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Studies on the Racemization of Amino Acids and Their Derivatives. II.[1] On the Deuterium–Hydrogen Exchange Reaction of Amino Acid Derivatives in a Medium of Deuterated Acetic Acid

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The hydrogen–deuterium exchange reaction at the α-carbon atom of 28 kinds of compounds which structurally related to α-amino acids was carried out in a medium of [carboxy-^{2}H]acetic acid. Some special features which have never been observed in usual base- or acid-catalyzed hydrogen–deuterium exchange reaction or racemization were recognized in the reaction in acetic acid medium. Especially, the presence of free amino-group in the molecule very much facilitated the racemization of amino acid derivatives, whether their carboxyl group was substituted or not. In addition, it was also found that acetate anion displayed a catalytic role as a base in a medium of acetic acid.

The mechanism of the racemization in acetic acid was also discussed.

It has been well known that the acid–catalyst such as hydrochloric acid or other mineral acids, does not display so significant role in the racemization reaction of α-amino acids and their derivatives as the base–catalyst does.[3]

In 1959, however, Mitsuno, *et al.*[4] reported the facile recemization of α-amino acids which was observed in media of lower homologs of fatty acids, such as acetic acid, propionic acid and others. Such an interesting behavior of α-amino acids in acetic acid solution leads us to investigate further this problem.

The racemization at a hydrogen–bearing asymmetric carbon center initially involves the carbanion formation by the abstraction of the proton from the asymmetric carbon in the question.

If the carbanion is formed in a deuterium–donating solvent, the rate of racemization is equal to that of hydrogen–deuterium exchange reaction.[1,5]

In our previous paper,[1] the relationship between the structure of α-amino acid derivatives and their racemizability in basic media was investigated by the aid of hydrogen–deuterium exchange reaction and thereby nuclear magnetic resonance (NMR) technique[6] was found to be an efficient tool to follow the hydrogen–deuterium exchange reaction.

Accordingly, in our present study, the racemization of several α-amino acids and their derivatives in a medium of deuterated acetic acid (AcOD) was investigated in terms of deuterium–hydrogen exchange reaction by the aid of NMR method in the similar manner as described in our previous paper.[1]

1) Part I: H. Matsuo, Y. Kawazoe, M. Sato, M. Ohnishi and T. Tatsuno, *Chem. Pharm. Bull.* (Tokyo), **15**, 391 (1967).
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3) A. Neuberger, *Adv. Protein Chem.*, **4**, 339 (1948).
4) H. Mitsuno and A. Sakiami, *Nippon Kagaku Kaishi*, **80**, 1035 (1959).
5) D.J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press, New York, 1965.
6) Y. Kawazoe and M. Ohnishi, *Chem. Pharm. Bull.* (Tokyo), **11**, 846 (1964).

Method

Deuteration reaction of amino acid derivatives in solutions of AcOD, D_2O–AcOD, D_2SO_4–D_2O and AcONa–AcOD was carried out and then deuterated amounts were determined by the NMR method which originally developed by Kawazoe, *et al.*[6]

General procedure is as follows: The tested sample exactly weighed was dissolved in a definite volume of above solvents in a sealed NMR sample tube, which was subjected to hydrogen–deuterium exchange reaction by heating at an appropriate temperature. After t hrs' reaction, the tubes were cooled in an ice–bath to quench the reaction and immediately submitted to NMR measurement directly (JNM-3H-60 Spectrometer, Japan Electron Optics Lab.). From the spectra thus obtained, the areal intensities of signals due to the exchanging α-proton (α-H^t) and the appropriate unexchangeable reference hydrogen (r-H^t) were integrated, the areal intensity ratio of α-proton *vs.* the reference signal (α-H^t/r-H^t) being determined. Therefore, the percentage of exchanged α-proton after t hrs' reaction (α-D^t) can be derived by the following equation:

$$\alpha\text{-}D^t = \left(1 - \frac{\alpha\text{-}H^t/\text{r-}H^t}{\alpha\text{-}H^0/\text{r-}H^0}\right) \times 100\ (\%) \qquad (1)$$

where α-D^t: deuteration percentage of α-proton after t hrs' reaction, α-H^t/r-H^t and α-H^0/r-H^0: areal intensity ratio after t hrs' reaction and at the initial step, respectively.

As the reference signal, the proton signal in the same molecule of the tested compound which was unexchangeable under the present conditions and well separated from others was selected. Otherwise, the exactly weighed amount of sodium formate was added as a reference into the tested solution after deuteration reaction.

Racemization reaction was followed polarimetrically by using Yanagimoto Direct Reading Polarimeter OR-20 (Na-D line).

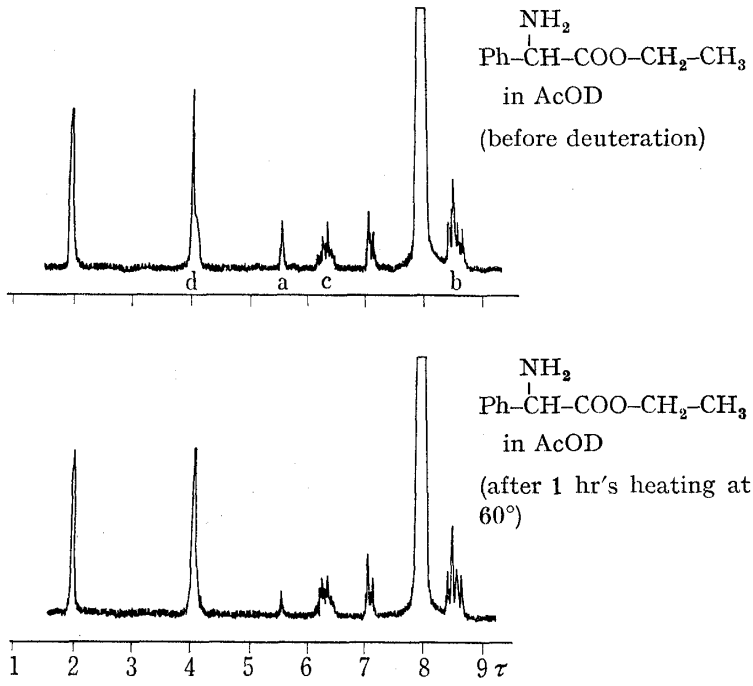


Fig. 1. NMR Spectra of Phenylglycine Ethyl Ester in AcOD before and after Deuteration

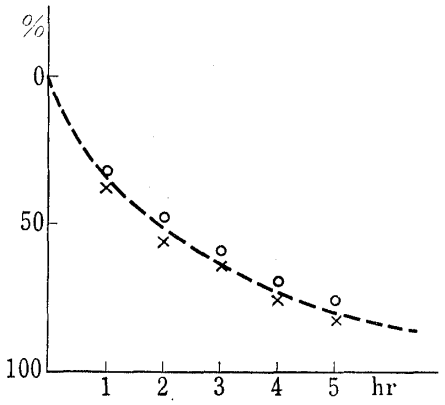


Fig. 2. Deuteration and Racemization of D-Phenylglycine Ethyl Ester (I) at 60°
(concentration: 10% in AcOD or AcOH)
—○—: deuteration
—×—: racemization

Result and Discussion

Parallelism between Hydrogen-Deuterium Exchange and Racemization

Racemization and deuterium exchange reaction of 10% solution of optically active D-phenylglycine ethyl ester (I) were carried out at 60°. The aliquots collected every one hour were submitted to NMR measurement and optical rotation measurement by the method described in experimental part. As clearly shown by the comparison of NMR spectrum after

reaction with that of compound (I) before reaction (Fig. 1), the deuterium exchange reaction at α-position of I can be followed in terms of the reducing areal intensity of α-proton signal (a) at τ 5.6 on NMR spectrum that was obtained after reaction. The percentage of exchanged α-proton after t hrs' reaction was calculated by equation 1 described in the preceding section. With the same aliquot used for NMR measurement, the percentage of racemization was measured polarimetrically.

These results which showed in Fig. 2, revealed the satisfactory parallelism between deuteration and racemization. Within experimental error, the percentage of racemization and deuteration was identical. Thus, it was found that the hydrogen–deuterium exchange reaction occured with the same rate of racemization for (I) in acetic acid medium, as well as the base–catalyzed reaction did as previously reported.

The behavior of optically inactive compounds in their deuteration must be the same as that of the corresponding optically active ones and therefore, the information of racemization can be drawn from the data of deuteration reaction of racemic compound in place of optically active one.

The Substitution Effect on the α-Deuteration of α-Amino Acids in AcOD Solution

For the preliminary estimation of the effect of substitution of caboxyl and amino groups of amino acids on their α-deuteration and racemization in AcOD solution, the deuteration reaction in a medium of AcOD was investigated with the following three groups of compounds:

a) **Propionic Acid Derivatives** Sodium propionate (II), ethyl ester (III), phenyl ester[7] (IV), benzyl ester (V), *p*-nitrophenyl ester[8] (VI), propionamide (VII), N,N-diethylpropionamide[1] (VIII) propionanilide (IX), and N-benzylpropionamide[1] (X).

b) **Phenylglycine Derivatives** Phenylglycine (XI), ethyl ester[1] (I), N-acetyl ethyl ester (XII), N-phthaloyl ethyl ester (XIII), N-acetyl-[1] (XIV), N-propionyl-[1] (XV), N-formyl- (XVI), N-phthaloyl-[1] (XVII), N-carbobenzyloxyphenylglycine (XVIII) and N-acetylphenylglycine amide (XIX).

c) **Alanine Derivatives** L-Alanine (XX), N-acetyl-[1] (XXI), N-carbobenzyloxy-[1] (XXII), N-phthaloyl-L-alanine[1] (XXIII), N-acyl-L-alanine amides (N-acetyl[1] (XXIV), N-carbobenzyloxy- (XXV), N-phthaloyl-[1] (XXVI)), L-phenylalanine amide[1] (XXVII) and N-acetyl-L-phenylalanine amide[1] (XXVIII).

After deuteration reaction of these compounds at 60°, 90°, 120°, 150° and 180° for one and/or two hours, the α-deuteration percentages (α-D^t) were determined in each sample. These results thus obtained were summarized in Tables I, II, and III. Considering the accuracy of NMR method, the following notations for deuteration percentages were used in a similar way previously reported for the base-catalyzed α-deuteration:[1] (—): negative, (±): less than 20%, (+): 20—40%, (++): 40—70%, (+++): more than 70%.

a) **α-Deuteration of Propionic Acid Derivatives (Table I)** For the purpose to elucidate the effect of carboxyl substitution, propionic acid derivatives (II—X) as des-amino models for alanine were compared in their attitudes toward α-deuteration reaction in AcOD solution. As seen from Table I, significant difference in the susceptibility to α-deuteration among tested compounds was not recognized. Therefore, the mode of carboxyl substitution did not display important role in α-deuteration reaction in AcOD, unlike in the case of base–catalyzed α-deuteration previously reported.

b) **α-Deuteration of Phenylglycine Derivatives (Table II)** In order to compare the effect of amino substitution, phenylglycine derivatives (I, XI—XIX) were submitted to α-deuteration reaction in AcOD and the results thus obtained are listed in Table II. As clearly

7) G.G.S. Dutton, *Can. J. Chem.*, **31**, 837 (1953).
8) S. Dahl, *J. Am. Leather Chemist's Assoc.*, **55**, 480 (1960).

Table I. $CH_3CH_2COX \xrightarrow[AcOD]{} CH_3CD_2COX$

Compound No.	X	temp. hr	60° 1	90° 1	120° 1	150° 1.5	180° 1
II	ONa		−	−	−	±	+
III	OEt		−	−	−	−	−
IV	OPh		−	−	−	−	−
V	OCH_2Ph		−	−	−	−	−
VI	OPh–NO_2 (p)		−	−	−	−	
VII	NH_2		−	−	−	±	+
VIII	NEt_2		−	−	−	−	±
IX	NHPh		−	−	−	−	+
X	$NHCH_2Ph$		−	−	−	±	+

substrate concn: 10%

Table II. PhGly Deriv. in AcOD

Compound No.		temp. hr	60° 1	60° 2	90° 1	90° 2	120° 1	120° 2	150° 1	150° 2	180° 1
XI	PhGly		##	##	##	###					
XII	Ac-PhGlyOEt		−	−	−	−	−	−	−	−	##
I	PhGlyOEt		+	##	##	###					
XIII	Phth-PhGlyOEt		−		−		−		−		
XIV	Ac-PhGly		−	−	−	−	−	−	±	##	
XV	Pr-PhGly		−	−	−	−	−	−	+	##	
XVI	For-PhGly		−	−	−	−	+	##	###		
XVII	Phth-PhGly		−	−	−	−	−	+	##		
XVIII	Cbz-PhGly		−	−	−	−	−	−	−	−	+
XIX	Ac-PhGlyNH_2		−		−		−		+		##

substrate concn: 10%

shown in this Table, it is evident that phenylglycine (XI) and its ethyl ester (I) are much more susceptible to α-deuteration than all others. Accordingly, it must be concluded that free amino group at α-position displays a strikingly accelerating effect on α-deuteration reaction and this effect considerably abolished by N-acylation.

c) α-Deuteration of Alanine Derivatives (Table III) The alanine derivatives (XX—XXVIII), which seemed to be less racemizable than those of phenylglycine by the methyl

Table III. Ala Deriv. in AcOD

Compound No.		temp. hr	60° 1	90° 1	120° 1	150° 1	180° 1
XX	Ala		−	−	+	##	###
XXI	Ac-Ala		−	−	−	−	+
XXII	Cbz-Ala		−	−	−	−	−
XXIII	Phth-Ala		−	−	−	−	−
XXIV	Ac-AlaNH_2		−	−	−	+	##
XXV	Cbz-AlaNH_2		−	−	−	±	##
XXVI	Phth-AlaNH_2		−	−	−	−	##
XXVII	PheNH_2		−	−	+		
XXVIII	Ac-PheNH_2		−	−	−	+	##

substrate concn : 10%

substitution in the place of phenyl group, were examined. The results are shown in Table III. Accelerating effect of free amino group which was evidently recognized in the cases of phenylglycine and ethyl ester (XI, I), was also observed in cases of alanine (XX) and phenylalanine amide (XXVII), both of which have a free amino group, even though it is not so dramatic as in the cases of I and XI.

The difference of susceptibility to α-deuteration between alanine and phenylglycine might be interpreted by the effect of phenyl group at α-position.

Further Approach to the Mechanistic Study on α-Deuteration in AcOD

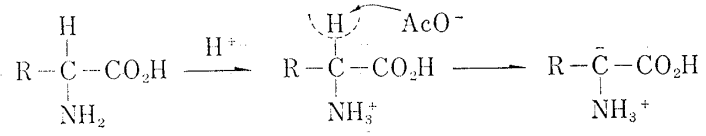
As was discussed in the preceding section, a clear-cut accelerating effect of free amino group on α-deuteration is suggestive for the elucidation of the mechanism of this reaction. In addition, the results listed in Table IV may give us further clue to this question.

Table IV.

No.	Compound	temp. / hr	60° 1	60° 2	90° 1	90° 2	120° 1	120° 2	150° 1	150° 2	180° 1
XI	PhGly in AcOD			+	##						
	in 50% D_2O–AcOD		−		−		##		##		
	in 10% D_2SO_4–D_2O		−		−		−		−		##
XIV	Ac-PhGly in AcOD		−	−	−	−	−	−	±	##	
	in 50% D_2O–AcOD		−	−	−	−	−	−	−	+	
	in 5.2% AcONa–AcOD		−		−		+		##		
XVIII	Cbz-PhGly in AcOD		−		−		−		−		+
	in 5.2% AcONa–AcOD		−		−		−		##		

substrate concn: 10%

As seen from the results of comparative experiments with phenylglycine under three different conditions, it was clear that dilution of acetic acid concentration by the addition of D_2O made the reaction rate considerably slower than that in 100% AcOD, even though it was still much higher than the rate of the reaction catalyzed by D_2SO_4, usual strong acid catalyst. These results showed that highly accelerating effect of free amino group on α-deuteration was unable to be explained only by the effect of protonation on amino group. Moreover, from the fact that acetic acid in nonaqueous state exhibited the strongest catalytic effect, it seemed to be likely that acetate anion, which probably formed from AcOD after protonation on free amino group, might play a significant role on α-deuteration in acetic acid. Further evidences for this explanation arised from four other experiments in Table IV. As seen from these data, the accelerating effect of added AcONa was observed even in the cases of N-acyl-phenylglycines (XIV, XVIII), which hardly racemized in 100% AcOD (see Table II). Accordingly, the following reaction mechanism seemed to be likely for the interpretation of the results obtained by our experiments.



In this mechanism, acetic acid will protonate free amino group to afford acetate anion, which probably displays as base-catalyst in the proton abstraction from α-carbon atom of amino acid.

The carbanion thus formed will be stabilized by the effect of protonated amino grouping at adjacent position.

The mechanism discussed above seemed to give satisfactory explanation for the special feature of AcOH–induced racemization of α-amino acid, such as its higher susceptibility to racemization in AcOH than that in mineral acid and a significant difference in substitution effect from those observed in base-catalyzed racemization.

Experimental

A) Preparation of Materials——N-Formyl-DL-phenylglycine (XVI): Prepared by the formylation of DL-phenylglycine with a mixture of acetic anhydride and formic acid. Colorless plates of mp 174° (from water). Yield: 79.2%. *Anal.* Calcd. for $C_9H_9O_3N$: C, 60.33; H, 5.06; N, 7.83. Found: C, 60.27; H, 4.89; N, 7.98.

N-Carbobenzoxy-DL-phenylglycine (XVIII): Prepared by usual carbobenzoxylation of DL-phenylglycine. Colorless needles of mp 123—124° (from ether–petr. ether). Yield, 70.7%. *Anal.* Calcd. for $C_{16}H_{15}O_4N$: C, 67.36; H, 5.31; N, 4.92. Found: C, 67.15; H, 5.01; N, 5.01.

N-Acetyl-DL-phenylglycine Ethyl Ester (XII): N-Acetyl-DL-phenylglycine was esterified by the action of $SOCl_2$ in a solution of anhydrous EtOH. Colorless needles of mp 63° (from ether–hexane). Yield: 55.8%. *Anal.* Calcd. for $C_{12}H_{15}O_3N$: C, 65.14; H, 6.84; N, 6.35. Found: C, 65.29; H, 6.67; N, 6.43.

N-Acetyl-DL-phenylgylcine Amide (XIX): Prepared from N-acetyl-DL-phenylglycine and ammonia by the mixed anhydride method using ethyl chloroformate. Colorless prisms of mp 233—234° (from EtOH–H_2O). Yield: 54.3%. *Anal.* Calcd. for $C_{10}H_{12}O_2N_2$: C, 62.81; H, 5.81; N, 14.67. Found: C, 62.14; H, 6.14; N, 14.50.

N-Phthaloyl-DL-phenylglycine Ethyl Ester (XIII): N-Phthaloyl-DL-phenylgycine was esterified by the action of anhyd. EtOH and $SOCl_2$. Colorless needles of mp 75.5° (from ether–hexane). Yield: 60.2%. *Anal.* Calcd. for $C_{18}H_{15}O_4N$: C, 70.18; H, 4.85; N, 4.49. Found: C, 69.22; H, 4.72; N, 4.38.

N-Carbobenzoxy-L-alanine Amide (XXV): Prepared from N-carbobenzoxy-L-alanine and ammonia by the mixed anhydride method using ethyl chloroformate. Colorless needles of mp 133° (from EtOH–hexane). Yield: 52.2%. $[\alpha]_D^{28}$ −1.84 (c=0.977, l=1 EtOH). *Anal.* Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.44; H, 6.35; N, 12.60. Found: C, 59.34; H, 6.06; N, 12.50.

B) Deuteration Experiment of D-I in AcOD——One half ml of 10% solution of D-I (500 mg) in AcOD (5.0 ml) was sealed in NMR sample tubes, which were thermostated at 60°. Each aliquot was sampled every one hour and then ice-cooled quickly to quench the reaction. NMR measurement was carried out using JNM-3H-60 Spectrometer (Japan Optics Labs.) at 60 Mc. Deuteration % (α-D^t) was calculated by the equation (1) in the same way as described in our previous paper.[1]

C) Racemization Experiment of D-I in AcOH——Each 5 ml portions of the AcOH solution containing D-I (10% concentration) were sealed in ampules, which were heated at 60° in the thermostat under the identical condition as above. Each aliquot was taken every an hour. After quenching the reaction by ice-cooling, 2 ml of 6N HCl was added to the resulting solution and then submitted to the measurement of optical rotation using Yanagimoto direct reading polarimeter OR-20 (Na-D line) at 22°.
Racemization % was calculated by the following equation.

$$\text{Racemization } \% = \frac{[\alpha]_D \text{ after } t \text{ hrs' reaction}}{[\alpha]_D \text{ at initial stage}} \times 100\ (\%)$$

The data obtained by experiment B and C described above were summarized in Fig. 2 and the following Table.

Time (hr)	0	1	2	3	4	5
Recemization (%)	0	62	44	35	24	18
Deuteration (%)	0	67	51	40	29	23

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