Journal of Chromatography, 257 (1983) 111–117
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,461

Note

High-performance liquid chromatographic separation of enantiomers on (*S*)-1-(α-naphthyl)ethylamine bonded to silica gel

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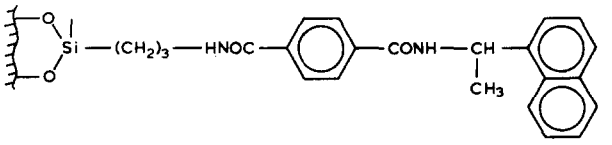
(Received October 18th, 1982)

It is well known that both gas and liquid chromatography on optically active stationary phases are very useful for the direct separation of enantiomers. In gas chromatography, Weinstein *et al.*[1] reported that it suffices for a chiral stationary phase to contain an amide group with an asymmetric carbon atom attached to the nitrogen atom [*e.g.* $RCONHCH(CH_3)R'$], in order to show selectivity in its interaction with the enantiomers of amides, and that the best efficiency is obtained when R′ is aromatic, particularly α-naphthyl, as in N-lauroyl-(*S*)-1-(α-naphthyl)ethylamine. We have reported[2,3] some N-acyl derivatives of (*R*)- or (*S*)-1-(α-naphthyl)ethylamine which have a high stereoselectivity for enantiomeric amides.

On the other hand, in liquid chromatography, Blaschke and Donow[4] prepared various polyacryl- and polymethacrylamides starting from chiral amine derivatives, and showed that racemates of mandelic acid or mandelamide can be separated to a considerable extent on these polymers. However, the solutes to which the method could be applied were limited, while the resolving power of these polymers is strongly influenced by the polymerization conditions.

In this study, we prepared two novel chiral stationary phases, I and II, comprised of (*S*)-1-(α-naphthyl)ethylamine chemically bonded to γ-aminopropyl silanized silica, and examined the direct separation of the derivatives of some racemic amines, amino acids and carboxylic acids by high-performance liquid chromatography.



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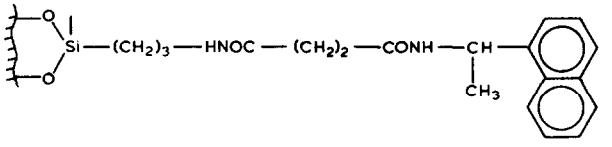

(II)

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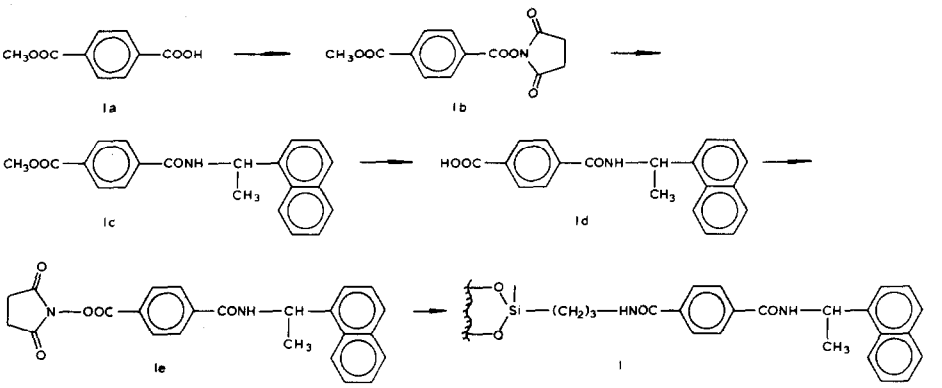
EXPERIMENTAL

Preparation of stationary phases

Phase I. This phase was prepared as shown in Scheme I and bonded onto LiChrosorb NH_2 (10 μm; E. Merck, Darmstadt, G.F.R.).



Scheme 1.

To a solution of 10 g of Ia (56 mmoles) and 6.5 g (56 mmoles) of N-hydroxysuccinimide (HOSu) in 100 ml of dry tetrahydrofuran (THF), 11.8 g (57 mmoles) of N,N′-dicyclohexylcarbodiimide (DCC) was slowly added at 0°C The mixture was kept at 5°C overnight. The precipitate was removed by filtration and washed with THF. The filtrate was concentrated under vacuum. The residue (Ib) was dissolved in 100 ml of dry THF. To this solution, 10 g (58 mmoles) of (*S*)-1-(α-naphthyl)ethylamine (*S*-NEA) was added, and the mixture was stirred at room temperature for 5 h and then at 50°C for 2 h. The solution was evaporated under vacuum and the residue was dissolved in ethyl acetate. This solution was washed successively with 1 *N* hydrochloric acid and water. The solvent was then removed under vacuum to afford the compound Ic.

To a solution of 10 g of Ic (30 mmoles) in 100 ml of methanol, 25 ml of 2 *N* sodium hydroxide was added and the mixture was kept at room temperature overnight. The organic phase was removed under reduced pressure. To the aqueous solution, 100 ml of water was added, and the pH of this solution was adjusted to 3–4 with 2 *N* hydrochloric acid, and the liberated Id was collected by filtration and washed with water.

To a solution of 10 g of Id (31 mmoles) and 3.6 g of HOSu (31 mmoles) in 100 ml of dry THF, 6.5 g of DCC (32 mmoles) was slowly added at 0°C. The mixture was kept at 5°C overnight. The precipitate was removed by filtration and washed with dry THF. The filtrate was concentrated under vacuum to afford the crude Ie which was then recrystallized from ethyl acetate–*n*-hexane.

To 2.5 g of LiChrosorb NH_2 (10 μm) in 30 ml of dry THF, 3 g of Ie (7.2 mmoles) was added, and the slurry was stirred slowly for 5 h at room temperature. After cooling, the modified silica (I) was collected and washed exhaustively with THF, methanol and ether and then dried under reduced pressure (found: C, 13.15; N, 1.73%).

Phase II. To 2.5 g of LiChrosorb NH_2 (10 μm, E. Merck) in 30 ml of water, 2.5 g of succinic anhydride (25 mmoles) was added with swirling. The pH of the solution was maintained at 4.0 with 2 *N* sodium hydroxide. The slurry was stirred at room temperature for 5 h. The silica was collected by filtration and washed with water, methanol and diethyl ether.

To 2.5 g of the above silica in 30 ml of THF, 3 g of 1,1′-carbonyldiimidazole (18 mmoles) was added at 0°C, and the mixture was gently stirred for 3 h after which time 3 g of *S*-NEA (18 mmoles) was added and the mixture gently stirred at room temperature for a further 5 h. The modified silica (II) was collected and washed exhaustively with THF, methanol and diethyl ether (found: C, 11.60; N, 1.94%).

Liquid chromatography

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

n-Hexane–isopropanol mixtures or n-hexane–1,2-dichloroethane–ethanol mixtures were used as mobile phases. Flow-rates typically of 1.0 ml/min were used at room temperature.

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

RESULTS AND DISCUSSION

The new phases are suitable for both normal- and reversed-phase systems, but in this study the properties were checked by utilizing only the normal-phase mode.

The chromatographic results are summarized in Tables I and II. It was found that the two novel phases I and II gave good chiral recognition for amines, amino acids and carboxylic acids. Typical chromatograms are shown in Figs. 1–3.

It is emphasized that amine and amino acid enantiomers were completely separated in the form of their N-3,5-dinitrobenzoyl derivatives, and carboxylic acid enantiomers were easily separated in the form of their 3,5-dinitroanilide derivatives. These phases contain a chiral amide group, which has the ability to serve as either a

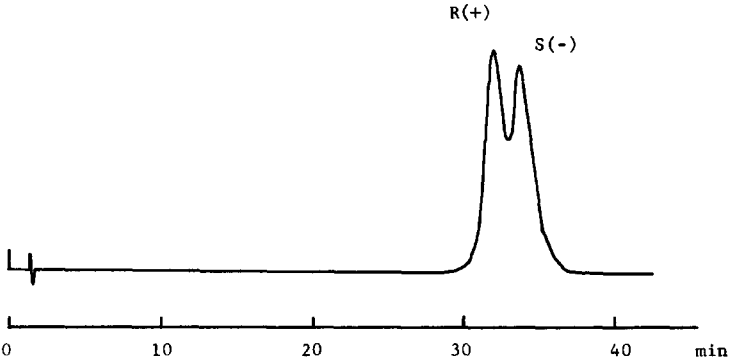
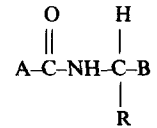


Fig. 1. Chromatographic separation of the enantiomers of racemic N-acetyl-1-(α-naphthyl)ethylamine on chiral stationary phase I. Chromatographic conditions as in Table I.

TABLE I

SEPARATION OF DERIVATIZED AMINES AND AMINO ACIDS ON CHIRAL STATIONARY PHASES



Compound A–C–NH–C–B			*Phase I*			*Phase II*		
A	*B*	*R*	α*	k_1'**	*Mobile phase***	α*	k_1'**	*Mobile phase****
CH_3	Phenyl	CH_3	1.05	8.14(*R*)	1	1.00	4.92	1
CH_3	α-Naphthyl	CH_3	1.09	10.79(*R*)	1	1.00	6.08	1
3,5-$(NO_2)_2C_6H_3$	C_2H_5	CH_3	1.04	4.85[§]	3	1.03	4.19[§]	2
3,5-$(NO_2)_2C_6H_3$	*n*-C_6H_{13}	CH_3	1.20	2.93[§]	3	1.09	2.30[§]	2
3,5-$(NO_2)_2C_6H_3$	Phenyl	CH_3	1.68	3.62(*R*)	5	1.63	2.04(*R*)	4
3,5-$(NO_2)_2C_6H_3$	α-Naphthyl	CH_3	2.25	4.23(*R*)	5	2.54	2.14(*R*)	4
3,5-$(NO_2)_2C_6H_3$	Phenyl	4-$CH_3C_6H_4CH_2$	1.15	6.23(*R*)	5	1.23	2.49(*R*)	4
3,5-$(NO_2)_2C_6H_3$	CO_2CH_3	CH_3	1.43	5.48(*D*)	3	1.40	4.40(*D*)	2
3,5-$(NO_2)_2C_6H_3$	$CONHC_4H_9$	CH_3	1.24	2.93(*D*)	3	1.13	4.51(*D*)	2
3,5-$(NO_2)_2C_6H_3$	CO_2CH_3	iso-C_3H_7	1.64	2.91(*D*)	3	1.56	1.60(*D*)	2
3,5-$(NO_2)_2C_6H_3$	$CONHC_4H_9$	iso-C_3H_7	1.55	1.13(*D*)	3	1.26	1.20(*D*)	2
3,5-$(NO_2)_2C_6H_3$	CO_2CH_3	$C_6H_5CH_2$	1.21	6.21(*D*)	3	1.24	3.26(*D*)	2
3,5-$(NO_2)_2C_6H_3$	$CONHC_4H_9$	$C_6H_5CH_2$	1.16	2.43(*D*)	3	1.04	2.33(*D*)	2

* The separation factor of the enantiomers, α, is the ratio of the capacity ratios of the enantiomers.

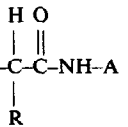
** k_1' is the capacity ratio for the initially eluted enantiomer.

*** Mobile phases: 1 = isopropanol–*n*-hexane (7:93); 2 = *n*-hexane–1,2-dichloroethane–ethanol (48:15:1); 3 = *n*-hexane–1,2-dichloroethane–ethanol (20:6:1); 4 = *n*-hexane–1,2-dichloroethane–ethanol (40:12:3); 5 = *n*-hexane–1,2-dichloroethane–ethanol (10:4:1).

[§] Elution orders have not been established.

TABLE II

SEPARATION OF THE ENANTIOMERS OF DERIVATIZED CARBOXYLIC ACIDS UPON CHIRAL STATIONARY PHASES

Compound 			*Phase I*			*Phase II*		
A	*B*	*R*	α*	k_1'**	*Mobile phase***	α*	k_1'**	*Mobile phase***
tert.-C_4H_9	4-ClC_6H_4	iso-C_3H_7	1.16	4.33(*S*)	6	1.00	2.69	6
3,5-$(NO_2)_2C_6H_3$	Phenyl	CH_3	1.93	6.35(*S*)	3	1.60	10.64(*S*)	2
3,5-$(NO_2)_2C_6H_3$	4-ClC_6H_4	iso-C_3H_7	1.71	4.93(*S*)	3	1.58	9.61(*S*)	2
3,5-$(NO_2)_2C_6H_3$	Br	*tert.*-C_4H_9	1.33	4.71(*R*)	3	1.26	10.32(*R*)	2

* See Table I.

** See Table I.

*** Mobile phases: 2 and 3 as in Table I; 6 = isopropanol–*n*-hexane (0.5:99.5).

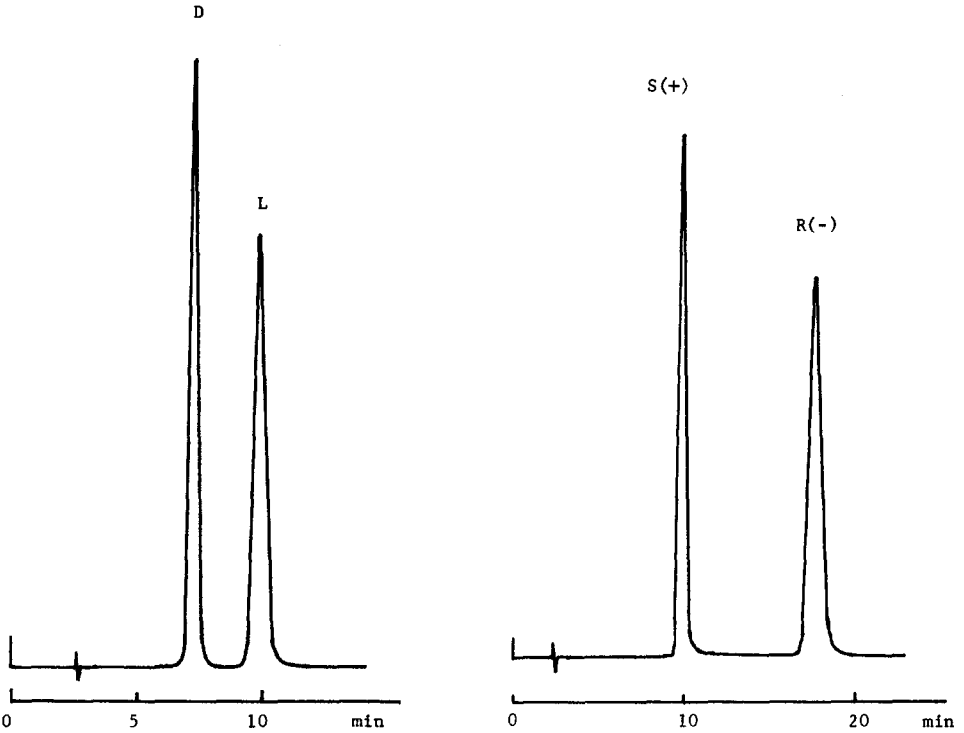


Fig. 2. Chromatographic separation of the enantiomers of racemic N-3,5-dinitrobenzoylvaline methyl ester on chiral stationary phase II. Chromatographic conditions as in Table I.

Fig. 3. Chromatographic separation of the enantiomers of racemic 2-phenylpropionic acid (3,5-dinitro)anilide on chiral stationary phase I. Chromatographic conditions as in Table II.

donor or an acceptor in hydrogen bonding, so that diastereoisomeric hydrogen bonding may contribute to the separation of amide enantiomers. However they did not show a high enantioselectivity for enantiomers of N-acetyl derivatives of amines and amino acids or alkylamide derivatives of carboxylic acids.

The surprising influence of the N-3,5-dinitrophenyl group suggests a contribution from a charge-transfer interaction in the separation of these enantiomers.

Recently, Pirkle and co-workers[5,6] achieved the separation of various enantiomers using stationary phases consisting of chiral 2,2,2-trifluoro-1-(9-anthryl)ethanol or N-(3,5-dinitrobenzoyl)phenylglycine bonded to silanized silica, and they indicated that the incorporation of a π–π donor–acceptor interaction to the diastereoisomeric hydrogen bonding is very effective for the chiral recognition.

As the α-naphthyl group in phases I and II can act as the π-base, the incorporation of a 3,5-dinitrophenyl group into the solutes should considerably extend the scope of these phases.

The elution order of the solute was determined by chromatography of samples whose configurations had been previously established. (*R*)-Enantiomers eluted first for amines and amino acids, while (*S*)-enantiomers eluted first for carboxylic acids, except in the case of 2-bromo-3,3-dimethylbutyric acid. These orders were consistent

with the orders obtained in gas chromatography using N-lauroyl-(*S*)-1-(α-naphthyl)ethylamine[1] or N,N′-[2,4-(6-ethoxy-1,3,5-triazine)diyl]bis-(L-valyl-L-valyl-L-valine isopropyl ester) (OA-300)[7–9] as the optically active stationary phase. This fact suggests that the chiral recognition mechanism is essentially similar in both gas and liquid chromatography.

We consider that the two novel phases, which can be simply prepared, are very useful for the separation of various amine, amino acid and carboxylic acid enantiomers in liquid chromatography.

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