Abbreviations

BSA	N,O-bis(trimethylsilyl) acetamide
TMS	Trimethylsilyl
MIT	L-Monoiodotyrosine
DIT	L-3,5-Diiodotyrosine
T_2	L-3,5-Diiodothyronine
T_3	L-3,5,3'-Triiodothyronine
T_4	L-Thyroxine
DBrT	L-3,5-Dibromotyrosine
TLC	Thin-layer chromatography
GC	Gas chromatography

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Rapid gas chromatographic separation of amino acid enantiomers using N-perfluoroacyl esters

Amino acid enantiomers can be resolved by gas chromatography using optically active stationary phases[1–5]. Stationary phases most commonly used are N-trifluoroacetyl (TFA)-L-valyl-L-valine cyclohexyl ester[3,5] and N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester[4]. N-TFA amino acid isopropyl esters have been utilized thus far as the most notable derivatives. However, the retention times were long, resulting in extended gas chromatographic runs. It was of interest to investigate the influence of perfluoroacyl derivatives of amino acid esters other than the trifluoroacetyl group with respect to retention times and resolution factors.

We wish to report the preparation of different N-pentafluoropropionyl (PFP), N-heptafluorobutyryl (HFB), and N-pentadecafluorooctanoyl (PDFO)-D,L-leucine

esters and their gas chromatographic behavior when used on the optically active stationary phase N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester.

Identical methods for the preparation of N-TFA and N-PFP leucine esters were used[4,6]. N-HFP and N-PDFO leucine esters were prepared by reaction of heptafluorobutyric or pentadecafluorooctanoic anhydride respectively in chloroform (ratios, 1 : 4) with the corresponding leucine ester in a sealed tube. Reaction time was 5 min at 100°. The gas chromatographic experiments were carried out with a Varian Aerograph 1200-1 instrument with attachments for capillary column and flame ionization detector. A 400 ft. × 0.02 in. I.D. stainless-steel column coated with N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester was used.

Table I reveals that N-PFP and N-HFB leucine esters are in general more volatile than the corresponding N-TFA derivatives. They require about 20%–27%

TABLE I

COMPARISON OF GAS CHROMATOGRAPHIC DATA OF DIFFERENT N-PERFLUOROACYL LEUCINE ESTERS
Column: 400 ft. × 0.02 in. I.D. stainless-steel capillary coated with N.TFA-L-phenylalanyl-L-leucine cyclohexyl ester, 110° isothermal, carrier gas He, pressure 10 p.s.i.

Leucine ester	*TFA*[a]	$r_{L/D}$	*PFP*[a]	$r_{L/D}$	*HFB*[a]	$r_{L/D}$	*PDFO*[a]	$r_{L/D}$	*Time reduction*[b] (%)	
									N-PFP	*N-HFB*
Methyl	D 6.10	1.082	4.45	1.090	4.90	1.091	12.40	1.100	26.9	19.3
	L 6.60		4.85		5.35		13.65			
Isopropyl	D 7.85	1.127	5.70	1.123	6.20	1.129	15.30	1.137	27.6	21.0
	L 8.85		6.40		7.00		17.40			
tert.-Butyl	D 7.40	1.176	5.40	1.167	6.00	1.142	15.35	1.117	27.1	19.5
	L 8.70		6.30		6.85		17.15			

[a] Corrected retention volumes.
[b] Reference compound is always the corresponding N-TFA-leucine ester, arbitrarily set at 100%.

less time, depending on the ester used. N-PFP-leucine isopropyl ester shows the highest reduction in retention time, 21.6%. N-HFB leucine esters are less volatile than N-PFP derivatives, but still more volatile than N-TFA derivatives. On the other hand, N-PDFO derivatives require twice as much time as the corresponding N-TFA derivatives. Apparently, the highest volatility is reached with the pentafluoroacyl derivatives.

However, more significant is the result that the reduction in retention time does not effect the resolution factors of the L- and D-enantiomers $r_{L/D}$. On the contrary, N-PFP-leucine isopropyl esters show the same or a slight increase in the resolution factor. Fig. 1 shows the separation of N-TFA, N-PFP, N-HFB, and N-PDFO-D,L-leucine isopropyl esters injected together on a 400 ft. column coated with N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester. All isomers are very well resolved with high efficiency in a short time.

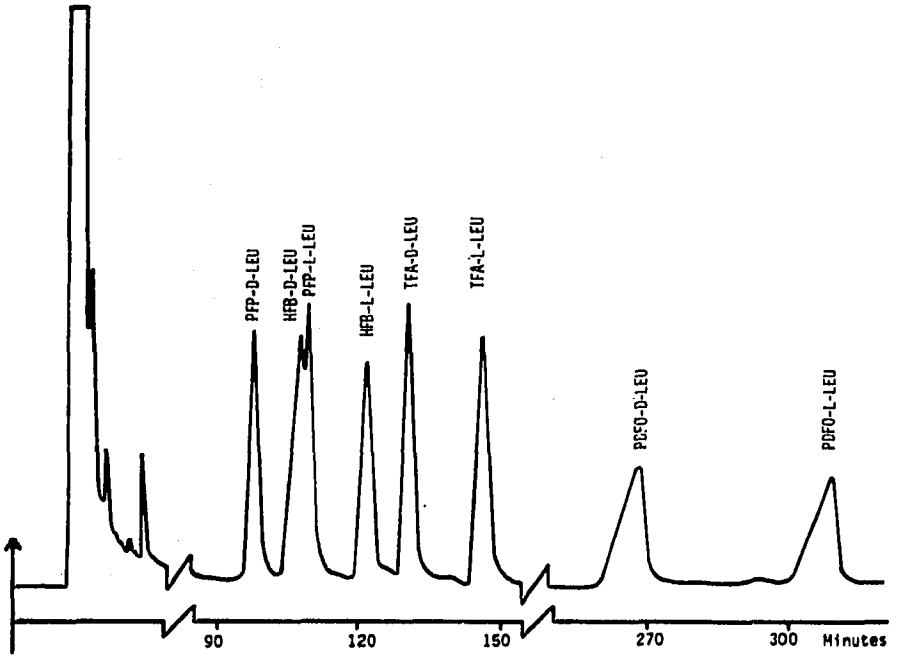

Fig. 1. Gas chromatogram of N-perfluoroacyl leucine isopropyl esters. Instrument, Varian-Aerograph 1200-1. Column, 400 ft. × 0.02 in. I.D., stainless-steel capillary, coated with N-TFA-L-phenylalanyl-L-leucine cyclohexyl ester. Temperature 110° isothermal, injector 190°, detector FID 280°. Carrier gas He, pressure 10 p.s.i.

The new derivatives, and especially PFP amino acid isopropyl esters, have two advantages. Due to their higher volatility, the PFP derivatives would allow the resolution of lower volatile amino acid enantiomers. On the other hand, a reduction in column temperature can be achieved in order to give better resolution of enantiomers. Further work on this subject is under investigation.

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