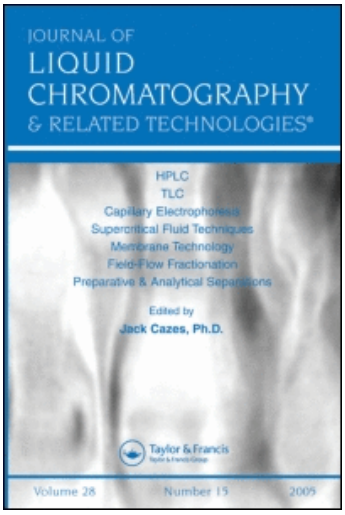

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Study of the Mechanism of Enantioseparation. IX. Effect of Temperature on Retention of Chiral Compounds on a Methylated Teicoplanin Chiral Stationary Phase

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Study of the Mechanism of Enantioseparation. IX. Effect of Temperature on Retention of Chiral Compounds on a Methylated Teicoplanin Chiral Stationary Phase

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ABSTRACT

The isocratic retention of enantiomers of chiral analytes (1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid, potential local anaesthetic drugs) was studied on a methylated teicoplanin

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CSP, at different temperatures (in the range of 0–50°C with 10°C increments) in the polar organic mode (the mobile phase composition consisted of methanol with 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine). The natural logarithms of the retention factors ($\ln k_i$) of the investigated chiral compounds depended linearly on the inverse of temperature ($1/T$). van't Hoff plots afforded thermodynamic parameters, such as the apparent change in enthalpy (ΔH_i), the apparent change in entropy (ΔS_i), and the apparent change in Gibbs free energy (ΔG_i) for the transfer of analyte between the mobile phase and the stationary phase. The thermodynamic parameters were calculated in order to promote an understanding of the thermodynamic driving forces for retention in this chromatographic system. Enthalpy–entropy compensation plots showed that all of the compounds in this study separate via the same enthalpy driven chiral recognition mechanism.

Key Words: Chiral separation; HPLC; Methylated teicoplanin CSP; Thermodynamic study; Enthalpy–entropy compensation; Chiral compounds; Alkoxysubstituted esters of phenylcarbamic acid.

INTRODUCTION

Temperature is a critical parameter in chromatography, and studying its effect on separations is key to understand the mechanism governing the chromatographic process. The effect of temperature on the resolution and selectivity factors for a set of chiral compounds is often interpreted using van't Hoff plots of the chromatographic data. Typical van't Hoff-type plots are constructed using either the logarithm of the retention factors (k_i) of an analyte, or the selectivity factors for the enantiomers versus the inverse of absolute temperature ($1/T$). For most separations, these plots are linear, indicating that the retention and/or selective processes governing the separation are unchanged over the temperature studied. Previous studies indicate that there are at least two significant temperature effects: the influence of viscosity, the diffusion coefficients of solutes, and a thermodynamic effect.[1–5]

Direct enantiomeric separations are based on the formation of reversible diastereoisomeric complexes, which are created by intermolecular interactions of the enantiomers with the chiral selector.[6] The formation process, for the R and S enantiomers, can be characterised by thermodynamic parameters (ΔG_i, ΔH_i, ΔS_i). These can be calculated for both enantiomers according to the equation

$$\Delta G_i = \Delta H_i - T\Delta S_i = -RT \ln K_i \quad (1)$$

where ΔG_i is the molar Gibbs free energy, K_i is the solute partition coefficient, R is the universal gas constant, and T is the temperature in degrees K.[5–15]

Many publications have considered the effect of temperature on the liquid chromatographic separation of enantiomers for different chiral stationary phases, such as those based on cyclodextrins, crown ethers, derivatised cellulose, macrocyclic glycopeptides, and other CSPs.[5,7,13,15–21]

The aim of the present paper was to investigate the effects of temperature on the enantioselective separations of 1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid on a methylated teicoplanin CSP. The thermodynamic data found from the linear dependencies of the natural logarithms of retention factors ($\ln k_i$) with $(1/T)$ were used to study some mechanistic aspects of the chiral recognition process.[5,9–11,15,19]

EXPERIMENTAL

Materials

The structures of 1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid are given in Fig. 1. All derivates of phenylcarbamic acid used in this study are listed in Table 1, and were prepared according to Pokorná and co-workers.[22] HPLC grade solvent (methanol) was obtained from Merck (Germany). Diethylamine and acetic acid were obtained from Lachema (Czech Republic).

Equipment

The HPLC chromatographic system, Hewlett Packard (series 1100), consisted of a quaternary solvent pump; an injection valve Rheodyne 7724i with a 20 μL sample loop; a switching valve, Valco, and a photodiode array detector.

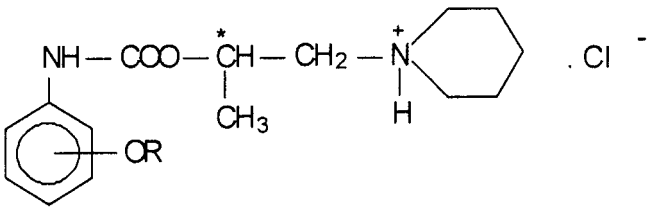

Figure 1. Structure of 1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid.

Table 1. Description and numbering of the 1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid derivates used in this study.

2-Position		3-Position		4-Position	
Analyte	R	Analyte	R	Analyte	R
1v	$-CH_3$	2v	$-CH_3$	3v	$-CH_3$
4v	$-C_2H_5$	5v	$-C_2H_5$	6v	$-C_2H_5$
10v	$-C_4H_9$	11v	$-C_4H_9$	12v	$-C_4H_9$
13v	$-C_5H_{11}$	14v	$-C_5H_{11}$	15v	$-C_5H_{11}$
16v	$-C_6H_{13}$	17v	$-C_6H_{13}$	18v	$-C_6H_{13}$
19v	$-C_7H_{15}$	20v	$-C_7H_{15}$	21v	$-C_7H_{15}$
28v	$-C_{10}H_{21}$	29v	$-C_{10}H_{21}$	30v	$-C_{10}H_{21}$

The column temperature was controlled in a column temperature box (LCT 5100, INGOS, Czech Republic).

Methods

A methylated teicoplanin CSP (150 × 5 μm^2 I.D., six methyl groups per teicoplanin aglycon molecule, the carboxy group is a methyl ester—according to the information of a procedures—Astec, USA) was used for the separation of enantiomers of the alkoxy-substituted esters of phenylcarbamic acid. The analytes were dissolved in methanol (concentration: 1 mg/mL). The analytes studied possess a UV absorption maximum at a wavelength of 240 nm, which was used for detection. Mobile phases were prepared by mixing methanol [100 mL (v)] with 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine. Separations were carried out at a flow rate of 1.0 mL/min. Thermodynamic data were obtained using isothermal conditions over a temperature range of 0–50°C at 10°C intervals. The precision of the controlled temperature was ±0.1°C. Higher temperatures were not used in order to protect the column from degradation. The elution of enantiomers is indicated in the text and tables with 1 (first eluted enantiomer) and 2 (second eluted enantiomer) labels, respectively.

RESULTS AND DISCUSSION

In this work, the separation of analytes (see Table 1) in the polar organic mode was carried out with a mobile phase of methanol containing 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine at different temperatures.

The results are presented in Table 2. This table lists the retention factors (k_1, k_2) measured for these analytes, all of which decrease with increasing column temperature. Also, the peak symmetry deteriorates with decreasing column temperature. Moreover, from Table 2, it is evident that the retention factors of these analytes decrease when the number of carbon atoms in the alkoxy chain increases in the range of C_1–C_{10}. This decrease in k_i with a methanol mobile phase is the characteristic behaviour when solvophobic interactions between the analytes and the CSP are dominant.

The resolution factors (R_{21}) of the enantiomers decrease with an increase in temperature in most cases. The data given in Table 2 show that the magnitude of the decrease of R_{21} depends on the position of the alkoxy group on the phenyl ring (e.g., in the 2-position, R_{21} varies from 3.0 to 0.4, in the 3-position, R_{21} varies from 1.9 to 1.0, and in the 4-position, R_{21} varies from 1.9 to 0.9). This indicates that steric interactions of the alkoxy chain influence chiral recognition only for the 2-alkoxy-substituted compounds. Considering the structure of these compounds, it is clear that the reason for this is the proximity of the 2-alkoxy substituent to the stereogenic center.

The values of the thermodynamic parameters depend on the structure of the compounds. In the case of these derivates of alkoxyphenylcarbamic acid, the main step in chiral recognition and enantioselective retention on the methylated teicoplanin CSP is probably the repulsion effect between the protonated amine of the analyte molecules and the ammonium group of the methylated teicoplanin molecule. Then, hydrogen bonding and steric interactions must also be considered.

Thermodynamic Parameters in the Polar Organic Mode

In order to calculate the thermodynamic parameters and acquire information of value for an understanding of enantiomeric retention, selectivity, and the separation mechanism of this CSP, van't Hoff plots were constructed using

$$\ln k_i = \frac{-\Delta H_i}{RT} + \frac{\Delta S_i}{R} + \ln \Phi \tag{2}$$

where k_i is the retention factor of a solute ($k_i = (t_R - t_M)/t_M$), ΔH_i is the interaction enthalpy of the solute in the chromatographic system, ΔS_i is the entropy of this solute, and Φ is the phase ratio of the chromatographic column ($\Phi = V_M/V_S$, where V_M is the column hold up volume and V_S is the volume of the chiral selector containing stationary phase).

Equation (2) shows that a plot of $\ln k_i$ versus $1/T$ should be linear with a slope of $\Delta H_i/R$ and an intercept of $\Delta S_i/R + \ln \Phi$, if ΔH_i is invariant with

Table 2. Dependences of enantiomer retention factors (k_1, k_2) and resolutions (R_{21}) for 2-alkoxy (A), 3-alkoxy (B), and 4-alkoxy (C) derivates of phenylcarbamic acid 1-methyl-2-piperidinoethylesters on temperature (see Experimental for details).

	Temperature																	
	273 K			283 K			293 K			303 K			313 K			323 K		
Analyte	k_1	k_2	R_{21}	k_1	k_2	R_{21}	k_1	k_2	R_{21}	k_1	k_2	R_{21}	k_1	k_2	R_{21}	k_1	k_2	R_{21}
2-Alkoxy derivates[a]																		
1v	35.52	43.18	2.1	27.51	32.32	2.1	24.24	27.78	1.9	21.86	24.62	1.7	18.25	20.34	1.6	16.91	18.38	1.3
4v	28.45	35.88	3.0	22.68	27.33	2.5	19.90	23.57	2.4	17.99	20.66	2.1	14.71	16.62	1.9	13.86	15.28	1.5
10v	21.62	25.39	2.0	16.74	19.01	1.6	15.96	17.91	1.6	14.25	15.57	1.3	11.74	12.68	1.1	11.15	11.85	0.8
13v	19.69	22.34	1.5	15.49	17.21	1.2	14.65	15.99	1.1	13.43	14.40	0.9	10.72	11.35	0.7	10.28	10.76	0.6
16v	18.16	19.91	1.1	14.22	15.41	1.0	13.50	14.36	0.8	12.61	13.25	0.7	10.81	11.25	0.6	9.55	9.86	0.4
19v	17.08	18.88	1.1	14.77	15.98	1.0	14.39	15.41	0.9	12.52	13.17	0.6	10.75	11.18	0.6	9.54	9.80	0.4
28v	13.34	14.76	1.1	11.63	12.63	1.0	1131	12.04	0.7	10.03	10.57	0.6	9.11	9.39	0.5	7.72	7.80	0.4
3-Alkoxy derivates[b]																		
2v	38.86	44.86	1.7	30.40	34.50	1.7	27.36	30.54	1.4	24.69	27.09	1.3	20.52	22.31	1.2	19.12	20.46	1.1
5v	32.12	36.65	1.5	27.04	30.52	1.5	24.02	26.64	1.3	21.84	23.87	1.2	18.04	19.55	1.2	16.83	17.94	1.0

11v	27.05	31.39	1.7	21.52	24.50	1.6	20.66	23.12	1.5	18.61	20.51	1.4	15.48	16.93	1.3	14.54	15.64	1.1
14v	25.12	29.14	1.7	20.21	23.19	1.6	19.23	21.72	1.5	17.96	19.95	1.4	15.81	17.39	1.3	13.37	14.84	1.1
17v	23.78	27.73	1.8	1973	22.78	1.7	18.25	20.48	1.5	17.01	18.96	1.4	14.71	16.17	1.4	13.08	14.16	1.1
20v	21.18	24.09	1.9	18.69	21.54	1.7	18.01	20.45	1.7	16.19	18.08	1.5	14.07	15.50	1.4	12.48	13.55	1.2
29v	16.69	19.80	1.9	14.85	17.22	1.7	14.49	16.48	1.6	13.00	14.58	1.5	11.62	12.84	1.3	10.21	11.16	1.1
4-Alkoxy derivates[c]																		
3v	42.88	49.32	1.6	34.14	38.78	1.5	30.58	33.99	1.3	27.62	30.26	1.2	22.88	24.89	1.2	21.42	22.89	1.0
6v	35.36	40.78	1.6	27.92	31.47	1.4	26.61	29.52	1.3	24.12	26.29	1.2	20.06	21.69	1.1	18.75	19.87	0.9
12v	29.90	34.80	1.7	23.64	26.95	1.5	22.71	25.39	1.4	20.46	22.45	1.3	17.16	18.64	1.1	16.13	17.24	1.0
15v	27.87	32.26	1.6	22.44	25.66	1.5	21.45	23.91	1.4	19.99	22.01	1.3	17.65	19.25	1.2	15.29	16.32	1.0
18v	25.07	29.31	1.6	21.95	25.04	1.5	20.32	22.62	1.3	18.97	20.85	1.3	16.44	17.87	1.1	14.65	15.63	0.9
21v	23.76	27.87	1.7	20.84	23.89	1.6	20.06	22.48	1.4	18.11	19.95	1.3	16.20	17.59	1.1	14.02	14.99	1.0
30v	20.35	24.41	1.9	18.06	20.72	1.5	17.56	19.76	1.4	15.89	17.58	1.3	14.09	15.34	1.1	12.54	13.46	1.0

[a]For $n = 3$: $k_1 \pm 0.28$, $k_2 \pm 0.14$, $R_{21} \pm 0.1$.
[b]For $n = 3$: $k_1 \pm 0.12$, $k_2 \pm 0.17$, $R_{21} \pm 0.1$.
[c]For $n = 3$: $k_1 \pm 0.12$, $k_2 \pm 0.15$, $R_{21} \pm 0.1$.

temperature. This provides a convenient way of calculating the thermodynamic constants, ΔH_i and ΔS_i, for a chromatographic system if the phase ratio is known or can be determined. Its determination is relatively easy in pure liquid–liquid chromatography, but it is much more complex in LC with chemically bonded stationary phases. Petér et al. have shown that the contribution of $\ln \Phi$ is usually lower than 2% for chemically bonded phases operated in all modes (PO, RP, NP).[5,7]

The dependence of the natural logarithm of the selectivity factor ($\ln \alpha$) on the reciprocal temperature ($1/T$) is given by the following relationship.

$$\ln \alpha = \frac{-\Delta(\Delta H_{2,1})}{RT} + \frac{\Delta(\Delta S_{2,1})}{R} \tag{3}$$

where $\Delta(\Delta H_{2,1})$ and $\Delta(\Delta S_{2,1})$ are the enthalpy and entropy differences characterising enantiomer interactions with mobile and chiral stationary phases.

The correlation coefficients of the van't Hoff plots [Eq. (2)] for the enantiomers of all compounds in this study indicated good linearity (correlation coefficients were higher than 0.982, see Table 3 and Fig. 2). Similar dependencies were obtained for all of the enantiomers in this study (see the $\Delta(\Delta H_{2,1})$, $\Delta(\Delta S_{2,1})$, $\Delta(\Delta G_{2,1})_{0^\circ C}$ values listed in Table 3). The isoenantioselective temperature (T_{iso}) can be defined as the ratio

$$T_{iso} = \frac{\Delta(\Delta H_{2,1})}{\Delta(\Delta S_{2,1})} \tag{4}$$

At a certain temperature [$\Delta(\Delta G_{2,1}) = 0$], the enantiomers are not separated. Above the enantioselectivity temperature, the elution order of the enantiomers can be reversed and the enantioselectivity is dominantly influenced by the entropic term. The isoenantioselective temperature determined in this work (380 ± 45 K) was higher than the working temperature range (273–323 K).

Multiple, simultaneous differential interactions between the chiral analyte and CSPs are necessary for chiral recognition and enantioseparation to take place. Among the more important molecular interactions for chiral recognition, are solvophobic, hydrogen bonding, π–π, dipolar, and steric repulsion types. Methylated teicoplanin chiral stationary phase has many functional groups, allowing it to take advantage of most of these interactions under certain conditions.

The thermodynamic parameters obtained in the polar organic mode are given in Table 3. The ΔH_i values calculated from the slopes of the plots of Eq. (2) were negative for all enantiomers. This indicates that the transfer of the enantiomers from the mobile to the chiral stationary phase is enthalpically favoured. The ΔH_i values are in the range of -7.511 to -12.126 J/mol for the 2-alkoxy-substituted derivates of phenylcarbamic acid (e.g., for the

Table 3. Thermodynamic data for the first and the second eluted enantiomers of 2- (A), 3- (B), and 4- (C) alkoxyphenylcarbamic acid 1-methyl-2-piperidinoethylesters (see Experimental for details).

Analyte	ΔH_1 (J mol)	ΔS_1 [J/(mol K)]	Correlation coefficient, r	ΔH_2 (J/mol)	ΔS_2 [J/(mol K)]	Correlation coefficient, r	$\Delta(\Delta H_{2,1})$ (J/mol)	$\Delta(\Delta S_{2,1})$ [J/(mol K)]	$\Delta(\Delta G_{2,1})_{0^\circ C}$ (J mol)	T_{iso}
Thermodynamic parameters 1[a]										
1v	−10,580	−11.40	0.992	−12,126	−13.49	0.993	−1,546	−2.09	−974	378
4v	−10,468	−10.73	0.993	−11,700	−13.30	0.994	−1,232	−2.57	−529	375
10v	−9,750	−10.10	0.950	−10,814	−12.60	0.986	−1,064	−2.50	−382	256
13v	−9,294	−9.50	0.982	−10,483	−12.00	0.985	−1,189	−2.50	−506	359
16v	−8,588	−7.80	0.982	−9,840	−10.90	0.986	−1,252	−3.10	−405	355
19v	−8,400	−7.40	0.984	−9,660	−9.90	0.988	−1,260	−2.50	−578	261
28v	−7,511	−5.87	0.987	−8,910	−8.90	0.987	−1,399	−3.03	−572	330
Thermodynamic parameters 2[b]										
2v	−10,121	−6.93	0.992	−11,216	−9.75	0.993	−1,096	−2.82	−325	388
5v	−9,487	−5.93	0.996	−10,489	−8.49	0.997	−1,002	−2.55	−305	393
11v	−8,782	−4.96	0.985	−98,58	−7.68	0.989	−1,076	−2.71	−335	396
14v	−8,228	−3.91	0.992	−93,28	−6.65	0.994	−1,100	−2.4	−353	402
17v	−7,995	−2.70	0.983	−9,023	−5.21	0.987	−1,029	−2.51	−343	410
20v	−7,483	−1.86	0.985	−8,676	−4.86	0.989	−1,192	−3.01	−372	397
29v	−6,925	−1.81	0.987	−8,142	−4.84	0.989	−1,217	−3.03	−391	402
Thermodynamic parameters 3[c]										
3v	−9,998	−5.61	0.993	−11,072	−8.36	0.995	−1,073	−2.76	−321	389
6v	−8,922	−3.84	0.986	−10,102	−7.11	0.989	−1,181	−3.27	−288	374
12v	−8,310	−2.20	0.985	−9,540	−5.56	0.989	−1,230	−3.36	−313	378
15v	−7,920	−1.56	0.982	−9,086	−4.60	0.987	−1,165	−3.03	−337	384

(*continued*)

Table 3. Continued.

Analyte	ΔH_1 (J mol)	ΔS_1 [J/(mol K)]	Correlation coefficient, r	ΔH_2 (J/mol)	ΔS_2 [J/(mol K)]	Correlation coefficient, r	$\Delta(\Delta H_{2,1})$ (J/mol)	$\Delta(\Delta S_{2,1})$ [J/(mol K)]	$\Delta(\Delta G_{2,1})_{0^\circ C}$ (J mol)	T_{iso}
18v	−7,539	−0.76	0.992	−8,829	−4.21	0.995	−1,290	−3.45	−347	374
21v	−7,266	−0.55	0.985	−8,606	−3.75	0.990	−1,340	−3.20	−466	372
30v	−6,880	−0.45	0.988	−8,196	−3.47	0.992	−1,316	−3.02	−389	396

[a]$\Delta H_1 \pm 145$ (J/mol); $\Delta H_2 \pm 150$ (J/mol); $\Delta S_1 \pm 0.21$ [J/(mol K)]; $\Delta S_2 \pm 0.18$ [J/(mol K)]; $\Delta(\Delta H_{2,1}) \pm 42$ (J/mol); $\Delta(\Delta S_{2,1}) \pm 0.15$ [J/(mol K)]; $\Delta(\Delta G_{2,1})_{0^\circ C} \pm 20$ (J/mol). Temperature reported to the nearest ± 10 K (for $n = 3$).
[b]$\Delta H_1 \pm 155$ (J/mol); $\Delta H_2 \pm 170$ (J/mol); $\Delta S_1 \pm 0.18$ [J/(mol K)]; $\Delta S_2 \pm 0.23$ [J/(mol .K)]; $\Delta(\Delta H_{2,1}) \pm 45$ (J/mol); $\Delta(\Delta S_{2,1}) \pm 0.19$ [J/(mol K)]; $\Delta(\Delta G_{2,1})_{0^\circ C} \pm 32$ (J/mol); Temperature reported to the nearest ± 12 K (for $n = 3$).
[c]$\Delta H_1 \pm 177$ (J/mol); $\Delta H_2 \pm 183$ (J/mol); $\Delta S_1 \pm 0.13$ [J/(mol K)]; $\Delta S_2 \pm 0.19$ [J/(mol K)]; $\Delta(\Delta H_{2,1}) \pm 62$ (J/mol); $\Delta(\Delta S_{2,1}) \pm 0.21$ [J/(mol K)]; $\Delta(\Delta G_{2,1})_{0^\circ C} \pm 43$ (J/mol); Temperature reported to the nearest ± 9 K. (for $n = 3$).

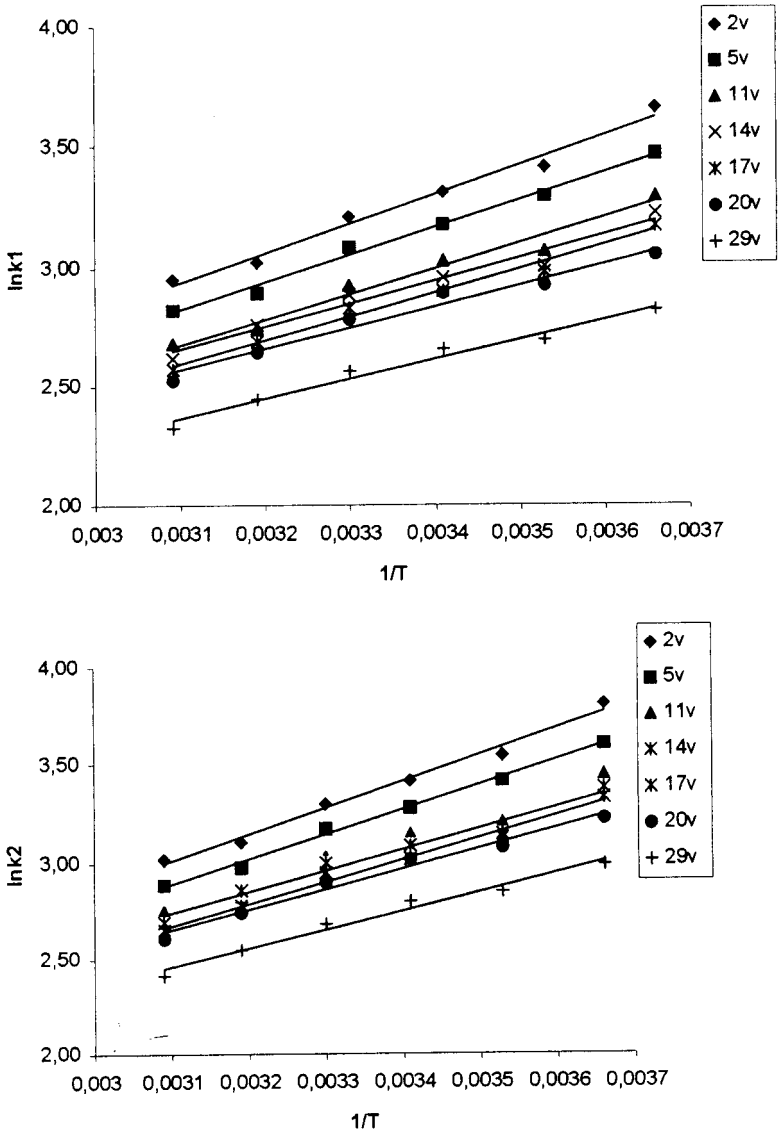

Figure 2. Dependence of natural logarithms of retention factors ($\ln k_i$) on the inverse of temperature ($1/T$) for 3-alkoxysubstituted esters of alkoxyphenylcarbamic acid (see Experimental for details).

3-derivatives the ΔH_i varies from -6.925 to $-11.216\,\mathrm{J/mol}$, and for the 4-derivatives the ΔH_i varied from -6.880 to $11.072\,\mathrm{J/mol}$). For all the investigated chiral compounds, the enthalpy change for the second eluted enantiomer is more negative than that of the first eluted enantiomer. This means, that the association between the second eluted enantiomer and the methylated teicoplanin chiral stationary phase is more favourable than for the first eluted enantiomer.

Moreover, from Table 3, this is evident that the entropy values (ΔS_i) were also negative in all cases. The ΔS_i of the first eluted enantiomer was always more positive than those for the second eluted enantiomer. Thus, in every case, the second eluted enantiomers always had more negative enthalpies and, at the same time, more negative entropy values. The two enantiomers must be solvated identically in the mobile phase (methanol with 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine), but may release a different number of solvent molecules when they associate with the methylated teicoplanin CSP. Therefore, this contribution to ΔS_i may not be identical for both enantiomers. Since the second eluted enantiomers have more negative ΔS_i values, they may have fewer degrees of freedom on the CSP (e.g., either they are less able to move or rotate, or a smaller number of solvent molecules may be displaced by the analyte when it associates with the methylated teicoplanin CSP).

Enthalpy–Entropy Compensation

A further thermodynamic approach for the analysis of physicochemical data used in this work was enthalpy–entropy compensation,[5,7] that can be expressed by the formula:

$$\Delta H_i = \beta \Delta S_i + \Delta G_\beta \tag{5}$$

where ΔG_β is the Gibbs free energy of the enantiomeric interactions in the chromatographic system at the compensation temperature (β).

Combination of Eqs. (2) and (5) leads to Eq. (6):

$$\ln k_i = \frac{-\Delta H_i}{R}\left(\frac{1}{T} - \frac{1}{\phi}\right) - \frac{\Delta G_\phi}{\phi R} + \ln \beta \tag{6}$$

which shows that plot of $\ln k_i$ on $-\Delta H_i$ can be used to determine the compensation temperature. If enthalpy–entropy compensation is observed, all compounds have the same free energy change, ΔG_β, at the compensation temperature β, and all compounds will have the same net retention at this temperature, although their temperature dependencies may differ.[11]

Figure 3 shows the enthalpy–entropy compensation plots ($\ln k_i$ versus $-\Delta H_i$) for analytes with alkoxy-substitution in the 3-positions ($T = 273\,\mathrm{K}$).

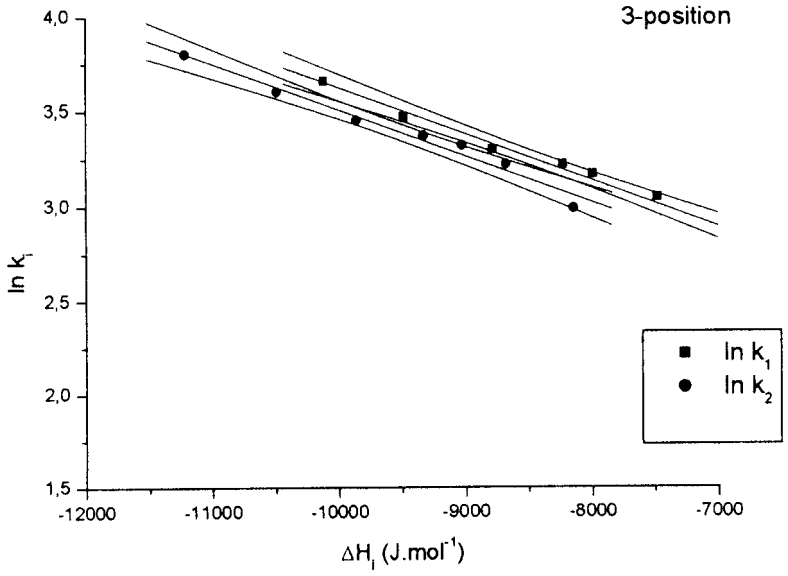

Figure 3. Plots of enthalpy–entropy compensation with the regression line and confidence intervals for the first (■) and second eluted (●) enantiomers of 1-methyl-2-piperidinoethyl esters of 3-alkoxyphenylcarbamic acid at a temperature of 273 K (see Experimental for details).

Similar dependencies were observed at other studied temperatures and for other substitution positions of the alkoxy chain.

Figure 4 shows the dependence of $\Delta(\Delta H_{2,1})$ on $T\Delta(\Delta S_{2,1})$ for the 3-alkoxy-substituted derivates of phenylcarbamic acid. The confidence interval at 90.0% probability shows no dependence of chiral recognition on the position of alkoxy-substituent on the phenyl ring, or the number of carbon atoms in the alkoxy chain (C_1–C_{10}). Similar conclusions were obtained for 2- and 4-alkoxy-substituted derivatives of phenylcarbamic acid.

Dependence of Enthalpy and Entropy on the Alkoxy Chain Carbon Number and its Position on the Phenyl Ring

The plots in Figs. 5 and 6 show that the enthalpy and entropy values (ΔH_i and ΔS_i) of these enantiomers increase (are more positive) with increasing length of the alkoxy chain. The dependence is linear for both

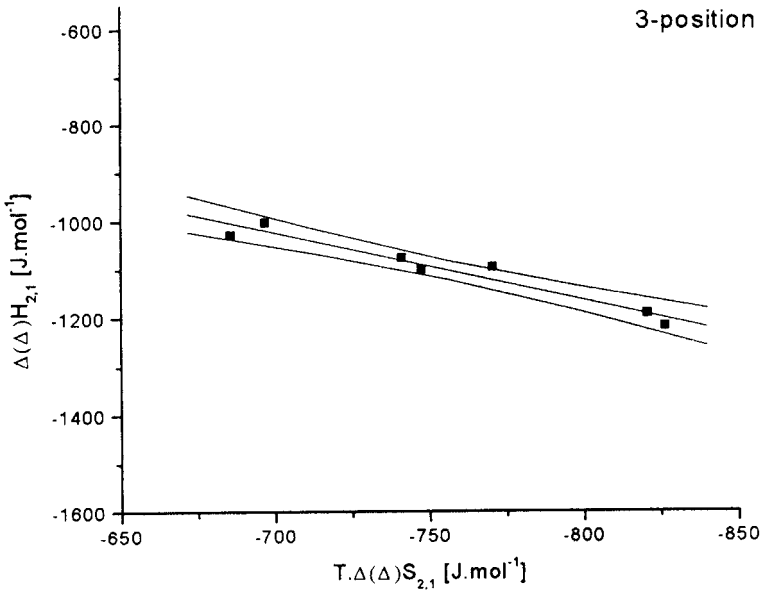

Figure 4. Dependence of $\Delta(\Delta H_{2,1})$ on $T\Delta(\Delta S_{2,1})$ for 3-alkoxyderivates of phenylcarbamic acid at 323 K (see Experimental for details).

enantiomers with alkoxy-substitution in the 3-position. Similar dependencies also were obtained for the 2- and 4-alkoxy-substituted analogues. The enantiomers with 10 carbon atoms in the alkoxy chain (C_{10}) have very poor retention (see Table 2), as a consequence of very weak interactions with the methylated teicoplanin CSP (the absolute value of $-\Delta H_i$ was the lowest, e.g., most positive). On the other hand, enantiomers with a single carbon atom in the alkoxy chain (C_1) had very high retention. In these cases, the volume of the molecule and also the orientation of the molecule, may play an important role in retention.

CONCLUSIONS

The effect of temperature on the retention of 1-methyl-2-piperidinethylesters of phenylcarbamic acid was studied. In the temperature range under study (0–50°C) van't Hoff plots [$\ln k_i = f(1/T)$] were linear. Changes in the enthalpies and entropies of solute transfer from mobile phase to methylated teicoplanin chiral stationary phase were determined. Using the Gibbs–Helmholtz

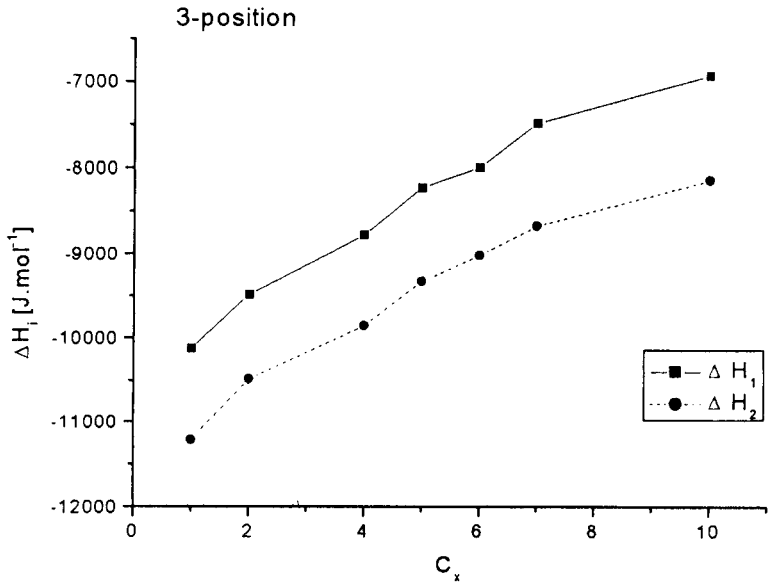

Figure 5. Dependence of the enthalpy (ΔH_i) on the number of carbon atoms (C_x) in the alkoxy chain attached to the 3-alkoxysubstituted esters of phenylcarbamic acid [for the first (■) and second eluted (●) enantiomers] (see Experimental for details).

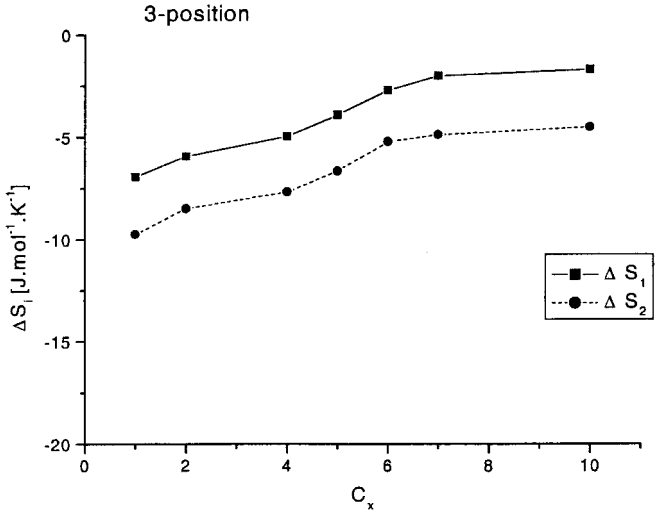

Figure 6. Dependence of the entropy (ΔS_i) on the number of carbon atoms (C_x) in the alkoxy chain attached to the 3-alkoxysubstituted esters of phenylcarbamic acid [for the first (■) and second eluted (●) enantiomers] (see Experimental for details).

equation ΔG_i, values were calculated. The absolute values of ΔH_i and ΔS_i decrease with increasing length of the alkoxy chain on benzene rings. From the statistical point of view, it seems that contributions from ΔH_i are statistically significant (level of probability $\alpha = 0.05$). The entropy of this system also plays an important role in the separation of enantiomers. Given the results, the number of carbon atoms in the alkoxy chain, only in position two (closest to the stereogenic center), plays a significant role on the chiral recognition of the studied enantiomers.

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