General Procedure for the Preparation of IIIa,b,c,d ——Method A: A mixture of IIb (2.6 g, 6.96 mM) and 2,3-dimethylbutadiene (1.0 g, 12.2 mM) in dry dioxane (10 ml) was heated at 95° for 16 hr. Removal of the solvent *in vacuo* gave a resignous matter. On recrystallization from *n*-hexane was obtained 1.4 g (44.1%) of IIId as colorless needles, mp 152—153°. Analytical data are given in Table I.

3-Benzyl-5-methoxycarbonylmethylidene-2-thioxo-4-thiazolidone (Ib)——a) A mixture of 3-benzyl-5-carboxymethyl-2-thioxo-4-thiazolidone (28.2 g, 0.100 M) and bromine (16.0 g, 0.100 M) in AcOH (70 ml) was heated under reflux for 30 min. After cooling precipitated crystals were collected and washed with water to give 23.7 g (84.6%) of 3-benzyl-5-carboxymethylidene-2-thioxo-4-thiazoldidone, mp 202—203°.

b) A stirred suspension of 3-benzyl-5-carboxymethylidine-2-thioxo-4-thiazolidone (23 g, 0.0825 M) in methanol (150 ml) was saturated with dry HCl. After cooling separated yellow needles were collected and washed with methanol to give 21.5 g (89.3%) of Ib, mp 147—149°.

In the same way Ia was prepared from 3-methyl-5-carboxymethyl-2-thioxo-4-thiazolidone in a 80.0% yield.

3-Benzyl-5-benzoylmethylidene-2-thioxo-4-thiazolidone (IId)——a) A mixture of 3-benzyl-2-thioxo-4-thiazolidone (2.2 g, 9.86 mM), phenylglyoxal (2.8 g, 18.7 mM) and NaOAc (0.5 g) in AcOH (20 ml) was heated on a boiling water bath for 30 min. After cooling separated yellow crystals were collected and washed with water to give 1.9 g (57.1%) of IIa, mp 177—179°. *Anal.* Calcd. for $C_{18}H_{13}O_2NS_2$: C, 63.72; H, 3.86; N, 4.15. Found: C, 63.82; H, 3.82; N, 4.13.

b) A mixture of 3-benzyl-5-benzoylmethyl-2-thioxo-4-thiazolidone (0.35 g, 1.03 mM) and bromine (0.20 g, 1.25 mM) in AcOH (10 ml) was heated on a boiling water bath for 5 min. Separated yellow crystals were collected and washed with ethanol to give 0.32 g (91.3%) of IIa, mp 175—177°. After recrystallization from AcOH it melts at 177—179°.

In the same way IIb was obtained from 3-benzyl-5-(*p*-chlorobenzoylmethyl)-2-thioxo-4-thiazolidone in a 95.0% yield as yellow leaflets, mp 251—252°. *Anal.* Calcd. for $C_{18}H_{12}O_2NS_2Cl$ (IIb): C, 57.82; H, 3.23; N, 3.75. Found: C, 57.65; H, 3.28; N, 3.50.

3-Benzyl-5-(*p*-chlorobenzoylmethyl)-2-thioxo-4-thiazolidone (Vb)——To a stirred solution of β-(*p*-chlorobenzoyl)-acrylic acid in ethanol (50 ml) was added triethylammonium N-benzyldithiocarbamate (5.8 g, 20.5 mM) at room temperature. Conc. HCl was added in three portions at the intervals of 15, 30, and 30 min. After additional stirring for 3 hr separated crystals were collected and washed with small portions of ethanol to give 6.5 g (86.5%) of Vb, mp 120—123°. After recrystallization from ethanol it melts 123—124°. *Anal.* Calcd. for $C_{18}H_{14}O_2NS_2Cl$: C, 57.51; H, 3.75; N, 3.73. Found: C, 57.24; H, 3.76; N, 3.51.

In the same way 3-benzyl-5-benzoylmethyl-2-thioxo-4-thiazolidone (Va) was prepared from β-benzoyl-acrylic acid and triethylammonium N-benzyldithiocarbamate in a 56.0% yield as pale yellow crystals, mp 126—128°. *Anal.* Calcd. for $C_{18}H_{15}O_2NS_2$: C, 63.34; H, 4.43; N, 4.10. Found: C, 63.10; H, 4.47; N, 4.20.

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Resolution of Racemic Amino Acids by Gas Chromatography. III.[1] *n*-Butyl Ester Derivatives

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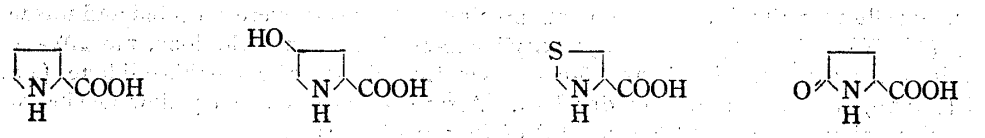
The preceding paper of this series reported the effect of N-perfluoroacyl groups and ester groups on the separation of four racemic amino acids (alanine, valine, leucine, and proline) and it was found that an increase in the separation factors of racemic amino acids, except

1) Part II: H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 8 (1974).
2) Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*

for proline, accompanied an increase in the number of CF_2 group in N-perfluoroacyl groups, and that esters of alkyl alcohols were more favorable than those of branched alcohols for the resolution of racemic amino acids. This paper deals with the resolution of the racemic amino acids by their conversion to *n*-butyl esters to see whether hydroxyproline, pyroglutamic acid, and 4-thiazolidinecarboxylic acid as the resolving agents can be used same as proline.



proline hydroxyproline 4-thiazolidinecarboxylic acid pyroglutamic acid

Chart 1

Experimental

Apparatus and Condition——A Hewlett-Packard Model 402 gas chromatograph equipped with dual flame ionization detectors 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 220°. Heliumn was used as the carrier gas at a flow rate of 60 ml/min.

Reagents and Materials——All the solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co. and Tokyo Kasei Co. Trifluoroacetic anhydride was purchased from Pierce Chemical Co. Hypovial was purchased from Pierce Chemical Co. The stationary phase, OV-1, was obtained from Analabs Inc. The support material, 100—120 mesh Supercoport, was obtained from Supelco. Inc. N-TFA[3]-L-HPC and N-TFA-L-TCAC were prepared in the same manner as N-TFA-L-PC.[4]

Preparation of Amino Acid Derivatives——Each amino acid *n*-butyl ester was prepared according to the method of Roach, *et al.*[5] Amino acid *n*-butyl ester derivatives were prepared in the same manner as described in the preceding paper.[4]

Separation Factors——Separation factors, α, 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

Hydroxyproline and 4-thiazolidinecarboxylic acid were found to be usable as the resolving agent for the resolution of racemic amino acids, while pyroglutamic acid could not be used because peaks of the racemic amino acids were not observed. It seems that proline-like derivative of pyroglutamic acid can not be formed due to the presence of a peptide bond in it.

The experimental data of relative retention times and separation factors of amino acid *n*-butyl ester derivatives are given in Table I.

The retention times of six racemic amino acid derivatives were found to increase with the change of the resolving agents in the order of proline<4-thiazolidinecarboxylic acid< hydroxyproline, and that the retention times of hydroxyproline derivatives were twice longer than those of the corresponding proline derivatives.

There are a number of reports concerning the elution of racemic amino acids in gas chromatography. It had been found[1,4] that D-amino acid derivatives except for basic amino acid derivatives had always shorter retention times than L-amino acid derivatives, and it was found that D-amino acid derivatives had also shorter retention times than L-amino acid derivatives when N–TFA–L–HPC and N–TFA–L–TCAC were used as the resolving agent. Some of the gas chromatograms obtained in this experiment are shown in Figs. 1 and 2.

3) Abbreviations: TFA; trifluoroacetyl, N-TFA-L-PC; N-TFA-L-prolyl chloride, N-TFA-L-HPC; N-TFA-L-hydroxyprolyl chloride, N-TFA-L-TCAC; N-TFA-L-4-thiazolidinecarboxylic acid chloride.

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

5) D. Roach and C.W. Gehrke, *J. Chromatog.*, **44**, 269 (1969).

Table I. Gas Chromatographic Data for Racemic Amino Acids on OV-1 at 220°

Resolving agent		Proline		4-Thiazolidine carboxylic acid		Hyydroxyproline	
Amino acid	Enantiomer	RRT[a]	(r L/D),	RRT,	(r L/D)	RRT,	(r L/D)
Alanine	D-	0.364	1.109	0.529	1.101	0.698	1.134
	L-	0.404		0.582		0.791	
Valine	D-	0.511	1.122	0.742	1.108	0.973	1.137
	L-	0.573		0.822		1.107	
Leucine	D-	0.618	1.086	0.916	1.068	1.196	1.089
	L-	0.671		0.978		1.302	
Proline	D-	0.902	1.108	1.258	1.131	1.627	1.134
	L-	1.000		1.422		1.845	
Methionine	D-	1.551	1.057	2.249	1.045	3.027	1.098
	L-	1.640		2.351		3.324	
Phenylalanine	D-	2.240	1.058	partially resolved		4.302	1.058
	L-	2.369				4.551	

a) RRT, reference compound is N-TFA-L-prolyl-L-proline n-butyl ester, t_R=5.84 min.

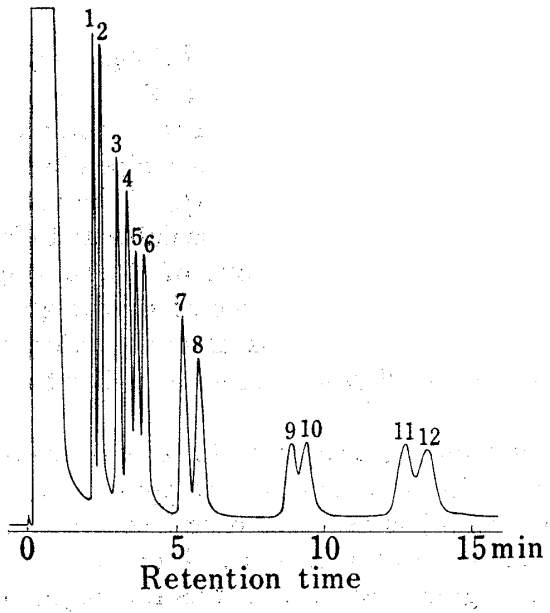


Fig. 1. Gas Chromatogram of N-TFA L-Prolyl Amino Acid n-Butyl Esters on OV-1

1: D-alanine, 2: L-alanine, 3: D-valine, 4: L-valine, 5: D-leucine, 6: L-leucine, 7: D-proline, 8: L-proline, 9: D-methionine, 10: L-methionine, 11: D-phenylalanine, 12: L-phenylalanine

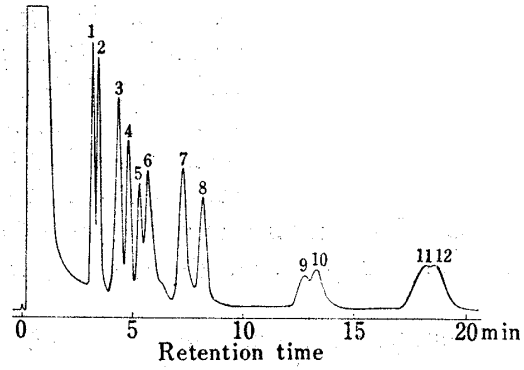


Fig. 2. Gas Chromatogram of N-TFA L-4-Thiazolidinecarboxylic Acid Derivatives

1: D-alanine, 2: L-alanine, 3: D-valine, 4: L-valine, 5: D-leucine, 6: L-leucine, 7: D-proline, 8: L-proline, 9: D-methionine, 10: L-methionine, 11: D-phenylalanine, 12: L-phenylalanine

We reported previously[1,4] that the separation factors of valine ester derivatives were the highest among the racemic amino acids examined. The present results also showed that the separation of valine n-butyl ester derivatives was better than those of other racemic amino acids studied.

Feibush[6] and Stern, *et al.*[7] reported that the bulk of the substituents associated with the asymmetric carbon affected the resolution of racemic amino acids. Examination of the effect of resolving agents on the resolution of racemic amino acids showed that the replace-

6) B. Feibush, *Anal. Chem.*, **43**, 1098 (1971).
7) R.L. Stern, B.L. Karger, W.J. Keane, and H.C. Rose, *J. Chromatog.*, **39**, 19 (1969).

ment of the carbon atom of the resolving agent by a sulfur atom decreased the separation factors of racemic amino acids except for proline derivatives, and the replacement of H atom attached to carbon atom by a OH group increased the separation factors of racemic amino acids. Proline derivative seems to be the best resolving agent because it has a desirable retention times with better separation factors.

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Isolation and Structure of α-1,3-Linked Glucan from the Hyphal Wall of *Phytophthora infestans*[1]

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Phytophthora infestans is a well-known phytopathogenic fungus which belongs to Oomycetes. Chemotaxonomically, it is interesting that the major component of the mycelial cell walls of Oomycetes is not chitin but glucan, and this is quite different from many other filamentous fungi.[3] Glucans from Phytophthora hyphal walls have been examined by a few workers. In *P. cinnamomi*,[4] two different β-glucans comprising nearly 90% of the mycelial wall were isolated and the major component was an extremely water-insoluble and highly branched glucan composed predominantly of β-1,3- and small amount of β-1,4-linkages. The minor component was cellulose. In the case of *P. hevea*[5] presence of similar glucans reported. These results were obtained from enzymic degradation and usual analytical procedures, and suggest that glucans from the hyphal wall of Phytophthora are similar to each other.

Recently, Hodgson, *et al.*[6] reported that water-soluble glucan from *P. infestans* consists of β-1,3-linkage and it completely inhibited local lesion development by potato virus X. However, water-insoluble component of the hyphal wall has not been described. In recent years, histochemical investigation on the hyphal walls of Oomycetes have been made in our laboratory, and an alkali-soluble and water-insoluble glucan was isolated as the major component from the hyphal wall of *P. infestans*. This glucan, contrary to expectation, is highly dextroratatory, $[\alpha]_D^{21}$ +217° (1N NaOH), and showed an absorption band at 844 cm^{-1} for α-glucosidic linkage in its infrared spectrum.

This glucan was methylated by the methods of Hakomori[7] and then of Purdie,[8] and after methanolysis of the methylated glucan was carried out the product was analyzed by gas–liquid chromatography (GLC). As shown in Fig. 1, methyl 2,4,6-tri-O-methyl- and a small amount of methyl 2,3,4,6-tetra-O-methyl-D-glucopyranosides were detected. The

1) This constitutes Part XIV of a series entitled "Studies on Fungal Polysaccharides." Part XIII: T. Miyazaki and Y. Naoi, *Chem. Pharm. Bull.* (Tokyo), **22**, 1360 (1974). A part of this work was presented at the 92nd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1973.
2) Location: *20–1, Kitashinjuku, 3-chome, Shinjuku-ku, Tokyo.*
3) S. Bartnicki-Garcia, *Annu. Rev. Microbiol.*, **22**, 87 (1968).
4) L.P.T.M. Zevenhuizen and S. Bartnicki-Garcia, *Biochemistry*, **8**, 1496 (1969).
5) M. Novaes-Ledieu and A. Jimenz-Martin, *J. Gen. Microbiol.*, **54**, 407 (1969).
6) F.A. Wood, R.P. Singh, and W.A. Hodgson, *Phytopathology*, **61**, 100 (1971).
7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
8) T. Purdie and J.C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).