxane solution (2% (v/v)) was used for the measurement. Buffers: pH 1—3, 0.1 M sodium citrate + 0.1 M HCl; pH 4—5, 0.1 M acetic acid + 0.1 M sodium acetate; pH 6—9, 0.1 M KH_2PO_4 + 0.05 M $Na_2B_4O_7$; pH 10—11, 0.05 M $Na_2B_4O_7$ + 0.05 M Na_2CO_3; pH 12—13, 0.1 M Na_2HPO_4 + 0.1 M NaOH.

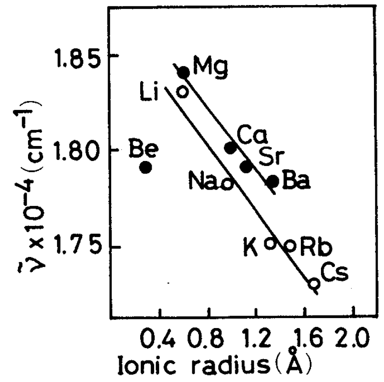

Fig. 2. $\tilde{\nu}$ *vs.* Ionic Radius

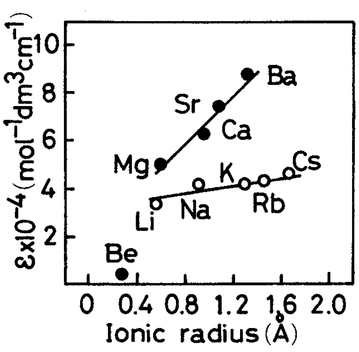

Fig. 3. ε *vs.* Ionic Radius

surface of the oxygen atoms is 1.6—1.8 Å, which is suitable to accomodate Li(I) or Mg(II). Such ions seem to be subject to rather strong interaction with the phenolate anion conjugated to the 2,4-dinitrophenylazo group. The anomalous behavior of Be(II) is believed to be largely caused by its extremely small ionic size.

Composition Ratios of the Complexes——As may be seen in Fig. 3 and Table II, larger molar absorptivities were observed for the alkaline earth metal complexes, as compared with

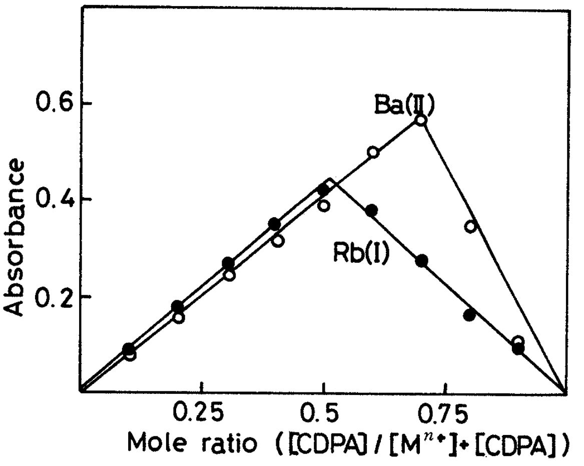

Fig. 4. Composition Ratios of Complexes

Table III. The Mole Ratio of Metal Ion to CDPA[a)]

Ion[b)]	([CDPA]+[M^{n+}])/mol dm^{-3}	Mole ratio Ion : CDPA
Li^+	5.0×10^{-5}	1 : 0.93
Na^+	5.0×10^{-5}	1 : 1.06
K^+	2.5×10^{-5}	1 : 0.93
Rb^+	2.5×10^{-5}	1 : 1.06
Cs^+	2.5×10^{-5}	1 : 1.00
Ca^{2+}	2.5×10^{-5}	1 : 2.33
Sr^{2+}	2.5×10^{-5}	1 : 2.28
Ba^{2+}	2.5×10^{-5}	1 : 2.33

a) Absorbance was measured at λ_{max} described in Table II, and square brackets denote the amount-of-substance concentration. *b)* Be^{2+} and Mg^{2+} did not give unambiguous mole ratios. Presumably, these findings are related to their smaller ionic radii than the others.

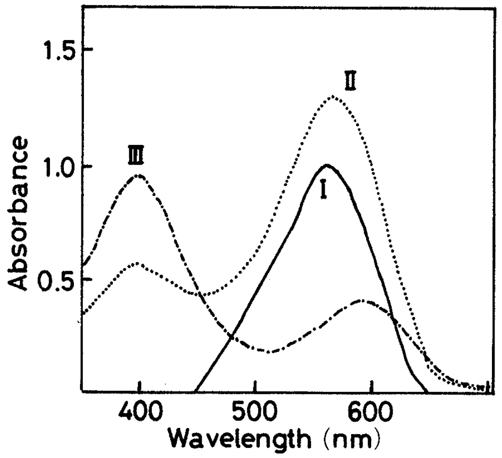

Fig. 5. Absorption Curves of CDPA–Li(I) Complex

I, CDPA–Li(I) complex (reference: blank solution); II, CDPA–Li(I) complex (reference: $CHCl_3$); III, blank solution (reference: $CHCl_3$).

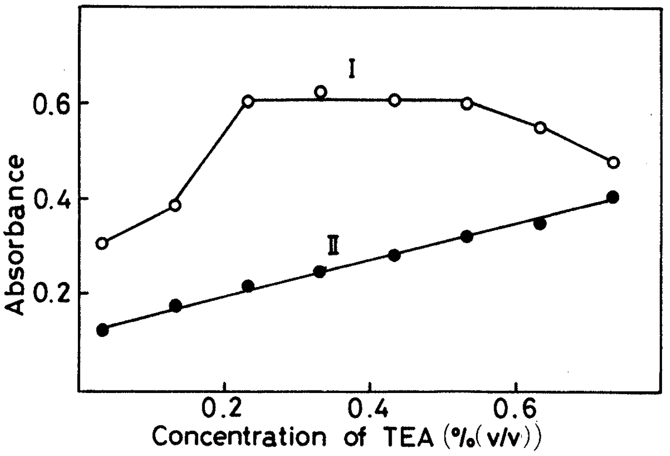

Fig. 6. Effect of TEA Concentration

Li(I): 1.44×10^{-5} M. I, CDPA–Li(I) complex (reference: blank solution); II, blank solution (reference: $CHCl_3$).

those of the alkali metal complexes. In order to explain the results, the mole ratios of metal to CDPA in $CHCl_3$–MeOH–TEA were examined spectrophotometrically by the continuous variation method (Fig. 4 and Table III). The alkaline earth metals were concluded to form 1 (metal): 2 (ligand) complexes while the alkali metal ions form 1 : 1 complexes.

Complexation of CDPA with Li(I) in the $CHCl_3$–DMSO–TEA System

Determination of Li(I)——The absorption spectra of the DCPA–Li(I) complex solution against $CHCl_3$ and the reagent blank prepared according to general procedure a) are recorded in Fig. 5. It was found that the longest wavelength maximum (λ_{max}) of the complex lies at 565 nm against the reagent blank.

The TEA concentration required for coloration of the CDPA–Li(I) complex was examined, and it was observed that the absorbance remained constant in the final concentration range of 0.3—0.6% (v/v) of TEA (Fig. 6). From this result, TEA was added to give 0.5% (v/v) concentration for subsequent study.

Next, the effect of CDPA concentration was examined. A constant absorbance was

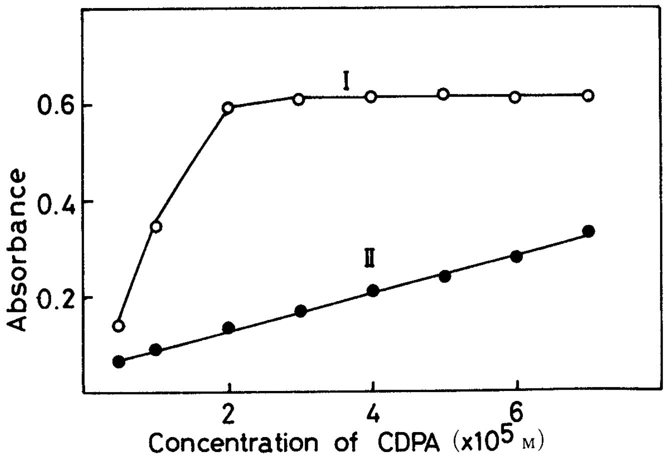

Fig. 7. Effect of CDPA Concentration

Li(I): 1.44×10^{-5} M. I, CDPA–Li(I) complex (reference: blank solution); II, blank solution (reference: $CHCl_3$).

Table IV. Effects of Various Ions on the Absorbance

Ion	Metal salt added[a]	Absorbance[b]
None	—	0.634
Rb^+	RbCl	0.658
Ca^{2+}	$CaCl_2$	0.643
Sr^{2+}	$SrCl_2$	0.641
Ba^{2+}	$BaCl_2$	0.685
Mg^{2+}	$MgCl_2$	0.643

Li (I): 1.44×10^{-5} M. *a*) Concentration of metal ion added: 1.44×10^{-6} M. *b*) At 565 nm.

Table V. Determination of Li_2CO_3 in Tablets

Tablet No.	Amount indicated in the tablet (mg)	Found[a] (mg)
1	200	193.2
2	200	200.0
3	200	210.8
4	200	201.6
5	200	188.8
Mean		198.9
Standard deviation		8.43
Coefficient of variation		4.24%

a) Mean of the three replicate experiments for each tablet.

obtained at more than 2×10^{-5} M CDPA concentration for 1.44×10^{-5} M Li(I) (Fig. 7). A 1 : 1.4 molar ratio of Li(I) to CDPA was found to be adequate.

The influence of DMSO (used as a solvent to dissolve the Li salt) was checked. The absorbance remained constant when the content of DMSO used was 4—6% (v/v).

The CDPA–Li(I) complex reacted with TEA, turning rapidly reddish-purple, and the resulting solution showed a constant absorbance for at least 90 min.

The calibration curve for Li(I) was obtained at 565 nm according to the standard method. Beer's law was obeyed in the concentration range of $(0.36—3.6) \times 10^{-5}$ M lithium (25—250 ppb). The apparent molar absorptivity estimated from the calibration curve was 4.3×10^4 dm^3 mol^{-1} cm^{-1}.

The influence of Rb(I), Ca(II), Sr(II), Ba(II), and Mg(II) on the determination of Li(I) was studied by adding 1.44×10^{-6} M of an ion in question to a solution containing 100 ppb (1.44×10^{-5} M) of Li(I). Na(I), K(I), Cs(I), and Be(II) could not be examined since their salts were insoluble in DMSO. The results are shown in Table IV, in which a positive error is indicated for all the cations listed.

The sensitivity of the proposed method is superior to those of the colorimetry based on thorin–Li(I) complex formation[9] and the recent spectrofluorometry based on the 1,8-dihydroxyanthraquinone–Li(I) reaction.[10] It was recently reported that a chromogenic crown ether, *i.e.*, *N*-(2-hydroxy-5-nitrobenzyl)aza-15-crown-5, is useful for determining 10—100 ppm of Li(I).[11]

For the determination of a commercial pharmaceutical preparation, a commercial tablet containing Li_2CO_3 (200 mg) was assayed according to general procedure b). The results obtained were satisfactory as illustrated in Table V.

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References and Notes

1) For recent reviews, see F. Vögtle, "Host Guest Complex Chemistry I and II," in "Topics in Current Chemistry," Vols. 98 and 101, ed. by F. L. Boschke, Springer-Verlag, Berlin, Heidelberg, New York, 1981 and 1982; G. W. Gokel and S. H. Korzeniowski, "Macrocyclic Polyether Syntheses," in "Reactivity and Structure Concepts in Organic Chemistry," Vol. 13, ed. by K. Hafner *et al.*, Springer-Verlag, Berlin, Heidelberg, New York, 1982.
2) T. Kaneda, K. Sugihara, H. Kamiya, and S. Misumi, *Tetrahedron Lett.*, **22**, 4407 (1981); K. Sugihara, T. Kaneda, and S. Misumi, *Heterocycles*, **18**, 57 (1982).
3) In the original solvent system ($CHCl_3$–pyridine),2) the spectra of these complexes could not be quantitatively measured because of the extremely poor solubility of the alkali and alkaline earth metal salts.
4) K. Nakashima, Y. Yamawaki, S. Nakatsuji, S. Akiyama, T. Kaneda, and S. Misumi, *Chem. Lett.*, **1983**, 1415.
5) The mechanism of the complexation and the subsequent coloration has already been investigated in the original solvent system; see ref. 2.
6) K. Nakashima, S. Nakatsuji, S. Akiyama, T. Kaneda, and S. Misumi, *Chem. Lett.*, **1982**, 1781.
7) *a*) S. Watanabe, "Richium-Kiso to Rinsho (Lithium-Basic and Clinical Medicine)," Ishiyaku Shuppan Co., Tokyo, 1983; *b*) D. Samuel and Z. Gottesfeld, *Endeavour*, **32**, 122 (1973); *c*) A. Amdisen, *Dan. Med. Bull.*, **22**, 277 (1957); *d*) M. Canessa, N. Adragna, H. S. Solomon, T. M. Connolly, and D. C. Tosteson, *N. Engl. J. Med.*, **302**, 722 (1980).
8) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases. A Laboratorial Manual," Methuen & Co., Ltd., London, 1962.
9) P. F. Thomason, *Anal. Chem.*, **28**, 1527 (1956).
10) M. R. Ceba, A. Fernandez-Gutierrez, and M. C. Mahedero, *Anal. Lett.*, **14**, 1579 (1981).
11) H. Nakamura, H. Sakka, M. Takagi, and K. Ueno, *Chem. Lett.*, **1981**, 1305.