UDC 616-006-085 : 547.786.1

76. Morizo Ishidate, Yoshio Sakurai, and Masahiro Torigoe : Studies on Carcinostatic Substances. XXXIV.*1 Anti-tumor Activity of 2,2-Bis(2-chloroethyl)isoxazolidinium Chloride and Related Compounds.

(*Iatrochemical Institute of Pharmacological Research Foundation**2)

In the course of investigation of N-oxides of nitrogen mustard, 2,2-bis(2-chloroethyl)-isoxazolidinium chloride was expectedly obtained by oxidation of N,N-bis(2-chloroethyl)-3-chloropropylamine with peracid. This compound drew interest of the present authors because it was found to be readily reduced by catalytic reduction to 3-bis(2-chloroethyl)-amino-1-propanol, which is one of the active bifunctional alkylating agents. As 2-chloroethyl group contained in this isoxazolidinium derivative is chemically inert as itself, it is regarded to be a new type of alkylating agent with latent activity, the so-called "masked compound."

The present paper deals with the preparation of and discussions on chemical and biological properties of 2,2-bis(2-chloroethyl)isoxazolidinium halide (I) and its related compounds, viz. 2-(2-chloroethyl)-2-methylisoxazolidinium halide (II), 2,2-diethylisoxazolidinium chloride (III), 2,2′-hexamethylene-bis[2-(2-chloroethyl)isoxazolidinium chloride](IV) and 1,1-bis(2-chloroethyl)pyrrolidinium chloride (V). Among the compounds, derivatives of isoxazolidinium compounds were generally prepared by oxidation with peracid of tertiary amines containing one 3-chloropropyl group according to the procedure described in the preceding papers.[1,2]

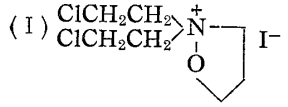
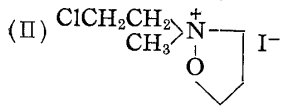
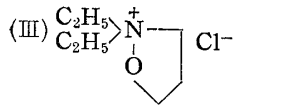
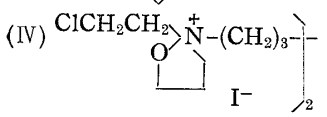
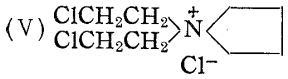
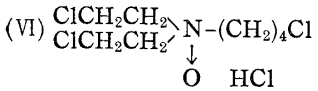
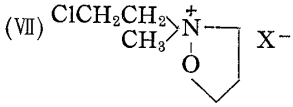
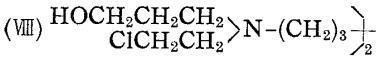
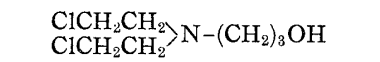
Thus, (I) was obtained by chlorination and oxidation of 3-bis(2-hydroxyethyl)amino-1-propanol which was prepared by heating a mixture of allyl alcohol and diethanolamine in the presence of sodium allyloxide for a long period. The corresponding hydroxyl intermediate in the preparation of (II) or (III) was obtained by heating respectively a mixture of glycidol and 2-methylaminoethanol or a mixture of diethylamine and allyl alcohol. (IV) was obtained by oxidation of N,N′-bis(2-chloroethyl)-N,N′-bis(3-chloropropyl)-1,6-hexanediamine. The corresposding hydroxyl intermediate was prepared by reaction of ethylene oxide with N,N′-bis(3-hydroxypropyl)-1,6-hexanediamine, which was synthesized by heating 3-amino-1-propanol with 1,6-dibromohexane. Crude N,N-bis(2-chloroethyl)amino-4-chlorobutylamine afforded its true N-oxide (VI) by the usual oxidation. However, if the former was subjected to distillation for the purpose of purification, it always gave the cyclized quaternary amine (V) alone. All these compounds, including 2-(2-chloroethyl)-2-methyl-isoxazolidinium halide*3 (VII), were examined for their chemical and biological activities, data of which are summarized in Table I.

As seen in Table I, the biological activity of (I) and (II) against Yoshida sarcoma was found to be very strong, in spite of the fact that the chemical reactivity, viz. Cl^- liberation and thiosulfate uptake, of these compounds in a neutral aqueous solution was extremely slow. In addition, cysteine uptake of (I) *in vitro*, by the procedure published earlier,[3] was also found to be only 0.2 molar equivalent at 26° during 24 hours, while N-methyl-2,2′-dichlorodiethylamine took up nearly 2 molar equivalents during the same period. From

*1 Part XXXIII : This Bulletin, **9**, 343 (1961).
*2 Designation now changed to Cancer Chemotherapy Section, Sasaki Institute. 26 Nishigahara 1-Chome, Kita-ku, Tokyo (石舘守三, 桜井欽夫, 鳥越政宏).
*3 The compound was kindly supplied by Mr. Sawatari, Yoshitomi Pharm. Ind., Ltd.

1) I. Aiko, S. Owari, M. Torigoe : Yakugaku Zasshi, **72**, 1297 (1952).
2) Y. Sakurai, M. Izumi : This Bulletin, **1**, 297 (1953).
3) M. Torigoe : *Ibid.*, **1**, 349 (1953).

Table I.

No.	Compound	$E_{1/2}$ v.s. S·C·E pH 3.5	Thiosulfate consumption (mole. equiv.) 2 hr.	Thiosulfate consumption (mole. equiv.) 24 hr.	Cl^- liberation (mole. equiv.) 2 hr.	Cl^- liberation (mole. equiv.) 24 hr.	Toxicity on rat LD_{50} (mg./kg.)	Antitumor activity against Yoshida sarcoma MTD (mg./kg.)	Antitumor activity against Yoshida sarcoma MED (mg./kg.)	Antitumor activity against Yoshida sarcoma MEC (mM)[a]
(I)		-0.48_8		0.1～0.2		0.2	7.5	5	0.1	10^{-2}
(II)		-0.46_0	0.14		0.27	1.39	3	1	0.1	10^{-3}
(III)		-0.81_1					150	100	—	
(IV)		-0.37_1	0.12	0.44	0.55	1.18	30	10	—	—
(V)							175	100	—	
(VI)							30	10	1	—
(VII)							75	50	—	
(VIII)										2.5×10^{-3}
										10^{-3}

a) Minimum effective concentration (MEC) in mM.
— No effect.

these results, it became clear that this compound was not reduced by thiosulfate or cysteine in such a condition but was readily reduced in animal body. It does not oxidize potassium iodide or ferrous sulfate at 37° but colorizes leuco Janus Green at pH 7.4 and 37°.

It could however be concluded that the antitumor activity of (I) or (II) should be due to the bifunctional alkylating activity of chlorine atoms present in β-position to nitrogen, because (III) and (VII), each of which has none or only one chlorine in the molecule, does not exhibit any antitumor activity. (V) is a very stable compound and is less toxic than (III). This fact shows that (V) is not capable of being activated *in vivo* despite the resemblance of its molecule to (I).

By the study on condition of activation of (I) and (II) with *in vivo*-cultured Yoshida sarcoma by the reported technique,[4] it was proved that activation velocity of (I) or (II) by the tumor is increased as the tumor cell population (number of cells/cc.) increases. This might be a proof that these compounds are masked type without question.

However, it still remains a matter of question why (IV) is not active as shown in Table I. The data showing its chemical reactivity, viz. Cl^- liberation and thiosulfate uptake, were found to be rather larger than those of (I) or (II), and yet it remained inactive through various antitumor screenings *in vitro* and *in vivo*.

(IV) was however reduced by hydrogen over palladium-carbon at room temperature, yielding N,N′-bis(2-chloroethyl)-N,N′-bis(3-hydroxypropyl)-1,6-hexanediamine (VIII), which

4) H. Imamura : *Ibid.*, 8, 449 (1960).

was determined to be active against Yoshida sarcoma *in vitro* as N,N′-dimethyl-N,N′-bis-(2-chloroethyl)-1,6-hexanedimine reported in the preceding paper of this series.[5]

Result of polarographic determination[6] of reduction potentials of the compounds is also demonstrated in Table I. Analyzing the polarograms of (I), it was found that $E_{1/2}$ was always constant between concentrations of 10^{-3} and $10^{-4}M$, and *id* was found to be proportional to the concentration. The elements of the wave almost satisfied the following equation at pH 3.5 :

$$E = E_{\frac{1}{2}} - \frac{0.059}{\alpha} \log \frac{i}{id-i}$$

It should however be noted that the value of α calculated from these data was as small as 0.38.

Half-wave potential of (IV) is shown in Table I as −0.37 volt and from this value it is also difficult to understand the ineffectiveness of this compound *in vivo*.

The previous experiment[1] has shown that 2-methyl-2-(2-chloroethyl)-1,2-oxazetidinium chloride transforms easily into N-(2-chloroethoxy)-N-methyl-2-chloroethylamine in a neutral solution. A neutral solution of (I) alone or with addition of excess of benzoate was incubated for many hours, but there was no formation of a transformed product. (I) was found to be so stable that it was recovered unchanged from the solution after a long incubation.

So far as known from the result of test of (I) on Yoshida sarcoma, the chemotherapeutic index (LD_{50}/MED) was as large as that of N,N-bis(2-chloroethyl)amine N-oxide, but toxicity of the former seemed to be enhanced by repeated administration. On this account, effect of life-span prolongation of (I) or (II) on tumor animals did not match that of the latter, as demonstrated in Figs. 1, 2, 3, and 4.

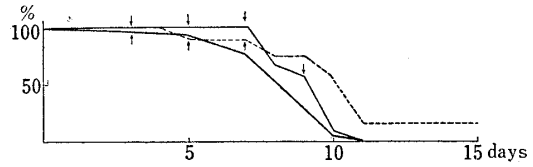


Fig. 1. Percentage Survival Diagram with (I) (Yoshida sarcoma)
—— Control
—— 2 mg./kg. i.p.×4
------ 0.5 mg./kg. i.p.×3

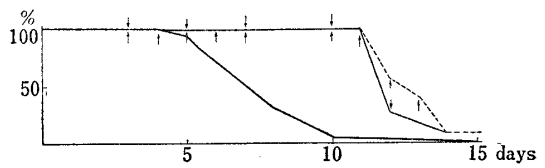


Fig. 2. Percentage Survival Diagram with (II) (Yoshida sarcoma)
—— Control
—— 0.5 mg./kg. i.p.×5
------ 0.2 mg./kg. i.p.×9

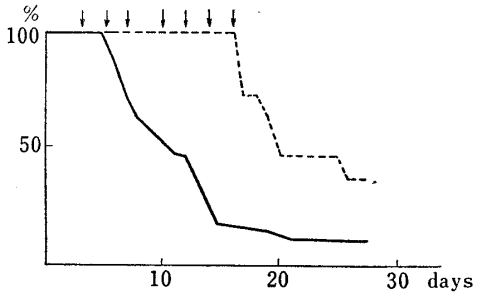


Fig. 3. Percentage Survival Diagram with (II) (AH 13)[a]
—— Control
------ 0.5 mg./kg. i.p.×7

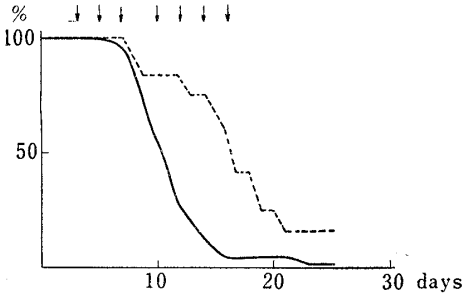


Fig. 4. Percentage Survival Diagram with (II) (AH 99)[a]
—— Control
------ 0.5 mg./kg. i.p.×7

a) Ascites rat hepatoma

5) M. Ishidate, Y. Sakurai, K. Maruyama : *Ibid.*, **6**, 164 (1958).
6) I. Aiko : *Ibid.*, **1**, 335 (1953).

Minimum effective dose (MED) and LD_{50} of (I) by oral administration on Yoshida sarcoma rat were determined as 5 mg./kg. It is also worth noting that (I) was similarly effective against rat ascites hepatoma AH-130 but ineffective against AH-7974, the former strain being susceptible to N-methyl-2,2'-dichlorodiethylamine and the latter resistant to the same agent. Comparison of leucopenia-inducing effect of (I) on normal rat with those of derivatives of nitrogen mustards was investigated and reported in 1960 by Ishidate, *et al.*[7]

In order to improve efficacy, trials to modify the molecule of (I) so as to control reducibility of C-O bond of this compound is now under progress. An attempt has not yet succeeded to prepare 2,2-bis(2-chloroethyl)-tetrahydro-1,2-oxazinium chloride by oxidation of N,N-bis(2-chloroethyl)-4-chlorobutylamine with peracid. The only product obtained by this reaction was the N-oxide of the starting material and, when the N-oxide was dissolved at pH 8 and incubated, the transformed product seemed to be only 2-(2-chloroethyl)-2-(4-chlorobutyl)-1,2-oxazetidinium chloride.

Experimental

3-Bis(2-hydroxyethyl)amino-1-propanol——A mixture of allyl alcohol (52 g.), diethanolamine (31.5 g.), and metallic Na (7 g.) was refluxed in an oil bath for 6 days. After removal of allyl alcohol *in vacuo*, the residue was added successively with H_2O (50 cc.), KCl (7.5 g.), and KOH (7.4 g.), and extracted continuously first with Et_2O and then with Me_2CO. The residue from the Me_2CO extract was submitted to vacuum distillation, $b.p._{1.7}$ 161～164°.*4 Yield, 9.6 g.

Picrylsulfonate : m.p. 156～160° (from Me_2CO+petr. ether) (sintering begins at 110°). *Anal.* Calcd. for $C_{13}H_{20}O_{12}N_4S$: C, 34.21; H, 4.42; N, 12.28. Found : C, 34.27; H, 4.32; N, 12.57.

N,N-Bis(2-chloroethyl)-3-chloropropylamine (IX)——The synthesis of this compound was based on the method of Wilson.[8]

Picrate : m.p. 87～89° (from EtOH). *Anal.* Calcd. for $C_{13}H_{17}O_7N_4Cl_3$: C, 34.87; H, 3.83; N, 12.52. Found : C, 35.14; H, 3.82; N, 12.86.

2,2-Bis(2-chloroethyl)isoxazolidinium (I) Picrate and Iodide——Into a mixture of Ac_2O (50.8 g.) and 30% H_2O_2 (45.3 g.), AcONa (9 g.) was dissolved with caution at 10～20°, followed by cautious addition of (IX) (25.5 g.) at 10°. The mixture was kept at that temperature for 3 hr. with stirring, then elevated to 30° for 1 hr., and finally held standing overnight at room temp. The reaction mixture was acidified strongly with conc. HCl and evaporated to dryness below 30° in a reduced pressure. The residue was extracted with hot Me_2CO and the solvent was evaporated *in vacuo*. The residue was converted to the picrate, m.p. 89～91° (from EtOH). *Anal.* Calcd. for $C_{13}H_{16}O_8N_4Cl_2$: C, 36.55; H, 3.78; N, 13.12. Found : C, 36.64; H, 3.42; N, 13.00.

The purified picrate was converted to a semi-crystalline ammonium chloride (I), which could not be purified. Yield : 12 g.

Iodide : Crude chloride, obtained from the picrate (5 g.), was dissolved in H_2O (11 cc.) and 50% (w/w) KI solution (3.8 g.) was added with cooling. Yellow precipitate was collected and recrystallized from MeOH-Et_2O to pale yellow needles melting at 103～104°. *Anal.* Calcd. for $C_7H_{14}ONCl_2I$: C, 25.79; H, 4.33; N, 4.30; Hal, 60.68. Found : C, 25.87; H, 4.22; N, 4.27; Hal, 60.63.

Catalytic Reduction of (I)——(I) (0.47 g.) dissolved in H_2O (9 cc.) was shaken with H_2 over Pd-C catalyst (2 cc. of 0.5% $PdCl_2$, 0.01 g. activated carbon) at room temperature. Within 40 min., 44 cc. of H_2 was absorbed. To one-half of the filtrate, 0.2*M* sodium picrate (5 cc.) was added and kept in an ice box. The precipitate was collected and recrystallized from EtOH-benzene or AcOEt-benzene mixture to yellow needles, m.p. 99～101°. Yield : 0.36 g. *Anal.* Calcd. for $C_{13}H_{18}O_8N_4Cl_2$: C, 36.38; H, 4.23; N, 13.06. Found : C, 36.54; H, 4.36; N, 12.96.

The remainder of the filtrate from hydrogenation was acidified with HCl and evaporated to dryness. A colorless oily residue (0.19 g.) so obtained was chlorinated at once with $SOCl_2$ (0.25 g.) in $CHCl_3$ (2 cc.). After standing overnight at room temperature, the mixture was refluxed on a water bath for 3 hr. The product was isolated as a picrate (0.21 g.) of m.p. 84～86°. After purification by recrystallization from EtOH, the substance melted at 87.5～88.5°, which showed no depression when mixed with the authentic sample of (IX) picrate.

*4 Jones, *et al.* (J. Chem. Soc., **1949**, 547) reported $b.p._{0.8}$ 167～169° for this compound.

7) M. Ishidate, Y. Sakurai, E. Matsui : This Bulletin, **8**, 89 (1960).

8) E. Wilson, M. Tishler : J. Am. Chem. Soc., **73**, 3635 (1951).

Reaction between (I) and Sodium Benzoate——(I) (0.7 g.) dissolved in H_2O (4 cc.) was shaken with freshly precipitated Ag_2CO_3 (prepared from 2.1 g. of $AgNO_3$ and 4.2 g. of K_2CO_3) and filtered. After BzONa (0.45 g.) was added to the filtrate, it was extracted continuously with Et_2O for 24 hr. Et_2O was removed from the extract by evaporation and the residue (0.1 g.) was added with dil. HCl and extracted with Et_2O to remove BzOH (0.035 g.). Attempt to isolate a basic substance as picrylsulfonate from the residue was unsuccessful. The aqueous layer was added with picric acid solution (0.68 g. of picric acid in 6 cc. of EtOH). The picrate precipitated instantly (0.54 g.) and melted at 88.5~90° (without further purification), showing no depression with the picrate of (I).

N,N-Diethyl-3-chloropropylamine (X)——It was prepared after the method of Gilman.[9] Its picrate melted at 67~68° (from EtOH). *Anal.* Calcd. for $C_{13}H_{19}O_7N_4Cl$: C, 41.22; H, 5.06; N, 14.79. Found: C, 41.31; H, 4.78; N, 14.78.

2,2-Diethylisoxazolidinium Picrate (III)——30% H_2O_2 (2 moles) was added at 15~17° with caution into Ac_2O (2 moles) within 10 min. The mixture was kept at the same temp. for 10 min. with stirring. Into this mixture, (X) (1 mole) in benzene was added at 17~20° with stirring during 30 min. After 3 hr.'s stirring, the mixture was acidified with dil. HCl and the aqueous layer was evaporated *in vacuo*. The residue was purified through the picrate, m.p. 167~176° (evolution of gas) (from EtOH). Yield, 37%. *Anal.* Calcd. for $C_{13}H_{18}O_8N_4$: C, 43.57; H, 5.06; N, 15.64. Found: C, 43.43; H, 4.79; N, 15.41.

Catalytic Reduction of (III)——(III) (0.33 g.) dissolved in water (6.6 cc.) was shaken with H_2 and Pd-C catalyst (2 cc. of 0.5% $PdCl_2$, 0.01 g. of activated carbon) at room temperature. Within 2 hr., 41 cc. of H_2 was absorbed (calcd. for 1 mole, 47.5 cc.). The filtrate was acidified with HCl and evaporated to dryness. Extraction of the residue with Me_2CO, drying, and evaporation of the extract yielded a crystalline residue (0.16 g.). After basification with 5*M* K_2CO_3, the substance was again transferred to Me_2CO. After removal of the solvent, there remained an oily residue (0.19 g.) which was converted to a picrate in Et_2O. Yield, 0.25 g., m.p. 73~74°. No depression was observed with the authentic specimen of 3-(diethylamino)-1-propanol, which was synthesized according to the procedure described by Gawron.[10] *Anal.* Calcd. for $C_{13}H_{20}O_8N_4$: C, 43.32; H, 5.59; N, 15.55. Found: C, 43.33; H, 5.56; N, 15.47.

4-Phthalimidobutyl Acetate——A mixture of potassium phthalimide (30.2 g.) and tetramethylenechlorohydrin (24.6 g.) was heated in an oil bath (190~200°) for 5.5 hr. After the reation mixture was dissolved in hot H_2O, the insoluble solid was collected and recrystallized from EtOH (30 cc.), m.p. 58~60°. Yield, 33 g.

4-Amino-1-butanol——The above phthalimide (32.3 g.) was refluxed with dil. H_2SO_4 (43 cc. of conc. H_2SO_4 + 78 cc. of H_2O) for 5 hr. in an oil bath. The reaction mixture was chilled overnight, the precipitated phthalic acid (18.5 g.) was filtered off, and washed with H_2O. After the filtrate was extracted with Et_2O, it was basified with 50% NaOH and the inorganic matter that precipitated was filtered by suction. The filtrate was continuously extracted with $CHCl_3$ for 10~20 hr. $CHCl_3$ was evaporated and 4-amino-1-butanol that remained was distilled at 109.5~109.8°/18 mm. Hg. Yield, 6.5 g.

4-Bis(2-hydroxyethyl) amino-1-butanol (XI)——Ethylene oxide (prepared from 6.05 g. of ethylenechlorohydrin) was passed through 25% aqueous solution of 4-amino-1-butanol (1.78 g.) with mechanical stirring at 5°±1°. The temperature of the solution was held at 8~10° for 2~3 hr. and then placed in a refrigerator overnight. Water was removed in a reduced pressure and the residue was subjected to vacuum distillation, $b.p_{0.03}$ 151~154°. Yield, 2.6 g. *Anal.* Calcd. for $C_8H_{19}O_3N$: C, 54.22; H, 10.80; N, 7.91. Found: C, 53.94; H, 10.13; N, 7.92.

N,N-Bis(2-chloroethyl)-4-chlorobutylamine (XII)——A mixture of $SOCl_2$ (268 cc.) and $CHCl_3$ (128 cc.) was added to a solution of (XI) (57 g.) in $CHCl_3$ (110 cc.) at 30°. After standing overnight at room temperature, the mixture was refluxed for 3 hr. After evaporaton of the reaction mixture *in vacuo*, a syrupy residue remained (93 g.). Attempt to isolate and purify its hydrochloride, perchlorate, picrate, picrylsulfonate, or chloroaurate did not materialize.

1,1-Bis(2-chloroethyl)pyrrolidinium Chloride (V)——The above crude hydrochloride (XII) (10 g.) was dissolved in H_2O (10 cc.) and washed well with Et_2O. After being basified with 5*N* NaOH, the free base was extracted with Et_2O and dried over Na_2SO_4 for 30 min. By distillation *in vacuo* (pressure: 1.5 mm. Hg, bath temp.: 150°), the base was completely trasformed into the quaternary ammonium base without evaporation and solidified in the distilling flask.. This solid was dissolved in H_2O (10 cc.) and warmed with activated charcoal (0.5 g.) with stirring for 30 min. Charcoal was removed by filtration and the filtrate was extracted with Et_2O. Aqueous layer was evaporated in a reduced pressure and a crystalline residue was obtained (5.1 g.), which decomposed at 209° after recrystal-

9) H. Gilman, D. A. Shirley: J. Am. Chem. Soc., **66**, 889 (1944).
10) O. Gawron, P. E. Spoerri: *Ibid.*, **67**, 514 (1945).

lization from dehyd. EtOH-Et_2O mixture. *Anal.* Calcd. for $C_8H_{16}NCl_3$: C, 41.31; H, 6.93; N, 6.02. Found : C, 41.39; H, 7.01; N, 6.20.
Picrate : m.p. 108~110° (from EtOH). *Anal.* Calcd. for $C_{14}H_{18}O_7N_4Cl_2$: C, 39.54; H, 4.27; N, 13.18. Found : C, 39.32; H, 4.08; H, 13.28.

N,N-Bis(2-chloroethyl)-4-chlorobutylamine N-Oxide Hydrochloride (VI)——Et_2O solution of (XII) (prepared from 4 g. of the crude hydrochloride) was oxidized by the procedure described for preparation of (III). The reaction product, converted to its picrate (3.4 g.) and purified, melted at 85.5~86.5° (from EtOH). *Anal.* Calcd. for $C_{14}H_{19}O_8N_4Cl_3$: C, 35.20; H, 4.01; N, 11.73. Found : C, 35.18; H, 4.12; N, 11.65.

This was again converted to the hydrochloride by the usual procedure and melted at 63~65° (from dehyd. EtOH-Et_2O). *Anal.* Calcd. for $C_8H_{17}ONCl_4$: C, 33.71; H, 6.01; N, 4.92. Found : C, 33.48; H, 6.14; N, 4·92.

3-(N-Methyl-2-hydroxyethylamino)-1,2-propanediol (XIII)——Glycidol (2.2 g.) was added dropwise into N-methyl-2-hydroxyethylamine (2.3 g.) at 90° with stirring and the mixture was kept at the same temperature for 30 min. The product was purified by vacuum distillation, $b.p_{0.15}$ 140°. *Anal.* Calcd. for $C_6H_{15}O_3N$: C, 48.30; H, 10.13; N, 9.39. Found : C, 47.85; H, 10.48; N, 9.00.

N-(2-Chloroethyl)-N-methyl-2,3-dichloropropylamine (XIV)——A solution of (XIII) (9.5 g.) in $CHCl_3$ (9.5 cc.) was added to a solution of $SOCl_2$ (68 g.) in $CHCl_3$ (68 cc.) at 25~30°. After refluxing for 2 hr. at 60~70°, both the solvent and excess $SOCl_2$ were evaporated. The residue was dissolved in H_2O and extracted with Et_2O. After addition of K_2CO_3 solution to the aqueous layer the free base of (XIV) was extracted with benzene and distilled *in vacuo*, $b.p_3$ 99°. *Anal.* Calcd. for $C_6H_{12}NCl_3$: C, 35.24; H, 5.91; N, 6.85. Found : C, 34.89; H, 5.53; N, 6.71.
Picrate : m.p. 90~94° (from MeOH). *Anal.* Calcd. for $C_{12}H_{15}O_7N_4Cl_3$: C, 33.24; H, 3.49; N, 12.92. Found : C, 33.00; H, 3.23; N, 13.04.

2-(2-Chloroethyl)-2-methyl-4-chloroisoxazolidinium Salts (II)——Ac_2O (1.6 g.) was added with stirring into 30% H_2O_2 (1.8 g.) at 35~40°. Into this mixture, a solution of (XIV) (1.6 g.) in benzene (3 cc.) was added with stirring at 27~28°. The mixture was kept under the same condition for 3 hr. and extracted with 10% HCl (7 cc.). The HCl layer was extracted with Et_2O and aqueous layer was evaporated to dryness *in vacuo*. The crude hydrochloride remained as a colorless syrup and was converted to a picrate of m.p. 120~122.5° (from MeOH). *Anal.* Calcd. for $C_{12}H_{14}O_8N_4Cl_2$: C, 34.88; H, 3.41; N, 13.56; Cl, 17.16. Found : C, 34.90; H, 3.39; N, 13.60; Cl, 17.44.
Iodide : The crude chloride was dissolved in a small amount of H_2O and 50% solution of KI was added. The iodide that precipitated was recrystallized from MeOH-Et_2O mixture, m.p. 81~82°. *Anal.* Calcld. for $C_6H_{12}ONCl_2I$: C, 23.10; H, 3.88; N, 4.49. Found : C, 23.11; H, 3.83; N, 4.31.

Catalytic Reduction of (II)——(II) (0.44 g.), converted from its pure picrate, was dissolved in H_2O (2 cc.) and shaken with H_2 at room temperature over Pd-C (prepared from 0.2 g. of activated carbon and 4 cc. of 0.5% $PdCl_2$), absorbing 40 cc. of H_2 within 1 hr. The filtrate was slightly acidified with HCl and 2*N* sodium picrate was added. The precipitated picrate was recrystallized from EtOH and dried over P_2O_5 *in vacuo* at 60~70° for 30 min. m.p. 79.5~82°. *Anal.* Calcd. for $C_{12}H_{16}O_8N_4Cl_2$: C, 34.71; H, 3.88; N, 13.50. Found : C, 34.94; H, 3.48; N, 13.27.

1,6-Dibromohexane (XV)——Into a mixture of 47% HBr (4.2 cc.), conc. H_2SO_4 (0.7 cc.), and 1,6-hexanediol (1.2 g.), conc. H_2SO_4 (1.1 cc.) was added slowly and the mixture was refluxed gently for 5 hr. Extraction was repeated 3 times with 3 cc. each of $CHCl_3$ and the extracts were combined, washed with dil. K_2CO_3, dried, and fractionated, $b.p_{14}$ 113~115°. Yield, 2.3 g.

3,3'-(Hexamethylenediamino)-di-1-propanol (XVI)——(XV) (11.3 g.) was added dropwise into 3-amino-1-propanol (33.6 g.) at 50~60° with stirring. After the mixture was kept at 25° for 24 hr., it was cooled, mixed with KOH (5.2 g.) dissolved in EtOH (23 cc.), and kept at 0° for several hr. The precipitated KBr was filtered off, and EtOH and unreacted aminopropanol were removed by distillation. The residue was purified by distillation but the distillate turned to a solid, $b.p_{0.15}$ 185~195°, m.p. 82~83.5° (from Me_2CO). *Anal.* Calcd. for $C_{12}H_{28}O_2N_2$: C, 62.02; H, 12.15; N, 12.06. Found : C, 62.34; H, 11.91; N, 12.12.

3,3'-[N,N'-Bis(2-hydroxyethyl)hexamethylenediamino]-di-1-propanol (XVII)——Into a mixture of (XVI) (4.1 g.) and H_2O (12.3 cc.), ethylene oxide (from 5.4 g. of ethylene chlorohydrin and 22 cc. of 5*N* NaOH) was passed through at 3~4°. After being kept at 8~10° for two days, H_2O was removed *in vacuo* and the residue was converted to the picrate, m.p. 132~133° (from EtOH). Yield, 12 g. *Anal.* Calcd. for $C_{28}H_{42}O_{18}N_8$: C, 43.18; H, 5.44; N, 14.39. Found : C, 43.49; H, 5.39; N, 14.19.

N,N'-Bis(2-chloroethyl)-N,N'-bis(3-chloropropyl)-1,6-hexanediamine (XVIII)——(XVII) (3.5 g.), obtained from the above pure picrate, was heated with $SOCl_2$ (25.2 g.) for 2 hr. at 60~70° Both the solvent and excess of $SOCl_2$ were evaporated and the residue was washed with Me_2CO. The resulting crystals (1.4 g.) were recrystallized from dehyd. EtOH, m.p. 166~168°. *Anal.* Calcd. for $C_{16}H_{34}N_2Cl_6$: C, 41.13; H, 7.33; N, 6.00. Found : C, 41.15; H, 7.20; N, 6.14.

2,2'-Hexamethylenebis[2-(2-chloroethyl)isoxazolidinium] Salts (IV)——Ac_2O (0.9 g.) was added with stirring into 30% H_2O_2 (1 g.) at 35~40°. Into the mixture, a benzene solution of (XVIII) (obtained from

1 g. of its hydrochloride) was added with stirring at 27~28°. After the mixture was kept under the same condition for 3 hr., it was extracted with 10% HCl (3.8 cc.). The acid layer was evaporated to dryness *in vacuo* to a syrupy residue (0.9 g.), which was converted to a picrate of m.p. 136~137° (from Me_2CO). *Anal.* Calcd. for $C_{28}H_{36}O_{16}N_8Cl_2$: C, 41.44; H, 4.47; N, 13.81. Found : C, 41.46; H, 4.63; N, 14.02.

The crude chloride (syrupy, 2.5 g.) was dissolved in H_2O (1 cc.) and 50% KI solution (2.5 g.) was added with cooling. The yellow precipitate (2.2 g.) was collected and recrystallized from MeOH or 0.1% HCl solution to pale yellow scales of the iodide, m.p. 137~138°. *Anal.* Calcd. for $C_{16}H_{32}O_2N_2Cl_2I_2$: C, 31.54; H, 5.29; N, 4.60. Found : C, 31.64; H, 5.21; N, 4.57.

Catalytic Reduction of (IV)——The iodide of the subject compound (0.61 g.) was suspended in hot H_2O (6 cc.) and shaken with AgCl (freshly prepared from 0.8 g. of $AgNO_3$) for 30 min. After AgI was filtered off and washed with hot H_2O (1 cc.) on the filter, the filtrate was shaken with H_2 at room temperature over Pd-C catalyst (prepared from 0.2 g. of charcoal and 4 cc. of 0.5% $PdCl_2$). During 1 hr., 41 cc. of H_2 was absorbed (91% of the calculated amount). From the filtrate of the reaction mixture, a picrate was isolated by additon of sodium picrate and recrystallized from MeOH, m.p. 132~135°; yield, 0.8 g. *Anal.* Calcd. for $C_{28}H_{40}O_{16}N_8Cl_2$: C, 41.23; H, 4.94; N, 13.74. Found : C, 41.23; H, 4.95; N, 13.98.

Determination of Cl^- Liberation and Thiosulfate Consumption in $NaHCO_3$-buffered Solution——Titrations were carried out by the procedure completely analogous to those described in the preceding report.[11]

The authors wish to express their gratitude to Prof. T. Yoshida, Dr. H. Satoh, and Mr. H. Imamura for their advices and technical collaboration, especially in the animal experiments. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education and from the Ministry of Health and Welfare, to which the anthors' thanks are due.

Summary

N,N-Bis(2-chloroethyl)isoxazolidinium chloride and its related compounds were prepared, and their latent anti-tumor activity was tested on Yoshida sarcoma.

(Received September 29, 1960)

11) M. Ishidate, *et al.* : This Bulletin, **6**, 164 (1958).