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High-performance ligand-exchange liquid chromatography of fluoro derivatives of alanine

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ABSTRACT

The separation of enantiomers of 3-fluoroalanine, 3,3-difluoroalanine and 3,3,3-trifluoroalanine on ChiralProCu, ChiralValCu and Chiral-1 Nucleosil columns was studied. The substitution of hydrogen for fluorine atoms results in a significant increase in the selectivity of separation of the enantiomers. The relationship between the number of fluorine atoms and the retention of enantiomers on different columns was studied. Optimum conditions for the separation of enantiomers were found.

INTRODUCTION

Fluorine analogues of amino acids have high biological activity depending on the absolute configuration of the molecule. High-performance liquid chromatography (HPLC) has been used successfully to separate the enantiomers of fluoroamino acids but no data concerning the effect of replacement of hydrogen atoms in molecules of amino acids by fluorine atoms on the retention and selectivity of the separation of enantiomers on chiral sorbents have been reported. The separation of some polyfluoro derivatives of alanine reversed-phase HPLC with a chiral mobile phase has been developed [1–3]. It was shown that the introduction of fluorine substituents into the molecule of amino acids leads to increased retention and selectivity of enantiomers [1]. The separation of some aromatic fluoroamino acids by ligand-exchange chromatography (LEC) has been described [4] and the chromatographic behaviour of α-trifluoromethyl-α-amino acids on different chiral sorbents has been studied [5]. It was shown that changes in the selectivity of the separation of enantiomers after the introduction of a CF_3 group into the molecule of amino acids depended greatly on the type of sorbent used.

The aims of this work were to separate the enantiomers of 3-fluoro, 3,3-difluoro and 3,3,3-trifluoro derivatives of alanine by LEC and to compare the selectivities of the separation of the enantiomers on different chiral sorbents.

EXPERIMENTAL

Chromatographic conditions

The experiments were performed on an LKB (Bromma, Sweden) liquid chromatographic system consisting of a Model 2150 HPLC pump, a Model 7410 injector, a Model 2140 rapid spectral detector set at 235 nm, a Model 2200 recording integrator and a Model 2155 column oven.

The columns used were (1) ChiralProCu = Si100, (2) ChiralValCu = Si100, both 5-μm (250 × 4.6 mm I.D.) (Serva, Heidelberg, Germany), and (3) Nucleosil Chiral-1, 5 μm (250 × 4.6 mm I.D.) (Macherey–Nagel, Düren, Germany). The mobile phases were 1–2.5 m*M* copper sulphate solutions at a flow-rate of 0.75 ml/min.

Materials

Stereoisomers of fluorine-containing amino acids were synthesized as described [6,7]. Copper sulphate was of analytical reagent grade. Water was doubly distilled and filtered before HPLC use.

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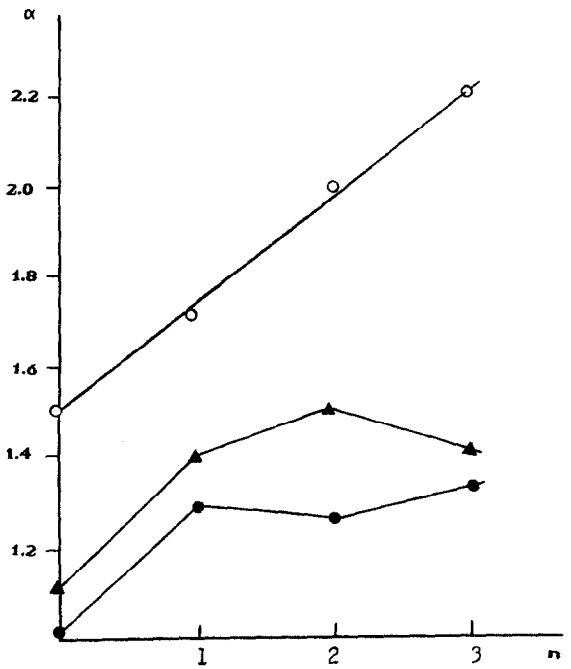

Fig. 1. Effect of the number of F atoms in the molecule of alanine (n) on selectivity of enantiomers separation. Column: ● = ChiralProCu, 5 μm (250 × 4.6 mm I.D.); ▲ = ChiralValCu, 5 μm (250 × 4.6 mm I.D.); ○ = Nucleosil Chiral-1, 5 μm (250 × 4.0 mm I.D.). Eluent, 2.5 mM $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; wavelength, 235 nm.

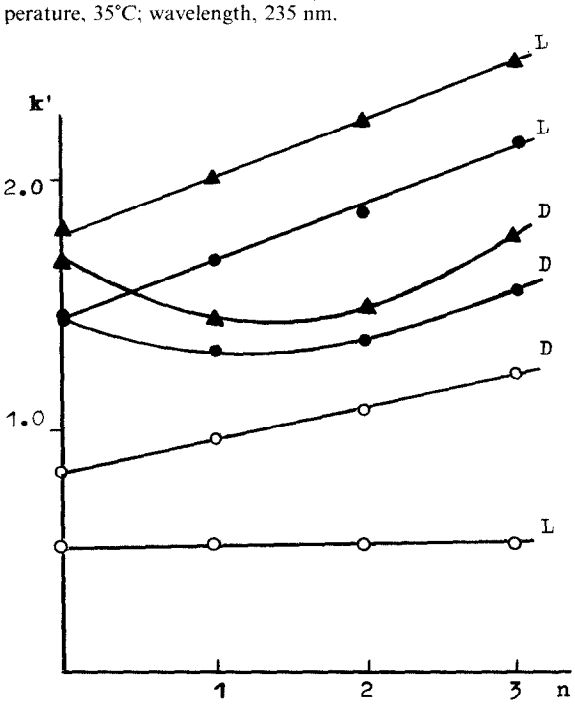

Fig. 2. Effect of the number of F atoms in the molecule of alanine (n) on the retention of enantiomers. Columns, eluent and conditions as in Fig. 1.

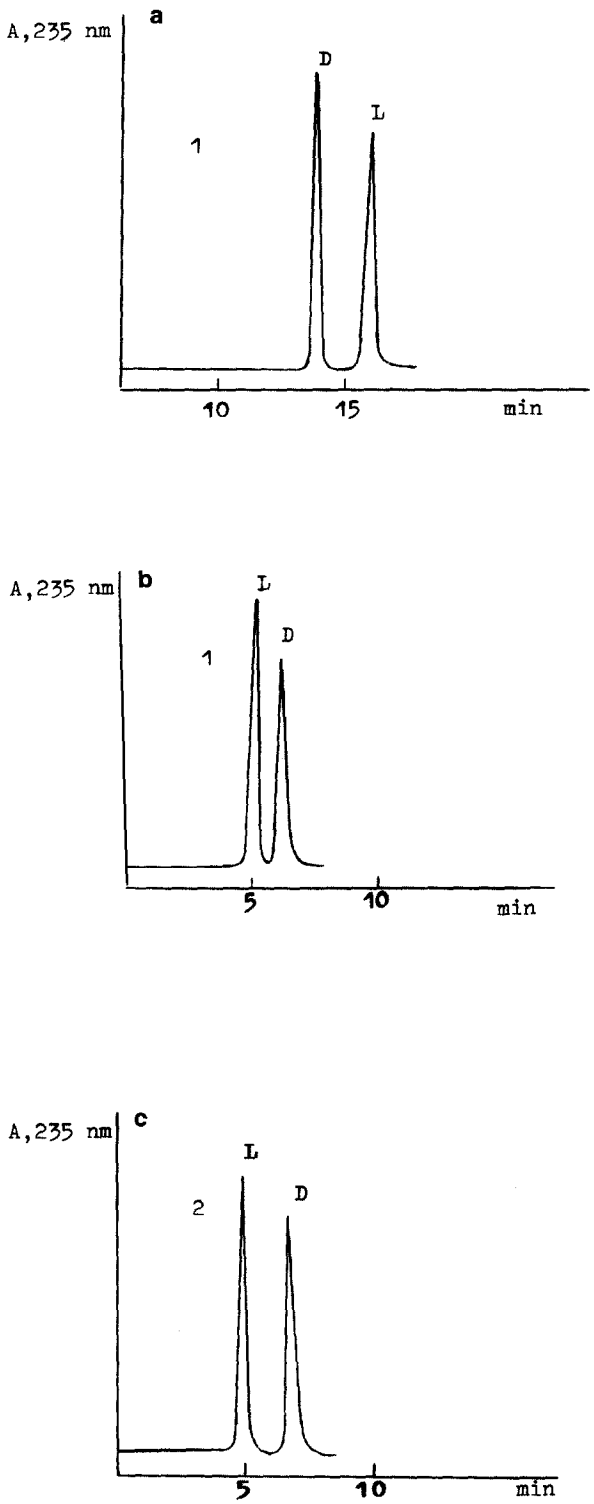

Fig. 3. Separation of enantiomers of (1) 3-fluoroalanine and (2) 3,3-difluoroalanine. Columns: (a) ChiralProCu, 5 μm (250 × 4.6 mm I.D.); (b and c) Nucleosil Chiral-1, 5 μm (250 × 4.0 I.D.). Eluent and conditions as in Fig. 1.

RESULTS AND DISCUSSION

The selectivity of the separation of amino acid enantiomers by LEC on chiral stationary phases depends substantially on the structure of ligand bonded [8]. Fig. 1 shows the dependence of the selectivity (α) of the separation of amino acids enantiomers on the number of atoms of fluorine in the molecule. The results show that for the sorbents studied there are considerable differences in the change in this parameter when H atoms are successively replaced with F atoms. For Nucleosil Chiral-1 the introduction of each F atom into the molecule of alanine leads to an increase in the selectivity of 0.23, whereas the introduction of only the first atom of fluorine significantly affects the selectivity of separation on ChiralProCu and ChiralValCu columns. The introduction of F atoms affects differently the retention of L- and D-isomers. For all the sorbents studied the plot of capacity factor (k') *vs.* number of F atoms (n) is a straight line of slope $0.15–0.25k'/n$ for more retained enantiomers (Fig. 2). The absence of any difference in the retention of L-isomers of the amino acids studied on the Nucleosil Chiral-1 column indicates that only a slight or no interaction occurs between the fluoro-containing part of the molecules and the surface ligand of the sorbent. It is also interesting that on columns 1 and 2 the order of elution of D- and L-isomers is the reverse of that with column 3 (Fig. 3). The order of elution on the Nucleosil Chiral-1 column ($k'_D > k'_L$) is characteristic of sorbents with a polymer covering a silica matrix [6].

Hence these experiments have shown that the enantiomers of all the fluoroamino acids studied can be successfully separated by LEC (Fig. 3). The efficiency of the columns is fairly high and the separation is easily achieved at ambient temperature, but some heating of the column enabled the efficiency of separation to be improved. The optimum concentration of Cu(II) ions in the mobile phase is 1.0–2.5 mM. The method may be useful both for the small-scale resolution of enantiomers and for monitoring the enantiomeric purity of fluoro derivatives of alanine.

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