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Note

Resolution of enantiomeric amino acid derivatives by high-performance liquid chromatography on chiral stationary phases*

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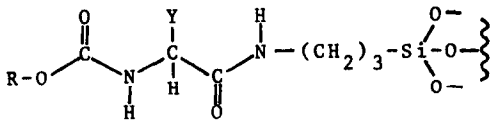
Separation of enantiomeric N-(3,5-dinitrobenzoyl)-α-amino acid esters was first reported by Ôi *et al.*[1]. Since then, several naturally occurring amino acids have been resolved as N-(3,5-dinitrobenzoyl)-derivatives on different chiral stationary phases[2–6]. Our interest in the resolution of enantiomeric proteinogenic amino acids has prompted us to look for suitable chiral stationary phases. Therefore, we tested the selectivity of the following chiral groups covalently bonded to silica gel: benzyloxycarbonyl-D-phenylglycyl-(3-triethoxysilyl)-*n*-propylamide (CSP I), benzyloxycarbonyl-L-phenylalanyl-(3-triethoxysilyl)-*n*-propylamide (CSP II), benzyloxycarbonyl-L-isoleucyl-(3-triethoxysilyl)-*n*-propylamide (CSP III), and *tert.*-butyloxycarbonyl-D-phenylglycyl-(3-triethoxysilyl)-*n*-propylamide (CSP IV).



CSP I: R = benzyl, Y = phenyl
CSP II: R = benzyl, Y = benzyl
CSP III: R = benzyl, Y = 2-butyl
CSP IV: R = *tert.*-butyl, Y = phenyl

The silanes were prepared in high enantiomeric purity from the corresponding protected amino acid and (3-triethoxysilyl)-*n*-propylamine via the mixed anhydride procedure[7]. Coupling of these silanes to silica gel yielded the respective stationary phases. Amino acids were converted into N-(3,5-dinitrobenzoyl)-α-amino acid-2-propyl esters before chromatography.

EXPERIMENTAL

Preparation of chiral silanes

The N-protected amino acid (50 mmol) was dissolved in 150 ml of absolute

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tetrahydrofuran in a dry nitrogen atmosphere. After coling to −15°C, N-methylmorpholine (50 mmol) and isobutylchloroformiate (50 mmol) were added dropwise with vigorous stirring for 5 min.

A solution of (3-triethoxysilyl)-*n*-propylamine (50 mmol) in 50 ml of absolute tetrahydrofuran was added. After the addition was complete, the mixture was stirred at 0°C for 1 h. The solvent was removed under vacuum and the residue was suspended in 150 ml of ethyl acetate. The organic layer was washed with a pH 2 buffer solution ($KHSO_4/K_2SO_4$)[8], saturated sodium bicarbonate solution and dried over magnesium sulphate. The solvent was removed under vacuum and the crude products were crystallized from toluene/hexane.

Benzyloxycarbonyl-D-phenylglycyl-(3-triethoxysilyl)-n-propylamide. Yield: 78%; m.p. 101°C; $[\alpha]_D^{23} = -44.6°$ (methanol). Calculated for $C_{25}H_{36}N_2O_6Si$: C 61.44%; H 7.43%; N 5.73%. Found: C 61.63%; H 7.39%; N 5.71%. ^1H NMR: δ = 0.52(m,2H), 1.19(t,9H), 1.56(p,2H), 3.26(d(tr),2H) 3.84(q,6H), 5.13(d,2H), 5.26(m,1H), 6.17(broad,1H), 7.47(m,10H).

Benzyloxycarbonyl-L-phenylalanyl-(3-triethoxysilyl)-n-propylamide. Yield: 57%; m.p. 82–83°C; $[\alpha]_D^{23} = -2.8°$ (methanol). Calculated for $C_{26}H_{38}N_2O_6Si$: C 62.00%; H 7.62%; N 5.98%. Found: C 62.13%; H 7.53%; N 5.64%. ^1H NMR: δ = 0.51(t,2H), 1.23(t,9H), 1.48(p,2H), 3.11(m,4H), 3.80(q,6H), 4.40(q,1H), 5.11(s,2H), 5.38(broad,1H), 5.83(broad,1H), 7.28(m,10H).

Benzyloxycarbonyl-L-isoleucyl-(3-triethoxysilyl)-n-propylamide. Yield: 67%; m.p. 130–132°C; $[\alpha]_D^{23} = -11.5°$ (methanol). Calculated for $C_{23}H_{40}N_2O_6Si$: C 58.94%; H 8.60%; N 5.98%. Found: C 58.99%; H 8.68%; N 5.98%. ^1H NMR: δ = 0.63(t,2H), 0.94(m,6H), 1.23(t,9H), 1.60(m,2H), 1.88(m,1H), 3.29(m,2H), 3.82(q,6H), 4.00(d(tr),1H), 5.10(s,2H), 5.44(d,1H).

tert-Butyloxycarbonyl-D-phenylglycyl-(3-triethoxysilyl)-n-propylamide. Yield: 62.2%; m.p. 89–90°C; $[\alpha]_D^{23} = -53.7°$ (methanol). Calculated for $C_{22}H_{38}N_2O_6Si$: C 58.12%; H 8.43%; N 6.16%. Found: C 58.00%; H 8.38%; N 6.15%. ^1H NMR: δ = 0.51(m,2H), 1.20(t,9H), 1.41(s,9H), 1.60(p,2H), 3.26(d(tr),2H), 3.02(q,6H), 5.09(m,1H), 5.88(broad,1H), 6.00(broad,1H), 6.88(m,5H).

*Grafting to silica gel**

3-g Portion of Silica gel (hydrated with 1 *M* hydrochloric acid at 90°C for 16 h and dried at 160°C, 0.1 Torr, for 12 h) and 12 mmol of the respective silane were refluxed with stirring for 72 h in 50 ml of dry toluene. The modified silica gel was filtered and washed with toluene and methylene chloride.

The amino acid content of the grafted silica gel was determined by the amino acid analysis according to Spackman *et al.*[9] after acid hydrolysis (6 *M* hydrochloric acid, 72 h, 110°C):

CSP I: 0.51 mmol phenylglycine/g silica gel
CSP II: 0.54 mmol phenylalanine/g silica gel
CSP III: 0.49 mmol isoleucine/g silica gel
CSP IV: 0.49 mmol phenylglycine/g silica gel

* LiChrosorb Si 100, 10 μm particle size, Merck (Darmstadt, FRG).

The chiral stationary phases were packed into 250 × 4.6 mm I.D. stainless-steel columns using conventional slurry techniques.

Derivatization procedure of amino acids

A 0.1 mmol aliquot of amino acid or an equivalent amount of peptide hydrolysate was stirred with 1 ml of 2.2 *M* hydrochloric acid in 2-propanol at 100°C for 4 h. Subsequently, the solution was evaporated to dryness. The residue was suspended in 1 ml of ethyl acetate, and N-methylmorpholine (0.2 mmol) and 3,5-dinitrobenzoyl chloride (0.1 mmol) were added. After stirring at room temperature for 3–4 h, the mixture was washed with saturated sodium bicarbonate solution and with a pH 2 buffer solution ($KHSO_4/K_2SO_4$)[8]. The organic layer was dried over magnesium sulphate and evaporated to dryness.

Chromatography

Experiments were carried out with a Perkin-Elmer series 3B liquid chromatograph equipped with a Perkin-Elmer LC-75 variable-wavelength detector. Solvents were glass-distilled before use. Solutes were injected with concentrations of 0.2–0.5 mg/ml using a 10-μl sample loop.

RESULTS AND DISCUSSION

It was found that only CSP I and CSP IV showed enantioselective adsorption, whereas CSP II and CSP III had no chiral selectivity for the amino acid derivatives employed in the present investigation. Table I summarizes some chromatographic results obtained with CSP I (comparable results were obtained with CSP IV).

Separation factors of all separations with these stationary phases were clearly

TABLE I

SEPARATION OF N-(3,5-DINITROBENZOYL)-α-AMINO ACID-2-PROPYLESTERS ON CSP I

α-Amino acid	*Separation factor, α*	*Capacity factor of 1st eluted enantiomer, k_1*	*Mobile phase: hexane–2-propanol*	*Absolute configuration of 1st eluted enantiomer*
Alanine	1.38	3.26	6:94	*R*
Valine	1.84	2.00	6:94	*R*
Leucine	1.69	1.78	6:94	*R*
Isoleucine	1.86	1.71	6:94	*R*
Serine	1.12	6.96	6:94	*R*
Threonine	1.28	1.28	6:94	*R*
Methionine	1.55	4.70	6:94	*R*
Lysine	1.29	5.03	20:80	*R*
Aspartic acid*	1.25	2.66	6:94	*R*
Glutamic acid*	1.45	2.77	6:94	*R*
Phenylglycine	1.43	3.79	6:94	*R*
Phenylalanine	1.55	3.66	6:94	*R*
Tyrosine	1.48	2.54	15:85	*R*
Proline	1.00	3.43	6:94	–

* As diisopropylesters.

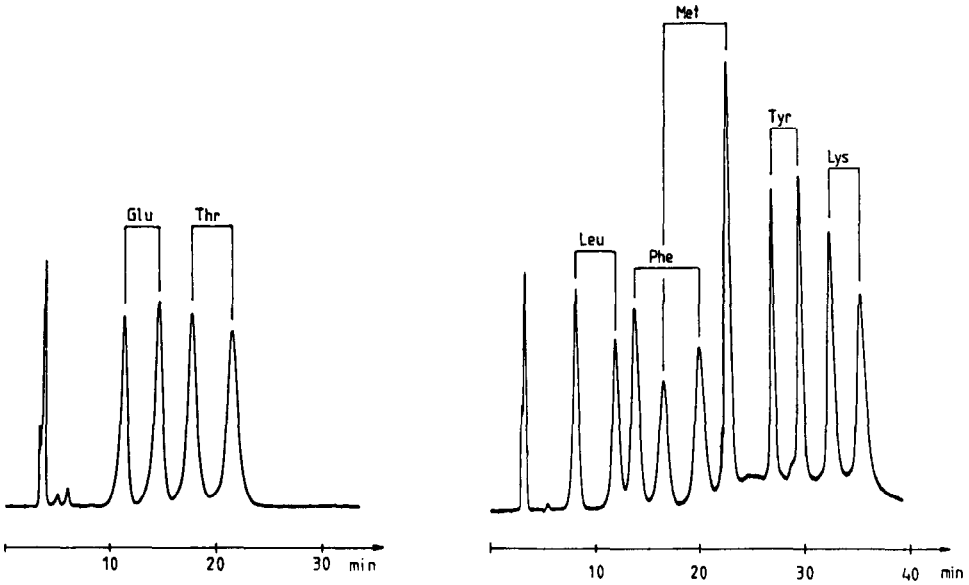

Fig. 1. Chromatographic separation of enantiomeric mixtures of N-(3,5-dinitrobenzoyl)-glutamic acid-di-2-propylester and N-(3,5-dinitrobenzoyl)-threonine-2-propylester on CSP IV. Mobile phase: hexane–2-propanol (94:6). The first peak of each amino acid derivative represents the D-enantiomer.

Fig. 2. Chromatographic separation of enantiomeric mixtures of some N-(3,5-dinitrobenzoyl)-α-amino acid-2-propylesters on CSP I. Mobile phase: 17 min of hexane–2-propanol (95:5), then 20 min of hexane–2-propanol (75:25). The first peak of each amino acid derivative represents the D-enantiomer.

different from 1.00. Figs. 1 and 2 show typical separations on phenylglycine-containing stationary phases.

The fact that, in all cases, the *R*-enantiomers were eluted first from the D-phenylglycine-containing stationary phases indicates that the reversible diastereomeric complex of the *S*-enantiomer with the chiral group of the stationary phase is stronger than that of the *R*-enantiomer.

For resolution to occur, there must be at least three simultaneous interactions between the chiral stationary phase and the solute, and one or more of them must be stereochemically dependent. The fact that only CSP I and CSP IV show enantioselectivity suggests that the phenyl group attached to the asymmetric carbon atom acts as a π-donor to the π-accepting 3,5-dinitrobenzoyl group of the solute. This kind of interaction was also observed by other authors in comparable cases[3,5,6,10,11].

Moreover, it is suggested that hydrogen bonding makes an additional contribution to enantioselective adsorption. Derivatives of proline, which are characteristically different from the other solutes only through the lack of the amide hydrogen, are not resolved on CSP I and CSP IV. So this N–H group seems to be a secondary binding site in the reversible adsorption complex.

Further experiments should give more information about the mechanism of adsorption; if the detailed structure of the adsorption complex is known, optimization of the separation system should be possible.

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