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Short communication

Enantiomer separation by gas and high-performance liquid chromatography with tripeptide derivatives as chiral stationary phases

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Abstract

Excellent enantiomer separation of a variety of racemic compounds, including alcohols, amines, amino alcohols, carboxylic acids, hydroxy acids and amino acids, was achieved by GC and HPLC with tripeptide derivatives, containing L-valyl-L-valyl-L-valine isopropyl ester as a chiral selector, bonded to amino silicone oil (CSP-3) and N-(2-aminoethyl)-3-aminopropyl silica gel (CSP-4) via a triazine ring, respectively. These results show that the hydrogen bonding association between solutes and chiral stationary phases (CSPs) can play an important role in chiral recognition in both GC and HPLC. The joint use of two CSPs is promising for the direct separation of racemic compounds.

1. Introduction

Enantiomer separations by gas chromatography (GC) on chiral stationary phases (CSPs) have made remarkable progress since the pioneering work by Gil-Av et al. [1], in which N-trifluoroacetyl (TFA)-DL-amino acid esters were resolved on N-TFA-L-amino acid esters using long capillary columns. Many amino acid derivatives have since been developed as CSPs, and it is believed that chiral recognition on these CSPs is mainly based on diastereomeric hydrogen bonding association [2].

We prepared some *s*-triazine derivatives of amino acid esters, dipeptide esters and tripeptide esters, and found that the enantioselectivity of N,N'-[2,4-(6-ethoxy-1,3,5,-triazine)diyl]bis(L-valyl-L-valyl-L-valine isopropyl ester) (CSP-1) was superior to that of the corresponding *s*-triazine derivative of L-valyl-L-valine isopropyl ester in the separation of racemic amino acids, amines and carboxylic acids [3]. This result was notable because Feibush and Gil-Av [2] had already indicated that N-TFA-tripeptide esters gave a lower enantioselectivity than the corresponding N-TFA-dipeptide ester phases. Recently, Lohmiller et al. [4] used a series of oligopeptides, L-(valine)$_n$-*tert*-butylamide ($n = 1$–4), linked to poly(β-methyl)siloxy-α-methylpropionic acid copolymer, for the enantiomer separation of N-TFA-amino acid esters, and showed that chiral recognition was most effective with $n = 1$ (known as Chirasil-Val [5]), as judged from the separation factors (α), the thermodynamic parameters and ^{1}H NMR data.

Although CSP-1 has good enantioselectivity and high thermal stability, its high melting point limited the working temperature. Therefore, we

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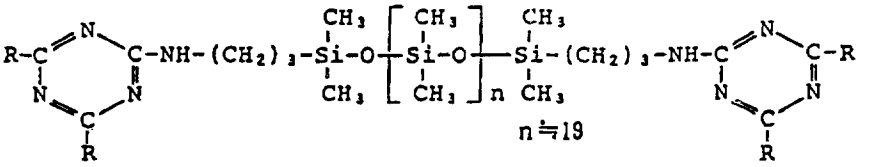
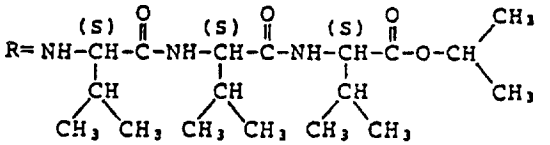


n≒19

[CSP-3]



prepared [6] N,N′-[2,4-(6-dioctylamino-1, 3,5-triazine)dilyl]bis(L-valyl-L-valyl-L-valine isopropyl ester) (CSP-2) to overcome this disadvantage. The melting point of CSP-2 is lower than that of CSP-1, but CSP-2 is still solid at room temperature, and it is difficult to obtain good efficiency and durability of the capillary column. In this study, we prepared a modified *s*-triazine derivative of L-valyl-L-valyl-L-valine isopropyl ester bonded to amino silicone oil (CSP-3), and examined its chromatographic properties.

In enantiomer separations by high-performance liquid chromatography (HPLC), it is also known that amino acid derivatives are effective as CSPs and the hydrogen bonding association is important for chiral recognition [7].

Previously, we reported [8] enantiomer separations by HPLC on an *s*-triazine derivative of L-valyl-L-valyl-L-valine isopropyl ester bonded to N-(2-aminoethyl)-3-aminopropyl silica gel (CSP-4), but the separation was limited to derivatized amino acids, carboxylic acids and alcohols, and the direct separation of underivatized racemic compounds was never examined. We report in this paper the direct enantiomer separation of various racemic compounds by HPLC on CSP-4.

2. Experimental

2.1. Preparation of CSPs

N,N′-[2,4-(6-Chloro-1,3,5,-triazine) diyl]bis(L-valyl-L-valyl-L-valine isopropyl ester) was prepared as described previously [3]. To solution of 1.25 g of this compound in 30 ml of dry dioxane, 0.85 g of amino silicone oil (KF865; Shinetsu Chemical, Tokyo, Japan) and 0.84 g of sodium hydrogencarbonate were added and the mixture was stirred under reflux for 36 h. The solution was filtered and the solvent was removed under reduced pressure. CSP-3 was obtained by column chromatographic purification of this crude product on silica gel with chloroform–acetone (8:2).

CSP-4 was prepared as described previously [8].

2.2. Gas chromatography

The experiments were carried out on a Shimadzu 14A gas chromatograph equipped with a flame ionization detector. A fused-silica capillary column (25 m × 0.25 mm I.D.) coated with CSP-3 using a 0.6% chloroform solution by the conventional dynamic method, and conditioned at 180°C with a helium stream, was used.

2.3. High-performance liquid chromatography

The experiments were carried out with a Shimadzu LC-5A high-performance liquid chromatograph equipped with a UV detector. A stainless-steel column (250 mm × 4.6 mm I.D.) was slurry packed with CSP-4 using a conven-

Table 1
Enantiomer separation by GC with CSP-3

Compound	T (°C)	t_R (min)	α
Pantolactone	110	14.7 (*S*)	1.102
Allethrolone	130	39.8 (*S*)	1.023
Methyl mandelate	90	57.0	1.035
cis-Chrysanthemic acid	120	63.0 (*S*)	1.027
trans-Chrysanthemic acid	120	70.6 (*R*)	1.027
2,2-Dimethylcyclopropanecarboxylic acid	100	23.3 (−)	1.060
2,2-Dimethylcyclopropanecarboxamide	120	12.7 (+)	1.079
O-Ethyl O-(3-trifluoromethylphenyl) N-isopropylphosphoramidothioate	120	75.3	1.032
Tetrahydrofurfuryl alcohol	60	12.9	1.070
3-Methoxy-1,2-propanediol	90	13.8	1.022

Carrier gas, helium; flow-rate, 0.8 ml/min.
T = column temperature; t_R = net retention time of first-eluted isomer; t_0 (dead time) = 1.4 min; α = separation factor.

tional technique. Other chemicals of analytical-reagent grade were purchased from Wako (Osaka, Japan). Some of the racemic compounds were kindly provided by Sumitomo Chemical (Osaka, Japan).

3. Results and discussion

The GC results are summarized in Table 1. Various racemic compounds were directly resolved on CSP-3 with a wide range of operating

Table 2
Enantiomer separation by HPLC with CSP-4

Compound	k'_1	α	Mobile phase[a]
Pantolactone	6.23	1.08 (+)	a
Allethrolone	9.25	1.03	b
Uniconaole	14.0	1.07 (−)	c
Diniconazole	9.25	1.09	c
trans-Chrysathemic acid	4.08	1.13 (*R*)	d
trans-3-(2,2-Dichlorovinyl)-2,2-cyclopropanecarboxylic acid	16.8	1.11	d
2-(4-Chlorophenyl)-3-methylbutylic acid	4.45	1.11	e
2-Phenoxypropionic acid	3.38	1.08	f
Mandelic acid	6.83	1.06	g
Ketamine	7.85	1.09	h
Salbutamol	5.84	1.06	i
Hexobarbital	4.06	1.13 (−)	f
Metoprolol	1.22	1.05	j
Phenylalanine	19.0	1.05	k
2-Pyrrolidone-5-carboxylic acid	14.3	1.09	l
O-Ethyl O-(2,4-dichlorophenyl) N-isopropylphosphoramidothioate	4.41	1.33	m
1,1′-Bi-2-naphthol	9.61	1.11	b

k'_1 = Capacity factor of first-eluted isomer; α = separation factor.
[a] Mobile phase: (a) hexane (H)–1,2-dichloroethane (D)–methanol (M) (100:20:1); (b) H–D–ethanol (E) (100:20:1); (c) H–D–E (200:20:1); (d) H–acetic acid (A) (1000:1); (e) H–D–E–A (490:9:5:1); (f) H–E–trifluoroacetic acid (T) (500:5:0.6); (g) H–D–M–T (400:90:10:1); (h) H–E–T (240:10:0.6); (i) H–D–M–T (240:150:15:1); (j) H–D–E–T (250:140:2.5:1); (k) H–D–M–T (250:220:10:1); (l) H–D–M–T (430:50:20:0.25); (m) H–E (500:1).

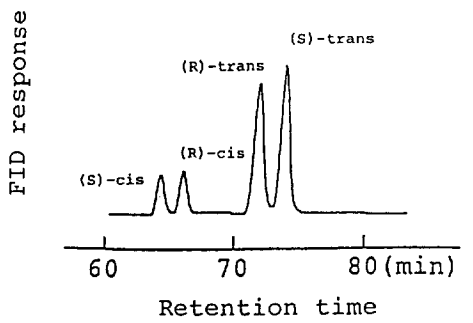


Fig. 1. GC separation of racemic *cis*–trans-chrysanthemic acid on a CSP-3 column. Chromatographic conditions as in Table 1.

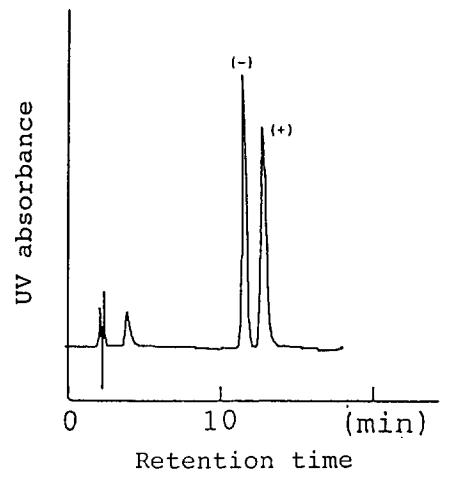


Fig. 2. HPLC separation of racemic hexobarbital on a CSP-4 column. Chromatographic conditions as in Table 2.

temperatures. A typical chromatogram is shown in Fig. 1. Four peaks of chrysanthemic acid were assigned using standard enantiomers given by Sumitomo Chemical.

The enantioselectivity and the operating temperature range of CSP-3 are comparable to those of CSP-2 [6], but it should be emphasized that CSP-3 is waxy at room temperature, is thermally highly stale and is soluble in many organic solvents. These properties can contribute to improving the efficiency and the durability of the capillary column.

The HPLC results are summarized in Table 2. The direct separation of underivatized racemic compounds, including alcohols, carboxylic acids, amines and amino alcohols, was accomplished using normal mobile phases. The addition of a small amount of acids and alcohols was effective in improving the peak shapes of carboxylic acids, amines and amino alcohols. A typical chromatogram is shown in Fig. 2.

As CSP-3 and CSP-4 contain the same chiral selector, these chromatographic results show that hydrogen bonding association can play an important role in chiral recognition in both GC and HPLC. The joint use of two CPSs is promising for the direct enantiomer separation of a wide range of racemic compounds.

Acknowledgement

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