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New chemically bonded chiral ligand-exchange chromatographic stationary phases

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ABSTRACT

The development of two new chemically bonded chiral ligand-exchange chromatographic phases is described. These phases were prepared by binding L-proline to silica gel via different spacer groups. The influence of the structure of the spacer on the enantioselectivity was studied. These phases showed enantioselectivity of enantiomer separation for many classes of substance that form chelate complexes, such as amino acids, dansylamino acids, dipeptides, hydroxy acids and barbiturates.

INTRODUCTION

Chiral ligand-exchange chromatography (LEC), introduced by Rogozhin and Davankov [1], has been found to be a powerful tool for the resolution of the enantiomers of chelating compounds [2]. In previous papers we described the development and application of chemically bonded chiral LEC phases of the type CSP I (Fig. 1) [3–5]. These phases were successfully used in the direct chiral resolution of amino acids [3–6] and their derivatives [7], thyroid hormones [8], hydroxy acids [9] and dipeptides [6]. This paper deals with the development of two new chiral LEC phases, CSP II and CSP III (Figs. 2 and 3). These phases showed improved enantioselectivity of the separation of amino acids, dipeptides and hydroxy acids. CSP III was found to be applicable also to the resolution of racemic barbiturates. The enantiomers of N-alkylated barbiturates are known to exhibit different pharmacological effects [10,11].

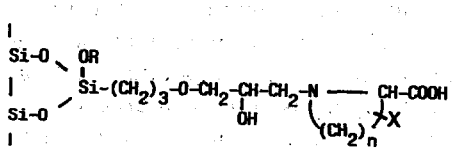

Fig. 1. Phases of the type CSP I. $n = 3–5$; X = H or OH.

EXPERIMENTAL

Apparatus

HPLC was performed using a Merck–Hitachi L-6200A intelligent pump and an L 4250 UV/Vis detector. Samples were injected by a Rheodyne Model 7161 six-port valve equipped with a 20-μl loop. Polarimetric detection was carried out with a Perkin-Elmer 241 MC polarimeter equipped with a micro flow cell of 100 mm path length and 0.65 mm I.D.

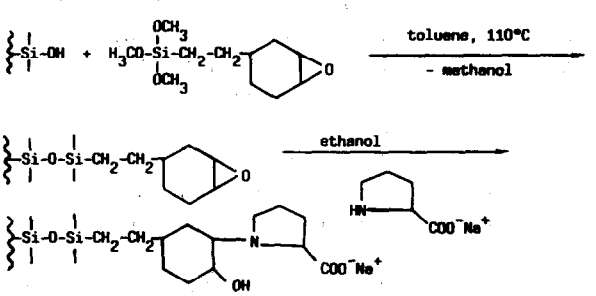

Fig. 2. Scheme for the synthesis of CSP II.

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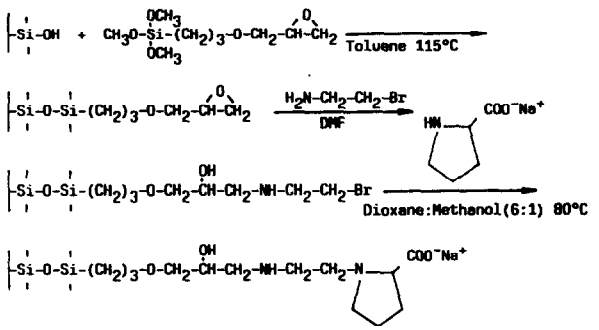

Fig. 3. Scheme for the synthesis of CSP III.

Chemicals and materials

L-Proline, potassium dihydrogenphosphate, LiChrosorb 100 (5 μm) and analytical-reagent grade solvents were purchased from Merck (Darmstadt, Germany), ammonium acetate, copper(II) sulphate and 2-aminoethyl bromide hydrobromide from Fluka (Buchs, Switzerland), 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane and trimethylmethoxysilane from Petrarch Systems (Bristol, PA, USA) and the racemates and enantiomers of amino acids, dansylamino acids, dipeptides and hydroxy acids from Sigma (Deisenhofen, Germany). Racemates and enantiomers of the barbiturates were a gift from Professor Knabe, University of the Saarland, Germany.

Preparation of the CSP II

Silica gel (4 g) was suspended in 20 ml of toluene and after adding 2.4 ml of 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane, the mixture was refluxed at 110°C with stirring for 6 h. The reflux condenser was kept at 65°C in order to remove the methanol formed in the reaction. The product was washed with toluene, methanol and acetone and dried at 50°C overnight. Endcapping was carried out by heating the product with 3 ml of trimethylmethoxysilane in dry toluene for 2 h. After washing with toluene, methanol and acetone, the product was dried for 2 h at 50°C. The product was shaken with 4.4 g of sodium prolinate dissolved in 40 ml of methanol for 48 h at room temperature. The modified silica was washed with methanol and loaded with copper(II) ions by shaking with a 15% solution of copper(II) sulphate. Elemental analysis: C 9.0, H 1.4, N 0.6%.

Preparation of CSP III

Silica gel (4 g) was reacted with 3-glycidoxypropyltrimethoxysilane according to the silanization procedure described above. The intermediate was then shaken with 8 g of aminoethyl bromide hydrobromide in 40 ml of dimethylformamide (DMF) for 24 h at room temperature. The resulting product was washed with DMF and dioxane-methanol (6:1) and loaded with copper(II) as described above. Elemental analysis: C 7, H 1.5, N 1.0%.

Columns

The CSP II and III materials were packed into stainless-steel columns (250 × 4.6 mm I.D.) by the descending slurry technique in methanol.

RESULTS AND DISCUSSION

The chiral LEC phases CSP II and III were examined in order to study the influence of the spacer's structure on the enantioselectivity. In CSP II, a cyclic group was introduced into the spacer with the aim of obtaining a more rigid structure element close to the chiral selector ligand to enhance chiral recognition. In addition to the chiral C atom in L-proline, there are three asymmetric centres in the spacer. As the enantiomers of the selector were not yet resolved, the influence of these additional asymmetric centers on the enantioselectivity could not be studied. Further, by opening of the cyclic epoxide, two geometrical isomers can be formed. This phase showed an improvement in the resolution of many amino acids with shorter retention times compared with CSP I, developed previously. In addition to amino acids, this phase showed enantioselectivity of enantiomer separation of a broad range of compounds such as dipeptides, hydroxy acids and barbiturates.

The proposed structure of the mixed complexes between L-proline as the fixed chiral selector and D- or L-phenylalinine is shown in Fig. 4. It can be assumed that the OH group on the cyclic spacer formed by opening of the cyclic epoxide participates in complex formation, thus enhanc-

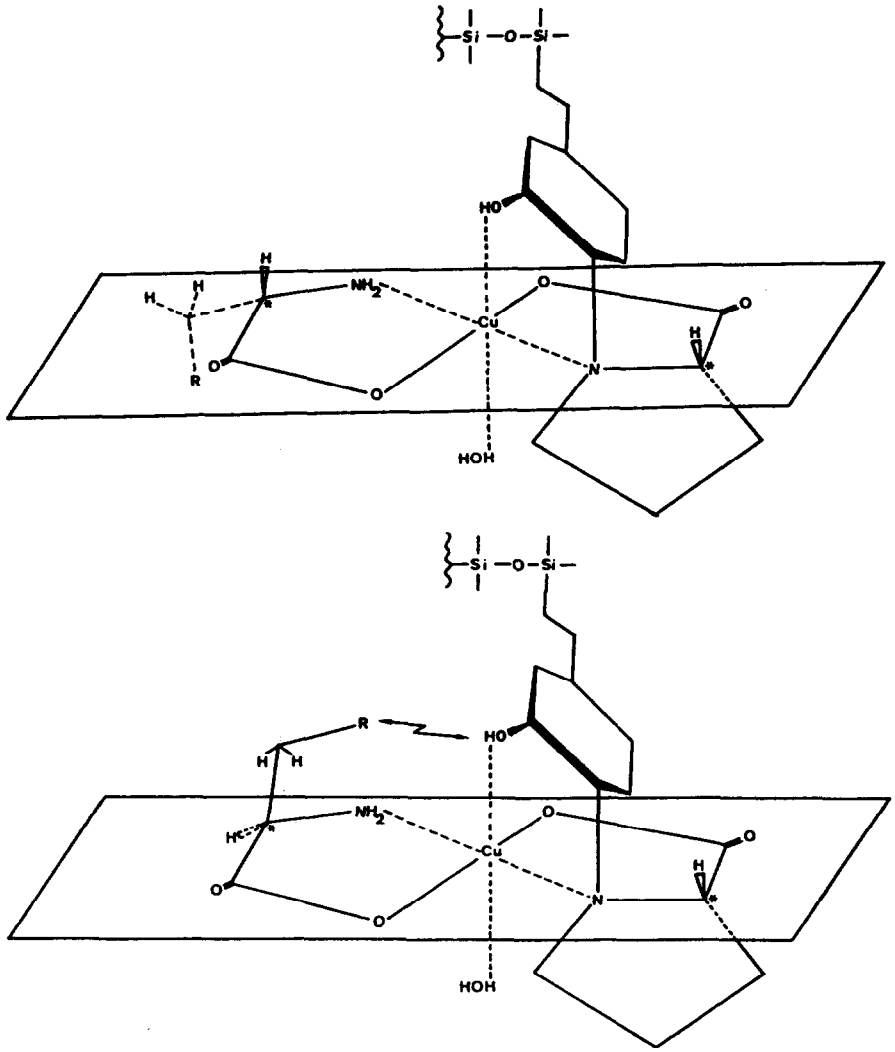

Fig. 4. Proposed structure of the mixed complexes between L-proline as the chiral selector ligand on CSP II and D- and L-phenylalanine.

ing the chiral recognition. Steric hindrance between the bulky substituent R in the D-form and the spacer group may lead to a weaker complexation compared with the L-form, where free rotation of the substituent R is possible. Thereby the elution order of D- before L-enantiomer can be explained. The reversed elution order for proline can be interpreted by the lack of the ability of the α-substituent in the L-form to rotate because of the cyclic structure.

In CSP III, an amino group as an additional complexing group was inserted in the spacer, forming an ethylenediamine structure. Participation of the nitrogen of this group in complex formation is possible, but has not yet been proved. This phase showed enantioselectivity for amino acids, dansylamino acids, glycyldipeptides and some hydroxy acids.

Amino acids and derivatives

Table I shows a comparison of the k' and α values of a series of amino acids on CSP II and III. Both phases showed a good resolution of amino acid enantiomers. The best results were

TABLE I

k' AND α VALUES FOR D,L-AMINO ACIDS ON CSP II AND CSP III

Conditions: mobile phase: (A) 0.05 *M* potassium dihydrogenphosphate (pH 4.6)–10^{-4} *M* copper(II) sulphate; (B) 10^{-4} *M* copper(II) sulphate; flow-rate, 1 ml/min; temperature, 50°C; detection, UV at 223 nm.

Amino acid	CSP II			CSP III		
	k'_D	k'_L	α	k'_D	k'_L	α
Alanine	0.9	0.9	1.0[a]	3.0	1.83	0.61[b]
Arginine	1.2	1.5	1.3[a]			
Asparagine	2.8	5.2	1.9[a]	3.17	4.33	1.37[a]
Aspartic acid	2.2	2.9	1.3[a]	1.87	2.25	1.20[a]
DOPA				5.20	16.0	3.08[a]
Ethionine				5.20	5.60	1.08[a]
Glutamic acid				2.90	2.10	0.72[b]
Histidine	1.9	5.5	2.8[a]	6.83	12.17	1.78[a]
Leucine	2.5	2.5	1.0[a]	5.00	2.50	0.50[b]
Methionine				1.88	2.35	1.25[a]
Norleucine	1.5	1.8	1.23[a]	10.0	6.50	0.65[b]
Norvaline	1.35	1.4	1.04[a]	9.00	4.50	0.50[b]
Proline	2.40	1.4	0.6[a]	2.17	1.17	0.54[a]
Phenylalanine	1.5	4.5	3.0[a]	5.4	11.0	2.04[a]
Phenylserine	8.0	14.8	1.85[a]			
Serine	2.1	3.6	1.7[a]	2.17	3.85	1.61[a]
Threonine	0.9	1.4	1.5[a]	3.33	5.0	1.50[a]
Tryptophan	3.7	13.7	3.7[a]	12.67	38.0	3.00[a]
Tyrosine				5.0	13.2	2.64[a]
Valine	1.8	3.3	1.9[a]	2.67	3.83	1.44[a]

[a] Mobile phase A.
[b] Mobile phase B.

obtained when using an acidic phosphate buffer as the mobile phase (Fig. 5). The influence of end-capping was investigated. End-capping did not effect the resolution but resulted in an improvement in peak shape and decreased tailing. The elution order was D-enantiomer before L-enantiomer on both phases except for proline, as also previously observed on CSP I.

When using water instead of the acidic buffer as the mobile phase, aliphatic amino acids such as alanine, leucine, norleucine and norvaline were resolved on CSP III. Surprisingly, a reverse elution order was observed in this instance.

By analogy with the results observed with the phases of the type CSP I [5], the replacement of L-proline with L-pipecolic acid as the selector ligand might result in a further improvement in the enantioselectivity for amino acids.

Dansylamino acids

In addition to free amino acids, amino acid derivatives, such as dansylamino acids, were also resolved. In Table II the k' and α values for some dansylamino acids on CSP III are given.

Dipeptides

Glycyl dipeptides were resolved on both phases with good results (Table III). Leucylleucine was resolved into four peaks on CSP II (Fig. 6) whereas for some other diastereomeric

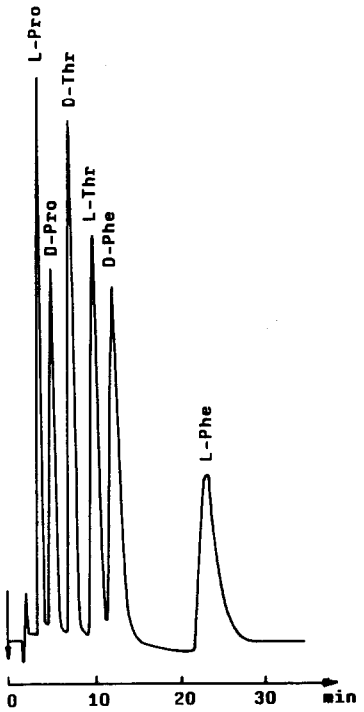

Fig. 5. Separation of a mixture of D,L-amino acid enantiomers on CSP III. Conditions: mobile phase, 0.05 *M* KH_2PO_4 (pH 4.6)–10^{-4} *M* $CuSO_4$; flow-rate, 1 ml/min; temperature, 50°C; detection, UV at 223 nm.

dipeptides only three peaks were observed (Table IV). Further optimization steps may result in complete resolution of the two possible enantiomers and two diastereoisomers, respectively.

TABLE II

k' AND α VALUES FOR DANSYL-AMINO ACIDS ON CSP III

Conditions: mobile phase, 0.05 *M* ammonium acetate (pH 7.5)–10^{-4} *M* copper(II) acetate; flow-rate 1 ml/min; detection, UV at 254 nm.

Compound	k'_D	k'_L	α
Dansylserine	4.87	6.00	1.23
Dansylvaline	2.17	2.76	1.27
Dansylnorvaline	2.66	3.77	1.42
Dansylphenylalanine	4.00	8.90	2.22

TABLE III

k' AND α VALUES FOR DIPEPTIDES ON CSP II AND CSP III

Conditions: mobile phase, 10^{-4} *M* copper(II) sulphate; flow-rate, 1 ml/min; temperature, 50°C; detection, UV at 223 nm.

Dipeptide	CSP II			CSP III		
	k'_D	k'_L	α	k'_D	k'_L	α
Gly–Leu	5.3	8.2	1.5	2.75	3.5	1.27
Gly–Val	3.8	5.7	1.5	2.42	3.0	1.24
Gly–Phe	2.7	5.0	1.9	2.0	2.83	1.42

Hydroxy acids

Table V shows the resolution of some examples of hydroxy acids on CSP II. As on phases of type CSP I containing L-hydroxyproline instead of L-proline as a chiral selector ligand, a marked increase in enantioselectivity for hydroxy acids

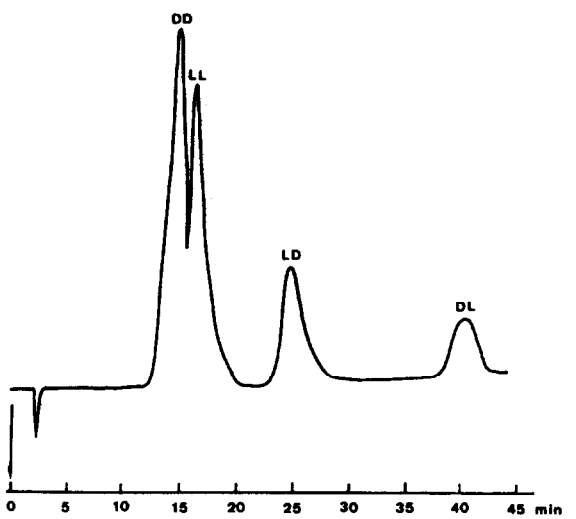

Fig. 6. Resolution of D,L-leucyl-D,L-leucine on CSP II. Conditions: mobile phase, 10^{-4} *M* $CuSO_4$; flow-rate, 1 ml/min; temperature, 50°C; detection, UV at 223 nm.

TABLE IV

RESOLUTION OF DIASTEREOMERIC DIPEPTIDES ON CSP II

Conditions as in Table III.

Dipeptide	k'_{DD}	k'_{LL}	k'_{LD}	k'_{DL}
Leu–Leu	5.0	5.7	9.7	16.7
Leu–Tyr	2.7	2.7	5.8	8.0
Ala–Val	2.2	2.2	3.3	6.0

was observed [9], a further improvement in resolution is to be expected by replacing L-proline by L-hydroxyproline in phases of type CSP II.

Barbiturates

CSP II showed enantioselectivity also for N-alkylated barbiturates (Table VI). The resolution increased with increasing pH, but the stability of the column decreased significantly. Up to pH 8 at room temperature the phase was found to be stable. Fig. 7 shows the resolution of the enantiomers of methylphenobarbital on CSP II.

Other compounds

Compounds having an amino alcohol structure could not be directly resolved on these phases. However, as we have shown previously [12], using a simple derivatization step with bromoacetic acid, enantiomers of amino aclohols such as adrenergic drugs or β-blockers can be resolved by LEC. We are currently investigating the separation of the derivatives of these drugs on these new phases.

TABLE V

k' AND α VALUES FOR D,L-HYDROXY ACIDS ON CSP II

Conditions as in Table III.

Hydroxy acid	k'_D	k'_L	α
Lactic acid	9.0	7.7	0.85
Mandelic acid	3.5	2.7	0.76
3-Hydroxymandelic acid	22.3	18.3	0.82
Malic acid[a]	0.94	1.17	1.25

[a] Mobile phase: 0.05 *M* KH_2PO_4.

TABLE VI

RESOLUTION OF N-ALKYLATED BARBITURATES ON CSP II

Conditions: mobile phase, 0.05 *M* ammonium acetate (pH 7.5)–10^{-4} *M* copper(II) acetate; flow-rate, 0.5 ml/min; detection, UV at 240 nm.

Barbiturate	k'_D	k'_L	α
Hexobarbital	1.42	1.78	1.26
Methylphenobarbital	1.14	1.43	1.25

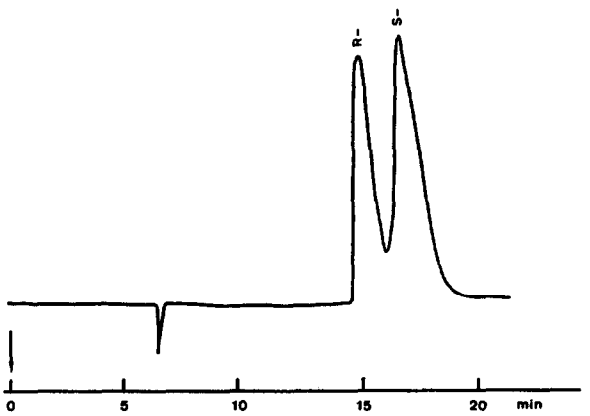

Fig. 7. Resolution of the enantiomers of methylphenobarbital on CSP II. Conditions: mobile phase, 0.05 *M* ammonium acetate buffer–10^{-4} *M* copper(II) acetate (pH 7); flow-rate, 0.5 ml/min; temperature, 23°C; detection, UV at 240 nm.

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