Notes

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Resolution of Racemic Amino Acids by Gas Chromatography. VI.[1] *l*-Menthyl Ester Derivatives

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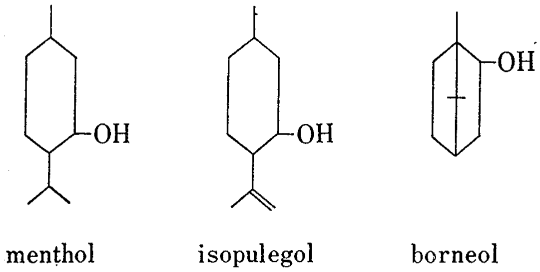
The resolution of racemic amino acids as their *l*-menthyl ester derivatives was carried out by gas chromatography. Seven stationary phases were studied for the chromatographic separation. The effect of N-perfluoroacyl groups and ester groups on the resolution of racemic amino acids was examined. Relation between separation factor and structure of amino acids has also been discussed.

It has already been reported that enantiomers by their conversion to diastereoisomers with *l*-menthol were resolved by gas chromatography. Murano[3] reported the resolution of diastereoisomeric esters of chrysanthemic acid with *l*-menthol and Westley, *et al.*[4] reported the resolution of racemic amino acids as their *l*-menthyloxycarbonyl diastereoisomeric derivatives. Vitt, *et al.*[5] and Halpern, *et al.*[6] also resolved the racemic amino acids as their N–TFA[7]-amino acid *l*-menthyl esters. However, the relation between separation factor and structure of racemic amino acids, and effect of N-perfluoroacyl groups on the resolution of racemic amino acids have not been disclosed. The present paper deals with the resolution of racemic amino acids by their conversion to N-TFA- amino acid *l*-methyl esters to examine the relation between separation factor and structure of amino acids. The author also wishes to report the resolution of racemic amino acids by their conversion to N-perfluoroacyl derivatives to examine whether *l*-borneol and *l*-isopulegol as the ester groups can be used in the manner as *l*-menthol.



Experimental

Apparatus——A Perkin-Elmer Model 900 gas chromatograph equipped with attachments for scot column and dual flame ionization detector was used. The stainless steel scot column of 100 ft × 0.02 in. i.d. coated with Apiezon L was used.

Reagents and Materials——All solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co., Tokyo Kasei Co., and K & K Laboratories, trifluoroacetic, pentafluoropropionic, heptafluorobutyric, and pentadecafluorooctanoic anhydrides from Pierce Chemical Co., and chlorodifluoro-

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[1] Part V: H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **23**, 217 (1975).
[2] Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
[3] A. Murano, *Agr. Biol. Chem.* (Tokyo), **36**, 917 (1972).
[4] J.W. Westley and B. Halpern, *J. Org. Chem.*, 33, 3978 (1968).
[5] S.V. Vitt, M.B. Saporowskaya, I.P. Gudkova, and V.B. Belikov, *Tetrahedron Letters.*, **1965**, 2575.
[6] B. Halpern and J.W. Westley, *Chem. Commun.*, **1965**, 421.
[7] Abbreviations: TFA, trifluoroacetyl; PFP, pentafluoropropionyl; HFB, heptafluorobutyryl; PDFO, pentadecafluorooctanoyl; CDF, chlorodifluoroacetyl; scot, support coated open tubular; PEGA, polyethylene glycol adipate; BDS, 1,4-butanediol succinate.

acetic anhydride from K & K Laboratories, and *l*-menthol, *l*-isopulegol and *l*-borneol from Aldrich Chemical Co. Scot columns were purchased from Perkin-Elmer Co. Hypovial was purchased from Pierce Chemical Co.

Preparation of Amino Acid Derivatives——Amino acid *l*-menthyl esters were prepared according to the procedure of Harada, *et al.*[8] N-Perfluoroacyl-amino acid *l*-menthyl esters were obtained by the known method.[9] N-Perfluoroacyl-amino acid *l*-bornyl esters were prepared in the same manner as *l*-menthyl ester derivatives.

Separation Factor——Separation factor, α, was calculated from the following definition:

$$\alpha = \frac{\text{retention time of D enantiomer}}{\text{retention time of L enantiomer}}$$

Result and Discussion

Stationary Phases

Seven stationary phases (Carbowax 20M, PEGA, BDS, Apiezon L, SE-30, SE-52, and Dexsil 300 GC) of scot columns were tested for the resolution of racemic amino acids.

The resolution of proline was poor on non-polar stationary phases (SE-30, SE-52 and Dexsil 300 GC) with tailing of the peaks, but it was much improved without tailing on polar stationary phases (BDS, Carbowax 20M and PEGA) and a non-polar stationary phase (Apiezon L). Apiezon L was used for the resolution of racemic amino acids, considering the separation of racemic amino acids and the asymmetric peaks. It is of interest that retention times increase in the order of valine, alanine, leucine and proline on Carbowax 20M, while in the order of alanine, valine, leucine and proline on Apiezon L.

Relation between Separation Factor and Structure of Amino Acids

The relationship between separation factor and structure of racemic amino acids was examined with N-TFA-amino acid *l*-methyl esters. It was found that, although the relative retention times of amino acids were the same as in the previous work,[10] relationship between separation factor and structure of amino acids was slightly different from that obtained in the previous work.[10] The separation factor increased with elongation of the side chain of amino acids in the case of α-amino-*n*-butyric acid, norvaline, norleucine, aspartic acid, and glutamic acid. With regard to amino acids possessing same number of carbons, the results were almost the same as in the previous work,[10] where the separation factors increased in the order of leucine, norleucine, isoleucine and *tert*-leucine. Attempts were then made to inspect the influence of the position of the amino group on the separation factor. It was found that amino acid having the amino group at the α-position was separated as previously,[10] whereas the β-amino acid could not be resolved. These experimental results indicate that, when amino acids are converted to diastereomers with N-TFA-L-proline derivatives or *l*-menthol, separation factor and retention time are dependent upon the side chain or the position of the amino group of amino acids, irrespective of whether the chiral group is introduced into the amino group or carboxylic group of amino acids. The experimental data of the relative retention times and separation factors of N-TFA-amino acid *l*-menthyl esters are summarized in Tables I, II and III.

Effect of Ester Groups and N-Perfluoroacyl Groups

l-Borneol was found to be useful as the ester group for the resolution of racemic amino acids, while *l*-isopulegol could not be used because many unknown peaks were accompanied. It seems that derivatization with *l*-isopulegol may not proceed so readily as with *l*-menthol due to the presence of a double bond.

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8) K. Harada and T. Hayakawa, *Bull. Chem. Soc. Japan*, **37**, 191 (1964).
9) D. Roach and C.W. Gehrke, *J. Chromatogr.*, **44**, 269 (1969).
10) H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2075 (1974).

Table I. Gas Chromatographic Data for Racemic Amino Acid as Their N-TFA *l*-Menthyl Esters at Different Temperatures

Amino acid	150°		170°	
	RRT[a]	α	RRT[a]	α
L-Alanine	0.550	1.117	0.580	1.116
D-Alanine	0.614		0.647	
L-α-Amino-*n*-butyric acid	0.735	1.109	0.765	1.099
D-α-Amino-*n*-butyric acid	0.815		0.840	
Glycine	0.751	—	0.765	—
L-Valine	0.823	1.117	0.849	1.099
D-Valine	0.920		0.933	
L-*tert*-Leucine	0.843	1.162	0.866	1.146
D-*tert*-Leucine	0.980		0.992	
L-Norvaline	1.000	1.120	1.000	1.118
D-Norvaline	1.120		1.118	
L-Leucine	1.129	1.121	1.118	1.105
D-Leucine	1.265		1.235	
L-β-Amino-*n*-butyric acid	1.129	1.000	1.126	1.000
D-β-Amino-*n*-butyric acid	1.129		1.126	
L-Isoleucine	1.181	1.153	1.168	1.129
D-Isoleucine	1.361		1.319	
L-Norleucine	1.482	1.130	1.420	1.124
D-Norleucine	1.675		1.597	
L-Proline	2.570	1.072	2.445	1.062
D-Proline	2.755		2.597	

a) RRT, relative retention time, reference compound is N-TFA-L-norvaline *l*-Menthyl ester, $t_{R_{150°}}$=12.45 min, $t_{R_{170°}}$=5.95 min

Table II. Gas Chromatographic Data for Racemic Amino Acid as Their N-TFA *l*-Menthyl Esters at 190°

Amino acid	RRT[a]	α
L-Methionine	0.927	1.079
D-Methionine	1.000	
L-Phenylalanine	1.466	1.081
D-Phenylalanine	1.584	

a) RRT, reference compound is N-TFA-D-methionine *l*-menthyl ester, t_R=10.95 min

Table III. Gas Chromatographic Data for Racemic Amino Acids as Their N-TFA *l*-Menthyl Esters at 215°

Amino acid	RRT[a]	α
L-Aspartic acid	0.622	1.000
D-Aspartic acid	0.622	
L-Glutamic acid	1.000	1.065
D-Glutamic acid	1.065	

a) RRT, reference compound is N-TFA-L-glutamic acid *l*-menthyl ester, t_R=38.75 min

The data of relative retention times and separation factors of N-perfluoroacyl-α-amino-*n*-butyric acid and leucine derivatives are given in Table IV and typical gas chromatograms are shown in Fig. 1 and 2.

There have been published numerous papers reporting the elution order of amino acids. It was found in the previous work[11] that, when amino acids were led to N-perfluoroacyl-L-

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[11] H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), 22, 1455 (1974).

prolyl-amino acid esters, D-amino acids were eluted out first, followed by L-amino acids, irrespective of whether the ester group was an optically active *l*-menthyl or inactive alkyl groups. In the present work the elution order of N-perfluoroacyl-amino acid *l*-menthyl esters (or *l*-bornyl esters) was found to be reversed, L-amino acids being eluted first, followed by D-amino acids. These facts suggest that the determining factor for the elution order of amino

Table IV. Comparison of Gas Chromatographic Data of Different N-Perfluoroacyl-Amino Acid Esters at 165°

Perfluoroacyl	Ester	Enantiomer	α-Amino-*n*-butyric acid		Leucine	
			RRT[a]	α	RRT	α
TFA	menthyl	L	0.698	1.108	1.038	1.109
		D	0.774		1.151	
	bornyl	L	0.799	1.059	1.214	1.052
		D	0.846		1.277	
PFP	menthyl	L	0.597	1.105	0.862	1.102
		D	0.660		0.950	
	bornyl	L	0.679	1.056	1.000	1.050
		D	0.717		1.050	
HFB	menthyl	L	0.635	1.099	0.912	1.097
		D	0.698		1.000	
	bornyl	L	0.723	1.052	1.057	1.042
		D	0.761		1.101	
PDFO	menthyl	L	1.157	1.092	1.597	1.091
		D	1.264		1.742	
	bornyl	L	partially resolved		partially resolved	
		D				
CDF	menthyl	L	1.717	1.110	2.484	1.111
		D	1.906		2.761	
	bornyl	L	1.962	1.061	2.950	1.053
		D	2.082		3.107	

[a] RRT, reference compound is N-HFB-D-leucine *l*-methyl ester, $t_R=7.95$ min

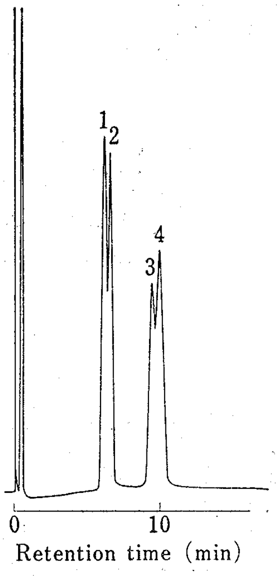

Fig. 1. Gas Chromatogram of N-TFA-Amino Acid *l*-Bornyl Esters at 165°

1: L-*α*-amino-*n*-butyric acid,
2: D-*α*-amino-*n*-butyric acid,
3: L-leucine,
4: D-leucine

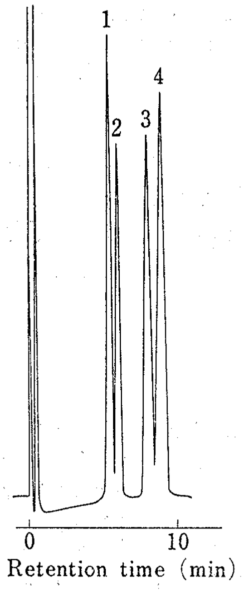

Fig. 2. Gas Chromatogram of N-TFA-Amino Acid *l*-Menthyl Esters at 165°

1: L-*α*- amino-*n*-butyric acid,
2: D-*α*-amino-*n*-butyric acid,
3: L-leucine,
4: D-leucine

acids is mainly due to the interaction between chiral centers of racemic amino acids and N-perfluoroacyl-L-prolyl group when L-prolyl residue is introduced into the amino group. On the other hand, the interaction between the optically active centers of racemic amino acids and *l*-menthyl ester group become the dominant factor when optically inactive group is introduced into the amino group instead of L-proline deivatives by the difference in mechanism of resolution from the former.

Retention times of two amino acid ester derivatives were found to increase with change of N-perfluoroacyl groups in the order of PFP, HFB, TFA, PDFO, and CDF. *l*-Menthyl ester derivatives had shorter retention times than *l*-bornyl ester derivatives.

A decrease in the number of CF_2 group in N-perfluoroacyl groups exerted an increase in the separation factors of racemic amino acids, and *l*-methyl ester derivatives exhibited larger separation factors than *l*-bornyl ester derivatives in all N-perfluoroacyl groups. *l*-Menthyl residue seems to be more suitable ester group in respects with the retention time and separation factor.

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Resolution of Racemic Amino Acids by Gas Chromatography. VII.[1] N-Trifluoroacetyl-Amino Acid Esters

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The gas chromatographic resolution of racemic amino acids as their N-trifluoroacetyl esters was carried out on an optically active stationary phase. Relation between the separation factor and structure of racemic amino acids, and relative retention times were studied. It is concluded that the separation factor of racemic amino acids depends on the relative size of the substituent on the asymmetric carbon and the position of amino group.

Following after the previous work, the present paper also deals with the resolution of racemic amino acids which are converted to N-trifluoroacetyl (TFA) amino acid esters with optically inactive reagents followed by gas chromatography using new agricultural chemicals[3] effective for preventing rice blast disease, N-lauroyl-L-valine and L-valine lauryl ester hydrochloride.

Experimental

Apparatus and Condition——A Perkin-Elmer Model 900 gas chromatograph equipped with attachments for capillary column and dual flame ionization detector was used. A stainless steel capillary column of 200 ft × 0.02 in. I.D. was cleaned as described by Koenig, *et al.*[4] and coated using 10% w/v solution of N-lauroyl-L-valyl-L-valine lauryl ester in methylene chloride, at 14 p.s.i. (dry nitrogen).

Reagents and Material——All solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co., Tokyo Kasei Co., and K & K Laboratories. N-Lauroyl-L-valine and L-valine

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[1] Part VI: H. Iwase, *Chem. Pharm. Bull.* (Tokyo), 23, 1604 (1975).

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[4] W.A. Koenig, W. Parr, H.A. Lichtenstein, E. Bayer, and J. Oró, *J. Chromatogr. Sci.*, 8, 183 (1970).