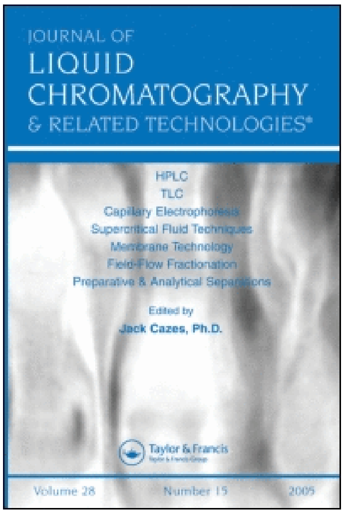

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# Optical Resolution of Some Derivatives of D,L-Amino Acids by Chiral Liquid Chromatography

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# OPTICAL RESOLUTION OF SOME DERIVATIVES OF D,L-AMINO ACIDS BY CHIRAL LIQUID CHROMATOGRAPHY

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## ABSTRACT

Enantioselective interaction of silica-immobilized bovine serum albumin (BSA) with some amino acid derivatives has been investigated by chiral HPLC. High enantiomeric separation factors ($\alpha > 5$) were found for N-(o-carboxybenzoyl)-alanine (Ia) and N-phthalimidothreonine (II). Both enantiomers of Ia were less retained than those of the precursor N-phtalimido-alanine (I). The N-benzenesulfonyl derivatives of serine and alanine were both optically resolved at low pH (5.8) with moderate $\alpha$-values (1.3 and 1.5 respectively).

## INTRODUCTION

Methods for the direct liquid chromatographic separation of enantiomers are rapidly developing (1, 2). In a series of papers we have called attention to the use of biopolymers as chiral stationary phases (CSP) and demonstrated the use of bovine serum albumin (BSA) covalently bound to a HPLC-silica support for optical resolution by HPLC (3-4). This CSP serves as a discriminator for a variety of racemic compounds

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and the process can be regarded as a "reversed" affinity chromatography. By using aqueous mobile phases we have been able to regulate retention via the buffer strength, pH or by the content of an organic modifier (e.g. 1-propanol). In this paper results from optical resolution studies of some different amino acid derivatives are described.

## EXPERIMENTAL

### Chromatography

A 4.6 x 150 mm steel column packed with the 10 µ BSA-silica for HPLC developed in our laboratory (columns available from Macherey-Nagel GmbH, Düren, GFR). The rest of the HPLC system was composed of an Altex model 110 A solvent pump, a Rheodyne model 7120 injection valve equipped with a 10 µl loop, an LDC Spectromonitor III variable-wavelength uv-detector and a Linear model 264 potentiomeric recorder. Peak area ratios were determined with the use of a Hewlett-Packard mod. 3390 A electronic integrator. All runs were performed isocratically with aqueous buffers as mobile phases containing 0-5% of organic modifier.

### Synthesis of the substituted amino acids

### Phthalimido amino acids

The amino acid (5.6 mmol) was dissolved in acetic acid (5 ml), sodium acetate (1.0 g) and phthalic anhydride (0.83 g, 5.6 mmol) were added and after refluxing the mixture for 30 min water (15 ml) was added and the solution was acidified with conc. hydrochloric acid. The product was extracted with chloroform (2 x 25 ml), dried with $MgSO_4$ and evaporated. Recrystallization from chloroform yielded white crystals. o-Carboxy-N-benzoyl-D, L-alanine (Ia) was isolated as a by-product from hydrolysis of the corresponding phthalimido compound.

(I) Phthalimido-D, L-Ala m.p 153-154°C (lit.(5) 160-162°C)
(II) Phthalimido-D, L-Thr m.p 108°C (lit.(5) 102-103°C)

### N-Benzenesulfonyl amino acids

The amino acid (5.6 mmol) was dissolved in 0.5 M sodium carbonate, (10 ml) benzenesulfonyl chloride (650 µl, 5.1 mmol) was then added and the mixture was acidified with conc. hydrochloric acid, extracted with diethyl ether and dried with $MgSO_4$. Evaporation yielded an oil which crystallized from a hot chloroform solution upon cooling.

(III) N-benzenesulfonyl-D, L-Ala m.p 121-123 °C (lit.(6) 126 °C)
(IV) N-benzenesulfonyl-D, L-Ser m.p 215-217 °C

## RESULTS AND DISCUSSION

The column performance is demonstrated by Fig. 1, which shows compounds II and IV, respectively, separated into enantiomeric pairs. Because particularly the separation of charged solutes have been shown earlier (4,7) to be strongly influenced by the pH of the mobile phase, such a study was performed using compound III. The result, represented by Fig. 2, clearly shows the rapidly increasing affinity of both enantiomers of III upon a decreasing pH. The effect is most pronounced, however, for most retained enantiomer which also leads to a significant increase in $\alpha$ ($= k'_2/k'_1$). As often found, a maximum $\alpha$-value will be obtained at a practical pH-extreme. The more hydrophobic phthalimido compounds I and II were found to be too retained in neutral buffers without organic modifier (1-propanol). However, only 2% of 1-propanol was necessary to produce useful k'-values. Although no exhaustive investigation of the influence of the mobile phase composition has been made, the conditions given in Table I were found to give $\alpha$-values high enough for complete optical resolution, except for III. The latter compound, however, is readily resolved by further lowering of the pH by changing from sodium- to ammonium dihydrogen/hydrogen phosphate buffers. With such buffers pH-values down to ca. 4.4 can be produced; conditions which have been found to be fully compatible with the column and often very useful.

A very large difference in retention of I as compared to Ia is evident from Table I. Most likely, this reflects the difference in hydrophobicity between the two compounds as hydrophobic interaction has been shown to be an important retentive factor. The same effect is found for the couple I/II, where II is the less hydrophobic due to the presence of a hydroxyl group.

The elution order of the phthalimido- (I,II) as well as the benzenesulfonyl- (III,IV) derivatives were as shown in Fig. 1, i.e. the L-form

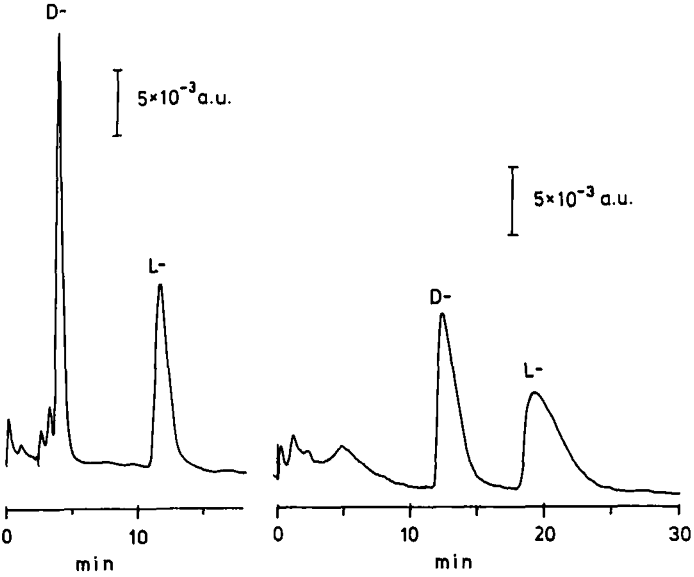

FIGURE 1. Optical Resolution of Phthalimido-D, L-threonine (0.5 mmol; left) and N-Benzenesulfonyl-D, L-serine (1.0 mmol; right). Conditions: Phosphate buffer (50 mM), 2.0% of 1-propanol, pH 7.10 (left); Phosphate buffer (50 mM), pH 5.78 (right); 1.0 ml/min; UV 225 nm/0.05.

TABLE 1

Capacity Ratios and Enantiomeric Separation Factors Obtained for Compounds I - IV in Various Mobile Phase Systems (A-C)

| System x) | pH | 1-propanol, % | compound no. | $k_1'$ | $k_2'$ | $\alpha$ |
|---|---|---|---|---|---|---|
| A | 7.2 | 2 | I | 7.1 | 14 | 2.0 |
| | | | Ia | 0.4 | 2.2 | 5.3 |
| B | 6.3 | 2 | II | 1.1 | 6.2 | 5.6 |
| C | 5.8 | 0 | III | 3.3 | 4.2 | 1.3 |
| | | | IV | 3.4 | 5.0 | 1.5 |

x) Phosphate buffers (50 mM), 0-2% of 1-propanol

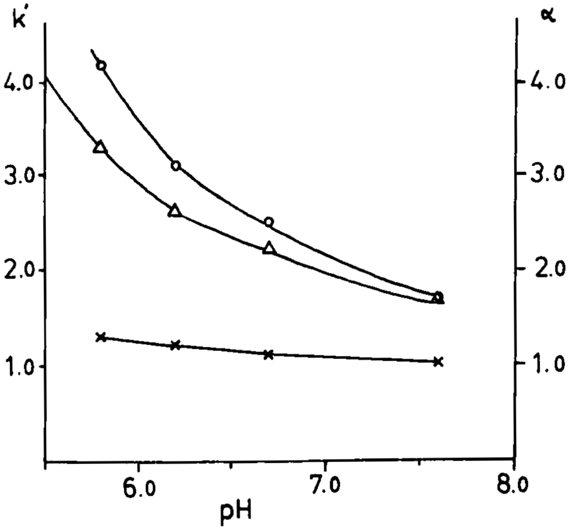

FIGURE 2. The Capacity Ratios (k′) and Enantiomeric Separation Factor (α) of N-Benzenesulfonyl-D, L-alanine as a Function of pH of the Mobile Phase (50 mM phosphate buffer). (Symbols: Δ = $k'_1$ , O = $k'_2$ , × = α ).

is the more retained enantiomer. The enantiomers of Ia, however, eluted in the reverse order, L- prior to D-, which is the same order as earlier found for N-benzoyl- and N-(p-nitrobenzoyl)- alanine (3,4).

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