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Note

Gas chromatographic separation of amino acid amide enantiomers on optically active stationary phases

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The gas chromatographic (GC) resolution of N-acyl-DL-amino acid esters on optically active stationary phases has been studied in considerable detail since the first success by Gil-Av *et al.*[1]. Many workers tried mainly the variation of chemical structure in chiral stationary phases to obtain high efficiency[2,3], but none used chemical variations of the solutes other than different ester groups or N-acyl groups. It was the purpose of the present research to examine the effect on the separation of amino acid enantiomers of converting the carboxyl group of the solute into amide.

EXPERIMENTAL

N-Trifluoroacetyl-(N-TFA)amino acid isopropylamides were prepared from the N-TFA-amino acid chlorides by treatment with isopropylamine.

GC was carried out with a Shimadzu GC-7A gas chromatograph equipped with a flame ionization detector. Chromatographic conditions used for the separation of optical isomers are summarized in Table I. N,N′-[2,4-(6-ethoxy-1,3,5-triazinediyl)] bis(L-valyl-L-valyl-L-valine isopropyl ester) (OA-300) and N,N′,N″-[2,4,6-(1,3,5-triazinetriyl)] tris(N^{α}-lauroyl-L-lysine-*tert.*-butylamide) (OA-400) were prepared as described previously[4,5].

RESULTS AND DISCUSSION

The results of the GC separation are given in Table I. In alanine, valine, leucine, methionine and phenylalanine, the separation factors for N-TFA-DL-amino acid isopropylamides were lower than those of N-TFA-DL-amino acid isopropyl esters. In contrast, it was noted that N-TFA-DL-proline isopropylamide was resolved with a high separation factor in spite of the fact that the same chromatographic conditions gave no detectable separation for N-TFA-DL-proline isopropyl ester. A typical chromatogram of N-TFA-DL-proline isopropylamide is shown in Fig. 1.

As is well known proline shows the lowest separation factor of all racemic amino acids in their N-acyl ester form, and this behaviour is thought to be due to the secondary amide group which has no hydrogen left on its nitrogen atom after acylation. The separation factor for N-TFA-DL-proline methyl ester is only 1.057 at 130°C on

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TABLE I

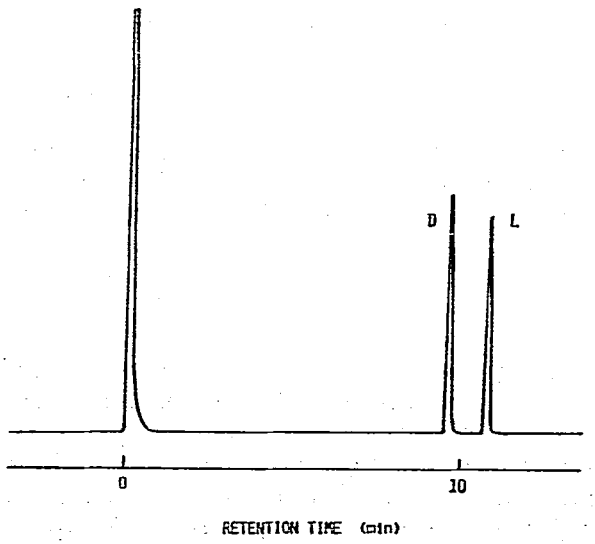
GAS CHROMATOGRAPHIC SEPARATION OF SOME AMINO ACID ENANTIOMERS

Glass capillary column, 40 m × 0.25 mm I.D. Carrier gas (helium) flow-rate, 0.6 ml/min. A = OA-300; B = OA-400.

Amino acid	*Temperature (°C)*	*Stationary phase*	*N-TFA isopropyl ester*			*N-TFA isopropylamide*		
			Retention time (min)*		$\alpha_{L/D}$	*Retention time* (min)*		$\alpha_{L/D}$
			D	*L*		*D*	*L*	
Alanine	180	A	1.00	1.07	1.07	13.80	14.30	1.036
Valine	180	A	1.12	1.19	1.06	18.02	18.67	1.036
Leucine	180	A	1.93	2.05	1.06	24.40	24.71	1.013
Proline	180	A	3.40	3.40	1.00	9.60	10.77	1.122
	130	B	6.85	6.95	1.02	27.53	35.10	1.275
	100	B	25.56	26.32	1.030	134.9	191.3	1.418
Methionine	180	A	9.88	10.50	1.063	92.50	95.10	1.028
Phenylalanine	180	A	12.48	13.20	1.058	118.0	120.0	1.017

* Time from solvent peak.

N-lauroyl-L-valine-*tert*.-butylamide[6], which is the most powerful chiral stationary phase known for GC separation of enantiomeric amino acid esters. In this study the excellent separation factor for N-TFA-DL-proline isopropylamide was observed on OA-300 although the column temperature was rather high. When a moderate temperature was used, very high separation factors were obtained on OA-400. Hitherto it has been necessary to use long capillary columns for resolution of DL-proline in the ester



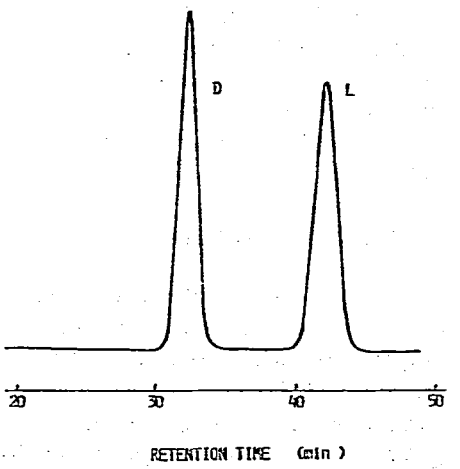


Fig. 1. Gas chromatogram of N-TFA-DL-proline isopropylamide. Column: glass capillary (40 m × 0.25 mm I.D.) coated with OA-300. Temperature: 180°C.

Fig. 2. Gas chromatogram of N-TFA-DL-proline isopropylamide. Column: 2 m × 3 mm I.D., containing Chromosorb W AW DMCS (100–120 mesh) coated with 5% of OA-400. Temperature: 130°C.

form, but such high separation factors allow the separation with a packed column as shown in Fig. 2.

Proline is used as a chiral reagent for conversion of the enantiomers of various alcohols, amines and amino acids into diastereomers and to resolve the optical isomers by GC using usual optically inactive stationary phases[7]; therefore, it is important to estimate its optical purity. This study enabled us to develop a new analytical method for the determination of optical isomers of proline. These results will be reported elsewhere.

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