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Direct separation of carboxylic acid enantiomers by high-performance liquid chromatography with amide and urea derivatives bonded to silica gel as chiral stationary phases

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Abstract

For the separation of carboxylic acid enantiomers by HPLC, the chromatographic properties of four chiral amide and urea derivatives [N-3,5-dinitrobenzoyl-D-1-(α-naphthyl)glycine, 3,5-dinitrophenylaminocarbonyl-D-phenylglycine, 3,5-dinitrophenylaminocarbonyl-L-valine and 3,5-dinitrophenylaminocarbonyl-L-*tert.*-leucine] covalently bonded to 3-aminopropylsilanized silica gel as chiral stationary phases were examined. Direct separation of various carboxylic acid enantiomers was accomplished with these chiral stationary phases. The influence of the composition of the mobile phase in these enantiomer separations was shown. Amino acid enantiomers were also well resolved in the form of their derivatives containing a free carboxylic acid group, such as *tert.*-butoxycarbonyl (t-BOC), benzyloxycarbonyl (Z), 9-fluorenylmethoxycarbonyl (FMOC) and 5-dimethylamino-1-naphthalenesulfonyl (dansyl) derivatives.

1. Introduction

Carboxylic acids are one of the most important classes of chiral compounds, and it is well known that high-performance liquid chromatography (HPLC) with chiral stationary phases (CSPs) is useful for the separation of carboxylic acid enantiomers. Derivatives of cyclodextrin, cellulose and protein have been used as CSPs for this purpose [1–3]. Brush-type CSPs are unsuitable for the direct separation of racemic carboxylic acids and these compounds have usually been resolved in the form of amide derivatives [4,5]. We have found [6] that the direct separation of some aromatic carboxylic acids was possible with N-3,5-dinitrobenzoyl-D-phenylglycine bonded to silica gel (I), which is a typical brush-type CSP developed by Pirkle and Finn [7], but its enantioselectivity was inadequate. Recently, Pirkle and Welch [8] reported the excellent enantiomer separation of naproxen and other anti-inflammatory drugs with an improved brush-type chiral stationary phase using normal mobile phases, although the separation of aliphatic carboxylic acids was not described.

In this paper, we report the direct separation of a variety of carboxylic acids, both aromatic and aliphatic, with brush-type CSPs II–V (Fig. 1) using aqueous mobile phases. CSP II is a modification of phase I. We previously reported [9] that N-3,5-dinitrobenzoyl-D-1-(α-naphthyl)gly-

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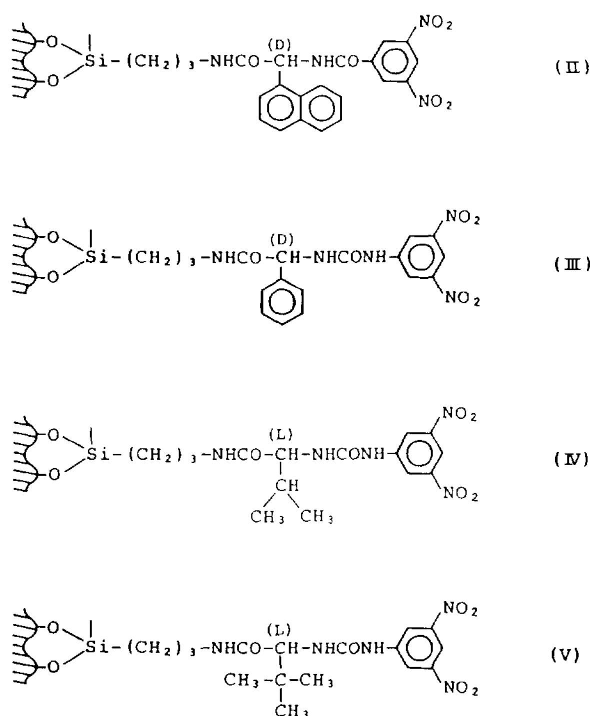

Fig. 1. Structures of CSPs.

cine ionically bonded to 3-aminopropylsilanized silica showed superior enantioselectivity to phase I in the separation of alcohol and ester racemates, but this phase was unsuitable for use under reversed-phase conditions. Therefore, we have prepared N-3,5-dinitrobenzoyl-D-1-(α-naphthyl)glycine covalently bonded to 3-aminopropylsilanized silica (II) for the separation of racemic carboxylic acids using aqueous mobile phases. CSP III is also a modification of phase I. We found previously [5,10] that various urea derivatives of amino acids bonded to silica gel were excellent CSPs for many racemic compounds, and this result showed that the urea group attached to the asymmetric carbon atom was effective in diastereomeric association for chiral recognition. Accordingly, we prepared a urea derivative (III) which contains the urea group instead of the amide group in phase I. Similar N-3,5-dinitrophenylurea derivatives (IV and V) derived from L-valine and L-*tert.*-leucine were also prepared. These CSPs are now commercially available as Sumichiral OA-2500 (CSP II), OA-3300 (CSP III), OA-3100 (CSP IV) and OA-3200 (CSP V).

2. Experimental

2.1. Chiral stationary phases

To prepare CSP II, N-3,5-dinitrobenzoyl-D-1-(α-naphthyl)glycine was synthesized by the procedure described previously [9]. It was covalently bonded to 3-aminopropylsilanized silica by the reported procedure [5].

CSP III was prepared using D-phenylglycine and 3,5-dinitrophenyl isocyanate instead of L-valine and alkyl isocyanate in the preparation of alkylurea derivatives of L-valine bonded to silica gel reported previously by paper [10]. 3,5-Dinitrophenyl isocyanate was obtained from 3,5-dinitroaniline (Aldrich, Milwaukee, WI, USA) by the reaction with phosgene.

CSPs IV and V were prepared as for CSP III but using L-valine and L-*tert.*-leucine, respectively, instead of D-phenylglycine.

Develosil-NH_2 (5 μm) (Nomura Chemical, Seto, Japan) was used as the starting silica gel. Grafting rates were calculated according to the C and N elemental analyses for each CSP: CSP II 0.42, CSP III 0.42, CSP IV 0.44 and CSP V 0.38 mmol/g.

2.2. Liquid chromatography

Stainless-steel columns (250 × 4.6 mm I.D.) were slurry packed with CSPs II–V using a conventional technique.

The experiments were carried out using a Waters Model 510 high-performance liquid chromatograph equipped with a variable-wavelength UV detector, operated at 230 and 254 nm. Solutes and solvents of analytical-reagent grade were purchased from Wako (Osaka, Japan). The structures of the racemic solutes used are shown in Fig. 2. Some compounds were kindly provided by Sumitomo Chemical (Osaka, Japan).

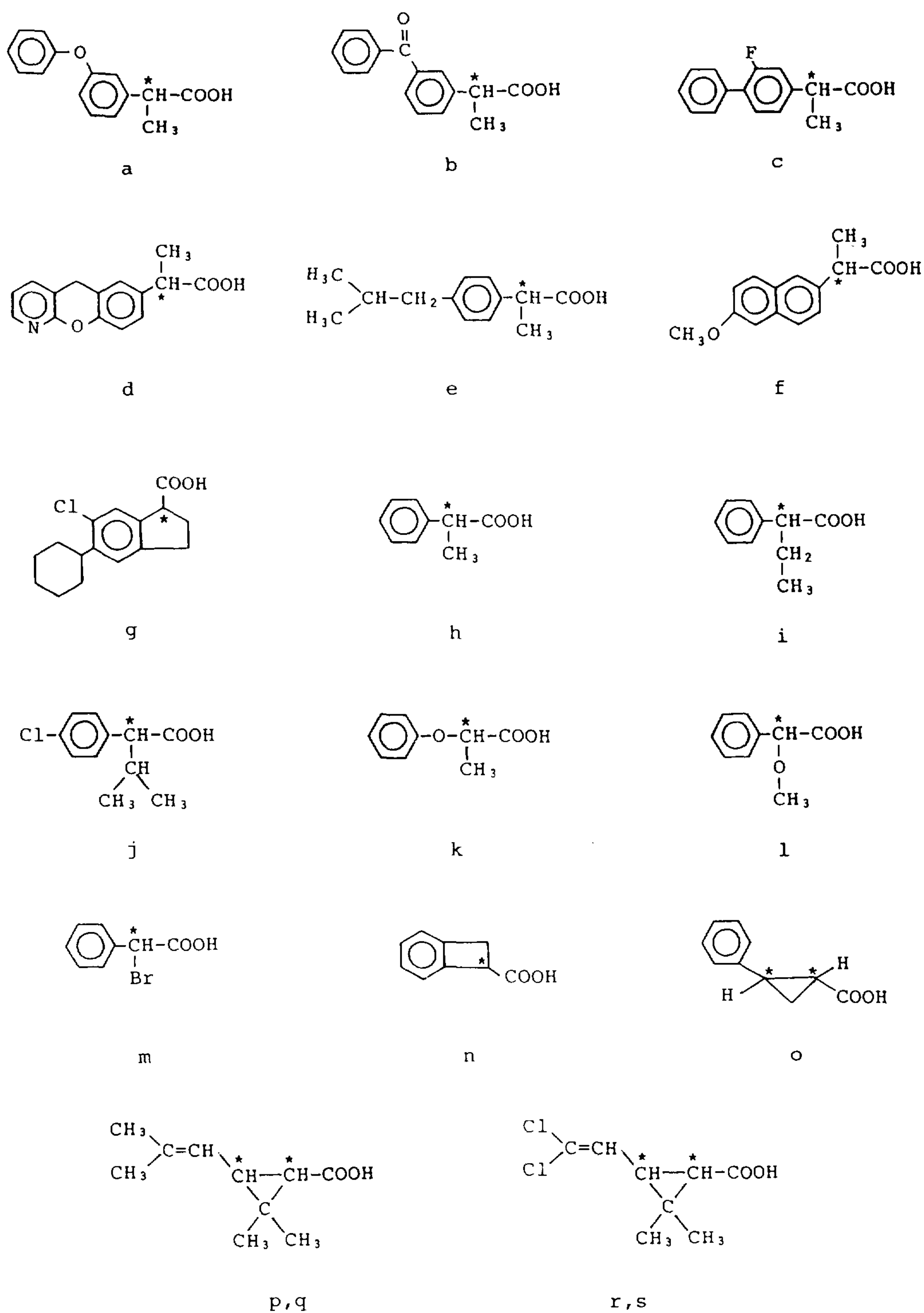

Fig. 2. Structures of racemic solutes.

3. Results and discussion

The chromatographic results are summarized in Table 1. A number of carboxylic acid enantiomers including pharmaceutical and agrochemical compounds were resolved directly with these CSPs under the reversed-phase conditions. Typical chromatograms of aromatic and aliphatic carboxylic acids are shown in Figs. 3 and 4.

Phase II shows superior enantioselectivity to phase I. This result showed that the naphthyl group of phase II plays a more effective role than the phenyl group of phase I for chiral recognition in racemic carboxylic acids and also in alcohol and ester enantiomers [9]. Phases III–V show very specific enantioselectivity and adequate separation was obtained for some carboxylic acids, such as clidanac (g), 2-(4-chlorophenyl)-3-methylbutyric acid (j), 2-phenoxypropionic acid (k), 1-benzocyclobutenecarboxylic acid (n) and *cis*-chrysanthemic acid (p). These carboxylic acid enantiomers were not or poorly resolved on phase II. These results show that the 3,5-dinitrophenylurea group attached to the asymmetric carbon atom is effective in the separation of carboxylic acid enantiomers and the effect of the structure of the amino acid moiety in chiral recognition is considerable.

Table 1
Enantiomer separation of carboxylic acids by HPLC

Compound	CSP II			CSP III			CSP IV			CSP V		
	k'_1	α	M	k'_1	α	M	k'_1	α	M	k'_1	α	M
(a) Fenoprofen	2.85	1.12	A	4.09	1.13	B	5.78	1.07	B	5.66	1.00	B
(b) Ketoprofen	2.27(−)	1.12	A	6.01	1.10	B	7.08	1.05	B	6.35	1.00	B
(c) Flurbiprofen	3.18	1.09	A	5.27	1.06	B	3.50	1.00	B	5.63	1.02	B
(d) Pranoprofen	4.46	1.14	A	10.84	1.04	A	16.60	1.00	B	12.84	1.07	B
(e) Ibuprofen	4.17	1.06	B	3.34	1.08	B	4.73	1.04	B	4.37	1.00	B
(f) Naproxen	5.25(−)	1.55	C	10.99	1.09	A	6.33	1.00	B	16.78	1.00	B
(g) Clidanac	4.22	1.03	A	4.42	1.17	B	6.85	1.56	B	5.26	1.55	B
(h) 2-Phenylpropionic acid	1.53	1.09	D	2.51	1.10	D	2.07	1.08	D	2.28	1.00	D
(i) 2-Phenylbutyric acid	2.01	1.08	D	3.54	1.13	D	3.20	1.10	D	3.21	1.07	D
(j) 2-(4-Chlorophenyl)-3-methylbutyric acid	3.10	1.00	D	9.91	1.16	D	5.40	1.06	D	7.35	1.00	D
(k) 2-Phenoxypropionic acid	1.15	1.00	D	2.58	1.34	D	2.14	1.00	D	2.34	1.05	D
(l) α-Methoxyphenylacetic acid	7.04	1.15	B	8.80	1.07	B	2.97	1.03	B	5.08	1.05	B
(m) α-Bromophenylacetic acid	6.22	1.11	B	5.48	1.00	B	2.08	1.06	B	3.48	1.05	B
(n) 1-Benzocyclobutene-carboxylic acid	5.87	1.00	B	6.03	1.07	B	2.12	1.10	B	3.28	1.10	B
(o) *trans*-2-Phenyl-1-cyclo-propanecarboxylic acid	4.68	1.08	B	4.37	1.00	B	1.94	1.00	B	2.49	1.00	B
(p) *cis*-Chrysanthemic acid	4.79	1.00	D	10.15	1.00	D	6.45(−)	1.05	D	7.45(−)	1.10	D
(q) *trans*-Chrysanthemic acid	3.88	1.06	D	6.11	1.16	D	3.81(−)	1.12	D	4.94(−)	1.16	D
(r) *cis*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid	6.58	1.09	D	10.67	1.07	D	7.67	1.08	D	8.91	1.08	D
(s) *trans*-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	4.68	1.10	D	6.44	1.17	D	3.87	1.10	D	5.00	1.12	D

Mobile phase (M): A = 0.02 *M* ammonium acetate in methanol; B = 0.01 *M* ammonium acetate in methanol; C = 0.05 *M* ammonium acetate in methanol; D = 0.1 *M* ammonium acetate in water–tetrahydrofuran (60:40). A flow rate of 1.0 ml/min was typically used for the 250 × 4.6 mm I.D. column at room temperature. An injection volume of 1 μl (5 mg/ml) was typically used. k'_1, k'_2 = Capacity factors of first- and second-eluted isomers, respectively; α = separation factor (k'_2/k'_1).

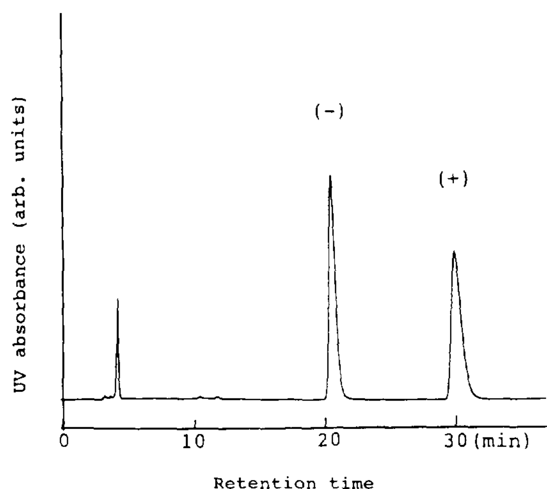

Fig. 3. Enantiomer separation of racemic naproxen (f) using a reversed mobile phase. Chromatographic conditions as in Table 1.

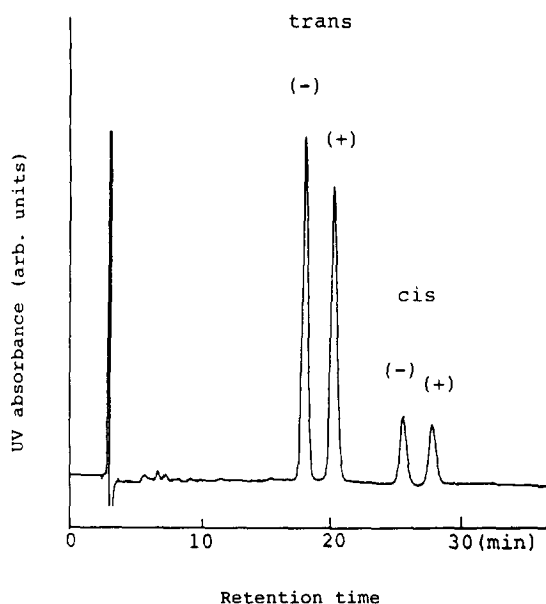

Fig. 4. Enantiomer separation of *cis*- and *trans*-chrysanthemic acid (p and q). Chromatographic conditions as in Table 1.

Table 2
Comparison of enantiomer separations using different mobile phase components

Compound	Stationary phase	k'_1	α	Mobile phase[a]
(b) Ketoprofen	CSP II	2.27	1.11	0.02 *M* NH_4AC in MeOH
		6.36	1.16	0.1 *M* NH_4AC in MeOH–H_2O (40:60)
		1.92	1.12	0.1 *M* NH_4AC in AN–H_2O (40:60)
		2.43	1.14	0.1 *M* NH_4AC in THF–H_2O (40:60)
		12.82	1.03	Hexane–DCE–EtOH–AA (190:9:1:1)
(f) Naproxen	CSP II	5.25	1.55	0.05 *M* NH_4AC in MeOH
		8.23	1.19	Hexane–DCE–EtOH–AA (190:9:1:1)
(g) Clidanac	CSP IV	6.85	1.56	0.01 *M* NH_4AC in MeOH
		4.01	1.19	Hexane–DCE–EtOH–AA (490:9:1:1)
(j) 2-(4-Chlorophenyl)-3-methylbutyric acid	CSP III	3.57	1.02	0.01 *M* NH_4AC in MeOH
		4.85	1.04	0.1 *M* NH_4AC in MeOH–H_2O (40:60)
		5.21	1.08	0.1 *M* NH_4AC in AN–H_2O (40:60)
		9.91	1.16	0.1 *M* NH_4AC in THF–H_2O (40:60)
		2.50	1.18	0.1 *M* NH_4AC in THF–H_2O (50:50)
		1.17	1.21	0.1 *M* NH_4AC in THF–H_2O (60:40)

A flow-rate of 1.0 ml/min was used for the 250 × 4.6 mm I.D. column at room temperature. An injection volume of 1 μl (5 mg/ml) was typically used. k'_1, k'_2 = Capacity factors of first- and second-eluted isomers, respectively; α = separation factor (k'_2/k'_1).

[a] NH_4AC = Ammonium acetate; MeOH = methanol; AN = acetonitrile; THF = tetrahydrofuran; DCE = 1,2-dichloroethane; EtOH = ethanol; AA = acetic acid.

It should be emphasized that these enantiomer separations were influenced by the composition of the mobile phase. Some of the data obtained are summarized in Table 2. An improvement of the separation was obtained by varying the aqueous mobile phase composition. For example, racemic 2-(4-chlorophenyl)-3-methylbutyric acid (j) was poorly resolved on phase III in methanol (containing 0.01 *M* ammonium acetate) or methanol–0.1 *M* ammonium acetate (40:60, v/v), but well resolved in tetrahydrofuran–0.1 *M* ammonium-acetate (40:60, v/v). Moreover, there was the increase in the separation factor as the tetrahydrofuran

Table 3
Enantiomer separation of amino acid derivatives by HPLC

Compound[a]	CSP III			CSP IV			CSP V		
	k'_1	α	M	k'_1	α	M	k'_1	α	M
N-Acetyl-									
alanine	2.30	1.12	A	3.71	1.00	B	3.16	1.12	A
valine	2.00	1.11	A	2.77	1.00	B	3.10	1.00	A
leucine	2.11	1.02	A	2.70	1.04	B	3.15	1.10	A
methionine	2.32	1.05	A	3.47	1.00	B	3.03	1.06	A
N-Benzoyl-									
valine	1.53	1.19	A	2.38	1.12	B	2.19	1.00	A
phenylalanine	2.41	1.17	A	4.12	1.07	B	2.83	1.06	A
glutamic acid	2.72	1.14	A	4.78	1.00	B	3.24	1.09	A
N-t-BOC-									
valine	1.04	1.10	A	1.09	1.00	A	2.32	1.00	A
leucine	1.13	1.09	A	0.99	1.00	A	2.45	1.04	A
phenylalanine	1.80	1.09	A	1.26	1.00	A	2.77	1.00	A
N-Z-									
alanine	2.77	1.05	A	4.14	1.04	B	3.33	1.16	A
valine	1.88	1.07	A	3.47	1.00	B	4.22	1.13	B
norvaline	2.19	1.09	A	4.00	1.00	B	4.61	1.13	B
leucine	2.10	1.07	A	3.85	1.05	B	3.23	1.17	A
serine	3.40	1.00	A	5.18	1.07	B	4.16	1.09	A
asparagine	5.68	1.00	A	6.60	1.12	B	9.94	1.11	B
N-FMOC-									
alanine	7.50(D)	1.17	A	3.18(D)	1.15	A	5.28(D)	1.19	A
valine	6.30	1.08	A	2.55	1.00	A	4.08	1.12	A
leucine	7.02	1.07	A	2.84	1.07	A	4.61	1.20	A
phenylalanine	8.76	1.08	A	3.52	1.00	A	5.41	1.10	A
N-Dansyl-									
valine	3.72	1.15	A	2.84(D)	1.21	A	5.21	1.28	A
norvaline	3.65	1.12	A	3.06(D)	1.15	A	6.58	1.24	A
threonine	4.59	1.18	A	4.09	1.17	A	8.42	1.15	A
Phenylalanine	5.65	1.12	A	4.53	1.17	A	8.34	1.27	A
tryptophan	15.92	1.12	A	9.41	1.16	A	16.93	1.16	A

Mobile phase (M): A = 0.01 *M* ammonium acetate in methanol; B = 0.005 *M* ammonium acetate in methanol; C = 0.03 *M* ammonium acetate in methanol. A flow-rate of 1.0 ml/min was used for the 250 × 4.6 mm I.D. column at room temperature. An injection volume of 1 μl (5 mg/ml) was typically used. k'_1, k'_2 = Capacity factors of first- and second-eluted isomers, respectively; α = separation factor (k'_2/k'_1).

[a] t-BOC- = *tert*.-Butoxycarbonyl-; Z- = benzyloxycarbonyl-; FMOC- = 9-fluorenylmethoxycarbonyl-; Dansyl- = 5-dimethylamino-1-naphthalenesulfonyl-.

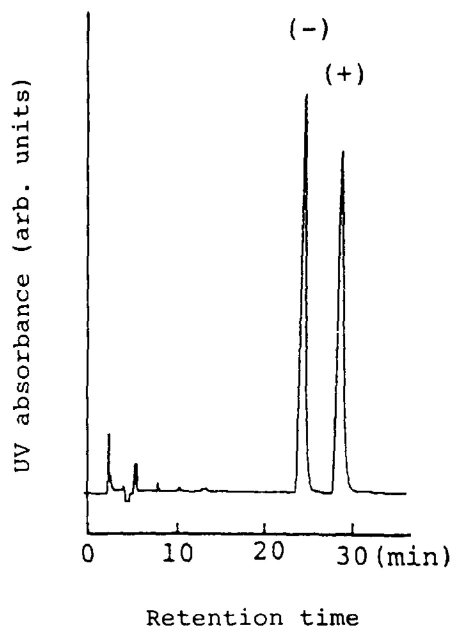

Fig. 5. Enantiomer separation of racemic naproxen (f) using a normal mobile phase. Chromatographic conditions as in Table 2.

content was increased in tetrahydrofuran–0.1 *M* ammonium acetate mobile phase. On the other hand, the influence was less in the separation of ketoprofen (b) enantiomers on phase II. The mechanism of the effect of the composition of the mobile phase is unclear and additional work is in progress. These CSPs were also effective using normal mobile phases. An example is shown in Fig. 5.

The results of the separation of amino acid enantiomers in the form of various derivatives containing free carboxylic acid groups are shown in Table 3. The enantiomer separation of *tert.*-butoxycarbonyl (t-BOC), benzyloxycarbonyl (Z) and 9-fluorenylmethoxycarbonyl (FMOC) derivatives of amino acid is significant for the peptide synthesis, and the separation of 5-dimethylamino-1-naphthalenesulfonyl (dansyl) derivatives of amino acids is valuable for the determination of small amounts of amino acids, as these derivatives show high sensitivity in UV or fluorescence detection. Hydroxy acid enantiomers were also resolved in the form of their derivatives containing free carboxylic acid groups.

These CSPs showed good durability under chromatographic conditions using the mobile phases shown in Tables 1 and 2. In conclusion, these novel amide and urea derivatives bonded to silica gel (CSPs II–V) are promising as chiral stationary phases for the separation of the enantiomers of various carboxylic acids and amino acids by HPLC.

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