SYNTHESIS AND HPLC ENANTIOSEPARATION OF DERIVATIVES OF THE 3-HYDROXYPHENYLETHANONE

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Within the framework of the study of the synthesis and high-performance liquid chromatography (HPLC) enantioseparation the series of 9 derivatives of 3-hydroxyphenylethanone was prepared by a well-tried method. The structure of the prepared compounds was confirmed on the basis of interpretation of the IR, UV, ^{1}H NMR and ^{13}C NMR spectra. An enantioseparation of prepared compounds was performed using HPLC on a native teicoplanin (Chirobiotic T) and the amylose tris (3,5-dimethylphenylcarbamate) (Chiralpak AD) chiral stationary phases, which is more suitable for the enantioseparation of all prepared compounds especially with heterocycles in the basic part of a molecule.

Keywords: aryloxyaminopropanol – hydroxyphenylethanone – enantioseparation – HPLC – Chirobiotic T – Chiralpak AD

INTRODUCTION

Aryloxyaminopropanol type compounds possess in their structure a single stereogenic centre and exist as stereoisomers. Their racemic compounds can be resolved to enantiomers by means of several analytical methods such as HPLC (Matchett et al., 1996, Park et al., 2000, Makamba et al. 1998; Haginaka et al., 1999, Henriksson et al., 1999, Sharma et al., 1995), GC (Gyllenhaal et al., 1985, Donnecke et al., 1996, Abe et al., 1995, Juvancz et al., 1993), TLC (Bhushan & Arora, 2003; Bhushan & Tanwar, 2008, Čižmáriková et al., 2010) or CE (Zhang et al., 2008, Beck & Neau, 2000; Proksa, 1999; Proksa & Čižmáriková, 2001). The most widely technique used for separation of the enantiomers have been HPLC on different chiral stationary phases (CSP) such as

β-cyclodextrin (Matchett et al., 1996; Park et al., 2000), immobilized proteins (Makamba et al., 1998, Haginaka et al., 1999, Henriksson et al., 1999), Pirkle-type phases (Petersen et al., 1997), and cellulose and amylose-based phases (Aboul-Enein & Bakr, 1998; Valentova et al., 2003). In our previous studies (Čižmáriková et al., 2003; Hroboňová et al., 2004, 2005) the enantioseparation of the racemic aryloxyaminopropanol type compounds was studied. In these papers HPLC techniques with chiral stationary phases based on macrocyclic antibiotics (vancomycin, teicoplanin, teicoplanin aglycone, permethylated teicoplanin aglycone), cyclodextrins-β and γ and amylose tris (3,5-dimethylphenylcarbamate) were used.

A common mobile phase that consisted of methanol/acetonitrile/acetic acid/triethylamine (45/55/0.3/0.2 v/v/v/v) (Bruchatá et al., 2010; Čižmáriková et al., 2003) was used in all cases of the enantioseparation using macrocyclic antibiotics. Comparing the separation on a teicoplanin column containing carbohydrate moieties (Chirobiotic T), a teicoplanin column without carbohydrate moieties (Chirobiotic TAG) and a methylated teicoplanin column without carbohydrate moieties (Chirobiotic TAG-methylated), the retention factors were increased in the order: T<TAG<TAG-methylated.

Poor separation of enantiomers was obtained on β and γ −cyclodextrins chiral stationary phases.

Enantioseparation using Chiralpak AD CSP with mobile phase hexane/ethanol/methanol/diethylamine was investigated at the novel series of aryloxyaminopropanol type compounds. The influence of mobile phase composition, particularly alcohol modifier content and composition, an analyte retention and separation were determined; the final composition being hexane/ethanol/methanol/diethylamine (85/3.75/11.25/0.1 v/v/v/v). The analyte structure, including the position and nature of aromatic substitution, steric bulk of the nitrogen alkyl substituent and length and bulk of the side chain were found to influence both retention and chiral discrimination.

EXPERIMENTAL DETAILS

The melting points were determined using a Kofler Micro Hot Stage and were quoted uncorrected. The purity of the prepared compounds was assessed using Silica gel plates UV 254 (Merck), and the solvent system of ethylacetate/diethylamine (9.5/0.5 v/v) was used. UV spectra were run on spectrophotometer GENESYS 10s UV-Vis in methanol. Concentration of compounds was about 10^{-4} mol.dm^{-3}. IR spectra were recorded using Nicolet 6700 (Termo Scientific). ^{1}H NMR and ^{13}C NMR were recorded on the Varian Gemini 2000 Spectrometer operating at 300 MHz for protons.

HPLC-chromatography
Instruments
HPLC studies were performed with a Hewlett Packard (series 1 100) HPLC-system consisting of a quaternary pump equipped with an injection valve (Rheodyne) and a diode array detector. The macrocyclic chiral stationary phase was Chirobiotic T (250

× 4 mm I.D. particle size 5 μm) (Advanced Separation Technologies. Inc. USA). The mobile phase was a mixture of methanol/acetonitrile/acetic acid/triethylamine (45/55/0.3/0.2 v/v/v/v). The separation was carried out at a flow rate of 1 ml.min^{-1} and column temperature was 23$^{°}$ C. The chromatograms were scanned at 270 nm. The injection volume was 20 μl. The analytes were dissolved in methanol (concentration 1 mg.ml^{-1}).

The experimental tasks for second studies were carried out using HPLC system AGILENT (series 1200), consisting of a quaternary pump and a diode detector.

HPLC was carried out using the chiral stationary phases (Chiralpak AD) based on the amylose tris (3,5-dimethylphenylcarbamate) (250x4.6 mm I.D. particle size 5 μm). The mobile phase consisted of hexane/ethanol/methanol/diethylamine (85/3.75/11.25/0.1 v/v/v/v). The samples for analysis were prepared as approximately 1 mg.ml^{-1} solution in mobile phase. Separation was carried out at a flow rate of 0.8 ml.min^{-1}, with a column temperature maintained at 25 ºC. The chromatograms were scanned at wavelength 267 nm ± 8 nm.

Chromatographic characteristics

The separation factor was expressed as $\alpha = k_1/k_2$, where k_1, k_2 are the retention factors for the first and second eluting enantiomer. The retention factor k was calculated as follows: $k_1 = (t_1-t_0)/ t_0$ and $k_2 = (t_2-t_0)/t_0$, where t_0 is the dead elution time and t_1 and t_2 are the elution times of enantiomers 1 and 2. The stereochemical resolution factor (R_s) of the first and the second eluting enantiomer was calculated as the ratio of the difference between the retention times t_1 and t_2 to the sum of the two peaks' widths w_1 and w_2: $R_S = 2 (t_2-t_1)/(w_1+ w_2)$.

Chemicals

All HPLC grade solvents were obtained from Merck (Germany).

Synthesis

[3-(2-hydroxy-3-alkylaminopropoxy)phenyl]ethanone and
[3-(2-hydroxy-3-heterocyclopropoxy)phenyl]ethanone.

To 0.15 mol of 3-hydroxyphenylethanone and 11 g 85 % KOH, 3 mol (235 ml) of chloromethyloxirane was gradually added. The mixture reacted for 4 h with stirring at the temperature of 50-55 °C. The produced KCl was sucked off and residual chloromethyloxirane was distilled off in a vacuum. The residue was extracted with chloroform and the organic layer was shaken with NaOH solution (2 mol.l^{-1}) and saturated NaCl solution. The chloroform solution was dried over anhydrous MgSO$_4$ and the chloroform was distilled off. Crude epoxide (60 %) was dissolved in ethanol (200 ml) and reacted with respective amine (20 ml). The mixture was kept at 30 °C for 3 h and then at a reflux for 4 h. The solvent and the unreacted amine were removed in vacuo, the residue was diluted with H$_2$O (100 ml) and the base was taken into diethylether. The extract was dried with K$_2$CO$_3$. Addition of an ether solution of fumaric or oxalic acid resulted in separation of the salt which was crystallized from an appropriate solvent (ethylacetate or propan-1-ol). Yield and physico-chemical parameters of prepared compounds are listed in Table 1.

Table 1. Physico-chemical parameters of prepared compounds
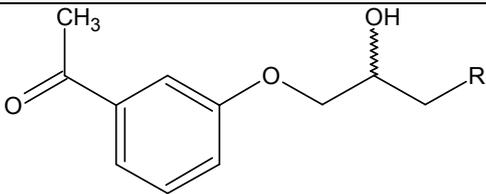
Compound Form of compound	R	Empirical formula Mr	M.p.[ºC] Solvent	Yield [%] R_F
I Base	Isopropylamino	$C_{14}H_{21}O_3N$ 251.33	64-65 Hexane	67 0.25
Ia Fumarate		$C_{28}H_{42}O_6N_2.C_4H_4O_4$ 618.74	139-142 ethyl acetate	0.26
II Base	*Tert*-butylamino	$C_{15}H_{23}O_3N$ 265.36	73-75 Hexane	70 0.38
IIa Fumarate		$C_{30}H_{46}O_6N_2.C_4H_4O_4$ 646,79	147-149 ethyl acetate	0.38
III Base	Isobutylamino	$C_{15}H_{23}O_3N$ 265.36	viscous oil	79 0.48
IIIa Fumarate		$C_{30}H_{46}O_6N_2.C_4H_4O_4$ 646.79	112-114 propan-2-ol	0.47
IV Base	Pyrrolidin-1-yl	$C_{15}H_{21}O_3N$ 263.34	viscous oil	78 0.52
Iva Fumarate		$C_{30}H_{42}O_6N_2.C_4H_4O_4$ 642.75	102-105 ethyl acetate	0.53
IVb Oxalate		$C_{30}H_{42}O_6N_2.C_2H_2O_4$ 616.71	139-141 ethyl acetate	0.51
V Base	Piperidino	$C_{16}H_{23}O_3N$ 277.37	84-86 a Hexane	66 0.66
Va Fumarate		$C_{32}H_{46}O_6N_2.C_4H_4O_4$ 670.79	165-7 ethyl acetate	0.67
VI Base	Azepan-1-yl	$C_{17}H_{25}O_3N$ 291.39	58-60 Hexane	63 0.78
Via Fumarate		$C_{34}H_{50}O_6N_2.C_4H_4O_4$ 698.85	169-172 ethyl acetate	0.77
VII Base	Morfolino	$C_{15}H_{21}O_4N$ 279.34	88-89 b Hexane	72 0.52
VIIa Fumarate		$C_{30}H_{38}O_8N_2.C_4H_4O_4$ 674.75	92-93	0.52

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VIII Base	4-Methyl- piperazine-1-yl	$C_{16}H_{24}O_3N_2$ 292.38	81-83 Hexane	76 0.71
VIIIa Fumarate		$C_{16}H_{24}O_4N_2$. $2C_4H_4O_4$ 524.52	158-160 ethyl acetate	0.71
IX Base	4-(2- Methoxyphenyl) piperazin-1-yl	$C_{22}H_{29}O_4N_2$. 372.47	72-74 Hexane	70 0.77
IXa Fumarate		$C_{22}H_{29}O_4N_2.C_4H_4O_4$ 488.54	172-173 propan-1-ol	0.78

M.p. melting point, R_F retardation factor
a)   m.p. 86; b) m.p. 91 according to Rastogi et al., (1973).

RESULTS AND DISCUSSION

The aim of this study was to prepare 9 derivatives of [1-alkylamino- or 1-heterocyclo-2-hydroxyphenyl]ethanone with branched alkylamino (isopropyl, *terc*-butyl and isobutyl) or heterocyclo (pyrolidin-1-yl, piperidino, azepan-1-yl, morpholino, 4-methylpiperazin-1-yl and 4-(2-methoxyphenylpiperazin-1-yl) in the basic part of molecule. The compounds were prepared by a two-step synthesis from 3-hydroxyphenylethanone. Oxirane intermediate prepared by the reaction of 3-hydroxyphenylethanone with chloromethyloxirane gives the final products by the reaction with appropriate amines. These were isolated in the form of free bases or salts with fumaric acid and as compounds with oxalic acid (Table 1).
The purity of the final products was checked by TLC using mobile phase ethylacetate/diethylamine (9.5/0.5 v/v). Structures of the prepared compounds were confirmed by IR, UV and NMR spectra (Table 2, 3, 4). The stretching vibrations of the characteristic groups in the IR spectra were ν(OH) 3139-3295 cm^{-1}, ν(NH) (base) 3073-3074 cm^{-1}, ν(C=C) 1562-1594 cm^{-1}, and ν(C$_{Ar}$-O-C$_{Alk}$) 1625-1682 cm^{-1} (Table 2).

Table 2. Values of the stretching vibration in IR spectra of the prepared bases

Compounds	ν(OH) [cm^{-1}]	ν(NH) [cm^{-1}]	ν(C=C) [cm^{-1}]	ν(C=O) [cm^{-1}]	ν(C$_{Ar}$-O-C$_{Alk}$) [cm^{-1}]
I	3281	3074	1594	1682	1267
II	3146	3073	1593	1673	1219
IIIa	3139		1562	1625 1683	1269
IVb	3295		1581	1680	1281
V	3154		1592	1682	1220
VIIa	3169		1591	1678	1268

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ν absorbtion maximum

The UV spectra of bases display bands corresponding to $\pi \rightarrow \pi^*$ transition at λ_{max} = 208 - 290 nm, log ε = 2.74 - 4.07 (Table 3).

Table 3. Values of λ_{max} and log ε in UV spectra, [ε]= $m^2.mol^{-1}$

Compounds	$\lambda_{max\ 1}$ [nm]	log ε_1	$\lambda_{max\ 2}$ [nm]	log ε_2	$\lambda_{max\ 3}$ [nm]	log ε_3
I	216	3.40	247	3.31	305	3.36
II	216	3.41	248	3.34	305	3.38
IIIa	215	3.83	247	3.67	304	3.67
IVa	215	3.72	247	3.60	303	3.64
VI	217	3.37	248	3.27	306	3.36
V	217	3.44	248	3.33	306	3.39
VIIa	217	3.86	249	3.72	306	3.78
IX	212	2.70	246	3.23	284	2.69
IXa	208	4.03	240	3.88	280	4.07

λ_{max} wave length, ε molar extinction coefficient

The structure of the aminopropanol chain was proofed by ^{1}H-NMR and ^{13}C-NMR spectra (Table 4, Table 5). Two HPLC methods with chiral stationary phases based on native teicoplanin (Chirobiotic T) and derivatised amylose (Chiralpak AD) were used for enantioseparation of racemic compounds in this work.

Table 4. ^{1}H NMR spectral data of bases δ [ppm] ($CDCl_3$, δ, TMS)

Compounds	δ [ppm] number of protons, multiplicity
I	1.09 (d, 6H, NH-CH-(C<u>H</u>$_3$)$_2$), 2.76 (m, 1H, NH-C<u>H</u>), 2.92 (m, 2H, Ar-O-C<u>H</u>$_2$), 2.82 (m, 1H, C<u>H</u>-OH), 2.46 (m, 2H, C<u>H</u>$_2$-NH), 2.59 (s, 3H, C<u>H</u>$_3$-CO), 7.14 (d, 1H, Ar-H^4), 7.26 (s, 1H, Ar-H^2), 7.36 (d, 1H, Ar-H^6), 7.53 (t, 1H, Ar-H^5)
Ia	1.38 (d, 6H, NH-CH-(C<u>H</u>$_3$)$_2$), 3.54 (m, 1H, NH-C<u>H</u>), 4.19 (m, 2H, Ar-O-C<u>H</u>$_2$), 4.35 (m, 1H, C<u>H</u>-OH), 3.35 (d, 2H, C<u>H</u>$_2$-NH), 2.65 (s, 3H, C<u>H</u>$_3$-CO), 7.27 (d, 1H, Ar-H^4), 7.31 (m, 1H, Ar-H^2), 7.63 (d, 1H, Ar-H^6), 7.51 (t, 1H, Ar-H^5), 6.51 (s, 2H, CH-COO$_{fumar}$)
II	1.13 (s, 9H, NH-C-(C<u>H</u>$_3$)$_3$), 4.03 (m, 2H, Ar-O-C<u>H</u>$_2$), 3.98 (m, 1H, C<u>H</u>-OH), 2.69 (d, 2H, C<u>H</u>$_2$-NH), 2.60 (s, 3H, C<u>H</u>$_3$-CO), 7.15 (d, 1H, Ar-H^4), 7.26 (s, 1H, Ar-H^2), 7.39 (m, 1H, Ar-H^6), 7.55 (t, 1H, Ar-H^5)+
IIa	1.43 (s, 9H, NH-C-(C<u>H</u>$_3$)$_3$), 4.19 (m, 2H, Ar-O-C<u>H</u>$_2$), 4.31 (m, 1H, C<u>H</u>-OH), 3.34 (d, 2H, C<u>H</u>$_2$-NH), 2.66 (s, 3H, C<u>H</u>$_3$-CO), 7.31 (d, 1H, Ar-H^4), 7.48 (s, 1H, Ar-H^2), 7.51 (m, 1H, Ar-H^6), 7.65 (m, 1H, Ar-H^5), 6.5 (s, 2H, CH-COO$_{fumar}$)
III	0.92 (d, 6H, CH-(C<u>H</u>$_3$)$_2$), 1.78 (m, 1H, C<u>H</u>-(CH$_3$)$_2$), 2.89 (d, 2H, NH-C<u>H</u>$_2$), 4.03 (m, 2H, Ar-O-C<u>H</u>$_2$), 2.48 (m, 1H, C<u>H</u>-OH), 3.10 (m, 2H, C<u>H</u>$_2$-NH), 2.58 (s, 3H, C<u>H</u>$_3$-CO), 7.13 (d, 1H, Ar-H^4), 7.27 (s, 1H, Ar-H^2), 7.35 (m, 1H, Ar-H^6), 7.52 (t, 1H, Ar-H^5)
IIIa	1.00 (m, 6H, CH-(C<u>H</u>$_3$)$_2$), 2.11 (m, 1H, C<u>H</u>-(CH$_3$)$_2$), 3.33 (m, 2H, NH-C<u>H</u>$_2$), 4.19 (m, 2H, Ar-O-C<u>H</u>$_2$), 4.39 (m, 1H, C<u>H</u>-OH), 2.85 (d, 2H, C<u>H</u>$_2$-NH), 2.65 (s, 3H, C<u>H</u>$_3$-CO), 7.27 (d, 1H, Ar-H^4), 7.46 (s, 1H, Ar-H^2), 7.51 (m, 1H, Ar-H^6),

	7.67 (t, 1H, Ar-H^5), 6.55 (s, 2H, CH-COO$_{fumar}$)
IV	2.08 (m, 4H, pyrH^{2,6}), 1.87 (m, 4H, pyrH^{3,5}), 1.83 (m, 2H, pyrH^4), 4.05 (m, 2H, Ar-O-CH$_2$), 3.37 (m, 1H, CH-OH), 2.72 (m, 2H, CH$_2$-Npyr), 2.81 (m, 3H, CH$_3$-CO), 7.12 (d, 1H, Ar-H^4), 7.27 (s, 1H, Ar-H^2), 7.36 (m, 1H, Ar-H^6), 7.53 (m, 1H, Ar-H^5)
VI	2.76 (m, 4H, azepH^{2,7}), 1.70 (m, 4H, azepH^{3,6}), 1.62 (m, 4H, azepH^{4,5}), 4.02 (m, 2H, Ar-O-CH$_2$), 2.56 (m, 1H, CH-OH), 2.60 (d, 2H, CH$_2$-Nazep), 2.59 (s, 3H, CH$_3$-CO), 7.50 (d, 1H, Ar-H^4), 7.26 (s, 1H, Ar-H^2), 7.13 (d, 1H, Ar-H^6), 7.36 (t, 1H, Ar-H^5)
VII	2.49 (m, 4H, morfH^{2,6}), 3.73 (m, 4H, morfH^{3,5}), 4.04 (m, 2H, Ar-O-CH$_2$), 3.38 (m, 1H, CH-OH), 2.68 (m, 2H, CH$_2$-Nmorf), 2.59 (m, 3H, CH$_3$-CO), 7.15 (d, 1H, Ar-H^4), 7.27 (s, 1H, Ar-H^2), 7.40 (m, 1H, Ar-H^6), 7.51 (m, 1H, Ar-H^5)
VIII	2.31 (s, 3H, CH$_3$-N$_{pip}$), 2.52 (m, 4H, pipH^{2,6}) 2.50 (m, 4H, pipH^{3,5}), 4.21 (m, 2H, Ar-O-CH$_2$), 4.03 (m, 1H, CH-OH), 2.64 (m, 2H, CH$_2$-NH), 2.39 (s, 3H, CH$_3$-CO), 7.15 (d, 1H, Ar-H^4), 7.25 (s, 1H, Ar-H^2), 7.52 (m, 1H, Ar-H^6), 7.39 (t, 1H, Ar-H^5)
VIIIa	2.22 (s, 3H, CH$_3$-N$_{pip}$), 4.15 (m, 4H, pipH^{2,6}) 2.84 (m, 4H, pipH^{3,5}), 4.16 (m, 2H, Ar-O-CH$_2$), 4.28 (m, 1H, CH-OH), 3.63 (m, 2H, CH$_2$-Npip), 2.66 (s, 3H, CH$_3$-CO), 7.27 (d, 1H, Ar-H^4), 7.47 (s, 1H, Ar-H^2), 7.49 (t, 1H, Ar-H^6), 7.64 (d, 1H, Ar-H^5), 6.55 (s, 2H, CH-COO$_{fumar}$)

δ chemical schift

Table 5. ^{13}C NMR spectral data of bases δ [ppm] (CDCl$_3$, δ, TMS)

Compounds	δ [ppm]
I	23.13 (NH-CH-(CH$_3$)$_2$), 26.89 (CO-CH$_3$), 49.08 (NH-CH-(CH$_3$)$_2$), 49.25 (CH$_2$-NH-), 68.49 (CH-OH), 70.89 (ArO-CH$_2$-), 113,32 (ArC2), 120.14 (ArC4), 121.52 (ArC6), 129.74(ArC5), 138.59 (ArC3), 159.05 (ArC1), 198 (Ar-CO)
Ia	20.75 (NH-CH-(CH$_3$)$_2$), 29.23 (CO-CH$_3$), 49.53 (NH-CH-(CH$_3$)$_2$), 53.94 (CH$_2$-NH-), 68.47 (ArO-CH$_2$-), 72.45 (CH-OH), 112.64 (ArC2), 123.45 (ArC4), 125.13 (ArC5), 133.03 (ArC6), 138.17 (CH=CH$_{fumar}$), 140.72 (ArC3), 160.96 (ArC1), 177.39 (COO$_{fumar}$), 206.25 (Ar-CO)
II	26.86 (CO-CH$_3$), 29.27 (C-(CH$_3$)$_3$), 44.66 (CH$_2$-NH-), 50.52 (C-(CH$_3$)$_3$), 68.69 (CH-OH), 70.9 (ArO-CH$_2$-), 113.34 (ArC2), 120.12 (ArC4), 121.44 (ArC6), 129.7 (ArC5), 138.57 (ArC3), 159.09 (ArC1), 197.99 (Ar-CO)
IIa	27.64 (CO-CH$_3$), 29.25 (C-(CH$_3$)$_3$), 46.74 (CH$_2$-NH-), 60.31 (C-(CH$_3$)$_3$), 68.76 (ArO-CH$_2$-), 72.51 (CH-OH), 116.34 (ArC2), 123.47 (ArC4), 125.14 (ArC5), 133.04 (ArC6), 138.17 (CH=CH$_{fumar}$), 140.76 (ArC3), 160.98 (ArC1), 177.41 (COO$_{fumar}$), 206.29 (Ar-CO)
III	20.67 (CH-(CH$_3$)$_2$), 26.85 (CO-CH$_3$), 28.36 (CH-(CH$_3$)$_2$), 51.82 (CH$_2$-NH-), 57.81 (CH$_2$-CH-(CH$_3$)$_2$), 67.98 (CH-OH), 70.86 (ArO-CH$_2$-), 113.33 (ArC2), 120.09 (ArC4), 121.47 (ArC6), 129.71 (ArC5), 138.54 (ArC3), 159.03 (ArC1), 198 (Ar-CO)
IIIa	21.93 (CH-(CH$_3$)$_2$), 28.22 (CO-CH$_3$), 29.31 (CH-(CH$_3$)$_2$), 52.71 (CH$_2$-NH-), 57.79 (CH$_2$-CH-(CH$_3$)$_2$), 68.1 (ArO-CH$_2$-), 72.58 (CH-OH), 116.47 (ArC2), 123.54 (ArC4), 125.22 (ArC5), 133.12 (ArC6), 138.04 (CH=CH$_{fumar}$), 140.85 (ArC3), 161.03 (ArC1), 176.52 (COO$_{fumar}$), 206.36 (Ar-CO)
IV	23.65 (C$_{pyr}^{3,4}$), 26.76 (CO-CH$_3$), 54.2 (CH$_2$-N$_{pyr}$), 58.34 (C$_{pyr}^{2,5}$), 67.23 (CH-

	OH), 70.67 (ArO-$\underline{C}H_2$-), 113.26 (ArC2), 121.31 (ArC4), 129.58 (ArC6), 129.77 (ArC5), 138.41 (ArC3), 158.98 (ArC1), 197.93 (Ar-CO)
VI	26.77 ($C_{pha}^{4,5}$), 26.92 (CO-$\underline{C}H_3$), 28.50 ($C_{pha}^{3,6}$), 55.82 ($C_{pha}^{2,7}$), 60.16 ($\underline{C}H_2$-NH-), 65.89 ($\underline{C}H$-OH), 70.58 (ArO-$\underline{C}H_2$-), 113.27 (ArC2), 120.04 (ArC4), 121.26 (ArC6), 129.57 (ArC5), 138.41 (ArC3), 159.08 (ArC1), 197.94 (Ar-CO)
Via	22.98 ($C_{pha}^{4,5}$), 26.76 (CO-$\underline{C}H_3$), 27.06 ($C_{pha}^{3,6}$), 55.93 ($C_{pha}^{2,7}$), 61,01 ($\underline{C}H_2$-NH-), 64.57 ($\underline{C}H$-OH), 70.13 (ArO-$\underline{C}H_2$-), 113.63 (ArC2), 120.83 (ArC4), 121.51 (ArC6), 129.73 (ArC5), 135.66 (ArC3), 138.5 (CH=CH$_{fumar}$), 158.49 (ArC1), 167.74 (COO$_{fumar}$), 197.79 (Ar-CO)
VII	26.74 (CO-$\underline{C}H_3$), 53.72 ($\underline{C}H_2$-N$_{morf}$), 60.88 ($C_{morf}^{2,6}$), 65.31 ($\underline{C}H$-OH), 66.94 ($C_{morf}^{3,5}$), 70.37 (ArO-$\underline{C}H_2$-), 113.1 (ArC2), 120.03 (ArC4), 121.44 (ArC6), 129.6 (ArC5), 138.42 (ArC3), 158.89 (ArC1), 197.85 (Ar-CO)
VIIIa	29.32 (CO-$\underline{C}H_3$), 45.90 (N$_{4pip}$-$\underline{C}H_3$), 52.99 ($C_{pip}^{2,6}$), 55.65 ($C_{pip}^{3,5}$), 61.76 ($\underline{C}H_2$-N$_{pip}$), 69.1 (ArO-$\underline{C}H_2$-), 73.32 ($\underline{C}H$-OH), 116.54 (ArC2), 123.64 (ArC4), 125.06 (ArC5), 133.08 (ArC6), 138.06 (CH=CH$_{fumar}$), 140.87 (ArC3), 161.27 (ArC1), 176.72 (COO$_{fumar}$), 221.5 (Ar-CO)

δ chemical schift

Native teicoplanin contains in its structure carbohydrate moieties with functional groups that permit hydrogen and π - π bonds, electrostatic interactions as well as hydrogen and steric repulsion hindrances.

In the mobile phase methanol/acetonitrile/acetic acid/trietylamine (45/55/0.3/0.2 v/v/v/v), the amount of the acid is relatively higher to the amount of base. Therefore, the ionisation of analytes is assured and ionic interactions of the stationary phase with functional groups are also probable. The presence of methanol in the mobile phase supports the production of hydrogen bonds, which have an effect on the resolution of enantiomers. The results of the enantioseparation are summarized in Table 6. It is evident that the character of substituent on the basic nitrogen has influence on enantioseparation of the prepared racemic compounds.

Table 6. Chromatographic data for the enantioseparation on teicoplanin bonded chiral stationary phase (Chirobiotic T)

Compound	t_1	k_1	α	R_s
Ia	18.58	3.67	1.20	2.01
IIa	16.84	3.23	1.20	2.62
IIIa	16.14	3.05	1.08	1.11
IV	6.87	0.73	1.38	1.23
VI	6.60	0.66	-	-
VIIa	5.10	0.28	1.17	0.50
VIIIa	4.13	0.04		
	6.45	1.62		

R_s stereochemical resolution factor, t_1 elution time for enantiomer 1, k_1 retention factor for enantiomer 1, α separation

Mobile phase: methanol/acetonitrile/acetic acid/trietylamine (45/55/0,3/0,2 v/v/v/v)

The results of the resolution showed that compounds with alkyl substituent (I-III) (isopropyl, *tert*-butyl, isobutyl) were effectively separated with resolution factors in the range 1.11-2.62 and selectivity factor in 1.08-1.38. Racemic compounds with heterocycles (V-IX) (morpholino, piperidino, azepan-1-yl, 4-methyl piperazine and 4-(2-methoxyphenyl)piperazine-1-yl) showed either any or poor resolution.

The second direct HPLC method was performed using polysaccharide derivatives as the chiral stationary phase. Amylose tris (3,5-dimethylphenylcarbamate) was used in our research, in which 3 hydroxy groups of amylose are substituted with carbamate moiety. The mechanism of chiral separation on the amylose tris (3,5-dimethylphenylcarbamate) is thought to involve the formation of complexes between the enantiomeric analytes and chiral cavities in the higher order structures of chiral stationary phase.

The initial analyte-phase interaction begin via hydrogen bond formation with the amide N-H and carbonyl groups of the carbamate moiety, followed by π - π and/or dipole-dipole interactions and formation of analyte-phase complexes without the structure of chiral stationary phase.

Mobile phase hexane/ethanol/methanol/diethylamine (85/3.75/11.25/0.1 v/v/v/v) was used for the enantioseparation. The presence of the alcohols had influence on the interactions and resolution by alteration of the steric environment of the chiral cavities. The addition of low concentration of the basic additive diethylamine improved the chromatography via interaction with chiral stationary phase.

The influence of the structure of alcohol modifier and its content in mobile phase was studied in the work of Valentova, (2003). From Table 7 and Figs. 1 - 4 it is evident that effective enantioseparation was achieved for all the prepared compounds with resolution factor 2.20-21.80 and selectivity factors 1.17-4.25.

Table 7. Chromatographic data for enantioseparation of prepared compounds on amylose tris(3,5-dimethylphenylcarbamate) bonded chiral stationary phase (Chiralpak AD)

Compounds	t_1	k_1	α	R_s
I	11.42	1.99	2.84	19.35
II	9.10	1.39	4.25	21.80
IIIa	16.45	3.33	2.18	17.12
IV	23.17	5.10	1.40	7.66
IV *	8,78	1.42	1.50	2.97
V	31.48	5.52	1.17	2.20
V*	34.93	4.72	1.16	3.34
VI	33.46	7.78	1.29	6.03
VII	46.84	11.26	1.38	9.70
VIII	29.14	6.65	1.68	10.78

*Mobile phase: hexane/ethanol/methanol/diethylamine (80/10/10/0.1 v/v/v/v)
R_s stereochemical resolution factor, t_1 elution time for enantiomer 1, k_1 retention factor for enantiomer 1, α separation factor

CONCLUSION

This study presents synthesis and HPLC enantioseparation of some newly synthesized derivatives of 3-hydroxyphenylethanone of the aryloxyaminopropanol type. The series of compounds were prepared by a well-tried method. Enantioseparation was performed by using two chiral stationary phases. Chiralpak AD was more suitable for enantioseparation of these types of compounds, especially with heterocycles in the basic part of a molecule, rather than antibiotic type teicoplanin and vancomycin of chiral stationary phase.

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SYNTÉZA A HPLC-ENANTIOSEPARÁCIA DERIVÁTOV 3-HYDROXYFENYLETANÓNU

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Ústav analytickej chémie

V rámci štúdia syntézy a HPLC enantioseparácie bola osvedčenou metódou pripravená séria 9 derivátov 3-hydroxyfenyletanónu aminopropanolového typu s rozvetvenými alkylmi a heterocyklami v bázickej časti molekuly. Štruktúra pripravených zlúčenín bola potvrdená interpretáciou IR, UV, ^{1}H NMR a ^{13}C NMR spektier. Enantioseparácia bola uskutočnená HPLC technikou na teikoplanínovej (Chirobiotic T) a na amylóze tris (3,5-dimetylfenylkarbamátovej) (Chiralpak AD) chirálnej stacionárnej fáze. Porovnaním získaných výsledkov sa potvrdilo, že chirálna stacionárna fáza založená na derivatizovanej amylóze je vhodnejšia na enantioseparačné delenie pripravených zlúčenín, hlavne s heterocyklom v bázickej časti molekuly.

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