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Elution orders in the separation of enantiomers by high-performance liquid chromatography with some chiral stationary phases

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ABSTRACT

Enantioselectivity and elution order in the separation of various racemic compounds by high-performance liquid chromatography with some urea derivatives containing two asymmetric carbon atoms attached to two nitrogen atoms of the urea group derived from (*S*)- or (*R*)-valine (Val) and (*S*)- or (*R*)-1-(α-naphthyl)ethylamine (NEA) as chiral stationary phases (CSPs) were investigated in order to explain the mechanism of enantiomer separation. The chromatographic results showed that two kinds of diastereometric interactions are produced and each of the two chiral centres may contribute to the chiral recognition. In the separation of racemic amino acid methyl esters, the Val component may control the chiral recognition for N-acetyl derivatives and the NEA component for N-3,5-dinitrobenzoyl derivatives. In the direct separation of various racemic alcohols and esters, the Val component may control mainly the chiral recognition. The NEA component may efficiently increase the enantioselectivity as shown in allethrolone, etc., but it may also decrease the enantioselectivity as shown in terallethrin, etc. We can assume that the overall enantioselectivity and elution order on these CSPs are determined by the combination of the structure effects and the chiral recognition mechanisms on two chiral components.

INTRODUCTION

In previous papers [1,2] we reported some urea derivatives of chiral amino acid and amines, such as N-(*tert.*-butylaminocarbonyl)-L-valylaminopropylsilica gel and (*R*)-1-(α-naphthyl)ethylaminocarbonylaminopropylsilica gel, were efficient for the separation of derivatives of racemic amino acid esters and amines. It was found that it is sufficient for a chiral stationary phase (CSP) to contain one asymmetric carbon atom attached to the nitrogen atom of the urea group in order to display enantioselectivity in its interaction with amide enantiomers.

During the course of our research to examine the effect of the structure of urea derivatives on enantioselectivity, we have found [3] that two novel CSPs derived from (*S*)- and (*R*)-1-(α-naphthyl)ethylamine (NEA) with (*S*)-valine (Val), which contain two asymmetric carbon atoms attached to two nitrogen atoms of the urea group, showed excellent enantioselectivity for the separation of various racemic compounds. The second chiral constituent improved the enantioselectivity of the urea derivatives, but the mechanism of separation with these phases had not been investigated.

In this study, we examined the enantioselectivity and elution order in separations by high-performance liquid chromatography (HPLC) with CSPs containing two asymmetric carbon atoms attached to the urea group in order to determine the relative contributions to the overall enantiomer separation of each of the two chiral centres in the urea derivatives.

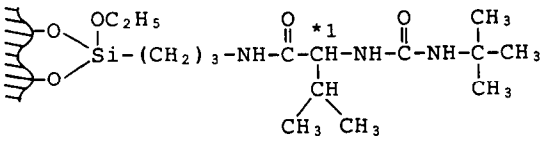
EXPERIMENTAL

Chiral stationary phases

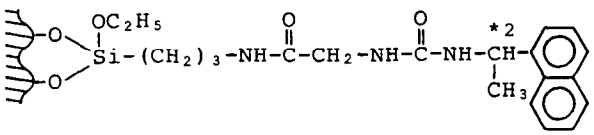
The structures of the CSPs used in this study are shown in Fig. 1. General procedures for the synthesis of CSPs derived from (*S*)- or (*R*)-Val and (*S*)- or (*R*)-NEA were given in previous papers [1–3]. The CSPs were obtained starting from γ-aminopropylsilica gel [Develosil-NH_2, 5 μm (Nomura Chemical, Seto, Japan) and LiChrosorb-NH_2, 5 μm (E. Merck, Darmstadt, F.R.G.)]. Grafting rates were calculated according to the C and N elemental analysis for each CSP: **1a** (0.50 mmol/g), **1b** (0.48 mmol/g), **2a** (0.53 mmol/g), **2b** (0.50 mmol/g), **3a** (0.37 mmol/g), **3b** (0.37 mmol/g), **4a** (0.41 mmol/g), **4b** (0.40 mmol/g).

Liquid chromatography

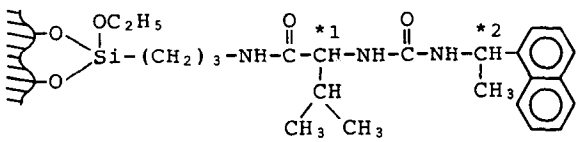
The experiments were carried out using a Waters Assoc. 510 high-performance liquid chromatograph equipped with a variable-wavelength UV detector operated at 230 and 254 nm. Stainless-steel columns (250 × 4 mm I.D.) were slurry packed using a conventional technique. The chromatographic conditions are given in Tables I–IV. Elution orders were determined by successive injection of racemic and enriched mixtures (in the *S* or *R* isomer) of test solutes.



*1
(S): CSP 1a
(R): CSP 1b



*2
(S): CSP 2a
(R): CSP 2b



*1 *2
(S),(S): CSP 3a
(R),(R): CSP 3b
(S),(R): CSP 4a
(R),(S): CSP 4b

Fig. 1. Structures of the CSPs.

Mobile phase

n-Hexane, 1,2-dichloroethane, ethanol and acetic acid of analytical-reagent grade were purchased from Wako (Osaka, Japan).

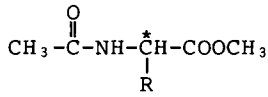
RESULTS AND DISCUSSION

The structures of the N-acetyl-(AC) and N-3,5-dinitrobenzoyl-(DNB) amino acid methyl esters, DNB-amines, O-3,5-dinitrophenylurethane (DNPU) derivatives of hydroxy acid methyl esters and DNPU derivatives of alcohols used are shown in Fig. 2. These derivatives were prepared in our laboratory [4,5]. The structures of various racemic compounds used for the direct separation are shown in Fig. 3. These compounds were kindly provided by Sumitomo Chemical (Osaka, Japan). HPLC results are summarized in Tables I–IV and typical chromatograms are shown in Figs. 4–7. The separation factor of the enantiomers, α, is the ratio of their capacity factors and k'_1 is the capacity factor for the initially eluted enantiomer.

As the configuration of CSPs **1b–4b** is opposite stereochemically to that of CSPs **1a–4a**, the results that inversion of elution orders and nearly identical magnitudes of the separation factors are observed between these CSPs are reasonable. As shown in Table I, in the separation of racemic AC-amino acid methyl esters the retention of *S* isomers was longer than that of *R* isomers on CSP **1a**, which contains (*S*)-Val as the chiral component, showing that the association between the *S* isomer and CSP **1a** was the more stable. It is natural that the retention of *R* isomers was longer on CSP **1b**, which contains (*R*)-Val. These separations may depend entirely on hydrogen bonding association and involve no other stronger complexations [2]. Racemic AC-amino acid methyl esters were hardly separated on CSPs **2a** and **2b**, indicating that the NEA component is insufficient for the separation of these enantiomers depending on the diastereomeric hydrogen bonding association.

The results that the retention of *S* isomers was longer than that of *R* isomers on CSPs **3a** and **4a** derived from (*S*)-Val with (*S*)- and (*R*)-NEA and the opposite elution order was obtained on CSPs **3b** and **4b** derived from (*R*)-Val with (*R*)- and (*S*)-NEA suggest that the chiral recognition may be controlled by the Val component in these separations.

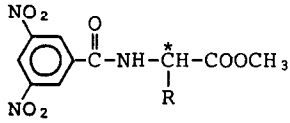
DNB-amino acid methyl esters were resolved not only with CSPs **1a** and **1b** but also with **2a** and **2b**, as shown in Table II. The elution orders of DNB- and AC-amino acid methyl esters on CSPs **1a** and **1b** are the same, but the separation factors of the DNB derivatives are smaller than those of the AC derivatives. This result suggests that AC derivatives are convenient stereochemically for the association with CSPs **1a** and **1b** by hydrogen bonding. On the other hand, DNB derivatives may easily associate with CSPs **2a** and **2b** by the combination of the π–π donor–acceptor interaction and hydrogen bonding. It was noted that the elution orders of AC- and DNB-amino acid methyl esters were clearly different on CSPs containing two asymmetric carbon atoms. Inversion of the elution orders was found in DNB derivatives on CSPs **3a** and **4a**, and also on CSPs **3b** and **4b**. The results that the retention of *S* isomers was longer than that of *R* isomers on CSPs **3a** and **4b** derived from (*S*)-NEA with (*S*)- and (*R*)-Val and the opposite elution order was obtained on CSPs **3b** and **4a** derived from (*R*)-NEA with (*S*)- and (*R*)-Val suggest that the NEA component mainly contributes to the chiral recognition in these separations.

(1) 

R=-CH_3 : (1a), (2a)

R=-CH() : (1b), (2b)

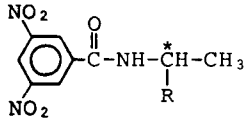
R=-CH_2-CH() : (1c), (2c)

(2) 

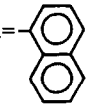
R=-CH_2-CH_2-S-CH_3 : (1d), (2d)

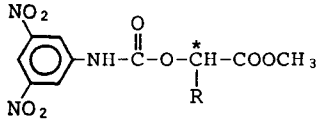
R=  : (1e), (2e)

R=-CH_2-  : (1f), (2f)

(3) 

R=  : (3a)

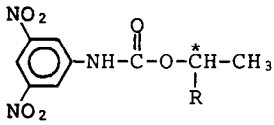
R=  : (3b)

(4) 

R=-CH_3 : (4a)

R=-CH_2-$COOCH_3$: (4b)

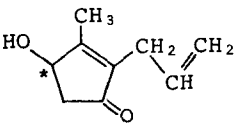
R=  : (4c)

(5) 

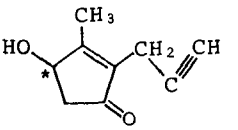
R=-$(CH_2)_5$-CH_3 : (5a)

R=  : (5b)

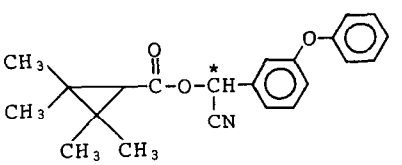
Fig. 2. Structures of N-acetyl-(AC) amino acid methyl esters (**1**), N-3,5-dinitrobenzoyl-(DNB) amino acid methyl esters (**2**), N-DNB-amines (**3**), O-3,5-dinitrophenylurethane (DNPU) derivatives of hydroxy acid methyl esters (**4**) and O-DNPU derivatives of alcohols (**5**).



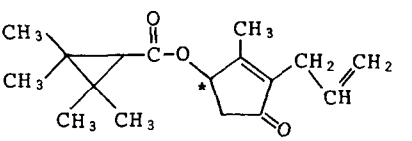
(a)



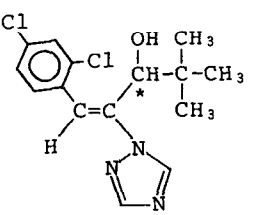
(b)



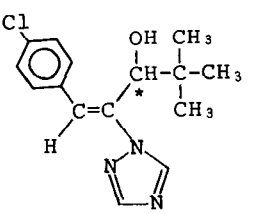
(c)



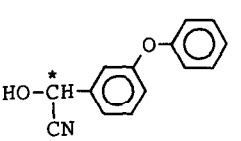
(d)



(e)



(f)



(g)

Fig. 3. Structures of various racemic compounds: allethrolone (**a**), propargyllone (**b**), fenpropathrin (**c**), terallethrin (**d**), diniconazole (**e**) uniconazole (**f**) and α-cyano-3-phenoxybenzyl alcohol (**g**).

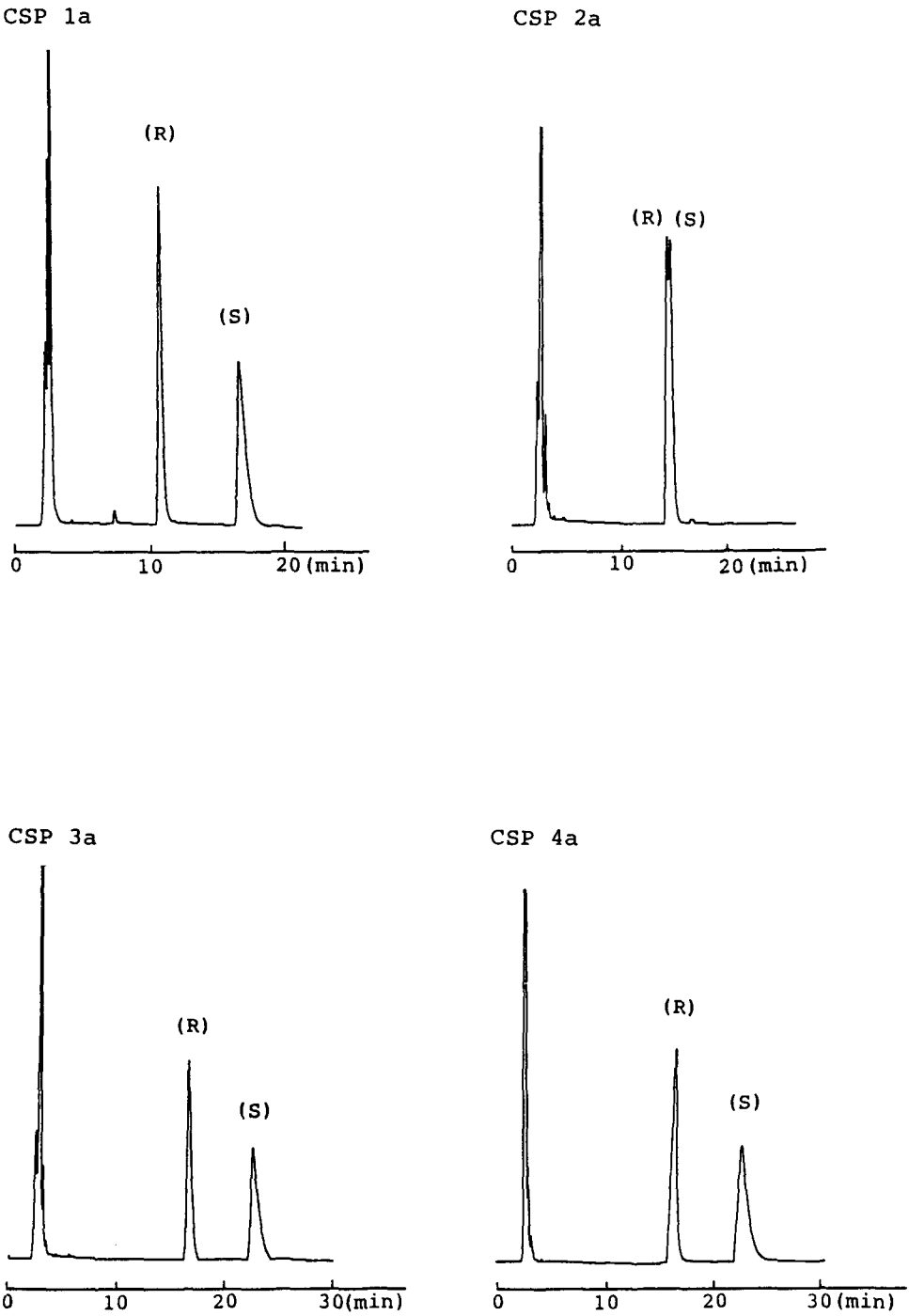


Fig. 4. Enantiomer separation of racemic N-acetylvaline methyl esters. Chromatographic conditions as in Table I.

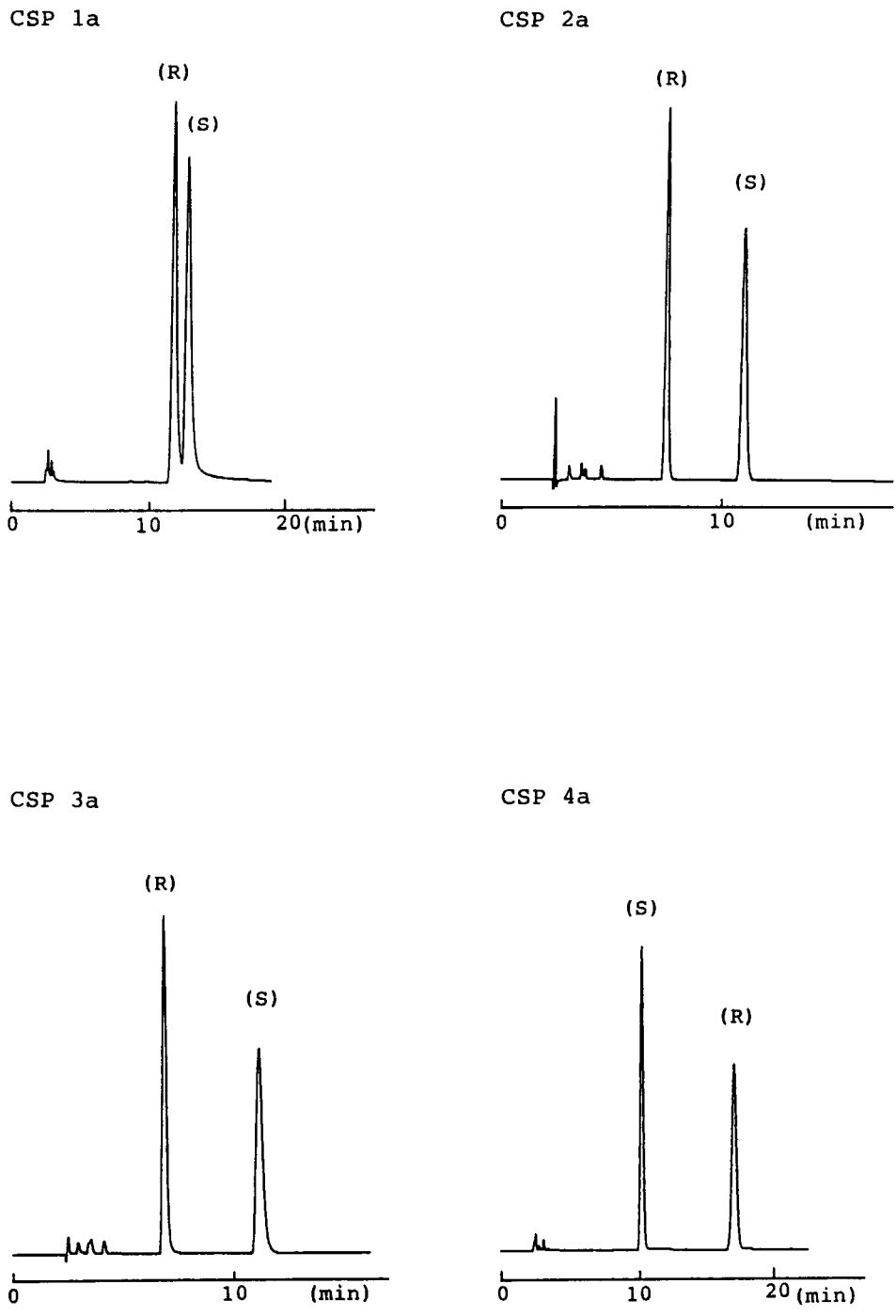


Fig. 5. Enantiomer separation of racemic N-3,5-dinitrobenzoylvaline methyl esters. Chromatographic conditions as in Table II.

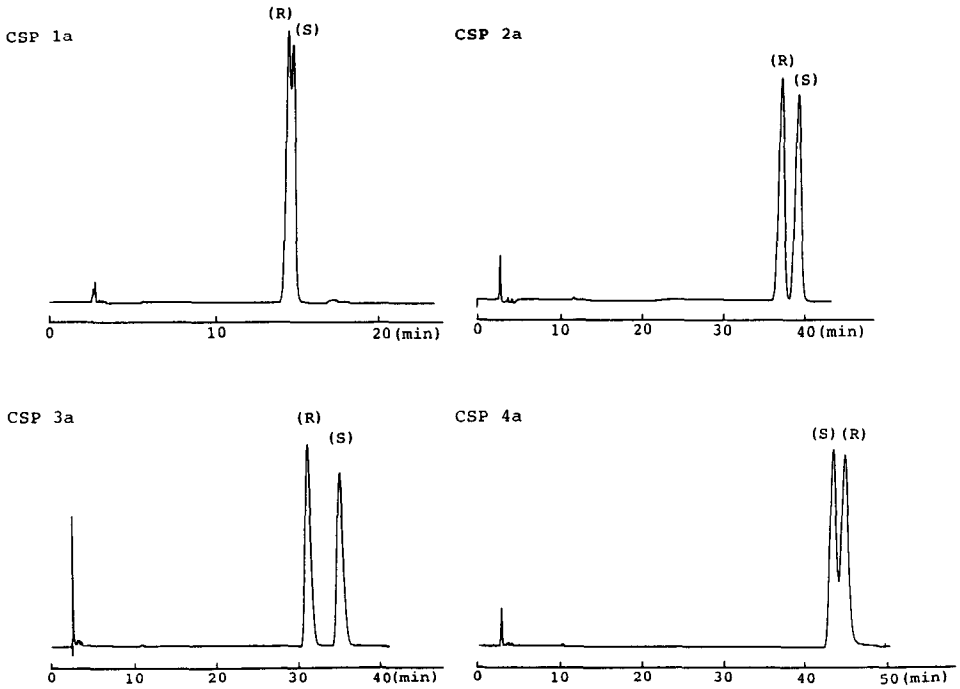


Fig. 6. Enantiomer separation of O-3,5-dinitrophenylurethane derivatives of racemic 2-octanol. Chromatographic conditions as in Table III.

In Table II the abnormal behaviour of DNB-phenylglycine methyl ester and DNB-phenylalanine methyl ester remains unclear, but it is assumed that the structure effect of the phenyl group may be introduced into the chiral recognition mechanism.

Similar elution orders were observed in the enantiomer separation of DNB-amines, DNPU-hydroxy acid methyl esters and DNPU alcohols, as shown in Table III. As these derivatives contain the 3,5-dinitrophenyl group, which can act as a π-acid, the π–π interaction may play an important role in the formation of the diastereomeric association complexes. The NEA component, which can act as a π-base in CSPs **3a**, **3b**, **4a** and **4b** may contribute to the chiral recognition in the separation of these derivatives and in the enantiomer separation of DNB-amino acid methyl esters.

In Table IV, it is emphasized that racemic allethrolone and propargyllone are well resolved directly with CSPs **3a**, **3b**, **4a** and **4b**, although these compounds were hardly resolved with CSPs **1a**, **1b**, **2a** and **2b**. These results show that the second chiral constituent in CSPs, which contain two chiral centres, may efficiently improve the enantioselectivity. Judging from the fact that the same elution order (*S,R*) is obtained on CSPs **3a** and **4a**, and *R,S* on CSP **3b** and **4b**, and also in the separation of AC-amino acid methyl ester enantiomers, the chiral recognition may be controlled by the Val component, and the NEA component may contribute to improve the enantioselectivity. The small difference between separation factors on CSPs **3a** and **3b** and those on CSPs **4a** and **4b** may depend on the combination of the configuration on two chiral components.

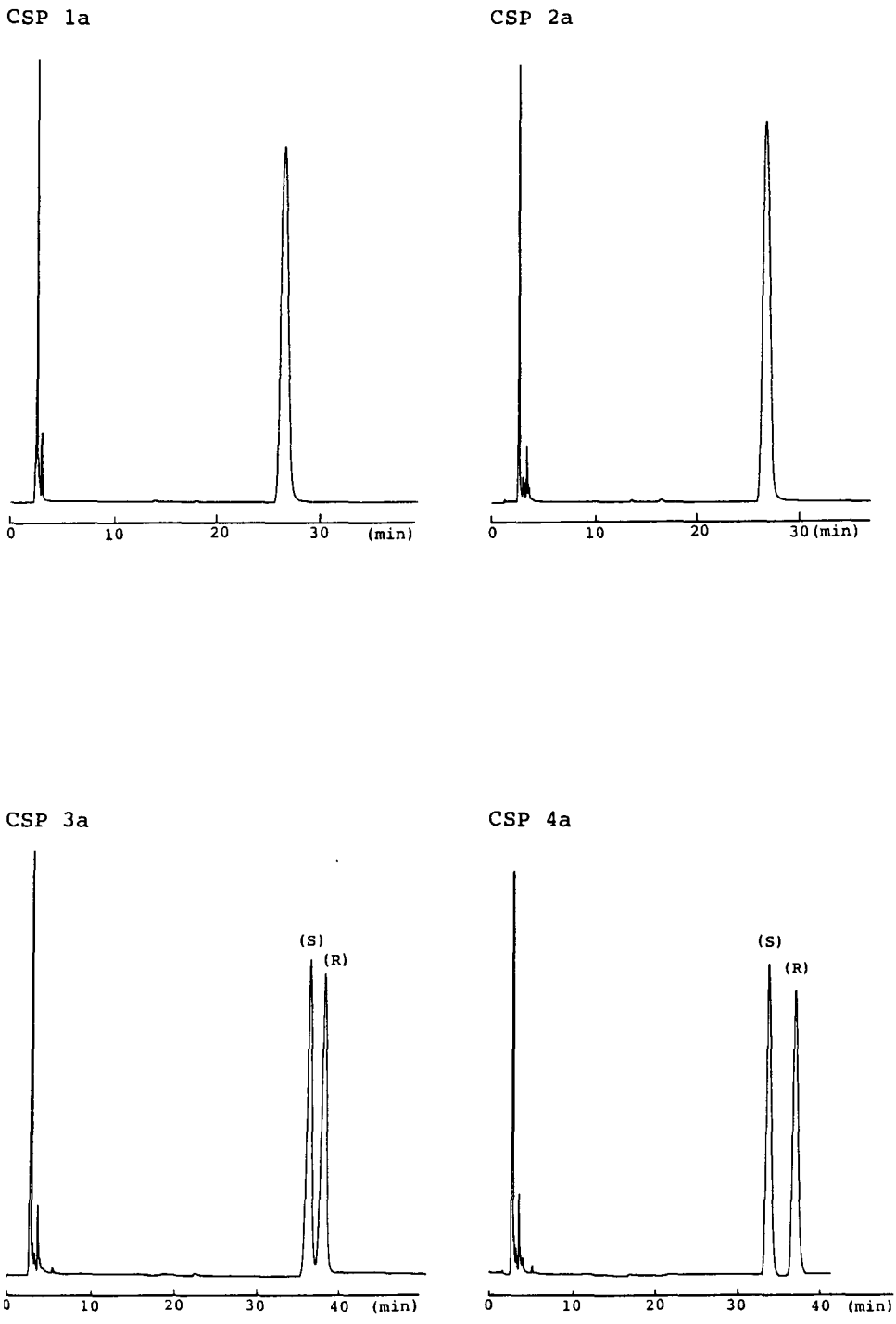


Fig. 7. Enantiomer separation of racemic allethrolone. Chromatographic conditions as in Table IV.

TABLE I

ENANTIOSELECTIVITIES AND ELUTION ORDERS OF N-ACETYLAMINO ACID METHYL ESTERS

Mobile phase (M): (A) *n*-hexane–1,2-dichloroethane–ethanol (40:10:1); (B) *n*-hexane–1,2-dichloroethane–ethanol (100:20:1). A flow-rate of 1.0 ml/min was used with a 250 × 4 mm I.D. column at room temperature.

N-AC-amino acid methyl ester[a]		CSP **1a**				CSP **1b**				CSP **2a**				CSP **2b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
1a	Alanine	2.72	1.24	*R,S*	A	2.82	1.27	*S,R*	A	3.20	1.00		A	3.94	1.00		A
1b	Valine	3.19	1.73	*R,S*	B	3.76	1.73	*S,R*	B	4.82	1.02	*R,S*	B	5.80	1.04	*S,R*	B
1c	Leucine	4.65	1.99	*R,S*	B	4.03	1.89	*S,R*	B	4.86	1.00		B	6.36	1.00		B
1d	Methionine	1.91	1.48	*R,S*	A	2.03	1.57	*S,R*	A	2.76	1.00		A	3.63	1.00		A
1e	Phenylglycine	3.71	1.39	*R,S*	B	3.99	1.39	*S,R*	B	4.92	1.00		B	6.98	1.00		B
1f	Phenylalanine	3.01	1.66	*R,S*	B	3.50	1.74	*S,R*	B	4.43	1.00		B	6.55	1.00		B
		CSP **3a**				CSP **3b**				CSP **4a**				CSP **4b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
1a	Alanine	3.67	1.17	*R,S*	A	3.90	1.21	*S,R*	A	3.87	1.16	*R,S*	A	3.35	1.17	*S,R*	A
1b	Valine	5.74	1.43	*R,S*	B	5.53	1.42	*S,R*	B	5.70	1.47	*R,S*	B	4.49	1.41	*S,R*	B
1c	Leucine	6.59	1.46	*R,S*	B	6.24	1.43	*S,R*	B	5.17	1.74	*R,S*	B	4.16	1.71	*S,R*	B
1d	Methionine	2.85	1.30	*R,S*	A	3.20	1.26	*S,R*	A	2.86	1.44	*R,S*	A	2.55	1.35	*S,R*	A
1e	Phenylglycine	5.87	1.20	*R,S*	B	5.96	1.20	*S,R*	B	4.95	1.30	*R,S*	B	4.84	1.28	*S,R*	B
1f	Phenylalanine	5.52	1.33	*R,S*	B	5.49	1.31	*S,R*	B	5.07	1.56	*R,S*	B	4.26	1.53	*S,R*	B

[a] See Fig. 2.

TABLE II

ENANTIOSELECTIVITIES AND ELUTION ORDERS OF N-3,5-DINITROBENZOYLAMINO ACID METHYL ESTERS

Mobile phase (M): (A) *n*-hexane–1,2-dichloroethane–ethanol (200:20:1); (B) *n*-hexane–1,2-dichloroethane–ethanol (40:10:1). A flow-rate of 1.0 ml/min was used with a 250 × 4 mm I.D. column at room temperature.

N-DNB-amino acid methyl ester[a]		CSP **1a**				CSP **1b**				CSP **2a**				CSP **2b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
2a	Alanine	12.66	1.11	*R,S*	A	11.91	1.09	*S,R*	A	5.27	1.69	*R,S*	B	7.25	1.71	*S,R*	B
2b	Valine	3.34	1.11	*R,S*	A	4.01	1.07	*S,R*	A	2.04	1.79	*R,S*	B	3.23	1.85	*S,R*	B
2c	Leucine	0.69	1.16	*R,S*	B	0.53	1.19	*S,R*	B	2.96	1.45	*R,S*	B	4.14	1.48	*S,R*	B
2d	Methionine	0.86	1.06	*R,S*	B	0.75	1.09	*S,R*	B	4.68	1.68	*R,S*	B	7.12	1.74	*S,R*	B
2e	Phenylglycine	4.63	1.00		A	4.38	1.00		A	4.85	1.11	*S,R*	B	6.82	1.09	*R,S*	B
2f	Phenylalanine	0.52	1.08	*R,S*	B	0.56	1.07	*S,R*	B	4.34	1.25	*R,S*	B	6.89	1.27	*S,R*	B
		CSP **3a**				CSP **3b**				CSP **4a**				CSP **4b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
2a	Alanine	4.50	2.19	*R,S*	B	5.57	2.07	*S,R*	B	6.22	1.73	*S,R*	B	6.14	1.81	*R,S*	B
2b	Valine	1.74	1.98	*R,S*	B	2.06	1.99	*S,R*	B	3.04	1.87	*S,R*	B	2.64	1.92	*R,S*	B
2c	Leucine	2.04	1.54	*R,S*	B	2.28	1.56	*S,R*	B	4.78	1.26	*S,R*	B	4.20	1.28	*R,S*	B
2d	Methionine	4.20	2.04	*R,S*	B	4.72	2.13	*S,R*	B	7.35	1.63	*S,R*	B	6.54	1.61	*R,S*	B
2e	Phenylglycine	3.44	1.29	*S,R*	B	4.02	1.30	*R,S*	B	6.04	1.30	*R,S*	B	5.66	1.25	*S,R*	B
2f	Phenylalanine	3.63	1.33	*R,S*	B	4.26	1.35	*S,R*	B	7.82	1.03	*R,S*	B	6.76	1.04	*S,R*	B

[a] See Fig. 2.

TABLE III

ENANTIOSELECTIVITIES AND ELUTION ORDERS OF DERIVATIVES OF AMINES, HYDROXY ACIDS AND ALCOHOLS

Mobile phase (M): (A) *n*-hexane–1,2-dichloroethane–ethanol (200:20:1); (B) *n*-hexane–1,2-dichloroethane–ethanol (80:20:3). (C) *n*-hexane–1,2-dichloroethane–ethanol (50:15:1); (D) *n*-hexane–1,2-dichloroethane–ethanol (100:20:1). A flow-rate of 1.0 ml/min was used with a 250 × 4 mm I.D. column at room temperature.

Solute[a]		CSP 1a				CSP 1b				CSP 2a				CSP 2b			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
3a	1-Phenylethylamine[b]	10.92	1.05	*R,S*	A	11.22	1.04	*S,R*	A	3.72	1.93	*R,S*	B	7.00	1.91	*S,R*	B
3b	1-(α-Naphthyl)-ethylamine[b]	10.45	1.10	*R,S*	A	9.33	1.11	*S,R*	A	3.89	2.85	*R,S*	B	6.57	3.07	*S,R*	B
4a	Lactic acid[c]	1.36	1.80	*R,S*	C	1.84	2.01	*S,R*	C	4.09	1.29	*R,S*	C	9.34	1.34	*S,R*	C
4b	Malic acid[c]	1.51	1.32	*R,S*	C	2.14	1.38	*S,R*	C	6.76	1.09	*R,S*	C	16.73	1.12	*S,R*	C
4c	Mandelic acid[c]	0.89	1.48	*R,S*	C	1.25	1.51	*S,R*	C	3.96	1.22	*R,S*	C	9.99	1.30	*S,R*	C
5a	2-Octanol[d]	4.06	1.02	*R,S*	A	4.66	1.05	*S,R*	A	12.19	1.06	*R,S*	A	28.99	1.06	*S,R*	A
5b	1-Phenylethanol[d]	2.75	1.03	*R,S*	D	2.96	1.05	*S,R*	D	10.16	1.16	*R,S*	D	22.35	1.22	*S,R*	D
		CSP 3a				CSP 3b				CSP 4a				CSP 4b			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
3a	1-Phenylethylamine[b]	2.28	1.96	*R,S*	B	3.29	2.02	*S,R*	B	3.37	2.50	*S,R*	B	4.23	2.41	*R,S*	B
3b	1-(α-Naphthyl)-ethylamine[b]	1.92	3.64	*R,S*	B	2.66	4.06	*S,R*	B	3.29	4.20	*S,R*	B	3.75	4.12	*R,S*	B
4a	Lactic acid[c]	3.41	1.83	*R,S*	C	4.47	1.87	*S,R*	C	4.58	1.07	*S,R*	C	7.84	1.08	*R,S*	C
4b	Malic acid[c]	4.99	1.23	*R,S*	C	8.44	1.27	*S,R*	C	5.32	1.06	*S,R*	C	9.53	1.08	*R,S*	C
4c	Mandelic acid[c]	3.27	1.50	*R,S*	C	4.46	1.60	*S,R*	C	3.90	1.34	*S,R*	C	6.31	1.36	*R,S*	C
5a	2-Octanol[d]	11.88	1.14	*R,S*	A	15.42	1.13	*S,R*	A	14.67	1.04	*S,R*	A	18.96	1.04	*R,S*	A
5b	1-Phenylethanol[d]	9.60	1.49	*R,S*	D	11.01	1.49	*S,R*	D	9.27	1.29	*S,R*	D	13.02	1.30	*R,S*	D

[a] See Fig. 2.
[b] Resolved as N-3,5-dinitrobenzoyl derivatives.
[c] Resolved as O-3,5-dinitrophenylurethane O-methyl ester derivatives.
[d] Resolved as O-3,5-dinitrophenylurethane derivatives.

TABLE IV

ENANTIOSELECTIVITIES AND ELUTION ORDERS OF VARIOUS RACEMIC COMPOUNDS

Mobile phase (M): (A) *n*-hexane–1,2-dichloroethane–ethanol (100:20:1); (B) *n*-hexane–1,2-dichloroethane–ethanol (500:10:0.05). (C) *n*-hexane–1,2-dichloroethane–ethanol (500:30:0.15); (D) *n*-hexane–1,2-dichloroethane–ethanol–acetic acid (500:150:5:0.6). A flow-rate of 1.0 ml/min was used with a 250 × 4 mm I.D. column at room temperature.

Solute[a]		CSP **1a**				CSP **1b**				CSP **2a**				CSP **2b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
a	Allethrolone	10.44	1.00		A	11.23	1.00		A	8.57	1.00		A	14.21	1.03	*S,R*	A
b	Propargyllone	14.00	1.00		A	15.93	1.00		A	12.31	1.00		A	23.00	1.03	*S,R*	A
c	Fenpropathrin	1.28	1.14	*R,S*	B	1.40	1.19	*S,R*	B	4.07	1.00		B	4.14	1.00		B
d	Terallethrin	2.41	1.17	*S,R*	C	1.88	1.22	*R,S*	C	7.20	1.00		C	3.26	1.04	*R,S*	C
e	Diniconazole	3.04	1.02	*R,S*	A	2.81	1.04	*S,R*	A	3.19	1.02	*S,R*	A	6.63	1.04	*R,S*	A
f	Uniconazole	4.45	1.05	*R,S*	A	3.96	1.08	*S,R*	A	4.21	1.00		A	8.58	1.00		A
g	α-Cyano-3-phenoxy-benzyl alcohol	2.93	1.03	*R,S*	D	3.67	1.02	*S,R*	D	4.11	1.04	*S,R*	D	8.90	1.04	*R,S*	D
		CSP **3a**				CSP **3b**				CSP **4a**				CSP **4b**			
		k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M	k'_1	α	Elution order	M
a	Allethrolone	12.21	1.05	*S,R*	A	12.89	1.05	*R,S*	A	11.86	1.10	*S,R*	A	13.58	1.11	*R,S*	A
b	Propargyllone	15.69	1.04	*S,R*	A	19.44	1.04	*R,S*	A	17.19	1.09	*S,R*	A	20.34	1.09	*R,S*	A
c	Fenpropathrin	4.22	1.11	*R,S*	B	3.88	1.10	*S,R*	B	3.03	1.04	*R,S*	B	1.66	1.05	*S,R*	B
d	Terallethrin	5.32	1.16	*S,R*	C	6.11	1.16	*R,S*	C	3.22	1.00		C	3.06	1.00		C
e	Diniconazole	3.99	1.22	*R,S*	A	4.73	1.17	*S,R*	A	3.32	1.27	*R,S*	A	3.22	1.26	*S,R*	A
f	Uniconazole	5.45	1.15	*R,S*	A	6.11	1.12	*S,R*	A	5.04	1.18	*R,S*	A	4.49	1.17	*S,R*	A
g	α-Cyano-3-phenoxy-benzyl alcohol	4.80	1.00		D	6.26	1.00		D	4.46	1.08	*R,S*	D	5.80	1.08	*S,R*	D

[a] See Fig. 3.

Racemic fenpropathrin and terallethrin were well resolved on CSPs **1a** and **1b**, but hardly resolved on CSPs **2a** and **2b**, in addition to racemic AC-amino acid methyl esters. Moreover, the same elution orders were obtained on CSPs **1a**, **3a** and **4a**, containing an (*S*)-Val component, and on CSPs **1b**, **3b** and **4b**, containing an (*R*)-Val component. These results show the Val component may control the chiral recognition in the separation of these compounds. The separation factors of fenpropathrin were very small on CSPs **4a** and **4b** and terallethrin was not resolved on CSPs **4a** and **4b**. Moreover, the separation factors of these compounds on CSPs **3a** and **3b** were smaller than those on CSPs **1a** and **1b**. These results suggest that the NEA component may decrease the enantioselectivity, and its structure effect may depend on the combination of the configuration.

In the separation of racemic diniconazole and uniconazole, the same elution order (*R, S*) was found on CSPs **1a**, **3a** and **4a**, and *S, R* on CSPs **1b**, **3b** and **4b**. Again, it is suggested the Val component may control the chiral recognition. However, it was noticed in the separation of these compounds that larger separation factors were obtained on CSPs **4a** and **4b** than on **3a** and **3b**, in contrast to the results with racemic fenpropathrin and terallethrin. In order to rationalize these results, the elution orders of racemic diniconazole on CSPs containing one chiral centre, *R, S* on CSP **1a**, *S, R* on CSP **1b**, *S, R* on CSP **2a** and *R, S* on CSP **2b**, offer helpful suggestions. We can assume that the enantioselectivity may be increased by the combinations of two chiral recognition mechanisms working in same stereochemical senses on two chiral components, and decreased by the combination of two mechanisms working in opposite stereochemical senses. For the enantiomer separation of diniconazole, the *S–S* or *R–R* configuration of the CSP may decrease the enantioselectivity and the *S–R* or *R–S* configuration of the CSP may increase the enantioselectivity.

In the separation of racemic α-cyano-3-phenoxybenzyl alcohol, very different enantioselectivity was found. A good separation was achieved on CSP **4a** or **4b**, but surprisingly no separation on CSP **3a** or **3b**. This result can also be rationalized by the above assumption. As the elution orders for this compound are *R, S* on CSP **1a**, *S, R* on CSP **1b**, *S, R* on CSP **2a** and *R, S* on CSP **2b**, the enantioselectivity may be increased in CSP **4a** and **4b** (*S–R* and *R–S* configuration) and decreased in CSP **3a** and **3b** (*S–S* and *R–R* configuration).

It was difficult to determine k' values from these results, as we prepared the chiral stationary phases using different aminopropylsilanized silica gels. However, both the retention mechanism and the chiral recognition mechanism are very important and interesting, and we intend to make further investigations of these mechanisms.

CONCLUSION

The enantioselectivity and the elution order in the HPLC separation of various racemic compounds with urea derivatives containing two asymmetric carbon atoms as CSPs were investigated, and it was demonstrated that the elution order and the magnitude of the separation factors can offer helpful suggestions for rationalizing the mechanism of enantiomer separation.

The chromatographic results indicate that two kinds of diastereomeric interactions are produced and each of the two chiral centres may contribute to the chiral

recognition. We can assume that the overall enantioselectivity and elution order on these CSPs are determined by a combination of the structure effects and the chiral recognition mechanisms on two chiral components.

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