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Note

High-performance liquid chromatographic separation of enantiomers on (*S*)-2-(4-chlorophenyl)isovaleric acid and its amide derivatives bonded to silica gel

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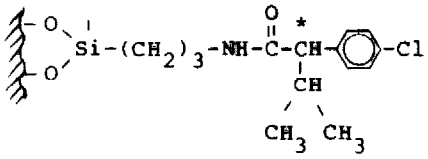
Recently we reported that some amide derivatives of (1*R*, 3*R*)-*trans*-chrysanthemic acid, which contain one or two asymmetric carbon atoms attached to carbon and nitrogen atoms of the amide group, are efficient for enantiomer separations not only by gas chromatography (GC)[1,2] but also by high-performance liquid chromatography (HPLC)[3].

In previous work[1] we found that N-(*S*)-2-(4-chlorophenyl)isovaleroyllaurylamine as well as N-(1*R*,3*R*)-*trans*-chrysanthemoyllaurylamine showed enantioselectivity for chiral amides in GC, suggesting that some amide derivatives of (*S*)-2-(4-chlorophenyl)isovaleric acid would also show enantioselectivity in HPLC.

In this work we prepared three novel chiral stationary phases I–III consisting of (*S*)-2-(4-chlorophenyl)isovaleric acid and its amide derivatives chemically bonded to γ-aminopropyl silanized silica, and the HPLC separation of various enantiomers on these phases was examined.



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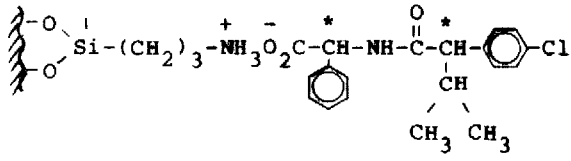

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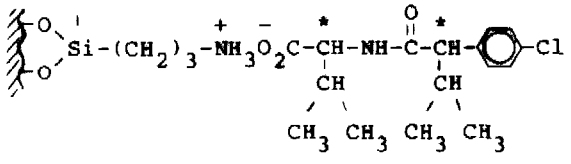

III

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TABLE I

HPLC SEPARATION OF ENANTIOMERS UPON CHIRAL STATIONARY PHASES

The separation factor of the enantiomers, α, is the ratio of their capacity factors. k', is the capacity factor for the initially eluted enantiomer. Mobile phases: 1 = *n*-hexane–1,2-dichloroethane–ethanol (100:20:1); 2 = *n*-hexane–1,2-dichloroethane–ethanol (48:15:1); 3 = isopropanol–*n*-hexane (5:95); 4 = isopropanol–*n*-hexane (0.5:99.5). Flow-rates of 1 ml/min were typically used for the 250 × 4 mm I.D. columns at room temperature.

Compound	*Phase I*			*Phase II*			*Phase III*		
	α	k'_1	*Mobile phase*	α	k'_1	*Mobile phase*	α	k'_1	*Mobile phase*
Amines									
N-Acetyl-1-phenylethylamine	1.07	6.88	1	1.00	2.14	2	1.00	1.96	2
N-Acetyl-1-(α-naphthyl)ethylamine	1.09	6.71	1	1.00	2.09	2	1.00	1.82	2
N-Acetyl-1-phenyl-2-(4-tolyl)ethylamine	1.00	4.04	1	1.00	1.50	2	1.00	1.29	2
N-3,5-Dinitrobenzoyl-1-phenylethylamine	1.23	2.38	1	1.00	2.47	2	1.09	2.54	2
N-3,5-Dinitrobenzoyl-1-(α-naphthyl)ethylamine	1.58	2.23	1	1.07	2.57	2	1.00	3.46	2
N-3,5-Dinitrobenzoyl-1-phenyl-2-(4-tolyl)ethylamine	1.13	1.83	1	1.18	1.75	2	1.20	1.88	2
N-3,5-Dinitrobenzoyl-*sec.*-butylamine	1.00	1.96	1	1.09	5.43	1	1.00	5.38	3
N-3,5-Dinitrobenzoyl-2-octylamine	1.24	1.09	1	1.27	3.45	1	1.27	3.50	3
Amino acids									
N-Acetylalanine methyl ester	1.02	7.85	1	1.00	4.93	1	1.05	5.89	1
N-3,5-Dinitrobenzoylalanine methyl ester	1.09	2.41	1	1.15	2.39	2	2.12	2.28	2
N-3,5-Dinitrobenzoylalanine *n*-butylamide	1.13	2.73	1	1.14	2.53	2	3.83	2.40	2
N-Acetylvaline methyl ester	1.04	3.19	1	1.00	2.25	1	1.00	3.16	1
N-3,5-Dinitrobenzoylvaline methyl ester	1.00	0.92	1	1.36	0.89	2	1.85	1.07	2
N-3,5-Dinitrobenzoylvaline *n*-butylamide	1.00	0.69	1	1.31	0.85	2	5.10	0.72	2
N-Acetylphenylalanine methyl ester	1.00	3.37	1	1.00	2.76	1	1.00	2.96	1
N-3,5-Dinitrobenzoylphenylalanine methyl ester	1.00	1.50	1	1.37	1.38	2	1.72	1.48	2
N-3,5-Dinitrobenzoylphenylalanine *n*-butylamide	1.00	1.00	1	1.30	1.30	2	5.10	1.20	2
Carboxylic acids									
2-Phenylpropionic acid isopropylamide	1.06	6.62	4	1.00	6.36	4	1.00	5.31	4
2-(4-Chlorophenyl)isovaleric acid *tert.*-butylamide	1.00	1.47	4	1.00	1.04	4	1.23	0.81	4
2-Phenylpropionic 3,5-dinitroanilide	1.29	2.62	1	1.23	4.00	2	1.32	2.96	2
2-(4-Chlorophenyl)isovaleric acid 3,5-dinitroanilide	1.14	2.05	1	1.10	3.25	2	1.34	2.57	2
1-Bromo-2,2-dimethylbutyric acid 3,5-dinitroanilide	1.06	2.73	1	1.93	3.51	2	1.08	3.44	2
trans-Chrysanthemic acid 3,5-dinitroanilide	1.07	1.76	1	1.22	2.25	2	1.08	1.77	2

EXPERIMENTAL

Preparation of chiral stationary phases

Phase I. A 3-g amount of (*S*)-2-(4-chlorophenyl)isovaleric acid chloride was coupled with 2.5 g of LiChrosorb NH_2 (10 μm) (E. Merck, Darmstadt, G.F.R.) by swirling gently in 30 ml dry tetrahydrofuran (THF) in the presence of 2 g of triethylamine at room temperature for 5 h and then at 50°C for 3 h. After cooling, modified silica I was collected by filtration and washed exhaustively with THF, methanol and diethyl ether and dried under vacuum. This silica contained 0.63 mmol of (*S*)-2-(4-chlorophenyl)isovaleric acid per g of support (based on C), 0.86 mmol/g (based on N).

A steel column (250 × 4 mm I.D.) was slurry packed with modified silica I using conventional techniques.

Phase II. To a solution of 9.5 g of D-phenylglycine in 32 ml 2 *M* NaOH and 20 ml diethyl ether, 17.5 g of (*S*)-2-(4-chlorophenyl)isovaleric acid chloride and 35 ml of 2 *M* sodium hydroxide solution were added very slowly with vigorous swirling at 0°C. When the addition was complete, swirling was continued for 2 h at room temperature. The solution was washed with diethyl ether, and the aqueous phase was acidified with 6 *M* hydrochloric acid and extracted with ethyl acetate. The extracts were washed with water, dried over anhydrous sodium sulphate and the solvent was removed under vacuum to afford 21 g of colorless crystalline N-(*S*)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine (m.p. 68–70°C). Calculated for $C_{19}H_{20}NO_3Cl$: C, 65.99; H, 5.83; N, 4.05; Cl, 10.25%. Found: C, 65.49; H, 5.99; N, 3.79; Cl, 10.41%.

To a slurry of 2.5 g LiChrosorb NH_2 (10 μm) in 20 ml of dry THF, 4 g of N-(*S*)-2-(4-chlorophenyl)isovaleroyl-D-phenylglycine were added and the mixture was stirred gently overnight at room temperature. This modified silica II was collected by filtration and washed exhaustively with THF, methanol and diethyl ether and dried under reduced pressure. It contained 0.43 mmol of N-(*S*)-2-(4-chlorophenyl)-isovaleroyl-D-phenylglycine per g of support (based on C), 0.55 mmol/g (based on N).

Phase III. N-(*S*)-2-(4-Chlorophenyl)isovaleroyl-L-valine was synthesized as for phase II but using L-valine instead of D-phenylglycine and formed colourless crystals (m.p. 134–137°C). Calculated for $C_{16}H_{22}NO_3Cl$: C, 61.62, H, 7.13; N, 4.49; Cl, 11.37%. Found: C, 61.79; H, 7.16; N, 4.13; Cl, 11.55%.

A steel column (250 × 4 mm I.D.) was slurry packed with LiChrosorb NH_2 (10 μm) using conventional techniques and a solution of 3 g of N-(*S*)-2-(4-chlorophenyl)isovaleroyl-L-valine in 30 ml of dry THF was pumped through the column. Finally the column was washed with THF.

Liquid chromatography

The experiments were carried out with a Shimadzu LC-3A high-performance liquid chromatograph equipped with a UVD-2 ultraviolet detector (254 nm). *n*-Hexane–isopropanol or *n*-hexane–1,2-dichloroethane–ethanol mixtures were used as mobile phases.

Various derivatized compounds as solutes were prepared by employing reagent-grade chemicals. Some compounds were synthesized in our laboratories.

RESULTS AND DISCUSSION

The chromatographic results are summarized in Table I. It was found that phase I had little enantioselectivity for amino acids, but enantiomers of amines and carboxylic acid derivatives were separated to a considerable extent, although this phase contains only one asymmetric carbon atom attached to the carbon atom of the amide group. An example of a chromatogram is shown in Fig. 1.

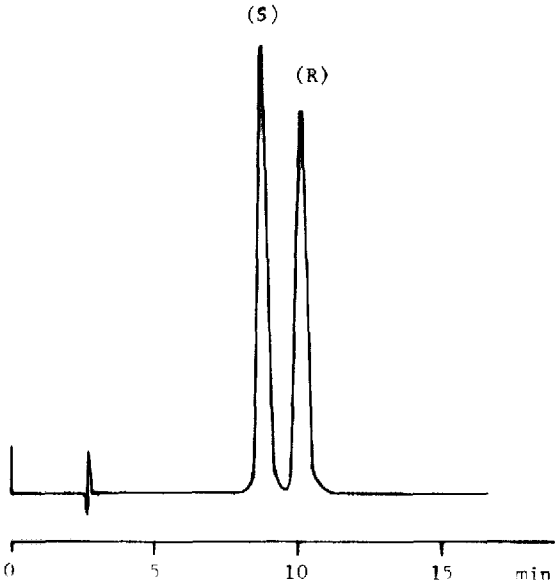

Fig. 1. Chromatographic separation of the enantiomers of racemic N-3,5-dinitrobenzoyl-1-phenylethylamine upon chiral stationary phase I. Chromatographic conditions as in Table I.

Phases II and III, which contain two asymmetric carbon atoms attached to both carbon and nitrogen atoms of the amide group, showed excellent enantioselectivity for N-3,5-dinitrobenzoyl derivatives of amine, amino acid ester or amide, and for carboxylic acid 3,5-dinitroanilides. It is emphasized that N-3,5-dinitrobenzoyl-DL-amino acid *n*-butylamides are separated with very high separation factors upon phase III. Typical chromatograms are shown in Figs. 2 and 3.

As these phases contain a chiral amide group, which has the ability to serve either as a donor or an accepter in hydrogen bonding, a diastereoisomeric hydrogen bonding association may contribute to the separation of amide enantiomers. Moreover, the fact that a 3,5-dinitrophenyl group is efficiently incorporated into the solutes suggests the additional contribution from a π–π donor–acceptor interaction in the separation of these enantiomers.

We consider that these stationary phases will be useful for the HPLC separation of amine, amino acid and carboxylic acid enantiomers.

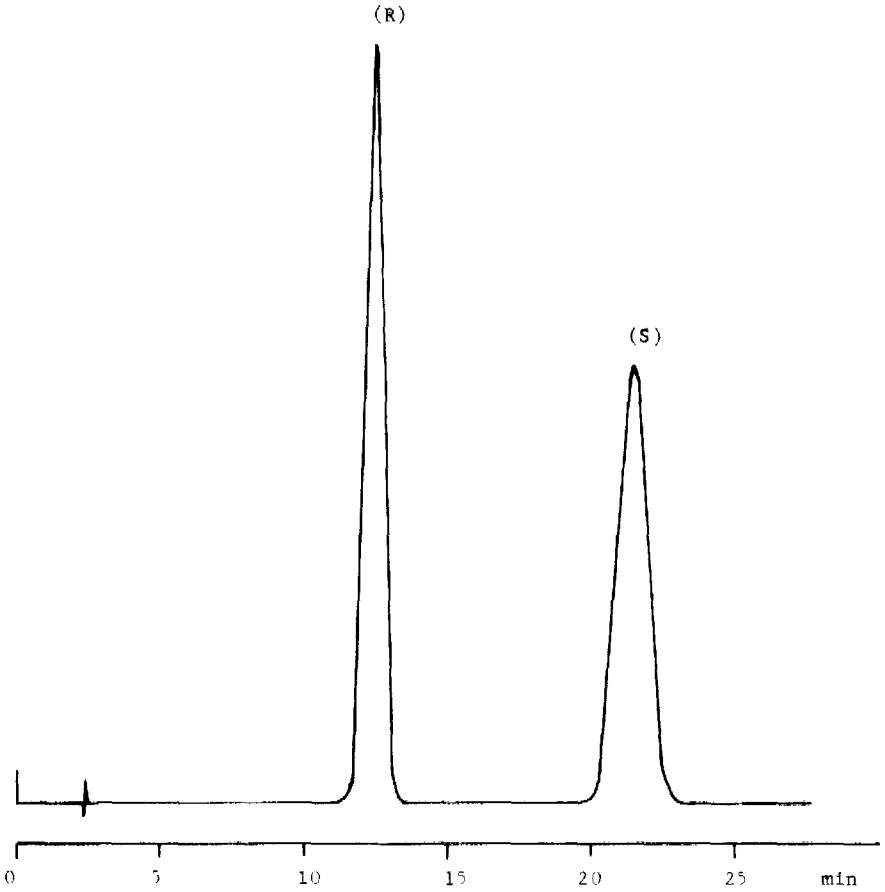

Fig. 2. Chromatographic separation of the enantiomers of racemic 1-bromo-2,2-dimethylbutyric acid 3,5-dinitroanilide upon chiral stationary phase II. Chromatographic conditions as in Table I.

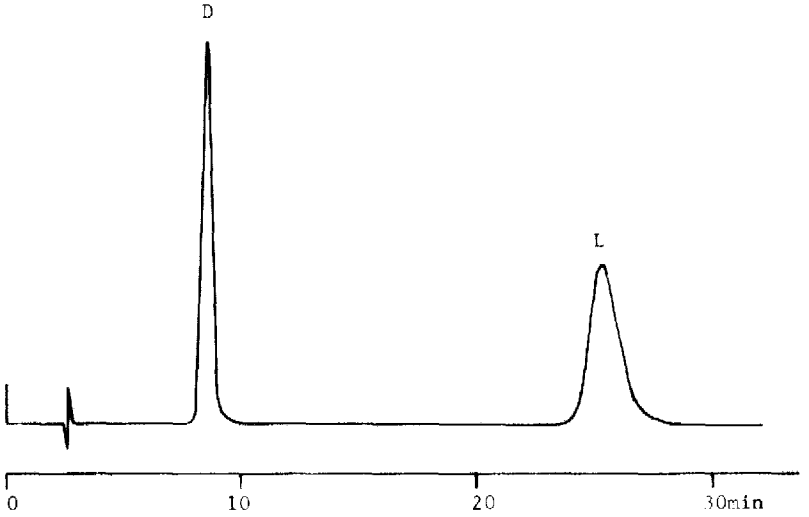

Fig. 3. Chromatographic separation of the enantiomers of racemic N-3,5-dinitrobenzoylalanine *n*-butylamide upon chiral stationary phase III. Chromatographic conditions as in Table I.

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