RACEMIZATION RATES OF 3,4-DIDEHYDRO-2-AMINO ACIDS

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The racemization rates of amino acids in acidic medium (acetic acid) were studied. The sensitivity to racemization decreases in the order (*E*)-3,4-didehydroornithine > (*E*)-N$^{\delta}$-Z-3,4-didehydroornithine ≫ (*Z*)-3,4-didehydronorvaline > ornithine, norvaline. (*E*)-3,4-Didehydroornithine is also relatively rapidly racemized on heating with 5M or 0·05M-HCl (100°C).

The synthesis of 3,4-didehydro-2-amino acids receives recently a growing attention[1]. These compounds exhibit interesting biological properties and may be used for the preparation of tritium-labelled amino acids and peptides. Only scarcely they have been synthesized in optically pure state[2] and their racemization has not been studied so far.

During the synthesis of (*E*)-3,4-didehydro-L-ornithine and its derivatives we observed unexpected racemization in an acid medium. Therefore, we compared the racemization rates of (*E*)-3,4-didehydroornithine (further dehydroornithine) and (*Z*)-3,4-didehydronorvaline (dehydronorvaline) with those of the corresponding saturated derivatives in acetic and hydrochloric acids. The effect of a substituent on the racemization rate was studied for (*E*)-N$^{\delta}$-Z-3,4-didehydroornithine.

The experimental conditions and the obtained rate constants are summarized in Table I. In most experiments the starting amino acids were mixtures of the D- and L-forms and the number of measurements was limited. For this reason, the rate constants in Table I may be regarded only as informative; they are, however, sufficient for comparison of the studied amino acids.

The rate constants (Table I) were calculated for a first-order reverse reaction kinetics according to the relationship $k = \frac{1}{2}t \ln [(L_0 - D_0)(L - D)]$, where L_0, D_0 are initial concentrations of the enantiomers and L, D are concentrations at the time t.

In acetic acid, phenylalanine has been shown (Yamada and coworkers[3]) to be the most readily racemized proteinogenic amino acid (35% racemization after 1 h in concentrated acetic acid at 100°C; $k = 6{\cdot}0 \cdot 10^{-5}\ s^{-1}$). The rate constant steeply drops with dilution of the acetic acid and at concentrations lower than 50% (100°C,

3 h) the racemization has not been polarimetrically detectable. Our experiment 21 agrees with this observation. The results of experiments 1–5 illustrate an extremely

TABLE I
Racemization experiments

Exp.	Medium	T, °C	Time, h	$D_{init.}$, %	$D_{fin.}$, %	k, s^{-1}
			(*E*)-3,4-Didehydroornithine			
1	AcOH, pH = 3·3	50	43	23	33	$1{\cdot}5 \cdot 10^{-6}$
2	0·5% AcOH, pH = 3	50	37	23	33	$1{\cdot}7 \cdot 10^{-6}$
3	15% AcOH	50	43	23	≧48	$\geqq 8 \cdot 10^{-6}$
4	AcOH, pH = 4	95	10 min	23	32	$3{\cdot}5 \cdot 10^{-4}$
5	15% AcOH	95	10 min	34	≧48	$\geqq 1{\cdot}7 \cdot 10^{-3}$
6	0·05M-HCl	100	2	23	44	$9{\cdot}5 \cdot 10^{-5}$
7	5M-HCl	100	1	23	35	$8{\cdot}0 \cdot 10^{-5}$
8	5M-HCl	100	2	23	43	$9{\cdot}0 \cdot 10^{-5}$
9	2·3% NH_4OH	45	1	23	23	—[a]
10	1% Na_2CO_3	45	1	23	23	—[a]
			(*E*)-N^{δ}-Benzyloxycarbonyl-3,4-didehydroornithine			
11	0·5% AcOH, pH = 3	50	40	9	9	—[a]
12	15% AcOH	50	43	5	21	$1{\cdot}5 \cdot 10^{-6}$
13	AcOH, pH = 4	95	10 min	5	6	$\leqq 5 \cdot 10^{-5}$
14	15% AcOH	95	10 min	5	35	$9{\cdot}0 \cdot 10^{-4}$
			(*Z*)-3,4-Didehydronorvaline			
15	0·5% AcOH	50	37	5	5	—[a]
16	15% AcOH	50	40	5	5	—[a]
17	15% AcOH	65	40	5	10	$4{\cdot}0 \cdot 10^{-7}$
18	5M-HCl	100	2	5	5	—[a]
			Norvaline			
19	15% AcOH	65	233	0·5	1·2	$\leqq 2 \cdot 10^{-8}$
			Ornithine			
20	15% AcOH	65	233	0·0	1·0	$\leqq 1{\cdot}8 \cdot 10^{-8}$
			Phenylalanine			
21	15% AcOH	65	233	0·2	0·3	$\leqq 1{\cdot}0 \cdot 10^{-8}$

[a] Sensitivity of the method did not allow determination of k.

fast racemization of dehydroornithine compared with ornithine (experiment 20) even in dilute acetic acid. Whereas dehydroornithine racemizes rapidly in 15% acetic acid at 50°C, racemization of ornithine at 65°C is only very slow (experiments 3 and 20). An evident, though much smaller, difference also exists between dehydronorvaline and norvaline (experiments 17 and 19). Derivatization of dehydroornithine by attachment of benzyloxycarbonyl group to the N^{δ} atom reduces the racemization rate, as follows from comparison of experiments 2 and 11, 3 and 12, 4 and 13, and 5 and 14.

The extraordinarily fast racemization of dehydroornithine in dilute acetic acid has also been verified by isotope exchange ^1H–^2H on the H—C^{α} bond (determined from decrease of the ^1H NMR signal) (Table II). As shown by ^2H NMR, deuterium was incorporated only into the α-position.

In hydrochloric acid, the racemization of alanine, valine, leucine and isoleucine has been thoroughly studied[4]. The rate constant for the most sensitive amino acid, leucine, amounts to $1{\cdot}5 \cdot 10^{-7}\ s^{-1}$ (105°C, 6M-HCl). As shown by our experiments 6, 7 and 8, dehydroornithine was racemized under comparable conditions at least 550 times faster than leucine, the racemization rate being practically the same in 0·05M-HCl (pH 1·3; region of the most difficult racemization of amino acids[5]) and in 5M-HCl.

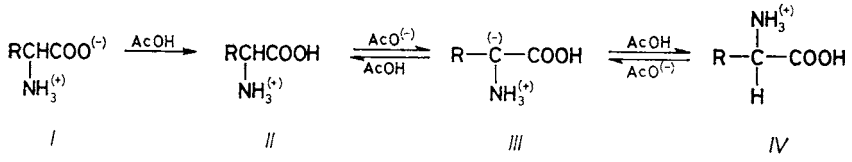
For dehydroornithine we also orientationally studied the danger of racemization in a weakly basic medium (experiments 9 and 10). Under the conditions used, we have found no enhanced propensity to racemization.

The suggested mechanism of racemization of amino acids in acetic acid[6] stresses the role of the acetate anion whose basicity causes cleavage of the C^{α}—H bond in the amino acid *II* under formation of the anion *III* (Scheme 1). The stabilization of the carbanion *III*, and thus acceleration of the racemization, is supported by delocalization of the negative charge at the α-carbon atom which is higher for R = = phenyl than for R = benzyl[7,8]. This agrees with the observed racemization rates which decrease in the order: 2-amino-2-phenylacetic acid > phenylalanine > alanine[3,6].

TABLE II

Isotope exchange in (*E*)-3,4-didehydro-D,L-ornithine

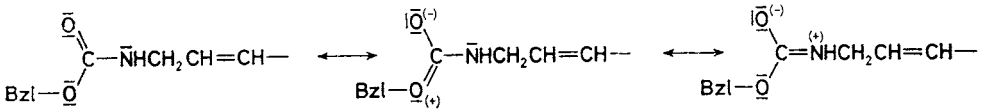
Exp.	Medium	*T*, °C	Time, min	C^{α}–^2H, %	*k*, s^{-1}
22	2% AcOD/D_2O	95	30	53 ± 5	$4 \cdot 10^{-4}$
23	5% AcOD/D_2O	95	50	88 ± 5	$7 \cdot 10^{-4}$



I *II* *III* *IV*

SCHEME 1

The above-mentioned conclusions[6-8] may serve as a basis for explanation of the racemization rates in acetic acid, found in the present study. In dehydroornithine the induction effect of the ammonium ion bonded to the C-5 atom enhances the influence of the C=C bond, compared with dehydronorvaline. In the N^{δ}-Z derivative *V* (Scheme 2) the protonation of the terminal nitrogen atom is suppressed (smaller -*I* effect) but the dipolar structure[9] *VII* stabilizes the carbanion *III* (compared with dehydronorvaline).



V *VI* *VII*

SCHEME 2

In 0·05M or 5M hydrochloric acid (unlike acetic acid, this medium does not contain any effective acceptor of H^+) the racemization of dehydroornithine is slower than in acetic acid but it is still unusually high compared with saturated amino acids. Neither the obtained results nor the available literature data (for saturated amino acids) lead to an unequivocal acceptable reaction mechanism.

EXPERIMENTAL

The gas-chromatographic separation of enantiomers was performed on a GC 3 700 instrument (Varian U.S.A.) equipped with flame-ionization detector, on a WCOT fused silica column (25 m × 0·22 mm) coated with Chirasil-L-Val (thickness 0·13 mm) at 110°C (D- and L-norvaline) and at 160°C (D- and L-ornithine and D- and L-phenylalanine). The mean quadratic deviation of the enantiomers determination was 0·17%. Proton NMR spectra were obtained at 400 MHz, ^{2}H NMR at 61·4 MHz, ^{13}C NMR at 100 MHz, on a Varian VXR-400 spectrometer (Varian, U.S.A.). The spectra are referenced to tetramethylsilane unless stated otherwise. Thin-layer chromatography (TLC) was performed on silica gel (Silufol sheets, Kavalier, Czechoslovakia) in 1-butanol–acetic acid–water (4 : 1 : 1). The preparation of (*Z*)-3,4-didehydro-L-norvaline and (*E*)-3,4-didehydro-L-ornithine is described elsewhere[10,11].

(*E*)-N^{α}-Ac-N^{δ}-Z-3,4-didehydro-D,L-ornithine (*I*)

(*E*)-N^{α}-Ac-3,4-didehydro-D,L-ornithine (containing 1/4 mol of crystal water)[11] (199 mg) was dissolved in a mixture of water (3·3 ml), dioxane (7 ml) and triethylamine (0·246 ml). Benzyl benzotriazol-1-yl-carbonate[12] (BZBC, 346 mg) was added under vigorous stirring at room temperature. The reaction was followed by TLC (starting compound $R_F = 0{\cdot}10$, product $R_F = 0{\cdot}75$). After 20 min another BZBC (60 mg) was added and the stirring was continued for 30 min. The mixture was concentrated under diminished pressure at room temperatur to a sirup. The product was isolated by chromatography on a silica gel column (1·5 × 8 cm). Elution with ethyl acetate–methanol (95 : 5) afforded 1-hydroxybenzotriazol, elution with 100% methanol gave the product *I* which crystallized as the dicyclohexylammonium salt from ethyl acetate; yield 285 mg (52%), m.p. 135°C. For $C_{27}H_{41}N_3O_5$ (487·3) calculated: 66·48% C, 8·48% H, 8·62% N; found: 66·09% C, 8·10% H, 8·26% N. ^{1}H NMR ($CDCl_3$): 1·08—1·36 mt, 10 H (DCHA); 1·64 mt, 2 H (DCHA); 1·77 mt, 4 H (DCHA); 1·96 mt, 4 H (DCHA); 2·02 s, 3 H (CH_3CO); 2·84 mt, 4 H (2 × CH, NH_2^+, DCHA); 3·80 t, 2 H (J = 5·3, H-5); 4·87 t, 1 H (J = = 5·1, H-2); 5·08 s, 2 H (OCH_2Ph); 5·38 brs, 1 H ($NHCH_2$); 5·66 dt, 1 H (J = 15·5, J = 5·3, H-4); 5·80 dd, 1 H (J = 15·5, J = 5·1, H-3); 6·83 d, 1 H (J = 5·1, NHCH); 7·34 mt, 5 H (Ph). ^{13}C NMR ($CDCl_3$): 23·45 q (CH_3CO); 24·96 t, 4 C (DCHA); 24·50 t, 2 C (DCHA); 31·04 t, 4 C (DCHA); 42·66 t (C-5); 53·02 d, 2 C, (DCHA); 56·56 d (C-2); 66·67 t (OCH_2Ph); 126·13 d (C-4 or C-3); 127·97 d (C-3 or C-4); 128·05 d (Ph); 128·59 d, 2 C (Ph); 130·66 d, 2 C (Ph); 136·61 s (Ph); 156·29 s (NHCOO); 169·27 s (CH_3CO); 173·90 s (C-1).

(*E*)-N^{δ}-Z-3,4-didehydro-L-ornithine (*II*)

The substituted ornithine *I* (176 mg) was dissolved in water (160 ml), cobalt(II) acetate (42 mg) was added and the solution was adjusted to pH 7·4 with aqueous ammonia. After addition of acylase *I* (Fluka, 90 mg), the solution was incubated at 37°C for 25 h. During the resolution the pH value was adjusted so the original value by addition of aqueous ammonia and acylase *I* (10 mg) was added four times. The reaction was monitored by TLC (product: $R_F = 0{\cdot}40$). The resolution took place only at the mentioned unusually low concentration of the substrate. After removal of the acylase by ultrafiltration, the aqueous solution was applied on Dowex 50 (H^+-form), the resin was washed with water and the amino acid was eluted with 3% aqueous ammonia. The eluate was concentrated under diminished pressure at 30°C to crystallization. Two fractions were obtained: 1. fraction (68 mg): L/D = 95/5, m.p. 193—195°C (turns brown at 180°C); 2. fraction (3 mg): L/D = 91/9; total yield 90%. For $C_{13}H_{16}N_2O_4$ (264·3) calculated: 59·08% C, 6·10% H, 10·60% N; found: 58·83% C, 6·32% H, 10·45% N. ^{1}H NMR (D_2O, acetone as internal reference): 3·825 dd, 2 H (J(4, 5) = 5·0, J(3, 5) = 1·6, H-5); 4·284 dd, 1 H (J(2, 3) = 8·1, J(2, 4) = 0·5, H-2); 5·156 s, 2 H (CH_2Ph); 5·719 ddt, 1 H (J(2,3) = 8·1, J(3, 4) = 15·5, J(3, 5) = = 1·6, H-3); 5·985 dtd, 1 H (J(3, 4) = 15·5, J(2, 4) = 0·5, J(4, 5) = 5·0, H-4); 7·456 mt, 5 H (Ph).

Racemization Experiments and Determination of L/D

The amino acid (0·5—1 mg) or its monohydrochloride was dissolved in the appropriate solution (0·8 ml). In experiments 1, 4, and 13 the pH was adjusted with acetic acid. The solution was then heated in a sealed ampoule as specified in Table I. Solutions in acetic acid were freeze-dried and solutions in 5M-HCl were evaporated in a desiccator at room temperature. Basic samples were neutralized with hydrochloric acid and then evaporated in a desiccator. Unsaturated amino acids were hydrogenated (wish simultaneous hydrogenolysis of the Z-group) in water (0·25 ml) over PdO (2 mg) for 30 min at room temperature and atmospheric pressure of hydrogen. The

catalyst was removed by centrifugation and the solution was evaporated. The amino acids were esterified with 3M methanolic HCl at 55°C for 1 h and then converted into their N-trifluoroacetyl derivatives as described in ref.[4].

Isotope Exchange (Experiments 22, 23)

A solution of (*E*)-3,4-didehydro-D,L-ornithine monohydrochloride (60 mg) in 99% D_2O (4 ml) was freeze-dried. The residue was treated in the same manner once more (exchange of labile hydrogen atoms). The amino acid was dissolved in 2% or 5% solution of [O–D]acetic acid in 99% D_2O (prepared by reaction of acetic anhydride with D_2O). The solution was heated as specified in Table II. After freeze-drying the samples were meazured by ^1H NMR or ^2H NMR spectroscopy in D_2O or H_2O with acetone or H_2O as the internal standards: 3·74 d, 2 H (J = 5·1, CH_2); 4·38 d, 1 H (J = 6·6, CH); 5·98–6·14 mt, 2 H (CH=CH). The ^2H NMR spectrum displayed only a signal at δ 4·38 ppm.

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REFERENCES

1. Castelhano A. L., Horne S., Taylor G. J., Billedeau R., Krantz A.: Tetrahedron *44*, 5451 (1988).
2. Williams R. M., Zhai W.: Tetrahedron *44*, 5425 (1988).
3. Yamada S., Hongo Ch., Yoshioka R., Chibata I.: J. Org. Chem. *48*, 843 (1983).
4. Nakaparksin S., Gil-Av E., Oro J.: Anal. Biochem. *33*, 374 (1970).
5. Bada J. L., Shou M.-Y. in: *Biochemistry of Amino Acids* (P. E. Hare, T. C. Hoering and K. King jr, Eds), p. 235. Wiley, New York 1980.
6. Matsuo H., Kawazoe Y., Sato M., Ohrishi M., Tatsuno T.: Chem. Pharm. Bull. *18*, 1788 (1970).
7. Neuberger A. in: *Advances in Protein Chemistry* (M. L. Anson and J. T. Edsall, Eds), Vol. 4, p. 298. Academic Press, New York 1948.
8. Smith G. G., Evans R. C. in: *Biogeochemistry of Amino Acids* (P. E. Hare, T. C. Hoering and K. King jr, Eds), p. 257. Wiley, New York 1980.
9. Bláha K. in: *Chemie organických sloučenin* (O. Červinka, Ed.), Vol. 2, p. 632. SNTL/ALFA Press, Prague 1987.
10. Havlíček L., Hanuš J.: *11th Radiochemical Conference, Mariánské Lázně, April 1987, Czechoslovakia*; Abstracts of papers: p. 73.
11. Havlíček L., Hanuš J.: Radioisotopy *29*, 157 (1988).
12. Kim S., Chang H.: Korean. Chem. Soc. *7*, 70 (1986).

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