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Note

Resolution of enantiomers of amino acid derivatives by high-performance liquid chromatography in a silica gel bonded chiral amide phase

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In the last few years[1–5] the separation of optical isomers by chromatographic methods has been developed into a promising analytical procedure. Favoured compounds for these investigations have been amino acids and their derivatives. Of particular interest has been chiral recognition by means of chiral stationary phases. For instance, long chain N-alkanoyl derivatives of *tert.*-butylvalinamide were coated as stationary phases on gas chromatographic (GC) supports (Chromosorbs)[6].

We prepared GC columns with N-stearoyl-*tert.*-butylvalinamide as stationary phase and obtained excellent separations for sufficiently volatile N-acyl amino acid esters up to column temperatures of 190°C. However, grafting of the valinamide at a styrene–divinylbenzene–methylacrylate copolymer (we used Cekachrom Typ 4, VEB Laborchemie Apolda) in order to increase the range of working temperatures gave unsatisfactory results. Limits to these GC separations are the thermal stability of the chiral phase and frequently also of the substrates to be separated. To get milder conditions for column and samples, we turned from GC to high-performance liquid chromatography (HPLC).

The purpose of this study was to bond *tert.*-butylvalinamide chemically on silica gel surfaces via the amino group and to prepare a new normal phase column for HPLC, which would especially be applicable to the resolution of less volatile amino acid derivatives. Hara and co-workers[8,9] have described a comparable phase using the carboxylic group instead of the amino group and a shorter spacer.

EXPERIMENTAL

Apparatus

Chromatography was performed using a HP 1084 B liquid chromatograph (Hewlett-Packard) with a fixed wavelength detector (254 nm). The column (stainless steel, 200 × 4 mm I.D.) was packed with a slurry of packing material at a pressure between 380 and 400 atm. LiChrosorb® Si 60 (pore diameter 60 Å, particle size 10 μm; E. Merck) and Nucleosil® 100-5 (spherical, pore diameter 100 Å, particle size 5 μm; Macherey, Nagel & Co.) were used as supports. The solvents, 2-propanol, p.a.,

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and *n*-heptane, p.a., were purified by distillation controlled by UV absorption measurement.

Preparation of the chiral phase

tert.-Butyl-L-valinamide (I). This compound was synthesized according to Saeed *et al.*[10].

But-3-enecarboxylic acid hydroxysuccinimido ester (II). Equimolar amounts of but-3-enecarboxylic acid, N-hydroxysuccinimide and dicyclohexylcarbodiimide were stirred in chloroform for 72 h at room temperature. After filtration of the dicyclohexylurea and removal of the chloroform, the residue was recrystallized twice from hexane and gave satisfactory mass spectral (MS), IR and elemental analyses; m.p. 45°C.

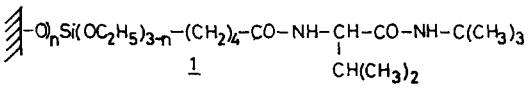
N-But-3-enoyl-tert.-butylvalinamide (III) (in analogy to Beitler and Feibush[11]*).* Equimolar amounts of compounds I, II and NEt_3 were stirred in chloroform for 12 h at 0°C and then for 36 h at 20°C. Solvents were removed and the residue was dissolved in diethyl ether–chloroform (1:1) and filtered in the presence of a small amount of charcoal. The organic phase was washed with 2% hydrochloric acid, followed by water, 5% sodium hydrogen carbonate solution and finally water. After drying over sodium sulphate the solvents were removed, yielding a colourless solid, yield 87%, m.p. 178–184°C. It was characterized by IR, MS and elemental analysis: found 11.11% N (calc. 11.01%); $[\alpha]_D^{22} = -22.1°$ in chloroform (c = 2 g per 100 ml).

Hydrosilylation of III (in analogy to Berendsen et al.[12]*).* One gram (0.004 mol) of III and 2 g (0.012 mol) triethoxysilane were refluxed (4 h) in 40 ml dry toluene with 0.1 mol % hexachloroplatinic acid as catalyst. Toluene and silane were removed under vacuum and the residue used without purification. All operations were performed in an argon atmosphere.

Grafting to silica gel (in accordance with Hara and co-workers[8,9]*).* Three grams of silica gel (activated for 8 h at 150°C in a stream of argon) and 1 g (about 0.0024 mol) hydrosilylation product were stirred and refluxed in 40 ml dry toluene for 16 h. Toluene and ethanol were removed by azeotropic distillation within 2 h, 80 ml toluene were added and the distillation continued for 3 h. The silica gel phase was filtered on a G4-glass funnel and extracted fifteen times each with boiling toluene, hexane, 2-propanol and diethyl ether, then dried under vacuum. Elemental analyses: LiChrosorb®, 0.65% N (8.5% chiral component); Nucleosil®, 0.93, 1.03% N (about 12% chiral component).

RESULTS

The bonding between the chiral phase and the silica gel surface may be represented in the following manner:



Obviously we have to assume a two-point hydrogen bonding interaction between the grafted and dissolved chiral components, which is probably more pronounced in less polar than in aqueous solutions. Furthermore, reversed-phase methods with buffered

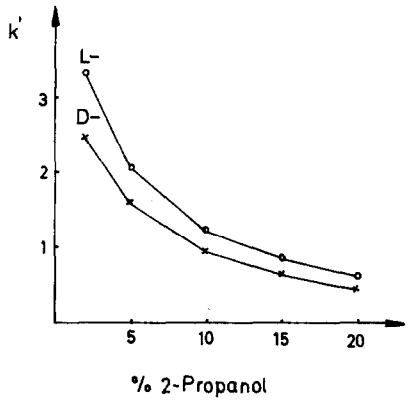


Fig. 1. Correlation between the concentration of 2-propanol in *n*-heptane as mobile phase and the capacity factor, k', for L- and D-N-benzoyl-3,4-dimethoxyphenylalanine ethyl ester on a chiral Nucleosil 100-5 phase.

aqueous eluents lead to a hydrolysis of the remaining SiOEt groups and to difficulties in the collection and isolation of the separated samples. Therefore, we applied normal phase HPLC using heptane–2-propanol as mobile phase and N-acetyl or N-benzoyl amino acid methyl esters as substrates.

Fig. 1 shows the influence of the composition of the mobile phase on the chiral recognition. Best results were achieved with low 2-propanol contents; 5% 2-propanol gave a good compromise between efficiency and speed of separation. Our results are collected in Table I.

TABLE I

SEPARATION OF ENANTIOMERS OF N-ACETYL OR N-BENZOYL AMINO ACID METHYL ESTERS UPON THE CHIRAL PHASE 1 ON NUCLEOSIL 100-5

Mobile phase: *n*-heptane–2-propanol (95:5); flow-rate 1 ml/min. Temperature: 25°C. Column: 200 × 4 mm I.D. All data after reversed-phase experiment (see text). Amount injected: *ca.* 5 μg unless stated otherwise. $k'_L = (t_{R_L} - t_0)/t_0$; $k'_D = (t_{R_D} - t_0)/t_0$; $\alpha_{L,D} = k'_L/k'_D$; where t_R = retention time and t_0 = dead time[13].

No.	*Amino acid derivative*	k'_L	k'_D	$\alpha_{L,D}$
1	N-Benzoylalanine methyl ester	1.93	1.66	1.16
2	N-Benzoylnorvaline methyl ester	0.93	0.77	1.21
3	N-Benzoylvaline methyl ester	0.68	0.62	1.11
4	N,O-Dibenzoyltyrosine methyl ester	1.26	1.0	1.26
5	N-Acetylphenylalanine methyl ester*	4.72	4.22	1.12**
6	N-Benzoyl-3,4-dimethoxyphenylalanine methyl ester	3.27	2.82	1.16***
7	N-Benzoyl-3,4-dimethoxyphenylalanine ethyl ester	2.03	1.61	1.26
8	N-Benzoyl-3-methoxy-4-acetoxyphenylalanine methyl ester	1.69	1.31	1.29

* *ca.* 20 μg injected.

** $\alpha_{L,D}$ = 1.19 before reversed-phase experiment.

*** $\alpha_{L,D}$ = 1.21 before reversed-phase experiment (see text).

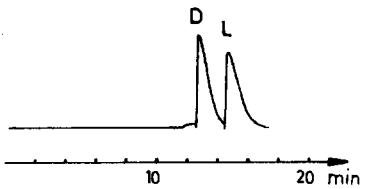


Fig. 2. Resolution of a racemic mixture of N-acetylphenylalanine methyl ester (20 μg) on a chiral Nucleosil 100-5 phase. Chromatographic conditions as in Table I.

In all examples the D-enantiomers were less strongly retained than the corresponding L-enantiomers. N-Benzoylvaline methyl ester showed the weakest interaction with the stationary phase and the poorest separation. Fig. 2 demonstrates a typical separation.

Only the Nucleosil phase gave satisfactory chiral recognition owing to its greater accessibility (smaller particle size and larger pore diameter) than in the Li-Chrosorb phase. Compared with the results of Hara and co-workers[8,9] our new chiral phase has remarkably shorter retention times and better separation factors for N-acyl amino acid methyl esters.

We also attempted a direct separation of amino acids with a hydrophilic mobile phase[7,14], acetonitrile–0.1 *M* ammonium acetate in the presence of Cu^{2+}, but a significant increase of pressure occurred with loss of chiral differentiation. The column could be regenerated stepwise and gave normal-phase chromatography separations of slightly lower quality (see Table I).

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