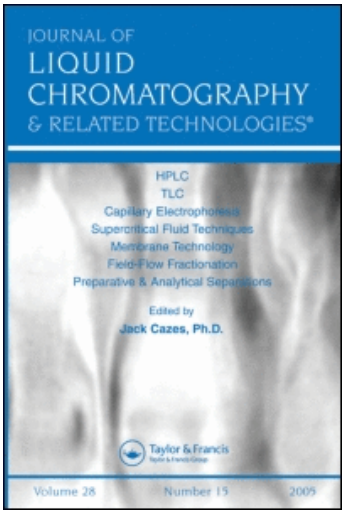

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Study of the Mechanism of Enantioseparation. X. Comparison Study of Thermodynamic Parameters on Separation of Phenylcarbamic Acid Derivatives Using Vancomycin and Teicoplanin CSPs

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Study of the Mechanism of Enantioseparation. X. Comparison Study of Thermodynamic Parameters on Separation of Phenylcarbamic Acid Derivatives Using Vancomycin and Teicoplanin CSPs

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ABSTRACT

The enantiomers of 1-methyl-2-piperidinoethylesters of 3- and 4-alkoxy-phenylcarbamic acid were separated on vancomycin (CHIROBIOTIC V)

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and teicoplanin (CHIROBIOTIC T) columns isothermally in the range of 0–50°C at 10°C increments, using methanol containing 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine as the mobile phase. Lower temperatures produced the expected increase in retention of the studied enantiomers and improved their separation. The retention factors k_i (as well as the resolutions R_{ij}) were higher on the teicoplanin chiral stationary phase (CSP) compared with the vancomycin CSP. Van't Hoff plots (dependence of $\ln k_i$ on $1/T$) were linear within the temperature interval studied, and they also were used to determine thermodynamic data, such as the (ΔH_i) and (ΔS_i) of transfer of the enantiomers between mobile and stationary phases. From these results, it is evident that the prolongation of carbon atoms in the alkoxy-chain has a major influence on the values of $\Delta(\Delta S_{2,1})$ using the vancomycin CSP, and on the values of $\Delta(\Delta H_{2,1})$ using the teicoplanin CSP. However, when the alkoxy-substituent on the phenylcarbamic acid ring system is in the 3- and 4-positions, there seems to be little difference in the thermodynamic parameters.

Key Words: Chiral separation; HPLC; Glycopeptide antibiotics; Thermodynamic study; Alkoxysubstituted esters of phenylcarbamic acid; Local anaesthetics.

INTRODUCTION

The use of macrocyclic glycopeptide antibiotics as chiral selectors was introduced in 1994 by Armstrong and co-workers.[1,2] Due to their structural characteristics, the macrocyclic compounds combine the chiral properties of several different chiral stationary phase (CSPs). Vancomycin (CHIROBIOTIC V) or teicoplanin (CHIROBIOTIC T) contains from 18 to 20 stereogenic centres, three or four inclusion cavities, and ionizable groups. The aglycones of vancomycin and teicoplanin contain two chloro-substituted aromatic rings. The vancomycin macrocyclic glycopeptide is smaller than the related teicoplanin molecule. It consists of three macrocyclic rings and an attached disaccharide consisting of D-glucose and vancosamine. A teicoplanin macromolecule has three attached monosaccharides, two of which are D-glucosamine and one of which is D-mannose. Teicoplanin has one unique characteristic: ithas a hydrophobic acyl side chain attached to a 2-amino-2-deoxy-β-D-glycopyranosyl moiety. Consequently, teicoplanin is surface active and it aggregates to form micelles.[3] The complexity of the selector structure is responsible for the fact that several different chiral recognition mechanisms are possible. Some studies have been carried out on the basis of the enantiomer–macrocycle interaction for teicoplanin CSPs. Both Berthod et al.[4] and Péter and co-workers[5,6] have found that interaction with the ammonium

group is on the aglycone of the chiral selector involved in the enantioseparation of many amino acids and analogues. This primary interaction occurs with the carboxylate group of the solute. Furthermore, it was indicated that additional interactions must be present, including hydrophobic effects, steric repulsion, and hydrogen-bonding.[4–7]

There have been numerous studies of temperature effects on solute retention in liquid chromatography.[5,6,8–12] The dependence of the natural logarithms of retention and selectivity factors ($\ln k_i$, $\ln \alpha$, respectively) on the inverse of temperature ($1/T$) are routinely used to determine thermodynamic data that characterize the separation of enantiomers, and allows the study of some aspects of the chiral recognition processes.

It is generally accepted that the direct chromatographic enantioseparation is based on the formation of reversible diastereoisomeric associates that are created by intermolecular interactions of the enantiomers with the chiral selector.[13] This process, for the *R* and *S* enantiomers, can be characterised by thermodynamic data (ΔG_i, ΔH_i, ΔS_i). These can be calculated for both enantiomers according to the equation (Gibbs–Helmholtz equation):

$$\Delta G_i = \Delta H_i - T\Delta S_i = -RT \ln K_i \tag{1}$$

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 K.

The dependence of an analytes retention on the temperature can be expressed by the van't Hoff equation:

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

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 ($\Phi = V_M/V_S$).

Equation (2) shows that a plot of $\ln k_i$ vs. $1/T$ is 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 temperature. This simplifies the determination of ΔH_i and ΔS_i values of the separated analytes in a chromatographic system. There are, however, examples where non-linear dependences of $\ln k_i$ on $1/T$ might be observed, particularly for separations where the retention is influenced by mixed retention mechanisms.[14]

The $\Delta(\Delta H_{2,1})$ and $\Delta(\Delta S_{2,1})$ values for the separated enantiomers, can be determined from the differences ($\Delta H_2 - \Delta H_1$ and $\Delta S_2 - \Delta S_1$), or from the modified Eq. (2):

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

where α is the selectivity factor ($\alpha = k_2/k_1$) and the numbers 1 and 2 are the first and the second eluted enantiomer, respectively. If $\Delta(\Delta H_{2,1})$ is invariant with temperature, a straight line is obtained by plotting $\ln \alpha$ values on reversed temperature ($1/T$) with the slope $\Delta(\Delta H_{2,1})$ and the Y axis intercept $\Delta(\Delta S_{2,1})$. This usually occurs when there is no change in the retention mechanism as a function of temperature.

The aim of the present paper is to compare the thermodynamic parameters of the enantioseparations of 1-methyl-2-piperidinoethylesters of 3- and 4-alkoxyphenylcarbamic acid (potential local anaesthetic drugs) using vancomycin and teicoplanin CSPs. The thermodynamic data found from the linear dependencies of the natural logarithms of retention factors ($\ln k_i$) with ($1/T$), are used to compare some data obtained using vancomycin and teicoplanin CSPs. Mechanistic aspects of the chiral recognition process on these columns are discussed.

EXPERIMENTAL

Materials

The structures of the 1-methyl-2-piperidinoethylesters of 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.[15] HPLC grade solvent (methanol) was obtained from Merck (Germany). Diethylamine and acetic acid were of analytical grade and 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

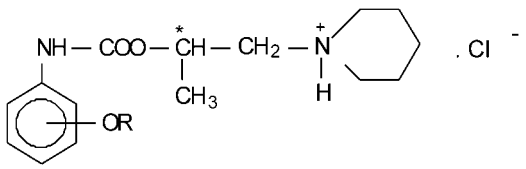

Figure 1. Structure of 1-methyl-2-piperidinoethylesters of 3- and 4-alkoxyphenylcarbamic acid.

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

3-Position		4-Position	
Analyte	R	Analyte	R
1_3	$-CH_3$	1_4	$-CH_3$
2_3	$-C_2H_5$	2_4	$-C_2H_5$
4_3	$-C_4H_9$	4_4	$-C_4H_9$
5_3	$-C_5H_{11}$	5_4	$-C_5H_{11}$
6_3	$-C_6H_{13}$	6_4	$-C_6H_{13}$
7_3	$-C_7H_{15}$	7_4	$-C_7H_{15}$

with a 20 μL sample loop; a switching valve, Valco, and a photodiode array detector. The column temperature was controlled in a column temperature box (LCT 5100, INGOS, Czech Republic).

Methods

A vancomycin (CHIROBIOTIC V) CSP (250 mm × 4.6 mm I.D., Astec, USA) and teicoplanin (CHIROBIOTIC T) CSP (250 mm × 4.6 mm I.D., Astec, USA) were used for the separation of enantiomers of the alkoxysubstituted esters of phenylcarbamic acid. The analytes were dissolved in methanol (concentration 1 mg/mL). The analytes studied possessed a UV absorption maximum at a wavelength of 240 nm that was used for detection. Mobile phases were prepared by mixing methanol 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, (a) first eluted enantiomer and (b) second eluted enantiomer, respectively.

RESULTS AND DISCUSSION

It has been found, that vancomycin and teicoplanin CSPs are able to resolve many compounds by HPLC.[1–6,16–21] In this work, the enantiomers of 1-methyl-2-piperidinoethylesters of 3- and 4-alkoxyphenylcarbamic acid

with different alkoxysubstituents (see Table 1) were separated on these columns isothermally, at different temperatures, using methanol containing 17.5 mmol/L acetic acid and 4.8 mmol/L diethylamine as mobile phase additives.

The retentions (k_1, k_2) and resolutions factors ($R_{2,1}$) determined for these enantiomers on vancomycin and teicoplanin CSPs are listed in Tables 2 and 3. The comparison of retention factors k_1, k_2 in these tables, show that all of the recorded values decrease with increasing temperature. Also, the peak symmetry deteriorates with decreasing column temperature. It is evident, that the retention values on the vancomycin CSP were lower in comparison with the teicoplanin CSP (Tables 2 and 3). This fact is associated with the structure of these CSPs, teicoplanin macromolecules consist of a nonyl carbon chain and they probably caused another kind of interaction (e.g., non-polar interaction) between teicoplanin CSP and the studied enantiomers. Consequently, the enantiomers have longer retentions on teicoplanin CSP. This decrease in k_i with a methanol mobile phase is the characteristic behaviour when solvophobic interactions between the analytes and the CSP are possible.

The resolution factors (R_{21}) of the enantiomers decrease by increasing temperature, in all cases. The data given in Tables 2 and 3 show that the magnitude of the decrease of R_{21} is independent on the position of the alkoxy group on the phenyl ring (e.g., in the 3-position R_{21} varies from 1.9 to <0.4, in the 4-position R_{21} varies from 2.2 to 0.9 on vancomycin CSP; and in the 3-position R_{21} varies from 2.7 to 0.6, in the 4-position R_{21} varies from 3.3 to 0.6 on teicoplanin CSP). It is important to note that there was no separation of the enantiomers with alkoxysubstitution in the 2-position. Considering the structure of these compounds, it was clear that the reason for this is the proximity of the 2-alkoxysubstituent to the stereogenic center.

Thermodynamic Parameters in the Polar Organic Mode

In order to calculate thermodynamic parameters and acquire information of value for an understanding of enantiomeric retention and the separation mechanism of this CSPs, van't Hoff plots were constructed using Eq. (2).

Effect of Temperature on Vancomycin CSP

The ΔH_i values calculated from the slopes of the plots of Eq. (2) were negative for all enantiomers. The ΔH_i values are in the range of -8153 J/mol to -10650 J/mol for the 3-alkoxysubstituted derivates of phenylcarbamic acid, and varied from -8422 J/mol to -10654 J/mol for the 4-alkoxy derivates. From Fig. 2, it is evident that the number of carbon atoms in

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

	Temperature (K)																	
	273			283			293			303			313			323		
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}
(A) 3-Alkoxy derivates[a]																		
1_3	2.55	2.85	1.2	2.14	2.29	0.8	1.89	1.98	0.7	1.73	1.73	0	1.50	1.50	0	1.39	1.39	0
2_3	2.22	2.51	1.4	1.90	2.08	1.0	1.70	1.82	0.7	1.51	1.58	0.6	1.37	1.37	0	1.27	1.27	0
4_3	1.97	2.30	1.7	1.66	1.85	1.2	1.47	1.61	1.0	1.32	1.42	0.7	1.18	1.23	<0.4	1.10	1.10	0
5_3	1.86	2.19	1.8	1.57	1.77	1.3	1.4	1.54	1.0	1.26	1.35	0.7	1.14	1.18	<0.4	0.95	1.05	<0.4
6_3	1.76	2.10	1.9	1.49	1.69	1.3	1.33	1.48	1.1	1.20	1.30	0.7	1.09	1.14	<0.4	0.89	1.00	<0.4
7_3	1.67	2.01	1.9	1.42	1.64	1.4	1.28	1.43	1.1	1.15	1.26	0.8	1.05	1.11	<0.4	0.85	0.94	<0.4
(B) 4-Alkoxy derivates[b]																		
1_4	2.62	3.01	1.6	2.18	2.41	1.1	1.95	2.1	0.8	1.74	1.84	0.6	1.58	1.58	0	1.44	1.44	0
2_4	2.38	2.73	1.5	1.98	2.18	1.0	1.77	1.91	0.8	1.58	1.66	0.7	1.44	1.44	0	1.32	1.32	0
4_4	2.07	2.51	2.2	1.71	1.98	1.5	1.53	1.73	1.2	1.38	1.51	0.9	1.22	1.31	0.6	1.14	1.20	<0.4
5_4	1.95	2.36	2.2	1.62	1.89	1.5	1.46	1.65	1.3	1.31	1.45	1.0	1.16	1.25	0.6	1.08	1.14	<0.4
6_4	1.84	2.26	2.2	1.55	1.82	1.6	1.4	1.59	1.3	1.25	1.39	1.0	1.12	1.21	0.6	1.01	1.09	<0.4
7_4	1.65	2.19	2.3	1.49	1.74	1.6	1.34	1.53	1.3	1.21	1.34	1.0	1.07	1.17	0.5	0.90	1.05	<0.4

[a]For $n = 3$: k_1 ± 0.12, k_2 ± 0.17, R_{21} ± 0.1.
[b]For $n = 3$: k_1 ± 0.14, k_2 ± 0.20, R_{21} ± 0.1.

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

	Temperature (K)																	
	273			283			293			303			313			323		
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}
(A) 3-Alkoxy derivates[a]																		
1_3	6.60	7.60	2.3	5.91	6.62	2.0	5.27	5.76	1.7	4.89	5.24	1.4	4.31	4.54	1.1	3.76	3.93	0.6
2_3	5.88	6.67	2.1	5.22	5.77	1.7	4.70	5.08	1.5	4.32	4.59	1.2	3.85	4.04	0.9	3.36	3.51	0.6
4_3	5.04	5.90	2.6	4.61	5.18	2.2	4.06	4.45	1.8	3.74	4.02	1.4	3.34	3.55	1.1	2.91	3.08	0.6
5_3	4.78	5.53	2.4	4.38	4.92	2.1	3.86	4.24	1.8	3.51	3.78	1.4	3.16	3.37	1.0	2.78	3.01	0.6
6_3	4.36	5.00	2.3	4.19	4.71	2.1	3.67	4.04	1.7	3.40	3.66	1.4	3.03	3.23	0.9	2.71	2.89	0.5
7_3	4.15	4.67	2.7	4.01	4.51	2.1	3.53	3.88	1.8	3.27	3.54	1.3	2.91	3.10	0.9	2.56	2.76	0.5
(B) 4-Alkoxy derivates[b]																		
1_4	7.50	9.31	3.3	6.66	7.95	2.9	5.98	6.89	2.6	5.49	6.17	2.2	4.87	5.26	1.5	4.25	4.55	1.3
2_4	6.84	8.22	2.8	6.06	7.03	2.4	5.44	6.12	2.1	4.99	5.48	1.8	4.44	4.78	1.5	3.87	4.09	1.0
4_4	5.97	7.05	2.5	5.44	6.21	2.2	4.77	5.30	1.9	4.39	4.76	1.5	3.91	4.18	1.2	3.42	3.60	0.8
5_4	5.56	6.37	2.2	5.20	5.91	2.2	4.55	5.04	1.8	4.19	4.54	1.5	3.71	3.95	1.1	3.27	3.44	0.7
6_4	5.30	5.90	2.5	4.99	5.68	2.2	4.37	4.84	1.8	4.04	4.37	1.4	3.57	3.79	1.1	3.17	3.34	0.7
7_4	5.01	5.63	2.5	4.81	5.47	2.2	4.22	4.67	1.8	3.89	4.21	1.4	3.44	3.65	1.1	3.05	3.22	0.6

[a]For $n = 3$: k_1 ± 0.12, k_2 ± 0.17, R_{21} ± 0.1.
[b]For $n = 3$: k_1 ± 0.13, k_2 ± 0.15, R_{21} ± 0.1.

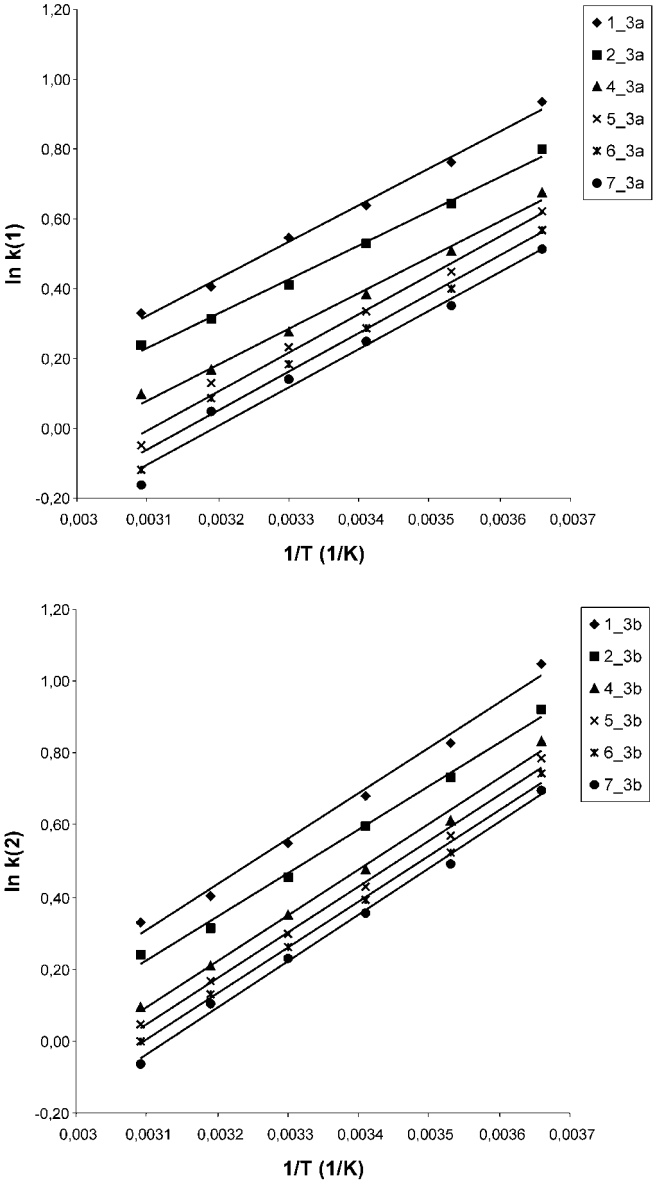

Figure 2. Dependence of natural logarithms of retention factors ($\ln k_i$) on the inverse of temperature ($1/T$) for 3-alkoxysubstituted esters of alkoxyphenylcarbamic acid using vancomycin CSP. (See Experimental for details.) Similar dependencies were obtained for the 4-alkoxysubstituted analogues.

alkoxychain have influenced change in the entropy values $\Delta(\Delta S_{2,1})$ but not on the entalpy values $\Delta(\Delta H_{2,1})$ (within statistical probability at a level of $\alpha = 0.05$), using a vancomycin CSP. The separation of enantiomers with alkoxysubstitution in the 3- and 4-positions show no significant differences in their thermodynamic parameters. The correlation coefficients (Fig. 2) of the van't Hoff plots (Eq. (2) for the enantiomers of all compounds in this study using the vancomycin CSP, indicated good linearity ($r > 0.995$). Thus, it can be concluded, that the steric effects probably play a significant role on chiral separation of alkoxysubstituted esters of phenylcarbamic acid enantiomers, using the vancomycin CSP.

Effect of Temperature on Teicoplanin CSP

From Fig. 3, it is evident that the entropy and enthalpy values obtained from the van't Hoff plots, done using data from the teicoplanin column, are lower in comparison with the values obtained on the vancomycin CSP. The number of carbon atoms in the alkoxychain of the phenylcarbamic acid has a significant influence on the change of the enthalpy values $\Delta(\Delta H_{2,1})$, and less influence on the change in entropy $\Delta(\Delta S_{2,1})$ using the teicoplanin CSP (level of probability $\alpha = 0.05$). As was found in the case of the vancomycin CSP, the separation of enantiomers with alkoxysubstituents in the 3- and 4-position have no significant influence on the values of thermodynamic parameters on the teicoplanin CSP. The correlation coefficients (Fig. 3) of the van't Hoff plots (Eq. (2) for the all enantiomers using the teicoplanin CSP indicated good linearity ($r > 0.996$). The repulsion effect between the protonated amine of the analyte molecules and the ammonium group of the teicoplanin macromolecules is probably important.[22,23] It can be concluded, that the energetic changes probably have greater influence on the separation of enantiomers. Besides the energetic changes, the influence of steric hindrances could be probably taken into consideration.

From Eq. (1), it follows that the isoenantioselective temperature (T_{iso}) can be defined as the ratio: $T_{iso} = \Delta(\Delta H_{2,1})/\Delta(\Delta S_{2,1})$. At a certain temperature ($\Delta(\Delta_{2,1}) = 0$) 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.[8] The isoenantioselective temperatures were (355 ± 30 K) on vancomycin and (365 ± 25 K) on teicoplanin CSPs. The isoenantioselective temperatures were higher than the working temperature range (273–323 K), and indicated that the separation mechanisms of 1-methyl-2-piperidinoethylesters of 3- and 4-alkoxyphenylcarbamic acid using vancomycin and teicoplanin CSPs are unchanged in the range of temperatures studied.

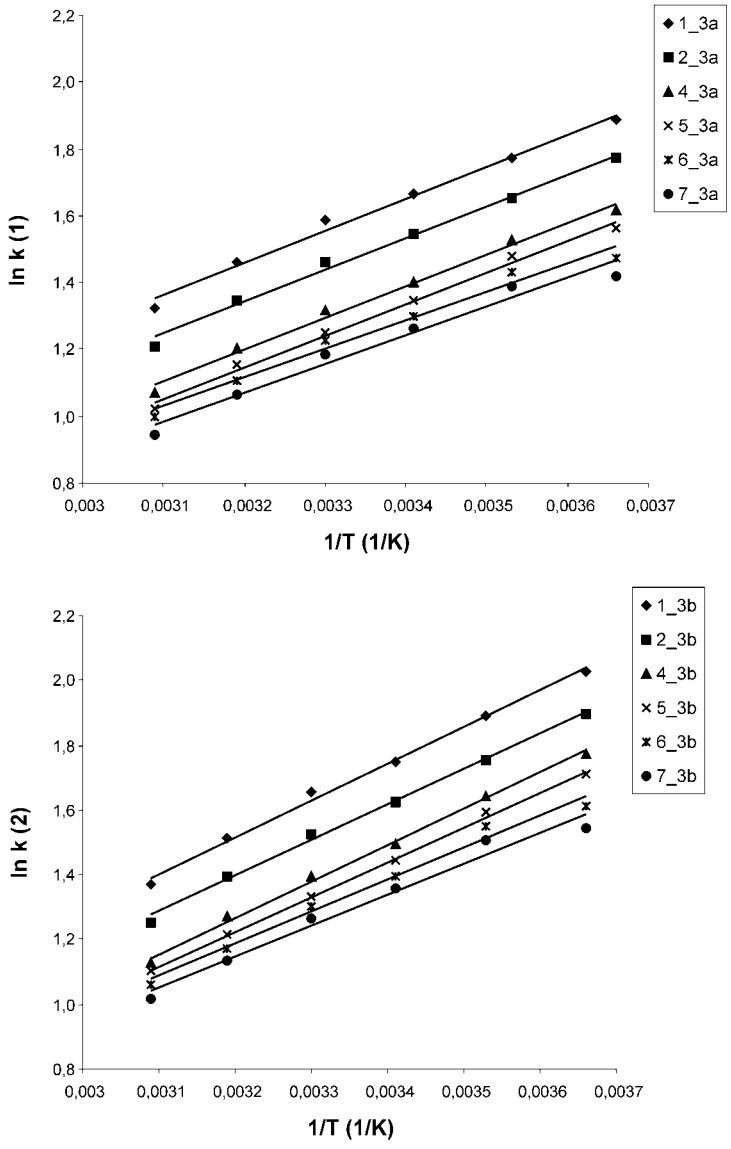

Figure 3. Dependence of natural logarithms of retention factors ($\ln k_i$) on the inverse of temperature ($1/T$) for 3-alkoxysubstituted esters of alkoxyphenylcarbamic acid using teicoplanin CSP. (See Experimental for details.) Similar dependencies were obtained for the 4-alkoxysubstituted analogues.

CONCLUSIONS

The effect of temperature on the retention of 1-methyl-2-piperidinethyl-esters of 3- and 4-alkoxyphenylcarbamic acid was studied. In the temperature range studied (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 vancomycin and teicoplanin CSPs were determined. From these results, it appears that the number of carbon atoms in the alkoxychain plays a significant role in the enantiomeric separation of the compounds studied, and has major influence on the thermodynamics. The change of enthalpy values $\Delta(\Delta H_{2,1})$ is most important when using teicoplanin, and the change of entropy values $\Delta(\Delta S_{2,1})$ is more significant on vancomycin CSPs. However, when the enantiomers with alkoxychains is in the 3-position, as well as in the 4-position, there seems to be little difference in the thermodynamic parameters using either the vancomycin or the teicoplanin CSP.

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