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122. **Morizo Ishidate, Yoshio Sakurai, and Isao Aiko :** Studies on Carcinostatic Substances. XXX.[*1] Syntheses of Derivatives of Nitrogen Mustard related to Amino Acid.

(*Iatrochemical Institute of Pharmacological Research Foundation*[*2]
and Yoshitomi Pharmaceutical Co., Ltd.[*3])

Recently, Izumi[1] reported on the preparation and properties of racemic N,N-bis(2-chloroethyl)-α-amino acids, which were proved to be potent in retarding the growth of Yoshida sarcoma or several rat ascites hepatomas. They however did not seem to maintain their concentration *in vivo* for a long time and observable effect on the tumor cells in the ascites was rather shorter than that of N-methyl-bis(2-chloroethyl)amine (HN_2) (No. 24).

It was therefore of interest to seek other kinds of more effective derivatives related to amino acid and this report dealts with the preparation of the new homologs of N,N-bis(2-chloroethyl)-α-amino acid and N^{ω},N^{ω}-bis(2-chloroethyl)-DL-ornithine (No. 536) and -DL-lysine (No. 539).

In 1954, Bergel, *et al.*[2] stated that L-isomer of *p*-[bis(2-chloroethyl)amino]phenylalanine exhibited a stronger anti-tumor activity than its D-isomer against the Walker rat carcinoma, while one of the present authors[3] could not find any difference in the activities of *d*- and *l*-2-[bis(2-chloroethyl)amino]propionamide (No. 244).

The fact seemed to suggest that the one optical isomer of 2-chloroethyl compounds of amino acid derivatives having at least one unsubstituted amino group at α-position might have a particular biological affinity to the malignant tissue than its antipode and this supposition led to the preparation of the afore-stated derivatives of diamino acids.

The preparation of these compounds was carried out by 2-hydroxyethylation of ω-amino-N^2-benzoylamino acid with ethylene oxide and the succeeding chlorination with thionyl chloride, or by 2-hydroxyethylation of the copper salt of α,ω-diamino acid and the succeeding benzoylation followed by treatment with thionyl chloride. Benzoyl group at N^2-position was hydrolyzed by hydrochloric acid at the final stage of procedure.

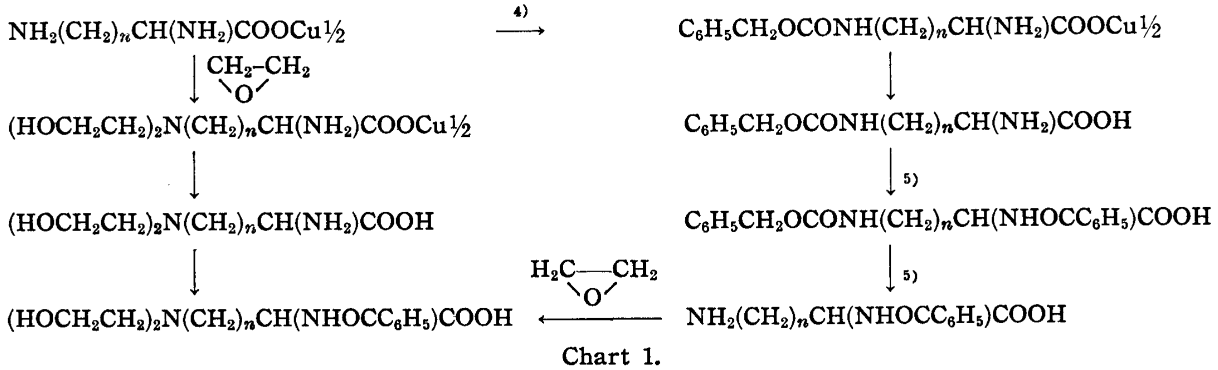


Chart 1.

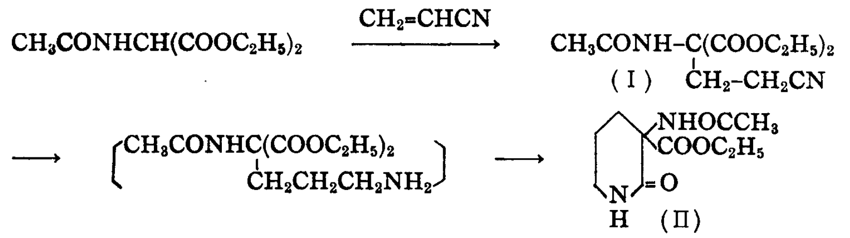
[*1] Part XXIX. This Bulletin, 8, 543(1960).
[*2] 26 Nishigahara 1-chome, Kita-ku, Tokyo (石舘守三, 桜井欽夫).
[*3] Yoshitomi-machi, Chikujo-gun, Fukuoka-ken (愛甲軍雄).
1) M. Izumi : This Bulletin, 2, 275(1954).
2) F. Bergel, J. A. Stock : J. Chem. Soc., **1954**, 2409.
3) I. Aiko, T. Gōno : This Bulletin, 5, 487(1938).
4) I. Schumann, R. A. Boissonnas : Helv. Chim. Acta, **35**, 2237(1952).
5) M. Bergmann, L. Zervas, W. F. Ross : J. Biol. Chem., **111**, 249(1935).

N^{ω},N^{ω}-Bis(2-hydroxyethyl)-N^2-benzoyl-lysine and -ornithine were prepared by the process shown in Chart 1.

Fischer[6] roported that N^2-benzoyllysine was obtained by partial hydrolysis of N^2,N^6-dibenzoyllysine, but there is still left a question whether it was really N-benzoyl derivative or not, because it was found in the present experiment that the dibenzoyl derivatives always yielded the N^{ω}-monobenzoyl by partial hydrolysis under various reaction conditions.

Furthermore, a reaction of N^2-benzoyllysine with phosphotungstic acid, which was used by Fischer for its identification, seemed not to be exclusively characteristic to N^2-benzoyl derivative alone.

In 1945, Albertson, *et al.*[7] reported that ethyl 2-oxo-3-acetamido-3-piperidinecarboxylate (II) was obtained by catalytic reduction of diethyl 2-acetamido-2-(2-cyanoethyl)-malonate (I) over Raney nickel.



$$CH_3CONHCH(COOC_2H_5)_2 \xrightarrow{CH_2=CHCN} \underset{\text{(I)}}{CH_3CONH\text{-}\underset{CH_2\text{-}CH_2CN}{C}(COOC_2H_5)_2} \longrightarrow \left[CH_3CONH\underset{CH_2CH_2CH_2NH_2}{C}(COOC_2H_5)_2 \right] \longrightarrow \text{(II)}$$

Expecting that the ester or amide of N^2-acetylornithine might be obtained by controlling the reaction condition, Albertson's experiment was repeated with some modifications, but it always resulted in the formation of a cyclized product alone, even when it was treated at low temperature or ethyl 2-acetamido-2-carbamoyl-4-cyanobutyrate was used instead as the starting material.

An attempt to prepare 2-amino-3-[bis(2-chloroethyl)amino]propionic acid by the Mannich reaction with nitromalonate, formaldehyde, and bis(2-chloroethyl)amine was not successful. Though the primary condensed product was obtained in fairly good yield, it decomposed during the succeeding reduction of its nitro group and hydrolysis of the ester. In another case, starting from acetamidomalonate or its hemiester, the Mannich reaction itself did not seem to proceed.

About higher homologs of N,N-bis(2-chloroethyl)glycine, N,N-bis(2-chloroethyl)-DL-alanine (No. 300) was already reported by Izumi.[1] Attempt was made but in vain to prepare some other homologs by the Strecker reaction, because acetone, benzaldehyde, and butyraldehyde gave no definite condensation products with bis(2-chloroethyl)amine and sodium cyanide. Only 2-[bis(2-chloroethyl)amino]butyronitrile was obtained by a similar procedure, but it did not go further than the amide by hydrolysis of its nitrile group and decomposed into the original components if it was treated under a more drastic conditions.

Another type of homolog which had the substituted amino group at 3-position was synthesized by the route shown in Chart 2.

The process (B) was applied with success to preparation of 4-[bis(2-chloroethyl)amino]-butyric acid (No. 529), using 4-chlorobutyronitrile in place of 3-chloropropionitrile.

However, it was not possible to prepare higher homologs of this type, viz. N-substituted 5-aminovaleric acid or N-substituted 6-aminocaproic acid, through a couple of reactions of the corresponding δ- or ε-amino acids, through 2-hydroxyethylation followed by chlorination, because of the difficulty in purification and crystallization of the product of the first reaction.

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6) E. Fischer : Ber., **35**, 3776(1902).
7) N.F. Albertson, S. Archer : J. Am. Chem. Soc., **67**, 2043(1945).

$$\text{(A)}\quad \underset{\text{(III)}}{(HOCH_2CH_2)_2NH} \xrightarrow{CH_2{=}CHCN} \underset{\text{(IV)}}{(HOCH_2CH_2)_2NCH_2CH_2CN^{8)}}$$

$$\longrightarrow \underset{\text{(No. 306)}}{(ClCH_2CH_2)_2NCH_2CH_2CN} \longrightarrow \underset{\text{(No. 301)}}{(ClCH_2CH_2)_2NCH_2CH_2CONH_2}$$

$$\longrightarrow \underset{\text{(No. 300)}}{(ClCH_2CH_2)_2NCH_2CH_2COOH}$$

$$\text{(B)}\quad \underset{\text{(III)}}{(HOCH_2CH_2)_2NH} \xrightarrow{ClCH_2CH_2CN} \underset{\text{(IV)}}{(HOCH_2CH_2)_2NCH_2CH_2CN}$$

$$\text{(C)}\quad (ClCH_2CH_2)_2NH + HCHO + CH_2(COOH)_2 \longrightarrow \underset{\text{(No. 300)}}{(ClCH_2CH_2)_2NCH_2CH_2COOH}$$

Chart 2.

Table I.

No.	Compound	m.p. (C°)	Biological effect[c] (mg./kg., i.p.) LD$_{50}$	MTD	MED	MEC
536	$(ClCH_2CH_2)_2N(CH_2)_3CH(NH_2)COOH$	108[d]	30	10	0.5	1×10^{-2}
539	$(ClCH_2CH_2)_2N(CH_2)_4CH(NH_2)COOH$	181～182[d][b]	3.0	1.0	0.5	1×10^{-3}
470	$(ClCH_2CH_2)_2NCH_2C(NO_2)(COOC_2H_5)_2$	119	175	100	10	
243	$(ClCH_2CH_2)_2NCH(CH_3)COOH$[a]	97[f]	30	10	0.05	1×10^{-2}
244	$(ClCH_2CH_2)_2NCH(CH_3)CONH_2$[a]	190[f]	3.0	1.0	0.01	5×10^{-3}
438	$(ClCH_2CH_2)_2NCH(C_2H_5)CONH_2$	155～156[f]	3.0	1.0	0.05	2.5×10^{-3}
300	$(ClCH_2CH_2)_2N(CH_2)_2COOH$	155～156[f]	7.5	5.0	0.1	5×10^{-2}
301	$(ClCH_2CH_2)_2N(CH_2)_2CONH_2$	208～209[e]	3.0	1.0	0.5	1×10^{-3}
768	$(ClCH_2CH_2)_2N(CH_2)_2COOC_2H_5$	122～123[f]				
306	$(ClCH_2CH_2)_2N(CH_2)_2CN$	95～97[f]	3.0	1.0	0.5	1×10^{-3}
529	$(ClCH_2CH_2)_2N(CH_2)_3COOH$	81～83[e]	3.0	1.0	0.1	
473	$(Cl\underset{CH_3}{C}HCH_2)_2NCH_2COOH$	194～195[e]	175	100	10	
453	$(ClCH_2CH_2)(Cl\overset{CH_3}{C}HCH_2)NCH_2COOH$	85～86[f][b]	30	10	0.1	1×10^{-1}
447	$(ClCH_2CH_2)(Cl\underset{CH_3}{C}HCH_2)NCH_2CONH_2$	160～161[f]	3.0	1.0	0.05	

a) Izumi's preparations.[1]
b) Monohydrate.
c) LD$_{50}$, maximum tolerance dose (MTD), and minimum effective dose (MED) on the Yoshida sarcoma rats. Minimum effective concentration (MEC) on the same tumor cultured *in vitro*.
d) Picrylsulfonate hydrochloride.
e) Picrylsulfonate.
f) Hydrochloride.

Anti-tumor activity against rats bearing Yoshida sarcoma or the same tumor cells cultured *in vitro* is given in Table I.

From the point of the ratio of acute toxicity (LD$_{50}$) to minimum effective dose (MED), both of bis(2-chloroethyl) derivatives of α,ω-diamino acid were not comparable to N,N-bis(2-chloroethyl)-DL-alanine (No. 300), although they were of course fully effective on this tumor either *in vivo* or *in vitro*. Resolution of the acids into optical isomers has not yet been effected.

The comparative biological inertness of the compound No. 470 seemed due to the -I effect of both nitro and ethoxycarbonyl groups which should hinder its immonium formation.

As for the derivatives of monoamino acid, it was of interest that the compounds Nos. 300 and 529 had a strong effect and toxicity rather close to that of HN$_2$. From this fact, it could be understood that carboxyl group located at β- or γ-position from nitrogen,

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8) F.C. Whitmore, *et al.*: J. Am. Chem. Soc., **66**, 725(1944).

different to that at α-position as in case of the compound No. 243, had far less influence upon the reactivity of 2-chloroethyl group. Table II shows comparison of the rates of hydrolysis and alkylation of these compounds *in vitro* which were determined by the method previously described.[9]

TABLE II.

No.	Compound	Cl^- liberation[a] (%)	H^+ liberation[b] (%)	$S_2O_3^{2-}$ uptake[c] (%)
24	$CH_3N(CH_2CH_2Cl)_2$	83	25	76
243	$(ClCH_2CH_2)_2NCH(CH_3)COOH$	77	86	16
300	$(ClCH_2CH_2)_2N(CH_2)_2COOH$	81	61	47

Incubation at 37°, 1 hr.

a) Sample, 0.1 m.mole/cc.; $NaHCO_3$, 0.8 m.mole/cc.

b) Sample, 0.1 m.mole/cc.; H^+ liberation rate was calculated from titration value of 0.1*N* NaOH necessary to maintain the solution continuously at pH 8.3.

c) Sample, 0.1 m.mole/cc.; $Na_2S_2O_3$, 0.2 m.mole/cc.; $NaHCO_3$, 0.8 m.mole/cc.

As seen in Table II, it was obvious that the chemical reactivity of No. 300 is between those of No. 243 and HN_2. Judging from the fact the compound No. 529 showed higher toxicity and smaller chemotherapeutic index (LD_{50}/MED) than No. 300, further attempt to prepare other higher homologs of these compounds was abandoned.

Looking through these compounds in the list, however, it is noted that some of the derivatives of carbonamide, Nos. 438, 447, and 244, exhibited a remarkably small MED and a fairly large chemotherapeutic index. Supported by this experience, preparation of simple peptides of N,N-bis(2-chloroethyl)amino acids was planned, and is now in progress.

Experimental

3-[Bis(2-chloroethyl)amino]propionitrile Hydrochloride (No. 306)—By chlorination with $SOCl_2$ of the crude 3-[bis(2-hydroxyethyl)amino]propionitrile (IV) which was prepared by cyanoethylation of diethanolamine (III) with acrylonitrile.[7] m.p. 95~97° (Me_2CO-EtOH). *Anal.* Calcd. for $C_7H_{13}N_2Cl_3$: C, 36.29; H, 5.66. Found: C, 35.98; H, 5.37.

Picrate: m.p. 154~156°. *Anal.* Calcd. for $C_{13}H_{15}O_7N_5Cl_2$: C, 36.81; H, 3.56; N, 16.51. Found: C, 36.69; H, 3.48; N, 16.28.

3-[Bis(2-chloroethyl)amino]propionamide Hydrogensulfate (No. 301)—Eighty grams of No. 306 was added into 200 g. of H_2SO_4 (90%) and, after being kept overnight at room temperature, the mixture was poured onto crushed ice. No. 301 separated instantly as white crystals, m.p. 173~174° (MeOH). Yield, 57%. *Anal.* Calcd. for $C_7H_{16}O_5N_2Cl_2S$: C, 27.02; H, 5.18; N, 9.00. Found: C, 26.80; H, 5.45; N, 8.74.

Picrylsulfonate: m.p. 208~209° (decomp.). Hydrochloride: m.p. 213°.

Ethyl 3-[Bis(2-chloroethyl)amino]propionate Hydrochloride (No. 768)—A solution of 20 g. of No. 306 or No. 301 dissolved in 180 cc. of EtOH was saturated with dry HCl gas. After being kept for 48 hr. at room temperature, the separated NH_4Cl was filtered off and the filtrate was evaporated to dryness. The residue was again dissolved in dehyd. Me_2CO, filtered, and the filtrate was evaporated. The residue was recrystallized from 99% EtOH; m.p. 122~123°. *Anal.* Calcd. for $C_9H_{18}O_2NCl_3$: C, 38.82; H, 6.52; N, 5.03. Found: C, 38.78; H, 6.45; N, 4.81.

3-[Bis(2-chloroethyl)amino]propionic Acid Hydrochloride (No. 300)—i) Into an aqueous solution of malonic acid (12 g. in 40 cc.), bis(2-chloroethyl)amine hydrochloride (18 g.) and 25.5% formaldehyde were added in drops. The reaction started immediately without heating and the mixture was kept standing overnight at room temperature. No. 300 separated as picrate from the reaction mixture and then converted to its hydrochloride.

ii) By hydrolysis of No. 306 with conc. HCl. m.p. 155~156° (90% Me_2CO). *Anal.* Calcd. for $C_7H_{12}O_2NCl_3$: C, 33.54; H, 5.63; N, 5.59. Found: C, 33.71; H, 5.90; N, 5.64.

The hydrochlorides obtained by the two different processes were quite identical and the mixed m.p. showed no depression.

Picrate: m.p. 135~137° (99% EtOH). *Anal.* Calcd. for $C_{13}H_{16}O_9N_4Cl_2$: C, 35.34; H, 3.63; N, 12.64. Found: C, 35.59; H, 4.12; N, 12.41.

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[9] I. Aiko, *et al.*: Yakugaku Zasshi, **72**, 1297 (1952).

4-[Bis(2-hydroxyethyl)amino]butyronitrile—Liquid, b.p$_4$ 205~215°. Yield, 55.5%.

4-[Bis(2-chloroethyl)amino]butyronitrile Picrate—By chlorination of the above nitrile with $SOCl_2$ in $CHCl_3$. m.p. 86~87°(MeOH). *Anal.* Calcd. for $C_{14}H_{17}O_7N_5Cl_2$: C, 38.37; H, 3.91. Found : C, 38.56; H, 3.71.

4-Bis[2-chloroethyl)amino]butyric Acid Picrylsulfonate (No. 529)—One part of the above nitrile was dissolved in 5 parts of conc. HCl and heated at 50~60° for 3 hr. The mixture was evaporated *in vacuo* and the residue was dissolved in MeOH. The insoluble part was removed by filtration and the filtrate was evaporated. The residue was then crystallized as picrylsulfonate, m.p. 81~82°. *Anal.* Calcd. for $C_{14}H_{18}O_{11}N_4Cl_2S$: C, 32.25; H, 3.48; N, 10.75. Found : C, 32.50; H, 3.68; N, 10.55.

2-[Bis(2-chloroethyl)amino]butyronitrile Hydrochloride—Prepared according to the procedure for the preparation of 2-[bis(2-chloroethyl)amino]propionitrile described by Izumi.[1]

Neither its hydrochloride nor picrate could be obtained in crystalline form and the crude nitrile hydrochloride was used as such in the following reaction.

2-[Bis(2-chloroethyl)amino]butyramide Hydrochloride (No. 438)—The above nitrile hydrochloride (10 g.) was added into H_2SO_4(conc. H_2SO_4 20 g. + H_2O 0.85 g.) under chilling and, after being kept overnight at room temperature, it was poured into ice-water (50 g.) under stirring. The solution was neutralized with $NaHCO_3$ and extracted with benzene. Dry HCl gas was then passed through the dried extract and the separated oily hydrochloride was added with AcOEt. White crystals of the subject compound was obtained and recrystallized from a mixture of EtOH and AcOEt; m.p. 155~156°. *Anal.* Calcd. for $C_8H_{17}ON_2Cl_3$: C, 36.45; H, 6.50, N, 10.63. Found : C, 36.48; H, 6.41; N, 10.54.

2-[N-(2-Chloroethyl)-2-chloropropylamino]acetamide Hydrochloride (No. 447)—The compounds Nos. 447, 453, and 473 were prepared by the Strecker reaction.[1] m.p. 160~161°(EtOH-AcOEt). *Anal.* Calcd. for $C_7H_{15}ON_2Cl_3$: C, 33.68; H, 6.06; N, 11.23. Found : C, 33.75; H, 6.07; N, 11.01.

N-(2-Chloroethyl)-N-(2-chloropropyl)glycine Hydrochloride Monohydrate (No. 453)—m.p. 85~86° (Me_2CO-AcOEt). *Anal.* Calcd. for $C_7H_{10}O_3NCl_3$: C, 31.29; H, 6.00; N, 5.21. Found : C, 31.06; H, 5.86; N, 5.15.

N,N-Bis(2-chloropropyl)glycine Picrylsulfonate (No. 473)—m.p. 194~195°(MeOH). *Anal.* Calcd. for $C_{14}H_{18}O_{11}N_4Cl_2S$: C, 32.25; H, 3.48. Found : C, 32.74; H, 4.12.

Diethyl 2-[Bis(2-chloroethyl)aminomethyl]-2-nitromalonate (No. 470)—37% Formaldehyde solution (19 g.) was added in drops under stirring into an Et_2O solution of diethyl nitromalonate (48 g.) and a solution of 20 g. of bis(2-chloroethyl)amine dissolved in 50 cc. of Et_2O was added slowly at 0°. The crystals separated after the mixture was kept standing for 1 week in an ice-bath. m.p. 119°(MeOH). *Anal.* Calcd. for $C_{12}H_{20}O_6N_2Cl_2$: C, 40.12; H, 5.61; N, 7.80. Found : C, 39.86; H, 5.71; N, 7.66.

Acetamidomalonamide—Diethyl acetamidomalonate (106 g.) was added into 455 cc. of conc. NH_4OH saturated with liq. NH_3 at 5°. The mixture was left at room temperature for 20 hr. in a sealed glass bottle. The separated crystals were collected, m.p. 207°(decomp.) (EtOH). Yield. 67.5 g. *Anal.* Calcd. for $C_5H_9O_3N_3$: C, 37.73; H, 5.70. Found : C, 38.03; H, 5.94.

2-Acetamido-2-(2-cyanoethyl)malonamide—Acrylonitrile (35 g.) was dropped into a mixture of the above malonamide (66 g.), 40% Triton B (benzyltrimethylammonium hydroxide) (8.3 g.), and water (355 cc.) at 35~38° under stirring. After the stirring was continued for 1 hr. at the same temperature, the mixture was filtered, neutralized exactly to pH 7, and chilled; m.p. 195°(EtOH). Yield, 76 g. *Anal.* Calcd. for $C_8H_{12}O_3N_4$: C, 45.28; H, 5.70. Found : C, 45.31; H, 5.90.

2-Oxo-3-acetamido-3-piperidinecarboxamide—A mixture of 2-acetamido-2-(2-cyanoethyl)malonamide (31.8 g.), Raney Ni (about 6 g.), and dehyd. MeOH (1600 cc.) was shaken in an autoclave with H_2 (73 atm.) at 65~70° for 3 hr. The catalyst was removed and the solvent was distilled off; m.p. 220° (decomp.) (H_2O). *Anal.* Calcd. for $C_8H_{13}O_3N_3$: C, 48.23; H, 6.58; N, 21.09. Found : C, 48.25; H, 6.73; N, 21.43.

When the procedure was repeated at room temperature instead of 70°, the same product alone was isolated.

DL-Ornithine Hydrochloride—i) The above carboxamide (20 g.) was refluxed with conc. HCl for 4 hr. The mixture was evaporated *in vacuo* to dryness and the residue was dissolved in EtOH (140 cc.). The insoluble substance was filtered off and the filtrate was added with 30% NH_4OH (140 cc.). The mixture was kept cooled in ice water for 1 hr. and the separated crystals were washed twice with cold 50% EtOH. The crystals were then suspended in EtOH (130 cc.), boiled for several minutes, and filtered. The crystals were collected and washed twice with EtOH (20 cc. each); m.p. 225°. Yield, 15 g.

ii) The same product was also obtained by the same procedure starting from ethyl 2-oxo-3-acetamido-3-piperidinecarboxylate (II).[6]

The two samples prepared from the different starting materials were proved to be identical by mixed m.p. determination or by paper chromatography (Rf. 0.1) using phenol saturated with 0.1% NH_4OH solution.

Copper Salt of DL-Ornithine—A mixture of DL-ornithine hydrochloride (14 g.), CuO powder (6.6

g.), and water (70 cc.) was heated for 1 hr. The deep blue solution was filtered and the filtrate was added with 1 mol. equiv. of NaOH solution. This was used as such for the following reaction.

2-Amino-5-[bis(2-hydroxyethyl)amino]valeric Acid—Ethylene oxide (14.6 g.) was bubbled through an aqueous solution of DL-ornithine copper salt, prepared from DL-ornithine hydrochloride (14 g.) by the above-described procedure, under stirring at 23～30°. The mixture was left standing overnight and then distilled *in vacuo* to remove excess ethylene oxide. The solution was acidified with HCl and H_2S was passed through it. After removal of CuS by filtration, the filtrate was neutralized with NaOH solution and evaporated *in vacuo*. The residue was extracted with MeOH to remove NaCl and the extract was again evaporated to dryness. The procedure was repeated once again. The final residue was a brown syrup, which was easily soluble in water, MeOH, and AcOH, soluble in EtOH, and sparingly soluble in Me_2CO and AcOEt. The crude product was used as such for the following reaction, because of difficulty in purification.

2-Benzamido-5-[bis(2-hydroxyethyl)amino]valeric Acid—The above compound was benzoylated using 1.3 mol. equiv. of BzCl according to the Shotten-Baumann method at pH range of 8.6～9.0 and below 30°. The reaction was continued for 1 hr. under severe stirring and then the mixture was made acid to Congo red. After removal of the separated BzOH by filtration, the filtrate was extracted with Et_2O and the residue, obtained by evaporation of water layer below 40°, was dissolved in EtOH. After removal of NaCl by fltration, the filtrate was again evaporated *in vacuo*. The crude syrupy residue was used for the next reaction without purification.

2-Amino-5-[bis(2-chloroethyl)amino]valeric Acid Picrylsulfonate Hydrochloride (No. **536**)—$SOCl_2$ (45 g.) was dropped under shaking at 40° into the $CHCl_3$ solution of the above compound (36 g. in 140 cc.). The temperature was gradually raised to the boiling point and refluxing was continued for 2.5 hr. After distillation, dry benzene was added to the residue and the solvent was evaporated *in vacuo*. The final residue was heated with conc. HCl (100 cc.) at 95° for 2.5 hr. in order to remove its benzoyl group. The reaction mixture was filtered and extracted with benzene to remove BzOH. By evaporation of the aqueous layer, dihydrochloride of the subject compound was obtained as a brown syrupy residue, which did not crystallize. It was dissolved in water and added with picrylsulfonic acid. After removing the first crop of picrylsulfonate hydrochloride which was rather crude and stained, the faint yellow leafy crystals precipitated; m.p. 108° (MeOH). *Anal.* Calcd. for $C_{15}H_{22}O_{11}N_5Cl_3S$: C, 30.70; H, 3.77; N, 11.93. Found : C, 30.47; H, 3.60; N, 11.60. It showed positive reactions with Dragendorff and Ninhydrin reagents.

DL-Lysine—Prepared according to the method of Ecke.[10] m.p. 259～260° (decomp.) (EtOH).

N^6-Benzyloxycarbonyl-DL-lysine—Prepared according to the method of Schumann, *et al.*[4] m.p. 253～255°. Purity was checked by paper chromatography using phenol-H_2O as a solvent system, in which a single spot was observed at Rf 0.95 (lysine, Rf. 0.43).

N^2-Benzoyl-N^6-benzyloxycarbonyl-DL-lysine—Prepared according to the method of Bergmann, *et al.*,[5] m.p. 131～133° (AcOH + H_2O). *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.28. Found : C, 65.28; H, 6.21; N, 7.36.

N^2-Benzoyl-DL-lysine—Obtained by catalytic reduction of the above benzyloxycarbonyl compound over Raney Ni in place of Pd in Bergmann's procedure.[5] m.p. 211～212°.

N^2-Benzoyl-N^6,N^6-bis(2-hydroxyethyl)-DL-lysine—Ethylene oxide (10 g.) was added to a mixture of α-benzoyl-DL-lysine (21 g.), $Ba(OH)_2$ (11 g.), and water (200 cc.). After being allowed to stand at 20° in a sealed tube for 2 days, it was neutralized with dil. H_2SO_4, filtered, and evaporated to dryness *in vacuo*. The yellow-brown resinous product was obtained and was used for the next reaction without purification.

N^6,N^6-Bis(2-chloroethyl)-DL-lysine Picrylsulfonate Hydrochloride Monohydrate (No. **539**)-Prepared by the procedure similar to the chlorination of No. 536; m.p. 181～182°. *Anal.* Calcd. for $C_{16}H_{26}O_{12}N_5Cl_3S$: C, 31.06; H, 4.23; N, 11.30. Found : C, 29.48; H, 3.50; N, 11.03. It showed positive Ninhydrin reaction and its copper salt precipitated instantly from the aqueous medium, but could not be purified further.

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Summary

Fourteen kinds of 2-chloroethyl derivatives of amino acids, including N^6,N^6-bis(2-chloroethyl)-DL-lysine (No. 539) and N^5,N^5-bis(2-chloroethyl)-DL-ornithine (No. 536), were newly synthesized and examined for their anti-tumor activity on Yoshida sarcoma *in vivo* and *in vitro*.

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