174 © 1982 *The Chemical Society of Japan* *Bull. Chem. Soc. Jpn.*, ***55***, *174—176 (1982)* [Vol. 55, No. 1

Studies on 2-Aziridinecarboxylic Acid. V.1) Formation of Dehydroamino Acid Containing Peptides *via* the Isomerization by NaI of 2-Aziridinecarboxylic Acid Containing Peptides

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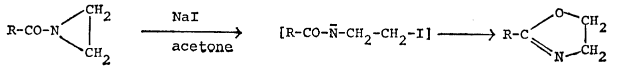
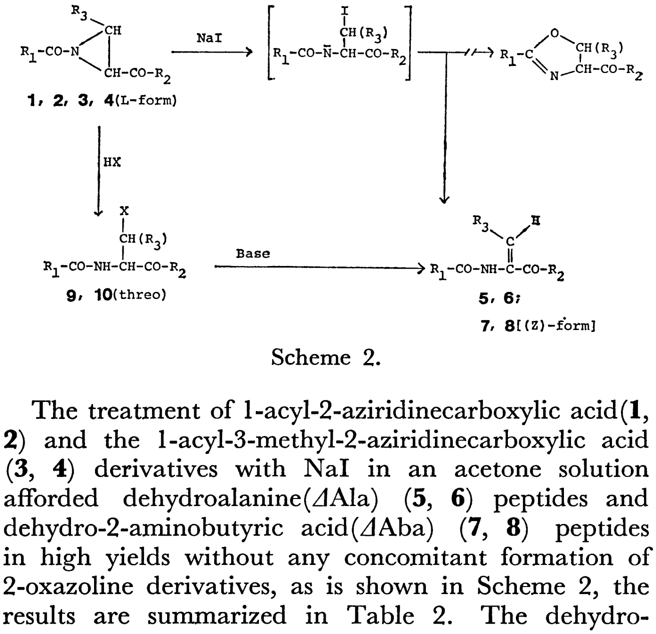
(Received March 25, 1981)

The 2-aziridinecarboxylic acid residue in peptides was isomerized with NaI in an acetone solution into the corresponding dehydroalanine or dehydro-2-aminobutyric acid derivatives, but not into the corresponding 2-oxazoline derivatives.

The isomerization of 1-acyl-2-aziridines into 2-aryl- or 2-alkyl-2-oxazolines by various nucleophiles has been investigated, and its mechanisms(Scheme 1) has been proposed.2) The isomerization of the 2-aziridinecarboxylic acid(Azyline, Azy) residue in peptides was investigated next. Two types of Azy-peptides, *e.g.* 1-acyl-2-aziridinecarboxylic acid(**1**, **2**) and 1-acyl-3-methyl-2-aziridinecarboxylic acid peptides(**3**, **4**), were synthesized according to the procedure described in the previous paper,3) as is summarized in Table 1.



Scheme 1.



Scheme 2.

The treatment of 1-acyl-2-aziridinecarboxylic acid(**1**, **2**) and the 1-acyl-3-methyl-2-aziridinecarboxylic acid (**3**, **4**) derivatives with NaI in an acetone solution afforded dehydroalanine(ΔAla) (**5**, **6**) peptides and dehydro-2-aminobutyric acid(ΔAba) (**7**, **8**) peptides in high yields without any concomitant formation of 2-oxazoline derivatives, as is shown in Scheme 2, the results are summarized in Table 2. The dehydroamino acid function of these products was readily characterized by the presence of vinyl protons in the NMR spectra, as is summarized in Table 3.

In the next approach, we tried to clarify the reaction mechanism of the isomerization of Azy residues and the structure of dehydro-2-aminobutyric acid derivatives. The treatment of 1-acyl-3-methyl-2-aziridinecarboxylic acid(**4**) and 1-acyl-2-aziridinecarboxylic acid peptide(**3**) with hydrogen halide readily afforded L-*threo*-2-amino-3-halogenobutyric acid(**9**, **10**) and β-halogenoalanine derivative(**11**) respectively. These derivatives can be considered as the corresponding intermediates in the isomerization reaction of Azy residues with NaI. The subsequent treatment of these derivatives(**9**, **10**) with a tertiary amine as a base gave the corresponding dehydroamino acid peptide(**8**), which were identical with the products obtained by the isomerization of the Azy peptides with NaI in an acetone solution. The results of the dehydrogen halide reaction are also summarized in Table 2.

Based on the above data, the mechanism of the isomerization of the Azy residue upon NaI treatment was thought to be the *trans*-E_2-elimination of a 2-amino-3-iodobutyric acid intermediate, followed by the direct elimination of an α-hydrogen atom. The NMR data of the dehydro-2-aminobutyric acid derivatives indicated that both the isomerization of Azy and the dehydrogen halide reaction of L-*threo*-2-amino-3-halogenobutyric acid [Thr(Cl) or Thr(I)] derivatives occurred *via* the stereospecific *trans*-E_2-elimination reaction. Thus, only dehydro-2-aminobutyric acid derivatives of the (*Z*)-geometory were prepared.

Experimental

Uncorrected melting points are reported. The homogeneity of the products was checked by thin-layer chromatography on silica-gel plates. The optical rotations were determined at the D line on a Perkin-Elmer 141 polarimeter. The NMR spectra were obtained with a Hitachi R-20B High-resolution NMR Spectrometer, the chemical shifts being obtained using TMS as the internal reference.

All the Azy peptides used in this study were synthesized by the method described in the literature;3) their properties and yields are summarized in Table 1.

*Z–Gly–ΔAla–Gly–OBzl(**5**).* Via *the Isomerization of Azy*: A solution of Z–Gly–L-Azy–Gly–OBzl(**1**) (200 mg, 0.47 mmol) and NaI (282 mg, 1.88 mmol) in acetone (5 ml) was refluxed for 2 h. After the solvent has been removed *in vacuo*, the residue was extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried over Na_2SO_4, and then concentrated *in vacuo*. The residue was crystallized from hexane; 154 mg (78.2%); mp 134—135 °C. NMR(DMSO-d_6) δ: 5.55 (1H m, $=CH_2$), 6.20 (1H m, $=CH_2$), 9.08 (1H bs, –NH–C=). Found: C, 61.53; H, 5.50; N, 10.04%. Calcd for $C_{22}H_{23}O_6N_3\cdot 1/2H_2O$: C, 60.82; H, 5.57; N, 9.63%.

*Z–L-Ala–ΔAla–Gly–OBzl(**6**).* Via *the Isomerization of Azy*: A solution of Z–L-Ala–L-Azy–Gly–OBzl(**2**) (200 mg, 0.46 mmol) and NaI (273 mg, 1.82 mmol) in acetone (5 ml) was refluxed for 7 h. Then the reaction mixture was worked-up as has been described above. The product was crystallized from ethyl acetate–hexane; 142 mg (71.2%); mp 128—131 °C, $[\alpha]^{25}_{D}$ −8.8 (*c* 1.0, DMF). NMR (DMSO-d_6) δ: 5.49 (1H m, $=CH_2$), 6.38 (1H d, J=2.0 Hz, $=CH_2$), 8.50

Table 1. Properties and yields of Azy peptides

Azy peptide	Yield/%[a]	Mp/°C	$[\alpha]^{23}_{D}$
Z-Gly-L-Azy-Gly-OBzl (**1**)	92.3	118—119	−52.5 (*c* 1.0, MeOH)
Z-L-Ala-L-Azy-Gly-OBzl (**2**)	83.0	128—128.5	−71.2 (*c* 1.0, $CHCl_3$)
Z-Gly-3-MeAzy-Gly-OBzl (**3**)	89.0	123.5—124.5	−58.2 (*c* 1.0, AcOEt)
Z-L-Ala-3-MeAzy-Gly-OBzl (**4**)	87.5	110—111	−59.7 (*c* 1.0, $CHCl_3$)

a) Coupled by the DCC method between Z-amino acid and Azy peptides.

Table 2. Properties and yields of dehydroamino acid derivatives

Peptide	Reagent	Product	React. time/h	Yield/%	Mp/°C	$[\alpha]^{23}_{D}$ (*c* 1.0, DMF)
Z-Gly-L-Azy-Gly-OBzl (**1**)	NaI	5	2[a]	78	134—135	—
Z-L-Ala-L-Azy-Gly-OBzl (**2**)	NaI	6	7[a]	71	128—131	−8.8
Z-Gly-3-MeAzy-Gly-OBzl (**3**)	NaI	7	5[a]	78	102—104	—
Z-L-Ala-3-MeAzy-Gly-OBzl (**4**)	NaI	8	2[a]	92	141—143	−8.7
Z-L-Ala-L-Thr(Cl)-Gly-OBzl (**9**)[d]	Dabco[c]	8	12	100	139—141	−9.0
Z-L-Ala-L-Thr(I)-Gly-OBzl (**10**)[e]	Dabco[c]	8	20[b]	92	141—142	−8.0

a) The reaction was carried out under refluxing in an acetone solution. b) 20 min. c) Dabco: 1,4-diazabicyclo-[2.2.2]octane. d) L-Thr(Cl): L-*threo*-2-amino-3-chlorobutyric acid. e) L-Thr(I): L-*threo*-2-amino-3-iodobutyric acid.

Table 3. NMR data of dehydroamino acid derivatives

Dehydroamino acid Peptide	NMR (δ, DMSO-d_6) =CH_2		-CH_3	-NH-
Z-Gly-ΔAla-Gly-OBzl (**5**)[a]	5.55 (m)	6.20 (m)	—	9.08 (bs)
Z-L-Ala-ΔAla-Gly-OBzl (**6**)[a]	5.49 (m)	6.38 (d)	—	8.50 (bs)
Z-Gly-ΔAba-Gly-OBzl (**7**)[b]	—	6.42 (q)	1.61 (d)	9.01 (bs)
Z-L-Ala-ΔAba-Gly-Obzl (**8**)[b]	—	6.44 (q)	1.60 (d)	9.08 (bs)

a) ΔAla: Dehydroalanine. b) ΔAba: (*Z*)-dehydro-2-aminobutyric acid.

(1H bs, -NH-CH=). Found: C, 62.76; H, 5.82; N, 9.70%. Calcd for $C_{23}H_{25}O_6N_3$: C, 62.86; H, 5.73; N, 9.56%.

Z-Gly-(Z)-ΔAba-Gly-OBzl (7). Via *the Isomerization of Azy*: A solution of Z-Gly-3-MeAzy-Gly-OBzl (**3**) (150 mg, 0.34 mmol) and NaI (205 mg, 1.36 mmol) in acetone (5 ml) was refluxed for 5 h. Then the reaction mixture was worked up as has been described above. The product was crystallized from ethyl acetate-hexane; 117 mg (78%); mp 102—104 °C. NMR (DMSO-d_6) δ: 1.61 (3H d, J=7.0 Hz, =$CHCH_3$), 6.42 (1H q, J=7.0 Hz, =CH-), 9.01 (1H bs, -NH-C=). Found: C, 61.94; H, 6.04; N, 9.73%. Calcd for $C_{23}H_{25}O_6N_3 \cdot 1/2H_2O$: C, 61.60; H, 5.84; N, 9.37%.

Z-L-Ala-(Z)-ΔAba-Gly-OBzl (8). Via *the Isomerization of Azy*: A solution of Z-L-Ala-3-MeAzy-Gly-OBzl (**4**) (150 mg, 0.33 mmol) and NaI (200 mg, 1.32 mmol) in acetone (5 ml) was refluxed for 2 h. Then the reaction mixture was worked up as has been described above. The product was crystallized from ethyl acetate-hexane; 139.6 mg (92.4%); mp 141—143 °C, $[\alpha]^{23}_{D}$ −8.7 (*c* 1.0, DMF). NMR (DMSO-d_6) δ: 1.60 (3H d, J=7.0 Hz, =$CHCH_3$), 6.44 (1H q, J=7.0 Hz, =CH-), 9.08 (1H bs, -NH-C=). Found: C, 63.25; H, 5.93; N, 9.01%. Calcd for $C_{24}H_{27}O_6N_3$: C, 63.56; H, 6.00; N, 9.27%.

Via *the β-Elimination of β-Halogeno Derivative*: *i*) A solution of Z-L-Ala-L-Thr(Cl)-Gly-OBzl (**9**) (150 mg, 0.3 mmol) and 1,4-diazabicyclo[2.2.2]octane (Dabco, 67 mg, 0.6 mmol) in ethyl acetate was stirred overnight at room temperature, and then water was added to the reaction mixture. The ethyl acetate solution was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was crystallized from ethyl acetate-ether-hexane; 136 mg (100%); mp 139—141 °C, $[\alpha]^{25}_{D}$ −9.0 (*c* 1.1, DMF).

ii) A solution of Z-L-Ala-L-Thr(I)-Gly-OBzl (**10**) (174 mg, 0.3 mmol) and Dabco (67 mg, 0.6 mmol) in ethyl acetate (10 ml) was stirred for 20 min at room temperature. The product was obtained as has been described above; 125 mg (91.9%); mp 141—142 °C, $[\alpha]^{25}_{D}$ −8.0 (*c* 1.0, DMF). The NMR data of these products were identical to those of the product (**8**) obtained *via* the isomerization of Azy.

Z-L-Ala-L-Thr(Cl)-Gly-OBzl (9): To a solution of Z-L-Ala-3-MeAzy-Gly-OBzl (**4**) (454 mg, 1 mmol) in acetone (10 ml) was added 1 M hydrochloric acid[†] (1 ml, 1 mmol) at 0 °C. After the reaction mixture had then been allowed to stand at 0 °C for 2 h, the precipitated crystals were filtered off, washed with water, and recrystallized from $CHCl_3$-ether; 478 mg (97.6%); mp 138—141 °C, $[\alpha]^{24}_{D}$ −1.8 (*c* 1.1, DMF). Found: C, 56.90; H, 5.51; N, 7.90; Cl, 6.70%. Calcd for $C_{24}H_{30}O_7N_3Cl$: C, 56.75; H, 5.95; N, 8.27; Cl, 6.98%.

Z-L-Ala-L-Thr(I)-Gly-OBzl (10): To a solution of Z-L-Ala-3-MeAzy-Gly-OBzl (**4**) (454 mg, 1 mmol) in acetone (10 ml) was added 1 M hydriodic acid (1 ml, 1 mmol) at 0 °C. After the reaction mixture had been allowed to stand at 0 °C for 30 min, water was added; then the precipitated crystals were filtered off, washed with water, and recrystallized from $CHCl_3$-ether; 567 mg (97.5%); mp 136—138 °C, $[\alpha]^{24}_{D}$ −10.4 (*c* 1.0, DMF). Found: C, 48.39; H, 4.83; N, 6.97; I, 20.86%. Calcd for $C_{24}H_{30}O_7N_3I$: C, 48.09; H, 5.04; N, 7.01; I, 21.17%.

Z-Gly-L-Ser(Cl)-Gly-OBzl (11): To a solution of Z-

[†] 1 M=1 mol dm^{-3}.

Gly–L-Azy–Gly–OBzl (**1**) (425 mg, 1 mmol) in acetone (10 ml) was added 1 M hydrochloric acid (1 ml, 1 mmol) at 0 °C. After the reaction mixture had been allowed to stand at 0 °C for 1 h, water was added; then the precipitated crystals were filtered off, washed with water, and recrystallized from $CHCl_3$–ether–hexane; 522 mg (100%); mp 143—145 °C, $[\alpha]_D^{24}$ −9.0 (*c* 1.0, DMF). Found: C, 55.39; H, 4.83; N, 8.97; Cl, 7.86%. Calcd for $C_{22}H_{24}O_6N_3Cl$: C, 55.52; H, 5.08; N, 8.83; Cl, 7.45%.

References

1) Part IV: T. Tanaka, K. Nakajima, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **53**, 1352 (1980). The abbreviations of the IUPAC–IUB commission (*J. Biol. Chem.*, **247**, 977 (1972)) are used. Z: benzyloxycarbonyl, OBzl: benzyl ester, "Azyline" is used as name of an L-2-aziridinecarboxylic acid, "Azy" being its abbreviation. 3-MeAzy: (2*S*,3*S*)-3-methyl-2-aziridinecarboxylic acid, ΔAla: dehydroalanine, ΔAba: dehydro-2-aminobutyric acid, Thr(Cl): *threo*-2-amino-3-chlorobutyric acid, Thr(I): *threo*-2-amino-3-iodobutyric acid, Ser(Cl): 2-amino-3-chloropropionic acid.

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