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Short communication

Chiral metal complexes XLII[☆]. Reversed-phase high-performance liquid chromatographic separation of racemic dipeptides as their ternary Co(III) complexes with a chiral triamine

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Abstract

The enantiomeric and diastereoisomeric dipeptides Gly–*R,S*-Val, Gly–*R,S*-Leu, Gly–*R,S*-Phe, *R,S*-Leu–Gly, *R,S*-Leu–*R,S*-Ala and *R,S*-Leu–*R,S*-Phe have been separated by RP-HPLC methods when coordinated in ternary Co(III) complexes $[Co(R,R\text{-benzet})(peptidato)]^{2+}$ [benzet = N-benzyl-*N'*-(2-picolyl-1,2-diaminocyclohexane)], on Amex Prepsil columns under a variety of conditions. A method is illustrated for the recovery of the pure peptides from the separated diastereoisomers. The method appears to be applicable for dipeptides, in general.

1. Introduction

Optically active α-amino acids are very important chiral reagents used in the preparation of a number of commercial pharmaceuticals. Twenty or so amino acids with the same relative configuration as *S*-alanine occur in free form in cellular tissues and fluids of living organisms and are the normal constituents of vegetable and animal proteins. These natural amino acids may be isolated for synthetic use (including the preparation of dipeptides) from appropriate organisms. Several non-proteinogenic amino acids of opposite hand to the above occur in certain biological systems, but the majority have to be synthesised, as do all unnatural amino acids.

Enantiomerically pure α-amino acids (which are essential for peptide synthesis) may be obtained directly by asymmetric synthesis, or more commonly, by the resolution of racemic products from syntheses using achiral or prochiral reagents. Resolution may be achieved by microbiological or chemical methods [1,2]. Chemi-

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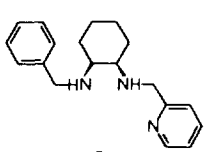
cal resolution involves the formation of diastereoisomeric salts or adducts which may be separated by fractional crystallisation or, now more frequently, chromatographic methods.

Both free and derivatised racemic amino acid and dipeptide mixtures have been resolved in the presence of chiral ligands and metal ions by high-performance liquid chromatography (HPLC). The asymmetric secondary ligand, commonly an amino acid derivative or a chiral polyamine, can be a component of the mobile phase [3,4], or be attached covalently to the stationary phase [5–8]. The metal ion is usually a labile, divalent transition metal such as Cu^{2+}, Ni^{2+} or Zn^{2+}, present as a constituent of the mobile phase. Diastereoisomers of inert Co(III) complexes containing a chiral ligand(s) and/or and asymmetric metal centre have been resolved previously by reversed-phase (RP) HPLC [9]. Ion-pairing anions such as *p*-toluenesulphonate, camphorsulphonate and diantimony tartrate, capable of interaction with the complexes and the hydrophobic stationary phase, are commonly used as mobile phase additives to effect or enhance resolution.

The resolution of dipeptides would be of interest, since resolution of the amino acid constituents used for the synthesis would not always be necessary. In this note, the separation of racemic dipeptides as their $[Co(R,R\text{-benzet})(dipeptidato)]^+$ [where benzet = N-benzyl-N′-(2-picolyl)-1,2-diaminocyclohexane, **I**] diastereomeric complexes by RP-HPLC is discussed. Baseline resolution has been achieved in short times without the need for any chiral additives.



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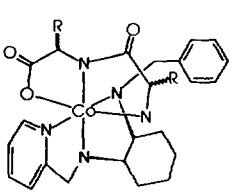

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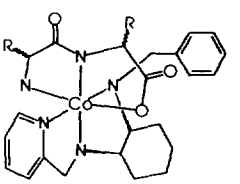

III

2. Experimental

2.1. Preparation of racemic dipeptide complexes

To a stirred suspension of $[Co(R,R\text{-benzet})Cl_3]$ [10] (0.15 g, 3.28×10^{-4} mol) in 5 cm^3 of water was added 1 mol equivalent of the appropriate dipeptide (Sigma–Aldrich). The mixture was warmed gently with stirring for 15 min, whereupon 4 mol equivalents of triethylamine were added. A deep purple solution immediately resulted. After stirring and warming for a further 45 min, the mixture was cooled to room temperature and applied to a CM-Sephadex column (40×1.5 cm) in the Na^+ cycle. The single purple-red band formed upon elution with 0.1 *M* aqueous NaCl was collected in bulk, and taken to dryness in vacuo at 35°C. The dry residue obtained was desalted twice with methanol to give a deep-purple solid after removal of the solvent in vacuo. ^1H NMR analysis of the dry residue confirmed all solids to be isomeric mixtures of the species represented in **II** and **III** [10].

2.2. Columns

Apex Prepsil ODS 15M (100×4.5 mm) and 5M (150×4.5 mm) columns were obtained from Jones Chromatography.

2.3. Apparatus

For the chromatographic runs using the phosphate-based mobile phase, a Merck Hitachi L-6200 intelligent pump was used to provide constant mobile phase flow, and a Merck Hitachi L-4200 variable wavelength detector operating at 254 or 480 nm was employed to monitor column eluent. The chromatographic data were recorded and processed with a JCL 6000 computer package (purchased from Jones Chromatography) in conjunction with a Walters personal computer.

For the runs using the *p*-toluenesulphonate-based mobile phase, Gilson 303 dual pumps were employed to control mobile phase flow in combination with a Gilson holochrome variable-wavelength UV–Vis detector operating at 280 or 480 nm. A BBC Coerz Meterawatt SE 120 chart recorder was used to record all the chromatograms.

2.4. Chromatographic procedures

Phosphate buffer solutions were prepared by adjusting a stock solution of NaH_2PO_4 (0.01 *M*) to the required pH using concentrated H_3PO_4, and an appropriate amount of HPLC grade acetonitrile and methanol was added thereto. The mixture was filtered through a micropore fibreglass filter paper, and degassed for 5 min under reduced pressure with constant stirring. All runs using this solvent system were performed under isocratic conditions.

p-Toluenesulphonate buffers were prepared in an analogous fashion to the phosphate buffer, but using a stock solution which was 25 m*M* in *p*-toluenesulphonic acid and adjusting to the required pH using sodium *p*-toluenesulphonate. Both isocratic and gradient elutions were performed using this mobile phase. The ternary Co(III) complexes were introduced by microsyringe as methanolic solutions.

2.5. Removal of glycylglycine from its ternary complex

C-[Co(*R*,*R*-benzet)(Gly–Gly)]$ClO_4 \cdot 2H_2O$ (160 mg) was dissolved in dilute HCl (0.001 M, 150 cm^3) and a potential difference of −1 V was applied for 5 h using an EG & G Princeton Applied Research Model 363 potentiostat (using an Hg working electrode and a Pt counter electrode). During this time, the red colour of the Co(III) complex gradually faded until a very pale pink solution remained. The solution was then neutralised and applied to a CM-Sephadex column (15 × 1.0 cm) in the Na^+ cycle. The dipeptide was eluted with water and shown to be present by ^1H NMR analysis of the residue remaining after removal of the water. Due to the small scale of the reduction, no further attempts were made to purify the dipeptide. This method is known to yield enantiomerically pure peptides, quantitatively [11].

3. Results and discussion

It has previously been shown that enantiomerically pure dipeptides give a single diastereoisomer when coordinated to the [Co(*R*,*R*-benzet)]$^{3+}$ nucleus [10]. Therefore, two isomers are to be expected for a racemic dipeptide containing a glycyl fragment, and four isomers for a dipeptide with two asymmetric centres. For Gly–Val, Gly–Leu, Gly–Phe, Leu–Gly, Leu–Ala and Leu–Phe, the diastereomeric products formed by

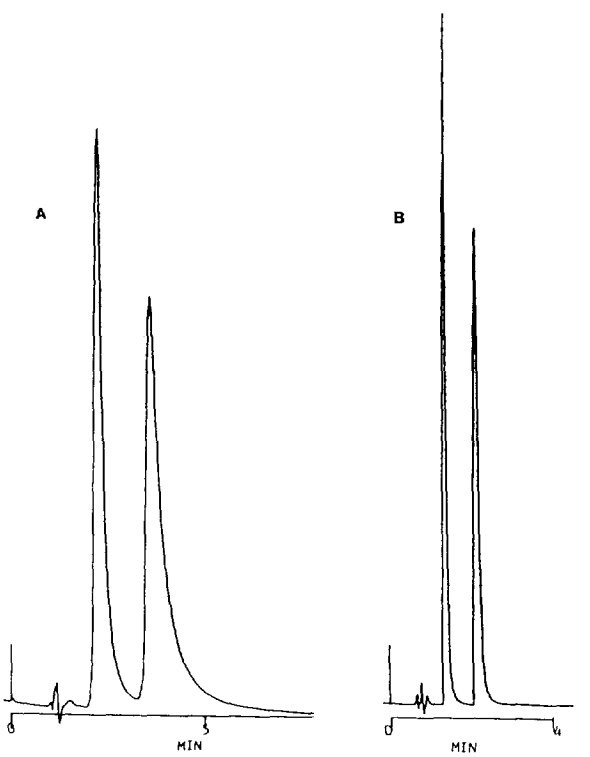

Fig. 1. Separation of the two diastereoisomers of (A) [Co(*R*,*R*-benzet)(Gly-*R*,*S*-Leu)]$^+$; 0.01 *M* phosphate–MeOH–MeCN (pH 4.5) mobile phase; 10 cm 15 μm C_{18} column, flow-rate 2.0 cm^3 min^{-1} and (B) [Co(*R*,*R*-benzet)(Gly-*R*,*S*-Val)]$^+$; 0.01 *M* phosphate–MeOH–MeCn (pH 4.5 mobile phase: 10 cm 5 μm C_{18} column, flow-rate 2.0 cm^3 min^{-1}.

reaction of [Co(*R*,*R*-benzet)Cl_3] and the racemic dipeptides were not separated by chromatography on CM-Sephadex using aqueous NaCl as eluent. However, all the isomers formed with any particular dipeptide have been completely resolved by RP-HPLC.

The separation of the two diastereoisomers of Gly–*R*,*S*-Val and Gly–*R*,*S*-Leu using an aqueous phosphate–MeCN–MeOH mobile phase is shown in Fig. 1. Retention times for these and the other complexes using this solvent system are listed in Table 1. Baseline resolution has been achieved in all cases, with the sole exception of Leu–Gly, within a short time (order of minutes).

In the pH range 2.0–5.0, little change was observed in retention times for the complexes, although the quality of the separation was reduced at the upper limit as the peaks became broadened. Optimum resolution and peak shape was achieved at a pH of 3.0. In a series of runs employing [Co(*R*,*R*-benzet)(Gly–*R*,*S*-Phe)], variation of retention times and separation factors with varying phosphate concentration and methanol proportion was assessed. Small decreases in retention times and separation were observed with increasing total PO_4^3-concentration, coinciding with improved peak shape and chromatographic profile. The slowest eluted diastereoisomer was observed as a broad hump at low MeOH proportion, but gradually sharpened as the ratio was increased.

For Gly–Val and Gly–Leu, the complex containing the dipeptide with the *R* stereochemistry is eluted first, an observation noted also for Leu–Gly. [Co(*R*,*R*-benzet)(*S*-Leu–*S*-Ala])$^+$ has the longest retention time of the diastereoisomers containing this dipeptide. Although the order of elution is only known completely for Gly–Val, Gly–Leu and Leu–GLy, the above observations imply that complexes of dipeptides with *S* chiral centres are more efficiently retained on the ODS column than their counterparts with the *R* stereochemistry. A pattern such as this is not entirely surprising, when it is considered that all the complexes are believed to possess a common topology at the metal centre ([OC-6-64-C]) [10]. Similar interactions would be expected within the series of complexes with the *S* stereo-

Table 1
Retention times (t_n) and their ratios for the [Co(*R*,*R*-benzet)(dipeptidato)]$^+$ complexes

Peptide	t_n (min)	t_2/t_1	t_3/t_2	t_4/t_3
Gly–*R*,*S*-Val	1.10	2.00		
	2.20			
Gly–*R*,*S*-Leu	1.86	1.86		
	3.46			
Gly–*R*,*S*-Phe	2.22	2.06		
	4.58			
R,*S*-Leu–Gly	2.09	2.67		
	2.67			
R,*S*-Leu–*R*,*S*-Ala	2.36	1.29	1.26	1.38
	3.04			
	3.84			
	5.29			
R,*S*-Leu–*R*,*S*-Phe	6.48	1.23	1.66	1.66
	8.00			
	13.28			
	22.08			

All runs performed under isocratic conditions using a 10 cm, 15 μm C_{18} silica column and an MeOH–MeCN–aqueous phosphate buffer (10:30:60) mobile phase initially 25 mmol in total PO_4^3 adjusted to pH 4.5; flow-rate 2.0 cm^3 min^{-1}. *n* refers to the number of the peak.

chemistry, and likewise the complexes containing *R* peptides. It is, however, not possible to ascertain the exact nature of the interactions leading to the observed separation, but the pendant benzene ring of the triamine and the side-chain of the dipeptide would be expected to have an affinity for the octadecyl residues of the stationary phase. In addition, the amine hydrogens and the hydrogen phosphate anions could form ion-pairs in solution.

Baseline separation was not fully achieved for Leu–Gly, and to a lesser extent for Leu–Ala, using the isocratic phosphate mobile phase. Improved resolution resulted when an aqueous *p*-toluenesulphonate solvent system was employed. Long retention times and fairly broad, but well-separated peaks were observed with isocratic elution. Gradient elution gave baseline separation in shorter times with improved peak shape, but with reduced values for ratios of retention times.

The dipeptides are not readily released from their Co(III) complexes by treatment with H_2S or aqueous Na_2CO_3, but may be recovered by an electrolytic method as outlined in the Experimental section. The small scale of the syntheses made it impractical to perform the reduction on all the complexes; details concerning the complex containing Gly–Gly are given as being typical. However, the method is applicable to all the complexes isolated and may be used for the resolution of other dipeptides not examined in this study [11].

In conclusion, several racemic dipeptides have been resolved by prior formation of ternary Co(III) complexes with the chiral triamine *R,R*-benzet and subsequent separation of resultant ternary species by RP-HPLC. Two aqueous mobile phases have been successfully employed, and conditions optimised in each case. Complete separation of the diastereoisomers has been achieved in all cases within minutes.

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