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Note

Complete resolution of DL-isoleucine by droplet counter-current chromatography

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Amino acid enantiomers have been resolved by column liquid chromatography using enantioselective differentiation of the copper complexes of chiral amino acids or their derivatives. The methods can be classified into three groups: (1) a chiral reagent is dissolved in the mobile phase and an achiral ion-exchange resin[1] or reversed phase silica gel[2] is used; (2) a chiral reagent is bound covalently to make a chiral stationary phase[3]; (3) a chiral reagent is coated on an achiral stationary phase[4,5]. In these instances, the surface of the solid stationary phase is considered to have a significant effect on the development of enantioselectivity and is involved in the mechanism of enantioselective differentiation.

Whereas the enantioselective discrimination of mixed ligand copper complexes is hardly observable in a homogeneous aqueous solution[6,7], we found an enantioselective distribution of neutral DL-amino acids in liquid–liquid two-phase system of *n*-butanol–water containing copper(II) ion and N-*n*-dodecyl-L-proline (C_{12}-Pro), and determined the distribution ratios of several neutral amino acid enantiomers in this two-phase system. By employing this two-phase system and droplet counter-current chromatography (DCC)[8], DL-isoleucine was completely resolved.

EXPERIMENTAL

Reagents

C_{12}-Pro was prepared as follows: *n*-dodecanal (0.2 mol) was dissolved in ethanol (150 ml). L-Proline (0.1 mol) and 5% palladium on carbon catalyst (1.5 g) were added to the solution as a suspension. The mixture was stirred with hydrogen until no L-proline was detected by thin-layer chromatography. The catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was washed with diethyl ether and recrystallized from diethyl ether. Analysis: calculated for $C_{17}H_{33}NO_2$, C 72.04, H 11.73, N 4.94; found, C 71.84, H 11.62, N 5.12%.

The other reagents were of analytical-reagent grade and purchased from commercial sources.

Preparation of aqueous and organic phases

The two-phase system was prepared by equilibrating equal volumes of 50 m*M*

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acetate buffer (pH 5.5) containing 1 m*M* copper(II) acetate and *n*-butanol containing 2 m*M* C_{12}-Pro at room temperature.

Determination of distribution ratios of amino acid enantiomers

Both 2 ml of the organic phase and the aqueous phase containing 0.1 m*M* DL-amino acid were placed in a centrifuge tube and the mixture was equilibrated by vortex mixing for 2 min. After centrifugation for 5 min at 3000 rpm, the concentrations of enantiomers in the aqueous phase were determined by ligand-exchange chromatography[2]. The distribution ratio is expressed by $D = (C_i - C_{aq})/C_{aq}$, where C_i is the initial concentration of amino acid and C_{aq} is the concentration of amino acid after equilibration. The separation factor is the ratio of distribution ratios of D- to L-amino acid.

Droplet counter-current chromatography

The column was made by connecting 400 pieces of Teflon tubing of two sizes, 40 cm × 4 mm and 50 cm × 1 mm I.D., alternatively in series. An aliquot of 20 ml of 1 m*M* DL-isoleucine in the mobile phase was injected into the column, and the separation was effected in the descending mode at a flow-rate of 1.1 ml/min. Fractions collected every 10 ml were analysed by ligand-exchange chromatography[2].

RESULTS AND DISCUSSION

Significantly higher enantioselectivity expressed in terms of separation factors was obtained for neutral amino acids than that previously observed in enantioselective solvent extraction[9–11] (Table I). In the system, essentially all of the copper(II) ions are extracted into the organic phase as a complex with C_{12}-Pro, which promotes the distribution of amino acid enantiomers into the organic phase.

Baseline resolution was achieved by using this solvent system and DCC (Fig. 1). DCC is support-free liquid–liquid partition chromatography in which no solid

TABLE I

DISTRIBUTION RATIOS AND SEPARATION FACTORS OF NEUTRAL AMINO ACID ENANTIOMERS

Amino acid	*Enantiomer*	*Distribution ratio*	*Separation factor*
Valine	D	0.71	1.69
	L	0.42	
Leucine	D	1.41	1.53
	L	0.92	
Isoleucine	D	1.43	2.01
	L	0.71	
Methionine	D	0.52	1.18
	L	0.44	
Tyrosine	D	0.97	1.05
	L	0.92	
Phenylalanine	D	2.33	1.29
	L	1.81	

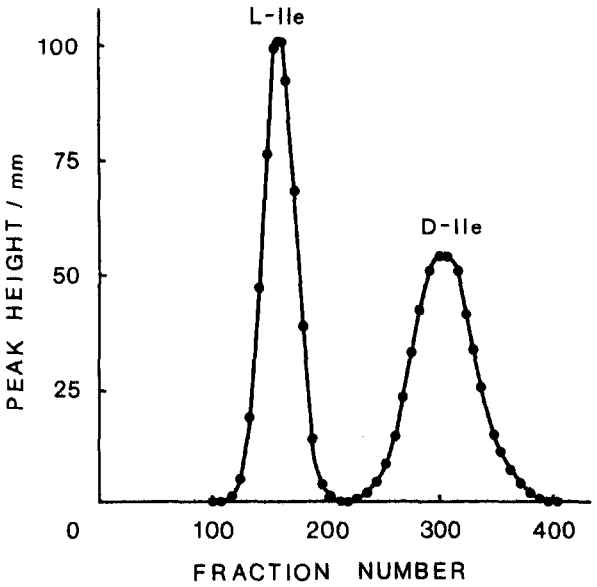

Fig. 1. Separation of DL-isoleucine by DCC. The stationary and mobile phases used were the organic and aqueous phases prepared by equilibrating equal volumes of 50 m*M* acetate buffer (pH 5.5) containing 1 m*M* copper(II) acetate and *n*-butanol containing 2 m*M* C_{12}-Pro. The column consisted of 400 pieces of Teflon tubing of two sizes, 40 cm × 4 mm I.D. and 50 cm × 1 mm I.D., alternately in series. The separation was effected in the descending mode at a flow-rate of 1.1 ml/min and 10-ml fractions were collected.

material is required to support the liquid stationary phase and separation occurs precisely according to the differences in distribution ratios. The capacity factors of D- and L-isoleucine observed were identical with those calculated from their distribution ratios and the volumes of the stationary and mobile phases in the column. This identity also implies that the distribution ratios of D- and L-isoleucine are constant under the chromatographic conditions used. However, if the concentration of DL-isoleucine is increased and exceeds that of the copper(II) ion, the distribution ratios decrease rapidly. Consequently, in order to achieve baseline resolution, the concentration of DL-amino acid to be resolved is kept at least below the concentration of copper(II) ions in the system.

As the present system does not include any surface of solid material, it is obvious that the enantioselective complexation can proceed in a two-phase solution, and the presence of a solid surface is not essential for the development of enantioselectivity. However, the separation factors obtained are smaller than those observed in high-performance liquid chromatography[4], even if the same chiral reagent is coated on ODS-silica gel[12]. Whether the surface of silica gel amplifies the intrinsic enantioselectivity of the complex or some other mechanism of enantioselective discrimination which involves the octadecyl chain or the surface of the silica gel matrix remains to be clarified.

In this experiment, 2.6 mg of DL-isoleucine was resolved. However, larger amounts can be resolved if the concentrations of chiral reagent and copper(II) ions in the organic phase are increased. In DCC, the number of theoretical plates is pro-

portional to the length of the column and DL-amino acids with lower separation factors will be resolved by increasing the column length.

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