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Note

High-performance liquid chromatographic separation of enantiomers on (1*R*,3*R*)-*trans*-chrysanthemic acid and its amide derivatives bonded to silica gel

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(1*R*,3*R*)-*trans*-Chrysanthemic acid is an important optically active constituent of various insecticidal pyrethroids. In our study of the direct separation of optical isomers by gas chromatography[1], we found that N-(1*R*,3*R*)-*trans*-chrysanthemoyl-laurylamine, which contains an asymmetric carbon atom attached to the carbon atom of the amide group, showed enantioselectivity for chiral amide compounds. We also found that N-(1*R*,3*R*)-*trans*-chrysanthemoyl-(*R*)-1-(α-naphthyl)ethylamine,which contains two asymmetric carbon atoms attached to both nitrogen and carbon atoms of the amide group, showed better enantioselectivity[2].

As it is well known that the chiral recognition mechanism is essentially same in gas and liquid chromatography, these results suggested that some derivatives of optically active chrysanthemic acid would be effective as chiral stationary phases in liquid chromatography and led us to this work.

In this study we prepared three novel chiral stationary phases (I, II and III), consisting of (1*R*,3*R*)-*trans*-chrysanthemic acid and its amide derivatives chemically bonded to γ-aminopropyl silanized silica, and the high-performanc liquid chromatographic separation of various enantiomers on these phases was examined.

EXPERIMENTAL

Preparation of chiral stationary phases

Phase I. (1*R*,3*R*)-*trans*-Chrysanthemic acid chloride (3 g, 16 mmol) was coupled with 2.5 g of LiChrosorb NH_2 (10 μm) (E. Merck, Darmstadt, G.F.R.) by swirling gently in 30 ml of 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, the modified silica I was collected by filtration and washed exhaustively with THF, methanol, and diethyl ether and dried under vacuum. Phase I contained 0.72 mmol of (1*R*,3*R*)-*trans*-chrysanthemic acid/g of support (based on C), 0.86 mmol/g (based on N).

Phase II. To a solution of 10 g of D-phenylglycine (66 mmol) in a mixture of 33 ml of 2 *N* sodium hydroxide solution and 20 ml of diethyl ether, 15 g of (1*R*,3*R*)-*trans*-chrysanthemic acid chloride (80 mmol) and 40 ml of 2 *N* sodium hydroxide solution were added very slowly with vigorous swirling at 0°C.

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TABLE I

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS ON CHIRAL STATIONARY PHASES

Compound	*Phase I*			*Phase II*			*Phase III*		
	α^{*}	k'^{**}	*Mobile phase****	α^{*}	k'^{**}	*Mobile phase****	α^{*}	k'^{**}	*Mobile phase****
Amines:									
N-Acetyl-1-phenylethylamine	1.05	4.22(*R*)	4	1.08	6.02(*R*)	4	1.00	4.32	4
N-Acetyl-1-(α-naphthyl)ethylamine	1.00	3.69	4	1.10	4.63(*R*)	4	1.00	3.72	4
N-3,5-Dinitrobenzoyl-1-(α-naphthyl)ethylamine	1.07	1.65(*S*)	4	1.07	8.21(*R*)	2	1.19	5.05(*R*)	4
N-3,5-Dinitrobenzoyl-1-phenyl-2-(4-tolyl)ethylamine	1.00	1.19	4	1.15	5.75(*R*)	2	1.14	3.92(*R*)	4
N-3,5-Dinitrobenzoyl-*sec*.-butylamine	1.00	2.02	4	1.12	4.24(*R*)	4	1.00	4.85	4
N-3,5-Dinitrobenzoyl-2-octylamine	1.10	0.92[§]	4	1.30	2.00[§]	4	1.10	2.92[§]	4
Amino acids:									
N-Acetylalanine methyl ester	1.00	5.50	4	1.00	5.82	4	1.06	5.55(D)	4
N-3,5-Dinitrobenzoylalanine methyl ester	1.00	2.38	4	1.91	6.81(D)	4	2.26	4.28(D)	4
N-3,5-Dinitrobenzoylalanine *n*-butylamide	1.04	2.11(L)	4	3.21	1.78(D)	5	2.02	2.04(D)	5
N-Acetylvaline methyl ester	1.00	2.14	4	1.07	2.53(L)	4	1.05	2.68(D)	4
N-3,5-Dinitrobenzoylvaline methyl ester	1.00	0.69	4	2.59	1.91(D)	4	1.90	1.54(D)	4
N-3,5-Dinitrobenzoylvaline *n*-butylamide	1.00	0.44	4	4.00	0.62(D)	5	1.75	0.56(D)	5

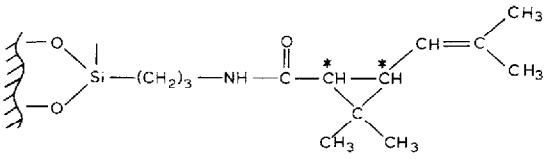
N-Acetylphenylalanine methyl ester	1.00	1.50	4	1.15	2.44(L)	4	1.00	3.00	4
N-3,5-Dinitrobenzoylphenylalanine methyl ester	1.00	1.04	4	1.96	3.42(D)	4	1.78	2.62(D)	4
N-3,5-Dinitrobenzoylijphenylalanine *n*-butylamide	1.00	0.49	4	3.35	0.99[§]	5	2.11	0.76[§]	5
Carboxylic acids:									
1-Methylphenylacetic acid isopropylamide	1.03	5.00(*S*)	1	1.00	6.00	1	1.00	4.65	1
1-Isopropyl-(4-chlorophenyl)acetic acid *tert.*-butylamide	1.19	0.84(*S*)	1	1.00	1.09	1	1.25	1.08 (*S*)	1
1-Methylphenylacertic acid 3,5-dinitroanilide	1.09	4.08(*R*)	4	1.07	8.33(*R*)	4	1.16	10.75(*S*)	4
1-Isopropyl-(4-chlorophenyl)acetic acid 3,5-dinitro-anilide	1.07	4.26(*S*)	4	1.00	7.41	4	1.16	18.48(*S*)	4
1-Bromo-2,2-dimethylbutyric acid 3,5-dinitroanilide	1.06	4.43(*S*)	4	1.25	6.25(*R*)	4	1.07	13.92(*S*)	4
trans-Chrysanthemic acid 3,5-dinitroanilide	1.07	2.23	4	1.07	3.71(*S*)	4	1.14	5.99(*R*)	4
Alcohols:									
1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308)	1.13	1.04(*R*)	3	1.50	1.69(*R*)	3	1.00	3.57	3
1-(4-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-07)	1.11	1.36(*R*)	3	1.31	2.33(*R*)	3	1.00	4.26	3

* The separation factor of the enantiomers (α) is the ratio of their capacity ratios.

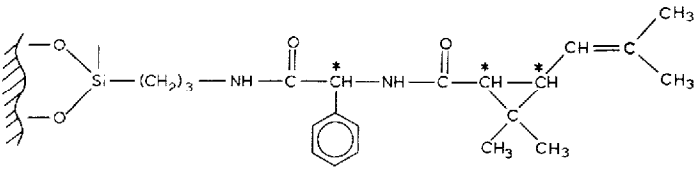
** k' is the capacity ratio for the initially eluted enantiomer.

*** Mobile phases: 1 = isopropanol–*n*-hexane (0.5:99.5); 2 = isopropanol–*n*-hexane (5:95); 3 = *n*-hexane–1,2-dichloroethane–ethanol (700:90:9); 4 = *n*-hexane–1,2-dichloroethane–ethanol (100:20:1); 5 = *n*-hexane–1,2-dichloroethane–ethanol (48:15:1).

§ Elution orders have not been established.



(I)



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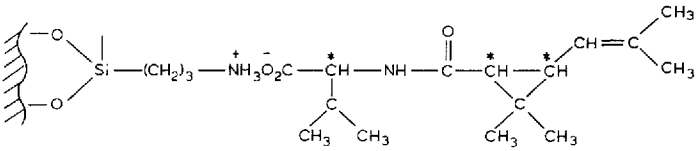

(III)

After the addition was completed, swirling was continued for 2 h at room temperature and the solution was washed with two 50-ml portions of diethyl ether. The aqueous phase was acidified with 6 *N* hydrochloric acid extracted with ethyl acetate. The extracts were washed with water and dried over anhydrous sodium sulphate and ethylacetate was removed under vacuum to afford 15 g of colourless crystalline N-(1*R*,3*R*)-*trans*-chrysanthemoyl-D-phenylglycine. (m.p. 76–80°C). Analysis: calculated for $C_{18}H_{23}NO_3$, C 71.74, H 7.70, N 4.65%; found, C 71.58, H 7.65, N 4.58%.

To a solution of 12 g of N-(1*R*,3*R*)-*trans*-chrysanthemoyl-D-phenylglycine (40 mmol) and 4.6 g of N-hydroxysuccinimide (40 mmol) in 100 ml of dry THF, 8.3 g of dicyclohexylcarbodiimide (40 mmol) in 20 ml of dry THF were added dropwise with vigorous swirling at 0°C. Swirling was continued for 2 h at 0°C and for 3 h at room temperature, then the solution was filtered and the solvent removed under vacuum.

The residue was dissolved in 150 ml of ethyl acetate. This solution was washed with water and dried over anhydrous sodium sulphate.

The solvent was then removed under reduced pressure to give the colourless crystalline N-hydroxysuccinimide ester of N-(1*R*,3*R*)-*trans*-chrysanthemoyl-D-phenylglycine. (m.p. 80–85°C). Analysis: calculated for $C_{22}H_{26}N_2O_5$, C 66.32, H 6.58, N 7.03%; found, C 66.53, H 6.31, N 6.99%.

To a slurry of 2.5 g of LiChrosorb NH_2 (10 μm) in 30 ml of dry THF, 6 g of the N-hydroxysuccinimide ester of N-(1R,3*R*)-*trans*-chrysanthemoyl-D-phenylglycine (15 mmol) were added, and the mixture was stirred gently for 5 h at room temperature, and for 5 h at 50°C.

After cooling, the modified silica II was collected by filtration and washed

exhaustively with THF, methanol, and diethyl ether and dried under reduced pressure. Phase II contained 0.56 mmol of N-(1*R*,3*R*)-*trans*-chrysanthemoyl-D-phenylglycine/g of support (based on C), 0.62 mmol/g (based on N).

Phase III. N-(1*R*,3*R*)-*trans*-Chrysanthemoyl-L-valine was synthesized as for phase II but using L-valine instead of D-phenylglycine, and was colourless and crystalline (m.p. 113–116°C). Analysis: calculated for $C_{15}H_{24}NO_3$, C 67.62, H 9.10, N 5.26%; found, C 67.33, H 9.46; N 5.19%.

To a slurry of 2.5 g of LiChrosorb NH_2 (10 μm) in 30 ml of THF, 4 g of L-valine (15 mmol) were added and the mixture was stirred gently overnight at room temperature.

The modified silica III was collected by filtration and washed exhaustively with THF, methanol, and diethyl ether and dried under reduced pressure. Phase III contained 0.52 mmol of N-(1*R*,3*R*)-*trans*-chrysanthemoyl-L-valine/g of support (based on C), 0.48 mmol/g (based on N).

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). Steel columns (250 × 4 mm I.D.) were slurry packed using conventional techniques.

n-Hexane–isopropanol and *n*-hexane–1,2-dichloroethane–ethanol mixtures were used as mobile phases. A flow-rate of 1.0 ml/min was typically used at room temperature. Various derivatized compounds as solutes were prepared by employing analytical-reagent grade chemicals. Some compounds were synthesized in our laboratories.

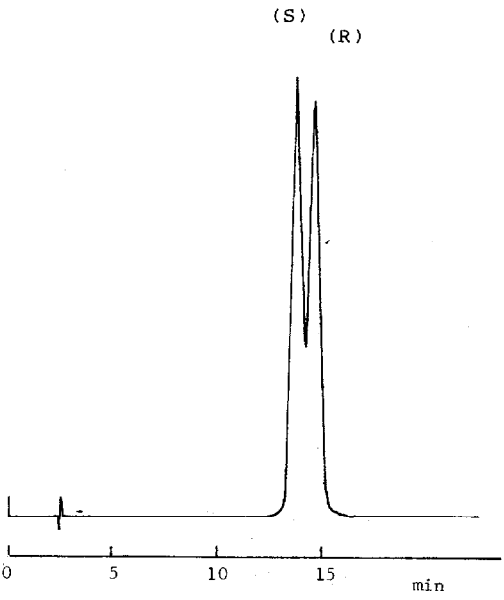

Fig. 1. Chromatographic separation of the enantiomers of racemic 1-isopropyl-(4-chlorophenyl)acetic acid 3,5-dinitroanilide on chiral stationary phase I. Chromatographic conditions as in Table I.

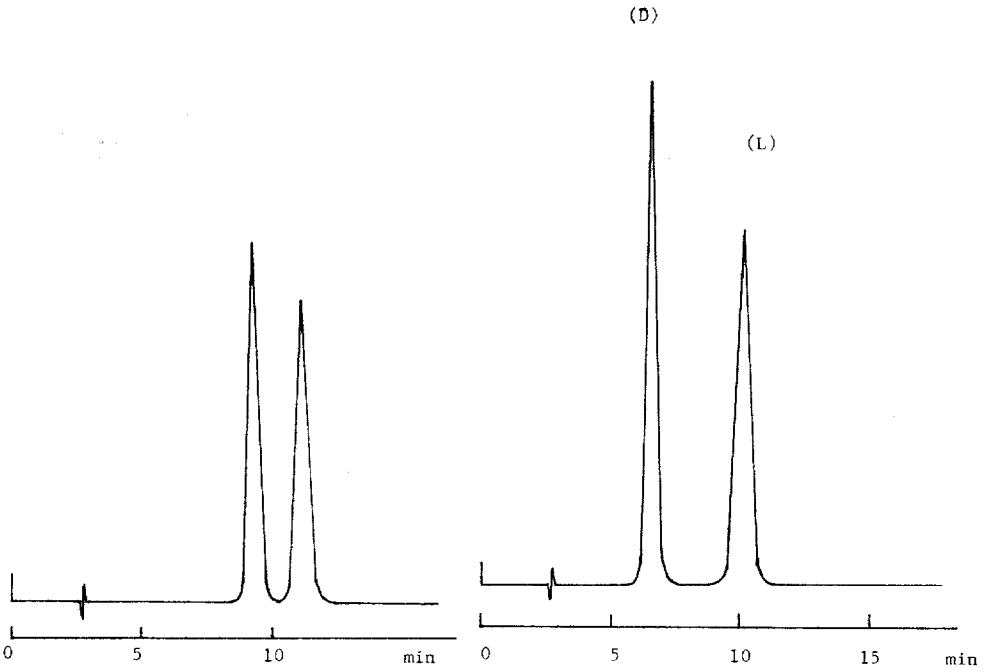

Fig. 2. Chromatographic separation of the enantiomers of racemic N-3,5-dinitrobenzoyl-2-octylamine on chiral stationary phase II. Chromatographic conditions as in Table I.

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

RESULTS AND DISCUSSION

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

Phases II and III, which contain two asymmetric carbon atoms attached to both carbon and nitrogen atoms of the amide group, showed better enantioselectivity than phase I. These phase can separate not only carboxylic acid enantiomers but also amine and amino acid enantiomers. Typical chromatograms are shown in Figs. 2 and 3.

It is especially emphasized that N-3,5-dinitrobenzoylderivatives of amino acids are separated with very high separation factors. For example, the separation factor was 4.0 for N-3,5-dinitrobenzoyl-DL-valine-*n*-butylamide on phase III.

Although many alcohol enantiomers were not resolved with these phases directly, complete separation of the enantiomers of the fungicide S-3308 [(±)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol], which shows excellent activity against powdery mildews, etc., and the plant growth retardant S-07

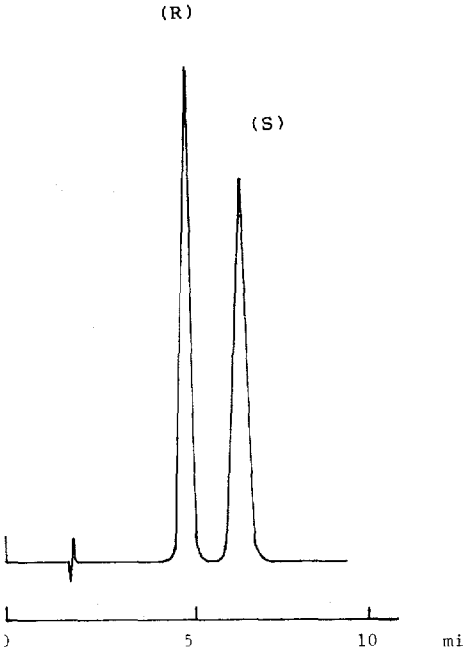

Fig. 4. Chromatographic separation of the enantiomers of racemic 1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazole-1-yl)-1-penten-3-ol on chiral stationary phase II. Chromatographic conditions as in Table I.

[(±)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-trizol-1-yl)-1-penten-3-ol] was accomplished. A typical chromatogram of racemic S-3308 is shown in Fig. 4.

In conclusion, the results show the (1*R*,3*R*)-*trans*-chrysanthemic acid derivatives are suitable for the separation of enantiomers and a second chiral constituent in optically active stationary phases can influence efficiently the chiral recognition in liquid chromatography as well as in gas chromatography.

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