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High-performance ligand-exchange chromatography of some amino acids containing two chiral centres

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ABSTRACT

The separation of 2*S*,3*S*-, 2*R*,3*R*-, 2*S*,3*R*- and 2*R*,3*S*-isomers of threonine, phenylserine and the *o*- and *p*-fluoro derivatives of the latter on chiral sorbents was studied. The sorbent ChiralProCu = Si100 gave a complete separation of the four isomers of the amino acids studied.

INTRODUCTION

High-performance ligand-exchange chromatography (HPLEC) is widely used to separate enantiomers of amino acids [1]. Sorbents containing residues of amino acids bonded to the surface of a silica matrix enable enantiomers of different amino acids to be separated [1,2]. Most papers, however, describe the separation of enantiomers that differ in the configuration of only one carbon atom. The separation of enantiomers of amino acids that contain two chiral centres is more complicated but highly desirable. Several methods for the synthesis of such amino acids have been developed [3–5] and the chromatographic separation of two pairs of enantiomers can be very useful for both analytical and preparative usage. The separation of a mixture of four enantiomers of isoleucine on a crown ether-based packing has been demonstrated, but only a very slight resolution of the *threo*- and *allo*-isomer pair was achieved [6]. The enantiomers of threonine were separated by reversed-phase high-performance liquid chromatography (HPLC) with a dynamic coating of a chiral crown ether but no separation of *allo*-threonine enantiomers was obtained [7].

The paper is intended to demonstrate the possibility of separating all four stereoisomers [(2*S*,3*S*)-L,L-, (2*R*,3*R*)-D,D-, (2*R*,3*S*)-D,L- and (2*S*,3*R*)-L,D-] of threonine, phenylserine and the *o*- and *p*-fluoro derivatives of the latter.

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 at 225 nm and a Model 2200 recording integrator. The column 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 1–5 m*M* copper sulphate solutions at a flow-rate of 0.75 ml/min.

Materials

Stereoisomers of fluorine-containing amino acids were obtained as described [8]. Natural amino acids (threonine and phenylserine) were supplied by Reakhim (Moscow, USSR) and Chemapol (Prague, Czechoslovavia). Copper sulphate (analytical-reagent grade) was used as received. Water was doubly distilled and filtered for HPLC use.

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TABLE I

RETENTION (k') OF ISOMERS OF α-AMINO-β-HYDROXY ACIDS ON CHIRAL COLUMNS

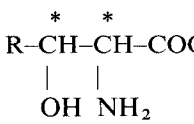
Eluent: 5 mM $CuSO_4$.

Compound	2*R*,3*R*			2*R*,3*S*			2*S*,3*R*			2*S*,3*S*		
	1[a]	2[a]	3[a]	1	2	3	1	2	3	1	2	3
o-F-PhSer	1.5	1.5	1.6	1.7	1.8	0.9	3.4	2.7	0.85	8.3	4.1	0.9
p-F-PhSer	1.6	1.5	1.3	1.8	1.7	0.9	3.4	2.6	0.88	8.3	4.0	0.85
PhSer	1.4	1.6	1.5	1.6	2.0	1.1	3.4	2.5	1.05	8.6	4.0	1.1
Thr	0.7	0.7	[b]	0.8	0.9	[b]	1.4	1.1	[b]	2.3	1.3	[b]

[a] Columns 1, 2 and 3 as described under Experimental.
[b] The k' values are too low to be determined exactly.

RESULTS AND DISCUSSION

The amino acids studied are α-amino-β-hydroxy acids. They contain two chiral carbon atoms which can be in the *S* or *R* configuration:



The 2*S*,3*R* (*S-threo*) configuration corresponds to natural phenylserine (R = C_6H_5) and threonine (R = CH_3). The configuration and the yield of the isomers of synthetic amino acids depend on the conditions of synthesis [3–5]. In the most complex case all four isomers are present simultaneously.

In searching for the optimum conditions for separation of the isomers, use was made of sorbents that contained residues of (*S*)-L-proline, (*S*)-L-hydroxyproline and (*S*)-L-valine bonded to the surface of the silica matrix.

The experiments showed that a proline column permits the complete separation of all isomers of the amino acids studied with the retention times increasing in the order 2*R*,3*R* < 2*R*,3*S* < 2*S*,3*R* < 2*S*,3*S*. It should be noted that the introduction of atoms of fluorine into the *ortho* and *para* positions of the phenyl ring has little effect on the capacity factors (k', Table I) and the selectivity of sepration of isomers (Table II). The threonine isomers have much lower retentions than phenylserine isomers. Mobile phases containing 5 mM and 1.25–2.5 mM

TABLE II

SELECTIVITY OF SEPARATION OF α-AMINO-β-HYDROXY ACID ISOMERS ON CHIRAL COLUMNS

Eluent: 5 mM $CuSO_4$.

Isomer pairs being separated	*o*-F-PhSer			*p*-F-PhSer			PhSer			Thr	
	1[a]	2[a]	3[a]	1	2	3	1	2	3	1	2
R,*R*/*R*,*S*	1.1	1.2	1.8	1.1	1.1	1.4	1.1	1.2	1.4	1.1	1.3
R,*R*/*S*,*R*	2.3	1.8	1.9	2.1	1.7	1.5	2.4	1.7	1.4	2.0	1.6
R,*R*/*S*,*S*	5.5	2.7	1.8	5.2	2.7	1.6	6.1	2.5	1.4	3.2	1.8
R,*S*/*S*,*R*	2.0	1.5	1.1	1.9	1.5	1.0	2.1	1.3	1.0	1.8	1.2
R,*S*/*S*,*S*	4.9	2.3	1.0	4.6	2.3	1.0	5.3	1.9	1.0	2.9	1.4
S,*R*/*S*,*S*	2.4	1.5	1.1	2.4	1.5	1.0	2.5	1.6	1.0	1.6	1.2

[a] Columns 1, 2 and 3 as described under Experimental.

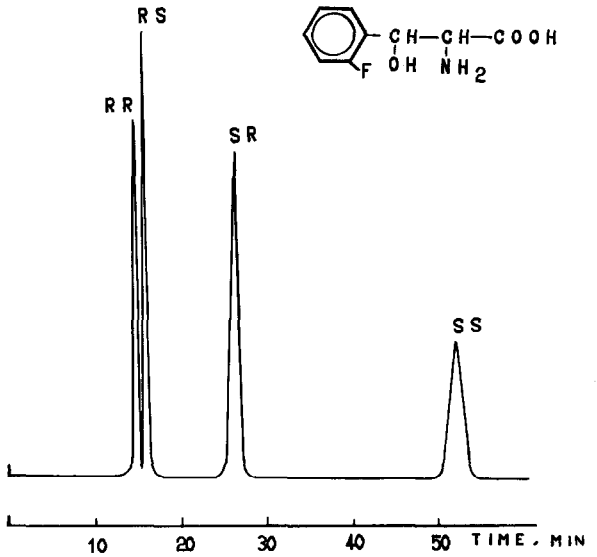


Fig. 1. Separation of isomers of *o*-fluorophenylserine. Column, ChiralProCu, 5 μm, 250 × 4.6 mm I.D.; eluent, 5 m*M* $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; wavelength, 225 nm.

copper sulphate are optimum for the separation of isomers of phenylserine and threonine, respectively (Figs. 1–3).

The order of elution of the isomers studied on valine and proline columns is the same whereas the selectivity of the separation of enantiomers on the valine column is much less than that on the proline columns but nevertheless the separation of four

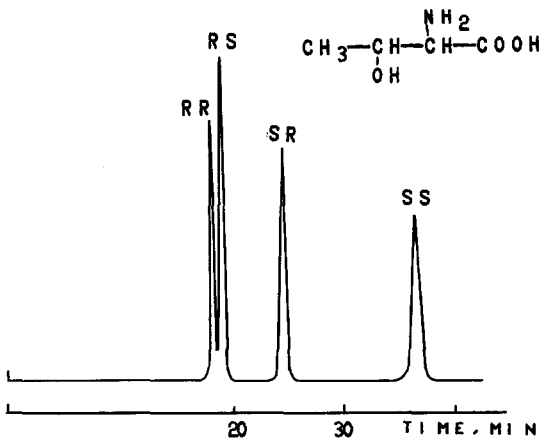


Fig. 2. Separation of isomers of threonine. Column, ChiralProCu, 5 μm, 250 × 4.6 mm I.D.; eluent, 2.5 m*M* $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; wavelength, 225 nm.

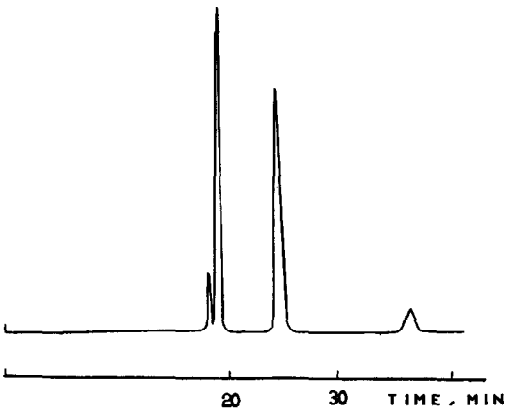


Fig. 3. Chromatogram of a threonine sample (Chemapol). Column, ChiralProCu, 5 μm, 250 × 4.6 mm I.D.; eluent, 1.5 m*M* $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; wavelength, 225 nm. The order of elution is as in Fig. 2.

isomers of the aromatic acids can be achieved (Fig. 4). It should be noted that a high column performance is needed for the separation of 2*R*,3*R*/2*R*,3*S* pairs on both the proline and valine columns. It is also interesting that the decrease in the selectivity of separation of enantiomers on the valine column as compared with the proline column is the result of a decrease in retention mainly of the 2*S*,3*R* and 2*S*,3*S* isomers (Tables I and II). The optimum concentrations of copper sulphate in the eluent are 4–5 m*M* for

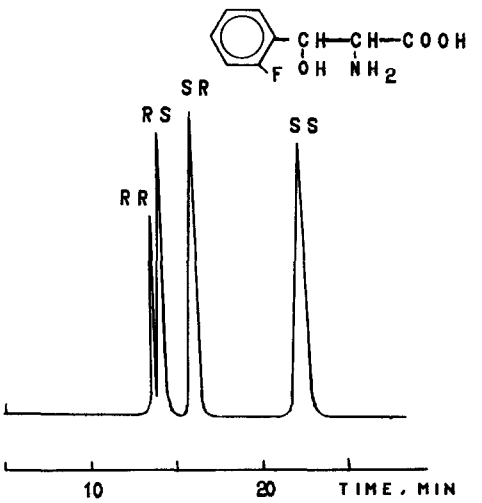


Fig. 4. Separation of isomers of *o*-fluorophenylserine. Column, ChiralValCu, 5 μm, 250 × 4.6 mm I.D.; eluent, 5 m*M* $CuSO_4$; flow-rate, 0.75 ml/min; temperature, 35°C; wavelength, 225 nm.

separation of isomers of phenylserine on the valine column. The retention and selectivity of the separation of threonine isomers on this column were not high enough for complete resolution to be obtained.

As can be seen from the results in Table I, the 3*S* isomers have the highest retention on the proline and valine sorbents. Moreover, if we consider the selectivity of separation of the 2*R*,3*R*/2*R*,3*S* and 2*R*, 3*R*/2*S*,3*R* and also the 2*S*,3*S*/2*R*,3*S* and 2*S*,3*S*/2*S*, 3*R* pairs (Table II), it can be seen that a change in the configuration of the α-carbon atom has a much greater effect on the selectivity of separation than that of the β-carbon atom. It can therefore be concluded that coordination interactions make a considerable contribution to the selectivity of separation of the isomers.

The order of elution and the selectivity of separation of enantiomers on the Nucleosil Chiral-1 hydroxyproline column are entirely different from those on the other two columns (Tables I and II). The capacity factors of isomers of aromatic amino acids increase in the order 2*S*,3*R* < 2*S*,3*S* = 2*R*, 3*S* < 2*R*,3*R*. In this instance it is impossible to separate all four isomers. The selectivity of the separation of the pairs 2*S*,3*R*/2*R*,3*S* and 2*S*,3*R*/2*S*, 3*S* is not high, but the selectivity of the separation of the pair 2*R*,3*S*/2*R*,3*R* is higher than that on the proline and valine columns. The replacement of hydrogen in the phenyl ring with fluorine has a weak effect on the retention of enantiomers on the hydroxyproline column. The retention times and selectivity of separation threonine isomers on this column were insufficient to obtain the resolution needed.

It is concluded that ChiralProCu = Si100 is an excellent sorbent for the simultaneous separation of all four isomers of amino acids containing two chiral centres. ChiralValCu = Si100 has similar possibilities for the separation of isomers of aromatic α-amino-β-hydroxy acids. The Nucleosil Chiral-1 hydroxyproline column is to be preferred for the separation of the 2*R*,3*R*/2*R*,3*S* pair. It should be noted that the possibility of separating all four isomers of such amino acids with two chiral centres has other useful applications in addition to analytical and preparative usages. As the capacity factors for enantiomers on ChiralProCu differ greatly and are weakly dependent on substituents in the phenyl ring, HPLEC may be effective in establishing the absolute configuration of the molecules. In many instances this problem is difficult to solve and needs expensive instrumentation and much effort.

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