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Characterization of Chiral Host−Guest Complexation in Fast Atom Bombardment Mass Spectrometry

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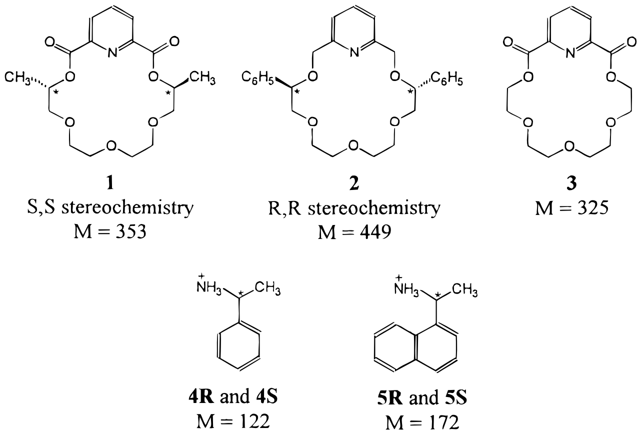
A new method has been developed for the characterization of complexion between host and guest molecules. Adduct formation between chiral crown ethers 1 and 2 and enantiomeric ammonium ions 4 and 5 was examined. The reference compound 3 (achiral host) was chosen to be similar in structure to the chiral crown ethers for quantitative measurements. Our approach is based on a formalism assuming an equilibrium: [chiral host + H]$^+$ + [achiral host + chiral guest]$^+$ $\rightleftharpoons$ [chiral host + chiral guest]$^+$ + [achiral host + H]$^+$. The equlibrium constant for this process was calculated using the relative peak intensities of the corresponding species in the FAB mass spectra. It was found that these provide significantly better reproducibility and more reliable results than the relative peak intensity method described before (Sawada, M.; et al. *J. Am. Chem. Soc.* 1992, *114,* 4405; 1993, *115,* 7381; *Org. Mass Spectrom.* 1993, *28,* 1525).[1−3] In the examples studied, the equilibrium constants corresponding to the formation of heterochiral adducts (*S,S-R* or *R,R-S*) were higher than those for the formation of homochiral aggregates (*S,S-S* or *R,R-R*).

Determination of the discrimination between guest enantiomers by a chiral host has always been a challenge for mass spectrometrists. The use of chiral crown ethers as host molecules and the investigation of their adduct formation with chiral guest compounds by mass spectrometry have gained a lot of interest during the last few years.[1−4] Sawada and co-workers[1] reported the use of modified carbohydrates and modified chiral crown ethers as host molecules (Chir) for the discrimination of enantiomeric alkylammonium ions (A). An internal standard compound (Ref) structurally related to the host was employed and the relative peak intensities (RPI) [$I_{(\mathrm{Chir+A^+})}/I_{(\mathrm{Ref+A^+})}$] were calculated for the relevant diastereomeric adduct ions. It was found that the studied heterochiral adducts (*RS* or *SR*) were more stable and gave higher RPI values than their homochiral (*RR* or *SS*) counterparts. A more detailed investigation[2] was also carried out to demonstrate the usefulness of the RPI method. The time dependence of RPI values was examined, and they were found to be nearly constant in a 1 min interval. The degree of chiral recognition ($\mathrm{RPI}_R/\mathrm{RPI}_S$) reached 1.6 in some cases. In another study,[3] *RR* and *SS* homochiral adducts were found to be more stable than *RS* or *SR* adducts. With the help of Fourier transform ion cyclotron resonance measurements[4] the equilibrium constants for the complexation between a chiral crown ether and the enantiomers of 1-(α-naphthyl)ethylammonium cation were determined. The heterochiral cluster was found to be more stable than the homochiral one, the stability difference being $\Delta\Delta G = 4.2 \pm 0.4$ kJ mol^{-1}.


1
S,S stereochemistry
M = 353

2
R,R stereochemistry
M = 449

3
M = 325

4R and **4S**
M = 122

5R and **5S**
M = 172

Figure 1. Chiral macrocycles and primary ammonium cations studied.

In the present paper we describe a new approach which provides a more reliable and reproducible method for the determination of the degree of chiral discrimination than the above-mentioned (RPI) technique. The usefulness of our methodology is demonstrated by two chiral crown ethers (**1** and **2**) as host molecules and two enantiomeric pairs of chiral ammonium salts (**4***R*, **4***S* and **5***R*, **5***S*] (see Figure 1) as guests.

EXPERIMENTAL SECTION

1 was prepared by a method different from that described in the literature.[5] It was prepared from dimethyl-2,6-pyridinedicar-

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Table 1. Relative Stability Constants of Crown Ether−Ammonium Cation Complexes[a]

	FAB		solution	
	1	**2**	**1**	**2**
4	1.00 ± 0.02	0.17 ± 0.01	1.0 ± 0.09	6.1 ± 0.09
5	0.92 ± 0.02	0.12 ± 0.01	1.2 ± 0.04	6.1 ± 0.09

[a] Mean values of homo- and heterochiral adducts are given relative to the complex **1**−**4**. These data are determined from the relative stability constants (K) measured in the present work, characterizing FAB conditions, and K values determined in methanol solution.[8]

boxylate and (2*S*,12*S*)-(+)-4,7,10-trioxatridecane-2,12-diol in the presence of 4 Å molecular sieves and a catalytic amount of sodium methoxide by base-catalyzed transesterification reaction as described for the preparation of the di-*tert*-butyl analogue.[6] **1** obtained by this base-catalyzed transesterification method gave the same optical rotation, melting point, and IR and ^{1}H NMR spectra as **1** prepared by the literature procedure.[5] **2**[6] and **3**[7] were prepared as described in the literature. The hydrogen perchlorate salts of **4** and **5** were prepared according to the method described.[8]

Fast atom bombardment mass spectra were recorded on a VG-ZAB-2SEQ (VG Analytical) reverse geometry mass spectrometer operating at an accelerating voltage of 8 kV. The sample was bombarded with a beam of Cs^+ ions having an energy of 30 keV. Scans were collected for 20 min in the mass range of 100−1000 Da using a 5 s/scan rate. The spectra were averaged for every minute, and the relative peak intensities listed by the data system were used to calculate the equilibrium constants.

The host (**1** and **2**), reference (**3**), and guest (perchlorate salts of **4***R* or **4***S* and **5***R* or **5***S*) compounds were dissolved in ethyl acetate (0.1 M). The solutions of host and reference compounds were mixed (1:1), and these mixtures were used for the studies. Glycerol (1 μL), the solution of the host + reference (1 μL), and solution of the guest compound (1 μL) were mixed on the FAB probe and introduced into the ion source. In the experiments, at least three parallel measurements were performed and the average K values were calculated. The results are summarized in Table 1.

RESULTS AND DISCUSSION

The adducts due to host−guest complexes gave reasonably abundant peaks in the FAB mass spectra. Figure 2 shows the mass spectrum obtained by mixing **1**, **3,** and **4***S*. The protonated molecules of the reference (Ref, an achiral host) and the chiral crown ether (Chir) give rise to peaks at m/z 326 and 354, respectively. m/z 364 and 392 are the potassium adducts due to K^+ traces in the sample, whereas the peaks at m/z 447 and 475 are due to the reference + amine guest (Ref + A^+) and to the chiral host + amine guest (Chir + A^+) adducts, respectively.

Methods for Data Evaluation. In earlier studies,[1−4] the relative peak intensity RPI = $I_{(\mathrm{Chir+A^+})}$ /$I_{(\mathrm{Ref+A^+})}$ values were calculated and used for the characterization of host−guest interaction phenomena. Our approach involves the assumption of an equilibrium between protonation and organic ammonium cation exchange, which can be described by the following equations:

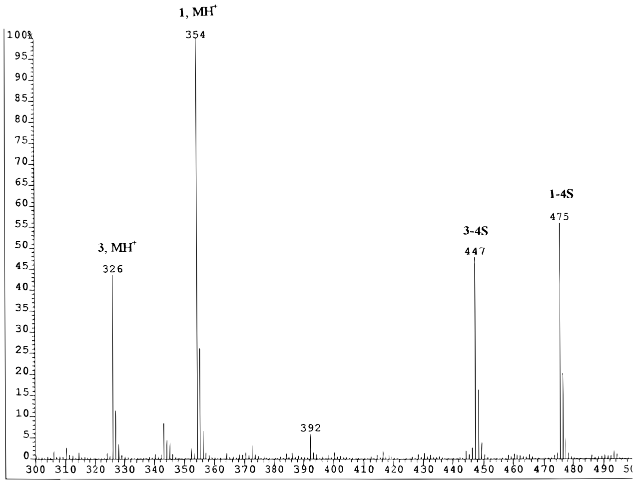

Figure 2. FAB mass spectrum obtained by mixing **1, 3**, and **4***S***.**

$$\mathrm{ChirH^+ + Ref \rightleftharpoons Chir + RefH^+} \qquad (1)$$

$$\mathrm{RefA^+ + Chir \rightleftharpoons Ref + ChirA^+} \qquad (2)$$

where Chir is the chiral crown ether, Ref is the achiral (reference) crown ether, and A^+ is the organic ammonium cation. The sum of these two processes is

$$\mathrm{ChirH^+ + RefA^+ \rightleftharpoons ChirA^+ + RefH^+} \qquad (3)$$

An equilibrium constant (K) can be defined corresponding to eq 3

$$K = \frac{[\mathrm{ChirA^+}][\mathrm{RefH^+}]}{[\mathrm{ChirH^+}][\mathrm{RefA^+}]} \qquad (4)$$

In eq 4, the brackets indicate concentrations. As only ionic species are present whose concentration is proportional to their relative peak abundances, K can be determined from the mass spectra.

Figure 3 shows the results of a typical experiment reproduced three times under identical conditions where the spectra were averaged for every minute between 1 and 20 min measurement time and the RPI and K values were calculated. It can clearly be seen that the K value remains constant (or shows a very slight linear time dependence) whereas RPI values show a relatively large scattering. We believe that this dramatic improvement shows the advantage of using an equilibrium constant instead of a somewhat arbitrary peak ratio. We do not claim that there is a true equilibrium in the FAB process (it may only approximate an equilibrium or a steady state), but using this formalism we get much more reproducible results. From another point of view, the scattering using RPI values is related to a change in the [$\mathrm{RefH^+}$]/[$\mathrm{ChirH^+}$] ratio. It is a typical effect in FAB that the spectra of mixtures show a time dependence; in the present case,

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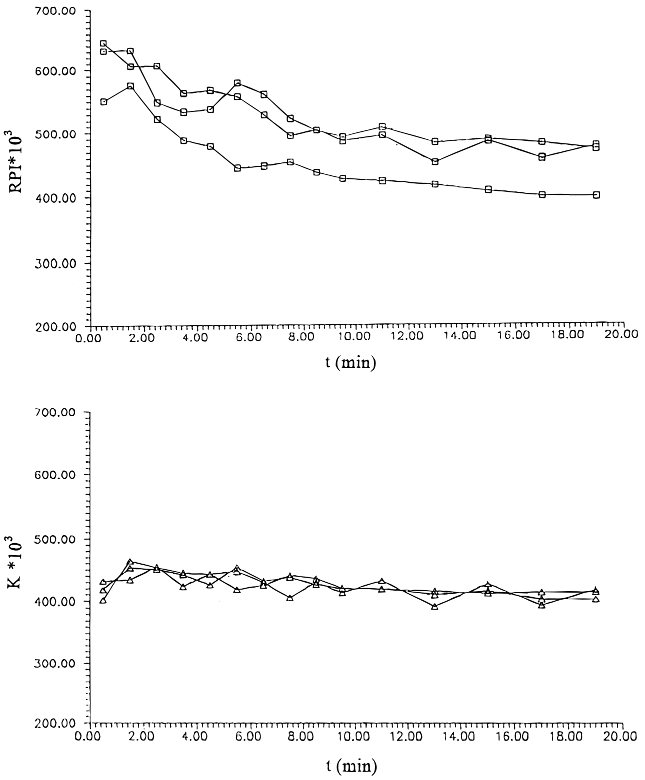

Figure 3. RPI and K values vs time. (RPI − [ChirA$^+$]/[RefA$^+$], K = [RH$^+$] [ChirA$^+$] / [RefA$^+$] [ChirH$^+$]); Chir = **1**; Ref = **3**; A = **5***S*.

the [RefH$^+$]/[ChirH$^+$] ratio changes. This ratio could change for several reasons, e.g., slightly different concentration of Ref and Chir in the matrix, surface effects (one of these compounds may be concentrated at the surface, and this may change during the experiment), etc. In experiments where very small differences have to be detected between the stabilities of different adduct species, it is very important to use a precise and reproducible method for data evaluation and sample characterization. As these results indicate, the equilibrium method developed here is much more suitable and accurate one for the study of host−guest complexation than the RPI method described before.[1]

When the concentrations of host + reference and those the guest were changed independently by ~1 order of magnitude, it was found that the calculated K values corresponding to a particular system changed only slightly (~3%). Thus, if only a very small amount of sample is available it is not necessary, that the reference compound and the chiral analyte be applied in the same concentration.

Comparison of *R* and *S* Enantiomers. As mentioned in the introduction, in some cases[3] homochiral and in others[1] heterochiral aggregates were found to give larger RPI values. In a study by Sawada et al.,[2] regardless of the configuration of the two applied crown ethers, $I_{(\mathrm{Chir+A}_R)^+}/I_{(\mathrm{Chir+A}_S)^+}$ ratios were found to be 1.2 for 1-(α-naphthyl)ethylammonium ion complexation. Our data show that heterochiral complexation is more favorable than the homochiral one; that is, K values corresponding to *S,S-R* and *R,R-S* were higher than the values of *S,S-S* and *R,R-R*, respectively. In Figure 4, the results of the **1**−**5***R* and **1**−**5***S* complexation experiments are shown. The K values exhibit a slight time dependence, but the two enantiomers of ammonium ions can unambiguously be distinguished, the two lines are well separated throughout the measurement.

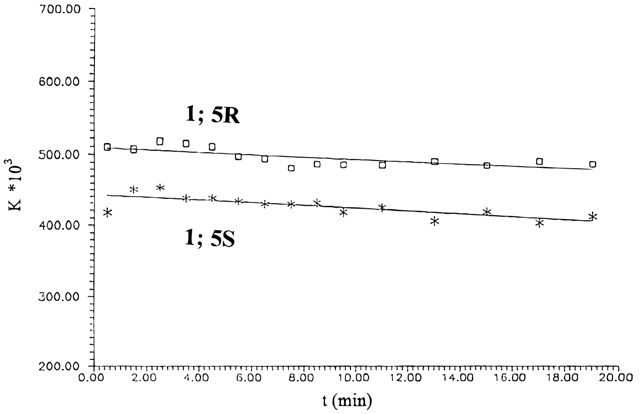

Figure 4. Comparison of enantiomers **5***R* and **5***S* using chiral crown ether **1**: $K_{SS\text{-}R}/K_{SS\text{-}S} = 1.17$.

Table 2. Chiral Discrimination (Ratios of Stability Constants, $K_{RR\text{-}S}/K_{RR\text{-}R}$ and $K_{SS\text{-}R}/K_{SS\text{-}S}$) for Hetero- vs Homochiral Complexes[a]

	FAB		solution	
	1	**2**	**1**	**2**
4	1.10 ± 0.02	1.29 ± 0.10	1.7 ± 0.31	1.4 ± 0.28
5	1.17 ± 0.02	1.70 ± 0.15	2.7 ± 0.19	1.5 ± 0.28

[a] FAB data refer to the present measurement of equilibrium constants (K); solution data were determined in methanol; the stability constants are taken from the literature.[8]

The stability of crown ether−ammonium cation complexes studied here is determined mainly by two attractive and one repulsive types of interactions:[8,9] three hydrogen bonds between the ammonium protons of the ammonium cation and the pyridine nitrogen and two alternating macroring oxygens of the crown ether; π−π interaction between the phenyl (**4**) or naphthyl (**5**) group of the ammonium cation and the pyridine ring of the crown ether; and steric hindrance between the ammonium cation and the methyl (**1**) or phenyl (**2**) substituents of the crown ether. In the complexes studied, this third interaction, steric hindrance, is the main difference. This is likely the primary cause of enantiomeric discrimination as well.

The stability of crown ether−ammonium cation complexes can be characterized by mass spectrometry using the relative equilibrium constant (K) described above. The precise mechanism and place [solution, selvedge (the relatively high-pressure gas close to the matrix surface), or gas phase] of the formation of these complexes under FAB conditions is, however, unclear. Ion abundances probably reflect steady state rather than true equilibrium conditions—so the equilibrium constants described in this paper should be regarded rather as an analytically useful characterization of the complexes and of chiral recognition than as true equilibrium values characterizing either the gas or the liquid phase. Even so, the stability of and chiral effects in the complexes studied under FAB conditions show a remarkable, and chemically very reasonable, dependence on the substituents. This behavior contrasts somewhat with that determined in solution (see Tables 1 and 2). This suggests that stabilities determined by FAB characterize complexes significantly isolated from their molecular environment, as in the gas phase, contrasting with that in solution

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where interactions with solvent molecules have a crucial role in determining stability constants.

As shown in Table 1, the stability of the complexes under FAB conditions depends very significantly on the size of the substituents both of the ammonium cation and of the crown ether (methyl vs phenyl). Steric hindrance between larger substituents results in lower stability in going from the phenyl (**4**) to the naphthyl (**5**) group of the ammonium cation and especially from methyl (**1**) to phenyl (**2**) substituents of the crown ether. This behavior contrasts with that in solution, where the difference in the stability of complexes with phenyl- and naphthylethylammonium cations is negligible.[8] In solution, the diphenyl-substituted crown ether (**2**) gives more stable complexes than the dimethyl-substituted one (**1**)—opposite to that observed in FAB. This undoubtedly reflects the effect of solvent molecules on complex stability and likely stereochemical behavior (enantioselectivity).

The heterochiral complexes (*S,S-R* or *R,R-S*) studied in the present paper are more stable than homochiral ones (*S,S-S* or *R,R-R*) both in FAB and in solution. (In the case of the complexes of **1**−**5***R* and **1**−**5***S* this order of stability was also observed in the crystalline state[10] .) This stability order is explained by steric repulsion between the substituents, destabilizing mainly the homochiral complex. Enantioselectivity is inversely proportional to the stability of the ammonium cation-crown ether complex in FAB: **2**−**5** is 8 times less stable with 7 times larger enantioselectivity than **1**−**4**. Substituent size has a smaller effect on the enantioselectivity in solution-phase equilibrium measurements,[8] and under such conditions, there is also little correlation between enantioselectivity and complex stability.

A possible explanation of this behavior is that, under FAB conditions (which is likely to be close to a solvent-free environment), optimum conformation of the crown ether, especially the plane of the phenyl substituent, could be fairly rigid. This changes during complex formation, decreasing complex stability in the case of phenyl, compared to that of the smaller (and spherically approximately symmetrical) methyl substituent. In one diastereomer (the heterochiral complex) the steric hindrance between substituents is smaller, so this complex has higher stability. The larger the substituents, the more important this repulsive effect is. This effect decreases stability but increases chiral discrimination at the same time.

In solution, the conformation of the crown ether, especially the plane of the phenyl substituent, is likely to be more flexible as the mobile solvent molecules can stabilize many possible conformations. This distorts both the “intrinsic” stability and the “intrinsic” chiral recognition between the two enantiomers. Therefore the correlation between substituent size and stability, between substituent size and chiral discrimination; and between stability and chiral discrimination becomes more difficult to rationalize in solution than in FAB conditions.

The study of chiral recognition by FAB has two main advantages compared to other studies: (1) the high sensitivity of mass spectrometry allows characterization using very small sample amounts, and (2) under FAB conditions, as the results in the present paper show, “intrinsic” chiral effects can be studied and good correlations between substituent size and complex stability and also between substituent size and enantioselectivity can be found.

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