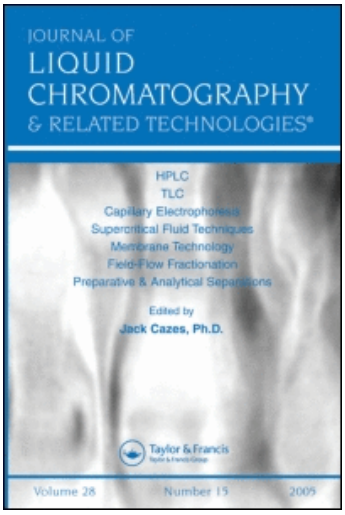

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MODIFICATION OF THE CHIRAL BONDING PROPERTIES OF TEICOPLANIN CHIRAL STATIONARY PHASE BY ORGANIC ADDITIVES. HPLC SEPARATION OF ENANTIOMERS OF ALKOXYSUBSTITUTED ESTERS OF PHENYLCARBAMIC ACID

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MODIFICATION OF THE CHIRAL BONDING PROPERTIES OF TEICOPLANIN CHIRAL STATIONARY PHASE BY ORGANIC ADDITIVES. HPLC SEPARATION OF ENANTIOMERS OF ALKOXYSUBSTITUTED ESTERS OF PHENYLCARBAMIC ACID

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ABSTRACT

The behaviour of teicoplanin-based chiral stationary phase (CHIROBIOTIC T) towards changes in organic and ionic modifiers in mobile phase was investigated in order to deduce suitable conditions for the liquid chromatographic enantioseparations of a series of alkoxysubstituted esters of phenylcarbamic acid. Methanol and acetonitrile were the non-ionic modifiers tested in the mobile phase, while different aliphatic carboxylic acids (formic acid, acetic acid,

propionic acid, hexanoic acid) and bases (triethylamine, trimethylamine, diethylamine) were used as ionic modifiers.

The influence of the nature and concentration of the modifiers on retention, selectivity, and resolution of enantiomers was investigated. Under these conditions, enantiomeric separations could be obtained for 3- and 4-alkoxysubstituted derivatives. The elution order of enantiomers was also determined.

INTRODUCTION

Macrocyclic antibiotics form one of the newest and perhaps most varied classes of chiral selectors. They contain a variety of functional groups which are ideal for providing multiple stereoselective interactions (such as hydrogen-bonding, hydrophobic, π-π, and dipolar associations with the amide linkages). They have been used successfully for the separation of enantiomers of biological and pharmacological importance by HPLC[1,2] and CE[3,4].

Alkoxysubstituted esters of phenylcarbamic acid are potential local anaesthetic drugs. The enantiomeric separation of derivatives of phenylcarbamic acid can be performed by means of different chromatographic techniques, TLC[5,6], HPLC[7-13]. In HPLC, numerous chiral stationary phases were used for the separation of enantiomers of phenylcarbamic acid derivatives. Some chiral stationary phases consisted of immobilised proteins, such as α_1-acid glycoprotein.[10,11]

Others were based on the use of cyclodextrins[12,13] or cellulose derivatives, especially cellulose tris-3,5-triphenylcarbamate[7], as a chiral selector. Some π-association type columns were also reported to be suitable for achieving chiral separation of alkoxysubstituted esters of phenylcarbamic acid.[8,9]

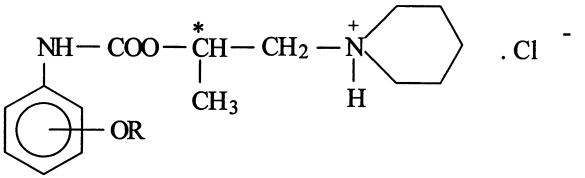
This paper deals with the HPLC enantioseparation of series of alkoxysubstituted derivatives of phenylcarbamic acid by using a teicoplanin-based chiral stationary phase. The influence of different parameters on enantioselectivity has been investigated. The effects of the mobile phase, the ratio of methanol/acetonitrile and addition of some ionic organic modifiers (aliphatic carboxylic acids and bases), have been studied in order to deduce the chiral separation mechanism.

EXPERIMENTAL

Materials

The analytes resolved in this study (1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid) were prepared according to Pokorna and col.[14] (Table 1). All HPLC grade solvents (methanol, acetonitrile) were obtained

Table 1. Chemical Structures of the Alkoxy-substituted Derivatives of Phenylcarbamic Acid That Were Examined



2-position		3-position		4-position	
Analyte Nr.	R	Analyte Nr.	R	Analyte Nr.	R
0	–				
1	$-CH_3$	2	$-CH_3$	3	$-CH_3$
4	$-C_2H_5$	5	$-C_2H_5$	6	$-C_2H_5$
7	$-C_3H_7$	8	$-C_3H_7$	9	$-C_3H_7$
10	$-C_4H_9$	11	$-C_4H_9$	12	$-C_4H_9$
13	$-C_5H_{11}$	14	$-C_5H_{11}$	15	$-C_5H_{11}$
16	$-C_6H_{13}$	17	$-C_6H_{13}$	18	$-C_6H_{13}$
19	$-C_7H_{15}$	19	$-C_7H_{15}$	20	$-C_7H_{15}$
22	$-C_8H_{17}$	23	$-C_8H_{17}$	23	$-C_8H_{17}$
25	$-C_9H_{19}$	26	$-C_9H_{19}$	27	$-C_9H_{19}$
28	$-C_{10}H_{21}$	29	$-C_{10}H_{21}$	30	$-C_{10}H_{21}$

from Merck (Germany). Triethylamine, diethylamine, trimethylamine, formic acid, acetic acid, propionic acid, hexanoic acid were obtained from Lachema (Czech Republic).

Instruments

A Chirobiotic T (250 x 4,6 mm I.D.) (Astec, USA) column was used for the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid. Separations were achieved using a Waters liquid chromatograph with photodiode array detector (Waters 990) and a NEC PowerMate 2 chromatographic data station. Separations were carried out at a flow rate of 0.8 mL/min at room temperature. The analytes were dissolved in methanol (concentration 1 mg/mL), and filtered with a 0.45 μm filter when necessary. Mobile phases were prepared by mixing methanol, acetonitrile, organic acid, and base. Compositions are listed in the appropriate tables and figures.

For the measurement of optical rotation, the polarimeter Polar LμP (Na lamp, λ=589 nm) (IBZ Messtechnik) was used. After the separation, the fractions of enantiomers were collected to measure their optical properties. The fractions of enantiomers were evaporated under a stream of nitrogen.

RESULTS AND DISCUSSION

The influence of the nature and concentration of ionic modifiers such as aliphatic carboxylic acids and bases on enantioseparation were investigated using derivatives of phenylcarbamic acid with different alkoxysubstitution in the 2-, 3-, and 4- positions (Table 1).

If the studied compounds have a sufficient number of functional groups capable of interacting with the stationary phase, then the best mobile phase choice often consists of polar organic solvents. This mobile phase system consists of methanol or acetonitrile or mixtures of methanol and acetonitrile, while acid and base are added in small quantities.[1]

No enantiomeric separation, and very high retentions, were observed for the studied analytes when the mobile phase consisted of methanol (retention factors were about 20 and more). The much smaller retention (retention factors were between 1 - 2) and, also, any enentiomeric separation was obtained when mobile phase consisted of methanol with addition of 17,5 mM acetic acid. This phenomena indicates strong cationic repulsive interaction between stationary phase and analyte ($–NH_3^+$, and $–NH_2^+$– groups in the macromolecules of teicoplanin and $–NH_2^+$– group in the molecules of the separated analytes) and does not support chiral separations. On the other hand, the presence of a small amount of base in this mobile phase has an influence on the retention and the enantioselectivity of separation.

The influence of base concentration (triethylamine) on the retention factor values of derivatives with alkoxysubstitution (C_1- C_5) in p-position is demonstrated in Figure 1 (the dependencies have similar tendency for other derivatives). It is evident, that an increase of the concentration of triethylamine in mobile phase (in the range 3.55 - 10.65 mM) caused lower retention of enantiomers, while the influence of base concentration on the enantiomer resolution was not so significant (Table 2). Decreasing the organic acid concentration in mobile phase (increase of concentration of triethylamine) produces a significant decrease in retention for analyte. With smaller amounts of organic acid in the mobile phase (relative to the base) the ionisation of analytes is suppressed and ion interaction of stationary phase with functional groups of derivatives of phenylcarbamic acid are also suppressed (lower resolution values).

Thus, it appears that a strong association between the carboxylic acid moiety on teicoplanin and the ammonium groups of these analytes is detrimental to chiral recognition.

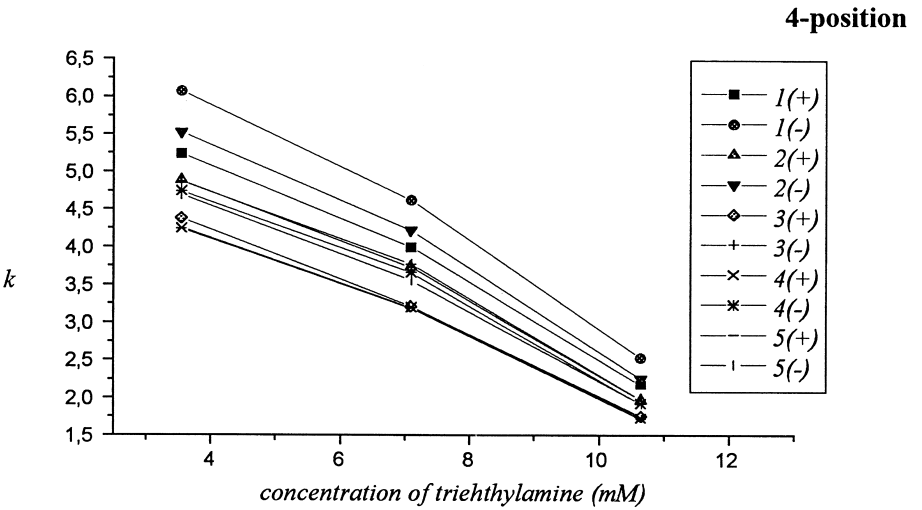


Figure 1. Influence of the concentration of triethylamine on retention factors of analytes with different number of carbon atoms in -OR (Cx). Chromatographic conditions: methanol containing 17.1 mM acetic acid and base (3.55 mM, 7.1 mM, 10.65 mM).

The influence of the nature of the base-additive (triethylamine, trimethylamine, diethylamine) on enantioseparation for derivatives with alkoxysubstitution (C_1 - C_5) in p-position is shown in Figure 2 (the dependencies for other derivatives are similar) with all of the bases being added at the same concentration (7.1 mM). The concentration of acetic acid in mobile phase was 17.5 mM. For these three ionic modifications, the retention factor values of phenylcarbamic acid derivatives have a tendency to decrease as the strength of bases increase [pKa (triethylamine) = 11.01; pKa (trimethylamine) = 9.81; pKa (diethylamine) = 10.49]. The resolution values obtained with diethylamine in mobile phase are higher for more compounds than in the case of triethylamine or trimethylamine (Table 3).

The influence of the carbon chain length of the aliphatic carboxylic acid on enantioseparation for derivatives with alkoxysubstitution (C_1 - C_5) in p-position is demonstrated in Figure 3 (the dependencies have similar tendency for other derivatives). The mobile phase consists of methanol, 17.5 mM organic acid, and 10 mM triethylamine. When acetic acid, propionic acid, and hexanoic acid were added to the mobile phase, the retention factors slowly decreased as the length of aliphatic chain increased The addition of formic acid also leads to a decrease of retention factor values of enantiomers.

It is interesting to note, however, that the highest resolution values were obtained by using acetic acid as the mobile phase modifier. The effect of concentration of acids on the value of retention factors is opposite to those observed for

Table 2. Influence of Concentration of Triethylamine on Resolution Values

Analyte Nr.	Number of C Atoms in -OR	Concentration of Triethylamine in Mobile Phase [mM]		
		3.55	7.10	10.65
0		1.4	1.5	1.4
2	1	1.1	1.2	1.0
5	2	0.9	1.0	0.7
8	3	1.0	1.0	0.8
11	4	1.1	1.2	0.7
14	5	0.9	1.0	0.6
17	6	0.9	0.9	0.7
19	7	0.8	0.7	0.6
23	8	0.8	0.8	0.6
26	9	0.6	0.6	0.6
29	10	0.6	0.6	0.5
3	1	2.1	2.3	1.5
6	2	1.5	1.8	1.2
9	3	1.2	1.3	0.9
12	4	1.5	1.7	0.9
15	5	1.4	1.5	0.8
18	6	1.2	1.3	0.8
20	7	1.3	1.3	0.8
23	8	1.4	1.3	0.7
27	9	1.2	1.2	0.9
30	10	1.3	1.5	1.0

Chromatographic conditions: methanol containing 17.5 mM acetic acid and (3.55 mM, 7.1 mM, 10.65 mM) triethylamine.

bases. When the acids at concentration 10 mM were added to the mobile phase, a decrease of the retention factor was observed, but there was no significant influence on the resolution values.

These observations indicate, that the addition of stronger acids to mobile phase produces the possibility of ion pair formation with the analytes, and the charge interactions with carboxylic or phenolic group in stationary phase are suppressed. The influence of the length of the aliphatic chain of carboxylic acid (steric interaction) has no significance. In the next set of experiments, 17.5 mM acetic acid was used as an ionic modifier. The separation of enantiomers of analyte 3 is shown in Figure 4.

The changing of organic modifier to acetonitrile had a negative effect on the enantioseparation. Similarly, as in the case of mobile phase consisting of

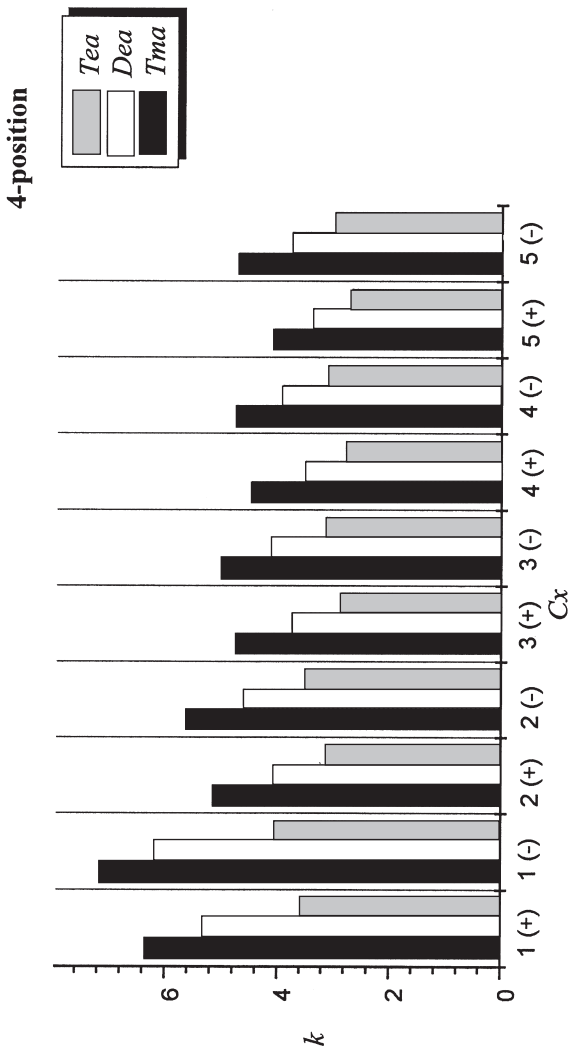


Figure 2. Influence of nature of bases on retention factors of analytes with different number of carbon atoms in -OR (Cx). Chromatographic conditions: methanol containing 17.5 mM acetic acid and 7.1 mM base. (TEA = triethylamine, TMA = trimethylamine, DEA = dimethylamine.)

Table 3. Influence of Nature of Bases on Resolution Values

Number of C Atoms in -OR	Position of OR	Diethylamine	Triethylamine	Trimethylamine
1	3	1.2	1.3	0.7
	4	1.8	1.7	1.5
2	3	1.0	0.9	0.7
	4	1.5	1.5	1.0
3	3	1.1	1.2	0.7
	4	1.2	1.3	0.9
4	3	1.2	1.0	1.0
	4	1.4	1.3	1.0
5	3	1.2	1.0	0.9
	4	1.3	1.4	1.0
6	3	1.2	0.9	0.9
	4	1.3	1.2	1.0
7	3	1.0	0.9	0.8
	4	1.3	1.2	1.1
8	3	1.2	0.8	1.0
	4	1.3	1.3	1.2
9	3	1.2	0.8	1.1
	4	1.2	1.1	1.2
10	3	1.1	0.7	1.0
	4	1.4	1.2	1.2

Chromatographic conditions: methanol containing 17.5 mM acetic acid and 7.1 mM base (diethylamine, triethylamine, trimethylamine).

methanol, high retention and no enantiomeric separation was achieved for the studied analytes when mobile phase consisted of acetonitrile or acetonitrile with addition of 17.5 mM acetic acid. The presence of a small amount of base (7.1 mM diethylamine) in mobile phase consisting of acetonitrile containing 17.5 mM acetic acid has an influence on the retention of analytes (the smaller retention factor values was obtain), and a very small effect on the efficiency of separation (poor enantioseparation was obtained). The addition of a large content of acetonitrile into mobile phase (methanol/acetonitrile containing 17.5 mM acetic acid and 7.1 mM diethylamine) had a negative effect on the resolution values (Table 4).

The influence of the concentration of acetonitrile in mobile phase on separation of enantiomers (retention factor value) for derivatives with alkoxysubstitution (C_1 - C_5) in p-position is demonstrated in Figure 5 (the dependencies have similar tendency for other derivatives). It should be noted, that the increasing of the acetonitrile content in the range 0-75% caused the decreasing of retention

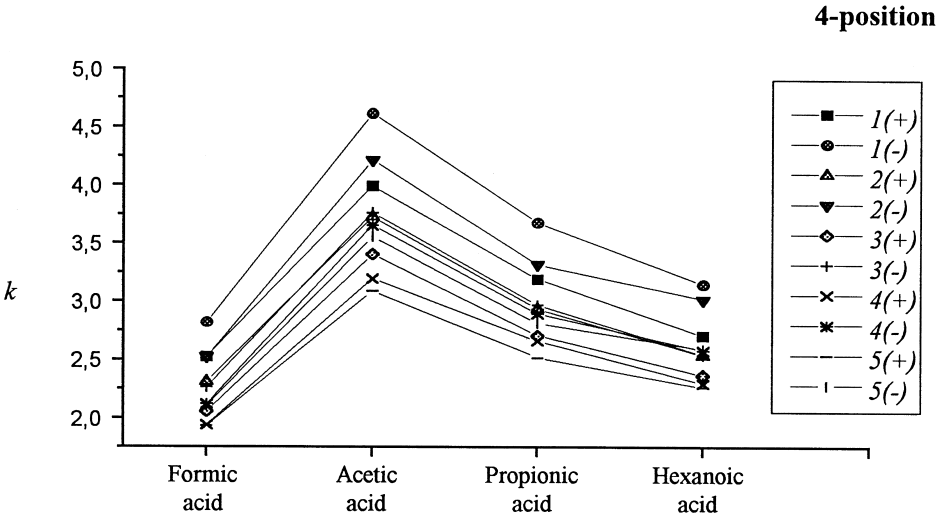


Figure 3. Influence of the nature of the aliphatic carboxylic acid on the retention factors of analytes with different number of carbon atoms in -OR. Chromatographic conditions: methanol containing 17.5 mM carboxylic acid and 7.1 mM triethylamine.

factor of analytes and resolution of enantioseparation. It can be supposed, that the hydrogen bonding interactions (the formation support in methanolic mobile phase) are favorable for the enantioseparation on teicoplanin-based chiral stationary phase. The highest values of resolution for more analytes were obtained by using mobile phase methanol/acetonitrile 75/25 (v/v) containing 17.5 mM acetic acid and 7.1 mM diethylamine. The separation of enantiomers of derivatives of phenylcarbamic acid nr. 3 is shown in Figure 6.

It seems that the asymmetric carbon atom environment has substantial influence on the efficiency of enantioseparation. The best separation of enantiomers (the highest values of R_s) was obtained for compounds without alkoxy-substitution (compound 0) or for compounds with alkoxysubstitution in 4-position. The poorest efficiency of enantioseparation was obtained for derivatives with alkoxysubstitution in 3-position, and no enantiomeric separations for analytes with alkoxysubstitution in the 2- position was observed. This is probably the effect of the shade of an asymmetric carbon atom by alkoxy-substituent and the effect of formation of intramolecular complexes. In all tested mobile phases, the number of carbon atoms in alkoxy- substituent had no significant effect on the retention factor values (1.5 - 4.0).

It can be observed that the retention factor values very slowly decrease as the number of carbon atoms in the -OR group increases, in the range C_1 - C_{10}. The poorest efficiency of enantioseparation (smaller R_s values) was obtained for

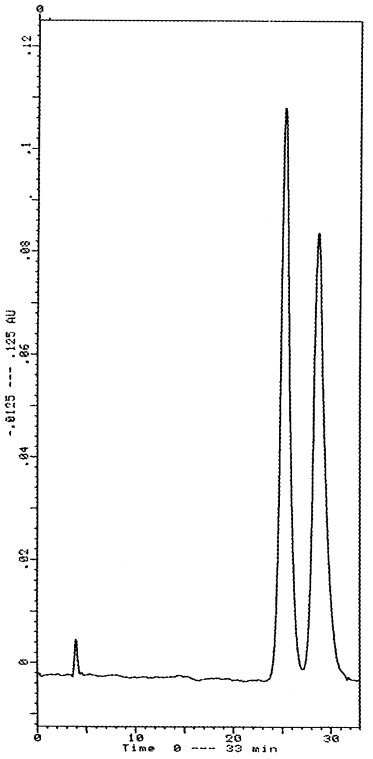


Figure 4. Chromatograms of enantioseparation of alkoxysubstituted ester of phenylcarbamic acid (compound nr. 3). Chromatographic conditions: methanol containing 17.5 mM acetic acid and 7.1 mM diethylamine.

analytes with longer alkoxysubstituents (C_5 - C_{10}). It should be noted, that good separation of 2-alkoxysubstituent enantiomers was achieved by using β-cyclodextrin and γ-cyclodextrin columns in reverse-phase mode.[13]

The influence of ionic strength on the enantiomer separation was also studied. According to the data in Table 5, it is obvious that the ionic strength has no significant influence on the resolution of enantiomers of alkoxysubstituted esters of phenylcarbamic acid.

The elution order of enantiomers was determined by measuring the optical rotation of each peak after HPLC separation for compounds 0, 2, and 3. The first eluted enantiomer for all tested analytes, rotates the plane of polarised light (wavelength 589 nm) to the right (+), and the second eluted enantiomer shows the opposite rotation. In the case of β-cyclodextrin chiral stationary phase, the elution order was the same (for compounds 0, 2, and 3).[13]

Table 4. Influence of the Concentration of Acetonitrile in Mobile Phase on Resolution Values

Number of C Atoms in -OR	Position of -OR	Ratio of Organic Modifiers in Mobile Phase Methanol/Acetonitrile (v/v)				
		100/0	75/25	50/50	25/75	0/100
0		1.3	1.7	0.9	1.0	< 0.4
1	3	1.2	1.1	0.7	0.6	0.5
	4	1.8	2.4	1.3	1.3	0.6
2	3	1.0	0.9	0.6	0.6	0.5
	4	1.5	2.1	1.0	1.2	0.6
3	3	1.1	0.9	0.5	0.5	< 0.4
	4	1.2	1.7	0.8	1.1	0.5
4	3	1.2	1.0	0.5	1.1	< 0.4
	4	1.4	1.8	0.9	1.2	< 0.4
5	3	1.2	1.0	0.5	0.7	< 0.4
	4	1.3	1.7	0.8	1.2	< 0.4
6	3	1.2	1.1	< 0.4	0.9	< 0.4
	4	1.3	1.6	0.8	1.1	< 0.4
7	3	1.0	0.9	< 0.4	0.6	< 0.4
	4	1.3	1.2	nm	1.0	< 0.4
8	3	1.2	0.9	< 0.4	0.7	< 0.4
	4	1.3	1.6	nm	1.0	< 0.4
9	3	1.2	0.9	< 0.4	0.8	< 0.4
	4	1.2	1.5	nm	1.1	< 0.4
10	3	1.1	0.7	< 0.4	0.6	< 0.4
	4	1.4	1.5	nm	1.0	< 0.4

Chromatographic conditions: methanol/acetonitrile containing 17.5 mM acetic acid and 7.1 mM diethylamine.

The different interaction of two enantiomeric forms with the stationary phase leading to chiral discrimination can be expressed as the difference in the free energy $-\Delta_{1,2}\Delta G°$ calculated from the separation factor α according to the equations:

$$-\Delta_{1,2}G° = \Delta_2 G° - \Delta_1 G°$$

$$-c_{1,2}\Delta G° = RT \ln k_2 / k_1 = RT \ln \alpha$$

The results given in Table 6, show the very small energy differences which is needed for chromatographic resolution of enantiomers of these alkoxysubstituted esters of phenylcarbamic acid. It is, therefore obvious, that binding of two

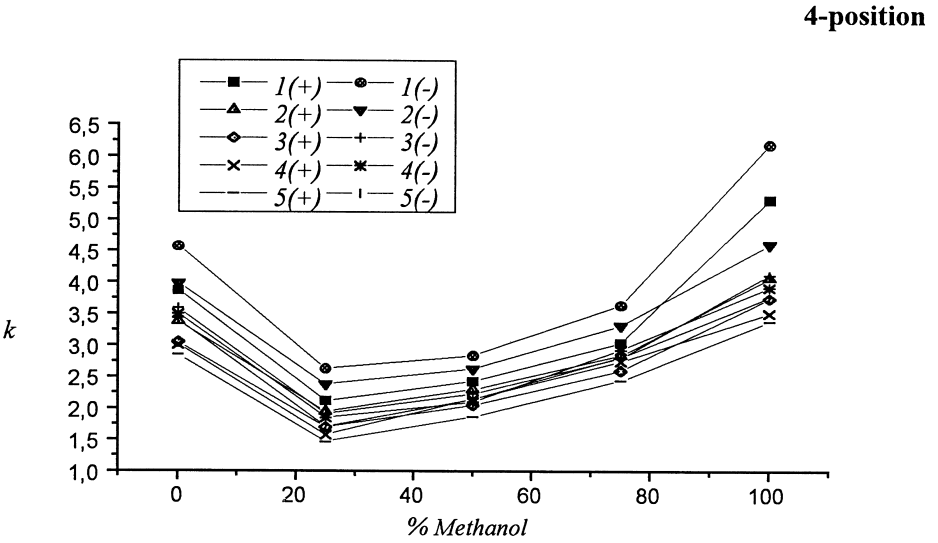


Figure 5. Influence of the concentration of acetonitrile on retention factors of analytes with different number of carbon atoms in -OR. Chromatographic conditions: methanol/acetonitrile containing 17.5 mM acetic acid and 7.1 mM diethylamine.

enantiomers to a given chiral site may involve different amounts of energy simply because one of the enantiomers, for steric reason, might be forced to adopt an energetically less favorable conformation.

In the mobile phase, methanol/acetonitrile 75/25 (v/v) containing 17.5 mM acetic acid and 7.1 mM diethylamine (where the best separation efficiency was obtain) the number of carbon atoms in alkoxy chain length (C_1 - C_{10}) had no significant effect on the differences in free energy (Table 6). The position of alkoxy-substitution has an effect on free energy of enantiomeric separation for the alkoxy-substituted esters of phenylcarbamic acid. The highest values of $\Delta_{1,2}\Delta G°$ (> 300 J/mole) was obtained for derivatives with 4-alkoxysubstitution, which also had the highest separation efficiencies.

CONCLUSION

The teicoplanin-based chiral stationary phase has the capability to separate enantiomers of alkoxysubstituted esters of phenylcarbamic acid. The separations can be accomplished in the polar-organic mode. According to the results of enantiomeric separations in different mobile phases using a teicoplanin column, it can be supposed that the interactions needed for chiral resolution of enantiomers involve:

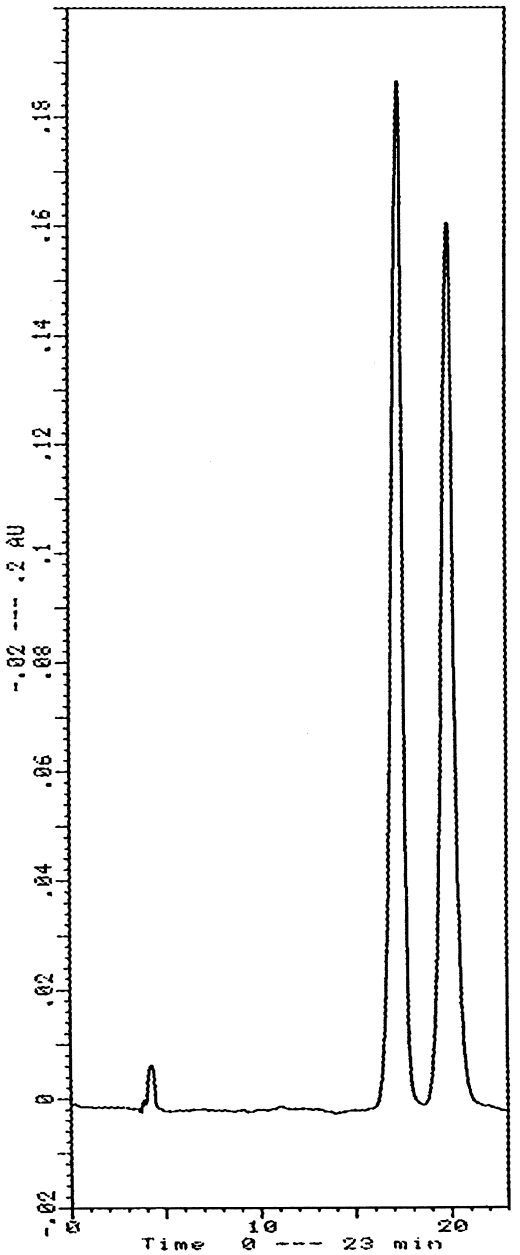


Figure 6. Chromatograms of enantioseparation of alkoxysubstituted ester of phenylcarbamic acid (compound nr. 3). Chromatographic conditions: methanol/acetonitrile 75/25 v/v containing 17.5 mM acetic acid and 7.1 mM diethylamine.

Table 5. The Influence of Ion Strength on the Separation of Enantiomers of Alkoxysubstituted Esters of Phenylcarbamic Acid

Analyte Nr.	Formic Acid			Acetic Acid			Propionic Acid			Hexanoic Acid		
	k_1	k_2	R_s	k_1	k_2	R_s	k_1	k_2	R_s	k_1	k_2	R_s
0	2.10	2.44	1.4	3.32	3.75	1.7	3.29	3.73	1.6	3.34	3.79	1.7
2	2.16	2.41	1.2	3.29	3.61	1.3	3.30	3.61	1.3	3.31	3.62	1.3
5	2.53	2.77	1.0	3.03	3.38	1.2	2.97	3.21	1.1	2.99	3.23	1.2
8	2.40	2.62	0.9	2.77	3.06	1.3	2.61	2.87	1.2	2.80	2.94	1.4
11	2.21	2.44	1.2	2.63	2.90	1.3	2.50	2.75	1.3	2.60	2.87	1.2
14	2.08	2.30	1.2	2.54	2.82	1.2	2.39	2.64	1.3	2.47	2.73	1.3
17	2.01	2.21	1.2	2.45	2.72	1.2	2.31	2.56	1.2	2.38	2.64	1.2
20	1.91	2.11	1.1	2.40	2.66	1.3	2.24	2.49	1.2	2.30	2.55	1.2
23	1.85	2.05	1.2	2.26	2.52	1.2	2.13	2.37	1.3	2.24	2.48	1.2
26	1.79	1.99	1.2	2.20	2.45	1.2	2.07	2.31	1.2	2.15	2.38	1.2
29	1.65	1.84	1.2	2.05	2.29	1.0	1.92	2.14	1.1	1.96	2.19	1.1
3	2.55	3.07	1.8	3.80	4.47	2.2	3.74	4.43	2.0	3.69	4.38	2.0
6	2.91	3.21	1.7	3.43	3.92	1.9	3.45	3.97	1.8	3.41	3.93	1.8
9	2.39	2.67	1.2	2.93	3.29	1.5	2.91	3.27	1.5	3.19	3.25	1.4
12	2.02	3.30	1.3	3.08	3.48	1.8	3.07	3.46	1.7	3.09	3.49	1.5
15	1.92	2.19	1.3	2.90	3.26	1.7	2.93	3.31	1.6	2.93	3.31	1.4
18	1.80	2.06	1.2	2.89	3.25	1.6	2.85	3.19	1.6	2.92	3.27	1.4
21	1.78	2.03	1.2	2.79	3.12	1.6	2.74	3.08	1.5	2.78	3.17	1.5
24	1.71	1.95	1.3	2.67	3.01	1.6	2.63	2.97	1.5	2.66	3.01	1.7
27	1.64	1.84	1.1	2.57	2.87	1.5	2.55	2.83	1.4	2.52	2.84	1.3
30	1.62	1.85	1.2	2.56	2.84	1.6	2.48	2.79	1.4	2.51	2.81	1.4

Chromatographic conditions: methanol containing 7.1 mM triethylamine and aliphatic carboxylic acid to obtain constant value of pH (1.7 mM formic acid, 17.5 mM acetic acid, 22.5 mM propionic acid, 23.1 mM hexanoic acid).

Table 6. Free Energy Differences Necessary for Enantioseparation of Alkoxysubstituted Esters of Phenylcarbamic Acid

Analyte Nr.	$-\Delta_{1.2}\Delta G^{o}$ J/mole	Analyte Nr.	$-\Delta_{1.2}\Delta G^{o}$ J/mole
0	321		
2	234	3	447
5	211	6	385
8	256	9	343
11	248	12	348
14	256	15	339
17	259	18	343
19	251	20	351
23	267	23	332
26	278	27	300
29	256	30	321

Chromatographic conditions: methanol/acetonitrile 75/25 v/v containing 17.5 mM acetic acid and 7.1 mM diethylamine. T=295 K; R=8.314 J/K.mole.

charge - charge interaction; the strong cation - cation interaction in acid mobile phase between analyte and stationary phase caused repulsion and small retention and no enantiomeric separation was observed;
hydrogen bonding interaction; the formation is supported in mobile phase containing methanol as organic modifier or in mobile phase consisting of methanol/acetonitril as organic modifier where the content of acetonitrile is smaller (25% or less);
steric interaction; asymmetric carbon atom environment has substantial influence on efficiency of enantioseparation (alkoxysubstituted esters of phenylcarbamic acid 2-position are not separated). The length of alkoxy chain has no significant influence on chiral separation.

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