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80. Yoshio Sakurai*1 and Eiichi Matsui*2: Preparation of Derivatives of Nitrogen Mustard having Structure of α-Amino Acid Amide.

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In 1954, a report was published by Ishidate, Sakurai, and Izumi[1] on preparation and antitumor effect of the derivatives of N-bis(2-chloroethyl)amino acid, of which N-bis(2-chloroethyl)-DL-alanine hydrochloride seemed to be the most promising. This compound was very stable as hydrochloride and also soluble in water either as hydrochloride or as free base. The effect on Yoshida sarcoma or ascites hepatoma bearing rats was found to be strong and, by clinical experiments, also proved to be effective against chronic leukemia, lymphoid and myeloid. Recently Dietrich, *et al.*[2] discussed about the clinical effect of this compound on various kinds of tumors.

In the course of study of derivatives of nitrogen mustard, it was lately found that the amides of these amino acid derivatives exhibited far better result in prolongating the life-span of Yoshida sarcoma bearing rats, although chemotherapeutic index (LD_{50}/MED*3) of the amides on Yoshida sarcoma by a single injection were rather poor than those of the original free amino acids, as shown in Table I.

Table I. $(CLCH_2CH_2)_2N-\underset{\underset{R}{|}}{CH}-COR'$

No.	R	R′	LD_{50} (mg./kg.)	MED (mg./kg.)	MEC (mM)	LD_{50}/MED
232	H	OH	1.5	0.5	1×10^{-1}	30
237	〃	NH_2	0.8	0.025	2.5×10^{-3}	32
243	CH_3	OH	13	0.05	1×10^{-2}	255
244	〃	NH_2	3	0.025	5×10^{-3}	120

The data in the Table I represents that the amides appeared more active in tissue culture screening than in animal test, that denoted the higher stability of the former in a neutral aqueous medium than the latter. The rapidly reacting alkylating agent often gave extremely small value of MED but not enough activity of prolongation of life-span of animals bearing ascitic tumor by intraperitoneal injection.

The present paper deals with the preparation of the compounds and discussion on their chemical reactivity. Detailed experiments with animal tumors will be published in recent future.

The compounds prepared are summarized in Table II. The method of synthesis is shown in Chart 1. Among the compounds listed in Table II, Nos. 818, 819, 820, and 828 of the glycine series, and No. 826 of the alanine series were found to have the far larger chemotherapeutic indexes (C. I.) on Yoshida sarcoma than the corresponding

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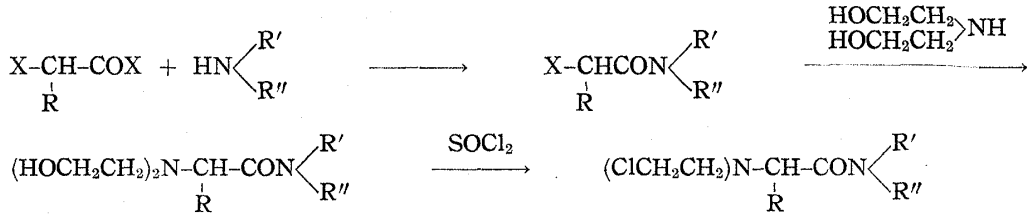
*3 Minimum Effective Dose
1) M. Ishidate, Y. Sakurai, M. Izumi: This Bulletin, **2**, 275 (1954).
2) F. S. Dietrich, *et al.*: Cancer Chemotherapy Reports, **23**, 31 (1962).



X=Cl, Br R, R′, R″=H, Alkyl, Aryl

Chart 1.

TABLE II. $ClCH_2CH_2$ HCl $ClCH_2CH_2$ >NCHCOR′ R

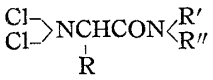
No.	R	R′	m.p. (°C)	LD_{50}	mg./kg. MTD	i.p. MED	m*M in vitro* MEC	LD_{50}/MED CI
232	H	-OH	76°	15	10	0.5	1×10^{-1}	30
137	〃	$-NH_2$	145°	0.8	0.5	0.025	2.5×10^{-3}	32
818	〃	$-N(C_6H_5)(CH_3)$	155～156° (decomp.)	20	10	0.1	1×10^{-3}	200
819	〃	$-N(C_2H_5)_2$	112～113°	7.5	5	0.05	2.5×10^{-3}	150
820	〃	-N O	191～192° (decomp.)	1.75	1	0.025	2.5×10^{-3}	70
927	〃	-N	159～160° (decomp.)	3.75	2.5	0.25	2.5×10^{-4}	15
825	〃	$-NH(CH_2)_2NH-$	218～219° (decomp.)	3.75	2.5	0.1	2.5×10^{-4}	37.5
828	〃	$-N(CH_2-CH_2)_2N-$	204～205° (decomp.)	7.5	5	0.05	5×10^{-4}	150
243	CH_3	-OH	97°	30	10	0.05	1×10^{-2}	600
244	〃	$-NH_2$	190°	3	1	0.025	5×10^{-3}	120
826	〃	$-N(C_6H_5)(CH_3)$	157° (decomp.)	75	50	0.5	5×10^{-3}	150
829	〃	$-N(C_2H_5)_2$	158～160° (pcs)				2.5×10^{-3}	
832	〃	-N O	178° (pcs) (decomp.)	17.5	10	0.5	2.5×10^{-3}	35
834	〃	-N	158～160° (pcs)	17.5	10	1	2.5×10^{-2}	17.5
835	〃	$-NH(CH_2)_2NH-$	158～160° (pcs) (decomp.)	17.5	10	0.25	2.5×10^{-3}	70.0

original amides, Nos. 237 and 244 and especially No. 818 exhibited a very strong life-span prolongating effect on the animals bearing the tumor by repeated intraperitoneal injections.

In Table III is presented the results of determination of velocity of Cl^--liberation and thiosulfate uptake at 37° in a neutral aqueous solution. As seen in this Table, the antitumor effect of the compounds seemed not to be necessarily depending on the velocity of formation of aziridinium intermediate or of alkylation. The certain physical and biological properties of the compounds like solubility, rate of distribution to the tissues, and velocity of excretion, etc., might be playing a fairly large role in exhibiting the final effect. To develop new derivatives of this series, the synthetic investigation of the derivatives of N-{2-[bis(2-chloroethyl)amino]acyl}amino acid is now progressing.

Table III. Thiosulfate Uptake in Sodium Carbonate Buffer at 37°

Compd. No.	R	R′	R″	Medium	Cl^- Liberation (mole equivalent (min.))				thiosulfate uptake (mole equivalent (min.))			
					10	30	60	120	10	30	60	120
237	H	H	H	H_2O	0.49	1.08	1.53	1.77	0.46	1.02	1.53	1.80
				50% Me_2CO	0	0.11	0.31	0.64	0.12	0.28	0.50	0.84
818	〃	CH_3	C_6H_5	〃	0.76	1.31	1.74	1.94	0.43	1.03	1.65	1.91
819	〃	C_2H_5	C_2H_5	〃	0.88	1.23	1.55	1.88	0.56	1.17	1.66	1.83
244	CH_3	H	H	H_2O	0.70	1.49	1.87	1.97	0.65	0.90	1.78	1.93
				50% Me_2CO	0.11	0.29	0.54	1.00	0.16	0.39	0.61	1.34
826	〃	CH_3	C_6H_5	〃	0.10	0.37	0.72	1.06	0.20	0.39	0.88	1.54



Experimental

Preparation of Halogenoacylamides——From halogenoacyl halogenide and amine by Schotten-Baumann's reaction at 5～−5°.

N-Methyl-2-chloroacetanilide——m.p. 70～71°(from MeOH). Yield, 21 g. from 15 g. of chloroacetyl chloride. *Anal.* Calcd. for $C_9H_{10}ONCl$: C, 58.86; H, 5.49; N, 7.63. Found: C, 58.88; H, 5.26; N, 7.61.

N,N-Diethyl-2-chloroacetamide——After acylation, the reaction mixture was extracted with AcOEt. b.p. 92～93°. Yield, 52.6 g. from 64 g. of chloroacetyl chloride.

2-Chloroacetomorpholide——Extracted from the reaction mixture with AcOEt. b.p. 130～139°. Yield, 30 g. from 40 g. of chloroacetyl chloride. *Anal.*Calcd. for $C_6H_{10}O_2NCl$: C, 44.05; H, 6.16; N, 8.56. Found: C, 43.93; H, 6.07; N, 8.21.

2-Chloroacetopiperidide——Extracted from the reaction mixture with AcOEt. b.p. 134～142°. Yield, 24 g. from 35 g. of chloroacetyl chloride. *Anal.* Calcd. for $C_7H_{12}ONCl$: C, 52.01; H, 7.48; N, 8.67. Found: C, 51.92; H, 7.39; N, 8.63.

N,N′-Ethylene-2,2′-dichlorobisacetamide——m.p. 173～174°(from MeOH). Yield, 16.5 g. from 38 g. of chloroacetyl chloride. *Anal.* Calcd. for $C_6H_{10}O_2N_2Cl_2$: C, 33.82; H, 4.73; N, 13.15. Found: C, 33.73; H, 4.75; N, 13.08.

1,4-Bis(2-chloroacetyl)piperazine——m.p. 136～137°(from EtOH). Yield, 25 g. from 67 g. of chloroacetyl chloride. *Anal.* Calcd. for $C_8H_{12}O_2N_2Cl_2$: C, 40.18; H, 5.06; N, 11.72. Found: C, 40.19; H, 4.99; N, 11.87.

N-Methyl-2-bromopropionanilide——Extracted with AcOEt from the reaction mixture. $b.p_{10}$ 155～158°. Yield, 20 g. from 25 g. of 2-bromopropionyl bromide. The product was used as such for the following synthesis without analysis.

N,N-Diethyl-2-bromopropionamide——$b.p_2$ 103～105°. Yield, 11.5 g. from 27 g. of 2-bromopropionyl bromide.

2-Bromopropionmorpholide——$b.p_6$ 151°. Yield, 36 g. from 65.5 g. of 2-bromopropionyl bromide. *Anal.* Calcd. for $C_7H_{12}O_2NBr$: C, 37.85 H, 5.45; N, 6.31. Found: C, 37.95; H, 5.59; N, 6.01.

2-Bromopropionpiperidide——$b.p_6$ 131～137°. Yield, 11.5 g. from 30 g. of 2-bromopropionyl bromide. *Anal.* Calcd. for $C_8H_{14}ONBr$: C, 43.65; H, 6.41; N, 6.36. Found: C, 43.20; H, 6.61; N, 6.12.

N,N′-Ethylene-2,2′-dibromobispropionamide——m.p. 205～206°(decomp.) (from EtOH). Yield, 10 g. from 58 g. of 2-bromopropionyl bromide. *Anal.* Calcd. for $C_8H_{14}O_2N_2Br_2$: C, 29.11; H, 4.28; N, 8.49. Found: C, 29.09; H, 4.12; N, 8.39.

Preparation of N{2-[bis(2-hydroxyethyl)amino]acyl}amino Acid Amide——Equal mole equivalent amount of 2-halogenoacylamide and diethanolamine were dissolved in dimethyl formamide. The mixture was added with an excess amount of anhyd. K_2CO_3 and warmed on water bath at 50～60° for several hr. The reaction mixture was filtered and the filtrate was evaporated to dryness. A small part of the syrupy residue was converted to picrate or picrylsulfonate for identification and the rest was used as such without purification.

N-Methyl-2-[bis(2-hydroxyethyl)amino]acetanilide——From 20 g. of N-Methyl-2-chloroacetanilide, 11 g. of diethanolamine, 10 g. of anhyd. K_2CO_3, and 100 ml. of dimethylformamide. Picrate: m.p. 98～100° (from 40% MeOH). *Anal.* Calcd. for $C_{19}H_{23}O_{10}N_5$: C, 47.40; H, 4.81; N, 14.55. Found: C, 47.58; H, 4.81; N, 14.52.

N,N-Diethyl-2-[bis(2-hydroxyethyl)amino]acetamide——From 16 g. of N,N-diethyl-2-chloroacetamide, 11 g. of diethanolamide, 10 g. of anhyd. K_2CO_3, and 100 ml. of dimethylformamide. Picryl sulfonate : m.p. 141~142° (from EtOH). *Anal.* Calcd. for $C_{16}H_{25}O_{12}N_5S$: C, 37.57; H, 4.93; N, 13.69. Found : C, 37.46; H, 4.86; N, 13.47.

2-[Bis(2-hydroxyethyl)amino]acetomorpholide——From 5 g. of 2-chloroacetmorpholide, 3.2 g. of diethanolamine, 8 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. Picryl sulfonate : m.p. 212~213° (decomp.) (from dil. MeOH). *Anal.* Calcd. for $C_{16}H_{23}O_{13}N_5S$: C, 36.57; H, 4.41; N, 13.33. Found : C, 36.61; H, 4.31; N, 13.53.

2-[Bis(2-hydroxyethyl)amino]acetpiperidide——From 5 g. of 2-chloroacetpiperidide, 3.2 g. of diethanolamine, 10 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. Picryl sulfonate : m.p. 159~160° (decomp.) (from EtOH). *Anal.* Calcd. for $C_{17}H_{25}O_{12}N_5S$: C, 39.00; H, 4.81; N, 13.38. Found : C, 38.91; H, 4.60; N, 13.12.

N,N-Ethylene-2,2′-bis[bis(2-hydroxyethyl)aminobisacetamide——From 5 g. of N,N-ethylene-2,2′-dichloro-bis-acetamide, 4.9 g. of diethanol amine, 13 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. The reaction mixture was heated at 60° for 18 hr. Picryl sulfonate : m.p. 210~211° (decomp.) (from H_2O). *Anal.* Calcd. for $C_{13}H_{18}O_{12}N_5S$: C, 33.34; H, 3.87; N, 14.95. Found : C, 32.71; H, 3.94; N, 14.88.

1,4-Bis{2-[bis(2-hydroxyethyl)amino]acetyl}piperazine——From 5 g. of 1,4-bis(chloroacetyl)piperazine, 4.2 g. of diethanolamine, 12 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. The reaction mixture was heated at 60° for 15 hr. Both the picrate and picryl sulfonate were not obtained as analitically pure samples and used as such for the next step of reaction.

N-Methyl-2-[bis(2-hydroxyethyl)amino]propionanilide——From 5 g. of N-methyl-2-bromopropionanilide, 2.1 g. of diethanolamine, 8 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. The reaction mixture was heated at 60° for 10 hr. Picryl sulfonate : m.p. 158~159° (from EtOH). *Anal.* Calcd. for $C_{20}H_{26}O_{12}N_5S$: C, 42.93; H, 4.50; N, 12.52. Found : C, 42.70; H, 4.49; N, 11.92.

N,N-Diethyl-2-[bis(2-hydroxyethyl)amino]propionamide——From 11.3 g. of N,N-diethyl-2-bromopropionamide, 5.5 g. of diethanolamine, 6 g. of anhyd. K_2CO_3, and 100 ml. of dimethylformamide. The reaction mixture was heated at 60° for 16 hr. Picryl sulfonate : m.p. over 200° (from H_2O). *Anal.* Calcd. for $C_{17}H_{21}O_{12}N_5S$: N, 13.33. Found : N, 12.98.

2-[Bis(2-hydroxyethyl)amino]propionmorpholide——From 5 g. of 2-bromopropionmorpholide, 2.2 g. of diethanolamine, 6 g. of anhyd. K_2CO_3, and 50 ml. of dimethylformamide. Picryl sulfonate : m.p. 206~207° (decomp.) (from 80% EtOH). *Anal.* Calcd. for $C_{17}H_{25}O_7N_5S$: C, 37.85; H, 4.67; N, 12.98. Found : C, 37.27; H, 4.63; N, 12.88.

2-[Bis(2-hydroxyethyl)amino]propionpiperidide——From 11 g. of 2-bromopropionpiperidide, 5.3 g. of diethanolamine, 10 g. of anhyd. K_2CO_3, and 100 ml. of dimethylformamide. Picryl sulfonate : m.p. 173~174° (from EtOH). *Anal.* Calcd. for $C_{18}H_{27}O_{12}N_5S$: C, 40.22; H, 5.06; N, 12.73. Found : C, 40.08; H, 5.09; N, 12.44.

N,N′-Ethylene-2,2′-bis[bis(2-hydroxyethyl)amino]bispropionamide——From 15.7 g. of N,N′-ethylene-2,2′-dibromobispropionamide, 10 g. of diethanolamine, 20 g. of anhyd. K_2CO_3, and 200 ml. of dimethylformamide. Picryl sulfonate : m.p. 160~161° (from dil. EtOH). *Anal.* Calcd. for $C_{14}H_{20}O_{12}N_5S$: C, 34.86; H, 4.18; N, 14.52. Found : C, 34.89; H, 4.09; N, 14.77.

Preparation of N{2-[Bis(2-chloroethyl)amino]acyl}amino Acid Amide——The above dihydroxylated intermediates were dissolved or suspended in $CHCl_3$ and $SOCl_2$ was added by drops into this mixture under chilling in ice water. The reaction mixture was then warmed at 50~60° for 6 to 12 hr. and the solvent and excess reagent were distilled off *in vacuo*. The residue crystallized in most cases after a long cooling in an icebox. Usually the compounds gave crystalline picrate or picryl sulfonate even when their hydrochloride remained uncrystallized for a long time.

N-Methyl-2-[bis(2-chloroethyl)amino]acetanilide——From 33 g. of the corresponding dihydroxylated intermediate, 20 ml. of $CHCl_3$, and 33 ml. of $SOCl_2$. Hydrochloride : m.p. 155~156° (decomp.) (from acetone). Yield, 10 g. *Anal.* Calcd. for $C_{13}H_{19}ON_2Cl_3$: C, 47.94; H, 5.88; N, 8.60. Found : C, 48.04; H, 5.80; N, 8.78.

N,N-Diethyl-2-[bis(2-chloroethyl)amino]acetamide——From 23 g. of the intermediate, 200 ml. of $CHCl_3$, and 25 ml. of $SOCl_2$. Hydrochloride : m.p. 112~113° (from acetone). Yield, 8 g. *Anal.* Calcd. for $C_{10}H_{21}ON_2Cl_3$: C, 41.18; H, 7.26; N, 9.61. Found : C, 41.18; H, 7.21; N, 9.65.

2-[Bis(2-chloroethyl)amino]acetomorpholide——From 42 g. of the intermediate, 300 ml. of $CHCl_3$, and 45 ml. of $SOCl_2$. Hydrochloride : m.p. 191~192° (decomp.) (from EtOH). Yield, 12 g. *Anal.* Calcd. for $C_{10}H_{19}O_2N_2Cl_3$: C, 39.30; H, 6.26; N, 9.17. Found : C, 39.30; H, 5.97; N, 9.00.

2[Bis(2-chloroethyl)amino]acetopiperidide——From 24 g. of the intermediate, 200 ml. of $CHCl_3$, and 35 ml. of $SOCl_2$. Hydrochloride : m.p. 159~160° (decomp.) (from AcOEt). Yield, 8 g. *Anal.* Calcd. for $C_{11}H_{21}ON_2Cl_3$: C, 43.51; H, 6.97; N, 9.23. Found : C, 43.37; H, 6.05; N, 9.13.

N,N′-Ethylene-2,2′-bis[bis(2-chloroethyl)amino]bisacetamide——From 13 g. of the intermediate, 150 ml. of $CHCl_3$, and 20 ml. of $SOCl_2$. Hydrochloride : m.p. 218~219° (decomp.) (from MeOH). Yield, 6 g. *Anal.* Calcd. for $C_{14}H_{28}O_2N_4Cl_6$: C, 33.82; H, 5.68; N, 11.30. Found : C, 33.62; H, 5.53; N, 11.29.

1,4-Bis{2-[bis(2-chloroethyl)amino]acetyl}piperazine——From 29 g. of the intermediate, 250 ml. of $CHCl_3$, and 60 ml. of $SOCl_2$. Hydrochloride: m.p. 204~205° (decomp.) (from MeOH). Yield, 5 g. *Anal.* Calcd. for $C_{16}H_{30}O_2N_4Cl_6$: C, 36.82; H, 5.78; N, 10.71. Found: C, 36.75; H, 5.77; N, 10.42.

N-Methyl-2-[bis(2-chloroethyl)amino]propionanilide——From 27 g. of the intermediate, 200 ml of $CHCl_3$, and 35 ml. of $SOCl_2$. Hydrochloride: m.p. 157~158° (decomp.) (from acetone). Yield, 13 g. *Anal.* Calcd. for $C_{14}H_{21}ON_2Cl_3$: C, 49.50; H, 6.23; N, 8.25. Found: C, 49.25; H, 5.57; N, 8.27.

N,N-Diethyl-2-[bis(2-chloroethyl)amino]propionamide——From 7 g. of the intermediate, 100 ml. of $CHCl_3$, and dissolved in EtOH and treated with active charcoal. Purified as picryl sulfonate: m.p. 158~160°. *Anal.* Calcd. for $C_{17}H_{25}O_{10}N_5Cl_2S$: C, 36.31; H, 4.48; N, 12.45. Found: C, 36.35; H, 4.49; N, 12.91.

2-[Bis(2-chloroethyl)amino]propionmorpholide——From 7 g. of the intermediate, 100 ml. of $CHCl_3$, and 10 ml. of $SOCl_2$. Purified as picryl sulfonate: m.p. 178° (decomp.) (from EtOH). *Anal.* Calcd. for $C_{17}H_{23}O_{11}N_5Cl_2S$: C, 35.42; H, 4.02; N, 21.15. Found: C, 35.47; H, 3.98; N, 12.58.

2-[Bis(2-chloroethyl)amino]propionpiperidide——From 17 g. of the intermediate, 200 ml. of $CHCl_3$, and 22 ml. of $SOCl_2$. Purified as picryl sulfonate: m.p. 155~158° (from EtOH). *Anal.* Calcd. for $C_{18}H_{25}O_{10}N_5Cl_2S$: C, 37.64; H, 4.39; N, 12.19. Found: C, 37.26; H, 4.94; N, 11.85.

N,N'-Ethylene-2,2'-bis[bis(2-chloroethyl)amino]bispropionamide——From 23 g. of the intermediate, 100 ml. of $CHCl_3$, and 25 ml. of $SOCl_2$. Crude picryl sulfonate: m.p. 158~160° (decomp.) (from MeOH). *Anal.* Calcd. for $C_{28}H_{36}O_{20}N_{10}Cl_4S_2$: C, 32.38; H, 3.49; N, 13.49. Found: C, 34.51; H, 3.34; N, 13.56.

Liberation Rate of Cl^-——A sample (0.5 mmol.) was dissolved in distilled water and added with $NaHCO_3$ (2 mmol.). The mixture was diluted with distilled water up to 50 ml. and kept in incubator at 37° for a required period. A portion (5 ml.) of the mixture was then titrated with 0.01*N* $AgNO_3$ under chilling. In cases of the less soluble samples, 50% acetone was used instead of distilled water.

Thiosulfate Consumption——A sample (0.5 mmol.) was dissolved in distilled water (15 ml.) and added with $NaHCO_3$ (2 mmol.). The mixture was then added with 0.1*N* $Na_2S_2O_3$ (10 ml.) and diluted immediately up to 50 ml. with distilled water. The final mixture was incubated at 37° for a required period and titrated with 0.02*N* I_2 solution. The samples insoluble in water was dissolved in 50% acetone.

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Summary

A number of new amide derivatives of bis(2-chloroethyl)-DL-glycine and bis(2-chloroethyl)-DL-alanine were prepared and tested for their *in vivo* and *in vitro* antitumor activity against Yoshida Sarcoma, and chemical properties.

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