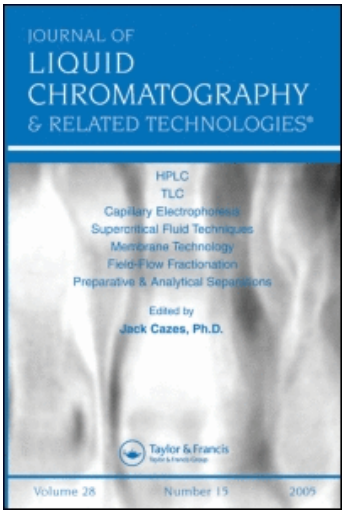

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CHIRAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF ENANTIOMERS OF α-METHYL α-AMINO ACIDS

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ABSTRACT

The enantiomeric separation by chiral high performance liquid chromatography of derivatized and underivatized α-methyl α-amino acids was achieved by two different methods. A cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase column (Daicel Chiralcel OD) was used to resolve N-benzyloxycarbonylated methyl esters. Excellent separations of enantiomers were achieved with all the α-methyl α-amino acids examined. A column packed with octadecylsilanized silica coated with N,S-dioctyl-D-penicillamine as a chiral ligand-exchange phase (Sumichiral OA-5000) was also used to resolve the underivatized amino acids. Excellent to good separations of enantiomers were achieved with a variety of underivatized α-methyl α-amino acids carrying aliphatic or aromatic side-chains by optimizing the amount of the organic component and the concentration of the copper(II) ion in the hydro-organic eluent.

INTRODUCTION

There has been a growing interest in homochiral α-alkylated α-amino acids, especially α-methyl α-amino acids, in recent years.[1] These amino acids

may act as enzyme inhibitors or antagonists of receptors. Introduction of α-alkylated residues into the naturally occurring peptide sequences leads to a restricted conformational freedom of the derived peptides. In addition, analogs bearing these residues are known to resist to hydrolysis by proteolytic enzymes. A number of methods using chiral auxiliaries or chiral phase transfer catalysts have been developed for the asymmetric synthesis of optically active α-alkylated amino acids.[2] In practice, however, enzymatic resolution procedures employing esterases, amidases, acylases, etc. have more often been utilized.[3] In this connection, rapid, simple, and accurate methods are required for the determination of enantiomeric purities of the products. High performance liquid chromatography (HPLC) has often been shown to meet this requirement.[4] In previous papers,[5,6] we reported two kinds of HPLC methods for the enantiomeric separation of non-protein α-amino acids of the general formula **2**. As the continuation of these works, we have examined the applicability of these procedures to the separation of enantiomers of α-methyl α-amino acids (**1**). (See Scheme 1).

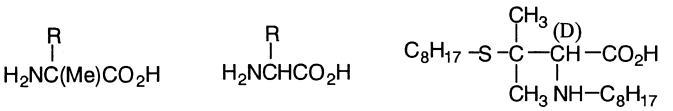
EXPERIMENTAL

Apparatus

The liquid chromatography system consisted of a Shimadzu (Japan) LC-10AS instrument, a Rheodyne (USA) 7725i sample injector, and a Shimadzu SPD-10A variable wavelength UV monitor. HPLC data were processed with a Shimadzu Chromatopac C-R6A data processor.

Chemicals

All the racemic α-methyl α-amino acids used in this study were purchased from Aldrich Chemical Co. (USA), Sigma Chemical Co. (USA), or Tokyo Chemical Industry (Japan). The amino acids were benzyloxycarbonylated and then esterified in the same manner as described in the previous paper.[5] Copper(II) sulfate pentahydrate and 2-propanol were purchased from Wako Chemical Co. (Japan).



Scheme 1. Structures.

HPLC Conditions

The enantiomeric separation of N-benzyloxycarbonylated α-methyl α-amino acid methyl esters was carried out using a Chiralcel OD column (4.6 mm i.d. × 250 mm) (Daicel Chemical Co., Japan) under the following conditions: mobile phase, hexane–2-propanol (9:1, v/v); flow rate, 1.0 mL min^{-1}; column temperature, 30°C; detection, UV at 254 nm. On the other hand, the column used for the resolution of underivatized α-methyl α-amino acids was Sumichiral OA-5000 (4.6 mm i.d. × 150 mm) (Sumika Chemical Analysis Service, Japan). The mobile phases included a 1–5 mM solution of copper(II) sulfate in water–2-propanol (95:5, 85:15, or 80:20, v/v) and an aqueous solution of copper(II) sulfate (1–5 mM). The flow rate was 1.0 mL min^{-1} unless otherwise noted. The column temperature was maintained at 30°C. UV absorbance was measured at 254 nm. The parameters used in the evaluation of enantiomeric separation by the above-mentioned columns are the separation factor, α, and the resolution, *Rs*:

$$a = k_2' / k_1'; \quad Rs = 2 \times (t_2 - t_1) / (W_1 + W_2),$$

where k_1' and k_2' are the capacity factors for the faster and slower eluting enantiomers, respectively, and W_1 and W_2 are the bandwidths of peaks of the faster and slower eluting enantiomers, respectively.

RESULTS AND DISCUSSION

We have already reported the high performance liquid chromatographic separation of enantiomeric N-protected amino acid esters on a cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase column (Daicel Chiralcel OD).[5] Of the derivatives examined, the methyl esters of the N-benzyloxycarbonyl (Z) derivatives of a number of non-protein α-amino acids showed excellent to good enantiomeric separation using hexane–2-propanol as a mobile phase. In general, amino acids carrying a bulky alkyl group near the α-carbon atom were resolved excellently. This suggests the applicability of this chiral stationary phase to the enantiomeric separation of α-substituted α-amino acids. Thus, the resolution of α-methyl α-amino acids (**1**) was examined in the form of their N-Z-protected methyl esters.

The results were compared with those for the corresponding α-H-amino acid (**2**) derivatives (Table 1). A typical chromatogram is shown in Figure 1. As expected, excellent resolutions were achieved with all the α-methyl amino acids examined, as evaluated by the values of both the separation factor (α) and the resolution (Rs). The α-methyl amino acid (**1**) derivatives generally eluted faster than the corresponding α-H-amino acid (**2**) derivatives, and moreover the former exhibited better resolutions than the latter with one exceptional case. Especially remarkable resolutions were attained with the aromatic α-methyl

Table 1

Resolution of α-Methyl α-Amino Acids (1) and the Corresponding α-H-Amino Acids (2) as Their N-Z-Protected Methyl Esters on a Cellulose Tris(3,5-Dimethylphenylcarbamate) Chiral Stationary Phase Column (Diacel Chiralcel OD)*

	1			2		
R	k_1'	α	Rs	k_1'	α	Rs
$MeS(CH_2)_2$	1.97	1.41	3.25	3.62	1.31	2.39
MeO_2CCH_2	3.04	2.53	9.17	5.12	1.37	2.43
$MeO_2C(CH_2)_2$	5.85	1.26	2.47	6.16	1.63	4.55
$PhCH_2$	2.07	3.92	10.01	3.63	1.18	1.79
3-Indolylmethyl	9.28	2.48	7.76	14.19	1.36	---

* See Experimental for details of chromatographic conditions.

amino acid derivatives. Although this chiral stationary phase was found to be useful for the enantiomeric analysis of α-methyl α-amino acids, two steps of derivatization, i.e., acylation and esterification, were necessary starting from free amino acids to be analyzed. A resolution method applicable directly to underivatized amino acids is unquestionably more convenient for the purpose.

The separation of enantiomers by means of ligand-exchange HPLC is a promising method developed in recent years,[7] and several columns have thus far become available for this purpose. We also have recently reported the enantiomeric separation of amino acids by a column packed with octadecylsilanized silica coated with N,S-dioctyl-D-penicillamine (**3**) as a chiral ligand-exchange phase (Sumichiral OA-5000).[6] The enantiomers of a variety of non-protein α-amino acids carrying aliphatic and aromatic side-chains were resolved well

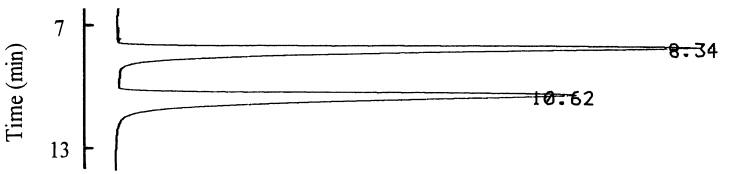


Figure 1. Resolution of N-benzyloxycarbonyl α-methylmethionine [1, R = $MeS(CH_2)_2$] methyl ester on Chiralcel OD. See Experimental for details of chromatographic conditions.

using this column. The enantiomeric separation of proteinogenic amino acids has also been achieved.[8]

These prompted us to examine the applicability of this ligand-exchange phase to the enantiomeric separation of α-methyl α-amino acids.[9] As the separation of enantiomers using the chiral ligand-exchange phase (**3**) is thought to be achieved by a combination of the ligand-exchange interaction and hydrophobic interaction with the stationary phase,[10] we examined several factors, mainly the effects of the amount of the organic component (2-propanol) and the concentration of the complexing metal ion (Cu^{2+}), which had been found to be the most significant in our previous work.[6] The results of the resolution of α-methyl α-amino acids (**1**) are summarized in Table 2. Most of the amino acids examined in this study were well resolved by choosing an appropriate hydro-organic eluent among the following A–F:

Table 2

Resolution of α-Methyl α-Amino Acids (1) on a Column Packed with Octadecylsilanized Silica Coated with N,S-Dioctyl-D-Penicillamine (3) as a Chiral Liqand-Exchange Phase (Sumichiral OA-5000)[a]

R	Mobile Phase	k_1'	α	Rs
$NH_2(CH_2)_3$	A[b]	0.32	3.94	11.10
4-Imidazolymethyl	A[b]	1.54	2.92	11.47
$HOCH_2$	B	0.86	1.90	3.40
	C	0.75	2.03	2.88
HO_2CCH_2	C	5.38	1.21	2.32
$MeS(CH_2)_2$	D	3.72	1.10	1.09
p-$HOPhCH_2$	D	4.95	1.12	1.30
$HO_2C(CH_2)_2$	D	6.93	1.43	5.26
m-$HOPhCH_2$	D	8.82	1.29	4.35
$(CH_3)_2CHCH_2$	E	1.54	1.56	3.29
$PhCH_2$	E	3.60	1.37	4.13
m-$MeOPhCH_2$	E	5.65	1.19	2.19
3-Indolylmethyl	E	12.71	1.28	3.75
	F	8.11	1.35	3.50

[a] Mobile phase: A, 1 mM $CuSO_4$ in water; B, 5 mM $CuSO_4$ in water; C, 1 mM $CuSO_4$ in water–2-propanol (95:5); D, 3 mM $CuSO_4$ in water-2-propanol (95:5); E, 5mM $CuSO_4$ in water–2-propanol (85:15), F, 4 mM $CuSO_4$ in water–2-propanol (80:20). For details of the other chromatographic conditions, see Experimental. [b] Flow rate, 0.2 mL/min^{-1}.

(A) 1 mM copper(II) sulfate in water;

(B) 5 mM copper(II) sulfate in water;

(C) 1 mM copper(II) sulfate in water–2-propanol (95:5);

(D) 3 mM copper(II) sulfate in water–2-propanol (95:5);

(E) 5 mM copper(II) sulfate in water–2-propanol (85:15);

(F) 5 mM copper(II) sulfate in water–2-propanol (80:20).

A typical chromatogram is shown in Figure 2. However, some α-methyl amino acids (e.g., α-methyl DOPA) could not be resolved under the present chromatographic conditions. The α-methyl amino acids were retained more strongly than the corresponding α-H-amino acids, probably due to an increase in the hydrophobicity by one methyl group. With α-methyl α-amino acids also, the influence of the organic composition in the eluent was generally more significant for the retention than that of the Cu^{2+} ion concentration: the smaller the amount of 2-propanol, the slower the elution.

The influence of the concentration of Cu^{2+} ion on the elution depends on the amount of organic component in the mobile phase: a more significant effect was observed in the eluent containing 5% of 2-propanol than in the eluent containing 15% of 2-propanol. The presence of a basic group in the side-chain reduces the retention, and a tolerable resolution was attained by employing mobile phase A (without 2-propanol). The presence of a hydroxyl group also weakens the retention and even impairs the resolution. In the case of α-methyl-

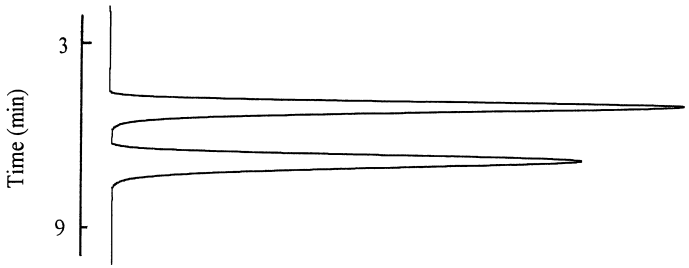


Figure 2. Resolution of α-methylleucine [R = $(CH_3)_2CHCH_2$] on Sumichiral OA-5000 using mobile phase E. See Experimental for details of chromatographic conditions.

tyrosine, no separation of enantiomers was observed using mobile phase E, though the parent tyrosine itself gave a good resolution under the same elution conditions (k_1' = 1.06, α = 1.39, Rs = 1.81).

A tolerable resolution was attained by employing mobile phase D. The presence of a carboxyl group in the side-chain also reduces the retention largely but little affects the resolution. With α-methyl amino acids bearing a large alkyl side-chain or an aromatic side-chain excellent resolutions were achieved using the mobile phase E which had been most preferably used for the resolution of α-H-amino acids.[6] With α-methyltryptophan the elution was slow using the mobile phase E, and the increase of proportion of 2-propanol up to 20% (mobile phase F) resulted in a good resolution in a much shorter time.

Taking the results described in the previous paper[6] into consideration, the ligand-exchange phase examined in this paper proved to be applicable to the separation of enantiomers of a wide variety of non-protein amino acids, including α-methyl α-amino acids.

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