Notes

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Resolution of Racemic Amino Acids by Gas Chromatography. V.[1] Tryptophan, Tyrosine and Related Compounds

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The resolution of tryptophan, tyrosine and related compounds as their trimethylsilylated N-trifluoroacetyl-L-prolyl amino acid *n*-butyl esters was studied by gas chromatography. It was found that tyrosine and 3,4 dihydroxyphenylalanine were resolved, while α-methyl-3,4 dihydroxyphenylalanine could not be resolved. The peaks of epinephrine, norepinephrine, metanephrine and normetanephrine, except for dopamine, were not observed. Tryptophan was not resolved completely.

In the preceding paper,[1] the author reported the resolution of 25 racemic amino acids to examine the relation between separation factors and structure of amino acids using L-proline derivative as a resolving agent, while the optical resolution of tryptophan, tyrosine, and related compounds were not included. As a part of studies on the resolution of racemic amino acids, this paper deals with the resolution of tryptophan, tyrosine, and related compounds which are the components of biologically significant materials.

Experimental

Apparatus and Conditions——A Hewlett-Packard Model 402 gas chromatograph equipped with dual flame ionization detector was used for the analysis of tryptophan. 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 and injection temperature were 230° and 250°, respectively. Helium was used as carrier gas at a flow rate of 60 ml/min, 40 p.s.i.

A Perkin-Elmer Model 900 gas chromatograph equipped with attachments for scot[3] column and dual flame ionization detector was used for the analysis of tyrosine and related compounds. A stainless steel scot column of 100 ft × 0.02 in. I.D. coated with Dexsil 300 GC was used. Column temperature and injection temperature were 225° and 250°, respectively. Helium was used as the carrier gas at a flow rate of 1 ml/min, 50 p.s.i.

Reagents and Materials——All solvents used in this study were of reagent grade. Amino acids were obtained from Ajinomoto Co. and Nakarai Chemical Co., dopamine hydrochloride from Wako Pure Chemical Co., epinephrine hydrochloride and norepinephrine hydrochloride from Tokyo Kasei Co., metanephrine hydrochloride from Sigma Chemical Co., normetanephrine hydrochloride from Tokyo Kasei Co. Trifluoroacetic anhydride and BSTFA with 1% TMCS were obtained from Pierce Chemical Co. Pyridine was used after drying over NaOH pellets. N-TFA-L-PC was prepared in the same manner as described previously.[4] Hypovial was obtained from Pierce Chemical Co. Scot column was purchased from Perkin-Elmer Co.

Preparation of Derivatives——Each amino acid *n*-butyl ester was prepared by the method of Roach, *et al.*[5] and Imai, *et al.*[6] N-TFA-L-prolyl amino acid *n*-butyl ester derivatives prepared in the same manner as described in the preceding paper[1] were mixed with 0.5 ml of pyridine and 0.5 ml of BSTFA with 1% TMCS, and allowed to stand for 15 min at room temperature. N-TFA-L-prolyl catecholamines prepared in

1) Part IV: H. Iwase, *Chem. Pharm. Bull.* (Tokyo), **22**, 2075 (1974).
2) Location: *1-1, Suzuki-cho, Kawasaki-ku, 210, Japan.*
3) Abbreviations: scot, support coated open tubular; N-TFA-L-PC, N-trifluoroacetyl-L-prolyl chloride; BSTFA, N.O-bis-(trimethylsilyl)trifluoroacetamide; TMCS, trimethylchlorosilane; RRT, relative retention time; DOPA, 3,4-dihydroxyphenylalanine, p.s.i: pound square inch.
4) H. Iwase and A. Murai, *Chem. Pharm. Bull.* (Tokyo), **22**, 8 (1974).
5) D. Roach and C.W. Gehrke, *J. Chromatogr.*, **44**, 269 (1969).
6) K. Imai, N. Arizumi, M. Wang, S. Yoshine, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **20**, 2436 (1972).

the similar manner as described in the previous paper[4] were mixed with 0.5 ml of pyridine and 0.5 ml of BSTFA with 1% TMCS and allowed to stand for 15 min at room temperature. A 0.3 μl portion of each solution was injected into gas chromatograph.

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

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

Results and Discussion

Tryptophan

L-Tryptophan was not identified as its N–TFA-L-prolyl *n*-butyl ester because many unknown overlapped peaks appeared in chromatogram. After trimethylsilylation of the indol group in N–TFA-L-prolyl-L-tryptophan *n*-butyl ester at room temperature, however, a single sharp peak was obtained with sufficient intensity. Trimethylsilylated N–TFA-L-prolyl-DL-tryptophan *n*-butyl esters were not resolved completely on OV-1.

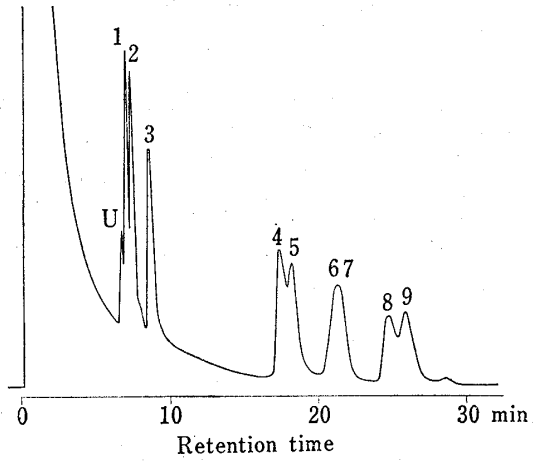

Fig. 1. Gas Chromatogram of N-TFA-L-Prolyl Amino Acid *n*-Butyl Esters on Scot Column coated with Dexsil 300GC

1: π-phenylalanine, 2: L-phenylalanine, 3: dopamine, 4: D-tyrosine, 5: L-tyrosine, 6: D-α-methyl DOPA, 7: L-α-methyl DOPA, 8: D-DOPA, 9: L-DOPA, U: unknown

Tyrosine and Related Compounds

Tyrosine and related compounds could not be resolved on OV-1 (dimethylsilicone) packed column. Tyrosine and related compounds, however, were almost resolved on Dexsil 300 GC (polycarborane siloxane) scot column. Gas chromatogram of tyrosine and related compounds is shown in Fig. 1.

As seen in Fig. 1, comparison of DOPA with α-methyl-DOPA in which the hydrogen atom bonded to asymmetric carbon of DOPA is substituted by a methyl group shows that the retention times of α-methyl-DOPA are shorter than those of DOPA.

The experimental data of relative retention times and separation factors of tyrosine and related compounds are given in Table I.

It can be seen in Table I that separation factor of phenylalanine is higher than those of tyrosine, α-methyl-DOPA and DOPA. Tyrosine gives smaller separation factor than DOPA. The preceding paper[1] reported that α-methyl amino acid (*e.g.*, isovaline) could not be resolved. The examination of the present work also shows that α-methyl-DOPA could not be resolved. Hydrogen atom attached to the asymmetric carbon may affect the resolution of enantiomers.

TABLE I. Gas Chromatographic Data for Racemic Amino Acids as Their N-TFA-L-Prolyl *n*-Butyl Esters at 225°

Compound	RRT[a]	$r_{L/D}$
D-Phenylalanine	0.382	1.065
L-Phenylalanine	0.407	
Dopamine	0.473	—
D-Tyrosine	0.959	1.042
L-Tyrosine	1.000	
D-α-Methyl DOPA	1.173	1.000
L-α-Methyl DOPA	1.173	
D-DOPA	1.363	1.046
L-DOPA	1.426	

a) RRT, reference compound is N-TFA-L-prolyl-L-tyrosine *n*-butyl ester, t_R=18.2 min.

A number of physiologically important catecholamines have been successfully analysed by gas chromatography.[7] Beckett, *et al.*[8] reported that catecholamine-like derivatives, ephedrine and related compounds, were resolved as their conversion to N–TFA-L-prolyl derivatives. Examination of the present results shows that the peaks of epinephrine, norepinephrine, metanephrine and normetanephrine, except for dopamine, are not observed. This may be considered that N–TFA-L-prolyl-catecholamines can not be trimethylsilylated because of steric interference, considering from the result that the peaks of trimethylsilylated N–TFA-L-prolyl dopamine are observed.

7) S. Kawai, T. Nagatsu, T. Imanari, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **14**, 618 (1966); K. Imai, M. Sugiura, and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 409 (1971); D.D. Clarke, S. Wilk, S.E. Gitlow, and M.J. Franklin, *J. Gas Chromatogr.*, **5**, 307 (1967); E.C. Horning, M.G. Horning, W.J.A. VandenHeuvel, K.L. Knox, B.G. Holmstedt, and C.J.W. Brooks, *Anal. Chem.*, **36**, 1546 (1964).
8) A.H. Beckett and H. Testa, *J. Pharm. Pharmac.*, **25**, 382 (1973).

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Reaction of N-(Alkoxymethyl)dialkylamines and N,N′-Methylene-bisdialkylamines with Isocyanides

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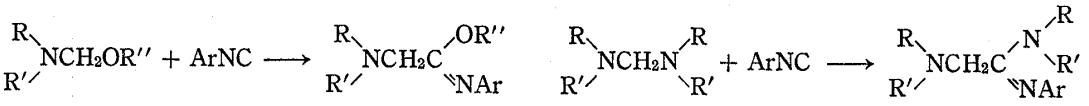
It has been found that N-(alkoxymethyl)dialkylamines and N,N′-methylenebisdialkylamines react with isocyanides, effecting 1,1-addition by suffering split of one of the methylene carbon-heteroatom bonds. The reactions furnish the corresponding imidates and amidines as the products which are not otherwise obtainable.

The representative pattern of the isocyanide reactions has been known as the 1,1-addition to the isocyanide carbon, where the presence of the copper catalyst is generally preferable. On inspection of these papers this addition reaction is limited to that of protic materials such as amine,[2] alcohol,[3] thioalcohol,[4] phosphine[5] and silane.[6] We have now found that, in the absence of the copper catalyst, N-(alkoxymethyl)dialkylamines and N,N′-methylenebisdialkylamines react with isocyanide resulting in the 1,1-adduct formation by suffering split of one of the methylene carbon-heteroatom bonds. A number of N-(alkoxymethyl)dialkylamines and N,N′-methylenebisdialkylamines were allowed to react with phenylisocyanides in 1:1 molar proportion by refluxing their toluene solution. The corresponding imidates and amidines were obtained as the 1,1-adduct product, which are shown in Table I with their yields. These



1) Location: *2-2-1, Oshika, Shizuoka.*
2) T. Saegusa, Y. Ito, S. Kobayashi, K. Hirota and H. Yoshioka, *Tetrahedron Letters*, **1966**, 6121.
3) T. Saegusa, Y. Ito, S. Kobayashi, N. Takeda and K. Hirota, *Tetrahedron Letters*, **1967**, 1273.
4) T. Saegusa, S. Kobayashi, K. Hirota, Y. Okumura and Y. Ito, *Bull. Chem. Soc. Japan*, **41**, 1638 (1968).
5) T. Saegusa, Y. Ito and S. Kobayashi, *Tetrahedron Letters*, **1968**, 935.
6) T. Saegusa, Y. Ito, S. Kobayashi and K. Hirota, *J. Am. Chem. Soc.*, **89**, 2240 (1967).