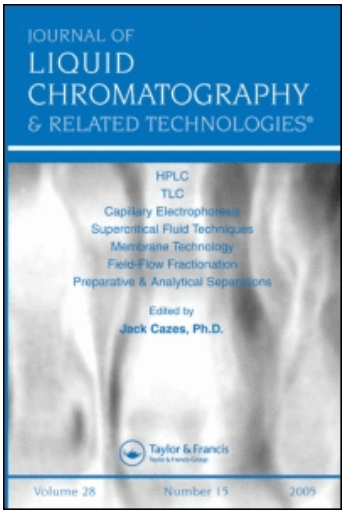

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ENANTIOMER SEPARATION BY HPLC ON REVERSED PHASE SILICA GEL COATED WITH COPPER(II) COMPLEXES OF (R,R)-TARTARIC ACID MONO-AMIDE DERIVATIVES

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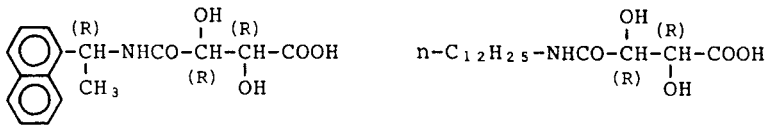
ABSTRACT

Two chiral ligand exchange stationary phases for HPLC, (R,R)-tartaric acid mono-(R)-1-(α-naphthyl)ethylamide [I] and (R,R)-tartaric acid mono-n-laurylamide [II], were prepared. The direct separation of a number of amino acid, hydroxy acid and amino alcohol enantiomers was achieved using ODS silica gel coated with [I] and [II], and water or hydro-organic eluents containing copper(II) ion as a mobile phase. The enantioselectivity of the phase [I] was superior than that of the phase [II]. The phase [I] is very promising as the chiral coating agent on reversed phase materials for the direct separation of a variety of enantiomers by ligand exchange HPLC.

INTRODUCTION

Chiral ligand exchange HPLC, as shown by Davankov and other workers (1-3), has proven to be a very powerful tool for enantiomer separation. In this technique various amino acids, amines and amino alcohols have been generally used as chiral ligands in either



[I] [II]

Fig.1. Structure of phases [I] and [II]

stationary phases or additives to the mobile phases (4-16). Hydroxy acids, such as (R,R)-tartaric acid and (S)-mandelic acid are also useful as covalently bonded chiral ligands as reported by Kicinski et al (17,18), and Lindner et al. (19) showed (R,R)-tartaric acid mono-n-octylamide was available as mobile phase additive in the presence of copper(II) or nickel(II) ion for enantiomer separation.

On the other hand, during the course of our research to examine the effect of the structure in the enantioselectivity of amide type chiral stationary phases, we (20,21) have found some chiral carboxylic acid amides which contain two asymmetric carbon atoms attached to the carbon and nitrogen atoms of the -NHCO- group showed excellent enantioselectivity.

In this study two (R,R)-tartaric acid mono amide derivatives [I] and [II] (Fig.1) were prepared and their chromatographic properties were examined in order to investigate the effect of structure in amine moiety for the enantiomeric separation by ligand exchange HPLC.

In chiral ligand exchange HPLC, the system which is composed of reversed phase packing coated with an appropriate resolving agent and a hydro-organic eluent containing the complexing metal ion is very advantageous as shown by Davankov et al (6). Therefore, the phase [I] and [II] were coated on the reversed phase silica gel and enantiomeric separation of racemic amino acids, hydroxy acids, amino alcohols was examined.

EXPERIMENTAL

Preparation of chiral stationary phases

Phase [I] : To a solution of 22.7g of (R,R)-O,O-diacetyl tartaric acid anhydride in tetrahydrofuran (300ml), an equimolar amount of (R)-1-(α-naphthyl)ethylamine in tetrahydrofuran (100ml) was added dropwise at room temperature. The solution was stirred for 4 hours at 60°C. After evaporating the solvent under reduced pressure, the residue was dissolved in dichloromethane and extracted with 1N potassium hydroxide. The aqueous solution was stirred for 3 hours at room temperature and acidified with 6N hydrochloric acid. A pale yellow product was filtered off and washed with water. After drying, it was used without further purification. The product was identified by IR and NMR spectroscopy; m.p. (decomposed) 107 - 109°C, $[\alpha]_D^{20}$ = +66.4° (C=0.2% in methanol).

Phase [II] : (R,R)-tartaric acid mono lauryl amide was prepared as for (R,R)-tartaric acid mono (R)-1-(α-naphthyl)ethylamide using n-laurylamine instead of (R)-1-(α-naphthyl)ethylamine. This compound was colourless crystalline. The product was identified by IR and NMR spectroscopy ; m.p. (decomposed) 147 - 150°C, $[\alpha]_D^{20}$ = +51.2° (C=0.2% in methanol).

Liquid chromatography

Commercially available SUMIPAX ODS columns (150 mm X 4.6 mmI.D.) packed with octadecylsilanized silica (5μm) were used. The coating of phase [I] and [II] on the reversed - phase support was accomplished by passing a 0.05% methanol-water (2:8, v/v) solution of [I] and [II] through the column followed by a 1 mM aqueous solution of copper(II) sulfate. The column coated with phase [I] is available from Sumika Chemical Analysis Service (Osaka, Japan) as SUMICHIRAL OA-6000. All chemicals and solvents of reagent grade were purchased from Wako (Osaka, Japan). The experiments were carried out using a Waters 510 high-performance liquid chromatograph equipped with a variable wavelength UV detector (operated at 254nm).

RESULTS AND DISCUSSION

The chromatographic results are sumarrized in TABLE I and II. The direct separation of many racemic amino acids, hydroxy acids, amino alcohols was accomplished by ligand exchange HPLC using octadecylsilanized silica gel coated with phase [I] and [II] and aqueous or hydro-organic solutions containing copper(II) ion as a mobile phase. It should be emphasized the remarkable difference in the enantioselectivity was found between phase [I] and [II] systems.

In the enantiomeric separation of amino acids, more than twenty racemic amino acids used in this study were well resolved at room temperature with phase [I]. Typical chromatograms are shown in Figs. 2 - 4. On the contrary, no separation was achieved in several amino acids with phase [II], although larger separation factors were obtained in some amino acids. It was noticed very large values of separation factors were obtained for hydrophobic amino acids, such as valine and norvaline, in the phase [I] system. This result suggested the enantioselectivity of the phase [I] was very sensitive to the steric effect of the alkyl group attached to the asymmetric carbon atom. It was also noticed in the phase [I] system excellent chromatograms were obtained for hydrophillic amino acids, such as ornithine and lysine, which were often difficult to resolve using the moderate flow rate as their capacity factors were too small. As a whole the phase [I] was very effective for the separation of a number of racemic amino acids, although the peak shape was rather broad in some amino acids unfortunately.

The apparent difference of the enantioselectivity was found between two systems with phase [I] and [II] in the separation of racemic hydroxy acids and amino alcohols as shown in TABLE II. Larger values of separation factors were obtained with phase [I] than with phase [II] in racemic hydroxy acids except in the case of glyceric acid. Racemic octopamine, normethanephrine and phenylalaninol were well resolved with phase [I], but not resolved with phase [II].

TABLE I ENANTIOMER SEPARATION OF AMINO ACIDS

Mobile phase : A = 0.5 mM copper(II) sulfate in water; B = 1 mM copper(II) sulfate in water; C = 2 mM copper(II) sulfate in water-acetonitrile(95:5); D = 2 mM copper(II) sulfate in water-acetonitrile(85:15). A flow-rate of 1 ml/min was used for the 150 mm x 4.6 mmI.D. column at room temperature. An injection volume of 5 µl(2mg/ml) was typically used. k'_1 is the capacity factor for the initially eluted enantiomer. The separation factor of the enantiomers, α, is the ratio of their capacity factors.

Amino acid	Phase [I]			Phase [II]		
	k'_1	α	Mobile phase	k'_1	α	Mobile phase
Ornithine	2.69(D)	1.32	A	0.75	1.00	A
Lysine	1.72(D)	2.31	B	0.51(D)	1.88	B
Asparagine	1.72(L)	1.49	B	0.73(L)	1.26	B
Alanine	2.39(D)	2.28	B	1.02(D)	2.28	B
Serine	2.71(D)	1.70	B	0.90(D)	2.02	B
Arginine	3.08(D)	4.60	B	1.09(D)	4.27	B
Threonine	3.20(D)	1.94	B	1.29(D)	2.30	B
Aspartic acid	3.32(L)	1.68	B	1.16	1.00	B
Allothreonine	3.38(D)	3.93	B	1.32(D)	5.39	B
Glutamine	3.38(D)	2.81	B	1.41(D)	2.41	B
Proline	4.14(L)	1.08	B	2.22(L)	1.65	B
2-Amino-n-butyric acid	1.21(D)	2.88	C	2.49(D)	3.43	B
Valine	1.85(D)	4.64	C	1.60(D)	1.96	C
Norvaline	2.19(D)	5.44	C	1.68(D)	2.20	C
Glutamic acid	3.19(D)	1.53	C	2.47	1.00	B
Tyrosine	4.48(D)	1.79	C	2.72(D)	1.41	C
Methionine	1.38(D)	2.60	D	2.29(D)	2.49	C
Isoleucine	1.46(D)	3.49	D	5.56(D)	2.21	C
Alloisoleucine	1.46(D)	2.79	D	5.56(D)	1.75	C
Leucine	1.65(D)	2.22	D	4.97(D)	1.66	C
Phenylglycine	2.09(D)	1.89	D	5.21(D)	1.18	C
tert-Leucine	2.18(D)	3.16	D	9.02(D)	1.73	C
Histidine	3.57(D)	2.09	D	2.69(D)	1.55	C
Phenylalanine	4.24(D)	2.70	D	1.86	1.00	D
Tryptophane	11.18(D)	1.24	D	3.38	1.00	D
1-Naphthylglycine	11.25(D)	1.46	D	4.67	1.00	D

TABLE II ENANTIOMER SEPARATION OF HYDROXY ACIDS AND AMINO ALCOHOLS

Mobile phase : A = 0.5 mM copper(II) sulfate in water; B = 1 mM copper(II) sulfate in water; C = 2 mM copper(II) sulfate in water-acetonitrile(95:5); D = 2 mM copper(II) sulfate in water-acetonitrile(85:15). A flow-rate of 1 ml/min was used for the 150 mm x 4.6 mmI.D. column at room temperature. An injection volume of 5 µl(2mg/ml) was typically used. k_1' is the capacity factor for the initially eluted enantiomer. The separation factor of the enantiomers, α, is the ratio of their capacity factors.

Amino acid	Phase [I]			Phase [II]		
	k_1'	α	Mobile phase	k_1'	α	Mobile phase
Lactic acid	2.63	1.60	B	1.53	1.34	B
Glyceric acid	5.69	1.33	B	1.91	1.47	B
2-Hydroxy-n-butyric acid	2.17	1.70	C	5.34	1.07	B
3-Hydroxy-n-butyric acid	4.79	1.23	B	2.96	1.09	B
Leucic acid	5.38	1.72	D	13.45	1.15	C
Mandelic acid	4.47	2.12	D	6.46	1.25	C
Malic acid	15.34	3.37	D	7.41	1.83	C
Octopamine	0.71	1.14	A	0.93	1.00	B
Norphenylephrine	1.79	1.20	A	1.89	1.06	B
Normethanephrine	1.51	1.20	A	1.69	1.00	B
p-Hydroxynorephedrine	1.58	1.53	A	1.81	1.08	B
Norephedrine	8.12	1.15	B	9.86	1.13	B
Phenylalaninol	12.55	1.21	B	14.61	1.00	B

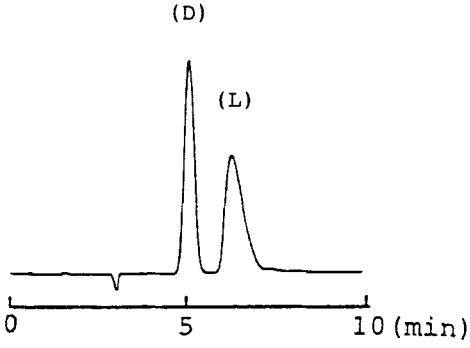


Fig.2. Enantiomer separation of racemic ornithine on phase [I]. Chromatographic conditions as in TABLE I.

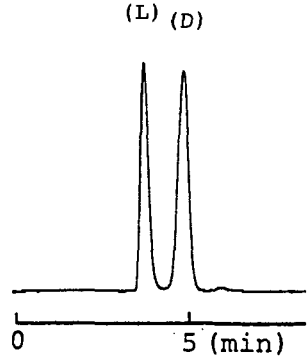


Fig.3. Enantiomer separation of racemic asparagine on phase [I]. Chromatographic conditions as in TABLE I.

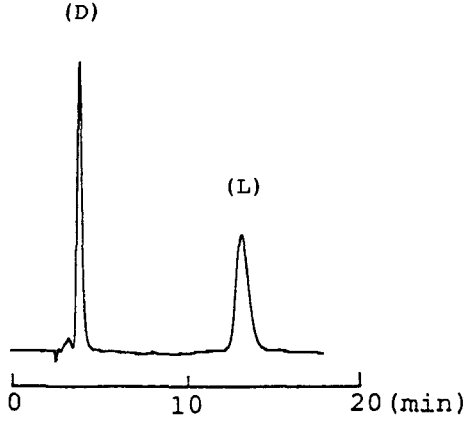


Fig.4. Enantiomer separation of racemic valine on phase [I]. Chromatographic conditions as in TABLE I.

It should be noticed there was no difference in the elution order of amino acid enantiomers between two systems with phase [I] and [II] as shown in TABLE I. These results suggest that (R,R)-tartaric acid component may play the important role for the chiral discrimination in both phases. The L-isomer was always more strongly retained than the D-isomer with the exception of proline, aspartic acid and asparagine. The inverse elution order of these amino acids is interesting, but the explanation of such chiral recognition mechanism needs further examination.

The results obtained in TABLE I and II clearly showed the enantioselectivity of (R,R)-tartaric acid mono amide derivatives was influenced by the structure of amine moiety. It is noticed (R)-1-(α-naphthyl)ethylamine component, which contain an asymmetric carbon atom attached to the nitrogen atom of the NHCO group and a naphthyl group, may contribute to improve the enantioselectivity. Unfortunately the structural factor for the improvement of the enantioselectivity is unclear yet, and we intend to make further investigation.

As far as column durability is concerned, it can be stated that 300 analysis of some amino acids on the column coated with phase [I] or [II] did not cause any change in their retention parameters, enantioselectivity or efficiency when water or water-acetonitrile (85/15, v/v) was used as the eluent.

In conclusion, the enantioselectivity of the phase [I] was superior than that of the phase [II], and the phase [I] is very promissing as the coating agent on reversed-phase materials for the direct separation of a number of enantiomers including amino acids, hydroxy acids and amino alcohols by chiral ligand exchange HPLC.

Recently, we (12,22) have reported the copper(II) complexes of N,S-dioctyl-(D)-penicillamine and N-salicyliden-(R)-2-amino-1,1-bis(2-butoxy-5-tert-butylphenyl)-3-phenylpropanol-1 were very efficient as the stationary phases for the separation of various enantiomers by chiral ligand exchange HPLC. Although the object of the optical resolution with these phases and the phase [I] overlaps largely, the complementary use of these copper(II) complex phases may contribute to accomplish the direct separation of a wide variety of enan-

tiomers, because three phases offer the characteristic enantioselectivity respectively.

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