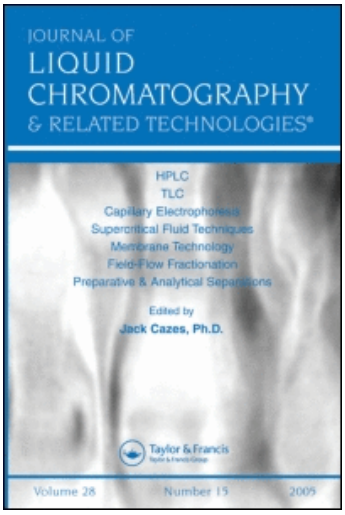

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ENANTIOMERIC RESOLUTION OF PROPRANOLOL AND ANALOGS ON TWO CELLULOSE (CHIRALCEL OF AND OC) AND ONE AMYLOSE (CHIRALPAK AD) CHIRAL STATIONARY PHASES

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ABSTRACT

The enantiomeric chiral separation of propranolol and several aryloxyaminopropan-2-ol analogs is studied using two cellulose-type chiral stationary phases (CSPs) namely Chiralcel OF and Chiralcel OC and one amylose type chiral stationary phase (CSP), Chiralpak AD. The results showed base-line separation for all the compounds studied using amylose tris (3,5-dimethylphenyl carbamate) known as Chiralpak AD in comparison to the cellulose tris (4-chlorophenylcarbamate) known as Chiralcel OF which showed a significant decrease in the selectivities for most of the compounds, while partial or no separation were obtained using cellulose tris (phenylcarbamate) known as Chiralcel OC.

It was observed that the chiral separation depends on the substitution pattern on the aryl group of the aryloxyaminopropan-2-ol i.e. 1-naphthyl, 2-naphthyl and phenyl group and the polarity on the basic nitrogen in the side chain which indicate that the substituents on the side chain did effect the interaction of the enantiomers with the polar carbamate moiety in the chiral stationary phase. Furthermore, the substituents on the phenylcarbamate group of the CSP does play a role in the enantiospecific recognition mechanisms.

INTRODUCTION

Propranolol is considered the model parent drug for non-selective β-adrenergic blockers and been used for the treatment of illnesses such as hypertension, angina pectoris, supraventricular, and ventricular arrhythmias, and reducing the frequency and intensity of migraine headaches,[1] it has various side effects such as mental depression, nausea, vomiting, light-headedness, and visual disturbances.[2,3] Recently increasing interest has been devoted to the synthesis of fluorinated medicinals since fluorine leads to a strong polarization of the molecules and increases their biological activity and enhances their pharmacological properties.[4,5]

In search for more potent and less toxic β-adrenoceptors antagonists, several propranolol analogs were synthesized as a racemic mixture which included three fluorinated analogs. The chemical structures of these analogs are shown in (Table 1). The synthesis and detailed pharmacological activity of these analogs will be presented elsewhere.

The enantiomeric separation of propranolol, chemically known as 1-isopropylamino-3-(1-naphthyloxy)-2-propanol and several of its analogs were investigated using cellulose tris (4-chlorophenylcarbamate), cellulose tris (phenylcarbamate), and amylose tris (3,5-dimethylphenyl carbamate) chiral stationary phases (CSPs), known as Chiralcel OF, Chiralcel OC, and Chiralpak AD respectively.

The aim of this study is to examine the effects of various substitution patterns in the side chain and polarity on the basic nitrogen (fluorinated versus nonfluorinated), and also the role of the aromatic group on the enantioselectivity and resolution using these CSPs in comparison to other chiral stationary phases used in earlier study by this laboratory[6] and elsewhere.[7]

EXPERIMENTAL

Apparatus

The HPLC system consisted of the following (All are products of Waters, Milford, MA, USA): a 501 solvent delivery pump, a Lambda Max 481 variable wavelength detector, a 746 Data Module, and a U6K Injector. The cellulose tris (4-chlorophenyl carbamate) column known as Chiralcel OF (Lot #30-092-90713), the cellulose tris (phenylcarbamate) column known as chiralcel OC, (Lot #34-05-90105) and the amylose tris (3,5-dimethylphenyl carbamate) column known as Chiralpak AD (Lot #15-31-01225), were obtained from Daicel Chemical Industries, Tokyo, Japan.

Chemicals

Racemic propranolol (Bath #BP5461) were kindly supplied by Berk Pharmaceuticals, (Leeds, England), all other analogs used in this study were synthesized in our laboratory. HPLC grade hexane, 2-propanol, and diethylamine, were obtained from Fisher Scientific (Fairlawn, NJ, USA). Absolute ethanol were purchased from Merck (Frankfurt, Germany).

Chromatographic Conditions

The mobile phase used for the enantiomeric separation for propranolol and all analogs on the Chiralpak AD column consists of a mixture of hexane: ethanol: diethylamine (95:5:0.5), while the mobile phase used for the Chiralcel OF and Chiralcel OC columns consists of hexane: 2-propanol: diethylamine (92:8:0.5). The flow rate was 0.8 mL/min at a pressure of 26 bar. Analysis was performed at 23°C and detection at 254 nm. The chart speed was 0.5 cm/min at an attenuation of 32.

Chromatographic Parameters

Capacity factors (k') were calculated using the equation $k'=(V_r-V_0/V_0)$, where V_r is the elution volume and V_0 is the void volume. The separation factor (α) is calculated using the equation $\alpha = k'_2/ k'_1$ where k'_2 and k'_1 are the capacity factors for the second and the first eluted peaks. The resolution factors

(R_s) were calculated using the equation $R_s=2xt_2-t_1/W_2+ W_1$ where t_1, and t_2 are the retention distances of peak 1 and peak 2 and W_1 and W_2 are the peak widths (in the same units as t_1, and t_2).

Determination of Elution Order

Peak identification for these analogs were established using Shodex OR-1 optical rotation detector (JM Sciences, NY, USA), with the same chromatographic conditions as described above. The results showed that (+)-R-enantiomer of these compounds eluted first followed by the (-)-S-enantiomer.

RESULTS AND DISCUSSION

The enantiomeric resolution of racemic propranolol and several other β-adrenergic blockers that belong to the aryloxyaminopropan-2 ol class were achieved using cellulose tris (3,5-dimethylphenyl carbamate) CSP known as Chiralcel OD under normal phase conditions.[8-15] Furthermore, a comparative study between liquid chromatographic (LC) method, and supercritical fluid chromatographic (SFC) method for the enantioseparation of some β-blockers including propranolol among other compounds were recently reported using the cellulose Chiralcel OD and amylose Chiralpak AD columns .[7]

Furthermore, direct enantiomeric separation of propranolol and several related analogs was recently reported using both normal and reversed phases of cellulose tris (3,5 dimethylphenyl carbamate) known as Chiralcel OD and OD-R respectively.[6] In the present study three polysaccharide-type CSPs, namely Chiralcel OF, Chiralcel OC, and Chiralpak AD were used for the enantiomeric separation of propranolol and several aryloxyaminopropan-2-ol derivatives for comparative studies and also to shed some light on the chiral recognition mechanisms for this type of CSPs.

Table 1 shows the chromatographic parameters for the enantioseparation of compounds 1-16 using the Chiralcel OF and Chiralcel AD-CSP's. A higher separation factor (α), and resolution factor (Rs) were obtained with Chiralpak AD column for all compounds in comparison to the values obtained for both parameters using Chiralcel OF column. The enantioseparation of these compounds on the Chiralcel OC column were less successful than those of the two previous phases, only partial separation ranged between 0-50% were obtained for all compounds under the same chromotographic conditions for both columns.

Table 1

Chemical Structures, Separation Factors (α) and Resolution Factors (RS) for Propranolol and Analogs Studied on Chiralcel OF and Chiralpak AD[a]

Compound	R1	Ar	Chiralpak AD α	Chiralpak AD Rs	Chiralcel OF α	Chiralcel OF Rs
A. 1-Naphthyloxyaminopropan-2-ol						
1 (Propranolol)	$CH(CH_3)_2$	1-naphthyl	1.50	2.77	1.35	1.75
2	$CH_2CH_2CH_2CH_3$	1-naphthyl	1.64	5.47	1.20	1.04
3	$CH_2CH_2CH_3$	1-naphthyl	1.41	3.64	1.22	1.15
4	CH_2CH_3	1-naphthyl	1.30	2.96	1.24	1.21
5	$C(CH_3)_3$	1-naphthyl	2.14	7.40	1.71	2.18
6	$CH_2CF_2CF_2CF_3$	1-naphthyl	1.45	2.72	NS[b]	NS
7	$CH_2CF_2CF_3$	1-naphthyl	1.31	3.70	NS[b]	NS
8	CH_2CF_3	1-naphthyl	1.44	4.30	1.50	1.57
B. 2-Naphthyloxyaminopropan-2-ol						
9	CH_2CH_3	2-naphthyl	1.66	6.32	1.13	0.72
10	$CH_2CH_2CH_3$	2-naphthyl	1.56	6.43	PS[c]	PS
11	$CH_2CH_2CH_2CH_3$	2-naphthyl	1.61	6.43	PS[c]	PS
12	$C(CH_3)_3$	2-naphthyl	1.44	4.10	1.35	1.95
C. Phenyloxyaminopropan-2-ol						
13	CH_2CH_3	phenyl	1.63	5.42	1.25	1.56
14	$CH_2CH_2CH_3$	phenyl	1.65	5.71	1.22	1.31
15	$CH_2CH_2CH_2CH_3$	phenyl	1.82	6.46	1.25	1.43
16	$C(CH_3)_3$	phenyl	1.60	3.93	1.43	1.67

[a] See chromatographic conditions under experimental.
[b] No separation.
[c] Partial separation.

The results obtained for the chromatographic parameters of these compounds using the Chiralpak AD column are in agreement with the previous work reported by Aboul-Enein et al.[6] on the Chiralcel OD column, and Bargmann-Leyder et al.[7] Accordingly, the following observations could be drawn:

- The resolution factor Rs (except for the fluorinated compounds) increases with increase of length and size of the alkyl side chain i.e. ethyl>propyl>*tert*.butyl. However, in the case of the fluorinated analogs (compounds 6-8); the resolution factor (Rs) increase with the decrease of the number of the fluorine atoms in the side chain. This is because of the decrease in basicity on the nitrogen due to the strong electron - withdrawing inductive effect and strong electronegativity exerted by the fluorine atoms in the polyfluorinated alkyl groups.

- The supposed helical structures of these polysaccharides derived CSPs are responsible for the different chromatographic behaviours exhibited by cellulose and amylose modified CSPs, 3/2 helical chain conformation for tris (phenylcarbamate) cellulose, and 4/1 helical chain conformation for tris (phenylcarbamate) amylose.[16,17]

- Yashima et al.[18] recently reported the influences of pore size of silical gel, coating amount, coating solvent and column temperature on the chiral discrimination property of cellulose (3,5 dimethylphenyl carbamate) - CDMPC.

- Each CDMPC polymer chain may exist separately in different and ordered structure at low loadings as compared with high loading on the silica gel. It is known that CDMPC form a lyotropic liquid crystal phase at high solution concentrations.[18] Accordingly, CDMPC coated on silica gel at higher concentration may have a conformation or orientation different from that of CDMPC coated at lower concentration. One would expect that the cellulose and amylose CSPs used in this study which are coated on macroporous silica gel have different conformation or orientation that affect the chiral recognition mechanism.

- Alcohols which are used as polar modifiers with Chiralpak AD play a role in the solvation of the polymer chains and in the competition for hydrogen-bonding donor and acceptor sites. The accessibility and/or geometry of the chiral cavities may be modified according to the alcohol's nature. Ethanol appears to be the most stereoselective polar modifier for the majority of the studied compounds.

- Indirect involvement of the chiral recognition mechanism indicates:

a) The stereoselectivity increases with the steric hindrance in the vicinity of the amine function.

b) The sites necessary for chiral discrimination to occur are the amine and hydroxyl functional groups; in the case of propranolol both are involved in stereoselective interactions, possibly through hydrogen bondings with the amide dipoles of the phenylcarbamate derivative of the CSP.

- In the case of Chiralcel OC column, the aromatic moiety seems to play a very minor role in the chiral recognition mechanism. This could be explained by:

a) The removal of the methyl groups on the phenylcarbamate group in the CSP may highly modify the secondary structure of the polymer and therefore the geometry of the chiral cavities.

b) The removal of these electron-donating methyl groups result in a decrease of the π-donor capability of the phenyl group and therefore the repulsion with the π-donor entity of the solute leading to a modification of the position of the solute in the chiral cavity.

Yashima and Okamoto recently examined the chiral recognition mechanisms of several derivatized polysaccharide CSPs and the influence of the substituents on the phenyl groups on the chiral resolving power based on chromotographic, computational, and spectroscopic methods.[19,20] It was reported that the introduction an electron-donating methyl group (as in Chiralpak AD) or an electron-withdrawing halogen at 4-position (as in Chiralcel OF) improved the resolutionability of many racemates. Our results are in agreement with these observations and substantiate these findings.

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