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Chromatographic Separation of Diastereomeric Schiff Base Copper(II), Nickel(II), and Zinc(II) Chelates from α-Amino Acid Racemates

EIKO TOYOTA, KUNIHIKO ITOH, HARUO SEKIZAKI, AND KAZUTAKA TANIZAWA[1]

Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-02, Japan

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Chromatographic separation of diastereomeric Schiff base copper(II), nickel(II), and zinc(II) chelates derived from optically active 3-substituted salicylaldehyde derivative and α-amino acids on a reverse-phase column was found to be successful. The separation of two elution peaks within a pair of enantiomers was excellent for most neutral amino acids. Optically pure α-amino acids were isolated from the chelates by the addition of EDTA · 2Na. The formation of bis(Schiff base)metal chelates with nickel(II) or zinc(II) was discussed.

INTRODUCTION

We were interested in the well-known chemical process of formation of a very stable chelate from α-amino acid, salicylaldehyde residue, and metal ion. This chelate formation process was reversed by the addition of ethylenediaminetetraacetic acid (EDTA). It is known that Schiff bases composed of α-amino acid and salicylaldehyde residue are not stable in aqueous media and they are dissociated into their original components. The process was successfully applied for the immobilization of protein to insoluble support, and thus a new method for protein immobilization was proposed (*1*, *2*).

This reversible process is expected to be applicable to the resolution of a racemic α-amino acid mixture if optically active salicylaldehyde derivatives were used. In a previous paper synthesis of optically active 5-substituted salicylaldehyde derivatives (Scheme 1, **D-I** and **L-III**) and chromatographic separation of diastereomeric Schiff base copper(II) chelates derived from various α-amino acids has been reported (*3*). The separation of diastereomeric chelates derived from **D-I** and **L-III** was found to be less than optimal. Thus, we attempted synthesis of 3-substituted salicylaldehyde derivatives (**D-II** and **L-IV**). It was assumed that the 3-substituted salicylaldehyde derivatives are promising for the purpose because two asymmetric carbon atoms included in the salicylaldehyde and α-amino acid residues are placed close to each

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[1] To whom correspondence should be addressed.

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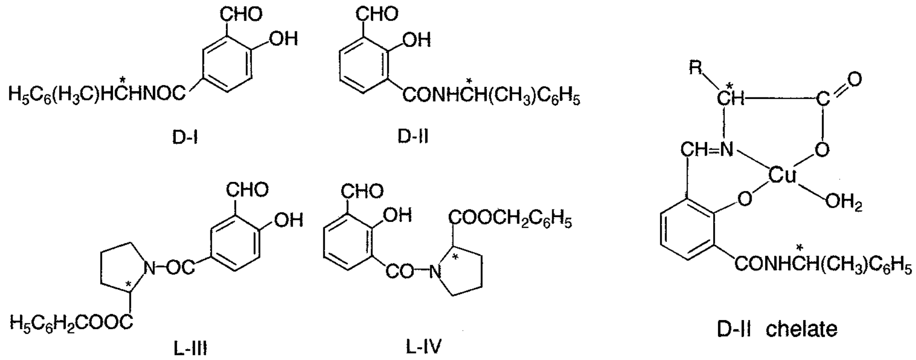

SCHEME 1

other in the structure of the resulting Schiff base copper(II) chelate (**D-II** chelate) (Scheme 1).

The present paper describes a method for separation of diastereomeric Schiff base copper(II), nickel(II), and zinc(II) chelates derived from optically active 3-substituted salicylaldehyde residue and various α-amino acids. In addition, a method for isolation of free D- and L-amino acids from the copper(II) chelates has been studied.

RESULTS AND DISCUSSION

The Schiff base metal(II) chelate solution was prepared by mixing equimolar solutions of aldehyde, α-amino acid, and metal ion. The formation of the chelate was confirmed by absorption spectra (*4*). Diastereomeric copper(II), nickel(II), and zinc(II) chelates derived from optically active salicylaldehyde derivatives and α-amino acid racemates were subjected to the high-performance liquid chromatography (HPLC). Retention times of the chelates together with Rs values (*5*), "separation index," were listed in Tables 1 and 2. The results show that the separation of peaks is satisfactory and that the method is applicable to the resolution of most neutral amino acid racemates.

Copper(*II*) *chelates.* A structure of Schiff base copper(II) chelates is shown in Scheme 1. The copper ion forms bonds to phenolic oxygen, imine nitrogen, and carboxy oxygen of terdentate Schiff base ligand and a water molecule.

Chromatographic separation of the copper(II) chelates was excellent for both cases with **D-II** and **L-IV,** whereas that of the chelates with **D-I** and **L-III** was partly successsful (*3*). It can be assumed that two asymmetric carbon atoms originated from the salicylaldehyde and α-amino acid residues are placed closer to each other when **D-II** and **L-IV** rather than **D-I** and **L-III** were employed. The elution pattern of the chelate solution is shown in Fig. 1. Peaks **A** and **B** were assigned as the chelates derived from L-Phe and D-Phe, respectively. The assignment was carried out by

TABLE 1
Retention Times and Rs Values of Copper(II) Chelates Derived from **D-II** and **L-IV**

		D-II chelate Retention time (min)[a]			**L-IV** chelate Retention time (min)[b]	
Amino acid	Rs	L-isomer	D-isomer	Rs	L-isomer	D-isomer
Ala	1.0	10.0	10.4		24.2	
Val	4.3	14.9	18.3	1.0	51.2	46.4
Leu	4.2	21.5	27.5	1.2	86.2	76.4
Ile	5.0	21.5	28.2	1.5	79.6	68.0
Met	1.7	13.8	15.6	0.6	41.2	39.2
Phe	3.2	22.7	26.8	1.8	94.9	81.2
Trp	1.0	16.6	17.5	0.8	77.6	73.0
Ser	0.8	7.7	7.9		17.4	
Thr		8.5			18.4	

[a] Medium, methanol : water = 60 : 40.
[b] Medium, methanol : water = 55 : 45.

determining the retention times of authentic chelates prepared from L-Phe or D-Phe. In the case of **D-II** chelates, the L-isomer was eluted before the corresponding D-isomer. This order was reversed for **L–IV** chelates. The Rs values of **L–IV** chelates were smaller than those of **D-II** chelates due to the tailing of chromatographic peaks. The Rs values of the **D-II** chelates derived from amino acids which have branched side chains on the α-carbon such as Val, Leu, and Ile were bigger than those of other amino acids. This may be attributed to the steric interaction between the

TABLE 2
Retention Times and Rs Values of Nickel(II) and Zinc(II) Chelates Derived from **D-II**

		Ni(II) chelate Retention time (min)[a]			Zn(II) chelate Retention time (min)[a]	
Amino acid	Rs	L-isomer	D-isomer	Rs	L-isomer	D-isomer
Ala	1.2	8.7	9.8	1.0	8.3	9.0
Val	1.2	14.2	20.3	1.2	12.2	15.8
Leu	2.2	26.2	34.8	2.6	20.1	27.7
Ile	2.0	22.5	34.5	2.7	19.1	26.0
Met	1.2	12.8	15.2	1.9	13.0	16.0
Phe	3.3	24.9	35.8	1.9	22.0	27.4
Trp	1.0	16.7	15.9	1.0	18.2	16.2
Ser		7.0			6.7	
Thr	1.0	7.9	8.9		7.3	

[a] Medium, methanol : water = 55 : 45.

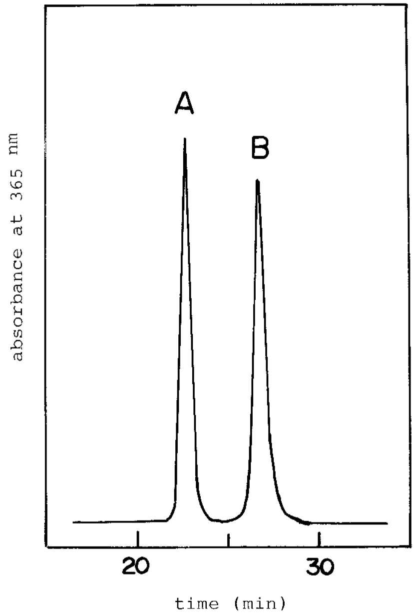

FIG. 1. Elution diagram of copper(II) chelates from **D-II** and DL-phenylalanine. Conditions as described under Experimental Procedures. Peaks A and B were assigned as chelates from L-Phe and D-Phe, respectively.

alkyl substituent on the α-carbon and *n*-octyl residue on the bonded phase silica gels (*6*).

Isolation of optically pure α-amino acid from diastereomeric copper(II) chelates was carried out in the following manner: Sample solution of diastereomeric [Cu(X-salal-DL-phe)(H_2O)], (X : $CONHCH(CH_3)C_6H_5$, salal: salicylaldehydato) was injected in the preparative HPLC system. The mobile phase of methanol–water (55 : 45) was used at 30°C. The flow rate was 1.0 ml/min. Elutions of [Cu(X-salal-L-phe)(H_2O)] (L-isomer) and [Cu(X-salal-D-phe)(H_2O)] (D-isomer) were observed at the retention times of around 24 min and 30 min, respectively. The eluates of L-isomer and D-isomer were then evaporated and were added to an aqueous solution of disodium dihydrogen ethylenediaminetetraacetic acid (EDTA · 2Na), respectively. The solution was subjected to Sephadex LH-20 column chromatography. The L-Phe or D-Phe was obtained from the eluate of methanol, and both amino acids completely retained their initial optical purity.

Nickel(II) and zinc(II) chelates. The maximum coordination number of Cu(II), Ni(II), and Zn(II) is six, and Schiff bases of salicylaldehyde with α-amino acids form terdentate ligand. Therefore, species of the type mono(terdentate)metal and bis(terdentate)metal are possible. If bis(terdentate)metal chelate is more stable than mono(terdentate)metal chelate, the former type will be formed predominantly. In the case of copper(II), only mono(terdentate Schiff base) chelate was present. Addition of nickel(II) or zinc(II) ion instead of copper(II) ion to the solution of