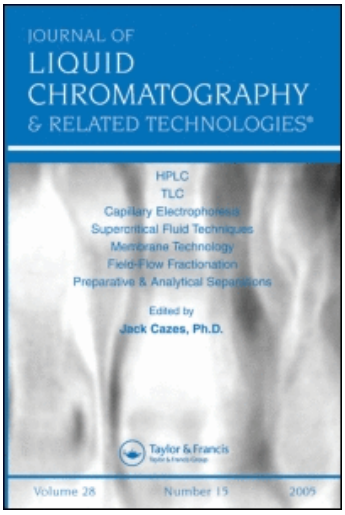

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Brian S. Kersten[a]
[a] Searle Research and Development, Skokie, Illinois

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HPLC CHIRAL OPTIMIZATION OF A UNIQUE β-AMINO ACID AND ITS ESTER

BRIAN S. KERSTEN
Searle Research and Development
4901 Searle Parkway
Skokie, Illinois 60077

ABSTRACT

Enantiomeric optimization of a unique ß-amino acid (ß-amino-3-pyridylpropionic acid) and its ethyl ester was achieved with a Daicel Crownpak CR (+) column requiring no derivatization. Using this column, the pH of the mobile phase was adjusted with perchloric acid and studied to determine the optimum pH. A plot of the capacity factor as a function of pH for the amines showed the critical pH required for chiral recognition. From this data, the retention of the amines shift from a hydrophobic non-enantioselective retention mechanism (pH > 2) to complex formation between the crown ether and the amines causing enantioselectivity (pH ≤ 2). At pH 1, lowering the column temperature from ambient to sub-ambient temperatures caused increased retention and enantiomeric separation of the amines. At pH 1 and a column temperature of ~7°C, concurrent enantiomeric baseline resolution (R_s > 1.5) of the ß-amino acid and ester was achieved. The addition of methanol under these conditions caused a decrease in retention and enantiomeric separation of the amines, especially for the ß-amino acid enantiomers.

INTRODUCTION

Currently, the development of chiral drugs has received more attention due to the Food & Drug Administration's (FDA) policy statement for the development of new chiral drugs [1]. Essentially, the policy allows the drug manufacturer to market the chiral drug as either a racemate or a single enantiomer. Interpretation of the policy will depend largely on individual FDA reviewers considering particular cases. However, it is clear from the policy that development of a racemate will require rigorous pharmacological, toxicological, and pharmacokinetic justification for FDA approval. This has prompted many drug manufacturers to develop single enantiomers or avoid asymmetric drugs altogether. While the latter is not always desirable, the former is becoming more feasible due to enantioselective syntheses and chromatographic techniques able to resolve enantiomers on the analytical, as well as the preparative scale. Thus, development of single enantiomers is becoming more prevalent in the drug industry.

Development of single enantiomers of unique ß-amino acids and their esters is on-going at Searle. These compounds are key intermediates in the synthesis of potent anti-platelet/anti-coagulant drugs. Several papers have been published on the enantiomeric separation of amino acids. The most utilized techniques have been pre-column derivatization with a chiral reagent followed by high-performance liquid chromatography

(HPLC) analysis [2-8], chiral ligand-exchange chromatography [9-13], and more recently, direct analysis by HPLC using chiral crown ethers as the stationary phase [14-22]. For an efficient enantiomeric separation, the latter's distinct advantage is no derivatization or special mobile phase requirements. This paper describes the utilization of a chiral crown ether stationary phase made by Daicel (Crownpak CR (+)) for the enantiomeric separation of a unique ß-amino acid and its ester. The effect of pH, temperature, and organic mobile phase modifier was investigated.

MATERIALS AND METHODS

Reagents and materials

ß-Amino-3-pyridylpropionic acid and ethyl ß-amino-3-pyridylpropionic ester were synthesized by the Discovery Department of Searle Research and Development. Both compounds were synthesized as hydrochloride salts. Mobile phase and diluting solvent constituents were obtained from the following vendors: Millipore Milli-Q water; methanol, Burdick & Jackson (Muskegon, MI, USA); perchloric acid, 70% A.C.S. reagent, Aldrich (Milwaukee, WI, USA).

Apparatus

The chromatographic system used for the development of the method consisted of the following: a Varian Model 2010

HPLC pump (Walnut Creek, CA, USA), a Varian Model 9090 autosampler, and a Kratos Model 783 UV detector (Foster City, CA, USA). The column was a Daicel Crownpak CR (+) (150 mm x 4 mm I.D., 5 μm particle size) obtained from Regis Chemical Company (Morton Grove, IL, USA) and cooled using a Jones Model 7950 temperature controller (Lakewood, CO, USA). The temperature of the column was measured with a Barnant 90 Model 600 type K thermocouple obtained from Barnant Company (Barrington, IL, USA). Chromatographic measurements (i.e., retention times, peak areas, etc.) were made with an in-house chromatographic data management system.

Chromatographic conditions

The mobile phase was prepared by adjusting the pH of the Milli-Q water to 1 using 70% perchloric acid and degassed for 5 min before use. Other pertinent HPLC parameters were as follows: column, Daicel Crownpak CR (+), 5 μm particle size; flow rate, 0.5 mL/min; injection volume, 10 μL; column temperature, ~7°C; detection, UV at 210 nm; total run time, 20 min.

Sample preparation

The ß-amino acid and ester samples were prepared by dissolving the compounds in Milli-Q water. Concentrations of approximately 1 mg/mL were used.

RESULTS AND DISCUSSION

Structural features

The absolute configuration of the ß-amino acid and its ethyl ester is shown in Figure 1. The synthesis of these compounds was presented elsewhere [23]. The structures show that these amines are chromophoric and therefore UV active. In addition, they have a free amine adjacent to the chiral center. These combined structural features make them ideal candidates for HPLC enantiomeric separation on a chiral crown ether column using UV detection.

Effect of pH

For chiral recognition to be achieved with the Daicel Crownpak CR (+) column, an acidic mobile phase was required for complex formation between the crown ether and the ammonium ion derived from the amine function. Perchloric acid was used to adjust the pH of the mobile phase owing to

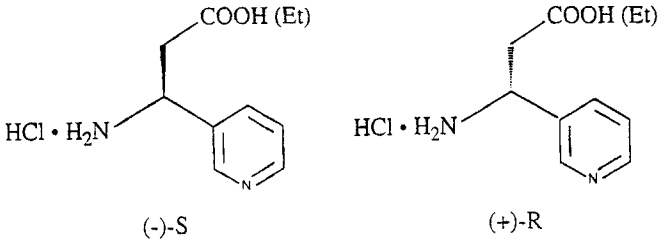

Figure 1. The absolute configurations of (+)-R-, (-)-S-ß-amino-3-pyridylpropionic acid and its ester.

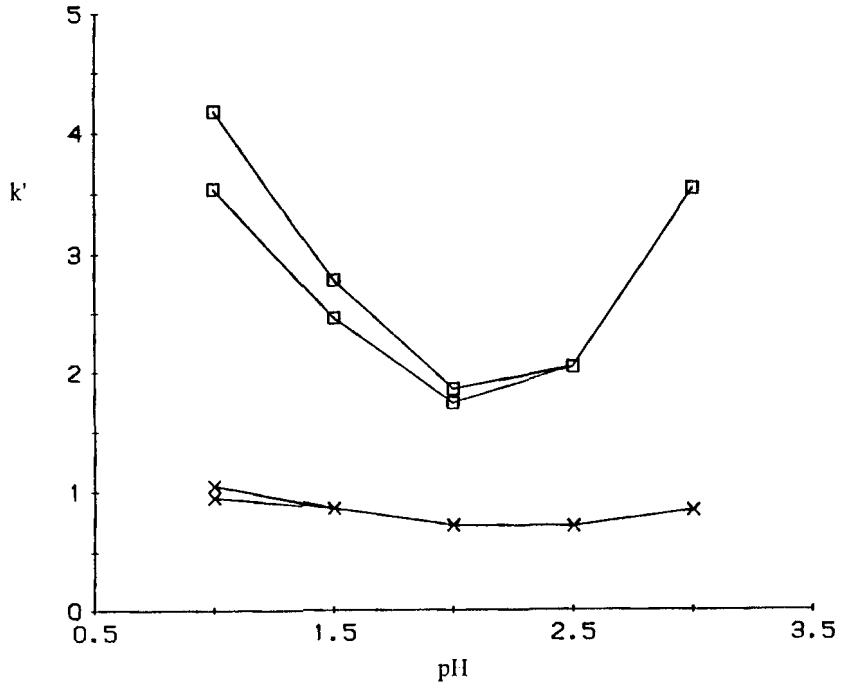

Figure 2. Capacity factor (k') as a function of pH for ß-amino acid (x) and its ester (□). The longest retained enantiomer being (-)-S.

its low UV-absorption. A plot of the capacity factor as a function of pH for the amines is shown in Figure 2. As the pH is decreased, retention of the amines decreased initially, with no evidence of chiral recognition. However, at pH 2, the ester enantiomers begin to resolve whereas the acid enantiomers do not. Below pH 2, the ester enantiomers are fully resolved. Only at pH 1 do the acid enantiomers show chiral recognition. Plotting the resolution (R_s) as a function of pH for these amines (refer to Figure 3) shows that at pH 1.5 the ester

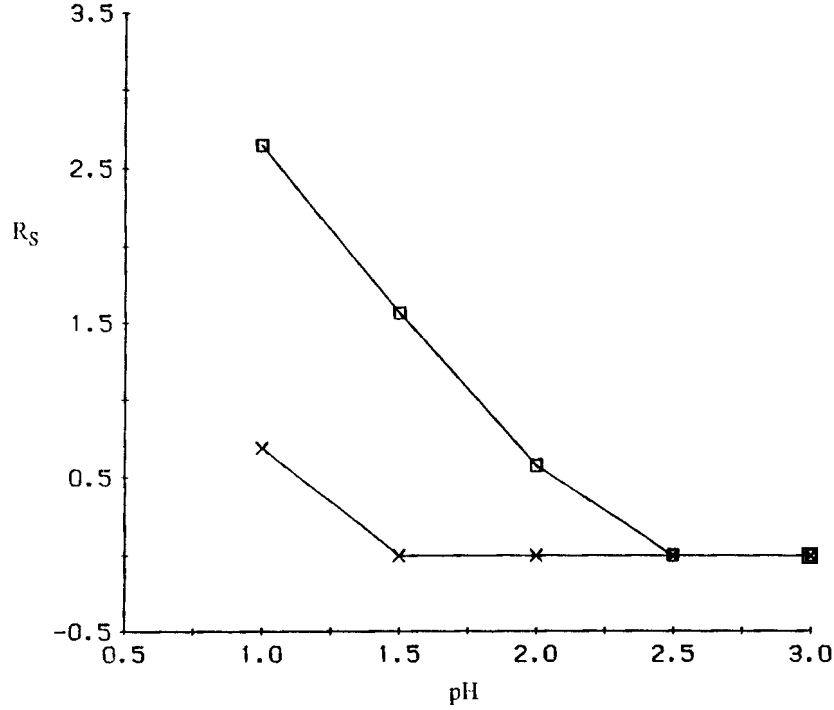

Figure 3. Resolution (R_s) as a function of pH for ß-amino acid and its ester. Symbols and enantiomeric elution same as in Figure 2.

enantiomers are baseline resolved, whereas the acid enantiomers show only partial resolution at pH 1.

This pH study illustrates the mechanism of the enantioselective retention of these amines. Shinbo et al. [20] reported that on a crown ether column, the amine is distributed between the mobile phase and stationary phase predominately by the following two reactions:

$$A^+ + X^- \rightleftharpoons [A^+X^-]_{sp} \quad (1)$$

$$A^+ + C_{sp} + X^- \rightleftharpoons [(AC)^+X^-]_{sp} \quad (2)$$

where A^+ is the protonated amine, X^- is the anion (perchlorate) in the mobile phase, C_{sp} is the crown ether, and $(AC)^+$ is the complex between the crown ether and the amine. The sp subscript indicates the stationary phase. Reaction (1) is non-enantioselective and contributes to the overall retention of the amines mainly through hydrophobic interactions. Reaction (2) determines the retention of the amines due to enantioselective recognition. Figure 2 illustrates these competing reactions. At pH 3, both the acid and ester show no enantiomeric resolution. The retention is determined mainly by reaction (1). Lowering the pH to 2.5 results in a significant decrease in retention for the ester and a slight decrease for the acid; however, there is still no chiral recognition. At pH 2, reaction (2) has begun to dominate over reaction (1), leading to chiral recognition for the ester enantiomers. The acid enantiomers are still unresolved at this pH. Continued lowering of the pH to 1.5 increases the chiral recognition of the ester enantiomers demonstrating that reaction (2) is clearly dominating. Finally at pH 1, the acid enantiomers are partially resolved. Since the manufacturer of the crown ether column does not recommend going below pH 1 due to column instability, the maximum separation allowed by pH was achieved at pH 1.

Effect of temperature

A pH of 1 provided adequate enantiomeric resolution of the ester enantiomers, but only partial resolution of the acid

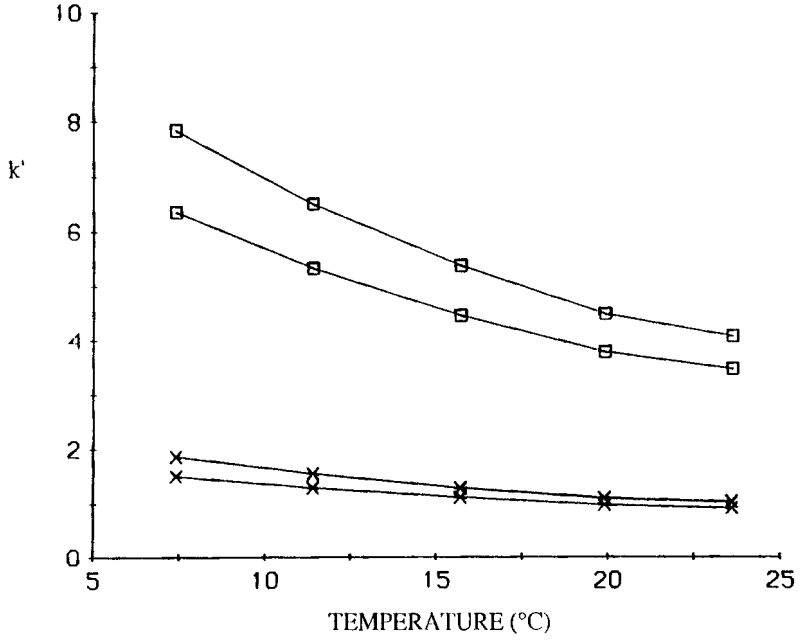

Figure 4. Capacity factor (k') as a function of temperature for ß-amino acid and its ester. Symbols and enantiomeric elution same as in Figure 2.

enantiomers. It had been reported [14,17,20,21] that lowering the temperature of a crown ether column leads to increased enantioselectivity. A plot of the capacity factor as a function of the temperature for the amines is shown in Figure 4. As the temperature decreased, the enantiomeric resolution increased for both the acid and ester enantiomers. Figure 5 is a plot of R_s as a function of temperature for the amines. Lowering the temperature dramatically affected the enantiomeric resolution of the acid. Resolution of the acid enantiomers more than doubled when going from ambient (R_s = 0.8) to approximately

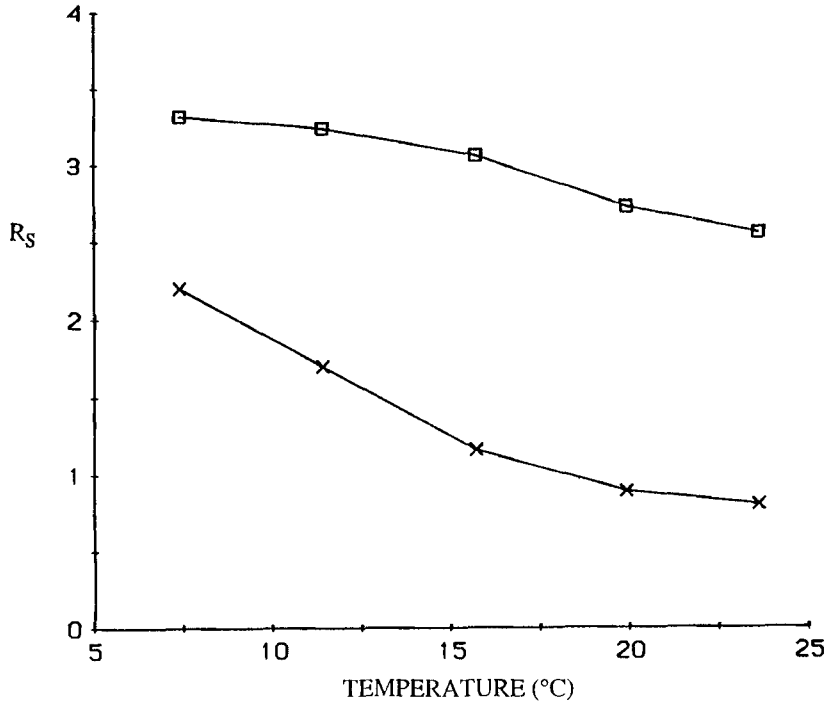

Figure 5. Resolution (R_s) as a function of temperature for ß-amino acid and its ester. Symbols and enantiomeric elution same as in Figure 2.

7°C (R_s = 2.2). The enantiomeric resolution increased slightly for the ester. Optimum HPLC enantiomeric resolution for the acid and ester enantiomers is illustrated in Figure 6 at pH 1 and a column temperature of ~7°C. The elution order of the enantiomers was confirmed by injection of the single enantiomers. The (+)-R-enantiomers eluted first for both the ß-amino acid and ester. This was consistent with the manufacturer's published report regarding elution order on the Crownpak CR (+) column [24]. Assuming a complex

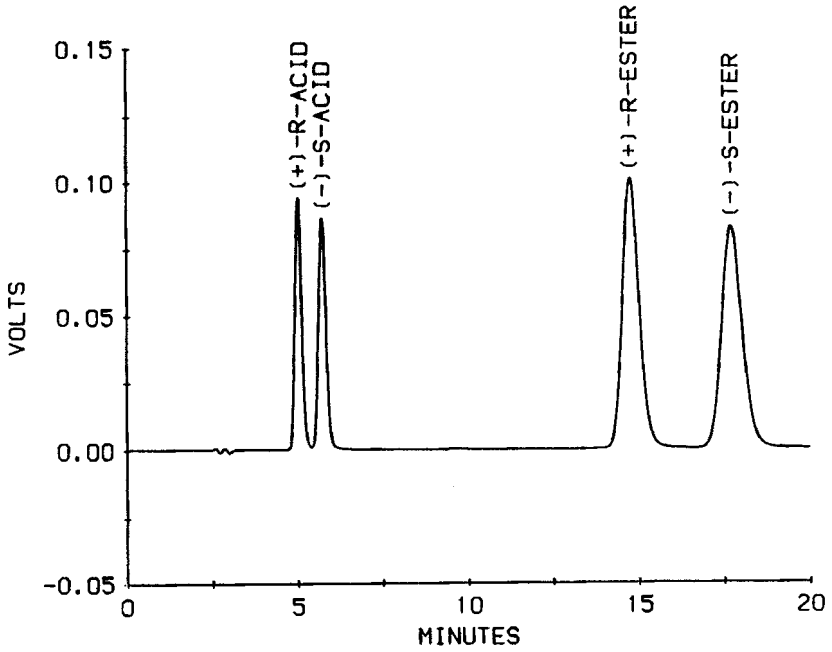

Figure 6. Optimum HPLC chiral separation of ß-amino acid and its ester. See Materials and Methods section for chromatographic conditions.

TABLE 1

Free-Energy Differences for the ß-Amino Acid and its Ester at Various Temperatures

	Δ(ΔG°), cal/mol	
Temperature, °C	Acid	Ester
23.6	-67	-93
19.9	-71	-101
15.7	-80	-109
11.4	-103	-112
7.4	-115	-115

stoichiometry of 1:1 between the crown ether and the amines, the free-energy difference can be calculated according to the following general equation [18]:

$$\Delta(\Delta G^\circ) = -RT\ln\alpha \quad (3)$$

where α is the selectivity coefficient. The free-energy differences for the ß-amino acid and ester at various temperatures are given in Table 1. The temperature dependence of $\Delta(\Delta G^\circ)$ indicates that the stability of the complex with the (-)-S-enantiomer depends to a large extent on the enthalpy term, whereas the less stable complex (i.e., (+)-R- enantiomer) is more determined by the entropy term [25]. In addition, when going from ambient to ~7°C, the acid undergoes a larger absolute change in its free-energy differences than the ester. This indicates that the stability of the crown ether-amine complex for the acid enantiomers is more dependent on the enthalpy term than the ester enantiomers. Thus, sub-ambient temperatures were required for baseline resolution of the acid enantiomers.

Effect of organic modifier

The effect of organic modifier (i.e., methanol) was also investigated. A plot of the capacity factor as a function of percent methanol for the amines is shown in Figure 7. As the percent methanol increased, a significant decrease in retention for the ester enantiomers and a slight decrease for

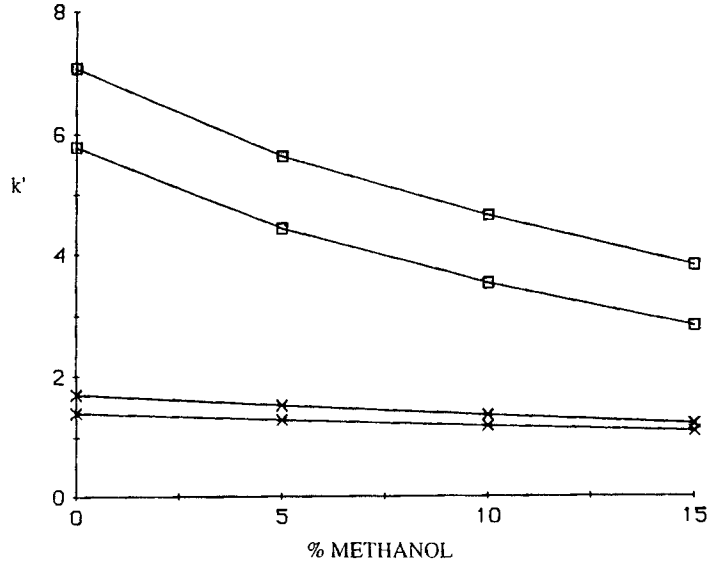

Figure 7. Capacity factor (k') as a function of percent methanol for ß-amino acid and its ester. Symbols and enantiomeric elution same as in Figure 2.

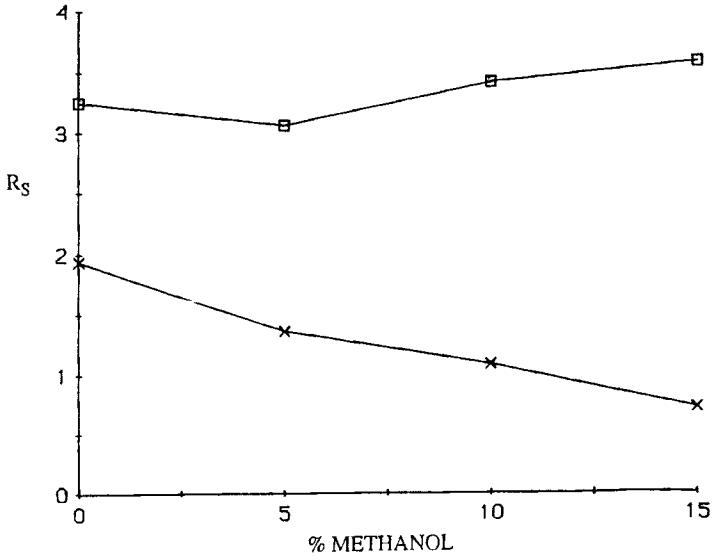

Figure 8. Resolution (R_s) as a function of percent methanol for ß-amino acid and its ester. Symbols and enantiomeric elution same as in Figure 2.

the acid enantiomers was obtained. Although resolution slightly increased for the ester enantiomers, a large loss in resolution for the acid enantiomers was observed as shown in Figure 8. Therefore, methanol was not added to the final method.

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