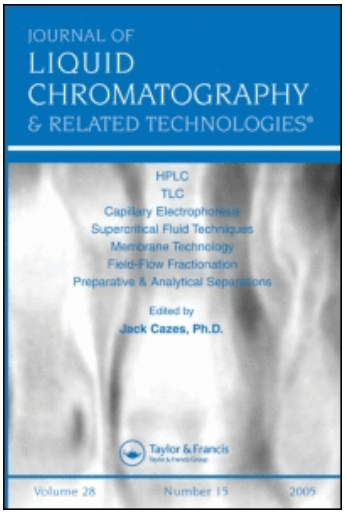


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

STUDY OF LOCAL ANAESTHETICS. CLVIII. CHROMATOGRAPHIC SEPARATION OF SOME DERIVATIVES OF SUBSTITUTED PHENYLCARBAMIC ACID ON A VANCOMYCIN-BASED STATIONARY PHASE

J. Ïungelová[a]; J. Lehotay[a]; K. Hrobonová[a]; J. Cizmárik[b]; D. W. Armstrong[c]
[a] Department of Analytical Chemistry, Faculty of Chemical Technology, Slovak University of Technology, Bratislava, Slovakia [b] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia [c] Department of Chemistry, Gilman Hall, Iowa State University, Ames, IA, U.S.A.

Online publication date: 05 July 2002

To cite this Article Ïungelová, J. , Lehotay, J. , Hrobonová, K. , Cizmárik, J. and Armstrong, D. W.(2002) 'STUDY OF LOCAL ANAESTHETICS. CLVIII. CHROMATOGRAPHIC SEPARATION OF SOME DERIVATIVES OF SUBSTITUTED PHENYLCARBAMIC ACID ON A VANCOMYCIN-BASED STATIONARY PHASE', Journal of Liquid Chromatography & Related Technologies, 25: 2, 299 — 312
To link to this Article: DOI: 10.1081/JLC-100108747
URL: http://dx.doi.org/10.1081/JLC-100108747

J. LIQ. CHROM. & REL. TECHNOL., 25(2), 299–312 (2002)

STUDY OF LOCAL ANAESTHETICS. CLVIII. CHROMATOGRAPHIC SEPARATION OF SOME DERIVATIVES OF SUBSTITUTED PHENYLCARBAMIC ACID ON A VANCOMYCIN-BASED STATIONARY PHASE

J. Ïungelová,[1] J. Lehotay,[1,*] K. Hrobonová,[1] J. Cizmárik,[2] and D. W. Armstrong[3]

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

ABSTRACT

Vancomycin, immobilized on silica, served as the chiral stationary phase (CSP) in this investigation with a polar organic solvent as the mobile phase in high performance liquid chromatography (HPLC). Enantiomers of alkoxysubstituted esters of phenylcarbamic acid (local anaesthetic drugs) were examined in order to evaluate the enantiorecognition process.

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

The chromatographic behavior was investigated in order to obtain a deeper insight into the enantiodiscrimination process. A variety of factors, including the nature and concentration of the ionic modifiers in the mobile phase were examined. Conditions for the enantioseparation of derivatives of phenylcarbamic acid were found. The interaction mechanism of the separations is discussed.

INTRODUCTION

Tremendous advances have occurred in the development of high efficiency enantiomeric separations using high performance liquid chromatography (HPLC) (1), gas chromatography (GC) (2), and capillary electrophoresis (CE) (3–5). These advances changed the nature of chiral pharmaceutical development. This occurred, in large part, because of the actions of government regulatory agencies that issued policies, which directly resulted from the advances in enantioselective HPLC (6).

HPLC has been a dominant method for the analysis of chiral molecules. Enantiomeric selectivity usually is achieved through the appropriate choices of a chiral stationary phase and mobile-phase conditions. The pioneering work of Davankov (7), Pirkle (8), Okamoto (9), and Armstrong (10–12), among others, has provided chromatographers with many choices of chiral stationary phases, as well as theoretical insights on different chiral recognition mechanisms.

Macrocyclic antibiotics (MA) represent a recent class of powerful chiral selectors. They contain numerous functional groups and many stereogenic centers. These sites offer the possibility of different simultaneous interactions, which allow the separations of a wide variety of racemic compounds. Different macrocyclic antibiotics were successfully used for both chromatographic and electrophoretic separations of various types of enantiomers. Chiral stationary phases based on the MA chiral selectors, vancomycin and teicoplanin, operate in all chromatographic separations modes, i.e. with normal-phase, reversed-phase, and polar-organic mobile phase (13).

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 including TLC (14,15) and HPLC (16–22).

In this study, a vancomycin-based CSP (Chirobiotic V) was used for the enantioseparation of alkoxysubstituted esters of phenylcarbamic acids using the polar-organic mode. The influence of different parameters on enantioresolution has been investigated. The effects of the mobile phase composition, the kind of

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

alcohol (methanol, ethanol and propanol) and acetonitrile, and the addition of some ionic organic modifiers (aliphatic carboxylic acids and bases) have been studied. The possible mechanism of enantiodiscrimination is discussed.

EXPERIMENTAL

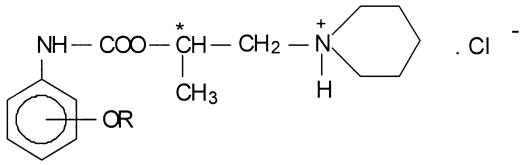
Materials

The analytes separated in this study (1-methyl-2-piperidinoethylesters of 2-, 3-, and 4-alkoxyphenylcarbamic acid) were prepared according to Pokorna and col.(23) (Table 1). All HPLC grade solvents (methanol, ethanol, propanol, and acetonitrile) were obtained from Merck (Germany). Triethylamine, diethylamine, trimethylamine, formic acid, acetic acid, propionic acid, hexanoic acid were obtained from Lachema (Czech Republic).

Instruments

A Chirobiotic V (250 × 4.6 mm I.D.) (Astec, USA) column was used for the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid.

Table 1. Chemical Structures of Alkoxy-Substituted Derivatives of Phenylcarbamic Acid



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	$-C_7H_{15}$	20	$-C_7H_{15}$	21	$-C_7H_{15}$
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	$-C_{10}H_{21}$

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Separations were achieved using a Waters liquid chromatograph with a 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 the stream of nitrogen.

RESULTS AND DISCUSSION

Methanol is one of the most commonly used mobile phase components in HPLC. Because of the more ionic characteristics of the macrocyclic glycopeptides, this solvent can be used as the main component of the polar organic mobile phase. Small amounts of acid and base modifiers are added to affect enantioseparations. This simple mobile phase has demonstrated broad selectivity and high efficiency with the glycopeptide chiral stationary phases and resulted in shorter analysis times. Statistically, more than 40% of the racemic compounds separated by vancomycin, teicoplanin, or ristocetin chiral stationary phases were done using this mobile phase (2–4).

To be resolved in this polar organic mode, analytes must have more than one functional group. The functional groups capable of interacting with the chiral stationary phases include hydroxyls, halogens, amines, carbonyl, and carboxyl groups, as well as oxidized forms of sulfur and phosphorus. At least one of these functional groups must be on or near the stereogenic center for a successful separation. Many important pharmaceuticals such as β-blockers, anticonvulsants, antidepressants, bronchodilators, anesthetics, and diuretics that have two or more of the above-mentioned functional groups, were well resolved on the macrocyclic glycopeptide chiral stationary phases. Recently, some of these compounds were used to compare the performance of mixed and coupled columns with that of the individual chiral stationary phases (25).

Enantiomers of alkoxysubstituted esters of phenylcarbamic acid with different alkoxysubstitution in the 2-, 3-, and 4-positions (Table 1) were separated on chiral stationary phases based on the macrocyclic antibiotics in the polar organic mode (26). Using the vancomycin column, no elution of the analytes in this study were achieved when the mobile phase consisted of methanol or acetonitrile. Reduced retention (retention factors between 0.1–0.6) and limited enantiomeric separations were obtained when the mobile phase consisted of

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

methanol or acetonitrile with the addition of 17.5 mmol/L acetic acid. This indicates strong cationic repulsive interaction between stationary phase and analyte, which does not support chiral separations. On the other hand, the presence of a small amount of base in a mobile phase consisting of methanol and 17.5 mmol/L acetic acid has a significant influence on the retention and the enantioresolution of the phenylcarbamic acid analytes used in this study. In mobile phases containing acetonitrile as the organic modifier, enantioseparations were not observed. Similar results were achieved in the case of teicoplanin chiral stationary phase (26).

The influence of the nature of the alcohol, used as the mobile phase, on the enantioresolution of the analytes, is indicated in Table 2 (the separation of enantiomers of analytes 0, 1, 2, and 3). The mobile phase consisted of an alcohol (methanol, ethanol and propanol), 17.5 mmol/L acetic acid, and 3.59 mmol/L diethylamine. When methanol, ethanol, and/or propanol were used as organic modifiers in mobile phase, the resolution values of the enantiomers decreases as the length of alcohol chain increases (Table 2). Ethanol and propanol produce lower enantioresolution and retention factors.

The influence of the nature of the base (triethylamine, trimethylamine, diethylamine) on the retention factors is shown in Fig. 1, with all of the bases being added at the same concentration (7.18 mmol/L). The concentration of acetic acid in the mobile phase was 17.5 mmol/L. For these three ionic modifiers, 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) (27)]. The influence on the resolution value of enantiomers is shown in Fig. 2. It is clear that the resolution values of enantiomers, obtained with trimethylamine in the mobile phase, are higher for compounds with alkoxysubstitution in the 3-position. In the case of compounds with alkoxysubstituents in position 4, it was statistically proven that

Table 2. Influence of the Kind of Alcohol That Constitutes the Mobile Phase, on Resolution Values

		R_s		
Number of C Atoms in −OR	Position of −OR	Methanol	Ethanol	Propanol
0		2.45	1.10	0
1	2	0.97	0	0
	3	1.96	0.67	0
	4	2.29	1.29	0

Chromatographic conditions: alcohol (methanol, ethanol and propanol) containing 17.5 mmol/L acetic acid and 3.59 mmol/L diethylamine.

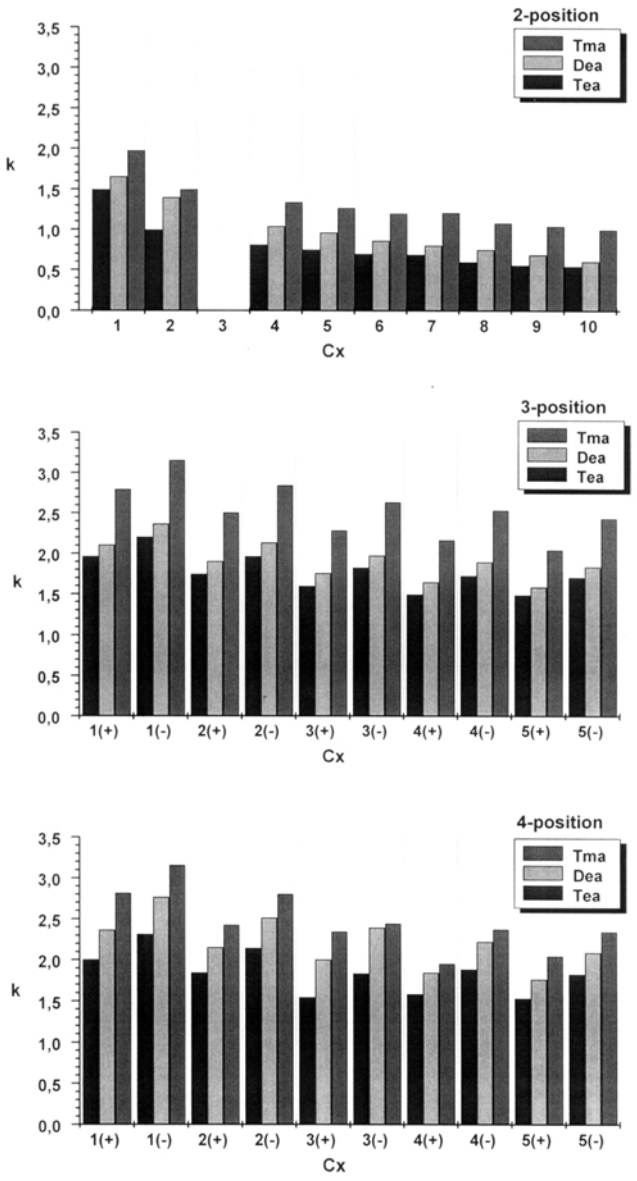


Figure 1. Influence of the type of organic amine additive on retention factors of substituted phenylcarbamic acids with different number of carbon atoms in −OR (Cx)* (see Table 1). Chromatographic conditions: methanol containing 17.5 mmol/L acetic acid and 7.18 mmol/L base (Tea = triethylamine, Tma = trimethylamine, Dea = diethylamine).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

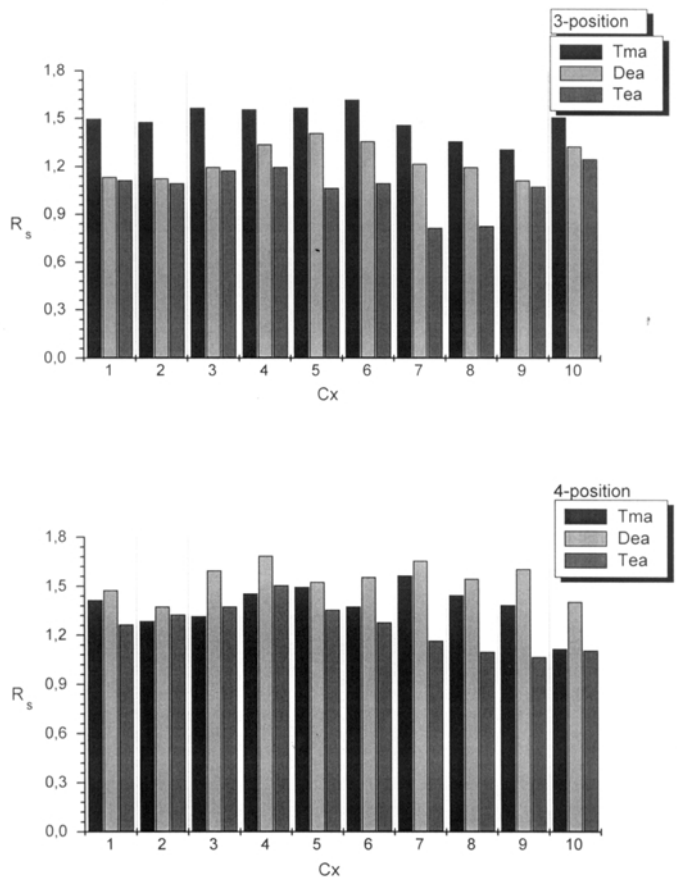


Figure 2. Influence of the type of organic amine additive on resolution values of substituted phenylcarbamic acids with different number of carbon atoms in −OR (Cx)* (see Table 1). Chromatographic conditions: methanol containing 17.5 mmol/L acetic acid and 7.18 mmol/L base (Tea = triethylamine, Tma = trimethylamine, Dea = diethylamine).

the type of the base (mentioned above) in the mobile phase is not significant under the conditions of this study.

The values of the retention factors on the vancomycin stationary phase (for the compounds listed in Fig. 1) are about half of that found for the same analytes on the teicoplanin column (26). These results indicate that the sorption properties of the teicoplanin and vancomycin stationary phases are different. The teicoplanin molecule has a nonyl hydrocarbon chain and this moiety would result in an increase of the lipophilicity of the teicoplanin surface. However, the teicoplanin

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

molecule also has more carbohydrate moieties and phenolic hydroxyl groups for hydrogen bonding. Interestingly, the resolution values for the enantiomeric separations on these two CSPs were similar.

The influence of the base concentration on the resolution values of these enantiomers was also studied (Table 3). The highest values of resolution were achieved at the 3.59 mmol/L concentration. Plots of retention factors versus the amount of base in the mobile phase (Fig. 3) gave similar profiles for each of the different alkoxysubstituted compounds. It is evident, that an increase in the concentration of diethylamine in the mobile phase (in the range 0.49–

Table 3. Influence of the Concentration of Diethylamine in the Mobile Phase on Resolution (R_s) Values

	Concentration of Diethylamine of Mobile Phase (mmol/L)					
Analyte Nr.	0.00	0.49	1.80	3.59	7.18	10.7
0	0	0.87	1.37	1.63	1.47	1.40
1	0	0.22	0.48	0.66	0.54	0.33
2	0	0.83	1.08	1.34	1.44	1.27
5	–	0.47	1.09	1.44	1.29	1.09
8	–	0.56	1.06	1.52	1.32	1.29
11	–	0.69	1.28	1.89	1.44	1.35
14	–	0.52	1.82	1.84	1.41	1.19
17	–	0.59	1.65	1.80	1.36	1.23
20	–	0.78	1.78	1.87	1.33	1.22
23	–	0.82	1.67	1.84	1.39	1.34
26	–	0.78	1.69	1.73	1.12	1.04
29	–	0.97	1.62	1.67	1.18	0.98
3	0	0.96	1.50	1.54	1.34	1.31
6	–	0.82	1.54	1.50	1.47	1.38
9	–	0.71	1.54	1.67	1.60	1.37
12	–	0.85	1.86	1.93	1.69	1.51
15	–	1.04	1.63	2.03	1.53	1.39
18	–	1.09	2.07	2.11	1.56	1.81
21	–	0.94	2.10	2.19	1.66	1.37
24	–	0.86	2.11	2.16	1.55	1.34
27	–	1.14	1.93	2.03	1.61	1.40
30	–	1.07	1.99	2.08	1.41	1.19

Chromatographic conditions: methanol containing 17.5 mmol/L acetic acid and different concentration diethylamine (0.49 mmol/L, 1.80 mmol/L, 3.59 mmol/L, 7.18 mmol/L, 10.7 mmol/L).

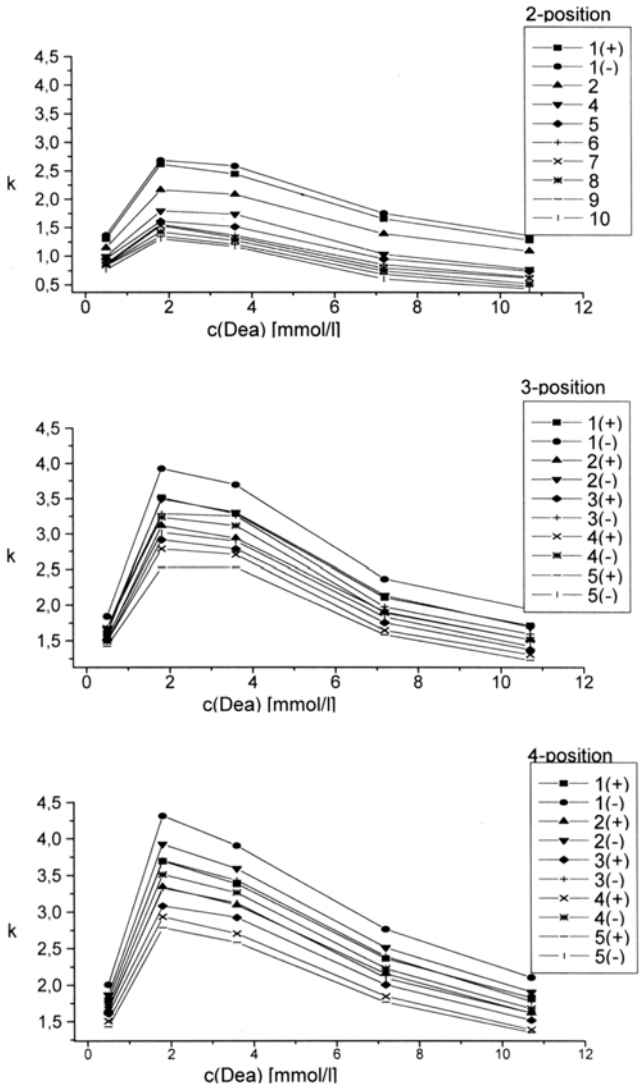


Figure 3. Influence of the concentration of diethylamine on retention factors of substituted phenylcarbamic acids with different number of carbon atoms in −OR (Cx)* (see Table 1). Chromatographic conditions: methanol containing 17.5 mmol/L acetic acid and (0.49 mmol/L, 1.80 mmol/L, 3.59 mmol/L, 7.18 mmol/L, 10.7 mmol/L) diethylamine.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

1.80 mmol/L) caused higher retention of these enantiomers. It is possible that repulsive charge interactions (in the original acidic environment) are involved. On the other hand, an increase in the concentration of diethylamine in the mobile phase, above 1.80 mmol/L, decreased the retention of the analytes. At this point, repulsive charge interactions are minimal, and other effects must come into play, including salt effects, because the ionic strength of the mobile phase increases.

The influence of the carbon chain length of the aliphatic carboxylic acid on enantioseparation was also studied. The mobile phase consisted of methanol, 17.5 mmol/L organic acid, and 7.18 mmol/L triethylamine. The retention factors slowly increased as the length of aliphatic chain was increased, but the resolution values were not significantly changed.

It is clear that the environment near the stereogenic center has a substantial influence on the resolution of the enantiomers. The best separation (i.e., highest values of R_s) was obtained for the compound without alkoxysubstitution (compound 0) and for compounds with alkoxysubstitution in the 4-position. Lower resolution of enantiomers was obtained for derivatives with the alkoxy-chain in the 3-position. No enantiomeric separations for analytes with alkoxysubstitution in the 2-position were observed, except for the single analyte with one carbon atom in alkoxy chain (compound 1). This probably can be attributed to the steric effect of the 2-alkoxy-substituent, which is near the stereogenic center. There may also be an effect due to the formation of intramolecular species. It can be observed that the

Table 4. 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	381		
2	288	3	350
5	291	6	366
8	382	9	394
11	423	12	461
14	434	15	465
17	417	18	469
20	432	21	468
23	439	24	440
26	394	27	377
29	371	30	426

Chromatographic conditions: methanol containing 3.59 mmol/L diethylamine and 17.5 mmol/L acetic acid.
$T = 295$ K.
$R = 8314$ J/K · mol.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

retention factors very slowly decrease as the number of carbon atoms in the −OR group increases, in the range of C_1–C_{10} (opposite to what is found in a reversed phase system). It can be seen that the number of carbon atoms in the alkoxy-substituent had no significant influence on the enantioseparation but its position on the aromatic ring is very important (Table 3).

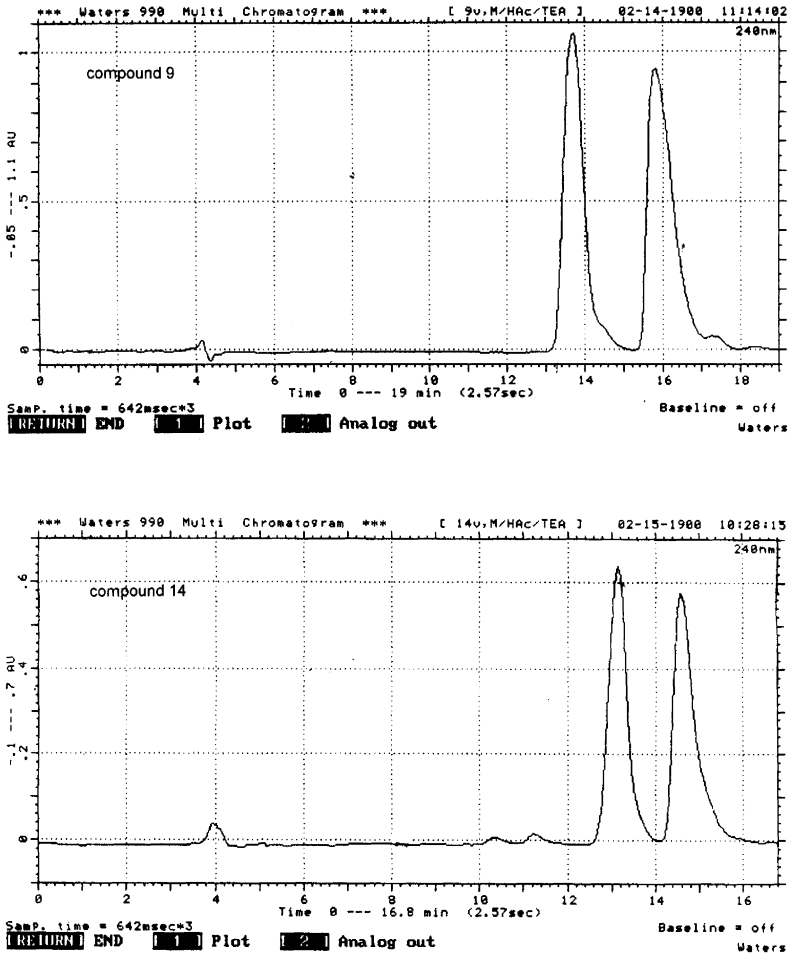


Figure 4. Chromatograms showing the enantioseparation of alkoxysubstituted esters of phenylcarbamic acid (compounds 9, 14). Chromatographic conditions: methanol containing 17.5 mmol/L acetic acid and 3.59 mmol/L diethylamine. *Similar dependences were obtained for analytes with number of carbon atoms C_6–C_{10} in −OR in the case of 3- and 4-position.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The different interactions of two enantiomeric forms of a molecule with the stationary phase leading to chiral discrimination can be expressed as a difference in the free energy $-\Delta_{1,2}\Delta G^o$ calculated from the selectivity coefficient α, according to the equations:

$$-\Delta_{1,2}\Delta G^o = \Delta_2 G^o - \Delta_1 G^o$$
$$-\Delta_{1,2}\Delta G^o = RT \ln k_2/k_1 = RT \ln \alpha$$

Values for the difference in free energy are shown in Table 4. It is obvious, that the binding of two enantiomers to a given site may involve different amounts of energy simply because one of the enantiomers, for steric reasons, might be forced to adopt an energetically less favourable conformation.

In a mobile phase of methanol containing 17.5 mmol/L acetic acid and 3.59 mmol/L diethylamine (where the best chiral resolution was obtained), the number of carbon atoms in the alkoxy chain (C_1–C_{10}) had no significant effect on the differences in free energy (Table 4). The position of alkoxysubstitution has an effect on free energy of enantiomeric separation. The highest values of $-\Delta_{1,2}\Delta G^o$ (> 350 J/mole) were obtained for derivatives with 4-alkoxysubstitution, which also had the highest separation selectivity.

Chromatograms of typical enantiomeric separations are shown in Figure 4.

CONCLUSION

The influence of the composition of the mobile phase on the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid by using vancomycin as a chiral stationary phase was studied. Different kinds and concentrations of aliphatic carboxylic acids and bases were used as ionic modifiers in the mobile phase (in the polar-organic mode). On the bases of the results it can be concluded that:

– repulsive charge interactions between the protonated amine of the analyte molecules and the amine groups of the vancomycine CSP are important.
– steric interaction, i.e., the position of the alkoxysubstituent in a molecule of phenylcarbamic acid derivatives, has an influence on chiral resolution. The length of alkoxy chain was not significant in comparison with the influence of the position of alkoxysubstituent on aromatic ring. It is clear that the steric crowding of the stereogenic carbon affects enantioresolution.

The vancomycin chiral stationary phase is suited for the separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The chiral recognition mechanism involves charge interactions, steric interactions, and, probably, hydrogen bonding/dipolar interactions as secondary effects.

ACKNOWLEDGMENTS

Support of this work by the National Institutes of Health, NIH RO1 GM53825-05, is gratefully acknowledged. KH and JL acknowledge the support of the Grant Agency of Slovak Republic (VEGA No. 1/6102/99) and The Agency for International Science and Technology Co-operation in Slovak Republic (Grant No. 002-98).

REFERENCES

1. Armstrong, D.W. LC-GC **May 1997**, S20.
2. Konig, W. *The Practive of Enantiomer Separations by Capillary Gas Chromatography*; Huthig Verlag: Heidelberg, 1989.
3. Kuhn, R.; Hoffstetter-Kuhn, S. Chromatographia **1992**, *34*, 505.
4. Snopek, J.; Jelinek, I.; Smolkova-Kenlemansova, E. J. Chromatogr. **1998**, *452*, 571.
5. Snopek, J.; Jelinek, I.; Smolkova-Kenlemansova, E. J. Chromatogr. **1992**, *609*, 1.
6. FDA. **1992**, *4*, 338.
7. Davankov, V.A.; Rogozhin, S.V. J. Chromatogr. **1971**, *60*, 280.
8. Pirkle, W.H.; Finn, J.M. J. Org. Chem. **1981**, *46*, 2935.
9. Okamoto, Y.; Kawashima, M.; Yamamoto, K.; Hatada, K. Chem. Lett. **1984**, *739*.
10. Armstrong, D.W.; DeMond, W. J. Chromatogr. Sci. **1984**, *22*, 411.
11. Armstrong, D.W.; Ward, T.J.; Armstrong, R.D.; Beesley, T.E. Science **1986**, *232*, 132.
12. Armstrong, D.W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill,C.; Chen, J.R. Anal. Chem. **1994**, *66*, 1473.
13. Tesarová, E.; Záruba, K.; Flieger, M. J. Chromatogr. A **1999**, *844*, 137.
14. Freitag, W.; Ney, K.H. J. Chromatogr. **1969**, *41*, 473.
15. Pirkle, W.; Hauske, R. J. Org. Chem. **1939**, *42*, 1939.
16. Okamoto, Y.; Cao, Z.K.; Aburatami, R.; Hatada, K. Bull. Chem. Soc. Jpn. **1987**, *60*, 3999.
17. Oi, N.; Kitahara, H. J. Chromatogr. **1983**, *265*, 117.
18. Oi, N.; Nagase, M.; Doi, T. J. Chromatogr. **1983**, *257*, 111.
19. Schill, G.; Wainer, I.W.; Barkan, S.A. J. Liq. Chromatogr. **1986**, *9*, 641.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

20. Hermansson, J.; Grahn, A. J. Chromatogr. A **1995**, *694*, 57.
21. Cizmárik, J.; Lehotay, J.; Hromuláková, K.; Pokorná, M.; Lacuška, M. Pharmazie **1997**, *52*, 5.
22. Pokorná, M.; Cizmárik, J. Pharmazie, *in press.*
23. Pokorná, M.; Cizmárik, J.; Sedlárová, E.; Racanská, E. Ces. a Slov. Farm. **1998**, *47*, 14.
24. *Chirobiotic Handbook*, 3rd Ed.; Advanced Separation Technologies, Inc.: Whippany, New Jersey, 1999.
25. Wang, X.A.; Lee, J.T.; Beesley, T.E. LC-GC **2000**, *18* (6), 626.
26. Lehotay, J.; Hrobonová, K.; Cizmárik, J.; Rencová, M.; Armstrong, D.W. J. Liq. Chromatogr., *in press.*
27. Lide, D.R. *Handbook of Chemistry and Physics*, 74th Ed.; 1993–1994; 8–3.

Received April 1, 2001
Accepted August 23, 2001
Manuscript 5547

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!