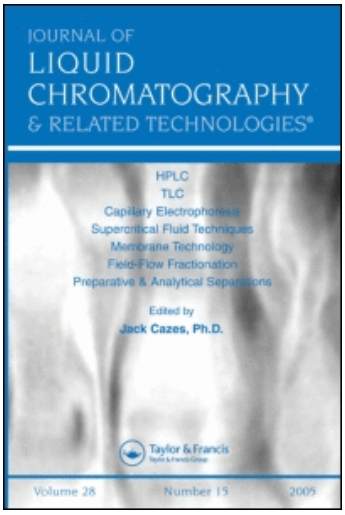

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STUDY OF MECHANISM OF ENANTIOSEPARATION. III. THE INFLUENCE OF CARBOHYDRATE MOIETIES OF TEICOPLANIN-BONDED CHIRAL STATIONARY PHASE ON THE SEPARATION OF SOME DERIVATES OF PHENYLCARBAMIC ACID

T. Rojkovicová[a]; J. Lehotay[a]; J. Dungelová[a]; J. Cizmárik[b]; D. W. Armstrong[c]
[a] Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic [b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic [c] Department of Chemistry, Iowa State University, Ames, IA, U.S.A.

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STUDY OF MECHANISM OF ENANTIOSEPARATION. III. THE INFLUENCE OF CARBOHYDRATE MOIETIES OF TEICOPLANIN-BONDED CHIRAL STATIONARY PHASE ON THE SEPARATION OF SOME DERIVATES OF PHENYLCARBAMIC ACID

T. Rojkovicová,[1] J. Lehotay,[1,*] J. Dungelová,[1] J. Cizmárik,[2] and D. W. Armstrong[3]

[1]Department of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovak Republic
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic
[3]Department of Chemistry, Gilman Hall, Iowa State University, Ames, IA 50011-3111, USA

ABSTRACT

Enantiomeric separation of alkoxysubstituted esters of phenylcarbamic acid (local anesthetic drugs) is carried out using high performance liquid chromatography (HPLC) with two chiral stationary phases. The teicoplanin chiral stationary phase—

*Corresponding author. E-mail: lehotay@cvt.stuba.sk

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chirobiotic T and the teicoplanin chiral stationary phase without the pendant carbohydrate moieties—chirobiotic teicoplanin aglycone (TAG) were used. The influence of the sugar units, the concentration of the ionic modifiers (diethylamine), the influence of the position and of number of carbon atoms in the analytic alkoxychain substituent, and the composition of the mobile phase; on retention factors and resolution of enantiomers were investigated. By comparison of chromatographic parameters obtained by using both chiral stationary phases, the influence of carbohydrate moieties on the mechanism of chiral separation could be evaluated. The interaction mechanism of the enantiomeric separations is discussed. Better separation, in terms of—greater values of R_{ij} of enantiomers—was achieved with the chirobiotic TAG column.

Key Words: Chirobiotic T; Chirobiotic TAG; Enantiomeric separation; HPLC; Chiral stationary phases; Alkoxysubstituted esters of phenylcarbamic acid; Local anesthetic drugs

INTRODUCTION

Chirality remains an important consideration for many compounds including pharmaceuticals, biological molecules, and agrochemicals to name a few.[1] In many cases, only one of the isomers is responsible for the desired activity, while the other isomer may exhibit no therapeutic value and may potentially cause unsuspected adverse effects.[1–3]

Alkoxysubstituted esters of phenylcarbamic acid form a group of potential drugs employed local anesthesias.[4,5] The enantiomeric separation of derivatives of phenylcarbamic acid can be performed by means of different chromatographic techniques, including TLC[6,7] and high performance liquid chromatography (HPLC).[8–14]

Macrocyclic glycopeptides, such as teicoplanin aglycone (TAG) and teicoplanin (i.e., teicoplanin without the carbohydrate moieties) represent a recent class of powerful chiral selectors. Their success can be attributed to the diversity of their structures that have multiple stereogenic centers and a variety of functional groups, which are known to provide multiple interactions necessary for enantioselectivity.[15,21] The role of teicoplanin's three moieties in chiral recognition can be examined by comparing the selectivity of the teicoplanin vs. its aglycone. It has been reported, that the pendant carbohydrate moieties can decrease the enantioselectivity for some compounds (such as amino acids) and enhance the enantioselectivity of other compounds.[18]

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From a chiral separations point of view, the sugar units of the native teicoplanin molecule may intervene in the chiral recognition process in at least three ways:

(a) steric hindrance, where the sugar units occupy room inside the "basket", which limits the access of other molecules to binding sites;
(b) blocking possible interaction sites on the aglycone, where two of the sugar moieties are linked through phenol hydroxyl groups and the third sugar moiety is linked through an alcohol group;
(c) offering competing interaction sites, since the carbohydrate moieties are themselves chiral and have hydroxyl, ether, and amido functional groups.[18]

Macrocyclic antibiotics show excellent enantioselectivity for a wide variety of compounds. Thus far, the glycopeptide macrocycles are the preferred chiral selectors of this class because of their broad application. Indeed, they offer a unique combination of structural features useful in the interaction with chiral analytes. They are able to associate through (i) ionic interactions; (ii) hydrogen bonding; (iii) π–π and (iv) dipole–dipole interactions; and (v) hydrophobic interactions via the cavity in the aglycone "basket" and for teicoplanin, the nonyl-tail chain.[19,20] Chiral stationary phases (CSPs) based on the macrocyclic antibiotics chiral selectors, teicoplanin and teicoplanin without carbohydrate moieties, operate in all chromatographic separations modes, i.e., polar-organic mobile phase, reversed-phase, normal-phase.[21]

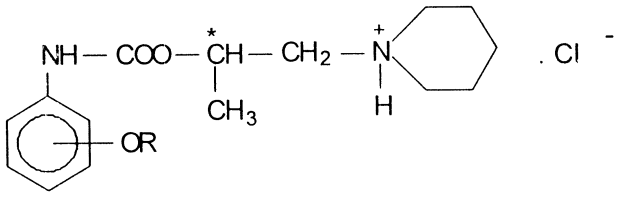
In this study, a teicoplanin (CSP-chirobiotic T) and teicoplanin without carbohydrate moieties (CSP-chirobiotic TAG), were used for the HPLC enantioseparation of series of alkoxysubstituted derivates of phenylcarbamic acid using the polar organic mode. The aim of this work was to directly compare the two columns under identical experimental conditions. The influence of the sugar moieties on the separation of 1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acids was evaluated.

EXPERIMENTAL

Materials

The analytes separated in this study (1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acids) were prepared according to Pokorna et al.[13] (Table 1). All HPLC grade solvents (methanol and acetonitrile) were obtained from Merck (Germany). Diethylamine and acetic acid were obtained from Lachema (Czech Republic).

Table 1. Chemical Structures of Alkoxysubstituted Derivate of Phenylcarbamic Acid



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	$-C_7H_{15}$
22v	$-C_8H_{17}$	26v	$-C_9H_{19}$	30v	$-C_{10}H_{21}$
25v	$-C_9H_{19}$	29v	$-C_{10}H_{21}$	—	—
28v	$-C_{10}H_{21}$	—	—	—	—

Equipment

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

Chromatography

A chirobiotic T and chirobiotic TAG columns ($250 \times 4{,}6$ mm I.D.) (Astec, USA) were used for the separation of enantiomers of the alkoxysubstituted esters of phenylcarbamic acid. Separations were carried out at a flow rate of 0.8 mL/min at ambient temperature. The analytes were dissolved in methanol (concentration 1 mg/mL). Mobile phases were prepared by mixing methanol, acetonitrile, an organic acid (acetic acid), and a base (diethylamine) in different ratios.

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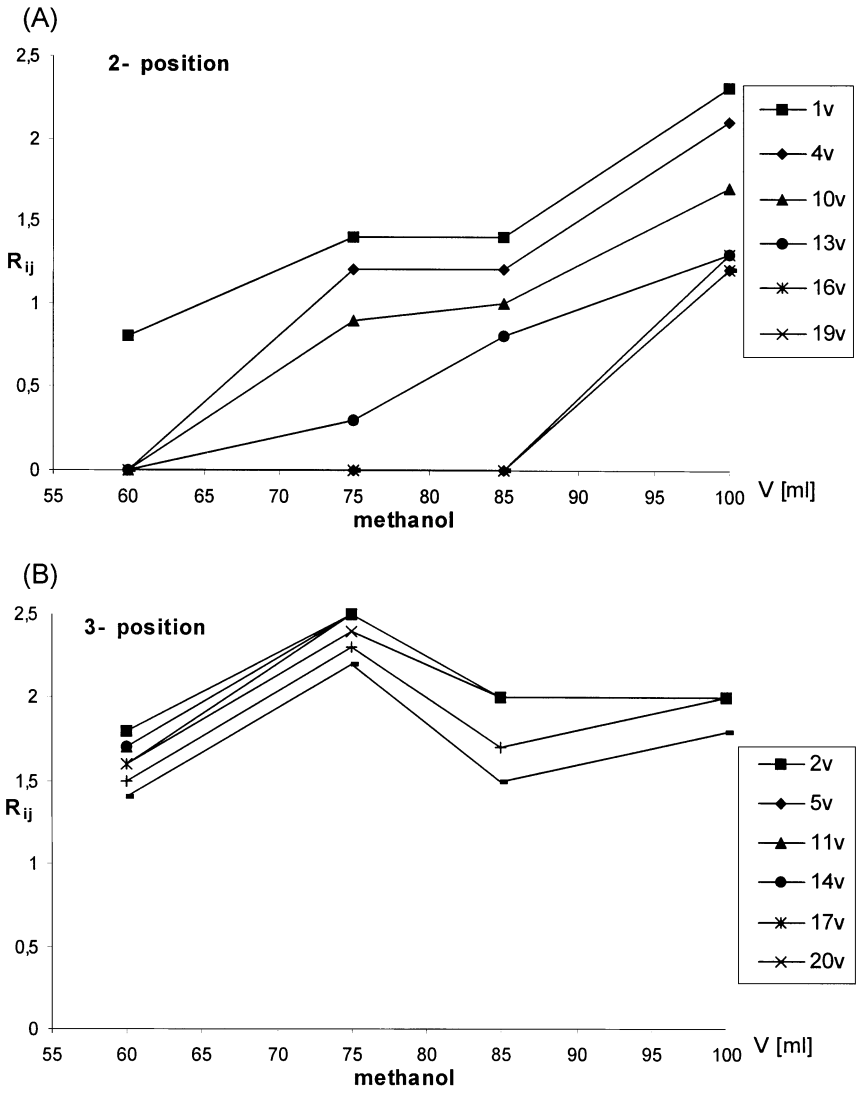

Figure 1. The dependence of resolution (R_{ij}) on methanol content in acetonitrile [final volume is 100 mL in every case] using the Chirobiotic TAG column. A—2-position; B—3-position and C—4-position. Mobile phase: methanol/acetonitrile 60–100/40–0 (v/v), 17.48 mmol/L, acetic acid and 9.57 mmol/L, diethylamine.

(continued)

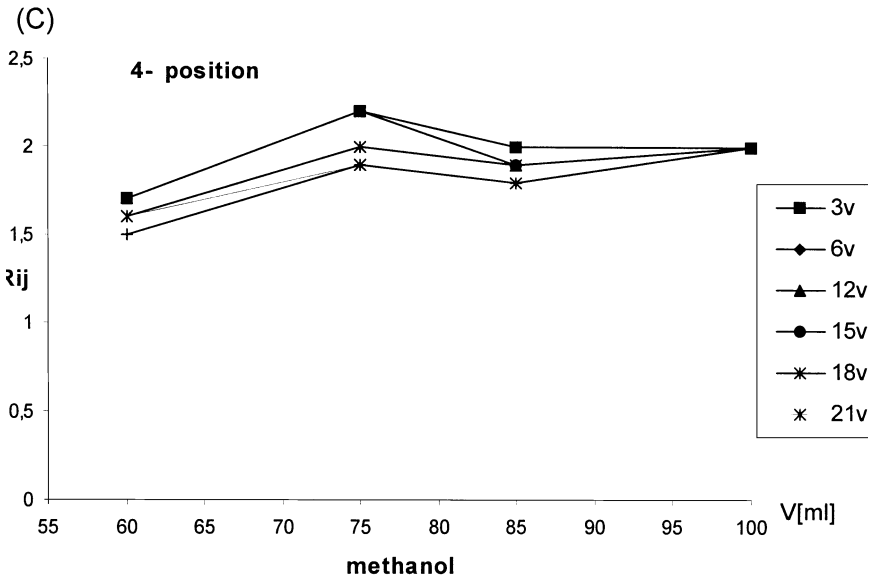

Figure 1. Continued.

RESULTS AND DISCUSSION

Influence of the Content of Methanol in Mobile Phase

The resolution of enantiomers by HPLC usually proceeds rapidly in the presence of an effective CSP. Due to the high selectivity requirements for chiral recognition, several different chiral phases may be needed to separate a variety of different structural types of molecules. The separation of enantiomers by HPLC using CSPs, is based on the formation of transient diasterometric complex between the enantiomorphs of the solute and chiral selector in the stationary phase.[22]

The separations were performed in the polar organic mode with either methanol, or mixtures of methanol and acetonitrite, as the main portion of the eluent. Small amounts of acetic acid and/or diethylamine were added to the mobile phase as well, to enhance the enantioselective separation. Methanol is a good hydrogen bond donor molecule, which can have a positive influence on the enantiomeric resolution (R_{ij}). This solvent is almost always used as the main component of the polar organic mobile phase (Fig. 1). It appears that it may successfully compete with many analytes for nonenantioselective binding sites.

Small amounts of acetic acid and diethylamine ionic modifiers are added to mobile phase. They serve to protonate or deprotonate both the analyte and chiral selector, which in turn, affects enantioselectivity.

Enantiomers of alkoxysubstituted esters of phenylcarbamic acid with different alkoxysubstitution (Table 1) were separated on CSPs based on a

Table 2. The Retention Factors (k) and Resolution (R_{ij}) of Alkoxy-substituted Derivates of Phenylcarbamic Acid Using the Chirobiotic T and TAG Column

	Chirobiotic T[a]			Chirobiotic TAG		
2-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
1v	2.27	2.27	0	4.33	4.70	1.4
4v	1.53	1.53	0	4.18	4.59	1.2
10v	1.00	1.00	0	3.75	3.97	0.9
13v	0.93	0.93	0	3.59	3.75	0.3
16v	0.94	0.94	0	3.50	3.68	0
19v	0.98	0.98	0	3.45	3.45	0
22v	0.79	0.79	0	3.14	3.14	0
25v	0.76	0.76	0	3.02	3.02	0
28v	0.73	0.73	0	2.32	2.32	0
3-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
2v	2.79	3.06	1.1	6.93	7.89	2.5
5v	2.54	2.87	1.0	6.14	7.23	2.5
11v	2.23	2.47	0.9	5.68	6.63	2.3
14v	1.79	2.00	1.4	5.36	6.36	2.5
17v	1.71	1.90	0.9	3.83	4.50	2.5
20v	1.65	1.84	0.7	3.74	4.45	2.4
26v	1.62	1.79	0.5	3.40	4.22	2.3
29v	1.22	1.58	0.5	3.09	3.73	2.2
4-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
3v	3.07	3.45	1.6	6.25	7.16	2.1
6v	2.85	3.27	1.3	5.82	6.63	1.8
12v	2.47	2.81	1.2	6.50	7.05	2.2
15v	2.17	2.48	1.1	5.74	6.67	2.3
18v	1.98	2.27	1.1	4.37	5.11	2.0
21v	1.96	2.24	1.1	4.31	5.09	1.9
30v	1.70	1.95	1.1	3.76	4.45	2.5

[a] k, R_{ij} were taken from the paper.[22]
Mobile phase: methanol/acetonitril 75/25 (v/v) and acetic acid/diethy-diethylamine 17.48 mmol/L/9.47 mmol/L.

macrocyclic glycopeptide (i.e., chirobiotic T column) in the polar organic mode (with a mobile phase containing methanol/acetonitrile (75/25, v/v) and acetic acid/diethylamine (17.48 mmol/L/9.57 mmol/L). At these conditions, the best separation of studied enantiomers on chirobiotic T column was achieved. The enantioseparation was obtained for derivates with alkoxysubstitution in

Table 3. The Retention Factors (k) and Resolution (R_{ij}) of Alkoxy-substituted Derivates of Phenylcarbamic Acid Using the Chirobiotic T and TAG Column

	Chirobiotic T[a]			Chirobiotic TAG		
2-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
1v	2.53	2.53	0	12.91	15.64	3.0
4v	2.29	2.29	0	11.09	13.95	3.0
10v	2.00	2.00	0	9.09	10.68	2.1
13v	1.93	1.93	0	8.50	9.64	1.4
16v	1.87	1.87	0	7.55	8.18	1.3
19v	1.60	1.60	0	7.09	7.91	1.3
22v	1.53	1.53	0	7.05	7.73	1.3
25v	1.52	1.52	0	6.55	6.82	1.3
28v	1.12	1.12	0	6.27	6.91	1.3
3-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
2v	3.58	3.96	1.1	14.64	16.45	2.0
5v	3.04	3.39	1.0	13.45	15.27	1.8
11v	2.57	2.84	0.9	11.73	13.36	1.7
14v	2.43	2.70	1.0	12.45	14.18	1.7
17v	2.27	2.43	1.0	12.09	13.45	1.7
20v	2.26	2.51	1.0	11.50	13.09	1.7
26v	2.04	2.28	0.9	10.45	11.77	1.5
29v	1.92	2.16	0.9	8.45	9.82	2.1
4-position	k_1	k_2	R_{ij}	k_1	k_2	R_{ij}
3v	5.28	6.05	1.2	17.05	18.86	1.9
6v	3.37	3.78	1.2	16.45	18.32	1.7
12v	3.05	3.41	1.2	14.18	16.00	1.7
15v	2.95	3.29	1.0	13.36	15.00	1.7
18v	2.68	3.07	1.1	13.05	14.41	1.7
21v	2.52	3.03	1.2	12.64	14.27	1.7
30v	2.06	2.33	1.1	10.18	11.64	1.9

[a]k, R_{ij} were taken from the paper.[22]
Mobile phase: methanol and acetic acid/diethylamine 17.48 mmol/L/4.79 mmol/L.

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3- and 4-position, and no enantiomeric separations for analytes with alkoxy-substitution in the 2-position was observed. This may have been due to the effect of shielding of the stereogenic center by the adjacent alkoxy-substituent and the effect of formation of intramolecular complexes.[23]

In Table 2, the values of the resolution (R_{ij}) and the retention factors (k) for the separation of enantiomers using the related Chirobiotic T and TAG columns, are presented. Higher values of R_{ij}, was observed for the 2- (mainly C_1–C_5), 3-, and 4-alkoxy-substituted analytes on the Chirobiotic TAG column, using the same mobile phase.

If only methanol is used as a mobile phase (Table 3) with acetic acid (17.48 mmol/L) and diethylamine (4.79 mmol/L), much higher retention factors were obtained in the case of the Chirobiotic TAG column. Better resolution was observed using the Chirobiotic TAG column for all compounds under study. Perhaps most interesting, was the fact that the 2-substituted analytes showed enhanced resolution on the TAG column with methanolic mobile phase, while the 3-and 4-substituted analytes were more poorly separated. This composition of mobile phase was used for further study of enantiomers.

Chromatograms of typical enantiomeric separation are shown in Figs. 2 and 3.

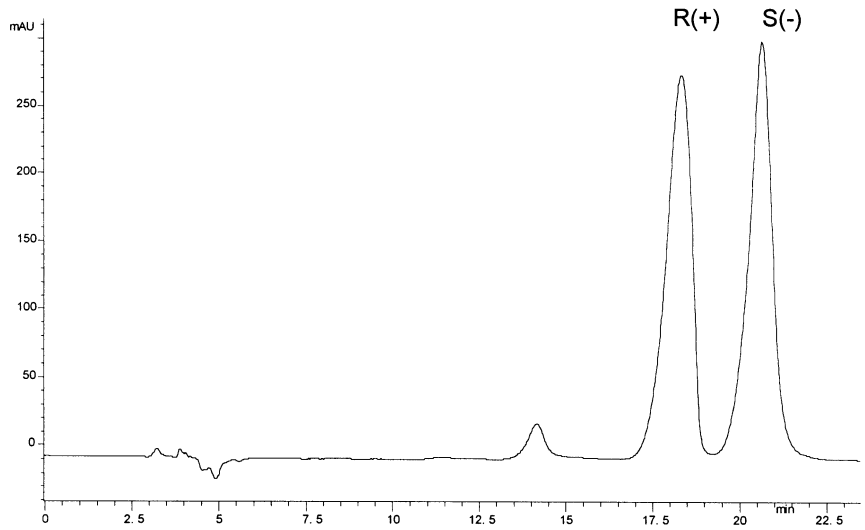

Figure 2. The enantioseparation of compound 3 v. Chromatographic conditions: mobile phase: methanol/acetonitrile 75/25 (v/v), acetic acid/diethylamine 17.48 mmol/L/ 9.57 mmol/L. stationary phase: Chirobiotic T; flow rate: 0.8 mL/min; temperature: 22°C.

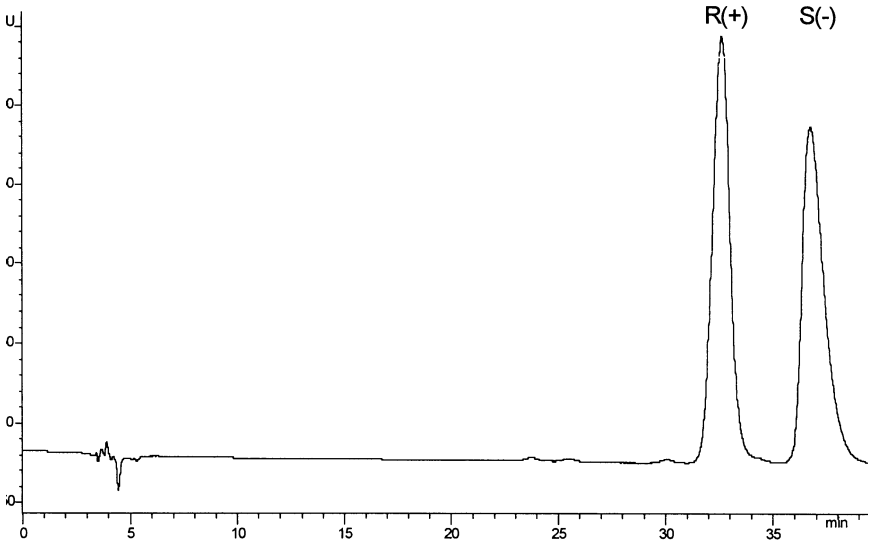

Figure 3. The enantioseparation of compound 3 v. Chromatographic conditions: mobile phase: methanol/acetonitrile 75/25 (v/v), acetic acid/diethylamine 17.48 mmol/L/ 9.57 mmol/L. stationary phase: Chirobiotic TAG; flow rate: 0.8 mL/min; temperature: 22°C.

Influence of Concentration of Acetic Acid and Diethylamine

The TAG is a weak amphoteric ion exchanger. From this point of view, the influence of acetic acid and diethylamine in mobile phase has an important role. Charge interactions, forming between quaternary nitrogen in the piperidine ring of the analyte and the stationary phase, can be governed by changing the ratio of acetic acid and diethylamine. From Fig. 4, it can be seen that the retention factors (k) decrease with concentrations of diethylamine over 2.39 mmol/L. The lowest of retention factors at zero concentration of diethylamine could be explained on the base of strong repulsive effects between the protonated amine of the analyte molecules and amine groups of the CSP (the piperidinoesters are in the form of cations). On the other hands the dependence of R_{ij} on Chirobiotic TAG column (Fig. 5) has a maximum in the range of diethylamine 2.39–4.79 mmol/L.

No enantiomeric separations were observed for the 4-substituted analytes when the mobile phase consisted of methanol/acetic acid/diethylamine (100 (v)/17.48 mmol/L/0 mmol/L). On the other hand, a partial separation of the 2-substituted and 3-substituted alkoxy analytes was observed (Table 4). An increase of the diethylamine concentration in the mobile phase (to about

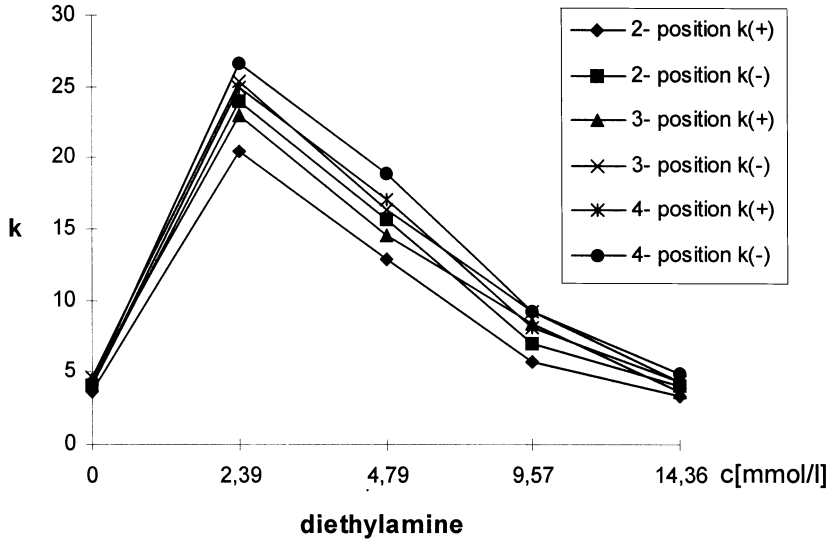

Figure 4. The dependence of retention factors on diethylamine content in mobile phase (methanol 100 (v)/acetic acid 17.48 mmol/L. and diethylamine (0–14.36 mmol/L) for compounds 1v, 2v, 3v.

2.39 mmol/L) decreased the retention factors of the studied analytes. An increase in the concentration of diethylamine at constant ionic strength (LiCl was added) still decreased the retention factors. This demonstrates, that it was the concentration (ratio) of acetic acid and diethylamine in mobile phase that has a substantial influence on the retention factors, and not the ionic strength of the mobile phase, which was constant. In comparison with the results obtained on the Chirobiotic T column,[23] it is clear that pendant sugar moieties have a definite influence on the interactions between these chiral analytes and the glycopeptide CSP.

Influence of Position of Alkoxy Chain

For this study, a mobile phase of methanol (100, v) acetic acid/ diethylamine (17.48 mmol/L/4.79 mmol/L) was used, because it produced the best separation of enantiomers (Table 3). Very good separations were observed for the 2-methoxy- and ethoxy substituted compounds. These results indicate, that the steric hindrance of the alkoxychain increases by prolongation of

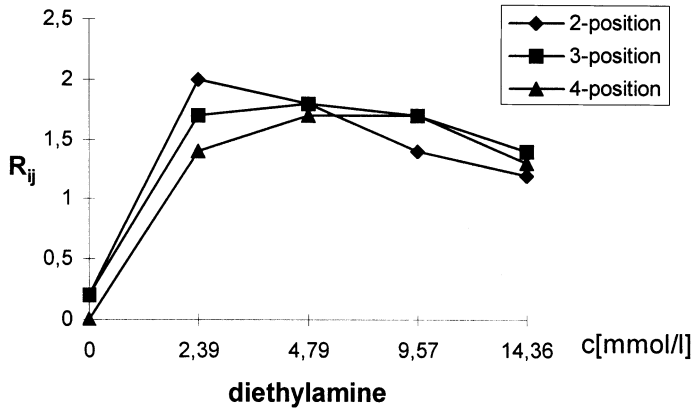

Figure 5. The dependence of resolution on diethylamine content in mobile phase (methanol 100 (v)/acetic acid 17.48 mmol/L and diethylamine (0–14.36 mmol/L for compounds 1v, 2v, 3v.

alkoxychain. In the case of 3- and 4-substituted analytes, the influence of the alkoxychain on the stereogenic center is much lower.

In comparison with the enantioseparation using Chirobiotic T column,[23] it is evident that much higher resolution was achieved in the case on the Chirobiotic TAG column. This indicates, that the sugar moieties had a delaterious effect on the separation of these compounds.

Influence of Number of Carbon Atoms in Alkoxy Chain

It can be observed, that the retention factors decrease as the number of carbon atoms in the -OR group increases in the range of C_1–C_{10}, with alkoxysubstitution in all positions (Fig. 6). The length of the alkoxychain does not influence the enantioseparation (R_{ij}) if the alkoxychain is in the 3- or 4-position. There is an influence of alkoxychan in length for the 2-substituted analytes, because it is very close to the stereogenic center, as was described above.

CONCLUSION

The influence of the composition of the mobile phase, the position, and the number of carbon atoms in the alkoxychain, on the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid, using two teicoplanin-based

Table 4. The Retention Factors (k) and Resolution (R_{ij}) of Alkoxysubstituted Derivates of Phenylcarbamic Acid Using the Chirobiotic TAG Column

	Chirobiotic TAG		
2-position	k_1	k_2	R_{ij}
1v	3.63	4.11	0.4
4v	3.50	4.02	0.4
10v	3.07	3.19	0.1
13v	2.89	2.90	0.2
16v	2.61	2.80	0.3
19v	2.57	2.69	0.2
22v	2.50	2.70	0.2
25v	2.46	2.50	0.1
28v	2.40	2.48	0.1
3-position	k_1	k_2	R_{ij}
2v	4.44	4.59	0.2
5v	3.52	3.80	0.2
11v	2.98	3.21	0.3
14v	2.85	3.11	0.3
17v	2.61	2.94	0.2
20v	2.56	2.89	0.2
26v	2.33	2.61	0.2
29v	2.50	2.55	0.1
4-position	k_1	k_2	R_{ij}
3v	4.06	4.06	0
6v	3.94	3.94	0
12v	3.87	0.387	0
15v	3.83	3.83	0
18v	3.16	3.16	0
21v	2.67	2.67	0
30v	2.56	2.56	0

Mobile phase: methanol and acetic acid 17.48 mmol/L.

CSPs (i.e., one with and one without carbohydrate moieties) was studied. The separation was accomplished in the polar organic mode. Given the results of these enantiomeric separations, the following conclusions have been reached:

Charge–charge interactions: repulsive charge interactions between the protonated amine of the analyte molecules and the Chirobiotic TAG CSP are important (small retention factors were observed).

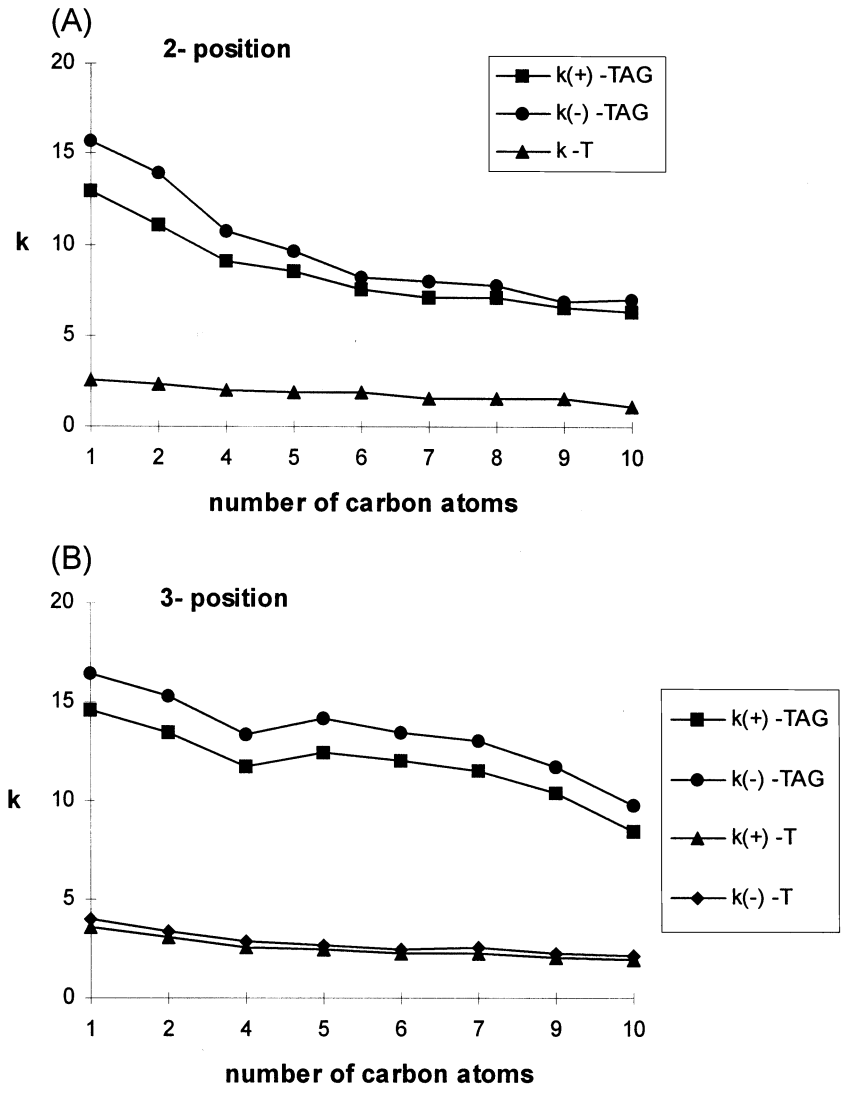

Figure 6. The dependence of the retention factors on the number of carbon atoms in alkoxychain. Mobile phase: methanol, 17.48 mmol/L acetic acid, and 4.79 mmol/L diethylamine. A: 2-position, B: 3-position, C: 4-position.

Steric interaction: environment near the stereogenic center has a substantial influence on the enantioseparation. Alkoxysubstituted esters of phenylcarbamic acid in 2-position are not separated in mobile phases containing methanol/acetonitril (75/25, v/v) and acetic acid (17.48 mmol/L), diethylamine (9.57 mmol/L). The position of the alkoxysubstituent in phenylcarbamic acid derivates can influence the chiral resolution. No enantiomeric separation was observed for the alkoxysubstituted esters of phenylcarbamic acid in the 4-position, when the mobile phase consisted of methanol/acetic acid (100 (v)/ 17.48 mmol/L). On the other hand, the separation was observed if the alkoxysubstitutions are in the 2-position and in the 3-position. The number of carbon atoms in the alkoxysubstituent has the influence if the alkoxy substituent is in the 2-position.

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