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Enantiomeric separation of underivatized aliphatic β-amino alcohols by ligand-exchange chromatgraphy using barbital as an additive to the mobile phase

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ABSTRACT

Underivatized aliphatic β-amino alcohols with a secondary alcohol moiety were separated into enantiomers by high-performance liquid chromatography using octadecylsilanized silica coated with N-*n*-dodecyl-L-hydroxyproline as the stationary phase and an aqueous solution containing copper(II) and barbital as the mobile phase.

INTRODUCTION

In previous papers we reported that underivatized aromatic β-amino alcohols (BAAs) could be separated into enantiomers by ligand-exchange chromatography (LEC) using a copper(II) solution as the mobile phase and octadecylsilanized silica gel (ODS) coated with N-*n*-dodecyl-L-hydroxyproline (C_{12}-Hyp) as the stationary phase [1,2]. On the other hand, for underivatized aliphatic BAAs, our attempts using this methodology were unsuccessful because of the low retention or a small separation factor.

In other studies, we found that not only aromatic but also aliphatic BAAs were separated into enantiomers by LEC using an ODS column and a chiral mobile phase containing copper(II), L-proline and barbital (BB). Although BB is a chiral, addition of BB to the mobile phase was critical for the separation [3].

In this work, BB addition was applied to the separation of underivatized aliphatic BAAs on a column packed with C_{12}-Hyp-coated ODS as a chiral stationary phase.

EXPERIMENTAL

Samples

The preparation of 1-amino-2-pentanol was accomplished by addition of ammonia to 1,2-epoxypentane according to the described method with minor modifications [4]. The epoxide was prepared by two methods: methylation of butyraldehyde using trimethylsulphonium iodide [5] or oxidation of 1-pentene with *m*-chloroperoxybenzoic acid [6]. The results of elemental analysis of the oxalate salt were as follows: calculated for $C_6H_{14}NO_3$, C 48.63, H 9.52, N 9.45; found, C 48.40, H 9.62, N 9.32%.

The preparation of chiral 1-amino-2-pentanol could not be accomplished by a fractional crystallization method using L-tartrate, dibenzoyl-L-tartrate, L-glutamate, *d*-camphor sulphonate or *d*-bromocamphorsulphonate as the chiral counter anion. However, it was achieved by using (*S*)-norvaline as the starting material to obtain the *S*-isomer as outlined in Fig. 1. The deamination

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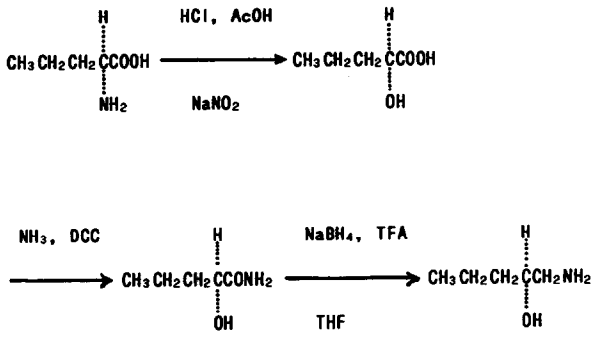

Fig. 1. Synthesis of (*S*)-1-amino-2-pentanol.

of (*S*)-norvaline [7] proceeds to (*S*)-2-hydroxyvaleric acid without racemization, as demonstrated by the chromatographic technique reported in a previous paper [8]. The solubility of lithium (*S*)-2-hydroxyvalerate is moderately low compared with the solubility of the sodium salt. Therefore, the reaction solution was poured into a cation-exchange column in the Li^+ form. The effluent from the column was concentrated and the lithium salt of the acid was obtained. After recrystallization, again using a cation-exchange column prepared in the acidic form, the lithium salt solution was converted into an acidic solution, which was dried and dissolved in chloroform. Using 1,3-dicyclohexcylcarbodiimide (DCC) and ammonia, the acid was derivatized to the amide [9]. Finally, the amide was reduced to the amine with sodium tetrahydroborate in trifluoroacetic acid–tetrahydrofuran [10]. After extraction with ethyl acetate, the amine was obtained as the oxalate salt. The results of elemental analysis of the oxalate salt were as follows: calculated for $C_6H_{14}NO_3$, C 48.63, H 9.52, N 9.45; found, C 48.46, H 9.38, N 9.37%; $[\alpha]_D^{27}$ + 7.7 (*c*, 0.60 in water).

The preparation of 1-amino-2-hexanol was accomplished by the method employed for the preparation of 1-amino-2-penatanol. 1,2-Epoxyhexene was commercially available. The results of elemental analysis of the oxalate salt were as follows: calculated for $C_7H_{16}NO_3$, C 51.90, H 9.95, N 8.33; found, C 51.83, H 9.94, N 8.64%.

(*S*)-1-Amino-2-hexanol was prepared according to the method employed for the preparation of (*S*)-1-amino-2-pentanol, but the results of elemental analysis were unsatisfactory. The crude product, however, was applicable for the determination of the elution order.

Other BAAs used were purchased from commercial sources.

Chromatography

The column used and the C_{12}-Hyp coating procedure have been described in a previous paper [1]. For the detection of BAAs a post-column reaction using *o*-phthalaldehyde to form a fluorophore was employed [1].

RESULTS AND DISCUSSION

The direct resolution of 1-amino-2-butanol, 1-amino-2-pentanol and 1-amino-2-hexanol, which contain an amino group attached to a primary carbon atom and a secondary alcohol group, was achieved. These 1-amino-2-ol type BAAs were all well resolved. Fig. 2 shows the chromatogram of 1-amino-2-pentanol. Conversely 2-amino-1-ol-type BAAs, which contain an amino group attached to a secondary carbon atom and a primary alcohol group, such as 2-amino-1-butanol and 2-amino-1-pentanol, could not be resolved under the conditions described in Fig. 2. A similar result, with the separation of 1-amino-2-ol-type aromatic BAAs being better than that of the corresponding 2-amino-1-ol-type aromatic BAAs on a column packed with C_{12}-Hyp-coated ODS, was reported in a previous paper [2]. These results indicate that the secondary alcohol group plays an important role in the separation mechanism in the present method. Fig. 3 shows that *trans*-2-aminocyclohexanol, which contains an amino group attached to a secondary carbon atom and a secondary alcohol group, could be separated even using a mobile phase containing no BB, but the separation was improved in the presence of BB. For 1-amino-2-propanol, only a partial separation was achieved even using three 15-cm columns in series and the mobile phase used in Fig. 2, because of the low retention.

We studied the influence of the BB, copper (II) and triethylamine(TEA) concentrations in the mobile phase and its pH on the separation of the three aliphatic 1-amino-2-ol-type BAA enantiomers.

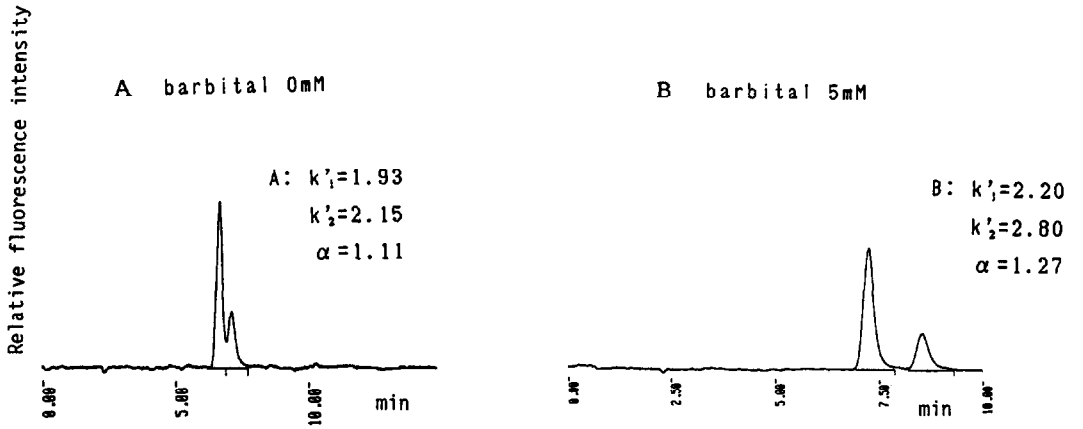

Fig. 2. (A) Chromatogram of 1-amino-2-pentanol. Column, Develosil ODS-5 coated with C_{12}-Hyp (150 mm × 4 mm I.D.); mobile phase, 12 m*M* copper(II) acetate and 20 m*M* TEA in 50 m*M* acetate buffer (pH 6.0, adjusted with acetic acid or NaOH); flow-rate, 0.5 ml/min; sample size, 20 μl, containing 0.1 μg of BAA (*S*/*R* = 3:1). Other conditions as in text. (B) Conditions as for (A) with the addition of 5 m*M* sodium barbital to the mobile phase.

The effect of BB concentration in the mobile phase on the capacity factor (k') and the separation factor (α) is shown in Fig. 4. The effect is concentration dependent in a non-linear and saturable manner. Maximum k' values were obtained at 5–10 m*M* BB, declining above 10 m*M* BB, but the α values above 10 m*M* BB remained approximately constant. BB can be replaced with other BB analogues such as amobarbital (Figs. 3 and 5).

The effect of addition of BB to the mobile phase on the separation of aromatic BAAs was also investigated. As shown in Figs. 5 and 6, the addition was effective for octopamine (1-amino-2-ol-type BAA) but not for phenylglycinol (2-amino-1-ol-type BAA). Although these structures are similar, the peaks of the octopamine enantiomers in Fig. 5 are tailing and those of phenylglycinol in Fig. 6 are fronting. The reason is not known.

Aldehydes and BAAs with a primary or secondary amine moiety can form an oxazolidine ring, and the oxazolidine could be separated into enantiomers [11]. BB has three carbonyl groups,

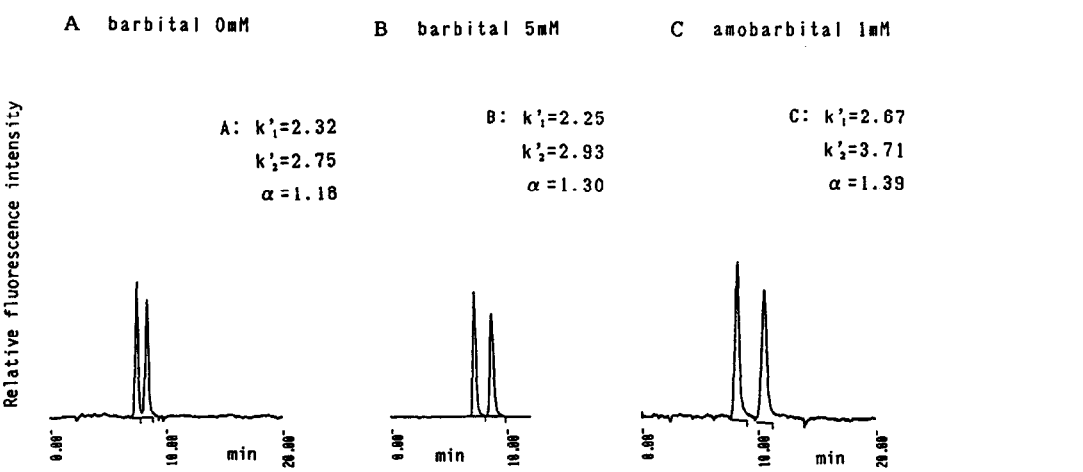

Fig. 3. (A) Chromatograms of *trans*-2-aminocyclohexanol. Conditions as in Fig. 2A. (B) Conditions as for (A) with the addition of 5 m*M* sodium barbital to the mobile phase. (C) Conditions as for (A) with the addition of 1 m*M* sodium amobarbital to the mobile phase.

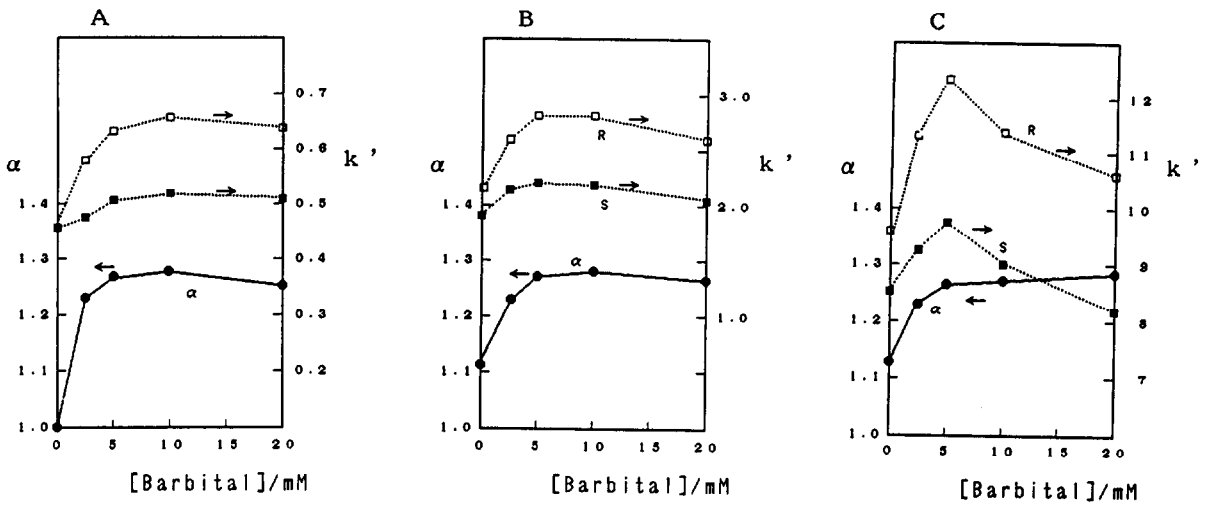

Fig. 4. Effect of BB concentration in the mobile phase on the enantiomeric separation of (A) 1-amino-2-butanol, (B) 1-amino-2-pentanol and (C) 1-amino-2-hexanol. Mobile phase, 8 m*M* copper(II) acetate, various concentrations of BB and 20 m*M* TEA in 50 m*M* acetate buffer (pH 6.0, adjusted with acetic acid or NaOH). Other conditions as in Fig. 2.

and the ring formation equilibrium between the BAA and BB may play some role in the separation. BAAs with a tertiary amine group cannot form such a ring; nevertheless, for the separation of 1-dimethylamino-2-propanol and 1-dimethylamino-2-butanol, a similar BB effect was also observed [12]. Hence oxazolidine formation does not seem to play a role in the separation. Although the exact role of BB is not clear, BB may coordinate with Cu(II). The use of BB as an additive is one of the advantages of the present method.

The separation was also strongly dependent on the copper(II) concentration, the TEA concentration and the pH of the mobile phase. Their influences on the separation of 1-amino-2-

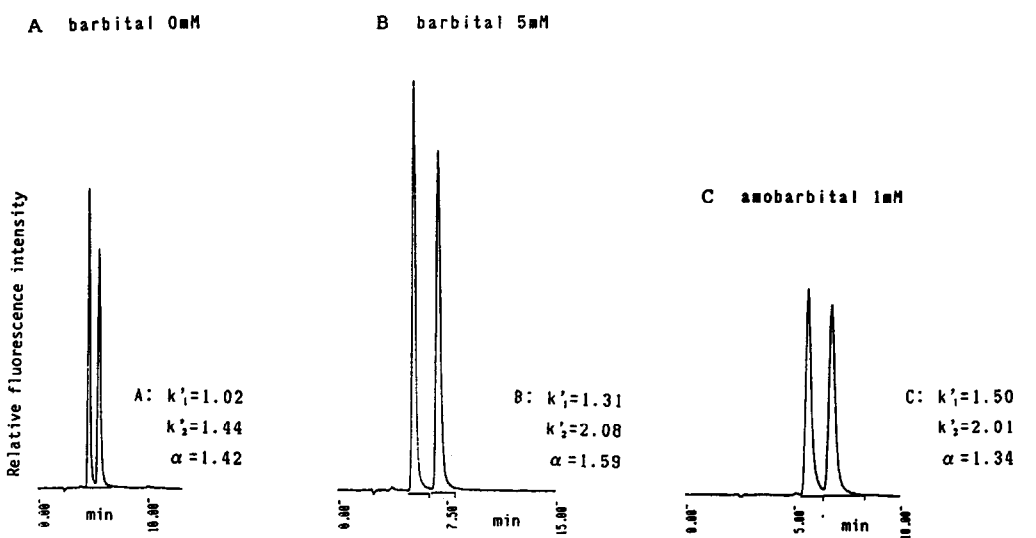

Fig. 5. (A) Chromatograms of octopamine. Conditions as in Fig. 2A. (B) Conditions as for (A) with the addition of 5 m*M* sodium barbital to the mobile phase. (C) Conditions as for (A) with the addition of 1 m*M* sodium amobarbital to the mobile phase.

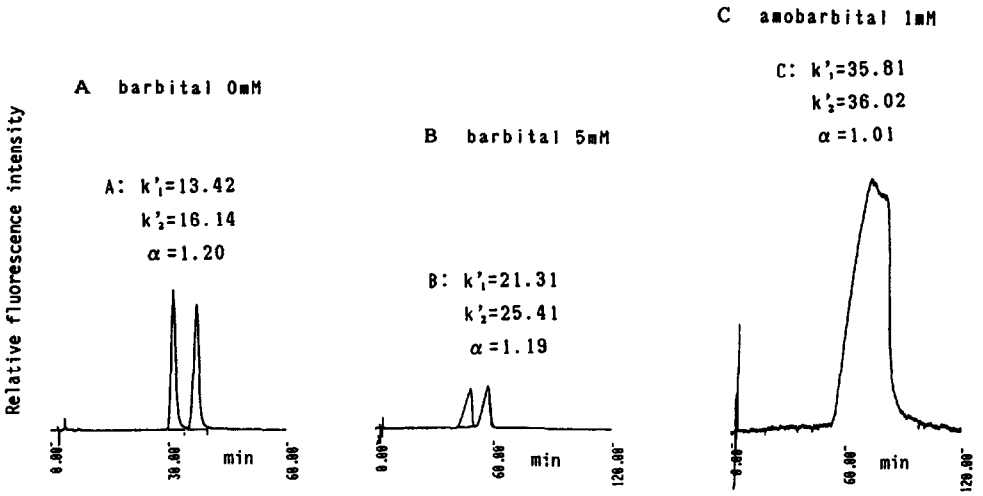

Fig. 6. (A) Chromatograms of phenylglycinol. Conditions as in Fig 2A. (B) Conditions as for (A) with the addition of 5 m*M* sodium barbital to the mobile phase. (C) Conditions as for (A) with the addition of 1 m*M* sodium amobarbital to the mobile phase.

butanol, 1-amino-2-pentanol and 1-amino-2-hexanol enantiomers were studied over the pH range 5–6 and with copper(II) concentrations in the range 1–16 m*M*. As shown in Figs. 7 and 8, increasing pH and copper(II) concentration of the mobile phase result in greater retention and better separation of the enantiomers. C_{12}-Hyp is an amino acid, and substantial complex formation with Cu^{2+} occurs at pH 5–6. With BAAs as solutes, the deprotonation of the BAAs is very low compared with that of the amino acid at this pH [13], and therefore the complex formation must increase as the pH increases. The strong dependence of retention and separation on pH 5–6 may be the reason. To avoid the precipitation of copper(II) hydroxide in the mobile phase, pH values above 6.2 were not studied.

The effect of TEA addition to the eluent on the k' and α values is shown in Fig. 9. It is well known that the addition of TEA to the mobile

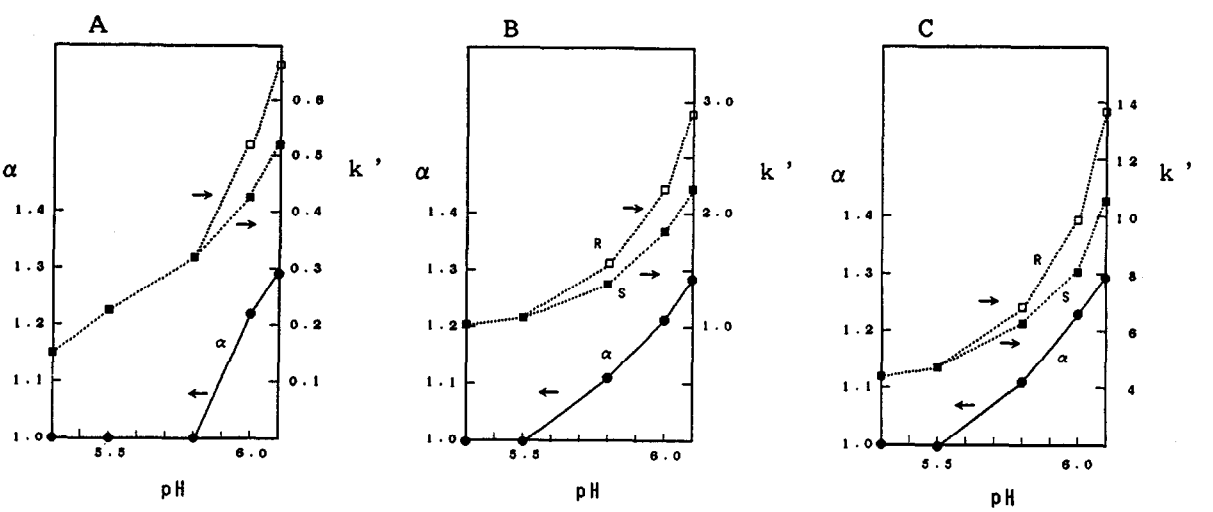

Fig. 7. Effect of pH of the mobile phase on the enantiomeric separation of (A) 1-amino-2-butanol, (B) 1-amino-2-pentanol and (C) 1-amino-2-hexanol. Mobile phase, 8 m*M* copper(II) acetate, 5 m*M* BB and 20 m*M* TEA in 50 m*M* acetate buffer (pH adjusted with acetic acid or NaOH). Other conditions as in Fig. 2.

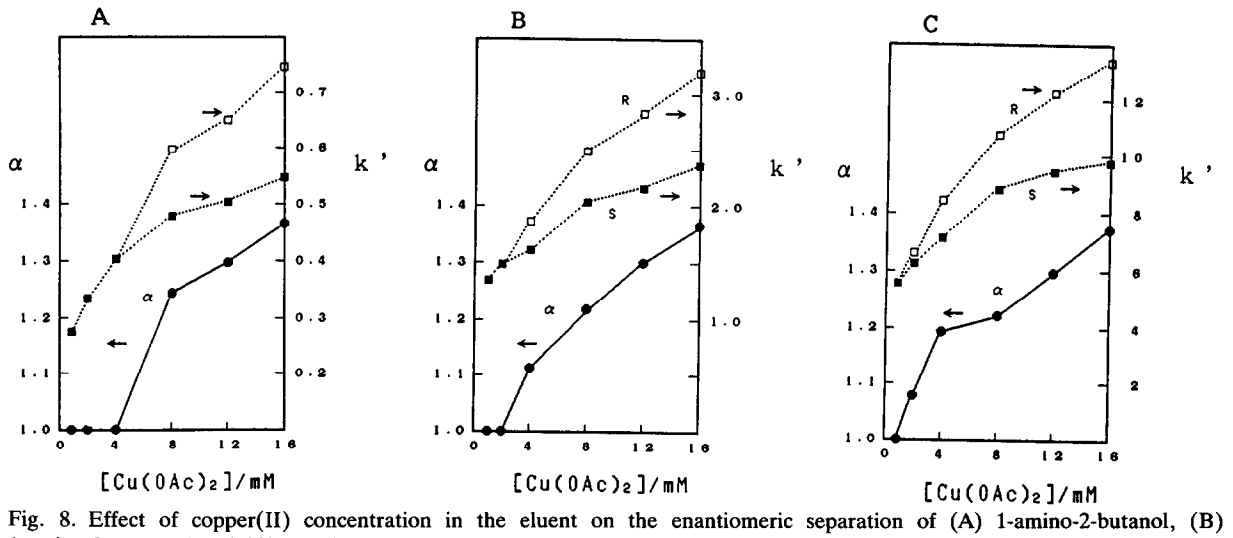

Fig. 8. Effect of copper(II) concentration in the eluent on the enantiomeric separation of (A) 1-amino-2-butanol, (B) 1-amino-2-pentanol and (C) 1-amino-2-hexanol. Mobile phase, various concentrations of copper(II) acetate, 5 m*M* BB and 20 m*M* TEA in 50 m*M* acetate buffer (pH 6.0, adjusted with acetic acid or NaOH). Other conditions as in Fig. 2.

phase improves the shape of amine peaks using a silica-based column. A 20 m*M* concentration of TEA resulted in an improvement in these separations, although it caused a decrease in the retention of the BAAs.

The final conditions selected were a mobile phase at pH 6 containing 8–16 m*M* copper(II), 5 m*M* barbital and 20 m*M* TEA, which provided a satisfactory separation of 1-amino-2-butanol, 1-amino-2-pentanol, 1-amino-2-hexanol and *trans*-2-aminocyclohexanol enantiomers. The results reported here show that the enantiomeric separation of underivatized aliphatic BAAs with a secondary alcohol moiety on a column packed with C_{12}-Hyp-coated ODS was improved by addition of BB to the mobile phase containing copper(II). Similarly, the separation of underivatized aromatic BAAs with a secondary alcohol

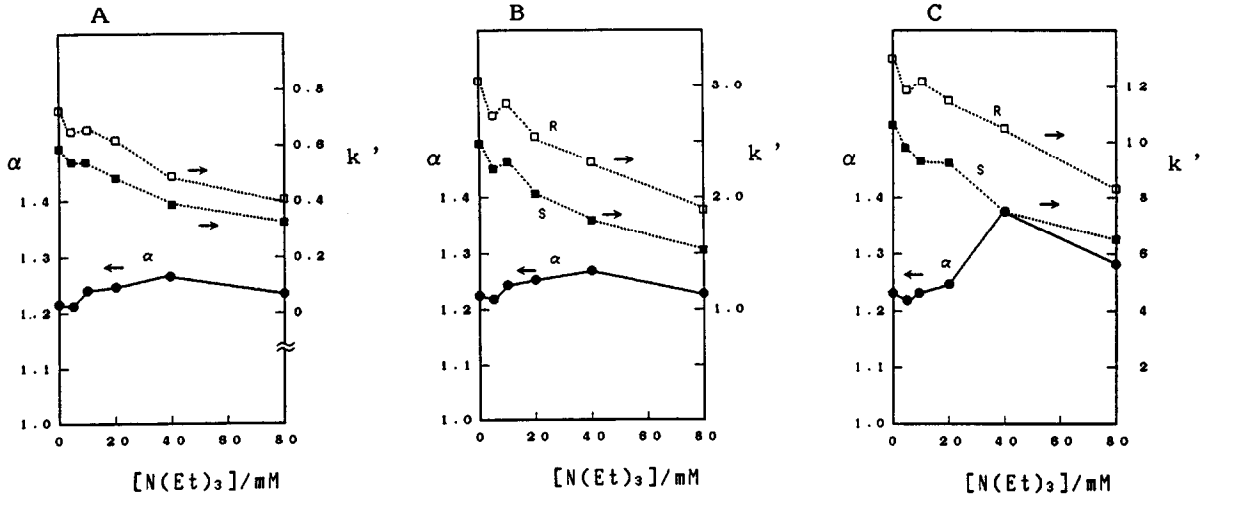

Fig. 9. Effect of TEA concentration in the eluent on the enantiomeric separation of (A) 1-amino-2-butanol, (B) 1-amino-2-pentanol and (C) 1-amino-2-hexanol. Mobile phase, 8 m*M* copper(II) acetate, 5 m*M* BB and various concentrations of TEA in 50 m*M* acetate buffer (pH 6.0, adjusted with acetic acid or NaOH). Other conditions as in Fig. 2.

moiety was improved. However, no improvements of the enantiomeric separation of 2-amino-1-ol-type BAAs, α-amino acids and β-amino acids were observed.

In subsequent work, we found that N-*n*-dodecylnorephedrine was also a useful coating reagent for ODS for the separation of both aliphatic 1-amino-2-ol- and 2-amino-1-ol-type BAAs into enantiomers. The details will be published elsewhere.

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