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.

[Chem. Pharm. Bull. 23(7)1608—1611(1975)]

UDC 547.466.05 : 543.544

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

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(Received July 7, 1974)

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

1) Part VI: H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **23**, 1604 (1975).
2) Location: *1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, 210, Japan.*
3) Y. Homma, Y. Shida, and T. Misato, *Ann. Phytopath. Soc. Japan*, **39**, 90 (1973); T. Shida, Y. Homma and T. Misato, *Agr. Biol. Chem.* (Tokyo), **37**, 1027 (1973).
4) W.A. Koenig, W. Parr, H.A. Lichtenstein, E. Bayer, and J. Oró, *J. Chromatogr. Sci.*, **8**, 183 (1970).

lauryl ester hydrochloride were obtained from Ajinomoto Co. Hypovial and trifluoroacetic anhydride were purchased from Pierce Chemical Co.

Preparation of Amino Acid Derivatives——N-TFA amino acid esters were prepared according to the method of Roach, *et al.*[5] N-lauroyl-L-valyl-L-valine lauryl ester was synthesized from N-lauroyl-L-valine and L-valine lauryl ester hydrochloride by the mixed anhydride method.[6]

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

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

Result and Discussion

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

Table I. Comparison of Gas Chromatographic Data of Different N-TFA-Amino Acid Esters at 135°, 30. p.s.i

Ester / Amino acid	iso-Propyl RRT	iso-Propyl α	*n*-Propyl RRT[a]	*n*-Propyl α	iso-Butyl RRT	iso-Butyl α	*n*-Butyl RRT	*n*-Butyl α
D-Isovaline	0.277	1.000	0.412	1.000	0.559	1.000	0.684	1.000
L-Isovaline	0.277		0.412		0.559		0.684	
D-Alanine	0.328	1.052	0.525	1.043	0.718	1.047	0.842	1.054
L-Alanine	0.345		0.548		0.751		0.887	
D-*tert*-Leucine	0.435	1.000	0.638	1.000	0.836	1.000	1.056	1.000
L-*tert*-Luecine	0.435		0.638		0.836		1.056	
D-Valine	0.446	1.038	0.684	1.033	0.898	1.037	1.136	1.040
L-Valine	0.463		0.706		0.932		1.181	
D-α-Amino-*n*-butyric acid	0.435	1.052	0.689	1.041	0.932	1.043	1.090	1.052
L-α-Amino-*n*-butyric acid	0.458		0.716		0.972		1.147	
Glycine	0.621	—	1.000	—	1.395	—	1.746	—
D-Isoleucine	0.706	1.032	1.068	1.037	1.395	1.032	1.751	1.039
L-Isoleucine	0.729		1.107		1.441		1.819	
L-Norvaline	0.701	1.040	1.113	1.041	1.463	1.042	1.723	1.046
D-Norvaline	0.729		1.158		1.525		1.802	
D-β-Amino-*n*-butyric acid	0.819	1.000	1.271	1.000	1.689	1.000	2.079	1.000
L-β-Amino-*n*-butyric acid	0.819		1.271		1.689		2.079	
D-β-Aminoisobutyric acid	0.864	1.000	1.299	1.000	1.723	1.000	2.141	1.000
L-β-Aminoisobutyric acid	0.864		1.299		1.723		2.141	
D-Leucine	0.927	1.054	1.486	1.053	1.949	1.052	2.288	1.057
L-Leucine	0.977		1.565		2.051		2.412	
D-Norleucine	1.102	1.051	1.746	1.049	2.299	1.044	2.695	1.054
L-Norleucine	1.158		1.831		2.401		2.841	
D-Proline	1.181	1.000	1.814	1.000	2.389	1.000	3.000	1.000
L-Proline	1.181		1.814		2.389		3.000	

a) RRT, relative retention time, reference compound is N-TFA-glycine *n*-propyl ester, t_R=8.85 min

In the previous work,[1] amino acids were converted to their diastereomers and the retention times were measured with straight-chain amino acids (norvaline and norleucine) and corresponding branched acids (valine, leucine, isoleucine and *tert*-leucine), and α-amino acid (α-amino-*n*-butyric acid) and β-amino acid (β-amino-*n*-butyric acid). In the present work examinations were similarly made.

It was reported previously[7] that 3,4-dihydroxyphenylalanine (DOPA) had longer retention times than α-methyl-DOPA. The present result also shows that α-amino-*n*-butyric acid has

5) D. Roach and C.W. Gehrke, *J. Chromatogr.*, **44**, 269 (1969).
6) D. Theodoropoulos and L.C. Craig, *J. Org. Chem.*, **20**, 1169 (1955).
7) H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **23**, 217 (1975).

longer retention times than isovaline where a methyl group is substituted for hydrogen on the asymmetric carbon in α-amino-*n*-butyric acid. It is interesting that an amino acid with larger molecular weight had been eluted earlier. This may be explained that the molecular shape of isovaline is somewhat more round than that of α-amino-*n*-butyric acid and hence the interaction of the former with the stationary phase is reduced to a certain extent.

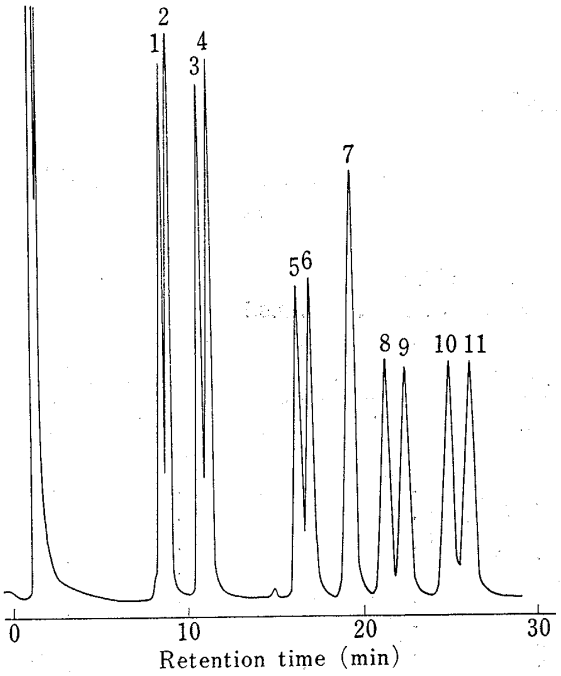

Fig. 1. Gas Chromatogram of N-TFA-Amino Acid *n*-Butyl Esters at 135°, 30 p.s.i.

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- and L-β-amino-*n*-butyric acids, 8: D-leucine, 9: L-leucine, 10: D-norleucine, 11: L-norleucine

There have been a number of reports concerning the elution order of racemic amino acids. The preceding paper[1] reported that L-amino acids, when converted to N-perfluoroacyl-amino acid *l*-menthyl esters (or *l*-bornyl esters), were eluted first, followed by D-amino acids. The present work showed that elution of N-TFA-amino acid esters was in the reverse order, D-amino acids being eluted first, followed by L-amino acids. A typical gas chromatogram of N-TFA-amino acid *n*-butyl esters is shown in Fig. 1.

Nakaparksin, *et al.*[8] reported that amino acid isopropyl ester derivatives were more favorable than *n*-propyl and *n*-butyl ester for the resolution of racemic amino acids on the optically active stationary phase. Lamkin, *et al.*[9] reported that with respect to volatility and gas chromatographic separation the most suitable derivatives of the natural protein amino acids were the N-TFA *n*-butyl esters. In this study the *n*-butyl ester derivatives were employed for the resolution of racemic amino acids because of their desirable retention times and separation factors.

Relationship between the separation factor and the relative size of the substituents in $CF_3CONH\overset{*}{C}HRCOOR'$ was examined from the data in Table I. The separation factors decreased with increasing degree of branching in such a way that they became smaller in the order of alanine, valine, and *tert*-leucine in all ester groups. The results on separation factors of amino acids with same carbon number, such as leucine, isoleucine, norleucine, and *tert*-leucine, were reversed to those obtained in the preceding work.[1]

Effect of the position of the amino group on resolution of amino acids was examined in the manner as previously described.[1] It was found that resolution was effected when the amino group was present at the α-position (α-amino-*n*-butyric acid) but not at the β-position (β-amino-*n*-butyric acid). The author reported[1,10] that α-methyl amino acids (*e.g.*, isovaline and α-methyl-DOPA) were not resolved. The present work also showed that isovaline was not resolved.

These results indicate that the retention times and separation factors are greatly affected by the side chain, degree of its branching, hydrogen on asymmetric carbon, and position of amino group in amino acids, irrespective of whether the amino acids are led to diastereomers with N-perfluoroacyl-L-proline,[10] *l*-menthol and *l*-borneol[1] or to derivatives formed in the

8) S. Nakaparksin, P. Birrell, E. Gil-Av, and J. Oró, *J. Chromatogr. Sci.*, **8**, 177 (1970).
9) W.M. Lamkin and C.W. Gehrke, *Anal. Chem.*, **37**, 383 (1965).
10) H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2075 (1974).

present work, though the different mechanisms are involved in the resolution between these two cases.

[Chem. Pharm. Bull. 23(7)1611—1612(1975)] UDC 547.291.04

Formic Acid Reduction. XXII.[1] Reaction of α,N-Diphenylnitrone with Formic Acid

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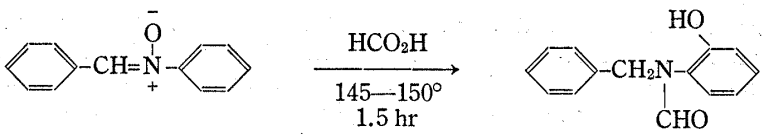
(Received October 16, 1974)

There has been found a formic acid reaction of α,N-diphenylnitrone which effects reduction together with introduction of hydroxyl group into a benzene ring, ortho to nitrogen function.

In a series of the formic acid reduction we now wish to describe our finding in the formic acid reaction of α,N-diphenylnitrone, which is of interest in effecting reduction together with specific introduction of hydroxyl group into a benzene ring, ortho to nitrogen function.



The reaction was carried out by heating a solution of the nitrone dissolved in 99% formic acid at 145—150° in a zirconium-lined autoclave. Treatment of the reaction mixture gave N-benzyl-*o*-hydroxyformanilide in 36% yield. By the use of TEAF, which has been known[3] as a distillable liquid formate given by $5HCO_2H \cdot 2NEt_3$, in place of formic acid, the reaction under ordinary pressure could be carried out similarly, but resulted in a less yield of the product.

Very recently the reaction of N-aryl nitrones with oxalyl chloride has been reported[4] to affect introduction of chloroglyoxalate grouping into ortho to the nitrogen. This paper suggests cyclic six centered transition state in intramolecular pathway for the reaction mecha-

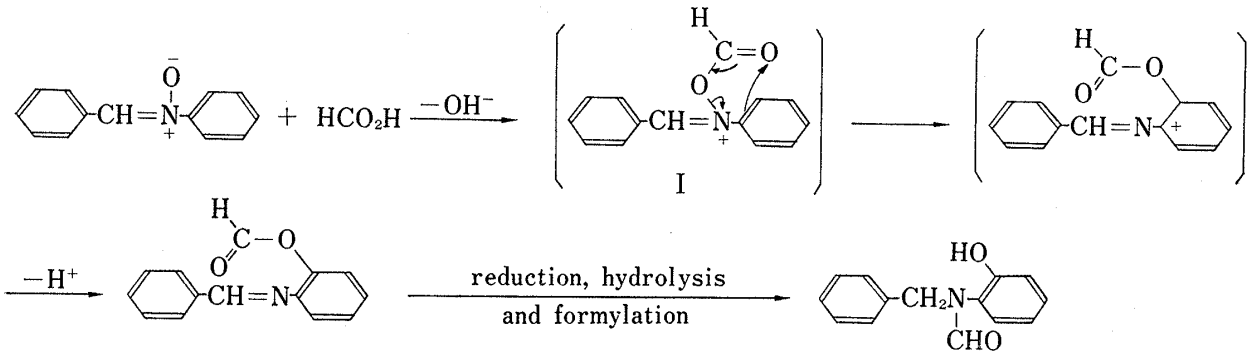


Chart 1

1) Part XXI: M. Sekiya and K. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1788 (1974).
2) Location: *2-2-1 Oshika, Sizuoka.*
3) K. Ito, *Yakugaku Zasshi*, **86**, 1166 (1966).
4) D. Liotta, A.D. Baker, N.L. Goldman, and R. Engel, *J. Org. Chem.*, **39**, 1975 (1974).