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Note

High-performance liquid chromatographic separation of enantiomers on *s*-triazine derivatives of a tripeptide ester and a chiral amine bonded to silica gel

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In our study of the direct separation of optical isomers by gas chromatography[1–4], we reported that some *s*-triazine derivatives of amino acid esters, dipeptide esters, tripeptide esters and amino acid amides showed excellent enantioselectivity for various chiral compounds.

As it is generally considered that the chiral recognition mechanism is essentially similar in both gas and liquid chromatography, these results suggested that some *s*-triazine derivatives of optically active compounds would be effective as chiral stationary phases in high-performance liquid chromatography (HPLC) and led us to this work.

In this study we prepared two novel chiral stationary phases, *s*-triazine derivatives of L-valyl-L-valyl-L-valine isopropylester and (*S*)-1-(α-naphthyl)ethylamine chemically bonded to silanized silica, and the HPLC separation of derivatives of various racemic compounds was examined on these phases.

EXPERIMENTAL

Preparation of stationary phase

Phase I was prepared as shown in Scheme 1.

L-Valyl-L-valyl-L-valine-isopropylrester was prepared as described previously[3]. To a mixture of 20 ml of dry dioxane and 2.2 g (0.012 mol) of 2,4,6-trichloro-1,3,5-triazine, a solution of L-valyl-L-valyl-L-valine isopropylester (1.07 g) in 30 ml of dry dioxane was added dropwise with stirring at 0°C. To this mixture, 4.0 g of anhydrous sodium carbonate were added at room temperature, and the swirling was continued. The solution was then filtered and the solvent removed under reduced pressure to give the colourless crystalline Ia.

To a solution of 8.3 g (0.01 mole) of Ia in 30 ml of dry dioxane, 2.0 g (0.009 mol) of N-(2-aminoethyl)-3-aminopropyltriethoxysilane and 1.5 g of anhydrous sodium carbonate were added and the mixture was stirred under reflux for 40 h. The solution was then filtered to remove the precipitate.

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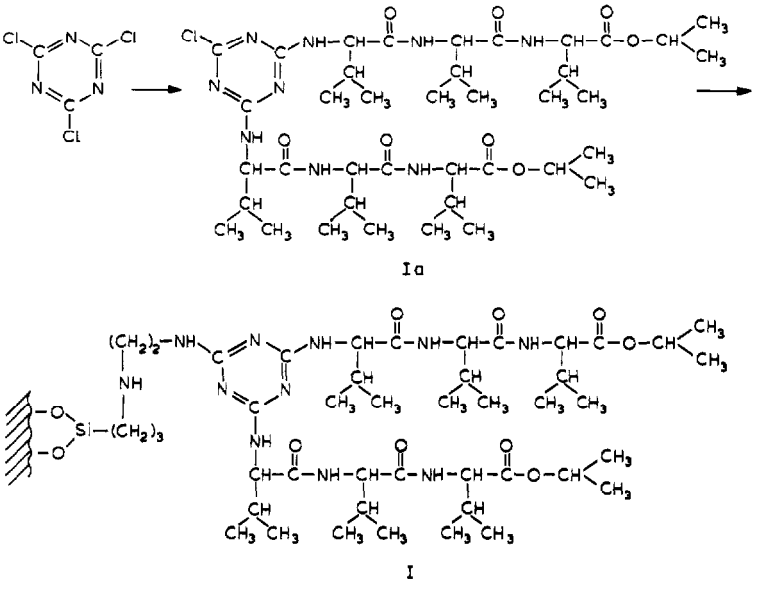
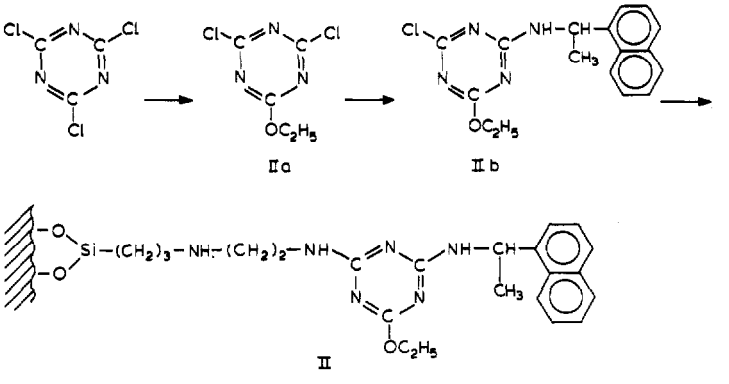


Scheme 1.

To this solution, 3 g of dried LiChrosorb Si 100 (10 μm) (E. Merck, Darmstadt, F.R.G.) were added, and the mixture was stirred slowly under reflux for 10 h. After cooling, the modified silica I was collected by filtration, washed exhaustively with dioxane, methanol and diethyl esther and dried under reduced pressure. Phase I contained 0.26 mmol of the *s*-triazine derivative of L-valyl-L-valyl-L-valine isopropylester per g of support (based on C and N).

Phase II was prepared as shown in Scheme 2.



Scheme 2.

To 50 ml of ethanol, 4.7 g of 2,4,6-trichloro-1,3,5-triazine and 2.5 g of sodium-carbonate were slowly added at 0°C. The mixture was stirred for 8 h at 3–5°C and added to 70 ml of ice-water. The precipitate Ia was collected by filtration, washed with ice-water and dried under reduced pressure. To a solution of 1.94 g of

IIa and 1.68 g of sodium bicarbonate in dry dioxane, 2.57 g of (S)-1-(α-naphthyl) ethylamine in 10 ml of dry dioxane were slowly added with swirling at 50°C under a stream of nitrogen. The mixture was kept at 50°C for 12 h. The precipitate was removed by filtration and the filtrate was concentrated under vacuum to afford the crude IIb which was then recrystallized from ethyl esther. To a solution of 2.9 g of IIb in 20 ml of dry toluene, 1.7 g of N-(2-aminoethyl)-3-aminopropyl-triethoxysilane and 1.0 g of sodium bicarbonate were added and stirred under reflux for 20 h. After the precipitate was removed by filtration, 2.5 g of LiChrosorb Si 100 (10 μm, E. Merck) were added to the filtrate. The mixture was stirred slowly for 20 h. After cooling, the modified silica II was collected, washed with toluene, chloroform, methanol and ether, and then dried under reduced pressure. This modified silica II contained 0.89 mmol of the *s*-triazine derivative of (*S*)-1-(α-naphthyl)ethylamine per g of support (based on C and N).

HPLC

The experiments were carried out with a Shimadzu LC-3A high-performance liquid chromatograph equipped with a UVD-2 UV detector (254 nm). Steel columns (250 × 4 mm I.D.) were slurry packed using conventional techniques.

TABLE I

HPLC SEPARATION OF DERIVATIZED ENANTIOMERS UPON CHIRAL STATIONARY PHASES

The separation factor of the enantiomers, α, is the ratio of their capacity factors. k'_1 is the capacity factor for the initially eluted enantiomer. Mobile phases: A = *n*-hexane–dichloromethane–ethanol (100:20:1); B = *n*-hexane–dichloromethane–ethanol (10:4:1); C = *n*-hexane–dichloromethane–ethanol (13:4:1); D = *n*-hexane–dichloromethane–ethanol (20:6:1); E = *n*-hexane–dichloromethane–ethanol (48:15:1).

Compound	*Phase I*			*Phase II*		
	α	k'_1	*Mobile phase*	α	k'_1	*Mobile phase*
*Amines**						
1-Phenylethylamine	1.14	5.58	A	1.46	1.53	B
1-(α-naphthyl)ethylamine	1.16	4.69	A	1.74	2.00	B
1-Phenyl-2-(4-tolyl)ethylamine	1.00	3.54	A	1.31	1.18	B
*Amino acids***						
Alanine	1.31	7.08	A	1.15	2.53	C
Valine	1.45	2.54	A	1.15	1.55	C
Phenylalanine	1.39	3.64	A	1.00	2.16	C
*Carboxylic acids****						
2-Phenylpropionic acid	1.00	12.18	A	2.17	4.17	D
2-(4-Chlorophenyl)isovaleric acid	1.09	11.27	A	1.98	3.22	D
2-Bromo-3,3-dimethylbutyric acid	1.10	12.34	A	1.54	3.20	D
Alcohols§						
1-Phenylethanol	1.03	7.04	A	1.10	2.81	D
1-(α-naphthyl)ethanol	1.07	7.51	A	1.10	3.58	D
Allethrolone	1.17	4.23	E	1.00	3.23	D

* Resolved as N-3,5-dinitrobenzoyl derivatives.
** Resolved as N-3,5-dinitrobenzoyl O-methylester derivatives.
*** Resolved as N-3,5-dinitroanilide derivatives.
§ Resolved as N-3,5-dinitrophenyl urethane derivatives.

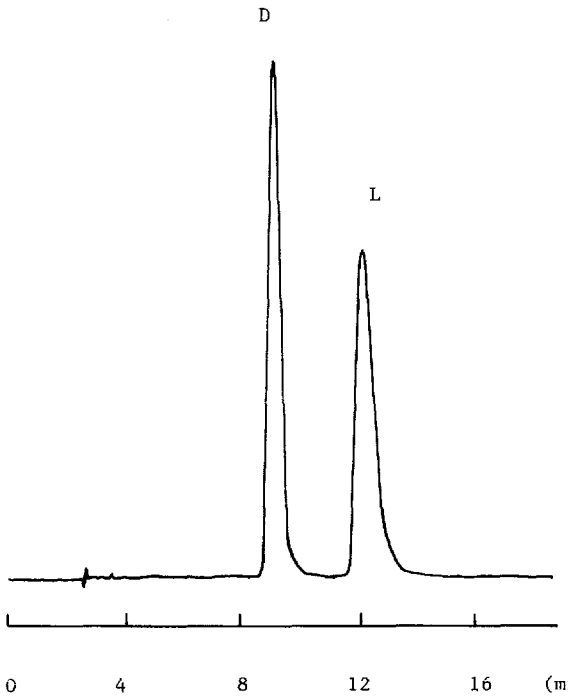


Fig. 1. Chromatographic separation of the enantiomers of racemic valine as N-3,5-dinitrobenzoyl methylester derivatives upon chiral stationary phase I. Chromatographic conditions as in Table I.

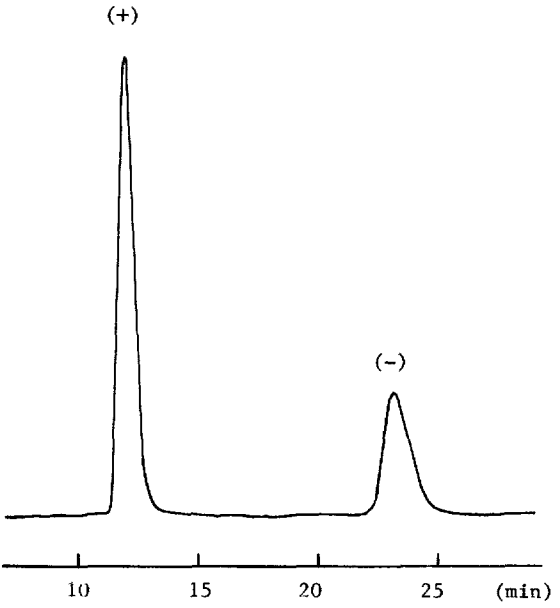


Fig. 2. Chromatographic separation of the enantiomers of racemic 2-phenyl propionic acid as N-3,5-dinitroanilide derivatives upon chiral stationary phase II. Chromatographic conditions as in Table I.

Various derivatized compounds for use as solutes were prepared from reagent-grade chemicals. Some materials were provided by colleagues in our laboratory.

RESULTS AND DISCUSSION

The chromatographic results are summarized in Table I.

It was found that two novel phases, I and II, gave good chiral recognition for derivatives of amines, amino acids, carboxylic acids, and alcohols. Typical chromatograms are shown in Figs. 1 and 2.

Phase I showed good enantioselectivity for derivatized enantiomers of amino acids. On the other hand, phase II showed excellent enantioselectivity for derivatives of amine and carboxylic acid enantiomers. In the case of alcohol enantiomers, phase II had little enantioselectivity for the derivatized allethrolone, and this racemic compound was separated to a considerable extent upon phase I.

In our previous investigation of chiral stationary phases for HPLC[5], we reported that some amide derivatives of chiral 1-(α-naphthyl)ethylamine bonded to silica gel gave good enantioselectivity. It is observed that phase II, which has the chiral 1-(α-naphthyl)ethyl group but no amide group, gives good chiral recognition as well as the amide-bonded stationary phase.

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