[Chem. Pharm. Bull. 15(4) 391~398 (1967)]

UDC 547.466.2.02.03 : 541.65

46. Hisayuki Matsuo,*1 Yutaka Kawazoe,*2 Motomu Sato,*1 Masako Ohnishi,*2 and Takashi Tatsuno*1 : Studies on the Racemization of Amino Acids and their Derivatives. I.*3 On the Deuterium-Hydrogen Exchange Reaction of Amino Acids Derivatives in Basic Media.

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(Received August 31, 1966)

In the field of the synthetic utilization of optically active amino acids, especially in the peptide syntheses, the racemization of amino acid derivatives has been one of the most important problems. A number of studies[1] have been reported in the connection with this point, but little is known of the effect of the structure of derivatives on their racemization[1a,2] and any conclusion drawn can only be of a qualitative nature. Therefore, we are interested in fundamental study to elucidate the relationship between the structure and racemizability of α-amino acids derivatives.

In general, the racemization of a hydrogen-bearing asymmetric carbon center involved initially the carbanion formation by the abstraction of the proton by the base-catalyst. If the carbanion was formed in the deuterium-donating solvent, the hydrogen-deuterium exchange reaction at this carbon center must be occurred.

Many years ago, Wilson and Ingold[3] found that the rates of hydrogen-deuterium exchange (k_e) and of racemization (k_α) of optically active 1-phenyl-2-methyl-1-butanone in a basic solution of deuterium oxide-dioxane were equal. A similar identity of rates was observed when optically active phenyl-*p*-tolyldeuterio-acetic acid[4] was heated in aqueous base. Recenly, Cram, *et al.*[5] observed that the ratio k_e/k_α was equal to unity in the base-catalyzed reaction of an optically active nitrile, an amide and an ester in a variety of solvents. These results were interpreted as involving proton-abstraction or protonation of an ambident carbanion which was stabilized by π-electron delocalisation in the presence of carbonyl-containing groups or equivalent attached to the anion and which was planar and long-lived enough to pass into symmetric solvent-envelopes.[6] Of the many investigations of the kinetics of proton-abstraction from carbon of the type described above, only leading references are mentioned here.

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*3 Presented in part at the 3rd Symposium on Peptide Chemistry held at Osaka University, Nov. 6, 1964 (Abstract Papers of the 3rd Symposium on Peptide Chemistry, **1964**, p. 30). This paper constitutes also Part Ⅳ of a series entitled by "Studies on Hydrogen Exchange" by Y. Kawazoe. Part Ⅲ: This Bulletin, **14**, 1413 (1966).

1) *a*) A. Neuberger : Adv. Protein Chem., **4**, 339 (1948).
b) G. T. Young : Coll. Czech. Commun. (Special Publication), **24**, 118 (1959).
c) Th. Wieland : Angew. Chem., **75**, 539 (1963).
d) B. Liberek : Tetrahedron Letters, **1963**, 925, 1103, 1479; **1964**, 159.
2) M. Bovarnick, H. T. Clarke : J. Am. Chem. Soc., **60**, 2426 (1938).
3) *a*) S. K. Hsu, C. K. Ingold, C. L. Wilson : J. Chem. Soc., **1938**, 78. *b*) C. L. Wilson : *Ibid.*, **1936**, 1550.
4) D. J. G. Ives, G. C. Wilks : *Ibid.*, **1938**, 1455.
5) D. J. Cram, B. Rickborn, C. A. Kingsbury, P. Haberfield : J. Am. Chem. Soc., **83**, 3678 (1961).
6) D. J. Cram : "Fundamentals of Carbanion Chemistry," Academic Press, N. Y. (1965).

Among compounds studied are nitroparaffines[7,8] and a number of ketones, nitriles, carboxylic acids, sulfones and amides.[9,10]

With amino acids derivatives which were considered to be analogous to the system above-mentioned, however, only information of a qualitative character is available.[1a]

For the preliminary survey, the investigation on the base-catalyzed deuterium-hydrogen exchange and racemization of several amino acids derivatives and related model compounds. The main objective which the present paper concerns is an approach to the estimation of the effect of substitution of α-amino acids on their hydrogen exchange reaction in basic media by the application of nuclear magnetic resonance (NMR) method.[11]

Method

Deuteration reaction of amino acids derivatives in basic solution of M sodium deuteroxide-deuterium oxide or sodium methoxide-deuterated methanol was carried out and then deuterated amounts were determined by the NMR method which recently developed by Kawazoe, *et al.*[11] This NMR technique has advantages to be carried out in a simpler manipulation than the conventional deuterium analysis and to detect the deuterated position directly from the recording chart to check the occurrence of unfavorable side-reaction.

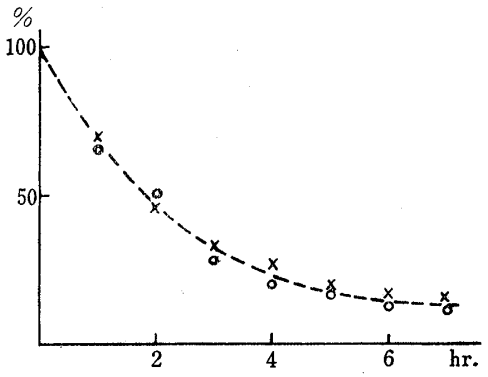


Fig. 1.

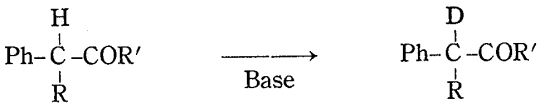
—○—○— deuteration reaction
—×—×— racemization reaction

General procedure is as follows :

The tested sample exacty weighed was dissolved in a definite volume of M NaOD-D_2O or M MeONa-MeOD 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 quickly in an ice-bath to quench the reaction and immediately subjected to NMR measurement directly (JNM-3H-60 Spectometer, 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 exchaged α-hydrogen after t-hrs' reaction can be derived by the following equation :

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

Table I. Parallelism between Deuteration and Racemization



Substrate No.	R	R'	Concn. (M)	Solvent	Base	Concn. (M)	Temp. (°C)	Time (hr.)	Rac. (%)	Exch. (%)
I	NH_2	OH	0.657	D_2O	NaOD	1.07	115	1	67	70
	〃	〃	0.657	〃	〃	1.07	115	2	48	46
	〃	〃	0.657	〃	〃	1.07	115	3	28	32
	〃	〃	0.657	〃	〃	1.07	115	4	23	20
	〃	〃	0.657	〃	〃	1.07	115	5	18	16
	〃	〃	0.657	〃	〃	1.07	115	6	11	13
II	〃	OEt	0.500	MeOD	MeONa	0.98	110	1	50	56
III	PhthN	OH	0.310	〃	〃	0.98	60	1.5	42	39

7) O. Reitz : Z. physik. Chem., **A 176**, 363 (1963).
8) R. P. Bell : "The Proton in Chemistry" Cornell Univ. Press, Ithaca, N. Y., p. 109 (1959).
9) R. G. Pearson, R. L. Dillon : J. Am. Chem. Soc., **75**, 2441 (1953).
10) K. F. Bonhoeffer, K. H. Geib, O. Reitz : J. Chem. Phys., **7**, 664 (1939).
11) Y. Kawazoe, M. Ohnishi : This Bulletin, **11**, 846 (1964).

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 used 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.

However, in the case of MeONa-MeOD system, it was found that the signal of $^{13}CH_3$ of $^{13}CH_3OD$ which was contained in the solvent in a definite concentration is possible to use as the effective reference. Some typical charts were shown in Figs. 2 to 4 (*vide supra*).

By this method, it was proved that the deuteration reaction could be followed with fairly sufficient reproducibility and in a simple manipulation. However, the accuracy of this method was not yet entirely examined and so, in the present paper, the obtained data were not subjected to kinetic treatment.*4 Racemization reaction was followed polarimetrically by using Yanagimoto direct-reading Polarimeter OR-20 (Na-D line).

Results and Discussion

Parallelism between α-Deuteration and Racemization (Table I) :

Base-catalyzed racemization and deuterium exchange reaction of optically active D-phenylglycine (I)[12] (115° in *M* NaOD-D_2O and in *M* NaOH-H_2O), D-phenylglycine ethyl ester (II)[13] (110° in *M* MeONa-MeOD and in MeONa-MeOH) and N-phthaloyl-D-phenylglycine (III) (60° in *M* MeONa-MeOD and in *M* MeONa-MeOH) were carried out and followed by the method described above.

As seen from Table I and Fig. 1 where results thus obtained were summarized, the sufficient parallelism between deuteration and racemization reactions was recognized in every case, as was expected. Within experimental error, the percentage racemized and exchanged were identical. Thus, it was found that the hydrogen-deuterium exchange reaction occurred with the same rate of racemization for (I), (II) and (III). The deuterium analysis values by NMR method were described in Table I without any correction.

Moreover, optically inactive DL-form of I showed that the same attitude as in the D-form and therefore, it was proved that the information of the racemization was also obtainable from the deuteration reaction of racemic compound in the place of optically active one.

Rough Estimation of the Effect Substitution of α-Amino Acids on their α-Deuteration in MeONa-MeOD Solution :

For the preliminary estimation of the effect of substitution of carboxyl and amino groups of amino acids on their α-deuteration and racemization, the base-catalyzed deuteration reaction of the following three groups of compounds were investigated in MeONa-MeOD solution. Tested compounds were as follows :

a) Propionic Acid Deirivatives :

Propionic acid Na-salt (IV), proponamide (V), N,N-diethylpropionamide (VI),[14] propionanilide (VII), N-benzylpropionamide (VIII),[15] γ-butyrolactam (IX), δ-valerolactam (X) and ethyl (XI) and benzyl propionate (XII).[16]

*4 Slight differences in the molar concentration and ionic character of the solutes, even in case where they are neutral molecules, may bring about a somewhat effect on the deuteration reaction rate. The result obtaind in the present study, therefore, should be re-examined for the kinetical works in taking account of these points.

12) Org. Syntheses, Coll. Vol. III, p. 84. See ref. 19).
13) G. Rosse : Ber., **91**, 2410 (1958).
14) V. Braun : Ber., **91**, 2410 (1958). T. W. Lynn : J. Am. Chem. Soc., **73**, 4284 (1951).
15) C. A. Duehler : *Ibid.*, **59**, 421 (1937).
16) M. Conrad, W. R. Hodgkinson : Ann., **193**, 311 (1878).

b) Phenylglycine Derivatives :

Phenylglycine (I) and N-acyl derivatives of I (N-propionyl- (XIII), N-acetyl- (XIV), N-phthaloyl- (III)) and ethyl ester of I (II). Phenylacetic acid (XV) and its ethyl ester (XVI) were used as models of des-N compound of I and II.

c) Alanine Derivatives :

L-Alanine (XVII), N-acetyl-L-alanine (XVIII),[17] L-alanine ethyl ester (XIX),[18] N-acetyl-L-alanineamide (XX) and N-phthaloyl-L-alanineamide (XXI).

After deuteration reaction of these compounds at 20°, 40°, 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 II, III, and IV. Considering the accuracy of NMR method and the present purpose, the following notation of deuteration percentages was used in these Tables :

(−) : negative, (±) : less than 20%, (+) : 20~40%, (++) : 40~70%, (+++) : more than 70%.

a) α-Deuteration of Propionic Acid Derivatives (Table II) :

In order to estimate the effect of carboxyl substitution, propionic acid derivatives and lactams (IV~XII), which seemed to be corresponding to des-amino compound of

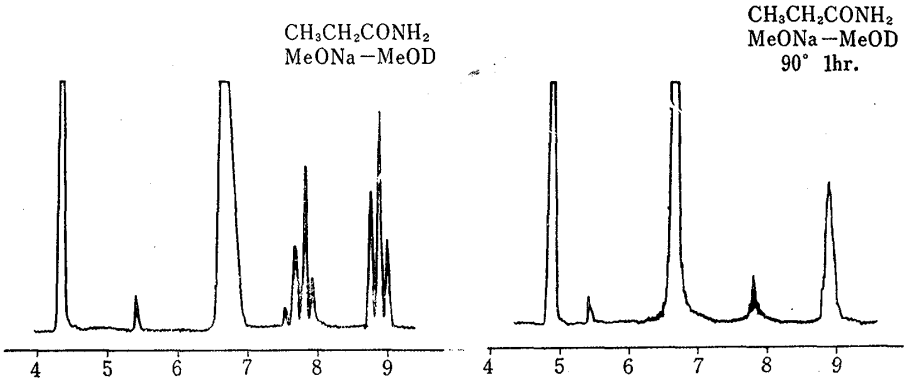
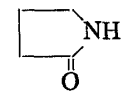
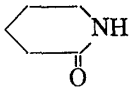


Fig. 2.

Table II. $CH_3CH_2COX \xrightarrow[(a: 1.14M,\ b: 1.03M)]{MeONa\text{-}MeOD} CH_3CD_2COX$

Compound No.	X	MeONa Concn.	60 (℃) 1 (hr.)	90 1	90 2	120 1	150 1	180 1
IV	ONa	b	−	−	−	−	+	
V	NH_2	a	−	+++	+++			
VI	NEt_2	〃	−	++	+++			
VII	NHPh	〃	−	+++				
VIII	$NHCH_2Ph$	〃	−	+++				
IX		b	−	+				
X		〃	−	++				
XI	OEt	〃	−	−	−	−	−	−
XII	OCH_2Ph	a	−	−	−	−	−	−

17) J. P. Greenstein : J. Biol. Chem., **194**, 455 (1952).
18) *Idem*; J. Am. Chem. Soc., **76**, 6054 (1954).

alanine, were prepared and subjected to deuteration reaction in MeONa-MeOD solution. The NMR spectra of the starting samples showed that α-CH_2 signals were quartet in the region of τ-value 7.5~8.0 and β-CH_3 signals triplet in τ-value 8.9~9.1. On the completion of α-deuteration, the former resulted into disappearance, while the latter collapsed into a singlet band around 9.0. After the reaction under the conditions showed in Table II, areal intensity of exchanging α-CH_2 signals were integrated and then α-D's were determined. These results thus obtained were shown in Table II. As seen from this Table, it was striking that all amides involving primary, secondary and tertiary ones (V to X) were very much readily exchangeable than others, *i.e.*, carboxylic acid and esters. This fact could not be explainable only by Neuberger's prediction[1a] that the negative charge present at carboxylate anion will oppose the dissociation of α-proton, while any substitution on the carboxyl group which abolish its charge would facillitate ionization of α-proton to undergo racemization. The further investigation on such a remarkable effect of amide are now in progress.

b) α-Deuteration of Phenylglycine Derivatives (Table III) :

Five kinds of the derivatives of phenylglycine, *i.e.*, I, II, XIII, XIV and III, which were considered the most racemizable of all other amino acids, were subjected to the

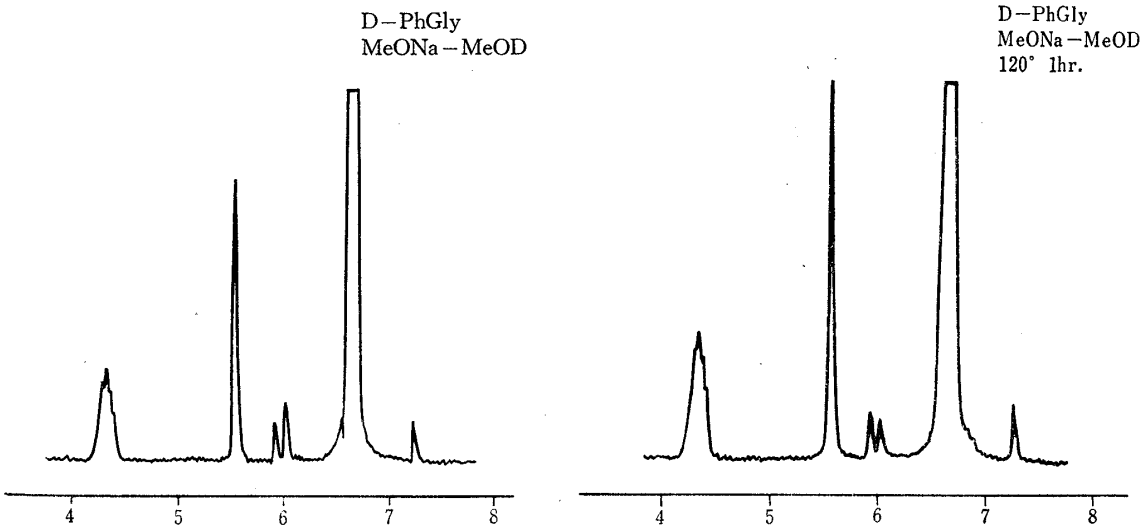


Fig. 3.

TABLE III. $Ph-\overset{\overset{H}{|}}{\underset{\underset{R}{|}}{C}}-COX \xrightarrow{MeONa\text{-}MeOD\ (0.98M)} Ph-\overset{\overset{D}{|}}{\underset{\underset{R}{|}}{C}}-COX$

Compound			60 (°C)		90		120	
No.	R	X	1 (hr.)	2	1	2	1	2
I	NH_2	OH	−	−	−	−	+	++
XIII	C_2H_5CONH	〃	±	+	+++	+++		
XIV	CH_3CONH	〃	−	−	+			
III	PhthN	〃	±	+	++	+++		
II	NH_2	OEt	−	−	−	±	++	+++
XV	H	OH	−		+			
XVI	〃	OEt	−		−			

α-deuteration as in the case described above and compared with those of phenylacetic acid (XV) and its ethyl ester (XVI) which correspond to the des-amino compound of I and II. Results were summarized in Table III. As shown in this Table, phenylglcine

(I) itself was fairly resistant to deuteration reaction rather than XV. From this fact, it was concluded that the α-amino group plays a retarding role on the base-catalyzed α-proton abstraction, which was removed by N-acylation. But, the delicate differences of this effect of various species of N-acyl group were not clear from the present data.

c) α-Deuteration of Alanine Derivatives (Table Ⅳ):

The alanine derivatives (XVII~XXI) which seemed to be less racemizable than those of phenylglycine by the methyl substitution in the place of phenyl group were examined.

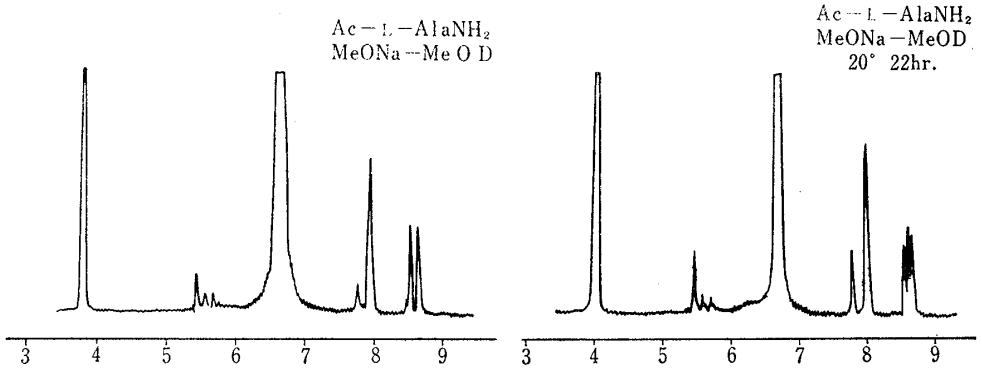


Fig. 4.

TABLE Ⅳ. $CH_3-\overset{H}{\underset{R}{C}}-COX \xrightarrow{MeONa\text{-}MeOD\ (1.12M)} CH_3-\overset{D}{\underset{R}{C}}-COX$

Compound			20 (℃)			40		60	90	120	150	180
No.	R	X	3 (hr.)	5	22	1	2	1	1	1	1	1
XVII	NH_2	OH	−			−		−	−	−	−	+
XIX	〃	OEt						−	−	−	−	+
XVIII	CH_3CONH	OH						−	−	−	−	+
XX	〃	NH_2	−	±	+	+	++	++				
XXI	PhthN	〃	±	+	++			+++				
V	H	〃						−	+++			

The results were shown in Table Ⅳ. As shown by the remarkable fact that N-acetylalanineamide (XX) was much more readily deuterated even at 20° than N-acetylalanine (XVIII), there exists the accelerating effect of carboxamide group also in this case. The effect of N-acetylation which was recognized in the case of phenylglycine series was not so clear but there was a similar tendency on the comparison of (XX) with propionamide (V).

Experimental

A) Preparation of Materials:

D-Phenylglycine (D-I)——Prepared by the optical resolution of DL-I, using *d*-camphorsulfonic acid by the method of Betti and Mayer.[19]

D- and DL-Phthaloylphenylglycine (D- and DL-III)——Prepared by the phthaloylation of D- and DL-I respectively by the method of Nefkens.[20] D-III: Colorless needles (from EtOH-H_2O). m.p. 186~187°, $[\alpha]_D^{31}$ −10.2 (c, 1.213, EtOH). Yield 72.5%. *Anal.* Calcd. for $C_{16}H_{11}O_4N$: C, 68.32; H, 3.94; N, 4.97. Found: C, 67.92; H, 3.88; N, 5.05. DL-III: Colorless powder (from EtOH-H_2O). m.p. 173~174°. Yield, 57.5%. *Anal.* Calcd. for $C_{16}H_{11}O_4N$: C, 68.32; H, 3.94; N, 4.97. Found: C, 68.29; H, 3.97; N, 5.12.

19) M. Betti, M. Mayer: Ber., **41**, 2071 (1908).
20) G. H. L. Nefkens: Rec. trav. chim. pay-bas; **79**, 688 (1961).

N-Propionyl-DL-phenylglycine (XIII)——Prepared from DL-I and propionyl chloride by Schotten-Baumann method. Colorless needles (from AcOEt). m.p. 152°. Yield, 68.8%. *Anal.* Calcd. for $C_{11}H_{13}O_3N$: C, 63.76; H, 6.32; N, 6.77. Found: C, 63.98; H, 6.19; N, 6.89.

N-Acetyl-DL-phenylglycine (XIV)——Prepared by acetylation of DL-I with Ac_2O and aq. NaOH. Colorless needles (from AcOEt). m.p. 196~197°. Yield, 93.2%. *Anal.* Calcd. for $C_{10}H_{11}O_3N$: C, 62.17; H, 5.74; N, 7.26. Found: C, 61.86; H, 5.60; N, 7.27.

N-Acetyl-L-alanineamide (XX)——Prepared from N-acetylalanine by the mixed anhydride method, using ethyl chloroformate and then bubbling by anhyd. NH_3 gas. Colorless prisms (from acetone). m.p. 162°. $[\alpha]_D^{27}$ −5.64 (c, 0.780, EtOH). Yield, 68.9%. *Anal.* Calcd. for $C_5H_{10}O_2N_2$: C, 46.15; H, 7.75; N, 21.52. Found: C, 46.17; H, 7.39; N, 21.43.

N-Phthaloyl-L-alanineamide (XXI)——Prepared from N-phthaloyl-L-alanine by the similar way as in the case of XX, described above. Colorless hexagonal prisms (from MeOH-H_2O). m.p. 202°. $[\alpha]_D^{17}$ +6.32 (c, 0.443, EtOH). Yield, 39%. *Anal.* Calcd. for $C_{11}H_{10}O_3N_2$: C, 60.53; H, 4.62; N, 12.83. Found: C, 60.72; H, 4.50; N, 12.89.

Methanol-d_1——Prepared by the method of Hobden, *et al.*[21]

B) Deuteration Experiment of D-I in NaOD-D_2O——One half ml. of the solution of D-I (497.1 g.) in 5.0 ml. of D_2O (99.7 atom%, Merck Co.), containing NaOD (218.6 mg.) was sealed in ten NMR sample tubes, which were thermostated at 115°. Each aliquot was sampled every an hour and then ice-cooled quickly to quench the reaction. After the addition of HCO_2Na exactly weighed as a reference, NMR measurement was carried out using JNM-3H-60 Spectrometer (Japan Electron Optics Labs.) at 60 Mc. From the spectra thus obtained, areal intensities of the signal of α-H of I (τ=6.0) and those of a reference signal were measured. Deuteration % (α-D^t) was calculated by the equation (1).

C) Racemization Experiment of D-I in NaOH-H_2O——Each 5-ml. portions of the solution of D-I (4.971 g.) in 50 ml. of 1.07*M* aq. NaOH were sealed in ampules, which were heated at 115° in the thermostat. Each aliquots were sampled every an hour and then ice-cooled quickly to quench the reaction. The content was acidified by addition of exactly measured 2 ml. of 6*N* HCl. Acidic solution thus obtained was subjected 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.

$$\text{Racemization } \% = \frac{[\alpha]_D \text{ after t-hrs. reaction}}{[\alpha]_D \text{ at initial stage}} \times 100\ (\%)$$

D) Deuteration Reaction in the Medium of MeONa-MeOD——Each 0.5 ml. of 10% solution of tested compounds in *M* MeONa-MeOD (1.14*M* for V, VI, VII, VIII, and XII; 1.03*M* for IV, IX, X, and XII; 0.98*M* for I, II, III, XIII, XIV, XV and XVI; 1.22*M* for XVII~XXI) was sealed in the NMR sample tubes and thermostated at various temperatures. Each aliquots were sampled after t-hrs' reaction and then ice-cooled quickly to quench the reaction. After addition of reference substance (HCO_2Na), if necessary, NMR measurements were carried out.

From the NMR spectra thus obtained, the deuteration % of α-H was calculated.

Conclusion

The sufficient parallelism between base-catalyzed α-deuteraion and racemization of α-amino acids and derivatives was recognized, using NMR method and polarimetry.

Therefore, it was revealed that the information of the racemization could be obtainable from the data of α-deuteration of the racemic form in simpler manipulation.

In order to examine the effect of substitution of amino and carboxyl groups in α-amino acid on their base-catalyzed racemization, α-deuteration was carried out to obtain the following results:

i) While free amino group has a retarding effect on base-catalyzed deuteration, compared with the corresponding des-amino compounds, N-acylation did not only abolish NH_2-retarding effect but also accelerated significantly α-deuteration reaction.

ii) Carboxylate anion has a considerably retarding effect as Neuberger predicted. But, the strikingly accelerating effect of carboxamide group cannot be explainable only by Neuberger's theory. On the other hand, ester group has not so much accelerating effect than carboxylate group does.

21) F. W. Hobden, E. F. Johnston, L. H. P. Weldon, C. L. Wilson: J. Chem. Soc., **1939**, 61.

iii) The order of racemizability of α-amino acid derivatives was as follows:

$$R'CONHCHCON\langle \gg R'CONHCHCO_2H > NH_2CHCO_2H$$

The authors are greatly indebted to Dr. W. Nakahara, Director of National Cancer Center Research Institute, and Professor Emeritus E. Ochiai and Professor S. Yamada of the University of Tokyo, for their kind encouragement throughout this work. Thanks are also due to the Central Analysis Room of the Institute of Physical and Chemical Research.