Racemization of Amino Acid Residues Fused in Thiazoline, Oxazoline, and Imidazoline Rings

Ken YONETANI, Yoshihiro HIROTSU, and Tetsuo SHIBA
Department of Chemistry, Faculty of Science, Osaka University, Toyonaka, Osaka 560
(Received June 9, 1975)

For the purpose of study on racemization of *exo* methine carbon atom of thiazoline, oxazoline or imidazoline ring, the following four compounds were synthesized from amino acid imino ether: 2-(1-benzyloxycarbonylaminoethyl)-2-thiazoline (I), methyl 2-(1-benzyloxycarbonylaminoethyl)-2-thiazoline-4-carboxylate (II), methyl 2-(1-benzyloxycarbonylaminoethyl)-2-oxazoline-4-carboxylate (III), and 2-(1-benzyloxycarbonylaminoethyl)-2-imidazoline (IV). A racemization rate of the alanine residue was measured from an optical rotation of 2,4-dinitrophenyl derivative obtained from the acid hydrolyzate and from hydrogen-deuterium exchange reaction at α-carbon atom. All these compounds were found to be racemized very easily particularly in the presence of proton source. From the experimental results, a plausible mechanism for racemization peculiar to such heterocyclic compounds through protonation-deprotonation process was presented.

Bacitracin A is a peptide antibiotic which involves a unique structure of the thiazoline ring in its molecule. Among peculiar chemical characters of this antibiotic, the racemization at the *N*-terminal isoleucine residue seems to be significantly important, since it is directly related to decrease of the biological activity against microorganisms. Thus, Konigsberg *et al.*[1,2] recognized that a "high potency" form of bacitracin A was converted to a "low potency" form on standing under weak acidic condition and both forms became nearly equal in amounts after two days. This interconversion between the two forms took place even under nearly neutral condition. Meanwhile, it was observed that L-isoleucine of *N*-terminal residue linked to the thiazoline ring in the "high potency" form was isomerized to D-alloisoleucine in the "low potency" one without any change in the rest of the molecule. This epimerization proceeded according to the first-order kinetics in weak acidic media. As pointed out in our previous paper,[3] we postulated that the ease of the isomerization in this case could be attributed to the particular position of the asymmetric center at C-2 *exo* methine of the thiazoline ring.

In the present paper, optical properties of the amino acid components of the peptides containing imidazoline or oxazoline ring as well as the thiazoline ring were investigated in order to clarify the reaction mechanism in more detail. Four model peptides *i.e.*, 2-(1-benzyloxycarbonylaminoethyl)-2-thiazoline (I), methyl 2-(1-benzyloxycarbonylaminoethyl)-2-thiazoline-4-carboxylate (II), methyl 2-(1-benzyloxycarbonylaminoethyl)-2-oxazoline-4-carboxylate (III), and 2-(1-benzyloxycarbonylaminoethyl)-2-imidazoline (IV) were synthesized by iminoether method[4] according to the scheme as outlined in Fig. 1.

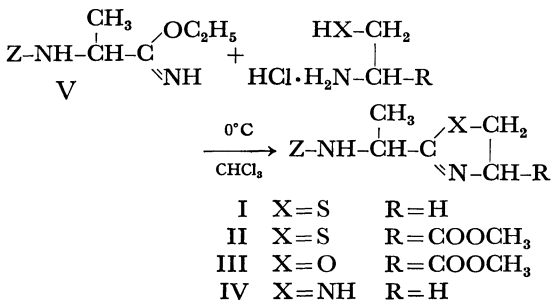

Fig. 1.

The thiazoline derivatives (I and II) were prepared by coupling of iminoether (V) derived from L-alanine with 2-aminoethanethiol hydrochloride and methyl L-cysteinate hydrochloride respectively in chloroform at 0 °C. Similarly, the oxazoline derivative (III) was synthesized by coupling of V with methyl L-serinate hydrochloride. The imidazoline derivative (IV) was also obtained by coupling of V with ethylenediamine in the same solvent at 0 °C.

An optical purity of the alanine residue in each compound was determined by means of the following two methods. First method was based on a measurement of an optical rotation of 2,4-dinitrophenyl (DNP)-alanine. Thus, acid hydrolyzate of the sample given was dinitrophenylated and DNP-alanine was isolated by preparative thin-layer chromatography (tlc). The optical rotation of DNP-alanine thus obtained was measured at 550 nm in aqueous sodium hydrogencarbonate solution and the concentration of the material in the same solution was calculated from UV absorbance at 360 nm compared with those of authentic DNP-L-alanine.[5]

The observed value of the optical purity of the alanine residue in thiazoline derivative (I) was found to be 48% by the DNP-method, whereas the same residue was completely racemized after standing of I at room temperature for a longer period than four weeks or even in the course of its purification procedure either by recrystallization or by silica gel column chromatography. When the oily thiazoline derivative (II) was purified by passing through silica gel column, the alanine residue in this compound was also perfectly racemized. In the case of oxazoline derivative (III), warming at 40 °C for 16 h caused complete racemization at the alanine residue, though the optical purity of this amino acid was maintained as L-form of 98% in the original compound III. When the oily oxazoline (III) was kept at around 20 °C for a couple of weeks, hydrolysis of III occurred by participation of moisture in air and crystals of optically pure benzyloxycarbonyl-alanylserine methyl ester corresponding to the open chain form of III was deposited. This finding may also mean that the racemization proceeded only in the

heterocyclic form. Although imidazoline derivative (IV) was obtained as crystals keeping an optical purity of 78% at the alanine residue, it was most easily racemized even during recrystallization. The racemization degrees in the above discussion were all obtained by the DNP method. However in the case of the compound I or IV where only one asymmetric center was involved, a racemization of the alanine residue can be determined simply by measurement of an optical rotation of the heterocyclic compound itself. Both methods can be applied to follow the progress of the racemization in the time course.

On the other hand, observation of NMR spectra of those compounds (I, II, III, and IV) in 0.5M CD_3OD solution indicated that the intensity of signal for methine proton on the alanine residue gradually decreased, and at the same time, the signal of methyl proton on the same residue changed to singlet from doublet. Hence, a rate of hydrogen-deuterium exchange on the α-carbon atom of the alanine residue could be calculated by comparison of integrated intensities of singlet and doublet signals due to methyl proton.

Discussion

The reaction times ($t_{1/2}$) of racemization and deuterium exchange reaction on the α-carbon atom of the *N*-terminal alanine residue of model peptides (I)—(IV) are summarized in Table 1. The reaction rates for those compounds at 30 °C are shown in Fig. 2.

Table 1. Reaction time ($t_{1/2}$, h) of racemization and deuterium exchange reaction

Compound	10 °C	30 °C	50 °C
I	1000[b]	140[a]	5[b]
		80[b]	
		22[c]	
II	550[b]	62[b]	8[b]
III	1300[b]	176	43
IV	3.2	0.5[a]	

a) Racemization (0.5 M CH_3OH solution). b) D-Exchange (0.5 M CD_3OD solution). c) D-Exchange (0.5 M solution in CD_3OD containing *ca.* 5% D_2O). The values with no mark represent the cases where both times for racemization and D-exchange reaction coincided each other.

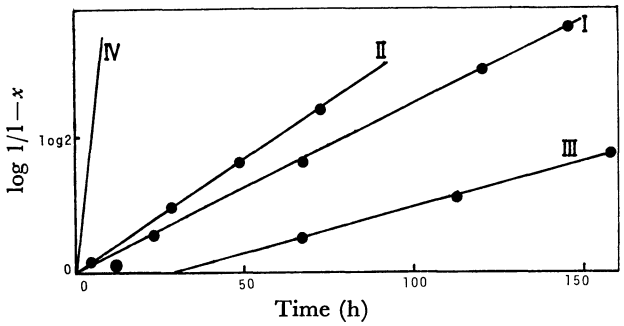

Fig. 2. The rates of racemization or D-exchange reaction at 30 °C.
For the Compound I, only the racemization rate is depicted.

Since an abscissa represents the time on a semilog scale, the straight lines in Fig. 2 indicate first-order kinetics either for racemization or deuterium exchang reaction. From the result in Table 1, it was shown that a higher temperature accelerates the reaction rate on each compound. The rates of racemization on oxazoline (III) and imidazoline (IV) are in agreement with those of deuterium exchange reaction with exception of the case of thiazoline derivative (I). The reaction rates on the compound I were further investigated as shown in Fig. 3. The reaction at 30 °C in CD_3OD

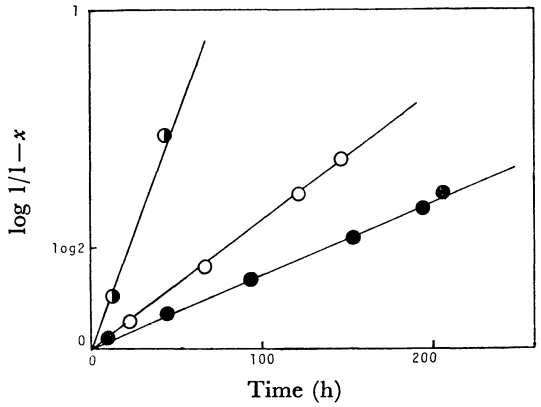

Fig. 3. The rate of racemization or D-exchange reaction in thiazoline compound (I) at 30 °C.
●: Racemization (0.5 M CH_3OH solution).
○: D-Exchange reaction (0.5 M CD_3OD solution).
◑: D-Exchange reaction (0.5 M solution in CD_3OD containing *ca.* 5% D_2O).

was shown to become faster in the presence of D_2O. This indicates that the reaction rate of thiazoline (I) is greately influenced by the presence of a trace amount of water or D_2O in the solvent. This effect may be a reason for discrepancy of reaction rates depending on the measurement condition in the case of the compound (I). Concerning a solvent effect for the racemization, the alanine residue of oxazoline (III) was partly racemized even at the stage of the coupling in methanol. On the other hand, no racemization occurred in chloroform in a preparation of III. This experimental fact suggests that the racemization depends on protic character of the solvent significantly. In this connection, an effect of the solvent was thoroughly investigated on imidazoline (IV) which was racemized most rapidly

Table 2. Racemization time ($t_{1/2}$) of imidazoline (IV) in 0.5 M solution at 30 °C

Solvent	$t_{1/2}$ min
CH_3OH	26
CH_3OH–$CHCl_3$ 1:1	68
$CHCl_3$	*ca.* 1000
$CHCl_3$+0.5 eq. AcOH	26
$CHCl_3$+2.5 eq. AcOH	137
$CHCl_3$+5 eq. AcOH	*ca.* 600
CH_3OH+0.5 eq. AcOH	28
CH_3OH–12 M HCl 1:1	∞
CH_3OH+1 eq. NEt_3	27
$CHCl_3$+1 eq. NEt_3	*ca.* 3000

among four model peptides. The racemization time ($t_{1/2}$) obtained in various media is shown in Table 2. The reaction rate was much faster in methanol as protic solvent than in chloroform as aprotic one. In chloroform containing a little amount of acetic acid, the reaction rate was as fast as in methanol. However, the rate decreased as the acid concentration increased, and racemization did not proceed eventually in strong acid media. From these results, it was proved that a deprotonation at the α-carbon atom of alanine residue is inhibited in acidic solvent, though the racemization requires the presence of proton. Furthermore, it seems unlikely that the racemization proceeds through proton abstraction directly at the α-carbon atom since the reaction was not catalyzed on addition of triethylamine as base. Consequently, the most plausible mechanism through protonated intermediate can be now presented for this racemization as depicted in Fig. 4. It should be

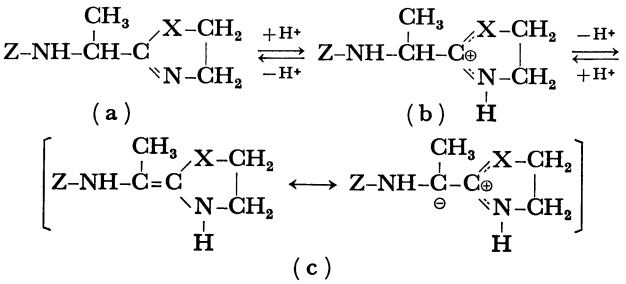

Fig. 4.

emphasized again that racemization and deuterium exchange reaction proceed in almost the same rates. From this fact it is assumed that protonation process from (c) to (b) is very rapid and the rate-determining step is deprotonation step from (b) to (c).[6,7] No effect of the base on racemization can be explained by assumption that either deprotonation from (b) to (a) proceeds predominantly or the protonation process from (a) to (b) might be inhibited. According to this mechanism, the reaction rate must be followed to first-order kinetics for the concentration of cation (b). This assumption is compatible with the results in model peptides I, II, and IV. Only exception is found in the rate of oxazoline (III) which showed the time lag for the reaction initiation. Although this phenomenon can not be elucidated unambiguously yet, an autocatalysis participated by some species formed in the process of deprotonation from (b) to (c) may be considered as one possibility since any protonation step should be rapid and seems to be improbable for explanation of such time lag.

Iminoether (V) used as starting material for the preparation of these model peptides was never racemized when it was treated under any coupling condition nor exchanged with deuterium at all even at 50 °C after 200 h. These results indicate that the racemization reaction is characteristic of heterocyclic structures such as thiazoline, oxazoline, and imidazoline rings. The cation protonated at N-3 of these heterocyclic compounds is stable owing to delocalization of the ring electron. The following deprotonation occurs concertedly with the shift of the inner double bond in the heterorings to the exocyclic position. Consequently, the racemization can take place in cases that the carbon atoms adjacent to C-2 of the heterorings are asymmetric. Therefore, it is useful to employ deuterium exchange as a detection method of racemization in these cases. It should be taken into consideration that racemization may occur always in the synthesis of such a heterocyclic compound, even when it is involved as an intermediate. At the same time, the possibility of electrophilic substitution on the carbanion adjacent to C-2 can be now suggested. For this purpose, the heterocyclic compound derived from glycine may be employed as substrate, and aldehyde or halide as electrophile. One can expect that the coupling product after hydrolysis is β-hydroxy-α-amino acid derivative or α-amino acid derivative respectively. A study along this line is in progress.

In conclusion, we not only clarified the racemization mechanism of heterocyclic compounds derived from amino acids but also pointed out the possibility for a new synthetic method of amino acid.

Experimental

All melting points are uncorrected. Nuclear magnetic resonances spectra were measured on a Varian T-60 spectrometer with tetramethylsilane as an internal standard. Optical rotations were observed with a JASCO ORD/UV-5 spectrophotometer. Ultraviolet spectra were obtained on a Hitachi 124 spectrophotometer. IR spectra were obtained on a JASCO IR-5 spectrophotometer.

Determination of Optical Purity of Alanine Residue. The sample (*ca.* 100 mg) was heated in 6M hydrochloric acid under reflux for 3 h. After evaporation *in vacuo*, the residue was dissolved in water (10 ml). Evaporation of the solvent was repeated. The hydrolyzate thus obtained was dissolved in 5% aqueous sodium hydrogencarbonate (12 ml). To the solution was added 1-fluoro-2,4-dinitrobenzene (*ca.* 200 mg) in 95% ethanol (24 ml). The reaction mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was dissolved in water (200 ml) and washed with ether. The aqueous layer was acidified with 6M hydrochloric acid to Congo red and extracted with ether. The ethereal extract was washed with water, dried over sodium sulfate and evaporated *in vacuo*. The residue was applied to preparative tlc on silica gel H to obtain DNP-alanine which was then dissolved in 1% aqueous sodium hydrogencarbonate (10 ml). The specific optical rotation of the solution was observed at 550 nm and the concentration of the same solution was calculated from the extinction coefficient at 360 nm: authentic DNP-L-alanine: $[\alpha]_{550}^{19}$ +197° (*c* 0.34, 1% $NaHCO_3$); $\lambda_{max}^{1\%\ NaHCO_3}$ 360 nm (ε 1.72×10^4).

2-(1-Benzyloxycarbonylaminoethyl)-2-thiazoline (I). To a solution of L-2-benzyloxycarbonylaminopropioimino ethyl ether[4] (3.00 g, 12 mmol) in chloroform (80 ml) was added 2-aminoethanethiol hydrochloride (1.8 g, 16 mmol) in anhydrous methanol (10 ml) with stirring at 0 °C under nitrogen atmosphere over a period of 2 h. The reaction mixture was stirred for additional 18 h and then concentrated *in vacuo* below 25 °C. The oily residue was dissolved in chloroform, washed with water and dried over magnesium sulfate. Evaporation of the solvent gave an oily material which was crystallized on addition of anhydrous *n*-hexane; yield, 2.06 g (64%); mp 51—53 °C; $[\alpha]_D^{28}$ −6.9° (*c* 1.5, CH_3OH); IR (Nujol): 1630 cm^{-1} (C=N), 1720, 1680 cm^{-1} (C=O); NMR (in $CDCl_3$): δ 7.4 (s, 5H), 5.8 (bd, 1H), 5.1 (s, 2H), 4.6 (q, 1H: J=7 Hz), 4.2 (t, 2H: J=8 Hz), 3.2 (t, 2H, J=8 Hz),

and 1.4 (d, 3H: J=7 Hz). The optical purity of the alanine residue in this material was found to be 84% by DNP method. When this sample was recrystallized from ethyl acetate–*n*-hexane, the obtained crystals were completely racemized: mp 93—95 °C; IR (Nujol): 1630 cm^{-1} (C=N), 1720 cm^{-1} (C=O); λ_{max}^{EtOH} 252 nm (ε 2.9×10³), $\lambda_{max}^{EtOH-12\ M\ HCl}$ 268 nm (ε 6.9×10³). Found: C, 59.08; H, 6.05; N, 10.43; S, 12.15%. Calcd for $C_{13}H_{16}O_2N_2S$: C, 59.06; H, 6.10; N, 10.60; S, 12.13%.

*Methyl 2-(1-Benzyloxycarbonylaminoethyl)-(*R*)-2-thiazoline-4-carboxylate (II).* To a solution of L-2-benzyloxycarbonylaminopropioimino ethyl ether (0.90 g, 3.6 mmol) in chloroform (40 ml) was added methyl L-cysteinate hydrochloride (0.70 g, 4.1 mmol) with stirring at 0 °C under nitrogen atmosphere. After the reaction mixture was stirred for 24 h, it was treated in a similar manner to that in preparation of I. The crude oily product (1.0 g) obtained was dissolved in anhydrous ether (10 ml) and subjected to silica gel (Merck silica gel 0.05—0.2 nm, 30 g) column. The column was eluted with anhydrous *n*-hexane–ether–acetone to give oily but pure II: yield, 0.80 g (69%); $[\alpha]_D^{23}$ +40.8° (*c* 2.7, CH_3OH); IR (Nujol): 1620 cm^{-1} (C=N); NMR (in $CDCl_3$): δ 7.4 (s, 5H), 5.6 (bd, 1H), 5.2 (s, 2H), 5.1 (t, 1H: J=9 Hz), 4.7 (q, 1H: J=7 Hz), 3.8 (s, 3H), 3.6 (d, 2H: J=9 Hz), and 1.5 (d, 3H: J=7 Hz). Found: C, 55.51; H, 5.51; N, 8.62; S, 9.78%. Calcd for $C_{15}H_{18}O_4N_2S$: C, 55.85; H, 5.63; N, 8.69; S, 9.95%. The alanine residue in II was found to be DL-form by DNP method.

*Methyl 2-(1-Benzyloxycarbonylaminoethyl)-(*S*)-2-oxazoline-4-carboxylate (III).* A solution of iminoether (V) (4.80 g, 19 mmol) and methyl L-serinate hydrochloride (3.80 g, 25 mmol) in chloroform (50 ml) was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*. Purification by silica gel column chromatography, gave an oily product III: yield, 4.15 g (72%); $[\alpha]_D^{13}$ +30.0° (*c* 3.3, CH_3OH); IR (Nujol): 1670 cm^{-1} (C=N); NMR (in $CDCl_3$): δ 7.3 (s, 5H), 5.8 (bd, 1H), 5.1 (s, 2H), 4.1—4.9 (m, 4H), 3.7 (s, 3H), and 1.4 (d, 3H: J=7 Hz). Found: C, 58.79; H, 6.07; N, 9.08%. Calcd for $C_{15}H_{18}O_5N_2$: C, 58.81; H, 5.92; N, 9.15%.

An optical purity of the alanine residue in III was shown to be 98% by DNP method. When the oily product III (1.5 g) itself was allowed to stand at room temperature for a couple of weeks in an open vessel, crystals of benzyloxycarbonyl-L-alanyl-L-serine methyl ester were formed. This open chain peptide was recrystallized from ethyl acetate–*n*-hexane: yield, 1.2 g (76%); mp 133—134 °C; $[\alpha]_D^{29}$ −18.9° (*c* 1.1, CH_3OH). (lit,[8]: L-L form: mp 134.5—135.5 °C; $[\alpha]_D^{28}$ −17.7° (*c* 3.43, CH_3OH)). IR (Nujol): 3500 cm^{-1} (OH); NMR (in $CDCl_3$): δ 7.4 (s, 5H), 7.3 (b, 1H), 5.8 (bd, 1H), 5.1 (s, 2H), 4.2—4.8 (m, 2H), 3.9 (d, 2H, J=4 Hz), 3.8 (s, 3H), 3.3 (s, 1H), and 1.4 (d, 3H: J=6 Hz). Found: C, 55.49; H, 6.24; N, 8.59%. Calcd for $C_{15}H_{20}O_6N_2$: C, 55.55; H, 6.22; N, 8.64%.

When the oxazoline (III) was prepared in methanol, an optical purity of the alanine residue was found to be 70—90% by DNP method.

2-(1-Benzyloxycarbonylaminoethyl)-2-imidazoline (IV). To a solution of iminoether (V) (3.60 g, 14.4 mmol) in chloroform (30 ml) was added ethylenediamine (1.10 g, 20 mmol). The reaction mixture was stirred at 0 °C for 158 h and then evaporated *in vacuo* below 25 °C. The residue was readily crystallized on addition of anhydrous ether. The crystals were collected and washed with anhydrous ether: yield, 1.44 g (41%); mp 100—103 °C; $[\alpha]_D^{23}$ −24.9° (*c* 2.1, CH_3OH); IR (Nujol): 1620 cm^{-1} (C=N); NMR (in $CDCl_3$): δ 7.4 (s, 5H), 6.1 (bd, 1H), 5.1 (s, 2H), 4.4 (q, 1H: J=7 Hz), 3.6 (s, 4H), and 1.4 (d, 3H: J=7 Hz).

An optical purity of the alanine residue in VI was found to be 78% by DNP method. On recrystallization from acetone–ether, IV was changed to completely racemized form: mp 111—113 °C. Found: C, 63.11; H, 7.09; N, 16.68%. Calcd for $C_{13}H_{17}O_2N_3$: C, 63.14; H, 6.93; N, 16.99%.

References

1) W. Konigsberg, R. H. Hill, and L. C. Craig., *J. Org. Chem.*, **26**, 3867 (1961).

2) W. Konigsberg and L. C. Craig, *J. Amer. Chem. Soc.*, **81**, 3452 (1959).

3) Y. Hirotsu, T. Shiba, and T. Kaneko, This Bulletin, **43**, 1870 (1970).

4) Y. Hirotsu, T. Shiba, and T. Kaneko, *ibid.*, **40**, 2945 (1967); **43**, 1564 (1970).

5) General and conventional application of combination of the measurements of optical rotation and UV absorbance of DNP amino acid to an evaluation of the optical purity of amino acid in minute amount will be reported soon elsewhere.

6) C. Hsu, C. Ingold, and C. Wilson, *J. Chem. Soc.*, **1938**, 78.

7) D. Cram, B. Bickborn, C. Kingsbury, and P. Haberfield, *J. Amer. Chem. Soc.*, **83**, 3678 (1961).

8) T. Kaneko, I. Takeuchi, and T. Inui, This Bulletin, **41**, 974 (1968).