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Resolution of Racemic Amino Acids by Gas Chromatography. II.[1] N-Perfluoroacyl-L-prolyl Derivatives

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The effect of N-perfluoroacyl groups and ester groups on the resolution of diastereoisomeric N-perfluoroacylprolyl-amino acid esters with respect to retention times and separation factor was investigated in detail on OV-1 column. Relation between separation factors of amino acids and CF_2 groups in N-perfluoroacylprolyl groups and CH_2 groups in primary alcohols was discussed.

It was concluded that the order of elution of racemic amino acids was not influenced by N-perfluoroacyl groups or the ester groups.

Gas chromatography has been proved to be a useful method for the resolution of amino acid enantiomers. Two different methods have been adopted; one using amino acid derivatives prepared with optically inactive reagents followed by chromatography on optically active stationary phases,[3] and the other using amino acid derivatives prepared with optically active reagents followed by chromatography on optically inactive stationary phases.[4]

In the preceding paper,[1] we reported the latter method in which N-TFA-L-PC[5] was used as a resolving agent to form the diastereomeric N-TFA-L-prolyl-amino acid methyl esters, and examined the reaction conditions, gas chromatographic conditions, and relation between separation factors and the structure of amino acids. This paper deals with the effect of ester group and that of N-perfluoroacyl-L-prolyl group on the separation of racemic amino acids (alanine, valine, leucine, and proline).

Experimental

Apparatus and Condition——A Hewlett-Packard Model 402 gas chromatograph equipped with dual flame ionization detector was used. A glass column of 5.5 ft × 1/4 in. O.D. packed with 5% OV-1 on 100—120 mesh Supelcoport was used. Column temperature was 250° for *l*-menthyl ester derivatives and 185° for the other ester derivatives.

Reagents——All the solvents used in this study were of reagent grade. Amino acids were purchased from Ajinomoto Co., trifluoroacetic, pentafluoropropionic, heptafluorobutyric, and pentadecafluorooctanoic anhydrides from Pierce Chemical Co., and *l*-menthol from Aldrich Chemical Co.

N-PFP-L-PC, N-HFB-L-PC, and N-PDFO-L-PC were prepared in the same manner as N-TFA-L-PC.[1]

Preparation of Amino Acid Derivatives——Each amino acid was esterified with thionyl chloride–methanol (1+9), 5% HCl–ethanol, isopropanol, *n*-propanol, *n*-butanol, and cyclopentanol. Amino acid *l*-menthyl

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3) *a*) W.A. Koenig, W. Parr, H.A. Lichtenstein, E. Bayer, and J. Oró, *J. Chromatog. Sci.*, **8**, 183 (1970); E. Gil-Av, B. Feibush, and R. Charles-Sigler, "Gas Chromatography 1966," A.B. Littlewood, ed., The Institute of Petroleum, London, 1967, p. 227; *b*) S. Nakaparksin, P. Birrell, E. Gil-Av, and J. Oró, *J. Chromatog. Sci.*, **8**, 177 (1970).
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5) Abbreviations; TFA, trifluoroacetyl; PFP, pentafluoropropionyl; HFB, heptafluorobutyryl; PDFO, pentadecafluorooctanonyl; N-TFA-L-PC, N-TFA-L-prolyl chloride; N-PFP-L-PC, N-PFP-L-prolyl chloride; N-HFB-L-PC, N-HFB-L-prolyl chloride; N-PDFO-L-PC, N-PDFO-L-prolyl chloride.

esters were prepared according to the method of Harada, *et al.*,[6] amino acid *tert*-butyl esters were prepared with isobutene,[7] and N-perfluoroacyl-L-prolyl amino acid esters in the same manner as described in the preceding paper.[1]

Separation Factors——Separation factors, α,(L/D) were calculated from the following definition:

$$\alpha = (t_{R_2} - t_a)/(t_{R_1} - t_a)$$

where t_{R_1} and t_{R_2} are the retention times (from injection) of the first and the second components, respectively, and t_a is the retention time of non-adsorbed species (methane).

Result and Discussion

The experimental data of relative retention times and separation factors of N-perfluoroacyl prolyl amino acid esters are given in Table I and II.

Retention times of amino acids investigated were found to increase with the change of ester groups in the order of methyl<ethyl<isopropyl<*tert*-butyl<*n*-propyl<*n*-butyl<cyclopentyl<*l*-menthyl in all N-perfluoroacyl derivatives.

The retention times of alanine, valine, and leucine ester derivatives, except for *l*-menthyl ester derivatives, were found to increase with the change of the N-perfluoroacyl groups in the order of PFP<TFA<HFB<PDFO, and those of *l*-menthyl ester derivatives increased in the order of PFP<HFB<TFA<PDFO. However, the retention times of proline ester derivatives were in the same order as those of alanine, valine, and leucine *l*-menthyl ester derivatives in all ester groups.

The retention times of N-PDFO derivatives were twice as much as those of the corresponding N-TFA derivatives on OV-1 and the peaks of N-PDFO derivatives were not sharp, as were the peaks of N-perfluoroacylamino acid cyclopentyl esters.

Feibush[8] reported that the separation factors of $CF_3CONH\overset{*}{C}HRCOOR'$ depended on the relative size of the substituents of the asymmetric carbon (H, R, and COOR'). In the present work, relation between the separation factors and the relative size of the substituents in $\underset{\underset{R'}{|}}{N}\overset{*}{C}ONH\overset{*}{C}HRCOOR''$ was examined and it was found that an increase in the separation factors of D- and L-isomers of alanine, valine, and leucine esters accompanied an increase in the number of CF_2 group in N-perfluoroacyl groups. The separation factors of proline *l*-menthyl ester derivatives was also found to increase in the order of TFA<PFP<HFB. On the other hand, a decrease in separation factors for proline esters, except for *l*-menthyl ester, accompanied an increase in the number of CF_2 group. This may be due to the fact that proline has no hydrogen left on its nitrogen atom after peptide-bond formation by reaction with the resolving agents.

The separation factors of N-TFA-prolyl-alanine, -valine, and -leucine, and -proline esters were found to increase in the order of leucine<proline<alanine<valine, and the separation factors of N-PFP and N-HFB-prolyl-amino acid derivatives, to increase in the order of proline<leucine<alanine<valine.

In the preceding work,[1] the separation factor of N-TFA-L-prolyl-DL-valine methyl ester was found to be the highest in racemic amino acid esters. The present results showed that valine gave the highest separation factor among the four amino acids on OV-1 column.

It was found that an increase in the separation factors of the four amino acids accompanied an increase in the number of CH_2 group of primary alcohol. The ester of primary alcohol (*n*-propyl) gave the higher separation factors than that of secondary alcohol (isopropyl). Primary alcohol group (*n*-butyl) also gave a higher separation factor than that of tertiary alcohol (*tert*-butyl). The separation factors of cyclopentyl ester derivatives were almost

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TABLE I. Comparison of Gas Chromatographic Data of Different N-Perfluoroacyl Prolyl Amino Acid Esters at 185°

Perfluoroacyl	Ester	Enantiomer	Alanine		Valine		Leucine		Proline	
			RRT[a]	rL/D	RRT	rL/D	RRT	rL/D	RRT	rL/D
TFA	methyl	D	0.259	1.139	0.408	1.142	0.537	1.081	0.805	1.103
		L	0.296		0.466		0.584		0.888	
	ethyl	D	0.329	1.141	0.513	1.148	0.667	1.082	0.986	1.109
		L	0.379		0.588		0.726		1.094	
	n-propyl	D	0.469	1.154	0.704	1.159	0.913	1.095	1.368	1.119
		L	0.542		0.815		1.000		1.531	
	isopropyl	D	0.383	1.123	0.581	1.130	0.740	1.078	1.094	1.106
		L	0.429		0.657		0.797		1.209	
	n-butyl	D	0.671	1.161	1.007	1.165	1.296	1.097	1.921	1.132
		L	0.779		1.173		1.422		2.173	
	tert-butyl	D	0.429	1.134	0.664	1.136	0.819	1.071	1.231	1.082
		L	0.487		0.755		0.877		1.332	
	cyclopentyl	D	1.209	1.149	1.765	1.151	2.238	1.092	3.332	1.130
		L	1.389		2.029		2.444		3.765	
PFP	methyl	D	0.256	1.141	0.399	1.164	0.523	1.103	0.747	1.092
		L	0.292		0.462		0.577		0.815	
	ethyl	D	0.299	1.151	0.480	1.165	0.661	1.109	0.906	1.096
		L	0.345		0.559		0.693		0.992	
	n-propyl	D	0.466	1.163	0.703	1.169	0.895	1.115	1.267	1.103
		L	0.542		0.812		0.998		1.397	
	isopropyl	D	0.365	1.137	0.566	1.140	0.707	1.097	1.004	1.079
		L	0.415		0.646		0.776		1.083	
	n-butyl	D	0.661	1.175	0.996	1.177	1.253	1.121	1.787	1.111
		L	0.776		1.173		1.404		1.985	
	tert-butyl	D	0.415	1.139	0.632	1.154	0.769	1.094	1.151	1.034
		L	0.473		0.729		0.841		1.191	
	cyclopentyl	D	1.137	1.156	1.693	1.158	2.083	1.118	3.007	1.112
		L	1.315		1.960		2.329		3.343	
HFB	methyl	D	0.283	1.146	0.443	1.168	0.559	1.109	0.797	1.081
		L	0.325		0.502		0.621		0.861	
	ethyl	D	0.359	1.152	0.538	1.174	0.693	1.118	0.982	1.092
		L	0.412		0.632		0.776		1.072	
	n-propyl	D	0.516	1.168	0.769	1.178	0.963	1.135	1.361	1.098
		L	0.602		0.906		1.094		1.494	
	isopropyl	D	0.386	1.149	0.595	1.158	0.747	1.111	1.064	1.078
		L	0.444		0.689		0.830		1.148	
	n-butyl	D	0.726	1.184	1.075	1.198	1.339	1.146	1.909	1.106
		L	0.859		1.288		1.534		2.111	
	tert-butyl	D	0.466	1.171	0.711	1.173	0.863	1.100	1.223	1.029
		L	0.545		0.834		0.949		1.259	
	cyclopentyl	D	1.265	1.174	1.881	1.197	2.355	1.134	3.299	1.109
		L	1.509		2.256		2.635		3.661	

a) Relative retention time, reference compound is N-TFA-L-prolyl-L-leucine *n*-propyl ester, t_R=7.15 min.

TABLE II. Comparsion of Gas Chromatographic Data of Different N-Perfluoroacyl Prolyl Amino Acid *l*-Menthyl Esters at 250°

Perfluoroacyl	Enantiomer	Alanine		Valine		Leucine		Proline	
		RRT[a]	rL/D	RRT	rL/D	RRT	rL/D	RRT	rL/D
TFA	D	0.692	1.078	0.901	1.081	1.018	1.027	1.462	1.043
	L	0.747		0.973		1.045		1.525	
PFP	D	0.665	1.082	0.837	1.097	0.946	1.057	1.281	1.049
	L	0.719		0.919		1.000		1.344	
HFB	D	0.683	1.093	0.873	1.104	0.957	1.085	1.308	1.059
	L	0.746		0.964		1.036		1.385	

a) RRT, reference compound is N-PFP-L-prolyl-L-leucine *l*-menthyl ester, t_R=5.53 min.

the same as those of *n*-propyl ester derivatives and the separation factors of *l*-menthyl ester derivatives were lower than those of other ester derivatives. The separation factors of N-perfluoroacyl-alanine and valine *tert*-butyl ester derivatives were higher than those of isopropyl ester derivatives. On the other hand, the separation factors of leucine and proline *tert*-butyl ester derivatives were lower than those of isopropyl ester derivatives.

It followed from above the observations that the esters of *n*-alkyl alcohols were more favorable than those of branched or cyclic chain alcohols for the resolution of racemic amino acids.

Nakaparksin, *et al.*[3b] reported that amino acid isopropyl ester derivatives were better esters than amino acid *n*-butyl ester derivatives for the separation of racemic amino acids because the former had reasonable retention times with good separation factors on capillary column coated with the optically active stationary phases.

However, we found that when racemic amino acids were converted into N-perfluoroacyl-prolyl-amino acid isopropyl esters and separated on OV-1, the separation factors of isopropyl ester derivatives were lower than those of *n*-butyl ester derivatives.

There have been a number of reports concerning the elution of racemic amino acids in gas chromatography. In the preceding paper,[1] we reported that D-amino acid derivatives except for basic amino acid derivatives had always shorter retention times than L-amino acid derivatives. It was found that D-amino acid derivatives also had shorter retention times than L-amino acid derivatives when N-perfluoroacyl-L-prolyl chloride was used as the resolving agent in all esters investigated on OV-1 column. It was concluded that the order of elution of racemic amino acids was not influenced by the N-perfluoroacyl groups or the ester groups.