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Note

Quantitation of the enantiomers of β-hydroxyamino acids by gas chromatographic resolution on an optically active packed column*

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The resolution of the optical antipodes of amino acids by gas chromatography (GC) on packed columns coated with a chiral phase is well established[1]. Whereas many monoamino acids are well resolved as their N-trifluoroacetyl (TFA) isopropyl esters and may therefore be used for determination of the enantiomeric ratio[2], this is not the case for the corresponding O,N-TFA derivatives of the β-hydroxyamino acids threonine, *allo*-threonine and serine. Several peaks are observed in the chromatograms of these amino acid derivatives[3,4], which were attributed to their partial decomposition in the column[5,6]. Pollock and Kawauchi[7] suggested an additional step in the derivatization to overcome the problem of degradation on the capillary column, but the partial separation which results is not suitable for exact determination of the enantiomeric ratio.

The lack of an appropriate method for the exact determination of the enantiomeric ratio is one of the major problems in the study of asymmetric synthesis of β-hydroxyamino acids by condensation of aldehydes with metal complexes of glycine[8]. In the present paper we report the quantitative separation of the enantiomers of serine, threonine and *allo*-threonine.

When formaldehyde is treated with coordinated glycine, hydroxymethylserine is obtained as the by-product[9]. This non-natural and achiral compound is therefore also included in the present study.

EXPERIMENTAL

Preparation of derivatives

The mixed N-TFA-O-acetyl isopropyl ester derivatives were prepared by using an analogous but simplified version of the method described by Pollock and Kawauchi[7]. The dried sample of the β-hydroxyamino acid (> 3 mg) was dissolved in 4 ml of 2 *M* HCl in isopropanol and refluxed for 45 min in an oil-bath at 110°C. After cooling, the mixture was evaporated on a rotary evaporator. Methylene chloride (3 ml) and 0.5 ml of trifluoroacetic anhydride were added, stirred at room temperature for 10 min and again evaporated to dryness. The product was dissolved in 4 ml

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freshly distilled methanol and allowed to stand at room temperature for 20 h in the case of threonine and for at least 7 h in the case of serine to complete methanolysis of the O-TFA group[7]. The mixture was evaporated to dryness, and 0.1 ml of pyridine, 2 ml of acetone and 0.2 ml of acetic anhydride were added. After stirring at room temperature for 10 min, the solvent and excess of reagent were removed under vacuum. Evaporation was repeated after the addition of 2 ml methylene chloride to ensure complete elimination of pyridine. The derivatives were finally dissolved in chloroform for chromatographic analysis.

Chromatography

Chromatograms were obtained with a DANI-3400 gas chromatograph equipped with a Hewlett-Packard 3990A integrator. Two optically active columns (3.75 m × 2 mm I.D.) coated with 10% SP-300 on Supelcoport stationary phase (100–120 mesh) were used. Oven temperature: 130°C. Nitrogen flow-rate: 20 ml/min.

Materials

Racemic and optically active amino acids, trifluoroacetic anhydride, methylene chloride, isopropanol, pyridine and acetone were analytical grade (Fluka). Acetic anhydride and solvents were distilled before use. Hydroxymethylserine was synthesized according to Akabori *et al.*[9].

RESULTS

Fig. 1 shows the chromatogram of a synthetic mixture of the N-TFA isopropyl ester of glycine and the N-TFA-O-acetyl isopropyl esters of (D,L)-threonine and of (D,L)-*allo*-threonine. The separation between the different amino acids as well as between the enantiomers of each amino acid is complete. A comparison with the corresponding N,O-diTFA-, N,O-diacetyl and N-TFA derivatives shows that these possible by-products were absent in the analyzed mixture and that the mixed N-TFA-O-acetyl derivatives are the only products from β-hydroxyamino acids detected in the chromatogram.

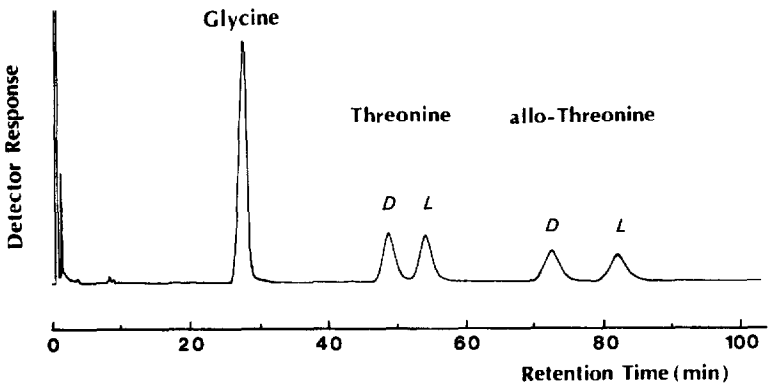

Fig. 1. Gas chromatogram of N-TFA, O-acetyl isopropyl esters of threonines, *allo*-threonines and the N-TFA isopropyl ester of glycine. For chromatographic conditions, see the Experimental section.

TABLE I

SEPARATION OF THE ENANTIOMERS OF N-TFA-O-ACETYL ISOPROPYL ESTERS OF THREONINES AND THE N-TFA ISOPROPYL ESTER OF GLYCINE

Amino acid	*Enantiomer*	*Retention time (min)*	*r (L/D)*
Glycine	–	27.4	
Threonine	D	48.2	
	L	53.5	1.11
allo-Threonine	D	71.7	
	L	81.3	1.13

The ratios of the retention times of the optical enantiomers (Table I) are of the same magnitude as observed for the amino acids without supplementary functional groups measured under similar conditions[2]. The proposed method therefore seems quite suitable for quantitative analysis of β-hydroxyamino acids. Fig. 2 shows the analysis of a reaction mixture containing serine obtained in an asymmetric synthesis by the reaction of formaldehyde with glycine in an optically active copper(II) complex. The ratio of the retention times of (L)- and (D)-serine, 1.09, is slightly lower than the corresponding values for the two threonines, but still allows quantitative separation of the enantiomers.

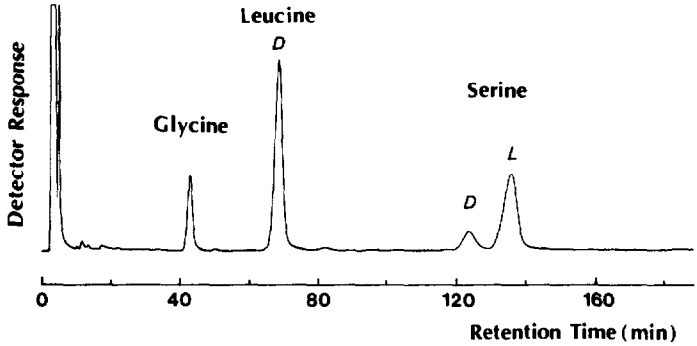

Fig. 2. Gas chromatogram of the N-TFA, O-acetyl isopropyl ester of serine, N-TFA isopropyl ester of glycine and (D)-leucine. Serine was obtained by the reaction of (2,6-bis [(3R)-3-phenyl-2-azabutyl]pyridine)(N-pyruvylideneglycinato)copper(II)[10,11] with formaldehyde. (D)-leucine was used as internal standard.

As for all other amino acids, the (L) form is more strongly retained on the column than the (D) form. As already mentioned, one of the problems in this condensation reaction is the formation of hydroxymethylserine as a secondary product. In order to check for the presence of this compound, a sample of pure hydroxymethylserine was analyzed under the same conditions as described for serine. Two peaks were observed with retention times of 214 and 233 min. The achiral hydroxymethylserine seems to form two different derivatives, but both can clearly be distinguished from serine.

The method developed has already been used successfully in our laboratory

for analysing a number of mixtures from the asymmetric synthesis of β-hydroxy-amino acids. A detailed description of the asymmetric synthesis of serine will be given elsewhere[10].

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