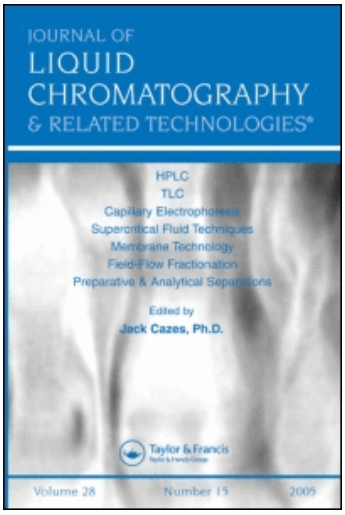

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STUDY OF THE MECHANISM OF ENANTIOSEPARATION. I. CHIRAL ANALYSIS OF ALKYLAMINO DERIVATIVES OF ARYLOXYPROPANOLS BY HPLC USING MACROCYCLIC ANTIBIOTICS AS CHIRAL SELECTORS

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STUDY OF THE MECHANISM OF ENANTIOSEPARATION. I. CHIRAL ANALYSIS OF ALKYLAMINO DERIVATIVES OF ARYLOXYPROPANOLS BY HPLC USING MACROCYCLIC ANTIBIOTICS AS CHIRAL SELECTORS

K. Hroboňová,[1] J. Lehotay,[1,*] R. Čižmáriková,[2] and D. W. Armstrong[3]

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ABSTRACT

The enantiomeric separation of alkylaminoderivatives of aryloxypropanols using macrocyclic bonded chiral stationary phases was studied. Teicoplanin and vancomycin chiral stationary phases were used to separate a large number of derivatives of aryloxypropanol enantiomers by HPLC in the polar-organic mode.

*Corresponding author.

By comparison of chromatographic parameters obtained by using both chiral stationary phases (CSPs), aspects of the enantioselective separation mechanism could be discerned. Originally, the polar organic mode was developed for chiral compounds that contained a minimum of two hydrogen bonding groups, resulting in a minimum of two hydrogen bonding interactions to the CSP. This work demonstrated that a combination of one hydrogen bonding interaction and one electrostatic interaction is equally effective. The environment (i.e., functional groups) nearest to the stereogenic center of the aryloxypropanols had the greatest effect on the enantioresolution. Teicoplanin CSPs produced the greatest $\Delta\Delta G°$'s and the best enantiomeric separations of these compounds. The site of a possible electrostatic interaction of these compounds is different from that found for amino acids.

INTRODUCTION

The separation of organic compounds with one or more stereogenic centres continues to be an important area of research, especially in pharmaceutical and environmental fields where many drugs and agrochemicals are racemic compounds. The stereochemistry of these compounds can have a dramatic effect on their properties, especially in biological contexts. A large number of pharmaceuticals are most effective as single enantiomers, and the opposite enantiomers are generally considered impurities.[1,2]

Macrocyclic antibiotics form one of the newest and perhaps most rapidly growing classes of chiral selectors. They are known to resolve a variety of racemic compounds.[3,4] Interactions such as hydrophobic, hydrogen bonding, dipole, π-π, steric repulsion, and ionic interactions can occur between these chiral stationary phases (CSPs) and a broad range of chiral analytes. These CSPs can be used in reversed phase, normal phase, and polar-organic separation modes.

Alkylaminoderivatives of aryloxypropanols form a group of drugs employed not only in the treatment of cardiovascular disorders but also for other medical conditions.[5,6] The activity of β-adrenoreceptor blocking agents are known to be strongly affected by their chirality. The enantiomeric separation of β-blocking drugs can be performed by means of different chromatographic techniques, including gas chromatography, thin layer chromatography, or with liquid chromatography, which is the most frequently used technique. In liquid chromatography, numerous chiral stationary phases were used for the separation of the enantiomers of β-blocking drugs. Some chiral stationary phases consisted of immobilised proteins such as bovine serum albumin,[7] α_1-acid glycoprotein,[8] ovomucoid,[9] and cellobiohydrolase.[10,11] Other ones were based on the use of

cyclodextrins,[12-14] amylose or cellulose derivatives, especially cellulose tris-(3,5-dimethylphenylcarbamate),[15-18] or macrocyclic antibiotics.[1,2,19-21] Some π-complex type CSPs were also reported to be suitable for achieving enantioseparation of β-blockers.[22-24] More recently, the enantioseparation of β-blocking drugs was also achieved in liquid chromatography by adding chiral selectors such as b-cyclodextrin[25] or (2R,3R)-di-n-butyltartrate[26] to the mobile phase, or by derivatization with chiral agent (acyl chlorides,[27] and isocyanates,[28,29] and anhydrides[30]).

In the present work, teicoplanin and vancomycin chiral stationary phases were used for the separation of alkylaminoderivatives of aryloxypropanols in the polar organic mode. The effect of the structure of the compounds on the selectivity and the resolution of enantiomers were studied.

EXPERIMENTAL

HPLC Analysis

Experiments were performed with a HPLC system from Hewlett Packard (series 1100) consisting of a quaternary pump equipped with a injection valve (Rheodyne) and diode array detector. The macrocyclic chiral stationary phases were Chirobiotic T and Chirobiotic V (250 x 4 mm I.D., particle size 5 μm) (Advanced Separation Technologies, Inc., USA). The mobile phase was a mixture of methanol and acetonitrile to which small amount of acetic acid and triethylamine was added (45/55/0,3/0,2 v/v/v/v). Separations were carried out at flow rate of 1 mL/min and the column temperature was maintained at 23°C. The chromatograms were scanned at wavelength 276 nm. The injection volume was 20 μL. The analytes were dissolved in methanol (concentration 1 mg/mL).

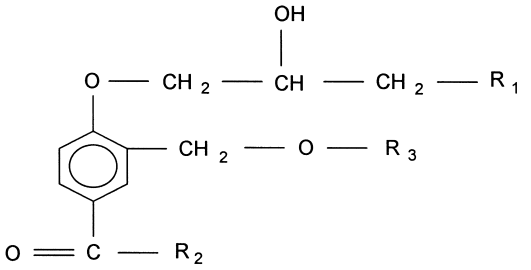
Chemicals

The racemic analytes resolved in this study were prepared according to Čižmáriková and col.[5,6] (Tables 1-3). All HPLC grade solvents (methanol, acetonitrile) were obtained from Merck (Germany). Triethylamine and acetic acid were obtained from Lachema (Czech Republic).

RESULTS AND DISCUSSION

The polar-organic mode was originally developed for enantioseparations on cyclodextrin CSPs.[12,31] Subsequently, this separation approach was found to be highly effective with macrocyclic glycopeptide CSPs,[2,20] as well as derivatized

Table 1. Chromatographic Data for the Enantioseparation of Derivatives of Aryloxypropanols on Vancomycin (V) and Teicoplanin (T) Bonded Chiral Stationary Phases (Chromatographic Conditions: See Experimental)



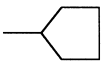
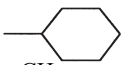
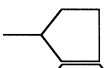
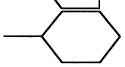
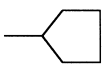
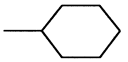
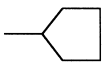
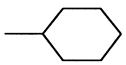
Nr.	R_1	R_2	R_3	k_1	α	R_s	$-\Delta_{1,2},\Delta G°$ [J/mol]	Col
1	$-NHCH(CH_3)_2$	$-CH_3$	$-CH_3$	2.52	1.08	1.27	186.9	V
				3.41	1.11	1.48	269.0	T
2			$-C_2H_5$	2.31	1.07	1.14	182.8	V
				3.09	1.12	1.58	282.3	T
3			$-C_3H_7$	2.17	1.08	1.26	187.1	V
				2.90	1.12	1.64	287.1	T
4			$-C_4H_9$	2.07	1.07	1.22	181.0	V
				2.76	1.13	1.55	295.8	T
5			$-C_5H_{11}$	1.97	1.08	1.01	181.5	V
				2.66	1.12	1.51	295.6	T
6			$-C_6H_{13}$	1.92	1.07	1.05	174.4	V
				2.59	1.13	1.61	291.1	T
7			$-C_7H_{15}$	1.86	1.07	1.06	171.5	V
				2.55	1.11	1.62	255.5	T
8			$-C_8H_{17}$	1.81	1.07	1.06	163.6	V
				2.44	1.12	1.58	293.3	T
9			$-CH(CH_3)_2$	2.12	1.07	1.14	183.9	V
				2.86	1.12	1.45	272.8	T
10			$-C_2H_3(CH_3)_2$	2.02	1.07	1.05	181.6	V
				2.72	1.12	1.51	289.1	T
11				2.27	1.07	1.26	187.9	V
				2.97	1.12	1.48	270.7	T
12				2.21	1.08	1.21	183.1	V
				2.92	1.12	1.55	270.7	T
13	$-NHCH(CH_3)_2$	$-C_2H_5$	$-CH_3$	2.26	1.07	1.18	183.1	V
				3.27	1.12	1.69	274.4	T
14			$-C_2H_5$	2.08	1.08	1.20	197.9	V
				2.98	1.12	1.68	285.0	T

Table 1. Continued

Nr.	R_1	R_2	R_3	k_1	α	R_s	$-\Delta_{1,2},\Delta G°$ [J/mol]	Col
15			$-C_3H_7$	1.97	1.07	1.16	174.0	V
				2.80	1.12	1.62	283.6	T
16	$-NHCH(CH_3)_2$	$-C_2H_5$	$-C_4H_9$	1.88	1.07	1.05	173.6	V
				2.68	1.13	1.76	292.8	T
17			$-C_5H_{11}$	1.82	1.07	1.05	166.4	V
				2.60	1.13	1.65	292.9	T
18			$-C_6H_{13}$	1.77	1.07	1.01	166.9	V
				2.52	1.13	1.56	290.0	T
19			$-C_7H$[15]	1.75	1.07	1.00	160.27	V
				2.49	1.11	1.51	270.3	T
20			$-C_9H_{19}$	1.72	1.08	1.01	163.4	V
				2.37	1.11	1.45	273.9	T
21			$-CH(CH_3)_2$	1.94	1.07	0.95	164.7	V
				2.78	1.11	1.36	264.9	T
22				2.02	1.09	1.05	211.8	V
				2.86	1.12	1.52	281.2	T
23				1.97	1.07	1.01	174.0	V
				2.81	1.11	1.50	271.8	T
24	$-NHC(CH_3)_3$	$-CH_3$	$-CH_3$	2.23	1.11	1.58	256.2	V
				3.07	1.17	2.07	395.0	T
25			$-C_2H_5$	2.06	1.10	1.53	232.9	V
				2.79	1.18	2.05	380.2	T
26			$-C_3H_7$	1.96	1.09	1.41	221.3	V
				2.62	1.14	2.08	322.1	T
27			$-C_4H_9$	1.88	1.09	1.33	218.9	V
				2.51	1.16	2.01	381.0	T
28			$-C_5H_{11}$	1.81	1.08	1.25	205.6	V
				2.40	1.17	2.02	381.9	T
29			$-C_6H_{13}$	1.78	1.08	1.12	196.5	V
				2.34	1.17	1.91	381.4	T
30			$-C_7H_{15}$	1.73	1.08	1.01	183.6	V
				2.27	1.16	2.01	380.9	T
31			$-C_8H_{17}$	1.70	1.06	0.95	159.6	V
				2.20	1.17	2.01	385.1	T
32			$-C_9H_{19}$	1.67	1.07	0.86	129.8	V
				2.14	1.17	2.01	392.4	T
33			$-CH(CH_3)_2$	1.92	1.09	1.31	210.5	V
				2.57	1.16	1.89	369.9	T
34			$-C_2H_3(CH_3)_2$	1.85	1.09	1.26	210.0	V
				2.47	1.17	2.04	389.1	T
35				2.00	1.09	1.21	205.6	V
				2.66	1.16	1.81	365.9	T

(continued)

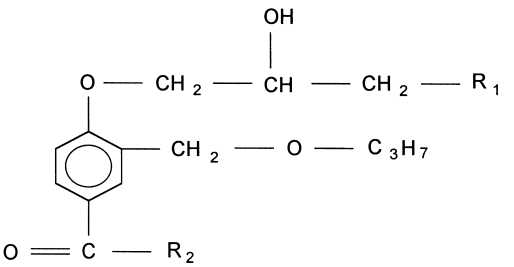
Table 1. Continued

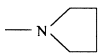
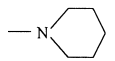
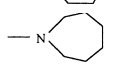
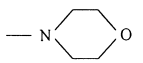
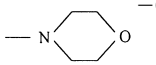
Nr.	R_1	R_2	R_3	k_1	α	R_s	$-\Delta_{1,2},\Delta G°$ [J/mol]	Col
36				2.29	1.07	1.20	186.9	V
				2.62	1.16	1.89	354.3	T
37	$-NHC(CH_3)_3$	$-C_2H_5$	$-CH_3$	2.14	1.11	1.54	260.3	V
				3.07	1.20	2.09	462.9	T
48			$-C_2H_5$	2.03	1.09	1.18	214.9	V
				2.83	1.19	2.10	381.5	T
39			$-C_3H_7$	1.91	1.09	1.40	243.5	V
				2.62	1.17	2.07	482.0	T
40			$-C_4H_9$	1.77	1.10	1.11	207.1	V
				2.48	1.16	2.01	356.0	T
41			$-C_5H_{11}$	1.69	1.07	1.06	179.4	V
				2.30	1.17	2.09	393.7	T
42			$-C_6H_{13}$	1.66	1.07	1.07	163.3	V
				2.25	1.17	2.09	399.7	T
43			$-C_7H_{15}$	1.64	1.07	1.01	155.8	V
				2.18	1.17	2.11	391.1	T
44			$-C_8H_{17}$	1.62	1.06	1.12	148.7	V
				2.13	1.17	2.10	400.7	T
45			$-C_9H_{19}$	1.58	1.07	1.01	156.6	V
				2.08	1.17	2.01	385.3	T
46			$-CH(CH_3)_2$	1.77	1.08	1.14	192.6	V
				2.43	1.16	1.92	371.2	T
47			$-C_2H_3(CH_3)_2$	1.72	1.07	1.11	176.4	V
				2.33	1.16	1.84	368.2	T
48				1.84	1.08	1.23	198.7	V
				2.53	1.16	1.79	369.3	T
49				1.80	1.08	1.21	189.5	V
				2.49	1.16	1.72	357.6	T

RSD for k ~ 2-4 %.

cellulosic and amylosic CSPs.[32,33] Compounds that can be enantioseparated in this mode have a minimum of two polar functional groups (e.g., -OH, -NH, -COOH, -N<, -SH, etc) capable of relatively strong interactions with the chiral stationary phase. These interactions are usually hydrogen bonds, but can sometimes be dipolar and/or electrostatic in nature (or some combination thereof). The mobile phase generally consists of methanol/acetonitrile mixtures with very small amounts of acid and base modifier. At least one of the analyte's polar functional groups must be on or near the stereogenic center. The other polar group can be

Table 2. Chromatographic Data for the Enantioseparation of Derivatives of Aryloxypropanols with Heterocyclic Nitrogen R_1 Substituents on Vancomycin (V) and Teicoplanin (T) Bonded Chiral Stationary Phases (Chromatographic Conditions: See Experimental)



Nr.	R_1	R_2	k_1	α	R_s	$-\Delta_{1,2}\Delta G°$ [J/mol]	Col
50		$-CH_3$	2.47	1.04	0.52	85.3	V
			3.53	1.05	0.77	132.1	T
51			2.18	—	—	—	V
			2.97	1.03	0.50	65.8	T
52			2.21	—	—	—	V
			3.02	1.04	0.52	86.0	T
53			0.17	—	—	—	V
			0.35	—	—	—	T
54		$-C_2H_5$	0.15	—	—	—	V
			0.30	—	—	—	T

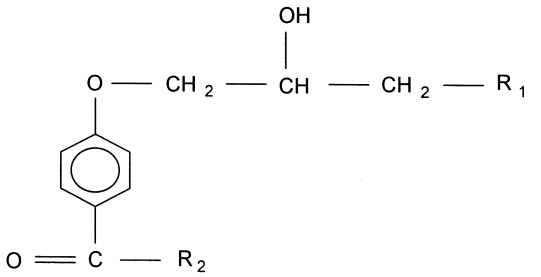
RSD for k ~ 2-4 %.

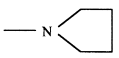
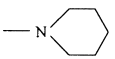
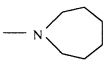
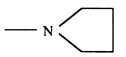
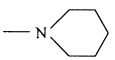
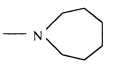
located anywhere in the molecule. In this mode it also is beneficial if the analyte has some steric bulk or an aromatic ring associated with it.[12,31]

In this study, a large number of racemic aromatic, amino alcohols were evaluated on teicoplanin and vancomycin CSPs. A common mobile phase consisting of methanol/acetonitrile/acetic acid/triethylamine (45/55/0,3/0,2 v/v/v/v) was used in all cases for comparison purposes. In the mobile phase, the amount of acid is higher relative to the amount of base. Therefore, the ionisation of analytes is assured and ion interaction of the stationary phase with functional groups of alkylaminoderivatives of aryloxypropanols is also probable.

The results of the separation of enantiomers of derivatives of aryloxypropanols are summarised in Tables 1-3. From Table 1, it is evident that in the tested

Table 3. Chromatographic Data for the Enantioseparation of Derivatives of Aryloxypropanols Without Substitution in 2-Position on Aromatic Ring on Vancomycin (V) and Teicoplanin (T) Bonded Chiral Stationary Phases (Chromatographic Conditions: See Experimental)



Nr.	R_1	R_2	k_1	α	R_s	$-\Delta_{1,2}\Delta G°$ J/mol	Column
55		$-CH_3$	3.09	1.04	0.61	86.67	V
			4.29	1.06	0.82	142.3	T
56			2.73	1.03	0.45	68.6	V
			3.65	1.05	0.67	127.8	T
57			2.67	1.04	0.42	85.0	V
			3.67	1.04	0.55	107.9	T
58	$-NHCH(CH_3)_2$		2.60	1.08	1.62	204.2	V
			3.77	1.14	1.84	325.9	T
59		$-C_2H_5$	2.86	1.04	0.63	93.4	V
			3.95	1.07	0.95	169.8	T
60			2.72	1.03	0.48	68.9	V
			3.64	1.05	0.67	121.8	T
61			2.53	1.04	0.45	85.3	V
			3.37	1.06	0.74	161.4	T
62	$-NHCH(CH_3)_2$		2.39	1.08	1.57	199.4	V
			3.38	1.14	1.78	331.4	T

RSD for k ~ 2-4 %.

mobile phase, the number of carbon atoms in the R_3 substituent has only a small effect on the retention factor values (k = 1,5 - 3,7). It can be observed, that the retention factor values decrease very slowly as the number of carbon atoms in the R_3 substituent increases in the range C_1 - C_9. The worst resolutions of enantiomers (R_s ~ 1) were obtained for analytes with longer alkoxysubstitution (C_6 - C_9) in the case of the vancomycin chiral stationary phase. The influence of the

length of the R_3 substituent on resolution is not significant for the teicoplanin chiral stationary phase. The cycloalkyl substitution (cyclopentyl-, cyclohexyl-) and branching of R_3 substituent have no significant influence on the selectivity and the resolution of enantiomers for either of the macrocyclic chiral stationary phases. The R_2 substituent also has no significant influence on the selectivity and the resolution for either macrocyclic chiral stationary phase (Table 1). Since the R_2 group is far from the stereogenic center, and far from both of the polar moieties of the analyte (which are responsible for the associative interactions with the CSPs) it can have little effect on enantioselectivity. The increase in length of the R_2 alkyl chain has a small effect on retention, resulting in decreased retention times.

Clearly, the type of nitrogen substitution in R_1 has the greatest influence on the enantioseparation. If the nitrogen is part of a heterocyclic ring, no separation (morpholino-) or very poor separations (piperidyl-, pyrolidyl-, perhydroazepinyl-) ($R_s < 0,8$) of enantiomers were obtained (Table 2). This is in stark contrast to compounds with alkylamino- R_2 substituents (Table 1). Branching of aminoalkyl substituents have a positive effect on the enantioselectivity.

As might be expected, it is the environment near the stereogenic center that exerts the greatest influence on enantioresolution. Higher R_s values were obtained for derivatives without substitution in the 2-position of the aromatic ring (Table 3) in comparison to the 2-substituted derivatives (Tables 2 and 1). This means that substituents in the 2-position of the aromatic ring, sterically hinder enantioselective interaction somewhat. When the 2-position is without substitution the enantiomers are more retained on the stationary phase and the highest resolutions for enantioseparation were obtained. Substitution in the 2-position on the aromatic ring only crowd the adjacent stereogenic centre environment somewhat, and slightly diminish interactions with the chiral stationary phase. For both vancomycin and teicoplanin bonded chiral stationary phases the length of the alkoxy-chain (the R_3 substituent) has no significant effect on the enantioseparation.

A similar influence of structure on the resolution of enantiomers was observed for alkoxysubstituted esters of phenylcarbamic acid separated on teicoplanin bonded chiral stationary phase in the polar-organic mode.[34]

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

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

The results given in Tables 1-3 show that only very small energy differences are needed for the chromatographic resolution of enantiomers of these

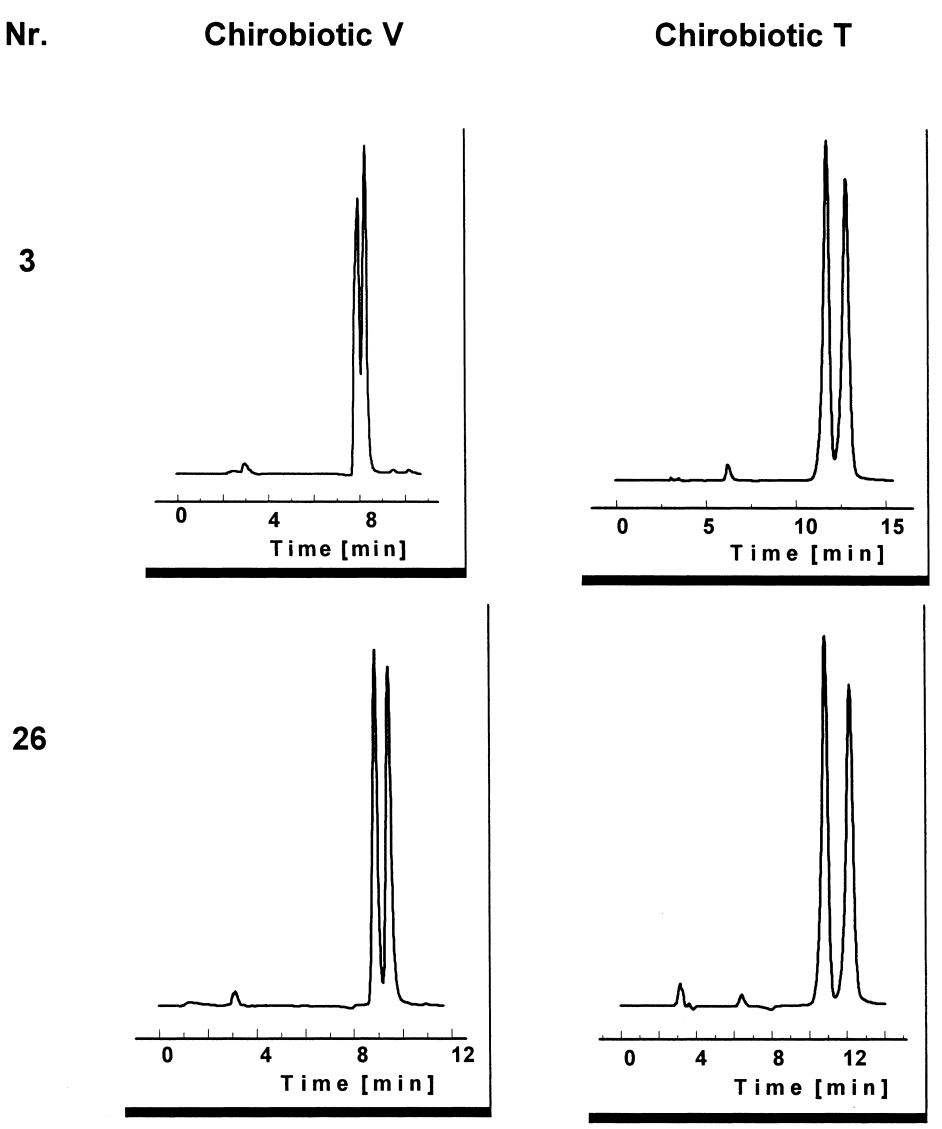


Figure 1. Chromatograms of separation of enantiomers of derivatives of aryloxypropanols on vancomycin and teicoplanin bonded chiral stationary phases. Chromatographic conditions: see experimental.

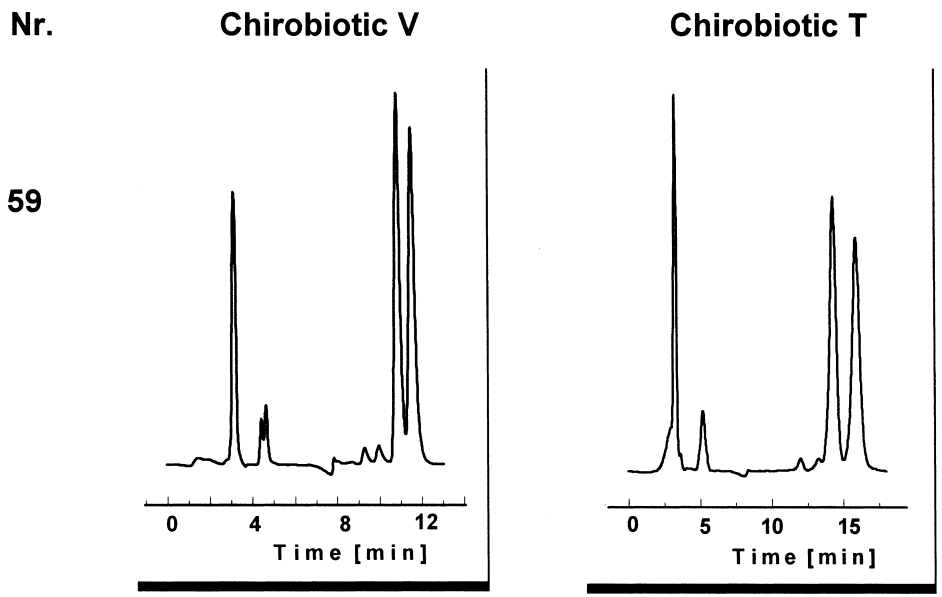


Figure 1. Continued.

alkylaminoderivatives of aryloxypropanols. It is, therefore, obvious that binding of two 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 favourable conformation. The number of carbon atoms in alkoxy- chain length in R_3 substituent (C_1 - C_9) had no significant effect on the free energy differences. The highest values of the free energy difference were obtained for teicoplanin-based chiral stationary phase, which also was reflected by their greater enantioresolutions.

The chromatograms of the separation of enantiomers of alkylaminoderivatives of aryloxypropanols on vancomycin- and teicoplanin-based chiral stationary phases are shown in Figure 1.

CONCLUSION

The teicoplanin- and vancomycin-bonded chiral stationary phases have the capability to separate enantiomeric derivatives of aryloxypropanols. The greatest resolution of these enantiomers was obtained on the teicoplanin chiral stationary phase. According to the results of enantiomeric separations using teicoplanin and vancomycin columns, it can be supposed that the interactions needed for chiral res-

olution of enantiomers involve charge interaction between functional groups of the macrocyclic antibiotic and analyte plus hydrogen bonding interactions; the formation of which is supported in mobile phases containing acetonitrile and methanol solvents. Steric interactions also contributes to enantioselectivity. The substituents in the 2-position of the aromatic ring influence the asymmetric carbon atom environment and have negative effect on the resolution of enantiomers. The substituents in 3-position of the aromatic ring have no significant effect on the enantioseparation. It seems that the type of nitrogen substituents in the hydrophilic part of molecule has dominant influence on the resolution of enantiomers.

One important aspect of this work is that it demonstrates that an electrostatic interaction can substitute for a hydrogen bonding interaction in the polar organic mode on macrocyclic glycopeptide CSPs. It appears that a pertinent associative interaction is between the protonated amine functional group of the analyte and the carboxylic acid group on the aglycone portion of the macrocyclic glycopeptide. This interaction site is different from that which has been defined for amino acids and peptides.

ACKNOWLEDGMENTS

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