

Correlation of Solution and Gas Phase Complexation Assessed by Electrospray Ionization Mass Spectrometry: Application to One-, Two-, and Three-Ring Macrocycles

Keshi Wang and George W. Gokel*

Bioorganic Chemistry Program and Department of Molecular Biology & Pharmacology, Washington University School of Medicine, Campus Box 8103, 660 South Euclid Avenue, St. Louis, Missouri 63110

Received January 2, 1996[®]

Electrospray ionization mass spectrometry (ESI-MS) was used to probe multiple cation complexation by $C_{12}H_{25}(N18N)(CH_2)_{12}(N18N)(CH_2)_{12}(N18N)C_{12}H_{25}$, **2**, and $(18N)CH_2C_6H_4CH_2(N18)$, **3**. Complexation of two cations ($2Na^+$, $2K^+$, or Na^+ and K^+) by **3** and three cations by **2** ($3Na^+$, $3K^+$, and mixtures) as well as mixed proton–metallic cation complexes of both were observed. The K^+/Na^+ cation-binding selectivity of 18-crown-6 was studied by ESI-MS of a methanol solution, and the selectivity profile was favorably compared with data obtained previously by ion-selective electrode techniques in the same solvent.

Introduction

During the past several years, we have attempted to develop relatively simple, synthetic organic models that would exhibit functional properties¹ of natural² cation-conducting channel systems.³ Our goal in this work has been to understand how selectivity is achieved in dynamic, membrane-spanning, cation-conducting systems. These model structures⁴ presumably remain stationary during cation transport unlike carrier compounds which move as a complex with the cation from one side of the membrane to the other. In addition to the obvious synthetic challenge of such model structures is the requirement to assess cation binding both in solutions of known polarity and in lipid bilayer membranes.⁵

Standard methods for assessing cation complexation in multiring macrocycles are problematic because multistate binding equilibria are inherently complicated to analyze. In channel model systems consisting of two or multiring macrocycles, the question of whether more than one ring is involved in complexation at any time is an important issue as it may bear directly on the mechanism of transport. In natural channels, flux has been attributed to cation occupancy of adjacent binding sites with fast displacement of cations by the nearest neighbor during transport. This transport mechanism requires the

multiple cation complexation situation that is difficult to quantitatively assess in solution.

Mass spectrometry has been shown to be a useful analytical tool for the assessment of cation–ligand interactions. It is of additional value in the present case because it is a rapid analytical method and very little sample is required. Practitioners in this field have been justifiably wary of adapting the technique to complexation studies because of the concern that the gas phase does not quantitatively reflect the solution phenomena under study.⁶ Their question is not whether the interactions apparent in the gas phase are possible or even reasonable in solution but whether the spectra accurately and precisely reflect solution phenomena. Certain assertions to the effect that the relationship is a valid one have been made in the literature,⁷ although the disparity between solution phase equilibria and charge state distributions for certain peptides has been described.⁸ We have recently used the Hammett linear free energy relationship to demonstrate a correlation between the solution and gas phase binding behavior of variously substituted dibenzyl ethers with Na^+ and K^+ cations.⁹ In the present report, we use the electrospray ionization (ESI-MS) method to assess the selectivity of 18-crown-6 binding the cations Na^+ and K^+ . The results are compared with the known selectivities determined by a variety of methods in the solution phase. We also expand our previous complexation studies (by fast atom bombardment, FAB-MS) of two-ring complexation¹⁰ to include three-ring macrocycles (by ESI-MS).¹¹

Results and Discussion

During the past several years, fast atom bombardment mass spectrometry has proved useful as a means to

* To whom correspondence should be addressed. Tel.: (314) 362-9297. FAX: (314) 362-9298 or 7058. E-mail: ggokel@pharmdec.wustl.edu.

[®] Abstract published in *Advance ACS Abstracts*, June 15, 1996.

(1) Gokel, G. W.; Medina, J. C.; Li, C. *Synlett* **1991**, 677–683.

(2) (a) Stein, W. D. *Channels, Carriers, and Pumps*; Academic Press: New York, 1990. (b) Hille, B. *Ionic Channels of Excitable Membranes*, 2nd ed.; Sinauer Associates: Sunderland, MA, 1992.

(3) (a) Nakano, A.; Xie, Q.; Mallen, J. V.; Echegoyen, L.; Gokel, G. W. *J. Am. Chem. Soc.* **1990**, *112*, 1287–1289. (b) Murillo, O.; Watanabe, S.; Nakano, A.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7665–7679.

(4) (a) Xie, Q.; Gokel, G. W.; Hernandez, J. C.; Echegoyen, L. *J. Am. Chem. Soc.* **1994**, *116*, 690–696. (b) Li, Y.; Gokel, G. W.; Hernandez, J. C.; Echegoyen, L. *J. Am. Chem. Soc.* **1994**, *116*, 3087–3096.

(5) (a) Deakyne, C. A.; Meot-ner, M. *J. Phys. Chem.* **1990**, *94*, 232. (b) Takayama, M.; Fukai, T.; Nomura, T.; Yamaguchi, T. *Org. Mass Spectrom.* **1991**, *26*, 655. (c) Zhang, H.; Chu, I.; Leming, S.; Dearden, D. *J. Am. Chem. Soc.* **1991**, *113*, 7415. (d) Takahashi, T.; Uchiyama, A.; Yamada, K.; Lynn, B. C.; Gokel, G. W. *Tetrahedron Lett.* **1992**, *33*, 3828. (e) Sawada, M.; Okumura, Y.; Shizuma, M.; Takai, Y.; Hidaka, Y.; Yamada, H.; Tanaka, T.; Kaneda, T.; Hirose, K.; Misumi, S.; Takahashi, S. *J. Am. Chem. Soc.* **1993**, *115*, 7381. (f) Takahashi, T.; Uchiyama, A.; Yamada, K.; Lynn, B. C.; Gokel, G. W. *Supramol. Chem.* **1993**, *2*, 177. (g) Brodbelt, J.; Maleknia, S. *J. Am. Chem. Soc.* **1993**, *115*, 2837. (h) Gross M. L.; Hu, P. *J. Am. Chem. Soc.* **1993**, *115*, 8821.

(6) (a) Wang, G.; Cole, R. B. *Org. Mass Spectrom.* **1994**, *29*, 419. (b) Langley, G. J.; Hamilton, D. G.; Grossel, M. C. *J. Chem. Soc., Perkin Trans. 2* **1995**, 929.

(7) Cheng, Z. L.; Siu, K. W. M.; Guevremont, R.; Berman, S. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 281.

(8) Wang, G.; Cole, R. B. *Org. Mass Spectrom.* **1994**, *29*, 419.

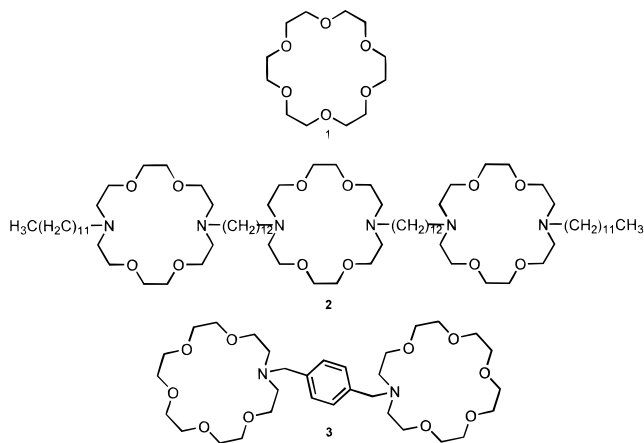
(9) Wang, K.; Han, X.; Gross, R. W.; Gokel, G. W. *J. Am. Chem. Soc.* **1995**, *117*, 7680–7686.

(10) Lynn, B. C.; Mallen, J. V.; Muñoz, S.; Kim, M.; Gokel, G. W. *Supramol. Chem.* **1993**, *1*, 195–197.

(11) A preliminary report of this aspect of the work has appeared: Wang, K.; Han, X.; Gross, R. W.; Gokel, G. W. *J. Chem. Soc., Chem. Commun.* **1995**, 641.

assess cation binding.¹² The importance of this developing technique is clear from recent reports of Johnstone *et al.*,¹³ Dearden *et al.*,¹⁴ Brodbelt *et al.*,¹⁵ Bowers,¹⁶ and others.¹⁷ We used this method to probe the binding properties of organometallic ligands.¹⁸ In order to assess whether corresponding results were obtained in solution and gas phase studies using the FAB-MS technique, we surveyed a series of 22 crown and lariat ethers.¹⁹ It proved possible in this case to correlate binding constants determined in the solution phase with peak intensities observed by FAB-MS. The relationship of binding strengths determined in solution and the gas phase by this method was "semi-quantitative" but the technique is of obvious value. Based in part upon this success, we extended studies to the compounds described below.

Compound Syntheses. Three compounds were required for the present study. 18-Crown-6, **1**, was prepared by the method described in *Organic Syntheses*.²⁰ Three-ring macrocycle **2** was prepared by the procedure recently detailed.^{3a} Two-ring ligand **3** was prepared by the reaction of α, α' -dibromo-*p*-xylene with 2 equiv of aza-18-crown-6 by a modification of the procedure reported by Sutherland and co-workers.²¹ The structures of compounds **1–3** are shown.



Electrospray Ionization Mass Spectrometry Method.

The fast atom bombardment method is ame-

nable to the study of molecular complexation because it involves desorption of the substance(s) under study by low level energy transfer from accelerated atoms that bombard the sample probe. Electrospray ionization is an even "softer" method for desorption, and very little molecular fragmentation is observed when this technique is used. Like FAB, ESI can be used to detect noncovalent interactions, but its "sensitivity" to such complexes is, in principle at least, greater. Moreover, a volatile solvent such as methanol or chloroform is amenable to ESI-MS whereas a less volatile matrix (commonly *m*-nitrobenzyl alcohol) is normally used in FAB-MS. The experiments described here were undertaken on a Finnigan mass spectrometer having an electrospray interface. Details of the electrospray ion source have been reported previously.²²

18-Crown-6 Complexation. One of the most extensively studied cation-complexing agents is 18-crown-6 (**1**), which binds a large array of metallic and organic ions. We used this host as a control compound to explore multi-crown ether cation binding. Sodium and potassium cations were used in this "calibration" study. In both cases, chloride was the counteranion.

The equilibrium constant for the reaction of 18-crown-6 with sodium cation in methanol is $10^{4.35}$ ($\log_{10} K_S = 4.35$) and $10^{6.08}$ for 18-crown-6 with potassium cation.²³ The binding constant ratio $[K_S(K^+)/K_S(Na^+)]$ is 54. Direct use of the relative mass peak intensities to assess the 18-crown-6 binding selectivity of K^+ over Na^+ presents an obvious problem. If the two cations were present in solution in equimolar concentrations and peak intensity quantitatively corresponds to cation binding strength, the ESI-MS peak intensity for $[1 \cdot K]^+$ would be 50-fold that of $[1 \cdot Na]^+$. The 50:1 peak ratio would introduce considerable error since the low peak intensity measurement for the latter would be inherently inaccurate. Therefore, a solution was used that contained a higher concentration ratio of Na^+ over K^+ .

For the present study, four anhydrous methanol solutions were prepared in which $[K^+] = 20 \mu M$ and $[Na^+]$ was varied so that $[Na^+]/[K^+]$ (as the chlorides) = 5, 10, 15, 20. In all cases, $[18\text{-crown-6}] = 2 \mu M$. Electrospray ionization mass spectra were determined in triplicate for each solution.²⁴ Under these circumstances, the ratio of K^+ to crown **1** was always 10 ($[K^+]:[1] = 10:1$) and the concentration of Na^+ was even higher (see Experimental Section). This experiment was designed so that competition between Na^+ and K^+ in the same solution could be reliably observed.

Isotopic Corrections Required for Binding Constant Studies. Normal isotopic distributions were assumed to be present in the macrocycles and cations used in the present study. Isotopic corrections were therefore applied to the experimental data prior to assessing the complexation constants. Five peaks are observed in a typical $Na^+/K^+/18\text{-crown-6}$ mass spectrum (see Figure 1). The observed peaks are $1 \cdot Na^+$ ($m/z = 287.0$), its isotope peak ($m/z = 288.0$), $1 \cdot K^+$ ($m/z = 303.0$), $1 \cdot K^+^{13}C$ isotope peak ($m/z = 304.1$), and $1 \cdot K^+^{41}K$ isotope peak ($m/z = 305.0$). In order to avoid systematic errors, especially for small peak intensities, theoretical isotopic corrections (see Experimental Section) were applied in order to

(12) (a) Malhotra, N.; Roepstorff, P.; Hansen, T. K.; Becher, J. *J. Am. Chem. Soc.* **1990**, *112*, 3709. (b) Zhang, H.; Chu, I.-H.; Leming, S.; Dearden, D. V. *J. Am. Chem. Soc.* **1991**, *113*, 7415. (c) Katritzky, A. R.; Malhotra, N.; Ramanathan, R.; Kemerait, R. C., Jr.; Zimmerman, J. A.; Eyler, J. R. *Rapid Commun. Mass Spectrom.* **1992**, *6*, 25. (d) Sawada, M.; Shizuma, M.; Takai, Y.; Yamada, H.; Kaneda, T.; Janafusa, T. *J. Am. Chem. Soc.* **1992**, *114*, 4405.

(13) (a) Johnstone, R. A. W.; Lewis, I. A. S. *J. Chem. Soc., Chem. Commun.* **1984**, 1268. (b) Johnstone, R. A. W.; Lewis, I. A. S. *Int. J. Mass Spectrom.* **1983**, *46*, 451. (c) Johnstone, R. A. W.; Lewis, I. A. S.; Rose, M. E. *Tetrahedron* **1983**, *39*, 1597.

(14) Zhang, H.; Chu, I.-H.; Leming, S.; Dearden, D. V. *J. Am. Chem. Soc.* **1991**, *113*, 7415.

(15) Liou, C.-C.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 543.

(16) Lee, S.; Wytenbach, van Helden, G.; Bowers, M. T. *J. Am. Chem. Soc.* **1995**, *117*, 10159.

(17) (a) Bonas, G.; Boss, C.; Vegnon, M. R. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 88. (b) Paker, D. *J. Chem. Soc., Chem. Commun.* **1985**, 1129. (c) Laali, K.; Lattimer, R. P. *J. Org. Chem.* **1989**, *54*, 496.

(18) (a) Medina, J. C.; Goodnow, T. T.; Rojas, M. T.; Atwood, J. L.; Lynn, B. C.; Kaifer, A. E.; Gokel, G. W. *J. Am. Chem. Soc.* **1992**, *114*, 10583–10595. (b) Medina, J. C.; Bert C. Lynn; Rojas, M. T.; Gokel, G. W.; Kaifer, A. E. *Supramol. Chem.* **1993**, *1*, 145–153.

(19) Gokel, G. W.; Trafton, J. E. *Cation Binding by Lariat Ethers. In Cation Binding by Macrocycles*; Marcel Dekker: New York, 1990; p 253.

(20) Gokel, G. W.; Cram, D. J.; Liotta, C. L.; Harris, H. P.; Cook, F. L. *Org. Synth.* **1977**, *57*, 30.

(21) Johnson M. R.; Sutherland, I. O.; Newton, R. F. *J. Chem. Soc., Perkin Trans. 1* **1980**, 586.

(22) Weintraub, S. T.; Pinckard, R. N.; Hail, M. *Rapid Commun. Mass Spectrom.* **1991**, *5*, 309.

(23) Gokel, G. W.; Goli, D. M.; Minganti, C.; Echeogoyen, L. *J. Am. Chem. Soc.* **1983**, *105*, 6786.

(24) Spectra for the $[Na^+/K^+] = 5$ case were measured once.

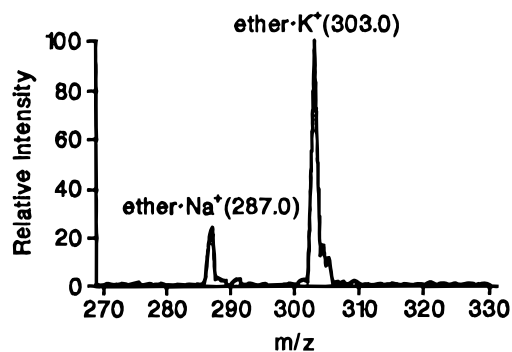


Figure 1. Electrospray ionization mass spectrum (ESI-MS) of 18-crown-6 binding Na^+ and K^+ . $[\text{Na}^+]/[\text{K}^+] = 10$. $[\text{K}^+] = 20 \mu\text{M}$. $[\text{18-Crown-6}] = 2 \mu\text{M}$.

Table 1. Peak Intensity Data for ESI-MS Analysis of 18-Crown-6 with Sodium and Potassium Cations^a

$[\text{Na}^+]/[\text{K}^+]$	obsd intensity values		corr'd intensity values	
	$I_{(\text{Na}^+)}$	$I_{(\text{K}^+)}$	$I_{(\text{Na}^+)}$	$I_{(\text{K}^+)}$
5	12	100	13	123.2
10	23 ± 1	100	26 ± 2	123.2
15	36 ± 3	100	41 ± 2	123.2
20 ^b	47 ± 2	100	54 ± 2	123.2

^a $[\text{K}^+] = 20 \mu\text{M}$, $[\text{18-crown-6}] = 2 \mu\text{M}$. ^b Methanol was used as solvent. "I" stands for peak intensity of crown-cation complex. ^b $[\text{K}^+] = 177408 \mu\text{M}$, $[\text{18-crown-6}] = 1 \mu\text{M}$.

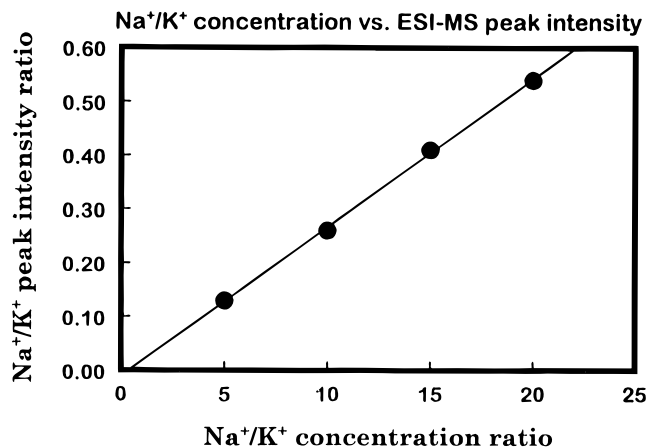


Figure 2. Na^+/K^+ concentration vs ESI-MS peak intensity.

obtain overall binding intensities. These corrections are reflected in all tabulated data (Table 1).

There are two issues that should be addressed for the simpler 18-crown-6 cation-binding case before considering the situation with two- or three-ring cation binders. First, it would be useful to establish the relationship between cation-crown complex peak intensity in the gas phase and the concentrations prepared in the solutions used in the experiment. Ideally, this should be a linear relationship. The second issue concerns the relationship between the cation selectivities observed for the gas phase compared to data obtained independently for the solution phase. The latter goes to the heart of the question, which is whether and to what extent the mass spectra reflect solution phenomena. The relationship between the intensity ratio observed in the mass spectrum and the cation concentration ratio in solution is plotted in Figure 2.

Assume that the crown-cation complex peak intensity (ESI-MS) is proportional to the concentration ratio in solution, *i.e.*, $I_{[\text{Na}^+]} \propto [\mathbf{1} \cdot \text{Na}^+]$ or $I_{[\text{Na}^+]} = C_1[\mathbf{1} \cdot \text{Na}^+]$, where

C_1 is a linear coefficient. If linear, the constant C_1 is the correlation factor between the gas phase spectra and solution phase. Similarly, $I_{[\text{K}^+]} = C_2[\mathbf{1} \cdot \text{K}^+]$. Therefore, $I_{[\text{Na}^+]} / I_{[\text{K}^+]} = [C_1 / C_2] [[\mathbf{1} \cdot \text{Na}^+] / [\mathbf{1} \cdot \text{K}^+]]$ or $I_{[\text{Na}^+]} / I_{[\text{K}^+]} = [C_1 / C_2] - [[K_S(\text{Na}^+) / K_S(\text{K}^+)][[\text{Na}^+] / [\text{K}^+]]$. It is apparent from Figure 2 that when the cation concentration ratio $[\text{Na}^+] / [\text{K}^+]$, increases, the crown-cation complex peak intensity ratio $I_{[\text{Na}^+]} / I_{[\text{K}^+]}$ increases linearly. Thus, the experimental data support the above assumption. Note that the Yintercept is close to zero. We infer from this that the above relationship is not only linear in the experimental range ($[\text{Na}^+] / [\text{K}^+] = 5-20$) but also in the range of $[\text{Na}^+] / [\text{K}^+] = 0-5$.

On the basis of the above assumption, the selectivity ratio $\text{K}^+/\text{Na}^+ = K_S(\text{K}^+) / K_S(\text{Na}^+) = [I_{[\text{K}^+]} / I_{[\text{Na}^+]}][[\text{Na}^+] / [\text{K}^+]] - [C_1 / C_2]$. The calculated results of $[I_{[\text{Na}^+]} / I_{[\text{K}^+]}]$ (see Table 1) for all four experiments fall in the range 46 ± 1 . Since $K_S(\text{K}^+) / K_S(\text{Na}^+) \approx 54$, the calculated value for $[C_1 / C_2]$ is 1.17, reasonably close to 1. If the gas phase spectra reflect solution phase phenomena and the peak intensity represents crown-cation binding strength in solution, $[C_1 / C_2]$ should equal 1, because in this case, peak intensity depends only on the concentrations of the complexes and not on their identities. We conclude from the above data, at least for the case of 18-crown-6 complexing Na^+ and K^+ , that the observation of solution complexation by the ESI-MS method is valid and nearly quantitative within the specified concentration range. Moreover, the relative peak intensity ratios can be used to estimate the crown ether cation-binding selectivity.

Analysis of Multiring Complexation. Two sets of experiments were done with each substrate. In the first, shown in Table 2 as A, a solution of either **2** or **3** (0.05 mM), KCl (0.05 mM), and NaCl (0.5 mM) was prepared in 10% v/v $\text{CHCl}_3:\text{CH}_3\text{OH}$. In the second set (B), **2** (0.05 mM), KCl (0.15 mM) and NaCl (0.5 mM) were dissolved in 10% v/v $\text{CHCl}_3:\text{CH}_3\text{OH}$. The masses and assignments of the observed peaks (compounds **2** and **3**) along with relative intensity data are recorded in Table 2.

The concentrations recorded in columns 1 and 2 in Table 2 reflect an effort to assess differences in sodium or potassium selectivities. When the Na^+ :ligand concentration ratio was 10:1, the base peak in the spectra of **2** or **3** (see column A in Table 2) was the complex between the ligand and a number of sodium cations equal to the number of macrorings present. Potassium complexation was certainly observed, but the spectrum was dominated by sodium-cation complexes. When the K^+ concentration was increased to equimolar with the number of cation-binding rings present in **2**, an ion having the composition $[\mathbf{2} \cdot 3\text{K}]^{3+}$ was prominent, although the base peak was $[\mathbf{2} \cdot 2\text{Na} \cdot \text{K}]^{3+}$. The latter is expected because of the differential in cation concentrations.

To our knowledge, the data presented herein constitute the first definitive evidence for triple cation complexation. In a previous study, we found that FAB-MS could detect the presence of certain dication complexes of tris(macrocyclics).¹¹ In the latter case, the three macrocyclic rings were separated by 1,6-hexylene spacers. Thus, in the extended conformation, the rings were closer together than they are in the present case and electrostatic repulsion could have been greater. The observation of $[\mathbf{3} \cdot 2\text{Na}]^{2+}$ is particularly notable since the macrorings of **3** are separated by a fairly rigid spacer that is expected to prevent the sort of ring-ring cooperativity that might be possible in **2**.

Table 2. Electrospray Ionization Mass Spectra for Compounds **2** and **3**^a

ion	<i>m/z</i>	rel int A ^b	rel int B ^c	ion	<i>m/z</i>	rel int A	rel int B
[2·3H] ³⁺	486	12	11	[2·3Na·Cl] ²⁺	780.5	31	20
[2·2H·Na] ³⁺	493.5	54	26	[2·H·2K·Cl] ²⁺	785.1	<5	16
[2·2H·K] ³⁺	498.6	17	28	[2·2Na·K·Cl] ²⁺	788.2	21	43
[2·H·2Na] ³⁺	500.7	98	43	[2·Na·2K·Cl] ²⁺	795.7	8	36
[2·H·Na·K] ³⁺	506.2	58	95	[2·3K·Cl] ²⁺	804.2	<5	28
[2·3Na] ³⁺	508.2	100	25	[2·Na] ⁺	1478.6	22	17
[2·H·2K] ³⁺	511.5	9	52				
[2·2Na·K] ³⁺	513.5	81	100	[3·2H] ²⁺	315.1	<5	
[2·Na·2K] ³⁺	518.7	17	83	[3·H·Na] ²⁺	326.2	5	
[2·3K] ³⁺	524.2	<5	31	[3·2Na] ²⁺	337.1	100	
[2·2H] ²⁺	729	12	10	[3·Na·K] ²⁺	345.1	25	
[2·H·Na] ²⁺	740.1	35	17	[3·2K] ²⁺	353.1	<5	
[2·H·K] ²⁺	747.9	9	16	[3·H] ⁺	629	<5	
[2·2Na] ²⁺	750.8	70	31	[3·Na] ⁺	651.6	18	
[2·Na·K] ²⁺	759	31	62	[3·K] ⁺	667.6	<5	
[2·2K] ²⁺	767	8	28	[3·2Na·Cl] ⁺	709.5	24	
[2·H·2Na·Cl] ²⁺	769.2	6	17	[3·Na·K·Cl] ⁺	725.4	9	
[2·H·Na·K·Cl] ²⁺	777.1	<5	26	[3·2K·Cl] ⁺	741.6	<5	

^a See Experimental Section for conditions. ^b Column A conditions: [2] = [3] = K⁺ = 0.05 mM; [Na⁺] = 0.5 mM. ^c Column B conditions: [2] = 0.05 mM, [K⁺] = 0.15 mM, and [Na⁺] = 0.5 mM.

Poly(ethylene glycol)s [PEG's, RO(CH₂CH₂O)_{*n*}R, R = H, CH₃] are capable of binding more than one cation simultaneously. Thus, in methanol solution, a plot of log *K*_S vs log MW increased linearly.²⁵ Nevertheless, the ability of the PEG's to catalyze phase-transfer reactions was not enhanced by the use of long chains. Instead, catalysis was correlated to the molarity of PEG chains available to transfer cations from the polar to the nonpolar phase. The present case is remarkable in this context as the macrocycle appears capable of binding three cations in the gas phase in the absence of a neutralizing anion or coordinating solvent. A perusal of Table 2 reveals that no peak observable above the base line indicates the presence of methanol.

No peak in the spectrum of **3** shows a relative intensity greater than 25% except [3·2Na]²⁺. Two peaks of a similar intensity (~25%, [3·Na·K]²⁺ and [3·2Na·Cl]⁺) have molecular weights that correspond to the inclusion of two cations.

Compound **2** possesses three diaza-18-crown-6 subunits, and the larger number of macrorings affords a larger number of possible cation complexes. Under conditions A, as noted in Table 2, the base peak is [2·3Na]³⁺. A peak observed at *m/z* 500.7 has the composition [2·H·2Na]³⁺ and a relative intensity of 98%. The relative intensity for the corresponding [2·H·2K]³⁺ ion is <10%. Peaks having relative intensities >50% and which contain two or three metallic cations are [2·H·Na·K]³⁺, [2·2Na·K]³⁺, and [2·2Na]²⁺. In addition, an ion corresponding to [2·2H·Na]³⁺ is observed with a similar, high relative intensity.

The presence of K⁺ in several of the major ions observed in the mass spectra of **2** and **3** suggests that this ion has a higher binding affinity for the diaza-18-crown-6 ring system than does Na⁺ since it is present in lower concentration than the latter. We have previously determined the cation complexation constants for *N,N*-di-*n*-nonyl-4,13-diaza-18-crown-6 with Na⁺ and K⁺ in anhydrous methanol solution. The values for log₁₀ *K*_S are, respectively, 2.99 and 3.70.²⁶ The corresponding values for the *n*-nonyl crown are 2.95 and 3.70. These solution phase data are consistent with the gas phase

observations presented here. At higher concentrations of K⁺ (Table 2, conditions B, [Na⁺]:[K⁺] = 10:3; conditions A, 10:1), five prominent ions are observed, and all of them contain K⁺. Two of the three most intense ions contain three metallic cations, with the base peak being [2·2Na·K]³⁺. Moreover, four of these five most intense ions are triply-charged.

It is also interesting to note that the chloride counterion appears in several ions. It is presumably coordinated to one or more sodium or potassium cations, although the ESI-MS technique does not afford structural information. It should be noted that chloride is observed only when more than two metallic cations are present; ions of the type [(**2** or **3**)·cation·Cl] would be neutral overall and not detected.

Conclusion

Two goals were accomplished in the present study. First, the K⁺/Na⁺ cation binding selectivity of the simple complexing agent 18-crown-6 is shown to be similar in methanol solution whether assessed by ion-selective electrode techniques or by electrospray mass spectrometry. This is an important validation for the ESI-MS technique as applied to crown ether chemistry and suggests that broader applicability to complexation phenomena may be justified. We also demonstrate by use of this mass spectrometric technique that three cations may be simultaneously bound by a tris(macrocylic) ligand. No previous study confirms this possibility, nor, to our knowledge, is there any obvious solution technique that can be used to determine cation-binding constants for such complex structures. The ESI-MS technique holds considerable promise for assessing cation interactions with binders that are too structurally complex to assess by other methods.

Experimental Section

¹H-NMR were recorded at 300 or 500 MHz in CDCl₃ solvents, and chemical shifts are reported in ppm (δ) downfield from internal (CH₃)₄Si. Melting points were determined on a capillary melting point device in open capillaries and are uncorrected. Thin layer chromatographic (TLC) analyses were performed on aluminum oxide 60 F-254 neutral (Type E) with a 0.2 mm layer thickness or on silica gel 60 F-254 with a 0.2 mm layer thickness. Flash chromatography columns were packed with silica gel, Merck grade 9385, 230–400 mesh 60 Å.

(25) Gokel, G. W.; Goli, D. M.; Schultz, R. A. *J. Org. Chem.* **1983**, *48*, 2837.

(26) Gatto, V. J.; Arnold, K. A.; Viscariello, A. M.; Miller, S. R.; Morgan, C. R.; Gokel, G. W. *J. Org. Chem.* **1986**, *51*, 5373.

All reactions were conducted under dry N₂ unless otherwise noted. All reagents were the best grade commercially available and were distilled, recrystallized, or used without further purification, as appropriate. Molecular distillation temperatures refer to the oven temperature of a Kugelrohr apparatus. Combustion analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are reported as percents.

18-Crown-6, 1, was prepared according to the *Organic Syntheses* procedure.²¹

N,N-Bis[12-[N-(N-dodecyl)diaza-18-crown-6]dodecyl]diaza-18-crown-6, 2, was prepared according to the published procedure and had analytical data that corresponded to published values.^{3a}

Synthesis of α,α' -Bis(aza-18-crown-6)-*p*-xylene. α,α' -Dibromo-*p*-xylene (264 mg, 1 mmol), 1-aza-18-crown-6 (553 mg, 2.1 mmol), and K₂CO₃ (1.5 mg, excess) were suspended in butyronitrile (30 mL). The reactants were heated under reflux for 4 h. The reaction mixture was cooled and filtered, and the filtrate was evaporated, giving the crude product which was purified by column chromatography (Al₂O₃) to afford a light yellow oil (500 mg, 80%): ¹H-NMR 2.79 (t, 8H, NCH₂CH₂O), 3.45–3.69 (m, 44H, OCH₂ + ArCH₂N), 7.25 (s, 4H, aryl-H); ESI-MS M + Na⁺ 651.6. The NMR data corresponded to published values.²³

Mass Spectra. ESI mass spectra were acquired on a triple quadrupole tandem mass spectrometer (Finnigan MAT TSQ 700, San Jose, CA) equipped with an electrospray interface (Analytica of Branford, Branford, CT). The detector of the instrument is an off-axis continuous dynode electron multiplier operable from –400 to –3000 V, with a variable post-acceleration/conversion dynode voltage from –3 to –20 kV for detection of positive ions. The electrospray ion source and its functional parts have been described in detail. For all experiments, both the electrospray needle and the skimmer were operated at ground potential, whereas the electrospray cham-

ber (i.e., cylindrical electrode) and metallized entrance of the glass capillary were operated at –3.5 kV. A +90 V potential was applied to the metallized exit of the glass capillary. A separate +175 V potential was placed on the tube lens for acquisition of the positive ions. The temperature of nitrogen drying gas as it entered the electrospray chamber was set at 100 °C, and the drying gas was held at a constant pressure of 25 psi. A 4-min period of signal averaging was employed for each spectrum.

Preparation of Samples. Anhydrous CH₃OH was used for the 18-crown-6–K⁺/Na⁺ cation-binding selectivity study. For studies involving **2** and **3**, the solvent was 10% CHCl₃ in CH₃OH (v/v). Solutions suitable for study were prepared (ambient temperature, ~23 °C) by mixing the appropriate individual stock solutions to afford the desired final concentration. The resulting solutions were vortexed and allowed to stand for ≥30 min prior to use. Sample solutions were infused directly into the ESI chamber by use of a syringe pump (flow rate of 2 μL/min).

Isotope Corrections. The following data were used in the isotope correction calculations: abundance, ¹²C, 100%; ¹³C, 1.11 (contribution to M + 1: 12 (carbons)1.11 = 13.32, contribution to M + 2 : 12 × (12 – 1) × 1.11/200 = 0.81); ¹H, 100; ²H, 0.015 (contribution to M + 1: 24(hydrogens) × 0.015 = 0.36); ¹⁶O, 100; ¹⁷O, 0.037 (contribution to M + 1: 6(oxygens) × 0.037 = 0.22); ¹⁸O, 0.038 (contribution to M + 2: 6 (oxygens) × 0.038 = 1.22); ³⁹K, 100; ⁴⁰K, 0.013; ⁴¹K, 7.22.

Acknowledgment. We thank the NIH for a grant (GM 36262) that supported this work. We also gratefully acknowledge the assistance of Drs. R. W. Gross and X. Han in the early stages of this work.

JO960023P