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Note

Resolution of amino acid enantiomers by glass capillary gas chromatography on easily prepared optically active stationary phases

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Resolution of amino acids on optically active stationary phases has become well-established. It was first demonstrated by Gil-Av *et al.*[1] by using N-trifluoroacetyl (TFA)-L-isoleucine lauryl ester as a stationary phase coated on a glass capillary column 100 m long. At present, dipeptide esters[2–4] and diamides[5–7] are the two major types of stationary liquids for the resolution of N(O,S)-perfluoroacyl amino acid esters.

Recently, Frank *et al.*[8] coupled N-isobutyryl-L-valine *tert.*-butylamide with polysiloxane to improve thermal stability and used this phase to resolve most protein amino acid enantiomers in a short time (*ca.* 28 min). An interesting stationary phase of the *s*-triazine type derived from L-valine has also been reported[9].

Although these phases have high thermal stability and are convenient for resolving less volatile amino acids, they appear to be less suitable for relatively more volatile amino acid mixtures. In addition, they are expensive and their preparation is difficult.

Here, we report the preparation of new, easily synthesized, optically active stationary phases of the dipeptide ester type by coupling N-lauroyl-L-valine with L-valine cyclohexyl ester or L-leucine cyclohexyl ester. The melting points for N-lauroyl-L-valyl-L-valine cyclohexyl ester and N-lauroyl-L-valyl-L-leucine cyclohexyl ester, are 85–87°C and 74–76°C respectively. These two phases were coated on glass capillary columns 17 m long and found to be stable up to 170°C in use. Sixteen protein amino acids, including tryptophan, were chromatographed within about 50 min.

EXPERIMENTAL

Apparatus

A Hitachi O63 gas chromatograph equipped with a flame ionization detector and an all-glass inlet splitter was used throughout this work.

Reagents and materials

All of the amino acids used were obtained from Wako (Osaka, Japan). Anhydrous HCl (>99.9%) was purchased from Seitetsu Kagaku (Osaka, Japan).

Isopropanol was first refluxed over BaO and then redistilled. Trifluoroacetic

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anhydride (TFAA) and pentafluoropropionic anhydride (PFPA) were obtained from Tokyo Kasei (Tokyo, Japan). Methylene chloride and chloroform were distilled before use. Anhydrous HCl was dissolved in isopropanol and its normality verified by titration.

N-Lauroyl-L-valyl-L-valine cyclohexyl ester (Lau-Val-Val) was synthesized as follows. First, N-lauroyl-L-valine was prepared by the Schotten–Baumann reaction[10] and L-valine cyclohexyl ester hydrogen chloride by Fischer's method[11]. Next, N-lauroyl-L-valine (10 mmole), 1-hydroxybenzotriazole (10 mmole) and dicyclohexylcarbodiimide (11 mmole) were dissolved in 40 ml of chloroform. After cooling the mixture to about 2°C, L-valine cyclohexyl ester hydrogen chloride (10 mmole) and N-ethylmorpholine (10 mmole) were added and the mixture stirred for 2 h, and then for another 6 h at room temperature.

The precipitate was filtered off, the filtrate evaporated *in vacuo* and the residue dissolved in 30 ml of ethyl acetate. The solution was filtered, and extracted successively with 5% sodium bicarbonate and (twice) with water. The ethyl acetate layer was dried over sodium sulphate overnight and the solvent removed *in vacuo*. The final compound was recrystallized twice from petroleum ether. Its yield was 42% and its melting point 85–87°C.

N-Lauroyl-L-valyl-L-leucine cyclohexyl ester (Lau-Val-Leu) was prepared in the same manner. Its yield was 69% and its melting point 74–76°C.

The optical purity of Lau-Val-Leu was determined by gas chromatography (GC) after hydrolysis in 6 *N* HCl at 120°C for 10 h and conversion of the hydrolysis products into their TFA-isopropyl esters. According to this method, the optical purities of L-valine and L-leucine were determined to be 82% and 97% respectively. The optical purity of the valine residue at the amide side of Lau-Val-Val could not be determined accurately because hydrolysis yielded a mixture of the valine residues in the lauroyl end and the cyclohexyl ester end.

Preparation of glass capillary columns

Coiled glass capillaries were drawn from Pyrex tubing on a home-made drawing machine[12]. Glass capillaries (17m × 0.25 mm I.D.) were pretreated with 0.5% HF solution for 1 h and silanized with 3-aminopropyltriethoxysilane. Coating was performed by the dynamic method. A methylene chloride solution containing about 12% of these phases was passed through the pretreated capillary. Finally, the capillary columns were conditioned at 170°C overnight with a flow of helium as carrier gas.

Sample preparation

All amino acid derivatives were prepared according to the usual method. About 0.5 mg of the individual DL-amino acids or their mixtures were heated in 2 *N* HCl–isopropanol at 110°C for 1 h. After evaporating the HCl and isopropanol, 2 ml of methylene chloride were added to the residue which was then cooled to about −10°C.

Finally, 0.2 ml of TFAA or PFPA were added to the solution and left to stand for 1 h at room temperature. Solvent and excess of reagents were evaporated and the final product was dissolved in 0.2–0.5 ml of chloroform.

RESULTS AND DISCUSSION

Table I shows the retention data and resolution factors of sixteen (N,O)-TFA-amino acid isopropyl esters on Lau-Val-Val (17 m × 0.25 mm) and Lau-Val-Leu (17 m × 0.25 mm). Most of the amino acids can be resolved on both phases, with the former phase giving somewhat higher resolution factors than the latter. Proline, however, could not be completely resolved.

TABLE I

RETENTION TIMES AND RESOLUTION FACTORS OF N(O)-TFA-AMINO ACID ISOPROPYL ESTERS

Temp. = column temperature; t_R = retention time; r(L/D) = resolution factor calculated from corrected retention times; nd = not determined.

Amino acid		*Lau-Val-Val*			*Lau-Val-Leu*		
		Temp. (°C)	*t_R (min)*	*r(L/D)*	*Temp. (°C)*	*t_R (min)*	*r(L/D)*
Ala	D	110	4.6	1.174	110	3.2	1.163
	L		5.3			3.6	
Val	D	110	6.6	1.158	110	4.7	1.135
	L		7.6			5.2	
Thr	D	110	7.3	1.132	110	4.9	1.123
	L		8.1			5.5	
Gly		110	9.4	—	110	6.2	—
allo-Ile	D	110	9.8	1.175	110	6.8	1.161
	L		11.3			7.8	
Ile	D	110	10.8	1.150	110	7.4	1.139
	L		12.3			8.4	
Leu	D	110	14.5	1.228	130	4.3	1.161
	L		17.6			4.9	
Ser	D	110	15.2	1.097	130	4.3	1.074
	L		16.6			4.6	
Pro	D	110	16.0	1.022	130	5.1	1.000
	L		16.3			5.1	
Asp	D	150	8.5	1.035	150	5.7	1.030
	L		8.8			5.9	
Met	D	150	14.8	1.108	150	9.8	1.103
	L		16.3			10.7	
Glu	D	170	8.9	1.064	170	6.1	1.061
	L		9.4			6.5	
Phe	D	170	9.7	1.072	170	6.7	1.070
	L		10.4			7.1	
Tyr	D		nd		170	14.5	1.086
	L					15.7	
Orn	D		nd		170	32.9	1.078
	L					35.4	
Lys	D		nd		170	47.7	1.080
	L					51.5	

Fig. 1 shows a typical chromatogram of sixteen amino acid mixtures on Lau-Val-Leu with temperature programming. Alanine, valine, threonine, glycine, *allo*-isoleucine, isoleucine, methionine, tyrosine, ornithine and lysine are well resolved. Overlapping is obserbed for D-leucine and D-serine, proline and L-leucine and L-

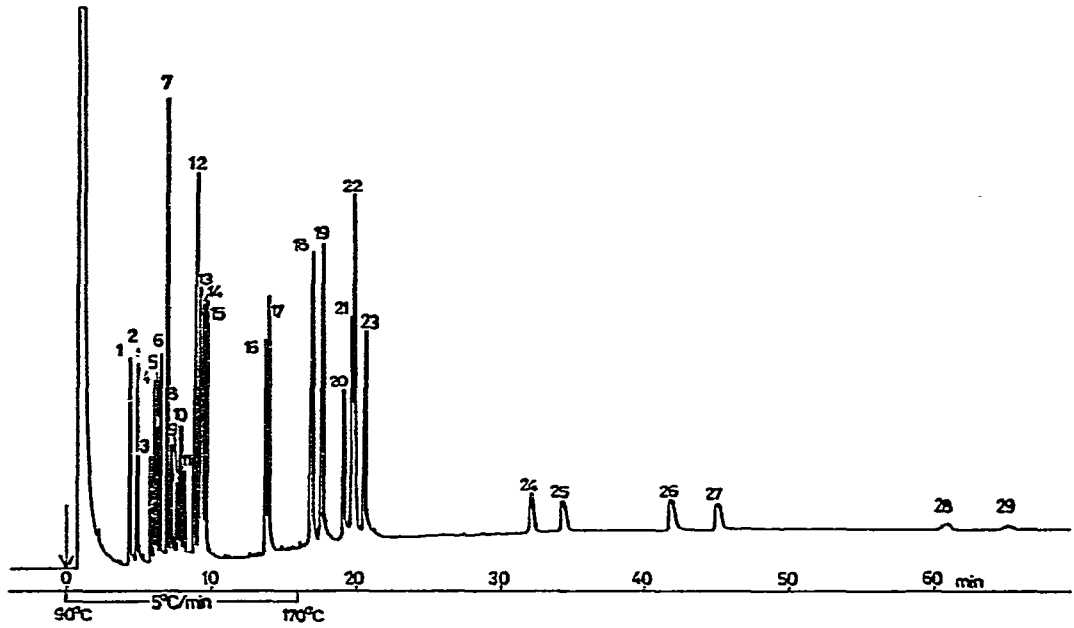

Fig. 1. Chromatogram of N(O)-TFA isopropyl esters of racemic amino acid mixtures with temperature programming. Column: Lau-Val-Leu (17 m × 0.25 mm). Carrier gas: helium. Peaks: 1 = D-Ala; 2 = L-Ala; 3 = D-Val; 4 = D-Thr; 5 = L-Val; 6 = L-Thr; 7 = Gly; 8 = D-*allo*-Ile; 9 = D-Ile; 10 = L-*allo*-Ile; 11 = L-Ile; 12 = D-Leu + D-Ser; 13 = L-Ser; 14 = DL-Pro; 15 = L-Leu; 16 = D-Asp; 17 = L-Asp; 18 = D-Met; 19 = L-Met; 20 = D-Glu; 21 = L-Glu; 22 = D-Phe; 23 = L-Phe; 24 = D-Tyr; 25 = L-Tyr; 26 = D-Orn; 27 = L-Orn; 28 = D-Lys; 29 = L-Lys.

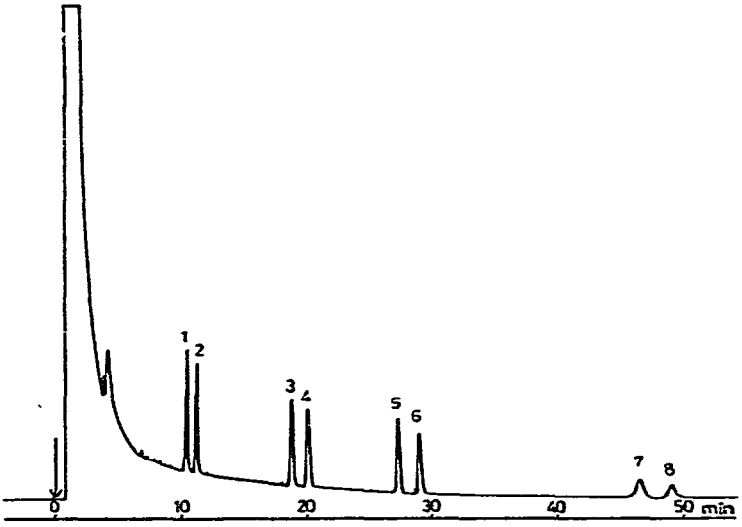

Fig. 2. Chromatogram of N(O)-PFP isopropyl esters of racemic amino acid mixtures. Column: Lau-Val-Leu (17 m × 0.25 mm). Column temperature: 170°C. Carrier gas: helium. Peaks: 1 = D-Tyr; 2 = L-Tyr; 3 = D-Orn; 4 = L-Orn; 5 = D-Lys; 6 = L-Lys; 7 = D-Trp; 8 = L-Trp.

glutamic acid and D-phenylalanine. Tryptophan exhibited very long retention times and the peak maxima of its TFA-isopropyl ester derivatives could not be accurately located. Fig. 2 shows a chromatogram of the N(O)-PFP isopropyl ester derivatives of tyrosine ornithine, lysine and tryptophan on Lau-Val-Leu at 170°C. The last peak of L-tryptophan is eluted within 50 min.

Comparing Lau-Val-Val with Lau-Val-Leu, more peak overlapping is found on the former.

It can be concluded that Lau-Val-Leu is of superior selectivity.

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