Journal of Chromatography A, 661 (1994) 51–54
Elsevier Science B.V., Amsterdam

CHROMSYMP. 2966

Ligand-exchange high-performance liquid chromatography of fluorine-containing phenylglycine and phenylalanine

S.V. Galushko*, I.P. Shishkina and V.A. Soloshonok

Institute of Bioorganic Chemistry and Oil Chemistry, Academy of Sciences of the Ukraine, 253660 Kiev-94 (Ukraine)

ABSTRACT

The relationships between the number of fluorine atoms, their position in the aromatic ring of fluorine-containing phenylglycine and phenylalanine and the selectivity of the separation of enantiomers on Chiral ProCu, Chiral ValCu and Chiral-1 (hydroxyproline) columns were studied. Optimum conditions for the separation of enantiomers were established.

INTRODUCTION

The replacement of hydrogen atoms by fluorine atoms in natural compounds in general and amino acids in particular is a very useful approach in the design of biologically active compounds [1].

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 only a few 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 relationship between the number of fluorine atoms and the retention of enantiomers of alanine derivatives on different chiral columns has been studied [2]. The separation of some aromatic fluoroamino acids by ligand-exchange chromatography (LEC) has been described [3]. It has been shown that the introduction of fluorine atoms into the *ortho* and *para* positions of the phenyl ring has little effect on the capacity factors and the selectivity of separation of phenylserine isomers [4].

The aim of this work was to separate the enantiomers of different fluoro derivatives of phenylalanine and phenylglycine and to compare the selectivity 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 (I) Chiral ProCu=Si100, (II) Chiral ValCu=Si100, both 5 μm (250 × 4.6 mm I.D.) (Serva, Heidelberg, Germany) and (III) Nucleosil Chiral-1, 5 μm (250 × 4.6 mm I.D.) (Macherey–Nagel, Düren, Germany). The mobile phases were 2–5 m*M*

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(93)E1109-D

copper(II) sulphate solutions at a flow-rate of 0.75 ml/min.

Materials

Racemic fluorine-containing phenylglycines and phenylalanines were purchased from Fluka (Buchs, Switzerland). Individual enantiomers of fluorine-containing phenylglycines were prepared by biocatalytic separation of their corresponding racemic N-phenylacetyl derivatives [5], and enantiopure fluorinated phenylalanines were prepared by asymmetric synthesis using chiral nucleophylic glycine template [6].

Copper(II) sulphate was of analytical-reagent grade. Water was doubly distilled and filtered before HPLC use.

RESULTS AND DISCUSSION

The selectivity of the separation of the enantiomers of phenylalanine (Phe) and phenylglycine (PhGly) derivatives depends substantially on the structures of both the ligand and the enantiomers being separated (Table I). As can be seen from Table I, the introduction of a fluorine atom into the *ortho* position of the aromatic ring of PhGly increases the selectivity of enantiomer separation on all the sorbents studied. The introduction of a fluorine atom in the *meta* and *para* positions of PhGly leads to a considerable decrease in the selectivity of separation of enantiomers on column III and to a complete loss of selectivity on column I. Addition of methanol to the mobile phase (0–30%, v/v) has only a slight effect on the selectivity of enantiomer separation but leads to an appreciable increase in efficiency. Columns II and III allow the complete separation of enantiomers of fluoro derivatives of PhGly with a mobile phase containing 2.5–5 m*M* copper(II) sulphate (Fig. 1a and b) and 0–20% (v/v) methanol.

The introduction of a methylene group into the molecule on going from PhGly to Phe derivatives results in a significant increase in capacity factors (Table I). In this case the maximum selectivity can be achieved on column I. It should be noted that the replacement of one hydrogen atom by a fluorine atom in any position on the phenyl ring of Phe derivatives hardly affects the selectivity of separation of enantiomers (Table I). The replacement of hydrogen by two and five atoms of fluorine in the aromatic ring results in a decrease in retention of the enantiomers on columns I and II. An increase in the number of fluorine atoms in the aromatic ring affects differently the selectivity of separation of enantiomers. For column I the introduction of each F atom into the molecule of Phe

TABLE I

SEPARATION OF AROMATIC FLUORINE-CONTAINING AMINO ACIDS

$n = 4$. For k' and α values, S.D. = 0.2 and 0.1 respectively; $r = 0.987$.

Compounds	Column I		Column II		Column III	
	k'_L	α	k'_L	α	k'_L	α
4F-DL-α-PhGly	2.1	1.0	2.5	1.2	0.9	1.8
3F-DL-α-PhGly	1.9	1.0	2.2	1.2	0.9	1.8
2F-DL-α-PhGly	2.4	1.3	2.7	1.4	0.9	2.8
DL-α-PhGly	2.3	1.1	2.7	1.2	0.8	2.4
4F-DL-α-Phe	4.8	2.3	4.6	1.5	1.3	1.6
3F-DL-α-Phe	5.1	2.0	4.6	1.4	1.5	1.6
2F-DL-α-Phe	5.3	2.0	4.6	1.3	1.6	1.5
DL-α-Phe	4.6	2.3	3.6	1.3	1.4	1.6
$3CF_3$-DL-α-Phe	6.2	2.2	4.8	1.4	2.3	2.0
3-Di-F-DL-α-Phe	4.4	1.9	3.7	1.7	1.7	1.7
1,2,3,4,5-Penta-F-DL-α-Phe	2.3	1.2	2.7	1.2	1.5	1.6

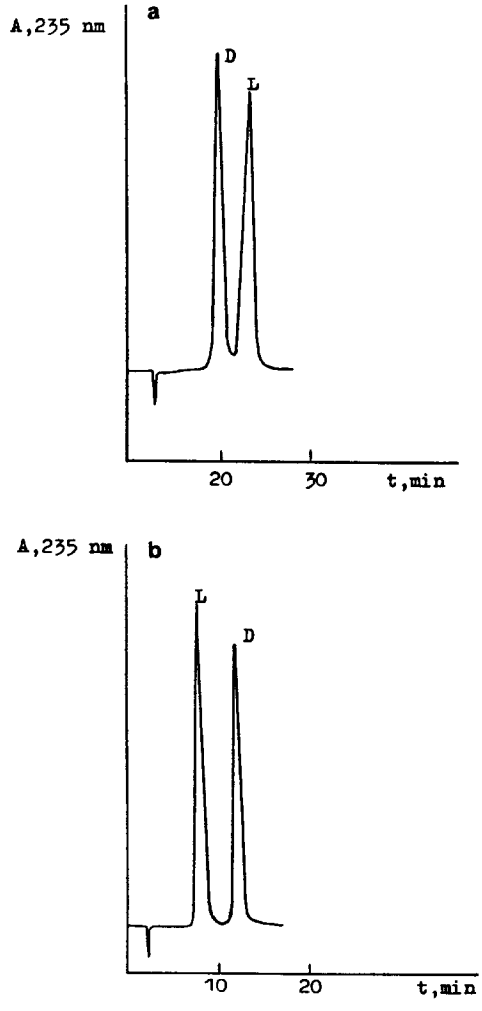

Fig. 1. Separation of enantiomers of (a) 4-fluoro-DL-α-phenylglycine and (b) 3-fluoro-DL-α-phenylglycine. Mobile phase, 5.0 m*M* $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; detection wavelength, 235 nm. Column: (a) Chiral ValCu, 5 μm (250 × 4.6 mm I.D.); (b) Nucleosil Chiral-1, 5 μm (250 × 4.0 mm I.D.).

leads to a decrease in the selectivity of separation of 0.2, whereas for column III no effect is observed (Fig. 2). It has been found that a CF_3 group in the α-position in Phe exerts a considerable influence on both the retention and the selectivity of the separation of enantiomers [7]. Introduction of a CF_3 group into the phenyl ring of Phe has little effect on the retention and selectivity. It seems that only a slight interaction occurs between the CF_3 group in a *meta* position

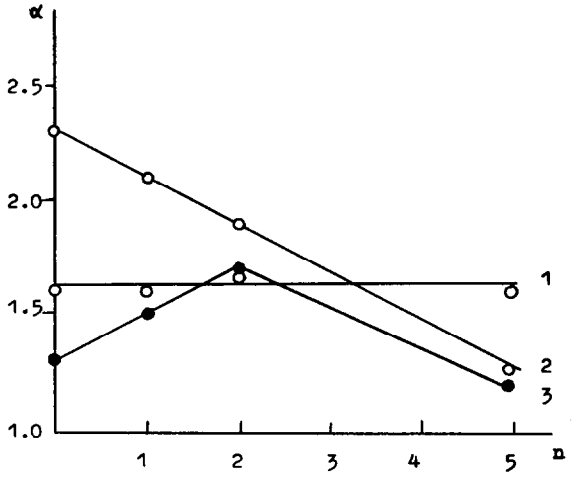

Fig. 2. Effect of the number of fluorine atoms in the molecule of phenylalanine (*n*) on selectivity of enantiomer separations (α). Column: 1 = Nucleosil Chiral-1, 5 μm (250 × 4.0 mm I.D.); 2 = Chiral ProCu, 5 μm (250 × 4.6 mm I.D.); 3 = Chiral ValCu, 5 μm (250 × 4.6 mm I.D.).

on the phenyl ring of Phe and the surface ligand of the sorbent.

A mobile phase containing 2.5–5 m*M* copper(II) sulphate and 0–20% (v/v) methanol is optimum for separating fluoro derivatives of phenylalanine (Fig. 3).

CONCLUSIONS

The replacement of hydrogen atoms with fluorine atoms in the aromatic ring affects the

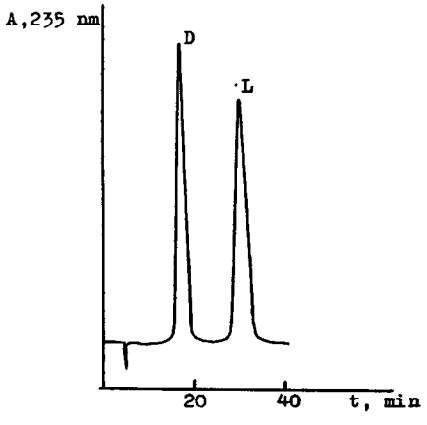

Fig. 3. Separation of enantiomers of 2-fluoro-DL-α-phenylalanine. Column, Chiral ProCu, 5 μm (250 × 4.6 mm I.D.); eluent and conditions as in Fig. 1.

selectivity of separation of enantiomers to a greater extent for phenylglycine derivatives than for phenylalanine derivatives.

Chiral ProCu=Si100 is to be preferred for the separation of monosubstituted derivatives of phenylalanine.

A Nucleosil Chiral-1 (hydroxyproline) column is to be preferred for the separation of monosubstituted derivatives of phenylglycine and pentafluoro derivatives of phenylalanine.

All columns studied have similar possibilities for the separation of enantiomers of 2,6-difluoro derivatives of phenylalanine.

REFERENCES

1 J.T. Welch, *Flourine in Bioorganic Chemistry*, Wiley, New York, 1991.
2 S.V. Galushko, I.P. Shishkina, I.I. Gerus and M.T. Kolycheva, *J. Chromatogr.*, 600 (1992) 83.
3 J.R. Gerson and M.I. Adam, *J. Chromatogr.*, 325 (1985) 103.
4 S.V. Galushko, I.P. Shishkina and V.A. Soloshonok, *J. Chromatogr.*, 592 (1990) 345.
5 V.F. Soloshonok, I.Yu. Galaev, V.G. Svedas, E.V. Kozlova, U.V. Kotik, I.P. Shishkina, S.V. Galushko, A.V. Rozhenko and V.P. Kukhar, *Bioorg. Khim.* 19 (1993) 103.
6 V.P. Kukhar, Yu.N. Belokon', V.A. Soloshonok, N.A. Svistunova, A.V. Rozhenko and N.A. Kuz'mina, *Synthesis*, 1 (1993) 117.
7 S.V. Galushko, I.P. Shishkina, V.A. Soloshonok and V.P. Kukhar, *J. Chromatogr.*, 511 (1990) 115.