Journal of Chromatography A, 666 (1994) 457–462
Elsevier Science B.V., Amsterdam

CHROM. 25 478

Short Communication

Enantiomer separation by high-performance liquid chromatography with (*R*,*R*)-tartaric acid mono-amide derivatives as bifunctional chiral selectors

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ABSTRACT

Three mono-amide derivatives of (*R*,*R*)-tartaric acid derived from (*R*)-1-(α-naphthyl)ethylamine (I), (*S*)-valine-(*S*)-1-(α-naphthyl)ethylamide (II) and (*R*)-valine-(*R*)-1-(α-naphthyl)ethylamide (III) were prepared and their chromatographic properties as chiral selectors were examined by HPLC. The enantiomeric separation of derivatives of racemic amino acids, amines, carboxylic acids and alcohols was achieved with these chiral selectors ionically bonded to 3-aminopropyl silica gel using organic mobile phases. In this separation the chiral amine or amino acid amide moiety may play an important role in chiral recognition. On the other hand, the direct separation of racemic amino acids and hydroxy acids was accomplished with these chiral selectors in the form of copper(II) complexes coated on the reversed-phase materials using aqueous mobile phases containing copper(II) ions. In this separation the (*R*,*R*)-tartaric acid moiety may contribute to the chiral recognition. These results clearly show that these (*R*,*R*)-tartaric acid mono-amide derivatives can act as bifunctional chiral selectors, and they are promising for the enantiomeric separation of a wide range of racemic compounds by HPLC.

INTRODUCTION

A great number of chiral selectors have been developed by many researchers for enantiomer separation by high-performance liquid chromatography (HPLC). These chiral selectors are generally divided into several types according to their chemical structures and to the molecular interactions involved in the chiral recognition process [1]. For instance, N-acyl amino acid derivatives belong to the hydrogen bonding–charge transfer type [2,3]. Copper(II) complexes of amino acids belong to the ligand-exchange type [4,5]. Crown-ether derivatives and cyclodextrin derivatives belong to the inclusion type [6,7]. Polymer-type chiral selectors including synthetic and natural polymers, such as polyacrylamide [8], protein [9] and cellulose derivatives [10,11], are also valuable.

Recently we [12] found that (*R*,*R*)-tartaric acid mono-(*R*)-1-(α-naphthyl)ethylamide(I) is an efficient chiral coating agent on reversed-phase materials for the direct separation of amino acid enantiomers by ligand-exchange HPLC using aqueous mobile phases. On the other hand, we [13] previously reported that some N-acyl derivatives of (*R*)-1-(α-naphthyl)ethylamine bonded to silica gel are efficient chiral selectors of the hydrogen bonding–charge transfer type for the enantiomeric separation of derivatives of racemic amino acids, amines and carboxylic acids. These

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0021-9673/94/$07.00
SSDI 0021-9673(93)E0863-P

results suggested that selector I could act as a bifunctional chiral selector, offering the functions of both hydrogen bonding–charge transfer type and ligand exchange-type chiral selectors, and led us to this work. We have also prepared two novel chiral selectors, (II) and (III), composed of (*R*,*R*)-tartaric acid mono-amide derivatives, and examined their chromatographic properties in order to investigate the effect of the structure of the chiral amine moiety in these selectors on enantiomer separation by HPLC.

EXPERIMENTAL

Preparation of chiral selectors

Selector I was prepared by the procedure described previously [12].

Selector II was prepared in the same way as selector I but using (*S*)-valine-(*S*)-1-(α-naphthyl)ethylamide instead of (*R*)-1-(α-naphthyl)-ethylamine. This compound was colourless and crystalline and identified by IR and NMR spectroscopy: m.p. (decomposed) 175–179°C, $[\alpha]_D^{20} = +33.0°$ (C = 0.2%, in methanol).

Selector III was prepared in the same way as selector II using (*R*)-valine(*R*)-1-(α-naphthyl)-ethylamide instead of (*S*)-valine-(*S*)-1-(α-naphthyl)ethylamide. This compound was colourless and crystalline and identified by IR and NMR spectroscopy: m.p. 213–214°C, $[\alpha]_D^{20} = +64.0°$ (C = 0.2%, in methanol).

The chemical structures of chiral selectors I, II and III are shown in Fig. 1.

Liquid chromatography

Columns Ia, IIa and IIIa. Mixing 3-aminopropyl silanized silica (Sumipax-NH_2, 5 μm, Sumika Chemical Analysis Service, Osaka, Japan) with chiral selectors I, II and III in methanol for 5 h affords ionically bonded CSPs. A typical example of an ionically bonded CSP was shown by Pirkle and Finn [3]. Stainless-steel columns (250 × 4 mm I.D.) were slurry packed with these CSPs using a conventional technique.

Columns Ib, IIb and IIIb. Sumipax ODS columns (150 × 4.6 mm I.D.) packed with octadecylsilanized silica (5 μm) were used. The

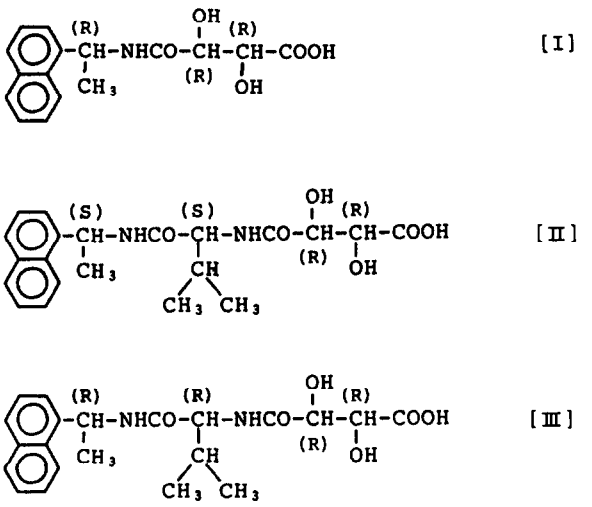

Fig. 1. Structures of chiral selectors.

coating of chiral selectors I, II and III on the reversed-phase support was accomplished by passing a 0.1% methanol–water (60:40, v/v) solution of I, II and III through the column followed by a 1 m*M* aqueous solution of copper(II) sulphate. All chemicals and solvents of reagent grade were purchased from Wako (Osaka, Japan). The experiments were carried out using a Waters Model 510 high-performance

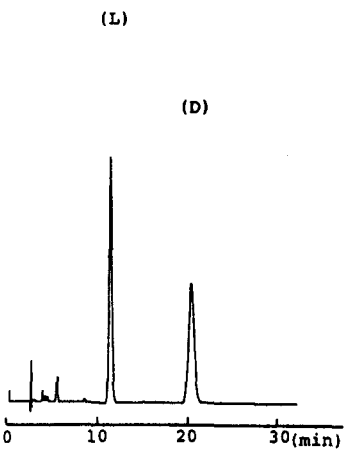

Fig. 2. HPLC separation of racemic N-3,5-dinitrobenzoyl-valine methylester with column Ia. Chromatographic conditions as in Table I.

liquid chromatograph equipped with a variable-wavelength UV detector.

RESULTS AND DISCUSSION

The results of enantiomer separation by HPLC with column Ia using organic mobile phases are shown in Table I. Adequate separation of racemic amino acids, amines, carboxylic acids and alcohols in the form of their derivatives was achieved, and some racemic compounds were resolved directly. A typical chromatogram is shown in Fig. 2.

As no separation of these enantiomers was found with (*R*,*R*)-tartaric acid mono lauryl amide as a chiral selector in our complementary

TABLE I

ENANTIOMER SEPARATIONS BY HPLC WITH COLUMN Ia

Mobile phase (M): A = *n*-hexane–1,2-dichloroethane–ethanol (50:10:1); B = *n*-hexane–1,2-dichloroethane–ethanol (100:20:1). A flow-rate of 1.0 ml/min was used for the 250 × 4 mm I.D. column at room temperature. An injection volume of 1 μl (2 mg/ml) was typically used. k_1', k_2' = Capacity factors of first- and second-eluted isomer; α = separation factor (k_2'/k_1'); R_s = resolution $[1.18(t_2 - t_1)/(Wh_1 + Wh_2)]$; t_1, t_2 and Wh_1, Wh_2 = retention times and half-widths of first- and second-eluted isomers.

Compounds	Column Ia				
	k_1'	k_2'	α	R_s	M
Amino acids[a]					
Valine	3.88(L)	7.03(D)	1.81	11.60	A
Alanine	10.65(L)	18.16(D)	1.71	8.84	A
Leucine	4.52(L)	7.76(D)	1.72	8.67	A
Phenylalanine	10.49(L)	12.27(D)	1.17	2.76	A
Amines[b]					
1-Phenylethylamine	12.77(*S*)	28.80(*R*)	2.26	13.47	A
1-(α-Naphthyl)ethylamine	14.53(*S*)	47.02(*R*)	3.24	19.92	A
2-Octylamine	3.15	3.79	1.20	2.69	A
Alcohols[c]					
1-Phenylethanol	7.78(*S*)	9.34(*R*)	1.20	3.28	A
1-(α-Naphthyl)ethanol	9.65	12.35	1.28	4.41	A
2-Octanol	3.01(*S*)	3.16(*R*)	1.05	0.31	A
Carboxylic acids[d]					
2-Phenylpropionic acid	16.81(*R*)	41.20(*S*)	2.45	16.69	A
2-(4-Chlorophenyl)-3-methylbutyric acid	13.72(*R*)	34.22(*S*)	2.49	16.09	A
Others					
Pantolactone	5.67(*S*)	5.91(*R*)	1.04	0.31	A
Diniconazole[e]	5.85(*R*)	6.73(*S*)	1.15	2.59	B
Uniconazole[f]	5.28(*R*)	5.63(*S*)	1.07	1.02	B
2,2,2-Trifluoro-1-(9-anthryl)ethanol	4.42(*S*)	4.65(*R*)	1.05	0.86	B

[a] Resolved as N-3,5-dinitrobenzoyl O-methylester derivatives.
[b] Resolved as N-3,5-dinitrobenzoyl derivatives.
[c] Resolved as O-3,5-dinitrophenyl urethane derivatives.
[d] Resolved as O-3,5-dinitroanilide.
[e] 1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol.
[f] 1-(4-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol.

experiment, it was believed that the (*R*)-1-(α-naphthyl)-ethylamine moiety in I may contribute to the chiral recognition. The fact that the (*R*,*R*)-tartaric acid moiety in I plays an important role in the chiral recognition, as reported previously by us [12] clearly shows that I can act as a bifunctional chiral selector.

The chromatographic results obtained with columns IIa and IIIa are summarized in Table II. The efficient separation of racemic N-3,5-dinitrobenzoyl amino acid methyl esters, N-3,5-dinitrobenzoyl amines, 3,5-dinitroanilides of carboxylic acids, 3,5-dinitrophenyl urethane derivatives of alcohols and some other racemic compounds was achieved using organic mobile phases. In this separation hydrogen bonding and charge transfer interaction may contribute as well as in the enantiomer separation with column Ia.

The direct separation of amino acid, hydroxy

TABLE II

ENANTIOMER SEPARATIONS BY HPLC WITH COLUMNS IIa AND IIIa

Mobile phase (M): A = *n*-hexane–1,2-dichloroethane–ethanol (50:10:1); B = *n*-hexane–1,2-dichloroethane–ethanol (100:20:1). A flow-rate of 1.0 ml/min was used for the 250 × 4 mm I.D. column at room temperature. An injection volume of 1 μl (2 mg/ml) was typically used. k_1', k_2' = Capacity factors of first- and second-eluted isomer; α = separation factor (k_2'/k_1'); R_s = resolution $[1.18(t_2 - t_1)/(Wh_1 + Wh_2)]$; t_1, t_2 and Wh_1, Wh_2 = retention times and half-widths of first- and second-eluted isomers.

Compounds	Column IIa					Column IIIa				
	k_1'	k_2'	α	R_s	M	k_1'	k_2'	α	R_s	M
Amino acids[a]										
Valine	2.92(D)	6.42(L)	2.20	12.88	A	3.23(L)	6.08(D)	1.88	13.99	A
Alanine	7.58(D)	12.43(L)	1.64	10.18	A	8.58(L)	14.30(D)	1.67	11.67	A
Leucine	3.46(D)	7.05(L)	2.04	15.06	A	4.00(L)	6.92(D)	1.73	11.94	A
Phenylalanine	6.14(D)	9.03(L)	1.47	6.80	A	6.62(L)	9.68(D)	1.46	8.67	A
Amines[b]										
1-Phenylethylamine	8.61(*R*)	16.39(*S*)	1.90	11.74	A	9.10(*S*)	17.02(*R*)	1.87	15.24	A
1-(α-Naphthyl)ethylamine	9.21(*R*)	30.27(*S*)	3.29	20.66	A	8.67(*S*)	33.94(*R*)	3.91	28.17	A
2-Octylamine	4.66	5.03	1.08	1.25	A	4.37		1.00		A
Alcohols[c]										
1-Phenylethanol	6.59(*R*)	8.92(*S*)	1.31	5.72	A	6.64(*S*)	8.70(*R*)	1.31	6.92	A
1-(α-Naphthyl)ethanol	7.43	10.66	1.43	6.56	A	7.44	10.24	1.38	8.23	A
2-Octanol	3.09(*R*)	3.20(*S*)	1.04	0.31	A	3.03(*S*)	3.18(*R*)	1.05	0.93	A
Carboxylic acids[d]										
2-Phenylpropionic acid	14.35(*S*)	36.39(*R*)	2.54	16.22	A	16.06(*R*)	38.90(*S*)	2.42	17.50	A
2-(4-Chlorophenyl)-3-methylbutyric acid	13.34(*S*)	27.97(*R*)	2.10	12.67	A	14.35(*R*)	27.93(*S*)	1.95	15.61	A
Others										
1,1'-Binaphthol	6.56(*S*)	7.08(*R*)	1.08	1.28	A	12.19		1.00		A
Diniconazole[e]	6.42(*S*)	6.77(*R*)	1.05	1.00	B	7.10		1.00		B
Uniconazole[f]	7.92(*S*)	8.13(*R*)	1.03	0.52	B	9.14		1.00		B
2,2,2-Trifluoro-1-(9-anthryl)ethanol	4.10(*R*)	4.34(*S*)	1.06	1.09	B	6.98(*S*)	7.31(*R*)	1.05	1.22	B

[a] Resolved as N-3,5-dinitrobenzoyl O-methylester derivatives.
[b] Resolved as N-3,5-dinitrobenzoyl derivatives.
[c] Resolved as O-3,5-dinitrophenyl urethane derivatives.
[d] Resolved as 3,5-dinitroanilide.
[e] 1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol.
[f] 1-(4-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol.

acid and amino alcohol enantiomers was accomplished with columns IIb and IIIb using aqueous or hydro-organic mobile phases containing copper(II) ion, as shown in Table III. A typical example is shown in Fig. 3. It is believed that the (*R*,*R*)-tartaric acid moiety in II and III as well as in I, plays an important role in enantiomer separation in the form of copper(II) complexes, and it is clear that II and III can act as bifunctional chiral selectors. Separation factors obtained with column IIb are generally superior to those obtained with column IIIb, but a few interesting values were found with IIIb for some amino acids, such as proline. It was especially noticed that efficient separation of racemic β-amino acids, such as 3-amino-*n*-butyric acid,

TABLE III

ENANTIOMER SEPARATIONS BY HPLC WITH COLUMNS IIb AND IIIb

Mobile phase (M): A = 1 m*M* copper(II) sulphate in water; B = 2 m*M* copper(II) sulphate in water–acetonitrile (95:5); C = 2 m*M* copper(II) sulphate in water–acetonitrile (90:10); D = 2 m*M* copper(II) sulphate in water–acetonitrile (85:15). A flow-rate of 1.0 ml/min was used for the 150 × 4.6 mm I.D. column at room temperature. An injection volume of 5 μl (2 mg/ml) was typically used. k'_1, k'_2 = Capacity factors of first- and second-eluted isomer; α = separation factor (k'_2/k'_1); R_s = resolution $[1.18(t_2 - t_1)/(Wh_1 + Wh_2)]$; t_1, t_2 and Wh_1, Wh_2 = retention times and half-widths of first- and second-eluted isomers.

Compounds	Column IIb					Column IIIb				
	k'_1	k'_2	α	R_s	M	k'_1	k'_2	α	R_s	M
Amino acids										
Ornithine	6.83(D)	14.42(L)	2.11	5.90	A	0.03		1.00		A
Lysine	6.63(D)	25.78(L)	3.89	8.53	A	0.15(D)	0.21(L)	1.40	0.29	A
Asparagine	6.09(L)	9.36(D)	1.54	4.08	A	0.35		1.00		A
Alanine	9.22(D)	19.31(L)	2.09	8.00	A	0.60		1.00		A
Serine	10.32(D)	18.42(L)	1.78	6.07	A	0.38(D)	0.77(L)	2.03	3.24	A
Arginine	2.10(D)	9.42(L)	4.49	10.76	B	0.46(D)	1.40(L)	3.04	4.57	A
Threonine	4.80(D)	9.45(L)	1.97	5.63	A	0.61(D)	1.41(L)	2.31	5.49	A
Glutamine	2.42(D)	5.94(L)	2.45	8.07	B	0.94		1.00		A
Proline	0.82(L)	1.28(D)	1.56	3.74	B	1.14(L)	4.71(D)	4.13	6.13	A
Valine	1.30(D)	5.68(L)	4.37	10.02	C	6.32(L)	10.66(D)	1.69	3.44	A
Tyrosine	2.66(D)	4.30(L)	1.62	3.76	C	3.75(L)	5.29(D)	1.41	2.65	B
Methionine	2.29(D)	9.18(L)	4.01	10.92	C	2.19(D)	3.29(L)	1.50	4.15	B
Leucine	1.85(D)	4.05(L)	2.19	5.67	D	5.44		1.00		B
Histidine	4.93(D)	7.28(L)	1.48	1.14	D	2.21		1.00		B
Phenylalanine	4.45(D)	11.48(L)	2.58	7.42	D	14.69(L)	20.00(D)	1.36	2.58	B
Tryptophan	11.17(D)	14.89(L)	1.33	2.25	D	7.36(D)	10.97(L)	1.49	3.30	D
DOPA[a]	5.10(D)	10.27(L)	2.01	6.65	B	2.27		1.00		B
3-Amino-*n*-butyric acid	2.11	2.74	1.30	1.73	A	0.68		1.00		A
Hydroxy acids										
Lactic acid	4.96(D)	9.14(L)	1.84	6.39	A	1.41		1.00		A
Glyceric acid	9.34	10.32	1.10	0.29	A	1.64	2.04	1.24	0.74	A
2-Hydroxy-*n*-butyric acid	2.94	6.37	2.17	7.85	B	3.13	4.80	1.53	2.66	A
Mandelic acid	6.60(D)	15.82(L)	2.40	4.99	D	7.63		1.00		B
3-Hydroxy-*n*-butyric acid	7.69	9.39	1.22	1.95	A	2.56		1.00		A
Amino alcohols										
Octopamine	0.90	1.24	1.38	1.39	A	0.89		1.00		A
Norphenylephrine	1.68	1.91	1.14	0.96	A	1.94		1.00		A
Normethanephrine	1.31	1.49	1.14	0.33	A	1.65		1.00		A
p-Hydroxynorephedrine	1.29	1.66	1.29	1.52	A	1.77		1.00		A

[a] 3-(3,4-Dihydroxyphenyl)alanine.

Fig. 3. HPLC separation of racemic valine with column IIb. Chromatographic conditions as in Table III.

which were poorly resolved with Ib and IIIb, was accomplished with IIb. These results clearly show that the enantioselectivity of (*R*,*R*)-tartaric acid mono-amide derivatives in ligand-exchange HPLC was influenced remarkably by the structure of amine moiety [12].

It should be noted that the durability of the column was influenced by the mobile phase, and recommended compositions of mobile phases are given in Tables I–III. When these eluents were used, about 300 analyses did not cause any change in their retention parameters, enantioselectivity or efficiency on each column.

In conclusion (*R*,*R*)-tartaric acid mono-amide derivatives, I, II and III, are useful bifunctional chiral selectors. They can offer two different functions which are contained in the hydrogen bonding–charge transfer type and in the ligand-exchange type selectors. We consider these results are important to make clear the molecular interactions involved to the chiral recognition, and these chiral selectors are promising for the enantiomer separation of a wide range of racemic compounds by HPLC.

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