A Novel Chiral Stationary Phase for Optical Resolution of Amino Acids and Their Derivatives by Ligand-Exchange High-Performance Liquid Chromatography

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Synopsis. A novel chiral stationary phase, [(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethylamino]acetic acid, was prepared from (−)-norephedrine, and its sodium salt was bound to silica gel pretreated with [3-(glycidyloxy)propyl]trimethoxysilane. The chiral stationary phase was found to be effective for the optical resolution of amino acids and their derivatives by ligand-exchange high-performance liquid chromatography using aq copper(II) sulfate solution as an eluent.

The chromatographic resolution of amino acids is achieved by ligand-exchange chromatography using copper(II) complexes of chiral stationary phases (CSPs). Gübitz and his co-workers developed practically useful packings for high-performance liquid chromatography (HPLC), in which copper(II) complexes of various amino acids are covalently bonded to silica gel pretreated with [3-(glycidyloxy)-propyl]trimethoxysilane.[1-5] Among these CSPs, L-hydroxyproline shows the best chiral recognition ability. Moreover, recently we developed and reported new CSPs consisting of optically active *erythro*- and *threo*-2-carboxymethylamino-1,2-diphenylethanol moieties.[6,7] These results indicate that hydroxyl group in the chiral amino acid moieties may play an important role for the chiral recognition and that optically active 2-aminoalkanols would be applicable as chiral moieties for CSPs.

On the basis of this consideration, we prepared a novel CSP, [(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethylamino]acetic acid, from (−)-norephedrine and applied it for ligand-exchange HPLC.

Sodium [(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethylamino]acetate (sodium salt of N-carboxymethylated norephedrine) (**2**) was easily prepared by ethoxycarbonylmethylation of (−)-norephedrine (**1**) with ethyl bromoacetate, followed by hydrolysis with aq NaOH solution. Without further purification, the sodium salt was allowed to react with silica gel pretreated with [3-(glycidyloxy)propyl]trimethoxysilane to give a new covalently-bonded CSP (**3**). The CSP was converted to copper(II) complex (**4**) by treatment with aq $CuSO_4$ solution. The silica gel, bonded with the CSP, was packed in a stainless steel column by the ascending slurry technique.

A number of amino acids and *N*-benzyloxycarbonylated (Z-) amino acids could be resolved on the copper(II) complex of this CSP by means of ligand-exchange HPLC using an aq copper(II) sulfate solution as a mobile phase. But, this CSP could not resolve amino acids such as alanine, leucine, methionine, phenylglycine, glutamine, glutamic acid, citrulline, and histidine. Typical chromatograms for the resolution of amino acids by HPLC are shown in Fig. 1, and the corresponding chromatographic parameters are given in the Table 1.

The CSPs, derived from *erythro*- and *threo*-2-amino-1,2-diphenylethanol, have quite different chiral recognition ability each other for amino acids and their derivatives, namely, amino acids and their derivatives resolved can be devided into two groups.[6,7] But, **4** could resolve amino acids and their derivatives of the two groups, although the scope of application and the α values obtained are smaller than those showed when *erythro*- and *threo*-2-amino-1,2-diphenylethanol moieties were used as CSPs.[6,7] This difference in the chiral recognition should result from the difference of the substituent at C2. In the case of *erythro*- and *threo*-2-amino-1,2-diphenylethanol, the

Table 1. Chromatographic Parameters for the Resolution of Amino Acids and N-Benzyloxycarbonylated Amino Acids

Racemate	Capacity factor k'_L	Capacity factor k'_D	Separation factor α	Resolution factor R_s
Valine	7.50	8.28	1.10	1.10
Isoleucine	8.94	9.90	1.11	1.15
tert-Leucine	6.58	7.65	1.16	1.00
Phenylalanine	10.18	11.60	1.14	1.15
Tyrosine	8.28	10.13	1.22	1.51
Proline	9.73	8.74	1.11	0.87
Asparagine	4.39	3.75	1.17	1.38
Aspartic acid	5.22	6.05	1.16	1.06
Serine	4.40	4.72	1.07	0.56
Threonine	4.61	5.04	1.09	0.83
Tryptophan	16.20	18.67	1.15	0.83
Arginine HCl	9.05	9.75	1.08	0.55
Lysine HCl	7.43	7.15	1.04	—
Z-Phenylalanine	9.92	9.10	1.09	—
Z-Alanine	3.56	3.34	1.07	—
Z-Valine	4.74	4.36	1.09	—
Z-Asparagine	3.50	3.17	1.10	0.95

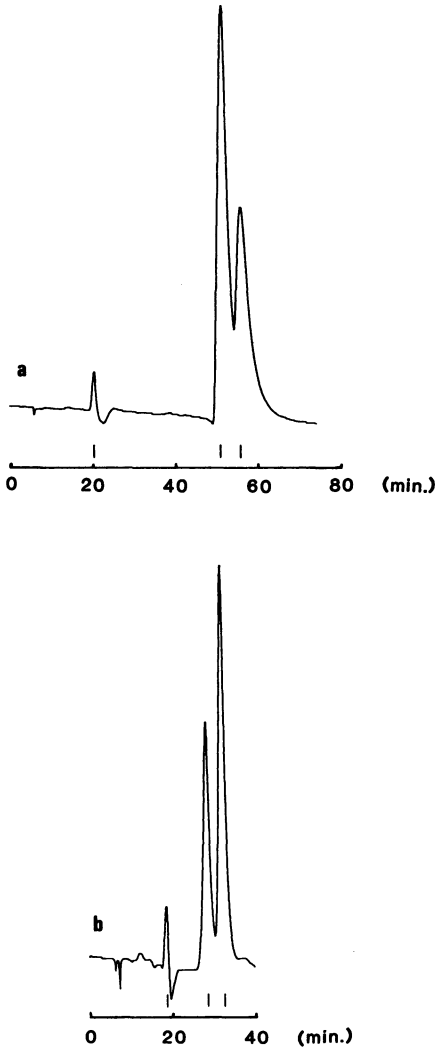

Fig. 1. The HPLC Chromatograms of (a) valine and (b) asparagine (D/L=1/2).

π–π interaction between the phenyl groups at C1 and C2 play an important role to fix the comformation of the copper(II) chelates. On the other hand, the chelate of **4**, in which the π–π interaction is absent, would be flexible. The flexibility would result in the samller α values for amino acids and their derivatives in the two groups.

Moreover, the CSP, derived from (*R*)-2-amino-2-phenylethanol showed no chiral recognition ability, indicating that the phenyl group at C1 plays also an important role for the chiral recognition.

Experimental

Materials. (−)-Norephedrine was purchased from Aldrich Chemical Company, silica gel, Develosil 100-5 from Nomura Chemical Co., [3-(glycidyloxy)propyl]trimethoxysilane from Shin-Etsu Chemical Co., amino acids from the Peptide Institute, and *N*-benzyloxycarbonylated amino acids from Sigma Chemical Company.

All solvents were of reagent grade.

Instruments. Melting point was measured by a Laboratory Devices MEL-TEMP apparatus and is uncorrected. IR spectra were recorded on a JASCO IR-810 spectrometer. ^{1}H NMR was recorded on a JOEL JNM-PMX60 at 60 MHz using TMS as an internal standard. A JASCO TRIROTAR-V, a Rheodyne 7125 injector, a JASCO UVIDEC-100-IV UV detector, and a NIHON CROMATO CDS 86 Ver. 2.7 data processor were used for HPLC. The column temperature was maintained at 35 °C by a JASCO TU-100 column oven.

Ethyl [(1*R*,2*S*)-2-Hydroxy-1-methyl-2-phenylethylamino]-acetate. To a solution of (−)-norephedrine (1.21 g, 8 mmol) in dichloromethane (10 ml) was added drop by drop a solution of ethyl bromoacetate (1.50 g, 9 mmol) in dichloromethane (10 ml) in a period of 30 min at room temperature. After stirring at the temperature for additional 2.5 h, triethylamine (1.2 ml, about 9 mmol) was added to the solution, and the reaction mixture was stirred for 2.5 h. Then, the solvent was evaporated, and benzene (ca. 100 ml) was added to the residue. The benzene solution was washed with water and sat. NaCl solution and dried with Na_2SO_4. After evaporation of the solvent under reduced pressure, crude product was purified by silica-gel chromatography

(eluent: hexane/ethyl acetate=1/1) to give ethyl [(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethylamino]acetate in 78% yield. Glassy oil. $[\alpha]_D^{22.4}$ +2.4° (*c* 0.414, EtOH). IR (KBr): 3470, 1747, 1455, 1241, 1148, and 703 cm^{-1}. ^1H NMR ($CDCl_3$): 0.78 (d, 3H, *J*=7 Hz), 1.22 (t, 3H, *J*=7 Hz), 2.74 (m, 3H), 3.30 (s, 2H), 4.08 (q, 2H, *J*=7 Hz), 4.57 (d, 1H, *J*=4 Hz), and 7.23 (s, 5H).

Calcd for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90%. Found: C, 65.43; H, 8.02; N, 5.94%.

Sodium [(1*R*,2*S*)-2-Hydroxy-1-methyl-2-phenylethylamino]-acetate (2). To a solution of ethyl [(1*R*,2*S*)-2-hydroxy-1-methyl-2-phenylethylamino]acetate (1.66 g, 7 mmol) was added 7.2 ml of 1 $mol \cdot dm^{-3}$ NaOH solution at room temperature, and the solution was stirred overnight at room temperature. Evaporation of the solvent gave 1.60 g of slightly yellow-colored solid mass. The mass was identified to be **2** by IR spectroscopy. IR (KBr): 3400, 1600, 1415, 1000, 760, 700 cm^{-1}.

Coating on Silica Gel. A suspension of silica gel (50 g), dried for 3 h at 120 °C, in dry benzene (500 ml) was refluxed, and the benzene (ca. 50 ml) was distilled off. Then, [3-(glycidyloxy)propyl]trimethoxysilane (25 ml) was added to the suspension. The suspension was refluxed for 8 h with removal of methanol formed from the mixture. After cooling, the benzene was removed by filtration, and the silica gel was thoroughly washed with dichloromethane. To a solution of **2** in methanol (20 ml) was added the silica gel (7.0 g), and the mixture was stirred for 6 d at 30 °C. The CSP-bonded silica gel was collected by filtration, washed with methanol, and poured into 1 w/v% $CuSO_4$ solution (150 ml). The copper(II)-loaded silica gel was collected by filtration and washed thoroughly with 2.5×10^{-4} $mol \cdot dm^{-3}$ $CuSO_4$ solution. Elemental analysis for the copper-loaded silica gel gave C, 6.00; H, 1.35; N, 0.32% (C, 4.20; H, 1.25% for the silanized silica gel).

Chromatography. The copper-loaded silica gel, suspended in 2.5×10^{-4} $mol \cdot dm^{-3}$ $CuSO_4$ solution, was packed in a stainless steel column (25×0.46 (i.d.) cm) by the slurry technique under the pressure of 250 $kg \cdot cm^{-2}$.

The chromatography was carried out at 35 °C at a flow-rate of 0.5 $ml \cdot min^{-1}$ using 2.5×10^{-4} $mol \cdot dm^{-3}$ $CuSO_4$ solution as a mobile phase. The dead volume of the column was estimated to be 3.00 cm^3 with water.

The *k'* values of unresolved amino acids were as follows; alanine (5.96), leucine (9.35), methionine (7.95), phenylglycine (8.77), glutamine (10.53), glutamic acid (9.68), citrulline (6.10), and histidine (13.67).

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