

solvent. Recrystallization from EtOH gave II (190 mg) as colorless leaflets. mp 210—212°.  $[\alpha]_D^{18} + 3.6^\circ$  ( $c=0.14$ ,  $\text{CHCl}_3$ ). *Anal.* Calcd. for  $\text{C}_{39}\text{H}_{49}\text{O}_{10}\text{N}$ : C, 67.71; H, 7.14; N, 2.02. Found: C, 67.49; H, 7.42; N, 2.24. NMR (5% solution in  $\text{CDCl}_3$ )  $\delta$ : 0.68 (3H, s, 18- $\text{CH}_3$ ), 3.72 (1H, d,  $J=5$  cps, 17 $\beta$ -H), 4.12 (2H, nm,  $W_{1/2}=7.2$  cps, 6'-H), 4.62 (1H, d,  $J=7.8$  cps, 1'-H), 4.95 (2H, s,  $\text{C}_6\text{H}_5\text{CH}_2$ -), 7.27 (5H, s,  $\text{C}_6\text{H}_5\text{CH}_2$ -).

**3-Hydroxyestra-1,3,5(10)-trien-17 $\alpha$ -yl-2'-acetamido-2'-deoxy-3',4',6'-tri-O-acetyl- $\beta$ -D-glucopyranoside (IIIa)**—A solution of II (163 mg) in AcOEt (10 ml) was shaken with 5% Pd/C (100 mg) overnight under a stream of  $\text{H}_2$  gas at room temperature. After removal of the catalyst by filtration the filtrate was concentrated *in vacuo* to give a crystalline product. Recrystallization from acetone-hexane gave IIIa (151 mg) as colorless needles. mp 245—246°.  $[\alpha]_D^{20} - 42.7^\circ$  ( $c=0.12$ , MeOH). *Anal.* Calcd. for  $\text{C}_{32}\text{H}_{43}\text{O}_{10}\text{N}$ : C, 63.88; H, 7.20; N, 2.33. Found: C, 63.91; H, 7.14; N, 2.49. NMR (5% solution in  $\text{CDCl}_3$ )  $\delta$ : 0.67 (3H, s, 18- $\text{CH}_3$ ), 4.13 (2H, nm,  $W_{1/2}=7$  cps, 6'-H), 4.47 (1H, d,  $J=8$  cps, 1'-H).

**3-Acetoxyestra-1,3,5(10)-trien-17 $\alpha$ -yl-2'-acetamido-2'-deoxy-3',4',6'-tri-O-acetyl- $\beta$ -D-glucopyranoside (IIIb)**—IIIa (25 mg) was treated with pyridine and  $\text{Ac}_2\text{O}$  at room temperature overnight. Usual work-up followed by recrystallization from acetone-hexane gave IIIb (27 mg) as colorless needles. mp 145—147°.  $[\alpha]_D^{19} - 13.6^\circ$  ( $c=0.11$ ,  $\text{CHCl}_3$ ). *Anal.* Calcd. for  $\text{C}_{34}\text{H}_{45}\text{O}_{11}\text{N}$ : C, 63.44; H, 7.05; N, 2.17. Found: C, 63.35; H, 7.08; N, 2.23. NMR (5% solution in  $\text{CDCl}_3$ )  $\delta$ : 0.70 (3H, s, 18- $\text{CH}_3$ ), 3.73 (1H, d,  $J=5$  cps, 17 $\beta$ -H), 4.17 (2H, nm,  $W_{1/2}=7$  cps, 6'-H), 4.66 (1H, d,  $J=7.5$  cps, 1'-H).

**3-Hydroxyestra-1,3,5(10)-trien-17 $\alpha$ -yl-2'-acetamido-2'-deoxy- $\beta$ -D-glucopyranoside (IV)**—To a solution of IIIa (57 mg) in MeOH (3 ml) was added 1N methanolic KOH (0.35 ml) and allowed to stand at room temperature overnight. To the resulting solution was added Amberlite IR-120 H-form (*ca.* 1 ml) and stirred for 10 min. The reaction mixture was filtered and washed with MeOH. The filtrate and washings were combined and concentrated *in vacuo*. Recrystallization of the crude product from aq. MeOH gave IV (40 mg) as colorless needles. mp 170—177°.  $[\alpha]_D^{19} - 20.0^\circ$  ( $c=0.10$ , MeOH). *Anal.* Calcd. for  $\text{C}_{26}\text{H}_{37}\text{O}_7\text{N} \cdot \frac{1}{2}\text{H}_2\text{O}$ : C, 64.44; H, 7.90; N, 2.89. Found: C, 64.51; H, 8.27; N, 2.98. TLC (Silica gel G) *Rf*: 0.35 (AcOEt/MeOH (5:1)), 0.61 ( $\text{CHCl}_3/\text{EtOH}$  (7:3)). IR spectra and TLC comparison with the metabolite isolated from the rabbit urine<sup>3)</sup> showed the identity of two samples.

**Acknowledgement** The authors are grateful to Prof. D.S. Layne, Ottawa University, for generous gift of precious sample and comparison data. They are also indebted to all the staffs of central analytical laboratory of this Institute for elemental analyses and spectral measurements.

[Chem. Pharm. Bull.]  
[19(10)2185—2189(1971)]

UDC 547.466.1.057 : 547.96.057

## Studies on Peptides. XXXIII.<sup>1,2)</sup> $\text{N}^\epsilon$ - $\beta,\beta,\beta$ -Trichloroethyloxycarbonyllysine

HARUAKI YAJIMA, HIDEHIKO WATANABE and MICHIKO OKAMOTO

*Faculty of Pharmaceutical Sciences, Kyoto University<sup>3)</sup>*

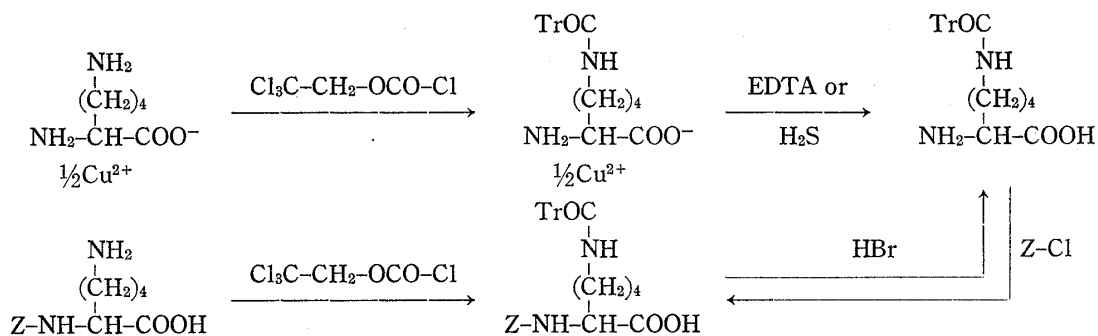
(Received May 10, 1971)

The  $\beta,\beta,\beta$ -trichloroethyloxycarbonyl (TrOC) group was first introduced by Woodward, *et al.*<sup>4)</sup> as an amino protecting group in the synthesis of cephalosporin C. Later Windholz and Johnston<sup>5)</sup> studied its applicability in organic synthesis. This masking group is resistant to the action of HCl in dioxane or trifluoroacetic acid (TFA) but can be removed under very mild conditions by treatment with Zn in acetic acid or boiling methanol for a short period.

- 1) Part XXXII: H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), 19, 1905 (1971).
- 2) Amino acids, peptides and their derivatives mentioned in this communication are of the L-configuration. Abbreviations used are those recommended by IUPAC-IUB Commission of Biochemistry Nomenclature in July, 1965 and July 1966; *Biochemistry*, 5, 2485 (1966); *ibid.*, 6, 362 (1967). Z(OMe) = *p*-methoxybenzyloxycarbonyl, Z = benzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, OBzl = benzyl ester.
- 3) Location: *Sakyo-ku, Kyoto*.
- 4) R.B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranganathan and H. Vorbrüggen, *J. Am. Chem. Soc.*, 88, 852 (1966).
- 5) T.B. Windholz and D.B.R. Johnston, *Tetrahedron Letters*, 1967, 2555.

Taking advantage of this unique property of the protecting group, we have recently prepared TrOC-hydrazide and applied it to the synthesis of Z(OMe)-Glu(OBzl)-NHNH<sub>2</sub> and Z(OMe)-Asp(OBzl)-NHNH<sub>2</sub>.<sup>6)</sup> The way of synthesizing peptide hydrazides containing Glu(OBzl) or Asp(OBzl) was thus introduced.

In the present investigation, we have prepared N<sup>ε</sup>-β,β,β-trichloroethyloxycarbonyllysine [H-Lys(TrOC)-OH] and examined its application to the peptide synthesis. The TrOC group was introduced to the ε-amino function of lysine through its Cu-chelate<sup>7)</sup> with trichloroethyl chloroformate<sup>5)</sup> in the presence of sodium carbonate. The use of a strong base, such as sodium hydroxide, should be avoided, since this chloroformate has a tendency to form a bis-compound, bis-(trichloroethyl)-carbonate. The resulting Cu complex of H-Lys(TrOC)-OH was then treated with either disodium ethylenediamine tetraacetate (EDTA) or H<sub>2</sub>S. H-Lys(TrOC)-OH thus obtained is a crystalline compound which can be recrystallized from 30% acetic acid. This compound could be prepared alternatively by the reaction of trichloroethyl chloroformate with Z-Lys-OH<sup>8)</sup> followed by debenzoyloxycarbonylation with HBr in acetic acid. The intermediate, Z-Lys(TrOC)-OH, was prepared also by benzyloxycarbonylation of H-Lys(TrOC)-OH and characterized as its dicyclohexylamine (DCHA) salt.



Besides Z-derivative of H-Lys(TrOC)-OH, two other N<sup>α</sup>-protected derivatives, Z(OMe)-Lys(TrOC)-OH and Boc-Lys(TrOC)-OH, were prepared by the azidoformate procedure.<sup>9)</sup>

As an example, Z-Lys(TrOC)-OH was condensed with H-Ala-Gly-OMe by dicyclohexylcarbodiimide (DCC) to give Z-Lys(TrOC)-Ala-Gly-OMe. Exposure of this protected tripeptide ester to the action of sodium hydroxide or hydrazine hydrate gave Z-Lys(TrOC)-Ala-Gly-OH or Z-Lys(TrOC)-Ala-Gly-NHNH<sub>2</sub> respectively. The TrOC group which was once introduced to the amino function seems to be stable under basic conditions required frequently in the peptide synthesis. In order to examine the stability of the TrOC group to the action of HF,<sup>10)</sup> Z-Lys(TrOC)-Ala-Gly-OH was treated with HF at 0° for 30 min. No degradation of the TrOC group occurred and H-Lys(TrOC)-Ala-Gly-OH was obtained in quantitative yield. Alternatively, this partially protected tripeptide was obtained after treatment of the same starting material, Z-Lys(TrOC)-Ala-Gly-OH, with HBr in AcOH. Treatment of <sup>3</sup>H-Lys(TrOC)-Ala-Gly-OH with Zn in methanol followed by column chromatography on carboxy methyl cellulose (CMC) afforded the free tripeptide, H-Lys-Ala-Gly-OH, in an analytically pure form.

From the above experiments, the stability of the TrOC group to the action of strong acids, such as HBr or HF, was confirmed. Although the earlier investigators mentioned that

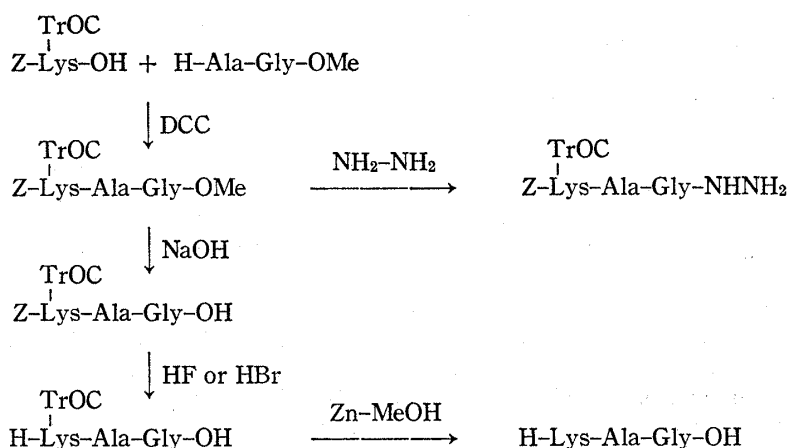
6) H. Yajima and Y. Kiso, *Chem. Pharm. Bull.* (Tokyo), **19**, 420 (1971).

7) A. Neuberger and F. Sanger, *Biochem. J.*, **37**, 515 (1943).

8) B. Bezas and L. Zervas, *J. Am. Chem. Soc.*, **83**, 719 (1961).

9) L.A. Carpino, *J. Am. Chem. Soc.*, **79**, 4427 (1957); L.A. Carpino, B.A. Carpino, P.J. Crowley, C.A. Giza and P.H. Terry, *Org. Synth.*, **44**, 15 (1964); F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).

10) S. Sakakibara and Y. Shimonishi, *Bull. Chem. Soc. Japan*, **38**, 1412 (1965); S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada and H. Sugihara, *ibid.*, **40**, 2164 (1967).



this masking group was not affected by exposure to hydrogen in the presence of Pd,<sup>5)</sup> when H-Lys(TrOC)-OH was placed over a Pd catalyst in acetic acid, formation of H-Lys-OH was noted by thin-layer chromatography. Aside from this discrepancy, this group attached at the  $\epsilon$ -amino function of H-Lys-OH seems to survive under various conditions employed in the synthesis of peptides. Further application of H-Lys(TrOC)-OH to the peptide synthesis will be reported in future.

### Experimental

General experimental methods employed are essentially the same as described in the Part XXII<sup>11)</sup> of this series. On thin-layer chromatography (Kiesel gel G, Merck), *R<sub>f</sub>* values refer to the following solvent systems; *R<sub>f1</sub>*: CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (40:15:5), *R<sub>f2</sub>*: *n*:BuOH-AcOH-pyridine-H<sub>2</sub>O (4:1:1:2). For detection of N<sup>α</sup>-protected derivatives on thin-layer chromatography, 27% HBr in AcOH and ninhydrin were sprayed and the plate was heated in an oven at 80° for 20 min.

**H-Lys(TrOC)-OH**—a) According to Neuburger and Sanger,<sup>7)</sup> a solution of H-Lys-OH monohydrochloride (9.14 g) in H<sub>2</sub>O (180 ml) was heated in the presence of basic copper carbonate (21.60 g) under reflux for 3 hr and then the solution was filtered. To the filtrate containing the Cu-chelate of H-Lys-OH, TrOC-Cl (15.90 g) prepared according to Windholz and Johnston<sup>5)</sup> (bp 111–117°/131 mmHg, lit.<sup>5)</sup> bp 75–76°/60 mmHg) and a solution of Na<sub>2</sub>CO<sub>3</sub> (13.30 g) in H<sub>2</sub>O (40 ml) were added alternatively during a period of 30 min and the mixture was stirred in an ice-bath for 3 hr. The blue powder formed during the reaction was collected by filtration; yield 15.29 g (87%). a-1) The Cu-complex obtained above was suspended in a solution of EDTA (16.10 g) in H<sub>2</sub>O (180 ml) and the mixture was heated at 80° for 3 hr until the blue-colored complex disappeared. The solution was chilled with ice. The crystalline product was collected by filtration and recrystallized from 30% AcOH; yield 12.40 g (78%), mp 218–220°, [ $\alpha$ ]<sub>D</sub><sup>20</sup> +13.9° (*c*=0.2, 50% AcOH). *R<sub>f2</sub>*: 0.76. *Anal.* Calcd. for C<sub>9</sub>H<sub>15</sub>O<sub>4</sub>N<sub>2</sub>Cl<sub>3</sub>: C, 33.6; H, 4.7; N, 8.7. Found: C, 33.4; H, 4.8; N, 8.6. a-2) Through a boiling solution of the Cu-complex of H-Lys(TrOC)-OH, prepared as described above (1.35 g) in H<sub>2</sub>O (20 ml), H<sub>2</sub>S was bubbled for 3 hr and the hot solution was filtered to remove CuS. The filtrate was condensed *in vacuo* and the residue was recrystallized from 30% AcOH; yield 1.04 g (64%), mp 217–220°, *R<sub>f2</sub>*: 0.75.

b) Z-Lys(TrOC)-OH, obtained as described below, (0.42 g) was treated with 27% HBr in AcOH (3 ml) at room temperature for 60 min and the solvent was evaporated *in vacuo*. The residue was dissolved in a small amount of H<sub>2</sub>O and the pH of the solution was adjusted to 8 with 5% NH<sub>4</sub>OH. The fine precipitate thus obtained was collected by filtration and recrystallized from 30% AcOH; yield 0.22 g (76%), mp 218–220°. *R<sub>f2</sub>*: 0.76. IR spectra of the product was identical with those obtained in a-1.

**Bis-(Trichloroethyl) Carbonate**—To a solution of Cu-complex of H-Lys-OH (prepared from 1.83 g of the hydrochloride), 1N NaOH (25 ml) and TrOC-Cl (3.18 g) were added alternatively and stirring was continued in an ice-bath for 2 hr. The precipitate formed during the reaction was collected by filtration (2.70 g) and then treated with EDTA (0.7 g) in H<sub>2</sub>O (8 ml) as described above. When the resulting crude product was recrystallized from 30% AcOH, and insoluble compound was isolated; 0.88 g, mp 88–91°. IR: 1765 cm<sup>-1</sup> (-CO-). *Anal.* Calcd. for C<sub>5</sub>H<sub>4</sub>O<sub>3</sub>Cl<sub>6</sub>: C, 18.5; H, 1.2. Found: C, 18.7; H, 1.2. From the above 30% AcOH, H-Lys(TrOC)-OH was obtained; yield 1.68 g (52%), mp 212–215°.

**Z-Lys(TrOC)-OH·DCHA Salt**—a) To a solution of H-Lys(TrOC)-OH (2.60 g) in ice-cooled 1N NaOH (8 ml), Z-Cl (2.70 g) and 1N NaOH (16 ml) were added alternatively. The solution was stirred in an ice-bath for 3 hr and then washed with ether. The aqueous phase was acidified with 5N HCl and the resulting precipi-

11) H. Yajima, Y. Okada, H. Kawatani and N. Mizokami, *Chem. Pharm. Bull.* (Tokyo), **17**, 1229 (1969).

pitape was extracted with AcOEt, which after washing with a solution of NaCl, was dried over  $\text{Na}_2\text{SO}_4$  and then evaporated to give an oily residue; yield 3.10 g (84%).  $R_{f_1}$  0.44,  $R_{f_2}$  0.87. In the usual manner, the oily product was converted to the corresponding DCHA salt, which was recrystallized from MeOH and ether; mp 162—165°,  $[\alpha]_D^{25} + 2.9^\circ$  ( $c=1.1$ , MeOH). *Anal.* Calcd. for  $\text{C}_{17}\text{H}_{21}\text{O}_6\text{N}_2\text{Cl}_3 \cdot \text{C}_{12}\text{H}_{23}\text{N}$ : C, 54.7; H, 7.0; N, 6.6. Found: C, 54.8; H, 7.0; N, 6.8.

b) Z-Lys-OH (3.19 g), prepared according to Bezas and Zervas,<sup>9</sup> was dissolved in 2N NaOH (5.6 ml). To this ice-cooled solution, TrOC-Cl (3.59 g) and 1N  $\text{Na}_2\text{CO}_3$  (20 ml) were added and the mixture was stirred in an ice-bath for 2 hr. The solution was washed with ether and then acidified with 1N HCl. The resulting precipitate was isolated as described above; yield 4.70 g (89%).  $R_{f_1}$ : 0.44,  $R_{f_2}$ : 0.87.

**Z(OMe)-Lys(TrOC)-OH·DCHA Salt**—To a solution of H-Lys(TrOC)-OH (2.50 g) and  $(\text{C}_2\text{H}_5)_3\text{N}$  (2.2 ml) in  $\text{H}_2\text{O}$  (10 ml), *p*-methoxybenzyl azidoformate (1.70 g) in dioxane (10 ml) was added and the mixture was stirred at room temperature for 24 hr. The solvent was evaporated and the residue was acidified with 10% citric acid and the resulting precipitate was extracted with AcOEt, which was washed with  $\text{H}_2\text{O}$ , dried over  $\text{Na}_2\text{SO}_4$  and then evaporated to give an oily product. It was dissolved in ether and converted to the corresponding DCHA Salt, which was recrystallized from MeOH and ether; yield 2.62 g (52%), mp 138—140°,  $[\alpha]_D^{25} + 4.2^\circ$  ( $c=1.0$ , MeOH). *Anal.* Calcd. for  $\text{C}_{18}\text{H}_{23}\text{O}_7\text{N}_2\text{Cl}_3 \cdot \text{C}_{12}\text{H}_{23}\text{N}$ : C, 54.0; H, 7.0; N, 6.3. Found: C, 53.8; H, 6.9; N, 6.1.

**Boc-Lys(TrOC)-OH**—To a solution of H-Lys(TrOC)-OH (3.22 g) and  $(\text{C}_2\text{H}_5)_3\text{N}$  (3.1 ml) in  $\text{H}_2\text{O}$  (15 ml), *tert*-butyl azidoformate (1.77 g) in dioxane (15 ml) was added and the mixture was stirred at room temperature for 48 hr. The solvent was evaporated and to the residue,  $\text{H}_2\text{O}$  and ether were added. The oily material was separated in the aqueous phase. The entire aqueous phase containing the oily precipitate was then acidified with 10% citric acid. The resulting precipitate was extracted with AcOEt, which after washing with  $\text{H}_2\text{O}$ , was dried over  $\text{Na}_2\text{SO}_4$  and then evaporated to give an oily residue. Treatment of the residue with petroleum ether afforded the crystalline mass; yield 4.40 g (99%), mp 94—98°,  $[\alpha]_D^{25} - 9.8^\circ$  ( $c=1.0$ , MeOH). *Anal.* Calcd. for  $\text{C}_{14}\text{H}_{23}\text{O}_6\text{N}_2\text{Cl}_3$ : C, 39.9; H, 5.5; N, 6.6. Found: C, 39.7; H, 5.7; N, 6.6.

**Z-Lys(TrOC)-Ala-Gly-OMe**—DCC (2.26 g) was added to a mixture of Z-Lys(TrOC)-OH (4.70 g), HCl·H-Ala-Gly-OMe (2.15 g), prepared from the corresponding Z-derivative<sup>12</sup> by catalytic hydrogenation, and  $(\text{C}_2\text{H}_5)_3\text{N}$  (1.4 ml) in DMF (20 ml). After the solution was stirred at room temperature for 18 hr, the solvent was evaporated and the residue was dissolved in AcOEt, which was washed successively with 2N HCl, 10%  $\text{Na}_2\text{CO}_3$  and  $\text{H}_2\text{O}$  (saturated with NaCl), dried over  $\text{Na}_2\text{SO}_4$  and then evaporated. Addition of ether to the residue afforded solid, which was recrystallized from AcOEt and ether; yield 3.82 g (64%), mp 105—107°.  $[\alpha]_D^{25} - 23.1^\circ$  ( $c=0.7$ , MeOH).  $R_{f_1}$ : 0.85. *Anal.* Calcd. for  $\text{C}_{23}\text{H}_{31}\text{O}_8\text{N}_4\text{Cl}_3$ : C, 46.2; H, 5.2; N, 9.4. Found: C, 46.4; H, 5.3; N, 9.3.

**Z-Lys(TrOC)-Ala-Gly-OH**—To a solution of Z-Lys(TrOC)-Ala-Gly-OMe (3.11 g) in MeOH (20 ml), 1N NaOH (10.4 ml) was added and the solution was stirred at room temperature for 30 min. The solution was neutralized with AcOH and the solvent was evaporated. The residue was again dissolved in 5%  $\text{Na}_2\text{CO}_3$ . The aqueous phase, after washing with AcOEt, was acidified with 2N HCl and the resulting precipitate was extracted with AcOEt, which was washed with  $\text{H}_2\text{O}$  (saturated with NaCl), dried over  $\text{Na}_2\text{SO}_4$  and then evaporated. Addition of petroleum ether to the residue gave solid, which was recrystallized from AcOEt and petroleum ether; yield 2.41 g (80%), mp 125—126°,  $[\alpha]_D^{25} - 3.3^\circ$  ( $c=0.8$ , MeOH).  $R_{f_1}$ : 0.20. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{29}\text{O}_8\text{N}_4\text{Cl}_3$ : C, 45.3; H, 5.0; N, 9.6. Found: C, 45.3; H, 5.3; N, 9.7.

**Z-Lys(TrOC)-Ala-Gly-NHNH<sub>2</sub>**—To a solution of Z-Lys(TrOC)-Ala-Gly-OMe (0.60 g) in MeOH (2 ml), 80%  $\text{NH}_2\text{NH}_2$  hydrate (0.6 ml) was added. The gelatinous mass formed on standing was collected and recrystallized from hot MeOH; yield 0.52 g (86%), mp 112—115°.  $R_{f_2}$ : 0.52. *Anal.* Calcd. for  $\text{C}_{22}\text{H}_{31}\text{O}_7\text{N}_6\text{Cl}_3 \cdot 1/2 \text{H}_2\text{O}$ : C, 43.5; H, 5.3; N, 13.9. Found: C, 43.3; H, 5.3; N, 14.6.

**H-Lys(TrOC)-Ala-Gly-OH**—a) Z-Lys(TrOC)-Ala-Gly-OH (1.0 g) was treated with HF (approximately 10 ml) in the presence of anisole (1.0 ml) in an ice-bath for 30 min. After evaporation of the HF *in vacuo*, the residue was dissolved in  $\text{H}_2\text{O}$  (20 ml), which was washed with ether and then treated with Amberlite CG-4B (acetate cycle, approximately 5 g) for 30 min. The resin was removed by filtration and the filtrate was condensed. Addition of EtOH to the residue afforded solid, which was recrystallized from  $\text{H}_2\text{O}$  and EtOH; yield 0.71 g (92%), mp 202—203°.  $R_{f_1}$ : 0.03,  $R_{f_2}$ : 0.56.  $[\alpha]_D^{25} + 7.0^\circ$  ( $c=0.6$ , 10% AcOH). *Anal.* Calcd. for  $\text{C}_{14}\text{H}_{23}\text{O}_6\text{N}_4\text{Cl}_3$ : C, 37.4; H, 5.2; N, 12.5. Found: C, 37.4; H, 5.4; N, 12.3.

b) Z-Lys(TrOC)-Ala-Gly-OH (1.17 g) was treated with 25% HBr in AcOH (2.5 ml) at room temperature for 60 min and dry ether was added. The resulting precipitate was purified by treatment with Amberlite CG-4B and recrystallization from  $\text{H}_2\text{O}$  and EtOH as stated above; yield 0.56 g (62%), mp 202—203°. Identity of the products obtained in a) and b) was established by comparison of their  $R_f$  values and mixed mp.

**H-Lys-Ala-Gly-OH**—To a solution of H-Lys(TrOC)-Ala-Gly-OH (0.90 g) in 70% aqueous MeOH (14 ml), Zn powder (2 g) was added. After the solution was heated under reflux for 3 hr, the solution was filtered and the filtrate was evaporated.  $R_{f_2}$  0.13 and 0.56 (faint spot). The residue was dissolved in  $\text{H}_2\text{O}$  (200 ml) and the solution was applied to a column of CMC (3 × 27 cm), which was washed with  $\text{H}_2\text{O}$

12) N. F. Albertson and F. C. Kay, *J. Am. Chem. Soc.*, **75**, 5323 (1953).

(1100 ml). Gradient elution was then established by addition of 0.05M, pH 6.0 pyridine acetate buffer (1500 ml) through a mixing flask containing H<sub>2</sub>O (300 ml). Individual fractions of 10 ml each were collected. The ninhydrin positive fractions in the gradient eluate (tube 51—121) were collected and the solvent was evaporated. Addition of EtOH to the residue afforded powders; yield 0.34 g (63%), mp 217—220°,  $[\alpha]_D^{19} - 7.7^\circ$  ( $c=0.3$ , 10% AcOH).  $Rf_2$  0.13. Amino acid ratios in an acid hydrolysate Lys<sub>1.01</sub>Ala<sub>1.00</sub>Gly<sub>1.00</sub> (average recovery 87%). *Anal.* Calcd. for C<sub>11</sub>H<sub>22</sub>O<sub>4</sub>N<sub>4</sub>·CH<sub>3</sub>COOH: C, 46.7; H, 7.8; N, 16.8. Found: C, 46.3; H, 7.9; N, 17.1.

**Acknowledgement** The authors are indebted to Prof. S. Uyeo for his encouragement during the course of this investigation. The authors also wish to express their deep appreciation to Dr. M. Fujino and Mr. M. Nishimura of Takeda Research Laboratory for elemental analysis. They wish to extend their sincere appreciation to Dr. T.Y. Liu of Brookhaven National Laboratory for his advice in the preparation of this manuscript.

[Chem. Pharm. Bull.]  
19(10)2189—2192(1971)

UDC 547.963.32.04 : 546.133.1.04

## Reaction of Sodium Hypochlorite with Nucleic Acids and Their Constituents

HIKOYA HAYATSU, SHOE-KUNG PAN and TYUNOSIN UKITA

*Faculty of Pharmaceutical Sciences, University of Tokyo<sup>1)</sup>*

(Received June 2, 1971)

Sodium hypochlorite has been used as a disinfectant of drinking water. Bacteria and viruses are rapidly killed in hypochlorite solutions containing several-tenth parts per million (ppm) available chlorine. In order to elucidate the mechanism of the disinfection by hypochlorite, it seems important to know how nucleic acid molecules react with hypochlorite, since the mechanism still remains obscure.<sup>2)</sup> Such knowledge may also enable one to evaluate potential mutagenic toxicity of this chemical.

Little has been reported concerning the chemical reaction between nucleic acids and aqueous chlorine. Prat, *et al.*<sup>3)</sup> have isolated 5-chlorinated pyrimidines from an acid-hydrolyzate of bacteria that had been treated with hypochlorite. In this communication we wish to report a preliminary survey of the reaction of sodium hypochlorite with purines and pyrimidines constituting DNA and RNA.

Calf thymus DNA and yeast transfer RNA were treated with sodium hypochlorite at pH 7 and 37°, and the changes in their ultraviolet absorptions at 260 m $\mu$  were followed as a function of time of the treatment. As Fig. 1 shows, rapid decreases in the absorbance of both DNA and RNA were observed even with 10 ppm chlorine. On treatment with 100 ppm chlorine for 1 hr, the absorbance of either of DNA and RNA decreased to about half of the original value. No significant difference in the reaction rate was observed between double-stranded and heat-denatured DNA's. This indicates that a higher order structure does not protect the DNA from the attack of the agent. The decrease in the absorbance must have resulted from the breakdown of the purine and/or pyrimidine bases constituting the nucleic acids. A similar experiment was carried out using nucleotide or nucleoside as the substrate of the reaction, and the results are summarized in Table I. All of the bases re-

1) Location: *Bunkyo-ku, Tokyo.*

2) L. Friberg and E. Hammarström, *Acta Path. Microb. Scand.*, **38**, 127 (1956); L. Friberg, *ibid.*, **38**, 135 (1956); A.M. Cook and W.R.L. Brown, *J. Pharm. Pharmacol.*, **16**, 611 (1964); M.A. Benarde, W.B. Snow, V.P. Olivieri, and B. Davidson, *Appl. Microbiol.*, **15**, 257 (1967).

3) R. Prat, C. Nofre, and A. Cier, *Compt. Rend.*, **260** (18) (Groupe 13), 4859 (1965); *idem*, *Ann. Inst. Past.*, **114**, 595 (1968).