[Chem. Pharm. Bull. 22(9)2075—2080(1974)] UDC 547.466.05 : 543.544

Resolution of Racemic Amino Acids by Gas Chromatography. IV.[1] N-Trifluoroacetyl-L-prolyl Amino Acid *n*-Butyl Esters

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(Received February 19, 1974)

The structural effect of the amino acids on the resolution of enantiomers and the relative retention times were studied by gas chromatography on OV-1 and polyethylene glycol adipate as the stationary phases. Relation between the separation factors and structure of racemic amino acids was discussed. It was concluded that the separation factors of racemic amino acids depend on the relative size of the substituents on the asymmetric carbon and the position of amino group.

Gas chromatography has been used for the resolution of amino acid enantiomers which are converted to form the diastereoisomers prepared with optically active reagents followed by gas chromatography on an optically inactive stationary phase,[3] or to form suitable derivatives prepared with optically inactive reagents followed by gas chromatography on an optically active stationary phase.[4]

In continuation of the previous work[1,3a,b] this paper reports the resolution of racemic amino acids. The previous papers reported that effect of resolving agents,[1] N-perfluoroacyl groups and ester groups,[3b] on the resolution of racemic amino acids and the relation between separation factors and structure of amino acids.[3a] This paper deals with the relative retention times and relation between the separation factors and structure of 25 racemic amino acids and glycine which are converted to N-TFA[5]-L-prolyl amino acid *n*-butyl esters.

Experimental

Apparatus and Conditions——A Hewlett-Packard Model 402 gas chromatograph equipped with a dual flame ionization detector was used. Two glass columns of 5.5 ft×1/4 in O.D. packed with 5% OV-1 on 100—120 mesh Supelcoport, and 4 ft×1/4 in O.D. packed with 2% PEGA, stabilized grade, were used. Helium was used as the carrier gas at a flow rate of 60 ml/min.

Reagents and Material——All solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co., Sigma Chemical Co., K & K Laboratories, and Tokyo Kasei Co. Trifluoroacetic anhydride, N,O-bis-(trimethylsilyl)trifluoroacetamide, trimethylchlorosilane, and acetonitrile were purchased from Pierce Chemical Co. N-TFA-L-PC was prepared in the same manner as described in the previous paper.[3a] Hypovial was obtained from Pierce Chemical Co.

Preparation of Amino Acid Derivatives——Amino acid *n*-butyl esters were prepared according to the method of Roach, *et al.*[6] N-TFA-L-prolyl amino acid *n*-butyl esters were prepared according to the method reported previously.[3a]

1) Part III: H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **22**, 1663 (1974).
2) Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
3) *a*) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 8 (1974); *b*) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 1455 (1974); *c*) B. Halpern and J.W. Westley, *Biochem. Biophys. Res. Commun.*, **19**, 361 (1965); B. Halpern and J.W. Westley, *Chem. Commun.*, **1965**, 246; E. Gil-Av, R. Charles, and G. Fischer, *J. Chromatogr.*, **17**, 408 (1965); E. Gil-Av, R. Charles-Sigler, G. Fischer and D. Nurok, *J. Gas Chromatogr.*, **4**, 51 (1966); F. Raulin and B.N. Khare, *J. Chromatogr.*, **75**, 13 (1973).
4) S. Nakaparksin, P. Birrell, E. Gil-Av, and J. Oró, *J. Chromatogr. Sci.*, **8**, 177 (1970); W.A. Koenig, W. Parr, H.A. Lichtenstein, E. Bayer and J. Oró, *J. Chromatogr. Sci.*, **8**, 183 (1970); K. Grohmann and W. Parr, *Chromatographia*, **5**, 18 (1972); W. Parr and P.Y. Howard, *J. Chromatogr.*, **67**, 227 (1972).
5) Abbreviations: TFA, trifluoroacetyl; N-TFA-L-PC, N-TFA-L-prolyl chloride; PEGA, polyethylene glycol adipate.
6) D. Roach and C.W. Gehrke, *J. Chromatogr.*, **44**, 269 (1969).

Table I. Gas Chromatographic Data for Racemic Amino Acids as Their N-TFA-L-Prolyl Amino Acid *n*-Butyl Esters with 5% OV-1 on Supelcoport at 210°

Amino acid	Structure	Emantiomer	RRT[a]	(r L/D)
Alanine	CH_3–CH(NH$_2$)–COOH	D-	0.519	1.124
		L-	0.583	
Glycine	H–CH(NH$_2$)–COOH	—	0.615	—
α-Amino-*n*-buturic acid	CH_3CH_2–CH(NH$_2$)–COOH	D-	0.658	1.114
		L-	0.733	
Isovaline	CH_3CH_2–C(CH_3)(NH$_2$)–COOH	D-	0.689	1.000
		L-	0.689	
β-Amino-*n*-butyric acid	CH_3CH(NH$_2$)–CH_2–COOH	D-	0.743	1.086
		L-	0.806	
Valine	$(CH_3)_2$CH–CH(NH$_2$)–COOH	D-	0.743	1.137
		L-	0.845	
Norvaline	$CH_3CH_2CH_2$–CH(NH$_2$)–COOH	D-	0.818	1.105
		L-	0.904	
β-Amino-iso-butyric acid	CH_2(NH$_2$)–CH(CH_3)–COOH	D-	0.845	1.000
		L-	0.845	
tert-Leucine	CH_3–C(CH_3)(NH$_2$)–CH(CH_3)–COOH	D-	0.818	1.144
		L-	0.936	
Leucine	$(CH_3)_2$CH–CH_2–CH(NH$_2$)–COOH	D-	0.914	1.094
		L-	1.000	
Isoleucine	CH_3CH_2(CH_3)CH–CH(NH$_2$)–COOH	D-	0.979	1.098
		L-	1.075	
Norleucine	$CH_3CH_2CH_2CH_2$–CH(NH$_2$)–COOH	D-	1.096	1.088
		L-	1.198	
Serine	HO–CH_2–CH(NH$_2$)–COOH	D-	1.214	1.097
		L-	1.332	
Threonine	CH_3CH(OH)–CH(NH$_2$)–COOH	D-	1.278	1.100
		L-	1.406	
Proline		D-	1.332	1.104
		L-	1.471	
Penicillamine	$(CH_3)_2$C(SH)–CH(NH$_2$)–COOH	D-	1.427	1.075
		L-	1.535	
Pipecolic acid		D-	1.834	1.087
		L-	1.995	
α-Amino-*n*-caprylic acid	$CH_3CH_2CH_2CH_2CH_2CH_2$–CH(NH$_2$)–COOH	D-	2.016	1.074
		L-	2.166	
Methionine	$CH_3SCH_2CH_2$–CH(NH$_2$)–COOH	D-	2.326	1.062
		L-	2.471	
Phenylglycine	C_6H_5–CH(NH$_2$)–COOH	D-	2.588	1.000
		L-	2.588	
Ethionine	$CH_3CH_2SCH_2CH_2$–CH(NH$_2$)–COOH	D-	2.919	1.064
		L-	3.107	
Phenylalanine	$C_6H_5CH_2$–CH(NH$_2$)–COOH	D-	3.417	1.063
		L-	3.631	

a) Relative retention time, reference compound is N-TFA-L-prolyl-L-leucine *n*-butyl ester. t_R=4.92 min

Separation Factors——Separation factors, α, were calculated from the following equation:

$$\alpha = (tR_2 - t_a)/(tR_1 - t_a)$$

were t_{R1} and t_{R2} 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 the relative retention times and separation factors of N-TFA-L-prolyl amino acid *n*-butyl esters are given in Tables I, II, and III.

Table II. Gas Chromatographic Data for Racemic Amino Acids as Their N-TFA L-Prolyl Amino Acid *n*-Butyl Esters with 2% PEGA on Supelcoport at 220°

Amino acid	Enantiomer	RRT[a]	(r L/D)
tert-Leucine	D-	0.325	1.204
	L-	0.391	
Threonine	D-	0.351	1.151
	L-	0.404	
Serine	D-	0.431	1.153
	L-	0.497	
Isoleucine	D-	0.444	1.209
	L-	0.536	
β-Amino-*n*-butyric acid	D-	0.536	1.148
	L-	0.616	
β-Amino-iso-butyric acid	D-	0.576	1.069
	L-	0.616	
Glycine	—	0.788	—
α-Amino-*n*-caprylic acid	D-	0.947	1.168
	L-	1.106	
Penicillamine	D-	1.039	1.102
	L-	1.146	
Proline	D-	1.000	1.252
	L-	1.583	
Pipecolic acid	D-	1.252	1.106
	L-	1.384	
Phenylglycine	D-	2.325	1.105
	L-	2.569	
Methionine	D-	2.404	1.127
	L-	2.709	
Ethionine	D-	2.815	1.132
	L-	3.185	
Aspartic acid	D-	2.848	1.044
	L-	2.974	
Glutamic acid	D-	4.563	1.120
	L-	5.113	

a) RRT, reference compound is N-TFA-L-prolyl-D-proline *n*-butyl ester. t_R=4.05 min

Table III. Gas Chromatographic Data for Racemic Amino Acids as Their N-TFA L-Prolyl Amino Acid *n*-Butyl Esters with 5% OV-1 on Supelcoport at 290°

Amino acid	Structure	Enantiomer	RRT[a]	(r L/D)
Ornithine	$NH_2(CH_2)_3$–CH(NH$_2$)–COOH	L-	0.812	0.953
		D-	0.852	
Lysine	$NH_2(CH_2)_4$–CH(NH$_2$)–COOH	L-	1.000	0.936
		D-	1.068	

a) RRT, reference compound is N-TFA-L-prolyl-L-lysine *n*-butyl ester. t_R=6.35 min

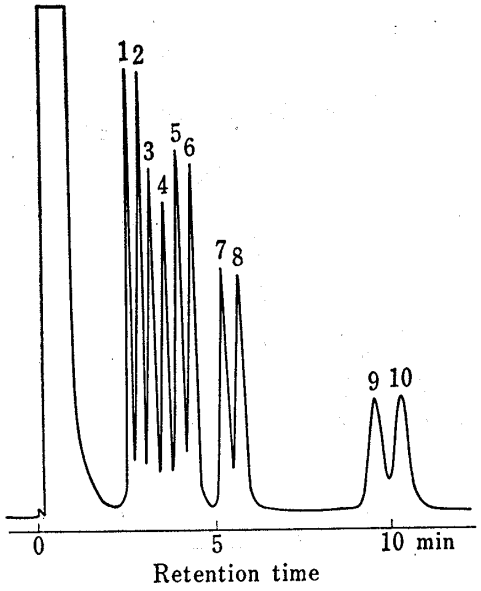


Fig. 1. Gas Chromatogram of N–TFA–L-Prolyl Amino Acid *n*-Butyl Esters on OV-1

1: D-alanine, 2: L-alanine, 3: D-α-amino-*n*-butyric acid, 4: L-α-amino-*n*-butyric acid, 5: D-norvaline, 6: L-norvaline, 7: D-Norleucine, 8: L-norleucine, 9: D-α-amino-*n*-caprylic acid, 10: L-α-amino-*n*-caprylic acid

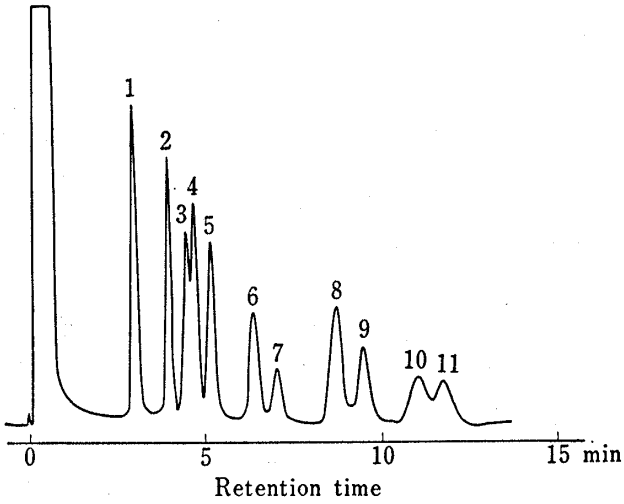


Fig. 2. Gas Chromatogram of N–TFA–L-Prolyl Amino Acid *n*-Butyl Esters on OV-1

1: glycine, 2: D-*tert*-leucine, 3: L-*tert*-leucine, 4: D-isoleucine, 5: L-isoleucine, 6: D-proline, 7: L-proline, 8: D-pipecolic acid, 9: L-pipecolic acid, 10: D-methionine, 11: L-methionine

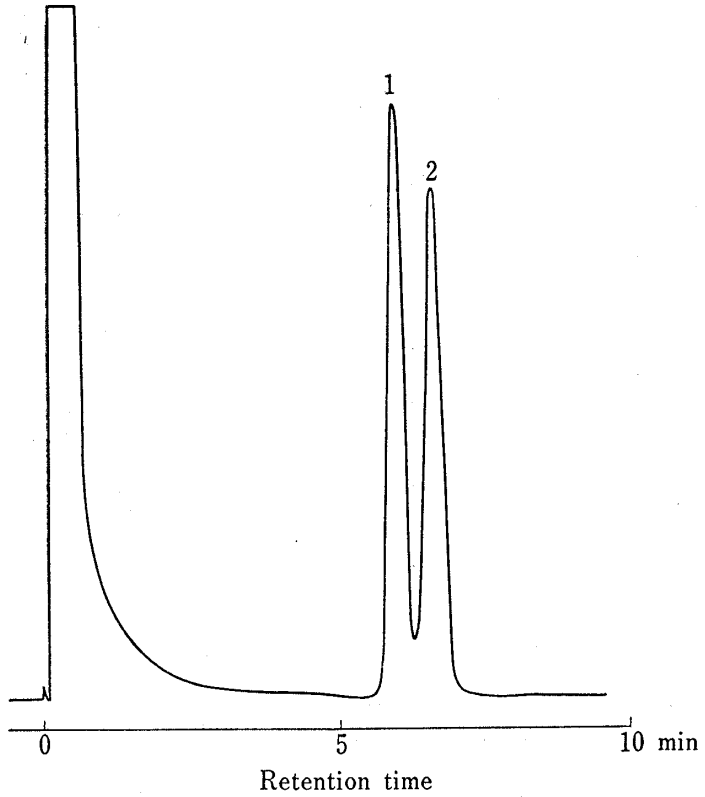


Fig. 3. Gas Chromatogram of N–TFA–L-Prolyl Amino Acid *n*-Butyl Esters on OV-1

1: D-threonine, 2: L-threonine

As can be seen from Table I, it is of interest that normal chain amino acid derivatives (norvaline and norleucine) have longer retention times than the corresponding branched amino acid derivatives (valine, leucine, isoleucine, and *tert*-leucine). The retention times of α-amino-*n*-butyric acid, β-amino-*n*-butyric acid, and β-aminoisobutyric acid increase in this order. The gas chromatograms of amino acid derivatives are shown in Fig. 1, 2, 3, 4, and 5.

Rose, *et al.*[7] and Stern, *et al.*[8] studied the separation of diastereomeric esters of acetylated lactic acid and diastereoisomeric methylalkylcarbinyl α-haloalkanoates on the optically inactive stationary phases, and Feibush[9] investigated the separation of stereoisomers of amino acids on an optically active stationary

7) H.C. Rose, R.L. Stern, and D.L. Karger, *Anal. Chem.*, **38**, 469 (1966).
8) R.L. Stern, B.L. Karger, W.J. Keane, and H.C. Rose, *J. Chromatogr.*, **39**, 17 (1969).
9) B. Feibush, *Anal. Chem.*, **43**, 1098 (1971).

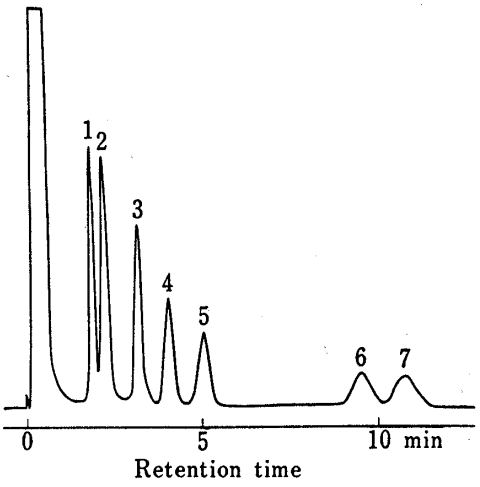


Fig. 4. Gas Chromatogram of N-TFA-L-Prolyl Amino Acid *n*-Butyl Esters on PEGA

1: D-isoleucine, 2: L-isoleucine, 3: glycine, 4: D-proline, 5: L-proline, 6: D-methionine, 7: L-methionine

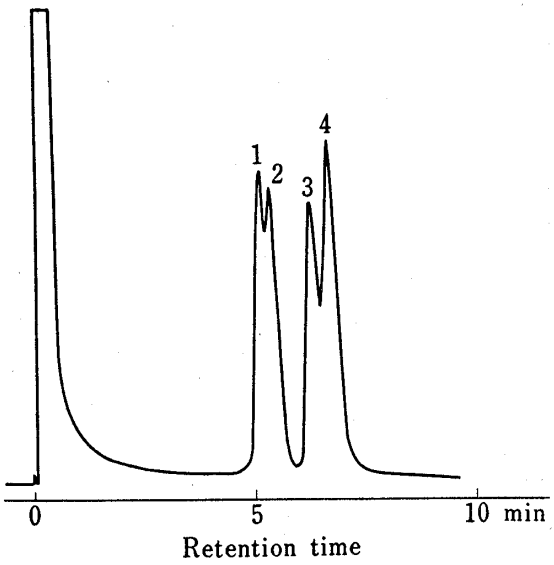


Fig. 5. Gas Chromatogram of N-TFA-L-Prolyl Amino Acid *n*-Butyl Esters on OV-1

1: L-ornithine, 2: D-ornithine, 3: L-lysine, 4: D-lysine

phase. These workers reported that the bulk of the substituents associated with the asymmetric carbon affected the resolution of the enantiomers. In the present work, relation between the separation factors and the relative size of the substituents in N-TFA-L-prolyl amino acid *n*-butyl esters on OV-1 and PEGA as the stationary phases was examined.

Relation between the separation factors and the structure of amino acids was studied from the data in Tables I, II, and III. The variational series deals with simple insertion of methylene groups between the initial α- and β-carbons. Derivatives of DL-alanine, α-amino-*n*-butyric acid, norvaline, norleucine, α-amino-*n*-caprylic acid, valine, leucine, proline, pipecolic acid, methionine, ethionine, phenylglycine, phenylalanine, aspartic acid, glutamic acid, ornithine, and lysine were chosen for this examination. For this homologous side chain modification, it was found that an increase in the separation factors of racemic amino acids on OV-1 was accompanied with a decrease in the number of methylene group for alanine, α-amino-*n*-butyric acid, norvaline, norleucine, and α-amino-*n*-caprylic acid. This was true for valine and leucine on OV-1, and results were also the same for proline and pipecolic acid on both OV-1 and PEGA. On the other hand, it was found that a slight increase in the separation factors was accompanied with an increase in the number of methylene group for methionine and ethionine on both stationary phases, and also for phenylglycine and phenylalanine on OV-1, but was reverse on PEGA. Table II shows that an increase in the separation factors on PEGA is accompanied with an increase in the number of methylene group for aspartic acid and glutamic acid.

Data in Table III indicate that a decrease in the separation factors of D- and L-isomers of ornithine and lysine is accompanied with an increase in the number of methylene group. However, as shown in Fig. 5, resolution of these basic amino acids becomes better with an increase in the number of methylene group. This may be due to the fact that basic amino acids have two amino groups which are masked by N-TFA-L-PC.

A comparsion was then made by replacing the proton at the β-carbon with methyl groups. Derivatives of DL-alanine, valine, and *tert*-leucine were chosen for this examination. For these homologues, it was found that separation factors on OV-1 increased with the increase in the degree of branching. The separation factors were found to increase in the order of alanine, valine, and *tert*-leucine.

An examination was made on the influence of the alkyl group attached to the asymmetric carbon on the solute having five and six carbon atoms in their molecule. The substituent

is primary in norleucine, secondary in isoleucine, and tertiary in *tert*-leucine. In addition, leucine which has an isobutyl group attached to the α-carbon was also investigated. An increase in the degree of branching of the side chain is also accompanied with an increase in the value of separation factors. The separation factors were found to increase in the order of norleucine, leucine, isoleucine, and *tert*-leucine on OV-1, and to increase in the order of norleucine=leucine, *tert*-leucine, and isoleucine on PEGA. Isoleucine and *tert*-leucine can be resolved on both stationary phases. Norleucine and leucine can be resolved on OV-1, but not on PEGA. This may be due to the difference in the side chain of these amino acids. The separation factors of amino acids having five carbon atoms were found to increase in the order of isovaline, norvaline, and valine on OV-1.

The separation factors of phenylglycine and phenylalanine were smaller than those of alanine and α-amino-*n*-butyric acid on OV-1, and this may be due to the fact that phenylglycine and phenylalanine have a bulky phenyl group and benzyl group attached to their α-carbon, respectively.

The separation factor of α-amino-*n*-butyric acid was larger than that of isovaline, and this may be due to the H atom attached to the asymmetric carbon affecting the resolution of the enantiomers.

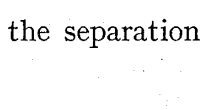
Effect of the position of amino group on the resolution of the enantiomers was also examined. α-Amino-*n*-butyric acid has a larger separation factor than β-amino-*n*-butyric acid on OV-1, and β-amino-*n*-butyric acid has a larger separation factor than β-aminoisobutyric acid on OV-1. On the other hand, the separation factors on PEGA are found to increase in the order of α-amino-*n*-butyric acid, β-aminoisobutyric acid, and β-amino-*n*-butyric acid.

Effect of the alkyl group attached to the asymmetric carbon on the solutes seems to be fairly complex and much dependent on the position of branching and amino group.

Penicillamine[9] was converted to 5,5-dimethylthiazolidine-4-carboxylic acid derivatives and resolved by reaction with N-TFA-L-PC. In the present work, it was converted to N-TFA-L-prolyl derivatives and was resolved on OV-1 and PEGA.

Serine and threonine have lower separation factors than alanine and α-amino-*n*-butyric acid on OV-1, but are reverse on PEGA. As described above, the separation of enantiomers differs from each other when OV-1 or PEGA is used. This may be due to the difference in the interaction between the solutes and stationary phases.

The previous papers[1,3a] reported that the separation factors of valine derivatives were the highest among the racemic amino acids examined. The present results show that the separation factors of *tert*-leucine derivatives is the best among racemic amino acids on OV-1, and proline derivatives is the best among enantiomers on PEGA.

It is concluded that the separation factors of 

relative size of the substituents on the asymmetric carbons, and that they seem to be influenced by the resolving agents,[1] N-perfluoroacyl groups,[3b] ester groups,[3b] and the structure of amino acids.

10) B. Halpern and J.W. Westley, *Tetrahedron Lett.*, **1966**, 2283.