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132. Yutaka Kuwada: Studies on Carcinostatic Substances. XXXI.*1 Anti-tumor Action of 1-Dialkylamino-2,3-dichloropropanes and 2-Dialkylamino-1,3-dichloropropanes.

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In 1956, Haddow and Ross[1] reported the notable observation that 2-chloroethyl methanesulfonate exhibited anti-tumor activity against Walker rat carcinoma. This compound possessed one extremely active alkylating group in the form of methanesulfonate at one end and one chlorine atom at the other end of the molecule. It has, however, been known that a chlorine atom is not active enough to induce biological effect on tumor unless it was located at a position β to nitrogen or sulfur atom. It was therefore suggested by the English workers that a methanesulfonic acid group of this compound reacted primarily with amino or mercapto group of the cell constituents and the newly formed 2-chloroethylamino or 2-chloroethylthio group takes part in the second reaction, thus completing the bifunctional alkylation. However, it seemed rather contradictory to find in the same paper that ethyl methanesulfonate itself was found to be similarly active against the same tumor and therefore the mode of action of this type of compound was not yet clearly explained.

In the present series of work, the two compounds in question were prepared according to the method reported by Haddow[1] and their anti-tumor activity was tested, but later examinations of these compounds on Yoshida sarcoma rats gave contrary result. They neither affected the tumor by any means with dosage up to LD_{50} nor consumed more than 1 molar equivalent of thiosulfate *in vitro* even after 24-hour incubation.

For the purpose of investigating the possible bifunctional alkylation of this type of compounds, this paper deals with the preparation of several derivatives of 1-dialkylamino-2,3-dichloropropane and the examination of their chemical and biological properties.

It is well known that a chlorine atom in γ-position of nitrogen is not only biologically inactive but also inert against alkylating reaction at 37° with thiosulfate in a neutral aqueous solution. From this aspect, 1-dialkylamino-2,3-dichloropropanes would be regarded as one of typical monofunctional agents, but there was an anticipation that it might react bifunctionally in a solution, in which amino or mercapto group was present in abundance, according to the following reaction scheme:

$$R_2N\text{-}CH_2\text{-}\underset{Cl}{CH}\text{-}\underset{Cl}{CH_2} \xrightarrow{HY} R_2N\text{-}CH_2\text{-}\underset{Y}{CH}\text{-}\underset{Cl}{CH_2} \xrightarrow{HY} R_2N\text{-}CH_2\text{-}\underset{Y}{CH}\text{-}\underset{Y}{CH_2}$$

Y : R-NH- or R-S-

If the primary alkylation occurred with the primary amino group of the cell constituents, 2-chloroethylated secondary amine might be formed, which was usually anticipated to have only a weak activity in tumor inhibition contrary to the tertiary amine derivatives. According to Torigoe,[2] however, the alkylation of L-cysteine by nitrogen mustard proceeded preferentially with mercapto group than amino or carboxyl group. It could therefore be supposed that the alkylation proceeds more easily with mercapto group than the

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*1 This paper constitutes a part of a series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XXX: This Bulletin, 8, 732(1960).

*2 26 Nishigahara 1-Chome, Kita-ku, Tokyo (桑田 胖).

1) A. Haddow, W. C. J. Ross: Nature, **177**, 995(1956).

2) M. Torigoe: This Bulletin, **1**, 349(1953).

others even in a more complex reaction medium *in vivo*, and that the strong activity of 2-chloroethyl group attached to β-position of sulfur atom is without question. The series of compounds prepared are listed in Table I.

Table I.

	Compound	*in vitro*[b] (mg./kg.)				*in vivo*[c]	
		CE	LD_{50}	MTD	MED	CE	MEC (m*M*)
(I)	$(CH_3)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	+	175	100	100	−	
(II)	$(C_2H_5)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	+	175	100	50	+	2.5×10^{-4}
(III)	$(C_2H_5)_2N\cdot CH_2\cdot CHBr\cdot CH_2Br$	+	175	100	50		
(IV)	$(C_4H_9)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	−	175	100		−	
(V)	$O(CH_2CH_2)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	+	175	100	50	−	
(VI)	$(C_6H_{11})_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	−	375	250		−	
(VII)	$(C_6H_5CH_2)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$	−	750	500		−	
(VIII)	[a]					−	
(IX)	$(ClCH_2CH_2)_2N\cdot CH_2\cdot CHCl\cdot CH_2Cl$					+	2.5×10^{-4}
(X)	$CH_3N(CH_2CHCl\cdot CH_2Cl)_2$	+	175	100	50	+	5×10^{-3}
(XI)	$C_2H_5N(CH_2CHCl\cdot CH_2Cl)_2$						
(XII)	$(C_2H_5)_2N\cdot CH(CH_2Cl)_2$	+	175	100	50	+	2.5×10^{-2}
(XIII)	$(CH_3)_2N\cdot C(CH_3)(CH_2Cl)_2$	−	175	100		−	
(XIV)	$CH_3SO_2\cdot O\cdot C_2H_5$	−	350	200			
(XV)	$CH_3SO_2\cdot O\cdot CH_2\cdot CH_2Cl$	−	75	50			

All animal tests were carried out with the hydrochloride on Yoshida sarcoma.
a) 2,2-Diethyl-4-chloroisoxazolidinium chloride.
b) Yoshida sarcoma (i. p.). CE : Cytomorphological effect.
MTD : Maximum tolerance dose. MED : Minimum effective dose.
c) Yoshida sarcoma. MEC : Minimum effective concentration.

Preparation of the corresponding dihydroxylamine was carried out by the reaction of glycidol with dialkylamine and chlorination of the vicinal two hydroxyl groups was attained by boiling with thionyl chloride. The other procedure of preparation was the addition of chlorine or bromine to dialkylallylamine. In order to obtain the N-oxides of the corresponding chloro derivatives, 1-diethylamino-2,3-dichloropropane (II) was oxidized with peracid but resulted in formation of 2,2-diethyl-4-chloroisoxazolidinium chloride alone instead of the N-oxide of (II) and this quaternary salt was proved to have no anti-tumor activity on account of being reduced *in vivo* to 1-diethylamino-2-chloro-3-hydroxypropane.

Most of the hydrochlorides of 1-dialkylamino-2,3-dichloropropane were crystalline and had no vesicant activity on the skin or mucous membrane. The compounds liberated approximately 1 molar equivalent of halogen ion within 2 hours at 37° in an aqueous solution. On the contrary, the thiosulfate uptake of the same compounds reached 1.5 molar equivalents or more within 2 hours (in case of (II) and (III), approximately 2 molar equivalents), as shown in Table II, and these values were regarded to be sufficient to expect anti-tumor activity from these compounds.

Table II. Thiosulfate Consumption (A)[a] and Cl^- Liberation (B)[a]

		10 min.	2 hr.	24 hr.			10 min.	2 hr.	24 hr.
(I)	(A)	0.09	1.07	1.83	(V)	(A)	0.00	0.14	1.54
	(B)	0.02	0.44	1.31		(B)	0.00	0.17	
(II)	(A)	0.39	1.72	1.88	(XII)	(A)	1.07	1.90	1.92
	(B)	0.41	1.10	1.50		(B)	0.46	1.02	1.49
(III)	(A)	1.70	1.89	1.89	(XIII)	(A)	0.98	1.73	1.73
	(B)	1.04	1.24			(B)	0.51	1.09	1.97
(IV)	(A)	0.12	1.39	1.86	(XIV)	(A)[b]	0.07	0.19	0.78
	(B)	0.04	1.02		(XV)	(A)[b]	0.00	0.06	0.74

a) Molar equivalent values in $NaHCO_3$-buffered solution.
b) Acetone-water (1:1) was used as the solvent.

The animal experiments showed however that, in spite of the bifunctional alkylating activity of the compounds which seemed comparable to that of the ordinary tertiary bis-(2-chloroethyl)amine, these 2,3-dichloropropyl derivatives were only slightly or not effective against the tumor, as indicated in Table I. The reason for it is not yet clear but it seemed to suggest that, in order for this kind of compounds to exhibit anti-tumor activity, it might be necessary to have not only the bifunctional activity but also a certain distance between the two reactive chlorine atoms.

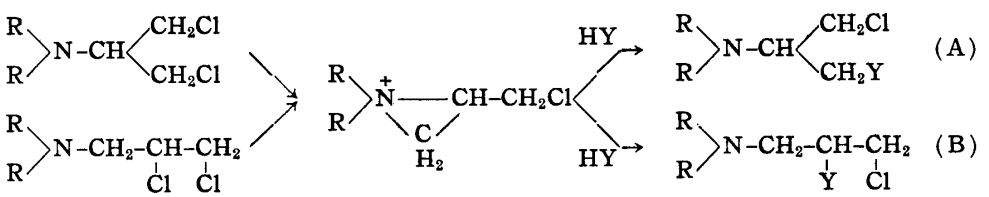
A similar observation was reported in 1957 by Ishidate, *et al.*[3] that anti-tumor activity of disulfonate of polymethyleneglycol slowed down almost to zero if the methylene bridge was cut down to ethylenic C-C chain.

From this point of view, the investigation was extended to 2-dialkylamino-1,3-dichloropropanes, because they had intermediate distance of the two chlorine atoms between that of the above stated 2,3-dichloropropylamines and ordinary nitrogen mustards.

2-Dialkylamino-1,3-dichloropropanes were synthesized by chlorination of the corresponding dihydroxylamine. 2-Dialkylamino-1,3-propanediol was produced by reduction of diethyl diethylaminomalonate with lithium aluminium hydride and 2-dimethylamino-2-methyl-1,3-propanediol by methylation of 2-amino-2-methyl-1,3-propanediol. As shown in Table II, they consumed approximately 2 molar equivalents of thiosulfate within 2 hours but their Cl^- liberation seemed to be slower than the ordinary N-alkyl-bis(2-chloroethyl)-amines. Although a promising anti-tumor efficacy of these compounds was anticipated from the rate of alkylation *in vitro*, the experiment on tumor animals resulted in disappointment. (XII) was only weakly effective and (XIII) almost ineffective. Even in this case the distance in question was not yet long enough.

In 1958, Preussmann[4] alluded to the anti-tumor activity of (XIII), stating that it was almost inert in biological activity and the inertness might be due to the strong basic character of amino group of these compounds. He stated that they might remain *in vivo* in the form of a salt instead of the free base. It is believed, however, that the problem of distance between two chlorine atoms should not be neglected, because these compounds have high alkylating activity in a neutral aqueous solution.

As had been reported by Cromwell, *et al.*[5] in 1955, 2-piperidino-1,3-dichloropropane hydrochloride underwent rearrangement to 1-piperidino-2,3-dichloropropane hydrochloride on being heated to its melting point. He also stated that 1-dimethylamino-2,3-dichloropropane afforded 1-dimethylamino-2,3-dipiperidinopropane when the former was refluxed in ethanol with an excess of piperidine for 40 hours, but whether the product was dimethylamino-1,3-dipiperidinopropane or 1-dimethylamino-2,3-dipiperidinopropane they did not make clear. In connection with their results, it might be supposed that 2-dialkylamino-1,3-dichloropropane and 1-dialkylamino-2,3-dichloropropane might behave quite similarly even in milder condition as that *in vivo*, and a proposed scheme of this reactions is shown as follows :



Y : OH^- (hydrolysis), $S_2O_3^-$ (thiosulfate uptake)

3) M. Ishidate, Y. Sakurai, S. Owari : This Bulletin, **5**, 203(1957).
4) R. Preussmann : Arzneimittel Forschung, **8**, 638(1958).
5) N. H. Cromwell, A. Hassner : J. Am. Chem. Soc., **77**, 1568(1955).

In order to solve this question, periodic acid oxidation was carried out on the hydrolysis products of both compounds (I and XIII), obtained by keeping the dichloro compounds in a neutral aqueous solution at 37° for 4 days, and the results indicated the presence of nearly equal amounts of the 1,2-glycol in both hydrolysates, as presented in Table III.

Table III. Periodic Acid Oxidation of the Hydrolysate of (I) and (XIII)

Compound	HIO_4 Uptake (mol. equiv.)	
	4 hr.	24 hr.
(I)	0.48	0.48
(XIII)	0.39	0.48

These analytical results seemed to agree with the fact that they had just the same grade of toxicity and efficacy on Yoshida sarcoma rats. However, comparing the data of Cl^- liberation and thiosulfate uptake of each type of the compounds, it seemed still early to conclude that the two different kinds of dialkylamino-dichloropropane should react by the common mode of alkylation *in vivo* when administered to animals bearing the tumor.

At any rate, the trial to improve the selective action of nitrogen mustard derivatives on tumor by restricting their alkylation process or mode was not successful.

In 1956, Larionov[6] reported that N,N-bis(2-chloroethyl)-2-chloropropylamine hydrochloride seemed to have a better efficacy on some experimental tumors than the original N-methyl-bis(2-chloroethyl)amine hydrochloride. Several new derivatives of nitrogen mustard having 2-chloropropyl group and also their N-oxides were added to the present investigation, especially from interest on the correlation between the alkylating activity of chlorine attached to secondary carbon and the tumor inhibiting action. As shown in Tables IV and V, it was concluded, however, that the velocity of formation of the intermediate (aziridinium form) and the following alkylation of both types of 2-chloroalkyl group were found to be not so widely different as expected. Only (XX) was worth noting, because it had an extremely large chemotherapeutic index (LD_{50}/MED). As regards the concrete animal experiment on this compound, the investigation has not yet been completed.

Table IV.

	Compound	*in vivo* (mg./kg.)				*in vitro*	
		CE	LD_{50}	MTD	MED	CE	MEC (m*M*)
(XVI)	$(C_2H_5)_2N\cdot CH_2\cdot CHCl\cdot CH_3$	−	175	100		−	
(XVII)	$CH_3N(CH_2\cdot CHCl\cdot CH_3)_2$	+	3	1	0.5	+	10^{-3}
(XVIII)	its oxide	+	300	100	5	+	10^{-1}
(XIX)	$HN(CH_2\cdot CHCl\cdot CH_3)_2$	+	100	50	10	+	2.5×10^{-3}
(XX)	$N(CH_2\cdot CHCl\cdot CH_3)_3$	+	30	10	0.01	+	5×10^{-3}
(XXI)	$CH_3\cdot CHCl\cdot CH_2\cdot N(CH_2\cdot CH_2Cl)_2$	+	0.3	0.1	0.01	+	5×10^{-3}
(XXII)	its oxide	+	3	1	0.5	+	2.5×10^{-3}
(XXIII)	$[(CH_3\cdot CHCl\cdot CH_2)_2N\cdot CH_2-]_2$	+	30	10	0.05	+	2.5×10^{-3}
(XXIV)	$(C_2H_5)_2N\cdot CH_2\cdot CH_2Cl$	−	(30∼50)			−	
(XXV)	$CH_3\cdot N(CH_2\cdot CH_2Cl)_2$	+	1.6	1.0	0.05	+	2.5×10^{-4}
(XXVI)	its oxide	+	80	40	1	−	
(XXVII)	$HN(CH_2\cdot CH_2Cl)_2$	+	300	100	50	+	1×10^{-2}
(XXVIII)	$N(CH_2\cdot CH_2Cl)_3$	+	0.75	0.5	0.01	+	5×10^{-4}
(XXIX)	$[(ClCH_2\cdot CH_2)_2N\cdot CH_2-]_2$	+	2	1	0.1	+	5×10^{-3}

The same abbreviations as in Table I are used.

6) L. F. Larionov: Brit. J. Cancer, **10**, 26(1956); Acta Unio Internationalis Contra Cancrum, **15**, 171(1959).

Table V. Thiosulfate Consumption (A)* and Cl^- Liberation (B)*

		10 min.	2 hr.	24 hr.			10 min.	2 hr.	24 hr.
(XVI)	(A)	0.07	0.75	0.94	(XXIII)	(A)	0.46	3.17	3.48
	(B)	0.59	0.93	0.96		(B)	0.68	2.60	3.36
(XVII)	(A)	1.32	1.90	1.94	(XXIV)	(A)	0.18	0.93	0.99
	(B)	0.98	1.54	1.96		(B)	0.38	0.90	0.96
(XVIII)	(A)	0.13	0.86	1.26	(XXV)	(A)	0.92	1.88	1.88
	(B)	0.07	0.89	1.38		(B)	1.11	1.54	2.00
(XIX)	(A)	0.13	0.76	0.83	(XXVI)	(A)	0.02	0.98	1.69
	(B)	0.75	0.98	1.17		(B)	0.24	0.79	1.59
(XX)	(A)	1.32	2.37	2.50	(XXVII)	(A)	0.00	0.03	0.04
	(B)	0.26	0.91	2.15		(B)	0.79	0.97	0.98
(XXI)	(A)	0.70	2.92	2.92	(XXVIII)	(A)	1.44	2.89	2.99
	(B)	1.09	2.78	2.88		(B)	1.04	2.52	2.82
(XXII)	(A)	0.96	1.86	1.90	(XXIX)	(A)	0.28	1.60	3.60
	(B)	0.51	1.01	1.41		(B)	1.51	2.75	3.36

* Molar equivalent values in $NaHCO_3$-buffered solution at 37°.

Table VI. Miscellaneous $RN(CH_2 \cdot CH_2 \cdot Cl)_2$

	R	*in vivo* (mg./kg.)			
		CE	LD_{50}	MTD	MED
(XXXI)	$C_6H_5CH(CH_3)$-	+	30	10	0.1
(XXXII)	C_6H_{13}-	+	30	10	0.1
(XXXIII)	C_7H_{15}-	+	30	10	1
(XXXIV)	$C_{18}H_{37}$-	−	30	10	

The same abbreviations as in Table I are used.

Experimental

Preparation of 1-Dialkylaminopropane-2,3-diol—The diols were generally prepared by warming a mixture of the corresponding secondary amines with the molar equivalent amount of glycidol under stirring.

1-Morpholinopropane-2,3-diol[*3]—Pale yellow oil, $b.p_6$ 152~154°.

1-Dicyclohexylaminopropane-2,3-diol—From dicyclohexylamine (36 g.) and glycidol (100 g.), 44 g. of the diol was obtained, white prisms, m.p. 74~75°, from ligroine. *Anal.* Calcd. for $C_{15}H_{29}O_2N$: C, 70.54; H, 11.45; N, 5.48. Found : C, 70.43; H, 11.36; N, 5.51.

1-Dibenzylaminopropane-2,3-diol—From dibenzylamine (25 g.) and glycidol (10 g.), 32 g. of the diol was obtained as white needles, m.p. 47~48°, from ligroine. *Anal.* Calcd. for $C_{17}H_{21}O_2N$: C, 75.24; H, 7.80; N, 5.16. Found : C, 75.16; H, 8.10; N, 5.13.

Preparation of 1-Dialkylamino-2,3-dichloropropane—A mixed solution of 1-dialkylaminopropane-2,3-diol and $SOCl_2$ in $CHCl_3$ was refluxed for about 2 hr. After removing the solvent under a reduced pressure, the residue was purified by recrystallization or by conversion into the picrate if necessary.

1-Dimethylamino-2,3-dichloropropane Hydrochloride[7] (I)—White plates, m.p. 165~166°, from EtOH. Picrate, m.p. 101~102°.

1-Diethylamino-2,3-dichloropropane Hydrochloride (II)—From a mixture of 1-diethylaminopropane-2,3-diol (29 g.), $CHCl_3$ (40 cc.), and $SOCl_2$ (60 cc.), 65 g. of the picrate of (II) was obtained as yellow plates, m.p. 96~97°, from EtOH. *Anal.* Calcd. for $C_{13}H_{18}O_7N_4Cl_2$: C, 37.78; H, 4.39; N, 13.56. Found: C, 37.89; H, 4.34; N, 13.75.

It showed no m.p. depression with the picrate obtained by chlorination of N,N-diethyl-allylamine. The hydrochloride recovered from the picrate was recrystallized from Me_2CO to white prisms, but its m.p. was not determined because of its hygroscopic property. *Anal.* Calcd. for $C_7H_{16}NCl_3$: C, 38.11; H, 7.31; N, 6.35. Found : C, 38.17; H, 6.94; N, 6.31.

*3 cf. Swiss Pat. 223, 161 (C. A., **43**, 1812(1949)). It was prepared from morpholine and ethylenechlorohydrin but the physical constants were not given.

7) L. A. Amundsen, L. S. Pitts : J. Am. Chem. Soc., **73**, 1494(1951).

1-Dibutylamino-2,3-dichloropropane Hydrochloride (IV)—From a mixture of 1-dibutylaminopropane-2,3-diol (30 g.), $CHCl_3$(60 cc.), and $SOCl_2$(30 cc.), 28 g. of the free base of (IV) was obtained. $b.p_7$ 120～124°. *Anal.* Calcd. for $C_{11}H_{23}NCl_2$: C, 55.00; H, 9.65; N, 5.83. Found : C, 55.56; H, 9.50; N, 5.80.

The hydrochloride was very hygroscopic crystals and could not be completely purified. Picrate: Yellow prisms, m.p. 94～95°, from EtOH. *Anal.* Calcd. for $C_{17}H_{26}O_7N_4Cl_2$: C, 43.51; H, 5.58; N, 11.94. Found : C, 43.58; H, 5.54; N, 11.93.

1-Morpholino-2,3-dichloropropane Hydrochloride (V)—A crude hydrochloride, obtained from morpholinopropane-2,3-diol (15 g.) and $SOCl_2$(45 cc.), was purified as the picrate of yellow needles, m.p. 89～90°, from dil. EtOH. Yield, 30 g. *Anal.* Calcd. for $C_{13}H_{16}O_8N_4Cl_2$: C, 36.55; H, 3.77; N, 13.12. Found : C, 36.85; H, 3.68; N, 13.25.

Hydrochloride : Very hygroscopic white crystals, m.p. 115°. It decomposed at 190°. *Anal.* Calcd. for $C_7H_{14}ONCl_3$: C, 35.84; H, 6.02; N, 5.97. Found : C, 36.18; H, 6.11; N, 5.97. Free base: $b.p_8$ 104～106°.

1-Dicyclohexylamino-2,3-dichloropropane Hydrochloride (VI)—From a mixture of 1-dicyclohexylaminopropane-2,3-diol (39 g.), $CHCl_3$(110 cc.), and $SOCl_2$(50 cc.), 60 g. of the picrate of (VI) was obtained as yellow prisms, m.p. 129～130°, from EtOH. *Anal.* Calcd. for $C_{21}H_{30}O_7N_4Cl_2$: C, 43.38; H, 5.80; N, 10.75. Found : C, 43.86; H, 5.69; N, 10.79.

Hydrochloride : White plates, m.p. 159～160°, from Me_2CO. *Anal.* Calcd. for $C_{15}H_{23}NCl_3$: C, 54.80; H, 8.58; N, 4.26. Found : C, 54.61; H, 8.48; N, 4.39.

1-Dibenzylamino-2,3-dichloropropane Hydrochloride[5] (VII)—White prisms, m.p. 154～155°, from EtOH.

1-Diethylamino-2,3-dibromopropane Hydrochloride (III)—N,N-Diethyl-allylamine (1.5 g.) dissolved in CCl_4(5 cc.) was treated under cooling with Br_2(2 g.) dissolved in CCl_4(10 cc.). The mixture was kept standing for 2 days at room temperature. After removing the solvent under a reduced pressure, the residue was purified as the picrate of yellow plates, m.p. 89～90°, from 50% EtOH. Yield, 4 g. *Anal.* Calcd. for $C_{13}H_{18}O_7N_4Br_2$: N, 11.16. Found : N, 11.16, 10.89.

Picrylsulfonate : Pale yellow needles, m.p. 143～144°, from dil. EtOH. *Anal.* Calcd. for $C_{13}H_{18}O_9N_4Br_2S$: C, 27.58; H, 3.20; N, 9.90. Found : C, 27.98; H, 3.28; N, 9.94. For the animal experiment, the syrupy hydrochloride was prepared from the picrate, which could not be crystallized.

Oxidation of 1-Diethylamino-2,3-dichloropropane— A benzene solution of the free base of (II) (33 g.) was oxidized with 30% H_2O_2(20 cc.) and Ac_2O(18 g.) by the method previously reported.[8]

The product (VIII) was purified as the picrate of yellow needles, m.p. 145～146°, from EtOH. Yield, 22 g. It was positive to the Beilstein halogen test but negative to KI-starch reagent and was identified with 2,2-diethyl-4-chloroisoxazolidinium salt by analysis. *Anal.* Calcd. for $C_{13}H_{17}O_8N_4Cl$: C, 39.75; H, 4.36; N, 14.26. Found : C, 39.74; H, 4.27; N, 13.91.

N,N-Bis(2-hydroxyethyl)-2,3-dihydroxypropylamine—Prepared by heating a mixture of diethanolamine (21 g.), glycidol (15 g.), and a small amount of H_2O, $b.p_{0.02}$ 190～195°. Yield, 38 g. *Anal.* Calcd. for $C_7H_{17}O_4N$: C, 46.91; H, 9.56; N, 7.82. Found : C, 46.47; H, 9.10; N, 7.68.

N,N-Bis(2-chloroethyl)-2,3-dichloropropylamine Hydrochloride (IX)—The above tetra-ol (10 g.) dissolved in $CHCl_3$(50 cc.) was treated with $SOCl_2$(40 cc.). After removing the solvent and reagent, the residue was basified with NaOH and extracted with Et_2O. Distillation of the extract gave the free base of (IX) as a yellow oil, $b.p_7$ 145～146°. Yield, 7 g.

Picrate : Yellow needles, m.p. 67～68°, from AcOEt-ligroine. *Anal.* Cacld. for $C_{13}H_{16}O_7N_4Cl_4$: N, 11.62. Found : N, 11.40.

Picrylsulfonate : White needles, m.p. 157～158°, from EtOH. *Anal.* Calcd. for $C_{13}H_{16}O_9N_4Cl_4S$: C, 28.59; H, 2.95; N, 10.26. Found : C, 28.82; H, 2.67; N, 10.05. The hydrochloride was not recrystallized.

N-Methyl-bis(2,3-dichloropropyl)amine Hydrochloride (X)—The crude N-methyl-bis(2,3-dihydroxypropyl)amine (10 g.) in $CHCl_3$(30 cc.) was chlorinated with $SOCl_2$(40 cc.) by the usual procedure. After chlorination and evaporation of the solvent and reagent, the residue was basified with NaOH and extracted with Et_2O. Free amine, $b.p_6$ 141～142°. Yield, 9 g.

Hydrochloride : Hygroscopic crystals, m.p. 123～124°, from Me_2CO. *Anal.* Calcd. for $C_7H_{14}NCl_5$: C, 29.04; H, 4.87; N, 4.84. Found : C, 29.43; H, 4.87; N, 4.64.

Picrate : Yellow plates, m.p. 78～79°, from H_2O. *Anal.* Calcd. for $C_{13}H_{14}O_7N_4Cl_4$: C, 32.38; H, 3.35; N, 11.62. Found : C, 32.28; H, 3.40; N, 11.71.

N-Ethyl-bis(2,3-dihydroxypropyl)amine—From glycidol (30 g.), diethylamine (10 g.), and a small quantity of H_2O, 33 g. of the tetra-ol was obtained, $b.p_{0.02}$ 175～176°.

N-Ethyl-bis(2,3-dichloropropyl)amine Hydrochloride (XI)—A mixture of the tetrahydroxy-amine (14 g.) and $CHCl_3$(50 cc.) was treated with $SOCl_2$(45 cc.) by the usual procedure. Basification of the chlorinated product and subsequent extraction with Et_2O yielded the free base as a colorless oil,

8) Y. Sakurai, M. Izumi : This Bulletin, **1**, 300(1953).

b.p_7 137～139°. Yield, 11 g. *Anal.* Calcd. for $C_8H_{15}NCl_4$: C, 35.98; H, 5.66; N, 5.25. Found: C, 36.17; H, 5.33; N, 5.35. The hydrochloride has not so far crystallized.

Diethyl Diethylaminomalonate—It was prepared by the reaction of diethylamine (36 g.) and diethyl bromomalonate (58 g.) in EtOH (100 cc.), b.p_8 110～113°. Yield, 25 g. *Anal.* Calcd. for $C_{11}H_{21}O_4N$: N, 6.06. Found: N, 5.92.

2-Diethylaminopropane-1,3-diol—A dry Et_2O solution (100 cc.) of the above ester (10 g.) was dropped into a dry Et_2O solution of $LiAlH_4$ (5 g.) and the mixture was refluxed for 1 hr. with stirring. After cooling and decomposition of excess of $LiAlH_4$ with H_2O, Et_2O was evaporated *in vacuo* and the residue was extracted with hot MeOH. The MeOH extract was added with MeOH solution of oxalic acid and the precipitate was filtered off. Concentration of the filtrate *in vacuo*, separated an orange-yellow oil. An adequate amount of $Ba(OH)_2$ solution was added and the precipitate was filtered off. The residue obtained by evaporation of the filtrate was extracted with hot EtOH. The pale yellow viscous oil was obtained by evaporation of the extract, b.$p_{0.1}$ 110°. Yield, 3.5 g. *Anal.* Calcd. for $C_7H_{17}O_2N$: N, 9.52. Found: N, 8.90.

1,3-Dichloro-2-diethylaminopropane Hydrochloride (XII)—A mixture of the above dihydroxyamine (3 g.) and $CHCl_3$ (20 cc.) was treated with $SOCl_2$ (8 cc.). The chlorinated product was purified as a picrate of yellow plates, m.p. 91～92°, from EtOH. *Anal.* Calcd. for $C_{13}H_{18}O_7N_4Cl_2$: C, 37.78; H, 4.39; N, 13.56. Found: C, 37.99; H, 4.33; N, 13.24. The hydrochloride was crystalline but hygroscopic and could not be purified.

1,3-Dichloro-2-dimethylamino-2-methylpropane Hydrochloride[*4] **(XIII)**—Hygroscopic white plates, m.p. 126～127°, from Me_2CO.

Picrate: Yellow prisms, m.p. 156～157°, from EtOH.

2-Chloroethyl Methanesulfonate[1] **(XV)**—Methanesulfonyl chloride (3.6 g.) was dropped at 0° into a solution of ethylenechlorohydrin (6 g.) in dehyd. pyridine (13 cc.). After filtering off the precipitated pyridine hydrochloride, the filtrate was dissolved in Et_2O. The solution was washed with HCl and H_2O, and dried over anhyd. Na_2SO_4. After evaporation of the solvent, the residue was distilled *in vacuo*. Colorless oil, b.p_{15} 138～139°. Yield, 3 g.

N,N-Diethyl-2-chloropropylamine Hydrochloride[*5] **(XVI)**—Very hygroscopic crystals, m.p. 100～101°, from Me_2CO. *Anal.* Calcd. for $C_{13}H_{19}O_7N_4Cl$: C, 41.22; H, 5.06; N, 14.79. Found: C, 41.27; H, 4.71; N, 14.55.

Picrate: m.p. 126～127°, from EtOH.

N-Methyl-bis(2-chloropropyl)amine Hydrochloride[*6] **(XVII)**—White prisms, m.p. 104°, from Me_2CO. *Anal.* Calcd. for $C_7H_{16}ONCl_3$: C, 35.54; H, 6.82; N, 5.92. Found: C, 35.54; H, 6.74; N, 6.04.

Picrate: Yellow prisms, m.p. 107°, from EtOH.

N-Methyl-bis(2-chloropropyl)amine N-Oxide Hydrochloride (XVIII)—AcONa (0.9 g.) was dissolved in a mixture of Ac_2O (4.3 g.) and 30% H_2O_2 (5 g.) with caution under cooling. Into this solution, (XVII) (2.5 g.) was added in small portions at 10° and the mixture was kept at the same temperature for 3 hr. The temperature was then raised to 30° during 1 hr. under stirring. After acidification with conc. HCl to strong acidity, the reaction mixture was evaporated to dryness under a reduced pressure below 30° and extracted with hot Me_2CO. Removal of Me_2CO gave the N-oxide hydrochloride. It was purified by converting it to a picrate. Picrate: Yellow prisms, m.p. 109～110°, from EtOH. Yield, 3.5 g. *Anal.* Calcd. for $C_{13}H_{15}O_8N_4Cl_3$: C, 36.63; H, 3,55; N, 13.15. Found: C, 36.58; H, 3.77; N, 13.07.

Hydrochloride: White plates, m.p. 117～118°, from Me_2CO. *Anal.* Calcd. for $C_7H_{16}ONCl_3$: C, 35.54. H, 6.82; N, 5.92. Found: C, 35.54; H, 6.74; N, 6.04.

N,N-Bis(2-chloropropyl)amine Hydrochloride (XIX)—White prisms, m.p. 200～201°, from EtOH.

N,N,N-Tris(2-chloropropyl)amine Hydrochloride[9] **(XX)**—White prisms, m.p. 126～127°,[*7] from Me_2CO. *Anal.* Calcd. for $C_9H_{19}NCl_4$: C, 38.18; H, 6.76; N, 4.95. Found: C, 37.86; H, 6.57; N, 4.81.

Picrate: Yellow prisms, m.p. 127～128°, from EtOH.

N,N-Bis(2-chloroethyl)-2-chloropropylamine Hydrochloride[10] **(XXI)**—White prisms, m.p. 72～73°, from EtOH. Picrate: Yellow plates, m.p. 80～81°, from EtOH. Free base: Colorless oil, b.p_5 106～107°.

N,N-Bis(2-chloroethyl)-2-chloropropylamine N-Oxide Hydrochloride (XXII)—Oxidation was carried out as in the case of (XVIII). The product was purified as the picrate of yellow plates, m.p. 102～

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*4 E. R. H. Jones and W. Wilson (J. Chem. Soc., **1949**, 550) reported the free base as b.$p_{2.3}$ 52～53° and the picrate as m.p. 158°.

*5 R. C. Ederfield, *et al.* (J. Am. Chem. Soc., **68**, 1579(1946)) reported the free base as b.p_{50} 79～80°.

*6 W. E. Handy and N. Rydon (J. Chem. Soc., **1947**, 513) reported the free base as b.p_6 103～104°.

*7 E. Wilson, *et al.* (*loc. cit.*) reported its hemihydrate as m.p. 112～113°.

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

10) A. H. Ford Moore, A. G. Lidstone, W. A. Walters: J. Chem. Soc., **1946**, 819.

103°, from EtOH. Yield, 30%. *Anal.* Calcd. for $C_{13}H_{16}O_8N_4Cl_3$: C, 33.75; H, 3.49; N, 12.11. Found : C, 33.73, 33.73; H, 3.49, 3.55; N, 12.11, 11.91.
Hydrochloride : White needles, m.p. 111~112°, from Me_2CO. *Anal.* Calcd. for $C_7H_{15}ONCl_3$: C, 31.02; H, 5.58; N, 5.17. Found : C, 31.01; H, 5.48; N, 5.15.

In the course of reaction, an oily substance separated, which was extracted with Et_2O. After evaporation of Et_2O, a pale yellow oily residue was distilled at 122~124°, which was assumed by analysis to be N-bis(2-chloroethyl)-O-2-chloropropylhydroxylamine or N-2-chloropropyl-N-2-chloroethyl-O-2-chloroethylhydroxylamine. *Anal.* Calcd. for $C_7H_{14}ONCl_3$: C, 35.84; H, 6.02; N, 5.97. Found : C, 35.60; H, 5.71; N, 5.87.

Tetrakis(2-hydroxypropyl)ethylenediamine—A mixture of diisopropanolamine (50 g.), ethylene dibromide (35.3 g.), and anhyd. K_2CO_3 (26 g.) was heated at 100~120° for 30 hr. Extraction with hot EtOH and distillation of the extract yielded a viscous pale yellow oil, b.$p_{0.05}$ 165°. Yield, 35 g. *Anal.* Calcd. for $C_{14}H_{32}O_4N_2$: N, 9.58. Found : N, 9.36.

Tetrakis(2-chloropropyl)ethylenediamine Dihydrochloride (XXIII)—The above tetra-ol (50 g.), dissolved in $CHCl_3$ (60 cc.), was added with $SOCl_2$ (90 cc.) dissolved in $CHCl_3$ (60 cc.), and the mixture was boiled for 1 hr. After evaporation to dryness, the residue obtained was recrystallized from EtOH-Et_2O to white needles, m.p. 159~160°. Yield, 30 g. *Anal.* Calcd. for $C_{14}H_{30}N_2Cl_6$: C, 38.29; H, 6.89; N, 6.38. Found : C, 38.66; H, 6.83; N, 6.57.
Picrate : Yellow prisms, m.p. 125~126°, from EtOH. *Anal.* Calcd. for $C_{26}H_{34}O_{18}N_4Cl_4$: C, 37.88; H, 4.16; N, 13.60. Found : C, 37.89; H, 4.02; N, 13.63.

2,4-Dinitro-N,N-bis(2-chloroethyl)aniline[*8] **(XXX)**—It was prepared by stirring 2,4-dinitrofluorobenzene (0.4 g.) dissolved in EtOH, with N,N-bis(2-chloroethyl)amine hydrochloride (0.2 g.) and $NaHCO_3$ (0.6 g.) for 2 hr. Yellow needles, m.p. 119°, from MeOH. Yield, 0.2 g.

N,N-Bis(2-chloroethyl)-α-methylbenzylamine Hydrochloride (XXXI)—N-(α-Methylbenzyl)diethanolamine (20 g.) was heated with $SOCl_2$ (36 g.) and $CHCl_3$ (50 cc.). The product was purified as a picrate of yellow plates, m.p. 130~131°, from EtOH. Yield, 58 g. *Anal.* Calcd. for $C_{18}H_{20}O_7N_4Cl_2$: C, 45.48; H, 4.24; N, 11.79. Found : C, 45.41; H, 4.26; N, 11.87.
Hydrochloride : Converted from the picrate, could not be completely purified.

Determination of Cl^- Liberation and Thiosulfate Consumption in $NaHCO_3$-buffered Solution—Titrations were carried out by the procedures described in the preceding paper.[11]

Periodic Acid Oxidation of the Hydrolysate of (I) and (XIII)—Each sample (2.5 m. mole) was dissolved in H_2O (10 cc.) containing $NaHCO_3$ (5 m. mole). The mixture was kept standing at 37° for 4 days until they were almost completely hydrolyzed. Hydrolysis rate was checked by titration of Cl^- liberation in the solution. The solution was then neutralized to pH 7 with addition of dil. H_2SO_4 and added with 3 cc. of 0.54*M* HIO_4 solution. After 4 or 24 hr. it was added with an excess of 0.1*N* As_2O_3 solution and back-titrated with 0.1*N* I_2 solution. Molar equivalent values of HIO_4 consumption, indicated in Table III, were calculated from the amount of actually hydrolyzed products, determined by Cl^- liberation rate.

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Summary

1-Dialkylamino-2,3-dichloropropanes, 2-dialkylamino-1,3-dichloropropanes, and several new derivatives of nitrogen mustard having 2-chloropropyl group were synthesized and tested on Yoshida sarcoma. Mode of alkylation and hydrolysis *in vitro* of the former two types of compound were also discussed.

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[*8] W. C. Ross (J. Chem. Soc., **1949**, 183) prepared it by chlorination of 2,4-dinitro-N,N-bis(2-hydroxyethyl)aniline.

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