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Direct enantiomeric separation of β-amino acids and β-amino alcohols by ligand-exchange chromatography

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ABSTRACT

Three underivatized β-amino acids and various aromatic β-amino alcohols were separated into enantiomers by high-performance liquid chromatography using octadecylsilanized silica coated with N-*n*-dodecyl-L-hydroxyproline as the stationary phase and acetate buffer containing copper(II) as the mobile phase.

INTRODUCTION

Although ligand-exchange chromatography (LEC) is very effective for enantiomeric separations of chelating reagents, the application of this technique has been mainly focused on α-amino acids and α-hydroxycarboxylic acids [1]. There have been only a few studies on the direct enantiomeric separation of β-amino alcohols with phenol moieties by LEC [2,3]. For indirect enantiomeric separations of β-amino alcohols, two widely applicable LEC methods by which aromatic and aliphatic β-amino alcohols can be separated have been published [4–6]. Lam and Malikin [4] showed that reversed-phase chromatography with a chiral mobile phase containing Cu(II)–L-proline is applicable to the derivatives of various amino alcohol with *o*-phthaldehyde and a chiral thiol. Gelber and co-workers [5,6] employed L-proline grafted silica gel as a stationary phase and copper(II) solution as a mobile phase for the separation. In their method, the stationary phase was laboratory-made and the β-amino alcohols were derivatized to Schiff bases in order to improve the coordination power to copper(II) under acidic conditions. Gelber *et al.* [5] considered the direct resolution of underivatized β-amino alcohols by LEC using a silica matrix packing to be impracticable, because complexation between copper(II) and a β-amino alcohol requires basic conditions, which would cause a deterioration of column stability.

In a previous paper [7], we reported that underivatized β-amino alcohols which have a 2-amino-1-phenylethanol moiety could be separated into enantiomers by LEC independent of the phenol moiety of the structure. For this process we used octadecylsilanized silica gel (ODS) coated with N-*n*-dodeceyl-L-hydroxyproline (C_{12}-Hyp) and a mobile phase containing copper(II) at pH 6.0 We have now

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attempted to extend the applicability of this method to the β-amino acids and aromatic β-amino alcohols listed in Tables I and II, respectively.

In 1979, Davankov *et al.* [8] reported a method for the enantiomeric separation of β-amino acids; this was applicable to compound **3**. Their method is not applicable for analytical purposes, but can be used for preparative purposes. In 1986, Lindner and Hirschbock [3] reported the enantiomeric separation of compound **2** with an ODS column and a chiral mobile phase containing copper(II) and an L-tartaric acid derivative. They reported that β-amino acids were difficult to separate by LEC. More recently, Griffith *et al.* [9] reported separations of aliphatic β-amino acids which were derivatized to the N-3,5-dinitrobenzoyl alkyl esters followed by chromatography on a Pirkle-type column. It should be noted that the increase in the excretion of compound **1** has been of interest in connection with cancer markers, lead poisoning, etc. [10,11].

The structure of the β-amino alcohols shown in Table II can be divided into three types. Type I compounds (**4, 5, 6, 9, 10**), contain an amino group attached to a primary carbon atom and a secondary alcohol group. Conversely, type II compounds (**7, 8**), contain an amino group attached to a secondary carbon atom and a primary alcohol. Type III compounds (**11, 12**), which have two asymmetric carbon atoms, contain an amino group attached to a secondary carbon atom and a secondary alcohol group.

Many sympathomimetic amines have type I structures [12]. For example, many β-adrenergic antagonists (β-blockers) used therapeutically have a 2-amino-3-aryloxy-2-propanol component (compounds **9** and **10**), while many β-adrenergic agonists have a 2-amino-1-phenylethanol component. As might be expected for such useful compounds, separation methods have been the subject of a number of studies. For the β-blockers, a number of enantiomeric separation methods, using high-performance liquid chromatography, have been published [13–17]. For the agonists, partial resolution by LEC [2,3], and complete resolution by ion-pair chromatography have been published [18]. Type II compounds derived from α-amino acid esters have been used in studies of the inhibition of enzymatic reactions, *e.g.*, compound **5** has been used in the binding of phenylalanine to phenylalanine tRNA synthetase [19,20]. Type III compounds **11** and **12** have *erythro* and *threo* configurations, respectively, and have been used as chiral counter ions [21] for the fractional crystallization of acidic compounds and as catalysts of asymmetric reactions [21].

In this paper, we demonstrate that β-amino acids and various β-amino alcohols, including noradrenaline, phenylglycinol, 1-amino-3-phenyl-2-propanol and 2-amino-1,2-diphenylethanol, can be separated into enantiomers by LEC, using ODS coated with C_{12}-Hyp as the stationary phase.

EXPERIMENTAL

Samples

Compounds **1–5** were purchased from commercial sources. Compounds **7** and **8** were prepared by reduction of the appropriate amino acid esters [22]. Compounds **6, 9** and **10** were prepared by addition of ammonia or methylamine to the corresponding epoxides [23,24]. Compounds **11** and **12** were prepared by reduction of α-benzoin oxime [25]. To determine the elution order of enantomers, chiral samples were used. Chiral compounds **4** and **7** were commercially available. Chiral compound **8** was prepared from L-phenylalanine. Chiral compounds **5, 9–12** were obtained by

fractional crystallization methods [25–28]. Chiral compound **6** was prepared by reduction of (*S*)-phenyllactamide [29], which was derived from (*S*)-L-phenyllactic acid (commercially available). The compounds gave correct analytical data.

Chromatography

The column packing was Develosil ODS-5 (Nomura Chemical, Gifu, Japan), with a length of either 5 or 15 cm and I.D. 4 mm. Four 15-cm columns in series were employed for compounds **1**, **2** and **4**, because these compounds have smaller separation factors (α) and/or capacity factors (k'). A 5-cm column was employed for compounds **3**, **11** and **12**. For the other sample compounds a 15-cm column was employed. The C_{12}-Hyp coating procedure and the eluent used have been described in a previous paper [7]. For the detection of compounds **11** and **12**, a UV monitor operated at 280 nm was used [7]. For the other solutes a spectrofluorometer using postcolumn reaction was used [7].

RESULTS AND DISCUSSION

β-Amino acids

Capacity factors and separation factors of the β-amino acids studied are listed in Table I. The chromatogram of compound **1** is shown in Fig. 1. We found that compound **3** could be completely separated on a 5-cm column. The large k' and α values of compound **3** suggest that hydrophobicity or the bulkiness of the phenyl group on the asymmetric centre of the compound plays an important role in the separation. For the more weakly retained compounds **1** and **2**, longer columns (four 15-cm columns) were applied.

β-Amino alcohols

Capacity factors and separation factors of the β-amino alcohols studied are listed in Table II. Because of its small k', long columns (four 15-cm columns) were used for noradrenaline (compound **4**). The chromatogram is shown in Fig. 2. Adrenaline, which is N-methylated noradrenaline, could not be separated by this method. The

TABLE I

CHEMICAL STRUCTURES, CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF THE β-AMINO ACIDS STUDIED

$H_2NCH(R^1)CH(R^2)COOH$

Compound[a]	R^1	R^2	EF[b]	k'_1[c]	α
1	H	CH_3	ND[d]	0.49	1.32
2	CH_3	H	ND	0.55	1.10
3	C_6H_5	H	ND	28.0	1.49

[a] Names of compounds: **1** = 3-amino-2-methylpropionic acid (3-aminoisobutyric acid); **2** = 3-aminobutyric acid; **3** = 3-amino-3-phenylpropionic acid.

[b] Configuration of the first-eluted enantiomer.

[c] Capacity factor of the first-eluted enantiomer.

[d] ND = Not determined.

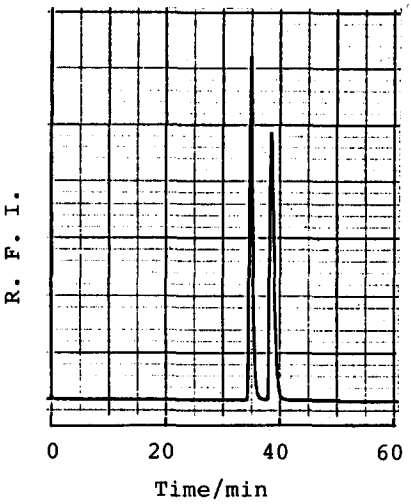

Fig. 1. Chromatogram of compound **1** (see Table I). Conditions: column length, 60 cm (15 cm × 4); flow-rate, 0.2 ml/min; sample size, 5 μg; eluent, 0.05 *M* acetate buffer (pH 6.0) containing 8 m*M* copper(II). R.F.I. = Relative fluorescence intensity.

chromatogram of compound **9** on a 15-cm column is shown in Fig. 3. Compound **10**, which is N-methylated **9**, gave a smaller α value than did compound **9**. The same trends were observed in the α values of the other two pairs of type 1 compounds [7].

The chromatogram of compound **11** is shown in Fig. 4. Both the k' and the α value of the *threo* form (compound **12**) are larger than those of the *erythro* form

TABLE II

CHEMICAL STRUCTURES, CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) OF THE β-AMINO ALCOHOLS STUDIED

$R^3HNCH(R^1)CH(R^2)OH$

Compound[a]	R^1	R^2	R^3	EF[b]	k'[c]	α
4	H	$C_6H_3(OH)_2$	H	*R*	1.28	1.14
5	H	C_6H_5	H	*S*	9.20	1.98
6	H	$CH_2C_6H_5$	H	*S*	21.1	1.26
7	C_6H_5	H	H	*R*	13.3	1.13
8	$CH_2C_6H_5$	H	H	ND[d]	27.0	(1.0)
9	H	$CH_2OC_6H_5$	H	*S*	24.6	1.63
10	H	$CH_2OC_6H_5$	CH_3	*S*	26.9	1.23
11	C_6H_5	C_6H_5	H	(1*S*,2*R*)	14.0	1.30
12	C_6H_5	C_6H_5	H	(1*S*,2*S*)	31.5	1.42

[a] Names of compounds: **4** = 2-amino-1-(3,4-dihydroxyphenyl)-ethanol (noradrenaline); **5** = 2-amino-1-phenylethanol (phenylethanolamine); **6** = 1-amino-3-phenyl-2-propanol; **7** = 2-amino-2-phenylethanol (phenylglycinol); **8** = 2-amino-3-phenylpropanol (phenylalaninol); **9** = 1-amino-3-phenoxy-2-propanol; **10** = 1-methylamino-3-phenoxy-2-propanol; **11** = (1*RS*,2*SR*)-2-amino-1,2-diphenylethanol (*erythro* form); **12** = (1*RS*,2*RS*)-2-amino-1,2-diphenylethanol (*threo* form).

[b–d] As Table I.

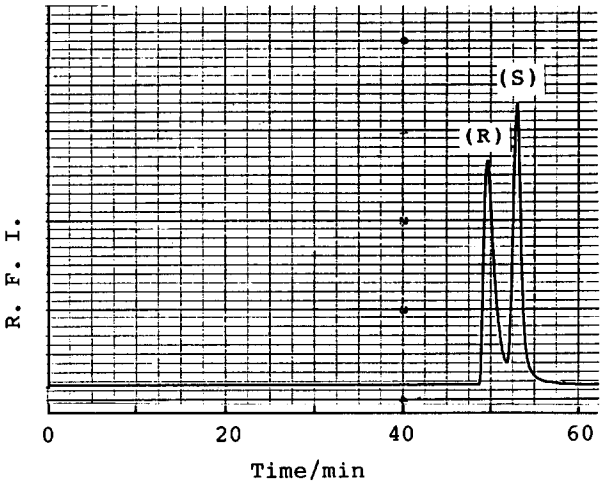

Fig. 2. Chromatogram of compound **4** (see Table II). Conditions: sample size, 0.5 μg; other conditions as in Fig. 1.

(compound **11**). Also, in a previous paper [7] we reported that both the k' and the α values of norpseudoephedrine (*threo* form) are larger than those of norephedrine (*erythro* form). The eluent used was a mixture of acetonitrile and acetate buffer containing copper(II). Using an eluent containing no acetonitrile, compounds **11** and **12** gave large k' values compared with those of other solutes used in this work. To shorten the analysis time, an eluent containing acetonitrile was used. Lowering the acetonitrile concentration in the eluent resulted in longer retentions and better resolution of the enantiomers. Even on 5-cm columns, the resolutions of compounds **11** and **12** were adequate. Because the adsorption of C_{12}-Hyp is dependent mainly on the hydrophobic interaction between the dodecyl group of C_{12}-Hyp and the octadecyl

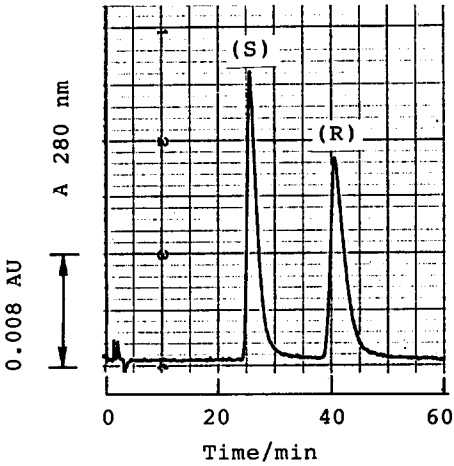

Fig. 3. Chromatogram of compound **9** (see Table II). Conditions: column length, 15 cm; flow-rate, 0.4 ml/min; sample size, 5 μg; eluent, 0.05 *M* acetate buffer (pH 6.0) containing 8 m*M* copper(II).

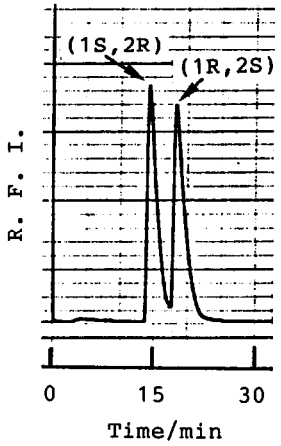

Fig. 4. Chromatogram of compound **11** (see Table II). Conditions: column length, 5 cm; flow-rate, 0.4 ml/min; sample size, 5 μg; eluent, acetonitrile–0.05 *M* acetate buffer (pH 5.75) containing 1 m*M* copper(II) (17.5:82.5, v/v).

group of ODS, the "bleeding" of C_{12}-Hyp from the column might be caused by an acetonitrile-rich eluent. To test the stability of the coating against the eluent used, 3 l of the eluent were delivered to the column. Subsequently no change in the retention of these compounds was observed, indicating that the stability of the coating was sufficient.

Concerning the positions of amino and alcohol groups in the structure, compounds **5** and **7** are geometric isomers of compounds **6** and **8**, respectively. Chromatograms of these compounds are shown Fig. 5. Comparing the α values of type I compounds (**5** and **7**) with those of type II compounds (**6** and **8**), the proposed method was found to be more convenient for the separation of type I compounds in the

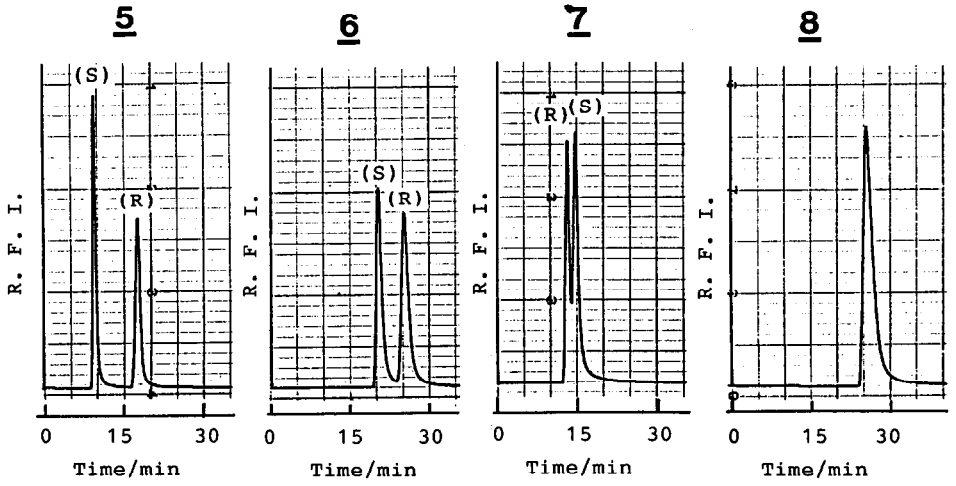

Fig. 5. Chromatograms of compounds **5**, **6**, **7** and **8** (see Table II). Conditions as in Fig. 3.

corresponding pair. For both the pair of type I compounds (**5** and **6**) and the pair of type II compounds (**6** and **7**), the α values of the compounds which have a phenyl group attached to the asymmetric carbon were larger than those of the compounds which have a benzyl group attached to the asymmetric carbon. These results indicate that the long distance between the bulky phenyl group and the chiral carbon atom was disadvantageous for the separation. Aliphatic β-amino alcohols with no aromatic group, such as 1-amino-2-butanol and 2-amino-1-butanol, could not be separated by this method, except for *trans*-2-aminocyclohexanol, which could be completely resolved using an eluent with a higher copper(II) concentration (12 m*M*, at pH 6).

Compound **8** and aliphatic β-amino alcohols could not be separated by the proposed method. However, we were able to accomplish a complete separation by LEC using ODS columns and a chiral mobile phase containing copper(II) and L-proline. These results will be published elsewhere.

As preliminary experiments have shown, the method possesses several advantages: precolumn derivatization is not required; the eluent is aqueous; β-amino acids and a variety of aromatic β-amino alcohols can be separated into enantiomers; and a commercially available column can be used.

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