Unusual Temperature-Induced Retention Behavior of Constrained β-Amino Acid Enantiomers on the Zwitterionic Chiral Stationary Phases ZWIX(+) and ZWIX(–)

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ABSTRACT The effects of temperature on the chiral recognition of cyclic β-amino acid enantiomers on zwitterionic [Chiralpak ZWIX(+) and ZWIX(–)] chiral stationary phases were investigated. Experiments were performed at different mobile phase compositions and under 10°C column temperature increments in the temperature range 10–50°C. Apparent thermodynamic parameters and T_{iso} values were calculated from plots of ln k and ln α versus 1/T, respectively. Unusual temperature behavior was observed, especially on the ZWIX(–) column, where the application of MeOH/MeCN (50/50 v/v) containing 25 mM triethylamine and 50 mM formic acid as mobile phase led to nonlinear van't Hoff plots and increasing retention time with increasing temperature. On both columns, both enthalpically and entropically driven separations were observed. *Chirality 26:385–393, 2014.* © 2014 Wiley Periodicals, Inc.

KEY WORDS: column liquid chromatography; enantiomer separation; monoterpene-based 2-aminocarboxylic acids; cinchona-alkaloid-based columns; temperature effect

INTRODUCTION

β-Amino acids and their foldameric oligomers are currently of significant interest,[1,2] since they are key building blocks of numerous bioactive molecules.[3–6] Icofungipen (PLD-118; (1*R*,2*S*)-2-amino-4-methylenecyclopentanecarboxylic acid), a β-amino acid, disturbs the biosynthesis of proteins in *Candida albicans*.[7] The monoterpene-based β-amino acids are excellent building blocks for syntheses of monoterpene-fused saturated 1,3-heterocycles, β-lactam-based peptidomimetics or β-peptidic foldamers.[8]

The synthesis of β-amino acids demands analytical methods, which can be used to elucidate the stereochemistry and enantiomeric excess of the final product. One of the most frequently applied techniques is enantioselective high-performance liquid chromatography (HPLC). For the enantioseparation of β-amino acids, new types of chiral derivatizing agents and chiral stationary phases (CSPs) have been applied, and the results of that work can be found in the literature.[9–18]

Enantiomer separations are inherently challenging, because enantiomers have exactly the same properties in an anisotropic environment. Enantioseparation is usually achieved through the formation of transitional diastereomeric complexes between the analytes (selectand enantiomers, SAs) and the chiral selector (SO) as part of the CSP. In most cases, the stereoselective interactions are sensitive to temperature. It has also been observed that there are both achiral and chiral contributions to retention that can vary with a wide variety of experimental parameters.[19–21] Accordingly, the column temperature often has to be optimized in enantioselective HPLC separations and kept well controlled.[22–25]

In investigations of the thermodynamic functions of enantioselective adsorption, van't Hoff plots may facilitate an interpretation of the mechanistic aspects of chiral recognition:

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (1)$$

where k is the retention factor, ΔH° is the standard enthalpy of transfer of the solute from the mobile phase to the stationary phase, ΔS° is the standard entropy of transfer of the solute from the mobile phase to the stationary phase, R is the universal gas constant, T is temperature (Kelvin) and ϕ is the ratio of the volumes of the stationary (V_S) and mobile (V_M) phases of the column. Since the value of ϕ is often not known, the $\Delta S^\circ *$ values [$\Delta S^\circ * = \Delta S^\circ + R \ln \phi$] calculated from the intercepts of the plots via Eq. (1) are generally used. Equation (1) reveals that a plot of ln k vs. 1/T is linear, with slope $-\Delta H^\circ/R$ and intercept $\Delta S^\circ/R + \ln \phi$, if ΔH° is invariant with temperature. In chiral chromatography, however, the van't Hoff plot often deviates from linearity; this is an indication of a mixed retention mechanism.

When solute molecules are transferred from the mobile phase to the stationary phase, the process is enthalpically favorable, but entropically unfavorable. If the solute is retained by the stationary phase, its freedom and entropy decrease; the entropy change, ΔS°, must be negative. On the other hand, when the molecule is adsorbed to the SO of the stationary phase, energy is used during the interaction, and the enthalpy ΔH^o must therefore also be negative.

Change of the temperature of the enantioseparation process usually results in variation of the conformation of the SO of the CSP and/or the SAs. The structural modification of the SAs resulting from the temperature change modifies the geometrical positions of the interaction sites within the SOs and the SAs. This also depends on the mobile phase composition, influencing the overall binding mechanism observed, which are manifested in different retention characteristics. The conformation change

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may be reversible or irreversible, depending on the characteristics of the SO of the CSP and the SAs. Changes in temperature may result in nonlinear variation of the pK_a values of chargeable SAs.[26] The temperature-related changes in conformation, together with the change in the enthalpy or entropy of adsorption, can generate a nonlinear van't Hoff plot.[26–31]

Chromatographic chiral separations are determined by the difference in free energy $\Delta(\Delta G°)$ of adsorption of the enantiomers:

$$\Delta(\Delta G°) = -RT \ln \alpha = \Delta(\Delta H°) - T\,\Delta(\Delta S°) \quad (2)$$

where α is the enantioselectivity factor. If $\Delta(\Delta H°)$ is constant within the given temperature range, the slope of a plot of $\ln \alpha$ vs. $1/T$ is $-\Delta(\Delta H°)/R$ and the intercept is $\Delta(\Delta S°)$.

Equation (2) clearly reveals that the stereoselectivity increases as the temperature decreases. At lower temperatures, the effectiveness of intramolecular interactions increases and therefore improves the enantioselectivity. Stronger interactions between the SA and the SO of the CSP are manifested in more negative $\Delta H°$ values, which is favorable because of the more exothermic process. The hypothesis is valid when conditions such as the structures of intermediate associates, the solvation of the SO and SA, and the configurations of the SA and SO are constant throughout the temperature range studied. On the other hand, the weaker intramolecular interactions that occur at higher temperature have an energetically positive effect on the $\Delta S°$ values. A less negative entropy indicates an increase in the number of degrees of freedom of the SAs in the mobile phase. In some cases, the gain in the change of entropy with increasing temperature is more significant than the loss in the change of enthalpy. From an enantioselectivity standpoint, this means that the selectivity increases with increasing temperature and a new set of $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ contributes to the overall observed enantioselectivity.

Newly developed *Cinchona* alkaloid-based zwitterionic ion-exchange-type chiral SOs and the related CSPs operate best under slightly acidic polar-ionic (PI) mobile phase conditions. Three modes of ion-exchange modes can be envisioned: an anion-exchange mode for the separation of chiral acids, a cation-exchange mode for the separation of chiral amines, and a zwitterionic ion-exchange mode for the enantioseparation of ampholytic compounds possessing both positive and negative charges.[32–35]

The present article describes the temperature-dependent enantioseparation of five monoterpene-based 2-aminocarboxylic acids representing bulky β-amino acid enantiomers (Fig. 1) in PI mode on *Cinchona* alkaloid-based zwitterionic CSPs (Fig. 2). The effects of temperature under different conditions on the retention, enantioselectivity, and thermodynamic parameters are discussed. The sequence of elution of the enantiomers was determined.

MATERIALS AND METHODS

Chemicals and Synthesis

The enantiomers of monoterpene-based β-amino acids were prepared by methods published previously.[36–42] Addition of chlorosulfonyl isocyanate to the corresponding chiral monoterpene afforded β-lactams in highly regio- and stereospecific reactions; this was followed by treatment with hydrochloric acid, which resulted in the corresponding *cis*-β-amino acids (**1a,b, 2a,b** and **3a,b**).[39,41] Acid-catalyzed ethanolysis of the β-lactams derived from apopinene (**1a** and **1b)** led to *cis*-β-amino esters, which underwent base-catalyzed isomerization, followed by hydrolysis,

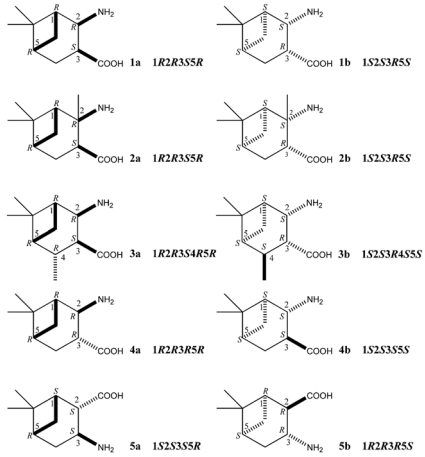


Fig. 1. Structures of the investigated analytes.

to afford the corresponding *trans* enantiomers **4a** and **4b** in excellent yields.[40] The regioisomeric *trans* apopinane-based β-amino acids **5a** and **5b** were prepared by stereoselective Michael addition of lithium dibenzylamide to (–)- and (+)-*tert*-butyl myrtenate, followed by catalytic debenzylation and hydrolysis of the resulting β-amino esters.[42]

Methanol (MeOH) and acetonitrile (MeCN) of HPLC grade were from Merck (Darmstadt, Germany). Triethylamine (TEA), propylamine (PRA), formic acid (FA), and glacial acetic acid (AcOH) of analytical reagent grade were purchased from Sigma-Aldrich (St. Louis, MO).

Instruments and Chromatography

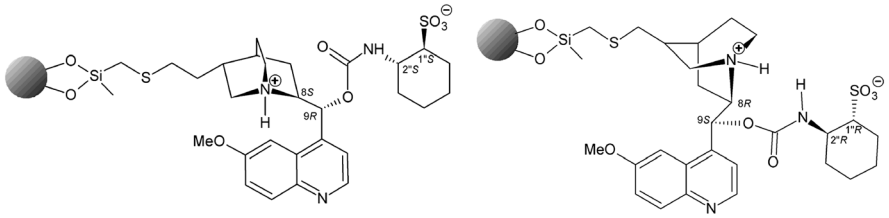
The apparatus for chromatography comprised a Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, and Empower 2 data manager software (Waters Chromatography, Milford, MA). The columns were thermostated in a Spark Mistral column thermostat (Spark Holland, Emmen, The Netherlands). The alternative 1100 Series HPLC system consisted of a solvent degasser, a pump, an autosampler, a column thermostat, a multiwavelength UV-VIS detector (all from Agilent Technologies, Waldbronn, Germany), and a corona charged aerosol detector from ESA Biosciences (Chelmsford, MA). Data acquisition and analysis were carried out with ChemStation chromatographic data manager software from Agilent Technologies. The precision of temperature adjustment was ±0.1°C.

The Chiralpak ZWIX(+) and ZWIX(–) columns (150×3.0 mm I.D., 3-μm particle size for both columns) were provided by Chiral Technologies Europe (Illkirch, France).

Chromatography was performed in isocratic mode at a flow rate of 0.6 ml min^{-1}; column temperature was varied in 10ºC increments between 10 and 50°C. Detection was accomplished by UV and corona discharge detection. The void volume of the columns (t_0) was determined by injecting a methanolic solution of acetone. Solutions of SAs were made in MeOH in the concentration range 0.5–1.0 mg ml^{-1}.

RESULTS AND DISCUSSION

In order to investigate the effects of temperature on the chromatographic parameters, a variable-temperature study was carried out on the *Cinchona* alkaloid-based ZWIX(+) and ZWIX(–) CSPs over the temperature range 10–50°C. Experimental data for all SAs on both columns in the mobile phases MeOH/MeCN (50/50 v/v) containing **(a)** 25 mM TEA and 50 mM FA, **(b)** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM AcOH, **(c)** MeOH/MeCN (50/50 v/v) containing 25 mM PRA and 50 mM AcOH, and **(d)** MeOH/MeCN (25/75 v/v) containing 25 mM PRA and 50 mM AcOH are listed in Tables 1 and 2.



Chiralpak ZWIX(+)™
(8*S*, 9*R*)
(1"*S*, 2"*S*)

Chiralpak ZWIX(-)™
(8*R*, 9*S*)
(1"*R*, 2"*R*)

Fig. 2. Structures of Chiralpak ZWIX(+) and ZWIX(–) CSPs.

The tabulated data indicate that the retention, expressed as retention factor k, in most cases decreased with increasing temperature. Transfer of the SA from the mobile phase to the stationary phase is generally an exothermic process and consequently k (and α) decreases with increasing temperature. However, on the ZWIX(–) column with mobile phase **a**, MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA, the behavior of SAs **2**, **4,** and **5** was unusual; with increasing temperature, k (and α) increased. Such unusual behavior was recently observed for an entirely different chiral chromatographic system by Chankvetadze and colleagues[43] and for achiral separations by Adlof and Lis,[44] Wu et al.,[45] and Yogo et al.[46]

The unexpected behavior described here occurred only when the mobile phase contained FA and only for SAs **2, 4,** and **5** on ZWIX(–) CSP. In the studied temperature range, the conformation and solvation of the ZWIX(–) column SO probably do not change significantly. However, for SAs **2, 4,** and **5,** either the solvation process, or the structure of the intermediate associates, may be different. A possible explanation for this behavior may be the co-ion effect in the presence of FA. The extent of protonation of SAs **2, 4,** and **5** may decrease at higher temperature, and the interaction between the negatively charged carboxy group of the SA and the partially charged tertiary amino group of the quinuclidine ring may be strengthened, resulting in an increased level of retention. (The ion-pairing ability of the SO may also be affected by the temperature variance.) However, further studies are required for a better understanding of the mechanism of this behavior.

The changes observed in the selectivity and resolution with temperature were not consistent. In most cases, α and R_S decreased with increasing temperature. However, on ZWIX(+) for SAs **2** and **3** in mobile phases **a, b,** and **c**, and for SA **4** in mobile phase **a**, and on ZWIX(–) CSP for SA **2** in mobile phases **a, b,** and **c**, and for SAs **4** and **5** in mobile phase **a**, α and R_S increased with increasing temperature.

Since the effect of temperature on the enantiomer separation demands a complex interpretation, an extensive study relating to the thermodynamics of this system was carried out. Accurate chromatographic data were utilized to construct van't Hoff plots (Eqs. (1) and (2)), and the thermodynamic parameters for the individual enantiomers were calculated from the slopes and intercepts of these plots.

As a general trend, van't Hoff analysis of the retention factors (ln k vs. 1/T) gave linear plots, as indicated by the correlation coefficients in Tables 3 and 4. However, for SAs **2** and **5** on ZWIX(–) with mobile phase **a**, nonlinear plots were observed (Fig. 3). In contrast, the ln α vs. 1/T plots (Eq. (2)) exhibited linear behavior over the temperature range 20–50 °C, as depicted in Figure 3. These observations demonstrate that the factors causing nonlinearity for the relationship ln k vs. 1/T affect the retentions of the two enantiomers to nearly the same extent, suggesting that the origin of this phenomenon is relatively unrelated to the chiral recognition process. Below 20°C, the slope increased, i.e., ΔH° and ΔS° became more positive.

The ΔH° and $\Delta S^{\circ *}$ values for the enantiomers were negative on both ZWIX(+) and ZWIX(–) with the exception of SA **4** on ZWIX(–) with mobile phase **a** (Tables 3 and 4; due to the nonlinearity of the van't Hoff plots, ΔH° and $\Delta S^{\circ *}$ values for SAs **2** and **5** with eluent **a** were not calculated). These values indicate that solute transfer from the mobile phase to the stationary phase is enthalpically favorable but entropically unfavorable. Further, the absolute values of $-\Delta H^\circ$ and $-\Delta S^{\circ *}$ for the first-eluting enantiomer were usually smaller than those for the second-eluting enantiomer. Since the second-eluting enantiomers had larger $-\Delta S^{\circ *}$ values, they probably had fewer degrees of freedom on the CSP, i.e., the SO-SA associates were more stable due to a stronger intermolecular interaction or were less able to move or rotate. Multiple simultaneous interactions between the chiral SA and the SO appeared somewhat more likely for the second-eluting enantiomers than for the first-eluting enantiomers. This indicates that enantioselectivity is preferentially expressed by the behavior of the second peak, a combination with nonstereoselective interaction of both enantiomers. In other words, the retention of the first peak relates to SO-SA ion pair formation, without the onset of additional SO-SA interactions causing chiral discrimination.

On ZWIX(+) SAs, **2** and **3** at all mobile phase compositions, and SA **4** with mobile phase **a**, while on ZWIX(–), SA **2** with mobile phases **b** and **c** exhibited smaller $-\Delta H^\circ$ and $-S^{\circ *}$ values for the second-eluting enantiomers (SAs **2, 4,** and **5** exhibited unusual behavior with mobile phase **a**). The smaller $-\Delta H^\circ$ data for the enantiomers mean that the interactions between the SA and the SO are energetically less favorable with increasing temperature. The smaller $-\Delta S^{\circ *}$ indicated that the second enantiomer had more freedom in the stationary phase.

It was also observed that the absolute values of $-\Delta H^\circ_1$ and $-\Delta H^\circ_2$, and in parallel $-\Delta S^{\circ *}_1$ and $-\Delta S^{\circ *}_2$ for the ZWIX(–) column, were in most cases smaller than those for ZWIX(+); it seems that the interactions between the SAs and the SO were less favorable on ZWIX(–), but the difference in interaction of the two enantiomers with the SO does not follow this trend.

TABLE 1. Temperature dependence of retention factor of first eluting enantiomer (k_1), separation factor (α) and resolution (R_S) of monoterpene-based 2-amino carboxylic acids on Chiralpak ZWIX(+) CSP

			Temperature (°C)					
Compound	Mobile phase	k, α, R_S	10	20	30	40	50	Elution sequence
1	**a**	k_1	4.32	4.08	3.84	3.55	3.33	a<b
		α	1.24	1.20	1.17	1.14	1.11	
		R_S	2.50	2.40	2.30	1.60	1.50	
	b	k_1	3.66	3.27	2.76	2.38	2.15	a<b
		α	1.26	1.24	1.23	1.21	1.19	
		R_S	2.70	2.50	2.40	1.80	1.10	
	c	k_1	2.62	2.31	2.12	1.99	1.83	a<b
		α	1.27	1.23	1.21	1.18	1.14	
		R_S	2.30	2.30	2.20	2.10	2.00	
2	**a**	k_1	3.24	3.07	2.93	2.81	2.71	a<b
		α	1.00	1.00	1.02	1.02	1.03	
		R_S	0.00	0.00	0.30	0.30	0.50	
	b	k_1	3.14	2.78	2.35	2.19	1.94	a<b
		α	1.00	1.00	1.05	1.05	1.08	
		R_S	0.00	0.00	0.30	0.40	0.60	
	c	k_1	2.19	2.01	1.80	1.68	1.58	a<b
		α	1.00	1.00	1.06	1.07	1.07	
		R_S	0.00	0.00	0.30	0.40	0.50	
3	**a**	k_1	4.92	4.60	4.32	4.03	3.80	a<b
		α	1.03	1.04	1.05	1.06	1.06	
		R_S	0.50	0.60	0.60	0.70	0.80	
	b	k_1	4.20	3.74	3.26	2.73	2.41	a<b
		α	1.08	1.09	1.11	1.13	1.15	
		R_S	0.60	0.90	0.70	1.00	1.10	
	c	k_1	3.45	3.06	2.74	2.45	2.16	a<b
		α	1.03	1.05	1.06	1.06	1.07	
		R_S	0.40	0.60	0.70	0.80	1.00	
4	**a**	k_1	11.08	9.88	9.03	8.40	7.79	b<a
		α	1.03	1.04	1.06	1.07	1.08	
		R_S	0.30	0.30	0.30	0.40	0.50	
	b	k_1	12.60	11.08	9.70	8.43	7.38	b<a
		α	1.14	1.12	1.11	1.10	1.10	
		R_S	1.60	1.60	1.50	1.40	1.00	
	c	k_1	9.32	8.19	7.23	6.41	5.86	b<a
		α	1.14	1.13	1.12	1.11	1.10	
		R_S	1.80	1.90	1.70	1.70	1.20	
5	**a**	k_1	10.53	9.75	8.95	8.35	7.83	-
		α	1.04	1.00	1.00	1.00	1.00	
		R_S	0.40	0.00	0.00	0.00	0.00	
	b	k_1	11.25	10.10	8.99	7.71	6.94	-
		α	1.00	1.00	1.00	1.00	1.00	
		R_S	0.00	0.00	0.00	0.00	0.00	
	c	k_1	6.95	6.00	5.45	4.94	4.51	-
		α	1.00	1.00	1.00	1.00	1.00	
		R_S	0.00	0.00	0.00	0.00	0.00	
	d	k_1	18.05	16.11	14.54	13.13	11.98	a<b
		α	1.11	1.10	1.09	1.09	1.08	
		R_S	2.00	1.90	2.00	1.80	1.60	

Chromatographic conditions: column, Chiralpak ZWIX(+); mobile phase, **a,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA, **b**, MeOH/MeCN=50/50 (v/v) containing 25 mM TEA and 50 mM AcOH, **c,** MeOH/MeCN=50/50 (v/v) containing 25 mM PRA and 50 mM AcOH, **d,** MeOH/MeCN=25/75 (v/v) containing 25 mM PRA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 nm.

The differences in the changes in enthalpy and entropy, -Δ(ΔH°) and -Δ(ΔS°), are also presented in Tables 3 and 4. The -Δ(ΔH°) values ranged from −1.6 to 2.1 kJ mol^{-1} on ZWIX(+), and from −1.9 to 2.7 kJ mol^{-1} on ZWIX(–). The trend in the change in -Δ(ΔS°) is similar to that in -Δ(ΔH°). Under the conditions where Δ(ΔH°) has negative values, Δ(ΔS°) was also negative and the largest positive Δ(ΔH°) was accompanied by the largest positive Δ(ΔS°). The interactions of **1** with ZWIX(+) and ZWIX(–) were characterized by the highest -Δ(ΔH°) and -Δ(ΔS°) values with eluent composition **a**.

When the selectivity increased with increasing temperature, Δ(ΔH°) and Δ(ΔS°) were positive. In these cases, the change in the adsorption enthalpy with increasing temperature had a positive effect on the enantioselectivity. On the other hand, the positive Δ(ΔS°) compensated the positive Δ(ΔH°) and

TABLE 2. Temperature dependence of retention factor of first eluting enantiomer (k_1), separation factor (α) and resolution (R_S) of monoterpene-based 2-amino carboxylic acids on Chiralpak ZWIX(-) CSP

			Temperature (°C)					
Compound	Mobile phase	k, α, R_S	10	20	30	40	50	Elution sequence
1	**a**	k_1	4.23	4.02	3.84	3.67	3.51	b<a
		α	1.55	1.50	1.45	1.39	1.35	
		R_S	3.80	3.40	3.30	3.30	3.30	
	b	k_1	3.89	3.72	3.58	3.43	3.28	b<a
		α	1.47	1.44	1.41	1.37	1.35	
		R_S	3.30	3.20	3.20	2.80	2.60	
	c	k_1	2.39	2.21	2.11	1.99	1.88	b<a
		α	1.44	1.40	1.37	1.35	1.33	
		R_S	2.90	2.80	2.60	2.50	2.50	
2	**a**	k_1	2.83	2.91	2.96	3.01	2.99	b<a
		α	1.00	1.11	1.16	1.18	1.22	
		R_S	0.00	0.50	1.40	1.40	2.20	
	b	k_1	3.51	3.32	3.16	3.01	2.83	b<a
		α	1.11	1.12	1.13	1.14	1.15	
		R_S	0.60	1.00	1.60	1.40	1.80	
	c	k_1	2.07	1.94	1.85	1.75	1.67	b<a
		α	1.08	1.10	1.11	1.13	1.13	
		R_S	0.50	0.80	1.00	1.20	1.30	
3	**a**	k_1	5.29	4.90	4.70	4.50	4.23	b<a
		α	1.41	1.38	1.34	1.32	1.30	
		R_S	2.80	2.70	2.60	2.50	2.60	
	b	k_1	4.58	4.35	4.12	3.92	3.76	b<a
		α	1.52	1.48	1.46	1.43	1.39	
		R_S	2.90	3.00	3.00	3.00	3.00	
	c	k_1	3.80	3.60	3.37	3.15	2.93	b<a
		α	1.42	1.38	1.36	1.34	1.32	
		R_S	3.90	3.70	3.70	3.50	3.30	
4	**a**	k_1	7.64	7.77	7.91	7.99	8.10	a<b
		α	1.07	1.09	1.10	1.12	1.13	
		R_S	0.50	0.50	0.60	0.70	0.80	
	b	k_1	11.89	10.92	10.15	9.50	8.60	a<b
		α	1.37	1.33	1.31	1.29	1.28	
		R_S	2.60	2.40	2.10	2.10	2.00	
	c	k_1	9.70	9.01	8.21	7.71	7.22	a<b
		α	1.22	1.20	1.19	1.18	1.17	
		R_S	2.10	2.10	2.10	2.00	2.00	
5	**a**	k_1	6.70	7.40	7.40	7.53	7.46	b<a
		α	1.00	1.00	1.06	1.13	1.17	
		R_S	0.00	0.00	0.60	0.90	1.20	
	b	k_1	9.11	8.24	7.68	7.21	6.79	b<a
		α	1.35	1.32	1.30	1.28	1.26	
		R_S	4.50	2.50	2.10	2.40	1.80	
	c	k_1	5.34	5.00	4.76	4.52	4.30	b<a
		α	1.24	1.22	1.20	1.19	1.18	
		R_S	1.70	1.70	1.70	1.60	1.60	

Chromatographic conditions: column, Chiralpak ZWIX(-)™; mobile phase, **a,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA, **b**, MeOH/MeCN=50/50 (v/v) containing 25 mM TEA and 50 mM AcOH, **c,** MeOH/MeCN=50/50 (v/v) containing 25 mM PRA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 nm.

resulted in a negative $\Delta(\Delta G°)$. In eluent system **a,** containing FA as acid modifier, the largest positive $\Delta(\Delta H°)$ and especially $\Delta(\Delta S°)$ were obtained for SAs **2, 4,** and **5**; here, not only the selectivity, but also the retention increased with increasing temperature. However, for SAs **2** and **5** in eluent system **a**, the nonlinear van't Hoff plots over the entire temperature range were attributed to the change in retention mechanism. Thermodynamically, this unusual behavior may be attributed to the largest positive $\Delta(\Delta S°)$ values, indicating the importance of the entropy contribution to the chiral separation.

The thermodynamic parameter $-\Delta(\Delta G°)_{298}$ suggests that, both on ZWIX(+) and on ZWIX(–), mobile phase **b,** i.e., MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM AcOH, induced the binding to the SO more efficiently, as reflected by the larger $-\Delta(\Delta G°)$ values (SA **5** was not separable on ZWIX(+) under these conditions).

From the $-T\Delta(\Delta S°)$ data for some SAs, the positive $\Delta(\Delta S°)$ on both CSPs compensated for the positive $\Delta(\Delta H°)$ and resulted in a $-\Delta(\Delta G°)$ value (Tables 3 and 4). For these SAs in this temperature range, enantioresolution is entropically driven, and the selectivity increases with increasing temperature, as depicted for SA **2** in Figure 4.

The data were used to calculate the temperature T_{iso}, at which the enantioselectivity and the elution sequence change

TABLE 3. Thermodynamic parameters, ΔH°, ΔS°*, Δ(ΔH°), Δ(ΔS°), TxΔ(ΔS°), Δ(ΔG°), correlation coefficients (R^2) and T_{iso} temperature of monoterpene-based 2-aminocarboxylic acids on ZWIX(+) column

Analyte	Mobile phase	Stereo-isomer	-ΔH° (kJ mol^{-1})	-ΔS°* (J mol^{-1} K^{-1})	Correlation coefficients (R^2)	-Δ(ΔH°) (kJ mol^{-1})	-Δ(ΔS°) (J mol^{-1} K^{-1})	-TxΔ(ΔS°)$_{298K}$ (kJ mol^{-1})	-Δ(ΔG°)$_{298K}$ (kJ mol^{-1})	T_{iso} (°C)
1	**a**	1	5.0±0.2	5.5±0.8	0.9929	2.1	5.6	1.7	0.4	105
		2	7.1±0.2	10.9±0.8	0.9964					
	b	1	10.5±0.5	26.2±1.7	0.9930	1.0	1.3	0.4	0.6	395
		2	11.5±0.5	27.7±1.6	0.9956					
	c	1	6.7±0.3	15.6±1.1	0.9919	1.9	4.9	1.5	0.4	115
		2	8.6±0.4	20.5±1.2	0.9950					
2	**a**	1	3.4±0.1	2.3±0.3	0.9938	-0.5	-1.9	-0.6	0.1	-10
		2	2.9±0.1	0.4±0.1	0.9979					
	b	1	9.1±0.5	22.8±1.7	0.9967	-1.5	-5.2	-1.5	0.1	15
		2	7.6±0.3	17.6±1.1	0.9991					
	c	1	6.4±0.3	16.1±1.0	0.9929	-1.6	-5.7	-1.7	0.1	8
		2	4.8±0.2	10.4±0.6	0.9963					
3	**a**	1	4.9±0.1	4.1±0.3	0.9991	-0.5	-2.0	-0.6	0.1	-23
		2	4.4±0.1	2.1±0.3	0.9991					
	b	1	10.9±0.6	26.2±1.9	0.9906	-1.3	-5.1	-1.5	0.2	-18
		2	9.6±0.5	21.1±1.8	0.9910					
	c	1	8.8±0.2	20.7±0.8	0.9976	-0.7	-2.8	-0.8	0.1	-23
		2	8.1±0.3	17.9±0.9	0.9967					
4	**a**	1	6.6±0.2	3.5±0.8	0.9950	-0.9	-3.4	-1.0	0.1	-8
		2	5.7±0.1	0.1±0.1	0.9985					
	b	1	10.2±0.3	14.8±1.0	0.9974	0.7	1.4	0.4	0.2	210
		2	10.9±0.3	16.2±0.9	0.9981					
	c	1	8.9±0.2	13.1±0.6	0.9988	0.7	1.2	0.4	0.3	270
		2	9.6±0.2	14.3±0.6	0.9990					
5	**a**	1	5.7±0.1	0.5±0.1	0.9991	0.7	2.2	0.6*	0.1*	45
		2	6.4±0.2	2.7±0.8	0.9962					
	b	1	9.4±0.5	12.9±1.5	0.9928	-	-	-	-	-
		2	9.4±0.5	12.9±1.5	0.9928					
	c	1	8.1±0.3	12.6±1.0	0.9958	-	-	-	-	-
		2	8.1±0.3	12.6±1.0	0.9958					
	d	1	7.8±0.04	3.5±0.1	0.9990	0.4	0.7	0.2	0.2	300
		2	8.2±0.05	4.2±0.2	0.9989					

Chromatographic conditions: column, Chiralpak ZWIX(+)™; mobile phase, **a,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA, **b,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM AcOH, **c,** MeOH/MeCN (50/50 v/v) containing 25 mM PRA and 50 mM AcOH, **d,** MeOH/MeCN (25/75 v/v) containing 25 mM PRA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 nm; $\Delta S^{\circ *} = \Delta S^{\circ} + R \ln \phi$, where ϕ is reversal of the phase ratio; R^2, correlation coefficient of van't Hoff plot, ln k – 1/T curves; T_{iso}, temperature of ln k – 1/T curves where enantioselectivity cancels;
*283 *K*; errors have been calculated as the standard errors of the slopes and intercepts of the fitted lines, applying the regression analysis of the Excel program.

TABLE 4. Thermodynamic parameters, ΔH°, ΔS°*, Δ(ΔH°), Δ(ΔS°), TxΔ(ΔS°), Δ(ΔG°), correlation coefficients (R^2) and T_{iso} temperature of monoterpene-based 2-aminocarboxylic acids on ZWIX(-) column

Analyte	Mobile phase	Stereo-isomer	-ΔH° (kJ mol^{-1})	-ΔS°* (J mol^{-1} K^{-1})	Correlation coefficients (R^2)	-Δ(ΔH°) (kJ mol^{-1})	-Δ(ΔS°) (J mol^{-1} K^{-1})	-TxΔ(ΔS°)$_{298K}$ (kJ mol^{-1})	-Δ(ΔG°)$_{298K}$ (kJ mol^{-1})	T_{iso} (°C)
1	**a**	1	3.5±0.03	0.5±0.1	0.9998	2.7	5.6	1.7	1.0	209
		2	6.2±0.1	6.1±0.4	0.9990					
	b	1	3.2±0.1	0.1±0.1	0.9971	1.7	2.6	0.8	0.9	380
		2	4.9±0.1	2.7±0.4	0.9979					
	c	1	4.4±0.1	8.3±0.5	0.9966	1.5	2.5	0.7	0.8	330
		2	5.9±0.2	10.8±0.7	0.9966					
2	**a**	1	-	-	0.9895**	-2.2*	-8.7*	-2.6*	0.4*	-
		2	-	-						
	b	1	4.0±0.2	3.8±0.5	0.9950	-0.5	-2.9	-0.9	0.4	-100
		2	3.5±0.1	0.9±0.2	0.9925					
	c	1	4.0±0.1	8.2±0.2	0.9976	-0.9	-4.0	-1.2	0.3	-48
		2	3.1±0.1	4.2±0.3	0.9986					
3	**a**	1	4.1±0.1	0.6±0.3	0.9906	1.6	2.7	0.8	0.8	319
		2	5.7±0.2	3.3±0.7	0.9954					
	b	1	3.8±0.04	0.6±0.1	0.9996	1.6	2.3	0.7	0.9	420
		2	5.4±0.1	2.9±0.2	0.9995					
	c	1	4.9±0.1	6.3±0.3	0.9974	1.5	2.1	0.6	0.9	440
		2	6.4±0.2	8.4±0.7	0.9969					
4	**a**	1	-1.1±0.05	-20.8±0.2	0.9944	-1.0	-4.3	-1.3	0.3	-41
		2	-2.1±0.03	-25.1±0.1	0.9994					
	b	1	6.0±0.1	0.5±0.2	0.9990	1.3	2.1	0.6	0.7	345
		2	7.3±0.2	2.6±0.5	0.9965					
	c	1	5.7±0.1	1.2±0.2	0.9996	0.8	1.1	0.3	0.5	450
		2	6.5±0.1	2.3±0.5	0.9986					
5	**a**	1	-	-	0.9937**	-3.9*	-13.9*	-4.2*	0.4*	-
		2	-	-						
	b	1	5.5±0.1	1.1±0.2	0.9920	1.2	1.8	0.5	0.7	390
		2	6.7±0.2	2.9±0.8	0.9990					
	c	1	4.1±0.1	0.4±0.2	0.9977	1.0	1.8	0.5	0.5	280
		2	5.1±0.1	2.2±0.4	0.9987					

Chromatographic conditions: column, Chiralpak ZWIX(-)™; mobile phase, **a,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA, **b,** MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM AcOH, **c,** MeOH/MeCN (50/50 v/v) containing 25 mM PRA and 50 mM AcOH; flow rate, 0.6 ml min^{-1}; detection, 215 nm; ΔS°* = ΔS° +R ln ϕ, where ϕ is reversal of the phase ratio; R^2, correlation coefficient of van't Hoff plot, ln k – 1/T curves; T_{iso}, temperature of ln k – 1/T curves where enantioselectivity cancels,

*temperature range 20-50 °C;

**correlation coefficient of ln α vs. 1/T curves; errors have been calculated as the standard errors of the slopes and intercepts of the fitted lines, applying the regression analysis of the Excel program.

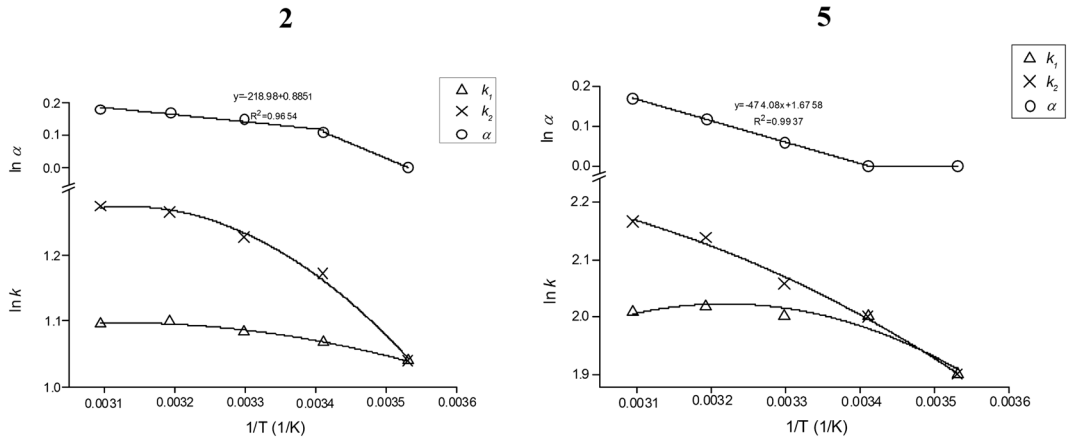


Fig. 3. Nonlinear van't Hoff plots of ln k vs. 1/T and ln α vs. 1/T for analytes 2 and 5 on ZWIX(–) CSP. Chromatographic conditions: column: ZWIX(–); mobile phase: MeOH/MeCN (50/50 v/v) containing 25 mM TEA and 50 mM FA; flow rate: 0.6 ml min^{-1}; detection: 215 nm; temperature range: 10–50°C.

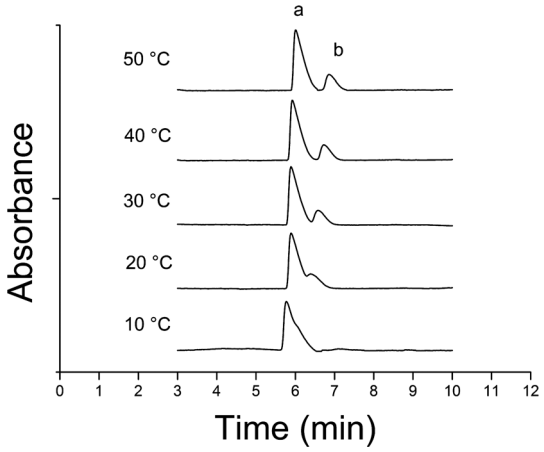


Fig. 4. Temperature dependence of the separation of analyte 2 (for chromatographic conditions, see Fig. 3).

(Tables 3 and 4). In most cases, T_{iso} was considerably higher than room temperature; enthalpically driven enantioseparation was obtained. When T_{iso} was obtained at lower than the ambient temperature, positive Δ(ΔH°) and Δ(ΔS°) were observed and the selectivity increased with increasing temperature. These enantioseparations were entropically driven.

Elution Sequence of Monoterpene-Based 2-Aminocarboxylic Acid Enantiomers

The Chiralpak ZWIX(+) and ZWIX(–) CSPs (see Fig. 2) are actually diastereomers but behave like pseudo-enantiomers.[32] As a consequence, on change from the quinine- to the quinidine-based CSP, reversal of the sequence of the elution of monoterpene-based 2-aminocarboxylic acids between ZWIX(+) and ZWIX(–) was observed in all cases.

CONCLUSIONS

The temperature effects for the separations of the enantiomers of monoterpene-based 2-aminocarboxylic acids (bulky β-amino acids) were investigated by using *Cinchona* alkaloid-based zwitterionic chiral SOs and CSPs: Chiralpak ZWIX(+) and ZWIX(–). In some cases, unexpected temperature behavior was observed: The selectivity, and in special cases, the retention time increased with increasing temperature. This unusual behavior was discussed on the basis of the thermodynamic data. The values of thermodynamic parameters such as the changes in enthalpy, Δ(ΔH°), entropy, Δ(ΔS°), and Gibbs energy, Δ(ΔG°), depended on the molecular structures of the SAs and the chiral SOs investigated.

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