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HPLC Separation of enantiomers of some potential β-blockers of the aryloxyaminopropanol type using macrocyclic antibiotic chiral stationary phases

Studies of the mechanism of enantioseparation, part XI

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Received April 11, 2005, accepted July 13, 2005

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Pharmazie 60: 888–891 (2005)

The macrocyclic antibiotic type chiral stationary phases based on native vancomycin, teicoplanin and teicoplanin aglycone (Chirobiotic V, Chirobiotic T and Chirobiotic TAG) were used for the HPLC separation of enantiomers of potential β-blockers of the aryloxyaminopropanol type with a morpholino moiety in the hydrophilic part of the molecule. The chromatographic results presented include: retention, separation and resolution factors along with the enantioselective free energy difference corresponding to the separation of the enantiomers. Comparison of the results obtained on the three macrocyclic chiral stationary phases showed that in most cases the teicoplanin aglycone is responsible for enantioseparation of morpholino derivatives. By application of these chiral stationary phase highest resolution factors were achieved with a polar organic mobile phase system.

1. Introduction

Macrocyclic glycopeptides were introduced in 1994 by Armstrong as a new class of chiral selectors for different separation techniques (Armstrong 1994, 1995). This class of chiral stationary phases (CSP) is used especially in the separation of enantiomers containing an ionizable group at or close to the stereogenic center (Wang 2000). The structure of macrocyclic antibiotics contains functional groups that permit enantiomers to interact through π-π, hydrogen bonding, electrostatic interaction, as well as hydrophobic interactions and steric or repulsive hindrances. The macrocyclic part of the glycopeptides can interact with solutes forming inclusion complexes. In liquid chromatography (HPLC), these CSPs can work with polar hydro-organic phases (reversed phase mode), low polar alkane-alcohol mobile phases (normal phase mode) and polar organic mobile phases (non-aqueous organic solvents) with traces of acid and base modifiers to adjust the chiral selector ionization state (polar organic mode or polar ionic mode) (Chirobiotic Handbook 2002).

β-Blockers of the aryloxyaminopropanol type are drugs with a single stereogenic center and exhibit a chiral structure. From the viewpoint of β-adrenolytic activity, (*S*)-(−)-enantiomers are several times more effective. Of the β-blockers of the arylaminopropanol type (*S*)-(−)-penbutolol, (*S*)-(−)-timolol and (*S*)-(−)-levobunolol are used as pure enantiomers (Čižmáriková 2002). Several chiral stationary phases have been used for the separation of enantiomers of β-blocking drug substances: chiral phases consisting of immobilized proteins (Henriksson 1999), β-cyclodextrin (Park 2000), Pirkle-type phases (Petersen 1997), and cellulose-based phases (Sharma 1995).

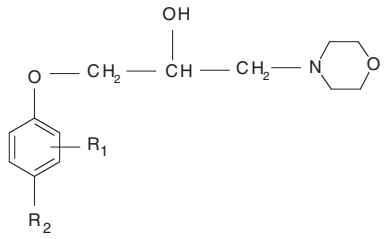
In the present paper, three macrocyclic antibiotic type CSPs, based on native vancomycin, teicoplanin and teicoplanin aglycone were used for the HPLC separation of enantiomers of 15 potential β-blockers of the aryloxyaminopropanol type. This study may contribute to the investigation of the influence of the saccharide parts of CSP on separation of enantiomers.

2. Investigations, results and discussion

The analytes under study (Table 1) can be arranged into three classes. The first class includes compounds without R_1 substitution in 2- or 3-position on aromatic ring. The next two classes include derivatives with 2- or 3-acetyl-substitution or 2-metoxyalkyl substitution. The derivatives with 2-metoxyalkyl substituent include the compounds containing unbranching, branching, saturated and nonsaturated alkyl chain. All relevant separation data on these three classes of compounds are given in Table 1 and Figs. 1 and 2. This includes the retention factors, separation factors, resolution factors and difference in enantioselective free energy of analytes for several mobile and stationary phases.

All studied compounds were evaluated with several polar organic mobile phases (methanol-acetic acid-triethylamine). Table 1 lists only the chromatographic results obtained when enantiomeric separation was achieved (teicoplanin aglykone as CSP, methanol/acetic acid/triethyl/amine 100/0,025/0,017 as mobile phase).

Table 1: Retention factors (k_1), separation factors (α), resolution (R_s) and difference in free energies ($-\Delta(\Delta G^\circ)$) for enantiomeric separation of studied morpholino derivatives of atyloxyaminopropanol on teicoplanin aglycone chiral stationary phase in the polar organic mode



Compd.	R_1	R_2	k_1	α	R_s	$-\Delta(\Delta G^\circ)$ (J · mol^{-1})
1	2-H	4-$COCH_3$	8.16	1.07	1.47	172
2	2-H	4-COC_2H_5	6.13	1.06	1.14	147
3	2-$COCH_3$	4-H	4.48	1.00	0	0
4	3-$COCH_3$	4-H	6.85	1.04	0.79	99
5	2-CH_2OCH_3	4-$COCH_3$	4.70	1.05	0.97	123
6	2-$CH_2OC_2H_5$	4-$COCH_3$	4.32	1.05	0.86	122
7	2-$CH_2OC_4H_9$	4-$COCH_3$	3.20	1.06	1.07	147
8	2-$CH_2OC_5H_{11}$	4-$COCH_3$	5.07	1.08	1.28	194
9	2-$CH_2OC_2H_5$	4-COC_2H_5	4.39	1.08	1.22	196
10	2-$CH_2OC_3H_7$	4-COC_2H_5	4.34	1.07	1.18	170
11	2-$CH_2OC_5H_{11}$	4-COC_2H_5	4.44	1.08	1.21	194
12	2-$CH_2OCH_2CH{=}CH_2$	4-$COCH_3$	6.39	1.07	1.24	170
13	2-$CH_2OCH_2CH{=}CH_2$	4-COC_2H_5	5.99	1.08	1.01	194
14	2-$CH_2O(CH_2)_2OCH_3$	4-$COCH_3$	5.96	1.06	1.04	147
15	2-$CH_2OCH(CH_3)_2$	4-$COCH_3$	3.80	1.05	0.83	123

The chromatographic conditions: mobile phase, methanol/acetic acid/triethylamine 100/0.025/0.017 (v/v/v); flow rate 0.7 ml/min; column temperature 30 °C; UV detection at 276 nm; injection volume 20 µl

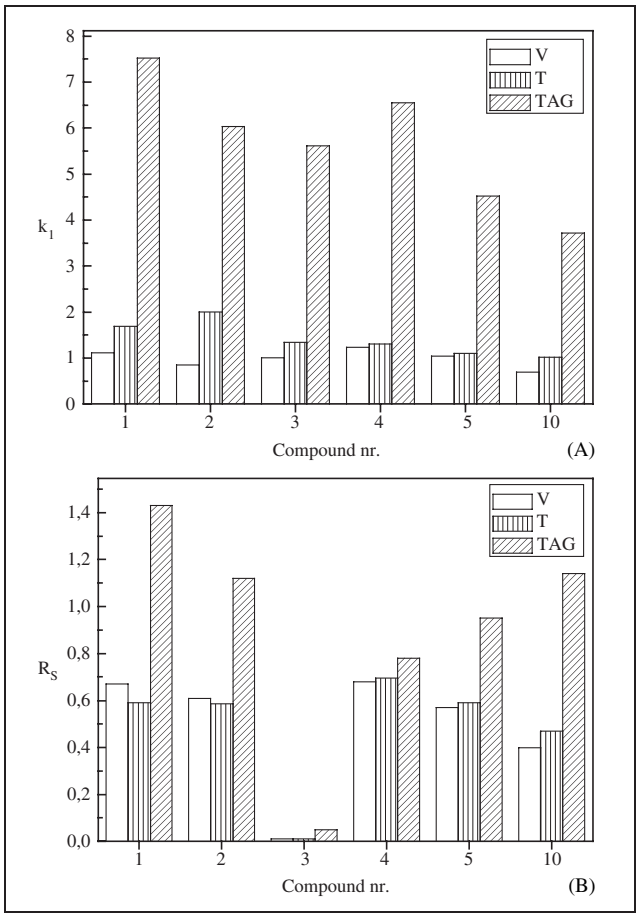


Fig. 1: Retention factors (A) and resolution factors (B) of some compounds obtained on vancomycin (V), teicoplanin (T), and teicoplanin aglycone (TAG) chiral stationary phases.
Chromatographic conditions: mobile phase, methanol/acetic acid/triethylamine 100/0.02/0,01 (v/v/v) for other conditions see Table 1

In this paper antibiotic type CSPs Chirobiotic V, Chirobiotic T and Chirobiotic TAG were tested for the HPLC separation of enantiomers of morpholino-derivatives of the aryloxyaminopropanol.

For the vancomycin macromolecule, the basket has nine hydroxyl groups (of which three are phenolic groups), two amine groups (one primary and one secondary) and one carboxylic group. It has the very low row of six amide linkages, and the five aromatic rings. Polar groups of the free teicoplanin antibiotic include 14 hydroxy groups (of which four are phenolic groups), one carboxylic group, and one amino group. Its apolar groups are nine methylene units of its sugar alkyl chain, the row of six amide linkages in the macrocyclic part of the molecule and seven benzene rings. The molecule of teicoplanin aglycone contains seven polar hydroxy groups (of which six are phenols), the amino and carboxylic acid groups, the apolar row of six amide linkages, and the seven aromatic rings. It lacks the apolar alkyl chain connected to the sugar (Armstrong 1994, 1995). It is evident, that the polarities of these CSPs are difficult to evaluate by considering their molecular structures.

The relative polarities of stationary phases used were evaluated by comparing the retention factors of the aryloxyaminopropanol molecules studied. With the same mobile phase, the retention factors of the first eluted enantiomers increased in the order of the macrocyclic stationary phase:

$$k_1(\text{vancomycin}) < k_1(\text{teicoplanin}) < k_1(\text{teicoplanin aglycone})$$

Fig. 1A shows the retention factors for some compounds obtained on tested stationary phases (for other compounds similar results were obtained). Since the retention factors for the first eluted enantiomer differ considerably on the native antibiotics and aglycone stationary phases, it can be presumed that the relative polarity of the teicoplanin aglycone stationary phase differs from the teicoplanin and vancomycin stationary phases, which have relatively similar polarities.

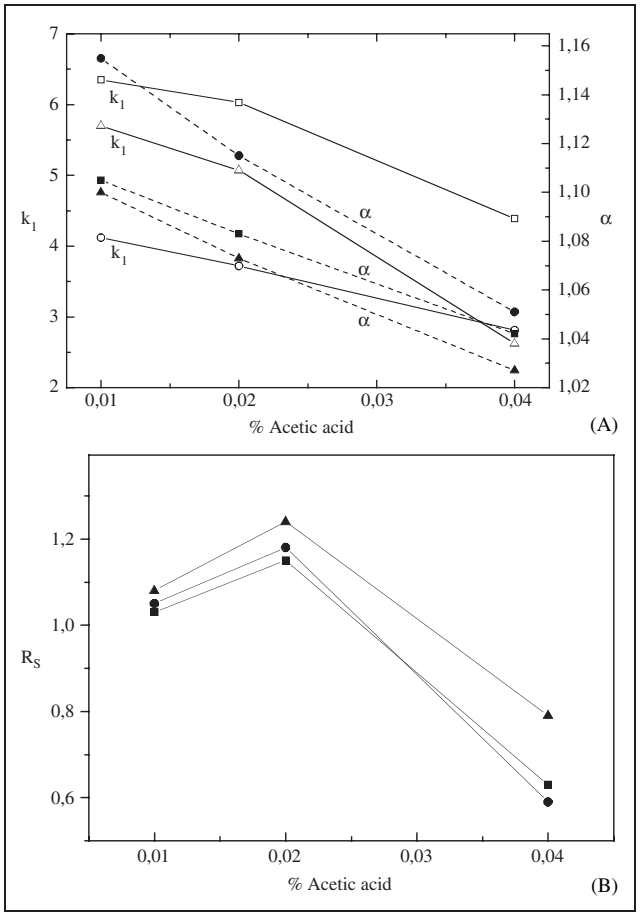


Fig. 2: Retention factors and separation factors (A) and resolution factors (B) vs. content of the acetic acid in mobile phase for teicoplanin aglycone chiral stationary phase.
The chromatographic conditions: mobile phase, methanol/acetic acid/triethylamine 100/x/0,01 (v/v/v) for other conditions see Table 1.
Legend: compound **2** (■, □), compound **8** (▲, △), compound **10** (●, ○)

Table 1 lists the chromatographic results of enantioseparations of the studied compounds obtained at 30 °C on the teicoplanin aglycone CSPs. The separation of enantiomers was obtained on the native teicoplanin and vancomycin CSP only for four compounds (**1**, **2**, **4**, **5**, **10**). The resolution factors were 0–0.70 for native teicoplanin CSP and 0–0.68 for vancomycin CSP (Fig. 1B). In the case of the other derivatives studied no enantioseparation was obtained. The separation factors obtained on the aglycone CSP were in the range 1.00–1.08. The highest α values observed correspond to the highest difference in enantioselective free energy, which is indicative of the good enantiorecognition capability of chiral selector. Table 1 shows that the resolution factors associated with these separation factors are in the interval 0–1.47. A change in the mobile phase composition (the content of acetic acid in the interval 0.01–0.04% (Fig. 2); the content of acetic acid and triethyamine while their ratio was constant (Table 2) produced relatively small variations in separation factors due to the weak dissociation of compounds in the mobile phase used, but on the other side it influenced the resolution of enantiomers. Maximum resolution factor values were obtained at about 0.02% acetic acid and 0.01% triethylamine in polar organic mobile phase (Fig. 2B).
The more effective separations (the highest values of the resolution factors) were obtained for derivatives without substitution in the 2-position on the aromatic ring (compounds **1**, **2**). The number of carbon atoms and branching

Table 2: Chromatographic results of selected compounds obtained on the teicoplanin aglycone chiral stationary phase and different mobile phase composition

Compd.		Methanol/acetic acid/triethylamine v/v/v		
		100/0.025/0.017	100/0.05/0.035	100/0.1/0.07
1	k_1	8.16	4.49	3.10
	α	1.08	1.07	1.05
	R_s	1.47	0.94	0.63
2	k_1	6.13	3.20	2.45
	α	1.06	1.05	1.04
	R_s	1.14	0.62	0.49
10	k_1	4.34	2.16	2.11
	α	1.07	1.05	1.05
	R_s	1.18	0.64	0.53

For chromatographic conditions: see Table 1

in the R_1 substituent has only a small effect on the retention factor values and no significant effect on the resolution factors. The R_2 substituent also has no significant influence on the selectivity and on the resolution. The increase in the length of the alkyl chain has a small effect on the retention.
Previously (Hrobonova 2001) we reported that any separation of morpholino derivatives was obtained on vancomycin and teicoplanin CSP in a polar organic mobile phase system. As it is documented in Table 1 and Fig. 1, the studied analytes are better resolved by the aglycone CSP than by the native teicoplanin CSP. Fig. 3 shows the chro-

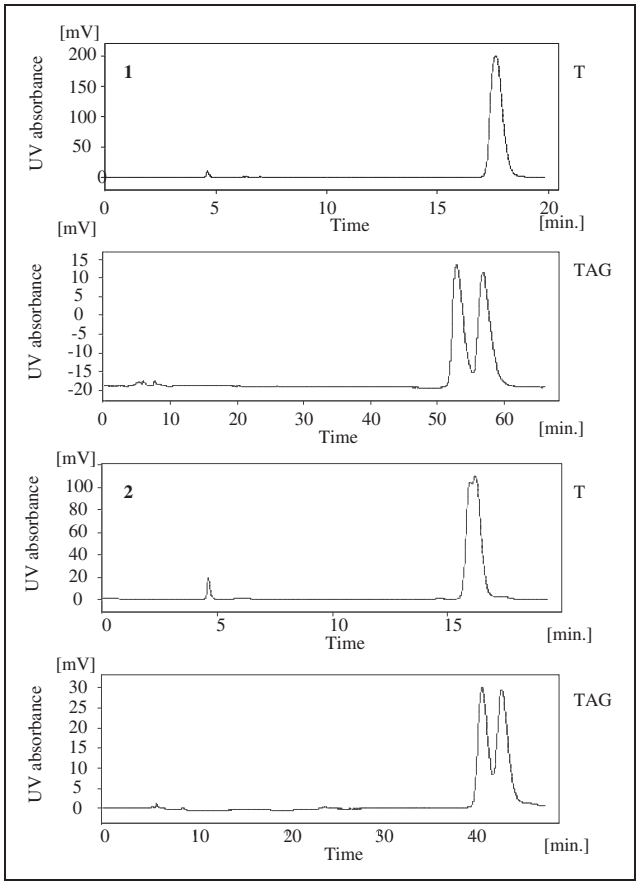


Fig. 3: Separation of enantiomers of compounds **1** and **2** on teicoplanin (T) and teicoplanin aglycon (TAG) chiral stationary phases in the polar organic mode.
Chromatographic conditions: mobile phase, methanol/acetic acid/triethylamine 100/0,025/0,017 (v/v/v) flow rate 0,7 ml/min column temperature 30 °C UV detection at 276 nm injection volume 20 µl

matograms of compounds **1** and **2** on the two CSPs. For the studied compounds, the differences in enantioselective free energy are 2–6 times higher on the aglycone CSP than on the teicoplanin CSP. This energy differences mean that the sugar units decrease the enantiorecognition of the β-blockers of the aryloxyaminopropanol type with morpholino moiety in the basic part of the molecule. The role of the sugar units was pronounced in the case of almost all compounds under study with exception of compound **3** where enantioseparation was not achieved. Compounds **3** and **4** documented that the environment near the stereogenic center influenced the enantioseparation (Čižmáriková 2003; Hroboňová 2001). Substituent in the 2-position on the aromatic ring (compound **3**) sterically hinders enantioselective interaction. When the 2-position is without substitution (compound **4**) the enantiomers are more retained on the stationary phase (the highest value of retention factor for compound **4**) and the highest value of resolution for enantioseparation was obtained.

From the chiral separation point of view, the saccharide moieties of the native teicoplanin may intervene in the chiral recognition process in at least three ways (Berthod 2000): i) by steric hindrance, which limits the access of other molecules to binding sites, ii) by blocking of possible interaction centers on the aglycone, iii) by offering competing interaction sites. The free energy difference between the two CSPs is probably due to the effect of steric hindrance, but the other two possibilities should be considered as well because some of the derivatives (compds. **1**, **2**, **4**, **5**) are partially resolved on the teicoplanin CSP, and the enantioresolution is enhanced on the agycone CSP (Table 1). The absence of sugar units in the aglycone teicoplanin molecule gives the possibility of interactions as soon as charge interaction between chiral selector and analyte and electrostatic interactions between the protonated amine functional group of the analyte and carboxylic acid group on the aglycone portion of the glycopeptide participate on enantiorecognition.

3. Experimental

3.1. Materials

The analytes (Table 1) were prepared according to Čižmáriková et al. (1985, 2003). Methanol of HPLC grade was obtained from Merck (Germany). Triethylamine and acetic acid of analytical-reagent grade were also obtained from Merck (Germany).

3.2. Instruments

The macrocyclic chiral stationary phases Chirobiotic TAG (250 × 4,6 mm I.D. 5 μm), Chirobiotic T (250 × 4,6 mm I.D. 5 μm) and Chirobiotic V (250 × 4,6 mm I.D. 5 μm) (Astec, USA) were used for the separation of enantiomers of studied compounds.

Experiments were performed with a Hewlett Packard (series 1100) HPLC system consisting of a quaternary pump equipped with an injection valve (Rheodyne), diode array detector and thermostat. The mobile phase was a mixture of methanol to which acetic acid and triethylamine were added. All the separations were carried out at a flow rate of 0,7 ml/min and the column temperature was 30 °C. The chromatograms were scanned at the wavelength of 276 nm. The injection volume was 20 μl. The analytes were dissolved in methanol (concentration 1 mg/ml), and filtered through a 0,45 μm filter when necessary. The retention time of solvent peak (methanol) was used for the determination of the dead time for all types of column.

3.3. Chromatographic characteristics

The separation factor (α) was expressed as

$$\alpha = k_2/k_1 \quad (1)$$

where k_2, k_1 are the retention factors for the first and second eluting enantiomer. The retention factors k_1 and k_2 were calculated as follows:

$$k_1 = (t_{R1} - t_0)/t_0 \quad \text{and} \quad k_2 = (t_{R2} - t_0)/t_0 \quad (2)$$

where t_0, t_{R1}, and t_{R2} are the dead elution time and elution time of enantiomers **1** and **2**.

The resolution (R_s) of the first and second eluting enantiomers was calculated by the ratio of the difference between the elution times t_{R1} and t_{R2} to the arithmetic mean of the two peak widths w_1 and w_2.

$$R_s = 2(t_{R2} - t_{R1})/(w_1 + w_2) \quad (3)$$

The difference in the free energy ($-\Delta(\Delta G^\circ)$) was calculated from the separation factor according to the following equation:

$$-\Delta(\Delta G^\circ) = RT \ln \alpha \quad (4)$$

Acknowledgement: Support from the Grant agency Slovak Republic (grants 1/2460/05, 1/0508/03, and 1/1198/04) and International project (SR-USA) (035/2001), is gratefully acknowledged. The authors are thanks to the D.W. Armstrong for the donation of macrocyclic antibiotics chiral stationary phases.

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