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VERSITA

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SYNTHESIS OF NEW COMPOUNDS OF THE ARYLOXYAMINOPROPANOL TYPE AND THEIR HPLC ENANTIOSEPARATION

SYNTÉZA NOVÝCH ZLÚČENÍN ARYLOXYAMINOPROPANOLOVÉHO TYPU A ICH HPLC ENANTIOSEPARÁCIA

Original research article

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Abstract This article describes a preparation of some new compounds of the aryloxyaminopropanol type derived from 4-hydroxyphenylpropan-1-one with phenylamino, cyclohexylamino and isobutylamino group in the hydrophilic part and methoxymethyl or ethoxymethyl substituent in the lipophilic part of the molecule. The purity of the prepared compounds was checked by thin-layer chromatography and the structure was confirmed on the basis of interpretation of the IR, UV, ^{1}H NMR and ^{13}C NMR spectra. An enantioseparation of the prepared compounds was performed by using high-performance liquid chromatography on an amylase tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and native teicoplanin (Chirobiotic T). The chromatographic results such as retention, separation and resolution factors have shown that Chiralpak AD is more suitable for enantioseparation of some of the prepared compounds.

Slovak abstract V rámci štúdia syntézy a HPLC enantioseparácie bola osvedčenou metódou pripravená séria 4 derivátov aryloxyaminopropanolového typu s cyklohexylamino, fenylamino a izobutylaminoskupinou ako súčasť hydrofilnej časti molekuly. Finálne látky boli izolované vo forme voľných báz a solí s kyselinou fumarovou resp. oxálovou. Čistota pripravených zlúčenín bola kontrolovaná chromatografiou na tenkej vrstve a ich štruktúra bola potvrdená interpretáciou IČ, UV, ^{1}H NMR a ^{13}C NMR spektier. Enantioseparácia bola uskutočnená priamou HPLC technikou na amylóze tris(3,5-dimetylfenylkarbamátovej) (Chiralpak AD) a na teikoplanínovej (Chirobiotic T) 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 rozvetveným alkylom (izobutylom) na bázickom dusíku.

Keywords *aryloxyaminopropanol – enantioseparation – HPLC – Chirobiotic T – Chiralpak AD*

Kľúčové slová: *aryloxyaminopropanoly – enantioseparácia – HPLC – Chirobiotic T – Chiralpak AD*

INTRODUCTION

In previous works, some new compounds of the aryloxyaminopropanol type derived from 4 hydroxypropiophenones with isopropyl and *tert*-butyl group in the hydrophilic part of the molecule were prepared (Čižmáriková et al., 1990a, 2003). Their anti-izoprenaline activity and anti-arrhythmic activity (Čižmáriková & Kozlovský, 1994a,b) depend on the substitution of the hydrophilic and lipophilic parts of the molecule. In addition, the prepared compounds with higher alkoxymethyl (octyloxymethyl and nonyloxymethyl) group in position 3 of the aromatic ring have shown antimicrobial and local anaesthetic activities (Čižmáriková et al., 1990b). In many papers, compounds with isopropyl and *tert*-butyl group in the hydrophilic part of molecule are active *beta*-adrenolytics (Čižmáriková & Račanská, 1998; Bruchatá & Čižmáriková, 2010; Kečkéšová & Sedlárová, 2010). Lower *beta*-adrenolytic activity was observed in the compounds with other alkyl groups such as isobutyl and diethyl groups (Griffith, 2003). The large variety of different aromatic rings and substituents on nitrogen atom leads to compounds with combined pharmacological properties with affinity to both types of adrenergic receptor, α and β (Bruchatá & Čižmáriková, 2010).

In the work (Mosti et al., 2000), compound with cyclohexylamino moiety gives antiarrhythmic, local anaesthetic and analgesic activity. Compounds of aryloxyaminopropanol type possess in

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their structure a single stereogenic centre and exist as stereoisomers. The most widely used technique for the separation of their enantiomers was high-performance liquid chromatography (HPLC) (Wang et al., 2008) on different chiral stationary phases such as cyclodextrin (Wang & Ching, 2002; Zhang et al., 2008; Hroboňová et al., 2004), immobilised proteins (Fulde & Frahm, 1999; Fornstedt et al., 1999), Amberlite XAD-4 (Agrawal & Patel, 2005) and cellulose and amylase-based phases (Aboul-Enein & Ali, 2001; Valentova et al., 2003). Indirect enantioseparation using derivatisation agents is less suitable at these compounds (Bojarsky, 2002, Bojarsky et al., 2005).

This paper was oriented on a preparation, characterisation of the new prepared derivatives of aryloxyaminopropanol type and its enantioseparation at the Chiralpak AD and Chirobiotic T columns. Chromatographic parameters such as separation, retention and resolution factors were calculated.

EXPERIMENTAL

Chemicals

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

Synthesis

(3-Chloromethyl-4-hydroxyphenyl)propan-1-one and (3-alkoxymethylphenyl-4-hydroxyphenyl)propan-1-one were prepared according to Čižmáriková et al. (2002).

[4-(3-Alkylamino-2-hydroxypropoxy)phenyl]propan-1-one and [4-(3-cycloalkylamino-2-hydroxypropoxy)phenyl] propan-1-one were prepared according to Čižmáriková et al. (2012).

Instruments

The melting points were determined using a Kofler Micro Hot Stage and were quoted uncorrected. The purity of the prepared compounds was assessed using Silufol® UV 254 (Merck) sheets in the solvent system ethyl acetate/diethylamine (9.5:0.5, v/v). UV spectra were run on spectrophotometer GENESYS 10s UV–Vis in methanol. Concentration of compounds was about 10^{-1} mol·m^{-3}. IR spectra were recorded using Nicolet 6700 (Thermo Scientific). ^{1}H NMR and ^{13}C NMR were recorded on the Varian Gemini 2000 Spectrometer operating at 300 MHz for protons and 75 MHz for carbons. Elementary analysis was carried out on FLESCH 2000 (Thermo Scientific) within 0.3 % of the theoretical values.

HPLC analysis

HPLC was carried out using the chiral stationary phases (Chiralpak AD) based on the amylase tris(3,5-dimethylphenylcarbamate) (0.46 × 25). The mobile phases consisted of hexane/ethanol/methanol/diethylamine 85:7.5:11.25:0.1, v/v/v/v (A) and hexane/ethanol/methanol/diethylamine 85:7.5:7.5:0.1, v/v/v/v (B). Samples for analysis were prepared as approximately 1 mg·ml^{-1} solution in methanol. Separations were carried out at a flow rate of 0.8 ml·min^{-1} and the column temperature was maintained at 25°C. Chromatograms were scanned at a wavelength of 267 ± 8 nm.

HPLC studies were performed using 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 mm × 4 mm LD 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 using Hewlett Packard (series 1 100) at 270 nm. The injection volume was 20 µl. The analyte was dissolved in methanol (concentration 1 mg·ml^{-1}). Secondary studies were carried out using HPLC system AGILENT 1200, consisting of a quaternary pump and a diode detector.

Chromatographic characteristics

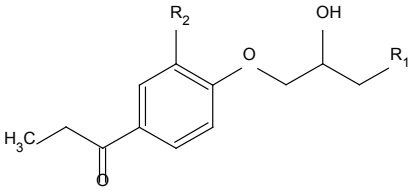
The separation factor was expressed as $\alpha = k_1/k_2$, where k_1, k_2 are retention factors for the first and second eluting enantiomers. The retention factors k' were calculated as follows: $k_1 = (t_1 - t_0)/t_0$ and $k_2 = (t_2 - t_0)/t_0$, where t_0, t_1 and t_2 are the dead elution time and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

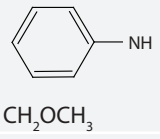
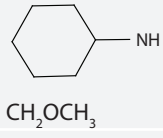
RESULTS AND DISCUSSION

The aim of this study was to prepare four derivatives of aryloxyaminopropanol type with different groups in hydrophilic and lipophilic parts of a molecule. The compound without side alkoxymethyl chain was prepared by a two-step synthesis and those with alkoxymethyl group by a four-step synthesis from 4-hydroxyphenylpropan-1-one. Oxirane intermediates prepared by the reaction of 4-hydroxyphenylpropan-1-one with epichlorohydrin gave final products by the reaction with appropriate amine (phenylamine, cyclohexylamine and isobutylamine). These were isolated in the form of free bases or salts with fumaric acid and with oxalic acid (Table 1). Compound I was isolated only in the form of free base; salts with fumaric and oxalic acid could not be prepared due to increased basicity of its nitrogen atom. The purity of the final products was checked by thin-layer chromatography using ethyl acetate/diethylamine as the mobile phase (Table 1). The structures of prepared compounds were confirmed by IR, UV and ^{1}H NMR, ^{13}C NMR spectra (Tables 2–4). The stretching vibration of the characteristic group in the IR spectra were ν(OH) 3166–3400 cm^{-1}, ν(NH) (base) 3073–3274 cm^{-1}, ν(C=C) 1578–1604 cm^{-1}, ν(C=O) 1668–1682 cm^{-1}, ν(C_{Al} OC_{Ar}) 1251–1276 cm^{-1} (Table 2).

The UV spectra of bases display two bands corresponding to π→π* transition at λ_{max} = 202–270 nm, log ε = 3.86–4.80 (Table 3). Compound I with two aromatic rings gives four bands corresponding to π→π* transition (Table 3).

Table 1. Physico-chemical parameters of prepared compounds.



Compound Form of compound	R^1 R^2	Empirical formula Mr	M.p. (°C) Solvent	Yield (%) R_F
I Base	 CH_2OCH_3	$C_{20}H_{25}O_4N$ 251.33	67–69 Hexane	62 0.73
II Base	 CH_2OCH_3	$C_{20}H_{31}O_4N$ 349.47	82–87 Hexane	40 0.79
IIa Fumarate		$C_{40}H_{62}O_8N_2 \cdot C_4H_4O_4$ 646,79	117–120 Ethylacetate	0.78
III Base	$(CH_3)_2CHCH_2NH$ H	$C_{16}H_{23}O_3N$ 279.02	57–59 Hexane	66 0.66
IIIa Fumarate		$C_{16}H_{23}O_3N \cdot C_4H_4O_4$ 331.46	164–167 Ethylacetate	0.66
IIIb Oxalate		$C_{16}H_{23}O_3N \cdot C_2H_2O_2$	159–160 Ethanol	0.65
IV Base	$(CH_3)_2CHCH_2NH$ $CH_3CH_2OCH_2$	$C_{19}H_{31}O_4N$ 337.44	Viscous oil	79 0.48
IVa Fumarate		$C_{38}H_{66}O_8N_2 \cdot C_4H_4O_4$ 646.79	154-156 propan-2-ol	0.47

M.p. melting point, R_F retardation factor

^{1}H NMR and ^{13}C NMR spectra of free bases showed the proof of the final structure proton and carbon signals of the aminopropanol chain (Tables 4 and 5).

In this work, a direct HPLC method was used for enantioseparation of prepared racemic compounds using chiral stationary phases based on derivatised amylose (Chiralpak AD) and native teicoplanin (Chirobiotic T). Mobile phases A hexane/ethanol/methanol/diethylamine (85:3.75:11.25:0.1, v/v/v/v) and B hexane/ethanol/methanol/diethylamine (85:7.75:7.75:0.1, v/v/v/v) were used for the separations on the Chiralpak AD column. From Table 6 it is evident that good enantioseparations were achieved for all prepared compounds. The obtained separation factors were in the interval from 1.16 to 1.41 and resolution factors in the interval 2.36–6.35.

In the case of compounds III, IIIa and IIIb in the B mobile phase, all forms of compound have similar values of selectivity factor and the values of resolution factors were in order oxalate>base>fumarate. Comparing the type of the mobile phase, greater value of Rs was obtained for the compound IIa in the form of base at the B mobile phase (Table 6). The results of the enantioresolution on the Chirobiotic T in polar organic separation mode (mobile phase methanol/acetonitrile/acetic acid/triethylamine 45:55:0.3:0.2, v/v/v/v) has shown that compounds with phenylamino and cyclohexylamino (I and II) are very well separated with resolution factor values in the range 1.93–2.36 and selectivity factors in the range 1.13–1.15. Comparing the enantioseparations of racemic compound with isobutyl substituent (compound III) on Chiralpak AD and on Chirobiotic T chiral stationary phases, lower enantioresolution was obtained (α = 1.07 and R_s = 0.92) on teicoplanin stationary phase (Table 7 and Figs 1 and 2).

The mechanism of the separation on the both chiral phases is based on the interaction between chiral selector (derivatised amylase or native teicoplanin) and analyte by forming complexes between the enantiomeric analytes and chiral cavities. Detailed mechanism of enantioseparation was discussed in the papers Čižmáriková et al. (2012), Valentova et al. (2003) and Hroboňová et al. (2001). Our study of pharmacological activities of the prepared compounds continues, and the results will be published in the next paper.

CONCLUSION

In this paper, four new compounds of the aryloxyaminopropanol type were prepared from 4-hydroxyphenylpropan-1-one by two- and four-step syntheses. Chemical names of these compounds are: 1-[4-(2-hydroxy-3-phenylaminopropoxy)phenyl]propan-1-one, 1-[3-(methoxymethyl)-4-(2-hydroxy-3-cyclohexylaminopropoxy)phenyl]propan-1-one, 1-[4-(2-hydroxy-3-isobutylaminopropoxy)phenyl]propan-1-one and 1-[3-(ethoxymethyl)-4-(2-hydroxy-3-isobutylaminopropoxy)phenyl]propan-1-one. An enantioseparation of the prepared compounds was performed by using HPLC on an amylase tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and native teicoplanin (Chirobiotic T). The chromatographic results such as retention factor, separation factor α= 1.16–1.41) and resolution factor (R_s = 2.36–6.35) have shown that Chiralpak AD is more suitable for enantioseparation of some of the prepared compounds than Chirobiotic T.

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Table 2. Values of stretching vibrations in IR spectra of prepared bases.

Compounds	ν(OH) (cm^1)	ν(NH) (cm^1)	ν(C=C) (cm^1)	ν(C=O) (cm^1)	ν(ArOalk) (cm^1)
I	3166	3274	1602	1675	1265
II	3283	3073	1600	1670	1276
III	3298*		1578 1604	1679	1257
IIIa	3298		1600	1679	1251
IIIb	3400		1600	1668	1254
Iva	3270		1600	1682	1255

ν, absorbtion maximum.
*ν (OH, NH).

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

Compounds	$\lambda_{max\,1}$ (nm)	Log ε_1	$\lambda_{max\,2}$ (nm)	Log ε_2	$\lambda_{max\,3}$ (nm)	Log ε_3	$\lambda_{max\,4}$ (nm)	Log ε_4
I	205	4.47	220	4.23	252	4.23	270	4.24
II	203	4.00	216	3.86			269	3.96
III	202	4.58					269	4.41
IIIa	203	4.40					269	4.64
IVa	203	4.80					269	4.62

λ_{max}, wave length; ε, molar extinction coefficient.

Table 4. ^{1}H NMR spectral data of compounds δ (ppm) (TMS).

Compounds Solvent	δ (ppm) (multiplicity and number of protons)
I $CDCl_3$	1.19–1.24 (t, 3H, $C\underline{H}_3CH_2$), 2.94–3.00 (q, 2H, $CH_3C\underline{H}_2$), 3.26–3.32 (m, 2H, $C\underline{H}_2NH$), 3.40 (s, 3H, $OC\underline{H}_3$), 4.11–4.13 (m, 2H, $C\underline{H}_2CH$), 4.24–4.28 (m, 1H, $CH_2C\underline{H}$), 4.48–4.59 (m, 2H, $C\underline{H}_2OCH_3$), 6.66–6.69 (d, 2H, $C^{2ANL}\underline{H}$, $C^{6ANL}\underline{H}$), 6.71–6.77 (t, 1H, $C^{4ANL}\underline{H}$), 6.91–6.94 (d, 1H, $C^{5AR}\underline{H}$), 7.17–7.22 (t, 2H, $C^{3ANL}\underline{H}$, $C^{5ANL}\underline{H}$), 7.93–7.96 (m, 2H, $C^{2AR}\underline{H}$, $C^{6AR}\underline{H}$)
II $CDCl_3$	1.04–1.13 (t, 3H, $C{=}OCH_2C\underline{H}_3$), 1.16–1.25 (m, 6H, $C^{3,4,5\text{-}CHex}\underline{H}_2$), 1.60–1.75 (m, 4H, $C^{2,6\text{-}CHex}\underline{H}_2$), 2.40–2.46 (m, 1H, $C^{1\text{-}CHex}\underline{H}$), 2.70–2.77 (q, 2H, $C{=}OC\underline{H}_2$), 2.91–2.96 (m, 2H, $C\underline{H}_2NH$), 2.99 (s, 3H, $OC\underline{H}_3$), 3.55–3.62 (m, 1H, $C\underline{H}OH$), 3.97–4.02 (m, 2H, $OC\underline{H}_2CH$), 4.07–4.10 (m, 2H, $C\underline{H}_2OCH_3$), 6.93–6.96 (d, 1H, $C^{5\text{-}AR}\underline{H}$), 7.92–7.95 (d, 2H, $C^{2,6\text{-}AR}\underline{H}$)
III $CDCl_3$	0.91 (d, 6H, $CH\text{-}(C\underline{H}_3)_2$), 1.75 (m, 1H, $C\underline{H}\text{-}(CH_3)_2$), 2.45 (m, 2H, $NHC\underline{H}_2CH$), 2.76 (m, 2H, $CHOH\text{-}C\underline{H}_2$), 2.85 (m, 2H, $CO\text{-}C\underline{H}_2$), 4.05 (m, 3H, $O\text{-}C\underline{H}_2C\underline{H}$).
IVa D_2O	1.02–1.05 (d, 6H, $CH(C\underline{H}_3)_2$), 1.13–1.18 (t, 3H, $C{=}OCH_2C\underline{H}_3$), 1.22–1.26 (t, 3H, $OCH_2C\underline{H}_3$), 2.05–2.14 (m, 1H, $C\underline{H}(CH_3)_2$), 3.00–3.02 (m, 2H, $C\underline{H}_2CH(CH_3)_2$), 3.05–3.09 (m, 2H, $CHOHC\underline{H}_2NH$), 3.27–3.34 (m, 2H, $C{=}OC\underline{H}_2$), 3.64–3.71 (q, 2H, $OC\underline{H}_2CH_3$), 4.16–4.27 (m, 2H, $OC\underline{H}_2CH$), 4.38–4.45 (m, 1H, $C\underline{H}OH$), 4.83 (s, 2H, $C^{AR}C\underline{H}_2$), 6.08 (s, 1H, $C^{fum}\underline{H}COO$), 6.52 (s, 1H, $C^{fum}\underline{H}COOH$), 7.07–7.10 (d, 1H, $C^{3AR}\underline{H}$), 7.98–8.01 (d, H, $C^{2AR}\underline{H}$, $C^{6AR}\underline{H}$)

δ, chemical shift.

Table 5. ^{13}C NMR spectral data of compounds δ (ppm) (TMS).

Compounds	δ (ppm)
I $CDCl_3$	8.39 ($\underline{C}H_3CH_2$), 31.52 ($CH_3\underline{C}H_2$), 46.30 ($CH_2\underline{C}H$), 58.23 ($O\underline{C}H_3$), 68.62 ($CH_2\underline{C}H$), 70.55 ($O\underline{C}H_2CH$), 71.74 ($\underline{C}H_2OCH_3$), 112.22 (C^{5AR}), 113.21 ($C^{2,6ANL}$), 114.19 (C^{3AR}), 118.02 (C^{4ANL}), 126.68 (C^{1AR}), 129.34 ($C^{3,5ANL}$), 130.28 ($C^{2,6AR}$), 148.07 (C^{1ANL}), 160.80 (C^{4AR}), 199.49 (C=O)
II $CDCl_3$	8.42 ($C=OCH_2\underline{C}H_3$), 25.00 ($C^{3,5\text{-}CHex}$), 26.05 ($C^{4\text{-}CHex}$), 31.43 ($C=O\underline{C}H_2CH_3$), 33.72 ($C^{6\text{-}CHex}$), 34.02 ($C^{2\text{-}CHex}$), 48.55 ($\underline{C}H_2NH$), 56.72 ($C^{1\text{-}CHex}$), 68.21 ($O\underline{C}H_3$), 68.94 ($\underline{C}HOH$), 70.62 ($O\underline{C}H_2CH$), 76.59 ($\underline{C}H_2OCH_3$), 114.21 ($C^{3,5\text{-}AR}$), 130.21 ($C^{2,6\text{-}AR}$), 162.43 ($C^{1\text{-}AR}$), 183.18 ($C^{4\text{-}AR}$), 199.49 (C=O)
III $CDCl_3$	20.54 ($CH(\underline{C}H_3)_2$), 26.38 ($\underline{C}H(CH_3)_2$), 28.45 ($COC\underline{H}_2$), 51.53 ($CHOH\text{-}\underline{C}H_2$), 57.75 ($\underline{C}H_2CH(CH_3)_2$), 67.78 ($\underline{C}HOH$), 70.63 ($O\text{-}\underline{C}H_2$), 114.22 ($C_{Ar}3,5$), 130.60 ($C_{Ar}2,6$), 162.62 ($C_{Ar}4$), 196.81 (CO)
IVa D_2O	10.93 ($C=OCH_2\underline{C}H_3$), 21.86, 21.98 ($CH(\underline{C}H_3)_2$), 28.15 ($\underline{C}H(CH_3)_2$), 34.45 ($C=O\underline{C}H_2CH_3$), 52.59 ($CHOH\underline{C}H_2NH$), 57.73 ($\underline{C}H_2CH(CH_3)_2$), 67.96 ($O\underline{C}H_2CH$), 72.37 ($\underline{C}HOH$), 117.32 ($C^{5AR}$), 132.58 ($C^{1AR}$), 133.69 ($C^{2,6AR}$), 138.21 ($C^{3AR}$), 165.26 ($C^{4AR}$), 183.55 ($C^{fum}OO$), 208.31 (C=O)

δ, chemical shift.

Table 6. Chromatographic data for enantioseparation of prepared compounds on amylase tris(3,5-dimethylphenylcarbamate) bonded chiral stationary phase (Chiralpak AD).

Compounds	t_1	k_1	α	R_s	Mobile phase
I	41.10	10.14	1.37	5.70	A
I	58.58	14.46	1.36	2.39	B
II	38.07	8.97	1.30	3.14	A
IIa	33.07	7.91	1.36	6.35	B
III	27.98	6.93	1.33	4.90	B
IIIa	27.91	6.51	1.41	4.28	B
IIIb	27.91	6.84	1.32	5.67	B
IVa	29.40	6.74	1.16	2.36	B

*Mobile phase: A hexane/ethanol/methanol/diethylamine (85:3.75:11.25:0.1, v/v/v/v) and
B hexane/ethanol/methanol/diethylamine (85:7.75:7.75:0.1, 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.

Table 7. Chromatographic data for the enantioseparation on teicoplanin-bonded chiral stationary phase (Chirobiotic T).

Compound	t_1	k_1	α	R_s
I	21.43	3.87	1.15	2.36
IIa	17.84	3.05	1.13	1.93
IIIa	19.65	3.47	1.07	0.92

Mobile phase: methanol/acetonitrile/acetic acid/triethylamine (45:55:0.3:0.2, 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.

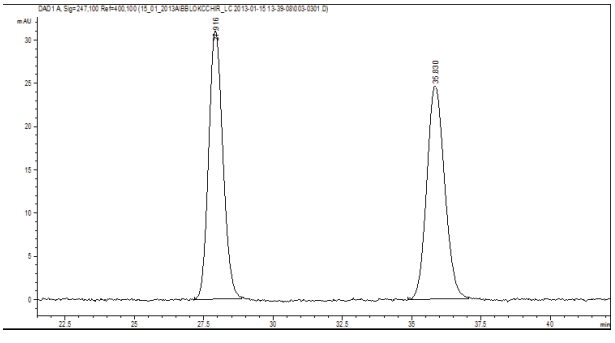

Fig. 1. Separation of enantiomers of compound IIIa. Column: Chiralpac AD; Mobile phase: hexane/ethanol/methanol/dietylamine (85:7.5:7.5:0.1, v/v/v/v).

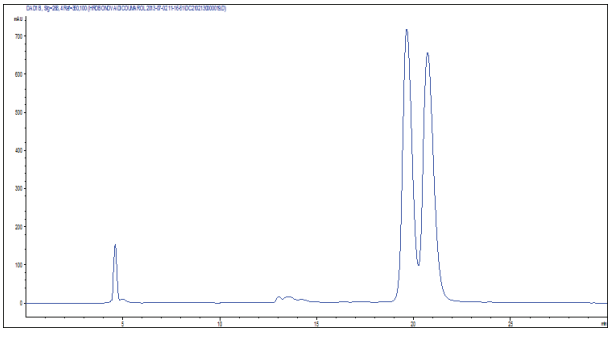

Fig. 2. Separation of enantiomers of compound IIIa.Column: Chirobiotic T; Mobile phase: methanol/acetonitrile/acetic acid/triethylamine (45:55:0.3:0.2, v/v/v/v).

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