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Studies on the Racemization of Amino Acids and Their Derivatives. III[1] The Effect of Alkyl-, Aralkyl- and Aryl-Side Chain at α-Position of Amino Acids on Their Base-Catalyzed Racemization

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Kinetical study was carried out in the base-catalyzed racemization reaction of N-benzoyl anilide derivatives of six neutral amino acids, such as alanine, α-aminobutyric acid, valine, leucine, phenylalanine and phenylglycine.

From the comparison of rate-constants thus obtained, it was concluded that the susceptibility of amino acids tested to the racemization may be expressed in term of acidity of α-proton related to the electronnegativity of alkyl-, aralkyl- and aryl-side chains at α-carbon.

As Neuberger stated,[3] racemization of amino acid derivatives can be classified in the following two categories[4—6] ; the one is a mechanism through oxazolone intermediate, followed by the proton-replacement at its 4-position, which is often observed in the peptide-coupling reaction, while the other is the base-catalyzed racemization through the planar carbanion which formed by the direct proton-abstraction from α-position in amino acids by the attack of the base.

The oxazolone mechanism has extensively been investigated by many workers.[7] On the contrary, the quantitative information related to the base-catalyzed racemization of amino acid derivatives is limited to those of special amino acids, such as Ser,[8] Thr,[4—6] CySH,[4—6] β-cyanoalanine,[9] *etc.*, even though the carbanion mechanism has recently been established for the base-catalyzed racemization of compounds containing an amide, ester, or nitrile group adjacent to an asymmetric carbon.[10]

In order to obtain general aspect on the susceptibility of amino acids to their base-catalyzed racemization, which was often encountered in the preparative works, the kinetical study was carried out in the base-catalyzed racemization of derivatives of neutral amino acids ($\underset{\displaystyle\mathrm{R}}{\mathrm{NH_2\text{-}\overset{|}{C}H\text{-}COOH}}$), such as alanine (Ala, R: Me), α-aminobutyric acid (But, R: Et), valine (Val, R: iso-Pro), leucine (Leu, R: iso-Bu), phenylalanine (Phe, R: $PhCH_2$) and phenylglycine (Phgly, R: Ph) and the effect of alkyl-, aralkyl- and aryl-substituents (R) at α-position on the base-catalyzed racemization was investigated, which are described in the present paper.

In our previous study[11] on the effect of substitution at amino and carboxyl groups in amino acids on their base-catalyzed racemization, N-acyl carboxamide type of compounds were found to be fairly racemizable under mild base-catalyzed conditions.

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1) Part II: H. Matsuo, M. Sato, T. Tatsuno, and Y. Kawazoe, *Chem. Pharm. Bull.* (Tokyo) **18**, 1788 (1970). A part of this paper was presented at the 21st Annual Meeting of Pharmaceutical Society of Japan at Tokushima (1965).

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3) A. Neuberger, *Adv. in Protein Chem.*, **4**, 339 (1948).

4) E. Schröder and K. Lübke "The Peptides," Vol. 1, Academic Press, New York, 1966.

5) M. Bodanszky and M.A. Ondetti, "*Peptide Synthesis*," Intersciences Publishers, New York, 1966.

6) N. Nakamizo, *Protein, Nucleic Acid, Enzyme*, **12**, 586 (1967).

7) See references in 4—6).

8) Z. Bohak and E. Katchalski, *Biochemistry*, **2**, 228 (1963).

9) B. Liberek, *Tetrahedron Letters*, **1963**, 1479; **1964**, 159; B. Liberek, *Roczniki Chem.*, **40**, 597, 781, 941 (1966).

10) D.J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press, New York, 1965.

11) H. Matsuo, Y. Kawazoe, M. Sato, M. Ohnishi, and T. Tatsuno, *Chem. Pharm. Bull.* (Tokyo), **15**, 2015 (1967).

Accordingly, kinetic behavior of anilides of N-benzoyl-L-Ala, -L-But, -L-Val, -L-Leu, -L-Phe and -L-Phgly was examined under the identical reaction conditions in the presence of sodium ethoxide as a base in a solution of ethanol–dimethyl sulfoxide (DMSO)(1:1v/v).

Experimental

Preparation of N-Benzoyl-L-amino Acid Anilides——Usual coupling reaction of N-benzoyl-amino acid and aniline was found to be accompanied by considerable racemization.

Preparations of all substrates were carried out by the enzymatic method according to Albertson[12] and Uchio,[13] starting from the corresponding N-benzoyl-DL-amino acids, which were synthesized by the described manner.[14]

Benzoyl-DL-amino acid (40 mmoles) was dissolved in a solution of 1N NaOH (12 ml), 2M NaOAc (22 ml), aniline (4 ml) and 0.25M citrate buffer (pH 5.0; 30 ml) and pH of the solution was adjusted to 5.0 by adding appropriate amount of acetic acid. To this solution, was added KCN-activated papain solution in citrate buffer (pH 5.0) and the whole was incubated at 39—40° overnight.

Precipitated optically active L-anilide was filtered on the funnel and washed with 10% HCl, water, 5% Na_2CO_3 and water, successively. Recrystallization from MeOH afforded optically pure N-benzoyl-L-amino acid anilide, whose physical constants were listed in Table I.

Table I. Physical Constants of N-Benzoyl-L-amino Acid Anilides (Numbers in Parenthesis showed the Reported Values[12])

Amino acid	mp (°C)	$[\alpha]_D$
Ala	178—179 (175—176)	− 9°[a] (−13.2°)[b]
But	169—170 (170—171)	− 16°[a] (−19°)[b]
Val	220 (217—218)	− 37°[a] (−36°)[b]
Leu	220 (216—217)	− 23°[a] (−28°)[b]
Phe	224—225 (221—222)	+ 25°[a] (+27°)[b]
Phgly[c]	223—224	+144°[a]

a) $c=1$, in pyridine, $t=27°$
b) $c=5$, in pyridine, $t=29—31°$
c) *Anal.* Calcd. for $C_{21}H_{18}O_2N_2$: N, 8.47. Found: N, 8.42

Preparation of Kinetic Solution——EtOH–DMSO (1:1 v/v), containing desired amount of NaOEt was prepared as follows; concentrated stock solution of NaOEt in EtOH (3—4 moles/liter) was prepared by adding a carefully weighed amount of clean metallic sodium to anhydrous EtOH placed in a dry pre-weighed flask under an atmosphere of pure nitrogen gas. After reaction was complete, the flask was weighed and the weights of NaOEt and EtOH were determined. To the resulted solution, was added anhydrous redistilled DMSO of the equal volume to that of EtOH determined as above, to afford a stock solution of EtOH–DMSO (1:1 v/v) containing desired amount of NaOEt. On use, the stock solution prepared above was diluted by adding exact volume of EtOH–DMSO (1:1 v/v) to make a kinetic solution with desired concentration. Base concentration was determined by adding water, followed by titrating the base with standard 0.01N acid to the phenolphthalein end point.

Racemization of Benzoyl-L-amino Acid Anilides in the System of EtOH-DMSO Containing NaOEt——A substrate (1 mmole) was dissolved in 20 ml of the kinetic solution containing 0.103 mole/liter of NaOEt, which prepared as above, at room temperature to give the sample solution containing 0.05 mole/liter of a substrate. Each 2 ml portion was sealed in 10 ml ampules under nitrogen flushing and submitted to the racemization reaction by heating at 50±0.1° in the thermostat.

With varying time intervals, the ampule was taken out of the bath, then ice-cooled to quench the reaction and immediately subjected to the polarimetric measurement by the aid of Yanagimoto Direct Reading Polarimeter OR-20 (tube length: 1 cm) using Na D-line. The polarimeter could be read with ease to ±0.001°. For each point, three observations of optical rotation were made and the average value of the three optical observations were recorded.

All of the rates examined gave clear straight line plots of time *vs.* logarithm of rotation. Data obtained in typical run were recorded in Table II. In the case of Phe, kinetic runs were also made in the presence

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12) N.E. Albertson, *J. Am. Chem. Soc.*, **73**, 452 (1951).
13) H. Uchio, *Nippon Kagaku Kaishi*, **81**, 316, 1851 (1960); **82**, 104 (1961).
14) J.P. Greenstein and M. Winitz, "*Chemistry of Amino Acids*," Vol. 1, John Wiley & Sons, Inc., New York, 1961.

of 0.021 and 0.012M NaOEt for the purpose of getting specific first order rate constants. Results were listed in Table III and Fig. 1. The rates and half-life values were determined by a simple graphic method and by calculation using equations (1) and (2).

$$K = 1/t \cdot \ln D_0/D_t = 2.303/t \cdot \log D_0/D_t \qquad \text{Eq. 1}$$

$$t_{1/2} = \ln 2/K \qquad \text{Eq. 2}$$

where D_0 : α_D at 0-min's reaction
D_t : α_D at t-mins' reaction
K : rate constant
$t_{1/2}$: half-life time (min)

Results obtained by two ways showed satisfactory agreement as shown in Table IV.

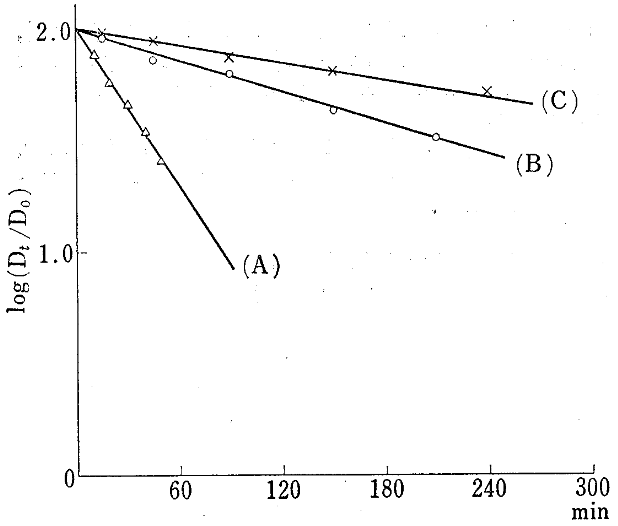

Fig. 1. Kinetics of Racemization of N-Benzoyl-L-Phe Anilide at 50° in EtOH–DMSO (1:1 v/v) Solution in the Presence of NaOEt

concentration of substrate: 0.050 mole/liter
concentration of NaOEt:
(A) 0.103 mole/liter (B) 0.021 mole/liter
(C) 0.012 mole/liter
D_t and D_0: α_D at time t and 0 min, respectively

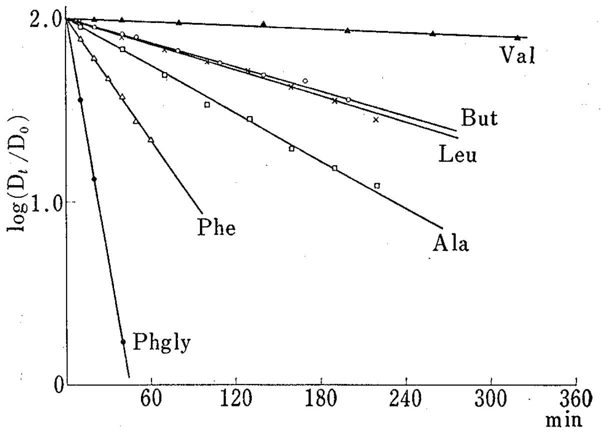

Fig. 2. Kinetics of Racemization of N-Benzoyl-L-amino Acid Anilides at 50° in EtOH–DMSO (1:1 v/v) Solution in the Presence of NaOEt (0.103 mole/liter)

D_t and D_0: α_D at time t and 0 min, respectively

Table II. Racemization at 50° of N-Benzoyl-L-Phe Anilide in EtOH–DMSO (1:1 v/v) Solution, 0.050M in Substrate, 0.103M in EtONa

Time (min)	D_t	$K_{obs} \times 10^2$ (min^{-1})
0	0.083°	
10	0.067°	2.199
20	0.050°	2.564
30	0.039°	2.538
40	0.030°	2.559
50	0.022°	2.667
		Average 2.55

Table III. Specific First-Order Rate Constant for the Racemization of N-Benzoyl-Phe Anilide at 50° in EtOH–DMSO (1:1 v/v) 0.050M in Substrate

Concentration of NaOEt(mole/liter)	$K \times 10^2$ (min^{-1})	$K \times 10^2$/[NaOEt] (min^{-1}mole^{-1} liter)	$t_{1/2}$ (min)
0.103	2.55	24.76	27.2
0.021	0.51	24.3	136.3
0.012	0.29	24.2	241.5

Table IV. Rate Constants and Half-Life Times for the Racemization of N-Benzoyl-L-amino Acid Anilides at 50° in EtOH-DMSO (1:1 v/v), 0.050 mole/liter in Substrates, and 0.103 mole/liter in NaOEt

Substrates (Amino acids)	$t_{1/2}$ (min) Obtained by graphic method	$K\times 10^2$ (min^{-1})	$t_{1/2}$ (min) Obtained by using Eqs. 1 and 2	$K\times 10^2$ (min^{-1})
Phgly	6.3	11.00	6.7	10.39
Phe	27	2.56	27.2	2.55
Ala	70	0.990	67.7	1.023
Leu	128	0.541	129.9	0.533
But	135	0.514	135.6	0.511
Val	909	0.0762	917	0.0756

Result and Discussion

Racemization of N-Benzoyl-L-phenylalanine Anilide

In order to investigate the kinetics of the racemization of anilides of N-benzoyl-L-amino acids in a homogeneous system, three solutions of 0.05 mole/liter N-benzoyl-L-Phe anilide in EtOH-DMSO(1:1v/v) containing 0.012, 0.021 and 0.103 M EtONa, were heated at 50° and the decrease in the optical rotation with time was followed polarimetrically, respectively.

The results obtained are given in Tables II and III, and Fig. 1. In all the experiments recorded, the rotation reached to half value within 4 hours. The data presented in Fig. 1 and Table II, show that the change in optical rotation with time follows first-order kinetics. It may thus be described by Eq. 3,

$$\frac{da}{dt}=-K\alpha \qquad \text{Eq. 3}$$

where α denotes the optical rotation of the substrate at any given instant and K is the specific rate constant of the reaction. As shown in Table III, the rate constant of racemization was found to be proportional to the concentration of NaOEt used. It is thus obvious that the reaction is base-catalyzed. An average value of K/[base] is 24.4×10^{-2} (mole^{-1}, liter, min^{-1}). The base-catalyzed racemization of amino acid derivative is most likely due to the ionization of the hydrogen at the asymmetric α-carbon and the formation of a symmetric ambident carbanion through the similar mechanism which has recently proposed by Cram[10] for the base-catalyzed racemization of compounds containing carbonyl or its equivalent group adjacent to the asymmetric center.

Effect of α-Substituents (R) in Amino Acids on Their Rates of Racemization

In order to investigate the relationship between the structure and the relative susceptibility to racemization, the rates constants for the racemization of N-benzoyl anilides of six optically active amino acids (Ala, But, Val, Leu, Phe, and Phgly having different α-substituent (R), such as Me, Et, *iso*-Pro, *iso*-Bu, $PhCH_2$- and Ph-, respectively) were measured under the identical conditions at 50° in a solution of EtOH-DMSO (1:1 v/v) containing 0.05 mole/liter of substrates in the presence of 1.03 M NaOEt as a base.

As shown in Fig. 2, all of the rates examined gave clear linearity in the plots of time *vs.* logarithm of rotation, indicating first order kinetics similar to the case of Phe described in the preceding section. As seen from Table IV showing the rates constants and half-life times for all substrates, the relative susceptibilities to the racemization increases in the following order;

Val<But<Leu<Ala<Phe<Phgly

R: *iso*-Pro<Et<*iso*-Bu<Me<$PhCH_2$<Ph

These results lead us to anticipate that the racemizability may be expressed in term of acidity of α-proton related to the electron-negativity of α-substituent (R).

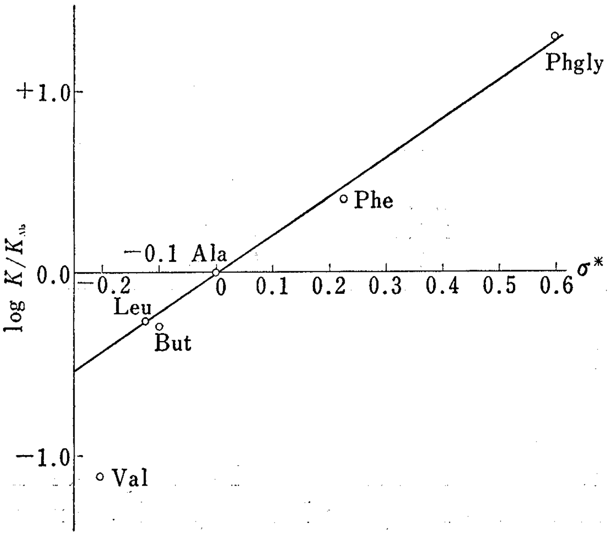

Fig. 3. Plot of log $K/_{Ala}$ against σ^*

In order to correlate the racemization-susceptibility to the structure of the substrates, *i.e.*, the electron-negativity of R, it is convenient to maintain the term log K/K_{Ala} for a series of substrates examined as referring to the rate constant for Ala and to plot log K/K_{Ala} against Taft's polar substituent constant (σ^*),[15] as shown in Fig. 3.

The polar substituent constants (σ^*) used in Fig. 3, must be valid for the open-chain aliphatic compounds according to Taft.[15] Fig. 3 shows satisfactory linearity except the case of Val, whose α-substituent *iso*-Pro group seems to exert a considerable steric effect on the racemization.

Accordingly, the relative susceptibility of a series of amino acid examined to the base-catalyzed racemization is proportional to the electron-negativity of α-substituent (R), unless the α-substituent exert the steric effect.

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[15] R.W. Taft, Jr., *J. Am. Chem. Soc.*, **75**, 4231, 4534, 4538 (1953).