Effect of Temperature on Retention of Cyclic β-Amino Acid Enantiomers on a Chiral Crown Ether Stationary Phase

A. Péter* and **G. Török**
Department of Inorganic and Analytical Chemistry, Attila József University, P.O. Box 440, H-6701, Szeged, Hungary

F. Fülöp
Institute of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701, Szeged, Hungary

Abstract

The isocratic retention of cyclic β-amino acids is studied on a chiral crown ether column (Crownpak CR[+]) at different temperatures. The natural logarithm of the retention factor (ln *k*) depends linearly on the inverse of temperature (1/*T*). van't Hoff plots afford thermodynamic parameters, such as the apparent change of enthalpy ($\Delta H°$) and the apparent change of entropy ($\Delta S°$) for the transfer of analyte from the mobile phase to the stationary phase. The information obtained from the thermodynamic study is discussed, and the qualitative relationships between the structure and the thermodynamic data are evaluated.

Introduction

Numerous studies have been published on the effect of temperature on retention in reversed-phase high-performance liquid chromatography (HPLC). The general conclusion of these papers is that the main effect is a decrease in retention with an increase in temperature. A good choice of temperature can assist in improving column efficiency, sensitivity, and reproducibility. The effect of temperature on column efficiency can be ascribed to a decrease in viscosity of the solvent. The relationship between retention and temperature can be derived from the van't Hoff equation.

In most investigations, the van't Hoff plots had positive slopes, which indicated a negative enthalpy change for the transfer of analyte from the mobile phase to the stationary phase (1–19). On the other hand, nonlinear van't Hoff plots have been observed for reversed-phase stationary phases (8,11,17,20). Cole and Dorsey (17) interpreted this nonlinearity as indicative of a change in the retention mechanism.

This temperature dependence plays an important role in chiral chromatography. On a chiral crown ether stationary phase, the degree of chiral recognition strongly depends on the temperature of the separation process: it is greater when the temperature is lower (21–35). Chiral recognition, the ability to discriminate between enantiomers, relies on the formation of two diastereomeric inclusion complexes between the ammonium ion moiety of the amine or the amino acid (guest) and the chiral crown ether entity of the stationary phase (host). Thus it is necessary to carry out chromatography in a highly acidic medium to ensure the presence of an ammonium moiety on amines and/or amino acids. The proposed scheme of complex formation with the chiral selector of Crownpak CR(+) is shown in Figure 1 (29). In other reports, the temperature dependence of the enantiomeric separation was measured experimentally, and the free energy difference $\Delta(\Delta G°)$ values for the complex formation of different enantiomers were established (22,23,32). It was found that using the standard CR(+) column, the stability of the complex with the (–)-*S* enantiomer depended to a large extent on the enthalpy term, whereas the less stable complex (i.e., the [+]-*R* enantiomer) was determined by the entropy term. It should be mentioned that the stability of the crown ether–enantiomer complex and thus the elution order depend on the configuration of the chiral crown ether. Because the column is available in either configuration (CR[+] or CR[–]) and they have the opposite enantioselectivity, the elution order can be reversed by altering the columns.

The aim of the present paper was to investigate the effect of temperature on enantiomeric separations on a chiral crown ether stationary phase (Crownpak CR[+]). The model compounds investigated are the enantiomers of different cyclic β-amino acids: 2-aminocyclopentane-1-carboxylic acid (ACPC), 2-aminocyclohexane-1-carboxylic acid (ACHC), 2-amino-4-cyclohexene-1-carboxylic acid (ACHC-ene), 3-aminobicyclo[2.2.1]heptane-2-carboxylic acid (ABHC), and 3-amino-5-bicyclo[2.2.1]heptene-2-carboxylic acid (ABHC-ene). The separations of some of these compounds by reversed-phase HPLC were discussed earlier (36,37).

The temperature dependence of the retention of these analytes was studied using perchloric acid as an eluent. Retention data obtained at different temperatures allow an evaluation of the variations in enthalpy ($\Delta H°$) and entropy ($\Delta S°$), which are related to the process of transfer of the analyte from the mobile to the stationary phase (15–17,38,39), and also allow a comparison with the data obtained by conventional reversed-phase chromatography.

* Author to whom correspondence should be addressed.

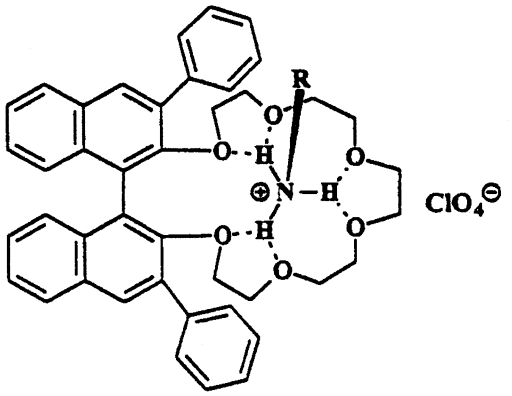

Figure 1. The chiral crown ether utilized in the column interacting with an ammonium group.

Experimental

Apparatus

HPLC measurements were performed on a Waters system consisting of an M-600 low-pressure gradient pump, an M-996 photodiode array detector, and a Millennium 2010 Chromatography Manager Data System (Waters Chromatography, Milford, MA). A Rheodyne model 7125 injector (Cotati, CA) with 10-µL sample loops was also used.

The column used for analytical separations was a Crownpak CR(+) (150 × 4-mm i.d., 5-µm particle size) (Daicel Chemical Industries, Tokyo, Japan). The column was temperautre-controlled with an MK 70 thermostat (Mechanik Prüfgeräte, Medlingen, Germany). The accuracy of temperature adjustment was ± 0.1°C.

The chromatographic system was conditioned by passing the eluent through the column until a stable baseline signal and reproducible retention factors were obtained for the subsequent injections. This procedure was always followed when a new mobile phase or temperature was chosen.

Spectrophotometric detection was performed at 205 nm for ACPC, at 195 nm for ACHC, at 205 nm for ACHC-ene, and at 210 nm for ABHC and ABHC-ene. A Radelkis OP/20811 pH-meter (Budapest, Hungary) equipped with a combined glass-calomel electrode was employed for pH measurement.

Chemicals and reagents

The following racemic enantiomers were prepared in our laboratory (Figure 2): *cis*-(1*S*,2*R* and 1*R*,2*S*)- and *trans*-(1*S*,2*S* and 1*R*,2*R*)-2-aminocyclopentane-1-carboxylic acids (ACPC) (I), *cis*-(1*S*,2*R* and 1*R*,2*S*)- and *trans*(1*S*,2*S* and 1*R*,2*R*)-2-aminocyclohexane-1-carboxylic acids (ACHC) (II), *cis*-(1*S*,2*R* and 1*R*,2*S*)- and *trans*-(1*S*,2*S* and 1*R*,2*R*)-2-amino-4-cyclohexene-1-carboxylic acids (ACHC-ene) (III), *diexo*-(1*S*,2*R*,3*S*,4*R* and 1*R*,2*S*,3*R*,4*S*)- and *diendo*-(1*S*,2*S*,3*R*,4*R* and 1*R*,2*R*,3*S*,4*S*)-(-3-aminobicyclo[2.2.1]heptane-2-carboxylic acids (ABHC) (IV), and *diexo*-(1*S*,2*R*,3*S*,4*R* and 1*R*,2*S*,3*R*,4*S*)- and *diendo*-(1*S*,2*S*,3*R*,4*R* and 1*R*,2*R*,3*S*,4*S*)-3-amino-5-bicyclo[2.2.1]heptene-2-carboxylic acids (ABHC-ene) (V).

For preparation of enantiomers I–V, lipase PS-catalyzed selective *N*acylation of the ethyl esters of the racemic β-amino acids was used (40).

Lipase PS (*Pseudomonas cepacia*) was obtained from Amano Pharmaceuticals (Nagoya, Japan) and was immobilized on Celit. Perchloric acid and other chemicals

Table I. Natural Logarithms of the Retention Factors as a Function of Temperature*

Analyte	Temperature (K)	ln *k*			
ACPC		(1*S*,2*R*)	(1*R*,2*S*)	(1*S*,2*S*)	(1*R*,2*R*)
	298	0.358	0.476	0.761	1.176
	293	0.438	0.582	0.904	1.358
	288	0.542	0.708	1.082	1.579
	283	0.647	0.842	1.278	1.826
	278	0.761	0.978	1.477	2.074
ACHC		(1*S*,2*R*)	(1*R*,2*S*)	(1*S*,2*S*)	(1*R*,2*R*)
	298	1.188	1.289	1.411	1.643
	293	1.264	1.386	1.500	1.770
	288	1.348	1.495	1.600	1.917
	283	1.435	1.615	1.708	2.073
	278	1.569	1.760	1.862	2.260
ACHC-ene		(1*S*,2*R*)	(1*R*,2*S*)	(1*S*,2*S*)	(1*R*,2*R*)
	298	0.718	0.718	1.314	1.575
	293	0.846	0.846	1.493	1.783
	288	0.932	0.932	1.619	1.959
	283	1.058	1.058	1.787	2.166
	278	1.258	1.258	2.024	2.426
ABHC		*diendo*-		*diexo*-	
		(1*S*,2*S*,3*R*,4*R*)	(1*R*,2*R*,3*S*,4*S*)	(1*R*,2*S*,3*R*,4*S*)	(1*S*,2*R*,3*S*,4*R*)
	298	1.297	1.348	1.552	1.579
	293	1.350	1.406	1.604	1.644
	288	1.429	1.496	1.682	1.730
	283	1.500	1.577	1.754	1.810
	278	1.571	1.660	1.826	1.895
ABHC-ene		*diendo*-		*diexo*-	
		(1*S*,2*S*,3*R*,4*R*)	(1*R*,2*R*,3*S*,4*S*)	(1*R*,2*S*,3*R*,4*S*)	(1*S*,2*R*,3*S*,4*R*)
	298	1.051	1.051	1.335	1.394
	293	1.125	1.125	1.406	1.479
	288	1.156	1.156	1.479	1.560
	283	1.267	1.267	1.554	1.645
	278	1.340	1.340	1.663	1.735

* Conditions: stationary phase, Crownpak CR(+); mobile phase, perchloric acid (pH 1.0); flow rate, 0.5 mL/min.

were analytical-grade reagents from Merck (Darmstadt, Germany). Ultrapure water from a Millipore Milli-Q system (Milford, MA) was used for the preparation of all the solutions. The hydrogen ion concentration of the perchloric acid was checked by pH measurement and by potentiometric titration with standardized sodium hydroxide.

Retention data

The retention factors (k) were calculated via the equation

$$k = (t_R - t_o)/t_o \qquad \text{Eq 1}$$

where t_R is the retention time of the enantiomer peak maximum and t_o is the void time. The void times were measured by injecting perchloric acid with a higher pH than that of the eluent.

Results and Discussion

The retention of different β-amino acid enantiomers was studied at different temperatures in the range of 5–25°C. Table I shows the natural logarithms of the retention factors (ln k) obtained at 5, 10, 15, 20, and 25°C for the enantiomers studied. For all the analytes investigated, the retention decreased as the temperature was increased. Higher temperatures resulted in decreasing resolutions (R_S), and in the case of *cis*-ACHC-ene and *diendo*-ABHC-ene, the enantiomers coeluted, and lower temperatures did not improve the separation of the unresolved enantiomers.

To demonstrate the relationship between the retention factor (k') and temperature for a given mobile phase, van't Hoff plots were constructed. The chromatographic retention, expressed by the retention factor (k'), was related to the thermodynamic equilibrium constant (K) according to the following equation:

$$k' = K\phi \qquad \text{Eq 2}$$

where ϕ is the phase ratio of the column (the volume of the stationary phase divided by the volume of the mobile phase). The free energy change for the process is expressed as:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K = -RT \ln (k/\phi) \qquad \text{Eq 3}$$

Therefore:

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \phi \qquad \text{Eq 4}$$

where ΔG° is the standard free energy of transfer of the analyte from the mobile phase to the stationary phase, ΔH° is the associated change in enthalpy, ΔS° is the associated change in entropy, R is the gas constant, and T is the absolute temperature. ΔH° and ΔS° can be derived from the slope and the intercept, respectively, and ln k is generally a linear function of 1/T.

In the present study, all the plots of ln k versus 1/T can be fitted by straight lines whose correlation coefficients are reported in Table II (all squares of the correlation coefficient [r^2] were greater than 0.98). The linear behavior of the plots suggests that, in the temperature range investigated, the retention process proceeds through only one kind of mechanism.

The slopes of the plots yielded the ΔH° values listed in Table II. The negative values indicated that transfer of the enantiomers from the mobile phase to the stationary phase was enthalpically favored. The values obtained (between –2.30 and –7.50 kcal/mol) are similar to those generally reported for the conventional reversed-phase mechanism (around –3 kcal/mol) (17,38).

The change in the enthalpy of transfer for the analyte from the mobile to the stationary phase depends on the number of ring carbons in the cyclic β-amino acids. In the ACPC, ACHC, and ABHC series, $-\Delta H^\circ$ decreases with an increase in the number of ring carbons, due to the steric effects. Furthermore, the unsaturated compounds have larger $-\Delta H^\circ$ values than the saturated ones (ACHC–ACHC-ene and ABHC–ABHC-ene), probably because of π–π interactions with the chiral selector. It is also known that in general, the more rigid a chiral compound is, the easier it is to resolve its enantiomers.

The enthalpy changes ($-\Delta H^\circ$) for the *trans*-diastereomers are larger than those for the *cis*-compounds; the complex formation of the *trans*-diastereomers with the chiral selector seems to be more favorable. The steric hindrance of the *cis*- enantiomers inhibits the formation of more stable complexes. For a given diastereomeric

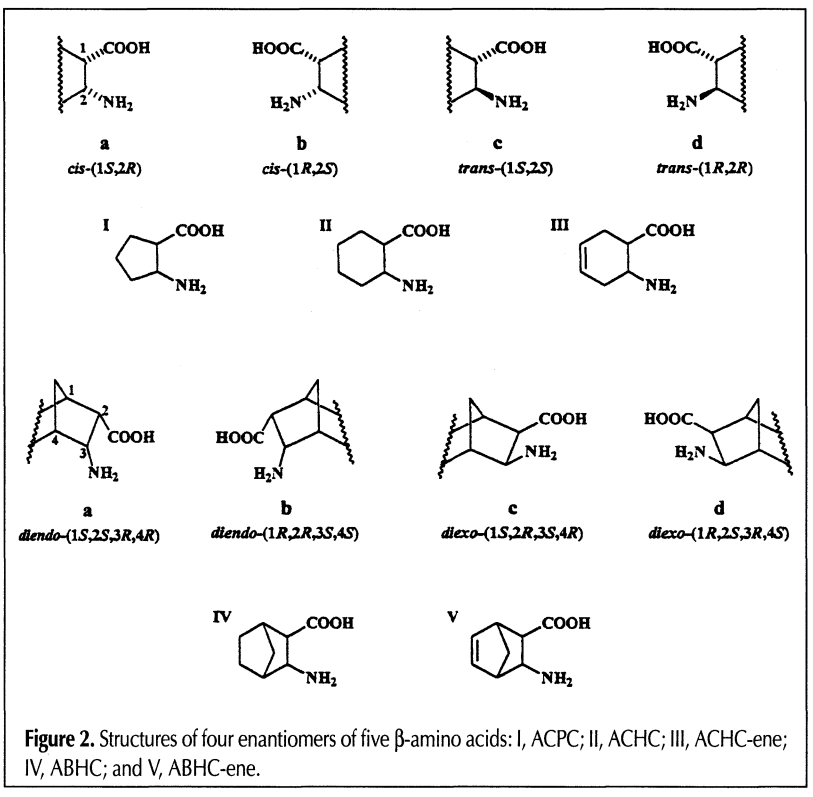

Figure 2. Structures of four enantiomers of five β-amino acids: I, ACPC; II, ACHC; III, ACHC-ene; IV, ABHC; and V, ABHC-ene.

pair, the enantiomer with the R configuration at the carbon atom adjacent to the carboxyl group has a higher $-\Delta H°$ value than that of the enantiomer with the S configuration at the carbon atom adjacent to the same carbon. It is interesting that the configuration of the carbon atom adjacent to the amino group is not the determining factor in the enthalpy evaluation. One would expect that the configuration of the stereogenic center (carbon) to which the amino group (that forms the inclusion complex) is attached is the most important, whereas the configuration of the adjacent carbon bearing the carboxyl group is secondarily important. The stabilities of the complexes of the two enantiomers are determined not only by the ionic interactions between the amino group and the oxygen atoms of the crown ether (Figure 2), but also by the interaction of the ring bearing the carboxyl group with the planar part of the chiral selector.

With regard to the evaluation of the entropy variation involved in the retention process, the intercept ($b = \Delta S°/R + \ln \phi$) of Equation 3 can provide $\Delta S°$ if the phase ratio ($\phi = V_S/V_M$) is known. Although determination of the phase ratio is relatively easy in pure liquid–liquid chromatography, the situation is much more complex in reversed-phase chromatography with chemically bonded materials. In any case, the choice of ϕ must be in agreement with the definition of K. For reversed-phase chromatography, Melander and Horváth (41) suggested the expression as the ratio of the surface area of the adsorbent (m^2) divided by the column dead volume (cm^3). Davydov et al. used the mass of material (g) in the column divided by the column dead volume (cm^3), as is usual in adsorption chromatography (42). Dorsey et al. (17,43) proposed different equations for evaluation of the phase ratio. These equations make use of technical data on the stationary phase packing material, such as the percentage of carbon load, the bonded packing weight, the bonded alkyl chain density, and the number of carbons in the alkyl ligand. These data are not always provided by the manufacturers and are often difficult to obtain. Sander and Field (39) proposed a f value of 0.384, and Yamamoto et al. (44) reported ϕ values ranging between 0.351 and 0.819 for different C18 columns. Other workers (45) chose a f value of 0,536, which is the average of the values given by Yamamoto (44). Some investigation simplified the problem by simply choosing a ϕ value of 1 (45). Any uncertainty in the phase ratio affects $\Delta S°$ equally, and thus trends in $\Delta S°$ as a function of molecular structure are unaffected.

Table II. Enthalpy ($-\Delta H°$), Entropy ($-\Delta S°$), $-T\Delta S°$, and Temperature Compensation (β) Values for the Process of Analyte Transfer from the Mobile Phase to the Stationary Phase

Analyte	$-\Delta H°$ (kcal/mol)	$-\Delta S°$* (kcal/mol)	$-T\Delta S°$*† (cal/mol K)	$\beta = \frac{\Delta H°}{\Delta S°}$	r^2‡
ACPC					
(1*S*,2*S*)	5.98	17.70	5.27	337.8	0.9996
(1*R*,2*R*)	7.50	21.97	6.55	341.2	0.9994
(1*S*,2*R*)	3.36	9.70	2.89	346.6	0.9998
(1*R*,2*S*)	4.18	12.22	3.64	342.4	0.9996
ACHC					
(1*S*,2*S*)	3.69	8.70	2.59	423.5	0.9966
(1*R*,2*R*)	5.09	12.97	3.87	392.8	0.9996
(1*S*,2*R*)	3.10	7.17	2.14	432.2	0.9956
(1*R*,2*S*)	3.88	9.60	2.86	404.5	0.9994
ACHC-ene					
(1*S*,2*S*)	5.68	15.57	4.64	364.8	0.9938
(1*R*,2*R*)	6.90	19.15	5.70	360.4	0.9980
(1*S*,2*R*)	4.29	12.11	3.61	354.5	0.9874
(1*R*,2*S*)	4.29	12.11	3.61	354.5	0.9874
ABHC					
diendo-(1*S*,2*S*,3*R*,4*R*)	2.31	4.29	1.28	538.2	0.9980
(1*R*,2*R*,3*S*,4*S*)	2.63	5.27	1.57	498.8	0.9982
diexo- (1*R*,2*S*,3*R*,4*S*)	2.31	3.79	1.13	609.7	0.9984
(1*S*,2*R*,3*S*,4*R*)	2.64	4.84	1.44	545.6	0.9992
ABHC-ene					
diendo-(1*S*,2*S*,3*R*,4*R*)	2.38	5.03	1.50	473.7	0.9809
(1*R*,2*R*,3*S*,4*S*)	2.38	5.03	1.50	473.7	0.9809
diexo- (1*R*,2*S*,3*R*,4*S*)	2.67	5.42	1.62	492.2	0.9978
(1*S*,2*R*,3*S*,4*R*)	2.80	5.73	1.71	488.4	0.9978

* Calculated with $\phi = 0.638$.
† Calculated at 298 K.
‡ r^2 value for the linear fit of the van't Hoff plot.

Because the ϕ value and the technical data are not available for the Crownpak CR(+) column, the method of Jandera et al. (46) was chosen for the estimation of ϕ.

They used the following definition of the phase ratio:

$$\phi = (V_G - V_M)V_M \qquad \text{Eq 5}$$

where V_G and V_M are the geometric (empty column) and dead volumes of the column, respectively. The phase ratio as defined in Equation 4 is a dimensionless quantity. This is the upper limit for the estimation of ϕ and implies that the distribution constant is to be understood as the ratio of the solute concentration in the bulk volume of the packing material (i.e., support + bonded phase) to that in the mobile phase in the column. When the geometry of the column and the dead volume were taken into account, ϕ was calculated as 0.638. The $\Delta S°$ values evaluated on this basis are listed in Table II.

The $\Delta S°$ values are negative, and this supports the idea that the analyte is more ordered on the stationary phase than in the mobile phase (47). Even with $\phi \approx 0.2$, one of the smallest values mentioned in the literature (39), $\Delta S°$ remains negative. $-\Delta S°$ was observed to be less negative for ABHC and ABHC-ene than for the cycloalkane amino acids. This means that,

probably because of the two-ring systems, the analyte is less ordered on the stationary phase. A possible explanation for the more negative values observed for the cycloalkane compounds can be that the complex formation on the surface of the stationary phase leads to a more ordered situation in the retention process. The primary interaction is always electrostatic but beside this there may also be some secondary hydrophobic interactions with the rest of the crown ether (aromatic part). The same situation can be observed from comparison of the $-\Delta S°$ values for *trans*- and *cis*- diastereomers or unsaturated and saturated compounds (ACHC-ene versus ACHC and ABHC-ene versus ABHC). In the former case, the more negative $\Delta S°$ is due to the favorable steric effect. In the latter case, it is due to secondary hydrophobic interactions, as explained earlier. For most of the solutes analyzed (ACPC, ACHC, and ACHC-ene), the magnitude of $\Delta H°$ was comparable with that of $T\Delta S°$ calculated for 298 K (Table II). In conventional reversed-phase chromatography, the role of entropy and enthalpy in transfer of analyte from the mobile phase to the stationary phase, and therefore in the retention process, depends on both the nature of the analyte and the stationary phase. Because complex formation with the chiral selector is the factor governing retention here, the role of the stationary phase results in a decrease in entropy and represents an increase in the order of the system. In order to establish whether these differences or similarities are really significant, enthalpy–entropy compensation has been applied to chromatographic systems to ascertain the retention mechanism (19,39,44,48,49). One method of evaluating enthalpy–entropy compensation is to determine the compensation temperature (13) according to Equation 6 (19,50):

$$\beta = \Delta H°/\Delta S° \qquad \text{Eq 6}$$

where $\Delta S°$ was calculated with a ϕ value of 0.638. The values of β for the different analytes studied are reported in Table II. According to the literature (6,7,17), this result seems to indicate that the mechanisms of retention for these analytes are similar (17).

The mechanism proposed for chiral recognition by Shinbo et al. (24,25) was based on ion-pair and ternary complex formation:

$$A^+ + X^- = \overline{AX} \qquad \text{Eq 7}$$

$$\overline{A^+} + \overline{C} + X^- = \overline{ACX} \qquad \text{Eq 8}$$

where A^+ is the protonated amino acid, X^- is the anion present in the mobile phase, and *C*, *AX*, and *ACX* are the crown ether, the ion pair formed between A^+ and X^-, and the ternary complex formed between A^+, *C*, and X^-, respectively. The bars above the letters denote the stationary phase. In reality, this so-called mechanism tells one nothing about chiral recognition.

Pioneering work was done in investigation of the mechanism of chiral recognition by Guiochon et al. (51,52) and Armstrong et al. (53–55). Guiochon investigated the separation of propranolol enantiomers on cellulase protein immobilized on silica gel and differentiated two types of sites on the surface. Type I sites included all molecular interactions between the analyte molecules and atoms or group of atoms belonging to the surface. This includes van der Waals or dispersive interactions as well as the simple polar interactions involving Debye or Keesom forces, and even single hydrogen bond interactions. These types of interactions are generally responsible for retention on conventional, non-enantioselective phases. On Type II sites, selective interactions that are responsible for the separation of enantiomers take place.

It has been shown that for chiral recognition, a minimum of three simultaneous interactions involving three of the groups attached to the chiral center are necessary. These interactions usually involve the formation of hydrogen bonds or polar interactions (56) in addition to the primary interaction with the chiral recognition site of the stationary phase. With ligands as complex as proteins, more than one chiral recognition site may exist.

Armstrong et al. (53,54) explained the chiral recognition of amino acid enantiomers on chiral antibiotics and vancomycin and teicoplanin stationary phases. The teicoplanin molecule contains several characteristic moieties that seem to be involved in amino acid interactions. They are: (*a*) those of an ionic character, including a cationic site ($-NH_3^+$) and an anionic site ($-COO^-$); (*b*) additional polar groups with three sugar moieties and four phenolic groups; and (*c*) the apolar aglycone "basket" and the ninecarbon side-chain. The strong charge–charge interactions between the teicoplanin ammonium group and the carboxylate group of the amino acid are responsible for the amino acid net retention behavior. The polar, apolar, and steric interactions with the amino acid *R* group affect both retention and selectivity.

In analogy to the work of Guichon and Armstrong, it can be supposed that on a crown ether stationary phase, the crown ether cavity with oxygen atoms is the most available and logical site for the initial docking of the ammonium group of the amino acid. The stability of this complex governs the non-enantioselective retention. This interaction is of essential importance in a three-point interaction, and its lack in the case of the secondary amino acids led to unresolved peaks of enantiomers. Secondary interactions (hydrophobic, steric, and possibly some dipolar interactions) with the chiral part of the selector play an important role in chiral recognition.

In the ACPC, ACHC, and ABHC series, $-\Delta H°$ and $-\Delta S°$ decrease with an increasing number of ring carbons. This means that, probably because of the increasing ring systems, the analyte is less ordered on the stationary phase, which resulted in purer selectivity (the selectivity factor [α] decreased with an increasing number of ring carbons). The effect of polar interaction could be observed by comparing the behavior of saturated and unsaturated compounds. The unsaturated compounds (ACHC-ene and ABHC-ene) have larger $-\Delta H°$ and $-\Delta S°$ values than the saturated ones (ACHC and ABHC), indicating the effect of rigidity, which can increase steric interactions in chiral recognition even though they can decrease retention. A good example of the steric interaction is the behavior of *cis*- and *trans*- isomers. The steric hindrance of the *cis*-enantiomers inhibits the secondary interactions with the chiral selector, which resulted in smaller $-\Delta H°$ and $-\Delta S°$ values and selectivity factors as compared to the *trans*- ones.

The results obtained on the crown ether stationary phase indicate that the primary docking site is the cavity of the crown

ether, where complex formation takes place with the ammonium ion of the primary amino acid. Secondary polar, apolar, and steric interactions make further contributions to retention and selectivity.

Conclusion

The effect of temperature on retention demonstrated that the enantiomers of cyclic β-amino acids can be separated by using subambient temperature. Linear van't Hoff plots were observed in the studied temperature range, and the apparent changes of entropy were obtained. The values of the thermodynamic parameters depended on the structures of the analytes.

Acknowledgments

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