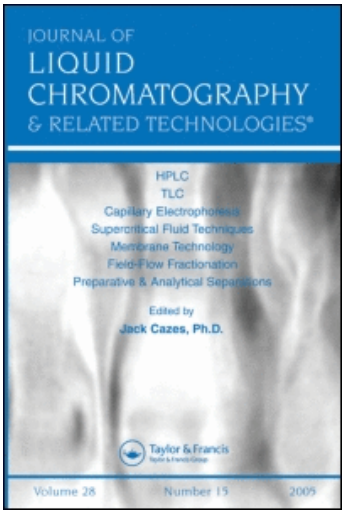

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Direct Liquid Chromatographic Separation of 2,3-Dihydro-2-ethylbenzofuran-2-carboxylic Acid Enantiomers Using a Cellulose Carbamate-Based Chiral Stationary Phase Column

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DIRECT LIQUID CHROMATOGRAPHIC SEPARATION OF 2,3-DIHYDRO-2-ETHYL-BENZOFURAN-2-CARBOXYLIC ACID ENANTIOMERS USING A CELLULOSE CARBAMATE-BASED CHIRAL STATIONARY PHASE COLUMN

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ABSTRACT

Direct liquid chromatographic separation of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid enantiomers on a commercial cellulose carbamate-based chiral stationary phase column is described. The addition of a small amount of organic acid in the mobile phase is required for the elution and separation of both enantiomers. Better peak shapes are obtained when trifluoroacetic acid is used. The method allows the detection of the undesired (+)-enantiomer down to a level of 0.5 % and can be used to measure the enantiomeric purity of (-)-enantiomer after resolution.

INTRODUCTION

Efaroxan, a chiral compound, has been extensively investigated as a potential anti-diabetic agent (1). 2,3-Dihydro-2-ethylbenzofuran-2-carboxylic acid

is used in a synthesis of efaroxan (2). It is convenient and cheaper when a resolution is carried out early in a synthesis. The development of an analytical method to measure the enantiomeric purity of the carboxylic acid is required.

A literature survey indicated that resolution of racemic carboxylic acids can be achieved using HPLC chiral stationary phases after conversion of the acids into either esters or amides (3-8). Derivatization procedures have many drawbacks (7-11). Recent efforts have focused on development of direct resolution of carboxylic acids on various chiral stationary phases and examples have been documented (7,8,11-22).

In this communication, the direct HPLC separation of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid (as shown in Figure 1) is described using a chiral stationary phase column and is compared to derivatization.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a Hitachi L-6200 intelligent pump, a Micromeritics 728 autosampler and a Valco injector with a 20 μl loop, a Kratos Spectroflow 757 variable wavelength UV absorbance detector, and a Hitachi D-2000 Chromatointegrator. The analytical column was a Chiralcel OF (250 x 4.6 mm I.D., 10 micron particle size) column and was purchased from Chiral Technologies, Inc, Exton, PA.

Chemicals

Hexane and 2-propanol (HPLC grades) were obtained from EM Science, Gibbstown, NJ. Formic acid (88%) and acetic acid (reagent grade) were purchased

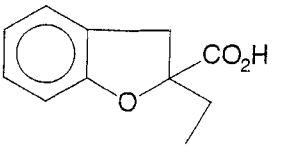

FIGURE 1. The structure of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid.

from J.T. Baker Inc., Phillipsburg, NJ. Trifluoroacetic acid (99%) was obtained from Aldrich Chemical Company, Milwaukee, WI. (-)-2,3-Dihydro-2-ethylbenzofuran-2-carboxylic acid, 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid and the corresponding amide, methyl, 1-propyl, and 1-pentyl esters were prepared at the Parke-Davis Pharmaceutical Research Division, Holland, MI (23).

Chromatographic Conditions

The mobile phase for the amide and esters was hexane/2-propanol. An organic acid modifier, 0.01-0.1 %, was used to separate 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid enantiomers. These modifiers were trifluoroacetic acid, formic acid or acetic acid. Flow rate was 1.0 mL/min. The UV detection wavelength was 280nm. Sample amount for the enantiomeric purity of (-)-2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid was about 0.11 μmole. Column void volume was determined by injection of tri-t-butylbenzene (15,24).

RESULTS AND DISCUSSION

The enantiomeric separation of the amide and esters of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid enantiomers can be achieved using hexane/2-propanol as mobile phase. As shown in Table 1, good separation and resolution were observed. However, this system did not separate the parent acid enantiomers.

TABLE 1. Enantiomeric Separations for 2,3-Dihydro-2-ethylbenzofuran-2-carboxylic Acid Derivatives

Compound	k'_1	α	R_s
methyl ester	1.73	2.08	6.95
1-propyl ester	1.20	2.45	6.92
1-pentyl ester	0.99	2.54	6.92
amide	5.46	1.46	3.72

k'_1 is the capacity factor of first eluted enantiomer; α is the stereoselectivity; R_s is the resolution factor.

HPLC conditions: Mobile phase = 2 % 2-propanol in hexane for esters and 10 % 2-propanol in hexane for amide; flow rate = 1.0 mL/min.; detector wavelength = 280 nm; detector sensitivity = 0.01 AUFS;
column temperature = room temperature.

TABLE 2. Effect of Trifluoroacetic Acid Modifier on Resolution of 2,3-Dihydro-2-ethylbenzofuran-2-carboxylic Acid Enantiomers

% Modifier	k'_1	α	R_s
0.01	0.57	8.99	9.59
0.05	0.55	9.15	9.74
0.10	0.55	9.07	9.81

k'_1 is the capacity factor of first eluted enantiomer; α is the stereoselectivity; R_s is the resolution factor.

HPLC Conditions: Mobile phase = 20 % 2-propanol in hexane with added amount of trifluoroacetic acid modifier; flow rate = 1.0 mL/min.; detector wavelength = 280 nm; detector sensitivity = 0.01 AUFS; column temperature = room temperature.

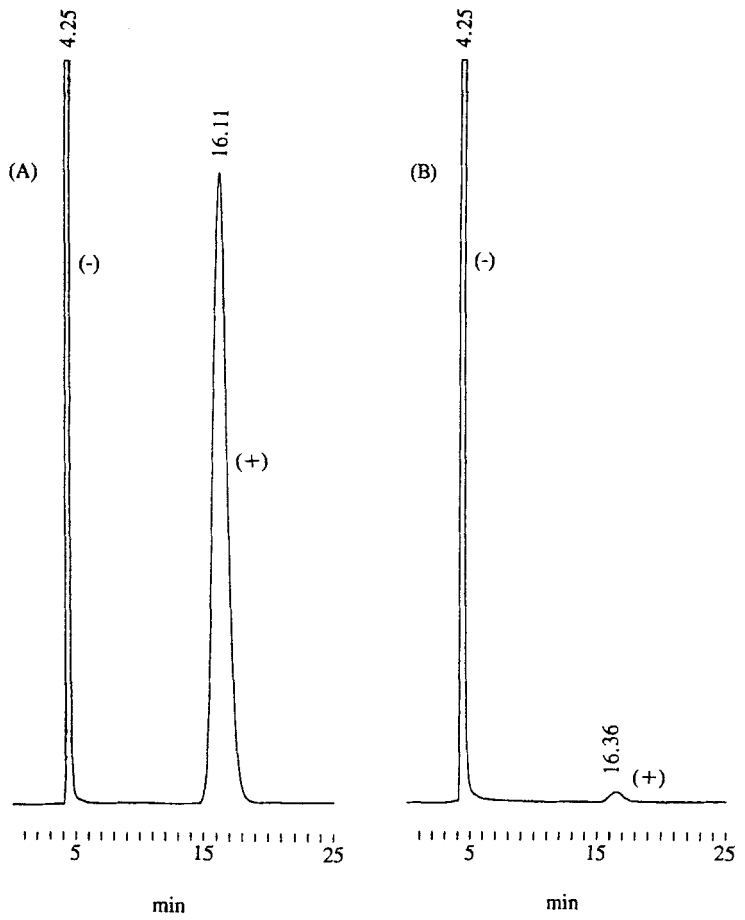

FIGURE 2. Enantiomeric separation of (A) racemic 2,3-dihydro-2-ethyl-benzofuran-2-carboxylic acid and (B) (-)-2,3-dihydro-2-ethyl-benzofuran-2-carboxylic acid after resolution. Mobile phase = 20% 2-propanol in hexane with 0.10 % trifluoroacetic acid added; flow rate = 1.0 mL/min.; detector wavelength = 280 nm; detector sensitivity = 0.01 AUFS; column temperature = room temperature; sample amount injected = 20 μg.

The addition of an organic acid to the mobile phase to effect the elution of carboxylic acid containing compounds from a tris-(3,5-dimethylphenyl carbamate) derivative of cellulose and amylose based chiral columns has been reported earlier (15,19). The same strategy was applied in this study. Acetic acid, formic acid and trifluoroacetic acid were each added as a modifier to the mobile phase. In each case, separation of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid enantiomers was reasonably good. Nevertheless, moderate tailing of peak shapes were observed associated with the use of both acetic and formic acid. Much improved peak shapes as well as better separation and resolution (α = 9.07, R_s = 9.81) were seen when the added modifier was trifluoroacetic acid.

The amount of trifluoroacetic acid in the mobile phase was varied to study the concentration effect on the resolution of 2,3-dihydro-2-ethylbenzofuran-2-carboxylic acid enantiomers. The results shown in Table 2 indicate there was not a significant change in resolution between 0.01 and 0.1 % of trifluoroacetic acid.

Finally, chromatograms of a racemic sample and a typical sample after resolution are shown in Figure 2. The enantiomers were readily resolved without derivatization. The method has been used to screen a number of chiral agents for large scale resolution. Moreover, the method allows the detection of the undesired (+)-enantiomer down to a level of 0.5 % and has been used routinely to check the enantiomeric purity of (-)-2,3-dihydro-ethylbenzofuran-2-carboxylic acid after resolution.

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