

N-Alkyloxycarbonyl Isobutylamides as Readily Prepared Diamide Derivatives of Amino Acids for Separation of Enantiomeric Isomers by Chiral Phase Capillary Gas Chromatography

Iwao Abe,* Masatoshi Kawazuma, Noriteru Fujimoto, and Taketoshi Nakahara

Department of Applied Chemistry, College of Engineering, University of Osaka Prefecture, 1-1, Gakuen-cho, Sakai, Osaka 593

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Amino acid diamide derivatives of N-alkyloxycarbonyl isobutylamides have been prepared from corresponding N-alkyloxycarbonyl 2,2,2-trifluoroethyl esters and provided for use in separation of enantiomeric isomers by chiral phase capillary gas chromatography. The substitution reaction of 2,2,2-trifluoroethyl ester with isobutylamine was found to proceed almost completely within 5 min at room temperature. All the diamide derivatives prepared have increased the separation factor including Pro.

The method is well documented for an analysis of amino acid enantiomers on capillary columns coated with chiral stationary phases in gas chromatography (GC).^{1,2} Among many chiral stationary phases in GC, Chirasil-Val is known as one of the most thoroughly studied phase that is frequently used for the separation of amino acid enantiomers, either singly or in mixtures. Chirasil-Val is a statistical copolymer of dimethylsiloxane and (2-carboxypropyl)methylsiloxane coupled with chiral selector of L-valine-*t*-butylamide exhibits chiral recognition through hydrogen bonding, and capable of separating all protein amino acid enantiomeric pairs almost completely in the form of N(O)-perfluoroacyl isopropyl esters.³ However, drawback is an incomplete separation of the enantiomeric pair of Pro that possesses no amide hydrogen in its N-perfluoroacyl alkyl ester derivative results in an inadequate enantioselective interaction with Chirasil-Val.

In previous papers,^{4,5} we have reported a rapid method for derivatizing amino acids into N(O)-alkyloxycarbonyl alkyl esters for enantiomer analysis. However, Pro didn't give any tendency to be separated.

In this paper, we would like to report a convenient method for preparing diamide derivatives of amino acids from N-alkyloxycarbonyl 2,2,2-trifluoroethyl esters by nucleophilic substitution of the ester group with isobutylamine.

The derivatives were prepared in a similar manner before amidation step described in the literature.⁵ DL-Amino acid standard solution (100 μ l) containing each amino acid to a concentration of 2.5 μ mol/ml was pipetted into a vial and 2,2,2-

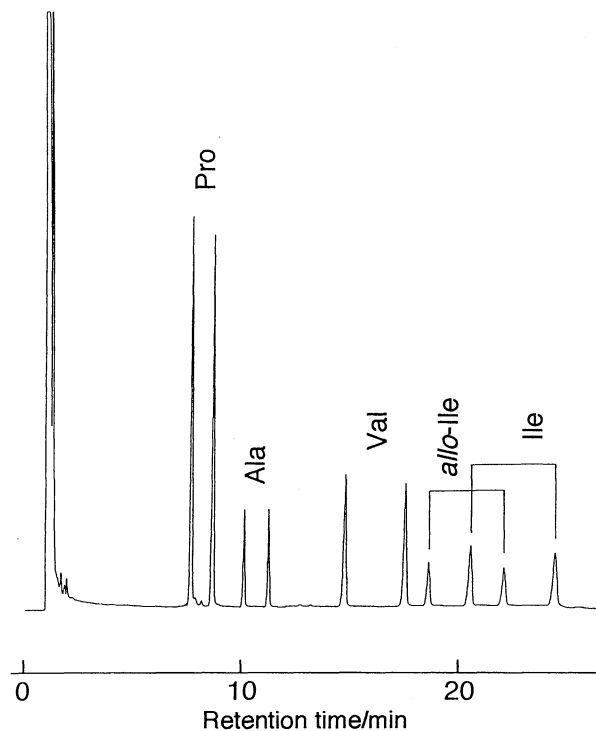


Figure 1. Gas chromatogram of N-isobutyloxycarbonyl isobutylamides of amino acid enantiomeric mixture. Column: Chirasil-Val capillary column (20 m x 0.25 mm i.d.); Column temp: 170 °C, isothermal; Carrier gas: He, 1.3 kg/cm²; Detector: FID; Split ratio: 1:40. For each amino acid enantiomeric pair, the L-enantiomer eluted faster except Pro which elute D-enantiomer fast.

trifluoroethanol/pyridine (3:1, v/v) mixture (50 μ l) was added. Next, alkyl chloroformate (8 μ l) was added to the vial, capped immediately, and the vial was shaken vigorously for 10 s. Amino acid derivatives of N-alkyloxycarbonyl 2,2,2-trifluoroethyl ester were formed which was added ethyl ether (200 μ l) followed by

Table 1. Separation data for amino acid enantiomeric derivatives on Chirasil-Val capillary GC column

Amino acid	Derivative A ^a			Derivative B ^b			Derivative C ^c		
	Retention time/min		Sf ^d	Retention time/min		Sf ^d	Retention time/min		Sf ^d
	1st peak	2nd peak		1st peak	2nd peak		1st peak	2nd peak	
Pro	2.51	2.51	1.000	3.28	3.54	1.109	7.80	8.79	1.144
Ala	2.03	2.09	1.061	4.66	5.00	1.091	10.23	11.29	1.114
Val	2.47	2.55	1.051	6.16	7.09	1.176	14.93	17.70	1.199
allo-Ile	3.14	3.28	1.064	7.60	8.69	1.163	18.78	22.24	1.194
Ile	3.42	3.54	1.048	8.33	9.64	1.177	20.69	24.62	1.199
Leu	4.21	4.47	1.080	8.66	9.68	1.132	NE ^e		—

^a N-2,2,2-Trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl ester; column temp: 120 °C, isothermal. ^b N-2,2,2-Trifluoroethoxycarbonyl isobutylamide; column temp: 170 °C, isothermal. ^c N-Isobutyloxycarbonyl isobutylamide; column temp: 170 °C, isothermal. ^d Separation factor (corrected, air-peak retention time: 0.94 min). ^e Not examined. For other conditions, see text.

shaking to extract the derivatives into organic layer. The ethereal layer was transferred into another vial by a Pasteur pipet, and about 0.2 g of anhydrous sodium sulfate was added and thoroughly shaken for dehydration. After standing for 10 min, the ether solution was transferred into another vial and evaporated to dryness with gentle stream of argon gas at room temperature. The residue was added 200 μ l of freshly distilled isobutylamine, capped tightly, ultrasonicated for 5 s, and left stand at room temperature for 5 min with occasional shaking. The excess reagent was evaporated to complete dryness. The residue was dissolved in 30 μ l of dichloromethane and about 0.5 μ l of the solution was injected into the GC.

Table 1 represents the retention times and separation factors of N-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl ester, N-2,2,2-trifluoroethoxycarbonyl isobutylamide, and N-isobutyloxycarbonyl isobutylamide derivatives of amino acid enantiomers, respectively. All amino acids examined in this study showed increased the separation factors by substituting 2,2,2-trifluoroethyl ester group with isobutylamine. Figure 1 shows a typical GC of N-isobutyloxycarbonyl isobutylamide derivatives of Pro, Ala, Val, *allo*-Ile, and Ile enantiomeric mixture. As easily recognized from Figure 1, all the amino acid enantiomeric pairs could be separated completely including Pro. Pro enantiomer was not able to be separated at all in the form of N-2,2,2-trifluoroethoxycarbonyl 2',2',2'-trifluoroethyl ester. Interestingly, the elution order reversed the enantiomer of all amino acids except Pro by substituting the ester group with isobutylamine. In addition to say, the method has accompanied by no appreciable racemization according to the test by using each amino acid of L-form. In enantiomer separation of Pro, it has been reported

previously that the separation factor increased considerably when converted into N-trifluoroacetyl isopropyl⁶ or *t*-butyl⁷ amide derivative. However, suitable derivatization method applicable to microanalysis has been not yet developed. This study has given a simple conversion method of amino acids into diamide derivatives which afford to separate the derivatives into enantiomeric pair completely on Chirasil-Val capillary column.

Studies on the substitution reaction with a variety of amines and application to various types of carboxyl-containing compounds are currently under way.

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References and Notes

- 1 W. A. König, "The Practice of Enantiomer Separation by Capillary Gas Chromatography," Hüthig Publisher, Heidelberg(1987).
- 2 S. G. Allenmark, "Chromatographic Enantioseparation," Ellis Horwood, Chichester(1988).
- 3 G. J. Nicholson, H. Frank, and E. Bayer, *J. High Resol. Chromatogr. & Chromatogr. Commun.*, **2**, 411(1979).
- 4 I. Abe, T. Nishiyama, and T. Nakahara, *Anal. Sci.*, **10**, 501(1994).
- 5 I. Abe, N. Fujimoto, and T. Nakahara, *Chem. Lett.*, 113(1995).
- 6 N. Oi, M. Horiba, and H. Kitahara, *J. Chromatogr.*, **202**, 299(1980).
- 7 S.-C. Chang, R. Charles, and E. Gil-Av, *J. Chromatogr.*, **202**, 247(1980).