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Journal of Chromatography A, 719 (1996) 315–320

JOURNAL OF CHROMATOGRAPHY A

High-performance liquid chromatographic separation of β-amino alcohols
II. Separation of *trans*-2-(dialkylamino)cyclohexanols on an amylose-based chiral stationary phase

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First received 10 February 1995; revised manuscript received 30 June 1995; accepted 30 June 1995

Abstract

Direct enantiomeric separations of racemic mixtures of *trans*-2-(dialkylamino)cyclohexanols were achieved with a variety of alcohol-modified pentane mobile phases and a Chiralpak AD chiral stationary phase. The effects of the aliphatic component and the alcohol modifier in the mobile phase were studied independently. A variety of alcohol modifiers were investigated that introduced steric factors or affected hydrogen bonding. Ring-size effects of the substituents were noted.

Keywords: Enantiomer separation; β-Amino alcohol; *trans*-2-(Dialkylamino)cyclohexanols

1. Introduction

Enantiomerically pure β-amino alcohols are important pharmacological agents in medicinal chemistry [1,2]. Therapeutic activity of these molecules can be divided into three categories based on their pharmacological action: vaso constrictors, antihypertensive agents, and beta (β) blockers [3]. Many of the β-blockers are marketed as racemic mixtures, but their mode of action is enantioselective [4,5]. The (*S*)-enantiomers are often 50 to 500-fold more active than their antipode [6]. The binding affinity to the β-receptor has been reported to range from 10 to 1000 for atenolol and pindolol, respectively [1].

The preparation [7–9] and use of β-amino alcohols in organic syntheses has been increasing [9]. Many important transformations of prochiral substrates into chiral compounds of high enantiomeric purity have been achieved using a catalytic amount of an enantiomerically pure β-amino alcohol as a chiral auxiliary [9,10].

There are several methods available for the synthesis of racemic β-amino alcohols [11]. Enantiomerically pure β-amino alcohols are usually obtained either from amino acids or by resolution procedures [12]. The only general asymmetric syntheses of β-amino alcohols cur-

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SSDI 0021-9673(95)00736-9

rently available are the homogeneous asymmetric hydrogenation of α-amino ketones, using (*R*)-(+)- and (*S*)-(−)-2,2′-bis(diphenylphosphine)-1,1′-binaphthyl ruthenium (BINAP-Ru) complexes with hydrogen pressures of 50–100 atm (1 atm = 101 325 Pa) [13], and the asymmetric reduction of α-amino ketones with the chiral borohydride, K-Glucoride [7].

A general synthesis for the preparation of racemic β-amino alcohols via the hydroboration/oxidation of enamines has been developed [11]. This procedure was extended to the preparation of enantiomerically enriched β-amino alcohols via the asymmetric hydroboration of enamines using diisopinocampheylborane at 0°C in tetrahydrofuran (THF) generating enantiomeric excesses ranging from 50 to 86% [14].

Accurate determination of the enantiomeric purity of β-amino alcohols is essential to assess their effectiveness as both therapeutic agents and chiral auxiliaries. Many chromatographic techniques have been employed for the analysis of derivatized and underivatized β-amino alcohols, but the use of high-performance liquid chromatographic (HPLC) procedures predominates [15]. Pirkle and Burke [16] recently described an N-3,5-dinitrobenzoyl-α-amino phosphonate chiral stationary phase (CSP) that was developed specifically for the separation of β-blockers. Many direct separations of amino alcohols have also been achieved on modified cellulose and amylose CSPs [17–21].

Papers describing enantiomeric separations using cellulose or amylose-based CSPs frequently include discussions of the solute–CSP interactions and recognition mechanisms. Hydrogen bonding, dipole, and π–π interactions have been identified [17–25] as important interactive forces that may be used to form the diastereomeric solute–CSP complexes which yield the separations. Enantiomeric discrimination may also be influenced by the degree of steric fit in the "chiral cavity" of the CSP [25,26]. The composition of the mobile phase is an important factor in these separations. Most of the separations have been obtained using normal-phase conditions with an aliphatic carrier and alcohol modifier in the mobile phase. The type and concentration of the alcohol in the mobile phase has a significant effect on some separations [17,21–26].

This article describes the synthesis and direct enantiomeric separation of *trans*-2-(dialkylamino)cyclohexanols using a 3,5-dimethylphenyl carbamate modified amylose CSP (Chiralpak AD, Chiral Technologies).

2. Experimental

2.1. Synthesis

The following reagents were purchased from the Aldrich Chemical Company and used without further purification: cyclohexene oxide, 1-(pyrrolidino)cyclohexene, piperdine, hexamethyleneimine, heptamethyleneimine, cyclohexanone, and borane methyl sulfide (BMS). All new compounds gave satisfactory C, H, N analyses, and their structures were further confirmed by ^{1}H and ^{13}C NMR spectrometry and FT-IR spectroscopy.

2.2. Preparation of the trans-2-(dialkylamino) cycloalkanols

The compounds were prepared by the neat reaction of the appropriate secondary amine and cycloalkene oxide at reflux [11] or by the hydroboration of the appropriate 1-(dialkyl amino)cycloalkene with borane methyl sulfide (BMS) followed by methanolysis and oxidation of the dimethylboronate ester with basic 30% hydrogen peroxide [11].

2.3. Materials

HPLC-grade pentane, hexane, *n*-heptane, methanol (MeOH), and 2-propanol (IPA) were purchased from Fisher Scientific. Absolute ethanol (EtOH) was obtained from Quantum Chemical Corporation, USI Division. Cyclohexanol, 1-octanol, *tert.*-amyl alcohol, 2,2,2-trifluoroethanol, and 1,1,1,3,3,3-hexafluoro-2-propanol were purchased from Aldrich Chemical and were ≥99% pure. Mobile phases were prepared by blending appropriate volumes of liquids in a 1-l graduated

cylinder and mixing with a stirring bar. The mobile phases were neither vacuum degassed nor sparged with helium before or during their use.

2.4. Instrumentation

Chromatographic separations were achieved using a liquid chromatograph constructed from the following components: a Milton Roy reciprocating piston pump operating at 1–2 ml/min, a free-flow pulse dampener (Alltech Associates, Deerfield, IL, USA), a Rheodyne 7125 injector, a 250 × 4.6 mm I.D. Chiralpak AD column from Chiral Technologies, and a Kratos 773 UV absorbance detector. The detector output was stored and reprocessed using a Perkin-Elmer Nelson ACCESS*CHROM data system. Note: some chromatographic systems do not pump pentane-based mobile phases very effectively. We have, in most cases, overcome cavitation and bubble formation with the use of a low-pressure (8 psig) solvent handling system and backpressure regulator (Alltech Associates, Deerfield, IL, USA).

2.5. Chromatography

A series of structurally similar amino alcohols and various mobile phases were used to investigate solute–CSP interactions affecting the separation of amino alcohols on an amylose-based CSP. Racemic mixtures of each compound were analyzed individually and then combined with the other compounds of the series and reanalyzed each time the composition of the mobile phase was changed.

Capacity factors and resolutions were computed from the retention times and peak widths. Column dead-times (t_0) were calculated from the retention time of the first positive-going baseline upset. The t_0 values were also verified by injecting solutions containing 1,3,5-tri-*tert.*-butylbenzene. The capacity factors for the first peak of each pair of enantiomers (k'_1) and resolution (R_s) values are provided in Table 1. Mobile phase, substituent, and CSP effects are described below.

3. Results and discussion

3.1. Mobile phase effects

Hydrocarbon component

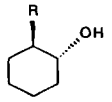
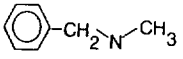
The effect of the aliphatic component in the mobile phase has received little attention. Recently, we determined the effect of the hydrocarbon components in the mobile phase on the separation of some (*R,S*)-1-(dialkylamino)-2-alkanols [27]. Three hydrocarbons, pentane, hexane and heptane, were evaluated. Pentane was selected to permit the use of a variety of alcohols in the mobile phase. Methanol (MeOH) has limited solubility in hexane and higher alkanes but is miscible in all proportions with pentane. Pentane is also a convenient co-solvent for ethanol (EtOH) and isopropanol (IPA).

Mobile phases containing 5% ethanol and 95% of either *n*-pentane, hexane, or heptane were used again in this study to evaluate the effect of the hydrocarbon component in the mobile phase on the separation of *trans*-2-(1-hexamethyleneimino)cyclohexanol and *trans*-2-(1-heptamethyleneimino)cyclohexanol (compounds C and D of Table 1). The results were consistent with our earlier findings [27]. No significant improvement was obtained by using a specific hydrocarbon in the mobile phase. Elution order and peak shape were unaffected. Even though retention times were slightly longer with pentane, resolution for compounds C and D was equivalent in all cases regardless of the hydrocarbon component used in the mobile phase.

Alcohol component

The effect of the alcohol modifier (5% by volume) in the mobile phase on retention and resolution was complex (Table 1 and Fig. 1). Elution order of each enantiomer was not determined during this study. We have, however, found ([27] and other, unpublished results) that the *R*- or *R,R*-enantiomer will elute before the *S*- or *S,S*-enantiomer. The capacity factors for each compound in Table 1 show a trend which is related to the type of alcohol component used in a pentane-based mobile phase. The retention was the largest when MeOH was used as the alcohol

Table 1
Chromatographic parameters for *trans*-2-(dialkylamino)cyclohexanols

		MeOH			EtOH			IPA		
	R-group	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
A		3.01	1.29	1.43	2.60	1.17	1.24	1.33	1.04	0.00
B		6.41	1.00	0.00	4.61	1.03	0.22	1.60	1.52	3.70
C		6.52	1.97	8.95	5.61	1.81	6.52	1.50	2.06	7.04
D		1.97	1.52	5.36	1.50	1.49	1.31	0.56	1.38	2.64
E		3.57	1.29	2.30	2.09	1.21	2.98	1.75	1.00	0.00

Mobile phase composition: alcohol–pentane (5:95, v/v).

component in the mobile phase and smallest when it was IPA.

There were differences in physical state and resolution among the cyclohexanols. The piperidino cyclohexanol (compound B, Table 1) was a solid at room temperature; all of the other substituted cyclohexanols were liquids. Resolution was the largest for the 5, 7 and 8-membered cyclic amines (compounds A, C, and D of Table 1) when MeOH was used as the alcohol component in the mobile phase and smallest when IPA was used in the mobile phase. The hexamethyleneimino cyclohexanol (compound C of Table 1) always had the largest resolution of the cyclohexanols, regardless of the alcohol component in the mobile phase. In contrast, the resolution of compound B was the largest with IPA in the mobile phase and smallest when MeOH was the alcohol component of the mobile phase. That trend was opposite all of the other cyclohexanols, which had the largest resolution using MeOH as the modifier and the smallest resolution using IPA as the modifier.

Peak tailing for the pyrrolidino-substituted cyclohexanol increased as the polarity of the alcohol modifier decreased (Fig. 1). The pyrrolidino group is the smallest and the most basic of the amine substituents. Ring size, basicity of the solute, and interactions with the silica surface of the CSP may play a role in peak shape.

Specific type of alcohol

The alcohol component of the mobile phase affects enantiomeric separations obtained with cellulosic and amylosic CSPs [17,21–26]. We investigated the effect of the alcohol on the separation of *trans*-2-(1-hexamethyleneimino)-cyclohexanol and *trans*-2-(1-heptamethyleneimino)cyclohexanol, compounds C and D of Table 1, by using six different alcohol-modified pentane mobile phases. The modifiers included

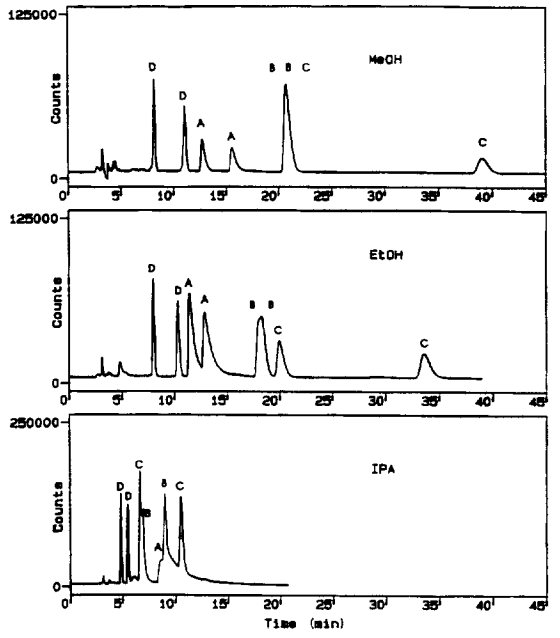


Fig. 1. Effect of MeOH, EtOH, and IPA in a pentane-based mobile phase on the direct enantiomeric separation of some *trans*-2-(dialkylamino)cyclohexanols. Chromatographic conditions: mobile phase, 95% pentane and 5% MeOH (A), EtOH (B), or IPA (C); flow-rate, 1 ml/min; injection volume, 10 μl; analytical column, Chiralpak AD; UV detection at 210 nm.

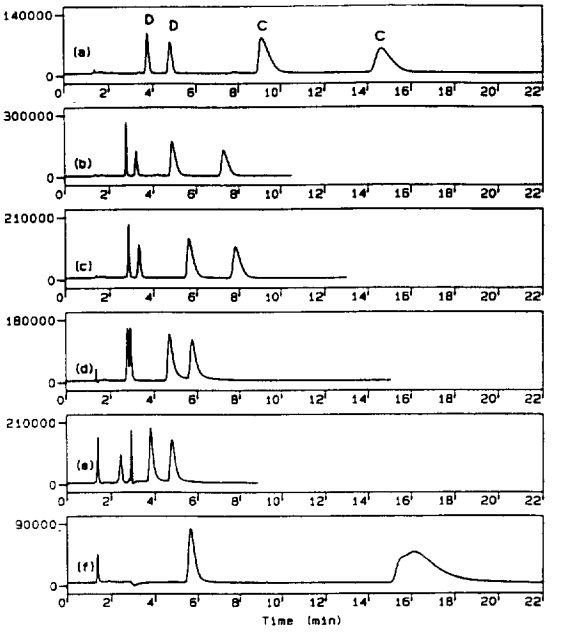


Fig. 2. Effect of alcohol modifiers in a pentane-based mobile phase on the direct enantiomeric separation of *trans*-2-(1-hexamethyleneimino)cyclohexanol and *trans*-2-(1-heptamethyleneimino)cyclohexanol. Chromatographic conditions: mobile phase, 95% pentane and (a) 5% EtOH, (b) 1.5% 2,2,2-trifluoroethanol and 3.5% EtOH (c) 1.5% 1,1,1,3,3,3-hexafluoropropanol and 3.5% EtOH, (d) 5% *tert.*-amyl alcohol, (e) 5% cyclohexanol, (f) 5% 1-octanol; flow-rate, 2 ml/min; injection volume, 10 μl; analytical column, Chiralpak AD; UV detection at 210 nm.

two aliphatic alcohols, EtOH and 1-octanol, two unusual modifiers, cyclohexanol and *tert.*-amyl alcohol, chosen for their steric bulk, and two fluorinated alcohols, chosen for their potential ability to influence hydrogen bonding interactions. The composition of the mobile phases was based on volume and not molarity, as reported by Wainer et al. [26]. Therefore, the number of alcohol molecules available for solvation or competing for active sites was not constant in all mobile phases and may have influenced some of the results.

The effect of the alcohol modifiers is shown in Fig. 2. Composition of the alcohol modifiers in the mobile phase totaled 5 vol.%. The amount of fluorinated alcohol added to some mobile phases was held at 1.5%, due to the quantity of fluorinated alcohol available in our laboratory at the time of the experiments.

The following conclusions regarding changes in retention or resolution were drawn from comparisons to the separation obtained with the mobile phase containing EtOH–pentane (5:95): retention was reduced when fluorinated alcohols, *tert.*-amyl alcohol, or cyclohexanol were used and increased when 1-octanol was used. The best resolution was obtained when EtOH was present; the worst with 1-octanol.

The bulky alcohol modifiers, *tert.*-amyl alcohol and cyclohexanol, affected both retention and resolution. The loss of resolution for *trans*-2-(1-hexamethyleneimino)cyclohexanol (compound C) was greater than for *trans*-2-(1-heptamethyleneimino)cyclohexanol (compound B). Peak shapes were unaffected. The use of 1.5% (v/v) of the fluorinated alcohols reduced reten-

tion and affected resolution. Resolution was reduced when hexafluoroisopropanol was added to the mobile phase, but was unaffected when trifluoroethanol was present. The fluorinated alcohols increased the eluotropic strength of the mobile phase without eliminating the hydrogen bonding essential for chiral recognition.

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