Host–Guest Complexation. 12. Total Optical Resolution of Amine and Amino Ester Salts by Chromatography[1,2]

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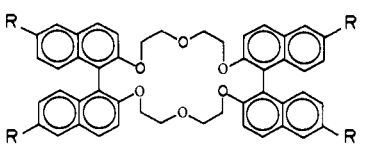
Abstract: This paper reports two new methods for the optical resolution of racemates of primary amine salts, particularly those of amino esters (guests or G). The first method involved liquid–liquid chromatography with H_2O–$NaPF_6$ or H_2O–$LiPF_6$ solutions supported on Celite or silica gel as the stationary phase. The mobile phase consisted of $CHCl_3$ solutions of (*R,R*)-2,3,4,5,13,14,15,16-tetra(1,2-naphtho)-1,6,9,12,17,20-hexaoxacyclodocosa-2,4,13,15-tetraene (bis(dinaphthyl)-22-crown-6) as host (H). The appearance of the H·G complex in the column eluate was monitored conductometrically. The following separation factors (α) for enantiomers were observed: for α-phenylethylammonium hexafluorophosphate 1.48 at 25 °C and 1.76 at 0 °C; for methyl phenylglycinate·HPF_6 2.48 at −13 °C; for methyl *p*-hydroxyphenylglycinate·HPF_6 3.6 at −15 °C. These values correlate well with the enantiomer distribution constant values observed in one-plate extractions of the same guests in water by the same hosts in $CHCl_3$. The direction of the stereochemical bias in complexation in the one-plate and chromatographic separations also matched. The second method utilized liquid–solid chromatography at 25 °C. The same (*R,R*)-bis(dinaphthyl)-22-crown-6 host was attached to silica gel through Ar–$Si(CH_3)_2O$–Si bonds at the 6 position of the naphthalene rings to provide a solid phase in which each host site had an average molecular weight of ~17 000. This position is remote from the complexing site of the host. The mobile phase consisted of $CHCl_3$ or CH_2Cl_2 solutions of primary amine salts as guests and 18-crown-6, ethanol, or isopropyl alcohol as carriers. The appearance of salt in the column eluate was monitored conductometrically. For α-phenylethylammonium hexafluorophosphate in $CHCl_3$, α = 1.6; for methyl phenylglycinate·HPF_6 in $CHCl_3$, α = 1.52; for isopropyl phenylglycinate·HCl in CH_2Cl_2, α = 2.9; for methyl *p*-hydroxyphenylglycinate·HCl in $CHCl_3$, α = 2.4; for methyl valinate·HCl in $CHCl_3$, α = 1.73; for methyl phenylalaninate·HCl in $CHCl_3$, α = 4.4; for methyl tyrosinate·HCl in $CHCl_3$, α = 2.3; and for methyl tryptophanate·HCl in $CHCl_3$, α = 6.4. The *direction* and *extent* of the stereochemical bias in complexation at the solid–liquid interface also correlate with those observed in one plate H_2O–$CHCl_3$ extractions. Baseline separations between enantiomers were observed only for the ester salts of the most bulky guests, *p*-hydroxyphenylglycine, phenylalanine, tyrosine, and tryptophane. Minima were observed between peaks for the other guests. Models for the host–guest *complexes are discussed.*

Gas–liquid chromatography has been developed to a fine art for the analytical separation of volatile racemates into their enantiomers. In the most successful separations, derivatives of amino acid racemates have been completely resolved by passing them through long capillary columns of very high plate value which contained optically active peptide-derived liquid phases.[4] Liquid–solid chromatography for preparative optical resolution with optically active, naturally occurring polymers or inorganic surfaces has been reviewed.[5] Optically active complexing agents (e.g., Newman's chiral π-acid[6a,b]) have been successfully used for chromatographically resolving π-base hydrocarbons,[6c,d] particularly the helicenes.[6e] By attachment of L-arginine to Sephadex, a solid phase was obtained which was used to chromatographically resolve 3,4-dihydroxyphenylalanine. A complementary placement of ion-pairing sites between the bound resolving site and one enantiomer of the racemate was probably responsible for the observed resolution.[6f] Countercurrent extraction in liquid–liquid ion exchange between optically active amine salts and racemic sodium mandelate or *N*-acetylalaninate led to complete resolution of the former substances.[7] Separation factors, α (the ratio between the retention volume of the more highly bound and that of the lesser bound enantiomer), as high as 1.6 were observed;[6f] however, most were less than 1.3.[4-7]

In papers 7–9 in this series, we described the syntheses of optically active host compounds designed to complex differentially and predictably the enantiomers of amine salts as guests, particularly those of amino acids and esters.[8] One of the hosts was a polyether containing two 1,1′-dinaphthyl units of established absolute configuration incorporated into a macrocycle by substitution at their 2,2′ positions (**1**).[8a] Feasibility in attaching substituents in the 6,6′ positions remote from the central binding site was established.[8b] In complexation experiments in distributing amine and amino ester salts between water and $CHCl_3$ solutions of (*S,S*)-**1**, enantiomer distribution constants (EDC) (eq 1 and 2) that ranged between 1.7 and 5.0 were observed.[9b]



1, R = H
2, R = $Si(CH_3)_2OCH_3$
3, R = Br

This paper describes the use of systems such as **1** and **2** to preparatively resolve three amine or amino ester salt racemates by chromatography. In one method, (*R,R*)-**1** in an organic mobile phase was used to elute differentially enantiomeric salts in a water phase absorbed on a stationary solid phase. In a second method, (*R,R*)-**1** was covalently attached at its 6 position to silica gel and used in liquid–solid chromatographic columns to resolve six amine or amino ester salts.

Results and Discussion

Resolution by Liquid–Liquid Chromatography. The solid phases consisted of Celite or silica gel on which were absorbed half to two times their weights of aqueous solutions of $NaPF_6$ or $LiPF_6$. Racemic amine hydrobromide or hydrochloride (25 to 200 mg) in a minimum of salt solution absorbed on a minimum of solid phase was placed at the top of each column. The mobile phase was a solution of optically pure (*R,R*)-**1**[10] in $CHCl_3$ or CH_2Cl_2. The appearance of complexed salt in the column eluate was monitored by a conductivity cell whose relative conductance was linear in the concentration of complex (see Figure 1).

The salts of the racemates of α-phenylethylamine, methyl phenylglycinate, and methyl *p*-hydroxyphenylglycinate were resolved. Figures 2–4 are plots of the relative conductance vs. milliliters of the column eluates for the three guest compounds.

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Table I. Liquid-Liquid Chromatographic Runs with Host (*R,R*)-**1** in $CHCl_3$ in the Mobile Phase and Racemic Guest in the Aqueous Stationary Phase

Run[a] no.	*T*, °C	G($R^*NH_3PF_6$) RNH_3^+	Amt, mg	Separation factor α	EDC (K_A/K_B)	Resolution R_s	Band integrals (A/B)	Theor plates *N*	$([H]/[G])_A$	$([H]/[G])_B$	M_H/M_G	Configuration of a more complex enantiomer
1	0	$C_6H_5CH(CH_3)NH_3^+$	200	1.8	1.8	0.6	0.94	24	4.5	8	16	*S*
2	25	$C_6H_5CH(CH_3)NH_3^+$	25	1.5	1.5	0.6		19	65	136	155	*S*
3	−13	$C_6H_5CH(CO_2CH_3)NH_3^+$	100	2.5	2.5	1.25	1.08	34	2.1	3.3	9	D
4	−15	p-$HOC_6H_4CH(CO_2CH_3)NH_3^+$	108	3.6	5	1.57	0.83	34	9	25	10	D
5[b]	−15	p-$HOC_6H_4CH(CO_2CH_3)NH_3^+$	100	2.4		1.28	0.93	74	2.7	4.1	5	D

[a] See Experimental Section for details. [b] Same as run 4 except CH_2Cl_2 was substituted for $CHCl_3$.

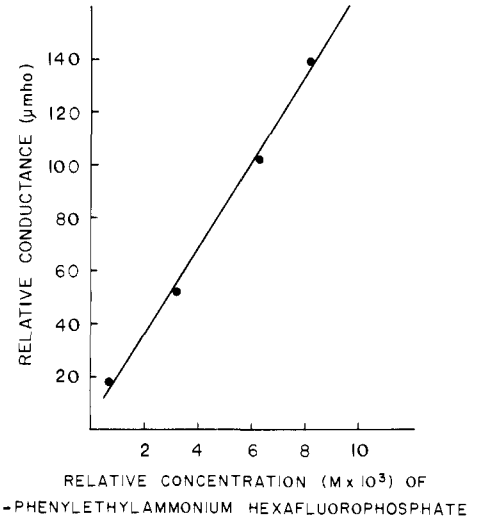


Figure 1. Plot of relative conductance vs. concentration of alkyl ammonium salt in $CHCl_3$ ~0.0375 M in (+)-(*R,R*)-**1**.

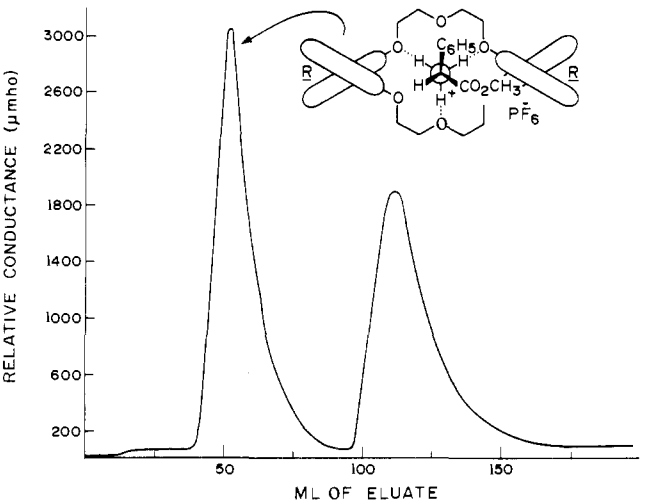


Figure 3. Chromatographic optical resolution by (*R,R*)-**1** of methyl phenylglycinate hexafluorophosphate salt.

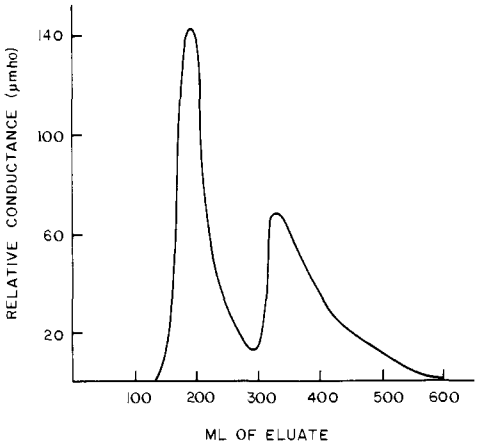


Figure 2. Chromatographic optical resolution by (*R,R*)-**1** of α-phenylethylammonium hexafluorophosphate.

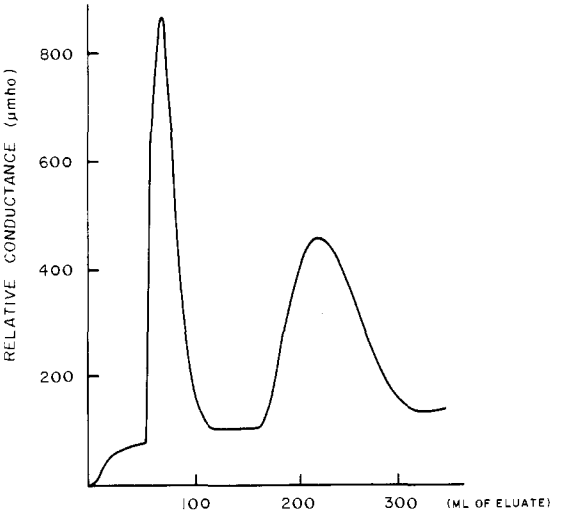


Figure 4. Chromatographic optical resolution by (*R,R*)-**1** of methyl *p*-hydroxyphenylglycinate hexafluorophosphate salt.

Pure enantiomers were recovered from each peak of Figures 2-4. Rotations were equal in magnitude and opposite in sign, and their configurations were determined by reference to authentic materials (see Experimental Section). Table I provides values for the column parameters,[11] and the Experimental

Section describes the columns and conditions used. Figures 2, 3, and 4 correspond to runs 1, 3, and 4, respectively.

Relationships between the enantiomer distribution constants (EDC) obtained in one-plate extractions and the separation factors (α) for the enantiomers in the multiplate extractions on these columns are indicated in eq 1–5. Equations 1 and 2

$$K_A = [G_A]_{CHCl_3}/[G_A]_{H_2O}; K_B = [G_B]_{CHCl_3}/[G_B]_{H_2O} \quad (1)$$

$$EDC = K_A/K_B \quad (2)$$

define EDC in terms of equilibrium guest concentrations of the more complexed enantiomer (A) in $CHCl_3$, the less complexed enantiomer (B) in $CHCl_3$, the corresponding concentrations of A and B in the aqueous phase, and the distribution constants (K) of each enantiomer between the two phases.[9b] Equations 3 and 4 pertain to the (multiplate) chromatographic

$$\alpha = \frac{V_{RB} - V_M}{V_{RA} - V_M} \quad (3)$$

$$V_{RA} = V_M + (V_s/K_A); V_{RB} = V_M + (V_s/K_B) \quad (4)$$

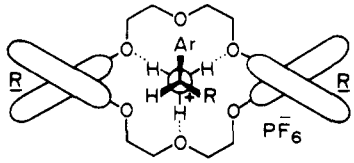
separations, and involve the following definitions:[11] V_M is the free volume of the column; V_s is the volume of the stationary aqueous phase; V_{RA} is the retention volume of the better-complexed, faster-moving component A; V_{RB} is the retention volume of the slower moving component B. Equation 5 relates the separation factors (α) obtained from the chromatographic runs to the EDC values obtained from the one-plate distribution experiments.[9b] Equation 6 defines the number of theoretical plates (N) of the column. Equation 7 defines the resolution (R_s) which provides a useful measure of the degree of separation of components A and B. In eq 6 and 7, W_A is the band width of component A and W_B is the band width of B measured in terms of the volumes at the baseline of the plot of relative conductivity vs. eluent volume.

$$\alpha = K_A/K_B = EDC \quad (5)$$

$$N = 16(V_{RA})^2/W_A^2 \quad (6)$$

$$R_s = 2\frac{V_{RB} - V_{RA}}{W_B + W_A} \quad (7)$$

With $C_6H_5CH(CH_3)NH_3PF_6$ and $C_6H_5CH(CO_2CH_3)NH_3PF_6$ salts as guests (runs 1–3), the observed separation factors (α) approximately equaled the EDC values observed in the one-plate extraction experiments,[9b] as required by eq 5. Thus, in these runs the columns exhibited the characteristics of liquid–liquid countercurrent extraction in which guest salt equilibrated between the two phases as it passed down the column. In run 4, the more hydrophilic *p*-$HOC_6H_4CH(CO_2CH_3)NH_3PF_6$ salt was used. Equilibrium does not appear to have been reached throughout the column, since $\alpha = 3.6$ and EDC = 5. The greater band width for component B over that for A also points to the nonideality of the column for this run. In runs 1–5, as anticipated from CPK molecular model examination and the one-plate extraction experiments,[9b] the first eluted enantiomer A possessed the configuration indicated by the guest in the structure of complex **4**. Thus, the configuration of the more stable diastereomeric complex is predictable based on stereoelectronic grounds. The ratio of the peak areas (A/B), which ideally should be unity, varied from 0.83 to 1.08. This was probably due to slight changes in the conductivity cell characteristics during a run. The baseline separation of enantiomers observed in runs 3–5 (e.g., see Figures 3 and 4) correlates with the resolution factors (R_s) that ranged from 1.25 to 1.57. The near baseline separation of enantiomers of Figure 2 indicates that in these runs, $R_s = 0.6$ is high enough to resolve most, but not all, of the sample. Interestingly, the theoretical plates (N), with $CHCl_3$ as the mobile phase, varied only between 19 and 34 in runs 1–4, although the column diameters, character of the solid support, concentrations of the MPF_6 salt in H_2O, and concentrations of host in the mobile phase varied (see Experimental Section). When the mobile phase was switched from $CHCl_3$ to CH_2Cl_2 (runs 4 and 5), N increased from 34 to 74, α decreased from 3.6 to 2.4, and R_s decreased from 1.56 to 1.28. Thus, chiral recognition, as measured by α, decreases in changing from $CHCl_3$ to CH_2Cl_2. This observation correlates with EDC measurements made in one-plate extraction experiments that involved other hosts and guests.[12]



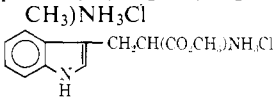
4a, Ar = C_6H_5, R = CH_3
4b, Ar = C_6H_5, R = CO_2CH_3
4c, Ar = *p*-HOC_6H_4, R = CO_2CH_3

This type of chromatography allows another interesting parameter to be calculated. The concentrations of host [H] used throughout each run were constant and known. The values of guest salt concentration [G] in the organic phase at the peak maxima of the plots were estimated from the total moles of each enantiomeric guest loaded on each column, the total integral of each band, and the integral of a small volume taken straddling the peak maximum of each band. The ratios, $([H]/[G])_A$ and $([H]/[G])_B$, were estimated and appear in Table I. Control experiments in one-plate extractions demonstrated that essentially all G in the $CHCl_3$ layer was complexed.[9b] If maximum use was being made of the host, the $([H]/[G])_A$ value would approach unity. Also, the greater the chiral recognition, the smaller should be the ratio of $([H]/[G])_A/([H]/[G])_B$. With $C_6H_5CH(CH_3)NH_3PF_6$ as guest, $([H]/[G])_A$ changed from 4.5 to 65 and $([H]/[G])_B$ from 8 to 136, respectively, when the temperature was changed from 0 to 25 °C in runs 1 and 2. Obviously, both enantiomers are more poorly complexed at the higher temperature. Similar temperature effects were observed in one-plate extraction experiments involving the same systems.[9b] With $C_6H_5CH(CO_2CH_3)NH_3PF_6$ as guest in run 3, $([H]/[G])_A = 2.1$ and $([H]/[G])_B = 3.3$. In this run, the host was more effectively used. With the more hydrophilic *p*-$HOC_6H_4CH(CO_2CH_3)NH_3PF_6$ of runs 4 and 5, the values increased. In $CHCl_3$, $([H]/[G])_A = 9$ and $([H]/[G])_B = 25$ (run 4), whereas in the more polar CH_2Cl_2 solvent, $([H]/[G])_A = 2.7$ and $([H]/[G])_B = 4.1$.

A practical question about these columns arises as to how much host is needed to resolve a given amount of guest. An indication is given by the ratio (M_H/M_G) between the moles of host (M_H) and moles of guest (M_G) measured between the points at which guest first appears in the eluate and the point at which it vanishes. The values range from 155 to 5 (Table I). In runs 1 and 2 in which temperature was changed from 0 to 25 °C, the ratio changed from 16 to 155 for $C_6H_5CH(CH_3)NH_3PF_6$. The respective values of 9 and 10 for runs 3 and 4 which involved $C_6H_5CH(CO_2CH_3)NH_3PF_6$ and *p*-$HOC_6H_4CH(CO_2CH_3)NH_3PF_6$ salts in $CHCl_3$ at −13 and −15 °C are somewhat higher than the value of 5 obtained in CH_2Cl_2 at −15 °C for the latter salt in run 5. Since baseline separation was observed in run 5, that chromatographic run was the most practical overall, even though the α value was somewhat lower in CH_2Cl_2 than in $CHCl_3$.

These examples demonstrate that one-plate extraction experiments based on designed chiral recognition in complexation can be easily translated into total optical resolutions using liquid-liquid chromatography, provided enough host is available.

Table II. Optical Resolution at 25 °C of Amine Salts by Solid-Liquid Chromatography on a 14 g Silica Gel Column Containing 0.82 mmol (768 mg) of (*R,R*)-Host

Run no.	G(R*NH$_3$X) RNH$_3$X	Amt, mg	Mobile phase	Carrier concn, M[a]	H/G[b]	Separation factor α	Resolution R_s	Band integrals A/B[c]	Theor plates N	Configuration of more complex enantiomer
1	$C_6H_5CH(CH_3)NH_3PF_6$	8.0	CH_2Cl_2	4.4×10^{-5}	21	1.5	~0.3	~0.9	≈60	*S*
2	$C_6H_5CH(CH_3)NH_3PF_6$	2.0	$CHCl_3$	2.0×10^{-4}	84	1.7	~0.6	~1	≈50	*S*
3	$C_6H_5CH(CO_2CH_3)NH_3Cl$	6.0	CH_2Cl_2	4.4×10^{-5}	20	1.2	~0.2	~1	≈70	D
4	$C_6H_5CH(CO_2CH_3)NH_3PF_6$	5.0	$CHCl_3$	2.0×10^{-4}	50	1.4	~0.3	~1	≈80	D
5	$C_6H_5CH(CO_2CH_3)NH_3PF_6$	2.0	$CHCl_3$	0.8% (v/v)[d]	128	1.6	0.45	1	90	D
6	$C_6H_5CH(CO_2CH(CH_3)_2)$-NH_3Cl	2.3	CH_2Cl_2	5% (v/v)[e]	80	2.9	2.3	1	50	D
7	*p*-$HOC_6H_4CH(CO_2CH_3)$-NH_3Cl	2.7	$CHCl_3$	10% (v/v)[e]	70	6.4	2.4	1	50	D
8	$(CH_3)_2CHCH(CO_2CH_3)$-NH_3Cl	6.0	$CHCl_3$	1.0×10^{-2}	23	1.7	1.4	1.1	30	L
9	$C_6H_5CH_2CH(CO_2CH_3)$-NH_3Cl	5.9	$CHCl_3$	5.0×10^{-3}	23	4.4	3.1	1.2	290	L
10	*p*-$HOC_6H_4CH_2CH(CO_2$-$CH_3)NH_3Cl$	2.4	$CHCl_3$	10% (v/v)[e]	82	2.3	0.9	1.0	50	L
11		3.0	$CHCl_3$	2.0×10^{-4}	70	6.4	2.5	1.1	40	D

[a] 18-Crown-6, unless indicated otherwise. [b] Moles of host to moles of guest. [c] A is the better complexed diastereomer. [d] Ethanol. [e] Isopropyl alcohol.

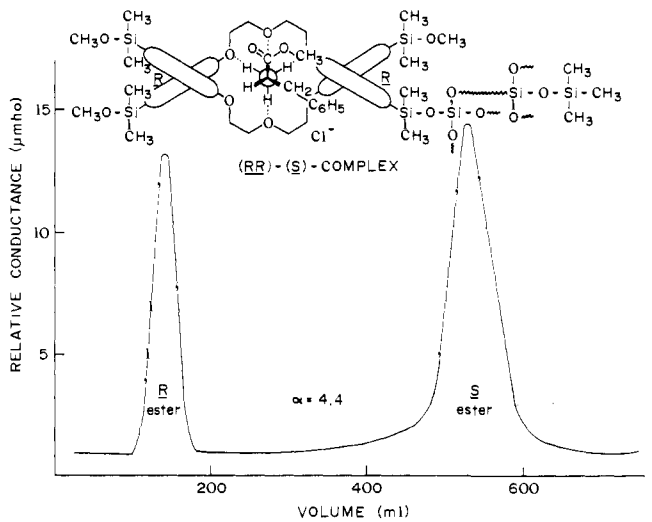


Figure 5. Chromatographic optical resolution by host-bound silica of methyl phenylalaninate hydrochloride salt.

Resolution by Liquid–Solid Chromatography. The previous section describes fractional elution of racemate guests from a stationary liquid phase by a mobile liquid phase containing an optically active host that differentially complexed and lipophilized the enantiomers. This section describes the covalent attachment of the same optically active host to silica gel to form a stationary solid phase used to resolve, by selective complexation, racemic guests from a mobile liquid phase. The syntheses of (*R,S*)-**2** and optically pure (*R,R*)-**3** have been described previously.[8b] The latter compound served as the starting material for attachment of host to silica gel.

Treatment of tetrabromide (*R,R*)-**3** with butyllithium in dry glyme at −75 °C, followed by dichlorodimethylsilane, provided the corresponding tetrakis(dimethylchlorosilyl) derivative. This substance was treated first with dry carbon-free silica gel and then with dry methanol to convert the unused $ArSi(CH_3)_2Cl$ functions to $ArSi(CH_3)_2OCH_3$ groups. The resulting silica gel bound host was shown, by combustion, to be 3.94% by weight. If, as is statistically probable, each cycle was covalently bound at only one site and the other three were capped with CH_3O groups, the product would contain 0.059 mmol of host per gram. Each host site, therefore, had an average molecular weight of 17 000. This material was then treated with excess $(CH_3)_3SiCl$ to destroy all sterically available SiOH groups remaining on the silica gel to give the product used in the chromatographic runs. This material was 4.65% by weight in carbon (combustion analysis) or 0.20 mmol of $(CH_3)_3Si$ groups per gram.

Chromatographic runs were made with racemic $C_6H_5CH(CH_3)NH_3PF_6$, $C_6H_5CH(CO_2CH_3)NH_3Cl$ and PF_6 salts, $C_6H_5CH(CO_2CH(CH_3)_2)NH_3Cl$, *p*-$HOC_6H_4CH(CO_2CH_3)NH_3Cl$, $(CH_3)_2CHCH(CO_2CH_3)$-NH_3Cl, $C_6H_5CH_2CH(CO_2CH_3)NH_3Cl$, *p*-$HOC_6H_4$-$CH_2CH(CO_2CH_3)NH_3Cl$, and $C_8H_6NCH_2CH(CO_2CH_3)$-NH_3Cl (methyl tryptophanate hydrochloride). These guest salts were dissolved in CH_2Cl_2 or $CHCl_3$ containing carriers (solubilizers) such as 18-crown-6, ethanol, or isopropyl alcohol. The column eluates were passed through the flow conductivity cell described in the Experimental Section. Plots of relative conductance vs. milliliters of eluate gave curves for each run. Table II summarizes the results.

In runs 1, 3, 4, and 6, the configurational identities of the enantiomers in the bands were established by comparing their signs of rotation with those of authentic samples. In runs 7–11, the identifications of the bands were made by comparing their retention volumes with those of pure enantiomers put through the same column under the same conditions as their racemates. The separation factors, α, were calculated from the elution plots. Figure 5 illustrates one resolution (run 9) which involved $C_6H_5CH_2CH(CO_2CH_3)NH_3Cl$ as guest. Baseline separation was observed only between the enantiomers of the ester salts of *p*-hydroxyphenylglycine, phenylalanine, tyrosine, and tryptophane. Minima were observed in the other runs. Preliminary runs made with host-bound silica gel untreated with $(CH_3)_3SiCl$ gave poorer separations, and bad tailing was observed. With the treated material, some tailing was observed for the more hydrophilic alkylammonium salts but less for the more lipophilic. These facts suggest that even with the treated material, SiOH groups are still available for interacting with

the more polar guests. The superposition of nonstereoselective and stereoselective complexation on the chromatograms should cause band overlap. Although CH_2Cl_2, as the mobile phase, gave less tailing, it also gave lower separation factors (compare runs 1 and 2 and 3 and 4).

Effects of Structure, Medium, Temperature, and Counterion on Complexation. The direction and extent of configurational bias in complexation has been determined between host **1** and a variety of RNH_3^+ guests in seven media: ($CHCl_3$, CH_2Cl_2, Celite-H_2O-$CHCl_3$, silica gel-H_2O-$CHCl_3$, silica gel-H_2O-CH_2Cl_2, silica gel-$CHCl_3$, and silica gel-CH_2Cl_2 at a variety of temperatures and with several counterions.[9b] Comparisons provide generalizations and conclusions. In runs 1 and 2 on silica gel-CH_2Cl_2 and silica gel-$CHCl_3$ (Table II) with $C_6H_5CH(CH_3)NH_3PF_6$ as guest at 25 °C, α values of 1.5 and 1.7 were obtained, respectively. The more stable diastereomeric complex possessed the (*R,R*)-(*S*) configuration. In runs 1 and 2 (Table I) in $CHCl_3$ with the same guest salt, α was 1.5 and 1.8 at 0 and 25 °C, respectively, and again, the more stable complex possessed the (*RR*)-(*S*) configuration. In run 4 on silica gel-$CHCl_3$ (Table II) with $C_6H_5CH(CO_2CH_3)NH_3PF_6$ at 25 °C, α = 1.4, and the more stable diastereomeric complex possessed the (*RR*)-(D) configuration. In run 3 (Table I) in $CHCl_3$, the same salt at −13 °C gave α = 2.5, and the more stable diastereomeric complex had the (*RR*)-(D) configuration. In run 6 (Table II) in silica gel-CH_2Cl_2, $C_6H_5CH(CO_2CH(CH_3)_2)NH_3Cl$ at 25 °C gave α = 2.9, and the (*RR*)-(D) complex was the more stable. In the one-plate extraction from water into $CDCl_3$ (Table III, run 1 in ref 9b), $C_6H_5CH(CO_2CH(CH_3)_2)NH_3PF_6$ at 25 °C gave EDC = 4.0 and the (*SS*)-(L) diastereomer was the more stable. In run 7 of Table II at 25 °C, *p*-$HOC_6H_4CH(CO_2CH_3)NH_3Cl$ on silica gel-$CHCl_3$ gave α = 6.4. In run 4 (Table II) in $CHCl_3$ at −15 °C, *p*-$HOC_6H_4CH(CO_2CH_3)NH_3PF_6$ gave 3.6. The same salt in one-plate extractions at −16 °C gave EDC = 5.0 (Table II, run 7).[9b] In all three types of experiments with the *p*-hydroxyphenylglycine ester salts, the (*RR*)-(D) diastereomer was the more stable. In runs 8 and 9 (Table II) at 25 °C, $(CH_3)_2CHCH(CO_2CH_3)NH_3Cl$ and $C_6H_5CH_2CH(CO_2CH_3)NH_3Cl$ salts with silica gel-$CHCl_3$ gave α values of 1.7 and 4.4, respectively. In each case, the (*RR*)-(L) diastereomeric complexes were the more stable. In experiments involving one-plate extractions into $CDCl_3$ at −10 and −1 °C (Table II, runs 9 and 11, in ref 9b), $(CH_3)_2CHCH(CO_2CH_3)NH_3PF_6$ and $C_6H_5CH_2CH(CO_2CH_3)NH_3PF_6$ salts gave EDC values of 1.5 and 1.8, respectively, and the (*RR*)-(L) complexes were the more stable.

In these three types of experiments (one-plate extractions,[9b] liquid-liquid chromatography, and liquid-solid chromatography), the complexing part of the host possessed the same structure. Six different media for complexing, and several different temperatures were involved. The above comparisons provide the following important generalizations and conclusions.

(1) *The configurations of the more stable diastereomeric complexes were independent of the media in which complexation occurred and were independent of the counterion and the temperature, and yet they were dependent on the structural relationships between host and guest.* Thus, the premise that examination of scale molecular models of complexes is a valid starting point for the design of host-guest relationships and for the interpretation of results appears sound to the extent that data are available.

(2) *The degree of chiral recognition depends on structure, environment, temperature, and counterion.* The data suggest that chiral recognition is greatest under the following conditions: when the cavities of the host are the most completely filled by the guest; when $CHCl_3$, silica gel-H_2O-$CHCl_3$, or silica gel-$CHCl_3$ provides the environment for complexation; when the temperature is lower; and when the counterion competes the least effectively with the host for hydrogen bonding sites of RNH_3^+ guest.[9b]

The conclusion involving the counterions requires elaboration. In experiments involving extraction or dissolution in $CDCl_3$, $C_6H_5CH(CH_3)NH_3^+Br^-$ or -I^- gave little or no chiral recognition.[9b] However, in runs 7 and 11 of Table II, methyl tyrosinate hydrochloride and methyl tryptophanate hydrochloride salt at the silica gel-$CHCl_3$ interface gave the highest chiral recognition yet observed with this simple system (α = 6.4). The difference in free energy between the two diastereomers for each salt is about −1.1 kcal/mol. Furthermore, hydrochloride salts of the other esters could be used successfully in the silica gel chromatograms, but not in the extraction, dissolution, or liquid-liquid chromatographic experiments.

A possible explanation is as follows. The tailing of the bands from the chromatograms suggested that some SiOH groups remained on the $(CH_3)_3SiCl$ treated silica gel. These sites may have provided hydrogen bonds to the Cl^- ions of low enough energy to free RNH_3^+ from hydrogen bonding to Cl^-. This allowed the host to bind in a tripod arrangement, the three RNH_3^+ hydrogen bonding sites that provide the complex with its high degree of structural organization and chiral recognition.

One of the objectives of these investigations is to be able to predict and rationalize on the basis of complementary placement of binding sites and steric barriers the direction of chiral bias in complexation. Plausible reasons have been given for the bias of (*R,R*)-**1** complexing (*S*)-$C_6H_5CH(CH_3)NH_3PF_6$, D-$C_6H_5CH(CO_2CH_3)NH_3PF_6$, D-$HOC_6H_4CH(CO_2CH_3)NH_3PF_6$, L-$(CH_3)_2CHCH(CO_2CH_3)NH_3PF_6$, and L-$C_6H_5CH_2CH(CO_2CH_3)NH_3PF_6$.[9b] The configurational bias of (*R,R*)-host toward D-methyl tryptophanate salt in run 9 of Table II contrasts with that of (*R,R*)-host toward L-methylphenylalaninate of run 8 and requires comment. Attractive forces between the C_6H_5 group acting as a π acid and a naphthalene as a π base arranged in parallel planes provided a key part of the explanation for the direction of the bias toward the methyl phenylalaninate salt guest.[9b] The greater steric requirements of the indolyl as compared to the phenyl group explains the switch in configurational bias between runs 8 and 9. A molecular model of the complex between (*R,R*)-**1** and D-methyl tryptophanate salt suggests that the large $C_8H_6CH_2$ group needs one entire cavity for its disposition. The (*R,R*)-(L) complex is difficult to construct. The π acid to π base attraction between the aromatic rings present in host and guest, which was invoked to explain the stability of the (*R,R*)-**1**·L-methyl phenylalaninate complex, must be missing in the corresponding tryptophanate complex.

Experimental Section

General. All solvents were reagent grade and were distilled before use. Chloroform was freed of ethanol by washing it six times with water containing a trace of H_2SO_4 and dried over $MgSO_4$. Optical rotations were determined at 25 °C in thermostated 1-dm cells with a Perkin-Elmer polarimeter Model 141. NMR spectra were taken on a Varian T-60 Spectrophotometer with Me_4Si as internal standard. All solutions for chromatography were carefully filtered before use. The sources and properties of the various amine and amino ester salts used here have been described,[9b] except for the hydrochloride methyl ester salt of racemic and L-tryptophane, which were purchased from Sigma and stored at 0 °. Salts $LiPF_6$ and $NaPF_6$ were purchased from Ventron (98+% pure). Hexafluorophosphoric acid diethyl etherate (Aldrich) was used to prepare aqueous HPF_6 solutions or amine salt solutions. The syntheses and properties of optically pure host (*R,R*)-**1** and tetrabromide (*R,R*)-**3** have been reported.[8a,10] The jacketed chromatographic columns were insulated with two layers of Presstite insulation tape (Virginia Chemicals) and were cooled by circulating constant temperature ethylene glycol-water through their jackets.

Liquid-Liquid Chromatographic Runs. The solid supports for the

liquid-liquid chromatographic columns were Celite (Johns-Manville Purified) or silica gel (Davidson No. 56, surface area 285 m^2/g, pore volume of 1.20 mL/g, and average pore diameter of 168 Å).

Run 1. The stationary phase was prepared by rolling (8 h) 90 g of Celite, dried to constant weight at 180 °C at 50 μm, with 39 mL of $CHCl_3$-saturated distilled water containing 6.52 g of $NaPF_6$ (0.94 M). This not quite moist material was dry packed in small portions onto a 57 by a 2.5 (i.d.) cm jacketed chromatographic column. The stationary phase was 29% water, 5% salt, and 66% Celite by weight. A solution of 0.75 mL of $CHCl_3$ saturated with distilled water containing 0.200 g of racemic α-phenylethylammonium bromide and 0.175 g of $NaPF_6$ was mixed with 1.6 g of Celite. This material was packed at the top of the column (0.75-cm band). The column was cooled to 0.5 °C and was developed by gravity flow with a 0.0375 M solution of (*R,R*)-**1** of $[\alpha]^{25}_{578}$ +221° (*c* 1.0, CH_2Cl_2) in $CHCl_3$ saturated with water at 0 °C. The free volume (V_M) was 164 mL, as shown by the amount of solution added before eluate appeared. Fractions of the column eluate were collected, the flow rate being 0.3 mL/min. The relative conductivity of each fraction was measured with a dip cell with a constant of about 0.19 cm^{-1} and an Industrial Instruments Inc. conductivity null bridge instrument. Figure 2 provides a plot of milliliters of eluate vs. relative conductivity of eluate for run 1. Fractions 8–12 (120 mL) were combined and washed with three 40-ml portions of water containing one drop of concentrated hydrochloric acid per 100 mL of water. The amine salt in this aqueous solution was converted to its tosylamide derivative to give 117 mg (42%) of material nonoptically fractionated during derivatization (control experiment), $[\alpha]^{25}_{546}$ −102° (*c* 1.5, CH_2Cl_2). Fractions 19–28 (120 mL) were combined and similarly converted to the tosylamide derivative to give 84 mg (30%) of material nonoptically fractionated during preparation, $[\alpha]^{25}_{546}$ +101° (*c* 1.5, CH_2Cl_2). A sample of (−)-(*S*)-α-phenylethylamine, $[\alpha]^{25}_{D}$ −40.8° (neat, 1 dm) of maximum rotation[13] when converted to its tosylamide derivative on the same scale by the same method gave a 90% yield of nonpurified material, $[\alpha]^{25}_{546}$ −102° (*c* 1.5, CH_2Cl_2). A much larger sample prepared and recrystallized four times from dichloromethane–ether gave mp 99–100 °C, $[\alpha]^{25}_{546}$ −108° (*c* 0.9, CH_2Cl_2), and $[\alpha]^{25}_{D}$ −79.7° (*c* 2.0, C_6H_6), essentially identical to literature values.[16] Thus, a minimum of 84% of the original (*S*) salt put on the column was eluted as optically pure (*S*) salt in the first peak of run 1, and a minimum of 60% of the original (*R*) salt was eluted optically pure in the second peak. The amine salt in fraction 16, which occurred immediately after the conductivity minimum in Figure 2, was converted to its tosylamide derivative (4.9 mg) and had a rotation of $[\alpha]^{25}_{546}$ +14° (*c* 0.5, CH_2Cl_2). Thus, the material eluted at this crossover point was only 14% optically pure in (*R*) salt. An estimate of the relative peak areas (planimeter) of Figure 2 gave (area A)/(area B) = 0.94. Table I records the parameters derived from the plot for run 1.

The other chromatographic runs were monitored by passing the column eluate through a conductivity cell constructed as follows. Teflon tubing, ⅛ in. o.d., was used for all connections. Two brass plates held apart by a slotted Teflon gasket were held together by two outer Teflon plates. The five-layered apparatus was secured with six nylon bolts around the perimeter. Tapped holes in one face of the cell provided inlet and outlet ports. The cell volume was about 0.1 mL, and the cell constant was 0.017 cm^{-1}. The conductance of flow through cell was continuously monitored with a Philips PR 9501 direct-reading conductivity bridge attached to a strip-chart recorder. The linearity of conductance of the cell with the concentration of complexed α-phenylethylammonium hexafluorophosphate in 0.0375 M solutions of (*R,R*)-**1** in $CHCl_3$ is indicated in Figure 1 for concentrations of salt ranging from 0.80 to 8.24 × 10^{-3} M.

Run 2. A 60 by 0.76 (i.d.) cm stainless steel jacketed and insulated chromatographic column was dry packed in small portions, with tamping, with 34% (by weight) silica gel on which was absorbed 9% $NaPF_6$ (0.94 M) in 57% distilled water. This support was prepared by shaking the dried silica gel (see above) with the aqueous $NaPF_6$ salt solution for 15 h. A 0.0375 M solution of (*R,R*)-**1** in $CHCl_3$ saturated with distilled water was pumped through the column with a Milton-Roy Mini-pump, Model 3365-290, whose stroke length was varied between 6 and 20% to attain the proper pressure drop (30 psi) and flow rate (0.15 mL/min). Although tiny air bubbles were noted in all runs, they did not interfere with detection. The free volume (V_M) was determined by measuring the volume of solution pumped into the dry-packed column before eluate appeared. The column was equilibrated with the $CHCl_3$–host solution at 25 °C. Racemic α-phenylethylammonium bromide (25 mg) dissolved in 0.5 mL of the $CHCl_3$–host solution was introduced onto the column through a SV 8031 Chromatronix Sample Injection Valve Block. Table I reports the results.

Run 3. The chromatographic column, 60 by 0.76 (i.d.) cm, was packed with 40% (by weight) silica gel and 19% $NaPF_6$ (2.4M) in 41% H_2O. The $CHCl_3$ solution of the host (0.0375 M) was saturated with water at the temperature of the run (−13 °C) before use. The racemic methyl phenylglycinate hydrochloride (100 mg) to be resolved was dissolved in a minimum of the aqueous $NaPF_6$ solution used as the stationary phase. This solution was mixed with sufficient silica gel to provide slightly damp-appearing material. This solid was loaded onto the top 3 cm of the column after removal of an equivalent amount of column packing material. The column was cooled to −13 °C. At a constant temperature bath of −21.5 °C in a test run, the coolant returning to the bath was −20.4 °C. Run 3 was made with a pressure drop of 80 psi (0.50 mL/min). Table I reports the column parameters, and Figure 3 records the volume-conductance plot. The column eluate was divided into three fractions: fraction A (0–90 mL), a second fraction consisting of a few drops of eluate which gave a negative ninhydrin test, and fraction B (90–200 mL). Both A and B gave very strong ninhydrin tests. Fraction A was washed with three 20-mL portions of water. The combined aqueous layers were washed with CH_2Cl_2 to remove all traces of host, and the pH of the aqueous layer was adjusted to 9 with dilute aqueous NH_4OH. The free amino ester was extracted into CH_2Cl_2, the solution was dried with $MgSO_4$, concentrated under vacuum, and transferred to a tared vial. The concentrated solution was evaporated to dryness and the traces of CH_2Cl_2 were removed under high vacuum. The vial was weighed, the contents were quantitatively transferred into a 2-mL volumetric flask with CH_2Cl_2, and the rotation of the contents was determined. Fraction B was submitted to the same procedure. As a control, 38 mg of D-methyl phenylglycinate hydrochloride of 97.2% optical purity,[15] $[\alpha]^{25}_{546}$ −155.0° (*c* 1, CH_3OH) was subjected to the same procedure to give 29.1 mg (93%) of the amino ester, $[\alpha]^{25}_{546}$ −181° (*c* 2.9, CH_2Cl_2). Optically pure amino ester prepared on a large scale gave $[\alpha]^{25}_{546}$ −185° (*c* 2.4, CH_2Cl_2). From fraction A was obtained 40% of the total racemic amino ester salt initially used, $[\alpha]^{25}_{546}$ −180° (*c* 2.4, CH_2Cl_2). From B was obtained a 38% recovery, $[\alpha]^{25}_{546}$ +180° (*c* 2.4, CH_2Cl_2).

Run 4. Due to the greater hydrophilicity of the methyl *p*-hydroxyphenylglycinate salt, a 4 M solution of $LiPF_6$ was used as the stationary phase. The $LiPF_6$ salted the guest out of the stationary aqueous phase yet did not complex with the host. The $LiPF_6$ solution was prepared by adding the salt, with stirring, to water at such a rate that the temperature never exceeded 0 °C. The addition rate was controlled to maintain the temperature near 0 °C. After addition, the pH of solution was raised to 4 by adding an appropriate amount of 5 M LiOH. Finally, the solution was cooled to −15 °C, filtered, and shaken with dried silica gel as before. A sample of the resulting material underwent no visible change on extended refrigeration at −15 °C. However, a sample of 4 M $LiPF_6$ in water at −15 °C deposited a small amount of LiF after several days, but maintained its pH. It finally became acidic by hydrolysis of the PF_6^-. A 60 by 0.76 (i.d.) cm column was packed, as in run 3, with 41% (by weight) silica gel and 26% $LiPF_6$ (4.0 M, pH 6) in 33% H_2O. The 108 mg of racemic methyl *p*-hydroxyphenylglycinate hydrochloride salt was loaded on the column as in run 3. The mobile phase was a 0.0750 M solution of (*RR*)-**1** in $CHCl_3$ equilibrated with water at −15 °C. The pressure drop during the run was 22 psi (0.52 mL/min). Table I records the results and Figure 4 records the conductance-volume plot.

The column eluate was collected in 25-mL fractions which were analyzed for amino ester by ninhydrin. These tests qualitatively paralleled the conductivity measurements. Fraction 6 collected between 125 and 150 mL gave a negative test for the amino ester, whereas fractions 5 and 7 gave strong positive tests. Fractions 1–5 were combined to give A and fractions 7–12 to give B.

The amino esters in fractions A and B were isolated as follows. Each solution was extracted with two 20-mL portions of 0.1 N aqueous HCl solution and the combined aqueous extracts were washed with CH_2Cl_2 to remove traces of (*R,R*)-**1**. Each aqueous solution was adjusted to pH 9 with Na_2CO_3 and extracted six times with ethyl acetate. Ninhydrin tests indicated extraction was nearly complete. The ethyl acetate solution from A was concentrated at 30 °C. The residue was dissolved in 0.1 N aqueous HCl solution and extracted as before with ethyl acetate, which was again evaporated, finally in a tared vial to

give 24.1 mg (54%) of the free amine. Similar treatment of the solution from B gave 15.2 mg (34%) of free amine. The amine from each source was transferred quantitatively, using 1.0 N aqueous HCl, into a 2 mL volumetric flask after having been filtered from a small amount of insoluble material. The following specific rotations were calculated, assuming quantitative conversion of the amino ester to its corresponding hydrochloride salt: from band A, $[\alpha]^{25}_{589}$ −105°, $[\alpha]^{25}_{578}$ −110°, $[\alpha]^{25}_{546}$ −127° (*c* 1.44, 1 N HCl); from band B, $[\alpha]^{25}_{589}$ +105°, $[\alpha]^{25}_{578}$ +111°, $[\alpha]^{25}_{546}$ +127° (*c* 0.92, 1 N HCl). The hydrochloride salt of D-methyl *p*-hydroxyphenylglycinate was prepared from a sample of D-*p*-hydroxyphenylglycine of 98.3% optical purity of rotations: $[\alpha]^{25}_{589}$ −106.2°, $[\alpha]^{25}_{578}$ −110.7°, $[\alpha]^{25}_{546}$ −127.9°, $[\alpha]^{25}_{436}$ −231.8° (*c* 1.03, H_2O), lit.[16] $[\alpha]^{25}_{589}$ −108° (*c* 1.0, H_2O). The amino ester salt produced gave $[\alpha]^{25}_{589}$ −141.8°, $[\alpha]^{25}_{578}$ −147.8°, $[\alpha]^{25}_{546}$ −171.1°, $[\alpha]^{25}_{436}$ −317.6° (*c* 0.99, CH_3OH) (which compares with a sample provided by the Upjohn Co., $[\alpha]^{25}_{546}$ −172.8° (*c* 1.0, CH_3OH)) and $[\alpha]^{25}_{589}$ −121.1°, $[\alpha]^{25}_{578}$ −125.9°, $[\alpha]^{25}_{546}$ −145.5°, and $[\alpha]^{25}_{436}$ −267.3° (*c* 0.99, 1 N HCl). A 54-mg sample of this prepared ester salt was treated according to the procedure outlined for the isolation of material from bands A and B to give 32.3 mg (72%) of the isolated amino ester: $[\alpha]^{25}_{589}$ −103°, $[\alpha]^{25}_{578}$ −107°, $[\alpha]^{25}_{546}$ −124° (*c* 1.6, 1 N HCl).

These experiments indicate that bands A and B are 87% chemically pure and ~100% optically pure since the 98.3% optically pure material when submitted to the same isolation procedure gives an optical rotation of only 85% of the maximum.

Run 5. The procedure for run 5 was identical to that for run 4 except that CH_2Cl_2 was substituted for $CHCl_3$. Table I records the results.

Host (*R,R*)-**1** used in runs 1–5 was recovered in the following manner: The organic layers containing (*R,R*)-**1** were extracted with several portions of dilute HCl and dried with $MgSO_4$, and the solvent was removed under reduced pressure to give ~95% of (*R,R*)-**1** $[\alpha]^{25}_{578}$ +221° (*c* 1.0, CH_2Cl_2). This rotation is the same as that of host (*R,R*)-**1** used in the five runs.

Preparation of Silica Gel Bound Host. To 2.11 g of optically pure (*R,R*)-2,3,4,5,13,14,15,16-tetra-1,2-(6-bromonaphtho)-1,6,9,12,-17,20-hexaoxacyclodocosa-2,4,13,15-tetraene ((*R,R*)-**3**)[8b] in 200 mL of dry glyme (freshly distilled from CaH_2) and stirred under pure N_2 (passed through concentrated H_2SO_4 and a tower of KOH pellets) at −75 °C was added a trace of triphenylmethane indicator and 10 mL of BuLi (2.2 M in hexane) dropwise. A pink color resulted. After 2 h of stirring, the reaction mixture was added to 12 g of dichlorodimethylsilane stirred under N_2 at −75 °C. The mixture was then stirred at 25 °C for 4 h, cooled, and filtered and the solids were washed with dry glyme. Volatile components were evaporated at 0.1 mmHg, and the residue was dissolved in 15 mL of dry (P_2O_5) $CHCl_3$. The residue was filtered onto a stirred slurry of 40.0 g of Davidson Grade 56 silica gel (200–325 mesh, pore volume 1.20 mL/g, surface area 285 m^2/g, average pore diameter 168 Å), and dried at 300 °C for 24 h under argon to constant weight. The mixture was stirred for 10 h while the evolved HCl was purged with pure N_2. It was then filtered and the filtrate was washed successively with dry pure $CHCl_3$, CH_3OH, C_6H_6, and $CHCl_3$. This material was then dried at 90 °C at 0.01 mmHg pressure for 18 h to a constant weight of 41.6 g. Combustion of this solid gave 3.94% carbon and 0.58% hydrogen by weight. This material was mixed with 200 mL of dry glyme (freshly distilled from CaH_2). To this mixture, stirred under dry pure N_2 at −70 °C was added 10 g of trimethylchlorosilane. The liberated HCl was swept out of the reaction flask with dry nitrogen as the mixture, with stirring, was allowed to come to 25 °C. After stirring for 5 h at 25 °C, the silica gel was filtered and washed successively with dry (P_2O_5)$CHCl_3$, CH_3OH, C_6H_6, and $CHCl_3$. This final product was dried at 90 °C at 0.01 mmHg pressure for 24 h to give 41.7 g of solid. Combustion analysis gave 4.65% by weight of carbon and 0.77 of hydrogen, corresponding to 0.059 mmol of host per g of material. This material absorbed hydroxylic solvents such as methanol to only a small extent, but 2.6 g of it absorbed 6.6 g of $CHCl_3$ before appearing wet.

The filtrate and washings from the reaction of the lithiated host with dichlorodimethylsilane were combined and evaporated. The brown residue was dissolved in CH_2Cl_2, and the solution was water washed, dried, and evaporated to give a foam. This material was chromatographed on silica gel with CH_2Cl_2, and the product was crystallized from benzene–cyclohexane (1:3 v:v) to give 0.10 g of optically pure (*R,R*)-**1**, mp 123–126 °C, which, after drying out the solvent of crystallization, gave $[\alpha]^{25}_{578}$ +220° (*c* 1.0, CH_2Cl_2), which compares with the original (*R,R*)-**1** (starting material for preparing (*R,R*)-**2**), $[\alpha]^{25}_{578}$ +221° (*c* 1.0, CH_2Cl_2)).

The recovery of optically pure (*R,R*)-**1** from the sequence, (*R,R*)-**1** → (*R,R*)-**2** → lithiated material → (*R,R*)-**1** demonstrates that no racemization occurred during any of these reactions, that tetralithiated host existed at one point in the sequence, and that this material scavenged enough H^+ from the glass, medium, and reagents to produce (*R,R*)-**1**. By implication, host bound on the silica gel is also optically pure.

Solid–Liquid Chromatographic Runs on Silica Gel Bound Host. A 60 cm by 0.75 cm (i.d.) stainless steel column was dry packed with 14.0 g of silica gel containing 76.8 mg (0.82 mmol) of (*R,R*) host (see previous section). The column was equilibrated with the elution medium of $CHCl_3$ or CH_2Cl_2 containing the salt carrier, usually 18-crown-6[17] at concentrations ranging from 10^{-2} to 4.4×10^{-5} M, 0.8% ethanol (by volume), or 5–10% isopropyl alcohol. The jacketed column was insulated with three layers of Presstite insulation tape, glass wool, and thin aluminum foil and was maintained at 25 °C by circulating ethylene glycol–water solution from a constant temperature bath through the column's jacket. A constant flow rate of solvent through the column was maintained by use of a Milton-Roy Mini-Pump, whose percent stroke was varied between 5 and 20% to attain a pressure drop of 300 to 975 psi and a flow rate between 0.42 and 1.0 mL/min. The same column was used for all runs, and the column was flushed and stored under pure dry methanol when not in use. The column did not noticeably deteriorate over about a 9-month period. Pure, freshly distilled solvents were used. The racemic amine salt dissolved in a measured amount of the mobile phase was pumped into the top of the column through a valve device (0.50 mL volume) interposed between the pump and the column. Between runs, the column was washed with several column volumes of methanol, then chloroform, and finally the carrier solution. The column eluates were passed through the same conductivity cell described above, which was connected to a Philips PR 9501 direct reading conductivity meter, which in turn was connected to a 1.0 mV strip-chart recorder.

For each run, plots of relative conductance vs. the volume of eluate were made, and from the retention volumes of the two maxima observed for the two enantiomers, the separation factors (α) were calculated. The V_M values for the column varied from 23 to 26 mL.

In runs preliminary to runs 1–6 using the same salts, the column was deliberately overloaded with 20–45 mg of guest. The signs of rotations of the better bound enantiomer (band A, or last appearing) and of the less bound enantiomer (band B, or first appearing) were determined polarimetrically, and their absolute configurations were assigned accordingly. In runs 7–11, the retention volumes of either one (runs 10 and 11) or both enantiomeric guests (runs 7–9) were determined immediately after the runs were made and under the same conditions. The configurations of bands A and B were assigned accordingly. Excellent correspondence was observed for the retention volumes for the single enantiomer and the corresponding enantiomers when racemates were separated.

For runs 1–11 of Table II, the flow rates in milliliters per minute and the pressure drops over the column in psi were respectively: 1, 1.0, 900; 2, 0.6, 975; 3, 1.0, 900; 4, 0.6, 400; 5, 0.5, 300; 6, 0.5, 600; 7, 0.5, 650; 8, 0.5, 600; 9, 0.6, 975; 10, 0.5, 650; and 11, 0.6, 600.

The racemic methyl ester hydrochloride salts of serine and methionine in $CHCl_3$–10% (v:v) isopropyl alcohol were also run through the column, but only one peak was observed. However, fractions were cut, and their signs of rotation were determined. The D-serine and L-methionine derivatives were better complexed by the silica gel bound (*R,R*) host.

References and Notes

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Thermodynamic Studies of the Cyclodextrin-Accelerated Cleavage of Phenyl Esters

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Abstract: The α-cyclodextrin-accelerated cleavage of several (*p*-methyl, *m*-methyl, *p*-nitro, *m*-nitro, and *m*-chloro) phenyl acetates was examined thermodynamically from 15 to 70 °C. The activation enthalpy and entropy for the α-cyclodextrin reactions as well as for the alkaline hydrolyses were determined. The logarithm of the magnitude of the acceleration by α-cyclodextrin over that for the alkaline hydrolyses linearly increases with decreasing difference of the activation enthalpy and entropy between the α-cyclodextrin reactions and the corresponding alkaline hydrolyses. The activation enthalpy is responsible for stereospecific acceleration by α-cyclodextrin; however, the activation entropy partly compensates the activation enthalpy. The enthalpy-controlled stereospecific acceleration by cyclodextrins is attributed to stereospecific complex formation between the cyclodextrins and the phenyl acetates. Cyclodextrins are good enzyme models, especially suited to examine the effect of enzyme–substrate complexation on enzymatic acceleration.

Introduction

Cyclodextrins (CD) have served as good models of serine proteases as reviewed recently by the present authors.[1] CDs accelerate the cleavages of phenyl esters[2,3] and acylimidazoles.[4] Furthermore, they catalyze the hydrolyses of amides such as acetanilides[5] and penicillins.[6] One of the characteristics of the CD-accelerated cleavage of esters and amides is that the reaction pathway is initiated by binding, followed by acylation of CD, and deacylation, which is identical with the pathway used by serine proteases. The CD-accelerated cleavages of phenyl acetates are highly stereospecific, with the meta-substituted compounds being cleaved much faster than the corresponding para-substituted compounds by a factor of 30–200.[2] However, it is still unknown whether activation enthalpy or entropy is responsible for the stereospecific acceleration by CD.

In this paper, thermodynamic studies on the CD-accelerated cleavages of phenyl acetates are described. The rate constants and the dissociation constants of the CD–phenyl acetate complexes were determined at various temperatures from 15 to 70 °C. The activation parameters for the cleavage of the esters and the thermodynamic parameters for complex formation are shown. Relations between the magnitude of acceleration of ester cleavage by CD and the activation parameters are described.

Experimental Section

Materials. α-Cyclodextrin (α-CD) was purified by recrystallization from water. Phenyl acetates were purchased from Eastman Kodak Co. or were synthesized by the method of Spasov.[7,8] All water used for the kinetic studies was doubly distilled.

Kinetics. The hydrolyses of phenyl esters were followed by the appearance of phenols at 300 (*p*-tolyl, *m*-tolyl, and *m*-chlorophenyl acetates) or 400 nm (*p*-nitrophenyl and *m*-nitrophenyl acetates) on a Cary Model 14 PM spectrophotometer equipped with a thermostated cell compartment. The observed rate constants of the cleavage of phenyl esters in the presence (k_{obsd}) and absence (k_{un}) of added α-CD were determined by a usual first-order equation. The values of k_c (the rate constant of the α-CD-accelerated cleavage of phenyl esters) and K_d (the dissociation constant of the α-CD–phenyl acetate complexes) were determined from the Y intercept and the slope of the plot of $(k_{obsd} - k_{un})$ vs. $(k_{obsd} - k_{un})/[\alpha\text{-CD}]$. Plots were carried out at pH 10.6 carbonate buffer, I = 0.2 M, for the cleavage of *m*- and *p*-nitrophenyl acetates or at pH 10.0 carbonate buffer, I = 0.2 M, for the cleavage of *m*-tolyl, *p*-tolyl, and *m*-chlorophenyl acetates.

The rate constant of the alkaline hydrolysis (k_{OH}) was determined from k_{un} and the ion product of water (K_w) at various temperatures.[9] The value of the limiting value of k_c ($k_{c(lim)}$), which refers to complete ionization of α-CD, was calculated by use of k_c and the values of the K_a of α-CD, where K_a of α-CD was taken as 12.1 at 25 °C[3] and it was assumed that K_a varied with the temperature in a fashion exactly parallel to the variation of K_w with the temperature.[10]

From the values of $k_{c(lim)}$ and k_{OH} at various temperatures, the activation enthalpy ($\Delta H^{\ddagger}$) and activation entropy ($\Delta S^{\ddagger}$) were evaluated.[11]

The enthalpy change (ΔH_f) and entropy change (ΔS_f) of the formation of the complexes between α-CD and phenyl acetates were also determined from the kinetically determined K_d's at various temperatures.

Results

Table I shows the $k_{c(lim)}$ values of the α-CD-accelerated cleavages of phenyl acetates. The plots of $k_{c(lim)}$ and k_{OH} vs. $1/T$ (Arrhenius plots) exhibited fair straight lines, from which the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of both the α-CD-accelerated cleavages and

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