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Enantiomer separation by high-performance liquid chromatography with copper(II) complexes of Schiff bases as chiral stationary phases

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ABSTRACT

Copper(II) complexes of Schiff bases of chiral amino alcohols were examined as chiral ligand-exchange phases for high-performance liquid chromatography. The direct separation of a large number of amino alcohol, amine, amino acid and hydroxy acid enantiomers was accomplished using octadecylsilanized silica coated with the binuclear copper(II) complex of N-salicylidene-(*R*)-2-amino-1,1-bis(2-butoxy-5-*tert.*-butylphenyl)-3-phenyl-1-propanol (**1**) and water or water–organic eluents containing copper(II) ion as mobile phases. The interaction of various enantiomers with the copper(II) complex **1** for chiral discrimination was discussed.

INTRODUCTION

Chiral ligand-exchange high-performance liquid chromatography (HPLC), as shown by Davankov and co-workers [1–3], is a very powerful tool for enantiomer separations. In this technique various amino acids and their derivatives have mainly been used as ligands in either chiral stationary phases or as chiral additives to the mobile phases [4–12]. It has also been shown that non-amino acid-type chiral ligands, such as L-2-isopropyl-4-octyldiethylenetriamine, (−)-*trans*-1,2-cyclohexyldiamine, (1*R*,2*S*)- or (1*S*,2*S*)-2-carboxymethylamino-1,2-diphenylethanol, (*R*,*R*)-tartaric acid and (*S*)-mandelic acid, can be used [13–19].

Schiff bases are well known to form stable metal complexes, but they have never been utilized as ligands for chiral ligand-exchange HPLC. Gelber and co-workers [20,21] reported a method for the enantiomeric resolution of primary amino alcohols involving derivatization to the salicylaldehyde Schiff base followed by ligand-exchange HPLC with an L-proline-bonded stationary phase. This result suggested that Schiff bases of chiral amino alcohols may be valuable as chiral ligands for enantiomer separations, and we have achieved [22] efficient separations of amino alcohol, amino acid and amine enantiomers by HPLC on reversed-phase silica gel coated with the copper(II) complex of N-salicylidene-(*R*)-2-amino-1,1-bis(2-butoxy-5-*tert.*-butylphenyl)-3-phenyl-1-propanol (**1**).

In this paper we report enantiomer separations by HPCL with the copper(II) complexes of Schiff bases **1** and **2** [23,24] as chiral stationary phases. These copper(II) complexes are known to be effective catalysts for practical asymmetric syntheses.

EXPERIMENTAL

Two binuclear copper(II) complexes of N-salicylidene-(*R*)-2-amino-1,1-bis(2-butoxy-5-*tert.*-butylphenyl)-3-phenyl-1-propanol (**1**) and N-salicylidene-(*R*)-2-amino-1,1-bis(5-*tert.*-butyl-2-octyloxyphenyl)-1-propanol (**2**) were kindly provided by Dr. T. Aratani (Sumitomo Chemical). The

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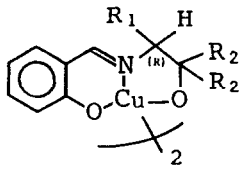

1 R_1 = benzyl
R_2 = 2-butoxy-5-*tert.*-butylphenyl

2 R_1 = methyl
R_2 = 5-*tert.*-butyl-2-octyloxyphenyl

coating of phases **1** and **2** on Sumipax ODS columns (150 mm × 4.6 mm I.D.) packed with octadecylsilanized silica (5 μm) was accomplished by passing a 0.05% tetrahydrofuran–water (50:50) solution of **1** and **2** through the column followed by a 1 m*M* aqueous solution of copper(II) acetate. The column coated with phase **1** is available from Sumika Chemical Analysis Service (Osaka, Japan) as Sumichiral OA-5500. All chemicals and solvents were of analytical-reagent grade from Wako (Osaka, Japan). Some samples were provided by Sumitomo Chemical. The experiments were carried out using a Waters Model 510 high-performance liquid chromatograph equipped with a variable-wavelength UV detector.

RESULTS AND DISCUSSION

The HPLC separation of six racemic compounds was tested with the two copper(II) complexes **1** and **2** under the same chromatographic conditions in order to compare their enantioselectivities. As shown in Table I, approximate separation factors (α) were obtained, and further experiments were performed with the complex **1**, which is more convenient to prepare. The results of enantiomer separations of various racemic compounds with **1** are summarized in Table II; the structures of the racemic amino alcohols and amines are shown in Fig. 1. Typical chromatograms are shown in Figs. 2–5.

Racemic amino alcohols such as phenylalaninol and 2-amino-1-phenylethanol, which contain either an amino group or a hydroxyl group directly attached to the asymmetric carbon atom, were efficiently resolved, and no enantiomeric separation was obtained for racemic 1-phenylethylamine and 1-phenylethanol in a complementary experiment. These results show that both amino and hydroxyl groups in amino alcohols may play an important cooperative role in the interaction with phase **1** for chiral discrimination. The fact that the efficient enantiomer separation of racemic amines such as 1,2-diphenylethylamine and ketamine, which contain several aromatic or polar groups, was accomplished suggests similar complexation with **1** in

TABLE I

ENANTIOMER SEPARATIONS BY HPLC WITH PHASES 1 AND 2

Mobile phase: (A) 1 m*M* copper(II) sulphate in water; (B) 2 m*M* copper(II) sulphate in water–acetonitrile (85:15). A flow-rate of 1 ml/min was typically used for the 150 × 4.6 mm I.D. columns 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 isomers, respectively; α = separation factor (k'_2/k'_1).

Compound	Phase **1**				Phase **2**			
	k'_1	k'_2	α	Mobile phase	k'_1	k'_2	α	Mobile phase
2-Amino-1-phenylethanol	4.50	5.36	1.19	A	2.64	3.14	1.19	A
Octopamine	1.46	1.90	1.30	A	0.59	0.76	1.29	A
Phenylglycinol	2.78	3.75	1.35	A	1.63	1.84	1.13	A
1,2-Diphenylethylamine	7.42	12.17	1.64	B	4.62	7.54	1.63	A
Phenylglycine	6.54 (D)	8.10 (L)	1.24	A	11.85 (D)	16.68 (L)	1.45	A
Tyrosine	6.18 (D)	12.73 (L)	2.06	A	6.69 (D)	32.76 (L)	4.90	A

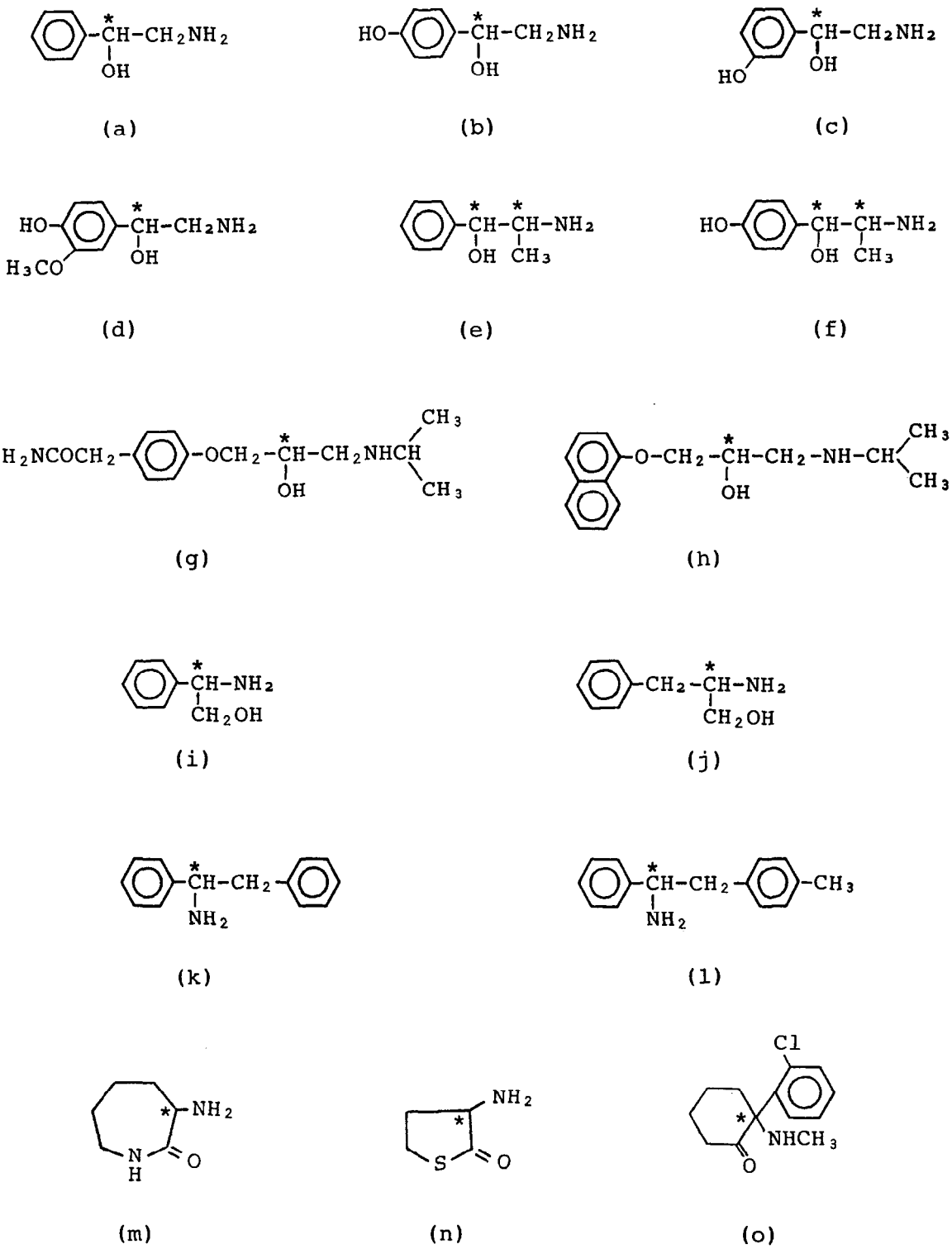

Fig. 1. Structures of amino alcohols and amines: (a) 2-amino-1-phenylethanol; (b) octopamine; (c) norphenylephrine; (d) normethanephrine; (e) norephedrine; (f) *p*-hydroxynorephedrine; (g) atenolol; (h) propranolol; (i) phenylglycinol; (j) phenylalaninol; (k) 1,2-diphenylethylamine; (l) 1-phenyl-2-(*p*-tolyl)ethylamine; (m) α-amino-ε-caprolactam; (n) homocysteine thiolactone; (o) ketamine.

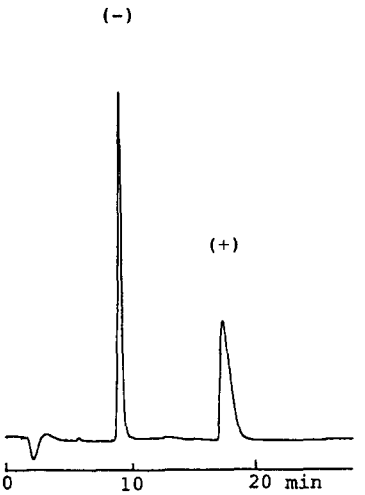

Fig. 2. HPLC separation of racemic phenylalaninol with phase **1**. Chromatographic conditions as in Table II.

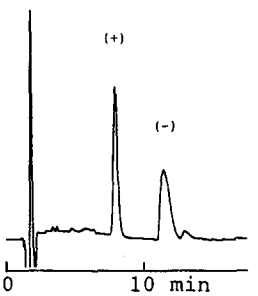

Fig. 3. HPLC separation of racemic 1-phenyl-2-(*p*-tolyl)ethylamine with phase **1**. Chromatographic conditions as in Table II.

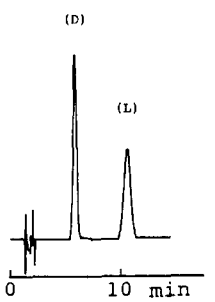

Fig. 4. HPLC separation of racemic tryptophan with phase **1**. Chromatographic conditions as in Table II.

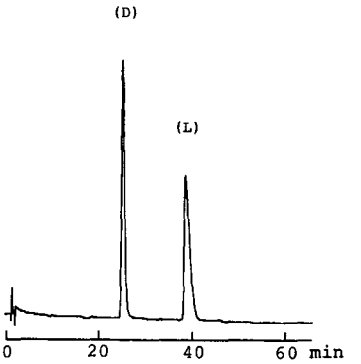

Fig. 5. HPLC separation of racemic lactic acid with phase **1**. Chromatographic conditions as in Table II.

racemic amino alcohols was effective for the chiral discrimination in these amino compounds.

The separation of amino acid and hydroxy acid enantiomers was not always adequate, but superior separations were obtained with several amino acids and hydroxy acids. Some racemic β-amino acids and β-hydroxy acids were also resolved.

A very interesting result was foud in the elution order of amino acid enantiomers. D-Isomers were retained strongly for valine, leucine, isolucine, etc., and L-isomers for serine, tyrosine histidine, tryptophan etc. The interaction between these amino acids and phase **1** is unclear, but it is noted that amino acids in the latter class contain some polar functional groups other than amino and carboxylic acid groups attached to the asymmetric carbon atom. We consider that such polar groups may produce a difference in the interaction to form the complex with **1**, and the inversion of the elution order of enantiomers may depend on the nature of the polar groups.

It should be noted that the durability of the column was influenced by the mobile phase. The compositions of the mobile phases were as given in Table II. The column conditioning by passing a 1 m*M* aqueous solution of copper(II) acetate after daily use was effective in preventing changes in the retention parameters, enantioselectivity or efficiency of the column.

The use of the copper(II) complex **2** as a chiral stationary phase in gas chromatography was reported previously [25–27]. It is expected that the use

TABLE II

ENANTIOMER SEPARATIONS BY HPLC WITH PHASE 1

Mobile phase: (A) 1 m*M* copper(II) sulphate in water; (B) 2 m*M* copper(II) sulphate in water–acetonitrile (85:15); (C) 2 m*M* copper(II) sulphate in water–acetonitrile (80:20). A flow-rate of 1 ml/min was typically 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 isomers, respectively; α = separation factor (k'_2/k'_1).

Compound	k'_1	k'_2	α	Mobile phase
Amino alcohols				
Norphenylephrine	2.90	3.57	1.23	A
Normetanephrine	6.25	7.19	1.15	A
Norephedrine	3.85	4.27	1.11	A
p-Hydroxynorephedrine	1.56	1.76	1.13	A
Atenolol	5.98	6.40	1.07	A
Propranolol	9.85	10.44	1.06	B
Phenylalaninol	5.43	11.07	2.04	A
Amines				
1-Phenyl-2-(*p*-tolyl)ethylamine	17.60	28.51	1.62	C
α-Amino-ε-caprolactam	0.70	1.34	1.91	A
Homocysteine thiolactone	6.03	7.19	1.19	A
Ketamine	1.47	1.85	1.26	B
Amino acids				
Serine	0.21 (D)	0.25 (L)	1.19	A
Allothreonine	0.32 (D)	0.55 (L)	1.72	A
Proline	0.69 (L)	0.84 (D)	1.22	A
Valine	1.40 (L)	1.81 (D)	1.29	A
Methionine	3.04 (D)	3.95 (L)	1.30	A
Allo-isoleucine	3.19 (L)	3.82 (D)	1.20	A
Histidine	3.82 (D)	4.51 (L)	1.18	A
tert.-Leucine	5.45 (L)	7.30 (D)	1.34	A
Leucine	5.66 (L)	6.17 (D)	1.09	A
Aspartic acid	5.87 (L)	6.52 (D)	1.11	A
Isoleucine	6.39 (L)	7.34 (D)	1.15	A
Phenylalanine	2.18 (D)	3.79 (L)	1.74	B
Tryptophan	3.21 (D)	6.58 (L)	2.05	B
3-Aminobutyric acid	1.91	2.29	1.20	A
3-Amino-2-methylpropionic acid	2.87	3.09	1.08	A
Hydroxy acids				
Lactic acid	17.36	27.06	1.56	B
Glyceric acid	13.61	15.40	1.13	B
2-Hydroxybutyric acid[a]	41.86	91.59	2.19	B
3-Hydroxybutyric acid[b]	53.53	62.07	1.16	B

[a] Column: 10 mm × 4 mm I.D.
[b] Column: 50 mm × 4.6 mm I.D.

of both HPLC and GC with copper(II) complexes of Schiff bases of chiral amino alcohols may extend the scope of enantiomer separations to a wide range of racemic compounds.

CONCLUSIONS

The copper(II) complexes of Schiff bases of chiral amino alcohols **1** and **2** are very promising as coat-

ing agents on reversed-phase material for the direct enantiomer separation of racemic amino alcohols, amines, amino acids and hydroxy acids by ligand-exchange HPLC. It is suggested that amino or hydroxyl groups attached to the asymmetric carbon atom and other polar functional groups may play an important cooperative role in the complexation with phase **1** or **2** for chiral discrimination.

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