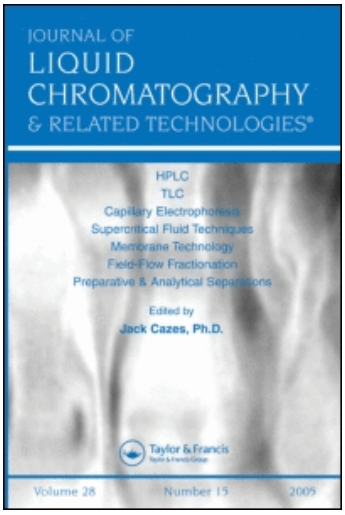

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Determination of the Enantiomeric Composition of Chiral Aminoalcohols Using Chiral Derivatization and Reversed-Phase Liquid Chromatography

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DETERMINATION OF THE ENANTIOMERIC COMPOSITION OF CHIRAL AMINOALCOHOLS USING CHIRAL DERIVATIZATION AND REVERSED-PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

A variety of racemic aminoalcohols were derivatized with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate. The resulting two diastereomeric derivatives of each racemate were separated on octadecylsilane liquid-chromatographic columns using methanol-water mixtures as mobile phase and detection at 254 nm. The procedure is simple, rapid and convenient, and may be generally applicable to the determination of the enantiomeric composition of aminoalcohols.

INTRODUCTION

Chiral aminoalcohols are important as chiral synthons (1-3), as physiologically active agents (4), as pharmaceuticals (5,6), etc. The gas-

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chromatographic resolution of the enantiomers of aminoalcohols--mainly those of pharmaceutical interest--have been studied extensively by Konig and coworkers (7-10). Investigations of the liquid-chromatographic (LC) resolution of aminoalcohol enantiomers have focused primarily on propranolol (10-15) and ephedrine (16-18) and closely related compounds of pharmacological interest. Pirkle et al. described the design and preparation of chiral LC stationary phases capable of resolving the enantiomers of several aminoalcohols as their N-3,5-dinitrobenzoyl derivatives (19,20).

In the present report, a variety of enantiomeric aminoalcohols are shown to be separable via derivatization with a chiral reagent and resolution on traditional (non-chiral) LC columns.

EXPERIMENTAL

Chemicals

The following compounds were obtained from the Aldrich Chemical Company (Milwaukee, WI): cyclohexylamine, 2-amino-1-phenylethanol, L-(+)- and DL-2-amino-3-methyl-1-butanol, (R)-(-)- and (±)-1-amino-2-propanol, D-(-)-phenylgycinol, and 2-butylamino-1-propanol. Fluka Chemical Corporation (Hauppauge, NY) was the source of the following compounds: (R)-phenyloxirane, D- and L-leucinol, L-(+)-phenylglycinol, and D- and L-phenylalaninol. Samples of 2-cyclohexylamino-1-phenylethanol and DL-2-benzylamino-1-propanol were purchased from Pfaltz and Bauer, Inc. (Stamford, CT). The following compounds were also obtained from commercial sources: 2,3,4,6-tetra-O-acetyl-β-D-gluco pyranosyl isothiocyanate (TAGIT), Polysciences, Inc. (Warrington, PA);

methanol and acetonitrile (distilled-in-glass grade), Burdick and Jackson Laboratories (Muskegon, MI); ammonium phosphate monobasic, J.T. Baker (Phillipsburg, NJ). Glass-distilled water was used.

(R)-1-Phenyl-2-cyclohexylaminoethanol

Cyclohexylamine (44 mg, 0.44 mmol), (R)-phenyloxirane (50 mg, 0.42 mmol), and methanol (50 µl) were mixed in vial, the vial tightly capped and heated in a heating block at 50° for 72 hrs. The solvent was then evaporated in a stream of nitrogen.

Chromatography

A Waters Assoc. (Milford, MA) LC system consisting of a model M-6000 solvent delivery system, a model U6K injector, and a model 440 absorbance detector was used. Separations were carried out on a Beckmann (Berkeley, CA) 150 x 4.6 mm I.D. column packed with Ultrasphere ODS of 5-µm particle size. The mobile phases were prepared by first vacuum-filtering methanol and water separately, followed by mixing the two components in the appropriate ratios (see below). The mobile phase was delivered at 1.0 ml/min, and the column effluent was monitored at 254 nm. The detector output was recorded using a Hewlett-Packard (Avondale, PA) model 3390A electronic integrator.

Derivatization

The aminoalcohol (0.3-0.5 mg) was placed in a test tube. Acetonitrile (200 µl) containing 2 mg TAGIT was added, and the contents of the tube were swirl-mixed (Vortex) and the tube was capped and allowed to stand at room temperature for 30 min. Acetonitrile (200 µl) and 0.02 M

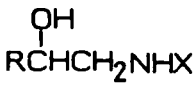
ammonium phosphate buffer (50 µl, pH 3.0) were added, the tube was briefly vortex-mixed, and aliquots (2-4 µl) were injected into the LC system.

RESULTS AND DISCUSSION

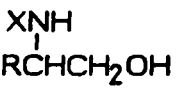
Previous studies (14,17) in our laboratory showed that derivatization of racemic propranolol, ephedrine, and related compounds with TAGIT yielded diastereomeric derivatives which could be readily separated by reversed-phase LC using octadecylsilane columns. Similar results were obtained for the aminoalcohols epinephrine and norepinephrine by Nimura et al. (21). These observations suggested that derivatization with TAGIT may have general utility in the LC separation of enantiomeric aminoalcohols. The isothiocyanato group of the reagent reacts selectively and rapidly with the primary or secondary amino group to give the corresponding thiourea derivatives (14,17,21).

The structures of the aminoalcohols studied are shown in Figure 1. Both primary (4-10) and secondary (1-3) alcohols were included. Conversely, in compounds 1-3 the amino group is attached to a primary carbon atom, while in 4-10 the attachment is to a secondary carbon atom. Compounds 3,9 and 10 contain a secondary amino group.

The separation of the diastereomeric derivatives of enantiomeric aminoalcohols is summarized in Table 1. With the exception of compound 1, the diastereomeric derivatives of each aminoalcohol were completely resolved, as indicated by the values ($R > 1.5$) of the peak resolution R (Table 1), and even 1 was nearly baseline-resolved, with $R = 1.42$. In



	R	X
1	CH_3	H
2	C_6H_5	H
3	C_6H_5	cyclohexyl



	R	X
4	CH_3	H
5	$(CH_3)_2CH$	H
6	$(CH_3)_2CHCH_2$	H
7	C_6H_5	H
8	$C_6H_5CH_2$	H
9	CH_3	$CH_3(CH_2)_3$
10	CH_3	$C_6H_5CH_2$

FIGURE 1 - Chemical structures of the compounds studied

Figure 2 the resolution of 10 is shown. As was found in earlier work (14,17), the TAGIT derivatives generally displayed good chromatographic peak shapes.

Individual enantiomers for six of the racemates were available from commercial sources, and one was synthesized. It was found that the R enantiomers of compounds 4-8--all primary alcohols--provided the diastereomers which were more strongly retained than the corresponding epimers (Table 1). For the secondary alcohols 1 and 3, on the other hand, the reverse was true: the more strongly retained diastereomer was derived from the S enantiomers (Table 1).

TABLE 1
Separation of Enantiomeric Aminoalcohols as Their Derivatives Formed with TAGIT

Compound[a]	Mobile Phase[b]	α[c]	R[d]	t_R (min)	Config[e]
1	35:65	1.10	1.42	25.17, 27.54	(S)-(+)
2	52:48	1.11	1.64	11.99, 13.16	N.D.
3	70:30	1.40	4.40	13.14, 17.90	(S)
4	40:60	1.29	2.40	10.32, 12.93	(R)-(-)
5	50:50	1.49	6.04	8.30 11.72	(R)-(-)
6	52:48	1.40	3.38	11.60 15.72	(R)-(-)
7	52:48	1.32	2.99	7.90, 9.96	(R)-(-)
8	55:45	1.28	2.89	12.26, 15.34	(R)-(+)
9	57:43	1.23	3.27	12.38, 14.91	N.D.
10	52:48	1.22	3.50	17.78, 21.33	N.D.

[a]See Fig. 1

[b]Proportions of volumes of methanol and water, respectively, mixed. See Experimental for other chromatographic conditions.

[c]Separation factor (22).

[d]Peak resolution (23).

[e]Configuration of aminoalcohol enantiomer yielding the more strongly retained derivative. N.D. = not determined.

(R)-1-Phenyl-2-cyclohexylaminoethanol was synthesized from (R)-phenyloxirane via ring-opening aminolysis with cyclohexylamine. This procedure provides the basis for the LC determination of the enantiomeric composition of chiral epoxides as developed recently in our laboratory (24).

In summary, the procedure described possesses several advantages: a variety of different types of aminoalcohols are amenable to resolution; only commercially available reagents (25) and chromatographic columns

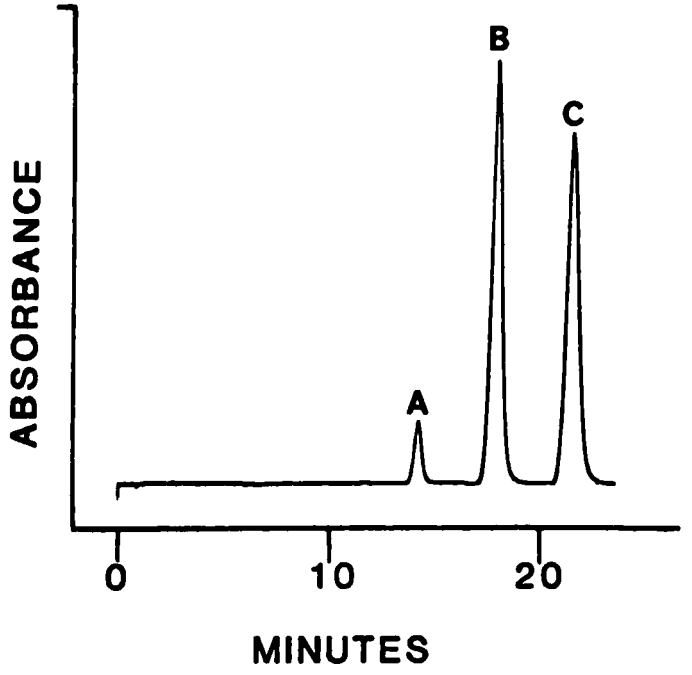


FIGURE 2 - The resolution of compound 10. A: excess TAGIT; B,C: derivatives of 10.

are required; the derivatization procedure is simple and rapid; good separation of the diastereomeric derivatives is obtained. It is concluded that the procedure may be generally useful in the resolution of enantiomeric aminoalcohols.

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