Binding Properties of Neutral Diamide Ligands for Alkaline-Earth Cations†

Tze-Chein Wun, Robert Bittman,* and Irving J. Borowitz

ABSTRACT: The complexation of a series of aromatic and alicyclic *N,N,N′,N′*-tetra-*n*-propyl amides of 1,2-ethylenedioxydiacetic acids with group IIA metal-ion bromides in anhydrous methanol was investigated by ultraviolet absorption spectroscopy. These synthetic ligands were previously found to show selectivity toward divalent over monovalent cations with respect to extraction of ions into bulk organic phase (Borowitz, I. J., Lin, W-O., Wun, T-C., Bittman, R., Weiss, L., Diakiw, V., and Borowitz, G. B. (1977), *Tetrahedron,* in press). At low concentrations, ligands bearing benzene and naphthalene rings form 1:1 ligand to divalent cation complexes with each of the alkaline-earth metals, but ligands in the cyclohexyl series are stoichiometrically bound to cations in more than one type of complex. Binding isotherms obtained by Scatchard analysis and by the method of continuous variation revealed ligand to divalent ion mole ratios of 2:1, 3:2, and 4:3 for binding of *N,N,N′,N′*-tetra-*n*-propyl-*cis*-1,2-cyclohexanedioxydiacetamide with Ca^{2+}, Sr^{2+}, and Ba^{2+}, respectively. In contrast, Scatchard analysis of ultraviolet spectral changes showed that a 1:1 complex is formed between this ligand and Na^+ with an apparent association constant of $56 \pm 2\ M^{-1}$; the constant for binding with K^+ was smaller ($11\ M^{-1}$). The order of apparent association equilibrium constants for complexation of group IIA cations with this series of neutral ligands was $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$; for example, for *N,N,N′,N′*-tetra-*n*-propyl-1,2-phenylenedioxydiacetamide the apparent binding constants at 25 °C were $7.33 \pm 0.25 \times 10^4\ M^{-1}$ for Ca^{2+}, $1.23 \pm 0.03 \times 10^4$ for Sr^{2+}, $4.42 \pm 0.09 \times 10^3$ for Ba^{2+}, and $4.04 \pm 0.24 \times 10^2$ for Mg^{2+}. The divalent cation binding properties of these synthetic diamide ligands are discussed in relation to those of other synthetic ligands and of two naturally occurring ligands.

For the design of synthetic multidentate compounds suitable for selective complexation with a large array of metal ions, some definitive information is available concerning the relationship between the structure of coordinating sites and selectivity toward different univalent cations (e.g., Pedersen, 1967, 1970; Frensdorff, 1971; Pedersen and Frensdorff, 1972; Christensen et al., 1974; Vögtle and Weber, 1974; Koenig et al., 1976). Little information is available, however, regarding the structural features in ligands that govern selective complex formation with divalent metal ions (Morf and Simon, 1973; Lehn and Sauvage, 1975). We have recently accomplished the synthesis of a series of neutral 1,2-ethylenedioxydiacetamide ligands, and investigated their relative selectivities toward various metal ions with respect to solubilization of group IA and group IIA cations in bulk organic phase (Borowitz et al., 1977). In this paper, we describe quantitative measurements of the interaction of these ligands with alkaline-earth metal ions.

Experimental Section

Materials

Anhydrous $CaBr_2$, $SrBr_2$, $BaBr_2$ (obtained from ROC/RIC), and $Ba(SCN)_2$ (obtained from Alfa Inorganics) were used as received and kept under nitrogen in a dessicator. $MgBr_2 \cdot 6H_2O$ was supplied by Fisher Scientific Co. Anhydrous methanol (Matheson, Coleman & Bell) was spectral grade. The ligands were synthesized as described previously (Ammann et al., 1975; Borowitz et al., 1977). Figure 1 shows the structure of the *N,N,N′,N′*-tetra-*n*-propyl amides of 1,2-phenylenedioxydiacetic acid (P-PR), 2,3-naphthalenedioxydiacetic acid (N-PR), and *cis*- and *trans*-1,2-cyclohexanedioxydiacetic acids (*c*-C-PR and *t*-C-PR). The structure of *N*-methyl-*N*-octadecyl-1,2-phenylenedioxydiacetamide (P-18) is also shown.

Methods

Measurement of Metal Ion Binding to Ligands. Absorption spectra were measured using a Cary 14 spectrophotometer. Spectral titrations were carried out at room temperature (20 °C) using a 0–0.1 slidewire and a cell of 10-cm path length and 28-mL capacity, or a 1-cm cell of 6-mL capacity and a 0–0.1 or 0–1 slidewire. Concentrated solutions of ligands and metal bromides were prepared in methanol. Aliquots of these solutions were added by microsyringe to methanolic solutions of ligands. The total volume of added cation solution was less than 0.2 mL. The absorbance changes were corrected for the absorption of the metal bromide in the absence of ligands. Titration of N-PR was carried out at 324 nm. Titrations of P-PR and P-18 were conducted at 280 nm for $CaBr_2$ and $SrBr_2$ and at 283 nm for $BaBr_2$. Titrations of *c*-C-PR and *t*-C-PR were done at 230 nm.

The stoichiometry of the binding of metal ions to the ligands was determined by the method of continuous variation (Job, 1928). The Job plots were obtained by plotting the absorbance change vs. the mole fraction of metal ion relative to the sum of the cation and ligand concentrations; during the titration, the sum of the cation and ligand concentrations was maintained constant. The sum of the concentrations and the range of ratios over which the relative concentrations were varied are indicated in the figure captions. The mole fraction at which the maximum absorbance change occurs and the ratio of the slopes of the linear portions of the two lines give the stoichiometry of the binding.

† From the Department of Chemistry, Queens College of The City University of New York, Flushing, New York 11367 (T-C.W. and R.B.), and the Department of Chemistry, Yeshiva University, New York (I.J.B.). *Received September 1, 1976.* This work was supported by Grants HL 16746 and 16660 from the National Institutes of Health and from the Research Corporation.

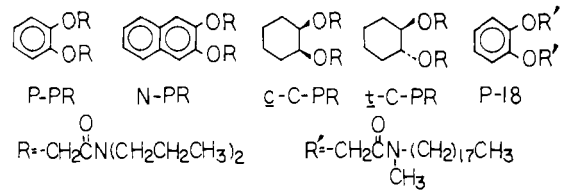

FIGURE 1: Structures of P-PR, N-PR, *c*-C-PR, *t*-C-PR, and P-18.

Apparent association constants, K_{app}, for metal ion binding to ligands were determined from Scatchard plots ([949(/ The concentration of ligands was maintained constant in the titration. Binding isotherms were constructed from data obtained by two methods. In method A, the stoichiometry of binding is known to be 1:1 from the Job plot. The Scatchard equation, $r/[c] = K_{app}(n - r)$, is used to obtain K_{app}, by plotting $r/[c]$ vs. r, where r is the molar ratio of cation bound to the total ligand, [c] is the molar concentration of free cation, and n is the number of moles of cation bound per mole of ionophore (1.0). The value of r is calculated from $r = \Delta A/\Delta A_m$, where ΔA is the absorbance change and ΔA_m is the maximum absorbance change. The latter is determined at saturating concentrations of metal ions. The value of [c] is calculated from $[c] = [c]_T - (\Delta A/\Delta A_m)[L]$, where $[c]_T$ is the total cation concentration added, and [L] is the ligand concentration. The value of K_{app} is calculated from the binding isotherm by an iterative procedure. An initial value of ΔA_m is obtained from experiments where cation is in large excess. This value is used to calculate [c] by the above equation. A slightly different value of ΔA_m is obtained from the intercept of a double-reciprocal plot of ΔA vs. [c]. This ΔA_m is then used to recalculate r and [c]. The procedure is repeated until the best fit is obtained for the extrapolated line of the Scatchard plot to a value of unity. (The x intercept of the extrapolated straight line of the Scatchard plot gives the stoichiometry of binding, which was determined from Job plot data to be 1 mol of cation bound per mol of ligand.) The negative of the slope gives K_{app}. The values of K_{app} and n are determined by a least-squares analysis. Note that the beginning part of the titration gives data points at low r values. Method B was used when the stoichiometry of binding was found to be other than 1:1 in the Job plot; the Scatchard equation, $R/[L] = K_{app}(N - R)$ is used to obtain K_{app} by plotting $R/[L]$ vs. R. Here, R is the molar ratio of ligand to the cation added, [L] is the molar concentration of free ligand, and N is the number of moles of ligand bound per mole of cation. The value of R is calculated from $R = [L]_T(\Delta A/\Delta A_m)/[C]_T$, where $[L]_T$ is the total ligand concentration. $[C]_T$ is the total concentration of cation. The value of [L] is calculated from $[L] = [L]_T - [L]_T(\Delta A/\Delta A_m)$. Note that the beginning part of the titration corresponds to data points at large R values in this plot.

Results

UV[1] *Spectra.* The absorption spectra of P-PR, P-18, and N-PR are shown in Figure 2A,B,C (solid curves). Upon addition of $CaBr_2$ or $Ba(SCN)_2$, the spectra changed with distinctive isosbestic point(s). Addition of other divalent salts ($SrBr_2$, $BaBr_2$, $MgBr_2$) caused similar changes (spectra not shown). Addition of divalent cations to *c*-C-PR (Figure 2E) and *t*-C-PR (spectrum not shown) caused decreases in extinction coefficients at shorter wavelengths.

Stoichiometry and Binding Constants of P-PR-M^{2+} and

[1] Abbreviation used: UV, ultraviolet.

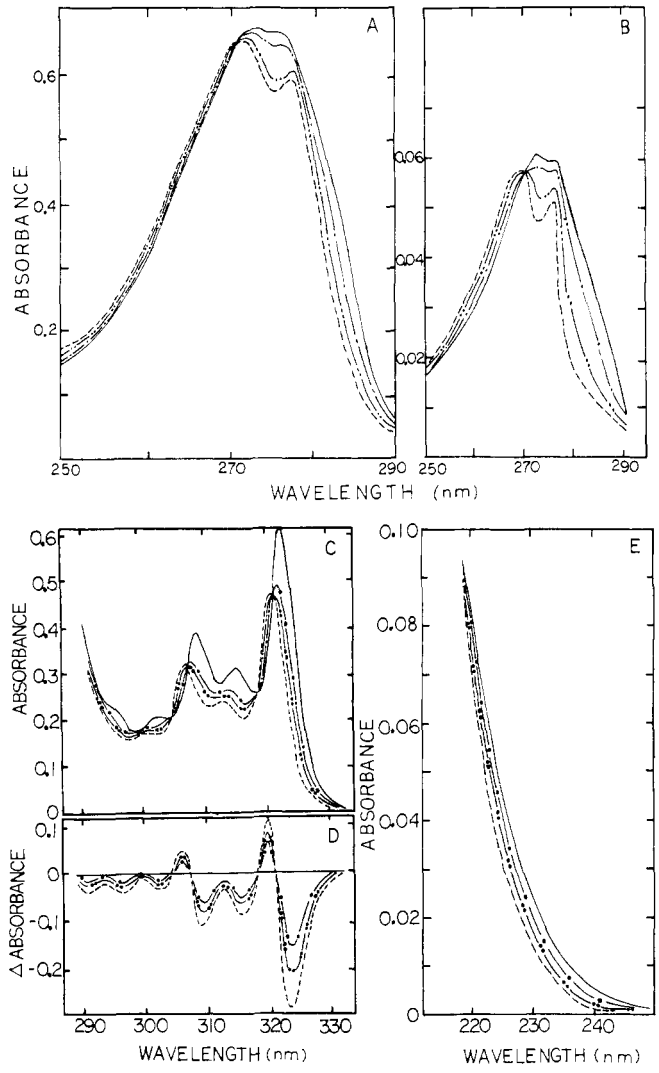

FIGURE 2: UV spectra of P-PR, P-18, N-PR, and *c*-C-PR in methanol and the effect of addition of divalent cations. (A) (—) Spectrum of 3.37×10^{-4} M P-PR; (—•—) spectrum after addition of 8.73×10^{-5} M $CaBr_2$; (—••—) addition of 1.75×10^{-4} M $CaBr_2$; (- - -) addition of 8.73×10^{-4} $CaBr_2$. (B) (—) Spectrum of 2.80×10^{-5} M P-18; (—•—) spectrum after addition of 1.33×10^{-5} M $CaBr_2$; (—••—) addition of 5.32×10^{-5} M $CaBr_2$; (- - -) addition of 2.39×10^{-4} M $CaBr_2$. (C) (—) Spectrum of 2.06×10^{-4} M N-PR; (—•—) spectrum after addition of 3.23×10^{-4} M $Ba(SCN)_2$; (—••—) addition of 6.46×10^{-4} M $Ba(SCN)_2$; (- - -) addition of 2.91×10^{-3} M $Ba(SCN)_2$. (D) The difference spectra of N-PR and its complexes with $Ba(SCN)_2$. The sample cuvette contained 3.23×10^{-4} M (—•—), 6.46×10^{-4} M (—••—), or 2.91×10^{-3} M (- - -) $Ba(SCN)_2$ plus 2.06×10^{-4} M N-PR. The reference cuvette lacked $Ba(SCN)_2$. (E) (—) Spectrum of 3.44×10^{-5} M *c*-C-PR; (—•—) spectrum after addition of 6.46×10^{-6} M $CaBr_2$; (—••—) addition of 1.29×10^{-5} M $CaBr_2$; (- - -) addition of 2.91×10^{-5} M $CaBr_2$.

N-PR-M^{2+} Complexation. Job and Scatchard plots of the titrations of metal-ion binding to P-PR and N-PR showed that a single class of binding sites is present over the concentration ranges of the ligands and the metal ions used (Figure 3). The Scatchard plots shown in Figure 3B for the interaction of $CaBr_2$ with P-PR and N-PR in methanol are similar to those obtained for the binding of $SrBr_2$ and $BaBr_2$ to these ligands. The number of binding sites is 1.0 mol of M^{2+}/mol of ligand. The binding of $MgBr_2$ to N-PR shows other than 1:1 complexation at the beginning of titration, but the molar ratio in the latter part of titration (approaching saturation) appears to be 1.0 (Figure 3C).

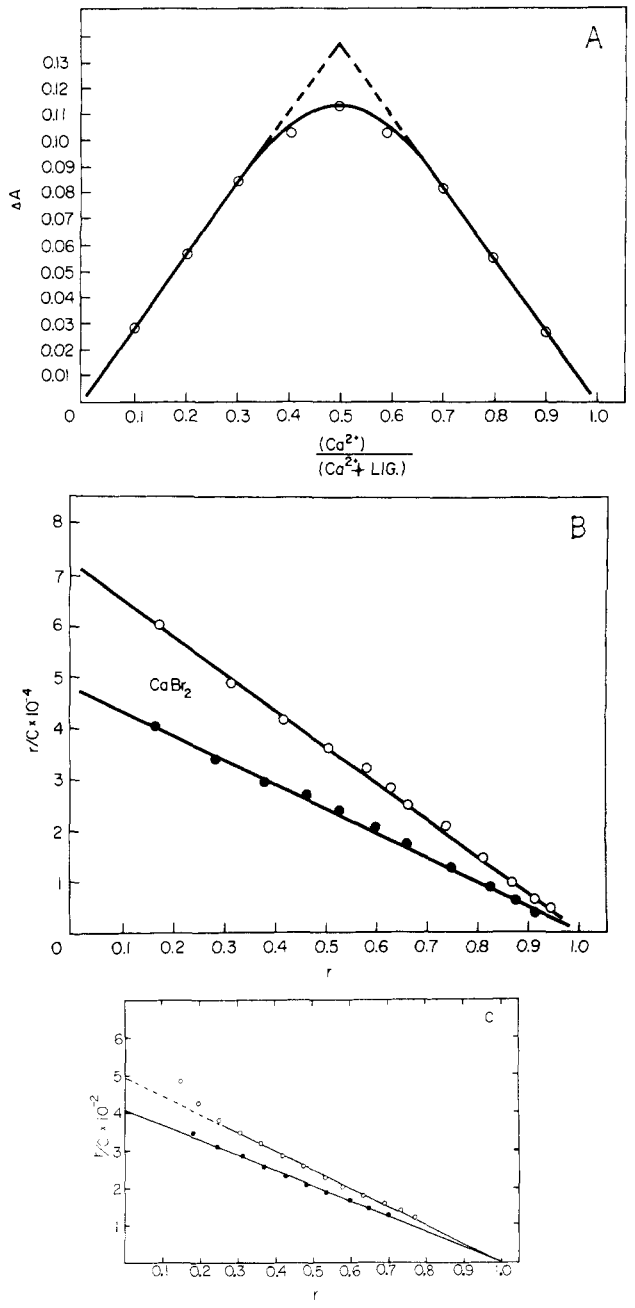

FIGURE 3: Interaction of $CaBr_2$ and $MgBr_2$ with P-PR and N-PR. (A) Job plot for the interaction of P-PR with $CaBr_2$ in methanol. The sum of the concentrations of $CaBr_2$ and P-Pr was kept constant at 2.45×10^{-4} M. The change in absorbance was measured at 280 nm. (B) Scatchard plot of the binding of $CaBr_2$ to (○) P-PR and (●) N-PR in methanol. In the titration with P-PR (1.13×10^{-5} M), the concentration of $CaBr_2$ was varied from 5.0×10^{-6} to 2.87×10^{-4} M; for N-PR (5.53×10^{-6} M), the concentration of $CaBr_2$ was varied from 4.95×10^{-6} to 2.85×10^{-4} M. The values of r, [C], and K_{app} were calculated as described under Methods. (C) Scatchard plot of the binding of $MgBr_2$ to (○) P-PR and (●) N-PR in methanol. In the titration with P-PR (1.13×10^{-5} M), the concentration of $MgBr_2$ was varied from 5.31×10^{-4} to 5.36×10^{-3} M; for N-PR (5.53×10^{-6} M), the concentration of $MgBr_2$ was varied from 8.39×10^{-4} to 4.89×10^{-3} M.

When higher ligand concentrations are used, deviation from 1:1 stoichiometry is observed. A Scatchard plot of Ca^{2+} binding to P-PR at a ligand concentration of 6.6×10^{-4} M shows that at the beginning of the titration species higher than 1:1 complexes are present in the solution (Figure 4); however, the stoichiometry approaches a molar ratio of 1.0 at the end of the titration. For N-PR, deviation from Beer's law was found

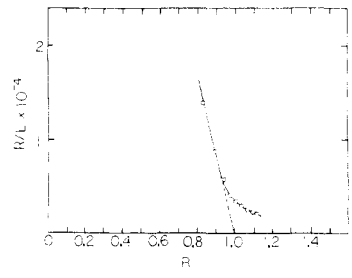

FIGURE 4: Scatchard plot of the binding of $CaBr_2$ to P-PR in methanol. The concentration of P-PR was 6.60×10^{-4} M. The concentration of $CaBr_2$ was varied from 6.25×10^{-5} to 1.53×10^{-3} M.

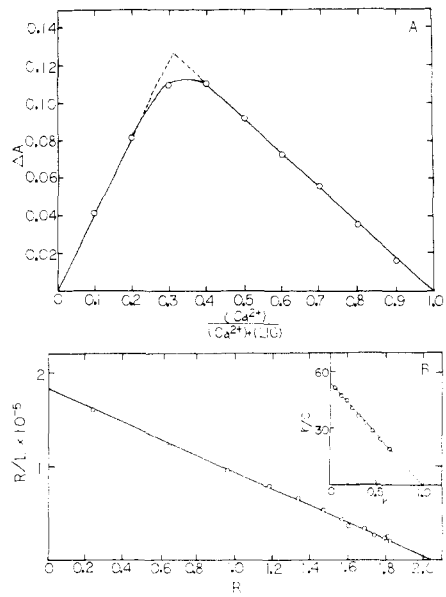

FIGURE 5: Interaction of $CaBr_2$ and NaBr with c-C-PR. (A) Job plot for the interaction of *c*-C-PR with $CaBr_2$ in methanol. The sum of the concentrations of $CaBr_2$ and *c*-C-PR was kept constant at 2.30×10^{-4} M. The change in absorbance was measured at 230 nm. (B) Scatchard plot of the binding of $CaBr_2$ to *c*-C-PR in methanol. The concentration of *c*-C-PR was 9.63×10^{-5} M. The concentration of $CaBr_2$ was varied from 4.06×10^{-6} M to 3.94×10^{-4} M. Insert: Scatchard plot of the binding of NaBr to *c*-C-PR in methanol. The concentration of *c*-C-PR was 9.63×10^{-5} M. The concentration of NaBr was varied from 1.18×10^{-3} to 3.46×10^{-2} M.

in the concentration range of 10^{-4}–10^{-3} M, suggesting possible aggregation of ligand. The Job plot is asymmetrical and the Scatchard plot shows deviation from 1:1 complexation (data not shown) at this concentration.

Complexation of c-C-PR and t-C-PR with M^{2+} and c-C-PR with Na^+ and K^+ in Methanol. The Job plot of the binding of $CaBr_2$ to *c*-C-PR shows a 2:1 ligand to ion ratio (Figure 5A). The Scatchard plot (Figure 5B) confirms this stoichiometry and gives a binding constant of $8.70 \pm 0.24 \times 10^4$ M^{-1}, which is the highest for this series of ligands.

The Scatchard plot of Na^+-*c*-C-PR binding (insert, Figure 5B) shows that a 1:1 complex is formed and gives an apparent association constant of 56 ± 2 M^{-1}. Scatchard analysis of the titration data for the interaction of *c*-C-PR with K^+ gives a binding constant of approximately 11 M^{-1}. (Because of the limited solubility of KBr in methanol, only the beginning part of the titration could be performed.) Thus the association of *c*-C-PR with Na^+ is stronger than that with K^+, but with each of these cations the magnitude of the binding constant is low.

Figure 6A shows the Job plot of the binding of $SrBr_2$ to *c*-

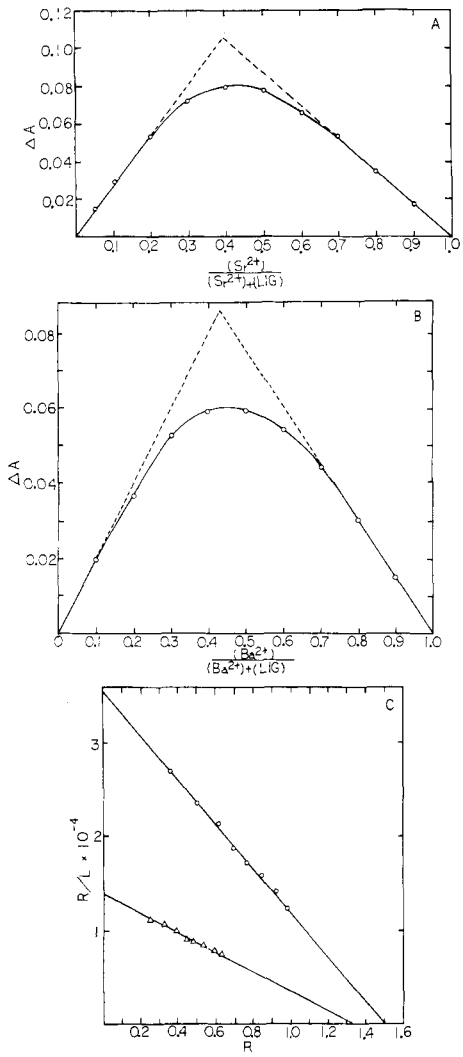

FIGURE 6: Interaction of $SrBr_2$ and $BaBr_2$ with *c*-C-PR. (A) Job plot for the interaction of *c*-C-PR with $SrBr_2$ in methanol. The sum of the concentrations of $SrBr_2$ and *c*-C-PR was kept constant at 2.33×10^{-4} M. The change in absorbance was measured at 230 nm. (B) Job plot for the interaction of *c*-C-PR with $BaBr_2$ in methanol. The sum of the concentrations of $BaBr_2$ and *c*-C-PR was kept constant at 3.19×10^{-4} M. The change in absorbance was measured at 230 nm. (C) Scatchard plot of the binding of (○) $SrBr_2$ and (●) $BaBr_2$ to *c*-C-PR in methanol. The concentration of *c*-C-PR was 9.63×10^{-5} M. The concentration of $SrBr_2$ was varied from 1.70×10^{-5} to 6.62×10^{-4} M. The concentration of $BaBr_2$ was varied from 1.62×10^{-5} to 9.31×10^{-5} M.

C-PR. The maximum absorption change occurs at a Sr^{2+} mole ratio of 0.4 and the slope ratio is 1.5. Hence, the ligand to ion ratio of *c*-C-PR–Sr^{2+} binding is 1.5:1. Figure 6B shows the Job plot of the interaction of $BaBr_2$ with *c*-C-PR, giving a slope ratio of 1.3:1. The Scatchard plot (Figure 6C) confirms these stoichiometries and gives binding constants of $2.26 \pm 0.10 \times 10^4$ M^{-1} for $SrBr_2$ and $1.07 \pm 0.09 \times 10^4$ M^{-1} for $BaBr_2$.

Figure 7A shows the Job plot of *t*-C-PR–Ca^{2+} binding. The maximum absorption change occurs at a Ca^{2+} mole ratio of 0.5, but the curve is asymmetrical with a slope ratio of 1.5. The Scatchard plot (Figure 7B) confirms these parameters, showing that the ligand to ion ratio is 1.5:1 at the beginning part of the titration and 1:1 at saturation.

Complexation of P-18 with Ca^{2+}. Figure 8 shows the Scatchard plot of the binding of $CaBr_2$ to P-18. The ligand to cation mole ratio is 0.38:1. It is possible that self-association of P-18 may account for this behavior even though P-18 may have the same binding sites as the other ligands.

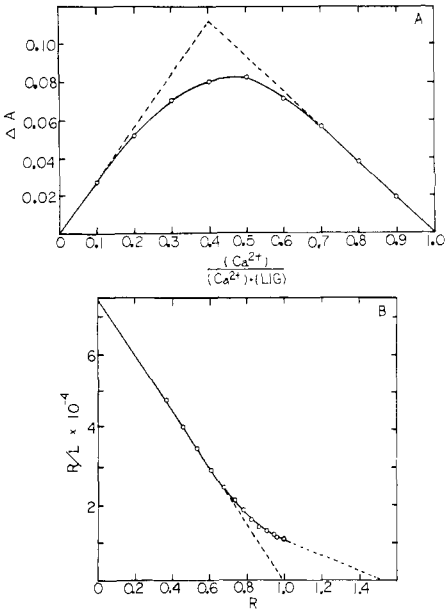

FIGURE 7: Interaction of $CaBr_2$ with *t*-C-PR. (A) Job plot for the interaction of *t*-C-PR with $CaBr_2$ in methanol. The sum of the concentrations of $CaBr_2$ and *t*-C-PR was kept constant at 2.95×10^{-4} M. The change in absorbance was measured at 230 nm. (B) Scatchard plot of the binding of $CaBr_2$ to *t*-C-PR in methanol. The concentration of *t*-C-PR was 9.63×10^{-5} M. The concentration of $CaBr_2$ was varied from 7.08×10^{-6} to 2.41×10^{-4} M.

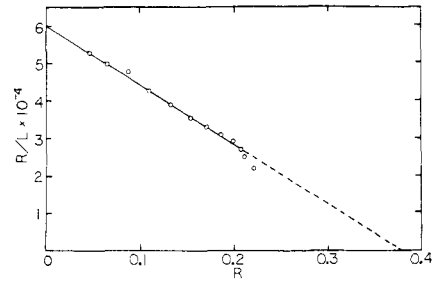

FIGURE 8: Scatchard plot of the binding of $CaBr_2$ to P-18 in methanol. The concentration of P-18 was 1.02×10^{-5} M. The concentration of $CaBr_2$ was varied from 1.31×10^{-6} to 2.01×10^{-4} M.

A summary of the binding constants of these ligands with divalent cations in methanol shows that for P-Pr, N-Pr, and *c*-C-PR the order of binding with divalent cations is $Ca^{2+} > Sr^{2+} > Ba^{2+} > (> Mg^{2+})$ (Table I). For each divalent cation (except Mg^{2+}), the order of affinity toward ligand has the general trend of *c*-C-PR > P-PR ≃ N-PR.

Discussion

The combined use of the methods of continuous variation and of Scatchard plots provides data concerning the stoichiometry and binding constant of complexation in solution.

At low concentrations, the aromatic ligands, P-PR and N-PR, form 1:1 ligand–divalent cation complexes with all of the alkaline-earth metals in methanol, whereas with ligands in the cyclohexyl series a 2:1 ligand–ion complex is formed with Ca^{2+} by *c*-C-Pr, and both 3:2 and 1:1 complexes are formed with Ca^{2+} by *t*-C-PR. In addition, the stoichiometry of the complex formed by *c*-C-PR depends on the divalent cation (Table I). Variations in ion-binding stoichiometries have been reported for other ionophores. Cyclic polyethers, which form 1:1 complexes with many cations (Pedersen, 1967), also form complexes having polyether to metal mole ratios of 2:1 and 3:2,

TABLE I: Stoichiometry and Binding Constants for Interaction of Ligands and Divalent Cations in Methanol.

Ligand	Ligand Concn (M)	Salt	Molar Ratio of Ligand-M^{2+}	K_{app}[a]
P-PR	1.13×10^{-5}	$CaBr_2$	1:1	$7.33 \pm 0.25 \times 10^4$
	6.60×10^{-4}	$CaBr_2$	1.35:1 and 1:1	
	1.13×10^{-5}	$SrBr_2$	1:1	$1.23 \pm 0.03 \times 10^4$
	1.13×10^{-5}	$BaBr_2$	1:1	$4.42 \pm 0.09 \times 10^3$
	1.13×10^{-5}	$MgBr_2$	1:1	$4.04 \pm 0.24 \times 10^2$
N-PR	5.53×10^{-6}	$CaBr_2$	1:1	$4.81 \pm 0.21 \times 10^4$
	5.53×10^{-6}	$SrBr_2$	1:1	$1.09 \pm 0.03 \times 10^4$
	5.53×10^{-6}	$BaBr_2$	1:1	$3.98 \pm 0.08 \times 10^3$
	5.25×10^{-6}	$MgBr_2$	1:1	$4.70 \pm 0.28 \times 10^2$
c-C-PR	9.63×10^{-5}	$CaBr_2$	2.08:1	$8.70 \pm 0.24 \times 10^4$
	9.63×10^{-5}	$SrBr_2$	1.54:1	$2.26 \pm 0.10 \times 10^4$
	9.63×10^{-5}	$BaBr_2$	1.31:1	$1.07 \pm 0.09 \times 10^4$
t-C-PR	9.63×10^{-5}	$CaBr_2$	1.5:1 and 1:1	$7.72 \pm 0.34 \times 10^4$ [b]
P-18	1.02×10^{-5}	$CaBr_2$	0.38:1	$1.60 \pm 0.08 \times 10^5$

[a] The units of K_{app} are M^{-1} for complexation with P-PR and N-PR, M^{-2} for Ca^{2+}-*c*-C-PR, and noninteger values for the other complexes. The error limits represent 90% confidence levels. [b] The binding constant is for the 1:1 complex.

depending on the sizes of the cavity and cations (Pedersen, 1970; Frensdorff, 1971). The carboxylic ionophorous antibiotic A23187 forms 2:1 ligand–cation complexes with alkaline-earth metals in ethanol (Pfeiffer et al., 1974; Deber and Pfeiffer, 1976) and in the crystalline state (Smith and Duax, 1976), but 1:1 as well as nonintegral mole ratios have been reported with these cations under other conditions (Alpha and Brady, 1973, Case et al., 1974). The polyether, carboxylic ionophorous antibiotic X537A also forms 2:1 and 1:1 ionophore–divalent cation complexes (Degani and Friedman, 1974; Caswell and Pressman, 1972; Johnson et al., 1970). The depsipeptide monovalent ionophore valinomycin forms both 1:1 and 2:1 ionophore–K^+ complexes in ethanol, depending on the cyclopeptide concentration (Ivanov, 1975). Both 1:1 and 2:1 complexation of ligand with $CaCl_2$ was reported with an acyclic ligand (a derivative of dioxaoctanediacetamide) at high ligand concentration (Büchi and Pretsch, 1975). This variation in mole ratios in complexes formed by the synthetic ligands and well-known ionophores suggests that ion-binding properties are sensitive to changes in ligand–ligand interactions, ligand conformation, and cation size and charge. Studies on the possible participation of the aromatic ring in the interaction of cation with our ligands are in progress. These studies may clarify the origin of the differences found in stoichiometry for aromatic and nonaromatic ligands.

The relatively small difference in the apparent binding constants of P-PR and C-PR for Ca^{2+} may arise because opposing factors in P-PR partially offset each other. Relative to C-PR, P-PR has a higher dipole moment because of colinearity of the side chains. This effect is expected to increase the association constants (Simon et al., 1973). Opposed to this is the lower basicity of the coordinating sites in the aromatic ligand. This tends to weaken the interaction with cations, as shown for dibenzo-18-crown-6 relative to the dicyclohexyl analogue (Pedersen and Frensdorff, 1972).

The fact that *c*-C-PR forms a 2:1 complex with Ca^{2+} (Figure 5) suggests a possible correlation between ion-binding stoichiometry of neutral carrier molecules and their ion-transporting activity. We have found that *c*-C-PR transports Ca^{2+} across artificial membranes faster than N-PR and P-PR, which form 1:1 complexes with Ca^{2+} (Wun and Bittman, 1977). The ion in a 2:1 ligand–cation complex may be better shielded from the hydrophobic membrane interior than cation in a 1:1 complex, resulting in more facile transmembrane ion transport.

Complex formation in solution may involve various species. In an equilibrium of the type $nL + M \rightleftharpoons L_nM$, where L and M are interacting components, species such as LM, L_2M . . . L_{n-1} and L_nM may coexist in solution. At high ligand concentration and high ligand to cation ratio, complexes having ligand to cation mole ratios higher than 1:1 may exist in solution, causing deviations from 1:1 stoichiometry at the beginning of some of the titrations (Figures 4 and 7). However, a simple 1:1 stoichiometric relationship is found when titrations are performed at lower ligand concentrations (Figure 3). The $MgBr_2$ used has 6 molecules of hydration. The low association constant of ligand–Mg^2 binding (Table I) and the deviation from 1:1 stoichiometry for N-PR–Mg^{2+} interaction (Figure 3C) are not likely to arise from the small amount of H_2O (~0.07% at the end of titration) present; further addition of H_2O to a final concentration of 0.14% in the titrated solution did not cause appreciable dissociation of the complex, as judged from the absorbance of the solution.

The order of apparent binding strengths for complexation with divalent cations ($Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$) found for each ligand (Table I) may indicate that a field-strength effect is operative, i.e., stronger binding by the smaller and more intensely charged ions. This suggests that in complex formation ion–dipole interaction between the cation and the negative dipoles of the ligand is important. Magnesium ion may deviate because its small radius does not allow efficient "packing" of all of the necessary chelating sites (the "radius-ratio" effect as described by Williams, 1970).

The order of binding affinity obtained for the synthetic ligands toward alkaline-earth cations ($Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$) differs from the ion complexation order found for X537A in methanol and in two-phase extraction studies ($Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$) (Degani et al., 1973; Degani and Friedman, 1974; Pressman, 1973), A23187 in aqueous media ($Ca^{2+} > Mg^{2+} \gg Sr^{2+} \gg Ba^{2+}$) (Pfeiffer et al., 1974), and polyanionic acyclic ligands in aqueous media (generally, $Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$) (Sillen and Martell, 1964, 1971). It also differs from the selectivity sequences displayed by two types of electrically neutral polycyclic ligands that form inclusion complexes with cations, the diazapolyoxamacrobicyclic ligands ("cryptands") and the cyclic polyethers. Cryptands having large cavity size display the sequence $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$ (Lehn and Sauvage, 1975). Dicyclohexyl-18-crown-6 in aqueous solution has the selectivity sequence $Ba^{2+} > Sr^{2+} \gg Ca^{2+}, Mg^{2+}$ (Izatt et al., 1971). The order we found is the same as the selectivity sequence obtained in liquid-membrane electrodes for a dioxaoctanediacetamide ligand (Kirsch and Simon, 1976); however, the order of binding affinity found for the interaction in ethanol of the alkaline-earth ions with the latter ligand differs from this sequence. The ordering $Ca^{2+} > Sr^{2+} > Ba^{2+} \gg Mg^{2+}$ is also found in aqueous solution for proteins such as troponin and some extracellular enzymes (Williams, 1970) and for ligands such as 3-oxadiacetic acid and 1,2-bis(carboxymethoxy)ethane (Miyazaki et al., 1974) and 1,2-phenylenedioxydiacetic acid (Suzuki et al., 1968). The ligands we describe here appear to be novel in that they display a moderate degree of selectivity for Ca^{2+} over the other alkaline-earth metal ions while retaining a marked selectivity for divalent vs. monovalent cations.

Since the existence of selective complexation does not necessitate high stability, it is also of interest to compare the stability constants of the complexes formed by our ligands with those of natural and other synthetic ligands. The apparent binding constants for association of the synthetic ligands with Ca^{2+} in methanol (Table I) are comparable, and even slightly higher, than that found for X537A-Ca^{2+} complexation in methanol (Degani et al., 1973), in 80% ethanol and 50% methanol (Caswell and Pressman, 1972), and in absolute ethanol (Cornelius et al., 1974). Our ligands form stronger complexes with alkaline-earth cations in methanol than do the dioxaoctanediacetamide ligands bearing *N*-phenyl or *N*-(ω-carbethoxyundecyl) groups in ethanol (Kirsch and Simon, 1976). However, the macrobicyclic cryptands form much tighter complexes with these ions in 95% methanol (Lehn and Sauvage, 1975).

References

Alpha, S. R., and Brady, A. H. (1973), *J. Am. Chem. Soc. 95,* 7043–7049.

Ammann, D., Bissig, R., Güggi, M., Pretsch, E., Simon, W., Borowitz, I. J., and Weiss, L. (1975), *Helv. Chim. Acta 58,* 1535–1548.

Borowitz, I. J., Lin, W-O., Wun, T-C., Bittman, R., Weiss, L., Diakiw, V., and Borowitz, G. B. (1977), *Tetrahedron* (in press).

Büchi, R., and Pretsch, E. (1975), *Helv. Chim. Acta 58,* 1573–1583.

Case, G. D., Vanderkooi, J. M., and Scarpa, A. (1974), *Arch. Biochem. Biophys. 162,* 174–185.

Caswell, A. H., and Pressman, B. C. (1972), *Biochem. Biophys. Res. Commun. 49,* 292–298.

Christensen, J. J., Eatough, D. J., and Izatt, R. M. (1974), *Chem. Rev. 74,* 351–384.

Cornelius, G., Gärtner, W., and Haynes, D. H. (1974), *Biochemistry 13,* 3052–3057.

Deber, C. M., and Pfeiffer, D. R. (1976), *Biochemistry 15,* 132–140.

Degani, H., and Friedman, H. L. (1974), *Biochemistry 13,* 5022–5031.

Degani, H., Friedman, H. L., Navon, G., and Kosower, E. M. (1973), *J. Chem. Soc., Chem. Commun.,* 431–432.

Frensdorff, H. K. (1971), *J. Am. Chem. Soc. 93,* 600–606.

Ivanov, V. T. (1975), *Ann. N.Y. Acad. Sci. 264,* 221–243.

Izatt, R. M., Nelson, D. P., Rytting, J. H., Haymore, B. L., and Christensen, J. J. (1971), *J. Am. Chem. Soc. 93,* 1619–1623.

Job, P. (1928), *Ann. Chim. 9,* 113–203.

Johnson, S. M., Herrin, J., Liu, S. J., and Paul, I. C. (1970), *J. Am. Chem. Soc. 92,* 4428–4435.

Kirsch, N. N. L., and Simon, W. (1976), *Helv. Chim. Acta 59,* 357–363.

Koenig, K. E., Helgeson, R. C., and Cram, D. J. (1976), *J. Am. Chem. Soc. 98,* 4018–4020.

Lehn, J. M., and Sauvage, J. P. (1975), *J. Am. Chem. Soc. 97,* 6700–6707.

Miyazaki, M., Shimoishi, Y., Miyata, H., and Toei, K. (1974), *J. Inorg. Nucl. Chem. 36,* 2033–2038.

Morf, W. E., and Simon, W. (1973), *Helv. Chim. Acta 54,* 2683–2704.

Pedersen, C. J. (1967), *J. Am. Chem. Soc. 89,* 7017–7036.

Pedersen, C. J. (1970), *J. Am. Chem. Soc. 92,* 386–391.

Pedersen, C. J., and Frensdorff, H. (1972), *Angew. Chem. Int. Ed. Engl. 11,* 16–25.

Pfeiffer, D. R., Reed, P. W., and Lardy, H. A. (1974), *Biochemistry 13,* 4007–4014.

Pressman, B. C. (1973), *Fed. Proc., Fed Am. Soc. Exp. Biol. 32,* 1698–1703.

Scatchard, G. (1949), *Ann. N.Y. Acad. Sci. 51,* 660–672.

Sillen, L. G., and Martell, A. E. (1964), *Chem. Soc., Spec. Publ. 17.*

Sillen, L. G., and Martell, A. E. (1971), *Chem. Soc., Spec. Publ. 25.*

Simon, W., Morf, W. E., and Meier, P. Ch. (1973), *Struct. Bonding (Berlin) 16,* 113–160.

Smith, G. D., and Duax, W. L. (1976), *J. AM/ Chem. Soc. 98,* 1578–1580.

Suzuki, K., Hattori, T., and Yamasaki, K. (1968), *J. Inorg. Nucl. Chem. 30,* 161–166.

Vögtle, F., and Weber, E. (1974), *Angew. Chem. Int. Ed. Engl. 13,* 149–150.

Williams, R. J. P. (1970), *Q. Rev., Chem. Soc. 23,* 331–365.

Wun, T-C., and Bittman, R. (1977), *Biochemistry 16* (following paper in this issue).